AN ABSTRACT OF THE DISSERTATION OF

Sahar Abd El-Sattar Fahmy for the degree of Doctor of Philosophy in Pharmacy presented on July 8, 2004.

Title: Development of Novel Spray Coated Soft Elastic Gelatin Capsule Sustained Release Formulations of Nifedipine, Bioavailability and Bioequivalence of Verapamil HCL Controlled Release Formulations, Pharmacokinetics of Terbinafine after Single Oral Doses in Raptors.

Abstract approved:

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J. Mark Christensen James W. Ayres

This dissertation describes the development of a new sustained release formulation of nifedipine. The new formulation was developed by coating commercially available immediate release soft elastic gelatin capsules using a spray coating technique with two different polymeric combinations. Dissolution studies were conducted and showed that controlled release of nifedipine was obtained by increasing the ratio of the water insoluble polymer in the coat and increasing the percent weight gain of the coating. Simulated plasma concentration versus time profiles after administration of 30 mg dose of selected formulations showed a

prolonged nifedipine release with concentrations above the minimum effective concentration for up to 12 hours.

Bioavailability and bioequivalence of tableted test formulation of verapamil HCL was determined in 8 volunteers and compared to Covera HS® under fed and fasting conditions. The 90% confidence intervals for individual percent ratios of the C_{max}, AUC_{0-58} and $AUC_{0-\infty}$ were not within the range of 80 - 125% in both fed and fasted states, suggesting that these formulations are not bioequivalent. However, the bioavailability of verapamil from the new formulation was higher in the fed state but this effect was not statistically significant.

Pharmacokinetics of terbinafine administered orally at single doses of 15, 30, 60 and 120 mg were determined in raptors to recommend an appropriate dosing scheduled for terbinafine in the treatment of Aspergillosis. Calculation of steady state trough terbinafine plasma concentration after administration of daily doses of 15 or 30 mg /day showed that 30 mg daily dose of terbinafine administered orally in raptors produces a steady state trough terbinafine plasma concentration above the minimum inhibitory concentration (MIC) of (0.8 - 1.6) μg/ml against *aspregillus fumigatus*. From the data, 30 mg per day oral dose of terbinafine should be the recommended dose for treatment of aspergillosis in raptors. Approximate pharmacokinetic linearity of terbinafine was demonstrated for AUC_{0-t} in the dose range of 15 - 120 mg while non-linearity for C_{max} in the same dose range was demonstrated using the power model.

Development of Novel Spray Coated Soft Elastic Gelatin Capsule Sustained Release Formulations of Nifedipine, Bioavailability and Bioequivalence of Verapamil HCL Controlled Release Formulations, Pharmacokinetics of Terbinafine after Single Oral Doses in Raptors.

by
Sahar Abd El-Sattar Fahmy

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Sahar Abd El-Sattar Fahmy, Author

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF APPENDIX FIGURES

LIST OF APPENDIX FIGURES (Continued)

LIST OF APPENDIX FIGURES (Continued)

LIST OF APPENDIX FIGURES (Continued)

LIST OF APPENDIX FIGURES (Continued)

LIST OF APPENDIX TABLES

DEDICATION

To my Dad
who taught me that
education is a big aim you live for

(1) Development of Novel Spray Coated Soft Elastic Gelatin Capsule Sustained Release Formulations of Nifedipine, (2) Bioavailability and Bioequivalence of Verapamil HCL Controlled Release Formulations, (3) Pharmacokinetics of Terbinafine after Single Oral Doses in Raptors.

INTRODUCTION

There is always an increasing demand for the production of new sustained release drug delivery systems. Sustained release drug delivery systems designed for oral administration have numerous benefits over conventional dosage forms; maintain therapeutic concentrations of drug with narrow fluctuation, reduce frequency of dose administration, increases patient compliance, and minimize adverse side effects while reducing health care costs. Also, testing the efficiency of these delivery systems *in vivo* is an important step in drug development. This thesis describes the development of a new sustained release delivery system of nifedipine using commercially available immediate release soft elastic gelatin capsules and *in vivo* testing of a developed sustained release system of verapamil HCL.

Chapter 1 describes the formulation and *in vitro* evaluation of a new coated commercially available soft elastic gelatin capsule using nifedipine as a model drug. Coated capsules of nifedipine were formulated by coating the immediate release, commercially available, soft elastic gelatin capsules using a Fluid-bed

spray coater with different polymeric combinations. Sustained release action of the newly coated capsule formulations of nifedipine were evaluated by conducting dissolution studies in three different dissolution media to investigate pH effects on drug release. Convolution of the *in vitro* dissolution data is also described in this chapter.

Chapter 2 describes bioavailability and bioequivalence studies of single 240 mg doses of developed controlled release formulations of verapamil HCL and a marketed product of verapamil HCL, Covera HS® as a reference. Evaluation was done in crossover studies with 8 subjects under fed and fasting conditions. Statistical analysis was performed for testing bioequivalence based on the two one-sided t-tests which involves calculation of a 90% confidence interval for the ratio of averages of pharmacokinetic measures (maximum drug concentration, C_{max} and the area under the plasma concentration versus time curve, AUC, after log transformation) for the test and reference products.

Chapter 3 describes the pharmacokinetics of terbinafine in raptors following single oral doses of 15, 30, 60 and 120 mg to determine the appropriate dosing in raptors for the treatment of aspergillosis. Pharmacokinetic analysis was done with non-compartmental analysis using WinNonlin® program (Pharsight Version 3.2). Dose proportionality of pharmacokinetics of terbinafine in raptors following single oral dosing in the range of 15 - 120 mg was also determined for the pharmacokinetic parameters, C_{max}, and AUC from time zero to time t (AUC_{0-t}).

CHAPTER 1

Development of Novel Spray Coated Soft Elastic Gelatin Capsule Sustained Release Formulations of Nifedipine

Sahar A. Fahmy, J. Mark Christensen and James W. Ayres

ABSTRACT

Release of nifedipine from coated commercially available immediate release soft elastic gelatin capsules was investigated. Capsules were spray coated using two different polymeric combinations, Surelease® (ethyl cellulose) and Opadry® (hydroxypropyl-methylcellulose) or pectin at different coating loads.

In vitro drug release studies were conducted in three different dissolution media to investigate pH effect on nifedipine release. Release rate of nifedipine decreased as coating load of Surelease® increased. Nifedipine release from coated capsules depends on the amount of Surelease® in the coat, and thickness of the applied coat. There was a pH dependent effect on drug release from Surelease® Opadry® and Surelease® pectin formulations with no drug release in gastric fluid for all tested Surelease® pectin formulations. The effect of pectinolytic enzymes on nifedipine release from Surelease® pectin coated formulations was also investigated. There was no difference in release rate of nifedipine with and without pectinolytic enzymes because the release rate was about the same after enzyme addition.

Convolution of *in vitro* dissolution data was used to simulate expected plasma concentration versus time profiles after oral administration of a 30 mg dose of nifedipine following administration of commercially available sustained release formulations and new formulations of nifedipine. The expected plasma

concentrations obtained by convolution simulation of tested formulations were quite different from that of commercially available sustained release formulations of nifedipine. However, the tested formulations provided release profiles of nifedipine that are very promising in terms of desirable sustained release formulations.

INTRODUCTION

Sustained release delivery systems have been extensively investigated over the past years. These systems provide numerous benefits over immediate release dosage forms that do not control rate of drug input. Frequent administration of immediate release dosage forms of short half-life drugs is required to maintain drug concentrations in the therapeutic range. As a result, drug concentrations fluctuate considerably in blood or tissues over time. Sustained release dosage systems are designed to release drug over an extended period of time to achieve a desirable pharmacodynamic response. Sustained release systems can maintain therapeutic concentrations of drug within narrow fluctuation, reduce frequency of dose administration, increase patient compliance, and minimize adverse side effects while reducing health care costs (1).

The concentration profile of drug in blood versus time depends on the formulation technology used in sustained release dosage form production, which may generate different release patterns, resulting in different pharmacological and pharmacokinetic responses in blood or tissues. The basic techniques of developing sustained release systems use (1):

a) Insoluble, slowly eroding, or swelling matrices.

b) Polymer –coated Tablets, pellets, or granules.

c) Osmotically driven systems.

d) Systems controlled by ion exchange mechanisms

e) Various combinations of these approaches.

Formulation of sustained release dosage forms based on application of a film coat around the surface of unit dosage forms represents a simple way to provide sustained release of drug. The mechanism of drug release depends upon the materials used in both the unit dosage form and the film coat.

Soft elastic gelatin capsules

The formulation of drug into soft elastic gelatin capsules (SEG) has been used for many years as an immediate release oral dosage form. SEG capsules has several advantages; it can increase bioavailability of hydrophobic drugs as the drug can be incorporated in a liquid form, improve stability of drugs that are susceptible to oxidation or hydrolysis, eliminate many problems associated with tablet manufacturing including lack of content or weight uniformity and poor compaction (2). Table 1.01 shows a list of some commercially available products in the form of SEG capsules.

Coating of SEG capsules has been reported to mask unpleasant taste, to improve appearance (i.e. Nifedipine capsules 10 and 20 mg, Purpac Pharmaceutical Co.), and to control site of action (i.e. Mentothicone enteric coated SEG capsules, Pharco). Recently, research has been published involving conversion of liquid

filled SEG capsules into controlled release dosage forms by application of a composite wall on the surface of immediate release SEG capsules. This wall is composed of a barrier layer formed over the surface of the gelatin capsules and then an expandable layer formed over the barrier layer, and a final coating layer that is a semi-permeable layer formed over the expandable layer. Drug release occurred through an orifice in the external layer formed by mechanical or laser drilling (3). Production of such systems is complicated and costly. Therefore, conversion of an immediate release SEG capsules to a sustained release formulation by applying a diffusional barrier membrane in only one extra step starting with commercially available, marked SEG capsules and commonly used polymeric materials is reported herein, and represents a significant advance on the industrial scale. Some quite unexpected results were obtained.

Table 1.01: List of some of commercially available SEG capsules (4).

Commercial name	Active ingredient	Half-life	Manufacturing company
Depakene® capsules	Valproic acid 250 mg	9-16 hours	Abbott laboratories
Hytrin®	Terazosin HCL 1, 2, 5 and10 mg	~12 hours	Abbott laboratories
Adalat®	Nifedipine 10 and 20 mg	~2 hours	Bayer corporation
Nifedipine capsules	Nifedipine 10 and 20 mg	~2 hours	Purpac Pharmaceutical
Tessalon®	Benzonatate 100 (perles) and 200 mg (Cap.)	Not available	Forest Pharmaceuticals
Agenerase® capsules	Amprenavir 50 and 150 mg	7.1-10.6 hours	Glaxo Wellcome
Lanoxicaps®	Digoxin soln in cap.0.05, 0.1 and 0.2 mg	1.5-2 days	Glaxo Wellcome
Maximum strength Mylanta Gas® soft gel	Calcium carbonate, Magnesium hydroxide	Not available	J & J Merk Consumer
Motrin® gelcaps	Ibuprofen 200 mg	2 hours	McNeil Consumer healthcare
Tylenol® gelcaps	Acetaminophen 500 mg	1-3 hours	McNeil Consumer healthcare
Maximum strength Tylenol® allergy sinus	Acetaminophen + Chlorpheniramine maleate	Not available	McNeil Consumer healthcare
Neoral® SEG capsules	Cyclosporine 25 and 100 mg	8.4 hours	Novartis Pharmaceuticals
Procardia®	Nifedipine 10 and 20 mg	~2 hours	Pfizer

Table 1.01: List of some of commercially available SEG capsules (continued).

Commercial name	Active ingredient	Half-life	Manufacturing company
Accutane®	Isotretinoin 10, 20 and 40 mg	21 hours	Roche
Rocaltrol®	Calcitriol 0.5 and 0.25 mcg	5-8 hours	Roche
Vesanoid®	Tretinoin 10 mg	0.5-2 hours	Roche
Chromagen OB®	Prenatal multivitamin/ mineral	-	Savage lab.
Prometrium®	Progesterone 100 and 200 mg	-	Solvay Pharmaceutical
Atromid-S®	Clofibrate 500 mg	18 hours	Wheth-Ayerst Pharmaceuticals
Mentothicone enteric coated SEG capsules	Simethicone 100 mg Peppermint oil 80 mg	-	Pharco
Sandimmune®	Cyclosporine 25 and 100 mg	19 hours	Novartis Pharmaceuticals
Calderol® SEG capsules	Calcifediol 20 and 50 mcg	16 days	Organon Inc.
Indomethacin capsules	Indomethacin 50 mg	4.5 hours	Pharco

Film coating

"Film coating consists mainly of polymers, which are applied to the cores in the form of solutions or dispersions in which other excipients are dissolved or suspended" (5).

Coated pharmaceutical dosage forms were first developed using sugar coating for the purpose of masking unpleasant tastes and to improve appearance. With advanced technology, coating has other important applications: protect the drug against atmosphere, increase mechanical stability during manufacture, packaging and shipment, protect the drug against the influence of gastric fluid in the stomach, and to control site of action of drug.

Controlled release of drug from oral dosage forms can be influenced by film coating, which also depends upon type of polymeric materials used in the coat. Controlled release of drug is influenced by application of permeable or semi-permeable coating. In permeable coating, water can diffuse into the drug-loaded core. If the drug is water soluble, it can permeate through the coat and drug release is controlled by permeability of the film membrane. However, if the film coat is permeable to water but not to the drug, drug release occurs through pores in the film coat under the influence of the osmotic pressure developed in the core. Controlled release by applying semi-permeable membrane can be achieved by formulation of an osmotically active system in which the tablet core is coated with a semi-permeable membrane, and drug release occurs through a laser drilled orifice by internally generated pressure.

Model Drug: Nifedipine

Nifedipine was chosen as a model drug. Nifedipine is a calcium channel blocker known to be effective in treatment of stable, variant and unstable angina, mild to severe hypertension and Ray-nauds phenomenon (8). Conventional dosage forms of nifedipine must be dosed either twice daily (tablet) or three times a day (SEG capsules). Subsequent drug absorption is rapid and this coupled with a short elimination half life (2 - 5 hours) (6-8), results in significant fluctuation of peak and trough concentrations. Due to this pharmacokinetic profile, nifedipine conventional dosage forms may produce side effects such as tachycardia and flushing in some patients (8).

Controlling nifedipine delivery can alter this pharmacokinetic profile and provide constant plasma concentrations with minimal fluctuation. Nifedipine is a water insoluble drug, solubility = 10 μg/ml, so it represents a challenge for development of sustained release formulations of nifedipine (9).

Nifedipine sustained release formulations are available on the market as either a matrix tablet in which drug is dispersed in a polymeric matrix and release occurs by erosion (Adalat CC®, Bayer) (10), or an osmotic pump tablet in which drug is released in a zero- order manner (Procardia XL®, Pfizer). However, there is no sustained release dosage form of nifedipine in a capsule form (11).

Figure 1.01 illustrates the configuration of the novel delivery system described herein, and mechanism of drug release upon ingestion. Upon contact with

the gastrointestinal (G.I.) fluid, the coat is activated and the water soluble polymers form pores or holes in the coat which allow passage of the G.I. fluid to come inside the core containing drug, and drug comes out through these pores by diffusion.

Goals of this research were: a) to produce a sustained release action dosage form from a marketed immediate release dosage formulation in a process which is easy to manufacture, by applying a coating layer around an immediate release SEG capsule using a combination of polymeric materials. Two polymeric combinations were studied, ethylcellulose (Surelease®) as a water insoluble polymer with hydroxypropyl methylcellulose (Opadry®), or pectin, as water-soluble polymers with different ratios; b) to study the effect of gastric pretreatment on drug release from this new delivery system on both polymeric combinations; and c) to investigate the effect of pectinolytic enzymes on drug release from Surelease® pectin polymeric coated capsules.

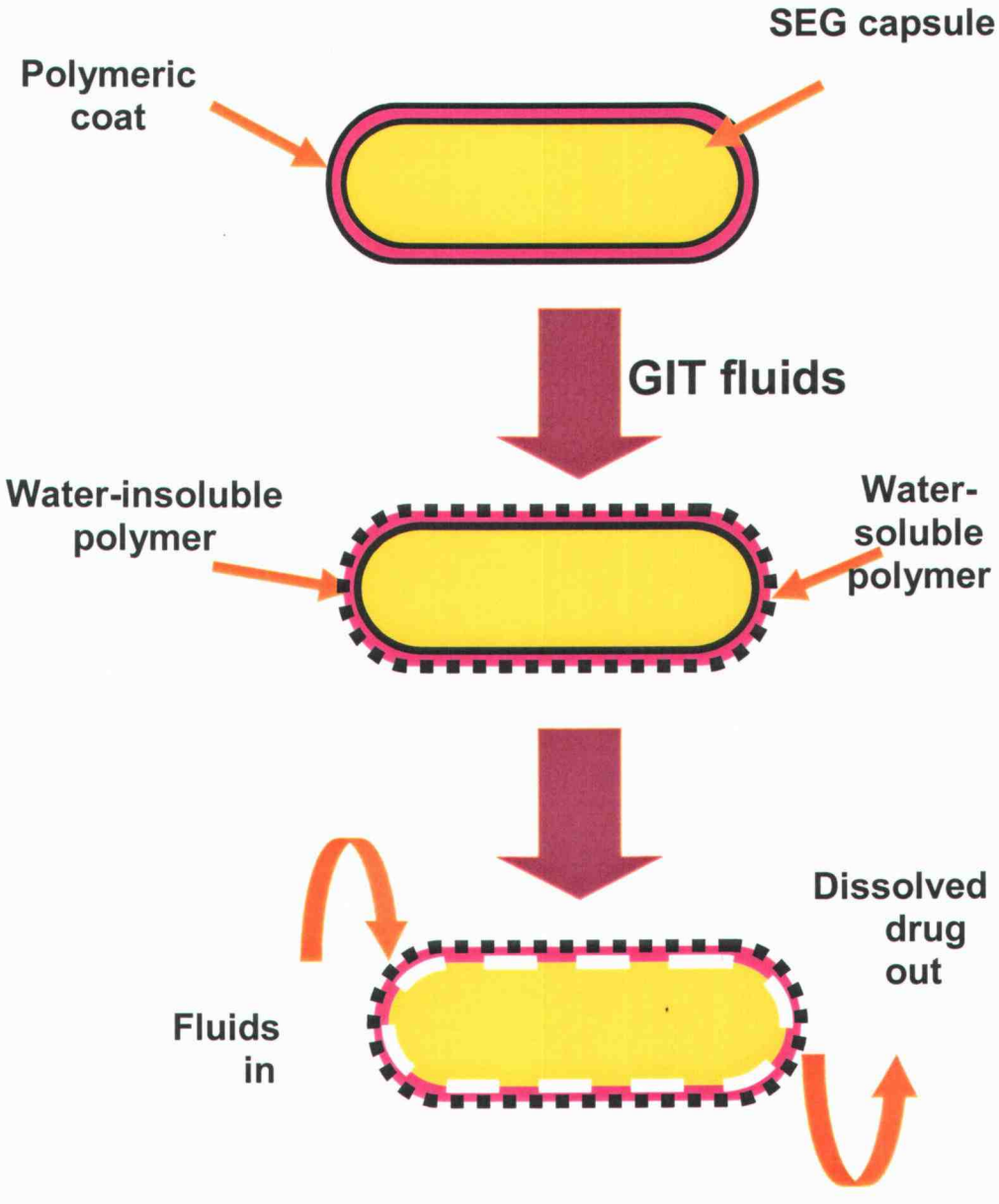

Figure 1.01: Schematic representation of drug release from coated immediate release SEG capsule formulation.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were purchased from standard sources. Nifedipine, poly-oxyethylene sorbitan monooleate (Tween 80, Sigma Chemicals Co., St. Louis, MO), nifedipine capsules, USP, 10 mg dose (Purpac Pharmaceutical Co., NJ), ethylcellulose aqueous dispersion- Surelease®, and hydroxypropyl methylcellulose (HPMC)-based coating formula-Opadry® (Colorcon, West Point, PA), Pectin PE 100 (Spectrum Quality Products INC, Gardena, CA- New Brunswick, NJ), acetonitrile, methanol (HPLC grade), sodium hydroxide, sodium phosphate mono-basic monohydrate, tri-basic sodium phosphate, sodium chloride, hydrochloric acid (Fisher Scientific, Fair Lawn, NY), Pectinex Ultra SP-L (gift supplied by Novo Nordisk Biochem., North America Inc., Franklinton, NC) was used to mimic pectinolytic enzymes in the colon. Water was distilled deionized water using Milli-Q reagent water system (Millipore, Bedford, MA).

METHODS

Coat preparation

Two different polymeric combinations were studied, Surelease® with Opadry® or pectin. Several coat formulations containing different amounts of polymeric materials were prepared (see Tables 1.02 and 1.03 for comparisons).

Film Coat suspension preparation

1. Weigh exact amount of Opadry® or pectin into a 400 ml beaker.
2. Measure exact volume of distilled water and transfer it into the beaker to form 20% w/v Opadry® solution or 5% w/v pectin solution. Stir until a homogenous solution is produced.
3. Based on manufacturer recommendations, Surelease® was diluted with distilled water to give (1:1) ratio. Measure exact volume of Surelease® in a graduated cylinder and transfer into the same beaker.
4. Gently stir for at least 30 minutes to ensure homogeneity of the coating solution.

Surelease® Opadry® Combination

Four coat formulations were prepared with different polymeric materials ratios which are described in Table 1.02. A 20% w/v solution of Opadry® in distilled water was prepared.

Table 1.02: Compositions of Surelease® Opadry® coat formulations

Formula	Component	Volume (ml)	Amount of solid (g)
O1	Surelease®	60	14.9
	Opadry®	75	15
	Water	60	solids ratio 1:1
O2	Surelease®	55	13.64
	Opadry®	45	8.75
	Water	55	solids ratio 3:2
O3	Surelease®	50	12.4
	Opadry®	32.5	6.5
	Water	50	solids ratio 2:1
O4	Surelease®	62.5	15.5
	Opadry®	31.25	6.25
	Water	62.5	solids ratio 3.5:1.5

Surelease® pectin combination

Two coat formulations were prepared with different Surelease® pectin ratios which are described in Table 1.03. A 5% w/v solution of pectin USP in distilled water was prepared.

Table 1.03: Composition of Surelease® pectin coat formulations.

Formula	Copmonent	Volume (ml)	Amount of solid (g)
P1	Surelease®	50	12.4
	5% pectin	130	6.43
	Water	50	solids ratio 2:1
P2	Surelease®	100	24.8
	5% pectin	100	5
	Water	100	solids ratio 5:1

Coating process

A number of 9-27 commercially available SEG nifedipine capsules and placebo sugar filled hard gelatin capsules as "filler" for the chamber to give a total capsule weight of 50 gm were placed into a Fluid-bed spray coater chamber (Strea-1, Nitro- Aeromatic, Columbia, MD) with a modified Wurster column insert (Figure 1.02) and pre-warmed for 5 minutes to equilibrate with the coating temperature (60 °C). Coating solution was delivered by peristaltic pump (Rabbit® peristaltic pump, Gilson Medical Electronics, Middleton, WI) with a flow rate of 1.75 ml/min and was applied through a 1.0 mm spray nozzle. Coating was performed at 60 °C inlet air temperature and 50 °C outlet air temperature. Air pressure was maintained at 10 - 15 psi to ensure continuous cyclic flow of capsules inside the chamber. During the coating process, coating solution was continuously stirred gently to ensure homogeneity of the solution.

Coating solution was applied onto the capsules to provide different percent weight gains. Percent weight gain is the actual weight gained relative to the weight of uncoated nifedipine SEG capsules.

Figure 1.02: A laboratory Aromatic Strea-1 fluidized bed coater.

Dissolution studies

Dissolution of coated SEG nifedipine capsules was conducted using the dissolution tester shown in Figure 1.03 (Dissolution Apparatus VK 7000®, Vankel Industries, Inc., Cary, NC). All tests were conducted in 1 liter dissolution medium maintained at 37 ± 0.5 °C (Heater VK 750 D® Vankel Industries, Inc., Cary, NC) with a paddle speed of 75 rpm. Dissolution studies of coated SEG nifedipine capsules were performed in triplicate and carried out in three different dissolution media:

1. Simulated gastric fluid (SGF) containing 1% Tween 80 for 2 hours, then pH was adjusted to 7.4 using 0.2 M tri-basic sodium phosphate for 22 hours.
2. Simulated intestinal fluid (SIF) containing 1% Tween 80 for 24 hours (pH 7.4).
3. Distilled water containing 1% Tween 80 for 24 hours.

The effect of pectinolytic enzymes on nifedipine release from Surelease® pectin coated capsules was studied by conducting dissolution studies in simulated gastric fluid for 2 hours followed by simulated intestinal fluid for 4 hours (pH adjusted to 7.4 using 0.2 M tri-basic sodium phosphate), then 3 ml of Pectinex ultra-SPL was added to each dissolution vessel for the remaining 24 hours.

Three ml samples of dissolution medium were collected without replacement at 5, 15, 30, and 45 min and 1, 1.5, 2, 3, 4, 5, 7, 9, 11, 13, 15, 17, 20

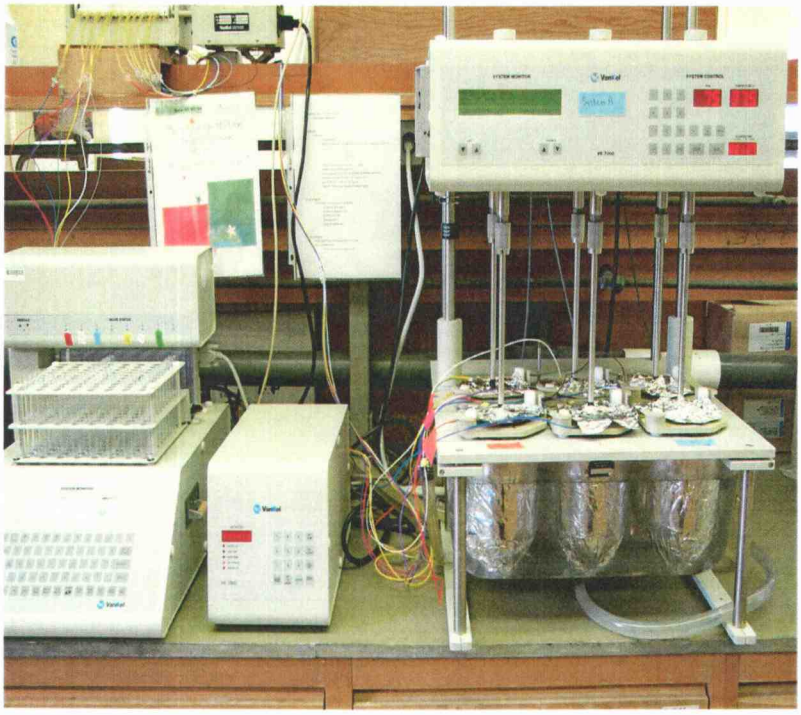

Figure 1.03: A Vankel dissolution tester.

and 24 hours using a computerized auto-sampler (VK 8000®, Vankel industries, Inc., Cary, NC) with peristaltic pump (VK 810®, Vankel industries, Inc., Cary, NC) with tube filter tips 70 micron.

Because nifedipine is a light sensitive drug, all dissolution studies were shielded from light. Dissolution vessels were amber colored wrapped with aluminum foil and the auto-sampler unit was covered with a cardboard box. Also, efforts were made with the sample handling to keep away from direct light as much as possible. Nifedipine analysis was conducted using high performance liquid chromatography (HPLC). HPLC results show minimal nifedipine degradation during these studies.

Chromatographic conditions

The HPLC column was a reverse phase micro particulate C_{18} (Prosphere C_{18}, particle size 5 μm, 250X4.6 mm, Alltech Associates, Inc., Deerfield, IL) preceded by a C_{18} guard cartridge (ODS, 4 x 3 mm, Phenomenex, CA).

The HPLC analytical method for nifedipine is similar to that in USP 23 (12) with some modification. Eluent was acetonitile: methanol: water in the ratio of 35:17:48. Mobile phase was prepared by mixing exact volumes of acetonitile, methanol, and water. Water used in the preparation of mobile phase was filtered under vacuum through a 0.2 μm filter. Mobile phase was degassed before use. The

flow rate was 0.8 ml/min in a HPLC integrated system composed of a delivery pump, UV detector, and automatic sampler injector (LC Module I integrated system, Waters Associates, Milford, MA) connected to an integrator (CR 501 Chromatopac, Schimadzu Corp., Kyoto, Japan). The UV detector was set at 240 nm wavelength.

Sample preparation

Samples were filtered through 0.45 microns filters, diluted with mobile phase in a ratio of 1:4, then vortex mixed for 30 seconds. Then 100 µl of this mixture was transferred into an HPLC vial containing 100 µl mobile phase and vortex mixed for 30 seconds. A volume of 100 µl was injected into the HPLC column.

Standard solution preparation

Nifedipine standard stock solution was prepared to contain 100 µg/ml of nifedipine by dissolving 10 mg nifedipine in 100 ml methanol in 100 ml amber colored volumetric flasks. This stock solution was diluted to prepare a second stock

solution of 10 μg/ml with mobile phase. The second stock solution was serially diluted with mobile phase to contain 100, 200, 400, 500, 1000, 1500, and 2000 ng/ml of nifedipine. All solutions were prepared in amber colored volumetric flasks and refrigerated unless in use. Freshly prepared standard solutions were prepared from time to time. It is reported that nifedipine standard solution, if prepared under light protection conditions and stored in a refrigerator will be stable for at least 3 months (13). Standard solutions of nifedipine were injected into HPLC with each run of samples. A standard curve was constructed by plotting peak area against nifedipine concentration. A typical standard curve of nifedipine is shown in Figure 1.04.

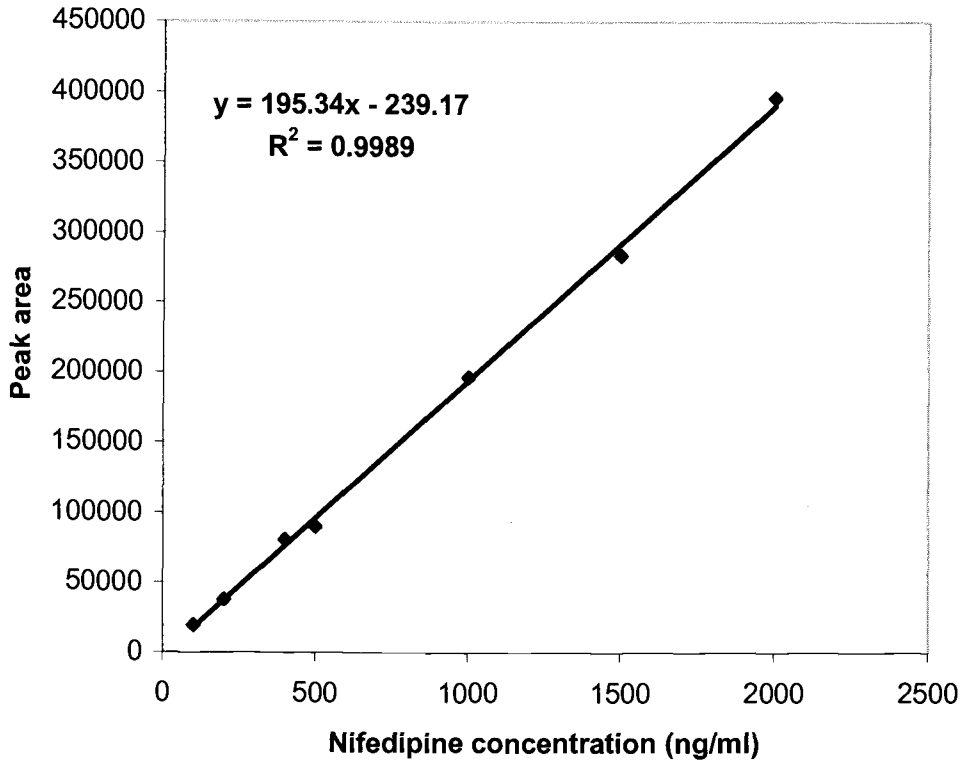

Figure 1.04: A representative standard curve of nifedipine.

Convolution Analysis

Convolution of *in vitro* dissolution profiles for certain nifedipine coated SEG capsule formulations and commercially available nifedipine formulations were conducted to predict plasma concentration versus time profiles, assuming these formulations are administered *in vivo*. The dissolution-time profile from nifedipine SEG capsules coated with the O3 coat formulation with 13% weight gain, nifedipine SEG capsules coated with P1 coat formulation with 14% weight gain, Procardia XL® 30 mg dose, Adalat CC® 30 mg dose and immediate release SEG nifedipine capsules were convolved to produce simulated plasma concentration-time profiles using a spread sheet. Simulated plasma concentration time profiles were compared with published data of plasma concentration time profiles for commercially available sustained release formulations of nifedipine (8).

RESULTS AND DISCUSSION

Difficulties often arise during coating of SEG capsules. It has been reported that problems associated with coating of SEG capsules were generally related to the physical properties of gelatin: capsules smooth surfaces, and their flexibility or elasticity. Application of aqueous solution as a coating solution caused solubilization of gelatin that composed the capsule shell which lead to softness and stickiness of capsules in the coating chamber (14). To overcome these difficulties, several trials have been made in search of optimum conditions for coating SEG capsules. Pre-warming of capsules before coating is an important step which increases temperature of the filled liquid to that of the bed temperature and allows coating to dry more uniformly which resulted in a homogenous film around the surface of the capsules. If the capsules were not pre-warmed, the capsule is cold and after completion of the coating process, the outer layers dry faster than the inner layers causing bubble formation in the film (14).

An excellent drying temperature was 60 °C, which allows fast drying of the film coat formed around the surface of the gelatin capsules. At lower temperatures, the drying process was not fast enough to prevent solubilization of the gelatin shell. At higher temperatures, the distortion temperature of the gelatin shell can be reached (which is reported to be 80 °C) causing deformation of the gelatin shell

(15). A good flow rate for the equipment used was 1.75 ml/min. Higher flow rates produced stickiness of capsules due to incomplete drying of the film coat applied.

Plasticizers are an especially necessary component in the coating solutions, which were already included in the commercially available aqueous dispersion of ethyl-cellulose (Surelease®) and in Opadry®. Plasticizer helps produce a smooth film, reduce brittleness, increase strength, and reduce tear resistance of the film coat (16). It was observed that coating solutions with higher pectin ratios produce flakes in the coating chamber, thought to be mainly due to low amount of plasticizers in these solutions.

Coating applied to the SEG capsules was stable, and adhered to the surface without cracking during the coating process. However, the shape of the capsules appeared expanded after the coating process, Figure 1.05.

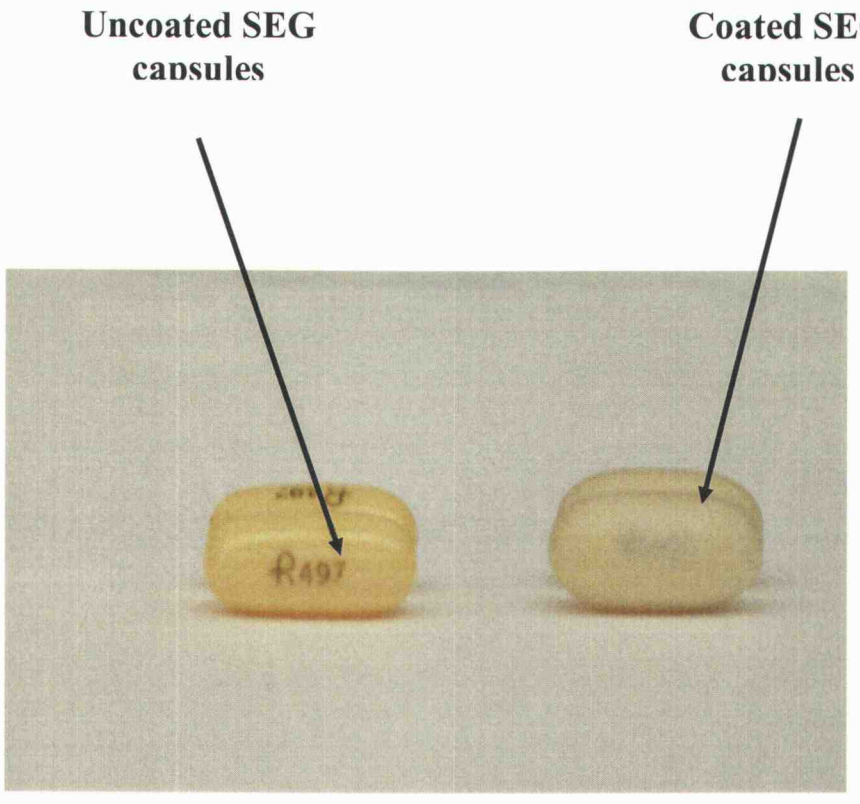

Figure 1.05: picture showing uncoated and Surelease® Opadry® coated SEG nifedipine capsules.

In vitro evaluation of coat performance

Dissolution studies of coated nifedipine SEG capsules and commercially available nifedipine formulations were conducted in three different dissolution media: simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours; simulated intestinal fluid; and water containing 1% Tween 80 to study pH effect on drug release from these formulations. Dissolution profiles of some commercially available nifedipine formulations are shown in Figures 1.06 (a, b, and c). Hundred percent drug release occurred from uncoated nifedipine SEG capsules in gastric fluid within 15 minutes. Nifedipine release from matrix tablet (Adalat CC®, Bayer) occurred by erosion and continues until 100% drug release after 11 hours with no lag time, and drug release was independent of pH of dissolution media used in the dissolution studies. Nifedipine release from osmotic pump tablets (Procardia XL®, Pfizer) has two hours lag time followed by zero-order drug release that continues until 24 hours, and is also independent of pH.

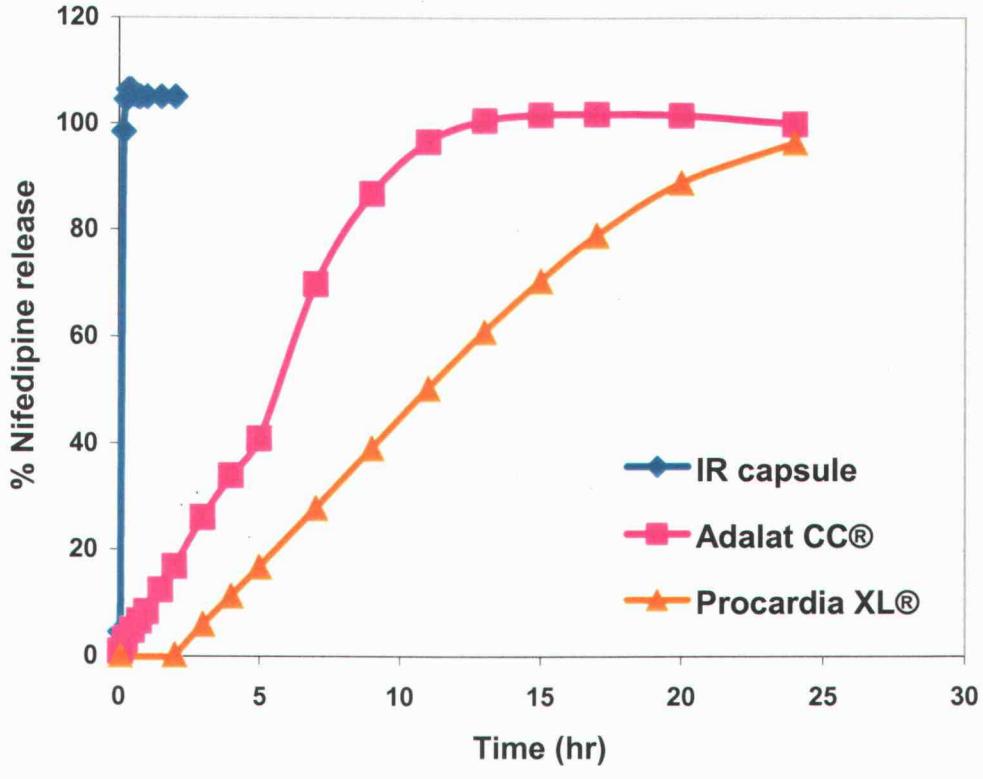

Figure 1.06a: Mean dissolution profiles of immediate release (IR) and sustained release nifedipine from commercially available formulations in simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours containing 1% Tween 80. (error bar represents standard deviation, n=3)

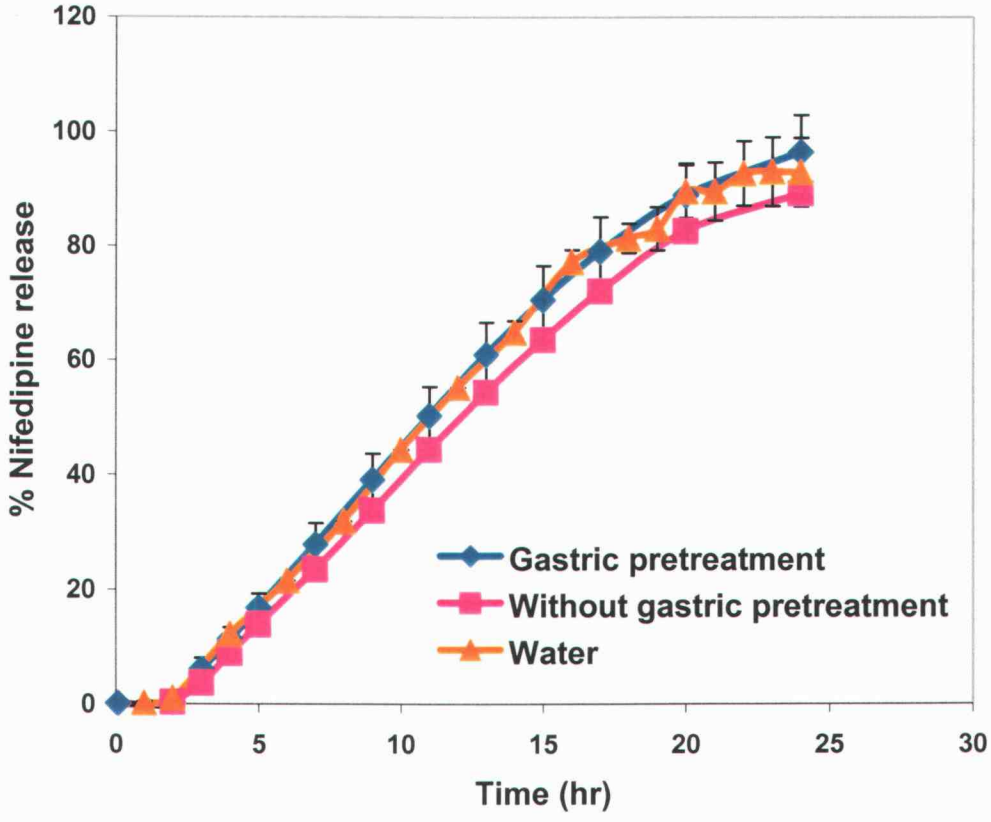

Figure 1.06b: Mean dissolution profiles of nifedipine from osmotic pump tablet (Procardia XL®) over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—◆—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n=3).

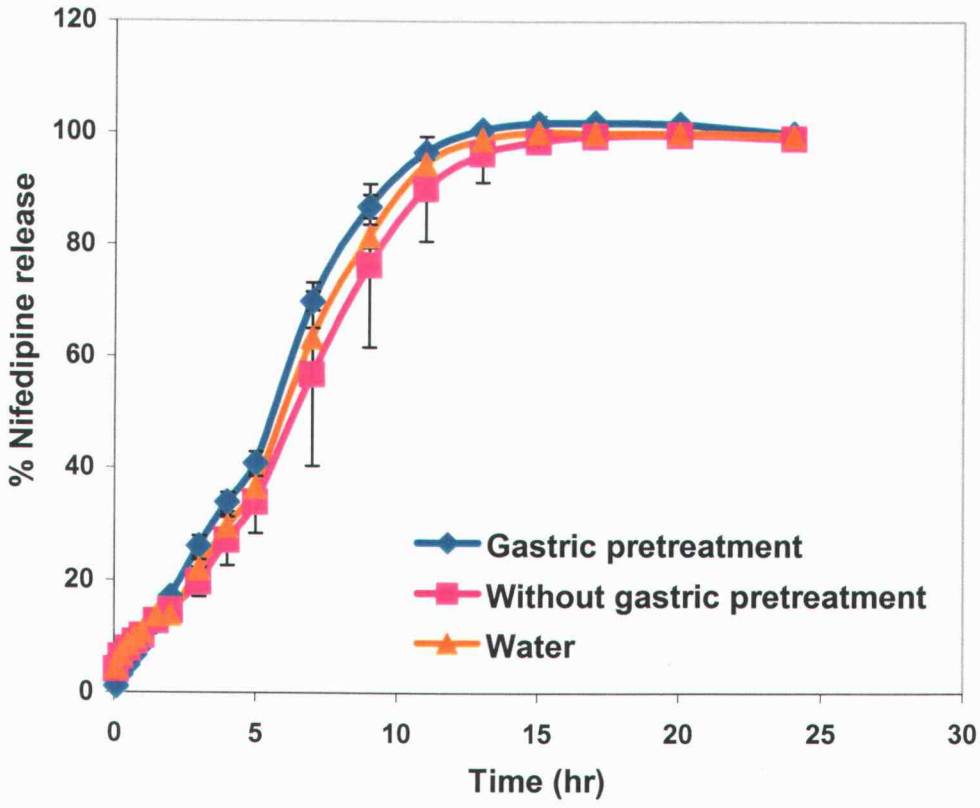

Figure 1.06c: Mean dissolution profiles of nifedipine from matrix tablet (Adalat CC®) over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—♦—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n=3).

Surelease® Opadry® combination

Nifedipine release from SEG capsules coated with four different coat formulations O1, O2, O3 and O4 with different weight gain was performed in three different dissolution media in triplicate, in simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours, in simulated intestinal fluid, and in water containing 1% Tween 80.

Release from coat formulation (O1)

Percent drug released from nifedipine SEG capsules coated with 1:1 ratio with 16% actual weight gain is shown in Figure 1.07. Dissolution experiments were in triplicate in three different dissolution media. Table 1.04 shows differences in percent drug release from the O1 coated capsules in these different dissolution media.

Table 1.04: Differences in drug release from nifedipine O1 SEG coated capsules in different dissolution media (n = 3).

Formula O1	*Gastric fluid /intestinal fluid	Intestinal fluid	Water
Lag time	-	-	-
Time of 50% drug release	5 min	5 min	5 min
Time of 100% drug release	15 min	15 min	15 min
% Released after 24 hours	100 %	100 %	100 %

*Simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours.

By comparison to Figure 1.06a, it is clear there is no difference in drug release in the three dissolution media from that of uncoated SEG capsules, which indicates ineffective polymer coating. Hundred percent drug release occurred within 15 - 20 minutes for coated capsules. Generally, as Surelease® is a water insoluble polymer it is the rate controlling membrane. With higher HPMC ratios, HPMC particles in the film coat provide a point of entry for dissolution media to enter the capsules which increases dissolution rate of drug.

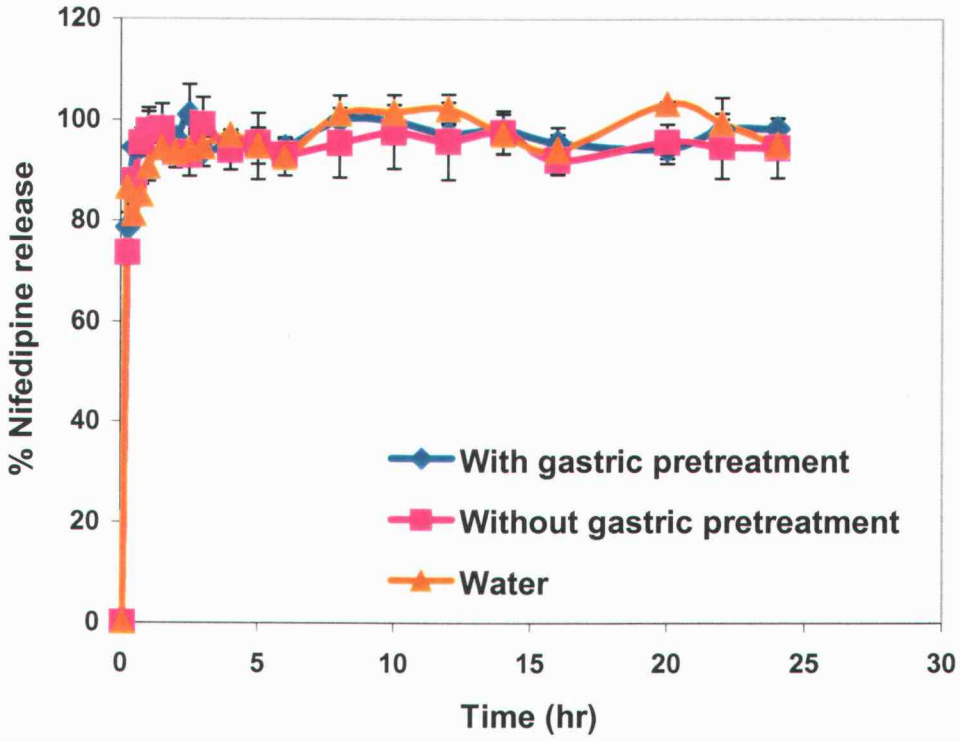

Figure 1.07: Mean nifedipine release from O1 coated SEG capsules with 16% actual weight gain over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—♦—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n=3).

Release from coat formulation (O2)

Percent nifedipine released at different times from nifedipine coated SEG with 14%, 17% and 21% coat weight gains of O2 coat formulation are shown in Figures 1.08 (a, b, c and d). Table 1.05 shows some differences in drug release from SEG capsules coated with O2 coat formulation with different weight gains in three different dissolution media.

As shown in Figure 1.08a, capsules coated with 14% O2 coat formulation showed 100% nifedipine release within 4 hours with 0.5 hour lag time, attributed to higher Surelease® ratio in O2 formula than in O1. Drug release from 14% O2 formula was the same in the three dissolution media, which indicates no pH dependent effect on drug release (see Figure 1.08a). Note that the rate of drug release following the lag time was quite rapid.

As shown in Figure 1.08b, 17% weight gain did not produce a significant difference in drug release when compared to the 14% weight gain of O2 coat formulation in intestinal fluid or water; but, the dissolution profile was quite different for gastric fluid pretreatment for 2 hours followed by intestinal fluid, wherein nifedipine release was delayed with 71% drug release within 5 hours and average slow rate of release thereafter to provide 92% drug release within 24 hours.

As shown in Figure 1.08c, with the 21% weight gain O2 formula, lag time increased from 0.5 hour to 0.75 hour. Drug release in intestinal fluid and water was complete within 5 hours. With gastric pretreatment, 67% of nifedipine was released

after 5 hours period, followed by a very slow release rate with only 86% drug released during the 24 hours. This indicated a pH effect on nifedipine release from 21% O2 formula. This pH effect for Surelease® HPMC containing films on dosage forms is surprising because the solubility of ethylcellulose (Surelease®) has been reported to be pH independent (17). However, it was reported that release rates of theophylline, phenylpropanolamine HCL, propranolol HCL (17), ketoprofen, and nicardipine HCL (18) from Surelease® coated beads were pH dependent. Release rate from Surelease® coated beads (with high percent coating) were controlled by diffusion through the coating film and therefore based on the concentration of non-ionized form of drugs. Nifedipine, with unknown pKa, has a faster rate of drug release from O2 coat formulation with 21% actual weight gain in a basic medium than in an acidic medium. Thus suggesting that nifedipine is expected to be in the ionized form in an acidic medium with less diffusion through the film coat while nifedipine will be in the non-ionized form in a basic medium.

Figure 1.08d shows drug dissolution curves for O2 formulations are quite different from commercially available products. It can be noted that drug release from the 21% O2 formulation is promising in terms of relatively rapid release over the first 5 hours followed by sustained release which is desirable in many cases.

Table 1.05: Differences in drug release from nifedipine O2 SEG coated capsules in different dissolution media (n = 3).

Formula O2	*Gastric fluid/ intestinal fluid	Intestinal fluid	Water
14 % weight gain			
Lag time	0.5 hours	0.5 hours	0.5 hours
Time of 50% drug release	1.5 hours	1.25 hours	1.5 hours
Time of 100% drug release	4 hours	4 hours	4 hours
% Released after 24 hours	100 %	100 %	100 %
17% weight gain			
Lag time	0.5 hours	0.5 hours	0.5 hours
Time of 50% drug release	2 hours	1.5 hours	1.5 hours
Time of 100% drug release	Not determined	5 hours	5 hours
% Released after 24 hours	92%	100%	100%
21% weight gain			
Lag time	0.75 hours	0.75 hours	0.75 hours
Time of 50% drug release	3.5 hours	2 hours	2 hours
Time of 100% drug release	Not determined	5 hours	5 hours
% Released after 24 hours	86%	100%	100%

*Simulated gastric fluid pretreatment for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours.

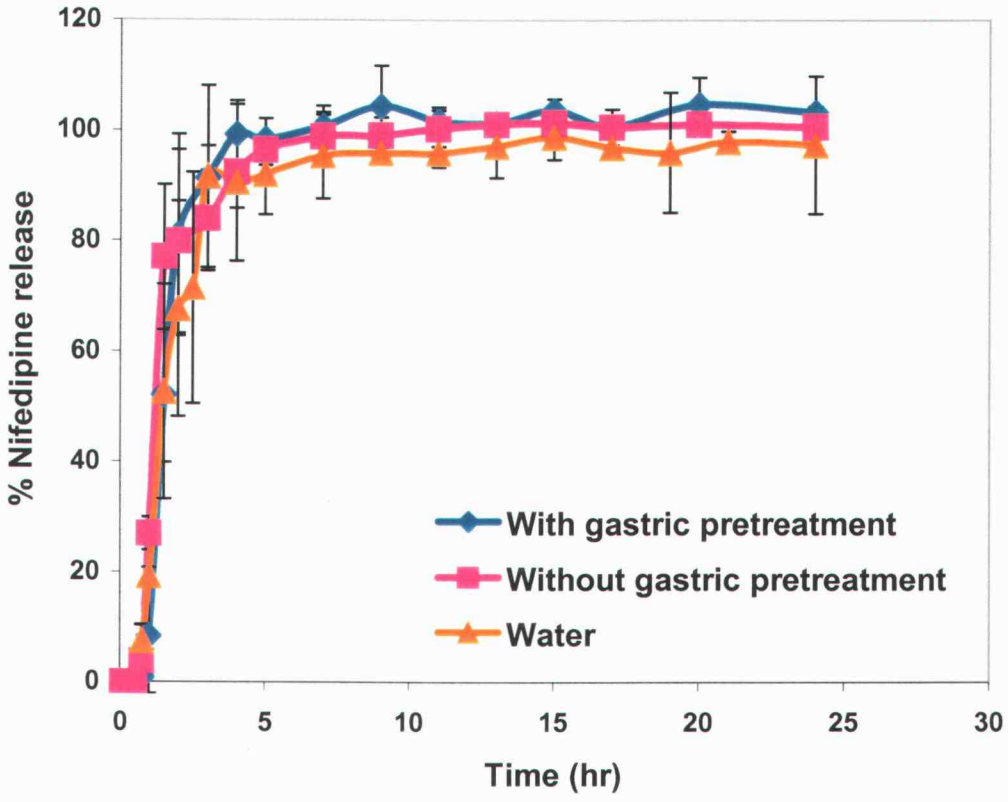

Figure 1.08a: Mean nifedipine release from O2 coated SEG capsules with 14% actual weight gain over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—◆—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n = 3).

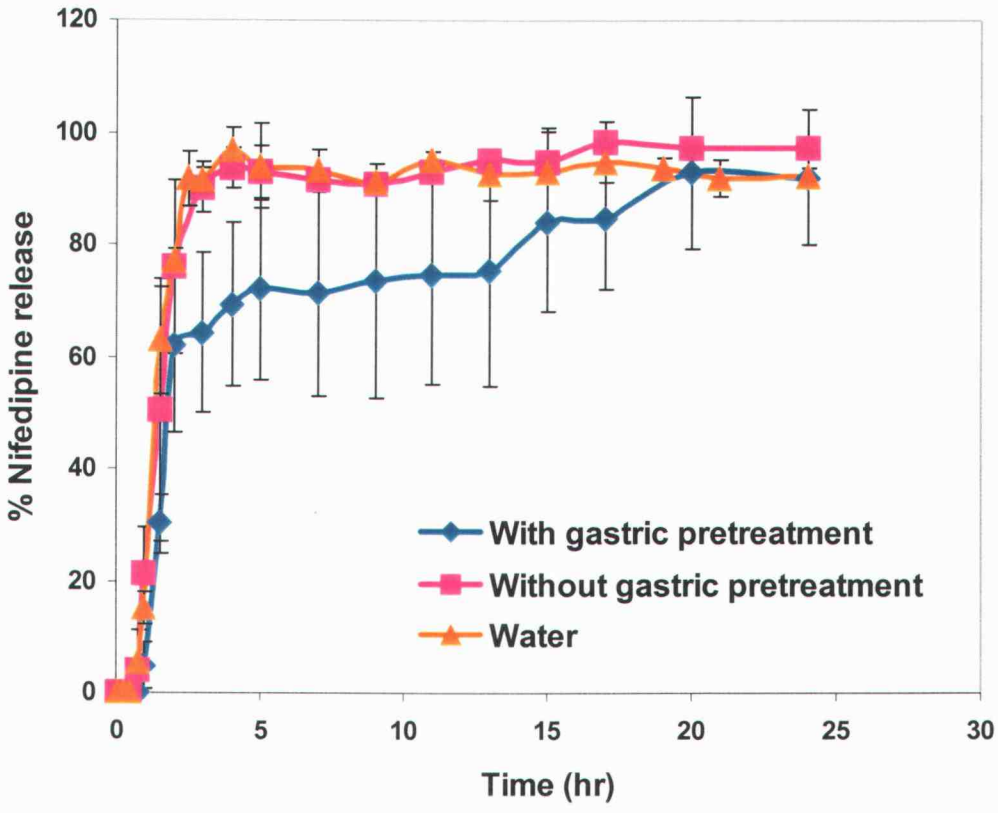

Figure 1.08b: Mean nifedipine release from O2 coated SEG capsules with 17% actual weight gain over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—◆—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n = 3).

