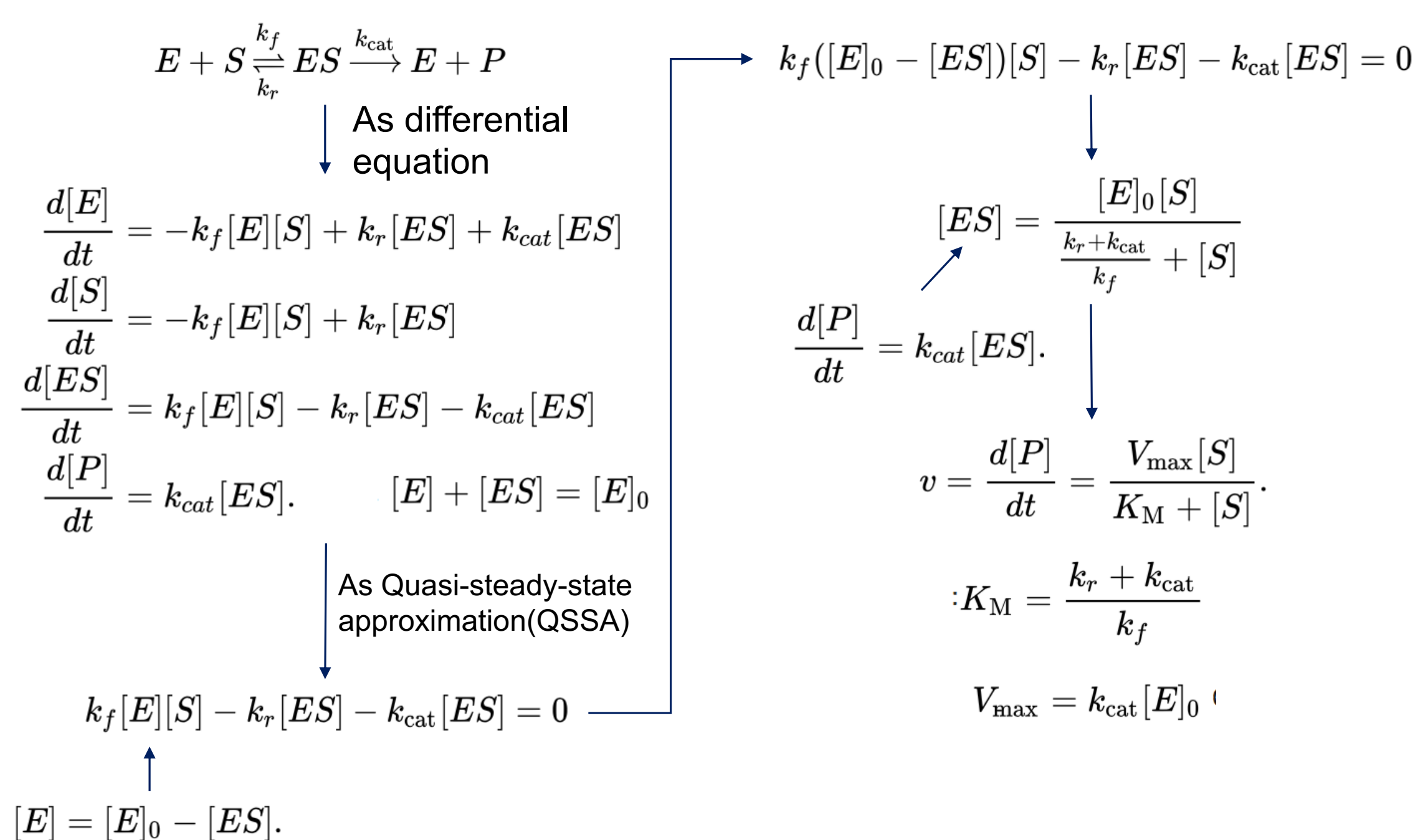


Objectives

The procedure for prediction of in vivo hepatic clearance using in vitro kinetic data from liver microsomes is well-established and is in wide use. The approach derives from calculation of intrinsic clearance (CL_{int}) based on Michaelis-Menten (MM) kinetics, with the assumption that total enzyme concentration at the active site (E_0) was much lower than the Michaelis constant (K_M). However, in drugs with very low K_M , predicted results could be inappropriate because the above assumption failed to hold. The main aim of this study is to afford a comparison of standard and new in vivo hepatic clearance methods using drugs with low K_M and suggestion of new strategies for appropriate prediction in these drugs.

