Phytosterols by HPLC with the Thermo Scientific Corona *ultra* Charged Aerosol Detection

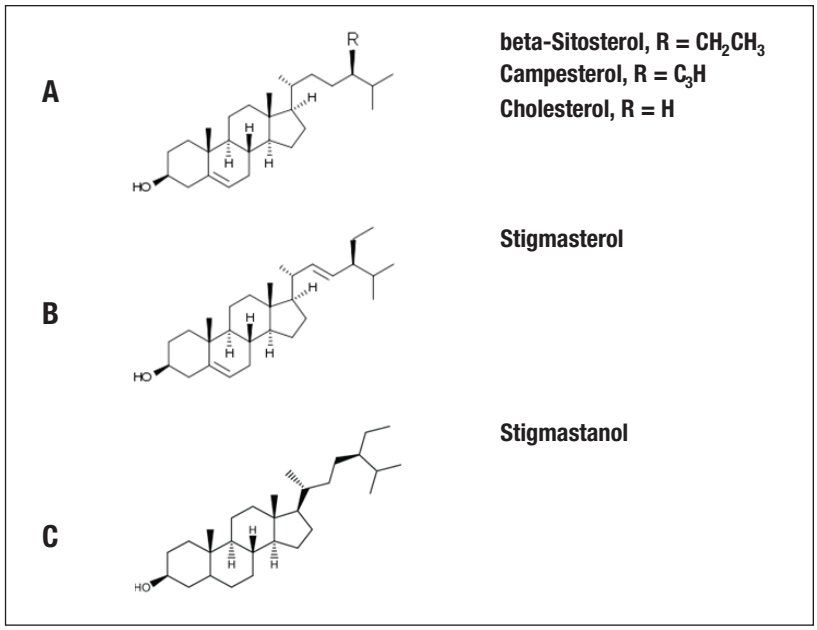

Figure 1. Chemical structures of the five phytosterol compounds measured in this study.

Phytosterols are a group of naturally occurring steroid alcohols found in plants and their oils. Phytosterols are a key structural component of plant cell membranes, assuming the role that cholesterol plays in mammalian cells. There is considerable interest in phytosterols as a dietary supplement because they are reported to lower cholesterol levels and have a positive impact on cardiovascular diseases. However, recent research has suggested that phytosterols supplementation may aggravate atherosclerosis and lead to aortic valve stenosis.

Phytosterols are commonly measured by using gas chromatography (GC). However, this approach is time consuming requiring saponification of the sample, a number of extractions, and derivatization. Presented here is a simplified method, using reversed-phase high-pressure liquid chromatography (HPLC) and charged aerosol detection (Thermo Scientific Dionex Corona *ultra* Charged Aerosol Detector). The Corona™ *ultra* Charged Aerosol Detector is a sensitive, mass-based detector that is sufficiently sensitive to quantify analytes in the low nanograms on-column range. The Corona also has response factors that are similar across many compounds, allowing for analytes' mass-percent values to be estimated using peak area-percent.

The chemical structures of five standards, campesterol, cholesterol, stigmasterol, beta-sitosterol, and stigmastanol, are shown in Figure 1. The structures of these compounds are very similar, with only small changes in alkyl content and/or unsaturation, yet resolution was still possible using a reversed-phase column. These standards and a sample of red palm oil were dissolved in methanol/chloroform and analyzed directly, using RP-HPLC-charged aerosol detector.

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The HPLC-charged aerosol detector method is simple to implement, shows good linearity and sensitivity, and is capable of measuring numerous phytosterols in plant extracts. This approach can be used to examine product purity, supplement content, and possible adulteration.

Method Parameters

Column:	C8, 150 × 4.6 mm, 2.7 µm, 50 °C
Gas:	Nitrogen
Gas Pressure:	35.0 psi
Nebulizer Heater:	30 °C
Filter:	Medium
Mobile Phase A:	Methanol/water/acetic acid (750:250:4)
Mobile Phase B:	Acetone/methanol/tetrahydrofuran/acetic acid (500:375:125:4)
Gradient:	See Table 1
Flow Rate:	0.8 mL/min
Run Time:	25–35 min
Injection Volume:	5 µL at 20 °C
Sample Solvent:	Methanol/chloroform (1:1)

Table 1. UHPLC gradient

Time	%A	%B
0.0	100.0	0.0
1.0	100.0	0.0
3.0	70.0	30.0
20.0	62.0	28.0
20.1	100.0	0.0
25.0	100.0	0.0

Results and Discussion

The method was able to completely resolve all five phytosterols in under 15 minutes. Standards (Sigma-Aldrich, St. Louis, USA) were analyzed by using a shorter gradient (0%B for 5 minutes at a time of 20.1 minutes). However, the full gradient is required for many biological samples in order to elute the highly retained hydrophobic compounds.

