Protein Binding Methodologies and Relevance for DDI - An Industry Perspective

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WORLDWIDE RESEARCH & DEVELOPMENT

SCIENCE FOR LIFE-CHANGING IMPACT

Concerns of Regulatory Agencies on PPB in DDI Prediction

- Set a lower limit of PPB f_u to 1% for DDI
- If experimental f_u < 1%, the cutoff f_u value of 1% should be used for DDI prediction
- On top of the 10- or 50-fold safety margin

J Pharm Sci, 2017, 106(12): 3442-3452

J Pharm Sci, 2019, Online

Regulatory DDI Draft Guidelines on Plasma Protein Binding

FDA DDI Draft Guideline (2017) $I_{max,u}$

*Considering uncertainties in the protein binding measurements, the unbound fraction in plasma should be set to 1% (fraction unbound in the plasma ($f_{u,p}$) = 0.01) if experimentally determined to be < 1%.

EMA DDI Draft Guideline (2012)

Hepatic (and renal) exposure

If the enzyme studied is mainly available in the liver, or the kidney/another organ with main drug input from the systemic circulation, the concentration range should allow determination of a Ki which is ≤ 50-fold the mean unbound Cmax obtained during treatment with the highest dose. In this estimation, when an estimation of fu is used, figures lower than 1% should not be used due to the uncertainties in the estimation. Thus, as an example, if the free fraction has been estimated to be 0.5% *in vitro* or *ex vivo*, a 1% free fraction should be used.

Japanese DDI Draft Guideline (PMDA) (2014)

maximum total blood concentration (unbound + bound forms) of the inhibitor in the steady state. When the protein binding ratio in blood is high (99% or higher), making measurement difficult, $f_{u,b}$ = 0.01 should be used. $[I]_g$ may also be estimated with the equation $[I]_g = F_a \times k_a$

Rationales of Setting 1% f_u Cutoff

- Perceived uncertainties in measuring f_u values of highly bound compounds
- Historically sub-optimal methods
  - Purity of radio-labeled materials in radiometric methods
  - Ultrafiltration (high nonspecific binding)
- Err on the conservative side for DDI prediction to avoid false negatives

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Example for Renal Transporter DDI

- EMA DDI guideline to predict OAT1/3 DDI risk (DDI risk if $K_i \leq$ 50-fold $C_{max,u}$)
  - f_u = 0.008, no DDI risk
  - f_u = 0.01, potential DDI risk
- AUCR calculation (mechanistic model): negligible (~1). Clinically relevant DDI was not predicted.
- Assessor disagreed and potential DDI with OAT1 and OAT3 substrates was included on the label.

1% f_u cutoff predicts higher DDI risk for highly bound compounds

Mol Pharm, 2013, 10, 4207-4215. J Pharm Sci, 2017, 106(12): 3442-3452.

Example for OATP1B1 DDI Prediction

OATP1B1 IC_{50} (µM)	f_u Measured	R-value Predicted with lower limit f_u of 0.01	R-value Predicted with Measured f_u	Observed Clinical DDI (AUC fold)
0.17	0.002	6.1	2.0	1.8

$$R = 1 + ((f_{u,p} \times I_{in,max})/IC_{50}) \geq 1.1$$

$$I_{in,max} = (I_{max} + (F_a F_g \times k_a \times Dose))/Q_h/R_B$$

- **Using f_u 1% cutoff overpredicts clinical DDI for highly bound compounds**

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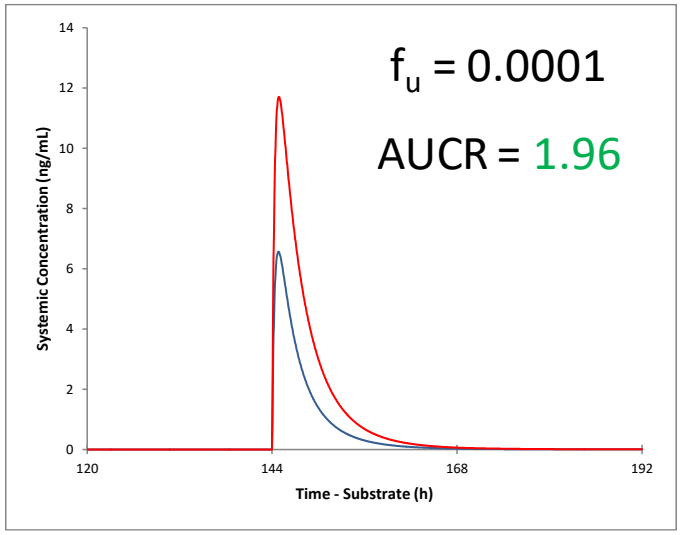
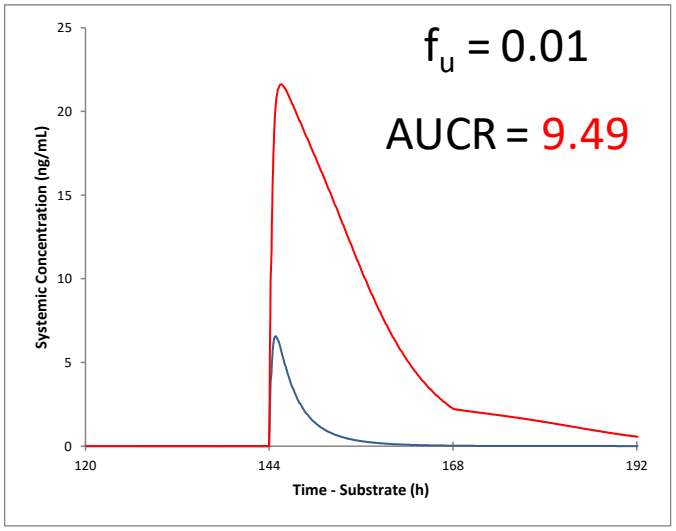
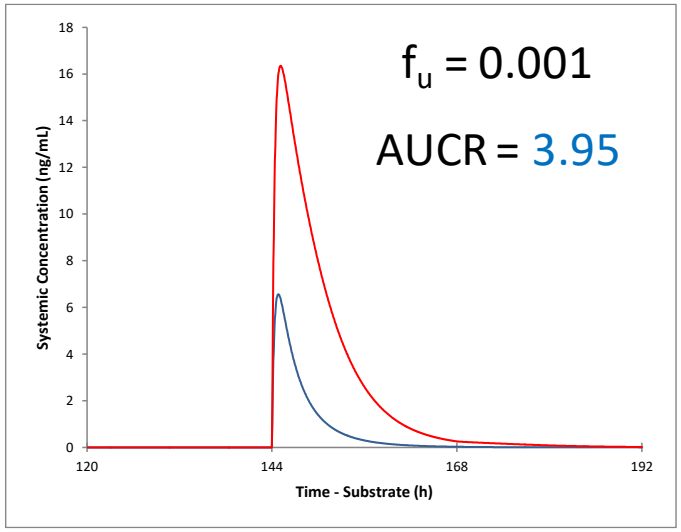
Implications of Setting Lower Limit of f_u to 1%

Inhibition of 3A, Midazolam







- Cause overprediction of DDI
- Lead to unnecessary and expensive clinical studies
- More stringent inclusion / exclusion criteria
- 1% f_u cutoff is somewhat arbitrary and unscientific

J Pharm Sci, 2017, 106(12): 3442-3452.

