Extraction of a Range of Acidic, Basic and Neutral Drugs from Plasma Using ISOLUTE® PLD+ Plates Prior to LC-MS/MS Analysis

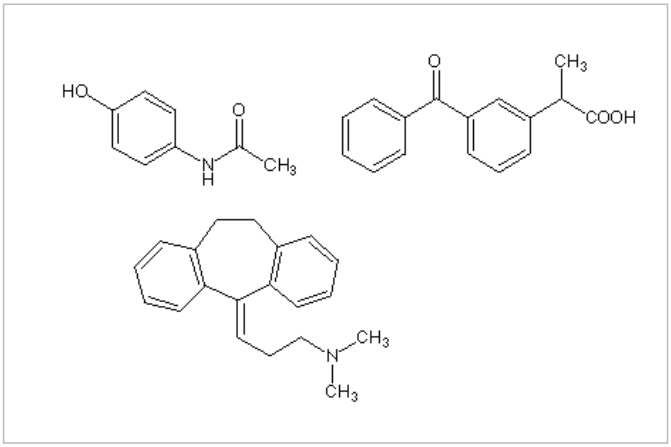

Figure 1. Structures of acetaminophen (neutral), ketoprofen (acidic) and amitryptiline (basic): examples of the broad range of analytes extracted in this application

Introduction

This application note describes the use of the ISOLUTE® PLD+ Protein and Phospholipid Removal Plate for clean-up of a range of acidic, basic and neutral drugs from plasma.

ISOLUTE® PLD+ Protein and Phospholipid Removal Plates provide very effective but extremely simple sample clean-up for LC-MS/MS analysis. Requiring next to no method development, ISOLUTE PLD+ can be integrated quickly and easily into routine workflow, increasing productivity and reducing instrument downtime. ISOLUTE PLD+ plates remove >99% of plasma proteins and phospholipids, the main causes of ion suppression, leading to cleaner extracts and increased sensitivity (signal to noise) for a broad range of analytes.

Analytes

Acetaminophen, amitriptyline, atenolol, bretylium tosylate, brompheniramine, fluoxetine, metoprolol, mianserin, naltrexone, procainamide, quinidine, ranitidine, salbutamol, sulindac, p-toluamide and ketoprofen.

Sample Preparation Procedure

Format:	**ISOLUTE® PLD+ Protein and Phospholipid Removal Plate, 50 mg, part number 918-0050-P01**
Sample Pre-treatment:	If required, spike plasma samples with appropriate internal standards (typically 10 µL volume) and mix thoroughly. **Note:** no internal standards were used in this study.

Sample Clean up:

Ensure collection plate is in position before processing

Step 1:	Dispense acetonitrile (400 µL) into each well
Step 2:	Dispense plasma (100 µL) into each well. Mix thoroughly using vortex mixing for 30 s or repeat aspirate/dispense steps
Step 3:	Apply vacuum (–0.2 bar) or positive pressure (2–3 psi) until sample is fully eluted (5 min). For extremely viscous samples e.g. dog plasma, the vacuum/positive pressure required for adequate flows may be higher.
Post Extraction:	Evaporate to dryness (SPE Dry, 40°C, 40 mins)
Reconstitution:	Reconsititute in 0.1% Formic acid aq/MeOH (80/20, v/v, 200 µL) prior to analysis

Biotage®

HPLC Conditions

Instrument: Waters 2795 Liquid Handling Unit

Column: Phenomenex Kinetex XB-C18 (50 x 2.1mm, 2.6 µ)

Mobile Phase: A: 0.1% Formic acid aq (v/v)

B: MeCN

Flow Rate: 0.3 mL/min

Table 1. Gradient Conditions

Time	% A	% B	Curve
0	90	10	1
4	26	74	6
4.4	90	10	11

Injection Volume: 10 µL

Sample Temperature: 20 °C

Column Temperature: Ambient

Mass Spectrometry Conditions

Instrument: Waters Ultima Pt Triple Quadrupole Mass Spectrometer

Desolvation Temperature: 350 °C

Ion Source Temperature: 100 °C

Collision Cell Pressure: 2.7 e^{-3} mbar

Positive ions were acquired in the multiple reaction monitoring (MRM) mode.

