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Limited sampling strategy of partial area-under-the-concentration-time-curves to estimate midazolam systemic clearance for cytochrome P450 3A phenotyping

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Abstract

Objective—Intravenous (IV) midazolam is the preferred cytochrome P450 (CYP) 3A probe for phenotyping, with systemic clearance (CL) estimating hepatic CYP3A activity. A limited sampling strategy was conducted to determine if partial area-under-the-concentration-time-curves (AUCs) could reliably estimate midazolam systemic CL during conditions of CYP3A baseline activity, inhibition and induction/activation.

Methods—Midazolam plasma concentrations during CYP3A baseline (n=93), inhibition (n=40), and induction/activation (n=33) were obtained from seven studies in healthy adults. Non-compartmental analysis determined observed CL (CL_{obs}) and partial AUCs. Linear regression equations were derived from partial AUCs to estimate CL (CL_{pred}) during CYP3A baseline, inhibition and induction/activation. Pre-established criterion for linear regression analysis was r^2 ≥0.9. CL_{pred} was compared to CL_{obs}, and relative bias and precision were assessed using percent mean prediction error (%MPE) and percent mean absolute error (%MAE).

Results—During CYP3A baseline and inhibition, all evaluated partial AUCs failed to meet criterion of r^2 ≥0.9 and/or %MAE <15%. During CYP3A induction/activation, equations derived from partial AUCs from 0 to 1 hour ($AUC_{0–1}$), $AUC_{0–2}$, and $AUC_{0–4}$ were acceptable, with good precision and minimal bias. These equations provided the same conclusions regarding equivalency testing compared to intense sampling.

Conclusions—During CYP3A induction/activation, but not baseline or inhibition, midazolam partial $AUC_{0–1}$, $AUC_{0–2}$, and $AUC_{0–4}$ reliably estimated systemic CL, and consequently hepatic CYP3A activity in healthy adults.

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Keywords

CYP3A; partial AUC; limited sampling; midazolam; phenotyping

Phenotyping is used to determine real time, *in vivo* drug metabolizing enzyme activity and assess pharmacokinetic-mediated drug-drug interactions (DDIs)[1, 2]. Of the drug metabolizing enzymes, cytochrome P450 (CYP) 3A plays a substantial role in clinically significant DDIs as more than 50% of the drugs available on the market are subject to CYP3A4 and CYP3A5 pathways[3]. The preferred CYP3A probe for phenotyping is midazolam[2] with intravenous (IV) midazolam exclusively assessing hepatic CYP3A activity[1, 4]. Systemic (or total body) clearance (CL) of IV midazolam strongly correlates with hepatic CYP3A content ($r = 0.93$, $p < 0.001$)[5] and is commonly used as a surrogate for hepatic CYP3A activity.

Limited sampling strategy is a methodology that estimates systemic CL or area-under-the-concentration-time-curve (AUC) from a small number of plasma samples, ranging from 1 to 4. This method alleviates the cost and inconvenience of intense sampling, while maintaining acceptable precision and minimal bias. Limited sampling strategy has been adopted for clinical use for cyclosporine[6], tacrolimus[7], and mycophenolic acid[8]. With regards to IV midazolam, several limited sampling strategies have been examined. Single-point sampling strategies have been proposed, but timepoints vary regarding the optimal post-dose timepoint(s)[9–11]. Metabolic ratios of 1-hydroxymidazolam to midazolam, as well as two- and three-point limited sampling models (LSMs) have also been suggested[11], but conflicting results have been reported[12, 13].