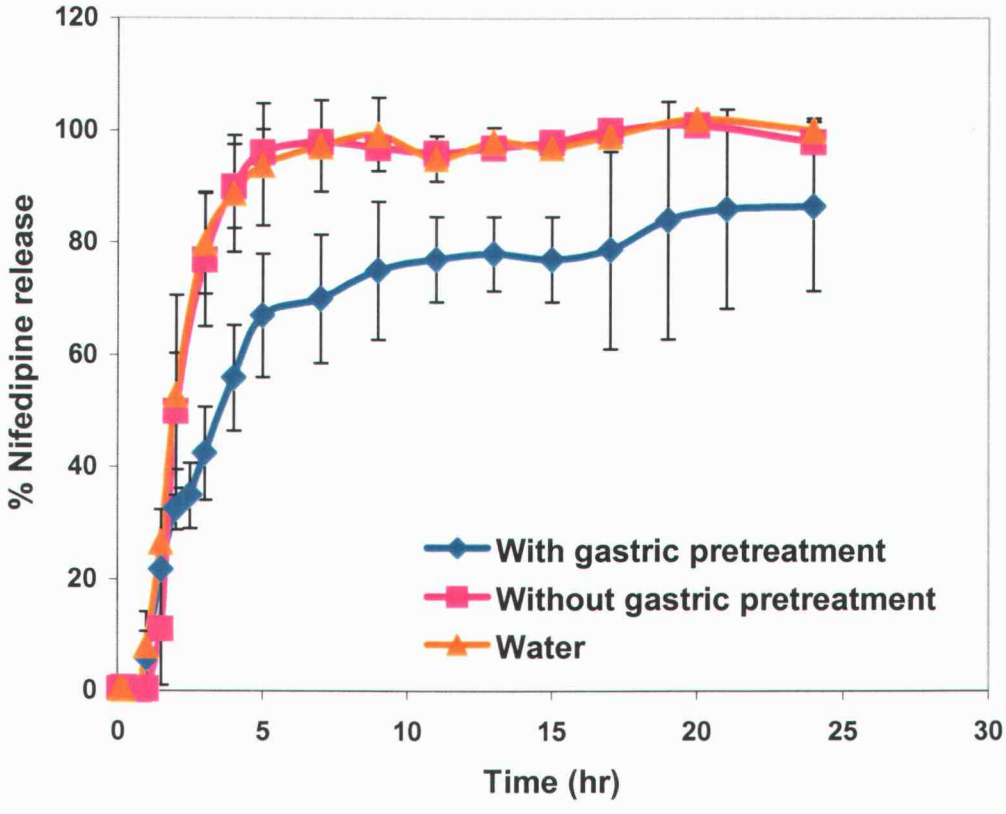

Figure 1.08c: Mean nifedipine release from O2 coated SEG capsules with 21% actual weight gain over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—◆—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n = 3).

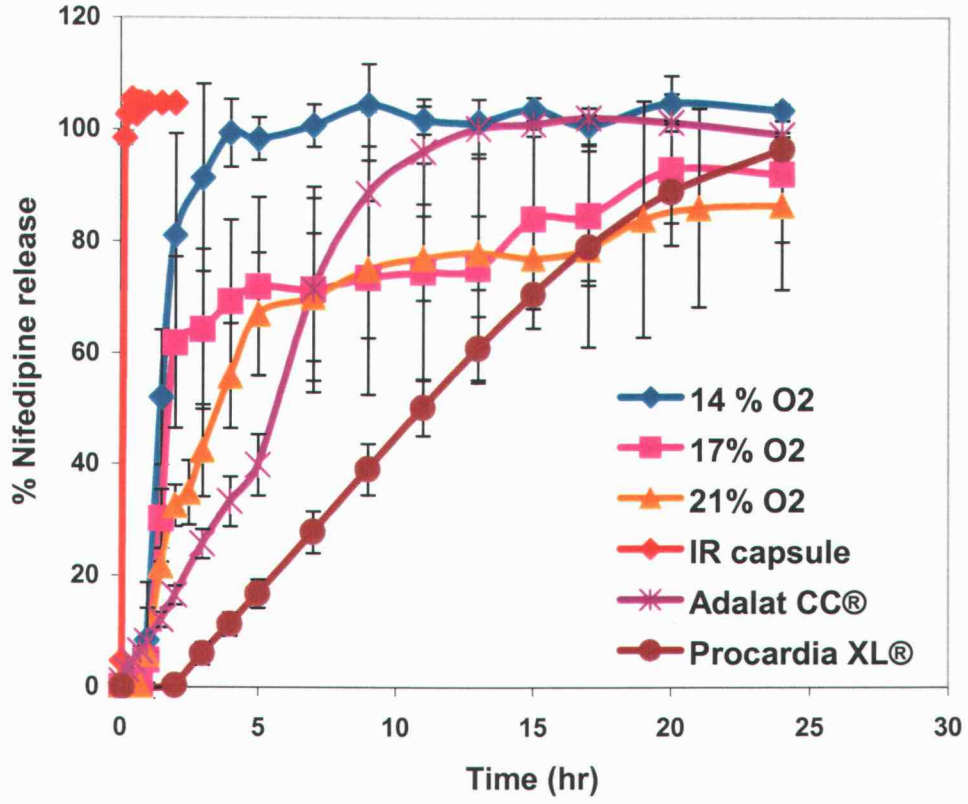

Figure 1.08d: Nifedipine release from commercially available formulae (immediate release, IR, and sustained release) and O2 SEG coat formulations in simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours (error bar represents standard deviation, n = 3).

Release from coat formulation (O3)

Percent drug released at different times from nifedipine SEG capsules coated with O3 coat formulation (contains less HPMC relative to O2 formulation) with 13%, 18% and 21% coat weight gains are shown in Figures 1.09 (a, b, c, and d). Table 1.06 shows some differences in drug release from capsules coated with O3 coat formulation with different weight gains in three different dissolution media.

Capsules coated with 13% O3 coat released about 100% nifedipine within 4 hours with 1 hour lag time which was attributed to higher Surelease® ratio (2:1) in O3 than in O2. Nifedipine release from the 13% O3 formula was different in the three dissolution media indicating there was a pH dependent effect on nifedipine release as shown in Figure (1.09a). With gastric pretreatment, nifedipine release was delayed and only 90% of nifedipine was released during 24 hours, compared to 100% drug release in either intestinal fluid or water dissolution media without pretreatment in gastric fluid. With higher percent weight gain of coating, it is expected that this effect will be more pronounced.

Eighteen percent actual weight gain of O3 coat formula resulted in 1.5 hour lag time with gastric pretreatment and 88% drug released after 24 hours compared to 1 hr lag time and 100% drug release in intestinal fluid and in water (Figure 1.09b). Twenty one percent actual weight gain of O3 coat formula resulted in a longer lag time (2 hours with gastric pretreatment) and 69% drug released during

24 hours dissolution compared to 1.5 hour lag time and 85% drug release in 24 hours dissolution in intestinal fluid and in water (Figure 1.09c). Dissolution of drug from new O3 formulations is compared to Adalat CC® and Procardia XL® in Figure 1.09d.

Figure 1.10 shows the shape of nifedipine capsules coated with 21% O3 coat formulation after 24 hour dissolution. Notice that the capsule was intact and the surface was irregular or wrinkled. In Figure 1.11, one can see a cross section of the nifedipine capsules coated with 21% O3 coat formulation after 24 hours dissolution. Also, nifedipine precipitated into yellow crystals inside the capsule shell.

With O3 coat formulations, all the coating percent weight gains studied resulted in drug precipitation inside the coated SEG. pH effect was also observed in 13%, 18% and 21% weight gain of coating. The precipitation of nifedipine was an unexpected and surprising finding and will be discussed in more detail later in "Nifedipine release from Surelease® Opadry® combinations" section.

Table 1.06: Differences in drug release from nifedipine O3 SEG coated capsules in different dissolution media (n = 3).

Formula O3	*Gastric fluid/ intestinal fluid	Intestinal fluid	Water
13 % weight gain			
Lag time	1 hr	0.5 hour	0.5 hour
Time of 50% drug release	3 hours	1.25 hours	1.5 hours
Time of 100% drug release	Not determined	4 hours	4 hours
% Released after 24 hours	90 %	97 %	96 %
17% weight gain			
Lag time	1.5 hours	1 hour	1.5 hours
Time of 50% drug release	4 hours	1.75 hours	2.5 hours
Time of 100% drug release	Not determined	4 hours	4 hours
% Drug released after 24 hours	88%	100%	100%
21% weight gain			
Lag time	2 hours	1.5 hours	1.5 hours
Time of 50% drug release	9 hours	3.5 hours	5 hours
Time of 100% drug release	Not determined	Not determined	Not determined
% Released after 24 hours	69%	85%	82%

*Simulated gastric fluid pretreatment for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours.

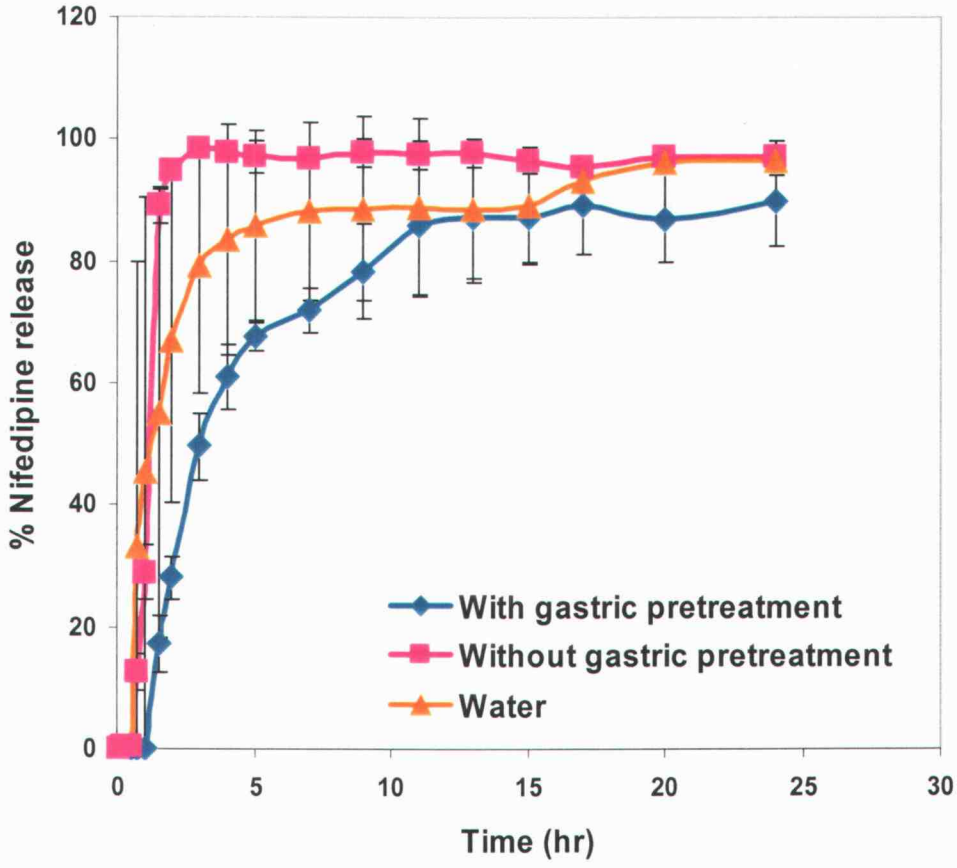

Figure 1.09a: Mean nifedipine release from O3 coated SEG capsules with 13% actual weight gain over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—♦—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n = 3).

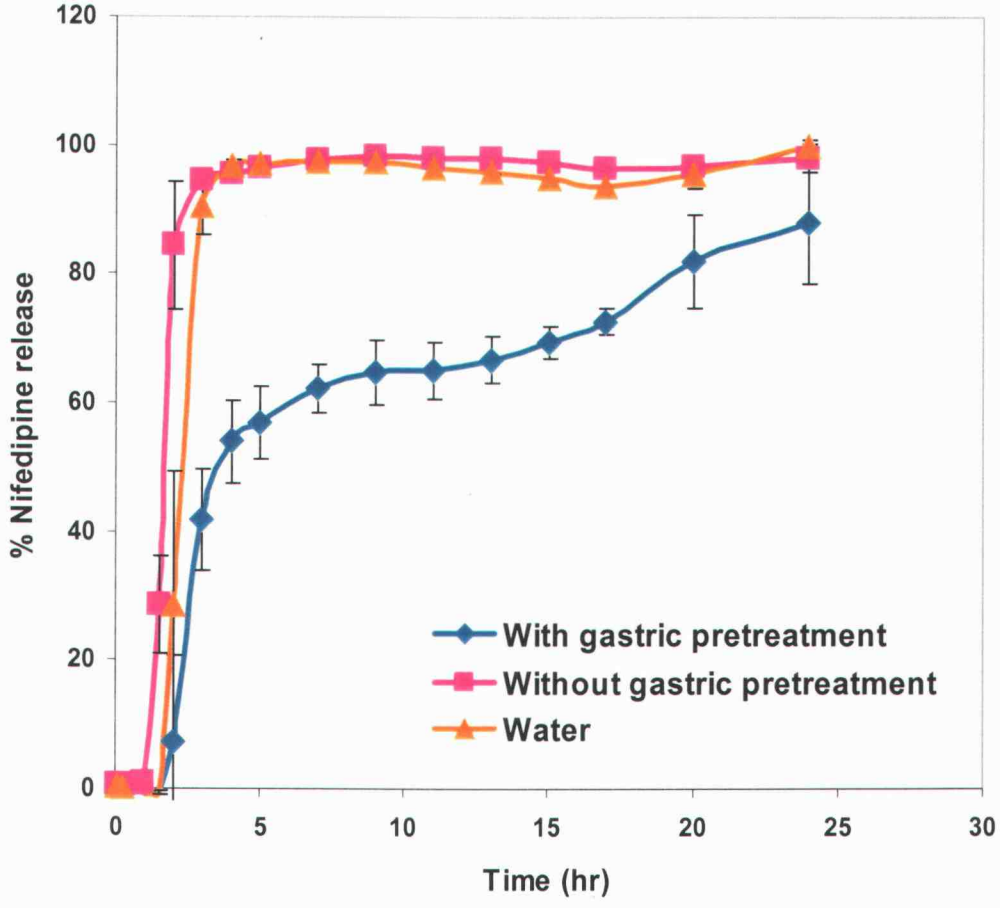

Figure 1.09b: Mean nifedipine release from O3 coated SEG capsules with 18% actual weight gain over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—♦—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n = 3).

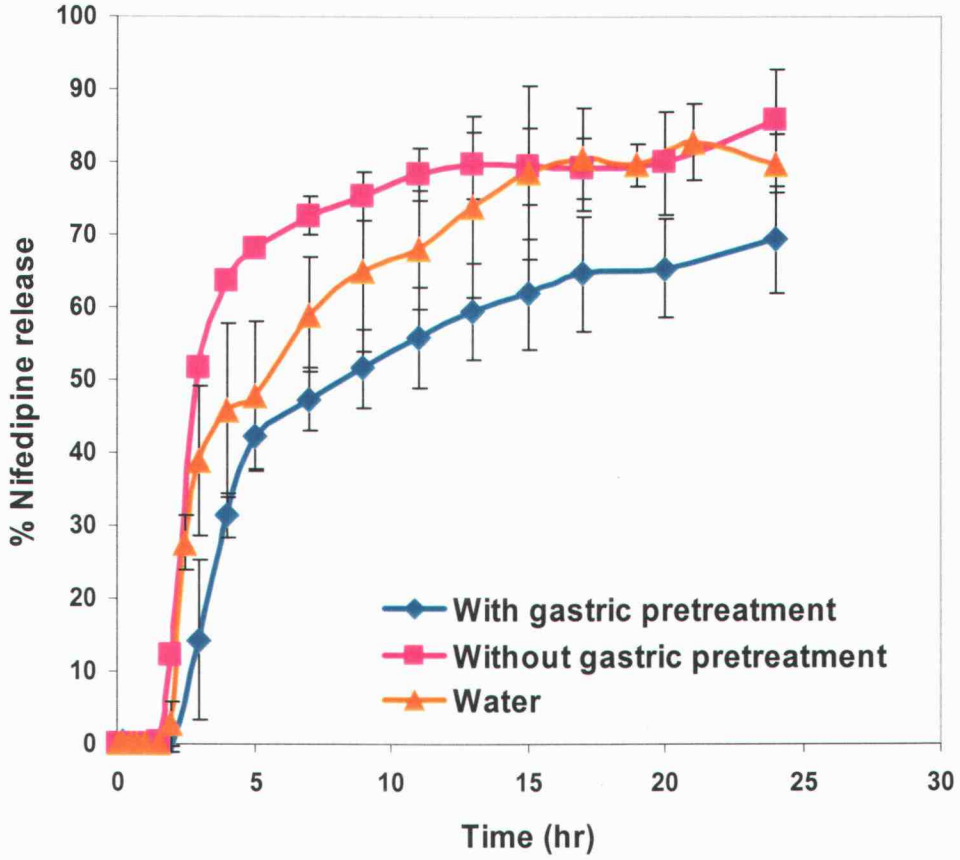

Figure 1.09c: Mean nifedipine release from O3 coated SEG capsules with 21% actual weight gain over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—◆—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n = 3).

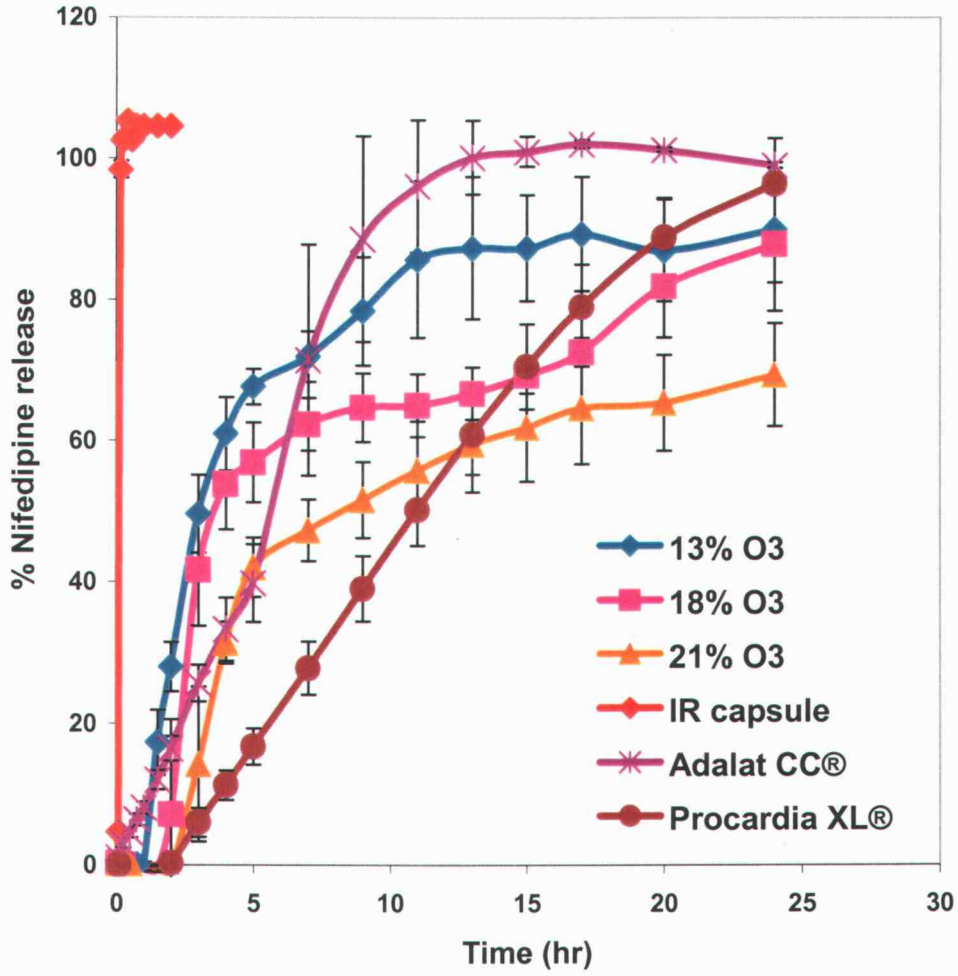

Figure 1.09d: Mean nifedipine release from commercially available formulae (immediate release, IR, and sustained release) and O3 SEG coat formulations in simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours (error bar represents standard deviation, n = 3).

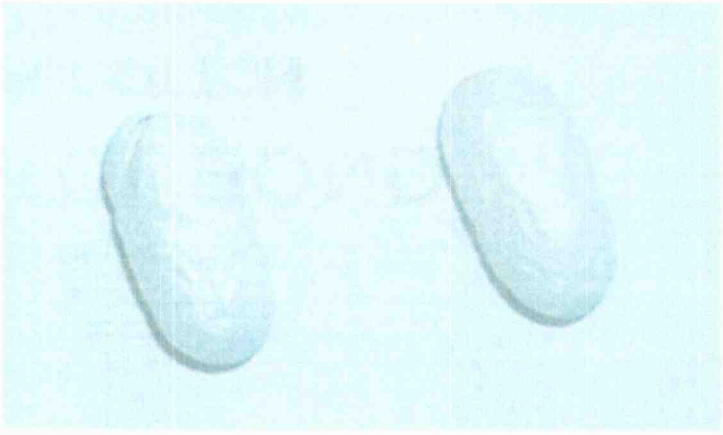

Figure 1.10: Shape of nifedipine SEG capsules coated with O3 coat formulation after 24 hour dissolution.

Figure 1.11: Cross section of nifedipine SEG capsules coated with O3 coat formulation after 24 hour dissolution.

Release from coat formulation (O4)

Percent drug released from nifedipine SEG capsules coated with O4 coat (least amount of HPMC tested) formulation with 15% actual weight gain at different time periods is shown in Figures 1.12 (a-b). Table 1.07 shows differences in percent nifedipine release from coated SEG capsules in the different dissolution media.

Table 1.07: Differences in drug release from nifedipine O4 SEG coated capsules in different dissolution media (n = 3).

Formula O4	*Gastric fluid /intestinal fluid	Intestinal fluid	Water
Lag time	2 hours	1.5 hours	1.5 hours
Time of 50% drug release	24 hours	3.5 hours	5 hours
Time of 100% drug release	Not determined	Not determined	Not determined
% Released after 24 hours	51 %	85 %	80 %

*Simulated gastric fluid pretreatment for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours.

Increasing Surelease® ratio (3.5: 1.5) in O4 coat formulation decreased % drug released. With low HPMC ratio there are fewer holes or "pores" developed in the coat around the surface of the capsules which allows a small volume of dissolution media to enter into the core. Because nifedipine is a water insoluble drug, nifedipine precipitated inside the capsules when the water entered thereby trapping the drug inside the SEG capsule such that nifedipine could not diffuse out of the SEG capsule. In the O4 formulation, nifedipine release had a 2 hour lag time with 51% nifedipine released during the 24 hour period with gastric pretreatment compared to 1.5 hours lag time and 85% drug release in simulated intestinal fluid, which also indicates a pH effect on drug release. Figure 1.12b shows that the dissolution profile of the O4 formulation was quite different from commercially available sustained release products of nifedipine (Adalat CC® and Procardia XL®).

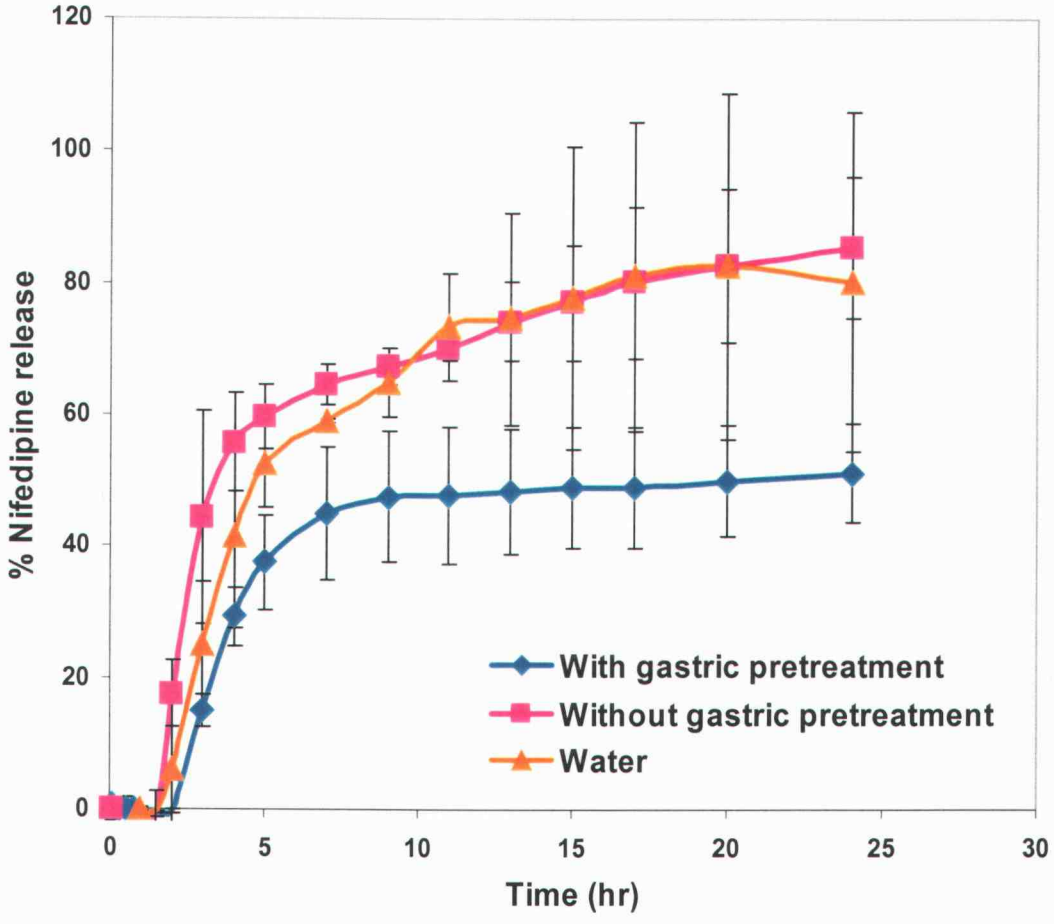

Figure 1.12a: Mean nifedipine release from O4 coated SEG capsules with 15% actual weight gain over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—◆—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n = 3).

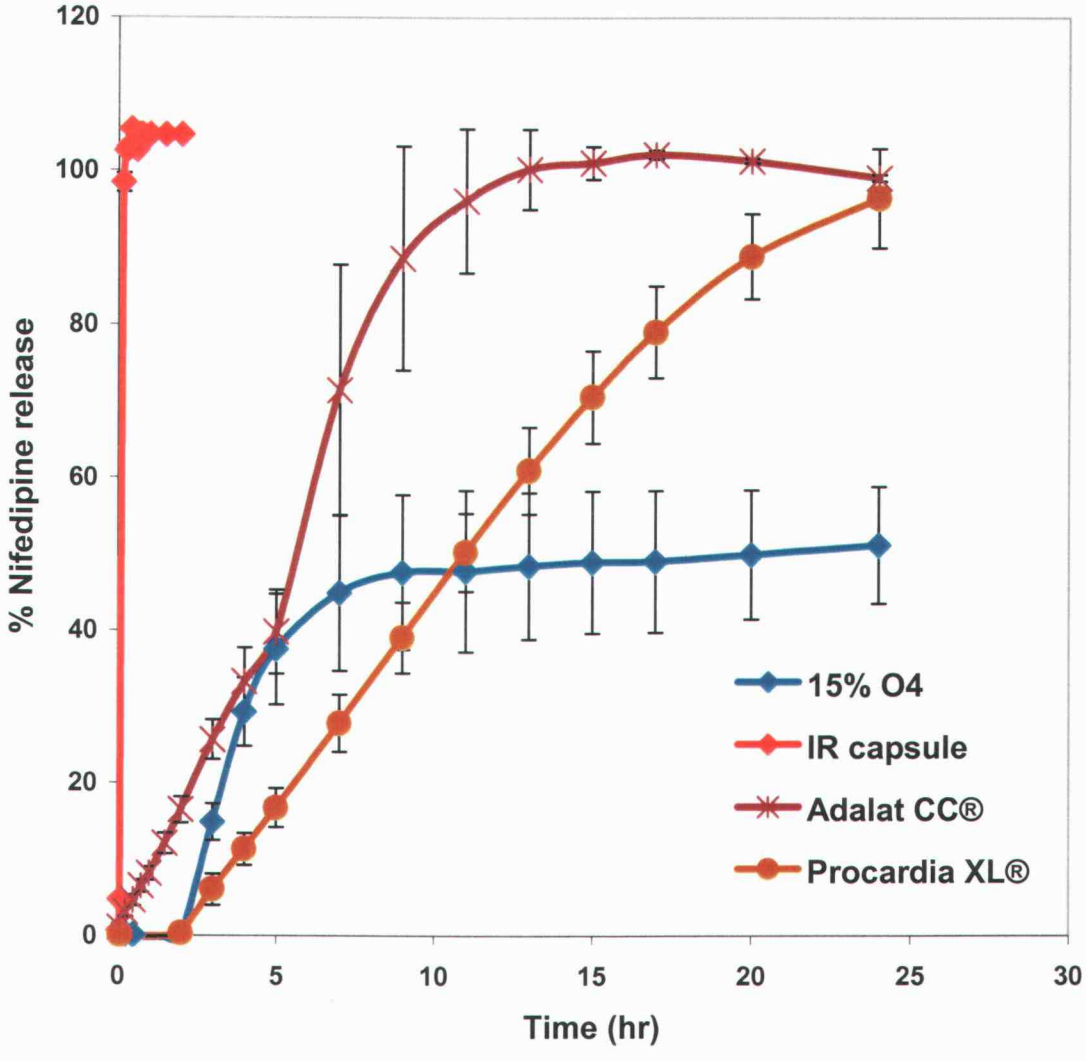

Figure 1.12b: Mean nifedipine release from commercially available formulae (immediate release, IR, and sustained release) and SEG O4 coat formulations in gastric fluid for 2 hours followed by intestinal fluid for 22 hours (error bar represents standard deviation, n = 3).

Nifedipine release from Surelease® Opadry® combinations

A summary of dissolution profiles obtained from all Surelease® Opadry® coated SEG capsules in simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours, simulated intestinal fluid, and water containing 1% Tween are presented in Figures 1.13, 1.14, and 1.15, respectively.

Opadry®, a water soluble polymer used in these coating formulations, forms holes in the non-porous film formed by the water insoluble polymer Surelease®. Pores act as a point of entry for dissolution fluid into the capsule core and as points of exit for dissolved drug into dissolution media. Drug release occurs through the hydrated polymeric gel. An increase in coat thickness was accompanied by a decrease in drug release rate. The decreased drug release rate was complicated by the drug precipitation effects that occurred inside the SEG capsule when the water flows too slowly into and back out of the coated SEG capsule. Table 1.08 shows occurrence of nifedipine precipitation inside the SEG capsules and degree of precipitation in the three dissolution media studied.

During the coating process, the application of multiple coats increased the weight gain, and thickness of the film formed around the surface of the capsules. Holes in thin films are gradually blocked. Therefore, the greater the coating thickness the slower the drug dissolution rate. It was also discovered that the

formulations investigated could be modified to produce lag time in drug release. Lag time correlated with film thickness.

By increasing the percent coat weight gain, there are an increase in the diffusion path length and diffusional resistance for dissolution media to enter the core and for dissolved drug to come out from the core. This partially accounts for the much lower release rates for capsules with higher coating levels along with drug precipitation inside the SEG in some cases. As small volumes of dissolution media entered the core, nifedipine will precipitate inside the coated SEG for some formulations and make it difficult for drug to be released, especially with coat formulations containing higher ratios of Surelease®.

Table 1.08: Nifedipine precipitation inside the SEG capsules with various coatings of polymer in different dissolution media.

SEG formulations	*Gastric fluid/ intestinal fluid	Intestinal fluid	Water
16% O1	-	-	-
14% O2	-	-	-
17% O2	+	-	-
21% O2	+	-	-
13% O3	+	-	-
18% O3	+	-	-
21% O3	+++	++	++
15% O4	+++	++	++

Degree of drug precipitation inside the SEG core:
-: No drug crystals +: Low drug crystals
++: Medium drug crystals +++: High drug crystals

*Simulated gastric fluid pretreatment for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours.

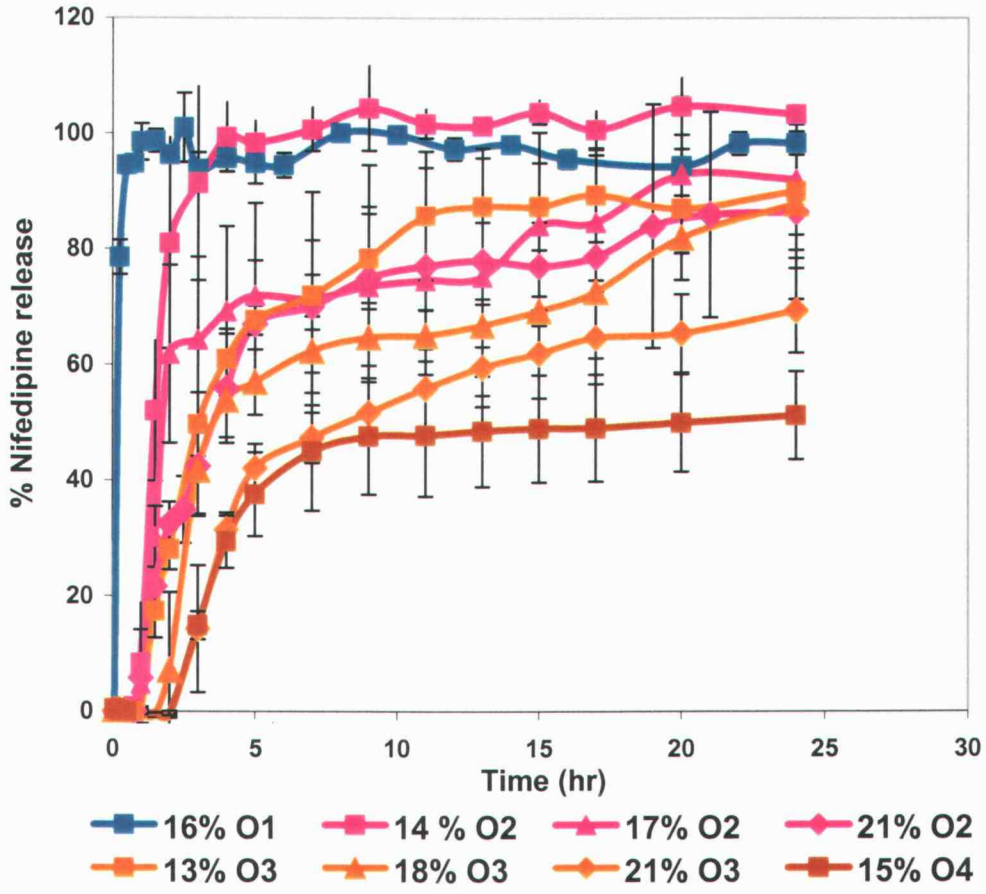

Figure1.13: Mean nifedipine release from SEG capsules coated with O1, O2, O3 and O4 coat formulations in simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours containing 1% Tween 80 (error bar represents standard deviation, n = 3).

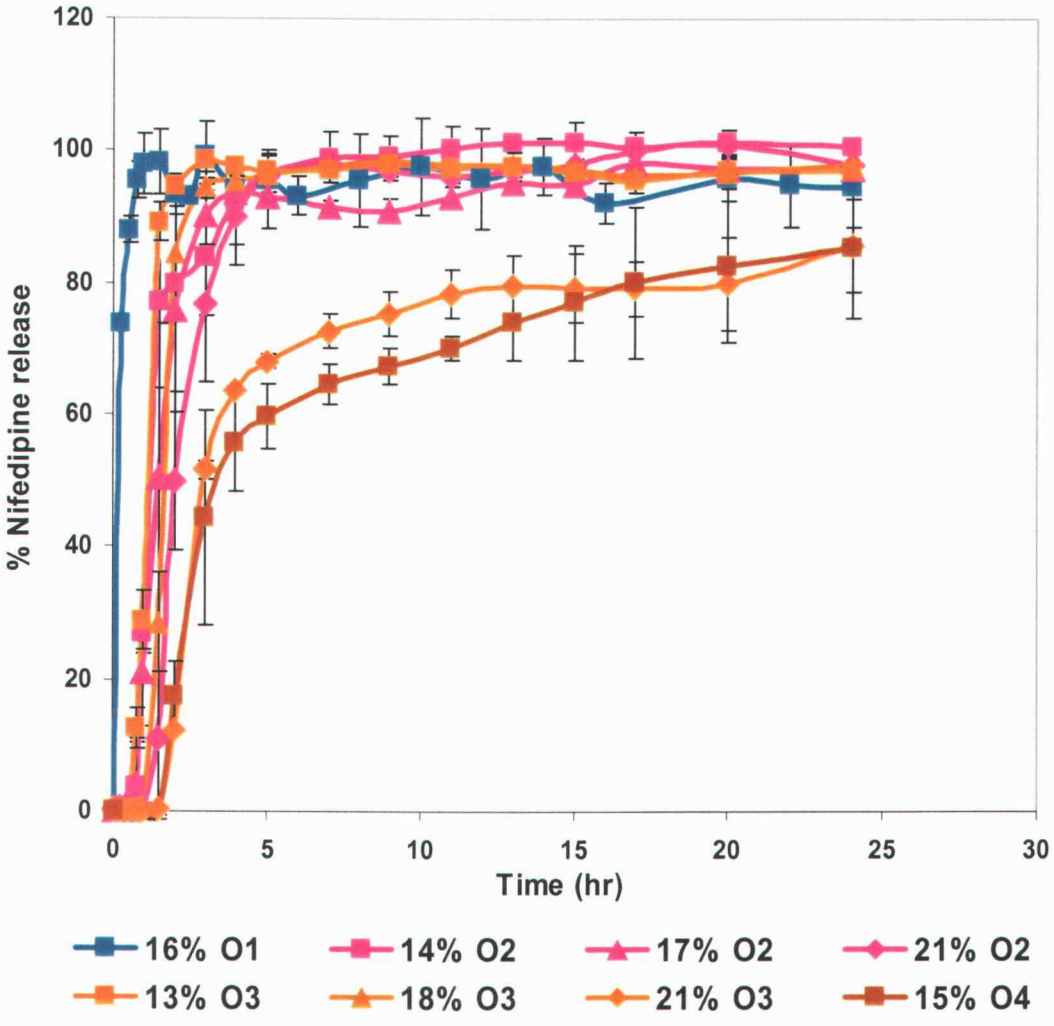

Figure1.14: Mean nifedipine release from SEG capsules coated with O1, O2, O3 and O4 coat formulations in simulated intestinal fluid containing 1% Tween 80 (error bar represents standard deviation, n = 3).

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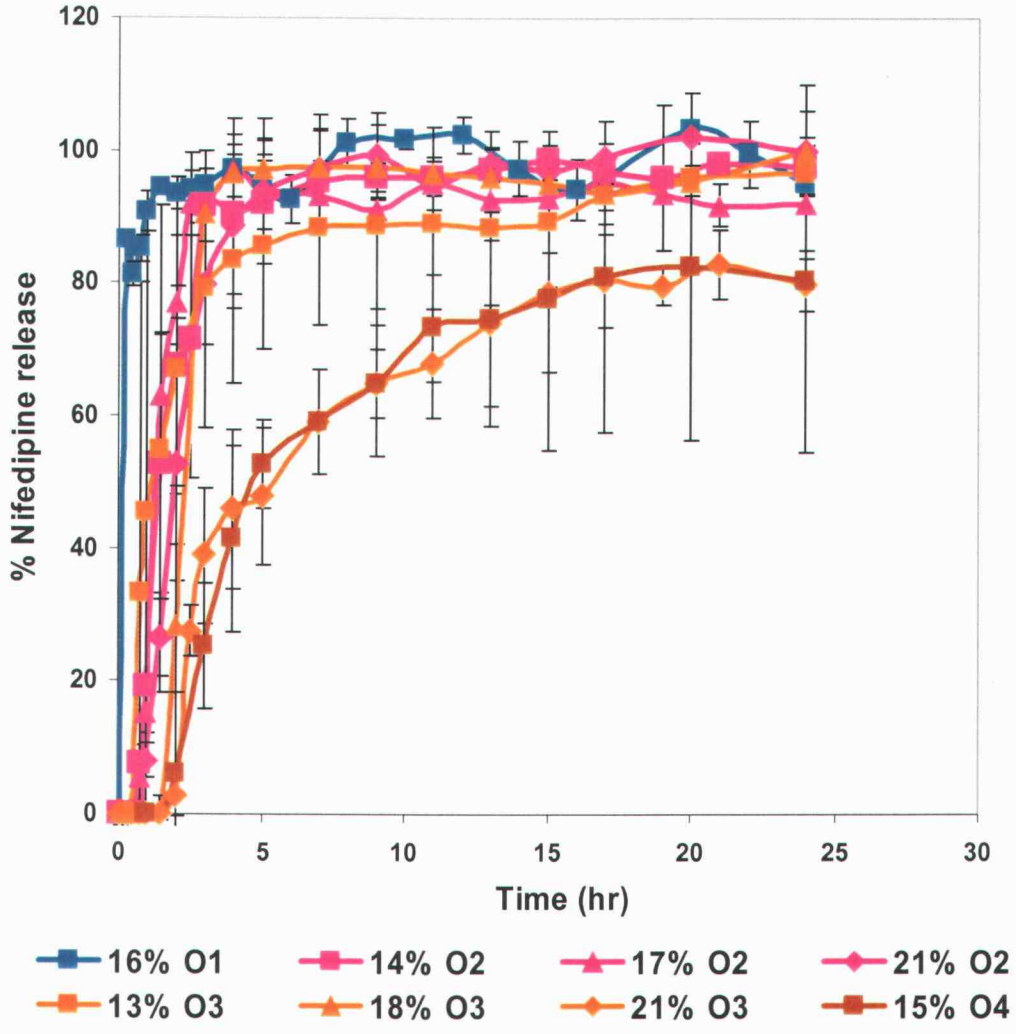

Figure1.15: Mean nifedipine release from SEG capsules coated with O1, O2, O3 and O4 coat formulations in water containing 1% Tween 80 (error bar represents standard deviation, n = 3).

Surelease® pectin coat formulations

Two coat formulations containing pectin (P1 and P2) have been studied with different coating weight gains.

Release from coat formulation (P1)

Pectin is an anionic polysaccharide water soluble polymer. It is non-ionized and less soluble in gastric fluid (at lower pH). Gastric pretreatment of pectin containing films results in low pectin solubility and prevents drug release in gastric fluid (19). With higher percent weight gains of Surelease® pectin, more film coat was applied which may have blocked holes or pores in the thinner film coats, which may have apparently caused a gradual reduction in the dissolution rate of nifedipine.

Percent drug released at different time periods from nifedipine SEG capsules coated with Surelease® pectin (2:1 solid ratio) with 14%, 17% and 21% actual coating weight gains are shown in Figures 1.16 (a, b, c and d). Table 1.09 shows some of the differences in drug release from SEG capsules coated with P1 coat with different coating weight gains in three different dissolution media.

In Figure 1.16a, SEG capsules coated with 14% P1 coat formulation showed 100% drug release within 2 - 4 hours with 15 minutes lag time in intestinal fluid and in water. Gastric fluid pretreatment for 2 hours prolonged the lag time to 2 hours and 100% drug release occurred during 15 hour period. With the P1 formulations, the release rate of nifedipine continued to be slowed after adjusting pH to intestinal fluid even though pH was neutral. This suggests that pH effected nifedipine release from SEG capsule. But with P1 formulation containing pectin, increasing the percent weight gain did not produce a difference in drug release when comparing 14% and 17% weight gain. In Figure 1.16b, the 17% weight gain produced a lag time of 30 minutes in intestinal fluid and water compared to 2 hours in gastric fluid followed by intestinal fluid for 22 hours. Hundred percent drug release occurred within 2 hours in the intestinal fluid and in water but it took around 15 hours with gastric pretreatment for 2 hours to get 100% release. In Figure 1.16c, 24% weight gain produced a lag time of 30 minutes in the intestinal fluid and in water compared to 2 hours with gastric pretreatment for 2 hours. Nifedipine release was nearly identical with 14% and 17% coating weight gains with P1 coat formulation. Lastly, Figure 1.16d shows dissolution curves of new P1 formulations is quite different from commercially available products of nifedipine, Adalat CC® and Procardia XL®.

Table 1.09: Differences in drug release from nifedipine P1 SEG coated capsules in different dissolution media.

Formula O2	*Gastric/intestinal	Intestinal	Water
14 % weight gain			
Lag time	2 hours	0.25 hour	0.25 hour
Time of 100% drug release	15 hours	2 hours	4 hours
% released after 24 hours	100 %	100 %	100 %
17% weight gain			
Lag time	2 hours	0.5 hour	0.5 hour
Time of 100% drug release	15 hours	2 hours	2 hours
% released after 24 hours	100%	100%	100%
24% weight gain			
Lag time	2 hours	0.5 hour	0.5 hour
Time of 100% drug release	13 hours	4 hours	2 hours
% released after 24 hours	100%	100%	100%

*Simulated gastric fluid pretreatment for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours.

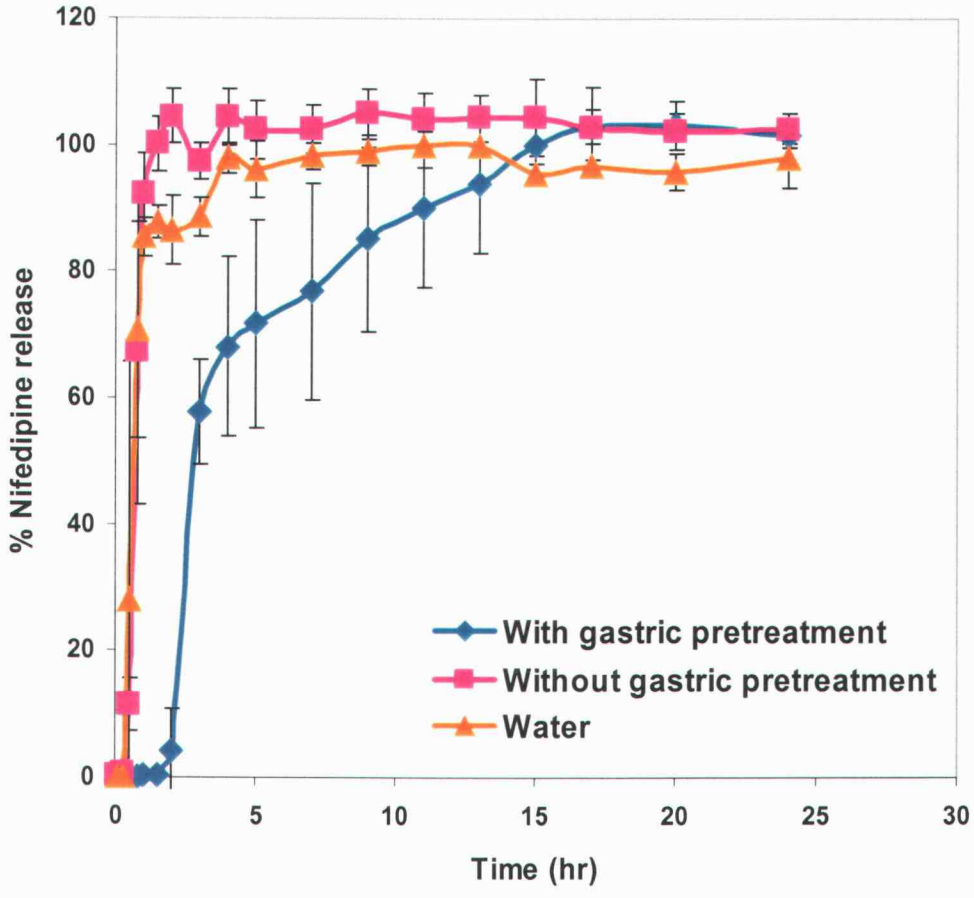

Figure 1.16a: Mean nifedipine release from P1 coated SEG capsules with 14% actual weight gain over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—♦—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n = 3).

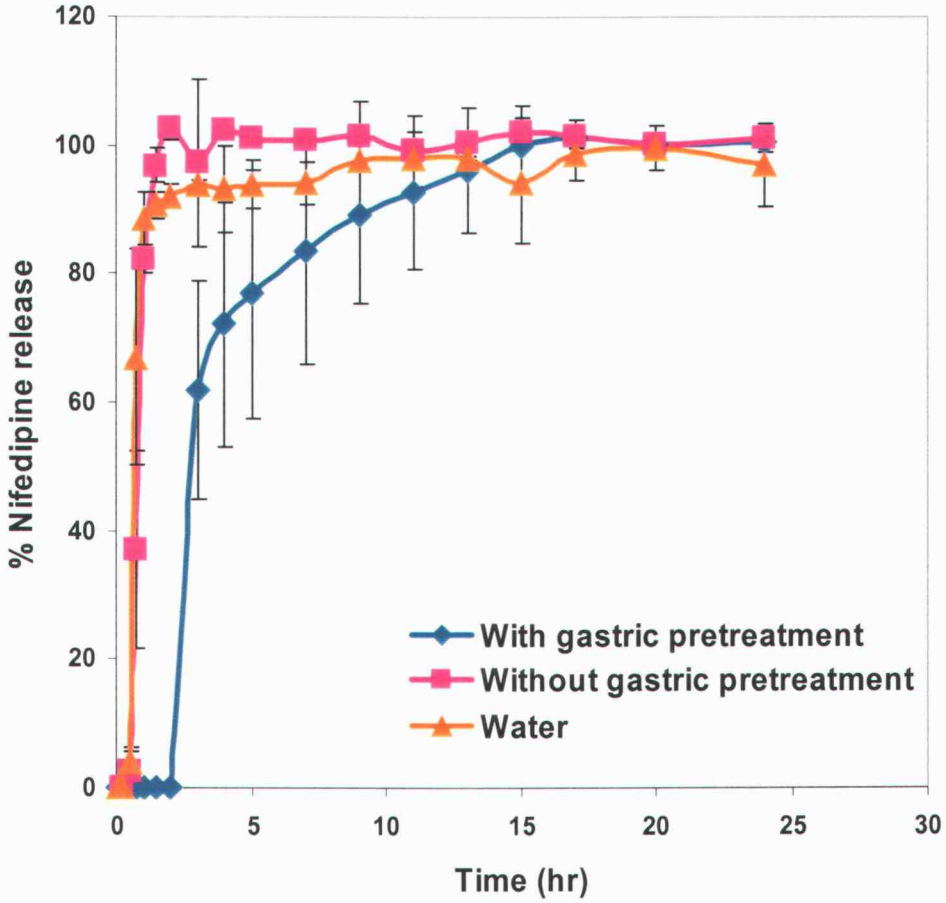

Figure 1.16b: Mean nifedipine release from P1 coated SEG capsules with 17% actual weight gain over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—♦—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n = 3).

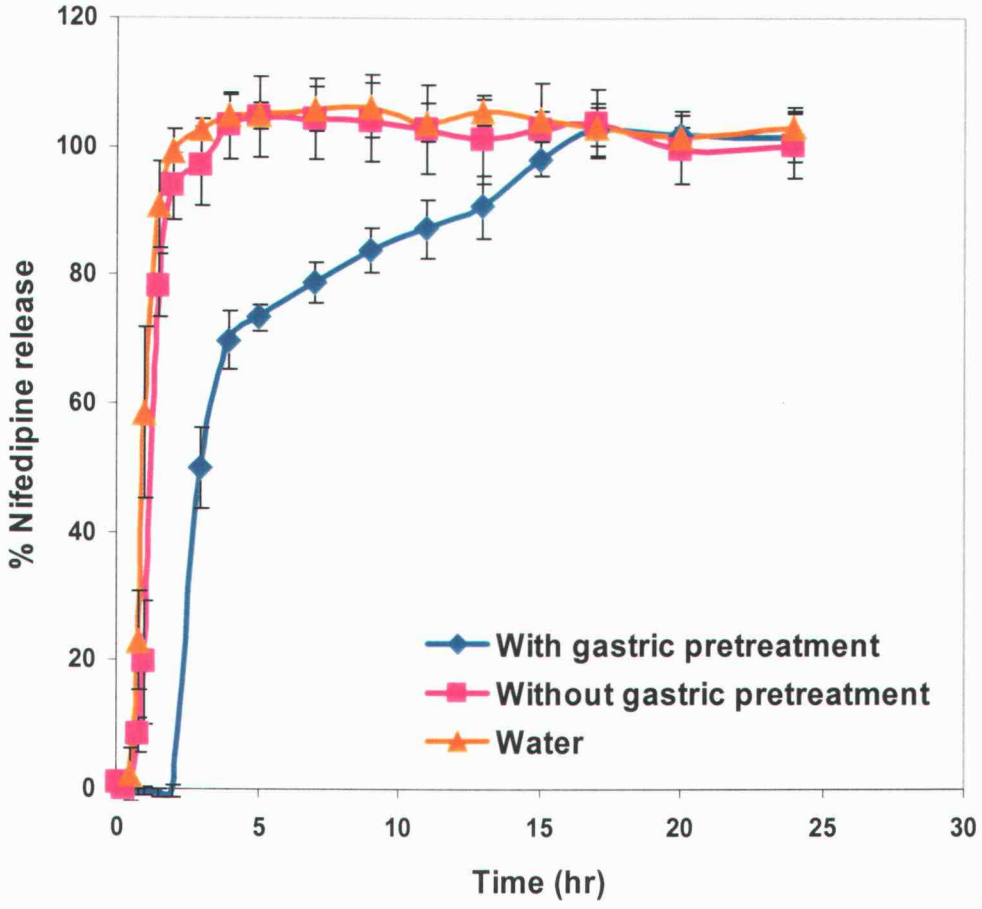

Figure 1.16c: Mean nifedipine release from P1 coated SEG capsules with 24% actual weight gain over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—♦—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n = 3).

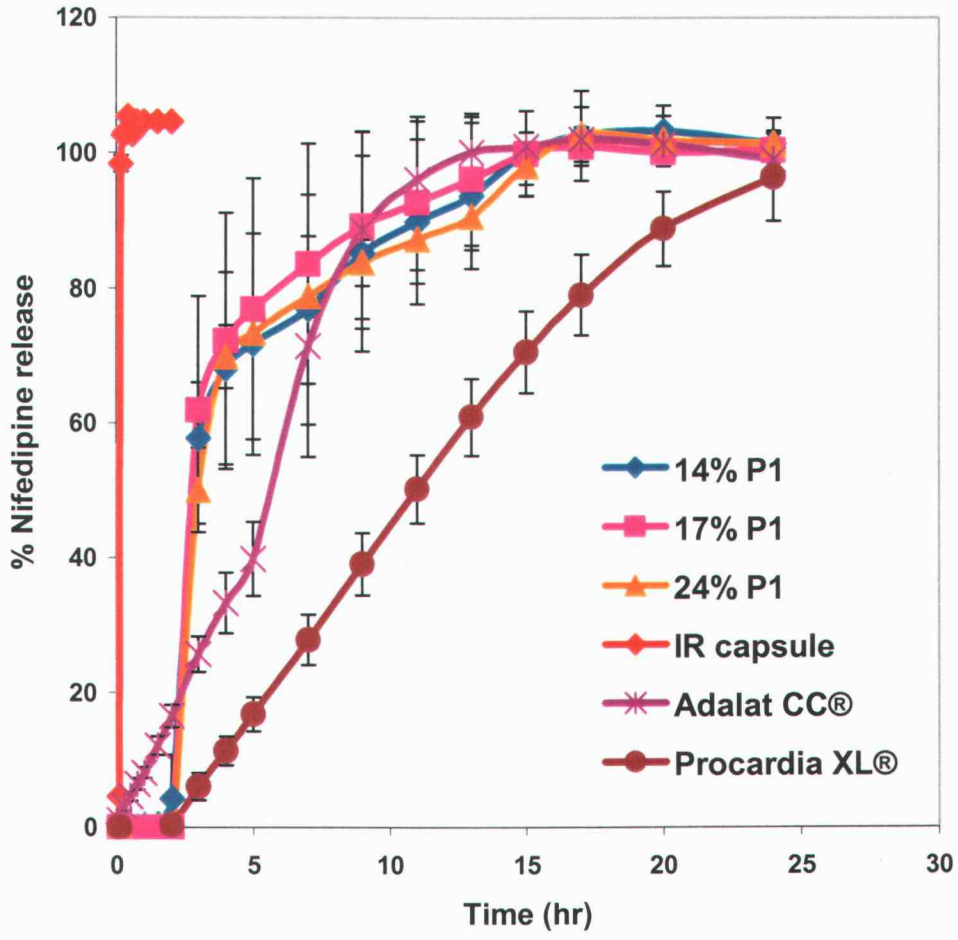

Figure 1.16d: Mean nifedipine release from commercially available formulae (immediate release, IR, and sustained release) and P1 SEG coat formulations in simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours. (error bar represents standard deviation, n = 3).

Release from coat formulation (P2)

Percent drug released at different time periods from nifedipine SEG capsules coated with P2, Surelease® pectin (5:1 solid ratio) with 12% and 17% actual coating weight gains are shown in Figures 1.19 (a, b, and c). Table 1.10 shows some differences in drug release from SEG capsules coated with P2 coat formulation with different coating weight gains in three different dissolution media. Collective dissolution profiles of SEG capsules coated with studied Surelease® pectin coat formulations in the three different dissolution media are shown in Figures 1.20 (a, b, and c).

Nifedipine release from SEG capsules coated with P2 coat formulation with 12% actual coating weight gain did not release 100% drug over a 24 hour period. Dissolution with gastric fluid pretreatment showed 3 hours lag time and 61% drug release over a 24 hour period compared to 1.5 hours lag time and 95% and 89% in intestinal fluid and in water, respectively (see Figure 1.19a).

With 17% weight gain, dissolution with gastric fluid pretreatment for 2 hours showed 4 hours lag time with 38% drug release over a 24 hour period compared to 2 hours lag time and 68% and 73% drug release after 24 hours dissolution in intestinal fluid and in water, respectively (Figure 1.19b). Dissolution profiles of the P2 coat formulations are shown in Figure 1.19c are quite different from commercial products of nifedipine.

With SEG capsules coated with P2 coat formulation, uniform coat was obtained with slight change in shape of the SEG capsules after coating (Figure 1.17a). Hundred percent drug release was not obtained for any P2 coat formulation for the weight gains studied over the 24 hour period. Nifedipine precipitation occurred inside the SEG capsule shell coated with P2 coat formulation in all dissolution media as seen previously. Nifedipine precipitation may be explained by assuming a lower pectin ratio in this coat formulation produced fewer holes in the film coat, which allowed a small volume of dissolution fluid to enter the SEG capsule. With the high nifedipine concentration inside the capsules, some nifedipine precipitated unable to diffuse through the film coated SEG capsule.

Also, a gel formed inside the SEG capsule retaining nifedipine inside but not in the form of yellow crystals as seen previously with the dissolution studies in the gastric fluid for 2 hours and then in the intestinal fluid (Figure 1.17b). Gelation of pectin has been reported to occur by two different mechanisms: a) high methoxyl pectins gel in the presence of high concentration of soluble solids and low pH and b) low methoxyl pectins gel in the presence of divalent cations (20). The pectin used in the P1 and P2 coatings is low methoxyl pectin, which may have gelled in the presence of the polyvalent cations. To investigate possible mechanisms for the gelation occurrence inside the SEG capsule shell during dissolution studies, an experiment was performed by emptying contents of 5 immediate release nifedipine SEG capsules to each of 5 beakers containing:

1. 5 ml simulated gastric fluid.

