

# APPLICATIONS

## A Rapid, Automatable Solution for Phosphatidylethanol (PEth) LC-MS/MS Analysis in Whole Blood

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### Overview

Phosphatidylethanol (PEth) has emerged as an ethanol specific marker for alcohol consumption. PEths are phospholipids with very similar structures, which makes them challenging to separate chromatographically. In this fast analysis, a Kinetex<sup>®</sup> Core-Shell Biphenyl LC column was used with a direct, in-plate phospholipid enrichment and cleanup for PEth from whole blood. The automatable LC-MS/MS workflow, including sample preparation and column selection, greatly simplifies the analysis while achieving excellent analytical performance.

### Sample Preparation

<b>Sample:</b>	200 µL whole blood
<b>Add:</b>	1 mL Acetonitrile
<b>Centrifuge:</b>	@ 13,500 rpm for 10 min
<b>96-Well Plate:</b>	Phree <sup>™</sup> Phospholipid Removal Plate
<b>Part No.:</b>	<a href="#">8E-S133-TGB</a>
<b>Dispense:</b>	900 µL of supernatant into each well
<b>Wash Plate:</b>	Place a collection plate below the Phree plate to collect wash fraction
<b>Wash:</b>	2x 1 mL of acetonitrile into each well and apply vacuum, positive pressure, or centrifuge
<b>Elute Plate:</b>	Place a new collection plate below the Phree plate to collection elution fraction
<b>Elute:</b>	1 mL of 1 % Ammonia in 2-propanol and apply vacuum, positive pressure, or centrifuge
<b>Dry Down:</b>	Under a stream of nitrogen @ 40 °C
<b>Reconstitute:</b>	50 µL of 30:70 Water/Acetonitrile (v/v)
<b>Inject:</b>	10 µL onto LC-MS/MS

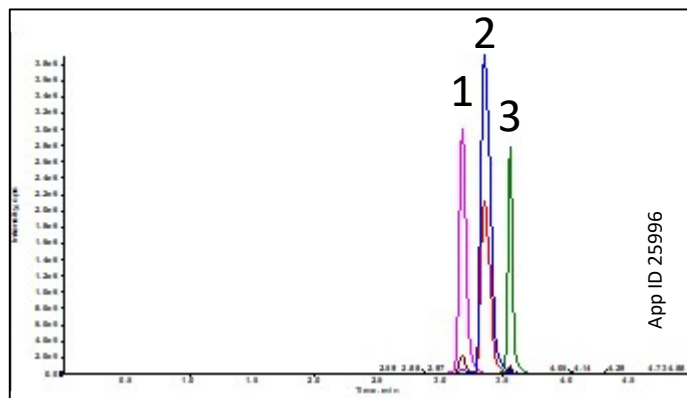
### Linearity Based on Standards in Neat Matrix

PEth	16:0/18:1	18:1/18:1	16:0/16:0
<b>Range (ng/mL)</b>	23.4 - 750	9.4 - 300	9.4 - 300
<b>LLOQ (ng/mL)</b>	3	0.6	0.6
<b>R<sup>2</sup></b>	0.9955	0.9923	0.9971

### Recovery Value (%), CVs (%)

PEth	16:0/18:1	18:1/18:1	16:0/16:0
<b>CV (n=8)</b>	3.6	3.8	4.1
<b>Recovery (%)</b>	98.1	99.2	107.3

<b>Column:</b>	Kinetex 2.6 µm Biphenyl	
<b>Dimension:</b>	50 x 2.1 mm	
<b>Part No.:</b>	<a href="#">00B-4622-AN</a>	
<b>Mobile Phase</b>	A: 10 mM Ammonium Acetate in water B: Methanol	
<b>Gradient:</b>	<b>Time (min)</b>	<b>%B</b>
	0.0	10
	0.1	75
	5.0	90
	5.1	100
	5.5	100
	6.0	10
<b>Flow Rate:</b>	0.6 mL/min	
<b>Temperature:</b>	20 °C	
<b>Detection:</b>	MS/MS (MRM, SCIEX <sup>®</sup> 4000 QTRAP <sup>®</sup> )	
<b>Sample:</b>	1. PEth 16:0 /16:0 2. PEth 16:0 /18:1 3. PEth 18:1 /18:1	



### Highlights of the Workflow

1. Kinetex Core-Shell Biphenyl LC Column
  - a. Efficiently resolved the PEth homologs
  - b. Simple mobile phase solution
2. Phree Phospholipid Removal Plate
  - a. Effectively retains the PEth from complex matrix
  - b. Further cleanups prolonged LC column usage and reduces the risk for contaminants on the mass spectrometer

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