Table 2. MRM Conditions

Function	Compound	MRM Transition	Cone Voltage (V)	Collision Energy (eV)
1	Procainamide	236.10 > 163.10	35	15
	Salbutamol	240.00 > 148.00	35	15
	Atenolol	267.20 > 190.20	55	18
	Ranitidine	315.10 > 176.00	35	16
2	Acetaminophen	152.10 > 110.10	40	12
	Bretylium	242.10 > 169.00	35	15
	Quinidine	325.10 > 160.00	35	25
	Naltrexone	342.10 > 324.10	40	19
3	p-toluamide	136.00 > 93.00	35	10
	Metoprolol	268.10 > 116.10	35	17
	Brompheniramine	319.10 > 274.00	35	15
4	Mianserin	265.00 > 208.00	35	19
5	Amitriptyline	278.10 > 233.00	35	15
	Fluoxetine	310.00 > 148.00	35	8
6	Ketoprofen	255.10 > 209.10	35	11
	Sulindac	357.00 > 233.00	50	25

Results

Good analyte recovery, reproducibility and extract cleanliness were achieved for a broad range of analytes using ISOLUTE® PLD+ Protein and Phospholipid Removal Plates, allowing quantitation of analytes at low levels. **Table 3** shows recovery and reproducibility (RSD generally <10%) for the range of analytes. **Figure 2** illustrates the MRM chromatogram for amitryptilline at a concentration of 20 pg/mL in plasma (s/n 57:1).

Analyte Recovery

Table 3. Recoveries of analytes spiked at a concentration of 20 ng/mL in human plasma

Analyte	Functionality	pK_a*	logP*	% Recovery	% RSD (n=7)
Ketoprofen	Acidic	4.2	2.8	67.8	6.7
Sulindac	Acidic	4	3.59	74.5	4.1
Atenolol	Basic	9.1	0.16	74.0	5.8
Ranitidine	Basic	8.8	0.27	64.0	11.8
Procainamide	Basic	9.4	0.88	67.8	4.1
Salbutamol	Basic	9.4	1.31	73.9	6.2
Naltrexone	Basic	9.2	1.8	85.6	5.2
Metoprolol	Basic	10.8	1.88	77.3	4.8
Quinidine	Basic	9.28	2.88	75.1	5.0
Amitriptyline	Basic	9.4	3.1	75.6	4.5
Mianserin	Basic	8.3	3.6	67.0	2.2
Brompheniramine	Basic	9.2/3.6	4.06	61.1	3.0
Fluoxetine	Basic	9.5	4.2	83.1	3.5
Bretylium	Quat	N/A	1.17	60.3	7.4
Acetaminophen	Neutral	N/A	0.34	82.6	3.7

*pK and logP values were obtained from the literature, or values were calculated if not available

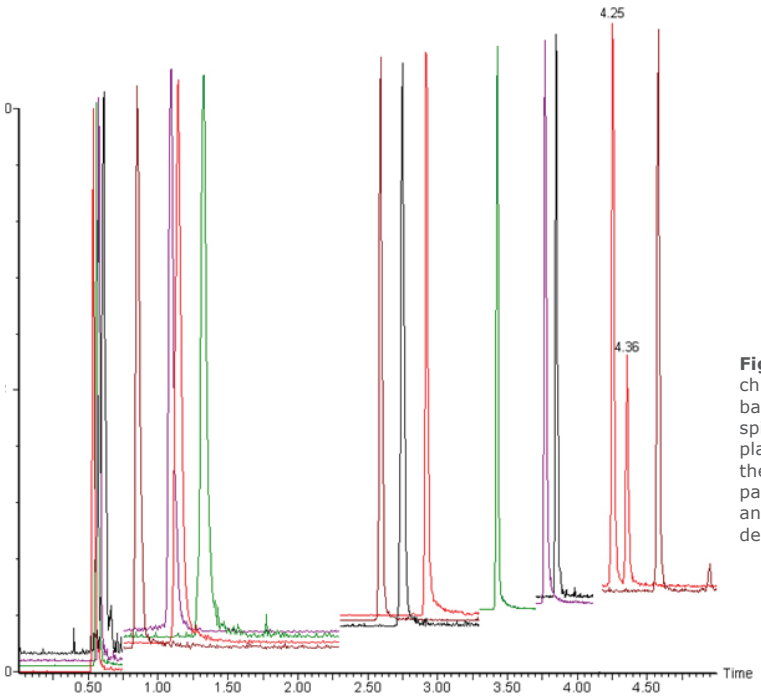

Figure 2. Offset MRM chromatogram of acidic, basic and neutral drugs spiked at 20 ng/mL into plasma and extracted using the protocol described on page 1. Analyte elution order and analytical conditions as described in **Table 2.**