Katzenmaier et al. proposed an alternative limited sampling strategy of a partial AUC to estimate intestinal and hepatic CYP3A activity with oral midazolam[14, 15]. These studies recommended a partial AUC from 2 to 4 hours ($AUC_{2–4}$) to estimate metabolic CL[14, 15]. In addition, Tai et al. reported a partial $AUC_{0–4}$ and a partial $AUC_{1–4}$ could reliably estimate apparent oral CL during conditions of CYP3A induction with rifampin and Ginkgo biloba extract[16]. Whether this limited sampling strategy is applicable with IV midazolam in estimating hepatic CYP3A activity is unknown. The purpose of this study was to determine if a limited sampling strategy using partial AUCs could estimate systemic CL and thus hepatic CYP3A activity with IV midazolam. The second objective was to perform equivalence testing to determine if the LSMs reproduce the same conclusions (e.g., equivalence or lack of equivalence) as those derived from intense sampling during conditions of CYP3A inhibition and induction/activation.

MATERIALS AND METHODS

Study subjects

This study was granted Institutional Review Board exemption by the University of California, San Diego, Human Research Protections Program. Midazolam plasma concentration data from seven published studies were obtained[4, 17–22] (Table 1). One subject’s profile under CYP3A baseline conditions was excluded due to a >100-fold

difference for a single midazolam concentration that was determined to be erroneous. The final sample size was 93 subjects. Demographic information was provided in the majority of studies (Table 1), and included a mix of healthy men and women, with an age range of 18 to 50 years and weight range of 52 to 102.3 kg. Healthy status was determined by medical history, physical examination, and blood and urine laboratory tests. Subjects received IV bolus midazolam alone (baseline) or in combination with CYP3A inhibitors ketoconazole, itraconazole or aprepitant, or CYP3A inducers/activators rifampin or pleconaril (Table 1). Midazolam doses ranged across studies from 0.025 mg/kg to 2 mg.

Sample collection and assay

Eight to 15 plasma samples per subject were collected at various study-specific time points between 0 and 24 hours post-dose (Table 1). Assay methods for plasma detection included HPLC-MS, GC, and LC-MS-MS. The lower limit of quantitation ranged from 0.1 to 1 ng/mL, while inter- and intraassay precision and accuracy was <15%. Details of each assay are provided elsewhere[4, 17–22].

Data analysis

The current study determined novel LSMs of partial AUCs (independent variable) to estimate systemic CL (dependent variable). In contrast, a portion of the data were used to evaluate previously published LSMs of plasma concentrations (independent variable) to estimate AUC (dependent variable)[12]. Due to differences in drug distribution based on IV bolus versus IV infusion[23, 24], the current study included only IV bolus data. Noncompartmental analysis (WinNonlin v 5.2.1, Pharsight Corporation, Cary, NC, USA) of the concentration-time data was performed to calculate observed AUC infinity (AUC_{inf}), observed clearance (CL_{obs}) and partial AUCs, utilizing a linear trapezoidal method for calculation of AUC and software-determined best fit for elimination phase (λ). AUC values were dose-normalized to 1 mg. Partial AUCs (0–1, 0–2, 0–4, 1–2, 1–4, 2–4, and 2–6 hour intervals) were selected based on previous studies of partial AUCs for midazolam[14]. Midazolam systemic CL and dose-normalized partial AUCs were log transformed prior to linear regression analysis.

Linear regression analyses were performed with SAS version 8 (SAS Institute, Cary, NC, USA). During CYP3A baseline conditions, a limited sampling strategy was performed, which entailed randomizing subject data (n=93) into a training (n=30) and validation (n=63) set. Linear regression equations to estimate systemic CL, as a function of partial AUCs, were derived from the training set. Preset criteria for selecting linear regression equations was a coefficient of determination (r^2) greater than or equal to 0.9[25]. The resulting equations were used to calculate individual CL (CL_{pred}) estimates from validation set data. Relative bias and precision, reported as percentages, were assessed using percent mean prediction error (%MPE) and percent mean absolute error (%MAE), respectively. Acceptable limits were defined as %MPE of −15% to +15%, and %MAE <15%[16].