Method

Canonical Model



therefore

$$v = \frac{dP}{dt} = \frac{k_{cat}E_0[S]}{[S] + K_M} \xrightarrow{\text{In liver } [S] \ll K_M} \frac{dP}{dt} = \frac{k_{cat}E_0[S]}{[S] + K_M} \approx \frac{k_{cat}E_0[S]}{K_M} = CL_{int}E_0[S]$$

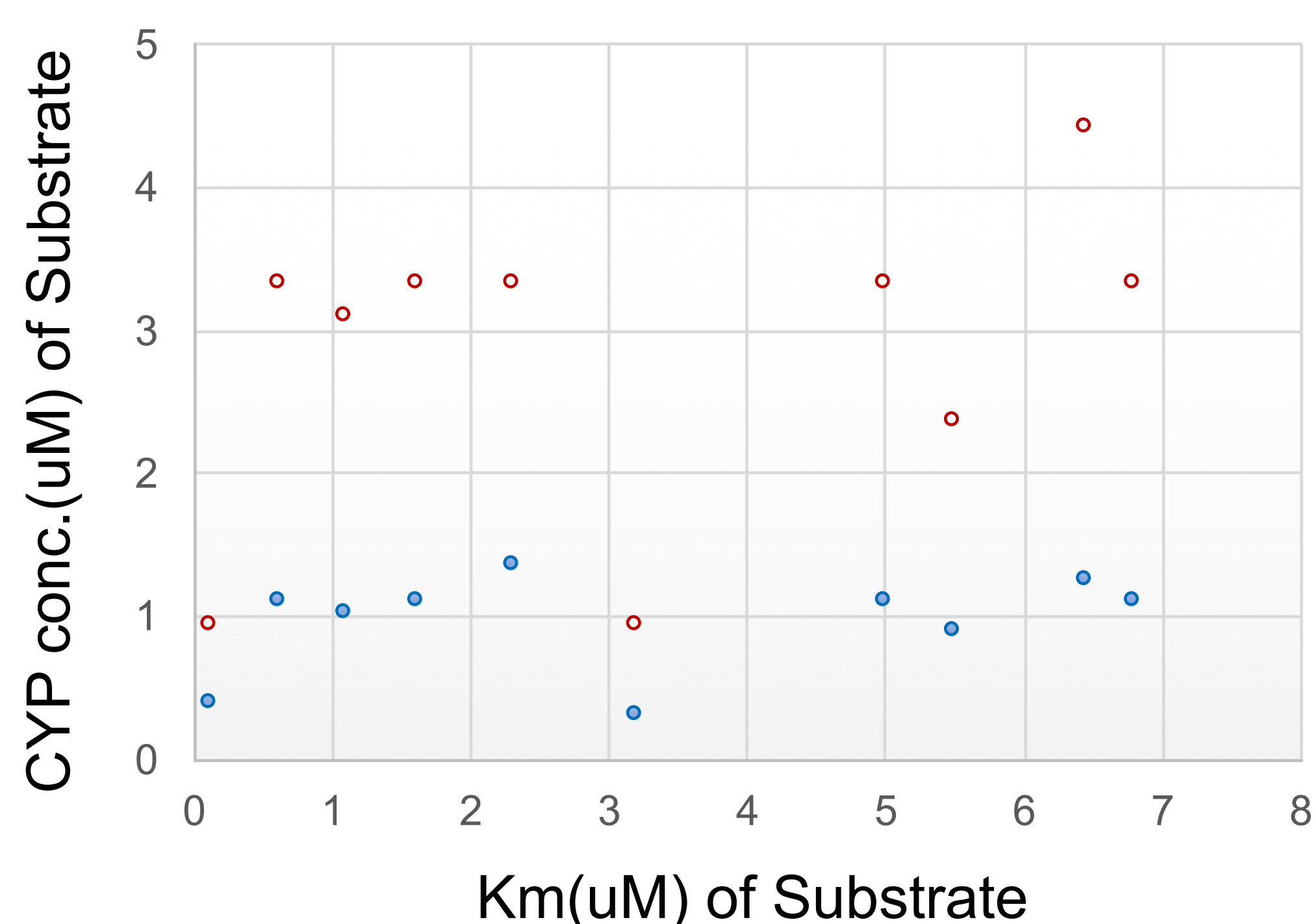
In case of HLM
 E_0 = mg protein
 k_{cat} = pmol/min/mg protein

