

PHOSPHATIDYLETHANOL – PETH.

An alcohol marker.

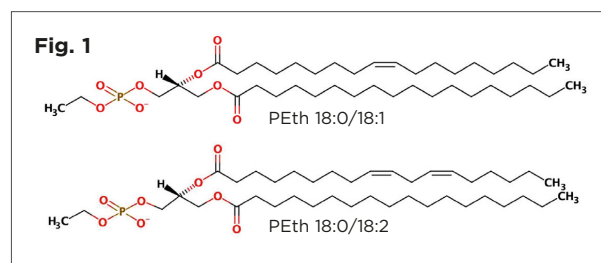
WHAT IS PETH?

Phosphatidylethanol (PEth) is an atypical phospholipid that can be found in the cell membrane of red blood cells. It is formed and incorporated into the cell membranes only in the presence of ethanol. Phospholipase D converts the phosphatidylcholine in red blood cells via a transphosphatidyltransfer reaction into PEth. This reaction is highly specific for the presence of ethanol in the bloodstream.

Up to 50 different PEth homologues occur naturally. Each has different abundancies and half-life times. Those molecules differ in the lengths and composition of the fatty chains. PEth 16:0/18:1 (also known as POPEth) and PEth 16:0/18:2 (PLPEth) are two of the most abundant homologues and are therefore predestined for quantification of PEth.

Advantages of PEth.

PEth reflects the direct and cumulative alcohol intake and is unaffected by recent short-term abstinence. The molecules are integrated into the cell membranes of red blood cells, where they remain stable over time. That stability offers a detection window of up to 4 weeks after the consumption of alcohol.



PEth LC-MS measurement sample prep.

As phosphatidylethanol is incorporated in red blood cells, whole blood is used to determine the PEth concentration. Whole blood is a complex matrix that can be challenging to work with, requiring thorough sample preparation and clean-up to ensure a smooth and consistent LC-MS measurement. Using a deproteinization solution to remove unwanted blood components and proteins to reduce any interference. A sample clean-up will also help to prevent system clogging and will enhance the lifetime of the LCMS-column.

Calibrators and controls.

Using calibrators and controls is essential in every LCMS measurement. While calibrators define the signal-to-concentration relationship and establish traceability, controls provide a continuous performance check and monitor the total analytical process. Each Tecan Tec-Trace® Calibrator and Control is matrix-matched to compensate for matrix effects and will be shipped lyophilized to ensure product stability.

Internal Standard.

An internal standard (IS) is an isotopically labeled analogue of the target compound, in this case POPEth-D5 and PLPEth-D5. The internal standard normalizes each sample individually. During sample preparation, calibrators and controls will be spiked with this internal standard, too. This ensures reproducible results while monitoring deviations in the methods for each sample. The internal standard is already added to the deproteinisation solution, making sample preparation fast while reducing pitfalls in sample handling.

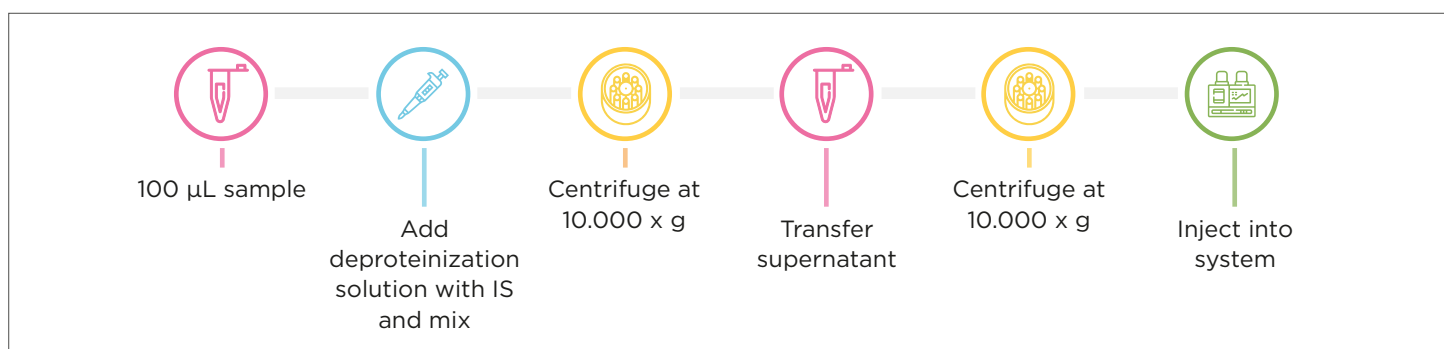
Fig. 1: Adapted from: Marisa Henriques Maria, Benedicte Marie Jørgenrud, Thomas Berg, Determination of eight phosphatidylethanol homologues in blood by reversed phase liquid chromatography–tandem mass spectrometry – How to avoid co-elution of phosphatidylethanol and unwanted phospholipids, *Journal of Chromatography A*, Volume 1684, 2022, 463566, ISSN 0021-9673, <https://doi.org/10.1016/j.chroma.2022.463566>.



METHOD AND PRODUCTS.

Matrix	Whole blood
Analytes	PEth 16:0/18:1 (POPEth - 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol) PEth 16:0/18:2 (PLPEth - 1-Palmitoyl-2-linoleoyl-sn-glycero-3-phosphoethanol)
LC-MS runtime	5 Minutes
Injection	2 - 20 μ L

Sample Preparation.



PEth - LC-MS products.

LC-MS Kit PEth **30269139*** - for 200 assays

Including	Mobile Phase I	(500 mL)
	Mobile Phase II	(500 mL)
	Deproteinization solution with internal standard	(2 x 82 mL)
	Calibrator set with 6 calibrator levels	(6 x 2 x 250 μ L)
	Autosampler washing solution	(500 mL)

Tec Trace PEth **30267543***

Calibrator set with 6 calibrator levels (6 x 2 x 250 μ L)

30267544*

Control set with 3 levels (3 x 3 x 250 μ L)

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