Research Article

Determination of Phytosterols in vegetable oils by GC-MS method

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Abstract

In this study, a method for determination of six phytosterols by gas chromatography-mass spectrometry with derivatization in vegetable oils was validated. The samples were hydrolyzed in an alkaline media at 70°C for 60 min. Then, the samples were performed liquid-liquid extraction with toluene. The phytosterols are derivatized to trimethylsilyl ethers and then analyzed by gas chromatography-mass spectrometry. The limit of detection and limit of quantification was 5 and 15 mg/kg, respectively. Recoveries of six phytosterols were between 93.5% and 101%.

Keywords: *GC-MS, phytosterols, vegetable oils.*

1. INTRODUCTION

Phytosterols are plant compounds that have similar chemical structures and biological functions as cholesterol. The most common phytosterols are sitosterol, campesterol, stigmasterol. Phytosterols have properties and biological effects, such as hypocholesterolemic, anti-inflammatory, anti-oxidative, and anti-tumor. The daily doses are 2-3 g of phytosterols to reduce both the total cholesterol and low-density lipoprotein cholesterol levels in the blood by 10% [6]. They are widely used in pharmaceuticals, nutritional supplements, and cosmetics [9].

In recent years, a few analytical methods have been developed for the determination of phytosterols for vegetable oils, nuts, foods. A method for the determination of cholesterol and four phytosterols in foods without derivatization by gas chromatography-tandem mass spectrometry was developed by Yan-Zong Chen et al. Limit of quantification was 2 mg/kg. The recoveries of cholesterol and four phytosterols from general food were between 91 and 100% [5].

Maria et al. developed the method for the determination of sterols in vegetable oils by ultraperformance liquid chromatography with atmospheric pressure chemical ionization mass spectrometry detection. The limits of detection of six sterols were from 0.03 to 0.07 µg/mL. The content of 14 sterols in eight vegetable oils such as avocado oil, corn oil, extra virgin olive, grapeseed oil, hazelnut oil, peanut oil, soybean oil, and sunflower oil was established. The content of beta-sitosterol was the main sterols in all cases [1].

Our study aimed to validate a simple and accurate method for the determinations of six phytosterols including campesterol, beta-sitosterol, stigmasterol, Δ7-stigmasterol, Δ5-avenasterol, Δ7-avenasterol in some vegetable oils and investigated the phytosterols contents and composition of phytosterols of different kinds of vegetable oils.

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2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Campesterol, beta-sitosterol, stigmasterol, Δ5-avenasterol, Δ7-avenasterol, Δ7-stigmasterol were obtained from Toronto (Canada). 5α-Cholestane used as an internal standard was purchased from Sigma-Aldrich. Toluene, dimethylformamide, hexamethyldisilane, potassium hydroxide, trimethylchlorosilane, sodium sulfate, acetone ethanol, n-heptane were obtained from Merck (Germany).

2.2. Instruments

A Trace 1310 Thermo GC equipped with an ISQ 7000 Thermo mass detector was used for the identification of phytosterols from vegetable oils. The analytes were separated using a TG-5MS Thermo column (30m × 0.25 mm ID × 0.25μm). The oven temperature program was set as follows: the initial temperature was 50°C, held for 1 min, and then the temperature ramped at 40°C/min to 280°C and was held for 20 min. The injection volume was 1 μL in splitless mode. Helium was used as the carrier gas at a flow rate of 1 mL/min. The temperatures of the injector, transfer line, and ion source were set to 260, 280, and 250°C, respectively. The mass scan range was 50 - 600 m/z. No mass spectrum was collected during the solvent delay for the first 6 min of each run. The scan was performed by selected ion monitoring mode (SIM).

Other equipment included magnetic stirrer-hot plate with variable speed and heat controls, rotary evaporator with glass condenser flask between concentration flask and metal shaft. Glasswares used included 250-mL Erlenmeyer flasks, 500-mL separatory funnel, volumetric flasks, glass funnels, and graduated cylinders.

2.3. Sample preparation

Some vegetable oils were collected from the markets such as soybean oil, rapeseed oil, sunflower oil, rice bran oil, palm oil. All oil samples were store in darkness at 4°C for further analysis.

The sample preparation referred to AOAC Official methods 2007.03 [4] with some modifications.

