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Identification of 48 homologues of phosphatidylethanol in blood by LC-ESI-MS/MS

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Abstract Phosphatidylethanol (PEth) is an abnormal phospholipid carrying two fatty acid chains. It is only formed in the presence of ethanol via the action of phospholipase D (PLD). Its use as a biomarker for alcohol consumption is currently under investigation. Previous methods for the analysis of PEth included high-performance liquid chromatography (HPLC) coupled to an evaporative light scattering detector (ELSD), which is unspecific for the different homologues—improved methods are now based on time of flight mass spectrometry (TOF-MS) and tandem mass spectrometry (MS/MS). The intention of this work was to identify as many homologues of PEth as possible. A screening procedure using multiple-reaction monitoring (MRM) for the identified homologues has subsequently been established. For our investigations, autopsy blood samples collected from heavy drinkers were used. Phosphatidylpropanol 16:0/18:1 (internal standard) was added to the blood samples prior to liquid–liquid extraction using borate buffer (pH 9), 2-propanol and n-hexane. After evaporation, the samples were redissolved in the mobile phase and injected into the LC-MS/MS system. Compounds were separated on a Luna Phenyl Hexyl column (50 mm×2 mm, 3 μm) by gradient elution, using 2 mM ammonium acetate and methanol/acetone (95/5; v/v). A total of 48 homologues of PEth could be identified by using precursor ion and enhanced product ion scans (EPI).

Keywords Alcohol · Phosphatidylethanol · Phospholipids · Homologues · LC-MS/MS · Biomarker

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Introduction

Harmful drinking is a main problem in contemporary society and still has a great importance in traffic medicine enquiries. There are several direct markers of alcohol consumption to detect very recent alcohol exposure, such as the blood alcohol concentration (BAC), ethyl glucuronide (EtG), and ethyl sulfate (EtS) [1, 2]. Markers with a wider window of detection are usually also divided into indirect and direct markers. Gamma-glutamyltransferase (GGT) and carbohydrate-deficient transferring (CDT) enzymes, for example, are indirect markers mainly elevated in blood as a consequence of liver cell lesion. Fatty acid ethyl esters (FAEE) and phosphatidylethanol (PEth) are direct markers which are formed in vivo if ethanol is present. The sensitivity of the direct markers, especially that of PEth is much higher than of indirect markers, even after

moderate drinking (40 g ethanol/day). It is close to 100% whereas that for GGT and CDT it is only about 40% [3].

PEth is an abnormal phospholipid with fatty acid chains (two per molecule) in sn-1 and sn-2 position and phosphoethanol as the head group. It can extrahepatically be formed in membranes of red blood cells via a transphosphatidylation reaction, catalyzed by the ubiquitous enzyme phospholipase D (PLD) [4–6]. Regularly, if ethanol is absent, phosphatidic acid is formed. If ethanol is available, it can act as a co-substrate of the enzyme instead of water just like other primary short-chain alcohols [7]. Because of a higher PLD specificity constant of ethanol (about 250 times higher than water) PEth is formed [8]. It is measurable up to 29 days after the last intake of alcohol in heavy drinkers. The mean

half life is about 4 days [4, 9] and it is stable for 3 weeks in refrigerator or freezer at −80 °C, whereas at −20 °C PEth is still formed in the presence of ethanol [10]. Both Ruelland et al. and Welti et al. have shown that PLD in *Arabidopsis* cells is activated after exposure to cold, which can serve as an explanation for that occurrence [11, 12].

Most studies about PEth were performed using an evaporative light scattering detector (ELSD) system or nonaqueous capillary electrophoresis (NACE) coupled to UV-detection [13, 14]. These methods have the disadvantage of long run-times. In addition, they are unable to separate the homologues of PEth and to identify all individual components.

