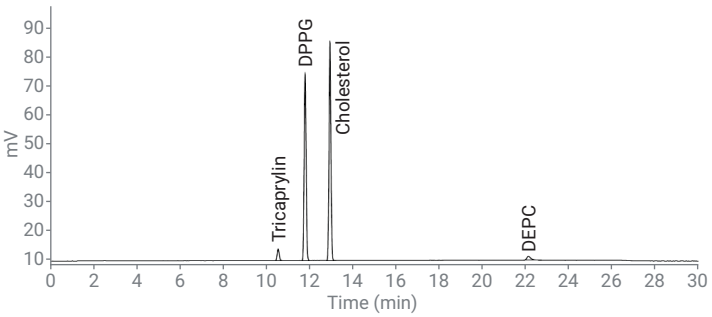
Application Note

Pharma & Biopharma

A Sensitive Detection Technique for Analysis of Lipids in Liposomal Formulations Using an Agilent 1290 Infinity II ELSD



Author

Kriti Tyagi
Agilent Technologies, Inc.

Abstract

This Application Note demonstrates the use of an Agilent 1290 Infinity II evaporative light scattering detector (ELSD) for achieving excellent detection limits for lipids present in liposomal formulations. Quantitation of these formulations, commonly used as drug carrier systems in pharmaceutical applications, is of utmost importance because these concentrations affect both the release mechanism and stability of the drug. Due to an absence of chromophores in lipids, ELSD is an ideal detection technique for their quantification. The 1290 Infinity II ELSD's novel PMT technology provides the sensitivity required for quantifying lipids in liposomal formulations. Simultaneously, subambient operation with evaporation down to 10 °C delivers maximum sensitivity for compounds with significant volatility below 30 °C. With this method, excellent sensitivity of up to 1 µg/mL for lipids was achieved using the 1290 Infinity II ELSD.

Introduction

Lipids have advanced pharmaceutical drug delivery research due to their ability to enhance the delivery and targeting of a wide range of drugs and vaccines. Liposome structural components are phospholipids incorporated with sterols such as cholesterol to influence membrane permeability. Drugs are encapsulated in liposomes to enhance the therapeutic indexes of various agents mainly through changes in their pharmacokinetics and pharmacodynamics. Most liposomes consist of neutral lipids as a main component as well as alternating ratios of charged lipids, modified lipids, or adjuvants. Phosphatidylcholines (PC) belong to the neutral lipid class; phosphatidylglycerols (PG) belong to the anionic lipid class, and are widely applied in drug delivery formulations.

This Application Note tests detection limits for four lipids used in liposomal formulations: tricaprylin, dierucoylphosphatidylcholine (DEPC), dipalmitoylphosphatidylglycerol (DPPG), and cholesterol. These lipids are generally used in combination with anesthetic drug molecules. Evaporative light scattering detection (ELSD) is an ideal technique for lipid analysis due to its sensitivity as well as its flexibility to allow the use of gradients unlike those used in RID. The 1290 Infinity II ELSD comprises an optical unit consisting of a blue laser light source coupled with a high-gain photomultiplier and digital signal processing. This setup results in noise reduction and enhanced signal. Compared to previously used LED light sources, this results in increased sensitivity.

The latest version of Agilent OpenLab CDS software was used for data analysis. OpenLab CDS software is equipped with tools to provide time-saving steps in the analysis, interpretation, and reporting of workflows to identify key information and improve turnaround time.

Experimental

Instrumentation

An Agilent 1260 Infinity II LC was used for experimentation, and consisted of the following modules:

- Agilent 1260 Infinity II binary pump (G7112B)
- Agilent 1260 Infinity II vialsampler with integrated column compartment (G7129A)
- Agilent 1290 Infinity II ELSD (G4261B)

Solvent and samples

Methanol was LC/MS Chromasolv grade (Honeywell, Charlotte, NC, USA). Ammonium acetate was LC/MS grade (Sigma-Aldrich, St. Louis, MO, USA). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipore, Burlington, MA, USA).

Software

Agilent OpenLab CDS workstation version 2.3.0 (M8413AA)

Sample preparation

Stock solutions of 1,000 µg/mL were prepared separately for the four lipids in methanol and stored at 2 to 8 °C. For the calibration standard mix, the solutions were prepared at 20, 40, 60, 80, and 100 µg/mL. The limit of detection (LOD) solution was prepared at 1 µg/mL each for tricaprylin, cholesterol, and DPPG, and at 1.5 µg/mL for DEPC.

