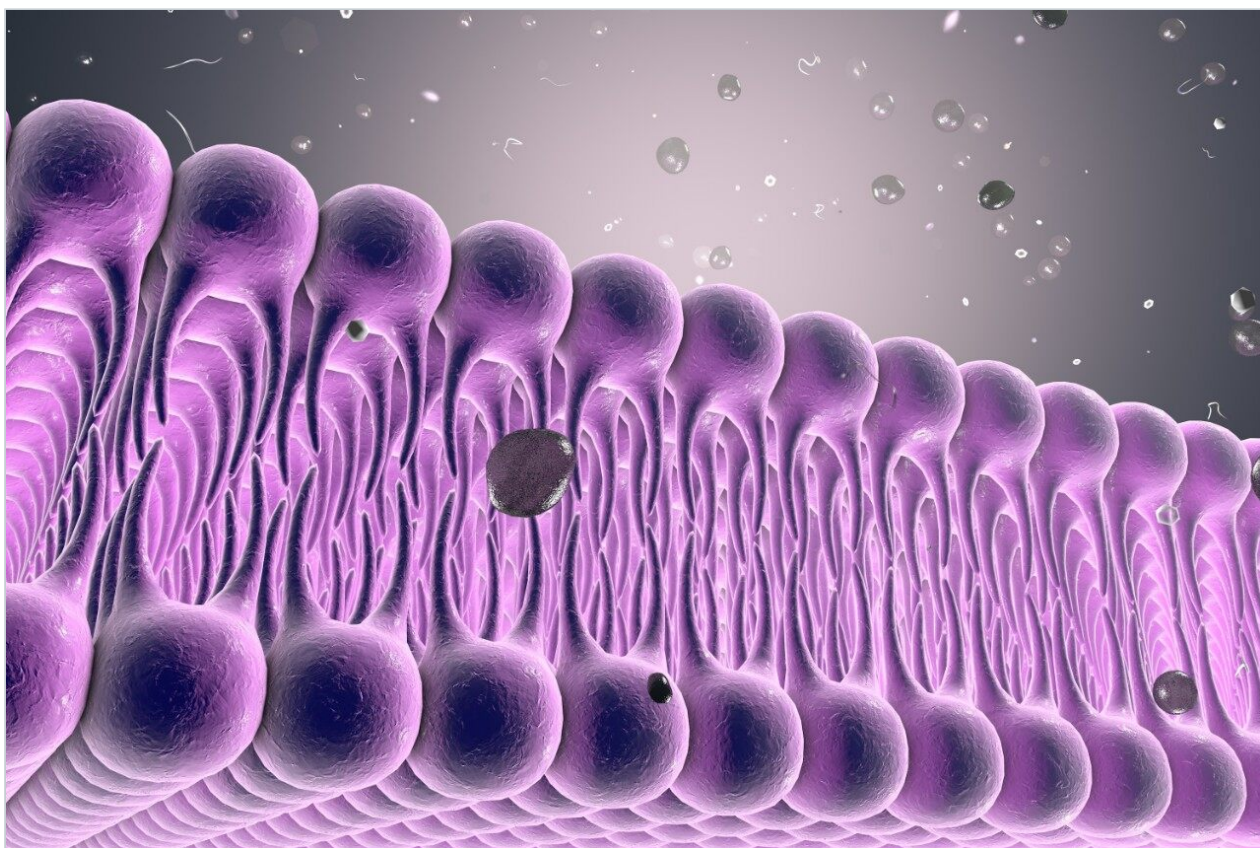


## A Rapid Approach to Phospholipid Profiling using UPLC-MS<sup>E</sup>

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### Abstract

This application note shows the application of MS<sup>E</sup> combined with UltraPerformance LC in determining the correct identification of a phospholipid, extracted from protein precipitated rat plasma.

## Introduction

Traditional methods for determining correct structural information often involve techniques such as directed MS/MS, NMR, or comparison to an authentic standard. All of these techniques involve additional analysis time, specific instrumentation, and in the case of authentic standards, compound availability.

