Technical note **no: 24 – 01.**

IMPACT OF THE REGIOISOMERIC PURITY IN PHOSPHATIDYLETHANOL ON CALIBRATION AND QC SAMPLES

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INTRODUCTION

Phosphatidylethanol (PEth) is a group of phospholipids formed through enzymatic reaction between ethanol and phosphatidylcholine on the cell membrane. The chemical structure of PEth consists of a phosphoethanol head with the attachment of two fatty acid chains to the glycerol backbone. The variety of attached fatty acids give rise to at least 48 homologues of PEth in which PEth-16:0/18:1 (palmitic acid/oleic acid) is the predominant molecule extracted from human erythrocytes. PEth is a direct biomarker for alcohol consumption with a detection window of 3-4 weeks in blood due to its slow breakdown in human body.

In humans only one regioisomeric form of PEth 16:0/18:1 is found. However, synthetic derived PEth can have two isomeric forms. This was first noticed by Luginbuhl et al. 2021, that performed an analyze of the regioisomeric properties of PEth from different vendors.

The impact of regioisomeric purity can be significant for the quantification of Peth with LC-MS/MS and will lead to either a too high or a too low result. This is due to the different fragmentation pattern obtained depending on the positions of the two different fatty acid chains of Peth.

Considering and addressing regioisomeric purity of PEth is crucial for accurately analyzing and interpreting data in LC-MS/MS applications.

OBJECTIVES

To compare PEth 16:0/18:1 from two producers, Cerilliant and Avanti, in the quantification of PEth in human blood samples.

COMPARISON STUDY

The MassDetect™ PEth kit was used in the study.
MS/MS instrument: API 5500 from Sciex

Two calibration curves in blood were prepared from Avanti and Cerilliant materials, respectively.
Two QC levels in blood from ACQ science were used, 0.075 µM (endogenous) and 0.44 µM (spiked with ENZO).
The MS/MS parameters were separately optimized for Avanti and Cerilliant material, respectively. The major fragments for PEth are m/z 281 (18:1) and 255 (16:0), see table 1.

Table 1

Vendor	m/z	Ion mode	Fragment	DP	CE	CXP
Cerilliant	701	negative	281	70	38	19
Cerilliant	701	negative	255	70	56	27
Avanti	701	negative	281	105	50	33
Avanti	701	negative	255	105	52	13

As could be seen in table 1 there are a significant difference in MS-parameters for PEth from the two

producers. The same concentration of PEth have been used for optimization, which indicates that there must be a difference in the composition of the two PEth materials.

Endogenous PEth consists of the regioisomer with 16:0 at the sn1 and 18:1 at the sn2 position, see figure 1. In agreement with Luginbuhl et al. we have seen that Cerilliant PEth has a comparable regioisomeric purity as endogenous material, as well as the ACQ control spiked with ENZO material. On the contrary PEth from Avanti shows a different regioisomeric purity.

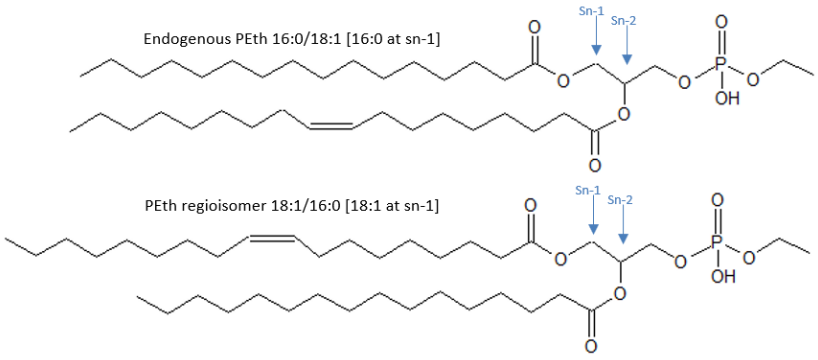

Figure 1

The relation between the two fragments (281 and 255) of PEth depends mainly on which of the two positions (sn-1 or sn-2) on the glycerol chain of the PEth molecule they are bond to. This due to the higher probability in the collision cell of acyl-cleavage occurring from the sn-2 position than from sn-1.

Consequently, the observed relation between the two fragments 281 and 255 (the ion ratio) will vary depending on the regioisomeric purity of PEth from different producers/vendors.

Samples from calibration curves from both Cerilliant and Avanti, as well as control samples from ACQ has been compared in respect to ion ratios (area ratio of 281 / area ratio of 255). All samples have been analyzed using the parameters optimized for PEth from Cerilliant, see table 2.

Table 2

Calibration levels/samples (µM PEth)	Spiking solution	Ion Ratio (281/255)	Spiking solution	Ion Ratio (281/255)
< 0.02	Endogenous	3.8	Endogenous	3.7
0.02	Cerilliant	4.1	Avanti	3.4
0.05	Cerilliant	4.0	Avanti	3.4
0.1	Cerilliant	3.9	Avanti	3.0
0.2	Cerilliant	4.1	Avanti	3.0
0.5	Cerilliant	4.1	Avanti	3.0
1	Cerilliant	4.0	Avanti	3.0
0.075 (ACQ)	Endogenous	4.8		
0.44 (ACQ)	ENZO	4.4		

It has been found that in blood samples with very low levels below the quantification limit (< 0.02 µM) the ion ratio may be distorted. Both PEth from Cerilliant and ENZO have comparable ion ratios with endogenous PEth and thereof similar regioisomeric purity. However, the ion ratio is significantly lower for the Avanti PEth.

To investigate the impact of regioisomeric purity on the quantification of PEth in blood samples, a comparison of measured levels of the ACQ controls was performed, see table 3.

Table 3

ACQ nominal levels (µM)	Measured PEth levels (µM)	Calibration curve used
0.075	0.080 (n=7)	Cerilliant
0.44	0.45 (n=7)	Cerilliant
0.075	0.12 (n=5)	Avanti
0.44	0.67 (n=5)	Avanti

The results in table 3 are in line with the differences in ion ratios shown (table 2), resulting in an over estimation of the concentrations of the ACQ controls when using the Avanti standard curve (with different/lower ion ratio).

In contrary, using the Cerilliant standard curve (with comparable ion ratio) in the quantification a good correlation with the nominal levels of the ACQ controls were achieved.

CONCLUSION

Our study confirms the results reported by Luginbuhl et al. (2021) that there is a difference in regioisomeric purity between Cerilliant and Avanti PEth.

Both the fact that significant difference was found in the optimization parameters as well as the ion ratios (fragment 281/255) confirms different regioisomeric purity of the two producers PEth.

The results from the comparison with the ACQ controls confirms the importance of the regioisomeric purity of PEth. Furthermore, ion ratios are similar for both endogenous, Cerilliant and ENZO material (fig.3.), but not for Avanti. Measured concentrations also confirms that to achieve an accurate concentration for the control samples a regiospecific purity is crucial (table 3).

Finally, the regioisomeric purity of PEth is crucial for accurately analyzing and interpreting data in LC-MS/MS applications.

REFERENCES

Luginbuhl et al. Journal of Analytical Toxicology, 2021;45:76-83