

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, which 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.

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

Experimental Condition

Column: Unifinepak C18 (2.0 mmID x 150 mmL, 1.9 μm)

Eluent : A; Acetonitrile/Water (95/5), B; THF

Flow rate: 0.5 mL/min

Column temp.: 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)

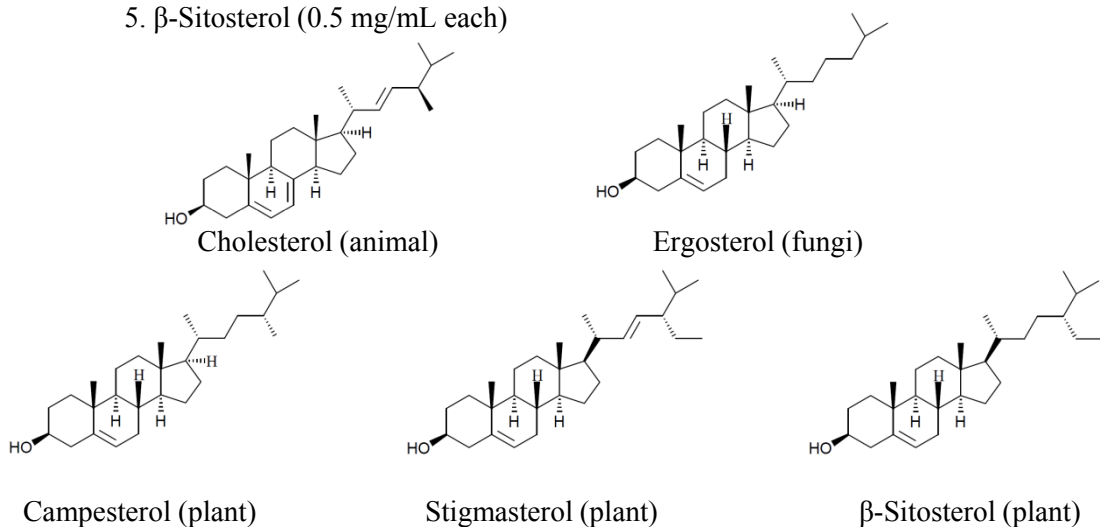


Fig.1 Structural formula of sterols

Result

Fig.2 shows the 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 μ m particles) completely separates campesterol (peak 3) and stigmasterol (peak 4) with baseline resolution.

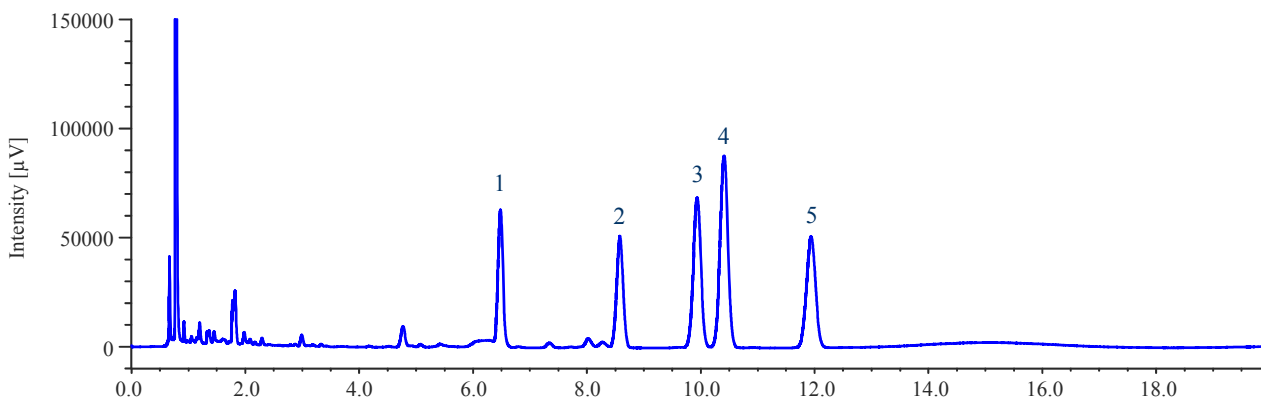


Fig.2 Chromatogram of the standard sample of sterols

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

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.