MS<sup>E</sup> is a technique whereby both precursor and fragment mass spectra are simultaneously acquired by alternating between high and low collision energy during a single chromatographic run.<sup>1</sup> Here we show the application of MS<sup>E</sup> combined with UltraPerformance LC in determining the correct identification of a phospholipid, extracted from protein precipitated rat plasma. A database search, based on the monoisotopic mass, resulted in two hits. Without further discriminating information, the correct chemical structure assignment would be ambiguous. However, by using the additional information provided by the UPLC-MS<sup>E</sup> technique, the diagnostic fragments from the phospholipid head group can be used to provide a unique assignment.

Possible Glycerophospholipid Structures

Exact Mass:  Mass Tolerance:   [New Search](#)

C=Number of Carbons; DB=Number of double bonds; sn1('1),sn2...=MS/MS Product Ions (neutral loss)

Mass	C	DB	Abbrev.	M-sn1+H	M-sn1-H2O+H	M-sn2+H	M-sn2-H2O+H	sn1 acid(-)	sn2 acid(-)	HG	Formula
496.3403	16	0	<a href="#">16:0/0:0</a>	258.1106	240.1	482.361	464.3504	255.2324	30.982	GPCho	C <sub>24</sub> H <sub>50</sub> NO <sub>7</sub> P
496.3403	19	0	<a href="#">19:0/0:0</a>	216.0637	198.0531	482.361	464.3504	297.2794	30.982	GPEtn	C <sub>24</sub> H <sub>50</sub> NO <sub>7</sub> P

Figure 1. Database results showing the existence of two phospholipids with the same monoisotopic mass.

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## Experimental

### Sample Preparation

Standards were obtained from Avanti Polar Lipids, Inc. (AL, USA). Rat plasma was obtained from Equitech-Bio Inc. (TX, USA) and precipitated with Acetonitrile, 2:1.

### LC Conditions

LC system:	Waters ACQUITY UPLC System
Mobile phase A:	20 mM Ammonium Acetate, pH 5.0
Mobile phase B:	Acetonitrile/Acetone (9:1)
Gradient:	35-95% B/10 min
Flow rate:	600 $\mu$ L/min
Column temp:	60.0 $^{\circ}$ C
Column:	ACQUITY UPLC BEH C <sub>8</sub> , 2.1 x 100 mm, 1.7 $\mu$ m

### MS Conditions

MS system:	Waters Q-ToF Premier Mass Spectrometer
Ionization mode:	ESI Positive
Capillary voltage:	3200 V
Cone voltage:	35 V
Desolvation temp:	400 $^{\circ}$ C

Desolvation gas:	800 L/Hr
Source temp:	120 °C
Acquisition range:	100-1000 <i>m/z</i>
Collision energies:	High 25 V, Low 5 V

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## Results and Discussion

Figure 2 shows the chemical structure of 1-oleoyl-2- hydroxy-sn-glycero-3-phosphocholine with known losses (2a) and the corresponding MS<sup>E</sup> spectra (2b).