Phospholipid Removal

The effective protein and phospholipid removal obtained using ISOLUTE PLD+ Protein and Phospholipid Removal Plates provided clean extracts with very low matrix effects. Residual phospholipids were investigated to provide an indication of extract cleanliness. We investigated the most abundant phospholipids (selected from full scan, SIR and precursor ion scanning experiments) using MRM transitions monitoring the common 184 product ion. **Figure 3** demonstrates phospholipid content comparing protein precipitated plasma, solvent blank and the final extraction protocol.

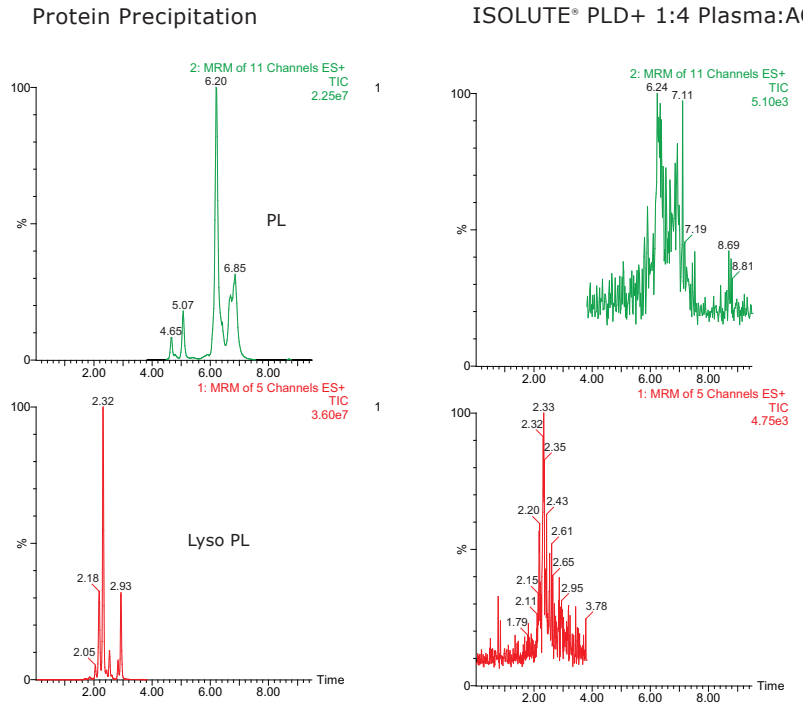

Figure 3. MRM TICs showing phospholipid content of plasma prepared by a) protein precipitation at a 1:4 ratio of plasma : acetonitrile and b) ISOLUTE® PLD+ under the same conditions. >99% of plasma phospholipids are removed, allowing low level quantitation of analytes.

Conclusion

ISOLUTE® PLD+ Protein and Phospholipid Removal Plates are suitable for clean-up of a range of analytes with widely differing functionality and polarity characteristics from plasma, giving high recoveries, good reproducibility and excellent extract cleanliness.

Ordering Information

Part Number	Description	Quantity
918-0050-P01	ISOLUTE® PLD+ Protein and Phospholipid Removal Plate	1
Accessories		
121-5202	Collection plate, 1 mL	50
121-5203	Collection plate, 2 mL	50
121-5204	Piercable sealing cap	50
Vacuum Processing		
121-9600	Biotage® VacMaster™-96 sample Processing manifold	1
121-9601	VacMaster VCU-1 Vacuum Control Unit	1
121-9602	VacMaster VCU-2 Vacuum Control and Generation Unit	1
Positive Pressure Processing		
PPM-96	Biotage® PRESSURE+ Positive Pressure Manifold, 96 position	1

EUROPE
Main Office: +46 18 565900
Toll Free: +800 18 565710
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: + 46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
Outside US: +1 704 654 4900
us-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA
Tel: +86 21 2898 6655
Fax: +86 21 2898 6153
cn_order@biotage.com
cn-1-pointsupport@biotage.com

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