Phytosterol and Seed Oil Lipid Analysis by HPLC with UV, MS and Evaporative Light Scattering (ELS) Detection

Michael Woodman, Jerry Zweigenbaum
Agilent Technologies, Inc., Wilmington, Delaware, USA
HPLC2008 poster P-0513-T

Abstract

We have developed a method suitable for rapid separation of phytosterols in dietary supplements and fortified foods. UV and ELS detectors are used for routine monitoring. Single quadrupole MS detection was used during the method development phase of the project. In further development, and considering that seed oils are the primary phytosterol source, we concluded that the method and instrument configuration could logically be extended to allow general lipid analysis in various seed oils. This method, then, is also suitable for investigation of synthetic or naturally occurring antioxidants and seed oils including samples that have been saponified and/or esterified, such as in the development of green fuels like biodiesel and bio/petro blends.

Introduction

Phytosterols, as naturally occurring anticholesterol terpenes, have received considerable attention from nutritional supplement producers and consumers. While the efficacy of these materials for reducing serum cholesterol may be in question, there is nonetheless a strong need to monitor these relatively complex mixtures of compounds in the wake of increased consumption and increased scrutiny of supplements sold outside the scope of the USADA or FDA guidelines. 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. Because these compounds lack strong chromophores that benefit sensitive UV detection, we found it useful to add an Evaporative Light Scattering (ELS) detector to the configuration. These detectors are very effective for many non-volatile compounds and are especially popular with natural product analysts and development screening analysts.

Experimental

Agilent 1200 series Rapid Resolution LC, consisting of:
- G1379B micro vacuum degasser
- G1312B binary pump SL
- G1367C high performance autosampler SL
- G1316B column compartment SL
- G1315C UV/VIS Diode Array Detector SL
with 3 mm, 2 μL flow cell
- G4218A ELSD with standard nebulizer
ChemStation 32-bit version B.03.02

Figure 1. Photo of 1200 RRLC with ELSD.

Figure 1 shows the Agilent RRLC (Rapid Resolution LC) with the Agilent G4218A ELS detector. The ELSD is considered a destructive detector, with respect to recovery of effluent from the separation as might be appropriate for purification. When mass spectrometers, also similarly destructive, are present it is necessary to split the effluent into two or more streams depending on the specifics of the method and whether fraction collection is also desirable. ELSD’s are sensitive into the low nanogram range, generally, and that amount of mass introduced into an MS detector would often be excessive. Under those circumstances, an unequal split flow, normally after the UV detector (if present) is created such that relatively low flow is directed to the MS. To dilute the concentration and speed the detection, a make-up pump can be added, post-split in the MS inlet flow path. This allows the user to control and limit the total mass introduced in the MS and at the same time ensure rapid detection, coordinated with other detector signals on the system. The make-up solvent mixture can also be optimized to enhance detectability of target components, and might include different organic solvents (from that used for the optimal separation) and/or organic or inorganic pH modifiers that might also enhance detectability.

Table 1. Pertinent details of the typical phytosterol 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 plants or animal lipid fractions and was used as a retention marker. Beta-Sitosterol, Campesterol and Stigmasterol are typically the most abundant phyte’s extracted from oils. Brasicansterol is unique to rapeseed/canola.

<table>
<thead>
<tr>
<th>Name</th>
<th>Emp. Form.</th>
<th># C=C bonds</th>
<th>Calc. M. Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>C27H46O</td>
<td>1</td>
<td>386.366</td>
</tr>
<tr>
<td>ergosterol</td>
<td>C28H46O</td>
<td>(3) 2 conj. 1 isol.</td>
<td>390.360</td>
</tr>
<tr>
<td>brassicasterol</td>
<td>C28H50O4</td>
<td></td>
<td>398.398</td>
</tr>
<tr>
<td>Campesterol</td>
<td>C29H50O</td>
<td>2, non-conjugated</td>
<td>400.382</td>
</tr>
<tr>
<td>stigmasterol</td>
<td>C30H50O</td>
<td></td>
<td>402.398</td>
</tr>
<tr>
<td>Sitosterol</td>
<td>C28H50O</td>
<td>2, non-conjugated</td>
<td>412.382</td>
</tr>
<tr>
<td>beta-Sitosterol</td>
<td>C29H52O</td>
<td></td>
<td>414.398</td>
</tr>
<tr>
<td>b-Sitosterol</td>
<td>C30H52O</td>
<td>1</td>
<td>414.398</td>
</tr>
<tr>
<td>sitostAnol</td>
<td>C28H50O</td>
<td></td>
<td>420.398</td>
</tr>
<tr>
<td>Lanosterol</td>
<td>C29H52O</td>
<td>2, non-conjugated</td>
<td>426.398</td>
</tr>
</tbody>
</table>

Figure 2. beta-sitosterol C29H52O

Limited unsaturation 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

Figure 5. Separation of supplement product on Zorbax SB-C8 3.0 mm x 100 mm, 3 μm. Under these conditions, brassicasterol has virtually the same retention time as cholesterol. Lacking useful UV spectra, the use of MS detection provided confirmation.

Results and Discussion

Figure 6. Soy triglycerides and products of a slow saponification/esterification reaction, separated via a gradient optimized method. Conditions: A (75/25 ACN/water) to B (1/1 IPA/ACN) linearly at 15 minutes. All other conditions as listed for phytosterol method. 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 at these temperatures) caused significant baseline drift that was not observed in the ELSD. The UV signal is still useful, especially for the low levels of some of the less abundantly fatty acid methyl esters (FAME’s) produced during the saponification reaction. Sample taken at 90 minutes into ambient nmr 100mg/l, KCl/NaOH. Less than 10% TS remaining after 180 minutes.

Conclusion

The Agilent 1200 Rapid Resolution 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.