PRODUCT INSIGHTS

MassChrom® Phosphatidylethanol

Quantification of Phosphatidylethanol (PEth) as alcohol consumption marker

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FOR RESEARCH USE ONLY

Introduction

Phosphatidylethanols (PEth) serve as highly specific biomarkers for alcohol intake, as these abnormal phospholipids are synthesized in the body only in the presence of ethanol. In contrast to Ethyl Glucuronide (EtG), with its short urinary detection window of a few days, and Carbohydrate-Deficient Transferrin (CDT), which mainly reflects chronic alcohol use, PEth provides as a marker over an extended period.

While liquid chromatography tandem mass spectrometry (LC-MS/MS) can identify various PEth homologs, clinical testing typically targets the most prevalent forms, specifically PEth 16:0/18:1 and 16:0/18:2. Because PEth accumulates in erythrocytes and has a slow elimination rate, it can provide a detection window of several weeks. Furthermore, the faster synthesis rate and high abundance of PEth 16:0/18:2 enhance sensitivity for detecting recent alcohol consumption. Here, we describe a fast and robust method for the quantification of PEth 16:0/18:1 and 16:0/18:2 in whole blood.

Sample preparation is achieved through efficient extraction of analytes and the use of dedicated internal standards for all PEth homologs. The 6PLUS1® whole blood calibrator set covers the relevant calibration ranges for PEth and meets both forensic and clinical calibration requirements. Quality control is supported by the use of our two **MassCheck®** controls, which are designed to reflect the relevant decision limits.

Material and Methods

Sample Preparation

Prior to sample preparation, the Internal Standard Solution is prepared by mixing the Internal Standard Mix (order no. 63004) and the Extraction Reagent (Order No. 63005) in an appropriate ratio of 1:9.

For sample preparation, 100 µl of Internal Standard Solution is pipetted into a 1.5 mL reaction vial. Subsequently, 20 µl of sample/calibrator/**MassCheck®** control is added and vortexed for 5 seconds, followed by centrifugation for 10 minutes at 15,000 g. The resulting supernatant is transferred into an autosampler vial.

LC-MS/MS

Injection volume of 1–20 µl is used. Chromatographic separation is performed on an Analytical Column (order no. 63100) at a column temperature of 40 °C. The analytes are separated under isocratic conditions at a flow rate of 0.4 ml/min using Mobile Phase (order no. 63001).

Electrospray ionization in negative ion mode is used. The multiple reaction monitoring (MRM) transitions used for quantification are listed in Table 1 (see page 2).

Analyte	Corresponding ISTD MRM	MRM 1	MRM 2
PEth 16:0/18:1	706.5 → 281.1	701.5 → 281.1	701.5 → 255.1
PEth 16:0/18:2	704.5 → 281.1	699.5 → 279.1	699.5 → 255.1

Table 1: Multiple reaction monitoring transitions of Phosphatidylethanol 16:0/18:1 and 16:0/18:2, together with their respective internal standards.

Quantification

A full calibration of the analysis system for each series of measurements is performed with 6PLUS1® multi level calibrator (order no. 63039). Calibration curves are constructed by calculating the analyte to internal standard (ISTD) peak area ratio on the y axis against calibrator concentrations on the x axis. Then a calibration curve is plotted for both PEth homolouges using linear regression and 1/x weighting.

Results

The run time is 3.5 min (see chromatogram in fig. 1). The calibration range of 10-1000 ng/mL covers the forensic and clinical relevant concentrations (table 2).

