Novel Approach to Determine Plasma Protein Binding for Labile Compounds Using Equilibrium Dialysis

Piyun Chao[1], Shurra Johnghar[1], Hui Wu[1], Himanshu Arora[1], Gary (Ganfeng) Wang[1], Yajie Zhang[1], Libo Xu[1], Jennifer Thomas[2], Eyad Kalawy Fansa[3], Faith Stevison[3]

[1]WuXi AppTec, DMPK department, 6 Cedarbrook Dr., Cranbury, NJ 08512, United States; [2]Certara, New Jersey, United States; [3]Constellation Pharmaceuticals, Inc., a MorphoSys Company, Boston, MA, United States

Poster#: P96

Objective

Equilibrium dialysis (ED) is one of the commonly used techniques for determining plasma protein binding (PPB) of drug molecules and their metabolites. ED is usually conducted at 37°C for 4-6 hours or longer (18-24 hours). For compounds with stability concerns, equilibrium cannot be reached under typical ED incubation conditions due to continuous degradation/ metabolism. At lower temperature, rates of degradation and/or enzymatic reactions decrease and therefore compounds may better remain stable for ED. The objective was to evaluate a novel ED approach for PPB determination of labile compounds, at a lower temperature with pre-saturation technique[1] (ED with initial incubation conditions close to equilibrium).

Method

- Compound (cpd) A and B were initially found unstable in human plasma at room temperature at timepoints beyond 2h (data not shown). To obtain the acceptable incubation conditions for PPB evaluations, stability of labile compounds (cpd A & B) was first evaluated in buffer at 37°C, then further evaluated in buffer and in diluted (10%) and undiluted plasma from mouse, rat, dog, monkey and human at 4°C.
- Since both cpd A & B demonstrated sufficient stability in buffer and in plasma (diluted and undiluted) at 4°C, the feasibility of PPB evaluation was first investigated by studying the time required to reach equilibrium utilizing ED method at 4°C. The time required to reach equilibrium for each compound was evaluated in undiluted and diluted human plasma using High Throughput Dialysis (HTD) devices at 4°C for up to 6-hr to obtain approximate PPB values. Compounds with known PPB, i.e., warfarin (high protein bound) and propranolol (medium protein bound, preferentially to α-1 acid glycoprotein), were also evaluated in undiluted human plasma at 4°C.
- An additional time-to-equilibrium assay was performed for cpd A & B, and warfarin and propranolol with the initial conditions close to equilibrium i.e., spiking the buffer side of the HTD devices with the approximate equilibrium concentration identified in the previous experiment (pre-saturation), at 4°C for up to 6-hr to determine the optimal incubation conditions to reach equilibrium.
- The PPB for cpd A & B at 0.1, 1 and 10 µM in plasma from all species was then determined using ED under the established pre-saturation incubation conditions at 4°C.

