Buffer-Free High-pH LC-MS/MS Method to Determine Phosphatidylethanol and 20 Drugs in Blood

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1. Background

Alcohol, medicinal, and illicit drugs have significant health and economic impacts. Therefore, reliable determination of these substances is essential in forensic and pharmacological studies. While most LC-MS/MS methods use acidic mobile phases, basic mobile phases offer advantages for many compounds, such as enhanced retention, increased sensitivity, improved peak shapes [1].

2. Objective

This study aimed to develop a sensitive, accurate and precise LC-MS/MS method for the quantification of the alcohol biomarker phosphatidylethanol 16:0/18:1 (PEth 16:0/18:1) and 20 additional drugs and metabolites [2].

3. Methodology

Whole blood was prepared by 96-well supported liquid extraction (96-SLE) and then analysed by reversed phase LC-MS/MS (Figure 1). LC-MS/MS analyses were performed on an Acquity BEH C18 column (50 x 2.1mm, 1.7 µm particles). The injected volume was 1µL. The mobile phase composition was 0.025% ammonia, pH 10.7 (0.025% NH_3) and methanol (MeOH). See Fig. 1.

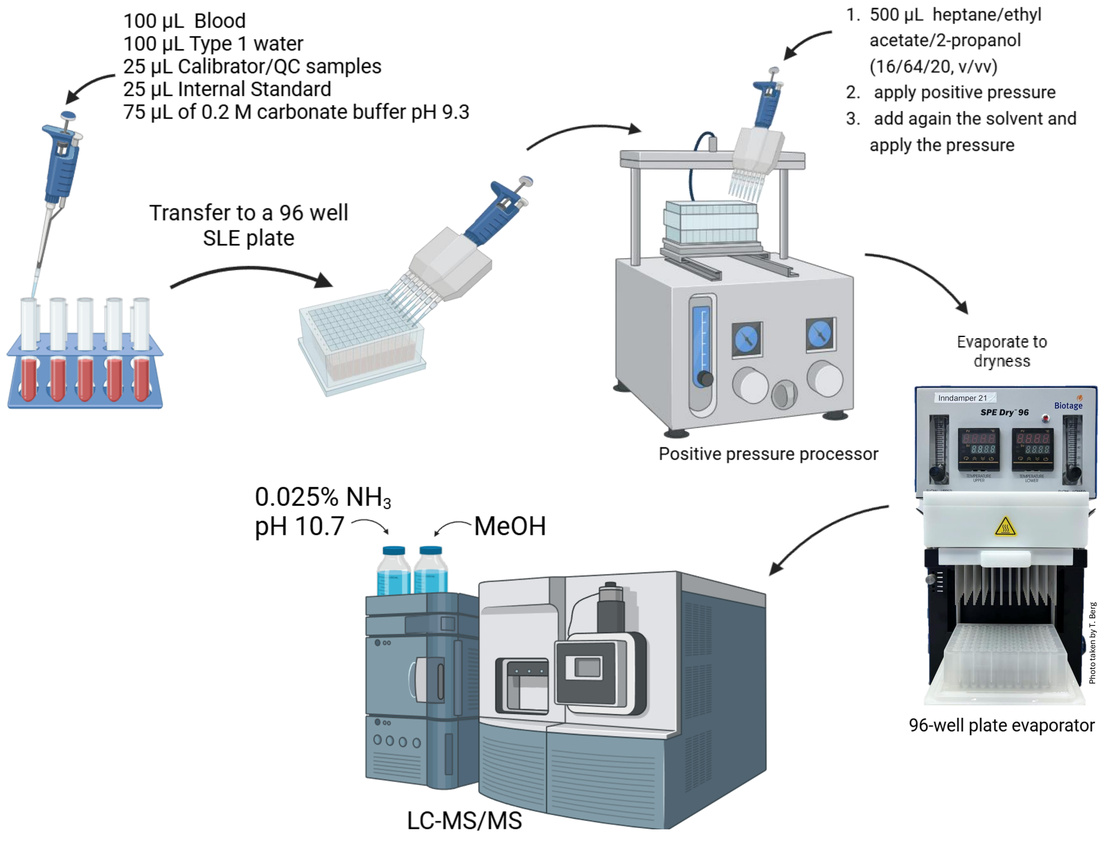

Figure 1: Schematic of the experimental procedure, 96-well supported-liquid extraction (SLE) and instrument analysis by LC-MS/MS (created on Biorender)

4. Results and Discussion

A sensitive, accurate and precise LC-MS/MS method for the determination of phosphatidylethanol (PEth) 16:0/18:1 and 20 drugs and metabolites was developed and validated (see data in Table 1). Stable isotope labelled internal standards were used for all compounds.

- Increased retention for most compounds in a basic mobile phase (Fig. 2.)
- Retention for PEth 16:0/18:1, THC and benzodiazepines were unaffected by mobile phase pH (Fig. 2).
- Buffer-free basic mobile phase (0.025% ammonia, pH 10.7) effectively separated PEths from unwanted phospholipids and avoided co-elution (Fig. 3).
- The method was validated with accuracy and precision within ±20 %, and matrix effects were minimal (Table 1).