Until now, there is not much information about the fatty acids, which form the homologues (nomenclature of the

Fig. 1 a Total ion chromatogram of an enhanced product ion scan of [M−H]⁻ 701.5 with the corresponding mass spectrum; b chemical structure of PEth 16:0/18:1 and of fragment ions
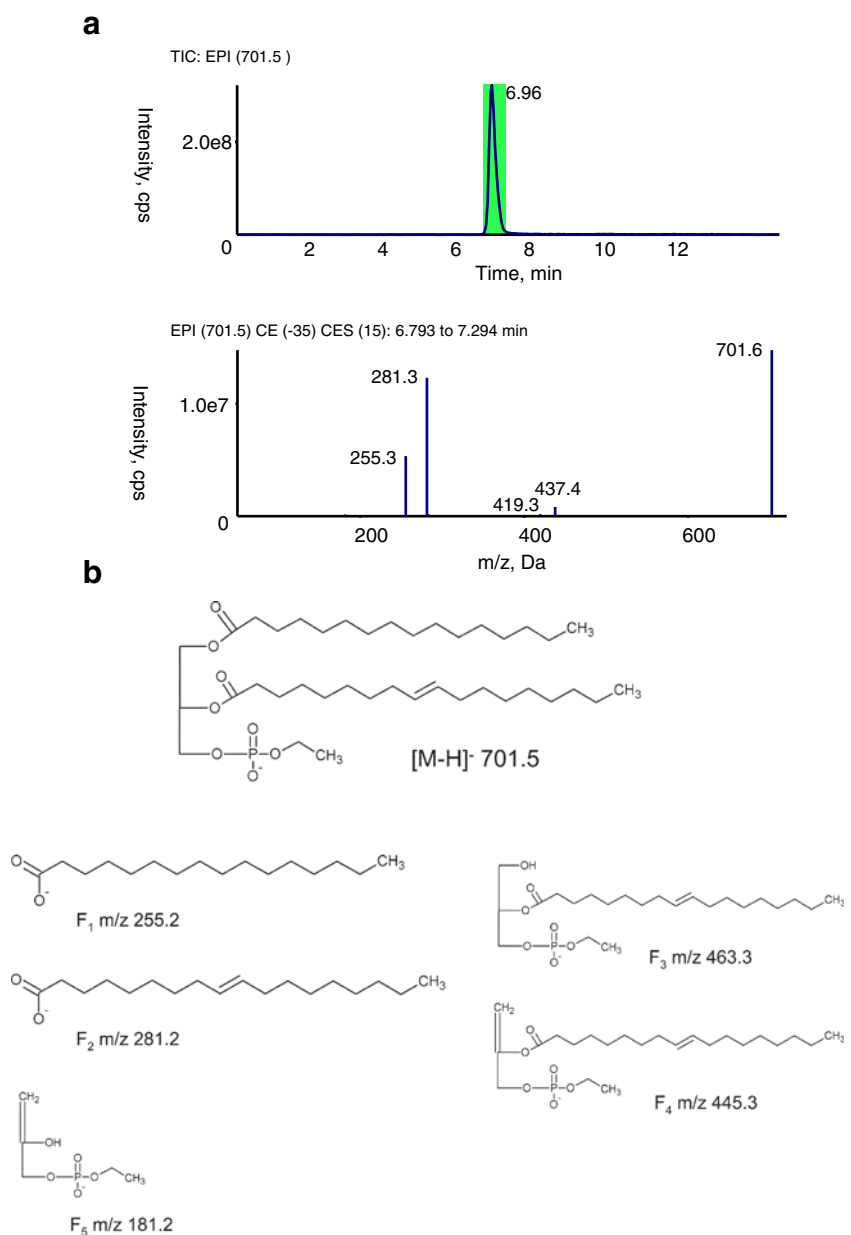
fatty acids is based on the number of carbons and the number of double bonds, e.g., oleic acid 18:1) and only a few of them have been identified yet [15, 16]. Therefore, the aim of the present paper was the identification of the highest number of PEth homologues, which can be used as alcohol consumption markers as well as to study the individual variability of this group of compounds.

Materials

Standard solutions of 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanol (sodium salt; PEth 16:0/16:0), 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanol (sodium salt; PEth 18:1/18:1) and 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphopropanol (sodium salt; PProp 18:1/18:1, internal standard (IS)) were obtained from Avanti Polar Lipids, Inc. (Alabama, USA). 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol (PEth 16:0/18:1) was received from BIOMOL Research Laboratories (Lörrach, Germany).

