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# A Nomogram for Valproic Acid and the Effect of Missed Doses

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# A NOMOGRAM FOR VALPROIC ACID AND THE EFFECT OF MISSED DOSES

A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Sciences at Virginia Commonwealth University.

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Dedication

To my parents, siblings and friends,

Without your support this would've been impossible.

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# Table of Contents

Page

ACKNOWLEDGEMENTS ..... iii

LIST OF TABLES ..... v

LIST OF FIGURES ..... vi

ABSTRACT ..... viii

1 INTRODUCTION AND LITERATURE REVIEW ..... 1

2 THE USE OF MONTE CARLO SIMULATIONS TO STUDY THE EFFECT OF POOR COMPLIANCE ON THE STEADY STATE CONCENTRATIONS OF VALPROIC ACID FOLLOWING ADMINISTRATION OF ENTERIC-COATED AND EXTENDED RELEASE DIVALPROEX SODIUM FORMULATIONS ..... 26

3 A RATIONAL DOSING NOMOGRAM FOR VALPROIC ACID BASED ON NONLINEAR PLASMA PROTEIN BINDING ..... 57

4 THE EFFECT OF DELAYED/MISSED DOSES FOLLOWING DEPAKOTE EXTENDED RELEASE ..... 90

5 CONCLUSIONS ..... 108

APPENDIXES

A CLINICAL STUDY PROTOCOL AND INFORMED CONSENT ..... 111

B HPLC ASSAY FOR THE DETERMINATION OF (-)-D-β-DIOXOLANE-THYMINE, A POTENTIAL ANTI-HIV AGENT, IN RAT PLASMA ..... 137

C REPRESENTATIVE CODES AND OUTPUT FILES ..... 153

# List of Tables

Page

Table 1.1: Pharmacokinetics of VPA in healthy adults, patients with epilepsy and healthy elderly.....24

Table 2.1: Summary of simulations scenarios for once daily ER.....43

Table 2.2: Summary of simulations scenarios for twice daily DR. .....44

Table 2.3: Dosing recommendations for ER based on free VPA. .....45

Table 2.4: Dosing recommendations for DR based on free VPA.....46

Table 3.1: Predictions for percent increase in dose based on individual estimates .....76

Table 3.2: Predictions for percent increase in dose based on population estimates .....77

Table 3.3: Root mean squared errors (RMSEs) and mean absolute errors (MAEs) for individual and population predictions.....78

Table 3.4: Dosing nomogram for VPA concentrations between 20-100 mg/l .....79

Table 3.5: Dosing nomogram for VPA concentrations between 100-200 mg/l .....80

Table 3.6: Individual plasma protein binding parameter estimates for a one saturable site model.....81

Table 3.7: Trough concentrations for nine volunteers .....82

Table 3.8: Volunteers demographics .....83

Table 3.9: Concomitant medication and adverse events at study conclusion .....84

Table 4.1: Parameters for original and revised simulation models.....102

Table B.1: Assay specifications for DOT .....149

# List of Figures

Page

Figure 1.1: Chemical structure of valproic acid.....25

Figure 2.1: Simulation scenarios for one missed dose on a once daily regimen. While at steady state, next dose was taken 6, 12, 18 hours late and then missed. In the last scenario missed dose is replaced.....47

Figure 2.2: Unbound (upper panel) and total (lower panel) VPA concentrations following single 1000 mg dose administration of ER (F=0.89) and DR (F=1) .....48

Figure 2.3: Unbound (upper panel) and total (lower panel) VPA concentrations following 500 mg bid of DR using average adult parameters. Different missed doses scenarios are shown .....49

Figure 2.4: Unbound (upper panel) and total (lower panel) VPA concentrations following 1000 mg QD of ER using average adult parameters. Different missed doses scenarios are shown .....50

Figure 2.5: Unbound and total VPA levels following administration of 2500 mg daily of the ER preparation. ER dose on day 7 was administered 6 h late. .....51

Figure 2.6: Unbound and total VPA levels following administration of 2500 mg daily of the ER preparation. ER dose on day 7 was missed.....52

Figure 2.7: Unbound and total VPA levels following administration of 2500 mg daily of the ER preparation. ER dose on day 7 was administered 18 hours late.....53

Figure 2.8: Unbound and total VPA levels following administration of 2500 mg daily of the ER preparation. ER dose on day 7 was missed. Dose was doubled on day 8.....54

Figure 2.9: Structural and statistical simulation model for the ER formulation .....55

Figure 2.10: Structural and statistical simulation model for the DR formulation .....56

Figure 3.1: Dose escalation Schematic .....85

Figure 3.2: Fraction bound as a function of total VPA concentrations (mcg/ml) for nine volunteers. The fitted line represents the population fit in NONMEM .....86

Figure 3.3: Simulations of bound versus unbound VPA concentrations (mcg/ml) for nine volunteers using parameters obtained from a one site saturable binding model .....87

Figure 3.4: Difference between individual predictions and nominal increases in dose.....88

Figure 3.5: Difference between population model predictions and percent increases in dose .....89

Figure 4.1: Missed doses schematic.....103

Figure 4.2: Structural and statistical model used to simulate missed doses .....104

Figure 4.3: Simulated total (upper panel) and free (lower panel) VPA concentrations for 100 subjects when a 1000 mg is taken 6 h late. Results for group one are superimposed (subjects 005, 007, 008).....105

Figure 4.4: Simulated total (upper panel) and free (lower panel) VPA concentrations for 100 subjects when a 1000 mg is taken 12 h late. Results for group two are superimposed (subjects 002, 003, 004).....106

Figure 4.5: Simulated total (upper panel) and free (lower panel) VPA concentrations for 100 subjects when a 1000 mg is missed followed by dose double-up. Results for group three are superimposed (subjects 001, 006, 009).....107

Figure B.1: Chemical Structure of (-)-D-β-dioxolane-thymine.....150

Figure B.2: Chromatograms of (a) blank rat plasma, (b) rat plasma with (1) 5 μg/ml of DOT and (2) 50 μg/ml internal standard added, (c) a rat plasma sample collected 1 h after intravenous administration of DOT to one rat and (d) a rat plasma sample collected 1 h after oral administration of DOT to a second rat .....151

Figure B.3: Concentration time-profiles following intravenous (circles) and oral administration (squares) of DOT to rats .....152

# Abstract

A NOMOGRAM FOR VALPROIC ACID AND THE EFFECT OF MISSED DOSES

By ALAA M. AHMAD, Ph.D.

A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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**Background**. Clinicians are divided on dosing recommendations when a dose is delayed or missed. For a neuropsychiatric agent like valproic acid (VPA), rational dosing recommendations are of particular importance. VPA is subject to therapeutic monitoring using total concentrations. Due to non-linear binding of VPA to plasma proteins, current dose titration schemes for VPA are empirical. The objectives of this research were to 1- study the effect of missed/delayed doses on steady state concentrations of VPA and 2- design a nomogram that can be used for dose titration based on total VPA concentrations.

**Methods.** 1- A simulation study was conducted to test for different poor compliance scenarios. The effect of missed doses was quantified and used to derive dosing recommendations. 2- A clinical study was carried out in healthy volunteers. Nine volunteers were administered 500, 750 and 1000 mg VPA in a dose escalation study. A nomogram was developed using *in vitro* plasma protein binding data in all volunteers and tested using dose escalation data. Several delayed/missed doses scenarios were tested in order to validate the simulation model. 3- A revised simulation model was developed using combined information from plasma protein binding and pharmacokinetic analysis of clinical study data.

**Results and Discussion.** Simulation study: Dosing recommendations in the case of a missed or delayed dose are both formulation and dose dependent. Results from the clinical study validated the simulation model and the revised simulation model properly incorporated intra and inter individual variabilities.

VPA nomogram: A one-site saturable binding model provided an adequate description of the binding of VPA to albumin. A dosing nomogram for VPA was constructed. To avoid the risk of achieving toxic concentrations, the dose should not be increased by more than 2 fold at a time. The nomogram should be used in conjunction with patient history and clinical response.

**Conclusions.** This research provides dosing recommendations to the clinicians to counsel patients taking preparations of VPA in the event of a missed dose. The use and

validation of VPA nomogram will foster the rational use of VPA for the treatment of epilepsy and its role in other neuropsychiatric disorders.

# CHAPTER 1

# INTRODUCTION AND LITERATURE REIVEW

## Epilepsy

About two million Americans have epilepsy (2-4%); of the 125,000 new cases that develop each year, up to 50% are in children and adolescents [1]. Epilepsy is a chronic condition of various etiologies characterized by a predisposition to recurrent, usually spontaneous, epileptic seizures. While a single seizure does not constitute epilepsy, two initial seizures occurring within a 24-hour period are considered to have the same significance as a single seizure. However, a single seizure accompanied by evidence of a cortical lesion (e.g. abnormalities on neurologic examination such as mental retardation or on neuro-imaging) or a single seizure accompanied by epileptiform abnormalities on electroencephalography (EEG) could serve as basis for the diagnosis of epilepsy. Therefore; an epileptic seizure is an abnormal and excessive discharge of brain neurons involving hyper-synchrony accompanied by some behavioral change. An Epileptic syndrome has been defined as "a clinical entity with relatively consistent clinical features, including seizure type(s), etiology, EEG features, neurologic status, prognosis, and, in some cases, response to specific antiepileptic drugs "[2].

There are several types of seizure according to the international classification of epilepsies [3].

Simple partial (focal) seizure is caused by a local cortical discharge, which results in seizure symptoms appropriate to the function of the discharging area of the brain without impairment of consciousness. Simple partial seizures may consist of motor, sensory, autonomic, or psychic signs or symptoms, or combinations of these.

Complex partial (psychomotor, temporal lobe) seizure. The crucial distinction between simple partial seizures and complex partial seizures is that consciousness is impaired in the latter and not in the former. Impaired consciousness is defined as the inability to respond normally to exogenous stimuli, owing to altered awareness or responsiveness. At the onset of a complex partial seizure, any of the symptoms or signs (motor, sensory, autonomic, or psychic) of a simple partial seizure might happen without impairment of consciousness providing an aura. The central feature of a complex partial seizure is impairment of consciousness, which may occur with or without a preceding simple partial aura. No other symptoms or signs maybe present during the period of impaired consciousness or automatisms might appear (i.e., unconscious acts that are that "automatic" and of which the patient has no recollection). The attack characteristically ends gradually, with a period of postictal drowsiness or confusion.

Absence (petite mal) seizures. These consist of sudden onset and cessation of impaired responsiveness, accompanied by a unique 3-Hz spike-and-wave EEG pattern. No aura is present, and little or no postictal symptomatology occurs. The majority of absence seizures last 10 seconds or less and may be accompanied by clonic, atonic or tonic components, automatisms, or autonomic components. Absence seizures usually first manifest between the ages of 5 and 12 years and often stop spontaneously in the teens.

Myoclonic seizures. Consist of brief, sudden muscle contractions that maybe generalized or localized, symmetric or asymmetric, synchronous or asynchronous. No loss of consciousness is generally detectable.

Tonic seizures. Consists of a sudden increase is muscle tone in the axial or extremity muscles, or both, producing a number of characteristic postures. Consciousness is usually partially or completely lost. Prominent autonomic phenomena occur. Postictal alteration of consciousness is usually brief but it may last several minutes. Tonic seizures are relatively rare and usually begin between 1 and 7 years of age.

Atonic seizures. Consist of sudden loss of muscle tone. The loss of muscle tone may be confined to a group of muscles, such as the neck, resulting in a head drop. Alternatively, atonic seizures may involve all trunk muscles, leading to a fall to the ground.

Clonic seizures. Occur almost extensively in early childhood. The attack begins with loss or impairment of consciousness associated with a sudden hypotonia or a brief, generalized tonic spasm. This is followed by 1 minute to several minutes of bilateral jerks, which are often asymmetric and may appear predominantly in one limb. During the attack, the amplitude, frequency and spatial distribution of these jerks may vary greatly from moment to moment. In other children, particularly those aged 1 to 3 years the jerks remain bilateral and synchronous throughout the attack. Postictally, recovery may be rapid, or a prolonged period of confusion or coma may ensue.

Tonic-clonic (grand mal) seizures. Before the tonic phase of a tonic-clonic seizure, bilateral jerks of the extremities or focal seizure activity might occur. The onset of the seizure is marked by loss of consciousness and increased muscle tone (tonic phase), which usually results in a rigid flexed posture at first, and a then a rigid extended posture. This is followed by bilateral rhythmic jerks that become further apart (clonic phase). Prominent autonomic phenomena are observable during the tonic and clonic phases. In the postictal phase, increased muscle tone occurs first, followed by flaccidity. Incontinence may occur. The patient awakens by passing through the stages of coma, confusional state, and drowsiness.

Some epilepsies remain unclassified due to lack of data. Another classification of seizures is based on etiologies: idiopathic, symptomatic or familial. There is also a

classification of epilepsy syndromes. A complete clinical discussion of seizure types and diagnosis can be found in many references [3, 4].

## Valproic Acid (VPA)

VPA was first used in France in 1967. It is effective against most seizures. It is also used in bipolar disorders and for migraine prophylaxis [5, 6].

## Chemistry

VPA (molecular weight = 144.2 g/mol), also called 2-propylpentanoic acid or dipropylacetic acid [7], is a C-8 branched chain fatty acid; see chemical structure in figure 1.1. Divalproex sodium is a common therapeutic form of the drug (e.g. Depakote), it's a coordination compound between sodium VPA and it's sodium salt in a 1:1 molar ratio. The free acid has a P$k_a$ of 4.56-4.8 [8].

## Routes of Elimination

Phase 1. The metabolism of VPA is complex due to the fact that several of the metabolites are formed by more than one route [9-14]. For example, 2-ene VPA, 3-OH VPA and 3-keto VPA [15] all result from β-oxidation (mitochondria). On the other hand, CYP-450 dependent (ω-, (ω-1)- and (ω-2)-hydroxylation transforms VPA to 5-OH-VPA, 4-OH-VPA, and 3-OH-VPA, respectively [16-18]. Some researchers have suggested CYP-450 accounts for a large percentage of VPA transformation to 3-OH-VPA [19, 20]. Some examples of desaturation (formation of double bonds) resulting from β-oxidation are 2-

ene-VPA, 3-ene-VPA 2,3`- diene-VPA and 2,4-diene-VPA [19-21]. The 2,4-diene-VPA, thought to play a role in the hepatotoxicity of VPA [21-25], is a product of the pathway that coverts 4-ene-VPA-CoA ester to the corresponding 2,4-diene-VPA-CoA. Experiments with human cDNA-expressed P450 isoforms have shown that multiple human CYP-450 isoforms are involved in the desaturation of VPA. These include CYP2C9 and CYP2A6 [25] as well as CYP2B6 [26].

Phase 2. Glucuronidation is the major pathway of VPA metabolism in several animal species [27] and in humans [28-30].

**Pharmacokinetics**

Many studies were conducted to characterize the disposition of VPA [31-39]. Table 1.1 summarizes the pharmacokinetic parameters of valproic acid in adult volunteers, patients with epilepsy and healthy elderly. The pharmacokinetics of VPA are unchanged in patients with epilepsy. Therefore, studies in healthy volunteers can be extrapolated to patients with epilepsy. Patients on polytherapy have increased clearance and require higher doses than monotherapy patients.

**Plasma Protein Binding (PPB)**

VPA PPB has been demonstrated very early. Most authors favor the presence of one saturable binding site on albumin [40-42]. A recent paper [43] looked at the PPB of VPA following rapid infusions (*ex vivo*). The authors used a two-saturable biding site model to

characterize the binding of VPA to albumin. The analysis assumed the presence of a high capacity, low affinity site ($N_1$=1.54± 0.108, $K_{a1}$ "association rate constant" =11.9±1.99 $mM^{-1}$, equivalent to a dissociation rate constant $K_d$= 0.084 mM or 12 mg/l) and a second low capacity, high affinity site ($N_2$= 0.194±0.0783, $K_{a2}$=164±141 $mM^{-1}$ and $K_d$= 0.006 mM or 0.88 mg/l). There is significant doubt around the presence of the second binding site. The overall effect of PPB of VPA in terms of contribution of binding sites does not seem to differ whether the saturable binding is accounted for through one or two sites (computer simulations results). The presence of two sites versus one site will be challenged in an *in vitro* study to characterize the protein binding of VPA in human plasma.

Since the PPB of VPA is saturable, the free fraction increases with total concentration. This means that the clearance and volume of distribution of total VPA increase as the free fraction increases. Since there is no evidence of saturable metabolism, the pharmacokinetics of free VPA are linear. The clearance and volume of distribution of free VPA remain constant as the dose increases. Since VPA has is a low hepatic extraction ratio drug, as the free fraction increases; total VPA concentration decreases while the concentration of free VPA increases slightly [44].

**Concentration-Response Relationships**

It is recognized through clinical experience with VPA that there is not a clear correlation between concentration and clinical efficacy; mainly seizure control. This reflects the fact that VPA has a relatively narrow therapeutic index and might have a

different therapeutic index in each patient [45]. A therapeutic range of 50-100 mg/l based on total concentrations has been used for a while [46]. There is trend nowadays to broaden the therapeutic range to 50-150 mg/l [3] based on evidence from clinical practice that some subjects require concentrations in that range for therapeutic benefit. There have been recent attempts to find pharmacodynamic markers to relate VPA dose to its effect for use in therapeutic drug monitoring. Using VPA monotherapy, it was shown that there is no relationship between VPA dose and EEG changes, multiple sleep latency test (MSLT), critical flicker fusion test (CFF) and other neurophysiologic tests in juvenile myoclonic epilepsy [47, 48].

**Activity against Epilepsy**

In 1978, VPA was approved for absence seizures. Efficacy in the treatment of absence seizures was compared to ethosuximide [49, 50]. VPA and ethosuximide were equally effective in epileptics with who are being treated for the first time. VPA is also effective against convulsive seizures. Children with generalized tonic-clonic seizures often respond to monotherapy with VPA [51]. VPA is recognized as the drug of first choice against myoclonic seizures [52]. Use in the symptomatic generalized epilepsies (Lennox-Gastaut syndrome) is not as successful neither is the use for prevention of infantile spasms occurring as part of West's syndrome. VPA has some activity against partial seizures, febrile seizures and status epilepticus [53].

**Activity against other Conditions**

Migraine. Several double blind, placebo-controlled studies have shown that VPA is effective against migraine [54-56]. VPA reduces migraine attacks frequency, duration and intensity in some cases. The fact that migraine and epilepsy co-exist commonly in patients facilitated the discovery of the therapeutic benefit of VPA. FDA approved VPA for use against migraine in 1996 [57].

Other Conditions. This includes activity against acute mania, depression, bipolar disorder, anxiety disorders, schizoaffective disorders and behavioral problems associated with dementia in elderly. More empirical uses for VPA continue to arise [58].

**Mechanisms of Action**

Since VPA has a wide spectrum of activity against epilepsies and other neuro-psychiatric disorders, it is thought to act through a combination of mechanisms. There is no agreement on the exact mechanism of action of VPA. VPA modulates gamma amino butyric acid (GABA) turn over and potentiates GABAergic functions in different regions of the brain [59], limits depolarization-induced sustained repetitive firing by an effect on voltage-sensitive sodium ion channels [60] and has an effect on calcium conductance [61]. VPA has an effect on the neuronal excitation mediated by the NMDA subtype of glutamate receptors, which may play a role in its antiepileptic effect [62].

**Adverse Effects**

VPA is one of the better studied neuropsychiatric agents in terms of side effects. Side effects range from mild to sometimes severe effects. Due to the multiple mechanisms of action of VPA, much is still to be found about the causes of these adverse events, their relation to the VPA concentration and the nature of some idiosyncratic reactions some patients may be more susceptible to than others. The major side effects of VPA include liver toxicity, which may be fatal and teratogenic effects. The following summarizes the side effects often encountered in clinical practice [63].

Gastrointestinal (GI) side effects. Nausea, vomiting, and dyspepsia are among the most common. These symptoms might occur in up to 25% of the population but are reduced by the use of the controlled-release preparation as Depakote Extended release and delayed release (enteric coated). Usually seen at initiation of therapy.

Weight gain. A frequent side effect might be severe to require discontinuation of treatment. Can be counteracted by conscious reduction of caloric intake.

Hair change. Occasional, in terms of hair thinning or alopecia (hair loss).

Tremor. During long-term valproate therapy, seen in 10% of the patients. Rarely is sufficiently severe to limit treatment. Experience indicates that it's dose-related [64].

Other neurological side effects. Somnolence (drowsiness), acute confusional states and irritability; these are usually seen with poly therapy. Sedation alone occurs in 2% of the patients. Effects on cognition might be associated with higher doses.

Metabolic disturbances. Hyperammonemia is common after valproate administration, may or not be associated with hepatic (liver) dysfunction. VPA Concentrations higher than 100 mg/l increase incidence [65]. Other metabolic disturbances include hyperglycinemia and hyperglycineyurea, with no clinical symptoms.

Transient amenorrhea. In young women after initiation of valproate therapy, may persist up for a year.

Idiosynctratic reactions. Hematologic (blood) side effects such as neutropenia, bone morrow suppression, thrombocytopenia and inhibition of platelets in the clotting process.

Hepatotoxicity. Usually in the form of dose-related elevation in liver enzymes in 40% of the patients, but not associated with clinical symptoms and is usually transient. May be dose related. It's believed that it might reflect enzyme induction rather than hepatotoxicity.

Hepatic failure. Overall incidence of 1/10,000 [66]. The primary risk, however, is for children under 2 years of age receiving valproate as poly therapy. The risk in this group

has a rate of 1/500. For patients older than 2 years this risk declines to 1/12,000. When valproate was given as mono therapy the risk of fatality from liver failure is 1/37,000 for all mono therapy patients [67], although it was still 1/7,000 for ages 0-2 years. Patients on valproate mono therapy older than 2 years had a fatality rate of 1/45,000. Clinical features associated with hepatic toxicity are nausea, vomiting, anorexia, jaundice and lethargy sometimes accompanied by edema.

Pancreatitis. Acute and is occasionally fatal. Complications may include pericardial infusion, laparotomy and wound infection. This condition is usually reversible with withdrawal from valproate.

Teratogenicity. In the form of neural tube defect. The occurrence rate in children born to epileptic mothers taking valproate is approximately 1-2%. Most maternal exposures to valproate do not appear to have adverse outcomes. May be related to high VPA concentrations.

**Formulations and Routes of Administration**

VPA can be administered orally, intravenously and rectally. It's available as a capsule (Deproic), soft gelatin capsule (Depakene) of 250 mg and syrup (250mg / 5ml) as sodium salt. Intravenous formulation of sodium salt (Depacon, 100 mg / ml) is also available. One common therapeutic form is divalproex sodium; supplied as tablets (125, 250 and 500 mg) available as Depakote sprinkle capsules, delayed-release (DR) and

extended release (ER) tablets [5]. The ER formulation is formulated as a hydrophilic polymer matrix controlled-release tablet system to provide more consistent blood concentrations over 24 hours. This system results in the release of drug in the stomach, small intestine, and large intestine over an 18- to 24-hour period of time. The DR formulation is enteric coated and thus is stable in the stomach but dissolves in the small intestine and thus minimizes gastro-intestinal side effects associated with immediate-release forms of VPA.

**Dosing Recommendations**

For epilepsy, the recommended dose for monotherapy is 10-15 mg/kg daily. The dose is then increased as needed by 5-10 mg/kg/day increments, as tolerated. Doses in the range of 30-60 mg/kg/day are needed for Polytherapy [53].

For migraine, start with 250 mg/day and then increase to 500 mg/day and 750 mg/day if needed [68]. For other psychiatric conditions, start at 20-30 mg/kg/day and increase dose based on tolerance and response [58].

**Clinical Trial Simulation (CTS)**

CTS is being increasingly used as a fundamental tool in drug development. CTS aids in the design of clinical studies with an overall goal of improving the efficiency of drug development. With introduction of population-based modeling, a better understanding of the impact of variability encountered in clinical studies has evolved and now there are

mathematical methods to quantify this variability. CTS uses the models established in population modeling and the values obtained as quantifiers of the intra- and inter- subject variabilites to look at hypothetical scenarios both for pharmacokinetics and pharmacodynamics. CTS can also be used to improve the power of different studies through design optimization. As such, CTS is essentially an application of Monte Carlo Methods, which find uses in aerospace, engineering, physics and chemistry.

The simulation model consists of an Input-Output Model which is in this case is a Pharmacokinetic model. The pharmacokinetic model is called a structural model.
The element of parameter variability will be as a stochastic model. The stochastic model includes: a) a population parameter variability comprising between-subject and within-subject variability in model parameters. In practical terms, within-subject variability is largely defined by between-occasion variability but includes stochastic variation in parameters, such as clearance, that may occur within an occasion (within-occasion variability). A term often used for population parameter variability is inter-individual variability. b) Residual unexplained variability accounts for model misspecification and measurement error. A term often used for residual unknown variability is intra-individual variability [69].

The other part of the simulation model is called covariate distribution model, which determines the distribution of the demographic covariates in the trial subject sample (age,

weight etc.). The last part of the model is the trial execution model [69]. For example, the simulation protocol will incorporate different scenarios of poor compliance.

Technically speaking, In order to perform a Monte Carlo simulation, the sampling distribution of the model parameters (inputs) must be defined *a priori*, for example a normal distribution with mean $\mu$ and variance $\sigma^2$. Monte Carlo Simulation repeatedly simulates the model, each time drawing a different set of values (inputs) from the sampling distribution of the model parameters, the result of which is set of possible outcomes (outputs). The usual source for information on the distribution and covariance between the pharmacokinetic parameters is the result of a population pharmacokinetic analysis where an underlying structural pharmacokinetic model has been established and an assessment of the distribution; inter- and intra-individual variability of a set of pharmacokinetic parameters has been defined [70].

**Research Objectives**

This dissertation aimed to address the following:

First, the effect of missed doses on steady state concentrations of VPA (total and free) following the administration of Depakote extended release (ER) and Depakote delayed release (DR) was characterized using simulations.

Secondly, a nomogram for VPA that can be used to titrate VPA doses based on total concentrations to compensate for missed/delayed doses was developed. The nomogram was constructed using nonlinear plasma protein binding data and tested a in a clinical trial.

Thirdly, a simulation model that properly incorporates intra- and inter-individual variabilities in unbound and bound VPA was developed, validated and can be used to make dosing recommendation in the case of missed/delayed doses

**Clinical Significance**

Clinicians are divided on how to replace doses during therapy with VPA and it's unethical to withhold therapy from patients with epilepsy to explore the effect of missed and delayed doses. This research provides dosing recommendations to the clinicians to counsel patients taking preparations of VPA in the event of a missed dose. The use and validation of VPA nomogram will foster the rational use of VPA for the treatment of epilepsy and its role in other neuropsychiatric disorders.

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Table 1.1 Pharmacokinetics of VPA in healthy adults, patients with epilepsy and elderly.

| | | | | | | Clearance (L/h/kg) | |
|---|---|---|---|---|---|---|---|
| Category | Study Number of subjects | Mono/Poly Therapy | VPA Regimen | $V_d$ (L/kg) Total | $T_{1/2}$ (h) | Total VPA | Free VPA |
| Healthy adults (< 60 years) | Perucca et al.[31], n=6 | N/A | Single dose (800 mg) ORAL | 0.14± 0.02 | 13.0± 2.4 | 0.0077± 0.0015 | 0.127± 0.029 |
| | Bialer et al.[32, 33], n=6 | N/A | Single dose (1000 mg) ORAL | 0.14± 0.02 | 14.9± 2.4 | 0.0067± 0.0014 | 0.17± 0.46 |
| | Gugler et al.[34], n=6 | N/A | Steady state (1200) mg/day ORAL | 0.15± 0.02 | 15.9±2.6 | 0.0064± 0.0011 | - |
| | Bowdle et al.[35], n =6 | N/A | Steady State (500 mg/day) ORAL | 0.13± 0.02 | 13.6±2.8 | 0.0067± 0.0013 | 0.089± 0.071 |
| Patients with epilepsy (<60 years) | Miljkovic et al.[36], n=10 | Mono therapy | Single dose (900 mg) ORAL. | 0.20± 0.04 | 15.0± 4.0 | 0.0094± 0.0029 | - |
| | Sundqvist et al.[37], n=16 | Mono therapy | Steady State (500 mg b.i.d) ORAL | - | - | 0.011± 0.003 | 0.126± 0.044 |
| | | | Steady State (1000 mg b.i.d) ORAL | - | - | 0.015± 0.004 | 0.118± 0.067 |
| | Perucca et al.[38], n=6 | Poly therapy | Single dose (800 mg) I.V | 0.18± 0.03 | 9.0± 1.4 | 0.015± 0.006 | - |
| | | | Single dose (800 mg) ORAL | 0.18± 0.03 | 9.0± 1.2 | 0.0176± 0.0028 | - |
| | Schapel et al.[39], n=17 | Poly therapy | Single dose (600 mg) ORAL | 0.19± 0.09 | 9.3± 2.0 | 0.015± 0.0058 | - |
| Healthy elderly (>60 yrs) | Perucca et al.[31], n=6 | N/A | Single dose (800 mg) ORAL | 0.16± 0.02 | 15.3± 1.7 | 0.0075± 0.0022 | 0.078± 0.015 |

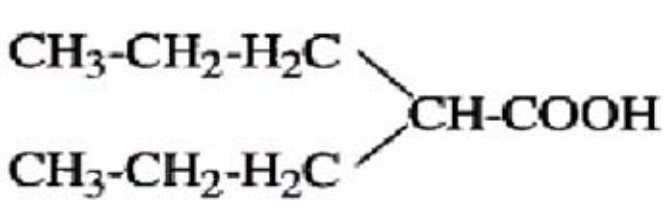


Figure 1.1 Chemical structure of valproic acid.

# THE USE OF MONTE CARLO SIMULATIONS TO STUDY THE EFFECT OF POOR COMPLIANCE ON THE STEADY STATE CONCENTRATIONS OF VALPROIC ACID FOLLOWING ADMINISTRATION OF ENTERIC-COATED AND EXTENDED RELEASE DIVALPROEX SODIUM FORMULATIONS

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**ABSTRACT**. Divalproex sodium extended-release (Depakote® ER) is a once daily (QD) formulation for valproic acid that was developed to improve patient compliance and reduce side effects compared to the standard twice-daily (BID) delayed release (DR) formulation (Depakote® tablets). However, there are concerns of potential sub-therapeutic concentrations following delayed or missed doses or toxic concentrations with replacement doses for the ER and DR formulations. Simulations can be used to investigate the effect of poor compliance on drug concentrations, which may not be possible to do in a study population due to ethical or practical reasons. Using Monte Carlo simulations, the effect of different patterns of poor compliance on ER QD and DR BID were systematically characterized. Non-linear binding of valproic acid to albumin was incorporated into the model, and the results were based on total and unbound VPA for comparison. The effect of poor compliance is less significant on DR BID compared to ER QD. Dosing recommendations in the case of a missed or delayed dose are both formulation and dose dependent. Since total VPA concentrations show higher inter-individual variability and tend to under-estimate the effect of poor compliance; the use of unbound VPA concentrations may offer an advantage in therapeutic monitoring.

**Introduction**

Valproic acid (VPA) is a broad-spectrum antiepileptic drug effective against a wide variety of seizures, including complex partial, tonic-clonic convulsive, absence and myoclonic seizures. It is also active against neonatal seizures, febrile seizures and status epilepticus. Valproic acid is also used in the treatment of bipolar disorders and for the prophylaxis of migraine headaches [1].

VPA is rapidly absorbed from its oral forms and has no first pass metabolism. It binds to plasma proteins and this binding is concentration dependent. As such, the free fraction of VPA increases from 10% at 40 mg/l to 18.5% at 130 mg/l [1]. The plasma protein binding of VPA decreases in the elderly, hepatic and renal impairment and in the presence of other medications that have higher binding affinity to the same binding site on albumin e.g. aspirin. This non-linear binding renders the kinetics of the total VPA non-linear i.e. the total concentration increases less than proportionally with the dose and the clearance and volume of distribution of total VPA increase with the free fraction. The kinetics of the unbound drug are linear. VPA is metabolized almost entirely by the liver. 30-50 % of the dose is appears in the urine as glucuronide conjugate. Mitochondrial β-oxidation is the other major pathway accounting for over 40% of the dose. Less than 15-20 % of the dose is eliminated by other oxidative mechanisms and less than 3% of the dose is eliminated as the unchanged drug in the urine [1]. Mean plasma clearance and volume of distribution for total valproate are 0.56 L/h and 11 L respectively. For free valproate mean plasma clearance and volume of distribution are 4.6 L/h and 92 L. Mean

terminal half-life for valproate mono therapy ranged from 9-16 hours following oral doses of 250-1000 mg [1].

Divalproex sodium extended-release (Depakote® ER) is a once daily (QD) VPA formulation that was developed to improve patient compliance and reduce side effects compared to the standard twice-daily (BID) delayed release (DR) formulation (Depakote® tablets). The ER formulation is formulated as a hydrophilic polymer matrix controlled-release tablet system to provide more consistent blood concentrations over 24 hours. This system results in the release of drug in the stomach, small intestine, and large intestine over an 18- to 24-hour period of time. The DR formulation is enteric coated and thus is stable in the stomach but dissolves in the small intestine and thus minimizes gastro-intestinal side effects associated with immediate-release forms of VPA.

Compliance with anti epileptic drug therapy is a major determinant of successful treatment [2, 3]. It has been shown that once daily dosing improves patients' adherence to medication and thus therapeutic outcomes [4]. Although once daily administration offers more convenience, there are concerns about using a QD regimen for an antiepileptic drug such as VPA. If a patient misses a dose, there is the possibility of attaining sub-therapeutic drug concentrations, which could lead to break-through seizures. Further, if the next dose is doubled, drug concentrations may rise above the therapeutic index leading to central nervous system (CNS) toxicity. This being the case, there is a need to

determine the effect of poor compliance on the VPA concentrations following administration of the ER and DR formulations.

