Evaluation of EVOLUTE™ CX Mixed-mode Cation Exchange SPE Resin

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Introduction

Matrix components, particularly salts, proteins and phospholipids, can lead to ion suppression resulting in inaccurate quantitation and reduced detection limits. Resin-based mixed-mode cation exchange SPE sorbents are widely used for the extraction of basic compounds from biological fluids. The dual retention mechanism allows a two stage interference wash protocol, which results in extremely clean extracts.
This poster will investigate the performance of EVOLUTE™ CX for the extraction of a wide range of basic drugs from plasma, showing high analyte recovery and advanced extract cleanliness. The analyte suite was selected to cover a variety of basic analytes with wide ranging polarities (LogP values). Extract cleanliness experiments were performed showing overall ion suppression, then individual matrix components examined in terms of protein and phospholipid removal.

Experimental Procedure

Reagents

All analytes, ammonium acetate, formic and acetic acids were purchased from Sigma Chemical Co. (Poole, UK). Blank human plasma was obtained through the Welsh Blood Service (Pontyclun, UK). Blank rat serum was obtained from the NCTR animal colony. Materials for the gel electrophoresis work were obtained from Invitrogen (Carlsbad, CA. USA). All solvents were HPLC grade from Fisher Scientific (Loughborough, UK) and Milli-Q water used throughout.

Sample Preparation

EVOLUTE™ CX Sample Preparation Procedure
Extractions were performed in the 25 mg 96-well format with blank human plasma/serum (100 µL)
Part Number 601-0025-P01
Sample Pre-treatment: Plasma sample diluted with 50 mM ammonium acetate buffer at pH 6 (1:3, v/v)
Column Conditioning: Methanol (1 mL)
Column Equilibration: 50 mM ammonium acetate buffer at pH 6 (1 mL)
Sample Loading: Pre-treated plasma sample (400 µL)
Interference Elution 1: 50 mM ammonium acetate buffer at pH 6 (1 mL)
Interference Elution 2: Methanol (1 mL)
Analyte Elution: 5% (v/v) NH_4OH in methanol (1 mL)

Experiment 1: Basic Analyte Recoveries

Blank human plasma was spiked at a concentration of 50 ng/mL and extracted using the SPE procedure above. The extracts were evaporated to dryness and reconstituted in 1 mL of 80:20 (v/v) H_2O/MeOH for subsequent LC-MS/MS analysis. Analysis was performed using MRM transitions for each analyte.

Experiment 2: Overall Ion Suppression FIA-MS/MS

Blank plasma SPE extracts were reconstituted in 1 mL of 50:45:5:0.1% H_2O/ACN/MeOH/Formic acid (v/v) mobile phase spiked with 1 µg/mL caffeine concentration. Both the MeOH interference wash and the elution step were investigated. The 2795 liquid handling unit delivered an isocratic mobile phase of 50:45:5:0.1% H_2O/ACN/MeOH/Formic acid (v/v) at 0.25 mL/min. Injection volumes were set to 5 µL and the MS set to monitor the MRM transition for caffeine (195>138, cone voltage 45 V and collision energy 16 eV).

Experiment 3: Gel Electrophoresis

Blank plasma SPE extracts were reconstituted in water and 5 µL serum equivalents removed. LDS sample buffer (4 µL) and reducing agent (2 µL) were added to each aliquot and boiled at ~100°C for 5 minutes, spun to pull down volume, and allowed to cool to room temperature. Electrophoresis was performed on two cells (running four gels simultaneously) using NuPAGE Novex 12% Bis-Tris mini gels with MOPS SDS running buffer at 200 V, 120 mA and 12.5 W. The total method time was set to 65 minutes to allow complete migration of the protein in each gel. 5 µL equivalent of each serum extract was compared to 0.5 µL equivalent of raw serum and 4 µL of the Benchmark Protein Ladder molecular weight marker in each gel.

Experiment 4: Phospholipid Removal

Blank plasma SPE extracts were reconstituted in 1 mL of 70:30 (v/v) H_2O/MeOH prior to analysis. Initial MS work was performed in the full scan mode (250-900 Da) to identify the most abundant phospholipid ions. MRM transitions were then employed for quantitative purposes using the 184 Da product ion.

