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Journal of Chromatography A, 935 (2001) 237–248

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Analysis of free and esterified sterols in fats and oils by flash chromatography, gas chromatography and electrospray tandem mass spectrometry

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Abstract

A method for the analysis of free and esterified sterols has been developed. Fat or oil samples were separated on solid-phase extraction silica gel columns into a sterol ester fraction, a fraction of triacylglycerols, and a free sterol fraction containing partial acylglycerols and residual triacylglycerols. Sterol esters and acylglycerols of the free sterol fraction were transesterified to methyl esters. The fatty acid methyl esters from sterol ester fraction and the free sterols from sterol ester fraction and free sterol fraction were determined by GLC. Precursor ion electrospray MS–MS of sterol fragment ions of sterol ester fractions were recorded and used for determination of sterol ester proportions in butterfat and vegetable oil samples. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Fats; Oils; Flash chromatography; Food analysis; Sterols

1. Introduction

A high cholesterol level in serum is considered an apparent risk factor for coronary heart disease. Thus, recent findings that plant sterols and sterol esters lower serum cholesterol levels, have focused special interest on the analytical methods applicable in their determination [1]. In the most widely used methods, a lipid extract is subjected to alkaline hydrolysis. After isolation of the unsaponifiables by extraction or solid-phase extraction (SPE) using normal- or reversed-phase columns, the sum of free and esterified sterols is determined by gas–liquid chromatography (GLC) of silylated samples [2]. In several studies, the fractions of free and esterified sterols have been separated by column chromatography on silica gel or Florisil or by thin-layer chromatography on silica gel plates [3,4]. Free sterols are silylated and determined by capillary GLC. Sterol esters are hydrolyzed under alkaline or acidic conditions, silylated and determined by capillary GLC [3]. Alternatively, intact sterol esters have been determined by high-temperature GLC on apolar and polar silicone columns [5–9]. On-line liquid chromatography–gas chromatography (LC–GC) after derivatization of free sterols to pivalic acid esters [10] or trimethylsilyl ethers have been reported [11–13] for transesterified edible oils [10–12] or vegetable oil methyl esters (diesel fuel substitutes) [13]. Using LC–GC, both free and total sterol esters [13] or the sum of free and esterified sterols [11] have been determined.

Ammonium adducts of sterol esters $[M+NH_4]^+$ have been used successfully for structural elucidation

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PII: S0021-9673(01)01315-2

of sterol esters by gas chromatography–chemical ionization mass spectrometry [14]. GC–MS using positive ion electron ionization and negative ion chemical ionization has been shown applicable in characterization of intact sterol esters [15]. Soft ionization with electrospray (ESI) allows also the formation of $[M+H]^+$ and $[M+NH_4]^+$ ions. Coupling of ESI with a triple quadrupole instrument and measurements in precursor ion mode enable the detection of compounds cleaving a specific group in collision with neutral gas. This offers a powerful tool for characterization of a sample mixture of closely-related compounds. Although tandem mass spectrometry has been used extensively in the structural analysis of triacylglycerols [16,17] and phospholipids (cf. literature cited in Ref. [18]), to our knowledge the only research paper dealing with identification and quantification of sterol esters by electrospray tandem mass spectrometry (in product ion, precursor ion and neutral loss modes) has been published very recently [18].

Our aim was to study the applicability of flash column chromatography on short silica gel columns to the separation of sterol esters and free sterols. We studied the possibility to determine the sterol composition and content of free sterols and sterol esters and the fatty acid composition of sterol esters using simple equipment and the transesterification of separated fractions. The sterol ester fraction was transesterified to methyl esters and sterols and esterified fatty acids were determined by GLC. The free sterol fraction was subjected to transesterification to degrade the diacylglycerols and the residual triacylglycerols, co-eluted with free sterols. Our objective was also to study the applicability of electrospray tandem mass spectrometry for structural characterization and quantification of intact sterol esters.

2. Experimental

2.1. Materials

Silica gel 60 for column chromatography was purchased from Merck (Darmstadt, Germany). Characteristics of the gel given by the manufacturer were: particle size range 0.040–0.063 mm, pore volume 0.71–0.78 ml/g, specific area 490 m^2/g, drying loss<9.0%, pH 6.5–7.5. All solvents used were purchased from Merck (Darmstadt, Germany) and Rathburn (Walkerburn, UK) and were analytical-reagent grade or HPLC grade. Lipid standards of 99% purity were from Sigma (St Louis, MO, USA), Nu Chek Prep (Elysian, MN, USA), Merck or Fluka (Buchs, Switzerland). Vegetable oils were bought in a local store. The preparation of modified butteroil using *Pseudomonas fluorescens* lipase-catalyzed interesterification is described elsewhere [19].

