DEVELOPMENT, ASSESSMENT AND OPTIMISATION OF ORAL FAMCICLOVIR FORMULATIONS FOR PAEDIATRIC USE

By

Laura Magnus

A Thesis Submitted to Rhodes University in

Fulfilment of the Requirements for the Degree of

MASTER OF SCIENCE (PHARMACY)

January 2012

Faculty of Pharmacy
Rhodes University
Grahamstown
South Africa

ABSTRACT

Many Active Pharmaceutical Ingredients (API) such as the antiviral agent famciclovir (FCV) are required for paediatric treatment but are not commercially available in age-appropriate dosage forms. It is common practice to prepare oral liquid dosage forms using commercially available tablets, capsules or powdered API and then dispersing or dissolving the crushed and/or powdered materials in a vehicle that the patient can swallow. Vehicles that are commonly used for this purpose include methylcellulose, syrup or combinations of these carriers where possible or commercially available suspending agents such as Ora-Sweet®, if available, can be used. However, several critical factors are overlooked when manufacturing extemporaneous formulations including, but not limited to, physical and chemical properties of the API, excipients, compatibility, stability and bioavailability issues.

A stability-indicating High Performance Liquid Chromatography (HPLC) method for the analysis of FCV was developed and validated according to the International Conference on Harmonization (ICH) guidelines. The method is sensitive, selective, precise, accurate and linear over the concentration range 2-120 µg/ml.

The stability of 25 mg/ml FCV formulations was assessed in vehicles manufactured from syrup simplex, hydroxypropyl methylcellulose (HPMC), Ora-Sweet® and an aqueous buffer (pH 6) following storage at 25 °C/60% RH and 40 °C/75% RH over six (6) to eight (8) weeks. The shelf life of the products was calculated as the longest period of storage for approximately 90% of the added FCV to be recovered.

Formulations were manufactured using syrup simplex or HPMC with methylparaben and propylparaben individually or in combination and with sodium metabisulphite, ascorbic acid or citric acid as antioxidants. The resultant products were subject to quality control analysis for API content, viscosity, pH and appearance and the resultant data were subject to statistical analysis. The degradation rates were calculated for each product and a degradation profile plotted. The degradation rates of FCV in extemporaneous formulations were compared to those of FCV manufactured using a commercially available suspending agent and a buffered vehicle.

FCV undergoes major degradation in the presence of sucrose, as observed for formulations in which the vehicle was syrup and Ora-Sweet®. FCV was found to be most stable when dissolved/dispersed in an HPMC vehicle incorporating sodium metabisulphite and a combination of parabens. The formulation that exhibited the maximum stability was manufactured using an aqueous solution buffered to pH 6. Due to the enhanced stability of FCV when added to a buffered vehicle a

formulation in which an HPMC vehicle buffered to pH 6 with sodium metabisulphite, methylparaben and propylparaben was selected for optimisation using a Central Composite Design approach (CCD). In this way it was possible to establish a relationship between input variables such as pH, % w/v HPMC, % w/v antioxidant and % w/v preservative and the responses selected for monitoring by means of response surface modelling. A quadratic model was found to be the most appropriate to describe the relationship between input and output variables.

Thirty batches of product were randomly manufactured according to the CCD and analysed to establish the stability in respect of viscosity, pH and the amount of FCV remaining following storage and the data were fitted to models using Design-Expert® software. A correlation between input variables and the responses was best described by a quadratic polynomial model. Analysis of Variance indicated that the response surface models were significant (P-value < 0.0001). The pH to which a FCV formulation was buffered was the most significant factor to effect the % drug content and the ultimate pH of the formulation, while the % w/v HPMC had the most significant effect on the viscosity of the product.

The optimum composition for the manufacture of an oral liquid FCV formulation was predicted using the optimisation function of the Design-Expert® software. A low % error of prediction was established, indicating that the model is robust and that RSM is an appropriate formulation optimisation tool as it has a high prognostic ability.

A liquid FCV formulation was developed, optimised and found to be suitable for its intended purpose. However further optimisation is required in respect of colourants, sweeteners and/or flavourants. The approach followed is useful in ensuring the development of quality products and can be applied in future.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the following people and organisations:

The Dean, Head of the Faculty of Pharmacy and my supervisor, Prof R.B. Walker for his continuous guidance, support and assistance throughout the course of this research in "fighting the battles and winning the war", as well as for the provision of facilities to conduct my research.

My parents, Ashley Magnus for his belief in me, continuous support, suggestions and words of wisdom from his experience as a masters student, even though "things have drastically changed" and Anne Magnus for her continuous love and encouragement, and for always telling me what a "star child" I am.

My brother, Scott Magnus for all his support and words of encouragement.

Dr S.M.M. Khamanga for his assistance with Statistical Design of Experiments and Response Surface Methodology and his guidance, support and encouragement throughout my studies.

Mr T. Samkange, for his technical assistance and Mr L. Purdon, Mr C. Nontyi, Ms T. Kent and Ms L. Emslie, for their assistance and availability to help throughout the duration of my studies.

Dr S. Robertson for his time and effort in the proofreading of this thesis.

The Henderson and DAAD scholarship programmes and the Research Committee of Rhodes University for financial assistance over the duration of this research.

My colleagues and friends in the Faculty of Pharmacy for their ongoing support, help and company; Ms Bianca Dagnolo, Mr Maynard Chiwakata, Ms Tatenda Munedzimwe, Ms Ayeshah Fauzee, Dr Kasongo wa Kasongo, Dr Adrienne Müller, Ms Henusha Jhundoo, Mr Loti King'ori, Mr Tawanda Dube, Mr Tendai Chanakira, Mr Farai Mhaka, Mr Chiluba Mwila and Ms Chiedza Zindove.

The staff of the Faculty of Pharmacy for their interest, kind words of encouragement and assistance during my time as a post graduate student.

My dear friends, Mr Jason Bartie, Ms Ashleigh Eales, Ms Jenna Berndt, Ms Lynne Dell, Ms Joni Griffin, Ms Kate Brook-Hart and Ms Tarryn Liddell for their love, friendship and continuous upliftment and motivation over the years.

STUDY OBJECTIVES

Famciclovir (FCV) is the parent drug of the antiviral agent penciclovir (PCV): an acyclic nucleoside analogue and potent inhibitor of DNA synthesis used for the treatment of herpes viral infections [1; 2]. FCV has an increased bioavailability compared to other antiviral agents such as acyclovir, is rapidly and extensively absorbed from the upper gastro-intestinal tract (GIT), and undergoes first past metabolism to form the active metabolite, PCV [1-3].

However, FCV is not available in a suitable dosage form for administration to paediatric patients [4]. Therefore there is a need to manufacture extemporaneous formulations of FCV. Consequently an oral liquid FCV formulation suitable for the administration of efficacious, therapeutic and safe doses to paediatric patients was developed and assessed.

The objectives of this study were:

1. To develop and validate a Reversed-Phase High Performance Liquid Chromatographic (RP-HPLC) method having the necessary sensitivity and selectivity to accurately and precisely quantitate FCV in the presence of excipients in pharmaceutical dosage forms.

2. To conduct preformulation studies to establish the compatibility between FCV and excipients used to manufacture Famvir® tablets, Ora-Sweet® and potential excipients for an extemporaneous oral formulation.

3. To conduct preliminary formulation studies to identify a suitable formulation in terms of stability and appearance for optimisation studies to be conducted using Central Composite Design and Response Surface Methodology approaches.

4. To assess the impact of formulation and composition variables on the stability of FCV formulations in terms of content, viscosity and pH.

5. To manufacture and assess the optimised formulation to establish the shelf-life in respect of FCV content, viscosity and pH.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

FAMCICLOVIR

1.1 INTRODUCTION

Famciclovir (FCV) is a guanosine analogue that is well absorbed from the gastrointestinal tract. FCV is the parent drug of penciclovir (PCV), a potent inhibitor of viral DNA synthesis. The introduction of acyclovir (ACV) resulted in a 'new era' in the treatment of herpes viral infections. ACV is highly effective when administered intravenously, however oral use requires frequent administration of large doses of the drug at frequent intervals to ensure effective therapy since absorption and bioavailability of ACV is poor. FCV was developed as there was a need for a prodrug approach to improve the absorption of this class of antiviral compounds. FCV is converted into PCV, the active compound that has a similar degree of antiviral activity to that of ACV [2; 3; 5].

PCV is 9-(4-hydroxy-3-hydroxy-methyl but-1-yl) and is a guanine derivative similar to acycloguanosine drugs such as ACV and ganciclovir. PCV is poorly absorbed following oral administration. To improve oral absorption mono- and di-carboxylic esters of PCV were synthesised. However, none of these exhibited any significant improvement in the extent of oral absorption. The physicochemical properties of PCV are directly influenced by the polar quinine ring (Figure 1.1) and in an attempt to improve on the oral absorption of the molecule, modifications of the structure of that ring were attempted. Initially a 6-deoxy derivative of PCV was synthesised as it is readily oxidised to form PCV. However this resulted in only a modest improvement in the extent of oral absorption of the molecule. Following further evaluation the diacetyl ester of the 6-deoxy derivative was synthesised to form FCV, which exhibited significantly better absorption and was also rapidly and efficiently converted to PCV [2; 3].

1.1 DESCRIPTION

1.2.1 Name, Formula, Molecular Weight and Chemical Structure

The chemical name for FCV is 2-[2-(2-amino-9*H*-purin-9-yl)ethyl]-1,3-propanediol diacetate [6; 7]. FCV, $C_{14}H_{19}N_5O_4$, occurs as a white to pale yellow solid powder with a molecular weight of 321.3 g/mol [6] and is an acyclic guanine analogue, shown in Figure 1.1.

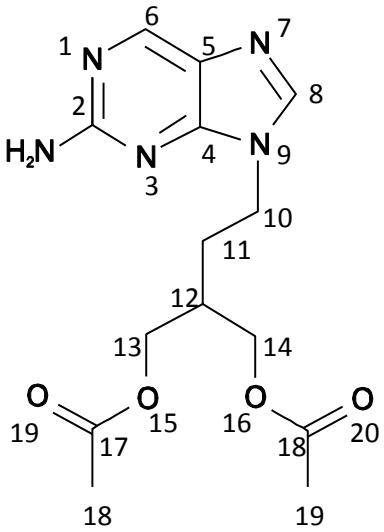


Figure 1.1 *Chemical structure of FCV ($C_{14}H_{19}N_5O_4$), MW=321.3 g/mol*

1.3 SYNTHESIS

The starting material most frequently used for the manufacture of PCV is 2-amino-6-chloropurine, which is converted to FCV, by alkylation, primarily at the N-7 and N-9 position of the purine moiety [8]. Several approaches involving changes to the C-6 substituent of 2-aminopurine to improve on the ratio of N-9:N-7 alkylation have been attempted [8]. However these reactions require multiple steps and 2-amino-6-chloro-purine is not suitable for large-scale application as it is highly mutagenic.

A schematic representation of the initial steps in the synthesis of PCV is given in Figure 1.2. Benzylation of the starting material, 2',3',5'-tri-O-acetylguanosine (I) results in the formation of a benzylated product as a bromide salt (II), which is hydrolysed in acid to form 7BnG.2HCl (III). To improve on the solubility of this compound acetylation and benzoylation are then performed, resulting in the formation of N-2-acetyl-7-benzylguanine (NAc7BnG) (IV).

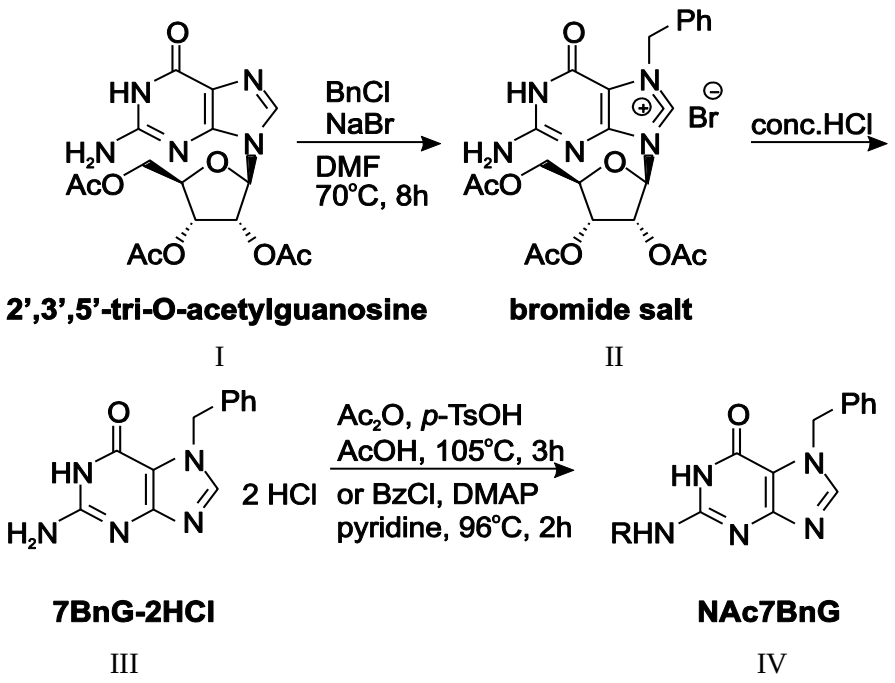


Figure 1.2 *Synthesis of NAcBnG from 2',3',5'-tri-O-acetylguanosine, adapted from [8]*

The coupling of NAC7BnG (IV) to a mesylate (V) side chain functionality at the N9 position under neutral conditions results in the production of PCV (VI) without the formation of an N7-alkylated by-product following removal of the N7 benzyl group by reduction. The coupling of NAc7BnG and the mesylate occurs selectively at the N9 position to produce the desired alkylated product within 8 hours of starting the reaction in N-methylpyrrolidone at a temperature of 120 °C. After conventional catalytic hydrogenolysis of the benzyl group and hydrolysis of the resulting acetate pure PCV is obtained. FCV is subsequently synthesised from PCV by conversion of PVC (VI) to Ac_2PCV (VII) by selective acetylation of the hydroxyl groups at positions 15 and 16. Following treatment of Ac_2PCV (VII) with phosphorus oxychloride, tetraethylammonium chloride and triethylamine at 80 °C, the intermediate 6ClFCV (VIII) is produced in a yield of 70%. The compound 6ClFCV (VIII) is then converted to FCV (IX) by dechlorination to yield 81% of the compound [9]. A graphical representation of this synthesis is shown in Figure 1.3.

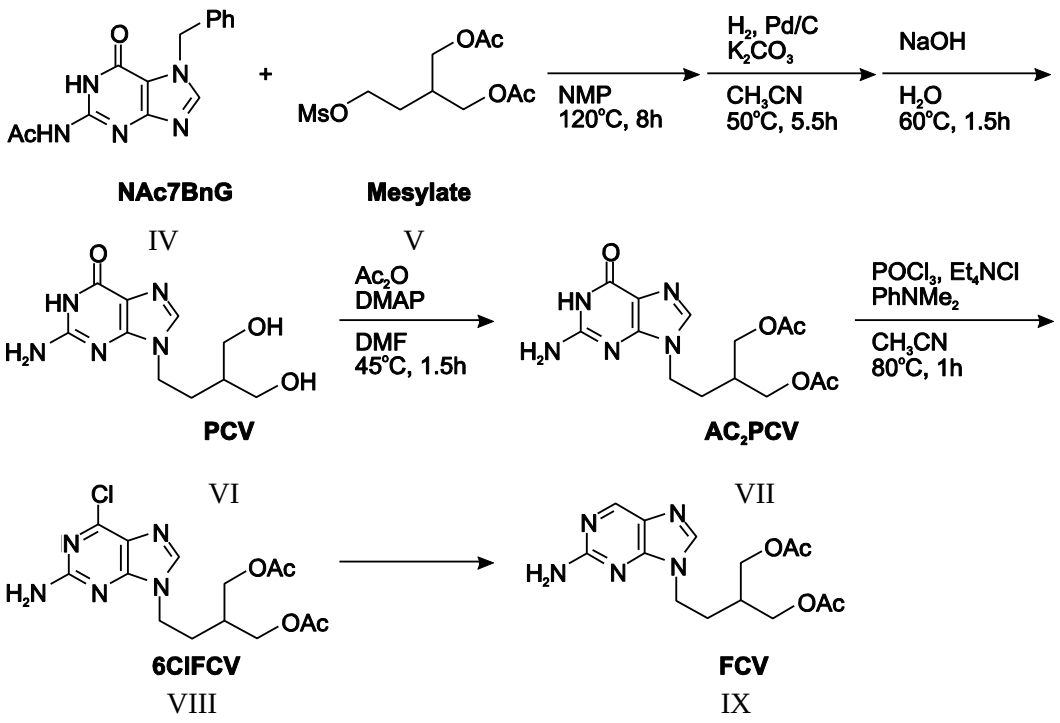


Figure 1.3 *Synthesis of FCV using NAC7BnG as a starting material, adapted from [8]*

An alternate route of FCV (IX) synthesis is to commence the process using another PCV derivative, Ac3PCV (X), which is synthesized by coupling NAc7BnG (IV) and the mesylate (V), followed by catalytic debenzylation at 50 °C. The product, Ac3PCV (X) is then converted to NAc6ClFCV (XI) by chlorination under acidic conditions in MeOH and in which the N-acetyl group is selectively deprotonated to produce 6ClFCV (VIII). The 6ClFCV (VIII) is then converted to FCV (IX) by dechlorination, as shown in Figure 1.4 [8].

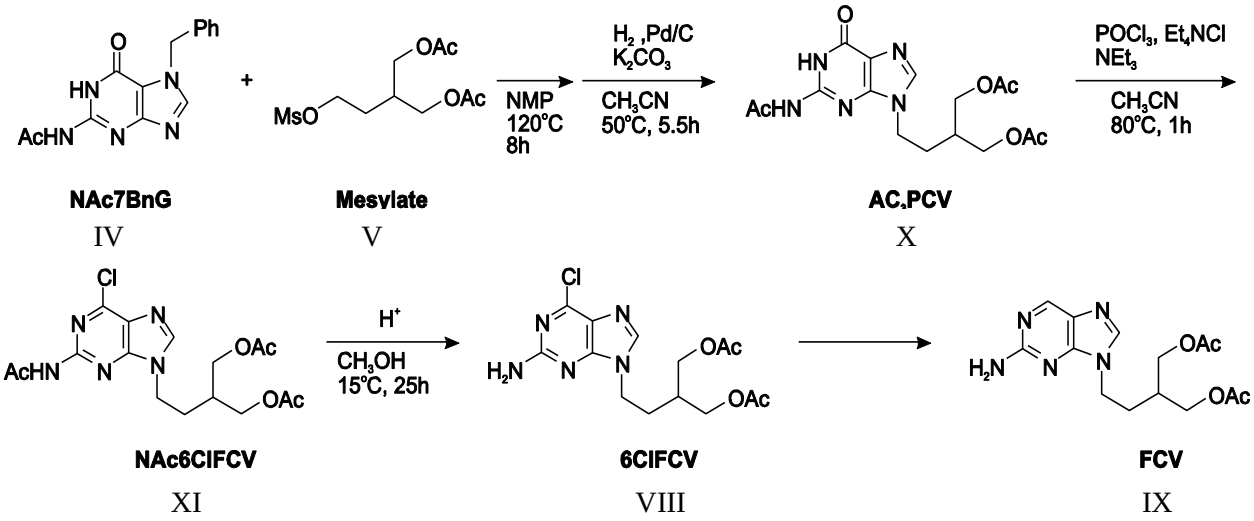


Figure 1.4 *Synthesis of FCV via the PCV derivative, AC_2PCV, adapted from [8]*

1.4 STRUCTURE ACTIVITY RELATIONSHIP

The commercially available antiviral nucleosides were discovered and developed on the basis that structural analogues of natural substrates are able to inhibit viruses. The newer antiviral agents were also developed as structural analogues of existing antiviral compounds to improve their efficacy and side effect profile [10].

FCV is converted to PCV and is then phosphorylated in herpes-infected cells to form PCV triphosphate. The antiviral activity of this compound is related to its incorporation by viral DNA polymerase into growing nucleotide chains in place of deoxy-gaunosine triphosphate. This results in early chain termination as the substrate is not recognised and hence viral DNA synthesis ceases [3]. The guanine functionality is responsible for the antiviral activity of this compound. The physicochemical properties of PCV are also a function of the polar guanine ring and the improved bioavailability of FCV compared to that of the parent compound, PCV. In addition the enhanced activity of the 6-deoxy derivative of PCV is attributed to the diacetyl ester of the 6-deoxy derivative[2].

1.5 PHYSICOCHEMICAL PROPERTIES

1.5.1 Partition Coefficient

The octanol:water and octanol:phosphate buffer (pH 7.4) partition coefficients are 1.09 and 2.08 respectively [6]

1.5.2 Dissociation Constant (pK_a)

The acid dissociation constant of FCV is 3.84 [11].

1.5.3 Solubility

FCV is freely soluble in acetone and methanol, and sparingly soluble in ethanol and isopropanol. FCV is freely soluble (> 25% w/v) in water at 25 °C but precipitates rapidly due to the formation of the sparingly soluble (2-3% w/v) monohydrate form [6; 11].

1.5.4 Melting Range

FCV has a melting range of 101-103.5 °C

1.5.5 Hygroscopicity

FCV is not hygroscopic in climatic conditions in which the relative humidity is < 85% [6].

1.5.6 Ultraviolet Absorption Spectrum (UV)

Ultraviolet (UV)-visible absorption spectroscopy is used to determine drug concentration to characterise chemical reactions and their kinetics and has been applied as a detection system for monitoring chromatographic separations [12; 13].

The absorption of UV or visible radiation involves the valence or outer electrons of a molecule and is dependent on the type and nature of chemical bonds in that molecule. The basis for selecting a wavelength applicable for detection of the analyte of interest in HPLC is a consequence of the maxima and minima in an absorption spectrum for that compound which results from different chromophoric functional groups in the molecule [14].

The ultraviolet (UV) spectrum of a 10 µg/ml solution of FCV in a mobile phase consisting of 0.05 M potassium phosphate buffer (pH 4) and ACN in a ratio of 84:16 % v/v was determined using a GBC UV/VIS 916 Spectrophotometer (GBC Scientific Equipment Pty Ltd, Dandenong, Melbourne, Australia) and is shown in Figure 1.5. The UV absorption spectrum shows a distinct maximum wavelength of absorption at 221.5 nm and is similar to what has been reported [15].

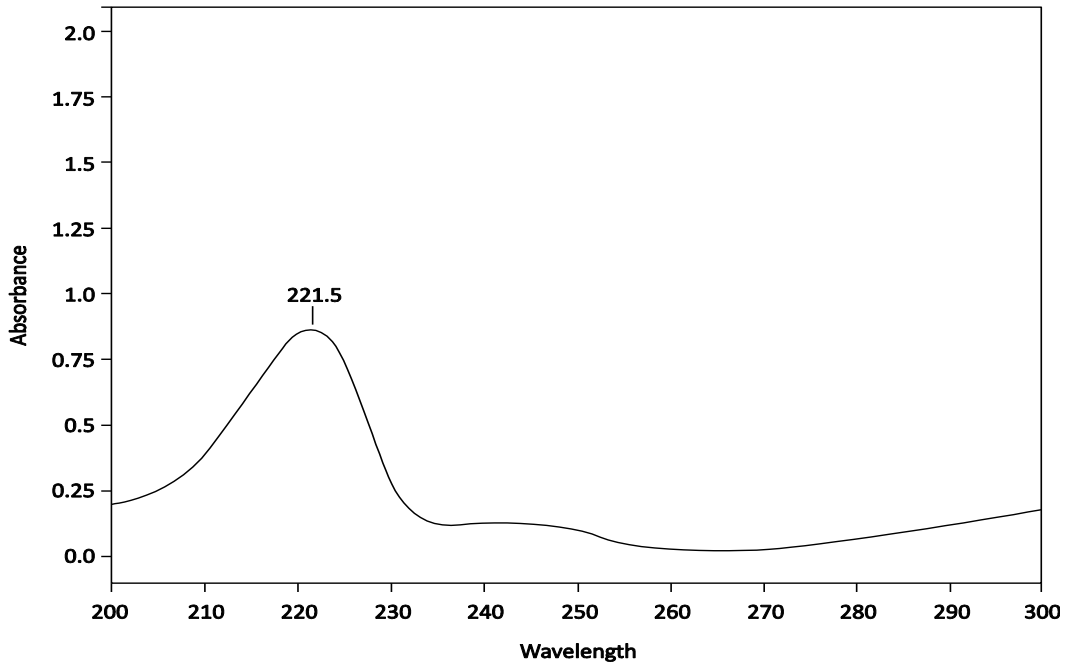


Figure 1.5 *UV Absorption spectrum of FCV in potassium phosphate buffer and ACN (84:16 % w/v)*

1.5.7 Infrared Absorption Spectrum (IR)

The vibrational spectrum of a molecule is a unique physical property of that molecule and can be used as a fingerprint for identification of the compound [16]. This approach is reliant on the fact that the structural features of a molecule, *viz*., the backbone or functional groups, result in characteristic and reproducible absorption bands in the IR spectrum of the compound. Furthermore this may indicate if there is a backbone in the molecular structure and whether the backbone is made up of linear or branched side chains. The IR spectrum also enables identification of the presence of unsaturated bonds and/or whether aromatic rings are present. It is also possible to determine whether specific functional groups are present and the orientation and location of the functional group in the molecule [16]. The IR spectrum of FCV shown in Figure 1.6 was generated using a Spectrum 100 FT-IR spectrometer (Perkin Elmer®, Beaconsfield, Buckinghamshire, England) from 4000 to 650 cm^{-1}.

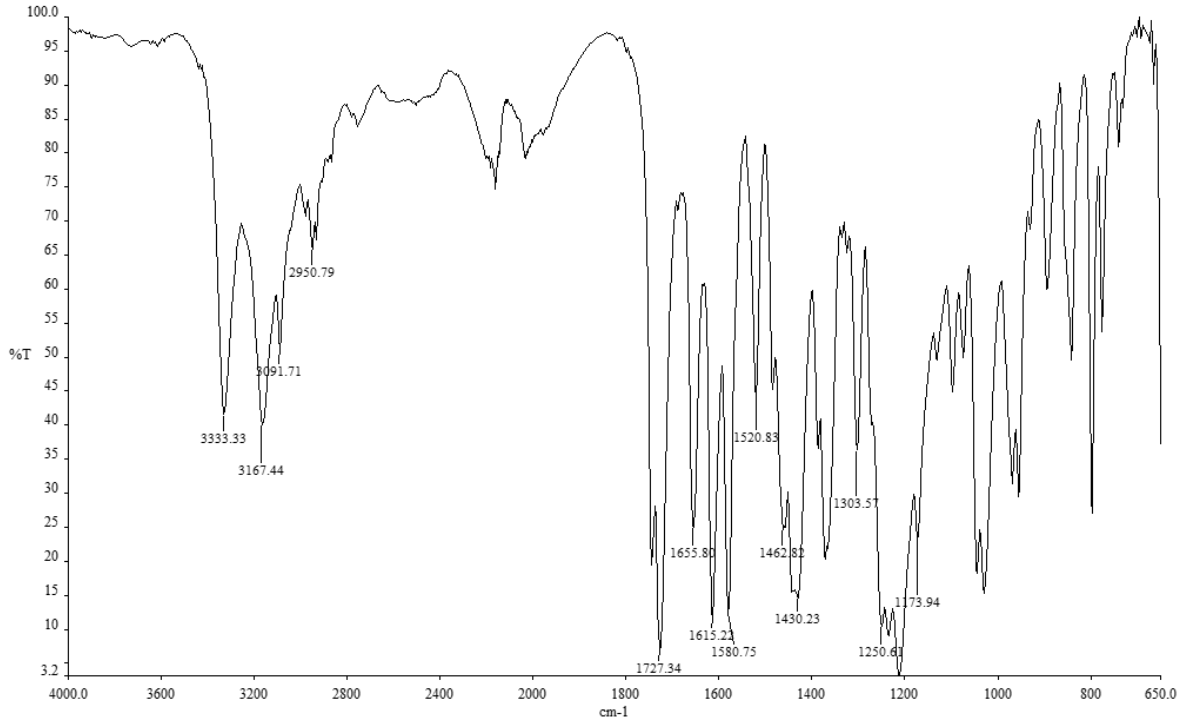


Figure 1.6 *IR spectrum of FCV*

The IR absorption spectrum shown in Figure 1.6 reveals characteristic absorption bands in the regions of 1300 -1000 cm^{-1} and 1750 – 1720 cm^{-1} which are attributed to the presence of an ester functional group. There is a strong, sharp band at 1724 cm^{-1} attributed to the carbonyl (C=O) bond of the functional groups present in the FCV molecule and the strong absorption peak at 1211 cm^{-1} and slightly weaker peaks at 1236 cm^{-1} and 1250 cm^{-1} can be attributed to the presence of C-O bond(s) which may appear as two or more bands in an IR spectrum.

In the 1470 – 1370 cm^{-1} and 2970- 2860 cm^{-1} regions there are two bands attributed to the bend and stretch bond of the methyl functional groups present in the FCV molecule, and in the 1485 – 1445 cm^{-1} and 2935 -2845 cm^{-1} regions there are two bands attributed to the bend and stretch bond of the ethyl functional groups present in the FCV molecule. Two medium to strong absorption bands observed in the 1510 – 1450 cm^{-1} and 1615 – 1580 cm^{-1} regions are attributed to the presence of aromatic double bonds. In the 3300 -3000 cm^{-1} regions there are two bands attributed to the presence of a primary amine functional group that are weak and sharp and are located at frequencies of 3163 cm^{-1} and 3331 cm^{-1}. Two bands are located in this region due to asymmetrical and symmetrical stretching of the N-H bond [17].

1.5.8 Nuclear Magnetic Resonance Spectrum (NMR)

NMR spectroscopy is a powerful technique for elucidating the structure and identifying functional groups in a molecule [18-20]. When a molecule is placed into a magnetic field the nuclei of the atoms resonate at a specific frequency as a result of the location of the atom in the molecule. The spinning charge of atoms behaves as an electric current and creates a magnetic field around the atom. The placement of a molecule in a strong external magnetic field causes magnetic nuclei such as ^{1}H and ^{13}C to spin, and the spin of the nuclei align with or against the magnetic field [20-22]. Following exposure to radiofrequency waves the nuclei 'spin-flip' from a lower energy state to a higher energy state on absorption of energy, and the resultant NMR spectrum is a display of the amplified energy absorption of the nuclei. Each peak that is observed has an exact position in the spectrum which is known as a chemical shift and is caused by the development of small local magnetic fields by electrons which shield neighbouring nuclei from the applied field [18; 21]. The NMR spectrum of FCV shown in Figure 1.7 was generated using a Bruker Avance 400 MHz NMR spectrometer (Bruker, Rheinstetten, Baden-Württemberg, Germany).

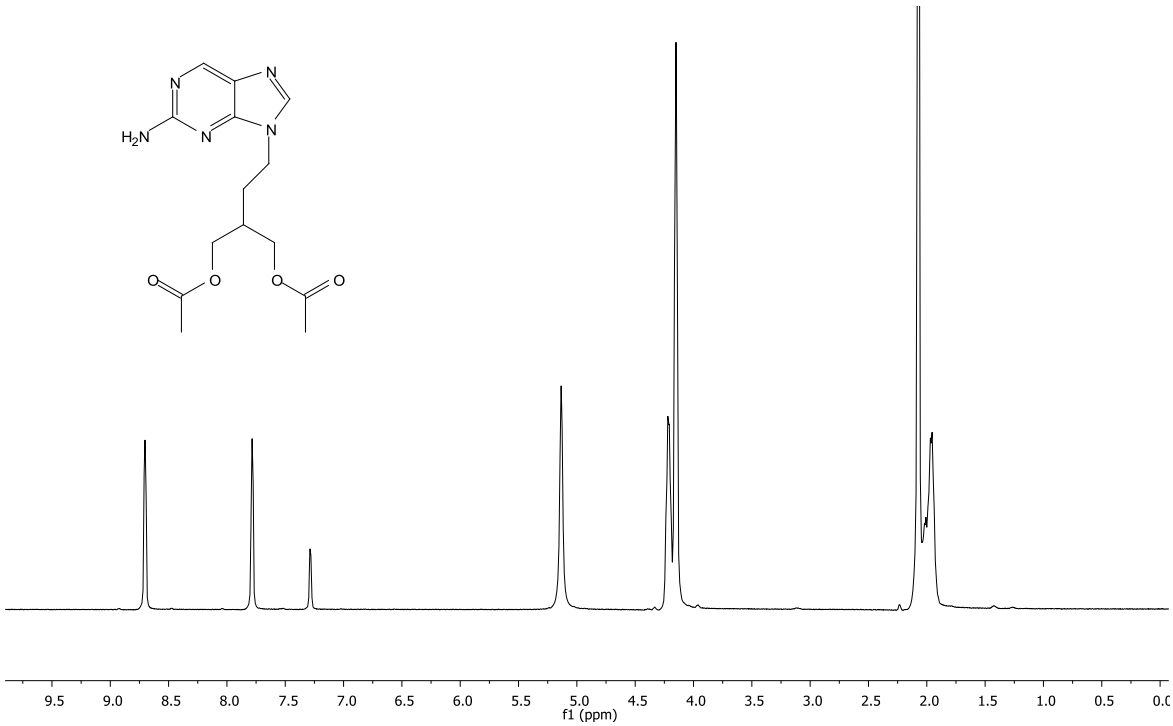


Figure 1.7 *Proton NMR spectrum of FCV*

A number of signals are observed at different ppm values, *viz.*, ^{1}H NMR - δ_H (400MHz, $CDCl_3$): 1.99 (d, 2H, -CH_2), 2.07 (s, 6H, -2CH_3); 4.19 (d, 4H, 2CH_2); 4.17 (t, 2H, -CH_2); 5.13(s, 2H, -NH_2) 7.78 (s, 1H); 8.70 (s, 1H). There is also a peak at 7.29 ppm which is attributed to the solvent used to prepare the sample; deuterated $CDCl_3$. Due to the resolution of the instrument, theoretical splitting of the peaks is not always observed, as shown by the doublet at 1.99 ppm which is expected to be a quartet.

1.6 STABILITY

1.6.1 Temperature

In aqueous media FCV is fairly stable and exhibits minimal degradation when heated to 80 °C for 8 hours, with 94.2% drug remaining following exposure [23]. This was confirmed by experiments conducted during validation of the RP-HPLC method developed for the analysis of FCV in these studies (Chapter two, *vide infra*). A solution of FCV (1 mg/ml) was heated to 50 °C for 8 hours under reflux conditions without evidence of degradation. However when heated to 60 °C for 8 hours under reflux conditions FCV (n=3) was found to undergo mild degradation with 95.19 % FCV remaining intact after 8 hours.

The thermal stability of FCV in powder form has also been reported. No degradation was observed when the compound was placed in a petri dish and stored in an oven for seven days [23].

1.6.2 pH Stability

Significant degradation of FCV was observed when the molecule was exposed to acidic and basic conditions [23; 24], with 86.0% remaining following exposure to 10 ml 0.1M HCl for 3 hours at unspecified ambient temperature and 87.5% following exposure to 10 ml 0.005 M NaOH [23]. When FCV was exposed to 1 ml of 1 M HCl at unspecified ambient temperature for 30 minutes 95.81% FCV remained. When analysed immediately after exposure to 1 ml of 0.1 M NaOH, 20.55% remained [24]. The degradation products shown in Figure 1.8 were observed with degradation product IV present in the highest concentration following degradation in acidic and basic conditions. Compounds III, V and VI were present following acidic degradation, and products III and V following basic degradation [23].

1.6.3 Oxidation

FCV was found to undergo significant degradation when 100 mg FCV was exposed to 10 ml of 10% v/v hydrogen peroxide solution for 5 hours at unspecified ambient temperature with 86.0 % FCV remaining after incubation [23]. The degradation products shown in Figure 1.8 were observed, with degradation product IV present in the highest concentration, followed by degradation products V and VI [23].

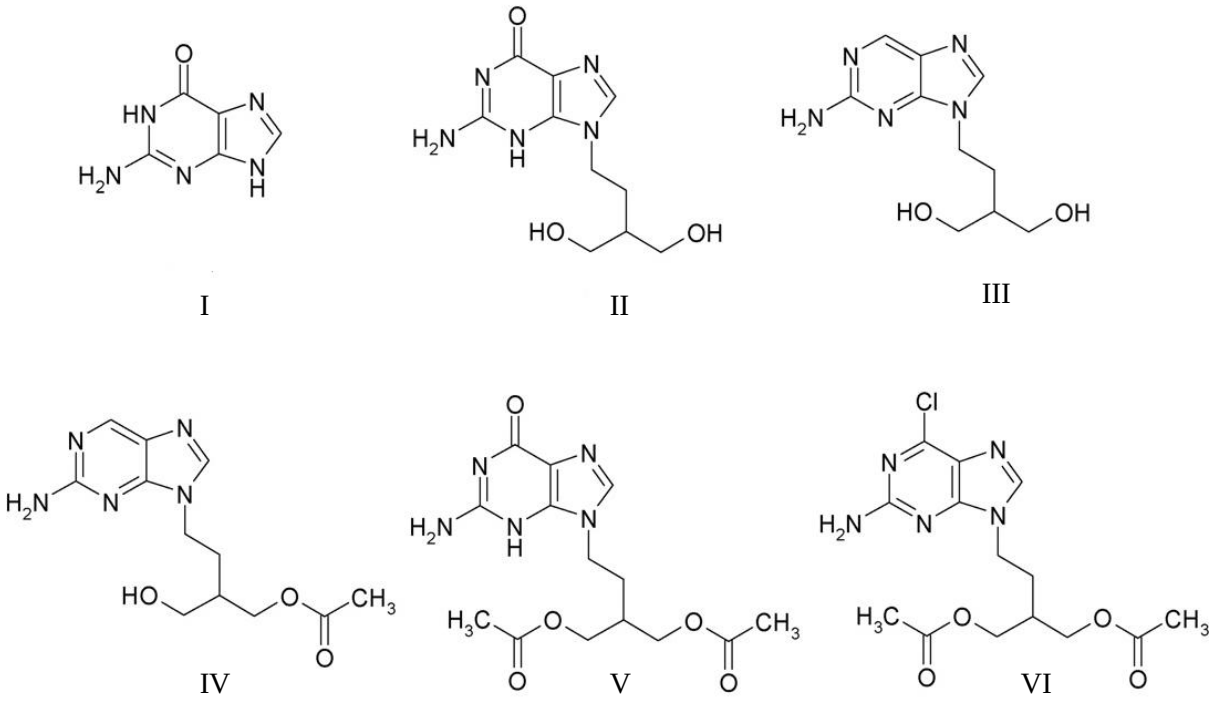


Figure 1.8 *Degradation products of FCV adapted from [23]*

1.7 CLINICAL PHARMACOLOGY

1.7.1 Mechanism of Action and Site of Bioconversion

FCV is stable in the contents of the human duodenum and is adequately absorbed from the small intestine [2; 5]. Following oral administration, FCV is rapidly converted by gastrointestinal and hepatic first pass metabolism to PCV, which exhibits inhibitory activity against the herpes simplex virus 1 (HSV-1) and 2 (HSV-2) in addition to the varicella zoster virus (VZV). Inhibition is effected by hydrolysis of one of the acetate ester groups of the molecule in the intestinal wall to yield the monoacetate form of 6-deoxy PCV and a small amount of the product following oxidation of the purine ring forming guanine. The pathways of the major and minor routes of conversion of FCV to PCV are shown in Figure 1.9. The removal of the second acetate functional group to form 6-deoxy PCV and subsequent oxidation of the purine ring to guanine occurs predominantly in the liver [2; 5; 25].

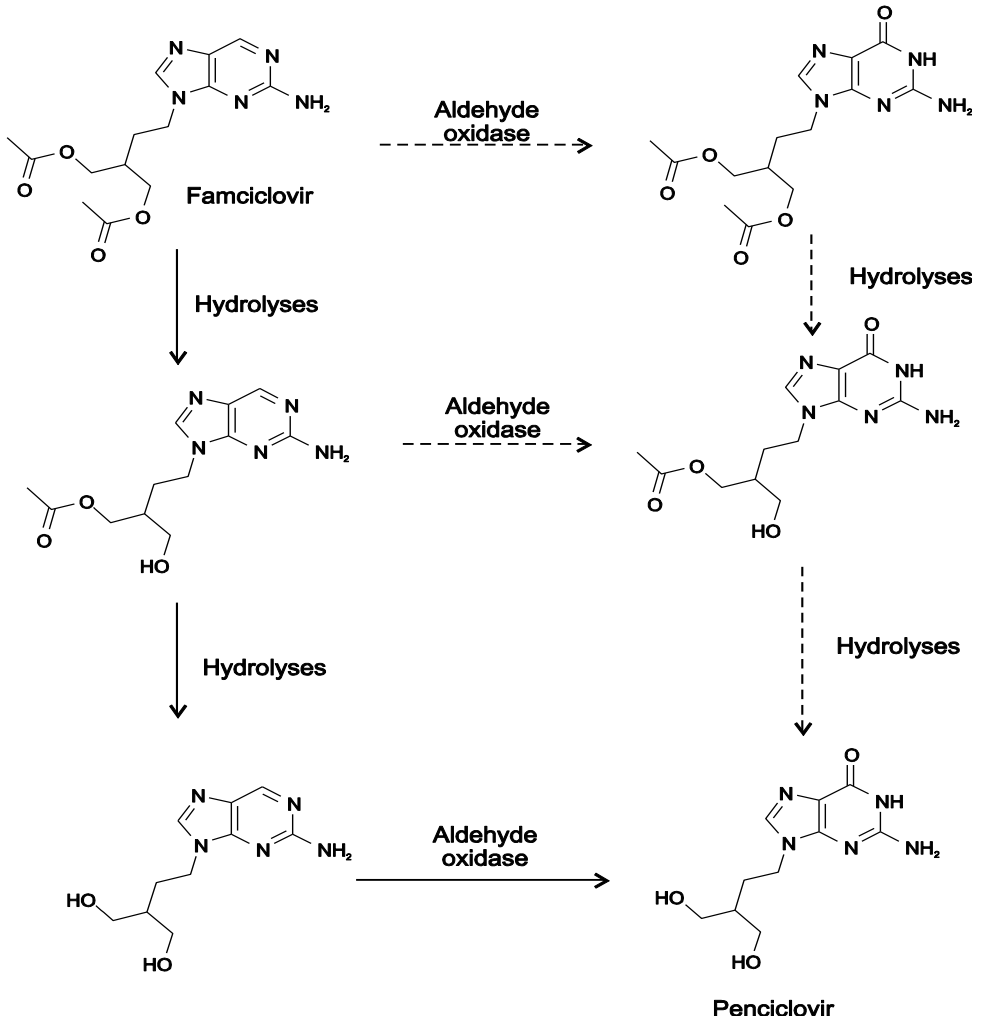


Figure 1.9 *Bioconversion of FCV into PCV showing the major (→) and minor (--->) routes of the process, adapted from [2].*

The rate determining step in the conversion of FCV to PCV is the oxidation of the purine ring to form guanine. Inhibition of the aldehyde oxidase enzyme delays the inhibition of the oxidation of FCV to form PCV significantly [2].

PCV is then converted to the monophosphate form, which in turn is converted to the active metabolite, PCV triphosphate [6]. Cells infected with herpes virus express the viral enzyme, thymidine kinase, which rapidly phosphorylates PCV. This enzyme is present only in infected cells, ensuring the specificity of PCV in treatment [3]. It has been demonstrated *in vitro* that PCV triphosphate inhibits HSV-1 and -2 DNA polymerase competitively with deoxyguanosine triphosphate and is incorporated into DNA, thus preventing significant elongation of these chains. Consequently, viral DNA synthesis, and therefore cellular replication, is selectively inhibited [6; 11; 26].

1.7.2 Indications

1.7.2.1. Herpes Labialis (Cold sores)

FCV is indicated for the treatment of recurrent *Herpes labialis* in immuno-comprimised patients [3; 6; 11]. *Herpes labialis* is characterised by unpredictable vesicular eruptions, which most commonly involve cutaneous and mucous membrane lesions of the peri-oral and oropharynx areas, respectively. The causative organism is HSV-1 which is estimated to affect one third of the world population [3; 27]. The primary episode is usually the most severe, occurs in childhood and is often associated with pain and an inability to eat or drink. Dormant HSV-1 is reactivated periodically and migrates from the sensory ganglia resulting in recurrent episodes that are experienced by many infected individuals [28]. The subsequent episodes tend to be less severe, of shorter duration and have minimal systemic involvement as compared to the primary episodes but are still associated with varying degrees of discomfort [3; 28].

1.7.2.2 Herpes Genitalis (Genital Herpes)

Genital herpes is caused by the HSV-2 as opposed to the HSV-1 variant and is characterised during onset by the presence of localized pain and tingling or burning sensations lasting up to 24 hours. Systemic signs and symptoms of the infection include headache, fever, malaise and inguinal lymphadenopathy within a few days of sexual contact with an infected individual.

The infection is characterised by the formation of vesicles of varying size that erupt on the affected area(s) to form irregular ulcers and/or erosion which eventually crust over. Clinical manifestations vary, from patients being asymptomatic to showing mild to severe lesions. Similar to *Herpes labialis*

infections the primary phases are generally more severe and recurrent episodes are experienced by many individuals [3; 28].

FCV is indicated for the treatment of recurrent episodes of genital herpes as well as for chronic suppressive therapy of recurrent episodes of the condition in immune-compromised patients [6; 7; 26]. The efficacy of FCV has not been established for the treatment of recurrent episodes of infection when therapy is not initiated within 6 hours following the onset of symptoms or appearance of lesions. The efficacy and safety of FCV for the suppression of recurrent genital herpes beyond a period of one year has also not yet been established [3; 6].

1.7.2.3 Herpes Zoster (Shingles)

Herpes zoster affects up to 20% of the population and is a result of the reactivation of dormant varicella-zoster viruses from infections earlier in life [3; 29]. The virus infects the skin and results in the development of a typical papular-vesicular rash. The virus migrates via the sensory nerve axons to the dorsal root and cranial nerve ganglia where it resides in a latent form. Reactivation occurs as a result of immunodeficiency associated with either disease or drug therapy. The reactivated virus travels along the axon resulting in the development of a unilateral papular-vesicular rash associated with dermatomal pain and/or abnormal sensation [3; 30]. FCV is indicated for the treatment of *Herpes zoster* in immuno-compromised patients, yet the efficacy of FCV has not been established when treatment has not been initiated within 72 hours following the appearance of the rash [3; 6; 11; 26].

1.7.2.4 Recurrent Herpes Labialis or Herpes Genitalis

FCV is indicated for the treatment of recurrent episodes of *Herpes labialis* or genital herpes in HIV-infected adults. However the efficacy of FCV when therapy is not initiated within 48 hours following the onset of symptoms or lesions has not yet been established [6; 26].

1.7.3 Resistance

Resistance to PCV may occur following mutation of the viral thymidine kinase enzyme through truncation of the thymidine kinase chain (thymidine kinase -negative or -deficient) or via the production of a thymidine kinase-altered enzyme [3; 6; 7; 26]. Mutations of the DNA polymerase genes where the enzyme has exhibited an altered ability to bind to the triphosphate form of the antiviral compound has also been reported [3]. This complexation process may result in complete or partial loss of thymidine kinase activity or alteration in the ability of viral thymidine kinase to phosphorylate the active pharmaceutical ingredient (API) without a loss in ability to phosphorylate thymidine [3; 6]. In patients who fail to respond to therapy, or experience recurrent viral shedding during therapy, there is a real possibility of viral resistance to PCV.

1.7.4 Cross Resistance

Cross-resistance has been observed with HSV DNA polymerase inhibitors. Thymidine kinase negative ACV resistant mutants are known to be resistant to PCV and are commonly encountered [6; 11]. In a recent study of thymidine kinase genes of 12 ACV resistant VSZ virus isolates, five (5) thymidine kinase genes were found to have a premature termination codon, resulting in the production of truncated proteins. Resistance to FCV has also been found in these thymidine kinase-deficient strains [3]. It has also been found in seven (7) other isolates that contained a single-nucleotide substitution which resulted in thymidine kinase production with an amino acid substitute at either the nucleoside, adenosine 5'-triphosphate or the intervening peptide chain binding sites. It is revealing that only one of these isolates exhibited sensitivity to PCV [3]. These data reveal that a change in a single amino acid in the thymidine kinase protein chain results in an altered ability of the thymidine kinase-dependent antiviral agent to bind to thymidine kinase, or the ability of the enzyme to phosphorylate the antiviral to produce the monophosphate form of the compound [3].

1.7.5 Dosing

1.7.5.1 Herpes Labialis

Treatment of *Herpes labialis* requires 1500 mg FCV administered as a single dose [6; 7]. Therapy should be initiated at the first sign or symptom of *Herpes labialis* infection, which may manifest as tingling, itching, burning, pain or as visible lesions.

1.7.5.2 Genital Herpes

Recurrent episodes of genital herpes require treatment with 1000 mg FCV administered twice daily for one (1) day. Therapy is initiated at the first sign or symptom of the recurrent episode with symptoms such as tingling, itching, burning, pain and/or lesions manifesting in patients. The recommended dosage of FCV for chronic suppressive therapy of recurrent episodes of genital herpes is 250 mg administered twice daily [6; 26].

1.7.5.3 Herpes Zoster

A dose of 500 mg FCV every 8 hours for seven (7) days is indicated for the treatment of *Herpes zoster*, and therapy must be initiated as soon as the diagnosis of *Herpes zoster* has been made [6; 26].

1.7.5.4 Recurrent Herpes Labialis or Herpes Genitalis

Treatment of recurrent *Herpes labialis* or *Herpes genitalis* in HIV-infected patients requires that

500 mg of FCV be administered twice daily for seven (7) days, therapy being initiated at the first sign or symptom of a recurrent episode of infection, which may manifest as tingling, itching, burning, pain and/or exhibiting lesions [6].

1.7.6 Dosage Forms

FCV is available only as 125 mg, 250 mg or 500 mg tablets. The excipients used to manufacture the tablets include hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, polyethylene glycol, sodium starch glycolate, and titanium dioxide. The white, film-coated tablets are round, biconvex with bevelled edges and are embossed with "FAMVIR" on one face and the strength indicated on the other face [6].

1.7.7 Drug Interactions

Studies have revealed that a single 500 mg dose of FCV can be safely co-administered with compounds such as cyclosporine, allopurinol, cimetidine, theophylline, zidovudine, emtricitabine, promethazine, and may be given shortly after magnesium or aluminium hydroxide without a clinically significant alteration in the pharmacokinetic profile of PCV. Multiple-dose administration of FCV (500 mg, three (3) times daily) with concomitant digoxin, which is also eliminated by glomerular filtration and active tubular secretion, revealed no clinically significant effects on the pharmacokinetics of PCV, however digoxin levels may be slightly raised [6]. An increase in the plasma concentrations of PCV may follow concurrent use with probenecid or other compounds eliminated from the biological system by active renal tubular secretion.

Aldehyde oxidase is the primary enzyme responsible for converting of 6-deoxy PCV to PCV, and interactions with compounds that are also metabolized by this enzyme or which may inhibit this enzyme may be observed [6]. Cimetidine and promethazine have been shown to inhibit the aldehyde oxidase enzyme *in vitro*, but clinical studies with FCV in combination with cimetidine and promethazine revealed no relevant effect(s) on the formation of PCV. However, raloxifene, a potent inhibitor of aldehyde oxidase *in vitro*, could potentially cause a decrease in the formation of PCV. Clinical studies have not been conducted to establish the magnitude of interaction, if any, between PCV and raloxifene [6].

1.7.8 Safety and Adverse Effects

FCV is safe and well tolerated during therapy [3]. The most common adverse effect associated with FCV is headache. Other common side effects include nausea and diarrhoea. Less common side effects include fatigue, migraine, pruritis, rash, dysmenorrhoea and abdominal pain [31].

1.7.9 Warning and Precautions

Cases of acute renal failure have been reported following administration of FCV in patients with underlying renal disease. This may be a consequence of these patients having received inappropriately high doses of FCV for their level of renal function. Dosage reduction when administering FCV is therefore recommended in patients with renal impairment.

1.7.10 Special Population Groups

1.7.10.1 Pregnancy

FCV is classed as a category B drug according to FDA, as there are no adequate and well-controlled studies documenting the use of FCV or PCV in pregnant women. Animal reproductive studies have not revealed adverse effects on foetal development following administration of FCV and PCV to rats and rabbits at doses up to 1000 mg/kg/day, which were 2.7 to 10.8 times (rats) and 1.4 to 5.4 times (rabbits) higher than the recommended maximum dose for human use. However, animal reproduction studies are not always valuable predictors of a human response and therefore FCV should be used with caution during pregnancy, and then only if absolutely necessary [6; 7].

1.7.10.2 Nursing Mothers

The administration of FCV to lactating rats resulted in the excretion of PCV in the breast milk at concentrations in excess of those observed in plasma [6; 7]. FCV use in nursing mothers should be avoided unless the potential risk(s) associated with treatment are outweighed by the potential benefits as it is not known whether FCV or PCV are excreted in human breast milk.

1.7.10.3 Paediatric Patients

The safety and efficacy of FCV in children under the age of 18 has not yet been established. However the pharmacokinetic and safety profile of FCV was studied in two (2) open-label studies following administration of experimental granules (25 mg/kg) mixed with Ora-Sweet®. These two (2) multicenter, open-label, single arm, two-phase clinical studies were performed to establish the single-dose pharmacokinetics and single and multiple-dose safety of use of a paediatric formulation of FCV in children, aged 1 to 12 years, presenting with either suspected or laboratory evidence of HSV or VZV infections [32].

Single oral doses of 12.5 mg/kg for children weighing < 40 kg and 500 mg for those > 40 kg were administered to 51 participants enrolled in the study. The pooled pharmacokinetic data established

that the average systemic exposure to PCV was similar to (6- to 12-year-olds) or slightly lower than (1- to < 6-year-olds) that observed in adults given a 500 mg single dose of FCV [32].

To optimise exposure in small children, a weight-based dosing regimen was developed and used in the seven (7) day multiple-dose safety phases of both studies in which 100 patients, with suspected or confirmed viral infections, were enrolled. A total of 55.3 % of HSV-infected patients that received FCV twice a day and 45.3% of VZV-infected patients receiving FCV three (3) times a day, experienced at least one (1) adverse event, most of which were gastrointestinal in nature [32].
Exploratory data analysis undertaken following a seven (7) day FCV dosing regimen showed resolution of the symptoms in 90.5% of the children with active HSV and 92.5% with VZV infections [32].

1.8 PHARMACOKINETICS

1.8.1 Absorption

Little or no FCV is detected in the plasma or urine following oral administration of the compound. The absolute bioavailability calculated following oral administration of 500 mg FCV and an intravenous dose of 400 mg PCV was 77 ± 8% [6; 7; 25; 26].

The concentration of PCV increases proportionally as the dose of FCV increases from 125 mg to 1000 mg [6; 7]. Data from these studies are summarised in Table 1.1, which lists the mean pharmacokinetic parameters of PCV following administration of a single dose of Famvir® tablet to healthy volunteers.

Table 1.1 *Mean Pharmacokinetic Parameters of PCV in Healthy Adult Subjects*

Dose (mg)	**AUC $_{0-\infty}$ (µg hr/ml)**	**C_{max} (µg/ml)**	**T_{max} (h)**
125	2.24	0.8	0.9
250	4.48	1.6	0.9
500	8.95	3.3	0.9
1000	17.9	6.6	0.9

Where,

$AUC_{0-\infty}$ = area under the plasma concentration-time profile extrapolated to infinity
C_{max} = maximum observed plasma concentration
T_{max} = time to reach C_{max}

A decrease of 18% in the maximum plasma concentration and a delay in T_{max} of approximately an hour was observed when FCV was administered 2 hours following a meal, compared to those observed when the compound was administrated 2 hours before a meal. No effect on the extent of systemic availability of PCV was observed and therefore FCV can be administered without regard to ingestion of food.

1.8.2 Distribution

Following administration of an intravenous dose of 400 mg of PCV over an hour, the volume of distribution of PCV was found to be 1.08 ± 0.17 L/kg and PCV was < 20% bound to plasma proteins over a concentration range of 0.1 to 20 μg/ml [6].

1.8.3 Metabolism

Following oral administration FCV is rapidly converted by first pass metabolism in the GIT and liver by deacetylation and oxidation to form PCV. Inactive metabolites formed include 6-deoxy PCV, monoacetylated PCV, and 6-deoxy monoacetylated PCV [5]. Little or no trace of FCV in the plasma or urine is observed [5].

An *in vitro* study in which human liver microsomes were used, revealed that the metabolism of FCV was not a function of the presence of CYP P450 enzyme and that the conversion of 6-deoxy PCV to PCV is catalysed by the enzyme aldehyde oxidase [5; 6].

1.8.4 Elimination

Following infusion of 5 mg/kg over one hour of a radio-labelled form of PCV, approximately 94% of the radioactivity was recovered in the urine over 24 hours, and 83% of the dose was excreted in the first 6 hours after dosing. PCV accounted for 91% of the radioactivity observed in the urine samples. Approximately 73% and 27% of the administered compounds were recovered in the urine and faeces, respectively over 72 hours following administration of a single 500 mg oral dose of radio-labelled FCV. PCV accounted for 82% of the excreted dose and 6-deoxy PCV accounted for 7% of the drug excreted in the urine, with approximately 60% of the administered dose being collected in the urine within the first 6 hours after dosing [6; 7].

Following intravenous administration of PCV to 48 healthy male volunteers the total plasma clearance of PCV was found to be 36.6±6.3 L/hr or 0.48±0.09 L/hr/kg, with PCV renal clearance accounting for 74.5±8.8% of the total plasma clearance of the compound [6; 7].

Following intravenous administration of PCV in healthy volunteers the plasma elimination half-life of PCV was calculated to be 2.0 ± 0.3 hours. After oral administration of 500 mg FCV, the plasma elimination half-life of PCV was found to be 2.3 ± 0.4 hours. In patients with *Herpes zoster,* following administration of single and repeated doses the elimination half-life was 2.8 ± 1.0 hours and 2.7 ± 1.0 hours, respectively. The plasma elimination half-life of PCV in patients with renal impairment was prolonged, and patients with a creatinine clearance of 20-39ml/min exhibited a plasma elimination half-life of 5-8 hours, whereas those with a creatinine clearance of < 20 ml/min had a plasma elimination half-life of between 3-24 hours. Approximately 73% of the drug is excreted unchanged in the urine and 27% in the faeces [33].

1.9 CONCLUSION

The search for prodrugs that are converted to a biologically active compound in order to improve on the oral absorption of ACV has led to the discovery of FCV [3; 34]. FCV is used in an oral formulation as a prodrug of PCV, a potent inhibitor of viral DNA synthesis, that is poorly absorbed. The physicochemical properties of PCV are influenced by modification of the polar guanine ring which is thought to lead to improved oral absorption of the drug [2]. FCV is a diacetyl derivative that was subsequently developed. The increased lipophilicity of FCV resulted in development of a prodrug with high oral bioavailability and the ability to penetrate the intestinal wall. FCV is readily converted to PCV via hydrolysis of one of the acetate functional groups to yield the monoacetate form of 6-deoxy PCV and a small amount of the oxidation product of the purine ring [2]. PCV is a potent inhibitor of HSV-1 and -2, and VZV as high concentrations are maintained in infected cells. The concentration in the cells results in effective inhibition of viral DNA polymerases and subsequent DNA synthesis.

Although ACV has been the drug of choice for paediatric patients requiring antiviral treatment, intravenous and high oral doses are required for effective therapy. Intravenous administration requires hospitalisation and oral ACV requires frequent administration due to low bioavailability. Consequently patients may not achieve maximum therapeutic benefit [32; 35]. Alternate therapies such as FCV are therefore desirable due to the relatively high intracellular concentrations of PCV achieved with a less frequent dosing regimen. FCV is not currently approved for paediatric use, but studies have been conducted to establish the single-dose pharmacokinetics and single and multiple dose safety of FCV in paediatric patients. A single oral dose of 12.5 mg/kg for children weighing below 40 kg, and 500 mg for those > 40 kg in mass resulted in an average systemic exposure to PCV similar to (6- to 12-year-olds) or slightly lower than (1- to < 6-year-olds) that observed in adults given a 500 mg dose of FCV [32].

CHAPTER TWO

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION

2.1 INTRODUCTION

2.1.1 Overview

The analysis of FCV in raw material and pharmaceutical dosage forms has not been included in any official pharmacopoeia [24; 36]. However several HPLC methods have been reported for the quantitative determination of FCV in raw material, pharmaceutical dosage forms [23], urine, blood and plasma samples [37]. In addition several spectrophotometric methods of analysis of FCV have also been published [38-40].

HPLC is a useful method of analysis since it offers high resolution and speed of analysis compared to other forms of liquid chromatography, with better reproducibility due to close control of parameters which affect the efficiency of a separation. The technique is also adaptable in respect of automation of instrumentation and quantitation [41].

The objective of these studies was to develop and validate a simple, sensitive, selective, stability-indicating HPLC method of analysis for the quantitation of FCV in pharmaceutical dosage forms.

2.1.2 Principles of HPLC

HPLC is a widely used analytical technique offering excellent performance and reliability [42].

Classical liquid chromatography has been used for many years. Originally a suitable adsorbent, for example alumina or silica was packed into a column which was then washed with a liquid or mobile phase. The separation is based on selective absorption of components of a mixture to the surface of the stationary phase. Weakly absorbed components will travel faster along the column than component(s) that are more strongly adsorbed. Separation of the compounds of interest is therefore achieved due to differential adsorption of the analytes to the stationary phase. This method of separation is termed adsorption or liquid solid chromatography [43; 44].

Other adsorption methods of separation depend on the choice of a liquid or a solid as the stationary phase, or the type of solid used [43]. Liquid-liquid chromatography makes use of a liquid stationary

phase that is coated onto a finely divided inert solid support and the separation is based on a difference in partitioning of the analytes between the two phases [43].

These methods have largely been replaced by bonded phase chromatography, which offers advantages over the traditional approaches as it is easier to conduct from an experimental point of view, has greater versatility, and produces rapid and reproducible results [43]. The terms normal and reversed phase are used to describe the adsorption of solutes for bonded phase separations. In normal phase liquid chromatography the stationary phase is polar, whereas the mobile phase is non-polar. In contrast, in reversed phase liquid chromatography the stationary and mobile phase(s) are non-polar and polar, respectively [43].

In bonded phase chromatography the stationary phase is primarily made up of a silica backbone that is bonded to different functional groups to provide a diverse range of stationary phases for analytical separation. The method most commonly used to prepare bonded phases from silica involves reaction of a silica base with substituted dimethylchlorosilane. Different kinds of bonded phases can be produced by varying the nature of the functional group(s) of the silylating agent. Other methods that have been used to manufacture bonded phase material include esterification of surface silanol groups with alcohol(s), or conversion of the silanol groups to Si-Cl using thionyl chloride and reaction of the product with an organo-metallic compound. The mobile phase is pumped through the column by means of a high pressure solvent delivery module, transferring the analyte of interest to a detection system monitored by display of a detector signal [45-47].

Chromatography involves the separation of the components of a mixture by differences in the equilibrium distribution (*K*) of the components between each phase as described by Equation 2.1:

$$K = \frac{C_s}{C_m} \qquad \textbf{\textit{Equation 2.1}}$$

Where,

K = equilibrium distribution
C_s = concentrations of the analyte in the stationary phase
C_m = concentration of the analyte in the mobile phase

The partitioning between the bulk and/or adsorption phases which contribute to the retention characteristics of a compound are described by the value *K* [46].

The interaction of an analyte with each of the phases determines the equilibrium distribution of the analyte between each phase. The relative strengths of these interactions are governed by the extent and type of interactive forces present in a system. Four (4) main types of force or interaction between

an analyte and solvent may be involved, *viz*. dispersive, dipole, hydrogen bonding and dielectric interactions [48]. A solute molecule having a dipole moment can interact selectively with other dipoles, causing intermolecular forces to come into play. Molecules may also interact with other molecules by hydrogen bonding, if it is a proton donor and/or acceptor. Molecules can also interact by much weaker dispersive forces such as hydrophobic or Van Der Waal's interactions , which rely on a specific molecule being polarised by another molecule [43; 49]. These are the forces that effect a separation in RP-HPLC [49].

In more general terms, polarity is defined as the ability of a sample or solvent molecule to interact by these forces and is used to describe the ability of a compound or compounds to interact in these ways. The higher the polarity of a molecule the greater the ability of the molecule to interact with other molecules [43; 48]. Molecules with polarities of similar strength may result in poor separation as the interaction of each molecule with each phase is likely to be similar. Hence, with stationary non-polar phases, a polar mobile phase is used to effect a separation, but when highly polar stationary phases, such as unmodified silica are used, mobile phase(s) of relatively low polarity are used [43].

2.2 LITERATURE REVIEW

A literature review was undertaken to establish the parameters that have been used for the quantitative analysis of FCV and the data are summarised in Table 2.1. FCV is a relatively new molecule that was approved by FDA in 1994 and there is limited published data relating to analysis of the molecule [50]. The initial HPLC conditions used for the development of a method of HPLC method for the analysis of FCV were selected on the basis of the published information.

Table 2.1 *Published HPLC methods of analysis for FCV*

Column	Mobile Phase	Buffer	Retention time (min)	Flow rate (ml/min)	Detection method (nm)	Reference
Inertsil® ODS 3V 250x4.6mm, 5µm	Gradient mixture of solvent A; Methanol:Buffer (20:80% v/v) and solvent B; Methanol:Buffer (80:20% v/v)	Phosphate, pH 6, 0.01 M	20	0.8	UV-215	[23]
RP C_{18} 250x4.6mm, 5µm	Methanol:Buffer (50:50 % v/v)	Not stated	2.5	1.0	UV- 242	[15]
Gemini® ODS C_{18} 250x4.6mm, 5µm	Acetonitrile: Buffer (5:95% v/v)	Phosphate, pH 2.5, 0.02 M	6.9	Not stated	UV- 314	[50]
Hypersil® BDS C_{18} 250x4.6mm, 5µm	Acetonitrile: Buffer (20:80% v/v)	Phosphate, pH 4, 0.05 M	6.9	1.0	UV-220	[24]
Symmetry C_{18} 250x4.6mm, 5µm	Methanol: Buffer (50:50% v/v)	Phosphate, pH 3.05	2.4	1.0	UV- 242	[51]
Kromasil® C_{18} 250 × 4.6mm, 5µm	Acetonitrile : NH_4OAc (80:20 % v/v)	Ammonium acetate, pH 7, 0.005 M	2.5	1.0	Unspecified UV	[52]
Nova-Pac® C_{18} 100 x 8mm	Gradient mixture of solvent A; Sodium hydrogen phosphate and solvent B; Methanol:Buffer (80:20% v/v)	Not stated	13.5	1.6	UV -254	[37]
Spherisorb® ODS C_{18} 250 x 4.6mm, 5 µm	Acetonitrile:Buffer (9:91 % v/v)	Ammonium acetate, pH 4.6, 0.5 mM	20	1.5	UV- 280	[37]

2.3 METHOD DEVELOPMENT AND OPTIMISATION

2.3.1 Introduction

Factors such as the physicochemical properties of a molecule and mode of detection of an analyte must be considered when developing an HPLC method [49]. Sample preparation is also an important consideration since centrifugation, filtration, sonication and dilution may affect the quality of a separation and impact on the recovery of the analyte(s) of interest [49; 53]. The stability of compounds in solution must also be established during early method development studies as without quality data all subsequent results, *viz*., dissolution, pharmacokinetic data amongst others becomes suspect if there is a degree of uncertainty around the data, resulting in challenges when attempting to compare subsequent analyses during method development [49; 53; 54]. The selection of an appropriate mobile phase is dependent on factors such as the ionogenic nature and/or the hydrophobicity of the analyte as these factors influence the selectivity of a method, in particular for ionisable compounds. The selection of an appropriate stationary phase is also important especially with regard to the stability of the bonded phase at the pH of the mobile phase selected for the analysis [49].

2.3.2 Materials and Reagents

FCV was purchased from Mttpharma (Pudong, Shanghai, China). HPLC grade Acetonitrile and methanol far UV Romil-SpS® were purchased from Romil® Ltd (Waterbeach, Cambridge, United Kingdom). Potassium dihydrogen orthophosphate, sodium hydroxide pellets and orthophosphoric acid were purchased from Merck Chemicals Ltd (Modderfontein, Gauteng, South Africa). HPLC grade water was prepared by reverse osmosis, using a Milli-RO® 15 water purification system (Millipore®, Bedford, Massachusetts, USA), consisting of a Super-C carbon cartridge, two Ion-X® ion-exchange cartridges and an Organex-Q® cartridge. The water was filtered through a 0.22 μm Millipak® stack filter (Millipore®, Bedford, Massachusetts, USA).

2.3.3 Column Selection

The column is regarded as ‘the heart of an HPLC separation’ [55; 56] and in order to develop a method that is rugged and reproducible the use of a stable, high-performance column is essential [55; 56]. The physicochemical properties such as solubility in a variety solvents, molecular weight and the ionic nature of the analyte have a direct impact on the choice of an analytical column [14; 55; 57]. The retention time of an analyte in an HPLC separation is in part a function of the packing material

used to produce the column and the dimensions of the column, and must be evaluated when selecting an appropriate stationary phase for a separation [14; 55].

2.3.3.1 Column Packing Material and Stationary Phases

A suitable HPLC packing material must be chemically stable, have sufficient mechanical strength to withstand high pressures and have a narrow particle size distribution. Porous silica phases meet these criteria and are commonly used in RP-HPLC. The material is able to withstand relatively high pressures of between 10,000 and 15,000 psi. One advantage of porous silica is that the particles are available in a range of sizes, shapes and degrees of porosity, further enhancing the flexibility for a specific separation [14; 55; 58]. In addition, silica-based columns are compatible with most organic and aqueous mobile phase solvents and are available in a wide range of bonded phases. Silica-based columns are often used for the separation of low molecular weight analytes using mobile phase solvents and samples over the pH range of 2 to 7.5 as they are reproducible, stable and versatile thereby ensuring consistency of results [14; 59; 60].

Commonly used bonded phases include C_1, C_4, C_8 and C_{18} based silica adsorbents. Stationary phases modified with methyl and butyl groups, *viz*., C_1 and C_4 respectively, have little application in HPLC and are used mainly for protein separation or purification, whereas octyl and octadecyl modified adsorbents, *viz*., C_8 and C_{18}, are commonly used for HPLC separations. Several functional groups including amino, cyano and diols amongst others may be bonded to the silica backbone for specific applications [57; 59].

2.3.3.2 Particle Type

Several particle types are available for HPLC applications. Porous microspheres are most commonly used for HPLC as they are highly efficient, durable, can accommodate a wide range of sample loads and are widely available. Furthermore, these particles are available in a variety of diameters, pore sizes and surface areas [14; 55]. Micro-pellicular particles have a solid core coated with a thin layer of the stationary phase and are usually available in 1.5 to 2.5 µm sizes that display a high level of efficiency for the analysis of macromolecules as a result of rapid mass transfer kinetics. However, micro-pellicular particles have a relatively low surface area and have limited sample load properties and are therefore best suited for analysis only as they overload easily and are not suitable for preparative liquid chromatography [46]. Perfusion particles have large pores ranging in size between 4000 and 8000 Å and also have small interconnected pores that form an internal network, resulting in the minimisation of band broadening. This is a consequence of the large porous particles mimicking

smaller particles in terms of column efficiency but with the added advantage that operating pressures are significantly reduced [14; 55].

2.3.3.3 Particle Size

Particle and pore size are important to achieve optimal separations. As the particle size of the packing material decreases the theoretical plate height of the column decreases. Consequently the column becomes less permeable and therefore a column of fixed length and small particle size will generate a theoretical plate count (N) that is indicative of greater column efficiency than columns with packing materials of larger particle size. However the use of smaller particles will result in increased resistance and consequently higher operating pressures at equivalent flow rates [58]. Spherical particles with diameters of 3, 5 or 10 μm are generally used in analytical HPLC columns. A column with a stationary phase with particles of 5μm diameter was selected for use in these studies.

2.3.3.4 Pore Size

To maximise the surface area and sample loading capacity of a column, *viz*., the amount of sample that can be separated on a specific column, the smallest possible pore diameter should be selected. Consequently the size and/or molecular weight of the analyte should be considered when selecting an appropriate pore size for the stationary phase. Low molecular weight solutes, i.e. molecules of molecular weight < 500, are best separated using packing materials with a pore diameter of between 50 and 130 Å and an associated surface areas of 200-400 m^2/g [58]. Larger surface area materials have greater capacity and longer analyte retention times and are often used for the analysis of large molecular weight molecules, as opposed to smaller surface area phases that offer faster equilibration times. Famciclovir has a molecular weight of 321.33 and therefore a column packed with particles of pore diameter of 120 Å was selected for these studies.

2.3.3.5 Column Dimensions

Following selection of a column with an appropriate stationary phase, the dimensions of the HPLC column should be selected for an optimal separation. The efficiency of a column increases with increased column length and small internal diameter. However there is a consequent increase in the operating back-pressure for a specific flow rate under such conditions [61].

Columns with an internal diameter of 2 mm to 4.6 mm and 3 cm to 50 cm in length are normally used for quantitative analysis as sample volumes are small. The most common internal diameter of columns used for the analysis of FCV is 4.6 mm as these operate at lower backpressures than that observed for smaller-diameter columns [61; 62]. However more solvent is consumed at operating

flow rates than with smaller-diameter columns [61]. This is a consequence of decreased flow rates that produce the same linear velocity of the analyte of interest throughout the column. The use of shorter columns has the added advantage that shorter analytical run times and lower back-pressures are possible, whereas separations on longer columns have inherently longer run times with higher resolution, as the number of theoretical plates (N) increases linearly with column length [61]. The standard length of columns used for RP-HPLC separations that achieve high separation efficiencies with low operating back pressures is 15 cm, and therefore a 15 cm column was selected for the development of an HPLC method for the analysis of FCV.

2.3.3.6 Guard Columns

A guard column can be used to protect a column from contaminants and also serves as an efficient in-line filter by retaining particulate matter from the sample and/or mobile phase. They are 10-20 mm in length and preferably contain packing materials that are similar or the same as that used in the analytical column. The guard column retains strong sample components including particulates and thus prevents these from damaging the analytical column. Guard columns should be replaced at regular intervals prior to becoming saturated with strongly retained sample components that then pass onto the analytical column. Guard columns are sometimes expensive, inconvenient and not always necessary. The use of guard columns with low volume and high efficiency columns (< 2 um) require special care due to extra-column peak broadening when small columns are used [55; 63]. For these reasons, a guard column was not deemed necessary for the analysis of FCV.

2.3.3.7 Choice of Column

A 150 mm x 4.6 mm i.d, 5 µm Phenomenex® ODS C_{18} column with a pore size of 120 Å was selected for the analysis of FCV.

2.3.4 Detection

Variations of detectors have been used in HPLC analysis, including ultraviolet (UV), fluorescence, electrochemical, refractive index, and more recently mass spectrometric detectors. The majority of compounds are readily analysed using UV-visible spectrophotometric detectors. The three (3) main types of UV-visible detectors are fixed wavelength, variable wavelength and diode array spectrophotometers. The most popular of these is the variable wavelength detector since detection is possible at wavelengths other than 254 nm. In many instances individual sample components have a high absorptivity at a number of different wavelengths and operation at a fixed or single wavelength results in a decreased sensitivity and in some cases sample components may be undetected [14; 58].

Compounds with one or more double bonds or those with unshared or unbonded electrons may be detected using UV spectrophotometry. Compounds that are readily analysed includes the olefins, aromatic materials and those containing C=0, C=S, -N-O and -N=N- functional groups [14; 64]. Information regarding the characteristic chromophoric groups and the wavelengths at which significant absorptivity is observed should be established. Furthermore the mobile phase selected for the separation should exhibit minimal absorption at the wavelength of detection and should not influence detection in any way. For example, changes in temperature or flow rate should have a minimal effect on the detector. The selection of a wavelength for analysis is a compromise between the optical properties of the analyte of interest and the solvent used for the separation [58].

Variable wavelength detectors are fitted with deuterium and tungsten lamp sources which serve to generate radiation in the ultraviolet and visible regions respectively. A monochromator is used to ensure that a specific wavelength is selected. The monochromator performs a similar function to a prism by refracting light. An exit slit permits light of a limited range of wavelengths to pass through the slit, thereby providing light of the desired wavelength for analysis [58].

Although UV detection has some limitations, this technique is used extensively as it provides the best combination of sensitivity, versatility and linearity. It can be accurately calibrated and the use of a single internal standard is acceptable for most HPLC separations [65].

The detection of FCV has primarily been achieved using UV detection as reflected by the data summarised in Table 2.1. The wavelength selected was 221 nm, established from the absorption spectrum shown in Figure 1.5 in §1.5.6.

2.3.5 Internal Standard Selection

The use of a standard for HPLC analyses can be for identification purposes, where the retention time of a reference standard is compared to the retention times of the components of a sample mixture that are unknown, or for calculating the peak height or area ratio which is then compared to a calibration curve to provide quantitative information. The standard can be used as an external or internal standard and is present to compensate for the inherent variability of a method, thereby increasing the accuracy and precision of that analytical method [66; 67].

2.3.5.1 External Standard

The use of an external standard requires that it be analysed independently of the sample(s) of interest, and the results of the chromatographic analyses are then compared. Quantitation is based on a comparison of the peak height/area of the external standard with the sample(s) of interest. The

chromatographic conditions must therefore be constant and the method is used when a suitable internal standard for analysis cannot be identified.

2.3.5.2 Internal Standard

The use of an internal standard is preferred and a calibration curve is produced by preparing and simultaneously analysing calibration standards of different concentrations of an analyte(s) with a fixed concentration of the internal standard [55]. The internal standard is usually a compound similar in structure to the analyte of interest. The internal standard should be well resolved from the other peaks so that accurate measurements can be made [68]. An internal standard is advantageous in analyses where sample loss may occur during sample preparation prior to quantitation. In such instances a known quantity of internal standard is added prior to any manipulation of the sample. The ratio of internal standard to analyte should remain constant as the same proportion of each compound should be lost during the manipulation process. Internal standards can also compensate for changes in sample volume or concentration due to instrumental variation.

The choice of internal standard for this study was based on the chemical structure of FCV. Initially ACV was selected as a possible internal standard since it is a synthetic purine nucleoside analogue derived from guanosine.

ACV has a much shorter retention time than FCV, as shown in Figure 2.1, since it is more polar than FCV due to the presence of an amide, alcohol and primary amine functional groups, as shown in Figure 2.2. This results in ACV preferentially partitioning into the mobile phase, as opposed to FCV which is retained on the hydrophobic stationary phase.

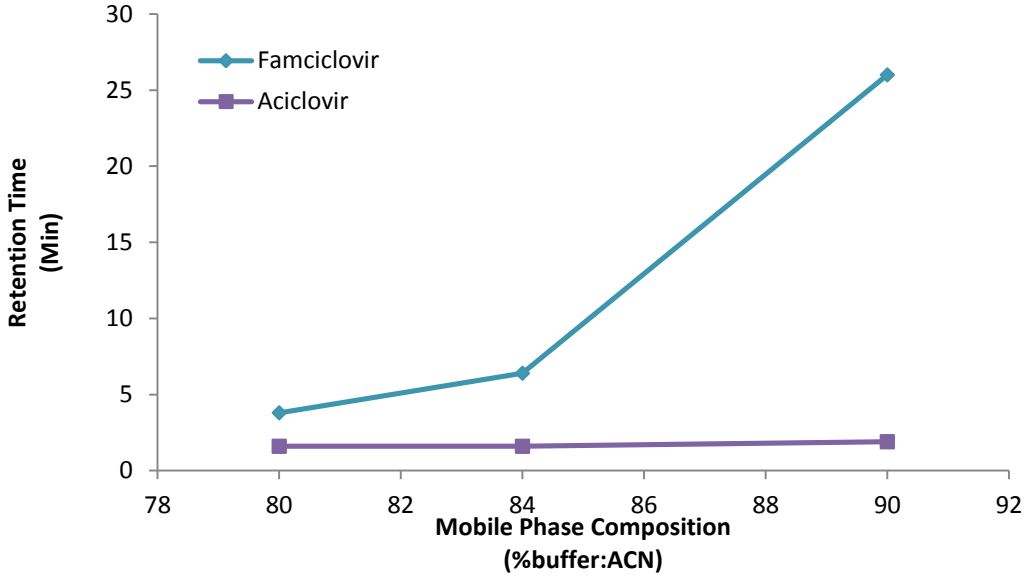


Figure 2.1 *Effect of mobile phase composition on retention time of FCV compared to ACV*

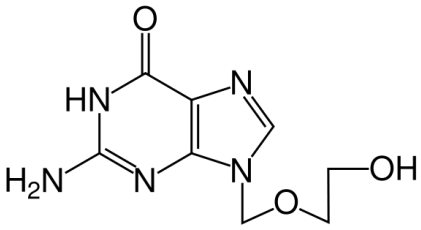


Figure2.2 *Structure of ACV ($C_8H_{11}N_5O_3$), MW=225.2 g/mol*

Due to the challenges of using ACV, zidovudine (AZT), a synthetic thymidine neucleoside analogue (Figure 2.3) was selected as an internal standard. AZT was chosen on the basis that it is less polar than ACV, but is slightly more polar than FCV due to the presence of an amide functional group. The lone pair of electrons on the nitrogen group is delocalised by the carbonyl group forming a partial double bond between the N and the carbon of the carbonyl functional group. In addition it has an alcohol functional group. The presence of the alcohol group imparts an ability to form hydrogen bonds, thereby altering the polarity of the molecule.

The AZT peak was well resolved from that of FCV, which eluted at 4 minutes under the specified conditions, resulting in a total run time of < 10 minutes for the analysis of each sample. Therefore AZT was chosen as the internal standard.

The concentration of AZT was 120 µg/ml to ensure a response of the internal standard that was half the height of the peak for the maximum expected concentration of FCV.

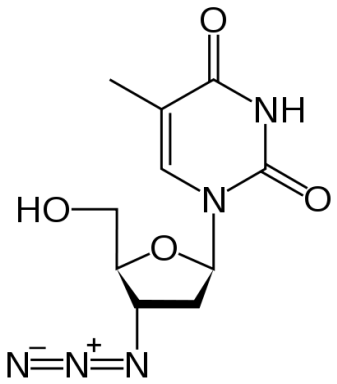


Figure 2.3 *Structure of AZT ($C_{10}H_{13}N_5O_4$), MW=267.2 g/mol*

2.3.6 Mobile Phase Selection

2.3.6.1 Introduction

The mobile phase composition can have a profound effect on the ease of analysis and sensitivity of RP-HPLC, as separations can be optimised through the composition [14; 55]. The resolution of a chromatographic separation, R, can be expressed in terms of three (3) parameters *viz.*, the capacity factor (k^1), selectivity (α) and column efficiency (N). These are directly related to experimental conditions and can be expressed by Equation 2.2.

$$Rs = \frac{1}{4}(\propto -1)\sqrt{N} * \frac{k^1}{(1+k^1)} \qquad \textbf{\textit{Equation 2.2}}$$

The resolution of a separation increases as the capacity factor increases and therefore the resolution of a chromatographic separation can be controlled by altering the solvent strength of the mobile phase. This can be achieved by changing the proportions of buffer and organic modifier used [46; 49; 55]. The stronger the polarity and strength of the solvent, the more likely it is to facilitate elution of an analyte from a column. If the capacity factor for a separation lies between 1 and 10 a change in resolution can be brought about by a change in the selectivity of the column. The selectivity can be enhanced by changing the composition of the mobile phase rather than the solvent strength [46]. Consequently, careful consideration of the composition and solvent strength of a mobile phase is vital for the development of a satisfactory RP-HPLC method [55].

All reagents and solvents used to prepare mobile phases should be of the highest quality, and although HPLC grade reagents cost more than lower grade reagents the difference in purity has a marked effect on the resultant separation, thus justifying the greater expense. Many lower grade reagent solvents are unsuitable for long term use in HPLC as they contain impurities that may produce spurious peaks in the resultant chromatograms [43].

2.3.6.2 Mobile Phase Composition

The amount and strength of buffer used in a mobile phase for RP-HPLC may affect a separation. As the buffer content increases and organic modifier content decreases there is likely to be a corresponding increase in the retention time of the analyte(s) of interest. This will occur as the relative distribution of a solute between two phases is determined by the interaction(s) of the solute species with each phase [43]. An increase in polarity as the content of the organic modifier is decreased results in preferential partitioning of an analyte onto the stationary phase rather than in the mobile phase. The extent to which an analyte is retained on the stationary phase is dependent on hydrophobic forces in the case of reversed-phase packing materials. In general, the more hydrophobic an analyte, the more likely it will be retained on the stationary phase due to the lack of an interaction of the non polar surface area of the molecule with the aqueous components of the mobile phase and preferred interaction with the hydrocarbon components of the stationary phase. However polar functional groups are generally well integrated into aqueous environments, resulting in very short retention times for molecules with this chemistry. The best initial choice of organic solvent for mobile phases is acetonitrile (ACN) as ACN-water mixtures have a much lower viscosity than methanol based systems, resulting in a lower column back pressure as well as higher theoretical plate numbers for the column. The effects of changes in mobile phase composition on the retention times of FCV and AZT are shown in Figure 2.4.

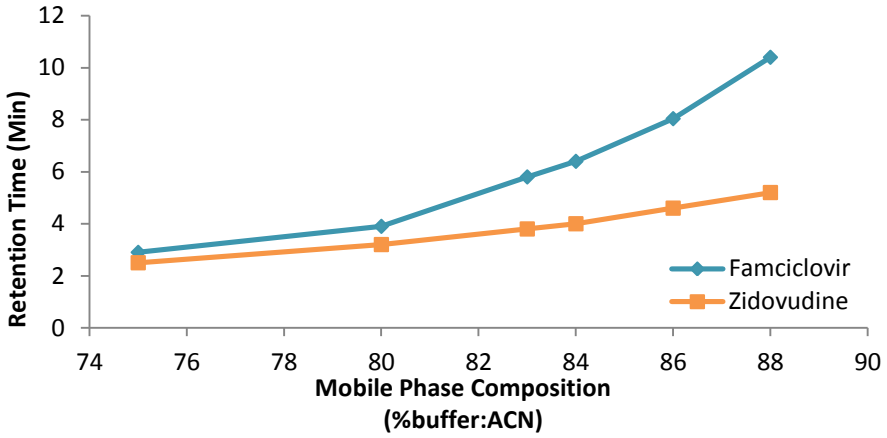


Figure 2.4 *Effect of mobile phase composition on the retention times of FCV and AZT*

There is an increase in the retention times for both analytes as the buffer composition increases and ACN content decreases.

2.3.6.3 Buffer Molarity and pH

To improve the selectivity and reproducibility of a separation it may be necessary to add a buffer to the mobile phase, with the added benefit of possibly improving peak shape. Buffers are used to control pH and the acid-base equilibrium of a solute in the mobile phase. Buffers are also used to influence the retention times of solutes, such as FCV, that ionise. The buffer should be able to control pH in the range 2-8 and have maximum buffer capacity. Furthermore buffers should be soluble, stable and compatible with the detection system used for the analysis [14; 55]. If the analyte of interest is ionisable, the pK_a of the material should be known or experimentally determined. The optimum pH to commence method development studies is one that falls within 2 pH units of the pK_a of the analyte of interest, as small changes in pH can have a pronounced effect on the resolution of a separation [55]. The optimum pH is usually determined by varying the pH of the buffer and monitoring the retention time of the analyte. The results of these studies generally produce a sigmoidal type of response if only one site of ionization is present in a molecule [49]. The presence of more than one site of ionization introduces competing effects on the retention and the overall effect on the retention is dependent on the relative hydrophobicities of the competing species, at a particular pH. For RP-HPLC separation a suitable buffer molarity is between 10 and 50 mM, assuming that the sample injected is relatively small and/or the sample is not heavily buffered at a pH value very different from the mobile phase pH. Buffer molarities greater than 50mM provide better buffer capacity, however may not be soluble in the mobile phase and may adversely affect the HPLC system [55]. A mobile phase with a low buffer capacity will result in poorly reproducible separations for a compound that partially ionises at the pH of the mobile phase, resulting in changes in retention time and distorted peaks [55].

2.3.6.4 Preparation of the Buffer

Buffers of different molarities were prepared to determine the optimal buffer molarity for HPLC analysis of FCV. Potassium dihydrogen phosphate (1.36, 3.40, 4.76, 6.80 and 10.21g) was accurately weighed and dissolved in HPLC grade water in a 1L A-grade volumetric flask. The solution was made up to volume with HPLC grade water to prepare phosphate buffers of 10, 25, 35, 50 and 75 mM. Buffers of different pH were also prepared using orthophosphoric acid to adjust and achieve the desired pH. HPLC grade water was used by means of a Milli-pore system, which removes traces of inorganic, organic, and particulate material from the water, thus preventing these from collecting on the non-polar stationary phase which could alter the nature of the stationary phase or possibly produce spurious peaks [43]. A Crison pH meter, Model GLP 21 (Crison Instruments, Alella, Barcelona, Spain) was used to measure the pH.

The retention time of an analyte in RP-HPLC increases with an increase in the hydrophobicity of the analyte. When an acid or base ionises, it becomes less hydrophobic than its base state, resulting in a reduction in the retention time for a separation. When the pH of the solvent used is < $pK_{a,}$ of FCV the molecule ionises and gains a proton, resulting in shorter retention times. As shown in Figure 2.5, as the pH of the buffer decreases to < 3.84 the retention time decreases. As shown in Figure 2.6 and Table 2.2 the peak shape was also affected by the pH of the buffer. At a pH < 4 tailing was more noticeable and the peaks were poorly resolved. Therefore the pH selected for use in further optimisation studies was pH 4. At this pH FCV has a retention time of 6.4 minutes and was well resolved from the solvent front and internal standard which has a retention time of four (4) minutes.

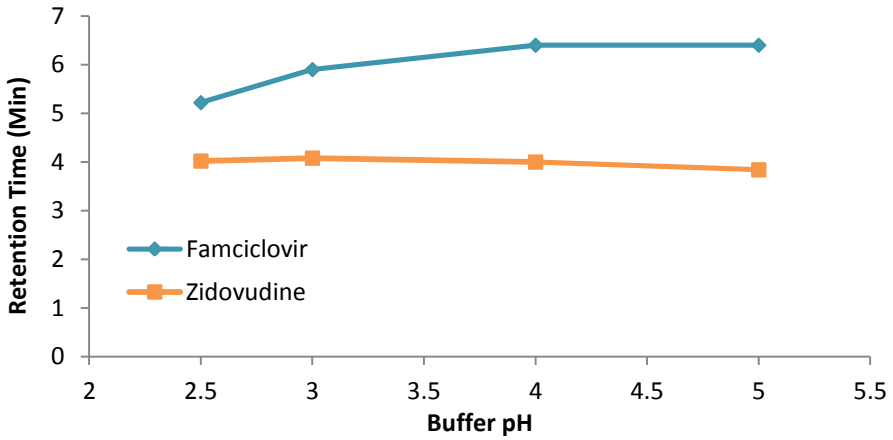


Figure 2.5 *Effect of buffer pH on retention time*

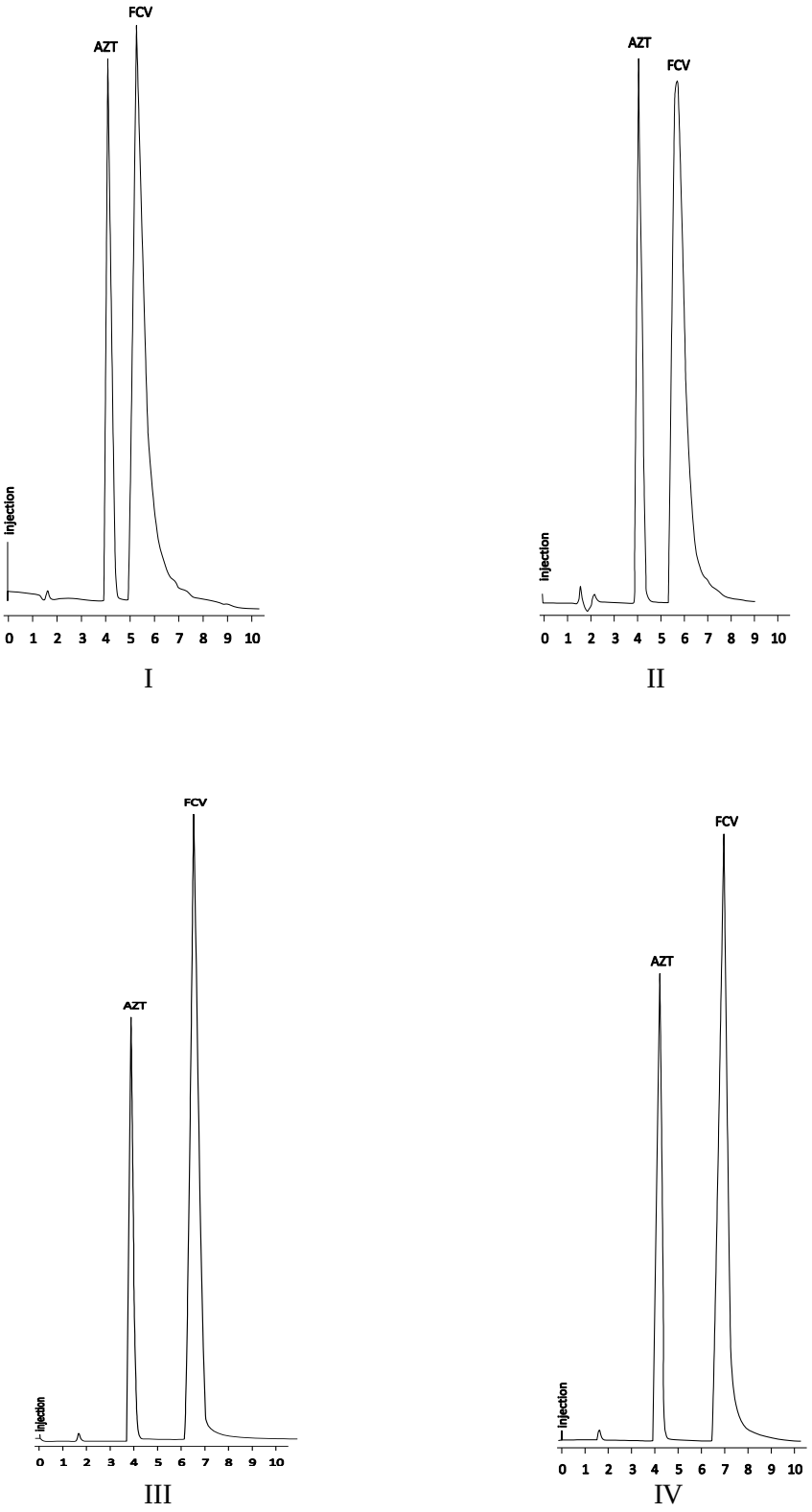


Figure 2.6 *The effect of pH on the peak shape of FCV at pH =2.5 (1), pH = 3 (II), pH = 4(III) and pH = 5 (IV) using a mobile phase comprised of 16% v/v ACN in 50 mM phosphate buffer*

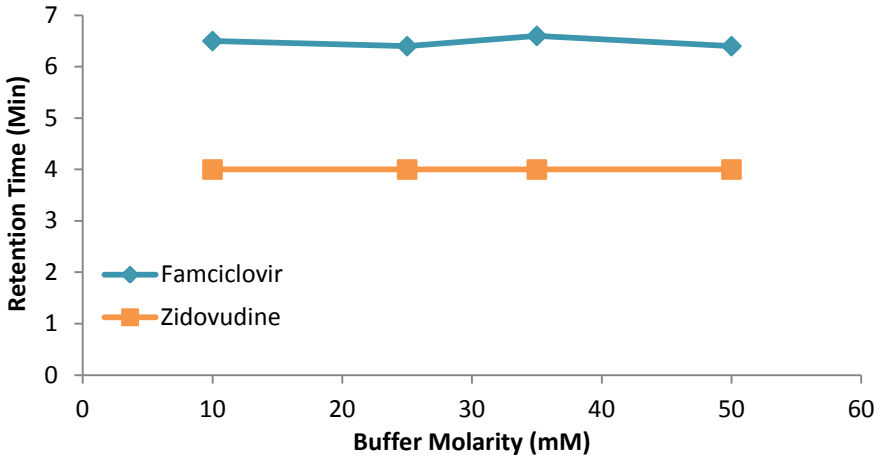


Figure 2.7 *The effect of buffer molarity on retention time*

An increase in buffer molarity, as shown in Figure 2.7, had no effect on the retention time of FCV. The data summarised in Table 2.2 reveal that at a higher molarity, *viz*., 35 and 50 mM, peak tailing was reduced. Therefore a buffer molarity of 50mM was selected for use as this resulted in peaks with limited tailing and the best resolution.

