# ELIMINATING MATRIX EFFECTS IN MASS SPECTROMETRY APPLICATIONS

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## OVERVIEW

The influence of gradient time, mobile phase pH, and evaporate and reconstitute versus dilute and shoot methods on matrix effects were studied. The nature of the matrix interferences was also studied.

## INTRODUCTION

The MS response obtained from an analyte in neat solution can differ significantly from that same analyte in a matrix. Matrix effects result from co-eluting matrix components that compete for ionization capacity. This competition will vary among samples, causing 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]. Previously, we have shown matrix effects from different sample matrices, different measuring techniques, and different sample preparation techniques such as SPE, liquid-liquid extraction and protein precipitation. In this work, we examined the contribution of the gradient time and mobile phase pH to matrix effects. We looked at specific matrix components remaining in the extracts by running the samples using neutral loss and precursor ion scanning LC/MS/MS (electrospray ionization)[4]. We also examined dilute and shoot versus evaporate and reconstitution of the extracts.

## METHODS

Column: ACQUITY UPLC™ BEH $C_{18}$ 2.1 x 50 mm, 1.7 µm
Mobile Phase A: 10 mM $NH_4HCO_3$, pH 10
or 0.1% HCOOH
Mobile Phase B: MeOH with 10 mM $NH_4HCO_3$, pH 10
or 0.1% HCOOH
Flow Rate: 0.6 mL/min

Gradient #1 ($t_g$ = 0.5 min)

| Time (min) | Profile %A | %B |
|---|---|---|
| 0.0 | 98 | 2 |
| 0.1 | 98 | 2 |
| 0.6 | 0 | 100 |
| 1.6 | 0 | 100 |
| 1.7 | 98 | 2 |
| 2.5 | 98 | 2 |

Gradient #2 ($t_g$ = 2 min)

| Time (min) | Profile %A | %B |
|---|---|---|
| 0.0 | 98 | 2 |
| 0.5 | 98 | 2 |
| 2.5 | 0 | 100 |
| 3.0 | 0 | 100 |
| 3.1 | 98 | 2 |
| 4.0 | 98 | 2 |

Instrumentation: Waters ACQUITY UPLC™ and Waters Micromass® Quattro Premier™
Note: For the neutral loss and precursor ion scanning experiments, a separate 4-min gradient from 98% aqueous to 100% organic, followed by a 1-min hold at the high organic, was employed to allow more time for analysis under these scanning conditions.

## Sample Preparation

1. Blank rat plasma samples were extracted using:
   Protein precipitation
   Oasis® MCX (Mixed-mode strong cation exchange)
2. Amitriptyline was spiked into the final eluates from these methods to a final concentration of 50 ng/mL.
3. A standard solution of amitriptyline was made using the same solvent composition as the extracts.

Protein Precipitation (PPT)
3:1 ACN to plasma
250 µL rat plasma was precipitated with 750 µL ACN, vortexed, centrifuged and the supernatant removed.

For dilute and shoot samples, the supernatant was dried down and reconstituted in 0.5 mL of 50/50 methanol/water containing 50 ng/mL of the standard. This resulted in a 2X dilution of the original sample. For the concentrated samples, the dried down supernatant was reconstituted in 100 µL , which resulted in a 2.5X concentration of the original sample.

SPE: Oasis® MCX (Mixed-mode cation exchange)
250 µL rat plasma was acidified and diluted with 250 µL 4% $H_3PO_4$ in water to disrupt protein binding
Wash 1: 2% HCOOH in water
Wash 2: 100% MeOH
Elute: 5% $NH_4OH$ in MeOH
The eluate was dried down and reconstituted in either 500 µL or 100 µL of 50/50 methanol/water containing 50 ng/mL of the standard, for the diluted and concentrated samples, respectively.
Matrix effects (ion suppression or enhancement) were measured for the analyte. The overall cleanliness of the extracts and the amount of biological matrix interferences in each sample were measured by LC/MS/MS in multiple reaction monitoring mode and by neutral loss and precursor ion scanning. Five MRM's were monitored to compare the amounts of 2 lysophosphatidylcholines (m/z 496 and m/z 524) and 3 phosphatidylcholines (m/z 704, 758, and 806.) The presence and identification of other phosphatidylcholines were examined by scanning for precursors of m/z 184, which corresponds to the loss of the phospholipid polar head group. The presence and identification of phosphatidylserines were examined by scanning for compounds exhibiting a neutral loss of 185 Da.[4]

## RESULTS AND DISCUSSION

(1) Comparison of PPT vs. Mixed-Mode SPE for Overall Cleanliness of Extract

Figure 1: Phosphatidylcholines by precursor ion scanning of m/z 184 ESI(+) in the extracted rat plasma matrices from PPT and Oasis® MCX.

