Automated Analysis of Phosphatidylethanol in Dried Blood Spots for Alcohol Consumption Behavior Assessment

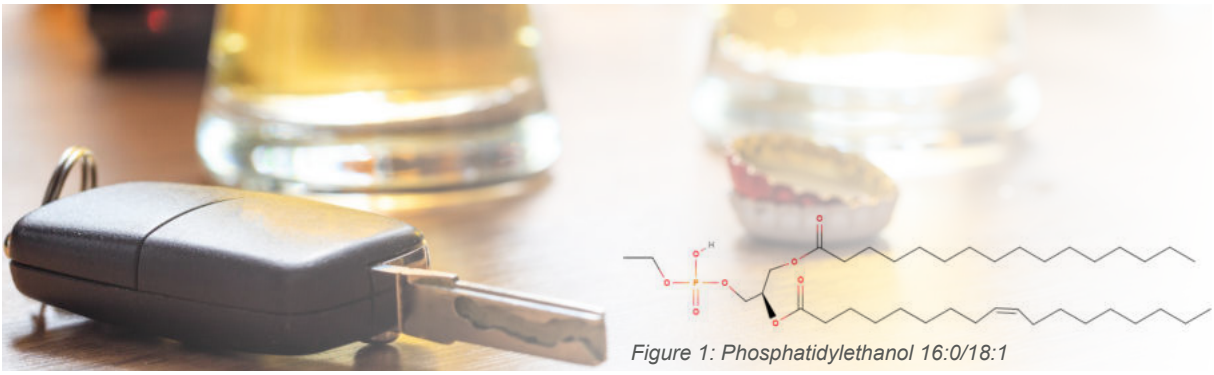

Figure 1: Phosphatidylethanol 16:0/18:1

Keywords

Alcoholism, driving aptitude assessment, family law, alcohol use disorder treatment, security environment, organ transplantations

Introduction

In the past decade, the analysis of the direct alcohol biomarker Phosphatidylethanol (PEth) to monitor alcohol consumption behavior has gained vast popularity. PEth is formed as an abnormal phospholipid after the consumption of alcohol. PEth itself is not a single molecule, but rather a group of molecules. PEth 16:0/18:1 and PEth 16:0/18:2 are the two most abundant PEth homologs in human blood and often measured together. The concentration of PEth 16:0/18:1 is frequently used to classify alcohol consumption behavior based on two threshold concentrations: A lower threshold is used to distinguish between light or no alcohol consumption and frequently set at 20 ng/mL (~0.03 µmol/L). An upper threshold is used to distinguish between substantial alcohol consumption and heavy alcohol consumption and set at ~200 ng/mL (~0.3 µmol/L). The analysis of PEth finds application during driving aptitude assessments, organ transplantations, alcohol use disorder treatment, and in the security environment. Compared to other direct or indirect alcohol markers (EtG, CDT, or GGT) PEth has been shown to have better sensitivity and specificity.

Scope

This fully automated DBS online-SPE-LC-MS/MS method allows to reliably quantify Phosphatidylethanol 16:0/18:1 and Phosphatidylethanol 16:0/18:2 within a run time of about 5 minutes per sample.

Hematocrit normalization can be integrated into the measurement method.

Recommended devices

- CAMAG DBS-MS 500 HCT
- 2x binary HPLC pumps allowing to run two independent gradients
- Sensitive ESI QqQ mass spectrometer (minimal requirement: 8050 Shimadzu, 5500 Sciex, or comparable)
- 6/2 or 10/2 HPLC switching valve
- Column oven

Samples

Dried blood spot samples with a minimum volume of 10 µL (≥ 6 mm Ø) on DBS AutoCollect cards (85 x 53 mm). Volumetric sampling is not required. Calibration and QC samples can be prepared in-house.