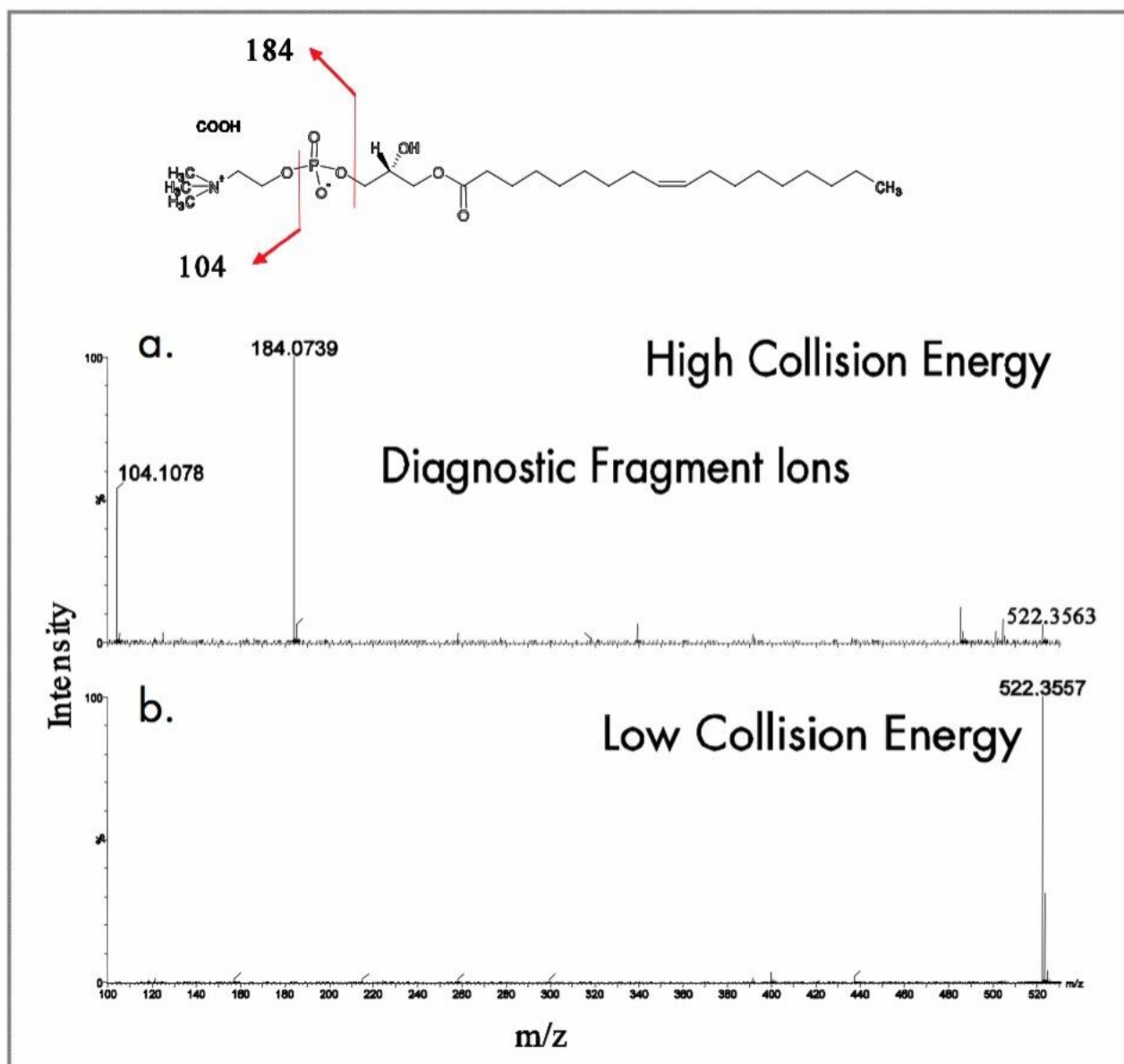


Figure 2. Chemical structure with known losses (a) and mass spectra of 1-oleoyl-2-hydroxy-sn-glycero-3-phosphocholine during the  $MS^E$  experiment (b).

Inspection of the  $MS^E$  spectra for this authentic standard shows the utility of acquiring both precursor and fragment mass spectra during the same chromatographic run while maintaining good mass accuracy. This method developed utilizing  $MS^E$  was then applied to investigate the phospholipid profiles of protein precipitated rat plasma. Lysophospholipids (LPL) and phospholipids (PL) are known to exist in the supernatant of protein precipitated plasma.<sup>2</sup> Figure 3 shows a representative separation of LPL and PL in the protein precipitated rat plasma sample (3a) and the  $MS^E$  spectra from a peak eluting with a retention time of 3.9 minutes and a precursor mass of 496.3397 (3b). High collision energy scans show major fragments

of 104.1078 and 184.0739  $m/z$  arising from the phospholipid head group. A database search of the monoisotopic precursor mass was done using the LIPID MAPS website and produced two possible matches within an error of 1.3 ppm: 2-lyso glycerophosphoethanolamine (2-lyso GPEtn) and 2-lyso glycerophosphocholine (2-lyso GPCho). These two matches produce different diagnostic fragments associated with their headgroup. 2-lyso GPCho is expected to produce ions of 184 and 104  $m/z$  while 2-lyso GPEtn is expected to produce ions of 141  $m/z$ .<sup>2,3</sup> The high collision spectra in Figure 3b shows the diagnostic fragments identifying 2-lyso GPCho as the correct structural match from the database.

