Maximizing the Chromatographic Resolution and Detection Content of Complex Plant Lipid Analyses with Optimized UHPLC Systems

Jerry Zweigenbaum, Michael Woodman, Agilent Technologies, Inc., Wilmington, Delaware, USA

Abstract

Recent UHPLC experiments on columns packed with materials in the 1.5-1.1um particle size range have shown great utility in the analysis of complex lipid mixtures. Maximized resolution requires the use of high column efficiency and, typically, shallow gradient profiles. Achieving high resolution while minimizing overall operating pressure and analysis time is also a critical part of method design. The highest resolution separation is a combination of column and mobile phase physics and chemistry with system dispersion (bandspreading) also optimized. Refined and unrefined triglyceride mixtures and phytosterols were acquired and subjected to a diversity of solvent and column configurations to explore high peak capacity separations. We typically use UV, ELSD and MS detection to enhance detection information.

Introduction

In this work we have exploited a broad range of resolution parameters on systems optimized for very high chromatographic resolution. We have also developed tools for method design and method translation to make these new technologies easier to use, and to significantly shorten the time required for method optimization.

Triglycerides play an important role for food research, biofuel, plant and animal physiology and lipidomics. High chromatographic resolution of intact triglycerides requires substantial effort to optimize the column and mobile phase choices and column operating temperature. The inherent sample complexity requires high peak capacity with relatively shallow gradients, typically under largely non-aqueous reversed phase conditions.

Phytosterols, as naturally occurring anticholesterologenic materials, have received considerable attention from nutritional supplement producers and consumers. MS detection was used to propose peak identity or confirmation, which was especially beneficial with these highly complex mixtures where many standards have limited availability and/or are very expensive.

Experimental

Agilent 1290 Infinity LC, consisting of: G4232A binary pump with integral vacuum degasser G4212A high performance autosampler G1316C thermo. column compartment SL G4226A UV-VIS Diode Array Detector with 10 nm, 1.5u flow cell G4211A ELSD with standard nebulizer G6140A MSD with ESI/APCI Multi-Mode source ChemStation 32-bit version B.04.02

Results and Discussion

Table 1. Pertinent details of the typical phytosteroids materials found in vegetable and/or animal fats and oils. Cholesterol occurs significantly in only one vegetable source – palm oil. Lanosterol does not typically occur in vegetable and/or animal fats and oils. Phytosterols were acquired and subjected to a diversity of solvent and column configurations to explore high peak capacity separations. We typically use UV, ELSD and MS detection to enhance detection information.

Figure 1. Photo of 1290 Infinity LC with ELSD.

Figure 2. beta-sitosterol C29H46O5 Limited unsuraturation or chromophoric functional groups limits UV performance in this compound class.

Figure 3. Optimized phytosterol separation on ZORBAX SB-C8 compared to some other ZORBAX ligands in the SB (StableBond) and XDB-Eclipse families.

Figure 4. below, peak identities based on the ZORBAX SB-C8 conditions, via positive ESI-MS. No post-UV addition was required to optimize the phytosterol response.

Figure 5. See triglycerides and products of a slow in vial FAME conversion reaction, separated via a gradient optimized method for gross composition. Conditions: 3x15mm SB-C8 40C 3.5um 1ml/min solvent A 75/25 ACN/wa, Solvent B 1/1 IPA/ACN, solvent A to 5 minutes, then to 100% B at 15 minutes to 17. Because of the low UV wavelength required for sensitive detection of these compounds, the IPA in solvent B (required to elute the di- and tri-glycerides of these temperatures) caused significant baseline drift that was not observed in the ELSD. The UV signal is still useful, especially for the few levels of some of the less abundant fatty acid methyl esters (FAME’s) produced during the saponification reaction. Sample taken at 90 minutes into ambient ren 100mg/l K2H2/MoOH. Less than 10% TG's remaining after 10B minutes.

Figure 6. High resolution separation of triglycerides using the Agilent 1290 Infinity LC and ZORBAX RRHD columns. Conditions: 30um, 0.2ml/min, 10-40% MTBE/ACN over 43 minutes. Up to 730 bar on ZORBAX SB-C18, 2.1x400mm, 1.8 μm, 20°C. Utilizing lower column temperature and C18 support with 1.8um particles, and with MTBE solvent to lower viscosity, a column set opting approximately 100,000 plates in actual use. For MS monitoring (not shown) APCI/MSD was particularly effective with post-column addition of 20μl/min MuOH/0.03M Ammonium Fomate.

Conclusion

The Agilent 1290 Infinity LC system equipped with ancillary Agilent ELS and MS detection provided a rapid separation with good resolution, good sensitivity and high confidence in the proposed identity of compounds where standards were not present. Future work includes expansion of the number of lipid classes, additional peak identity support, and recommended sample preparation and cleanup prior to HPLC analysis.