Results

- Both cpd A & B were not stable in buffer at 37°C (**Figure 1**) with 14% and 49% remaining at the end of 4-hr incubation. At 4°C, stability of both compounds improved significantly in buffer (**Figure 1**), and demonstrated stability in undiluted and diluted plasma, with 71.9% to 102% recoveries after a 2-hr incubation (**Table 1**).
- After dialyzing cpd A & B in undiluted and diluted (10%) human plasma in HTD devices at 4°C for up to 6-hr, the percent recovery ranged from 89.1% to 95.3% (**Table 2**) without apparent differences between the undiluted and diluted human plasma. Time-dependent increases of cpd A & B concentrations in the buffer side of HTD devices were observed over 6-hr (**Figure 2A**), indicating lack of equilibrium across the membrane. At 6-hr, the %unbound for cpd A & B were 2.64% to 2.78% and 3.16% to 4.12%, respectively. Therefore, in the next time-to-equilibrium assays, the dialysis was initiated with 95% of the total concentration being tested in undiluted plasma and 5% of the total concentration being tested in buffer (for both cpd A & B). Time-dependent increases of warfarin and propranolol concentrations in the buffer side of HTD devices were also observed over 4 to 6-hr (**Figure 2C**), indicating lack of equilibrium across the membrane for these two compounds as well. Therefore, in the next time-to-equilibrium assays, the dialysis was conducted with 99% in human plasma and 1% in buffer for warfarin; and 74% in human plasma and 26% in buffer for propranolol.
- Upon pre-saturation, there were no apparent time-dependent changes of concentrations of cpd A & B in buffer side of HTD over 6-hr at 4°C (**Figure 2B**), indicating equilibrium had been achieved. %Recovery for cpd A & B was similar as the 1st time-to-equilibrium assay (**Table 2**). Time-dependent decreases of warfarin concentrations in the buffer (receiver) chambers were observed from 1 to 4-hr incubations and remained relatively unchanged from 4 to 6-hr, with %unbound 0.44%-0.51% at 6-hr in two assays (**Figure 2D**). For propranolol, no apparent time-dependent changes of concentrations in dialysates, with %unbound of 26.7% - 27.1% (**Figure 2D**) at 6-hr in two assays.
- The PPB of cpd A & B at 3 concentrations in plasma from 5 species were then determined using the established low temperature incubation conditions with pre-saturation (4°C, 95% in plasma/5% in buffer, 4 hours). The PPB results are in **Table 3** and percent recovery ranged from 86.4% to 106% (**Table 4**).
- Comparing warfarin and propranolol PPB in human plasma determined at 4°C with pre-saturation conditions and under the conventional ED conditions at 37°C (**Table 5**), the determined PPB properties were comparable to WuXi AppTec historical data, indicating the described method may be useful for compounds unstable under conventional ED conditions.

Cpd	Plasma	% Remaining at 120 min, 4°C in Plasma from Various Species				
		Mouse	Rat	Dog	Monkey	Human
A	Diluted (10%)	101	101	102	90.5	88.2
	Undiluted	96.0	75.5	71.9	87.1	90.0
B	Diluted (10%)	86.2	93.3	85.0	100	86.9
	Undiluted	80.9	80.4	94.8	79.5	90.7

Table 1. Stability of Cpd A & B in diluted and undiluted plasma at 4°C

Cpd	Plasma	% Recovery in HTD dialysis (Human Plasma) at 4°C			
		1 hr	2 hr	4 hr	6 hr
A	Diluted (10%)	94.7	89.0	92.5	89.1
	Undiluted	107	97.7	94.3	89.6
	Undiluted*	97.6	96.6	89.3	84.4
B	Diluted (10%)	99.0	93.3	98.0	95.3
	Undiluted	100	98.1	101	99.4
	Undiluted*	93.9	96.7	93.6	99.2

Table 2. Cpd A & B %Recovery after dialyzing in diluted (10%) and undiluted human plasma at 4°C. * data from the 2nd time-to-equilibrium assay

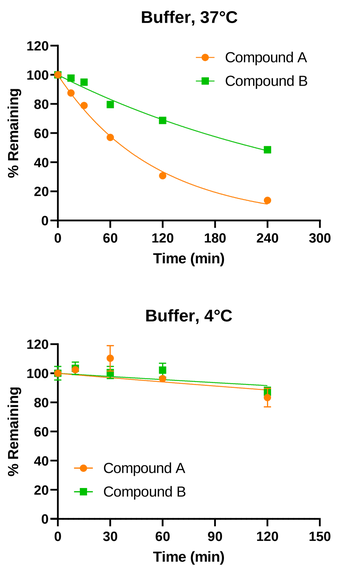

Figure 1. Stability of Cpd A & B in buffer at 37°C (top) and 4°C (bottom)

