Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 8
Open Access

Utilization of Physiologically based Biopharmaceutical Modelling and Simulations for Proposed Dissolution Specifications of Acetazolamide ER Capsules 500 mg

Pramod V Kadam[1], Dr K Lakshmi[2]

[1]Author, Research Scholar, Chettinad School of Pharmaceutical Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam – 603103 Tamilnadu, India

[2]Corresponding author, Chettinad School of Pharmaceutical Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam – 603103 Tamilnadu, India

Cite this paper as: Pramod V Kadam, Dr K Lakshmi (2024) Utilization of Physiologically based Biopharmaceutical Modelling and Simulations for Proposed Dissolution Specifications of Acetazolamide ER Capsules 500 mg ". *Frontiers in Health Informatics,* (8), 5453-5479

Abstract

This study investigates the application of physiologically based biopharmaceutics modeling (PBBM) to propose revised dissolution specifications for Acetazolamide Extended Release (ER) Capsules 500 mg which were different from the currently approved specifications. Acetazolamide is a carbonic anhydrase inhibitor which is utilized in the treatment of glaucoma and epilepsy, where extended-release formulations are designed to ensure sustained therapeutic levels.The research leverages PBBM to simulate gastrointestinal transit, drug release, and absorption dynamics, integrating comprehensive physiological, biochemical, and pharmacokinetic data. The model is meticulously calibrated and validated using existing clinical pharmacokinetic profiles. A range of dissolution profiles is simulated to determine optimal specifications that better align with in vivo performance compared to the approved standards.Results indicate that the proposed revised dissolution specifications demonstrate a closer correlation with in vivo drug release and absorption patterns, suggesting potential improvements in bioavailability and therapeutic efficacy. This study underscores the utility of PBBM in regulatory frameworks, offering a scientifically robust approach to revising dissolution specifications and ensuring enhanced drug quality and patient outcomes.

Keywords:- Biopharmaceutics modelling, Acetazolamide, Extended Release (ER) Capsules, Dissolution profiles, Specifications

1. Introduction

The generic version of acetazolamide extended release capsules 500 mg, was pharmaceutically equivalent to the innovator's Drug product of DIAMOX® SEQUELS® marketed by Duramed Pharmaceuticals, Inc. The present work has been done to demonstrate the proposed revised dissolution specification without compromising safety, efficacy and quality of the drug product using biopharmaceutics and fasting state PBPK modelling and simulations. The details of approved

dissolution specification and proposed dissolution specifications are listed in Table 1.

Table 1; Dissolution specification acetazolamide extended release capsules 500 mg

Time (h)	Approved Specification (% Drug release)	Proposed Specification I (% Drug release)	Proposed Specification II (% Drug release)
1	20-40	20-40	20-40
2	35-60	35-60	35-60
5	**70**-85	**65**-85	**67**-85
12	NLT 85	NLT 85	NLT 85

This work referred the various regulatory guidelines and documents.[1,2,3]

Acetazolamide is a carbonic anhydrase inhibitor which has been widely used to treat glaucoma[4]. The drug reduces intraocular pressure (IOP) by decreasing aqueous humor formation[5]. Acetazolamide is available as immediate release tablets (125 mg and 250 mg) and acetazolamide sodium injection is available for intravenous administration (500 mg; 100 mg/ml)[6]. The Bio-waiver monograph for acetazolamide immediate release solid oral dosage form was published in literature, which describes the possibilities for bio-waiver of immediate release dosage forms based on the physicochemical, biopharmaceutics and pharmacokinetics and formulation data[7]. The present work focuses on the PBBM model development and simulation in fasting state physiology and its application in post approval changes in dissolution specification limit for adopting the proposed revised dissolution specifications.

2. Objective

The objective is the development of PBPK model for biopharmaceutics application to demonstrate the biopharmaceutical quality of the acetazolamide extended release capsules 500 mg using the proposed revised dissolution specifications. This document provides the objective of fasting state PBPK model development and validation which may be useful in application of proposing dissolution specifications.

3. Methodology

GastroPlus version 9.7 (Simulation Plus, Inc.) was used to build the mechanistic pharmacokinetic model for acetazolamide to describe the in vivo pharmacokinetics of this compound, across the different studies in humans. The in-silico biopharmaceutical properties of acetazolamide were obtained from the literature. The experimental physicochemical data such as particle size, pH dependent solubility studies and dissolution studies were used to build the model for simulation studies.

3.1. Physicochemical, biopharmaceutics and pharmacological properties

Acetazolamide is chemically N-(5- sulfamoyl-1,3,4-thiadiazol-2-yl) acetamide and an enzyme inhibitor that acts specifically on carbonic anhydrase, the enzyme that catalyzes the reversible reaction involving the hydration of carbon dioxide and the dehydration of carbonic acid[5]. In the eye, this inhibitory action of acetazolamide decreases the secretion of aqueous humor and results in a drop in intraocular pressure, a reaction considered desirable in cases of glaucoma and even in certain non-glaucomatous conditions[5]. Evidence seems to indicate that acetazolamide has utility as an adjuvant in

treatment of certain dysfunctions of the central nervous system (e.g., epilepsy). Inhibition of carbonic anhydrase in this area appears to retard abnormal, paroxysmal, discharge from central nervous system neurons. The diuretic effect of acetazolamide is due to its action in the kidney on the reversible reaction involving hydration of carbon dioxide and dehydration of carbonic acid[8]. The result is renal loss of HCO3 ion, which carries out sodium, water, and potassium. Alkalinization of the urine and promotion of diuresis are thus affected. Alteration in ammonia. Acetazolamide shows dose related side effects, the most common of which are diuresis, gastrointestinal (GI) symptoms including cramping, epigastric burning, nausea, and diarrhoea and metabolic acidosis[9].

Acetazolamide is available in two forms (free base and sodium salt), free base is used in oral formulations such as immediate release tablets and extended release capsules, whereas, sodium salt is used in parenteral injection formulation. Acetazolamide exists in two polymorphic forms (form I and II), form I has slightly higher solubility and dissolution than form II, however, it is reported that there is no significant difference in bioavailability and/or clinical response[7]. Acetazolamide is a poorly soluble compound and has pH dependent solubility across the physiological pH range of 1.2 to 7.4. It has maximum solubility of above 2 mg/ml at pH above 7. The drug has negative log P value and has poor permeability in Caco2 cell line studies. Acetazolamide is classified as a BCS Class IV drug in a biopharmaceutical classification system based on the solubility and permeability data. However, some research shows that no firm conclusion could be obtained based on the available solubility and permeability data[7]. Despite poor solubility and permeability properties, the orally administered acetazolamide is completely absorbed from gastrointestinal tract[7]. The Martindale reports acetazolamide to be fairly rapidly absorbed with peak plasma concentrations occurring about 2 h after oral doses. The human first order absorption rate constant is reported to be 0.821 h. The drug has plasma half-life of 100 min and volume of distribution of the drug is about 20% of the body weight. About 80% of the drug is excreted by tubular secretion of the anionic species, and 70-90% of the administered dose is recovered unchanged within 24 h. The plasma concentrations of acetazolamide are proportional to dose, fall in the therapeutic range, and the drug can be detected for 6–12 h after a single dose administration. Food intake does not appear to influence absorption. Usual therapeutic serum acetazolamide concentration range is 10–20 mg/mL (for glaucoma 4–5 mg/mL), with variations in response from patient to patient[7]. The toxic plasma concentration for acetazolamide ranges from 25–30 mg/ml. According to the FDA definition for narrow therapeutic index, acetazolamide is a narrow therapeutic index drug, since there is a less than twofold difference between the highest reported toxic concentration (30 mg/mL) and the highest reported therapeutic plasma concentration (20 mg/mL)[7]. Kunka and Mattocks reported that acetazolamide follows a linear relationship between the AUC and the dose after intravenous bolus injections of ^{14}C-labeled acetazolamide, with doses ranging from 2 to 20 mg/kg, in rabbits[10].

Acetazolamide extended release capsules 500 mg are designed to provide prolonged action of inhibition of aqueous humour secretion for 18 to 24 h after each dose, whereas immediate release tablets act for only 8 to 12 h. This prolonged release effect of extended release capsules permit reduction in dosing frequency[11]. The reported oral bioavailability for acetazolamide extended release capsules 500

mg is 30-60%, when compared with suspension formulation. The peak plasma drug concentration achieves after 4 h of administration and the calculated elimination half-life is about 16 h. The observed peak plasma drug concentration is about 5-6 h with the inter subject variability of 26%. When co-administered with high fat breakfast, acetazolamide has increased the rate and extent of absorption with delayed Tmax[11]. However, there is no significant change in bioavailability and clinical response of acetazolamide extended release capsules 500 mg, when co-administered with food. Acetazolamide extended release capsule 500 mg is used in the treatment of chronic simple (open-angle) glaucoma, secondary glaucoma, and preoperatively in acute angle-closure glaucoma where delay of surgery is desired in order to lower intraocular pressure and is also indicated for the prevention or amelioration of symptoms associated with acute mountain sickness despite gradual ascent[11]. The in-silico and experimental physicochemical and biopharmaceutics properties are listed in Table 2. The below Table 2 summarizes the input parameters for PBPK model development

Table 2; Physicochemical and biopharmaceutics properties[7]

SN	Parameters	Values
1	Molecular weight	222.245
2	Log P	-0.26 - 1.13 (Clog P) 0.14 - 0.73 - 0.26 - 1.25 (Clog P)
3	Log D	-0.85
4	pKa	Strong acid: 7.2 Strong base: 6.93 (ChemAxon)[12] Strong base: - 3.3 (ChemAxon)[12] Strong base: 7.13 (Optimized based pH dependent solubility model) Strong base: 0.2 (Optimized based pH dependent solubility model)
5	Protein binding	% unbound – 10-30% % unbound – 5-10 % % unbound – 4.4 %
6	Solubility	6.47 mg/ml (ALOGPS)[12] Water (@25 °C) – 0.7 mg/ml pH 1.68(@25 °C) – 1.26 mg/ml pH 3.19(@25 °C) – 1.08 mg/ml pH 4.01(@25 °C) – 1.17 mg/ml pH 4.98(@25 °C) – 0.80 mg/ml pH 5.27(@25 °C) – 0.87 mg/ml pH 5.47(@25 °C) – 0.82 mg/ml pH 6.06(@25 °C) – 0.89 mg/ml pH 6.85(@25 °C) – 1.01 mg/ml pH 8.17(@25 °C) – 2.79 mg/ml pH 1.2(@37 °C) – 1.23 mg/ml

		pH 7.2(@37 °C) – 4.13 mg/ml pH 7.4(@37 °C) – 2.43 mg/ml
7	Particle size (micron)	D_{10}: 8.0 µ; D_{50}: 20 µ; D_{90}: 38 µ
8	Blood to plasma ratio	1.0[13]
9	Mean precipitation time	900 s
10	Drug particle density	1.2 g/ml (ADMET Predictor)
11	Diffusion co-efficient	0.75 x 10^{-5} cm^2/s (ADMET Predictor)
12	Polymorphism	Form I and II. The solubility and dissolution rate of Form I at 37 °C is about 1.1 times greater than those of Form II. This small relative difference in solubility is not presumed to significantly affect the bioavailability of acetazolamide.
13	Permeability	Caco2 – 0.2 x 10^{-6} cm/s[7] Caco2 – 0.5 x 10^{-6} cm/s[7]
14	ASF model	Opt log D Model SA/V 6.1(ADMET Predictor)
15	Duodenal solubility	2.12 mg/ml (Calculated by Gastroplus)
16	First pass effect	Not reported
17	Enterohepatic circulation	Negligible
18	Biliary excretion	Insignificant
19	PK linearity	Yes

3.2 Intravenous pharmacokinetic data modelling and Validation

Mean plasma concentration over time profile from IV PK data has been chosen for the optimization process. In the digitizing process, the web-browser-based digitizing software WebPlotDigitizer was used to extract numerical PK data from the plots. The demographic characteristics of the subjects such as age, body weight, sampling times and methodology was obtained from the literature. IV PK data were used to screen various compartmental and PBPK models using Gastroplus 9.7 software. The best-fit model was chosen and subjected to further validation and application. Table 3 summarizes the PK studies selected from literature for model building and validation.