Table 2.2 *Effect of mobile phase composition on retention time, resolution and tailing factor*

Phosphate Buffer %v/v	Organic Modifier %v/v	Buffer pH	Buffer Molarity (mM)	FCV Retention Time (min)	AZT Retention Time (min)	Tailing Factor	Resolution
Water:Methanol							
80	20	4	50	35.4	*	**	-
70	30	4	50	8.12	*	1.5	-
60	40	4	50	3.6	*	1	-
50	50	4	50	2.4	*	1	-
Water:Acetonitrile							
87	13	4	50	10.4	5.2	1.17	5.2
85	15	4	50	8.0	4.6	1	3.83
84	16	4	50	6.3	4.1	1	3.73
83	17	4	50	5.8	3.8	1	3.3
80	20	4	50	3.9	3.2	1	1.8
84	16	2.5	50	5.2	4.0	1.25	1.5
84	16	3	50	5.9	4.0	1.14	2.6
84	16	4	50	6.3	4.1	1	3.73
84	16	5	50	6.4	3.84	1	3.4
84	16	4	10	6.5	4	1.33	1.66
84	16	4	25	6.4	4	1.15	1.75
84	16	4	35	6.6	4.1	1	1.55
84	16	4	50	6.4	4	1	1.86

***no internal standard was included** ****Peak is undefined**

2.3.6.5 Preparation of the Mobile Phase

The mobile phase was prepared by accurately measuring 160 ml ACN and 840 ml phosphate buffer, of the required pH and molarity, *viz.*, pH = 4, 50 mM, into separate A-grade measuring cylinders. The individual components were then mixed and the mobile phase was degassed under vacuum using a Model A-2S Eyela Aspirator, (Tokyo Rikakikai Co., Bunkyo, Tokyo, Japan) and filtered through a 0.45 µm Durapore® HV membrane filter (Millipore, Cork, Munster, Ireland) into a 1000 ml Schott® Duran bottle (Schott Duran GmbH, Wertheim, Baden-Württemberg, Germany). Degassing of the mobile phase is necessary as gas in the mobile phase is a major cause of practical problems in HPLC analysis. The presence of air bubbles in the mobile phase in a system can for example collect in the pump or the detector cell. Due to the compressibility of air bubbles the volume of mobile phase delivered by the pump is therefore reduced, resulting in the reproducibility of delivery being affected. Due to flow variation the detector noise is also worse. Dissolved oxygen can interfere with UV absorbance detection at short wavelengths and therefore many problems of dissolved air can be avoided if the mobile phase is degassed prior to use [43].

2.3.6.6 Preparation of the Stock Solutions

Approximately 10 mg of FCV was accurately weighed and transferred into a 10 ml A-grade volumetric flask and made up to volume with HPLC grade water to produce a solution of final concentration 1mg/ml. The standard stock solution was serially diluted to prepare calibration standards of 2, 10, 20, 40, 60, 100 and 120 µg/ml.

A stock solution of the internal standard was prepared by accurately weighing approximately 24 mg of AZT into a 10 ml A-grade volumetric flask and making up to volume with HPLC grade water to produce a solution of concentration of 2.4 mg/ml. A 50 µl aliquot of the resultant solution was pipetted using an automated 100 µl pipette (Boeco Germany, Rödingsmarkt, Hamburg, Germany) directly into each sample vial that contained 1 ml FCV solution to achieve a final concentration of the internal standard of 120 µg/ml.

2.3.7 Chromatographic Conditions

The optimal chromatographic conditions selected for quantitative analysis of FCV are summarised in Table 2.3 and a typical chromatogram generated using the optimised conditions is shown in Figure 2.8.

Table 2.3 *Optimised chromatographic conditions for quantitation of FCV in pharmaceutical dosage forms*

Column	Phenomenex® ODS C_{18} (150 mm x 4.6 mm x 5 µm)
Mobile phase	0.05 M potassium phosphate buffer (pH=4) : ACN (84:16 v/v)
Flow rate	1 ml/min
Detection wavelength	221 nm
Backpressure	974 psi
Detection sensitivity	0.2 AUFS
Injection volume	20 µl
Chart speed	5 mm/min
Temperature	22 °C
FCV retention time	6.4 min
AZT retention time	4.0 min

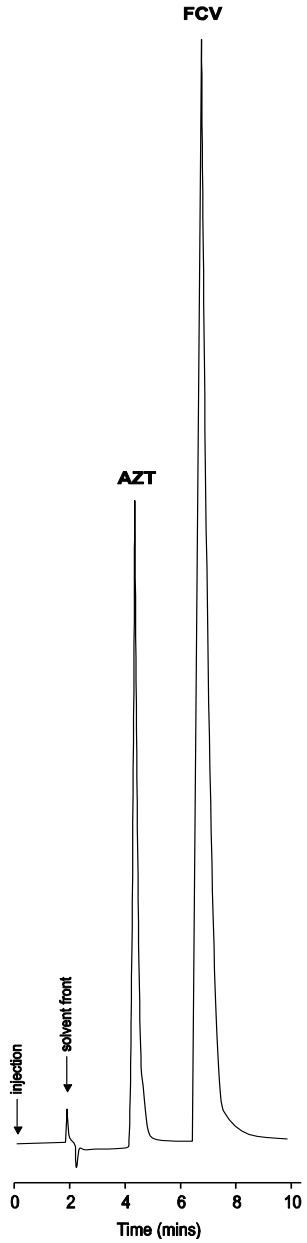


Figure 2.8 *Typical chromatogram showing the separation of AZT (4 min) and FCV (6.4 min)*

2.4 HPLC METHOD VALIDATION

2.4.1 Introduction

According to ICH Guidelines the objective of undertaking validation studies of analytical procedures is to demonstrate and ensure that the method is suitable for its intended purpose [69]. Typical validation parameters to be considered include accuracy, precision (including intra- and inter-day), specificity, limits of quantitation and detection in addition to linearity and range. The objective of the analytical procedure should be clearly understood as this is vital in establishing the validation parameters that must be evaluated. Revalidation may be necessary in certain circumstances, such as where a change in the synthetic procedure used to produce the analyte has been made, or where changes have occurred in the composition of a finished product or in an analytical procedure. The nature of any modification that has been made governs the extent of the revalidation studies that may be required [69; 70].

2.4.2 Linearity and Range

The linearity of an analytical method is defined as the ability of the method to generate test results that are directly proportional to the concentration of analyte within the sample over a specified concentration range [69; 71]. The linearity of an analytical method should be in agreement with the Beer-Lambert law, which states that at low concentrations the absorbance of an analyte is directly proportional to the concentration of that analyte [64; 72].

Linearity should be evaluated over the entire concentration range of an analytical procedure and may be demonstrated directly by the analysis of a series of dilutions of a standard stock solution of the analyte of interest. A plot of a signal, for example peak height as a function of the concentration of analyte, should be evaluated for linearity and if a linear relationship exists then an appropriate statistical method, such as least squares linear regression analysis, should be used to evaluate the test results [69].

The ICH guidelines specify that a minimum of five (5) samples of increasing concentration should be used to establish the linearity of a method across a specified range [69]. Therefore linearity was determined using a series of seven (7) FCV working standards and injecting five (n= 5) aliquot volumes over the concentration range 2-120 µg/ml. The lowest concentration represented the limit of quantitation. A calibration curve was plotted for peak height ratio versus concentration, and the correlation coefficient of the respective calibration curve was calculated using linear regression analysis. The resultant calibration curve is shown in Figure 2.9.

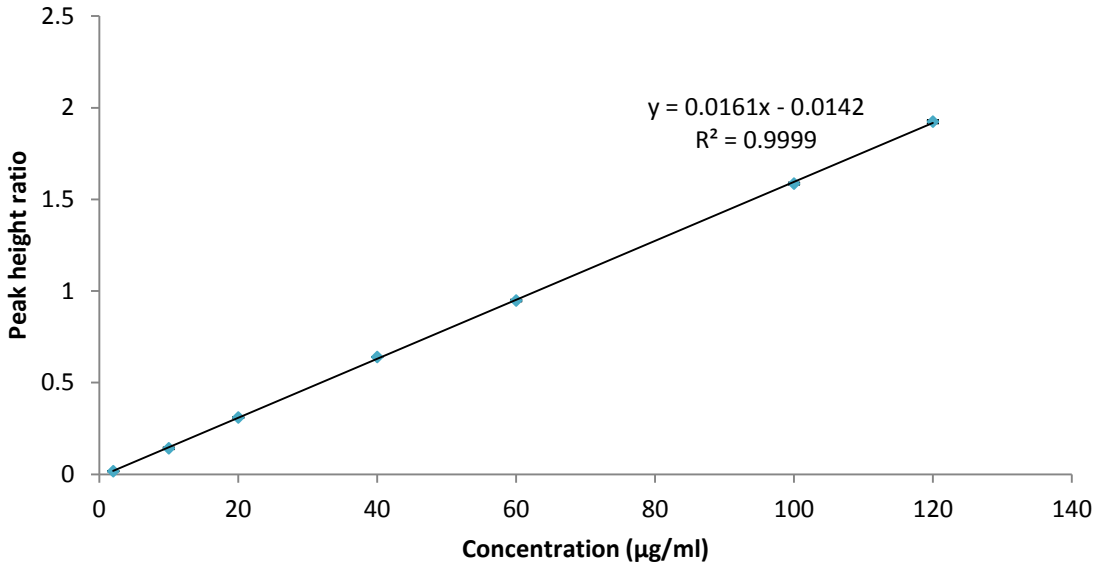


Figure 2.9 *Typical calibration curve for FCV over the concentration range 2 -120 µg/ml*

The calibration curve was found to be linear, with R^2 = 0.9999, the slope = 0.0161 and a y-intercept of -0.0142. The equation for the line was y = 0.0161x-0.0142. The linearity was accepted on the basis that a value for R^2 > 0.999 was obtained for the correlation coefficient [73] and the y-intercept was not significantly different from zero [74].

The specified range of a method is usually established by evaluating the linearity of a relationship and depends on the intended application of the analytical procedure. The range of an analytical method is defined as the interval between and including the upper and lower concentrations of an analyte in a sample for which it has been demonstrated that the analytical method has an appropriate level of precision, accuracy and linearity [69]. The method was found to be linear, with an appropriate level of precision, accuracy and linearity over the concentration range of 2 – 120 µg/ml.

2.4.3 Precision

The precision of a method is the extent to which individual test results, from multiple injections of a series of standards, agree under prescribed assay conditions [75]. According to the ICH guidelines [69] precision is considered at three (3) levels, *viz*. repeatability (intra-assay precision), intermediate precision (inter-assay precision) and reproducibility (inter-laboratory precision). The standard deviation (SD), percentage relative standard deviation (% RSD) or coefficient of variation of a series of measurements is recommended as a means of expressing the precision of an analytical method [69]. The limit of precision was set at a % RSD of < 5% in our laboratory.

2.4.3.1 Repeatability

The repeatability of a method expresses the precision of that method over a short period of time used by the same analyst under the same operating conditions. According to the ICH guidelines repeatability should be established from a minimum of nine (9) determinations covering the specified range of the method, for example three (3) concentrations in replicates of three (3) or from a minimum of six (6) analyses at 100% of the test or target concentration [69; 73]. It has been suggested that repeatability can be assessed by analysis of a minimum of three (3) different concentrations covering the low, medium and high range of a calibration curve with a minimum of five replicates (n=5) analysed at each concentration level [71].

Repeatability was evaluated using fifteen samples covering the complete specified range for the assay. The analyses were performed at concentrations of 6, 50 and 110 µg/ml in replicates of five (n=5). The % RSD of the peak height ratio at each concentration was then calculated [69]. Results from the repeatability studies are summarised in Table 2.4.

The data reveal low % RSD values of < 5% for all samples, indicating the precision and repeatability of measurements using this method, and the intra-day precision data reveal that the method is suitable for the analysis of FCV.

2.4.3.2 Intermediate Precision

The intermediate precision of a method expresses within-laboratory variation that may occur when different analysts or equipment are used for an analysis or when the analysis is performed on different days [69]. Intermediate precision evaluates the reliability of an analytical method, within the same laboratory but under different environmental conditions from those used during the development of the method. Such studies are undertaken in order to ensure that the method will produce the same results once the method development phase has been completed [76]. Analyses were performed in replicates of five (n=5) at three (3) different concentrations on three (3) consecutive days. Results of the intermediate precision studies are summarised in Table 2.4.

Table 2.4 *Repeatability and Intermediate precision results for FCV analysis*

	Concentration (µg/ml)	Mean peak height ratio (n=5)	Standard deviation	% RSD
	6	0.09	0.00045	0.51
Day 1	50	0.80	0.0045	0.56
	110	1.81	0.010	0.55
	6	0.08	0.00047	0.54
Day 2	50	0.78	0.0047	0.57
	110	1.77	0.0055	0.31
	6	0.08	0.00047	0.55
Day 3	50	0.81	0.0047	0.55
	110	1.79	0.0047	0.25
Average	6	0.08	0.020	1.12
	50	0.80	0.0153	1.92
	110	1.79	0.0032	3.80

The low % RSD values of < 5% indicate that the precision of the analytical procedure is more than adequate.

2.4.3.3 Reproducibility

The reproducibility of a method expresses inter-laboratory variation and is often used for the evaluation of inter-laboratory cross-over studies [55; 73]. Reproducibility was not evaluated in these studies as reproducibility is not normally evaluated if intermediate precision studies are performed [73] and the repeatability and intermediate precision are within limits to ensure that the analytical method is precise. Furthermore only one analyst using a single system conducted all studies within one laboratory.

2.4.4 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between a test result and a result that is accepted as the true value or is an accepted reference value [69]. Accuracy and precision are used to determine the error of an analytical method and are important criteria for the evaluation of the performance of an analytical method [71].

Several approaches can be used to establish the accuracy of a method. These include the analysis of control samples spiked with the analyte of interest, or by comparison of the results of an analytical method with those obtained using a reference method [71]. Accuracy should be assessed using a minimum of nine (9) analyses and over three (3) concentrations covering the specified range. By way of example three (3) different concentrations can be analysed in triplicate (n = 3). The accuracy of an analytical method should be reported as the percent recovery of a known amount of added analyte or

the difference between the mean value and the accepted true value for a sample reported with confidence intervals [69].

The percent bias (% Bias) is used to determine the extent of deviation of a mean value for a sample from the accepted true value for that sample. The % RSD and recovery were used to report the accuracy of the method. A % Bias and % RSD of < 5% and < 2%, respectively are the upper limits set in our laboratory as these parameters [77].

The accuracy of the methods was established at three (3) concentrations *viz*., low, medium and high in the range for this method. Each solution was injected in replicates (n=5) and the % Bias was calculated by interpolation of the data from those of a freshly constructed calibration curve. Accuracy data are summarised in Table 2.5.

Table 2.5 *Accuracy for FCV over the concentration range 2-120 µg/ml*

Theoretical concentration (µg/ml)	**Determined concentration (µg/ml)**	**% RSD**	**% Recovery**	**% Bias**
6.0	6.06	0.69	100.66	0.95
50.0	50.54	0.57	100.68	1.07
110.0	112.96	0.25	102.28	2.62

The data summarised in Table 2.6 reveal that the values for % RSD and % Bias are below the limits of 2% and 5% respectively, thereby indicating that the method is accurate for the analysis of FCV.

2.4.5 Limits of Quantitation (LOQ) and Detection (LOD)

The LOQ of an analytical method is defined as the lowest concentration of analyte that can be measured with the acceptable accuracy and precision under the stated experimental conditions [69; 71].

There are several ways of establishing the LOQ of an analytical method. Visual evaluation where samples of known concentration of an analyte are tested and the minimum level at which the analyte is reliably quantified is the LOQ and the signal-to-noise ratio approach in which the signals from samples of known concentration of an analyte are compared to responses of blank samples. The concentration of an analyte that can be reliably quantified with a signal-to-noise ratio of 1:10 is the LOQ [78]. The LOQ may also be based on the standard deviation of the response and the slope of the calibration curve [69]. The LOQ may also be ascertained directly from precision studies where decreasing concentrations of an analyte are tested. The % RSD is plotted against the corresponding

concentration and if a predefined limit for the % RSD is exceeded the corresponding concentration is then established as the LOQ.

The LOQ was established by evaluating the lowest concentration of analyte that resulted in a precision < 5% RSD [79]. Five (5) concentrations of the analyte were injected five (5) times (n=5) and were evaluated to establish the LOQ. These data are summarised in Table 2.6.

Table 2.6 *LOQ for FCV*

Concentration (µg/ml)	**Mean Peak Height Ratio (n=6)**	**Standard Deviation (µg/ml)**	**% RSD**
4	0.046	0.00017	0.36
3	0.034	0.00016	0.48
2	0.021	0.00020	0.96
1.5	0.016	0.0023	14.54
1	0.0049	0.0010	20.56

The LOQ was found to be 2 µg/ml, with an associated % RSD of < 5%. The LOD of an analytical method is defined as the lowest concentration of an analyte in a sample that can be detected but not quantitated with adequate accuracy and precision using the stated experimental conditions [69; 71] There are several ways of establishing the LOD of a method. One method of establishing the LOD is to establish the concentration at which the analyte can be reliably detected resulting in a signal-to-noise ratio of 3:1 or 2:1 [78]. However, by convention the LOD is usually 30% of the LOQ and for this method is therefore 0.67 µg/ml.

2.4.6 Specificity

A method is considered specific if it can be used to generate a response for a single analyte [71] Selectivity is therefore a more suitable term than specificity as it is almost impossible to develop a chromatographic assay for a compound dispersed in matrices that will respond to only the analyte of interest. Therefore this method is selective as it has the ability to accurately and quantitatively measure the concentration(s) of FCV in the presence of sample components which are expected to be present and that typically include impurities, degradation products, tablet and solution matrices, amongst others [69; 71; 73].

To demonstrate the selectivity of this method, commercially available Famvir® tablets (Novartis, Kempton Park, Gauteng, South Africa) were purchased from a local pharmacy and analysed. Forced degradation studies were also conducted on solutions of FCV in order to demonstrate the selectivity of the method in the presence of possible degradation products.

2.4.7 Assay of Famvir® Tablets

Twenty Famvir® tablets were accurately weighed using a Mettler Toledo analytical balance (Model AG135, Mettler Instruments, Greifensee, Zurich, Switzerland) and then crushed to a powder using a mortar and pestle. An aliquot of powder equivalent to the weight of one tablet was weighed into a 100 ml A-grade volumetric flask and dissolved in HPLC grade water. The solution was sonicated for 10 min with a Cole-Parmer Ultrasonic cleaner (Model 8845.30, Cole-Parmer, Chicago, Illinois, USA). A 5 ml aliquot of the resultant solution was filtered through a Millipore® Millex®-HV hydrophilic PVDF 0.45µm syringe filter. A 0.1 ml aliquot of the filtered solution was transferred to a 10 ml A-grade volumetric flask and spiked with the internal standard and made up to volume using HPLC grade water to produce a FCV solution of 125 µg/ml. The samples were analysed in triplicate (n=3).

The chromatograms developed during the assay of Famvir® tablets revealed that all peaks were well resolved from the solvent front and were free from interference from excipients present in the tablets. The results of the assay summarised in Table 2.7 reveal that the content of the Famvir® tablets were well within the pharmacopoeial limits of 98 -102 % as described in the pending United States Pharmacopeia (USP) monograph [80].

Table 2.7 *Assay of Famvir® tablets*

Brand Name	Label Claim	Mean Recovery (mg) ±SD	Mean % Recovery	% RSD
Famvir®	125 mg	123.13 mg ± 1.60	98.35 %	1.30 %

2.4.8 Stress Studies

Stability-indicating assays are defined as validated quantitative analytical procedures that are able to detect changes in the relevant properties of an API and/or drug product and can be used to measure the analyte of interest, accurately without interference from possible degradation products, process impurities, excipients and other potential impurities that could be present [81].

Testing of those features of a product that may be susceptible to change during storage and that are likely to influence the quality, safety and/or efficacy of the product must be undertaken using a validated stability-indicating method. Forced degradation studies should be performed on the analyte of interest in order to establish the inherent stability characteristics and degradation pathways of that material in order to confirm the suitability of the analytical method for the purpose for which it is intended [82].

Forced degradation studies were performed on a standard solution of FCV using stress conditions prescribed by the ICH. This approach necessitates the performance of forced degradation studies under different conditions including, pH, light, oxidation and heat to assess their impact on the stability of FCV [82; 83].

The response of a solution containing only FCV was compared to the response of a test sample of FCV generated by exposing the analyte to different stress conditions. All solutions were analysed with and without the presence of an internal standard in order to monitor the samples that had been subjected to the different conditions, qualitatively and quantitatively.

2.4.8.1 Sample Preparation

Approximately 10 mg FCV was accurately weighed and dissolved in HPLC grade water to produce a 1 mg/ml FCV solution. This was heated to 50 °C for 8 hours, under reflux conditions. A 1 ml aliquot of this solution was transferred to a 10 ml A-grade volumetric flask and made up to volume with HPLC grade water prior to analysis.

2.4.8.1.1 Hydrolytic Degradation Studies

No degradation following exposure of sample to hydrolytic conditions was observed. These experiments were repeated at a temperature of 60 °C for 8 hours and FCV was found to undergo mild degradation under these conditions. The resultant chromatogram is shown in Figure 2.10 (I). A degradation peak (A) was observed at a retention time of 2.2 minutes. Quantitative analysis of the samples revealed that approximately 5% of the FCV had degraded and that 95.19% FCV remained in solution, as shown in Figure 2.10 (II).

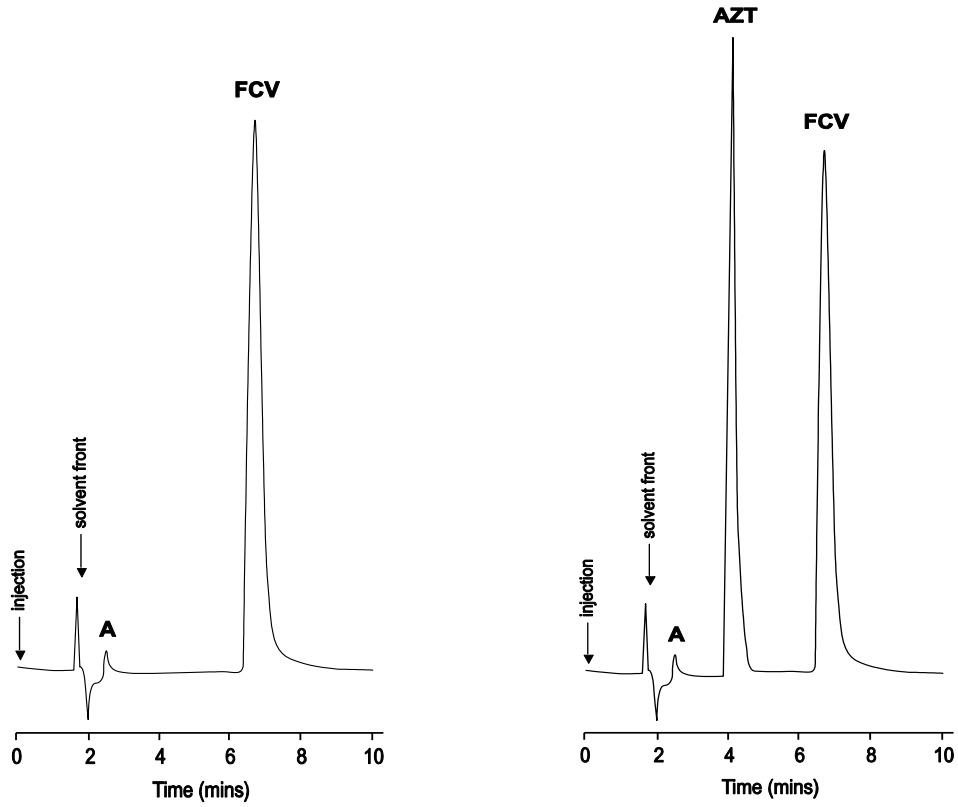
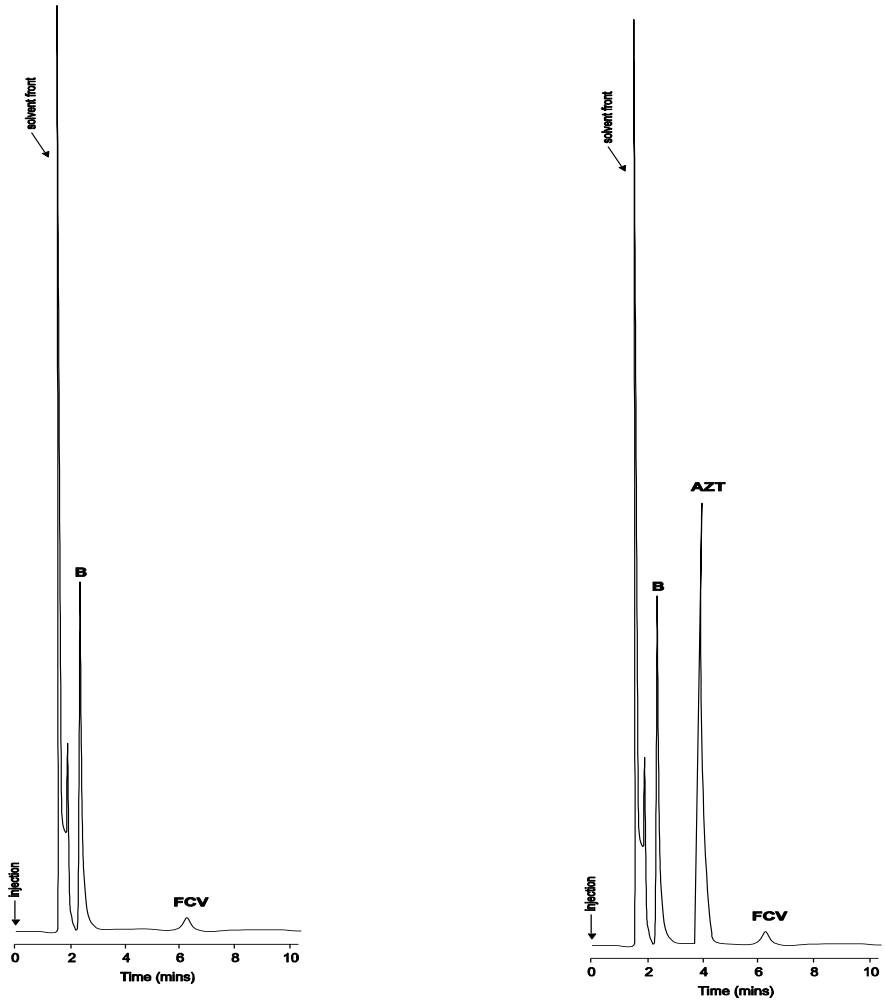


Figure 2.10 *Chromatographic separation following hydrolytic degradation without (I) and with (II) the presence of AZT*

2.4.8.1.2 Acid Degradation Studies

Significant degradation was observed following analysis of samples exposed to acidic conditions *viz.*, 0.1 M HCL, and revealed a degradation peak (B) that was well resolved from FCV, as shown in Figure 2.11 (I). Quantitative analysis revealed that approximately 94% of the FCV had degraded and that only 6.01% FCV remained in solution, as shown in Figure 2.11 (II). This experiment was repeated at 22 °C with analyses were performed at 30 minutes, 1 hour and 4 hours. Quantitative analysis revealed that approximately 31%, 57% and 67% FCV had degraded, and that 68.84%, 42.59% and 32.96% FCV remained in solution, respectively.



I II

Figure 2.11 *Chromatographic separation following exposure to 0.1 M HCl, when heated under reflux at 50 °C for 8 hours without (I) and with (II) AZT*

2.4.8.1.3 Base Degradation Studies

Complete degradation was observed following exposure of FCV to basic conditions *viz.*, 0.1M NaOH. The resultant degradation peak (C) was well resolved from FCV, as shown in Figure 2.12 (I). Quantitative analysis revealed that 100% FCV had degraded and 0% FCV remained in solution, as shown in Figure 2.12 (II). The experiment was repeated at 22 °C and samples were analysed after 15 minutes. Quantitative analysis revealed that 100% FCV had degraded and that 0% FCV remained in solution.

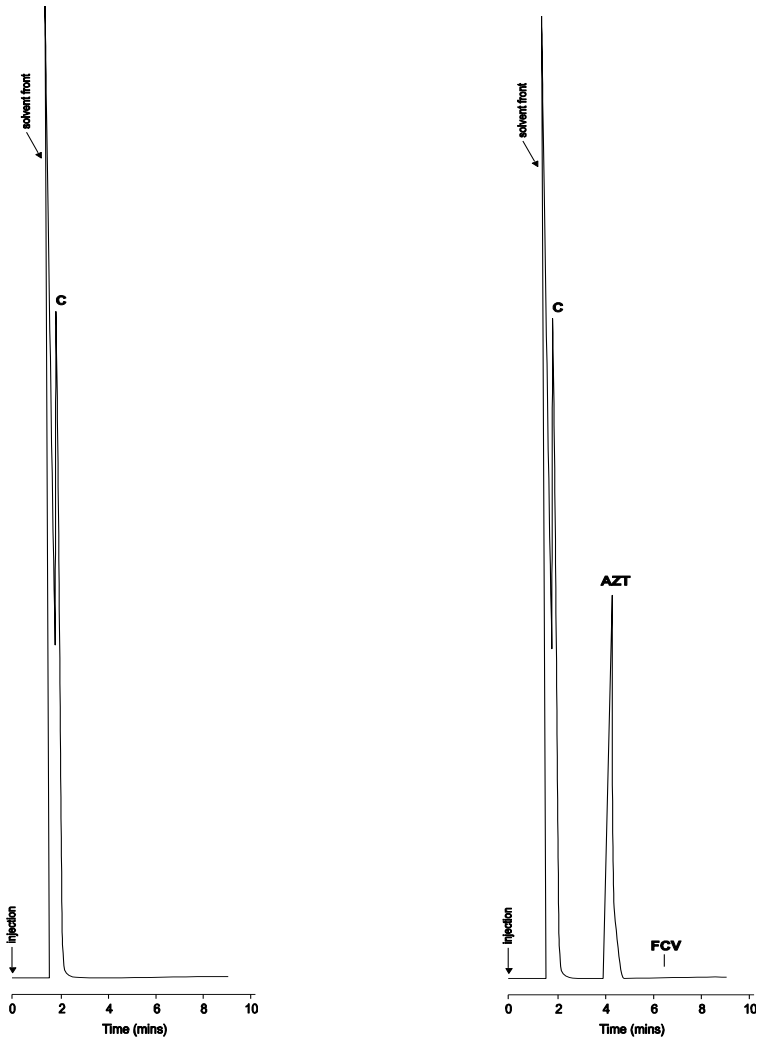


Figure 2.12 *Chromatographic separation following exposure of FCV to 0.1 M NaOH when heated under reflux to 50 °C for 8 hours, without (I) and with (II) AZT*

2.4.8.1.4 Oxidative Degradation Studies

Significant degradation of FCV was observed following analysis of samples exposed to oxidative conditions *viz.*, 3 % H_2O_2, with resultant degradation peaks (D, E, F and G) well resolved from FCV as shown in Figure 2.13 (I). Quantitative analysis revealed that approximately 49% FCV had degraded and that 51.23% FCV remained in solution, as shown in Figure 2.13 (II).

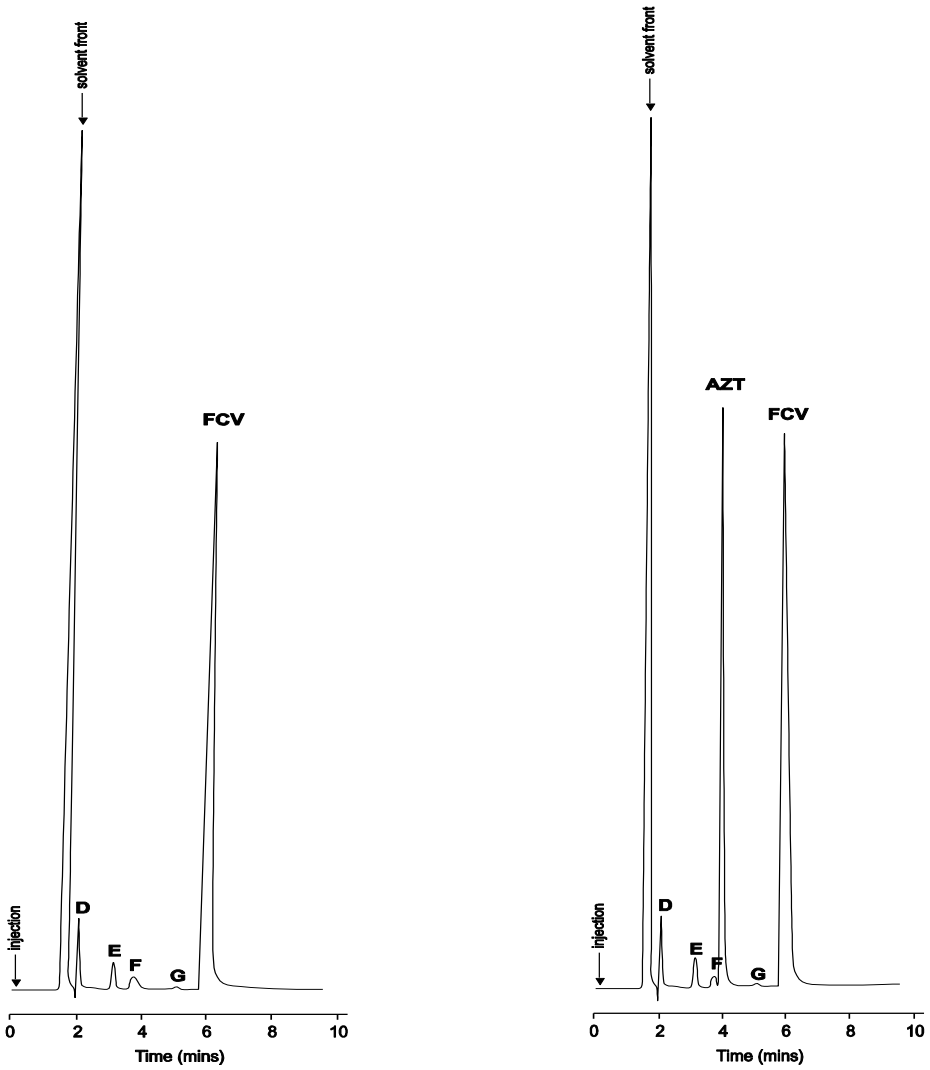


Figure 2.13 *Chromatographic separation following exposure of FCV to 3% H_2O_2 when heated under reflux conditions to 50 °C for 8 hours, without (I) and with (II) AZT*

2.4.8.2 Thermal Degradation Studies

FCV powder was incubated in an oven at 80 °C for 8 hours. Approximately 10 mg of this sample was then dissolved in 10 ml HPLC grade water. A 1 ml aliquot of this solution was transferred to a 10 ml A-grade volumetric flask and made up to volume. No degradation was observed. The experiment was repeated and samples were analysed after 8, 12 and 24 hours exposure. FCV was thus found to be stable under these conditions.

2.4.8.3 Photo Degradation Studies

Approximately 50 mg FCV was dissolved in HPLC grade water to produce a 1 mg/ml FCV solution. Five (5), 10 ml aliquots of this solution were transferred into clear glass ampoules using a needle and syringe and then sealed. The ampoules were stored in a photo-stability chamber and exposed to light (250 W/m^2) for 8 hours. A 1 ml aliquot was withdrawn from each ampoule diluted in a 10ml A-grade volumetric flask with HPLC grade water. No degradation of FCV was observed and FCV was considered to be stable under these conditions.

2.4.9 Sample Stability

2.4.9.1 Introduction

An integral part of any validation procedure requires that the stability of an analyte in solution be investigated, as an analyst must establish the conditions and length of time for which standard solutions can be stored, prior to and during use. To eliminate standards that do not represent true concentration the solutions should be freshly prepared until instability of standard solutions becomes evident [84; 85]. Long term stability assessment is usually performed by preparing samples at two (2) concentrations, covering the relevant concentration range [54; 85; 86] and comparing these to the absolute response of freshly prepared solutions with those following analysis of stored solutions [85]. The time between assessments may be variable and usually the first few evaluations are undertaken daily until the stability of the solutions has been established, after which testing may be extended to weekly analyses [85].

A statistical method for evaluation and reporting data from stability studies was used to interrogate stability data in these studies [54]. The statistical treatment of data takes into account the precision of analytical measurements and permits a certain degree of confidence to be assigned to conclusions relating to the stability of solutions and the differences in concentrations, if any, may be described as relevant and/or significant. The procedure for analysis involves replicate evaluation of the compound of interest in freshly prepared and stored samples on the same day. A 90% confidence interval (C.I) is constructed to establish the true percent change in concentration and the relative difference in response between the two sets of data being interrogated.

The relative difference in response is then used to calculate an upper (U.L) and lower limit (L.L) for the C.I. and the true percent change in response will lie within the limits with 90% certainty.

Timm *et al* [54] proposed that a change in response during storage may be statistically relevant only if both the U.L and L.L values of the confidence interval are > 10% or < -10%.

The possible outcomes that may result when the stability of stored samples is assessed using the approach by Timm *et al.* is shown in Figure 2.14, and indicates the relationship between significant and relevant change of response on storage.

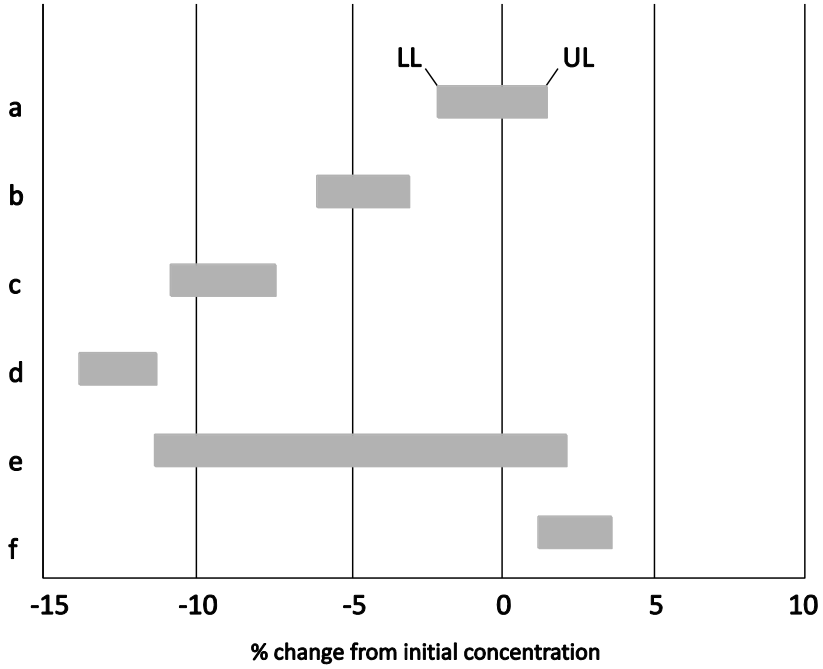


Figure 2.14 *Possible outcomes that could be generated using the Timm approach*

Where,

(a) change of response, not significant and not relevant
(b) decrease of response, significant, but not relevant
(c) decrease of response, significant and possibly relevant
(d) decrease of response, significant and relevant
(e) decrease of response, not significant, but possibly relevant
(f) increase of response, significant

The bars above the axis represent a 90% confidence interval for the percent change (Δ%) observed between stored and freshly prepared samples.

2.4.9.2 Method

The stability of stock solutions of FCV prepared as described in §2.3.6.6 using HPLC grade water was evaluated at low and high concentrations of 6 and 110 µg/ml, respectively, for two (2) weeks. The solutions were stored at 4°C and at 22°C. Aliquots of 60 µl and 1100 µl of the stock solution were pipetted into a 10 ml A-grade volumetric flask on day 0 and made up to volume with HPLC grade

water to produce solutions of 6 µg/ml and 110 µg/ml respectively. A 1 ml aliquot of each solution was transferred into a 1.5 ml clear glass vial for storage. Five (5) samples of each concentration were prepared on day 0, and analysed on days 1, 2, 3, 7 and 14 following storage. Fifty samples were analysed for each concentration. On each day of analysis a freshly prepared stock solution of FCV was used to prepare fresh samples for analysis and which were tested at the same time as the stored samples. All samples were spiked with 50 µl of the internal standard solution prior to analysis.

2.4.9.3 Results and Discussion

It is evident from the graphical representation in Figure 2.15 that no significant or relevant decrease in response was observed for the upper or the lower concentration of FCV stock solutions stored for a two (2) week period when stored at 4 °C. However storage at 22 °C revealed a decrease in response on day 7, which was considered significant but not relevant for the sample of low concentration. Storage for 14 days resulted in a decrease in response that was significant and relevant.

The 6 µg/ml sample showed an increase in response which was considered a result of experimental systematic error such as a weighing error on day 2. A significant increase in response is possible only in the extremely unlikely event of a compound producing a degradation product with the same retention time as the parent compound but which produces greater response in the detection system [54].

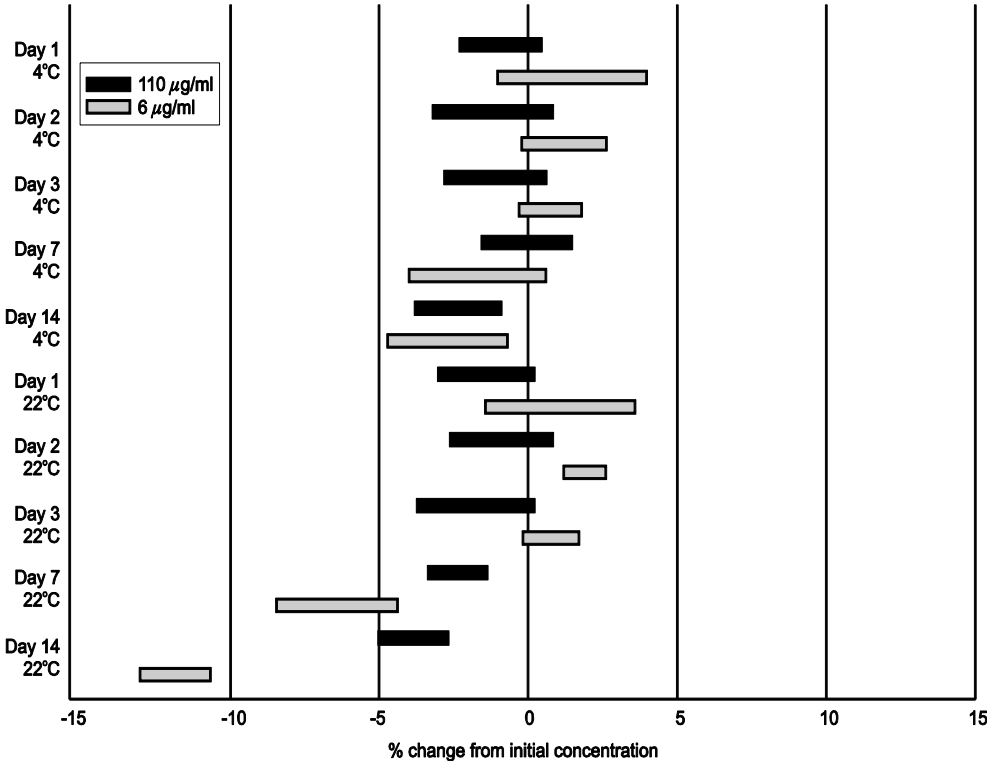


Figure 2.15 *Stability of the FCV stock solution following storage at 4 °C and 22 °C for 14 days*

2.5 CONCLUSION

A RP-HPLC has been developed, validated and successfully applied to the analysis of FCV in pharmaceutical dosage forms. The method was optimised to achieve a separation that was stability-indicating and FCV was resolved from degradation products and dosage form excipients.

The method was optimised using a Phenomenex® ODS C_{18} (150 mm x 4.6 mm x 5 µm) column by manipulation of the mobile phase composition. The eluant was monitored at 221 nm. The mobile phase composition was modified by altering the type and amount of organic modifier present and/or the amount, pH and molarity of the buffer used. FCV ionises and the retention time is affected by the mobile phase composition. A mobile phase consisting of 0.05 M potassium phosphate buffer (pH=4) and acetonitrile (84:16 v/v) at a flow rate of 1.0 $ml.min^{-1}$ was selected for use, as the peaks for FCV and the internal standard, AZT, were well resolved and adequately separated.

The method was validated for linearity, precision, accuracy, selectivity, LOQ and LOD by exposing FCV to forced degradation conditions as recommended in the ICH reference. The calibration curve for FCV was linear over the concentration range 2-120 µg/ml with a resultant R^2 value of 0.9999, slope of 0.0161 and a y-intercept value of -0.0142. The equation for the line was $y = 0.0161x - 0.0142$. The method was found to be precise and accurate, with % RSD and % Bias values of less than the stated limits of 2% and 5%, respectively. The LOQ and LOD were found to be 2 µg/ml and 0.6 µg/ml respectively. FCV was found to be stable under thermal and photolytic degradation conditions, whilst mild degradation of FCV was observed under neutral hydrolytic conditions. Significant degradation was observed under oxidative, acidic and basic degradation conditions.

The RP-HPLC method that was developed is simple, rapid, precise, accurate and selective, and can be applied to the quantitation of FCV in dosage forms.

2.6 MODIFICATION OF THE METHOD

2.6.1 Introduction

When selecting the initial chromatographic conditions for a separation, it is important that any degradation products formed during stress and stability testing be adequately retained and separated from the analyte of interest, the internal standard and other impurities by the HPLC method [49; 53; 70]. It is evident that this was achieved using the method described in §2.4. However additional degradation products were observed when FCV formulations were manufactured using syrup simplex, as the vehicle. A single degradation product with a retention time of 3.9 minutes was produced, and

this interfered with the integration of the internal standard, AZT, which had a retention time of 4.0 minutes as shown in Figure 2.16. Therefore a modified method of analysis for FCV was required.

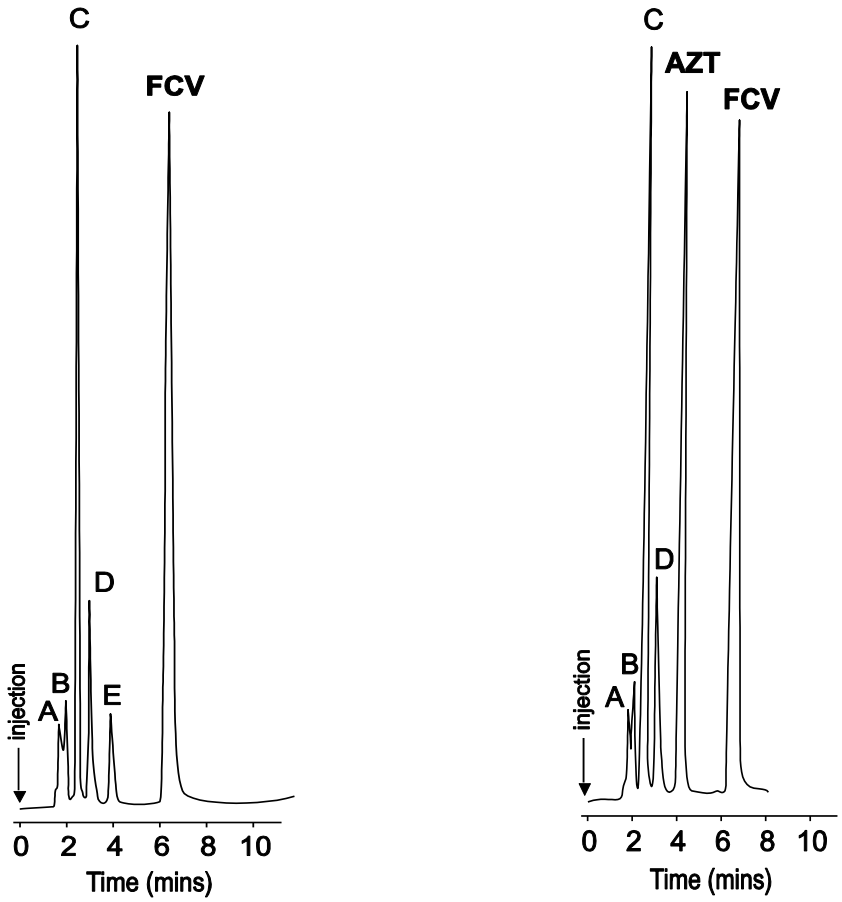


Figure 2.16 *Chromatograms showing degradation products of FCV (peaks A-E) (I) when syrup simplex was used as the vehicle and interference of the degradation product peak E with AZT (II) at a retention time of 4 min.*

2.6.2 Mobile Phase Composition

The mobile phase was prepared as described in §2.3.6.5, and the composition was varied in an attempt to separate the internal standard peak from the degradation product. Based on the fact that as the % buffer increases and the organic modifier and acetonitrile content decreases, the retention time of the analyte increases. The increase in retention time is however dependant on the polarity of the analyte. The effect of a change in mobile phase composition on the retention time of FCV, AZT and the degradation products is summarised in Table 2.8.

Table 2.8 *Effect of mobile phase composition on the retention time and resolution of AZT and an unidentified degradation product*

Phosphate Buffer % v/v	Organic Modifier % v/v	FCV Retention Time (min)	AZT Retention Time (min)	Degradation Product Retention Time (min)	Resolved Peaks
84	16	6.4	4.0	3.9	No
86	14	11.0	5.6	5.7	No
87	13	15.1	7.2	7.9	No
88	12	17.5	7.6	8.4	No

From the data summarised in Table 2.8, it is evident that changing the mobile phase composition was unsuccessful in separating the degradation product from the internal standard and an alternate internal standard was required.

2.6.3 Internal Standard Selection

An ideal internal standard should be chemically similar to the analyte of interest and must be resolved in order to accurately analyse the analyte in the presence of degradation products. As AZT was not adequately resolved from a degradation product using the method described in §2.3 a new internal standard was required [68]. Lamivudine (3TC) and stavudine (d4T) were assessed for suitability as possible internal standards as their chemical structure is similar to FCV and AZT.

3TC and d4T, similar to FCV and AZT, are nucleoside analogues and are more specifically analogues of cytidine and thymidine, shown in Figure 2.17.

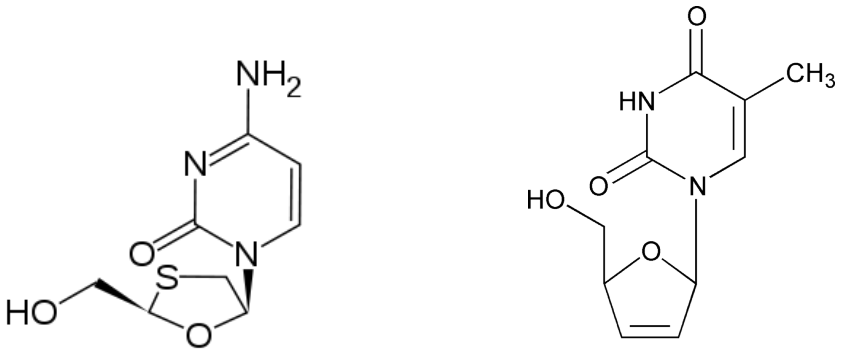


Figure 2.17 *Chemical structure of 3TC (I) ($C_8H_{11}N_3O_3S$), MW=229.3 g/ml and d4T (II) ($C_{10}H_{12}N_2O_4$), MW=224.213 g/mol*

3TC and d4T have retention times shorter than that of AZT as these compounds are more polar than AZT. 3TC has a retention time of 2.0 minutes and d4T a retention time of 2.1 minutes.

AZT and 3TC have amide and alcohol functional groups that increase their polarity, however 3TC has a primary amine rather than the ketone functional group that is present in AZT. Ketone functional groups add to the polarity of a molecule due to the carbonyl functionality exhibiting a large dipole moment and the presence of an electronegative oxygen, and amines have the ability to form hydrogen bonds, resulting in the molecule exhibiting polar characteristics. Therefore 3TC has a shorter retention time than AZT when using the specific mobile phase used in these studies, due to better partitioning of 3TC in the mobile phase and is less likely be retained on the stationary phase than AZT.

d4T has a structure very similar to that of AZT, however it does not contain an azide functional group. The azide functional group has a negatively charged N, resulting in an overall neutral charge in the molecule thereby increasing its lipophilic nature [87]. Therefore d4T exhibits a shorter retention time than AZT as the compound is more hydrophilic and elutes rapidly from a hydrophobic stationary phase.

Both potential internal standards exhibited retention times shorter than those observed for FCV, and the peaks could not be adequately separated from those of the degradation products generated in stability. Both compounds were therefore unsuitable for use as internal standards.

The simultaneous determination of AZT and nevirapine (NVP) using RP-HPLC has been reported by Marchei *et al* [88]. The two compounds were adequately separated using chromatographic conditions similar to those developed for the quantitation of FCV. NVP has a retention time of 9.5 minutes and AZT a retention time of 5.2 minutes. Due to the longer retention time of NVP and it being a heterocyclic compound it was considered suitable for further investigation as a possible internal standard. The structure of NVP is shown in Figure 2.18.

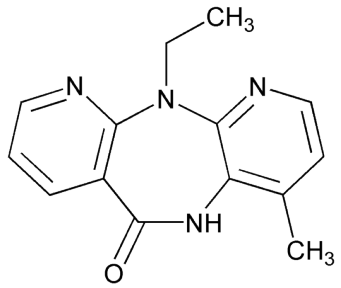


Figure 2.18 *Chemical structure of NVP ($C_{15}H_{14}N_4O$), MW=266.9 g/mol*

2.6.3.1 Preparation of the Internal Standard

The NVP internal standard solution was prepared by accurately weighing approximately 20 mg of NVP, which was transferred to a 10 ml A-grade volumetric flask. The powder was dissolved in 10 ml HPLC grade water to produce a solution of 2 mg/ml. A 50 µl aliquot was pipetted directly into an

HPLC vial together with 1 ml of a FCV solution to achieve a final concentration of the internal standard of 100 µg/ml.

2.6.4 Optimisation of Method

Optimisation studies were performed using the mobile phase prepared as described in §2.3.6.5. The separation was conducted at a flow rate of 1.5 ml/min and a solution of FCV and NVP were injected for analysis. The retention times were found to be 4.4 and 8.2 minutes for the FCV and NVP respectively. The peaks were well resolved and separated from the potential degradation products, as shown in Figure 2.20.

In an attempt to reduce the operating back-pressure on the column a flow rate of 1 ml/min was used, and in order to reduce the retention and run times, the mobile phase composition was adjusted to phosphate buffer (0.05 M, pH 4) 81% v/v and ACN 19% v/v. This mobile phase had reduced polarity and increased strength, resulting in shorter retention times for the compounds of interest.

The resultant chromatogram shown in Figure 2.19 reveals a retention time of 4.9 minutes and 8.6 minutes for FCV and NVP respectively. The compounds were well resolved and separated from any degradation product(s) that may be present in samples, as shown in Figure 2.20.

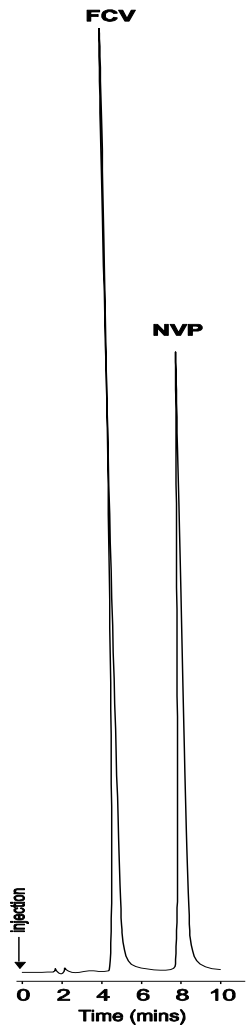


Figure 2.19 *Typical chromatogram of the separation of FCV (4.9 min) and NVP (8.6 min)*

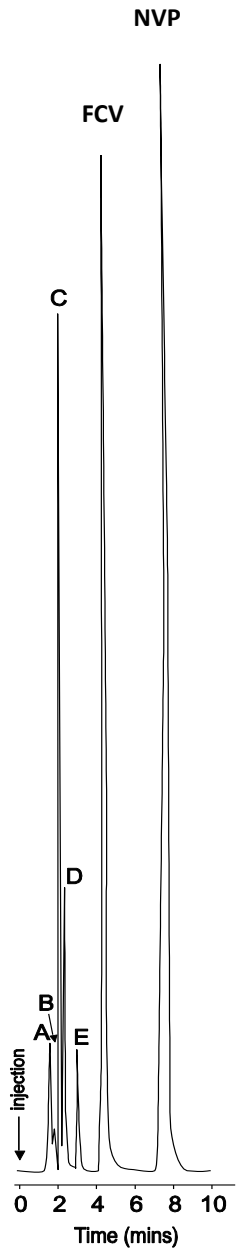


Figure 2.20 *Chromatogram showing complete resolution of FCV and NVP from degradation products*

2.7 RE-VALIDATION

2.7.1. Introduction

Following any major changes to an HPLC method, re-validation of the analytical procedure is essential and it must be established whether the changes warrant a complete or partial validation by addressing the validation parameters that are affected by the changes [49; 69].

Due to a change in mobile phase composition and internal standard, revalidation of the analytical method was required.

2.7.2 Linearity

Linearity was determined as described in §2.4.2. A calibration curve was plotted and the correlation coefficient of the respective calibration curve was established. The calibration standards were prepared as described in §2.3.6.6 and the resultant curve is shown in Figure 2.21.

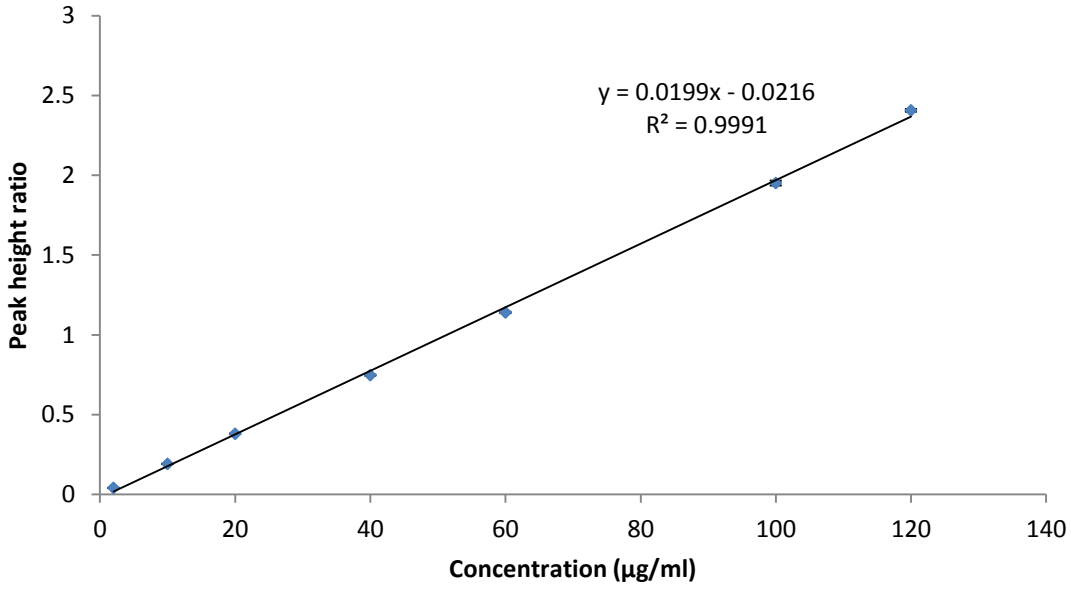


Figure 2.21 *Typical calibration curve of FCV over the concentration range 2-120 µg/ml using NVP as the internal standard*

The calibration curve was found to be linear over the concentration range tested, with a resultant R^2 value of 0.9991, slope of 0.0199 and a y-intercept value of -0.0216, with the resultant equation of the line of y = 0.0199x-0.0216.

2.7.3 Precision

Repeatability and intermediate precision were established as described in §2.4.3.

2.7.3.1 Repeatability

Intra-assay precision was performed at concentrations of 7, 50 and 110 µg/ml, as described in §2.4.3.1 in replicates (n=5). The % RSD of the peak height ratios was calculated and the results of repeatability studies are summarised in Table 2.9.

The data reveal low % RSD values of < 5% for all samples, indicating the precision and repeatability of measurements using this analytical method. The intra-day precision data reveal that the method is suitable for the analysis of FCV.

2.7.3.2 Intermediate Precision

Intermediate precision was performed at concentrations of 7, 50 and 110 µg/ml, as described in §2.4.3.2, with five replicates (n=5). The % RSD of the peak height ratio was then calculated. The results of intermediate precision studies are summarised in Table 2.9.

Table 2.9 *Repeatability and intermediate precision results for FCV analysis*

	Concentration (µg/ml)	**Mean Peak Height Ratio (n=5)**	**Standard Deviation (µg/ml)**	**% RSD**
	7	0.13	0.0003	0.26
Day 1	50	0.94	0.0020	0.21
	110	2.19	0.0013	0.06
	7	0.13	0.0004	0.32
Day 2	50	0.95	0.0019	0.20
	110	2.14	0.0137	0.64
	7	0.13	0.0025	1.98
Day 3	50	1.00	0.0212	2.12
	110	2.27	0.0442	1.95
Average	7	0.12	0.0642	4.01
	50	0.97	0.0313	3.24
	110	2.20	0.0051	2.92

The low % RSD values of < 5% for precision studies undertaken over three (3) consecutive days reveal that the analytical method is suitable and that inter-day precision is appropriate for this method.

2.7.4 Accuracy

The accuracy of the method was evaluated as described in §2.4.4 and established at three (3) concentrations, *viz.*, 7, 50 and 110 µg/ml. Each solution was analysed in replicates (n=5) and the % Bias was established following interpolation of the data from freshly constructed calibration curves. The data are summarised in Table 2.10.

Table 2.10 *Accuracy for FCV over the concentration range 2-120 µg/ml*

Theoretical concentration (µg/ml)	**Determined concentration (µg/ml)**	**% RSD**	**% Recovery**	**% Bias**
7	7.02	0.2677	1.0066	0.23
50	48.48	0.2068	1.0068	-3.14
110	111.16	0.0613	1.0228	1.05

The resultant values for % RSD and % Bias were below the limits of 2 and 5% set in our laboratory, indicating that the method is accurate.

2.7.5 Conclusion

A RP-HPLC method has been modified and re-validated and found to be simple, precise, accurate and selective and can be used for the quantitation of FCV in dosage forms. The method was re-validated for linearity and precision according to the ICH guidelines. The calibration curve for FCV was linear over the concentration range of 2 – 120 µg/ml with an R^2 value of 0.9991, a slope of 0.0199 and a y-intercept value of -0.0216. The equation for the line was Y = 0.0199x – 0.0216. The method was precise with intra-assay, intermediate precision and accuracy results with % RSD and % Bias values less than the stated limits of 2% and 5% respectively.

The method is suitable for its intended use and thus applicable in the analysis of FCV oral formulations, in the presence of formulation excipients.

CHAPTER THREE

PREFORMULATION

3.1 INTRODUCTION

Pharmaceutical dosage forms are usually comprised of the API and adjuvants or excipients that have no direct pharmacological action but are used in a formulation to improve the manufacture, stability, delivery of the drug and the aesthetic appeal of the formulation [89; 90]. During the development of an effective, reliable and stable dosage form it is vital that none of the excipients selected for use cause unintended effects or impact the efficacy of the product. It is therefore important that prior to formulation the API is adequately characterised in respect of its chemical and physical attributes, and that the effects of different excipients on the stability of the molecule are assessed with regard to mechanisms of degradation and modifications of the API [89; 91]. Many excipients are available for use in pharmaceutical formulations, however without understanding these materials it is unlikely that a successful, robust and stable formulation will be developed [92]. The necessary information is generated by conducting preformulation studies that include, but are not limited to, physical characterisation of the API in terms of particle size, solubility, pKa, pH, stability, incompatibility and toxicity, in addition to other characteristics relating to the nature of the API under investigation. By conducting appropriate preformulation studies an accurate prediction can be made of the potential challenges when combining an API with excipients to produce a dosage form. Preformulation studies therefore are vital for the development of a robust, high quality, safe, and effective dosage form [93].

3.2 PHYSICOCHEMICAL PROPERTIES

3.2.1 Particle Size and Morphology

True solutions are homogenous systems in which particles are < 1nm in diameter. Particle size is therefore not of special importance when formulating a solution, however the size and shape of particles of < 1 μm in diameter can affect their solubility and rate of solution [94]. Particle size and morphology are of greater importance for formulations in which the API is suspended. Suspensions are heterogeneous systems in which particles are normally > 100 nm in size and are therefore visible to the naked eye [94]. In terms of stability of a dispersion, a fine particle size is necessary to ensure a slow rate of sedimentation and better homogeneity within the dispersion, resulting in better predictability of suspension properties from batch to batch [95; 96]. Particles > 5 μm in diameter will impart grittiness to the product. This is especially undesirable for administration to paediatric populations. The particle size of an API may change on storage due to crystal growth, particularly if

there are fluctuations in the temperature during storage [95-97]. In a concentrated suspension particle-particle interactions may occur and result in an increase in the viscosity. Small particles can also alter viscosity of a vehicle due to increased surface area effects [95; 96].

3.2.2 pH and Solubility

The solubility of an API can be influenced by changing the pH of the vehicle in which it is dissolved or dispersed. However the pH selected for manufacture should not affect product characteristics, in particular chemical stability. The optimum pH for solubility does not always coincide with the pH at which the API exhibits optimum stability and often a compromise is required during formulation development to ensure that the stability and solubility of the API and excipients, in addition to the physiological compatibility and bioavailability of the product, are not affected [91; 97].

The solubility of an API is one of the most important physicochemical properties that must be characterised during preformulation studies [98]. For liquid dosage forms solubility data are essential to ensure the robustness of a finished product. One of the primary influences on the solubility of ionisable drugs is pH [98-100]. If a drug is weakly acidic or basic the solubility can be influenced by changes in pH, in accordance with the Henderson-Hasselbalch relationship, described by Equation 3.1 for weak acids and Equation 3.2 for weak bases. These equations relate pH, solubility and pK_a of an API. The equations reveal that the solubility of a weak base can be increased by a decrease in the pH of a solvent, and the converse is true for a weak acid [97; 101].

$$pH = pK_a + log\frac{s - s_0}{s_0} \qquad \textit{Equation 3.1}$$

$$pH = pK_a + log\frac{s_0}{s - s_0} \qquad \textit{Equation 3.2}$$

Where,

s = the overall solubility of the drug
s_0 = the solubility of the ionised form
pK_a = acid dissociation constant

3.2.3 pH and Stability

The stability of an API over a wide pH range should be established early during preformulation studies [91]. A common cause of instability is hydrolysis in aqueous solution and involves nucleophilic attack of labile bonds, for example the amide bond in the case of FCV. Several conditions catalyse degradation of drug molecules, including the presence of OH^- and H_3O^+ ions, particularly when present in high concentrations. Consequently many drugs are most stable in the pH range 4 - 8. Weakly acidic or basic drugs are highly susceptible to degradation in the ionised state as a result of increased solubility [97]. A plot of degradation rate versus pH whilst temperature, ionic strength and solvent composition are constant facilitates monitoring of the reactions in aqueous solution to establish the pH of maximum stability of the molecule [97].

3.2.4 Polymorphism and Solvatomorphism

Many organic compounds have the same elemental composition but different crystalline forms that may be amorphous, crystalline or hydrate [102]. The changes in crystalline form are defined as polymorphism and changes in the solvation state in combination with a different elemental composition is defined as solvatomorphism [102-104]. Different solid crystalline forms may exhibit different physical properties, thermal stability, dissolution characteristics and bioavailability [102; 105]. Amorphous and polymorphic forms or compounds generally exhibit differences in their thermal behaviour. The thermal behaviour of a material can be characterised by melting point determinations or using thermogravimetric analysis (TGA) and/or differential scanning calorimetry (DSC), and can be used to distinguish different polymorphic forms of a compound. A particular polymorphic form may also give rise to distinct spectroscopic properties that may be detected using x-ray powder diffraction (XRD), IR spectroscopy as described in §3.2.5, *vide infra* and C13 solid state NMR as described in §1.5.8 [105; 106].

3.2.5 Drug Excipient Compatibility

To develop a stable pharmaceutical formulation, drug-excipient compatibility studies must be undertaken to identify unfavourable combinations of API and excipients that may modify the chemical nature, stability and bioavailability of an API, resulting in altered therapeutic safety and efficacy [89; 90; 107; 108]. Many excipients contain reactive functional groups that may interact with an API, have the potential to cause degradation or alternatively contain residues or impurities that form degradation products that may interact with an API [90]. For example, the Maillard reaction that occurs between primary amine containing compounds and the hydroxyl functional group of reducing sugars which results in the formation of yellow-brown coloured by-products. Another example of incompatibility is observed with stearate salts such as magnesium stearate and/or sodium stearate

which are incorporated in tablets as lubricants and may result in ion-catalyzed degradation if an API that is susceptible to hydrolysis when crushed tablets are used in the preparation of extemporaneous formulations.

Several approaches have been proposed for screening the chemical compatibility of an API and excipients. However it is imperative to obtain rapid and reliable information about possible drug-excipient interactions, as the collection of real-time stability and compatibility data can be expensive and is time consuming [89; 107]. The computational approach is one in which comprehensive databases of reactive functional groups and drug-excipient compatibility can predict potential challenges. Another approach involves the storage of binary mixtures of API and excipients in a 1:1 ratio under stress conditions and analysis of samples using a stability-indicating HPLC assay. Alternatively, binary mixtures can be screened using IR or thermal methods of analysis such as DSC and TGA to determine if potential incompatibilities exist between the API and excipients [90; 109].

Insight gained into potential solid-state reactions between materials early in preformulation studies ensures that a rational approach can be followed, to avoid reactivity between an API and potential excipients and thus to establish stability of the API in the final product [89].

3.2.5.1 Infrared Spectroscopy (IR)

IR spectroscopy can be used to investigate potential incompatibilities between an API and excipients as it is a simple method for detecting changes in mixtures of drug and excipients. The disappearance or reduction in intensity of a peak and/or the appearance of new peak(s) may be evidence of a drug-excipient interaction and/or incompatibility [107].

Radiation of a molecule in the IR region of the electromagnetic spectrum causes bond vibrations in the exposed molecule. When the molecule is exposed to electromagnetic radiation of a specific frequency that matches the frequency of vibration of the molecule, energy is absorbed by a bond and the result is an increase in the amplitude of vibration. Energy of each frequency absorbed by a molecule corresponds to a specific molecular motion that can be determined by measuring the IR spectrum. Interpretation of these vibration responses permits the types of bond present in the molecule to be identified [110; 111].

3.2.5.2 Differential Scanning Calorimetry (DSC)

Thermal analysis can be used to investigate possible physicochemical interactions between the components of a formulation and can aid in the selection of suitable excipients that are chemically compatible with an API [97; 109]. DSC is a widely used thermal technique that provides a rapid and

easy method of obtaining information about materials of interest. DSC has many applications and advantages, such as low sample consumption, that make it suitable for detecting potential incompatibilities between an API and excipients during early product development studies. DSC may also form part of quality control procedures, where the presence or absence of a peak in the thermal analysis curve may be used to identify polymorphs which may be of particular relevance biologically. Different polymorphic forms may exhibit different physiological actions. An investigation into the potential reactivity between excipients that may be used in a formulation is vital in these studies [90; 107; 112; 113]. However DSC results are only indicative of potential stability or instability and do not provide information relating to the cause or nature of incompatibilities that may be observed [90; 113].

DSC measures heat and flow of energy into and out of a sample in relation to a reference as a function of increasing temperature [112; 114]. The sample is subject to a linear increase in temperature from an external source and heat flow is continuously monitored as the analysis proceeds [115]. The samples of interest and reference are held in a cell in which temperature sensors, one each for the sample and reference, are located, with an external heating element. The signal from the instrument is dependent on the difference(s) between the response of the test and reference cells. High sensitivity is achieved as the process represents a true thermal change in the materials, free from thermal effects that may influence the sensors [112].

Possible interactions between an API and excipients are revealed by the appearance of endothermic and/or exothermic peaks. A shift or disappearance in corresponding enthalpy values of materials is an indication of a possible incompatibility [116; 117]. However, differences in a DSC thermogram may sometimes be independent of a chemical incompatibility, and complementary analytical techniques should therefore be used to validate and facilitate interpretation of DSC data [117].

3.2.5.3 X-Ray Powder Diffraction (XRD)

Approximately 95% of all solid materials are crystalline and can therefore be classified according to the type of particles that occupy the crystal lattice and the type of intermolecular forces holding the lattice together [118; 119]. Exposure of a crystalline material to X-rays results in a diffraction pattern. XRD is classified as a 'fixed wavelength (λ), varying angle (θ)' technique in which variation of the θ is due to a large number of randomly oriented crystals within a sample such that some of the *hkl* planes or Miller indices in some powders will be orientated at the appropriate Bragg's angle for reflection [120]. The XRD pattern of a pure substance is therefore regarded as a fingerprint of that substance, and therefore XRD is ideally suited for the characterisation and identification of polycrystalline phases [118].

X-ray radiation is a form of energy that travels as electromagnetic waves and has a λ similar to that of an atom *viz.,* 10^{-10} m [119]. When X-rays pass through matter the radiation causes electrons in a molecule to oscillate at the same frequency as the incoming radiation. At almost all angles destructive interference will be observed where the combined waves are out of phase, with a result that no energy will leave the solid sample. However, if the atoms are in an organised plane as with crystalline materials, constructive interference will occur. The resultant waves will be in phase and produce a well defined X-ray beam emitted from the sample at characteristic angles based on the spaces between the atoms [118]. Such constructive interference patterns occur only when incident angles fulfil the Bragg condition shown in Equation 3.3 [120; 121].

$$n\lambda = 2d\ sin\theta \qquad \textbf{\textit{Equation 3.3}}$$

Where,

n = an interger
λ = the wavelength of the incident X-ray beam
d = the spacing between the planes in the atomic lattice
θ = the angle between the incident ray and the scattering planes

Plotting the angular positions versus intensity will result in a diffraction pattern characteristic of the sample under investigation [121; 122].

3.3 EXCIPIENTS

3.3.1 Excipients for Use in Paediatric Dosage Forms

Some common excipients are not suitable for use in children due to their toxicity and have resulted in adverse effects, some of which have been fatal. [123]. Therefore it is important to carefully consider potential excipients and the possibility of eliciting adverse events in this age group [124].

3.3.1.1 Vehicles for Oral Formulation

The use of methylcellulose-based solutions and syrup simplex as vehicles for oral suspensions has been widely reported [125-129]. Hydroxypropyl methylcellulose K100M (HPMC) and syrup simplex were therefore selected as potential vehicles for the manufacture of FCV formulations. HPMC based solutions can be considered as nearly ‘universal’ suspending agents as they are inert, non-toxic, non-irritating, pH-neutral and interactions between API and HPMC are relatively uncommon [130; 131]. HPMC is a viscosity enhancer or thickening agent stable in solution at pH 3-11and has adequate viscosity stability on long term storage [131].

Sucrose is the main excipient in syrup simplex and is a widely used vehicle for extemporaneous manufacture. It is also commonly found in commercially available suspending agents such as Ora-Sweet® and Ora-Blend® [126; 127]. Although sucrose is a widely used material there is a cause for concern about its use when treating patients with diabetes mellitus or other metabolic sugar disorders [131]. Sucrose solutions are stable and resist microbial contamination at high concentrations *viz.* > 60% w/w [131].

3.3.1.2 Antioxidants

Sodium metabisulphite, ascorbic and citric acid are used as antioxidants in formulations. Sodium metabisulphite is commonly used in oral dosage forms that are acidic at concentrations of 0.01-1.0% w/v. It is stable in solution and although it is primarily incorporated as an antioxidant it has some antimicrobial activity in oral dosage forms that contain syrup [131; 132]. Ascorbic acid is widely used as an antioxidant in aqueous formulations although it is relatively unstable in solution [131-133]. It is incorporated in oral formulations at concentrations between 0.01 and 1.0% w/v. Citric acid although widely used as a buffer component, has antioxidant activity at concentrations between 0.3 and 2.0% w/v, and is used in commercially available universal suspending agents such as Ora-Sweet® [126; 134].

3.3.1.3 Preservatives

The most common preservatives in oral liquid formulations used for over fifty years and administered safely to paediatric patients are methylparaben and propylparaben [135-138] Methylparaben and propylparaben are stable in solution and exhibit antimicrobial activity between pH 4 and 8 when incorporated at concentrations of between 0.1-0.2% w/v. The parabens have a broad spectrum of activity and the use of combinations of the parabens results in a synergistic antimicrobial effect [131; 136; 137].

3.4 METHODS

3.4.1 Scanning Electron Microscopy (SEM)

The particle size and morphology of FCV powder and crushed Famvir® tablets were determined using a Vega© Scanning Electron Microscope (Tescan, Vega LMU, Brno, Czechoslovakia Republic). Commercially available Famvir® tablets were crushed using a mortar and pestle prior to evaluation using SEM and small quantities of the powdered sample, of approximately 2 mg were dusted onto a graphite plate and gold coated for 20 minutes under vacuum. The samples were visualized using SEM at an accelerated voltage of 10 or 20 kV.

3.4.2 pH Solubility Profile

At 25 °C FCV is freely soluble (> 25% w/v) in water initially, but rapidly precipitates as a sparingly soluble (2-3% w/v) monohydrate salt [6; 11]. As precipitation of the compound occurs as a function of increased concentrations of FCV, a variation of the traditional saturation shake-flask method for solubility determination was used in these studies [139].

The pH solubility profile of FCV was constructed over the pH range 1.2 to 7.4 using a 50 mM phosphate buffer as solvent. Approximately 6.8 g of potassium dihydrogen orthophosphate was accurately weighed into a 1000 ml A-grade volumetric flask and made up to volume with HPLC-grade water. Aliquots (10 ml) of the resultant solution were harvested in triplicate (n=3) and the pH adjusted to 1.2, 2, 3, 4, 5, 6.8 and 7.4 using orthophosphoric acid or sodium hydroxide pellets. Approximately 450 mg of FCV was accurately weighed and added to each 10 ml A-grade volumetric flask. Thereafter FCV was added in 10 mg amounts and the solution was shaken following each addition. At the point of saturation, a 5 ml aliquot was filtered through a Millipore® Millex® HV hydrophilic PVDF 0.45 μm syringe filter and a 0.1 ml aliquot of the filtered solution was transferred into a 10 ml A-grade volumetric flask and made up to volume with HPLC grade water. A 1 ml aliquot of the resultant solution was pipetted into a sample vial and spiked with 50 μl internal standard solution prepared as described in §2.6.3.1 and analysed using the stability-indicating HPLC assay described in Chapter two.

3.4.3 pH Stability Profile

The pH stability profile for FCV was constructed over the pH range 1.2 - 12 using 50 mM phosphate buffer as solvent. Approximately 6.8 g potassium dihydrogen orthophosphate was accurately weighed into a 100 ml A-grade volumetric flask and made up to volume with HPLC-grade water. Aliquots (10 ml) of the resultant solution were harvested in triplicate (n=3) and the pH adjusted to 1.2, 2, 3, 4, 5, 6, 6.8, 7.4, 8, 10 and 12 using orthophosphoric acid or sodium hydroxide pellets. Approximately 20 mg FCV was accurately weighed and added to a 20 ml A-grade volumetric flask and made up to volume with HPLC-grade water. Aliquots (2500 μl) of this solution were pipetted into 25 ml A-grade volumetric flasks and made up to volume with buffered solution to achieve a concentration of 100 μl/ml. Aliquots (1000 μl) were pipetted from each volumetric flask at times of 0, 2, 6, 12, 24 and 48 hours into sample vials and spiked with 50 μl internal standard and analysed using the stability-indicating HPLC assay described in Chapter two. A plot of the logarithm of concentration of FCV versus pH at each time point resulted in a straight line with gradient *k*, the degradation rate constant. A plot of the logarithm of *k* was plotted versus pH to produce the pH stability profile for FCV.

3.4.4 Infrared Spectroscopy (IR)

The IR spectra of individual sample components and binary 1:1 mixture of API and excipients, *viz.* sodium metabisulphite, ascorbic acid, citric acid, propylparaben, methylparaben, HPMC, sucrose and potassium dihydrogen orthophosphate were generated using a Model 6.3.5.0176 Spectrum 100 FT-IR Spectrophotometer (Perkin Elmer®, Beaconsfield, Buckinghamshire, England). Binary mixtures of a 1:1 ratio of FCV and Famvir® excipients, *viz.* HPMC, lactose, sodium starch glycolate, magnesium stearate, titanium dioxide and polyethylene glycol were also generated. Binary mixtures of FCV and the excipients used in Ora-sweet®, *viz.* sorbitol, potassium sorbate and sodium phosphate of a 1:1 ratio were also generated. The powders were prepared by mixing the components using a mortar and pestle prior to analysis. A small quantity of the powder mixture was placed on a diamond crystal and analysed (n=4 scans) in the wavenumber range 650 and 4000 cm-1 at a resolution of 4 cm^{-1}.

3.4.5 Differential Scanning Calorimetry (DSC)

Samples of approximately 2 – 5 mg of individual materials and binary mixtures of API and excipients in a 1:1 ratio were accurately weighed prior to DSC analysis. DSC scans were performed between 25 and 250 °C using a Model DSC 7 (Perkin Elmer®, Norwalk, Connecticut, USA) with a PC control unit TAC 7 (Perkin Elmer®, Norwalk, Connecticut, USA) at a heating rate of 10 °C/min and nitrogen flow rate of 20 ml/min. Data analyses were performed using Pyris™ Manager software (Version 9.0, Perkin Elmer®, Norwalk, Connecticut, USA).

3.4.6 Nuclear Magnetic Resonance Spectroscopy (NMR)

Samples of approximately 0.5 g dissolved in approximately 1 ml of deuterated chloroform were used for NMR analyses. Correlation (COSY), proton and carbon spectroscopy were performed using a Bruker Avance 400 MHz NMR spectrometer (Bruker, Rheinstetten, Baden-Württemberg, Germany). The data were analysed using MestreNova software (Version 6.1.1-6384, MestreLab© Research, S.L., Santiago de Compostela, Galicia, Spain).

3.4.7 X-ray Diffraction (XRD)

X-ray powder diffraction patterns were generated at 22 °C using a Bruker D8 Discover X-ray diffractometer (Bruker AXS, Karlsruhe, Bundesland, Germany) with a proportional counter, and Cu-K radiation (1.5405 Å). Data for FCV and FCV monohydrate were collected and analysed in the 2θ angle range of 5 ° to 40 ° at a scanning rate of 1 °.min^{-1}. The filter time constant and the slit width were 2.5 s per step and 6.0 mm, respectively. Samples were placed on a silicon wafer slide and were

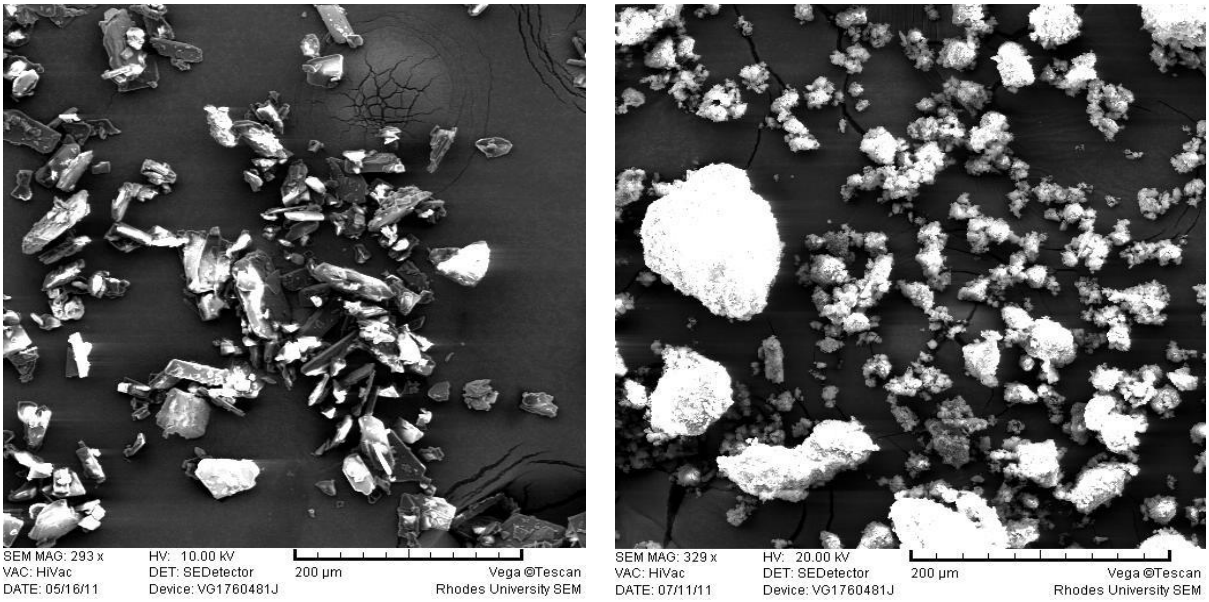
analysed and interpreted using XRD commander and EVA DIFFRAC.SUITE™ software (XRD commander version 2.61, Eva version 14.0, Bruker- AXS GmbH, Karlsruhe, Bundesland, Germany).

3.5 RESULTS AND DISCUSSION

3.5.1 Scanning Electron Microscopy (SEM)

The particle size of FCV powder and crushed Famvir® tablets was characterised by SEM to obtain information that would be potentially useful for formulation development. The SEM images are shown in Figure 3.1 and reveal that the particle size of FCV powder is similar in size ranging between approximately 21.05 and 77.19 μm in diameter (Feret's diameter) [140]. Particles of FCV powder were prism (polyhedral) in shape, whereas the crushed Famvir® tablets produced particles that differed in shape and size, raging between approximately 9.23 μm and 126.15 μm (Feret's diameter) probably due to the presence of tablet excipients.

A relatively narrow size distribution is desirable for stability as it results in a more uniform sedimentation rate and greater batch to batch reproducibility and uniformity [95; 96; 141]. Particles of different sizes, such as observed in the powder from crushed Famvir® tablets, will form a close-packed sediment with the smaller particles filling voids between the larger particles [95]. This may lead to difficultly in redispersion of sediment that would form when the suspension is stored.



I II

Figure 3.1 *SEM images of FCV API powder (I) and powder from crushed Famvir® tablets (II)*

3.5.2 pH Profile and Stability

The degradation of FCV in solution was found to be affected by pH. Degradation over a period of 48 hours in solutions of different pH is shown in Figure 3.2, and appeared to follow a first-order process. The equations that describe the degradation patterns for each pH are listed in Table 3.1with the associated degradation rate calculated for each pH value.

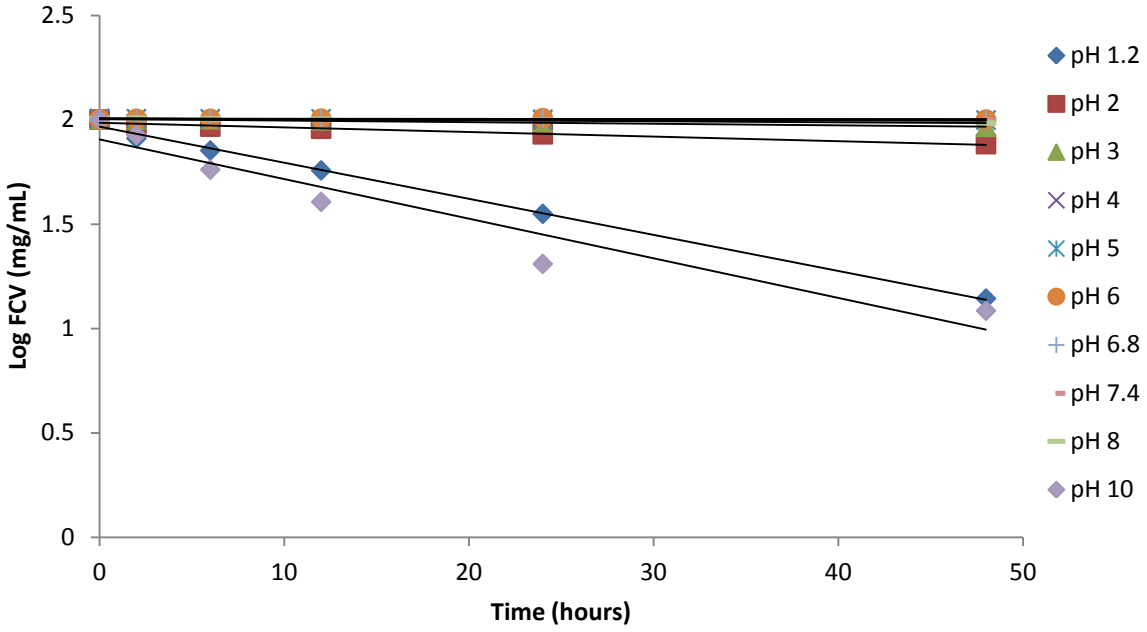


Figure 3.2 *Degradation profile of FCV over 48 hours*

Table 3.1 *Equation and associated degradation rates for FCV over the pH range 1.2 - 10*

pH	Equation	Degradation rate
1.2	Y= -0.0173x + 1.9669	0.0173.hours^{-1}
2	Y= -0.0022x + 1.9862	0.0022 .hours^{-1}
3	Y= -0.0008x + 2.0046	0.0008 .hours^{-1}
4	Y= -0.0002x + 2.006	0.0002 .hours^{-1}
5	Y= - 0.0001x + 2.0046	0.0001 .hours^{-1}
6	Y= -4e^{-05}x + 2.0042	4e^{-05}.hours^{-1}
6.8	Y= -6e^{-05}x + 2.0052	6e^{-05}.hours^{-1}
7.4	Y= - 0.0001x + 2.0028	0.0001.hours^{-1}
8	Y= -0.0005x + 2.0069	0.0005.hours^{-1}
10	Y= -0.019x + 1.9055	0.019.hours^{-1}

The plot of the logarithm of FCV concentration versus time at each pH value was found to be linear, indicating that degradation of FCV follows a first-order process. FCV was found to be most stable at a pH of approximately 6, as indicated by the resultant degradation rate of 4e^{-05}.hours^{-1}. As the concentrations of OH^- and H_3O^+ ions increase the rates of FCV degradation increased with resultant degradation rates of 0.0173.hours^{-1} and 0.019.hours^{-1} at a pH of approximately 1.2 and 10, respectively. FCV was found to undergo complete degradation at pH 12, where 0% FCV remained in

solution after two (2) hours of exposure of the drug to basic solution. FCV is a weak base and therefore an increase in degradation occurred at pH values < pK_a of FCV as a greater amount of FCV is in solution at low pH. A pH stability profile was constructed for FCV, and is shown in Figure 3.3. It is clear that the profile is V-shaped. A change in direction in the pH-rate curve in the region of ionisation of an API is expected since the conjugate acid and base are unlikely to undergo reactions at the same velocity [142].

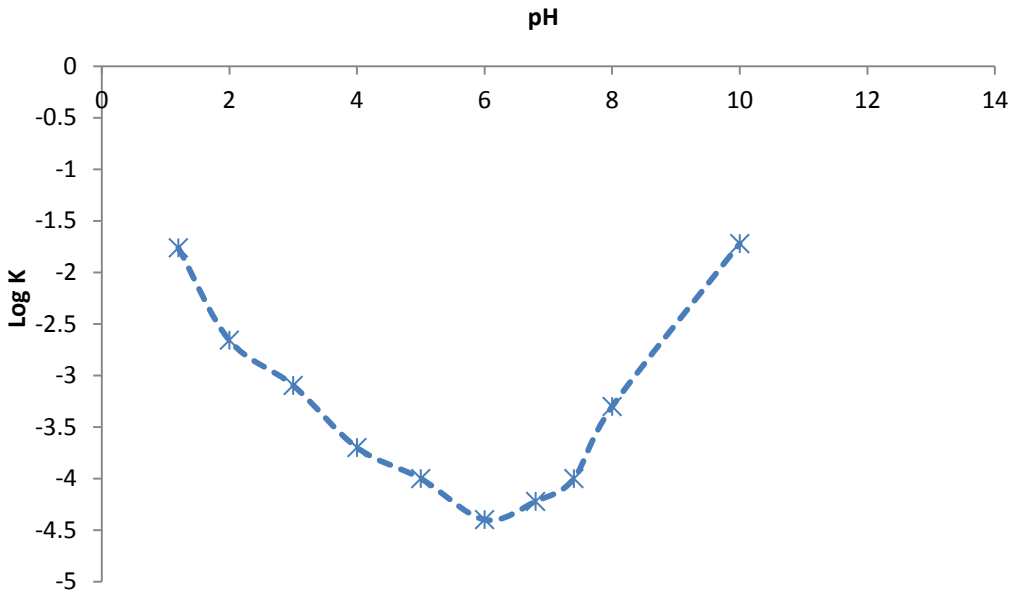


Figure 3.3 *pH stability profile of FCV*

It can be seen from the pH stability profile that FCV undergoes major degradation at extreme pH, viz. 1.2 and 10. The pH range of maximum stability of FCV was found to be between pH 4 and pH 7.4, with the maximum stability observed at pH 6

3.5.3 pH Solubility

3.5.3.1 pH Solubility Profile

The solubility of FCV was influenced by pH, as shown in Figure 3.4. At pH values < pK_a of FCV the API exhibited an increased solubility as compared to pH values > than 3.84. As the pH value decreases below 3.84 FCV ionises and is therefore more soluble.