HPLC method

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5 µm (p/n 959990-906)
Mobile Phase	Solution A: 315 mg of ammonium acetate in water Solution B: 315 mg of ammonium acetate in methanol
Gradient	Time (min) / Solution A (%) / Solution B (%) 0.0 / 10 / 90 15.0 / 0 / 100 23.0 / 0 / 100 23.1 / 10 / 90 30.0 / 10 / 90
Flow Rate	1.0 mL/min
Injection Volume	10 µL (100 µL for LOD solution)
Column Temperature	30 °C

Agilent 1290 Infinity ELSD method

Parameter	Value
Evaporator Temperature	35 °C
Nebulizer Temperature	35 °C
Gas Flow Rate	1.6 SLM
PMT Gain	1.0
Data Collection Rate	10 Hz
Smoothing	3.0 seconds

Results and discussion

As per the ELSD working principle, the higher the organic content in the mobile phase, the higher the amount of sample that enters the detector in the post nebulization stage. This method uses a methanol gradient between 90 and 100% to simultaneously enable sensitive detection in ELSD and separation of all four lipids in a single run. Various ELSD parameters were tested for optimizing the LOD level. To achieve the desired sensitivity, evaporator temperature, nebulizer temperature, and gas flow were tested at different settings. Best results were observed at a high gas flow rate of 1.6 SLM, and evaporator and nebulizer temperatures kept at 35 °C.

For determination of the LOD, all four lipids were measured at different concentrations. The LOD was achieved by injecting 100 µL of a 1 µg/mL solution of tricaprylin, cholesterol, and DPPG, and 1.5 µg/mL for DEPC, with signal-to-noise ratio (S/N) >3. (Figure 1). A series of calibration solutions were prepared to check the linearity of the 1290 Infinity II ELSD for these lipids. Linearity solutions were prepared at 20 µg/mL (level 1), 40 µg/mL (level 2), 60 µg/mL (level 3), 80 µg/mL (level 4), and 100 µg/mL (level 5) in methanol, and injected.

ELSD detectors generally provide a nonlinear response due to the correlation of concentration and particle size formed after the nebulization and evaporation of the mobile phase. A quadratic calibration curve was found to be the best fit for the calibration of all four lipids (Figure 2, Table 1).

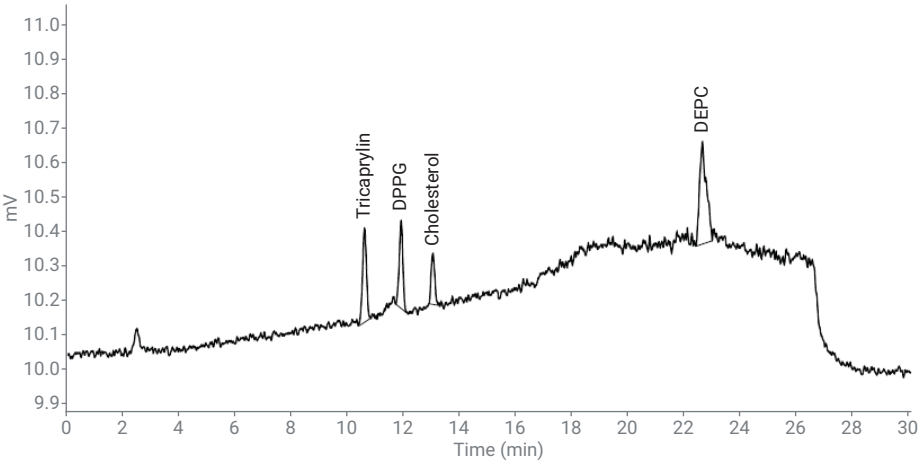


Figure 1. LOD standard mix for tricaprylin, DPPG, cholesterol, and DEPC.

