

PLASMA PROTEIN BINDING USING RAPID EQUILIBRIUM DIALYSIS

High throughput, cost-effective equilibrium dialysis method

Assessment of plasma protein binding by rapid equilibrium dialysis applies the gold standard equilibrium dialysis technique to a high throughput discovery setting to accurately determine the bound and unbound fractions of a test article in human or animal plasma. Only BD GentestSM Contract Research Services delivers a combination of industry leading proprietary products, advanced technology, expert guidance from renowned study directors and reliable, submission ready results. Together, these elements provide you with the most rapid path to more sound decision making in your drug discovery endeavors.

The **most rapid** path to more sound decision making.

PLASMA PROTEIN BINDING

The degree of plasma protein binding (PPB) determines which fraction of the total plasma concentration of a compound is bound to plasma proteins, and therefore, not available for interaction with its biological target (receptors, enzymes, transporters, etc.). Determination of the free and bound fractions of a test article in plasma is a critical parameter routinely determined during the drug discovery process to understand a test article's availability for interaction with its biological target.

HIGH THROUGHPUT, COST-EFFECTIVE, DISCOVERY SCREENING

PPB assays by rapid equilibrium dialysis with LC/MS/MS quantitation provides a cost-effective screening of large numbers of compounds with fast turnaround times. Rapid equilibrium dialysis accelerates the determination of unbound drug concentrations in plasma used for determining dosing for preclinical and clinical studies, drug efficacy, and drug-drug interaction testing.^[1]

EQUILIBRIUM DIALYSIS DELIVERS ACCURATE AND REPRODUCIBLE RESULTS

Results obtained at BD Biosciences using rapid equilibrium dialysis in human plasma are consistent with data reported in the literature. Results are highly reproducible over extended periods of time using various lots of plasma.

[More on reverse](#)

KEY FEATURES

- Provides a fast turnaround of 1-2 weeks and a cost-effective equilibrium dialysis method
- Provides highly reproducible results with low inter-plate, plate-to-plate, and assay-to-assay variability
- Available for human and various preclinical species (mouse, rat, dog, monkey, and others)
- Quantitation using LC/MS/MS
- Automated method validated on a Tecan[®] Freedom EVO[®] 200 liquid handling system
- Data reported as percent of test article bound to plasma proteins

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contact BD Biosciences at 888.334.5229 x2246 or 781.935.5115 x2246.
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PLASMA PROTEIN BINDING USING RAPID EQUILIBRIUM DIALYSIS

SPECIFICATIONS:

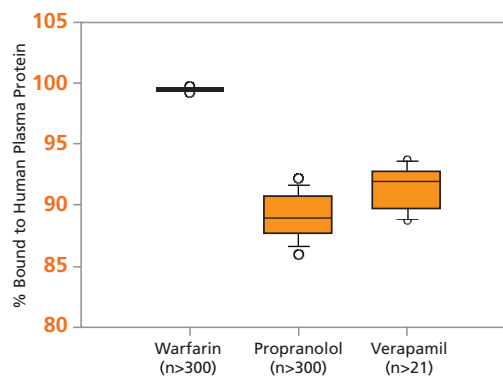
PROTEIN BINDING IN HUMAN PLASMA COMPARED WITH LITERATURE VALUES

Compound	PERCENT BOUND	
	Literature	Experimental
Amitriptyline	95%	95.4% (0.48%)
Atenolol	6-16%	4.0% (16.40%)
Bumetanide	93%	98.9% (0.19%)
Carbamazepine	76%	83.9% (3.85%)
Erythromycin	84%	79.1% (4.96%)
Haloperidol	92%	90.8% (1.20%)
Imipramine	89-92%	91.9% (0.89%)
Ketoprofen	>99%	99.6% (0.17%)
Metoprolol	12%	3.5% (32.00%)
Naproxen	99%	95.3% (1.73%)
Propranolol	81-93%	83.2% (3.95%)

Compound	PERCENT BOUND	
	Literature	Experimental
Terfenadine	97%	99.5% (0.30%)
Warfarin	99%	99.5% (0.08%)
Furosemide	91-99%	89.8% (1.23%)
Tolbutamide	96%	98.9% (0.36%)
Verapamil	90%	92.9% (1.83%)
Thioridazine	>95%	99.9% (0.04%)
Tacrine	75%	63.3% (6.21%)
Nadolol	30%	25.3% (8.11%)
Linezolid	31%	49.0% (15.60%)
Citalopram	80%	63.0% (2.58%)

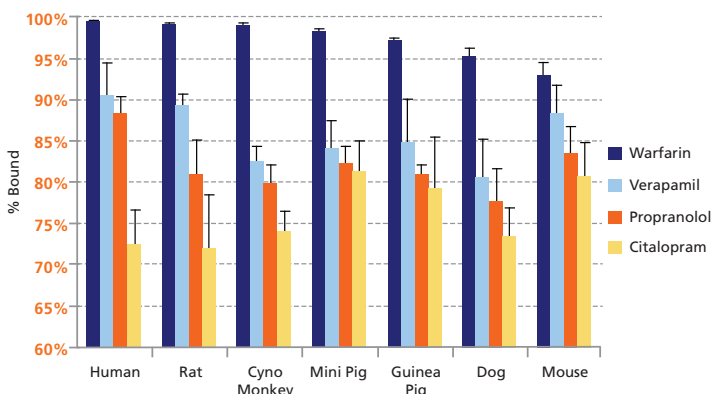
Comparison of % bound values obtained at BD Biosciences using rapid equilibrium dialysis in human plasma with literature data. Experimental values are means of replicates (N ≥ 4) with standard deviations in parentheses. Majority of literature values were collected from www.RXlist.com.

ASSAY PERFORMANCE FOR POSITIVE CONTROLS



Results (% bound) for warfarin, propranolol, and verapamil (10 μM each) in human plasma. Data points include intra-plate, inter-plate, and inter-day replicates and were obtained using various lots of plasma and rapid equilibrium dialysis devices over the course of approximately 2 years.

COMPARISON OF PROTEIN BINDING OF COMPARATOR COMPOUNDS IN HUMAN AND ANIMAL PLASMA



Comparison of mean % bound values across seven species at 10 μM final concentrations. Data represent the means of replicates (N ≥ 5) with standard deviations as error bars.

STUDY DESIGN SUMMARY

TEST SYSTEM:	Rapid equilibrium dialysis device with human and animal plasma.
CONCENTRATIONS:	Single or multiple, in duplicate.
ASSAY METHOD:	Dialysis against PBS buffer. Typical assay conditions are 3-5 hours at 37°C.
ANALYTICAL METHOD:	LC/MS/MS.
CONTROLS:	Warfarin (10 μM).
REPORT:	Percent bound and unbound fractions Excel-based data report.

References

[1] Waters, N.J., et al. (2008). Validation of a rapid equilibrium dialysis approach for the measurement of plasma protein binding. J Pharm Sci 97(10): 4586-95.