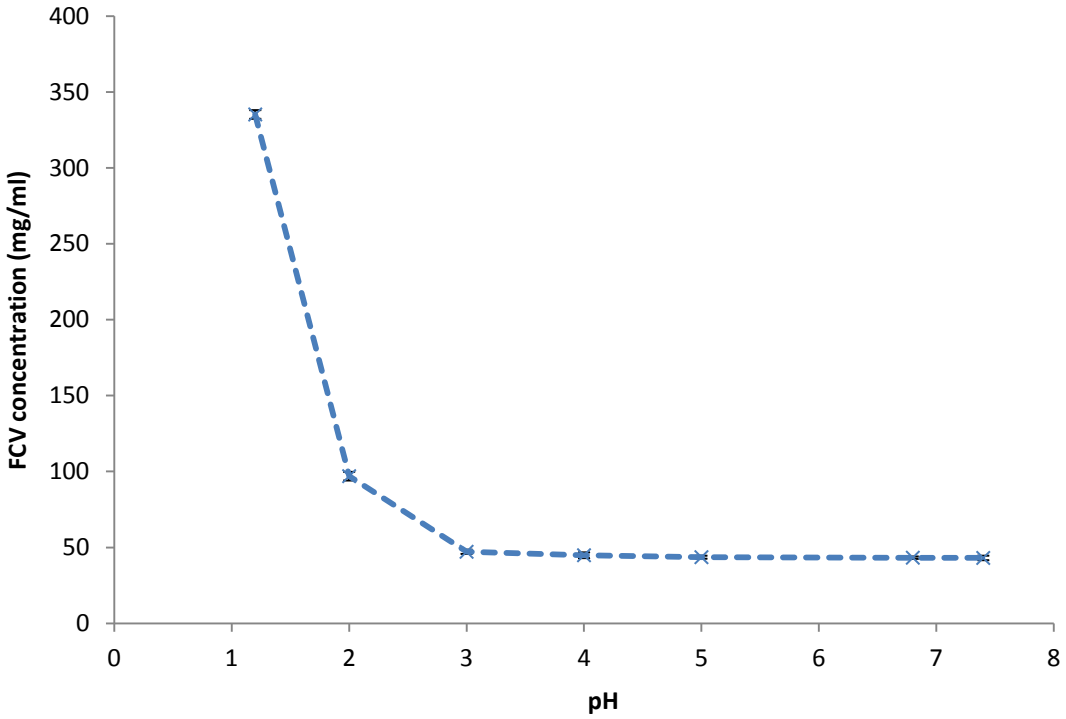


Figure 3.4 *pH solubility profile of FCV*

At the point of saturation FCV was found to precipitate out of solution to form FCV monohydrate as described in §3.5.5.

3.5.4 Polymorphism

3.5.4.1 FCV Crystalline Form

FCV exists in different crystalline forms that are described as anhydrous FCV or crystalline solid forms I, II and III. FCV primarily exists in the anhydrous form, which includes a mixture of solid crystalline forms I and II [105; 143].

FCV forms I and II cannot be distinguished from each other using DSC as both melt in the range 101-103.5 °C. Form III however can be characterised using DSC as it exhibits a melting endotherm at 84 °C and an additional endotherm at 100 °C. The DSC thermogram of FCV shown in Figure 3.5 shows a melting endotherm at 99 °C, in close agreement with the reported melting endotherm of 101 °C, confirming that FCV is comprised of crystalline forms I and/or II.

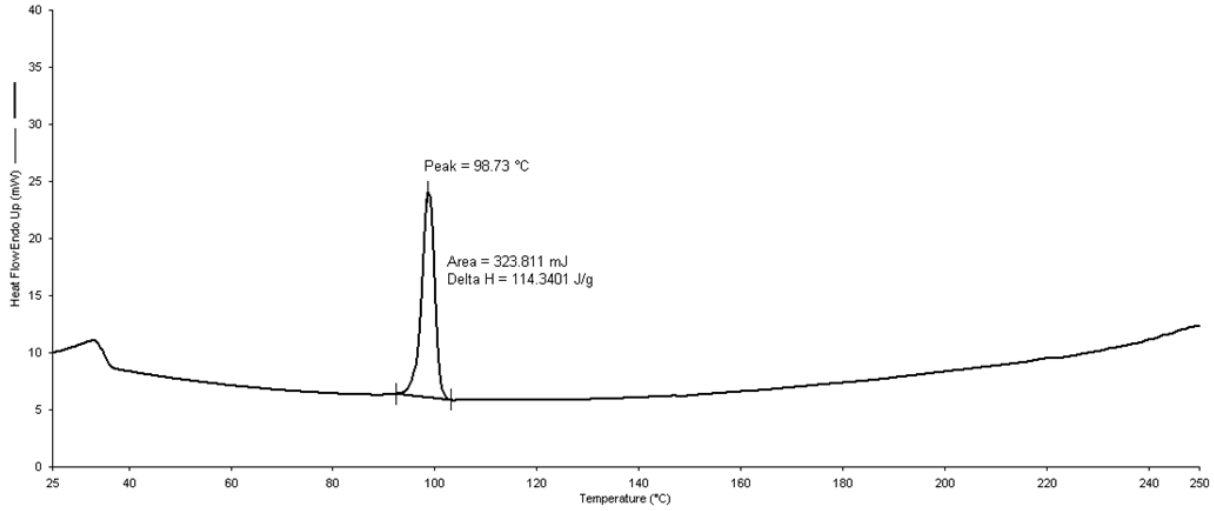


Figure 3.5 *DSC thermogram of FCV*

The X-ray diffraction pattern of FCV shown in Figure 3.6 reveals characteristic peaks at 10.48 °, 14.59 °, 15.66 °, 16.08 °, 17.15 °, 17.90 °, 19.58 °, 20.91 °, 21.31 °, 22.54 °, 24.00 °, 24.57 °, 25.76 °, 26.57 °, 28.85 ° and 32.71 °. These peaks are characteristic of FCV form I, which suggesting that the FCV sample used for these studies is comprised primarily of solid crystalline form I [105].

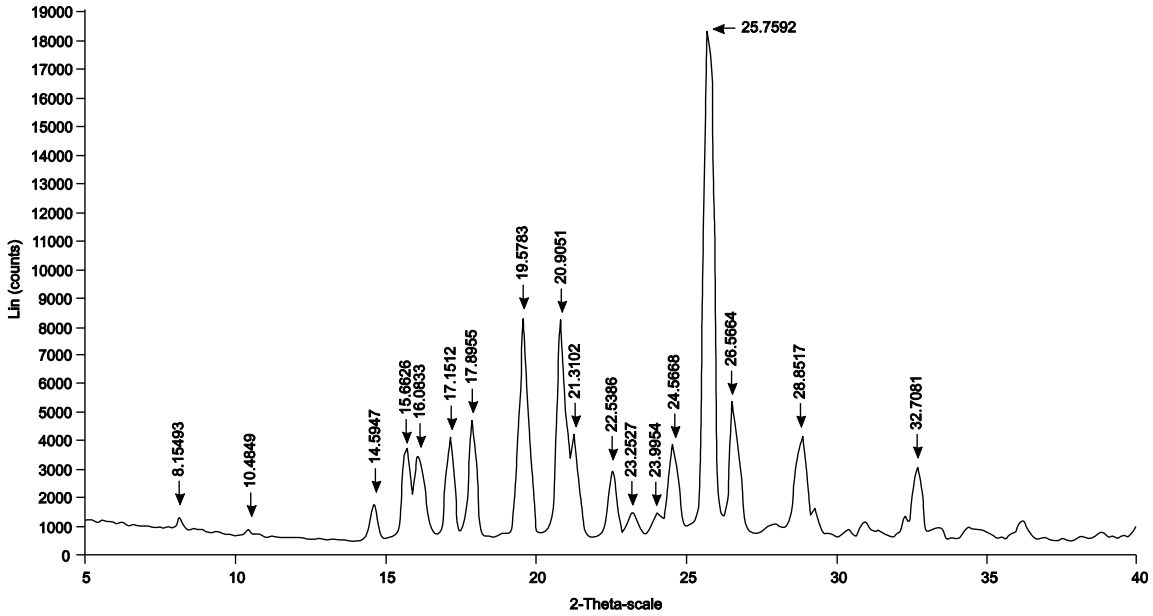


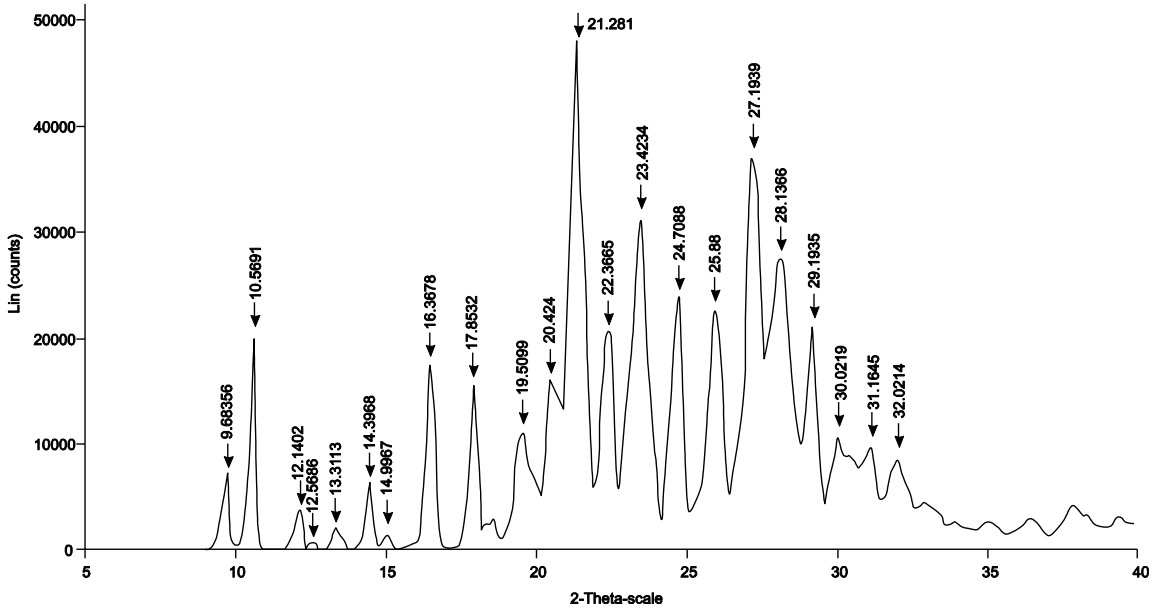
Figure 3.6 *X-ray powder diffraction pattern of FCV as a function of the diffraction angle 2θ*

3.5.5 Characterisation of FCV Monohydrate

The precipitate observed in solubility studies when saturation levels of FCV in solution were reached was thought to be FCV monohydrate. FCV monohydrate was collected by suction filtration using a Büchner funnel and side arm flask. The precipitate was dried and analysed using XRD, NMR, DSC and IR.

3.5.5.1 X-ray Diffraction (XRD)

The diffraction pattern of FCV monohydrate is shown in Figure 3.7 and reveals characteristic peaks at 9.68 °, 10.57 °, 14.40 °, 15.00 °, 16.37 °, 17.85 °, 19.51 °, 20.42 °, 21.28 °, 22.37 °, 23.42 °, 24.71 °, 25.88 °, 27.19 °, 28.14 ° and 32.02 °.



II

Figure 3.7 *X-ray powder diffraction pattern of FCV monohydrate as a function of the diffraction angle 2θ*

The strongest maximum intensities in the XRD pattern of FCV were observed at 19.58 °, 20.91 ° and 25.76°. The data are summarised in Table 3.2. The XRD pattern of FCV monohydrate revealed characteristic peaks of a similar intensity on the 2θ scale that were nearly super imposable on that observed for FCV (Figure 3.6). However slight differences were observed. The relative change in the diffraction intensity compared to FCV suggests a change in the quality of the crystals and/or crystal size and further investigation using other techniques *viz.*, NMR, DSC and IR was required to confirm the presence of the monohydrate form.

Table 3.2 *Characteristic peaks for FCV and FCV monohydrate from XRD patterns*

FCV		**FCV monohydrate**	
2 - Theta	**Lin (counts)**	**2 - Theta**	**Lin (counts)**
19.58 °	8500	19.51 °	11000
20.91 °	8500	20.42 °	1700
25.76 °	18500	25.88 °	22000

3.5.5.2 Nuclear Magnetic Resonance Spectroscopy (NMR)

As described in §1.5.8, the proton NMR spectrum of FCV produces a number of signals at different ppm values, *viz.*, ^{1}H NMR - δ_H (400MHz $CDCl_3$): 1.91 (q, 2H, -CH_2) 2.02 (s, 6H, -2CH_3); 4.10 (d, 4H, CH_2); 4.17 (t, 2H, -CH_2); 5.25(s, 2H, -NH_2) 7.74 (s, 1H); 8.65 (s, 1H). There is also a small peak at 7.29 ppm which can be attributed to the $CDCl_3$ solvent used to prepare the sample for analysis.

The proton NMR spectrum of FCV monohydrate is shown in Figure 3.8. It is very similar to that of FCV, and all peaks were present. However a singlet at 4.69 ppm and a doublet at 3.47 ppm were present that are not observed in the NMR spectrum of FCV. The singlet at 4.69 ppm is attributed to hydrolysis and the presence of an acidic group and the doublet at 3.47 ppm is attributed to a peak shift due to hydrolysis on formation of the precipitate. The carbon NMR spectra of FCV, as shown in Figure 3.9, reveals a number of signals at different ppm values, *viz.* ^{13}C NMR- δ_C (400MHz $CDCl_3$): 20.70 (C-18) 28.64 (C-11) 34.93 (C-12) 63.66 (C-13, C-14) 128.21 (C-3) 142.01 (C-8) 149.76 (C-6) 153.14(C-4) 159.84 (C-2) 170.74 (C-17, C-20) . These signals were identical for FCV monohydrate ± 0.5 ppm. FCV monohydrate showed no correlation from the singlet at 4.69 ppm and the doublet signal at 3.47 ppm, revealing these protons are not attached to hydrogen but are located in-between carbon atoms as shown in Figure 3.10.

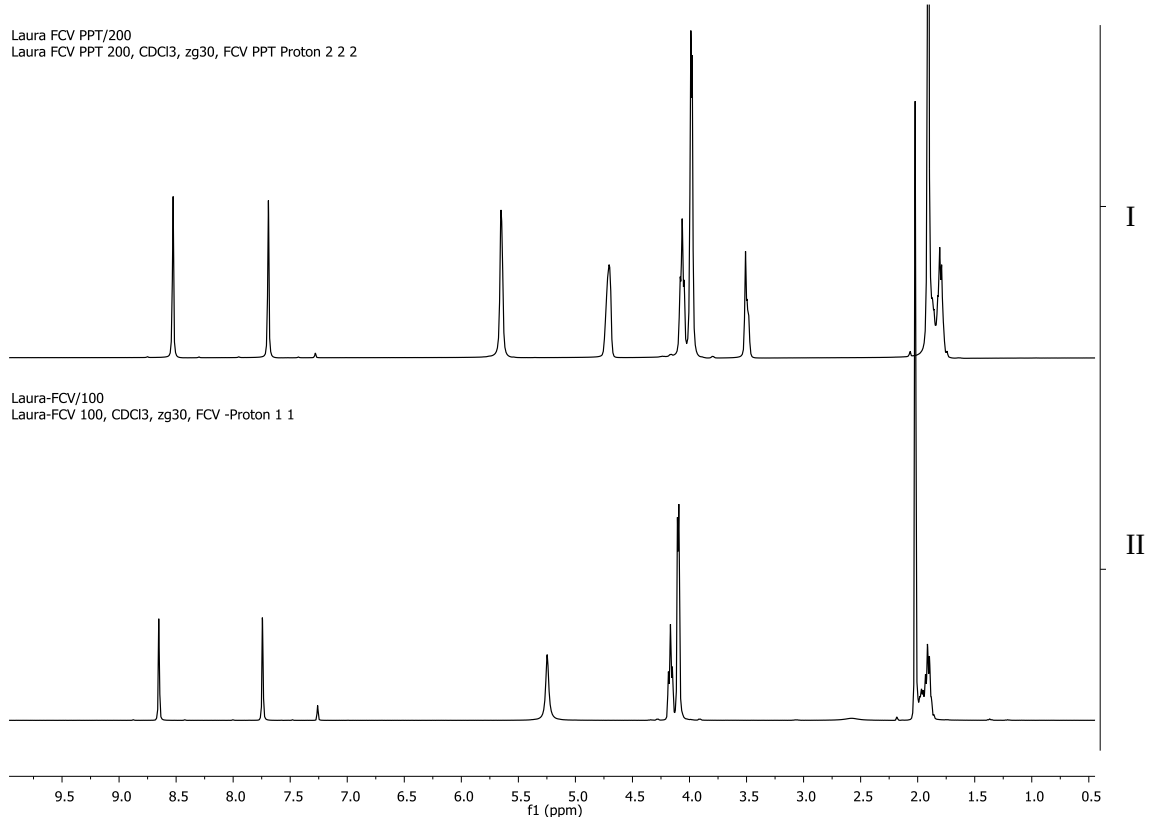


Figure 3.8 *NMR proton spectra of FCV precipitate (I) and FCV (II)*

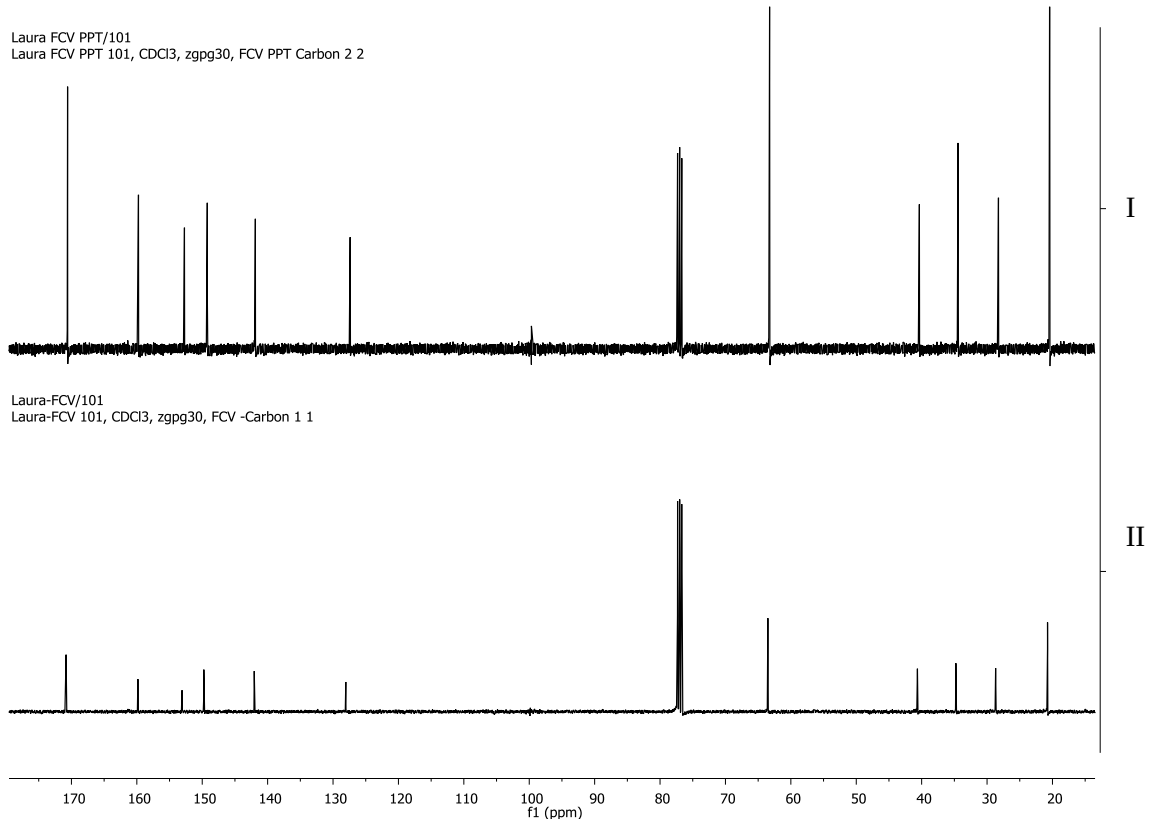


Figure 3.9 *NMR carbon spectra of FCV precipitate (I) and FCV (II)*

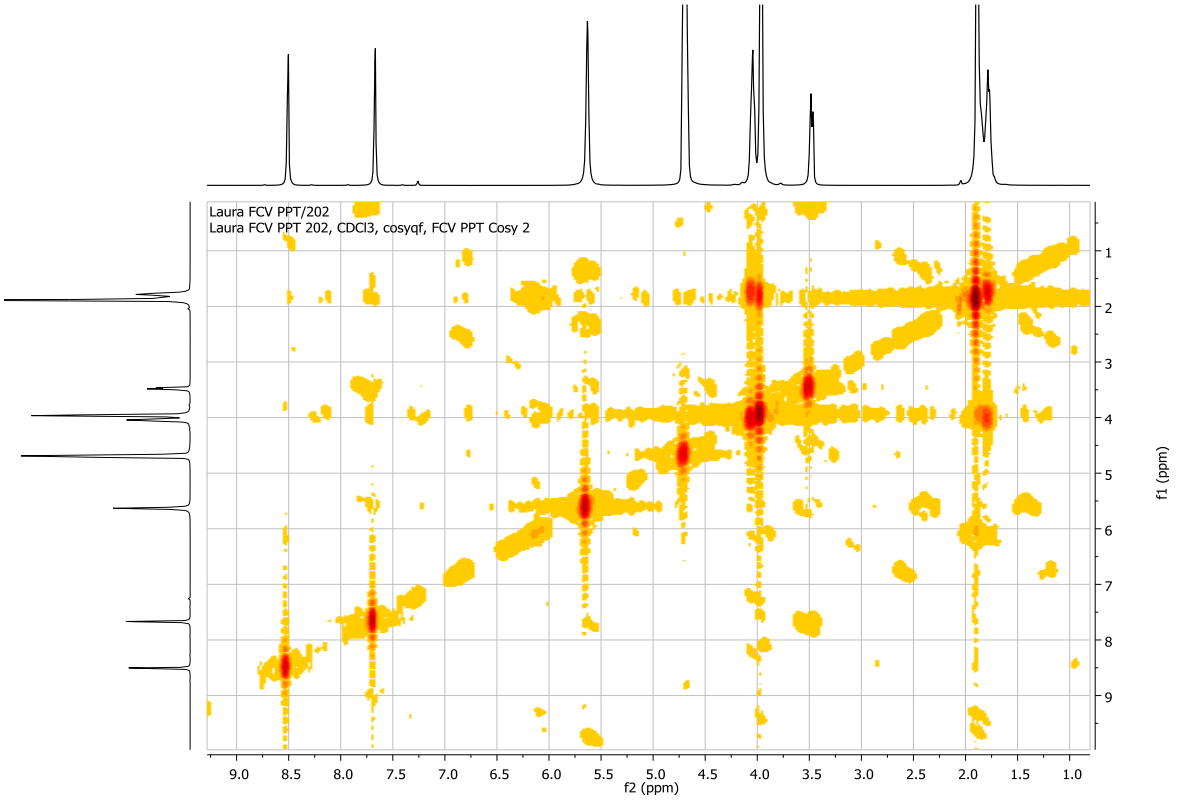


Figure 3.10 *COSY spectrum of FCV monohydrate*

3.5.5.3 Differential Scanning Calorimetry (DSC)

Thermal analysis for FCV monohydrate, as shown in Figure 3.11, reveals a lower melting point of 56 °C than for FCV. The melting point of FCV monohydrate has not been reported in the literature. However DSC alone cannot confirm the presence of the monohydrate but reveals that the precipitate is not FCV. A corresponding TGA experiment should be conducted to confirm the temperature range of volatile component loss [144].

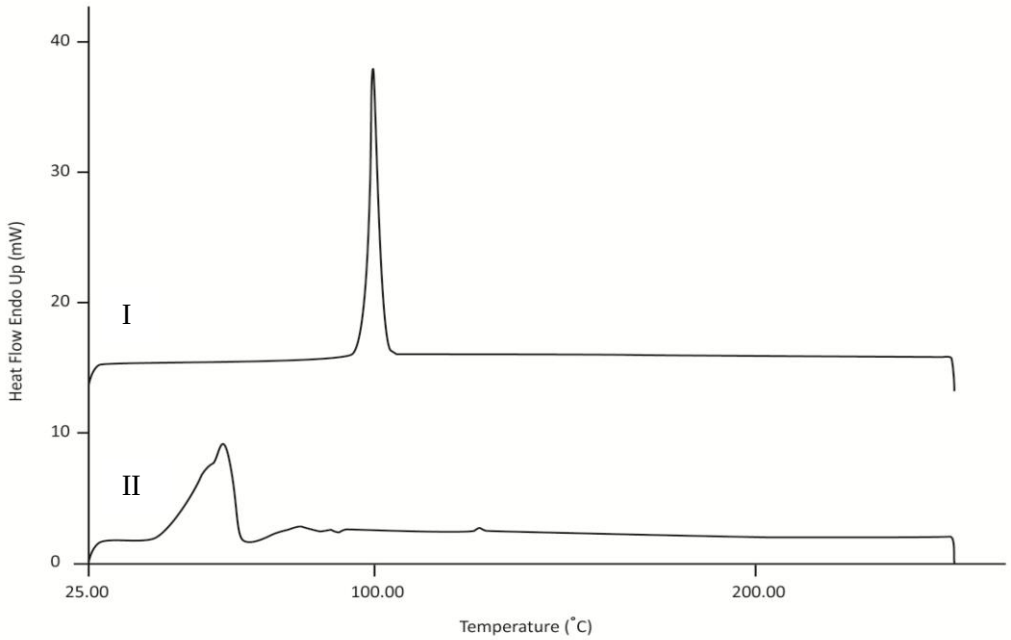


Figure 3.11 *DSC thermograms of FCV (I) and FCV monohydrate (II) generated at a heating rate of 10 °C/min*

3.5.5.4 Infrared Spectroscopy (IR)

The IR spectra for FCV and the monohydrate form are shown in Figure 3.12. The absorption band at 3164 cm^{-1} attributed to the amide functional group in FCV was not present in the FCV monohydrate spectrum. However the presence of a broad peak at 3570-3200 cm^{-1} may mask the absorption band of the amide functional group. The presence of this broad peak at this wavelength is a strong indication of the presence of a hydroxyl group in the molecule that may be due to the presence of water. On close examination of the IR spectra shown in Figure 3.12 the presence of the characteristic absorption bands for FCV as summarised in Table 3.3 in §3.5.6.1 *vide infra* were confirmed.

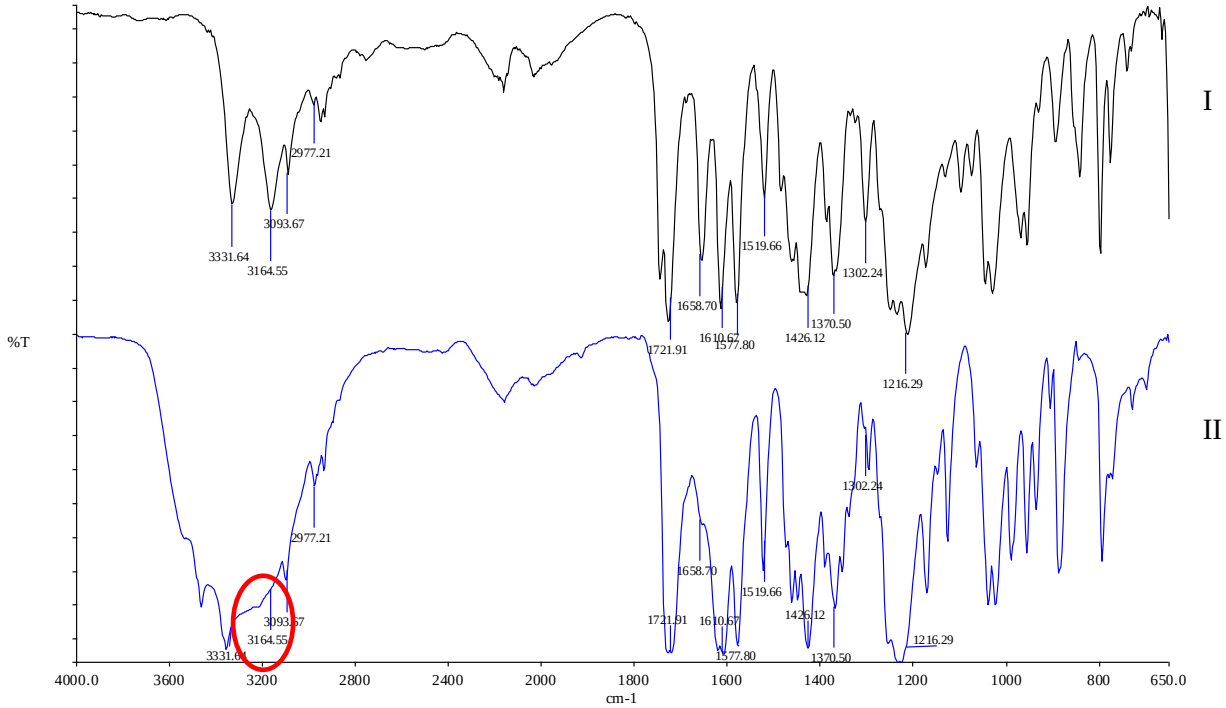


Figure 3.12 *IR spectra of FCV (I) and FCV monohydrate (II)*

3.5.6 Drug Excipients Compatibility Studies

3.5.6.1 Infrared Spectroscopy (IR)

The characteristic absorption band frequencies established from the IR spectrum of FCV shown in Figure 1.6 in §1.5.7 are listed in Table 3.3 and were used to interpret the IR spectra generated from 1:1 binary mixtures of FCV and potential excipients.

Table 3.3 *IR absorption band assignments[16]*

Functional Group	**Bond**	**Frequency (cm^{-1})**
Primary amine	N-H stretch	3400-3250
Primary amine	N-H stretch	3350-3320
Aromatics	C-H stretch	3100-3000
Methyl	C-H stretch	2970-2860
Ethyl	C-H stretch	2935-2845
Esters	C=O stretch	1750-1720
Imino group	C=N stretch	1690-1590
Primary amine	N-H bend	1650-1580
Aromatic (double bond)	C=C-C stretch	1615-1580
Aromatic (double bond)	C=C-C stretch	1510-1450
Ethyl	C-H bend	1485-1445
Methyl	C-H bend	1470-1370
Esters	C-O stretch	1300-1000

3.5.6.1.1 Screening of Potential Excipients for FCV Formulations

The IR spectra of binary mixtures of FCV and sodium metabisulphite, ascorbic acid, citric acid and methylparaben are shown in Figures 3.13 to 3.16 and reveal the presence of all characteristic bands for FCV with no indication of any potential incompatibility between the API and the excipients.

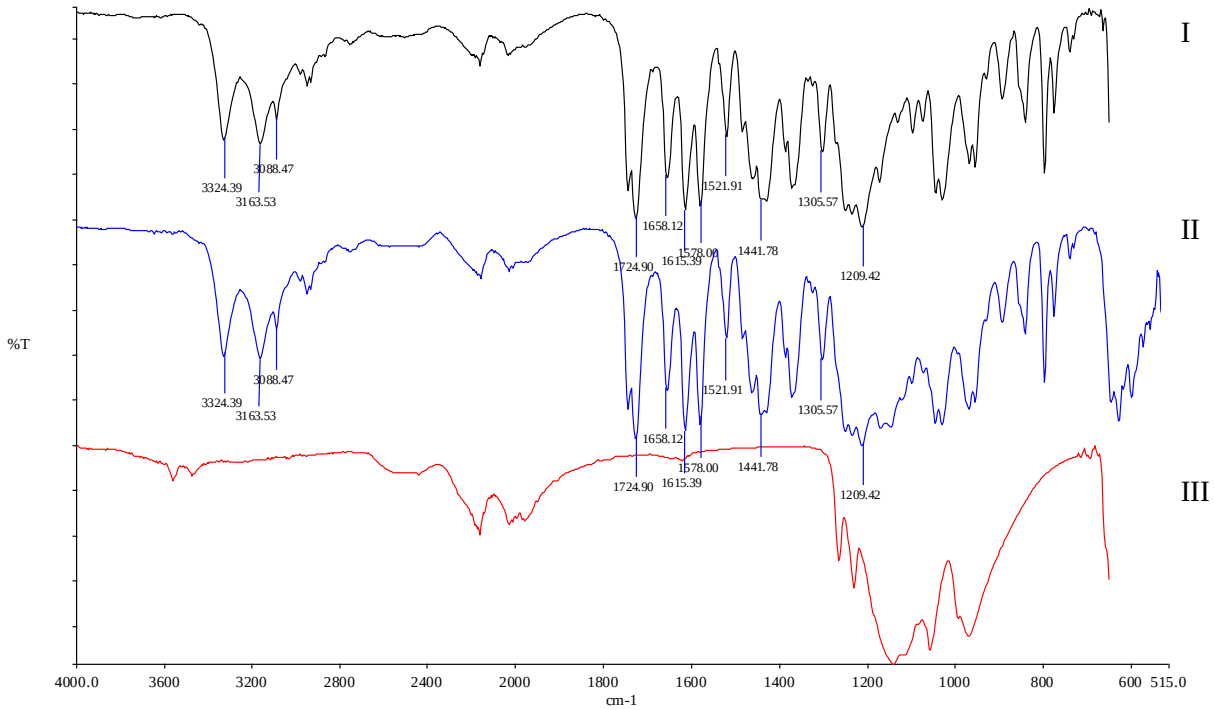


Figure 3.13 *IR spectrum of FCV (I), a binary mixture of FCV and sodium metabisulphite (II) and sodium metabisulphite (III)*

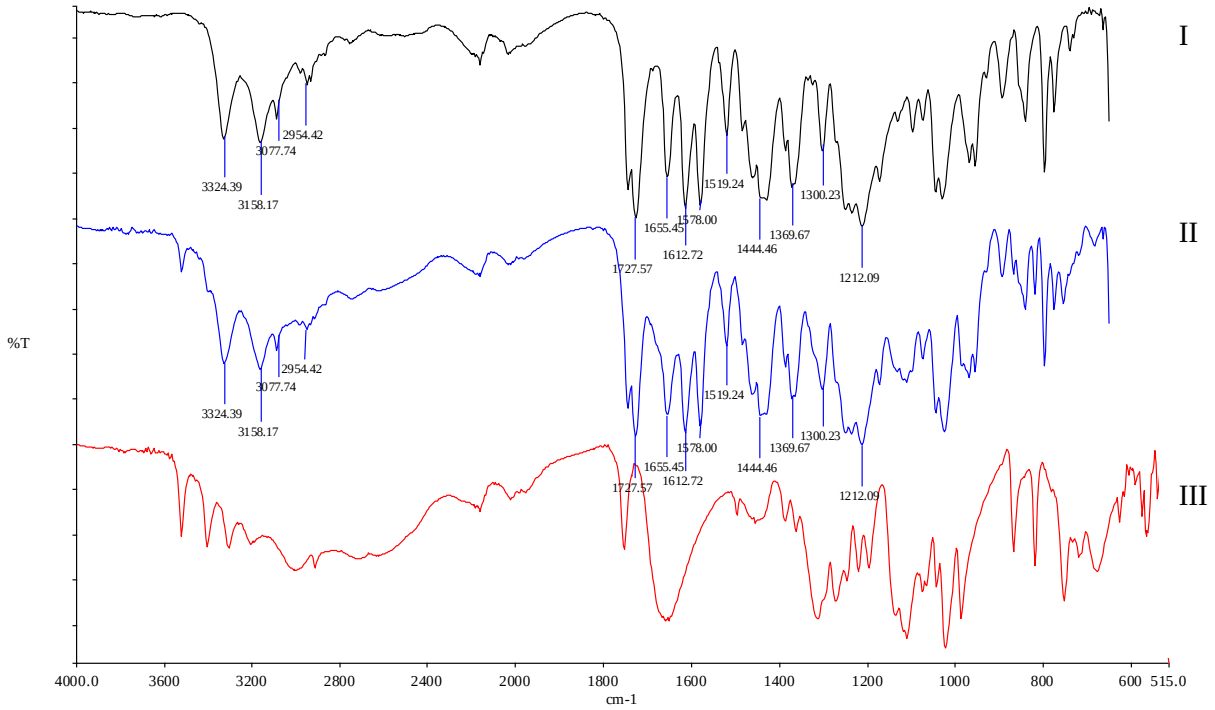


Figure 3.14 *IR spectrum of FCV (I), a binary mixture of FCV and ascorbic acid (II) and ascorbic acid (III)*

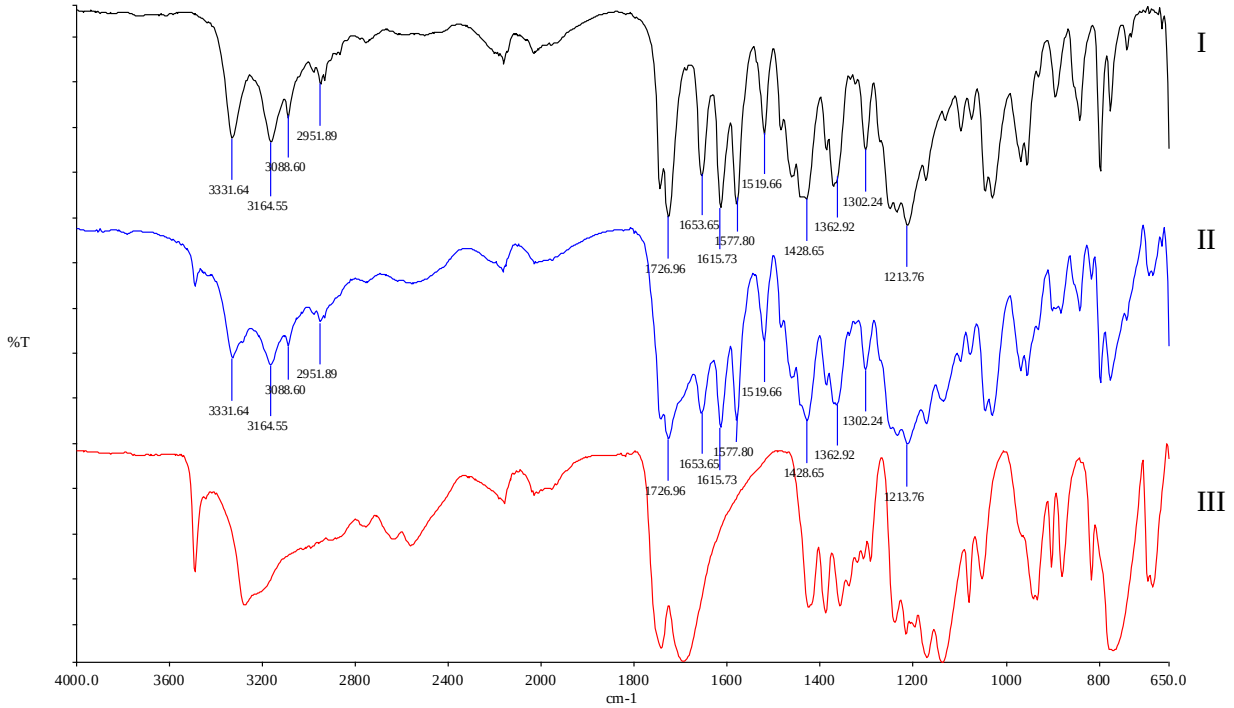


Figure 3.15 *IR spectrum of FCV (I), a binary mixture of FCV and citric acid (II) and citric acid (III)*

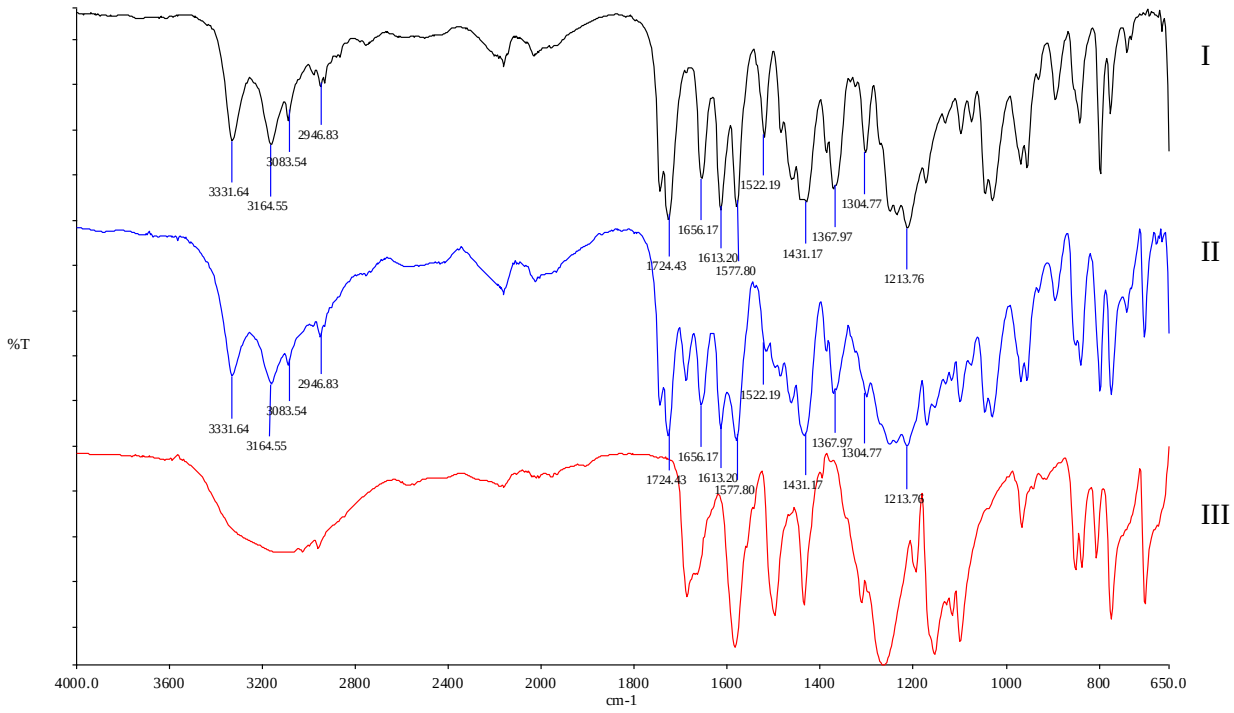


Figure 3.16 *IR spectrum of FCV (I), a binary mixture of FCV and methylparaben (II) and methylparaben (III)*

The IR spectrum of a binary mixture of FCV and propylparaben shown in Figure 3.17 reveals the presence of most of the typical band characteristics for FCV. However the absorption band at a frequency of 1520 cm^{-1} due to the unsaturated aromatic ring was not present. Close examination of the absorption band at 1520 cm-1 in Figure 3.17 reveals a broad band at the peak base that gradually

tapers suggesting that an overlap of two absorption bands has occurred between the C=C-C aromatic ring stretch of FCV at 1520 cm-1 and the C=C-C aromatic ring stretch of propylparaben at 1500 cm-1 as shown in the spectrum for the preservative. However, this is not thought to be evidence of an incompatibility.

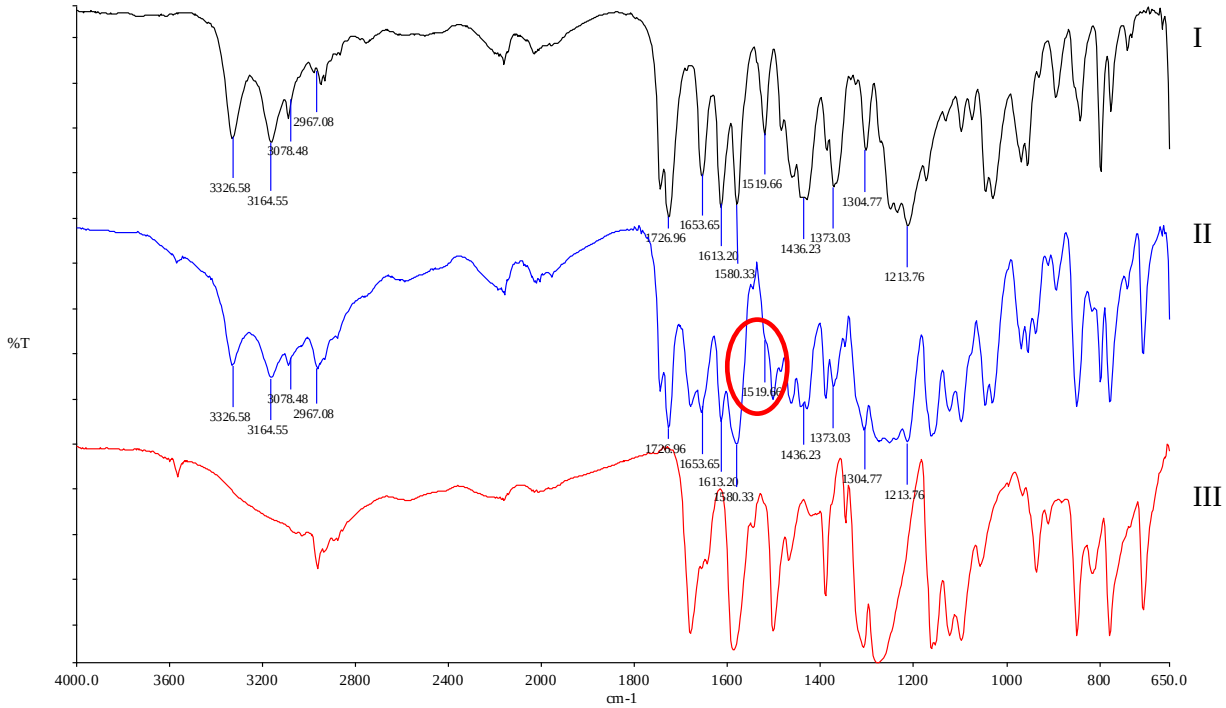


Figure 3.17 *IR spectrum of FCV (I), a binary mixture of FCV and propylparaben (II) and propylparaben (III)*

The IR spectra of binary mixtures of FCV and potassium orthophosphoric acid, HPMC and sucrose revealed all characteristic absorption bands of FCV and the individual excipients were present with no indication of any incompatibility, as shown in Figures 3.18 to 3.20.

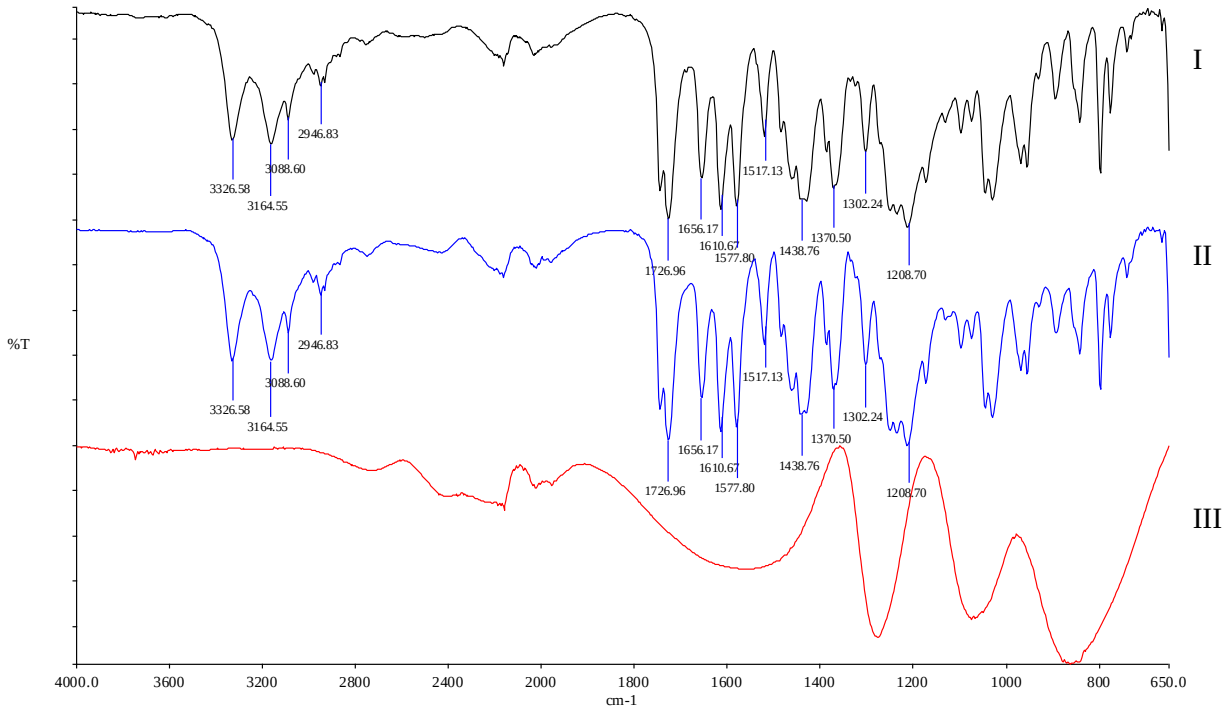


Figure 3.18 *IR spectrum of FCV (I), a binary mixture of FCV and potassium orthophosphoric acid (II) and potassium orthophosphoric acid (III)*

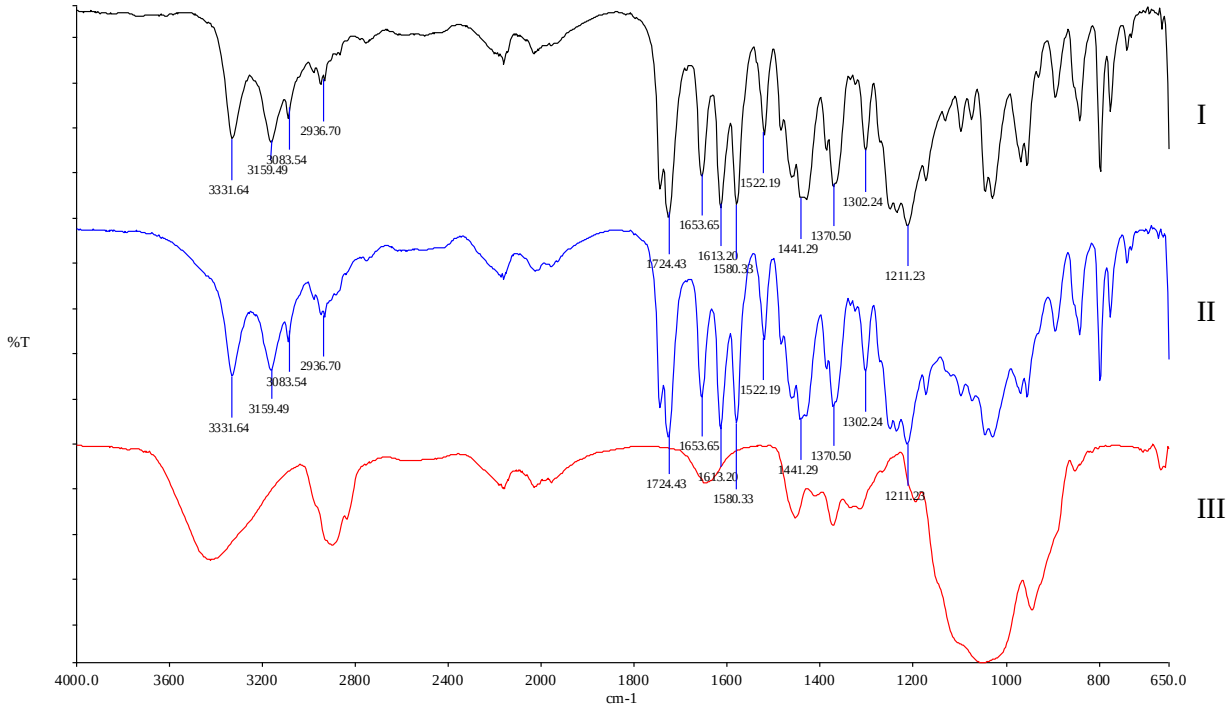


Figure 3.19 *IR spectrum of FCV (I), a binary mixture of FCV and HPMC (II) and HPMC (III)*

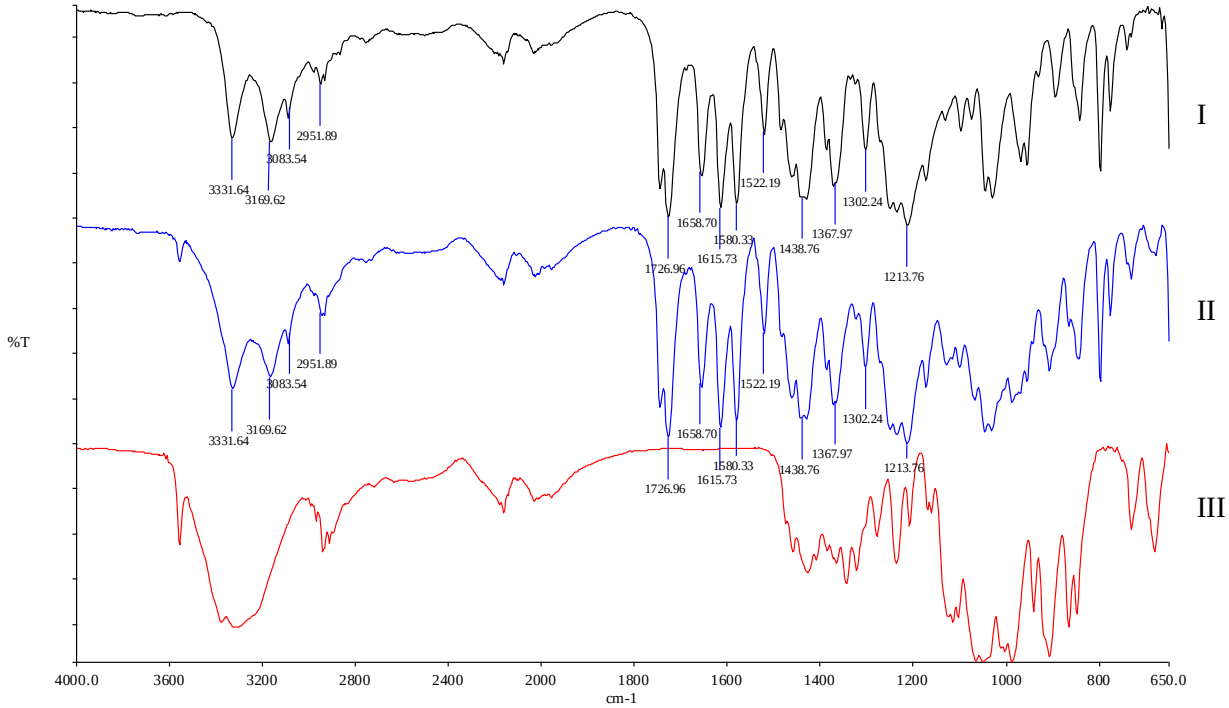


Figure 3.20 *IR spectrum of FCV (I), a binary mixture of FCV and sucrose (II) and sucrose (III)*

3.5.6.1.2 Screening of Excipients in Famvir® Tablets

IR spectra of FCV in binary mixtures with the excipients present in Famvir® tablets are shown in Figures 3.21 to 3.26. It is evident that all characteristic absorption bands for FCV and the respective excipients were present except in the case of magnesium stearate, as shown in Figure 3.24.

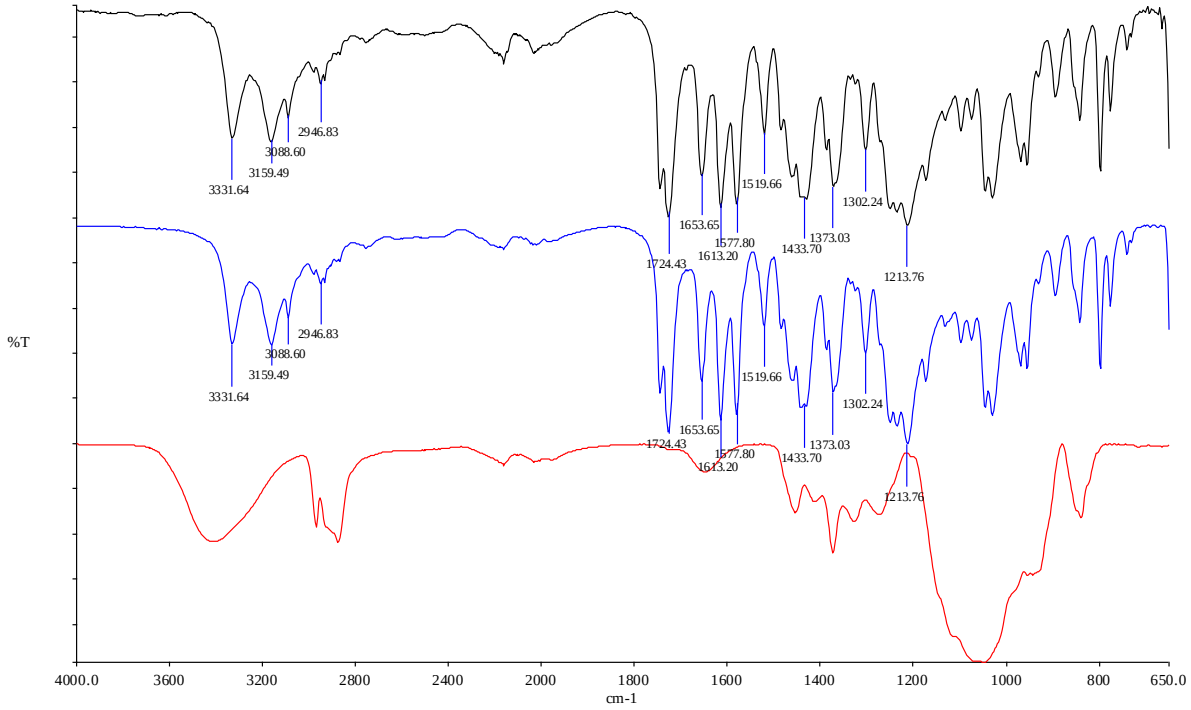


Figure 3.21 *IR spectrum of FCV (I), a binary mixture of FCV and HPC (II) and HPC (III)*

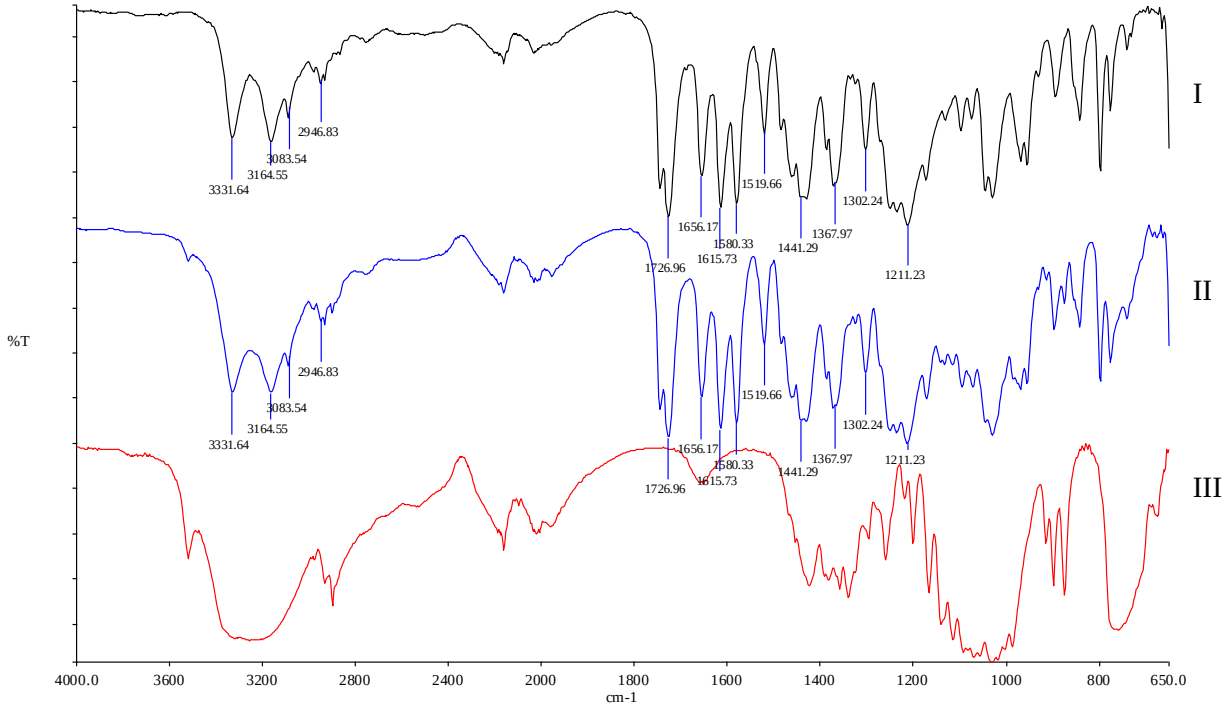


Figure 3.22 *IR spectrum of FCV (I), a binary mixture of FCV and lactose (II) and lactose (III)*

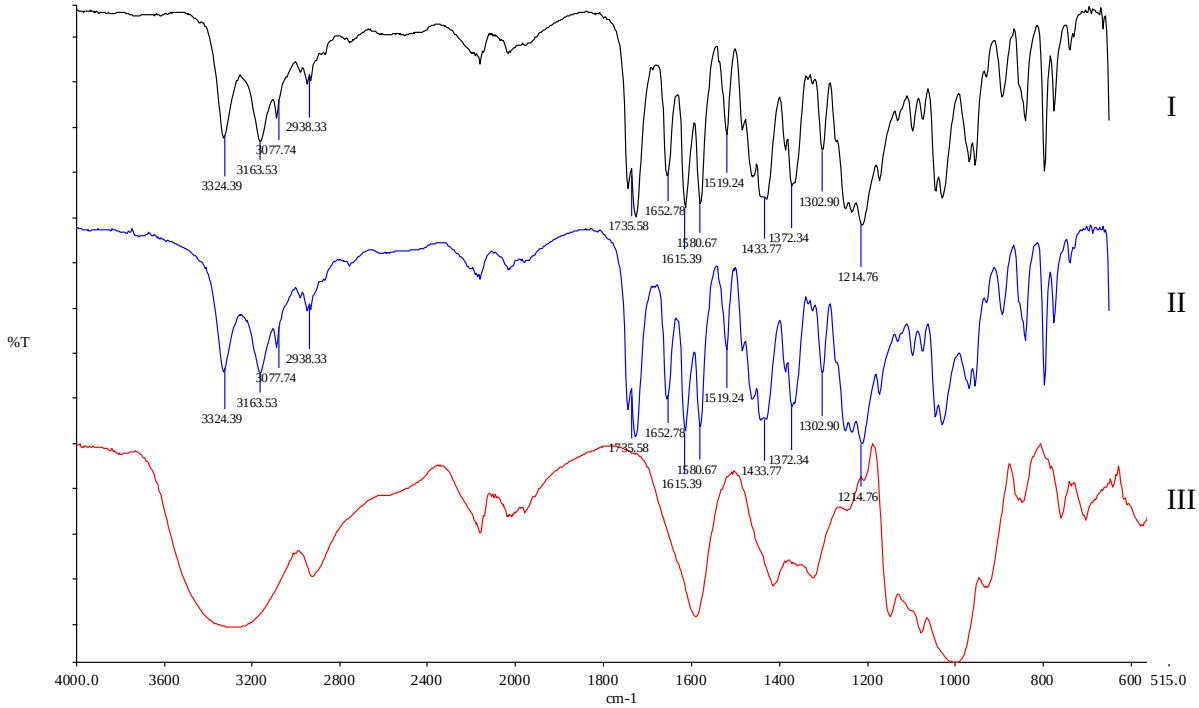


Figure 3.23 *IR spectrum of FCV (I), a binary mixture of FCV and sodium starch glycolate (II) and sodium starch glycolate (III)*

The IR spectrum of the binary mixture of FCV and magnesium stearate shown in Figure 3.24 reveals the presence of the absorption bands that are characteristic of FCV except the absorption band frequency at 1520 cm^{-1}. Inspection of the absorption band at 1520 cm^{-1} in Figure 3.24 reveals that a broad band is observed at the peak base that gradually tapers, suggesting consolidation of two absorption bands of the C=C-C aromatic ring stretch of FCV at 1520 cm^{-1} and the asymmetrical metal carboxylate stretch of magnesium stearate at 1577 cm^{-1}, as shown in the spectrum [145].

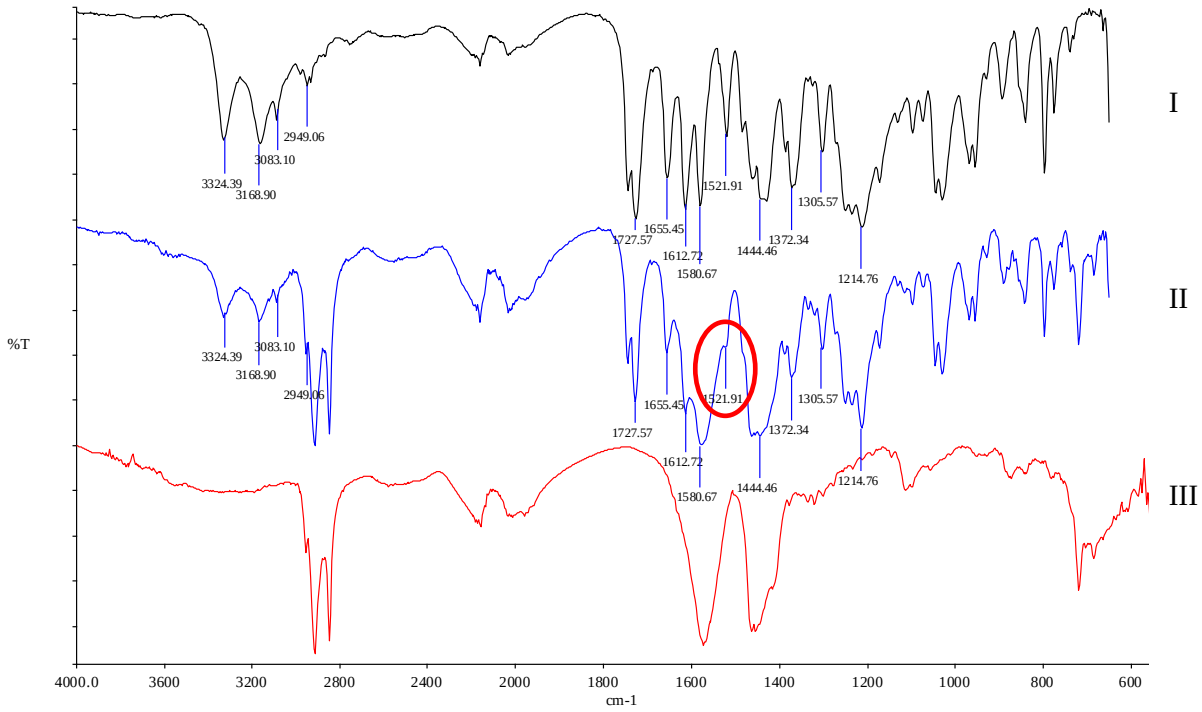


Figure 3.24 *IR spectrum of FCV (I), a binary mixture of FCV and magnesium stearate (II) and magnesium stearate (III)*

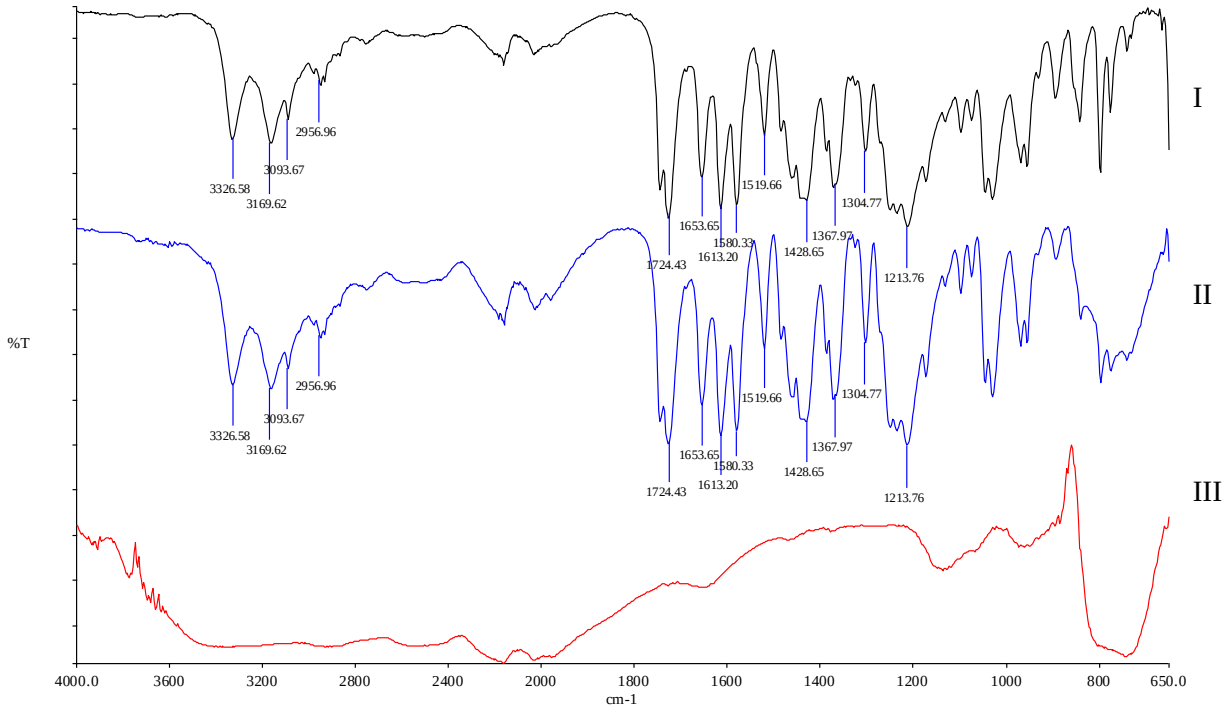


Figure 3.25 *IR spectrum of FCV (I), a binary mixture of FCV and titanium dioxide (II) and titanium dioxide (III)*

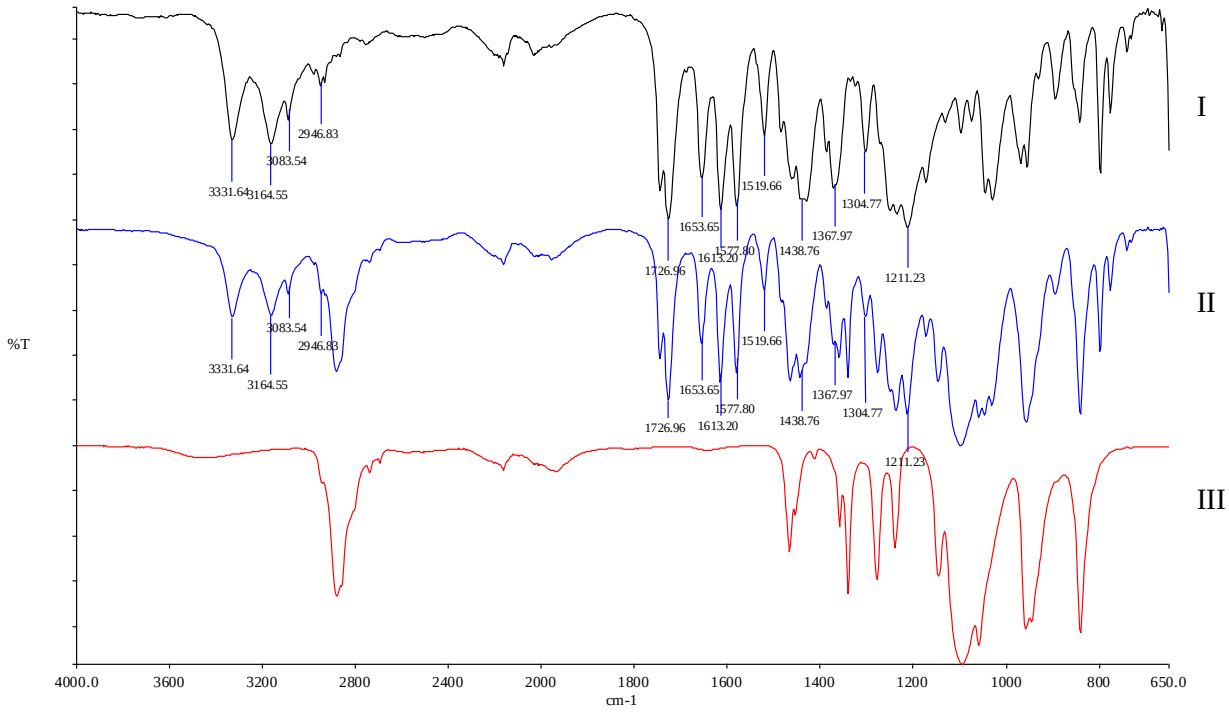


Figure 3.26 *IR spectrum of FCV (I), a binary mixture of FCV and polyethylene glycol (II) and polyethylene glycol (III)*

3.5.6.1.3 Screening of Excipients in Ora-Sweet®

The spectra of FCV in combination with the excipients used to manufacture Ora-Sweet® are shown in Figures 3.27 to 3.29. Several of the excipients used in Ora-Sweet®, *viz.* citric acid, methylparaben and sucrose have been discussed in §3.5.6.1.1 The spectra reveal the characteristic absorption peaks of FCV and relative excipients apart from that generated for the binary mixture of FCV and potassium sorbate shown in Figure 3.28.

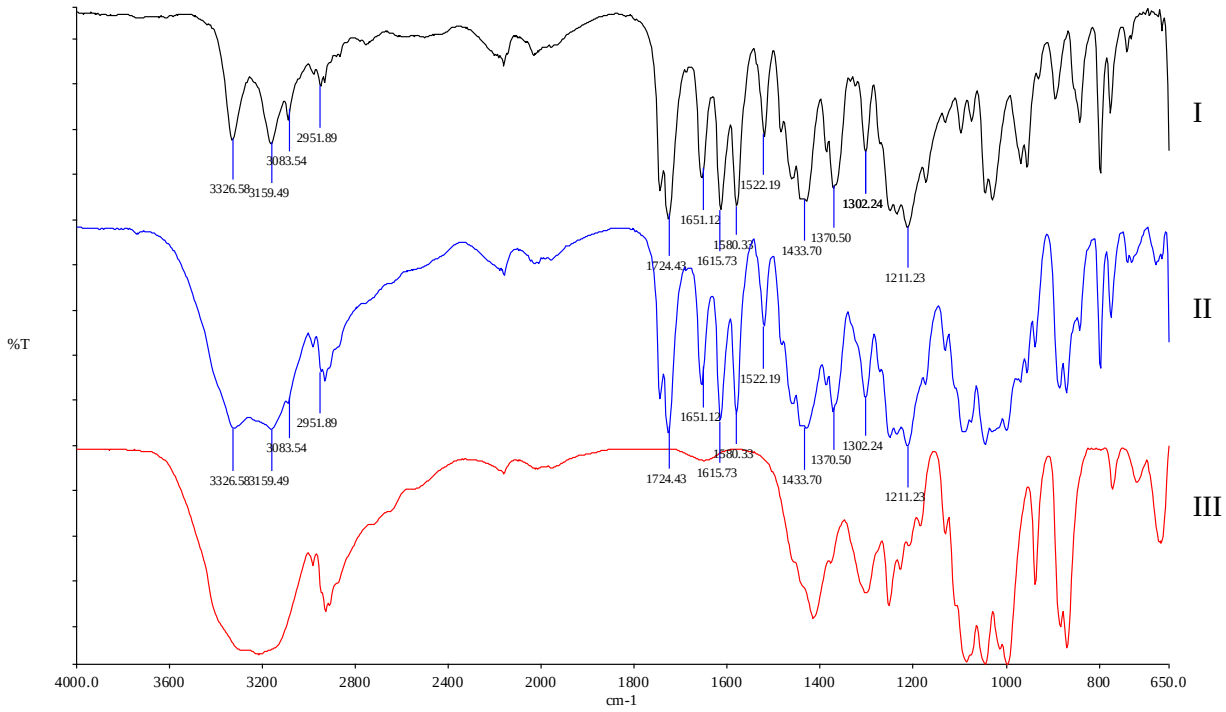


Figure 3.27 *IR spectrum of FCV (I), a binary mixture of FCV and sorbitol (II) and sorbitol (III)*

The IR spectrum of a binary mixture of FCV and potassium sorbate shown in Figure 3.28 was similar to that of FCV and revealed the presence of all characteristic absorption bands except for the absorption band at a frequency of 1520 cm^{-1}. Inspection of the absorption band at 1520 cm-1 in Figure 3.28 revealed that it was similar to that observed following analysis of the binary mixture of FCV and magnesium stearate. A broad band is observed at the peak base that has a slight indication of a peak and suggests that the two absorption bands have merged, *viz.* the C=C-C aromatic ring stretch of FCV at 1520 cm^{-1} and the asymmetrical metal carboxylate stretch of potassium sorbate at 1577 cm^{-1} as shown in the spectrum [145]. A DSC thermogram of a binary mixture of FCV and potassium sorbate did not indicate a potential incompatibility as the characteristic melting endotherms were evident as shown in Figure 3.46.

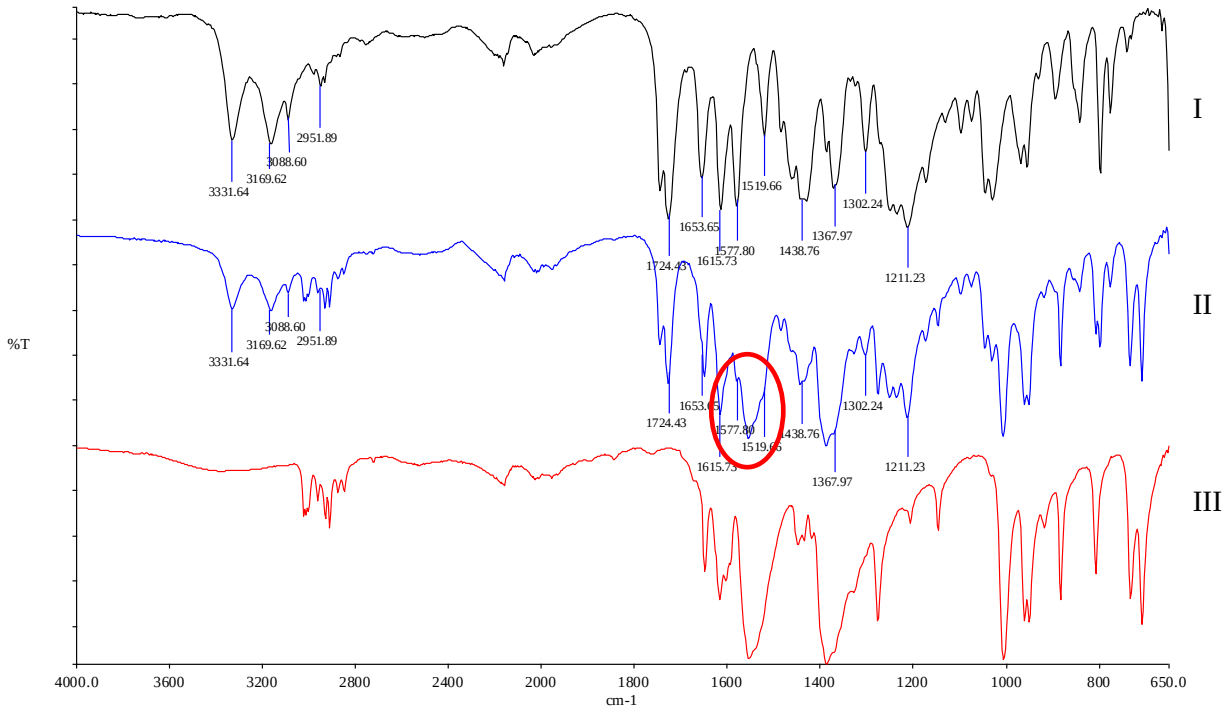


Figure 3.28 *IR spectrum of FCV (I), a binary mixture of FCV and potassium sorbate (II) and potassium sorbate (III)*

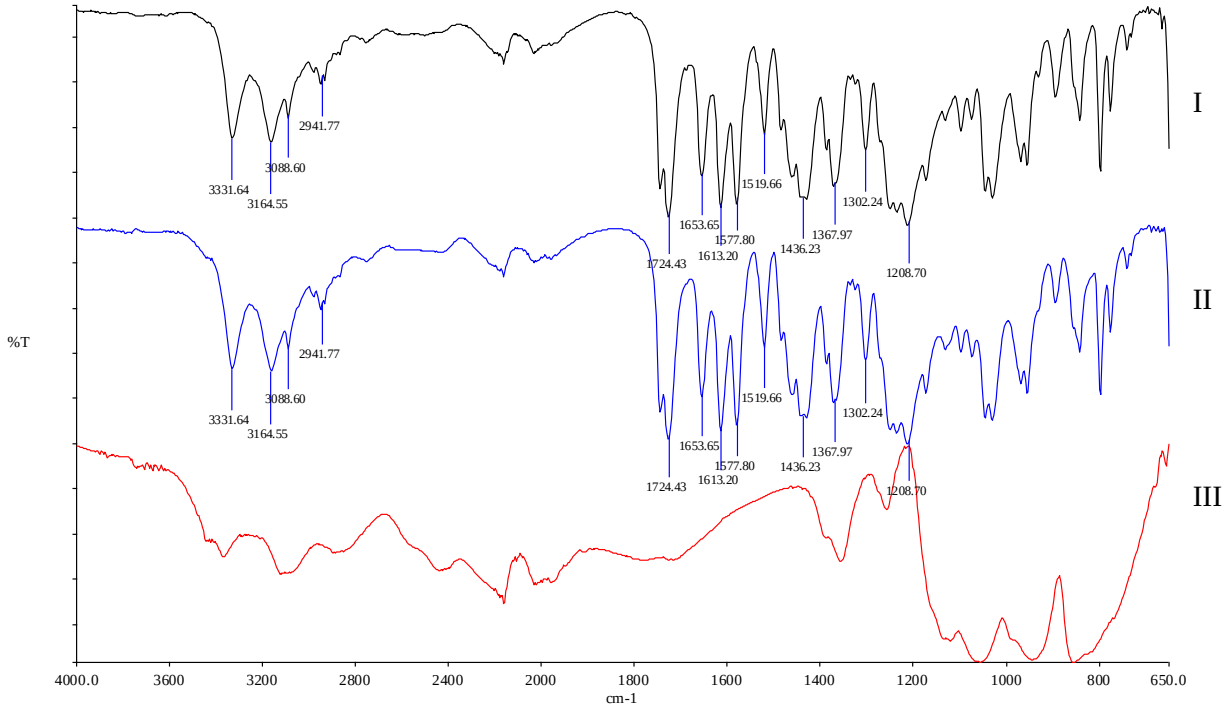


Figure 3.29 *IR spectrum of FCV (I), a binary mixture of FCV and sodium phosphate (II) and sodium phosphate (III)*

3.5.6.2 Differential Scanning Calorimetry (DSC)

3.5.6.2.1 Screening of Potential Excipients for FCV Formulations

The thermogram of the binary mixture of FCV and sodium metabisulphite is shown in Figure 3.30 (II) and reveals a sharp melting endotherm at 99 °C, indicative of FCV melting. This is in close agreement with the melting point of FCV shown in Figure 3.30 (I). There is also a broad endothermic peak at 190 °C suggesting decomposition of sodium metabisulphite that commences melting and decomposition at 150 °C as shown in Figure 3.30 (III).

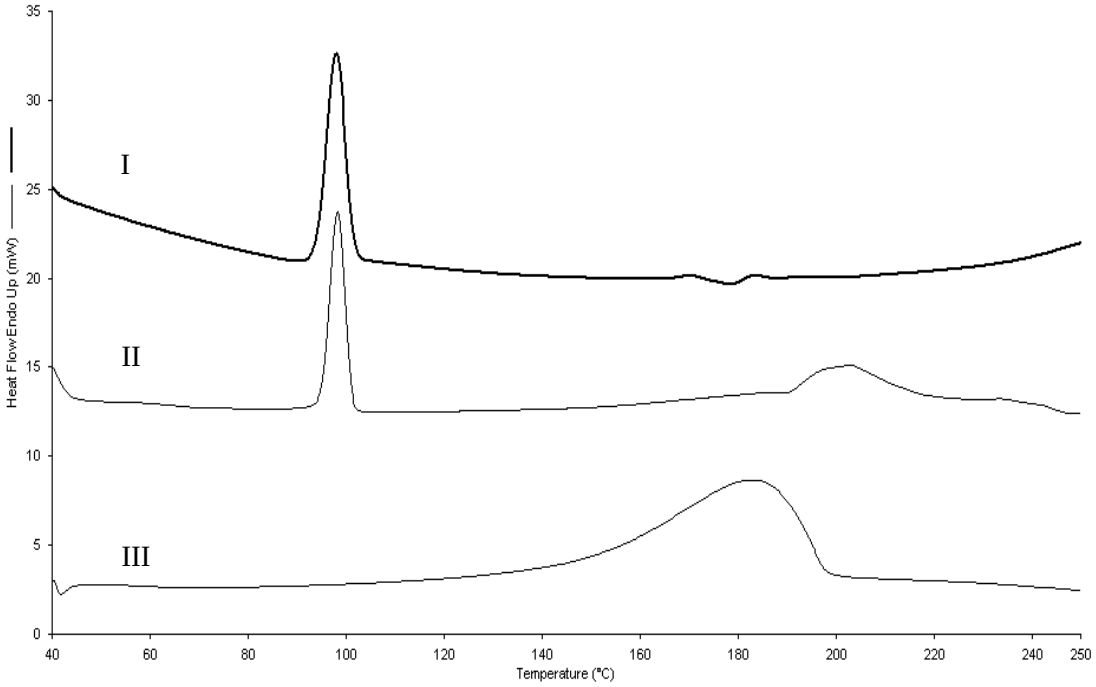


Figure 3.30 *DSC thermograms of FCV (I), a binary mixture of FCV and sodium metabisulphite (II) and sodium metabisulphite (III) at a heating rate of 10 °C/min*

The thermogram for ascorbic acid shown in Figure 3.31 (III) reveals a sharp endothermic peak at 190 °C indicative of the melting point of ascorbic acid. With melting there is also decomposition into furan derivatives and $\alpha\beta$-unsaturated cyclic ketones which are identified by the further endothermic changes above 190 °C [131; 146; 147]. The thermogram for the binary mixture of FCV and ascorbic acid in Figure 3.31 (II) does not reflect a sharp endothermic peak for FCV as observed in Figure 3.31 (I). Instead the presence of a flattened peak is evident, suggesting that an interaction between FCV and ascorbic acid may occur at high temperatures. On heating, ascorbic acid acts in a similar manner to reducing sugars in the Maillard reaction and degradation products are able to react with primary amines such as FCV to produce a large number of unwanted aromatic and polymeric products [148]. The IR scan of a binary mixture of these compounds shows all characteristic absorption bands for FCV and ascorbic acid, as seen in Figure 3.14. Therefore, due to the evidence of a potential interaction using thermal analysis, long term stability testing may be necessary for dosage forms in

which therapeutic amounts of FCV are included as described in §4.6.3.2, *vide infra*, where FCV formulations were subject to accelerated stability studies.

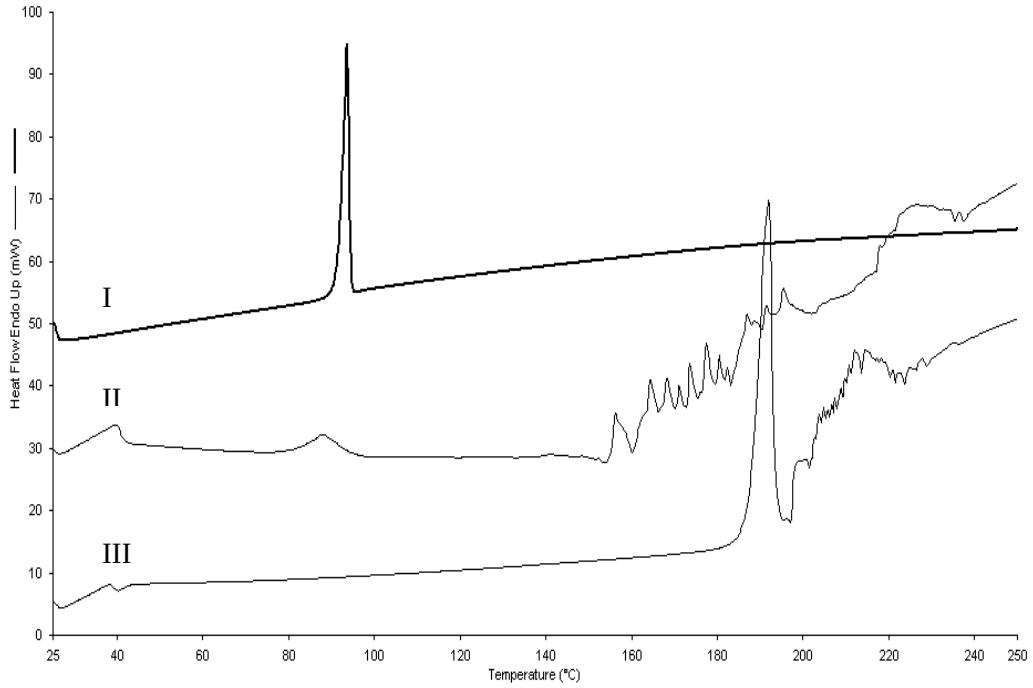


Figure 3.31 *DSC thermograms of FCV (I), a binary mixture of FCV and ascorbic acid (II) and ascorbic acid (III) at a heating rate of 10 °C/min*

The thermogram for citric acid (III) showed a sharp endothermic peak at 153 °C indicative of the melting point of citric acid. At 175 °C there are further endothermic events that are indicative of the dehydration of citric acid to produce aconitic acid and further heating results in the formation of methyl maleic anhydride [149]. Citric acid starts to decompose slowly at 148 °C but the decomposition rate significantly increases after melting at 153 °C has occurred. The thermogram for the binary mixture of FCV and citric acid (II) does not reflect a sharp endothermic peak for FCV but rather a flat, broad peak is evident, suggesting that FCV and citric acid may interact at high temperatures. The IR scan in Figure 3.15 of the binary mixture for these compounds shows the characteristic absorption bands for both compounds. Due to evidence of a potential interaction indicated by thermal analysis, long term stability testing may be necessary.

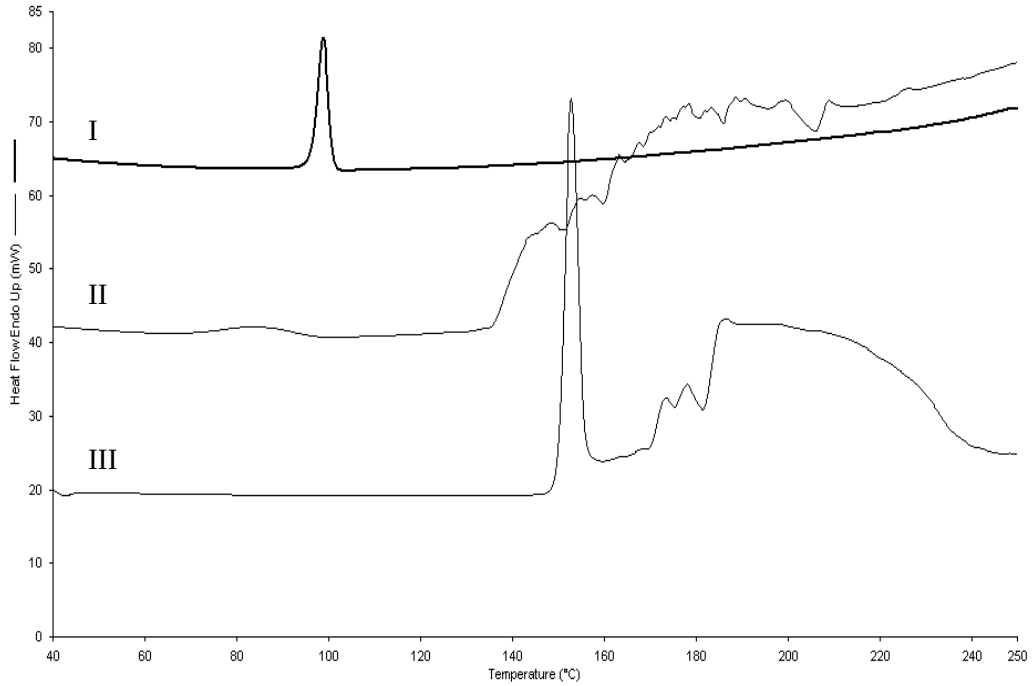


Figure 3.32 *DSC thermograms of FCV (I), a binary mixture of FCV and citric acid (II) and citric acid (III) at a heating rate of 10 °C/min*

The thermogram for a binary mixture of FCV and methylparaben shown in Figure 3.33 (II) reveals endothermic peaks at 85 °C and 99 °C indicative of melting events for methylparaben and FCV respectively. This is in close agreement with the melting point of FCV shown in Figure 3.33 (I) and methylparaben shown in Figure 3.33 (III).

