Biocompatible Solid Phase Micro Extraction (BioSPME): A Tool to Overcome Matrix Effect for Multi-Class Compound Methods

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Introduction

- It has been known for some time that the sensitivity and reproducibility of quantitative LC/MS/MS methods can be influenced by the presence of certain matrix components within a sample.
- These interferences impact analyte ionization through either suppression or enhancement mechanisms.
- Multi class, multi residue analysis requires the separation of many analytes with a broad range of chemical and physical properties (log P, pK_a, size, etc.).
- Under these conditions it becomes increasingly difficult to separate polar and non-polar interferences from analytes of interest via reversed phase liquid chromatography, thus signifying the need for improved sample preparation.
- The focus of this study was to determine the benefit of the combination of BioSPME and LC/MS/MS for multi class, multi residue analysis from complex samples, such as biological fluids.
- The quality of the quantitative methodologies was evaluated by comparing sensitivity, dynamic range, reproducibility, and robustness to traditional sample preparation techniques such as protein precipitation.

Table 1. Multi-class Compound List

Analyte	MW (g/mol)	Log P	pK_a	Class
Amiloride	229.05	-0.3	16.46, 3.29	Diuretic
Imidacloprid	255.05	0.57	11.12	Neonicotinoid
Nizatidine	331.11	1.1	6.83	Anti-histamine
Benzoylecgonine	289.13	1.71	3.15, 9.54	alkaloid
Nevirapine	266.12	2.5	10.37	NNRTI
Atrazine	215.09	2.61	1.7	Triazine
Buspirone	385.25	2.63	7.62	Azapirone
Imiquimod	240.14	2.7	5.4	Immune response modifier
Methapyrilene	261.13	2.87	8.76	Pyridine anti-histamine
Mirtazapine	265.16	2.9	6.67	Tricyclic antidepressant
Clarithromycin	747.48	3.16	8.99	Macrolide antibiotic
Mianserin	264.37	3.52	6.92	Tetracyclic antibiotic
Hydroquinidine	326.20	3.77		Alkaloid
Mesoridazine	386.15	3.9	19.36, 8.19	Piperidine neuroleptic
Haloperidol	375.14	4.3	8.66	Antipsychotic
Nefazodone	469.23	4.7	7.09	Phenylpiperazine antidepressant
Imipramine	280.19	4.8	9.4	Tricyclic antidepressant
Amitriptyline	277.18	5.1	9.4	Tricyclic antidepressant
Loratadine	382.15	5.2	4.33	Anti-histamine

Process

BioSPME is an equilibrium extraction technique in which the analyte of interest partitions between the sample matrix and the extraction coating on a BioSPME device. The extraction coating contains functionalized silica particles that are embedded within a proprietary biocompatible binder (Figure 1). The role of this binder is to reduce or eliminate the extraction of matrix interferences during immersion, without reducing analyte extraction. This allows for the isolation of target analytes, while minimizing the presence of matrix, resulting in a highly sensitive microextraction technique.

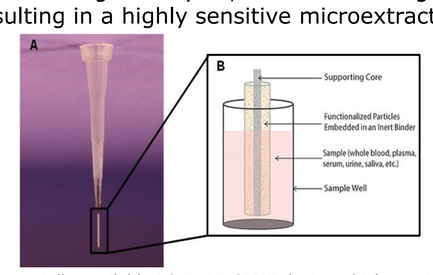

Figure 1. (A) A commercially available LC tip BioSPME device which consists of a coated fiber housed within a pipette tip. (B) A basic schematic of an extraction performed with a BioSPME fiber. The fiber is coated with functionalized particles that have been embedded within a proprietary binder. The binder allows the fiber to be placed directly within a biological fluid for sampling.

BioSPME

- Fibers were conditioned with 1 mL of 50:50 MeOH:Water for 20 min at 500 rpm.
- Following a 30 min equilibration period, spiked human plasma samples were extracted for 10 min at 500 rpm. Total sample volume was 1 mL.
- Fibers were washed for ~ 15 sec in 1 mL water at 500 rpm.
- Fibers were desorbed in 300 µL of methanol for 20 min at 500 rpm.
- Samples analyzed by LC/MS/MS

Protein Precipitation

- Following a 30 min equilibration period, a 100 µL aliquot of spiked human plasma samples was taken.
- Added 300 µL of acetonitrile and samples were vortexed for 5 min at 1000 rpm.
- Samples were centrifuged for 10 min at 10,000 rpm.
- Supernatant was transferred for analysis.
- Samples analyzed by LC/MS/MS