It is unethical to purposefully withhold doses from patients with epilepsy to determine the effect of poor compliance on drug concentrations. An alternative approach to studying poor compliance is to use computer simulations. To account for inter-individual variability, the simulations should generate a hypothetical population. This can be achieved using Monte Carlo simulations, which involve the deliberate use of random numbers in a calculation that has the structure of a stochastic process [5]. This stochastic process can be used to study the long-term effect in model variability on the outcome of the model [6]. Once the hypothetical population is generated, poor compliance scenarios can be introduced artificially by delaying doses from schedule, missing doses and adding make-up doses to the schedule. The effect of poor compliance on the population can then be studied quantitatively.

The purpose of this study was to study the effect of delaying, missing and replacing doses on VPA concentrations following both the ER and DR preparations using Monte Carlo simulations. The results will improve the clinicians' ability to consult patients if they delay or miss a dose of VPA.

## Methods

### *Study Design*

Each simulation included 100 hypothetical subjects. The random seed was set manually to (230, 240, 120, 100). The model parameters used were derived from an adult population and there were no covariate (age, weight, gender) distribution models for the virtual trial population. Subjects were assumed to be healthy and on valproate mono therapy. The simulations assumed that the extended release (ER) formulation was administered once daily and delayed-release (DR) preparation was administered twice daily. Unbound and total valproic acid concentrations were simulated from the time of dose administration to 280 h. The simulations were based on the administration of 1000 mg ER once daily, 500 mg DR twice daily, 2500 mg ER once daily and 1000 mg DR twice daily.

Hypothetical populations were generated at steady state for each drug administration schedule. Briefly, values for pharmacokinetic parameters means and estimates of variability were obtained from the literature and used to generate plausible parameters ranges based on a normal or lognormal distribution. The trial simulator software would then draw a set of parameters for each subject that defines its concentration time profile. Different poor-compliance scenarios were introduced to concentration-time profiles at steady state with one or two missed, delayed and replacement doses from schedule. For once-daily regimens, simulations' scenarios included doses taken 6, 12, 18, 24 hours late from schedule and then two doses taken 24

hours late (replacement dose for the missed dose), see Figure 2.1 for demonstration of doses. For twice-daily regimens, doses were simulated 3, 6, 9, 12 hours late from schedule and then two doses were simulated 12 hours late (replacement dose for the missed dose). More extreme cases where *two* doses are delayed at various time or missed were also simulated.

*Structural model*

A one-compartment model with first order elimination was used to simulate unbound VPA concentrations. The two formulations differ only in the input function: the ER formulation is accounted for through a zero order input over 22 hours with 89% bioavailability [7]. The DR formulation absorption is characterized by a 2 h lag time ($t_{lag}$= 2 h) followed by first order absorption rate ($k_a$= 0.1 $h^{-1}$). The bioavailability of the DR preparation is assumed to be complete (F=1) [8].

Equation 1 was used to simulate unbound VPA concentrations ($C_u$) following administration of the DR preparation and equation 2 was used to simulate $C_u$ following the ER formulation:

$$C_u = (k_a * D / V (k_a - CL_u/V_u) (e^{-CL_u/V_u t} - e^{-kat}) \quad \text{eq.1}$$

$$C_u = F*D/CL_u*T (e^{CL_u/V_u *T} - 1) e^{-CL_u/V_u t} \quad \text{eq.2}$$

where D is the dose, $V_u$ is the volume of distribution of unbound drug, $CL_u$ is the systemic clearance of unbound drug, F is bioavailability, $k_a$ is the first order absorption rate constant for DR and T is the duration of the zero order input for ER [9].

The following equation was used to calculate the total VPA concentrations ($C_t$) [7]:

$$C_t = C_u + (N_1K_1C_uP)/(1+K_1C_1) + (N_2K_2C_uP)/(1+K_2C_u) \quad \text{eq.3}$$

Where P is albumin concentration, $N_1$ and $K_1$ and $N_2$ and $K_2$ are the number of binding sites and equilibrium association constants for a low affinity - high capacity binding site and high affinity - low capacity binding site, respectively.

*Parameters and Parameter Distributions*

Random pharmacokinetic parameter means ±SD and *a priori* distributions of random model parameters were used for the simulations. Geometric means ± SD for $CL_u$ and $V_u$ were 5.04 ±1.00 L/h and 95.1 ± 19.0 L, respectively [1]. These parameters were assumed to by distributed log-normally. Protein binding parameters (obtained *ex vivo*) [5], assumed to be normally distributed, were: $N_1 = 1.54 \pm 0.108$, $K_1 = 11.9 \pm 1.99$ $mM^{-1}$, $N_2 = 0.194 \pm 0.0783$, $K_2 = 164 \pm 141$ $mM^{-1}$, and $P = 0.528 \pm 0.0528$ mM. Limits of ± 2 standard deviations were placed on all parameters for the simulations (this will allow inclusion of a reasonable ~ 95% of the population generated while avoiding contamination from outliers).

The simulations were performed using Pharsight® Trial Simulator ™ (Pharsight Corporation, Mountain View, CA) which uses Monte Carlo simulations based on a stochastic model to approximate the distribution of probable outcomes for a clinical trial [11].

*Summary of simulations' outcomes*

VPA concentration-versus time profiles were generated for each scenario. Drug concentrations were compared to the therapeutic range of valproic acid. Based on total VPA, a therapeutic range of 50-150 mg/l was assumed [1, 12]. The lower limit for the therapeutic range for unbound VPA was 5 mg/l; (at total concentrations of 50 mg/l, almost 90% of the binding sites are occupied [1], therefore the free fraction is = 10%). There is no accepted upper limit for the therapeutic range of unbound VPA. In order to assess the effect of missed or delayed doses, the simulations' outcomes were summarized by:

1- Number of subjects with sub-therapeutic concentrations after delayed or missed doses quantified as the percentage of subjects having total drug concentrations lower than 50 mg/l or unbound VPA concentrations less than 5 mg/l. Sub-therapeutic subjects at base line steady state were excluded from poor adherence scenarios.

2- The time range that subjects spent in the sub-therapeutic range or 'time at risk', in hours, which is essentially the duration of time where subjects might be at risk of breakthrough symptoms.

3- Number of subjects with drug concentrations above the upper limit of the therapeutic range quantified as the percent of subjects with total VPA concentrations exceeding 150 mg/l. This percentage reflects the probability of potential toxicity.

**Results**

Figure 2.2 shows the simulated unbound and total valproic acid concentrations following administration of 2500 mg daily of the ER preparation; ER dose on day 7 was administered six hours late from schedule (30 hours after the last dose on day six). Figure 2.3 shows the effect of missed dose followed by a double up on dose. The results of the scenarios for subjects taking 1000 and 2500 mg ER on a once daily regimen are summarized in Table 2.1. The percentage of subjects on ER 1000 mg that had sub-therapeutic concentrations due to poor compliance varied from (43-100%) with respect to $C_u$ (< 5mg/l) and from (28-100%) with respect to $C_{tot}$ (<50 mg/l). The mean times at risk varied from (6-60 h) with respect to $C_u$ and from (8-53 h) with respect to $C_{tot}$. None of the subjects on ER 2500 mg QD had sub-therapeutic concentrations even if one dose is delayed six hours from schedule. Almost 50% of the population had sub-therapeutic concentrations if one dose (ER 2500) is missed from schedule while all subjects will be sub-therapeutic if two doses are missed. The mean time at risk varied from (0-28 h). Regarding potential toxicity ($C_{tot}$ >150 mg/l), 52% of the population might experience toxic concentrations if two doses are taken 66 h after last dose while on ER 2500 mg QD.

Table 2.2 shows results for subjects taking 500 and 1000 mg DR on a twice-daily regimen. For DR 500 mg BID, the percentage of subjects that had sub-therapeutic concentrations due to poor compliance varied from (3-88 %) with respect to $C_u$ and from (3-77%) with respect to $C_{tot}$. The mean time at risk varied from (1-14 h). None of the subjects experienced sub-therapeutic concentrations if one dose is delayed or missed

from a DR 1000 mg BID regimen. However, if two doses are delayed from schedule, 1-24 % of the population might have sub-therapeutic concentrations. The mean time at risk varied from (2-6 h).

Dosing recommendations following missed doses of ER and DR VPA formulations are shown in Tables 2.3 and 2.4, respectively.

**Discussion**

Epilepsy is a chronic condition of various etiologies characterized by a predisposition to recurrent, usually spontaneous, epileptic seizures. About two million Americans have epilepsy; of the 125,000 new cases that develop each year, up to 50% are in children and adolescents [13]. Where there are several therapeutic alternatives for epilepsy, VPA remains one of the most widely used antiepileptic drugs. VPA is also used for other neuropsychiatric disorders such as migraine prophylaxis, bipolar disorder and mania.

Maintaining VPA concentration in the therapeutic range will determine the effectiveness of chronic therapy for patients with epilepsy. It is well recognized that patients will miss doses and that “ideal” steady state generated by computer models after multiple dosing is a rare finding in clinical practice. Although missing doses from the therapeutic regimen has been recognized as a random process with no specific patterns, it is possible to assume some general yet representative scenarios for missed doses that will

allow formation of dosing recommendations in the case a missed dose occur. In this study we introduced several representative and realistic scenarios to quantify the effect of missed doses on VPA concentration in the case of chronic monotherapy.

The effect of delayed or missed doses on steady state VPA concentrations depends on the dose and formulation administered. At a dosing regimen of ER 1000 mg QD, VPA concentrations in every subject in the hypothetical population hit the sub-therapeutic range when one dose was missed. This was due largely to steady state total drug concentrations for all subjects of less than 100 mg/l. For the higher dose of 2500 mg ER QD, the effect of delaying a dose was negligible for up to 12 hours after the last dose. Nevertheless, almost 50% of subjects had sub-therapeutic concentrations in the case of a missed dose while on 2500 mg QD.

The effect of delaying or missing two doses is more pronounced for both dose concentrations (ER 1000 and 2500 mg QD). For ER 1000 mg QD, there is a considerable risk of sub-therapeutic concentrations and the two missed doses must be replaced as soon as possible. Risk for sub-therapeutic concentrations for the 2500 mg QD is reduced since subjects have higher drug concentrations to begin with. Making up for two doses (one missed, one delayed) did show a significant risk of toxicity when two doses of ER 2500 mg are taken 54 h (14%), 60 h (32%) and 66 h (52%) after last dose, where numbers in brackets refer to percentage of subjects that have total VPA concentrations greater than 150 mg/l.

VPA concentrations after DR 500 mg BID were slightly higher for the overall population compared to ER 1000 mg QD because of the lower bioavailability of the ER formulation. It is the expectation that the effect of poor compliance in terms of delaying or missing a dose would be less pronounced on DR in terms of average times spent in the sub-therapeutic range or time at risk, which is the case.

On the higher DR dose of 1000 mg BID, there is virtually no risk of sub-therapeutic concentrations even after a dose is missed with no replacement. Dose double up did not result in toxicity. Taking two doses of DR 1000 mg at various times after the last dose up to 36 hours didn't elevate VPA concentrations to the toxic range.

We based our conclusions on both unbound and total VPA. Total and unbound VPA plasma concentrations can be determined using a commercially available fluorescence polarization immunoassay (FPAI) on the $TD_x$/$TD_x$ $FL_x$ system from Abbott Laboratories. Despite this, clinicians rely on total VPA concentrations to individualize therapy with valproic acid. The simulations showed that the times at risk based on total VPA are wider than the ones based on unbound VPA; reflecting the added variability arising from inter-individual variability in protein binding. It also seems that numbers of subjects in the sub-therapeutic range tended to be less when based on total VPA. This means that the effect of delayed and/or missed doses can be under-estimated when based on total VPA: patients may still seize but $C_{tot}$ seems to be in the therapeutic range. Since it is generally accepted that, the unbound form of the drug is associated with therapeutic

actions and adverse effects; unbound valproic acid concentrations may offer an advantage when used for therapeutic drug monitoring.

This simulation study has several limitations. Monte Carlo simulations generate hypothetical populations with certain characteristics based on a structural model. Intra- and inter-individual variability are introduced into the virtual trial population and do not necessarily reflect the overall population. Although the simulations performed scan a wide range, there are some subjects in real practice that are not necessarily accounted for. The introduction of covariates into the model will make inferences from these simulations more specific to certain sub populations. While the therapeutic range for VPA is believed to be between 50-150 mg/l based on total VPA, it should be realized that some patients with epilepsy have a higher minimally effective concentration. These patients usually require higher doses than regular doses. Unfortunately, there is no clear relationship between VPA concentrations and clinical effect that has been demonstrated so far [1]. This hindered the use of a PK/PD model in our simulations. Another important point to consider is that mono therapy or un-induced state was assumed. This study cannot be extrapolated to patients on poly-therapy.

**Summary**

This study reports a systematic investigation of the outcome of poor compliance on once daily ER and twice daily DR formulations using computer simulations. Higher doses of the ER preparation (2500 mg QD) can be used to provide adequate seizure

control with dose delays up to 12 hours. For unstable seizure patients, it is recommended that patients maintain a twice-daily regimen since twice-daily regimens are less susceptible to fluctuations in steady-state concentrations in the case of poor-compliance. Having a shorter dosing interval, twice daily regimens demonstrate better maintenance of drug concentrations in the case of delayed or missed doses.

*Appendix*

Derivation of eq 2: $C_u = F*D/CL_u*T\ (e^{CL_u/V_u*T} - 1)\ e^{-CL_u/V_u t}$ where

$CL_u/V_u = K$ and $F*D/CL_u*T = FK_o/V_uK$, where $K_o$ is the input rate in unit/time

This equation can be shown to account for pre and post release phases as follows:

- If t<T then release occurred for time=t

$C_u = FK_o/V_uK\ (e^{Kt} - 1)\ e^{-Kt}$, so $C_u = FK_o/V_uK\ (e^{(K-K)t} - e^{-Kt})$ which reduces to

$C_u = FK_o/V_uK\ (1 - e^{-Kt})$ this is also $= C_{max}$. It is clear that this same equation holds if t=T.

- If t>T, and t* is time after input ends (t=T+t*), now T is a constant referring to the duration of input

$C_u = FK_o/V_uK\ (e^{KT} - 1)\ e^{-K(T+t^*)}$) follows that

$C_u = FK_o/V_uK\ (e^{K(T-t^*-T)} - e^{-K(t+T^*)})$ and

$C_u = FK_o/V_uK\ (e^{K(-t^*)} - e^{-K(T+t^*)})$ so

$C_u = FK_o/V_uK\ (e^{K(-t^*)} - e^{-KT} * e^{-Kt^*})$ which after taking $e^{-Kt^*}$ as a common factor becomes

$C_u = FK_o/V_uK\ e^{K(-t^*)}\ (1 - e^{-KT})$ or $C_u = FK_o/V_uK\ (1 - e^{-KT})\ e^{-Kt^*}$ where

$FK_o/V_uK\ (1 - e^{-KT}) = C_{max}$

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Table 2.1 Summary of simulations scenarios for once daily ER

| ER 1000 mg | $C_u$ | | | $C_{tot}$ | | | |
|---|---|---|---|---|---|---|---|
| One dose taken 'x' hour after last dose | % Sub therapeutic (<5 mg/l) | Time at risk hours | | % Sub-therapeutic (<50 mg/l) | Time at risk hours | | % Subjects with $C_{tot}$ > 150 mg/l |
| | | Range | Mean | | Range | Mean | |
| 30 | 43 | 1-20 | 6 | 28 | 1-24 | 8 | 0 |
| 36 | 78 | 1-25 | 13 | 65 | 1-26 | 12 | 0 |
| 42 | 90 | 1-27 | 18 | 82 | 2-29 | 16 | 0 |
| 48, missed dose | 96 | 1-53 | 28 | 81 | 1-52 | 26 | 0 |
| 48, two doses | 96 | 1-29 | 20 | 83 | 1-32 | 19 | 0 |
| Two doses taken 'x' h after last dose | | | | | | | |
| 54 | 100 | 7-37 | 28 | 100 | 1-40 | 25 | 0 |
| 60 | 100 | 14-44 | 34 | 100 | 6-47 | 31 | 0 |
| 66 | 100 | 21-48 | 40 | 100 | 13-50 | 37 | 0 |
| 72, two doses | 100 | 28-54 | 46 | 100 | 9-59 | 43 | 0 |
| 72, one dose | 100 | 39-83 | 60 | 100 | 19-86 | 53 | 0 |
| ER 2500 mg | $C_u$ | | | $C_{tot}$ | | | |
| One dose taken 'x' hour after last dose | % Sub therapeutic (<5 mg/l) | Time at risk hours | | % Sub therapeutic (<50 mg/l) | Time at risk hours | | % Subjects with $C_{tot}$ > 150 mg/l |
| | | Range | Mean | | Range | Mean | |
| 30 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 36 | 3 | 1-3 | | 5 | 2-4 | 3 | 2 |
| 42 | 20 | 1-11 | 5 | 16 | 1-12 | 6 | 5 |
| 48, missed dose | 46 | 2-17 | 8 | 47 | 1-24 | 8 | 0 |
| 48, two doses | 51 | 1-17 | 7 | 46 | 1-17 | 7 | 8 |
| Two doses taken 'x' h after last dose | | | | | | | |
| 54 | 73 | 1-23 | 11 | 68 | 1-23 | 11 | 14 |
| 60 | 84 | 1-29 | 12 | 80 | 3-30 | 15 | 32 |
| 66 | 91 | 1-35 | 21 | 88 | 3-37 | 20 | 52 |
| 72, two doses | 91 | 1-41 | 26 | 92 | 1-46 | 25 | 3 |
| 72, one dose | 100 | 1-42 | 28 | 95 | 3-43 | 27 | 0 |

Table 2.2 Summary of simulation scenarios for twice daily DR.

| DR 500 BID | $C_u$ | | | $C_{tot}$ | | |
|---|---|---|---|---|---|---|
| One dose taken 'x' hour after last dose | | | | | | |
| | % Sub- therapeutic (<5 mg/l) | Time at risk hours | | % Sub- therapeutic (<50 mg/l) | Time at risk hours | |
| | | Range | Mean | | Range | Mean |
| 15 | 3 | N/A | 1 | 3 | 1-4 | 2.5 |
| 18 | 11 | 1-7 | 4 | 10 | 1-12 | 5 |
| 21 | 25 | 1-10 | 5 | 21 | 1-13 | 5 |
| 24, missed dose | 39 | 1-33 | 12 | 34 | 1-37 | 12 |
| 24, two doses | 39 | 1-13 | 6 | 29 | 1-16 | 7 |
| Two doses taken 'x' hour after last dose | | | | | | |
| 27 | 64 | 1-17 | 7 | 43 | 1-21 | 8 |
| 30 | 76 | 1-21 | 10 | 61 | 1-25 | 9 |
| 33 | 90 | 1-23 | 12 | 75 | 1-26 | 11 |
| 36 | 88 | 2-29 | 14 | 77 | 1-31 | 14 |
| DR 1000 BID | $C_u$ | | | $C_{tot}$ | | |
| One dose taken 'x' hour after last dose | % Sub therapeutic (<5 mg/l) | Time at risk hours | | % Sub therapeutic (<50 mg/l) | Time at risk hours | |
| | | Range | Mean | | Range | Mean |
| 15 | 0 | N/A | 0 | 0 | N/A | 0 |
| 18 | 0 | N/A | 0 | 0 | N/A | 0 |
| 21 | 0 | N/A | 0 | 0 | N/A | 0 |
| 24, missed dose | 0 | N/A | 0 | 0 | N/A | 0 |
| 24, two doses | 0 | N/A | 0 | 0 | N/A | 0 |
| Two doses taken 'x' hour after last dose | | | | | | |
| 27 | 1 | 2 | 2 | 4 | 2-3 | 2.2 |
| 30 | 5 | 1-6 | 3 | 19 | 1-8 | 4 |
| 33 | 19 | 1-9 | 3 | 15 | 1-11 | 5 |
| 36 | 24 | 1-11 | 6 | 22 | 1-13 | 6 |

Table 2.3 Dosing recommendations for ER based on free VPA.

| ER 1000 mg QD | |
|---|---|
| One dose taken ‘x’ hour after last dose | Dosing Recommendation |
| 30 | Take dose and resume dosing |
| 36 | Take dose and resume dosing |
| 42 | Take dose and resume dosing |
| 48, missed dose | Take make-up dose |
| 48, two doses | Take doses and resume dosing |
| Two doses taken ‘x’ h after last dose | |
| 54 | Take doses and resume dosing |
| 60 | Take doses and resume dosing |
| 66 | Take doses and resume dosing |
| 72, two doses | Take doses and resume dosing |
| 72, one dose | Take two doses and resume |
| ER 2500 mg QD | |
| One dose taken ‘x’ hour after last dose | |
| 30 | Take dose and resume dosing |
| 36 | Take dose and resume dosing |
| 42 | Take dose and resume dosing |
| 48, missed dose | Take dose and resume dosing |
| 48, two doses | Do not double the dose |
| Two doses taken ‘x’ h after last dose | |
| 54 | Risk of toxicity, take 1.5 dose |
| 60 | Risk of toxicity, take 1.5 dose |
| 66 | Risk of toxicity, take 1.5 dose |
| 72, two doses | Take doses and resume |
| 72, one dose | Take two doses and resume |

Table 2.4 Dosing recommendations for DR based on free VPA

| DR 500 mg BID | |
|---|---|
| One dose taken ‘x’ hour after last dose | Dosing Recommendation |
| 15 | Take dose and resume dosing |
| 18 | Take dose and resume dosing |
| 21 | Take dose and resume dosing |
| 24, missed dose | Take make-up dose |
| 24, two doses | Take doses and resume dosing |
| Two doses taken ‘x’ hour after last dose | |
| 27 | Take doses and resume dosing |
| 30 | Take doses and resume dosing |
| 33 | Take doses and resume dosing |
| 36 | Take doses and resume dosing |
| DR 1000 mg BID | |
| One dose taken ‘x’ hour after last dose | |
| 15 | Take dose and resume dosing |
| 18 | Take dose and resume dosing |
| 21 | Take dose and resume dosing |
| 24, missed dose | Take dose and resume dosing |
| 24, two doses | Take doses and resume dosing |
| Two doses taken ‘x’ hour after | |
| 27 | Take doses and resume dosing |
| 30 | Take doses and resume dosing |
| 33 | Take doses and resume dosing |
| 36 | Take doses and resume dosing |

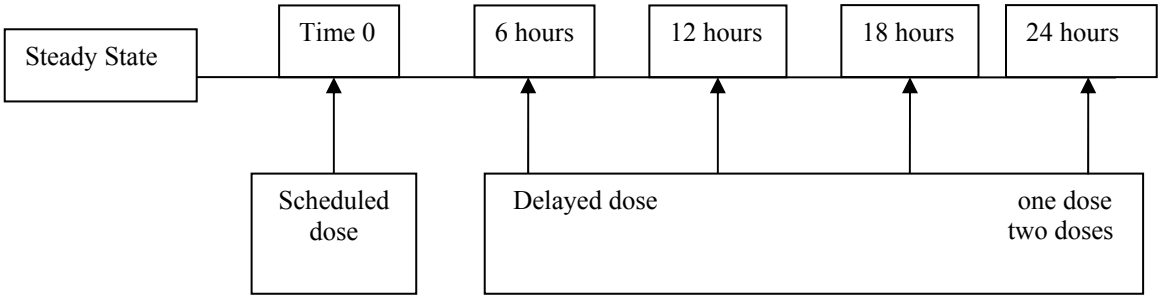


Figure 2.1 Simulation Scenarios for one missed dose on a once daily regimen. While at steady state, next dose was taken 6, 12, 18 hours late and then missed. In the last scenario missed dose is replaced.

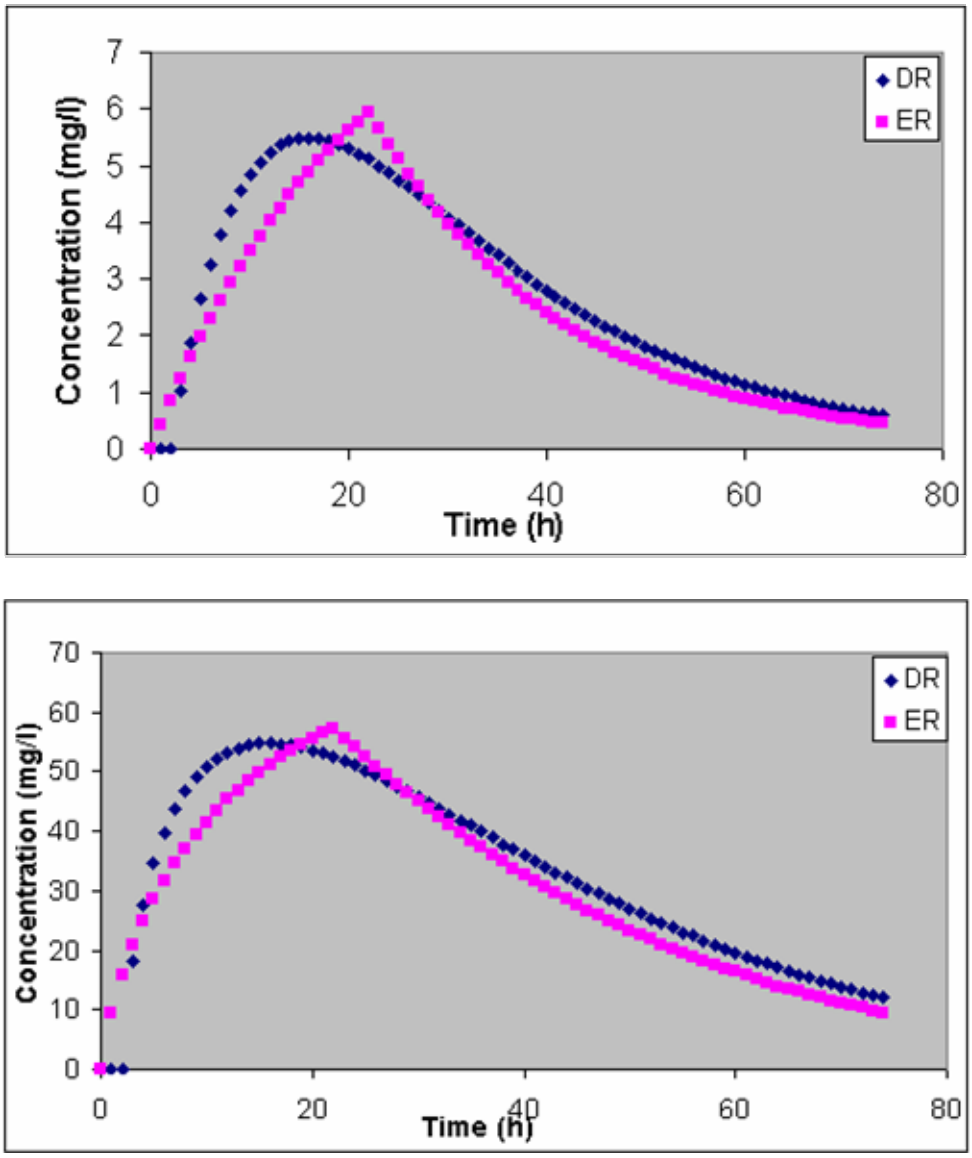


Figure 2.2 Unbound (upper panel) and total (lower panel) VPA concentrations following single 1000 mg dose administration of ER (F=0.89) and DR (F=1)

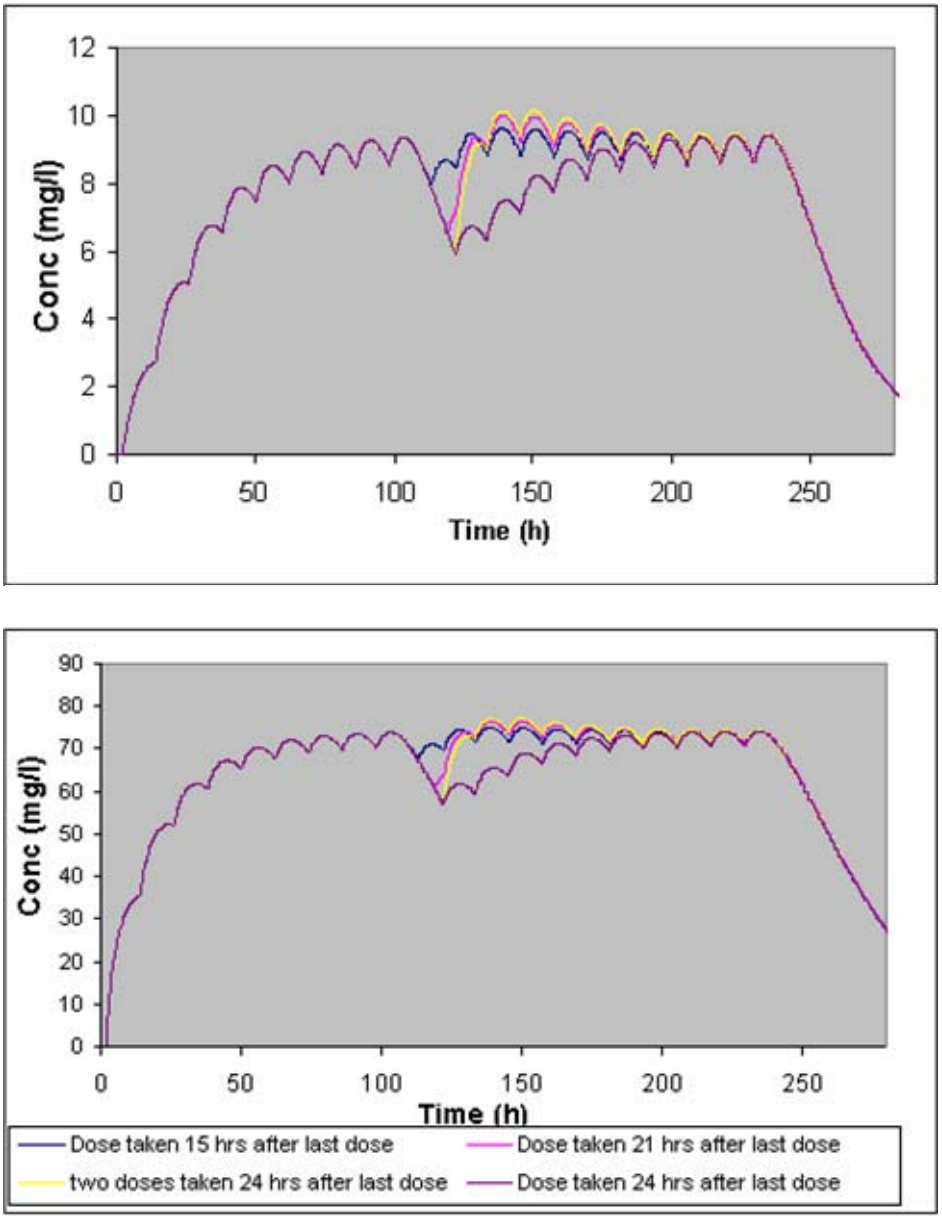


Figure 2.3 Unbound (upper panel) and total (lower panel) VPA concentrations following 500 mg bid of DR using average adult parameters. Different missed doses scenarios are shown.