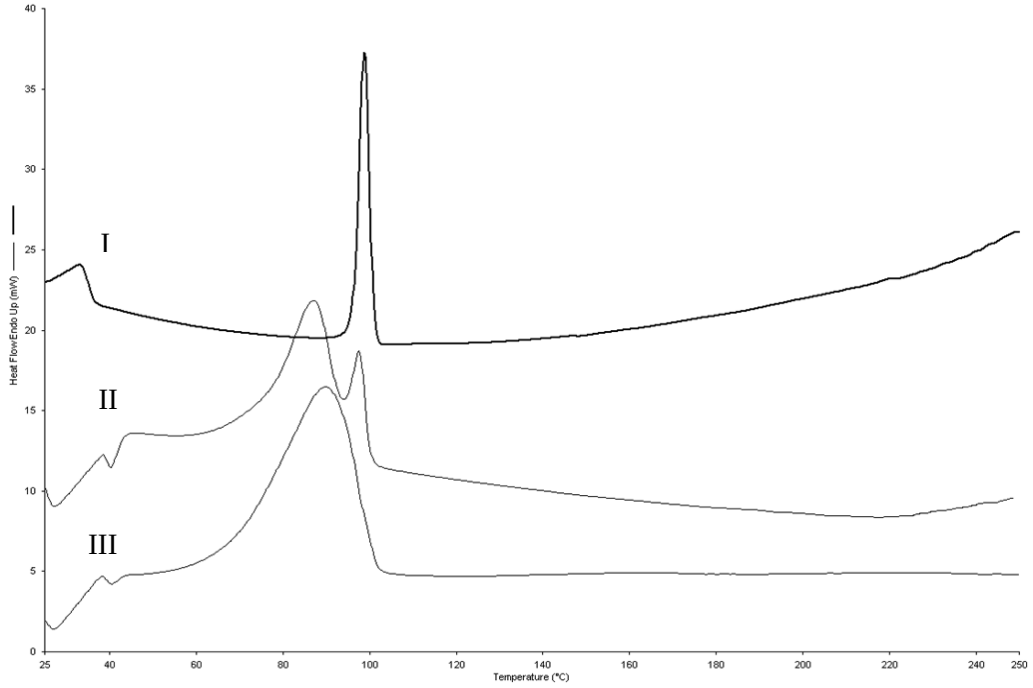


Figure 3.33 *DSC thermograms of FCV (I), a binary mixture of FCV and methylparaben (II) and methylparaben (III) at a heating rate of 10 °C/min*

Propylparaben is a hygroscopic powder that melts between 96 and 99 °C. The thermogram of propylparaben shown in Figure 3.34 (III) reveals the presence of a peak that can be attributed to a loss of water at 80 °C, and another flatter peak at 99 °C that can be attributed to the melting of propylparaben. A broad melting endotherm for FCV was observed in the thermogram for the binary mixture of FVC and propylparaben at a slightly lower temperature than the published melting point of FCV as seen in Figure 3.34 (II). A possible reason for the presence of this broad peak is the loss of moisture and melting of FCV occurring at a similar temperature. In addition, the presence of water in a sample may produce different results as water acts as a plasticizer and can reduce transition temperatures [150]. In contrast this may indicate a possible incompatibility on exposure to high temperatures. Propylparaben is however used in oral formulations at concentrations of 0.01-0.02 % w/v and it is unlikely that interactions occur at these concentrations.

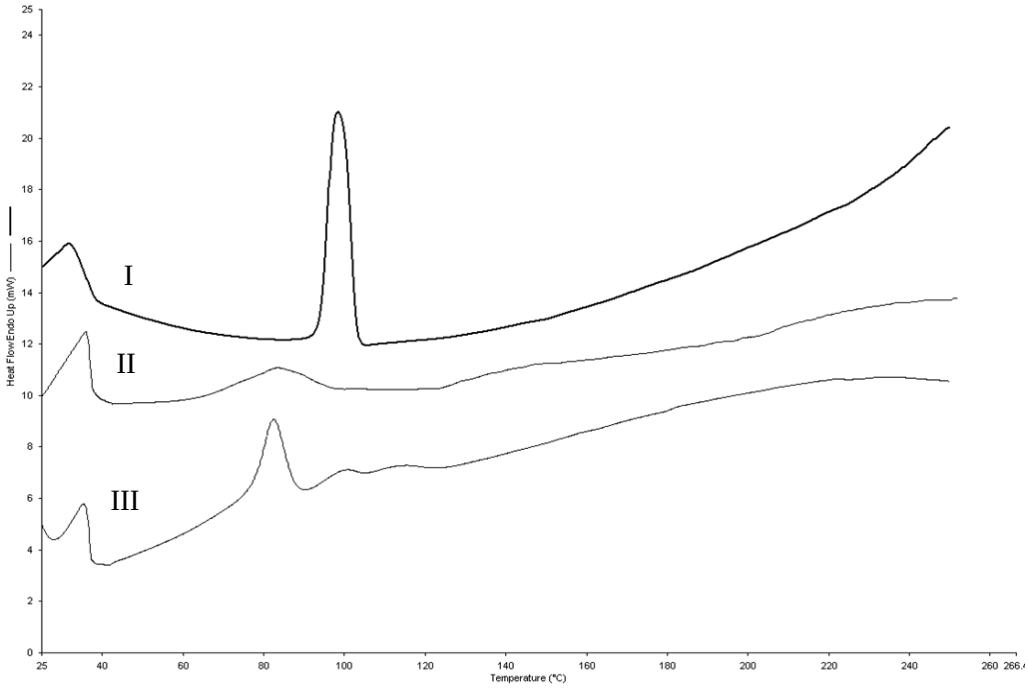


Figure 3.34 *DSC thermograms of FCV (I), a binary mixture of FCV and propylparaben (II) and propylparaben (III) at a heating rate of 10 °C/min*

The thermogram for the binary mixture of FCV and potassium dihydrogen orthophosphate shown in Figure 3.35 (II) reveals a sharp endothermic peak at 99 °C, indicative of FCV melting. Potassium dihydrogen orthophosphate undergoes a phase transition and decomposition to potassium pyrophosphate, observed by the endotherm peak at 220 °C as shown in Figure 3.35 (II) and Figure 3.35 (III) [151]. No indication of an interaction between FCV and potassium dihydrogen orthophosphate was observed.

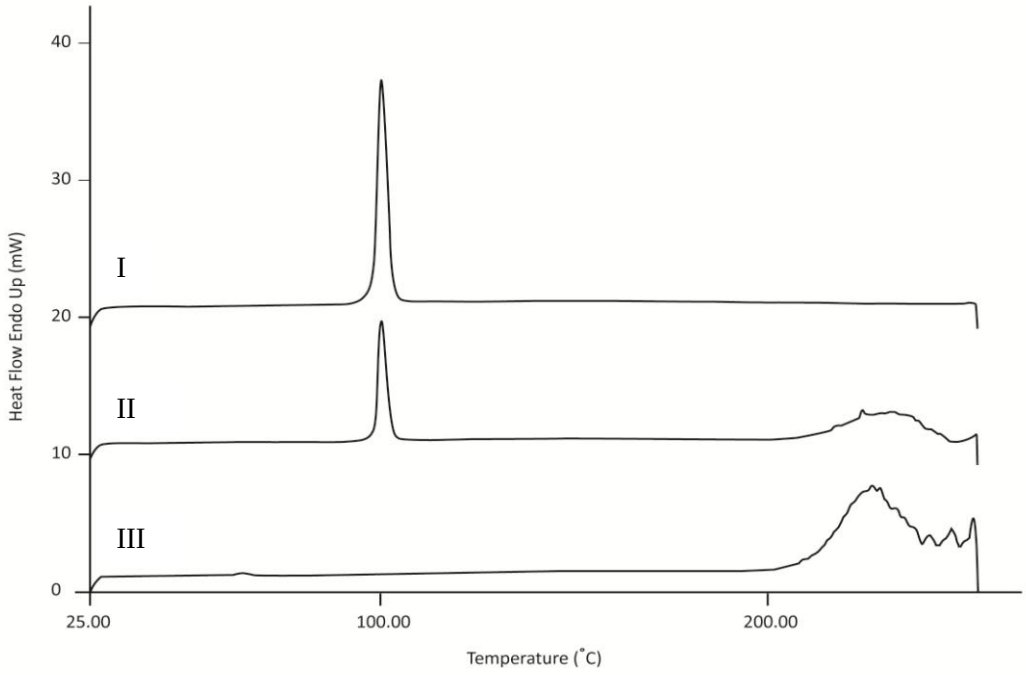


Figure 3.35 *DSC thermograms of FCV (I), a binary mixture of FCV and potassium dihydrogen orthophosphate (II) and potassium dihydrogen orthophosphate (III) at a heating rate of 10 °C/min*

Thermal analysis of a binary mixture of FCV and HPMC as shown in Figure 3.36 (II) reveals the presence of a sharp endothermic peak at 99 °C, indicative of FCV melting. This is in close agreement with the melting point of FCV observed in Figure 3.36 (I) and therefore it is unlikely that a reaction will occur between these two materials.

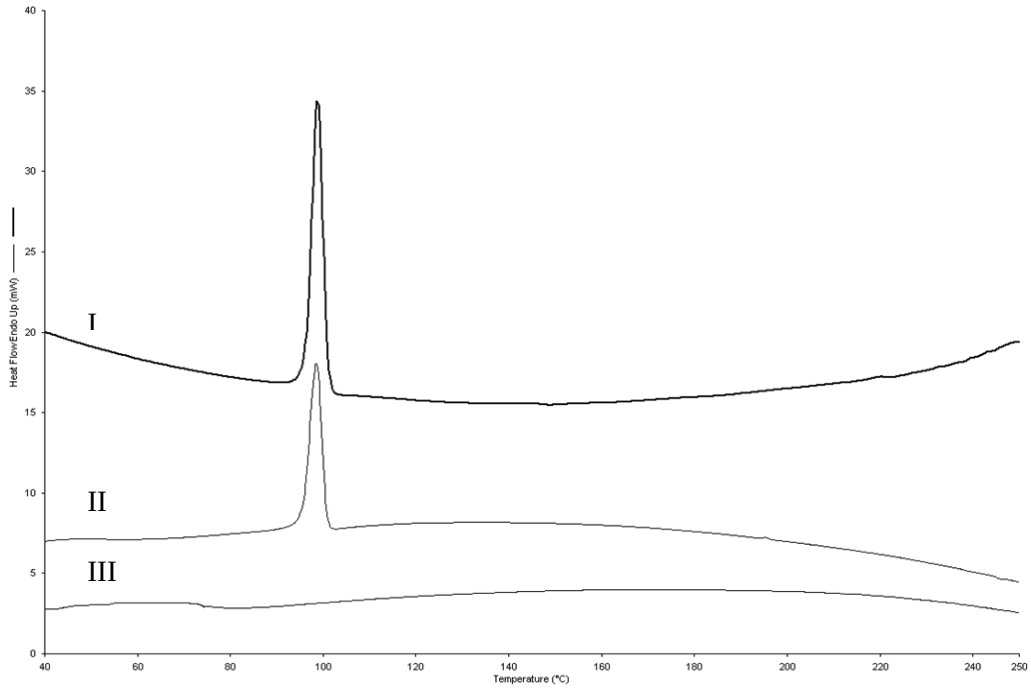


Figure 3.36 *DSC thermograms of FCV (I), a binary mixture of FCV and HPMC (II) and HPMC (III) at a heating rate of 10 °C/min*

The thermogram for the binary mixture of FCV and sucrose shown in Figure 3.37 (II) reveals a sharp endothermic peak at 99 °C, in close agreement with Figure 3.37 (I). Sucrose has a melting point of 160 to 186 °C, with decomposition as shown in Figure 3.37 (II) and Figure 3.37 (III) [131]. No indication of an interaction between FCV and sucrose was apparent.

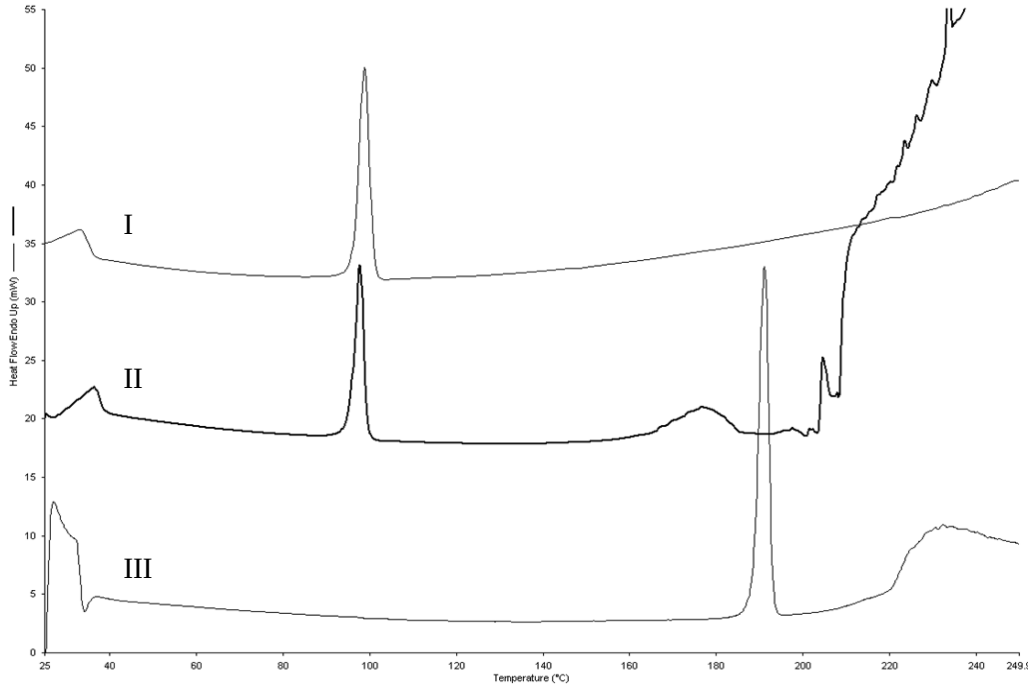


Figure 3.37 *DSC thermograms of FCV (I), a binary mixture of FCV and sucrose (II) and sucrose (III) at a heating rate of 10 °C/min*

3.5.6.2.2 Screening of Excipients in Famvir® tablets

Thermal analysis of a binary mixture of FCV and HPC as shown in Figure 3.38 (II) reveals a sharp endothermic peak at 99 °C, in agreement with the melting point of FCV shown in Figure 3.38 (I) and therefore no interaction between these two materials is expected.

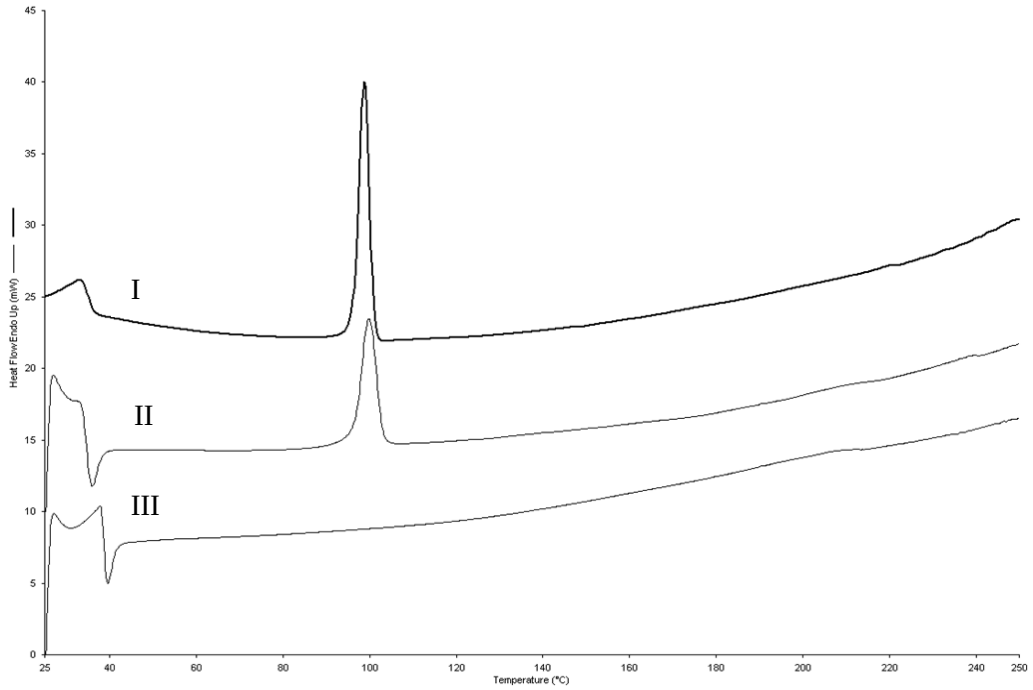


Figure 3.38 *DSC thermograms of FCV (I), a binary mixture of FCV and HPC (II) and HPC (III) at a heating rate of 10 °C/min*

DSC analysis of the binary mixture of FCV and lactose as shown in Figure 3.39 (II) reveals a sharp endothermic peak at 99 °C and at 138 °C, due to melting of FCV and the loss of moisture present in the lactose. At a temperature of 180 °C an additional endothermic change is observed due to melting and subsequent decomposition of lactose [108; 152]. This is also evident in Figure 3.39 (III), revealing endothermic changes that occur for pure lactose.

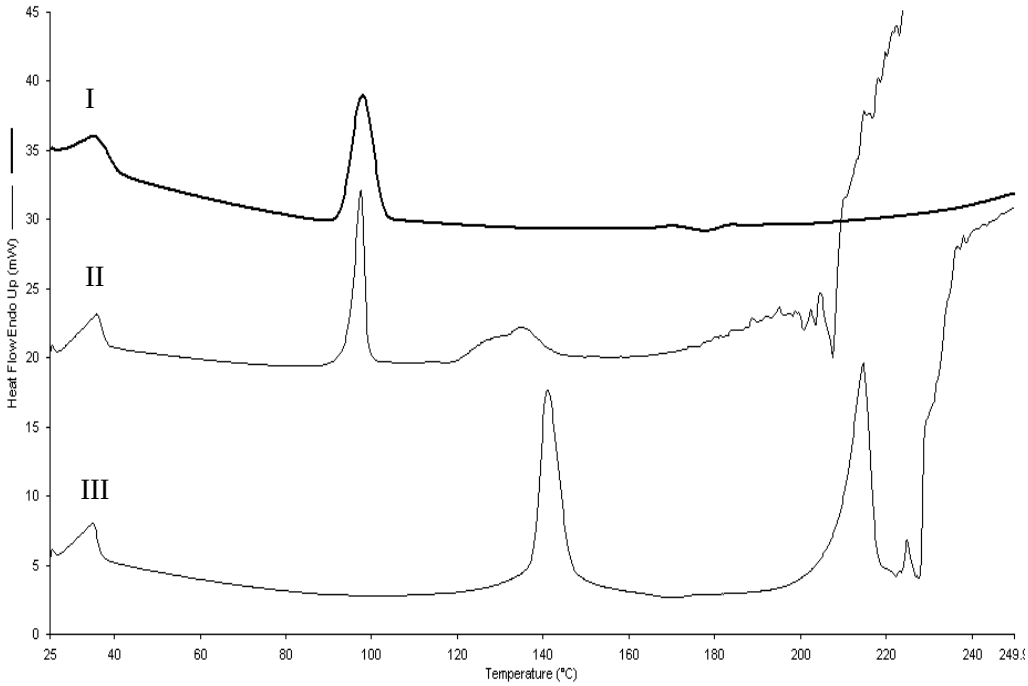


Figure 3.39 *DSC thermograms of FCV (I), a binary mixture of FCV and lactose (II) and lactose (III) at a heating rate of 10 °C/min*

Thermal analysis of the binary mixture of FCV and sodium starch glycolate as shown in Figure 3.40 (II) reveals no significant change in the melting point of FCV.

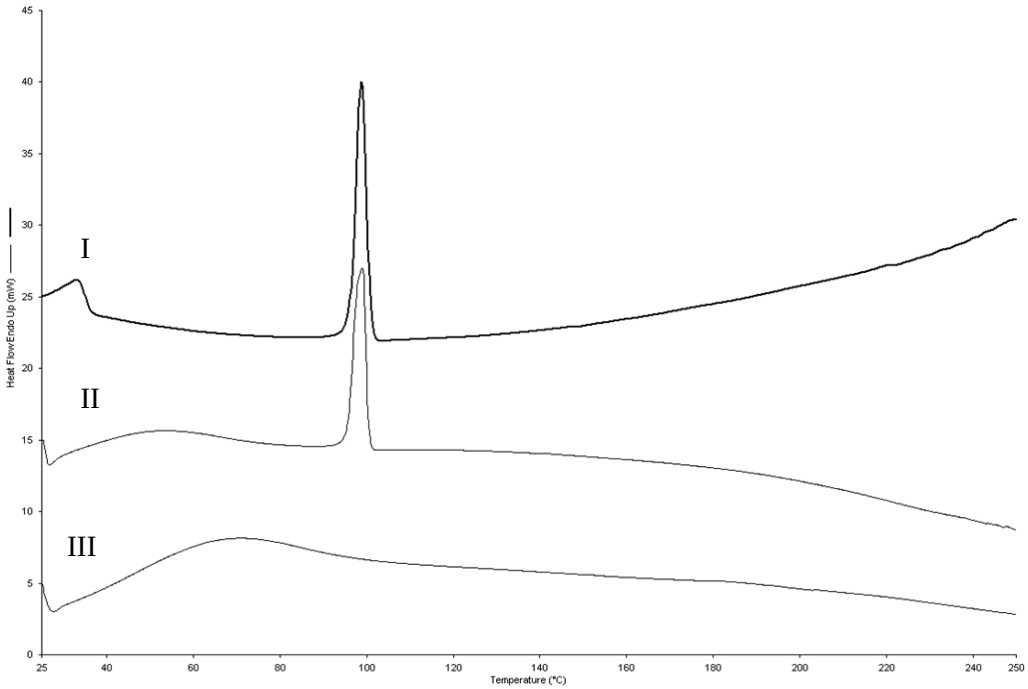


Figure 3.40 *DSC thermograms of FCV (I), a binary mixture of FCV and sodium starch glycolate (II) and sodium starch glycolate (III) at a heating rate of 10 °C/min*

A sharp endothermic peak at 99 °C was observed due to the melting of FCV in the binary mixture of FCV and magnesium stearate as shown in Figure 3.41 (II) and therefore an incompatibility between FCV and magnesium stearate was not expected. The thermogram of magnesium stearate shown in Figure 3.41 (III) reveals two endothermic peaks at 100 °C and 116 °C. The first peak is associated with the evaporation of bound water from the crystals. The second peak is associated with the melting of magnesium stearate [153; 154].

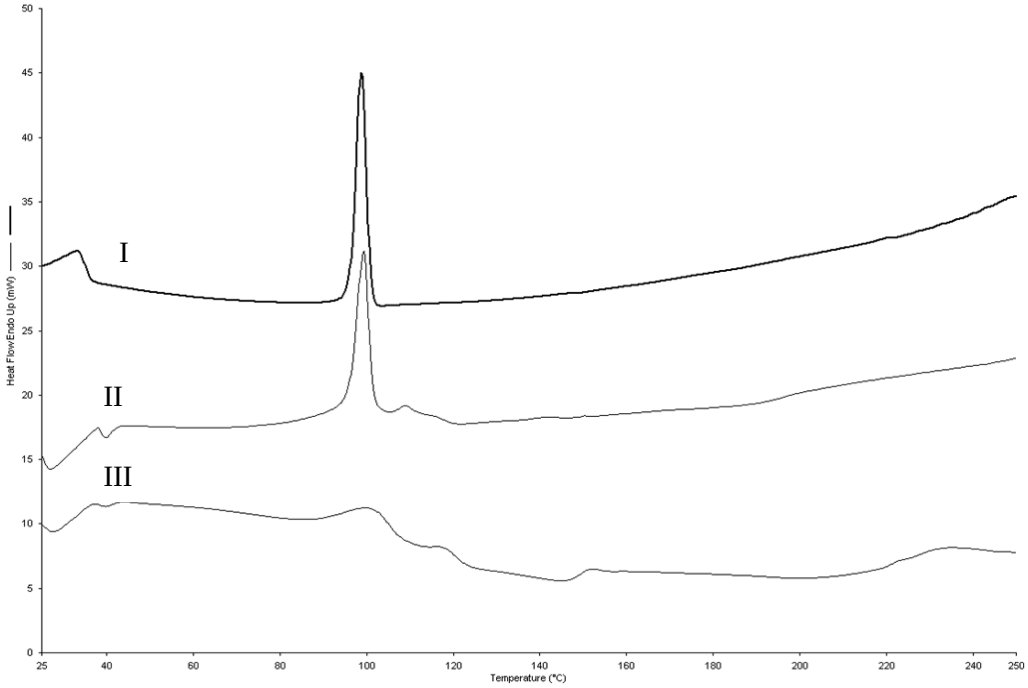


Figure 3.41 *DSC thermograms of FCV (I), a binary mixture of FCV and magnesium stearate (II) and magnesium stearate (III) at a heating rate of 10 °C/min*

DSC analysis of a binary mixture of FCV and titanium dioxide reveals no indication of a potential incompatibility as a sharp endothermic peak characteristic of FCV melting was observed at 99 °C.

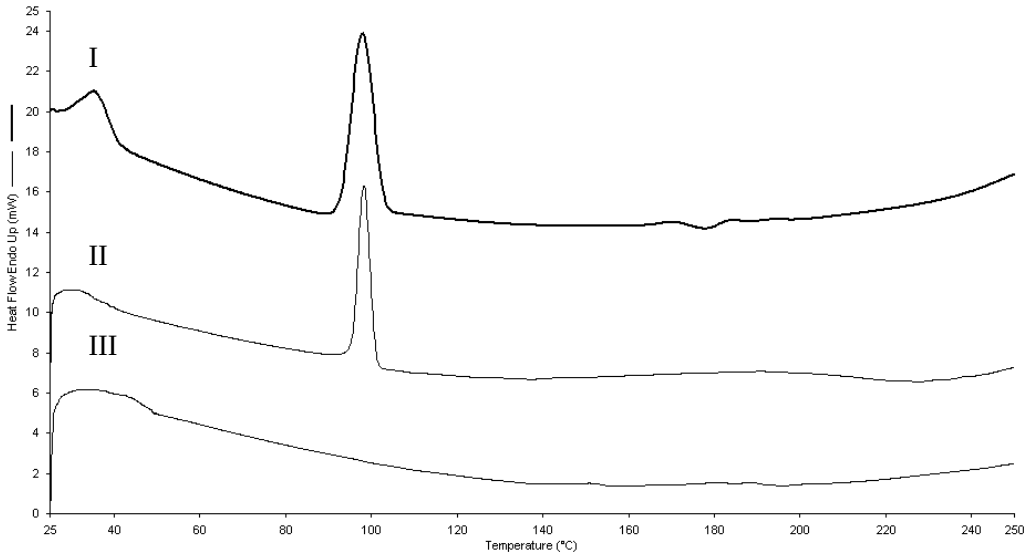


Figure 3.42 *DSC thermograms of FCV (I), a binary mixture of FCV and titanium dioxide (II) and titanium dioxide (III) at a heating rate of 10 °C/min*

A broad, flat peak was observed for the melting of FCV in the binary mixture of FCV and polyethylene glycol 4000, as shown in Figure 3.43 (II). A sharp endothermic peak was observed at 55 °C due to polyethylene glycol melting. It is likely that FCV partially dissolves in the molten polyethylene glycol resulting in a change in the shape of the melting endotherm for FCV [155; 156]. The IR spectrum of the binary mixture of FCV and polyethylene glycol shown in Figure 3.26 reveals no indication of an incompatibility.

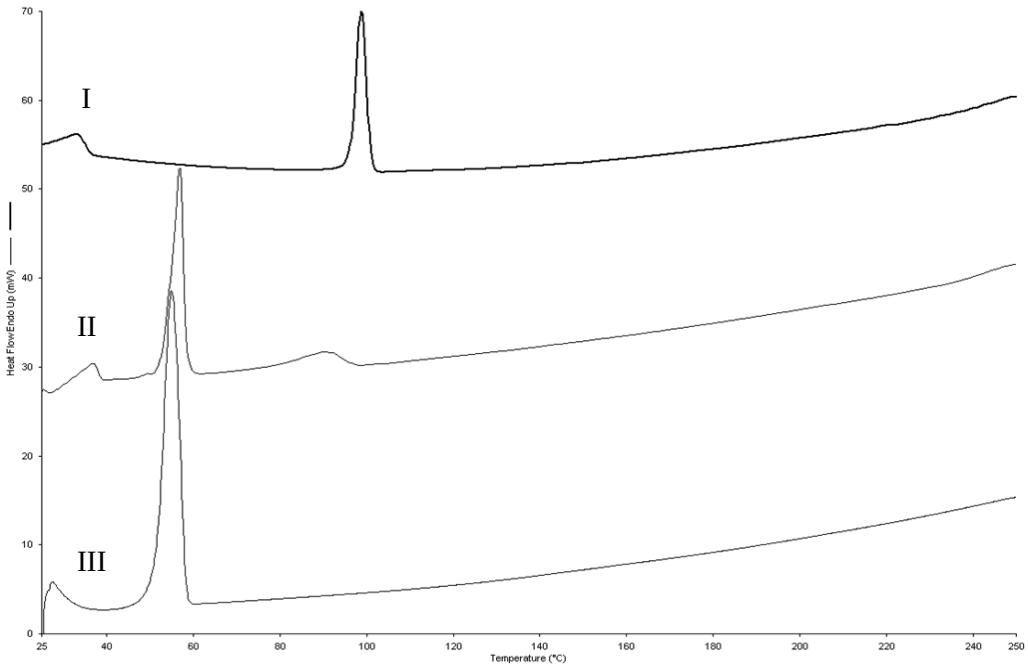


Figure 3.43 *DSC thermograms of FCV (I), a binary mixture of FCV and polyethylene glycol (II) and polyethylene glycol (III) at a heating rate of 10 °C/min*

3.5.6.2.3 Screening of Excipients in Ora-Sweet®

The melting point of sorbitol is 93 °C, as seen in Figure 3.44 (III), shown by a sharp endothermic peak at this temperature. The melting point of FCV observed in Figure 3.44 (I) is 99 °C, which is similar to that of sorbitol. As a result a single melting endotherm is observed. On closer examination of the peak slight shouldering on the left is noted and is indicative of the presence of two endothermic events. Due to the presence of both peaks there is no clear evidence of an interaction between FCV and sorbitol.

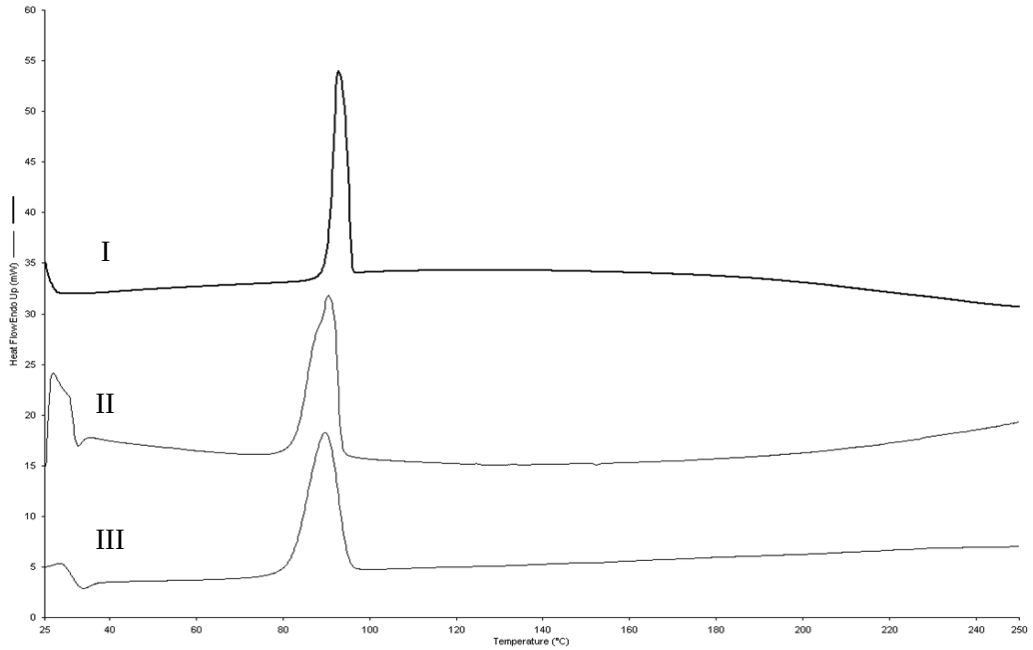


Figure 3.45 *DSC thermograms of FCV (I), a binary mixture of FCV and sorbitol (II) and sorbitol (III) at a heating rate of 10 °C/min*

No interaction was revealed when a binary mixture of FCV and potassium sorbate were subjected to DSC. A sharp endothermic peak at 99 °C was observed as shown in Figure 3.46 (II), due to the melting of FCV.

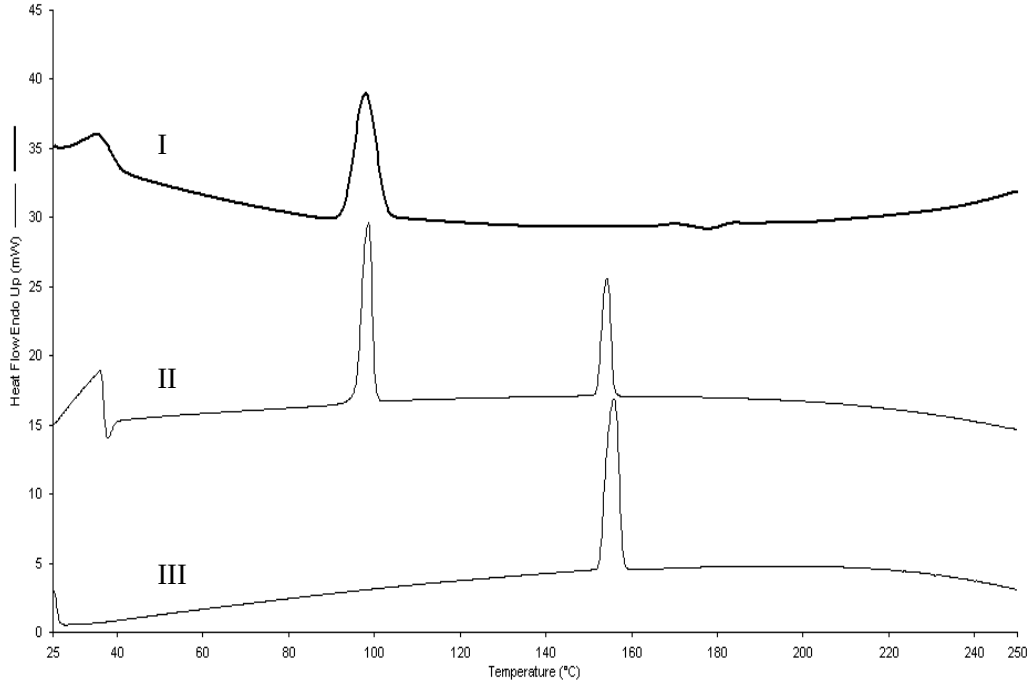


Figure 3.46 *DSC thermograms of FCV (I), a binary mixture of FCV and potassium sorbate (II) and potassium sorbate (III) at a heating rate of 10 °C/min*

The thermogram for the binary mixture of FCV and disodium phosphate shown in Figure 3.47 (II) reveals a sharp endothermic peak at 99 °C, indicative of FCV melting. The dodecahydrates in disodium phosphate fuse and are observed by the endotherm peak at 50-75 °C as shown in Figure 3.47 (II) and Figure 3.47 (III) [131]. No indication of an interaction between FCV and disodium phosphate was observed.

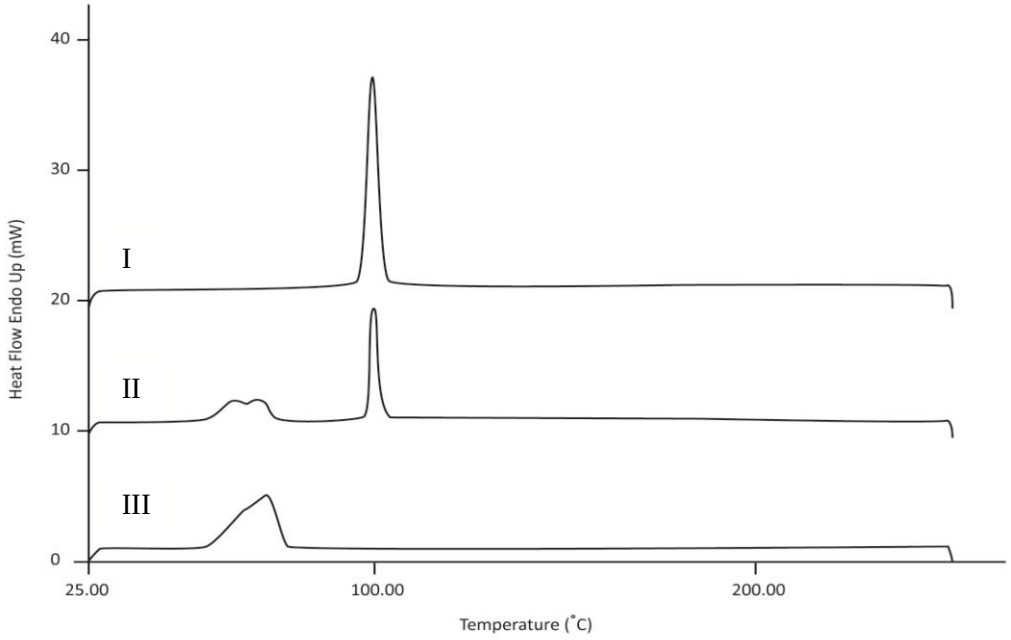


Figure 3.47 *DSC thermograms of FCV (I), a binary mixture of FCV and disodium phosphate (II) and disodium phosphate (III) at a heating rate of 10 °C/min*

3.6 CONCLUSION

Preformulation studies are essential during early drug product development studies to ensure that an API is chemically and physically characterised and the effects of potential formulation excipients on different types of degradation and modification of the API are characterised. The development of an effective, reliable and stable product requires selection of excipients that will not catalyse degradation or impair the efficacy of the API in any way.

Drug-excipient compatibility studies should be performed to identify potential unfavourable combinations of drugs and excipients that may result in physical and/or chemical interactions leading to an alteration in the chemical nature, stability and/or bioavailability of an API in a formulation.

Characterisation studies revealed the effect of pH on the solubility of FCV and the formation of FCV monohydrate that occurs on addition of excess FCV at the point where saturation solubility is reached.

The precipitate was further characterised using XRD, NMR, DSC and IR. The effects of pH on the stability of FCV suggest that a pH of approximately 6 is the point at which the drug is most stable and that degradation is pronounced at low and high pH.

Analyses of binary mixtures of FCV and the excipients used to manufacture Famvir® tablets, Ora-Sweet® and of potential excipients for an oral formulation were conducted using IR. These studies were undertaken to establish if instability of extemporaneous formulations could be attributed to specific components of the formulation. Overlapping and subsequent masking of some absorption bands of FCV were observed in binary mixtures of FCV and propylparaben, magnesium stearate and potassium sorbate. However, this is not conclusive evidence that an incompatibility exists between these materials. The results of DSC analysis suggest that possible interactions between FCV and ascorbic acid, citric acid and propylparaben may occur under the influence of high temperature. However no incompatibility between FCV and the same excipients was observed using IR.

It can be concluded that these preformulation studies have provided sufficient data to make a rational decision in respect of a potential composition for an oral formulation of FCV. Furthermore these data may be useful as a reference when discussing stability data derived following storage of extemporaneous formulations of FCV.

CHAPTER FOUR

DEVELOPMENT AND MANUFACTURE OF FAMCICLOVIR FORMULATIONS

4.1 INTRODUCTION

4.1.1 Paediatric Dosage Forms

Active Pharmaceutical Ingredients (API) such as FCV used for paediatric treatment are not commercially available in age-appropriate dosage forms. Therefore a common practice is the preparation of extemporaneous oral liquid dosage forms for individual patient use. The formulations are usually manufactured by manipulation of commercially available products such as tablets, capsules or powdered API and then dissolving or dispersing the material in a vehicle that patients can swallow relatively easily. The manipulation of adult dosage forms by crushing, reconstitution or division may lead to inaccurate dosing, degradation and loss of potency. Furthermore several factors including the physical and chemical properties of the API and excipients, compatibility of the API and excipients and potential stability, bioavailability, pharmacokinetic, pharmacodynamic, efficacy and tolerability issues are overlooked when manufacturing extemporaneous formulations [4; 125; 126; 157-159]. The age of the intended patient also needs to be considered as infants and children ≤ five (5) years have difficulty swallowing solid dosage forms. Solid dosage forms are also impractical as children require individual titrated dosages based on body weight or surface area (e.g. mg/kg or mg/m^2) [4; 126]. Ideally formulations for use in this population should be easy to swallow and have an acceptable taste, appearance and feel in the mouth. Furthermore they require simple instructions and should be easy to process. The formulation should be well tolerated and the drug and excipients used should be compatible so as to produce a formulation with a suitable stability and efficacy profile [4; 157].

4.2 CHALLENGES

4.2.1 Current Practice of Compounding Extemporaneous Preparations

The preparation of extemporaneous formulations involves the pulverization of tablets or emptying of the contents of a capsule into a mortar, adding a small quantity of a vehicle and mixing to form a paste. Thereafter additional vehicle is added in small portions, with mixing between each addition until the desired volume is reached, following which the formulation is packaged and labelled. When no stability data are available, the USP default expiry date is 14 days from manufacture. There is however no standard protocol for such activities [4].

Many compounds have limited aqueous solubility and therefore extemporaneously manufactured oral formulations are prepared for administration using carboxymethylcellulose, methylcellulose, syrup simplex-based or combinations of these as suspending agents. Commercially available suspending agents, Ora-Plus® or Ora-Sweet®, (Paddock Laboratories, Minneapolis, Minnesota) may also be used to manufacture extemporaneous formulations [4; 158]. However this product is not commercially available in South Africa.

4.2.2 Stability

Stability refers to the chemical and physical properties of a dosage form and where appropriate, the ability to maintain integrity against microbial contamination. It is important that extemporaneously manufactured formulations remain within specified physical, chemical and microbiological limits following storage over a prolonged period of time [159; 160].

Extemporaneous dosage forms can be complex due to the need to add excipients such as preservatives, viscosity enhancers, buffering, flavouring and suspending agents that are aimed at improving patient adherence and stability of the dosage form(s). However, there are often limited data to support the stability of the final liquid dosage form, where there is a potential for interaction between an API, vehicle and excipients [126].

The API must retain chemical integrity and labelled potency limits, and the shelf-life of an extemporaneously prepared oral liquid should be empirically evaluated or, where published information is available, based on that information for a particular formulation. Due to a lack of information on drug stability or limitations in published reports such as the design of the study, a conservative approach should be adopted when assigning a shelf life and expiry date to these dosage forms [160; 161]. A reduction of content to 90% of the initial value is generally regarded as the maximum acceptable reduction of content following storage and the time to reach this value is then designated the shelf-life of the product [126; 159; 160].

Physical stability including appearance, palatability, uniformity, dissolution, and suspendability of the formulation needs to be maintained in disperse systems such as suspensions, whilst initial clarity, colour, odour, taste and viscosity must be monitored for solutions [159; 160].

Resistance to microbial growth must be maintained in oral liquid products as a foul odour and/or turbidity of the formulation may adversely affect the palatability and appearance of the dosage form [161]. By-products of microbial metabolism may also result in a change in the pH of the formulation that may reduce the chemical stability or solubility of a drug [161]. Products that are manufactured

with aqueous vehicles allowing bacterial or fungal growth are particularly vulnerable to spoilage and require the use of an effective preservative system in the formulation [125; 159].

4.2.3 Bioavailability and Pharmacokinetics

Bioavailability and pharmacokinetic/pharmacodynamic studies are not commonly conducted for the majority of extemporaneously manufactured products due to a lack of financial resources and the complexity of performing these studies at health care facilities [4]. However generation of knowledge about the basic principles of dosing in paediatric patients and the differences in pharmacokinetics in patient sub groups is an extremely important consideration for the optimisation of treatment, in particular in children [162]. Children demonstrate age-related variations in anatomy and exhibit physiological differences and immaturity of enzyme systems and clearance mechanisms that can affect drug disposition and consequently the resultant biological response [157; 162]. Neonates have a body composition and organ development vastly different from that of older infants and possibly resulting in significant differences in absorption of drugs from the gastrointestinal tract, intramuscular injection site or through the skin. Significantly altered volumes of distribution are also observed within the first month of life as approximately 75% of the total body weight of a neonate is attributed to water. In adults the total body weight is comprised of 50-60% water. Water soluble drugs that are distributed in extracellular water therefore exhibit a decrease in the volume of distribution as age increases [162]. At birth the metabolic pathways responsible for the biotransformation of drugs are often underdeveloped. For example oxidative pathways are severely compromised at birth, but develop rapidly during the first week(s) of life. Therefore the efficacy and tolerability of an API should be closely monitored in patients requiring treatment with formulations that have been extemporaneously manufactured [4].

4.2.4 Effectiveness and Tolerability

Generally the effectiveness and tolerability of extemporaneous formulations have not been evaluated due to the high cost of studies of this nature as compared to conventional bioavailability, pharmacokinetic and pharmacodynamic studies. Consequently effectiveness and tolerability studies are unlikely to be performed for extemporaneous formulations [4; 163].

4.2.5 Limitations of Research

Most diseases are more prevalent in adult populations and therefore the majority of medications licensed for marketing are generally only indicated for adult populations at the time of approval [4]. Manufacturers may have little interest in conducting expensive Phase I - III studies for labelling in paediatric patients, especially due to a potential low return on investment. Paediatric patient

recruitment is challenging and involves ethical, practical and liability issues that include legal and regulatory requirements beyond those required for studies performed in adults [157; 158].

4.3 PHARMACEUTICAL CONSIDERATIONS WHEN MANUFACTURING EXTEMPORANEOUS FORMULATIONS

4.3.1 Excipients

Formulating stable liquid dosage forms requires the use of excipients to ensure dose uniformity, particularly if the drug is to be delivered as a suspension. These adjuvants promote chemical stability and prevent microbial growth during storage and use, in addition to improving the aesthetic appeal of the formulation. Excipients are not well regulated in many countries and some can be harmful to children [125]. The physicochemical characteristics of excipients incorporated in formulations are also key determinants of drug absorption and excipients used in formulations may have an effect on and influence the bioavailability of an API. For example sorbitol and mannitol when used in relatively large quantities in oral liquid dosage forms, reduce the bioavailability of an API that has low intestinal permeability as they tend to shorten intestinal transit times. The presence of PEG 400 can also influence transit time in the small intestine and may affect the absorption of APIs such as ranitidine which is thought to have a site specific absorption window [164].Careful evaluation of the physicochemical attributes and physiological compatibility when selecting excipients must be undertaken prior to use.

4.3.1.1 Preservatives

Preservatives are included in pharmaceutical solutions to control the microbial burden of a formulation and when a preservative is indicated, various factors should be considered [138; 165]. The preservative should:

i) inhibit the growth of micro-organisms that are likely to be present,
ii) possess a broad spectrum of antimicrobial activity,
iii) be sufficiently water soluble to provide a concentration of preservative that will inhibit growth,
iv) should be non-irritating and non-sensitising, and
v) be stable and compatible with other excipients used in the formulation in addition to the container and closure [4; 159; 165].

The most commonly used preservatives are methylparaben, propylparaben and butylparaben [138]. Other systems include benzoic acid or sodium benzoate [138]. However a preservative that may be

considered largely inert and appropriate for use in an adult may lead to life-threatening toxicity in paediatric patients following administration of multiple doses, as is seen with benzyl alcohol and benzoic acid that fall into this category [125; 126; 166]. Factors that directly impair the efficacy of the preservative such as the pH of the formulation and the presence of micelles and hydrophilic polymers must also be taken into account. The antimicrobial properties of a preservative are a function of the presence of unionised forms of the material. The degree of ionisation is affected by the pH of a formulation and therefore the selection of a preservative should be governed by the pH of the formulation [165].

The presence of micelles may decrease the available concentration of a preservative in solution since preservatives that exhibit lipophilic properties tend to partition into the core of a micelle. To address this challenge the amount of a preservative in a formulation must be increased.

The presence of hydrophilic polymers such as methylcellulose in vehicles has been shown to reduce the free concentration of preservative in formulations as some preservatives are able to interact with dissolved polymer. The most common approach to solving this challenge is to increase the amount of preservative in the formulation [165].

4.3.1.2 Antioxidants

To enhance the stability of an API that is susceptible to oxidation an antioxidant may be included in a formulation. Antioxidants are redox chemicals that form systems exhibiting a higher oxidative potential than the API, or that inhibit free radical-induced decomposition. In aqueous solutions antioxidants are oxidised in preference to the API, thus protecting the active material from decomposition. Typically low concentrations of antioxidants are used (< 0.2% w/w) and it is common for the concentration of an antioxidant in a final product to be noticeably lower than the initial concentration in a formulation due to antioxidant activity during the manufacture, packaging and storage of the dosage form [165].

4.3.1.3 Sweeteners and Flavourants

The unpleasant taste associated with an API that is dissolved or dispersed in a vehicle is often difficult to mask and requires the use of sweetening and/or flavouring agents to improve the aesthetic appeal of the formulation and ultimately patient adherence [4; 157; 163; 167]. Low molecular weight carbohydrates such as sucrose are widely used as sweetening agents. Sucrose has the advantage of being colourless, soluble in water, is stable over a pH range of approximately 4 to 8 and is able to mask the tastes of both salty and bitter drugs whilst maintaining a soothing effect in the mouth and

throat [97]. Polyhydric alcohols such as sorbitol and mannitol are alternate sweetening agents to sucrose when manufacturing formulation for use in diabetic patients. In addition artificial sweeteners can be used alone or in combination with sugars and alcohols to improve the taste of formulations. They are usually required in much smaller concentrations as they are intense sweeteners. They are highly water soluble and are chemically and physically stable over a wide pH range. However, liquid formulations containing large quantities of artificial sweeteners often have a noticeable bitter aftertaste that may lead to a decrease in patient adherence. Another important consideration when formulating dosage forms for paediatric use is the fact that taste perception may be cultural specific and changes with age [166].

4.3.1.4 Colourants

A colourant is any dye, pigment or other substance that can impart colour to a product [168]. Dyes are incorporated into a formulation for aesthetic purposes. When used in combination with excipients such as flavourants, the colour used should be complementary to improve the attractiveness of the final formulation [4; 97; 165]. The inclusion of colourants also allows for easy product identification and permits masking of strongly coloured degradation products that may be produced over time. A range of natural and synthetic dyes and colourants are available for use of which the former, tend to be more widely acceptable. Natural dyes include carotenoids, chlorophylls, anthocyanins and a miscellaneous group that includes riboflavines, caramel and extracts of red beetroot [97; 169]. Natural colourants and dyes may have limited availability and may vary in chemical composition. This may result in quality variations during manufacture. Synthetic colourants and dyes are more stable and result in brighter colours than those of natural origin [97]. As dyes and colourants are largely anionic or cationic they may be chemically incompatible with the API and/or other excipients in a formulation. Due to the wide scope and variation of colourants, certain colourants are approved in certain countries only whilst others have been banned. Several acts have been established to prohibit the use of poisonous and harmful colourants, which FDA is responsible to enforce. For example ‘The Food and Drug Act of 1906’ is in place to ensure only FDA approved dyes and colourants are selected for use in products [97; 168; 169].

4.3.1.5 Buffers

Buffers are used in formulations to control pH and consequently optimise the physicochemical performance of a product [165]. Buffering agents when dissolved in a solvent resist changes in pH when small amounts of an acid or base are added to the solution. The relationship between pH and buffering agents is given by Equation 4.1.

$$pH = pK_a + \log \frac{C_i}{C_u}$$ ***Equation 4.1***

Where,

pK_a = Acid dissociation constant
C_i = concentration of the protonated species
C_u = concentration of the unprotonated species

The pH of a solution will remain constant if the logarithm of the ratio $\frac{C_i}{C_u}$ remains constant. On addition of a small amount of base the conjugate acid will be converted to its salt form. If the concentration of the protonated and unprotonated species is reasonably high the effect of the change/addition will be negligible and the overall pH will remain unaltered. However on addition of a large volume of acid or base the change in the logarithm of the ratio $\frac{C_i}{C_u}$ becomes appreciable and the overall pH will be affected [97].

Selection of an appropriate buffer will depend on the pH and buffering capacity required in a formulation, in addition to the components of the buffer, excipients and API exhibiting the appropriate compatibility profile. Buffer salts commonly used in pharmaceutical formulations include acetates (acetic acid and sodium acetate), citrates (citric acid and sodium citrate) and phosphates (sodium phosphate and disodium phosphate) as they are physiologically compatible and produce solutions of pH that fall in the acceptable range for oral administration, *viz.*, pH 5 - 8 [165].

4.4 ORAL LIQUID FORMULATIONS

4.4.1 Solutions

Oral solutions allow the systemic absorption of an API following administration of the dose to the GIT. They may be formulated over a wide pH range due to the resilience of the GIT environment to administration of solutions of extreme pH but are typically formulated to produce a pH = 7. All components of a formulation should be soluble in the vehicle and no evidence of precipitation should be observed [165].

4.4.2 Syrups

Syrups contain high concentrations of sugar or sugar substitutes in an aqueous vehicle. Syrups traditionally include a flavouring agent, however unflavoured syrup is also a commonly used vehicle. Addition of other sweetening and viscosity enhancing agents is not necessary as syrups are inherently sweet and produce solutions of a moderate to high viscosity [165]. The addition of preservatives is often not required due to the high concentration of sucrose, consequent high osmotic pressure and

unavailability of water to support microbial growth. However in syrups in which sucrose has been partially substituted or is diluted prior to use, the addition of preservatives may be necessary [165; 170].

4.4.3 Commercially Available Vehicles for Oral Formulations

Ora-Sweet® is a commercially available vehicle used for the manufacture of extemporaneous oral products and simplifies the formulation process with respect to flavouring and sweetening of a product [134]. The use of Ora-Sweet® results in the manufacture of formulations that appeal to patients in terms of elegance and taste and permits the pharmacist to produce formulations efficiently. Ora-Sweet® is regarded as 'the modern version of simple syrup flavoured with a citrus-berry blend to produce a highly palatable taste' [134]. It can be used alone or in combination with other vehicles such as Ora-Plus®, simple syrup or methylcellulose solutions, and retains its flavouring properties when diluted up to 50 %. Ora-Sweet® is comprised of purified water, sucrose, glycerin, sorbitol and a flavouring system, and is buffered with citric acid and sodium phosphate. The vehicle is preserved using methylparaben and potassium sorbate [126; 134]. Ora-Sweet® has a pH of approximately 4.2 and is buffered to a slightly acidic pH to minimize oxidative degradation of medicinal agents that may be dissolved or dispersed in the vehicle [134].

4.5 METHODS

4.5.1 Materials

FCV was purchased from Mttpharma (Pudong, Shanghai, China). Sodium metabisulphite, ascorbic acid, citric acid, methyl-hydroxy benzoate sodium (methylparaben) and propyl-hydroxybenzoate sodium (propylparaben) were donated from Aspen Pharmacare (Port Elizabeth, Eastern Cape, South Africa). HPMC was purchased from Colorcon Ldt (Dartford, Kent, England). Ora-Sweet® was purchased from Paddock Laboratories, Inc. (Minneapolis, Minnesota, United States of America) Potassium dihydrogen orthophosphate, sodium hydroxide pellets and orthophosphoric acid were purchased from Merck Chemicals Ltd (Modderfontein, Gauteng, South Africa). Sugar was purchased from Huletts (Durban, Kwa-Zulu Natal, South Africa).

4.5.2 Manufacturing Equipment

Sucrose and HPMC were weighed using a Model PM4600 top-loading analytical balance (Mettler Instruments, Greifensee, Zurich, Switzerland) with a sensitivity of 0.01 g. All other raw materials were weighed using a using a Model AG135 Mettler Toledo analytical balance (Mettler Instruments,

Greifensee, Zurich, Switzerland) with a sensitivity of 0.01 mg. Syrup and HPMC were heated and stirred using a Model KH-4 Fried Electric hot plate and magnetic stirrer (Fried Electric, Haifa, Israel).

4.5.3 Method of Manufacture

4.5.3.1 Manufacturing Procedure

Extemporaneous manufacturing is a common practice and involves re-formulation of medicines into suitable dosage form for paediatric use [127]. A liquid dosage form is desirable as it allows for easy administration of a drug to children of all ages [127; 159]. Commercially available suspending agents are not always available and pharmacists are therefore faced with having to manufacture the vehicle in addition to the overall product as described in §4.5.3.1.1 - 4.5.3.1.4.

4.5.3.1.1 Manufacture of Syrup Simplex

The volume of syrup required for manufacture was established using the density of Syrup BP, calculated to be 1.33 g/ml) [171]. The relevant amount of sugar (667g) was accurately weighed and placed into a tared beaker, and 100g of distilled water was added.

The beaker was placed onto a hot plate and heated, with constant stirring, until all the sugar had dissolved. The beaker was removed from the heat and the syrup was cooled with constant stirring. The beaker containing syrup was weighed and distilled water was added to produce the correct volume of syrup.

4.5.3.1.2 Manufacture of 0.5% w/v HPMC Solution

The desired volume of the 0.5% w/v HPMC vehicle was established and the quantity of HPMC powder to be accurately weighed was calculated. The HPMC powder (0.5 g) was weighed and added in small quantities to distilled water (100 ml). The solution was gently heated on a hot plate with constant stirring until the entire 0.5 g had been added. The beaker was removed from the hot plate and the HPMC solution was cooled with constant stirring.

4.5.3.1.3 Preparation of 0.05M Buffer (pH=6)

The volume of buffer that was required was established and 6.8 g potassium dihydrogen orthophosphate was weighed and dissolved in 1000 ml distilled water in an A-grade volumetric flask to produce a 50 mM solution. The resultant solution was adjusted to pH 6 using sodium hydroxide pellets.

4.5.3.1.4 Manufacture of 25 mg/ml FCV Formulations

A schematic of the manufacturing process is shown in Figure 4.1. Ten (10) Famvir® 250 mg tablets were crushed using a mortar and pestle to produce a fine powder or 2.5 g of API was accurately weighed directly into a mortar. Aliquots (5 ml) of the relevant vehicle, *viz.* syrup simplex, HPMC, buffer or Ora-Sweet® were gradually added to the mortar and the powder triturated to form a paste. Approximately 5x10 ml aliquots of the vehicle were added to the mortar with thorough mixing between additions until a concentrated formulation had been produced. The mixture was then quantitatively transferred to an A-grade volumetric flask using 10 ml aliquots of the appropriate vehicle. The formulation was then made up to volume and mixed to produce solutions of 25 mg/ml. A dose concentration of 25 mg/ml was selected for formulation development based on the requirement to achieve a convenient dose volume of 5 ml for a 10 kg patient, that could easily be titrated to achieve a correct dose of 12.5 mg/kg. The solutions were packaged into 100 ml clear glass bottles. The batch manufacturing record summaries and results of testing are included in Appendix I and the formulae used for the extemporaneous preparation of oral FCV formulations are summarised in Table 4.1.

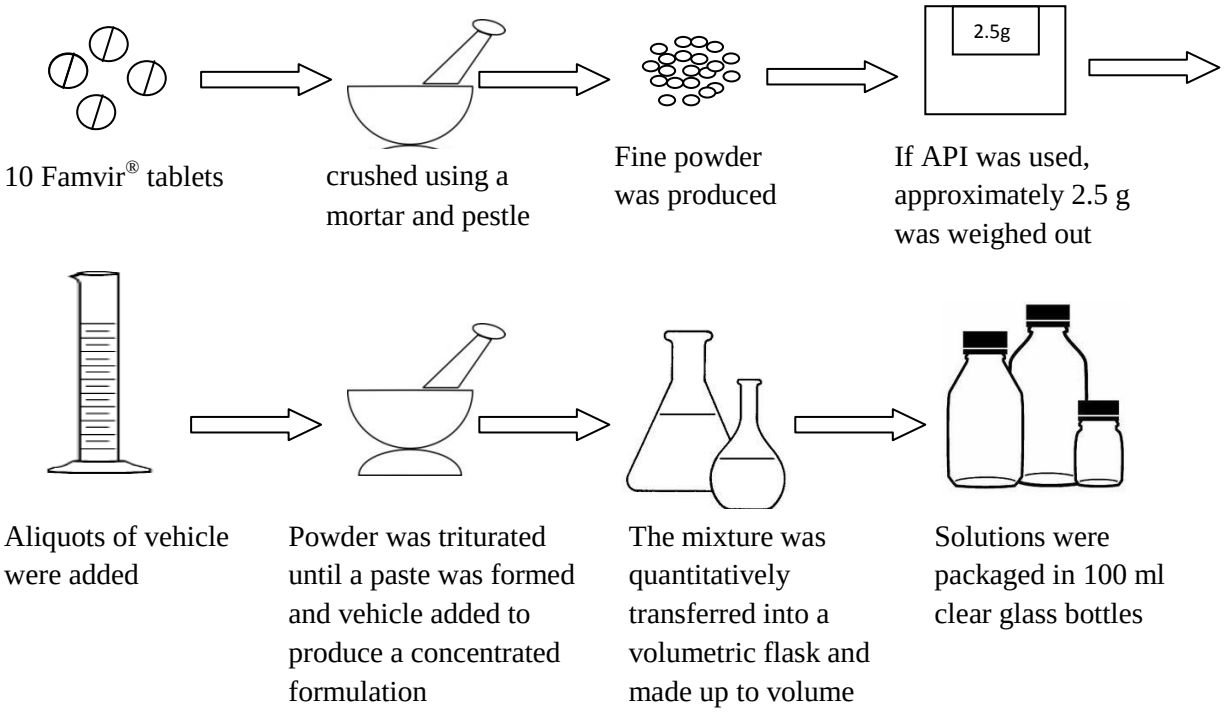


Figure 4.1 *Schematic representation of the manufacture of 25 mg/ml oral FCV formulations*

Table 4.1 *Formulae for extemporaneously manufactured 25 mg/ml FCV formulations*

	Vehicle (ml)				FCV form (mg/ml)		Antioxidant (% w/v)			Preservative (% w/v)		
Batch # FCV-	**Syrup**	**HPMC**	**Ora-Sweet®**	**Aqueous buffer**	**FCV powder**	**Famvir® crushed tablets**	**Sodium metabisulphite**	**Ascorbic acid**	**Citric acid**	**Methylparaben**	**Propylparaben**	***Methylparben and propylparaben**
01	100	-	-	-	25	-	-	-	-	-	-	-
02	100	-	-	-	-	25	-	-	-	-	-	-
03	-	100	-	-	25	-	-	-	-	-	-	-
04	-	100	-	-	-	25	-	-	-	-	-	-
05	-	-	100	-	25	-	-	-	-	-	-	-
06	-	-	100	-	-	25	-	-	-	-	-	-
07	100	-	-		25	-	0.05	-	-	-	-	0.1
08	100	-	-	-	25	-	-	0.05	-	-	-	0.1
09	100	-	-	-	25	-	-	-	1	-	-	0.1
10	-	100	-	-	25	-	0.05	-	-	-	-	0.1
11	-	100	-	-	25	-	-	0.05	-	-	-	0.1
12	-	100	-	-	25	-	-	-	1	-	-	0.1
13	100	-	-	-	25	-	0.05	-	-	0.1	-	-
14	100	-	-	-	25	-	-	0.05	-	0.1	-	-
15	100	-	-	-	25	-	-	-	1	0.1	-	-
16	-	100	-	-	25	-	0.05	-	-	0.1	-	-
17	-	100	-	-	25	-	-	0.05	-	0.1	-	-
18	-	100	-	-	25	-	-	-	1	0.1	-	-
19	100	-	-	-	25	-	0.05	-	-	-	0.1	-
20	100	-	-	-	25	-	-	0.05	-	-	0.1	-
21	100	-	-	-	25	-	-	-	1	-	0.1	-
22	-	100	-	-	25	-	0.05	-	-	-	0.1	-
23	-	100	-	-	25	-	-	0.05	-	-	0.1	-
24	-	100	-	-	25	-	-	-	1	-	0.1	-
25	-	-	-	100	25	-	-	-	-	-	-	-

*** Methylparaben = 0.08% w/v and propylparaben = 0.02% w/v**

4.5.4 Storage of Samples

Batches FCV-01-FCV-24 were stored under different conditions and periods of time, as summarised in Table 4.2. The formulations were stored until ≤ 90% of the content of FCV remained at the lowest temperature/humidity storage condition, *viz.* 4 °C or 25 °C/65% RH, so as to determine the shelf life for each batch. All formulations were exposed to the same level of light under the different storage conditions.

Table 4.2 *Storage conditions used for Batches FCV-01- FCV-24*

	Days of analysis			No. of formulations*
	4 °C	**25 °C/60% RH**	**40 °C/75% RH**	**100 ml**
FCV-01	-	0, 7, 14, 21, 28		5
FCV-02	-	0, 7, 14, 21, 28		5
FCV-03	-	0, 7, 14, 21, 28		5
FCV-04	-	0, 7, 14, 21, 28		5
FCV-05	0, 1, 2, 3, 4, 5, 6, 7,14, 21, 28, 42, 56, 84			3
FCV-06	0, 1, 2, 3, 4, 5, 6, 7,14, 21, 28, 42, 56, 84			3
FCV-07	-	0, 1, 2, 3, 4, 5, 6, 7, 14, 28, 42		3
FCV-08	-	0, 1, 2, 3, 4, 5, 6, 7, 14, 28, 42		3
FCV-09	-	0, 1, 2, 3, 4, 5, 6, 7, 14, 28, 42		3
FCV-10	-	0, 1, 2, 3, 4, 5, 6, 7, 14, 28, 42		3
FCV-11	-	0, 1, 2, 3, 4, 5, 6, 7, 14, 28, 42		3
FCV-12	-	0, 1, 2, 3, 4, 5, 6, 7, 14, 28, 42		3
FCV-13	-	0, 7, 14, 28, 42		3
FCV-14	-	0, 7, 14, 28, 42		3
FCV-15	-	0, 7, 14, 28, 42		3
FCV-16	-	0, 7, 14, 28, 42		3
FCV-17	-	0, 7, 14, 28, 42		3
FCV-18	-	0, 7, 14, 28, 42		3
FCV-19	-	0, 7, 14, 28, 42		3
FCV-20	-	0, 7, 14, 28, 42		3
FCV-21	-	0, 7, 14, 28, 42		3
FCV-22	-	0, 7, 14, 28, 42		3
FCV-23	-	0, 7, 14, 28, 42		3
FCV-24	-	0, 7, 14, 28, 42		3

**** Number of formulations stored at each temperature/humidity condition***

4.5.5.1 Assay of Oral Formulations

4.5.5.1.1 Mass Versus Volume

The analysis of viscous samples may be complicated as the viscous matrices are difficult to sample and transfer without loss of product [172]. The formulations that were manufactured in these studies had a relatively high viscosity compared to water, making sample preparation difficult and resulting in loss of formulation due to poor expulsion from the pipette tip. To determine the most accurate means

of sampling these formulations, accuracy of measurement was determined for sampling by mass and volume. The results are summarised in Table 4.3.

The density or specific gravity of the formulations was used to convert volume to mass and the densities of Syrup simplex and HPMC were calculated as 1.33 g/ml and 1.0 g/ml respectively.

Table 4.3 *Accuracy data for sampling by mass or volume for analysis of formulations with syrup simplex or HPMC (n=3)*

	Theoretical concentration (µg/ml)	**Measured concentration (µg/ml)**	**% RSD**	**% Bias**
		Syrup simplex		
Mass	100	99.29	2.20	-0.71
Volume	100	94.81	6.12	-5.48
		HPMC		
Mass	100	98.79	2.34	-1.22
Volume	100	94.23	4.35	-6.12

Sampling by mass was found to be more accurate to sampling by volume as the % RSD and % Bias were ≤ 5% in both cases. Consequently samples were weighed for analysis.

4.5.5.1.2 Method of Sampling

An aliquot equivalent to 100 µl was weighed directly into a 25 ml A-grade volumetric flask from each bottle, using a Model AG 135 Mettler Toledo analytical balance (Mettler Instruments, Greifensee, Zurich, Switzerland) and then made to volume with HPLC-grade water. The sample was then mixed on a Model G560E Scientific Industries vortex mixer (Scientific Industries Inc., Bohemia, New York, United States of America) for 30 seconds to produce a solution with a theoretical concentration of 100 µg/ml FCV. The resultant solution was filtered through a 0.45 µm Durapore® HV membrane filter (Millipore, Cork, Munster, Ireland) and a 1 ml aliquot was pipetted directly into a 1.5 ml sample vial to which 50 µl of the internal standard solution had been added. Each batch was analyzed in triplicate by a stability-indicating HPLC assay as described in Chapter two. The initial concentration of FCV was 25 mg/ml (100%), and sample concentrations were expressed as the mean percentage of the initial concentration remaining ± SD.

4.5.5.2 Viscosity

Approximately 100 ml of each formulation was transferred to a 200ml beaker and the viscosity of each formulation was determined using an RVDVI+ Viscometer (Brookfield Engineering Laboratories Inc., Stoughton, Massachusetts, USA) with either a RV2 or RV3 spindle at a rotational

speed of 100% so as to achieve a torque of greater than 10%. The viscosity and torque were determined in triplicate for each batch tested.

4.5.5.3 pH

The pH of each formulation was measure in triplicate using a Model GLP 21 digital Crison pH meter (Crison Instruments, Allela, Barcelona, Spain).

4.5.5.4 Appearance

Subjective assessment of the appearance and colour of each batch of product was made by observation against black and white backgrounds and any changes in colour or appearance noted.

4.6 RESULTS AND DISCUSSION

4.6.1 Syrup- and HPMC-based formulations of FCV API or crushed Famvir® tablets

4.6.1.1 Assay of Formulations

The % FCV remaining to be degraded following weekly analysis of batches FCV-01 - FCV-04 is summarised in Table 4.4.

Table 4.4 *FCV remaining in batches FCV-01 - FCV-04 (mean % ± SD)*

Batch #	Day 0	Day 7	Day 14	Day 21	Day 28
25 °C/60% RH					
FCV-01	100.00 ± 1.00	99.34 ± 2.63	96.59 ± 2.51	90.76 ± 3.12	84.64 ± 2.20
FCV-02	100.00 ± 2.44	99.65 ± 3.92	94.96 ± 1.56	87.77 ± 2.75	78.53 ± 3.92
FCV-03	100.00 ± 5.39	96.51 ± 0.29	96.04 ± 3.20	93.64 ± 0.87	90.80 ± 2.43
FCV-04	100.00 ± 4.01	96.57 ± 4.59	92.73 ± 4.44	89.71 ± 2.58	87.10 ± 3.28
40 °C/75% RH					
FCV-01	100.00 ± 3.39	95.86 ± 3.77	87.66 ± 0.53	85.10 ± 3.01	81.00 ± 3.06
FCV-02	100.00 ± 1.76	93.87 ± 4.66	87.93 ± 3.70	78.70 ± 5.92	71.36 ± 2.53
FCV-03	100.00 ± 1.38	95.07 ± 2.48	94.02 ± 1.46	93.35 ± 2.44	89.97 ± 4.93
FCV-04	100.00 ± 1.47	96.82 ± 2.86	89.55 ± 4.44	87.12 ± 3.22	84.90 ± 4.18

Interpretation of stability results was undertaken using the method described by Timm *et al.*, [54]. This approach permits an assessment of whether degradation that occurs following storage under the conditions specified is relevant and/or significant. The resulting data are compared to the data generated immediately following manufacture *viz.*, t = Day 0. The interpretation described in §2.4.9.1 was applied to the data generated for these stability experiments, and the resultant confidence intervals calculated following analysis of each formulation are shown in Figures 4.2-4.5. The data are reported as the percentage change from the initial amount of FCV determined on Day 0.

The data summarised in Figures 4.2 and 4.3 reveal that formulations manufactured using syrup simplex as the vehicle were stable following storage under both conditions for seven (7) days, and the change in FCV content was neither significant nor relevant. Following storage at 25 °C/60% RH a significant decrease in FCV content was observed for batches FCV-02 and FCV-01 between days 7 and 14 and 7 and 21 for FCV-02 and FCV-01, respectively. Following storage at 40 °C/75% RH a significant decrease in FCV content, that was possibly relevant, was observed 14 days of storage and after 21 days of storage a significant and relevant decrease was observed. Batch FCV-01, that was manufactured using FCV powder, was more stable than the batches manufactured using crushed tablets of FCV. However a decrease in FCV content that was both significant and relevant was observed for batches FCV-01 and FCV-02 following storage for 28 days at 25 °C/60% RH and 40 °C/75% RH.

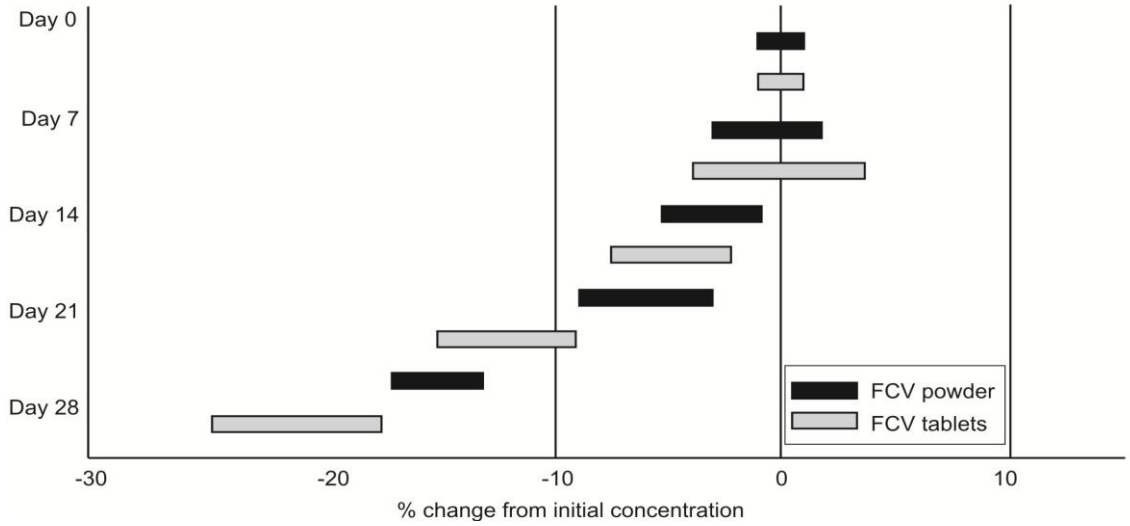


Figure 4.2 *Stability of FCV formulations using syrup simplex following storage at 25 °C/60% RH for 28 days*

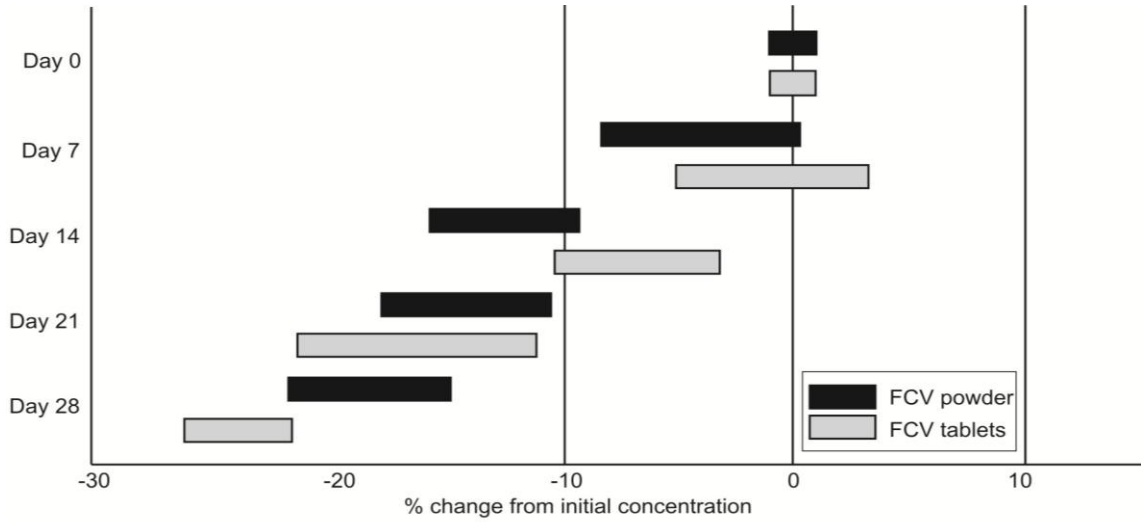


Figure 4.3 *Stability of FCV formulations using syrup simplex following storage at 40 °C/75% RH for 28 days*

The stability of FCV dissolved/dispersed in HPMC-based vehicles as shown in Figures 4.4 and 4.5 revealed that changes in content were neither significant nor relevant for the first seven (7) days when manufactured using crushed Famvir® tablets, *viz.* batch FCV-04, and for the first 14 days when used in pure powder form, *viz.* batch FCV-03, following storage at 25 °C/60% RH. Thereafter a significant and possibly relevant decrease in FCV content was observed after 28 days in batch FCV-03, and for batch FCV-04 a significant and relevant decrease in FCV content was observed after 28 days. At 40 °C/75% RH a similar trend was observed for batches FCV-03 and FCV-04, as shown in Figure 4.5.

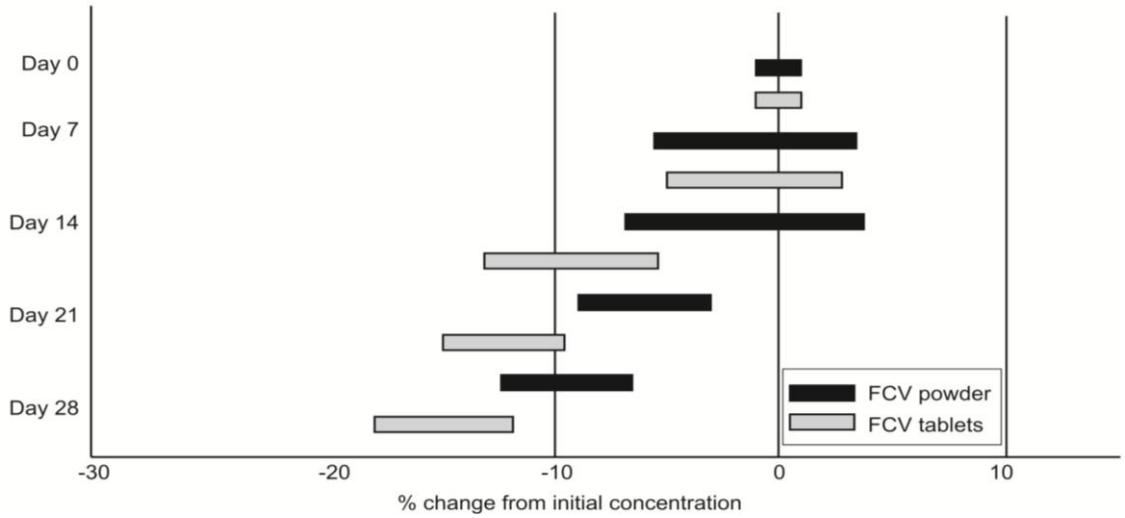


Figure 4.4 *Stability of FCV formulations using HPMC following storage at 25 °C/60% RH for 28 days*

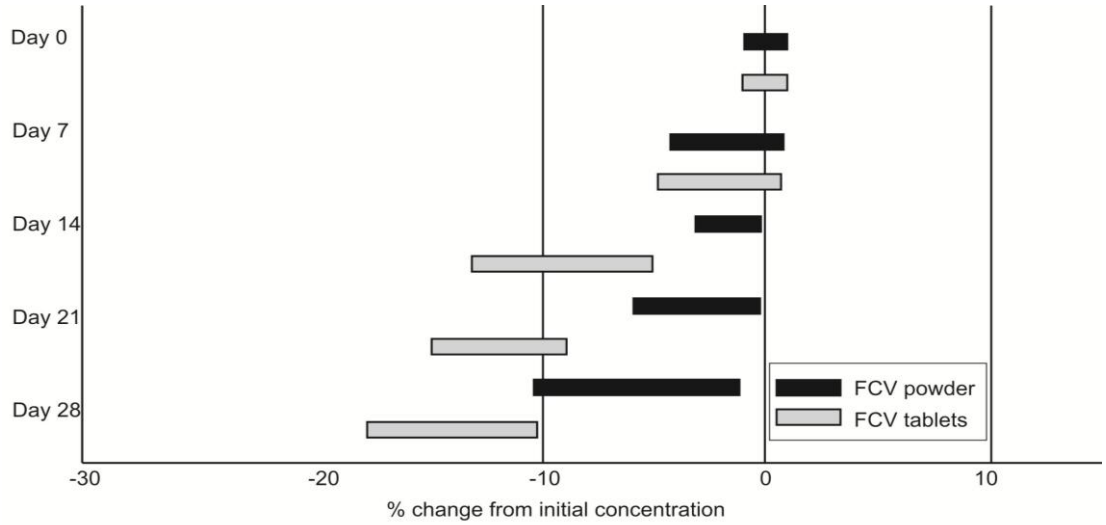


Figure 4.5 *Stability of FCV formulations using HPMC following storage at 40 °C/75% RH for 28 days*

FCV was found to be more stable in batches FCV-03 and FCV-04 that were manufactured using HPMC, than batches FCV-01 and FCV-02 in which syrup simplex was used. Syrup simplex is largely

composed of the disaccharide, non-reducing sucrose that undergoes pH dependent non-enzymatic hydrolysis into fructose and glucose, as shown in Figure 4.6 [173]. This is an acid-catalysed reaction, occurring under even slightly acidic conditions [173; 174]. A decrease in pH therefore results in an increase in the amount of fructose and glucose present and this consequently leads to FCV degradation.

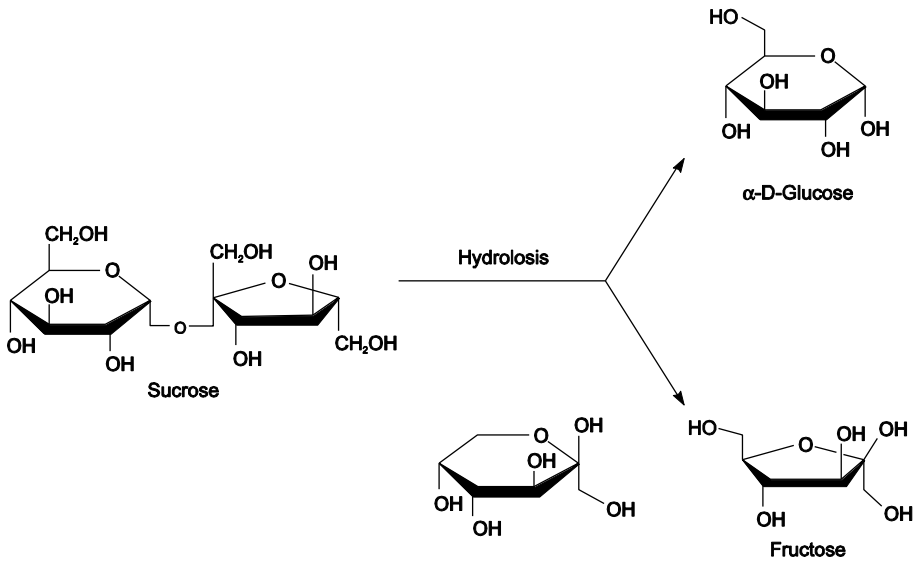


Figure 4.6 *Hydrolysis of sucrose into fructose and glucose, adapted and redrawn from [173]*

An incompatibility between a reducing sugar and primary amine-containing drugs such as FCV has been reported where aldehyde-amine addition leads to the formation of a Schiff-base [89; 90; 173; 175]. This sequence of reactions is known as the Maillard reaction and is responsible for a number of incompatibilities between API and excipients [89; 90; 173; 175].

As the pH increases, the fraction of free amine necessary for nucleophilic addition also increases. The pK_a of FCV is approximately 3.84 [11], suggesting that at a pH > 3.84 the majority of FCV exists in an un-protonated form (95-99%), resulting in the functional group being free for nucleophilic attack as shown in Figure 4.7 [173].

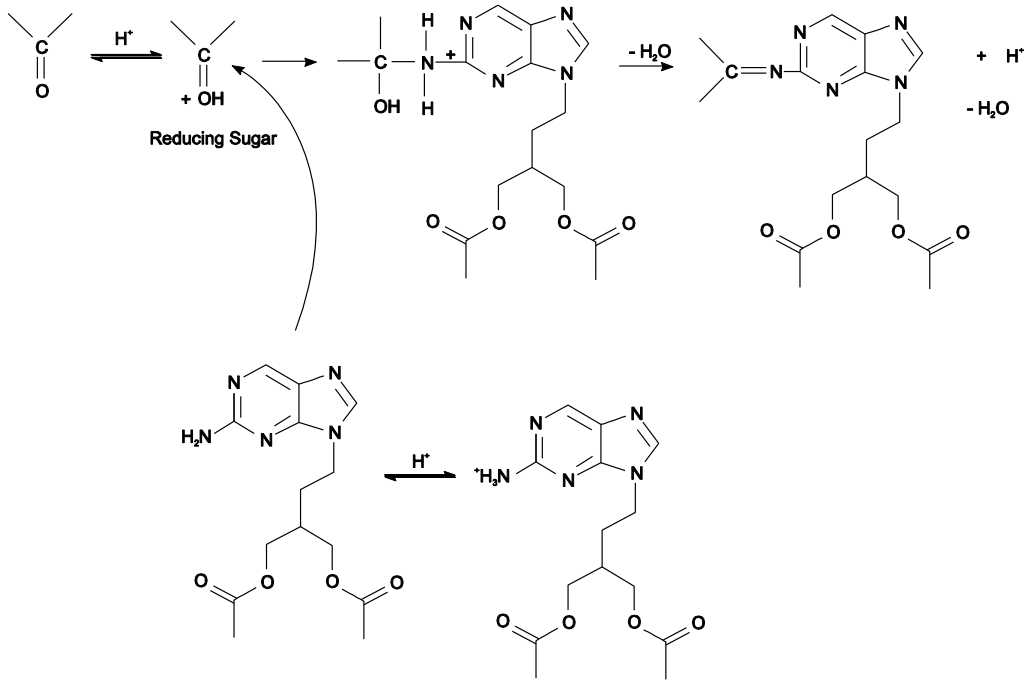


Figure 4.7 *Reaction mechanism of FCV with a reducing sugar, adapted and redrawn from [173]*

4.6.1.2 Appearance

Batches FCV-01 and FCV-02 were clear and pale yellow. At the end of the 28 day period the formulations were a dark shade of yellow/brown, as a possible result of degradation due to the Maillard reaction [174]. Batches FCV-03 and FCV-04 remained clear and colourless over the 28 day period. Batches FCV-02 and FCV-04 were manufactured using crushed Famvir® tablets. Each product revealed the presence of easily redispersed sediment. Sedimentation may have been due to the presence of insoluble excipients in the tablet.

4.6.2 Ora-Sweet®-Based Formulations

4.6.2.1 Assay of Formulation

Batches FCV-05 and FCV-06 were manufactured using FCV powder and crushed Famvir® tablets respectively, with Ora-Sweet® as the vehicle. Both batches exhibited degradation with < 90% FCV remaining following storage for 84 days at 4°C, 25 °C/60% RH and 40 °C/75% RH. The results listed in Table 4.5 summarise the degradation following storage at 4°C, 25 °C/60% RH and 40 °C/75% RH for a period of 84 days.

Table 4.5 *FCV remaining in batches FCV-05 and FCV-06 (mean % ± SD)*

Batch #	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
				4 °C			
FCV-05	100.00 ± 1.66	101.53 ± 0.26	99.31 ± 0.63	100.43 ± 1.75	100.34 ± 2.07	99.71 ± 2.60	100.72 ± 0.87
FCV-06	100.00 ± 2.00	98.41 ± 0.51	100.20±1.88	100.20 ± 2.82	99.48 ± 2.27	99.62 ± 1.18	99.09 ± 0.75
				25 °C/60% RH			
FCV-05	100.00 ± 0.75	98.57 ± 1.77	98.92 ± 2.27	98.76 ± 1.54	97.95 ± 2.14	98.25 ± 1.65	97.51 ± 2.86
FCV-06	100.00 ± 0.73	96.41 ± 0.44	96.99 ± 3.58	95.15 ± 2.61	95.18 ± 1.22	95.02 ± 0.92	94.24 ± 4.48
				40 °C/75% RH			
FCV-05	100.00 ± 1.02	99.33 ± 1.65	98.06 ± 0.97	96.98 ± 1.44	95.72 ± 1.46	93.60 ± 0.61	92.11 ± 1.63
FCV-06	100.00 ± 2.66	97.05 ± 0.82	96.47 ± 0.99	94.30 ± 1.41	93.08 ± 0.70	93.48 ± 0.40	91.86 ± 1.42
Batch #	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 42**	**Day 56**	**Day 84**
				4 °C			
FCV-05	99.54 ± 1.92	98.96 ± 0.43	98.85 ± 2.34	97.46 ± 3.24	94.51 ± 1.98	92.91 ± 0.50	89.22 ± 1.50
FCV-06	98.34 ± 4.35	98.27 ± 1.33	97.78 ± 2.26	95.76 ± 1.91	94.95 ± 0.78	92.98 ± 0.57	89.17 ± 1.94
				25 °C/60% RH			
FCV-05	96.94 ± 1.11	95.20 ± 0.32	92.28 ± 2.39	88.79 ± 1.25	84.93 ± 0.33	82.45 ± 0.65	71.16 ± 0.58
FCV-06	93.46 ± 3.35	92.39 ± 3.01	91.40 ± 3.94	87.32 ± 2.60	85.23 ± 1.09	82.92 ± 1.63	74.19 ± 2.48
				40 °C/75% RH			
FCV-05	89.97 ± 1.43	80.70 ± 0.84	72.33 ± 1.86	65.75 ± 0.89	56.05 ± 1.23	46.83 ± 1.15	31 82 ± 1.13
FCV-06	89.37 ± 2.22	80.28 ± 0.79	69.81 ± 1.50	65.03 ± 0.58	55.42 ± 1.07	49.39 ± 0.20	33.88 ± 0.12

Batches FCV-05 and FCV-06 were regarded as stable for a period of up to 56, 21 and 6 days when stored at 4°C, 25 °C/60% RH and 40 °C/75% RH respectively. Temperature and humidity were found to have a pronounced effect on FCV stability as shown in Figures 4.8 and 4.9, which revealed the degradation of FCV in batches FCV-05 and FCV-06 over an 84 day period.

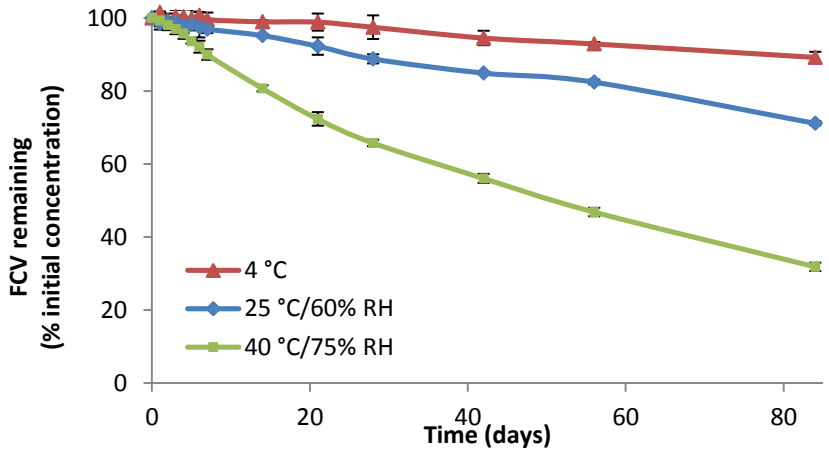


Figure 4.8 *Degradation of FCV in batch FCV-05 over an 84 day period*

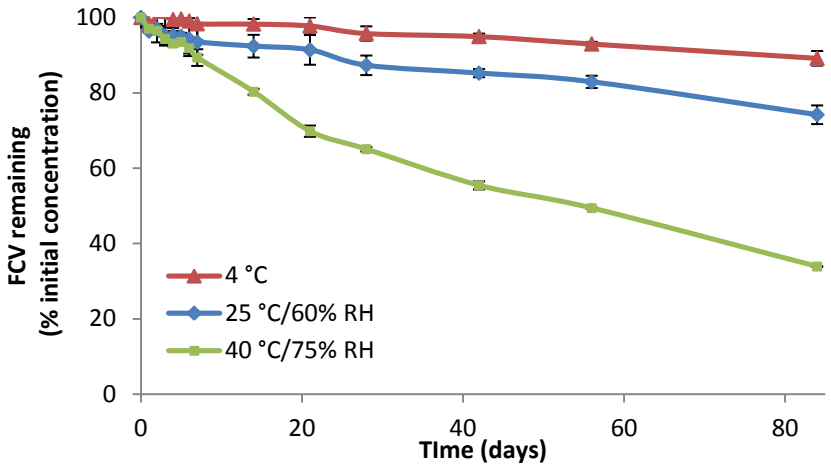


Figure 4.9 *Degradation of FCV in batch FCV-06 over an 84 day period*

FCV undergoes first-order degradation as described by the first-order rate law (Equation 4.2) and the integrated first-order rate law (Equation 4.3) [97].

$$(d[A])/dt = -k[A] \qquad \textbf{Equation 4.2}$$

$$\ln[A] = -kt + \ln[A]_0 \qquad \textbf{Equation 4.3}$$

Where,

A = concentration of the reactant
k = first-order rate constant
t = time

A plot of the natural logarithm of concentration of FCV versus time results in a straight line with a gradient k that is the degradation rate constant. The degradation rate profiles for FCV in batches FCV-05 and FCV-06 were calculated and are shown in Figures 4.10 and 4.11. The rate of degradation of FCV when used as a powder or crushed Famvir® tablets, batches FCV-05 and FCV-06 , were found to be similar, with degradation rates calculated to be 0.0014, 0.0039, 0.0137days^{-1} and 0.0013, 0.0031, 0.0128.days^{-1} respectively.