Boric acid and methanol (gradient grade, >99.8% purity) were supplied from J. T. Baker (Griesheim, Germany), isopropanol from Sigma-Aldrich (>99.8%), n-hexane (analytical grade, >99.0%), acetone (>99.8%), potassium chloride (>99.5%), sodium carbonate (>99.5%) and ammonium acetate (analytical grade, >98%) from Merck (Darmstadt, Germany).

Experimental

Sample preparation

For investigating homologues of PEth, a blood sample from an autopsy case was used. Several blood samples from dead and alive alcoholics were analyzed, but the one of the autopsy case was chosen, due to the higher concentration and number of homologues present. The following extraction procedure was performed: 10 μL IS (10 μg/mL), 100 μL borate buffer pH 9 (0.63 M), and 800 μL isopropanol were added to 300 μL blood. After vortexing and addition of 1,200 μL n-hexane, the mixture was agitated for 10 min and centrifuged (5 min, 16100×g). The supernatant was evaporated to dryness under a nitrogen stream at 40 °C. Samples were redissolved in 150 μl of the mobile phase and filtered through micro spin filters (Alltech, Germany) prior to injection of a 20 μL aliquot into the HPLC system.

HPLC

The Agilent 1100 Series HPLC system consisted of a G1312A binary pump, a G1313A autosampler and a G1379A degasser (Santa Clara CA, USA). Compounds were separated using a Luna Phenyl Hexyl column, 50 mm×2 mm, 3 μm (Phenomenex, Aschaffenburg, Germany). The mobile phases were: A, 2 mM ammonium acetate; B, methanol/acetone (95/5; v/v). The gradient elution mode started with 75% of solvent B, 0–3 min, 75% B; 3–8 min, 75–95%, linear; 8–12 min, 95% B; 12–13 min, 95–75% B, linear. The column was reequilibrated for 2 min. The flow rate was set to 0.4 mL/min and the total run time was 15 min.

ESI-MS

After chromatographic separation, the lipids were detected by a QTrap 2000 triple quadrupole linear ion trap mass spectrometer fitted with a TurboIonSpray interface (Applied Biosystems/Sciex, Darmstadt, Germany). Electrospray ionization was used in negative mode for precursor ion scans, EPI scans, and MRM. For all scan types, the following conditions were set: declustering potential −60 V, entrance potential −9 V, curtain gas 20 psi, collision gas 5 psi, ion spray voltage −4,200 V, ion source temperature 400 °C, ion source gas 1 40 psi and ion source gas 2 70 psi.

Operating with precursor ion scans, the first analyzer of the mass spectrometer had a scan range from m/z 600 to 850 using unit resolution. Collision energies extended from −50 to −55 V with a dwell time of 1.5 s.

For EPI scans, the time of the linear ion trapping was fixed to 50 ms with a scan rate of 4,000 amu/s. The lower limits for the scan range were m/z 50 and 100, respectively. The upper limits were conformed to the mass of the precursor ion [M−H]⁻. Collision energy spread (CES) and collision energies (CE) of −20, −35, and −50 eV were used. Using CES, one spectrum with information of three spectra, each with another CE, is
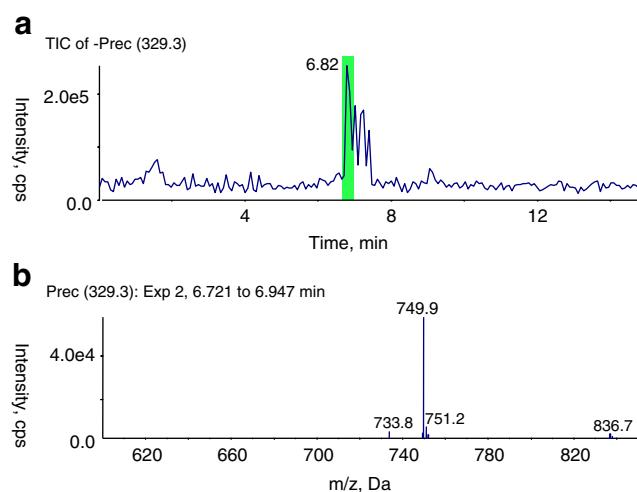