to keep the assumption of QSSA

$$E_0 \ll K_M + S$$

In case of Supersome
 E_0 = pmol CYP
 k_{cat} = pmol/min/pmol CYP

However, Does $E_0 \ll K_M + S$ hold in liver?

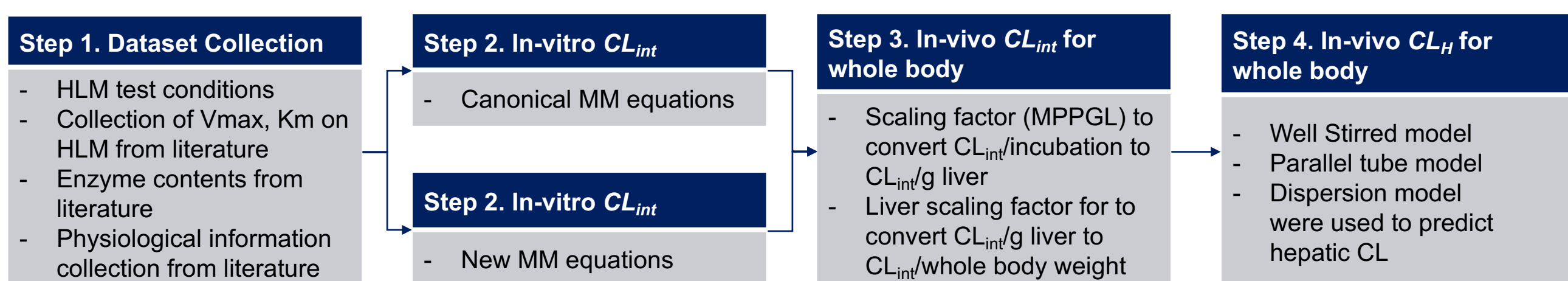


New approach to overcome $E_0 \ll K_M + S$ assumption

Another way to simplify the full MM model is based on the total quassi-steady-state approximation. According to those approximation, we can simplified full model as following equations:

$$\frac{dP}{dt} = k_{cat}C(\bar{S}) \approx \frac{k_{cat}E_0[\bar{S}]}{K_M + E_0} = CL_{int}E_0 \frac{K_M}{K_M + E_0} [\bar{S}]$$

