

# Phosphatidylcholine Profile Evaluation in Human Plasma at 8 seconds per Sample using the LDTD-MS/MS technique

Serge Auger, Jean Lacoursière and Pierre Picard  
Phytronix Technologies, Québec, CANADA;



## OVERVIEW

**Purpose**

- Optimization of an extraction process of Phosphatidylcholine profile evaluation in human plasma using LDTD-MS/MS.

**Method**

- Automated liquid-liquid extraction
- LazWell spotting
- Samples dried and analyzed by LDTD-MS/MS

**Analysis**

- Precision evaluation show %CV lower then 15%.
- Cross validation: Passing-Bablok regression shows a linearity of 0.9975 and all samples are within the confidence interval of 95% in the Bland and Altman plot.
- Samples analyzed with a runtime of 8 seconds using LDTD-MS/MS system**

## INTRODUCTION

Phosphatidylcholines (PCs) are the major constituents of cell membranes. There concentration may variate and can be useful as biomarkers for several diseases. This glycerophospholipid group contains a polar phosphocholine head connected to fatty acid sidechains via a glycerol backbone. Varying lengths and saturation of fatty acids may be observed. The fatty acids are connected via two types of chemical groups on a glycerol backbone: ester (acryl: a) or ether (e).

For this project, a generic extraction method is developed. Different phosphatidylcholine (PCaa, PCae and LPCa) in plasma are extracted. Quantification using Laser Diode Thermal Desorption and tandem mass spectrometry (LDTD-MS/MS) is chosen as a fast-analytical technique.

**LUXON Ionization Source:**

The Luxon Ion Source (Figure 1) is the second-generation sample introduction and ionization source based on the LDTD technology for mass spectrometry. The Luxon Ion Source uses a Fiber-Coupled Laser Diode (Figure 2) to obtain unmatched thermal uniformity giving more precision, accuracy and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High-efficiency protonation and strong resistance to ionic suppression characterize this type of ionization and is the result of the absence of solvent and mobile phase. This thermal desorption process yields high-intensity molecular ion signal in less than 1 second sample-to-sample and allows working with very small volumes.



Figure 1 Luxon Ion Source

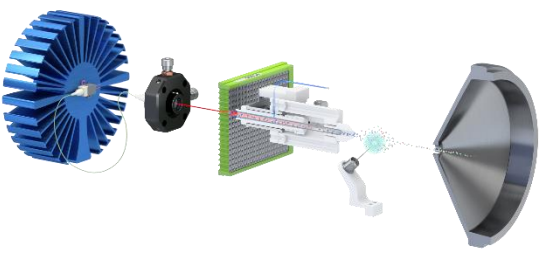


Figure 2 Schematic of the Luxon Ion Source

## METHOD

**Automated LLE extraction**

10 µL of plasma samples were extracted using the automated LLE extraction method. The robot scans the barcode of the sample and generates a batch file for the LDTD-MS/MS system. 9 µL of internal standard, 90 µL of methanol, 75 µL of water and 300 µL of MTBE are added in a deep-well extraction plate. Samples are vortexed and 5 µL of the upper layer phase are spotted in a coated LazWell plate. After evaporation, 5 µL of desorption solution (10 µg/mL BSA and 1 mM KH<sub>2</sub>PO<sub>4</sub> dilute in ACN:Water (80:20)) are added in the LazWell plate and dried before analysis by LDTD-MS/MS.



Figure 3 Azeo: Automated extraction system

**MRM transition:**

Positive ion mode was utilized for the quantification of phospholipids. A common loss associated to the polar phosphocholine head is used to generate the MRM method.