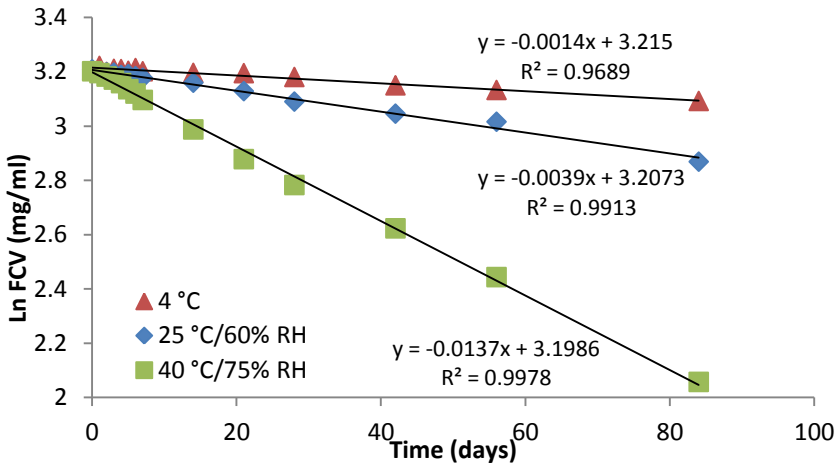


Figure 4.10 *Degradation rate profile of batch FCV-05 over an 84 day period*

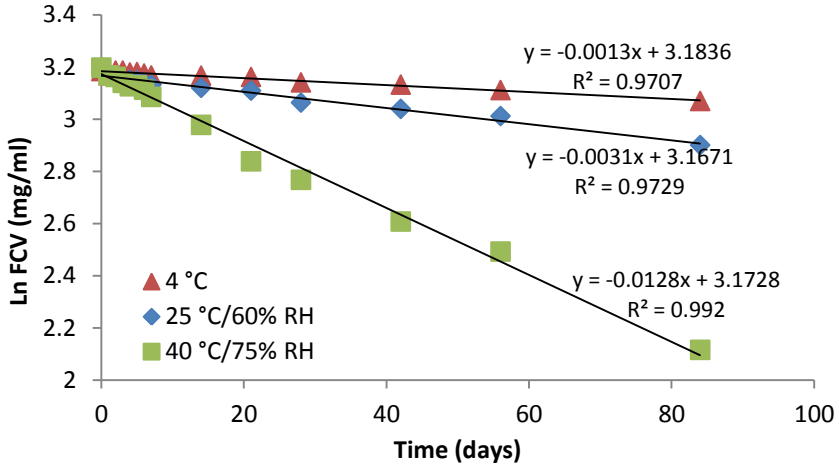


Figure 4.11 *Degradation rate profile of batch FCV-06 over an 84 day period*

The interpretation of data from stability studies was also undertaken as described in §4.6.1.1 using the method described by Timm *et al*, [54]. Batch FCV-05, manufactured using Ora-sweet® and FCV in powder form was more stable when stored at 4°C than when stored at the higher temperature and humidity conditions of 25 °C/60% RH and 40 °C/75% RH as shown in Figure 4.12 . The FCV content for batch FCV-05 showed a change in content that was neither significant nor relevant when stored at 4 °C for 28 days. When this batch was stored at 25 °C/60% RH and 40 °C /75% RH the change was neither significant nor relevant for only three (3) days and two (2) days respectively, after which a significant decrease in FCV content was observed. On days 28 and 21 following storage at 25 °C/60% RH and 40 °C /75% respectively, significant and relevant degradation of FCV was apparent.

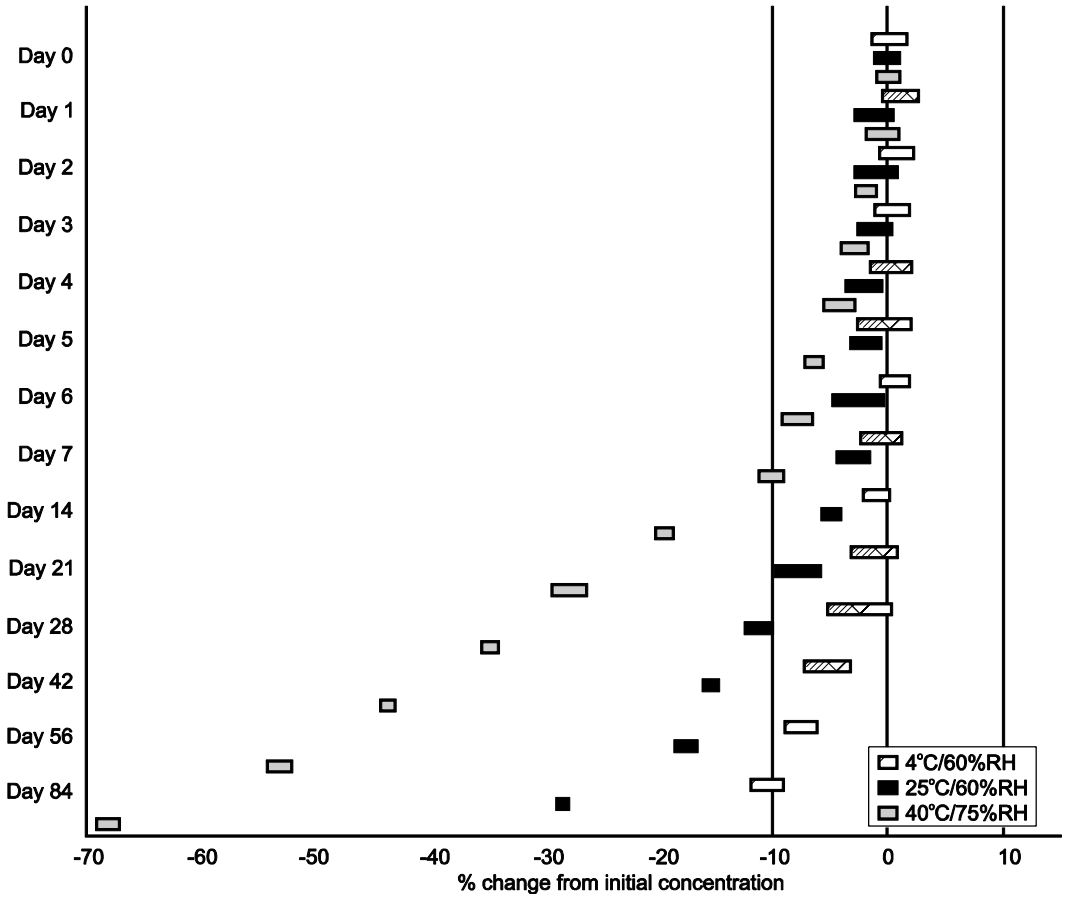


Figure 4.12 *Stability of FCV formulations (Batch FCV-05) incorporating FCV powder dispersed in Ora-Sweet* ®

Batch FCV-06 was manufactured using Ora-sweet® and FCV from crushed Famvir® tablets revealed a stability profile similar to those of the formulations manufactured using FCV powder. Batch FCV-06 was more stable following storage at 4°C than when stored at the higher temperature and humidity conditions of 25 °C/60% RH and 40 °C/75% RH, as shown in Figure 4.13. However, greater instability was observed for batch FCV-06 than for batch FCV-05 following storage at 4 °C: a significant decrease in FCV content was apparent after 14 days of storage. Furthermore following storage for 84 days significant and possibly relevant changes in content were also apparent. Storage of batch FCV-06 at 25 °C/60% RH and 40 °C /75% RH resulted in a decrease in FCV content that was considered significant and relevant on days 42 and 21 for each condition respectively.

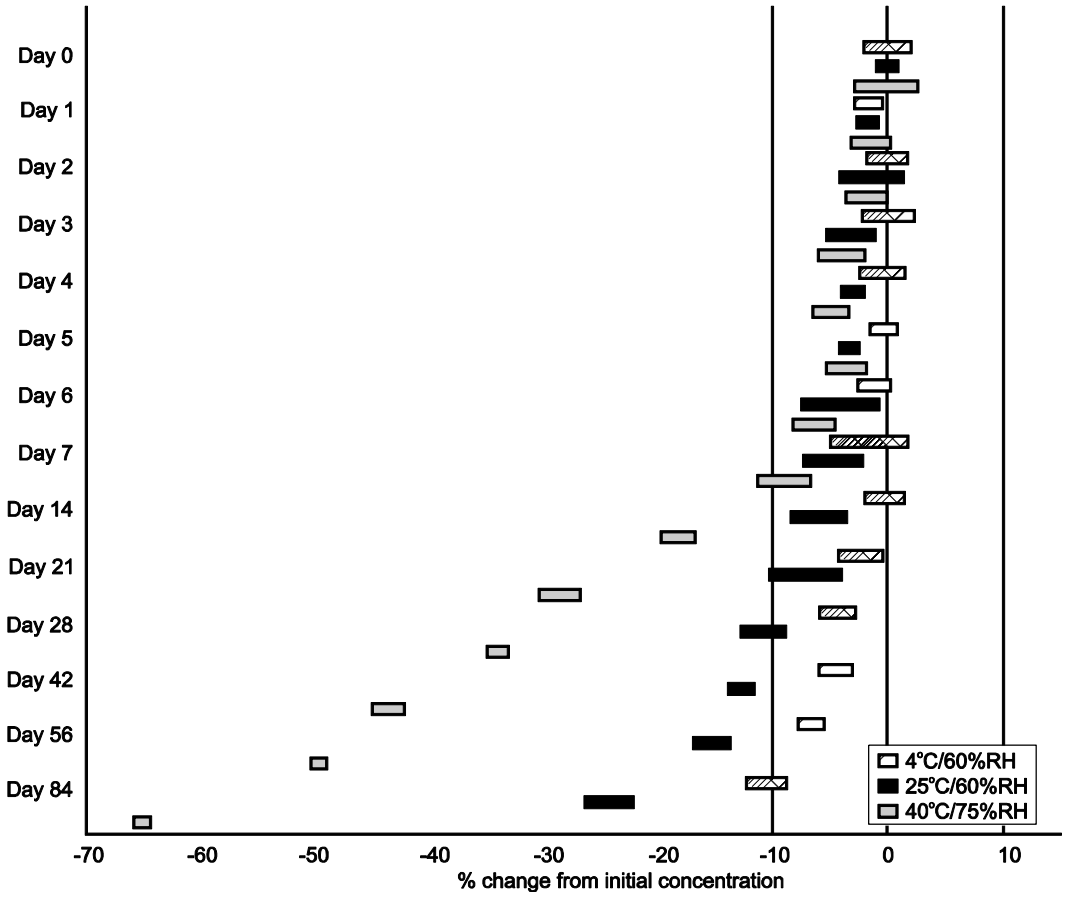


Figure 4.13 *Stability of FCV formulations (Batch FCV-06) using crushed Famvir® tablets dispersed in Ora-Sweet ®*

4.6.2.2 Viscosity

Formulations of low viscosity tend to pour more easily than those of higher viscosity, therefore the administration of oral formulations is likely to be affected by the viscosity. An ideal pharmaceutical suspension should have a high apparent viscosity at low rates of shear, such as when they are stored, to ensure particles remain suspended or settle very slowly over time. At high rates of shear, such as shaking of the product, the apparent viscosity should decrease sufficiently for the product to be easily poured from its container to facilitate administration of an appropriate dose [97]. The viscosity of pharmaceutical solutions and suspensions should be controlled to ensure accurate measurement of the volume to be dispensed and increasing the viscosity of formulations also may increase the palatability of the formulation in some cases [165]. Accordingly there is a range in which the viscosity of a formulation should fall to facilitate dose administration [165]. The viscosity of batches FCV-05 and FCV-06 was measured as described in §4.5.5.2 and the results are summarised in Table 4.6.

Table 4.6 Viscosity *of batches FCV-05 and FCV-06 following storage for 82 days (mean cP ± SD)*

Batch #	Day 0	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56	Day 82
				4 °C				
FCV-5	205.67±2.08	195.00±5.00	193.33±4.16	188.00±5.20	199.33±6.51	196.67±0.58	190.67±3.06	190.00±2.00
FCV-6	234.33±4.51	234.00±4.00	279.33±6.81	283.33±5.13	228.00±2.65	228.00±2.00	262.33±2.08	232.33±2.08
				25 °C/60% RH				
FCV-5	118.67±2.08	116.67±2.08	119.33±3.79	124.33±0.58	133.00±1.73	128.00±1.00	123.33±3.06	122.67±3.06
FCV-6	150.00±3.61	148.33±4.16	189.00±3.61	190.67±3.21	150.67±5.69	151.33±3.06	172.00±2.00	147.33±2.31
				40 °C/75% RH				
FCV-5	125.87±1.01	139.60±6.16	143.07±3.70	142.00±3.12	150.27±8.22	149.87±2.27	140.00±3.46	136.67±3.06
FCV-6	131.33±4.16	117.33±3.21	161.33±3.06	155.00±7.81	121.00±4.59	122.33±2.52	134.33±2.52	122.00±2.00

The changes in apparent viscosity of batches FCV-05 and FCV-06 are graphically shown in Figures 4.14 and 4.15. The viscosity of each batch remained constant over the 84 day period however the observed differences are a function of the ambient temperature and not a true reflection of changes in viscosity. Formulations following storage at 4 °C were shown to have a higher viscosity than those stored at 25 °C/60% RH and 40 °C/75% RH. At higher temperatures the average kinetic energy of the molecules in a liquid increases, thereby more easily overcoming the attractive forces that tend to hold the molecules together and thus resulting in a decrease in viscosity [176].

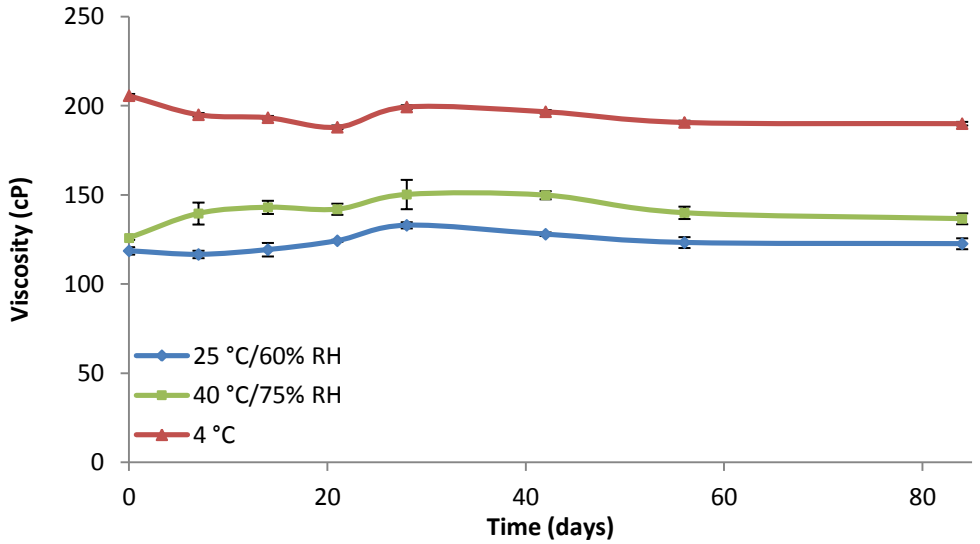


Figure 4.14 *Viscosity of batch FCV-05 over a period of 84 days*

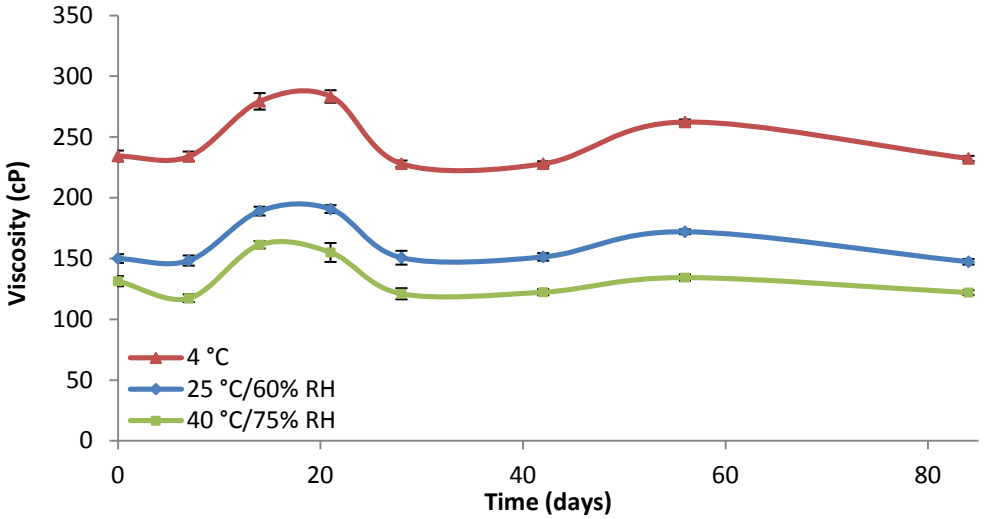


Figure 4.15 *Viscosity of batch FCV-06 over a period of 84 days*

4.6.2.3 pH

The pH of a formulation may have an effect on the stability and/or solubility of an ionisable compound and should be maintained within an acceptable range (pH 5 – 8) for dosage forms for oral administration [165]. The pH values of batches FCV-05 and FCV-06 are summarised in Table 4.7.

Table 4.7 *pH of batches FCV-05 and FCV-06 over 82 days*

Batch #	Day 0	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56	Day 82
				4 °C				
FCV-5	4.69 ± 0.01	4.64 ± 0.02	4.65 ± 0.03	4.63 ± 0.02	4.63 ± 0.01	4.61 ± 0.01	4.69 ± 0.01	4.65 ± 0.02
FCV-6	4.77 ± 0.02	4.76 ± 0.02	4.80 ± 0.02	4.72 ± 0.02	4.68 ± 0.01	4.7 ± 0.02	4.85 ± 0.02	4.82 ± 0.02
				25 °C/60% RH				
FCV-5	4.70 ± 0.02	4.74 ± 0.01	4.75 ± 0.02	4.75 ± 0.01	4.74 ± 0.02	4.74 ± 0.02	4.73 ± 0.01	4.68 ± 0.02
FCV-6	4.77 ± 0.02	4.76 ± 0.01	4.78 ± 0.02	4.78 ± 0.02	4.74 ± 0.02	4.77 ± 0.03	4.81 ± 0.03	4.81 ± 0.01
				40 °C/75% RH				
FCV-5	4.67 ± 0.01	4.62 ± 0.02	4.58 ± 0.02	4.56 ± 0.01	4.54 ± 0.02	4.58 ± 0.03	4.55 ± 0.02	4.53 ± 0.01
FCV-6	4.75 ± 0.03	4.72 ± 0.03	4.79 ± 0.03	4.75 ± 0.02	4.75 ± 0.01	4.71 ± 0.03	4.65 ± 0.06	4.68 ± 0.02

A paired sample Student t-test at a 5% level of significance was used to establish whether there was a significant difference between the initial and final pH of batches FCV-05 and FCV-06. The results are summarised in Table 4.8.

Table 4.8 *T-test results for changes in pH of batches FCV-05 and FCV-06*

Batch	**FCV-05**			**FCV-06**		
	P-Value	**Significance**		**P-value**	**Significance**	
4 °C	0.13	> 0.05	Not significant	0.08	> 0.05	Not significant
25 °C/60% RH	0.46	> 0.05	Not significant	0.17	> 0.05	Not significant
40 °C/75% RH	0.0043	< 0.05	Significant	0.029	< 0.05	Significant

The final pH of batches FCV-05 and FCV-06 was not significantly different from the initial pH following storage at 4 °C and 25 °C/60% RH. Ora-Sweet® contains a buffer system in which citric acid and sodium phosphate are used to maintain the pH at approximately 4.2 and therefore the pH was relatively constant over the 12 week study. Following storage at 40 °C/75% RH, batches FCV-05 and FCV-06 showed a significant difference between the initial and final pH values. Storage at higher temperature and humidity conditions clearly results in degradation of FCV, which could lead to the formation of degradation products subsequently causing a change in pH.

4.6.2.4 Appearance

Batches FCV-05 and FCV-06 were initially cloudy and pale orange. Storage at 4 °C revealed no change in colour, however slight crystallisation of the vehicle was observed following three (3) weeks of storage. Following storage at 25°C /60% RH and 40 °C/75% RH formulations became clear and exhibited an orange colour. Formulations stored at 40 °C/75% RH also darkened over time and the colour changed from orange to light brown at approximately five (5) weeks after manufacture. Following six (6) weeks of storage at 25 °C/60% RH, formulations started to discolour and become light brown in colour. Batch FCV-06 was manufactured using crushed Famvir® tablets and a sediment of insoluble tablet excipients, easily redispersed on shaking, was observed in each bottle.

4.6.3 Screening of Formulations

4.6.3.1 Introduction

Formulations were screened in terms of stability, viscosity, pH and appearance in order to determine the best combination of vehicle, antioxidant and preservative that would produce a formulation of FCV with maximum stability and be aesthetically pleasing. Furthermore the stable extemporaneous formulation should be easily manufactured by pharmacists and other health care providers to provide a safe and therapeutic dose to a paediatric patient.

4.6.3.2 Assay of Oral Formulation

Batches FCV-07 to FCV-24 were manufactured by incorporating FCV and different combinations of preservative(s) and antioxidant(s) as summarised in Table 4.1. The formulations were stored at 25 °C/60% RH and 40 °C/75% RH for a period of 42 days and were found to undergo different rates of degradation, as indicated in the summaries listed in Table 4.9 and 4.10.

Batches FCV-07 – FCV-12 used a combination of methylparaben and propylparaben as preservative and were analysed daily for the first seven (7) day period whereafter weekly analyses were performed. Daily analyses were undertaken to determine any initial changes in stability that may have occurred. Due to the similar degradation rates of formulations containing a combination of the parabens and those containing a single paraben, weekly analyses of batches FCV-13 - FCV-24 were deemed sufficient and appropriate.

Table 4.9 *FCV remaining in batches FCV-07 to FCV-12 (mean % ± SD) over a 42 day period*

Batch #	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 14	Day 28	Day 42
25 °C/60% RH											
FCV-07	100.00±1.20	93.48 ± 0.28	89.29 ± 0.81	87.97 ± 0.45	86.65 ± 0.24	84.33 ± 0.95	82.59 ± 0.95	80.88 ± 0.28	78.50 ± 0.68	74.51 ± 0.27	67.96 ± 1.13
FCV-08	100.00±0.38	95.26 ± 0.94	89.91 ± 0.79	88.28 ± 1.75	86.47 ± 0.47	85.79 ± 1.05	84.29 ± 0.83	84.10 ± 1.35	82.86 ± 1.27	78.32 ± 1.91	74.91 ± 0.60
FCV-09	100.00±1.65	99.68 ± 0.74	98.62 ± 0.43	99.07 ± 1.25	99.46 ± 0.62	98.01 ± 1.36	96.10 ± 0.90	95.90 ± 1.48	90.94 ± 1.19	83.75 ± 1.54	72.04 ± 0.52
FCV-10	100.00±1.57	98.17 ± 1.05	98.93 ± 0.88	98.68 ±0.41	98.50 ± 0.78	98.49 ± 0.40	96.22 ± 1.70	95.10 ± 2.52	94.46 ± 0.56	90.06 ± 1.63	89.05 ± 1.29
FCV-11	100.00±0.53	97.68 ± 0.16	97.15 ± 1.22	96.53 ± 2.19	97.55 ± 0.38	96.88 ±0.48	95.31 ± 0.71	94.72 ± 0.54	93.28 ± 0.82	90.66 ± 2.91	87.36 ± 1.35
FCV-12	100.00±1.08	100.06±0.85	100.19±1.21	99.31 ± 1.70	99.72 ± 0.24	97.65 ± 0.96	95.53 ± 0.43	95.53 ± 0.43	93.12 ± 0.99	80.90 ± 0.71	76.63 ± 1.24
40 °C/75% RH											
FCV-07	100.00±1.09	89.94 ± 0.88	82.94 ± 1.68	82.22 ± 1.34	80.65 ± 1.21	79.64 ± 1.03	78.83 ± 0.71	78.30 ± 1.06	73.28 ± 0.43	70.84 ± 0.42	64.87 ± 4.00
FCV-08	100.00±1.58	91.12 ± 1.12	88.25 ± 0.94	87.07 ± 2.13	86.15 ± 1.73	85.78 ± 1.38	83.36 ± 2.50	76.99 ± 0.36	79.11 ± 0.50	74.08 ± 0.50	68.66 ± 1.15
FCV-09	100.00±2.11	98.43 ± 1.56	96.35 ± 1.14	93.21 ± 1.08	87.67 ± 1.91	84.72 ± 0.37	79.82 ± 0.74	73.22 ± 1.53	52.91 ± 0.74	23.71 ± 1.15	13.02 ± 0.31
FCV-10	100.00±0.12	97.73 ± 2.13	97.05 ± 3.80	95.60 ± 1.28	95.95 ± 0.48	95.17 ± 2.69	95.14 ± 1.15	93.19 ± 1.87	89.64 ± 0.64	81.63 ± 1.30	78.32 ± 0.42
FCV-11	100.00±1.76	96.40 ± 1.34	95.64 ± 1.31	96.10 ± 0.25	95.83 ± 0.30	95.42 ± 1.10	92.63 ± 4.53	91.68 ± 0.81	86.71 ± 1.80	79.93 ± 1.85	75.08 ± 0.98
FCV-12	100.00±2.86	95.45 ± 0.25	95.69 ± 1.07	93.76 ± 0.91	93.94 ± 1.43	93.72 ± 0.26	89.61 ± 0.61	86.73 ± 1.02	72.50 ± 1.01	50.62 ± 0.99	38.62 ± 0.74

Table 4.10 *FCV remaining in batches FCV-13 to FCV-24 (mean % ± SD) over a period of 42 days*

Batch #	Day 0	Day 7	Day 14	Day 28	Day 42
			25 °C/60% RH		
FCV-13	100.00 ± 2.20	81.95 ± 1.29	77.73 ± 0.89	73.91 ± 0.72	66.99 ± 0.80
FCV-14	100.00 ± 1.79	88.88 ± 0.99	85.04 ± 1.65	77.04 ± 1.04	72.53 ± 0.99
FCV-15	100.00 ± 1.47	96.23 ± 0.47	94.59 ± 0.22	83.31 ± 0.16	75.43 ± 1.81
FCV-16	100.00 ± 1.15	96.85 ± 1.44	94.30 ± 0.77	88.80 ± 0.99	86.55 ± 0.07
FCV-17	100.00 ± 0.72	96.55 ± 0.93	92.19 ± 1.22	89.15 ± 0.90	86.71 ± 0.13
FCV-18	100.00 ± 2.61	94.29 ± 0.27	91.11 ± 0.89	81.48 ± 0.14	75.03 ± 0.01
FCV-19	100.00 ± 2.32	82.61 ± 0.90	79.53 ± 0.74	74.98 ± 1.60	69.76 ± 1.73
FCV-20	100.00 ± 0.71	87.62 ± 1.06	83.43 ± 1.92	79.98 ± 1.30	73.52 ± 1.42
FCV-21	100.00 ± 0.73	96.44 ± 0.07	94.39 ± 0.51	82.65 ± 0.58	72.32 ± 0.67
FCV-22	100.00 ± 3.63	94.88 ± 0.93	93.58 ± 1.61	90.28 ± 0.61	89.54 ± 2.24
FCV-23	100.00 ± 1.36	97.38 ± 0.26	95.87 ± 0.76	90.00 ± 1.02	87.93 ± 1.41
FCV-24	100.00 ± 2.62	87.26 ± 1.15	84.35 ± 1.42	77.20 ± 0.38	73.00 ± 2.16
			40 °C/75% RH		
FCV-13	100.00 ± 0.11	78.02 ± 0.92	74.75 ± 1.41	69.10 ± 0.50	63.21 ± 1.10
FCV-14	100.00 ± 2.43	81.36 ± 1.10	78.26 ± 1.39	73.21 ± 1.43	67.08 ± 1.36
FCV-15	100.00 ± 1.88	80.91 ± 1.14	53.73 ± 0.79	24.46 ± 3.20	13.59 ±0.49
FCV-16	100.00 ± 1.31	92.44 ± 1.66	89.65 ± 0.64	83.91 ± 1.82	77.44 ± 1.05
FCV-17	100.00 ± 1.47	91.81 ± 0.66	86.94 ± 0.82	80.54 ± 2.66	76.51 ± 1.02
FCV-18	100.00 ± 2.28	87.55 ± 2.09	73.71 ± 1.44	54.58 ± 0.78	39.18 ± 1.12
FCV-19	100.00 ± 1.62	77.74 ± 0.80	74.03 ± 0.82	71.37 ± 0.61	67.28 ± 1.36
FCV-20	100.00 ± 1.21	82.16 ± 1.26	80.81 ± 0.24	78.12 ± 1.24	71.49 ± 1.70
FCV-21	100.00 ± 0.93	83.29 ± 0.29	52.77 ± 0.50	24.17 ± 0.26	14.55 ± 0.37
FCV-22	100.00 ± 1.22	91.57 ± 1.80	85.13 ± 0.96	82.54 ± 1.65	79.82 ± 1.92
FCV-23	100.00 ± 2.55	92.49 ± 0.68	87.66 ± 1.59	83.21 ± 0.79	79.51 ± 1.42
FCV-24	100.00 ± 2.30	76.76 ± 1.47	64.93 ± 0.75	49.84 ± 1.51	36.43 ± 0.83

Figure 4.16 shows the degradation of FCV in batches FCV-07-FCV-12 manufactured using a different combination of vehicle and antioxidant over a period of 42 days.

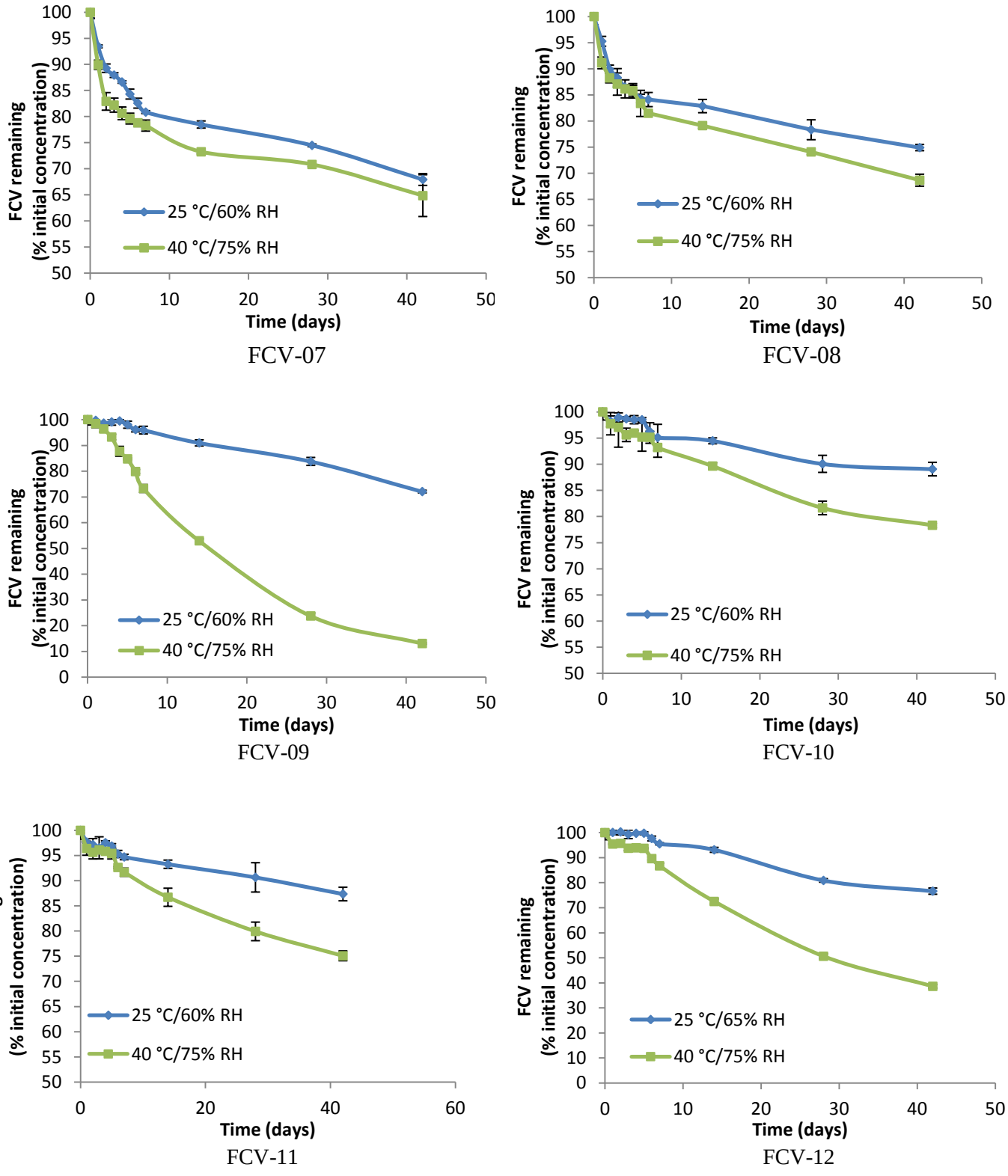


Figure 4.16 *Degradation of FCV in batches FCV-07-12 after storage at 25 °C/60% RH and 40 °C/75% RH*

Batches FCV-10, FCV-11 and FCV-12 manufactured with HPMC were found to be more stable than batches FCV-07, FCV-08 and FCV-09 in which syrup simplex was used, as discussed in §4.6.1.1.

Batches FCV-10 and FCV-11 were regarded as the most stable of the batches tested for seven (7) days and 28 days following storage at 40 °C/60% RH and 25 °C/60% RH respectively, and it is evident that approximately 90% FCV remained after those times. These two batches were preserved with a combination of methylparaben and propylparaben and included sodium metabisulphite or ascorbic acid as an antioxidant. Formulations incorporating sodium metabisulphite and ascorbic acid resulted in similar FCV degradation rates at both temperature/humidity storage conditions. However the formulations in which ascorbic acid was incorporated discoloured within a three (3) day period following storage at 40 °C/60% RH.

Batches FCV-09 and FCV-12 were manufactured using citric acid as an antioxidant. These formulations exhibited the greatest degree of FCV degradation. The significant degradation of FCV in the presence of citric acid is attributed largely to environmental pH as discussed in §4.6.3.4, *vide infra.*

The degradation of FCV in batches FCV-07 and FCV-08 is considered second-order as shown in Figure 4.17. Second-order reactions depend on the presence of a second-order reactant or two first-order reactants. It was assumed that the hydrolysis of sucrose to form glucose and fructose and the loss of FCV are first-order reactions that result in an overall second-order degradation reaction and rate that can be described by the second-order rate law (Equation 4.4) and the integrated second-order rate law (Equation 4.5) [173].

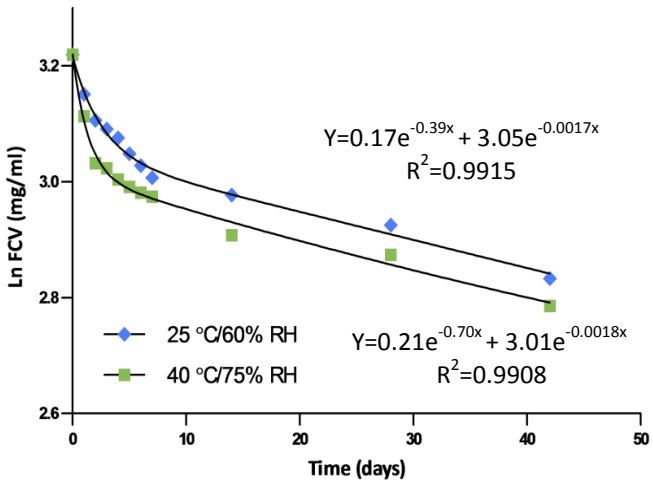
$$\frac{(d[A])}{dt} = -k[A][B] \qquad \textbf{Equation 4.4}$$

$$\ln\frac{[A]}{[B]} = \ln\frac{[A]_0}{[B]_0} + k(A_0 - B_0)t \qquad \textbf{Equation 4.5}$$

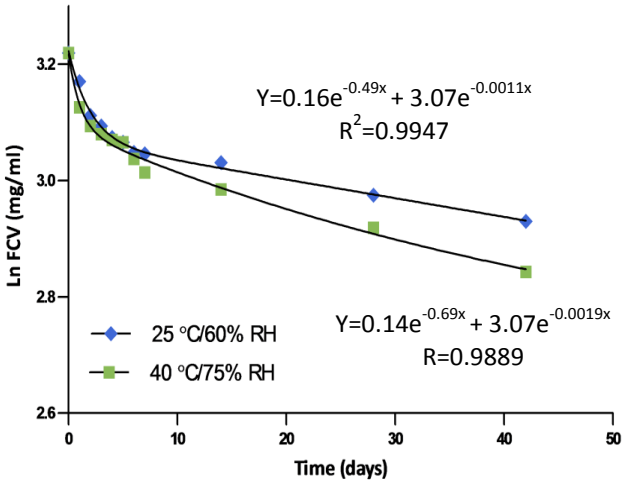
Where,

A = concentration of the first reactant
B = concentration of the second reactant
k = first-order rate constant
t = time

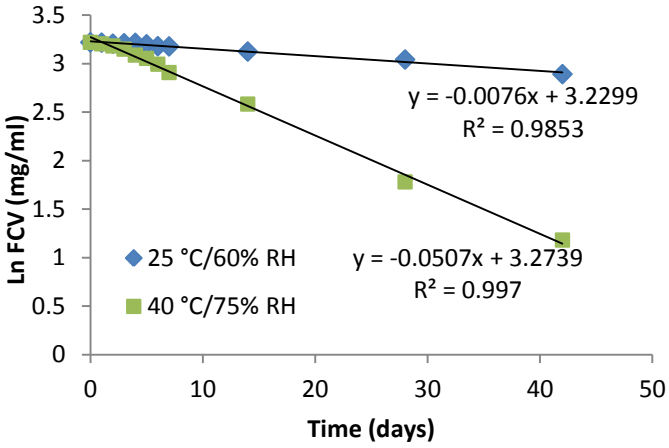
The degradation of FCV in batches FCV-09 - FCV-12 is considered first-order as described in §4.6.2.1 and is shown in Figure 4.17. FCV was most stable in batch FCV-11 with a degradation rate of 0.0028 $days^{-1}$ and 0.0058 $days^{-1}$ following storage at 25 °C/60% RH and 40 °C/75% RH, respectively. Alternatively Batch FCV-09 revealed the fastest degradation of FCV, particularly following storage at 40 °C/75% RH, with a degradation rate of -0.0507 $days^{-1}$.



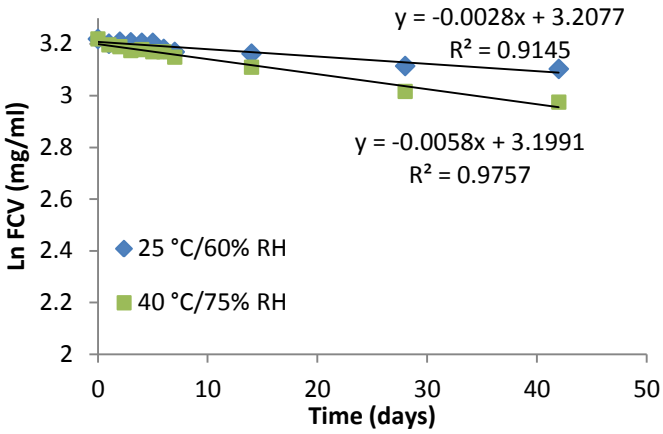
FCV-07



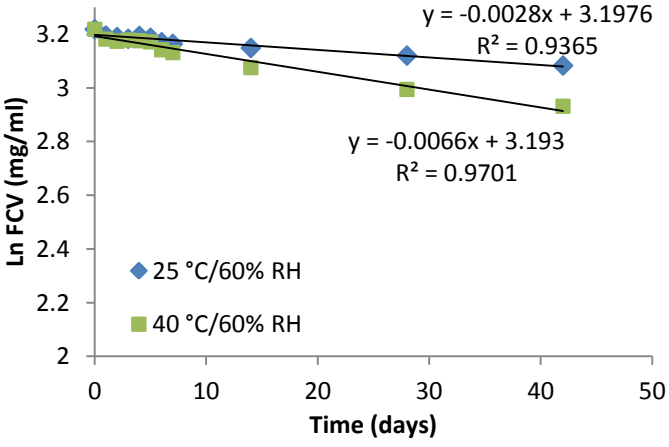
FCV-08



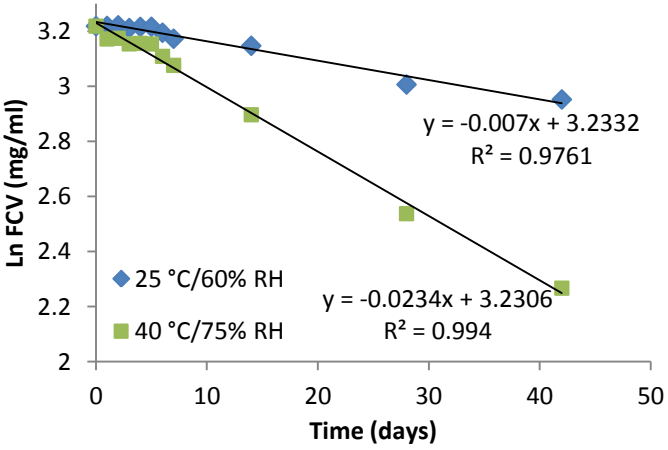
FCV-09



FCV-10



FCV-11



FCV- 12

*F**igure 4.17** Degradation rate profiles for batches FCV-07- FCV-12 following storage for a period of 42 days*

The interpretation of stability results was undertaken as described in §4.6.1.1. The upper and lower limits for the confidence intervals for batches FCV-07 – FCV-12 were calculated and are shown in Figures 4.18 – 4.23. The confidence intervals for batches FCV-13 - FCV-24 are included in Appendix I as part of the batch record summaries.

The instability of FCV in batches FCV-07 and FCV-08 following storage at 25 °C/60% RH and 40°C/75% RH was found to be significant and relevant and showed a significant and relevant decrease in FCV content within two (2) days following commencement of the study, as shown in Figures 4.18 and 4.19. Batch FCV-09 was initially more stable than batches FCV-07 and FCV-08 and a significant and relevant decrease in FCV content was observed only after five (5) and 28 days respectively, following storage at 40°C/75% RH and 25 °C/60% RH. However, the rate of degradation rapidly increases over time with an approximate change of 90% from the initial concentration following 42 days of storage at 40°C/75% RH.

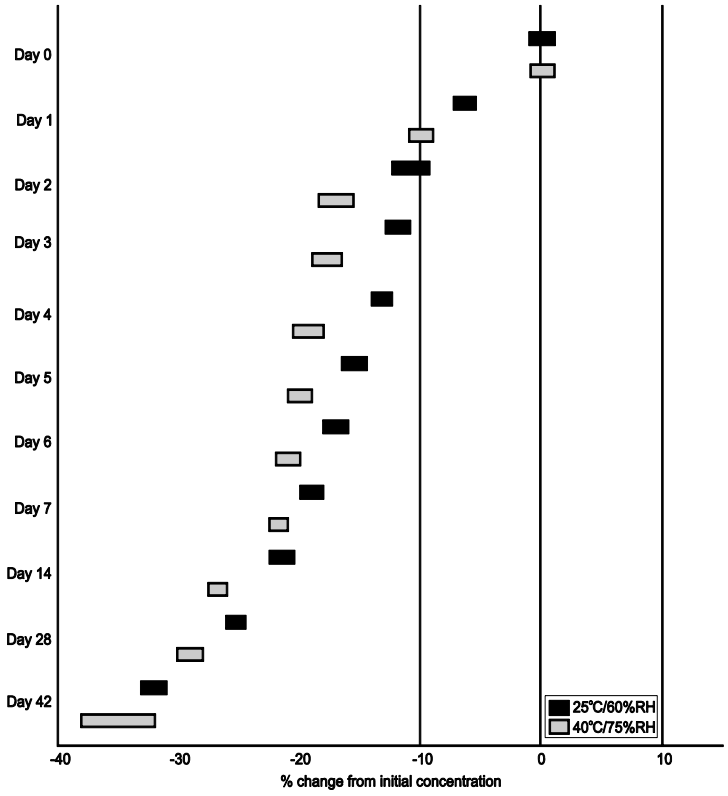


Figure 4.18 *Stability of FCV in formulations incorporating sodium metabisulphite, methylparaben and propylparaben in syrup simplex (Batch FCV-07)*

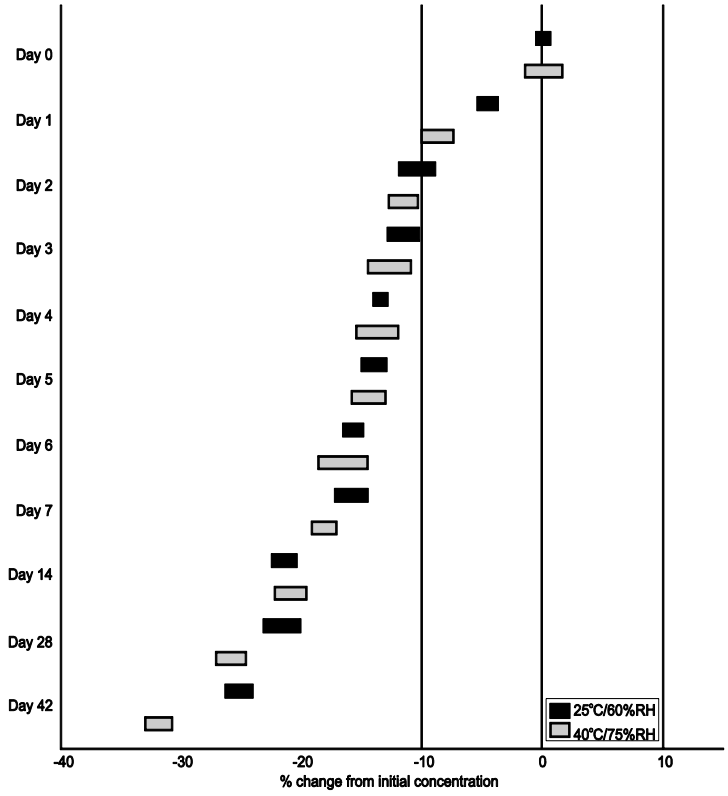


Figure 4.19 *Stability of FCV in formulations incorporating ascorbic acid, methylparaben and propylparaben in syrup simplex (Batch FCV-08)*

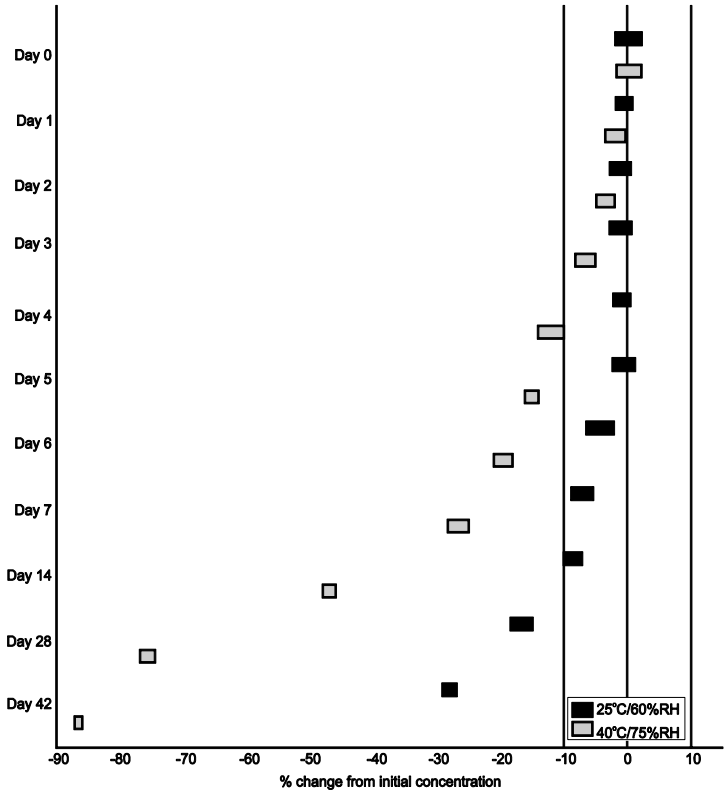


Figure 4.20 *Stability of FCV in formulations incorporating citric acid, methylparaben and propylparaben in syrup simplex (Batch FCV-09)*

In contrast, no relevant instability of FCV was observed for the first five (5) days in batch FCV-12 and the first seven (7) days in batches FCV-10 and FCV-11, as shown in Figures 4.21 – 4.23. However, within 14 days of commencing the study the observed decrease in FCV content was considered to be possibly relevant for batch FCV-10 and relevant for batches FCV-11 and FCV-12. Within 28 days following storage a significant and relevant decrease in FCV content had occurred in all batches except batch FCV-10, when a significant and possibly relevant decrease in FCV content was observed following storage at 25 °C/60% RH.

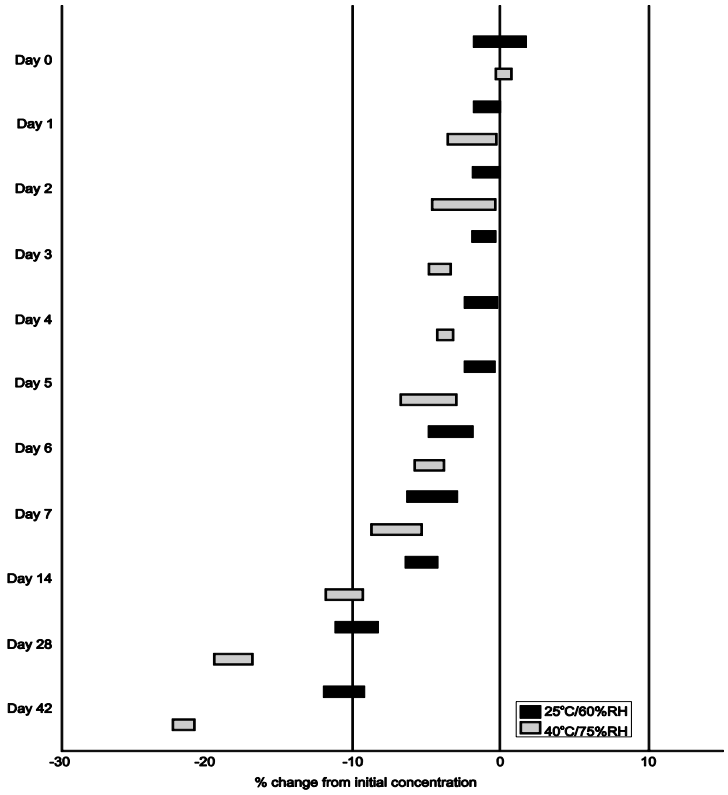


Figure 4.21 *Stability of FCV in formulations incorporating sodium metabisulphite, methylparaben and propylparaben in HPMC (Batch FCV-10)*

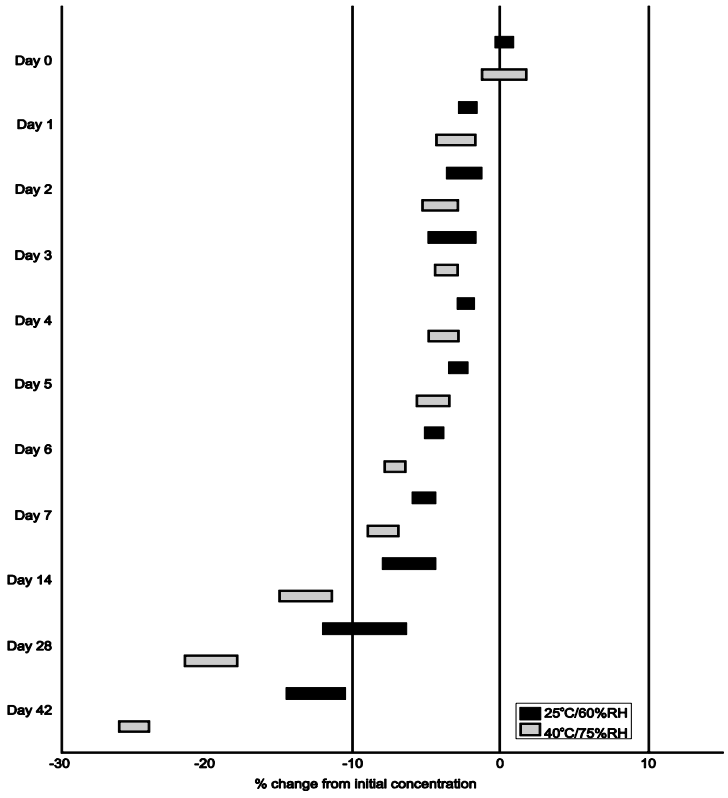


Figure 4.22 *Stability of FCV in formulations incorporating ascorbic acid, methylparaben and propylparaben in HPMC (Batch FCV-11)*

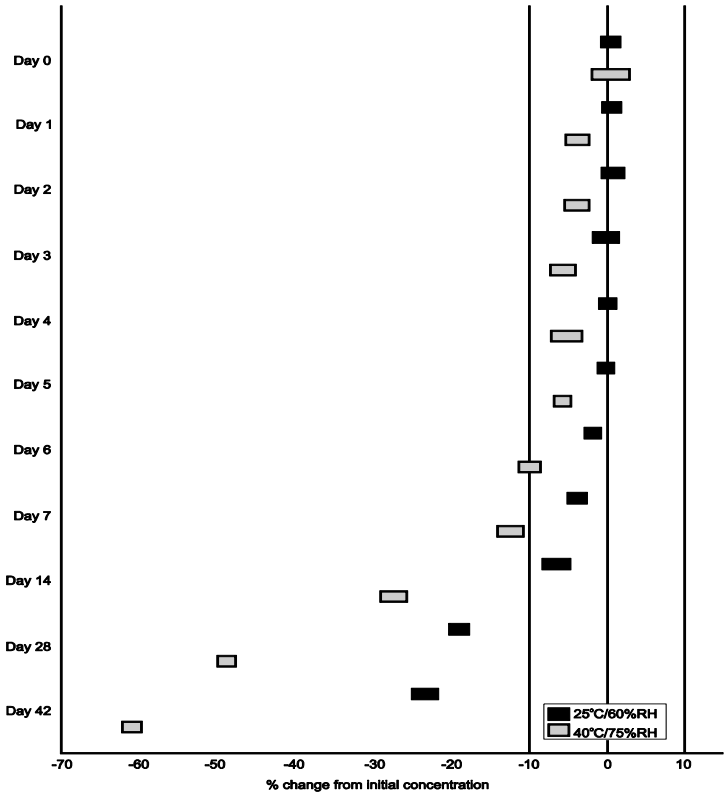


Figure 4.23 *Stability of FCV in formulations incorporating citric acid, methylparaben and propylparaben in HPMC (Batch FCV-12)*

4.6.3.3 Viscosity

Viscosity data for batches FCV-07- FCV-24 are listed in Table 4.11. It is evident that the viscosity of formulations containing HPMC was lower than the viscosities of those manufactured using syrup simplex. When stored under both test conditions *viz.*, 25 °C/65% RH and 40 °C/75% RH the viscosity of all formulations was found to be shear-thinning and a decrease in viscosity was observed over time. The viscosity of materials can be significantly affected by shear rate, shearing time, pressure and temperature. This decrease in viscosity was most noticeable for the product that was stored at 40 °C/75% RH [176]. The rapidly decreasing viscosity that is observed may be a result of instability in the structure of a system and deemed to be inappropriate by a patient.

The changes in viscosity of batches FCV-07 – FCV-12 are shown graphically in Figure 4.24. A decrease in viscosity was observed over the 42 day period for products in which syrup simplex and HPMC were used. The most significant change in viscosity was observed for batches FCV-08 and FCV-11, and is shown in Figures 4.34 and 4.37 respectively.

Table 4.11 *Viscosity of batches FCV-07-24over a 42 day period (mean cP ± SD)*

Batch #	Day 0	Day 7	Day 14	Day 28	Day 42	Day 0	Day 7	Day 14	Day 28	Day 42
	25 °C/60% RH					40 °C/75% RH				
FVC-07	487.67± 4.73	434.33± 5.27	384.00± 1.41	350.50± 2.12	351.00± 1.41	489.33± 4.93	408.33± 4.73	353.00± 5.00	331.00± 3.00	348.00± 1.41
FVC-08	527.00 ±1.00	398.00± 1.00	397.33± 6.81	395.67± 5.13	358.67± 1.52	528.00± 1.00	395.67± 4.16	393.00± 6.24	375.67± 5.03	353.67± 3.79
FVC-09	524.00 ±1.00	476.67± 2.89	440.67± 3.51	433.67± 4.04	401.33± 5.51	524.00± 1.00	422.67± 5.51	408.67± 5.69	405.33± 1.53	396.67± 6.03
FVC-10	135.00± 1.00	120.67± 1.15	109.00± 2.00	110.27± 0.46	108.13± 2.20	135.33± 0.58	114.00± 0.00	111.33± 2.81	92.13± 2.44	81.73± 2.66
FVC-11	139.33± 0.58	117.67± 1.53	107.60± 1.39	82.80 ± 1.83	67.73 ± 1.40	139.00± 1.00	87.00± 3.46	63.87± 2.34	42.00± 1.06	37.47± 0.46
FVC-12	139.33±0.58	133.67± 2.08	132.33± 0.58	129.33± 2.52	126.33± 2.52	139.00± 1.00	122.00± 1.00	118.67± 0.58	118.40± 2.43	100.67± 1.51
FVC-13	458.67± 1.15	412.67± 5.03	372.67± 2.08	346.00± 3.61	336.00± 3.46	456.00± 2.00	414.67± 3.51	366.67± 3.21	362.33± 4.73	365.67± 4.93
FVC-14	495.00± 2.65	442.33± 0.58	392.67± 1.53	352.00± 7.81	368.33± 2.08	494.33± 1.52	407.00± 1.00	395.67± 3.06	348.67± 5.77	342.33± 3.06
FVC-15	526.00± 4.36	460.67± 5.51	399.67± 3.06	368.00± 3.46	361.67± 3.79	520.00± 1.00	481.33± 6.11	443.00± 3.46	420.67± 3.06	366.67± 0.58
FVC-16	137.67± 0.58	127.00± 1.00	122.80± 7.11	114.27± 4.63	113.47± 2.05	138.33± 0.58	121.67± 0.58	112.3± 4.38	97.60± 1.70	97.60± 2.82
FVC-17	133.33± 2.31	112.87± 2.14	77.20± 4.39	64.40± 6.22	55.30± 0.92	133.00± 1.00	95.87± 7.16	61.30± 5.60	43.73± 0.99	41.73± 0.61
FVC-18	142.33± 2.08	135.67± 1.15	154.00± 1.67	154.27± 4.30	154.53± 5.43	143.00± 1.00	125.33± 1.15	134.27± 1.67	116.00± 2.43	107.07± 0.83
FVC-19	498.33± 1.53	469.67± 1.53	395.00± 2.00	325.00± 3.61	344.33± 5.50	498.00± 1.00	490.33± 1.15	435.33± 3.21	384.00± 4.58	443.33± 2.22
FVC-20	491.67± 2.08	422.00± 2.65	396.33± 3.21	314.33± 2.52	336.67± 4.53	492.00± 2.00	445.33± 8.39	449.33± 1.15	395.67± 2.08	450.00± 3.01
FVC-21	419.67± 4.51	382.33± 2.08	355.00± 4.36	337.00± 4.36	362.33± 0.84	421.33± 3.79	376.67± 0.58	350.00± 2.00	347.00± 1.73	335.33± 5.09
FVC-22	156.33± 6.51	145.67± 3.21	118.00± 2.65	115.00± 4.00	114.33± 0.19	154.67± 5.03	135.00± 5.20	103.00± 1.00	106.53± 6.60	105.30± 2.83
FVC-23	148.67± 7.77	90.13± 3.50	71.30± 4.35	52.53± 4.24	46.00± 2.83	149.67± 4.51	123.47± 3.26	66.67± 1.67	44.40± 0.80	41.20± 0.40
FVC-24	198.67± 0.58	190.00± 1.73	180.33± 3.21	177.33± 1.53	181.33± 1.39	197.67± 1.53	172.00± 3.61	155.33± 4.73	135.00± 3.61	115.33± 4.44

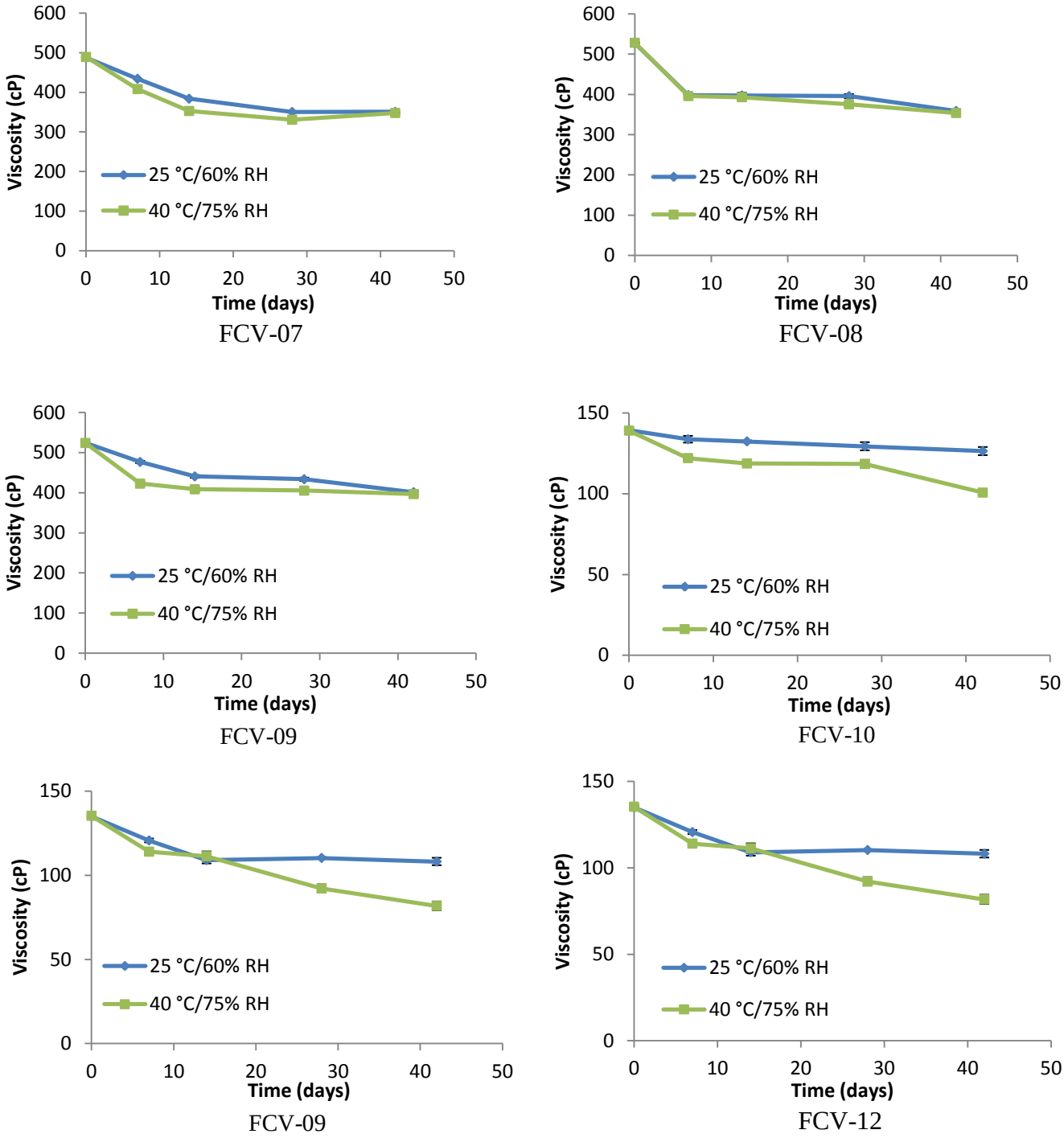


Figure 4.24 *Change in viscosity of batches FCV-07 - FCV-12*

4.6.3.4 pH

The pH of the formulations was expected to have an impact on the stability of FCV in each of the batches that were manufactured. The apparent pH for batches FCV-07 – FCV-12 are listed in Table 4.12.

Table 4.12 *pH for batches FCV-07 to FCV-12 over a period of 42 days*

Batch #	Day 0	Day 7	Day 14	Day 21	Day 28	Day 42
			25 °C/60% RH			
FVC-07	7.50 ± 0.01	6.55 ± 0.02	6.27 ± 0.01	6.05 ± 0.02	5.77 ± 0.01	5.43 ± 0.03
FVC-08	8.08 ± 0.06	6.10 ± 0.01	5.87 ± 0.00	5.76 ± 0.01	5.75 ± 0.01	5.64 ± 0.03
FVC-09	3.50 ± 0.01	3.46 ± 0.02	3.49 ± 0.03	3.45 ± 0.01	3.53 ± 0.01	3.21 ± 0.04
FVC-10	7.78 ± 0.02	6.47 ± 0.03	5.83 ± 0.01	5.63 ± 0.03	5.46 ± 0.04	5.32 ± 0.03
FVC-11	8.25 ± 0.01	6.32 ± 0.01	6.01 ± 0.02	6.01 ± 0.03	5.93 ± 0.02	5.84 ± 0.02
FVC-12	3.64 ± 0.01	3.54 ± 0.02	3.53 ± 0.02	3.56 ± 0.03	3.34 ± 0.03	3.29 ±0.01
			40 °C/75% RH			
FVC-07	7.50 ± 0.01	5.75 ± 0.02	5.39 ± 0.01	5.34 ± 0.01	5.42 ± 0.01	5.21 ± 0.01
FVC-08	8.14 ± 0.02	5.63 ± 0.00	5.36 ± 0.01	5.30 ± 0.01	5.23 ± 0.02	5.10 ± 0.01
FVC-09	3.52 ± 0.02	3.35 ± 0.01	3.10 ± 0.03	3.06 ± 0.01	3.07 ± 0.02	2.77 ± 0.05
FVC-10	7.79 ± 0.01	5.48 ± 0.01	5.11 ± 0.02	5.12 ± 0.02	5.03 ± 0.02	5.00 ± 0.02
FVC-11	8.25 ± 0.01	5.75 ± 0.02	5.47 ± 0.02	5.43 ± 0.02	5.29 ± 0.01	5.23 ± 0.02
FVC-12	3.63 ± 0.00	3.42 ± 0.01	3.22 ± 0.02	3.37 ± 0.02	3.14 ± 0.01	3.04 ± 0.01

The overall pH of each batch was largely determined by the type and amount of the excipients used to produce the formulation. A 1% w/v aqueous solution of citric acid has a pH of approximately 2.2 and would result in the pH of a final formulation being relatively acidic in comparison to when sodium metabisulphite or ascorbic acid were used, which result in the pH of a formulation falling between 3.5 and 5.0 and between 2.1 and 2.6 respectively, for 5% w/v aqueous solutions [131].

As summarised in Table 4.12, formulations in which citric acid was incorporated, *viz.* batches FCV-09 and FCV-12, were found to be the most acidic with a pH < 4. Formulations incorporating sodium metabisulphite and ascorbic acid, *viz,* batches FCV-07, FCV-08, FCV-10 and FCV-11, exhibited a pH that was neutral or slightly basic and became more acidic over the 42 day period.

Table 4.13 *T-test results for changes in pH in batches FCV-07to FCV-12*

Batch #	p-value	p-value	Summary	p-value	p-value	Summary
		25 °C/60% RH			**40 °C/75% RH**	
FCV-07	0.00004	< 0.0001	Significant	0.00001	< 0.0001	Significant
FCV-08	0.00043	< 0.0001	Significant	0.00002	< 0.0001	Significant
FCV-09	0.00138	< 0.05	Significant	0.00012	< 0.0001	Significant
FCV-10	0.00007	<0.0001	Significant	0.00002	< 0.0001	Significant
FCV-11	0.00002	<0.0001	Significant	0.00001	< 0.0001	Significant
FCV-12	0.00008	<0.0001	Significant	0.00013	< 0.0001	Significant

The final pH of the formulations was found to be different (P < 0.05) at a 5% level of significance from the initial pH. The data are summarised in Table 4.13. Changes in pH were attributed to the lack of presence of a buffer and factors such as degradation of the API, excipients and/or the vehicle. Excipients such as ascorbic acid undergo degradation in aqueous solution to form furoic acid,

tetrahydrofuran, furfural, 1-(2-furanyl)-ethanone and 1-(2-furanyl)-1-propanone, whereas citric acid and sodium metabisulphite are considered to be stable in solution [131; 147]. This could in part explain the more noticeable pH changes in formulations in which ascorbic acid has been incorporated.

A lowering of pH in sucrose solutions has been reported [173; 174] and is attributed to the generation of acidic degradation products such as formic and acetic acid [173]. Consequently any lowering of the pH may result in further conversion of sucrose into fructose and glucose, increasing the amount of reducing sugars present and possibly resulting in the degradation of FCV via the Maillard reaction [90; 174; 175].

4.6.3.5 Appearance

Batches FCV-07, FCV-13 and FCV-19 were initially clear and pale yellow. Crystallisation was observed after one (1) week, particularly in bottles of product that were stored at 25 °C/60% RH. Discolouration was observed following five (5) weeks of storage at 40 °C/75% RH. Batches FCV-08, FCV-14 and FCV-20 were initially also clear and pale yellow solutions, and crystallisation was also observed following one (1) week of storage at 25 °C/60% RH. Discolouration was observed following three (3) days of storage at 40 °C/75% RH. This was attributed to the presence of ascorbic acid in the formulation. This change in colour occurred prior to any noticeable decrease in purity of the formulation [146]. Ascorbic acid, although indicated for use as an antioxidant in aqueous pharmaceutical formulations, is relatively unstable in alkaline solutions and readily undergoes oxidation, accelerated by heat, thereby producing degradation products that cause a change in colour of the formulation [131; 146]. Batches FCV-09, FCV-15 and FCV-21 were initially clear and pale yellow and following storage for three (3) weeks a more intense yellow colour was observed. Following five (5) weeks of storage, further discolouration was observed as formulations were a darker yellow. Batches FCV-10, FCV-12, FCV-16, FCV-18, FCV-22 and FCV-24 remained clear and colourless throughout the 42 day storage period. Batches FCV-11, FCV-17 and FCV-23 were initially clear and colourless. A slight discolouration, producing a pale yellow solution, was observed after three (3) days of storage. This was most noticeable for batches stored at 40 °C/75% RH.

4.6.4 Buffered Formulations

4.6.4.1 Assay of Formulations

Degradation of FCV in an aqueous vehicle buffered to pH 6 is summarised in Table 4.14.

Table 4.14 *FCV remaining in batch FCV-25 (mean % ± SD) over a 42 day period*

Batch #	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
			25 °C/60% RH			
FCV-25	100.00 ± 1.66	99.76 ± 0.84	99.29 ± 1.40	98.91 ± 0.90	97.91 ± 0.92	97.32 ± 0.81
			40 °C/75% RH			
FCV-25	100.00 ± 1.04	99.30 ± 0.78	97.82 ± 2.33	97.07 ± 0.61	96.14 ± 1.09	94.80 ± 0.82
Batch #	**Day 6**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 42**
			25 °C/60% RH			
FCV-25	96.72 ± 0.91	95.98 ± 0.70	95.23 ± 1.69	94.36 ± 0.70	93.30 ± 1.12	90.81 ± 3.87
			40 °C/75% RH			
FCV-25	93.37 ± 0.41	92.49 ± 0.24	88.49 ± 1.52	87.11 ± 2.49	85.21 ± 1.92	80.40 ± 2.22

Formulations incorporating a buffer system exhibited lower rates and extent of degradation of FCV than when no buffer was present. Batch FCV-25 remained stable for a period of up to 42 and seven (7) days following storage at 25 °C/60% RH and 40 °C/75% RH respectively. The degradation profile of FCV when a buffer of pH 6 was used as the vehicle for the formulation is shown in Figure 4.25.

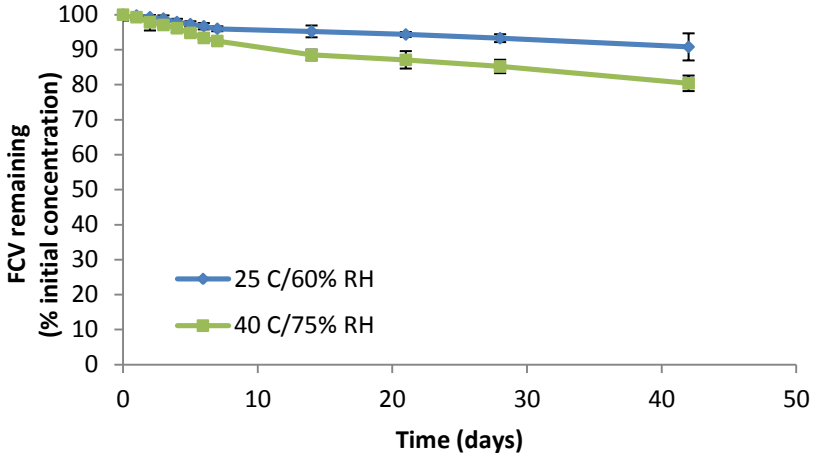


Figure 4.25 *Degradation of FCV in batch FCV-25 following storage at 25 °C/60% RH and 40 °C/75% RH*

The degradation rate profile of FCV in batch FCV-25 was plotted and the degradation rate calculated. It appears that FCV in this vehicle undergoes first-order degradation. The use of a buffered vehicle resulted in the slowest rate of degradation, with a degradation rate of 0.0022 $days^{-1}$ and 0.0051 $days^{-1}$ following storage at 25 °C/65% RH and 40 °C/75% RH respectively and is shown in Figure 4.26.

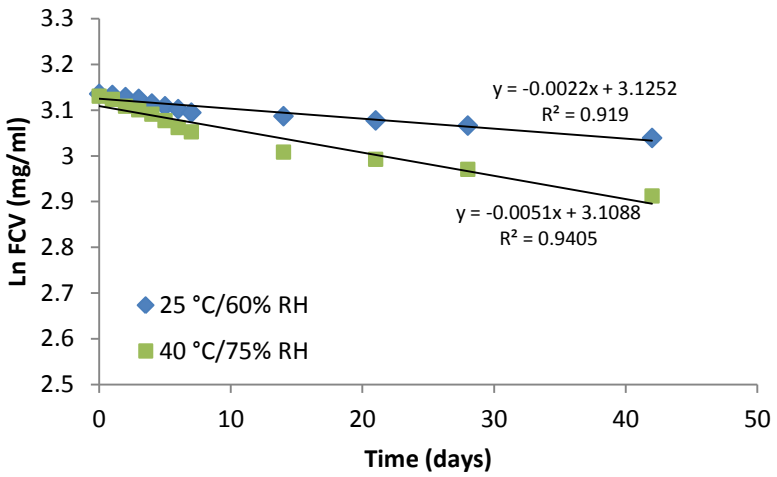


Figure 4.26 *Degradation rate profile of batch FCV-25 following storage at 25 °C/60% RH and 40 °C/75% RH*

As described in §3.5.2, FCV is most stable in an aqueous environment of pH 6. Dihydrogen orthophosphoric acid has a pK_a value of 6.8 and hence a phosphate buffer was selected to achieve and maintain a pH that would maximize the stability of FCV in solution.

The interpretation of the stability results was undertaken as previously described in §4.6.1.1 and the upper and lower limits of the confidence intervals for stability of FCV-25 were calculated. These are given in Figure 4.27.

It is evident that over a period of 28 days no significant or relevant degradation of FCV occurred in batch FCV-25, stored at 25 °C/60% RH. However on day 42 of storage a change in concentration, possibly relevant but not significant, was observed. However storage at 40 °C/75% RH revealed that FCV was unstable with a possibly relevant change observed on day 14. Relevant and significant changes were observed for longer storage periods under these conditions.

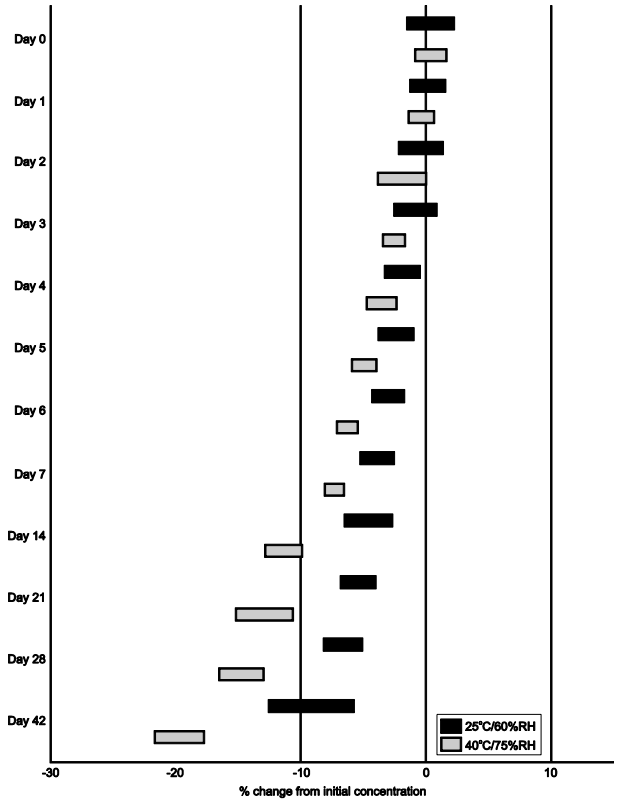


Figure 4.27 *Stability of FCV in formulations incorporating a buffer of pH 6 as the vehicle (batch FCV-25)*

4.6.4.2 Appearance

Batch FCV-25 was clear and colourless and the appearance remained unchanged over the 42 day storage period following storage at 25 °C/60% RH and 40°C/75% RH.

4.7 CONCLUSION

The lack of commercially available liquid dosage forms for paediatric use is an ongoing challenge that results in pharmacists having to prepare formulations by extemporaneous manufacture. The formulation of a stable liquid extemporaneous product is not simple due to the inherent instability of FCV in solution as the compound degrades readily to form several degradation products [23; 24]. It is a common practice to prepare extemporaneous formulations from a commercially available suspending agent by dissolving or dispersing the API in that suitable vehicle [4]. Commercially available suspending agents, also referred to as 'universal' suspending agents, are not readily available and may not always be appropriate. This was observed when Ora-Sweet® was used, and where an incompatibility between the vehicle and FCV, due to the Maillard reaction between the primary amine functional group of FCV and the presence of a reducing sugar occurred. This incompatibility reaction is likely to occur between any API that contains a primary amine functional

group and commercially available vehicles such as Ora-Sweet®. Furthermore vehicles that contain fructose, glucose or any other reducing sugar, or sugars such as sucrose that can be modified to form a reducing sugar, should also be avoided. Excipients such as lactose, found in many tablets, may also precipitate a Maillard reaction, leading to degradation of an amine-containing API [89; 90; 173; 175].

The addition and use of excipients such as citric acid and ascorbic acid, to improve adherence, stability and aesthetic appeal of a formulation, often lacks supporting and empirical data and therefore requires careful consideration prior to inclusion in a formulation. The potential physical and chemical interactions between an API and excipients that could negatively affect the stability, bioavailability and chemical nature of the API, and consequently the safety and therapeutic efficacy of the API, must be evaluated [89; 126]. Formulations manufactured from tablets may also contain excipients such as binders and disintegrants which, in addition to directly interacting with an API, may reduce the chemical stability of that API by altering the pH of a vehicle to a level at which the API is susceptible to rapid degradation [89; 161].

Therefore extemporaneously manufactured formulations of API that are stable in the solid state may be susceptible to reactions such as hydrolysis, oxidation and reduction in solution. The type and rate of reaction are commonly influenced by pH and in many cases a buffer should be included in the product [161]. The introduction of a buffer system into formulations under investigation for the production of paediatric formulation for FCV resulted in a noticeable decrease in the degradation rate of FCV.

Due to the degradation of FCV in formulations in which ascorbic and citric acid were used, as compared to those manufactured with sodium metabisulphite, this latter compound must be included in the product for optimisation. The use of methylparaben and propylparaben alone or in combination did not result in any noticeable difference in the degradation of FCV, and hence a combination of the two should be used in a formulation for optimisation as this will result in a synergistic preservative effect [131]. HPMC-based vehicles should be used as opposed to syrup simplex, and if possible FCV powder should be used to avoid possible degradation due to the presence of excipients such as lactose and magnesium stearate in the crushed tablets.

CHAPTER FIVE

THE APPLICATION OF RESPONSE SURFACE METHODOLOGY FOR THE OPTIMISATION OF FCV FORMULATIONS

5.1 INTRODUCTION

5.1.1 Quality by Design

Pharmaceutical development is aimed at designing and establishing a formulation and the associated manufacturing process that meets quality attributes that are required to achieve the therapeutic purpose and safety profile for that product. The FDA has therefore challenged the pharmaceutical industry to embrace the concept of Quality by Design (QbD) [91]. QbD is a systematic approach used in the development of pharmaceutical formulations that begins with predefined objectives to produce a high quality product from concept to final output. Emphasis is placed on the generation of knowledge of all aspects of a product, process controls and ensuring that an appropriate level of quality has been built into the product by design and one that is based on sound scientific evidence with the use of quality risk management [91; 92; 177; 178].

Pharmaceutical products and processes are complicated and multivariate in nature. An ideal QbD approach involves understanding multi-factorial relationships between formulation parameters, input variables and product quality attributes [179]. This generally requires the use of multivariate approaches, such as statistical design of experiments (DoE) to ensure the production of quality pharmaceutical products [178].

When QbD is implemented, all critical process variability must be identified, measured and understood so that they can be adequately controlled during the manufacturing process [180]. This will result in several advantages including, but are not limited to, a reduction in batch failure rates and associated operating costs, increased predictability regarding the quality and output of manufacturing, reduced raw material use, finished product inventory costs and decreased time from development to manufacture. Overall there will be a reduction in working capital requirements, cost of resources and time [181].

5.1.2 Pharmaceutical Optimisation

DOE and mathematical models have been used for the optimisation of pharmaceutical formulations over the past three decades [182-185] and are currently recommended by the ICH in the Pharmaceutical Development guideline, Q8 (R2) [177].

The design of an optimised pharmaceutical formulation encompasses multiple objectives. For decades this has been achieved through a trial and error approach in combination with previous experience and knowledge of pharmaceutical scientists. There are four (4) primary methods for the optimisation of a pharmaceutical formulation: i) one-factor-at-a-time, ii) direct optimisation, iii) non-systematic approaches and iv) the use of statistically designed experimental methods [184]. When using the traditional one-factor-at-a-time approach, one parameter is varied at a time in order to identify an optimum value for that parameter. Although a solution can be achieved using this method the realisation of a truly optimised composition is not always guaranteed. Not only is this approach uneconomical with respect to time, money and effort, but it is also often unsuccessful and unpredictable, and it cannot always be used to evaluate multiple responses or when strong interactions between input variables are likely to occur [184; 186; 187]. Direct optimisation methods are useful for establishing an experimental domain, particularly when combined with experimental design. Non-systematic approaches on the other hand are dependent on the knowledge and intuition of a formulator and involves changing several factors at a time [184]. Statistically designed experiments involve the establishment of a matrix so as to estimate coefficients using mathematical models that are then used to predict responses that are within predetermined formulation condition limits [184].

Formulation optimisation approaches that include DoE are extensively used for the development of pharmaceutical formulations. The use of a systematic approach is advantageous for a number of reasons, *viz.* fewer experiments are required to achieve an optimised formulation, problem tracing and rectification is simple and easy and potential interactions are easily detected. DoE and optimisation techniques are a cost-effective analytical tool to establish the best possible solution for a specific set of input variables [185; 186].

The use of DoE optimisation methodology involves several key elements, *viz.*, defining the study objectives, screening of possible input variables, experimental design, fitting of experimental data to mathematical model(s), mapping, generating graphic outcomes and validation using model-based response surface methodology (RSM) [186].

5.2 RESPONSE SURFACE METHODOLOGY

5.2.1 Introduction

RSM is defined as the collection of statistical and mathematical equations for the development, improvement and optimisation of pharmaceutical processes [188]. Through careful DoE and a limited number of experimental runs the objective is to optimise a response that is influenced by input

variables and to ascertain whether a relationship(s) exists between the input(s) and response(s) [189; 190].

RSM is widely used where input variables have the potential to influence the final characteristics of a formulation. The input variables, also referred to as independent variables, are subject to the control of the formulation scientist [188]. For the purpose of this study and the manufacture of FCV formulations, the input variables included HPMC concentration, pH of the final formulation and the concentrations of antioxidant and preservative used.

The performance measures or quality characteristics assessed following experimental design are called response variables [188]. The response variables for this study included assay, viscosity and pH.

5.2.1.1 Mathematical Modelling

The strategy of RSM involves the use of empirical polynomial models for the estimation of process performance, and results in the development of approximation models to predict a true response function of a specific process. Once the non-parametric models have been developed and an approximated response surface has been produced these can be used to solve an optimisation problem [189]. The polynomial equations may be first, second or third order in nature. First- or second-order models are most commonly applied to pharmaceutical systems [184].

When it is expected that the responses of interest vary only slightly across an experimental domain and when there is no interference by an interaction between factors and input variables, a first-order polynomial as shown in Equation 5.1, is usually selected for use[184]. Second-order polynomials as shown in Equations 5.2 and 5.3, are most commonly used for response surface methodology and process optimisation, which attempt to investigate up to five input factors [184].

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \varepsilon \qquad \textbf{Equation 5.1}$$

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \cdots + \varepsilon \qquad \textbf{Equation 5.2}$$

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \cdots + \varepsilon \qquad \textbf{Equation 5.3}$$

Where,

y = the estimated response
X_i = the input factors
β_0 = constant representing the intercept
β_i = coefficients of first-order terms
β_{ii} = coefficients of second-order terms
β_{ij} = coefficients of second-order interaction terms

5.2.1.2 Advantages of Response Surface Methodology (RSM)

The use of RSM is supported by regulatory agencies since it is a robust method and justifies the choice of input variables for the optimisation of a formulation. RSM generates a large amount of information from of a relatively small number of experiments, whereas other methods used are often time consuming and require a larger number of experiments to explain and classify the behaviour of a system [191; 192]. The former approach results in economic use of resources and provides a greater chance of finding optimum formulation conditions, particularly when several input factors exist. In addition, predictions can be made about future experiments that may be necessary. RSM allows a formulation scientist the flexibility to study interactions between factors, in contrast to studying one factor at a time which makes it a more rigid approach and is difficult to scale up production successfully [192].

5.2.1.3. Limitations of Response Surface Methodology (RSM)

The use of mathematical models to predict and map responses and optimise pharmaceutical formulations is restricted to instances where a relatively simple quadratic model can be applied with sufficient accuracy. Therefore the major drawback of RSM is the need to fit data to second-order polynomial models [184; 191]. Where this is not possible, the size of the domain may be reduced, a more complex or non-polynomial model may be used or alternatively the data may be converted into a form that can be explained using second-order models. For example logarithmic transformations or other linearization methods can be used. However, although such transformations may be useful it is difficult to achieve desirable results for all experimental systems. The transformation of responses or inputs is time consuming and the choice of which form of transformation is best for a specific set of data is a difficult one [191].

5.2.2 Central Composite Design (CCD)

Central Composite Design (Box-Wilson design) is most commonly used for generating response surfaces and can be used to develop second-order response models with several factors (*n) viz.*, $2 \leq n \leq 6$ [184; 193]. CCD has three (3) components. These are a full (2^n) or fractional ($2^{n-z} \geq n + 1$)

design where the factor levels are coded as either low (−1) or high (+1), axial points also referred to as "star" points on the axis of each variable at a distance of $\pm \alpha$ from the origin and a centre point which is replicated to take into account any experimental error of variance that may occur [187; 190; 193]. The simplest designs are 2^n full factorial designs where the number of experiments conducted is 2^n possible combinations of two (2) levels of n factors [184]. The total number of experiments to be performed in this type of design is generally the sum of 2^n factorial runs, $2n$ axial runs, and n centre runs. It can be established using Equation 5.4 [190].

$$N = 2^n + 2n + n_c \qquad \textbf{\textit{Equation 5.4}}$$

Where,

N= number of experimental runs
n = number of independent input variables

A diagrammatic representation of a CCD with only three factors is shown in Figure 5.1.

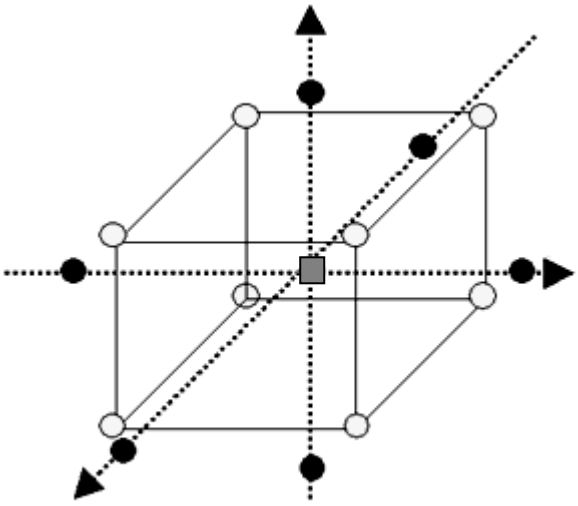


Figure 5.1 *CCD for three factors showing the factorial points (*O*), axial points (●) and centre point (■), adapted and redrawn from [184].*

As the number of factors, n, increase up to > five (5), the number of experiments to be conducted increases dramatically. To determine the main effects and their associated interactions, a fraction of the full design is often sufficient. This approach is known as a fractional factorial design [184; 190].

A 2^4 full factorial CCD comprised of four (4) input variables was selected for the optimisation of a FCV formulation. The result was that 30 experimental runs were to be conducted, comprising 16 factorial points, eight (8) axial points and six (6) centre point replicates.

5.3 MATERIALS AND METHODS

5.3.1 Materials

All the materials and equipment were the same as those listed and described in §4.5.1 and 4.5.2.

5.3.2 Experimental Design

A CCD for formulation optimisation was generated using Design-Expert® software (Version 7.0, Stat-Ease Inc., Minneapolis, USA). The input variables were concentration of HPMC (K100M) (X_1), pH to which a formulation was buffered (X_2), concentration of antioxidant (X_3) and the concentration of preservative (X_4) present. These are listed in Table 5.1. The input variables were selected for investigation at three (3) levels, *viz.*, -1, 0, +1. In addition experiments were conducted at the ± α levels.

Table 5.1. *Actual and coded values of the input variables used for CCD*

Variable	**Symbol**	**Real values of coded levels**				
		-α	**-1**	**0**	**+1**	**+α**
HPMC (% w/v)	X_1	0.25	0.5	0.75	1	1.25
Buffered pH	X_2	4	5	6	7	8
Antioxidant (% w/v)	X_3	0.01	0.03	0.05	0.07	0.08
Preservative (% w/v)	X_4	0.05	0.075	0.1	0.125	0.150

The different compositions that were manufactured and analysed in a randomised order using the CCD approach are listed in Table 5.2.

Table 5.2 *CCD for evaluating FCV formulation responses using defined input variables*

Run	Variables							
	% HPMC (K100M) (X_1)		Buffered pH (X_2)		% Sodium metabisulphite (X_3)		% Methylparaben and propylparaben* (X_4)	
	Level	%	Level	pH	Level	%	Level	%
1	0	0.75	0	6	0	0.05	0	0.100
2	-1	0.50	-1	5	+1	0.07	-1	0.075
3	-1	0.50	+1	7	+1	0.07	-1	0.075
4	+1	1.00	-1	5	-1	0.03	+1	0.125
5	-1	0.50	-1	5	-1	0.03	+1	0.125
6	+1	1.00	-1	5	+1	0.07	+1	0.125
7	+1	1.00	+1	7	+1	0.07	-1	0.075
8	+1	1.00	-1	5	-1	0.03	-1	0.075
9	+1	1.00	+1	7	-1	0.03	+1	0.125
10	0	0.75	+2	8	0	0.05	0	0.100
11	-1	0.50	+1	7	+1	0.07	+1	0.125
12	+1	1.00	-1	5	+1	0.07	-1	0.075
13	+1	1.00	+1	7	-1	0.03	-1	0.075
14	0	0.75	0	6	0	0.05	0	0.100
15	0	0.75	0	6	+2	0.09	0	0.100
16	0	0.75	0	6	0	0.05	0	0.100
17	+2	1.25	0	6	0	0.05	0	0.100
18	-1	0.50	-1	5	+1	0.07	+1	0.125
19	0	0.75	0	6	0	0.05	0	0.100
20	0	0.75	-2	4	0	0.05	0	0.100
21	-2	0.25	0	6	0	0.05	0	0.100
22	-1	0.50	-1	5	-1	0.03	-1	0.075
23	-1	0.50	+1	7	-1	0.03	-1	0.075
24	-1	0.50	+1	7	-1	0.03	+1	0.125
25	0	0.75	0	6	0	0.05	0	0.100
26	+1	1.00	+1	7	+1	0.07	+1	0.125
27	0	0.75	0	6	0	0.07	-2	0.050
28	0	0.75	0	6	-2	0.01	0	0.100
29	0	0.75	0	6	0	0.05	+2	0.150
30	0	0.75	0	6	0	0.05	0	0.100

***Methylparaben = 0.08% w/v and propylparaben = 0.02% w/v**

5.3.3 Manufacturing Procedure for FCV Formulations

The HPMC solutions were prepared by accurately weighing the correct amounts of powder using a Model PM4600 top-loading analytical balance (Mettler Instruments, Greifensee, Zurich, Switzerland) and manufactured as described in §4.5.3.1.2 to produce solutions of concentration 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50% w/v.

Batch sizes of 300 ml were prepared and decanted into 3x100 ml glass bottles for each formulation. Approximately 8.13 g FCV powder was accurately weighed using a Model PM4600 top-loading analytical balance (Mettler Instruments, Greifensee, Zurich, Switzerland) and transferred to a mortar. The appropriate amounts of sodium metabisulphite, methylparaben and propylparaben, as indicated in

Table 5.2, were accurately weighed directly into the mortar. Approximately 442.3 mg potassium dihydrogen orthophosphate was accurately weighed and added to the mortar to produce a 10 mM phosphate buffer, the lowest effective buffer molarity, when the vehicle was added. Aliquots (5ml) of the HPMC vehicle were added to the powders and mixed to form a paste. Approximately 200 ml of the vehicle was added in 20 ml aliquots, with thorough mixing between additions, to produce a concentrated formulation. The mixture was then quantitatively transferred to an A-grade volumetric flask using 10 ml aliquots of the vehicle. The formulation was then made up to volume to produce a dosage form containing 25 mg/ml FCV. The formulations were then transferred into a beaker where the pH was adjusted using a 0.5 M sodium hydroxide solution or orthophosphoric acid to achieve the desired pH. Solutions were then packaged in 100 ml clear glass bottles. The manufacturing batch record summary and results of quality control tests for all batches are included in Appendix II.

5.3.4 Storage of FCV Formulations

Three (3) 100 ml bottles of each batch were stored at 40 °C/ 75% RH. Three (3) bottles of 100 ml of formulations incorporating all four (4) variables at a coded level of -1, 0 and +1, *viz.*, run numbers 1, 22 and 26 were stored additionally at 25 °C/ 60% RH.

5.3.5 Physical Characterisation of FCV Formulations

The FCV formulations were analysed on days 0, 1, 3, 7, 14, 21, 28, 35, 42 and 56 following commencement of storage.

5.3.5.1 Assay

The FCV formulations were analysed for FCV content as described in §4.5.5.1.2.

5.3.5.2 Viscosity

The viscosity of the formulations was determined as described in §4.5.5.2. The type of spindle used to determine the viscosity of each formulation is indicated in Table 5.3.

Table 5.3 *Viscosity of formulations determined using different spindle sizes*

Spindle size	Formulations analysed
RV 2	21
RV 3	1-3, 5, 10, 11, 14-16, 18-20, 22-25, 27-30
RV 4	4, 6-9, 12-13, 26
RV 5	17

5.3.5.3 pH

The pH of each formulation was determined as described in §4.5.5.3.

