

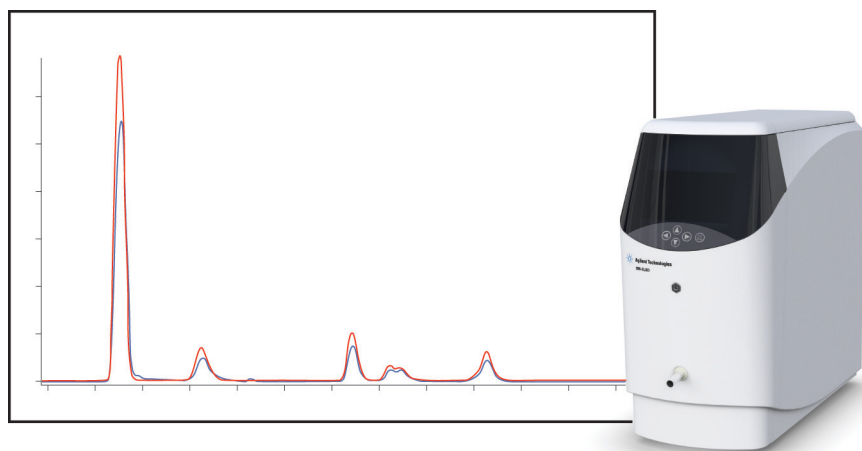
Sensitive analysis of polar lipids using HPLC with low temperature evaporative light scattering detection

Application Note

Food testing and agriculture, consumer products, pharmaceuticals

Author

Stephen Ball
Agilent Technologies, Inc.
Craven Arms, UK



Abstract

This Application Note demonstrates that the combination of HPLC with the Agilent 385-ELSD Evaporative Light Scattering Detector provides a better alternative for the sensitive analysis of polar lipids. Evaporative light scattering detection is universal and not dependent on the optical properties of the compound, making it ideal for analysis of lipids such as cholesterol and phosphatidylcholine that have very poor UV chromophores. ELS detection recognizes any compound that is less volatile than the mobile phase. This makes ELS detection fully compatible with HPLC elution gradients. ELS detection also eliminates the need for pre-analysis derivatization to enhance UV absorbance, facilitating rapid determination of lipids in complex matrices.



Agilent Technologies

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 amphiphilic, having a nonpolar hydrophobic tail attached to a polar hydrophilic head (Figures 1a and 1b).

Like most lipids, cholesterol and phosphatidylcholine exhibit very poor UV chromophores, which limit their sensitivity and the ability to run gradient elution because 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 difficult to apply to complex mixtures. The use of refractive index (RI) detection is also not possible because complex gradients

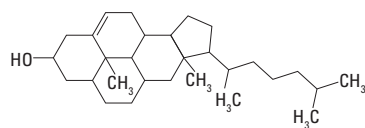


Figure 1a
Chemical structure of cholesterol.

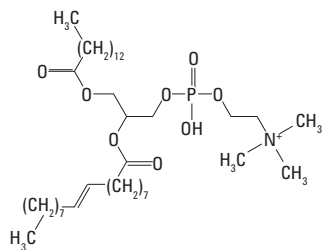


Figure 1b
Chemical structure of phosphatidylcholine.

are required to attain the necessary resolution of phospholipid mixtures. Evaporative light scattering (ELS) detection provides a better alternative for the analysis of polar lipids, as this Application Note shows. ELS detection recognizes any compound that is less volatile than the mobile phase. This makes ELS detection fully compatible with HPLC elution gradients. ELS detection also eliminates the need for pre-analysis derivatization to enhance UV absorbance, facilitating rapid determination of lipids in complex matrices.

Experimental

Instrumentation

Column: Silica, 250 × 4.6 mm, 3 μm
Detection: Agilent 385-ELSD Evaporative Light Scattering Detector
Nebulizer temp: 40 °C
Evaporation temp: 90 °C
Gas flow: 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 in 7 min, hold for 8 min;
100-0% B in 5 min, hold for 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 (about 90 °C) to maintain a stable baseline and maximize sensitivity.

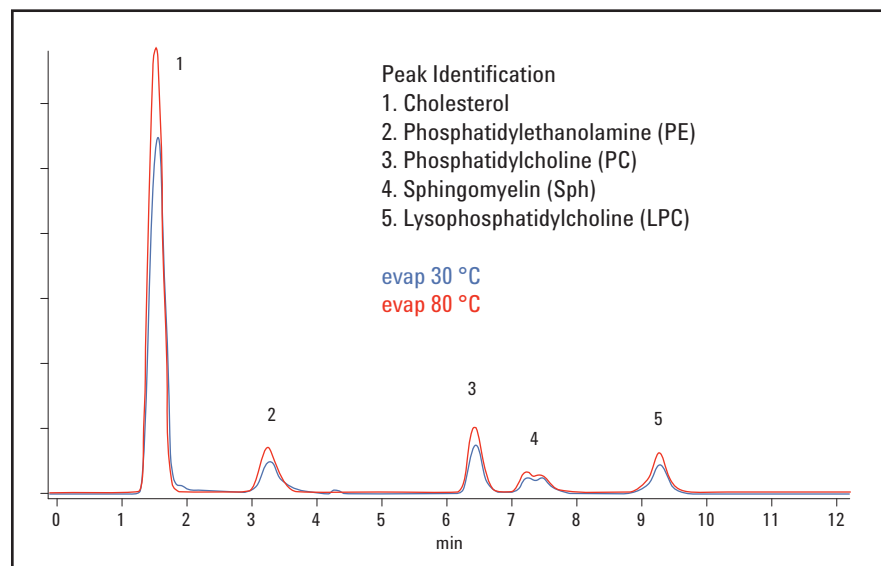


Figure 2
Separation of a 100 μg/mL polar lipid mixture at ambient temperature with the Agilent 385-Evaporative Light Scattering Detector.