The response of the phytosterols was linear over 3 orders of magnitude. Replicate chromatograms (n = 3) for different amounts of each phytosterol (4.9–313 ng o.c.) are shown in Figure 2. The method is also precise, with relative standard deviation (RSD) values less than 6% for all phytosterols greater than 10 ng o.c. A calibration plot, from 4.9–313 ng o.c. of each phytosterol is presented in Figure 3, and shows good linearity (R^2 = 0.997–0.999).

Sensitivity was evaluated by using the results from the 4.9 ng standard (Figure 4). The estimated limits of detection (LODs) (S/N = 3) and limits of quantification (LOQs) (S/N = 10) for the five phytosterols are presented in Table 2. Typical LODs and LOQs were <5 ng and <10 ng, respectively.

The method can be used to measure phytosterols in complex matrices. For example, red palm oil was dissolved in methanol/chloroform and analyzed directly. The sample chromatogram, overlaid with the 156 ng standard chromatogram, is presented in Figure 5. Only the phytosterol region is shown, for clarity. All five phytosterols were found in the palm oil sample. The method is compatible with mass spectrometry, which can be used to help identify other peaks in the chromatogram.

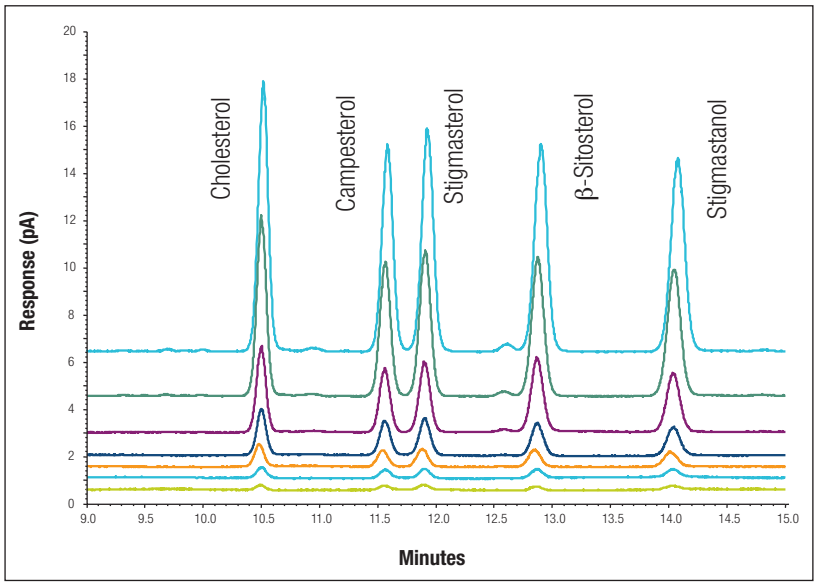

Figure 2. Phytosterols by RP-HPLC-charged aerosol detector from 4.9 to 313 ng o.c.

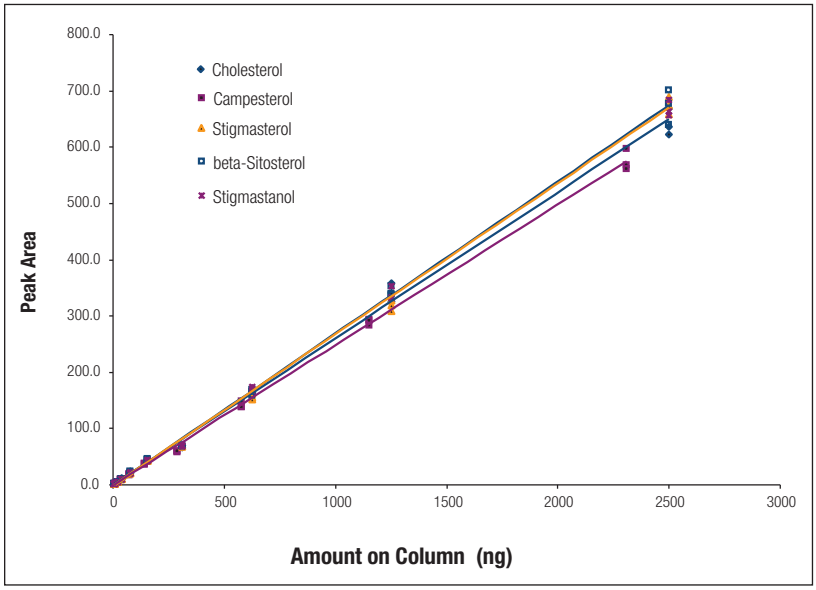

Figure 3. High-sensitivity analysis of phytosterols (4.9 ng each o.c.).

