

Bioequivalence Study Followed by Model Informed Dose Optimization of a Powder for Oral Suspension of 6-Mercaptopurine

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Abstract

AIMS 6 mercaptopurine (6MP) is the mainstay chemotherapy for acute lymphoblastic leukaemia (ALL) and is conventionally available as 50 mg tablets. This study aimed to evaluate the bioequivalence of a new 6MP Powder for Oral Suspension (PFOS) intended for paediatric use. Additionally, a virtual study with the obtained data was planned for determining a dose of the PFOS that matches tablet exposures and to confirm optimal drug levels in pediatrics. **METHODS** An open-label, randomized, two-treatment, two-period, two-sequence, single oral dose, crossover, bioequivalence study was conducted on 51 healthy subjects. A population pharmacokinetic (PopPK) model was developed using the data to perform simulations with various PFOS doses and select a bioequivalent dose. To simulate 6MP and 6 thioguanine (6TGN) exposures in pediatrics, a literature model for paediatric ALL patients, and allometrically scaled PK parameters were utilised. **RESULTS** The 6MP PFOS had 47% higher bioavailability compared to the reference product. Simulations using a two-compartmental PopPK model with dissolution and transit compartments showed that 40 mg of PFOS was found to be equivalent to the 50mg tablets. The simulated 6TGN concentrations in virtual paediatric patients were between 114 and 703.6 pmol/8x10⁸ RBCs, which was within the range of values reported in paediatric ALL studies. **CONCLUSION** The study demonstrates that 40 mg dose of 6MP PFOS 10 mg/mL has the same extent of absorption as the 50 mg tablet which can be precisely administered in pediatrics. The study also demonstrates the role of modelling and simulation to perform virtual bioequivalence and paediatric studies.

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ABSTRACT

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METHODS

An open-label, randomized, two-treatment, two-period, two-sequence, single oral dose, crossover, bioequivalence study was conducted on 51 healthy subjects. A population pharmacokinetic (PopPK) model was developed using the data to perform simulations with various PFOS doses and select a bioequivalent dose. To simulate 6MP and 6 thioguanine (6TGN) exposures in pediatrics, a literature model for paediatric ALL patients, and allometrically scaled PK parameters were utilised.

RESULTS

The 6MP PFOS had 47% higher bioavailability compared to the reference product. Simulations using a two-compartmental PopPK model with dissolution and transit compartments showed that 40 mg of PFOS was found to be equivalent to the 50mg tablets. The simulated 6TGN concentrations in virtual paediatric patients were between 114 and 703.6 pmol/8x10⁸ RBCs, which was within the range of values reported in paediatric ALL studies.

CONCLUSION

The study demonstrates that 40 mg dose of 6MP PFOS 10 mg/mL has the same extent of absorption as the 50 mg tablet which can be precisely administered in pediatrics. The study also demonstrates the role of modelling and simulation to perform virtual bioequivalence and paediatric studies.

What is already known about this subject:

- 50mg tablet formulation of 6 Mercaptopurine is extensively used in acute lymphoblastic leukaemia (ALL) in both children and adults.
- Significant differences in 6 Mercaptopurine exposures and response necessitate dosage adjustments.
- Suspensions are generally absorbed more readily than solid formulations, as they do not require disintegration.

What this study adds:

- Demonstrates an equivalent dose of a new 6MP liquid formulation to tablets.
- Emphasise the need to switch to formulations that enable precision dosing.

- Highlights the role of modelling and simulation in dose optimisation.

INTRODUCTION

Mercaptopurine is a prodrug of thioguanine, a purine analogue that antagonizes endogenous purines and inhibits RNA and protein synthesis in the S- phase of the cell cycle. The Food and Drug Administration (FDA) has authorized the use of mercaptopurine (6MP) as a component of combination therapy for acute lymphoblastic leukaemia (ALL) in both children and adults [1]. With the introduction of 6MP, complete remission to the tune of 90% has been reported in paediatric ALL, the most prevalent childhood cancer worldwide [2] [3]. The Institute for Safe Medication Practices (ISMP) has listed 6MP as a drug with narrow therapeutic index and has classified it as having a heightened risk of causing significant harm to the patient when used inappropriately [4] [1].

Oral 6MP has low and variable bioavailability, less than 20% on average, due to high first-pass metabolism in the intestinal mucosa and liver. The plasma concentrations exhibit significant inter- and intra-patient variability [5-8]. The oral formulation of 6MP is currently available as 50 mg tablets in India, while liquid suspension of 20 mg/mL is available in the United States and Europe. Dosing 6MP using the tablet formulation poses several challenges, particularly in children, owing to smaller body surface area. Splitting the tablet and/or alternate day dosing are often practiced to achieve the prescribed 6MP dose [9], although such manipulations are fraught with the potential for medication errors. Besides, the National Institute for Occupational Safety and Health (NIOSH), and the American Society of Health-System Pharmacists (ASHP) clearly identify crushing as an unsafe practice with a potential for increasing the risk of cytotoxic drug exposure to the preparator [10]. Additionally, factors such as the inability to swallow tablets, drug palatability, and giving crushed tablets mixed with various foods may also alter 6MP disposition in children [11]. To overcome these limitations, and to enable accurate dosage adjustments with age, genetic polymorphisms, body weight, body surface area, adverse outcomes, and so on, a powder for oral suspension (PFOS) dosage form of 6MP was developed.

