Reduction of Matrix Effects in LC-MS Using an Optimized SPE Sorbent for Bioanalytical Sample Preparation

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Introduction

Sample preparation is an essential technique prior to LC-MS/MS analysis of drugs in biological fluid samples as interfering matrix components can mask or otherwise interfere with the quantitation of the compound(s) of interest. Historically, non-polar generic polymer-based SPE products have been extensively used for the extraction of drugs from biological fluids. However, the non-selective nature of these polymers, while advantageous in enabling the simultaneous extraction of a wide range of drug types, can lead to the co-extraction of high levels of unwanted endogenous sample components. These components contaminate the final extract, causing matrix effects such as ion suppression or enhancement, which can adversely affect quantitation and sensitivity in LC-MS/MS analysis.

During the drug development process, studies are performed to evaluate the pharmacokinetic parameters of potential drug compounds. In addition to endogenous matrix components (proteins, salts, phospholipids etc), these samples can contain high levels of dosing vehicles used to solubilize test compounds in formulations. Dosing vehicles (e.g. PEG 400, Tween 80, Cremophore etc.) can also cause significant matrix effects, and are notoriously difficult to remove during the sample preparation procedure.

In this presentation, we have investigated the use of EVOLUTE™ ABN, a novel polymer based SPE sorbent, for reduction of matrix effects in LC-MS. Matrix effects due to endogenous plasma components (**Part 1**) and a range of dosing vehicles (**Part 2**) have been examined. The effectiveness of EVOLUTE ABN is compared with other polymer-based SPE sorbents.

1. Matrix Effects Due to Endogenous Sample Components

EVOLUTE™ ABN has an optimized pore structure designed to minimize the retention of endogenous plasma components during the SPE process, producing cleaner extracts for analysis.

The effect of extract cleanliness on ion suppression caused by plasma extracts was investigated using LC-MS/MS flow injection analysis (as described by *Bonfiglio et al* [1]).

Experimental Procedure

Sample Preparation Procedure

Blank human plasma samples (100 µL) were extracted using the EVOLUTE ABN generic method, as described below. As a comparison, plasma samples from the same pooled master batch of plasma were extracted using competitor polymer-based SPE products, using the manufacturer's recommended generic methods in each case.

Sample Preparation

Biotage

EVOLUTE ABN Generic SPE Method
SPE Column: EVOLUTE ABN 25 mg/1 mL (p/n 600-0002-A)
Sample Pre-treatment: Dilute plasma sample 1:3 (v/v) with aqueous formic acid (1 %, v/v). Mix thoroughly
Conditioning: Condition each column with MeOH (1 mL)
Equilibration: Aqueous formic acid (0.1%, v/v, 1 mL)
Sample Load: Load sample (400 µL diluted plasma)
Interference Wash: H2O/MeOH (95:5, v/v, 1 mL)
Analyte Elution: Methanol (500 µL)

Following extraction, the plasma extracts were evaporated to dryness and reconstituted in mobile phase spiked with caffeine at a concentration of 1 µg/mL. The MS/MS signal intensity observed from the spiked plasma sample extracts was then compared with that observed for pure mobile phase spiked at 1 µg/mL with caffeine.

FIA LC-MS/MS Conditions
Mobile Phase: H_2O/ACN/MeOH/0.1% formic (50:45:5 v/v)
Flow Rate: 0.25 mL/minute
Injection Volume: 5 µL
Instrument: Varian 1200L triple quadrupole
Ionization: Electrospray, +ve
Drying Gas Temp: 260°C
SRM Transition for Caffeine: m/z 195>138

Results

The signal intensity from spiked mobile phase relative to that from extracts produced using EVOLUTE ABN, Competitor A and Competitor C is shown below (**Figure 1**).

Sample	% Ion Suppression
Spiked mobile phase	0
EVOLUTE ABN	28
Competitor A	46
Competitor C	78

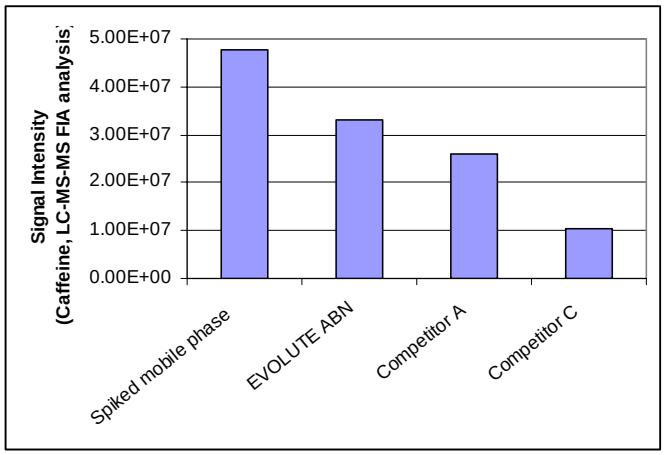

Figure 1. Caffeine signal intensity for plasma extracts compared to spiked mobile phase

Comments

Compared to spiked mobile phase, extracts from EVOLUTE ABN suppress the signal from caffeine by only 28 %. A and C exhibit 46 and 78 % ion suppression respectively.