Table 3: Summary of pharmacokinetic studies of acetazolamide under fasting condition

Sr No.	Study Objective	Subjects/ Route	Dose	Mean Age (year)	Mean Body weight (kg)	Sex	Sample size (n)	Reference
1	Influence of advanced age on disposition of acetazolamide	Healthy, IV bolus	325 mg	34.7	67	Male/ Female	4	14
2	Relative bioavailability of acetazolamide tablets	Healthy, Oral Solution, IR Tablets of different lots	250 mg	21-31	70-90	Male	4	15

3	Bioavailability of acetazolamide tablets	Healthy, oral IR tablets of 5 different lots	250 mg	21-40	70	Male	20	16
4.	Comparative bioavailability studies	Healthy, Oral ER capsules (test vs RLD)	500 mg	NA	NA	Male/ Female	39	17
5	Comparative bioavailability studies	Healthy, Oral ER capsules (test vs RLD)	500 mg	28	60	Male/ Female	36	18

3.3 Oral Pharmacokinetic data modelling and validation

Oral PK data reported from literature were used to build PK models. The similar procedure was used to extract the numerical PK data from plots. The demographic parameters such as age, sex, race and body weight were used to build the oral PK model. The distribution and elimination rate constants obtained from the validated IV PK model used as a basis to build oral PK data from literature. Opt log D Model SA/V 6.1 is used as an absorption scale factor for human fasted and fed state physiology. The advanced Compartmental Absorption Transit (ACAT) model is used for gastrointestinal PBPK and whole body PBPK and/or compartmental PK for predicting plasma drug concentration using Gastroplus 9.7 software. The pharmaceutical characterization data such as particle size distribution of API, drug particle density and drug substance solubility and dissolution profile of dosage form were incorporated.

3.4 Pharmaceutical properties of Acetazolamide Extended Release capsules 500 mg

Acetazolamide extended release capsules 500 mg consist of sustained release drug matrix pellets, which are encapsulated in hard gelatin capsules. Acetazolamide extended release capsules 500 mg of both reference and test products are manufactured using extrusion spheronization technology. Microcrystalline cellulose is used as matrix forming agent and sodium lauryl sulfate as wetting agent. The other pharmaceutical characteristics such as assay, related substance, pellet size and dissolution studies (release media and multimedia) are comparable for both test and reference products. The particle size distribution of input API, process impact on API particle size and API particle size in reference products is unknown.The API particle size in the test product is measured, monitored and controlled.

3.5 Bioequivalence studies for acetazolamide extended release capsules 500 mg

The study design consists of open-label, balanced, randomized, single-dose, two-treatment, two-sequence, two-period, two-way crossover bioequivalence study of acetazolamide extended release capsules 500 mg, (Test) with Diamox Sequels® (acetazolamide extended release capsules 500 mg, manufactured by Duramed Pharmaceuticals Inc, New York, USA (Reference) in healthy, adult, male and female, human Subjects under fasting condition. The primary objective of this study is to investigate the bioequivalence of test and reference formulation by means of rate and extent of absorption, in healthy, adult, male human subjects under fasting condition with at least seven days'

washout period between each administration. The secondary objective is to monitor the safety of the participating Subjects in this study determined by means of clinical biochemistry, physical examination and adverse drug reaction and serious adverse monitoring.

3.6 Simulation trials for acetazolamide extended release capsules 500 mg

A two-treatment, two-sequence, randomized, single-dose, crossover virtual bioequivalence trial simulation with PK endpoints in healthy subjects under fasting condition was conducted to mimic the in vivo BE study design. Groups of 36 virtual subjects were generated with demographics as close as possible to those expected in the in vivo BE studies in terms of age and gender of the subjects. The oral PBPK model development and validation was not possible because of variability in bioavailability study outcomes. Moreover, pharmaceutical factors such as disintegration time, dissolution profile, aging of the formulation and particle size of input drug substance particles are key elements, which influence the model parameters recorded. The bioavailability studies were conducted with less number of subjects (most frequently with 4 subjects). Acetazolamide has 25% intra subject CV and the study outcomes are not reliable because of low power of the study. However, the PBPK model was developed with in house pharmaceutical data and pharmacokinetic data with adequate statistical power. The dosage form selection for developing PBPK models is based on dosage form type, nature and drug release behaviour. CR integral was selected as a dosage form model because drug dissolution and release kinetics is predominantly governed by drug solubility in the pellet matrix. The solubilisation of the drug embedded matrix and subsequent erosion of the matrix results in drug release from dosage form. However, there is no evidence of complete dispersion of the pellets, subsequent disintegration of the pellets and release of the drug from undissolved particles. However, the transit time of the dosage form for matrix tablets are different from the pellet dosage forms. The pellets have longer residential time in small intestine and large intestine, and moreover, the drug pellet size, density are the factors, which are influencing the intestinal residential time, in vivo drug release and pharmacokinetic properties of the dosage forms[19,20]. Considering the pharmaceutical factors, the gastrointestinal physiology was optimized in order to get the best fit profile (Table 04). Moreover, the model consists of Johnson dissolution model, wherein in vitro drug release profile for both test and reference products obtained from release media was used to build the PBPK model. The test and reference products were subjected with 36 and 100 subject crossover bioequivalence trials and the observed values and predicted values are compared to calculate the % prediction error. Based on the prediction error, the PBPK model is further used to predict the pharmacokinetic profile of test product with different dissolution specifications.

Table 04; Fasting state gastrointestinal transit time: inbuilt vs optimized

GIT Compartments	Inbuilt Transit time (h)	Optimized Transit time (h)
Stomach	0.25	0.25
Duodenum	0.26	0.28
Jejunum 1	0.92	1.00
Jejunum 2	0.73	0.90

Ileum 1	0.57	0.90
Ileum 2	0.41	0.70
Ileum 3	0.29	0.41
Caecum	4.02	4.0
Ascending Colon	12.07	14.0

3.7 Virtual bioequivalence trials for acetazolamide extended-release capsules 500 mg

The validated PBPK model for acetazolamide extended-release capsules 500 mg is used for virtual bioequivalence trials with different dissolution profiles such as test product, reference product, approved profile (target), approved profile (lower limit), approved profile (upper limit), proposed profile 1 (lower limit) and proposed profile 2 (lower limit). There is a minor difference in composition between test and reference products. However, the quantitative difference between test and reference products have no significant influence on biopharmaceutical properties of dosage forms, especially, dissolution profiles of test and reference are similar in not only release media but also other dissolution media as well. Pharmaceutical design quality and characteristics are similar between test and reference products. Virtual bioequivalence studies for acetazolamide extended-release capsules 500 mg were performed with 36 and 100 subjects. The study design was conserved as randomized, single-dose, two-treatment, two-sequence, two-period, two-way crossover bioequivalence. The subject population groups (n=36 and n=100) were constant across the study, in order to minimize the population effect. The study details are listed in table 05.

Table 05; Details of virtual bioequivalence studies

Study ID	Test Product	Reference product	Number of subjects
1	Target Dissolution	Test	36
2	Upper limit Dissolution	Test	36
3	Approved lower limit dissolution	Test	36
4	Proposal 1 lower limit dissolution	Test	36
5	Proposal 2 lower limit dissolution	Test	36
6	Target Dissolution	Test	100
7	Upper limit Dissolution	Test	100
8	Approved lower limit dissolution	Test	100
9	Proposal 1 lower limit dissolution	Test	100
10	Proposal 2 lower limit dissolution	Test	100

3.8 Statistical Analysis

Statistical analysis was performed using GastroPlus 9.7 software. The statistical method considered was average bioequivalence with standard design as single-dose, two-treatment, two-sequence, two-period, two-way crossover for bioequivalence virtual BE simulation and the in vivo BE trial. Bioequivalence was established using pharmacokinetic metric parameters such as Cmax, AUC0-t and AUC0-∞ to the logarithmic scale. The 90% confidence intervals for geometric mean ratios of test and

reference within the limit of 80% to 125% is the statistical criteria for bioequivalence acceptance.

4 Results and discussion

4.1. Physicochemical, biopharmaceutics and pharmacokinetics (model development)

Acetazolamide is a predominantly weakly acidic compound with pH dependent poor solubility with pka of 7.2. However, the pH dependent solubility has limited role in in vivo solubilisation and bioavailability because the impact of pH on solubilisation with the physiological pH is insignificant. The solubility data obtained from the literature is used to build the PBPK model. The pH dependent solubility curve obtained from literature data and predicted pKa values are illustrated in figure 1. Acetazolamide is poorly lipophilic and hence lower log P, log D and in vitro permeability. Despite its poor solubility and permeability (considered as a BCS class IV molecule), it is a good absorption profile form human gastrointestinal tract. The selection of the other physicochemical and biopharmaceutical properties such as protein binding, blood to plasma ratio, mean precipitation time and diffusion coefficient were based on either *in silico* method or experimental values obtained from literature. The duodenal solubility of 2.120 mg/ml was obtained and the theoretical solubilisation ratio of 97.60. The experimental particle size data listed in table 8 was used for extended release dosage forms. However, the effect of nanoparticle solubility, effect of bile salts on diffusion coefficients were ignored because of the instant dissolution model. The biliary excretion of the drug is insignificant and entero hepatic cycle mode was not used to build the model. The reported pharmacokinetic linearity across the therapeutic dose range. The Opt log D Model SA/V 6.1 model was used for absorption scale factor. Table 6 illustrates model input parameters.

Table 6; Physicochemical and biopharmaceutics properties

SN	Parameters	Values
1	Molecular weight	222.245
2	Log P	No
3	Log D	-0.85
4	pKa	Strong base: 7.13 (Optimized based pH dependent solubility model) Strong base: 0.2 (Optimized based pH dependent solubility model)
5	Protein binding	% unbound – 4.4 %
6	Solubility	pH 1.2(@37 °C) – 1.23 mg/ml pH 7.2(@37 °C) – 4.13 mg/ml pH 7.4(@37 °C) – 2.43 mg/ml
7	Particle size (micron)	D_{10}: 8.0 µ; D_{50}: 20 µ; D_{90}: 38 µ
8	Blood to plasma ratio	1.0^{6}
9	Mean precipitation time	900 s
10	Drug particle density	1.2 g/ml (ADMET Predictor)
11	Diffusion co-efficient	0.75×10^{-5} cm^2/s (ADMET Predictor)
12	Polymorphism	No significance, hence polymorphic data is not used to build the model
13	Permeability	Caco2 – 0.5×10^{-6} cm/s[7]

14	ASF model	Opt log D Model SA/V 6.1(ADMET Predictor)
15	Duodenal solubility	2.12 mg/ml (Calculated by Gastroplus)
16	First pass effect	Not reported
17	Enterohepatic circulation	No
18	Biliary excretion	No
19	PK linearity	Yes

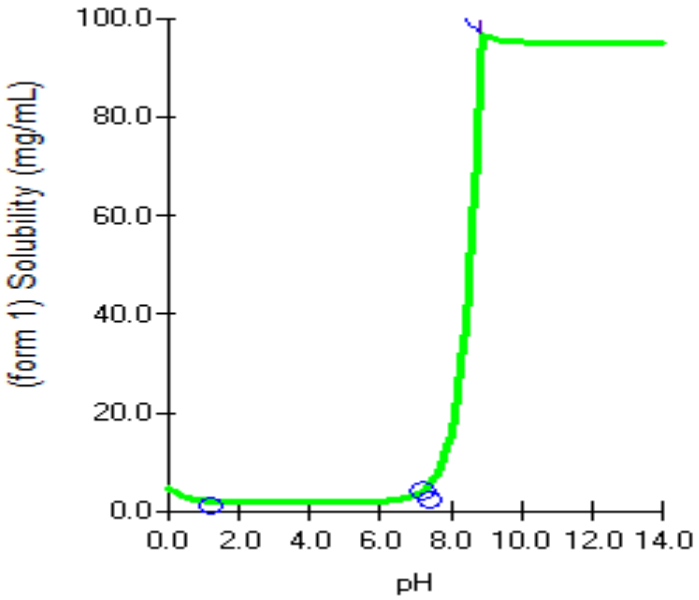

Figure 1; pH dependent solubility and fitted pKa values

4.2. Intravenous pharmacokinetic model development and validation

Intravenous pharmacokinetic data obtained from the literature was used to build the pharmacokinetic model and the developed model was further validated. Acetazolamide exhibits age dependent metabolism and clearance The elderly subjects have two-fold higher area under plasma drug concentration than healthy young male subjects and hence the pharmacokinetic study involved in young healthy male subjects was considered for pharmacokinetic model building. The disposition kinetics of acetazolamide after intravenous administration was fitted with various empirical compartment models and three compartment model was chosen based on the model validation and prediction analysis based on the default selection by Gastroplus software. Table 7 illustrates the pharmacokinetic parameters for modelling and simulation. The intravenous pharmacokinetic model validation data represented in table 8. Figure 2 and 3 illustrate the plasma drug concentration time profiles of intravenous pharmacokinetic model.