Fig. 2 **a** Total ion chromatogram of a precursor ion scan of m/z 329.3, blood from an autopsy case; **b** corresponding mass spectrum of the peak at a retention time of 6.82 min

Table 1 Twenty-nine precursor ion scans were performed based on the fragments in the left column of the table

fragment [m/z]	corresponding fatty acid	main precursor [m/z]
181.2		701.8
		699.8
		727.8
199.2	12:1	–
225.2	14:1	–
227.2	14:0	647.9
		673.9
251.3	16:3	–
253.2	16:1	674.0
		700.0
		702.0
255.2	16:0	702.0
		700.0
		674.0
279.2	18:2	700.0
		728.0
		726.0
281.2	18:1	701.9
		727.9
		729.9
283.3	18:0	730.0
		727.9
		752.0
301.3	20:5	721.9
		750.0
		747.9
303.2	20:4	724.0
		752.0
		750.0
305.5	20:3	726.0
		754.0
		752.0
307.3	20:2	728.0
		753.6
		755.7
309.3	20:1	756.4
		729.9
		777.9
311.3	20:0	–
327.3	22:6	747.8
		775.9
		774.2
329.3	22:5	749.9
		777.9
		762.1
331.3	22:4	752.0
		764.0
		778.0

Table 1 (continued)

fragment [m/z]	corresponding fatty acid	main precursor [m/z]
333.3	22:3	–
335.3	22:2	–
339.3	22:0	–
367.4	24:0	–
355.3	24:6	–
357.3	24:5	–
359.3	24:4	–
361.3	24:3	–
363.3	24:2	–
365.3	24:1	–

Corresponding fatty acids are listed in the middle column and main received precursors are shown in the right column

obtained. With a CE of −35 eV and CES of 15, the spectrum combines the spectra made with CE of −20, −35, and −50 eV.

The dwell time of each transition in the MRM mode was set to 15 ms to obtain a cycle time of 3.1 s. The CE for the non-fragmented precursor ion was set to −16 eV. For all transitions of precursor ion to product ions, the CE was −50 eV, except for PEth 16:0/16:0 and PEth 18:1/18:1, because all parameters were optimized for these two homologues, including the CE.

Results and discussion

Fragmentation of known PEths

The present investigations were based on the fragmentation of known homologues of PEth. Three of the homologues were available as reference material: PEth 16:0/16:0, PEth 18:1/18:1, and PEth 16:0/18:1.

In Fig. 1a, a chromatogram of an EPI scan of PEth 16:0/18:1 is shown, above its corresponding mass spectrum. Beside the precursor mass [M−H]⁻ 701.6, the main fragments m/z 255.3 and 281.3 were detected. These masses belonged to the fatty acid chains 16:0 and 18:1. The fragments m/z 437.4 (PEth minus one fatty acid chain, in this case 18:1, cf. F3 in Fig. 1b) and 419.3 (PEth minus one fatty acid chain minus water, cf. F4 in Fig. 1b) were much weaker.

Identification of unknown homologues of PEth

There is a lot of information about the molecular structure of other phospholipids and the composition of their fatty acid chains. Taking into account that PEth is formed from phosphatidylcholine (PC), this phospholipid was particularly relevant. Therefore, it was appropriate to look at the fatty acid moieties of PC with the expectation of finding those in PEth as well.

Table 2 Main precursors of Table 1 were used to perform enhanced product ion spectra

Precursor [m/z]	Main fragments [m/z]	Corresponding fatty acid	Possible combinations
645.5	225.2	14:1	14:0/16:1
	227.3	14:0	14:1/16:0
	253.2	16:1	
	255.2	16:0	
	409.2	14:0	
647.5	227.3	14:0	14:0/16:0
	255.3	16:0	
	437.3	16:0	
671.5	227.0	14:0	14:0/18:2
	251.2	16:2	16:0/16:2
	253.2	16:1	16:1/16:1
	255.2	16:0	
	279.1	18:2	
	281.3	18:1	
673.5	227.3	14:0	14:0/18:1
	253.3	16:1	16:0/16:1
	255.3	16:0	
	281.3	18:1	
	391.3	14:0	
	419.4	16:0	
	437.3	16:0	
	463.3	18:1	
695.5	227.2	14:0	14:0/20:4
	253.3	16:1	16:1/18:3
	277.2	18:3	
	303.4	20:4	
	391.2	14:0	
	409.2	14:0	
697.5	253.2	16:1	16:1/18:2
	255.2	16:0	16:0/18:3
	277.2	18:3	
	279.3	18:2	
	435.2	16:1	
	461.3	18:2	
699.5	253.3	16:1	16:0/18:2
	255.3	16:0	16:1/18:1
	279.3	18:2	
	281.2	18:1	
	419.3	16:0	
	437.3	16:0	
	461.3	18:2	
701.5	253.3	16:1	16:0/18:1
	255.3	16:0	16:1/18:0
	279.3	18:2	
	281.3	18:1	
	283.3	18:0	
703.5	255.2	16:0	16:0/18:0