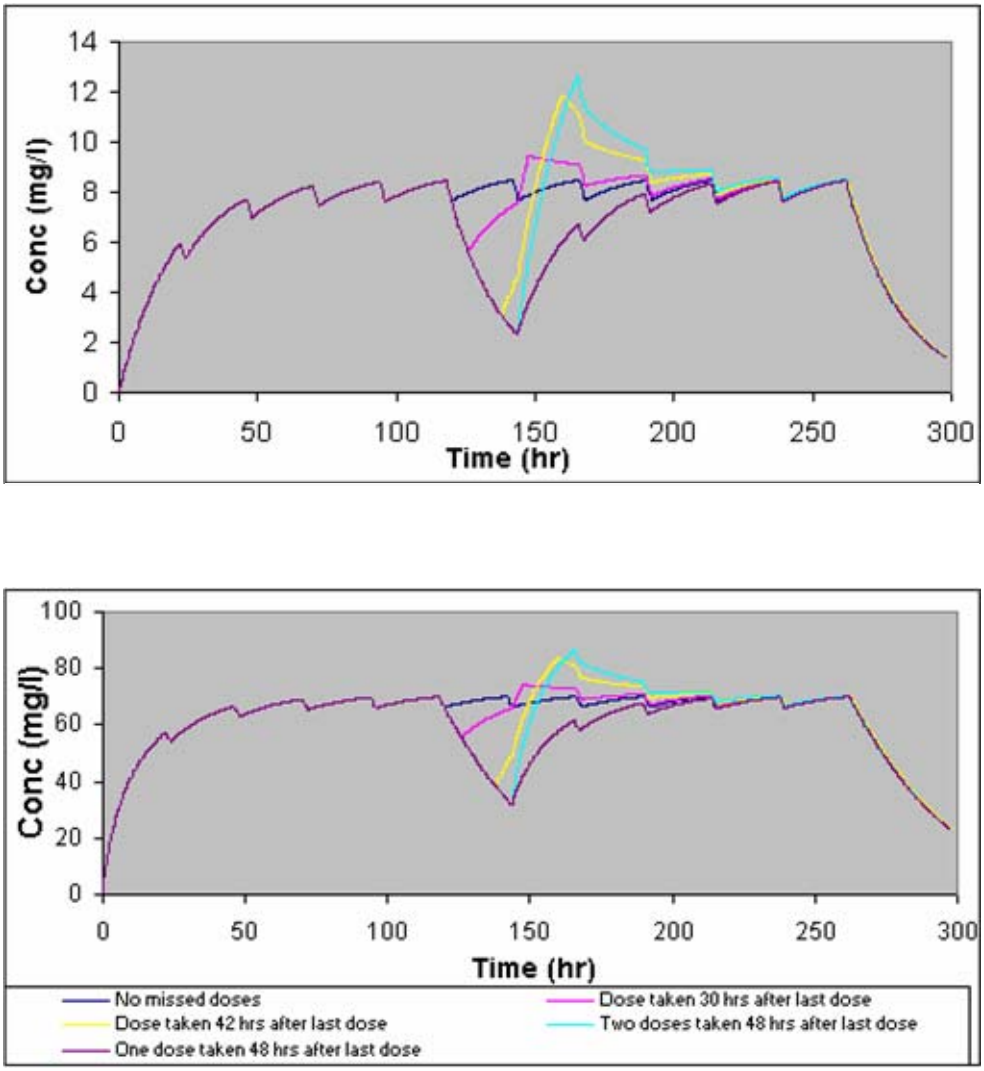


Figure 2.4 Unbound (upper panel) and total (lower panel) VPA concentrations following 1000 mg QD of ER using average adult parameters. Different missed doses scenarios are shown

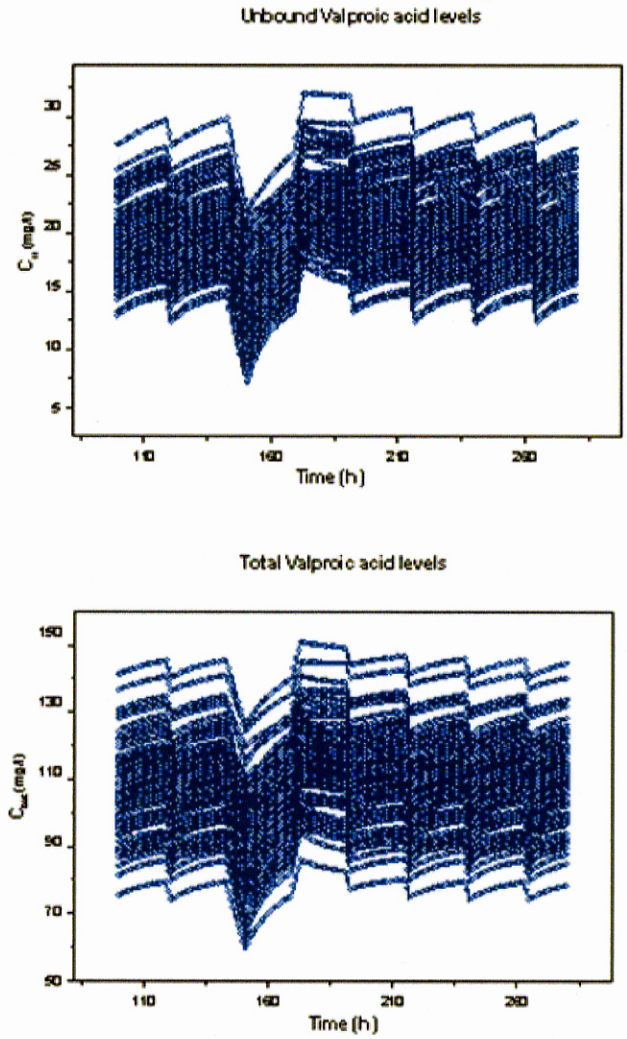


Figure 2.5 Unbound and total VPA levels following administration of 2500 mg daily of the ER preparation. ER dose on day 7 was administered 6 h late.

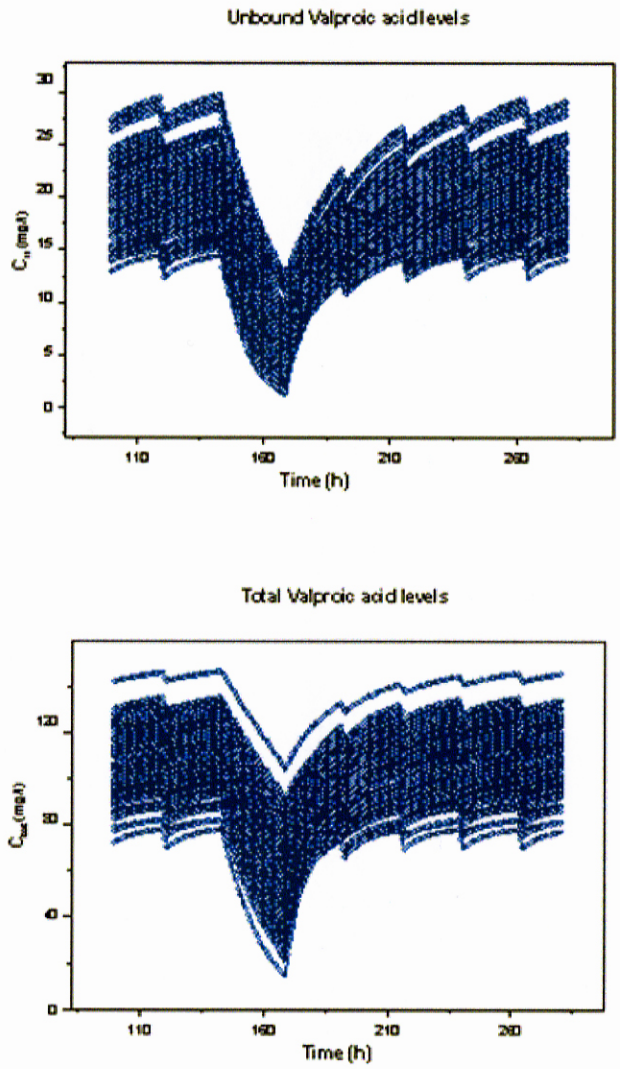


Figure 2.6 Unbound and total VPA levels following administration of 2500 mg daily of the ER preparation. ER dose on day 7 was missed.

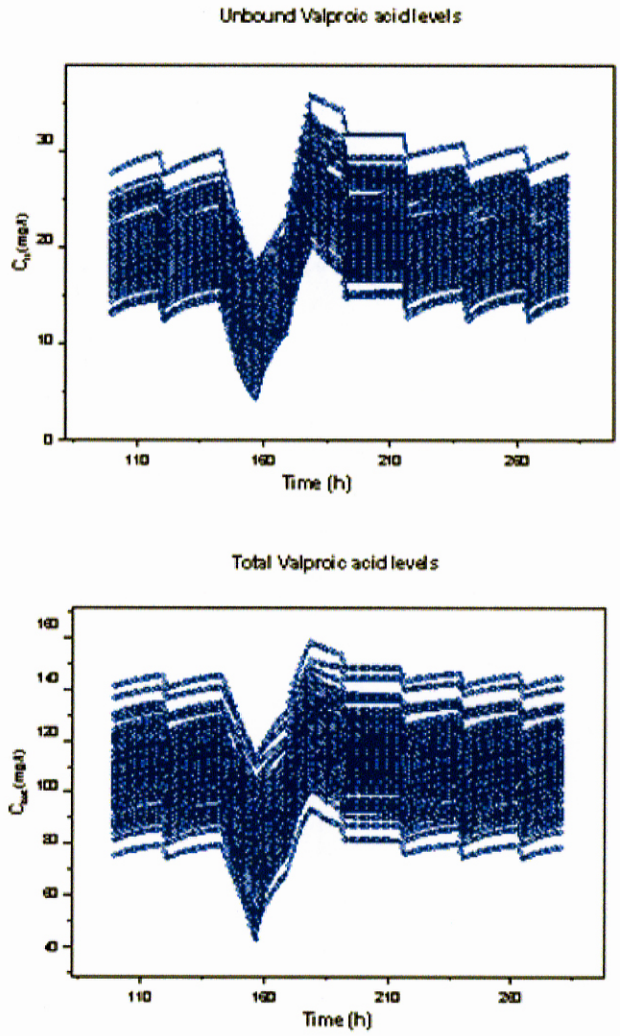


Figure 2.7 Unbound and total VPA levels following administration of 2500 mg daily of the ER preparation. ER dose on day 7 was administered 18 hours late.

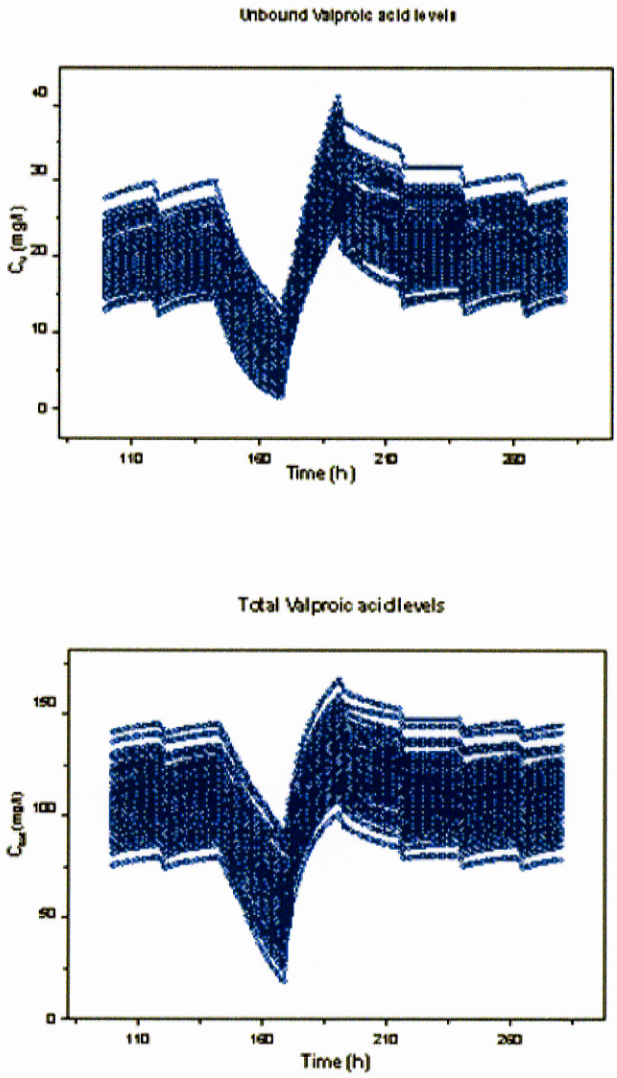


Figure 2.8 Unbound and total VPA levels following administration of 2500 mg daily of the ER preparation. ER dose on day 7 was missed. Dose was doubled on day 8.

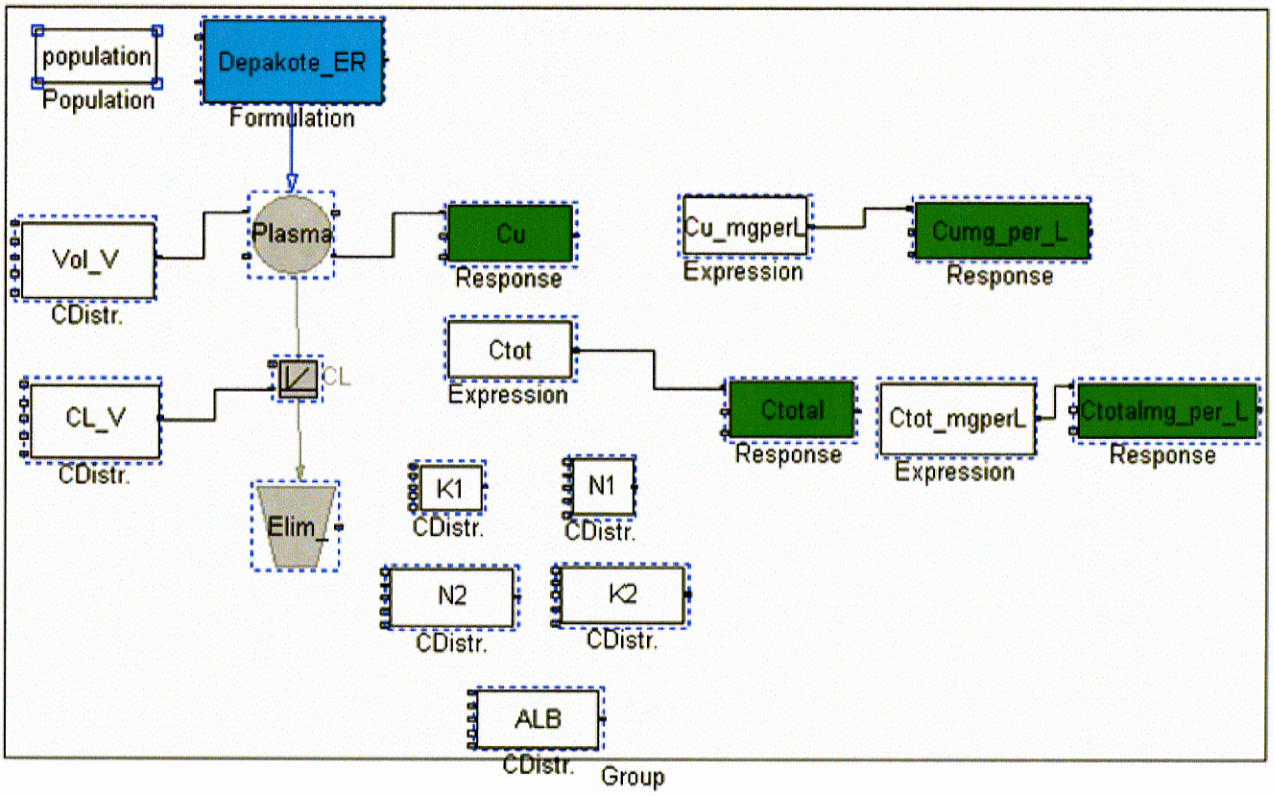


Figure 2.9 Structural and statistical simulation model for the ER formulation.

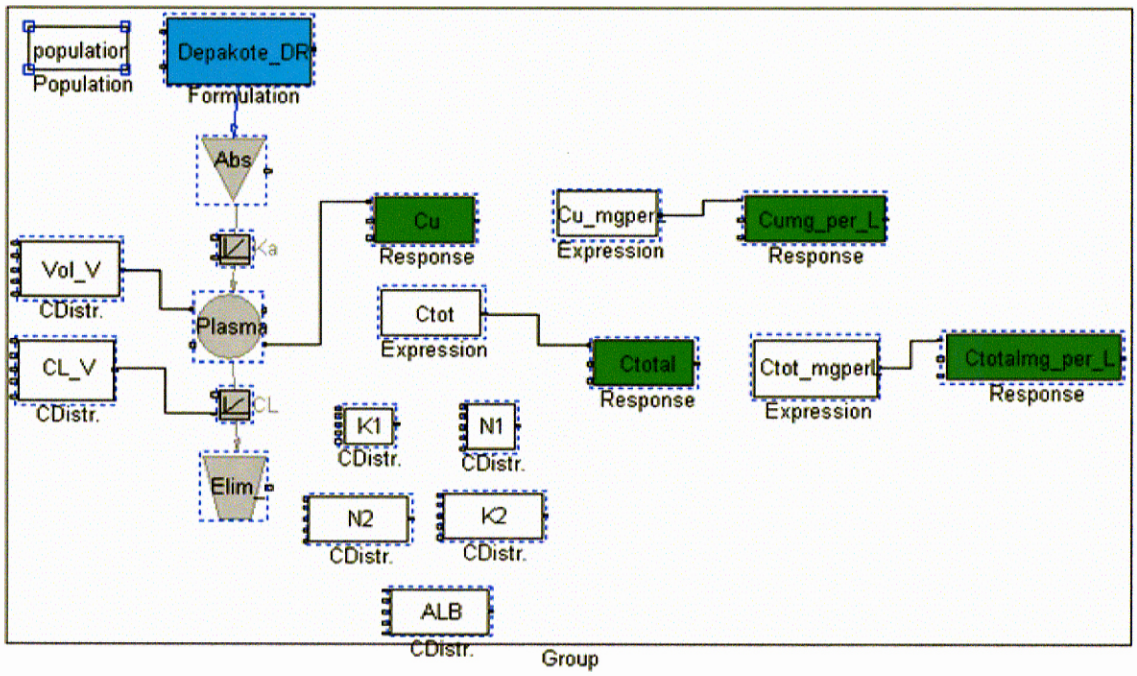


Figure 2.10 Structural and statistical simulation model for the DR formulation.

# A RATIONAL DOSING NOMOGRAM FOR VALPROIC ACID BASED ON NONLINEAR PLASMA PROTEIN BINDING

Alaa M. Ahmad, F. Douglas Boudinot, William H. Barr, Ronald C. Reed and William R. Garnett

**ABSTRACT**. Clinicians rely primarily on total valproic acid (VPA) concentrations for therapeutic monitoring. However, due to nonlinear plasma protein binding of VPA, dose titration is complicated by disproportionality between the total drug concentration and dose. The purpose of this report was to develop a nomogram for VPA based on total drug concentrations. Nine healthy volunteers were administered 500, 750 and 1000 mg VPA in a dose escalation study. Plasma protein binding of VPA was characterized for each volunteer *in vitro*. A one-site saturable binding model provided an adequate description of the binding of VPA to albumin and yielded (mean, % standard error) 2.3 (4.5%) binding sites (N) and an equilibrium association constant ($K_A$) of 6.7 (17.9%) (L/mM). Predictions for the increase in total concentration observed with dose escalation were based on individual and population pharmacokinetic parameters. There was close correlation between the predictions based on individual and population estimates. Using population estimates, a dosing nomogram for VPA was constructed. To minimize the risk of achieving toxic drug concentrations, the dose should not be increased more than 2 fold at a time. The nomogram should be used in conjunction with patient history and clinical response to aid clinicians in making informed decisions about dose-adjustments.

Key words: Valproic acid, protein binding, nomogram, dose titration.

**Introduction**

Valproic acid (VPA) is a broad-spectrum antiepileptic drug effective against a wide variety of seizure disorders, including complex partial, tonic-clonic convulsive, absence and myoclonic seizures. VPA also shows activity against neonatal seizures, febrile seizures and status epilepticus [1, 2]. VPA has been used in the treatment of bipolar disorders and for the prophylaxis of migraine headaches [3]. The drug is rapidly absorbed following oral administration with a bioavailability of greater than 85% [3]. VPA is metabolized almost entirely by the liver with 30-50 % of the dose appearing in urine as glucuronide conjugate and 40% of the dose undergoing mitochondrial β-oxidation. Less than 15-20 % of the dose is eliminated by other oxidative mechanisms and less than 3% of the dose is eliminated as unchanged drug in urine. There is no evidence of saturable metabolism. Mean total clearance and steady state volume of distribution for total valproate are 0.56 L/h and 11 L, respectively. For free valproate, mean unbound clearance and unbound volume of distribution are 4.6 L/h and 92 L, respectively. Mean terminal half-life for valproate mono therapy ranged from 9-16 hours following oral doses of 250-1000 mg [3].

VPA binds to plasma proteins in a concentration dependent fashion. As such, the free fraction increases from 10% at 40 mg/l to 18.5% at 130 mg/l [3]. The plasma protein binding of VPA decreases in the elderly, hepatic and renal impairment and in the presence of other medications that have higher binding affinity to the same binding site on albumin such as aspirin. This nonlinear binding renders the kinetics of the total drug

non-linear such that clearance and volume of distribution of total VPA increase with the free fraction. Thus total VPA concentrations increase less than proportionally with dose. To date, there is no generally accepted method to titrate the VPA dose based on total concentrations. Typically, VPA dosage adjustments are made empirically. This lengthens the process by which adequate seizure control and personal therapeutic range for each patient is achieved.

There are several preparations for VPA. Divalproex sodium extended-release (Depakote® ER) is a once daily (QD) preparation for VPA. It's formulated as a hydrophilic polymer matrix controlled-release tablet system to provide more consistent blood concentrations over 24 hours. This system results in the release of drug in the stomach, small intestine, and large intestine over an 18- to 24-hour period of time.

In this study, two dosing nomograms for VPA were constructed based on plasma protein binding data in nine healthy volunteers. A dose escalation study in the same volunteers was conducted to test the nomograms. The ER formulation was used in this study to provide once-daily dosing for convenience of administration. The nomograms can be used for all VPA preparations.

## Methods

This study was approved by the Institutional Review Board of the Virginia Commonwealth University Office of Research Subjects Protection and all volunteers provided signed informed consent to participate in the study.

### *Plasma Protein Binding*

One hundred milliliters (100 ml) of blood were obtained from each volunteer prior to the study. Blood was immediately centrifuged and plasma was obtained and stored at $^{-}70°C$. Plasma protein binding was characterized for each volunteer separately. Briefly, valproic acid (VPA, sodium salt, Sigma-Aldrich, St. Louis, MO) concentrations ranging from 25-2000 μg/ml were prepared in plasma and concentrations were confirmed by assay. Following incubation at 37°C for one hour, unbound valproic acid was separated by ultrafiltration; using a temperature-controlled (37°C) centrifuge (Eppendorf, Westbury, NY). Concentrations of VPA were determined using fluorescence polarization immunoassay on a $TD_x$ analyzer (Abbott Laboratories, Abbott Park, IL) after proper dilution. The calibration range for this assay was 12.5-150 μg/ml and 2-25 μg/ml for total and free valproate, respectively. Assay variability has been to shown to be less than 5 % [4]. Albumin and total protein concentrations were measured for each volunteer as part of serum chemistry panel.

To characterize the relationship between bound and unbound VPA, the change in bound VPA concentration after ultrafiltration was adjusted in a manner similar to the

correction for fluid shifts in equilibrium dialysis suggested by Boudinot and Jusko [5]. The correction results in a calculated bound fraction before ultrafiltration ($F_{Bi}$) = ($D_{Ta}$ - $D_F$) * $V_a$ /$V_i$ / ($D_{Ta}$ - $D_F$) * $V_a$ /$V_i$ + $D_F$ , where $D_{Ta}$ is the total concentration after ultrafiltration, $D_F$ is the free concentration and $V_i$ and $V_a$ are the volumes of the plasma before and after ultrafiltration, respectively. Ultrafiltrate volume was limited to 15% and this 0.85 was used as correction factor. Bound VPA ($D_B$) was calculated from $D_B = D_T - D_F$.

Three structural models were fitted to the individual plasma protein binding curves, a one site saturable binding model, a two site saturable binding model and a two-site model where one site is saturable and one is not. All modeling was performed using WinNonlin (Pharsight Corporation, Mountain View, CA). Models without weighting, with 1/Y (observed) or $1/Y_{hat}$ (predicted) as weights were tried. Models were compared based on visual inspection of predicted versus observed plots, precision of parameter estimates, residual plots, AIC (Akaike Information Criterion) and SBC (Schwarz Bayesian Criterion) for models with similar weighting schemes [6].

After determination of the structural model, a population model was developed using NONMEM (Globomax, Hanover, MD). Additive and exponential error models were used to describe the inter-individual variability in the number of binding sites and affinity rate constant. Similarly, additive and proportional error models were tried for residual variability. Different covariates (continuous and categorical) were tested on both N and $K_A$. The change in the objective function (Δobj) was used to judge whether a

N and $K_A$. The change in the objective function (Δobj) was used to judge whether a covariate has a significant effect on the fit. It is assumed that Δobj is approximately $\chi^2$ distributed and thus a reduction in the objective function of 3.84 units corresponds to a significance level of 0.05. The precision of parameter estimates with different methods of estimation (first order, first order conditional estimation with and without interaction) was compared and the method that provided best precision was used for estimation. Final model selection was based on the change in objective function for nested models, goodness of fit and residual plots and precision of parameter estimates for fixed and random effects [7].

*Dose Escalation Study*

Nine healthy volunteers were administered increasing doses of Depakote® extended release (ER) formulation. As shown in figure 3.1, during the first week, 500 mg of the ER formulation was taken once a day (QD). During the second week, the dose was increased to 750 mg QD and in the third week the dose was increased to 1000 mg QD. Two trough plasma samples were collected after steady state was reached at each dose.

*Nomogram*

The nomogram aims to quantify the nonlinear relationship between total VPA concentration and the dose assuming that unbound VPA is proportional to the dose. Using individual protein binding estimates, simulations were performed for each volunteer to predict the increase in dose needed to achieve the increase in total

concentration observed with dose escalation. Following this, predictions were based on the population model and compared to the predictions from individual estimates. All simulations were based on a one site saturable binding model, $D_B = (N * K_A * D_F * P) / (1 + K_A * D_F)$, where P is the albumin concentration, N is the number of binding sites and $K_A$ is the equilibrium association constant. The population estimates were used to construct the nomograms.

## Results

### *Plasma Protein Binding*

A one site saturable binding model provided the best fit to the plasma protein binding data for each of the volunteers. Figure 3.2 shows a plot of fraction bound as a function of total VPA concentration for all volunteers. A weighting factor of $1/Y_{hat}$ provided parameter estimates with best precision. Figure 3.3 shows a simulation for bound VPA as a function of unbound VPA concentrations using parameters obtained through modeling.

A one-site saturable binding model was used as a structural model for population model development. Due to the relatively small sample size, two random effects simultaneously on N and K introduced either additively or exponentially could not be estimated with good precision. From evaluation of the individual estimates, a random effect on K only that was estimated with reasonable precision (~ 31.0%) was retained. Thus, K was allowed to vary across volunteers while N was fixed. An exponential inter-

individual variability model was superior to the additive model in terms of precision of the random effect estimated and thus was retained. Residual variability was introduced using an additive and a proportional error model. The latter was used in the final model since there was an increase with variability indicated by residual plots with higher concentrations. The first order conditional estimation (FOCE) method provided superior estimates in terms of parameter precision compared to the first order method and the interaction option didn't provide further improvement and thus was not used.

Following this, different covariates including age, weight, body mass index, albumin and total protein concentrations and gender were introduced on both N and K and found insignificant ($\Delta obj < 3.84$), which is not surprising since the sample size is small.

The final population model predicted (mean, %standard error or %SE) an N of 2.3 (4.5%) and $K_A$ of 6.7 (17.9%) (L/mM). The inter-individual variability term for $K_A$ (ETA, exponential error model) was estimated to be 30.7% (%SE= 30.9%) and residual variability (proportional error model) was estimated to be 31.9% (%SE=14.8%). The FOCE method was used. The model didn't include any covariates. The fitted line in figure 2 represents final population model estimates.

*The VPA Nomogram*

Predictions were performed at the first dose increase (500 to 750 mg, 50% increase in dose), second dose increase (750 to 1000 mg, 33% increase in dose) and also from 500 to 1000 mg (100% increase in dose). Thus, for each volunteer, individual binding parameters were used to predict the needed increase in dose for each combination of trough levels. These individual predictions are summarized in table 3.1 and differences between the individual predictions and nominal percent increase in dose are illustrated in figure 3.4. Exact predictions have a difference of zero. Similarly, predictions based on population binding parameters were performed for each combination of trough levels for each volunteer and are shown in table 3.2. The differences between the population predictions and nominal percent increase in dose are depicted in figure 3.5.

To compare individual and population predictions, two performance measures for numeric prediction, root mean squared error (RMSE) and mean absolute error (MAE) were calculated at each dose increase (50%, 33% and 100%). These values are summarized in table 3.3. RMSE and MAE for individual and population predictions were not statistically significantly different (one tailed t-test, $P > 0.3$).

Using population estimates, a nomogram for VPA was developed. For purposes of illustration, the nomogram was divided into two dose-titration charts. The first chart shows dose titration at total concentrations between 20-100 μg/ml (Table 3.4) and the second chart acilitates dose-titration at total concentrations between 100-200 μg/ml (Table 3.5). One volunteer experienced increased gastrointestinal motility that resulted in

mal absorption of the compound, and therefore predictions were not performed for this volunteer.

**Discussion**

There are different techniques to measure unbound drug concentrations. In this study, ultrafiltration was used to separate unbound VPA. Ultrafiltration has been used and continues to be used increasingly because it simple, fast and efficient. Since there are concerns that the drug might be lost to the ultrafiltrate leading to a change in total concentration, it's been suggested to average total concentrations measured before and after ultrafiltration [8]. One other issue with the use of ultrafiltration is the change in protein concentration as plasma water is filtered. It has been suggested to limit the volume of the ultrafiltrate to 10-15% of initial plasma volume [8] to avoid considerable changes in protein concentration that might affect the binding. Thus, a correction for the change in bound concentration due to the change in protein concentration during ultrafiltration was developed. With this correction the calculated bound concentration takes into account the increase in protein concentration. In that sense, this correction is a more reasonable representation of the bound fraction that is not simply an average of pre and post ultrafiltration measurements.

Plasma protein binding of VPA has been reported previously. Yu [9] reported plasma protein binding parameters for VPA at steady state in children with epilepsy and

also in pooled sera of 10 healthy volunteers. One class of binding sites was detected both *in vitro* and *in vivo*. *In vitro*, N was 1.86 and $K_A$ was 8.03 L/mmol while *in vivo* studies yielded N and $K_A$ values of 2.48 and 4.98 L/mmol, respectively. Parameters obtained in vitro were not statistically significantly different from those generated *in vitro*. Cramer et al [10] studied the protein binding of VPA in patients with epilepsy on mono- and poly-therapy with valproate. The authors didn't estimate binding parameters but concluded that free valproate is more informative for therapeutic monitoring. In a later *in vivo* study with 37 patients the same group reported a binding site concentration (N*P) of 1.17 mmol/L and $K_A$ of 10.99 L/mmol for mono therapy patients and N*P of 1.04 mmol/L and $K_A$ of 13.3 L/mmol for mono and poly therapy patients combined [11]. The authors could not detect more than one saturable site but suggested the presence of a second non-saturable site. Another group [12] also reported *in vivo* estimates in nine healthy volunteers, where they suggested the presence of one saturable site (N =1 .80) with a $K_A$ value of 23.10 L/mmol. Most of the authors studied the binding of VPA at concentrations close to the therapeutic range (~ 40-200 μg/ml).

A recent paper [13] reported two saturable sites for valproic acid following rapid infusions in patients with epilepsy. Parameter estimates were $N_1$=1.54, $K_1$= 11.9 L/mmol and $N_2$=0.19, $K_2$=164 L/mmol. The affinity constant for the second site was not well estimated and there wasn't sufficient evidence to conclude that a second high affinity, low capacity binding site actually exists. One group [14] investigated the binding of VPA over a range of (0.56-2016) μg/ml using equilibrium dialysis, ultrafiltration and

ultracentrifugation reported the presence of two saturable sites, both estimated with good precision. However, there were discrepancies in the binding constants for the second class across methods.

In the present study, parameter estimates for a one saturable site model compare well with parameters values reported in the literature. A second binding site that might be operational at concentrations below the limit of detection of the assay (12.5 µg/ml) cannot be ruled out. However, if a second site does exist it has no clinical relevance since it is saturated at concentrations lower than therapeutic and probably represents binding to a protein other than albumin.

Nomograms can be designed to predict a prognostic outcome [15], calculate doses [16], derive useful clinical biomarkers [17] and predict disease progress [18]. VPA is widely used in neuropsychiatric disorders [3] and it's critical to maintain drug concentrations in the therapeutic range. Total and unbound VPA plasma concentrations can be determined using fluorescence polarization immunoassay on the $TD_x$/$TD_x$ $FL_x$ system. The unbound drug, in theory, is better correlated with the pharmacological effect than the total drug. Despite this, clinicians primarily rely on total VPA concentrations to individualize therapy. In epilepsy, most patients will respond to total concentrations between 50-150 µg/ml [1].

There are no reports in the literature that describe dose escalation studies for VPA with timed measurements. Such information is needed to test whether plasma protein binding can adequately account for the non-linear relationship between total concentrations and the dose. To obtain this information, we conducted a dose escalation study in nine healthy volunteers. This information coupled with plasma protein binding data was used to develop the VPA nomogram. Table 3.1 summarizes predictions for dose increase using individual plasma protein binding parameter estimates. There were considerable inter- and intra-individual variabilities apparent in the trough concentrations to which these discrepancies can be attributed. There are several reports on the population kinetics of VPA that attempt to quantify and explain this variability [19-21].

The second set of predictions, summarized in table 3.2, use population estimates. The purpose of performing similar predictions with population estimates was to assess whether the predictions from these estimates are reasonably close to the predictions based on individual parameters. Since there were no significant differences between individual and population predictions (based on RMSE and MAE); a dosing nomogram using population estimates was developed. The nomogram can also be used to decrease the dose if there is a need to lower the total concentration.

Since there is a risk of over predicting the needed increase in dose, it is not recommend increasing the dose by more than 2 fold at a time. The nomogram should be used in conjunction with patient history and clinical response. If there is information on

the expected concentrations in a certain patient with dose increase, this information should supplement the predictions provided by the nomogram. It should be noted that the nomograms could be used for all VPA preparations and regardless of whether patients are on enzyme-inducing or –inhibiting co-medication. The nomogram will always predict a percentage of the previously administered dose and not an absolute value.

In summary, two dosing charts for valproic acid were introduced to facilitate rational dose titration based on total concentrations. This will aid clinicians in maintaining VPA concentrations in the desired range and minimize the guessing involved in dose titration. The nomograms can be used for all VPA preparations.

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*Appendix*

*Correction of Bound VPA Concentrations After Ultrafiltration*

In order to characterize the relationship between bound and unbound VPA, a new method that adjusts for the change in bound VPA concentration after ultrafiltration was used. During ultrafiltration, plasma water passes through the membrane carrying the free drug. This leads to an increase in protein concentration. In a manner similar to the correction for fluid shifts in equilibrium dialysis suggested by Boudinot and Jusko [3], the change in bound drug concentration after ultrafiltration can be derived as follows:

Initial amount of protein ($AP_i$) = Amount of protein after ultrafiltration ($AP_a$)

Thus;

$$Vi * Pi = Va * Pa \qquad \text{Eq. 1}$$

assuming no loss of protein through the filter, where $V_i$ and $V_a$ are the initial plasma and retentate volumes, and $P_i$ and $P_a$ are the initial and post ultrafiltration protein concentrations, respectively. It follows then that

$$P_a = P_i * V_i / V_a \qquad \text{Eq. 2}$$

If there are no volume shifts, the bound concentration ($D_{Bi}$ ) generally can be described by (where n is the number of binding sites):

$$D_{Bi} = \sum_{m=1}^{n} (N * K * P_i * D_F) / (1 + K * D_F) \qquad \text{Eq. 3}$$

Where $N$ and $K$ are the number of binding sites and affinity constant, respectively, and $D_F$ is the free drug concentration. After ultrafiltration and with substituting $P_a$ from Eq. 2 the bound concentration ($DB_a$) can be written as:

$$DB_a = \sum_{m=1}^{n} (N * K* [P_i * V_i/V_a] * D_F) / (1 + K * D_F) \qquad \text{Eq. 4}$$

Assuming that $N$ and $K$ do not vary with changes in protein concentration $P$, then:

$$DB_a * V_a/V_i = \sum_{m=1}^{n} (N * K* P_i * D_F)/ (1 + K * D_F) = D_{Bi} \qquad \text{Eq. 5}$$

To calculate the fraction of bound drug before ultrafiltration ($F_{Bi}$) one can use:

$F_{Bi} = D_{Bi} / (D_{Bi} + D_F)$

Substituting for $D_{Bi}$ from Eq. 5, it follows that:

$F_{Bi} =(DB_a * V_a/V_i ) / (DB_a * V_a/V_i + D_F)$ Eq. 6

Since total drug concentration after ultrafiltration ($D_{Ta}$) as well as $D_F$ are determined experimentally, then:

$F_{Bi} = (D_{Ta} - D_F) * V_a/V_i / (D_{Ta} - D_F) * V_a/V_i + D_F$ Eq. 7

This calculated bound fraction value was used for modeling.