2. 5 ml simulated intestinal fluid.
3. 5 ml simulated gastric fluid + 5 ml 2.5% pectin solution.
4. 5 ml simulated intestinal fluid + 5 ml 2.5% pectin solution.

There was no gel formation observed upon addition of the capsule contents into either simulated gastric or simulated intestinal fluid. However, in the presence of pectin solution, an immediate gel formation occurred in the simulated gastric fluid and simulated intestinal fluid. The gel formed in the gastric fluid solidifies within 5 - 7 hours as shown in Figure 1.18. The contents of a SEG nifedipine capsule are nifedipine solution and excipients. The excipients are: peppermint oil, polyethylene glycol, yellow ferric oxide, and titanium dioxide. It has been reported that gelation of pectin occurs in the presence of divalent cation such as Ca^{2+}, Ba^{2+}, Sr^{2+}, Cd^{2+}, Ni^{2+}, or Pb^{2+} (21). The effect of divalent cation on the gelation of pectin may explain the gelation inside the SEG capsule shell. This gelation may be due to the effect of ferric (trivalent) cation or titanium (tetravalent) cation on the carboxylic acids of pectin. Gel formation inside SEG capsules coated with Surelease® pectin may be due to some pectin leaching from the coat and driven into the capsule core with the dissolution media resulting in gel formation inside the capsule core, which can eventually retard nifedipine diffusion from inside the capsule.

Higher variability was seen between the P2 coated capsules compared to the Surelease® HPMC formulations under the same conditions, which may be explained by inter-individual variability as each capsule depending mainly on the

gel formed inside the capsule. Capsules coated with P2 coat formulation showed a smoother surface after 24 hour dissolution period (Figure 1.17c) than those obtained from Surelease® Opadry® coat formulations (Figure 1.10), probably because pectin does not swell as much as occurred with Opardy® polymer. Figures 1.20 (a, b, and c) show collective dissolution curves of Surelease® pectin formulations in the three dissolution media.

Table 1.10: Differences in drug release from nifedipine SEG capsules coated with P1 coat formulation in different dissolution media.

Formula P2	*Gastric/intestinal	Intestinal	Water
12 % weight gain			
Lag time	3 hours	1.5 hours	1 hour
Time of 50% drug release	8 hours	3.5 hours	3 hours
Time of 100% drug release	Not determined	Not determined	Not determined
% released after 24 hours	61%	95%	89%
17% weight gain			
Lag time	4 hours	2 hours	1.5 hours
Time of 50% drug release	Not determined	6 hours	4.5 hours
Time of 100% drug release	Not determined	Not determined	Not determined
% released after 24 hours	38%	68%	73%

*Simulated gastric fluid pretreatment for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours.

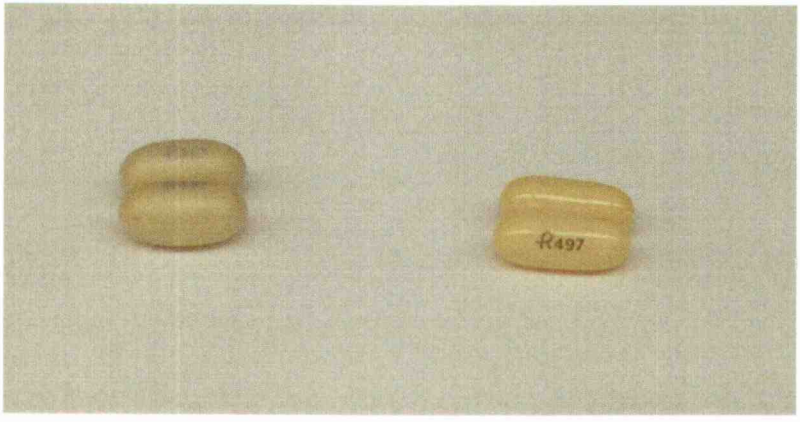

Figure 1.17a: Uncoated and SEG nifedipine capsules coated with P2 coat formulation.

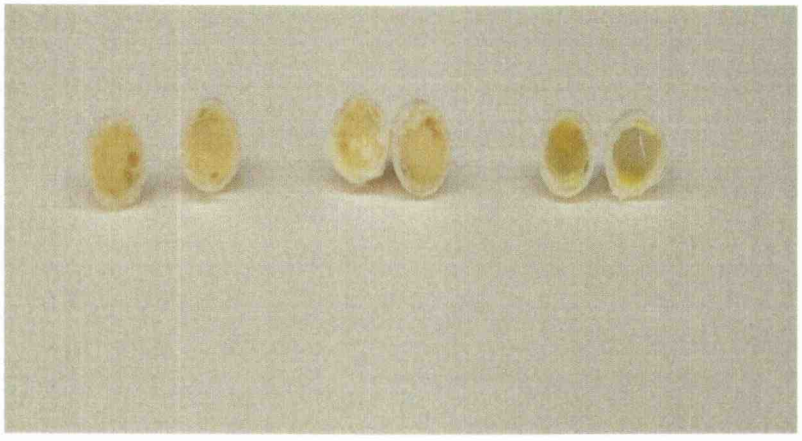

Figure 1.17b: Cross section of SEG capsules coated with P2 coat formulation after 24 hr dissolution respectively.

Figure 1.17c: SEG nifedipine capsules coated with P2 coat formulation following a 24 hour dissolution period showed yellow gel formation inside the capsule shell.

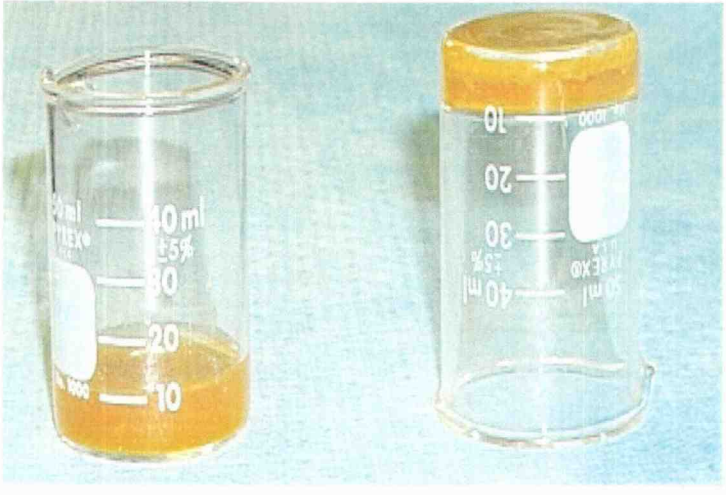

Figure 1.18: Gel formation occurred upon adding SEG capsule contents to a beaker containing 5 ml gastric fluid and 5 ml 2.5% pectin solution.

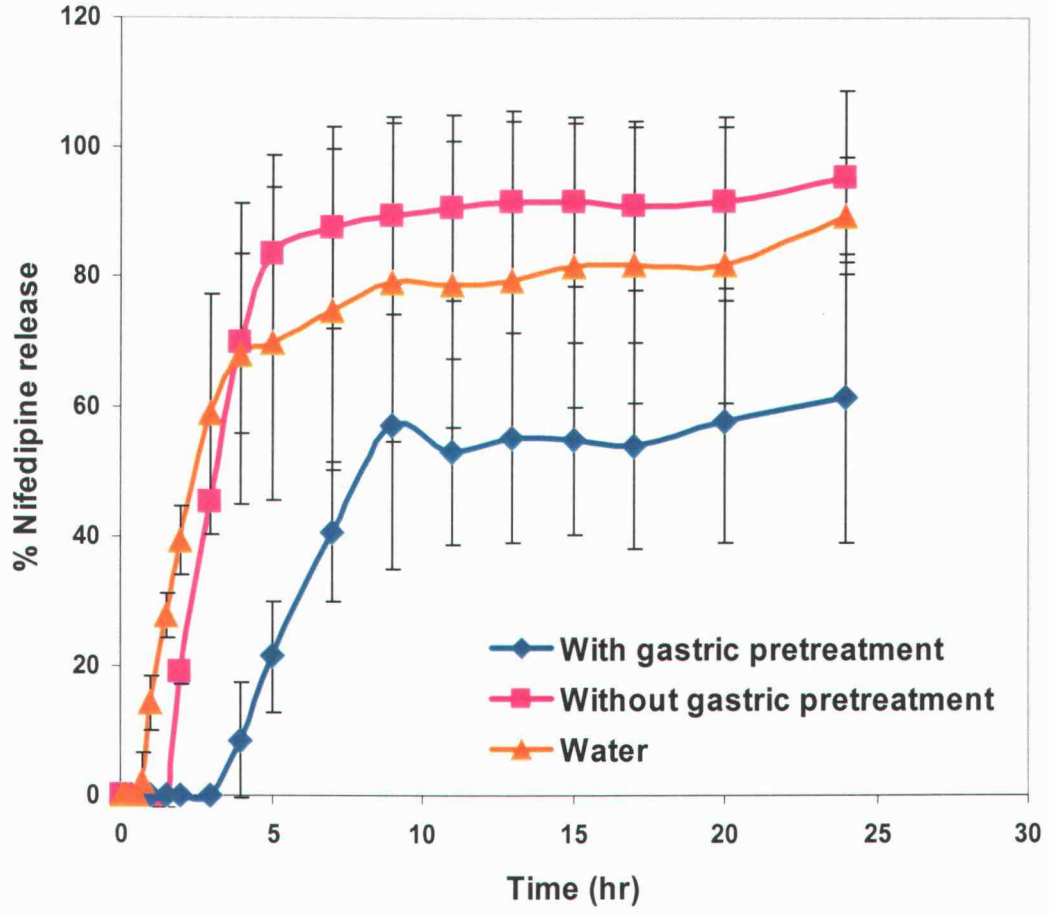

Figure 1.19a: Mean nifedipine release from P2 coated SEG capsules with 12% actual weight gain over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—♦—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n = 3).

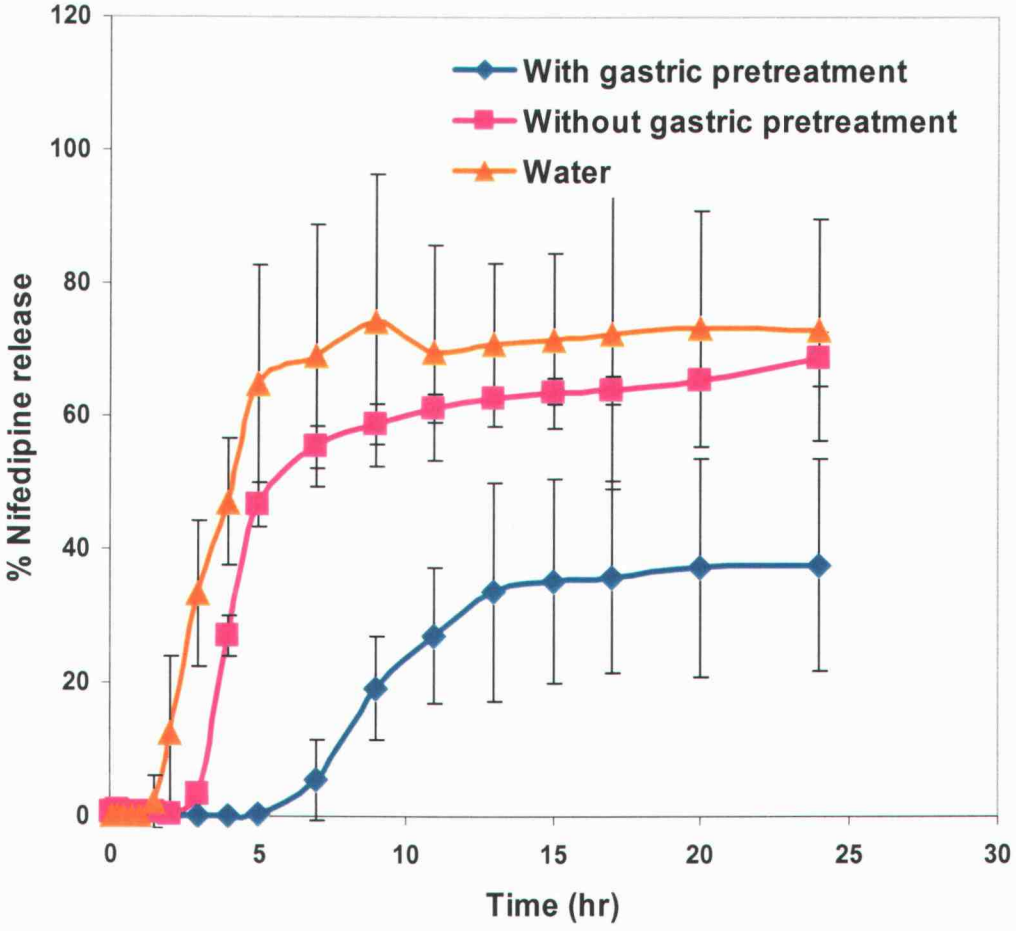

Figure 1.19b: Mean nifedipine release from P2 coated SEG capsules with 17% actual weight gain over 24 hours wherein dissolution occurred in simulated gastric fluid for 2 hours followed by intestinal fluid for 22 hours (—◆—); simulated intestinal fluid (—■—); and in water (—▲—) containing 1% Tween 80 (error bar represents standard deviation, n = 3).

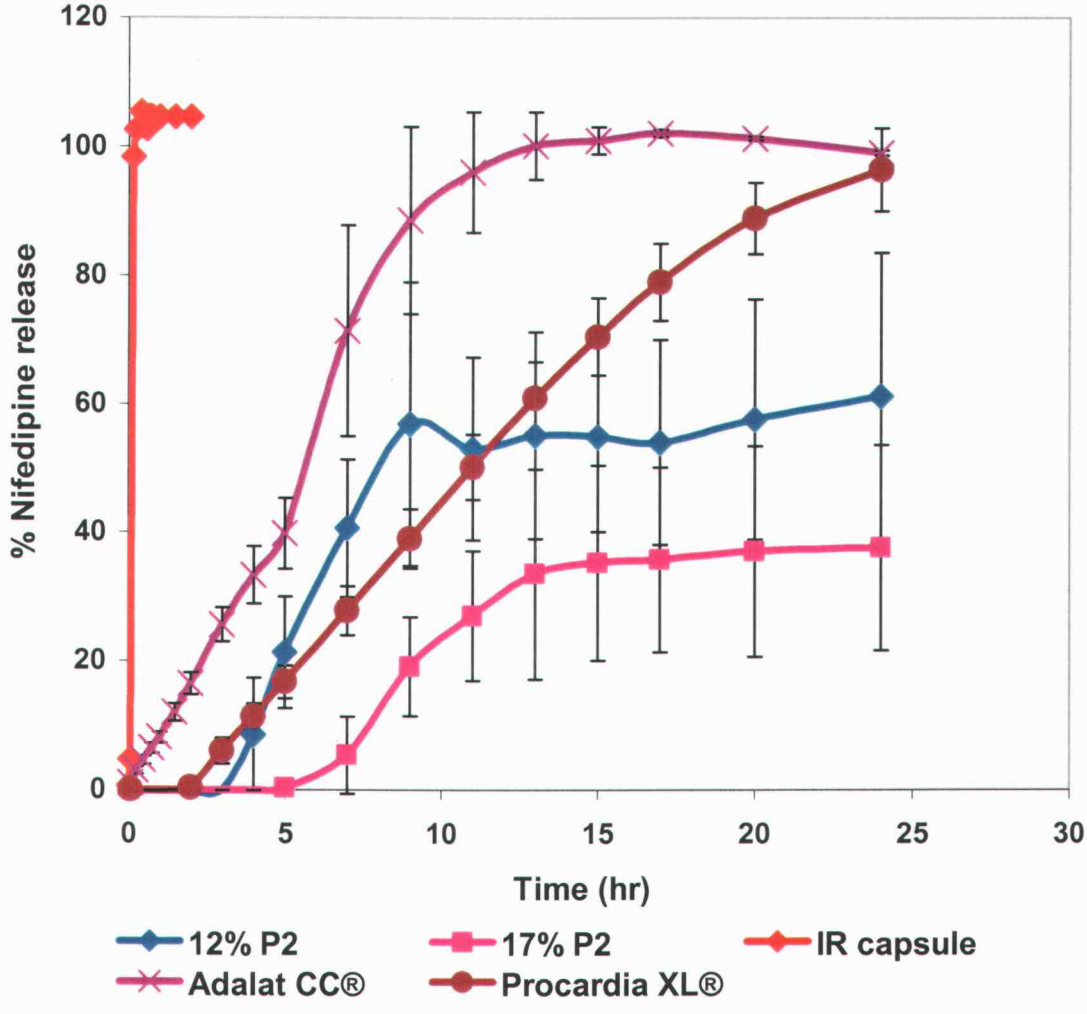

Figure 1.19c: Mean nifedipine release from commercially available formulae (immediate release and sustained release) and P2 SEG coated formulations in gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours. (error bar represents standard deviation, n = 3).

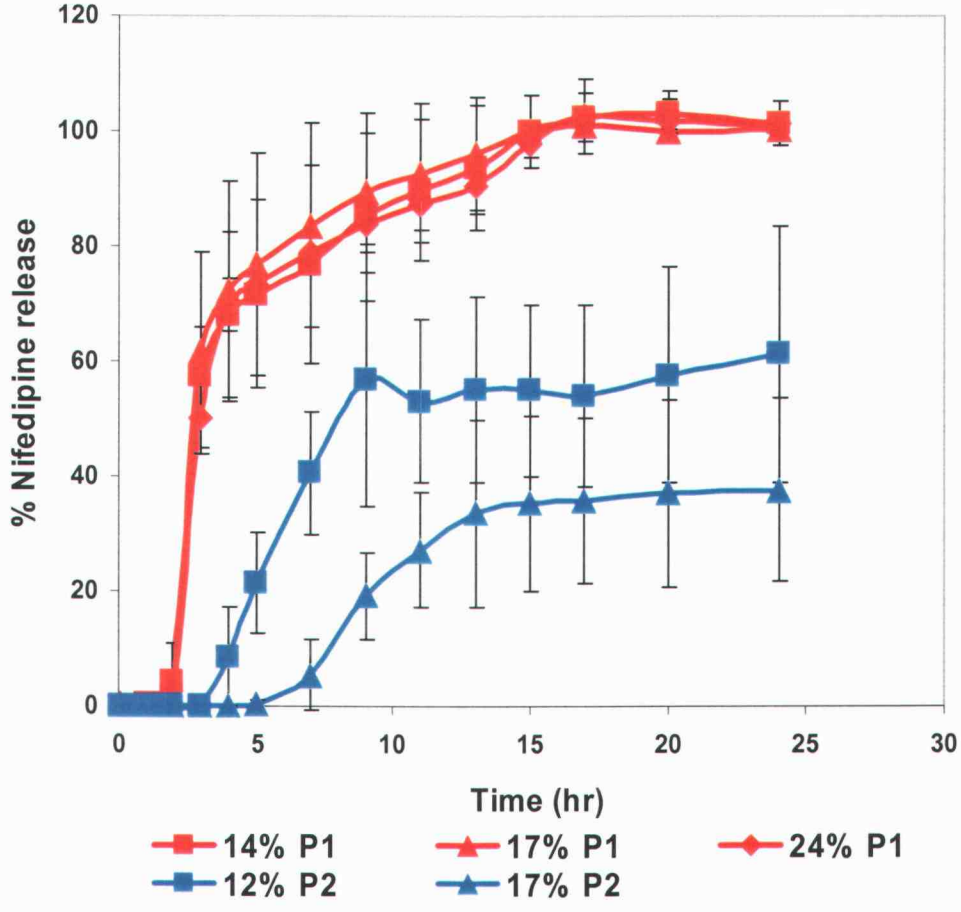

Figure 1.20a: Nifedipine release from SEG capsules coated with P1 & P2 coated formulations in gastric fluid for 2 hours followed by intestinal fluid for 22 hours. (error bar represents standard deviation, n = 3).

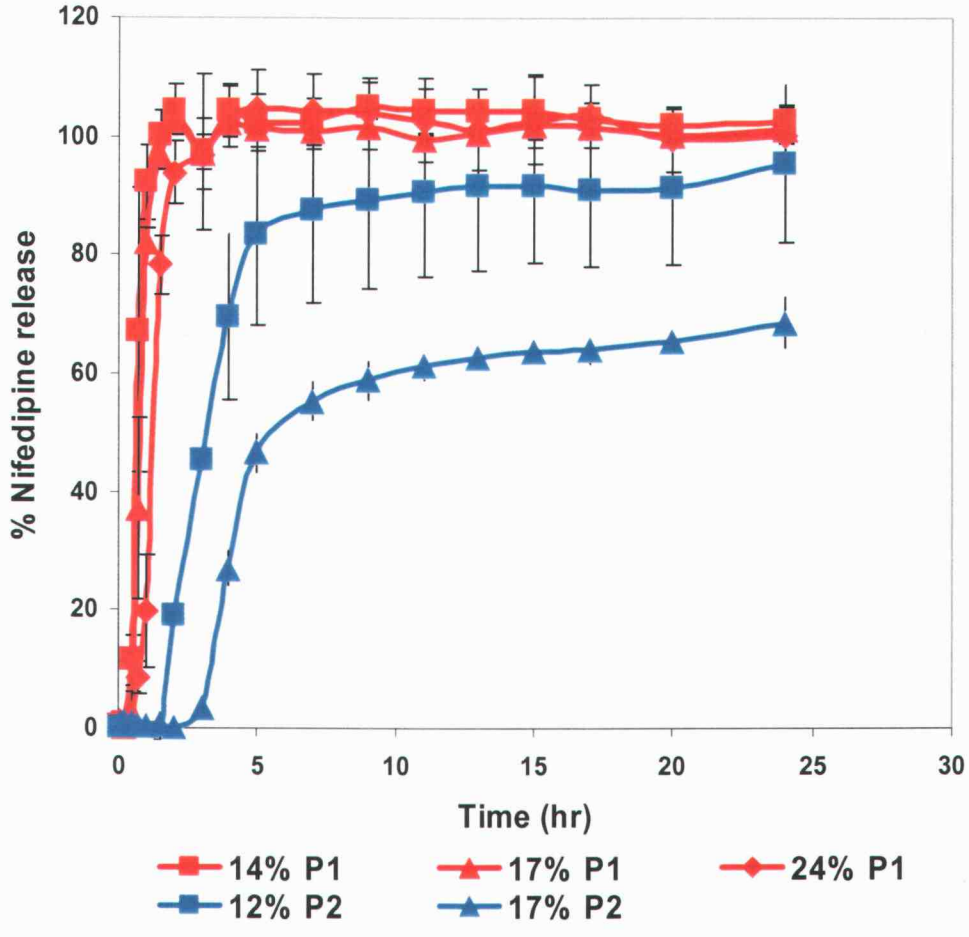

Figure 1.20b: Mean nifedipine release from SEG capsules coated with P1 & P2 coated formulations in intestinal fluid. (error bar represents standard deviation, n = 3).

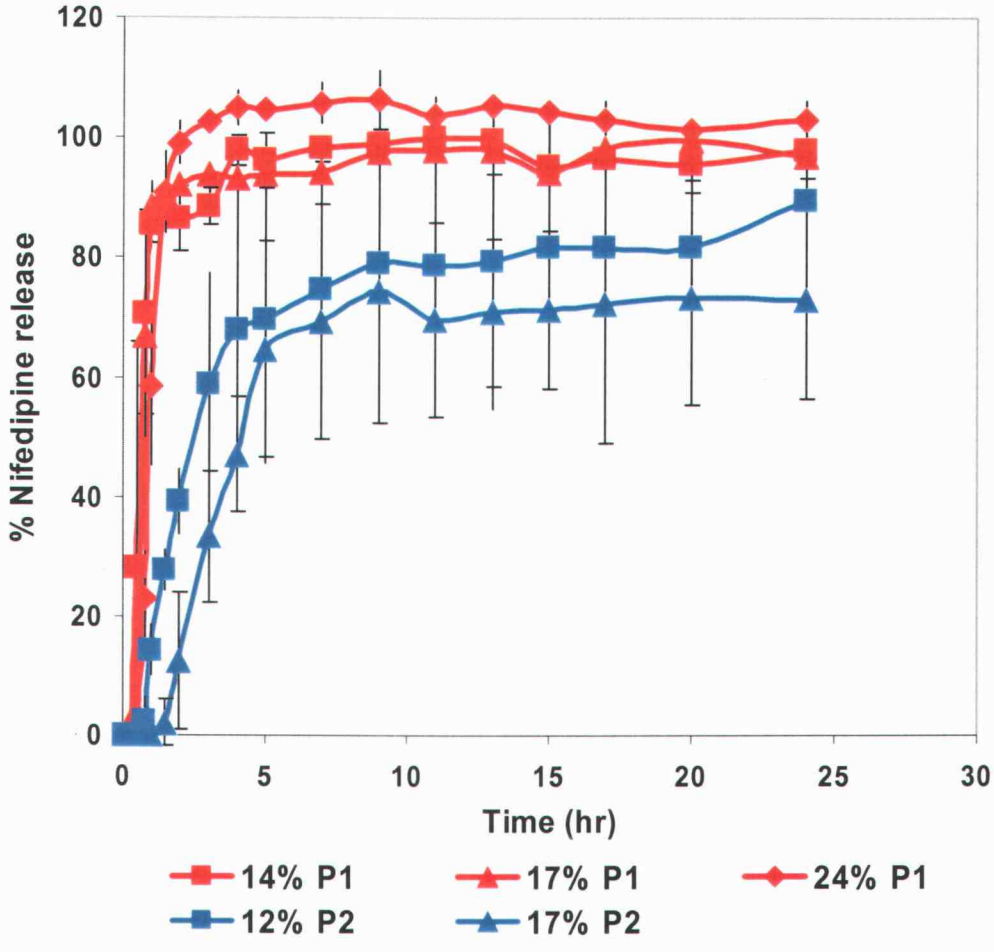

Figure 1.20c: Mean nifedipine release from SEG capsules coated with P1 & P2 coated formulations in water. (error bar represents standard deviation, n = 3).

Effect of pectinolytic enzyme on drug release from nifedipine capsules coated with Surelease® pectin combination

Effect of pectinolytic enzyme on drug release from P1 and P2 coated formulations was studied in dissolution studies in simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours (in triplicate). After 4 hours in intestinal fluid 3 ml Pectinex® Ultra enzyme was added to each dissolution vessel. Nifedipine release was compared to drug release under the same condition (in triplicate) but without the addition of the enzyme in the same experiment (see Figures 1.21 a-e).

Drug release from drug loaded beads coated with Surelease® pectin has been reported to be higher in the presence of pectinolytic enzymes than without enzymes addition in the dissolution media (19). Pectinolytic enzymes can attack pectin in the film coat, which creates holes that eventually increases drug release. This effect was not observed with P1 formulation because the release rate of nifedipine remained about the same after the addition of enzyme (after 6 hours dissolution). Only average drug release from SEG capsules coated with P2 coat formulation, 17% coating weight gain, showed the reverse effect; but, dissolution of nifedipine from these capsules had very high variability due to gelation and nifedipine precipitation inside the capsules. Because each capsule under the same condition behaved differently, the effect of the enzyme could not be distinguished in the P2 coating formulation.

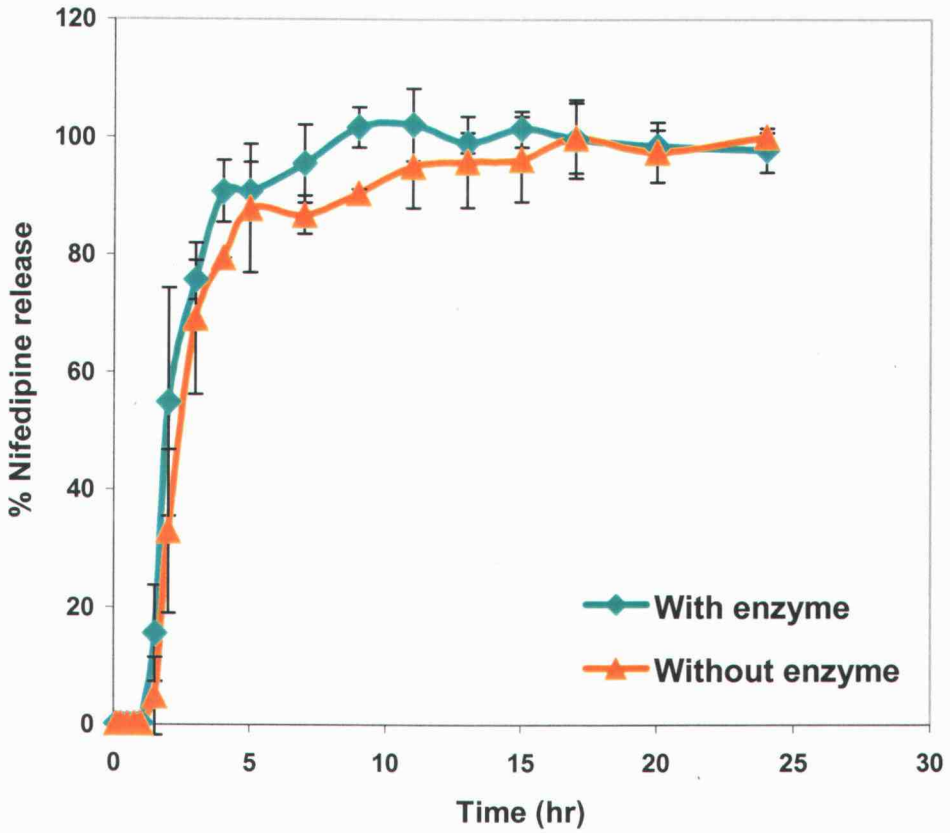

Figure 1.21a: Mean nifedipine release over 24 hours from P1 SEG coated capsules formulation with 14% weight gain in simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours. (error bar represents standard deviation, n = 3).

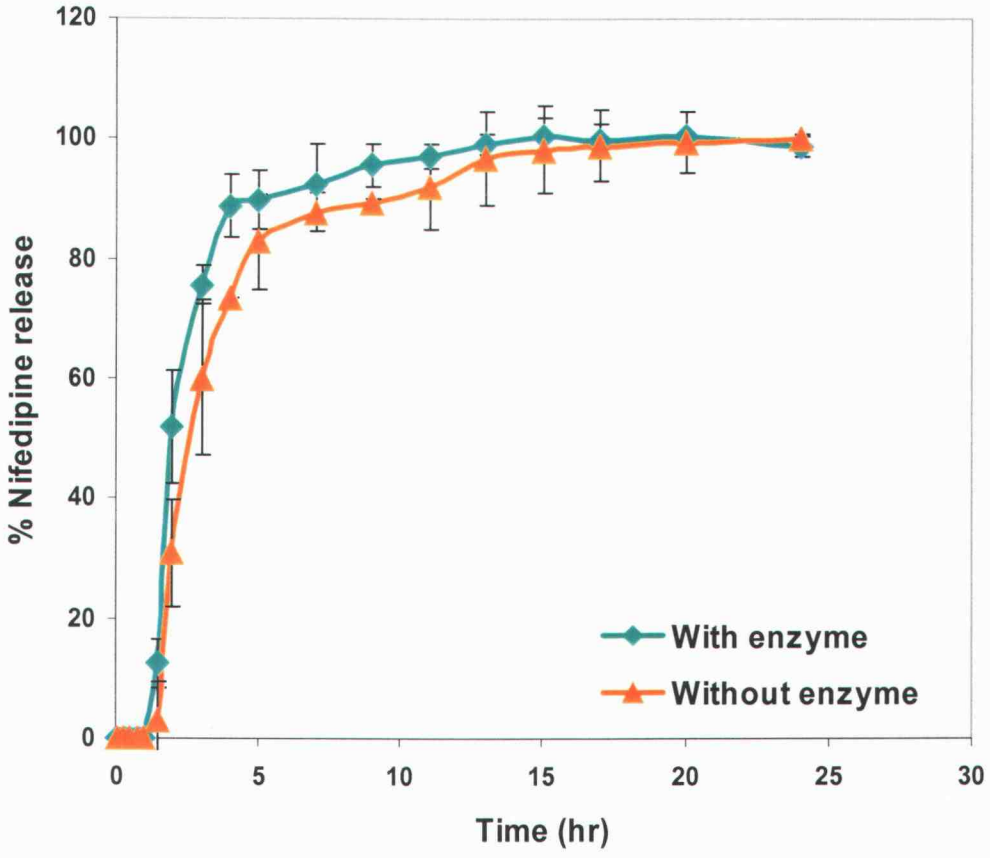

Figure 1.21b: Mean nifedipine release over 24 hours from P1 SEG coated capsules formulation with 17% weight gain in simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours (error bar represents standard deviation, n = 3).

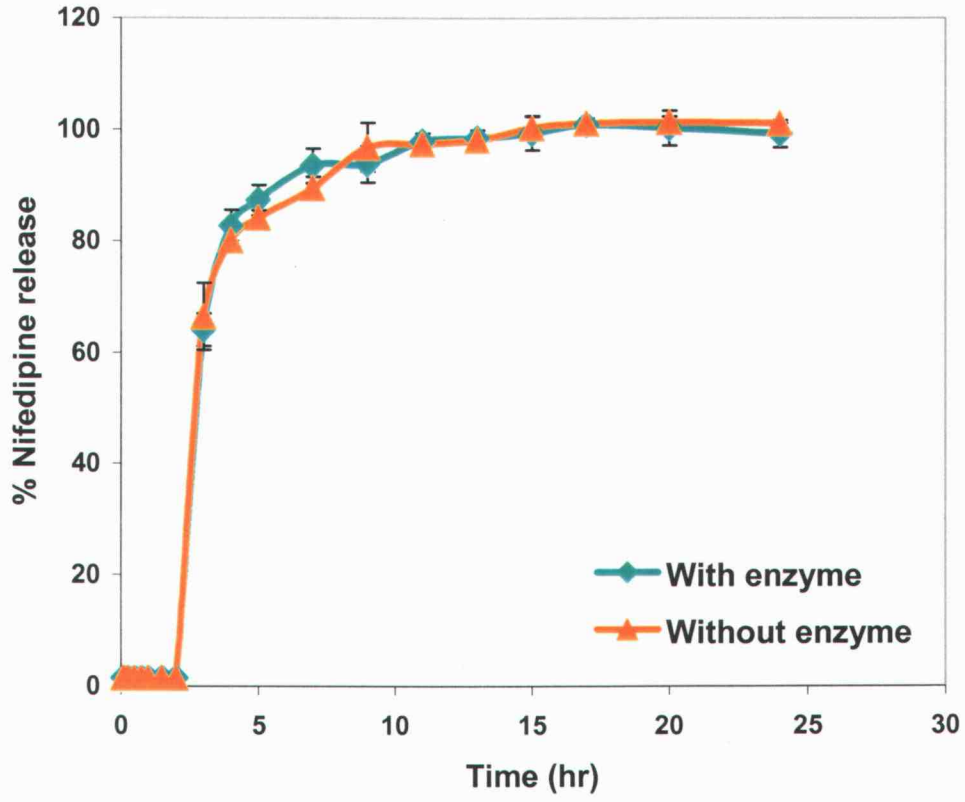

Figure 1.21c: Mean nifedipine release over 24 hours from P1 SEG coated capsules formulation with 24% weight gain in simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours (error bar represents standard deviation, n = 3).

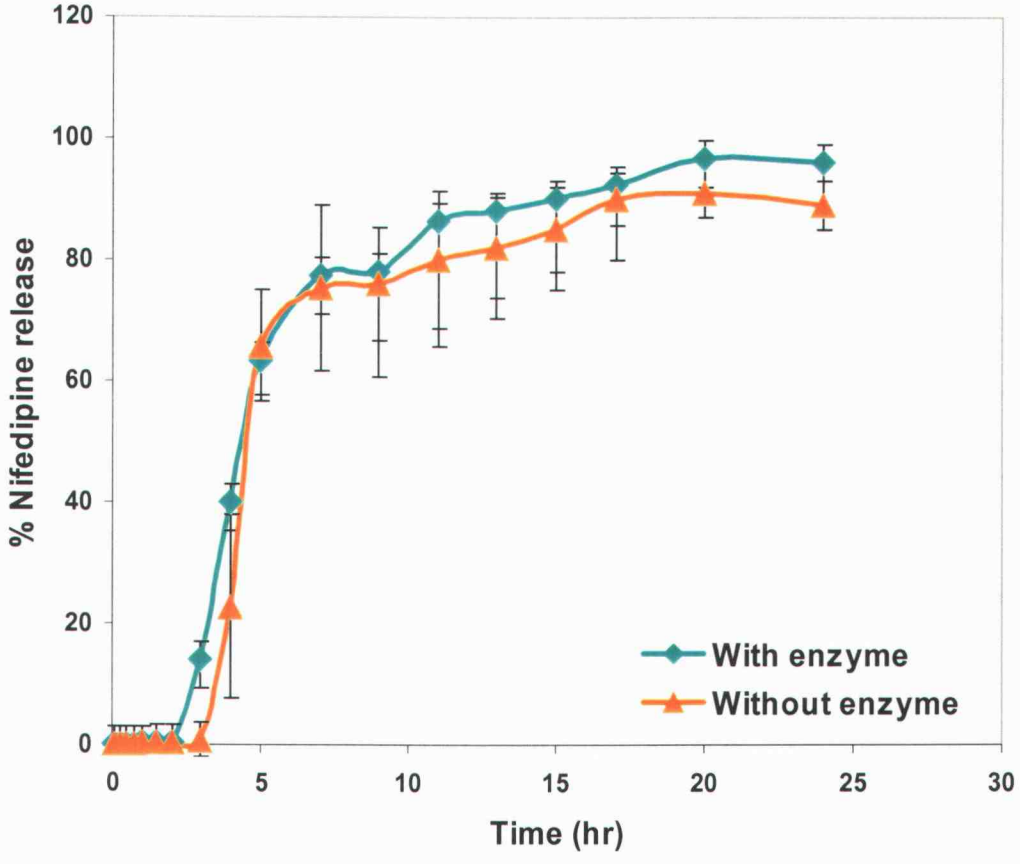

Figure 1.21d: Mean nifedipine release over 24 hours from P2 SEG coated capsules formulation with 12% weight gain in simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours (error bar represents standard deviation, n = 3).

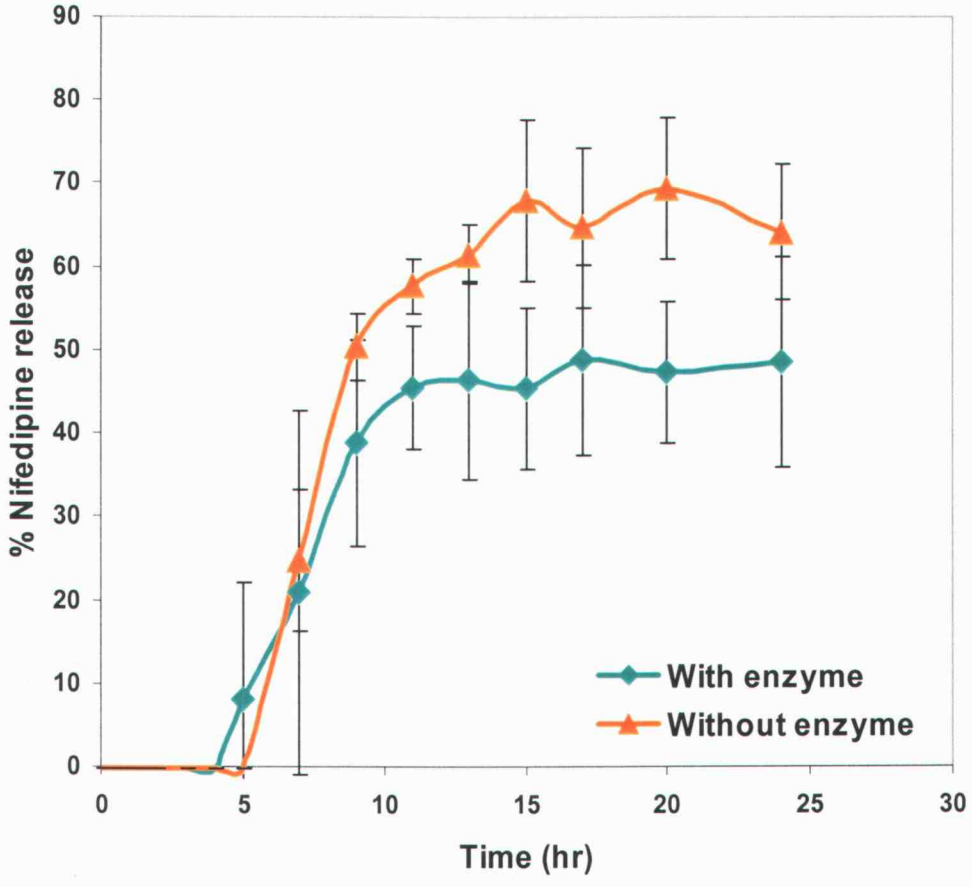

Figure 1.21e: Mean nifedipine release over 24 hours from P2 SEG coated capsules formulation with 17% weight gain in simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours (error bar represents standard deviation, n = 3).

Convolution analysis

Developing once daily formulations of antihypertensive drugs represents a challenge and should provide antihypertensive effect until the end of the dosing interval, as well as prolong duration of action through the daily time of peak hypertension. lood pressure (BP) is subjected to circadian rhythm. Its lowest level occurs during the sleep cycle and rises steeply during the early morning around 6 A.M. in the morning. One importance of giving a sustained release formula for hypertensive patients is to deliver the drug in a higher concentration during the time of greatest need (the early morning period). Coca et al (22) mentioned that most studies performed on effect of antihypertensive drugs on circadian rhythm showed most antihypertensive agents have relatively little effect on circadian rhythm of BP. However, there is a tendency for antihypertensive agents to decrease BP a little more during the day (22). They also observed that all calcium channel blockers investigated such as verapamil and nifedipine have been found to decrease BP without altering the circadian BP profile.

Simulation of expected nifedipine plasma concentration versus time profiles was calculated from dissolution data in simulated gastric fluid for 2 hours followed by pH adjustment to simulate intestinal fluid for 22 hours (convolution) for immediate release SEG nifedipine capsules (10, 20, and 30 mg doses), two commercially available sustained release dosage forms and some new SEG formulations reported herein. This helps guide formulation modification and in

recommending dosage regimens for hypertensive patients, especially for dosing in the morning versus night.

Simulation results showed that administration of 3 commercially available immediate release SEG capsules (10 mg each) resulted in a fast drug release with 267 ng/ml maximum concentration within 0.5 to 1 hour. Then, nifedipine starts to be eliminated from the body producing a drug concentration below the minimum effective concentration (15 ng/ml) (9) after 8 hours (Figure 1.22). The high peak drug concentration may result in adverse side effects, and the duration of action of nifedipine will not last over 8 hours. Simulation of expected nifedipine plasma concentration profile over time upon administration of commercially available nifedipine formulations and selected SEG coated formulations are presented in Figure 1.23. Simulation results obtained from the dissolution data of Procardia XL® were consistent with reported data (8). Procardia XL® ia an osmotic pump tablet that has a two hour lag time which is primarily the hydration time of the tablet before the plasma nifedipine concentration starts to rise. A plateau concentration is obtained after 6 to 8 hours, ~ 22 - 24 ng/ml, over the 24 hour dosing interval of a 30 mg tablet.

Nifedipine matrix tablet Adalat CC® did not have a lag time and a small peak was seen after 3 hours. The predicted nifedipine concentration increased until nifedipine plasma concentration reaches a maximum (50 ng/ml) after 7 hours following an oral dose of a 30 mg tablet.

Nifedipine SEG capsules coated with O3 coat formulation with 13% coating weight gain (30 mg dose) showed one hour lag time and drug release occurred with a maximum concentration of 55 ng/ml after 3 hours. Note that simulated curves are acceptably close to published data points. Nifedipine SEG capsules coated with P1 coat formulation with 14% coating weight gain (30 mg dose) showed one hour lag time and drug release occurred with a maximum concentration of 67 ng/ml after 4 hours. From these results, it was concluded that none of the tested dosage forms of coated nifedipine SEG capsules provided dissolution profiles that matched dissolution profiles from the commercially available tablets of nifedipine.

However, simulation for the new formulations provided a drug concentration above the minimum effective concentration around 6 A.M., which is the time of greatest need for antihypertensive effect in hypertensive patients and remained above the minimum effective concentration for more than 12 hours. These results suggest the new formulation is expected to be a successful sustained release product. These formulations did not duplicate Adalat CC® but they did prolong nifedipine release for up to 12 hours which was similar to Adalat CC® (Figure 1.23).

More research will be required to provide release patterns for nifedipine, after coating immediate release SEG nifedipine capsules, that are identical to commercially available sustained release nifedipine products. It should be noted, however, that some formulations (i.e., O3 coat formulation with 13% coating

weight gain and P1 coat formulation with 14% coating weight gain) provide nifedipine release profiles that are very promising in terms of desirable sustained release formulations, (Figures 1.09a and 1.16a).

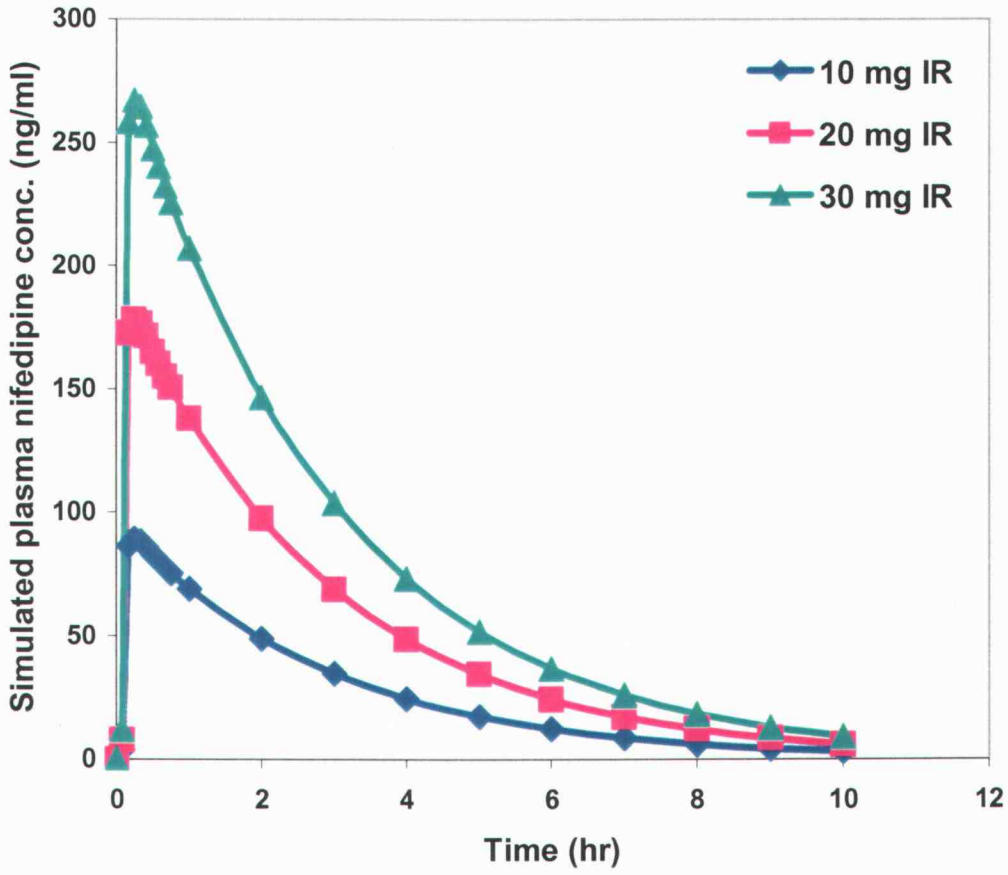

Figure 1.22: Simulated nifedipine plasma concentration after the administration of 10, 20 and 30 mg dose of immediate release (IR) SEG nifedipine capsules.

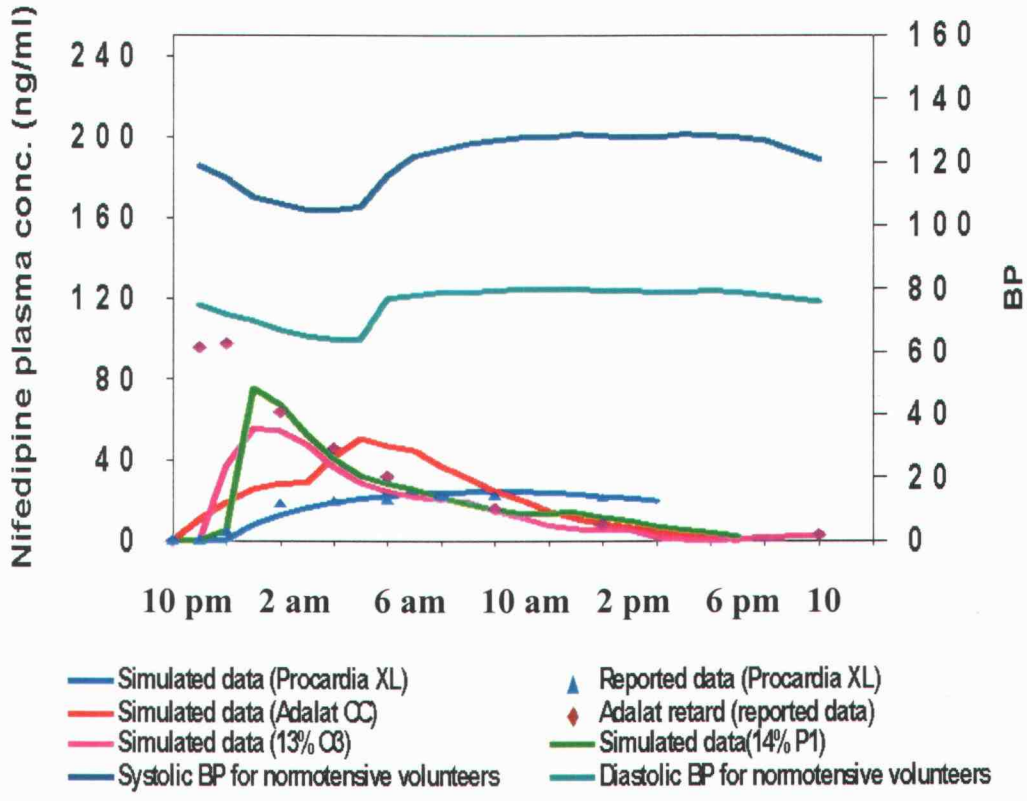

Figure 1.23: Simulated nifedipine plasma concentration after administration of commercially available and selected SEG formulations compared with reported data along with systolic and diastolic blood pressure (BP) from healthy volunteers*.

* Blood pressure (BP) pattern showing circadian rhythm obtained from 12 normotensive volunteers (23).

CONCLUSIONS

SEG capsules were successfully coated using a laboratory spray coater with a modified Wurster column insert. The coat was uniform and was applied onto the surface of the capsule without any cracking or flaking during the coating process. Sustained release action of nifedipine was obtained by coating immediate release SEG capsules with a combination of polymers Surelease® as a water insoluble polymer and Opadry® or pectin as a water soluble polymer. When the ratio of water soluble polymer to Surelease® is high (i.e. 1:1, 2:3, and 1:2 solid ratio), nifedipine release was relatively rapid, especially with the lower percent weight gains of coating. Nifedipine release depended mainly on the amount of water soluble polymer in the coat and thickness of the applied coat. Low amounts of coating had essentially no effect on the release rate of nifedipine and an increased amount of coating resulted in nifedipine precipitation inside the SEG capsule. Drug release "shut down" occurred after nifedipine precipitated with less than 50% drug released in the 24 hour period for many of the formulations studied. Nifedipine precipitation and SEG content gelling was attributed to the slow ingress of aqueous fluid into the capsules resulting in the dilution of the SEG contents with the dissolution fluid such that solubility of nifedipine was exceeded in capsule interior.

Unexpectedly, there was also a pH effect on drug release from nifedipine capsules coated with Surelease® Opadry® combination with O2, O3 and O4 coat

formulation. This effect was not detected with O1 coat formulation due to the rapid release of nifedipine. Surelease® pectin combination also showed a very surprising pH dependent effect with no drug release in gastric fluid. This effect was more pronounced than that of Surelease® Opadry® coat combinations. *In vitro* drug release studies in the presence of pectinolytic enzymes showed that there was no difference in release rate of nifedipine with and without pectinolytic enzymes because the drug release rate was about the same after enzyme addition (after 6 hours dissolution).

The release patterns of nifedipine were generally sigmoidal with a high curvature; thus, clearly indicating non-linear release. The expected plasma concentrations obtained by convolution simulation of tested formulations and commercially available sustained release formulations showed that more research is needed to obtain drug profiles equivalent to that of Adalat CC® matrix tablet or Procardia XL® osmotic pump tablet. However, simulated nifedipine plasma concentration for O3 coat formulation with 13% coating weight gain and P1 coat formulation with 14% coating weight gain provided sustained release of drug with a concentration above the minimum therapeutic concentration (15 ng/ml) at 6 A.M. in the morning which has been reported as the time of greatest need for antihypertensive effects. The tested formulations provided release profiles of nifedipine that are very promising in terms of desirable sustained release formulations.

Formulation of sustained release dosage forms with water insoluble drugs in a SEG capsule represents a challenge. The problem of the precipitation of nifedipine inside the SEG capsule must be solved to obtain desired drug release patterns. Further investigation is underway in our laboratory to obtain an appropriate polymer ratio that will produce a release profile of nifedipine that is comparable to the commercially available sustained release dosage forms and to elucidate the process of drug precipitation inside the SEG capsule.

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CHAPTER 2

Bioavailability and Bioequivalence of Verapamil HCL Controlled Release Formulations

Sahar A. Fahmy and James W. Ayres

ABSTRACT

The bioavailability of verapamil HCL from different verapamil controlled release formulations was tested in two pilot studies (I & II). Based on the results of these pilot studies, one formula (formula IV) was selected to be studied in a full crossover study to test bioequivalence compared to Covera-HS® as a reference product. Bioavailability and bioequivalence of verapamil HCL from a new sustained release tested formulation (treatment A, formula IV) and Covera-HS® (treatment B) were evaluated in two separate two way crossover studies in eight healthy volunteers under fed and fasting conditions. Subjects received a single 240 mg dose of each formulation with a wash-out period of one-week in a randomized, open label two treatment, two period crossover study either directly after a standard breakfast (fed study) or on an empty stomach (fasted study) beginning at 6 A.M. with different subjects involved in the two separate studies. In each study, blood samples were collected up to 58 hours after administration. Samples were analyzed for verapamil and its metabolite, norverapamil using a HPLC method. Non-compartmental pharmacokinetic (PK) data analysis was performed using WinNonlin® program to determine pharmacokinetic parameters of verapamil and norverapamil. Statistical analysis was done for testing bioequivalence based on the two one-sided t-tests which involves calculation of a 90% confidence interval (CI) for the ratio of averages of PK measures for the test and reference products.

In the fed study, the test /reference ratios for the pharmacokinetic parameters (PK) for verapamil were 1.246 (90% CI 1.017 - 1.52), 1.23 (90% CI 1.018 - 1.524) and 1.3 (90% CI 1.081 - 1.665) for AUC $_{0\text{-}58}$, $AUC_{0\text{-}\infty}$ and C_{max}, respectively. In the fasting study, the test /reference ratios for the PK parameters were 0.893 (90% CI 0.36 - 1.06), 0.972 (90% CI 0.354 - 1.22) and 0.998 (90% CI 0.49 - 1.49) for AUC $_{0\text{-}58}$, $AUC_{0\text{-}\infty}$ and C_{max}, respectively. PK measures of C_{max}, AUC $_{0\text{-}58}$, and $AUC_{0\text{-}\infty}$ were not within the generally accepted guidelines of 0.8 to 1.25 in either the fed or the fasting state. The PK parameters for the tested formulation were greater than the PK parameters for Covera HS® in the fed study and less than PK parameters for Covera HS® in the fasted study. However, the food effect was statistically insignificant for both formulations using standard two sample t-test after log transformation of data. These apparently conflicting outcomes may be due to the large variance associated with verapamil PK and the short gastrointestinal transit time for some subjects under fasting conditions. An *in vitro/in vivo* correlation (IVIVC) was established for the tested formulation to help evaluate formulation changes required to produce a product bioequivalent to Covera-HS®.

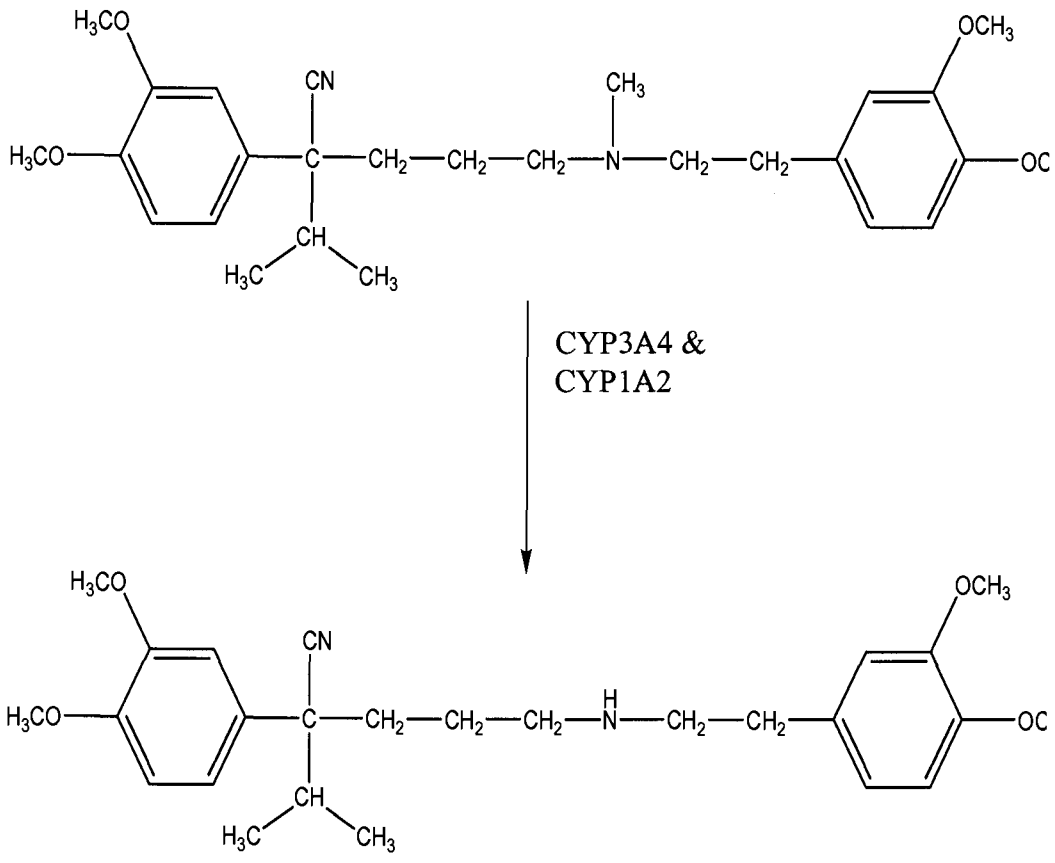
INTRODUCTION

Calcium channel blockers are synthetic drugs and comprise an increasing number of agents. The first generation of these compounds includes verapamil, nifedipine, and diltiazem. These compounds are different in their chemical structure and in their pharmacological action but all selectively antagonize calcium ion (Ca^{++}) movements which are responsible for excitation- contraction processes in the cardiovascular system (1). Fleckenstein et al were first in observing that verapamil and other calcium channel blockers mimicked the effect of calcium ions withdrawal on cardiac excitability in a reversible way (2).

