High resolution of complex lipids (triglycerides) using the Agilent 1290 Infinity LC and ZORBAX RRHT and RRHD 1.8 µm columns

Application Note

Lipid Analysis

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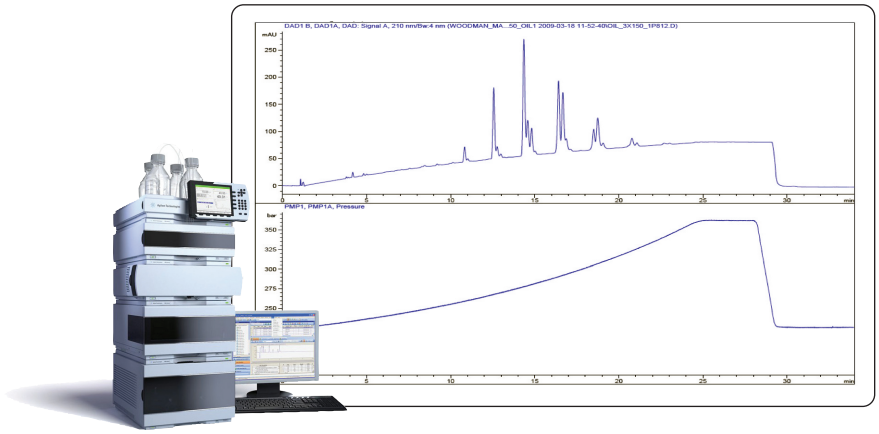

Abstract

The Agilent 1290 Infinity LC has significant capabilities for a wide range of HPLC and UHPLC applications. With a broader power range (that is, the combination of pressure and flow capabilities) than any other commercially available system, and the flexibility to operate a wide range of column dimensions and particle sizes, it is extremely useful for method transfer from any HPLC or UHPLC to the 1290 Infinity system. It allows the user to access capabilities not otherwise available.

Introduction

The typical HPLC resolution is shown by a separation of complex triglycerides in vegetable oil. Using a 24-min gradient and a 3.0 mm × 150 mm, 1.8 µm column, the analysis time of 35 min is typical; however, resolution is insufficient for good compositional investigation of the mixture. The separation of the main components is shown in Figure 1.

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The high resolution of the system is further demonstrated by separation on a much longer column, using more of the power range of the system. At 0.29 mL/min, incorporating a shallow gradient condition and an RRHD, 2.1 mm × 400 mm, 1.8 µm column, the separation is dramatically improved. The separation of the main components is shown in Figure 2. Subambient column temperature control, a standard feature of the Agilent Thermostatted Column Compartment, has significant advantages for many difficult isomer separations, including enantiomeric separations, and for shape-selective separations such as polycyclic aromatic hydrocarbons.

Configuration

- G4220A 1290 Infinity Binary Pump with Integrated Vacuum Degasser
- G4226A 1290 Infinity Autosampler
- G1316C 1290 Infinity Thermostatted Column Compartment
- G4212A 1290 Infinity Diode Array Detector

Conclusion

The high resolution and pressure capability of the system allows one to use high efficiency 2.1 mm id columns, generating approximately 97,000 theoretical plates and having approximately 400% lower solvent consumption compared to 4.6 mm id columns. With nearly 3 times higher efficiency, run time was increased by only about 80%. The low flow rate and high resolution facilitate the interfacing of the separation to high resolution TOF and QTOF mass spectrometers to produce high confidence peak identification and compositional information.

