Microplate liquid-liquid extraction: a rapid technique for routine analysis of phosphatidylethanol 16:0/18:1 in whole blood

Catalina Dumitrascu[1], Celine Gys[1] , Diona D'Hondt[2] , Werner Jacobs[2] , Hugo Neels[1] , Adrian Covaci[1] , Alexander L.N. van Nuijs[1]

Affiliations

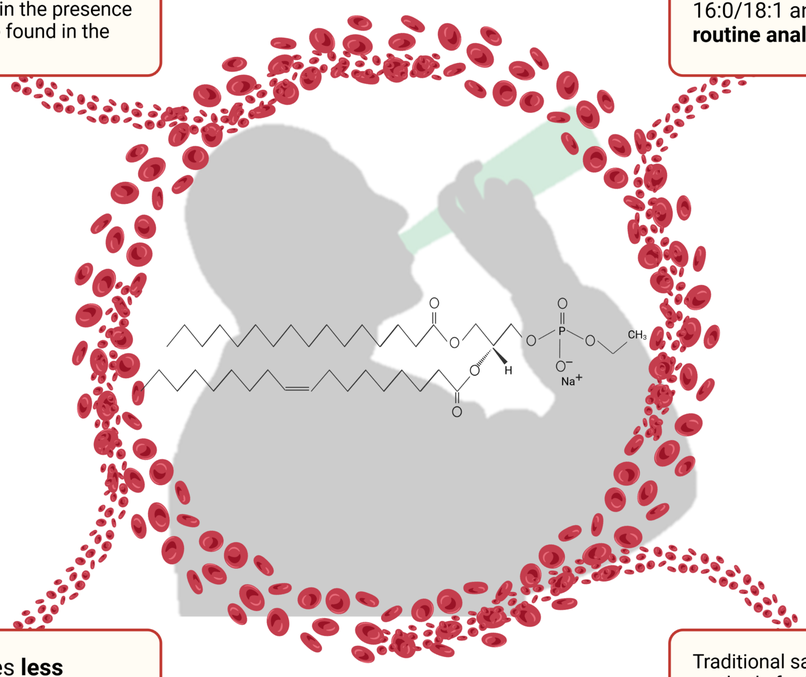
[1]Toxicological Centre, University of Antwerp, Antwerp, Belgium

[2]Department of Forensic Medicine and Pathology, University Hospital Antwerp, Antwerp, Belgium

Introduction

Phosphatidylethanol (PEth) 16:0/18:1 in whole blood is a **direct** alcohol consumption biomarker which is formed only in the presence of alcohol and can be found in the red blood cells