Table 7; Pharmacokinetic model parameters

PK parameters	Model output	PK parameters	Model output
Model	3 Compartmental	K_{12} (1/h)	1.0978

B/P ratio	1.0	K_{21} (1/h)	1.0215
Adj. Plasma % Fup	4.3998	V_2 (1/kg)	0.10397
Cl; (L/h)	2.182	K_{13} (1/h)	0.11617
Vc (L/kg)	0.09674	K_{31} (1/h)	0.06588
$T_{1/2}$ (h)	15.48	V_3 (1/kg)	0.17059

Table 8: Intravenous pharmacokinetic model validation

PK metrics	**Dose: 325 mg, IV bolus Single simulation**			**Dose: 325 mg, IV bolus n=12**		
	Digitalized	**Predicted**	**% PE**	**Observed**	**Predicted**	**% PE**
Cmax (ng/ml)	50.79	50.90	-0.22	-	51.0	-
AUC_{0-i} (ng.h/ml)	156.97	148.68	5.28	-	140.2	-
AUC_{0-t} (ng.h/ml)	153.4	144.56	5.76	140.8	136.0	3.41

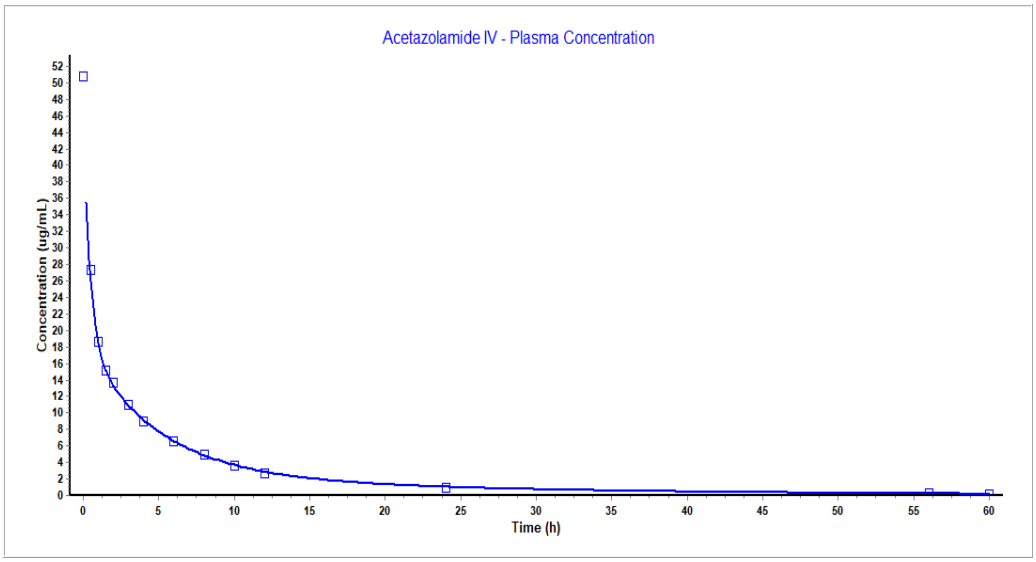

Figure 2; Single simulation of Intravenous PK profile (digitalized vs predicted)

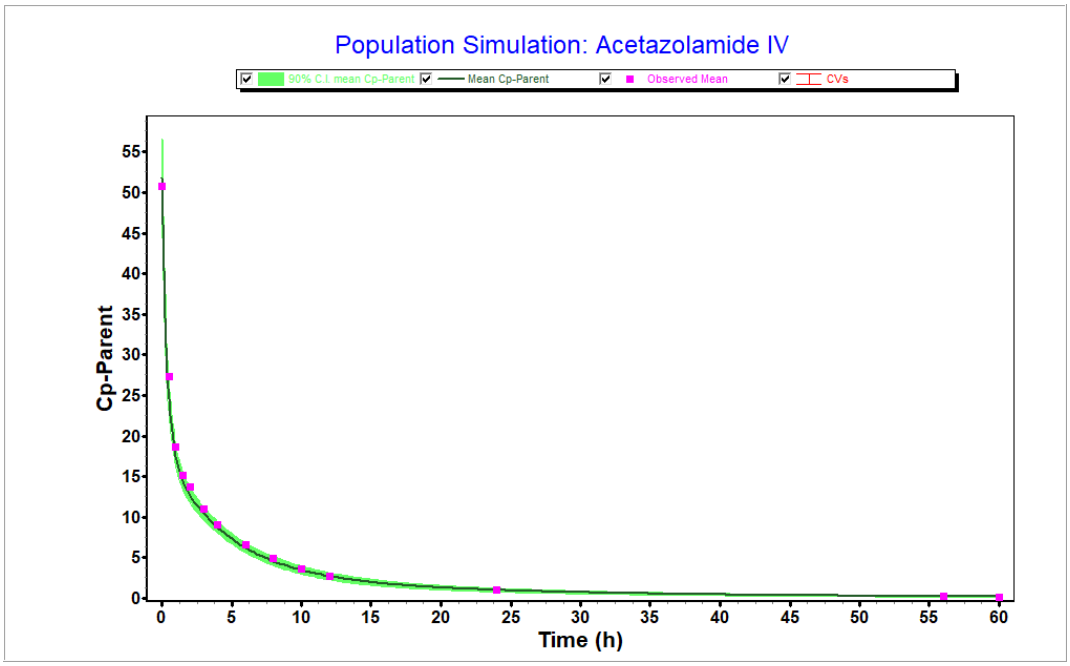

Figure 3; Simulations of Intravenous PK profile (digitalized vs predicted); n=12

4.3 Oral Pharmacokinetic data analysis for immediate release products

Oral pharmacokinetic data for acetazolamide immediate release tablets 250 mg were collected from various literature and summarized in table 5. The pharmacokinetic parameters of oral pharmacokinetic data obtained from literature is represented in table 9. The lot to lot variability in bioavailability of acetazolamide immediate release tablets 250 mg were reported in literature. The possible reasons are formulation factors and/or aging of the drug products. Several investigators examined the lot to lot variability of acetazolamide immediate release 250 mg products and established in vitro in vivo

correlation using disintegration and dissolution as surrogate markers for predicting the in vivo drug release and bioavailability. The peak plasma concentration ranged from 13.0 µg/ml to 21.5 µg/ml were observed with several lots of drug products administered in healthy subjects. This 40 percent difference was significant ($p < 0.05$) with one product having a lower peak concentration than all the other products. The solution exhibited a significantly higher peak concentration than immediate release tablets. The variability in the peak concentration was least for the solution with 19%, compared to a range of 27 % to 36 % for the tablets. A study by Yakatan et al, using five lots of acetazolamide tablets 250 mg and the observed peak concentrations ranging from 6.9 µg/ml to 11.4 µg/ml as compared to 13.0 µg/ml and 17.6 µg/ml for two lots from that manufacturer in the another study. Yakatan et al showed peak acetazolamide concentrations of 16-17 µg/ml with a 250 mg dose administered to two subjects, while Wallace found peak concentrations ranging from 10 to 18 µg/ml in a study of 5 subjects. Based on the study of Freedland et al report, the clinical significance of the observed differences in peak plasma concentrations may be minimal. These workers found that 63 mg oral doses of acetazolamide suspension, which resulted in peak plasma concentrations of 4-5 µg/ml, were as effective in lowering intraocular pressure. However, all these observations are from single dose studies, and the results might vary with chronic administration especially in clinical settings. Alm et al. determined the steady state plasma concentrations of acetazolamide in 40 patients after doses of 187.5, 375, 750, and 1000 mg. They found that mean plasma concentrations increased with increasing dosages but there were marked inter individual variations. The above stated literature review limits the development of reliable and validated model for acetazolamide immediate release tablets 250 mg. Especially, the vast difference in Cmax values between lots have limited the to develop the gastro intestinal permeability model for immediate release tablets. Hence, it was decided further to develop the pharmacokinetic model for acetazolamide extended release capsules 500 mg from the in house study.

Table 9; Oral Pharmacokinetics of acetazolamide[15, 16]

Formulation description	Cmax (µg/ml)	AUCt (µg.h/ml)	AUCi (µg.h/ml)	Tmax (h)	Subjects (n)
Oral solution, 250 mg (B.No IV solution Lot No 490376, Lederele lab)	21.5	102.4	113.0	0.6	12
250 mg IR tablets (Lot No: 485-636, Lederele lab)	13.0	100.7	113.9	2.4	12
250 mg IR tablets (Lot No: 6A001A, Warner)	20.2	114.5	114.5	1.0	12
250 mg IR tablets (Lot No: 485-586, Lederele lab)	17.6	115.5	115.5	2.0	12
250 mg IR tablets* (Lot No: 396-341, Brand: Diamox)	6.24	82.7	93.5	3.0	11
250 mg IR tablets* (Lot No: 401-351, Brand: Diamox)	8.79	91.73	108.18	2.06	11
250 mg IR tablets* (Lot No: 4469-680, Brand: Diamox)	9.0	95.8	105.6	4.15	12

250 mg IR tablets* (Lot No: 4469-324, Brand: Diamox)	11.2	101.4	116.8	1.86	12
250 mg IR tablets* (Lot No: 4469-475, Brand: Diamox)	7.8	84.7	105.6	2.9	12

* plot digitized data, all values are represented as mean

4.4 Pharmaceutical properties of acetazolamide extended release capsules

In vitro dissolution testing in release media is one of the critical quality attributes for acetazolamide extended release capsules. The dissolution method and profiles of test and reference products are shown in table 10. The dissolution data is screened for various dissolution models, particularly for Weibull models. Single Weibull model was selected based on the goodness of fit. Figure 4 depicts the observed vs modelled dissolution data. Dissolution modelled data was used for pharmacokinetic modelling and simulation of extended release capsules. The particle size of drug substance used to fabricate the test product is used as input parameter for the development of PBPK model and simulation for both test and reference products. Other pharmaceutical properties such as density, diffusion coefficients and diffusion layer thickness were obtained from default value used in gastroplus software. The dissolution specifications used in the PBPK model for virtual bioequivalence studies are shown in table 10.