Plasma Protein Binding Methods

- Equilibrium dialysis (IQ survey: 13 companies/14)
- Ultracentrifugation
- Ultrafiltration
- Equilibrium Gel Filtration
- Transil®
- HSA/AAG columns
- Off rate measurement (Biacore, charcoal)

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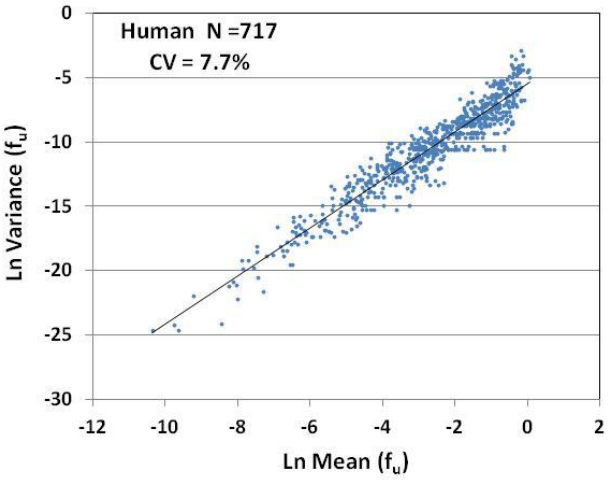
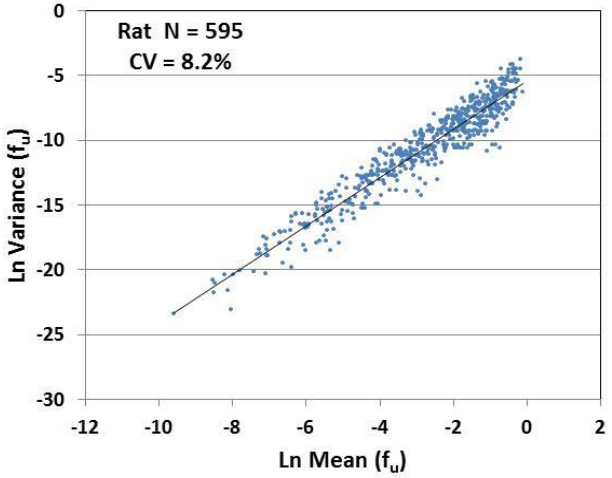
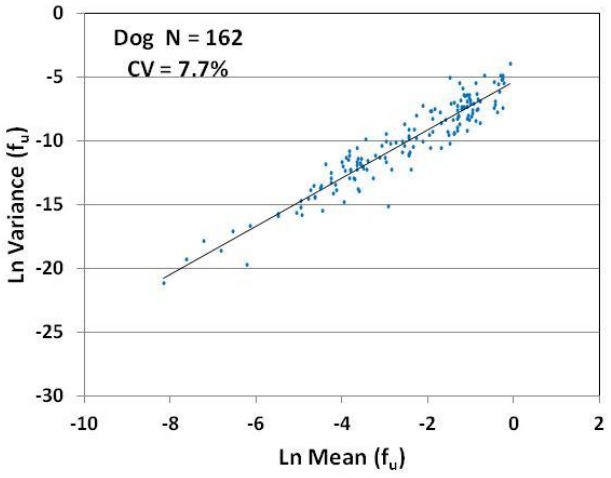
Challenging Compounds for PPB

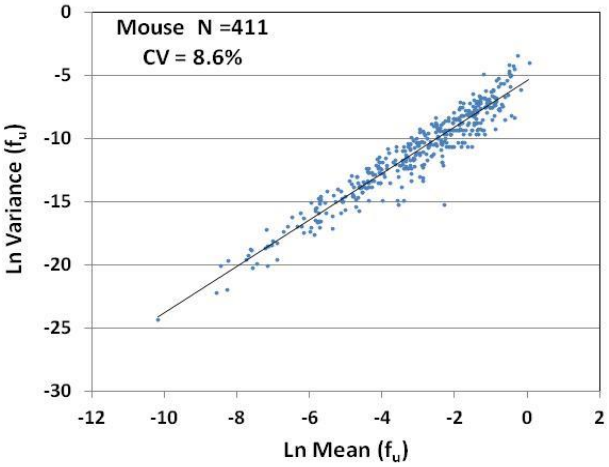
- Lipophilic acids
  - Liver targeting compounds
- Lipophilic insoluble compounds
  - Itraconazole, Amiodarone
- Large molecules (high MW)
  - Peptides, oligonucleotides
- Unstable compounds
  - ADC (Antibody Drug Conjugates), prodrugs