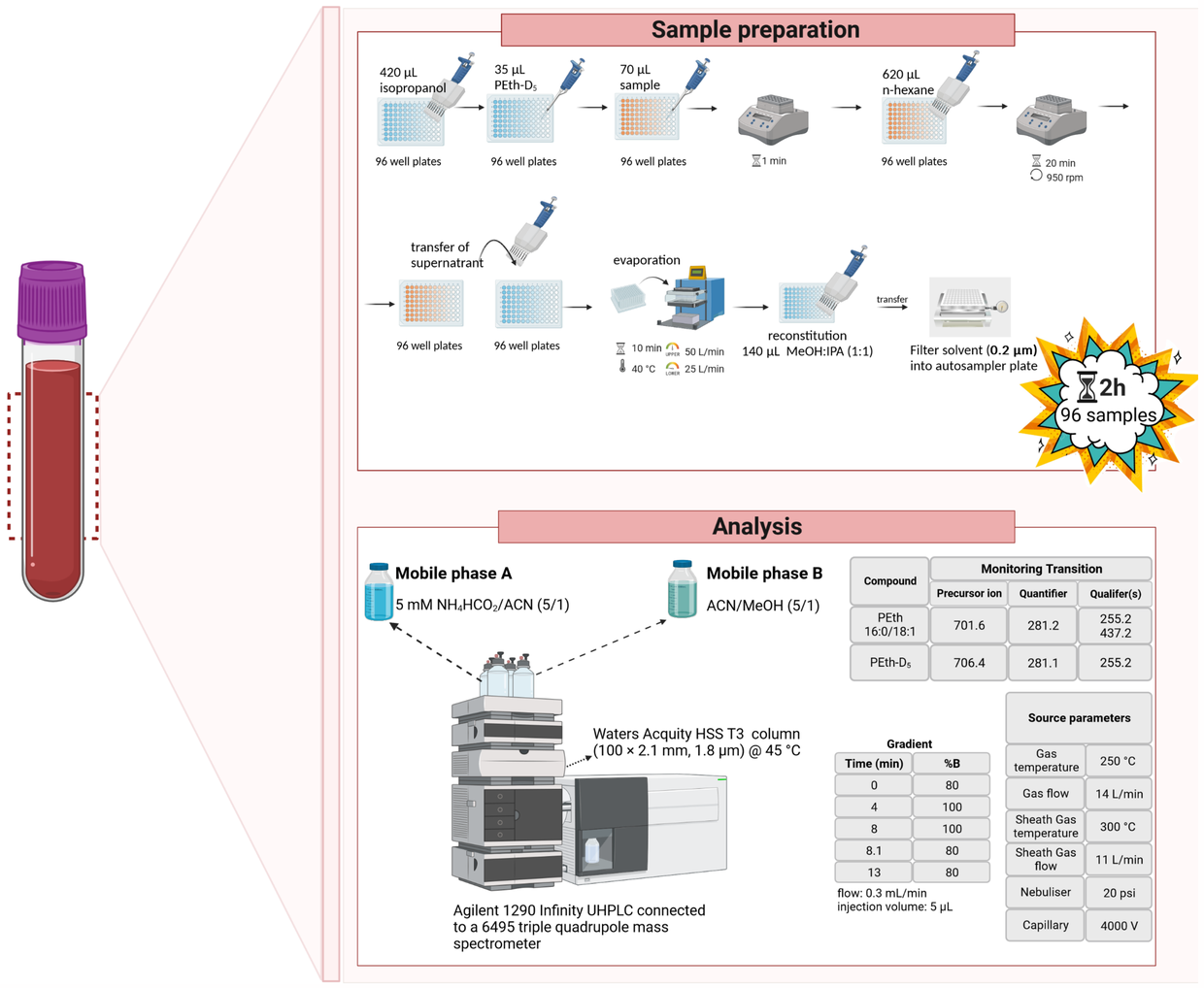
Clinical and forensic laboratories started to incorporate PEth 16:0/18:1 analyses into their **routine analysis**



A method that uses **less sample volume** and **solvents**, and is **less time-consuming** is of interest

Traditional sample preparation methods for PEth 16:0/18:1 analysis are often **time-consuming** and require the use of **large solvents** and **sample volumes**

Methodology



Compound	Monitoring Transition		
	Precursor ion	Quantifier	Qualifer(s)
PEth 16:0/18:1	701.6	281.2	255.2 437.2
PEth-D5	706.4	281.1	255.2

Gradient

Time (min)	%B
0	80
4	100
8	100
8.1	80
13	80

flow: 0.3 mL/min
injection volume: 5 µL

Source parameters	
Gas temperature	250 °C
Gas flow	14 L/min
Sheath Gas temperature	300 °C
Sheath Gas flow	11 L/min
Nebuliser	20 psi
Capillary	4000 V

The current methodology is based on previously published method. [1] The old method uses 30% more sample volume (100 µl), and 30% more solvents volumes (50 µl IS; 600 µl isopropanol; 900 µl hexane; 200 µl reconstitution solvent). Due to the 96 well plates, the multi-pipette, and the evaporation system, the sample preparation of 96 samples would take 50% less time. Therefore, this new method saves time, sample and solvent volumes when analysing a large amount of samples.

Results

Validation

- according to ICH guideline M10 on bioanalytical method validation and study sample analysis[2]

Calibration curve*

LLOQ: 5 ng/mL
ULOQ: 2000 ng/mL
weighting factor[3]: $1/x^2$

*matrix based

Carry-over

PEth 16:0/18:1: **17 %** (max. 20 % of LLOQ)
PEth-D_5: **0.3 %** (max. 5 % of LLOQ)

Matrix effect, recovery and process efficiency

Four* different sources of **blank whole blood** spiked at two levels

- QC low (QCL): 15 ng/mL
- QC high (QCH): 1500 ng/mL

Sample	Matrix effect (cv %; ±15 %)	Recovery (mean ± stdev, %)	Process efficiency (mean ± stdev, %)
QCL	5.7	98.2 ± 9.4	96.9 ± 10.6
QCH	8.9	107.7 ± 9.9	110.1 ± 6.6