Table 10; Mean dissolution profiles used for PBPK modelling and virtual bioequivalence

Time (h)	Test Mean (%)	RLD Mean (%)	Approved Specification			Proposed Specification	
			Target(%)	LSL(%)	USL(%)	LSL (%) – P1	LSL (%) – P2
1	29	31	30	20	40	20	20
2	46	49	47.5	35	60	35	35
5	78	80	77.5	70	85	65	67
12	102	101	100	85	NA*	85	85

* for modelling purpose, it is considered as 100%

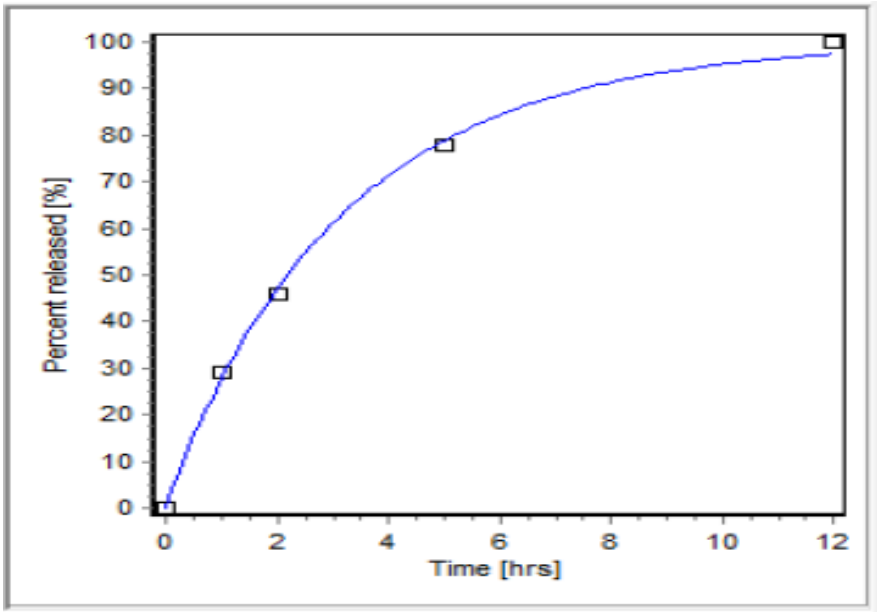

Figure 4 Observed vs modelled dissolution data

4.5. Bioequivalence studies for acetazolamide extended release capsules

The results of fasting state bioequivalence studies between test and reference product is summarized in table 11. The bioequivalence study result reveals the both test product and reference product meets bioequivalence criteria. Moreover, the study is adequately powered with enough subjects. The pharmacokinetic data obtained from bioequivalence study is used to build the PBPK model development and validation.

Table 11, Statistical summary of comparative bioavailability fasting data

PK parameters	Test	Reference	Geometric mean % T/R	90% LCI	90% UCI
AUC_{0-t} (ng.h/ml)	126026.80	120439.05	104.64	95.59	114.54
$AUC_{0-\infty}$ (ng.h/ml)	129391.09	124477.68	103.95	95.23	113.47
Cmax (ng/ml)	7619.26	7581.42	100.50	91.41	110.49

4.6 Simulation trials for acetazolamide extended release capsules 500 mg

The Advanced Compartmental Absorption and Transit (ACAT) model was used to predict the gastrointestinal absorption of acetazolamide extended release capsules 500 mg . The validated pharmacokinetic model obtained from intravenous pharmacokinetic data is used to build the disposition pharmacokinetics. The developed model was validated using the pharmacokinetic data obtained from the fasting state bioequivalence study of test and reference products. Pharmacokinetic simulations were performed using two sets of subject population (n=36; n=100) and the results are illustrated in figures 5 a-d. Virtual bioequivalence studies (n=36; n=100) were conducted between test and reference formulations (figure 6a and b). Table 12 a, b summarizes the pharmacokinetic parameters and geometric mean % T/R ratio of observed values and predicted values in 36 subjects virtual bioequivalence data, respectively. Table 13 summarizes the predicted pharmacokinetic parameters in 100 subjects virtual bioequivalence data The prediction errors for the test product is well within the 10 % for both Cmax and AUC (geometric mean and %T/R ratios). But, the reference product prediction for AUC is slightly higher (10.6 %) than the regulatory limit of 10%. However, virtual bioequivalence trials were performed using the test formulation and hence slightly higher prediction error of reference product has no impact on the virtual bioequivalence trials outcomes.

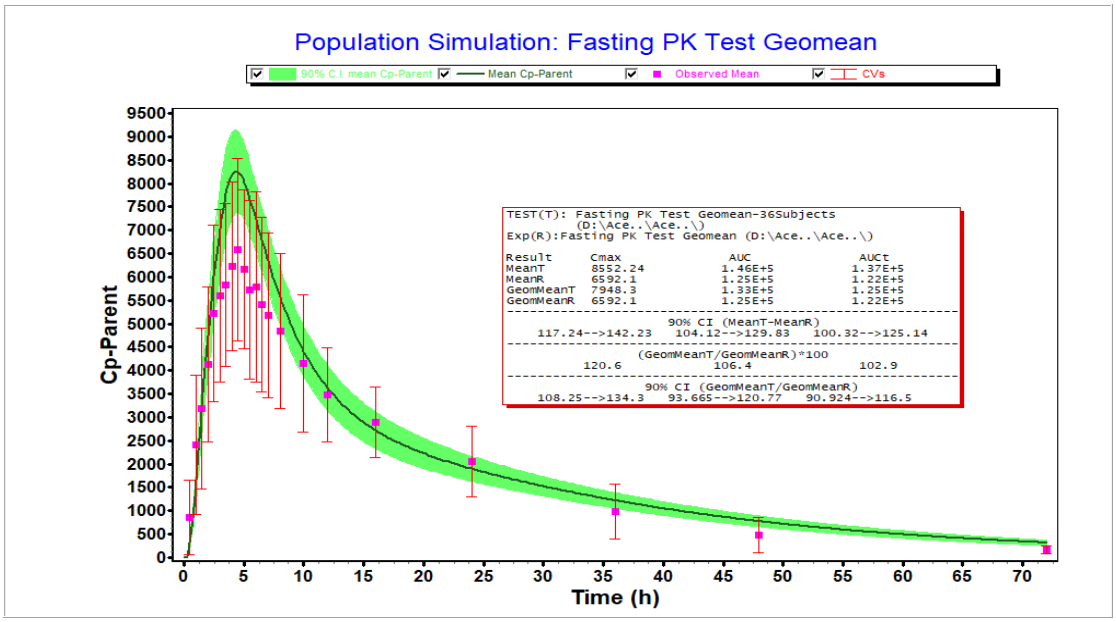

Figure 5a: PBPK modelling and simulation of test product observed vs predicted (n=36)

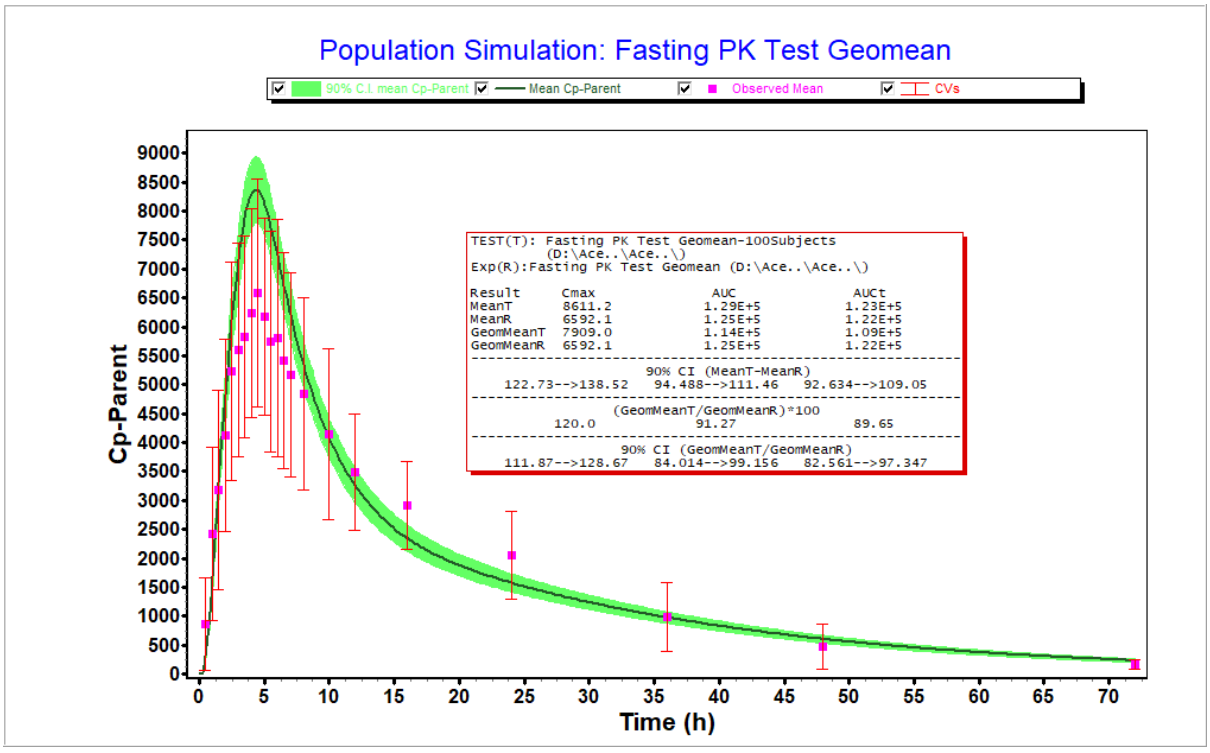

Figure 5b: PBPK modelling and simulation of test product observed vs predicted (n=100)

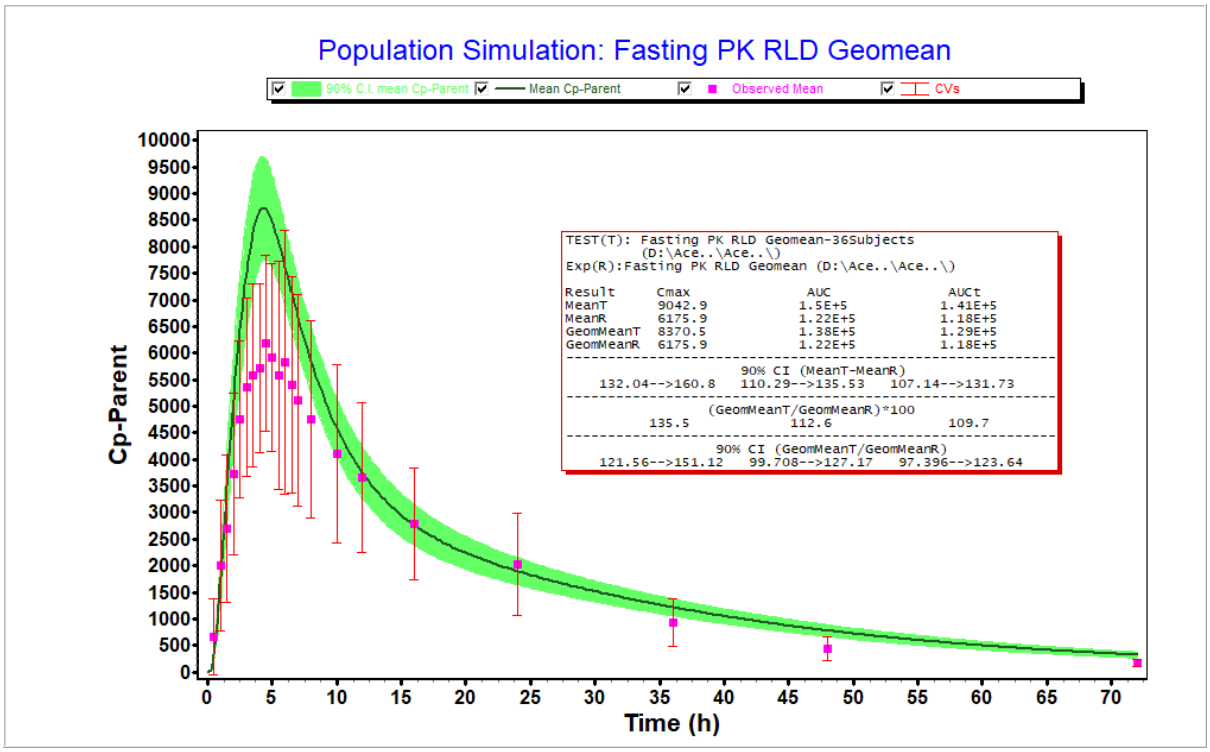

Figure 5c: PBPK modelling and simulation of reference product observed vs predicted (n=36)