Saponification: Each sample was weighed around 2.00 ± 0.1 g into a 250 mL Erlenmeyer flask, and added 50 mL of 20% KOH in ethanol. The mixture was placed in an oven at 70°C for 60 min, the mixture was gently shaken during the hydrolysis process. After that, the solution was left to cool down at room temperature.

Extraction: The saponified sample solution was transferred into a 500 mL separatory funnel and extracted twice by 60 mL toluene. The toluene fractions were combined and washed twice by 100 mL 5% NaCl in water. The remaining toluene layer was poured into a glass funnel containing a filter paper and 20 g anhydrous Na_2SO_4 into a 125 mL Erlenmeyer flask. The whole solution was transferred into a 100 mL round-bottomed flask and evaporate the contents to dry. The residue was resuspended by 10.0 mL dimethylformamide.

Derivatization: one milliliter of working standard or sample solution was placed in a 15 mL centrifuge tube. Then, 0.2 mL hexamethyldisilane and 0.1 mL trimethylchlorosilane were added into the tube and shaken vigorously on a vortex mixer for 30 secondes. The solution was left for 15 min at room temperature before adding 1.0 mL of 5α-cholestane IS solution and 10 mL

water. The mixture was shaken well and centrifuged for 2 min. Finally, the n-heptane layer was transferred into a vial and analyzed by GC-MS.

3. RESULTS AND DISCUSSION

3.1. Method validation

3.1.1. Specificity

Blank samples, spiked samples, and standard solutions were analyzed. Six phytosterols were detected on the GC-MS system. The concentration of phytosterols was measured by using the single ion monitoring mode (SIM). Each compound was identified by two ions that were shown in Table 1. Beta-sitosterol, Δ5-avenasterol, and Δ7-avenasterol were eluted at the nearby time. However, it does not affect the detection as the identification ions were different.

Table 1. *Identification ions and retention time of phytosterols*

Compounds	***Ions (m/z)***	***Retention time (min)***
Campesterol	343.47, 382.51	16.24
Stigmasterol	255.34, 394.53	16.75
Beta sitosterol	357.48, 396.53	17.78
Δ5-avenasterol	296.37, 386.47	17.95
Δ7-avenasterol	255.32, 343.39	17.92
Δ7-stigmaterol	351.47, 377.48	17.53

Figure 1 shown the chromatogram of a blank sample, a spiked sample, and a standard solution of beta-sitosterol. One peak was detected at the retention time of around 17.78 min in the chromatograms of the spiked sample and the standard solution. The chromatogram of the blank sample did not show any peak at that time. Overall, the specificity of the method meets the requirements of AOAC International.

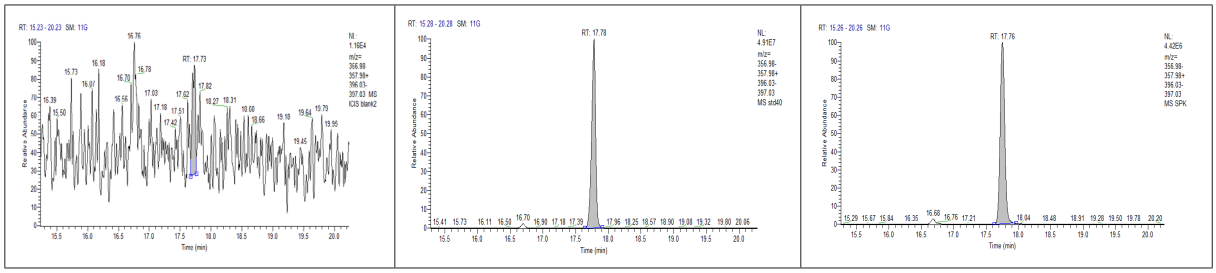

Figure 1. *Chromatogram of a blank sample, a spiked sample, and a standard solution of beta-sitosterol*

3.1.2. The linearity, limit of detection, limit of quantitation

The linearity of the chromatographic response was tested using five concentrations in the range of 1 - 20 mg/L. The TIC and SIM of six phytosterols at 20 mg/L are shown in Figure 2. The linear regression (R^2) for all the calibration curves used in this study was ≥ 0.995 (Table 2).