Ex: PC<sub>aa</sub> C40:0

- Q1: 846.7
- Commun loss of 183.1 amu
- Q3: 663.6

Final transition: 846.7 -> 663.6

**Instrumentation**

- Ion source: Phytronix Luxon S-960 Ion Source
- Mass spectrometer: Sciex, Q-Trap System 5500

**Luxon Parameters**

- Laser power pattern:
  - Increase laser power to 100% in 3 sec
  - Hold 3 seconds
  - Decrease laser power to 0%
- Carrier gas flow: 6 L/min (Air)

**Nomenclature:**

The following nomenclature will be used:

Ex: PC<sub>ae</sub> C32:1

- PC: Phosphatidylcholine head
- ae: Fatty acid chemical bonds: acryl (a) or ether (e)
- C32: Sum of Carbons on both fatty acid chains
- 1: Number of double bonds

**MS Parameters**

- APCI (+)
- Curtain: 20
- CE: 30
- Time: 20 msec
- MRM mode

Table 1 MRM transitions for some of the phosphatidylcholine

Compound	Q1 (Da)	Q3 (Da)
PCaa C40:0	846.7	663.6
PCaa C40:2	842.7	659.6
PCaa C40:4	838.6	655.5
PCaa C40:6	834.6	651.5
PCae C40:0	832.7	649.6
LPCa C20:0	552.4	369.3

## RESULTS

**Phospholipid analysis**

This method evaluates the phospholipid profile based on the sum of carbon, hydrogen, and oxygen atoms on the fatty acid sidechain. Also, the peak area corresponds to the sum of the potential isobaric phosphatidylcholine according to the following rules show in **Table 2**.

**Precision**

**Intra-run**

For the intra-run precision experiments, five different samples are analyzed in six replicates. **Table 3**, **Table 4** and **Table 5** show the intra-run results for PC<sub>aa</sub> C34:1, PC<sub>aa</sub> C34:2 and PC<sub>aa</sub> C36:2, respectively. No sample exceeds 15% CV. Similar results are obtained for the phospholipid transitions.

Table 3 Intra-run precision for PCaa C34:1

PC <sub>aa</sub> C34:1	M 1	M 2	M 3	M 4	M 5
Calc. conc (µM)	251.8	187.5	137.1	128.3	131.5
N	6	6	6	6	6
%CV	6.9	7.6	7.1	7.8	11.3

**Inter-run**

To determine the inter-run precision, five different samples are analyzed in three runs. **Table 6**, **Table 7** and **Table 8** show the inter-run results for PC<sub>aa</sub> C34:1, PC<sub>aa</sub> C34:2 and PC<sub>aa</sub> C36:2, respectively. No sample exceeds 15% CV. Similar results are obtained for the phospholipid transitions.

Table 6 Inter-run precision for PCaa C34:1

PC <sub>aa</sub> C34:1	M 1	M 2	M 3	M 4	M 5
Calc. conc (µM)	239.4	179.9	137.4	128.1	132.6
N	18	18	18	18	18
%CV	13.4	13.5	11.6	10.6	8.1

Table 4 Intra-run precision for PCaa C34:2

PC <sub>aa</sub> C34:2	M 1	M 2	M 3	M 4	M 5
Calc. conc (µM)	332.2	228.6	212.3	202.1	218.8
N	6	6	6	6	6
%CV	6.3	7.6	6.2	6.5	8.4

Table 7 Inter-run precision for PCaa C34:2

PC <sub>aa</sub> C34:2	M 1	M 2	M 3	M 4	M 5
Calc. conc (µM)	313.8	218.5	215.2	199.8	209.3
N	18	18	18	18	18
%CV	13.2	13.7	11.2	9.5	9.1

Table 5 Intra-run precision for PCaa C34:2

PC <sub>aa</sub> C36:2	M 1	M 2	M 3	M 4	M 5
Calc. conc (µM)	189.5	139.1	123.1	117.6	123.5
N	6	6	6	6	6
%CV	7.1	5.1	10.6	7.0	7.6

Table 8 Inter-run precision for PCaa C34:2

PC <sub>aa</sub> C36:2	M 1	M 2	M 3	M 4	M 5
Calc. conc (µM)	176.0	129.8	121.6	113.7	123.5
N	18	18	18	18	18
%CV	12.2	14.1	13.8	9.5	7.6

**Method comparison evaluation**

Plasma samples from healthy patients (N=24) have been tested with this method to correlate with results obtained with the assay reference kit Absolute*IDQ™* (N=220) reported by Quell et al<sup>1</sup> using flow injection MS quantification. The median concentration values were determined for 37 different phosphatidylcholines and used for the statistical evaluation. **Table 9** shows the phosphatidylcholines used for the method comparison.

