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Determination of Protein Binding by UPLC-MS/MS

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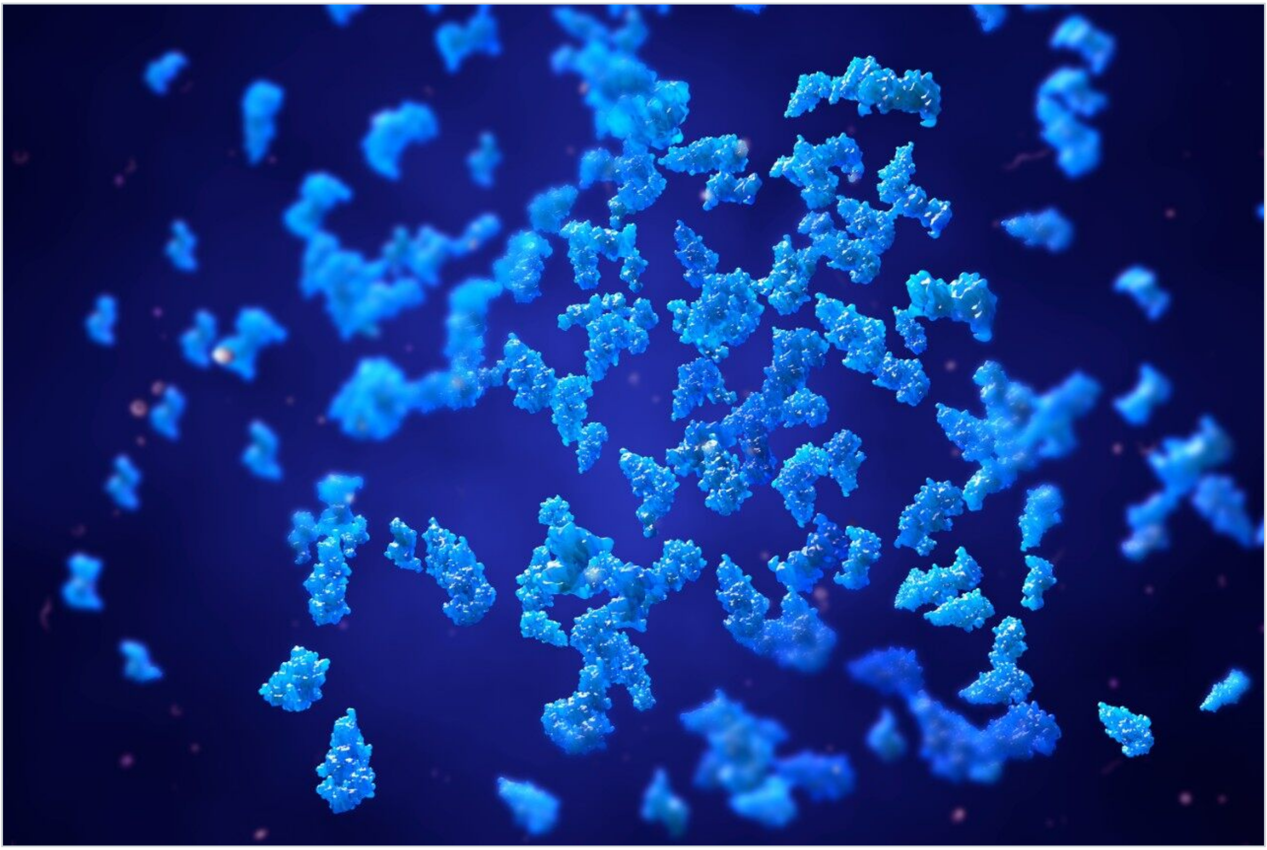


Abstract

In this application note, plasma protein binding was determined which helps in characterizing a drug's behavior and proper dosing in the discovery process.

Benefits

Using ProfileLynx and QuanOptimize Application Managers provided:

- Automated MS method development and data acquisition
- Single approach for data processing and report generation from multiple assays
- Complete automated analysis, processing, and reporting
- Increased laboratory throughput

Introduction

Plasma protein binding (PPB) can significantly affect the therapeutic action of a drug. PPB determines the extent and duration of action, as only unbound drug is thought to be available for passive diffusion to extravascular or tissue sites where therapeutic effects occur.

