Extraction of Analytes of Toxicological Interest from Plasma with Enhanced Matrix Removal-Lipid (EMR-Lipid) Material

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Abstract

A convenient analytical method for the determination of compounds from various classes in plasma involves the addition of acetonitrile to a small volume of plasma. The mixture is vortexed to elicit protein precipitation and centrifuged. The supernatant is transferred to a dispersive tube containing Enhanced Matrix Removal - Lipid (EMR – Lipid) sorbent to remove > 97% endogenous plasma lipid matrix components. EMR-Lipid removed substantially more lipids than other phospholipid removing sorbents tested. Analytes are isolated from spiked plasma samples with accuracies above 95 % and 6% RSDs on average. Combining protein precipitation with EMR - Lipid dispersive SPE in plasma offered separation for a variety of toxicological compounds, LOQs at 1 ng/mL or below based on method performance. The method is quick, easy and removes lipids that are known to remain on the column causing chromatographic anomalies and source contamination.

Introduction

Determination of compounds in biological matrices is commonly studied. Mass spectroscopy methods are the first choice for many applications based on their flexibility, selectivity, sensitivity, qualitative and quantitative capabilities. Analysis of compounds in biological samples requires sample preparation that can range from simple protein precipitation (PPT) to more complex solid- phase extraction (SPE).

Previously we described an extension of the work presented by Plössl *et al.* [1] for the determination of pharmaceuticals in whole blood employing a modified mini-extraction procedure and dispersive SPE cleanup with LC/MS/MS analysis [2]. Other researchers have also implemented a mini-QuEChERS approach with matrix cleanup by dispersive SPE [3,4,5]. The results are very promising but a major disadvantage noted by several authors was the lack of removal of lipids from the biological matrix. Co-extracted lipids can present major issues during analysis including contamination of the chromatographic system and MS ion suppression.

The experiments presented in this application note used plasma. A protein precipitation with acidified acetonitrile was performed, followed by cleanup with EMR-Lipid and enhanced post sample treatment (polish and dry step). The experiments were performed using twenty five different compounds with a broad range of hydrophobicity and pKa.

Results and Discussion

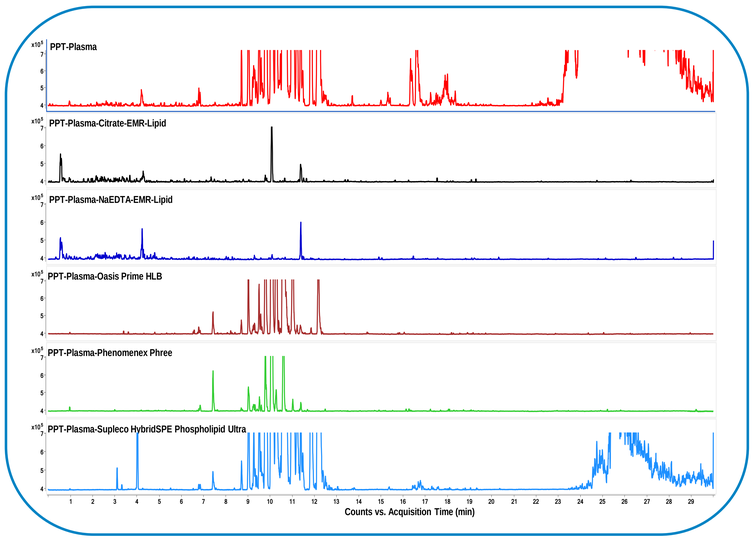

Figure 1. LC/MSMS product Ion m/z 184 for plasma sample after protein precipitation and various lipid removal sorbents.

Agilent Bond Elut EMR-Lipid selectively removes lipids through size exclusion and hydrophobic interactions without affecting analyte recovery. Phospholipids were monitored by LC/MSMS using precursor ion scan for product ion m/z 184 and MS1 from 100 to 1000. The chromatography run was extended out to 30 minutes to elute matrix co-extractives remaining on the column and possibly in the ion source. These wouldn't necessarily be apparent in a short fast gradient used for analyte analysis but can elute in subsequent injections causing analytical variability.

Experimental

Pre-spiked Plasma:
500 µL of plasma + QC (25 µL) + IS (25 µL) + 950 uL ACN (0.2% FA) in a 5 mL centrifuge tube
Vortex, centrifuge 5000 rpm, 5 min
Add 500 µL water to EMR-L 200 mg sorbent in a 5 mL centrifuge tube, vortex
Decant entire extract into EMR-L tube, vortex, centrifuge 5000 rpm, 5 min
Dispense extract into empty 5 mL tube, add 450 mg MgSO4, vortex immediately, centrifuge 5000 rpm, 5 min
Transfer supernatant to 2 mL tube containing ~125 mg MgSO4, vortex immediately, centrifuge 13,000 rpm, 3 min
Take 200 µL of final extract + 800 µL of water into a 2 mL AS vial, vortex, analyze

LC Conditions:
Column: Agilent Poroshell 120 EC-C18: guard 2.1 x 5 mm (p/n 821725-911), column 2.1 x 100 mm, 2.7 µm (p/n 695775-902)
Flow rate: 0.5 mL/min
Column temperature: 60 °C
Injection volume: 2 µL
Mobile phase: A: 5 mM ammonium formate, 0.01% formic acid
B: acetonitrile, 0.01% formic acid

