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A Systematic Approach to Reducing Matrix Effects in Bioanalytical Studies

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OVERVIEW

Several tools were assessed for their effectiveness in reducing matrix effects in bioanalytical methods. A cumulative strategy for maximum reduction of matrix effects is suggested.

INTRODUCTION

The MS response obtained from an analyte in neat solution can differ significantly from that same analyte in a matrix. Matrix effects, resulting from co-eluting matrix components that compete for ionization capacity, manifest themselves as suppression or enhancement of the analyte signal. Matrix effects can be infinitely variable and difficult to control or predict. They are caused by numerous factors, including but not limited to endogenous phospholipids. Other sources of variability in matrix effects include subject differences, possibly due to diet or other factors, and concentration of the endogenous phospholipids. The severity and nature, (suppression versus enhancement) of matrix effect observed can be a function of the concentration of the lipids at the elution time of the analyte. [5] Matrix effects can also be compounded by co-eluting metabolites, impurities or degradation products. All of the above can cause significant errors in the accuracy and precision of bioanalytical methods . These effects should be evaluated as a part of quantitative LC-ESI-MS/MS method development, validation and routine use. We believe that all available tools, or combinations of tools, should be employed to reduce these effects. In this work, we compare different sample preparation methods, investigate the influence of mobile phase pH on both matrix components and several basic analytes, and we compare HPLC and UPLC™ analysis for sensitivity and the presence of matrix effects. We chose to focus on basic analytes in a pharmaceutical bio-analysis environment for this research. The amount of specific matrix components, or classes of matrix components, remaining in the extracts was measured by LC/MS/MS (electrospray ionization) using multiple reaction monitoring (MRM). Upon high energy in-source CID, the lipids implicated in matrix effects yield a characteristic fragment ion at m/z 184, corresponding to the trimethylammonium-ethyl phosphate cation.[4] It is this ion that allows us to monitor multiple phospholipids and lysophospholipids with a single MRM transition, thus maximizing the duty cycle in the mass spectrometer. The relative levels of phospholipids present in our samples were measured by monitoring the transition 184.3 -> 184.3. The cone voltage was set to 90 V and the collision energy set to 3 eV so that no further fragmentation of the diagnostic ion would occur.

METHODS

Columns: ACQUITY UPLC ™ BEH C_{18} 2.1 x 50 mm, 1.7 µm or XTerra® MS C_{18} 2.1 x 50 mm, 3.5 µm
Mobile Phase A: H_2O with 10 mM $NH_4CH_3CO_2$, pH 9 or 0.1% HCOOH, pH ~2.7
Mobile Phase B:100% MeOH or 0.1% HCOOH in MeOH, pH ~2.7
Flow Rate: 0.6 mL/min
Gradient #1 (low pH): 98% A to 98% B over 1 or 2 min, hold 0.5 min, return to initial in 0.1 min and re-equilibrate for 0.4 min.
Gradient #2 (high pH): 50% A to 98% B over 1 or 2 min, hold 0.5 min, return to initial in 0.1 min and re-equilibrate for 0.4 min.
Injection Volume: 5 µL
Column Temperature: 50 °C
Sample Temperature: 15 °C
Instrumentation: Waters ACQUITY UPLC™ and Waters Quattro Premier™

Sample Preparation

Protein Precipitation (PPT)
300 ACN to 100 µL blank rat plasma
Vortexed and centrifuged at 13,000 rpm for 5 min.

SPE: Oasis® MCX (Mixed-mode cation exchanger)
Load: Blank rat plasma diluted and acidified 1:1 with 4% H_3PO_4 in water
Wash 1: 2% HCOOH in water
Wash 2: 100% MeOH
Elute: 5% NH_4OH in 90:10 MeOH:H_2O for Mix 1 or 5% NH_4OH in 100% MeOH for Mix 2

Mix 1 (risperidone, 9-OH risperidone, and clozapine) or Mix 2 (amitriptyline, terfenadine, and propranolol) were spiked into the final eluates from these methods to the appropriate final concentration, which ranged from 0.1 to 50 ng/mL. Several experiments were also carried out with imipramine at the same concentrations.
Standard (neat) solutions of these analytes were made using the same solvent compositions as the eluates.

RESULTS AND DISCUSSION

EFFECT OF SAMPLE PREPARATION

Figure 1. XIC's of the phospholipid transition, 184.3 > 184.3 from Oasis® MCX SPE and protein precipitated samples, low pH mobile phase, longer gradient.