The pharmacokinetics and the extent of absorption must be comparable to the tablet in order to consider the new formulation as an alternative option. The purpose of this study was to evaluate the pharmacokinetic equivalence between the PFOS of 6MP 10 mg/mL (Test) and Mercaptopurine USP Tablets 50mg (Reference) in healthy adult male subjects. Based on the pharmacokinetic profiles, the study also aimed to establish the optimal dose of the new formulation which would match the 50 mg reference tablet formulation in the extent of absorption within the BE criteria using a model-based approach and intended to extrapolate the exposure of the suggested dose in paediatric patients using an existing model in the literature.

METHODS

Bioequivalence Study

Subjects and study design

Eligible study participants were healthy male subjects aged between 18 to 45 years with a BMI of 18.5 to 30.0 kg/m². An informed consent form was obtained from all the study participants and the study was conducted in accordance with the Declaration of Helsinki and ICH-GCP guidelines. Important exclusion criteria included known hypersensitivity to 6MP, presence of TMPT polymorphisms, and consumption of xanthine-containing products. One-week preceding entry into the study. The study was designed as an open-label, balanced, randomized, single-dose, two-treatment, two-sequence, two-period, cross-over study (Figure 1). The test formulation was powder for oral suspension (PFOS) of 6MP 10 mg/mL (Dose: 5ml=50 mg) manufactured by IDRS Labs Private Ltd., India. The reference formulation was, Mercaptopurine USP tablets 50 mg (Dose: 50mg). The sample size was estimated to be 54 and eligible subjects were randomized using SAS 9.4 (North Carolina, United States). A 7-day washout interval was allowed between the two periods to ensure complete elimination of the administered drug. Subjects were fasted overnight for at least 10 hours before the scheduled time of dosing. A single oral dose of 5 mL of Oral Suspension i.e., Test Product (T) was administered orally using a graduated syringe in sitting posture and one tablet of the

reference product (R) was administered orally with 240 mL of water.

Determining the plasma 6MP concentration

Blood samples (5ml) were collected in pre-labelled vacutainers containing K₃EDTA as anticoagulant at pre-dose (0 h) and 0.08, 0.17, 0.25, 0.33, 0.50, 0.75, 1.00, 1.33, 1.67, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00 and 20.00 hours following drug administration in each period. The samples were centrifuged and stored at $-80 \pm 8^{\circ}\text{C}$ at the clinical site until analysis. A validated LC-ESI-MS/MS bioanalytical method was developed for the quantification of 6MP. The calibration curve extended from 1.0 ng/mL to 400.0 ng/mL. The intra- and inter-batch precision (% coefficient of variance [CV]) and mean accuracy (% bias) ranged from 1.75 to 3.85 and -1.69 to 2.13, respectively.

Data analysis plan

Test of bioequivalence

6MP pharmacokinetic parameters such as C_{max}, AUC_{0-t}, AUC_{0-∞}, T_{max}, AUC_% Extrap_obs, t_{1/2}, and Kel were calculated using plasma concentration versus time profile data of the investigational products in individual subjects using Non-Compartment Model of Phoenix WinNonlin(r) version 8.2 (Certara USA, Inc., Princeton, NJ). The ln-transformed pharmacokinetic parameters were analysed by analysis of variance (ANOVA) using PROC GLM in SAS Software, Version 9.4. Bioequivalence was concluded if the 90% CI of the GMR for the AUC_{0-t} and AUC_{0-∞} of 6MP was entirely within the bioequivalence limits of 0.80 – 1.25 (80.00% - 125.00%). C_{max} was not considered for establishing bioequivalence as the traditional suspension formulations have achieved higher peak plasma concentrations compared to solid dosage forms.

Development of a population model to evaluate the bioequivalent dose of the test formulation

The model-informed assessment followed various iterative steps as described in Figure 2.

Initially, a population pharmacokinetic model that best described the data of the clinical 6MP study was developed and validated. The developed model was then leveraged to perform clinical trial simulations using different doses of the test product to establish the optimal dose that has an identical drug exposure to that of the reference product. The model development, fitting as well as simulations were performed in Pumas^(r) version 2.0 (Maryland, USA).

Model development and validation

The study focused on developing an optimally parameterized PK model which can clearly capture the product characteristic as well as describe the observed clinical PK profiles accurately. Various compartmental models and the incorporation of product-specific characteristics such as in-vitro dissolution profiles (Supplementary Fig 1) of the test and reference products were tried iteratively to establish the best fit to the observed pharmacokinetic data.

The developed model was used to simulate the original clinical study of test and reference products in 51 virtual subjects and was assessed for comparability based on average bioequivalence. The confidence intervals of the Geometric mean ratio of C_{max} and AUC were derived and these results were compared with the corresponding BE results from observed clinical data for the reference and test product.