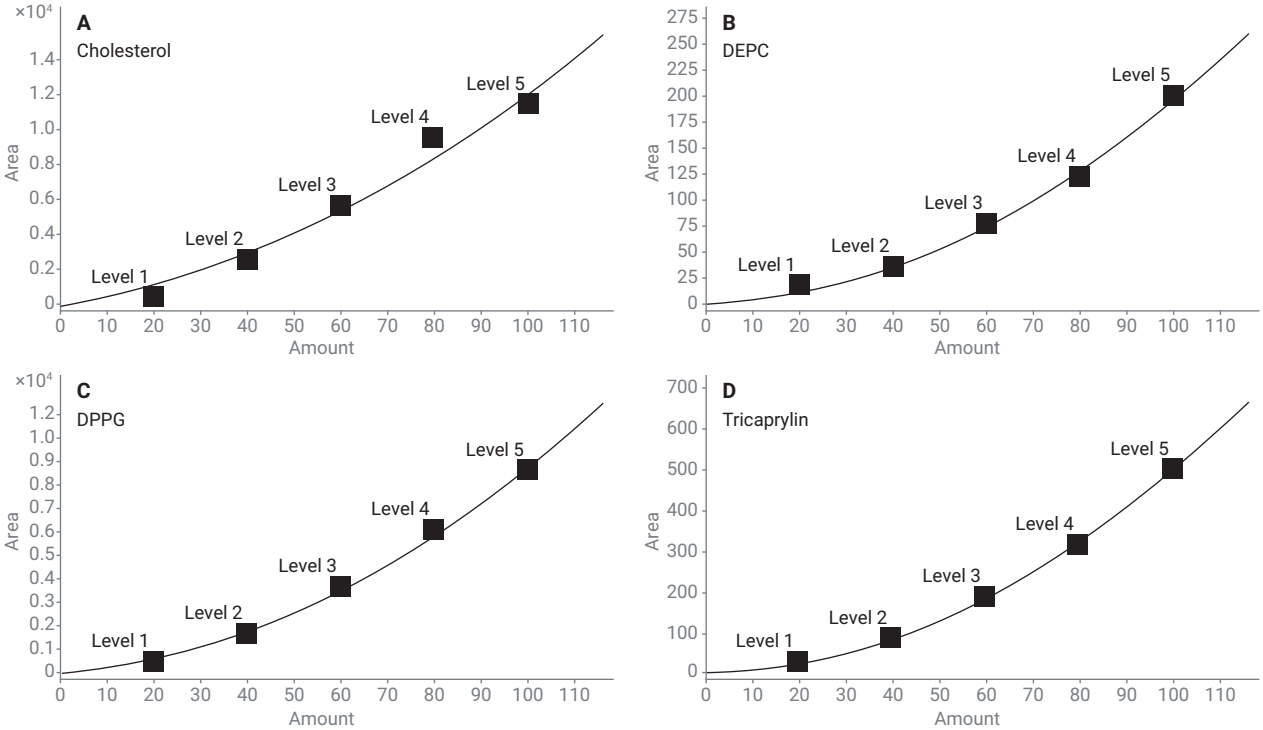


Figure 2. Calibration curves for cholesterol, DEPC, DPPG, and tricaprylin.

The %RSD for all four compounds was determined by injecting the solution at 100 µg/mL concentration in six replicates. Excellent %RSD of area and retention time were achieved for all compounds (Figure 3, Table 2).

Conclusion

The Agilent 1290 Infinity II ELSD is ideal for reproducible, sensitive detection of volatile and nonvolatile compounds that do not have a UV chromophore, such as lipids. This Application Note successfully demonstrates a unique reversed-phase technique for the separation of tricaprylin, DPPG, cholesterol, and DEPC in a single injection, achieving excellent sensitivity at 1 µg/mL using a 1290 Infinity II ELSD with an Agilent 1260 Infinity II LC.

References

1. Improved Sensitivity with the Agilent 1290 Infinity Evaporative Light Scattering Detector. *Agilent Technologies Technical Overview*, publication number 5991-2394EN.
2. Jeschek, D.; *et al.* A Versatile, Quantitative Analytical Method for Pharmaceutical Relevant Lipids in Drug Delivery Systems. *J. Pharmaceut. Biomed.* **2016**, *119*, 37–44.
3. Van Hoogevest, P.; Wendel, A. The Use of Natural and Synthetic Phospholipids as Pharmaceutical Excipients. *Eur. J. Lipid Sci. Technol.* **2014**, *116(9)*, 1088–1107.

Table 1. Correlation coefficients and S/N values.

No.	Analyte	R^2	S/N at LOD
1	Tricaprylin	0.999	8.0
2	DPPG	0.998	7.2
3	Cholesterol	0.991	4.7
4	DEPC	0.998	8.6

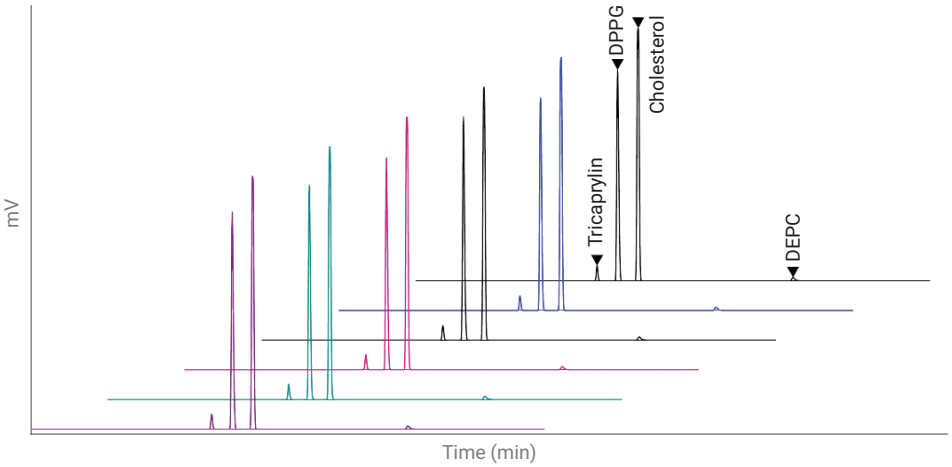


Figure 3. Six-injection repeatability study of a lipid standard at 100 µg/mL on the Agilent 1290 Infinity II ELSD.

Table 2. %RSD of RT and area for lipid standard mix.

No.	Analyte	%RSD RT	%RSD Area
1	Tricaprylin	0.02	1.51
2	DPPG	0.03	1.03
3	Cholesterol	0.04	0.68
4	DEPC	0.05	3.03

Acceptance criteria: not more than 5%.

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