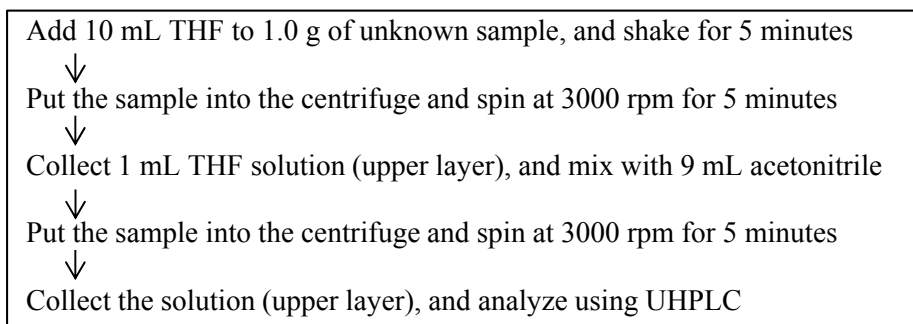


Fig. 3 Pretreatment procedure of a mayonnaise containing phytosterol

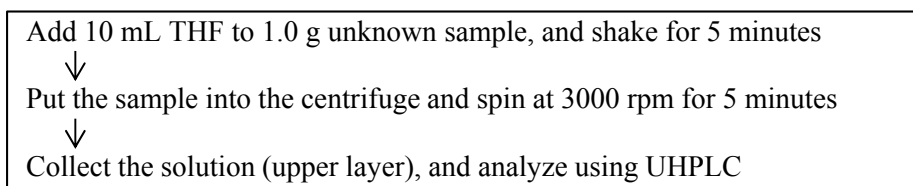


Fig. 4 Pretreatment procedure of common mayonnaise

Fig. 5 shows the chromatogram of a mayonnaise containing phytosterol. 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.

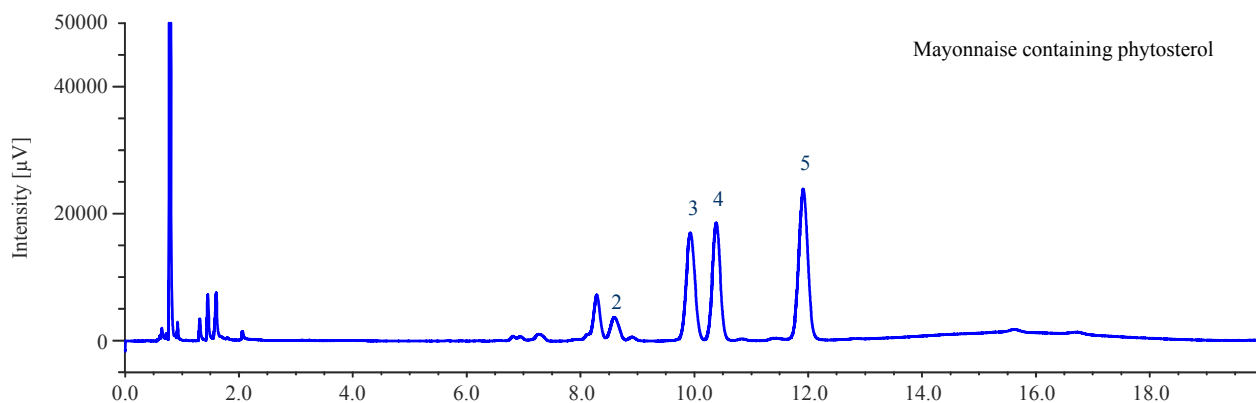


Fig.5 Chromatogram of mayonnaise containing phytosterol
2. Cholesterol, 3. Campesterol, 4. Stigmasterol, 5. β -Sitosterol

Fig. 6 shows the chromatogram of common mayonnaise. During pretreatment of the sample of common mayonnaise, 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.

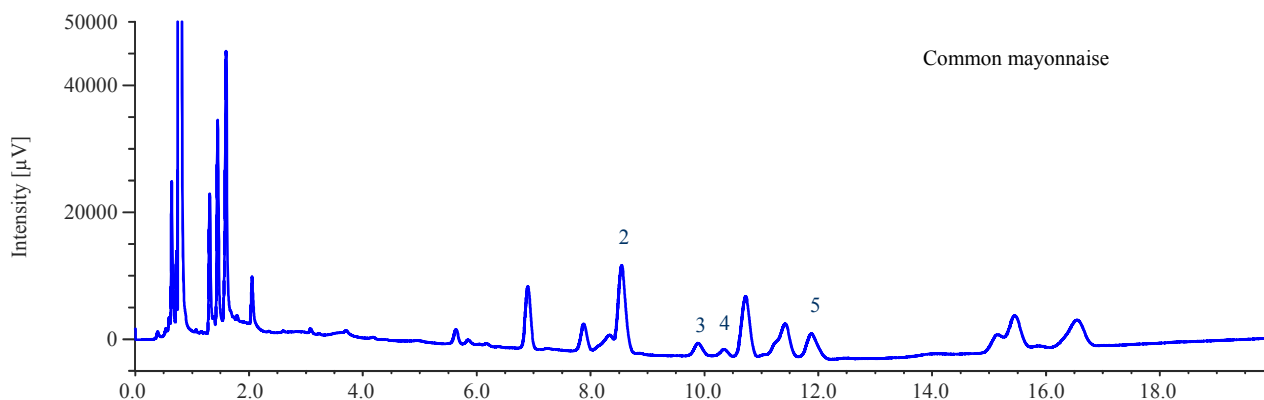


Fig.6 Chromatogram of common mayonnaise
2. Cholesterol, 3. Campesterol, 4. Stigmasterol, 5. β -Sitosterol