Lipidomic analysis of phosphatidylcholine species in a cerebrospinal fluid sample

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I. BACKGROUND

Lipidomics is a sub-discipline of metabolomics which is of high interest for clinical applications. Lipids are essential for biological systems and more data links them to specific roles in various diseases. In this experminet 4 phosphatidylcholine (PC) and 1 lysophosphatidylcholine (LPC) species are analysed quantitatively in cerebrospinal fluid samples (CSF) using HPLC-MS (ESI-Q-ToF) via external calibration with PC C17:0 C17:0 as an internal standard (*Analytical Chemistry* 2019).

II. METHODS AND MATERIALS

A. MATERIALS

1) Tool:

- Agilent HPLC system .
- Column C18 with guad column
- MS (Q-ToF)

2) Chemicals:

- methanol (provided by the institue)
- milliQ water (provided by the institute)
- 2-propanol (provided by the institue)
- acetic acid (provided by the institue)
- NH_4 acetate (provided by the institue)
- acetonitrile (provided by the institue)
- lipid stock solutions (provided by the institute)

B. METHODS

CSF proteins are removed by denaturation with methanol (1:10, final volume 1ml), vortexing (few times within 10 mins) and subsequent centrifugation (4000 rpm 5 min). The liquid phase is then transferred into a HPLC vial. The calibration solutions are prepared at various concentrations (see. Tab IV/c[ppm]) from a 50 ppm stock solution at a final volume of 1ml (diluted in methanol) (see. Tab 1.I).

c [ppb]	ratio	V_{total} sample	$V_{individual}$ sample
2000	$\frac{1}{25}$	200	40
1500	$\frac{1}{33}$	150	30
1000	$\frac{1}{50}$	100	20
500	$\frac{1}{100}$	60	12
20	$\frac{1}{25}$	200	

TABLE I: Generation of the calibration solutions. Every standard is filled up to 1ml with methanol, except for c_{500}. This solution is filled up to 1,2ml, where the additional 200ml serve for the dilution to 20 ppb.

HPLC-MS analysis is conducted on an Agilent HPLC system coupled with a quadrupole timeof-flight mass spectrometer (Q-ToF) coupled with positive mode ESI. The column is flushed for at least 20 mins with eluent mix time = 0 (Tab. II).The column temperature is 50 °C with an injection volume of 5 μL. The flow rate is set to 0,3 $\frac{mL}{min}$ and the post time is 15 min (*Analytical Chemistry* 2019).

time (min)	Eluent A (%)	Eluent B (%)
0	40	60
8	40	60
25	3	97
40	3	97

TABLE II:
Eluent A: (250 ml 60% water (150 ml) + 40% acetonitrile (100ml) + 10 mM NH4 acetate (≈ 193mg)+ 1 mM acetic acid (≈15mg).
Eluent B: (250 ml) 90% 2-propanol (225 ml) + 10% acetonitrile (25ml) + 10 mM NH4 acetate (≈ 193mg) + 1 mM acetic acid (≈ 15mg)

substances	formula	mass
LPC C17:0	C25H52NO7P	510,3554
PC C16:0 C16:0	C40H80NO8P	734,5694
PC C17:0 C17:0	C42H84NO8P	762,6007
PC C18:1 C18:1	C44H84NO8P	786,6007
PC C16:0 C18:1	C42H82NO8P	760,5851

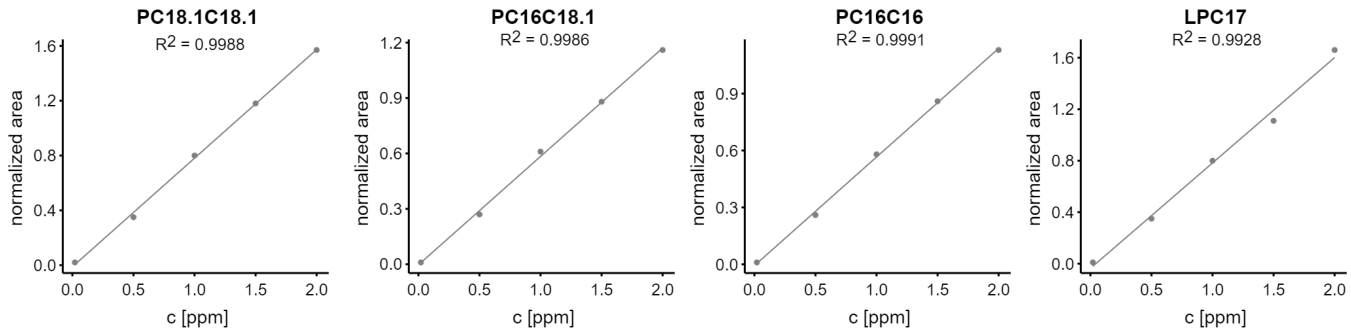
TABLE III: The Isotope distribution calculator created by Manura was used to calculate the exact protonated masses based on the chemical fomula (J. Manura and D. Manura 2009).

