APPLICATIONS

Simple and Robust LC-MS/MS Method of Phosphatidylethanol (PEth) in Whole Blood Using Luna® Omega Polar C18 Column

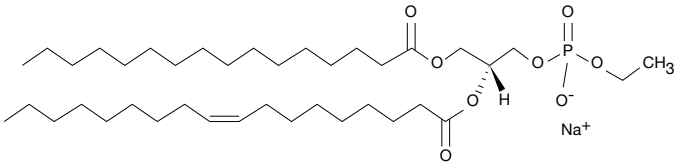
Xianrong (Jenny) Wei, Daniel Spurgin, and Sean Orlowicz
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Xianrong (Jenny) Wei
Senior Scientist
Jenny is a Senior Scientist in the Phenomenex PhenoLogix applications laboratory.

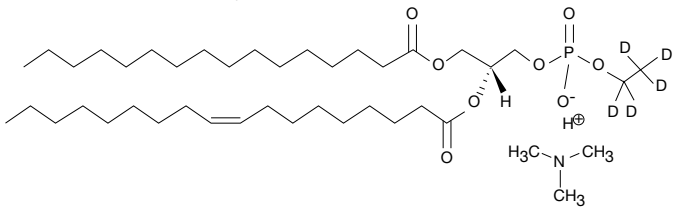
Introduction

Phosphatidylethanols (PEth) are types of phospholipids that form in the presence of ethanol. Unlike other metabolites of ethanol such as fatty acid ethyl esters (FAEE), ethyl glucuronide (EtG) and ethyl sulfate (EtS), the average half-life for PEth in circulation is around 4 days, which means that PEth may be detected weeks after ethanol has been consumed[1,2]. PEth is a phospholipid containing two fatty acids linked to a phosphoethanol. PEth 16:0/18:1 is the most abundant form and contains one palmitic acid and one oleic acid chain. It is one of nine PEth-homologues and is the focus of this study. In this study, we develop a LC-MS/ MS research assay including a simple extraction procedure with an internal standard using a reverse phase sub-2 µm Luna Omega Polar C18 column for the separation. The assay has been evaluated and meet ±15 % acceptance criteria.

Figure 1. Analyte Structures
Phosphatidylethanol (PEth) 16:0/18:1



d5-PEth 16:0/18:1 trimethylammonium salt



Materials

Analytical reference standard of Phosphatidylethanol 16:0/18:1 (PEth 16:0/18:1) was purchased from Avanti® Polar Lipids, Inc. (Alabaster, Alabama, USA); Internal standard of PEth 16:0/18:1 – d5 was obtained from redhot diagnostics AB(Södertälje, Sweden) and human blood was purchased from BioreclamationIVT® (Chastertown, MD, USA) respectively. All other chemicals were obtained from the Sigma-Aldrich® Company (St. Louis, MO). Water purification via Sartorius Arium® Comfort II (Goettinger, Germany)

Sample Extraction Procedure

Phosphatidylethanol (PEth) is extracted from 100 µL of whole blood by addition of 400 µL of isopropyl alcohol (IPA) containing internal standard (10 µg/mL). After thorough mixing, the mixture is centrifuged at 13,500 rpm for 10 minutes, then 200 µL aliquot of the supernatant transferred to a vial.

LC Conditions

Analytical Column:	Luna Omega 1.6 µm Polar C18
Dimension:	30 x 2.1 mm
Part No.:	00A-4748-AN
Recommended Guard:	SecurityGuard™ ULTRA
Part No:	AJ0-9505
Mobile Phase:	A: Water / IPA / Acetonitrile with 5 mM Ammonium formate (30:10:60) B: Water / IPA / Acetonitrile with 5 mM Ammonium formate (1:79:20)

Gradient:

Time (min)	% B
0	10
0.3	10
0.31	40
2	100
2.5	100
2.51	10
3.5	10

Flow Rate:	0.45 mL/min
Temperature:	60 °C
Injection Volume:	10 µL
Instrument:	Agilent® 1260
Detection	MS/MS (SCIEX Triple Quad™ 4500), 4500, ESI, Neg Polarity (700 °C)

Mass Transitions

ID	Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)
PEth 1	701.5	280.8	100
PEth 2*	701.5	254.9	100
PEth d5 1*	706.5	280.8	100
PEth d5 2	706.5	254.8	100

*Quantitation mass

Results and Discussion

To determine the appropriate LLOQ and linearity of the assay, we evaluated six individual lots of human blood to measure the average of endogenous level of PEth **(Table 1)**. Lots from female and/or under age 21 years old of human blood showed cleaner background of PEth. **Figure 3** shows the endogenous levels of two lots of blood. Red trace is from a 46 year old male and the blue trace is blank matrix from a 19 year old female. Lot 3 (blue) shows little to know endogenous levels and therefore can be used for the calibration standard and quality control sample preparation to ensure the assay accuracy, precision and linearity. **Figure 2**, showed the blank extraction solvent response, no interference or analyte presented.

