

Phosphatidylcholine Profile Evaluation in Human Plasma at 8 Seconds per Sample Using LDTD-MS/MS

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Introduction

Phosphatidylcholines (PCs) are the major constituents of cell membranes. They are used 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 bounds on a glycerol backbone: ester (acryl: a) or ether (e). The following nomenclature will be used:

Ex: PC_{ae} 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.

Our goal for this application note is to present a generic method that allows the quantitation of various phosphatidylcholine (PC_{aa}, PC_{ae} and LPC_a) in plasma in less than 8 seconds per sample. The automated sample preparation is reduced to a minimum to keep up with the analysis throughput.

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. Luxon Ion Source® uses 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 it 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 ionization source

Sample Preparation Method (Automated)

An automated system (Figure 3) is used to extract the samples using the following conditions:

- 10 µL of plasma sample
- 10 µL of internal standard at 12 µM in IPA:ACN:Water (75:20:5)
- 150 µL of Methanol
 - Vortex
- 500 µL of MTBE

- Vortex
- 150 µL of Water
 - Vortex and phase separation by gravity
- Spot 5 µL of the upper-layer phase on a coated LazWell™96 plate
 - Dry 2 minutes at room temperature with air flow
- Add 5 µL of the desorption solution
 - Evaporate to dryness



Figure 3 - Automated extraction system

Luxon-MS/MS Parameters

Luxon

Model: Luxon S-960, Phytronix

Carrier gas: 6 L/min (air)

Laser pattern:

- 3-second ramp to 100% power
- 3-second hold at 100% power

MS/MS

MS model: Q-Trap System® 5500, Sciex

Scan Time: 5 msec

CE: 30

Total run time: 8 seconds per sample

Ionization: APCI (positive)

Analysis Method: MRM mode

A common loss of 184 amu associated to the polar phosphocholine head is used to generate the MRM method. Some examples of the MRM transitions used can be found in Table 1.

Table 1 - MRM transitions for some of the phosphatidylcholine

Phosphatidylcholine	Q1	Q3
PC _{aa} C40:0	846.7	663.6
PC _{aa} C40:2	842.7	659.6
PC _{aa} C40:4	838.6	655.6
PC _{aa} C40:6	834.6	651.5
PC _{ae} C40:0	832.7	649.6
LPC _a C20:0	552.4	369.3

Results and Discussion

Phospholipids 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:

Table 2 - Potentially isobaric phosphatidylcholine

PC	Isobaric PC	Example	Empirical formula change
PC _{aa} C _{x:y}		PC _{aa} C20:0	
	PC _{aa} C _{x+1:y+7}	PC _{aa} C21:7	+ CH ₂ , -14H
	PC _{ao} C _{x+1:y}	PC _{ao} C21:0	+ CH ₂ , +2H, -O
	PC _{ao} C _{x+2:y+7}	PC _{ao} C22:7	+2 (CH ₂), -12H, -O
	LPC _a C _{x+1:y}	LPC _a C21:0	+ CH ₂ , +2H, -O

Precision

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_{aa} C34:1, PC_{aa} C34:2 and PC_{aa} 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 _{aa} 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

Table 4 - Intra-run precision for PCaa C34:2

PC _{aa} 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 5 - Intra-run precision for PCaa C36:2

PC _{aa} 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

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_{aa} C34:1, PC_{aa} C34:2 and PC_{aa} 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 _{aa} 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 7 - Inter-run precision for PCaa C34:2

PC _{aa} 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 8 - Inter-run precision for PCaa C36:2

PC _{aa} 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/DQ™ (N=220). 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 _{aa} C30:0	PC _{aa} C34:2	PC _{aa} C36:0	PC _{aa} C37:3	PC _{aa} C38:5	PC _{aa} C40:6
PC _{aa} C32:0	PC _{aa} C34:3	PC _{aa} C36:1	PC _{aa} C37:4	PC _{aa} C39:1	PC _{aa} C42:6
PC _{aa} C32:1	PC _{aa} C34:4	PC _{aa} C36:2	PC _{aa} C37:5	PC _{aa} C39:5	
PC _{aa} C32:2	PC _{aa} C35:1	PC _{aa} C36:3	PC _{aa} C37:6	PC _{aa} C39:6	
PC _{aa} C33:1	PC _{aa} C35:2	PC _{aa} C36:4	PC _{aa} C38:0	PC _{aa} C40:3	
PC _{aa} C33:2	PC _{aa} C35:3	PC _{aa} C36:5	PC _{aa} C38:3	PC _{aa} C40:4	
PC _{aa} C34:1	PC _{aa} C35:4	PC _{aa} C36:6	PC _{aa} C38:4	PC _{aa} C40:5	

The Passing-Bablok regression (Figure 4) reveals a correlation and no significant deviation from linearity. The Bland and Altman plot (Figure 5) shows the mean value bias of the two methods. All samples are within the confidence interval of 95%.

Method comparison

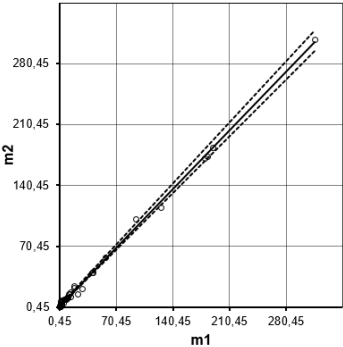


Figure 4 - Passing-Bablok regression curve

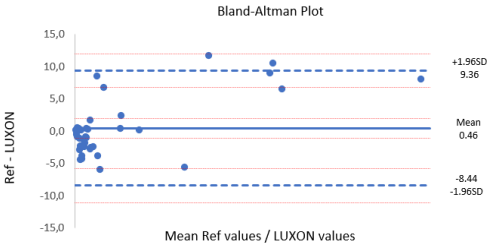


Figure 5 - Bland and Altman plot

Conclusion

A system combining Luxon Ion Source® with a Sciex 5500 Q-Trap mass spectrometer allows the ultra-fast (8 seconds per sample) phosphatidylcholine profiling in plasma samples using a simple and automated sample preparation method.