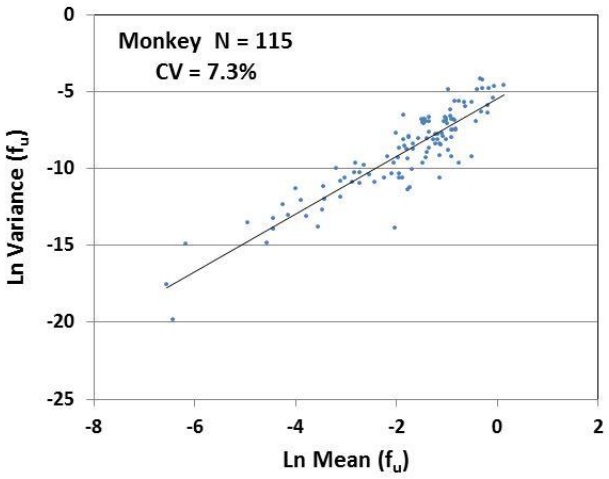
Precision of PPB: Intra-day Variability









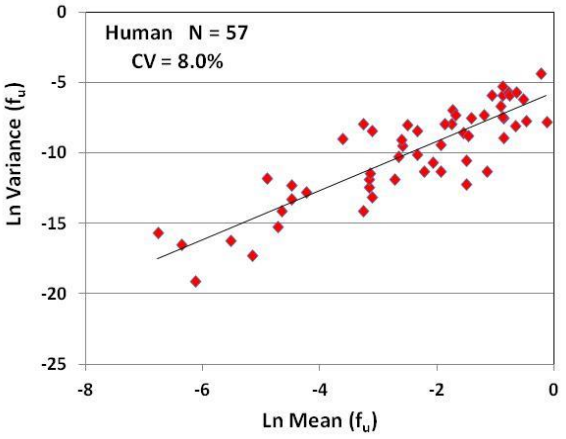


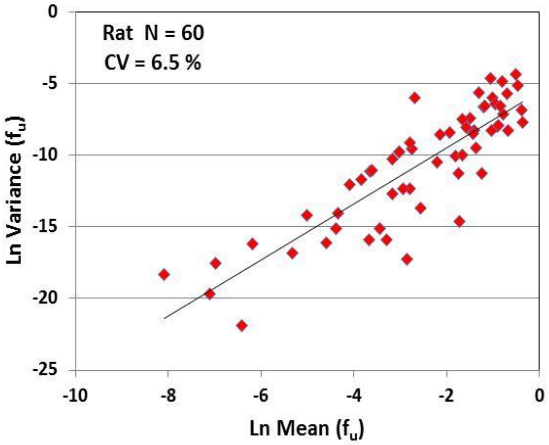
Intra-day precision is consistent (~8%) between high and low binding compounds