Since data show an increasing importance of PPB in characterizing a drug's behavior and proper dosing, PPB measurement needs to be made as early as possible in the discovery process. In vivo dose levels can be estimated from the determined fraction of unbound drug (fu); a drug that demonstrates high plasma protein binding indicates that an increase in dose might be necessary.

The classical method used to measure the level of protein binding is equilibrium dialysis. In equilibrium dialysis, a dialysis membrane with small pores allows molecules to diffuse through it. Once equilibrium has been reached, one can measure the amount of free analyte in the donor and receptor samples, and then determine the amount of bound analyte.

This process is laborious and time-consuming with the need to perform additional analytical steps, including radiolabeling. Given the detection speed and sensitivity of UPLC-MS/MS, the ACQUITY TQD System (Figure 1), used with specialized software, ProfileLynx and QuanOptimize Application Managers, is the ideal choice for analysis of PPB.

Figure 1. ACQUITY TQD System.

Experimental

A set of 22 commercially available drug compounds were randomly chosen to demonstrate the ProfileLynx Application Manager.

Rat samples were prepared at 5 µM in rat plasma. 500 µL of each sample was placed into the donor wells of the RED Device Inserts (Pierce) in the base plate. 750 µL of dialysis buffer was placed in each receptor well. The plate was gently shaken for 4 hours at 37 °C. 100 µL of plasma and 100 µL of phosphate buffer saline were added to each well of a 2 mL 96-well plate. For each compound, 100 µL of incubated donor and 100 µL of incubated receptor were added to separate wells. 700 µL of 90:10 acetonitrile/water was added to each well. The plate was shaken for 30 minutes, and then centrifuged for 20 minutes at 3000 RPM. The same process was repeated for human plasma.

These samples were analyzed by UPLC-MS/MS. The QuanOptimize Application Manager was used to automate the optimization of the MS multiple reaction monitoring (MRM) conditions for each compound.

LC Conditions

LC system:	ACQUITY TQD System
Column:	ACQUITY UPLC BEH C_{18} Column 2.1 x 50 mm,

	1.7 µm
Column temp.:	40 °C
Flow rate:	600 µL/min
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	0.1% Formic acid in acetonitrile
Gradient:	5 to 95% B/1.3 min