Sensitivity Assessment and selection of the optimal dose

The sensitivity of the model-based simulations was assessed based on its ability to distinguish the PK profiles between different doses. Since the doses of Mercaptopurine PFOS 10mg/mL are titrated based on body weight or surface area, exposure linearity can also be safely assumed within a 2-fold dose range. Therefore, only doses between 25 mg and 50 mg were simulated.

Doses of 25mg, 35mg, 37.5mg and 40 mg were simulated for the suspension in addition to the tablet dose of 50 mg. The simulations included the same pharmacokinetic variability of the Reference and Test products as observed in the clinical study. The simulated profiles for the test product corresponding to these doses

were compared with the simulated profile of Reference product and evaluated for the qualification of the BE criterion for the extent of absorption (AUC) and the dose of the 6MP PFOS which would match the exposure of the 6MP 50mg tablets was selected.

Extrapolation of PK of selected dose to pediatric ALL: Monte Carlo simulations in paediatric acute leukaemia patients using literature model

The PK established in healthy adults may not always reflect the PK profile in pediatrics with ALL. Thus, to confirm safe exposures of the 6MP test formulation in pediatrics, a simulation of 6MP and its metabolite exposures in children was performed by incorporating the formulation specific parameters derived from the healthy volunteer study and the available literature model. A literature search was performed using the search strategy; Mercaptopurine [MeSH] OR Mercaptopurine [tiab] OR "6-mercaptopurine" [tiab] AND Leukemia [MeSH] OR Leukemia [tiab] OR "Acute lymphoblastic leukaemia" [tiab] AND Pharmacokinetics [MeSH] OR Pharmacokinetic* [tiab] OR "Population pharmacokinetic*" [tiab] AND Pediatrics [MeSH] OR Pediatric* [tiab] OR Children [tiab] in 'PubMed'. Similar search was carried out on 'Embase', 'Web of Science' and 'Google Scholar' databases. The studies published in English before November 2022 that reported the PK of 6MP in paediatric patients with ALL were considered satisfactory. One population pharmacokinetic model for 6MP and its metabolites in pediatrics that evaluated the PopPK in 19 children with ALL with 150 plasma concentrations was identified and used for carrying out the simulations [12].

Monte Carlo simulation of 6MP and 6 thioguanine (6TGN) exposures in children was performed with a fixed absorption rate constant from the dissolution compartment of the developed Poppk model. The model was parameterised using allometrically scaled apparent clearance and weight normalized apparent volume of distribution derived from the non-compartmental analysis. Other relevant model parameters for the formation of 6TGN were used from the literature as the changes in 6MP concentrations after the introduction of the test drug disposition parameters shall ultimately reflect in the metabolite levels. The body weight for allometric scaling and the height for calculating the body surface area were randomly generated from the CDC growth charts. The BSA corresponding to the body weight and height of an individual that was included by the author as a covariate for 6TGN clearance was calculated using the Dubois formula. A per m² dosing equivalent to the standard 6MP dose of 50mg/m² that is routinely initiated in pediatrics which happened to be close to the median doses of the identified pharmacokinetic studies was scaled down as per the previous model recommendation on dose reduction and used for the simulations. TPMT mutation that was included in the literature model was not included as the adult study excluded TPMT polymorphism and the relatively rare incidence of it [13]. Pumas^(r) version 2.0 (Maryland, USA) was used to perform the simulations for 100 paediatric patients aged between 2-18 years of age with the body weight and height randomly sampled from the CDC growth charts, using the parameters (Supplementary table1). The interindividual variability of 40% was used for the parameters obtained from the healthy volunteer study due to the expectation of high variability in the clinical context and 33% for 6TGN clearance that was reported in the literature study. 6-TGN exposures were simulated for 30 days and the recommended dose was verified and considered optimal based on the attainment of concentrations within similar ranges published in the literature.

RESULTS

Bioequivalence Study

Demographic characteristics

A total of 54 healthy, adult, male human subjects were enrolled in the study and 3 subjects were withdrawn from the study during period 2 (2 found positive for drug abuse and 1 did not report to the facility). Body weights of the subjects were within the limit of normal range according to age between 20 and 44 years (Both inclusive) with a body mass index between 18.87 to 29.58 kg/m² (Both inclusive) with a body weight of not less than 51.00 kg. The demographics of the included subjects are summarised in supplementary table 2.

Pharmacokinetic parameters

The mean pharmacokinetic parameters estimated for Reference product (R) and Test product (T) are depicted in Table 1. The geometric least squares mean of Test Formulation (T) and Reference Formulation (R), its ratio (T/R)%, intra-subject variability, 90% confidence intervals the Geometric least square mean ratio (T/R) and power obtained from the analysis of ln-transformed parameters AUC_{0-t} and $AUC_{0-[\infty]}$ are summarized in Table 2 The GMR (90% CI) of the AUC_{last} between the test and the reference formulations was 1.46 (1.37–1.55). Thus, the test product (6 Mercaptopurine 10 mg/mL (at a dose of 5 mL = 50 mg) did not meet the bioequivalence criteria as the bioavailability of suspension was 46% higher compared to the tablet.