Comparisons between canonical method and new method



Well Stirred Model

Parallel tube Model

Dispersion Model

$$CL_H = \frac{Q_h \cdot f_{u-blood} \cdot \frac{CL_{int}}{f_{u-mic}}}{Q_h + f_{u-blood} \cdot \frac{CL_{int}}{f_{u-mic}}}$$

$$CL_H = Q_h \cdot [1 - e^{\left(\frac{-f_{u-blood} \cdot CL_{int}}{Q_h \cdot f_{u-mic}}\right)}]$$

$$CL_H = Q_h \cdot [1 - \frac{4a}{(1+a)^2 \cdot e^{(a-1)/2 \cdot D_n} - (1-a)^2 \cdot e^{-(a+1)/2 \cdot D_n}}]$$

$$D_n = 0.17$$

$$a = \sqrt{1 + 4 \cdot R_n \cdot D_n}$$

$$R_n = \frac{f_{u-blood} \cdot CL_{int}}{Q_h \cdot f_{u-mic}}$$

Results

Simulated results using canonical and new model

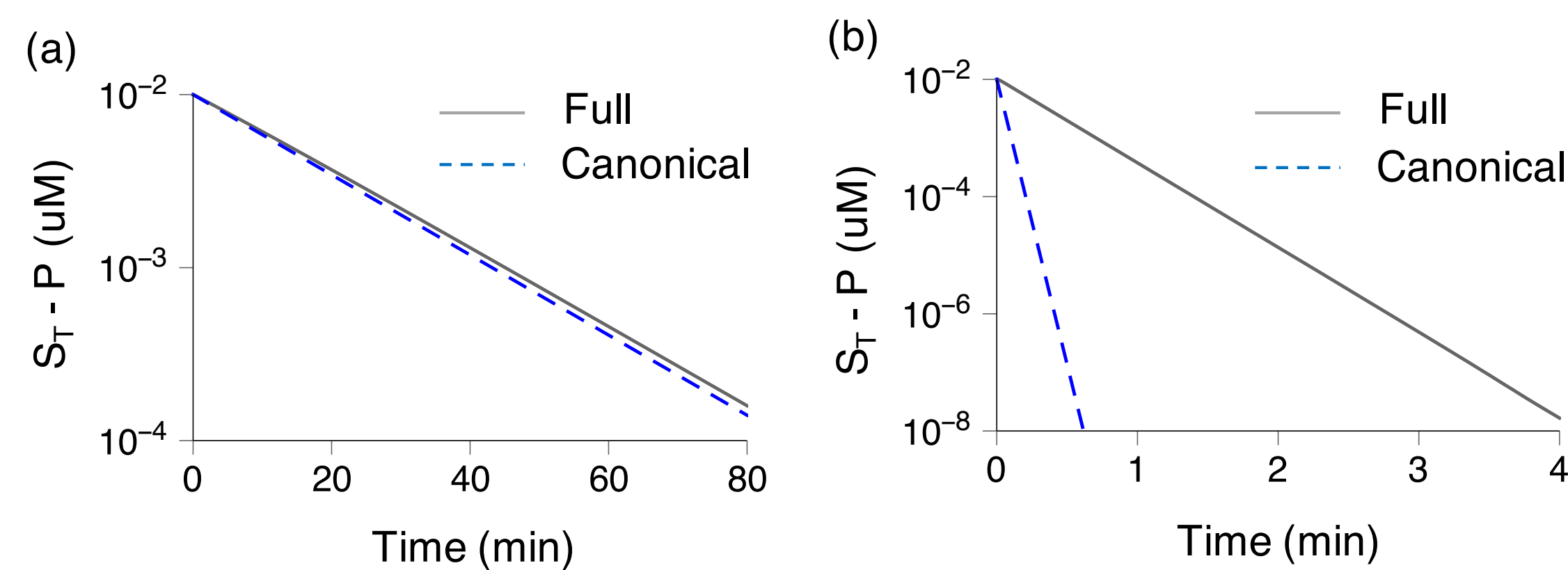


Figure 1 Simulated propafenone metabolism under in vitro (CYP2D6=0.0017uM) and in vivo (CYP2D6=0.95uM) conditions. The MM model accurately simulate the drug metabolism under in vitro condition (a), but not in vivo condition (b). For the simulations, $V_{max} = 3.72 \text{ pmol min}^{-1} \text{ pmol}^{-1} \text{ CYP}$, and $K_M = 0.12 \text{ uM}$ are used based on the experimental measurement. $k_f = 100 \text{ uM/min}$ and $k_b = 8.27/\text{min}$ are used for the full model simulation. The fraction of free form is not considered for the simplicity.