Results and Discussion (cont'd)

The assay was run on a Phenomenex Luna® Omega 1.6 µm Polar C18, 30 x 2.1 mm column with specified mobile phase and temperature which effectively ionizes the analytes while providing sufficient selectivity, separation and sensitivity **(Figure 4 and 5)**. The assay carryover was also evaluated and there is no significate carryover **(Figure 6)**. Challenging matrices such as whole blood sample can negatively impact column lifetime due to clogging, fouling, and creating robustness issues. For this reason an evaluation test of column lifetime was performed with the results showing very good reproducibility after at least 500 injections **(Figure 7)**.

The assay provides the dynamic range at 20–2000 ng/mL with the linearity (R^2) volume is greater than 0.99 **(Figure 8)**. The accuracy and precision were tested at three QCs levels at n=6, the low QC accuracy presented at 101% with CV% at 11.97; mid QC at 108.2 % with CV% at 5.11 and High QC at 92.8 % with CV% 7.61, respectively **(Table 1)**. The internal standard responses were also performed. The assay showed the consistent response of the internal standard, which was within ±25 % of mean of standards and QCs in the run **(Figure 9)**.

Table 1. Accuracy and Precision for 6 lots of blood

Analyte & IS ame	Low QC (60 ng/mL)	Mid QC (540 ng/mL)	High QC (1800 ng/mL)
Lot 1	62.2	560	1750
Lot 2	62.9	558	1500
Lot 3	46.8	591	1520
Lot 4	60.8	560	1700
Lot 5	61.7	627	1770
Lot 6	68.5	611	1780
Mean	60.5	585	1670
S.D.	7.24	29.8	127.1
%CV	11.97	5.11	7.61
%Theoretical	101	108.2	92.8
N	6	6	6

Figure 2. Representative chromatogram of blank extraction solvent (water/IPA 20:80)

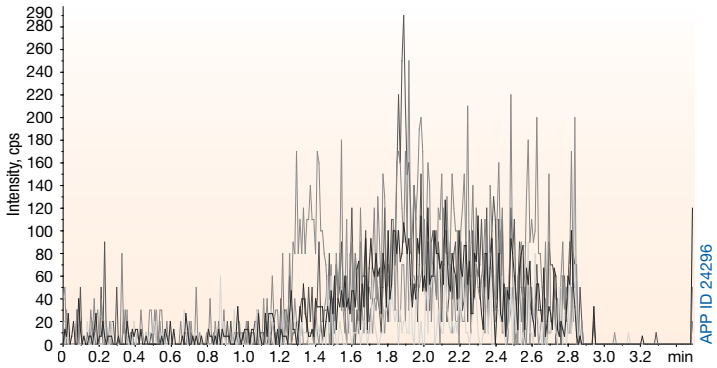


Figure 3. Representative chromatograms of endogenous level of PEth in blank human whole blood.

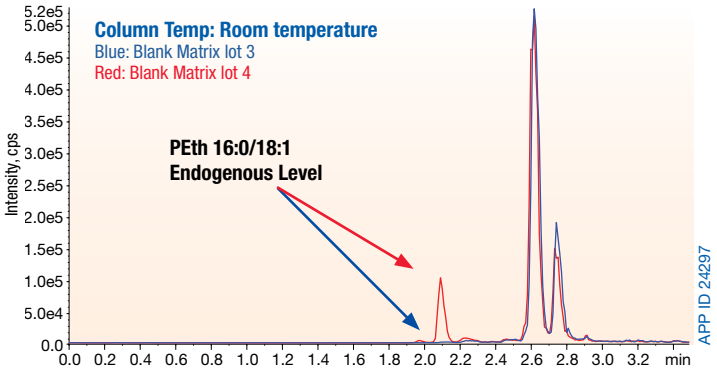


Figure 4. Representative chromatogram of LLOQ at 20 ng/mL in human whole blood at column temp 60 °C.

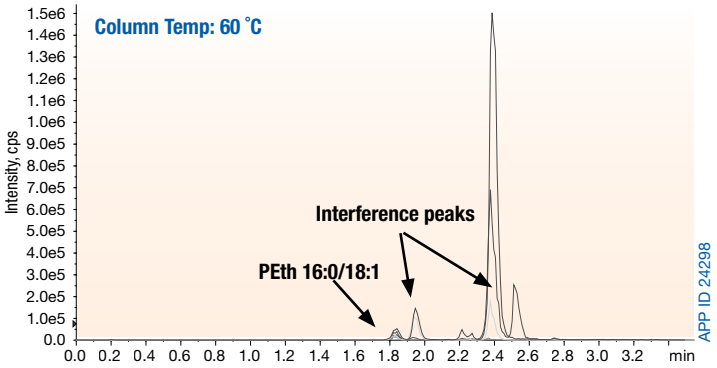


Figure 5. Representative chromatogram of ULOQ at 2000 ng/mL in human whole blood.