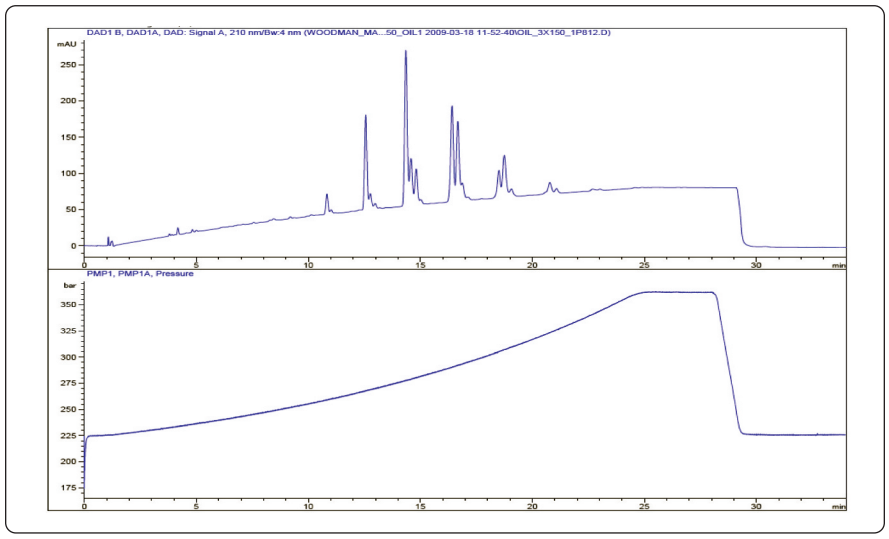

Figure 1
Analysis of vegetable oil components on the 1290 Infinity LC. Sample: soybean oil, 10 mg/mL, 10 µg on column. Conditions: 0.6 mL/min, 20% to 60% IPA vs. ACN at 24 min, hold to 30, run 35 min, ZORBAX RRHT StableBond C18, 3 mm × 150 mm 1.8, µm, 30 °C. Maximum operating pressure, 370 bar.

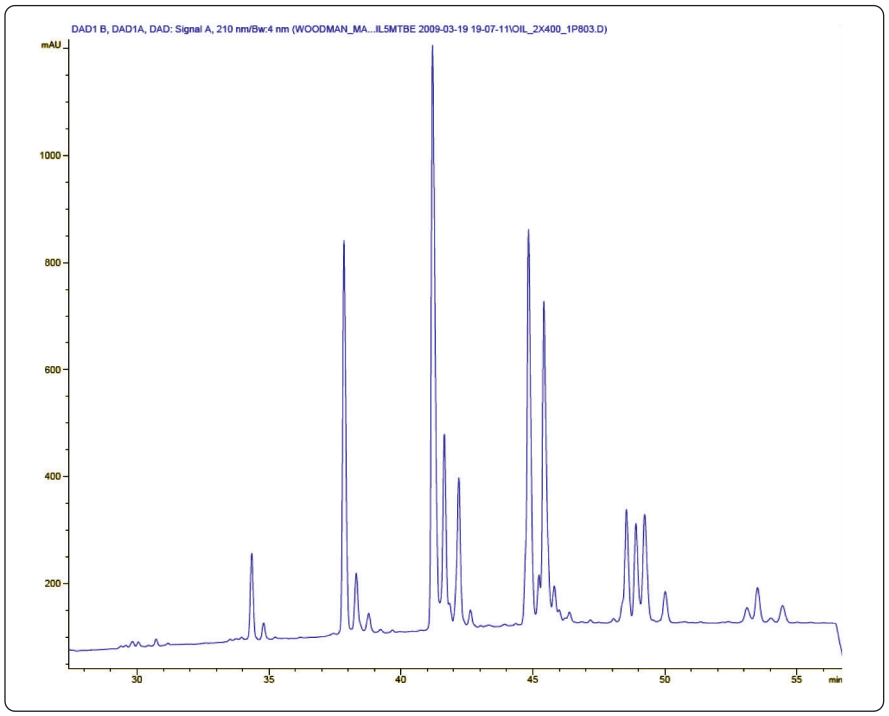

Figure 2
Analysis of soybean triglycerides on the 1290 Infinity LC. Sample: soybean oil, 10 mg/mL, 30 µg on column. Conditions: 0.29 mL/min, 10% to 40% MTBE vs. ACN at 42 minutes, hold to 55 minutes, run 60 minutes, 210 nm UV. ZORBAX RRHD StableBond C18, 2.1 mm × 400 mm (2–150 and 1–100 mm length in series), 1.8 µm, 20 °C. Operating pressure 730 bar.

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