The calcium channel blocker verapamil (2, 8-bis- (3, 4-dimethoxyphenyl)-6-methyl-2-isopropyl-6-azaoctanitrile) has been widely used in treatment of essential hypertension, angina pectoris, and cardiac arrhythmias. It is typically administered in the form of a racemic mixture and undergoes extensive and variable intestinal and hepatic metabolism. This first-pass metabolism is mediated through cytochrome P450 enzymes, mainly CYP3A4 and CYP 1A2. (3), which is responsible for inter-individual variability in plasma concentrations of verapamil and hence drug effects after oral administration of the same dose of the drug to a patient population. Norverapamil is an active metabolite of verapamil which possesses approximately 20% of the cardiovascular activity of verapamil. Therefore, it is considered a pharmacologically active metabolite. Other N-

dealkylated metabolites are devoid of any vasodilating effect (4). Chemical structure of verapamil and its active metabolite, norverapamil are shown in Figure 2.01. Oral bioavailability of verapamil reportedly ranged from 20 - 35% irrespective to dose. It has a terminal elimination half-life ranging from 2-8 hours (4-5).

Verapamil



Norverapamil

Figure 2.01: Chemical structures of verapamil and its metabolite, norverapamil (10).

Studies have demonstrated that the incidence of cardiovascular events is greatest in the early morning, which coincides with a quick surge of blood pressure (BP), occurring primarily ~ 6 A.M. (6). To control this increase in BP, to increase patient compliance, and decrease side effects, controlled release once daily dosage forms taken at bedtime are preferable to conventional immediate release dosage forms, which are taken three times a day. Development of once daily controlled-onset extended release system for verapamil, which is able to control BP for 24 hours, is beneficial to solve this problem. Covera-HS® (Searle, Chicago, IL) is a commercially available product of verapamil which is an osmotic pump tablet designed to be administered at bedtime. It has a 4 hour lag time followed by verapamil release from the osmotically active core through an artificial orifice (4), which provides maximal BP control during early-morning periods. In industry, preparation of osmotic pump tablets requires sophisticated techniques in manufacturing. Therefore, a new delivery system composed of a simple matrix tablet coated with a diffusional barrier membrane was developed to produce drug release that mimics the drug release from osmotic pump tablets.

A new bioequivalent product may provide an alternative therapy to patients with high efficacy, less side effects, and low cost. Two pharmaceutical alternatives or pharmaceutical equivalents are determined to be bioequivalent if there is no significant difference in the rate and extent to which the drug becomes available at the site of action upon administration of the same dose, under the same conditions in a well designed study (7). A crossover design is typically used in determination

of bioequivalence because each subject acts as their own control. According to Food and Drug Administration (FDA) guidelines, the new product will be considered to be bioequivalent to the reference if the 90% confidence interval (CI) of the test formulation is completely contained in the range of 80 to 125% of the reference formulation for the pharmacokinetic (PK) parameters $AUC_{0-\infty}$ and C_{max} using two one sided t-tests for bioequivalence (8).

OBJECTIVES

The objectives of this study were: 1) to compare bioavailability of Covera-HS® and a new-coated matrix tablet formulation made at College of Pharmacy, Oregon State University, when given as a single recommended therapeutic dose of 240 mg verapamil hydrochloride (HCL) to healthy volunteers under the condition of fed and fasting, 2) to study the effect of food on pharmacokinetics of verapamil HCL from both formulations, and 3) to develop an *in vitro/in vivo* correlation for Covera-HS® and the tested formulation.

Verapamil HCL is commercially available as Covera HS® in an osmotic pump tablet sustained release formulation. There is a need to develop new sustained release dosage forms for verapamil that will be bioequivalent to Covera HS®. Development of new sustained release oral dosage forms with a desired drug release profile *in vitro* does not always correlate with drug release profile *in vivo* due to differences in the two model systems. Several factors are involved, making the *in vivo* environment complex and difficult to be imitated *in vitro*. During early drug development, attempts are made to understand the behavior of a pharmaceutical dosage form *in vivo*, and the effect of food and gastrointestinal (G.I.) transit on drug absorption from a particular dosage form. Food intake can influence the pharmacokinetic and pharmacodynamic profile of some drugs, which may impact on clinical effect of these drugs. Food drug interactions may occur before/during gastrointestinal absorption, during distribution, during metabolism and/or elimination. However, food effect is more pronounced in the absorption and metabolism phases (9). Food –drug interaction is not well understood and cannot be predicted based on drug chemical entity.

Effect of food on drug absorption:

Food intake can delay, decrease or, increase drug absorption. Or, food may not have a significant effect on drug absorption depending on several factors: physicochemical properties of the drug, dosage form design, meal composition, time of meal in relation to time of drug administration, and physiological changes in the gastrointestinal (G.I.) tract due to food ingestion. Food intake can influence physiological environment of the G.I. tract by changing gastric emptying, increasing bile secretion, and stimulating gastric HCL secretion, which can affect drug absorption, depending on the physicochemical properties of that drug (9).

Effect of food on gastrointestinal (G.I.) motility:

Transit of pharmaceutical dosage forms through the G.I. tract depends on the presence or absence of food in the G.I. tract. Shargel et al. described G.I. motility in the fasting state as an alternating cycle of activity (migrating motor complex) initially with a quiescent stage followed by irregular contractions, then regular contractions with high magnitude of force, which is responsible for pushing any residual contents down the alimentary canal (10). In the fed state, the G.I. motility is characterized by gentle irregular contractions which are responsible for mixing and pushing the intestinal contents towards the colon in short segments.

These contractions will last in the stomach as long as food is present in the stomach (10).

Gastric emptying time:

Food intake delays gastric emptying rate which may decrease rate and possibly extent of drug absorption as it increases time for drug to reach to the duodenum where absorption of most drugs occurs. Gastric emptying depends mainly on the nature of the dosage form and presence of food in the stomach (10). Large single unit dosage forms such as one used in the current study often stay in the stomach up to 6 to 12 hours when taken with meals.

Intestinal motility and blood flow:

Intestinal motility can be expressed as peristaltic movements responsible for mixing intestinal contents together. Controlled release dosage forms should stay in the G.I. tract long enough for drug absorption to occur. Based on gamma scintigraphic studies, intestinal transit time was independent of dosage form administered or the fed state with mean value of 3 to 4 hours (11). For sustained release dosage forms that release drug over 14 hours, such as studied herein, drug

must be absorbed from the colon to obtain good bioavailability from the formulation.

Ingestion of food is demonstrated to increase splanchnic blood flow in which limited absorption of some compounds occurs via the splanchnic circulation. Drug molecules pass through epithelial cells lining the G.I. tract and enter the capillary network linked with the splanchnic circulation, then go to the portal circulation. Increasing splanchnic blood flow after food intake may have an effect on drug bioavailability as it decreases first-pass metabolism of some drugs, which results in increasing drug bioavailability (12). This effect has been reported for some adrenergic beta-blockers but not reported for verapamil.

The effect of food on drug absorption depends on physicochemical properties of the drug and the dosage formulation. Rate of drug release from a dosage form may be affected by food intake, which can influence the efficacy and safety of drugs from controlled release dosage forms. Drug release from hydrophilic matrix tablets is controlled by tablet erosion and drug diffusion through the gel layer formed around the tablet. The mechanical destructive force of the G.I. tract plays an important role in drug release from controlled release dosage form. Food intake increases the mechanical strength of contractions in the G.I. tract and may increase drug release rate. However, this increase depends on G.I. transit time and hardness of the tablet along different sections of the G.I. tract. Shameem et al found that there was no overall food effect on drug release from controlled release acetaminophen tablet which had been shown to erode with different rates (13).

However, their study showed that food promoted drug release *in vivo* for some subjects and inhibited drug release in others for tablets with slow release rate attributed to the high inter-subject variability in the G.I. destructive forces among subjects (13). In subjects with high drug release rate, the G.I. destructive forces response to food increased the tablet erosion rate. The delayed drug release rate in some subjects could be explained by the higher viscosity or absorption of fat components in the diet. They concluded that the G.I. destructive forces had an important role on the drug release from the erodable controlled release tablets. Covera HS® is not sensitive to G.I. contraction forces or erosion. Thus, formulations designed to be bioequivalent to Covera HS® should be designed to not be sensitive to G.I. contraction forces.

First-pass metabolism:

First-pass metabolism is a phenomenon characterized by pre-systemic removal of drugs after oral administration. The intestine and liver are the major sites for first-pass metabolism but first-pass metabolism could occur in the lung if it is administered via inhalation. Individual variation in first-pass metabolism has a high impact on pharmacokinetics of drugs that undergo first-pass metabolism. Inter-subject variability in bioavailability of drugs susceptible to first-pass metabolism is affected by age, sex, genetic polymorphism, enzyme induction and

inhibition, disease state and food intake (14). Verapamil undergoes first-pass metabolism mediated through cytochrome P450 enzymes, mainly CYP3A4 and CYP 1A2 activity (3), which is responsible for inter-individual variability in plasma concentrations and hence drug effects after oral administration of the same dose of the drug to a patient population. Understanding the impact of the physiological process and its impact on controlled release dosage formulation is important in drug formulation.

IN VITRO/ IN VIVO CORRELATION

Exploring a relationship between *in vitro* drug release profile and *in vivo* absorption that has the ability to predict expected drug bioavailability for a controlled release dosage form from its dissolution profile characteristics is an important part of the dosage form development process. *In vitro/in vivo* correlation (IVIVC) has been defined by Food and Drug Administration (FDA) as " a predictive mathematical model describing the relationship between an *in vitro* property of an extended release dosage form, e.g., drug dissolution profile and a relevant *in vivo* response, e.g., plasma drug concentration or amount of drug absorbed" (15). IVIVC can be categorized into four levels: 1) Level A correlation is a point to point relationship between *in vitro* dissolution and *in vivo* drug input rate

from a dosage form. Level A correlation is estimated by deconvolution of *in vivo* drug plasma profile to determine fraction of drug absorbed *in vivo*, and then compared with fraction dissolved *in vitro*. Level A correlation is usually a linear relationship but non linear relationships are also acceptable. 2) Level B correlation is the correlation of the mean *in vitro* drug dissolution time compared to either the mean *in vivo* dissolution time or the mean residence time derived by principles of statistical moment analysis. It is not a point-to-point correlation. 3) Level C correlation establishes a single point relationship between a dissolution parameter and a pharmacokinetic parameter. 4) Multiple C correlation correlates one or several pharmacokinetic parameters to the amount of drug dissolved at several time points of the dissolution profile of the drug. Level A correlation is considered the most informative and is recommended by FDA for regulatory purposes. It is possible to use the established correlation to employ *in vitro* testing for establishing effect of formulation changes on drug release profile *in vivo*. The main objective of developing IVIVC is to use *in vitro* dissolution testing as a surrogate for human bioequivalence studies, which reduces the number of bio-studies needed for initial approval processes as well as with certain scale up and post approval changes (15). IVIVC is also very useful when modifying drug product formulations to obtain new desirable *in vivo* outcomes if a known formulation is not producing a desired drug concentration versus time profile.

MATERIALS AND METHODS

Chemicals

Covera-HS® 240 mg tablets (Searle,Chicago, IL) was purchased from the Oregon State University's Student Health Pharmacy. Test formulations containing 240 mg verapamil hydrochloride were produced at College of Pharmacy, Oregon State University, Corvallis, OR. Verapamil HCL was a generous gift from TEVA Pharmaceuticals USA (Sellersville, PA).Normethyl-(±)-verapamil, methoxyverapamil HCL and phosphoric acid were purchased from Sigma Chemicals Co. (St. Louis, MO). Acetonitrile, methanol and potassium di-hydrogen phosphate were obtained from Fisher Chemicals (Fair Lawn, NJ). Boric acid, sodium hydroxide, and sodium citrate were purchased from Mallinckrodt Baker (Paris, KY) and potassium chloride was from Fluka Chemie (Buchs, Switzerland). Water was deionized using the Milli-Q® Reagent water system (Millipore, Bedford, MA).

Supplies

1. Catheter 18 gauge, 1.16 in, 1.3 x 30 mm (Instyle-W, Becton Dickinson Infusion Therapy systems, Inc., Sandy, Utah).
2. Normal saline IV flush syring, (Medefil Inc., Glendale height, IL).
3. Extension set (MPS Acacia- Brea, CA).
4. B-D posiflow, fluid capacity 0.06 ml (Becton Dickinson infusion therapy system Inc., Sandy, Utah).
5. IV preparation kit latex free contains: one povidone iodine, one alcohol wipe, two gauze sponges, one tegaderm dressing, one venipuncture information label, one roll of tape, and one non-latex tourniquet, (Acme Health care, Arden, NC).
6. 10 ml syringe (Becton Dickinson and Co., Rutherford, NJ).
7. 10 ml vacutainer® (Becton Dickinson and Co., Franklin Lakes, NJ).
8. 15 ml polyethylene centrifuge tubes (Fisher Chemicals, Lawn, NJ).
9. Alltima C18 3U column 150 x 4.6 mm (Alltech, Deerfield, IL).
10. Strata solid phase extraction (SPE) C18 column (Phenomenex, Torrance, CA).
11. Phenomenex C18 guard cartridge 4 mm L x 3.0 mm ID (Phenomenex Inc., Torrance, CA).

Volunteers:

Eligible volunteers identified for entry into the study were healthy subjects aged between 18 - 32 years. The study protocol was reviewed and approved by the OSU institutional Review Board (IRB) for the protection of human rights. All subjects were not taking any medications one week before or during the study. Subjects' demographic information participated in the full study under fasted and fed conditions is shown in Appendix A, Table A.01 and A.02, respectively.

METHODS

Study design

This research involved two pilot studies testing different coated matrix tablet formulations. Based on initial results, a full study was conducted to test a selected formulation in a crossover design under fed and fasting conditions.

Pilot study I was conducted in only four subjects in two different weeks, one month apart, under fed conditions. The purposes were to evaluate the biostudy processes and gather limited baseline pharmacokinetic data for Covera-HS® and

our two new verapamil formulations (formula I and II). Tablets compositions are shown in Tables (2.01 and 2.02) (16). The method of preparation can be found in reference 16. Formula I and II were round concave shaped tablets, 0.478 inches in diameter that have dissolution profiles similar to the dissolution profile of Covera HS® in intestinal fluid dissolution media. Two subjects were dosed twice orally with formula I (subjects #1 and #2), two subjects were dosed once each orally with formula II (subjects #3 and #4), and one subject (subject #5) was dosed twice with Covera-HS® (Searle) in a randomized order. Subject #3 received formula II during the first study week but was not available for the second treatment. He was withdrawn from the study and replaced by subject #4 in the second week of the study.

Table 2.01: Types and quantities of materials used in the preparation of matrix tablet formula I (wet granulation). (16)

Ingredients	Materials	% (w/w)
Active ingradient	Verapamil HCL (Tween 80 (1%)	33.56%
Hydrophilic polymer	HPMC E5	29.9%
Hydrophilic polymer	Pectin	4.98%
Filler	Lactose	14.53%
Filler	Expotab	14.53%
Lubricant	Stearic acid	2.49%
Tablet weight (mg)		722 mg

Table 2.02: Types and quantities of materials used in the preparation of matrix tablet formula II (direct compression). (16)

Ingredients	Materials	% (w/w)
Active ingradient	Verapamil HCL (Tween 80 (1%)	33.56%
Hydrophilic polymer	HPMC E5	29.9%
Hydrophilic polymer	Pectin	4.98%
Filler	Dicalcium phoaphate anhydrous	29.07%
Lubricant	Stearic acid	2.49%
Tablet weight (mg)		722 mg

Barrier film composition:

A film coat was applied onto the tablet core which contributed to a 9.5% weight gain for tablet formulation I and 6% weight gain for tablet formulation II. The film coat composition is shown in Table 2.03. (16)

Table 2.03: Film coat composition. (16)

Ingredients	Materials	Quantity
Rate controlling membrane	Surelease®	64 ml
Solvent	Deionized water	300 ml
Water soluble polymer	Opadry® Y-22-7719-A	10.125 g
Anti-sticking agent	Talc	21.307 g

Pilot study II was conducted in only six subjects in one week, under fed conditions. The tested formulations (III and IV) were prepared by the same method described in reference 16. Formula III and IV are coated matrix caplets (0.736 x 0.295 inches). Caplet composition is shown in Table 2.04. Caplet core was film coated with the same coat as used for formula I and II with 7% and 8% weight

gain, respectively. Verapamil release profiles from these matrix caplets were comparable to verapamil release from Covera HS® in a dissolution media of gastric fluid for 2 hrs followed by intestinal fluid (pH 7.4) for 22 hours. Three subjects received formula III and another three subjects received formula IV.

Table 2.04: Types and quantities of materials used in the preparation of matrix caplet formula III and IV *

Ingradients	Materials	% (W/W)
Active ingradient	Verapamil HCL (Tween 80 (1%)	29.29%
Hydrophilic polymer	HPMC E5	26.1%
Hydrophilic polymer	Pectin	4.35%
Filler	Spray-dried lactose	12.69%
Disintegrant	Explotab	12.69%
Lubricant	Stearic acid	2.18%
Binder	PVP-K30	12.69%
Tablet weight (mg)		827 mg

* Formula III and IV were prepared in the pharmaceutics lab at OSU by Dr. Angkana Tantituvanont.

Formula IV was chosen to be tested in a full study under fed and fasting conditions. Fed and fasted studies were open-label, single dose, crossover studies with two phases separated by a one-week wash-out period. Eight subjects were enrolled in the fed study and a different eight subjects were enrolled in the fasted study. Volunteers were randomly selected to be in one of the two sequence group (A - B, or B - A) and received one treatment during each phase; treatment A (240 mg-dose of verapamil HCL test formulation IV caplet) or treatment B (240 mg-dose verapamil HCL Covera HS® tablet).

Subjects fasted overnight and received a standard breakfast of one egg, a sausage, a biscuit, and 250 ml of orange juice. In the fed study, the dose was administered immediately following breakfast, with 200 ml of water. In the fasted study, the breakfast was provided two hours after taking the dose. All subjects drank 200 ml of water immediately before each blood sample was collected. Fluid intake for subjects was recorded during the first day of the study.

Blood sample collection

Volunteers were administered the dose of study medication under the supervision of the researcher immediately after a standard breakfast. During the first week of pilot study I, 10-ml blood samples were obtained immediately prior to dosing (zero time) and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16 and 28 hr after

dosing. During the second week of pilot study I, pilot study II and the crossover study, sample-withdrawing schedule was changed to be for a longer time. Ten ml blood samples were obtained at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 28, 34, 52, and 58 hr after dosing. Blood pressure measurements were obtained using a pressure cuff at each sample time.

Blood was collected via indwelling cannula secured in the volunteer's arm up to 16 hrs, and blood samples at 28 hrs and after were collected directly from the vein by venipuncture. Blood samples were immediately transferred into 4% sodium citrate anticoagulant tubes, placed on ice and centrifuged within 1 hour of collection. Supernatant plasma samples were collected by glass pipettes and frozen at -20 °C until time of analysis.

Drug analysis

Blood samples were analyzed for verapamil and its metabolite, norverapamil by solid phase extraction (SPE) followed by high performance liquid chromatography (HPLC) analysis with fluorometric detection. The method of analysis was modified based on the study of Verbesselt et al. (17).

Chromatographic conditions

The HPLC system was a Waters LC module I integrated system comprised of a solvent delivery system and an automatic sampler (Waters Associates, Milford, MA), connected to Perkin Elmer LC 240 Fluorescence detector (Perkin Elmer Corp., Norwalk, CT) and a Spectra-Physics model ChromJet integrator (Spectra-Physics Inc., San Jose, CA).

The column was a C18 reverse-phase column (Alltima C_{18} with 3 micron particle size, 150 mm x 4.6 mm column size, Alltech, Deerfield, IL) preceded by a C_{18} guard cartridge 4 mm L x 3.0 mm ID (Phenomenex Inc., Torrance, CA). The eluent was 0.05 M potassium di-basic phosphate (pH 3) and acetonitrile in the ratio of 65:35. The di-basic phosphate buffer was prepared by dissolving the exact amount of KH_2PO_4 in filtered water (filtered under vacuum through a 0.2 μm filter), then pH was adjusted to 3 using 50% phosphoric acid. The mobile phase was prepared by mixing exact volumes of 0.05 M potassium di-basic phosphate solution and acetonitrile. The mobile phase was degassed before use. The flow rate was maintained at 1 ml/min. The detector was a fixed wavelength spectrofluorometer with an excitation wavelength of 204 nm and an emission wavelength of 304 nm.

Standard solutions preparations

Verapamil and norverapamil were dissolved in methanol to give a stock solution of (1 mg/ml) for each analyte. The methanolic solution was first diluted to 10 μg/ml using vacuum filtered deionized Milli-Q water. This second stock solution was then serially diluted to prepare a concentration for spiking plasma samples (concentration range 1 - 250 ng/ml).

Internal standard

Methoxyverapamil HCL was used as an internal standard. The stock solution of methoxyverapamil HCL was prepared similarly to obtain a final concentration of 5 μg/ml. This concentration was chosen to achieve an appropriate peak area ratio.

Alkaline Borate buffer

Standard alkaline borate buffer was prepared according to the USP method for preparation of standard buffer solutions by dissolving a weighed amount of

boric acid and potassium chloride in an exact volume of distilled water, then pH was adjusted to 9 using 1 N sodium hydroxide.

Sample preparation

An aliquot of 100 µl internal standard solution (5 µg/ml) was added to a 1 ml aliquot of plasma with 1 ml borate buffer solution (pH 9) to alkalinize plasma. The mixture was vortexed for 30 seconds. Solid phase extraction (SPE) column (Strata C_{18} -200 mg, 3 ml) was preconditioned with 1 ml methanol followed by 1 ml distilled water under vacuum. After turning the vacuum off, the plasma mixture was applied to the preconditioned SPE column. The sample was aspirated slowly through the column under vacuum and the elute discarded. The column was then washed with 3 ml-distilled water twice, then with 250 µl acetonitrile to displace the remaining water from the SPE column. Verapamil, norverapamil, and the methoxyverapamil were eluted with two 250 µl aliquots of methanol, which were then collected into clean test tubes. The methanol was evaporated in a concentrator evaporator assembly (Joun RC 1010) at 55 °C (under vacuum and centrifugation at 2000 rpm). The residue was reconstituted in 100 µl mobile phase, vortexed for one minute, and analyzed by HPLC on an Alltima C18 3u (150 mm x 4.6 mm inner

diameter) column connected to a Phenomenex C18 guard cartridge. Injection volume was 50 µl.

Standard Curves

A good separation between verapamil and norverapamil was obtained as shown in Figure 2.02. Retention time was 10 minutes for the metabolite norverapamil, 11 minutes for verapamil, and 13 minutes for the internal standard methoxyverapamil.

Standard calibration curves for verapamil and norverapamil were constructed by plotting peak area ratio of verapamil or norverapamil to methoxyverapamil concentrations (Figures 2.03 and 2.04, respectively). Limits of quantification with the standard curve were 1 ng/ml to 250 ng/ml for both verapamil and norverapamil. Ranges of the coefficient of variation for standard curve assays were 5 - 10% and extraction recoveries were greater than 80%.

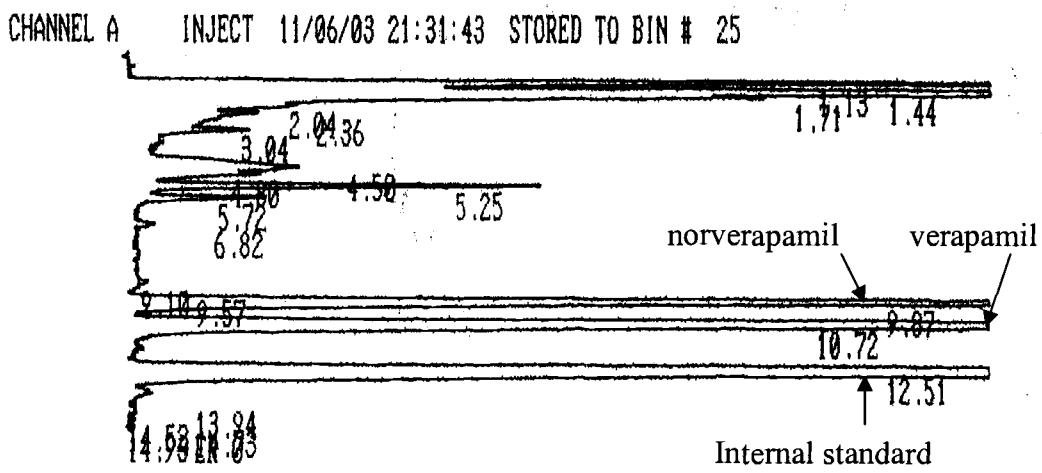


Figure 2.02: A representative HPLC chromatogram of plasma sample spiked with 100, 100 and 200 ng/ml of verapamil, norverapamil, and methoxyverapamil, respectively.

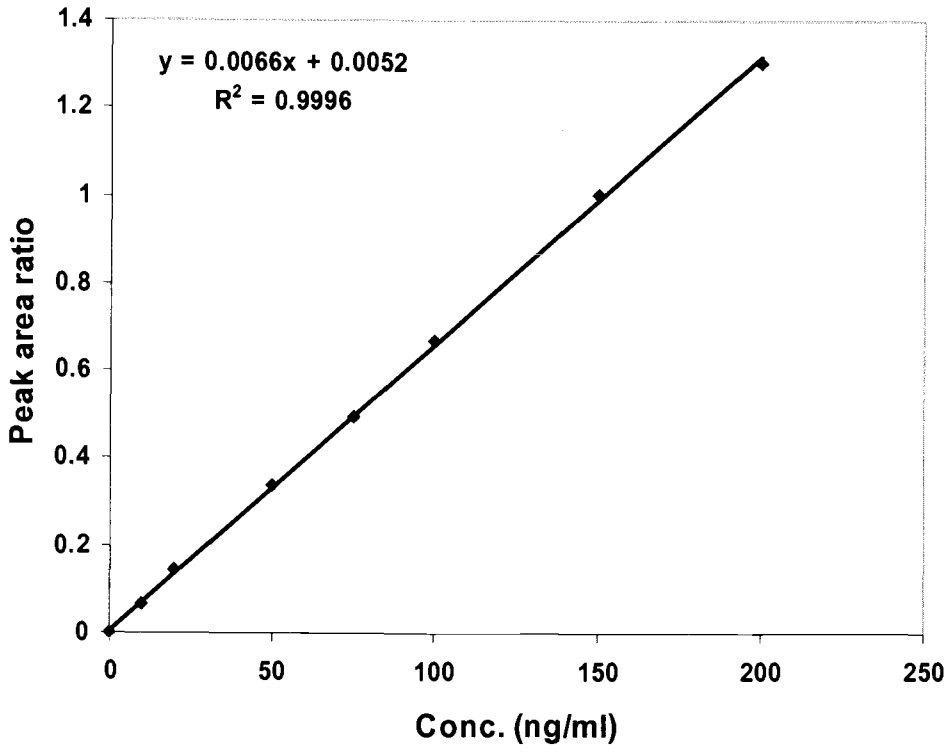


Figure 2.03: A representative standard curve for verapamil in plasma.

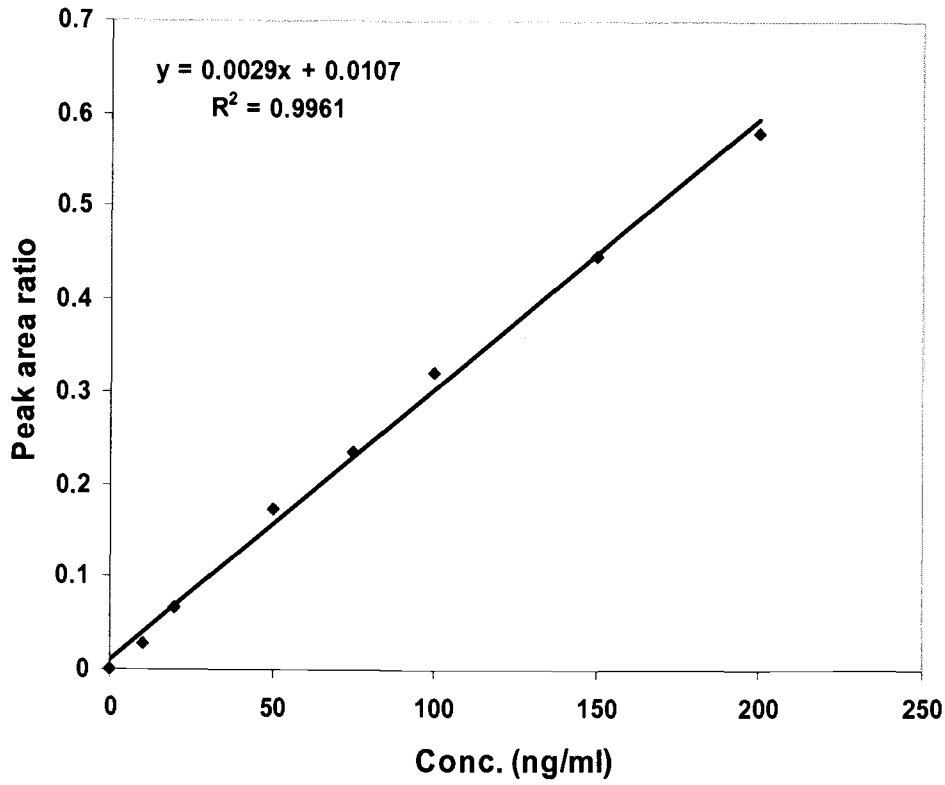


Figure 2.04: A representative standard curve for norverapamil in plasma

Blood pressure measurements

Blood pressure (BP) was monitored during the entire study to ensure the safety of the subjects and to compare the blood pressure pattern with verapamil plasma concentration with each time point. Immediately after each blood sample was withdrawn from the subjects, blood pressure was measured using a digital blood pressure cuff (Wrist blood pressure monitor, model HEM-637, Omron Health Care, Inc., IL). The normal blood pressure as defined by the World Health Organization (WHO) for an adult "is a systolic pressure equal to or below 140 mmHg together with a diastolic pressure equal to or below 90 mmHg" (18).

Pharmacokinetic analysis

The following pharmacokinetic parameters were evaluated for verapamil and norverapamil: a) maximum concentration of drug in plasma (C_{max}); b) time to C_{max} (T_{max}); c) area under the plasma concentration –time curves from 0 - 28 hrs post dose (AUC_{0-28}) or the area under the plasma concentration –time curves from 0-58 hours post-dose (AUC_{0-58}); and d) area under the plasma concentration –time curves from 0 - to infinity (∞) ($AUC_{0-\infty}$) using WinNonlin® (Pharsight, Version 3.2).

Deconvolution analysis and *in vitro/in vivo* Correlation (IVIVC)

Deconvolved input functions from biostudy data were determined using WinNonlin® (Pharsight, Version 3.2). Deconvolution generates an input function which is the cumulative amount absorbed *in vivo* versus time from an input response and the drug's characteristic impulse response function. The input response used was verapamil plasma concentration from the tested formulations and the impulse response used was verapamil plasma concentration after the administration of an intravenous bolus dose of verapamil, which was obtained from another study (5). The cumulative drug input over time predicted by deconvolution was used to show when verapamil absorption from the tablet formulation ends. Also, it helps in constructing an IVIVC.

IVIVC is a correlation between *in vitro* dissolution data and *in vivo* plasma concentrations. The *in vivo* amount absorbed was obtained after deconvolution of the mean verapamil plasma profile. The *in vitro* data was obtained from another study (16) based on tablet dissolution with gastric pretreatment for 2 hours followed by dissolution in intestinal fluid for 22 hours. The IVIVC was constructed using the amount of drug absorbed *in vivo* versus the amount of drug dissolved *in vitro* after time scaling.

Statistical analysis

The bioequivalence test was done using WinNonlin® (Pharsight Version 3.2) using log-transformed, $AUC_{0-\infty}$, AUC_{0-58} and C_{max}. The analysis of variance model included sequence, subjects nested within sequence, period, and drug formulation as factors. The significance of the sequence effect was tested using the subjects nested within sequence as the error term. Bioequivalence of the two formulations in each comparison was assessed by using the 90% confidence intervals (CI) method for the difference between drug formulation least-squares means. These values were calculated for the parameters $AUC_{0-\infty}$, AUC_{0-58}, and C_{max} using log-transformed data. If the 90% CI of the test formulation was completely contained in the range of 80 to 125% of the reference formulation, the two formulations would be considered bioequivalent for that pharmacokinetic parameter (8).

FDA guidelines recommendation for test of bioequivalence is to transform the pharmacokinetic (PK) measures to logarithmic scale because most biological data correspond more closely to a log-normal distribution than to a normal distribution, and variances will be independent of the mean after log transformation (19). PK measures for each individual subject were determined and the arithmetic means associated with standard deviations were calculated for the test and reference products. The geometric means (antilog of the mean of the log

transformed data) were calculated for C_{max} and AUC parameters. Two one-sided t-tests are recommended by FDA to evaluate bioequivalence between test and reference products (19). The two one-sided t-tests consist of two sets of one-sided hypotheses:

$H_{01} : \mu_T - \mu_R \leq \theta_1$

$H_{11} : \mu_T - \mu_R > \theta_1$

And

$H_{02} : \mu_T - \mu_R \geq \theta_2$

$H_{12} : \mu_T - \mu_R < \theta_2$

where,

μ_T = population average of PK parameter of the log transformed measure for the tested formulation.

μ_R = population average of PK parameter of the log transformed measure for the reference.

θ_1 and θ_2 are predetermined limits.

μ_T and μ_R are bioequivalent, if the two one sided tests procedures result in rejecting the null hypotheses H_{01} and H_{02} at a chosen nominal level of significance.

Assuming a normal distribution for the data, the two sets of one-sided hypotheses will be tested with an ordinary one-sided t-test. We conclude that μ_T and μ_R are bioequivalent if:

$$t_1 = \frac{(\overline{X_T} - \overline{X_R}) - \theta_1}{S\sqrt{2/n}} \geq t_{1-\alpha(\upsilon)}$$

$$t_2 = \frac{\theta_2 - (\overline{X_T} - \overline{X_R})}{S\sqrt{2/n}} \geq t_{1-\alpha(\upsilon)}$$

where,

S= square root of the error mean square obtained from crossover design analysis of variance.

υ = degrees of freedom associated with the error mean square.

$(\overline{X_T} - \overline{X_R})$ equals the difference between the observed average bioavailabilities of the test and reference, respectively. The two one-sided tests procedure provides equivalency if the ordinary (1-2α) confidence interval for μ_T and μ_R is completely contained in the predetermined equivalence interval (0.8 - 1.25) for log transformed data (20).

Bioequivalence will be established if the two null hypotheses were rejected, leading to the conclusion that there is no statistically significant difference between the average bioavailability of the test product compared to the reference.

Standard two-sample t-tests were done using SAS^{TM} statistical software (SAS Institute, Cary, NY) to test for differences between the mean of the PK parameters of both tested and reference formulations under the conditions of fed and fasting. This way, we test if there is any additional food effect on verapamil administration between the tested formulation and reference product.

RESULTS AND DISCUSSION

Pilot study I

The purposes of this study were to evaluate the biostudy processes and gather limited baseline PK data for two new verapamil formulations I and II. Results presented are obtained from only 4 subjects and two treatment periods. Two subjects (subjects #1 and #2) received formula I in both treatments of the study. Subject #3 received formula II in the first week and was no longer available afterwards. Subject #3 was replaced with another person (subject #4) in the second week of the study. One subject (subject #5) received Covera HS® (Searle, Chicago, IL) each of the two weeks. Mean verapamil and norverapamil plasma concentrations resulting from treatment with the three-verapamil formulations are presented in Figures 2.05 and 2.06, respectively and PK parameters of verapamil and norverapamil are shown in Tables 2.05 and 2.06 respectively. Individual curves for verapamil and norverapamil are shown in Appendix A Figures (A01 - A10).

As expected with such a small pilot study, the data are quite variable. Drug release from Covera HS® has a 4 hour lag time *in vivo* followed by drug release to produce a maximum concentration after 7 hours, and then the drug starts to decline. For formula I, the drug concentration versus time profile shows 2 - 3 hours lag time

followed by a first drug peak at about 4 - 5 hours and a second peak at a later time, and higher bioavailability than verapamil from Covera HS®. Verapamil from formula II shows a 2 hour lag time and the drug release pattern is quite different in the two weeks, which may be attributed to two different subjects receiving formula II.

Results show that AUC_{0-58} for formula I is higher than AUC_{0-58} for Covera-HS®. With formula II, AUC_{0-28} and AUC_{0-58} were comparable to that from Covera-HS® but C_{max} and T_{max} were significantly different and the shape of the drug concentration versus time curves are very different (Figure 2.05).

Figure 2.07 shows the mean cumulative amount of drug absorbed versus time (deconvolution) of the average biostudy data for the three-verapamil formulations. For Covera HS®, absorption of verapamil continued up to 16 hours, and then stopped. For formula I, absorption of drug continued up to 34 hours with much higher bioavailability. For formula II the absorption of drug stops after 28 hours.

In vitro dissolution study results for the new formulations in intestinal fluid only (pH 7.4) and the dissolution pattern were identical to that of Covera HS® (16). Further dissolution studies with gastric acid pretreatment for 2 hours followed by intestinal fluid pH 7.4 does not change the dissolution profile for Covera HS®, but it did change the dissolution pattern for the new formulations tested. Gastric pretreatment dramatically and unexpectedly decreased the rate of dissolution of verapamil HCL from formula I which suggests a pH effect on the polymeric

materials forming the tablet core of the tested formulations. This effect may lower drug release in the stomach and the upper part of the small intestine and allow more drug to deliver to the colon with higher drug bioavailability as the drug may bypass the first-pass metabolism, and the drug go directly to systemic circulation which makes more drug available at target receptors. This pilot study was conducted under fed conditions. Food intake may increase splenchnic blood flow, which has an effect on systemic clearance of some drugs. This possibility is consistent with higher bioavailability of verapamil from formula I as the AUC_{0-58} was double that from Covera HS®, and the C_{max} from formula II was delayed to 15-16 hours post dose. So, this new formulation is a new drug delivery system that may be useful for colonic targeting which requires further investigation. A crossover design is required for comparison of the new formulations and reference.

Norverapamil PK parameters were consistent with verapamil PK parameters for the three-verapamil formulations. Results showed that norverapamil AUC_{0-58} for formula I was almost twice AUC_{0-58} for Covera-HS®. Verapamil release from the new formulations tested showed two peaks, a small peak around 4-5 hours post dose and then a larger peak 15-16 hour post dose which also occurred with metabolite with peaks one hour after each verapamil peak. With formula II, AUC_{0-28} and AUC_{0-58} were comparable to that from Covera-HS® but C_{max} and T_{max} were significantly different.

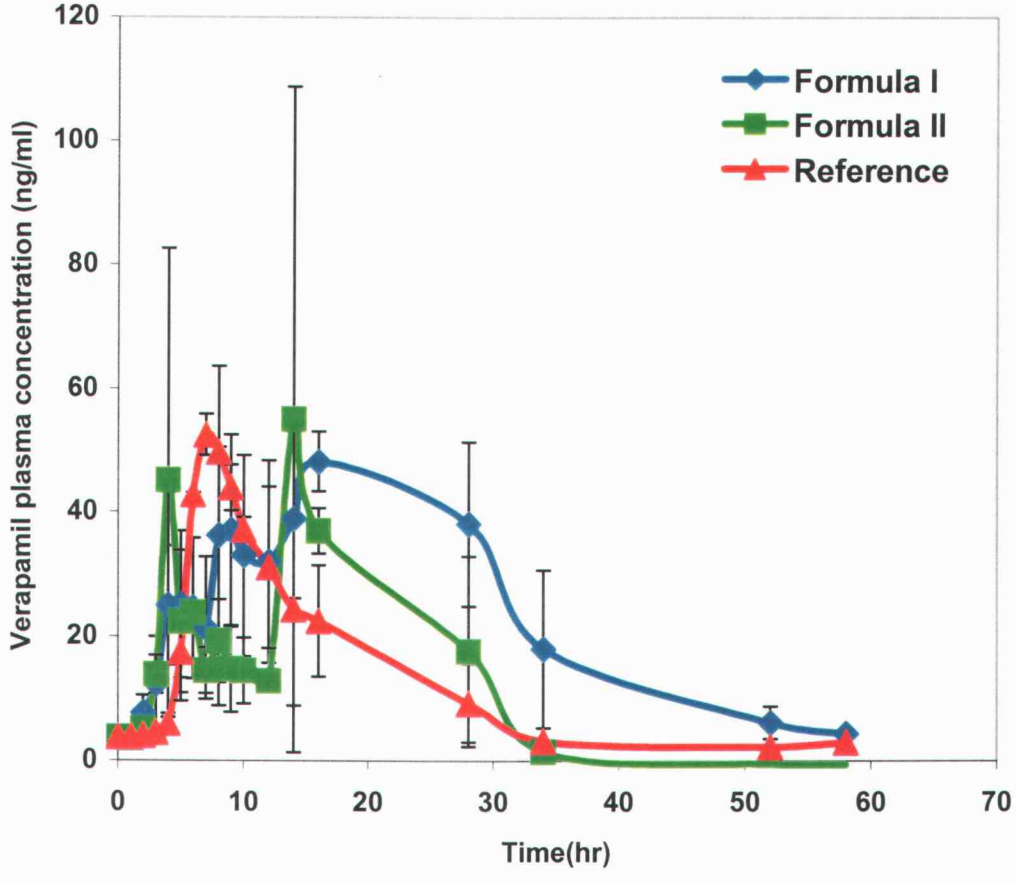


Figure 2.05: Mean verapamil plasma concentration-time curve after administration of three verapamil formulations under fed conditions (pilot study I, error bar represents standard deviation).

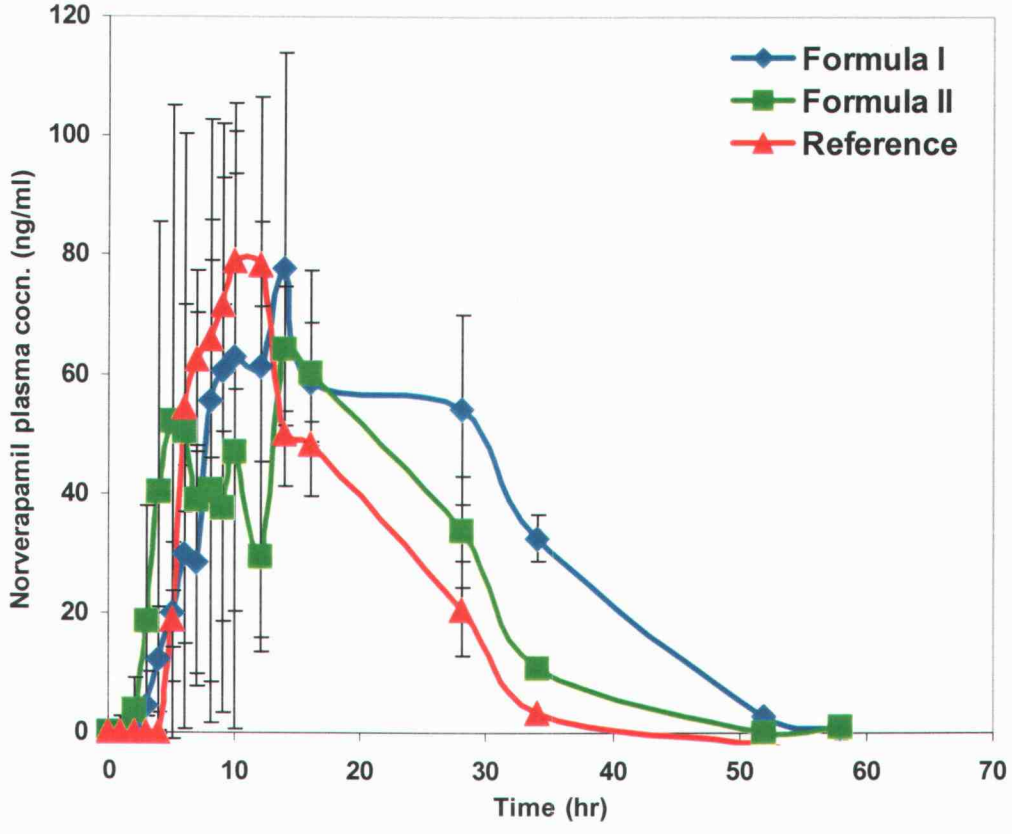


Figure 2.06: Mean norverapamil plasma concentration-time curve after administration of three verapamil formulations under fed conditions (pilot study I, error bar represents standard deviation).

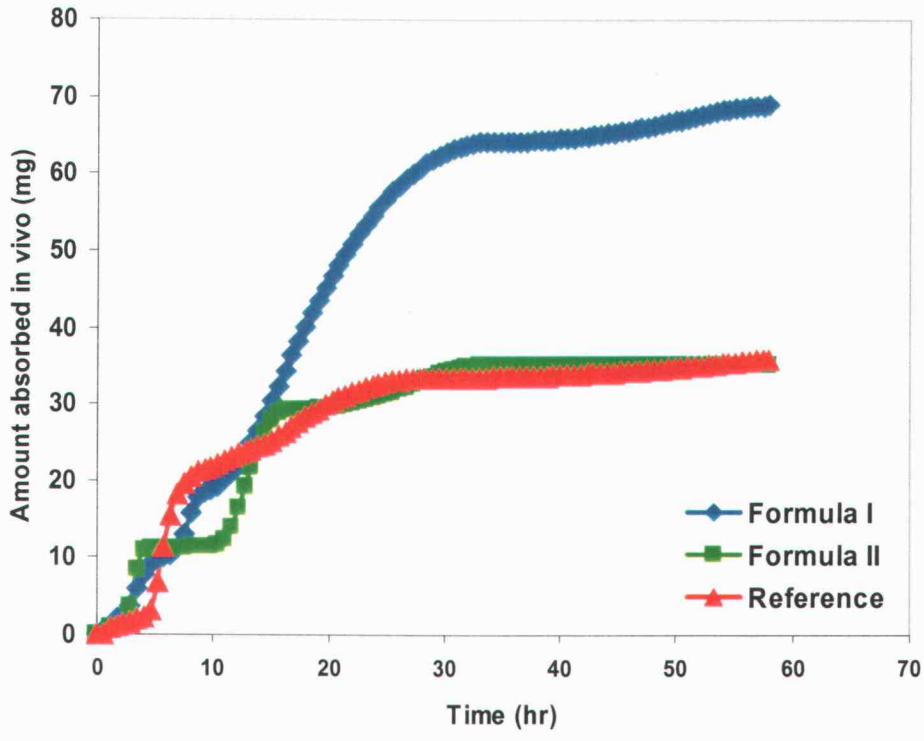


Figure 2.07: Mean cumulative amount of verapamil absorbed versus time deconvolved from average biostudy data for the three-verapamil formulations (pilot study I).

Table 2.05: Pharmacokinetic parameters of verapamil (pilot study I).

	AUC $_{0-28}$ (ng.hr/ml)	AUC $_{0-58}$ (ng.hr/ml)	C $_{max(1)}$ (ng/ml)	C $_{max(2)}$ (ng/ml)	T $_{max(1)}$ (hr)	T $_{max(2)}$ (hr)
Formula A	930.88	1192.89	32	57.76	4.75	16.5
SD	(157.54)	(94.6)	(8.12)	(12.49)	(0.96)	(8.38)
# Subjects	4	2	4	4	4	4
Formula B	697.70	615.26	47.25	63.73	5	15
SD	(157.65)		(34.43)	(41.47)	(1.41)	(1.41)
# Subjects	2	1	2	2	2	2
Covera HS	610.94	578.61	52.54		7	
SD	(173.55)		(3.34)		0	
# Subjects	2	1	2		2	

Table 2.06: Pharmacokinetic parameters for norverapamil (pilot study I).

	AUC$_{(0-28)}$ (ng.hr/ml)	AUC$_{(0-58)}$ (ng.hr/ml)	C $_{max(1)}$ (ng/ml)	C $_{max(2)}$ (ng/ml)	T $_{max(1)}$ (hr)	T $_{max(2)}$ (hr)
Formula A	1407.28	2387.50	33.59	82.57	6.5	16.5
SD	(602.84)	(760.40)	(17.74)	(32.27)	(1.29)	(7.90)
# subjects	4	2	4	4	4	4
Formula B	1017.63	1546.82	53.2	75	6	12
SD	(440.94)		(51.48)	(5.66)	(1.41)	(2.83)
# subjects	2	1	2	2	2	2
Covera	1102.56	1193.52	83.895		11	
SD	(61.67)		(14.84)		(1.41)	
# subjects	2	1	2		2	

Pilot study II:

This study was conducted on only six subjects, under fed conditions. The purposes were to evaluate the biostudy processes and to gather limited baseline PK data for two new verapamil formulations III and IV. The new tested formulations have *in vitro* dissolution profiles that are comparable to that of the reference after 2 hours gastric pretreatment followed by pH adjustment to 7.4. Three subjects were dosed once orally with formula III (7% coat) caplet, and three different subjects were dosed once with formula IV (8% coat) caplet in a randomized order.

Mean verapamil and norverapamil plasma concentrations resulting from treatment with tested verapamil formulations III and IV are shown in Figures 2.08 and 2.09, respectively and the PK parameters of verapamil and norverapamil are shown in Tables 2.07 and 2.08 respectively. Individual curves for verapamil and norverapamil are shown in Appendix A figures A11 - A14.

Results presented are obtained from only 6 subjects for only one-treatment period without crossover. As expected with such a small pilot study and high-variability drug, the subject data are quite variable. For formula III the average drug release profile shows 3 - 4 hour lag time followed by a first C_{max} of 43.24 ng/ml at about 5 hours and a second C_{max} of 73.7 ng/ml at about 18 hours. Drug release from formula IV shows a 3 - 4 hr lag time and the drug release pattern is quite similar to that of formula III, with an average first C_{max} of 62.9 ng/ml at about 5 hours and a second C_{max} of 163.48 ng/ml at 10 hours.

Both new formulations III and IV show prolonged drug absorption with verapamil concentration in plasma above 25 ng/ml for up to 36 hours, but drug concentration from Covera HS® are reported (21) to drop below 25 ng/ml at 18 hours (with 180 mg single dose) and fall rapidly thereafter. In an earlier pilot study in this lab, verapamil concentration in plasma decreased to below 25 ng/ml in 15 hours following a 240 mg dose in Covera HS®. Thus, total bioavailability from the new formulations is expected to be greater than that from Covera HS®.

Figure 2.10 shows the mean cumulative amount of drug absorbed versus time (deconvolution) of the average biostudy data for verapamil formulations (III and IV). For both formulations III and IV, absorption of the drug continued up to 34 hours with higher bioavailability than from reference product in an earlier pilot study.

In vitro dissolution of the tested formulations matched that from Covera HS®. *In vivo* drug release from the tested formulations suggest that after the film coat around the tablet core broke, initial drug release is rapid enough to produce a first peak around 4 - 7 hours in vivo, and then the G.I. fluid penetrate the matrix sufficiently to form a gluey core which slows drug release for some time and then drug release rate is increased over time which produces the second drug peak at later times.

In fact, this study was done on only 3 subjects per formulation and was compared to literature data from another study. This is not sufficient to determine bioequivalence because verapamil undergoes first-pass metabolism, shows high

variability, with drug metabolism depending on subject's body weight, age, gender and metabolic enzyme activity (14). To obtain conclusive bioavailability results new formulations must be studied in a crossover design with administration of Covera HS® and the new tested formulations in the same subjects with higher number of subjects involved in the study.

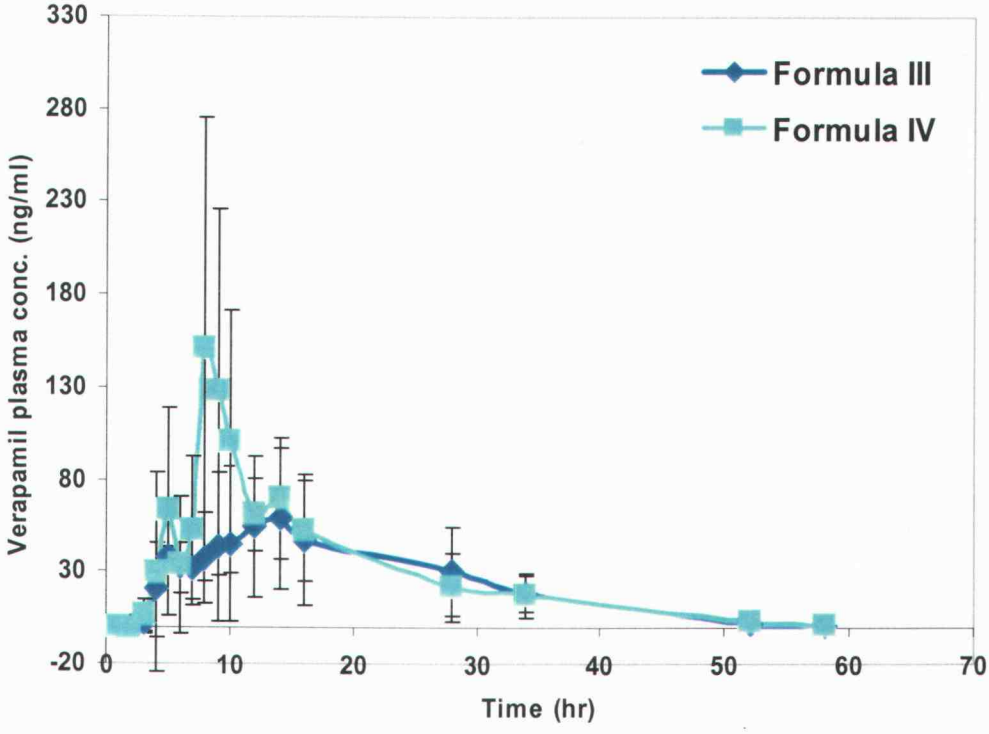


Figure 2.08: Mean verapamil plasma concentration-time curve after administration of formula III and IV under fed conditions in 3 subjects (pilot study II, error bar represents standard deviation).

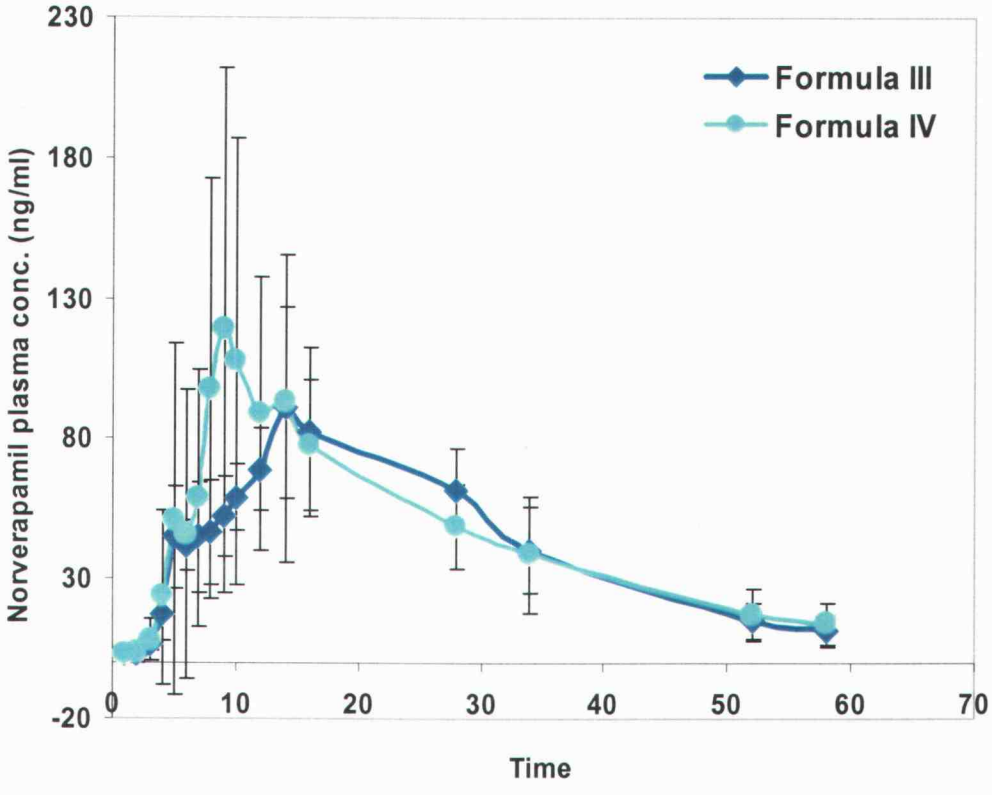


Figure 2.09: Mean norverapamil plasma concentration-time curve after administration of formula III and IV under fed conditions in 3 subjects (pilot study II, error bar represents standard deviation).

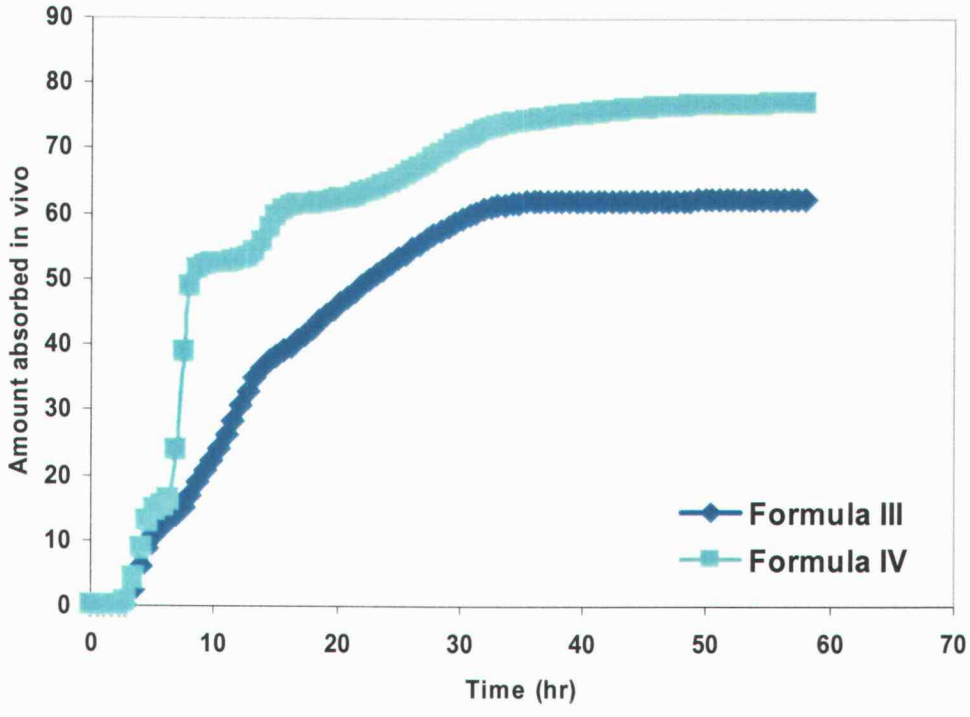


Figure 2.10: Mean cumulative amount of verapamil absorbed versus time deconvolved from average biostudy data for the tested formulations III and IV (pilot study II).