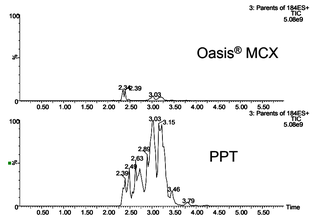
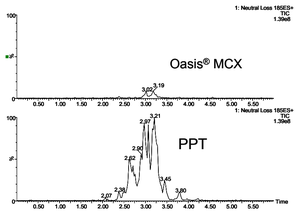



Figure 2: Phosphatidylserines by neutral loss scanning of 185 Da ESI(+) in the extracted rat plasma matrices from PPT and Oasis® MCX .




(2) Gradient Time and Mobile Phase pH Contributions

In the following examples, suppression or enhancement of the analyte signal is evaluated under shorter and longer gradients with low and high pH mobile phases. PPT and Oasis® MCX were used to prepare the samples. In Figures 3-6, the extracts were concentrated by drying down and reconstitution, which results in a concentration of the residual matrix components (i.e. a dirtier sample). The shorter gradient used results in co-elution of the analyte and residual matrix components. Even under the longer gradient conditions, samples prepared by PPT still suffer significant ion suppression. The effect of pH on the hydrophobic analyte retention time is demonstrated. Phospholipid retention times are relatively unaffected by pH.

Figure 3. Gradient #1, low pH. A) MRM transitions for 5 phospholipids and amitriptyline. B) XIC for amitriptyline and ion suppression values, calculated versus the neat standard solution.

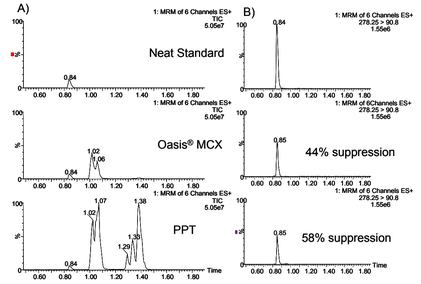



Figure 4. Gradient #1, high pH. A) MRM transitions for 5 phospholipids and amitriptyline. B) XIC for amitriptyline and ion suppression values, calculated versus the neat standard solution.

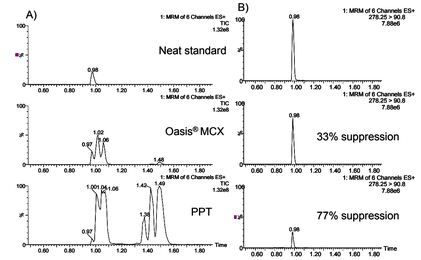



Figure 5. Gradient #2, low pH. A) MRM transitions for 5 phospholipids and amitriptyline. B) XIC for amitriptyline and ion suppression values, calculated versus the neat standard solution.

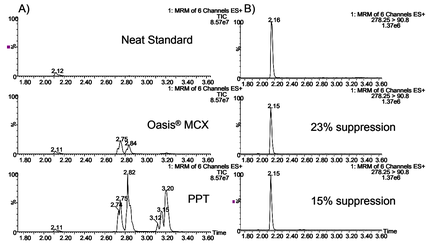



Figure 6. Gradient #2, high pH. A) MRM transitions for 5 phospholipids and amitriptyline. B) XIC for amitriptyline and ion suppression values, calculated versus the neat standard solution.

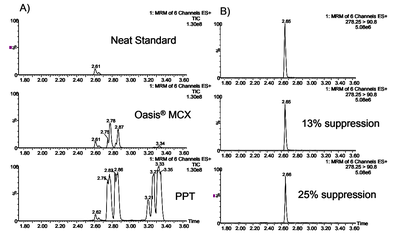
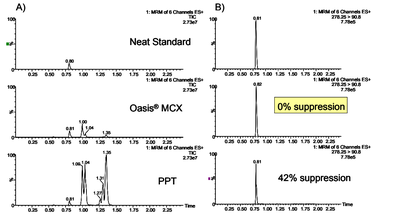



Figure 7. Gradient #1, low pH, diluted samples. A) MRM transitions for 5 phospholipids and amitriptyline. B) XIC for amitriptyline and ion suppression values, calculated versus the neat standard solution. Reduced matrix effects due to lower concentration of matrix components.




## CONCLUSIONS

- Mixed-mode SPE is the most effective sample preparation technique, resulting in the cleanest samples.
- For high-throughput analyses that utilize fast gradients, careful sample preparation is crucial for reducing matrix effects.
- pH is a very important consideration in manipulating analyte retention time relative to matrix interferences
- Clearly, mixed-mode SPE coupled with carefully chosen chromatographic conditions eliminates matrix effects.

(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.
(4). Taguchi, et al. J. Chromatography B 823 (2005) 26-36.



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