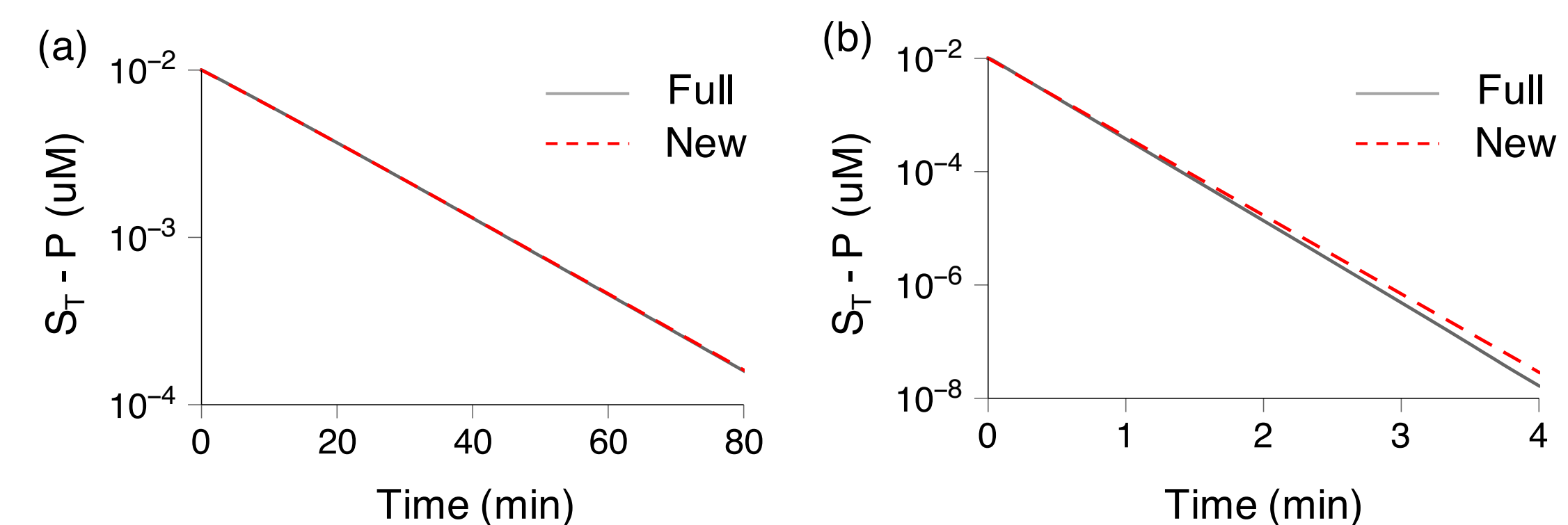


Figure 2 Simulated propafenone metabolism under in vitro (CYP2D6=0.0017uM) and in vivo (CYP2D6=0.95uM) conditions. The new model accurately simulates the drug metabolism under both in vitro (a) and in vivo conditions (b). See Fig. 1 for the values of parameters for the simulations.