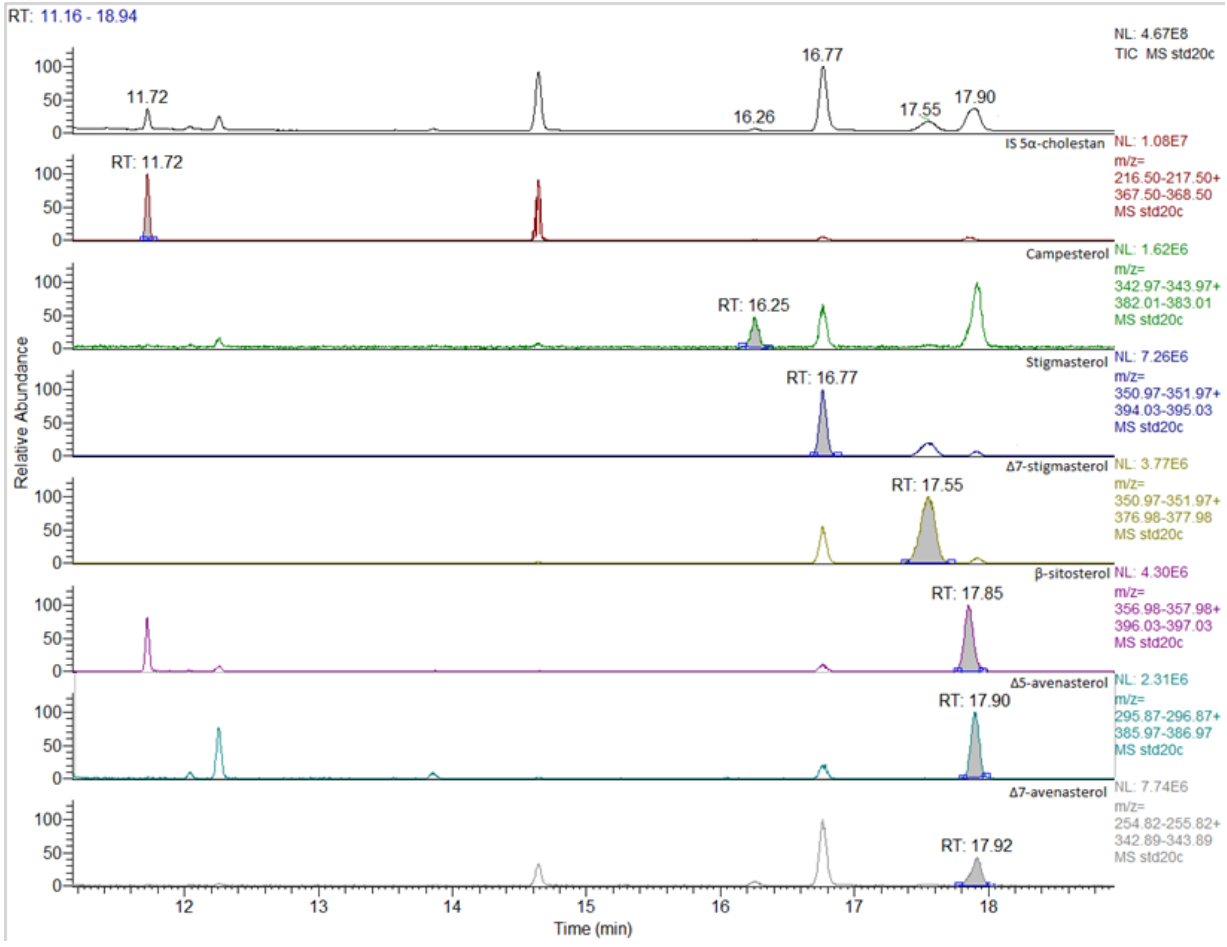

Figure 2. *Chromatogram of phytosterols standard solution 20 mg/L*

Limits of quantitation were evaluated by spiking the lowest concentration of six phytosterols in the blank samples. All signal to noise ratios of the analyte's peaks after sample treatment at a low spiked concentration have to be less than 10 for all double tests. LOQs were 15 mg/kg for six phytosterols. LODs were proposed 5 mg/kg for six phytosterols.

Table 2. *Linear correlation coefficients of the phytosterols content*

Compounds	*Linear equation*	*Correlation coefficient (R^2)*
Campesterol	y = 0.2342x - 0.1704	0.999
Stigmasterol	y = 0.0767x - 0.0432	0.999
Beta-sitosterol	y = 0.041x - 0.0352	0.998
Δ5-avenasterol	y = 0.0218x - 0.0201	0.996
Δ7-avenasterol	y = 0.0367x - 0.0275	0.998
Δ7-stigmasterol	y = 0.0594x - 0.0615	0.997

Oil samples were spiked with six standard phytosterols at low, middle, and high concentrations, and repeatability and recovery tests (n = 6) were performed to verify the precision and accuracy of the method. The recoveries and repeatabilities of six phytosterols were shown in Table 3.