Table 2.07: Pharmacokinetic parameters of verapamil (pilot study II).

	$AUC_{(0-58)}$ (ng.hr/ml)	$C_{max(1)}$ (ng/ml)	$C_{max(2)}$ (ng/ml)	$T_{max(1)}$ (hr)	$T_{max(2)}$ (hr)
Formula III	1334.78	43.24	73.7	5.33	18
SD	(634.82)	(7.53)	(20.46)	(1.53)	(8.72)
# Subjects	3	3	3	3	3
Formula IV	1613.98	62.91	163.48	5	10
SD	(495.89)	(56.53)	(103.32)	(0)	(3.46)
# Subjects	3	3	3	3	3

Table 2.08: Pharmacokinetic parameters of norverapamil (pilot study II).

.

	$AUC_{(0-58)}$ (ng.hr/ml)	$C_{max(1)}$ (ng/ml)	$C_{max(2)}$ (ng/ml)	$T_{max(1)}$ (hr)	$T_{max(2)}$ (hr)
Formula III	2501.06	60.83	104.93	7	18.67
SD	(185.07)	(25.86)	(35.41)	(2.65)	(8.08)
# subjects	3	3	3	3	3
Formula IV	2460.08	112.72	117.26	10	17
SD	(239.95)	(53.28)	(61.9)	(5.57)	(9.85)
# subjects	3	3	3	3	3

Full study

Formula IV was tested in a full crossover design study under fed and fasting conditions. These were open-label, single dose, crossover studies (under fed or fasting conditions) with two phases each separated by a 1-week wash-out interval.

Fed study

Results of the biostudy presented are for 8 subjects in the fed study who completed both test periods of the crossover study. Individual verapamil and norverapamil plasma concentrations resulting from treatment with the verapamil formulations in the fed state are shown in Appendix A (Figures A.15 - A.30). Figures 2.11 and 2.12 represent the average of verapamil and norverapamil plasma concentrations at each time point for each subject, respectively. The average C_{max} or AUC obtained from this average curve was not reported or used in the calculations. The reason for taking the mean of the individual PK parameters in the calculation is to take into account the individual variability of the subjects involved in the study.

Average PK parameters of verapamil and norverapamil resulting from the fed study are shown in Tables 2.09 and 2.10, respectively. Drug absorption profiles from formula IV and Covera HS® both exhibit 4 hour lag time. Mean peak plasma

verapamil concentration was 100 ng/ml after approximately 17 hours for formula IV compared to a mean peak plasma drug concentration of 76 ng/ml after approximately 10 hours from Covera HS®. Drug release from formula IV exhibits higher bioavailability (AUC) and C_{max} with a longer time to reach plasma peak concentration compared to Covera HS® in subjects who were fed immediately before taking the tablets. PK parameters from norverapamil mirrored those from verapamil. Individual deconvolved input functions are shown in Appendix A (Figures A.31 - A.38). Mean deconvolved input functions of verapamil from administration of the tested formulation and reference product are shown in Figure 2.13. Deconvolution results indicate that the amount of drug absorption from the two formulations was the same up to 9 hours, then drug input rate at 15 hours and up was higher from the tested formulation than from the reference (Table 2.11). Drug absorption occurred up to 30 hours from both formulations. Individual verapamil input rates indicate that input rate from tested formulation is slower than that from reference which indicates lower drug dissolution from IV tablet core in the first 10 - 15 hours until the tablet reaches the lower part of the small intestine or the colon (15 hours and up) at which higher drug input and higher drug absorption occurred resulting in a higher bioavailability from the tested formulation.

The tested formulation: Covera HS® ratios least-squares means for C_{max} and AUC_{0-58} (1.3 and 1.23 respectively) indicate a higher bioavailability of verapamil from the tested formulation compared to reference product (Table 2.12). Ninety percent CI for the ratios of the PK parameters fall above the recommended

confidence limits of 0.8 - 1.25 concluding non bioequivalence with higher bioavailability from the tested formulation (Figure 2.14). The same results were concluded from norverapamil data analysis. The tested formulation: Covera HS® ratios least-squares means for C_{max} and AUC $_{0\text{-}58}$ (1.2 and 1.13 respectively) indicate a higher bioavailability of norverapamil from the tested formulation compared to the reference product (Table 2.13).

There was a significant difference between C_{max} from the tested and reference formulations (p-value of 0.038) and marginally significant difference (due to small sample size) between AUC $_{0\text{-}58}$ and $AUC_{0\text{-}\infty}$ (p-value of 0.079 and 0.078, respectively) at a significance level of 5% when comparing formula IV and Covera HS®. These PK measures for the new formulation were not within the generally accepted guidelines of 0.8 to 1.25 with higher bioavailability from the new formulation under fed conditions.

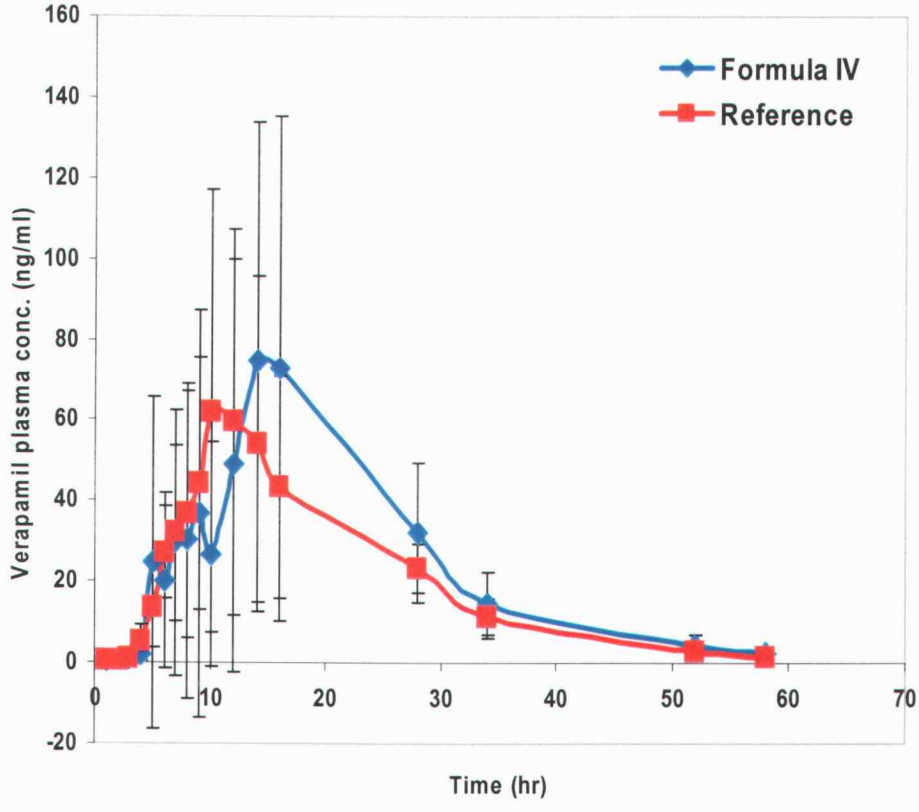


Figure 2.11: Mean verapamil plasma concentration-time curve after administration of tested formulation (formula IV) and Covera HS® (reference) under fed condition (error bar represents standard deviation, n = 8).

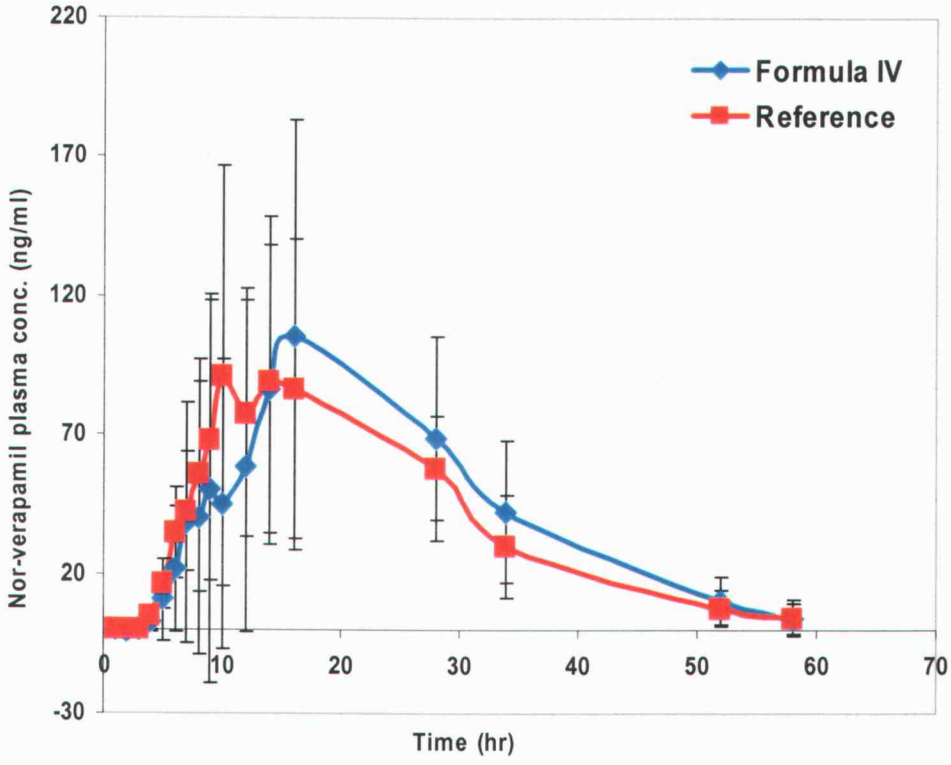


Figure 2.12: Mean norverapamil plasma concentration-time curve after administration of tested formulation (formula IV) and Covera HS® (reference) under fed condition (error bar represents standard deviation, n = 8).

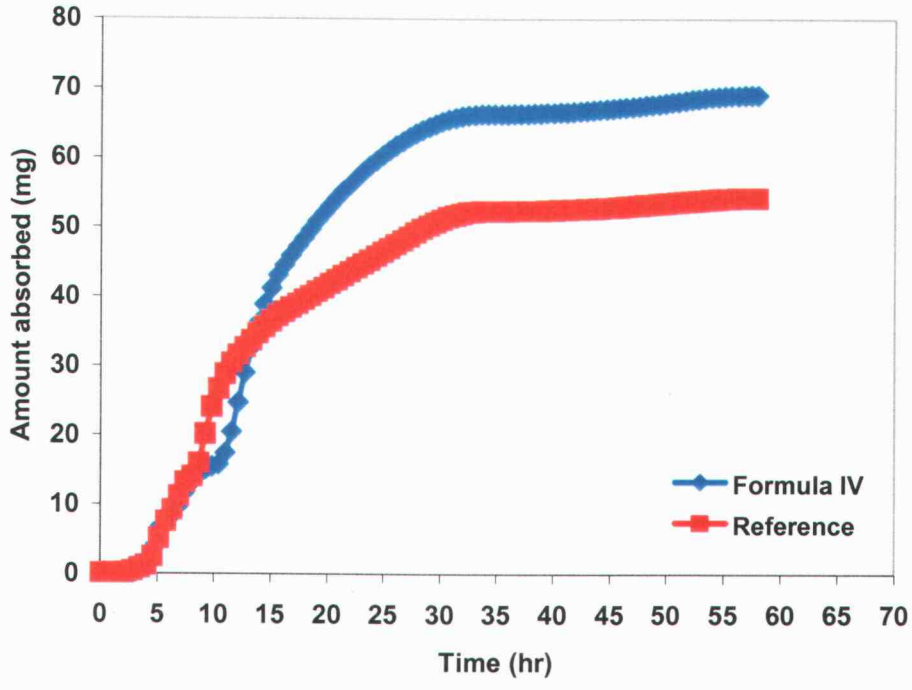
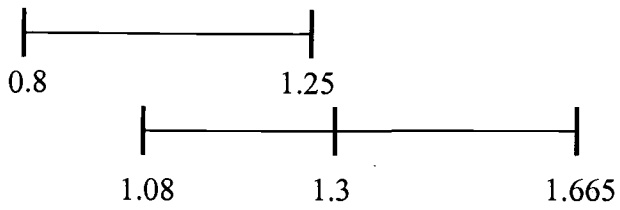


Figure 2.13: Mean cumulative amount of verapamil absorbed versus time deconvolved from average biostudy data for the tested formulation (formula IV) and Covera HS® (fed study).

C_{max}



AUC_{0-58}

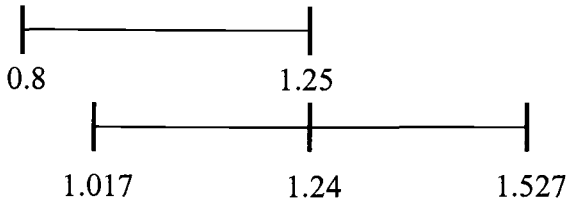


Figure 2.14: Ninety percent confidence intervals of pharmacokinetic parameters of verapamil from tested formulation (formula IV) under fed condition.

Table 2.9: Averages ± standard deviations of individual pharmacokinetic parameters of verapamil under fed conditions in eight subjects (WinNonlin® Version 3.2).

Pharmacokinetic parameter	Tested formulation	Reference
C_{max} (ng/ml)	100.26 ± 67.19	76.95 ± 57.82
T_{max} (hr)	16.93 ± 6.75	9.53 ± 3.3
AUC_{0-58} (hr.ng/ml)	1351.16 ±706.86	1084.35 ± 484.47
$AUC_{0-\infty}$ (hr.ng/ml)	1393.95 ± 698.63	1131.86 ± 507.74
Cl_F_obs (ml/hr)	212946.23 ± 98707.06	281706.25 ± 196977.9
Vz_F_obs (ml)	2780356.21 ± 2034587.85	4214713.1 ± 3727930.8
Lambda_z (1/hr)	0.0979 ± 0.0425	0.0766 ± 0.019
n	8	8

Table 2.10: Averages ± standard deviations of individual pharmacokinetic parameters of norverapamil under fed conditions in eight subjects (WinNonlin® Version 3.2).

Pharmacokinetic parameter	Tested formulation	Reference
C_{max} (ng/ml)	126.74 ± 73.29	105.13 ± 71.26
T_{max} (hr)	19.43 ± 7.08	12.03 ± 3.4
AUC_{0-58} (hr.ng/ml)	2416.4 ± 1302.01	2129.36 ± 991.75
$AUC_{0-\infty}$ (hr.ng/ml)	2508.12 ± 1315.88	2208.21 ± 1037.56
Cl_F_obs (ml/hr)	118258.6 ± 53701.77	152941.73 ± 122454.24
Vz_F_obs (ml)	1439144.35 ± 996002.96	2182393.92 ± 2128633.8
Lambda_z (1/hr)	0.092 ± 0.032	0.091 ± 0.045
n	8	8

Table 2.11: Comparison between individual verapamil input rate and area under the curve from 0-58 hours from tested formulation IV and reference product (fed study).

Time (hrs)	**3**		**5**		**10**		**24**		**58**		**AUC***	
Treat #	**IV**	**C**	**IV**	**C**	**IV**	**C**	**IV**	**C**	**IV**	**C**	**IV**	**C**
1	0	0	0.1	6.3	25	33	75	53	77	63	1441	1288
2	0	0	0	0	4	24	112	76	134	84	2469	1630
3	0	0.9	24	2.5	29	46	118	67	130	74	2283	1522
4	0.5	0	0.7	4	1.6	9.5	19	31	31	41	681	830
5	0.3	0.7	3	7	7.5	13	29	13	37	15	704	319
6	0.6	0.4	1.4	5.5	3.2	12	21	25	34	31	675	567
7	0	1.3	1.2	6.6	6.6	15	46	42	65	56	1147	1001
8	0.5	0	15	9.1	57	49	65	64	71	76	1407	1516
Mean	0.1	0.4	6.5	5	16	27	59	45	69	54	1351	1084

* Area under the curve in (hr*ng/ml)

IV: formula IV

C: Covera HS® (reference)

Table 2.12: Mean of verapamil pharmacokinetic parameters of the tested formulation and reference under fed conditions and their statistical analysis.

Treatment	**AUC_{0-58}**	**$AUC_{0-\infty}$**	**C_{max}**	**T_{max}**
	(ng.hr/mL)	(ng.hr/mL)	(ng/mL)	(hr)
Reference				
Mean	1084.35	1131.86	76.95	9.53
SD	(484.47)	(507.74)	(57.82)	(3.3)
N	8	8	8	8
Formula IV				
Mean	1351.16	1393.95	100.26	16.93
SD	(706.8)	(698.6)	(67.2)	(6.75)
N	8	8	8	8
Ratio	1.246	1.23	1.3	1.78
(90% C I)	(1.02–1.52)	(1.02-1.52)	(1.08-1.66)	

Table 2.13: Mean of norverapamil pharmacokinetic parameters of the tested formulation and reference under fed conditions and their statistical analysis.

Treatment	**AUC_{0-58}**	**$AUC_{0-\infty}$**	**C_{max}**	**T_{max}**
	(ng.hr/mL)	(ng.hr/mL)	(ng/mL)	(hr)
Reference				
Mean	2129.36	2208.21	105.13	12.03
SD	(991.75)	(1037.56)	(71.26)	(3.4)
N	8	8	8	8
Formula IV				
Mean	2416.4	2508.12	126.743	19.43
SD	(1302.01)	(1315.88)	(73.293)	(7.08)
N	8	8	8	8
Ratio	1.135	1.136	1.21	1.62
(90% C I)	(0.85-1.56)	(0.87-1.56)	(0.97-1.62)	

Fasted study:

Eight subjects participated in the fasted study. Subject #5 did not complete the two phases of the study. Therefore, results presented are for the 7 subjects who completed both test periods of the fasted crossover study. Individual verapamil and norverapamil plasma concentrations resulting from treatment with the verapamil formulations in the fasted state are shown in Appendix A (Figures A.39 - A.52) Mean verapamil and norverapamil plasma concentrations are shown in Figures 2.15 and 2.16 respectively.

Average PK parameters of verapamil and norverapamil resulting from the fasted study are shown in Tables 2.14 and 2.15 respectively. Verapamil release from both formula IV and Covera HS® showed a 3 hour lag time. Mean peak plasma verapamil concentration from formula IV was 52 ng/ml at 17 hours compared to mean verapamil plasma concentration of 52 ng/ml at approximately 13 hours for Covera HS®.

PK parameters from norverapamil mirrored those from verapamil. Individual deconvolved input functions of verapamil resulting from administration of tested formulation and reference product are shown in Appendix A (figures A.53 - A.59). Mean deconvolved input functions of verapamil resulting from administration of the test and reference products are shown in Figure 2.17 and indicated that drug input rate from the two formulations was the same as indicated

by the same slope for both formulations. Drug input continued up to 30 hours for reference product but was longer for the tested formulation. Individual verapamil input rates are shown in Table 2.16. Both tested formulation IV and reference exhibited 3 hour lag time followed by higher drug input rate from reference product than that from tested formulation IV. Drug input from tested formulation IV in subject #3 and #7 stopped after 10 hours which may indicate that the tested formulation left the body in a bowel movement without complete dissolution and absorption under fasting conditions as also indicated by lower bioavailability from the tested formulation compared to reference in these two subjects.

Despite this, the tested formulation : Covera HS® ratios least-squares means for C_{max} and AUC $_{0\text{-}58}$ were 0.998 and 0.89, respectively (Table 2.17), which are within 0.8 - 1.25 range, the 90% CI for the ratios of the PK parameters are wider than the recommended confidence limits of 0.8 - 1.25 (Figure 2.18) and requires a conclusion of non bioequivalence using FDA guidelines. Similar results were obtained from norverapamil data analysis. The tested formulation: Covera HS® ratios least-squares means for C_{max} and AUC $_{0\text{-}58}$ (0.71 and 0.91, respectively) indicate a lower bioavailability of norverapamil from the tested formulation compared to the reference product (Table 2.18).

Although there was no significant difference between C_{max}, AUC $_{0\text{-}58}$ and $AUC_{0\text{-}\infty}$ from both formulations at a significance level of 5% when comparing formula IV and Covera HS® (p-value = 0.61, 0.13 and 0.23, respectively), these PK

measures for the new formulation were not within the accepted guidelines of 0.8 to 1.25. Thus, this new formulation is expected to be bioequivalent to Covera HS® in fasted subjects in a larger study with larger sample size. The primary reason for non-equivalence is most likely high variability for the drug (as seen with both formulations) making it difficult to demonstrate bioequivalence even for equal C_{max} values (both 52 ng/ml).

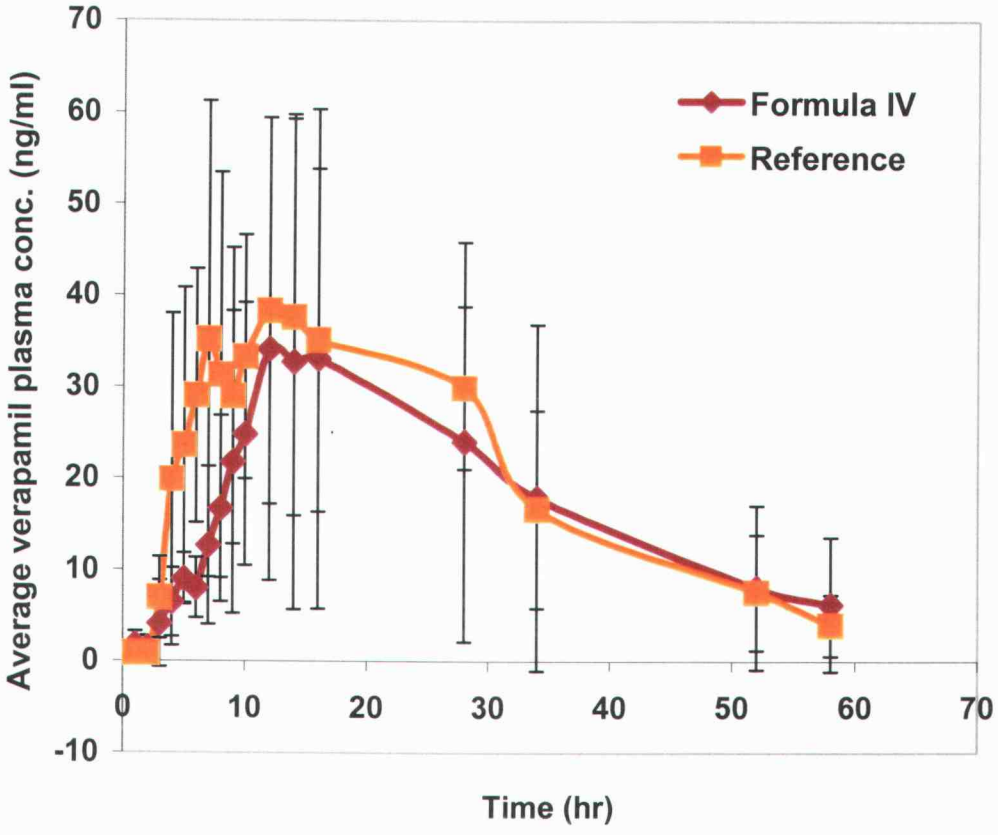


Figure 2.15: Mean verapamil plasma concentration-time curve after administration of tested formulation (formula IV) and Covera HS® (reference) under fasted condition (error bar represents standard deviation, n = 7).

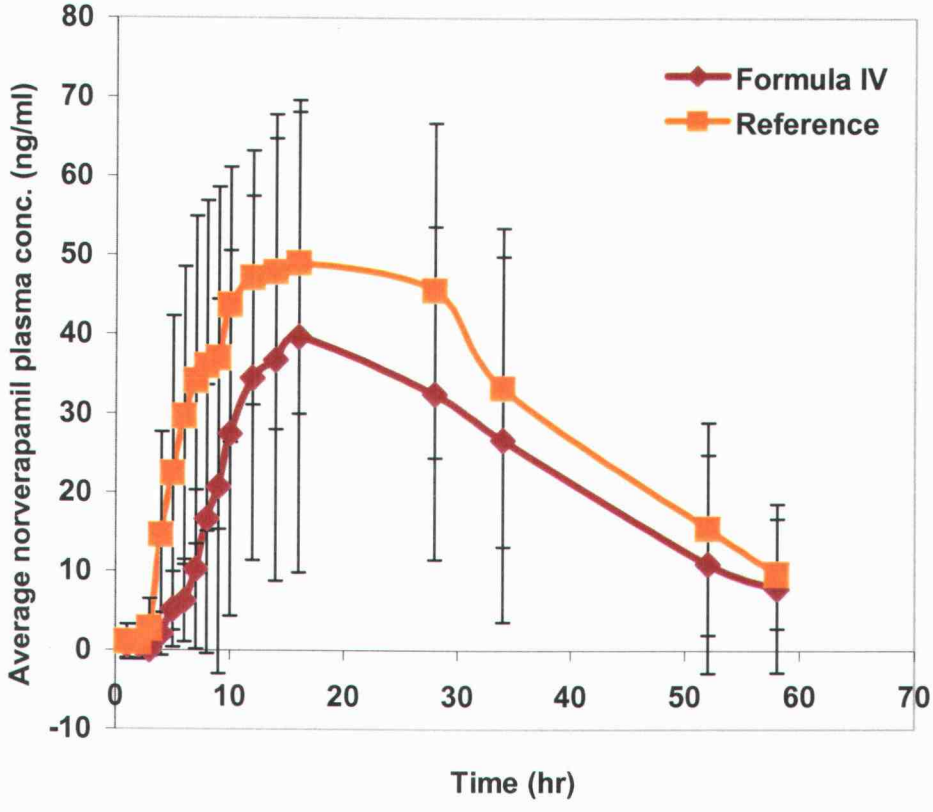


Figure 2.16: Mean norverapamil plasma concentration-time curve after administration of tested formulation (formula IV) and Covera HS® (reference) under fasted condition (error bar represents standard deviation, n = 7).

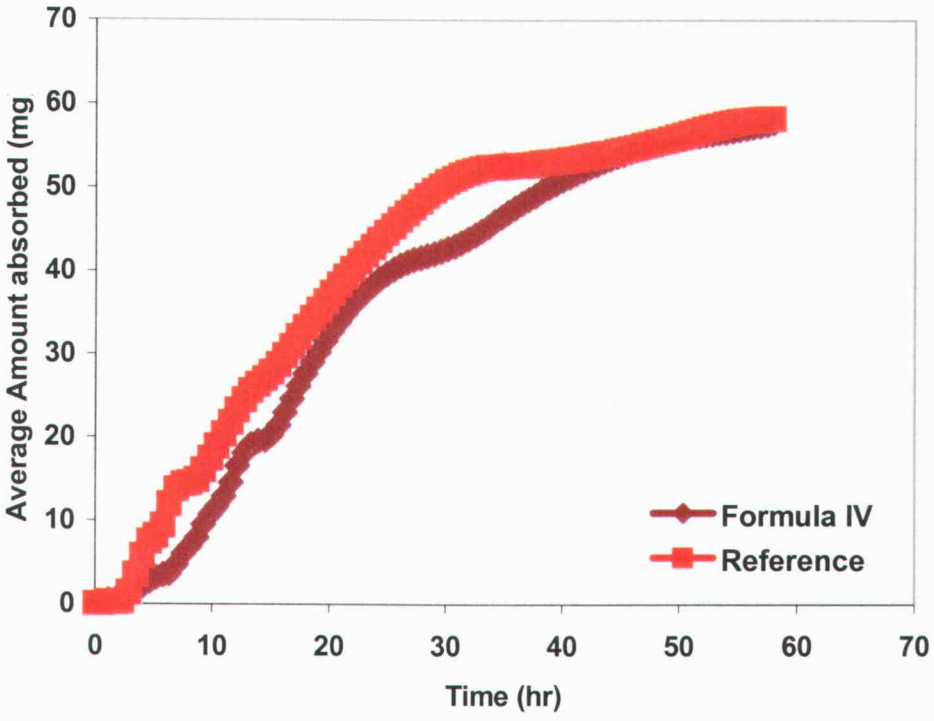
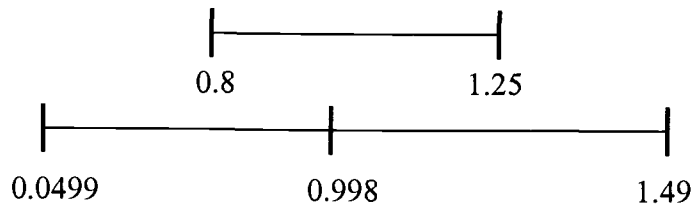


Figure 2.17: Mean cumulative amount of verapamil absorbed versus time deconvolved from average biostudy data for the tested formulation (formula IV) and Covera HS® (fasted study).

C_{max}



AUC_{0-58}

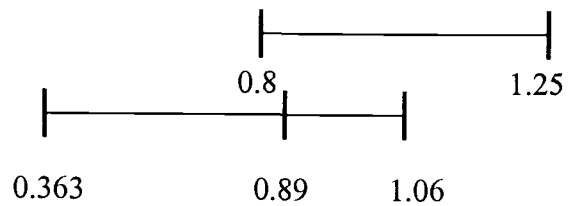


Figure 2.18: 90% confidence intervals of pharmacokinetic parameters of verapamil from tested formulation under fasted condition.

Table 2.14: Averages ± standard deviations of individual pharmacokinetic parameters of verapamil under fasteded conditions in seven subjects (WinNonlin® Version 3.2).

Pharmacokinetic parameter	Tested formulation	Reference
C_{max} (ng/ml)	52.41 ± 24.28	52.52 ± 19.42
T_{max} (hr)	17.03 ± 9.39	13 ± 7.77
AUC $_{0-58}$ (hr.ng/ml)	1000.23 ± 696.78	1120.15 ± 447.61
AUC $_{0-\infty}$ (hr.ng/ml)	1170.14 ± 871.89	1204.15 ± 489.48
Cl_F_obs (ml/hr)	844521.39 ± 1639781.04	255689.16 ± 198547.75
Vz_F_obs (ml)	5494961.75 ± 4393043.5	3703519.5 ± 1830161.2
Lambda_z (1/hr)	0.16 ± 0.28	0.067 ± 0.021
n	7	7

Table 2.15: Averages ± standard deviations of individual pharmacokinetic parameters of norverapamil under fasted conditions in seven subjects (WinNonlin® Version 3.2).

Pharmacokinetic parameter	Tested formulation	Reference
C_{max} (ng/ml)	57.68 ± 24.34	63.06 ± 5.42
T_{max} (hr)	18.04 ± 8.67	16.57 ± 8.9
AUC_{0-58} (hr.ng/ml)	1246.71 ± 691.5	1744.15 ± 551.9
$AUC_{0-\infty}$ (hr.ng/ml)	1471.48 ± 951.86	1979.41 ± 728.99
Cl_F_obs (ml/hr)	276798.14 ± 253576.42	157155.2 ± 123075.86
Vz_F_obs (ml)	5076184.87 ± 5943813.95	2486634.88 ± 966483.08
Lambda_z (1/hr)	0.063 ± 0.0296	0.0604 ±0.023
n	7	7

Table 2.16: Comparison between individual verapamil input rate and area under the curve from 0-58 hrs from tested formulation IV and reference product (fasted study).

Time (hrs)	**3**		**5**		**10**		**24**		**58**		**AUC***	
Treat #	**IV**	**C**	**IV**	**C**	**IV**	**C**	**IV**	**C**	**IV**	**C**	**IV**	**C**
1	1.1	3.6	2.7	11	7.7	33	37	71	93	80	1563	1592
2	0.8	3.2	2.7	6.2	17	10	48	31	54	56	1106	1100
3	0.9	1.4	2.5	7.8	20	20	21	41	24	50	450	991
4	2.4	0	4.9	1.5	11	11	72	49	117	70	2150	1260
6	0	1.7	2.8	5.7	7.6	19	26	62	46	91	887	1643
7	0.2	3.3	0.9	17	3.9	17	3.9	17	3.9	19	50	325
8	2.7	0.9	4	6.4	16	20	38	37	41	48	795	930
Mean	1.5	3.5	3.2	8.2	12	19	38	43	58	58	1000	1120

* Area under the curve in (hr*ng/ml)

IV: formula IV

C: Covera HS® (reference)

Table 2.17: Mean of verapamil pharmacokinetic parameters of the tested formulation and reference under fasted conditions and their statistical analysis.

Treatment	**AUC_{0-58}**	**$AUC_{0-\infty}$**	**C_{max}**	**T_{max}**
	(ng.hr/mL)	(ng.hr/mL)	(ng/mL)	(hr)
Reference				
Mean	1120.15	1204.15	52.51	13
SD	(447.6)	(489.47)	(19.42)	(7.77)
N	7	7	7	7
Formula IV				
Mean	1000.23	1170.14	52.42	17.04
SD	(696.78)	(871.89)	(24.29)	(9.4)
N	7	7	7	7
Ratio	0.893	0.972	0.998	1.31
(90% C I)	(0.363 -1.06)	(0.354 -1.22)	(0.4995-1.49)	

Table 2.18: Mean of norverapamil pharmacokinetic parameters of the tested formulation and reference under fasted conditions and their statistical analysis.

Treatment	**AUC_{0-58}**	**$AUC_{0-\infty}$**	**C_{max}**	**T_{max}**
	(ng.hr/mL)	(ng.hr/mL)	(ng/mL)	(hr)
Reference				
Mean	1744.15	1979.41	63.06	16.57
SD	(551.94)	(728.99)	(5.42)	(8.9)
N	7	7	7	7
Formula IV				
Mean	1246.71	1471.48	57.6838	18.036
SD	(691.49)	(951.85)	(24.34)	(8.67)
N	7	7	7	7
Ratio	0.71	0.743	0.91	1.09
(90% C I)	(0.44-0.85)	(0.43-0.95)	(0.53-1.23)	

Fed versus fasted

Statistical analyses for the difference between the mean of PK parameters resulting from the fed and the fasted studies for both the tested formulation and the reference product were done using standard two-sample t-test (SAS™ statistical software) after log transformation of data. For tested formulation IV, there was no convincing evidence that the mean of the PK parameters was different in the fed and the fasted study with p-values of 0.16 and 0.32 for C_{max} and $AUC_{0-\infty}$, respectively. For Covera HS®, the same conclusion was reached because of no convincing evidence that the mean of the PK parameters was different in the fed and the fasted study with a p-value of 0.6 and 0.8 for C_{max} and $AUC_{0-\infty}$ respectively. Concluding that there was no significant food effect on verapamil absorption which is in agreement with other published studies (22-23).

Marvola et al. (24) studied the effect of food on verapamil absorption from a single unit matrix tablet and the effect of food on G.I. transit time in six healthy volunteers. Based on tablet localization by radiography throughout the G.I. tract, they reported that an essential part of verapamil absorption from the matrix tablet occurred in the colon in the fasted state (24). Food intake delayed the transit time through the G.I. tract with a significant change of transit time between fed and fasting states. It has been reported that the transit time to reach the terminal ileum was 2 - 4 hours under fasting conditions and 4 - 24 hours in the fed state (24). Gastric emptying rate was delayed by food intake because the tablet remained in

the stomach at least 12 hours for 2 out of 6 subjects which confirms prolongation of gastric emptying rate after food intake. Thus, food intake can affect the G.I. transit of a matrix tablet and affects the absorption rate of verapamil.

DISCUSSION

Studies done on verapamil sustained release dosage formulations show high inter-individual and intra-individual variations that may be attributed to subject variability. In a study with verapamil sustained release (240 mg dose tablet) involving 6 subjects, the reported average peak verapamil concentration in plasma was 83.8 ng/ml, with average area under the curve of 1128.5 hr*ng/ml (25). The study was repeated under the same conditions in 12 subjects where the average C_{max} of 122.7 ng/ml and the AUC was 1619.5 hr*ng/ml (26). That was an increase of 46% in C_{max} and 44% in AUC when the study was repeated. Thus suggesting high variability in the PK parameters due to different subjects involved or small number of subjects involved in the study.

Drugs that undergo first-pass metabolism (like verapamil) are subject to high variability affected by several factors including age, sex, and ethnic background (14) which may explain the high variability of verapamil

pharmacokinetics from the tested formulation and the reference product under both fed and fasting conditions.

Drug release from the new matrix caplet studied herein was controlled by retained support platform membrane and erosion of the gel layer formed around the caplet core after contact with G.I. fluid, and drug diffusion through the gel layer. Drug release was affected by drug physicochemical properties and contractile intensity along the G.I. tract, which is subjected to inter-individual variability. Food intake is also another factor, which resulted in potential differences of drug release from matrix tablets between fed and fasting states. Increased agitation in the G.I. tract in the fed state was reported to exert more intense mechanical stress on the matrix tablet, which typically results in faster erosion rates from hydrophilic matrix tablets after food intake (27). Verapamil release form Covera HS® (osmotic pump tablet) was not expected to be affected by these variables as drug release reportedly is zero-order release, independent of pH, and food intake. It has been reported that the PK parameters of Covera HS® was not affected by consumption of a high fat meal just prior to dosing at night (28).

Verapamil input rate from tested formulation IV showed a slower drug input *in vivo* than *in vitro* which suggested that, after the film coat broke, the tablet took a longer time for hydration and dissolution from the tablet core which suggested incorporation or increased percentage of water soluble components in the tablet core to hasten drug release to produce a product that is bioequivalent to Covera HS®.

Higher verapamil bioavailability under the fed condition was obtained by slower drug dissolution in the stomach and the upper part of the small intestine and higher drug dissolution in the lower part of the small intestine and upper colon along with slow G.I. transit which may be successful drug delivery system for colonic targeting.

Sample size calculation

Based on results obtained from 8 subjects in the fed study and 7 subjects in the fasted study, sample size calculation was done based on the table by Diletti, et. al. (29) and are approximated by looking at the closest values for the mean ratios and percent coefficient of variation (%CV). The mean ratios and the %CV were calculated using the following formulas:

$\mu_T/\mu_R = e^{(\text{difference between log transformed means})}$

$\%CV = \sqrt{e^{MSE} - 1} * 100\,\%$

Tables 2.19 and 2.20 shows the minimum sample sizes needed for the ANOVA to detect a difference between the products with 80% power.

Table 2.19: Sample size calculated for tested formulation (formula IV) fed study:

PK Parameter	μ_T/μ_R	% CV	Sample Size
Ln-Transformed C_{max}	1.34	22.5	More than 368
Ln-Transformed AUC_{0-t}	1.25	21.1	More than 294

Table 2.20: Sample size calculated for tested formulation (formula IV) fasted study:

PK Parameter	μ_T/μ_R	% CV	Sample Size
Ln-Transformed C_{max}	0.92	53.7	More than 80
Ln-Transformed AUC_{0-t}	0.66	52.4	More than 292

In vitro/in vivo correlation (IVIVC)

In vitro/in vivo correlation was established using *in vitro* dissolution data for tested formulation IV and reference product (16) for dissolution in gastric fluid for 2 hours followed by intestinal fluid for 22 hours. Mean deconvolved input function resulting from tested formulation IV and reference product was obtained from the *in vivo* biostudy data from the fasted study as recommended by FDA (15). The time of maximum dissolution *in vitro* was not equivalent to time of complete absorption *in vivo*. Therefore, the time of maximum *in vitro* dissolution had to be scaled to be equivalent to that of *in vivo* deconvolved input (30).

Figures 2.19 and 2.20 show an *in vitro/in vivo* correlation between the amount of drug dissolved *in vitro* versus amount of drug absorbed *in vivo* before and after time scaling, respectively. Good linear relationship was observed with Covera HS® and slight nonlinearity was observed with formula IV. Non-linear relationship is also acceptable by FDA as level A correlation. Nearly the same regression equation holds for the correlation from both formulations, which suggest very similar drug release rates from both formulations. Slopes less than one suggest more rapid *in vitro* dissolution compared to *in vivo* absorption. The established correlation may be useful in dosage form development and reduce number of biostudies needed for formulation changes needed to produce a product bioequivalent to Covera HS®. Desired *in vitro* dissolution profile of a new

formulation obtained from IVIVC of formula IV along with the dissolution profile of formula IV and Covera HS® is shown in Figure 2.21.

Desired dissolution profile of a new formulation was obtained by using the *in vivo* input from Covera HS® as a desired *in vivo* input and back read the *in vitro* dissolution pattern using the established IVIVC for formula IV. The desired dissolution profile was drawn versus time after back time scaling (Figure 2.21). Diagram (2.22) shows how the desired dissolution pattern can be obtained using the established IVIVC correlation to determine which formulation changes can be made to obtain a release profile similar to Covera HS®. However, the expected dissolution profile obtained was very close to that of reference product. This was explained by very close mean *in vivo* input from formula IV and reference product in the fasted study.

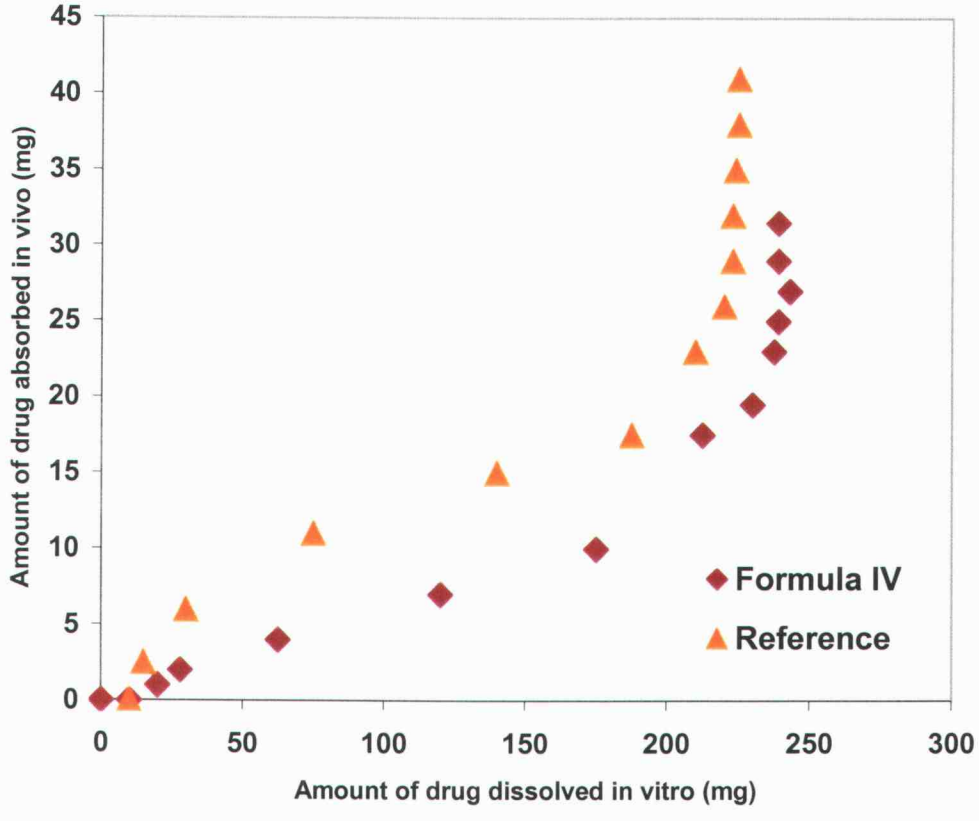


Figure 2.19: *In vitro in vivo* correlation for verapamil from tested formulation (formula IV) and Covera HS® (reference product) without time scaling.

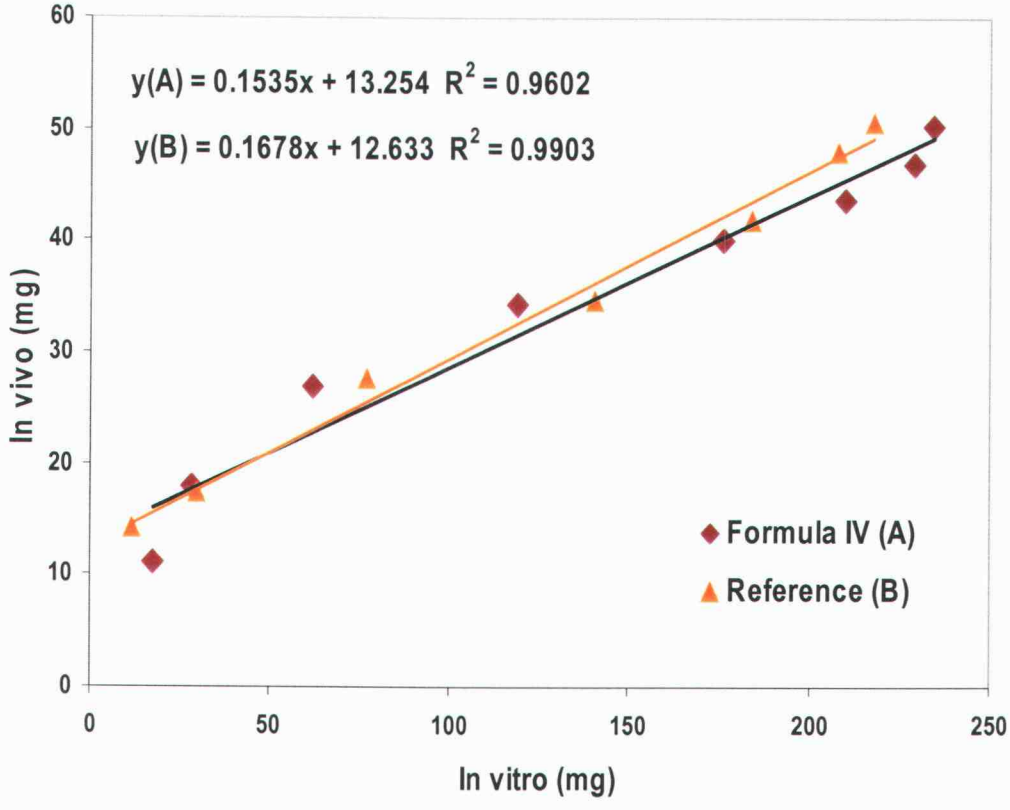


Figure 2.20: IVIVC for verapamil from tested formulation (formula IV) and Covera HS® (reference product) after time scaling.

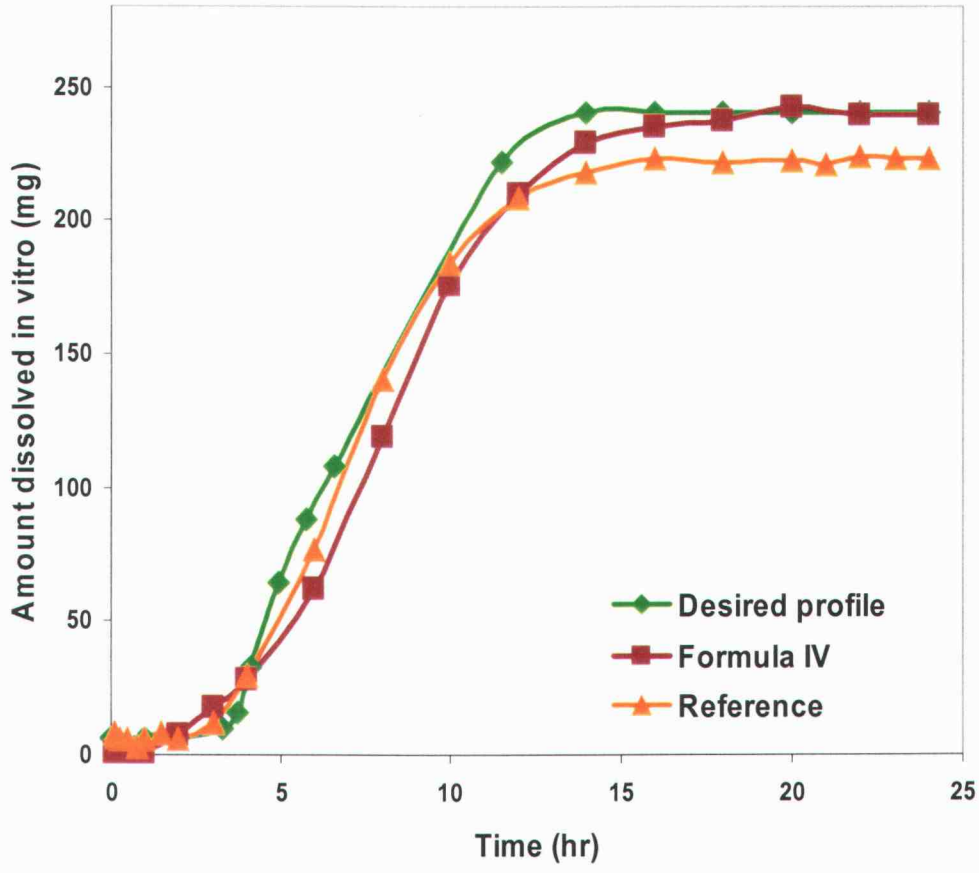


Figure 2.21: Desired dissolution profile obtained from the established IVIVC of formula IV.

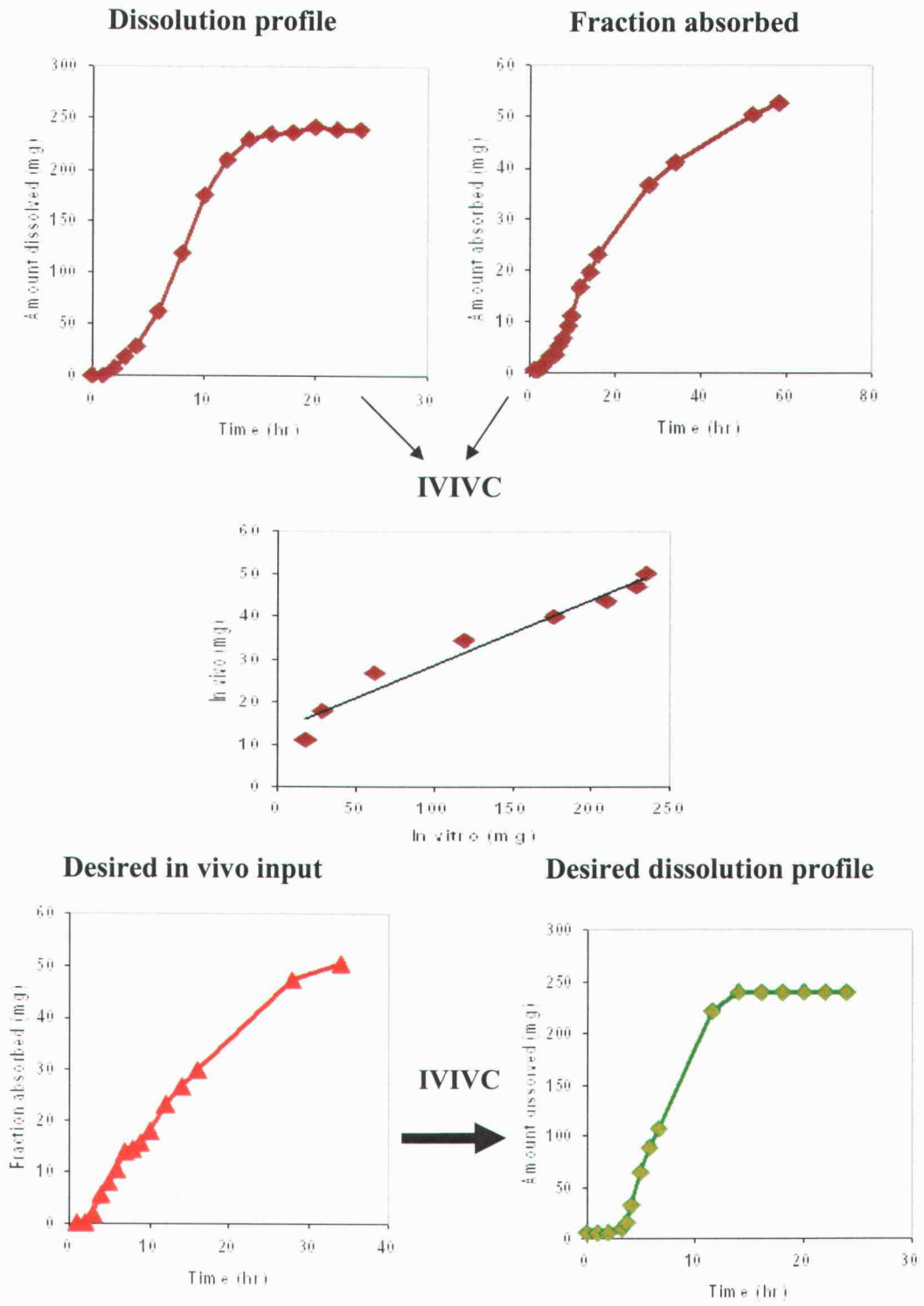


Figure 2.22: Diagram showing how the desired dissolution pattern was obtained using IVIVC.

Blood pressure measurements

Blood pressure measurement was done with each sample point for the subjects in the study. None of the subjects complained of any symptoms of hypotension. Figures (2.23 - 2.26) show mean verapamil concentration along with mean blood pressure measurements time curve resulting from administration of formula IV and reference product under both fed and fasting conditions. As shown in the figures, blood pressure pattern was consistent with verapamil plasma concentration in blood, i.e., blood pressure was slightly lower than baseline when verapamil plasma concentration in blood is high. Also, the blood pressure was lowest (and drug concentration highest) at about 5 or 6 P.M. in this study but circadian rhythm studies show blood pressure is typically at daytime maximum at this time.

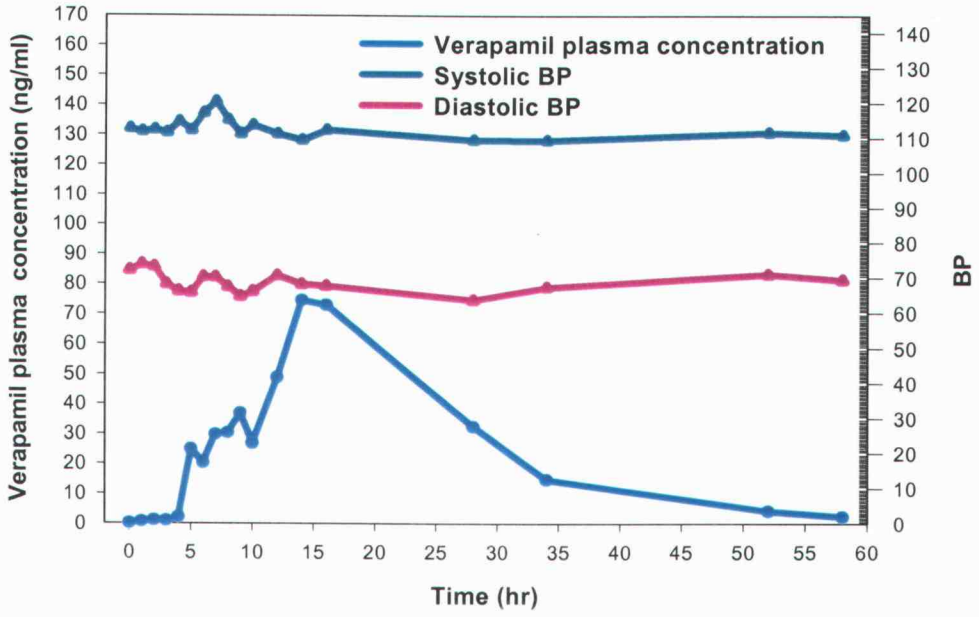


Figure 2.23: Mean verapamil plasma concentration and blood pressure (BP) versus time curves for tested formulation IV in the fed state (n=8).

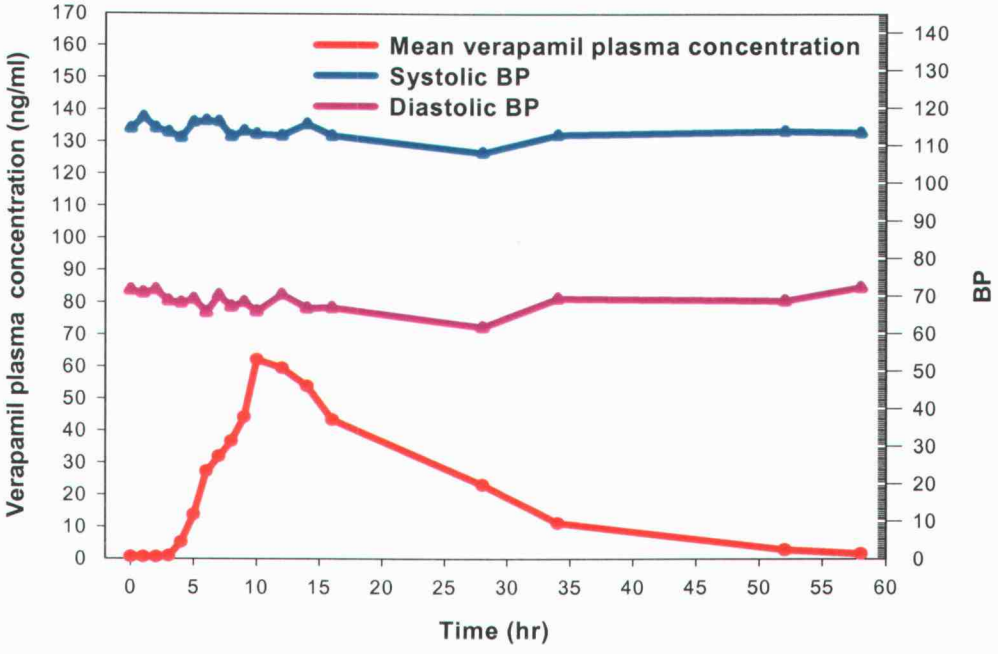


Figure 2.24: Mean verapamil plasma concentration and blood pressure (BP) versus time curves for Covera HS® in the fed state (n=8).

