Strategies for Improving Extract Cleanliness using Resin-based SPE

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Introduction

Generic resin-based SPE products are extensively used for the extraction of drugs from biological fluids. However, the non-selective nature of these resins, 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 such as proteins, salts and phospholipids. The optimized surface chemistry of EVOLUTE® ABN minimizes the amount of co-extracted matrix components giving lower ion suppression[1] and almost entirely eliminates problems associated with protein retention[2] (> 99% serum protein removed) compared to other resin-based sorbents. This poster demonstrates the use of EVOLUTE® ABN and addresses extract cleanliness problems through various washing and elution protocols. Slight modification of the generic method, depending on analyte properties can give significantly enhanced removal of matrix components and result in cleaner extracts and more reliable quantitation. Overall ion suppression data is shown along with specific data monitoring phospholipids (structures shown in Figure 1).

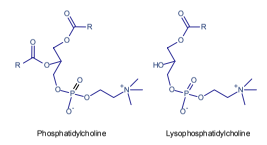


Figure 1. Phospholipid structures

Experimental Procedure

Reagents

Formic acid was purchased from Sigma Chemical Co. (Poole, UK). Blank human plasma was obtained through the Welsh Blood Service (Pontyclun, UK). All solvents were HPLC grade from Fisher Scientific (Loughborough, UK) and Milli-Q water used throughout.

Sample Preparation (Generic Method)

EVOLUTE® ABN Sample Preparation Procedure
Extractions were performed in the 25 mg 96-well format with blank human plasma (100 µL)
Part Number 600-0025-P01
Sample Pre-treatment: Plasma sample diluted (1:3, v/v) with aqueous formic acid (1%, v/v)
Column Conditioning: Methanol (1 mL)
Column Equilibration: Aqueous formic acid (0.1%, v/v, 1 mL)
Sample Loading: Pre-treated plasma sample (400 µL)
Interference Elution: Water/methanol (95:5, v/v, 1 mL)
Elution: Methanol (500 µL)

Experiment 1: Interference Wash Investigation

Interference Wash: Ranging from 100% Water to Water/Methanol or Water/Acetonitrile combinations up to 60:40, (v/v, 1 mL)
Elution: Either Methanol or Acetonitrile (500 µL)

Experiment 2: Elution Solvent Investigation

Interference Wash: Water/methanol (95:5, v/v, 1 mL)
Elution: Ranging from 100% Methanol or Acetonitrile to Methanol/Water or Acetonitrile/Water combinations up to 60/40 (v/v, 500 µL)

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. 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).

Phospholipid Removal

Blank plasma SPE extracts were reconstituted in 1 mL of 70:30 (v/v) H_2O/MeOH prior to analysis. The most abundant phospholipids were monitored in the MRM mode 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 ACN at flow rates of either 0.25 or 0.3 mL/min.
Gradient: Various gradients employed.
Injection Volume: 5 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 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

Both experiments were investigated for overall ion suppression and phospholipid removal.

Results

Figure 2. shows the overall ion suppression profile obtained from experiment 2 using ACN as the elution solvent. There was very little difference in suppression observed between the various elution solvent combinations. Full matrix factors for all experiments are detailed in Table 1.

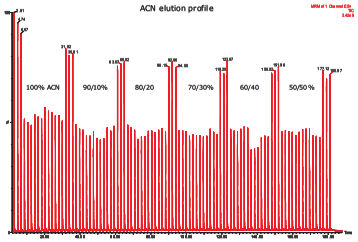


***Figure 2**. Overall ion suppression comparing various ACN elution solvent combinations (n=4)*

Table 1. Ion suppression matrix factors for the various wash and elution solvent combinations.

Wash Solvent Combination %	MeOH Wash Matrix Factor	ACN Wash Matrix Factor	Elution Solvent Combination %	MeOH Elution Matrix Factor	ACN Elution Matrix Factor
100 H_2O	0.80	0.83	**100**	0.65	0.66
95/5	0.91	0.73	**90/10**	0.69	0.70
90/10	0.94	0.76	**80/20**	0.79	0.73
80/20	0.88	0.72	**70/30**	0.79	0.71
70/30	0.85	0.69	**60/40**	0.78	0.72
60/40	0.80	0.73	**50/50**	0.75	0.73

Figure 3 shows phospholipid TIC's from a protein precipitated sample and EVOLUTE ABN comparing the generic method with modified wash and elution protocols. Figures 4 and 5 illustrate the amount of lyso phospholipid and higher molecular weight phospholipid removal comparing various wash and elution protocols from Experiments 1 and 2.

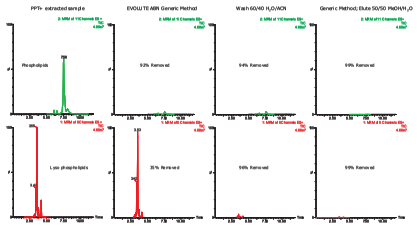
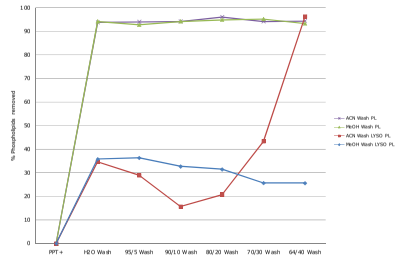


***Figure 3**. MRM TIC phospholipid comparison between plasma extracted with: PPT+; EVOLUTE ABN using the generic method; an advanced wash protocol; and an advanced elution protocol.*

***Figure 4.** Line graph comparing various wash protocols for lyso PL and PL removal*



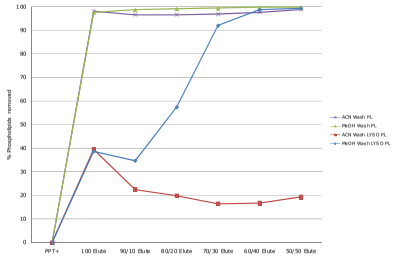


***Figure 5.** Line graph comparing various elution protocols for lyso PL and PL removal*

Conclusions

- Overall ion suppression work showed some similar trends as the phospholipid data although the results were not as pronounced and showed more variation.
- The EVOLUTE ABN generic method removes greater than 90% of PL's but only about 35% of the lyso PL's.
- Increasing the MeOH content in the wash solvent does not increase the amount of PL or lyso PL removal.
- When using ACN instead of MeOH in the wash solvent PL removal is greater than 90% while the lyso PL removal is still only around 30%. Advanced lyso PL removal is observed with ACN proportions greater than 30%. At 40% ACN lyso PL removal is greater than 95%.
- Elution solvent data shows that increasing the % H_2O in ACN elutions actually decreases the lyso PL removal to around 20%, however, PL removal is greater than 95% for all combinations.
- MeOH/H_2O elution solvent mixtures showed greater than 95% PL removal for all combinations. Combinations above 30% H_2O showed greater than 90% lyso PL removal.
- Depending on analyte properties modification of the generic method can dramatically reduce interfering endogenous matrix components resulting in cleaner extracts and more reliable quantitation.

References:
1. A New Polymer-based SPE Sorbent to Reduce Matrix Effects in Bioanalytical LC-MS/MS Presented at ASMS 2005. M.Cleeve *et al*

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