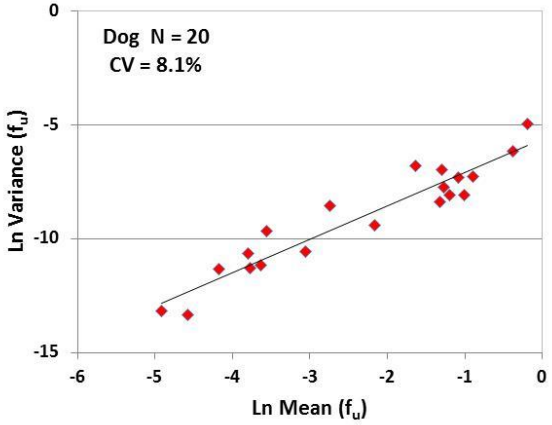
f_u range: 0.00005 - 1

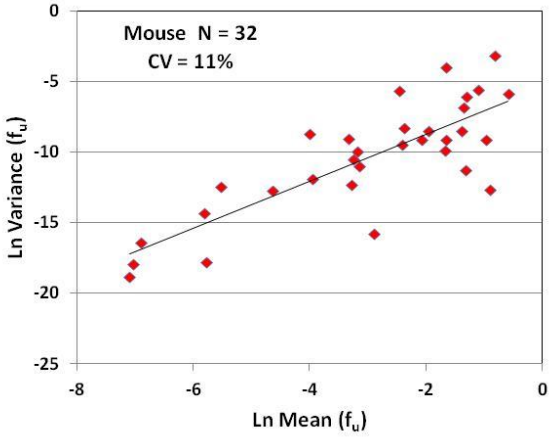
J. Pharm. Sci., 2015, 104(8):2627-36

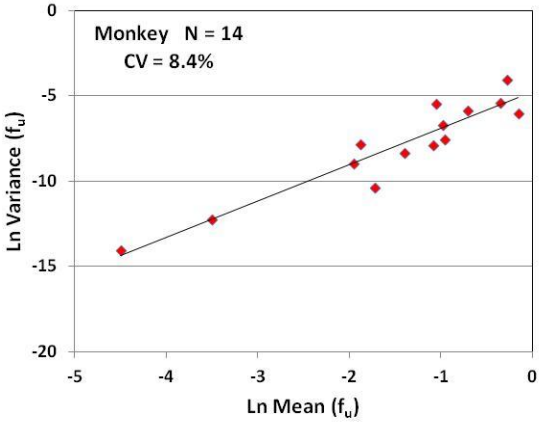
Precision of PPB: Inter-day Variability











Inter-day precision is consistent (6-11%) between high and low binding compounds

f_u range: 0.0003 - 1

J. Pharm. Sci., 2015, 104(8):2627-36

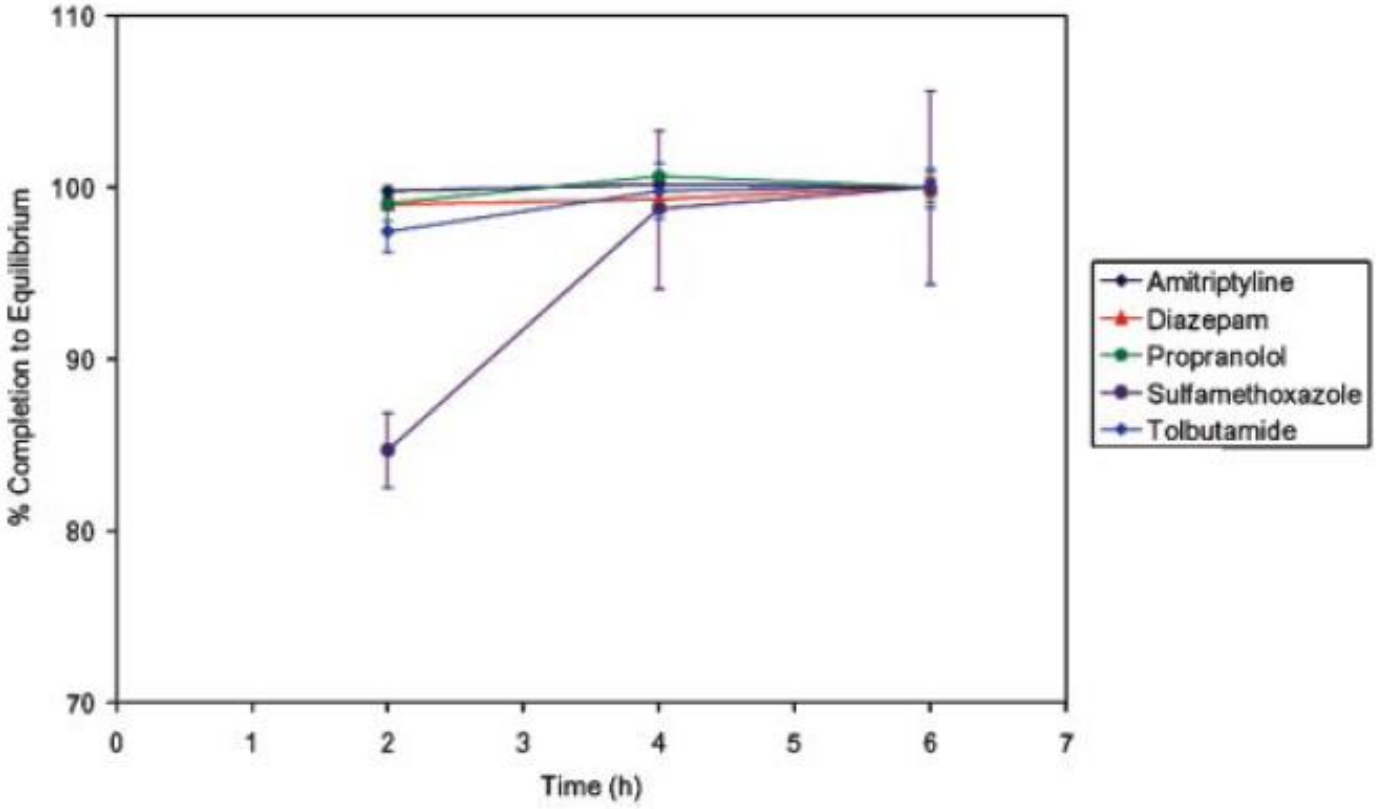
Precision of f_u Measurement

- CVs (coefficients of variation) are similar between highly bound and weakly bound compounds
- No bias of f_u values being more variable for highly bound compounds than weakly bound ones

Factors Governing PPB Precision

- Reproducibility of liquid transfer
- Reagent consistency
  - Plasma, pH, buffer
- Experimental conditions
  - Incubator temperature and humidity
  - Shaker speed
- Analytical sensitivity

Time to Equilibrium in RED

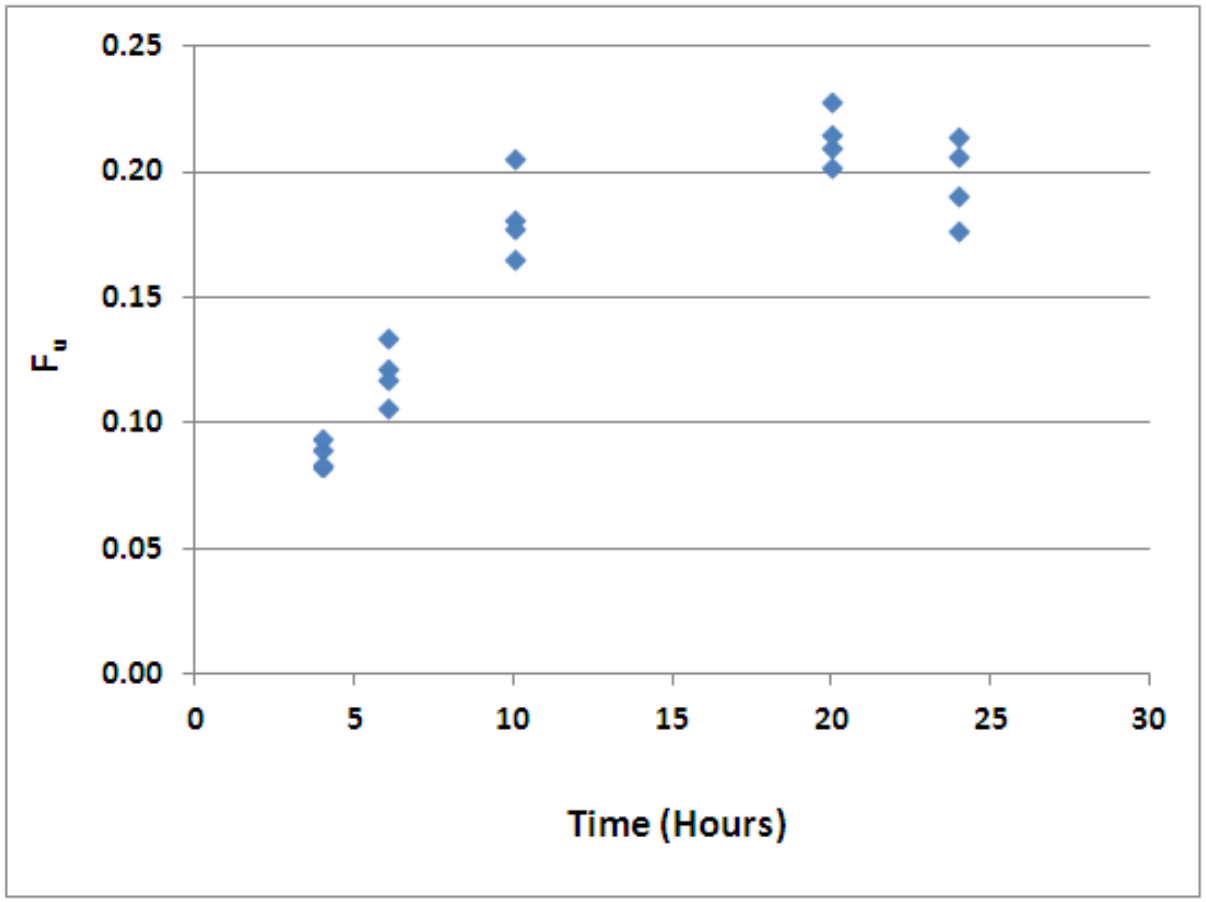


Standard protocol: 4 hour incubation

*RED = Rapid equilibrium dialysis device

Waters NJ, et al. J.Pharm.Sci. 2008, 97:4586-4595.

