Application Note 02340

Rapid Characterization of Phospholipids on the Varian 320-MS Triple Quadrupole LC/MS/MS with ACD Labs/MS Manager Software

Joseph Stork
Varian, Inc.

Introduction

Phospholipids are a class of lipids (fat-soluble, naturally-occurring molecules) that are a major component of all biological membranes. All phospholipids contain a diglyceride, a phosphate group, and a simple organic molecule such as choline. They form a lipid bilayer within a cell membrane. This short application note demonstrates how phospholipids can be identified in a mixture by triple quadrupole mass spectrometry and suspected structures confirmed by generating MS/MS spectra and submitting it to the ACD Labs/MS Manager software.

Instrumentation

- Varian 320-MS triple quadrupole mass spectrometer equipped with an electrospray source
- Built-in syringe infusion pump

Materials and Reagents

Methanol and water (Optima LC/MS grade) (part numbers A456-4 and W6-4, respectively) were purchased from Fisher Scientific (Pittsburgh, PA).

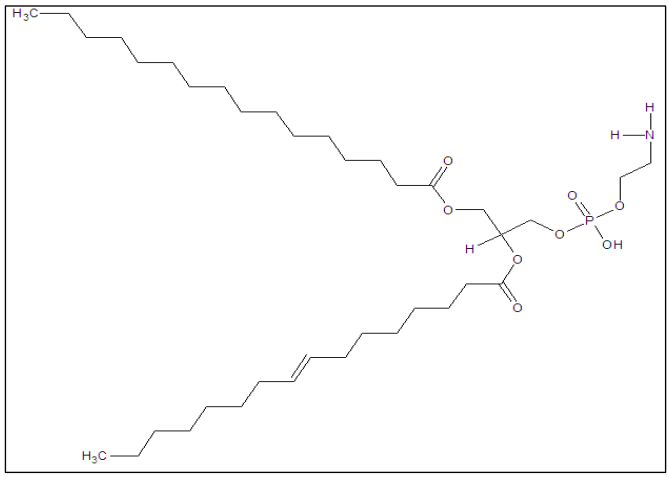

Figure 1. Chemical structure of DPPE.

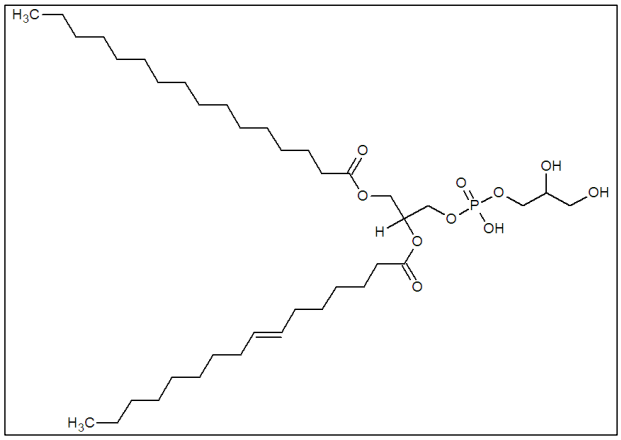

Figure 2. Chemical structure of POPG.

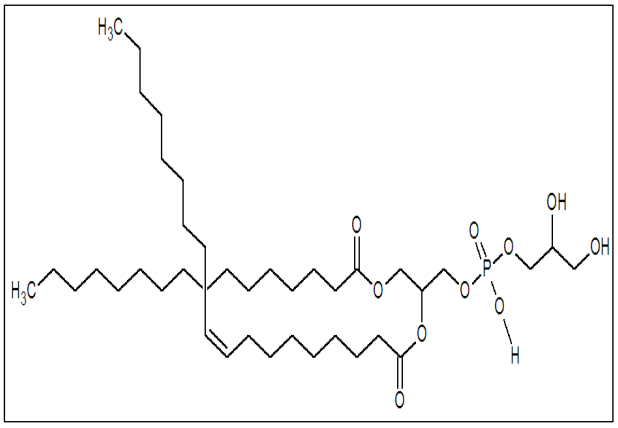

Figure 3. Chemical structure of DPPG.

Sample Preparation

Samples were provided as pure standards of: 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) (Figure 1), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (sodium salt) (POPG) (Figure 2), and 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (sodium salt) (DPPG) (Figure 3). They were prepared by suspending at a concentration of 1 ppm in 50:50 methanol:water for direct infusion into the mass spectrometer.