Analytical Method (Analytes)

column:Ascentis® Express RP Amide, 10 cm x 2.1 mm, 2.7 µm
mobile phase: (A) 5mM ammonium acetate, 0.1% acetic acid in water,
(B) 5 mM ammonium acetate in 95:5 acetonitrile:water
flow rate: 400 µL/min
column temp: 40 ºC
det.: MS/MS, ESI (+), MRM transitions
injection: 2 µL
gradient: 5%B for 1 min, 5%B to 55%B in 7 min, hold 55%B for 1 min, to 80%B in 2 min, 80%B to 5%B in 0.1 min and hold at 5%B for 1.4 min
instrument: Agilent® 1290 Infinity II with Agilent 6460 QQQ

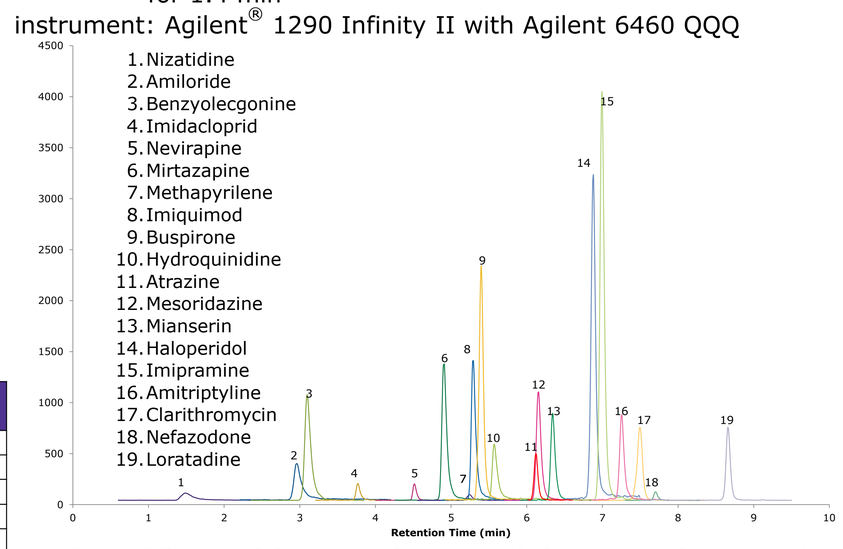

Figure 2 Representative chromatogram for Multi-class Analytes (1 ng/mL)

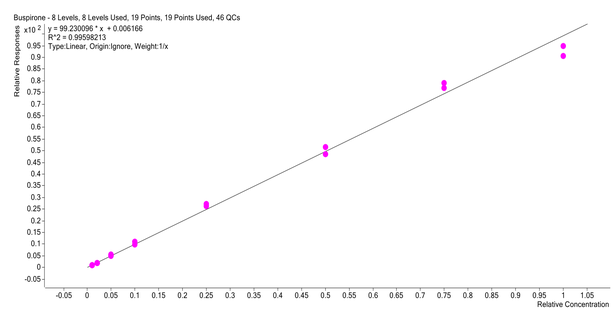

Figure 3 Representative calibration curve from 1 ng/mL to 1000 ng/mL

MRM Method (Phospholipids)

Table 2. MRM Transitions for Phospholipids

Analyte	Precursor (m/z)	Product (m/z)	Fragmentor (V)	Collision Energy (V)
Phospholipid 1	184	104	160	80
Phospholipid 2	496	184	160	80
Phospholipid 3	524	184	160	80
Phospholipid 4	70	184	160	80
Phospholipid 5	758	184	160	80
Phospholipid 6	786	184	160	80
Phospholipid 7	806	184	160	80

- Matrix blank samples were analyzed with the same LC conditions as the analytes to evaluate any co-elution of phospholipids.

LC Method (Phospholipids)

column: Ascentis® Express C18, 5 cm x 2.1 mm, 2.7 µm
mobile phase: (A) 5mM ammonium formate in 90:10 methanol:water
flow rate: 400 µL/min
column temp: 40 ºC
det.: MS/MS, ESI (+), MRM transitions
injection: 2 µL
instrument: Agilent® 1290 Infinity II with Agilent 6460 QQQ

- Matrix blank samples were analyzed with specific LC conditions to evaluate total presence of phospholipids after extraction.