MS Conditions

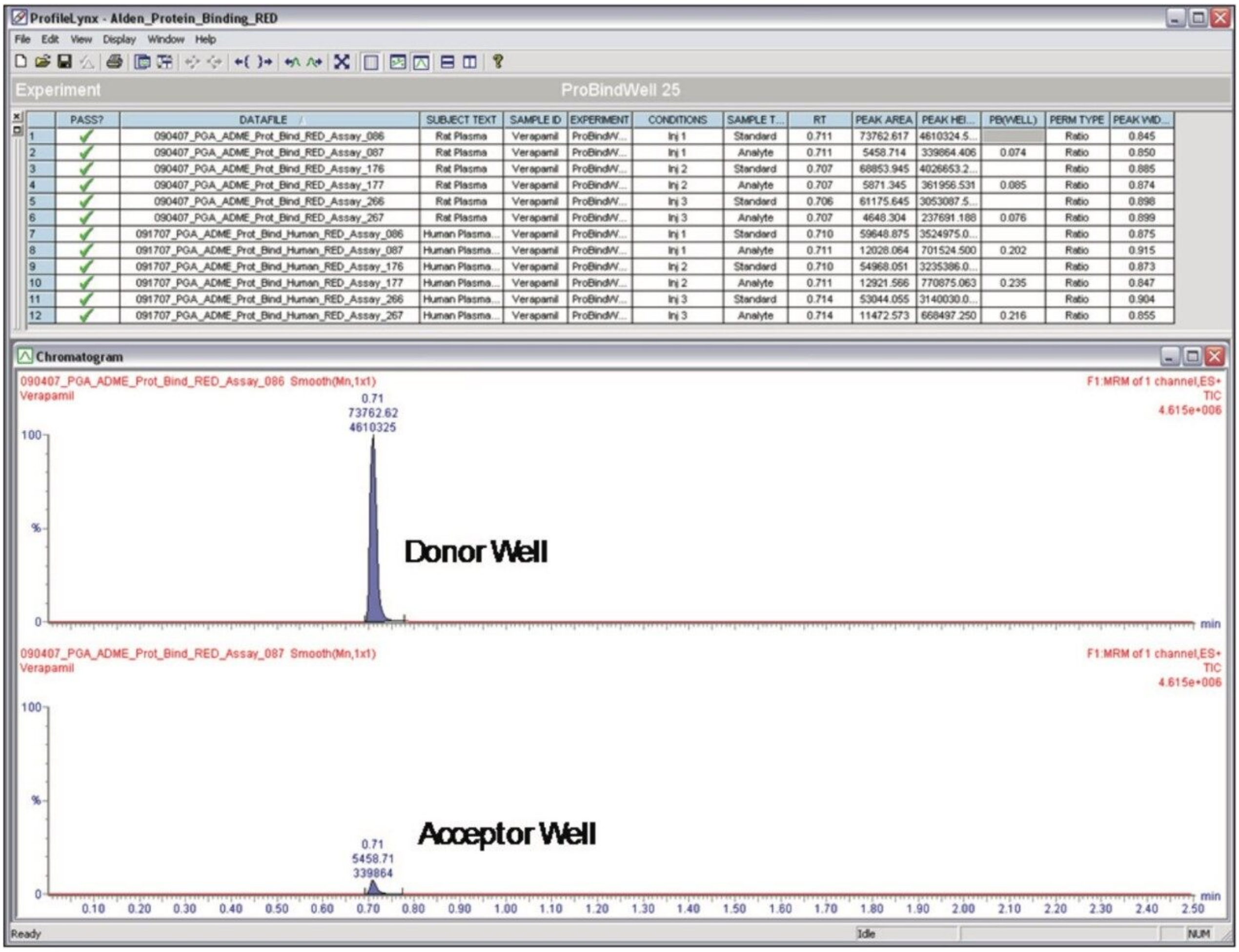
MS system:	TQ Detector
Ionization mode:	ESI positive
Capillary voltage:	3200 V
Source temp.:	150 °C
Desolvation temp.:	450 °C
Desolvation gas:	900 L/hr
Cone gas flow:	50 L/hr
Inter-scan delay:	20 ms
Inter-channel delay:	5 ms
Dwell:	200 ms
Acquisition range:	100 to 1000 *m/z*

Results and Discussion

Protein binding is calculated from the amount of a compound bound to protein substrate in a well, and is determined from the start and finish concentrations in a specified plate or plates. This is achieved by determining the ratio of the peak area of the analyte (receptor plate) to the peak area of the standard (donor plate). Compounds are designated as a standard or an analyte in the SampleType column. The standard and analyte are linked in the sample list with the Compound A column.

In the ProfileLynx browser, PPB is reported as a ratio of the peak area of the standard. Any PPB values outside of a specified minimum and maximum range were automatically flagged. For this experiment, the minimum was set at 50 and the maximum at 100. The interactive browser also allowed for the editing of peak integration. Peak assignments were easily changed and peak integrations were quickly optimized. Results were then exported in a format amenable to the corporate database.