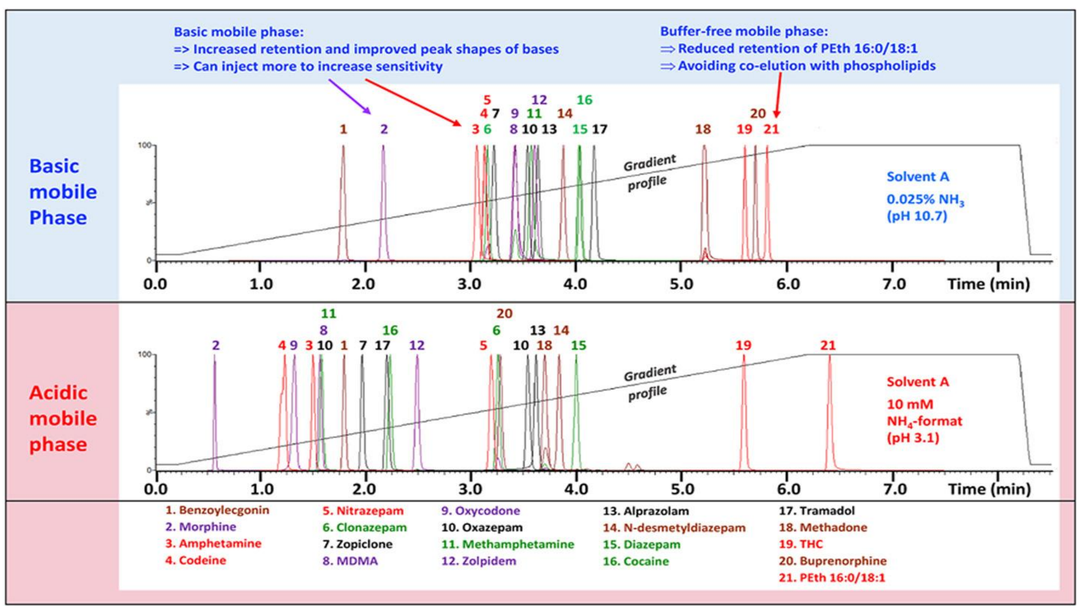

Figure 2: Chromatographic separation of the 21 compounds using a basic and an acidic mobile phase

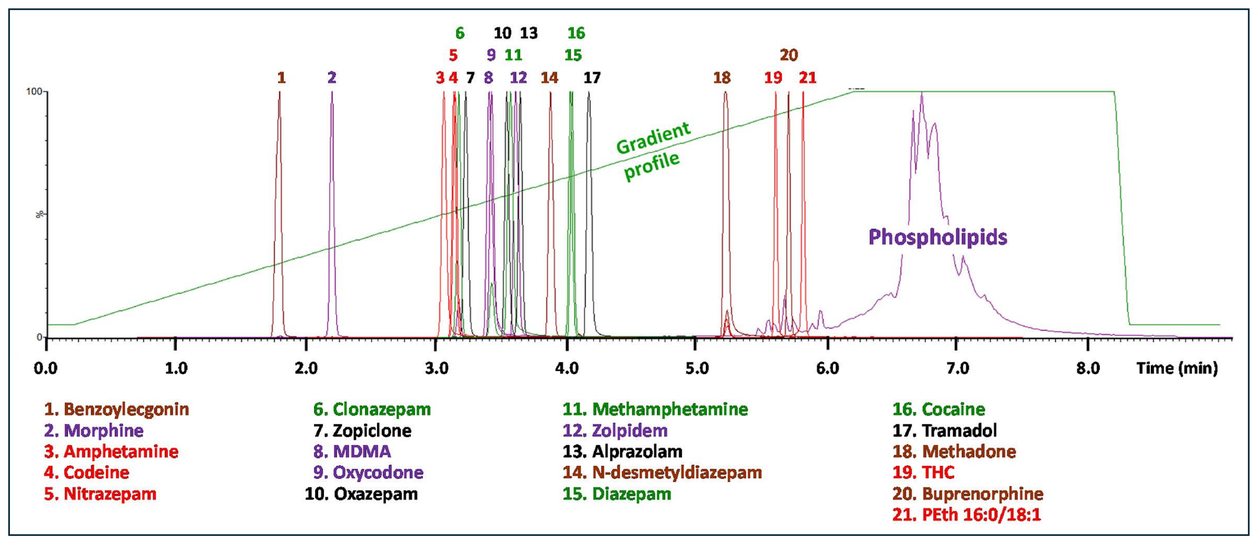

Figure 3: Chromatographic separation of the 21 compounds from the unwanted phospholipid background (purple broad peak, obtained by Parent Ion scan *m/z* of 184). All peaks are normalized to 100 % height. The phospholipid background got a much higher relative response than presented in the figure.