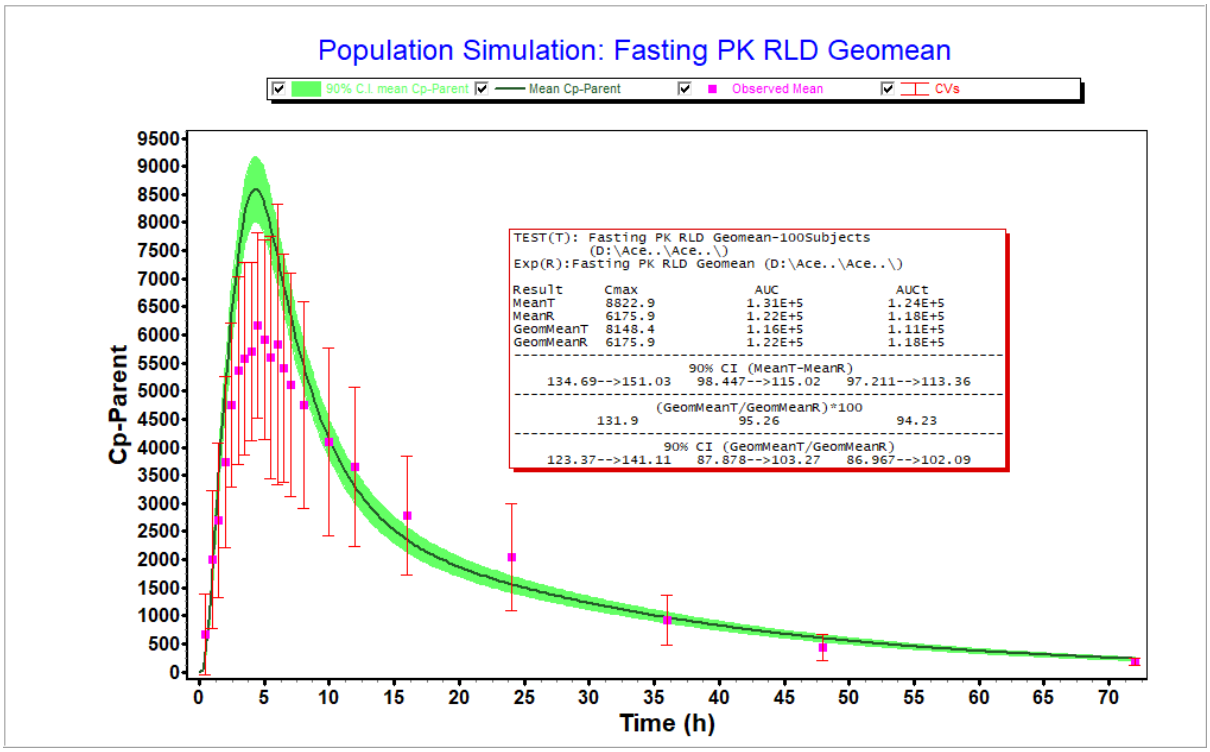

Figure 5d: PBPK modelling and simulation of reference product observed vs predicted (n=100)

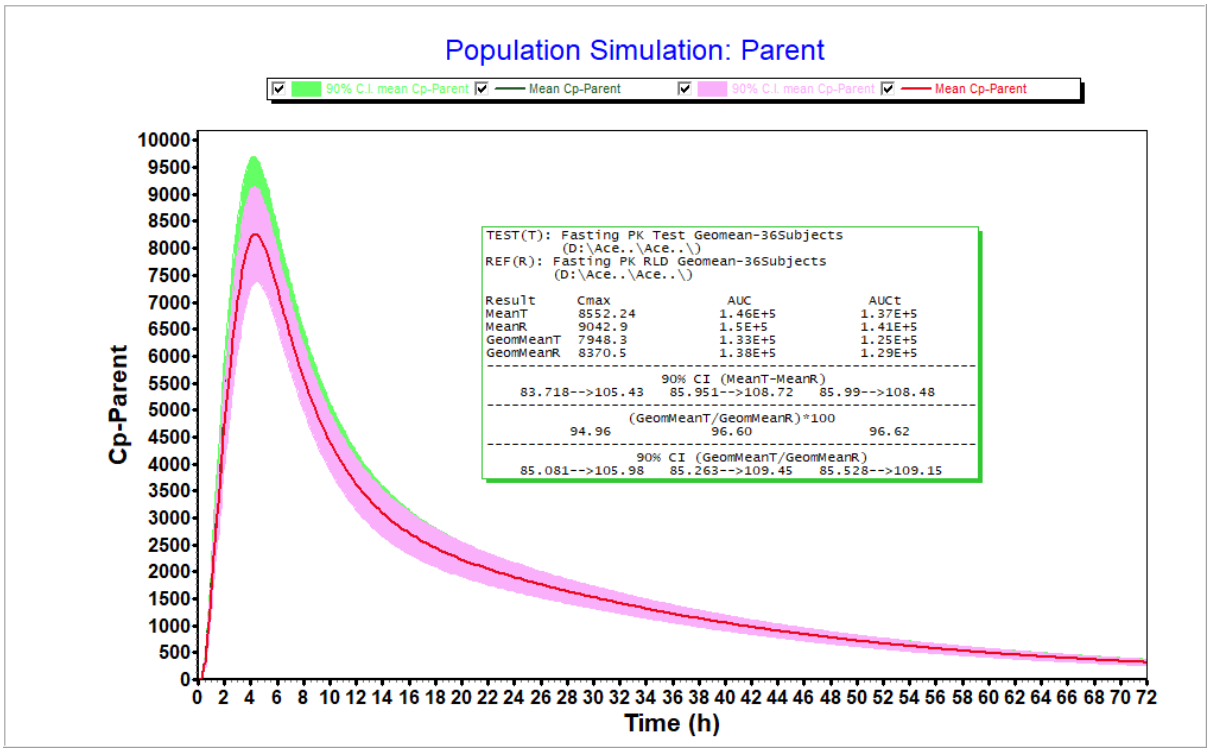

Figure 6a: Fasting state virtual bioequivalence test vs RLD (n=36)

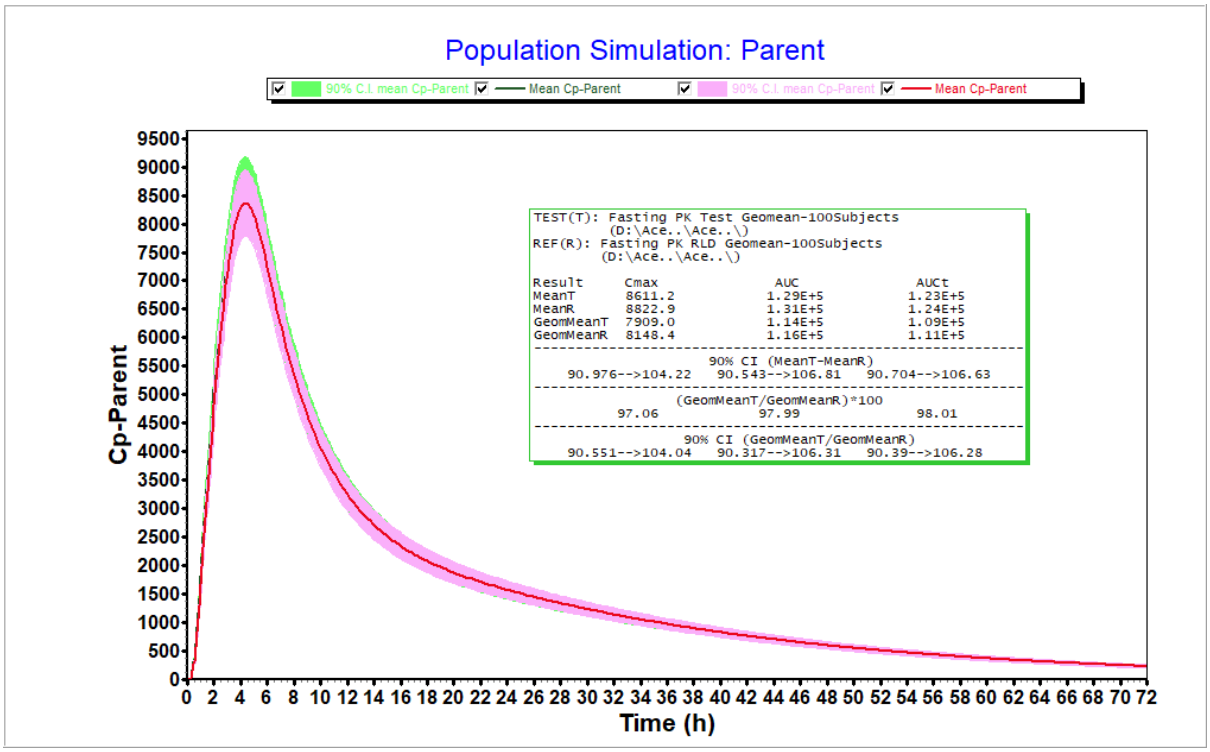

Figure 6b: Fasting state virtual bioequivalence test vs RLD (n=100)

Table 12 a; Statistical summary of fasting pharmacokinetics parameter predicted vs observed (n=36)

PK parameters	Test (Observed)	Test (Predicted)	% Prediction error	Reference (Observed)	Reference (Predicted)	% Prediction error
AUC_{0-t} (ng.h/ml)	126026.80	125100	0.7	120439.05	129400	-7.4
$AUC_{0-\infty}$ (ng.h/ml)	129391.09	133000	-2.8	124477.68	137700	-10.6
Cmax (ng/ml)	7619.26	79483	-4.3	7581.42	8370.5	-10.4

Table 12 b; Statistical summary of fasting pharmacokinetics parameter predicted vs observed (n=36)

PK Parameter	Cmax			AUC_{0-t}			$AUC_{0-\infty}$		
Statistical Parameters	Observed	Predicted	% Prediction error	Observed	Predicted	% Prediction error	Observed	Predicted	% Prediction error
% Geometric mean	100.5	94.96	5.5	104.64	96.62	7.7	103.95	96.6	7.1
90% LCI	91.41	85.08	6.9	95.59	85.53	10.5	95.23	85.26	10.5
90% UCI	110.49	105.98	4.1	114.54	109.15	4.7	113.47	109.45	3.5

Table 13; Statistical summary of comparative virtual bioavailability fasting data (n=100)

PK parameters	Test	Reference	Geometric mean % T/R	90% LCI	90% UCI
AUC_{0-t} (ng.h/ml)	109000	111000	98.01	90.4	106.3
$AUC_{0-\infty}$ (ng.h/ml)	114000	116000	97.99	90.3	106.3
Cmax (ng/ml)	7909	8148.4	97.06	90.5	104.0

4.7 Virtual bioequivalence trials for acetazolamide extended release capsules 500 mg

The plasma drug concentration profiles obtained from virtual bioequivalence are illustrated in figures 7a –h. Table 14a and b shows the predicted geometric mean ratios with confidence intervals for both simulations with 36 and 100 subjects, respectively. When virtual bioequivalence was carried out with 36 subjects, the 90% CI for upper confidence is outside the regulatory limits and both proposed lower

specifications are below the limit of accepted 90% confidence. This is probably due to the high variability of acetazolamide mg extended release capsules 500 . However, virtual bioequivalence studies with 100 subjects and confidence intervals are well within the limit, except for a few values. The targeted profile % T/R ratio 99.8%, whereas 90% UCI for upper specification limit is 126.6% and 90% LCI for proposed 1 lower specification limit is 78.7%. This could be due to high variability of Cmax, when compared with the rest of bioequivalence pharmacokinetic parameters. Further increasing the sample size, PK metric parameters could fall within the confidence interval of the assigned specifications. The confidence intervals for all the pharmacokinetic parameters are well within the limit for proposed 2 lower specifications. Hence, revising the dissolution specifications with proposed lower specification 2 would result in bioequivalent products with safety, efficacy and quality. Even with proposed lower limit 1 could be adopted as revised dissolution specification without compromising safety, efficacy and quality.