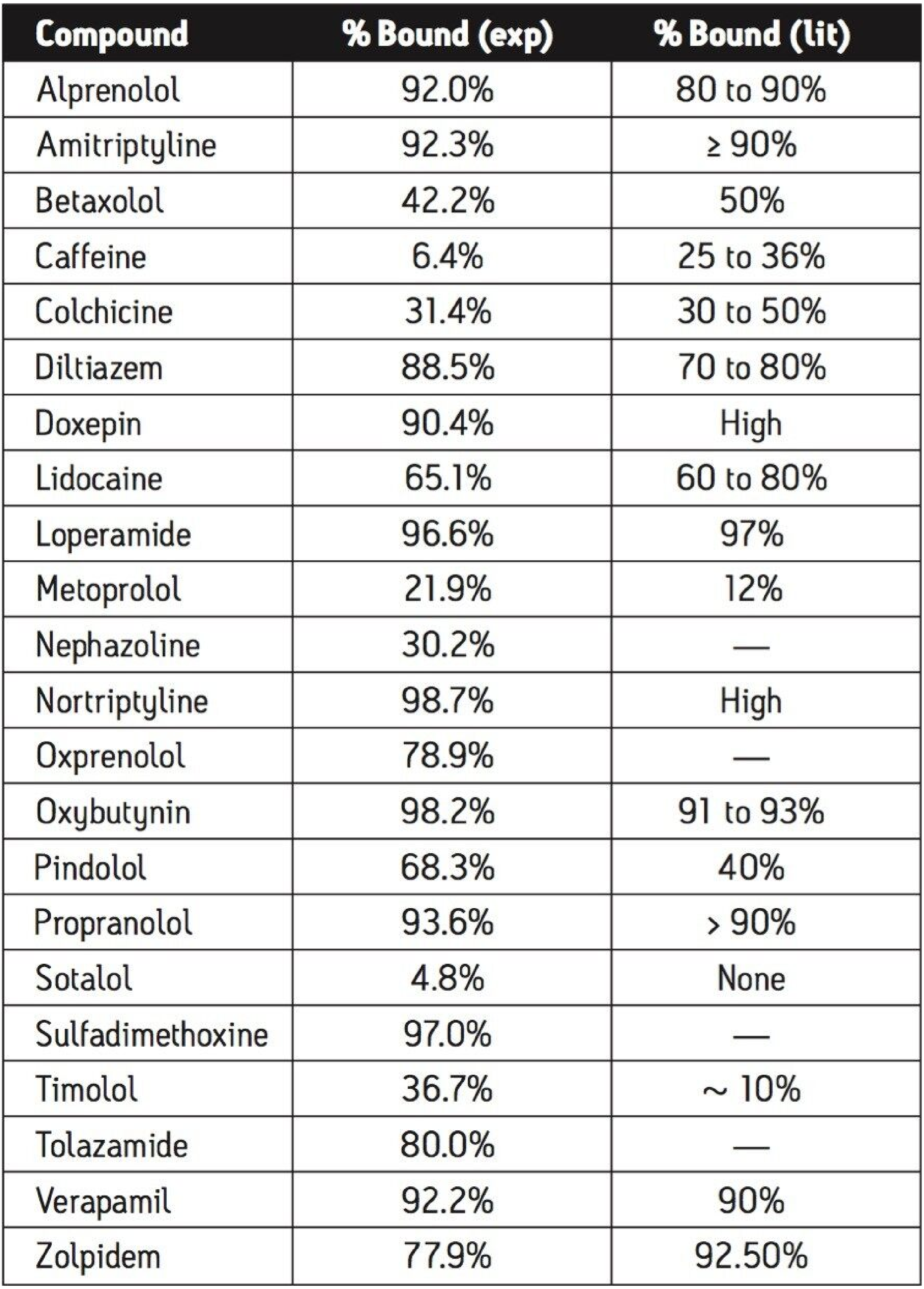
The example in Figure 2 demonstrates how protein binding results are displayed for Verapamil. The result column labeled PB(WELL) contains the fraction of free (unbound) compound. The percent of the bound compound is calculated by (1-PB(WELL))* 100%. The results for the entire set of compounds indicate a lower protein binding for compounds in human plasma than for the same compounds in rat plasma. Overall, the protein binding results are highly reproducible from injection to injection.



