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Agilent Technologies

Application Note SI-01224

Sensitive Polar Lipid Analysis by HPLC using Low Temperature ELSD

Stephen Bullock
Polymer Laboratories, now a part of Varian, Inc.

Introduction

Lipids are one of the major constituents of foods providing energy and essential lipid nutrients. Lipids are usually defined as soluble in organic solvents but insoluble in water. Consequently, there is a diverse range of lipid compounds including triglycerides, phospholipids, fatty acids, sterols, carotenoids and terpenes. Lipids are naturally occurring compounds and are used commercially in cosmetics, foodstuffs and pharmaceutical drug delivery. Cholesterol and selected phospholipids are amphophilic, having a non-polar hydrophobic tail attached to a polar hydrophilic head (see Figures 1a and 1b).

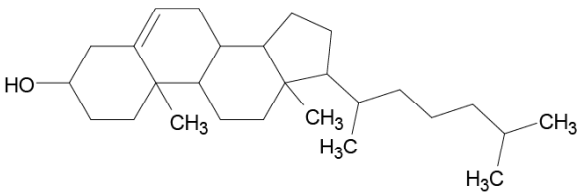

Figure 1a. Chemical structure of cholesterol.

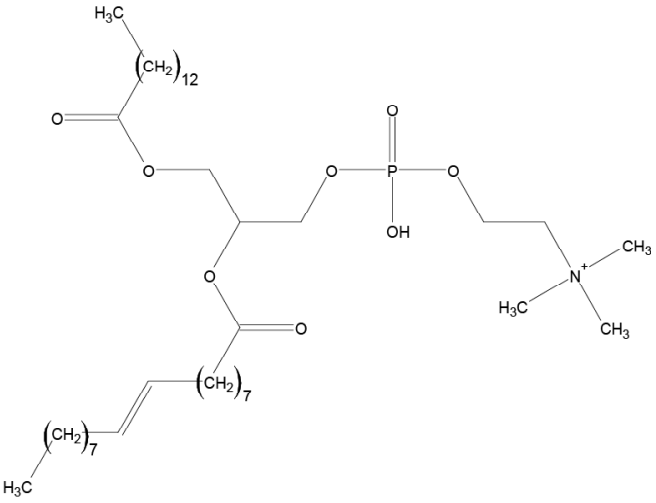

Figure 1b. Chemical structure of phosphatidylcholine.

Like most lipids, cholesterol and phosphatidylcholine exhibit very poor UV chromophores, which limit their sensitivity and the ability to run gradient elution on account of the need to analyze at short wavelengths. Consequently, lipids are often derivatized to enhance their absorbance in the UV range. However, this approach is time consuming and is difficult to apply to complex mixtures. The use of refractive index (RI) detection is also not possible because complex gradients are required to attain the necessary resolution of phospholipid mixtures. Evaporative light scattering detection (ELSD) provides a better alternative for the analysis of polar lipids, as this application shows. ELSD is universal and is not dependent on the optical properties of the compound. The detector recognizes any compound that is less volatile than the mobile phase, making it fully gradient compatible. ELSD also removes the need for derivatization, allowing the rapid determination of lipids in complex matrices.

Instrumentation

Column: Silica 3 µm, 250 x 4.6 mm
Detection: Varian ELSD (neb=40 °C, evap=90 °C, gas= 0.8 SLM)

Materials and Reagents

Eluent A: IPA/hexane/water/ammonia hydroxide (51.8/40/2/0.02)
Eluent B: IPA/hexane/water/ammonia hydroxide (51.8/40/8/0.2)

Conditions

Flow Rate: 0.3 mL/min
Injection Volume: 10 µL
Gradient: 0-100 % B 7 min, hold 8 min; 100-0 % B 5 min, hold 10 min

Results and Discussion

The separation of five polar lipids is shown in Figure 2, where a complex gradient of low boiling point eluents was used. Typically, ELS detection for such eluents is performed at high temperatures (ca 90 °C) in order to maintain a stable baseline and maximize sensitivity.