During CYP3A inhibition and induction/activation, data were not divided into training and validation sets due to small sample sizes. Novel linear regression equations to estimate midazolam systemic CL were derived from all subjects during CYP3A inhibition (n=40) and

from all subjects during CYP3A induction/activation (n=33). Preset criteria for selecting linear regression equations was a $r^2 \geq 0.9$[25]. Jackknife validation methods were used to determine CL_{pred} values from partial AUCs. This methodology is used for smaller sample sizes[25]. A regression equation was derived from $n - 1$ subjects and used to estimate midazolam CL for the nth subject, thus generating a slightly different regression equation for each subject. Relative bias and precision, reported as percentages, were assessed using %MPE and %MAE, respectively. Acceptable limits were defined as %MPE of −15% to +15%, and %MAE <15%[16].

To evaluate if the CL_{pred} equations were suitable to detect CYP3A inhibition or induction/activation, equivalence testing was performed exclusively with CL_{pred} equations that met all acceptable r^2, %MPE, and %MAE criteria. This method is used to assess equivalence in DDI studies and to evaluate bioequivalence for drugs[26–28]. General linear models that included subject, sequence, and treatment effects were performed and least squares geometric mean ratios (LS-GMR) of test/baseline were determined. Equivalence was concluded if the 90% confidence interval (CI) of the LS-GMR was within 0.8 to 1.25.

RESULTS

Pharmacokinetic parameters were consistent with those reported in the previously published studies. Mean (range) CL_{obs} and dose-normalized AUC_{inf} were 28.8 (9.8 – 54.3) L/h and 39.1 (18.4 – 102.5) ng▪hr/mL, 11.7 (3.4 – 23.8) L/h and 111.2 (42.0 – 282.0) ng▪hr/mL, and 49.7 (19.8 – 115.5) L/h and 25.4 (8.9 – 50.6) ng▪hr/mL for CYP3A baseline, inhibition, and induction/activation conditions, respectively.

During CYP3A baseline conditions, all of the evaluated partial AUC LSMs had unacceptable r^2, although a few had adequate bias and precision (Table 2). During CYP3A inhibition and regardless of inhibitor used, r^2 and precision (%MAE) all failed acceptance criteria for each evaluated LSM. In contrast, bias (%MPE) met acceptance criteria at < 15% for each model (Table 3). Consequently, equivalence testing was not performed during CYP3A inhibition.

During CYP3A induction/activation, partial $AUC_{0–1}$, $AUC_{0–2}$, and $AUC_{0–4}$ were acceptable estimates of CL_{pred}, with good precision and minimal bias (Table 4). During CYP3A induction with rifampin (Study 6c), LS-GMRs (90% CI) for CL_{pred} with $AUC_{0–1}$, $AUC_{0–3}$, and $AUC_{0–4}$ were 1.3 (1.18 – 1.41), 1.31 (1.19 – 1.43), and 1.32 (1.2 – 1.44), respectively. During CYP3A induction/activation with pleconaril (Study 3b) equivalence was observed utilizing CL_{pred} with $AUC_{0–1}$, $AUC_{0–2}$, and $AUC_{0–4}$. These results were consistent regarding equivalence testing compared to intense sampling (Table 5).

DISCUSSION

This was a study to determine if a previous limited sampling strategy of partial AUCs to estimate both hepatic and intestinal CYP3A activity could be applied to estimating solely hepatic CYP3A activity with IV midazolam. LSMs using partial $AUC_{0–1}$, $AUC_{0–3}$, and $AUC_{0–4}$ met acceptable r^2, %MAE, and %MPE criteria during CYP3A induction/activation, but not during CYP3A baseline and inhibition conditions. One possible reason the partial