Table 1: Precision and accuracy (n = 8 assays); Recovery and matrix effects corrected by the internal standards

Alprazolam					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
5	4	10	-15		
10	9	3	-12	87	106
20	17	3	-15		
240	224	6	-7	85	107
1200	1249	9	4		

Amphetamine					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
15	14	9	-10		
30	25	4	-18	85	105
60	51	16	-14		
720	690	7	-4	87	109
3600	3777	6	5		

Benzoylecgonin					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
5	5	6	-9		
10	9	10	-8	11	101
20	18	5	-12		
240	232	9	-3	10	101
1200	1240	6	3		

Buprenorphine					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
1	1	6	-11		
2	2	4	-7	54	98
4	3	7	-15		
48	48	10	0	56	100
240	236	10	-2		

Clonazepam					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
2	2	6	-18		
4	3	3	-13	87	101
8	7	2	-18		
96	86	5	-10	85	101
482	470	7	-2		

Codeine					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
5	5	4	4		
10	10	3	-3	91	96
20	18	2	-11		
240	228	5	-5	86	102
1200	1277	8	6		

Cocaine					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
5	5	8	-9		
10	9	6	-8	88	100
20	17	3	-13		
240	224	5	-7	86	101
1200	1097	4	-9		

Diazepam					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
10	9	7	-12		
20	18	3	-10	88	101
40	34	2	-14		
481	450	4	-6	86	102
2407	2513	10	4		

MDMA					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
15	15	7	-3		
30	29	3	-2	85	99
60	54	4	-10		
720	693	4	-4	84	101
3600	3863	9	7		

Methadone					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
15	15	5	2		
30	30	4	0	87	103
60	58	4	-4		
720	676	5	-6	86	104
3600	3055	7	-15		

Methamphetamine					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
15	14	6	-10		
30	26	10	-13	89	111
60	53	16	-12		
720	739	13	3	87	106
3600	4494	12	25		

Morphine					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
5	4	7	-14		
10	9	4	-11	80	99
20	17	5	-14		
240	242	6	1	75	101
1200	1184	9	-1		

N-desmethyldiazepam					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
10	9	6	-12		
20	19	3	-6	92	102
40	35	3	-12		
481	467	5	-3	86	102
2407	2579	8	7		

Nitrazepam					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
5	5	8	-2		
10	10	3	0	88	100
20	19	2	-5		
240	244	5	2	85	100
1200	1313	14	9		

Oxazepam					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
25	26	7	5		
50	53	5	6	93	100
101	101	3	0		
1202	1319	5	10	86	101
6012	6347	9	6		

Oxycodone					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
5	4	10	-13		
10	9	5	-8	88	99
25	17	3	-32		
240	225	5	-25	87	101
1500	1260	8	-16		

PEth 16:0/18:1					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
15	15	20	-1		
30	28	10	-7	79	107
60	51	6	-14		
720	750	7	4	76	105
3600	3912	18	9		

THC					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
1	1	4	3		
2	2	9	-6	67	103
4	3	3	-16		
48	42	6	-12	63	102
240	213	10	-11		

Tramadol					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
15	14	6	-7		
30	28	5	-7	87	105
60	52	3	-13		
720	676	5	-6	87	99
3600	2878	25	-20		

Zolpidem					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
10	9	10	-13		
20	18	3	-9	85	100
40	34	4	-14		
480	446	4	-7	88	101
2400	2526	13	5		

Zopiclone					
Theor. conc. (nM)	Calc. conc. (nM)	CV (RSD (%))	Accuracy (%)	Recovery (%) (n=4)	Corrected Matrix Effect (%) (n=8)
15	14	6	-4		
30	30	6	-2	87	98
60	55	2	-8		
720	711	5	-1	87	99
3600	3632	14	1		

5. Conclusion

- A precise, accurate and sensitive LC-MS/MS method for determination of PEth 16:0/18:1 and 20 drugs/metabolites in whole blood was developed and fully validated.
- A basic mobile phase increased retention and sensitivity for several compounds compared to using acidic mobile phase
- Buffer-free mobile phase used to improve separation of PEth 16:0/18:1 from unwanted phospholipids, reducing matrix effects.

References:

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[2] Jørgenrud B, McQuade T, Maria MH, Nilsson G, Berg T. Buffer-free high pH mobile phase LC-MS/MS for determination of the alcohol biomarker phosphatidylethanol 16:0/18:1 and 20 drugs and metabolites in whole blood. Talanta 2025;282:126964. https://doi.org/10.1016/j.talanta.2024.126964.

More information in the published paper

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ACKNOWLEDGMENTS

Centro de Química Estrutural is a Research Unit funded by Fundação para a Ciência e a Tecnologia through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by Fundação para a Ciência e a Tecnologia through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020). Marisa Henriques Maria is grateful to acknowledge her PhD grant no. 2022.10965.BDANA awarded by Fundação para a Ciência e a Tecnologia. CiiEM has provided support through Project 10.54499/UIDB/04585/2020, funded by FCT.