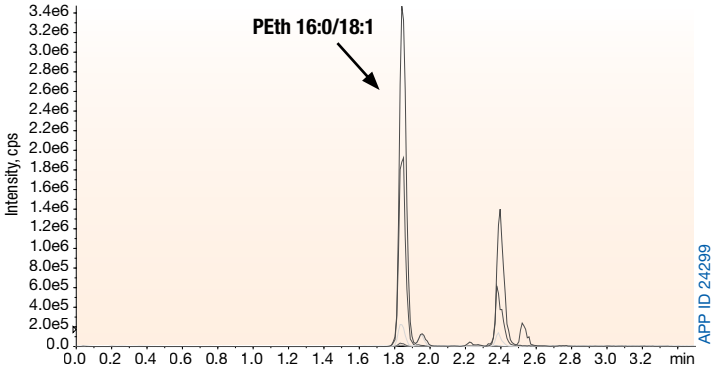


Figure 6. Representative chromatograms of carryover

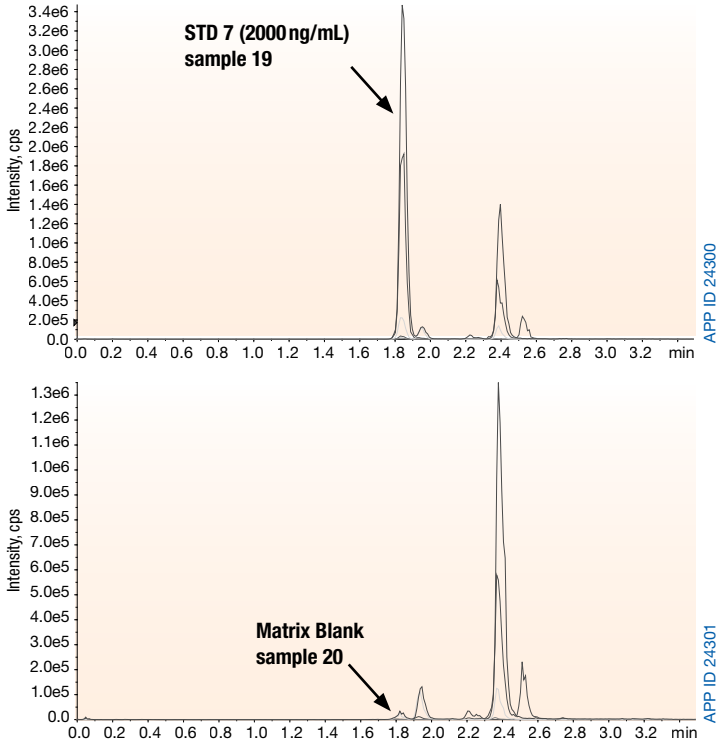


Figure 7. Representative chromatograms of reproducibility

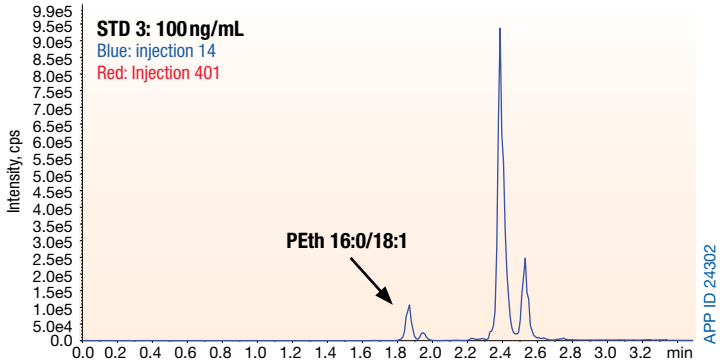


Figure 8. Representative calibration curve of PEth in human whole blood

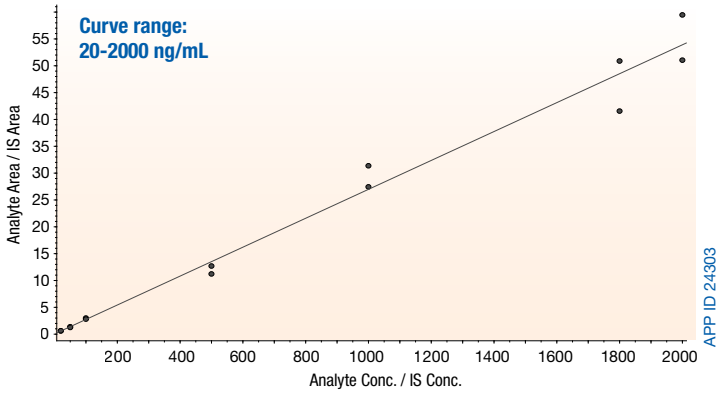
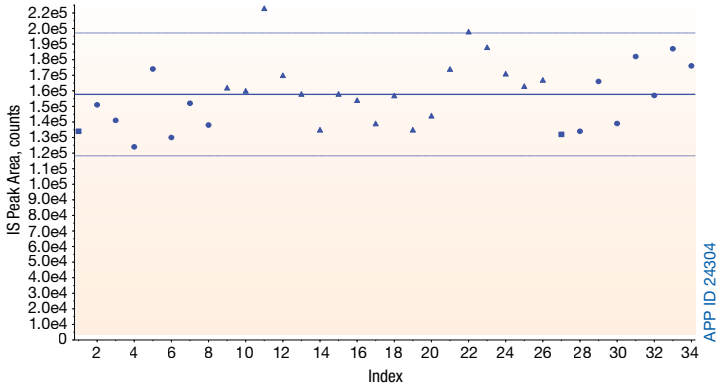


Figure 9. Representative response of isotope internal standard of PEth 16:0/18:1-d5



Conclusion

A simple and robust LC-MS/MS method is presented for the quantitation of PEth 16:0/18:1 in human whole blood using a Phenomenex Luna® Omega 1.6 µm Polar C18 UHPLC column. The assay provides selectivity, separation of matrix interference peaks, and great reproducibility. This assay is accurate, time saving, and automation friendly.

References

1. Steina Aradottir, Gulber Asanovska, Stefan Gjerss, Per Hasson and Christer Alling. Phosphatidylethanol (PEth) Concentrations in Blood Are Correlated To Reported Alcohol Intake in Alcohol- Dependent Patients. Alcohol & alcoholism Vol. 41, No. 4, pp.431-437, 2006
2. Guido Viel, Rafael Boscolo-Berto, Giovanni Cecchetto, Paolo Fais, Alessandro Nalesso and Santo Davide Ferrara. Phosphatidylethanol in Blood as a Marker of Chronic Alcohol Use: A Systematic Review and Meta-Analysis. Int J Mol Sci. 2012; 13(11): 14788-14812
3. Blomgren Anders, Hansson Therese, Isaksson Anders, Walther Lisa, Region Skane, Medical services. Fast and Robust LC-MS/MS Method for Determination of the alcohol Biomarker phosphatidylethanol (PEth) in whole blood using an Automated Extraction Procedure. Poster, 2013

guarantee

If Phenomenex products in this technical note do not provide at least equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

APPLICATIONS

Luna® Omega Polar C18 Ordering Information

1.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Polar C18	00A-4748-AN	00B-4748-AN	00D-4748-AN	00F-4748-AN	AJ0-9505