Population Model Development

The population model fitting in the observed healthy volunteer study data revealed that a two-compartment pharmacokinetic model adequately described the pharmacokinetics of 6MP after numerous iterative steps. The compartment-based pharmacokinetic model fit indicated significantly different absorption rates for the Test and the Reference product. Therefore, the cumulative fraction dissolved from the in-vitro dissolution study was fitted to a first-order exponential function to assess the rate of dissolution specific to the products which were then incorporated as fixed parameters specific to the formulation in the model. The final PK model described the pharmacokinetics of Mercaptopurine PFOS 10mg/mL after oral dosing with a dissolution and transit compartment followed by two compartmental pharmacokinetics. The PK model parameterized the volume of distribution of central and peripheral compartments (denoted as V1 and V2 respectively), clearance parameters from the central as well as inter-compartment (cl and Q respectively), and a first-order absorption rate (k_a). Figure 3 describes the schematic representation of the PK model

The final model parameters for the PK model fit into the 51 subject clinical data for Test and Reference products along with their inter-individual variabilities are illustrated in Table 3. Diagnostic plots for the population PK fit for Reference and test product indicate an acceptable fit of the model. The diagnostic plots of the model fit for the reference and test products are presented in supplementary figures 2 and 3 respectively.

Replication of BE results for Test and Reference product

The model fitted population parameters were used to simulate the PK of the Test and Reference products in 51 virtual subjects. The model simulation results matched the observed clinical data closely. Figure 4 shows the observed vs simulated concentration-time profiles for the test and reference. The C_{max} and AUC values calculated from the actual data and simulated data were comparable with a ratio ranging from 0.78 to 1.02 (Table 4). The BE assessment results for the model simulated data matched the results observed using clinical data. The 90 % CI of the geometric means were comparable (Supplementary table 3).

Optimal Dose Selection

The comparison of a range of doses that i.e., 25, 30, 35, 37.5, 40 and 50 mg that were simulated, clearly demonstrated the ability of the model to distinguish the PK profiles of each dose. The summary of pharmacokinetic profiles of various doses of Test product in comparison to Reference is presented in supplementary table 4. Figures 5 and 6 show the simulated C_{max} and AUC for different doses. The BE assessment that was performed for pharmacokinetic simulation data for different test doses with simulated data for Reference product at 50 mg revealed that only 40 mg dose-based simulation qualified the BE criterion for the extent of absorption (AUC) while all other doses failed. The statistical summary of BE evaluation of 40 mg Test (suspension) vs. Reference (50 mg Tablet) is presented in Table 5.

Evaluation of the selected dose reduction in pediatrics with Acute lymphoblastic leukaemia.

Monte Carlo simulations were performed to confirm safe exposures of the 6MP PFOS formulation in children with ALL. The model that was deployed for simulations described the kinetic parameters of 6MP and

6TGN using the following equations where $tvka$, $tvcl$, $tvvc$, $tvfm$, $tvclm$ and $tkme$ represent the population parameters and their inter-individual variability $etas(\eta)$ for the respective individual parameters.

$$Ka = tvka * \exp(\eta)$$

$$CL = tvcl * (WT/70)^{0.75} * \exp(\eta)$$

$$Vc = tvvc * (WT/70) * \exp(\eta)$$

$$FM = tvfm * (2.56)^{TPMT}$$

$$CLm = tvclm * (BSA)^{1.16} * \exp(\eta)$$

$$Kme = tvkme$$

The ODEs used to describe the drug and metabolite disposition in the gut and blood are as follows

$$\text{Depot}' = -Ka * \text{Depot}$$

$$\text{Central}' = Ka * \text{Depot} - CL/Vc * \text{Central}$$

$$\text{Metabolite}' = FM * Kme * \text{Central} - CLm * \text{Metabolite}$$

The dose of the suspension was scaled down by 20% to 40mg/m² from the standard dose of 50mg/m² as suggested by the previous virtual bioequivalence study. The median simulated 6TGN concentration was 288.0 (pmol/8x10⁸ RBCs) with a range between 114 and 703.6 pmol/8x10⁸ RBCs. While there is no consensus on the reference range for the therapeutic levels of 6-TGN, variable ranges of 6TGN have been reported, In a research by Chrzanoska et al., 6-TGN concentrations ranged from 60 to 833 pmol/8 x 10⁸ RBC in paediatric ALL patients receiving 6-MP dosed at 50 mg/m² [14], while in a study Rosdiana et al., 6-TGN concentrations ranged from 6 to 234.04 pmol/8 x 10⁸ RBC pmol/8 x 10⁸RBC [15]. Patients in the research by Bhatia et al received a larger dose of 6-MP (75 mg/m²/day), but 6-TGN levels remained the same at 0.3-714.1 pmol/8 x 10⁸ RBC [16]. A study by Zhou Y and colleagues reported a median 6TGN concentration of 217.13 pmol/8 x 10⁸ RBC with a range from 89.38–674.77 in the paediatric population receiving a median dose of 42.3mg/day [17]. Thus, the simulations revealed relatively safe and effective levels of 6TGN that were comparable with the previous studies (Figure 7).