$AUC_{0–1}$, $AUC_{0–3}$, and AUC_{0-} were not suitable during baseline CYP3A conditions is the influence of distribution early in the dosing interval. With regards to CYP3A inhibition, the difference is likely due to the impact of inhibitor concentration and time course on enzyme state. As systemic concentrations of a competitive CYP3A inhibitor decline through the dosing interval, even if at steady state, the degree of CYP3A inhibition will also vary over that time period. This may lead to a more pronounced inhibitory affect at the beginning of the dosing interval which tapers down as inhibitor concentration decreases. On the contrary, changing plasma concentrations of CYP3A inducers are less of a concern, as enzyme synthesis and degradation exert a larger impact on the enzyme activity than merely the inducer's current concentration, and are not expected to vary considerably over the period of a few hours[29,30]. Further, under enzyme induction, a greater extent of the midazolam exposure falls within the limited sampling window (partial AUC curve), and as partial AUCs approach AUC_{inf}, a higher correlation with midazolam clearance would be expected.

Although current results demonstrate that certain partial AUCs may reliably predict systemic clearance under CYP3A induction/activation conditions, this analysis does not provide conclusions regarding the optimal quantity of plasma midazolam measurements necessary to develop a reliable model based on partial AUC parameters, nor does it provide a recommendation for specific sampling timepoints. In the published studies selected for use, the quantity of plasma samples ranged from 8 to 15 total samples and between time zero to 24 hours post-dose. Multiple studies collected several samples between time zero and 1 or 2 hours post-dose, improving the ability to capture the midazolam maximum plasma concentration and better estimate a partial AUC from time zero to any time. The only acceptable models under any CYP3A condition were those using partial AUCs starting at time zero, as opposed to 1 or 2 hours post-dose.

Although the estimated systemic CL determined from partial $AUC_{0–1}$, $AUC_{0–3}$, and $AUC_{0–4}$ during CYP3A induction/activation are somewhat consistent with previous oral midazolam studies[14–16], the optimal partial AUC varied as Katzenmaier et al. recommended a partial $AUC_{2–4}$[14, 15]. Differences in the optimal partial AUCs between studies may be due to several reasons. In the current study, baseline CYP3A data from 93 subjects were divided into training (for model development) and validation (for model validation) sets. This is the recommended approach for limited sampling strategy[6, 25]. In contrast, Katzenmaier et al. utilized the same subjects (n = 12) for model development and validation[14]. This is believed to lead to an underestimation of bias and precision, since the same data pool was used for both model development and validation, as opposed to employing a separate validation dataset[31, 32]. In contrast, due to smaller sample sizes ($n \leq 40$) during CYP3A inhibition and induction conditions, jackknife methods were utilized for model validation. This method retains the separation of model development and validation, and was deemed the most rigorous method of evaluation for small sample sizes[25].

A second reason contributing to differences between study results could be the varying analytical methods used in the current study (Table 1) compared to a single assay method in previous studies[14, 15]. Analytical techniques and assay specificity are known sources of variance in drug concentrations. However, in the current study, the effect of different

analytical methods is likely non-contributory as others have reported acceptable precision and prediction of IV midazolam LSMs upon comparing different analytical assays[11].

A third reason for the difference in partial AUCs could be the selected pharmacokinetic parameter to determine CYP3A activity. Previous studies selected metabolic CL of 1-hydroxymidazolam to midazolam to estimate CYP3A activity[14, 15], with 1-hydroxymidazolam determined from a 24-hour urine collection. The authors' rationale for this pharmacokinetic parameter is that since this metabolite is primarily mediated by CYP3A and accounts for most of the administered midazolam dose, it is believed to more accurately represent CYP3A activity[14, 15]. In the current study, 1-hydroxymidazoam urine concentration data was not known and thus determining an observed metabolic CL and comparing these results to predicted metabolic CL would not be possible. Rather, the current study utilized systemic CL as this is an accepted parameter to estimate hepatic CYP3A activity with IV midazolam[2]. Lastly, this analysis included IV midazolam data, whereby results are applicable to estimation of hepatic CYP3A activity. Caution is warranted in that the conclusions from this study cannot be directly applied to previously published oral midazolam findings, which are subject to hepatic and intestinal CYP3A enzymes. Future studies are recommended to evaluate the preferred LSM of a partial AUC to estimate metabolic or systemic CL with IV midazolam.