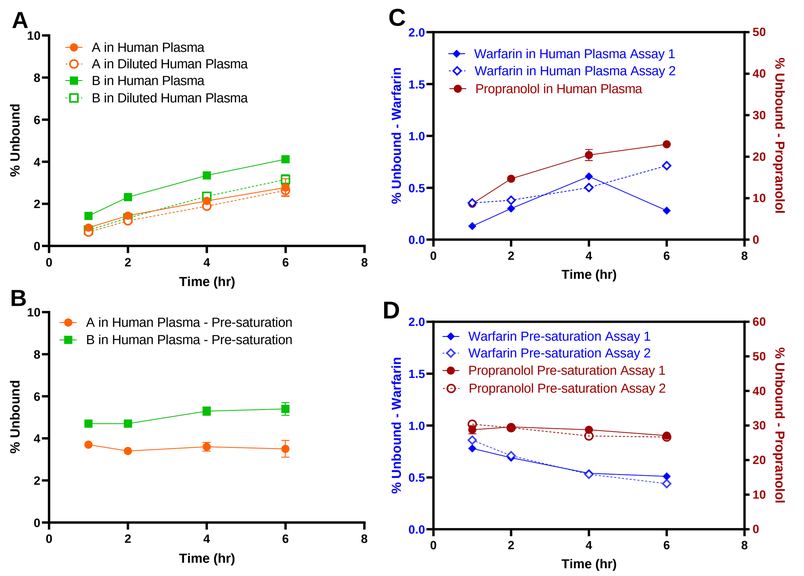

Figure 2. Time to equilibrium at 4°C for (A) cpd A & B in diluted and undiluted human plasma; (B) cpd A & B with pre-saturation in human plasma; (C) reference compounds warfarin and propranolol in human plasma; and (D) warfarin and propranolol in human plasma with pre-saturation

Cpd	Concentration (µM)	% Unbound in Plasma from Various Species				
		Mouse	Rat	Dog	Monkey	Human
A	0.1	2.62	2.38	2.40	2.79	2.47
	1	2.63	2.21	2.73	3.09	2.82
	10	2.58	2.33	3.06	3.48	2.64
B	0.1	3.67	2.49	4.63	6.05	5.27
	1	2.98	2.27	4.12	6.73	4.40
	10	3.21	2.40	4.31	5.93	4.61

Table 3. %Unbound of Cpd A & B determined using ED for 4 hr at 4°C, with pre-saturation condition (ca. 95% in plasma and 5% in buffer)

Cpd	Concentration (µM)	% Recovery in Plasma from Various Species at the end of Dialysis				
		Mouse	Rat	Dog	Monkey	Human
A	0.1	97.4	93.8	97.8	110	106
	1	102	101	93.0	106	102
	10	102	90.4	88.7	102	102
B	0.1	86.4	95.9	94.8	92.3	92.9
	1	93.7	94.6	92.7	95.8	98.7
	10	94.8	101	97.3	94.8	96.9

Table 4. Recovery of Cpd A & B after ED for 4 hr at 4°C, with pre-saturation condition (ca. 95% in plasma and 5% in buffer)

Cpd	Pre-saturation Condition	% Unbound in Human Plasma Determined using Equilibrium Dialysis at Different Temperatures (data in parenthesis are %recovery)	
		4°C (6-hr with pre-saturation)	37°C (4-18 hr, without pre-saturation)*
Warfarin (2 µM)	99% in plasma 1% in buffer	0.44% – 0.51% (99.1 – 99.6, 96.6 – 94.6)	0.51% – 2.34% (90.6% - 128%)
Propranolol (2 µM)	74% in plasma 26% in buffer	26.7% – 27.1% (89.1 – 93.1, 76.3-94.0)	24.0% – 25.8% (93.8% – 106%)

Table 5. %Unbound of warfarin and propranolol determined using ED at the end of 6-hr at 4°C, with pre-saturation condition, and at 37°C for 4-18-hr. *WuXi AppTec internal data from 2-8 studies.

Conclusion

For labile compounds, PPB can be determined using ED at low temperature, e.g., 4°C, if sufficiently stable under these conditions. For labile compounds requiring extended incubation times to achieve equilibrium at low temperature, the approximate equilibrium concentrations should first be estimated from a time-to-equilibrium incubation, pre-saturation conditions can then be utilized to reduce the time required to reach full equilibrium, and finally PPB can be measured under optimal pre-saturation ED conditions with acceptable percent recoveries.

Reference

[1] Riccardi K et al. 2015. Plasma Protein Binding of Challenging Compounds. J Pharm Sci 104:2627–2636