DISCUSSION

In this study, an enhanced bioavailability of the 10mg/ml liquid formulation was observed. The presence of a robust clinical data along with in-vitro dissolution profile of the drug enabled the development of a model that accurately matched the clinical profiles. This model facilitated the identification of a corrected dose of PFOS which is 20% lower than the conventional tablet. Additionally, exposures in children were simulated using an existing model from literature and the allometrically scaled parameter values from the adult data and were found to be acceptable. PFOS can facilitate flexible and precise dosage adjustment based on the patient's demographics and response to therapy which are crucial in establishing treatment success.

Maintenance of remission is a challenge in the context of high variability in the disposition of 6MP and consequently, the duration and depth of neutropenia between individuals. It is observed that the dose of 6MP in the maintenance therapy varies 5-fold among patients [18]. Interestingly, the PFOS had a lower pharmacokinetic variability compared to the tablet (55.87% versus 63.21%), although the bioavailability was higher. The increased bioavailability of the 10mg/ml liquid formulation can be attributed to various factors such as the type of formulation, constituents in the formulation, concentration, and viscosity of the suspension. An earlier study reported increased bioavailability with extemporaneously prepared 6MP liquid formulations compared to the tablet and notably, 6MP exposure in 5mg/ml solution was significantly higher compared to 50mg/ml solution in paediatrics with ALL [20]. Two other studies carried out with 6MP 20mg/ml liquid formulation either found comparable or lower oral bioavailability of the suspension compared to the tablet [21, 22]. In fact, our formulation has a 47% higher relative bioavailability compared to the 20 mg/ml liquid suspension, clearly demonstrating the effect of concentration and viscosity thereof

on bioavailability. The relatively small body sizes of children in India and other LMICs compared to their Western European or North American counterparts prompted us to develop a more dilute suspension.

The applications of model-informed drug development have been significantly increasing and virtual bioequivalence studies can assess the similarity and potential differences of pharmacokinetic and clinical performance between test and reference formulations based on the translational link between in-vitro, in-vivo, as well as physicochemical properties of the molecule to its clinical pharmacokinetic property [23]. The simulation results are encouraging and allometry is still considered as a reasonable choice that extrapolates adult estimates and predicts paediatric clearance within 25% - 50% for most drugs [24]. Of course, the 6MP exposure with the test formulation needs to be validated in paediatric ALL patients to confirm safe and effective exposures. A major limitation of the study is that the paediatric dosage simulation was based on only one population pharmacokinetic study in paediatric ALL patients conducted in a small cohort of 19 children [12]. Nevertheless, we believe that it is a reasonable pointer to the starting dose which could be further optimized based on routine blood cell count monitoring to compensate for the variable bioavailability and response. The palatability of the liquid formulations is a huge advantage as it can significantly improve adherence rates in pediatrics as shown by a survey on the acceptability of 6MP liquid formulation [25]. Importantly, the developed 6MP formulation will make it easier to choose an appropriate and constant treatment intensity of 6MP with precise dosing aided by lower pharmacokinetic variability of the PFOS compared to the tablets.

CONCLUSION

The study findings illustrate a higher bioavailability of the developed 10 mg/mL oral suspension dosage form compared to the 50mg tablet and exemplify that not all liquid 6-MP formulations are equal in terms of bioavailability of the parent drug. A starting dose 20% lower than the tablet formulation is proposed based on model informed dose optimization. Switching to PFOS can minimize sub-therapeutic or toxic exposures. However, dose modifications should be anticipated to maintain treatment intensity with close monitoring of the patient for blood counts and liver enzymes. The simulated exposures of 6MP according to the adjusted dosage regimens recommended model must be validated in real-time clinical conditions.

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CONFLICT OF INTEREST DISCLOSURE

Mahendra Joshi and Shivkumar Madki are Directors of IDRS Labs Pvt. Ltd. with financial interest in the product. Dr. Archana Khosa Kakkar is an employee of IDRS Labs Pvt. Ltd. and has financial interest in the product. The remaining authors have no relevant conflicts of interest to declare.

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Pharmacokinetic Parameters (Units)	Arithmetic Mean \pm SD (%CV) (N = 51)	Arithmetic Mean \pm SD (%CV) (N = 51)
	Reference Product (R)	Test Product (T)
C _{max} (ng/mL)	90.537 \pm 57.2242 (63.21%)	165.588 \pm 75.0941 (45.35%)
T _{max} (hr)	1.000 (0.500- 3.000)	0.500 (0.330- 1.330)
AUC _{0-t} (hr*ng/mL)	188.804 \pm 105.4906 (55.87%)	264.055 \pm 111.7610 (42.32%)
AUC _{0-∞} (hr*ng/mL)	191.800 \pm 105.9874 (55.26%)	267.163 \pm 111.6998 (41.81%)
k _{el} (1/hr)	0.542 \pm 0.1348 (24.86%)	0.569 \pm 0.1258 (22.12%)
t _{1/2} (hr)	1.405 \pm 0.5973 (42.50%)	1.286 \pm 0.3268 (25.41%)
AUC_%Extrap_obs (%)	1.846 \pm 0.8916 (48.30%)	1.382 \pm 0.9350 (67.64%)

