(Excerpt from Reporter 28.2)

Minimizing Phospholipid Matrix Effects in HILIC LC-MS using HybridSPE-Precipitation Small Volume Plates

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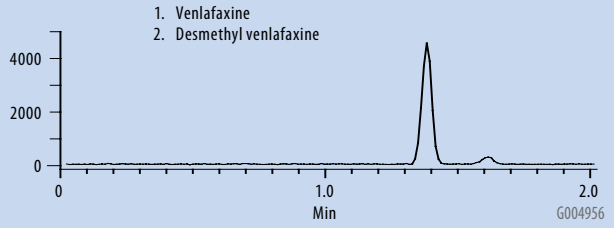
This article discusses phospholipid matrix effects in HILIC chromatography. HILIC chromatographic separations have become widely accepted in LC-MS applications due to increased retention of polar compounds and enhanced ionization of analytes. Though the retention mechanism of phospholipids in reversed-phase chromatography is different than in HILIC chromatography, phospholipid buildup still occurs in HILIC separations, causing poor analyte response. In this study, rat plasma samples spiked with venlafaxine and metabolite were processed using standard protein precipitation methods and compared with samples prepared using the HybridSPE-PPT Small Volume 96-well plate. Spiked sample concentrations ranged from 100 ng/mL to 1200 ng/mL in plasma. Analyte response was compared between the two sample prep techniques along with total amount of extracted phospholipid matrix. The phospholipid matrix was measured by scanning mass range 400-950 m/z.

Figure 1. HILIC Separation of Venlafaxine and Metabolite



Samples prepared using the HybridSPE Small Volume plate consisted of applying 20 µL of plasma to the plate, followed by 60 µL of 1% formic acid in acetonitrile. The plate was then vortexed for one minute, then placed on a vacuum manifold with 10" Hg vacuum for two minutes. The filtrate was collected and analyzed directly using an Ascentis Express HILIC column. Samples prepared using the standard protein precipitation method consisted of adding 100 µL of plasma to a centrifuge vial, followed by 300 µL of 1% formic acid in acetonitrile. Samples were then vortexed for one minute and centrifuged for two minutes at 15000 rpm. The supernatant was collected and analyzed directly.

Using a standard protein precipitation technique, significant phospholipid matrix interference occurred directly in the elution region of venlafaxine and metabolite. As depicted in Figure 2, a continued buildup of phospholipids occurred throughout the study. The blue trace represents phospholipid interference from the initial injection;

Figure 2. TIC Phospholipid Monitoring of Standard Protein Precipitation, initial injection (blue), 50th injection (red)

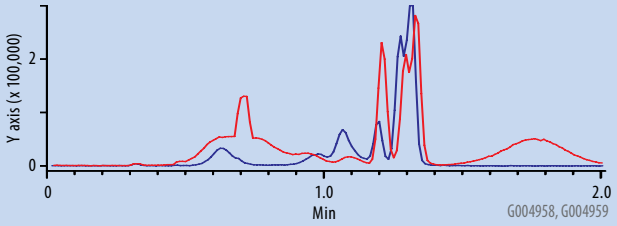
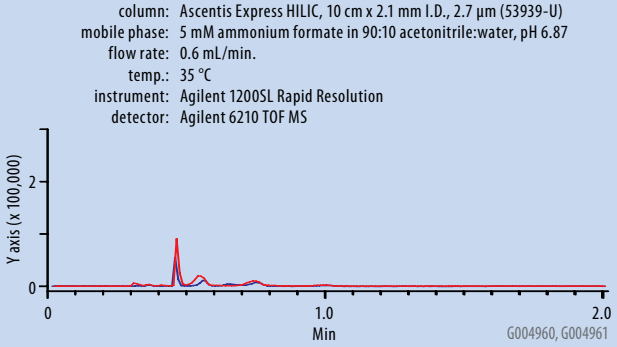


Figure 3.TIC Phospholipid Monitoring using HybridSPE Small Volume, initial injection (blue), 50th injection (red)



the trace in red represents the amount of phospholipid interference at the 50th injection. The continual increase in matrix buildup resulted in significant analyte suppression, even at the highest concentration level. When samples were prepared using the HybridSPE Small Volume technique, the phospholipid matrix interference was depleted, resulting in no matrix interference. As shown in Figure 3, no matrix interference is observed from the initial injection in blue to the final injection in red.

The HybridSPE Small Volume technique is a simple and effective method for sample preparation of small volume plasma samples, resulting in depletion of phospholipid matrix interference, eliminating the concern for matrix buildup in both reversed phase and HILIC separations.

Featured Products

Description	Cat. No.
HybridSPE-PPT Small Volume Plate, 15 mg/well	52794-U
Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm	53939-U