MS/MS Conditions

Ionization Mode:	ESI (negative)
Needle Voltage:	-5000 V
Shield Voltage:	600 V
Nebulizing Gas:	N_2 at 20 psi
Drying Gas:	N_2 at 350 °C at 25 psi
Capillary Voltage:	-130 V
Collision Energy::	DPPE 22 V
	POPG 27 V
	DPPG 28 V

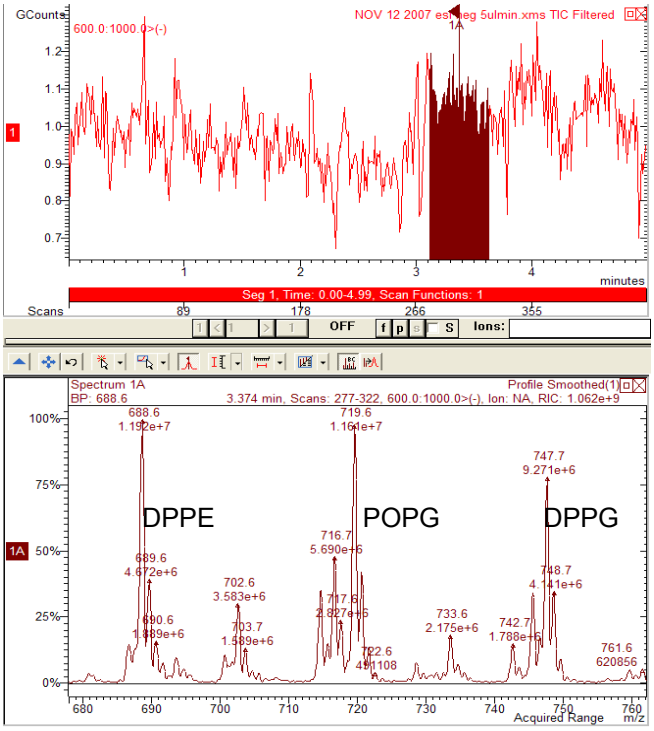

Figure 4. Top: Infused phospholipid mixture. Bottom: Full scan negative ion infused mixture (profile spectrum).

Discussion

Analysis of the mixture was performed by direct infusion with the built-in syringe pump at 10 µL/min. The ACD Labs/MS Manager software was used to characterize and confirm structures of phospholipids in the mixture. Figure 4 shows a full scan analysis of the mixture with corresponding spectra in profile mode. Figure 5 is the MS/MS product ion spectrum resulting from dissociation of precursor mass *m/z* 688, representing DPPE.

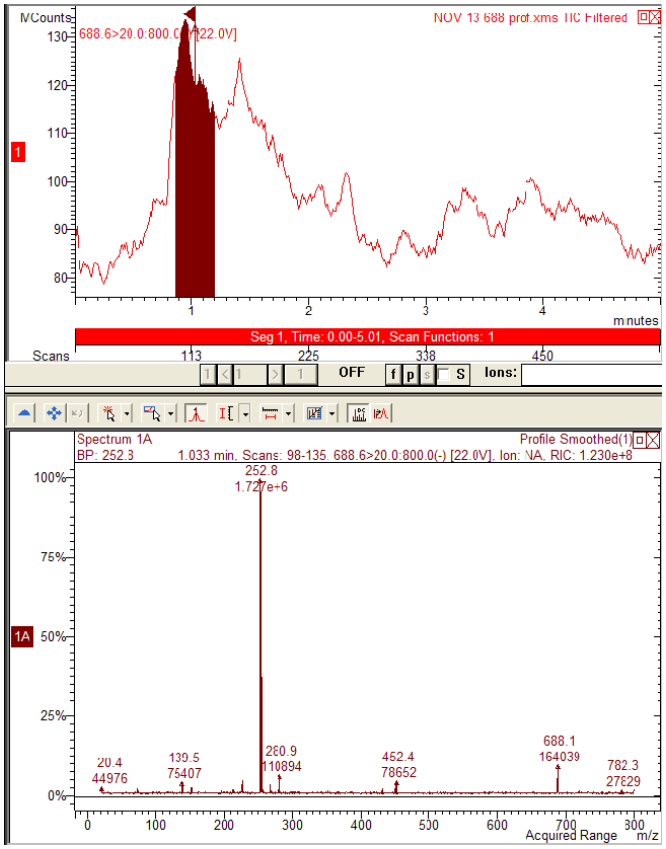

Figure 5. Top: Infused mixture, with DPPE. Bottom: MS/MS spectrum of precursor *m/z* 688.

The ACD Labs/MS Manager software allows the ability to draw the structure of a suspected peak (e.g., DPPE in Figure 6) and then attach it to the pertinent MS/MS spectrum so that predicted fragments can be matched to the actual data (colored in red). Clicking on the individual fragment in the table (in gray) shows the location in the structure in red. At the bottom of the display it indicates that 97.0% of the actual spectral peaks matched predicted fragments. Figures 7 and 8 show the results for DPPG and POPG, respectively; all with excellent match results.