Table 3.1 Predictions for percent increase in dose based on individual estimates.

| DOSE INCREASE | | | | | | |
|---|---|---|---|---|---|---|
| | 500-750 | | 750-1000 | | 500-1000 | |
| **Vol** | (50% increase in dose) | | (33% increase in dose) | | (100 % increase in dose) | |
| | Ctot from-to | Pred | Ctot from-to | Pred | Ctot from-to | Pred |
| 1 | 41-55 | 41% | 55-67 | 27% | 41-67 | 79% |
| | 43-55 | 32% | 55-64 | 20% | 41-64 | 69% |
| | | | | | 43-67 | 68% |
| | | | | | 43-64 | 59% |
| 2 | 25-50 | 130% | 38-51 | 40% | 25-51 | 134% |
| | 25-38 | 67% | 38-56 | 58% | 25-56 | 163% |
| | 27-50 | 107% | 50-56 | 15% | 27-51 | 110% |
| | 27-38 | 50% | | | 27-56 | 137% |
| 3 | 28-31 | 9% | 31-55 | 94% | 28-55 | 111% |
| | 28-32 | 16% | 31-73 | 173% | 28-73 | 197% |
| | 22-31 | 42% | 32-55 | 82% | 22-55 | 175% |
| | 22-32 | 51% | 32-73 | 156% | 22-73 | 287% |
| 4 | 30-49 | 73% | 49-70 | 53% | 30-70 | 165% |
| | 30-55 | 99% | 49-76 | 70% | 30-76 | 194% |
| | 34-49 | 51% | 55-70 | 33% | 34-70 | 131% |
| | 34-55 | 74% | 55-76 | 48% | 34-76 | 156% |
| 5 | 26-40 | 62% | 40-50 | 30% | 26-33 | 29% |
| | 26-38 | 53% | 38-50 | 38% | 26-50 | 111% |
| 6 | 45-56 | 32% | 56-76 | 49% | 45-76 | 95% |
| | 45-57 | 35% | 56-69 | 31% | 45-69 | 72% |
| | 39-56 | 55% | 57-76 | 45% | 39-76 | 130% |
| | 39-57 | 59% | 57-69 | 28% | 39-69 | 103% |
| 7 | 57-85 | 79% | 85-89 | 7% | 57-89 | 92% |
| | 57-80 | 62% | 80-89 | 18% | 57-79 | 62% |
| | 54-85 | 92% | | | 54-89 | 106% |
| | 54-80 | 74% | | | 54-79 | 74% |
| 8 | 27-52 | 107% | 52-59 | 16% | 27-59 | 140% |
| | 27-46 | 81% | 52-61 | 21% | 27-61 | 150% |
| | 29-52 | 93% | 46-59 | 33% | 29-59 | 125% |
| | 29-46 | 68% | 46-61 | 38% | 29-61 | 133% |

Table 3.2 Predictions for percent increase in dose based on population estimates.

| | **DOSE INCREASE** | | | | | |
|---|---|---|---|---|---|---|
| | 500-750 | | 750-1000 | | 500-1000 | |
| **Vol** | (50% increase in dose) | | (33% increase in dose) | | (100 % increase in dose) | |
| | Ctot from-to | Pred | Ctot from-to | Pred | Ctot from-to | Pred |
| 1 | 41-55 | 45% | 55-67 | 29% | 41-67 | 87% |
| | 43-55 | 37% | 55-64 | 21% | 41-64 | 75% |
| | | | | | 43-67 | 77% |
| | | | | | 43-64 | 66% |
| 2 | 25-50 | 126% | 38-51 | 43% | 25-51 | 131% |
| | 25-38 | 62% | 38-56 | 62% | 25-56 | 161% |
| | 27-50 | 105% | 50-56 | 16% | 27-51 | 109% |
| | 27-38 | 46% | | | 27-56 | 137% |
| 3 | 28-31 | 14% | 31-55 | 100% | 28-55 | 127% |
| | 28-32 | 18% | 31-73 | 190% | 28-73 | 229% |
| | 22-31 | 47% | 32-55 | 93% | 22-55 | 194% |
| | 22-32 | 53% | 32-73 | 179% | 22-73 | 326% |
| 4 | 30-49 | 79% | 49-70 | 59% | 30-70 | 185% |
| | 30-55 | 108% | 49-76 | 78% | 30-76 | 218% |
| | 34-49 | 54% | 55-70 | 37% | 34-70 | 145% |
| | 34-55 | 79% | 55-76 | 53% | 34-76 | 173% |
| 5 | 26-40 | 64% | 40-50 | 32% | 26-33 | 32% |
| | 26-38 | 54% | 38-50 | 40% | 26-50 | 115% |
| 6 | 45-56 | 31% | 56-76 | 50% | 45-76 | 96% |
| | 45-57 | 33% | 56-69 | 31% | 45-69 | 72% |
| | 39-56 | 57% | 57-76 | 47% | 39-76 | 135% |
| | 39-57 | 60% | 57-69 | 29% | 39-69 | 106% |
| 7 | 57-85 | 73% | 85-89 | 7% | 57-89 | 86% |
| | 57-80 | 59% | 80-89 | 17% | 57-79 | 56% |
| | 54-85 | 86% | | | 54-89 | 102% |
| | 54-80 | 70% | | | 54-79 | 67% |
| 8 | 27-52 | 116% | 52-59 | 17% | 27-59 | 153% |
| | 27-46 | 86% | 52-61 | 23% | 27-61 | 165% |
| | 29-52 | 102% | 46-59 | 36% | 29-59 | 137% |
| | 29-46 | 74% | 46-61 | 43% | 29-61 | 148% |

Table 3.3 Root mean squared errors (RMSEs) and mean absolute errors (MAEs) for individual and population predictions

| | Individual | | Population | |
|---|---|---|---|---|
| Dose increase | RMSE | MAE | RMSE | MAE |
| 50% | 0.31 | 0.24 | 0.32 | 0.25 |
| 33% | 0.44 | 0.26 | 0.51 | 0.30 |
| 100% | 0.56 | 0.42 | 0.67 | 0.49 |

Table 3.4 Dosing nomogram for total VPA concentrations between 20-100 mg/l.

**Titration up** →

| | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 | 95 | 100 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | **26** | 55 | 87 | 116 | 152 | 184 | 223 | 258 | 300 | 342 | 388 | 433 | 484 | 533 | 588 | 646 |
| 25 | **21** | **23** | 49 | 72 | 100 | 125 | 156 | 184 | 218 | 251 | 287 | 323 | 364 | 402 | 446 | 492 |
| 30 | 35 | **19** | **21** | 39 | 62 | 83 | 108 | 131 | 158 | 185 | 214 | 243 | 277 | 308 | 343 | 381 |
| 35 | 47 | 33 | **17** | **15** | 34 | 52 | 72 | 91 | 114 | 136 | 160 | 184 | 212 | 238 | 267 | 298 |
| 40 | 54 | 42 | 28 | **13** | **17** | 31 | 49 | 66 | 85 | 105 | 126 | 146 | 170 | 193 | 218 | 245 |
| 45 | 60 | 50 | 39 | 26 | **14** | **13** | 28 | 42 | 59 | 76 | 93 | 111 | 132 | 151 | 173 | 196 |
| 50 | 65 | 56 | 45 | 34 | 24 | **11** | **14** | 26 | 41 | 56 | 71 | 87 | 106 | 123 | 142 | 162 |
| 55 | 69 | 61 | 52 | 42 | 33 | 22 | **12** | **11** | 24 | 37 | 51 | 65 | 81 | 96 | 113 | 131 |
| 60 | 72 | 65 | 57 | 48 | 40 | 30 | 21 | **10** | **12** | 23 | 36 | 49 | 63 | 77 | 92 | 108 |
| 65 | 75 | 69 | 61 | 53 | 46 | 37 | 29 | 19 | **11** | **10** | 22 | 33 | 46 | 58 | 72 | 86 |
| 70 | 77 | 72 | 65 | 58 | 51 | 43 | 36 | 27 | 19 | **9** | **10** | 20 | 32 | 43 | 56 | 69 |
| 75 | 79 | 74 | 68 | 62 | 56 | 48 | 42 | 34 | 26 | 18 | **9** | **9** | 20 | 30 | 41 | 53 |
| 80 | 81 | 76 | 71 | 65 | 59 | 53 | 47 | 39 | 33 | 25 | 17 | **8** | **10** | 19 | 29 | 40 |
| 85 | 83 | 78 | 73 | 68 | 63 | 57 | 51 | 45 | 39 | 31 | 24 | 17 | **9** | **8** | 18 | 28 |
| 90 | 84 | 80 | 76 | 70 | 66 | 60 | 55 | 49 | 43 | 37 | 30 | 23 | 16 | **8** | **9** | 18 |
| 95 | 85 | 82 | 77 | 73 | 69 | 63 | 59 | 53 | 48 | 42 | 36 | 29 | 23 | 15 | **8** | **8** |
| 100 | 87 | 83 | 79 | 75 | 71 | 66 | 62 | 57 | 52 | 46 | 41 | 35 | 29 | 22 | 15 | **8** |
| | 95 | 90 | 85 | 80 | 75 | 70 | 65 | 60 | 55 | 50 | 45 | 40 | 35 | 30 | 25 | 20 |

↑ ↓

**Titration down** ←

Table 3.5 Dosing nomogram for total VPA concentrations between 100-200 mg/l.

**Titration up** →

| | 105 | 110 | 115 | 120 | 125 | 130 | 135 | 140 | 145 | 150 | 155 | 160 | 165 | 170 | 175 | 180 | 185 | 190 | 195 | 200 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 100 | **8** | 16 | 26 | 35 | 45 | 55 | 66 | 77 | 89 | 101 | 115 | 128 | 142 | 157 | 172 | 188 | 205 | 222 | 240 | 258 |
| 105 | **8** | **8** | 16 | 24 | 34 | 43 | 53 | 64 | 75 | 86 | 98 | 111 | 124 | 138 | 152 | 166 | 182 | 198 | 214 | 231 |
| 110 | 14 | **7** | **8** | 16 | 24 | 33 | 42 | 52 | 62 | 73 | 84 | 96 | 108 | 121 | 134 | 148 | 162 | 177 | 192 | 207 |
| 115 | 20 | 14 | **7** | **7** | 15 | 23 | 32 | 41 | 51 | 60 | 71 | 82 | 93 | 105 | 117 | 130 | 143 | 157 | 171 | 185 |
| 120 | 26 | 20 | 13 | **7** | **7** | 15 | 23 | 32 | 41 | 50 | 59 | 69 | 80 | 91 | 102 | 114 | 126 | 139 | 152 | 166 |
| 125 | 31 | 25 | 19 | 13 | **7** | **7** | 15 | 22 | 31 | 39 | 48 | 58 | 68 | 78 | 88 | 99 | 111 | 123 | 135 | 148 |
| 130 | 35 | 30 | 25 | 19 | 13 | **7** | **7** | 14 | 22 | 30 | 39 | 47 | 56 | 66 | 76 | 86 | 97 | 108 | 119 | 131 |
| 135 | 40 | 35 | 30 | 24 | 19 | 13 | **7** | **7** | 14 | 21 | 29 | 38 | 46 | 55 | 64 | 74 | 84 | 94 | 105 | 116 |
| 140 | 44 | 39 | 34 | 29 | 24 | 18 | 12 | **6** | **7** | 14 | 21 | 29 | 37 | 45 | 54 | 63 | 72 | 82 | 92 | 102 |
| 145 | 47 | 43 | 38 | 34 | 29 | 24 | 18 | 12 | **6** | **6** | 14 | 21 | 28 | 36 | 44 | 52 | 61 | 70 | 80 | 89 |
| 150 | 50 | 46 | 42 | 38 | 33 | 28 | 23 | 18 | 12 | **6** | **7** | 13 | 20 | 28 | 35 | 43 | 51 | 60 | 69 | 78 |
| 155 | 53 | 50 | 46 | 42 | 37 | 33 | 28 | 23 | 18 | 12 | **6** | **6** | 13 | 20 | 27 | 34 | 42 | 50 | 58 | 67 |
| 160 | 56 | 53 | 49 | 45 | 41 | 37 | 32 | 27 | 22 | 17 | 12 | **6** | **6** | 13 | 19 | 26 | 34 | 41 | 49 | 57 |
| 165 | 59 | 55 | 52 | 48 | 44 | 40 | 36 | 32 | 27 | 22 | 17 | 11 | **6** | **6** | 12 | 19 | 26 | 33 | 40 | 48 |
| 170 | 61 | 58 | 55 | 51 | 48 | 44 | 40 | 36 | 31 | 26 | 22 | 17 | 11 | **6** | **6** | 12 | 19 | 25 | 32 | 39 |
| 175 | 63 | 60 | 57 | 54 | 51 | 47 | 43 | 39 | 35 | 31 | 26 | 21 | 16 | 11 | **6** | **6** | 12 | 18 | 25 | 31 |
| 180 | 65 | 62 | 60 | 56 | 53 | 50 | 46 | 42 | 39 | 34 | 30 | 26 | 21 | 16 | 11 | **6** | **6** | 12 | 18 | 24 |
| 185 | 67 | 64 | 62 | 59 | 56 | 53 | 49 | 46 | 42 | 38 | 34 | 30 | 25 | 20 | 16 | 11 | **5** | **6** | 12 | 17 |
| 190 | 69 | 66 | 64 | 61 | 58 | 55 | 52 | 49 | 45 | 41 | 38 | 33 | 29 | 25 | 20 | 15 | 10 | **5** | **6** | 11 |
| 195 | 71 | 68 | 66 | 63 | 60 | 57 | 54 | 51 | 48 | 44 | 41 | 37 | 33 | 29 | 24 | 20 | 15 | 10 | **5** | **5** |
| 200 | 72 | 70 | 67 | 65 | 62 | 60 | 57 | 54 | 51 | 47 | 44 | 40 | 36 | 32 | 28 | 24 | 19 | 15 | 10 | **5** |
| | 195 | 190 | 185 | 180 | 175 | 170 | 165 | 160 | 155 | 150 | 145 | 140 | 135 | 130 | 125 | 120 | 115 | 110 | 105 | 100 |

**Titration down** ←

Table 3.6 Individual plasma protein binding parameter estimates for a one saturable site model.

| Volunteer | N<br>Mean (SE) | K (L/mM)<br>Mean (SE) |
|---|---|---|
| **001** | 2.5 (10.9%) | 3.4 (31.2%) |
| **002** | 2.4 (9.7%) | 9.1 (36.2%) |
| **003** | 3.3 (8.3%) | 4.5 (24.6%) |
| **004** | 3.1 (10.6%) | 3.7 (31.1%) |
| **005** | 2.5 (8.3%) | 5.6 (23.4%) |
| **006** | 2.6 (6.4%) | 5.5 (19.3%) |
| **007** | 2.2 (12.9%) | 7.4 (41.3%) |
| **008** | 2.6 (9.2%) | 3.0 (23.8%) |
| **009** | 2.0 (5.3%) | 8.8 (15.5%) |

Table 3.7 Trough concentrations (mg/l) for nine volunteers (total VPA).

| | Pre dose | Dose | | Dose | | Dose | |
|---|---|---|---|---|---|---|---|
| **Vol** | | **500** | | **750** | | **1000** | |
| | day1 | **day 7** | **day 8** | **day 14** | **day 15** | **day 21** | **day 22** |
| 1 | 0 | 40.9 | 43.1 | 40.1 | 55.0 | 67.2 | 64.3 |
| 2 | 0 | 24.8 | 27.1 | 50.3 | 38.2 | 50.5 | 55.9 |
| 3 | 0 | 28.0 | 21.8 | 30.5 | 31.8 | 55.1 | 73.0 |
| 4 | 0 | 30.0 | 33.5 | 49.3 | 55.1 | 70.0 | 76.0 |
| 5 | 0 | 26.2 | 26.5 | 40.0 | 38.0 | 32.5 | 49.5 |
| 6 | 0 | 44.7 | 39.0 | 56.1 | 57.0 | 75.6 | 69.0 |
| 7 | 0 | 57.0 | 53.6 | 84.5 | 79.7 | 89.2 | 78.9 |
| 8 | 0 | 27.3 | 29.0 | 51.7 | 46.2 | 59.1 | 60.6 |
| 9 | 0 | 19.2 | 20.0 | 8.8 | 9.6 | 9.4 | 14.4 |

Table 3.8 Volunteers demographics.

| Vol | Age (Y) | Weight (lb) | Height (in) | Race | Gender |
|---|---|---|---|---|---|
| 1 | 31 | 126 | 65.5 | C | F |
| 2 | 34 | 150 | 68.5 | C | F |
| 3 | 31 | 184 | 71.0 | AA | M |
| 4 | 35 | 186 | 71.0 | AA | M |
| 5 | 49 | 173 | 69.0 | C | M |
| 6 | 29 | 204 | 70.0 | AA | F |
| 7 | 38 | 175 | 65.0 | AA | F |
| 8 | 47 | 182 | 66.5 | C | M |
| 9 | 31 | 145 | 66.0 | H | M |

C: Caucasian, AA: African American, H: Hispanic
F: female, M: Male

Table 3.9 Concomitant medication and adverse events (AE) at study conclusion.

| Vol. # | Concomitant medications | AE |
|---|---|---|
| 1 | Tylenol, Immodium, Depo-Provera, Unisom | Runny nose |
| 2 | Ponstel, Yasmin | Hematoma from Stick |
| | | Rash, Dizziness, Headache |
| 3 | NONE | NONE |
| 4 | Alka-seltzer, Tylenol PM | Sore throat, cough |
| 5 | NONE | NONE |
| 6 | Orthotri-Cyclen, Nyquill | Sleepeliness, drowsiness |
| | | Breakthrough bleeding |
| 7 | NONE | Diarrhea, Stomach Cramps |
| 8 | NONE | NONE |
| 9 | NONE | Diarrhea |

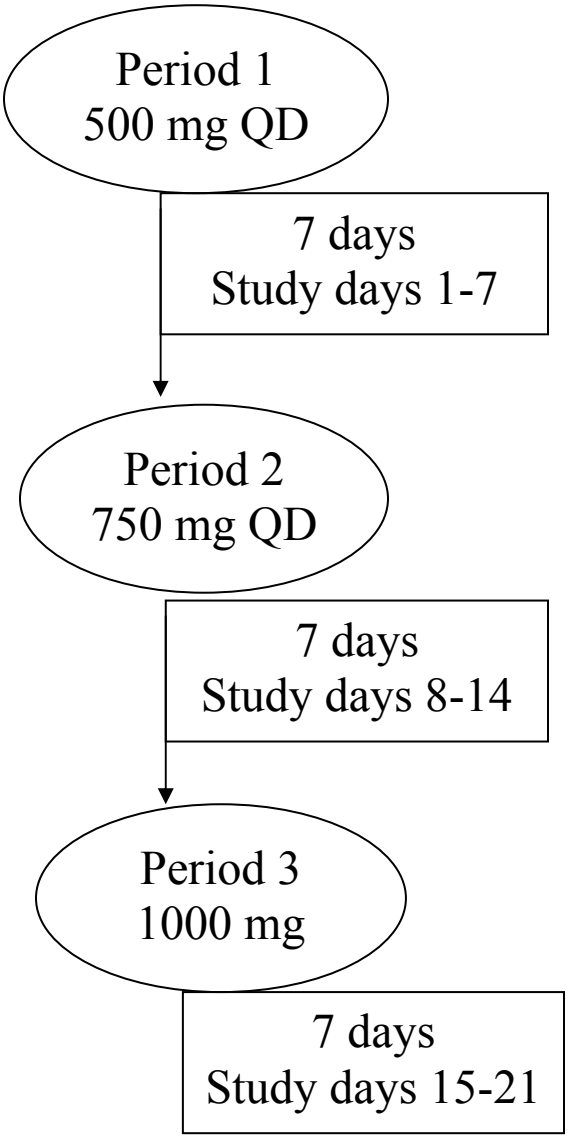


Figure 3.1 Dose escalation Schematic.

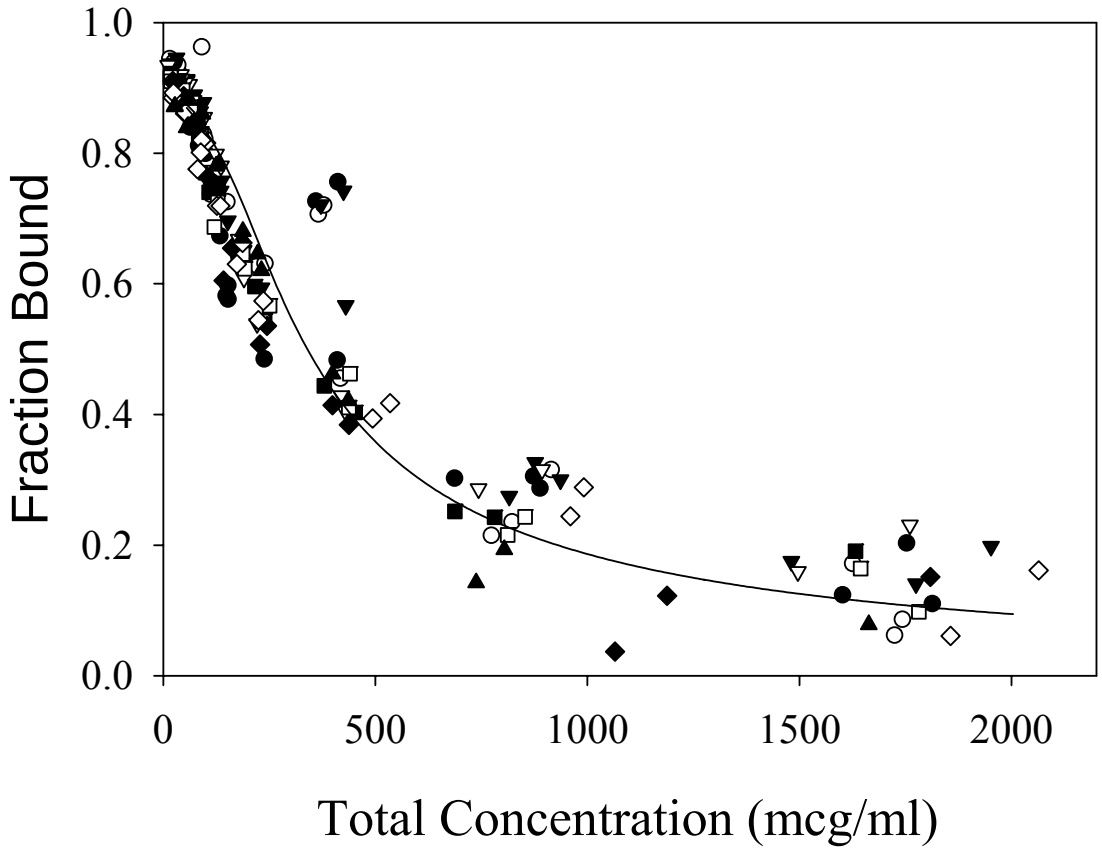


Figure 3.2 Fraction bound as a function of total VPA concentrations (mcg/ml) for nine volunteers. The fitted line represents the population fit in NONMEM.

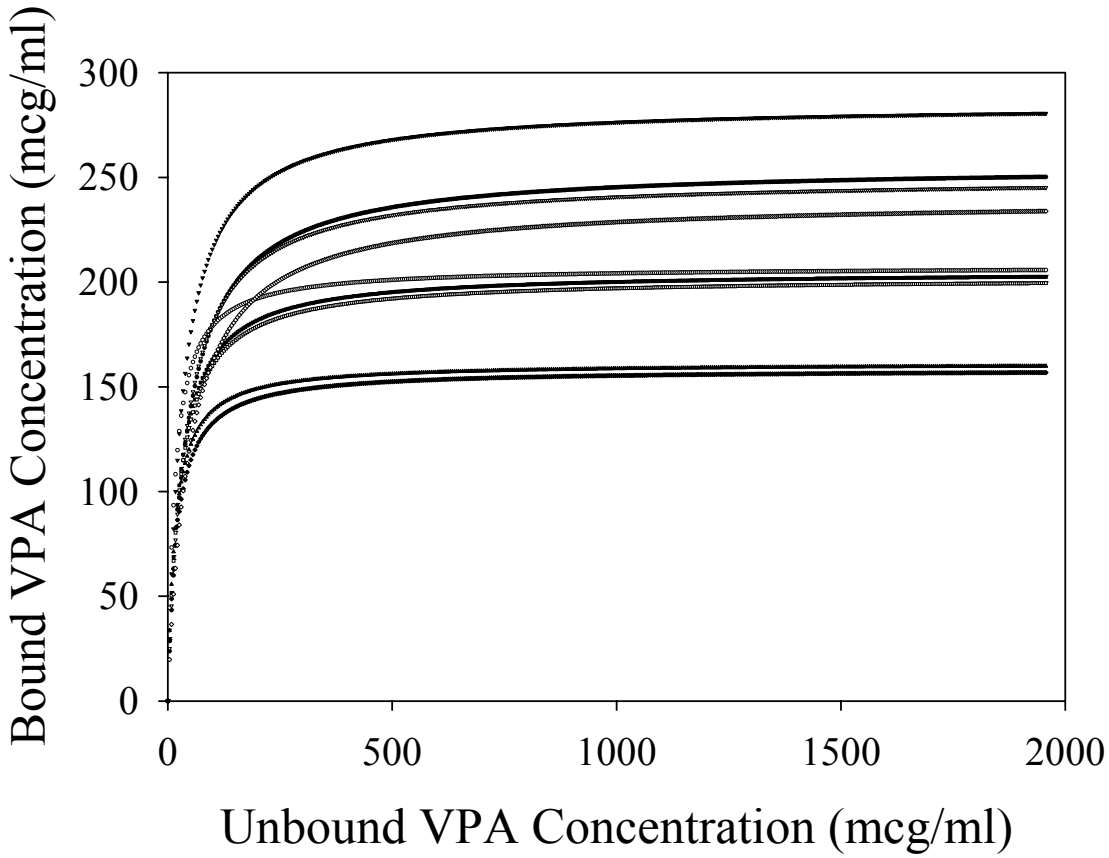


Figure 3.3 Simulations of bound versus unbound VPA concentrations (mcg/ml) for nine volunteers using parameters obtained from a one site saturable binding model.

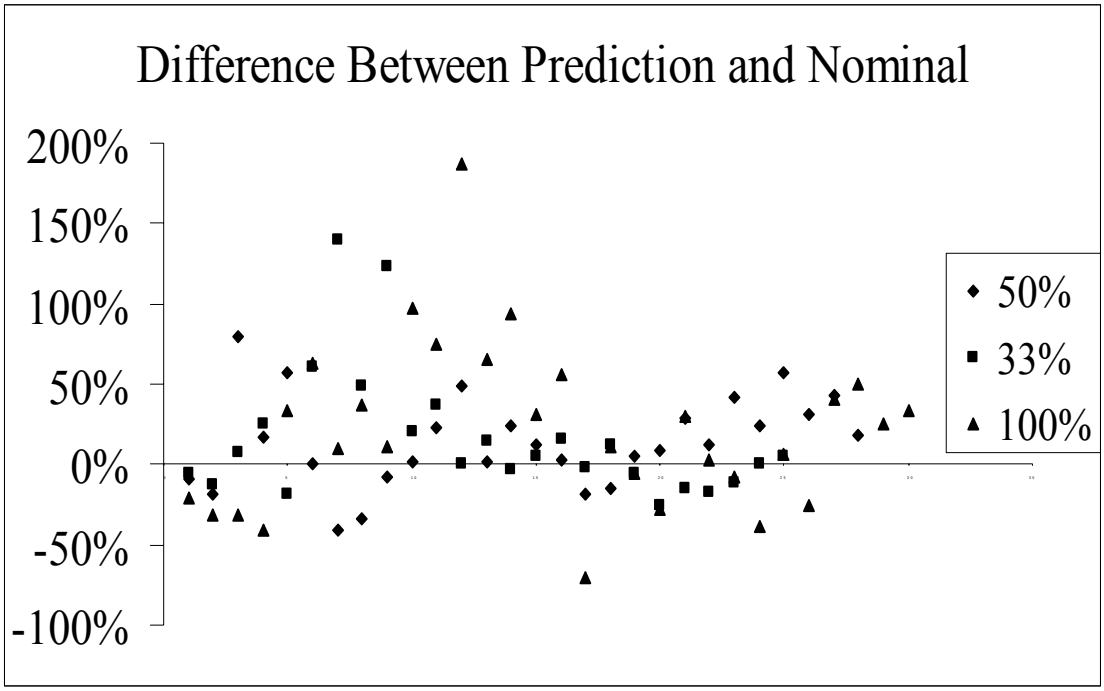


Figure 3.4 Difference between individual predictions and nominal increases in dose.

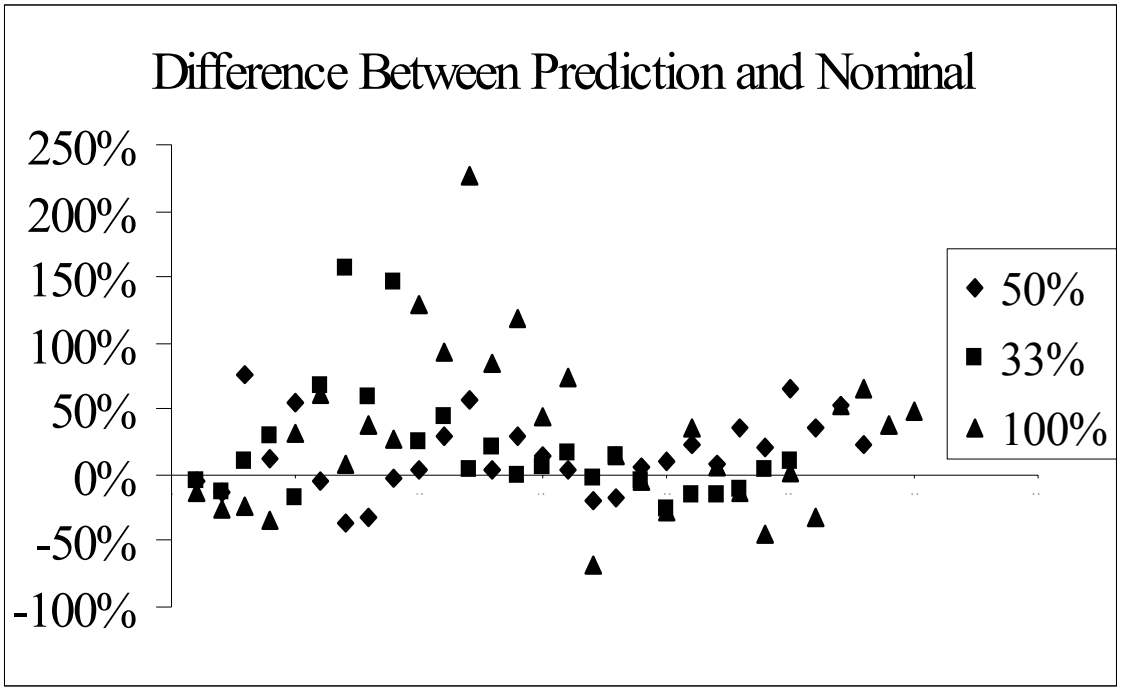


Figure 3.5 Difference between population model predictions and percent increases in dose.

# THE EFFECT OF DELAYED/MISSED DOSES ON VALPROIC ACID CONCENTRATIONS FOLLOWING DEPAKOTE® EXTENDED RELEASE

Alaa M. Ahmad, F. Douglas Boudinot, William H. Barr, Ronald C. Reed and William R. Garnett

**ABSTRACT**

Nine healthy volunteers were administered 500, 750 and 1000 mg Depakote® ER in a dose escalation study. After steady state at 1000 mg dose was reached, volunteers were randomized into three groups each subjected to a different delayed dose scenario. Groups one and two took a delayed dose (1000 mg) 6 h and 12 h respectively after the last scheduled dose. Group three took a daily dose plus replacement dose (2000 mg) 24 h after the last scheduled dose. Plasma protein binding of valproic acid (VPA) was characterized for each volunteer *in vitro*. Population pharmacokinetics models were developed for unbound VPA concentrations and for plasma protein binding. The combined information was used to construct a model to simulate missed dose scenarios for 100 subjects. The simulation model accounted for the effect of missed doses in the study volunteers and can be used to make dosing recommendations when patients miss a scheduled administration. At the higher dose of 2000 mg, the release from the ER preparation was not extended for 22 h. Although dose dumping has not been reported to date with Depakote® ER, it is not recommended to take more than two tablets (1000 mg) at once. A make-up dose can be taken 12 h after the daily dose if a dose is missed from a 1000 mg ER regimen.

Key words: valproic acid, missed doses, simulation, variability.

## Introduction

Valproic acid (VPA) is a broad-spectrum antiepileptic drug effective against a wide variety of seizure disorders, including complex partial, tonic-clonic convulsive, absence and myoclonic seizures [1, 2]. VPA has been used in the treatment of bipolar disorders and for the prophylaxis of migraine headaches. Other uses include management of acute mania, anxiety disorders and posttraumatic stress disorder. The drug has a good safety profile and most side effects are mild and transient. Two serious side effects are rare but fatal hepatotoxicity and teratogenicity [3].