The strengths of the current data analysis include the heterogeneity of the sample population and variety of enzymatic alterations. Although subjects were healthy volunteers, concentration-time data did include a mix of age, weight, and sex. The latter is particularly important as women have increased CYP3A4 activity compared to men[33]. Furthermore, the data used from multiple study sites reduces the impact of center specificity which may superficially limit variability in calculated pharmacokinetic parameters[34, 35]. Of course it is acknowledged that with a more heterogeneous population and data source, variability in midazolam clearance is likely increased as well. Thirdly, the analysis evaluated baseline, inhibition, and induction state models individually, and included non-baseline alterations to varying degrees, such as inhibition by strong (ketoconazole) and moderate (itraconazole) CYP3A inhibitors. This creates a more diverse real-world assessment of using partial AUCs to estimate systemic CL during various CYP3A perturbations. Considering that no models developed independently for two of the three states (baseline and inhibition) met acceptable model criteria, it was not reasonable to assume that a single model developed by combining concentration-time data from all three conditions would be useful or reliable. Therefore, partial AUCs to estimate systemic CL were only assessed according to enzymatic state.

Since intravenous midazolam has a relatively short elimination half-life ($t_{1/2}$) of approximately 3 hours[36] and therefore obtaining full pharmacokinetic profiles would not require excessively long clinic stays for healthy subjects, one might question the impetus to develop or validate LSMs for estimating hepatic CYP3A activity. However, even though comparatively a midazolam full pharmacokinetic profile seems more rapidly obtained, numerous plasma samples must be drawn over the course of several hours. The issue of cost for the sponsor, convenience for the staff, and comfort for the subject or patient still remains. As in many areas, the desire to find a simple, efficient, cost-effective alternative

should not be easily dismissed, no matter the degree of burden present in the traditional, full-scale approach.

Equivalence testing provided further evaluation to determine if the LSMs were able to detect CYP3A perturbations. For the LSMs during CYP3A induction/activation to be useful for DDI studies, the CL_{pred} equations should provide the same outcome (e.g., equivalence or lack of equivalence) compared to observed midazolam pharmacokinetic parameters obtained with intense sampling. The evaluated CL equations for $AUC_{0–1}$, $AUC_{0–2}$, and $AUC_{0–4}$ were able to detect CYP3A induction with rifampin (Study 6c). These results are consistent with those from LS-GMRs calculations using intense PK sampling (Table 5). With regards to pleconaril (Study 3b), equivalence was observed with intense sampling. The conclusion of equivalence remained consistent using the evaluated CL_{pred} equations for $AUC_{0–1}$, $AUC_{0–2}$, and $AUC_{0–4}$ (Table 5).

A limitation in the current study was that subjects were not genotyped for CYP3A4 and CYP3A5. In one study CYP3A4*1B homozygous subjects had smaller mean midazolam CL compared to CYP3A4*1 homozygous subjects (252 ± 53 vs. 310 ± 59 mL/min; $p = 0.02$)[37]. With regards to CYP3A5 polymorphisms, midazolam CL is similar in subjects genotyped as CYP3A5*1/*1 versus CYP3A5*1/*3[38]. Consequently, the contribution of CYP3A4 and CYP3A5 genetic polymorphisms on midazolam pharmacokinetics in the current study was not known.