The data processing was performed via R studio (V 1.2.1335) and visualized via graphrobot (R Core Team 2019, Wang 2019).

III. RESULTS

External standards are plotted on Fig. 1a 1b 1c 1d from the data in Tab. IV. The sample A contains all standards except for LPC C17:0, identified via their retention times (RT). LPC C17:0 (RT standard = 3,6588 ± 0,0089, RT sample = noise), PC C16:0 C16:0 (RT standard = 18,2588 ± 0,023 , RT sample = 18.229), PC C16:0 C18:1 (RT standard = 18.619, RT sample = 18.626), PC C17:0 C17:0 [internal standard] (RT standard = 20,5648 ± 0,014, RT sample = 20.545) and PC C18:1 C18:1 (RT standard = 19,784 ± 0,029 , RT sample = 20.545). According to the RT, the PC C18:1 C18:1 analyte is most likely a C-13 isotop of the Calculation of the concentration of the externally calibrated substance was performed via the respective regression equations. They follow a general linear context, with m = slope and t = intercept (Eq. 1). For the following calculations x = analyte concentration and y = peak area.

$$y = mx + t \tag{1}$$



(a) Slope 0.7925 Intercept -0.0116 C18-1 C18-1 external calibration

(b) Slope 0.5867 Intercept -0.0031 C16-0 C18-1 external calibration

(c) Slope 0.5726 Intercept -0.0069 C16-0 C16-0 external calibration

(d) Slope 0.8188 Intercept -0.0361 C17-0 external calibration

Fig. 1: External calibration curves generated via data from table IV

c [ppm]	integrated area	normalized area	c [ppm]	integrated area	normalized area
LPC17			PC16C16		
0.02	28349.02	0.01	0.02	25236.62	0.01
0.5	756602.78	0.35	0.5	564253.22	0.26
1.00	1743206.28	0.80	1.00	1267553.25	0.58
1.50	2714011.80	1.11	1.50	2082139.36	0.86
2.00	3906760.34	1.66	2.00	2675431.33	1.13
PC16C18.1			PC17C17		
0.02	27082.90	0.01	0.02	2148231.53	1.00
0.5	575372.05	0.27	0.5	2136474.07	1.00
1.00	1327240.06	0.61	1.00	2174692.03	1.00
1.50	2148995.64	0.88	1.50	2434508.91	1.00
2.00	2729674.42	1.16	2.00	2357667.70	1.00
PC18.1C18.1			sample A		
0.02	39106.12	0.02	LPC17	noise	noise
0.5	752876.52	0.35	PC16C16	1031055.67	0.54
1.00	1736993.78	0.80	PC16C18.1	991288.20	0.52
1.50	2864358.94	1.18	PC17C17	1902969.28	1.00
2.00	3696817.27	1.57	PC18.1C18.1	52979.59	0.03

TABLE IV: In order to establish a stable calibration curve, the normalized peak area calculated via $\frac{sample/standard}{internal\ \ standard}$

The slopes and intercepts of the regression lines can be found in the respective captions (Fig. 1a,1b,1c,1d).
The internal standard (PC17C17) integrated peak area is the divisor for the integrated areas of the sample A and the external standards. The calculated results are displayed in Table IV as normalized area. The sample data from Table IV is used to determine the unknown concentration of the sample via Equation 1 and consequently displayed in Table V.PC16C16 has a concentration of 9.58 ppm while PC16C18.1 has 8.93 ppm.

substance	normalized area	$c_{diluted}$ [ppm]	c [ppm]
PC16C16	0.54	0.96	9.58
PC16C18.1	0.52	0.89	8.93

TABLE V: Concentration of the substances present in sample A, via equation 1. The concentration of the diluted sample has to be multiplied with the dilution factor (10)

IV. DISCUSSION

In sample A, two of the four analytes are present with PC18.1C18.1 and LPC17:0 missing. The usage of an internal standard provided correction for possible matrix or drag-out effects. The method of choice was suitable for determination of the analyte compounds at the present concentrations. For more robust results, the samples could have been prepared in higher quantities (n = 3-8).

A. Experimental environment

The experiment was performed at TNF Turm JKU, Linz, Austria on 26.06.19 under the supervision of Bernd Reichl.

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