Differences between the canonical and new predictions under E_0 and K_M

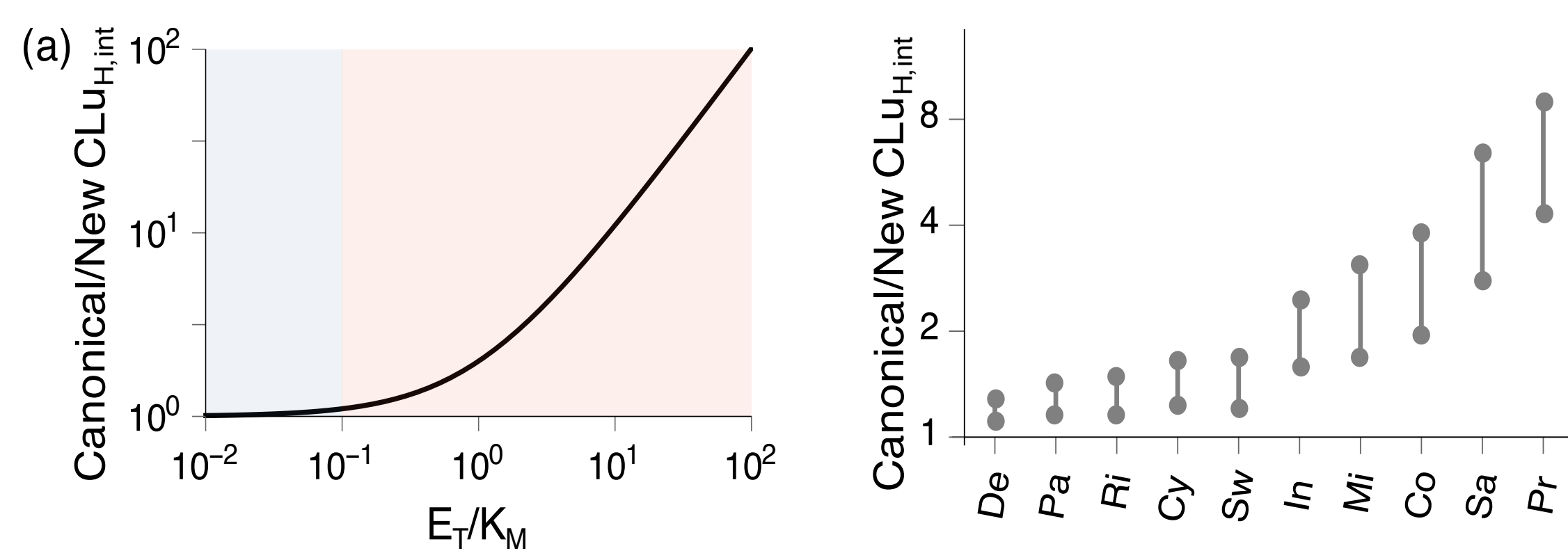


Figure 3 The ratio between $CL_{uH,int}$ predicted with the canonical and new approach. (a) Unless $E_T/K_M > 0.1$, the $CL_{uH,int}$ predicted with the canonical approach shows considerable error, which is highlighted as red color (a). (b) The predicted $CL_{uH,int}$ with the canonical approach is considerably larger than those with the new approach for various drugs whose K_M is not 10 fold higher than their major metabolic CYP concentration in liver: De=Dextromethorphan, Pa=Paclitaxel, Ri=Ritonavir, Cy=Cyclosporine, Sw= S-warfarin, In= Indinavir, Mi= Midazolam, Co= Coumarin, Sa= Saquinavir, Pr= Propafenone.