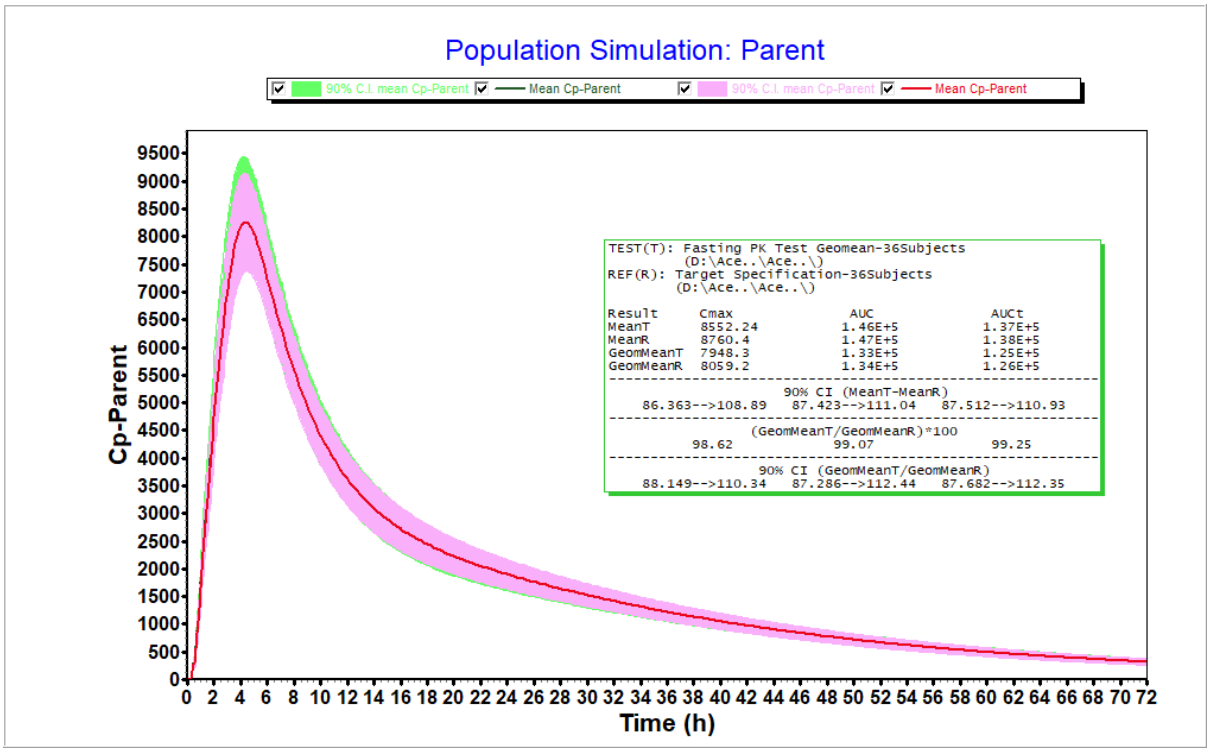

Figure 7a: Fasting state virtual bioequivalence test vs target specification (n=36)

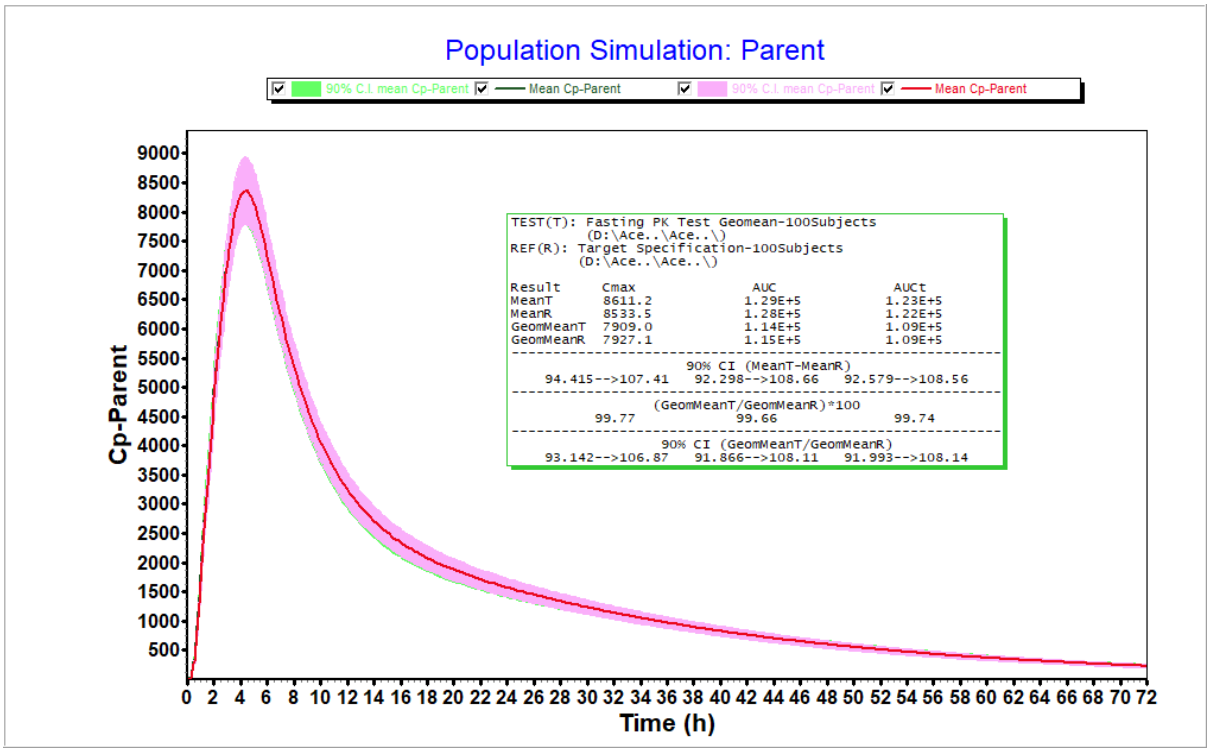

Figure 7b: Fasting state virtual bioequivalence test vs target specification (n=100)

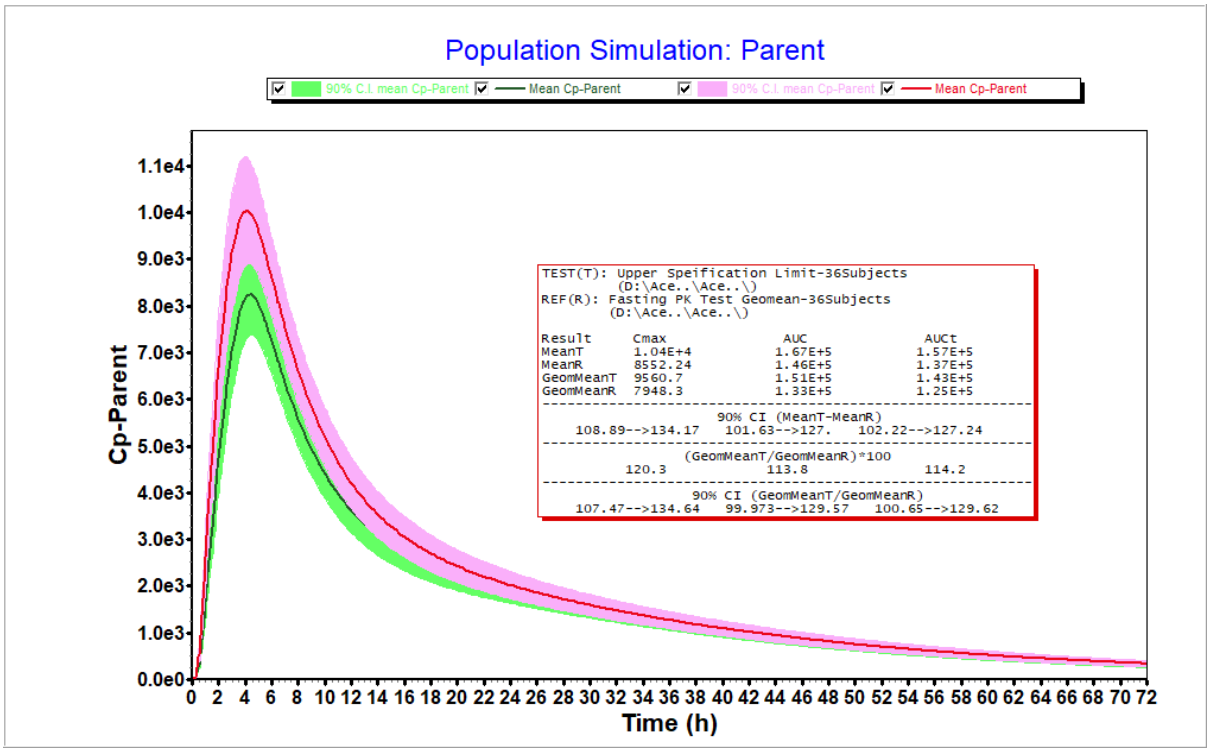

Figure 7c: Fasting state virtual bioequivalence test vs Upper limit specification (n=36)

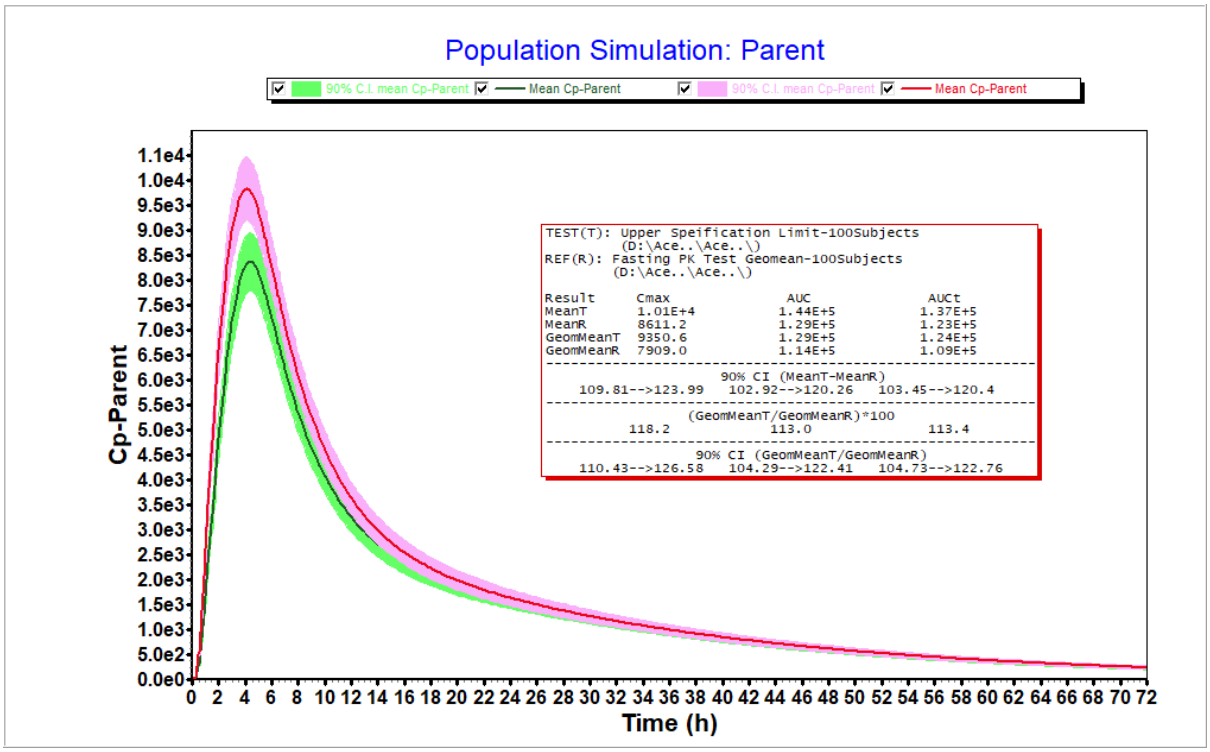

Figure 7d: Fasting state virtual bioequivalence test vs Upper limit specification (n=100)