The unique design of the Agilent 385-ELSD Evaporative Light Scattering Detector also facilitates analysis of lipid compounds 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 385-ELSD to polar lipids is shown in Figure 3, where a column loading of 100 μg gave signal-to-noise (S/N) ratios in the range of 30-150. Table 1 shows the S/N ratio values. By calibrating the Agilent 385-ELSD (Figure 4), ELS detection can also be used to accurately quantify lipid concentrations.

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

Table 1
Signal-to-noise ratios in the analysis of lipids using the Agilent 385-ELSD Evaporative Light Scattering Detector.

Conclusion

The Agilent 385-ELSD Evaporative Light Scattering Detector is universal and not dependent on the optical properties of the compound under consideration. Its unique configuration facilitates 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 Agilent 385-ELSD surpasses other ELS detectors for low temperature HPLC applications with semivolatile compounds. Its innovative design represents the next generation of ELS detection technology, providing optimum performance across a diverse range of HPLC applications. The unique gas control of the 385-ELSD facilitates 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 385-ELSD achieves superior performance compared to detectors from other vendors for the analysis of semivolatile compounds.

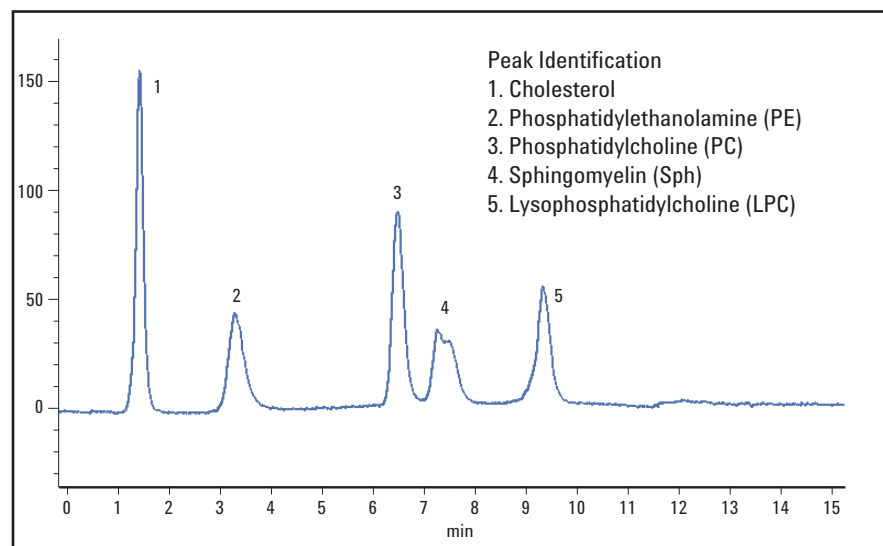


Figure 3
Excellent sensitivity of the Agilent 385-Evaporative Light Scattering Detector to polar lipids resulting from very low signal-to-noise ratios.

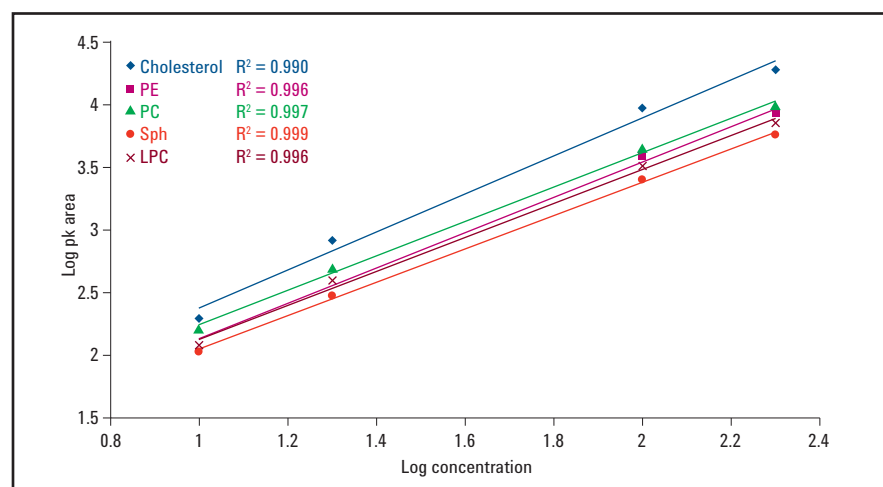


Figure 4
Calibration curve of individual polar lipids.

www.agilent.com/chem/elsd

© Agilent Technologies, Inc., 2012
Published in the USA, January 1, 2012
5990-8837EN



Agilent Technologies