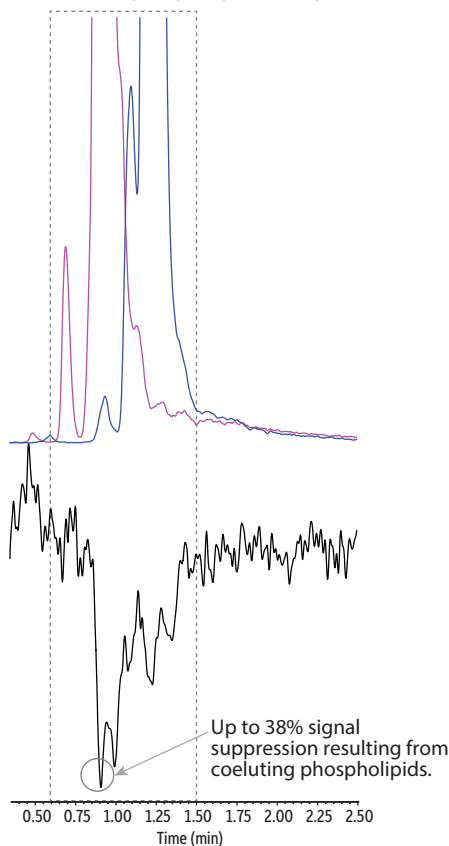


# Signal Suppression from Phospholipids

## A. Protein Precipitation Only

— Phosphatidylcholine 34:2  
 — Phosphatidylcholine 36:2  
 — Post-column infusion signal for nortriptyline without phospholipid cleanup



Peaks	tr (min)	Precursor Ion	Product Ion
1. Phosphatidylcholine 34:2	0.831	758.5	184.1
2. Phosphatidylcholine 36:2	1.119	786.5	184.1

**Column** Raptor Biphenyl (cat.# 9309232)  
**Dimensions:** 30 mm x 2.1 mm ID  
**Particle Size:** 1.8 µm  
**Pore Size:** 90 Å  
**Temp.:** 40 °C  
**Standard/Sample**  
**Diluent:** Methanol  
**Inj. Vol.:** 1 µL  
**Mobile Phase**  
**A:** 0.1% Formic acid in water  
**B:** 0.1% Formic acid in methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.75	13.3	86.7
2.50	0.75	13.3	86.7

**Detector** MS/MS  
**Ion Mode:** ESI+  
**Mode:** MRM  
**Instrument** UHPLC

**Sample Preparation** Proteins were precipitated out of plasma using a 3:1 1% formic acid in acetonitrile:human plasma mixture that was then centrifuged. The supernatant was then divided and cleanup was performed on half using a Resprep PLR SPE 96-well plate (cat.# 28301).

**Notes** Nortriptyline (15 ng/mL) was infused post-column at 60 µL/min in order to demonstrate signal suppression from coeluting phospholipids. To demonstrate the effect of cleanup on signal strength, 1 µL injections were made of protein crashed plasma samples both with and without Resprep PLR SPE cleanup.

## B. Phospholipid and Protein Removal (Resprep PLR SPE)

Post-column infusion — without phospholipid cleanup  
 signal for nortriptyline — with phospholipid cleanup