Table 2 (continued)

Precursor [m/z]	Main fragments [m/z]	Corresponding fatty acid	Possible combinations
	283.3	18:0	
	437.2	16:0	
	465.3	18:0	
721.5	253.3	16:1	16:0/20:5
	255.2	16:0	16:1/20:4
	301.2	20:5	
	303.3	20:4	
	417.4	16:1	
	435.4	16:1	
	437.4	16:0	
723.5	255.3	16:0	16:0/20:4
	279.3	18:2	18:2/18:2
	303.3	20:4	
	419.3	16:0	
	437.3	16:0	
	485.5	20:4	
725.7	255.3	16:0	16:0/20:3
	279.3	18:2	18:1/18:2
	281.3	18:1	
	305.3	20:3	
	437.4	16:0	
	463.3	18:1	
727.7	255.2	16:0	16:0/20:2
	279.2	18:2	18:0/18:2
	281.3	18:1	18:1/18:1
	283.3	18:0	
	307.3	20:2	
	463.4	18:1	
	465.4	18:0	
729.5	255.4	18:0	16:0/20:1
	281.3	18:1	18:0/18:1
	283.3	18:0	
	309.4	20:1	
	447.2	18:0	
	465.3	18:0	
736.5	281.3	18:1	
	303.3	20:4	
	305.4	20:3	
	329.3	22:5	
	331.3	22:4	
747.5	255.3	16:0	16:0/22:6
	279.2	18:2	18:2/20:4
	281.3	18:1	18:1/20:5
	301.3	20:5	
	303.1	20:4	
	327.4	22:6	
	419.4	16:0	
	437.4	16:0	

Table 2 (continued)

Precursor [m/z]	Main fragments [m/z]	Corresponding fatty acid	Possible combinations
749.5	255.2	16:0	16:0/22:5
	281.3	18:1	18:0/20:5
	283.3	18:0	18:1/20:4
	301.3	20:5	
	303.3	20:4	
	329.3	22:5	
	437.3	16:0	
	463.4	18:1	
751.5	255.2	16:0	16:0/22:4
	281.3	18:1	18:0/20:4
	283.3	18:0	18:1/20:3
	303.3	20:4	
	305.3	20:3	
	331.3	22:4	
	437.3	16:0	
	447.4	18:0	
	465.4	18:0	
	485.3	20:4	
753.5	255.5	16:0	16:0/22:3
	279.4	18:2	18:0/20:3
	281.3	18:1	18:1/20:2
	283.3	18:0	18:2/20:1
	305.4	20:3	
	307.3	20:2	
	309.4	20:1	
	333.3	22:3	
	465.2	18:0	
755.6	253.1	16:1	18:0/20:2
	255.2	16:0	18:1/20:1
	279.4	18:2	
	281.3	18:1	
	283.3	18:0	
	305.4	20:3	
	307.3	20:2	
	309.4	20:1	
	473.4	20:1	
	491.5	20:1	
757.5	281.4	18:1	18:1/20:0
773.5	279.3	18:2	18:1/22:6
	281.3	18:1	20:3/20:4
	283.3	18:0	
	303.1	20:4	
	305.3	20:3	
	327.3	22:6	
	417.5	16:1	
	463.5	18:1	
775.5	279.2	18:2	18:0/22:6
	281.4	18:1	18:1/22:5

Table 2 (continued)

