Fast analysis of fat soluble vitamins using the Agilent 1290 Infinity LC and ZORBAX RRHT and RRHD 1.8 µm columns

Application Note

Food Analysis

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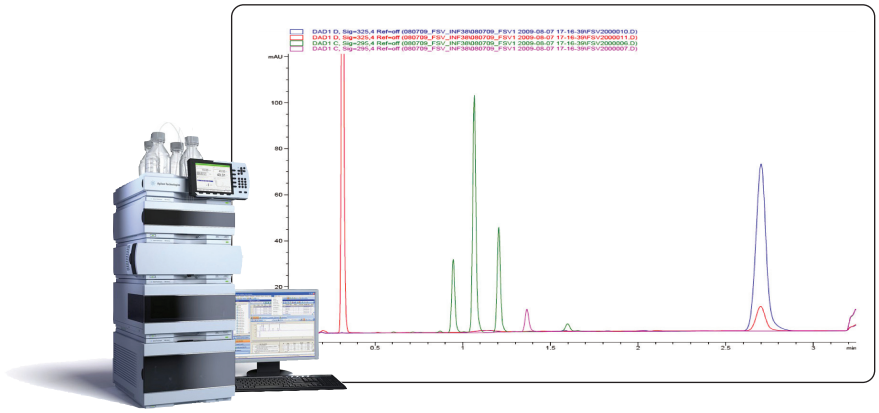

Abstract

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 LC system. It allows the user to access capabilities not otherwise available.

Introduction

The speed and high resolution are demonstrated by a separation of fat-soluble vitamin isomers and esters, at a high pressure and flow rate. At 2 mL/min, utilizing a simple 1-min gradient and a 3.0 x 50 mm, 1.8 µm column, the analysis time is only 3 min including the late eluting retinyl palmitate component. The separation of the main components is shown in Figure 1.

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The speed, resolution and flexibility of the system are further demonstrated by a separation of vitamins D2 and D3. At 2 mL/min, utilizing a simple isocratic condition and a 3.0 mm × 150 mm, 1.8 µm column, the analysis time is only 3 min. The separation of the main components, at three column temperatures including sub-ambient, is shown in Figure 2. Sub-ambient 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

Taking advantage of the combined high flow and high pressure capability of the system allows one to use high efficiency 3 mm id columns (having up to 40% higher efficiency than comparable 2.1 mm id columns) to produce rapid separations with remarkable resolution while conserving solvent over the use of 4.6 mm id columns.

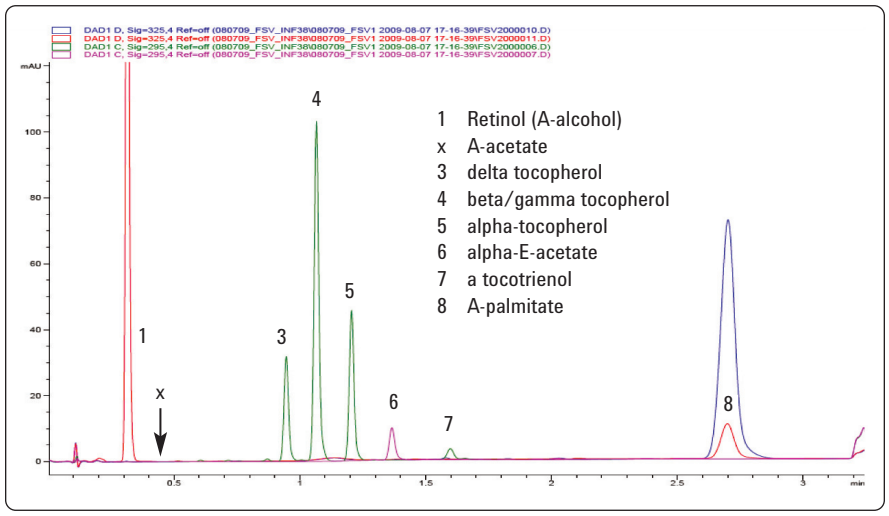

Figure 1
Analysis of important vitamins A and E components on the 1290 Infinity LC. Sample: solution of alcohols and esters of retinol and tocopherol. Conditions: 2.0 mL/min, 90% to 100% ACN at 1 min, hold to 3, run 4 min, ZORBAX RRHT StableBond C18, 3 mm × 50 mm, 1.8 µm, 45 °C.

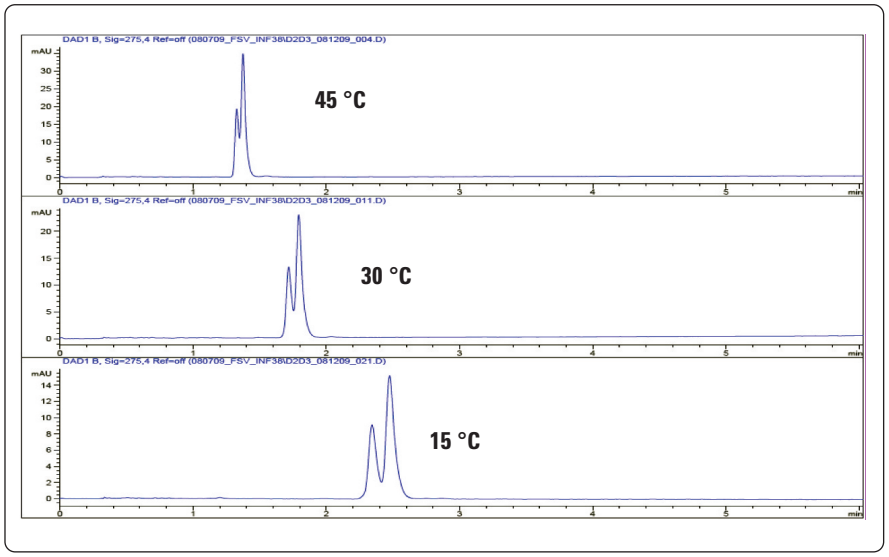

Figure 2
Analysis of vitamins D2 and D3 (order of elution) on the 1290 Infinity LC. Sample: standard mix (Sigma-Aldrich). Conditions: 2.0 mL/min, 75/25 ACN/MeOH isocratic, 280 nm UV ZORBAX RRHD StableBond C18, 3 mm × 150 mm, 1.8 µm, 45 °C, 30 °C and 15 °C.

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