Conclusion

The Varian 320-MS LC triple quadrupole mass spectrometer with ACD Labs/MS Manager software can be used to rapidly screen and characterize phospholipids in complex mixtures. These mixtures can be analyzed directly by infusion using the built-in syringe pump. The power of MS/MS provides excellent specificity for rapid screening of mixtures.

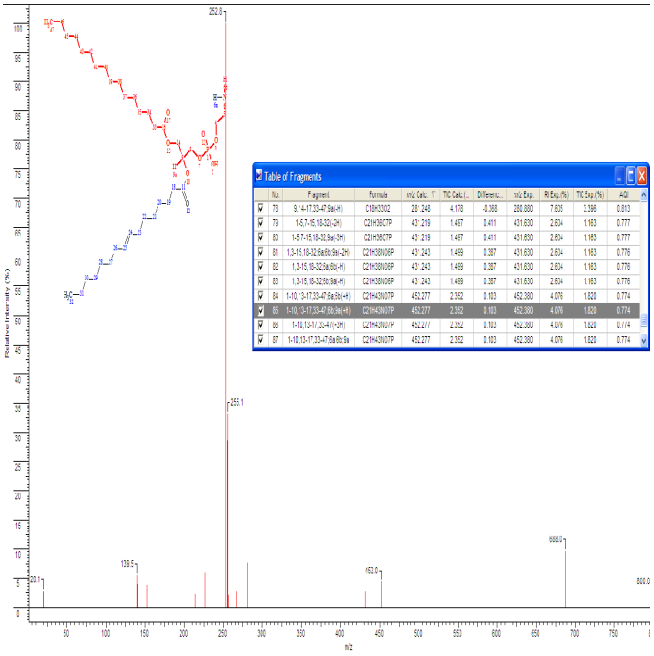

Figure 6. Structural confirmation of DPPE; 97% spectral match to predicted structure.

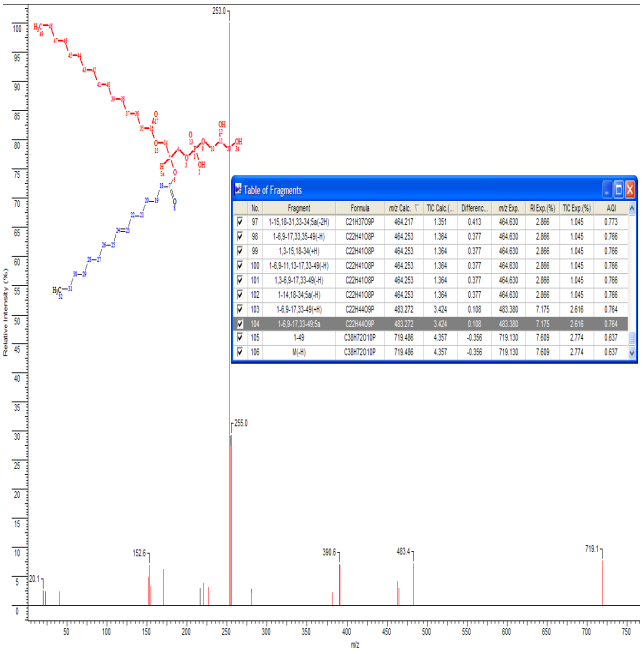

Figure 8. Structural confirmation of POPG; 93.1% spectral match to predicted structure.

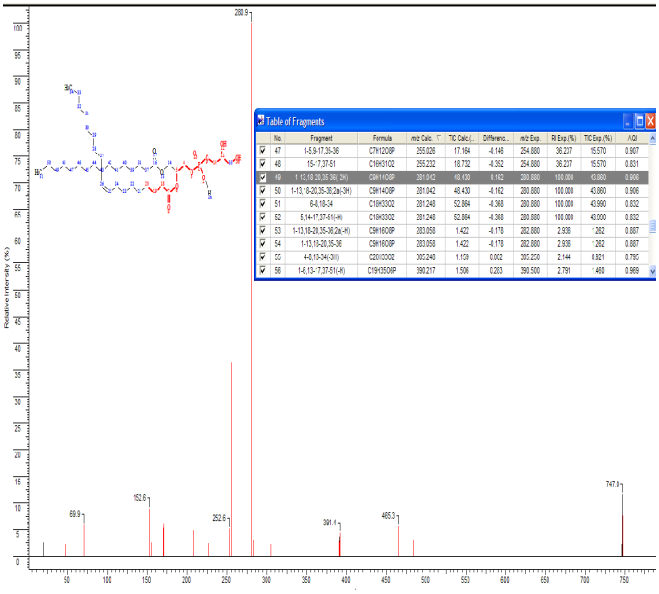

Figure 7. Structural confirmation of DPPG; 92.3% spectral match to predicted structure.

These data represent typical results.
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