2.2. Flash column chromatography

The 5.4×1.5 cm chromatographic column was prepared in a 15 ml Isolute filtration column tube with micro pore filters (International Sorbent Technology, Hengoed, UK) using a dry packing method [20]. Before introduction of the sample the column was solvated and equilibrated by introducing 50 ml of hexane through the sorbent. A flow of ca. 9 ml/min was achieved by applying vacuum to the lower end of column with Vac Elut Accessory (Analytichem International, Harbor City, CA, USA). Cholesteryl heptadecanoate was added as internal standard in fat and oil samples (100 mg). In addition, cholesterol was added as internal standard to vegetable oil samples. The sample was dissolved in 0.5 ml chloroform. A stepwise elution scheme was used in the fractionation (Table 1).

2.3. Transesterification and silylation

The sterol ester fraction was dissolved in 100 μl methyl *tert*.-butyl ether in a 12-ml screw cap vial and 6 μl 2 *M* sodium methoxide methanolic solution was added. After vigorous mixing in a Vortex mixer for 1 min the mixture was allowed to stand at ambient temperature for 40 min, then mixed again for 1 min in the Vortex mixer. To the vial were added 1 ml hexane and ca. 50 mg anhydrous sodium sulphate. The vial was mixed briefly in the Vortex mixer, and centrifuged at 700 *g*. Two 0.5-μl injections into the gas chromatograph were made for the analysis of fatty acid methyl esters. An additional 7 ml hexane, 1.2 μl glacial acetic acid, and ca. 0.4 g. anhydrous sodium sulphate were added, mixed briefly in the Vortex mixer, and centrifuged at 700 *g*. The solution was decanted to another 12-ml screw cap vial, in

Table 1
Stepwise elution scheme for separation of free sterol and sterol esters

Eluting solvent mixture	Volume (ml)	Lipids eluted
Dichloromethane–hexane (22:78, v/v)	40	Sterol esters
Dichloromethane–hexane (65:35, v/v)	60	Triacylglycerols
Acetone–dichloromethane (6:94, v/v)	40	Free sterols, diacylglycerols, and residual triacylglycerols

which secondary standard betulin or cholestanol in 3 ml chloroform had been added for the determination of the recovery. The sodium sulphate was washed twice with 0.5 ml hexane. After centrifugation the hexane washings were decanted to the other vial. Prior to the gas chromatographic analysis an aliquot of hexane–chloroform solution was evaporated to dryness in a gentle stream of nitrogen and equal volumes of N,O-bis(trimethylsilyl)trifluoroacetamide containing 1% of trimethylchlorosilane and dry pyridine were added. The sample was heated at 60°C for 30 min.

2.4. Gas chromatography

A Carlo Erba 5300 gas chromatograph (Milan, Italy) was used, equipped with a flame ionization detector, a constant pressure constant flow cp-cf module, and cold on-column injector. The carrier gas (hydrogen, 5.0 grade, Aga, Finland) inlet pressure was 0.100 MPa. The column used in the sterol analyses was a 30 m×0.25 mm I.D. fused-silica capillary coated with a 0.25-μm film of phenyl(5%)polydimethylsiloxane (DB-5; J&W Scientific, Folsom, CA, USA). The temperature program was: 1 min at 200°C, 10°C/min to 285°C (hold 1 min), 0.5°C/min to 295°C, and 5°C/min to 320°C (hold 10 min). For determination of mass correction factors, mixtures of cholesterol and betulin were analyzed. The column used in the analyses of fatty acid methyl esters was 30 m×0.25 mm I.D. fused-silica coated with 0.25-μm film of cross linked polyethylene glycol HP-Innowax (Hewlett-Packard, Avondale, PA, USA). The temperature program was 3 min at 50°C, 10°C/min to 195°C (hold 1 min), 1°C/min to 220°C, and 5°C/min to 240°C (hold 15 min). For determination of correction factors, two standard mixtures containing methyl esters 4:0, 6:0, 8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 18:1, 18:2, 18:3, 20:0, 22:0 and 16:0, 18:0, 18:1, 18:2, 18:3, 20:0 were analyzed. Data acquisition and integration were carried out with an SC Chromatography workstation (Version 1,2B, Sunicom, Helsinki, Finland). Mass correction factors for other saturated fatty acid methyl esters were calculated using the non-linear regression equation $1/y=a+b/x$ ($r^2=0.999$, fit standard error=0.0173, y=mass correction factor, x=number of acyl group carbons, $a=1.160$, $b=-3.105$. Molar correction factors for other chain lengths of saturated fatty acid methyl esters were calculated using the regression equation $1/y=a+bx$ ($r^2=0.999$, fit standard error 0.0442, y=molar correction factor, x=number of acyl group carbons, $a=-0.163$, $b=0.0746$).