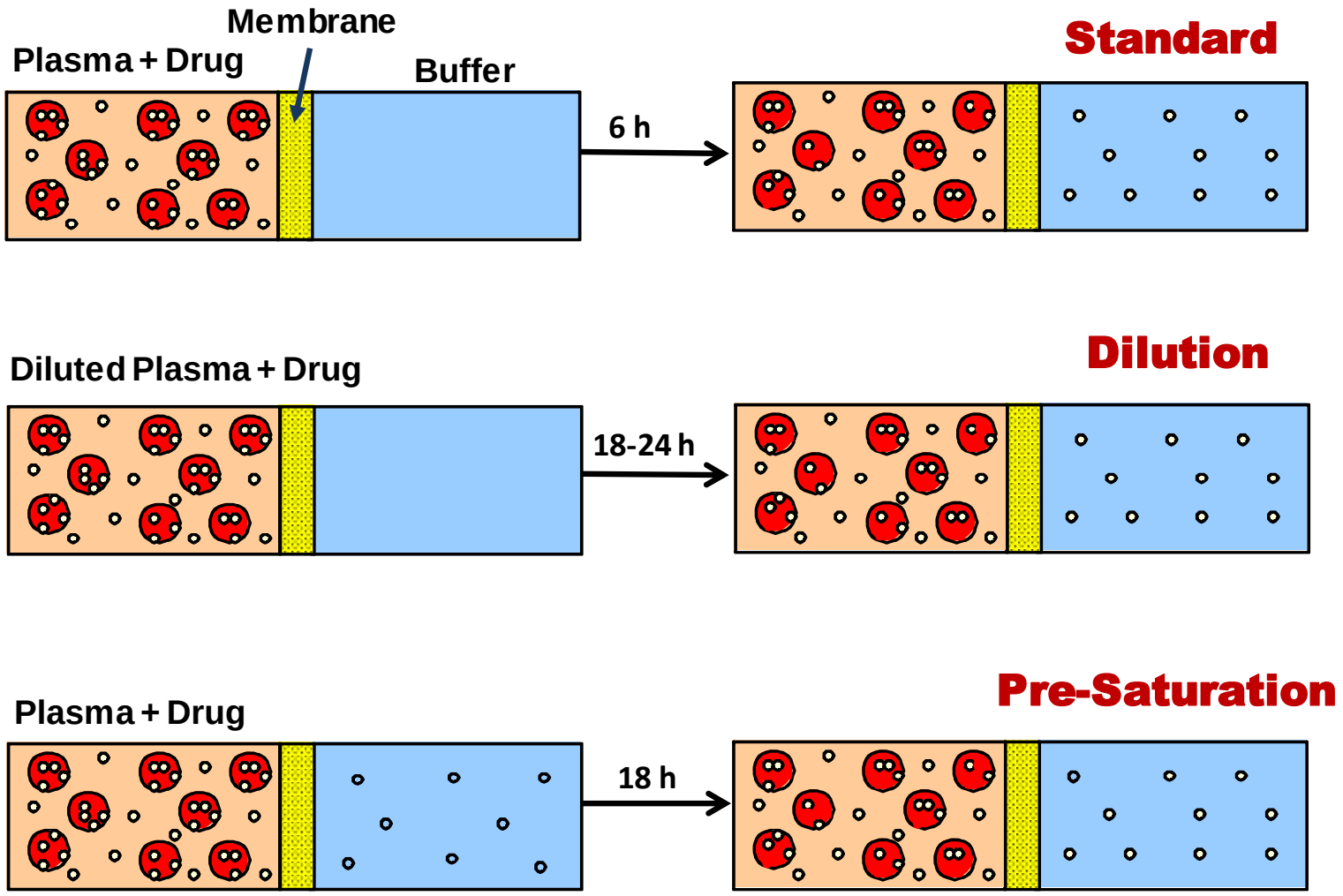
Time to Equilibrium



It takes longer for challenging compounds to achieve equilibrium (high MW, low f_u, high nonspecific binding)

J Pharm Sci, 2017, 106(12): 3442-3452.

Schematic of Plasma Protein Binding Methods



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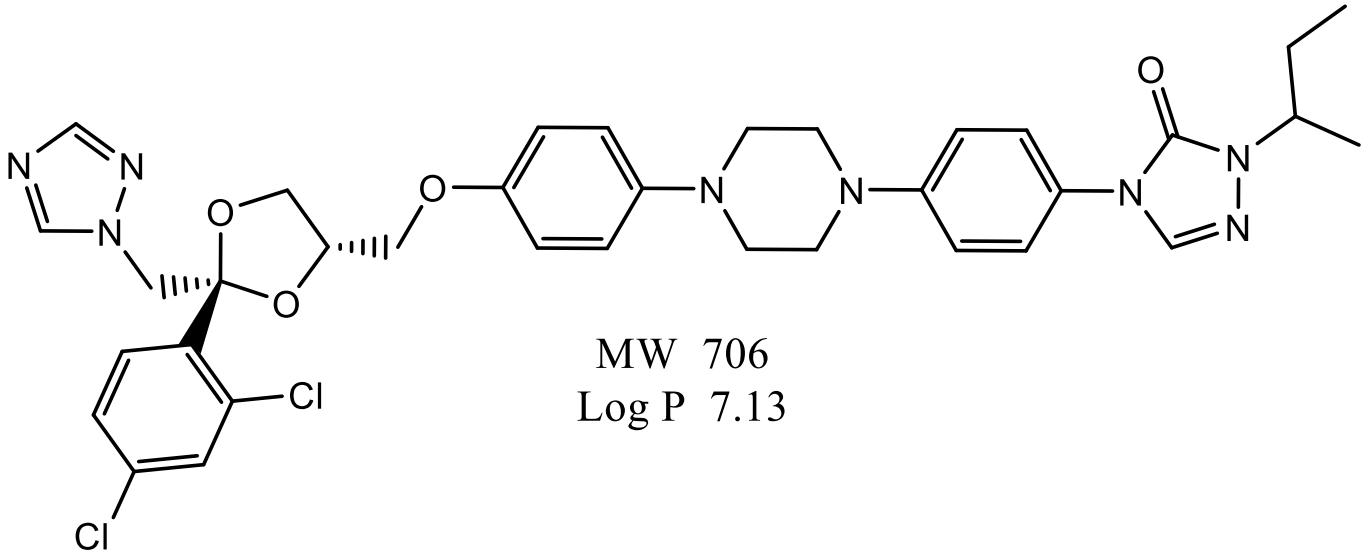
Itraconazole – Method Comparison



Method	f_u ± SD (Human Plasma)
Standard RED	Not Measurable
Dilution Method	0.0020 ± 0.0002
Pre-saturation Method	0.0021 ± 0.0004
Literature	0.0020