CONCLUSIONS

Midazolam systemic CL was accurately estimated using limited sampling strategy of partial AUCs only during conditions of CYP3A induction/activation with rifampin and pleconaril. The systemic CL equations derived from $AUC_{0–1}$, $AUC_{0–2}$, and $AUC_{0–4}$ were able to detect a lack of equivalence with rifampin and equivalence with pleconaril. These conclusions were consistent compared to intense sampling. In contrast, during CYP3A baseline and inhibition conditions with ketoconazole, itraconazole, and aprepitant, no LSMs yielded satisfactory reliability, as determined by r^2 or precision (%MAE). The study findings suggest that a general LSM of a partial AUC to estimate hepatic CYP3A activity with IV midazolam, which can be used reliably under multiple conditions during drug development or everyday clinical care, has yet to be developed. Additional research is warranted to examine other methodological approaches (e.g. Bayesian parameter estimation, D-optimal sampling) that are appropriate to estimate *in vivo* hepatic CYP3A activity with IV midazolam as a probe drug.

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Table 1

Summary of published intravenous midazolam studies for data analysis.

Study	n (M/F)	Age (years)	Weight (kg)	Midazolam dose	Inhibitor or inducer/activator	Assay	Collection Timepoint (h)	Reference
1	14 (6/7)	19–50	55–96	0.025 mg/kg	none	LC/MS/MS	0, 0.083, 0.5, 1, 2, 4, 5, 6	17
2	11 (8/3)	24–45	67–102	0.025 mg/kg	none	LC/MS/MS	0, 0.083, 0.5, 1, 2, 4, 5, 6	21
3a	14 (8/6)	36.1[a]	55–98	1 mg	none	HPLC-MS	0, 0.083, 0.5, 1, 2, 4, 5, 6	18
3b				1 mg	Pleconaril 400 mg 3 times daily × 16 doses	HPLC-MS	0, 0.083, 0.5, 1, 2, 4, 5, 6	18
4a	11 (3/8)	20–36	N/A	2 mg	none	LC/MS/MS	0, 0.03, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24	19
4b	12 (3/9)				Aprepitant 125 mg ×1 dose	LC/MS/MS	0, 0.03, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24	19
5a	9 (6/3)	19–41	54–87	2 mg	none	GC	0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 24	4
5b				2 mg	Ketoconazole 200 mg X3 doses	GC	0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 24	4
6a	19 (17/2)	21–28	52–81	1 mg	none	LC-MS-MS	0, 0.17, 0.33, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12	20
6b					Itraconazole 200 mg once daily × 4 days	LC-MS-MS	0, 0.17, 0.33, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12	20
6c					Rifampin 600 mg once daily × 10 days	LC-MS-MS	0, 0.17, 0.33, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12	20
7	16 (14/2)	18–42	61–95	1mg	none	HPLC-MS	0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 1012, 24	22

[a] Mean age

Abbreviations: GC, gas chromatography; HPLC, high performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; N/A, not applicable.

Table 2

Midazolam clearance equations and assessment of bias and precision during CYP3A baseline conditions

Equation[a]	Predicted CL (L/h)	r^2	%MPE	%MAE
$-0.64*\log AUC_{0-1} + 2.19$	26.3	0.58	−1.5	16.5
$-0.82*\log AUC_{0-2} + 2.53$	26.3	0.69	−2.3	13.1
$-0.96*\log AUC_{0-4} + 2.82$	26.6	0.8	−1.7	10.6
$-0.75*\log AUC_{1-2} + 2.03$	27.3	0.5	7.4	20.2
$-0.80*\log AUC_{1-4} + 2.32$	27.8	0.59	9.1	18.1
$-0.78*\log AUC_{2-4} + 2.06$	28.4	0.63	11	19.2
$-0.79*\log AUC_{2-6} + 2.2$	28.5	0.68	11.4	19.6
Acceptable criteria	--	**≥ 0.9**	**−15% to +15%**	**< 15%**

[a] Equations were derived from training set data (n = 30).

Abbreviations: AUC_{X-Y}, area under the concentration time curve from time X to Y; r^2, coefficient of determination; %MAE, relative percent mean absolute error; %MPE, relative percent mean prediction error.