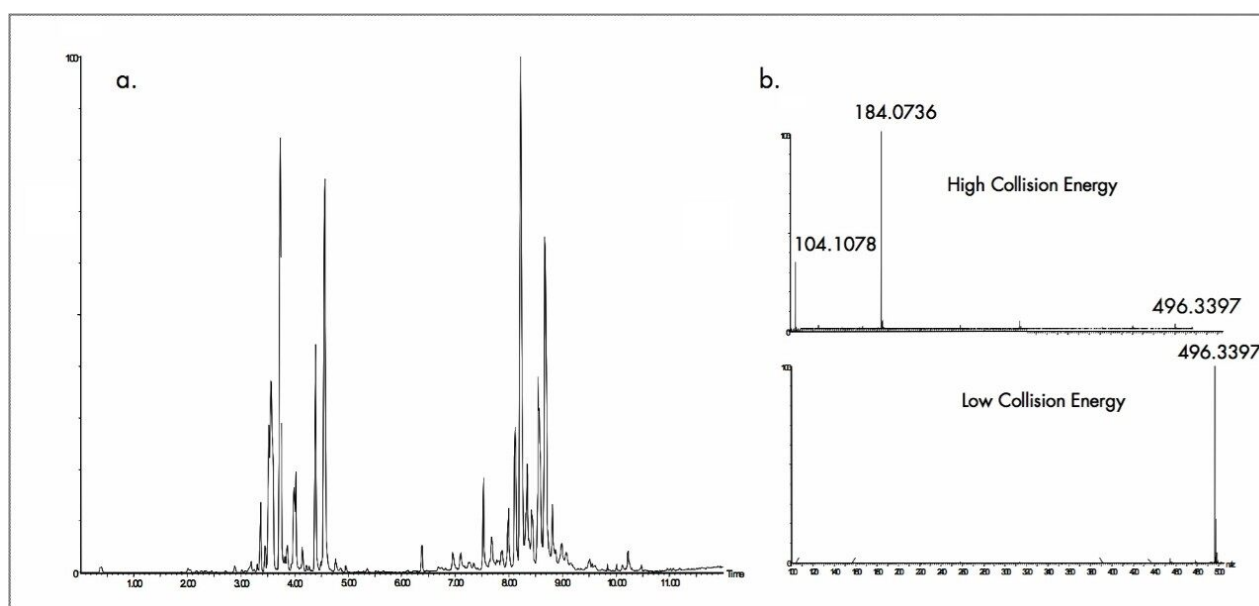


Figure 3. ESI positive UPLC-MS<sup>E</sup> separation of protein precipitated rat plasma (a) and MS<sup>E</sup> spectra of peak eluting at 3.9 minutes (b).

## Conclusion

The use of the MS<sup>E</sup> technique employed on the Q-ToF Premier Mass Spectrometer illustrates that accurate precursor and fragment mass information can be acquired in a single UPLC-MS analysis. A database search of a selected precursor ion acquired under low collision energy returned two monoisotopic isomer matches. An ambiguous database result would normally lead to targeted MS/MS experiments or analysis of authentic standards, depending on their availability. However, diagnostic fragment ions obtained from high collision energy scans during the MS<sup>E</sup> experiment provided unique structural fragment information needed to

definitively confirm the correct structural identity from the database without additional UPLC-MS analysis. We have further shown the application of this technique for the profiling of phospholipids in protein precipitated rat plasma.

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## References

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