There are several preparations for VPA. Divalproex sodium extended-release (Depakote® ER) is a once daily (QD) preparation for VPA. It's formulated as a hydrophilic polymer matrix controlled-release tablet system to provide more consistent blood concentrations over 24 hours. This system results in the release of drug in the stomach, small intestine, and large intestine over an 18- to 24-hour period of time.

The ER preparation was developed to provide convenient QD dosing and reduce side effects (mostly gastro-intestinal) associated with faster release preparations. Since VPA is used widely in different neuropsychiatric disorders, there are concerns about the effect of a missed or delayed dose from a once-daily regimen. We addressed this issue systematically in a previous paper using simulations [4].

As a follow-up for the previous simulations, a study was conducted in nine healthy volunteers. The objective of this study was to explore the effect of delayed/missed doses on steady state concentrations following the administration of Depakote® (ER) formulation and to validate the simulation model that accounts for delayed/missed doses.

## Methods

The Institutional Review Board of the Virginia Commonwealth University Office of Research Subjects Protection approved this study, and all volunteers provided signed informed consent prior to participation.

### *Clinical Study*

Nine healthy volunteers were administered 500, 750 and 1000 mg VPA in a dose escalation study. Two trough plasma samples were collected after steady state was reached at each dose. On day 22 of the study, all volunteers were expected to be at steady state concentrations at a 1000 mg QD of the ER formulation. Volunteers were pre randomized, group one took their next scheduled dose 6 hours late, group two took their next dose 12 hours late; while group three missed their dose completely on day 22 (figure 4.1). On day 23 of the study, groups one and two took 1000 mg, while group three took the daily dose plus replacement dose (2000 mg). All volunteers took 1000 mg on day 24. Drug administration was then reduced gradually over the course of four days to safely remove volunteers from the valproic acid regimen. Serial blood sampling was performed

for 24 hours around the delayed dose and plasma was stored at ¯70 °C till analysis. Prior to analysis, an aliquot of each plasma sample was incubated at 37 °C for one hour and unbound valproic acid was separated by ultrafiltration using a temperature-controlled (37 °C) centrifuge (Eppendorf, Westbury, NY). Free and total valproic acid concentrations were determined using fluorescence polarization immunoassay; on a $TD_x$ analyzer (Abbott Laboratories, Abbott Park, IL). The calibration range for this assay was 12.5-150 μg/ml and 2-25 μg/ml for total and free valproate, respectively. Assay variability has been to shown to be less than 5 % [5].

*Population Model Development*

The purpose of this analysis was to obtain estimates for clearance (CL/F) and volume of distribution (Vd/F) and their inter- and intra-individual variabilities using NONMEM (Globomax, Hanover, MD). A one compartment model was used as a structural model. Inter-individual variabilities in CL/F and Vd/F ($\eta_{CL}$ and $\eta_{Vd}$ ) were modeled as normally distributed parameters with a mean of zero and variances $\omega^2_{CL}$ and $\omega^2_{Vd}$, respectively, using exponential error models. Residual variability was modeled using a proportional error model as a normally distributed parameter with mean of zero and variance $\sigma^2$. The first order conditional estimation method with interaction was used for estimation [6].

Covariates were incorporated using linear models. The change in the objective function (Δobj) was used to judge whether a covariate explains some of the random variability in CL or Vd. It is assumed that Δobj is approximately $\chi^2$ distributed and thus a reduction in the objective function of 3.84 units corresponds to a significance level of 0.05 [6]. Final model selection was based on the change in objective function for nested models, goodness of fit and residual plots and precision of parameter estimates for fixed and random effects.

*Simulations*

Once estimates for CL/F, Vd/F and their intra- and inter-individual variabilities were obtained, a simulation model was constructed in the Trial Simulator $^{TM}$ (Pharsight Corporation, Mountain View, CA). The model assumed that the kinetics of unbound VPA were linear and thus started with unbound parameters. Total VPA concentrations were simulated using a plasma protein binding model. Development of the plasma protein binding model for VPA was based on data from the same nine volunteers and is reported elsewhere [7]. A one site saturable binding model was used that incorporates inter-individual variability in the equilibrium association constant $K_A$. Unbound VPA concentrations ($C_u$) were simulated according to $C_u = F*D/CL_u*T\ (e^{CL_u/V_u*T} - 1)\ e^{-CL_u/V_u t}$, where D is the dose, $V_u$ is the volume of distribution of unbound drug, $CL_u$ is the systemic clearance of unbound drug, F is bioavailability and T is the duration of the zero order input [8]. T was fixed to 22 h and F to 0.89 [9].Total VPA concentrations ($C_t$) was

calculated using $C_t = C_u + (N K C_u P)/(1+KC_u)$ Where P is albumin concentration, N and $K_A$ are the number of binding sites and equilibrium association constant, respectively.

The simulation model was used to simulate the delayed doses scenarios in the clinical study. Unbound and total VPA steady state concentrations following multiple doses of 1000 mg daily of ER were generated for 100 hypothetical subjects. Concentration time profiles were generated for delaying the dose by 6 h (group1), 12 h (group 2) and 24 h (group3). In the third scenario (24 late, missed dose), a replacement dose was given (dose double-up).

**Results**

*Clinical Study*

All volunteers completed the study. One volunteer experienced increased gastrointestinal motility that resulted in mal absorption of the compound, and thus concentration decreased with dose escalation. The data for this volunteer were excluded from population model development.

*Population model*

Dosing, concentration-time data and covariates (age, weight, body mass index and gender) for eight volunteers were used for analysis (24 days). None of the covariates tested resulted in a significant reduction in the objective function from the base model and thus the final model included no covariates. The final model estimated a CL/F of 5.4

(0.05%) L/h and a Vd/F of 186 L (0.1%). The inter-individual variabilities in CL/F and Vd/F were 25.6% and 15.9% respectively. The residual variability was estimated to be 23.4%.

Estimates for plasma protein binding parameters obtained previously [7] were N = 2.3 (4.5%) and $K_A$ = 6.7 (17.9%) (L/mM). The inter-individual variability term for $K_A$ ($\eta_K$, exponential error model) was estimated to be 30.7% and residual variability (proportional error model) was estimated to be 31.9%.

*Simulation Study*

The simulation model is shown in figure 4.2. Parameter means and their variabilities were obtained from this study and the previous analysis of plasma protein binding data [7].

Figures 4.3-4.5 show results for simulation of 100 individuals under different scenarios. Results for the three groups in this study are superimposed on the corresponding scenario. Figure 4.3 shows total and unbound concentrations, respectively, for group one which took a dose 6 hours after the scheduled dose. Figure 4.4 shows total and unbound concentrations for group two which took a dose 12 hours after the scheduled dose. Results for group three (missed dose followed by replacement dose) are shown in figure 4.5 for total and unbound concentrations. For group three, it seems that the release of VPA from the ER formulation was not extended for 24 hours at this higher

dose. Also volunteer 007 (group 1) appeared to have a higher free concentration that what's expected. This couldn't be attributed to medical history or disease state. Plasma protein binding curve for 007 was not significantly different from other volunteers.

**Discussion**

Previously, a systematic characterization of the effect of missed doses following the administration of Depakote® ER using simulations was performed [4]. The simulations suggested that at a daily dose of 1000 mg, a delayed or missed dose should be replaced as soon as possible with no risk of toxicity in the case of dose double-up. The previous simulation model did not include estimates for residual variability and estimates for random variability in system parameters were obtained from the literature. In this study, we were able to obtain estimates for clearance and volume of distribution of unbound VPA and their inter-individual variabilities. An estimate for residual variability accounting for model misspecification, measurement error and intra-individual variability was also obtained and incorporated into the current simulation model. Further in the previous simulation model estimates for plasma protein binding parameters and their variabilities were not obtained in the same population as CL and Vd of the unbound drug. In this study, plasma protein binding was characterized *in vitro* for each volunteer and estimates for plasma protein binding parameters and the inter-individual variability in the equilibrium binding association constant were obtained. An estimate for the residual variability in plasma protein binding curves was used in the current simulation model. With this, possible sources of the variability in the study population

were accounted for. Unfortunately, due to the small sample size, covariate trends could not be detected. Therefore, the simulation model didn't include any covariates.

The simulation model developed in this study accounts for the effect of delayed doses in the three groups for both unbound and bound VPA. The previous model didn't completely account for all individuals (results not shown) because it lacked specification of residual variabilities.

It is apparent from the results of this study that the ER preparation does not always provide extended release over 22 hours. Although the release rate was fixed to 22 hours in the simulation model, the population model estimated ~ 43% inter-subject variability in the duration parameter. Most of this variability is contributed by group three, for whom the release was extended to 12 hours at best and much less for volunteer one. There is no reason to believe that the release from the ER preparation should differ at higher doses [10], apart from the increase surface area, which might not totally account for the enhanced release. To ensure patient safety, taking four ER tablets (2000 mg) at once is not recommended. Patients are advised to take 1000 mg and then another 1000 mg after 12 hours if a dose is completely missed from a 1000 mg QD ER regimen. A dose taken 6 or 12 h late from a once-daily ER regimen should be replaced as soon as remembered.

In summary, a simulation model that appropriately incorporates information on the inter-subject variabilities in unbound and bound VPA and their residual variabilities was developed and validated in healthy volunteers. This model can be used to derive dosing recommendations in the case of delayed/missed doses.

*Comparison between original and revised models*

Table 4.1 lists parameters estimates and their standard deviations used in original and revised simulations models. Clearance estimates are comparable in both models while volume of distribution is almost double in revised model. A different plasma protein binding model was used in each model; the original model employed a two-site saturable binding model. It can be seen that the affinity rate constant ($K_2$) is not estimated with good precision (COV=80%). The revised model employed a one-site saturable binding model and parameters were well estimated. During the process the identifying the structural model (that lead to the revised one-site model), a two site model was tested but parameters were not estimated with good precision and convergence couldn't be achieved for several volunteers. Being the case, a one-site model was used in the revised simulation model to account for PPB of VPA to albumin. Albumin concentrations were similar in both models.

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Table 4.1 Parameters for original and revised simulation models.

| Parameter (SD) | Original model | Revised model |
|---|---|---|
| $CL_u$ (L/h) | 5.04 ±1.00 | 5.4 ± 0.0027 |
| $Vd_u$ (L) | 95.1 ± 19.0 | 186 ± 0.186 |
| $N_1$ | 1.54 ± 0.108 | N= 2.3 ± 0.1 |
| $K_1$ (L/mmol) | 11.9 ± 1.99 | K= 6.7 ± 1.2 |
| $N_2$ | 0.194 ± 0.0783 | - |
| $K_2$ (L/mmol) | 164 ± 141 | - |
| P (mmol/L) | 0.528 ± 0.0528 | 0.57 ± 0.06 |

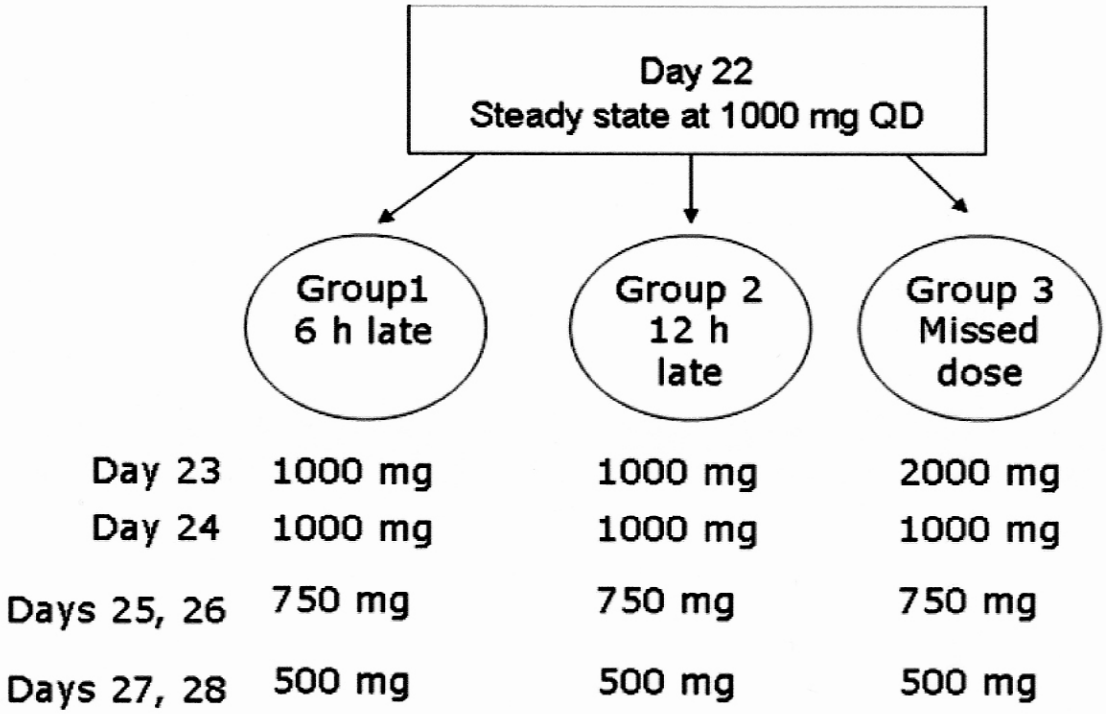


Figure 4.1 Missed doses schematic.

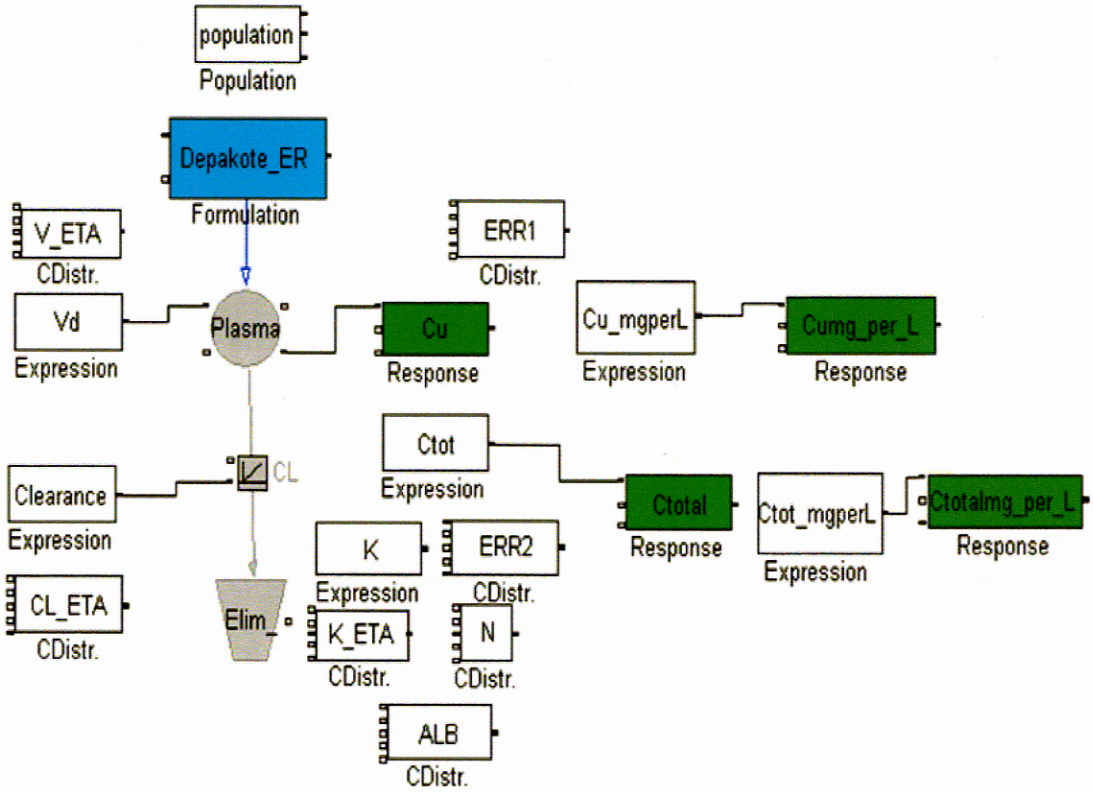


Figure 4.2 Structural and statistical model used to simulate missed doses.

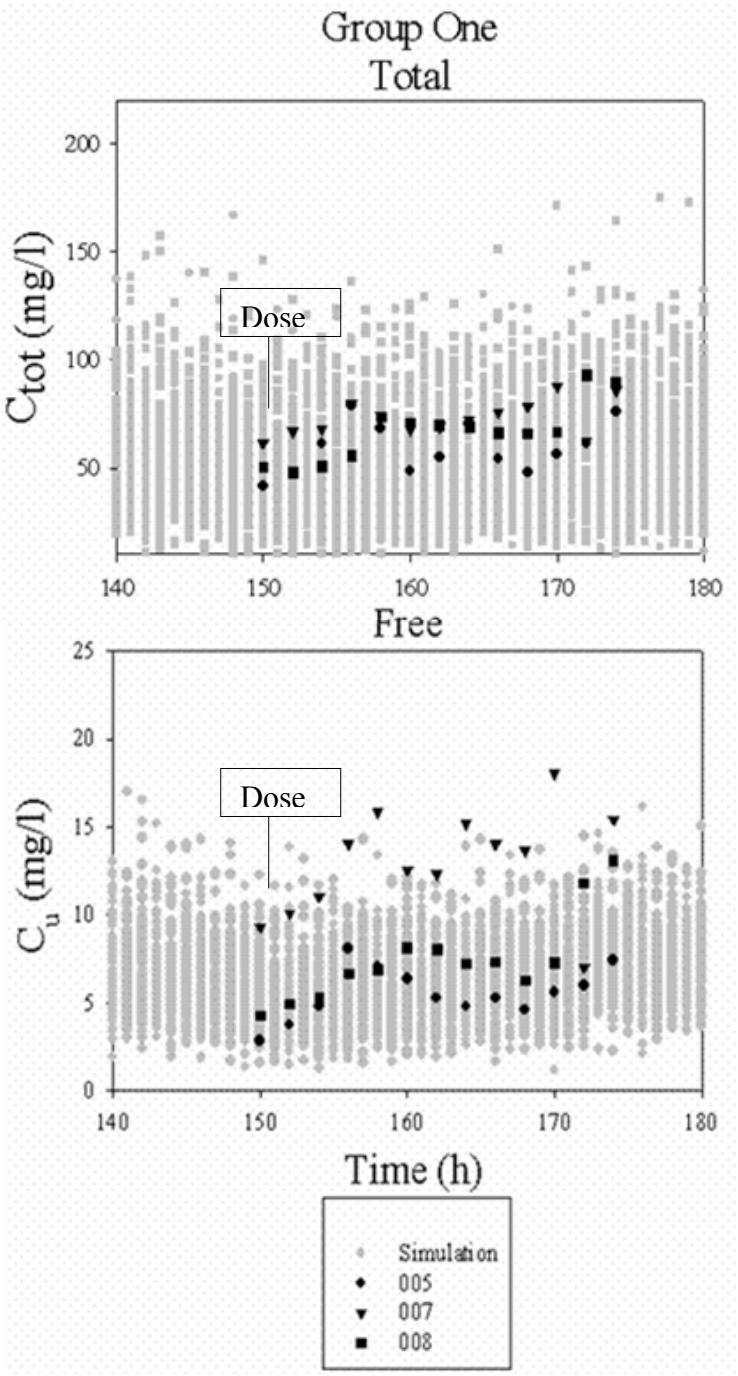


Figure 4.3 Simulated total (upper panel) and free (lower panel) VPA concentrations for 100 subjects when a 1000 mg is taken 6 h late. Results for group one are superimposed (subjects 005, 007, 008).

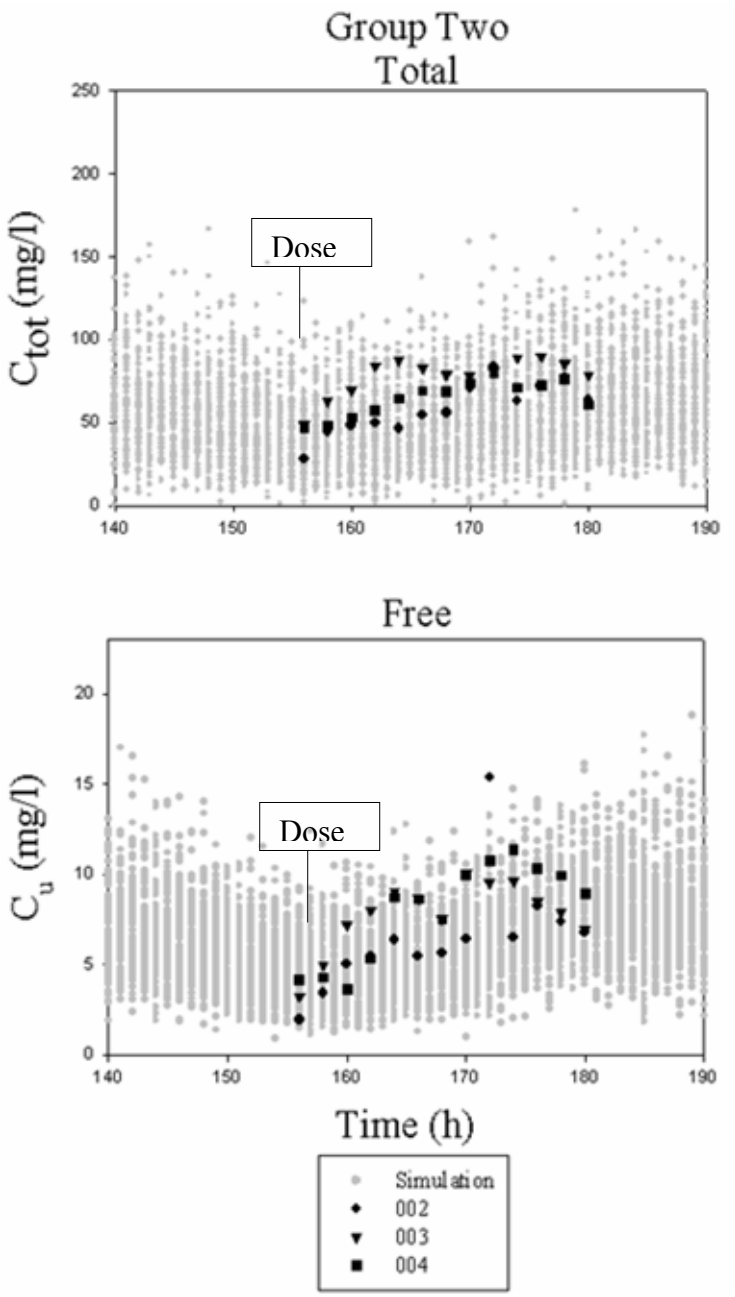


Figure 4.4 Simulated total (upper panel) and free (lower panel) VPA concentrations for 100 subjects when a 1000 mg is taken 12 h late. Results for group two are superimposed (subjects 002, 003, 004).

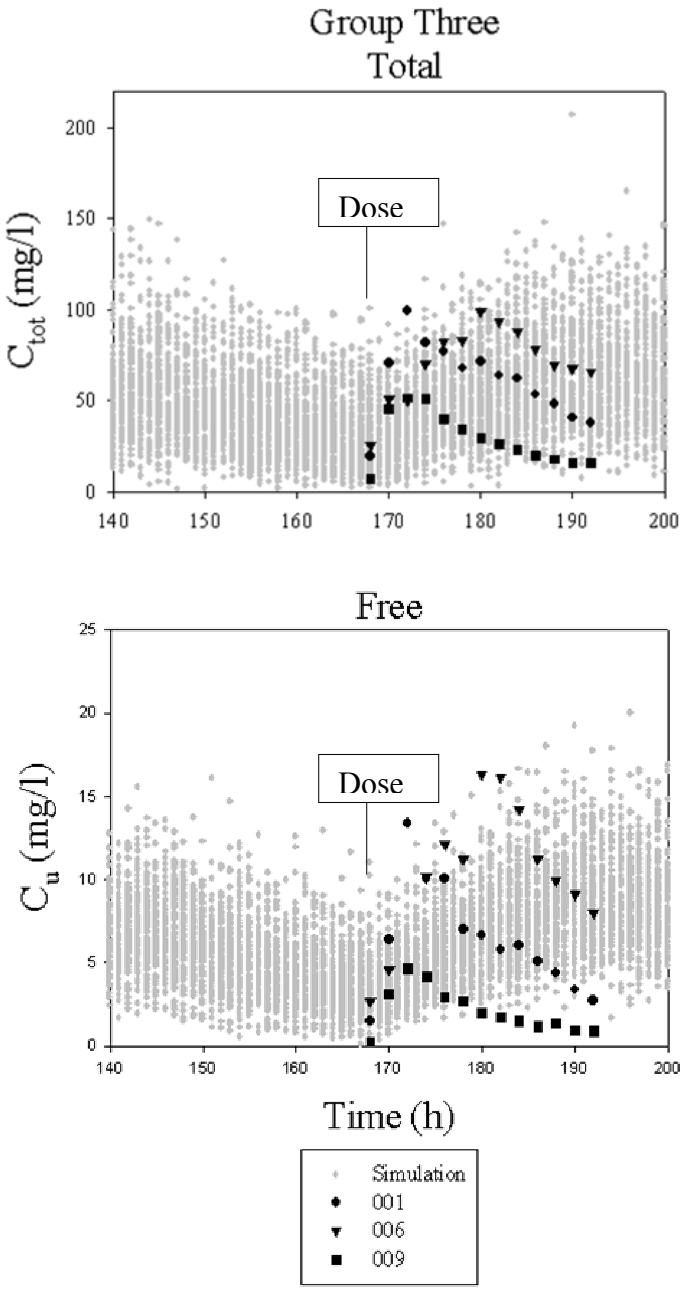


Figure 4.5 Simulated total (upper panel) and free (lower panel) VPA concentrations for 100 subjects when a 1000 mg is missed followed by dose double-up. Results for group three are superimposed (subjects 001, 006, 009).

# CONCLUSIONS

Using Monte Carlo simulations, the effect of different patterns of poor compliance on ER QD and DR BID were systematically characterized. Non-linear binding of VPA to albumin was incorporated into the model, and the results were based on total and unbound VPA for comparison. The effect of poor compliance is less significant on DR BID compared to ER QD. Dosing recommendations in the case of a missed or delayed dose are both formulation and dose dependent. Since total VPA concentrations show higher inter-individual variability and tend to under-estimate the effect of poor compliance; the use of unbound VPA concentrations may offer an advantage in therapeutic monitoring.

The VPA nomogram is based on non-linear plasma protein binding data and quantitates the non-linear relationship between total concentrations and the dose. To minimize the risk of achieving toxic drug concentrations, the dose should not be increased more than 2 fold at a time. The nomogram should be used in conjunction with patient history and clinical response to aid clinicians in making informed decisions about dose-adjustments. It should be noted that the nomogram can be used even if patients are on enzyme-inducing or inhibiting co-medications. The nomogram will always predict a

percentage of the previously administered dose and not an absolute value. Disease (e.g. liver) or physiological conditions (e.g. pregnancy) that lead to reduced binding will result in lower total concentrations. In this case the nomogram might under predict the needed increase in dose, which means that dose titration might take longer but there is reduced risk of achieving toxic concentrations. The Effect of drugs that displace VPA from plasma protein binding sites is more complex. In this case, factors to consider are the concentration of the displacer and its comparative affinity to VPA binding site. Since therapeutic VPA concentrations are in the order of 50-150 mg/l, presence of displacers (e.g. aspirin) might not affect the use of the nomogram in a clinically significant manner.

Population pharmacokinetics models were developed for unbound VPA concentrations and for plasma protein binding. The combined information was used to construct a model to simulate missed dose scenarios for 100 subjects. The simulation model accounted for the effect of missed doses in the study volunteers and can be used to make dosing recommendations when patients miss a scheduled administration. At the higher dose of 2000 mg, the release from the ER preparation was not extended for 22 h. Although dose dumping has not been reported to date with Depakote® ER, it is not recommended to take more than two tablets (1000 mg) at once. A make-up dose can be taken 12 h after the daily dose if a dose is missed from a 1000 mg ER regimen. If a 1000 mg dose is taken 6 or 12 h late from schedule, then it should replaced as soon as possible.

**Clinical Significance**

Clinicians are divided on how to replace doses during therapy with VPA and it's unethical to withhold therapy from patients with epilepsy to explore the effect of missed and delayed doses. This research provides dosing recommendations to the clinicians to counsel patients taking preparations of VPA in the event of a missed dose. The use and validation of VPA nomogram will foster the rational use of VPA for the treatment of epilepsy and its role in other neuropsychiatric disorders.

APPENDIX A

# CLINICAL STUDY PROTOCOL

Title: A Prospective study to test a nomogram for valproic acid, a neuro psychiatric agent, and to explore the effect of poor compliance on Depakote® extended release, a commercial preparation for valproic acid

Study Site: Center for Drug Studies, School of Pharmacy, Virginia Commonwealth University, Richmond, VA

Study Design: Open Label, Dose escalation and missed doses in healthy volunteers

Principal Investigator: William R. Garnett, Pharm.D, Departments of Pharmacy and Neurology, VCU Medical Center, Richmond, VA.

Medical Investigator: Larry Morton, MD. Department of Neurology.

Co-investigators

F. Douglas Boudinot, Ph.D. Department of Pharmaceutics. (Supervision and data analysis)

William H. Barr PharmD., Ph.D., Department of Pharmacy, Center for drug studies. (Supervision)

Alaa Ahmad, B.S. Pharm., Department of Pharmaceutics. (Analytical, in vitro study, data analysis, manuscript write-up)

**Contents**

Abbreviations
1.0 Background
2.0 Study Objectives
3.0 Study Design
- 3.1 Subject Selection
- 3.2 Screening
- 3.3 Study Assessment and Procedures
  - 3.3.1 Dose Escalation
  - 3.3.2 Missed Doses
  - 3.3.3 Dose Tapering
  - 3.3.4 Exit Procedures
- 3.4 Meals
- 3.5 Drug Storage, Dispensing and Methods of Administration
- 3.6 Sample Collection, Handling and Storage
- 3.7 Data Collection Schedule
- 3.8 Analytical Methodology
- 3.9 Statistical Procedures
  - 3.9.1 Sample Size
  - 3.9.2 Randomization and Blinding
  - 3.9.3 Demographics and Patient Characteristics

4.0 Data Analysis
5.0 Duration of Study
6.0 Recruitment for All Groups
7.0 Adverse Events
8.0 Data Collection Management
- 8.1 Source Documents / Retention of Data

9.0 Protection Of Human Subjects
- 9.1 Ethical Regulations
- 9.2 Subject Safety and Confidentiality
- 9.3 Protection of Human Subjects

10.0 Publication of Results
11.0 Biohazard Containment
12.0 References
13.0 Investigator Signature
14.0 Informed Consent

Appendix 1 VPA nomogram
Appendix 2 Sampling Schematic
Appendix 3 Event Schedule

ABBREVIATIONS

| | |
|---|---|
| AE | adverse event |
| BP | blood pressure |
| CBC | complete blood count |
| CNS | central nervous system |
| C-T | Concentration Time |
| DR | Delayed Release |
| DSMC | Data Safety Monitoring Committee |
| ECG | electrocardiogram |
| ER | Extended Release |
| FDA | Federal Drug Administration |
| GCP | Good Clinical Practice |
| G1 | Group 1 |
| G2 | Group 2 |
| G3 | Group 3 |
| hCG | urine pregnancy test |
| HR | heart rate |
| IRB | Internal Review Board |
| n | number (e.g., number of volunteers) |
| U/A | urinary analysis (Lab Tests) |
| VCU | Virginia Commonwealth University |
| VPA | valporic acid |

## 1.0 Background

Since its first clinical use in France in 1967, valproic acid (VPA), rapidly became a major anti-epileptic drug worldwide. VPA is recognized as a highly effective first-line drug against the generalized seizures encountered in idiopathic or primary generalized epilepsy, including absence, generalized tonic-clonic and myoclonic seizures. It is also used in bipolar disorders and for the prophylaxis of migraine [1, 2]. Valproic acid has an interesting pharmacokinetic /pharmacodynamic profile. It binds extensively to plasma proteins; at therapeutic concentrations, bound VPA comprises 90% of total VPA. This binding is also non-linear; binding site(s) saturate at higher doses affecting the clearance and volume of distribution of total VPA. The kinetics of unbound VPA, however, remains linear. This means that unbound or free VPA levels increases proportionally with dose while total VPA levels increases less than proportionally. It is widely accepted that the free form of the drug is the one that is associated with pharmacological actions and toxic effects. Although the analytical means to measure free VPA are available [1], total VPA levels are most commonly used in therapeutic drug monitoring during therapy with VPA. This has various implications. Of interest to our research is the effect of poor compliance, in terms of delayed and/or missed doses from schedule, on two commercially available preparations for VPA. These are the Depakote® extended release and delayed release formulations (ER and DR respectively). We have recently completed a systematic characterization of the effect of poor compliance on the ER & DR preparations [3]. The manuscript for this work, which involved Monte Carlo simulations, is in preparation. We have concluded from our simulations that Unbound VPA levels should be used for therapeutic monitoring since total levels show higher inter-individual variability and can under-estimate the effect of poor compliance. We plan to test the model used for the simulations in terms of its predictive power for the effect of poor compliance on the ER preparation.

Recognizing that total VPA levels are still in use for therapeutic drug monitoring (although unbound VPA levels are more informative as stated earlier), we have developed a nomogram (Appendix 1) that can be used to predict percent (%) increase or decrease in dose needed to escalate or decrease total VPA levels [4]. This nomogram was developed using simulations based on a two-saturable binding site model for the plasma protein binding of VPA [5, 6]. Before recommending the nonmogram for clinical use, it is necessary to test the nomogram in a clinical study. Since the VPA nomogram is based on deterministic simulations, we plan to characterize the plasma protein binding of VPA in order to account for inter-individual variability in protein binding. This mixed effect modeling will allow individualizing the therapeutic drug monitoring of VPA based on total levels.

