



TRANSIL High Protein Binding Assay

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23.01.2009



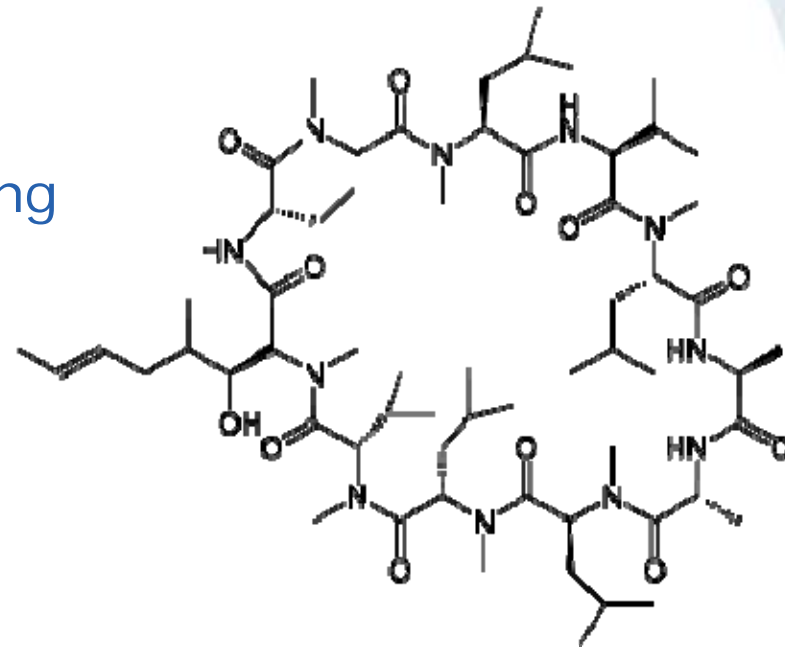
TRANSIL High Protein Binding Assay

Key Features

- TRANSIL based “erythrocyte” partitioning
- Discrimination of compounds with $f_u < 1\%$
- Works well with lipophilic compounds
- No bias through unspecific binding
- Full range of species available

Example: Cyclosporine A

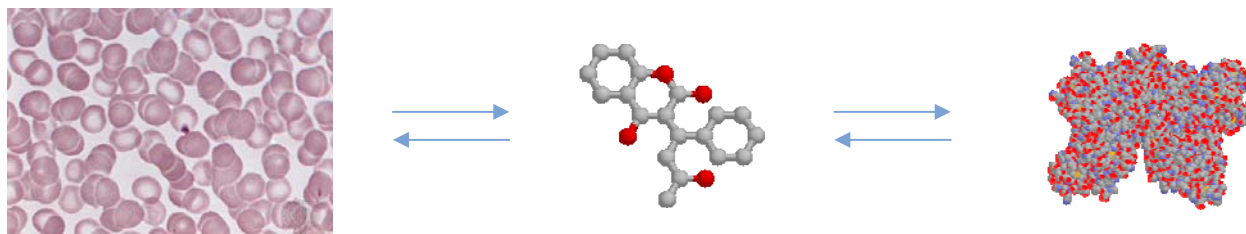
- Dialysis
 - Recovery: < 10%
 - Free fraction: 0%
- TRANSIL HSA & AGP Binding
 - Recovery: 80%
 - Free fraction: 30%
- Ultracentrifugation
 - Free fraction: 1%
- TRANSIL High Protein Binding
 - Free fraction: 1%



Protein Binding Assays

Assay	Unspecific Binding	Analytics fu < 1%
• Equilibrium Dialysis	☹️	☹️
• Ultrafiltration	☹️	☹️
• Ultracentrifugation	😊	☹️
• TRANSIL Protein Affinities	☹️/😊	😊
• Erythrocyte partitioning	😊	😊

Rational: Erythrocyte Partitioning Assay



by Garrett & Hunt (1974), *J. pharm. Sci* 63:1056-1064

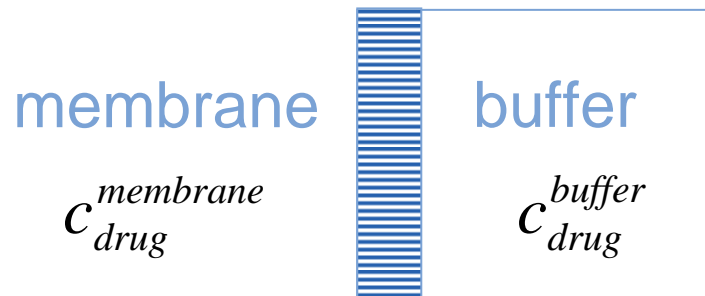
- Compounds bind to plasma proteins & blood cells
- Compounds are stabilized in solution by lipid fraction
- Partitioning alleviates analytical problem