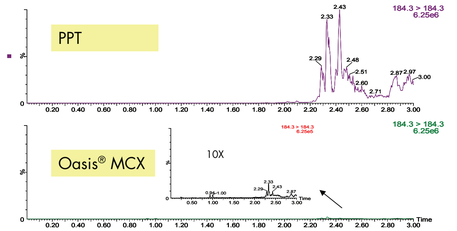
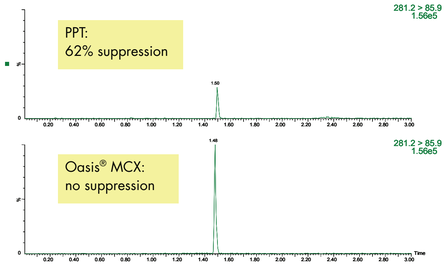


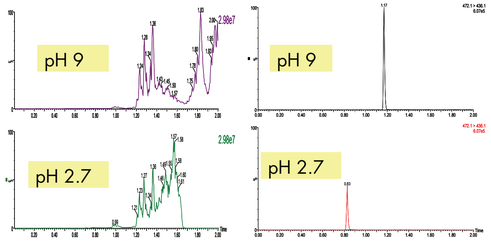
Figure 1 shows the difference in phospholipid levels in samples prepared by PPT and SPE. The signal for 5 ng/mL imipramine was suppressed by approximately 62% in the ACN PPT sample and showed no suppression in the Oasis® MCX SPE sample. Additionally, Oasis® MCX removes up to 90% more of the 5 phospholipids monitored than PPT does.

Figure 2. A comparison of matrix effects in PPT versus Oasis® MCX SPE prepared samples. 5 ng/mL imipramine



EFFECT OF MOBILE PHASE PH

Figure 3. A comparison between low and high pH for the retention of phospholipids (left) and terfenadine (right).



As shown in Figure 3, retention of the earliest eluting phospholipids is independent of pH, while retention of ionizable bases can change significantly from low to high pH. We can utilize this behavior to help minimize matrix effects by "moving" the analytes of interest relative to matrix components with pH. In addition, sensitivity of many ionizable bases improves dramatically at high pH. At high pH, bases are neutral, retain longer via reversed-phase retention and therefore require higher organic solvent for elution. Sensitivity for terfenadine at high pH is 2.3X that observed at low pH.

EFFECT OF LC SYSTEM TYPE

Figure 4. HPLC chromatogram at high pH of a 0.1 ng/mL terfenadine sample in plasma, prepared by PPT.

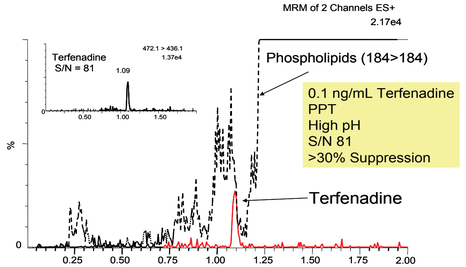
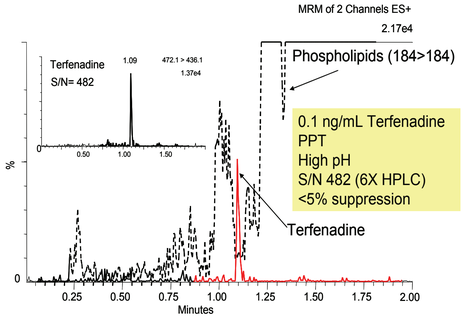


Figure 5. UPLC™ chromatogram at high pH of a 0.1 ng/mL terfenadine sample in plasma, prepared by PPT.

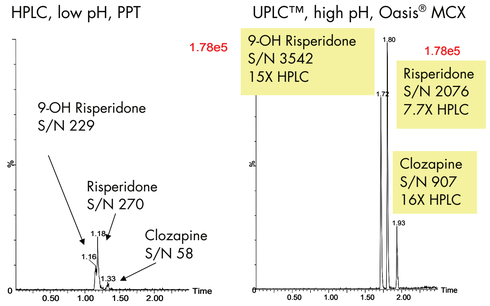


As shown in Figures 4 and 5, the UPLC™ data clearly show better separation between terfenadine and phospholipids. The increased resolution afforded by UPLC™ was enough to adequately separate the analyte from interferences resulting in a significant reduction in ion suppression. The terfenadine signal was suppressed by >30% in the HPLC run and suffered only minimal suppression (<5%) in the UPLC™ run. Additionally, the signal-to-noise for 0.1 ng/mL of terfenadine is six times higher in UPLC™ than in HPLC.
Note: Although suppression been reduced with UPLC, the concentration of phospholipids in the PPT extracts depicted above is still significant, and can dramatically reduce column lifetime and method robustness.

CUMULATIVE BENEFITS

Figure 5 demonstrates the cumulative improvement obtained by utilizing all of the previous tools we've described. The chromatogram on the left represents a typical plasma sample assay, run under conditions that are common practice in a variety of laboratories today: low pH mobile phase, PPT sample prep and HPLC. The chromatogram on the right represents a plasma sample treated and analyzed according to the tools we've described: high ph mobile phase, mixed-mode SPE, and UPLC™. The net improvement in signal-to-noise ranges from almost 8X to 16X!

Figure 5: Combining all of the previous tools to analyze 1 ng/mL



CONCLUSIONS

- Use mobile phase pH to move compounds away from interferences and improve retention, resolution and sensitivity
- Mixed-mode solid phase extraction results in cleaner extracts than PPT, and improves method robustness by reducing build-up on LC columns
- UPLC™ provides increased resolution from endogenous compounds and closely related compounds over HPLC
- UPLC™ improves S/N over HPLC, which improves LOD/LLOQ, helping to compensate for ion suppression.
- The combination of mixed-mode SPE and UPLC™ with high pH for ionizable bases is the best solution for minimizing matrix effects in bioanalytical analyses.

REFERENCES

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