Precursor [m/z]	Main fragments [m/z]	Corresponding fatty acid	Possible combinations
	283.3	18:0	20:2/20:4
	303.3	20:4	
	307.3	20:2	
	327.3	22:6	
	329.3	22:5	
	465.3	18:0	
777.5	281.4	18:1	18:0/22:5
	283.4	18:0	18:1/22:4
	303.2	20:4	20:1/20:4
	309.3	20:1	
	329.3	22:5	
	331.4	22:4	
	463.3	18:1	
	465.4	18:0	
	491.3	22:6	
779.6	255.4	16:0	16:0/24:4
	277.2	18:3	18:0/22:4
	279.3	18:2	18:1/22:3
	281.2	18:1	20:1/20:3
	283.4	18:0	
	303.2	20:4	
	305.4	20:3	
	309.4	20:1	
	331.3	22:4	
	333.2	22:3	
	359.3	24:4	
	465.2	18:0	

In the second column, the main fragments are listed, which resulted from the precursor. Collision energy spread (CES) and collision energies (CE) of −20, −35, and −50 eV were used to obtain these fragments. In the right column, the possible combinations of fatty acid chains, which had to be confirmed, are shown

With the clues of the fatty acid chains from PC and the knowledge of the fragmentation of known PEth compounds, a series of precursor ion scans was performed.

For precursor ion scanning, the first analyzer of the mass spectrometer scans in a chosen mass range, while the second analyzer is focused on the mass-to-charge-ratio of a selected ion. Thus, the precursor ion scan produces a spectrum of all precursors whose fragmentation results in the selected product ion.

An example for this scan mode is shown in Fig. 2. The total ion chromatogram of the product ion m/z 329.3 (corresponding to the fatty acid 22:5) indicated four precursor ions, all of them were chromatographically separated. In the mass spectrum below (Fig. 2b), a precursor ion of the mass [M−H]⁻ 749.5 was detected at a
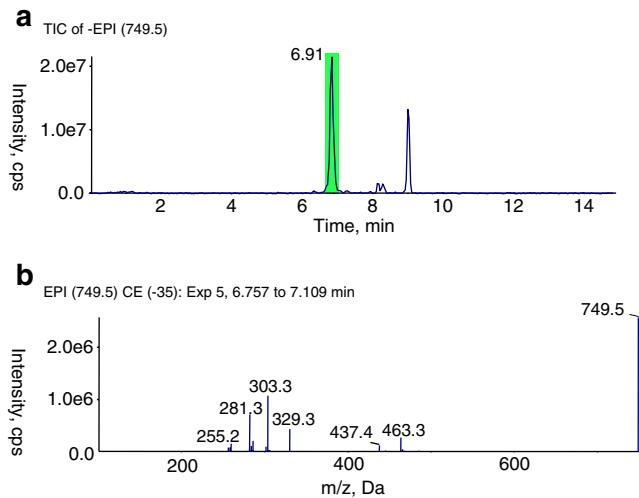
Fig. 3 a Total ion chromatogram of an enhanced product ion scan of [M−H]⁻ 749.5, blood from an autopsy case; **b** corresponding mass spectrum of the peak at a retention time of 6.91 min, main fragments are: m/z 255.2, 281.3, 303.3, 329.3, 437.4, 463.3

retention time of 6.82 minutes. All chosen precursor ion scans, which were carried out, are listed in Table 1.

