Application Note

UHPLC - 410009X

Analysis of sterols by Ultra High-Performance Liquid Chromatography

Introduction

Sterols can be derived from plant material - plant sterol (phytosterol), or from animals - animal sterol (zoosterol). A commonly occurring zoosterol is cholesterol, this molecule is an important component found in animal cell membranes.

Recent research has revealed that there is no significant relationship between the consumption of dietary cholesterol and serum (blood) cholesterol levels. However, in general, dietary patterns that are lower in cholesterol are recommended for reducing the risks of cardiovascular disease.

Phytosterol inhibits the absorption of cholesterol by internal organs, so it helps in reducing physiological cholesterol levels. Therefore, the analysis and quantitation of sterols is important in food production and quality control.

This application note illustrates the analysis of several different sterol standard samples (four types of phytosterols and cholesterol (shown in Fig. 1)) using a UHPLC column with 1.9 µm particles. Additionally, a mayonnaise containing phytosterol and a conventional mayonnaise have also been analyzed

Keywords

Sterol, UHPLC, Phytosterol, Cholesterol, Unifinepak C18, UV detector, HPLC

JASCO LC-4000 UHPLC system
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Experimental

Experimental conditions	
Column	Unifinepak C18 (2.0 mm ID x 150 mmL, 1.9 µm)
Eluent	A; Acetonitrile/Water (95/5), B; THF
Flow rate	0.5 mL/min
Column temperature	40°C
Detection	UV detection (Wavelength: 278 nm)
Injection Volume	1 µL
Standard Sample	1. Ergosterol 2. Cholesterol 3. Campesterol 4. Stigmasterol 5. β-Sitosterol (0.5 mg/mL each)

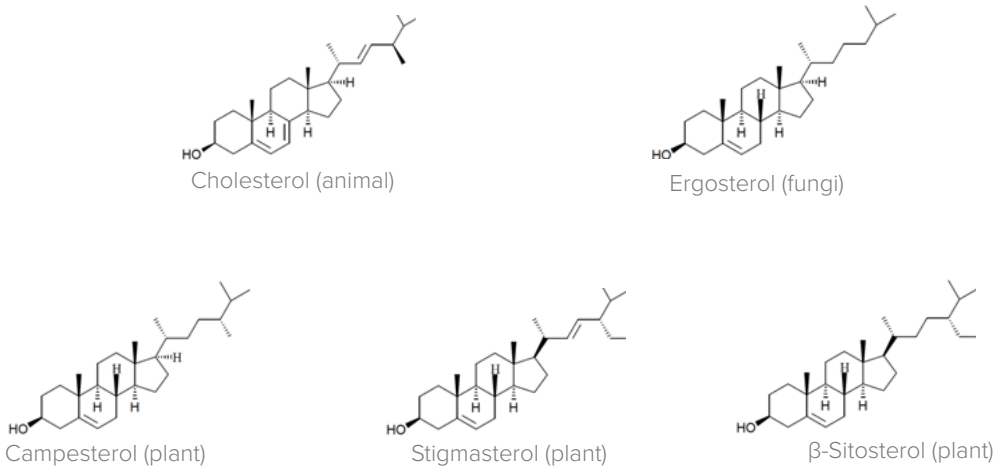

Fig. 1 Structural formula of sterols

Results

Fig.2 Chromatogram of sterol standard sample. THF solutions of 10 mg/mL sterols were prepared as stock solutions, and diluted with acetonitrile to prepare 0.5 mg/mL standard samples.

As shown in Fig.2, a high resolution UHPLC column (2.0 mmID x 150 mmL, 1.9 µm particles) completely separates campesterol (peak 3) and stigmasterol (peak 4) with baseline resolution.

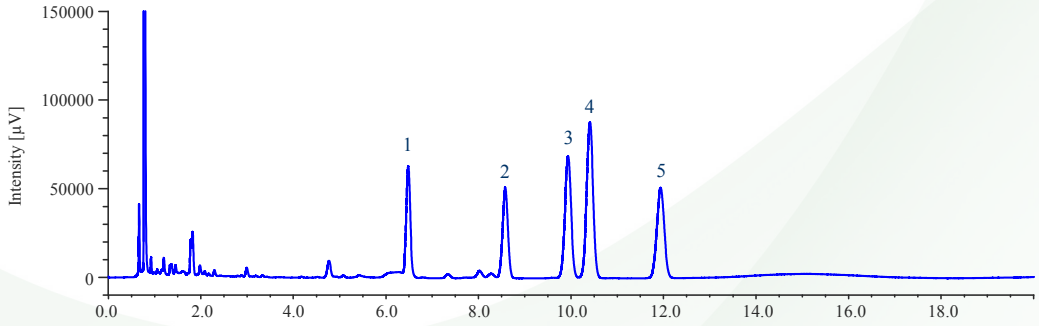

Fig.2 Chromatogram of the standard sample of sterols

1. Ergosterol, 2. Cholesterol, 3. Campesterol, 4. Stigmasterol, 5. β-Sitosterol (0.5 mg/mL each)

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Next, two mayonnaise samples were measured; the first contained phytosterol, and the second a more commonly used mayonnaise. Figs. 3 and 4 show the pretreatment procedure of each sample. As shown in the Figs. 3 and 4, the pretreatment procedures are different for each sample.