	PASS?	DATAFILE	SUBJECT TEXT	SAMPLE ID	EXPERIMENT	CONDITIONS	SAMPLE T...	RT	PEAK AREA	PEAK HEI...	PB(WELL)	PERM TYPE	PEAK WID...
1	✓	090407_PGA_ADME_Prot_Bind_RED_Assay_086	Rat Plasma	Verapamil	ProBindW...	Inj 1	Standard	0.711	73762.617	4610324.5...		Ratio	0.845
2	✓	090407_PGA_ADME_Prot_Bind_RED_Assay_087	Rat Plasma	Verapamil	ProBindW...	Inj 1	Analyte	0.711	5458.714	339864.406	0.074	Ratio	0.850
3	✓	090407_PGA_ADME_Prot_Bind_RED_Assay_176	Rat Plasma	Verapamil	ProBindW...	Inj 2	Standard	0.707	68853.945	4026653.2...		Ratio	0.885
4	✓	090407_PGA_ADME_Prot_Bind_RED_Assay_177	Rat Plasma	Verapamil	ProBindW...	Inj 2	Analyte	0.707	5871.345	361956.531	0.085	Ratio	0.874
5	✓	090407_PGA_ADME_Prot_Bind_RED_Assay_266	Rat Plasma	Verapamil	ProBindW...	Inj 3	Standard	0.706	61175.645	3053087.5...		Ratio	0.898
6	✓	090407_PGA_ADME_Prot_Bind_RED_Assay_267	Rat Plasma	Verapamil	ProBindW...	Inj 3	Analyte	0.707	4648.304	237691.188	0.076	Ratio	0.899
7	✓	091707_PGA_ADME_Prot_Bind_Human_RED_Assay_086	Human Plasma...	Verapamil	ProBindW...	Inj 1	Standard	0.710	59648.875	3524975.0...		Ratio	0.875
8	✓	091707_PGA_ADME_Prot_Bind_Human_RED_Assay_087	Human Plasma...	Verapamil	ProBindW...	Inj 1	Analyte	0.711	12028.064	701524.500	0.202	Ratio	0.915
9	✓	091707_PGA_ADME_Prot_Bind_Human_RED_Assay_176	Human Plasma...	Verapamil	ProBindW...	Inj 2	Standard	0.710	54968.051	3235386.0...		Ratio	0.873
10	✓	091707_PGA_ADME_Prot_Bind_Human_RED_Assay_177	Human Plasma...	Verapamil	ProBindW...	Inj 2	Analyte	0.711	12921.566	770875.063	0.235	Ratio	0.847
11	✓	091707_PGA_ADME_Prot_Bind_Human_RED_Assay_266	Human Plasma...	Verapamil	ProBindW...	Inj 3	Standard	0.714	53044.055	3140030.0...		Ratio	0.904
12	✓	091707_PGA_ADME_Prot_Bind_Human_RED_Assay_267	Human Plasma...	Verapamil	ProBindW...	Inj 3	Analyte	0.714	11472.573	668497.250	0.216	Ratio	0.855

Figure 2. ProfileLynx browser showing protein binding results for Verapamil.

Table 1 lists the average experimental values for protein binding of the sample compounds in rat plasma, and the literature values for protein binding of these compounds (www.DrugBank.ca). Experimental values agree closely for the majority of the compounds analyzed, particularly for compounds with a high degree of binding.



Compound	% Bound (exp)	% Bound (lit)
Alprenolol	92.0%	80 to 90%
Amitriptyline	92.3%	≥ 90%
Betaxolol	42.2%	50%
Caffeine	6.4%	25 to 36%
Colchicine	31.4%	30 to 50%
Diltiazem	88.5%	70 to 80%
Doxepin	90.4%	High
Lidocaine	65.1%	60 to 80%
Loperamide	96.6%	97%
Metoprolol	21.9%	12%
Nephazoline	30.2%	—
Nortriptyline	98.7%	High
Oxprenolol	78.9%	—
Oxybutynin	98.2%	91 to 93%
Pindolol	68.3%	40%
Propranolol	93.6%	> 90%
Sotalol	4.8%	None
Sulfadimethoxine	97.0%	—
Timolol	36.7%	~ 10%
Tolazamide	80.0%	—
Verapamil	92.2%	90%
Zolpidem	77.9%	92.50%

Table 1. Protein binding results in rat plasma.

Table 2 lists the average experimental values for protein binding of the sample compounds in human plasma

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Featured Products

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QuanOptimize <https://www.waters.com/534330>

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