By means of the evaluation of the precursor ion scan data, EPI scans of possible homologues of PEth with masses from m/z 645.5 to 779.5 (Table 2) were performed.
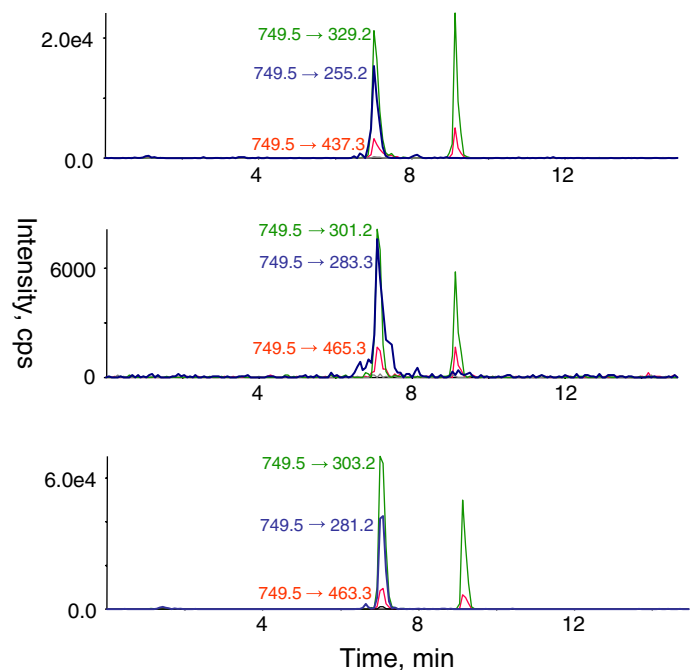
For an enhanced product ion scan, the first analyzer of the mass spectrometer selects a specific precursor m/z, while the fragments in a chosen mass range are collected in the linear ion trap prior to their detection. Thus, a spectrum of all product ions after fragmentation of one selected precursor ion is produced.

Taking up the example of the precursor scan, in which a precursor with the mass m/z 749.5 was detected, an EPI scan with this mass (Fig. 3) was carried out. In this case, as well as in other cases, a number of product ions were detected: m/z 255.2, 281.3, 283.3, 301.3, 303.3, 329.3, 437.3, 463.4. Observing the corresponding fatty acid chains (Table 2), it was obvious, that these fragments were not indicative for only one homologue, but for several homologues of PEth. These homologues are listed in the right column of Table 2. For the precursor [M−H]⁻ 749.5 with its product ions, three different homologues are possible: PEth 16:0/22:5, PEth 18:0/20:5, and PEth 18:1/20:4. The product ions m/z 437.3 and 463.4 are associated to the fragments F2 and F3 and verify the respective homologue. Mass spectra of the enhanced product ion scans are shown in Online Resource 1.

Having the clues for many homologues of PEth, the last step was to confirm these. For that reason, a MRM-method was developed.

Using MRM, both analyzers of the mass spectrometer are set to detect a specific transition from precursor to product ions, which increases sensitivity and selectivity.

Continuing the previous example, an extracted ion chromatogram of the MRM-method is shown in Fig. 4. Three different homologues of PEth with the same precursor mass [M−H]⁻ 749.5 were found using MS/MS without chromatographic base-line separation.

The final method contains 155 transitions. For each homologue, the non-fragmented precursor [M−H]⁻ was detected in Q3 as well as both fatty acid moieties as product ions of the respective precursor (Table 3). It cannot be excluded, that there is another structurally related compound

Fig. 4 Extracted chromatogram of the MRM-method for the confirmation of PEth 16:0/22:5 with transitions m/z 749.5—329.2, 255.2, 437.3; PEth 18:0/20:5 with transitions m/z 749.5—301.2, 283.3, 465.3 and PEth 18:1/20:4 with transitions m/z 749.5—303.2, 281.2, 463.3

Table 3 MRM transitions of the 48 homologues of PEth; for each homologue the non-fragmented precursor ion and both fatty acid moieties as transitions 1 and 2 were utilized