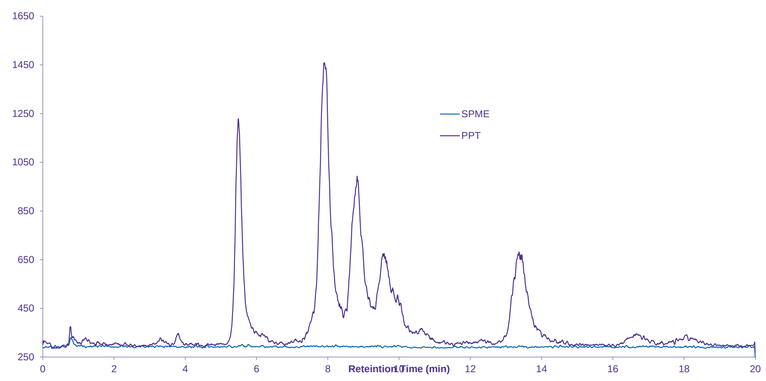

Figure 5 Total Phospholipids from human plasma after extraction

Results

- Recoveries ranged from 95.9-112% with % RSD's < 6% for BioSPME and 95.8-113% with %RSD's < 3% for protein precipitation.

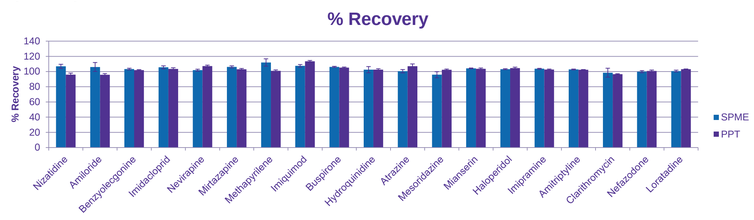

Figure 5. Recoveries and %RSD values for spiked human plasma samples

- The effect the matrix had on the analyte response was also evaluated by spiking analyte into extracted human plasma blank samples and comparing the response to analyte spiked into solvent.
- Matrix effects were less than 5% for the BioSPME extracts; where as the protein precipitation observed greater than 50% matrix effects (50% ion suppression) for nevirapine along with the majority of the analytes seeing more than 20% ion suppression.

$$\%\ \text{Matrix Effects} = \frac{\text{Analyte in presence of matrix}}{\text{Analyte in absence of matrix}} - 1 \times 100\%$$

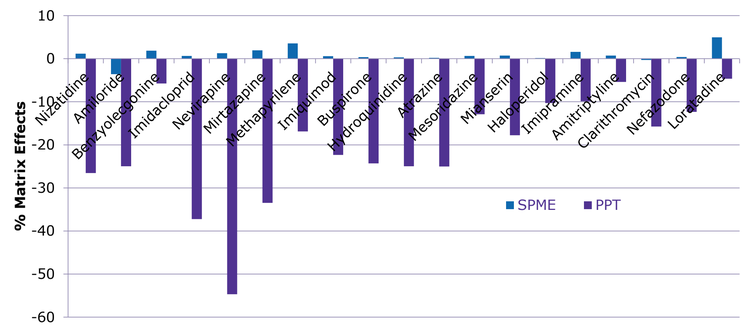

Figure 6 Matrix Effects from human plasma samples

- For the protein precipitation extracts, significant phospholipids were detected in the analyte retention region, especially in the 3-5 minute range where the majority of analytes eluted.

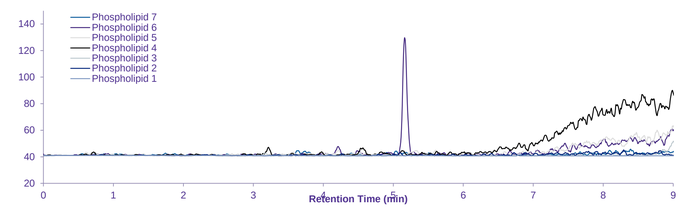

Figure 7 Phospholipids from human plasma using protein precipitation

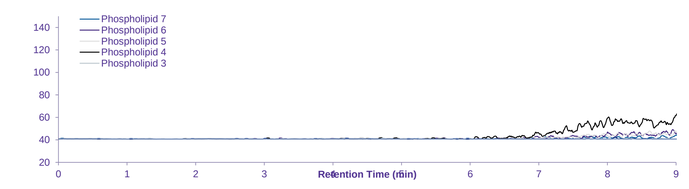

Figure 8 Phospholipids from human plasma using BioSPME

Summary

- Biocompatible solid phase microextraction fibers demonstrated significant advantages over traditional protein precipitation methods for human plasma samples.
- Using the BioSPME technique, multi-class compounds were able to be extracted and analyzed with minimal matrix effects present.
- The protein precipitation method demonstrated substantial matrix suppression for most analytes which may reduce the robustness of the analytical method.
- The protein precipitation method also introduced significant phospholipids onto the LC column. These were not observed in the extracts from the BioSPME sample preparation.
- By eliminating the presence of matrix constituents, the BioSPME method proves to be the better choice for method robustness and sample cleanliness.

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