5.3.5.4 Appearance

The appearance of the formulations was monitored as described in §4.5.5.4

5.4 RESULTS AND DISCUSSION

The results for the assay of each formulation, viscosity and pH are summarised in Tables 5.4 -5.6 No change in appearance was observed and the formulations remained clear over the 56 day storage period, with the exception of formulation 20, which showed a yellow discolouration after 42 days.

Table 5.4 *Assay (% ± SD) results for FCV (Y_1)*

Run	FCV content (% of initial concentration)									
	Day 0	**Day 1**	**Day 3**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 42**	**Day 56**
1	100.00 ± 3.51	99.97 ± 2.31	98.19 ± 2.63	96.02 ± 2.40	94.37 ± 1.43	92.16 ± 1.81	90.38 ± 1.36	87.28 ± 0.80	83.82 ± 1.36	81.26 ± 1.36
2	100.00 ± 0.56	99.94 ± 0.88	97.96 ± 0.09	94.58 ± 0.52	91.35 ± 1.48	88.73 ± 0.86	86.00 ± 0.30	81.14 ± 0.44	78.17 ± 0.91	73.90 ± 1.16
3	100.00 ± 0.85	99.74 ± 0.17	95.59 ± 0.88	91.46 ± 0.14	88.58 ± 0.91	85.02 ± 1.46	80.76 ± 0.85	75.08 ± 0.36	71.97 ± 0.63	65.61 ± 1.09
4	100.00 ± 1.09	100.21±1.46	97.14 ± 1.25	94.56 ± 1.06	90.66 ± 0.44	88.22 ± 0.56	84.63 ± 0.26	81.52 ± 1.13	78.07 ± 1.18	73.37 ± 0.68
5	100.00 ± 0.43	98.24 ± 0.27	95.64 ± 0.55	95.10 ± 1.30	92.70 ± 0.42	91.07 ± 0.58	86.87 ± 0.78	81.54 ± 0.43	74.91 ± 0.81	74.91 ± 0.37
6	100.00 ± 0.22	99.63 ± 0.34	97.65 ± 0.71	94.70 ± 0.50	93.34 ± 0.76	90.89 ± 0.50	86.76 ± 0.56	81.49 ± 1.08	76.68 ± 0.50	72.58 ± 1.02
7	100.00 ± 0.35	100.24±1.83	98.27 ± 1.69	92.20 ± 0.16	88.60 ± 0.92	83.63 ± 1.11	78.37 ± 2.50	73.55 ± 2.67	70.09 ± 1.33	63.17 ± 0.58
8	100.00 ± 1.27	99.93 ± 0.25	98.28 ± 0.40	94.54 ± 0.49	93.28 ± 0.85	92.89 ± 0.82	90.31 ± 0.10	85.15 ± 0.31	83.12 ± 1.23	80.05 ± 2.06
9	100.00 ± 0.43	100.22±0.43	96.51 ± 0.89	90.11 ± 0.33	86.90 ± 0.42	83.10 ± 0.48	73.84 ± 1.49	69.79 ± 1.94	67.80 ± 1.38	62.29 ± 1.26
10	100.00 ± 1.02	94.45 ± 1.92	87.40 ± 2.50	77.12 ± 2.11	65.86 ± 1.42	57.81 ± 1.55	47.36 ± 2.61	42.06 ± 2.14	35.61 ± 1.82	23.57 ± 1.02
11	100.00 ± 0.41	99.73 ± 1.11	96.69 ± 0.89	90.79 ± 0.17	88.95 ± 1.28	85.43 ± 1.13	76.62 ± 0.65	75.23 ± 0.85	71.59 ± 1.26	66.73 ± 1.43
12	100.00 ± 2.16	99.52 ± 1.34	97.53 ± 0.73	94.79 ± 1.61	93.16 ± 0.66	90.78 ± 1.00	86.83 ± 2.17	84.96 ± 0.46	81.74 ± 2.62	79.61 ± 0.42
13	100.00 ± 1.07	99.63 ± 0.43	98.24 ± 0.68	93.05 ± 1.58	88.26 ± 2.08	86.87 ± 0.96	80.02 ± 1.72	75.70 ± 2.19	68.16 ± 1.26	61.15 ± 0.51
14	100.00 ± 0.90	100.13±0.16	99.22 ± 1.24	96.64 ± 0.82	94.89 ± 0.53	92.90 ± 0.80	90.07 ± 0.47	86.59 ± 0.09	84.19 ± 0.49	82.67 ± 0.71
15	100.00 ± 0.63	99.79 ± 1.51	98.08 ± 1.43	96.32 ± 0.91	92.85 ± 0.47	91.81 ± 0.45	90.18 ± 0.45	87.49 ± 0.23	84.82 ± 1.00	82.08 ± 1.11
16	100.00 ± 1.58	100.33±0.96	97.86 ± 0.65	97.13 ± 0.34	94.86 ± 1.15	92.43 ± 2.54	90.74 ± 1.26	88.30 ± 1.61	83.89 ± 0.76	81.52 ± 1.11
17	100.00 ± 0.47	98.63 ± 0.22	98.45 ± 2.25	96.08 ± 0.99	94.34 ± 1.38	92.48 ± 2.22	92.24 ± 1.38	91.06 ± 0.87	89.26 ± 0.77	85.81 ± 0.15
18	100.00 ± 0.09	100.56±0.55	98.87 ± 1.07	93.08 ± 1.62	90.24 ± 0.23	86.65 ± 0.38	84.81 ± 1.09	79.00 ± 0.44	76.98 ± 0.77	73.17 ± 0.72
19	100.00 ± 1.37	99.62 ± 1.88	97.74 ± 2.47	96.74 ± 3.03	94.65 ± 2.83	92.37 ± 2.26	90.53 ± 1.64	86.91 ± 2.63	84.37 ± 1.39	81.17 ± 1.25
20	100.00 ± 0.62	100.14±0.83	95.12 ± 0.69	90.10 ± 0.06	84.70 ± 2.05	79.97 ± 0.84	72.15 ± 0.77	63.73 ± 0.56	59.00 ± 0.89	51.00 ± 0.85
21	100.00 ± 0.68	100.00±0.84	98.51 ± 1.70	96.62 ± 0.77	94.97 ± 0.76	94.46 ± 0.88	90.13 ± 0.65	86.72 ± 0.70	83.33 ± 0.70	80.80 ± 1.06
22	100.00 ± 0.72	99.24 ± 2.87	98.64 ± 2.46	95.60 ± 2.24	93.37 ± 2.38	88.75 ± 2.23	83.96 ± 1.71	81.35 ± 0.84	77.16 ± 1.13	74.84 ± 1.52
23	100.00 ± 0.61	97.67 ± 0.84	96.29 ± 2.55	92.08 ± 0.51	89.20 ± 2.56	83.45 ± 0.46	76.68 ± 0.45	71.88 ± 0.32	69.24 ± 3.35	60.86 ± 2.57
24	100.00 ± 0.67	97.65 ± 0.37	95.03 ± 0.94	92.30 ± 0.56	89.16 ± 0.76	85.85 ± 0.35	77.61 ± 1.66	73.15 ± 0.54	69.90 ± 2.07	61.00 ± 2.09
25	100.00 ± 1.30	99.99 ± 1.21	97.28 ± 1.47	96.39 ± 0.22	94.50 ± 2.87	93.58 ± 3.23	90.27 ± 1.66	88.27 ± 0.95	85.35 ± 1.81	83.19 ± 2.89
26	100.00 ± 0.95	98.77 ± 0.28	96.39 ± 1.49	90.44 ± 0.39	87.12 ± 0.63	83.89 ± 0.84	79.76 ± 0.76	72.49 ± 0.61	69.78 ± 2.86	62.57 ± 2.75
27	100.00 ± 0.96	99.83 ± 0.80	98.94 ± 2.98	97.45 ± 0.54	93.16 ± 0.67	91.20 ± 0.66	90.80 ± 0.33	88.56 ± 0.12	85.50 ± 1.71	82.14 ± 0.29
28	100.00 ± 2.30	100.07±1.21	99.06 ± 1.01	96.21 ± 0.38	94.31 ± 0.33	93.05 ± 0.61	91.60 ± 0.54	89.41 ± 2.20	85.80 ± 0.52	83.11 ± 1.91
29	100.00 ± 0.59	99.25 ± 0.34	99.07 ± 0.91	96.27 ± 0.67	93.31 ± 0.68	91.28 ± 0.44	89.83 ± 0.25	87.65 ± 0.79	84.71 ± 1.43	80.64 ± 0.62
30	100.00 ± 1.07	99.07 ± 0.73	98.91 ± 0.50	96.30 ± 0.55	92.91 ± 1.48	91.60 ± 2.09	90.31 ± 0.48	88.85 ± 1.17	86.04 ± 0.81	81.52 ± 1.11

Table 5.5 *Viscosity (cP ± SD) of FCV formulations (Y_2)*

Run	Viscosity (cP)									
	Day 0	**Day 1**	**Day 3**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 42**	**Day 56**
1	634.33 ± 0.58	566.00 ± 5.20	492.67 ± 2.31	480.67 ± 5.13	402.00 ± 3.00	379.33 ± 9.02	334.00 ± 7.21	282.00 ± 2.65	277.00 ± 6.56	271.67 ± 6.03
2	199.67 ± 2.52	164.00 ± 6.00	152.67 ± 5.03	135.33 ± 4.73	128.00 ± 3.00	117.33 ± 3.06	105.47 ± 1.22	103.67 ±2.08	93.67 ± 2.08	91.00 ± 1.00
3	203.33 ± 1.53	165.67 ± 3.06	149.33 ± 5.03	136.33 ± 2.08	128.33 ± 2.52	119.00 ± 5.57	112.93 ± 2.05	107.33 ± 2.52	94.33 ± 3.21	92.33 ± 3.21
4	1151.33 ± 6.43	1097.33 ± 23.69	953.33 ± 6.43	801.33 ± 4.16	738.00 ± 8.00	703.67 ± 6.66	660.67 ± 9.02	595.33 ± 5.03	584.00 ± 8.00	558.67 ± 7.57
5	199.00 ± 2.65	177.00 ± 2.65	158.00 ± 6.56	125.33 ± 3.51	119.00 ± 4.58	111.67 ± 3.79	105.33 ± 4.16	103.67 ± 3.21	103.27 ± 2.87	96.33 ± 2.08
6	1144.67 ± 8.08	1130.00 ± 12.49	1006.00 ± 22.72	876.00 ± 24.25	794.67 ± 5.03	731.67 ± 3.51	669.33 ± 18.15	596.67 ± 6.11	582.00 ± 9.17	576.00 ± 5.29
7	1171.33 ± 12.86	1060.67 ± 16.04	1021.33 ± 15.80	964.67 ± 22.91	902.00 ± 16.37	739.33 ± 29.48	687.33 ± 3.06	666.00 ± 11.14	658.00 ± 15.87	648.67 ± 14.57
8	1205.33 ± 6.11	1037.33 ± 15.14	1018.67 ± 8.08	901.67 ± 7.63	822.00 ± 24.57	703.33 ± 4.16	667.33 ± 3.06	659.33 ± 9.02	612.67 ± 8.32	604.33 ± 6.66
9	1209.33 ± 4.62	1060.00 ± 10.58	1006.00 ± 25.06	970.67 ± 10.07	970.00 ± 5.29	818.00 ± 8.72	698.00 ± 13.86	688.00 ± 19.29	655.33 ± 6.43	639.67 ± 13.80
10	607.33 ± 2.08	520.33 ± 4.93	490.67 ± 5.51	480.33 ± 6.51	419.67 ± 1.53	402.00 ± 10.44	406.33 ± 10.69	379.67 ± 4.51	372.33 ± 10.79	355.00 ± 2.00
11	202.33 ± 6.81	167.67 ± 4.04	161.00 ± 2.00	149.67 ± 2.08	145.33 ± 3.79	119.00 ± 2.65	108.67 ± 2.52	104.33 ± 4.51	102.33 ± 2.52	96.00 ± 2.65
12	1194.00 ± 9.165	1042.67 ± 22.03	1014.00 ± 19.08	876.00 ± 15.10	820.67 ± 8.32	724.33 ± 8.14	680.67 ± 14.47	656.67 ± 12.06	622.00 ± 14.00	604.67 ± 6.43
13	1467.33 ± 23.69	1208.67 ± 9.02	1076.67 ± 15.14	1028.67 ± 3.06	876.00 ± 19.29	927.33 ± 7.02	879.33 ± 10.07	787.33 ± 8.33	726.00 ± 6.00	694.67 ± 4.16
14	709.67 ± 3.21	551.00 ± 1.011	494.67 ± 9.87	473.67 ± 4.04	391.33 ± 14.57	349.00 ± 6.56	347.67 ± 8.74	336.67 ± 10.60	299.33 ± 4.16	277.00 ± 6.56
15	704.00 ± 3.61	553.33 ± 10.50	521.00 ± 10.54	483.67 ± 4.51	399.00 ± 3.61	351.67 ± 4.73	338.00 ± 7.21	335.67 ± 4.04	296.67 ± 8.32	288.67 ± 4.04
16	709.67 ± 2.08	539.33 ± 2.08	498.33 ± 8.62	466.67 ± 4.16	401.67 ± 6.51	349.33 ± 4.04	342.33 ± 5.51	336.00 ± 6.08	294.67 ± 6.11	281.00 ± 9.64
17	2881.33± 26.63	2368.00± 32.00	2245.3 ± 42.39	2013.3 ± 38.02	1518.67 ± 11.72	1432.00 ± 16.00	1324.00± 24.33	1312.00±10.58	1287.33±9.45	1230.67±9.45
18	233.33 ± 2.89	190.00 ± 8.00	178.67 ± 5.03	150.67 ± 4.04	131.67 ± 3.51	128.00 ± 2.00	128.00 ± 4.58	121.00 ± 2.65	112.67 ± 2.52	100.67 ± 3.06
19	643.33 ± 9.71	531.33 ± 3.21	518.00 ± 7.00	449.00 ± 8.54	393.00 ± 13.08	351.00 ± 7.94	329.67 ± 4.73	320.67 ± 5.03	303.00 ± 5.57	290.67 ± 3.06
20	653.67 ± 3.21	535.67 ± 4.04	519.33 ± 7.02	491.67 ± 6.81	466.67 ± 7.57	448.33 ± 9.61	461.67 ± 5.86	427.33 ± 8.02	392.00 ± 4.00	370.67 ± 3.06
21	58.93 ± 1.01	53.07 ± 0.61	51.33 ± 1.29	47.33 ± 0.83	40.80 ± 1.06	40.27 ± 1.51	40.80 ± 1.05	40.13 ± 0.23	39.33 ± 1.15	38.00 ± 2.00
22	197.00 ± 6.56	166.33 ± 5.51	156.33 ± 5.51	131.33 ± 8.50	115.33 ± 4.16	108.67 ± 4.51	101.67 ± 2.08	99.00 ± 3.61	96.33 ± 1.53	92.00 ± 2.00
23	197.33 ± 5.51	167.67 ± 7.51	160.67 ± 3.06	141.33 ± 2.52	124.00 ± 6.56	120.67 ± 1.53	109.33 ± 4.16	105.33 ± 3.05	97.33 ± 1.15	92.67 ± 2.31
24	194.00 ± 4.00	180.67 ± 4.04	175.67 ± 2.89	142.33 ± 5.51	126.33 ± 4.04	118.67 ± 1.53	109.00 ± 1.00	110.33 7.77	102.00 ± 2.00	98.00 ± 2.00
25	682.33 ± 4.93	546.67 ± 6.66	515.67 ± 5.13	478.00 ± 17.44	403.67 ± 4.16	344.33 ± 4.04	333.33 ± 2.52	323.67 ± 3.21	303.00 ± 3.00	292.67 ± 3.06
26	1454.00 ± 5.29	1201.33 ± 4.16	1142.00 ± 13.86	1056.67±21.57	966.00 ± 8.72	876.00 ± 3.46	814.67 ± 7.02	784.00 ± 8.72	732.00 ± 5.29	701.33 ± 3.06
27	687.00 ± 2.65	552.33 ± 3.06	508.00 ± 9.85	479.33 ± 15.14	402.67 ± 3.21	340.67 ± 5.13	330.67 ± 3.06	323.33 ± 3.06	300.67 ± 3.06	291.33 ± 2.31
28	684.00 ± 4.58	551.00 ± 5.67	509.00 ± 1.73	478.33 ± 13.50	428.33 ± 13.61	418.67 ± 17.47	405.33 ± 7.37	388.00 ± 4.00	357.00 ± 2.65	327.67 ± 2.52
29	689.00 ± 3.61	558.33 ± 1.53	511.33 ± 2.52	469.00 ± 17.52	400.00 ± 11.79	360.33 ± 21.46	334.33 ± 10.41	322.00 ± 5.29	336.00 ± 53.78	294.00 ± 4.00
30	684.67 ± 4.04	550.67 ± 3.79	504.33 ± 7.64	475.33 ± 17.62	401.00 ± 5.57	341.33 ± 3.21	320.67 ± 7.02	318.67 ± 7.02	303.33 ± 4.16	290.00 ± 2.00

Table 5.6 *pH (mean± SD) of FCV formulations (Y_3)*

Run	pH									
	Day 0	**Day 1**	**Day 3**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 42**	**Day 56**
1	6.02 ±0.01	5.99 ±0.05	5.86 ± 0.03	5.77 ± 0.02	5.75 ± 0.04	5.73 ± 0.02	5.73 ± 0.01	5.74 ± 0.03	5.74 ± 0.03	5.74 ± 0.02
2	4.98 ± 0.01	5.02 ± 0.05	4.88 ± 0.02	4.85 ± 0.04	4.84 ± 0.01	4.82 ± 0.01	4.80 ± 0.01	4.82 ± 0.02	4.76 ± 0.02	4.76 ± 0.02
3	7.00 ± 0.00	6.96 ± 0.02	6.85 ± 0.03	6.80 ± 0.04	6.75 ± 0.03	6.73 ± 0.03	6.71 ± 0.02	6.70 ± 0.01	6.71 ± 0.01	6.69 ± 0.01
4	5.03 ± 0.01	4.93 ± 0.02	4.89 ± 0.02	4.85 ± 0.02	4.86 ± 0.01	4.86 ± 0.02	4.86 ± 0.02	4.85 ± 0.03	4.81 ± 0.03	4.80 ± 0.02
5	4.96 ± 0.06	4.93 ± 0.02	4.92 ± 0.04	4.92 ± 0.01	4.88 ± 0.03	4.88 ± 0.03	4.87 ± 0.03	4.86 ± 0.03	4.84 ± 0.04	4.82 ± 0.03
6	4.98 ± 0.01	4.95 ± 0.03	4.89 ± 0.02	4.89 ± 0.03	4.81 ± 0.03	4.77 ± 0.03	4.78 ± 0.02	4.78 ± 0.02	4.77 ± 0.01	4.77 ± 0.02
7	6.99 ± 0.01	6.84 ± 0.01	6.74 ± 0.07	6.74 ± 0.07	6.74 ± 0.00	6.74 ± 0.06	6.78 ± 0.05	6.78 ± 0.01	6.77 ± 0.05	6.76 ± 0.02
8	5.05 ± 0.01	4.87 ± 0.03	4.86 ± 0.06	4.89 ± 0.03	4.89 ± 0.03	4.82 ± 0.03	4.80 ± 0.03	4.73 ± 0.01	4.73 ± 0.03	4.75 ± 0.03
9	6.95 ± 0.02	6.84 ± 0.04	6.84 ± 0.02	6.80 ± 0.04	6.80 ± 0.03	6.79 ± 0.01	6.80 ± 0.04	6.78 ± 0.04	6.79 ± 0.02	6.76 ± 0.03
10	7.95 ± 0.00	7.92 ± 0.02	7.95 ± 0.02	7.84 ± 0.05	7.73 ± 0.05	7.74 ± 0.05	7.67 ± 0.07	7.70 ± 0.07	7.63 ± 0.03	7.62 ± 0.02
11	7.04 ± 0.04	7.03 ± 0.02	6.73 ± 0.02	6.71 ± 0.04	6.75 ± 0.01	6.72 ± 0.02	6.74 ± 0.04	6.75 ± 0.04	6.73 ± 0.03	6.72 ± 0.02
12	5.02 ± 0.08	4.92 ± 0.03	4.87 ± 0.06	4.83 ± 0.04	4.84 ± 0.04	4.84 ± 0.04	4.84 ± 0.02	4.85 ± 0.01	4.77 ± 0.03	4.74 ± 0.04
13	6.98 ± 0.01	6.94 ± 0.03	6.73 ± 0.02	6.72 ± 0.04	6.75 ± 0.04	6.76 ± 0.06	6.76 ± 0.03	6.76 ± 0.03	6.78 ± 0.03	6.72 ± 0.02
14	6.09 ± 0.00	5.85 ± 0.01	5.80 ± 0.07	5.82 ± 0.03	5.85 ± 0.04	5.74 ± 0.02	5.76 ± 0.04	5.76 ± 0.03	5.73 ± 0.01	5.71 ± 0.01
15	6.06 ± 0.01	5.84 ± 0.01	5.87 ± 0.01	5.84 ± 0.04	5.81 ± 0.05	5.76 ± 0.06	5.78 ± 0.01	5.76 ± 0.02	5.76 ± 0.02	5.74 ± 0.01
16	6.03 ± 0.01	5.88 ± 0.01	5.86 ± 0.03	5.83 ± 0.04	5.79 ± 0.03	5.79 ± 0.03	5.78 ± 0.02	5.75 ± 0.02	5.75 ± 0.02	5.76 ± 0.03
17	6.08 ± 0.01	5.97 ± 0.03	5.93 ± 0.07	5.84 ± 0.04	5.71 ± 0.01	5.76 ± 0.05	5.76 ± 0.03	5.74 ± 0.02	5.76 ± 0.02	5.74 ± 0.01
18	5.00 ± 0.02	4.89 ± 0.01	4.74 ± 0.01	4.75 ± 0.02	4.73 ± 0.02	4.76 ± 0.04	4.79 ± 0.01	4.75 ± 0.03	4.78 ± 0.01	4.72 ± 0.02
19	6.07 ± 0.04	5.89 ± 0.03	5.86 ± 0.01	5.87 ± 0.03	5.89 ± 0.06	5.85 ± 0.02	5.85 ± 0.01	5.82 ± 0.02	5.78 ± 0.01	5.75 ± 0.03
20	4.07 ± 0.03	3.92 ± 0.03	3.93 ± 0.03	3.75 ± 0.03	3.78 ± 0.05	3.77 ± 0.05	3.76 ± 0.02	3.74 ± 0.03	3.75 ± 0.01	3.75 ± 0.01
21	6.04 ± 0.04	5.88 ± 0.02	5.77 ± 0.02	5.76 ± 0.03	5.79 ± 0.05	5.79 ± 0.03	5.81 ± 0.04	5.76 ± 0.03	5.77 ± 0.01	5.76 ± 0.03
22	5.02 ± 0.02	4.92 ± 0.02	4.87 ± 0.01	4.75 ± 0.02	4.78 ± 0.01	4.79 ± 0.05	4.78 ± 0.01	4.76 ± 0.05	4.76 ± 0.02	4.74 ± 0.02
23	7.00 ± 0.02	6.82 ± 0.02	6.78 ± 0.04	6.78 ± 0.03	6.72 ± 0.02	6.78 ± 0.04	6.72 ± 0.04	6.74 ± 0.03	6.70 ± 0.02	6.67 ± 0.02
24	7.04 ± 0.00	6.87 ± 0.02	6.74 ± 0.01	6.73 ± 0.03	6.77 ± 0.03	6.80 ± 0.02	6.81 ± 0.04	6.77 ± 0.03	6.73 ± 0.01	6.69 ± 0.01
25	6.06 ± 0.02	5.86 ± 0.02	5.83 ± 0.04	5.86 ± 0.03	5.85 ± 0.05	5.82 ± 0.02	5.82 ± 0.03	5.82 ± 0.02	5.81 ± 0.02	5.78 ± 0.02
26	7.02 ± 0.04	6.82 ± 0.02	6.79 ± 0.03	6.82 ± 0.05	6.80 ± 0.02	6.78 ± 0.03	6.80 ± 0.02	6.79 ± 0.02	6.78 ± 0.01	6.76 ± 0.02
27	5.98 ± 0.06	5.86 ± 0.03	5.84 ± 0.02	5.85 ± 0.04	5.84 ± 0.02	5.79 ± 0.01	5.81 ± 0.06	5.78 ± 0.02	5.78 ±0.03	5.76 ± 0.03
28	6.07 ± 0.03	5.98 ± 0.02	5.83 ± 0.02	5.86 ± 0.05	5.84 ± 0.04	5.77 ± 0.02	5.78 ± 0.05	5.80 ± 0.02	5.77 ± 0.04	5.75 ± 0.02
29	6.02 ± 0.02	5.88 ± 0.02	5.84 ± 0.05	5.87 ± 0.04	5.82 ± 0.04	5.78 ± 0.02	5.79 ± 0.02	5.78 ± 0.04	5.75 ± 0.03	5.74 ± 0.02
30	6.09 ± 0.01	5.97 ± 0.03	5.87 ± 0.06	5.86 ± 0.02	5.81 ± 0.01	5.81 ± 0.03	5.83 ± 0.02	5.79 ± 0.01	5.79 ± 0.03	5.78 ± 0.02

The degradation of FCV in formulations in which all variables represented the centre points of the CCD at a coded level of 0, *viz.*, runs 1, 14, 16, 19, 25 and 30 representing excipient levels for *HPMC*, sodium metabisulphite, methylparaben and propylparaben of 0.75% w/v, 0.05% w/v and 0.1% w/v respectively and buffered to pH = 6 and were monitored over an eight (8) week period. The degradation profiles are shown in Figure 5.2. It is clear that FCV undergoes faster and greater degradation when stored at 40 °C/75% RH, with approximately 81.26% remaining at the end of the test period. Although degradation of FCV was observed when samples were stored at 25 °C/60% RH, the formulations were still considered acceptable as > 90% FCV remained after an eight (8) week period.

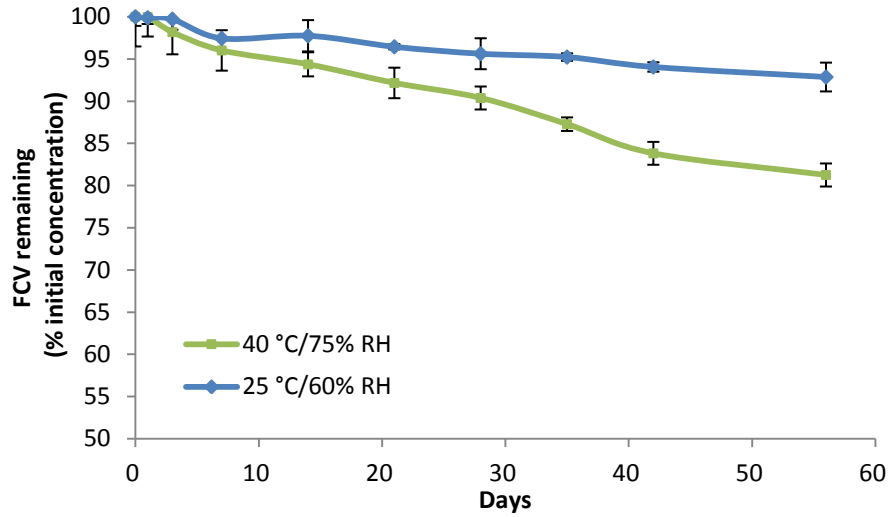


Figure 5.2 *Degradation of FCV in formulations where variables were used at a coded level of 0 (Run 1) following storage at 40 °C/75% RH and 25 °C/60% RH for an 8 week period*

The degradation rate profiles shown in Figure 5.3 reveal that FCV degrades by first-order kinetics. A plot of the natural logarithm of concentration of FCV versus time resulted in a straight line, with degradation rate constants of 0.0038.days^{-1} and 0.0013.days^{-1} following storage at 40 °C/75% RH and 25 °C/60% RH, respectively. These degradation rates are much slower than those determined for formulations manufactured with the variables at coded levels of +1 and -1, and that are shown in Figures 5.5 and 5.7 respectively.

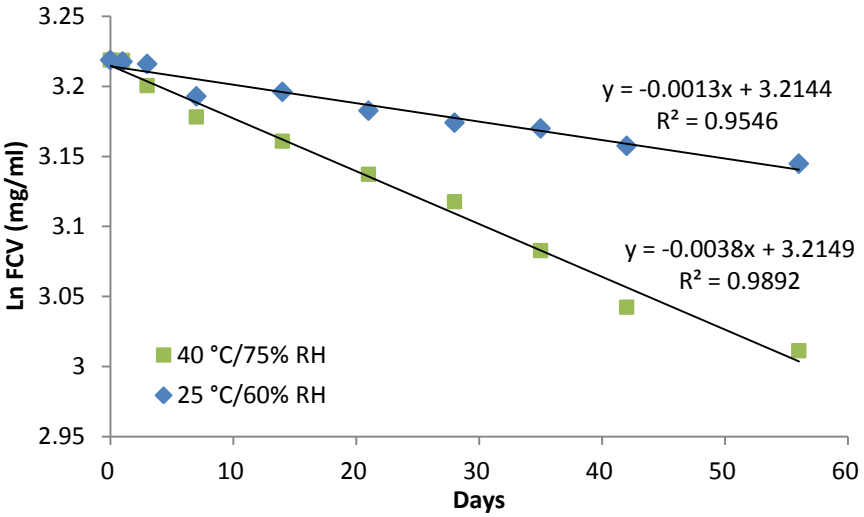


Figure 5.3 *Degradation rate of FCV in formulations where variables were used at a coded level of 0 (Run 1) following storage at 40 °C/75% RH and 25 °C/60% RH for an 8 week period*

The degradation of FCV in a formulation in which all variables represented a coded level of -1 *viz.*, run 22 representing excipient levels for HPMC, sodium metabisulphite, methylparaben and propylparaben of 0.50% w/v, 0.025% w/v and 0.075% w/v, respectively and buffered to a pH = 5 was monitored over a period of eight (8) weeks. The degradation profiles are shown in Figure 5.4. FCV undergoes fast, significant and relevant degradation when stored at 40 °C/75% RH and 25 °C/65% RH with approximately 74.84% and 90.03 % remaining, respectively at the end of the test period.

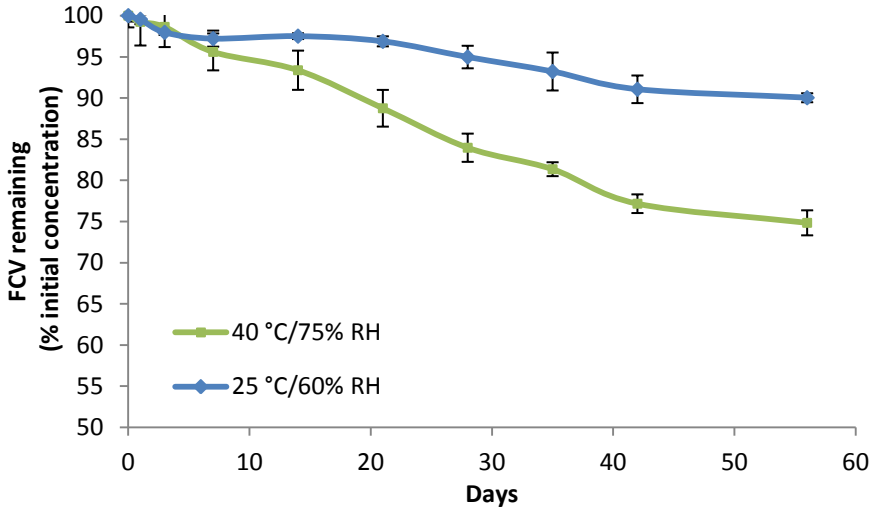


Figure 5.4 *Degradation of FCV in formulations where variables were used at a coded level of -1 (Run 22) following storage at 40 °C/75% RH and 25 °C/60% RH for an 8 week period*

The degradation rate profiles for FCV formulations that incorporated all variables at a coded level of

-1 is shown in Figure 5.5. FCV degraded faster than formulations in which the variables were used at coded levels of 0, with degradation rates of 0.0056.days^{-1} and 0.0018.days^{-1} resulting following storage at 40 °C/75% RH and 25 °C/60% RH, respectively.

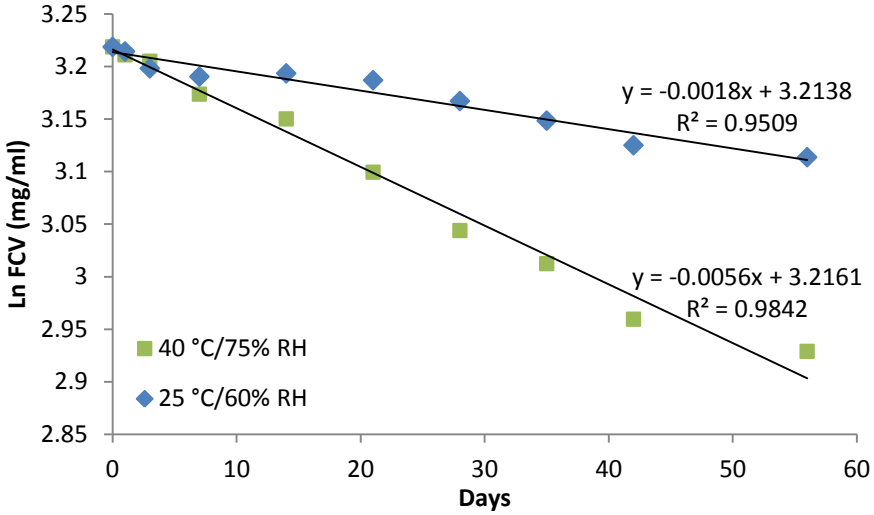


Figure 5.5 *Degradation rate of FCV in formulations where variables were used at a coded level of -1 (Run 22) following storage at 40 °C/75% RH and 25 °C/60% RH for an 8 week period*

The degradation of FCV in a formulation in which all variables represented a coded level of +1 *viz.*, run 26, representing excipient levels for HPMC, sodium metabisulphite, methylparaben and propylparaben of 1.00% w/v, 0.075% w/v and 0.125% w/v, respectively and buffered to a pH = 7 was monitored over a period of eight (8) weeks. The degradation profiles are shown in Figure 5.6. It is clear that FCV undergoes degradation rapidly following storage at 40 °C/75% RH and 25 °C/60% RH, with approximately 62.57 % and 86.88 % remaining respectively at the end of the test period.

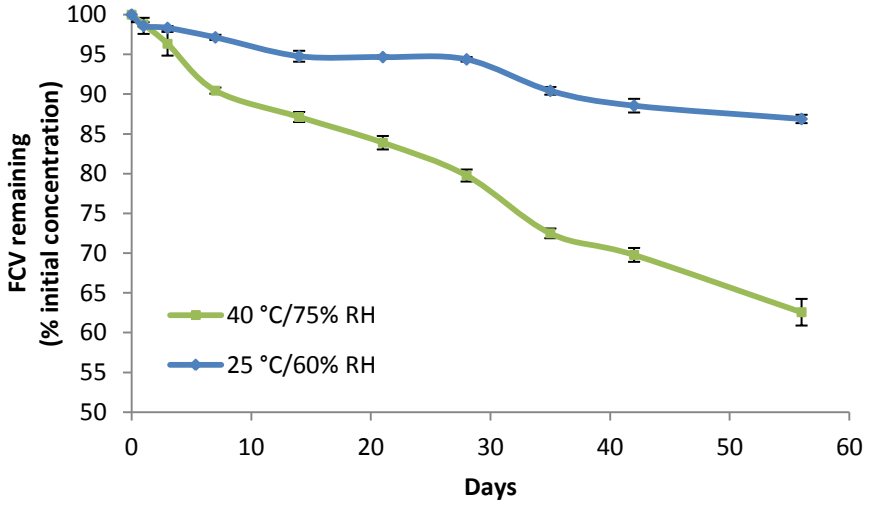


Figure 5.6 *Degradation of FCV in formulations where variables were used at a coded level of +1 (Run 26) following storage at 40 °C/75% RH and 25 °C/60% RH for an 8 week period*

The degradation rate profile for the FCV formulation that incorporated all variables at a coded level of +1 is shown in Figure 5.7. FCV degraded faster than the other formulations, with a rate of 0.0082.days^{-1} and 0.0024.days^{-1} following storage at 40 °C/75% RH and 25 °C/60% RH, respectively.

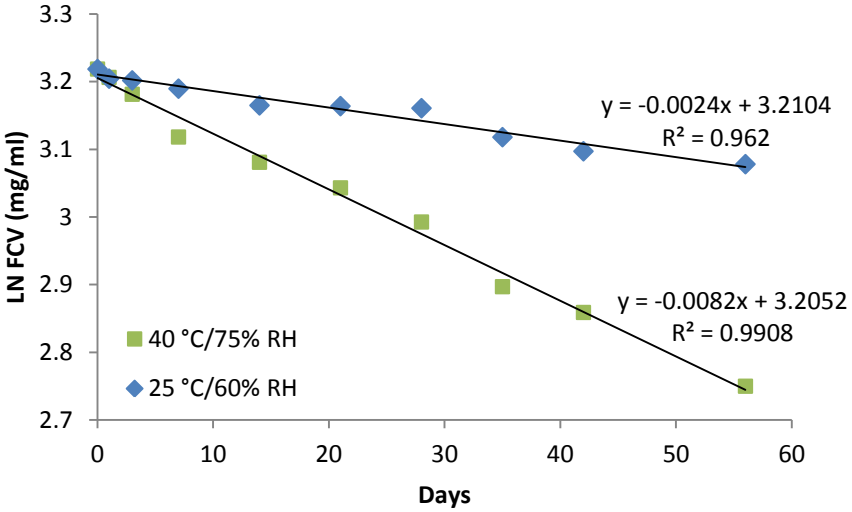


Figure 5.7 *Degradation rate of FCV in formulations where variables were used at a coded level of +1 (Run 26) following storage at 40 °C/75% RH and 25 °C/60% RH for an 8 week period*

5.5 RESPONSE SURFACE PLOT ANALYSIS

Design-Expert® software (Version 7.0, Stat-Ease Inc., Minneapolis, USA) was used to analyse the data by fitting to linear, two factorial, quadratic and cuboidal models. The best fit model that showed a correlation between an input variable and a resultant response was described by a quadratic polynomial equation. Subsequently an Analysis of Variance (ANOVA) conducted at a 0.05 level of significance, and Regression Coefficients (R^2) analysis were used to evaluate the data. Response models were used to depict the relationship(s) between an input variable and a response. Normal probability plots of studentized residuals were constructed to evaluate the goodness of fit of the proposed model and to determine whether the assumption of normality of errors was appropriate. Contour and 3-dimensional (3D) response surface plots for each response were used to depict the data graphically.

5.5.1 Quadratic Polynomial Equations and Regression Coefficients

The final empirical models in terms of coded factors for % drug content (Y_1), viscosity (Y_2) and pH (Y_3) are shown in Equations 5.5 -5.7. These models were based on the results obtained following storage of the formulations at 40 °C/75% RH for 28 days, and in which approximately 90% FCV remained in the most stable formulation. Statistical analysis of the results obtained following storage at 40 °C/75% RH for 0, 1, 3, 7, 14, 21, 42 and 56 days are reported in Appendix III.

% drug content (Y_1) = $90.37 + 0.47X_1 - 4.85X_2 + 0.14X_3 - 0.59X_4 + 0.0451X_1^2 - 7.81X_2^2 - 0.018X_3^2 - 0.17X_4^2 - 0.41X_1X_2 + 6.250E\text{-}003X_1X_3 - 0.56X_1X_4 - 0.56X_2X_3 - 0.26X_2X_4 + 0.26X_3X_4$ ***Equation 5.5***

Viscosity (Y_2) = $335.45 + 310.14X_1 + 9.87X_2 - 6.59X_3 - 1.79X_4 + 80.58X_1^2 + 15.15X_2^2 + 2.90X_3^2 - 6.89X_4^2 + 25.12X_1X_2 - 5.19X_1X_3 - 5.85X_1X_4 - 7.53X_2X_3 - 4.17X_2X_4 + 19.93X_3X_4$ ***Equation 5.6***

pH (Y_3) = $5.78 - 0.017X_1 + 0.97X_2 - 8.33E\text{-}003X_3 + 8.333E\text{-}003X_4 + 6.250E\text{-}003X_1^2 - 6.250E\text{-}003X_2^2 + 6.250E\text{-}003X_3^2 + 6.250E\text{-}003X_4^2 + 0.013X_1X_2 + 0.013X_1X_3 - 0.012X_1X_4 - 0.000X_2X_3 + 0.000X_2X_4 - 0.025X_3X_4$ ***Equation 5.7***

The quadratic polynomial model contains regression parameters that include terms for the main effects, coefficients for quadratic main effects and coefficients for two factor interactive effects i.e. those that show the manner in which the response varies when two factors are changed simultaneously. Second degree terms are included in order to investigate any non-linearity in the data [194]. A positive (+) sign in front of a term indicates a synergistic effect and a negative (-) sign indicates an antagonistic effect on the response. The value of the coefficient is indicative of the magnitude of the effect [194].

Linear regression is used to calculate the coefficients of the model that are plotted as a cumulative distribution of a normal plot [184]. A linear plot supports the adequacy of the model. The presence of deviations may indicate that the model is inappropriate and that transformation of the data may be required, or that errors exist within the data set [195].

The quadratic model was regarded as appropriate, based on the regression coefficient and standard deviation values as summarised in Table 5.7.

Table 5.7 *Summary of statistical data for the appropriate response surface models*

	Quadratic Response Surface Model							
	SD*	**F-value**	**Prob>F**	**R^{2*}**	**Adj R^{2*}**	**Pred R^{2*}**	**Adeq Prec***	**C.V (%)***
Y_1	2.11	37.70	<0.0001	0.9724	0.9466	0.8413	28.229	2.51
Y_2	51.27	68.50	<0.0001	0.9846	0.9702	0.9121	34.22	12.54
Y_3	0.037	1203.14	<0.0001	0.9991	0.9983	0.9965	149.755	0.63

***SD= Standard deviation, R^2 = Regression coefficient, Adj R^2 = Adjusted R^2, Pred R^2 = Predicted R^2, Adeq Prec = Adequate precision, C.V (%) = coefficient of variation**

The results from the statistical summary shown in Table 5.7 reveal that the R^2 values for the three (3) models were > 0.9, indicating that a good correlation exists between the experimental and predicted responses, with only 2.76, 1.54, and 0.90% not able to be explained by the model. The predicted R^2 values are in reasonable agreement with the adjusted R^2 values, thereby confirming the reliability of the model [194]. In addition, high values *viz.*, > 4 for adequate precision indicate an adequate signal while the relatively low coefficient of variation values indicate better precision and reliability of the experiments that were performed [194]. The F-value indicates the significance of the regression coefficient while the P-value signifies the pattern of interaction between the factors. The high F-

values and low probability P-values shown in Table 5.7 indicate the significance of the three (3) models with more significant corresponding coefficient terms producing a greater F-value and low P-value, respectively [196; 197].

5.5.2 Analysis of Variance and Normal Plots of Residuals

ANOVA was used to estimate the effects of the main input variables and their interactive effects on each response *viz.*, % drug content, viscosity and pH. Normal plots of residuals were constructed to determine the normality of the data. Graphical analysis permits visualisation of the data and in this case the points fall fairly close to the straight line, confirming the data are normally distributed [197; 198]. Studentized residuals were used to overcome differences in variance of the residuals at the different input variable values. A summary of the ANOVA of the response surface quadratic models at a 0.05 level of significance for the three (3) responses is given in Tables 5.8 - 5.10 respectively. Normal plots of residuals are shown in Figures 5.8 -5.10.

5.3.3.1 Analysis of Variance and Normal Plots of Residuals for % Drug Content

An F- value of 37.70 was observed when ANOVA was used for the response surface quadratic model for % drug content. This indicates that the model was significant and this was confirmed by the Prob> F value of < 0.0001. The data shown in Table 5.8 revealed that the significant factors that affected response Y_1 were the antagonistic effects of the linear contribution of X_2 and the quadratic contribution of X_2 as the corresponding value of Prob> F< 0.05. The terms X_1X_3, X_4, X_1^2, X_3^2, X_4^2, X_1X_2 , X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4 were shown to have an insignificant effect on the response, with a value of Prob> F of > 0.1.

Table 5.8 *ANOVA of the response surface quadratic model for % drug content*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob>F
Model	2340.69	14	167.19	37.70	< 0.0001
X_1	5.32	1	5.32	1.20	0.2906
X_2	563.57	1	563.57	127.09	< 0.0001
X_3	0.45	1	0.45	0.10	0.7535
X_4	8.28	1	8.28	1.87	0.1918
$X1^2$	2.64	1	2.64	0.60	0.9128
X_2^2	6.250E-004	1	6.250E-004	1.409E-004	< 0.0001
X_3^2	4.95	1	4.95	1.12	0.9655
X_4^2	4.95	1	4.95	1.12	0.6825
X_1X_2	1.05	1	1.05	0.24	0.4523
X_1X_3	1.05	1	1.05	0.24	0.9907
X_1X_4	0.055	1	0.055	0.012	0.3074
X_2X_3	1670.98	1	1670.98	376.82	0.3074
X_2X_4	8.601E-003	1	8.601E-003	1.940E-003	0.6335
X_3X_4	0.77	1	0.77	0.17	0.6335
Residual	66.52	15	4.43	-	-

Values of "Prob>F" less than 0.0500 indicate model terms are significant.

The normal probability plot of residuals revealed the data to be normally distributed, as seen by the relatively linear distribution of the data points in Figure 5.8. Both the maximum and minimum values for % drug content fall approximately on the straight line.

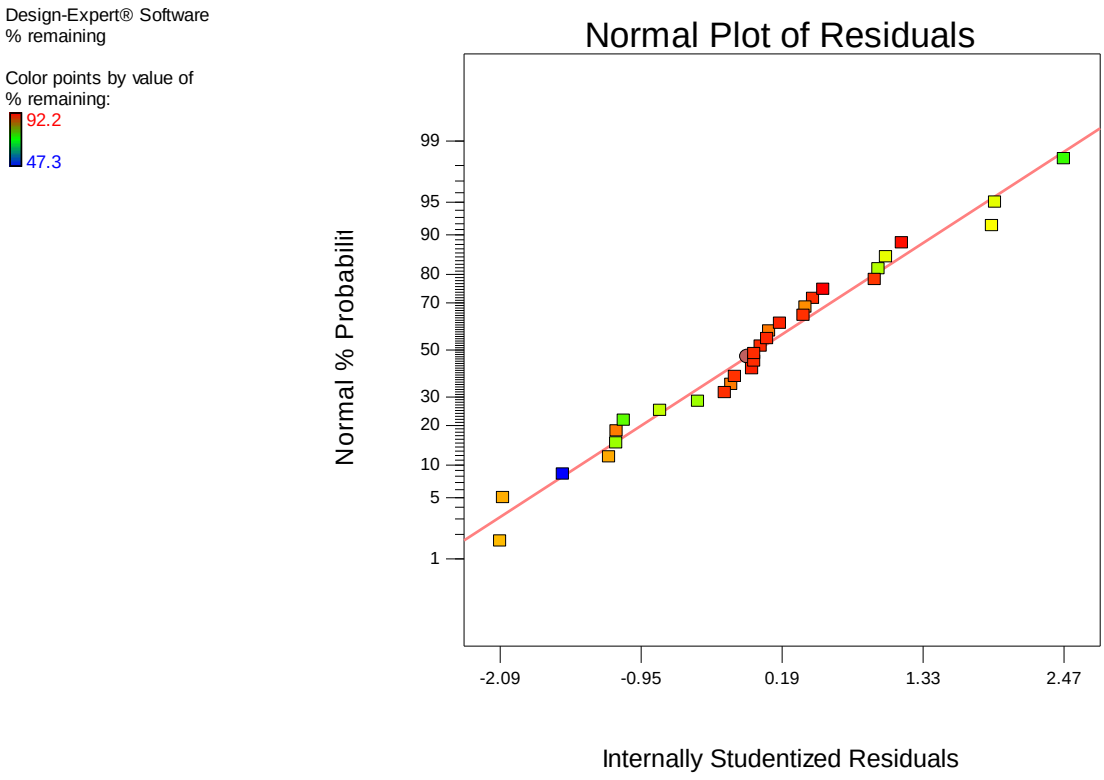


Figure 5.8 *Normal probability plot of studentized residuals for % drug content*

5.3.3.2 Analysis of Variance and Normal Plots of Residuals for Viscosity

An F-value of 68.50 was observed for the ANOVA of the response surface quadratic model for viscosity that also indicates the model was significant and was confirmed by a value of Prob> F of < 0.0001. The data shown in Table 5.9 revealed the significant factors that affect Y_2 were the synergistic effects of the linear contribution and quadratic contribution of X_1 as the corresponding value of Prob> F was < 0.05. The terms X_2, X_3, X_4, X_2^2, X_3^2, X_4^2, X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4 were shown to have an insignificant effect on the response, with a resultant value of Prob> F of > 0.1.

Table 5.9 *ANOVA of the response surface quadratic model for viscosity*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob>F
Model	2.521E+006	14	1.800E+005	68.50	< 0.0001
$\mathbf{X_1}$	2.308E+006	1	2.308E+006	878.37	< 0.0001
$\mathbf{X_2}$	2336.23	1	2336.23	0.89	0.3607
$\mathbf{X_3}$	1043.46	1	1043.46	0.40	0.5381
$\mathbf{X_4}$	77.29	1	77.29	0.029	0.8661
$\mathbf{X1^2}$	1.781E+005	1	1.781E+005	67.77	< 0.0001
$\mathbf{X_2^2}$	6293.66	1	6293.66	2.39	0.1426
$\mathbf{X_3^2}$	230.13	1	230.13	0.088	0.7714
$\mathbf{X_4^2}$	1303.86	1	1303.86	0.50	0.4920
$\mathbf{X_1X_2}$	10093.72	1	10093.72	3.84	0.0689
$\mathbf{X_1X_3}$	431.29	1	431.29	0.16	0.6911
$\mathbf{X_1X_4}$	547.44	1	547.44	0.21	0.6546
$\mathbf{X_2X_3}$	907.97	1	907.97	0.35	0.5654
$\mathbf{X_2X_4}$	278.64	1	278.64	0.11	0.7492
$\mathbf{X_3X_4}$	6357.27	1	6357.27	2.42	0.1407
Residual	39421.65	15	2628.11	-	-

Values of "Prob>F" less than 0.0500 indicate model terms are significant.

The normal probability plot of residuals reveals that the data are normally distributed as a relatively linear distribution was observed. This is shown by Figure 5.9, with both the maximum and minimum values for viscosity falling on or about a straight line.

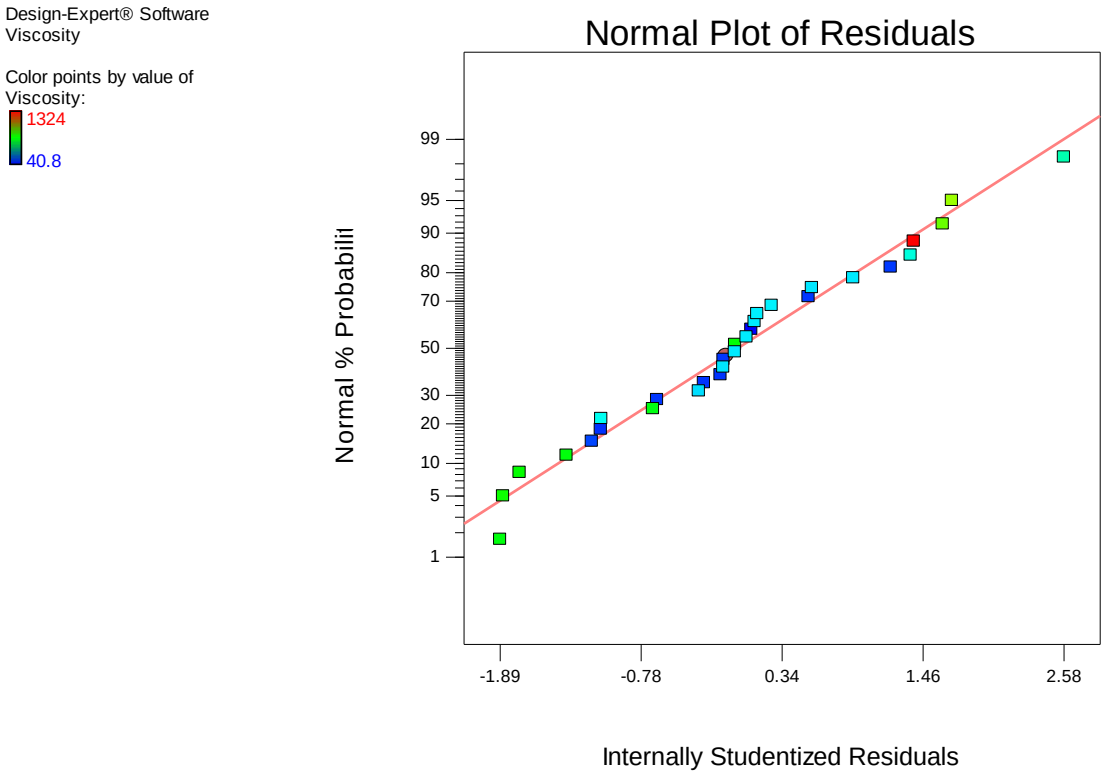


Figure 5.9 *Normal probability plot of studentized residuals for viscosity*

5.3.3.3 Analysis of Variance and Normal Plots of Residuals for pH

An F-value of 1203.14 was observed when ANOVA was used to evaluate the response surface quadratic model for pH, indicating that the model was significant. This was confirmed by the value of Prob> F of < 0.0001.The data shown in Table 5.10 reveal that the significant factors that affect Y_3 were the linear contributions of X_1 and X_2 with antagonistic and synergistic effects respectively, and the interactive effects of X_3X_4, as the corresponding value of Prob> F was < 0.05. The term X_2 had the most significant effect on the response as indicated by a value of Prob> F of < 0.0001. The terms X_3, X_4, X_2^2, X_3^2, X_4^2, X_1X_2 , X_1X_3, X_1X_4, X_2X_3 and X_2X_4 were shown to have an insignificant effect on the response, with a resultant value of Prob> F of > 0.1.

Table 5.10 *ANOVA of the response surface quadratic model for pH*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob>F
Model	22.46	14	1.60	1203.14	< 0.0001
X_1	6.667E-003	1	6.667E-003	5.00	0.0410
X_2	22.43	1	22.43	16820.00	< 0.0001
X_3	1.667E-003	1	1.667E-003	1.25	0.2811
X_4	1.667E-003	1	1.667E-003	1.25	0.2811
$X1^2$	1.071E-003	1	1.071E-003	0.80	0.3842
X_2^2	1.071E-003	1	1.071E-003	0.80	0.3842
X_3^2	1.071E-003	1	1.071E-003	0.80	0.3842
X_4^2	1.071E-003	1	1.071E-003	0.80	0.3842
X_1X_2	2.500E-003	1	2.500E-003	1.88	0.1911
X_1X_3	2.500E-003	1	2.500E-003	1.88	0.1911
X_1X_4	2.500E-003	1	2.500E-003	1.88	0.1911
X_2X_3	0.000	1	0.000	0.000	1.0000
X_2X_4	0.000	1	0.000	0.000	1.0000
X_3X_4	0.010	1	0.010	7.50	0.0152
Residual	0.020	15	1.333E-003	-	-

Values of "Prob>F" less than 0.0500 indicate model terms are significant.

The normal probability plot of residuals shows that the data are normally distributed with a relatively linear distribution as shown by Figure 5.10. Both the maximum and minimum values for pH fall approximately on a straight line.

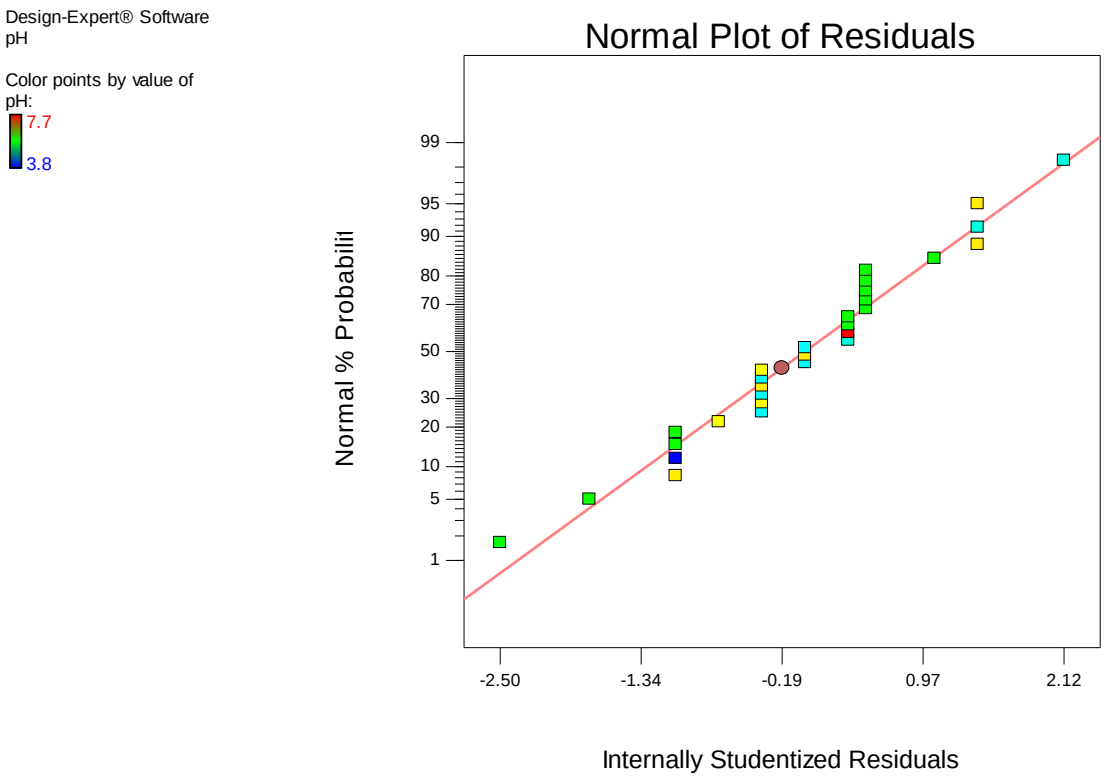


Figure 5.10 *Normal probability plot of studentized residuals for pH*

5.5.3 Contour and Response Surface Plots

To gain insight into the relationship between each variable and the associated responses, and to produce graphic representation, contour (2D) and response (3D) surface plots were generated based on the relevant response surface quadratic model.

5.5.3.1 % Drug Content

The % drug content (Y_1) in each formulation was most significantly affected by the effect of pH to which a formulation had been buffered (X_2), as suggested by a high F-value of 127.09 and low P-value of < 0.0001. The other input variables, *viz.* % w/v HPMC (X_1), antioxidant (X_3) and preservative (X_4) did not have a significant effect on FCV content, as suggested by the F-values of 1.20, 0.10 and 0.187 respectively and Prob> F values of > 0.05.

As shown in the contour plot in Figure 5.11 and the response surface plot in Figure 5.12, FCV was most stable when the formulations were buffered to pH 6. A maximum of 92.2% FCV remained, following storage of the formulations suggesting that FCV is most stable at this pH. In formulations buffered to more acidic or basic pH than 6 the degradation of FCV was evident. This finding suggests that FCV undergoes acid- and base- catalysed degradation [23; 24]. It is clear from the surface plots that degradation of FCV is more pronounced at pH > 7.

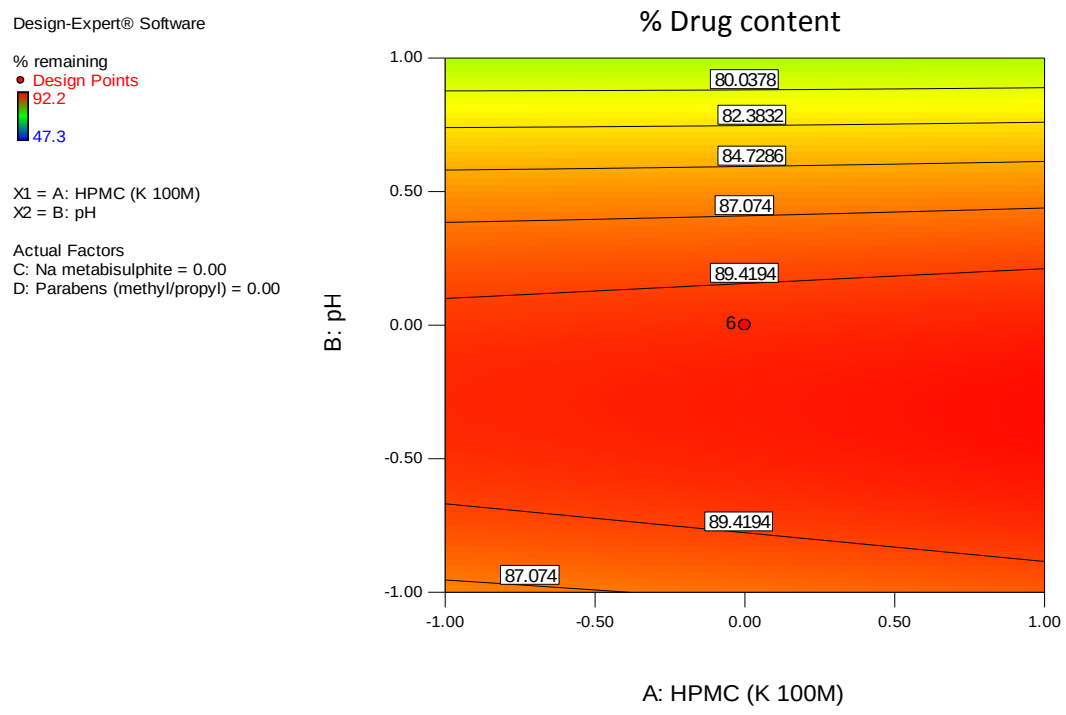


Figure 5.11 *Contour plot showing the effect of pH and HPMC concentration on % drug content*

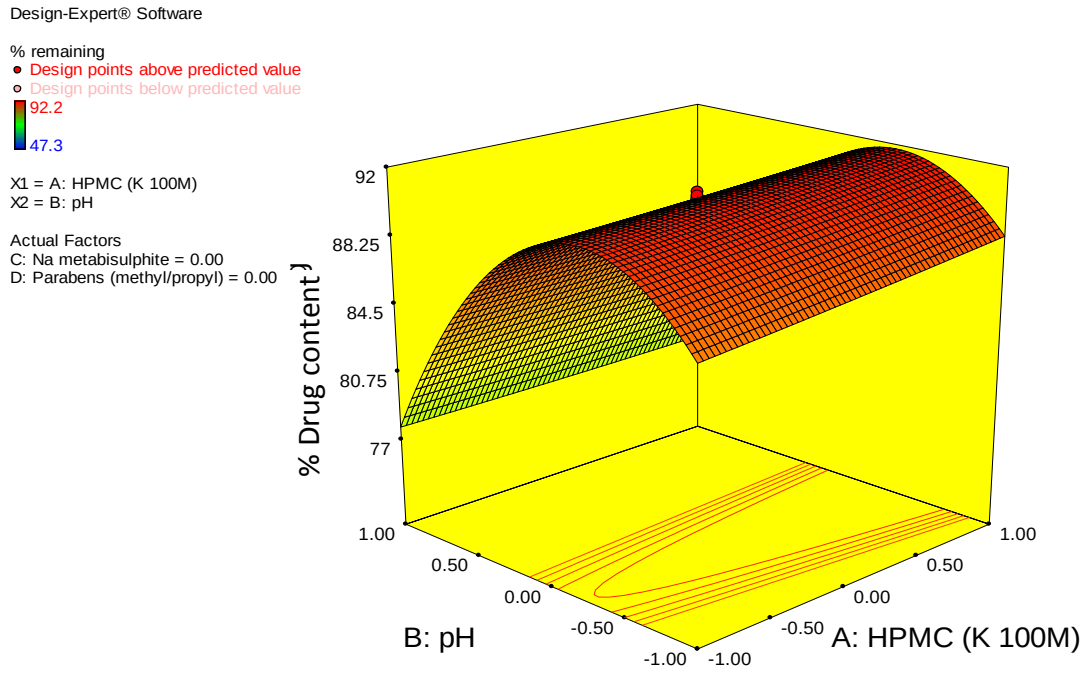


Figure 5.12 *3D response surface plot showing the effect of pH and HPMC concentration on % drug content*

The concentrations of sodium metabisulphite, methylparaben and propylparaben did not have a significant effect on FCV content. As shown in the contour plot in Figure 5.13, no significant change in the % drug content was observed with a change in the concentration of antioxidant or preservative incorporated into the formulation as the % FCV ranged between 89.48% and 90.61%. The response surface plot shown in Figure 5.14 reveals that changes in concentration of the antioxidant and preservative in the formulation had no significant impact on the stability of FCV.

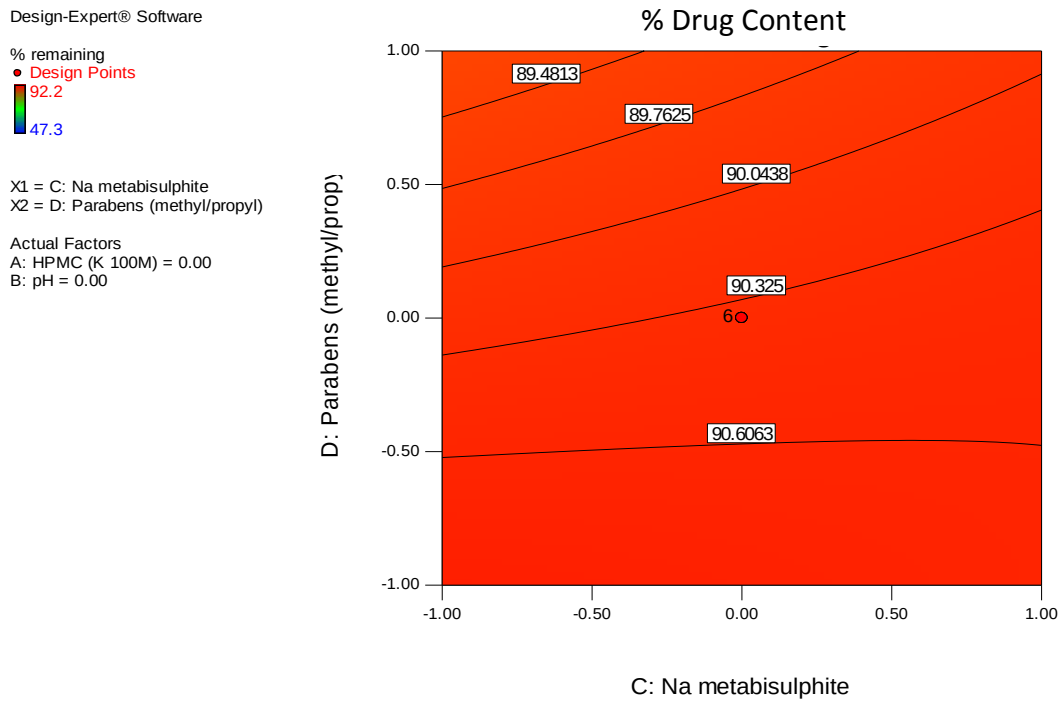


Figure 5.13 *Contour plot showing the effect of sodium metabisulphite, methylparaben and propylparaben concentrations on the % drug content*

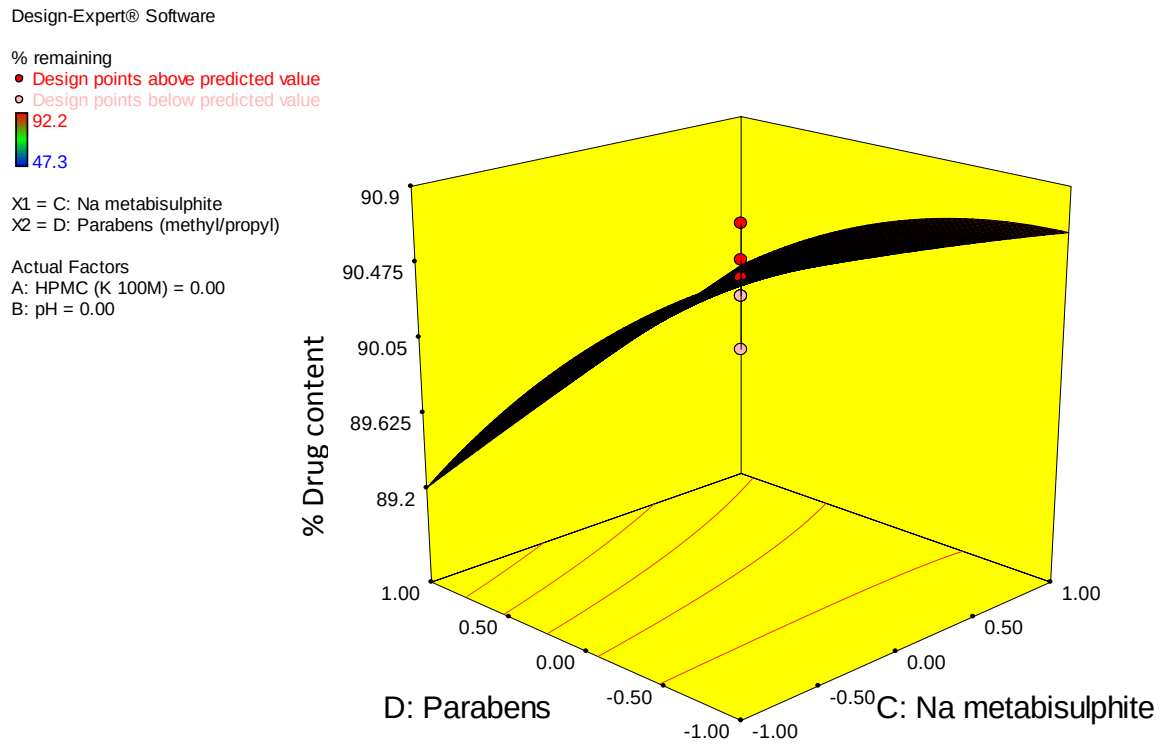


Figure 5.14 *3D response surface plot showing the effects of sodium metabisulphite, methylparaben and propylparaben concentrations on the % drug content*

5.5.3.2 Viscosity

As expected, the viscosity (Y_2) of the formulation was affected by the concentration of HPMC (X_1) that was used. This is indicated by the F-value of 878.37 and P-value of < 0.0001. The other input variables *viz.*, pH to which the formulations were buffered (X_2), % w/v antioxidant (X_2) and preservative (X_4) did not have a significant effect on the viscosity of the products, as indicated by F-values of 0.89, 0.40 and 0.029 respectively and Prob> F values of > 0.05.

The contour and response surface plots shown in Figures 5.15 and 5.16 show the effect of % w/v HPMC and pH on the viscosity of the formulation. The concentration of HPMC incorporated into the formulation significantly affected the final viscosity, and an increase in HPMC resulted in the production of more viscous formulations and a viscosity increase from 214.42 to 663.92 cP. High concentrations of HPMC result in a greater degree of swelling of the polymer and the formation of colloidal solutions that are thickening agents [131; 199]. The hydrodynamic volume of the polymer is determined by the chain stiffness, expansion of the polymer coil and space requirements that affect the viscosity and flow properties of the polymer solution [199]. The pH to which formulations were buffered had no significant effect on viscosity, as no change in viscosity was observed at a pH of 5, 6 and 7.

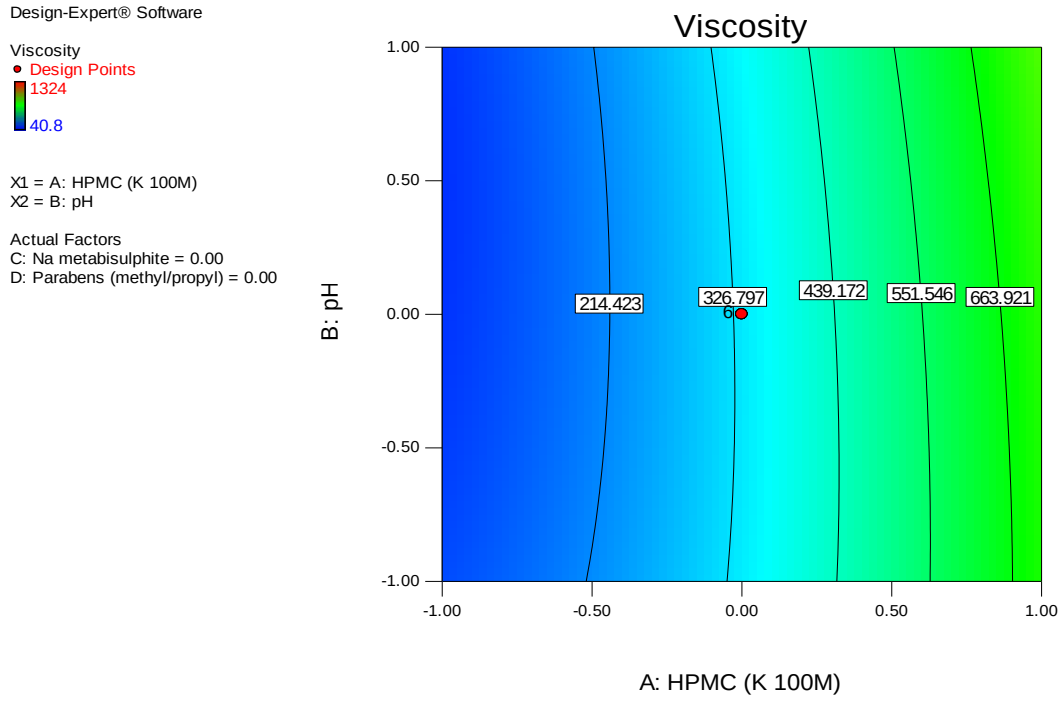


Figure 5.15 *Contour plots showing the effect of pH and HPMC concentration on viscosity*

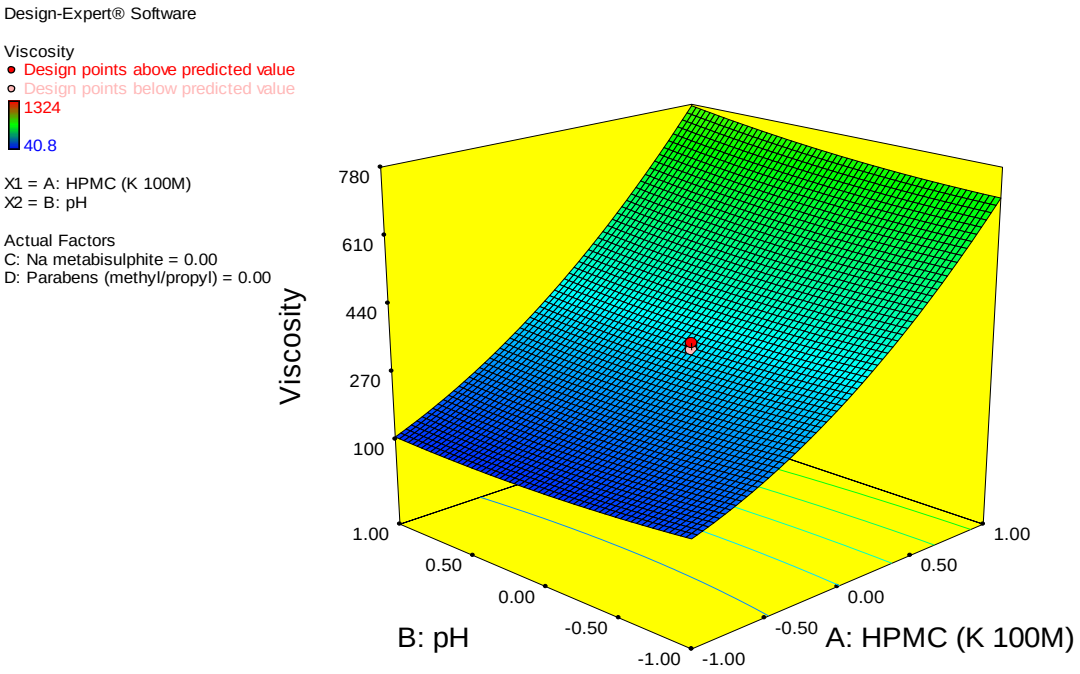


Figure 5.16 *3D response surface plot showing the effects of pH and HPMC concentration on viscosity*

The concentration of sodium metabisulphite, methylparaben and propylparaben that were incorporated into the formulation had no significant effect on the viscosity of the formulations, as shown in the countour and respose surface plots in Figures 5.17 and 5.18. The viscosity of the formulations was essentially unchanged. The difference in viscosity was 26.53 cP and 35.37 cP on incorporation of the minimum and maximum amount of antioxidant and preservative respectively.

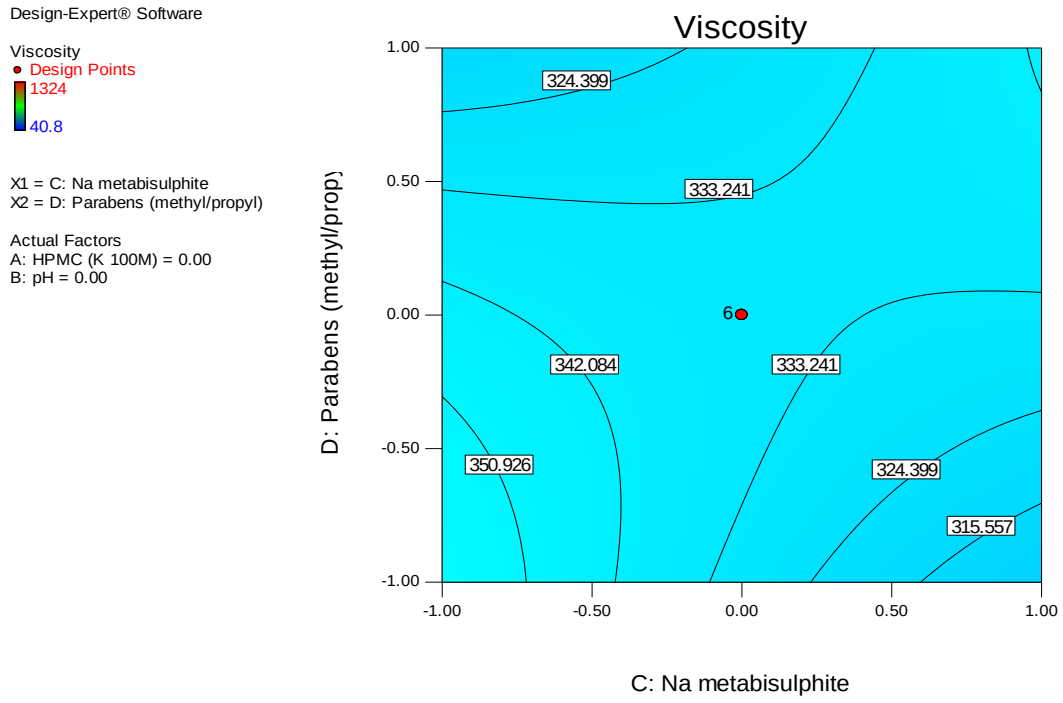


Figure 5.17 *Contour plot showing the effect of sodium metabisulphite, methylparaben and propylparaben concentrations on viscosity*

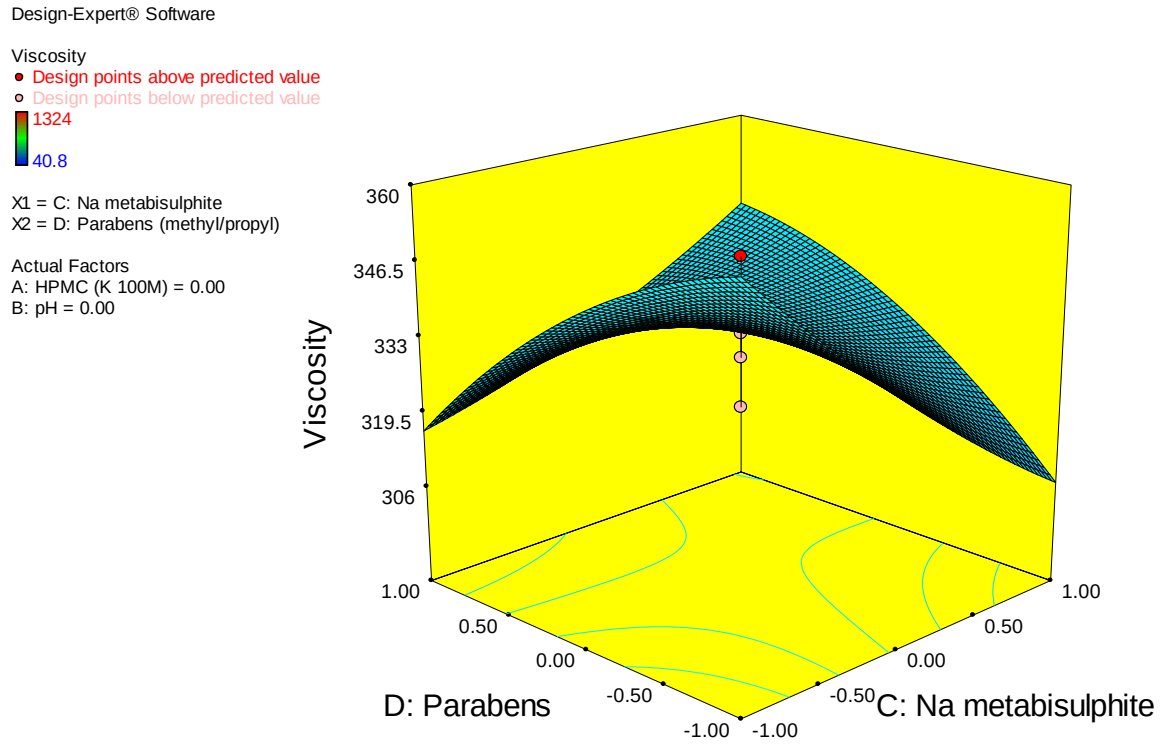


Figure 5.18 *3D response surface plot showing the effects of sodium metabisulphite, methylparaben and propylparaben concentrations on viscosity*

5.5.3.3 pH

The ultimate pH (Y_3) of the formulation was primarily affected by the pH to which the formulations were buffered (X_2), as indicated by an F-value of 16820.00 and P-value of < 0.0001. Interrogation of the quadratic polynomial equation reveals that the input variable pH (X_2) had a synergistic effect on the overall pH of the formulation (Y_3). Viscosity (X_1) was also shown to have a significant synergistic effect on the overall pH of the formulation (Y_3), with an F-value of 0.0410. The interactive effect of the input variables, % w/v antioxidant (X_3) and preservative (X_4) were shown to have a significant antagonistic effect on the overall pH (Y_3), with an F-value of 0.0152. The F-values of the other input variables, % w/v antioxidant (X_3) and preservative (X_4) were shown to have the least significant effect on pH, with an F-value of 1.25 and a Prob> F value > 0.05.

The contour and response surface plots shown in Figures 5.19 and 5.20 show the effect of % w/v HPMC and buffer pH on the overall pH of the formulation. Changes in concentration of HPMC incorporated into the formulation had little effect on the overall pH, as shown in Figure 5.19 by the relatively straight lines representing 0.5%, 0.75% and 1.0% HPMC, respectively. Figure 5.5 also shows this relationship, in which the pH remains relatively constant at coded values of -1.00, 0.00 and +1.00 of HPMC. As expected, the pH to which the formulations were originally buffered had the most significant effect on the overall pH of the product. This is shown in Figure 5.20, where the pH ranged between 5.12 and 6.45.

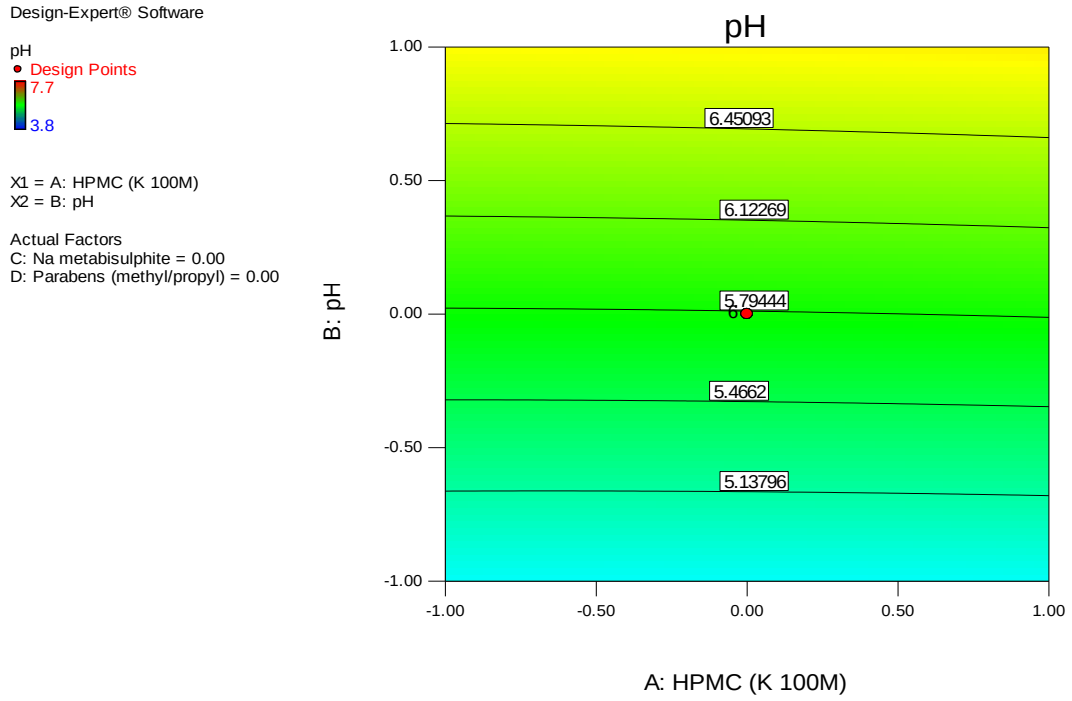


Figure 5.19 *Contour plot showing the effect of pH and HPMC concentration on pH*

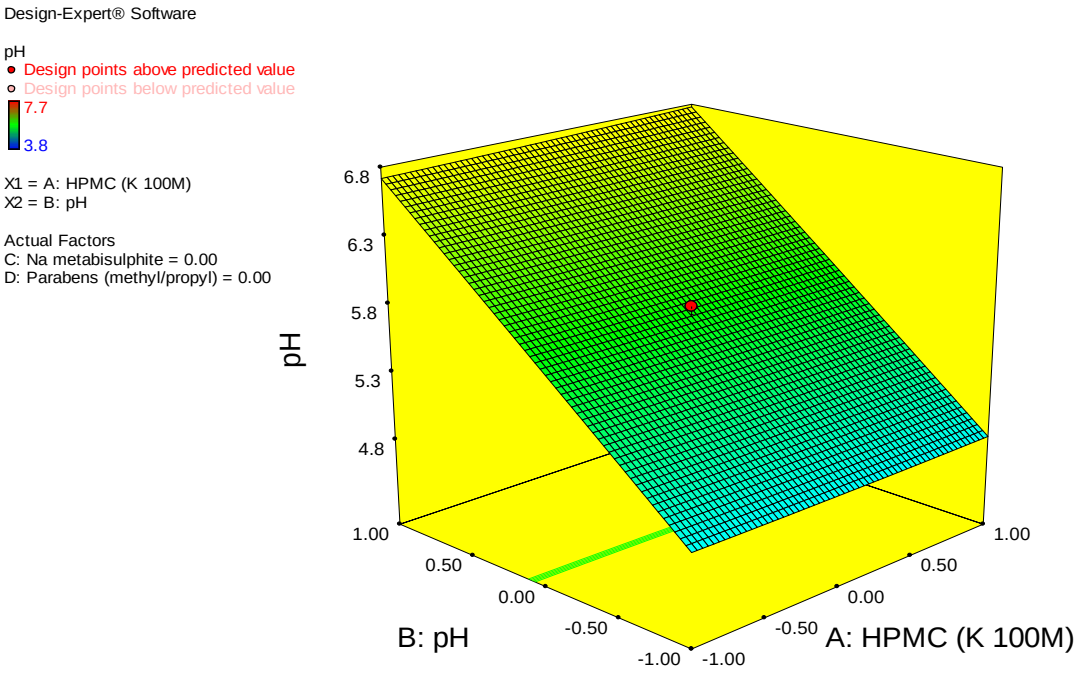


Figure 5.20 *3D response surface plot showing the effects of pH and HPMC concentration on pH*

The concentrations of sodium metabisulphite, methylparaben and propylparaben that were incorporated into the formulations had no noticeable effect on the overall pH of the products, as shown in the countour and respose surface plots in Figures 5.21 and 5.22. The overall pH of the formulation exhbited a difference of only 1.99 pH units, when the maximum and minimum amounts of the antioxidant and preservative were used.

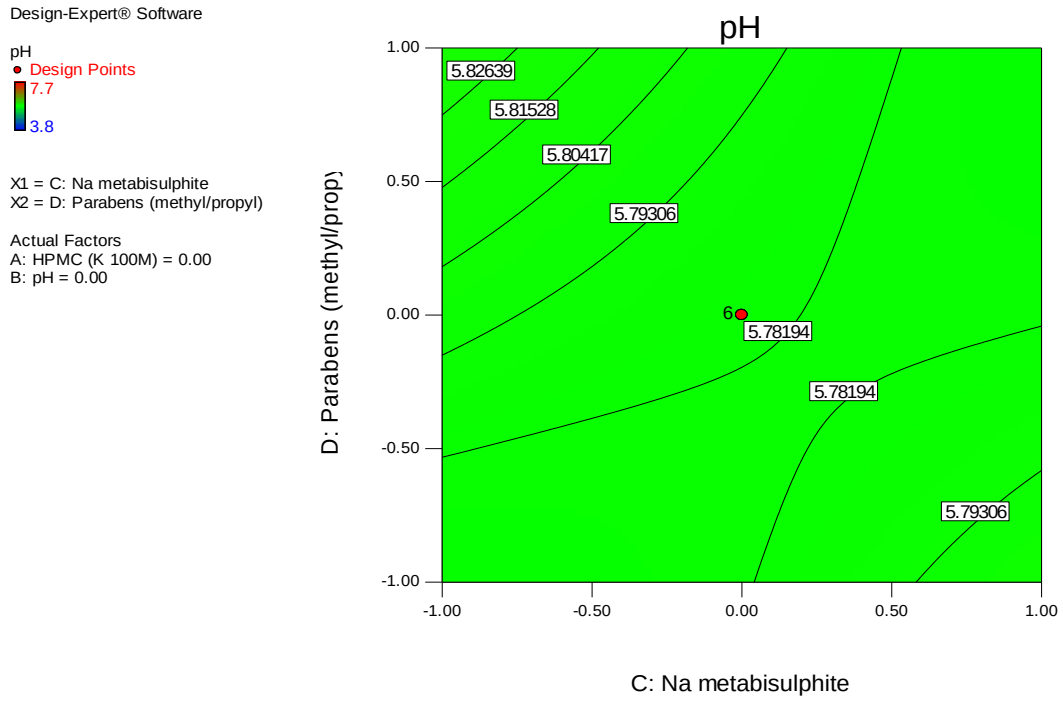


Figure 5.21 *Contour plot showing the effect of sodium metabisulphite, methylparaben and propylparaben concentrations on pH*

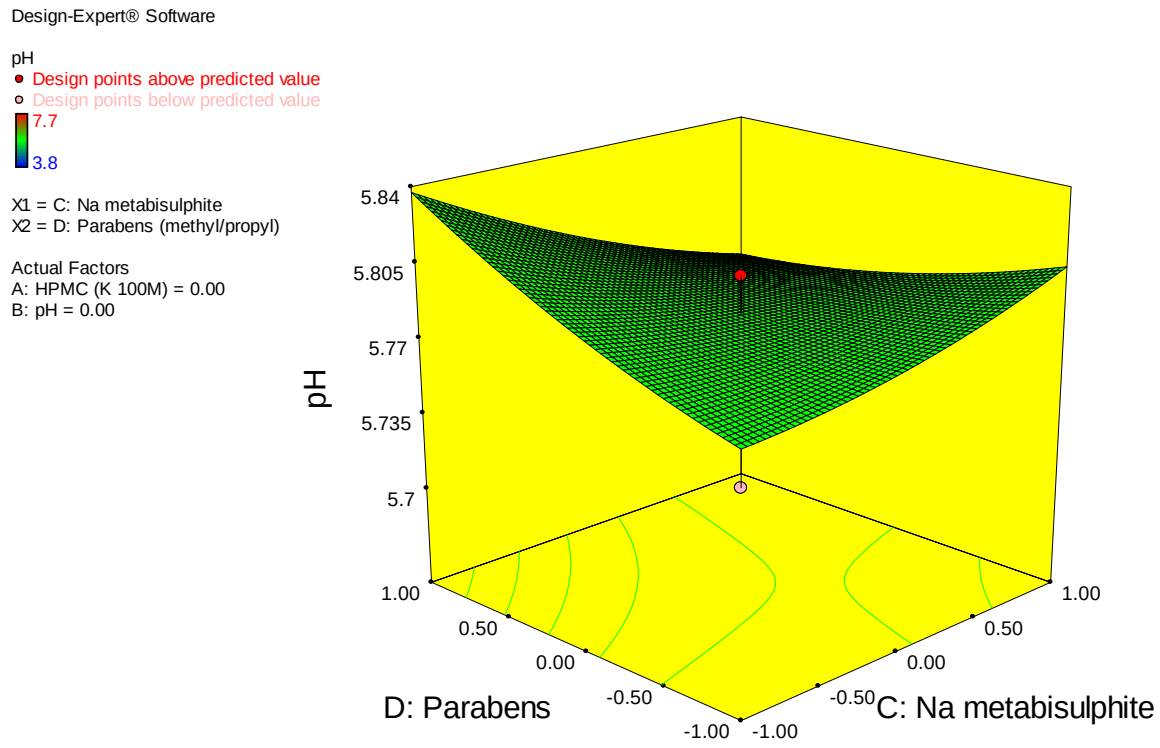


Figure 5.22 *3D response surface plot showing the effect of sodium metabisulphite, methylparaben and propylparaben concentrations on pH*

5.6 FORMULATION OPTIMISATION

The composition for the manufacture of an oral formulation of FCV was predicted using the optimisation function of Design-Expert® software. Several methods are available for use for the optimisation of a formulation, but numerical optimisation using a desirability approach was selected as the preferred method of choice. The individual responses are combined into a single composite response which is then optimised. The desirability approach attempts to satisfy the requirements of each response without compromising any one of the individual requirements to any extent [185; 200].