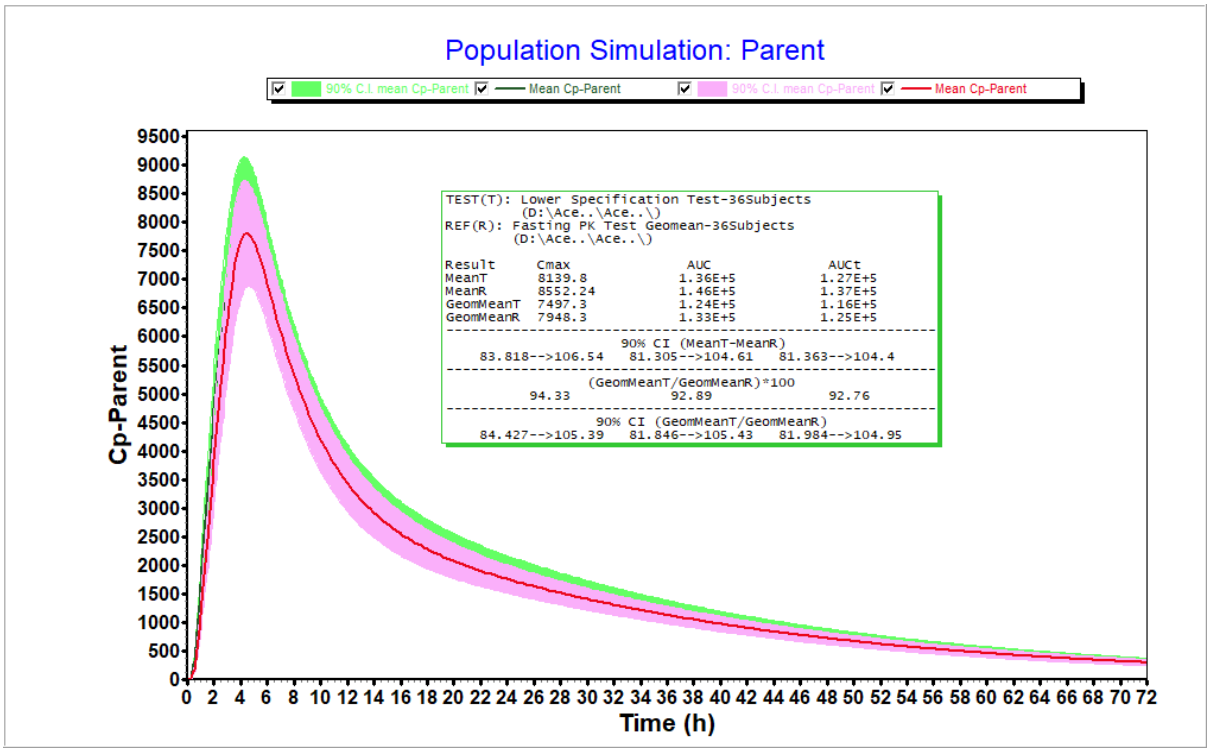

Figure 7e: Fasting state virtual bioequivalence test vs Lower limit specification (n=36)

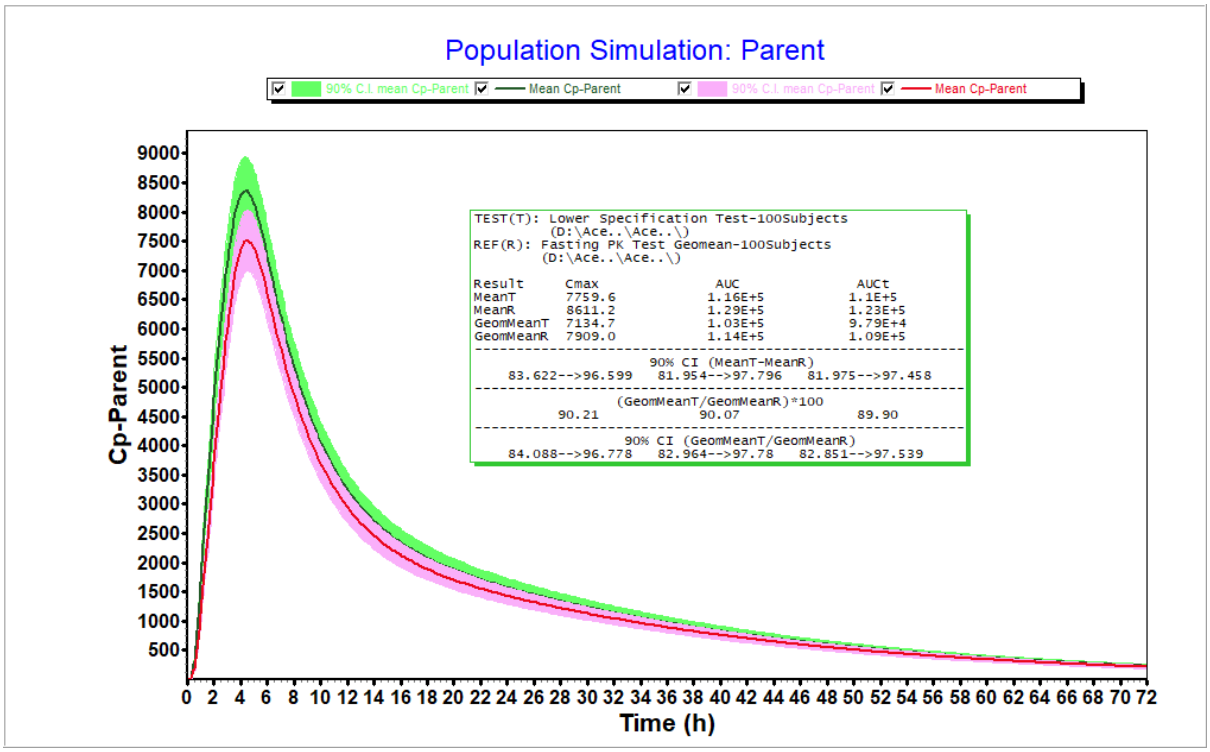

Figure 7f: Fasting state virtual bioequivalence test vs Lower limit specification (n=100)

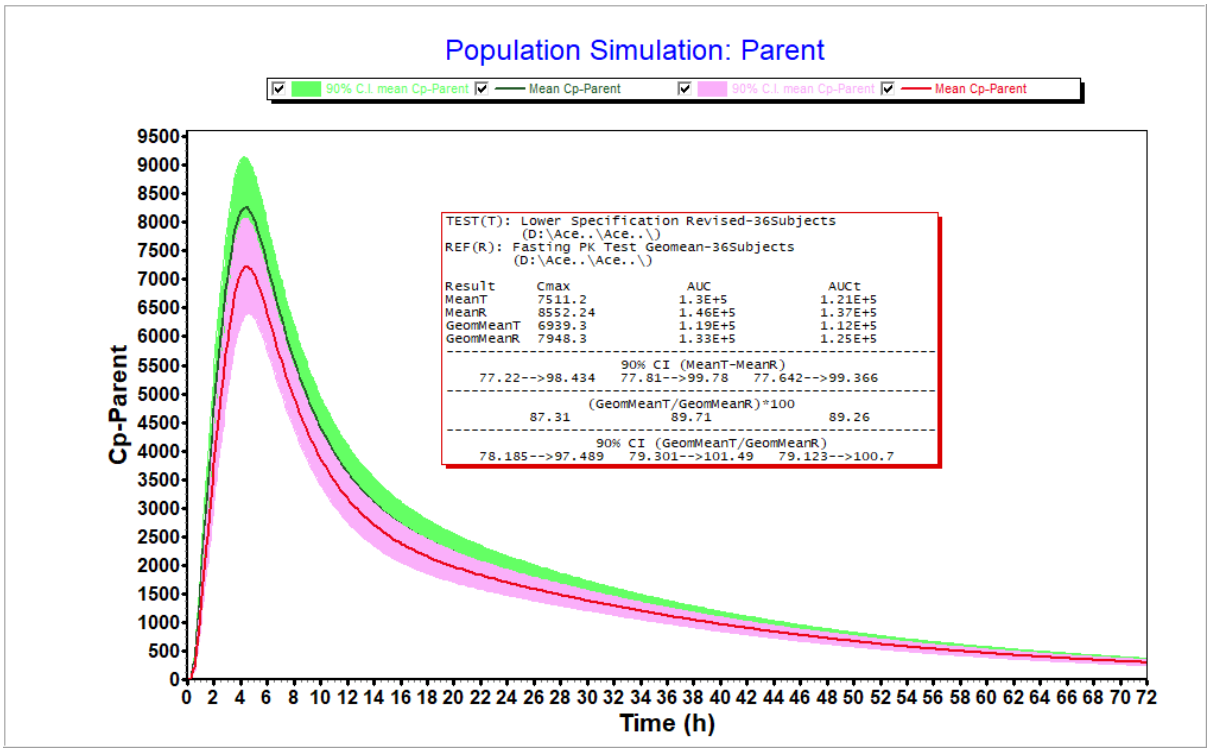

Figure 7g: Fasting state virtual bioequivalence test vs Proposed (1) Lower limit specification

(n=36)

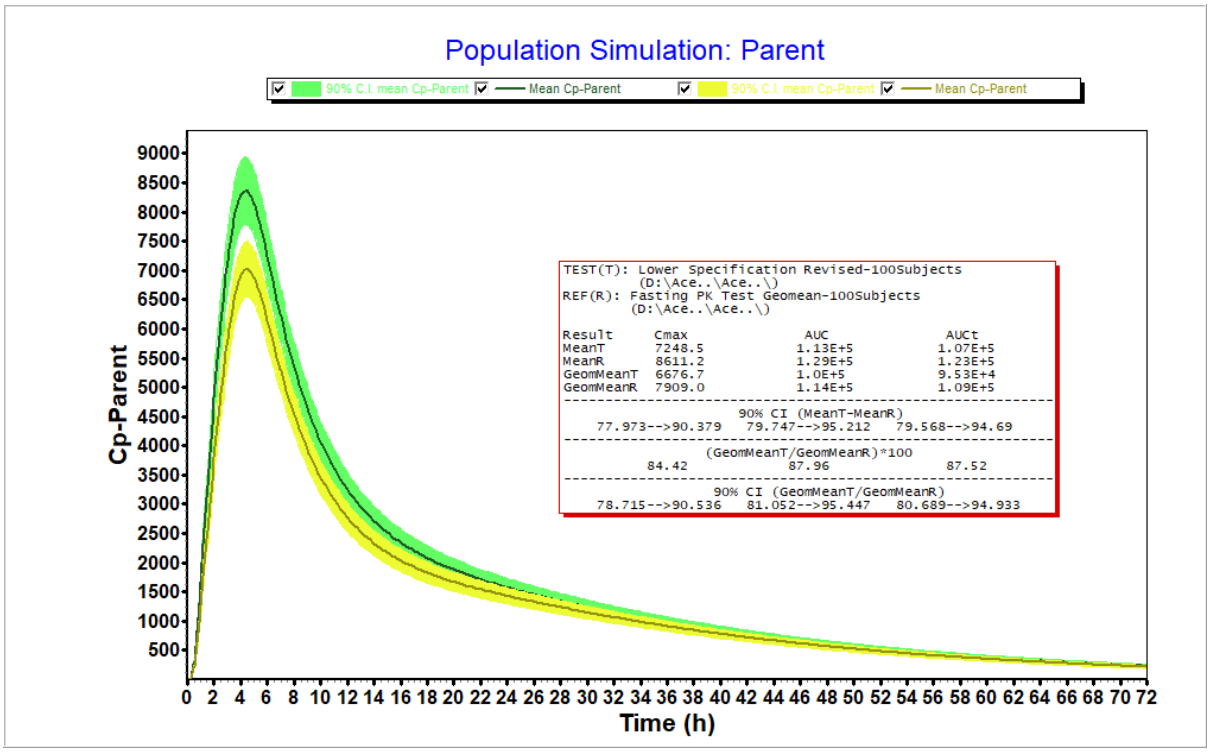

Figure 7h: Fasting state virtual bioequivalence test vs Proposed (1) Lower limit specification (n=100)

