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.

MS² 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. Here we show the application of MS² 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/MSE technique, the diagnostic fragments from the phospholipid head group can be used to provide a unique assignment.

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 µL/min
Column Temp: 60.0 °C
Column: ACQUITY UPLC BEH C₈
2.1 x 100 mm, 1.7 µm

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 °C
Desolvation Gas: 800 L/Hr
Source Temp: 120 °C
Acquisition Range: 100-1000 m/z
Collision Energies: High 25 V, Low 5 V

Possible Glycerophospholipid Structures

Figure 1. Database results showing the existence of two phospholipids with the same monoisotopic mass.
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 MS² spectra (2b).

Inspection of the MS² 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 MS² 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. Figure 3 shows a representative separation of LPL and PL in the protein precipitated rat plasma sample (3a) and the MS² 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. 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/MS² separation of protein precipitated rat plasma (a) and MS² spectra of peak eluting at 3.9 minutes (b).
CONCLUSION

The use of the MS² 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 MS² 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.

REFERENCES