The targeted solution was to achieve a FCV content (Y_1) that remained as close to 100% as possible, a viscosity (Y_2) and pH (Y_3) that were suitable to facilitate oral administration and that exhibited appropriate stability of the product. The predicted values for % w/v HPMC (X_1), pH (X_2), and % w/v antioxidant (X_3) and preservative (X_4) and the desired formulation responses *viz.*, % drug content (Y_1), viscosity (Y_2) and pH (Y_3) generated from the numerical optimisation process are summarised in Tables 5.11 and 5.12.

Table 5.11 *Predicted values for input variables*

Input Variables				
X_1 (%)	***X_2 (pH)***	***X_3 (%)***	***X_4 (%)***	***Desirability***
0.070	5.75	0.03	0.05	0.66

To achieve an optimised response the predicted values for the input variables resulted in a desirability of 0.66. This relatively low value is due to the optimised response being based on the data following analysis conducted on day 28 of this study. It may be difficult to achieve 100% drug content following 28 days of storage at 40 °C/75% RH due to the inherent instability of FCV, and a value close to 100% drug content was therefore considered appropriate. The optimised FCV formulation was manufactured by buffering the formulation to pH = 5.75 and incorporating 0.07% w/v HPMC, 0.03% w/v antioxidant and 0.05% w/v preservative. The optimised formulation following storage at 25 °C/60% RH and 40 °C/75% RH for 28 days was found to have 96.16% and 91.54 % FVC remaining, a viscosity of 379 cP and 295 cP and a pH of 5.56 and 5.54, respectively as summarised in Table 5.12.

Table 5.12 *Responses monitored for a period of 28 days*

	Day 0	Day 7	Day 14	Day 21	Day 28
			25 °C/60% RH		
Y_1	100 ± 1.86	99.81 ± 3.22	98.46 ± 3.20	97.64 ± 3.20	96.16 ± 0.83
Y_2	471.67 ± 6.51	452.33 ± 9.29	420.67 ± 2.08	391.00 ± 12.12	379.00 ± 5.00
Y_3	5.76 ± 0.017	5.72 ± 0.02	5.64 ± 0.02	5.59 ± 0.01	5.56 ± 0.02
			40 °C/75% RH		
Y_1	100.00 ± 1.57	99.30 ± 0.10	96.59 ± 1.54	94.38 ± 0.97	91.54 ± 0.79
Y_2	472.00 ± 5.29	408.33 ± 3.51	365.00 ± 5.56	331.00 ± 5.29	295.00 ± 5.29
Y_3	5.76 ± 0.025	5.71 ± 0.01	5.61 ± 0.01	5.57 ± 0.03	5.54 ± 0.03

The predicted response variables based on results following storage at 40 °C/75% RH for 28 days are summarised in Table 5.13, in addition to the % predicted error of the experimental and predicted response variables.

Table 5.13 *Predicted response variables and % predicted error*

Predicted response Variables			**% Prediction error**		
Y_1 (%)	**Y_2 (cP)**	**Y_3 (pH)**	**Y_1 (%)**	**Y_2 (cP)**	**Y_3 (pH)**
91.48	300	5.53	0.066 %	1.67 %	-0.18 %

FCV was found to be more stable in the optimised formulation following storage at 40 °C/75% RH, in contrast to formulations used as part of the CCD as seen in Figure 5. 23. The degradation rates of FCV calculated following storage at 25 °C/60% RH and 40 °C/75% RH were 0.0012 and 0.0029 $days^{-1}$ respectively.

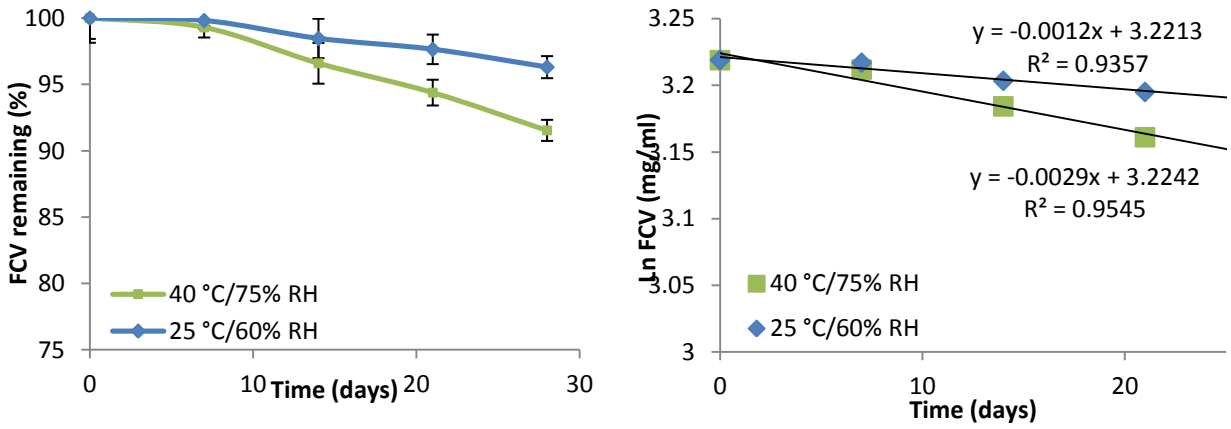


Figure 5.23 *Degradation and degradation rate plots of FCV in the optimised formulation following storage at 25 °C/60% RH and 40 °C/75% RH for 28 days.*

5.7 CONCLUSION

CCD was successfully used and applied to optimise a FCV formulation for oral administration to paediatric patients. CCD was selected as the optimisation tool as it has an advantage over traditional approaches to product development, is robust and highly efficient in generating critical information for the optimisation of a formulation from a relatively small number of experiments [191]. The intention of CCD was to identify a composition to formulate a stable product that could easily be administered to paediatric patients via the oral route. The input variables selected for evaluation were % w/v HPMC, pH and the % w/v antioxidant and preservative to be incorporated. Thirty experiments were conducted and each formulation was assessed in terms of the % drug content, viscosity and pH. With the aid of Design-Expert® software (version 7.0, Stat-Ease, Inc., Minneapolis, USA), the data were fitted to a quadratic polynomial equation that best described the data and showed a high degree of correlation between the input variables and responses monitored. ANOVA was used to analyse the data and normal plots of residuals, 3D response surface plots and contour plots were used to depict and interpret the data. ANOVA proved that the response models for % drug content, viscosity and pH were significant. The data for each model were found to be normally distributed with R^2 values of > 0.9, indicating a good correlation between the experimental and predicted responses, with only 2.76, 1.54, and 0.90% not explained by the model for % drug content, viscosity and pH respectively. In addition the > 4 value for adequate precision indicates that an adequate signal was achieved, while the relatively low coefficient of variation values indicate better precision and reliability of the experiments that were performed. ANOVA was conducted at a 5% level of significance and revealed that the % drug content in the formulation was significantly affected by the pH of the formulation and was not affected by the % w/v HPMC, antioxidant and preservatives. The viscosity of the formulation was significantly affected by the % w/v HPMC incorporated into the formulation. The pH to which a formulation was buffered, % w/v antioxidant and preservative content had no significant effect on the viscosity of the formulation. The overall pH of a formulation was significantly affected by the pH to which a formulation was originally buffered and the viscosity of a formulation had a significant effect on pH. The interactive effects of the input variables such as % w/v antioxidant and preservative were also shown to have a significant and antagonistic effect on the overall pH of the formulation. An optimised formulation was manufactured and placed on stability to identify the shelf life and possessed a viscosity and pH that were appropriate for oral administration. The low % prediction error indicated the robustness of the model and the suitability of RSM as an optimisation tool for formulation development, due to the high prognostic ability of RSM.

CHAPTER SIX

CONCLUSIONS

FCV is an antiviral agent that has an advantage over other antiviral agents such as ACV as it has high bioavailability and subsequently high intracellular concentrations. FCV is a pro-drug of PCV and is readily converted into the active form via hydrolysis of the acetate functional groups and oxidation [2; 5]. PCV is converted by the enzyme thymidine kinase into the monophosphate form, which in turn is phosphorylated to form PCV-triphosphate. PCV-triphosphate competes with deoxyguanosine triphosphate that inhibits HSV-1 and -2 DNA polymerase, resulting in the termination of DNA chain elongation. Herpes viral DNA synthesis and therefore cellular replication is therefore selectively inhibited [6; 11; 26].

A simple, rapid, precise, accurate and selective HPLC method was developed and validated according to ICH guidelines and was successfully applied to the quantitation of FCV in dosage forms. This method was further optimised, re-validated and used for the quantitation of FCV in syrup simplex in the presence of degradation products. The HPLC method was found to be linear over the concentration range of 2–120 μg/ml and demonstrated intra-assay and intermediate precision with % RSD values of ≤ 5% for both parameters. The method was found to be accurate with % RSD and % Bias determined to be ≤ 5% and ≤ 2% respectively. In addition the method was found to be selective and consequently stability-indicating as FCV was readily detected and quantified in the presence of excipients and degradation products.

Access to age-appropriate dosage forms is essential when administering medicines to provide effective and well tolerated therapy for paediatric patients [4]. The range of commercially available paediatric oral formulations is limited due to the specialised use of these products and the lack of incentives for product development, manufacture and registration of formulations for these patients [161; 201]. Paediatricians are therefore faced with having to prescribe agents for children where data regarding the dose, efficacy and safety have not been established. Therefore pharmacists are faced with the challenge of formulating extemporaneous liquid dosage forms for oral administration by dissolving or dispersing commercially available tablets, capsules or powdered API in a vehicle that patients can easily swallow [4; 201]. However several factors are often overlooked with this practice. These involve chemical, physical and microbial stability, bioavailability and compatibility issues [4; 160; 161].

Scientific understanding to support the manufacture of quality products is gained through information and knowledge generated during preformulation studies [69]. Preformulation studies therefore require that a selected API is chemically and physically characterised and evaluates the effect of excipients on the stability and/or modification of an API [89; 91]. The effect of pH on the solubility and stability of FCV and the formation of a precipitate was observed at concentrations in excess of the saturation solubility of FCV. Analytical techniques such as IR, NMR and XRD were used in an attempt to elucidate the nature of the precipitate, which was thought to be FCV monohydrate.

IR and DSC were used to investigate the compatibility between FCV and excipients found in Ora-Sweet®, Famvir® tablets and of excipients that may be suitable for use in extemporaneous manufacture of a FCV product. The preformulation studies provided evidence and data in respect of the suitability of some excipients and thus their suitability for use in the manufacture of an extemporaneous FCV formulation.

The stability of extemporaneously manufactured FCV formulations was studied. FCV formulations (25 mg/ml) were manufactured using syrup simplex or HPMC vehicles with crushed Famvir® tablets or API powder. The stability-indicating HPLC method was used to analyse FCV content and the interpretation of the stability results was undertaken using the method described by Timm *et al.*, [54]. This approach permits an assessment as to whether or not the degradation that had occurred and the different amounts of FCV remaining to be degraded, following storage at 25 °C/60% RH and 40 °C/75% RH, was relevant and/or significant. The resulting data were compared to the data generated immediately following manufacture *viz.*, t = Day 0. The most significant degradation following storage at 25 °C/60% RH and 40 °C/75% RH was observed when syrup simplex and crushed Famvir® tablets were used with approximately 78.53 ± 3.92 % and 71.36 ± 2.53 % remaining to be degraded, following 28 days of storage. FCV was most stable following storage at 25 °C/60% RH and 40 °C/75% RH when HPMC and FCV powder were used with approximately 90.80 ± 2.43 % and 87.61 ± 4.93 % remaining after the 28 day storage period.

Formulations were also manufactured using a commercially available vehicle, Ora-Sweet® to compare the stability of FCV in products made with non-commercial vehicles. The formulations incorporated FCV from Famvir® tablets or API powder and were assessed in terms of % drug content, pH, viscosity and appearance following storage at 4 °C, 25 °C/60% RH and 40 °C/75% RH. Although formulations containing Ora-Sweet® are recommended to be stored at 4 °C, this is not always practical in a South African setting as not all patients have access to a refrigerator [202]. Following storage at 40 °C/75% RH a significant and relevant decrease in FCV content was observed after 14 days and approximately 80.70 ± 0.84 and 80.28 ± 0.79% remained when FCV powder and Famvir® tablets were used respectively. Formulations were more stable following storage at 4°C, as they

revealed a FCV content > 90% for up to 56 days. The initial and final viscosity of the formulations remained unchanged and results from a paired sample Student T-test at a 5% level of significance revealed no significant change in pH over an 84 day storage period, except following storage at 40 °C/75% RH. The clarity of the solutions increased following storage at 40 °C/75% RH, and slight crystallisation was observed after three (3) weeks when stored at 4 °C. Discolouration was observed after five (5) and six (6) weeks following storage at 40 °C/75% RH and 25 °C/60% RH respectively. Incorporation of a preservative and antioxidant into FCV formulations was deemed necessary to improve the physical, chemical and microbial stability of the products. Therefore the formulations were manufactured using different combinations of HPMC or syrup simplex, methylparaben and/or propylparaben and sodium metabisulphite, ascorbic acid or citric acid. Formulations in which HPMC was used were more stable than formulations manufactured using syrup simplex. No differences in FCV stability were observed when formulations were manufactured using methylparaben and propylparaben alone or when in combination. A combination of methylparaben and propylparaben was therefore selected for use as the preservatives in the formulation selected for optimisation, as it has been established that the use of a combination of parabens results in improved preservative activity due to synergistic effects [131]. The incorporation of citric acid into the formulation revealed the most significant degradation of FCV due to the acidic pH of the formulations (pH < 4). FCV is known to undergo acid catalysed degradation [23; 24]. Discolouration was also observed, particularly following storage at 40 °C/75% RH. Formulations incorporating sodium metabisulphite were more stable than those incorporating ascorbic or citric acid. HPMC-based formulations incorporating sodium metabisulphite remained clear and colourless throughout the duration of the study. HPMC-based formulations that incorporated ascorbic acid discoloured slightly to pale yellow observed after three (3) days of storage. The discolouration intensified as the length of the study increased and was most noticeable following storage at 40 °C/75% RH. Discolouration was observed after five (5) weeks in all formulations incorporating syrup simplex and following storage at 25 °C/60% RH crystallisation was observed in formulations containing ascorbic acid or sodium metabisulphite. The formulation selected for optimisation was manufactured using HPMC, sodium metabisulphite methylparaben and propylparaben. This resulted in approximately 89.05 ± 1.29 and 78.32 ± 0.42 % FCV remaining following storage at 25 °C/60% RH and 40 °C/75% RH respectively for a period of 42 days.

Due to the effect of pH on the stability of FCV the use of a buffer system was investigated. FCV formulations were manufactured using a phosphate buffer of pH 6 (50 mM) and the FCV content was assessed using HPLC. Incorporation of a buffer increased the stability of FCV, and when no buffer was used instability was observed. The formulations were regarded as stable following storage at 25 °C/60% RH with approximately 90.81 ± 3.87% remaining for a period of 42 days.

A Central Composite Design approach was used to investigate the effect of input variables on product performance. Through careful design of experiments, and consequently a limited number of investigations, the objective was to determine the effect of four (4) independent variables on formulation responses and to ascertain if a relationship existed between the input and output variables. Thirty formulations were manufactured using input variables of % w/v HPMC, pH, and % w/v antioxidant and preservative. The resultant formulations were analysed in terms of % drug content, viscosity and overall pH of the formulation. Design-Expert® software (version 7.0, Stat-Ease, Inc., Minneapolis, USA) revealed that the correlation between the variables and the formulation responses was best described by a quadratic polynomial model. ANOVA and regression coefficients were used to analyse the data statistically and normal probability plots of residuals, 2D contour plots and 3D response surface plots were used to graphically depict and facilitate interpretation of the data. ANOVA showed the response models for % drug content, viscosity and pH were significant with P-values of < 0.0001. The data for each model were found to be normally distributed, with R^2 values of > 0.9 that indicated a good correlation between the experimental and predicted responses existed with only 2.76, 1.54, and 0.90% not explained by the model for % drug content, viscosity and pH respectively. ANOVA of the quadratic polynomial model determined at a 5% level of significance revealed that the % drug content was influenced significantly by the pH. A buffer of pH 6 resulted in the maximum FCV stability with a decrease in stability as the pH increased and decreased above and below this value. This was confirmed by the contour and response surface plots. ANOVA also revealed that viscosity was significantly affected by the synergistic effect of % w/v HPMC and the overall pH of the formulation was affected most significantly by the pH to which a formulation was originally buffered and the significant synergistic effect of viscosity on pH .The optimal composition for the manufacture of a FCV formulation was predicted using the optimisation function of Design-Expert® software. Optimisation aimed at achieving a maximum FCV content, viscosity appropriate for ease of administration and a pH within an acceptable range for oral administration. The low % prediction error indicates the robustness of the model and the suitability of RSM as an optimisation tool due to the high prognostic ability of RSM.

The studies reported in this thesis look at several important aspects that must be addressed when any formulation is manufactured extemporaneously. The information includes material relating to selection of a suitable vehicle, and appropriate excipients to develop a stable dosage form for oral administration. The need for stability data is highlighted, and it cannot be assumed that “universal” suspending agents will always be an appropriate vehicle. Rather, each API needs to be addressed individually and stability and other studies must be conducted before the product is dispensed to a patient. Pharmacists practicing in resource-limited environments should engage with other health care providers and refer to the literature to identify possible interactions. Future studies that would be necessary for the progression of this project would be an evaluation of the incorporation of suitable

colouring, flavouring and sweetening agents to improve the aesthetic and palatability properties of the final formulation.

The lack of age-appropriate commercially available liquid dosage forms for paediatric use is an ongoing challenge. Physicians, pharmacists and other health care providers, through consultation, should develop a prioritised list of paediatric formulations that may be required. Mechanisms for funding and incentive programmes should be established in order for studies to be conducted to develop effective and well tolerated high-priority extemporaneous formulations [201; 203]. Data regarding the manufacture and use of extemporaneous formulations should be well documented and shared amongst health care professionals through presentations at national meetings, publications and postings on reputable web-sites. In addition, manufacturers with a knowledge of drug stability should make such information freely available through package information leaflets, by way of example [4]. Data relating to the effectiveness and tolerability of extemporaneous formulations should also be documented as most extemporaneous formulations have not been subject to clinical studies [4].

Pharmacists, if faced with the challenge of not having a suitable product for administration to paediatric patients should first consider a therapeutic alternative in liquid form that may be chemically different to the molecule of choice, but clinically similar in its effect. If this is not possible, an extemporaneous formulation can be prepared, however the relevant pharmacopoeial formulary should be consulted or literature on a suitable stabile formula must be sought. If no suitable formula is available, the pharmacist must develop a formula based on sound scientific principles with due consideration of the stability of the API. Storage and packaging should also be considered and a suitable shelf life assigned to the formulation. Possible interactions between excipients and the API, particularly if tablets or capsules have been used as the source of the API should be researched and clarified [126].

APPENDIX I

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 1100 ml**
Date of Manufacture: 03 October 2010 **Batch Number: FCV-01**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

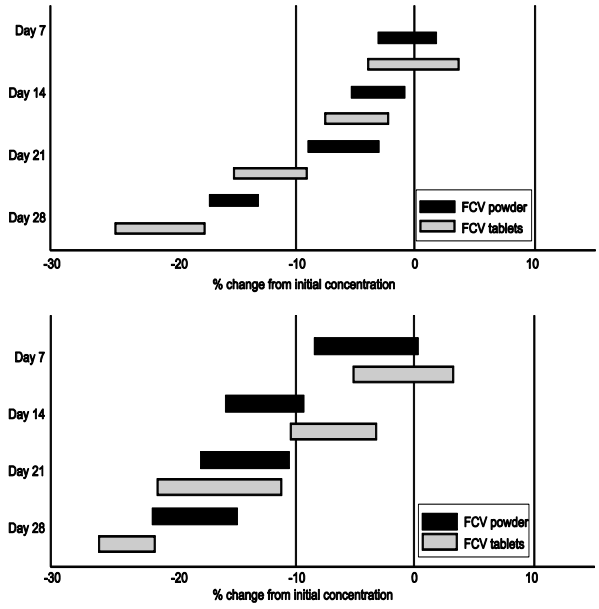
Raw Material	Original Formula	Working Formula	Receiving Number
FCV (pure API powder)	2.5 g	16.25 g	RM000252
Syrup BP	100 ml	650 ml	-

Method of Manufacture:

1. Accurately weigh 27.5 g of FCV and transfer to a mortar.
2. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.1.
3. Transfer the product into a measuring cylinder and make up to a volume of 1100 ml.
4. Transfer the formulation into 10x100 ml bottles.
5. Analyse the formulations on days 0, 7, 14 and 28.

Results Following Testing:

Day	0	7	14	21	28
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 1.00	99.34 ± 2.63	96.59 ± 2.51	90.76 ± 3.12	84.64 ± 2.20
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 3.39	95.86 ± 3.77	87.66 ± 0.53	85.10 ± 3.01	81.00 ± 3.06



Stability of FCV at 40 °C/75% RH from day 7-day 28

Visual Observations

- Significant and relevant FCV degradation observed on day 21
- Crystallisation of syrup observed following one (1) week of storage
- No colour changes were observed over the 28 day period

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 1100 ml**
Date of Manufacture: 03 October 2010 **Batch Number: FCV-02**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

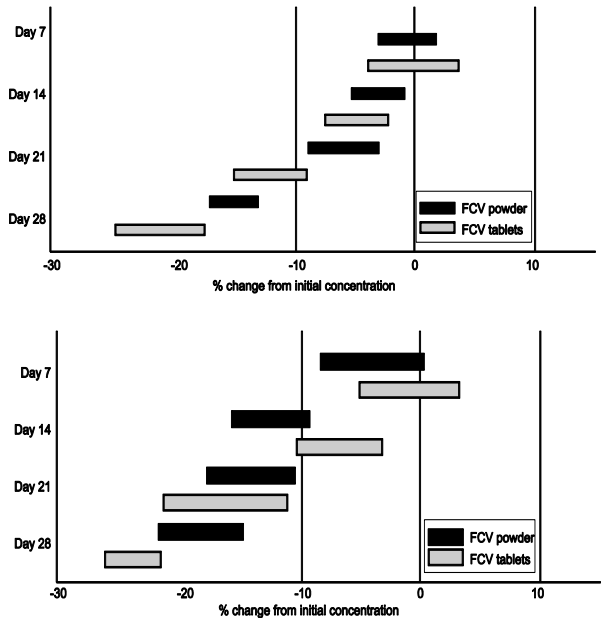
Raw Material	Original Formula	Working Formula	Receiving Number
FCV (Famvir®)	2.5 g	16.25 g	B8067A
Syrup BP	100 ml	650 ml	-

Method of Manufacture:

1. Accurately weigh 27.5 g of FCV and transfer to a mortar.
2. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.1.
3. Transfer the product into a measuring cylinder and make up to a volume of 1100 ml.
4. Transfer the formulation into 10x100 ml bottles.
5. Analyse the formulations on days 0, 7, 14 and 28.

Results Following Testing:

Day	0	7	14	21	28
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 2.44	99.65 ± 3.92	94.96 ± 1.56	87.77 ± 2.75	78.53 ± 3.92
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 1.76	93.87 ± 4.66	87.93 ± 3.70	78.70 ± 5.92	71.36 ± 2.53



Stability of FCV at 40 °C/75% RH from day 7-day 28

Visual Observations

- Significant and relevant FCV degradation observed on day 21
- Crystallisation of syrup observed following one (1) week of storage
- No colour changes were observed over the 28 day period

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 1100 ml**
Date of Manufacture: 03 October 2010 **Batch Number: FCV-03**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

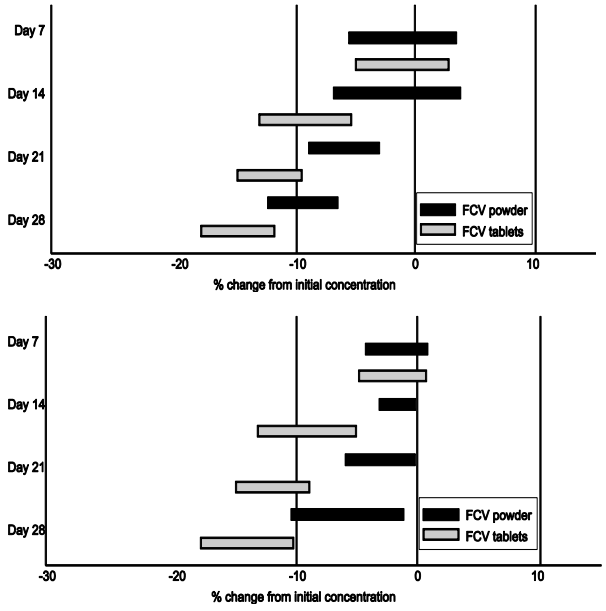
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
HPMC	100 ml	650 ml	RM000062

Method of Manufacture:

1. Accurately weigh 27.5 g of FCV and transfer to a mortar.
2. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.2.
3. Transfer the product into a measuring cylinder and make up to a volume of 1100 ml.
4. Transfer the formulation into 10x100 ml bottles.
5. Analyse the formulations on days 0, 7, 14 and 28.

Results Following Testing:

Day	0	7	14	21	28
			25 °C/60 %RH		
Content(mg/ml)	100.00 ± 5.39	96.51 ± 0.29	96.04 ± 3.20	93.64 ± 0.87	90.80 ± 2.43
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 1.38	95.07 ± 2.48	94.02 ± 1.46	93.35 ± 2.44	89.97 ± 4.93



Stability of FCV at 40 °C/75% RH from day 7-day 28

Visual Observations

- Significant and relevant FCV degradation observed on day 28
- Formulations remained clear over the 28 day period
- No colour changes were observed over the 28 day period

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 1100 ml**
Date of Manufacture: 03 October 2010 **Batch Number: FCV-04**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

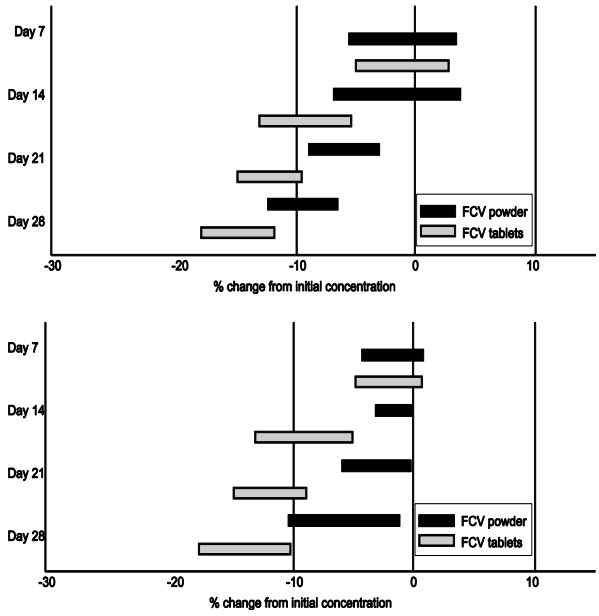
Raw Material	Original Formula	Working Formula	Receiving Number
FCV (Famvir®)	2.5 g	16.25 g	B8067A
HPMC	100 ml	650 ml	RM000062

Method of Manufacture:

1. Accurately weigh 27.5 g of FCV and transfer to a mortar.
2. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.2.
3. Transfer the product into a measuring cylinder and make up to a volume of 1100 ml.
4. Transfer the formulation into 10x100 ml bottles.
5. Analyse the formulations on days 0, 7, 14 and 28.

Results Following Testing:

Day	0	7	14	21	28
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 4.01	96.57 ± 4.59	92.73 ± 4.44	89.71 ± 2.58	87.10 ± 3.28
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 1.47	96.82 ± 2.86	89.55 ± 4.44	87.12 ± 3.22	84.90 ± 4.18



Stability of FCV at 40 °C/75% RH from day 7-day 28

Visual Observations

- Significant and relevant FCV degradation observed on day 28
- Formulations remained clear over the 28 day period
- No colour changes were observed over the 28 day period

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 02 June 2011 **Batch Number: 05**
Storage Conditions: 4 °C , 25 °C/60% RH and 40 °C/75% RH

Formula:

Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	25 g	RM000252
Ora-Sweet®	100 ml	1000 ml	RM000271

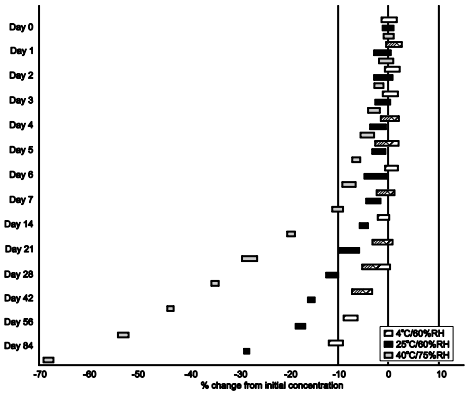
Method of Manufacture:

1. Accurately weigh 25 g of FCV and transfer to a mortar.
2. Gradually incorporate the vehicle, Ora-Sweet®.
3. Transfer the product into a measuring cylinder and make up to a volume of 1000 ml.
4. Transfer the formulation into 9x100 ml bottles.
5. Analyse the formulations on days 0, 1, 2, 3, 4, 5, 6, 7, 14, 21 , 28, 42, 56 and 84.

Results Following Testing:

Batch #	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
				4 °C			
Content (mg/ml)	100.00 ± 1.66	101.53 ± 0.26	99.31 ± 0.63	100.43 ± 1.75	100.34 ± 2.07	99.71 ± 2.60	100.72 ± 0.87
Viscosity (cP)	205.67±2.08	-	-	-	-	-	-
				25 °C/60% RH			
Content (mg/ml)	100.00 ± 0.75	98.57 ± 1.77	98.92 ± 2.27	98.76 ± 1.54	97.95 ± 2.14	98.25 ± 1.65	97.51 ± 2.86
Viscosity (cP)	118.67±2.08	-	-	-	-	-	-
				40 °C/75% RH			
Content (mg/ml)	100.00 ± 1.02	99.33 ± 1.65	98.06 ± 0.97	96.98 ± 1.44	95.72 ± 1.46	93.60 ± 0.61	92.11 ± 1.63
Viscosity (cP)	125.87±1.01	-	-	-	-	-	-

Batch #	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56	Day 84
				4 °C			
Content (mg/ml)	99.54 ± 1.92	98.96 ± 0.43	98.85 ± 2.34	97.46 ± 3.24	94.51 ± 1.98	92.91 ± 0.50	89.22 ± 1.50
Viscosity (cP)	205.67±2.08	195.00±5.00	193.33±4.16	188.00±5.20	199.33±6.51	196.67±0.58	190.67±3.06
				25 °C/60% RH			
Content (mg/ml)	96.94 ± 1.11	95.20 ± 0.32	92.28 ± 2.39	88.79 ± 1.25	84.93 ± 0.33	82.45 ± 0.65	71.16 ± 0.58
Viscosity (cP)	118.67±2.08	116.67±2.08	119.33±3.79	124.33±0.58	133.00±1.73	128.00±1.00	123.33±3.06
				40 °C/75% RH			
Content (mg/ml)	89.97 ± 1.43	80.70 ± 0.84	72.33 ± 1.86	65.75 ± 0.89	56.05 ± 1.23	46.83 ± 1.15	31 82 ± 1.13
Viscosity (cP)	125.87±1.01	139.60±6.16	143.07±3.70	142.00±3.12	150.27±8.22	149.87±2.27	140.00±3.46



Stability of FCV from day 0-day 84

Visual Observations

- Crystallisation of syrup observed following three (3) week of storage at 4°C
- Colour changes were observed following five (5) and six (6) weeks of storage at 40 °C/75% RH and 25 °C/60% RH respectively

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 15 June 2011 **Batch Number: 06**
Storage Conditions: 4 °C , 25 °C/60% RH and 40 °C/75% RH

Formula:

Raw Material	Original Formula	Working Formula	Receiving Number
FCV (Famvir®)	2.5 g	25 g	B80004
Ora-Sweet®	100 ml	1000 ml	RM000271

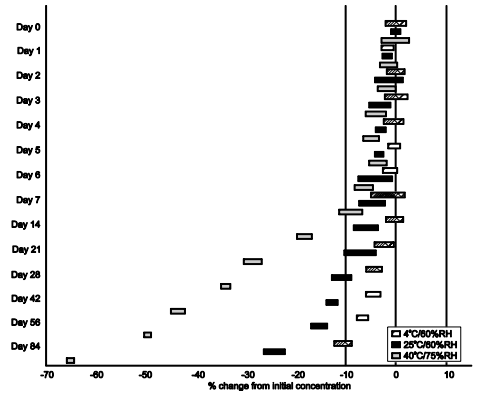
Method of Manufacture:

1. Transfer 100 Famvir® tablets to a mortar and size reduce tablets using a pestle.
2. Gradually incorporate the vehicle, Ora-Sweet®.
3. Transfer the product into a measuring cylinder and make up to a volume of 1000 ml.
4. Transfer the formulation into 9x100 ml bottles.
5. Analyse the formulations on days 0, 1, 2, 3, 4, 5, 6, 7, 14, 21 , 28, 42, 56 and 84.

Results Following Testing:

Batch #	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
				4 °C			
Content (mg/ml)	100.00 ± 2.00	98.41 ± 0.51	100.20±1.88	100.20 ± 2.82	99.48 ± 2.27	99.62 ± 1.18	99.09 ± 0.75
Viscosity (cP)	234.33±4.51	-	-	-	-	-	-
				25 °C/60% RH			
Content (mg/ml)	100.00 ± 0.73	96.41 ± 0.44	96.99 ± 3.58	95.15 ± 2.61	95.18 ± 1.22	95.02 ± 0.92	94.24 ± 4.48
Viscosity (cP)	150.00±3.61	-	-	-	-	-	-
				40 °C/75% RH			
Content (mg/ml)	100.00 ± 2.66	97.05 ± 0.82	96.47 ± 0.99	94.30 ± 1.41	93.08 ± 0.70	93.48 ± 0.40	91.86 ± 1.42
Viscosity (cP)	131.33±4.16	-	-	-	-	-	-

Batch #	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56	Day 84
				4 °C			
Content (mg/ml)	98.34 ± 4.35	98.27 ± 1.33	97.78 ± 2.26	95.76 ± 1.91	94.95 ± 0.78	92.98 ± 0.57	89.17 ± 1.94
Viscosity (cP)	234.00±4.00	279.33±6.81	283.33±5.13	228.00±2.65	228.00±2.00	262.33±2.08	232.33±2.08
				25 °C/60% RH			
Content (mg/ml)	93.46 ± 3.35	92.39 ± 3.01	91.40 ± 3.94	87.32 ± 2.60	85.23 ± 1.09	82.92 ± 1.63	74.19 ± 2.48
Viscosity (cP)	148.33±4.16	189.00±3.61	190.67±3.21	150.67±5.69	151.33±3.06	172.00±2.00	147.33±2.31
				40 °C/75% RH			
Content (mg/ml)	89.37 ± 2.22	80.28 ± 0.79	69.81 ± 1.50	65.03 ± 0.58	55.42 ± 1.07	49.39 ± 0.20	33.88 ± 0.12
Viscosity (cP)	117.33±3.21	161.33±3.06	155.00±7.81	121.00±4.59	122.33±2.52	134.33±2.52	122.00±2.00



Stability of FCV from day 0-day 84

Visual Observations

- Crystallisation of syrup observed following three (3) week of storage at 4°C
- Colour changes were observed following five (5) and six (6) weeks of storage at 40 °C/75% RH and 25 °C/60% RH respectively
- Sediment of insoluble excipients present

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 03 April 2011 **Batch Number: FCV-07**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

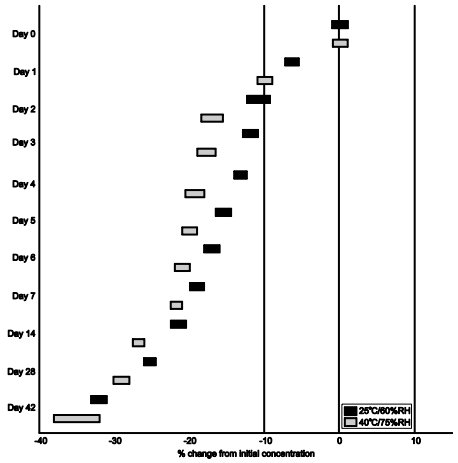
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Methylparaben and propylparaben	0.1 %	0.65g	X061903/X062223
Sodium Metabisulphite	0.05%	0.325 g	RM000265
Syrup BP	100 ml	650 ml	-

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.1.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 1, 2, 3, 4, 5, 6, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ±1.20	80.88 ± 0.28	78.50 ± 0.68	74.51 ± 0.27	67.96 ± 1.13
Viscosity (cP)	487.67 ± 4.73	434.33 ± 5.27	384.00 ± 1.41	350.50 ± 2.12	351.00 ± 1.41
pH	7.50 ± 0.01	6.55 ± 0.02	6.27 ± 0.01	6.05 ± 0.02	5.77 ± 0.01
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 1.09	78.30 ± 1.06	73.28 ± 0.43	70.84 ± 0.42	64.87 ± 4.00
Viscosity (cP)	489.33 ± 4.93	408.33 ± 4.73	353.00 ± 5.00	331.00 ± 3.00	348.00 ± 1.41
pH	7.50 ± 0.01	5.75 ± 0.02	5.39 ± 0.01	5.34 ± 0.01	5.42 ± 0.01



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on day two (2)
- Crystallisation of syrup observed following one (1) week of storage
- Colour changes were observed following five (5) weeks of storage

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 03 April 2011 **Batch Number: FCV-08**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

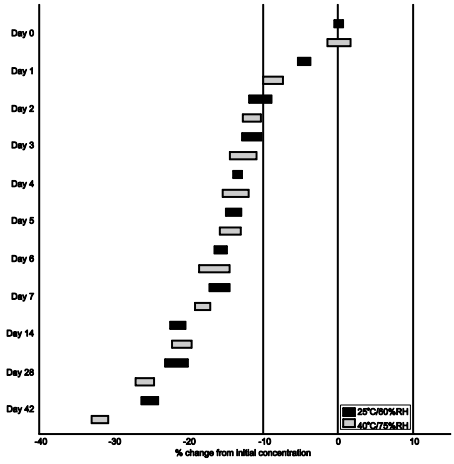
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Methylparaben and propylparaben	0.1 %	0.65g	X061903/X062223
Ascorbic acid	0.05%	0.325 g	RM000265
Syrup BP	100 ml	650 ml	-

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.1.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 1, 2, 3, 4, 5, 6, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 0.38	79.90 ± 1.35	82.86 ± 1.27	78.32 ± 1.91	74.91 ± 0.60
Viscosity (cP)	527.00 ±1.00	398.00 ± 1.00	397.33 ± 6.81	395.67 ± 5.13	358.67 ± 1.52
pH	8.08 ± 0.06	6.10 ± 0.01	5.87 ± 0.00	5.76 ± 0.01	5.75 ± 0.01
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 1.58	76.99 ± 0.36	79.11 ± 0.50	74.08 ± 0.50	68.66 ± 1.15
Viscosity (cP)	528.00 ± 1.00	395.67 ± 4.16	393.00 ± 6.24	375.67 ± 5.03	353.67 ± 3.79
pH	8.14 ± 0.02	5.63 ± 0.00	5.36 ± 0.01	5.30 ± 0.01	5.23 ± 0.02



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on day two (2)
- Crystallisation of syrup observed following one (1) week of storage
- Initial colour changes were observed following three (3) days of storage at 40 °C/75% RH

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 03 April 2011 **Batch Number: FCV-09**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

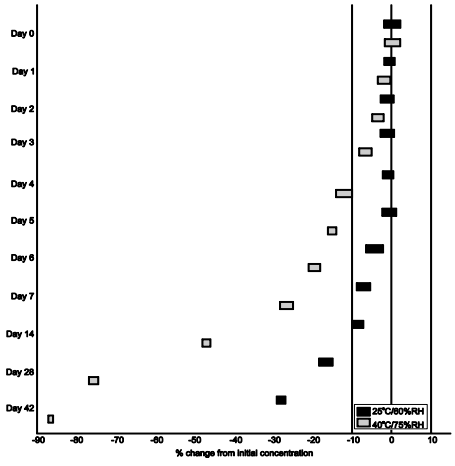
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Methylparaben and propylparaben	0.1 %	0.65g	X061903/X062223
Citric acid	1.0%	6.5 g	RM000265
Syrup BP	100 ml	650 ml	-

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.1.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 1, 2, 3, 4, 5, 6, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 1.65	95.90 ± 1.48	90.94 ± 1.19	83.75 ± 1.54	72.04 ± 0.52
Viscosity (cP)	524.00 ±1.00	476.67 ± 2.89	440.67 ± 3.51	433.67 ± 4.04	401.33 ± 5.51
pH	3.50 ± 0.01	3.46 ± 0.02	3.49 ± 0.03	3.45 ± 0.01	3.53 ± 0.01
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 2.11	73.22 ± 1.53	52.91 ± 0.74	23.71 ± 1.15	13.02 ± 0.31
Viscosity (cP)	524.00 ± 1.00	422.67 ± 5.51	408.67 ± 5.69	405.33 ± 1.53	396.67 ± 6.03
pH	3.52 ± 0.02	3.35 ± 0.01	3.10 ± 0.03	3.06 ± 0.01	3.07 ± 0.02



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on days five (5) and 28 following storage at 40 °C/75% RH and 25 °C/60% RH respectively
- Colour changes were observed after five (5) weeks of storage

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 04 April 2011 **Batch Number: FCV-10**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

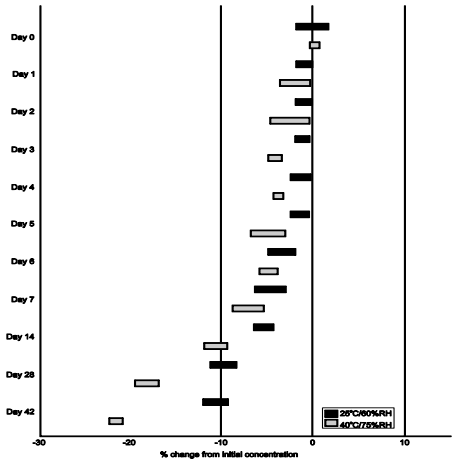
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Methylparaben and propylparaben	0.1 %	0.65g	X061903/X062223
Sodium Metabisulphite	0.05%	0.325 g	RM000265
HPMC	100 ml	650 ml	RM000062

Method of Manufacture: of manufacture: :

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.2.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 1, 2, 3, 4, 5, 6, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 1.57	95.10 ± 2.52	94.46 ± 0.56	90.06 ± 1.63	89.05 ± 1.29
Viscosity (cP)	135.00 ± 1.00	120.67 ± 1.15	109.00 ± 2.00	110.27 ± 0.46	108.13 ± 2.20
pH	7.78 ± 0.02	6.47 ± 0.03	5.83 ± 0.01	5.63 ± 0.03	5.46 ± 0.04
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 0.12	93.19 ± 1.87	89.64 ± 0.64	81.63 ± 1.30	78.32 ± 0.42
Viscosity (cP)	135.33 ± 0.58	114.00 ± 0.00	111.33 ± 2.81	92.13 ± 2.44	81.73 ± 2.66
pH	7.79 ± 0.01	5.48 ± 0.01	5.11 ± 0.02	5.12 ± 0.02	5.03 ± 0.02



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on day 28 following storage at 40 °C/75% RH
- No colour changes were observed

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 04 April 2011 **Batch Number: FCV-11**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

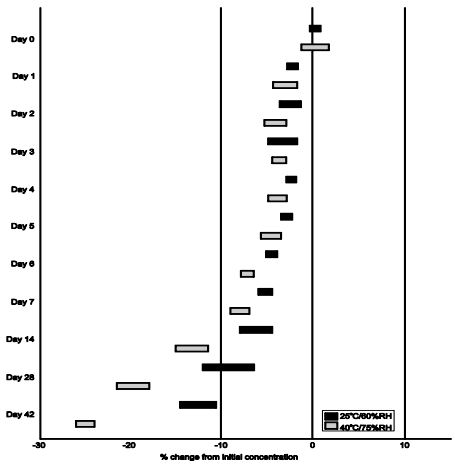
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Methylparaben and propylparaben	0.1 %	0.65g	X061903/X062223
Ascorbic acid	0.05%	0.325 g	RM000265
HPMC	100 ml	650 ml	RM000062

Method of Manufacture: of manufacture: :

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.2.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 1, 2, 3, 4, 5, 6, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 0.53	94.72 ± 0.54	93.28 ± 0.82	90.66 ± 2.91	87.36 ± 1.35
Viscosity (cP)	139.33 ± 0.58	117.67 ± 1.53	107.60 ± 1.39	82.80 ± 1.83	67.73 ± 1.40
pH	8.25 ± 0.01	6.32 ± 0.01	6.01 ± 0.02	6.01 ± 0.03	5.93 ± 0.02
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 1.76	91.68 ± 0.81	86.71 ± 1.80	79.93 ± 1.85	75.08 ± 0.98
Viscosity (cP)	139.00 ± 1.00	87.00 ± 3.46	63.87 ± 2.34	42.00 ± 1.06	37.47 ± 0.46
pH	8.25 ± 0.01	5.75 ± 0.02	5.47 ± 0.02	5.43 ± 0.02	5.29 ± 0.01



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on day 28 after storage at 25 °C/75% RH and 40 °C/75% RH
- Initial colour changes were observed on day Three (3) following storage at 40 °C/75% RH

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 04 April 2011 **Batch Number: FCV-12**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

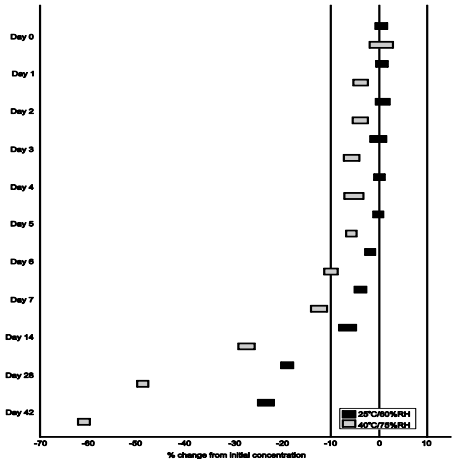
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Methylparaben and propylparaben	0.1 %	0.65g	X061903/X062223
Citric acid	1.0%	6.5 g	RM000265
HPMC	100 ml	650 ml	RM000062

Method of Manufacture: of manufacture: :

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.2.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 1, 2, 3, 4, 5, 6, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 1.08	95.53 ± 0.43	93.12 ± 0.99	80.90 ± 0.71	76.63 ± 1.24
Viscosity (cP)	139.33 ± 0.58	133.67 ± 2.08	132.33 ± 0.58	129.33 ± 2.52	126.33 ± 2.52
pH	3.64 ± 0.01	3.54 ± 0.02	3.53 ± 0.02	3.56 ± 0.03	3.34 ± 0.03
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 2.86	86.73 ± 1.02	72.50 ± 1.01	50.62 ± 0.99	38.62 ± 0.74
Viscosity (cP)	139.00 ± 1.00	122.00 ± 1.00	118.67± 0.58	118.40 ± 2.43	100.67 ± 1.51
pH	3.63 ± 0.00	3.42 ± 0.01	3.22 ± 0.02	3.37 ± 0.02	3.14 ± 0.01



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on days seven (7) and 14 following storage at 40 °C/75% RH and 25 °C/60% RH respectively
- No colour changes were observed

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 05 April 2011 **Batch Number: FCV-13**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

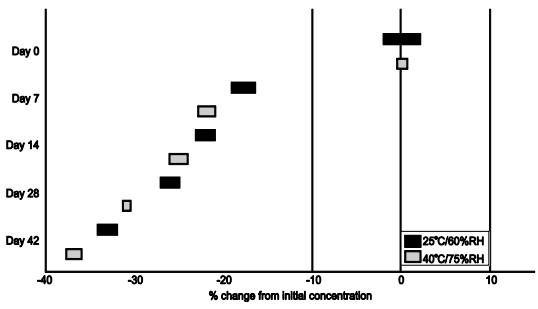
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Methylparaben	0.1 %	0.65g	X061903
Sodium metabisulphite	0.05%	0.325 g	RM000265
Syrup BP	100 ml	650 ml	-

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.1.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 2.20	81.95 ± 1.29	77.73 ± 0.89	73.91 ± 0.72	66.99 ± 0.80
Viscosity (cP)	458.67 ± 1.15	412.67 ± 5.03	372.67 ± 2.08	346.00 ± 3.61	336.00 ± 3.46
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 0.11	78.02 ± 0.92	74.75 ± 1.41	69.10 ± 0.50	63.21 ± 1.10
Viscosity (cP)	456.00 ± 2.00	414.67 ± 3.51	366.67 ± 3.21	362.33 ± 4.73	365.67 ± 4.93



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on day seven (7) following storage at 40 °C/75% RH and 25 °C/60% RH
- Crystallisation of syrup observed following one (1) week of storage
- Colour changes were observed following five (5) weeks of storage

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 05 April 2011 **Batch Number: FCV-14**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

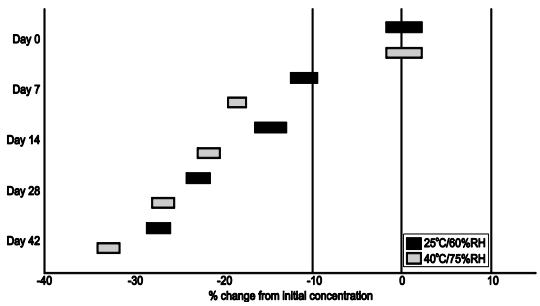
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Methylparaben	0.1 %	0.65g	X061903
Ascorbic acid	0.05%	0.325 g	RM000265
Syrup BP	100 ml	650 ml	-

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.1.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 1.79	88.88 ± 0.99	85.04 ± 1.65	77.04 ± 1.04	72.53 ± 0.99
Viscosity (cP)	495.00 ± 2.65	442.33 ± 0.58	392.67 ± 1.53	352.00 ± 7.81	368.33 ± 2.08
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 2.43	81.36 ± 1.10	78.26 ± 1.39	73.21 ± 1.43	67.08 ± 1.36
Viscosity (cP)	494.33 ± 1.52	407.00 ± 1.00	395.67 ± 3.06	348.67 ± 5.77	342.33 ± 3.06



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on days seven (7) and 14 following storage at 40 °C/75% RH and 25 °C/60% RH Respectively
- Crystallisation of syrup observed after one (1) week of storage
- Initial colour changes were observed on day three (3) following storage at 40 °C/75% RH

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 05 April 2011 **Batch Number: FCV-15**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

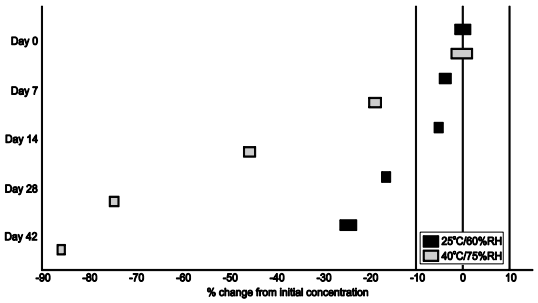
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Methylparaben	0.1 %	0.65g	X061903
Citric acid	1.0%	6.5 g	RM000265
Syrup BP	100 ml	650 ml	-

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.1.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 1.79	88.88 ± 0.99	85.04 ± 1.65	77.04 ± 1.04	72.53 ± 0.99
Viscosity (cP)	495.00 ± 2.65	442.33 ± 0.58	392.67 ± 1.53	352.00 ± 7.81	368.33 ± 2.08
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 2.43	81.36 ± 1.10	78.26 ± 1.39	73.21 ± 1.43	67.08 ± 1.36
Viscosity (cP)	494.33 ± 1.52	407.00 ± 1.00	395.67 ± 3.06	348.67 ± 5.77	342.33 ± 3.06



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on days seven (7) and 14 following storage at 40 °C/75% RH and 25 °C/60% RH respectively
- Colour changes were observed after five (5) weeks of storage

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 05 April 2011 **Batch Number: FCV-16**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

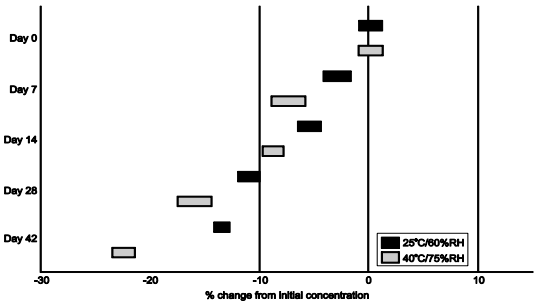
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Methylparaben	0.1 %	0.65g	X061903
Sodium metabisulphite	0.05%	0.325 g	RM000265
HPMC	100 ml	650 ml	RM000062

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.2.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 1.15	96.85 ± 1.44	94.30 ± 0.77	88.80 ± 0.99	86.55 ± 0.07
Viscosity (cP)	137.67 ± 0.58	127.00 ± 1.00	122.80 ± 7.11	114.27 ± 4.63	113.47 ± 2.05
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 1.31	92.44 ± 1.66	89.65 ± 0.64	83.91 ± 1.82	77.44 ± 1.05
Viscosity (cP)	138.33 ± 0.58	121.67 ± 0.58	112.3 ± 4.38	97.60 ± 1.70	97.60 ± 2.82



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on days 14 and 28 following storage at 25 °C/75% RH and 40 °C/75% RH respectively
- No colour changes were observed

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 06 April 2011 **Batch Number: FCV-17**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

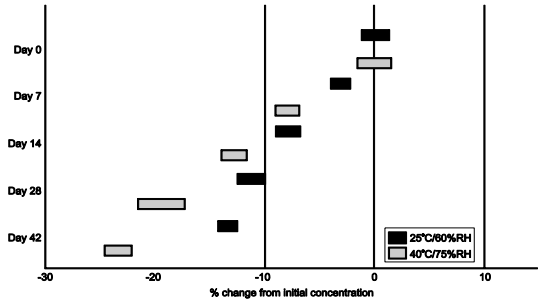
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Methylparaben	0.1 %	0.65g	X061903
Ascorbic acid	0.05%	0.325 g	RM000265
HPMC	100 ml	650 ml	RM000062

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.2.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 0.72	96.55 ± 0.93	92.19 ± 1.22	89.15 ± 0.90	86.71 ± 0.13
Viscosity (cP)	133.33 ± 2.31	112.87 ± 2.14	77.20 ± 4.39	64.40 ± 6.22	55.30 ± 0.92
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 1.47	91.81 ± 0.66	86.94 ± 0.82	80.54 ± 2.66	76.51 ± 1.02
Viscosity (cP)	133.00 ± 1.00	95.87 ± 7.16	61.30 ± 5.60	43.73 ± 0.99	41.73 ± 0.61



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on day 14 following storage at 25 °C/75% RH and 40 °C/75% RH
- Initial colour changes were observed on day three (3) following storage at 40 °C/75% RH

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 06 April 2011 **Batch Number: FCV-18**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

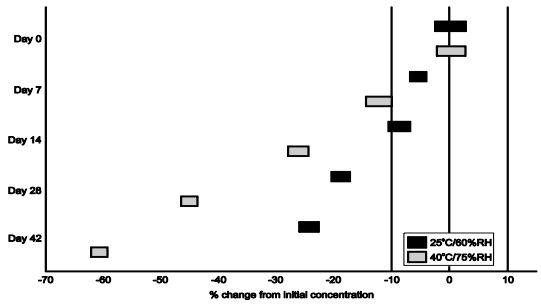
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Methylparaben	0.1 %	0.65g	X061903
Citric acid	1.0%	6.5 g	RM000265
HPMC	100 ml	650 ml	RM000062

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.2.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 2.61	94.29 ± 0.27	91.11 ± 0.89	81.48 ± 0.14	75.03 ± 0.01
Viscosity (cP)	142.33 ± 2.08	135.67 ± 1.15	154.00 ± 1.67	154.27 ± 4.30	154.53 ± 5.43
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 2.28	87.55 ± 2.09	73.71 ± 1.44	54.58 ± 0.78	39.18 ± 1.12
Viscosity (cP)	143.00 ± 1.00	125.33 ± 1.15	134.27 ± 1.67	116.00 ± 2.43	107.07 ± 0.83



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on day14 after storage at 40 °C/75% RH and 25 °C/60% RH respectively
- No colour changes were observed

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 06 April 2011 **Batch Number: FCV-19**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

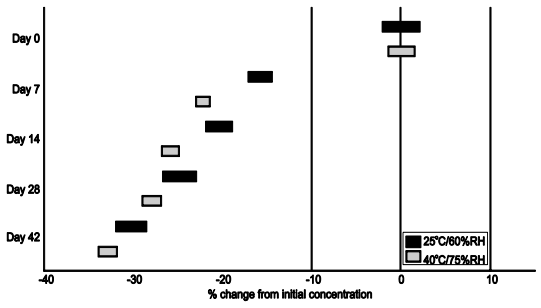
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Propylparaben	0.1 %	0.65g	X062223
Sodium metabisulphite	0.05%	0.325 g	RM000265
Syrup BP	100 ml	650 ml	-

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.1.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 2.32	82.61 ± 0.90	79.53 ± 0.74	74.98 ± 1.60	69.76 ± 1.73
Viscosity (cP)	498.33 ± 1.53	469.67 ± 1.53	395.00 ± 2.00	325.00 ± 3.61	344.33 ± 5.50
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 1.62	77.74 ± 0.80	74.03 ± 0.82	71.37 ± 0.61	67.28 ± 1.36
Viscosity (cP)	498.00 ± 1.00	490.33 ± 1.15	435.33 ± 3.21	384.00 ± 4.58	443.33 ± 2.22



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on day seven (7) following storage at 40 °C/75% RH and 25 °C/60% RH
- Crystallisation of syrup observed after one (1) week of storage
- Colour changes were observed after five (5) weeks of storage

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 06 April 2011 **Batch Number: FCV-20**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

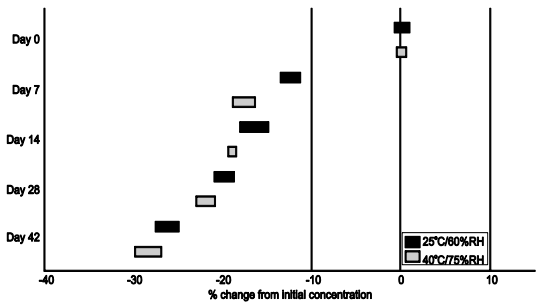
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Propylparaben	0.1 %	0.65g	X062223
Ascorbic acid	0.05%	0.325 g	RM000265
Syrup BP	100 ml	650 ml	-

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.1.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 0.71	87.62 ± 1.06	83.43 ± 1.92	79.98 ± 1.30	73.52 ± 1.42
Viscosity (cP)	491.67 ± 2.08	422.00 ± 2.65	396.33 ± 3.21	314.33 ± 2.52	336.67 ± 4.53
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 1.21	82.16 ± 1.26	80.81 ± 0.24	78.12 ± 1.24	71.49 ± 1.70
Viscosity (cP)	492.00 ± 2.00	445.33 ± 8.39	449.33 ± 1.15	395.67 ± 2.08	450.00 ± 3.01



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on day seven (7) following storage at 40 °C/75% RH and 25 °C/60% RH
- Crystallisation of syrup observed following one (1) week of storage
- Initial colour changes were observed Following three (3) days of storage at 40 °C/75% RH

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 07 April 2011 **Batch Number: FCV-21**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

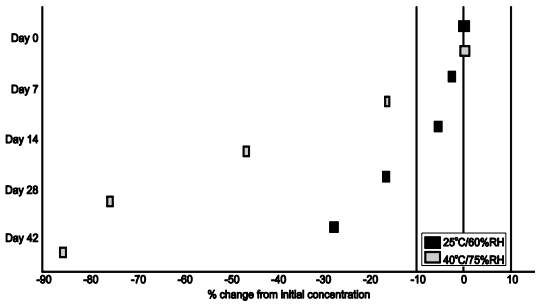
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Propylparaben	0.1 %	0.65g	X062223
Citric acid	1.0%	6.5 g	RM000265
Syrup BP	100 ml	650 ml	-

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.1.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 0.73	96.44 ± 0.07	94.39 ± 0.51	82.65 ± 0.58	72.32 ± 0.67
Viscosity (cP)	419.67 ± 4.51	382.33 ± 2.08	355.00 ± 4.36	337.00 ± 4.36	362.33 ± 0.84
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 0.93	83.29 ± 0.29	52.77 ± 0.50	24.17 ± 0.26	14.55 ± 0.37
Viscosity (cP)	421.33 ± 3.79	376.67 ± 0.58	350.00 ± 2.00	347.00 ± 1.73	335.33 ± 5.09



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on days five (5) and 28 following storage at 40 °C/75% RH and 25 °C/60% RH respectively
- Colour changes were observed following five (5) weeks of storage

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 07 April 2011 **Batch Number: FCV-22**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

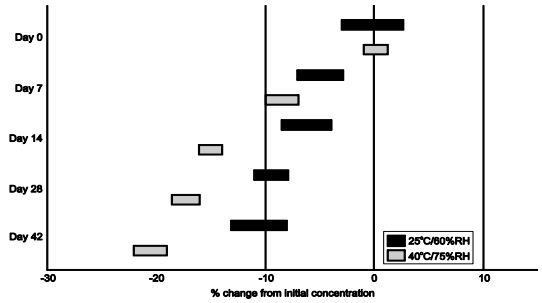
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Propylparaben	0.1 %	0.65g	X062223
Sodium metabisulphite	0.05%	0.325 g	RM000265
HPMC	100 ml	650 ml	RM000062

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.2.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 3.63	94.88 ± 0.93	93.58 ± 1.61	90.28 ± 0.61	89.54 ± 2.24
Viscosity (cP)	156.33± 6.51	145.67± 3.21	118.00± 2.65	115.00± 4.00	114.33± 0.19
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 1.22	91.57 ± 1.80	85.13 ± 0.96	82.54 ± 1.65	79.82 ± 1.92
Viscosity (cP)	154.67± 5.03	135.00± 5.20	103.00± 1.00	106.53± 6.60	105.30± 2.83



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on day 28 following storage at 40 °C/75% RH
- No colour changes were observed

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 07 April 2011 **Batch Number: FCV-23**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

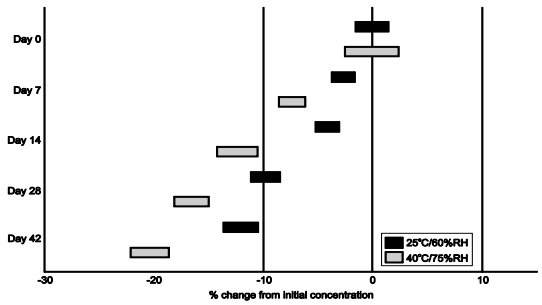
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Propylparaben	0.1 %	0.65g	X062223
Ascorbic acid	0.05%	0.325 g	RM000265
HPMC	100 ml	650 ml	RM000062

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.2.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 1.36	97.38 ± 0.26	95.87 ± 0.76	90.00 ± 1.02	87.93 ± 1.41
Viscosity (cP)	148.67± 7.77	90.13± 3.50	71.30± 4.35	52.53± 4.24	46.00± 2.83
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 2.55	92.49 ± 0.68	87.66 ± 1.59	83.21 ± 0.79	79.51 ± 1.42
Viscosity (cP)	149.67± 4.51	123.47± 3.26	66.67± 1.67	44.40± 0.80	41.20± 0.40



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on days 14 and 28 following storage at 40 °C/75% RH and 25 °C/75% RH respectively
- Initial colour changes were observed on day three (3) following storage at 40 °C/75% RH

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 07 April 2011 **Batch Number: FCV-24**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

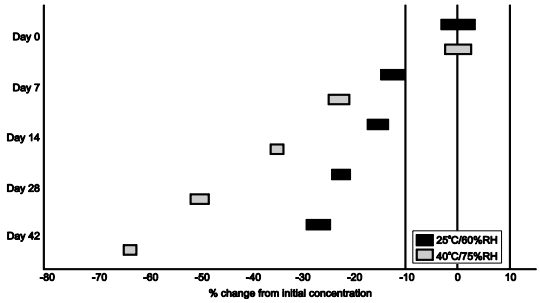
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Propylparaben	0.1 %	0.65g	X062223
Citric acid	1.0%	6.25 g	RM000265
HPMC	100 ml	650 ml	RM000062

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Add the other excipients.
3. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.2.
4. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
5. Transfer the formulation into 6x100 ml bottles.
6. Analyse the formulations on days 0, 7, 14, 28 and 42.

Results Following Testing:

Day	0	7	14	28	42
			25 °C/60% RH		
Content(mg/ml)	100.00 ± 2.62	87.26 ± 1.15	84.35 ± 1.42	77.20 ± 0.38	73.00 ± 2.16
Viscosity (cP)	198.67± 0.58	190.00± 1.73	180.33± 3.21	177.33± 1.53	181.33± 1.39
			40 °C/75% RH		
Content(mg/ml)	100.00 ± 2.30	76.76 ± 1.47	64.93 ± 0.75	49.84 ± 1.51	36.43 ± 0.83
Viscosity (cP)	197.67± 1.53	172.00± 3.61	155.33± 4.73	135.00± 3.61	115.33± 4.44



Stability of FCV from day 0-day 42

Visual Observations

- Significant and relevant FCV degradation observed on day seven (7) following storage at 40 °C/75% RH and 25 °C/60% RH
- No colour changes were observed

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus
Product: 25 mg/ml FCV suspension **Batch Size: 650 ml**
Date of Manufacture: 13 May 2011 **Batch Number: FCV-25**
Storage Conditions: 25 °C/60% RH and 40 °C/75% RH

Formula:

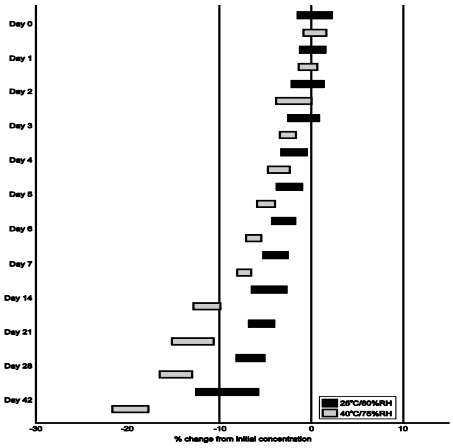
Raw Material	Original Formula	Working Formula	Receiving Number
FCV	2.5 g	16.25 g	RM000252
Phosphate buffer (50 mM; pH 6)	100 ml	650 ml	-

Method of Manufacture:

1. Accurately weigh 16.25 g of FCV and transfer to a mortar.
2. Gradually incorporate the vehicle, manufactured as described in §4.5.3.1.3.
3. Transfer the product into a measuring cylinder and make up to a volume of 650 ml.
4. Transfer the formulation into 6x100 ml bottles.
5. Analyse the formulations on days 0, 7, 14, 21, 28 and 42.

Results Following Testing:

Day	0	1	2	3	4	5
			25 °C/60% RH			
Content	100.00 ± 1.66	99.76 ± 0.84	99.29 ± 1.40	98.91 ± 0.90	97.91 ± 0.92	97.32 ± 0.81
			40 °C/75%RH			
Content	100.00 ± 1.04	99.30 ± 0.78	97.82 ± 2.33	97.07 ± 0.61	96.14 ± 1.09	94.80 ± 0.82
Day	**6**	**7**	**14**	**21**	**28**	**42**
			25 °C/60% RH			
Content	96.72 ± 0.91	95.98 ± 0.70	95.23 ± 1.69	94.36 ± 0.70	93.30 ± 1.12	90.81 ± 3.87
			40 °C/75%RH			
Content	93.37 ± 0.41	92.49 ± 0.24	88.49 ± 1.52	87.11 ± 2.49	85.21 ± 1.92	80.40 ± 2.22



Stability of FCV from day 0-day 42

Visual Observations

- No changes in appearance were observed over the period of 42 days

All raw data is available on request.

APPENDIX II

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 28 July 2011 **Run Number: 1**

Storage Conditions: 40 °C/75% RH **ID Number: 0**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.05%	162.5 mg	RM000265
Methylparaben and propylparaben	0.1%	325.0 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Level:

pH	6
% HPMC	0.75%
% Sodium metabisulphite	0.05%
% Methylparaben and propylparaben	0.1%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar
2. Add the other excipients
3. Gradually incorporate the HPMC
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 6 using orthophosphoric acid
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results following testing

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 3.51	99.97 ± 2.31	98.19 ± 2.63	96.02 ± 2.40	94.37 ± 1.43
Viscosity (cP)	634.33 ± 0.58	566.00 ± 5.20	492.67 ± 2.31	480.67 ± 5.13	402.00 ± 3.00
pH	6.02 ± 0.01	5.99 ± 0.05	5.86 ± 0.03	5.77 ± 0.02	5.75 ± 0.04
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	92.16 ± 1.81	90.38 ± 1.36	87.28 ± 0.80	83.82 ± 1.36	81.26 ± 1.36
Viscosity (cP)	379.33 ± 9.02	334.00 ± 7.21	282.00 ± 2.65	277.00 ± 6.56	271.67 ± 6.03
pH	5.73 ± 0.02	5.73 ± 0.01	5.74 ± 0.03	5.74 ± 0.03	5.74 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 28 July 2011 **Run Number: 2**

Storage Conditions: 40 °C/75% RH **ID Number: 5**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.07%	227.5 mg	RM000265
Methyl paraben and propyl paraben	0.075%	243.75 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Level:

pH	5
% HPMC	0.5%
% Sodium metabisulphite	0.07%
% Methylparaben and propylparaben	0.075%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar
2. Add the other excipients
3. Gradually incorporate the HPMC
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 5 using orthophosphoric acid
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results following testing

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.56	99.94 ± 0.88	97.96 ± 0.09	94.58 ± 0.52	91.35 ± 1.48
Viscosity (cP)	199.67 ± 2.52	164.00 ± 6.00	152.67 ± 5.03	135.33 ± 4.73	128.00 ± 3.00
pH	4.98 ± 0.01	5.02 ± 0.05	4.88 ± 0.02	4.85 ± 0.04	4.84 ± 0.01
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	88.73 ± 0.86	86.00 ± 0.30	81.14 ± 0.44	78.17 ± 0.91	73.90 ± 1.16
Viscosity (cP)	117.33 ± 3.06	105.47 ± 1.22	103.67 ±2.08	93.67 ± 2.08	91.00 ± 1.00
pH	4.82 ± 0.01	4.80 ± 0.01	4.82 ± 0.02	4.76 ± 0.02	4.76 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 28 July 2011 **Run Number: 3**

Storage Conditions: 40 °C/75% RH **ID Number: 7**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.07%	227.5 mg	RM000265
Methyl paraben and propyl paraben	0.075%	243.75 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Level:

pH	7
% HPMC	0.5%
% Sodium metabisulphite	0.07%
% Methylparaben and propylparaben	0.075%

Method:

6. Accurately weigh 8.125 g of FCV and transfer into a mortar.
7. Add the other excipients.
8. Gradually incorporate the HPMC.
9. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
10. Measure the pH and adjust to pH 7 using sodium hydroxide.
11. Transfer the formulation into 3x100 ml bottles.
12. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results following testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.85	99.74 ± 0.17	95.59 ± 0.88	91.46 ± 0.14	88.58 ± 0.91
Viscosity (cP)	203.33 ± 1.53	165.67 ± 3.06	149.33 ± 5.03	136.33 ± 2.08	128.33 ± 2.52
pH	7.00 ± 0.00	6.96 ± 0.02	6.85 ± 0.03	6.80 ± 0.04	6.75 ± 0.03
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	85.02 ± 1.46	80.76 ± 0.85	75.08 ± 0.36	71.97 ± 0.63	65.61 ± 1.09
Viscosity (cP)	119.00 ± 5.57	112.93 ± 2.05	107.33 ± 2.52	94.33 ± 3.21	92.33 ± 3.21
pH	6.73 ± 0.03	6.71 ± 0.02	6.70 ± 0.01	6.71 ± 0.01	6.69 ± 0.01

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 28 July 2011 **Run Number: 4**

Storage Conditions: 40 °C/75% RH **ID Number: 10**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.03%	97.5 mg	RM000265
Methyl paraben and propyl paraben	0.125%	406.25 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Level:

pH	5
% HPMC	1%
% Sodium metabisulphite	0.03%
% Methylparaben and propylparaben	0.125%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 5 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results following testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 1.09	100.21±1.46	97.14 ± 1.25	94.56 ± 1.06	90.66 ± 0.44
Viscosity (cP)	1151.33 ± 6.43	1097.33 ± 23.69	953.33 ± 6.43	801.33 ± 4.16	738.00 ± 8.00
pH	5.03 ± 0.01	4.93 ± 0.02	4.89 ± 0.02	4.85 ± 0.02	4.86 ± 0.01
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	88.22 ± 0.56	84.63 ± 0.26	81.52 ± 1.13	78.07 ± 1.18	73.37 ± 0.68
Viscosity (cP)	703.67 ± 6.66	660.67 ± 9.02	595.33 ± 5.03	584.00 ± 8.00	558.67 ± 7.57
pH	4.86 ± 0.02	4.86 ± 0.02	4.85 ± 0.03	4.81 ± 0.03	4.80 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 28 July 2011 **Run Number: 5**

Storage Conditions: 40 °C/75% RH **ID Number: 9**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.03%	97.5 mg	RM000265
Methyl paraben and propyl paraben	0.125%	406.25 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Level:

pH	5
% HPMC	0.5%
% Sodium metabisulphite	0.03%
% Methylparaben and propylparaben	0.125%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 5 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results following testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.43	98.24 ± 0.27	95.64 ± 0.55	95.10 ± 1.30	92.70 ± 0.42
Viscosity (cP)	199.00 ± 2.65	177.00 ± 2.65	158.00 ± 6.56	125.33 ± 3.51	119.00 ± 4.58
pH	4.96 ± 0.06	4.93 ± 0.02	4.92 ± 0.04	4.92 ± 0.01	4.88 ± 0.03
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	91.07 ± 0.58	86.87 ± 0.78	81.54 ± 0.43	74.91 ± 0.81	74.91 ± 0.37
Viscosity (cP)	111.67 ± 3.79	105.33 ± 4.16	103.67 ± 3.21	103.27 ± 2.87	96.33 ± 2.08
pH	4.88 ± 0.03	4.87 ± 0.03	4.86 ± 0.03	4.84 ± 0.04	4.82 ± 0.03

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 28 July 2011 **Run Number: 6**

Storage Conditions: 40 °C/75% RH **ID Number: 14**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.07%	227.5 mg	RM000265
Methyl paraben and propyl paraben	0.125%	406.25 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Level:

pH	5
% HPMC	1%
% Sodium metabisulphite	0.07%
% Methylparaben and propylparaben	0.125%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 5 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.22	99.63 ± 0.34	97.65 ± 0.71	94.70 ± 0.50	93.34 ± 0.76
Viscosity (cP)	1144.67 ± 8.08	1130.00 ± 12.49	1006.00 ± 22.72	876.00 ± 24.25	794.67 ± 5.03
pH	4.98 ± 0.01	4.95 ± 0.03	4.89 ± 0.02	4.89 ± 0.03	4.81 ± 0.03
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	90.89 ± 0.50	86.76 ± 0.56	81.49 ± 1.08	76.68 ± 0.50	72.58 ± 1.02
Viscosity (cP)	731.67 ± 3.51	669.33 ± 18.15	596.67 ± 6.11	582.00 ± 9.17	576.00 ± 5.29
pH	4.77 ± 0.03	4.78 ± 0.02	4.78 ± 0.02	4.77 ± 0.01	4.77 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 02 August 2011 **Run Number: 7**

Storage Conditions: 40 °C/75% RH **ID Number: 8**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.07%	227.5 mg	RM000265
Methyl paraben and propyl paraben	0.075%	243.75 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	7
% HPMC	1%
% Sodium metabisulphite	0.07%
% Methylparaben and propylparaben	0.075%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 7 using sodium hydroxide.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.35	100.24±1.83	98.27 ± 1.69	92.20 ± 0.16	88.60 ± 0.92
Viscosity (cP)	1171.33 ± 12.86	1060.67 ± 16.04	1021.33 ± 15.80	964.67 ± 22.91	902.00± 16.37
pH	6.99 ± 0.01	6.84 ± 0.01	6.74 ± 0.07	6.74 ± 0.07	6.74 ± 0.00
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	83.63 ± 1.11	78.37 ± 2.50	73.55 ± 2.67	70.09 ± 1.33	63.17 ± 0.58
Viscosity (cP)	739.33 ± 29.48	687.33 ± 3.06	666.00 ± 11.14	658.00 ± 15.87	648.67± 14.57
pH	6.74 ± 0.06	6.78 ± 0.05	6.78 ± 0.01	6.77 ± 0.05	6.76 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 02 August 2011 **Run Number: 8**

Storage Conditions: 40 °C/75% RH **ID Number: 2**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.03%	97.5 mg	RM000265
Methyl paraben and propyl paraben	0.075%	243.75 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	5
% HPMC	1%
% Sodium metabisulphite	0.03%
% Methylparaben and propylparaben	0.075%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 7 using sodium hydroxide.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 1.27	99.93 ± 0.25	98.28 ± 0.40	94.54 ± 0.49	93.28 ± 0.85
Viscosity (cP)	1205.33 ± 6.11	1037.33 ± 15.14	1018.67 ± 8.08	901.67 ± 7.63	822.00 ± 24.57
pH	5.05 ± 0.01	4.87 ± 0.03	4.86 ± 0.06	4.89 ± 0.03	4.89 ± 0.03
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	92.89 ± 0.82	90.31 ± 0.10	85.15 ± 0.31	83.12 ± 1.23	80.05 ± 2.06
Viscosity (cP)	703.33 ± 4.16	667.33 ± 3.06	659.33 ± 9.02	612.67 ± 8.32	604.33 ± 6.66
pH	4.82 ± 0.03	4.80 ± 0.03	4.73 ± 0.01	4.73 ± 0.03	4.75 ± 0.03

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 28 July 2011 **Run Number: 9**

Storage Conditions: 40 °C/75% RH **ID Number: 12**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.03%	97.5 mg	RM000265
Methyl paraben and propyl paraben	0.125%	406.25 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	7
% HPMC	1%
% Sodium metabisulphite	0.03%
% Methylparaben and propylparaben	0.125%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 7 using sodium hydroxide.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.43	100.22±0.43	96.51 ± 0.89	90.11 ± 0.33	86.90 ± 0.42
Viscosity (cP)	1209.33 ± 4.62	1060.00 ±10.58	1006.00± 25.06	970.67± 10.07	970.00 ± 5.29
pH	6.95 ± 0.02	6.84 ± 0.04	6.84 ± 0.02	6.80 ± 0.04	6.80 ± 0.03
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	83.10 ± 0.48	73.84 ± 1.49	69.79 ± 1.94	67.80 ± 1.38	62.29 ± 1.26
Viscosity (cP)	818.00 ± 8.72	698.00 ± 13.86	688.00 ± 19.29	655.33 ± 6.43	639.67±13.80
pH	6.79 ± 0.01	6.80 ± 0.04	6.78 ± 0.04	6.79 ± 0.02	6.76 ± 0.03

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 28 July 2011 **Run Number: 10**

Storage Conditions: 40 °C/75% RH **ID Number: 20**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.05%	162.5 mg	RM000265
Methyl paraben and propyl paraben	0.10%	325.0 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	8
% HPMC	0.75%
% Sodium metabisulphite	0.05%
% Methylparaben and propylparaben	0.10%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 8 using sodium hydroxide.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 1.02	94.45 ± 1.92	87.40 ± 2.50	77.12 ± 2.11	65.86 ± 1.42
Viscosity (cP)	607.33 ± 2.08	520.33 ± 4.93	490.67 ± 5.51	480.33 ± 6.51	419.67 ± 1.53
pH	7.95 ± 0.00	7.92 ± 0.02	7.95 ± 0.02	7.84 ± 0.05	7.73 ± 0.05
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	57.81 ± 1.55	47.36 ± 2.61	42.06 ± 2.14	35.61 ± 1.82	23.57 ± 1.02
Viscosity (cP)	402.00 ±10.44	406.33 ± 10.69	379.67 ± 4.51	372.33 ±10.79	355.00 ± 2.00
pH	7.74 ± 0.05	7.67 ± 0.07	7.70 ± 0.07	7.63 ± 0.03	7.62 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 28 July 2011 **Run Number: 11**

Storage Conditions: 40 °C/75% RH **ID Number: 15**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.07%	227.5 mg	RM000265
Methyl paraben and propyl paraben	0.125%	406.25 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	7
% HPMC	0.50%
% Sodium metabisulphite	0.07%
% Methylparaben and propylparaben	0.125%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 7 using sodium hydroxide.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.41	99.73 ± 1.11	96.69 ± 0.89	90.79 ± 0.17	88.95 ± 1.28
Viscosity (cP)	202.33 ± 6.81	167.67 ± 4.04	161.00 ± 2.00	149.67 ± 2.08	145.33 ± 3.79
pH	7.04 ± 0.04	7.03 ± 0.02	6.73 ± 0.02	6.71 ± 0.04	6.75 ± 0.01
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	85.43 ± 1.13	76.62 ± 0.65	75.23 ± 0.85	71.59 ± 1.26	66.73 ± 1.43
Viscosity (cP)	119.00 ± 2.65	108.67 ± 2.52	104.33 ± 4.51	102.33 ± 2.52	96.00 ± 2.65
pH	6.72 ± 0.02	6.74 ± 0.04	6.75 ± 0.04	6.73 ± 0.03	6.72 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 28 July 2011 **Run Number: 12**

Storage Conditions: 40 °C/75% RH **ID Number: 06**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.07%	227.5 mg	RM000265
Methyl paraben and propyl paraben	0.075%	243.75 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	5
% HPMC	1.00%
% Sodium metabisulphite	0.07%
% Methylparaben and propylparaben	0.075%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 5 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 2.16	99.52 ± 1.34	97.53 ± 0.73	94.79 ± 1.61	93.16 ± 0.66
Viscosity (cP)	1194.00 ± 9.165	1042.67 ± 22.03	1014.00 ± 19.08	876.00 ± 15.10	820.67 ± 8.32
pH	5.02 ± 0.08	4.92 ± 0.03	4.87 ± 0.06	4.83 ± 0.04	4.84 ± 0.04
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	90.78 ± 1.00	86.83 ± 2.17	84.96 ± 0.46	81.74 ± 2.62	79.61 ± 0.42
Viscosity (cP)	724.33 ± 8.14	680.67 ± 14.47	656.67 ± 12.06	622.00 ± 14.00	604.67 ± 6.43
pH	4.84 ± 0.04	4.84 ± 0.02	4.85 ± 0.01	4.77 ± 0.03	4.74 ± 0.04

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 07 August 2011 **Run Number: 13**

Storage Conditions: 40 °C/75% RH **ID Number: 04**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.03%	97.5 mg	RM000265
Methyl paraben and propyl paraben	0.075%	243.75 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	7
% HPMC	1.00%
% Sodium metabisulphite	0.03%
% Methylparaben and propylparaben	0.075%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 7 using sodium hydroxide.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 1.07	99.63 ± 0.43	98.24 ± 0.68	93.05 ± 1.58	88.26 ± 2.08
Viscosity (cP)	1467.33 ± 23.69	1208.67 ± 9.02	1076.67 ± 15.14	1028.67 ± 3.06	876.00 ± 19.29
pH	6.98 ± 0.01	6.94 ± 0.03	6.73 ± 0.02	6.72 ± 0.04	6.75 ± 0.04
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	86.87 ± 0.96	80.02 ± 1.72	75.70 ± 2.19	68.16 ± 1.26	61.15 ± 0.51
Viscosity (cP)	927.33 ± 7.02	879.33 ± 10.07	787.33 ± 8.33	726.00 ± 6.00	694.67 ± 4.16
pH	6.76 ± 0.06	6.76 ± 0.03	6.76 ± 0.03	6.78 ± 0.03	6.72 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 07 August 2011 **Run Number: 14**

Storage Conditions: 40 °C/75% RH **ID Number: 00**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.05%	162.5 mg	RM000265
Methyl paraben and propyl paraben	0.10%	325.0 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	6
% HPMC	0.75%
% Sodium metabisulphite	0.05%
% Methylparaben and propylparaben	0.10%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 6 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.90	100.13 ± 0.16	99.22 ± 1.24	96.64 ± 0.82	94.89 ± 0.53
Viscosity (cP)	709.67 ± 3.21	551.00 ± 1.011	494.67 ± 9.87	473.67 ± 4.04	391.33 ± 14.57
pH	6.09 ± 0.00	5.85 ± 0.01	5.80 ± 0.07	5.82 ± 0.03	5.85 ± 0.04
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	92.90 ± 0.80	90.07 ± 0.47	86.59 ± 0.09	84.19 ± 0.49	82.67 ± 0.71
Viscosity (cP)	349.00 ± 6.56	347.67 ± 8.74	336.67 ± 10.60	299.33 ± 4.16	277.00 ± 6.56
pH	5.74 ± 0.02	5.76 ± 0.04	5.76 ± 0.03	5.73 ± 0.01	5.71 ± 0.01

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 07 August 2011 **Run Number: 15**

Storage Conditions: 40 °C/75% RH **ID Number: 22**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.09%	292.5 mg	RM000265
Methyl paraben and propyl paraben	0.10%	325.0 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	6
% HPMC	0.75%
% Sodium metabisulphite	0.09%
% Methylparaben and propylparaben	0.10%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 6 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.63	99.79 ± 1.51	98.08 ± 1.43	96.32 ± 0.91	92.85 ± 0.47
Viscosity (cP)	704.00 ± 3.61	553.33 ± 10.50	521.00 ± 10.54	483.67 ± 4.51	399.00 ± 3.61
pH	6.06 ± 0.01	5.84 ± 0.01	5.87 ± 0.01	5.84 ± 0.04	5.81 ± 0.05
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	91.81 ± 0.45	90.18 ± 0.45	87.49 ± 0.23	84.82 ± 1.00	82.08 ± 1.11
Viscosity (cP)	351.67 ± 4.73	338.00 ± 7.21	335.67 ± 4.04	296.67 ± 8.32	288.67 ± 4.04
pH	5.76 ± 0.06	5.78 ± 0.01	5.76 ± 0.02	5.76 ± 0.02	5.74 ± 0.01

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 07 August 2011 **Run Number: 16**

Storage Conditions: 40 °C/75% RH **ID Number: 00**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.05%	162.5 mg	RM000265
Methyl paraben and propyl paraben	0.10%	325.0 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	6
% HPMC	0.75%
% Sodium metabisulphite	0.05%
% Methylparaben and propylparaben	0.10%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 6 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 1.58	100.33±0.96	97.86 ± 0.65	97.13 ± 0.34	94.86 ± 1.15
Viscosity (cP)	709.67 ± 2.08	539.33 ± 2.08	498.33 ± 8.62	466.67 ± 4.16	401.67 ± 6.51
pH	6.03 ± 0.01	5.88 ± 0.01	5.86 ± 0.03	5.83 ± 0.04	5.79 ± 0.03
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	92.43 ± 2.54	90.74 ± 1.26	88.30 ± 1.61	83.89 ± 0.76	81.52 ± 1.11
Viscosity (cP)	349.33 ± 4.04	342.33 ± 5.51	336.00 ± 6.08	294.67 ± 6.11	281.00 ± 9.64
pH	5.79 ± 0.03	5.78 ± 0.02	5.75 ± 0.02	5.75 ± 0.02	5.76 ± 0.03

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 07 August 2011 **Run Number: 17**

Storage Conditions: 40 °C/75% RH **ID Number: 18**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.05%	162.5 mg	RM000265
Methyl paraben and propyl paraben	0.10%	325.0 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	6
% HPMC	1.25%
% Sodium metabisulphite	0.05%
% Methylparaben and propylparaben	0.10%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 6 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.47	98.63 ± 0.22	98.45 ± 2.25	96.08 ± 0.99	94.34 ± 1.38
Viscosity (cP)	2881.33 ± 26.63	2368.00 ± 32.00	2245.3 ± 42.39	2013.3 ± 38.02	1518.67 ± 11.72
pH	6.08 ± 0.01	5.97 ± 0.03	5.93 ± 0.07	5.84 ± 0.04	5.71 ± 0.01
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	92.48 ± 2.22	92.24 ± 1.38	91.06 ± 0.87	89.26 ± 0.77	85.81 ± 0.15
Viscosity (cP)	1432.00 ± 16.00	1324.00 ± 24.33	1312.00 ± 10.58	1287.33 ± 9.45	1230.67 ± 9.45
pH	5.76 ± 0.05	5.76 ± 0.03	5.74 ± 0.02	5.76 ± 0.02	5.74 ± 0.01

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 07 August 2011 **Run Number: 18**

Storage Conditions: 40 °C/75% RH **ID Number: 13**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.07%	227.5 mg	RM000265
Methyl paraben and propyl paraben	0.125%	406.25 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	5
% HPMC	0.50%
% Sodium metabisulphite	0.07%
% Methylparaben and propylparaben	0.125%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 5 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.09	100.56 ± 0.55	98.87 ± 1.07	93.08 ± 1.62	90.24 ± 0.23
Viscosity (cP)	233.33 ± 2.89	190.00 ± 8.00	178.67 ± 5.03	150.67 ± 4.04	131.67 ± 3.51
pH	5.00 ± 0.02	4.89 ± 0.01	4.74 ± 0.01	4.75 ± 0.02	4.73 ± 0.02
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	86.65 ± 0.38	84.81 ± 1.09	79.00 ± 0.44	76.98 ± 0.77	73.17 ± 0.72
Viscosity (cP)	128.00 ± 2.00	128.00 ± 4.58	121.00 ± 2.65	112.67 ± 2.52	100.67 ± 3.06
pH	4.76 ± 0.04	4.79 ± 0.01	4.75 ± 0.03	4.78 ± 0.01	4.72 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 12 August 2011 **Run Number: 19**

Storage Conditions: 40 °C/75% RH **ID Number: 00**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.05%	162.5 mg	RM000265
Methyl paraben and propyl paraben	0.10%	325.0 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	6
% HPMC	0.75%
% Sodium metabisulphite	0.05%
% Methylparaben and propylparaben	0.10%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 6 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 1.37	99.62 ± 1.88	97.74 ± 2.47	96.74 ± 3.03	94.65 ± 2.83
Viscosity (cP)	643.33 ± 9.71	531.33 ± 3.21	518.00 ± 7.00	449.00 ± 8.54	393.00 ± 13.08
pH	6.07 ± 0.04	5.89 ± 0.03	5.86 ± 0.01	5.87 ± 0.03	5.89 ± 0.06
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	92.37 ± 2.26	90.53 ± 1.64	86.91 ± 2.63	84.37 ± 1.39	81.17 ± 1.25
Viscosity (cP)	351.00 ± 7.94	329.67 ± 4.73	320.67 ± 5.03	303.00 ± 5.57	290.67 ± 3.06
pH	5.85 ± 0.02	5.85 ± 0.01	5.82 ± 0.02	5.78 ± 0.01	5.75 ± 0.03

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 12 August 2011 **Run Number: 20**

Storage Conditions: 40 °C/75% RH **ID Number: 19**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.05%	162.5 mg	RM000265
Methyl paraben and propyl paraben	0.10%	325.0 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	4
% HPMC	0.75%
% Sodium metabisulphite	0.05%
% Methylparaben and propylparaben	0.10%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 4 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.62	100.14±0.83	95.12 ± 0.69	90.10 ± 0.06	84.70 ± 2.05
Viscosity (cP)	653.67 ± 3.21	535.67 ± 4.04	519.33 ± 7.02	491.67 ± 6.81	466.67 ± 7.57
pH	4.07 ± 0.03	3.92 ± 0.03	3.93 ± 0.03	3.75 ± 0.03	3.78 ± 0.05
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	79.97 ± 0.84	72.15 ± 0.77	63.73 ± 0.56	59.00 ± 0.89	51.00 ± 0.85
Viscosity (cP)	448.33 ± 9.61	461.67 ± 5.86	427.33 ± 8.02	392.00 ± 4.00	370.67 ± 3.06
pH	3.77 ± 0.05	3.76 ± 0.02	3.74 ± 0.03	3.75 ± 0.01	3.75 ± 0.01

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 12 August 2011 **Run Number: 21**

Storage Conditions: 40 °C/75% RH **ID Number: 17**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.05%	162.5 mg	RM000265
Methyl paraben and propyl paraben	0.10%	325.0 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	6
% HPMC	0.25%
% Sodium metabisulphite	0.05%
% Methylparaben and propylparaben	0.10%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 6 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.68	100.00±0.84	98.51 ± 1.70	96.62 ± 0.77	94.97 ± 0.76
Viscosity (cP)	58.93 ± 1.01	53.07 ± 0.61	51.33 ± 1.29	47.33 ± 0.83	40.80 ± 1.06
pH	6.04 ± 0.04	5.88 ± 0.02	5.77 ± 0.02	5.76 ± 0.03	5.79 ± 0.05
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	94.46 ± 0.88	90.13 ± 0.65	86.72 ± 0.70	83.33 ± 0.70	80.80 ± 1.06
Viscosity (cP)	40.27 ± 1.51	40.80 ± 1.05	40.13 ± 0.23	39.33 ± 1.15	38.00 ± 2.00
pH	5.79 ± 0.03	5.81 ± 0.04	5.76 ± 0.03	5.77 ± 0.01	5.76 ± 0.03

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 12 August 2011 **Run Number: 22**

Storage Conditions: 40 °C/75% RH **ID Number: 01**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.03%	97.50 mg	RM000265
Methyl paraben and propyl paraben	0.075%	243.75 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	5
% HPMC	0.50%
% Sodium metabisulphite	0.03%
% Methylparaben and propylparaben	0.075%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 5 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.72	99.24 ± 2.87	98.64 ± 2.46	95.60 ± 2.24	93.37 ± 2.38
Viscosity (cP)	197.00 ± 6.56	166.33 ± 5.51	156.33 ± 5.51	131.33 ± 8.50	115.33 ± 4.16
pH	5.02 ± 0.02	4.92 ± 0.02	4.87 ± 0.01	4.75 ± 0.02	4.78 ± 0.01
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	88.75 ± 2.23	83.96 ± 1.71	81.35 ± 0.84	77.16 ± 1.13	74.84 ± 1.52
Viscosity (cP)	108.67 ± 4.51	101.67 ± 2.08	99.00 ± 3.61	96.33 ± 1.53	92.00 ± 2.00
pH	4.79 ± 0.05	4.78 ± 0.01	4.76 ± 0.05	4.76 ± 0.02	4.74 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 12 August 2011 **Run Number: 23**

Storage Conditions: 40 °C/75% RH **ID Number: 03**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.03%	97.50 mg	RM000265
Methyl paraben and propyl paraben	0.075%	243.75 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	7
% HPMC	0.50%
% Sodium metabisulphite	0.03%
% Methylparaben and propylparaben	0.075%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 5 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.61	97.67 ± 0.84	96.29 ± 2.55	92.08 ± 0.51	89.20 ± 2.56
Viscosity (cP)	197.33 ± 5.51	167.67 ± 7.51	160.67 ± 3.06	141.33 ± 2.52	124.00 ± 6.56
pH	7.00 ± 0.02	6.82 ± 0.02	6.78 ± 0.04	6.78 ± 0.03	6.72 ± 0.02
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	83.45 ± 0.46	76.68 ± 0.45	71.88 ± 0.32	69.24 ± 3.35	60.86 ± 2.57
Viscosity (cP)	120.67 ± 1.53	109.33 ± 4.16	105.33 ± 3.05	97.33 ± 1.15	92.67 ± 2.31
pH	6.78 ± 0.04	6.72 ± 0.04	6.74 ± 0.03	6.70 ± 0.02	6.67 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 12 August 2011 **Run Number: 24**

Storage Conditions: 40 °C/75% RH **ID Number: 11**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.03%	97.50 mg	RM000265
Methyl paraben and propyl paraben	0.125%	406.25 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	7
% HPMC	0.50%
% Sodium metabisulphite	0.03%
% Methylparaben and propylparaben	0.125%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 5 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.67	97.65 ± 0.37	95.03 ± 0.94	92.30 ± 0.56	89.16 ± 0.76
Viscosity (cP)	194.00 ± 4.00	180.67 ± 4.04	175.67 ± 2.89	142.33 ± 5.51	126.33 ± 4.04
pH	7.04 ± 0.00	6.87 ± 0.02	6.74 ± 0.01	6.73 ± 0.03	6.77 ± 0.03
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	85.85 ± 0.35	77.61 ± 1.66	73.15 ± 0.54	69.90 ± 2.07	61.00 ± 2.09
Viscosity (cP)	118.67 ± 1.53	109.00 ± 1.00	110.33 7.77	102.00 ± 2.00	98.00 ± 2.00
pH	6.80 ± 0.02	6.81 ± 0.04	6.77 ± 0.03	6.73 ± 0.01	6.69 ± 0.01

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 17 August 2011 **Run Number: 25**

Storage Conditions: 40 °C/75% RH **ID Number: 00**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.05%	162.5 mg	RM000265
Methyl paraben and propyl paraben	0.10%	325.0 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	6
% HPMC	0.75%
% Sodium metabisulphite	0.05%
% Methylparaben and propylparaben	0.10%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 6 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 1.30	99.99 ± 1.21	97.28 ± 1.47	96.39 ± 0.22	94.50 ± 2.87
Viscosity (cP)	682.33 ± 4.93	546.67 ± 6.66	515.67 ± 5.13	478.00 ± 17.44	403.67 ± 4.16
pH	6.06 ± 0.02	5.86 ± 0.02	5.83 ± 0.04	5.86 ± 0.03	5.85 ± 0.05
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	93.58 ± 3.23	90.27 ± 1.66	88.27 ± 0.95	85.35 ± 1.81	83.19 ± 2.89
Viscosity (cP)	344.33 ± 4.04	333.33 ± 2.52	323.67 ± 3.21	303.00 ± 3.00	292.67 ± 3.06
pH	5.82 ± 0.02	5.82 ± 0.03	5.82 ± 0.02	5.81 ± 0.02	5.78 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 17 August 2011 **Run Number: 26**

Storage Conditions: 40 °C/75% RH **ID Number: 16**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.07%	227.5 mg	RM000265
Methyl paraben and propyl paraben	0.125%	406.25 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	7
% HPMC	1.00%
% Sodium metabisulphite	0.07%
% Methylparaben and propylparaben	0.125%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 7 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.95	98.77 ± 0.28	96.39 ± 1.49	90.44 ± 0.39	87.12 ± 0.63
Viscosity (cP)	1454.00 ± 5.29	1201.33 ± 4.16	1142.00 ± 13.86	1056.67 ± 21.57	966.00 ± 8.72
pH	7.02 ± 0.04	6.82 ± 0.02	6.79 ± 0.03	6.82 ± 0.05	6.80 ± 0.02
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	83.89 ± 0.84	79.76 ± 0.76	72.49 ± 0.61	69.78 ± 2.86	62.57 ± 2.75
Viscosity (cP)	876.00 ± 3.46	814.67 ± 7.02	784.00 ± 8.72	732.00 ± 5.29	701.33 ± 3.06
pH	6.78 ± 0.03	6.80 ± 0.02	6.79 ± 0.02	6.78 ± 0.01	6.76 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 17 August 2011 **Run Number: 27**

Storage Conditions: 40 °C/75% RH **ID Number: 23**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.05%	162.5 mg	RM000265
Methyl paraben and propyl paraben	0.05%	162.5 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	6
% HPMC	0.75%
% Sodium metabisulphite	0.05%
% Methylparaben and propylparaben	0.05%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 6 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	**0**	**1**	**3**	**7**	**14**
Content(mg/ml)	100.00 ± 0.96	99.83 ± 0.80	98.94 ± 2.98	97.45 ± 0.54	93.16 ± 0.67
Viscosity (cP)	687.00 ± 2.65	552.33 ± 3.06	508.00 ± 9.85	479.33 ± 15.14	402.67 ± 3.21
pH	5.98 ± 0.06	5.86 ± 0.03	5.84 ± 0.02	5.85 ± 0.04	5.84 ± 0.02
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	91.20 ± 0.66	90.80 ± 0.33	88.56 ± 0.12	85.50 ± 1.71	82.14 ± 0.29
Viscosity (cP)	340.67 ± 5.13	330.67 ± 3.06	323.33 ± 3.06	300.67 ± 3.06	291.33 ± 2.31
pH	5.79 ± 0.01	5.81 ± 0.06	5.78 ± 0.02	5.78 ±0.03	5.76 ± 0.03

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 17 August 2011 **Run Number: 28**

Storage Conditions: 40 °C/75% RH **ID Number: 21**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.01%	32.5 mg	RM000265
Methyl paraben and propyl paraben	0.10%	325.0 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	6
% HPMC	0.75%
% Sodium metabisulphite	0.01%
% Methylparaben and propylparaben	0.10%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 6 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

	0	**1**	**3**	**7**	**14**
Content(mg/ml)	100.00 ± 2.30	100.07 ± 1.21	99.06 ± 1.01	96.21 ± 0.38	94.31 ± 0.33
Viscosity (cP)	684.00 ± 4.58	551.00 ± 5.67	509.00 ± 1.73	478.33 ± 13.50	428.33±13.61
pH	6.07 ± 0.03	5.98 ± 0.02	5.83 ± 0.02	5.86 ± 0.05	5.84 ± 0.04
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	93.05 ± 0.61	91.60 ± 0.54	89.41 ± 2.20	85.80 ± 0.52	83.11 ± 1.91
Viscosity (cP)	418.67 ± 17.47	405.33 ± 7.37	388.00 ± 4.00	357.00 ± 2.65	327.67 ± 2.52
pH	5.77 ± 0.02	5.78 ± 0.05	5.80 ± 0.02	5.77 ± 0.04	5.75 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 17 August 2011 **Run Number: 29**

Storage Conditions: 40 °C/75% RH **ID Number: 24**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.05%	162.5 mg	RM000265
Methyl paraben and propyl paraben	0.15%	487.5 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	6
% HPMC	0.75%
% Sodium metabisulphite	0.05%
% Methylparaben and propylparaben	0.15%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 6 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 0.59	99.25 ± 0.34	99.07 ± 0.91	96.27 ± 0.67	93.31 ± 0.68
Viscosity (cP)	689.00 ± 3.61	558.33 ± 1.53	511.33 ± 2.52	469.00 ± 17.52	400.00 ± 11.79
pH	6.02 ± 0.02	5.88 ± 0.02	5.84 ± 0.05	5.87 ± 0.04	5.82 ± 0.04
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	91.28 ± 0.44	89.83 ± 0.25	87.65 ± 0.79	84.71 ± 1.43	80.64 ± 0.62
Viscosity (cP)	360.33 ± 21.46	334.33 ± 10.41	322.00 ± 5.29	336.00 ± 53.78	294.00 ± 4.00
pH	5.78 ± 0.02	5.79 ± 0.02	5.78 ± 0.04	5.75 ± 0.03	5.74 ± 0.02

Rhodes University
Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140
BATCH RECORD SUMMARY

Formulator: Laura Magnus

Product: 25 mg/ml FCV suspension **Batch Size: 325 ml**

Date of Manufacture: 17 August 2011 **Run Number: 30**

Storage Conditions: 40 °C/75% RH **ID Number: 00**

Formula:

Raw Material	Original Formula	Working Formula	Raw Material Number
FCV	2.5 g	8.125 g	RM000252
Sodium Metabisulphite	0.05%	162.5 mg	RM000265
Methyl paraben and propyl paraben	0.10%	325.0 mg	X061903/X062223
Potassium dihydrogen orthophosphate	0.68 g	442.3 mg	SAAR5043600EM
HPMC (K100M)	100 ml	325 ml	RM000062

CCD Defined Levels:

pH	6
% HPMC	0.75%
% Sodium metabisulphite	0.05%
% Methylparaben and propylparaben	0.10%

Method:

1. Accurately weigh 8.125 g of FCV and transfer into a mortar.
2. Add the other excipients.
3. Gradually incorporate the HPMC.
4. Transfer the product into a measuring cylinder and make up to a volume of 325 ml.
5. Measure the pH and adjust to pH 6 using orthophosphoric acid.
6. Transfer the formulation into 3x100 ml bottles.
7. Analyse the formulations of days 0, 1, 3, 7, 14, 21, 35, 42 and 56.