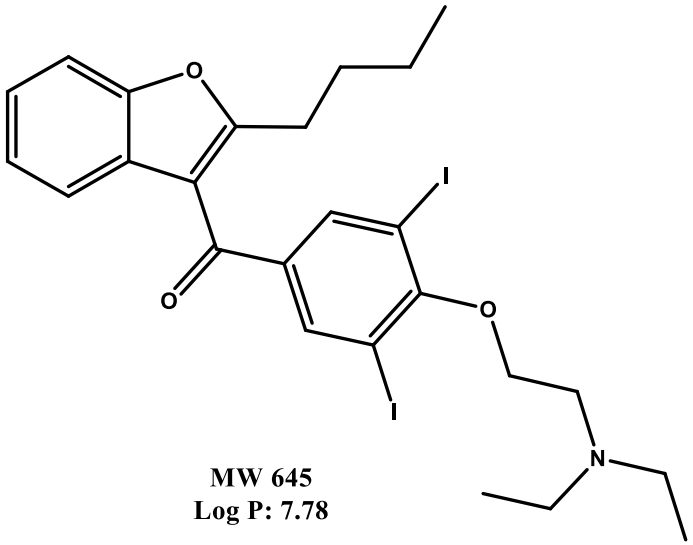
J. Pharm. Sci., 2015, 104(8):2627-36

F_u of Itraconazole and Metabolites

Compounds	MW	Log P	f_u	% CV
Itraconazole	706	7.13	0.0020	9.4
Hydroxy-Itraconazole	722	6.11	0.017	14
Keto-Itraconazole	720	5.60	0.010	12
N-Desalkyl-Itraconazole	650	5.75	0.011	13

J. Pharm. Sci., 2015, 104(8):2627-36

Amiodarone – Method Comparison



MW 645
Log P: 7.78

Method	f_u (Human Plasma)
Standard RED	Not Measurable
Dilution Method	0.00014 ± 0.00002
Pre-saturation Method	0.00021 ± 0.00006
Literature	0.0002

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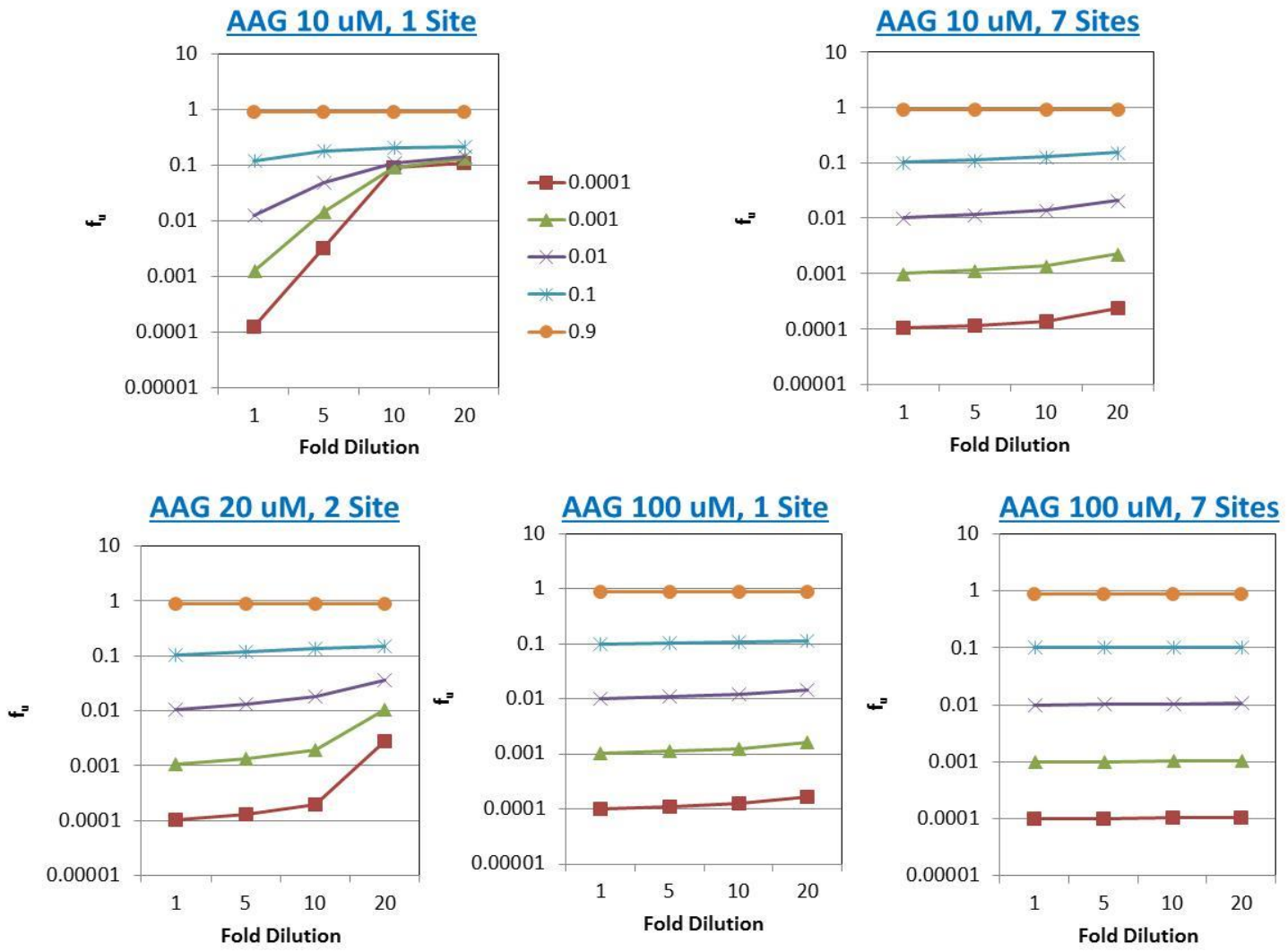
Major Plasma Proteins

- Human Serum Albumin (HSA)
  - 600 μM
  - At least 6 primary binding sites with high affinity
  - A large number of secondary binding sites with low affinity
  - Binds to acids, bases and neutrals

- α1-Acid Glycoprotein (AAG)
  - 20-30 μM
  - Acute phase protein: 10 -100 μM
  - Up to 7 binding sites
  - Binds to bases, also neutrals and acids

Effect of AAG Concentration and Number of Binding Sites on f_u Determination



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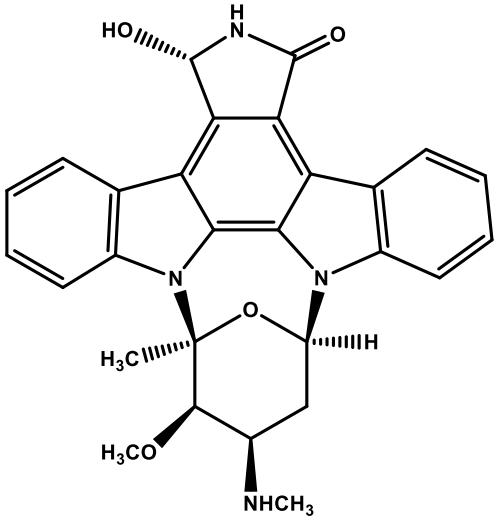
UCN-01 – Method Comparison