Standards

- PEth 16:0/18:1 (1 mg/mL in MeOH, certified reference material, Cerilliant, cat. no. P-114)
- PEth 16:0/18:2 (1 mg/mL in MeOH, certified reference material, Cerilliant, cat. no. P-115)
- Internal standard PEth 16:0/18:1-d5 and 16:0/18:2-d5 (commercial or prepared in-house)

Chromatography

Columns

- Reverse-phase **analytical column**, C5, 5 µm particle size, 50 mm × 2 mm (Phenomenex, cat. no. 00B-4043-B0) equipped with a Krud Katcher ULTRA HPLC inline filter
- Reverse-phase **trapping column**, Polar-RP, 4 µm particle size, 20 mm × 2 mm, cartridge column (Phenomenex, cat. no. 00M-4336-B0-CE)
- Cartridge holder for 20 mm × 2 mm trapping column (Phenomenex, cat. no. CH0-5845)

Reagents

	Reagent name	Reagent content
LC-MS mobile phases	A1, aqueous trapping column B1, organic trapping column A2, aqueous analytical column B2, organic analytical column	H2O/0.1% (vol/vol) FA H2O/MeCN, 30/70, 2 mM NH_4Ac H2O /MeCN, 30/70, 0.6 mM NH_4Ac 2-propanol
CAMAG DBS-MS 500 HCT solutions	E1, extraction solution R1, rinsing solution R2, rinsing solution R3, rinsing solution IS1, internal standard spray solution	H2O/MeCN/2-propanol/FA, 34.5/15/50/0.05 2-propanol/H2O, 85/15, 13 mM NH_4Ac H2O/MeCN, 50/50 2-propanol PEth 16:0/18:1-d5 and PEth 16:0/18:2-d5 in 2-propanol

*MeCN =acetonitrile, FA=formic acid, NH_4Ac= ammonium acetate

NOTE: The presented results are to be regarded as examples only!

Please contact CAMAG for more application notes and products!

LC-gradient

Trapping column LC program

Trapping column, Polar RP				
Time [min]	Mobile Phase A1 [%]	Mobile Phase B1 [%]	Flow [mL/min]	Comment
0	100	0	0.5	Start MS, Start Pump
0.250	100	0		
0.251	0	100		
2.5	0	100		
2.6	100	0		
5	100	0		End of program

Analytical column LC program

Analytical column, C5				
Time [min]	Mobile Phase A2 [%]	Mobile Phase B2 [%]	Flow [mL/min]	Comment
0	100	0	0.5	Start MS, Start
2.00	100	0		Start Gradient
4	75	25		
4.1	0	100		
5	0	100		End of program

Experimental setup

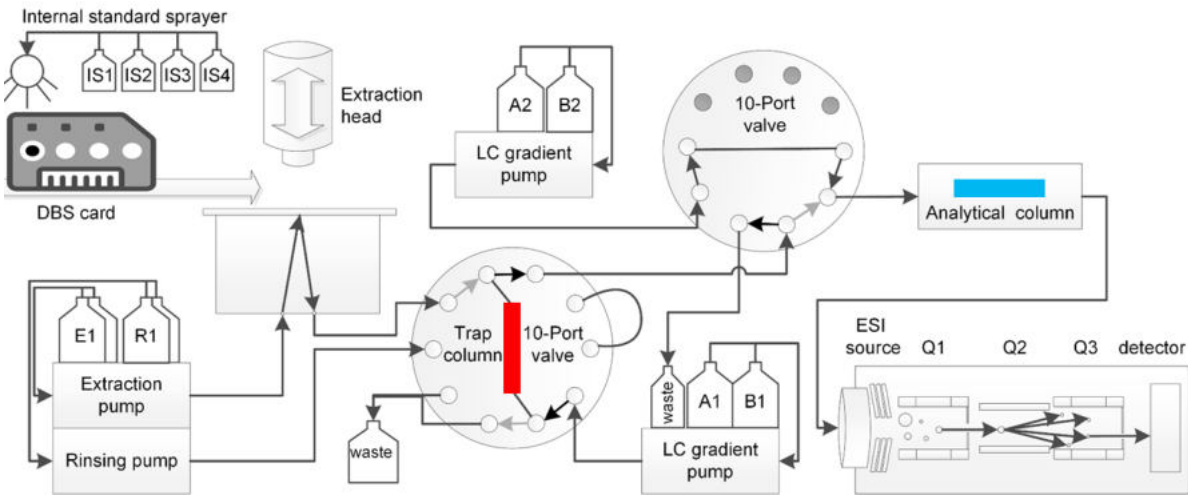

Figure 2: Schematic drawing of the CAMAG-DBS-MS 500 HCT being coupled to an LC-MS/MS system to perform online SPE extraction using two columns.