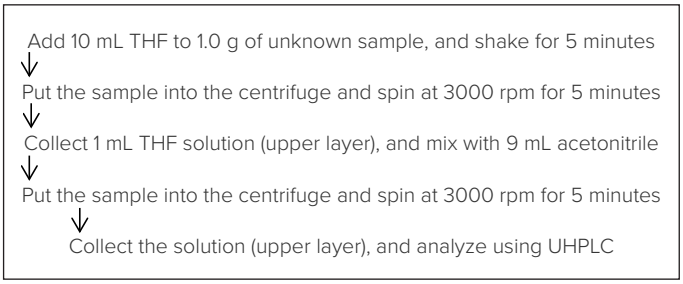

Fig. 3 Pretreatment procedure of a sample containing phytosterol

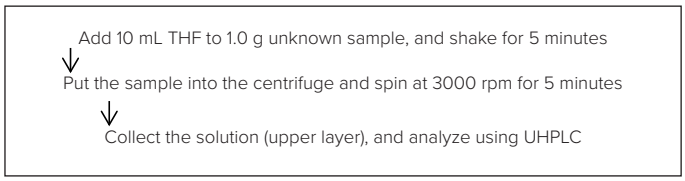

Fig. 4 Pretreatment procedure of common mayonnaise

As shown in Fig. 5, UHPLC could be used to clearly identify several different sterols - phytosterol; campesterol, stigmasterol, and β-sitosterol.

In this measurement, components which are strongly retained are included in this sample, these are difficult to remove during pre-treatment. Therefore, the column must be flushed with THF (B solvent) between sample measurements.

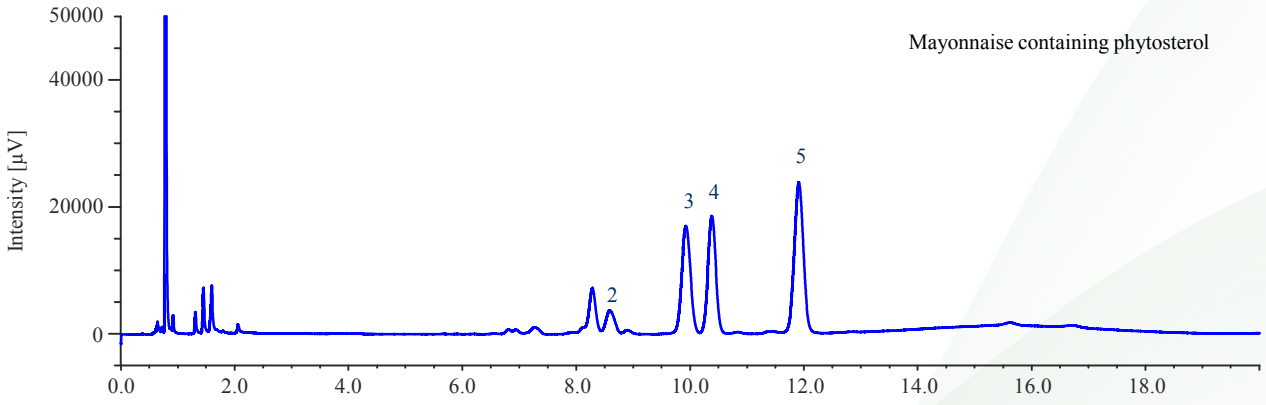

Fig.5 Chromatogram of sample containing phytosterol

2. Cholesterol, 3. Campesterol, 4. Stigmasterol, 5. β-Sitosterol

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During pretreatment of the sample of common mayonnasie, dilution with acetonitrile was not performed. Therefore, it should be noted that the standard sample concentration in Fig. 6 is ten times higher than that in Fig. 5.

Fig. 6 indicates that a low level of phytosterol is present in the common mayonnaise. This may be due to plant oil being used in the production of this mayonnaise.

These results show that the UHPLC is a fast, accurate and reliable method for the analysis of sterols.

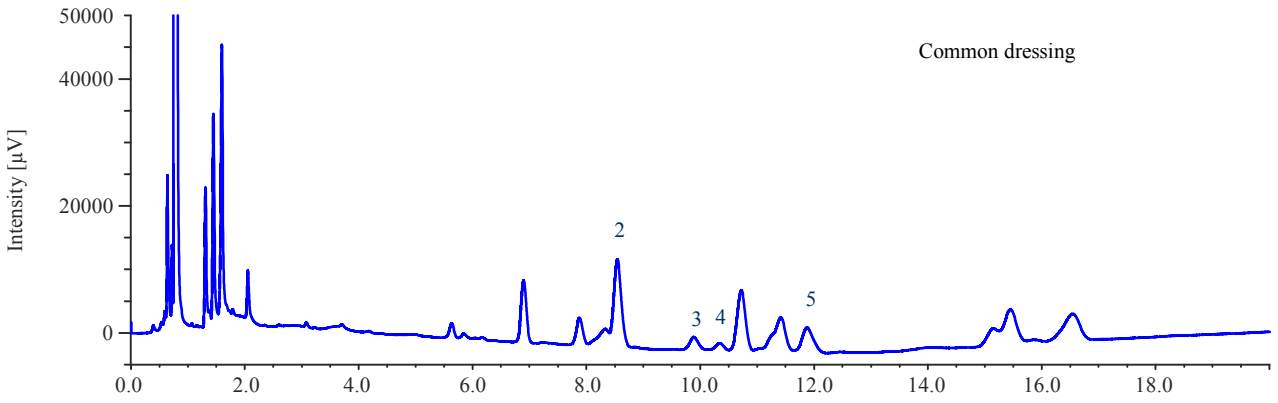

Fig.6 Chromatogram of common semi-solid dressing

2. Cholesterol, 3. Campesterol, 4. Stigmasterol, 5. β-Sitosterol

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