## 2.0 Study Objectives

1. To test the VPA nomogram. This will allow individualizing the therapeutic monitoring of VPA based on total levels.

2. To determine if VPA levels after missed doses in the population under study can be predicted from the simulations. This means that the population model used for the stochastic simulations is adequate, valid and can be used to make predictions regarding the steady state levels in the case of missed doses.
3. To characterize the plasma protein binding of VPA *in vitro.* Additional knowledge obtained will be used to modify the nomogram to account for inter-individual variability.

## 3.0 Study Design

This is an open-label, dose escalation, and missed doses study that will be performed in healthy volunteers (n=9). One of the study objectives is to develop a model for the binding parameters involved in the nonlinear binding of Valproic acid to albumin. This will be achieved by obtaining blank blood samples from subjects within one week before drug administration starts. Drug Administration will last up to 28 days; subjects will be housed in the Clinical Study Unit for 3 overnight stays during the course of the study. The study drug is a commercially available preparation of Valproic acid; the Depakote® extended release preparation (Depakote ER). The basic design of the study will involve escalating the dose of the ER preparation at three dosing levels: 500 mg, 750 mg & 1000 mg, all given once daily; to achieve steady state at each dosing level. The purpose of the dose escalation is to test the VPA nomogram (appendix 1). Two blood samples will be taken to confirm steady state at each dosing level. After dose escalation, subjects will be randomized into three groups (on day 21: G1, G2, and G3), each of which will miss a scheduled ER dose at a certain time. Thirteen (13) blood samples will be withdrawn from subjects in each group, following the missed dose, to characterize the concentration-time (C-T) profile of both free and total VPA. The C-T profile following missed doses is needed to test the outcome of a stochastic model for missed doses. After the completion of blood sampling for missed doses, Depakote dose will be tapered off as a precautionary measure. Subjects will be monitored for side effects during all phases of the study and vital signs recorded.

### 3.1 Subject Selection

**Inclusion criteria**

1. Males or females, in the age range of 18-55 years (inclusive).
2. Healthy volunteers with no clinically relevant abnormalities as determined by: medical history, Physical examination, blood chemistry, complete blood count (CBC), and urine analysis.
3. Negative test for drugs of abuse (LIST)
4. Subjects must be within normal limits of weight (± 30%).

5. Subjects not taking any other medications during the study that might produce drug-drug interactions with the valproic acid.
6. Due to teratogenic effects of VPA, women of childbearing potential will be included in the study provided that they are not nursing and practice an acceptable form of contraception. A urine hCG test will be performed to rule out pregnancy.
7. Subjects must be non-smokers.

**Exclusion criteria**

1. Subjects with known hypersensitivity to the valproic acid.
2. Subjects with liver function tests those are greater than two times the upper normal limit or active liver disease.
3. Subjects with known urea cycle disorders
4. Donation or receipt of whole blood or blood products within 3 months of study day 1.
5. Subjects with albumin concentration < 0.35 g/dl.
6. Participation in a clinical study for an investigational drug, device, or procedure for a period of 2 months prior to the first day of the study.
7. Subjects with any disease states that might affect the pharmacokinetics of VPA.
8. History of vaso-vagal reaction or "feeling faint" during blood collections.
9. A hematocrit level below 40% for males and females.
10. Any personnel involved with the study at the investigational site
11. Unable to refrain from alcohol the duration of the study and within 48 hours of entry during the inpatient phase.

## 3.2 Screening

Prior to the initiation of the study, subjects will be fully informed of the study plan, procedures and risks involved in participating in the study, and will be asked to sign and date the Informed Consent Form (ICF). Once a subject has agreed to participate and signed the informed consent, they will be screened (screening window is 28 days), in order to establish eligibility. The volunteers will receive a medical examination, consisting of a medical history, brief physical examination, 12-lead ECG, Vital Signs (BP, HR) and laboratory tests (Chemistries (SMA-18), CBC with Hematocrit, U/A, HIV, Urine Drug Screen, Urine Pregnancy test for females). Albumin concentration will be measured because it is an important determinant of the kinetics of VPA. The normal range for albumin concentration is 3.4-5.4 g/dl or 0.4857-0.7714 mM.

## 3.3 Study Assessments and Procedures

Eligible subjects (based on screening procedures described above and in appendix 3) are required to donate 100 ml of blood within one week before drug administration starts.

Blank blood will be centrifuged to obtain plasma and stored at -70 ° C. Blank plasma from study subjects will be used to characterize the plasma protein binding of VPA *in vitro*. Briefly, serial concentrations of VPA ranging from 0-200 mg/l will be prepared in plasma. Following mixing under 37 ° C for 1 hour, Unbound VPA will be determined using ultra filtration. Bound VPA will be calculated as: nominal concentration – unbound concentration. Data analysis is described below.

Details of study procedure are described in appendix 3. Dose Escalation, Missed doses, and Dose Tapering Phases are described below.

3.3 .1 Dose Escalation **(See study schema: appendix 2)**

Subjects will be administered three different doses to reach steady state:

1. 500 mg once daily (8:00 am) for seven days to achieve steady state (Study days 1-7).
2. 750 mg once daily (8:00 am) for seven days to achieve steady state (Study days 8-14)
3. 1000 mg once daily (8:00 am) for seven days to achieve steady state (Study days 15-21)

Note: All blood samples are 7 ml each. Both free and total VPA will be determined. The purpose of dose escalation is to test the nomogram. In order to confirm steady state, two blood samples will be taken from each subject. The two samples will measure the trough level (Minimum concentration, $C_{min}$) and will be taken before the daily doses (8:00 am) on the following study days: 1, 7, 8, 14, 15, 21 and 22.

3.3 .2 Missed doses

On day 21 of the study, subjects will be randomized into 3 groups each consisting of 3 subjects:
1- Group 1 (G1): will take 1000 mg ER at 14:00 (six (6) hours late from schedule)
2- Group 2 (G2): will take 1000 mg ER at 20:00 (twelve (12) hours late from schedule)
3- Group 3 (G3): will not take any dose i.e. miss ER dose

Serial blood sampling will be conducted for each group as detailed in appendix 2.
**Total number of samples withdrawn from each subject is 20**.

3.3.3 Dose tapering

ER dose will be tapered off for each subject after completion of the Pharmacokinetic Sampling (Day 22-24) as shown in the study schema (appendix 2).

3.3.4 Exit Procedure

At the completion of the inpatient phase of the study procedures, the volunteer will have the following exit procedures: brief physical exam, ECG, Vital Signs (BP, HR) and Adverse Event evaluation. Volunteers will be discharged when it's clear that there are no complications**.** The Medical Investigator or his designee will authorize release of the volunteer. Volunteers will return after dose tapering for a follow-up visit (Vital Signs (BP, HR) and Adverse Event evaluation).

## 3.4 Meals

There will be no food or water restriction. Standardized meals will be served to subjects while on site (Breakfast will be served 2 hours post dose, other meals during missed doses will be served at the designated times).

| | Breakfast | Lunch | Dinner |
|---|---|---|---|
| Day 21 | | | G1, G2, G3 |
| Day 22 | G1, G2, G3 | G1, G2, G3 | G1, G2, G3 |
| Day 23 | G1, G2, G3 | G1, G2, G3 | G1, G2, G3 |
| Day 24 | G1, G2, G3 | | |

## 3.5 Drug Storage, Dispensing and Methods of Administration

Depakote ER preparation will be stored at room temperature and constant humidity conditions in the CDS pharmacy. CDS pharmacy technicians and investigators will be responsible for the preparation and dispensing to study subjects each period and while subjects are in the CDS. Study drug will be administered orally with 100 ml water. Subjects are required to call in to the unit every morning to ensure compliance for outpatient dosing.

## 3.6 Sample Collection, Handling and Storage

Samples will be collected in suitable heparinized glass or plastic collection centrifuge tubes. Blood will be centrifuged in a refrigerated centrifuge (4 $^{o}$C) at 3000 rpm (1500g) for 10 minutes. Plasma will be harvested into Teflon capped, silanized tubes labeled (Study #, Subject #, Collection Time, Date), and stored in an alarmed –70$^{o}$C freezer. All collection, centrifuge and storage times will be immediately recorded on the appropriate source documents.

## 3.7 Timed Event Schedule

See Appendix 3.

## 3.8 Analytical Methodology

Total and unbound VPA plasma concentrations will be determined using a commercially available fluorescence polarization immunoassay (FPAI) on the $TD_x$/$TD_x$ $FL_x$ system (Abbott Laboratories, Abbott Park, IL, USA). Ultra filtration will be used to separate unbound drug. Reports in the literature almost invariably report a coefficient of variation of less than 10 % for the assay. The lower limits of quantification for total and unbound VPA in plasma were 12.5 and 2 mg/L, respectively [5].

## 3.9 Statistical Considerations

### 3.9.1 Sample size

No formal power calculations were performed. This study is a carried out as proof of concept for predictions provided by the stochastic and deterministic simulations.

### 3.9.2 Randomization and Blinding

On day 22 of the study, subjects will be randomized into 3 groups each consisting of 3 subjects:
1- Group 1 (G1): will take 1000 mg ER at 14:00 (six (6) hours late from schedule)
2- Group 2 (G2): will take 1000 mg ER at 20:00 (twelve (12) hours late from schedule)
3- Group 3 (G3): will not take any dose i.e. miss ER dose

Blinding is irrelevant in this study.

### 3.9.3 Demographics and patient characteristics

Demographics and important patient variables will be summarized for all subjects.

## 4.0 Data Analysis

1- Blood samples taken during dose escalation (two at each dosing level) will be used to test the nomogram based on total levels.
To illustrate how the nomogram works, here is an example:
To elevate total VPA levels from 50 mg/l to 75 mg/l we predict that dose should be increased by 104% [4]. The nomogram is shown below.

2- Plasma protein binding of VPA will be characterized using blank plasma taken from the study subjects before the study conduct. Free and total VPA will be measured to calculate bound VPA. Protein binding models incorporating one saturable site, two saturable sites, and two sites: one saturable, one non-saturable will be challenged to achieve best fit.
These models are:
a- One saturable site: $C_b = (NKC_uP)/(1+KC_u)$

b- Two saturable sites: $C_b = (N_1K_1C_uP)/ (1+K_1C_u) + (N_2K_2C_uP)/ (1+K_2C_u)$
c- Two sites: one saturable, one non-saturable:

$C_b = (N_1K_1C_uP)/(1+K_1C_u)+[(N_2K_2)C_uP]$

Common to all equations, bound VPA will be calculated from:
$C_b = C_{tot} - C_u$
Where:
$C_b$ = bound VPA
$C_u$ = Unbound VPA
$C_{tot}$ = Total VPA
P is albumin concentration (will be measured chemically)
$N_1$ & $N_2$ represent the number of binding sites per class of binding site; $K_1$ & $K_2$ are the affinity (association) constants for the two binding sites. N, K are the corresponding parameters for one-binding site.
3- Trough levels after the missed doses and $C_{max}$'s after dose double-up will be used to test the predictions of the stochastic simulations.

## 5.0 Duration of Study

This study will last approximately 28 days. Subjects will be taking study drug during the out patient phase to reach steady state for each dose escalation, prior to each dose escalation subjects will enter the CDS unit for a trough level (PK blood sample). On the evening of day 21 subjects will enter the CDS unit and remain for the Pharmacokinetic portion of the study. Each subject will spend approximately 36-48 hours at the VCU Center for Drug Studies including three overnight stays. See Timed Events Schedule, Appendix 3.

## 6.0 Recruitment for All Groups

Volunteers will first be recruited from the CDS database. If necessary, advertising will also be used to recruit volunteers.

### Subject Withdrawal or Dropout

All volunteers are free to withdraw from the study at any time for any reason, and without prejudice to further treatment. Volunteers may also be withdrawn from the study by the Medical Investigator Dr. Larry Morton. If a subject withdraws from the study prior to completion of all parts of the study, collection of data should be as complete as possible. The reason for withdrawal must be recorded in the CRF.

Any subject who has been randomized but does not complete the study may be replaced and a new randomization number must be used for the replacement subject.

An attempt will be made to get 9 completed volunteers. However, no more than 12 volunteers will be entered into the study.

## 7.0 Adverse Events

Any adverse event, including both observed and volunteered problems, complaints, or symptoms are to be recorded on the Adverse Event page of the CRF. The need to capture this information is not dependent upon whether the adverse event is associated with the use of the treatment. In order to avoid vague, ambiguous, or colloquial expressions, the adverse event should be recorded in standard medical terminology. Each adverse event is to be evaluated for onset, duration, frequency, intensity, severity, relationship to study product, action taken, and the outcome.

Adverse Event Definitions

The investigator will be asked to identify any adverse events in accordance with Good Clinical Practice (GCP). An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial, whether or not the event is considered study product related.

A serious adverse event (SAE) is defined as any adverse drug experience occurring at any dose that results in any of the following outcome:

- death,
- a life-threatening adverse drug experience,
- inpatient hospitalization,
- results in a persistent or significant disability/incapacity,
- a congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Any adverse experience, the specificity or severity of which is not consistent with risk information described in the general investigational plan, is considered an unexpected adverse event.

### a) Definitions of Intensity

The intensity of an Adverse Event is a relative estimate made by the investigator.

| | |
|---|---|
| Mild | The adverse event is transient and easily tolerated by the patient and requires no special treatment |
| Moderate | The adverse event causes the patient discomfort that may be ameliorated by simple therapeutic measures |
| Severe | The adverse event is incapacitating, simple therapeutics do not ameliorate the event. |

b) Relationship to Study Treatment

The investigator will determine the relation of each adverse event to the study product using one of the following categories:

| | |
|---|---|
| Definite | The adverse event – including laboratory test abnormality - has a timely relationship to administration of the study product and there is no apparent, potential alternate etiology |
| Possible | The adverse event – including laboratory test abnormality - has a timely relationship to administration of the treatment and there is an apparent, potential alternate etiology |
| Not Related | The adverse event is related to an etiology other than administration of study product |
| Not Assessable | An adverse event for which sufficient information is not available to allow a reasonable assessment. The assessment should be performed later, if possible. |

c) Definitions of Action Taken

The action taken as a result of the adverse event will be classified in one of the following categories:

| | |
|---|---|
| None | No action taken |
| Other therapy | Drug or other therapy was initiated to counteract the adverse event |

Emergency Contacts

The investigator will report the SAE to the Institutional Review Board (IRB).

Copies of the adverse event forms are located in the CRF booklet for each patient.

## 8.0 Data Collection Management

Any treatment-emergent signs and symptoms (TESS), defined as adverse events (AEs) that have first occurred or worsened in severity after initiation of the study procedures, will be reported and recorded on the CRFs.

### 8.1 Source Documents / Retention of Data

The principal investigator will prepare and maintain adequate and accurate records for each volunteer receiving either treatment or study procedure. Source documents such as hospital, clinic or office records, laboratory reports, study worksheets and the signed informed consent must be included in the investigator's files with the volunteer's records.

## 9.0 Protection Of Human Subjects

### 9.1 Ethical Regulations

The study will be carried out in accordance with the International Code on Harmonization (ICH) Guidelines for Good Clinical Practice (January 17, 1997) and with the Declaration of Helsinki, revised version, 2000 Edinburgh, as well as per the U.S. Code of Federal Regulations.

### 9.2 Subject Safety and Confidentiality

Data Safety Monitoring Board

The Investigators will be responsible for data and safety monitoring during the study. The review will include protocol compliance with inclusion/exclusion criteria, gender and minority, and adverse events. The protocol will undergo its initial review by the study team after at least 3 subjects have been enrolled, with follow-up review at the end of the study since this study is not high risk.

Subject safety

All subjects will be monitored for side effects. Subjects who show signs of toxicity or intolerance will be withdrawn from the study. Volume of blood withdrawn from subjects is 233 ml (~1/2 pint).

Inclusion of Women

There will be no restriction regarding inclusion of women in the study. However, Women of childbearing potential will be included provided that they are not nursing and practice an adequate and acceptable form of birth control. General inclusion criteria involve the recruitment of healthy women ages 18-55.

Inclusion of Minorities

Minorities will be included in the study. The percentage will depend on recruitment. General inclusion criteria involve the recruitment of healthy subjects ages 18-55.

Inclusion of Children

Children will not be included in the study since the research topic is not relevant to children.

9.3 Protection of Human Subjects

Risks to subjects

As mentioned above, blood samples will be taken from subjects to determine the concentration of VPA. Personal information including medical history will be taken at the patient interview to determine eligibility for the study and will not be disclosed to any other parties other than immediate study personnel. We do not expect the subjects to encounter undue health risks when taking the Depakote ER; subjects will be monitored for side effects and vitals signs during the study.

Adequacy of protection against risks

Information will be provided to prospective Subjects on the study goal and anticipated side effects. Each subject will sign a consent form after eligibility is established.

Potential benefits of the proposed research to the subjects and others and importance of the knowledge to be gained

Information obtained from the study will help avoid undue side effects for epileptic patients receiving the ER preparation. We expect that the outcome of the study will be to provide information that will help minimize the occurrence of break-through seizures and

CNS toxicity. The subjects are not expected to be at significant risk during the course of the study.

Internal Review Board (IRB)

As required, the protocol will be reviewed and approved by the VCU designated IRB (VCU IRB) before volunteers are enrolled in the study. The Center for Drug Studies (CDS) complies strictly with the IRB approved protocol for study implementation, recruitment and advertising. In addition, the CDS follows GCP guidelines through their Standard Operating Polices. CDS is fully compliant with confidentiality issues now required by the HIPPA regulations.

Subject Privacy

The investigator confirms and upholds the principle of the patient's right to protection against invasion of privacy. The data will be blinded correspondingly in all data analyses. No personal information will be disclosed to individuals other than direct study personnel. The principal investigator is responsible for keeping a Confidential Subject Identification list of all volunteers enrolled; including randomization numbers, and will maintain records of last known address and phone number**.**

Individual patient medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited.

Informed Consent

The investigator will ensure that each patient is given full and adequate verbal and written information about the nature, purpose, possible harm and benefit of the study. Volunteers must also be notified that they are free to discontinue their participation in the study at any time. The volunteer should have the opportunity to ask questions and be given time for consideration. The investigator or designee is responsible for obtaining signed informed consent from all volunteers prior to study initiation.

The original signed informed consent will be retained with the study records, and a copy of the signed informed consent form will be given to the volunteers.

## 10.0 Publication Of Results

The data obtained from this study will be used as part of the Ph.D. dissertation for Alaa Ahmad. Manuscripts preparation will be a collaborative effort between the PI and all Co-investigators.

## 11.0 Biohazard Containment

Any materials used in this study that may present a biohazard, including but not limited to used syringes, needles, devices, blood samples, shall be handled using appropriate care to prevent transmission of potential infectious diseases. All such material will be discarded in compliance with all applicable institutional, local and other applicable rules and regulations.
Special precautions will be made to prevent aerosolization of blood during the collection process.

## *12.0 References*

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2) Jeavons P.M., Clark J.E., Maheshwari M.C. Treatment of generalized epilepsies of childhood and adolescence with sodium valproate (Epilim). *Dev Med Child Neurol* 1977; 19:9-25.

3) Ahmad A.M., Boudinot F.D., Barr W.H., Baker J.R., Reed R.C., Garnett W.R.**.** Effect of poor compliance on the steady state levels of Valproic acid following administration of the Depakote® ER and Depakote® DR preparations: Monte Carlo simulations. Manuscript in preparation.

4) Ahmad A.M., Boudinot F.D., Reed R.C., Garnett W.R.. A Nomogram for Valproic acid: Prediction of % increase or decrease in Valproic acid dose needed to escalate or reduce total levels using simulations. Manuscript in preparation.

5) Cloyd J.C., Dutta S., Cao G., Walch J.K., Collins S.D., Granneman G.R. The Depakon Study Group. Valproate unbound fraction and distribution volume following rapid infusions in patients with epilepsy. *Epilepsy Research* 53 (2003) 19-27.

6) Dutta S., Cloyd J.C., Granneman R., Collins S.D.. Oral/intravenous maintenance dosing of valproate following intravenous loading: a simulation. *Epilepsy Research* 53 (2003) 29 -38.

7) Scheyer R.D., Cramer J.A., Toftness B.R., Hochholzer J.M. and Mattson R.H. In vivo Determination of valproate binding constants during sole and mutli-drug therapy. *Therapeutic Drug Monitoring* 12: 117-123 © Raven press, Ltd. New York.

## 13.0 Investigator Signature Agreement To Protocol

I hereby declare that I have read the protocol and agree to the terms of this study protocol. I will conduct the study according to the procedures specified in the study protocol, and according to the principles of Good Clinical Practice.

_____

Printed Name of Investigator

_____ _____

Signature of Investigator Date

## 14. Informed Consent

### Investigators

Principal Investigator: Dr. William R. Garnett, Pharm.D, Departments of Pharmacy of Neurology, VCU Medical Center, Richmond, VA.

Dr. Larry Morton, MD. Department of Neurology. (Medical Investigator)

Co-investigators

Dr. F. Douglas Boudinot, Ph.D. Department of Pharmaceutics.

Dr. William H. Barr, PharmD., Ph.D.,Department of Pharmacy, Center for Drug Studies.

Alaa Ahmad, B.S. Pharm., Department of Pharmaceutics.

1. **Title of Research**

A Prospective study to test a nomogram for valproic acid, a neuro psychiatric agent, and to explore the effect of poor compliance on Depakote® extended release, a commercial preparation for valproic acid

2. **Introduction**

Valproic acid is a drug used to treat different neuro pschychiatric disorders including epilepsy, migraine and bipolar disorder. Depakote extended release (ER) is a new preparation for Valproic acid that can be taken once daily. The usual dose is approximately 1000 mg to 2500 mg per day, with some patients requiring higher doses. This study is being conducted to test a nomogram for Valproic acid. A nomogram is a chart that can aid in individualizing therapy with valproic acid to achieve better therapeutic outcome. This study will also explore the effect of poor compliance (i.e. missed doses) on valproic acid levels in the body. Since the study drug binds to albumin, a protein in your blood, we will characterize this binding using blood samples and use the data obtained to modify the nomogram to account for inter-individual differences.
The screening phase precedes drug administration and might last up to 28 days. Within one week before drug administration starts, each eligible subject (based on screening) will be asked to donate 100 ml of blood. After screening is completed, drug administration will start and continue for 28 days according to the study schedule. Your total commitment to this study is therefore approximately two months, including the screening window. It is anticipated that at least nine, but no more than twelve volunteers, ages 18-55, will be enrolled in this study.

3. **Description of the study**

To qualify for this research study, you will have to provide written consent to participate in the research study and successfully pass an outpatient screening examination (Screening window is 28 days). Before you enter the study, your health will be assessed by a complete medical history including family history, physical examination, blood pressure and heart rate, laboratory screening (blood chemistries, complete blood cell count, urinalysis), and an electrocardiogram (ECG) (a measure of the electrical activity of the heart), to determine if you have any medical condition that would prevent you from participating in this research study. The outpatient screening examination will include a urine test for drugs of abuse, and an alcohol breath test, and will be repeated again on the day you are admitted to the study before your first treatment dose. Eligible subjects; based on screening, are required to donate 100 ml of blood within one week before drug administration starts.

Throughout this study, multiple blood samples will be taken either by direct venipuncture (insertion of a small needle into a vein in the arm) or through a heparin lock (a small plastic catheter inserted into a vein in your arm used to take small blood samples) at frequent. The total blood volume collected throughout the study will be approximately 250 ml (~ ½ pint). This is close to half the amount you would give at a single blood donation (450ml, less than one pint).

Drug administration will start after at least nine (9) eligible subjects have been recruited, signed their informed consent form and donated 100 ml of blood.
You are required to come to the clinic on day one (1) of the drug administration period to have a baseline blood sample taken and be given a dose of (500 mg) Depakote on the unit. You will also be given five (5) more doses (500 mg each) to take for the next five (5) days (study days 2-6), which you will take at home. You are required to call the unit on those days to document that you have taken your dose. On day seven (7), you will come for a blood sample prior to 8:00 am and take (500 mg) dose at the study unit. First dose increase, on day 8, you will come back to the clinic for another blood sample prior to 8:00 am and you will given (750 mg) dose plus five (5) more (750 mg) doses for the next five (5) days of the study (study days 8-13). You are required to call the unit each time you take your dose. On day 14, you will come to the clinic for a blood sample prior to 8:00 am and be given (750 mg) dose. Second dose increase, on day 15, you will come for another blood sample prior to 8:00 am and be given (1000 mg) dose on the unit, plus five (5) more (1000 mg) doses to take on days (16-20). You must call the unit each time you take your dose. On day 21, you will come for a blood sample prior to 8:00 am and take (1000 mg) dose on the unit.

You will come back in the evening of day 21 to start the in-patient phase of the study and the study investigators will assign you to one of three groups.

On day 22, a blood sample will be taken at 8:00 am.
Group 1 will take (1000 mg) at 14:00, Group 2 will take (1000 mg) at 20:00, and Group 3 will not take any dose on day 22.
Group1 and Group 2 will have blood samples (13 total) starting on day 22 for 24 hours from the time they take their missed dose (14:00 (day 22) to 14:00 (day 23) for Group 1 and 20:00 (day 22) to 20:00 (day 23) for Group 2). On day 23, Group 1 and Group 2 will take (1000 mg) at 8:00 am
On day 23, Group 3 will take (2000 mg) at 8:00 am and have blood samples (13 total) for 24 hours (from 8:00 (day 23)-8:00 (day 24)).

On day 24, all subjects will take (1000 mg) at 8:00 am on the unit. All subjects will have exit procedures done before they leave the unit. These procedures include a physical exam, vital signs, 12-lead ECG, hematology, chemistry, urine analysis and adverse events assessment. The medical investigator or his designee will authorize your release.

You will be given two (750 mg) and two (500 mg) doses to take over the next 4 days. On days 25 and 26 of the study, you will take (750 mg) at 8:00 am. On days 27 & 28, you will take (500 mg) at 8:00 am. You are required to call the unit to document when you take your study medication as before. You should complete this study in approximately 28 days. You will have to return to the unit within one week after your last dose for a follow-up visit; at this time you will have Vital Signs and Adverse Event evaluation.

Over the course of the study you will have to spend approximately two days and three nights at the Center for Drug Studies, Clinical Study Unit. While you are on the Clinical Study Unit, you will eat the food provided by the investigators at times specified, and will abide by the rules of the Clinical Study Unit. Portions of the inpatient screening examination will be repeated at the end of the study and are requirements for completing the study.

This study is being conducted at the Center for Drug Studies, Virginia Commonwealth University, VCU Medical Center, by Dr. William Garnett, Pharm.D, Departments of Pharmacy and Neurology, Dr. Douglas Boudinot, Ph.D, Department of Pharmaceutics, Dr. William Barr, Pharm.D, Ph.D, Department of Pharmacy, Alaa Ahmad, Ph.D candidate, Department of Pharmaceutics and Dr. Larry Morton, M.D., Department of Neurology.

Dr. Larry Morton is the first investigator that you should contact in the event of a medical emergency.

3. Benefits

You are being asked to participate in this study as a volunteer. This study is of no direct medical benefit to you. There will be no charge to you for the screening examination and the results will be made available to you if you want them.

In order to achieve the total number of volunteers to complete the study per protocol, it is often necessary to recruit, screen additional volunteers (alternates). If you qualify as a volunteer and check in to the Clinical Studies Unit, but *do not receive the study drug*, you will receive a $100.00 payment. You will be paid $ $1,240.00 for full completion of the study. Conditions for early withdrawal are described in Section 10.

4. Alternative Therapy

There is no therapeutic benefit to you in this study. Your participation is entirely voluntary.

5. Risks, Inconveniences, Discomforts

The most common side effects for valproic acid are nausea, dyspepsia, diarrhea, vomiting, abdominal pain, somnolence, edema, weight gain, tremors and hair loss. Other side effects may occur.
There may be some local discomfort, pain or bruising at the site of the venipuncture. Rarely, fainting or infection may occur. In the event that an indwelling catheter (heparin lock) cannot be inserted, it may be necessary to obtain sample(s) by venipuncture (directly inserting needle into vein of arm to withdraw blood sample).

LIST OTHER RISKS ASSOCIATED WITH THE STUDY.

You will be informed of any changes in the study and of any new risks, which become evident. You will be questioned for any symptoms, which you may experience during the study. You should report immediately any subjective feelings, symptoms, or changes, which you experience during the study.

6. Cost of Participation

There will be no charge to you for any laboratory tests, physical examination, study drug, any over-the-counter products provided, or housing that relates to the conduct of this study.

This is not a time consuming study and should not interfere with your employment, school, or other activities. Participation should not interfere with your normal activities after this study, but this cannot be guaranteed because some side effects may occur.

7. Research Related Injury

In the event of physical and/or mental injury resulting from your participation in this research project, Virginia Commonwealth University and VCU Medical Center Hospitals will not provide compensation. If injury occurs, medical treatment will be available at the

VCU Medical Center Hospitals. If you are injured during this research study, and the injury is a direct result of the effects of the study drug or procedures, the cost of reasonable emergency medical treatment will be paid by the Study investigator provided that you have not violated the stipulations of the protocol (for example, alcohol consumption, other drugs, or failure to follow the instructions of the investigators). However, such reimbursement by the sponsor shall be limited to medical expenses not otherwise covered by existing health care programs or insurance policies. No other compensation will be provided by the study investigator. It is necessary that you notify the investigators in person or in writing of the nature of expenses to be covered. Financial compensation for items such as lost wages; disability or discomfort is not available.

8. Pregnancy

Every effort will be made to have females enter this study on an equal basis with male volunteers. Females who are on adequate form of birth control will be entered into this study. Female volunteers participating in this study should not be nursing or pregnant. If pregnancy were to occur, there is a risk of birth defects. A pregnancy test will be performed on female volunteers prior to administration of the study medication. A positive pregnancy test will exclude you from participation in the study.

9. Confidentiality of Records

In connection with this study, it is important for the investigators, as well as your personal physician and the Institutional Review Board (IRB) to be able to inspect your medical records. Therefore, you authorize the investigators to release your medical records to the study investigators, the FDA, and the IRB. The results of this study may also be used for medical and scientific publications, but your identity will not be disclosed.

10. Withdrawal

Your participation in this study is entirely voluntary. The investigators will answer any questions you may have about the study. You are free to withdraw your consent and discontinue participation at any time. If you decide to withdraw from this study, you should contact the Principal Investigator, Dr. William Garnett. Discontinuation will in no way affect the care you receive now or in the future at this institution. Your doctor may also withdraw you for medical reasons. If during the course of the study your medical condition changes or you experience adverse side effects, your participation may be terminated by the investigator without your consent. Any significant new findings which develop during the course of the research study that may affect your willingness to participate will be provided to you. If you are withdrawn from the study for medical reasons, based upon the judgment of the medical investigator, or elect to withdraw, you will receive a prorated compensation based upon the usable information obtained prior to your withdrawal. If you are removed from the study for any of the following reasons, you

will receive no compensation: (1) Failure to give an accurate history; (2) Failure to follow the guidelines of the study; (3) Failure to follow the rules of the unit. If you should discontinue participation in this study, you understand that a physical examination, vital signs, an ECG and an evaluation of your laboratory data (complete blood count, blood chemistries and urinalysis) will be repeated for your safety as part of the required study termination procedures.

11. Current Telephone Numbers

You have been given the opportunity to ask questions that you may have. If you have questions or if you experience any side effects, you can contact the investigators at:

| | Work | Home | Pager/Cell |
|---|---|---|---|
| Dr. William Garnett | 828-8328 | 804-378-4222 | 804-997-9050 |
| Alaa Ahmad | 828-6215 | 804-262-7889 | 804-502-0467 |
| Dr. Larry Morton | 828-0422 | | 828-4999 ext. 3594 |
| Clinical Study Unit | 828-5522 | | |

In the case of a medical emergency, you should contact Dr. Larry Morton at the above number.

12. Subjects Rights Information

If you want additional information about your rights as a research subject, you may contact the Chairman of the Committee on the Conduct of Human Research at Virginia Commonwealth University/VCU Medical Center at (804) 828-0868.

You will be given a copy of this consent form to keep.

Your signature below indicates that you voluntarily consent to participate in this study under the conditions disclosed in this document.