Table 1- Mean PK parameters for the reference and test formulations

PK Parameters (Unit)	Geometric Least Square Means and Its Ratio (N=51)	Geometric Least Square Means and Its Ratio (N=51)	Geometric Least Square Means and Its Ratio (N=51)	Intra-subject CV (%)	90% Confidence Interval	Power (%)
	Test Product (T)	Reference Product (R)	(T/R) (%)			
AUC _{0-t} (hr*ng/mL)	244.908	166.462	147.13	18.80	138.29% - 156.52%	100.00
AUC _{0-∞} (hr*ng/mL)	248.357	169.604	146.43	18.62	137.72% - 155.69%	100.00

Table 2- Summary of BE assessment

Parameter	Value in test formulation (CV%)	Value in reference formulation (CV%)
Ka (hr ⁻¹)	21.15 (10.2)	5.53 (15.1)
Cl/F (L/hr)	205.1 (17.1)	307.01 (13.9)
Q/F (L/hr)	41.8 (20.5)	73.80 (7.1)
V1/F (L)	17.3 (14.36)	70.66 (13.5)
V2/F (L)	103.9 (3.6)	147.88 (4.0)

Table 3: Parameter estimates of the final PK model. Ka- Absorption rate constant, Cl/F- Apparent clearance from central compartment, Q/F -Apparent inter compartmental clearance, V1/F – Apparent volume of central compartment, V2/F – Apparent volume of peripheral compartment

Group	Cmax (ng/mL)	AUC (ng.hr/mL)	AUCinf (ng.hr/mL)
Reference_observed-50mg	76.95	166.66	169.80
Reference_simulated-50mg	75.42	211.35	213.68
Test_observed-50mg	152.04	245.06	248.50
Test_simulated-50mg	148.68	292.42	298.90

Table 4: Comparison of PK parameter estimates of Reference and Test product (observed vs. Predicted)

Parameters	F values	P values	PE (%)	Lower 90%CI	Upper 90%CI
Cmax	7.891	0.007	-	-	-
AUC0-t	1.185	0.282	-	-	-
AUC0-inf	0.83	0.742	-	-	-
ln(Cmax)	0.789	0.795	135.963	116.164	159.137
ln(AUC0-t)	0.76	0.83	97.982	82.772	115.986
ln(AUC0-inf)	0.773	0.815	98.152	82.902	116.207

Table 5: The BE result summary of 40 mg Test (suspension) vs. Reference (50 mg Tablet)