Table 9 Phosphatidylcholines used for method comparison

PC <sub>aa</sub> C30:0	PC <sub>aa</sub> C34:2	PC <sub>aa</sub> C36:0	PC <sub>aa</sub> C37:3	PC <sub>aa</sub> C38:5	PC <sub>aa</sub> C40:6
PC <sub>aa</sub> C32:0	PC <sub>aa</sub> C34:3	PC <sub>aa</sub> C36:1	PC <sub>aa</sub> C37:4	PC <sub>aa</sub> C39:1	PC <sub>aa</sub> C42:6
PC <sub>aa</sub> C32:1	PC <sub>aa</sub> C34:4	PC <sub>aa</sub> C36:2	PC <sub>aa</sub> C37:5	PC <sub>aa</sub> C39:5	
PC <sub>aa</sub> C32:2	PC <sub>aa</sub> C35:1	PC <sub>aa</sub> C36:3	PC <sub>aa</sub> C37:6	PC <sub>aa</sub> C39:6	
PC <sub>aa</sub> C33:1	PC <sub>aa</sub> C35:2	PC <sub>aa</sub> C36:4	PC <sub>aa</sub> C38:0	PC <sub>aa</sub> C40:3	
PC <sub>aa</sub> C33:2	PC <sub>aa</sub> C35:3	PC <sub>aa</sub> C36:5	PC <sub>aa</sub> C38:3	PC <sub>aa</sub> C40:4	
PC <sub>aa</sub> C34:1	PC <sub>aa</sub> C35:4	PC <sub>aa</sub> C36:6	PC <sub>aa</sub> C38:4	PC <sub>aa</sub> C40:5	

1) Quell et al., Metabolites 2019, 9,109

## CONCLUSION

- Efficient Liquid-Liquid Extraction is used to extract phosphatidylcholine.
- High-throughput profile evaluation using LDTD-MS/MS
- Precision lower than 15 %CV and method comparison evaluation within the acceptance criteria.
- Sample-to-sample analysis of **8 seconds**

The Passing-Bablok regression (**Figure 3**) reveals a correlation (0.9975) and no significant deviation from linearity. The Bland and Altman plot (**Figure 4**) shows the mean value bias of the two methods. All samples are within the confidence interval of 95%.

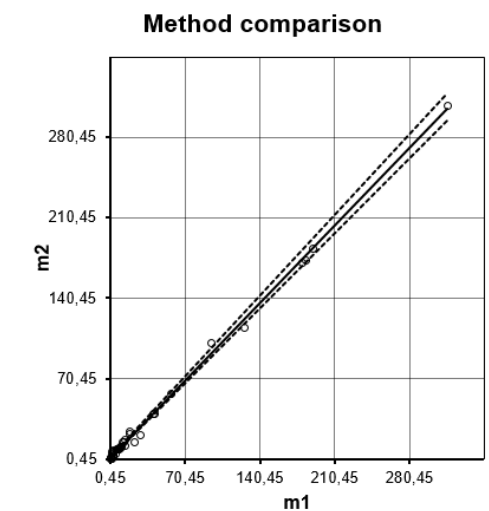


Figure 3 Passing-Bablok regression curve

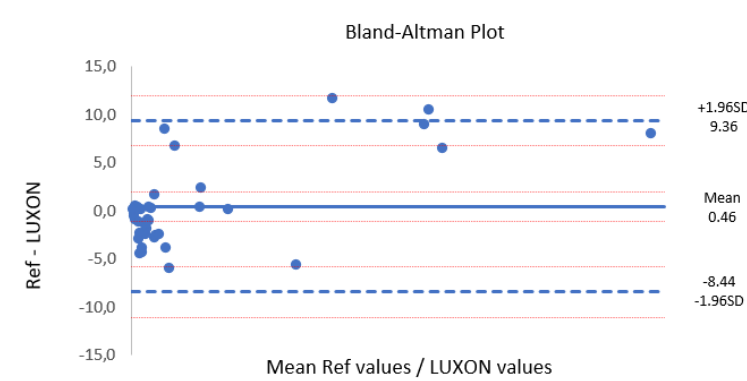


Figure 4 Bland and Altman plot