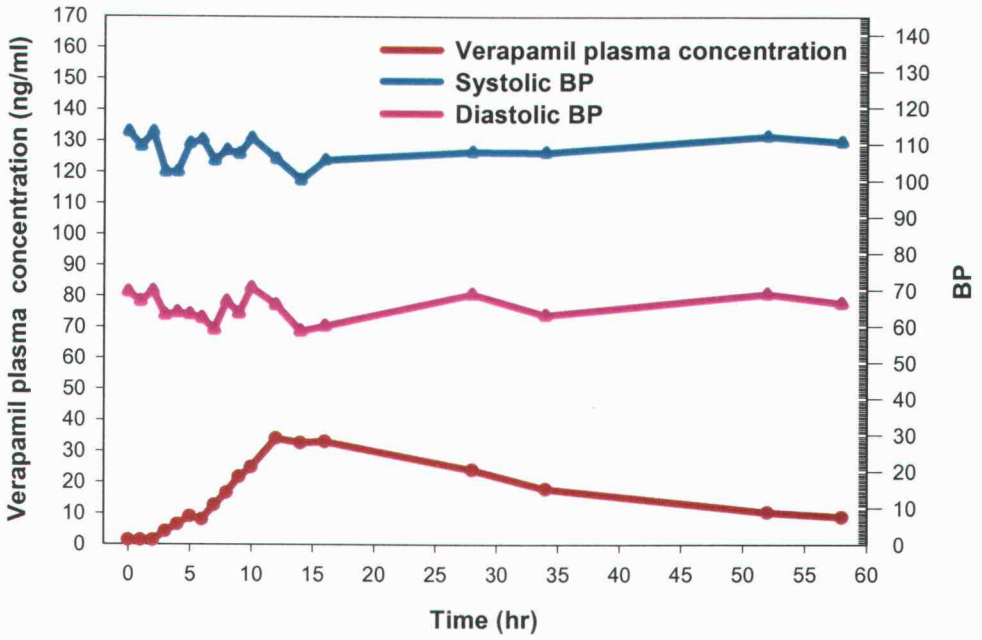


Figure 2.25: Mean verapamil plasma concentration and blood pressure (BP) versus time curves for tested formulation IV in the fasted state (n=7).

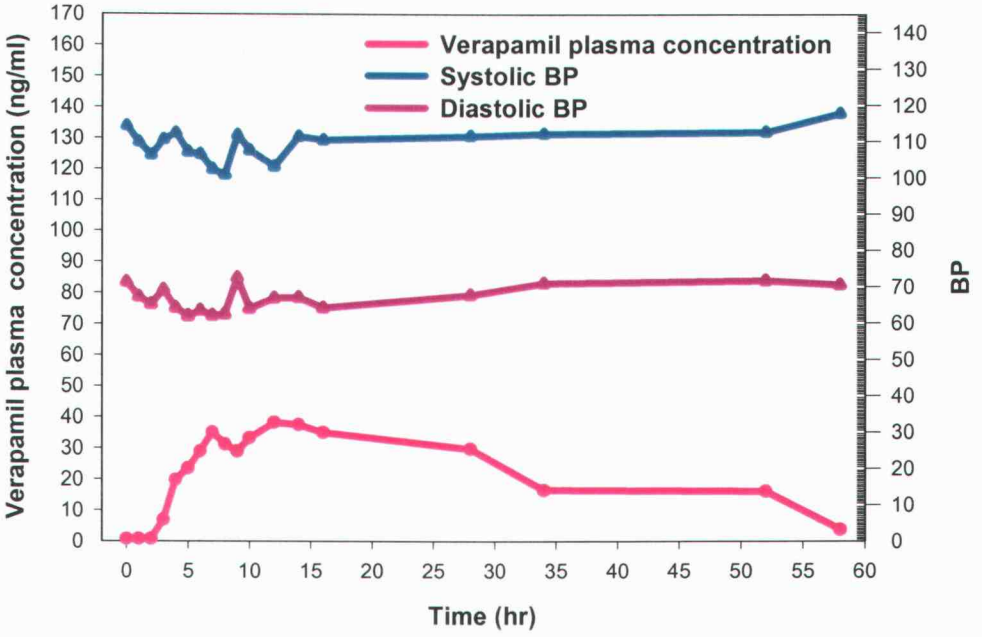


Figure 2.26: Mean verapamil plasma concentration and blood pressure (BP) versus time curves for Covera HS® in the fasted state (n=7).

CONCLUSIONS

Pharmacokinetic measures of AUC_{0-58}, $AUC_{0-\infty}$, and C_{max} for the new formulation (formula IV) were not within the generally accepted guidelines of 0.8 to 1.25 in either fed or fasting states. The new formulation showed higher bioavailability in the fed state and lower bioavailability in the fasted state compared to Covera HS®. The data were quite variable which was attributed to inter-subject variability characteristic of verapamil.

In the fed study, test /reference ratios for the PK parameters for verapamil were 1.246 (90% CI 1.017 - 1.52), 1.23 (90% CI 1.018 - 1.524) and 1.3 (90% CI 1.081 - 1.665) for AUC_{0-58}, $AUC_{0-\infty}$ and C_{max}, respectively. In the fasting study the test /reference ratios for the PK parameters were 0.893 (90% CI 0.36 - 1.06), 0.972 (90% CI 0.354 - 1.22) and 0.998 (90% CI 0.49 - 1.49) for AUC_{0-58}, $AUC_{0-\infty}$ and C_{max}, respectively. Due to small sample size and high drug variability it was difficult to demonstrate bioequivalence. Results showed higher bioavailability of verapamil from tested formulation (formula IV) in the fed state than in the fasted state but the difference was statistically insignificant. An *in vitro/in vivo* correlation was established for the tested formulation (IV) and the reference product to help future formulation changes required in the product development process.

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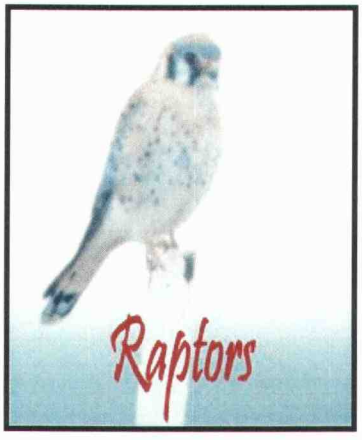
CHAPTER 3

Pharmacokinetics of Terbinafine after Single Oral Doses in Raptors.



Sahar A. Fahmy and J. Mark Christensen

ABSTRACT

The objectives of this study were to determine appropriate oral dose of terbinafine for treatment of aspergillosis in raptors and to determine pharmacokinetics and dose proportionality of terbinafine in raptors following single oral doses of 15, 30, 60 and 120 mg. In the study, ten raptors participated in 15, 30 and 60 mg terbinafine studies with a wash-out period of two weeks between each dose. And, only 6 raptors were in a 120 mg dose study with a 8 weeks wash-out period. Blood samples were taken over 24 hours and analyzed for terbinafine plasma concentrations using HPLC assay. Pharmacokinetic analysis included non-compartmental analysis using WinNonlin® program (Pharsight Version 3.2). Means and standard deviations of peak terbinafine plasma concentration, C_{max}, time to reach C_{max} (T_{max}), area under plasma concentration versus time curve from time zero to time t (AUC_{0-t}), apparent elimination rate constant (Lambda_z) following the administration of 15, 30, 60 and 120 mg doses were, 0.31 ± 0.24, 1.21 ± 0.4, 2.02 ± 0.75 and 4.1 ± 1.9 mg/l, 5.4 ± 2.98, 3.4 ± 0.96, 5.1 ± 3.5 and 3.7 ± 0.8 hours, 3.8 ± 2.2, 11.5 ± 3.4, 24.8 ± 11.3 and 37.9 ± 17.1 hr.mg/l, 0.047 ± 0.02, 0.033 ± 0.01, 0.058 ± 0.02 and 0.035 ± 0.01 hr^{-1}, respectively. Drug accumulation was demonstrated after oral dose administration which was confirmed by presence of drug in the first plasma sample of the following dose, and also a decreasing volume of distribution (Vd/F) following administration of doses with two weeks wash-out

period as follows: 76.83 ± 38.06 L for 15 mg dose which was administered first, 55.2 ± 17.4 L for 30 mg dose which was administered second, and 42.38 ± 25.4 L for 60 mg dose which was administered last. This indicates long-lasting retention of terbinafine in the deep tissues in raptors because it is a lipophilic and the two week wash-out period was not enough time to completely eliminate drug from the body. A long third elimination rate constant was demonstrated for 15 and 30 mg dose studies and was estimated to be 162.27 ± 78.23 and 147.1 ± 65.6 hours for 15 and 30 mg dose, respectively. Approximate linearity of terbinafine pharmacokinetics was demonstrated for AUC_{0-t} in the dose ranges of 15 - 120 mg after eliminating the effect of previous dose on subsequent plasma concentrations while non-linearity for C_{max} in the same dose range was demonstrated using the power model. Calculation of steady state trough terbinafine plasma concentration after administration of daily doses of 15 or 30 mg /day showed that 30 mg daily dose of terbinafine administered orally in raptors produces a steady state trough terbinafine plasma concentration above the minimum inhibitory concentration (MIC) of (0.8-1.6) μg/ml against *aspregillus fumigatus*. From the data, 30 mg per day oral dose of terbinafine should be the recommended dose for treatment of aspergillosis in raptors.

INTRODUCTION

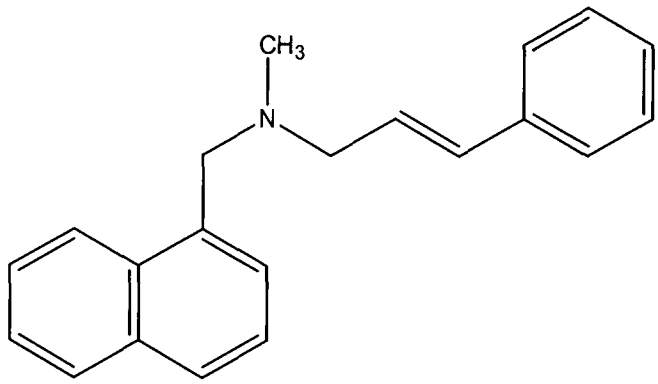
Aspergillosis is the most commonly occurring avian mycotic infection. The disease occurs as a result of inhalation of spores, affecting the respiratory system, and typically causes chronic, debilitating disease and mortality. This fungal infection may occur in individuals of any avian species, especially those held in captivity including raptors, goshawks (*Accipter gentiles*), gyrfalcons (*Falco rusticolus*), immature red-tailed hawks (*Buteo jamaicensis*), golden eagles (*Aquila chrysaetos*), and snowy owls (*Nyctea scandiaca*). These species seem more likely to develop the disease. Several forms of the disease are recognized: an acute form, which occurs due to exposure to a large number of spores. Acute Aspergillosis, also known as brooder pneumonia, had a rapid onset and may be characterized by anorexia, dyspnea, or sudden death. Chronic forms of the disease include focal lesions in the lung, pericardium, trachea, or syrinx associated with host immuno-supression. The birds suffer from dyspnea, lethargy, or depression (1).

Aspergillus fumigatus is the most common species causing aspergillosis (about 95%) followed by *Aspergillus flavus*, then other species such as *Aspergillus niger*, *terreus*, and *nidulans* (2). A variety of drugs have been used for the treatment of aspergillosis. The most commonly used agents are itraconazole and amphotericin B.

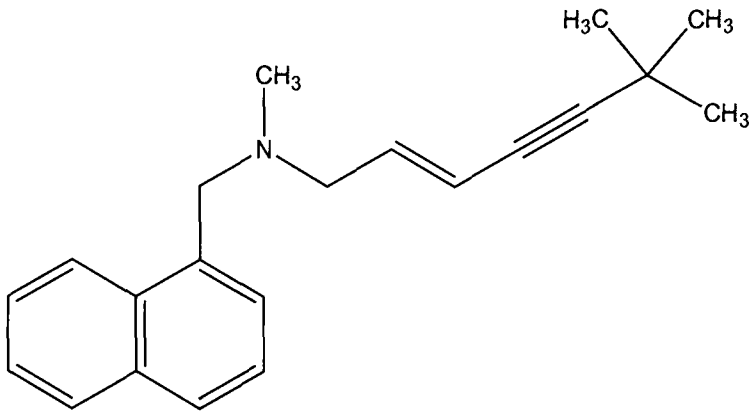
Amphotericin B remains the gold standard among anti-fungal agents for the treatment of aspergillosis. Combinations of amphotericin B with other anti-fungal agents are effective in the treatment of known cases of Aspergillosis. Amphotericin B (available in the form of intravenous, topical and liposomal formulation) is limited in its use by associated adverse events, which may require discontinuation of treatment. Liposomal formulation is less toxic but very costly.

Itraconazole is the only anti-fungal drug that is available in a tablet form and it has associated problems of inconsistent absorption, which is affected by food ingestion, nausea, and abdominal pain. Both amphotericin B and itraconazole have been associated with drug interaction (2).

The increase of aspregillar infection and ineffective treatment as well as adverse events associated with currently used drugs require introduction of new classes of anti-fungal agents to be used alone or in combination with other anti-fungal agents for treatment of aspergillosis. Terbinafine (Lamisil®, Novartis, East Hanover, NJ) belongs to a new class of anti-fungal agents, the allyl-amine derivatives, which have been developed by modification of naftifine, a topical antimyotic. Naftifine provided the starting point for synthesis of other compounds to develop more potent, orally active anti-fungal agents (3). Chemical structures of terbinafine and its parent compound niftifine are shown in Figure 3.01.



Naftifine



Terbinafine

Figure 3.01: Structure of parent compound naftifine and terbinafine.

Terbinafine is active against a wide range of fungi *in vitro*. Table 3.01 shows spectrum of activity of terbinafine along with minimum inhibitory concentration for each species tested *in vitro*. Terbinafine exerts fungicidal action with higher activity against dermatophytes indicating a high specificity towards these organisms.

Table 3.01: Spectrum and minimum inhibitory concentration (MIC) of anti-fungal activity of terbinafine (3).

Species	MIC (μg/ml)
Trichophyton spp.	0.0015-0.006
Epidermophyton floccosum	0.0015-0.006
Microsporum canis	0.006-0.01
Aspregillus spp.	0.02-1.56
Sporothrix schenckii	0.1-0.4
Candida albicans	6.25-100
Candida spp.	0.1 to > 100

Terbinafine exhibits a novel mode of action by inhibiting ergosterol biosynthesis at the point of squalene epoxidation with greater potency than naftifine, which has a similar mechanism of action (4). Terbinafine's mode of action is selective to the fungal cell without affecting cholesterol biosynthesis in mammalian cells, which involves squalene oxidation. A study done in rat liver cells showed that mammalian epoxidase is 3 - 4 orders of magnitude less sensitive to terbinafine than is the fungal enzyme. Terbinafine's mechanism of action is different from that of azole compounds, which inhibit lanosterol 14 α-demethylation process, which is a cytochrome P450 mediated step (5). This gives another advantage for the use of terbinafine as less significant interference with metabolism of other drugs may occur compared to itraconazole (6). Comparison between some anti-fungal agents based on their *in vitro* minimum inhibitory concentration is shown in Table 3.02 (7).

Table 3.02: Minimum inhibitory concentration (MIC_{50}) of terbinafine obtained against *Aspregillus fumigatus* compared to standard anti-mycotic agents (7).

Compound	MIC_{50}, μg/ml (range)
Terbinafine	0.8 (0.05-1.56)
Ketoconazole	17.6 (0.8-25)
Amphotericin B	1.56 (0.8-1.56)
Econazole	0.28 (0.1-0.8)
Miconazole	1.56 (0.4-3.13)
Clotrimazole	1.11 (0.1-1.56)

An *in vitro* study was done to compare minimum inhibitory concentration, MIC, of terbinafine and amphotericin B against three *Aspregillus* species. The study showed that amphotericin B was active against *A. fumigatus* at one-forth the concentration of terbinafine. However, MIC_{90} of terbinafine never exceed 1.6 μg/ml. Terbinafine was active against *A. flavus* and *A. niger* at one-forth the concentration of amphotericin B as shown in Table 3.03 (8).

Table 3.03: *In vitro* activity of terbinafine and amphotericin B against *Aspregillus* species (8).

Species	Terbinafine*	Amphotericin B*
A. fumigatus	0.8 - 1.6	0.2 - 0.4
A. flavus	0.025 - 0.4	0.8 - 1.6
A. niger	0.025 - 0.4	0.4 - 0.8

* MIC in μg/ml.

Terbinafine demonstrated a higher activity against *A. niger* and *A. flavus* which is consistent with other *in vitro* studies which reported terbinafine inhibitory antimycotic activity occurs at lower concentrations than amphotericin B (9). Terbinafine is a promising new drug which should be tested in animal models for the treatment of pulmonary aspergillosis.

Several pharmacokinetic studies have been conducted to determine absorption, distribution, metabolism and elimination of terbinafine after oral administration in humans and some animal species such as dogs, rats and rabbits. In animal species, terbinafine is well absorbed with > 60% in rats, > 85% in mice, and > 46% in dogs. Because terbinafine is a lipophilic drug, it is highly distributed in fat tissues and binds highly to plasma proteins (about 99%) in dog and rabbit (10).

Terbinafine is extensively metabolized by similar metabolic pathways in all tested animal species including human. All metabolites observed lack the antifungal activity of the parent compound.

Pharmacokinetic studies conducted in human showed that terbinafine is well absorbed with > 70% bioavailability after an oral dose of 250 mg. Maximum drug concentrations of 0.8 - 1.5 μg /ml are reached 2 hours after drug administration. Terbinafine plasma concentration time profile in human was biphasic or triphasic, a very rapid distribution phase with distribution half life of 1.5 hours, an initial elimination phase with elimination half life of 22 hours, and a slower elimination phase could be detected, when radio-labeled drug was used, with elimination half life of 99 hours after single oral dose of 250 mg in men. The slow elimination of terbinafine may be explained by strong drug lipophilicity causing high distribution of drug to skin and adipose tissue which slowly releases drug back into the central compartment. Volume of distribution was greater than 2000 L. The total plasma clearance was 1250 ml/min, with ~ 80% of the drug excreted as metabolites in urine (10).

In multiple dose studies conducted in humans, terbinafine elimination was demonstrated to be multiphasic, being initially fast followed by slow elimination phases with mean terminal elimination half lives estimated to be ranging from 165 ± 49 hours (11), 293 ± 165 hours (12), 16.5 ± 2.8 days (13) and 3 weeks (14-15). The slowest elimination of terbinafine was observed from the dermis-epidermis and from keratinic tissues such as hair and nails after administration of 250 mg dose in

multiple dose studies in human (13, 15). Long-term studies following terbinafine in human demonstrate that tissues involved in the slow terminal elimination phase contribute a relatively small portion to the total body exposure. Compartmental analysis of terbinafine data obtained from a multiple dose study in human was performed and the data fit well with a three compartment open model with zero order input and first order output from the central compartment (14).

Effective terbinafine therapy requires adequate concentration of drug at the target organs, which should be above the minimum inhibitory concentration. Dosage schedules in avian species have been largely derived by using doses recommended for mammalian species. Prediction of the efficacy of these dosage schedules has not been possible owing to the failure of most avian species to respond to attempts at treatment of cases of aspergillosis. Therefore, knowledge of pharmacokinetics of the drug in the targeted species is very important in determining the most appropriate dose to treat a disease.

The objectives of this study are: a) to determine an appropriate dose for terbinafine that yield sufficiently high plasma concentrations to inhibit the growth of *A. fumigatus* in avian species, b) Investigate dose dependency of the pharmacokinetic parameters of terbinafine following single oral doses of 15, 30, 60 and 120 mg in raptors.

STUDY PROTOCOL

Ten healthy raptors housed in Seneca Park Zoo (222 Saint Paul Str., Rochester, NY, 14621, USA) were used in the study. Terbinafine dose was given orally to ten birds for the 15, 30 and 60 mg dose studies and to 6 birds for the 120 mg dose study. The 15, 30 and 60 mg oral doses were administered in the same birds with a wash-out period of 2 weeks while 120 mg dose study was conducted in 6 birds with wash-out period of 8 weeks. Blood samples were collected at –5, 15, 30, 45 minutes 1, 2, 4, 10, 12, and 24 hours post-administration. Plasma samples were stored at –4 °C until time of analysis. Terbinafine was determined in plasma samples using a HPLC method. Sample analysis was conducted in School of Veterinary Medicine, Polton Center, University of Pennsylvania Kentte Square, Pennsylvania, USA.

Determination of terbinafine plasma concentrations

Determination of terbinafine in plasma was performed using reversed phase HPLC following on-line solid phase extraction on a C_2 pre-column (16). Plasma samples were thawed at room temperature, vortex mixed for 10 seconds and then

centrifuged for 5 minutes at 1000 g. In polystyrene tubes, 0.75 ml of plasma was transferred with the addition of 50 μl of IW 85-190 hydrochloride as internal standard, 25 μl H_3PO_4 (85%) and 0.75 ml of an ethanol-2-propanol mixture (75:25 v/v). Samples were vortex mixed for 10 seconds then chilled on crushed ice for 30 minutes. Samples were centrifuged again for 15 minutes at 1000 g. 0.4 ml of the supernatant was transferred to a glass vial, then 0.4 ml of 0.01 M phosphate buffer (pH =5) was added and vortex mixed for 10 seconds. 250 μl was injected onto the pre-column for extraction. Terbinafine adsorbed on the pre-column, and then the column was washed with 20 mM KH_2PO_4 and 0.25% triethylamine (pH adjusted to 3.8). Terbinafine was transferred to the analytical column for separation by mobile phase which is composed of 55: 45 of acetonitrile and 20 mM KH_2PO_4, 0.125% triethylamine in water (pH adjusted to 3.8), respectively. Terbinafine retention time was 36 minutes and was detected at 244 nm wavelength using UV detector.

Pharmacokinetic analysis

Plasma terbinafine concentrations time profiles were analysed by utilizing non-compartmental approaches using WinNonlin® (Pharsight, Version 3.2). Maximum drug concentration, C_{max}, and time to reach maximum drug concentration, T_{max}, were determined directly from the data. Area under the curve,

AUC, was calculated using linear trapezoidal rule method. Total body clearance (CL_T /F) was calculated according to

$$CL_T / F = \frac{Dose}{AUC}$$

Mean residence time (MRT) is calculated according to

$$MRT = \frac{AUMC}{AUC}$$ where AUMC is the area under the first momentum curve which was also determined using the linear trapezoidal rule.

Terminal elimination half-life was calculated according to

$$t_{1/2} = \frac{0.693}{\lambda}$$ where λ is the elimination rate constant.

For 15 mg, 30 mg, and 60 mg dose studies it was noticed that the wash-out period of two weeks was not enough duration to completely eliminate drug from the body in all raptors. Terbinafine plasma profiles were corrected by subtracting the concentration carried over from the previous dose (C') from the measured concentration at each sampling points (C_m) (17):

$$C_{corrected} = C_m - C' \quad \text{where,} \quad C' = C_o e^{-\lambda_z t}$$

$$\lambda_z = \frac{\ln C_{24(lastdose)} - \ln C_{o(nextdose)}}{\Delta t}$$

λ_z is the elimination rate constant from the previous dose.

Assuming terbinafine was administered on a once daily dose basis after administration of 15 and 30 mg doses, terbinafine accumulation can be calculated according to the following equation (18):

$$\text{Accumulation factor} = \frac{1}{1 - e^{-kT}}$$

Where, k is the elimination rate constant, and T is the dosing interval.

Due to limited sampling time points in the terminal elimination phase, compartmental analysis was not conducted. To obtain best estimates of the absorption rate constant, observed rapid distribution rate constant, and slower elimination rate constant, a stripping method was done for individual data. This is a commonly used technique to solve a curve into its exponential components (19). The equation selected to describe terbinafine plasma profiles from time zero to 24 hours is a two compartment open model equation:

$$C = Ne^{-K_a t} + Le^{-\alpha t} + Me^{-\beta t}$$

where, K_a is the absorption rate constant, α is the first elimination rate constant, β is the second elimination rate constant, and L, M and N are coefficients.

Statistical analysis

Dose proportionality of terbinafine was determined AUC_{0-t} and C_{max} with respect to the AUC_{0-t} value and C_{max} value of the lowest dose (15 mg) according to the following equations:

$$R = \frac{AUC_{15,30,60or120mg}}{AUC_{15mg}} \quad \text{and} \quad R = \frac{C\max_{15,30,60or120mg}}{C\max_{15mg}}$$

where R is the dose proportionality ratio. If dose proportionality is linear, ratios for the four doses should not be statistically different from 1: 2: 4: 8. The linearity was tested using a lack of fit F-test (20). In addition, a power function relationship was used to describe the relationship between AUC_{0-t}, C_{max} and dose.

$$AUC = a(Dose)^b \quad \text{and} \quad C\max = a(Dose)^b$$

where a represents the coefficient, and b represents the exponent of the power function determined by regression. If the AUC_{0-t} dose relationship is linear, then the exponent b should be equal to unity. Linearity was indicated if 95% confidence interval for the exponent b included the value of 1.0 (21).

Following oral administration of terbinafine at four different doses, statistical comparisons of mean plasma concentrations at each sampling time and estimates of the pharmacokinetic parameters among the four doses were made using ANOVA. Statistical software SAS™ (SAS Institute, Cary, NC) was used in the analysis.

RESULTS AND DISCUSSION

Ten raptors were enrolled in the study and finished three treatments (15, 30 and 60 mg doses) and six birds were enrolled in 120 mg dose study. Mean and individual terbinafine plasma concentrations data after administration of 15, 30, 60 and 120 mg doses are shown in Tables 3.04 (a-d).

Mean and individual terbinafine plasma concentration time profiles after administration of 15, 30, 60 and 120 mg doses are shown in Figures 3.02, 3.03, 3.04, 3.05 and 3.06, respectively. A correction was performed to obtain more accurate terbinafine concentrations for 30 mg and 60 mg dose studies to eliminate the effect from previous dose. Elimination rate constant used in the calculation was determined using the last sampling point from the first dose and the first sampling point from the second dose which resulted in a very small third elimination rate constant. This third elimination constant was calculated along with elimination half-lives for 15 mg and 30 mg doses only (Tables 3.04b and 3.04c, respectively). 120 mg dose study was conducted with a longer wash-out period as indicated by no drug being present in the first sample at zero time in all subjects.

Mean pharmacokinetic parameters following administration of 15, 30, 60 and 120 mg doses are shown in Table 3.05a and individual pharmacokinetic parameters of raptors after administration of terbinafine oral doses of 15, 30, 60, and 120 mg doses are shown in Tables 3.05b, 3.05c 3.05d, and 3.05e, respectively.

Means and standard deviations of peak terbinafine plasma concentration, C_{max}, time to reach peak plasma concentration, T_{max}, area under plasma concentration versus time curve from time zero to time t, AUC_{0-t}, apparent elimination rate constant and mean residence time following the administration of 15, 30, 60 and 120 mg doses were, 0.31 ± 0.24, 1.21 ± 0.4, 2.02 ± 0.75 and 4.1 ± 1.9 mg/l, 5.4 ± 2.98, 3.4 ± 0.96, 5.1 ± 3.5 and 3.7 ± 0.8 hours, 3.8 ±2.2, 11.5 ± 3.4, 24.8 ± 11.3 and 37.9 ± 17.1 hr.mg/l, 0.047 ± 0.02, 0.033 ± 0.01, 0.058 ± 0.02 and 0.035 ± 0.01 hr^{-1} and 10.26 ± 1.38, 8.6 ± 1.4, 9.6 ± 0.75 and 8.5 ± 1.4, respectively.

Earlier studies of pharmacokinetic parameters of terbinafine in humans showed that it is a highly lipophilic drug, and highly distributed in skin and adipose tissues with a very long elimination half life of up to 3 weeks (11-15). Two weeks wash-out period in the current study was not enough to completely eliminate drug from the body and deep tissues as shown by a decreasing volume of distribution (Vd) as the study progressed from dose to dose. A Vd of 76.83 ± 38.06 L for the 15 mg dose was observed for the first dose administered to raptors and then Vd declined to 55.2 ± 17.4 L for 30 mg dose administered 2 weeks after the 15 mg dose, and finally declined to 42.38 ± 25.4 L for 60 mg dose which was administered last. Volume of distribution of 120 mg dose (67.8 ± 22.5 L) was near the value of the 15 mg dose as the 120 mg dose had enough wash-out period before being dosed.

Two elimination phases were observed from plasma concentration time data. The first elimination phase was rapid followed by a slower elimination phase.

These two phases are more pronounced in the 120 mg oral dose plasma terbinafine profiles in all subjects. This is comparable to terbinafine distribution and elimination in human (10).

Accumulation of terbinafine was calculated based on 15 and 30 mg daily dose in raptors using the third elimination rate constants and 24 hour dosing interval. The calculation gave a steady state trough terbinafine plasma concentration of 1.10 and 2.12 μg/ml for 15 and 30 mg daily doses, respectively. The calculated steady state trough terbinafine plasma concentration for 30 mg daily dose was above the MIC against *A. fumagatus* which suggests that a 30 mg daily dose should be the recommended daily for treatment of aspergillosis in raptors.

Due to the short length of the sampling time and short wash-out period between doses, definitive pharmacokinetic analysis could not be performed. Several attempts were made to fit the data with compartmental modeling. Fitting the data to a one compartment open model yielded a poor fit. Data fitting to a two-compartment open model gave erratic results as there are too few data points in the two elimination phases.

Following oral administration of terbinafine at four different doses, there were no significant differences between mean plasma concentrations at each sampling time and the estimated pharmacokinetic parameters (C_{max} and AUC_{0-t}) among the four doses after dose normalization to the 15 mg dose.

Table 3.04a: Mean and individual terbinafine plasma concentrations (μg/ml) after administration of 15 mg oral dose in raptor (n=10).

Time (hrs)	0	0.5	1	2	4	10	12	24
dark	0.00	0.00	0.00	0.259	0.846	0.425	0.272	0.124
green	0.00	0.00	0.00	0.256	0.505	0.133	0.127	0.111
gw	0.00	0.00	0.00	0.058	0.088	0.101	0.099	0.061
mag	0.00	0.011	0.116	0.281	0.506	0.366	0.206	0.124
no	0.00	0.079	0.09	0.093	0.234	0.139	0.13	0.08
pur	0.00	0.051	0.082	0.306	0.36	0.331	0.227	0.098
red	0.00	0.00	0.027	0.045	0.078	0.08	0.083	0.051
ry	0.00	0.031	0.171	0.158	0.242	0.106	0.073	0.045
silver	0.00	0.00	0.022	0.038	0.107	0.096	0.07	0.043
yg	0.00	0.00	0.00	0.01	0.163	0.16	0.143	0.10
Mean	**0.00**	**0.017**	**0.051**	**0.151**	**0.313**	**0.194**	**0.143**	**0.084**
SD	0.00	0.028	0.06	0.115	0.245	0.128	0.069	0.031

Table 3.04b: Mean and individual terbinafine plasma concentrations (μg/ml) after administration of 30 mg oral dose in raptor (n=10).

Time (hrs)	0	0.5	1	2	4	10	12	24
dark	0.00	0.346	0.849	1.325	1.319	0.3786	0.430	0.229
green	0.00	0.091	0.093	0.610	0.570	0.441	0.312	0.222
gw	0.00	0.035	0.048	0.844	1.385	0.210	0.179	0.115
mag	0.00	0.254	0.566	0.646	1.072	0.729	0.555	0.387
no	0.00	0.049	0.093	0.349	1.036	0.272	0.326	0.239
pur	0.00	0.073	0.099	0.127	1.382	0.267	0.183	0.155
red	0.00	1.445	1.812	1.886	1.604	0.785	0.342	0.295
ry	0.00	1.319	0.154	0.729	1.650	0.266	0.191	0.153
silver	0.00	0.00	0.00	0.032	0.774	0.294	0.286	0.217
yg	0.00	0.051	0.106	0.818	1.004	0.667	0.337	0.240
Mean	**0.00**	**0.249**	**0.372**	**0.737**	**1.179**	**0.431**	**0.314**	**0.226**
SD	0.00	0.433	0.576	0.550	0.350	0.215	0.117	0.077

Table 3.04c: Mean and individual terbinafine plasma concentrations (μg/ml) after administration of 60 mg oral dose in raptor (n=10).

Time (hrs)	0	0.5	1	2	4	10	12	24
dark	0.00	0.208	0.644	1.102	2.073	1.005	1.512	0.489
green	0.00	0.475	0.879	0.837	0.727	0.682	0.644	0.232
gw	0.00	0.001	0.407	1.415	1.096	20.76	0.207	0.148
mag	0.00	0.001	0.017	0.495	1.021	1.714	0.681	0.301
no	0.00	0.00	0.00	0.134	2.011	0.871	0.883	0.598
rg	0.00	0.367	1.230	2.350	1.700	0.620	-	0.497
red	0.00	0.089	0.867	3.084	1.995	2.947	-	0.8315
ry	0.00	0.00	0.735	2.349	3.280	2.261	1.381	0.814
silver	0.00	0.00	0.00	1.040	1.230	0.678	0.738	0.282
yg	0.00	0.00	0.188	0.835	1.381	1.503	0.974	0.473
Mean	**0.00**	**0.114**	**0.498**	**1.364**	**1.651**	**1.435**	**0.878**	**0.467**
SD	0.00	0.176	0.439	0.937	0.737	0.801	0.419	0.233

Table 3.04d: Mean and individual terbinafine plasma concentrations (μg/ml) after administration of 120 mg oral dose in raptor (n=6).

Time (hrs)	0	0.5	1	2	4	10	12	24
dark	0.00	0.00	3.00	5.40	5.46	1.40	1.12	0.88
rg	0.00	0.00	1.18	5.46	7.42	2.35	1.53	1.20
mag	0.00	0.73	2.32	3.99	3.11	0.61	0.48	0.37
no	0.00	0.00	0.10	1.52	3.07	1.42	1.15	0.91
lav	0.00	0.00	0.46	0.97	2.26	1.41	1.13	0.55
silver	0.00	0.00	0.00	0.07	2.61	0.86	0.59	0.44
Mean	**0.00**	**0.121**	**1.176**	**2.901**	**3.988**	**1.341**	**1.00**	**0.725**
SD	0.00	0.298	1.24	2.35	2.022	0.599	0.393	0.323

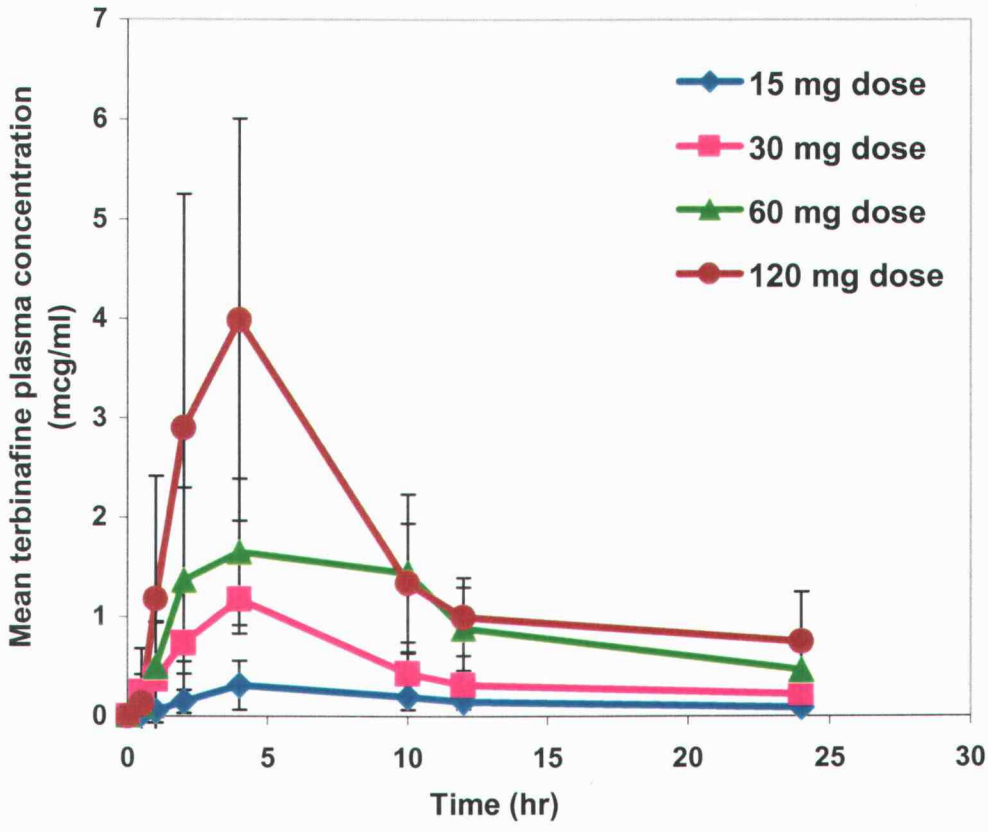


Figure 3.02: Mean terbinafine plasma concentrations after administration of oral doses of 15, 30, 60 and 120 mg in raptors (n= 10, 10, 10 and 6, respectively, error bar represents standard deviation).

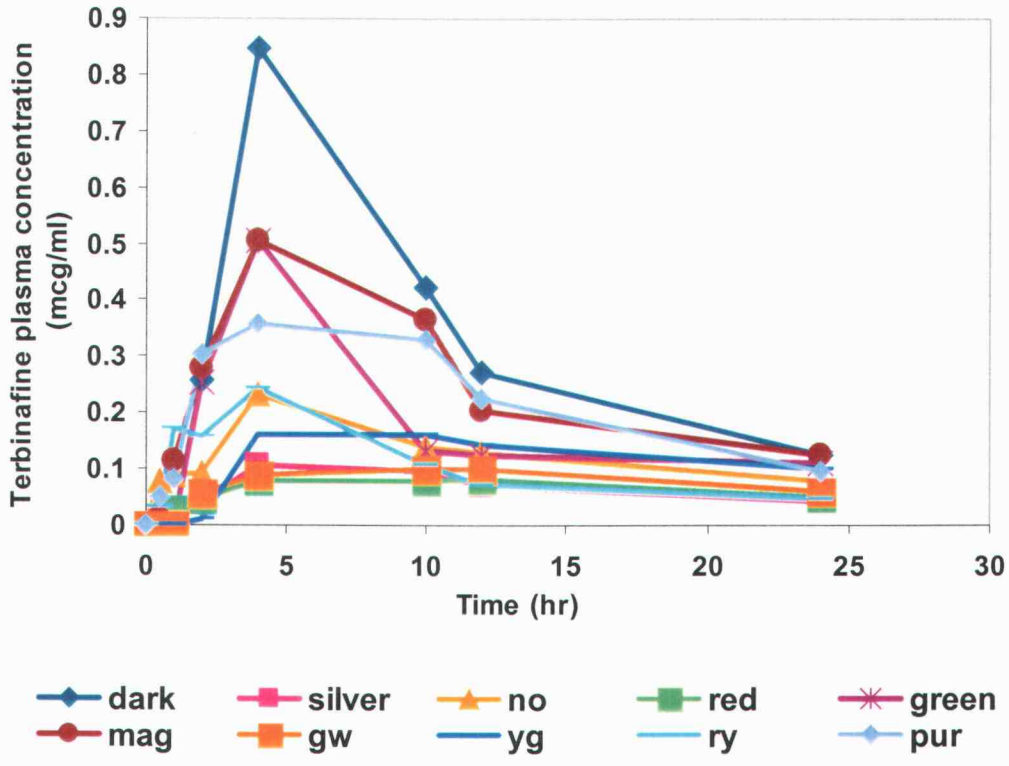


Figure 3.03: Terbinafine plasma concentrations after administration of 15 mg oral dose in raptors (n= 10).

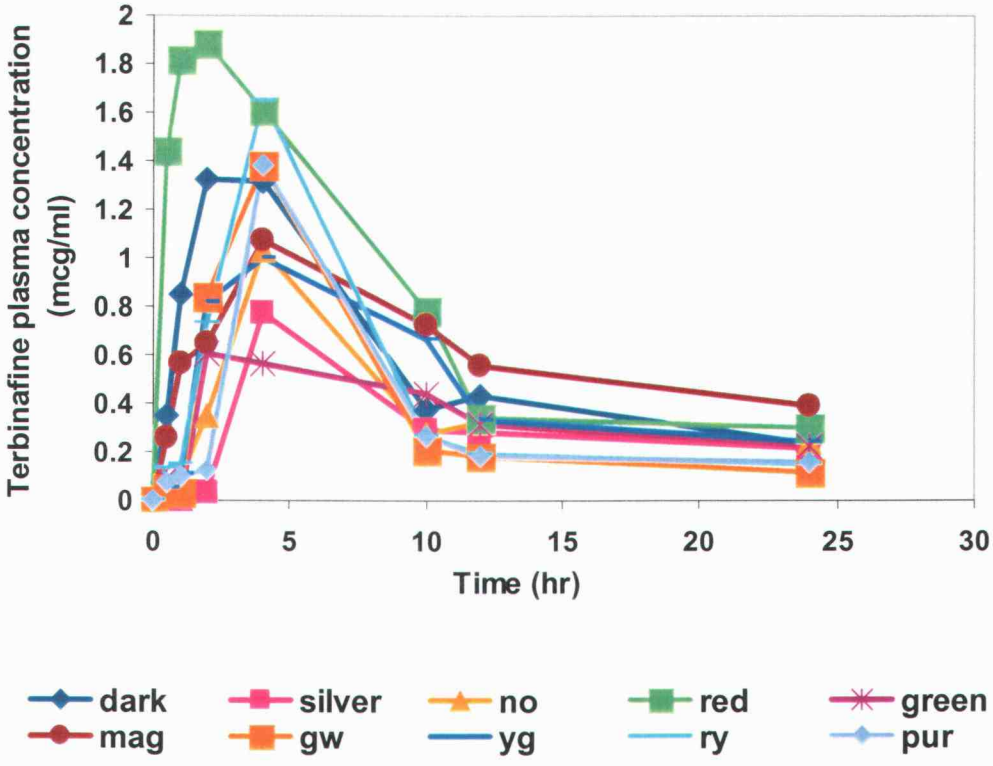


Figure 3.04: Terbinafine plasma concentrations after administration of 30 mg oral dose in raptors (n= 10).

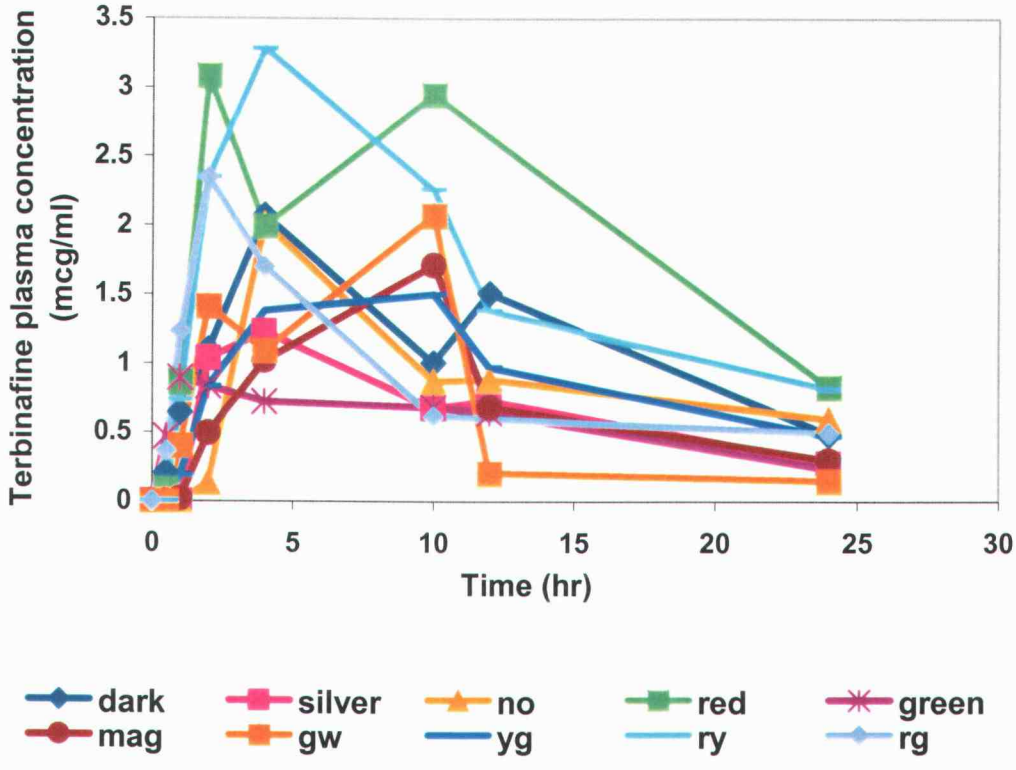


Figure 3.05: Terbinafine plasma concentrations after administration of 60 mg oral dose in raptors (n= 10).

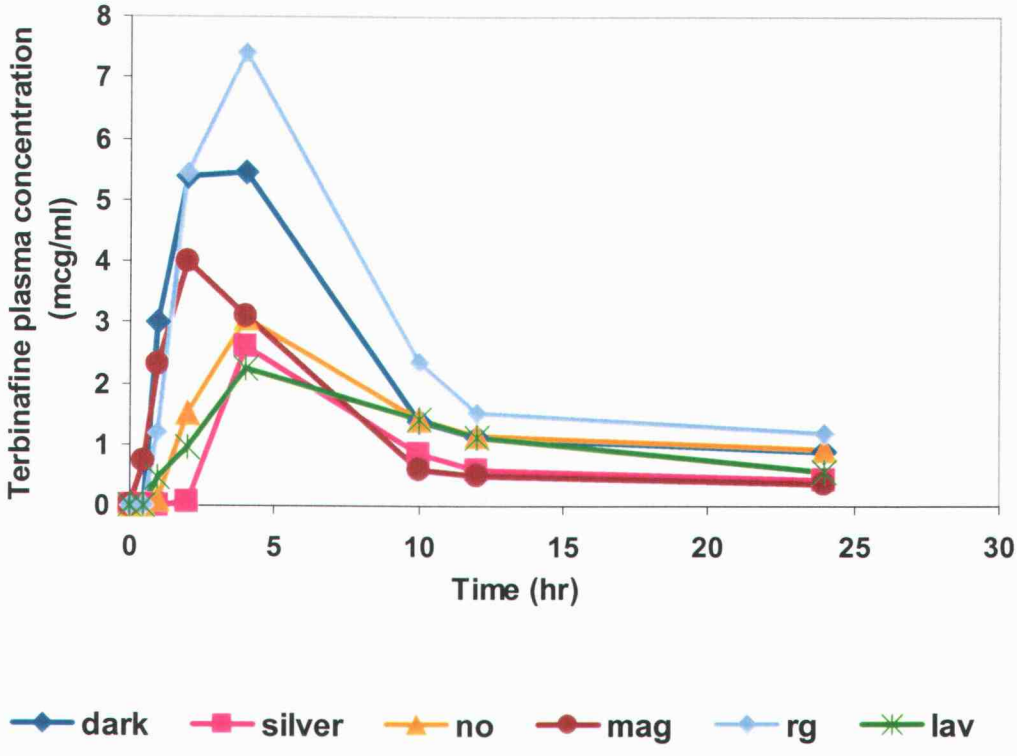


Figure 3.06: Terbinafine plasma concentrations after administration of 120 mg oral dose in raptors (n= 6).

Table 3.05a: Mean terbinafine noncompartmental pharmacokinetic parameters after oral administration of 15, 30, 60 and 120 mg doses in raptors.

	15 mg dose	30 mg dose	60 mg dose	120 mg dose
Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Cmax (μg/ml)	0.31 ± 0.24	1.21 ± 0.4	2.02 ± 0.75	4.1 ± 1.9
Tmax (hr)	5.4 ± 2.98	3.4 ± 0.96	5.1 ± 3.5	3.7 ± 0.8
AUC_{0-t} (hr.mg/l)	3.8 ± 2.2	11.5 ± 3.4	24.8 ± 11.3	37.9 ± 17.1
$AUC_{0-\infty}$ (hr.mg/l)	6.24 ± 3.57	20.27 ± 9.07	35.3 ± 15.39	63.75 ± 35.89
$AUMC_{0-t}$ (hr^2.mg/l)	37.34 ± 18.68	97.6 ± 28.7	239.2 ± 112	315.5 ± 132.3
$AUMC_{0-\infty}$ (hr^2.mg/l)	204.5 ± 280.03	754.3 ± 788.3	847.8 ± 814.7	1935.4 ± 1629.6
Cl/F (l/hr)	3.2 ± 1.69	1.67 ± 0.5	2.03 ± 0.89	2.38 ± 1.1
Cl/F (l/hr)*	2.4 ±1.46	1.48 ± 0.72	1.69 ± 0.75	1.88 ± 1.18
Half life (hr)	19.05 ± 13.7	25.8 ± 14.7	15.9 ± 11.7	22.4 ± 7.9
Half life (hr)*	14.7 ± 6.67	20.8 ± 8.7	11.8 ± 5.03	19.6 ± 9.9
Lambda_z (1/hr)	0.047 ± 0.02	0.033 ± 0.01	0.058 ± 0.02	0.035 ± 0.01
Vz_F (l)	76.83 ± 38.06	55.2 ± 17.4	42.38 ± 25.4	67.8 ± 22.5
MRT (hr)	10.26 ± 1.38	8.6 ± 1.4	9.6 ± 0.75	8.5 ± 1.4

* Harmonic mean

Table 3.05b: Individual terbinafine noncompartmental pharmacokinetic parameters after oral administration of 15 mg dose in raptors (n=10).

Subject	T_{max} (hr)	C_{max} (µg/ml)	AUC_{0-t} (hr.mg/l)	K_{el} (hr^{-1})	$t_{1/2}$ (hr)	Vz (l)	CL (l/hr)	$AUMC_{0-t}$ (hr^2.mg/l)	MRT_{0-t} (hr)	$AUC_{0-\infty}$ (hr.mg/l)	K_{el}* (hr^{-1})	$t_{1/2}$* (hr)
dark	4	0.85	8.12	0.081	8.65	19.4	1.56	72.01	8.9	9.7	0.0078	88.8
green	4	0.505	4.49	0.012	56.24	90.2	1.1	40.8	9.09	13.5	0.0014	495
gw	10	0.101	1.905	0.04	17.5	109.5	4.34	22.8	11.96	2.3	0.0031	223.5
mag	4	0.51	6.19	0.04	16.4	38.9	1.65	58.8	9.5	9.1	0.0041	169.02
no	4	0.23	3.13	0.04	17.5	73.47	2.96	32.15	10.27	4.47	0.0039	177.7
pur	4	0.36	5.5	0.08	8.55	27.7	2.25	53.2	9.7	6.7	0.0043	161.2
red	12	0.08	1.61	0.04	17.5	129.9	5.15	19.01	11.8	2.2	0.0055	126
ry	4	0.242	2.56	0.053	13.03	82.7	4.4	21.4	8.37	2.97	0.0061	113.6
silver	4	0.11	1.64	0.05	13.6	119.08	6.07	17.8	10.88	2.1	0.0012	577.5
yg	4	0.16	2.91	0.03	21.5	77.5	2.5	35.45	12.2	6.01	0.0053	130.7
Mean	5.4	0.31	3.80	0.047	19.05	76.83	3.2	37.34	10.26	6.24	0.0043	226.3
SD	2.98	0.24	2.2	0.02	13.7	38.06	1.69	18.68	1.38	3.58	0.002	168.7
Harm_mean				0.036	14.7		2.4				0.0031	162.27
Pseudo_SD				0.032	6.67		1.46				0.002	78.23

* Third elimination rate extrapolated from the last sampling time point and the first sampling time from the next dose.

Table 3.05c: Individual terbinafine noncompartmental pharmacokinetic parameters after oral administration of 30 mg dose in raptors (n=10).

Subject	T_{max} (hr)	C_{max} (μg/ml)	AUC_{0-t} (hr.mg/l)	K_{el} (hr^{-1})	$t_{1/2}$ (hr)	Vz (l)	CL (l/hr)	$AUMC_{0-t}$ (hr^2.mg/l)	MRT_{0-t} (hr)	$AUC_{0-\infty}$ (hr.mg/l)	K_{el}* (hr^{-1})	$t_{1/2}$* (hr)
dark	2	1.33	13.98	0.042	16.6	36.97	1.53	110.12	7.9	19.5	0.0038	182.4
green	2	0.61	8.55	0.042	16.6	51.86	2.16	87.01	10.2	13.9	0.0057	121.6
gw	4	1.38	9.65	0.041	16.9	58.75	2.41	64.8	6.7	12.45	0.0072	96.2
mag	4	1.07	14.9	0.04	17.4	30.72	1.22	151.27	10.12	24.7	0.007	99
no	4	1.04	9.58	0.015	46.5	78.4	1.17	90.57	9.5	25.7	0.0024	288.7
pur	4	1.38	9.12	0.03	22.9	69.6	2.1	71.12	7.8	14.3	0.0059	117.5
red	2	1.89	18.64	0.012	56.68	57.3	0.7	135.8	7.3	42.8	0.0059	117.5
ry	4	1.65	11.2	0.032	21.6	58.5	1.87	77.6	6.9	16.01	0.0035	198
silver	4	0.77	7.63	0.022	31.05	77.4	1.73	79.6	10.4	17.4	0.001	693
yg	4	1.01	11.8	0.057	12.09	32.67	1.87	108.3	9.2	16.02		
Mean	3.4	1.21	11.5	0.033	25.8	55.21	1.67	97.6	8.6	20.28	0.0047	212.6
SD	0.96	0.39	3.42	0.014	14.69	17.37	0.52	28.4	1.44	9.06	0.002	190.6
Harm_mean				0.027	20.8		1.48				0.0033	147.1
Pseudo_SD				0.016	8.7		0.72				0.0038	65.6

* Third elimination rate extrapolated from the last sampling time point and the first sampling time from the next dose.

Table 3.05d: Individual terbinafine noncompartmental pharmacokinetic parameters after oral administration of 60 mg dose in raptors (n=10).

Subject	T_{max} (hr)	C_{max} (μg/ml)	AUC_{0-t} (hr.mg/l)	K_{el} (hr^{-1})	$t_{1/2}$ (hr)	Vz (l)	CL (l/hr)	$AUMC_{0-t}$ (hr^2.mg/l)	MRT_{0-t} (hr)	$AUC_{0-\infty}$ (hr.mg/l)
dark	4	2.07	28.1	0.066	10.44	25.26	1.67	274.9	9.8	35.5
green	1	0.89	13.72	0.085	8.2	42.9	3.64	129.9	9.5	16.5
Gw	10	2.07	17.46	0.028	25.09	95.06	2.6	143.99	8.2	22.8
Mag	10	1.71	18.28	0.068	10.17	38.78	2.64	187.03	10.2	22.7
no	4	2.01	21.5	0.029	24.1	49.1	1.41	227.8	10.6	42.3
rg	2	2.35	21.11	0.016	43.88	72.25	1.14	180.7	8.6	52.6
red	2	3.08	48.6	0.09	7.67	11.5	1.04	476.4	9.8	57.8
ry	4	3.28	40.8	0.063	11.03	17.76	1.12	383.9	9.4	53.8
silver	4	1.23	16.06	0.068	10.11	43.36	2.97	152.7	9.5	20.19
Yg	10	1.5	22.59	0.075	9.3	27.88	2.08	234.9	10.4	28.9
Mean	5.1	2.02	24.8	0.058	15.99	42.38	2.03	239.2	9.6	35.3
SD	3.5	0.75	11.3	0.025	11.67	25.36	0.89	112.2	0.75	15.4
Harm_mean				0.043	11.78		1.69			
Pseudo_SD				0.036	5.03		0.75			

Table 3.05e: Individual terbinafine noncompartmental pharmacokinetic parameters after oral administration of 120 mg dose in raptors (n=6).

Subject	T_{max} (hr)	C_{max} (μg/ml)	AUC_{0-t} (hr.mg/l)	K_{el} (hr^{-1})	$t_{1/2}$ (hr)	Vz (l)	CL (l/hr)	$AUMC_{0-t}$ (hr^2.mg/l)	MRT_{0-t} (hr)	$AUC_{0-\infty}$ (hr.mg/l)
dark	4	5.46	50.9	0.03	24.23	51.36	1.47	382.6	7.5	81.7
lav	4	2.26	27.69	0.06	10.7	51.23	3.3	269.9	9.7	36.2
mag	2	3.99	28.55	0.03	22.5	96	2.95	181.6	6.4	40.5
no	4	3.07	33.8	0.03	25.2	65.2	1.79	338.2	9.9	66.9
rg	4	7.42	66.1	0.02	34.23	47.3	0.96	531.3	8.04	125.4
silver	4	2.61	20.76	0.04	17.46	94.94	3.76	189.2	9.12	31.8
Mean	3.67	4.14	37.96	0.035	22.39	67.8	2.4	315.5	8.5	63.75
SD	0.82	1.97	17.12	0.016	7.9	22.5	1.12	132.29	1.40	35.9
Harm_mean				0.033	19.6		1.88			
Pseuod_SD				0.031	9.87		1.18			

Curve stripping:

Terbinafine plasma profiles in raptors are similar to in humans in that they are characterized by rapid absorption, followed by a rapid initial elimination phase, and then a slower second elimination phase. To get an estimate of absorption rate constant Ka, first elimination rate constant (α) and second elimination phase constant (β) and associated half lives, curve stripping was performed for individual data sets for all doses administered to raptors.

Curve stripping could not be done for some subjects (especially with 60 mg dose) as the two elimination phases could not be defined from terbinafine plasma profiles. Mean and individual pharmacokinetic parameters obtained by curve stripping are summarized in Tables 3.06 (a-e). Absorption of terbinafine following the four oral doses was rapid with ~ 1 - 1.5 hour absorption half-life. The first elimination phase was rapid with an associated half-life of ~ 2 - 3.6 hours. The second elimination phase is comparatively slow with a half-life of a wider range (16 - 26 hours) following administration of the four doses of terbinafine in raptors.

Table 3.06a: Mean absorption rate, mean first elimination rate and mean second elimination rate constants along with associated mean half-lives after administration of 15, 30, 60 and 120 mg doses in raptors.

	15 mg dose	30 mg dose	60 mg dose	120 mg dose
Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
N	0.86 ± 0.46	5.35 ± 5.4	4.74 ± 3.43	20.69 ± 16.1
K_a (hr^{-1})	0.62 ± 0.21	0.78 ± 0.61	0.63 ± 0.12	0.59 ± 0.21
K_a_HL (hr)	1.26 ± 0.55	1.42 ± 1.11	1.13 ±0.2	1.33 ± 0.49
K_a_HL (hr)*	1.11 ± 0.37	0.88 ± 0.78	1.1 ± 0.21	1.18 ± 0.44
L	0.51 ± 0.23	4.71 ± 5.42	4.83 ± 2.87	19.87 ± 16.4
K_α (hr^{-1})	0.29 ± 0.19	0.37 ± 0.22	0.30 ± 0.17	0.45 ± 0.17
K_α _HL (hr)	3.58 ± 2.9	3.59 ± 3.88	2.94 ± 1.69	1.82 ± 0.93
K_α _HL (hr)*	2.35 ± 1.77	1.87 ± 1.12	2.303 ± 1.49	1.54 ± 0.58
M	0.29 ± 0.25	0.52 ± 0.25	2.32 ± 1.99	1.62 ± 0.58
K_β (hr^{-1})	0.046 ± 0.02	0.033 ± 0.01	0.059 ± 0.03	0.034 ± 0.01
K_β _HL (hr)	19.4 ± 13.3	25.78 ± 14.6	15.99 ± 11.7	22.6 ± 7.8
K_β _HL (hr)*	14.98 ± 7.13	20.82 ± 8.63	11.78 ± 5.03	20.13 ± 9.02

* Harmonic mean

Table 3.06b: Absorption rate, first elimination rate and second elimination rate constants along with associated half-lives after administration of 15 mg dose in raptors.