Results

The feasibility of the automated analysis was successfully validated, has been applied to real case samples, and was subsequently transferred to routine PEth analysis. The obtained results are comparable to the widely applied manual extraction of PEth in DBS or the analysis of PEth in liquid blood samples. Automation with the DBS MS-500 HCT includes the possibility of sample identification by a barcode label on the DBS card – with a link to a laboratory information system (LIMS). PEth is stable on DBS cards for several weeks at room temperature and there is no post-sampling formation or degradation as observed in liquid blood or other micro sampling devices.

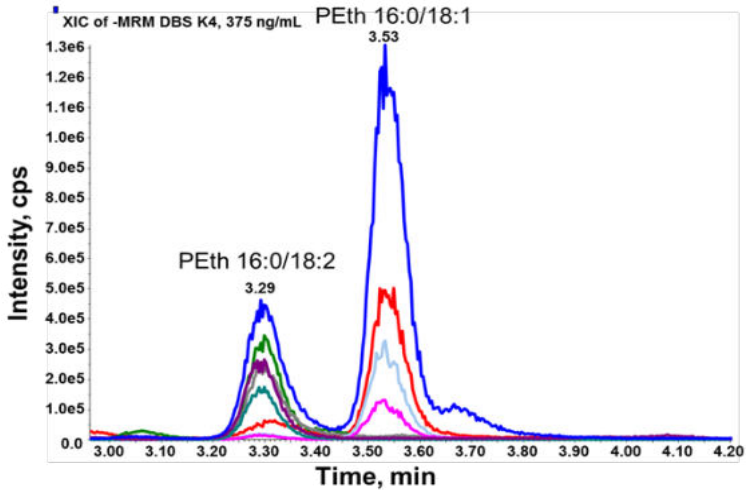

Figure 3: Chromatogram for the analysis of a DBS calibrator sample. The two PEth homologs can be baseline separated.

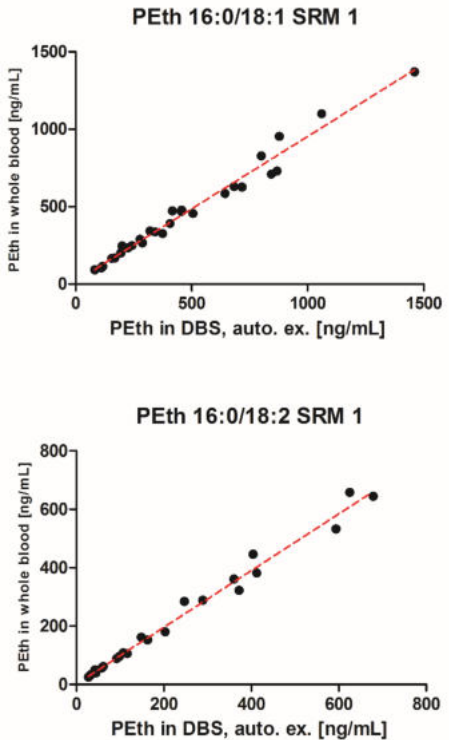

Figure 4: Comparison study between liquid whole blood analysis and fully automated DBS analysis shows excellent agreement.

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Literature

Luginbühl M, Stöth F, Schröck A. *et al*. Quantitative determination of phosphatidylethanol in dried blood spots for monitoring alcohol abstinence. *Nature Protocols*. 2021;16: 283–308. doi:10.1038/s41596-020-00416-x

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