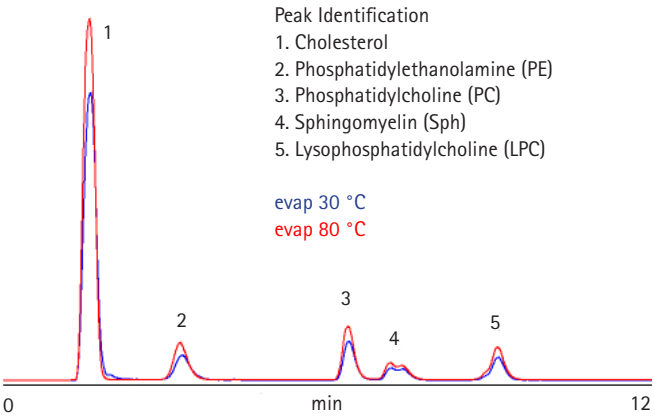

Figure 2. Separation of a 100 µg/mL polar lipid mixture at ambient temperature with the Varian ELSD.

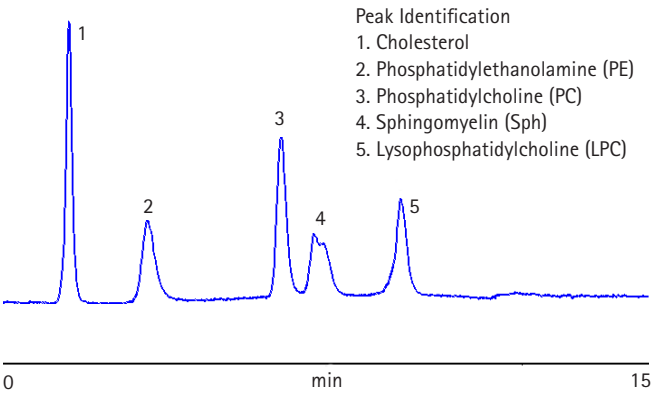

Figure 3. Excellent sensitivity of the Varian ELSD to polar lipids resulting from very low signal to noise ratios.

The unique design of the Varian ELSD also allows lipid compounds to be analyzed at ambient temperatures, thus minimizing the loss of thermally labile species. This has distinct advantages when analyzing complex mixtures, because it gives a better representation of the sample composition. The sensitivity of the Varian ELSD to polar lipids is shown in Figure 3, where a column loading of 100 µg gave signal to noise ratios in the range of 30-150. Table 1 shows the S/N ratio values.

Table 1. Signal to noise ratios in the analysis of lipids using the Varian ELSD.

Lipid	S/N Ratio
1. Cholesterol	158
2. Phosphatidylethanolamine	45
3. Phosphatidylcholine	88
4. Sphingomyelin	33
5. Lysophosphatidylcholine	54

By calibrating the Varian ELS detector (Figure 4), ELSD can also be used to accurately quantify lipid concentrations.

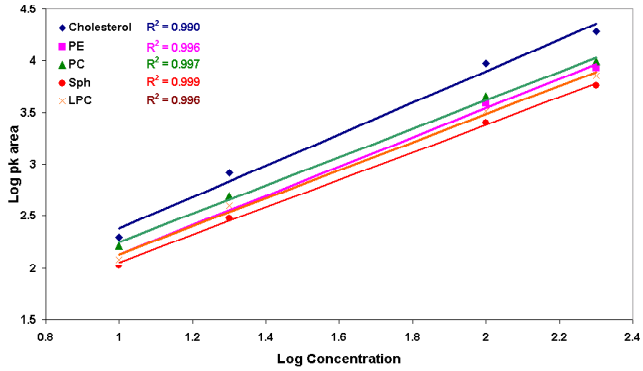

Figure 4. Calibration curve of individual polar lipids.

Conclusion

The Varian evaporative light scattering detector is universal and is not dependent on the optical properties of the compound under consideration. Its unique configuration allows determination under ambient conditions to minimize losses of heat sensitive compounds. Consequently, its good discriminating power and sensitivity is well suited to compounds such as lipids that possess weak or no UV chromophores. The Varian ELSD surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The Varian ELSD's unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100 % water at a flow rate of 5 mL/min can be removed at 30 °C. The novel design of the Varian provides superior performance compared to detectors from other vendors for the analysis of semi-volatile compounds.

These data represent typical results. For further information, contact your local Varian Sales Office.

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