Name	N	K_a	K_a (HL)	L	K_α	K_α (HL)	M	K_β	K_β (HL)
dark	1.58	0.29	2.41	0.68	0.25	2.71	0.83	0.08	8.64
silver	0.23	0.86	0.80	0.36	0.21	3.37	0.14	0.05	13.56
no	1.01	0.59	1.17	0.84	0.67	1.03	0.21	0.04	17.5
green	1.098	0.49	1.4	0.71	0.17	4.14	0.15	0.01	55
mag	0.42	0.69	1.01	0.29	0.07	9.73	0.34	0.04	16.34
ry	0.65	0.56	1.23	0.49	0.37	1.87	0.16	0.05	12.95
pur	1.04	0.88	0.79	0.22	0.32	2.17	0.67	0.08	8.53
red	-	-	-	-	-	-	0.12	0.03	20.08
gw	-	-	-	-	-	-	0.15	0.04	18.68
yg	-	-	-	-	-	-	0.21	0.03	22.79
Mean	**0.86**	**0.62**	**1.26**	**0.51**	**0.29**	**3.58**	**0.29**	**0.046**	**19.4**
SD	0.46	0.21	0.55	0.24	0.19	2.89	0.25	0.021	13.34

(-): could not be calculated

Table 3.06c: Absorption rate, first elimination rate and second elimination rate constants along with associated half-lives after administration of 30 mg dose in raptors.

Name	N	K_a	K_a (HL)	L	K_α	K_α (HL)	M	K_β	K_β (HL)
dark	6.78	0.776	0.893	5.79	0.05	1.38	0.63	0.041	16.66
silver	5.94	0.48	1.45	5.58	0.64	1.08	0.38	0.023	30.13
no	3.74	0.44	1.57	3.31	0.38	1.79	0.35	0.015	46.2
green	1.317	2.05	0.34	0.085	0.05	13.25	0.59	0.042	16.65
mag	1.46	0.55	1.26	0.53	0.21	3.23	0.99	0.04	17.37
ry	17.67	0.69	1.01	16.98	0.63	1.1	0.32	0.032	21.66
pur	11.03	0.56	1.24	10.75	0.57	1.22	0.31	0.03	22.94
red	2.42	1.73	0.4	2.17	0.16	4.35	0.397	0.012	56.8
gw	1.8	0.4	1.72	1.53	0.45	1.54	0.28	0.04	17.32
yg	1.33	0.16	4.32	0.42	0.1	6.95	0.91	0.057	12.09
Mean	**5.35**	**0.78**	**1.42**	**4.71**	**0.37**	**3.59**	**0.52**	**0.033**	**25.78**
SD	5.35	0.61	1.12	5.42	0.22	3.88	0.25	0.014	14.6

Table 3.06d: Absorption rate, first elimination rate and second elimination rate constants along with associated half-lives after administration of 60 mg dose in raptors.

Name	N	K_a	K_a (HL)	L	K_α	K_α (HL)	M	K_β	K_β (HL)
dark	-	-	-	-	-	-	2.51	0.66	10.44
silver	2.11	0.76	0.92	5.28	0.293	2.365	1.49	0.068	10.12
no	10.57	0.49	1.42	9.41	0.58	1.2	1.2	0.029	24.15
green	-	-	-	-	-	-	1.79	0.085	8.15
mag	-	-	-	-	-	-	1.54	0.068	10.19
ry	4.92	0.59	1.17	4.62	0.276	2.51	3.58	0.063	11.03
rg	3.57	0.76	0.91	2.66	0.24	2.88	0.73	0.016	43.86
red	-	-	-	-	-	-	7.27	0.09	7.67
gw	2.52	0.56	1.24	2.18	0.12	5.75	0.29	0.028	25.1
yg	-	-	-	-	-	-	2.78	0.075	9.24
Mean	**4.74**	**0.63**	**1.13**	**4.83**	**0.30**	**2.94**	**2.32**	**0.059**	**15.99**
SD	3.44	0.12	0.22	2.87	0.17	1.69	1.99	0.026	11.67

(-): could not be calculated

Table 3.06e: Absorption rate, first elimination rate and second elimination rate constants along with associated half-lives after administration of 120 mg dose in raptors.

Name	N	K_a	K_a (HL)	L	K_α	K_α (HL)	M	K_β	K_β (HL)
dark	48.94	0.81	0.86	44.3	0.61	1.14	1.73	0.03	24.23
silver	11.19	0.398	1.74	10.1	0.45	1.53	1.11	0.04	17.46
no	11.70	0.53	1.31	9.33	0.45	1.52	1.74	0.027	25.2
lav	3.45	0.34	2.07	0.94	0.19	3.58	2.32	0.06	11.55
rg	25.6	0.59	1.17	21.2	0.34	2.07	2.09	0.02	34.65
mag	23.26	0.86	0.81	33.34	0.65	1.06	0.76	0.031	22.5
Mean	**20.69**	**0.589**	**1.33**	**19.87**	**0.45**	**1.82**	**1.63**	**0.034**	**22.6**
SD	16.1	0.21	0.5	16.4	0.17	0.94	0.58	0.014	7.78

Terbinafine dose proportionality:

Terbinafine dose proportionality after administration of oral doses of 15, 30, 60 and 120 mg doses was assessed with the power model. Also a lack of fit F-test was performed for deviation from linearity for dose normalized AUC_{0-t} and C_{max}.

Comparisons of individual AUC_{0-t} and C_{max} following administration of four doses of terbinafine along with the power model for the relationship between AUC_{0-t} and C_{max} versus dose are shown in Figures 3.07 and 3.08, respectively.

An approximate linear relationship was observed between AUC_{0-t} and dose which is supported by lack of statistically significant deviation from linearity in the dose normalized AUC_{0-t} (p-value > 0.1). Using the power model, the exponent of dose for AUC [$0.164*Dose^{1.17}$] was not significantly different from unity with 95% confidence interval of this exponent including one (0.95 - 1.39). Whereas the relationship between C_{max} and dose shows nonlinearity using the power model with exponent of dose for C_{max} [$0.0092*Dose^{1.3}$] wherein the 95% confidence interval of this exponent does not include one (1.03 - 1.57). C_{max} may be increasing disproportionately due to saturation of metabolic enzymes at the highest dose (120 mg) in which the value of C_{max} is higher than expected. Also fewer numbers of subjects were involved in the 120 mg dose study with one bird involved was not participant in the other doses studies.

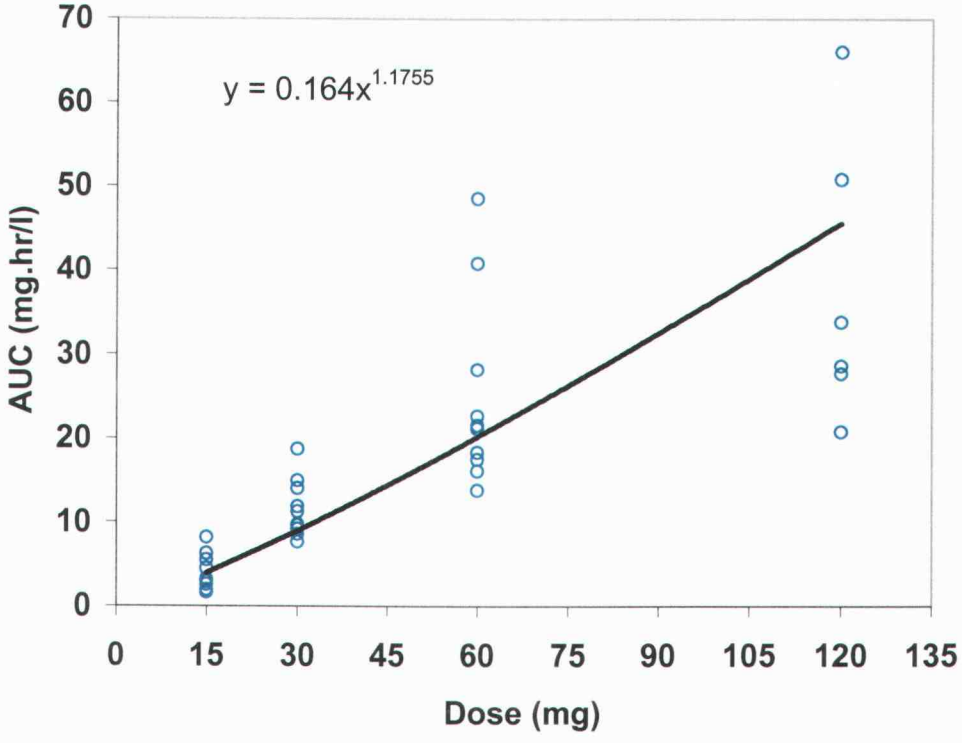


Figure 3.07: Individual terbinafine AUC_{0-t} versus dose following the administration of single oral doses of 15, 30, 60 and 120 mg with fitted power function.

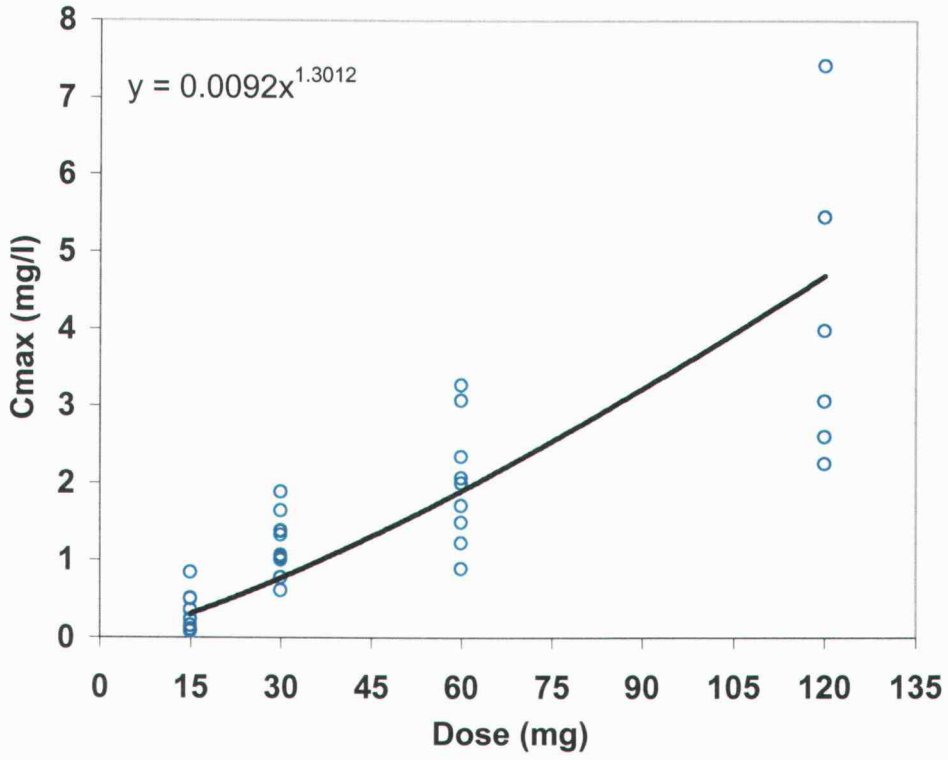


Figure 3.08: Individual terbinafine C_{max} versus dose following the administration of single oral doses of 15, 30, 60 and 120 mg with fitted power function.

CONCLUSIONS

From the data, it is recommended that a 30 mg per day oral dose of terbinafine be used for the treatment of aspergillosis in raptors. Disposition of terbinafine after oral administration of 15, 30, 60 and 120 mg in raptors showed rapid absorption and a rapid first elimination phase followed by a slow second elimination phase similar to disposition of terbinafine in humans. Curve stripping gave an estimate of absorption rate constant, first elimination rate constant and second elimination rate constant. Absorption rate constants were 0.62 ± 0.21, 0.78 ± 0.61, 0.63 ± 0.12 and 0.59 ± 0.21 hr^{-1}, first elimination rate constants were 0.29 ± 0.19, 0.37 ± 0.22, 0.21 ± 0.21 and 0.45 ± 0.17 $hr^{-1}$and second elimination rate constants were 0.046 ± 0.02, 0.033 ± 0.01, 0.059 ± 0.02, 0.034 ± 0.01 hr^{-1} after administration of 15, 30, 60 and 120 mg terbinafine oral dose, respectively..

Terbinafine pharmacokinetic analysis was performed using non-compartmental analysis. Mean PK parameters of : peak terbinafine plasma concentration ,C_{max}, time to reach peak plasma concentration, T_{max}, area under plasma concentration versus time curve from time zero to time t, AUC_{0-t}, and mean residence times following the administration of 15, 30, 60 and 120 mg doses were, 0.31 ± 0.24, 1.21 ± 0.4, 2.02 ± 0.75 and 4.1 ± 1.9 mg/l, 5.4 ± 2.98, 3.4 ± 0.96, 5.1 ±

3.5 and 3.7 ± 0.8 hours, 3.8 ± 2.2, 11.5 ± 3.4, 24.8 ± 11.3 and 37.9 ± 17.1 hr.mg/l, and 10.26 ± 1.38, 8.6 ± 1.4, 9.6 ± 0.75 and 8.5 ± 1.4, respectively.

Two weeks wash-out period was not enough to completely eliminate the effect of previous doses on subsequent doses plasma concentrations which was confirmed by the presence of drug in the first sample of the following dose and also a decrease in volume of distribution (Vd/F) following administration of oral doses of terbinafine. This indicates long lasting accumulation of terbinafine in the deep tissues in raptors which could be expected as terbinafine is a lipophilic drug. Approximate linearity of pharmacokinetics of terbinafine was demonstrated for AUC_{0-t} to dose in the dose range of 15 - 120 mg while non-linearity for C_{max} in the same dose range was demonstrated using the power model. Calculation of steady state trough terbinafine plasma concentration after administration of daily doses of 15 or 30 mg/day in raptors showed that 30 mg dose produces a steady state trough terbinafine plasma concentration above the MIC of 0.8 - 1.6 µg/ml for *aspregillus fumigatus*. A larger study should be conducted with longer sampling times and a longer wash-out period should be performed so valid pharmacokinetic analysis of terbinafine in raptors can be performed.

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CONCLUSIONS

CONCLUSIONS

A new sustained release capsule formulation of nifedipine was successfully developed by coating an immediate release commercially available soft elastic gelatin capsule by applying the coat around the surface of SEG capsules using Fluid-bed spray coater. The coating was performed in single step which does not unduly lengthen manufacturing. Sustained release action of nifedipine was obtained by coating immediate release SEG capsules with a combination of polymers Surelease® as a water insoluble polymer and Opadry® or pectin as a water soluble polymer. Drug release depends mainly on the amount of water soluble polymer in the coat and thickness of the applied coat. Unexpectedly, there was a pH dependent effect on drug release from nifedipine SEG capsules coated with some Surelease® Opadry® combinations. Surprisingly, Surelease® pectin combinations also showed pH dependent effect with no drug release in gastric fluid. Simulated nifedipine plasma concentrations of selected tested formulations provided sustained release of drug with concentrations above the minimum therapeutic concentration (15 ng/ml) at 6 A.M. in the morning which is the time of greatest need for antihypertensive effect. The tested formulations provided drug release profiles that are very promising in terms of desirable sustained release formulations. More research is needed to obtain drug profiles similar to that of commercially available products of nifedipine.

Pharmacokinetic measures of AUC_{0-58}, $AUC_{0-\infty}$, and C_{max} for the new formulation (formula IV) were not within the generally accepted guidelines of 0.8 to 1.25 in either fed or fasting states. The new formulation showed higher bioavailability in the fed state and lower bioavailability in the fasted state compared to Covera HS®. The data are quite variable which is attributed to inter-subject variability characteristic of verapamil. A good linear *in vitro/in vivo* correlation (IVIVC) was established for the tested formulation (IV) and the reference product to help future formulation changes required in the product development process.

The disposition of terbinafine after oral administration of 15, 30, 60 and 120 mg in raptors showed rapid absorption, a fast first elimination phase followed by slow second elimination phase similar to terbinafine's disposition in human. Accumulation of terbinafine in the deep tissues occurred which is demonstrated by long half-life of elimination as it is a lipophilic drug. Approximate pharmacokinetic linearity of terbinafine was demonstrated for AUC_{0-t} in the dose ranges of 15 - 120 mg while non-linearity for C_{max} in the same dose range was demonstrated using the power model. Calculation of steady state trough terbinafine plasma concentration after administration of daily doses of 15 or 30 mg/day showed that 30 mg daily dose produces a steady state plasma concentration above the MIC of (0.8-1.6) µg/ml against *aspregillus fumigatus* which may be an appropriate dose for the treatment of aspergillosis in raptors. Due to small sample size and short sampling time period and short wash-out period between doses, definitive pharmacokinetic analysis could not be done. Another study should be conducted with longer

sampling times and a longer wash-out period between doses to better validate pharmacokinetic behavior of terbinafine in raptors.

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Appendix A

Table A.1: Demographic information of subjects participated in the full study under fasting conditions.

	Sex	Age (yrs)	Weight (lb)	Height	*Ethnic background
Subject #1	male	21	190	6'	Caucasian
Subject #2	male	21	195	6'	White
Subject #3	male	21	150	5'9"	White caucasian
Subject #4	male	19	165	5'11"	White European
Subject #6	male	22	140	5'11"	Chinese / caucasian
Subject #7	male	34	115	5'2"	Chinese
Subject #8	male	25	170	5'11"	Caucasian

Table A.2: Demographic information of subjects participated in the full study under fed conditions.

	Sex	Age (yrs)	Weight (lb)	Height	*Ethnic background
Subject #1	male	23	170	6'	Caucasian
Subject #2	male	31	170	6'2"	Asian
Subject #3	male	21	140	6'	White
Subject #4	male	31	185	6'	Caucasian
Subject #5	male	20	140	5'11"	White
Subject #6	male	21	195	5'11"	Asian
Subject #7	male	20	195	5'11"	White
Subject #8	female	19	135	5'5"	Caucasian

* Ethnic background: Self identified by subjects.

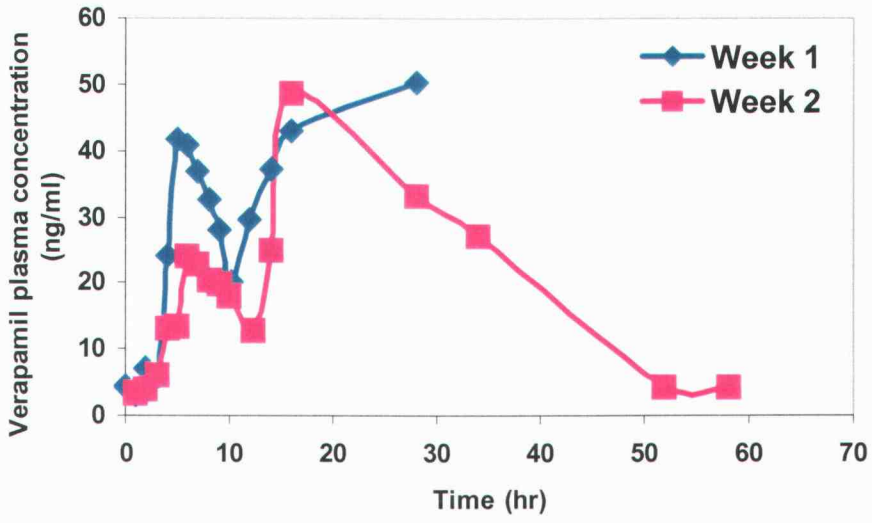

Figure A.1: Verapamil plasma concentration-time curve for subject 1 after administration of formula I under fed conditions.

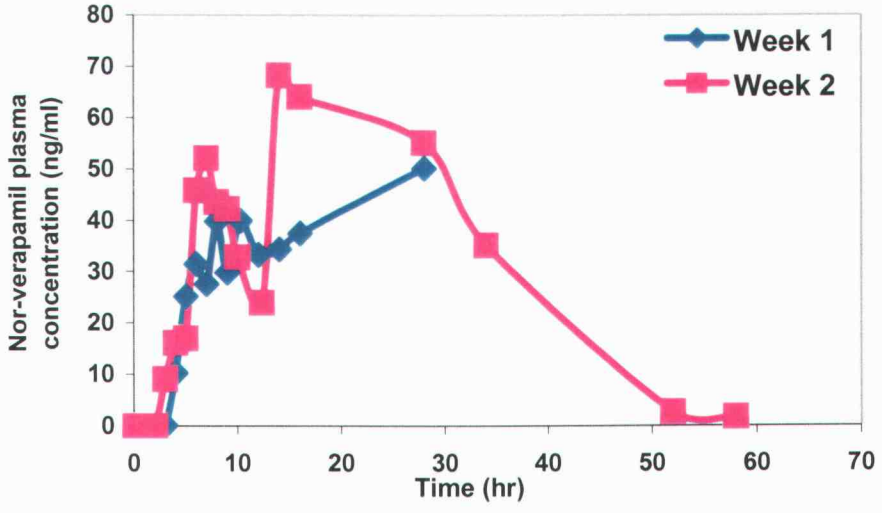

Figure A.2: Norverapamil plasma concentration -time curve for subject 1 after administration of formula I under fed conditions.

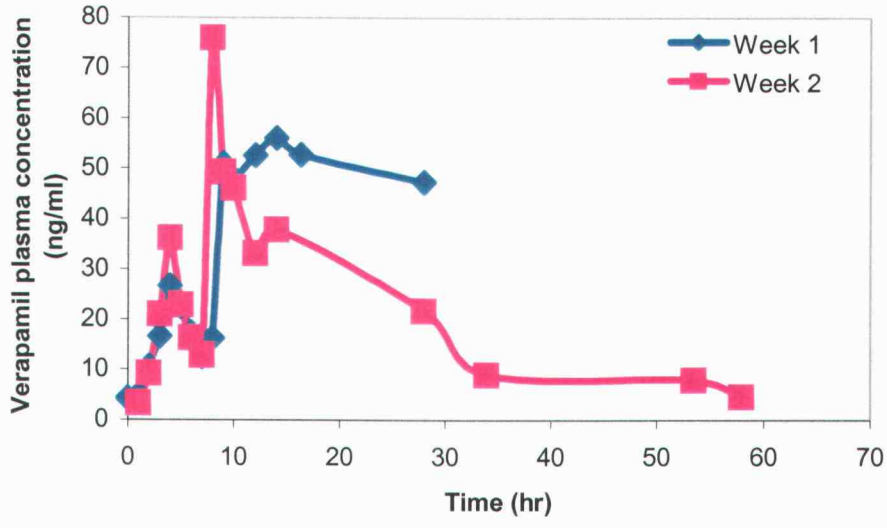

Figure A.3: Verapamil plasma concentration-time curve for subject 2 after administration of formula I under fed conditions.

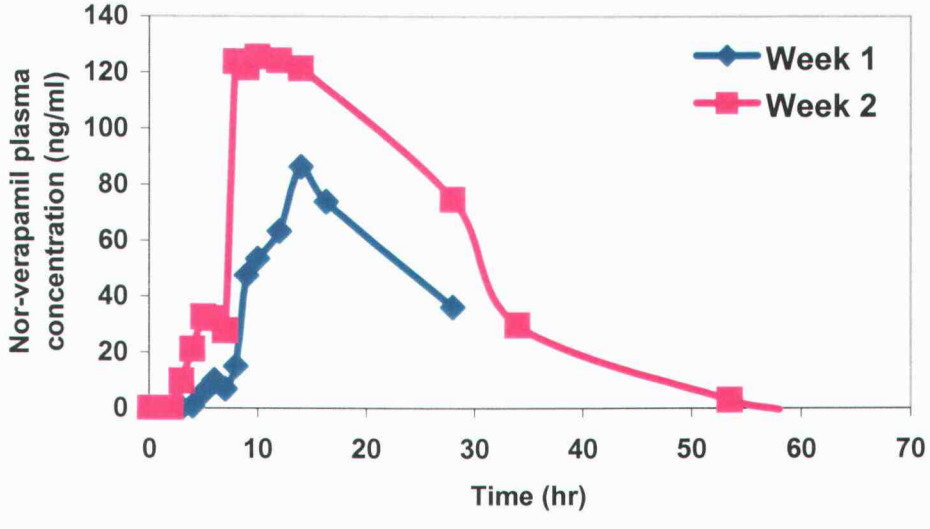

Figure A.4: Norverapamil plasma concentration-time curve for subject 2 after administration of formula I under fed conditions.

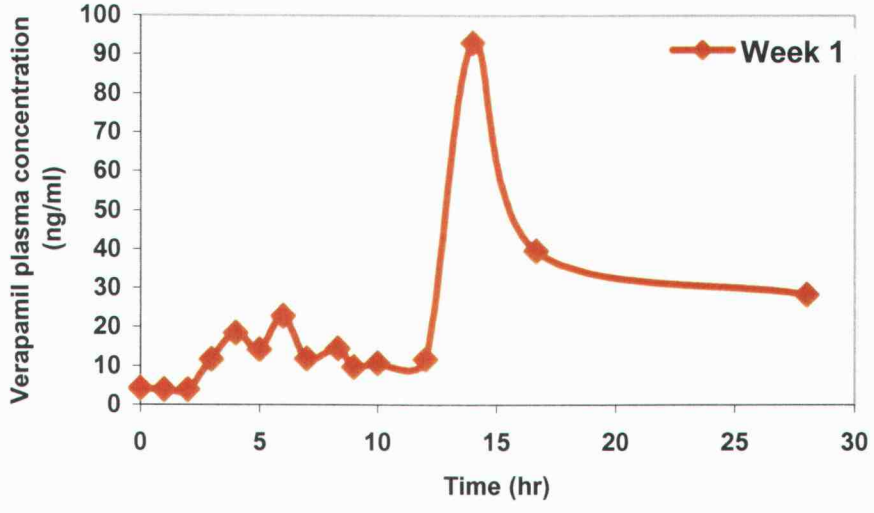

Figure A.5: Verapamil plasma concentration-time curve for subject 3 after administration of formula II under fed conditions.

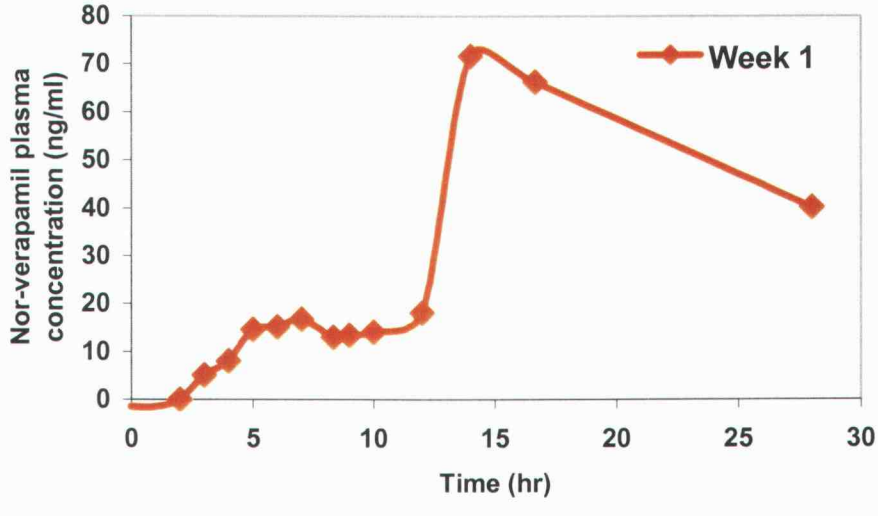

Figure A.6: Norverapamil plasma concentration-time curve for subject 3 after administration of formula II under fed conditions.

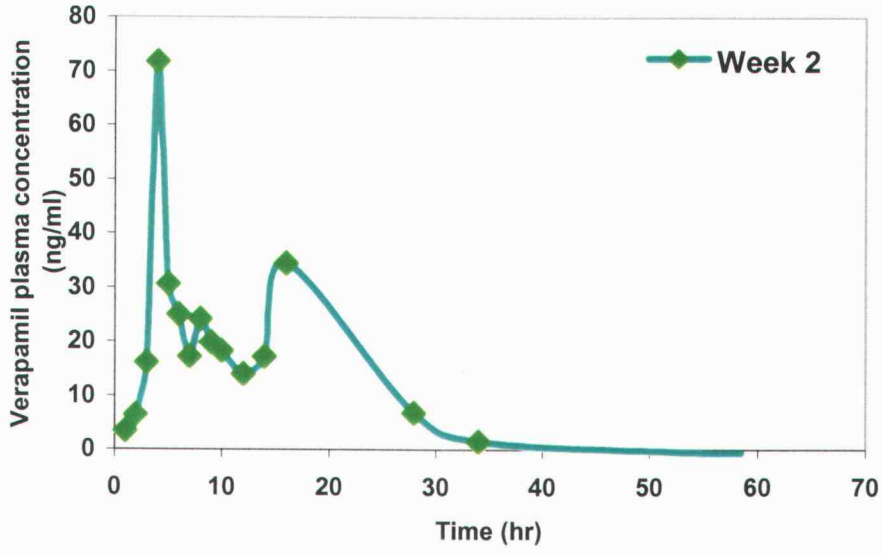

Figure A.7: Verapamil plasma concentration-time curve for subject 4 after administration of formula II under fed conditions.

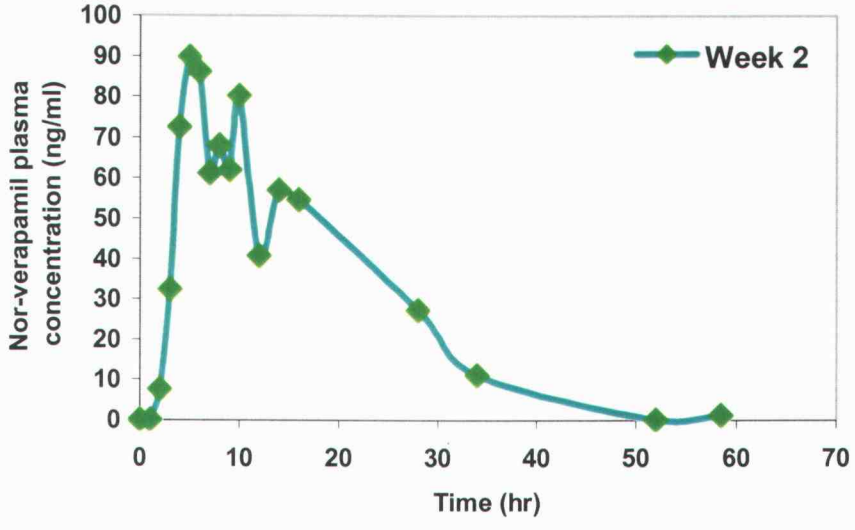

Figure A.8: Norverapamil plasma concentration-time curve for subject 4 after administration of formula II under fed conditions.

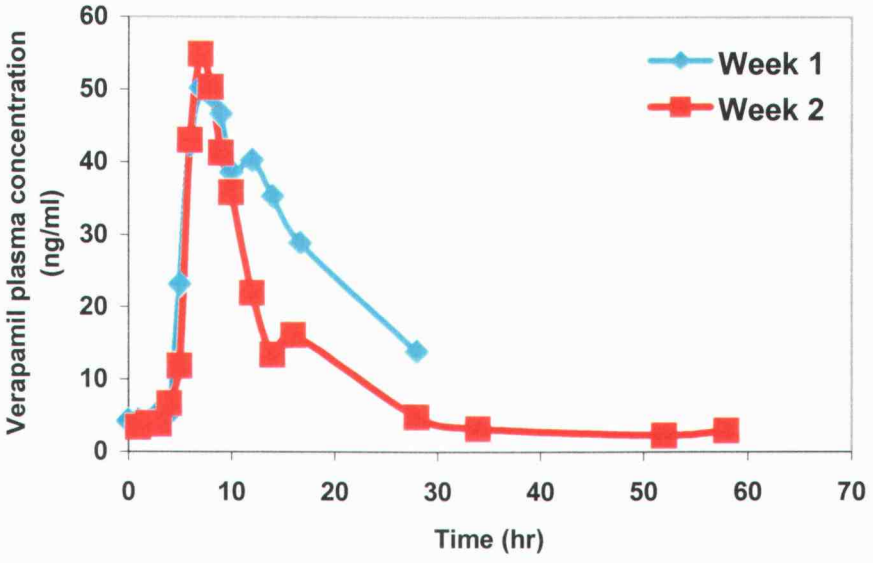

Figure A.9: Verapamil plasma concentration-time curve for subject 5 after administration of reference under fed conditions.

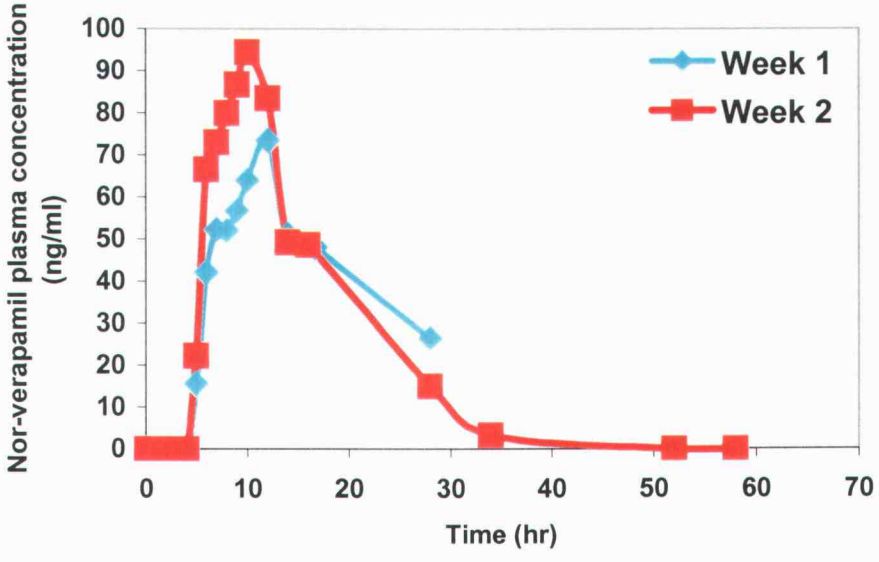

Figure A.10: Norverapamil plasma concentration-time curve for subject 5 after administration of reference under fed conditions.

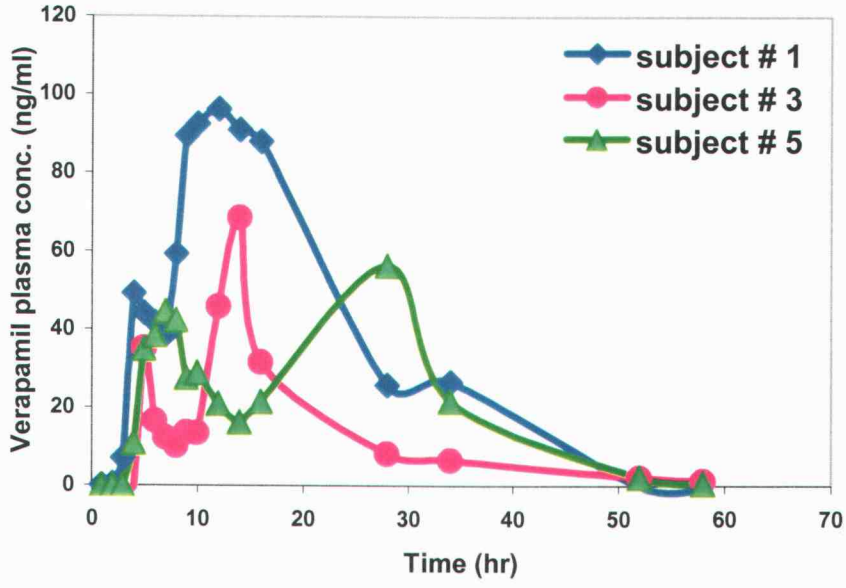

Figure A.11: Verapamil plasma concentration-time curve for subject 1, 3 and 5 after administration of formula III under fed conditions (pilot study II).

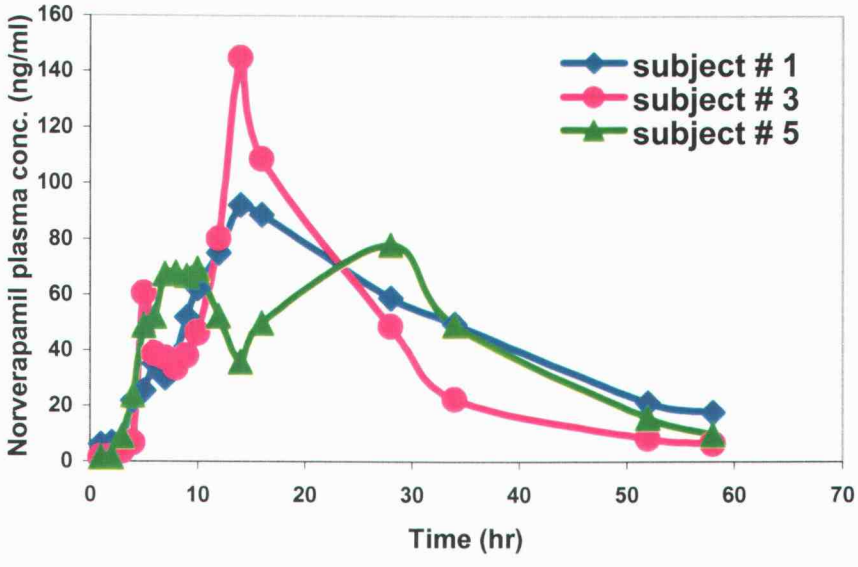

Figure A.12: Verapamil plasma concentration-time curve for subject 1, 3 and 5 after administration of formula III under fed conditions (pilot study II).

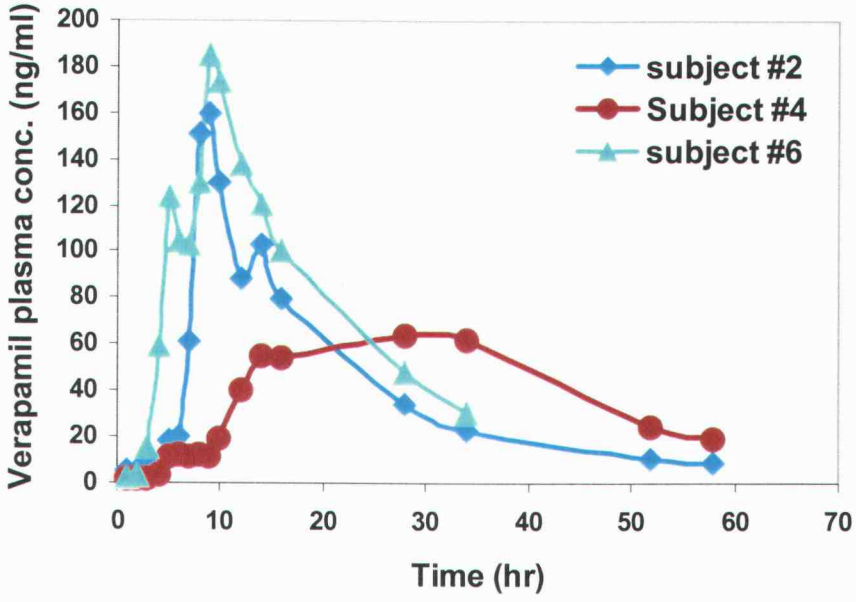

Figure A.13: Verapamil plasma concentration-time curve for subject 2, 4 and 6 after administration of formula IV under fed conditions (pilot study II).

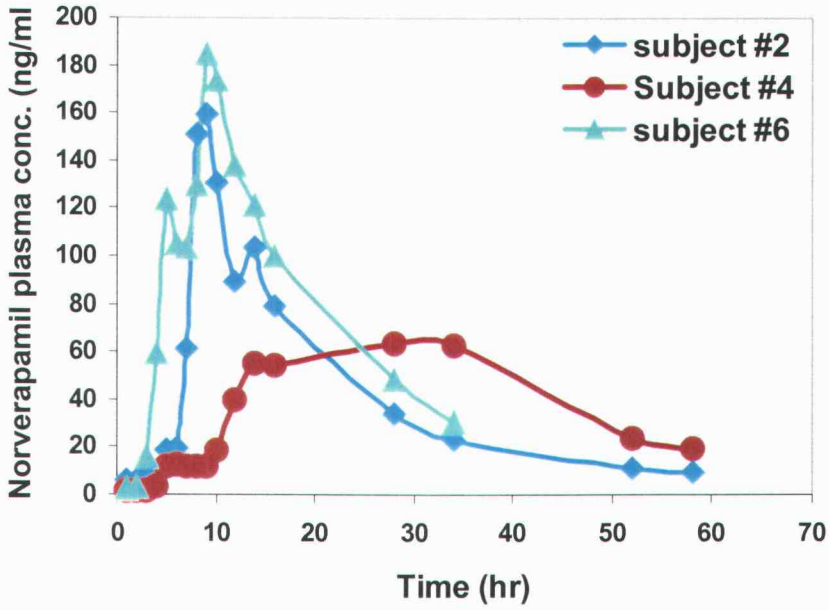

Figure A.14: Norverapamil plasma concentration-time curve for subject 2, 4 and 6 after administration of formula IV under fed conditions (pilot study II).

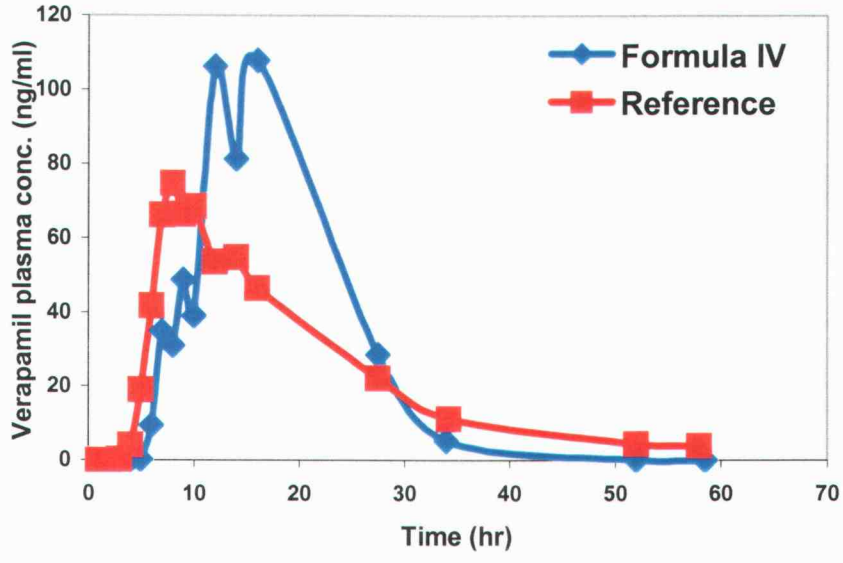

Figure A.15: Verapamil plasma concentration-time in subject #1 (fed study).

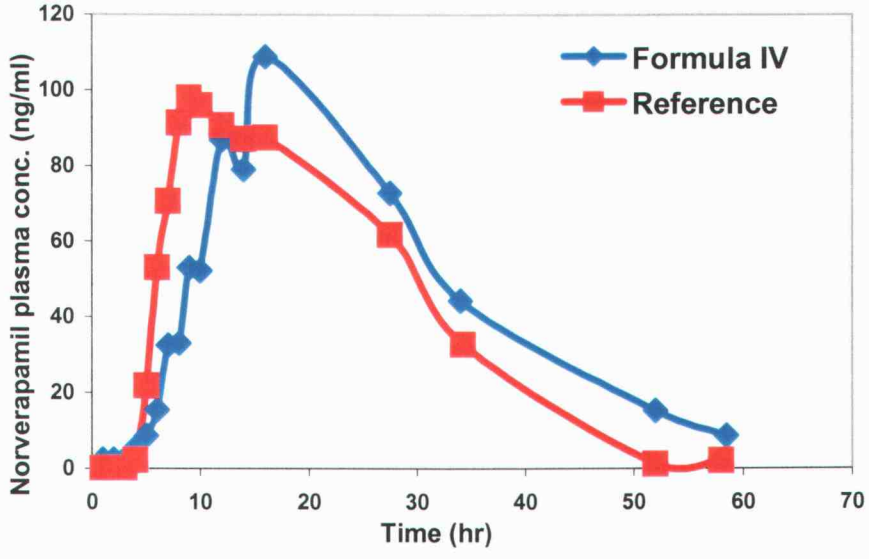

Figure A.16: Norverapamil plasma concentration-time in subject #1 (fed study).

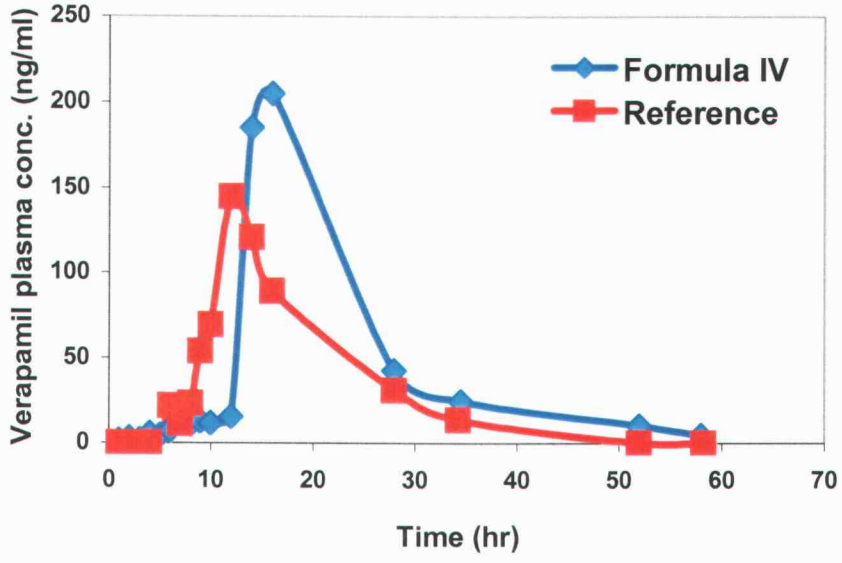

Figure A.17: Verapamil plasma concentration-time in subject #2 (fed study).

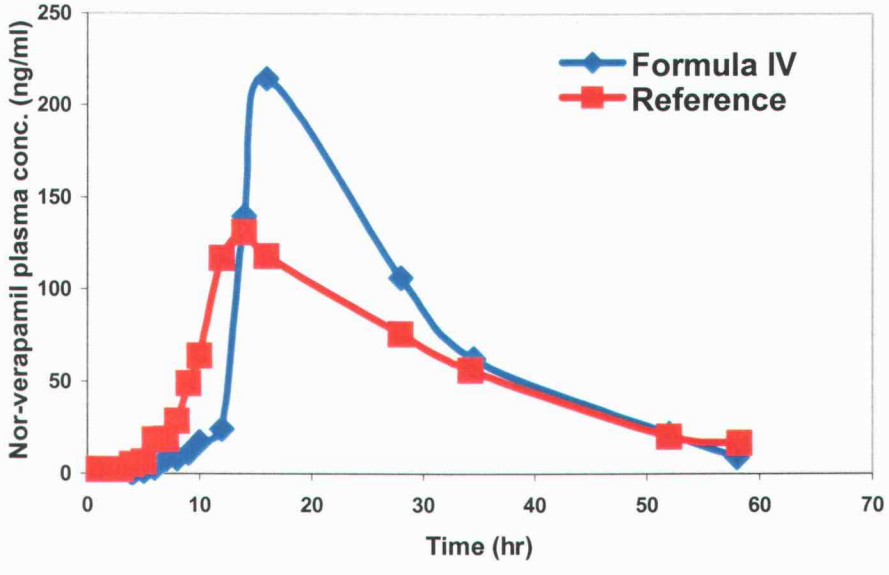

Figure A.18: Norverapamil plasma concentration-time in subject #2 (fed study).

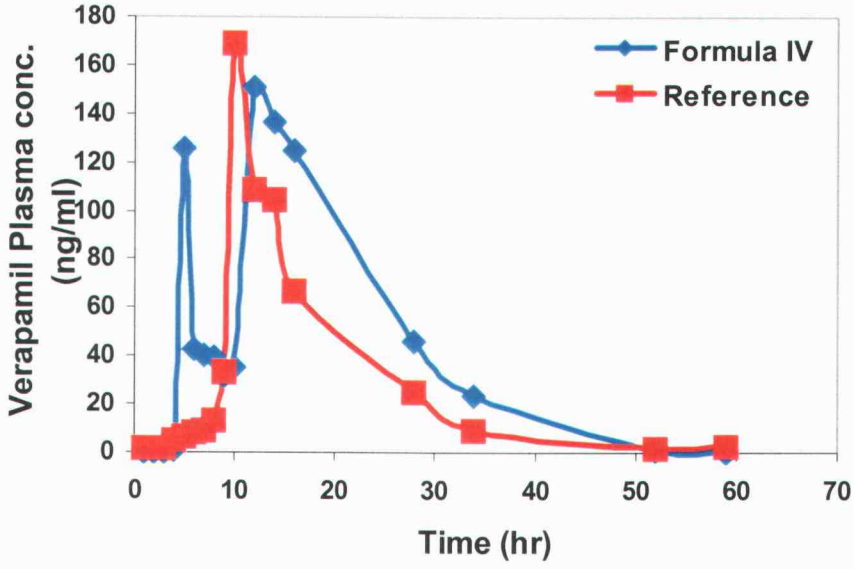

Figure A.19: Verapamil plasma concentration-time in subject #3 (fed study).

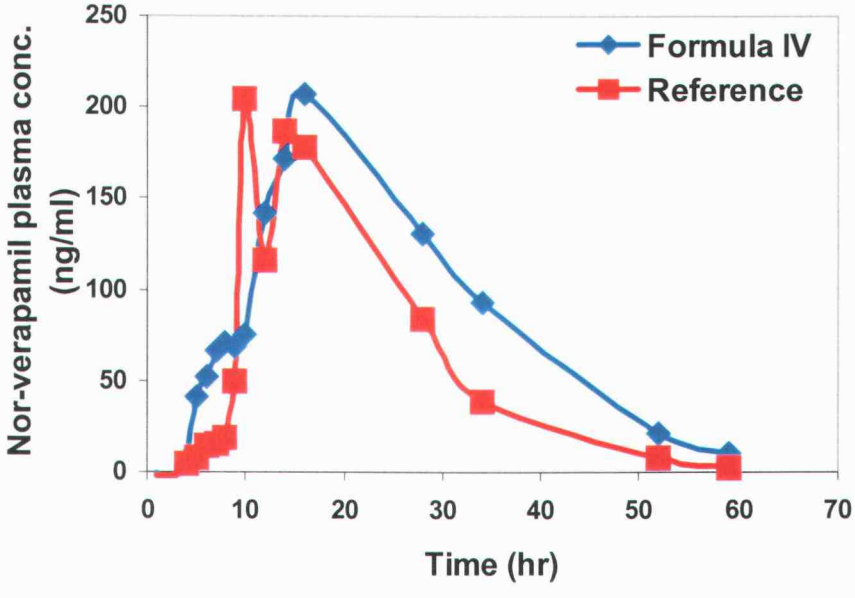

Figure A.20: Norverapamil plasma concentration-time in subject #3 (fed study).

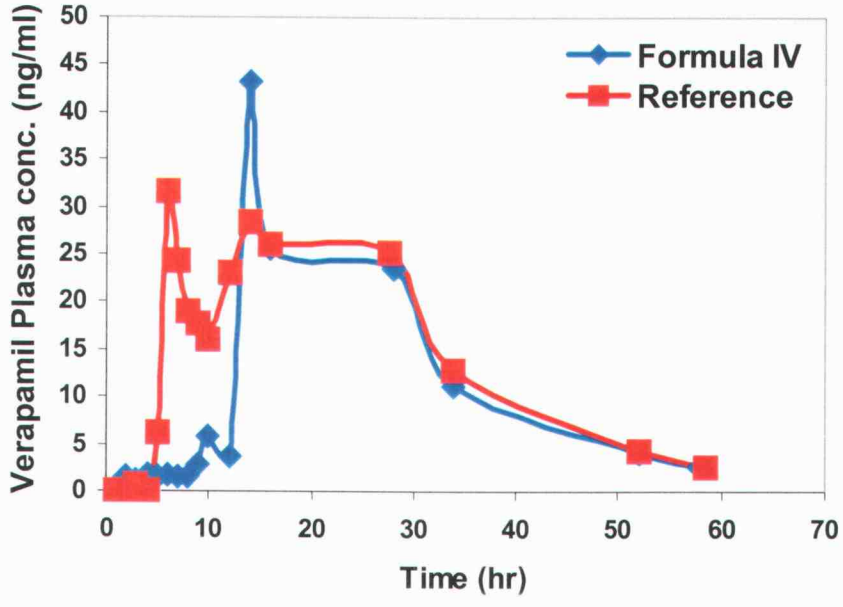

Figure A.21: Verapamil plasma concentration-time in subject #4 (fed study).

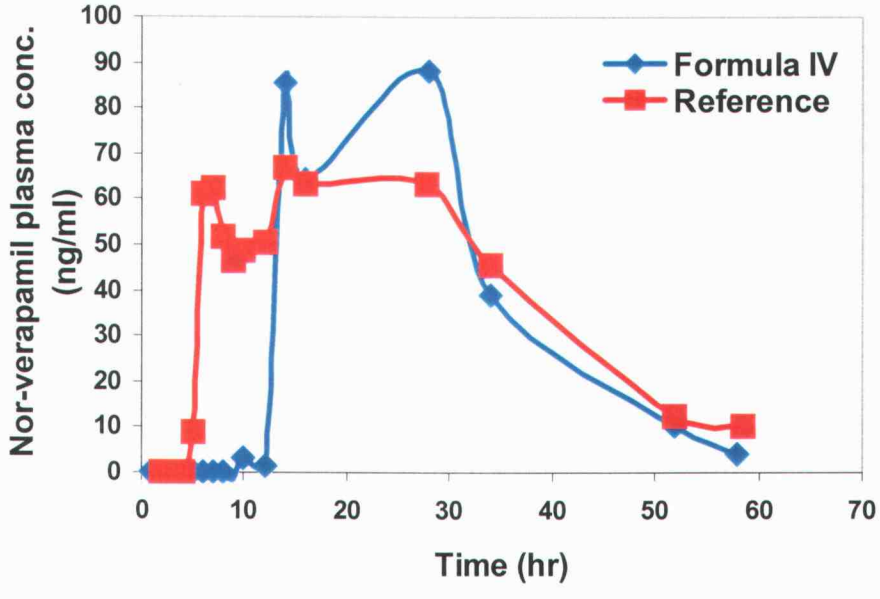

Figure A.22: Norverapamil plasma concentration-time in subject #4 (fed study).

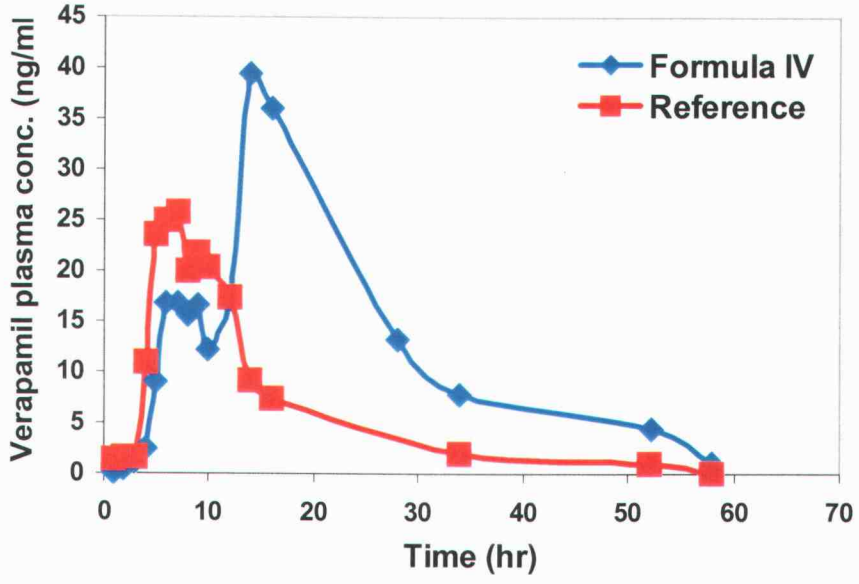

Figure A.23: Verapamil plasma concentration-time in subject #5 (fed study).

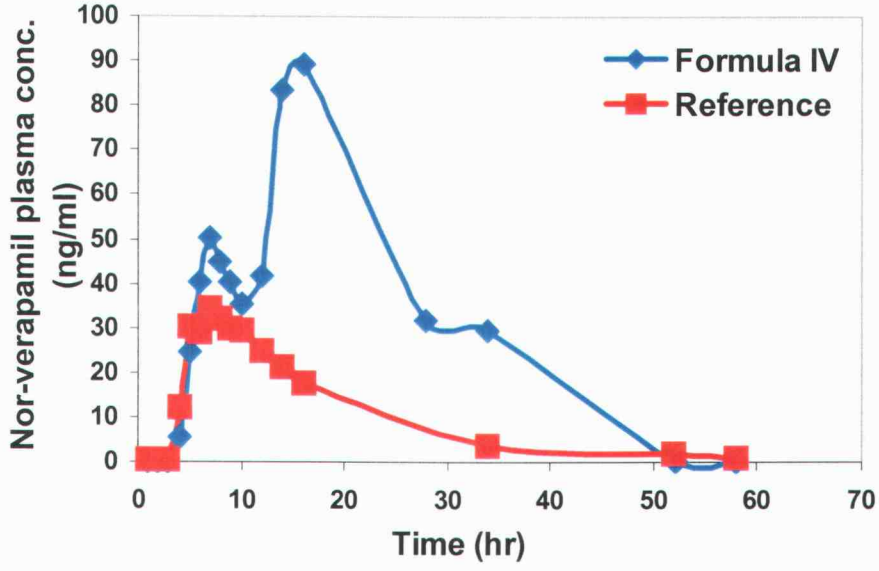

Figure A.24: Norverapamil plasma concentration-time in subject #5 (fed study).

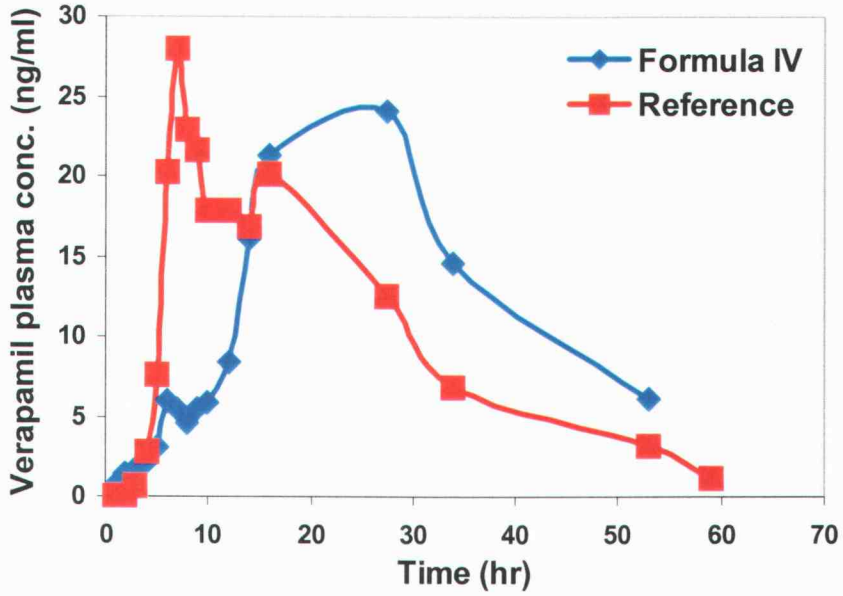

Figure A.25: Verapamil plasma concentration-time in subject #6 (fed study).