Results Following Testing:

Day	0	1	3	7	14
Content(mg/ml)	100.00 ± 1.07	99.07 ± 0.73	98.91 ± 0.50	96.30 ± 0.55	92.91 ± 1.48
Viscosity (cP)	684.67 ± 4.04	550.67 ± 3.79	504.33 ± 7.64	475.33 ± 17.62	401.00 ± 5.57
pH	6.09 ± 0.01	5.97 ± 0.03	5.87 ± 0.06	5.86 ± 0.02	5.81 ± 0.01
	21	**28**	**35**	**42**	**56**
Content(mg/ml)	91.60 ± 2.09	90.31 ± 0.48	88.85 ± 1.17	86.04 ± 0.81	81.52 ± 1.11
Viscosity (cP)	341.33 ± 3.21	320.67 ± 7.02	318.67 ± 7.02	303.33 ± 4.16	290.00 ± 2.00
pH	5.81 ± 0.03	5.83 ± 0.02	5.79 ± 0.01	5.79 ± 0.03	5.78 ± 0.02

All raw data is available on request.

APPENDIX III

ANOVA of response surface models

Definitions

Std. Dev = Standard deviation
C.V = Coefficient of variance
Adeq Precision = Adequate precision
Adj R^2 = Adjusted R^2 value
Pred R^2 = Predicted R^2 value

Values of "Prob > F" > 0.0500 indicate model terms are significant.

Table A1 *ANOVA of the response surface quadratic model for viscosity on day 0*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	9.251E+006	14	6.608E+005	27.52	< 0.0001
X_1	8.184E+006	1	8.184E+006	340.86	< 0.0001
X_2	9560.04	1	9560.04	0.40	0.5375
X_3	22.04	1	22.04	9.180E-004	0.9762
X_4	77.04	1	77.04	3.209E-003	0.9556
$X1^2$	8.315E+005	1	8.315E+005	34.63	< 0.0001
X_2^2	35075.86	1	35075.86	1.46	0.2455
X_3^2	10846.07	1	10846.07	0.45	0.5117
X_4^2	12544.07	1	12544.07	0.52	0.4809
X_1X_2	25520.06	1	25520.06	1.06	0.3189
X_1X_3	885.06	1	885.06	0.037	0.8503
X_1X_4	742.56	1	742.56	0.031	0.8628
X_2X_3	203.06	1	203.06	8.457E-003	0.9279
X_2X_4	495.06	1	495.06	0.021	0.8877
X_3X_4	20952.56	1	20952.56	0.87	0.3650
Residual	3.602E+005	15	24010.15	-	-
Std. Dev.	154.95	-	-	-	-
C.V.%	20.52	-	-	-	-
Adeq Precision	22.024	-	-	-	-
R^2	0.9625	-	-	-	-
Adj R^2	0.9276	-	-	-	-
Pred R^2	0.7865	-	-	-	-

Table A2 *ANOVA of the response surface quadratic model for pH on day 0*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	23.47	14	1.68	776.06	< 0.0001
X_1	2.817E-003	1	2.817E-003	1.30	0.2714
X_2	23.44	1	23.44	10850.57	< 0.0001
X_3	1.667E-005	1	1.667E-005	7.714E-003	0.9312
X_4	1.500E-004	1	1.500E-004	0.069	0.7958
$X1^2$	2.201E-003	1	2.201E-003	1.02	0.3288
X_2^2	6.001E-003	1	6.001E-003	2.78	0.1163
X_3^2	1.440E-004	1	1.440E-004	0.067	0.7998
X_4^2	9.430E-003	1	9.430E-003	4.36	0.0541
X_1X_2	4.225E-003	1	4.225E-003	1.96	0.1823
X_1X_3	0.000	1	0.000	0.000	1.000
X_1X_4	6.250E-004	1	6.250E-004	0.29	0.5986
X_2X_3	1.600E-003	1	1.600E-003	0.74	0.4030
X_2X_4	2.025E-003	1	2.025E-003	0.94	0.3483
X_3X_4	9.000E-004	1	9.000E-004	0.42	0.5284
Residual	0.032	15	2.161E-003	-	-
Std. Dev.	0.046	-	-	-	-
C.V.%	0.77	-	-	-	-
Adeq Precision	120.281	-	-	-	-
R^2	0.9986	-	-	-	-
Adj R^2	0.9973	-	-	-	-
Pred R^2	0.9931	-	-	-	-

Table A3 *ANOVA of the response surface quadratic model for FCV content on day 0*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	0.000	14	0.000		
X_1	0.000	1	0.000		
X_2	0.000	1	0.000		
X_3	0.000	1	0.000		
X_4	0.000	1	0.000		
$X1^2$	0.000	1	0.000		
X_2^2	0.000	1	0.000		
X_3^2	0.000	1	0.000		
X_4^2	0.000	1	0.000		
X_1X_2	0.000	1	0.000		
X_1X_3	0.000	1	0.000		
X_1X_4	0.000	1	0.000		
X_2X_3	0.000	1	0.000		
X_2X_4	0.000	1	0.000		
X_3X_4	0.000	1	0.000		
Residual	0.000	15	0.000	-	-
Std. Dev.	0.000	-	-	-	-
C.V.%	0.000	-	-	-	-
Adeq Precision	0.000	-	-	-	-
R^2	-	-	-	-	-
Adj R^2	-	-	-	-	-
Pred R^2	-	-	-	-	-

Table A4 *ANOVA of the response surface quadratic model for viscosity after one (1) day*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	6.616E+006	14	4.726E+005	9.48	< 0.0001
X_1	5.247E+006	1	5.247E+006	105.28	< 0.0001
X_2	5.247E+006	1	5.247E+006	105.28	< 0.0001
X_3	33635.35	1	33635.35	0.67	0.4242
X_4	18286.10	1	18286.10	0.37	0.5537
$X1^2$	7.140E+005	1	7.140E+005	14.33	0.0018
X_2^2	2426.76	1	2426.76	0.049	0.8283
X_3^2	295.29	1	295.29	5.925E-003	0.9397
X_4^2	175.73	1	175.73	3.526E-003	0.9534
X_1X_2	95943.51	1	95943.51	1.93	0.1856
X_1X_3	84261.03	1	84261.03	1.69	0.2131
X_1X_4	67224.82	1	67224.82	1.35	0.2636
X_2X_3	71421.23	1	71421.23	1.43	0.2498
X_2X_4	42126.54	1	42126.54	0.85	0.3724
X_3X_4	1.309E+005	1	1.309E+005	2.63	0.1259
Residual	66.52	15	4.43	-	-
Std. Dev.	223.24	-	-	-	-
C.V.%	38.07	-	-	-	-
Adeq Precision	14.010	-	-	-	-
R^2	0.8985	-	-	-	-
Adj R^2	0.8037	-	-	-	-
Pred R^2	0.5115	-	-	-	-

Table A5 *ANOVA of the response surface quadratic model for pH after one (1) day*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	23.34	14	1.67	312.27	< 0.0001
X_1	6.000E-004	1	6.000E-004	0.11	0.7421
X_2	23.32	1	23.32	4368.85	< 0.0001
X_3	1.500E-004	1	1.500E-004	0.028	0.8691
X_4	6.667E-005	1	6.667E-005	0.012	0.9125
$X1^2$	8.048E-004	1	8.048E-004	0.15	0.7033
X_2^2	4.762E-004	1	4.762E-004	0.089	0.7693
X_3^2	7.619E-005	1	7.619E-005	0.014	0.9065
X_4^2	1.905E-003	1	1.905E-003	0.36	0.5592
X_1X_2	2.025E-003	1	2.025E-003	0.38	0.5472
X_1X_3	9.025E-003	1	9.025E-003	1.69	0.2132
X_1X_4	0.000	1	0.000	0.000	1.0000
X_2X_3	2.500E-005	1	2.500E-005	4.683E-003	0.9463
X_2X_4	0.000	1	0.000	0.000	1.0000
X_3X_4	1.000E-004	1	1.000E-004	0.019	0.8930
Residual	0.080	15	5.339E-003	-	-
Std. Dev.	0.073	-	-	-	-
C.V.%	1.24	-	-	-	-
Adeq Precision	76.323	-	-	-	-
R^2	0.9966	-	-	-	-
Adj R^2	0.9934	-	-	-	-
Pred R^2	0.9835	-	-	-	-

Table A6 *ANOVA of the response surface quadratic model for FCV content after one (1) day*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	5.675E+005	14	40537.91	1.07	0.4485
X_1	31348.56	1	31348.56	0.83	0.3778
X_2	42616.18	1	42616.18	1.12	0.3060
X_3	31061.53	1	31061.53	0.82	0.3799
X_4	31256.11	1	31256.11	0.82	0.3785
$X1^2$	1636.77	1	1636.77	0.043	0.8383
X_2^2	1857.77	1	1857.77	0.049	0.8279
X_3^2	1572.27	1	1572.27	0.041	0.8414
X_4^2	1612.50	1	1612.50	0.042	0.8395
X_1X_2	61813.15	1	61813.15	1.63	0.2213
X_1X_3	80427.54	1	80427.54	2.12	0.1661
X_1X_4	79145.16	1	79145.16	2.09	0.1693
X_2X_3	62751.50	1	62751.50	1.65	0.2180
X_2X_4	62706.42	1	62706.42	1.65	0.2181
X_3X_4	79725.76	1	79725.76	2.10	0.1678
Residual	5.692E+005	15	37948.72	-	-
Std. Dev.	194.80	-	-	-	-
C.V.%	127.68	-	-	-	-
Adeq Precision	5.005	-	-	-	-
R^2	0.4993	-	-	-	-
Adj R^2	0.0319	-	-	-	-
Pred R^2	194.80	-	-	-	-

Table A7 *ANOVA of the response surface quadratic model for viscosity after three (3) days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	6.043E+006	14	4.317E+005	59.24	< 0.0001
X_1	5.353E+006	1	5.353E+006	734.70	< 0.0001
X_2	1617.04	1	1617.04	0.22	0.6444
X_3	876.04	1	876.04	0.12	0.7336
X_4	63.38	1	63.38	8.698E-003	0.9269
$X1^2$	6.073E+005	1	6.073E+005	83.35	< 0.0001
X_2^2	3997.86	1	3997.86	0.55	0.4703
X_3^2	2448.36	1	2448.36	0.34	0.5707
X_4^2	3212.86	1	3212.86	0.44	0.5168
X_1X_2	4000.56	1	4000.56	0.55	0.4702
X_1X_3	1207.56	1	1207.56	0.17	0.6897
X_1X_4	370.56	1	370.56	0.051	0.8246
X_2X_3	5.06	1	5.06	6.948E-004	0.9793
X_2X_4	945.56	1	945.56	0.13	0.7237
X_3X_4	4522.56	1	4522.56	0.62	0.4431
Residual	1.093E+005	15	7286.51	-	-
Std. Dev.	85.36	-	-	-	-
C.V.%	14.31	-	-	-	-
Adeq Precision	32.097	-	-	-	-
R^2	0.9822	-	-	-	-
Adj R^2	0.9657	-	-	-	-
Pred R^2	0.8981	-	-	-	-

Table A8 *ANOVA of the response surface quadratic model for pH after three (3) days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	22.77	14	1.63	465.28	< 0.0001
X_1	7.704E-003	1	7.704E-003	2.20	0.1583
X_2	22.72	1	22.72	6500.03	< 0.0001
X_3	2.042E-004	1	2.042E-004	0.058	0.8123
X_4	1.042E-004	1	1.042E-004	0.030	0.8652
$X1^2$	1.857E-003	1	1.857E-003	0.53	0.4772
X_2^2	6.607E-003	1	6.607E-003	1.89	0.1893
X_3^2	1.857E-003	1	1.857E-003	0.53	0.4772
X_4^2	3.157E-003	1	3.157E-003	0.90	0.3569
X_1X_2	7.562E-004	1	7.562E-004	0.22	0.6485
X_1X_3	5.063E-004	1	5.063E-004	0.14	0.7088
X_1X_4	0.014	1	0.014	3.95	0.0654
X_2X_3	2.256E-003	1	2.256E-003	0.65	0.4343
X_2X_4	1.562E-004	1	1.562E-004	0.045	0.8354
X_3X_4	7.656E-003	1	7.656E-003	2.19	0.1595
Residual	0.052	15	3.495E-003	-	-
Std. Dev.	0.059	-	-	-	-
C.V.%	1.01	-	-	-	-
Adeq Precision	93.095	-	-	-	-
R^2	0.9977	-	-	-	-
Adj R^2	0.9956	-	-	-	-
Pred R^2	0.9875	-	-	-	-

Table A9 *ANOVA of the response surface quadratic model for FCV content after three (3) days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	124.08	14	8.86	6.62	0.0004
X_1	1.10	1	1.10	0.82	0.3797
X_2	24.34	1	24.34	18.19	0.0007
X_3	0.055	1	0.055	0.041	0.8419
X_4	1.85	1	1.85	1.39	0.2575
$X1^2$	0.44	1	0.44	0.33	0.5745
X_2^2	77.37	1	77.37	57.82	< 0.0001
X_3^2	0.61	1	0.61	0.46	0.5094
X_4^2	1.83	1	1.83	1.36	0.2610
X_1X_2	2.47	1	2.47	1.85	0.1941
X_1X_3	0.95	1	0.95	0.71	0.4137
X_1X_4	0.36	1	0.36	0.27	0.6101
X_2X_3	0.14	1	0.14	0.10	0.7519
X_2X_4	0.033	1	0.033	0.025	0.8767
X_3X_4	1.83	1	1.83	1.36	0.2610
Residual	20.07	15	1.34	-	-
Std. Dev.	1.16	-	-	-	-
C.V.%	1.19	-	-	-	-
Adeq Precision	12.617	-	-	-	-
R^2	0.8608	-	-	-	-
Adj R^2	0.7308	-	-	-	-
Pred R^2	0.2800	-	-	-	-

Table A10 *ANOVA of the response surface quadratic model for viscosity after seven (7) days*

Day 7 - Viscosity					
Source	**Sum of squares**	**Degree of Freedom**	**Mean Square**	**F-value**	**Prob >F**
Model	4.952E+006	14	3.537E+005	55.59	< 0.0001
X_1	4.418E+006	1	4.418E+006	694.27	< 0.0001
X_2	13490.04	1	13490.04	2.12	0.1660
X_3	551.04	1	551.04	0.087	0.7726
X_4	165.38	1	165.38	0.026	0.8741
$X1^2$	4.377E+005	1	4.377E+005	68.78	< 0.0001
X_2^2	2568.57	1	2568.57	0.40	0.5348
X_3^2	3275.00	1	3275.00	0.51	0.4841
X_4^2	4408.00	1	4408.00	0.69	0.4183
X_1X_2	18157.56	1	18157.56	2.85	0.1118
X_1X_3	85.56	1	85.56	0.013	0.9092
X_1X_4	540.56	1	540.56	0.085	0.7747
X_2X_3	189.06	1	189.06	0.030	0.8655
X_2X_4	1207.56	1	1207.56	0.19	0.6693
X_3X_4	5076.56	1	5076.56	0.80	0.3859
Residual	95449.42	15	6363.29	-	-
Std. Dev.	79.77	-	-	-	-
C.V.%	14.63	-	-	-	-
Adeq Precision	30.848	-	-	-	-
R^2	0.9811	-	-	-	-
Adj R^2	0.9634	-	-	-	-
Pred R^2	0.8917	-	-	-	-

Table A11 *ANOVA of the response surface quadratic model for pH after seven (7) days*

Source	**Sum of squares**	**Degree of Freedom**	**Mean Square**	**F-value**	**Prob >F**
Model	23.14	14	1.65	437.12	< 0.0001
X_1	7.004E-003	1	7.004E-003	1.85	0.1936
X_2	23.11	1	23.11	6112.44	< 0.0001
X_3	3.375E-004	1	3.375E-004	0.089	0.7692
X_4	9.375E-004	1	9.375E-004	0.25	0.6257
$X1^2$	4.503E-003	1	4.503E-003	1.19	0.2923
X_2^2	5.424E-003	1	5.424E-003	1.43	0.2496
X_3^2	2.679E-006	1	2.679E-006	7.085E-004	0.9791
X_4^2	1.312E-004	1	1.312E-004	0.035	0.8547
X_1X_2	1.056E-003	1	1.056E-003	0.28	0.6048
X_1X_3	5.063E-004	1	5.063E-004	0.13	0.7195
X_1X_4	3.906E-003	1	3.906E-003	1.03	0.3255
X_2X_3	1.056E-003	1	1.056E-003	0.28	0.6048
X_2X_4	3.063E-004	1	3.063E-004	0.081	0.7798
X_3X_4	2.756E-003	1	2.756E-003	0.73	0.4066
Residual	0.057	15	3.781E-003	-	-
Std. Dev.	0.061	-	-	-	-
C.V.%	1.06	-	-	-	-
Adeq Precision	90.277	-	-	-	-
R^2	0.9976	-	-	-	-
Adj R^2	0.9953	-	-	-	-
Pred R^2	0.9872	-	-	-	-

Table A12 *ANOVA of the response surface quadratic model for FCV content after seven (7) days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	412.17	14	29.44	20.05	< 0.0001
X_1	1.10	1	1.10	0.75	0.4011
X_2	106.22	1	106.22	72.33	< 0.0001
X_3	1.08	1	1.08	0.74	0.4047
X_4	3.82	1	3.82	2.60	0.1278
$X1^2$	1.84	1	1.84	1.25	0.2805
X_2^2	285.55	1	285.55	194.45	< 0.0001
X_3^2	0.11	1	0.11	0.074	0.7897
X_4^2	0.20	1	0.20	0.14	0.7155
X_1X_2	0.074	1	0.074	0.051	0.8251
X_1X_3	1.62	1	1.62	1.10	0.3103
X_1X_4	0.34	1	0.34	0.23	0.6377
X_2X_3	6.250E-006	1	6.250E-006	4.256E-006	0.9984
X_2X_4	0.60	1	0.60	0.41	0.5334
X_3X_4	0.043	1	0.043	0.029	0.8663
Residual	22.03	15	1.47	-	-
Std. Dev.	1.21	-	-	-	-
C.V.%	1.29	-	-	-	-
Adeq Precision	21.304	-	-	-	-
R^2	0.9493	-	-	-	-
Adj R^2	0.9019	-	-	-	-
Pred R^2	0.7152	-	-	-	-

Table A13 *ANOVA of the response surface quadratic model for viscosity after 14 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	3.522E+006	14	2.516E+005	188.82	< 0.0001
X_1	3.246E+006	1	3.246E+006	2436.47	< 0.0001
X_2	9243.38	1	9243.38	6.94	0.0188
X_3	198.38	1	198.38	0.15	0.7050
X_4	198.38	1	198.38	0.15	0.7050
$X1^2$	2.354E+005	1	2.354E+005	176.65	< 0.0001
X_2^2	1986.57	1	1986.57	1.49	0.2409
X_3^2	35.36	1	35.36	0.027	0.8728
X_4^2	95.36	1	95.36	0.072	0.7927
X_1X_2	16192.56	1	16192.56	12.15	0.0033
X_1X_3	52.56	1	52.56	0.039	0.8452
X_1X_4	27.56	1	27.56	0.021	0.8876
X_2X_3	85.56	1	85.56	0.064	0.8034
X_2X_4	4865.06	1	4865.06	3.65	0.0753
X_3X_4	115.56	1	115.56	0.087	0.7724
Residual	19986.92	15	1332.46	-	-
Std. Dev.	36.50	-	-	-	-
C.V.%	7.62	-	-	-	-
Adeq Precision	56.997	-	-	-	-
R^2	0.9944	-	-	-	-
Adj R^2	0.9891	-	-	-	-
Pred R^2	0.9677	-	-	-	-

Table A14 *ANOVA of the response surface quadratic model for pH after 14 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	22.67	14	1.62	738.03	< 0.0001
X_1	5.042E-004	1	5.042E-004	0.23	0.6386
X_2	22.64	1	22.64	10319.50	< 0.0001
X_3	2.604E-003	1	2.604E-003	1.19	0.2931
X_4	2.042E-004	1	2.042E-004	0.093	0.7645
$X1^2$	7.150E-003	1	7.150E-003	3.26	0.0911
X_2^2	8.300E-003	1	8.300E-003	3.78	0.0708
X_3^2	5.030E-005	1	5.030E-005	0.023	0.8817
X_4^2	1.860E-004	1	1.860E-004	0.085	0.7749
X_1X_2	5.063E-004	1	5.063E-004	0.23	0.6379
X_1X_3	5.625E-005	1	5.625E-005	0.026	0.8749
X_1X_4	5.625E-005	1	5.625E-005	0.026	0.8749
X_2X_3	2.256E-003	1	2.256E-003	1.03	0.3266
X_2X_4	2.756E-003	1	2.756E-003	1.26	0.2800
X_3X_4	3.906E-003	1	3.906E-003	1.78	0.2020
Residual	0.033	15	2.194E-003	-	-
Std. Dev.	0.047	-	-	-	-
C.V.%	0.81	-	-	-	-
Adeq Precision	117.300	-	-	-	-
R^2	0.9986	-	-	-	-
Adj R^2	0.9972	-	-	-	-
Pred R^2	0.9940	-	-	-	-

Table A15 *ANOVA of the response surface quadratic model for FCV content after 14 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	824.08	14	58.86	16.02	< 0.0001
X_1	0.51	1	0.51	0.14	0.7154
X_2	198.55	1	198.55	54.04	< 0.0001
X_3	1.10	1	1.10	0.30	0.5929
X_4	1.71	1	1.71	0.47	0.5053
$X1^2$	1.84	1	1.84	0.50	0.4903
X_2^2	576.58	1	576.58	156.92	< 0.0001
X_3^2	2.686E-003	1	2.686E-003	7.310E-004	0.9788
X_4^2	0.25	1	0.25	0.069	0.7964
X_1X_2	3.79	1	3.79	1.03	0.3257
X_1X_3	4.44	1	4.44	1.21	0.2889
X_1X_4	0.92	1	0.92	0.25	0.6247
X_2X_3	0.17	1	0.17	0.045	0.8345
X_2X_4	0.19	1	0.19	0.051	0.8245
X_3X_4	0.45	1	0.45	0.12	0.7325
Residual	55.12	15	3.67	-	-
Std. Dev.	1.92	-	-	-	-
C.V.%	2.11	-	-	-	-
Adeq Precision	18.73	-	-	-	-
R^2	0.9373	-	-	-	-
Adj R^2	0.8788	-	-	-	-
Pred R^2	0.6524	-	-	-	-

Table A16 *ANOVA of the response surface quadratic model for viscosity after 21 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	2.981E+006	14	2.129E+005	107.71	< 0.0001
X_1	2.709E+006	1	2.709E+006	1370.28	< 0.0001
X_2	7279.82	1	7279.82	3.68	0.0742
X_3	1546.74	1	1546.74	0.78	0.3904
X_4	308.24	1	308.24	0.16	0.6985
$X1^2$	2.300E+005	1	2.300E+005	116.33	< 0.0001
X_2^2	5238.25	1	5238.25	2.65	0.1244
X_3^2	426.76	1	426.76	0.22	0.6489
X_4^2	633.41	1	633.41	0.32	0.5797
X_1X_2	14721.58	1	14721.58	7.45	0.0155
X_1X_3	667.32	1	667.32	0.34	0.5699
X_1X_4	38.04	1	38.04	0.019	0.8915
X_2X_3	2618.11	1	2618.11	1.32	0.2678
X_2X_4	0.69	1	0.69	3.506E-004	0.9853
X_3X_4	4312.22	1	4312.22	2.18	0.1604
Residual	29655.21	15	1977.01	-	-
Std. Dev.	44.46	-	-	-	-
C.V.%	10.20	-	-	-	-
Adeq Precision	42.744	-	-	-	-
R^2	0.9902	-	-	-	-
Adj R^2	0.9810	-	-	-	-
Pred R^2	0.9446	-	-	-	-

Table A17 *ANOVA of the response surface quadratic model for pH after 21 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	23.04	14	1.65	1091.31	< 0.0001
X_1	4.167E-006	1	4.167E-006	2.763E-003	0.9588
X_2	23.03	1	23.03	15270.20	< 0.0001
X_3	4.537E-003	1	4.537E-003	3.01	0.1033
X_4	1.042E-004	1	1.042E-004	0.069	0.7963
$X1^2$	5.250E-006	1	5.250E-006	3.481E-003	0.9537
X_2^2	8.110E-004	1	8.110E-004	0.54	0.4747
X_3^2	2.367E-004	1	2.367E-004	0.16	0.6976
X_4^2	1.167E-004	1	1.167E-004	0.077	0.7847
X_1X_2	6.250E-006	1	6.250E-006	4.144E-003	0.9495
X_1X_3	7.562E-004	1	7.562E-004	0.50	0.4897
X_1X_4	6.250E-006	1	6.250E-006	4.144E-003	0.9495
X_2X_3	6.250E-006	1	6.250E-006	4.144E-003	0.9495
X_2X_4	3.062E-004	1	3.062E-004	0.20	0.6587
X_3X_4	5.256E-003	1	5.256E-003	3.49	0.0816
Residual	0.023	15	1.508E-003	-	-
Std. Dev.	0.039	-	-	-	-
C.V.%	0.67	-	-	-	-
Adeq Precision	142.689	-	-	-	-
R^2	0.9990	-	-	-	-
Adj R^2	0.9981	-	-	-	-
Pred R^2	0.9963	-	-	-	-

Table A18 *ANOVA of the response surface quadratic model for FCV content after 21 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	1300.39	14	92.88	18.12	< 0.0001
X_1	0.076	1	0.076	0.015	0.9047
X_2	301.54	1	301.54	58.84	< 0.0001
X_3	2.44	1	2.44	0.48	0.5009
X_4	0.99	1	0.99	0.19	0.6668
$X1^2$	3.48	1	3.48	0.68	0.4226
X_2^2	919.09	1	919.09	179.34	< 0.0001
X_3^2	0.25	1	0.25	0.050	0.8266
X_4^2	1.11	1	1.11	0.22	0.6484
X_1X_2	6.06	1	6.06	1.18	0.2939
X_1X_3	0.12	1	0.12	0.024	0.8783
X_1X_4	7.74	1	7.74	1.51	0.2380
X_2X_3	0.42	1	0.42	0.082	0.7788
X_2X_4	0.81	1	0.81	0.16	0.6958
X_3X_4	0.37	1	0.37	0.072	0.7921
Residual	76.87	15	5.12	-	-
Std. Dev.	2.26	-	-	-	-
C.V.%	2.57	-	-	-	-
Adeq Precision	19.854	-	-	-	-
R^2	0.9442	-	-	-	-
Adj R^2	0.8921	-	-	-	-
Pred R^2	0.6856	-	-	-	-

Table A19 *ANOVA of the response surface quadratic model for viscosity after 35 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	2.328E+006	14	1.663E+005	65.79	< 0.0001
X_1	2.114E+006	1	2.114E+006	836.32	< 0.0001
X_2	5797.04	1	5797.04	2.29	0.1507
X_3	513.38	1	513.38	0.20	0.6587
X_4	287.04	1	287.04	0.11	0.7408
$X1^2$	1.779E+005	1	1.779E+005	70.37	< 0.0001
X_2^2	6723.24	1	6723.24	2.66	0.1237
X_3^2	113.17	1	113.17	0.045	0.8353
X_4^2	1687.53	1	1687.53	0.67	0.4267
X_1X_2	10972.56	1	10972.56	4.34	0.0547
X_1X_3	115.56	1	115.56	0.046	0.8336
X_1X_4	1040.06	1	1040.06	0.41	0.5309
X_2X_3	162.56	1	162.56	0.064	0.8033
X_2X_4	945.56	1	945.56	0.37	0.5500
X_3X_4	3164.06	1	3164.06	1.25	0.2808
Residual	37916.92	15	2527.79	-	-
Std. Dev.	50.28	-	-	-	-
C.V.%	12.83	-	-	-	-
Adeq Precision	33.393	-	-	-	-
R^2	0.9840	-	-	-	-
Adj R^2	0.9690	-	-	-	-
Pred R^2	0.9114	-	-	-	-

Table A20 *ANOVA of the response surface quadratic model for pH after 35 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	23.76	14	1.70	7.78	0.0002
X_1	9.375E-004	1	9.375E-004	4.296E-003	0.9486
X_2	23.19	1	23.19	106.25	< 0.0001
X_3	5.042E-004	1	5.042E-004	2.310E-003	0.9623
X_4	1.504E-003	1	1.504E-003	6.893E-003	0.9349
$X1^2$	0.20	1	0.20	0.93	0.3495
X_2^2	0.25	1	0.25	1.13	0.3042
X_3^2	0.18	1	0.18	0.80	0.3846
X_4^2	0.18	1	0.18	0.80	0.3846
X_1X_2	1.056E-003	1	1.056E-003	4.840E-003	0.9455
X_1X_3	2.256E-003	1	2.256E-003	0.010	0.9204
X_1X_4	5.625E-005	1	5.625E-005	2.578E-004	0.9874
X_2X_3	5.625E-005	1	5.625E-005	2.578E-004	0.9874
X_2X_4	5.625E-005	1	5.625E-005	2.578E-004	0.9874
X_3X_4	7.656E-003	1	7.656E-003	0.035	0.8539
Residual	3.27	15	0.22	-	-
Std. Dev.	0.47	-	-	-	-
C.V.%	8.00	-	-	-	-
Adeq Precision	11.902	-	-	-	-
R^2	0.8789	-	-	-	-
Adj R^2	0.7659	-	-	-	-
Pred R^2	0.8234	-	-	-	-

Table A21 *ANOVA of the response surface quadratic model for FCV content after 35 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	2901.00	14	207.21	64.39	< 0.0001
X_1	9.33	1	9.33	2.90	0.1093
X_2	528.47	1	528.47	164.22	< 0.0001
X_3	0.040	1	0.040	0.012	0.9127
X_4	11.23	1	11.23	3.49	0.0814
$X1^2$	9.011E-003	1	9.011E-003	2.800E-003	0.9585
X_2^2	2230.05	1	2230.05	692.97	< 0.0001
X_3^2	0.45	1	0.45	0.14	0.7136
X_4^2	1.26	1	1.26	0.39	0.5408
X_1X_2	12.08	1	12.08	3.75	0.0718
X_1X_3	0.30	1	0.30	0.094	0.7634
X_1X_4	11.46	1	11.46	3.56	0.0787
X_2X_3	4.84	1	4.84	1.50	0.2390
X_2X_4	0.77	1	0.77	0.24	0.6328
X_3X_4	0.15	1	0.15	0.047	0.8308
Residual	48.27	15	3.22	-	-
Std. Dev.	1.79	-	-	-	-
C.V.%	2.24	-	-	-	-
Adeq Precision	36.758	-	-	-	-
R^2	0.9836	-	-	-	-
Adj R^2	0.9684	-	-	-	-
Pred R^2	0.9117	-	-	-	-

Table A22 *ANOVA of the response surface quadratic model for viscosity after 42 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	2.181E+006	14	1.558E+005	73.19	< 0.0001
X_1	1.965E+006	1	1.965E+006	922.93	< 0.0001
X_2	4240.04	1	4240.04	1.99	0.1786
X_3	408.38	1	408.38	0.19	0.6676
X_4	77.04	1	77.04	0.036	0.8517
$X1^2$	1.906E+005	1	1.906E+005	89.54	< 0.0001
X_2^2	4717.50	1	4717.50	2.22	0.1573
X_3^2	11.07	1	11.07	5.202E-003	0.9435
X_4^2	209.00	1	209.00	0.098	0.7583
X_1X_2	9072.56	1	9072.56	4.26	0.0567
X_1X_3	7.56	1	7.56	3.552E-003	0.9533
X_1X_4	689.06	1	689.06	0.32	0.5778
X_2X_3	5.06	1	5.06	2.378E-003	0.9618
X_2X_4	217.56	1	217.56	0.10	0.7536
X_3X_4	1387.56	1	1387.56	0.65	0.4321
Residual	31933.25	15	2128.88	-	-
Std. Dev.	46.14	-	-	-	-
C.V.%	12.43	-	-	-	-
Adeq Precision	35.080	-	-	-	-
R^2	0.9856	-	-	-	-
Adj R^2	0.9721	-	-	-	-
Pred R^2	0.9179	-	-	-	-

Table A23 *ANOVA of the response surface quadratic model for pH after 42 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	23.05	14	1.65	2324.62	< 0.0001
X_1	9.375E-004	1	9.375E-004	1.32	0.2680
X_2	23.03	1	23.03	32512.95	< 0.0001
X_3	3.375E-004	1	3.375E-004	0.48	0.5006
X_4	2.204E-003	1	2.204E-003	3.11	0.0981
$X1^2$	8.601E-005	1	8.601E-005	0.12	0.7323
X_2^2	7.907E-003	1	7.907E-003	11.16	0.0045
X_3^2	8.601E-005	1	8.601E-005	0.12	0.7323
X_4^2	8.360E-004	1	8.360E-004	1.18	0.2945
X_1X_2	6.006E-003	1	6.006E-003	8.48	0.0107
X_1X_3	6.250E-006	1	6.250E-006	8.824E-003	0.9264
X_1X_4	1.562E-004	1	1.562E-004	0.22	0.6453
X_2X_3	5.625E-005	1	5.625E-005	0.079	0.7819
X_2X_4	7.562E-004	1	7.562E-004	1.07	0.3179
X_3X_4	1.806E-003	1	1.806E-003	2.55	0.1311
Residual	0.011	15	7.083E-004	-	-
Std. Dev.	0.027	-	-	-	-
C.V.%	0.46	-	-	-	-
Adeq Precision	208.208	-	-	-	-
R^2	0.9995	-	-	-	-
Adj R^2	0.9991	-	-	-	-
Pred R^2	0.9983	-	-	-	-

Table A24 *ANOVA of the response surface quadratic model for FCV content after 42 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	3295.76	14	235.41	72.88	< 0.0001
X_1	7.33	1	7.33	2.27	0.1528
X_2	593.62	1	593.62	183.77	< 0.0001
X_3	0.24	1	0.24	0.073	0.7906
X_4	5.41	1	5.41	1.68	0.2150
$X1^2$	0.59	1	0.59	0.18	0.6760
X_2^2	2528.48	1	2528.48	782.75	< 0.0001
X_3^2	0.28	1	0.28	0.087	0.7720
X_4^2	0.63	1	0.63	0.19	0.6657
X_1X_2	14.33	1	14.33	4.44	0.0525
X_1X_3	0.31	1	0.31	0.097	0.7596
X_1X_4	8.85	1	8.85	2.74	0.1186
X_2X_3	9.46	1	9.46	2.93	0.1077
X_2X_4	5.11	1	5.11	1.58	0.2278
X_3X_4	1.03	1	1.03	0.32	0.5806
Residual	48.45	15	3.23	-	-
Std. Dev.	1.80	-	-	-	-
C.V.%	2.34	-	-	-	-
Adeq Precision	39.376	-	-	-	-
R^2	0.9855	-	-	-	-
Adj R^2	0.9720	-	-	-	-
Pred R^2	0.9217	-	-	-	-

Table A25 *ANOVA of the response surface quadratic model for viscosity after 56 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	2.052E+006	14	1.466E+005	111.41	< 0.0001
X_1	1.848E+006	1	1.848E+006	1404.23	< 0.0001
X_2	3978.38	1	3978.38	3.02	0.1025
X_3	84.38	1	84.38	0.064	0.8035
X_4	117.04	1	117.04	0.089	0.7696
$X1^2$	1.807E+005	1	1.807E+005	137.37	< 0.0001
X_2^2	4945.00	1	4945.00	3.76	0.0716
X_3^2	1.07	1	1.07	8.166E-004	0.9776
X_4^2	483.36	1	483.36	0.37	0.5535
X_1X_2	7353.06	1	7353.06	5.59	0.0320
X_1X_3	60.06	1	60.06	0.046	0.8337
X_1X_4	540.56	1	540.56	0.41	0.5312
X_2X_3	14.06	1	14.06	0.011	0.9190
X_2X_4	264.06	1	264.06	0.20	0.6606
X_3X_4	1040.06	1	1040.06	0.79	0.3880
Residual	19736.08	15	1315.74	-	-
Std. Dev.	36.27	-	-	-	-
C.V.%	10.19	-	-	-	-
Adeq Precision	43.270	-	-	-	-
R^2	0.9905	-	-	-	-
Adj R^2	0.9816	-	-	-	-
Pred R^2	0.9459	-	-	-	-

Table A26 *ANOVA of the response surface quadratic model for pH after 56 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	22.83	14	1.63	2073.26	< 0.0001
X_1	1.667E-003	1	1.667E-003	2.12	0.1661
X_2	22.82	1	22.82	29002.12	< 0.0001
X_3	1.500E-004	1	1.500E-004	0.19	0.6686
X_4	1.350E-003	1	1.350E-003	1.72	0.2099
$X1^2$	1.905E-005	1	1.905E-005	0.024	0.8784
X_2^2	6.519E-003	1	6.519E-003	8.29	0.0115
X_3^2	4.762E-006	1	4.762E-006	6.053E-003	0.9390
X_4^2	1.905E-005	1	1.905E-005	0.024	0.8784
X_1X_2	3.025E-003	1	3.025E-003	3.85	0.0687
X_1X_3	1.000E-004	1	1.000E-004	0.13	0.7264
X_1X_4	2.500E-005	1	2.500E-005	0.032	0.8609
X_2X_3	3.025E-003	1	3.025E-003	3.85	0.0687
X_2X_4	1.000E-004	1	1.000E-004	0.13	0.7264
X_3X_4	2.025E-003	1	2.025E-003	2.57	0.1295
Residual	0.012	15	7.867E-004	-	-
Std. Dev.	0.028	-	-	-	-
C.V.%	0.49	-	-	-	-
Adeq Precision	196.646	-	-	-	-
R^2	0.9995	-	-	-	-
Adj R^2	0.9990	-	-	-	-
Pred R^2	0.9977	-	-	-	-

Table A27 *ANOVA of the response surface quadratic model for FCV content after 56 days*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	4830.42	14	345.03	95.61	< 0.0001
X_1	7.88	1	7.88	2.18	0.1602
X_2	986.50	1	986.50	273.37	< 0.0001
X_3	1.90	1	1.90	0.53	0.4794
X_4	10.15	1	10.15	2.81	0.1142
$X1^2$	6.786E-003	1	6.786E-003	1.881E-003	0.9660
X_2^2	3620.66	1	3620.66	1003.34	< 0.0001
X_3^2	0.71	1	0.71	0.20	0.6644
X_4^2	5.88	1	5.88	1.63	0.2212
X_1X_2	11.95	1	11.95	3.31	0.0888
X_1X_3	2.81	1	2.81	0.78	0.3911
X_1X_4	11.82	1	11.82	3.27	0.0904
X_2X_3	17.37	1	17.37	4.81	0.0444
X_2X_4	16.30	1	16.30	4.52	0.0506
X_3X_4	0.22	1	0.22	0.062	0.8069
Residual	54.13	15	3.61	-	-
Std. Dev.	1.90	-	-	-	-
C.V.%	2.63	-	-	-	-
Adeq Precision	44.659	-	-	-	-
R^2	0.9889	-	-	-	-
Adj R^2	0.9786	-	-	-	-
Pred R^2	0.9393	-	-	-	-

All raw data is available on request.

1. Georgiev, V., "Herpes Simplex Virus," *Opportunistic Infections.* Humana Press, Inc., New Jersey, USA, 2003, pp. 33-51.

2. Gudmundsson, O. S. and Antman, M., "Case Study: Famciclovir: A Prodrug of Penciclovir," *Prodrugs*, edited by V. J. Stella, R. T. Borchardt, M. J. Hageman, R. Oliyai, H. Maag, and J. W. Tilley, 5 ed. Biotechnology: Pharmaceutical Aspects, Springer, New York, USA, 2007, pp. 1231-1239.

3. LaRussa, P. S., "Famciclovir," *Seminars in Pediatric Infectious Diseases*, Vol. 7, No. 2, 1996, pp. 138-144.

4. Nahata, M. C. and Allen, J., "Extemporaneous drug formulations," *Clinical Therapeutics*, Vol. 30, No. 11, 2008, pp. 2112-2119.

5. Bacon, T. H., "Famciclovir, from the bench to the patient - a comprehensive review of preclinical data," *International Journal of Antimicrobial Agents*, Vol. 7, No. 2, 1996, pp. 119-134.

6. "Drugs Information Online, Drugs.com, Famciclovir," 2011, http://www.drugs.com/pro/famciclovir.html [cited 25 May 2011].

7. "Daily Med,Current Medication Information: Famciclovir Tablets," 2011, http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?id=40350 [cited 3 March 2011].

8. Torii, T., Shiragami, H., Yamashita, K., Suzuki, Y., Hijiya, T., Kashiwagi, T., and Izawa, K., "Practical syntheses of penciclovir and famciclovir from N2-acetyl-7-benzylguanine," *Tetrahedron*, Vol. 62, No. 24, 2006, pp. 5709-5716.

9. Choudary, B. M., Geen, G. R., Kincey, P. M., Parratt, M. J., Robert, J., Dales, M., Johnson, G. P., O'Donnell, S., Tudor, D. W., and Woods, N., "A Direct Approach to the Synthesis of Famciclovir and Penciclovir," *Nucleosides and Nucleotides*, Vol. 15, No. 5, 1996, pp. 981-994.

10. Herdewijn, P., "Structural requirements for antiviral activity in nucleosides," *Drug Discovery Today*, Vol. 2, No. 6, 1997, pp. 235-242.

11. "Prescribing Information, Famvir," 2001, C:\Users\user\Documents\Masters work\Lit review doc-Novartis.htm [cited 11 May 2011].

12. Edwards, A. A. and Alexander, B. D., "Organic Applications of UV-Visible Absorption Spectroscopy," *Encyclopedia of Spectroscopy and Spectrometry.*, edited by L. John Academic Press, Oxford, UK, 2010, pp. 2030-2039.

13. Gault, V. A. and McClenaghan, N. H., "Applications of Spectroscopy," *Understanding Bioanalytical Chemistry*. John Wiley & Sons, Ltd., West Sussex, UK, 2009, pp. 99-122.

14. Ahunja, S., "Selectivity and Detectability Optimization in HPLC," John Wiley & Sons, New York, USA, 1989, pp. 75-91.

15. Vishnumulaka, S., Medicherla, N. R., Rao, A., and Sinubabu, G. E., "Development and Validation of LC Method for the Determination of Famciclovir in Pharmaceutical

Formulation Using an Experimental Design," *E-Journal of Chemistry*, Vol. 5, No. 1, 2008, pp. 58-67.

16. Coates, J., "Interpretation of Spectra, a Practical Approach," *Encyclopedia of Analytical Chemistry*. John Wiley & Sons Ltd., West Sussex, UK, 2000, pp. 10815-10837.

17. Socrates, G., *Infrared and Raman Characteristic Group Frequencies*, 3rd ed., John Wiley & Sons, Ltd., West Sussex, UK, 2001.

18. McMurry, J., *Organic chemistry: a biological approach*, Thomson Learning Inc., California, USA, 2007, pp. 484-486.

19. Lindon, J. C., "NMR spectroscopy: Analytical applications from chemistry to the clinic," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 4, No. 2, 1986, pp. 137-145.

20. Jacobson, N. E., "Fundamentals of NMR Spectroscopy in Liquids," *NMR Spectroscopy Explained.* John Wiley & Sons, Inc., New Jersey, USA, 2007, pp. 1-38.

21. Bakhmutov, V. I., "How and Why Nuclei Relax," *Practical NMR Relaxation for Chemists.* John Wiley & Sons Ltd., West Sussex, UK, 2004, pp. 1-18.

22. Willock, D. J., "Symmetry Elements and Operations," *Molecular Symmetry*. John Wiley & Sons, Ltd., West Sussex, UK, 2009, pp. 1-24.

23. Raman, N. V. V. S., Harikrishna, K. A., Prasad, A. V. S. S., Reddy, K. R., and Ramakrishna, K., "Development and validation of a stability-indicating RP-LC method for famciclovir," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 50, No. 5, 2009, pp. 797-802.

24. Narasimha Raju, C., Panda, G., and Nageswara Rao, G., "Stability Indicating LC Assay Method for the Determination of Famciclovir in Bulk Drug and Pharmaceutical Dosage Forms," *Chromatographia*, Vol. 68, No. 9, 2008, pp. 837-841.

25. Gnann, J., "What is in the Pipeline for VZV Infections," *Clinical Management of Herpes Viruses.*, edited by S. L. Sacks, S. E. Straus, R. J. Whitley, and P. D. Griffiths IOS Press., Amsterdam, Netherlands, 1995, pp. 253-264.

26. Paintsil, E. and Cheng, Y. C., "Antiviral Agents," *Encyclopedia of Microbiology.*, edited by S. Moselio Academic Press, Oxford, UK, 2009, pp. 223-257.

27. Boon, R., Goodman, J. J., Martinez, J., Marks, G. L., Gamble, M., and Welch, C., "Penciclovir cream for the treatment of sunlight-induced herpes simplex labialis: A randomized, double-blind, placebo-controlled trial," *Clinical Therapeutics*, Vol. 22, No. 1, 2000, pp. 76-90.

28. Fatahzadeh, M. and Schwartz, R. A., "Human herpes simplex virus infections: Epidemiology, pathogenesis, symptomatology, diagnosis, and management," *Journal of the American Academy of Dermatology*, Vol. 57, No. 5, 2007, pp. 737-763.

29. Tyring, S. K., "Advances in the treatment of herpesvirus infection: the role of famciclovir," *Clinical Therapeutics*, Vol. 20, No. 4, 2007, pp. 661-670.

30. Schmader, K. E. and Dworkin, R. H., "Natural History and Treatment of Herpes Zoster," *The Journal of Pain*, Vol. 9, No. 1, Supplement 1, 2008, pp. 3-9.

31. *Martindale: The Complete Drug Reference*, 33rd ed., The Pharmaceutical Press., London, UK, 2002, pp. 620-621.

32. Saez-Llorens, X., Yogev, R., Arguedas, A., Rodriguez, A., Spigarelli, M. G., De, L. C., Bomgaars, L., Roberts, M., Abrams, B., Zhou, W., Looby, M., Kaiser, G., and Hamed, K., "Pharmacokinetics and safety of famciclovir in children with herpes simplex or varicella-zoster virus infection," *Antimicrobial Agents and Chemotherapy*, Vol. 53, No. 5, 2009, pp. 1912-1920.

33. "Merck Manuals Online Medical Library, Famciclovir," 2009, http://www.merckmanuals.com/professional/print/lexicomp/famciclovir.html [cited 24 May 2011].

34. Chakrabarty, A., Tyring, S. K., Beutner, K., and Rauser, M., "Recent Clinical Experience with Famciclovir - A 'Third Generation' Nucleoside Prodrug," *Antiviral Chemistry and Chemotherapy*, Vol. 15, No. 5, 2004, pp. 251-253.

35. Crocetti, M., Barone, M. A., and Oski, F. A., "Viral Infections," *Oski's Essential Pediatrics*. Lippincott Williams & Wilkins, Philadelphia, USA, 2004, pp. 342-343.

36. Nesalin, J. A. J., Babu, C. J. G., Kumar, V., and Mani, T., "Validated Spectrophotometric Estimation of Famciclovir in Tablet Dosage Form," *E-Journal of Chemistry*, Vol. 6, No. 3, 2009, pp. 780-784.

37. Lunn, G., *HPLC Method for Pharmaceutical Analysis*, Vol. 3, John Wiley and Sons, Inc., USA, 2000, pp. 187-189.

38. Gouda, A. A., Shafey, Z. E., Hossny, N., and El-Azzazy, R., "Spectrophotometric determination of hyoscine butylbromide and famciclovir in pure form and in pharmaceutical formulations," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, Vol. 70, No. 4, 2008, pp. 785-792.

39. Vamsi, K. M., Madhavi, L. P. V., and Gowri, S. D., "Spectrophotometric Determination of Famciclovir and Racecodoctril Using 2,6-dichloroquinone-Chlorimide," *E-Journal of Chemistry*, Vol. 4, No. 1, 2007, pp. 50-52.

40. Nizamuddin, S., Gurupadayya, B. M., Ravi, M. C., Manohara, Y. N., and Appala Raju, S., "Spectrophotometric Estimation of Famciclovir in Bulk and Tablet Dosage Form," *Indian Journal of Pharmaceutical Sciences*, Vol. 69, No. 3, 2007, pp. 451-453.

41. McMaster, M. C., "Advantages and Disadvantages of HPLC," *HPLC, a Practical User's Guide*, 2nd ed. John Wiley & Sons Inc., New Jersey, USA, 2007, pp. 3-14.

42. Dong, M. W., *Handbook of Pharmaceutical Analysis by HPLC*, 6 ed., Elsevier Inc., London, UK, 2005.

43. Lindsay, S., *High Performance Liquid Chromatography*, John Wiley & Sons, London, UK, 1987.

44. Braithwaite, A. and Smith, F. J., "High Performance Liquid Chromatography," *Chromatographic Methods.*, 5th ed. Springer, New York, USA, 1996, pp. 258-365.

45. Heftmann, E., "Column Liquid Chromatography," *Chromatography: Fundamentals and Applications of Chromatography.*, 6th ed. Elsevier B.D., Amsterdam, The Netherlands, 2004, pp. 95-138.

46. Hamilton, R. J. and Sewell, P. A., *Introduction to High Performance Liquid Chromatography*, 2nd ed., Chapman and Hall Ltd, New York, USA, 1982.

47. Cserhati, T., "Liquid Chromatography," *Multivariate Method in Chromatography: A Practical Guide.* John Wiley & Sons, Ltd., West Sussex, UK, 2008, pp. 113-264.

48. Smith, R. M., "Retention and Selectivity in Liquid Chromatography," Elsevier Science B.V., Amsterdam, The Netherlands, 1995, pp. 62.

49. Kazakevish, Y. and LoBrutto, R., "HPLC for Pharmaceutical Scientists," John Wiley & Sons, Inc., New Jersey, USA, 2007, pp. 458.

50. Subrahmanyam, K. V., "Rp-HPLC Ion Pair Method Development, Validation And Stability Indicating Assay For Famciclovir," *Latest Reviews*, Vol. 5, No. 2, 2007.

51. Srinubabu, G., Sudharani, B., Sridhar, L., and Rao, J. L. N. S., "Development and Validation of Liquid Chromatographic and UV Derivative Spectrophotometric Methods for the Determination of Famciclovir in Pharmaceutical Dosage Forms," *Chem.Pharm.Bull*, Vol. 54, 2006, pp. 819-822.

52. Bryant, D. K., Kingswood, M. D., and Belenguer, A., "Determination of liquid chromatographic peak purity by electrospray ionization mass spectrometry," *Journal of Chromatography A*, Vol. 721, No. 1, 1996, pp. 41-51.

53. Huynh-Ba, K., "Handbook of Stability Testing in Pharmaceutical Development, Regulations, Methodologies, and Best Practices," Springer Science+Business Media, New York, USA, 2009, pp. 154.

54. Timm, U., Wall, M., and Dell, D., "A New Approach for Dealing with the Stability of Drugs in Biological Fluids," *Journal of Pharmaceutical Sciences*, Vol. 74, No. 9, 1985, pp. 972-977.

55. Snyder, L. R., Kirkland, J. J., and Glajch, J. L., *Practical HPLC Method Development*, John Wiley & Sons, Inc., Canada, 1997.

56. Okafo, G. N. and Roberts, J. K., "Development of Achiral Separation Methods," *Pharmaceutical Analysis.* Blackwell Publishing Ltd., Oxford, UK, 2003, pp. 31-73.

57. Pryde, A. and Gilbert, M. T., "The Practice of HPLC," *Applications of High Performance Liquid Chromatography.* Chapman and Hall Ltd., London, UK, 1979, pp. 24-40.

58. Nielsen, S., "Food Analysis," Springer Science + Business Media, New York, USA, 2010, pp. 503-505.

59. Troy, D. B., "Remington: The Science and Practice of Pharmacy,", 2 ed. Lippincot Williams & Wilkins, Maryland, USA, 2011, pp. 615-616.

60. Dong, M. W., "Modern HPLC for Practising Scientists," John Wiley & Sons, Inc., Hoboken, New Jersey, 2006, pp. 70-72.

61. Bidlingmeyer, B. A., "Experiment 3: Effect of Column Length and Recycle," *Practical HPLC Methodology and Applications.* John Wiley & Sons, inc., New York, USA, 1992, pp. 345-357.

62. Bigelow, J., "Bioanalytical Tools for Analysis," *Pharmacology: Principles and Practice.*, edited by M. Hacker, K. Bachmann, and W. Messer Elsevier, Inc., Oxford, UK, 2009, pp. 279-302.

63. Parris, N. A., "LC Instrumentation," *Journal of Chromatography Library*, 2nd ed. Vol. 27, Instrumental Liquid Chromatography, Elsevier Science Publishers B.V., Amsterdam, The Netherlands, 1984, pp. 90.

64. Anderson, R. J., Bendell, D. J., and Groundwater, P. W., "Ultra Violet-Visible Spectroscopy," *Organic Spectroscopic Analysis*. The Royal Society of Chemistry, Cambridge, UK, 2004, pp. 7-23.

65. Scott, R. P. W., "Liquid Chromatography Detectors," Elsevier Science Publishers, B.V., Amsterdam, The Netherlands, 1986, pp. 235.

66. Hammarstrand, K., "Internal Standard in Gas Chromatography," *Varian Instrument Applications*, Vol. 10, No. 1, 1976, pp. 10-11.

67. Michael, L. G., "A comparison of internal and external standardization in amino acid analysis," *Analytical Biochemistry*, Vol. 150, No. 1, 1985, pp. 174-177.

68. Barna, A. B., Furr, H. C., Olson, J. A., and van Breeman, R. B., "Vitamin A and Carotenoids," *Modern Chromatographic Analysis of Vitamins, Revised and Expanded.*, edited by A. P. de Leenheer, W. E. Lambert, and J. F. Van Bocxlaer Vol. 84, Chromatographic Science, Marcel Dekker, Inc., New York, USA, 2000, pp. 1-68.

69. "International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1)," 2004, http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html [cited 6 June 2010].

70. Medina, C., "Validating Analytical Methods for Pharmaceutical Applications," *Compliance Handbook for Pharmaceuticals, Medical Devices and Biologics.* Marcel Dekker Inc., New York, USA, 2005, pp. 98-151.

71. Rosing, H., Man, W. Y., Doyle, E., Bult, A., and Beijnen, J. H., "Bioanalytical Liquid Chromatographic Method Validation. A Review of Current Practice and Procedures," *Journal of Liquid Chromatography and Related Technology*, Vol. 23, No. 3, 2000, pp. 329-354.

72. Haught, R. and Fabris, M., "Inorganic Monitors," *Online Monitoring for Drinking Water Utilities.* Awwa Research Foundation, Colorado, USA, 2002, pp. 133-162.

73. Shabir, G. A., "Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization," *Journal of Chromatography A*, Vol. 987, No. 1-2, 2003, pp. 57-66.

74. Green, J. M., "Peer Reviewed: A Practical Guide to Analytical Method Validation," *Analytical Chemistry*, Vol. 68, No. 9, 1996, pp. 305A-309A.

75. Shah, V., Midha, K., Findlay, J., Hill, H., Hulse, J., McGilveray, I., McKay, G., Miller, K., Patnaik, R., Powell, M., Tonelli, A., Viswanathan, C. T., and Yacobi, A., "Bioanalytical

Method Validation - A Revisit with a Decade of Progress," *Pharmaceutical Research*, Vol. 17, No. 12, 2000, pp. 1551-1557.

76. Peters, F. T., Drummer, O. H., and Musshoff, F., "Validation of new methods," *Forensic Science International*, Vol. 165, No. 2-3, 2007, pp. 216-224.

77. Carr, G. P. and Wahlich, J. C., "A practical approach to method validation in pharmaceutical analysis," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 8, No. 8-12, 1990, pp. 613-618.

78. Ermer, J., "Validation in pharmaceutical analysis. Part I: An integrated approach," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 24, No. 5-6, 2001, pp. 755-767.

79. Paino, T. C. and Moore, A. D., "Determination of the LOD and LOQ of an HPLC Method Using Four Different Techniques," *Pharmaceutical Technology*, Vol. 23, No. 10, 1999, pp. 82-90.

80. "Authorised USP Pending Monograph, Version 1," 2009, http://www.usp.org/pdf/EN/pendingStandards/2010-04-m3749.pdf [cited 20 November 2010].

81. Alsante, K. M., Ando, A., Brown, R., Ensing, J., Hatajik, T. D., Kong, W., and Tsuda, Y., "The role of degradant profiling in active pharmaceutical ingredients and drug products," *Advanced Drug Delivery Reviews*, Vol. 59, No. 1, 2007, pp. 29-37.

82. Bakshi, M. and Singh, S., "Development of validated stability-indicating assay methods--critical review," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 28, No. 6, 2002, pp. 1011-1040.

83. Bakshi, M., Singh, B., Singh, A., and Singh, S., "The ICH guidance in practice: stress degradation studies on ornidazole and development of a validated stability-indicating assay," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 26, No. 5-6, 2001, pp. 891-897.

84. Buick, A. R., Doig, M. V., Jeal, S. C., Land, G. S., and McDowall, R. D., "Method Validation in the Bioanalytical Laboratory," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 8, No. 8-12, 1990, pp. 629-637.

85. Nowatzke, W. and Woolf, E., "Best Practices During Bioanalytical Method Validation for the Characterization of Assay Reagents and the Evaluation of Analyte Stability in Assay Standards, Quality Controls, and Study Samples," *The AAPS Journal*, Vol. 9, No. 2, 2007, pp. 117-122.

86. Hoffman, D., Kringle, R., Singer, J., and McDougall, S., "Statistical methods for assessing long-term analyte stability in biological matrices," *Journal of Chromatography B*, Vol. 877, No. 23, 2009, pp. 2262-2269.

87. Novak, I. and Kovac, B., "Photoelectron Spectra of Important Drug Molecules: Zidovudine and Artemisinine," *The Journal of Organic Chemistry*, Vol. 68, No. 14, 2003, pp. 5777-5779.

88. Marchei, E., Valvo, L., Pacifici, R., Pellegrini, M., Tossini, G., and Zuccaro, P., "Simultaneous determination of zidovudine and nevirapine in human plasma by RP-LC," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 29, No. 6, 2002, pp. 1081-1088.

89. Bharate, S. S., Bharate, S. B., and Baja, A. N., "Interactions and Incompatibilities of Pharmaceutical Excipients with Active Pharmaceutical Ingredients: A Comprehensive Review," *Review Paper*, 2010.

90. Crowley, P. and Martini, L. G., "Drug-Excipient Interactions," *Pharmaceutical Technology Magazine*, No. March, 2001.

91. Ng, K. and Rajagopalan, N., "Application of Quality by Design and Risk Assessment Principles for the Development of Formulation Design Space," *Quality by Design for Biopharmaceuticals: Principles and Case Studies.*, edited by A. S. Rathore and R. Mahtre John Wiley & Sons., New Jersey, USA, 2009.

92. Moreton, R. C., "Commonly Used Excipients in Pharmaceutical Suspensions," *Pharmaceutical Suspensions:From Formulation Development to Manufacturing.*, edited by A. K. Kulshreshtha, O. N. Singh, and M. Wall Springer, New York, USA, 2010, pp. 67-102.

93. Ahuja, S., "Overview of Pharmaceutical Analysis," *Handbook of Modern Pharmaceutical Analysis.*, edited by S. Ahuja and S. Scypinski, 2nd ed. Elsevier Inc., Massachusetts, USA, 2011, pp. 1-9.

94. Rachna Sagar, "Elements, Compounds and Mixtures," *Chemistry.* Rachna Sagar PVT. LTD., New Delhi, India, 2004, pp. 32-42.

95. Swarbrick, J., Rubino, J. T., and Rubino, O. P., *Coarse Dispersions*, 21st ed., Lippincott Williams and Wilkins, Maryland, USA, 1885.

96. Nutan, M. T. H. and Reddy, I. K., "General Principles of Suspensions," *Pharmaceutical Suspensions: From Formulation Development to Manufacturing.*, edited by A. K. Kulshreshtha, O. N. Singh, and M. Wall Springer Science + Business Media, LLC., New York, USA, 2010, pp. 39-66.

97. Aulton, M. E., *Pharmaceutics, the science of dosage form design*, Churchill Livingstone, Broadway, NY, 1988, pp. 62-80.

98. Tong, W. Q., "Practical Aspects of Solubility Determination in Pharmaceutical Preformulation," *Solvent Sytems and Their Selction in Pharmaceuticals and Biopharmaceuticals.*, edited by P. Augustijns and M. Brewster Biotechnology:Pharmaceutical Aspects, Springer, New York, USA, 2007, pp. 137-149.

99. Lawrence, A. T. and Attwood, D., "The Solubility of Drugs," *Physicochemical Principles of Pharmacy*, 4th ed. Pharmaceutical Press, London, UK, 2006, pp. 150.

100. Stella, V. J., "Formulation Challenges with Prodrugs," *Prodrugs: Challenges and Rewards.*, edited by V. J. Stella, R. T. Borchardt, M. J. Hageman, R. Oliyai, H. Maag, and J. W. Tilley Springer Science + Business Media, LLC., New York, USA, 2007, pp. 385-409.

101. Sandmann, B. J., "Solubility and Partition Phenomena," *Applied Physical Pharmacy.*, edited by M. M. Amiji and B. J. Sandmann The McGraw-Hill Companies, Inc., New York, USA, 2003, pp. 161-198.

102. Brittain, H. G. and Medek, A., "Polymorphic and Salvatomorphic Impurities," *Handbook of Isolation and Characterization of Impurities in Pharmaceuticals.*, edited by S. Ahuja and K. M. Alsante Vol. 5, Separation Science and Technology, Elsevier Science, San Diego, USA, 2003, pp. 39-74.

103. Barich, D. H., Zell, M. T., and Munson, E. J., "Physicochemical Properties, Formulation and Drug Delivery," *Drug Delivery: Principles and Applications.*, edited by B. Wang, T. Siahaan, and R. Soltero John Wiley & Son, Inc., New Jersey, USA, 2005, pp. 57-72.

104. Brittain, H. G., "X-ray Diffraction and X-ray Fluorescence," *Comprehensive Analytical Science.*, edited by D. Barcelo, 1st ed. Vol. 47, Modern Instrumental Analysis, Elsevier, B.V., Oxford, UK, 2006, pp. 177-226.

105. Dolitzsky, B.-Z.. Crystalline Solid Famciclovir Forms and Preparation Thereof. Teva Pharmaceutical Industries Limited. EP/EP03749164[EP1532151A2]. 5-25-2005. Ref Type: Patent

106. Caira, M. R., "Polymorphism," *Recent Advances, Techniques and Applications.*, edited by M. E. Brown and P. K. Gallagher Vol. 5, Handbook of Thermal Analysis and Calorimetry., Elsevier B.V., Oxford, UK., 2008, pp. 597-630.

107. Liltorp, K., Larsen, T. G., Willumsen, B., and Holm, R., "Solid state compatibility studies with tablet excipients using non thermal methods," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 55, No. 3, 2011, pp. 424-428.

108. Monajjemzadeh, F., Hassanzadeh, D., Valizadeh, H., Siahi-Shadbad, M. R., Mojarrad, J. S., Robertson, T. A., and Roberts, M. S., "Compatibility studies of acyclovir and lactose in physical mixtures and commercial tablets," *European Journal of Pharmaceutics and Biopharmaceutics*, Vol. 73, No. 3, 2009, pp. 404-413.

109. Balestrieri, F., Magri, A. D., Magri, A. L., Marini, D., and Sacchini, A., "Application of differential scanning calorimetry to the study of drug-excipient compatibility," *Thermochimica Acta*, Vol. 285, No. 2, 1996, pp. 337-345.

110. McMurry, J., "Structure Determination: Mass Spectroscopy and Infrared Spectroscopy," *Organic Chemistry.*, 6th ed. Brooks/Cole-Thompson Learning, California, USA, 2004, pp. 394-423.

111. Pedersen, S. F. and Myers, A. M., "Structure Determination," *Understanding the Principles of Organic Chemistry: A Laboratory Course.* Brooks/Cole-Cenage Learning, California, USA, 2011, pp. 141-199.

112. Laye, P. J., "Differential Thermal Analysis and Differential Scanning Calorimetry," *Principles of Thermal Analysis and Calorimetry* The Royal Society of Chemistry, Cambridge, UK, 2002, pp. 55-92.

113. Chieng, N., Rades, T., and Aaltonen, J., "An overview of recent studies on the analysis of pharmaceutical polymorphs," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 55, No. 4, 2011, pp. 618-644.

114. Gabbot, P., "A Practical Introduction to Differential Scanning Calorimetry," *Principles and Applications of Thermal Analysis.* Blackwell Publishing Ltd, Oxford, UK, 2008, pp. 1-50.

115. Giron, D., "Applications of thermal analysis in the pharmaceutical industry," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 4, No. 6, 1986, pp. 755-770.

116. Tita, B., Fulias, A., Bandur, G., Marian, E., and Tita, D., "Compatibility study between ketoprofen and pharmaceutical excipients used in solid dosage forms," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 56, No. 2, 2011, pp. 221-227.

117. Tomassetti, M., Catalani, A., Rossi, V., and Vecchio, S., "Thermal analysis study of the interactions between acetaminophen and excipients in solid dosage forms and in some binary mixtures," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 37, No. 5, 2005, pp. 949-955.

118. Scintag Inc., "Basics of X-ray Diffraction," *Providing Solutions to Your Diffraction Needs.* Scintag, Inc., California, USA, 1999, pp. 1-25.

119. Myers, R. L., "Matter," *The Basics of Physics.* Greenwood Press, Connecticut, USA, 2006, pp. 101-125.

120. Hammond, C., "X-ray Diffraction of Polycrystalline Materials," *The Basics of Crystallogrpahy and Diffraction.*, 3rd ed. Oxford University Press Inc., New York, USA, 2009, pp. 243-268.

121. Kalantar-Zadeh, K. and Fry, B., "Characterization Techniques for Nanoparticles," *Nanotechnology - Enabled Sensors.* Springer Science + Business Media, Melbourne, Australia, 2008, pp. 237-239.

122. Cornell, R. M. and Schwertmann, U., "Characterization," *The Iron Oxides.* Wiley-VCH, Verlag GmbH & co., Baden-Württemberg, Germany, 2003, pp. 139-184.

123. Ernest, T. B., Elder, D. P., Martini, L. G., Roberts, M., and Ford, J. L., "Developing paediatric medicines: identifying the needs and recognizing the challenges," *Journal of Pharmacy and Pharmacology*, Vol. 59, No. 8, 2007, pp. 1043-1055.

124. Nunn, T. and Williams, J., "Formulation of Medicine for Children," *British Journal of Clinical Pharmacology*, Vol. 59, No. 6, 2005, pp. 674-676.

125. Standing, J. F. and Tuleu, C., "Paediatric formulations--Getting to the heart of the problem," *International Journal of Pharmaceutics*, Vol. 300, No. 1-2, 2005, pp. 56-66.

126. Glass, B. D. and Haywood, A., "Stability considerations in liquid dosage forms extemporaneously prepared from commercially available products," *Journal of Pharmacy & Pharmaceutical Sciences*, Vol. 9, No. 3, 2006, pp. 398-426.

127. Garg, A., Garg, S., and She, R. W., "Development of an extemporaneous oral liquid formulation of oxandrolone and its stability evaluation," *Burns*, Vol. 37, No. 7, 2011, pp. 1150-1153.

128. Nahata, M. C. and Morosco, R. S., "Stability of Lisinopril in Two Liquid Dosage Forms," *The Annals of Pharmacotherapy*, Vol. 38, No. 4, 2004, pp. 396-399.

129. Nahata, M. C., "Development of two stable oral suspensions for gabapentin," *Pediatric Neurology*, Vol. 20, No. 3, 1999, pp. 195-197.

130. Pygall, S. R., Griffiths, P. C., Wolf, B., Timmins, P., and Melia, C. D., "Solution interactions of diclofenac sodium and meclofenamic acid sodium with hydroxypropyl methylcellulose (HPMC)," *International Journal of Pharmaceutics*, Vol. 405, No. 1-2, 2011, pp. 55-62.

131. Rowe, R. C., Sheskey, P. J., and Quinn, M. E., *Handbook of Pharmaceutical Excipients*, 6th ed., Pharmaceutical Press, London, UK, 2009.

132. Michael, R. and Richards, E., "Ophthalmic Products," *Pharmaceutical Practice.*, 4th ed. Elsevier Ltd., Philadelphia, USA, 2004, pp. 429-446.

133. Damian, F., Fabian, J., Friend, D. R., and Kiser, P. F., "Approaches to improve the stability of the antiviral agent UC781 in aqueous solutions," *International Journal of Pharmaceutics*, Vol. 396, No. 1-2, 2010, pp. 1-10.

134. Paddock Laboratories Inc., "Ora-Sweet, Flavoured Syrup Vehicle," 2010, http://www.stobec.com/documents/data/8196.pdf [cited 1 June 2011].

135. Durgin, J. M. and Hanan, Z. I., "Pharmaceutical Dosage Forms," *Pharmacy Practice for Technicians.* Delmar Cengage Learning., New York, USA, 2010, pp. 197-228.

136. Soni, M. G., Taylor, S. L., Greenberg, N. A., and Burdock, G. A., "Evaluation of the health aspects of methyl paraben: a review of the published literature," *Food and Chemical Toxicology*, Vol. 40, No. 10, 2002, pp. 1335-1373.

137. Soni, M. G., Burdock, G. A., Taylor, S. L., and Greenberg, N. A., "Safety assessment of propyl paraben: a review of the published literature," *Food and Chemical Toxicology*, Vol. 39, No. 6, 2001, pp. 513-532.

138. McNally, G. P. and Railkar, A. M., "Formulation of Pediatric Dosage Forms," *Pediatric Drug Development, Concepts and Applications.*, edited by A. E. Mulberg, S. A. Silber, and J. N. Van Den Anker John Wiley & Sons, Inc., New Jersey, USA, 2009, pp. 553-566.

139. Baka, E., Comer, J. E. A., and Takβcs-Novβk, K., "Study of equilibrium solubility measurement by saturation shake-flask method using hydrochlorothiazide as model compound," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 46, No. 2, 2008, pp. 335-341.

140. Walton, W. H., "Feret's Statistical Diameter as a Measurement of Particle Size," *Nature*, Vol. 162, No. 4113, 1948, pp. 329-330.

141. Halbert, G., "Pharmaceutical Development," *The Textbook of Pharmaceutical Medicine.*, edited by J. P. Griffin, 6th ed. John Wiley & Sons, Ltd., West Sussex, UK, 1993, pp. 81-100.

142. Connors, K. A., "Phenomena for Study," *Chemical Kinetics: The Study of Reaction Rates in Solution.* VCH Publishers, Inc., New York, USA, 1990, pp. 245-310.

143. Eisen-Nevo, H. and Maidan-Hanoch, D.. Drying Process for Preparing Crystalline Solid Famciclovir. Teva Pharmaceuticals USA, Inc. 12/291951[US Patent 20090076270]. 3-19-2009.
Ref Type: Patent

144. Storey, R. A., "Thermal Analysis - Conventional Techniques," *Solid State Characterization of Pharmaceuticals.* Blackwell Publishing., West Sussex, UK, 2011, pp. 135-186.

145. Phaechamud, T., Charoenteeraboon, J., and Mahadlek, J., "Characterization and in-vitro Drug Release of a Chitosan-Magnesium Stearate Monolithic Matrix System," *Asian Journal of Pharmaceutical Sciences*, Vol. 4, No. 5, 2009, pp. 265-276.

146. Shephard, A. B., Nichols, S. C., and Braithwaite, A., "Moisture induced solid phase degradation of l-ascorbic acid: Part 1: a kinetic study using tristimulus colorimetry and a quantitative HPLC assay," *Talanta*, Vol. 48, No. 3, 1999, pp. 585-593.

147. Kumar, V., Shah, R. P., Malik, S., and Singh, S., "Compatibility of atenolol with excipients: LC–MS/TOF characterization of degradation/interaction products, and mechanisms of their

formation," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 49, No. 4, 2009, pp. 880-888.

148. Vernin, G., Chakib, S., Rogacheva, S. M., Obretenov, T. D., and Parkanyi, C., "Thermal decomposition of ascorbic acid," *Carbohydrate Research*, Vol. 305, No. 1, 1997, pp. 1-15.

149. Barbooti, M. M. and Al-Sammerrai, D. A., "Thermal decomposition of citric acid," *Thermochimica Acta*, Vol. 98, No. 0, 1986, pp. 119-126.

150. Thomas, L. C., "Interpreting Unexpected Results and Transitions in DSC Results," *Technical Publications TA-039, Ta Instruments*, 2011.

151. Abdel-Kader, A., Ammar, A. A., and Saleh, S. I., "High-temperature Phase Transition in Potassium Dihydrogen Phosphate Crystals," *Thermochimica Acta*, Vol. 167, No. 2, 1990, pp. 225-233.

152. Islam, M. I. U. and Langrish, T. A. G., "An investigation into lactose crystallization under high temperature conditions during spray drying," *Food Research International*, Vol. 43, No. 1, 2010, pp. 46-56.

153. Dubinsksaya, V., Polyakov, N., Suponitskii, Y., Dement'eva, N., and Bykov, V., "Studies of moisture exchange between stearic acid, calcium stearate, and magnesium stearate," *Pharmaceutical Chemistry Journal*, Vol. 44, No. 2, 2010, pp. 89-93.

154. Rajala, R. and Laine, E., "The effect of moisture on the structure of magnesium stearate," *Thermochimica Acta*, Vol. 248, No. 0, 1995, pp. 177-188.

155. Biswal, S., Sahoo, J., and Murthy, P., "Characterisation of Gliclazide - PEG 8000 Solid Dispersions," *Tropical Journal of Pharmaceutical Research*, Vol. 8, No. 5, 2009, pp. 417-424.

156. Yamashita, K., Nakate, T., Okimoto, K., Ohike, A., Tokunaga, Y., Ibuki, R., Higaki, K., and Kimura, T., "Establishment of new preparation method for solid dispersion formulation of tacrolimus," *International Journal of Pharmaceutics*, Vol. 267, No. 1-2, 2003, pp. 79-91.

157. M.De Matas, C.Okwelogu, and P.York, "Paediatric Dosage Forms," *Chemistry Today*, Vol. 27, No. 5, 2009, pp. 20-24.

158. Freed, A. L., Silbering, S. B., Kolodsick, K. J., Rossi, D. T., Mahjour, M., and Kingsmill, C. A., "The development and stability assessment of extemporaneous pediatric formulations of Accupril," *International Journal of Pharmaceutics*, Vol. 304, No. 1-2, 2005, pp. 135-144.

159. Loyd, V., "Dosage form design and development," *Clinical Therapeutics*, Vol. 30, No. 11, 2008, pp. 2102-2111.

160. Helin-Tanninen, M., "Extemporaneous Preparation of Pediatric Oral Formulations," 2008, http://epublications.uef.fi/pub/urn_nbn_fi_uef-20100060/urn_nbn_fi_uef-20100060.pdf [cited 25 October 2011].

161. Woods, D. J., "Extemporaneous Formulations of Oral Liquids, a Guide," 1994, http://www.pharminfotech.co.nz/manual/Formulation/extemprep.pdf [cited 25 June 2011].

162. Eksborg, S., "The pharmacokinetics of antiviral therapy in paediatric patients," *Herpes : the journal of the IHMF*, Vol. 10, No. 3, 2003, pp. 66-71.

163. Cram, A., Breitkreutz, J., Desset-Brethes, S., Nunn, T., and Tuleu, C., "Challenges of developing palatable oral paediatric formulations," *International Journal of Pharmaceutics*, Vol. 365, No. 1-2, 2009, pp. 1-3.

164. Yariez, J. A., Brocks, D. R., Forrest, L. M., and Davis, N. M., "Pharmacokinetic Behavious of Orally Administered Drugs," *Oral Bioavailability* John Wiley & Sons, Inc, Hoboken, New Jersey, 2011, pp. 183-220.

165. Pharmapress, "Pharmaceutical solutions for oral administration," 2008, http://www.pharmpress.com/files/docs/FT_pharm_dosage_sample.pdf [cited 23 July 2011].

166. Buck.M.L, "A Guide to Pharmaceutical Excipients," *Pediatric Pharmacotherapy*, Vol. 2, No. 9, 1996.

167. Kairuz, C., Chhim, S., Hasan, F., Kumar, K., Lal, A., Patel, R., Singh, R., Dogra, M., and Garg, S., "Extemporaneous Compounding in a Sample of New Zealand Hospitals: A Retrospective Survey," *The New Zealand Medical Journal*, Vol. 120, No. 1251, 2007.

168. Barrows, J. N., Lipman, A. L., and Bailey, C. J., "Color Additives: FDA's Regulatory Process and Historical Perspectives," *Food Safety Magazine*, No. October/November, 2003.

169. Pifferi, G. and Restani, P., "The safety of pharmaceutical excipients," *Il Farmaco*, Vol. 58, No. 8, 2003, pp. 541-550.

170. Kearsley, M. W. and Dziedzic, S. Z., "Physical and Chemical Properties of Glucose Syrups," *Handbook of Starch Hydrolysis Products and Their Derivatives* Springer, London, 1995, pp. 129-154.

171. *British Pharmacopoeia*, Vol. 3, The Stationary Office, London, UK, 2010, pp. 3243.

172. Warne, T. M., "Analysis of Viscous Oils," *Manual on Hydrocarbon Analysis.*, edited by A. W. Drews, 6th ed. American Society for Testing and Material, West Conshohockern, PA, 1998, pp. 25-30.

173. Desai, D., Rao, V., Guo, H., Li, D., and Bolgar, M., "Stability of low concentrations of guanine-based antivirals in sucrose or maltitol solutions," *International Journal of Pharmaceutics*, Vol. 342, No. 1-2, 2007, pp. 87-94.

174. Yu, J., Chou, C. C., and Saska, M., "The Behavior of Invert Sugar in Sugar Processing," 2011, http://www.esugartech.com/SIT_2011_Behavior_of_Invert_Sugar_in_Sugar_Processing.pdf [cited 20 July 2011].

175. Fathima, N., Mamatha, T., Qureshi, H. K., Anitha, N., and Rao, J. V., "Drug-Excipient Interaction and Its Importance in Dosage Form Development," *Journal of Applied Pharmaceutical Science*, Vol. 1, No. 06, 2011, pp. 66-71.

176. Fresno Contreras, M. J., Ramirez Diguez, A., and Jimenez Soriano, M. M., "Viscosity and temperature relationship in ethanol/water mixtures gelified with Carbopol Ultrez(TM) 10," *Il Farmaco*, Vol. 56, No. 5-7, 2001, pp. 443-445.

177. "International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Pharmaceutical Development Q8 (R2)," 2009, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.pdf [cited 22 August 2011].

178. Wu, H., White, M., and Khan, M. A., "Quality-by-Design (QbD): An integrated process analytical technology (PAT) approach for a dynamic pharmaceutical co-precipitation process characterization and process design space development," *International Journal of Pharmaceutics*, Vol. 405, No. 1-2, 2011, pp. 63-78.

179. Bondi, J. and Drennen III, J. K., "Quality by Design and the Importance of PAT in QbD," *Separation Science and Technology*
Handbook of Modern Pharmaceutical Analysis, edited by Satinder Ahuja and Stephen Scypinski, Volume 10 ed. Academic Press, 2011, pp. 195-224.

180. Xu, X., Khan, M. A., and Burgess, D. J., "A quality by design (QbD) case study on liposomes containing hydrophilic API: I. Formulation, processing design and risk assessment," *International Journal of Pharmaceutics*, Vol. 419, No. 1-2, 2011, pp. 52-59.

181. Neway, J. O., "Quality by Design is Essential in the New U.S. Regulatory Environment," *Next Generation Pharmaceutical Magazine*, No. 10, October, 2007.

182. Fonner, D. E., Buck, J. R., and Banker, G. S., "Mathematical optimization techniques in drug product design and process analysis," *Journal of Pharmaceutical Sciences*, Vol. 59, No. 11, 1970, pp. 1587-1596.

183. Schwartz, J. B., Flamholz, J. R., and Press, R. H., "Computer optimization of pharmaceutical formulations I: General procedure," *Journal of Pharmaceutical Sciences*, Vol. 62, No. 7, 1973, pp. 1165-1170.

184. Lewis, G., "Optimization Methods," *Encylopedia of Pharmaceutical Technology.*, edited by J. Swarbrick and J. C. Boylan Marcel Dekker, Inc, New York, 2002, pp. 1922.

185. Podczeck, F., "Aims and Objectives of Experimental Design and Optimization in Formulation and Process Development," *Rational Design and Formulation*, 3rd ed. Vol. 2, Pharmaceutical Dosage Forms: Tablets., Informa Healthcare USA, Inc., New York, USA, 2007, pp. 105-136.

186. Singh, B., Kumar, R., and Ahuja, N., "Optimizing drug delivery systems using systematic "design of experiments." Part I: fundamental aspects," *Critical Reviews in Therapeutic Drug Carrier Systems*, Vol. 22, No. 1, 2005, pp. 27-105.

187. Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., and Escaleira, L. A. l., "Response surface methodology (RSM) as a tool for optimization in analytical chemistry," *Talanta*, Vol. 76, No. 5, 2008, pp. 965-977.

188. Carley, K. M., Kamneva, N. Y., and Reminga, J., "Response Surface Methodology, CASOS Technical Report," Carnegie Mellon University, 2004.

189. Khayet, M., Cojocaru, C., and Essalhi, M., "Artificial neural network modeling and response surface methodology of desalination by reverse osmosis," *Journal of Membrane Science*, Vol. 368, No. 1-2, 2011, pp. 202-214.

190. Singh, K. P., Gupta, S., Singh, A. K., and Sinha, S., "Experimental design and response surface modeling for optimization of Rhodamine B removal from water by magnetic nanocomposite," *Chemical Engineering Journal*, Vol. 165, No. 1, 2010, pp. 151-160.

191. Bas, D. and BoyacI, I. H., "Modeling and optimization I: Usability of response surface methodology," *Journal of Food Engineering*, Vol. 78, No. 3, 2007, pp. 836-845.

192. Vogel, H. C. and Todaro, C. L., "Statistical Methods for Fermentation Optimization," *Fermentation and Biochemical Enginering Handbook.* Noyes Publications., New Jersey, USA, 1997, pp. 161-180.

193. Khayet, M., Seman, M. N. A., and Hilal, N., "Response surface modelling and optimization of composite nanofiltration modified membranes," *Journal of Membrane Science*, Vol. 349, No. 1-2, 2010, pp. 113-122.

194. Ahuja, M., Yadav, M., and Kumar, S., "Application of response surface methodology to formulation of ionotropically gelled gum cordia/gellan beads," *Carbohydrate Polymers*, Vol. 80, No. 1, 2010, pp. 161-167.

195. Lewis, G., Mathieu, D., and Phan, R. T. L., "Variability and Quality, Analysing and Minimizing Variation," *Pharmaceutical Experimental Design.* Marcel Dekker, Inc., New York, USA, 2005, pp. 289-319.

196. Jo, M. S., Rene, E. R., Kim, S. H., and Park, H. S., "An analysis of synergistic and antagonistic behavior during BTEX removal in batch system using response surface methodology," *Journal of Hazardous Materials*, Vol. 152, No. 3, 2008, pp. 1276-1284.

197. Gottipati, R. and Mishra, S., "Process optimization of adsorption of Cr(VI) on activated carbons prepared from plant precursors by a two-level full factorial design," *Chemical Engineering Journal*, Vol. 160, No. 1, 2010, pp. 99-107.

198. Rahmanian, B., Pakizeh, M., Mansoori, S. A. A., and Abedini, R., "Application of experimental design approach and artificial neural network (ANN) for the determination of potential micellar-enhanced ultrafiltration process," *Journal of Hazardous Materials*, Vol. 187, No. 1-3, 2011, pp. 67-74.

199. Clasen, C. and Kulicke, W. M., "Determination of viscoelastic and rheo-optical material functions of water-soluble cellulose derivatives," *Progress in Polymer Science*, Vol. 26, No. 9, 2001, pp. 1839-1919.

200. Smith, W. F., "Optimisation," *Experimental Design for Formulation.* ASA-SIAM, Philadelphia, USA, 2005, pp. 277-296.

201. Lai, W. W., Vetter, V. L., Richmond, M., Li, J. S., Saul, J. P., Mital, S., Colan, S. D., Newburger, J. W., Sleeper, L. A., McCrindle, B. W., Minich, L. L., Goldmuntz, E., Marino, B. S., Williams, I. A., Pearson, G. D., Evans, F., Scott, J. D., and Cohen, M. S., "Clinical Research Careers: Reports from a NHLBI Pediatric Heart Network Clinical Research Skills Development Conference," *American Heart Journal*, Vol. 161, No. 1, 2011, pp. 13-67.

202. Davies, M. A., Boulle, A., Fakir, T., Nuttall, J., and Eley, B., "Adherence to antiretroviral therapy in young children in Cape Town, South Africa, measured by medication return and caregiver self-report: a prospective cohort study," *BMC Pediatrics*, Vol. 8, No. 1, 2008, pp. 34.

203. Breitkreutz, J., "European perspectives on pediatric formulations," *Clinical Therapeutics*, Vol. 30, No. 11, 2008, pp. 2146-2154.