Figures

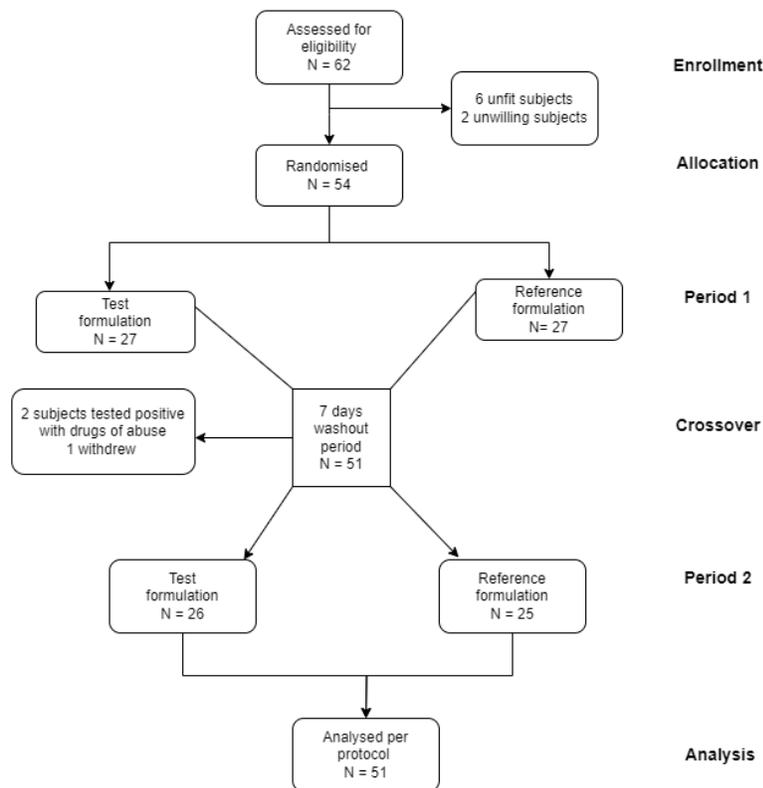


Figure 1: Study Schema

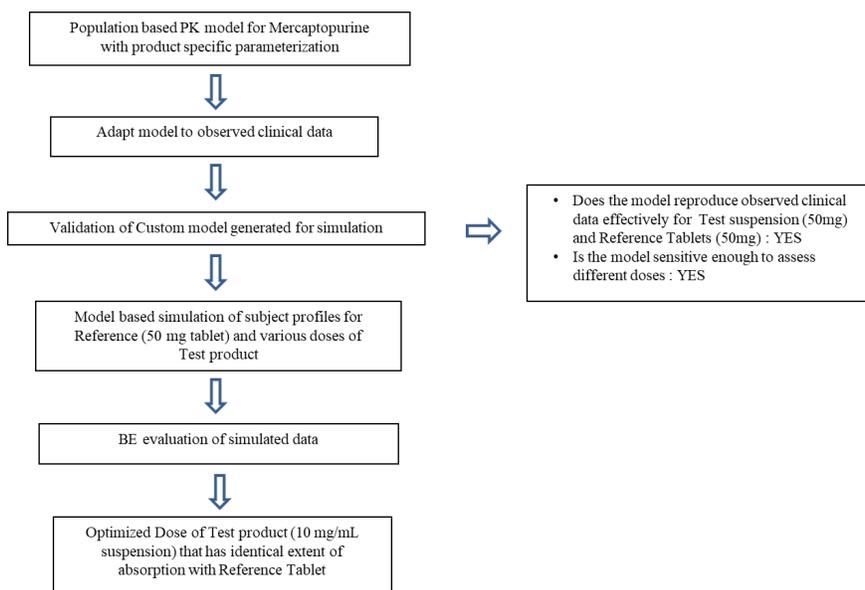


Fig 2: Developmental strategy for model-based simulation of mercaptopurine PFOS 10mg/mL.

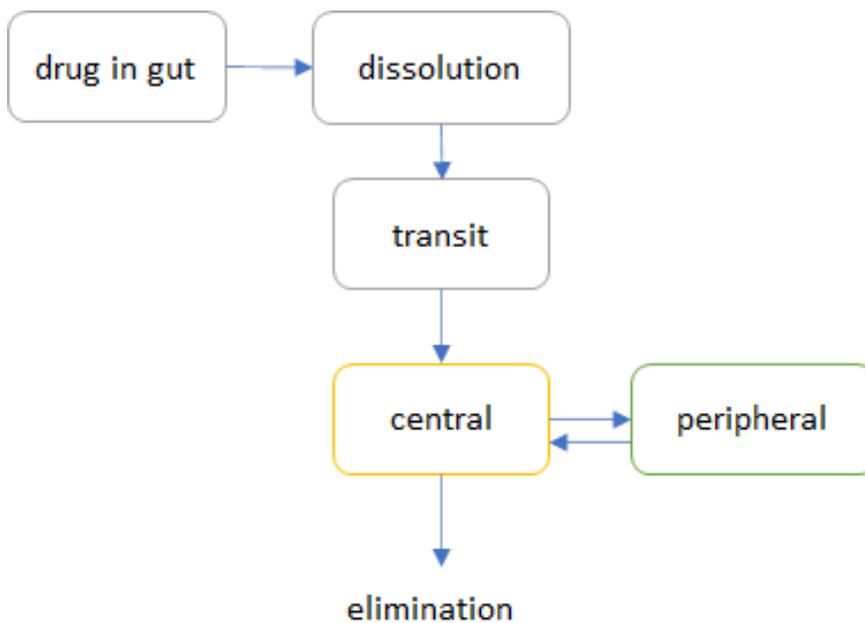


Figure 3A

$$\begin{aligned} \frac{dDepot(t)}{dt} &= (-1 + e^{-kd \cdot t}) \cdot Depot(t) \\ \frac{dDissol(t)}{dt} &= (1 - e^{-kd \cdot t}) \cdot Depot(t) - kt \cdot Dissol(t) \\ \frac{dtransit(t)}{dt} &= kt \cdot Dissol(t) - Ka \cdot transit(t) \\ \frac{dCentral(t)}{dt} &= Ka \cdot transit(t) + k_{21} \cdot Peripheral(t) - (k + k_{12}) \cdot Central(t) \\ \frac{dPeripheral(t)}{dt} &= k_{12} \cdot Central(t) - k_{21} \cdot Peripheral(t) \end{aligned}$$

Figure 3B

Fig 3: PK model developed (A) for 6MP & differential equations used (B)