Sample Preparation

Biotage

2. Matrix Effects due to Dosing Vehicles

Dosing vehicles are used to help solubilize test compounds in dose formulations. They generally have non-ionic surfactant-like characteristics, and are often present at high concentrations relative to the test drug compound, in biological fluid samples. When biological fluid samples contain dosing vehicles, there is an additional challenge to overcome during sample clean-up. Using human plasma samples spiked with common dosing vehicles, the contribution of the dosing vehicle to any matrix effects following sample clean-up was evaluated.

EVOLUTE ABN was used to extract plasma samples spiked with the following commonly used dose vehicles, and matrix effects from those extracts were compared using FIA LC-MS/MS: **Carboxymethylcellulose (CMC), Cremophore, PEG 400, Tween 80**. The results were compared with matrix effects from extracts produced using a competitor polymer-based SPE sorbent.

Experimental Procedure

Samples: Pooled human plasma samples were spiked with each dosing vehicle at a concentration of 50 µg/mL.

The same procedure was used as in **Section 1**. As a comparison, plasma samples from the same pooled master batch of spiked plasma were extracted using a competitor polymer-based SPE product, using the manufacturer's recommended generic method.

Following extraction, the extracts were evaporated to dryness (40 °C) and reconstituted in mobile phase spiked with caffeine at a concentration of 100 ng / mL. The FIA LC-MS/MS signal intensity observed from the extracts was then compared with that observed for mobile phase spiked at the same concentration (100 ng / mL) with caffeine.

FIA LC-MS/MS Conditions
Mobile Phase: H20:ACN:MeOH:0.1% formic (50:45:5 v/v)
Flow Rate: 0.25 mL/minute
Injection Volume: 10 µL
Instrument: Waters Quattro Ultima
Ionization: Electrospray, +ve
DesolvationTemp: 350°C
SRM Transition for Caffeine: m/z 195>138

Results

a) Matrix Effects Due to Different Dose Vehicles (samples extracted using EVOLUTE ABN):

Note: The matrix effect observed for extracts of plasma with dose vehicle compared to blank plasma (i.e. the contribution of the dosing vehicle to the total matrix effect) is reported.

Sample	Additional Suppression
Plasma + CMC	-5% (5% enhancement)
Plasma + Cremophor	-0.3% (0.3% enhancement)
Plasma + PEG 400	15%
Plasma + Tween 80	29%

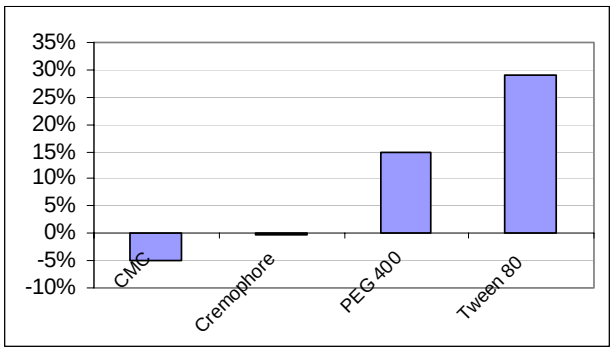

Figure 2. Contribution of dosing vehicle to total matrix effect (spiked plasma samples extracted using EVOLUTE ABN)

Comments

There is significant variation in the contribution of different dosing vehicles to total matrix effects from spiked plasma when extracted using EVOLUTE ABN.

b) Comparison of Polymeric SPE Sorbents

Note: The matrix effect observed for extracts of plasma + dose vehicle compared to blank plasma (i.e the contribution of the dosing vehicle to the total matrix effect) is reported.

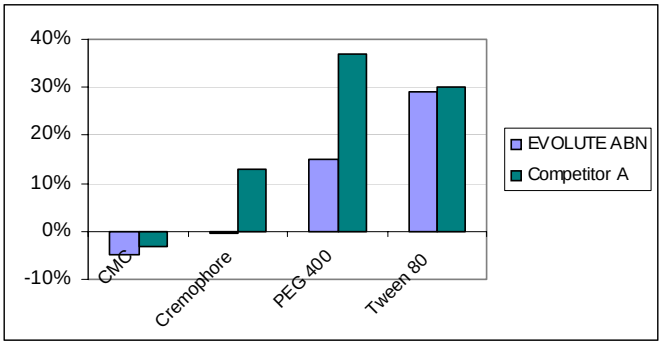

Figure 3. Contribution of dosing vehicle to total matrix effect (spiked plasma samples extracted using EVOLUTE ABN compared to competitor A)

Comments

Extracts produced using EVOLUTE ABN show a reduced contribution to matrix effects for some dose vehicles compared to extracts produced using competitor A (particularly PEG 400 (37% vs. 15% additional suppression) and Cremophore (-0.3% vs 13% additional suppression)).

For other dose vehicles (Tween 80 and CMC), the dose vehicle matrix effect is similar for both EVOLUTE ABN and competitor A extracts.

Overall Conclusions

Biological fluid extracts produced using EVOLUTE ABN exhibit reduced matrix effects due to endogenous sample components compared with other polymer-based SPE products.

Dose vehicles were found to make a significant contribution to total matrix effects from spiked biological fluids.

EVOLUTE ABN is more effective at reducing the matrix effects caused by dose vehicles than a polymer-based competitor SPE product.

References:

1. R. Bonfiglio, R.C. King, T.V. Olah, K. Mwerkle, Rapid Commum. Mass Spectrom. 13 (1999) 1175-1185.

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Sample Preparation

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