MATRIX EFFECTS ELIMINATED BY A SIMPLE AND FAST SAMPLE PREPARATION METHOD - MIXED-MODE SPE

Waters

Ziling Lu, Diane M. Diehl and Jeffrey R. Mazzeo
Waters Corporation , Milford, Massachusetts USA

OVERVIEW

A thorough examination of the causes of matrix effects from biological samples results in a clear-cut methodology for removing these interferences.

INTRODUCTION

Matrix effects can cause significant errors in the accuracy and precision of bioanalytical methods[1]. Some researchers have identified phospholipids as causing significant interferences in LC/MS/MS analyses[2], and others have demonstrated the degree of ion suppression and enhancement of matrix components in LC/MS[3]. Additionally, in an effort to create high-throughput methods, other researchers have suggested eliminating the chromatographic separation entirely. We had two goals for our research: 1. To understand the effects of residual matrix components on the analyte signal. 2. To determine which sample preparation method provides the cleanest extracts.

METHODS

Column: XTerra® MS C_{18} 2.1 x 20 mm *IS™*, 3.5 µm
Mobile Phase A: H_2O with 10 mM NH_4HCO_3, pH 10
Mobile Phase B: MeOH with 10 mM NH_4HCO_3, pH 10
Flow Rate: 0.4 mL/min

Gradient #1:

Time (min)	Profile %A	%B
0.0	95	5
5.0	5	95
7.0	5	95
7.5	95	5
10.0	95	5

Gradient #2:

Time (min)	Profile %A	%B
0.0	95	5
1.5	5	95
3.2	5	95
3.4	95	5
5.0	95	5

Instrumentation: Waters 2777 Sample Manager, Waters 1525µ Binary HPLC Pump, Waters Micromass® Quattro Premier™

Sample Preparation

1. Blank rat plasma samples were extracted using:
   Protein precipitation
   Liquid-liquid extraction
   Oasis® HLB (Reversed-phase)
   Oasis® MCX (Mixed-mode strong cation exchange)
2. Propranolol was spiked into the final eluants from these methods to a final concentration of 200 ng/mL.
3. For some samples, terfenadine and atenolol were spiked into the eluants at concentrations of 20 ng/mL and 400 ng/mL, respectively.
4. The eluants were diluted with water to reduce the organic solvent portion to 37.5%. These diluted samples were then directly injected into the LC/MS system.
5. Standard solutions of these analytes were made using the same solvent compositions as the extracts.

Protein Precipitation (PPT)
3:1 ACN to plasma

Liquid-Liquid Extraction (LLE)
3:1 MTBE to plasma

SPE: Oasis® MCX (Mixed-mode cation exchanger)
Wash 1: 0.1 N HCl
Wash 2: MeOH
Elute: 5% NH_4OH in MeOH

SPE: Oasis® HLB – 2D Optimized Method
Wash 1: 5% MeOH in H_2O
Wash 2: 40% MeOH with 2% NH_4OH in H_2O
Wash 3: H_2O
Elute: 70% MeOH with 2% FA

The overall cleanliness of the extracts and the amount of biological matrix interferences in each sample were measured by: (1) LC/MS in full scan mode, and (2) LC/MS in multiple reaction monitoring mode.

RESULTS AND DISCUSSION

(1) LC/MS in Full Scan Mode

Figure 1: ESI(+) TIC of the extracted rat plasma matrices from the clean-up procedures. The level of remaining matrix was highest in PPT. The 2D Oasis® HLB SPE samples were much cleaner, but the Oasis® MCX extracts were the cleanest.

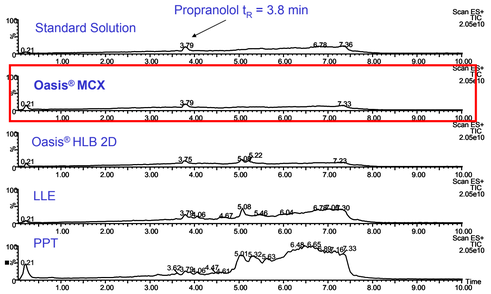
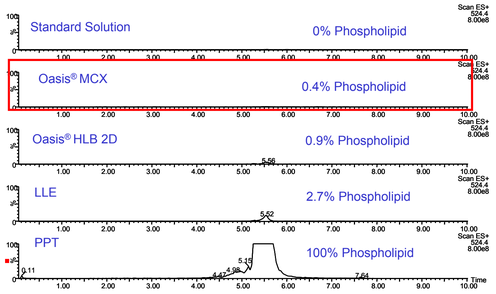