Measure of Fraction Unbound

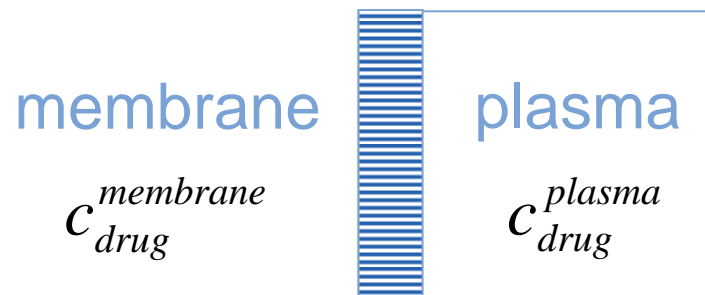


$$f_u = \frac{C_{drug}^{buffer}}{C_{drug}^{plasma}}$$

Measure of Membrane Affinity



$$MA_{buffer} = \frac{c_{drug}^{membrane}}{c_{drug}^{buffer}}$$

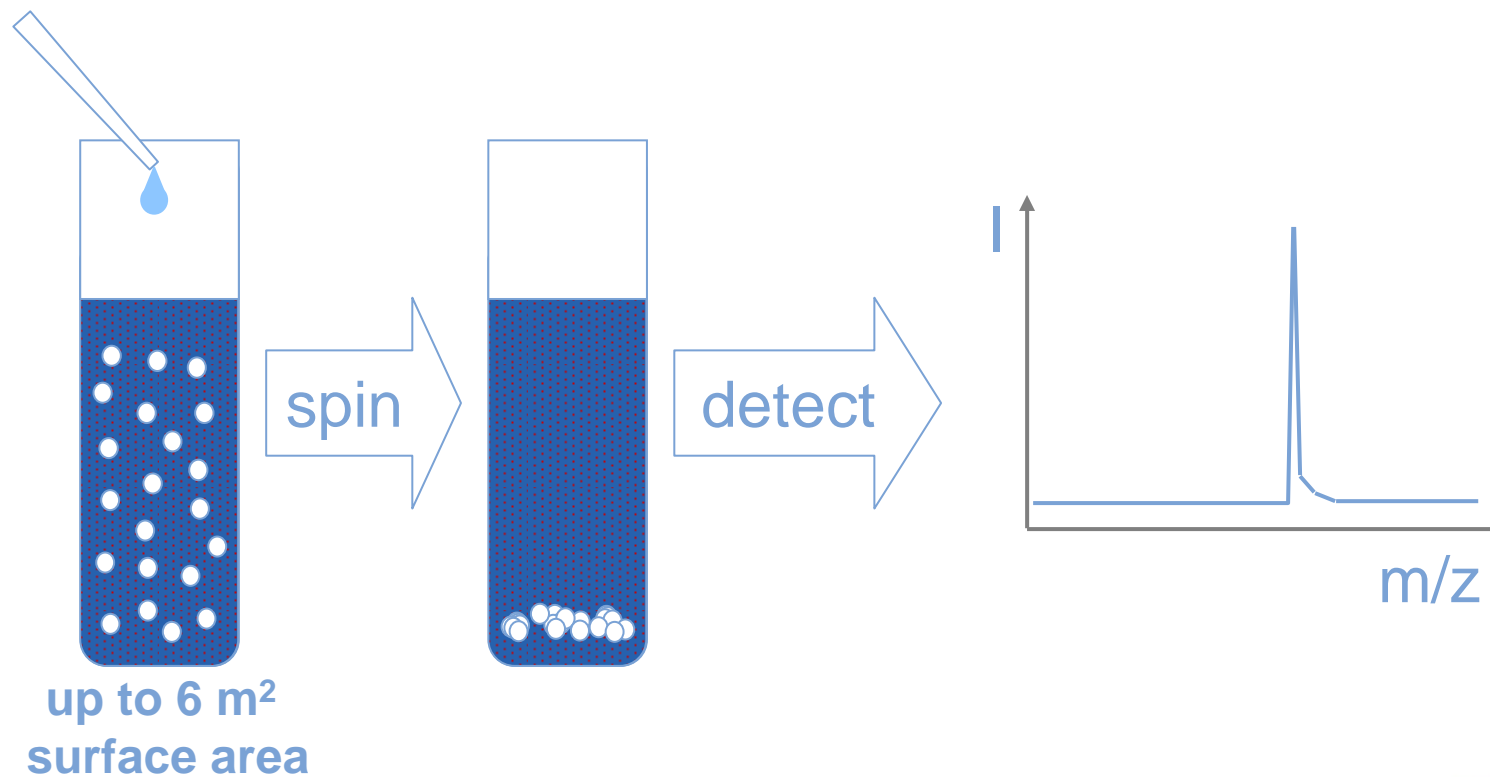


$$MA_{plasma} = \frac{c_{drug}^{membrane}}{c_{drug}^{plasma}}$$

New Measure of Fraction Unbound

$$f_u = \frac{C_{drug}^{buffer}}{C_{drug}^{plasma}} = \frac{MA_{plasma}}{MA_{buffer}} = \frac{\frac{C_{drug}^{plasma}}{\cancel{C_{drug}^{membrane}}}}{\frac{\cancel{C_{drug}^{membrane}}}{C_{drug}^{buffer}}}$$

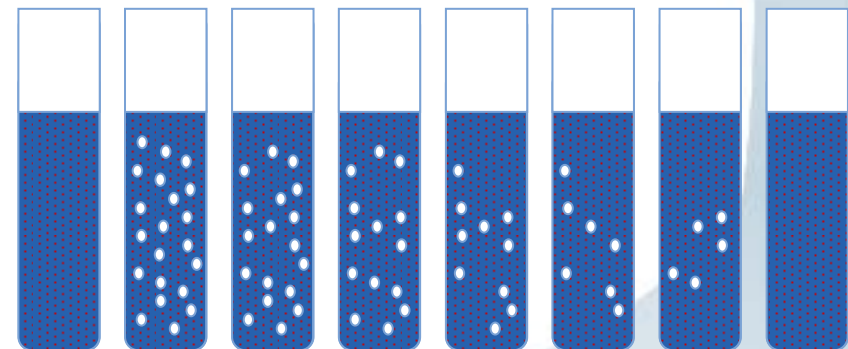
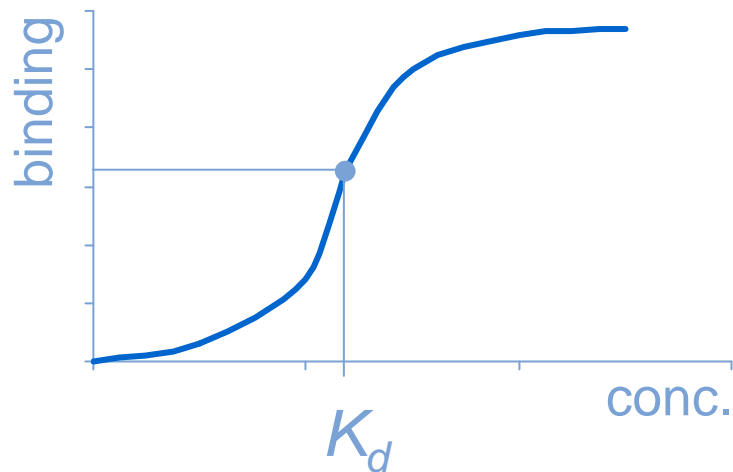
TRANSIL Technology Basic Principle



TRANSIL Technology

Estimation of Affinities

- 2 blanks to correct for non-specific binding
- 6 different bead concentrations to determine conc. dependent binding
- Estimation of logMA
- Calculation of f_u