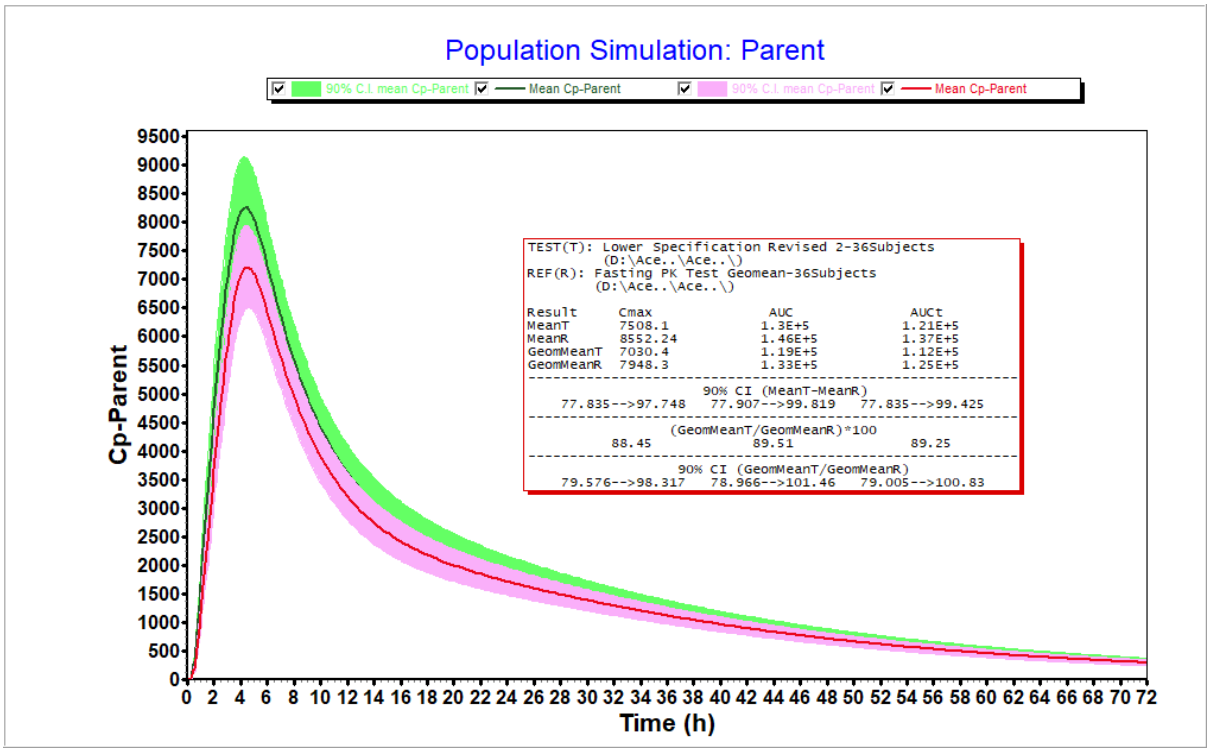

Figure 7i: Fasting state virtual bioequivalence test vs Proposed (2) Lower limit specification (n=36)

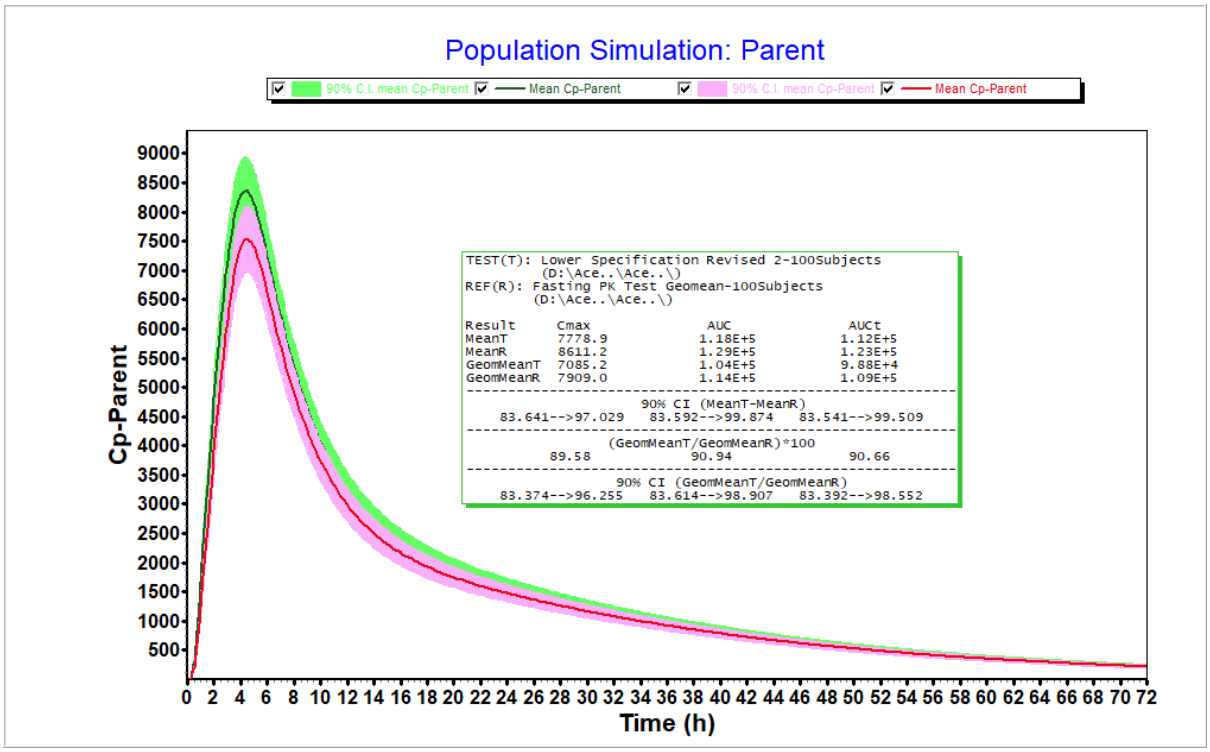

Figure 7j: Fasting state virtual bioequivalence test vs Proposed (2) Lower limit specification (n=100)

Table 14 a; Statistical summary of virtual BE data from different dissolution specification (n=36)

Specifications	Upper Spec.			Target Spec			Lower Spec.			Lower Spec. Proposal 1			Lower Spec. Proposal 2		
PK parameters	% T/R	90% LCI	90% UCI	% T/R	90% LCI	90% UCI	% T/R	90% LCI	90% UCI	% T/R	90% LCI	90% UCI	% T/R	90% LCI	90% UCI
Cmax (ng/ml)	120.3	107.5	**134.6**	98.6	88.1	110.3	94.3	84.4	105.4	87.3	**78.2**	97.5	88.5	**79.6**	98.3
AUC0−∞ (ng.h/ml)	113.8	99.9	**129.6**	99.1	87.3	112.4	92.9	81.8	105.4	89.7	**79.3**	101.5	89.5	**78.9**	101.5
AUC0-t (ng.h/ml)	114.2	100.6	**129.6**	99.3	87.7	112.4	92.8	81.9	104.9	89.3	**79.1**	100.7	89.3	**79.0**	100.8

Bold values are outside the regulatory limit

Table 14 b; Statistical summary of virtual BE data from different dissolution specification (n=100)

Specifications	Upper Spec.			Target Spec			Lower Spec.			Lower Spec. Proposal 1			Lower Spec. Proposal 2		
PK parameters	% T/R	90% LCI	90% UCI	% T/R	90% LCI	90% UCI	% T/R	90% LCI	90% UCI	% T/R	90% LCI	90% UCI	% T/R	90% LCI	90% UCI
Cmax (ng/ml)	118.2	110.4	**126.6**	99.8	93.1	106.9	90.2	84.1	96.9	84.4	**78.7**	90.5	89.5	83.4	96.3

AUC0−∞ (ng.h/ml)	113.0	104.2	122.4	99.7	91.9	108.1	90.1	82.9	97.8	87.9	81.1	95.4	90.9	83.6	98.9
AUC0-t (ng.h/ml)	113.4	104.7	122.7	99.7	91.9	108.1	89.9	82.9	97.5	87.5	80.7	94.9	90.7	83.4	98.6

Bold values are outside the regulatory limit

5. Summary

The present study demonstrates the fasting state PBPK model development for acetazolamide extended release capsules 500 mg using in vitro dissolution data. The developed model was validated using pharmacokinetic data obtained from the bioequivalence study. The proposed dissolution specifications (especially for lower specification limit at 5 h) along with approved dissolution specification was used to predict the pharmacokinetic profile as well as establish the bioequivalence with already approved product. Both dissolution specifications were meeting the BE criteria (n=100) and hence proposed dissolution specifications could be adopted without compromising the safety, efficacy and quality of the drug product. We may choose the desired dissolution specification (either proposal 1 or 2) of acetazolamide extended release capsules 500 mg based on the above stated biopharmaceutics and PBPK modelling and simulation data supported with virtual BE outcomes.

References

1. Technical and regulatory considerations for pharmaceutical product life cycle management, Q12, Final version, adopted on November 2019, ICH harmonized guideline, International council for harmonization of technical requirements for pharmaceuticals for human use. Downloaded from: https://database.ich.org/sites/ default/files/Q12_Guideline_Step4_2019_1119.pdf
2. The use of physiological based pharmacokinetic analyses – Biopharmaceutical applications for oral drug product development, manufacturing changes, and control, Guidance for Industry, Draft guidance issued on October 2020 and published by Center for Drug Evaluation and Research, Food Drug Administration, U. S. Department of Health and Human Services. Downloaded from: https://www.fda.gov /media/142500/download
3. Physiological based pharmacokinetic analyses – Format and content, Guidance for Industry, Draft guidance issued on August 2018 and published by Center for Drug Evaluation and Research, Food Drug Administration, U. S. Department of Health and Human Services. Downloaded from: https://www.fda.gov/media/101469/download
4. Diamox® Sequels® (Acetazolamide extended release capsules) Label, Downloaded from: https://www.accessdata.fda.gov/drugsatfda docs/label/2005/12945s037,038 lbl.pdf
5. Acetazolamide: Mechanism of action, Downloaded from: https://www. openanesthesia.org/ acetazolamide _mechanism_of_action/
6. Active ingredient search of acetazolamide in orange book, Downloaded from: https://www.accessdata.fda.gov/scripts/cder/ob/search_product.cfm
7. G. E. Granero, M. R. Longhi, C. Becker et.al. Biowaiver monographs for immediate release solid oral dosage forms: acetazolamide, 2008, J. Pharm. Sci., 97, 9, 3691-8.
8. https://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=85975cf0-1286-4af9-ac7f-94bb2483bf09&type=display

9. C. N. Schmickl, R. L. Owens, J. E. Orr et.al. Side effects of acetazolamide: a systematic review and meta-analysis assessing overall risk and dose dependence, 2020, BMJ Open Resp. Res., 7, e0005557, 1-13.
10. R. L. Kunka, A. M. Mattocks, Nonlinear model for acetazolamide, 1979, J. Pharm. Sci., 68, 342–6.
11. https://www.rxlist.com/diamox-sequels-drug.htm
12. https://go.drugbank.com/drugs/DB00819
13. P. Poulin, F. P. Theil, Prediction of Pharmacokinetics Prior to In Vivo Studies.1. Mechanism-Based Prediction of Volume of Distribution, 2002, J. Pharm. Sci., 91, 01, 129-156.
14. D. J. Chapron, K. A. Sweeney, P. U. Feig et.al, Influence of advanced age on the disposition of acetazolamide, 1985, Br. J. Clin. Pharmac., 19, 363-371.
15. A. B. Straughn, R. Gollamudi, M. C. Meyer. Relative bioavailability of acetazolamide tablets, 1982, Biopharm. Drug Disposit., 3, 75-82
16. G. J. Yakatan, E. L. Frome, R. G. Leonard et. al. Bioavailability of acetazolamide tablets, 1978, J. Pharm. Sci., 67, 2, 252-256
17. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/012945Orig1s038.pdf
18. In-house BE study, data available in ANDA file
19. H. Bechgaard, K. Ladefoged. Distribution of pellets in the gastrointestinal tract. The influence on transit time exerted by the density or diameter of pellets, 1978, J. Pharm. Pharmacol., 30, 11, 690-2.
20. G. M. Clarke, J. M. Newton, M. B. Short. Comparative gastrointestinal transit of pellet system of varying density, 1995, Int. J. Pharm., 114, 1, 1-11.