Accuracy and precision of predicted values from canonical and new methods

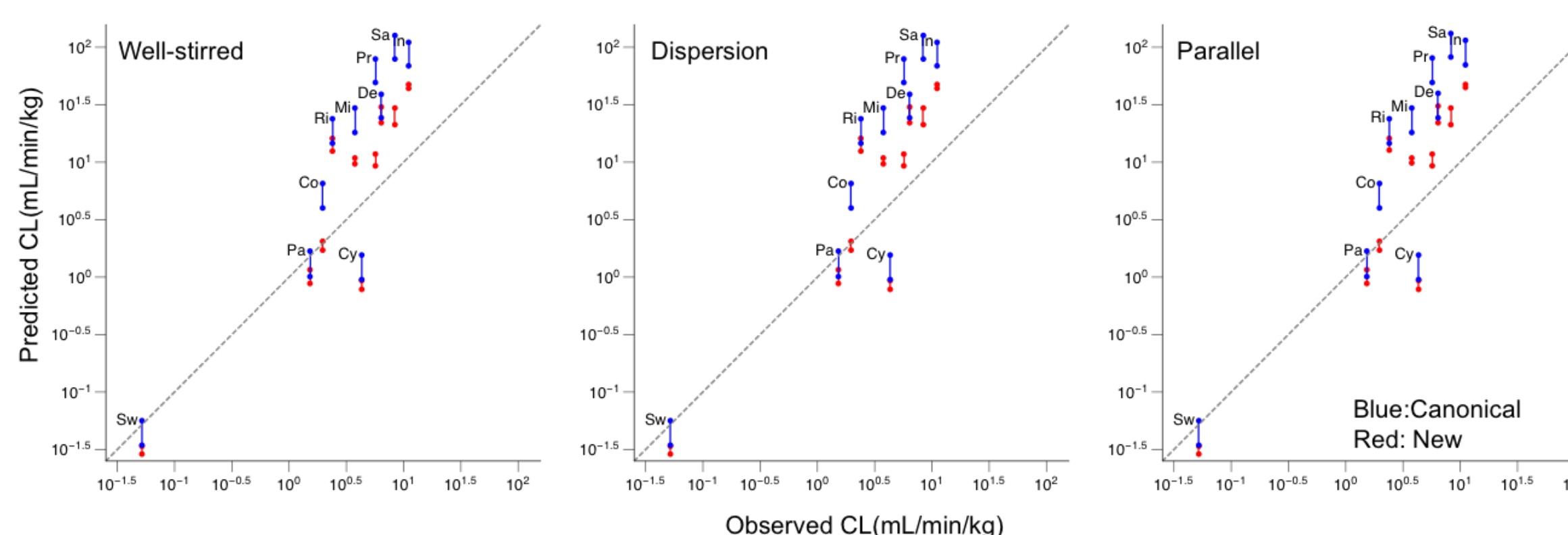


Figure 4 The new approach provides more accurate prediction for CL_{uH} than canonical approach. (a) Relationships between measured hepatic drug clearance rates (CL_H) and values predicted by either canonical or new approach. Here, well-stirred model is used to estimate CL_H (see Method for details). (b) Precision error of predicted CL_H (the predicted/measured CL_H ratio) with the canonical and new approach. See Fig. 3 for the abbreviation of drug name.

	Well-stirred model		Parallel tube model		Dispersion model	
	Canonical	New	Canonical	New	Canonical	New
AFE	3.23	1.60	3.27	1.61	3.26	1.60
GMFE	4.51	2.70	4.57	2.71	4.55	2.70
RMSE	45.8	14.6	47.5	14.8	47.0	14.7
R-RMSE	6.34	2.32	6.53	2.34	6.47	2.34

AFE, Average fold error; GMFE, Geometric mean fold error; RMSE, Root mean squared error; R-RMSE, Relative root mean squared error

Discussion

Since CYP enzyme saturation can occurs easily for drugs with lower K_M value, prediction of drug clearance in vivo from in vitro data becomes challenging with the canonical approach as pharmacokinetic behavior of drugs do not follow conventional Michaelis-Menten kinetics. Indeed, the canonical approach fails to estimate CL_H for drugs if their K_M is not 10-fold higher than the concentration of their major metabolic CYP concentrations in the liver. Thus, we propose an alternative approach considering the enzyme saturation and leading accurate estimation of CL_H for drugs regardless of their K_M . In this study, drugs whose metabolism dominated by a single CYP isoform is considered. For drugs metabolized by multiple CYPs, the canonical approach estimates the contribution of each CYP to net metabolism with the V_{max}/K_M and then combines them to estimate global CL_H . Similarly, our approach can be extended for drugs with multiple metabolic pathways by simply combining the estimated contribution of each CYP to metabolism with $V_{max}/(K_M + E_0)$. It would be an interesting future work to investigate how this extended approach improves the estimation of drug clearance.