Analysis	
Time	%B
0.0	10
0.5	15
3.0	50
4.0	95
6.0	95

Program for lipid evaluation	
Time	%B
0.00	5
3.00	30
17.00	100
30.00	100

MS Conditions: Agilent 6490 LC/MSMS
Ion-Source: AJS ESI, Positive
Dynamic MRM
Gas Temp (°C): 120
Gas Flow (L/min): 14
Nebulizer (psi): 40
Sheath Gas Temp: 400
Sheath Gas Flow: 12
Capillary (V): 3000

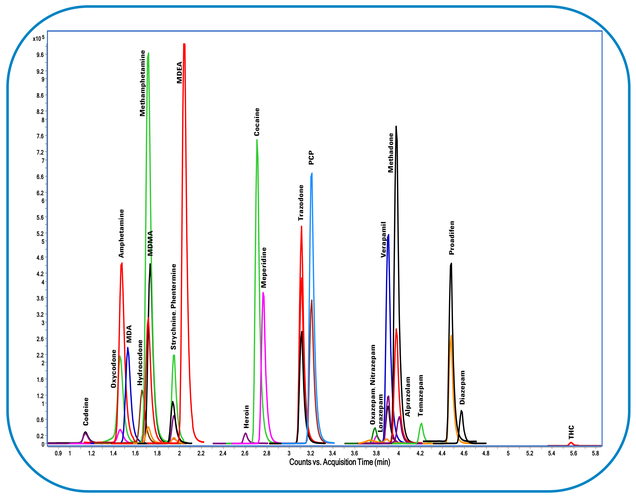

Figure 2. LC/MSMS MRM chromatogram of 10 ng/mL spiked plasma after protein precipitation and EMR-L dSPE cleanup.

Table 1. Linear Correlation Coefficient and LOQ for 25 Compounds

Compound	R^2	LOQ ng/mL	Compound	R^2	LOQ ng/mL
Codeine	0.9989	1.0	Trazodone	0.9983	0.5
Oxycodone	0.9988	0.5	PCP	0.9991	0.5
Amphetamine	0.9991	0.5	Nitrazepam	0.9919	0.5
MDA	0.9916	0.5	Oxazepam	0.9966	1.0
Hydrocodone	0.9984	0.5	Lorazepam	0.9964	5.0
Methamphetamine	0.9983	0.5	Verapamil	0.9915	0.5
MDMA	0.9989	0.5	Methadone	0.9736	0.5
Strychnine	0.9993	0.5	Alprazolam	0.9939	0.5
Phentermine	0.9990	0.5	Temazepam	0.9961	0.5
MDEA	0.9857	0.5	Proadifen	0.9988	0.5
Heroine	0.9967	0.5	Diazepam	0.9996	0.5
Cocaine	0.9766	0.5	THC	0.9929	5.0
Meperidine	0.9952	0.5			

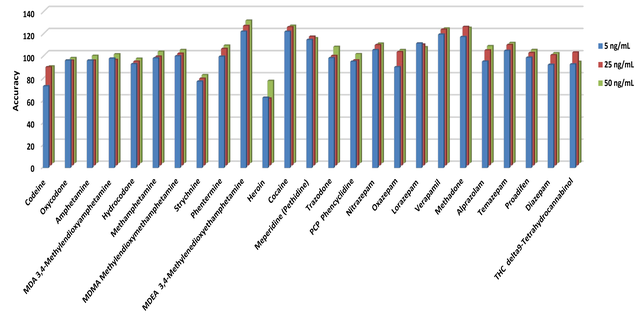

Figure 3. Accuracy for the 25 Extracted Compounds in Plasma

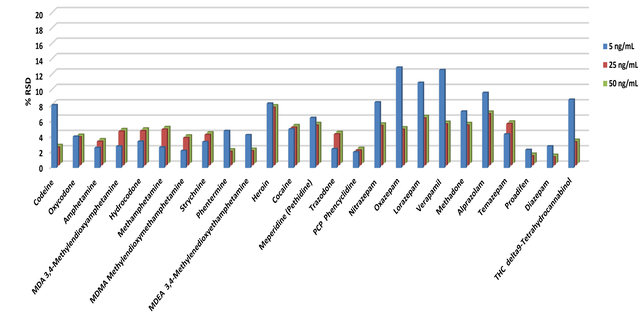

Figure 4. Relative Standard Deviation (RSDs) for the 25 Compounds in Plasma

Conclusion

A simple and inexpensive sample clean-up method using protein precipitation and EMR-Lipid dispersive cleanup has been developed for plasma prior to LC/MSMS for a range of compounds. The main advantages of this new method is a cleaner extract with significant overall lipid removal compared to standard PPT and commercially available lipid removal products, excellent recoveries and single digit %RSDs. All of the compounds with average recoveries at 5 ng/mL greater than 95% and RSD < 6%. Heroin was the only compound with relatively lower recoveries 65% on average but RSDs below 8%. Substantially cleaner extracts without the need for additional instrumentation or glassware, offer an easy and user-friendly sample preparation. This approach for the extraction of compounds from plasma is easily implemented into laboratories without extensive expertise in sample preparation techniques since it only involves spiking, vortexing and centrifugation.

References

1. F. Plössl, M. Giera, F. Bracher, *J. Chrom. A.* 1135, 19-26 (2006).
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