_____ _____
Subjects Signature Date

_____ _____
Subjects Printed Name Date

_____ _____
Witness Date

_____ _____
Investigators Signature Date

# *Appendix 1*

Nomogram for % increase in dose needed to titrate total VPA upwards

| Ctot | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 | 95 | 100 | 105 | 110 | 115 | 120 | 125 | 130 | 135 | 140 | 145 | 150 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 50 | 17 | 36 | 57 | 79 | 104 | 131 | 160 | 192 | 227 | 265 | 306 | 350 | 398 | 449 | 504 | 562 | 623 | 688 | 756 | 826 |
| 55 | 15 | 16 | 34 | 53 | 74 | 97 | 122 | 149 | 178 | 211 | 246 | 283 | 324 | 368 | 414 | 464 | 516 | 572 | 630 | 690 |
| 60 | 27 | 14 | 15 | 32 | 50 | 69 | 91 | 115 | 140 | 168 | 198 | 230 | 266 | 303 | 343 | 386 | 431 | 479 | 529 | 581 |
| 65 | 36 | 25 | 13 | 14 | 30 | 47 | 66 | 86 | 108 | 133 | 159 | 187 | 218 | 250 | 285 | 322 | 362 | 403 | 447 | 492 |
| 70 | 44 | 35 | 24 | 13 | 14 | 29 | 45 | 63 | 82 | 103 | 126 | 151 | 178 | 206 | 237 | 269 | 304 | 340 | 378 | 418 |
| 75 | 51 | 42 | 33 | 23 | 12 | 13 | 27 | 43 | 60 | 79 | 99 | 121 | 144 | 169 | 196 | 225 | 255 | 287 | 320 | 355 |
| 80 | 57 | 49 | 41 | 32 | 22 | 12 | 13 | 27 | 42 | 58 | 76 | 95 | 116 | 138 | 162 | 187 | 213 | 242 | 271 | 302 |
| 85 | 62 | 55 | 48 | 40 | 31 | 22 | 11 | 12 | 26 | 40 | 56 | 73 | 91 | 111 | 132 | 155 | 178 | 203 | 230 | 257 |
| 90 | 66 | 60 | 53 | 46 | 39 | 30 | 21 | 11 | 12 | 25 | 39 | 54 | 70 | 88 | 107 | 127 | 148 | 170 | 193 | 218 |
| 95 | 69 | 64 | 58 | 52 | 45 | 38 | 29 | 20 | 11 | 12 | 24 | 38 | 52 | 68 | 85 | 102 | 121 | 142 | 162 | 184 |
| 100 | 73 | 68 | 63 | 57 | 51 | 44 | 37 | 29 | 20 | 10 | 11 | 23 | 37 | 51 | 65 | 82 | 98 | 116 | 135 | 154 |
| 105 | 75 | 71 | 66 | 61 | 56 | 50 | 43 | 36 | 28 | 19 | 10 | 11 | 23 | 35 | 49 | 63 | 78 | 94 | 111 | 127 |
| 110 | 78 | 74 | 70 | 65 | 60 | 55 | 49 | 42 | 35 | 27 | 19 | 10 | 11 | 22 | 34 | 48 | 61 | 75 | 90 | 106 |
| 115 | 80 | 76 | 73 | 69 | 64 | 59 | 54 | 48 | 41 | 34 | 27 | 18 | 10 | 10 | 21 | 33 | 45 | 58 | 72 | 86 |
| 120 | 82 | 79 | 75 | 71 | 67 | 63 | 58 | 53 | 47 | 40 | 34 | 26 | 18 | 9 | 10 | 21 | 32 | 44 | 56 | 69 |
| 125 | 83 | 81 | 77 | 74 | 70 | 66 | 62 | 57 | 52 | 46 | 40 | 33 | 25 | 17 | 9 | 10 | 20 | 31 | 42 | 54 |
| 130 | 85 | 82 | 79 | 76 | 73 | 69 | 65 | 61 | 56 | 51 | 45 | 39 | 32 | 25 | 17 | 9 | 9 | 19 | 29 | 40 |
| 135 | 86 | 84 | 81 | 78 | 75 | 72 | 68 | 64 | 60 | 55 | 50 | 44 | 38 | 31 | 24 | 17 | 9 | 9 | 18 | 28 |
| 140 | 87 | 85 | 83 | 80 | 77 | 73 | 71 | 67 | 63 | 59 | 54 | 49 | 43 | 37 | 30 | 23 | 16 | 8 | 9 | 18 |
| 145 | 88 | 86 | 84 | 82 | 79 | 76 | 73 | 70 | 66 | 62 | 57 | 53 | 47 | 42 | 36 | 30 | 23 | 16 | 8 | 8 |
| 150 | 89 | 87 | 85 | 83 | 81 | 78 | 75 | 72 | 69 | 65 | 61 | 56 | 51 | 46 | 41 | 35 | 29 | 22 | 15 | 8 |
| Ctot | 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 | 95 | 100 | 105 | 110 | 115 | 120 | 125 | 130 | 135 | 140 | 145 |

Nomogram for % decrease in dose needed to taper off

**Valproic acid nomogram. The nomogram is based on a two-site binding equation.**

## *Appendix 2 (Study Schema)*

| Day | ER Dose (mg) | Time of dose | Blood sampling (nominal time) |
|---|---|---|---|
| **1** | 500 | 8:00 am | Baseline level |
| **2** | 500 | 8:00 am | ------ |
| **3** | 500 | 8:00 am | ------ |
| **4** | 500 | 8:00 am | ------ |
| **5** | 500 | 8:00 am | ------ |
| **6** | 500 | 8:00 am | ------ |
| **7** | 500 | 8:00 am | Trough at 8:00 am |
| **8** | 750 | 8:00 am | Trough at 8:00 am |
| **9** | 750 | 8:00 am | ------ |
| **10** | 750 | 8:00 am | ------ |
| **11** | 750 | 8:00 am | ------ |
| **12** | 750 | 8:00 am | ------ |
| **13** | 750 | 8:00 am | ------ |
| **14** | 750 | 8:00 am | Trough at 8:00 am |
| **15** | 1000 | 8:00 am | Trough at 8:00 am |
| **16** | 1000 | 8:00 am | ------ |
| **17** | 1000 | 8:00 am | ------ |
| **18** | 1000 | 8:00 am | ------ |
| **19** | 1000 | 8:00 am | ------ |
| **20** | 1000 | 8:00 am | ------ |
| **21** | 1000 | 8:00 am | Trough at 8:00 am |
| **22 G1** | 1000 | 14:00 pm | Trough at 8:00 am, 14:00, 16:00, 18:00, 20:00, 22:00, 00:00. |
| **G2** | 1000 | 20:00 pm | Trough at 8:00 am, 20:00, 22:00, 00:00. |
| **G3** | Missed | ---------- | Trough at 8:00 am |
| **23 G1** | 1000 | 8:00 am | 02:00, 04:00, 06:00, 08:00, 10:00, 12:00, 14:00. |
| **G2** | 1000 | 8:00 am | 02:00, 04:00, 06:00, 08:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00 |
| **G3** | 2000 | 8:00 am | 08:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00, 22:00, 00:00. |
| **24 G1** | 1000 | 8:00 am | ------ |
| **G2** | 1000 | 8:00 am | ------ |
| **G3** | 1000 | 8:00 am | 02:00,04:00, 06:00, 08:00. |
| **25 G1** | 750 | 8:00 am | ------ |
| **G2** | 750 | 8:00 am | ------ |
| **G3** | 750 | 8:00 am | ------ |
| **26 G1** | 750 | 8:00 am | ------ |
| **G2** | 750 | 8:00 am | ------ |
| **G3** | 750 | 8:00 am | ------ |
| **27 G1** | 500 | 8:00 am | ------ |
| **G2** | 500 | 8:00 am | ------ |
| **G3** | 500 | 8:00 am | ------ |
| **28 G1** | 500 | 8:00 am | ------ |
| **G2** | 500 | 8:00 am | ------ |
| **G3** | 500 | 8:00 am | ------ |

# Appendix 3

| Schedule of Events | | | |
|---|---|---|---|
| **Study Procedures** | **Pre-Study Screening** | **Study Days** | **Exit /Early Withdrawal** |
| | **-28 to – 1** | **1-28** | |
| **Informed Consent** | **X** | | |
| **Demographics** | **X** | | |
| **Medical History** | **X** | | |
| **Medication History** | **X** | | |
| **Physical Exam** [a] | **X** | | **X** |
| **Vital Signs** [b] | **X** | **X** | **X** |
| **12-Lead ECG** [c] | **X** | | **X** |
| **Hematology** [d] | **X** | **X** | **X** |
| **Chemistry** [d] | **X** | **X** | **X** |
| **(HCG) Serum Pregnancy (if applicable)** [d] | **X** | | |
| **Urine Drug Screen** [d] | **X** | | |
| **HIV test** [d] | **X** | | |
| **Urinalysis** [d] | **X** | **X** | **X** |
| **Alcohol breathalyzer** [e] | **X** | | |
| **PK sampling (plasma)** [f] | | **X** | |
| **Drug Administration** [g] | | **X** | |
| **Adverse Event Assessment** [h] | | **X** | **X** |
| **Concomitant Medication Assessment** [h] | | **X** | **X** |

a) Physical Exam will be done at screening and exit from study unit.

b) Vital Signs (BP/HR) will be done at screening and prior to dosing on days 7, 8, 14,15, 21 22, 23,24 and at exit

c) 12-Lead ECG will be done at screening and exit from study unit.

d) Labs (CBC, Chem, hCG, UDS, U/A, HIV will be done at screening, Labs (CBC, Chem, U/A) will be done at exit from study unit.

e) Alcohol breath test will be done at screening (for 100ml-blood work and day 1) and upon entry day 21

f) PK blood sampling will be done prior to dosing (trough level) on days 1, 7, 8, 14, 15,21,22 all subjects

**Group 1**: (3 subjects) will have additional samples at 0hr, 6hr, 8hr, 10hr, 12hr, 14hr, 16hr, 18hr, 20hr, 22hr, 24hr, 26hr, 28hr, 30hr on day 22-23

**Group 2:** (3 subjects) will have additional samples at 0hr, 12hr, 14hr, 16hr, 18hr, 20hr, 22hr, 24hr, 26hr,28hr,30hr, 32hr, 34hr, 36hr on days 22-23

**Group 3:** (3 subjects) will have additional samples at 0hr, 2hr, 4hr, 6hr, 8hr, 10hr, 12hr, 14hr, 16hr, 18hr, 20hr, 22hr, 24hr, on days 23-24

g) Outpatient dosing:

Days 2- 6(500mg), days 9- 13(750mg), days 16- 20(1000mg), days 24(1000mg), day 25-26(750mg), day 27-28(500mg).

Inpatient dosing:

Days 1, 7(500mg), days 8, 14 (750) 15 (1000 mg), days 21, 22, 23, 24 (1000 to 2000mg).

h) Adverse Events and Concomitant Medication will be monitored continuously from screening throughout the study.

# APPENDIX B

## HPLC ASSAY FOR THE DETERMINATION OF (-)-D-β-DIOXOLANE-THYMINE, A POETENTIAL ANTI-HIV AGENT, IN RAT PLASMA

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**Abstract**

(-)-D-β-Dioxolane-thymine (DOT) is a nucleoside analog with potent *in vitro* anti-HIV activity. A simple, rapid, accurate and reproducible reversed-phase HPLC method was developed to determine DOT in rat plasma. The extraction recovery of DOT was greater than 96%. DOT & D4T (internal standard) eluted at 6.9 and 9.8 minutes respectively. The limit of quantitation of the assay was 0.25 μg/ml. Intra-day and inter-day precision and accuracy were acceptable. The assay was applied for investigation of preliminary disposition of DOT in rats after intravenous and oral administration.

*Keywords:* (-)-D-β-Dioxolane-thymine, high performance liquid chromatography (HPLC),

## 1. Introduction

Acquired immune deficiency syndrome (AIDS) is caused by infection with the human immunodeficiency virus (HIV) [1]. An estimated 850,000-950,000 persons in the United States are living with human immunodeficiency virus (HIV), including 180,000-280,000 who do not know they are infected [2]. In 2003**,** the estimated number of diagnoses of AIDS in the United States was 43,171. Adult and adolescent AIDS cases totaled 43,112 with 31,614 cases in males and 11,498 cases in females. Also in 2003, there were 59 AIDS cases estimated in children under age 13 [3]. Sixteen anti-viral agents have been approved for the treatment of HIV infection including the nucleoside/nucleotide reverse transcriptase (RT) inhibitors (ABC) abacavir, ddC (zalcitabine), ddI (didanosine), d4T (stavudine), 3TC (lamivudine), ZDV (zidovudine) and tenofovir, the non-nucleoside RT inhibitors delavirdine, nevirapine and efavirenz and the protease inhibitors ritonavir, saquinavir, indinavir, amprenavir, nelfinavir and lopinavir [4]. The natural history of HIV infection has been dramatically modified by the use of multi-drug highly active antiretroviral therapy (HAART) regimens. The recommended HAART regimens are all built around a backbone of two nucleoside analogs with either a protease inhibitor or non-nucleoside reverse transcriptase inhibitor. However, AIDS is still incurable, and current HAART regimens are unable to eradicate HIV infection. Thus, there are continuous efforts to develop more effective anti-HIV agents [5].

(-)-D-β-Dioxolane-thymine, DOT, (Figure B.1) is a nucleoside analog with potent *in vitro* anti-HIV activity. Racemic dioxolane-thymine, (±)-DT has been shown to provide a 50% protective effect against the infectivity and cytopathic effect of HIV-1 at 20 μM. In contrast, AZT exerts a 50% protective effect at approximately 0.5 μM and a 100% protective effect at 5 μM. AZT anti-retroviral activity, however, is accompanied by substantia+l *in vitro* toxicity: 11% at 5 μM and 50% at 50 μM [6]. The relative lack of toxicity exhibited by (±)-DT warranted further exploration of this compound and its enantiomers. The asymmetric synthesis of enantiomerically pure (-)-(1`R, 4`R)-dioxolane-thymine and its anti-retroviral activity was reported by Chung K. Chu et al [7]. The anti-HIV activities of (±)-DT and its enantiomers in peripheral blood mononuclear cells (PBM) expressed as $EC_{50}$ were 4.81 μM for (+)-L-β-dioxolane-thymine, 0.39 μM for (-)-D-β-dioxolane-thymine and 0.009 μM for (±)-DT, while that for AZT is 0.002 μM [8].

Characterization of the preclinical pharmacokinetics of these potential antiviral agents is an integral part of drug development. Thus, development of an analytical methodology is essential for conducting preclinical investigations [9]. High-performance liquid chromatography has been shown to be an efficient, simple and relatively inexpensive technique for quantitating nucleoside analogs in biological samples [10-14]. The purpose of this study was to develop an HPLC assay for determination of (-)-D-β-dioxolane-thymine (DOT) in rat plasma. This assay can be used for the quantitation of DOT after administration to rats to characterize the disposition in this animal model.

## 2. Experimental

### *2.1. Chemicals*

(-)-D-β-Dioxolane-thymine was synthesized as previously described [7]. The chemical purity of DOT, as assessed by high performance liquid chromatography (HPLC) and spectral analysis, was greater than 99%. Internal standard, d4T, was purchased from Sigma-Aldrich Co. (St. Louis, Mo), acetonitrile (UV grade) was obtained from Burdick & Jackson (Muskegon, MI), sodium acetate trihydrate (HPLC grade) was obtained from Fisher Scientific (Fair Lawn, NJ), acetic acid (reagent grade) and octanol (reagent grade) were obtained from J.T Baker (Phillipsburg, NJ). All other reagents, analytical grade, were also obtained from J.T Baker (Phillipsburg, NJ).

### *2.2. $\lambda_{max}$, pKa and partition coefficient*

The wavelength of maximum absorption, $\lambda_{max}$ of DOT was determined by spectrophotometric scanning initially using a Perkin Elmer UV/VIS Spectrometer (Norwalk, CT) and confirmed with Shimadzu SPD-10A diode array detector (Columbia, MD). The pKa of DOT was estimated by preparing 8 μM solutions of DOT in buffers of varying pHs and measuring absorbance at $\lambda_{max}$. Absorbance was plotted against pH to determine the acidity constant at the inflection point of the curve. Mutually saturated water and octanol solutions were used for the partition coefficient determination at 37 °C.

DOT (100 µl of 1 µg/µl stock) was dissolved in 4ml water. This was mixed with 4.1 ml octanol and shaken for 24 h in a horizontal shaker (Lab-line Instruments Inc., Melrose, IL). After equilibration, the water phase was separated and the partition coefficient (PC) calculated from PC = (Co-Ce / Ce) where Co is the initial concentration in the aqueous phase and Ce is the concentration in water at equilibrium.

*2.3. Preparation of Standards*

Stock solutions of 1000 µg/ml, 250, 100, 25, 10 and 0.5 µg/ml DOT were prepared in distilled water. Stock solutions were added to rat plasma to obtain calibration concentrations of 0.25, 1, 3, 5, 10, 25 and 50 µg/ml DOT.

*2.4. Extraction Procedure*

Extraction was achieved using a two-step centrifugation procedure. To 100 µl rat plasma in a polypropylene micro centrifuge tube were added 50 µl of d4T (50 µg/ml) as internal standard and 100 µl perchloric acid (2 M) to precipitate Plasma proteins. Tubes were vigorously mixed for 30 seconds and centrifuged at 13,000 rpm for 3 minutes. The supernatant was then transferred to a clean centrifuge tube and 100 µl (2 M) potassium hydroxide was added, samples mixed for 30 seconds and then centrifuged for 3 minutes at 13,000 rpm. Two hundred microliters (200 µl) of the supernatant was transferred to a 2 ml vial and injected onto the HPLC column. Volumes of injection were variable depending on the concentration.

*2.5. Chromatography*

A Shimadzu (Columbia, MD) HPLC system consisting of a SCL-10A System Controller, LC-10AT liquid chromatograph, DGU-14A degasser, SPD-10A diode array detector and SIL-10AD auto injector was used. The system was equipped with Shimadzu EZ start software package V7.2. The separation employed reversed-phase liquid chromatography using a Hypersil™ 5 μm BDS C18 analytical Column (4.6 x 250mm, Phase Separations, Franklin, MA) protected by a C18 guard column. The mobile phase consisted of 5% acetonitrile in 40 mM sodium acetate trihydrate with 100 μl of 5% acetic acid added to each liter of the mobile phase to optimize band spacing. The eluants were detected at 266 nm. The chromatography was performed at room temperature (23 ° C).

*2.6. Quantitation*

Concentrations of DOT were determined from the slope of calibration plots of the peak-area ratio of DOT/internal standard versus the calibration standard concentrations. Slopes were determined using linear regression analysis with a weighting factor of 1/concentration. Shimadzu EZ start software package (v7.2) was used for regression.

*2.7. Assay Specifications*

The extraction recovery of DOT was assessed at plasma concentrations of 0.25, 5.0 and 50 μg/ml. The recovery of internal standard was assessed at 50 μg/ml, at which it

was used for the assay. Five plasma samples containing drug and internal standard were extracted and injected. Five injections of the same amount of compound in distilled water were directly injected. The percentage recovery was calculated from (100 x peak $area_{extracted}$/peak $area_{unextracted}$).

The intra- and inter-day precision and accuracy of the analytical method were determined by analysis of five plasma samples containing 0.25, 5.0, 50 µg/ml concentrations. Assay precision was determined by calculating relative standard deviations (%RSD) for each drug concentration. Accuracy was calculated by comparing measured concentrations to the known values and expressed as % mean accuracy.

*2.7. Experimental Design*

Adult male Sprague Dawley rats (Hilltop Lab. Animals, Inc. Scottdale, PA) were used for the study. Animals were housed in a 12-h light/12-h dark, constant temperature (22°C) environment with free access to standard laboratory chow and water in the Virginia Commonwealth University Animal Care Facility, which is fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). Rats were acclimatized to this environment for at least one week before the study. Animal studies were approved by the Virginia Commonwealth University Animal Care and Use Committee and conducted in accordance with guidelines established by the Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

External jugular vein cannulas were implanted under (ketamine : acepromazine : xylazine; 50:3.3:3.3 mg/kg) anesthesia the day before the experiment. Animals were fasted overnight, however, water was allowed *ad libitum*. DOT was administered intravenously to one rat by bolus injection via the jugular vein cannula and orally to one rat by gastric gavage. The animals were housed in metabolism cages following drug administration. Blood samples (0.25 ml) were collected from the cannulas into heparinized tubes prior to and at 0.083, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8 drug administration. Blood volume was replaced with normal saline (0.5-0.75 ml). Blood samples were immediately centrifuged and plasma was frozen at -20°C until analysis.

**3. Results and Discussion**

The purpose of this study was to develop an analytical HPLC method for determination of DOT in rat plasma. The physicochemical characteristics of DOT were evaluated. Spectrophotometric scanning indicated that DOT has a $\lambda_{max}$ of 266 nm, an acidity constant of 9.4 and an oil/water partition coefficient of 0.2. An isocratic HPLC method achieved adequate separation of DOT from the internal standard and any plasma peaks.

Chromatograms of (a) blank rat plasma, (b) rat plasma containing DOT internal standard d4T, (c) a rat plasma sample collected 1 h after intravenous administration of DOT and (d) a rat plasma sample collected 1 h after oral administration of DOT are shown in Figure B.2. DOT and d4T eluted at 6.9 and 9.8 min, respectively. The assay

specifications including extraction recovery, assay precision and accuracy are presented in Table B.1. The extraction recovery of DOT averaged 98% (96-100%). The limit of quantitation of the assay was 0.25 μg/ml. A weighting factor of 1/concentration yielded a linear calibration curve from 0.25-50 μg/ml [y=0.042 (0.000329) x + 0.001645 (0.001337), r=0.9999), n=7]. Intra-day and inter-day relative standard deviations of the assay were less than 15%. The accuracy of the method was greater than 95%.

Plasma concentrations of DOT following intravenous and oral administration to rats determined by the assay described are illustrated in Figure B.3.

In summary, the determination of DOT in rat plasma by the HPLC developed in the present study is simple, rapid, accurate and reproducible. The limit of quantitation of this method is sufficiently sensitive to characterize the preclinical pharmacokinetics of DOT in rats.

**Acknowledgments**

The authors will like to thank Dr. Leslie Edinoboro and Dr. Masahiro Sakagami for their technical assistance.

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**Table B.1** Assay specifications for DOT.

| Concentration (μg/ml) | Recovery (%) (Mean ± SD) | Precision | | Accuracy | |
|---|---|---|---|---|---|
| | | Intra-day | Inter-day | Intra-day | Inter-day |
| 0.25 | 100.0 ± 2.6 | 3.5 | 14.2 | 97.2 | 106.8 |
| 5 | 96.0 ± 1.8 | 5.7 | 8.2 | 100.5 | 99.9 |
| 50 | 98.3 ± 7.4 | 9.2 | 9.4 | 111.1 | 98.2 |

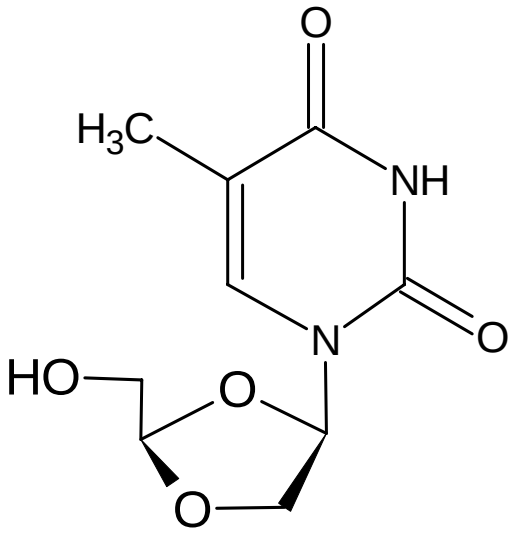



Figure B.1 Chemical Structure of (-)-D-β-dioxolane-thymine.

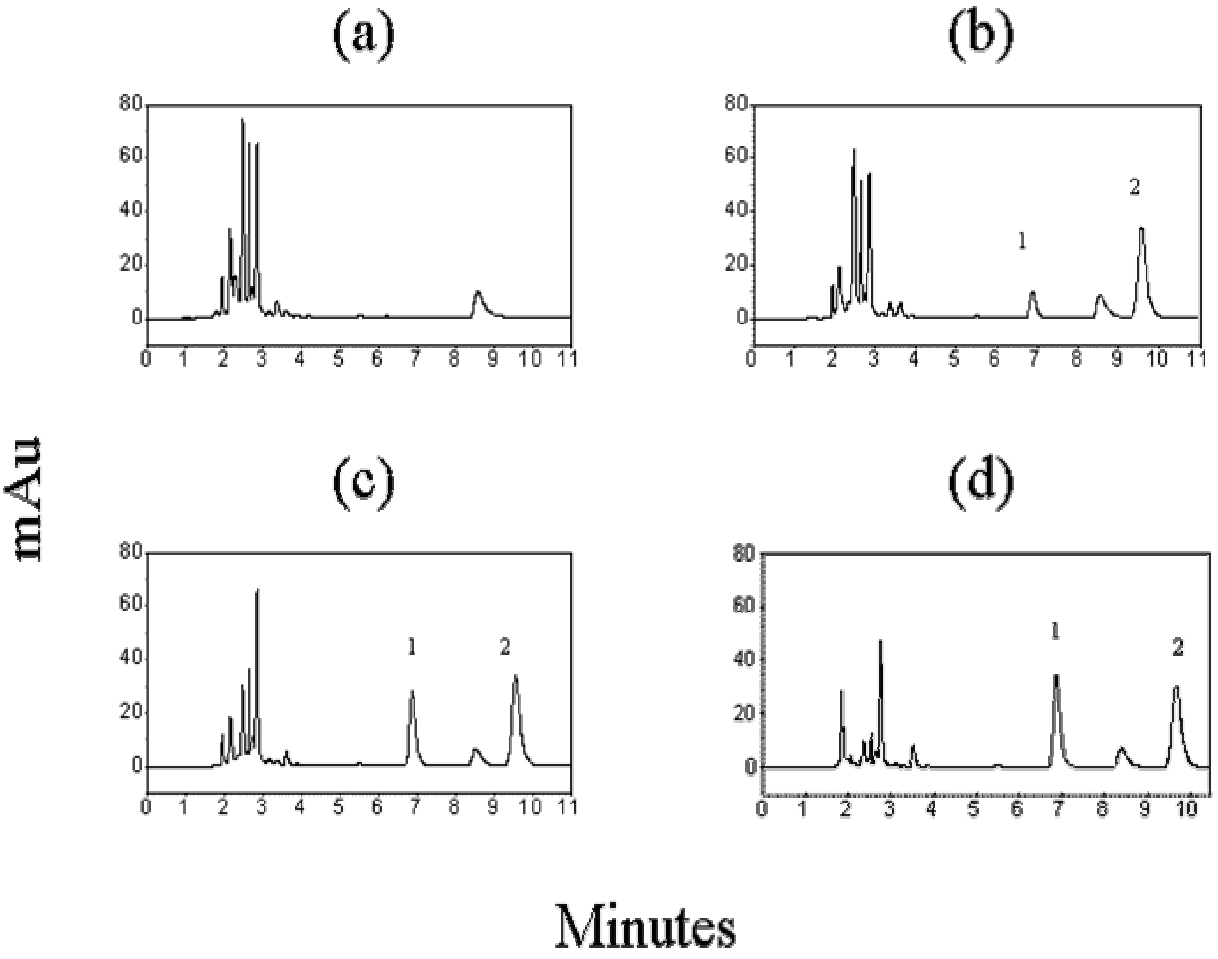



Figure B.2 Chromatograms of (a) blank rat plasma, (b) rat plasma with (1) 5 μg/ml of DOT and (2) 50 μg/ml internal standard added, (c) a rat plasma sample collected 1 h after intravenous administration of DOT to one rat and (d) a rat plasma sample collected 1 h after oral administration of DOT to a second rat.

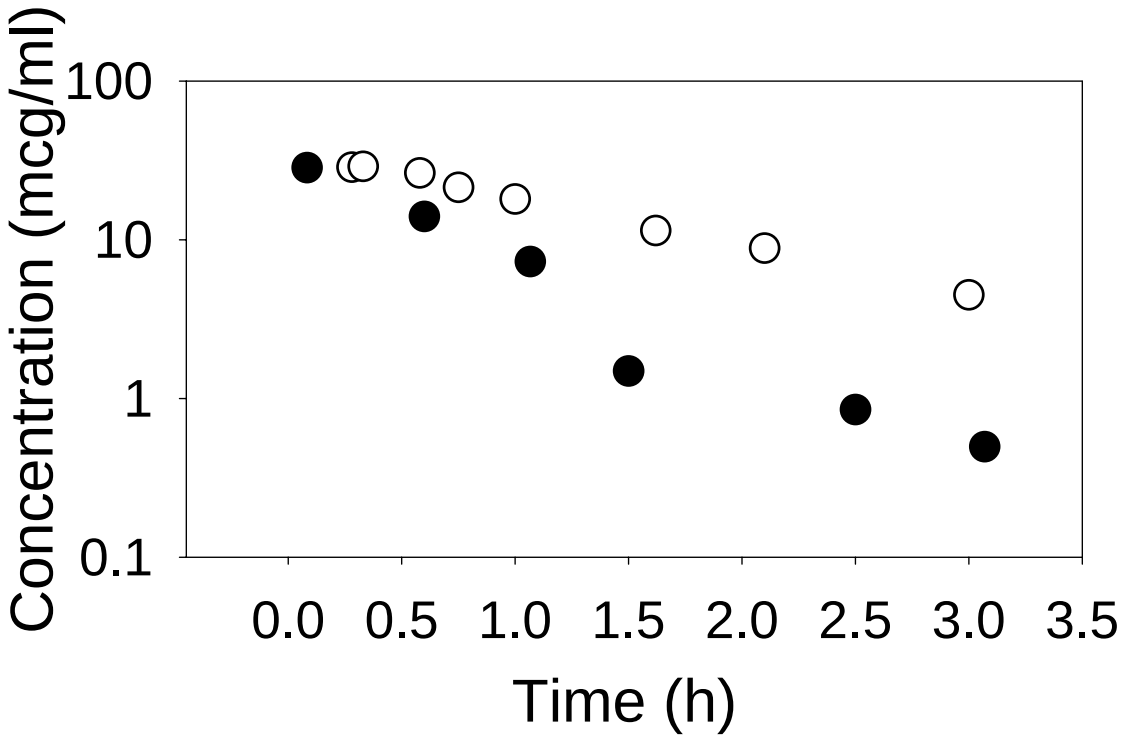



Figure B.3 Concentration time-profiles following intravenous (circles) and oral administration (squares) of DOT to rats.