Table 3. *Within-day precisions and recoveries of selected phytosterols*

Compounds	*Spiking amount (mg/100g)*	*Within-day precision (RSD, %)*	*Mean Recovery (%)*
Campesterol	15	4.0	98.1
	30	3.2	100
	60	4.1	100
Stigmasterol	15	4.2	99.3
	30	3.0	96.4
	60	2.2	93.5
Beta sitosterol	15	2.6	99.6
	30	2.9	101
	60	2.1	95.7
Δ5-avenasterol	15	3.3	96.2
	30	2.6	98.5
	60	3.8	99.4
Δ7-avenasterol	15	3.9	96.8
	30	3.5	100
	60	3.7	100
Δ7-stigmaterol	15	4.2	97.8
	30	3.4	99.4
	60	3.6	100

The relative standard deviation (RSD) of the repeatability for determination of the selected phytosterols ranges from 2.1 to 4.2%, which indicates that the precision of the analytical method is acceptable over the three concentration levels. The mean recoveries range from 93.5 to 101%. The relative standard deviation and recovery are in the allowed range AOAC [3].

3.4. Application to real vegetable oil samples

In this study, five types of vegetable oil were analyzed using the developed method. The contents of six phytosterols were shown in Table 4.

Table 4. *Contents of six phytosterols in vegetable oils*

Oil type	*Soybean oil (mg/100g)*	*Sunflower oil (mg/100g)*	*Rice bran oil (mg/100g)*	*Palm oil (mg/100g)*	*Rapeseed oil (mg/100g)*
Campesterol	47.5	42.2	130	27.1	33.0
Stigmasterol	54.1	82.7	6.65	96.1	33.3
Beta-sitosterol	825	850	1,064	218	992
Δ5-avenasterol	67.9	45.4	25.2	107	28.1
Δ7-avenasterol	143	136	189	40.7	27.7
Δ7-stigmaterol	127	135	22.9	51.6	76.3
Total	1,264	1,291	1,438	540	1,190

Contents of beta-sitosterol in the oil samples are highest among all the analytes. Beta-sitosterol in soybean oil, sunflower oil, rice oil, and canola oil accounts for over 50% of the total phytosterol content (six types), the results are in line with other previous studies [1, 8], the comparation results are shown in Table 5. The total phytosterol content was lowest in palm oils, highest in rice oil, and similar in the other tested oils. The concentrations of each phytosterol in palm oil are far different.

Table 5. *Proportions of beta-sitosterol in total phytosterols fraction in some studies*

Oil type	***Soybean oil***		***Sunflower oil***			***Rapeseed oil***	
Lab	Our study	[1]	Our study	[1]	[8]	Our study	[8]
Fraction (%)	65.2	56.7	65.8	69.6	78.7	83.3	81.2

4. CONCLUSION

The gas chromatography-mass spectrometry method was validated for the simultaneous analysis of six phytosterols in vegetable oils. The method has a high specificity and accuracy, meeting the requirements of AOAC. The method has been used for the determination of phytosterols in five kinds of edible oils on the local market. These results can be used for further studies for the determination of the composition of other oils and substances of the phytosterol group, contributing to a better understanding of the importance of phytosterols for human health.

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Xác định Phytosterols trong dầu thực vật bằng sắc ký khí khối phổ GC-MS

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Tóm tắt

Trong nghiên cứu này, phương pháp sắc ký khí khối phổ kết hợp với việc dẫn xuất hóa đã được thẩm định và áp dụng để phân tích đồng thời sáu chất nhóm phytosterol trong dầu thực vật . Mẫu được thủy phân trong tủ ấm 70°C trong 60 phút, rồi được chiết với toluen. Các chất nhóm phytosterol được dẫn xuất với trimethysilyl ether trước khi phân tích trên hệ thống sắc ký khí khối phổ. Giới hạn phát hiện và giới hạn định lượng của phương pháp lần lượt là 5 và 15 mg/kg. Độ thu hồi của cả sáu chất nhóm phytosterol trong khoảng từ 93,5 đến 101%.

Từ khóa: *GC-MS, phytosterols, dầu thực vật.*