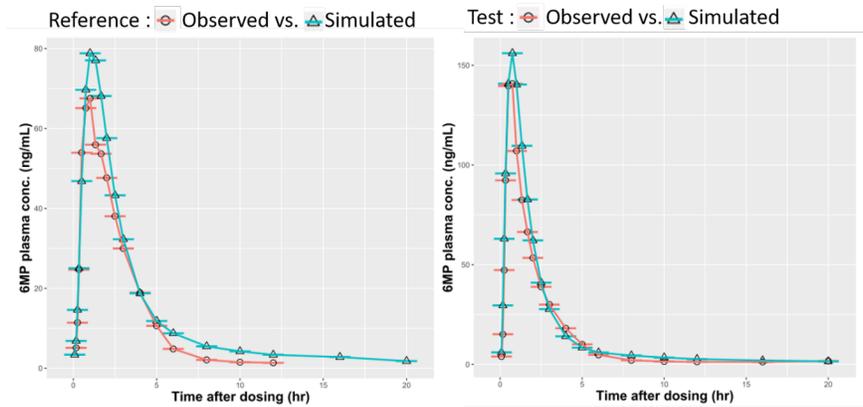


Fig 4: Average PK profiles of Test and Reference product: Observed vs. Simulated

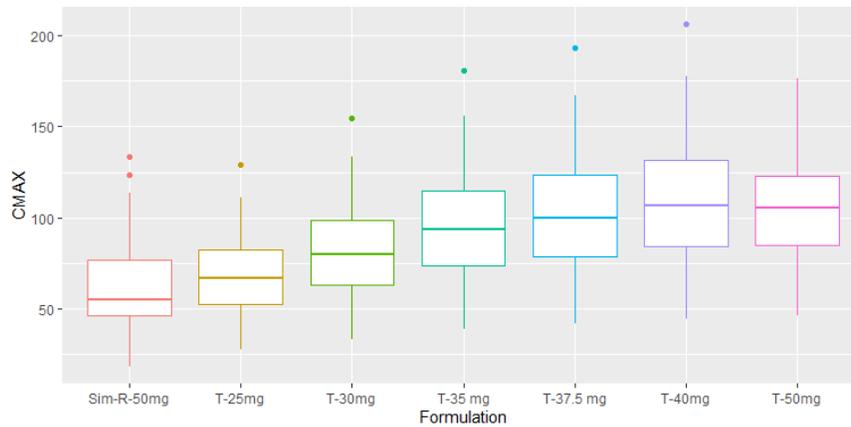


Fig 5: Boxplot of population spread of Cmax for various Test doses. The horizontal middle line indicates median and the box depicts upper and lower quantiles.

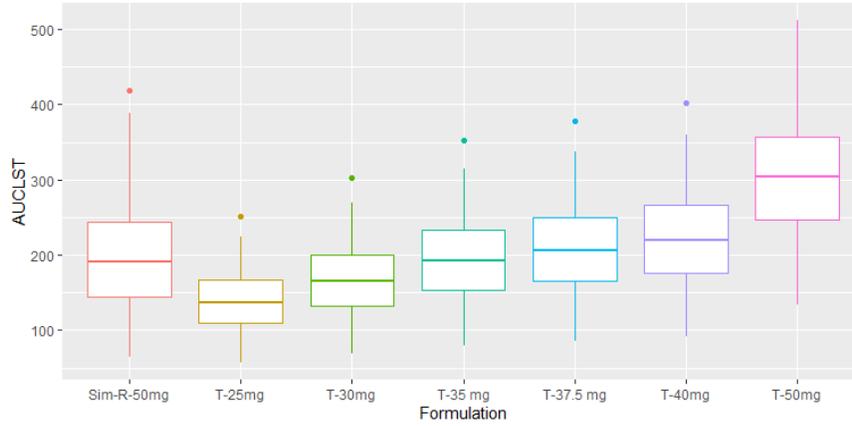


Fig 6: Boxplot of population spread of AUC last for various Test doses. The horizontal middle line indicates median and the box depicts upper and lower quantiles.

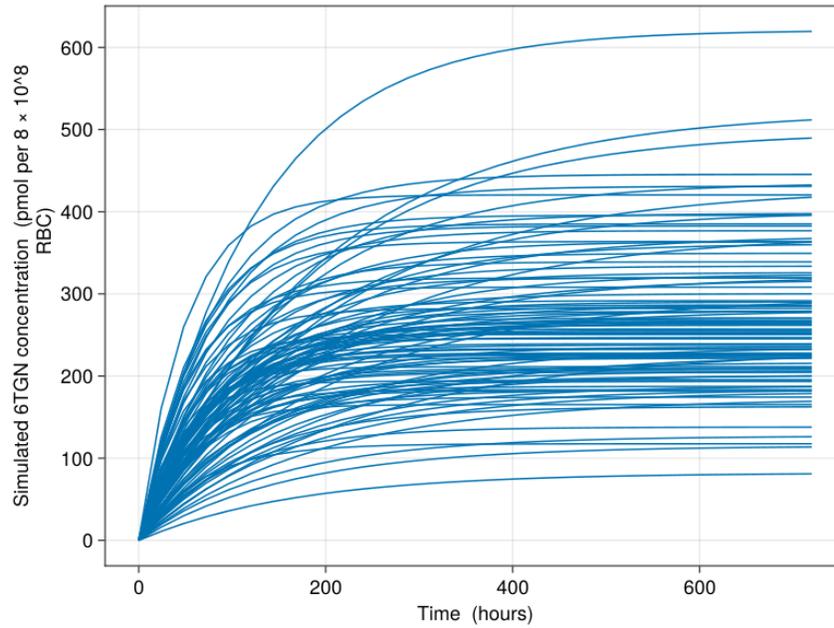


Fig 7: Simulated blood concentrations of 6TGN in pediatrics with acute lymphoblastic leukemia.