# APPENDIX C

WINNONLIN OUTPUT

# WEIGHT 1/Yhat
# 001
# WinNonlin Compartmental Modeling Analysis

Version 4.0.1 Build 200210171634

## User-defined ASCII model:

```
MODEL
remark   ***************************************************
remark   Developer:
remark   Model Date:       02-02-2005
remark   Model Version:    1.0
remark   ***************************************************
remark
remark - define model-specific commands
COMMANDS
NFUNCTIONS 1
NPARAMETERS 2
PNAMES  'N', 'K'
END
remark - define temporary variables
TEMPORARY
CU=X
END
remark - define algebraic functions
FUNCTION 1
F= N*K*CU*0.714/(1+K*CU)
END
remark - define any secondary parameters
remark - end of model
EOM
```

## Settings for analysis:

Input Workbook: C:\Program Files\Pharsight\WinNonlin\VPAPPB\001.pwo
Input Worksheet: Sheet1
Input Sort Keys: [none]
Gauss-Newton (Levenberg and Hartley) method used
**Iterative reweighting used during minimization process**

Convergence criteria of 0.0001 used during minimization process
50 maximum iterations allowed during minimization process

**Input data:**

| CU (mmol/l) | CBbe | CBaf | Dbi-calc |
|---|---|---|---|
| 0.011388889 | 0.125277778 | 0.156041667 | 0.129514583 |
| 0.015138889 | 0.157708333 | 0.205902778 | 0.170899306 |
| 0.035277778 | 0.303958333 | 0.354375 | 0.29413125 |
| 0.035833333 | 0.318819444 | 0.355972222 | 0.295456944 |
| 0.057847222 | 0.367916667 | 0.465555556 | 0.386411111 |
| 0.070694444 | 0.412361111 | 0.447013889 | 0.371021528 |
| 0.108402778 | 0.519722222 | 0.562777778 | 0.467105556 |
| 0.137916667 | 0.549444444 | 0.663333333 | 0.550566667 |
| 0.193055556 | 0.717222222 | 0.720416667 | 0.597945833 |
| 0.301736111 | 0.587986111 | 0.750208333 | 0.622672917 |
| 0.423958333 | 0.685347222 | 0.759791667 | 0.630627083 |
| 0.429513889 | 0.707569444 | 0.719791667 | 0.597427083 |
| 0.447916667 | 0.741527778 | 0.734166667 | 0.609358333 |
| 0.682638889 | 2.004861111 | 2.186805556 | 1.815048611 |
| 0.695833333 | 1.854166667 | 2.600694444 | 2.158576389 |
| 0.850694444 | 0.9 | 0.965138889 | 0.801065278 |
| 1.470833333 | 1.272916667 | 1.658333333 | 1.376416667 |
| 3.326388889 | 1.606944444 | 1.7375 | 1.442125 |
| 4.211805556 | 1.172916667 | 2.232638889 | 1.853090278 |
| 4.395833333 | 1.4 | 2.134722222 | 1.771819444 |
| 9.694444444 | 0.419444444 | 2.980555556 | 2.473861111 |
| 9.743055556 | 1.466666667 | 1.658333333 | 1.376416667 |
| 11.20138889 | 0.747222222 | 1.673611111 | 1.389097222 |

**Output data:**

**Initial Parameters**

| Parameter | Value | Lower | Upper |
|---|---|---|---|
| N | 1 | 0 | 10 |
| K | 11 | 0 | 110 |

**Minimization Process**

| Iteration | Weighted_SS | N | K |
|---|---|---|---|
| 0 | 17.0566 | 1 | 11 |
| 1 | 3.78824 | 1.918 | 10.46 |
| 2 | 2.82727 | 2.19 | 4.663 |
| 3 | 2.62827 | 2.457 | 3.429 |
| 3 | 2.62826 | 2.459 | 3.424 |

**Final Parameters**

| N | N_StdErr | N_CV% | N_Univa | N_Univa | N_Planar | N_Planar | K | K_StdEr | K_CV% | K_Univa | K_Univa | K_Plana | K_Plana |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

| or | | | rCI_Lower | rCI_Upper | CI_Lower | CI_Upper | ror | | | rCI_Lower | rCI_Upper | rCI_Lower | rCI_Upper |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2.458627 | 0.268531 | 10.92 | 1.900191 | 3.017063 | 1.734195 | 3.183060 | 3.424069 | 1.066849 | 31.16 | 1.205452 | 5.642685 | 0.545964 | 6.302174 |

**Correlation Matrix**

| Parameter | N | K |
|---|---|---|
| N | 1 | |
| K | -0.725898 | 1 |

**Eigenvalues**

| Number | Value |
|---|---|
| 1 | 3.795 |
| 2 | 0.1063 |

**Condition Numbers**

| Iteration | Rank | Condition |
|---|---|---|
| 0 | 2 | 4.994653 |
| 1 | 2 | 4.046633 |
| 2 | 2 | 3.139938 |
| 3 | 2 | 2.910889 |

**Variance-Covariance Matrix**

| Parameter | N | K |
|---|---|---|
| N | 7.21E-02 | |
| K | -0.207957 | 1.13817 |

**Summary Table**

| CBbe | CBaf | CU_obs (mmol/l) | Dbi-calc_obs | CU (mmol/l) | Dbi-calc | Predicted | Residual | Weight | SE_Yhat | Standard_Res |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.125277778 | 0.156041667 | 0.011388889 | 0.129514583 | 0.0114 | 0.1295 | 0.0659 | 0.0636 | 15.1629 | 0.0154 | 0.7106 |
| 0.157708333 | 0.205902778 | 0.015138889 | 0.170899306 | 0.0151 | 0.1709 | 0.0865 | 0.0844 | 11.5480 | 0.0199 | 0.8258 |
| 0.303958333 | 0.354375 | 0.035277778 | 0.294103125 | 0.0353 | 0.2941 | 0.1892 | 0.1049 | 5.2808 | 0.0402 | 0.7061 |
| 0.318819444 | 0.355972222 | 0.035833333 | 0.295456944 | 0.0358 | 0.2955 | 0.1918 | 0.1036 | 5.2078 | 0.0407 | 0.6926 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.3679 | 0.4655 | 0.0578 | 0.3864 | 0.0578 | 0.3864 | 0.2902 | 0.0962 | 3.4427 | 0.0568 | 0.5285 |
| 16667 | 55556 | 47222 | 11111 | | | | | | | |
| 0.4123 | 0.4470 | 0.0706 | 0.3710 | 0.0707 | 0.3710 | 0.3421 | 0.0289 | 2.9206 | 0.0641 | 0.1468 |
| 61111 | 13889 | 94444 | 21528 | | | | | | | |
| 0.5197 | 0.5627 | 0.1084 | 0.4671 | 0.1084 | 0.4671 | 0.4752 | - | 2.1028 | 0.0788 | - |
| 22222 | 77778 | 02778 | 05556 | | | | 0.0081 | | | 0.0351 |
| 0.5494 | 0.6633 | 0.1379 | 0.5505 | 0.1379 | 0.5506 | 0.5631 | - | 1.7747 | 0.0857 | - |
| 44444 | 33333 | 16667 | 66667 | | | | 0.0125 | | | 0.0498 |
| 0.7172 | 0.7204 | 0.1930 | 0.5979 | 0.1931 | 0.5979 | 0.6986 | - | 1.4305 | 0.0920 | - |
| 22222 | 16667 | 55556 | 45833 | | | | 0.1007 | | | 0.3581 |
| 0.5879 | 0.7502 | 0.3017 | 0.6226 | 0.3017 | 0.6227 | 0.8920 | - | 1.1205 | 0.0940 | - |
| 86111 | 08333 | 36111 | 72917 | | | | 0.2694 | | | 0.8399 |
| 0.6853 | 0.7597 | 0.4239 | 0.6306 | 0.4240 | 0.6306 | 1.0394 | - | 0.9617 | 0.0925 | - |
| 47222 | 91667 | 58333 | 27083 | | | | 0.4088 | | | 1.1724 |
| 0.7075 | 0.7197 | 0.4295 | 0.5974 | 0.4295 | 0.5974 | 1.0449 | - | 0.9566 | 0.0925 | - |
| 69444 | 91667 | 13889 | 27083 | | | | 0.4475 | | | 1.2797 |
| 0.7415 | 0.7341 | 0.4479 | 0.6093 | 0.4479 | 0.6094 | 1.0626 | - | 0.9407 | 0.0923 | - |
| 27778 | 66667 | 16667 | 58333 | | | | 0.4533 | | | 1.2844 |
| 2.0048 | 2.1868 | 0.6826 | 1.8150 | 0.6826 | 1.8150 | 1.2295 | 0.5856 | 0.8131 | 0.0939 | 1.5373 |
| 61111 | 05556 | 38889 | 48611 | | | | | | | |
| 1.8541 | 2.6006 | 0.6958 | 2.1585 | 0.6958 | 2.1586 | 1.2365 | 0.9221 | 0.8085 | 0.0942 | 2.4138 |
| 66667 | 94444 | 33333 | 76389 | | | | | | | |
| 0.9 | 0.9651 | 0.8506 | 0.8010 | 0.8507 | 0.8011 | 1.3068 | - | 0.7650 | 0.0981 | - |
| | 38889 | 94444 | 65278 | | | | 0.5058 | | | 1.2889 |
| 1.2729 | 1.6583 | 1.4708 | 1.3764 | 1.4708 | 1.3764 | 1.4646 | - | 0.6826 | 0.1172 | - |
| 16667 | 33333 | 33333 | 16667 | | | | 0.0882 | | | 0.2142 |
| 1.6069 | 1.7375 | 3.3263 | 1.4421 | 3.3264 | 1.4421 | 1.6138 | - | 0.6196 | 0.1494 | - |
| 44444 | | 88889 | 25 | | | | 0.1716 | | | 0.4049 |
| 1.1729 | 2.2326 | 4.2118 | 1.8530 | 4.2118 | 1.8531 | 1.6416 | 0.2115 | 0.6091 | 0.1568 | 0.4972 |
| 16667 | 38889 | 05556 | 90278 | | | | | | | |
| 1.4 | 2.1347 | 4.3958 | 1.7718 | 4.3958 | 1.7718 | 1.6461 | 0.1257 | 0.6075 | 0.1581 | 0.2955 |
| | 22222 | 33333 | 19444 | | | | | | | |
| 0.4194 | 2.9805 | 9.6944 | 2.4738 | 9.6944 | 2.4739 | 1.7041 | 0.7697 | 0.5868 | 0.1751 | 1.8012 |
| 44444 | 55556 | 44444 | 61111 | | | | | | | |
| 1.4666 | 1.6583 | 9.7430 | 1.3764 | 9.7431 | 1.3764 | 1.7044 | - | 0.5867 | 0.1752 | - |
| 66667 | 33333 | 55556 | 16667 | | | | 0.3280 | | | 0.7674 |
| 0.7472 | 1.6736 | 11.201 | 1.3890 | 11.201 | 1.3891 | 1.7109 | - | 0.5845 | 0.1772 | - |
| 22222 | 11111 | 38889 | 97222 | 4 | | | 0.3218 | | | 0.7527 |

## Diagnostics

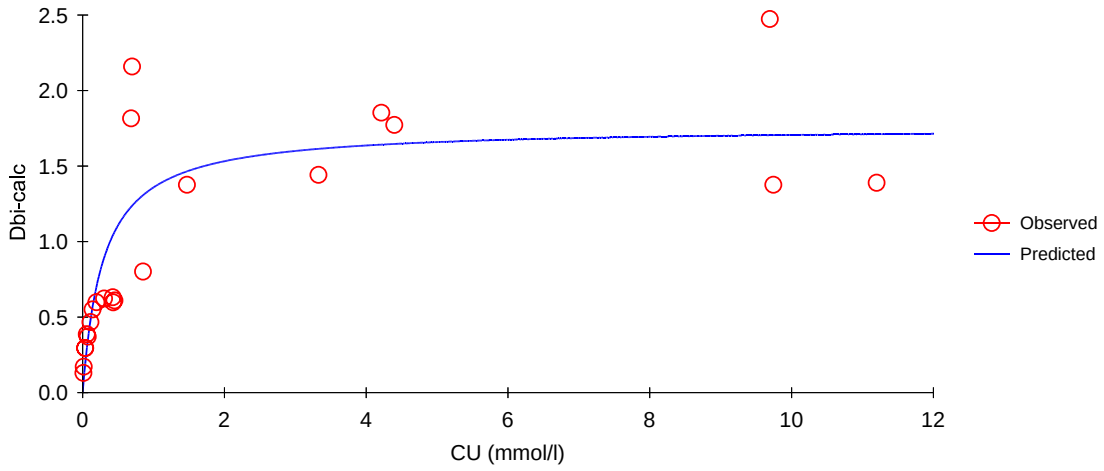
| Function | Item | Value |
|---|---|---|
| 1 | CSS | 10.596 |
| 1 | WCSS | 13.728 |
| 1 | SSR | 3.04904 |
| 1 | WSSR | 2.62737 |
| 1 | S | 0.353713 |
| 1 | DF | 21 |
| 1 | CORR_(OBS,PRED) | 0.844 |
| 1 | AIC | 26.21762 |
| 1 | SBC | 28.48861 |

## Partial Derivatives

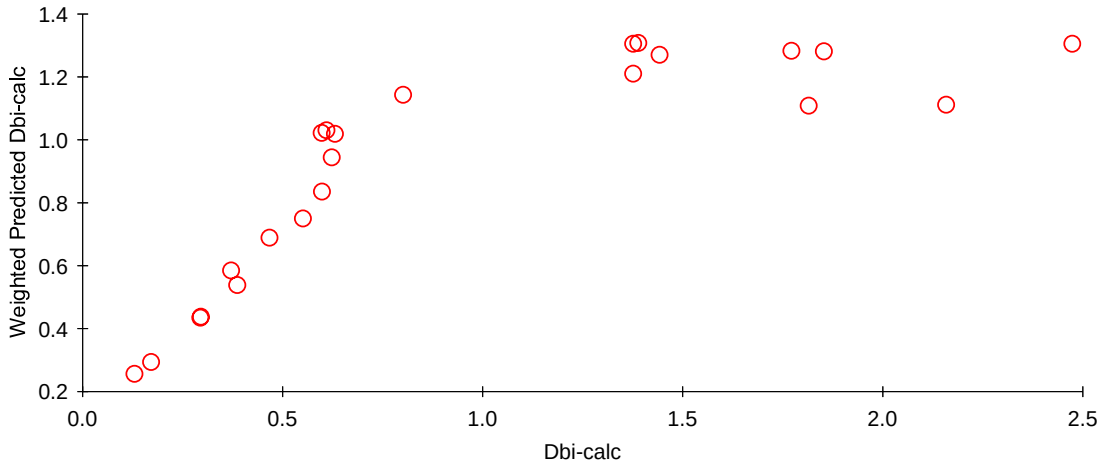
| Function | Time (mmol/l) | N | K |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 1 | 1.14E-02 | 0.10434956 | 0.07211385 |
| 1 | 1.51E-02 | 0.11957209 | 0.08162460 |
| 1 | 3.53E-02 | 0.17682545 | 0.11327810 |
| 1 | 3.58E-02 | 0.17806129 | 0.11387645 |
| 1 | 5.78E-02 | 0.21900630 | 0.13124649 |
| 1 | 7.07E-02 | 0.23778139 | 0.13744921 |
| 1 | 0.108402778 | 0.28024002 | 0.14673293 |
| 1 | 0.137916667 | 0.30505391 | 0.14875778 |
| 1 | 0.193055556 | 0.33978849 | 0.14685663 |
| 1 | 0.301736111 | 0.38395849 | 0.13556623 |
| 1 | 0.423958333 | 0.41446533 | 0.12135264 |
| 1 | 0.429513889 | 0.41556300 | 0.12073703 |
| 1 | 0.447916667 | 0.41906192 | 0.11872502 |
| 1 | 0.682638889 | 0.45076292 | 0.09694776 |
| 1 | 0.695833333 | 0.45204895 | 0.09592561 |
| 1 | 0.850694444 | 0.46472702 | 0.08525010 |
| 1 | 1.470833333 | 0.49198929 | 0.05850038 |
| 1 | 3.326388889 | 0.51643017 | 0.02991566 |
| 1 | 4.211805556 | 0.52086796 | 0.02424086 |
| 1 | 4.395833333 | 0.52157640 | 0.02332090 |
| 1 | 9.694444444 | 0.53068969 | 0.01113845 |
| 1 | 9.743055556 | 0.53072842 | 0.01108530 |
| 1 | 11.20138889 | 0.53173674 | 0.00969712 |

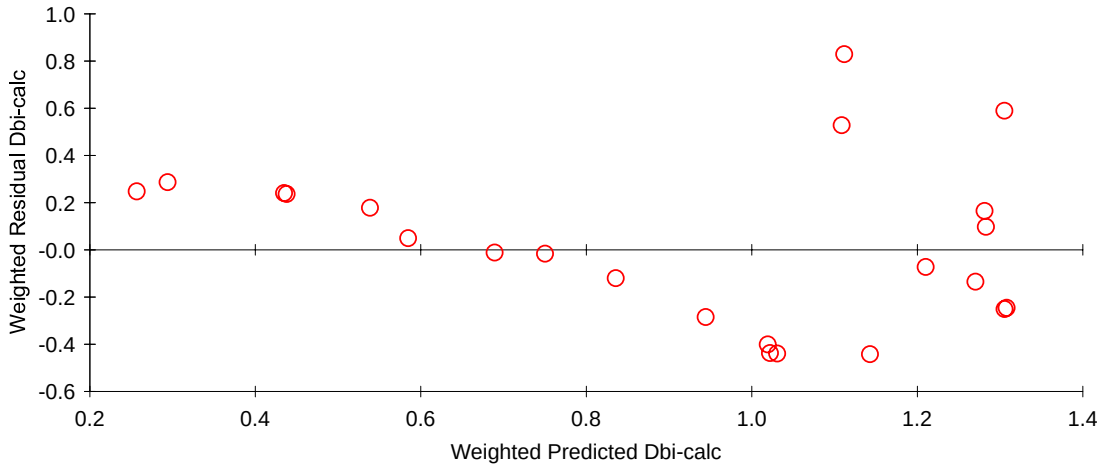
## X vs. Observed Y and Predicted Y



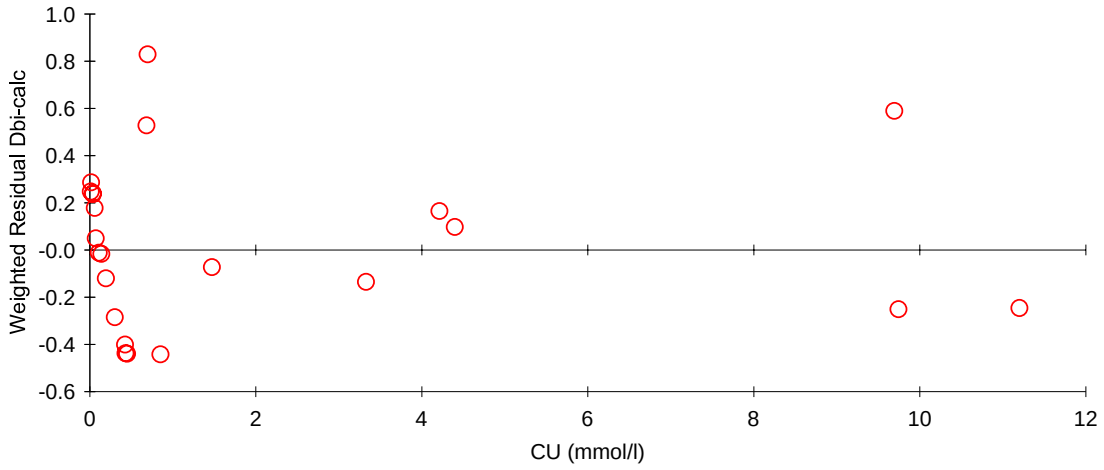
**Observed Y vs. Weighted Predicted Y**



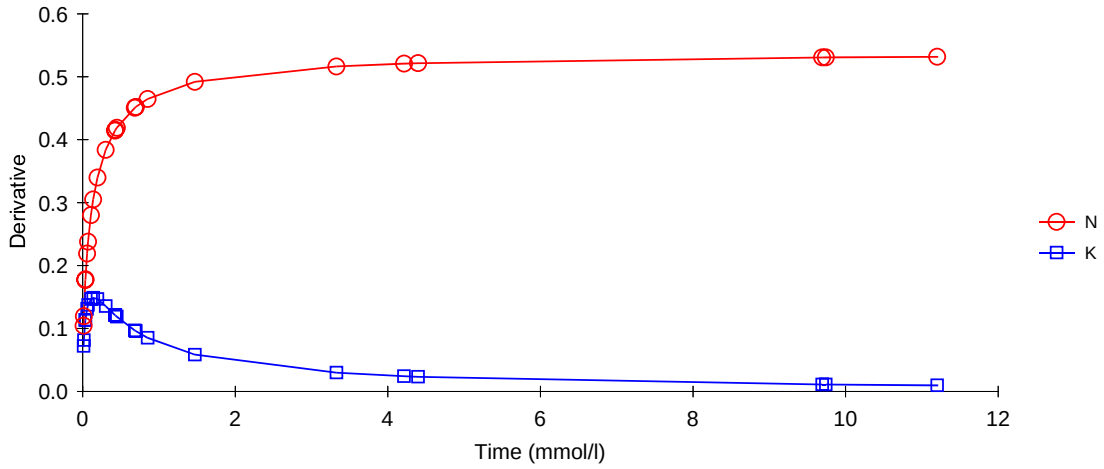

**Weighted Predicted Y vs. Weighted Residual Y**



## X vs. Weighted Residual Y




## Partial Derivatives



NONMEM CODES

```
$PROB PPB DATA FROM NINE VOLUNTEERS
$DATA ..\PPB.csv IGNORE #
$INPUT ID CU CBBE CBAF DBI=DV ALB GEN AGE WT HT BMI PROT
$PRED
IF(GEN.EQ.1)  I2 = 1              ;SET IND. FOR FEMALE
IF(GEN.EQ.2)  I2 = 0              ;SET IND. FOR MALE
N=THETA(1)*EXP(ETA(1))            ;NUMBER OF BINDING SITES
K=THETA(2)*EXP(ETA(2))            ;AFFINITY CONSTANT, WITH
INTERINDIVIDUAL VARIABILITY
F=((N*K*CU*ALB)/(1+K*CU))         ;ONE SATURABLE BINDING SITE
DEL=0
IF (F.EQ.0) DEL=0.000025; PREVENTS IWRES FROM BLOWING UP FOR F=0
IPRED=F
W=IPRED+DEL
IRES=DV-IPRED
IWRES=IRES/W
Y=W*(1+ERR(1))
$THETA
(0, 2)        ; N
(0, 10)       ; K
$OMEGA
0 FIXED
0.1
$SIGMA
0.1
$EST NOABORT SIG=3 MAX=2000 PRINT=1 METHOD = 1; FOCE METHOD
$COV
$SCATTER PRED VS DV UNIT
$SCATTER IPRED VS DV UNIT
$SCATTER PRED VS RES
$SCATTER IPRED VS IWRES UNIT
$SCATTER WRES VS ALB
$SCATTER WRES VS GEN
$SCATTER WRES VS AGE
$SCATTER PRED VS WRES
$TABLE ID CU N K ALB AGE GEN WT BMI PROT IPRED IWRES
ETA(2) FIRSTONLY NOPRINT FILE=base.fit
$TABLE ID CU N K ALB AGE GEN WT BMI PROT IPRED IWRES
ETA(2) NOPRINT FILE=PPB.fit
```

```
$PROB PK DATA FROM NINE VOLUNTEERS
$DATA ..\PK-9.csv IGNORE #
$INPUT ID DAY=DROP TIME DV AMT DOSE RATE EVID MDV ALB GEN AGE
WT HT BMI PROT

$SUBROUTINE  ADVAN6  TOL=3 ; SET UP DIFFERENTIAL EQUATION MODE
$MODEL COMP(CENTRAL,DEFDOSE) ; JUST ONE COMPARTMENT

$PK ; DEFINE BASIC PK RELATIONSHIPS
  CL = THETA(1)*EXP(ETA(1))
  V = THETA(2)*EXP(ETA(2))
  D1 =THETA(3)*EXP(ETA(3))
   K= CL/V

$DES ; DEFINE DIFFERENTIAL EQUATIONS
  DADT(1) =  -A(1) * K

$ERROR ; COMPUTE DV FROM STATE VARIABLES
Y= A(1)/V * (1+EPS(1))
;F= A(1)/V
;DEL=0
;IF (F.EQ.0) DEL=0.000025;  PREVENTS IWRES FROM BLOWING UP FOR F=0
;IPRED=F
;W=IPRED+DEL
;IRES=DV-IPRED
;IWRES=IRES/W
;Y=W*(1+ERR(1))

$THETA (1, 20)           ;CL
$THETA (2,150)           ;V
$THETA (0.1,8)          ;DURATION
;$THETA (1, 12)          ;
;$THETA (-0.1,0.18,0.55)  ;CL_AGE
;$THETA (0.6,1,2)        ;CL_SMK
;$THETA (-0.1,0.01,2)    ;CL_CGLF
;$THETA (0.25,1,3)       ;F_GEN
$OMEGA  0.1          ;CL
$OMEGA  0.2          ;V
$OMEGA  0 FIX          ;DUR
;$OMEGA BLOCK (2) 0.1 0.15 0.5 ;CL-V
$SIGMA 0.15              ;PROP
;MSFI=
$EST NOABORT MAXEVAL=4000 SIGDIGITS=3 PRINT=5 METH=1 INTER
$COV
```

```
;$SCATTER PRED VS DV UNIT
;$SCATTER IPRED VS DV UNIT
;$SCATTER PRED VS RES
;$SCATTER IPRED VS IWRES UNIT
;$SCATTER WRES VS GEN
;$SCATTER WRES VS AGE
;$SCATTER PRED VS WRES
;$TABLE ID DV AGE GEN WT BMI IPRED IWRES FIRSTONLY NOPRINT
FILE=PK1.FIT
;$TABLE ID DV AGE GEN WT BMI IPRED IWRES NOPRINT FILE=PK.FIT
```

NONMEM OUTPUT

```
1NONLINEAR MIXED EFFECTS MODEL PROGRAM (NONMEM)    DOUBLE
PRECISION NONMEM    VERSION V LEVEL 1.1
 DEVELOPED AND PROGRAMMED BY STUART BEAL AND LEWIS SHEINER

 PROBLEM NO.:         1
 PK DATA FROM NINE VOLUNTEERS
0DATA CHECKOUT RUN:              NO
 DATA SET LOCATED ON UNIT NO.:    2
 THIS UNIT TO BE REWOUND:         NO
 NO. OF DATA RECS IN DATA SET:  336
 NO. OF DATA ITEMS IN DATA SET:  14
 ID DATA ITEM IS DATA ITEM NO.:   1
 DEP VARIABLE IS DATA ITEM NO.:   3
 MDV DATA ITEM IS DATA ITEM NO.:  7
0INDICES PASSED TO SUBROUTINE PRED:
  6  2  4  5  0  0  0  0  0
  0  0
0LABELS FOR DATA ITEMS:
   ID   TIME     DV    AMT   RATE   EVID    MDV    ALB    GEN
  AGE     WT     HT    BMI   PROT
0FORMAT FOR DATA:
 (E2.0,2E6.0,E5.0,E3.0,2E2.0,E7.0,E2.0,E3.0,E4.0,2E5.0,E4.0)

 TOT. NO. OF OBS RECS:     152
 TOT. NO. OF INDIVIDUALS:    8
0LENGTH OF THETA:  3
0OMEGA HAS SIMPLE DIAGONAL FORM WITH DIMENSION:  3
0SIGMA HAS SIMPLE DIAGONAL FORM WITH DIMENSION:  1
0INITIAL ESTIMATE OF THETA:
 LOWER BOUND    INITIAL EST    UPPER BOUND
  0.1000E+01     0.9000E+01     0.1000E+07
  0.2000E+01     0.1860E+03     0.1000E+07
  0.1000E+00     0.1000E+02     0.1000E+07
0INITIAL ESTIMATE OF OMEGA:
 0.1000E+00
 0.0000E+00   0.1000E+00
 0.0000E+00   0.0000E+00   0.1000E+00
0INITIAL ESTIMATE OF SIGMA:
 0.8000E-01
0ESTIMATION STEP OMITTED:           NO
 CONDITIONAL ESTIMATES USED:       YES
 CENTERED ETA:                     NO
 EPS-ETA INTERACTION:              YES
 LAPLACIAN OBJ. FUNC.:             NO
```

```
 NO. OF FUNCT. EVALS. ALLOWED:    4000
 NO. OF SIG. FIGURES REQUIRED:    3
 INTERMEDIATE PRINTOUT:           YES
 ESTIMATE OUTPUT TO MSF:          NO
 ABORT WITH PRED EXIT CODE 1:     NO
0COVARIANCE STEP OMITTED:    NO
 EIGENVLS. PRINTED:    NO
 SPECIAL COMPUTATION:  NO
 COMPRESSED FORMAT:    NO
1DOUBLE PRECISION PREDPP VERSION IV LEVEL 1.1

 GENERAL NONLINEAR KINETICS MODEL (ADVAN6)
0MODEL SUBROUTINE USER-SUPPLIED - ID NO. 9999
0MAXIMUM NO. OF BASIC PK PARAMETERS:   1
0COMPARTMENT ATTRIBUTES
 COMPT. NO.   FUNCTION   INITIAL    ON/OFF      DOSE      DEFAULT
DEFAULT
                  STATUS     ALLOWED    ALLOWED    FOR DOSE   FOR OBS.
   1         CENTRAL      ON         YES        YES        YES        YES
   2         OUTPUT       OFF        YES        NO         NO         NO
0NRD VALUE FROM SUBROUTINE TOL:   3
1
 ADDITIONAL PK PARAMETERS - ASSIGNMENT OF ROWS IN GG
 COMPT. NO.                     INDICES
          SCALE     BIOAVAIL.   ZERO-ORDER  ZERO-ORDER  ABSORB
                    FRACTION    RATE       DURATION    LAG
   1         *         *         *          2          *
   2         *         -         -          -          -
         - PARAMETER IS NOT ALLOWED FOR THIS MODEL
         * PARAMETER IS NOT SUPPLIED BY PK SUBROUTINE;
           WILL DEFAULT TO ONE IF APPLICABLE
0DATA ITEM INDICES USED BY PRED ARE:
   EVENT ID DATA ITEM IS DATA ITEM NO.:      6
   TIME DATA ITEM IS DATA ITEM NO.:          2
   DOSE AMOUNT DATA ITEM IS DATA ITEM NO.:   4
   DOSE RATE DATA ITEM IS DATA ITEM NO.:     5

0PK SUBROUTINE CALLED WITH EVERY EVENT RECORD.
 PK SUBROUTINE NOT CALLED AT NONEVENT (ADDITIONAL OR LAGGED)
DOSE TIMES.
0ERROR SUBROUTINE CALLED WITH EVERY EVENT RECORD.
0DES SUBROUTINE USES COMPACT STORAGE MODE
1
 MONITORING OF SEARCH:
```

0ITERATION NO.: 0 OBJECTIVE VALUE: 0.3568E+03 NO. OF FUNC. EVALS.: 6
CUMULATIVE NO. OF FUNC. EVALS.: 6
PARAMETER: 0.1000E+00 0.1000E+00 0.1000E+00 0.1000E+00 0.1000E+00 0.1000E+00 0.1000E+00
GRADIENT: 0.4468E+03 -0.7039E+03 0.3495E+03 -0.3117E+03 0.3577E+02 -0.1645E+03 0.4818E+03
0ITERATION NO.: 5 OBJECTIVE VALUE: 0.3488E+03 NO. OF FUNC. EVALS.:20
CUMULATIVE NO. OF FUNC. EVALS.: 61
PARAMETER: 0.7053E-01 0.1179E+00 0.1037E+00 0.3067E+00 0.2052E+00 0.2469E+00 0.8598E-01
GRADIENT: -0.4564E+04 -0.4544E+03 -0.5192E+03 0.4704E+02 0.3883E+02 0.1258E+02 0.8373E+03
0ITERATION NO.: 10 OBJECTIVE VALUE: 0.3423E+03 NO. OF FUNC. EVALS.: 8
CUMULATIVE NO. OF FUNC. EVALS.: 123
PARAMETER: 0.7053E-01 0.1165E+00 0.1037E+00 0.1276E+00 0.2034E+00 0.2421E+00 0.8579E-01
GRADIENT: 0.1342E+04 -0.4894E+04 -0.5387E+04 0.6220E+02 0.1448E+02 0.3025E+02 -0.7310E+04
0ITERATION NO.: 15 OBJECTIVE VALUE: 0.3405E+03 NO. OF FUNC. EVALS.:18
CUMULATIVE NO. OF FUNC. EVALS.: 192
PARAMETER: 0.7052E-01 0.1164E+00 0.1037E+00 0.1059E+00 0.2032E+00 0.2415E+00 0.8581E-01
GRADIENT: -0.1233E+05 -0.6500E+04 -0.1202E+05 0.4236E+02 0.1406E+02 0.2611E+02 0.1262E+05
0ITERATION NO.: 17 OBJECTIVE VALUE: 0.3405E+03 NO. OF FUNC. EVALS.:16
CUMULATIVE NO. OF FUNC. EVALS.: 227
PARAMETER: 0.7052E-01 0.1164E+00 0.1037E+00 0.1058E+00 0.2032E+00 0.2415E+00 0.8581E-01
GRADIENT: -0.1307E+05 -0.1081E+05 -0.1202E+05 -0.1203E+05 0.3174E+04 -0.5337E+04 0.1262E+05
0MINIMIZATION SUCCESSFUL
NO. OF FUNCTION EVALUATIONS USED: 227
NO. OF SIG. DIGITS IN FINAL EST.: 3.3

ETABAR IS THE ARITHMETIC MEAN OF THE ETA-ESTIMATES,
AND THE P-VALUE IS GIVEN FOR THE NULL HYPOTHESIS THAT THE TRUE MEAN IS 0.

ETABAR: 0.83E-01 -0.47E+00 -0.15E+00

P VAL.: 0.37E+00 0.58E-04 0.37E+00
1

**********
MINIMUM VALUE OF OBJECTIVE FUNCTION**********
********** 340.479 **********

********** FINAL PARAMETER ESTIMATE**********

THETA - VECTOR OF FIXED EFFECTS PARAMETERS **********

TH 1 TH 2 TH 3

4.98E+00 2.51E+02 1.07E+01

OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS ********

ETA1 ETA2 ETA3

ETA1
+ 1.12E-01

ETA2
+ 0.00E+00 4.13E-01

ETA3
+ 0.00E+00 0.00E+00 5.83E-01

SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS ****

EPS1

EPS1
+ 5.89E-02

********** STANDARD ERROR OF ESTIMATE**********

THETA - VECTOR OF FIXED EFFECTS PARAMETERS *********

TH 1 TH 2 TH 3

6.96E-03 3.67E-01 2.12E-02

OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS ********

ETA1 ETA2 ETA3

ETA1
+ 4.37E-04

ETA2
+ ..... 1.46E-03

ETA3
+ ..... ..... 1.50E-03

SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS ****

EPS1

EPS1
+ 1.93E-04

********** COVARIANCE MATRIX OF ESTIMATE **********

TH 1 TH 2 TH 3 OM11 OM12 OM13 OM22 OM23 OM33 SG11

TH 1

+ 4.85E-05

TH 2
+ 2.55E-03 1.35E-01

TH 3
+ 1.45E-04 7.66E-03 4.50E-04

OM11
+ 3.02E-06 1.60E-04 9.22E-06 1.91E-07

OM12
+ ..... ..... ..... ..... .....

OM13
+ ..... ..... ..... ..... ..... .....

OM22
+ -1.01E-05 -5.32E-04 -3.08E-05 -6.36E-07 ..... ..... 2.12E-06

OM23
+ ..... ..... ..... ..... ..... ..... ..... .....

OM33
+ 1.04E-05 5.51E-04 3.17E-05 6.56E-07 ..... ..... -2.19E-06 ..... 2.26E-06

SG11
+ -1.32E-06 -6.98E-05 -4.10E-06 -8.40E-08 ..... ..... 2.80E-07 ..... -2.89E-07 3.73E-08

**********CORRELATION MATRIX**********

TH 1 TH 2 TH 3 OM11 OM12 OM13 OM22 OM23 OM33 SG11

TH 1
+ 1.00E+00

```
 TH 2
+       9.98E-01  1.00E+00

 TH 3
+       9.82E-01  9.83E-01  1.00E+00

 OM11
+       9.95E-01  9.96E-01  9.95E-01  1.00E+00

 OM12
+       ......... ......... ......... ......... .........

 OM13
+       ......... ......... ......... ......... ......... .........

 OM22
+       -9.94E-01 -9.95E-01 -9.96E-01 -1.00E+00 ......... .........  1.00E+00

 OM23
+       ......... ......... ......... ......... ......... ......... ......... .........

 OM33
+       9.97E-01  9.98E-01  9.93E-01  1.00E+00 ......... ......... -9.99E-01 .........  1.00E+00

 SG11
+       -9.83E-01 -9.83E-01 -9.99E-01 -9.95E-01 ......... .........  9.97E-01 ......... -9.93E-01
1.00E+00
```

********** INVERSE COVARIANCE MATRIX OF ESTIMATE ************

```
          TH 1      TH 2      TH 3      OM11      OM12      OM13      OM22      OM23
OM33      SG11

 TH 1
+       2.27E+08

 TH 2
```

+ -1.15E+07 1.71E+06

TH 3
+ 9.08E+07 5.95E+06 1.42E+08

OM11
+ 2.45E+10 -5.08E+09 -2.72E+10 1.60E+13

OM12
+ ..... ..... ..... ..... .....

OM13
+ ..... ..... ..... ..... ..... .....

OM22
+ 1.20E+10 -8.42E+08 2.84E+09 2.08E+12 ..... ..... 7.06E+11

OM23
+ ..... ..... ..... ..... ..... ..... ..... .....

OM33
+ 5.33E+09 4.96E+07 5.52E+09 -5.82E+11 ..... ..... 2.35E+11 .....
2.45E+11

SG11
+ 2.65E+09 -1.29E+09 -9.84E+09 4.23E+12 ..... ..... 3.50E+11 ..... -
2.89E+11 1.27E+12

# VITA

Alaa M. Ahmad was born on 05/08/1978 in Amman, Jordan. He received his B.Sc. in pharmacy in 06/2001 from the University of Jordan. He joined the department of pharmaceutical and biomedical sciences at the University of Georgia in 08/2001 and then the department of pharmaceutics at Virginia Commonwealth University in 08/2002.He presented 5 abstracts in national meetings and wrote 4 manuscripts. He received his Ph.D. in pharmaceutical sciences with concentration in pharmaceutics in May 2005.