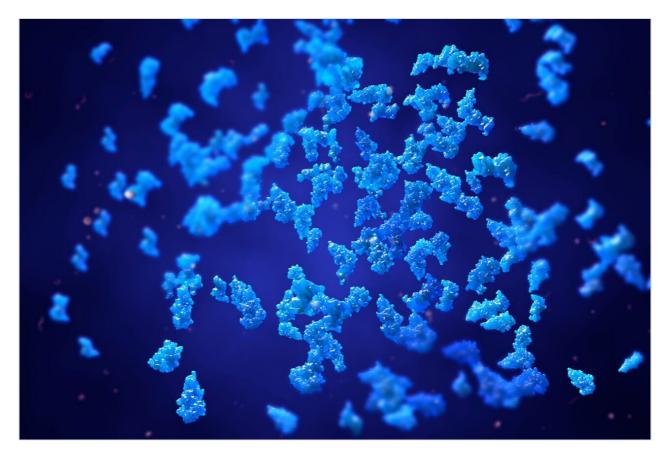
Waters™

응용 자료

Determination of Protein Binding by UPLC-MS/MS

Darcy Shave, Peter G. Alden

Waters Corporation



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 μ M in rat plasma. 500 μ L of each sample was placed into the donor wells of the RED Device Inserts (Pierce) in the base plate. 750 μ L of dialysis buffer was placed in each receptor well. The plate was gently shaken for 4 hours at 37 °C. 100 μ L of plasma and 100 μ L of phosphate buffer saline were added to each well of a 2 mL 96-well plate. For each compound, 100 μ L of incubated donor and 100 μ L of incubated to each well to separate wells. 700 μ L 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 <i>m/z</i>

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.

ProfileLynx -	Alden_Protein_Binding	_RED												
	Display Window Help													
S . (😂 🔯 🖼 🔗 🌾 📢	()+ +n n+ X 🗆 🖂		2										
xperiment				1	ProBindW	/eli 25								
PASS?	p	ATAFILE	SUBJECT TEXT	SAMPLEID	EXPERIMENT	CONDITIONS	SAMPLE T	RT	PEAK AREA	PEAK HEL	PROVELL	PERM TYPE	PEAK WD	
1		E Prot Bind RED Assay 086	Rat Plasma	Verapami	ProBindW	Ini 1	Standard	0.711	73762.617	4610324.5	T D(THELE)	Ratio	0.845	-
2		Prot Bind RED_Assay_087	Rat Plasma	Verapami	ProBind/V.	Ini 1	Analyte	0.711	5458.714	339864.406	0.074	Ratio	0.850	-
3		E_Prot_Bind_RED_Assay_176	Ret Plasma	Verapanil	ProBindW	Inj 2	Standard	0.707	68853.945	4026653.2		Ratio	0.885	1
4	090407_PGA_ADM	E_Prot_Bind_RED_Assay_177	Rat Plasma	Verapamil	ProBind/V	Inj 2	Analyte	0.707	5871.345	361956.531	0.085	Ratio	0.874	1
5	090407_PGA_ADM	E_Prot_Bind_RED_Assay_266	Ret Plasma	Verapami	ProBind/V	Inj 3	Standard	0.706	61175.645	3053087.5		Ratio	0.898	
6	090407_PGA_ADM8	E_Prot_Bind_RED_Assay_267	Rat Plasma	Verapamil	ProBind/V	Inj 3	Analyte	0.707	4648.304	237691.188	0.076	Ratio	0.899	
7 1	091707_PGA_ADME_Pr	ot_Bind_Human_RED_Assay_086	Human Plasma	Verapamil	ProBind/V	inj 1	Standard	0.710	59648.875	3524975.0		Ratio	0.875	
3 🗸		ot_Bind_Human_RED_Assay_087	Human Plasma	Verapamil	ProBind/V	inj 1	Analyte	0.711	12028.064	701524.500	0.202	Ratio	0.915	
		ot_Bind_Human_RED_Assay_176	Human Plasma	Verapamil	ProBindW	Inj 2	Standard	0.710	54968.051	3235386.0		Ratio	0.873	-
0		ot_Bind_Human_RED_Assay_177	Human Plasma	Verapamil	ProBindW	Inj 2	Analyte	0.711	12921.566	770875.063	0.235	Ratio	0.847	-
1		ot_Bind_Human_RED_Assay_266	Human Plasma	Verapami	ProBindW	Inj 3	Standard	0.714		3140030.0	0.010	Ratio	0.904	-
2	091707_PGA_ADME_Pr	ot_Bind_Human_RED_Assay_267	Human Plasma	Verapamil	ProBindW	Inj 3	Analyte	0.714	11472.573	668497.250	0.216	Ratio	0.855	_
Chromatogra	am												1	_0
ANT DOA AD	DME Prot Bind RED Ass	au 000 Consolit/Mo tut)										E 1.	MRM of 1 ch	annal F
		73762.62 4610325												
б-		4610325	Donor	Mell										
0407_PGA_AD	DME_Prot_Bind_RED_Ass	4610325	Donor	Mell	entre entre terr	-in-in-in-in-in-in-in-in-in-in-in-in-in-			12000 [1000 [1000				MRM of 1 ch	n
0	DME_Prot_Bind_RED_Ass	4610325	Donor	Nell	eden Freedon	-too too too to				Internetion	danaturalm		MRM of 1 ch	nannel,E
%- - - - - - - - - - - - - - - - - - -	DME_Prot_Bind_RED_Ass	4610325			éll.						.lto		MRM of 1 ch	nannel,E
90407_PGA_AC erapamii 000		4610325 4610325 () () () () () () () () () () () () ()	Accept	tor W	Minaritanian							F1	MRM of 1 ch	iannet,E asnet 1 .615e+0
0		4610325 4610325 gy_087 Smooth(Mn,1x1)	Accept		Minaritanian	1.30 1.40	1.50 1.8() 1.70	1.80 1.	90 2.00	2.10		MRM of 1 ch	

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

- · 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

Featured Products

ProfileLynx <https://www.waters.com/513819> QuanOptimize <https://www.waters.com/534330>

720002610, May 2008

© 2021 Waters Corporation. All Rights Reserved.