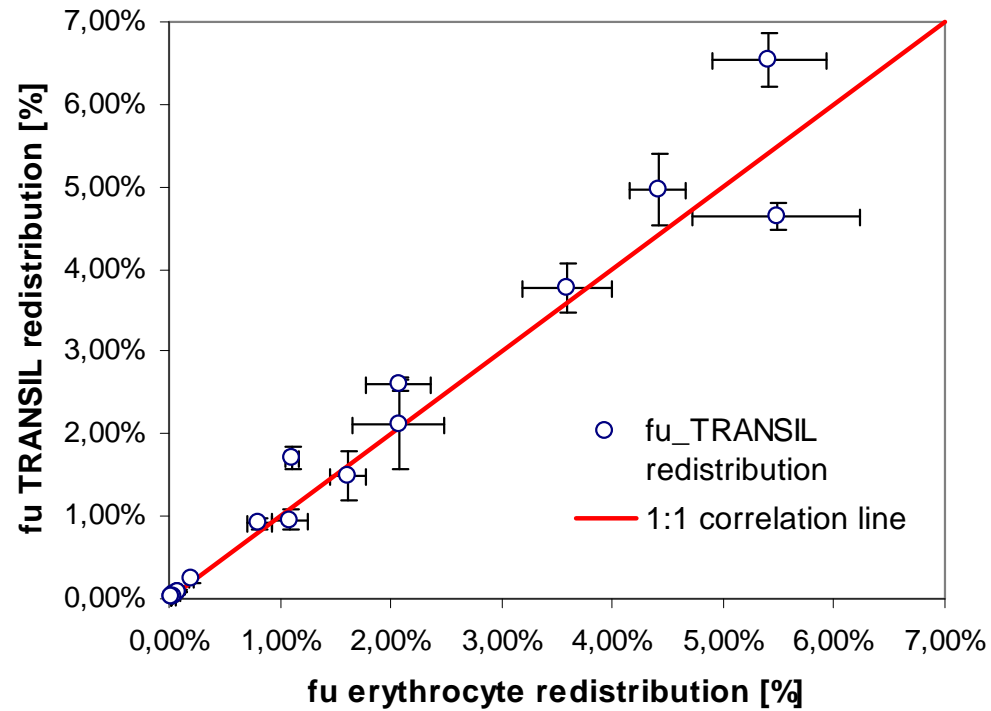
Provisions Against Unspecific Binding

- Use of silanized glass vials
- Low conc. of heat denatured HSA
- Presence of full plasma
- Presence of lipid phase

Assay Steps

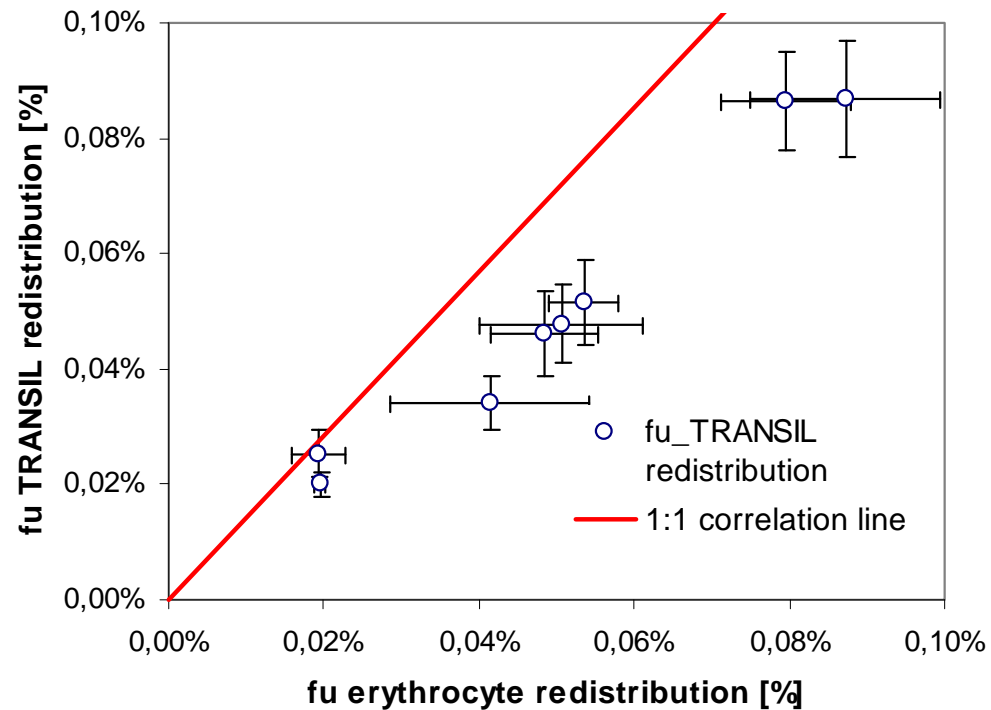
- Incubation in 96-well plates with removable silanized glass vials
- Centrifugation
- Direct injection of buffer (10 μ g/ml heat inactivated HSA) or plasma supernatant on a Chromolith column

Validation Data II: Erythrocyte Partitioning



Data by J. Schuhmacher at Bayer HealthCare

Validation Data III: Erythrocyte Partitioning



Data by J. Schuhmacher at Bayer HealthCare

Validation Data: Well Behaved Compounds

	conc. mg/ml	f_u Dialysis	f_u E.P.	f_u TRANSIL	Anova
Amitriptyline	45	0.150 ± 0.002	0.153 ± 0.006	0.151 ± 0.004	NS
Imipramine	130	0.145 ± 0.015	0.163 ± 0.004	0.154 ± 0.007	NS
Qunidine	60	0.151 ± 0.018	0.172 ± 0.016	0.170 ± 0.005	NS
Lidocaine	8000	0.47 ± 0.018	0.48 ± 0.011	0.48 ± 0.020	NS
Propanolol	70	0.137 ± 0.018	0.129 ± 0.007	0.131 ± 0.010	NS

Conclusion

- Accurate protein binding data for all compounds
- Discrimination of compounds with $f_u < 1\%$
- Works well with lipophilic compounds & peptides
- No bias through unspecific binding
- Complete high through-put assay scheme

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