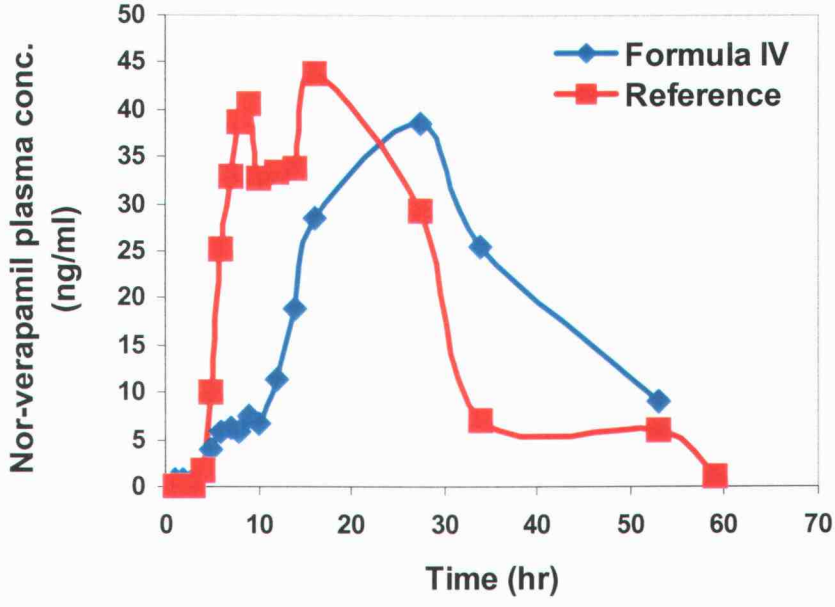

Figure A.26: Norverapamil plasma concentration-time in subject #6 (fed study).

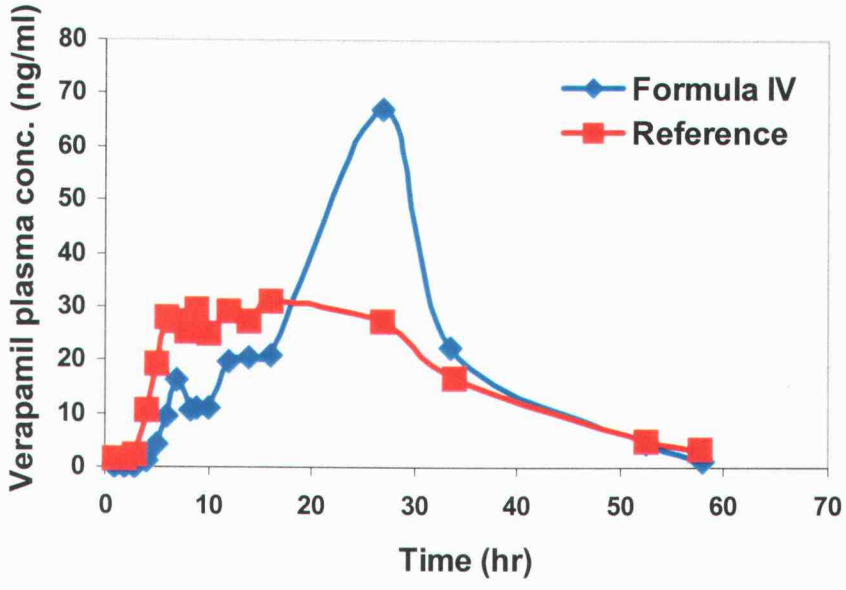

Figure A.27: Verapamil plasma concentration-time in subject #7 (fed study).

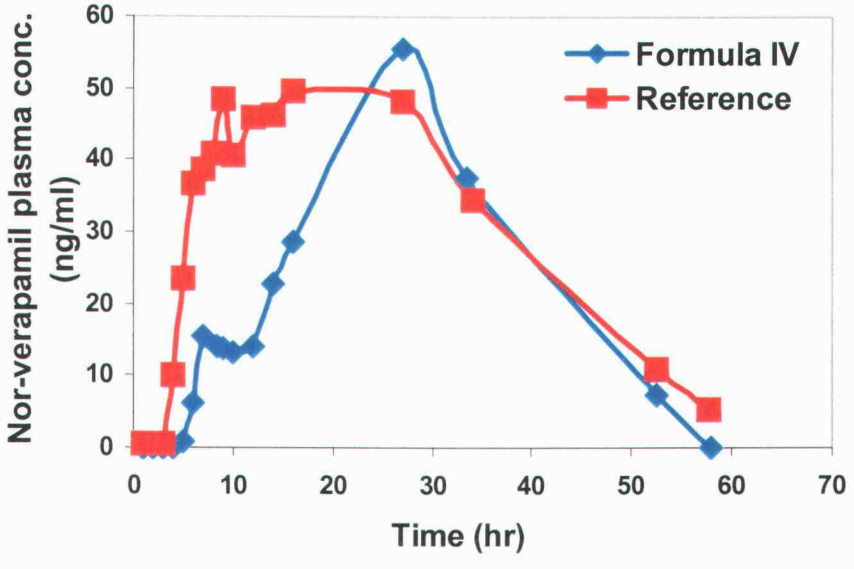

Figure A.28: Norverapamil plasma concentration-time in subject #7 (fed study).

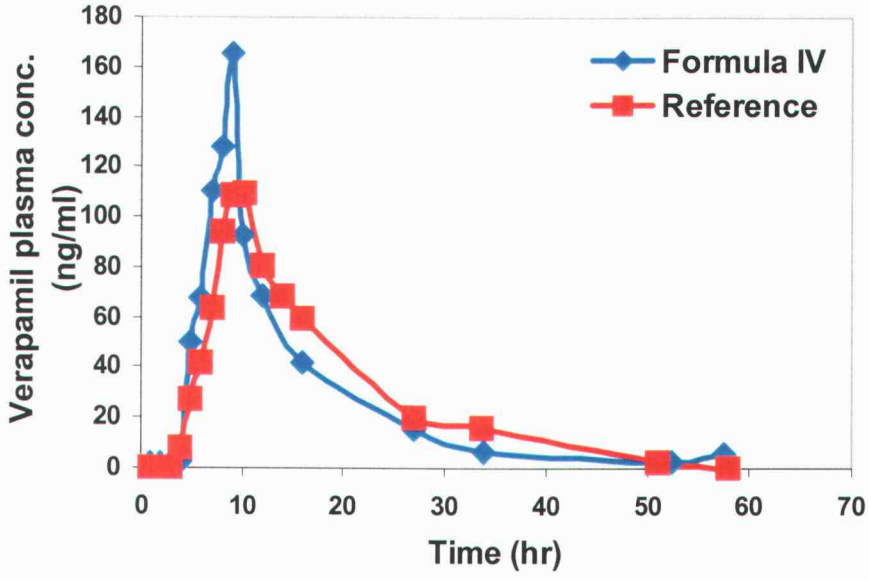

Figure A.29: Verapamil plasma concentration-time in subject #8 (fed study).

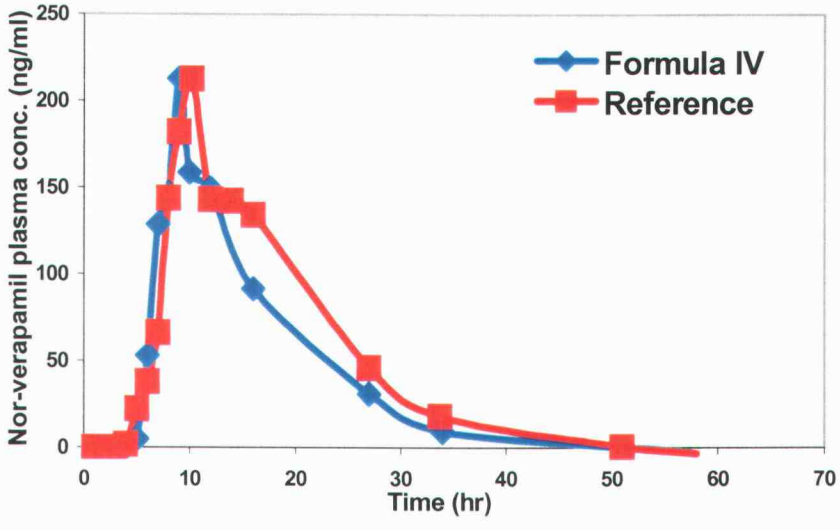

Figure A.30: Norverapamil plasma concentration-time in subject #8 (fed study).

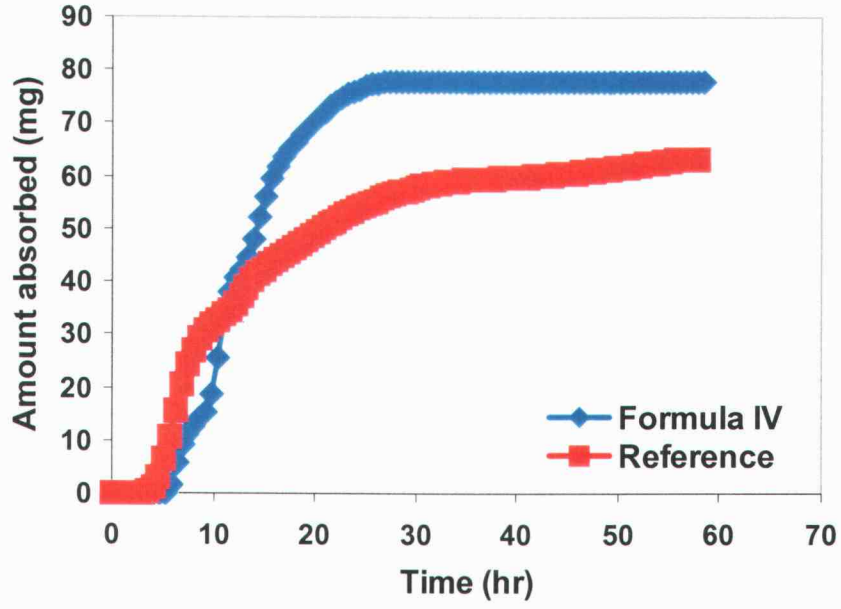

Figure A.31: Deconvolved input function of verapamil from subject #1 (fed study).

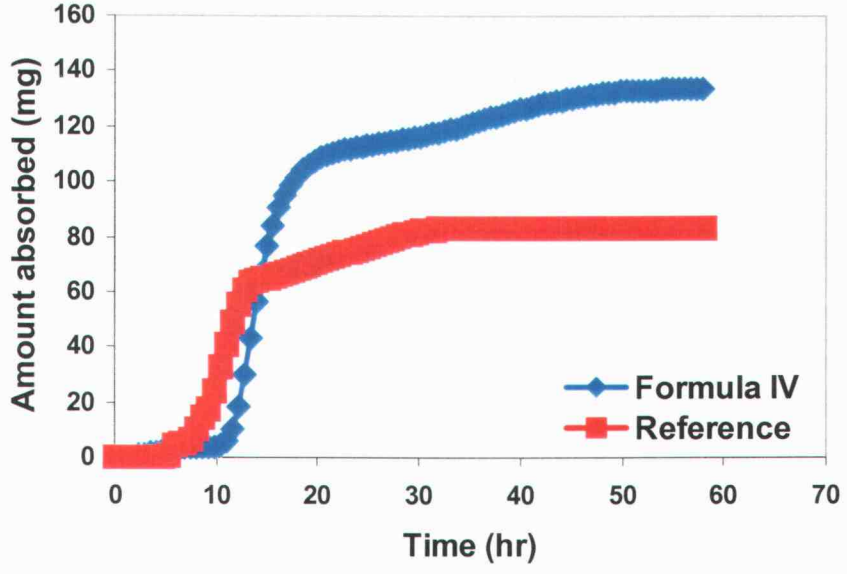

Figure A.32: Deconvolved input function of verapamil from subject #2 (fed study).

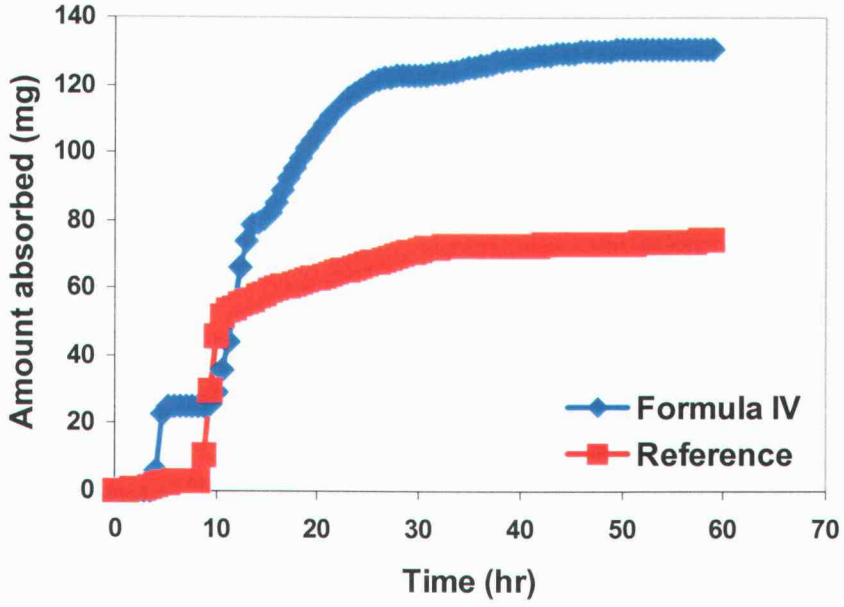

Figure A.33: Deconvolved input function of verapamil from subject #3 (fed study).

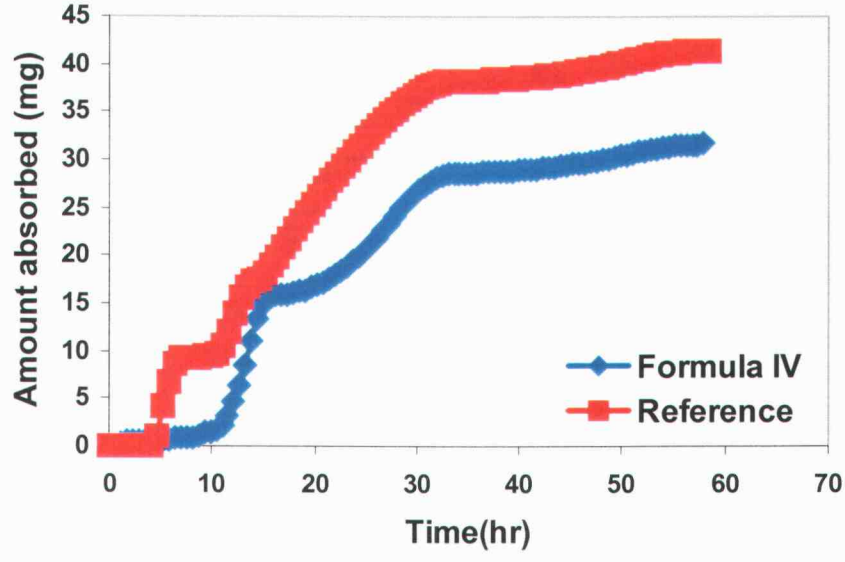

Figure A.34: Deconvolved input function of verapamil from subject #4 (fed study).

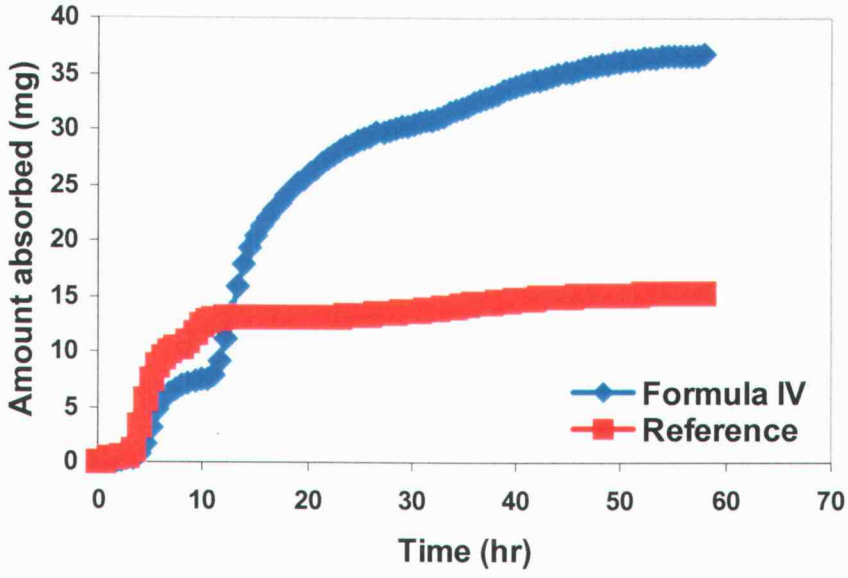

Figure A.35: Deconvolved input function of verapamil from subject #5 (fed study).

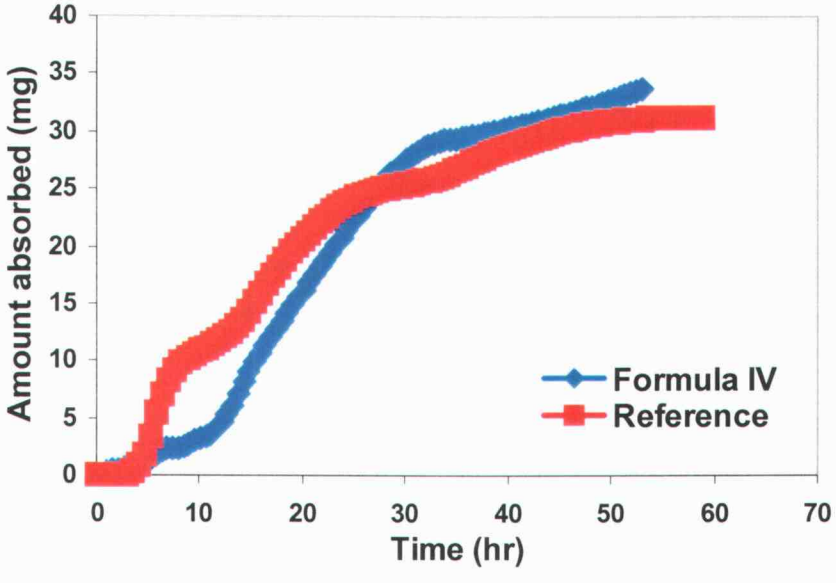

Figure A.36: Deconvolved input function of verapamil from subject #6 (fed study).

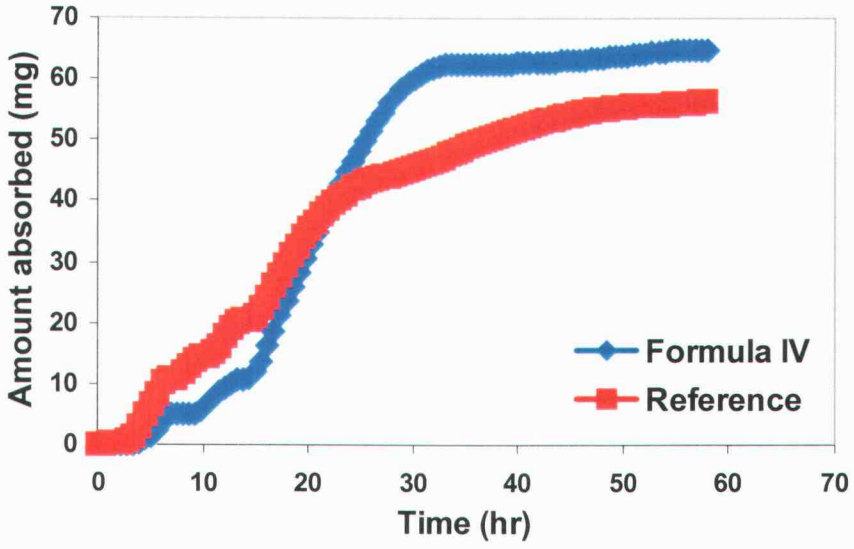

Figure A.37: Deconvolved input function of verapamil from subject #7 (fed study).

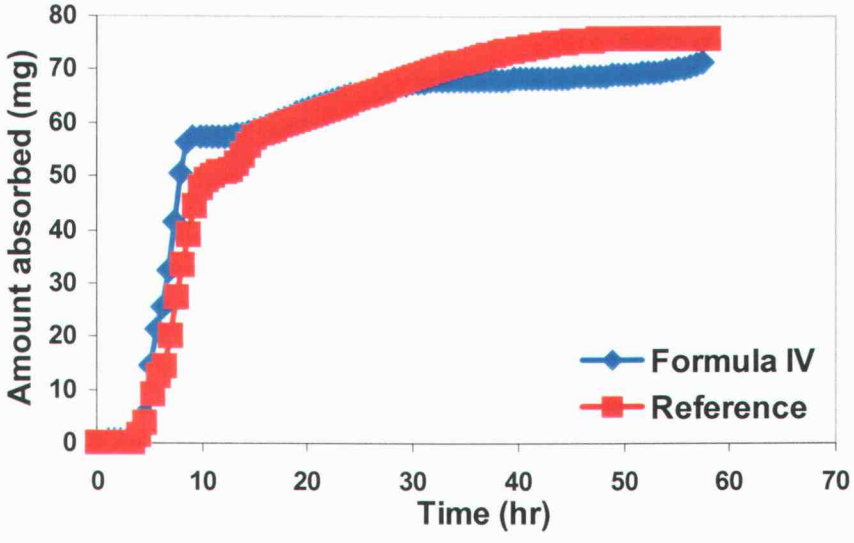

Figure A.38: Deconvolved input function of verapamil from subject #8 (fed study).

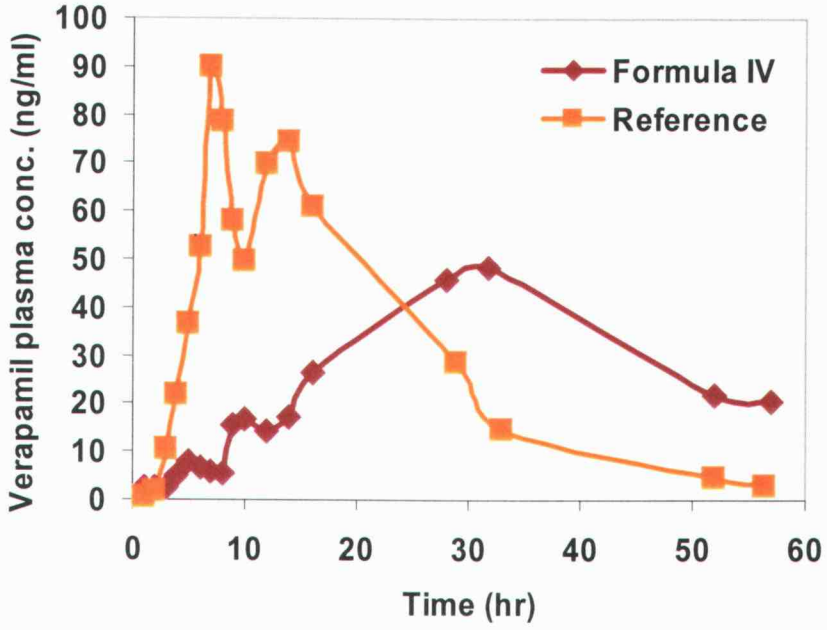

Figure A.39 Verapamil plasma concentration-time in subject #1 (fasted study).

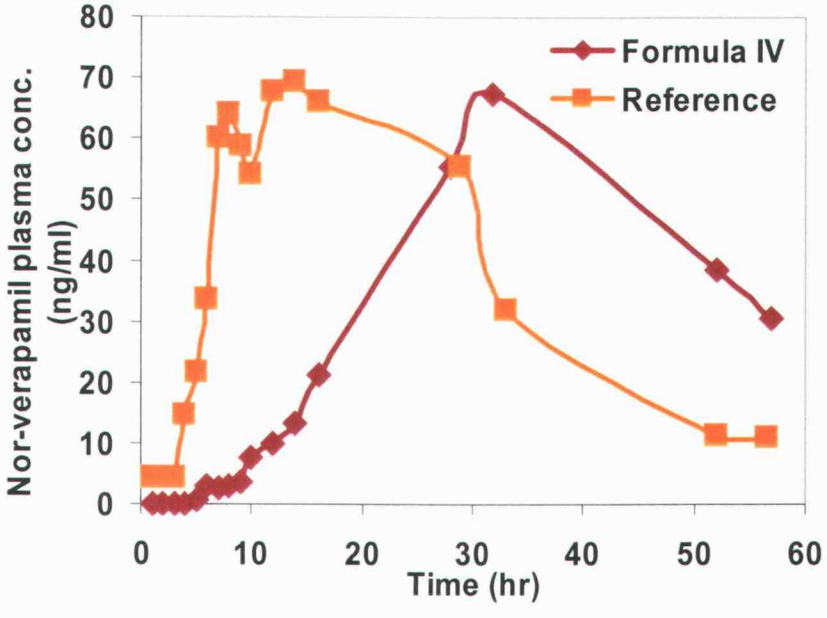

Figure A.40: Norverapamil plasma concentration-time in subject #1 (fasted study).

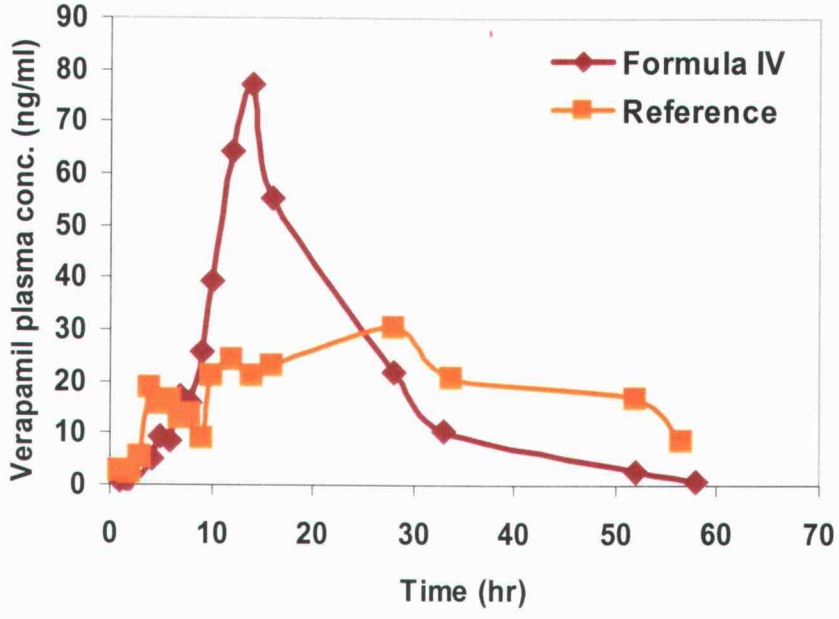

Figure A.41: Verapamil plasma concentration-time in subject #2 (fasted study).

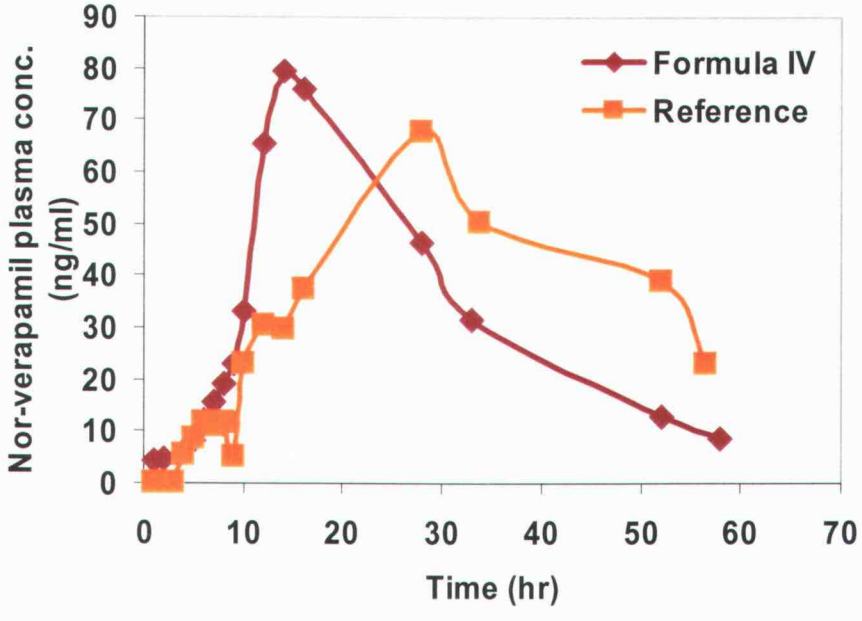

Figure A.42: Norverapamil plasma concentration-time in subject #2 (fasted study).

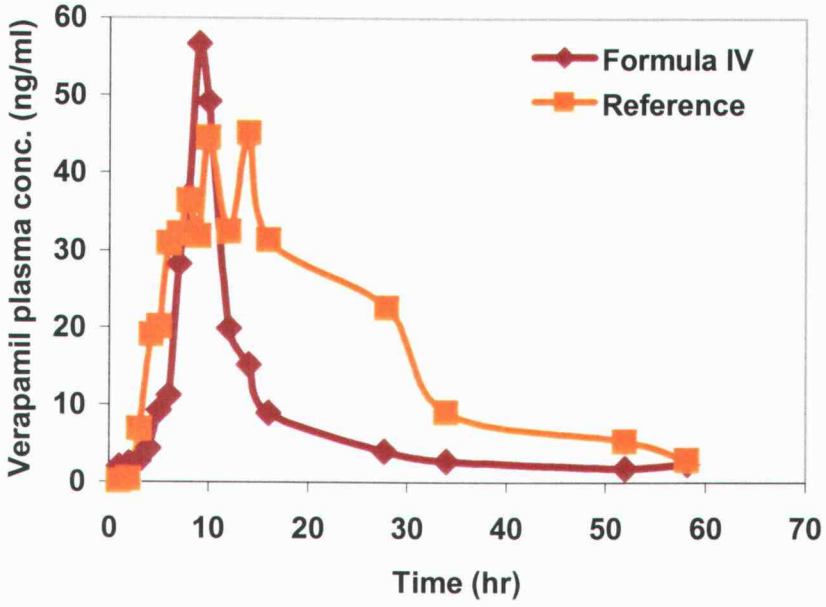

Figure A.43: Verapamil plasma concentration-time in subject #3(fasted study).

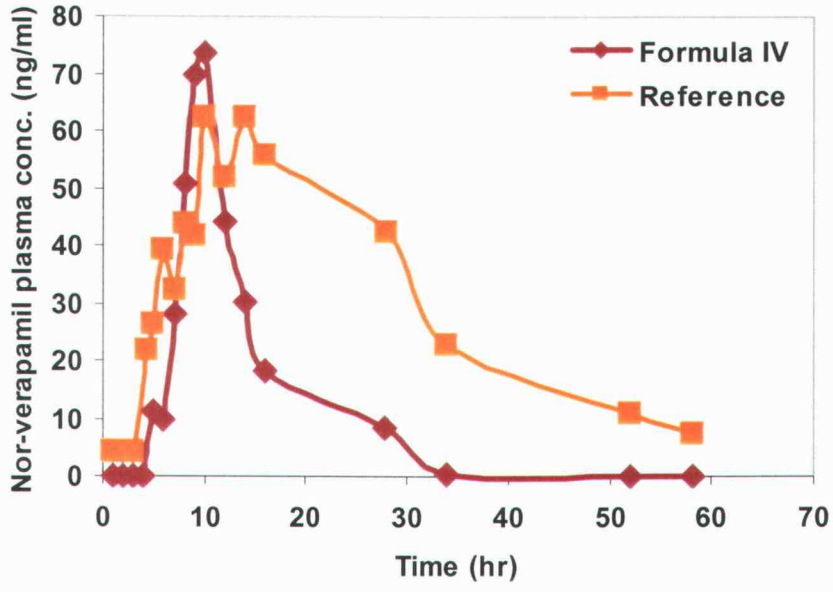

Figure A.44: Norverapamil plasma concentration-time in subject #3(fasted study).

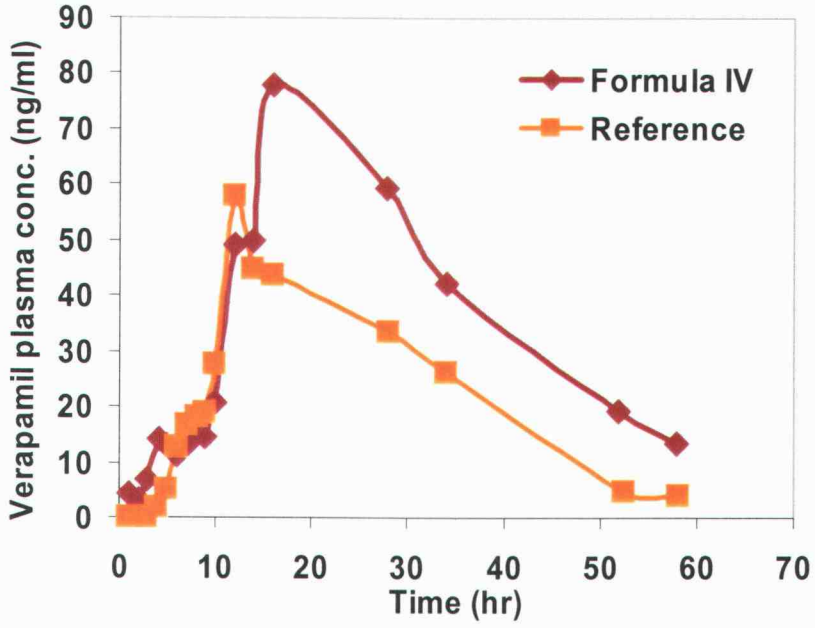

Figure A.45: Verapamil plasma concentration-time in subject #4 (fasted study).

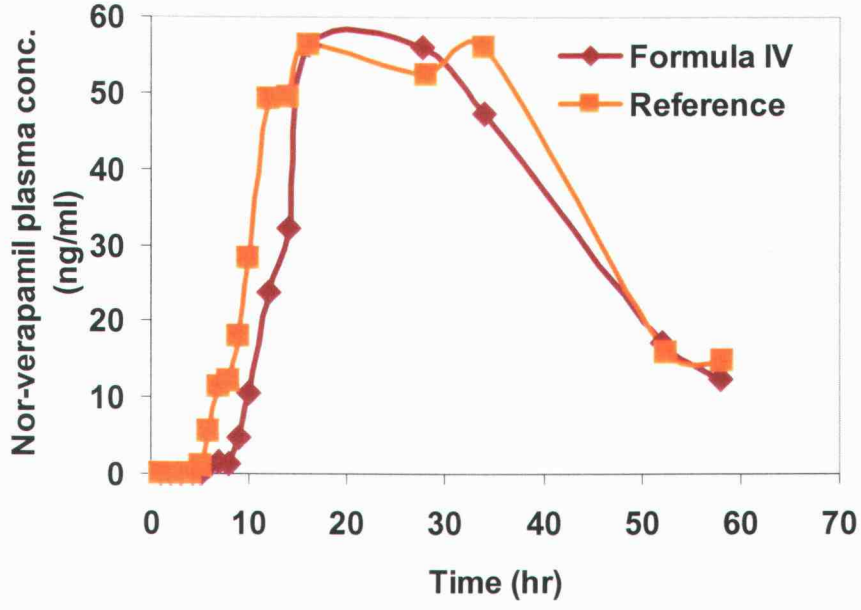

Figure A.46: Norverapamil plasma concentration-time in subject #4 (fasted study).

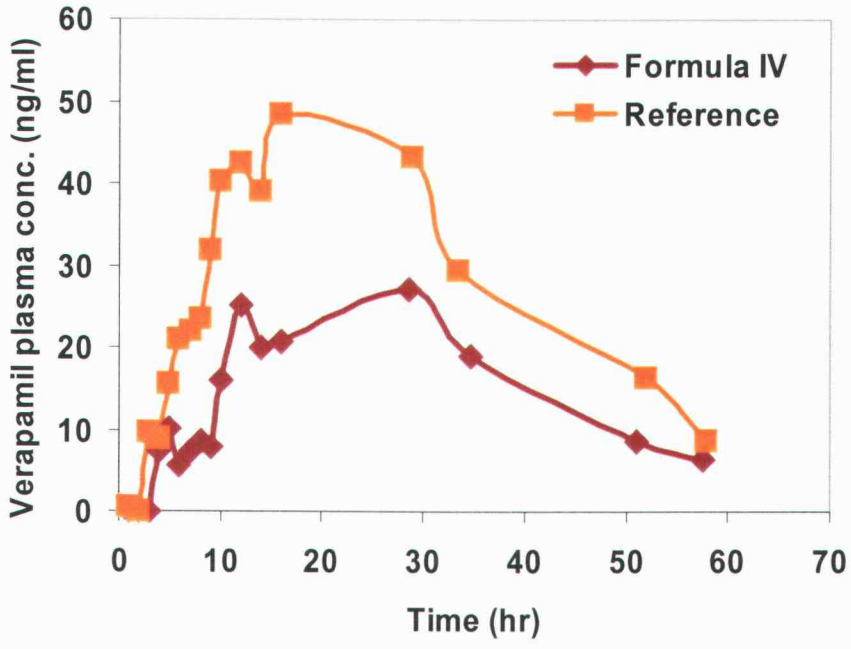

Figure A.47: Verapamil plasma concentration-time in subject #6 (fasted study).

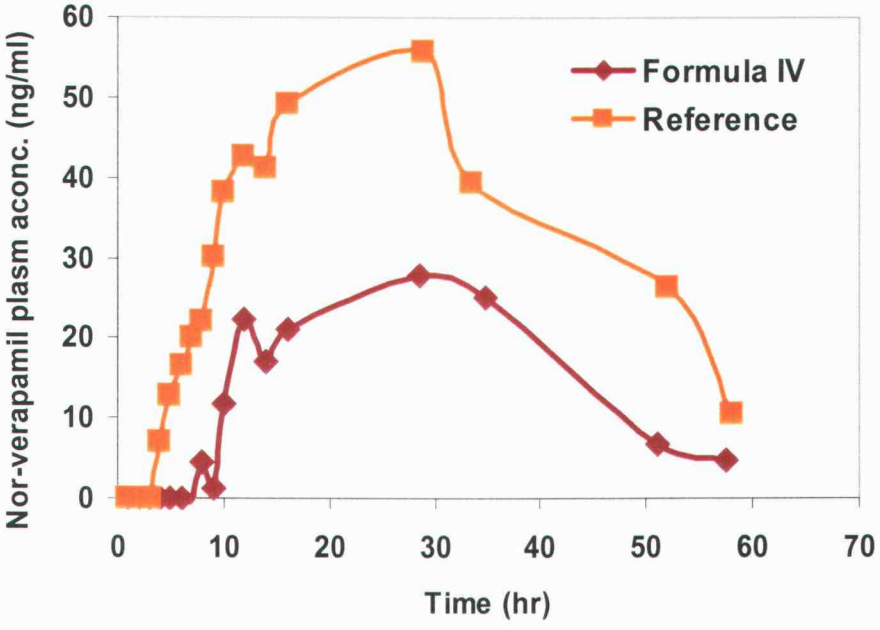

Figure A.48: Norverapamil plasma concentration-time in subject #6 (fasted study).

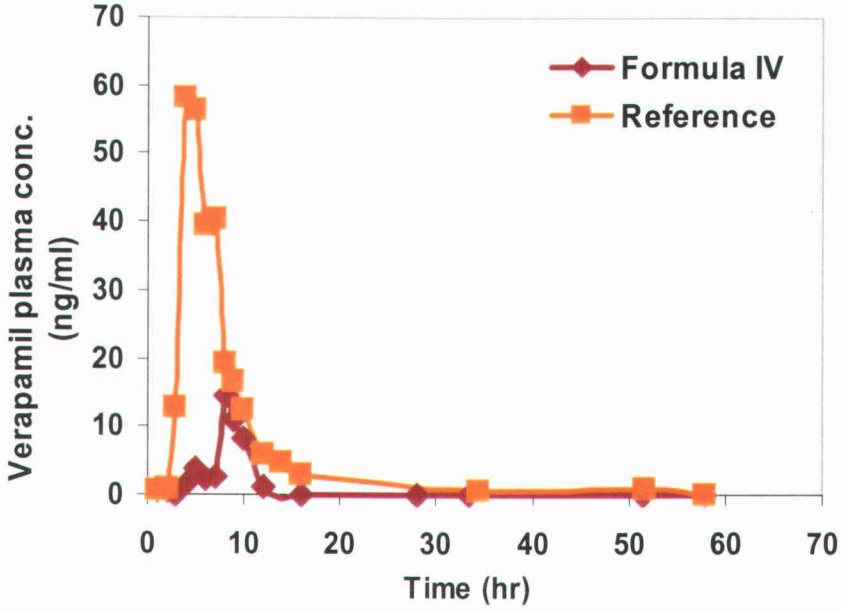

Figure A.49: Verapamil plasma concentration-time in subject #7 (fasted study).

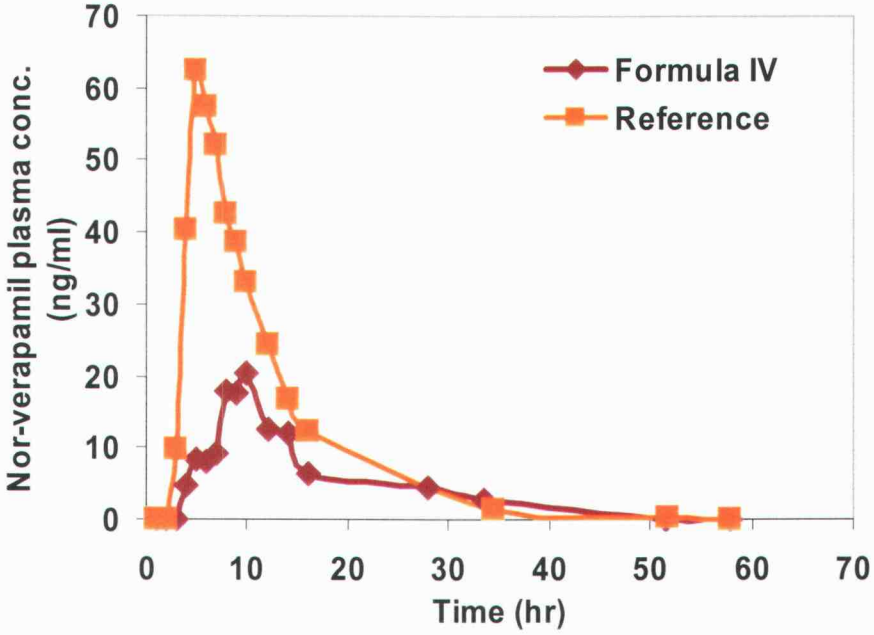

Figure A.50: Norverapamil plasma concentration-time in subject #7 (fasted study).

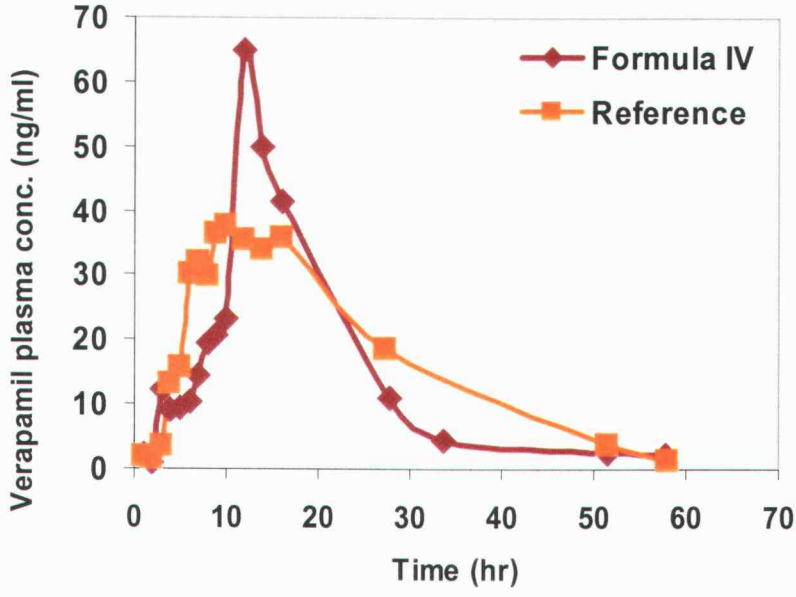

Figure A.51: Verapamil plasma concentration-time in subject #8 (fasted study).

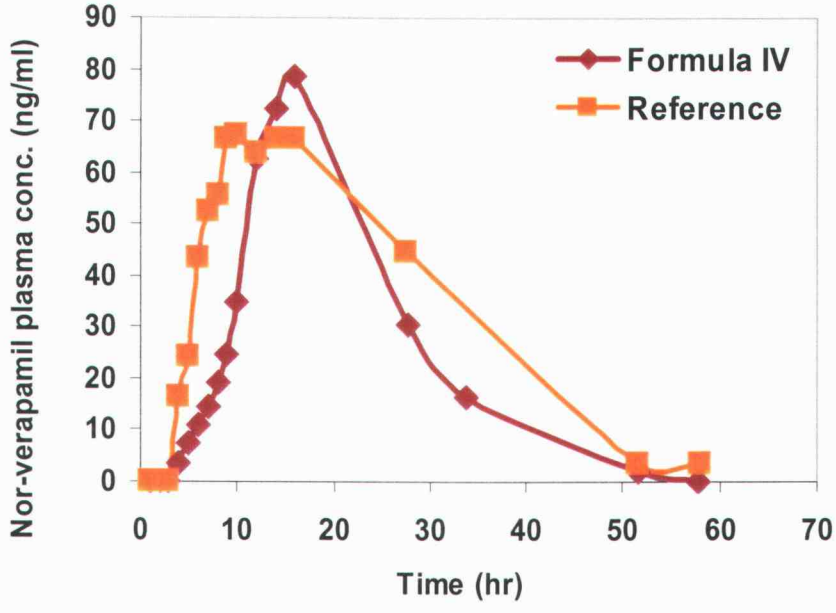

Figure A.52: Norverapamil plasma concentration-time in subject #8 (fasted study).

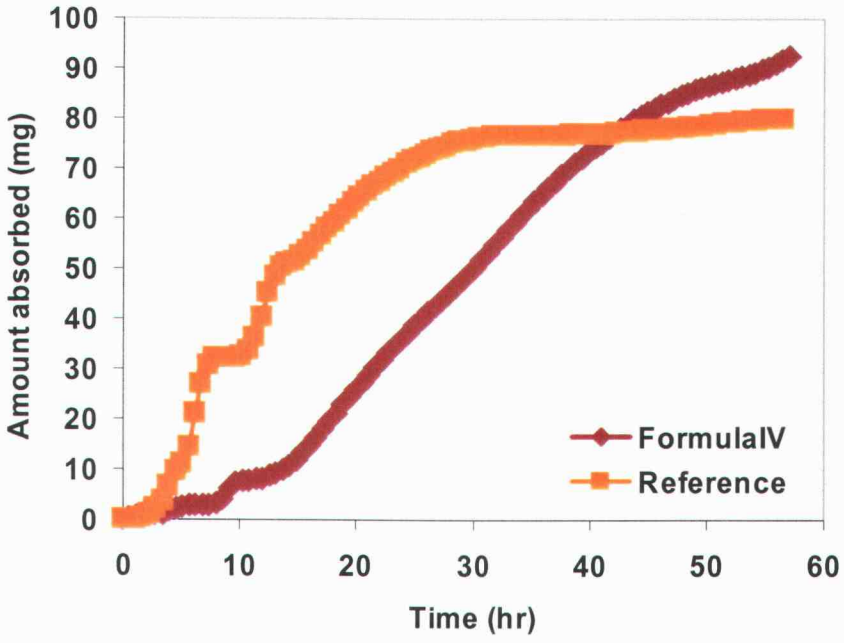

Figure A.53: Deconvolved input function of verapamil from subject #1 (fasted study).

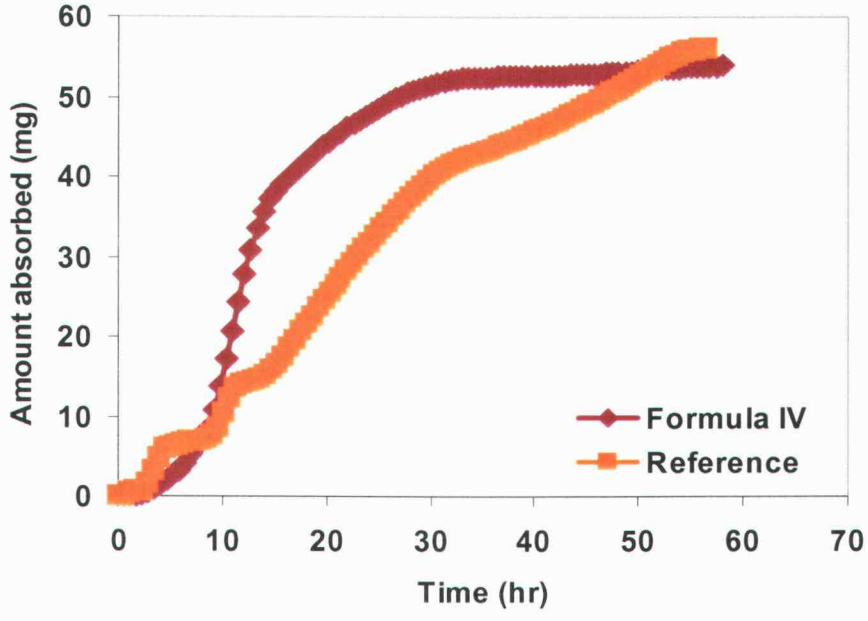

Figure A.54: Deconvolved input function of verapamil from subject #2 (fasted study).

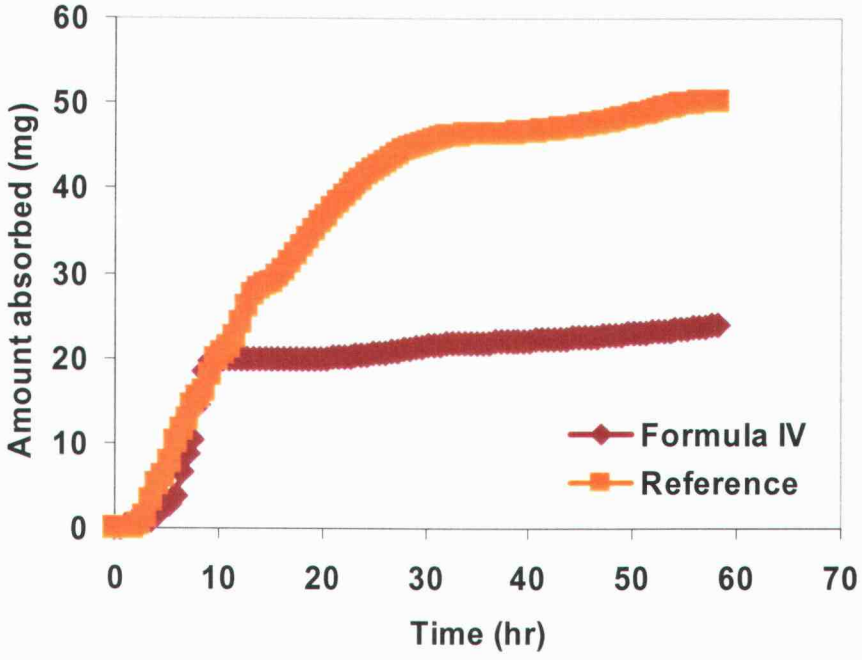

Figure A.55: Deconvolved input function of verapamil from subject #3 (fasted study).

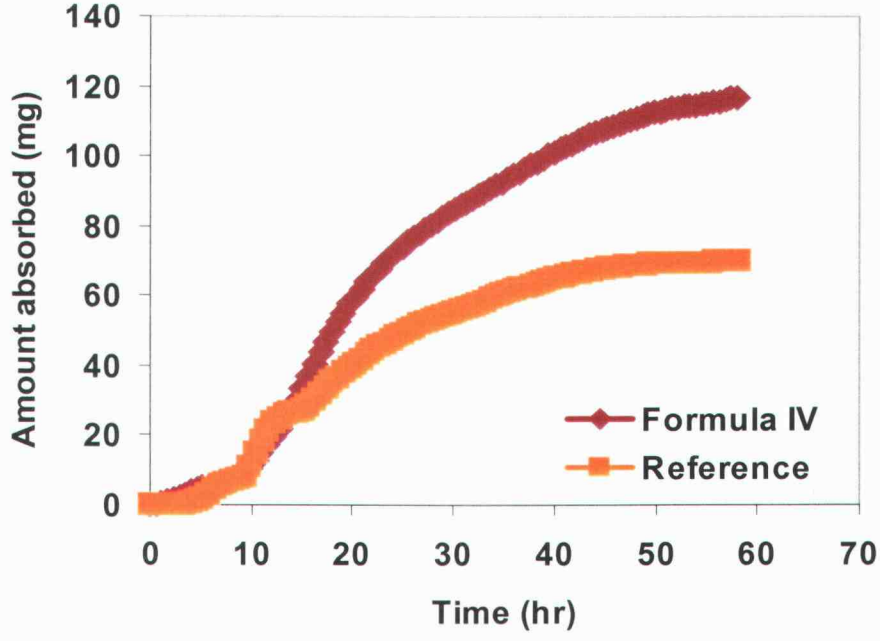

Figure A.56: Deconvolved input function of verapamil from subject #4 (fasted study).

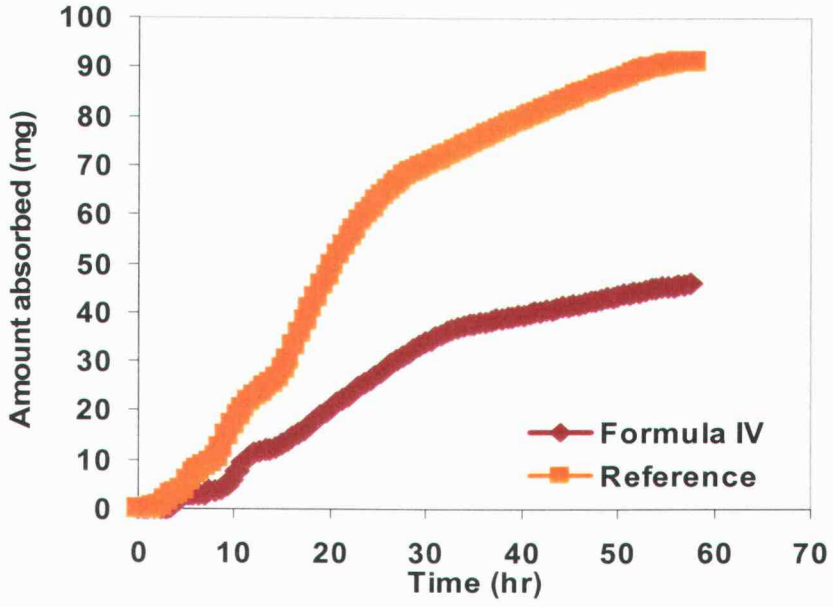

Figure A.57: Deconvolved input function of verapamil from subject #6 (fasted study).

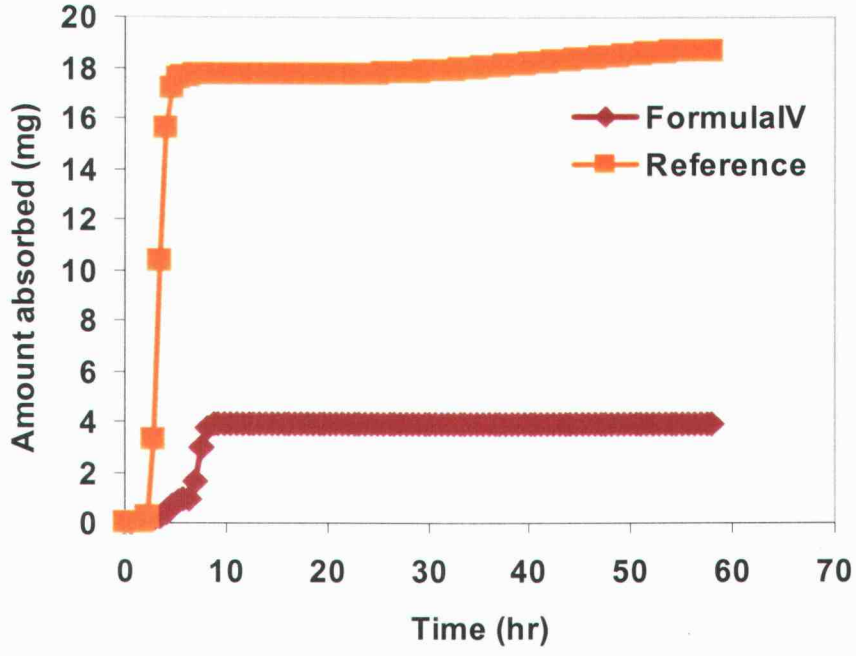

Figure A.58: Deconvolved input function of verapamil from subject #7 (fasted study).

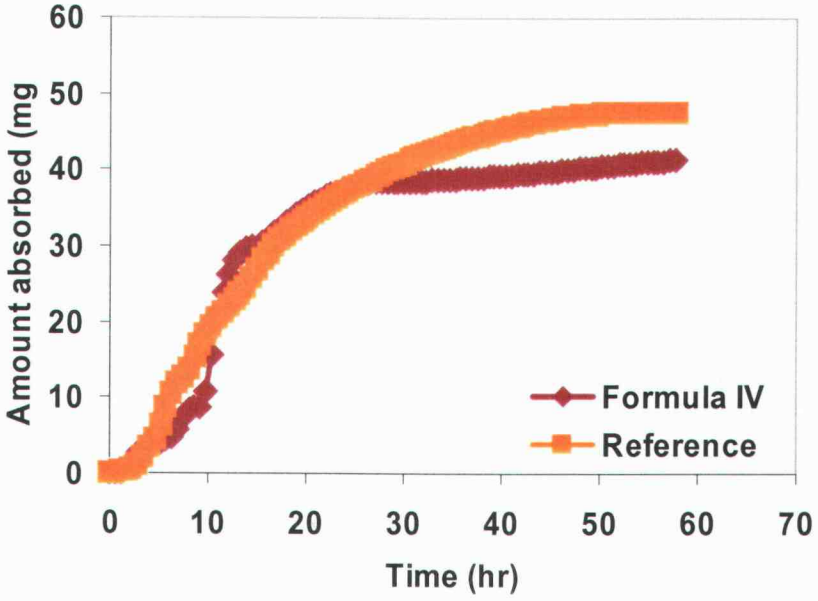

Figure A.59: Deconvolved input function of verapamil from subject #8 (fasted study).