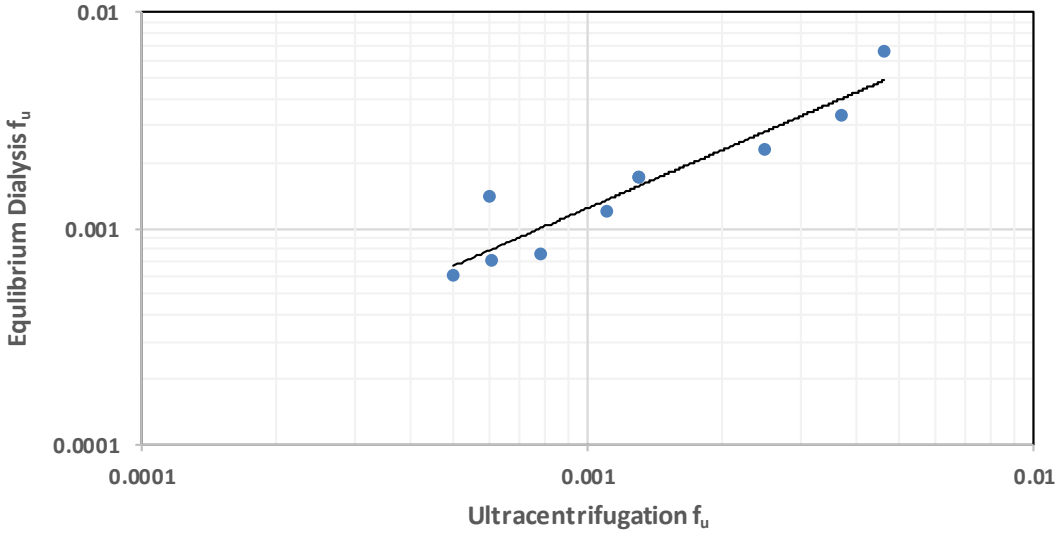


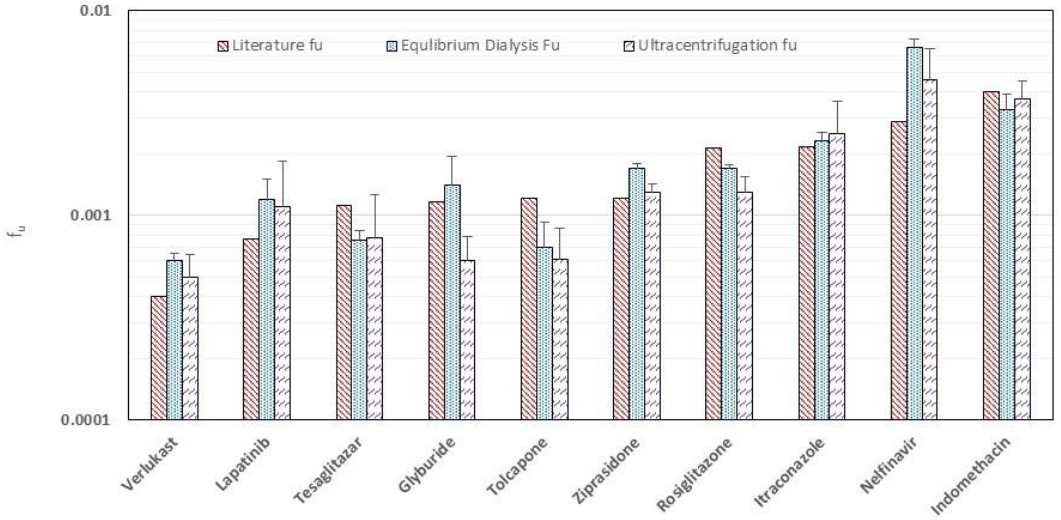
MW 483
Log P: 2.76

Method	f_u (Human Plasma)
Standard RED	Not Measurable
Dilution Method	0.0019 ± 0.0002
Pre-saturation Method	0.0013 ± 0.0003
Literature	0.0022

J. Pharm. Sci., 2015, 104(8):2627-36

Orthogonal Methods for Highly Bound Compounds





- Equilibrium dialysis and ultracentrifugation gave similar f_u
- F_u of highly bound compounds can be measured accurately
- Measured f_u values should be used for DDI prediction

J. Pharm. Sci., 2019, online

IQ Human Plasma f_u for Warfarin and Itraconazole

Companies	(±) Warfarin f_u
a	0.017
b	0.013
c-1	0.008
c-2	0.005
d-1	0.018
d-2	0.013
e-1	0.008
e-2	0.013
f	0.0097
g	0.0092
h	0.009
I-1	0.010
I-2	0.010
Mean	0.011
% CV*	30

Companies	Itraconazole f_u
a	0.0016
b	0.0020
c	0.0010
d	0.0017
e	0.0016
f	0.0014
g	0.0022
h	0.0015
I	0.0017
J	0.0007
K	0.0005 (undiluted), 0.0013 (diluted)
Mean	0.0015
% CV*	31

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Plasma Protein Binding of Unstable Compounds

- Enzymatic hydrolysis
- pH instability
- Light sensitivity
- Chiral conversion

Stabilize compounds before measuring PPB

Hydrolase Inhibitors to Stabilize Prodrug

Species	Inhibitor	f_u (%CV)
Human	None	0.21 (13%)
	AEBSF	0.24 (3%)
	PMSF	0.28 (15%)
Rat	AEBSF	0.26 (10%)
	PMSF	0.26 (5%)