Figure 2: Extracted ion chromatogram for one of the phospholipids implicated in matrix effects. The percentages refer to the amount of phospholipids present, setting PPT at 100%. Clearly, the mixed-mode ion exchange material results in the cleanest extracts.



m/z 524.4, 1-Stearoyl-2-hydroxy-*sn*-glycero-3-phosphocholine

In the following examples, atenolol (polar) and terfenadine (hydrophobic) were added to the sample mixture. PPT and Oasis® MCX were used to prepare the samples. These extracts were dried and reconstituted, which results in a concentration of both the analytes and the residual matrix components (i.e. a dirtier sample).

Figure 3: Samples prepared with protein precipitation have significant levels of ion suppression for the hydrophobic terfenadine (due to co-elution with residual matrix components), with slight suppression for the propranolol, and no suppression for atenolol.

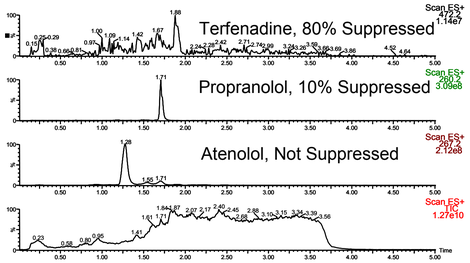
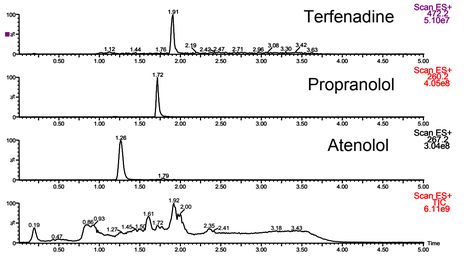


Figure 4: Using mixed-mode SPE for sample preparation results in no ion suppression for all three analytes.



(2) LC/MS in Multiple Reaction Monitoring Mode (MRM)

Residual matrix components are usually not monitored in MRM, resulting in an unrealistic picture of how clean or dirty a sample is. We investigated the MRM for the analytes, as well as several known phospholipid interferences. The samples are the same as used for the LC/MS full scan analyses – i.e. we already know the relative amounts of residual matrix components in each sample.

Figure 5: MRM transition for the phospholipid interference m/z 758.4 → 184.3 as indicated in Ref. 2. PPT and LLE prepared samples contain the greatest levels of this phospholipid. The SPE prepared samples contain virtually no levels of this phospholipid.

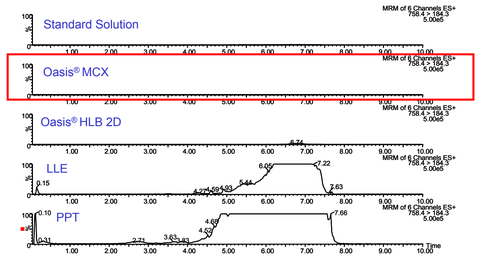
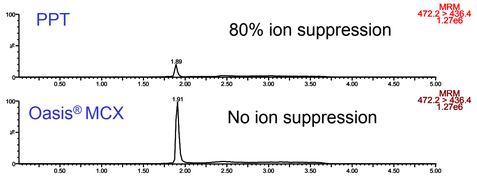


Figure 6: The same sample used to generate the data in Figures 3 and 4 was run under MRM mode. The same levels of ion suppression are observed as in full scan mode.

Terfenadine— Same samples as Figures 3 & 4



CONCLUSIONS

- Protein precipitation is the least effective sample preparation method, resulting in significant ion suppression.
- Mixed-mode SPE (Oasis® MCX) results in the cleanest extracts due to the selectivity of the ion-exchange mechanism for ionizable analytes.
- A combination of LC/MS^n with mixed-mode SPE is the best methodology for removing matrix interferences.

(1). Avery, M. J. *Rapid Commun. Mass Spectrom.* 2003, (17)197-201.
(2). Bennett, P.; Van Horne, KC. Poster 6006, AAPS 2003.
(3). Mallet, C. R.; Lu, Z.; Mazzeo, J. R. *Rapid Commun. Mass Spectrom.* 2004, (18) 49-58.

TO DOWNLOAD A COPY OF THIS POSTER VISIT WWW.WATERS.COM/POSTERS

©2005 Waters Corporation