5 µm Minibore Columns (mm)					SecurityGuard Cartridges (mm)
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	4 x 2.0* 10/pk
Polar C18	00A-4754-AN	00B-4754-AN	00D-4754-AN	00F-4754-AN	AJ0-7600
				for ID:	2.0 - 3.0 mm

5 µm MidBore™ Columns (mm)				SecurityGuard Cartridges (mm)
Phases	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0* 10/pk
Polar C18	00B-4754-Y0	00D-4754-Y0	00F-4754-Y0	AJ0-7600
			for ID:	2.0 - 3.0 mm

5 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 2.0* 10/pk
Polar C18	00B-4754-E0	00D-4754-E0	00F-4754-E0	00G-4754-E0	AJ0-7601
				for ID:	3.1-8.0 mm

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000
* SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

Australia
t: +61 (0)2-9428-6444
f: +61 (0)2-9428-6445
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
f: +43 (0)1-319-1300
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
f: +1 (310) 328-7768
info@phenomenex.com

China
t: +86 400-606-8099
f: +86 (0)22 2532-1033
phen@agela.com

Denmark
t: +45 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
f: +33 (0)1 30 09 21 11
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
f: +49 (0)6021-58830-11
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
f: +91 (0)40-3012 2411
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
f: +39 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
f: +64 (0)9-4780952
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: +1 (800) 541-HPLC
f: +1 (310) 328-7768
info@phenomenex.com

Spain
t: +34 91-413-8613
f: +34 91-413-2290
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
f: +44 (0)1625-501796
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

All other countries
Corporate Office USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

www.phenomenex.com
Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com

TN53390617_W