HPLC Conditons

Instrument: Waters 2795 Liquid Handling System (Waters Assoc., Milford, MA, USA).
Column: Luna Phenyl-Hexyl 5 µm analytical column (50 x 2.0 mm id) or Zorbax Eclipse XDB C18 3.5 µm analytical column (100 x 2.1 mm id).
Mobile Phase: 0.1% formic acid aq and either MeCN or 0.1% formic acid in MeCN at flow rates of either 0.25 or 0.3 mL/min.
Gradient: Various gradients employed.
Injection Volume: 5-15 µL
Temperature: Ambient

Mass Spectrometry

Instrument: Quattro Ultima Pt triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Positive ions were acquired in the full scan and multiple reaction monitoring modes (MRM).
Desolvation Temperature: 350 °C
Ion Source Temperature: 100 °C
Collision Gas Pressure for MRMs: 2.6 x 10^{-3} mbar

Results

Figure 1. shows the recovery chart for the basic analytes used in this study. The recoveries for all analytes were in excess of 90% with corresponding RSD's below 10%.

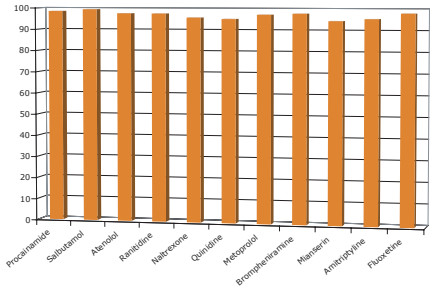

Figure 1. *Basic analyte recovery profile (n=7)*

Figure 2. shows overall ion suppression experiments, comparing the MeOH interference wash and elution steps.

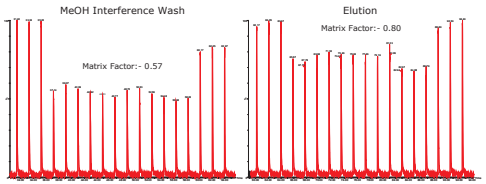

Figure 2. *Overall ion suppression comparing MeOH interference wash and elution steps (n=4)*

Protein removal in both the MeOH interference wash and elution steps was demonstrated using gel electrophoresis and the results are shown in ***Figure 3***.

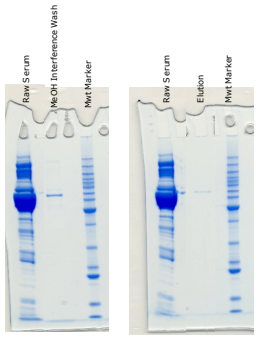

Figure 3. *Gel electrophoresis protein profile comparing MeOH interference wash and elution steps*

The final experiment investigating the amount of phospholipid removal is shown in ***Figure 4***.

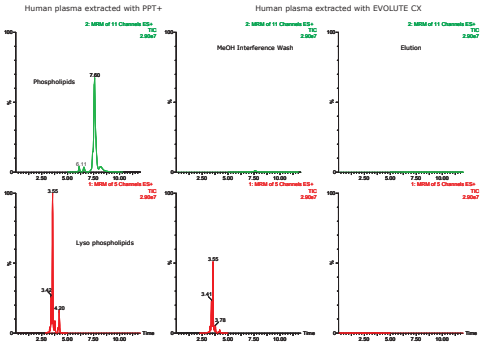

Figure 4. *MRM TIC phospholipid comparison between plasma extracted with PPT+ and the MeOH interference wash and elution steps from an EVOLUTE CX extracted sample.*

Conclusions

- Excellent recoveries of a wide range of basic analytes (>90%) with corresponding RSD's <10%.
- Overall ion suppression using FIA-MS/MS shows matrix factors of 0.57 and 0.8 for the interference wash and elution steps, respectively.
- Gel electrophoresis data showed > 99% serum protein removed in both the MeOH interference wash and elution steps. This indicates that proteins are removed during the sample load and/or the first interference wash step.
- Approximately 70% of the lyso PLs and >95% of the larger chain PLs are removed prior to the MeOH interference wash step.
- EVOLUTE™ CX gave >98% overall phospholipid removal in the final extract.

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