*The guideline requires 6 different sources but the use of fewer sources may be acceptable in the case of rare matrice. [2]

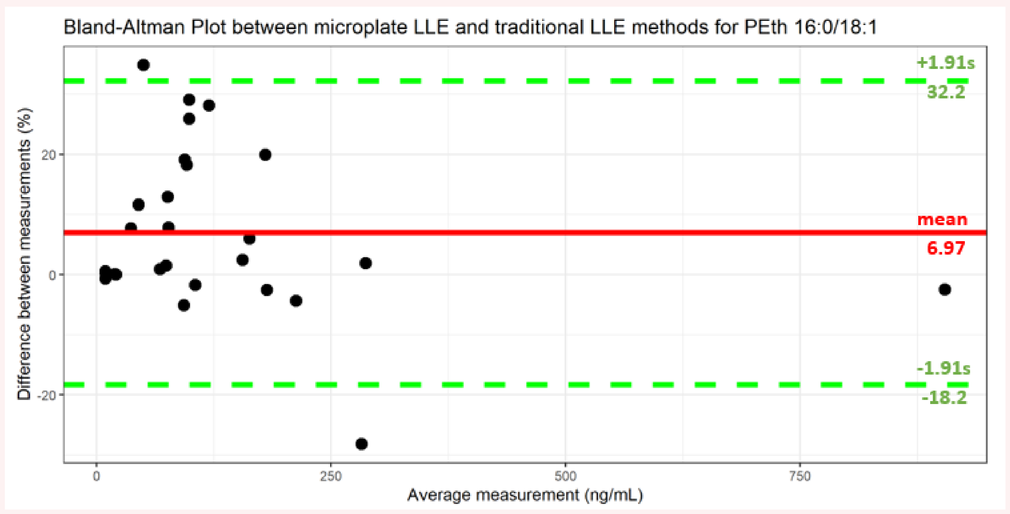
Accuracy and precision*

- LLOQ: 5 ng/mL
- QC low (QCL): 15 ng/mL
- QC medium (QCM): 150 ng/mL
- QC high (QCH): 1500 ng/mL

Sample	inter-day analysis		intra-day analysis	
	accuracy (%)	precision (cv %)	accuracy (%)	precision (cv %)
LLOQ	102.4	1.6	103.1	2.8
QCL	97.2	4.1	98.3	3.5
QCM	102.5	5.6	100.3	1.2
QCH	101.5	1.1	101.7	2.6

*Accuracy: ±15% (QCL, QCM, QCH) and ±20% (LLOQ)
Precision: <15% (QCL, QCM, QCH) and <20% (LLOQ)

Applicability



The microplate liquid-liquid extraction (LLE) method was compared with the previously validated traditional LLE approach using authentical samples. Our findings demonstrate an agreement between the two methods, confirming that microplate LLE is a reliable and efficient alternative for routine analysis. These results highlight the potential of microplate LLE as a successful technique in routine laboratories.

Conclusions

- **validated 96 well plate method**
- **30% less solvent** and **sample volume** compared to **traditional liquid-liquid extraction**
- **50% more time efficient** compared to **traditional liquid-liquid extraction**
- **96 well plate method** is an **efficient technique** for **routine laboratories**

References

[1] C. Dumitrascu et al., "Stability of phosphatidylethanol 16:0/18:1 in authentic and spiked whole blood," Drug Test. Anal., vol. 13, no. 6, pp. 1219–1222, Jun. 2021, doi: 10.1002/dta.2995.

[2] European Medicines Agency, " ICH guideline M10 on bioanalytical method validation and study sample analysis " 2022.

[3] H. Gu et al., "Selecting the correct weighting factors for linear and quadratic calibration curves with least-squares regression algorithm in bioanalytical LC-MS/MS assays and impacts of using incorrect weighting factors on curve stability, data quality, and assay perfomance", Anal. Chem., 2014, 86, 8959–8966.

Toxicological Centre
Toxicological Centre
catalina.dumitrascu@uantwerpen.be
University of Antwerp
Toxicological Centre