AEBSF 2 mg/mL, PMSF 2 mM

Ensure inhibitors do not interfere with PPB

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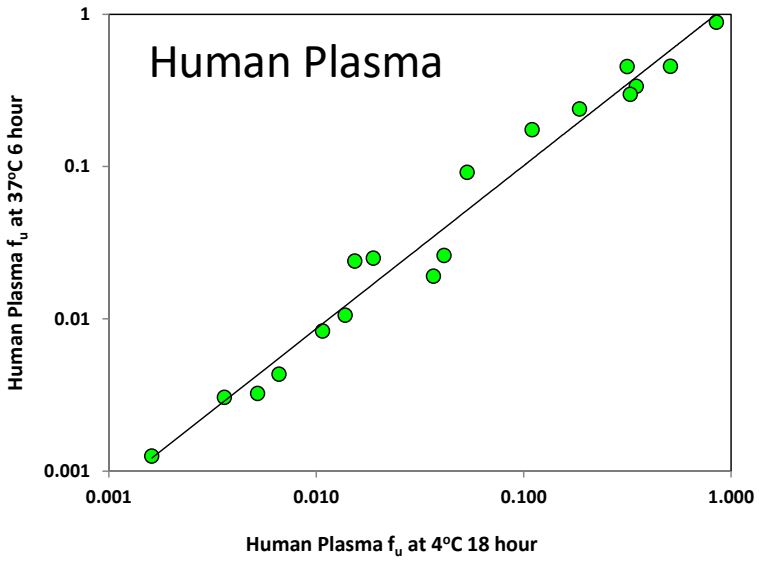
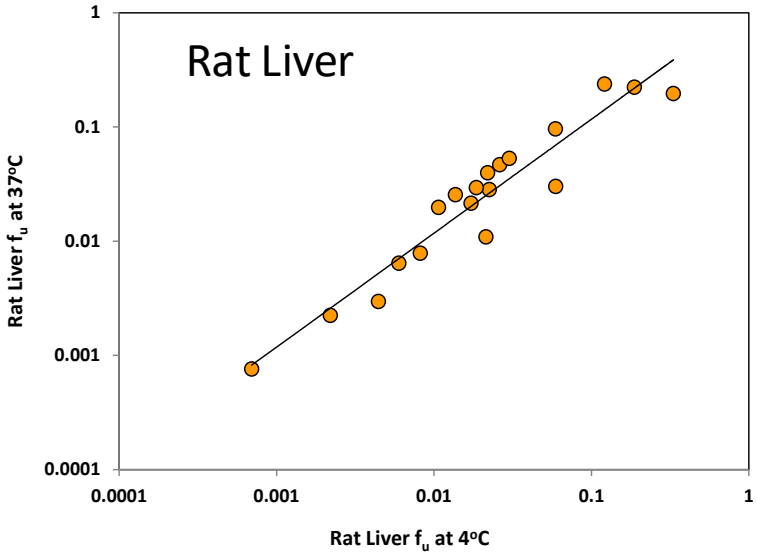
No Effects of Temperature of f_u

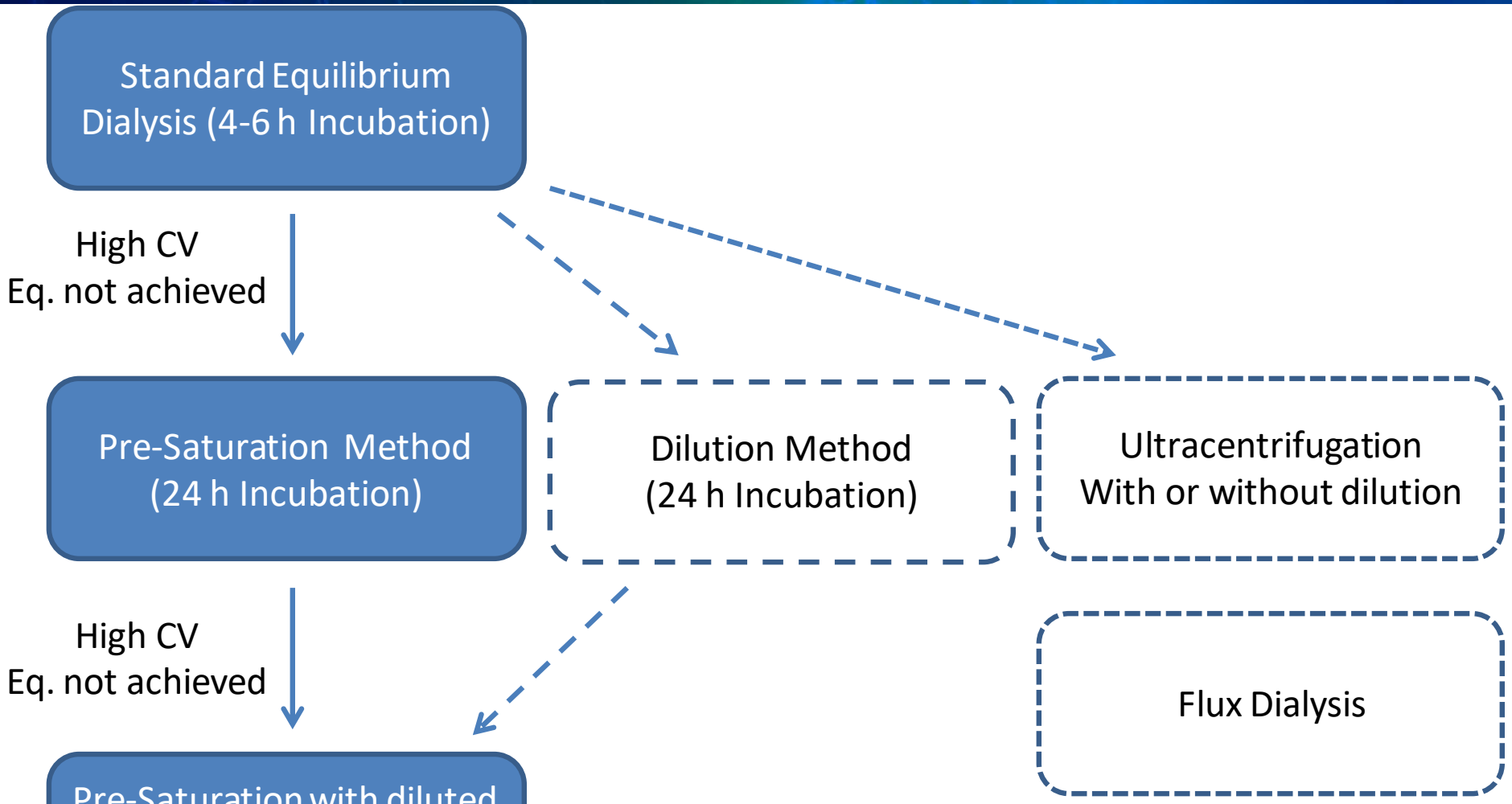




- **Plasma protein binding and liver binding are similar at 37°C and 4°C**
- **Binding at 4°C can be used to measure PPB for unstable compounds**

Biopharmaceutics Drug Disposition, 2018, 39(9), 437-442

PPB Methods for Highly Bound Compounds



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DMD, 2018, 46(4):458-469.

Conclusions

- Setting 1% reportable lower limit of PPB is overly conservative and can lead over-prediction of DDI
- PPB below 1% can be measured accurately when appreciate assay conditions are used and these values should be used for DDI prediction
- New methodologies continue to evolve to address challenging compounds (highly bound, unstable)