Does the Flux Competition Method Add Significant Value in Species Difference Protein Binding Evaluation?

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Background

The competition method is frequently evaluated in drug development to compare the fraction unbound (f_u) differences between two species. In the experiment, compound is spiked in plasma on both sides of membrane and dialyzed for a long time to reach equilibrium (defined as equilibrium competition). Kalvass et al.[1] reported the flux dialysis in 2018 in which the flux dialysis-based competition method (defined as flux competition) was compared with the equilibrium competition method. According to theoretical analysis, it was reported that flux competition showed the benefits of allowing for the calculation of actual fu values and not requiring equilibrium to be reached. The objective of this study is to evaluate whether the flux competition method has significant advantages over the equilibrium competition method in species difference PPB evaluation.

Methods

- Eleven compounds with moderate or minimal species differences in f_u, as well as large (>3-fold ratio) species differences were selected for validation.
- In the equilibrium competition, compounds spiked at both sides of the equilibrium dialysis device were tested at 2 μM in human plasma and plasma of a second species (rat or dog) for multiple timepoints up to 48 hr or 96 hr.
- In the flux competition method, compounds were spiked at one side of the device and incubated for up to 120 hr, and the experiments were carried out with spiking on different sides.
- Equilibrium dialysis of these compounds in the plasma of both species were performed to obtain control data. The spiked human or a second species (rat or dog) plasma was dialyzed against PBS for 4 hr.
- For equilibrium competition, when the system reached equilibrium, the f_u ratio between the 2 species was calculated using the following **Equation 1** [1].

$$\frac{f_u(2)}{f_u(1)} = \frac{\frac{C_u(2)}{C_t(2)}}{\frac{C_u(1)}{C_t(1)}} = \frac{C_t(1)}{C_t(2)} \qquad \textbf{Equation 1}$$

- For flux competition, according to the kinetic model explained in Kalvass's paper [2], $\frac{C_{receiver}}{C_{donor}}$, denoted as R, can be fitted with incubation time. When the system reaches a steady state where $f_{u,\ receiver} \times C_{receiver}$ is equal to $f_{u,\ donor} \times C_{donor}$, the R_{eq} (which is equal to the f_u ratios of donor versus receiver side) can be obtained by nonlinear curve fitting for **Equation 2**. k is the exponential rate constant.

$$R = Req \times (1\text{-}e^{-k \times t}) \qquad \textbf{Equation 2}$$

Results

Table 1. F_u ratios of 11 compounds in both competition and equilibrium dialysis method

For 7 of the 11 compounds, comparable f_u ratios were obtained from both competition methods, with f_u ratios within 2-fold of the mean. For 4 of the 11 compounds, inconsistent data were observed between the two flux competition experiments: compound spiked in human side and in animal plasma side. Besides, 3 of the f_u ratio spiked in human plasma were significantly larger than when spiked in animal plasma, as well as the data from the equilibrium competition method. All of the 11 compounds, the f_u ratios of the equilibrium competition and the equilibrium dialysis are within 2-fold. Data represents the mean of 3 replicate incubation samples.

Compound	The second species	F_u human/f_u second species				
		Equilibrium Competition Method	Flux Competition Method (Spike in human plasma side)	Flux Competition Method (Spike in the second species plasma side)	Mean of the 3 data obtained from competition Method	In-house equilibrium dialysis method
Sildenafil	Dog	0.4	0.62	0.35	0.46	0.51
Prazosin	Dog	0.29	0.35	0.22	0.28	0.23
Tasulosin	Rat	0.41	0.33	0.22	0.32	0.49
Rosuvastatin Calcium	Rat	2.07	1.91	1.96	1.98	2.27
Topotecan	Rat	1.01	1.06	1.05	1.04	1.20
Warfarin	Dog	0.28	0.31	0.17	0.25	0.14
Nirmatrelvir	Dog	7.11	11.66	10.19	9.65	9.89
Cefpiramide	Dog	0.06	0.12	0.05	0.08	0.04
Pimozide	Dog	2.08	197.58	2.42	67.36	1.81
Amiodarone	Dog	1.39	34.98	0.07	12.15	1.84
Nifedipine	Rat	6.8	98.93	7.44	37.72	7.16