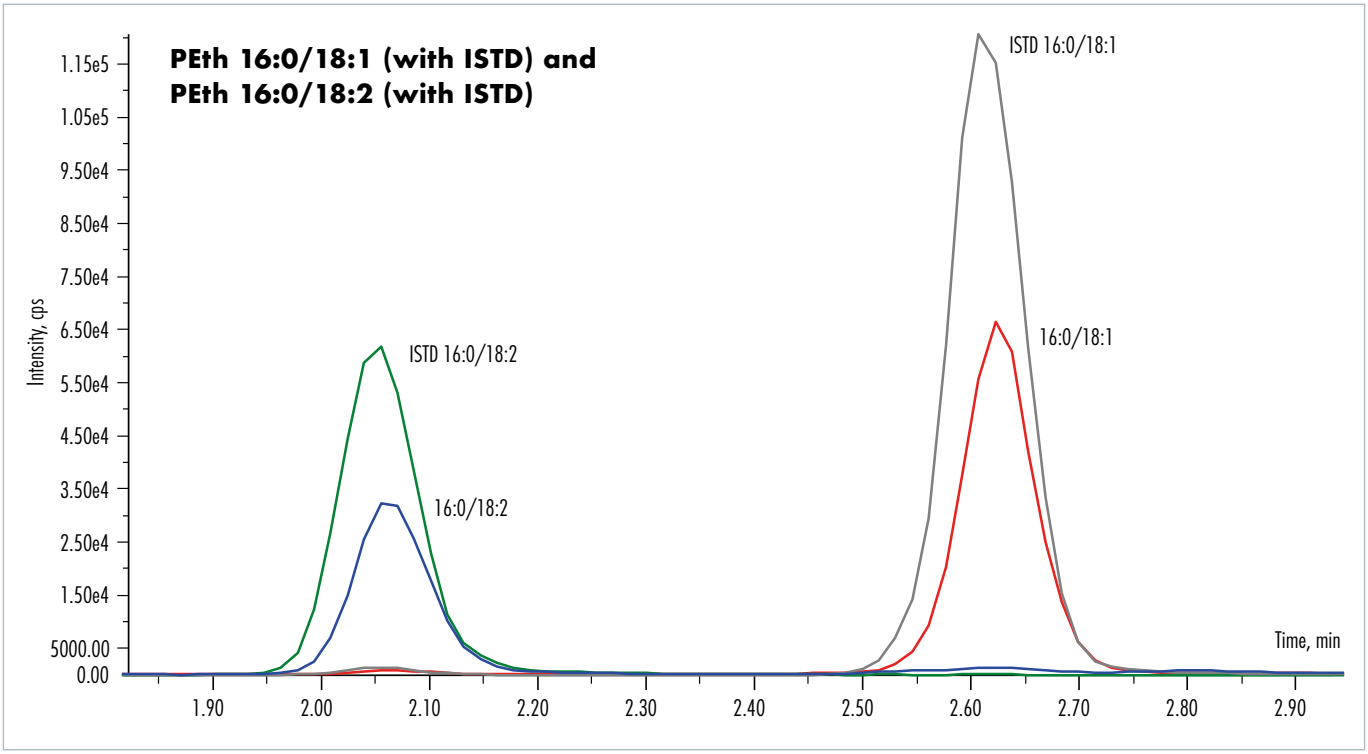

Fig. 1: Chromatogram of phosphatidylethanol 16:0/18:1 and 16:0/18:2 at a concentration of 300 ng/mL each, with their respective internal standards.

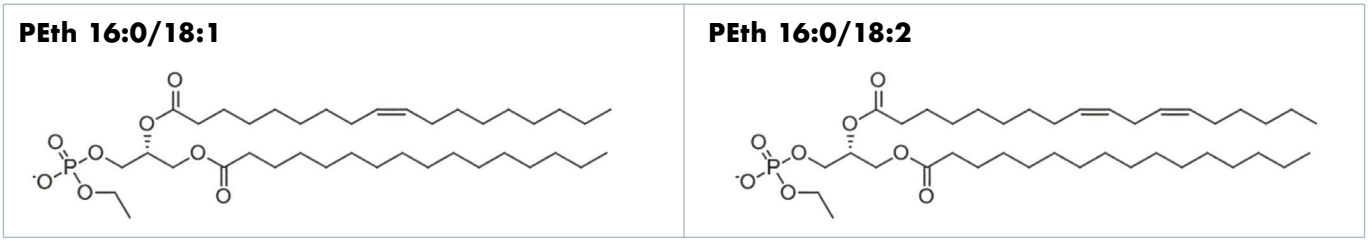

Fig. 2: Chemical structure of Phosphatidylethanol 16:0/18:1 and 16:0/18:2

Category	PEth 16:0/18:1 Concentration	Interpretation
Abstinence / Very Light Alcohol Intake	< 20 ng/mL	Shows abstinence or minimal consumption; suggests no relevant recent alcohol use.
Moderate / Social Alcohol Consumption	20–200 ng/mL	Indicates low to moderate or social drinking; cannot be interpreted as heavy or chronic use; reflects a range of consumption behaviors.
Sustained Heavy Alcohol Use	> 200 ng/mL	Strongly suggestive of sustained heavy drinking

Table 2: Interpretation of Phosphatidylethanol 16:0/18:1 values

Conclusion

This product insight describes a protocol for the determination of PEth 16:0/18:1 and PEth 16:0/18:2 in whole blood using Chromsystems components. Suitable products for implementation in your laboratory will soon be available from Chromsystems.

Outlook

Further research will focus on developing a reliable protocol for automated sample preparation using 96-well plates and liquid handling systems.

Ordering information

Product	Order no.
Mobile Phase	63001
Rinsing Solution	63009
Internal Standard Mix	63004
Extraction Reagent	63005
Tuning Mix, Analytes and Internal Standards	63015
Analytical Column	63100

Product	Order no.
6PLUS1® Multilevel Whole Blood Calibrator Set **MassChrom®** Phosphatidylethanol (PEth)	63039
MassCheck® Phosphatidylethanol (PEth) Whole Blood Control Set Level I	6310
MassCheck® Phosphatidylethanol (PEth) Whole Blood Control Set Level II	6320

Please note:
These products are currently being prepared for sale and are not yet available to order.
For further information, please contact your sales representative.

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