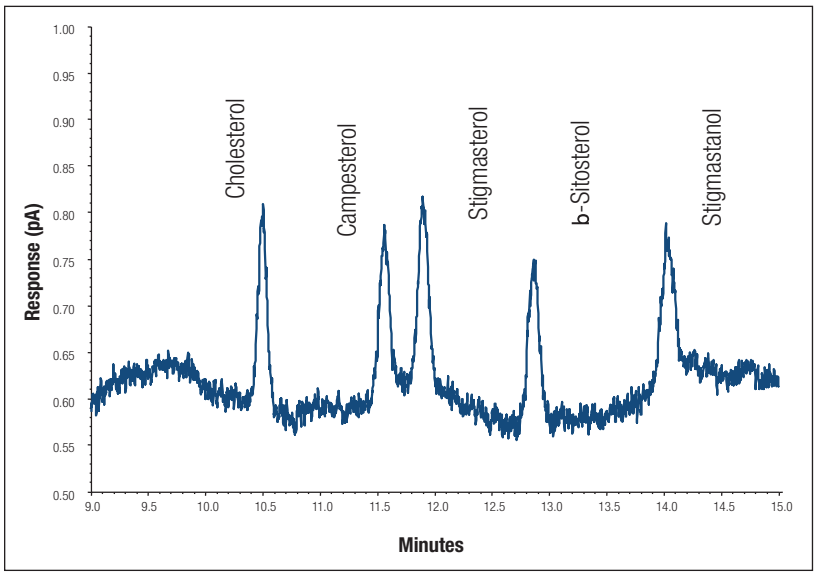

Figure 4. Calibration plots for phytosterols.

Table 2. LOD and LOQ values for the five phytosterols

Phytosterol	LOD (ng)	LOQ (ng)
Cholesterol	2	6
Campesterol	2	7
Stigmasterol	2	7
beta-Sitosterol	2	8
Stigmastanol	3	9

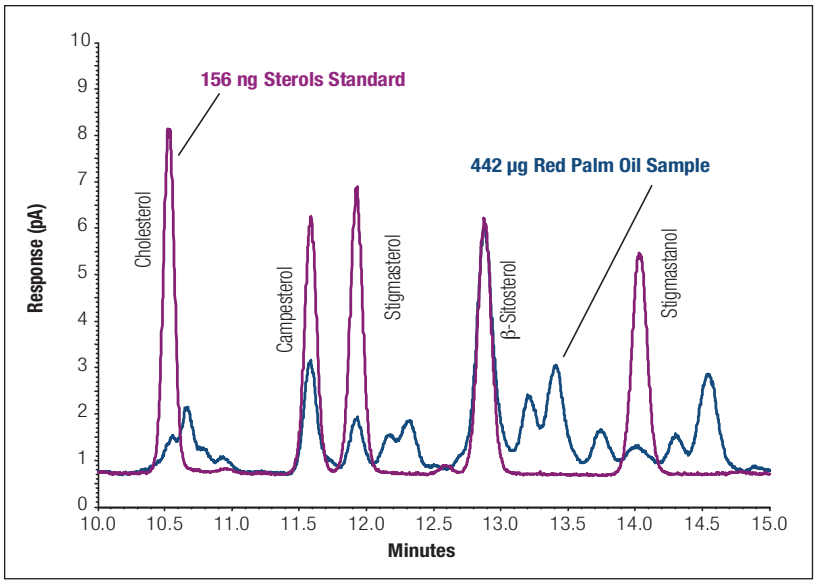

Figure 5. HPLC-charged aerosol detector chromatogram of red palm oil (442 µg) overlaid with chromatogram of phytosterol standard (156 ng o.c.).

Conclusion

This application note describes a simple, direct analytical method for the separation and measurement of five phytosterol compounds. The method is sensitive down to 3 ng (o.c.), precise, and linear over three orders of magnitude. The method does not require derivatization or extensive sample preparation. Samples are simply diluted prior to analysis.

The practical use of the method is illustrated using red palm oil. All five phytosterols could be measured in this complex matrix.

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