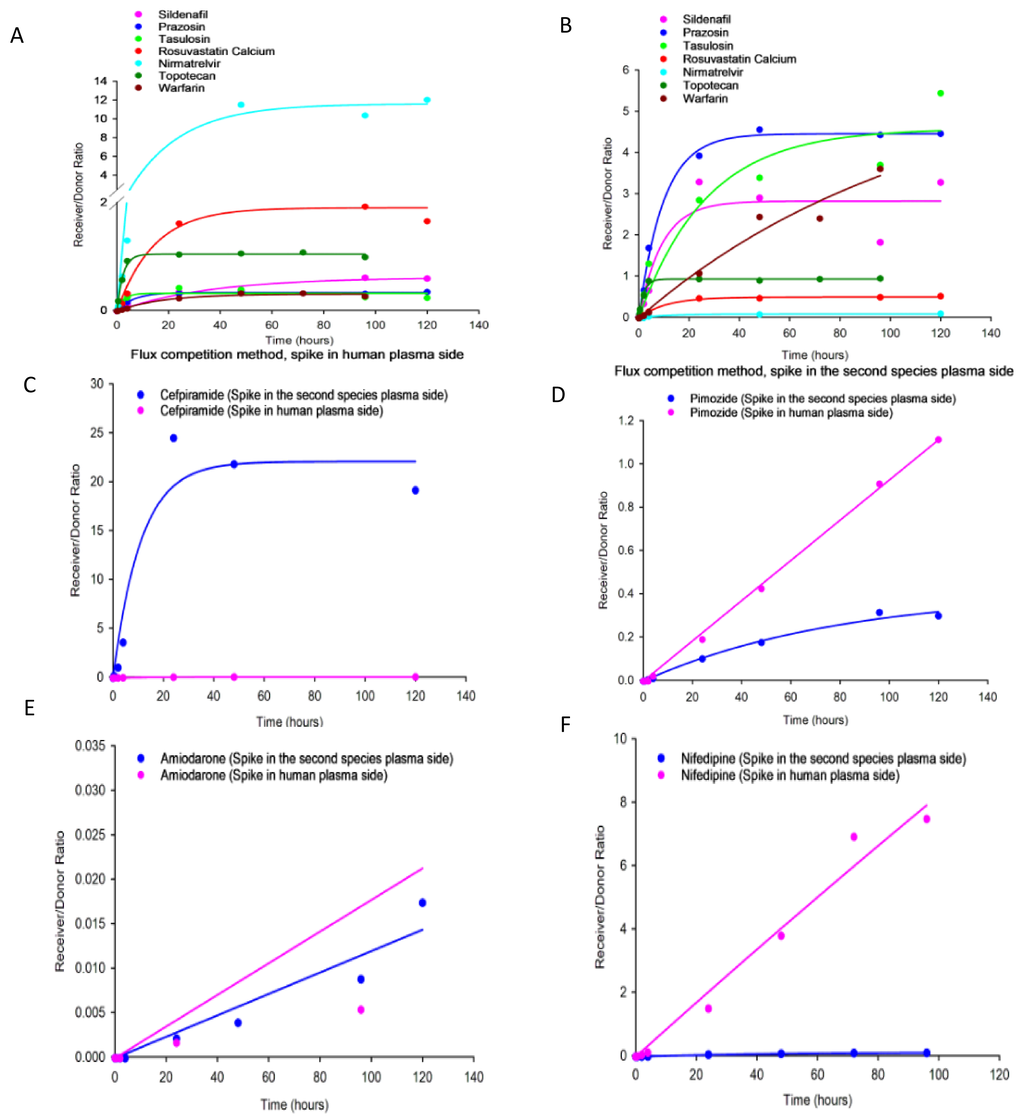

Figure 1. Receiver/donor ratio-time curves of the 11 compounds.
A and B: the 7 compounds that exhibited consistent data. All the curves reached a plateau.
C: for cefpiramide, both curves reached a plateau but the obtained f_u ratios showed a 2.5-fold difference.
D, E and F: for pimozide, amiodarone, and nifedipine, the receiver/donor ratio-time curves did not reach a plateau within 120 hr of incubation.

Table 2. Parameters R_{eq} and k obtained by nonlinear curve fitting.

These 11 compounds had a total of 22 sets of data. Most of the P values from statistical analysis were lower than 0.05. For the below sets of data, the p values were greater than 0.05 for R_{eq} and k, indicating that the fitted curves were not significant. We further calculated the R_{slope} values using linear regression from the early times points of the R-t curve, and similar f_u ratios were obtained (data not shown).

Spike in human plasma side			
Pimozide		Coefficient	P
	R_{eq}	197.5807	0.96
	K	4.71E-05	0.96
Amiodarone		Coefficient	P
	R_{eq}	34.9766	1
	K	5.08E-06	1
Nifedipine		Coefficient	P
	R_{eq}	98.9304	0.84
	K	0.0009	0.84
Spike in the second species plasma side			
Amiodarone		Coefficient	P
	R_{eq}	13.9958	1
	K	8.59E-06	1

Conclusion

- Reaching equilibrium is required to obtain high-quality data for f_u estimations on both sides.
- Per test compounds in this investigational study, the flux competition method does not necessarily add more value to competition PPB evaluation due to the much longer time required to reach equilibrium compared to the equilibrium competition method.

References

[1] J. Cory Kalvass, et al. Drug Metab Dispos. 2018; 46(4):458-469.
[2] Di L, et al. J Pharm Sci. 2017; 106(12):3442-3452.

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