Table 3

Midazolam clearance equations and assessment of bias and precision during CYP3A inhibition conditions with ketoconazole, itraconazole, and aprepitant

Equation[a]	Predicted CL (L/h)	r^2	%MPE	%MAE
$-0.56*\log AUC_{0-1} + 1.74$	10.5	0.16	12.7	44.7
$-0.98*\log AUC_{0-2} + 2.47$	10.7	0.33	10.3	38.3
$-1.31*\log AUC_{0-4} + 3.18$	11.1	0.56	6.8	28.9
$-1.29*\log AUC_{1-2} + 2.29$	11.1	0.60	5.9	28.3
$-1.23*\log AUC_{1-4} + 2.71$	11.3	0.69	4.6	25.
$-1.13*\log AUC_{2-4} + 2.3$	11.3	0.72	4.2	23.9
$-1.1*\log AUC_{2-6} + 2.5$	11.4	0.77	3.4	21.8
Acceptable Criteria	--	**≥0.9**	**−15% to +15%**	**<15%**

[a] Equations were derived from jackknife analysis (n = 40).

Abbreviations: AUC_{X-Y}, area under the concentration time curve from time X to Y; r^2, coefficient of determination; %MAE, relative percent mean absolute error; %MPE, relative percent mean prediction error.

Table 4

Midazolam clearance equations and assessment of bias and precision during CYP3A activation/induction conditions with rifampin and pleconaril

Equation[a]	Predicted CL (L/h)	r^2	%MPE	%MAE
$-0.86*\log AUC_{0-1} + 2.63$	48.8	***0.92***	***1***	***12.2***
$-0.95*\log AUC_{0-2} + 2.84$	49.3	***0.97***	***0.4***	***7.9***
$-1*\log AUC_{0-4} + 2.96$	49.5	***0.99***	***0.1***	***4.5***
$-0.94*\log AUC_{1-2} + 2.13$	47.4	0.58	5.9	27.7
$-0.9*\log AUC_{1-4} + 2.35$	47.6	0.62	5.3	26.4
$-0.82*\log AUC_{2-4} + 2.01$	47.7	0.64	5	25.2
$-0.72*\log AUC_{2-6} + 2.06$	47.6	0.6	5.5	27.2
Acceptable Criteria	--	**≥0.9**	**−15% to +15%**	**<15%**

[a] Equations were derived from jackknife analysis (n = 33).

Abbreviations: AUC_{X-Y}, area under the concentration time curve from time X to Y; r^2, coefficient of determination; %MAE, relative percent mean absolute error; %MPE, relative percent mean prediction error.

Table 5

Least squares geometric mean ratios and 90% confidence intervals utilizing observed and estimated systemic midazolam clearance during CYP3A induction/activation with pleconaril (Study 3b) and rifampin (Study 6c).

Study	LS-GMR	90% CI
3b (n=14)	1.13[a]	1.1 – 1.15
	1.18[b]	1.14 – 1.21
	1.16[c]	1.13 – 1.19
	1.15[d]	1.12 – 1.18
6c (n=19)	1.34[a]	1.21 – 1.46[e]
	1.3[b]	1.18 – 1.41[e]
	1.31[c]	1.19 – 1.43[e]
	1.32[d]	1.2 – 1.44[e]

[a] For each study, LS-GMR of observed $CL_{INDUCTION}$ / observed $CL_{BASELINE}$

[b] For each study, LS-GMR of estimated $CL_{INDUCTION}$ / observed $CL_{BASELINE}$; utilized $AUC_{0–1}$

[c] For each study, LS-GMR of estimated $CL_{INDUCTION}$ / observed $CL_{BASELINE}$; utilized $AUC_{0–2}$

[d] For each study, LS-GMR of estimated $CL_{INDUCTION}$ / observed $CL_{BASELINE}$; utilized $AUC_{0–4}$

[e] Lack of bioequivalence, the 90% CI is outside the 0.8–1.25 range.

Abbreviations: 90% CI, ninety percent confidence interval; LS-GMR, least squares geometric mean ratio.