Fatty acid moieties	Precursor [M−H]⁻	Non-fragmented precursor m/z	Transition 1 m/z	Transition 2 m/z
14:0/16:0	647.5	647.5	227.2	255.2
14:0/18:1	673.5	673.5	227.2	281.2
14:0/18:2	671.5	671.5	227.2	279.2
14:0/20:4	695.5	695.5	227.2	303.2
16:0/16:0	675.5	675.5	255.2	437.3
16:0/16:1	673.5	673.5	255.2	253.2
16:0/17:1	687.5	687.5	255.2	267.2
16:0/18:0	703.5	703.5	255.2	283.2
16:0/18:1	701.5	701.5	255.2	281.2
16:0/18:2	699.5	699.5	255.2	279.2
16:0/18:3	697.5	697.5	255.2	277.2
16:0/20:2	727.7	727.7	255.2	307.2
16:0/20:3	725.7	725.7	255.2	305.2
16:0/20:4	723.5	723.5	255.2	303.2
16:0/20:5	721.5	721.5	255.2	301.2
16:0/22:4	751.5	751.5	255.2	331.3
16:0/22:5	749.5	749.5	255.2	329.2
16:0/22:6	747.5	747.5	255.2	327.2
16:1/14:0	645.5	645.5	253.2	227.2
16:1/16:1	671.5	671.5	253.2	435.3
16:1/18:0	701.5	701.5	253.2	283.2
16:1/18:1	699.5	699.5	253.2	281.2
16:1/18:2	697.5	697.5	279.2	253.2
16:1/20:4	721.5	721.5	253.2	303.2
17:0/18:1	715.5	715.5	281.2	269.2
17:0/18:2	713.5	713.5	279.2	269.2
18:0/18:1	729.5	729.5	283.2	281.2
18:0/18:2	727.7	727.7	283.2	279.2
18:0/20:3	753.5	753.5	283.2	305.2
18:0/20:4	751.5	751.5	283.2	303.2
18:0/20:5	749.5	749.5	283.2	301.2
18:0/22:4	779.6	779.6	283.2	331.3
18:0/22:5	777.5	777.5	283.2	329.2
18:0/22:6	775.5	775.5	283.2	327.2
18:1/18:1	727.7	727.7	281.2	463.3
18:1/18:2	725.7	725.7	281.2	279.2
18:1/20:1	755.6	755.6	281.2	309.3
18:1/20:2	753.5	753.5	281.2	307.2
18:1/20:3	751.5	751.5	281.2	305.2
18:1/20:4	749.5	749.5	281.2	303.2
18:1/22:4	777.5	777.5	281.2	331.3
18:1/22:5	775.5	775.5	281.2	329.2
18:1/22:6	773.5	773.5	281.2	327.2
18:2/18:2	723.5	723.5	279.2	461.3
18:2/20:1	753.5	753.5	279.2	309.3
18:2/20:4	747.5	747.5	279.2	303.2
20:1/20:4	777.5	777.5	309.3	303.2
20:2/20:4	775.5	775.5	307.2	303.2

with the same transitions and the same retention time which does not present a PEth homologue. Further quantitation and unequivocal identification would require the synthesis of reference compounds, which are not commercially available.

Nevertheless, 48 homologues of PEth were assumed to be present in an authentic blood sample. Also, homologues with an odd number of carbons were discovered in blood. Odd numbered fatty acids are much rarer than even numbered fatty acids in blood. A hint of the presence of an odd number fatty acid moiety was reported by Ekroos et al. [17], who found phosphatidylcholine with C17 as a fatty acid moiety in red blood cells.

Conclusions

The use of PEth as an alcohol consumption marker is still under evaluation. Most analysis performed till now did not show good sensitivity. Reported limits of detection (LOD) values for PEth were 0.4 μmol/L and 0.8 μmol/L using NACE and ELSD, respectively. The developed method for the determination of PEth homologues via LC-MS/MS considerably increases the sensitivity of the analytical procedure with an LOD of about 0.04 μmol/L for standard PEths.

After the application of the described methodology, 48 homologues of PEth were identified. To each one, a pair of fatty acids could be related, although the position of each fatty acid chain could not be assessed.

Until now, many of the identified compounds have not been described in the literature and have not been aim of any investigations. The identification of new homologues of PEth increases the ability of using them as alcohol consumption biomarkers. Although quantitation was not possible due to the lack of reference materials, it was observed, that the most abundant homologues were PEth 16:0/18:1 and PEth 16:0/18:2. The area ratio from PEth 16:0/18:1 is nearly 10 times higher than PEth 16:0/16:0 and 18:1/18:1. At this point further investigations are necessary to compare the abundance and the distribution of PEth homologues in blood samples from alcoholics, social drinkers and healthy persons tending to hazardous drinking. A further aspect is the nutrition. It plays an important role in the formation of phospholipids, because their fatty acids mainly origin from the food ingestion. Therefore it will be helpful to determine the different homologues of PC and compare the distribution of fatty acid chains with those of PEth.

Currently, quantitation of three PEth compounds is possible. It would be a further progress, if other homologues will be available as reference material, because this is the limiting factor for quantitation of PEth at this moment.

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