2.5. Tandem mass spectrometry

The mass spectrometer was a Perkin-Elmer Sciex API 3000 (Sciex, Toronto, Canada) triple quadrupole LC–MS–MS instrument, equipped with an electrospray ion source. Synthetic air (4.8 grade, Woikoski, Finland) was used as nebulizing gas at a flow-rate of 0.92 l/min. Curtain and collision gas was nitrogen (99.999%, Woikoski) used at a flow-rate of 0.95 l/min and instrument parameter 4, respectively. Ion spray voltage was 5 kV, declustering potential 50 V and collision offset voltage 50 V. For recording direct inlet electrospray mass spectra, aliquots of sterol ester fractions of untreated and lipase-modified butterfat, and vegetable oils were dissolved in 10 m*M* ammonium acetate in chloroform–methanol (1:1) to the final internal standard (cholesteryl heptadecanoate) concentration 10 μg/ml [18]. In addition, a similar solution of standard mixture containing

Table 2
Precursor ion tandem mass spectrum of cholesterol ester standard mixture. Precursors of cholesterol fragment ion m/z 369

Cholesterol ester	$[M+NH_4]^+$ m/z	Intensity	Concentration (μg/ml)
CE4:0	474	354 000	9.95
CE8:0	530	659 000	10.65
CE12:0	587	912 000	10.15
CE13:0	601	1 030 000	10.65
CE16:1	641	2 850 000	16.6
CE16:0	643	1 330 000	10.25
CE17:0	657	1 150 000	10
CE18:3	665	3 030 000	12.1
CE18:2	667	2 620 000	11.65
CE18:1	669	2 040 000	10.8
CE18:0	671	1 300 000	10.3

saturated cholesterol esters 4:0, 8:0, 12:0, 13:0, 16:0, 17:0, 18:0, and unsaturated cholesterol esters 16:1, 18:1, 18:2, and 18:3 was prepared (Table 2). In order to record mass spectra, the sterol ester samples were introduced continuously to the ion source with a microsyringe pump (Harvard Apparatus) at a rate of 10 μl/min. For quantification, precursor ion scans of the same samples were measured in positive ion mode over the scan range of m/z 200–1200. The spectra were recorded with a scan speed of 30 s/scan and 10 spectra per each experiment were accumulated. The data were processed with PE Sciex Analyst 1.1 version software.

The precursor ion spectra of standard mixture revealed that response depended on the chain length and unsaturation (Table 2). Correction factors were determined by calculating the concentration/intensity ratio and normalizing with respect to the internal standard (cholesteryl heptadecanoate). Molar and mass correction factors for other chain-lengths were determined by non-linear regression using equation $y=a+b/x$ ($r^2=0.997$, fit standard error 0.0781, $y=$ molar correction factor, $x=$number of acyl group carbons, $a=-0.148$, $b=18.843$ for molar correction factors, and $r^2=0.996$, fit standard error 0.0568, $a=0.0771$, $b=0.0581$) for mass correction factors. The correction factors for unsaturated sterol esters not present in the standard mixture were calculated from those of the respective saturated ester by multiplying it by the ratio of correction factors of 18:1/18:0, 18:2/18:0 or 18:3/18:0.

3. Results and discussion

3.1. Fractionation and transesterification

The SPE of free and esterified sterols is a simplified version of the separation of lipids in several fractions (to be published). When fractionating butterfat on a short column, a part of sterols co-elute among the most polar short-chain triacylglycerols. On the other hand, a part of sterols co-elute with diacylglycerols. The aim was to develop a simple fractionation method capable of separating a pure sterol ester fraction, a fraction containing the major part of triacylglycerols, and a free sterol fraction containing partial acylglycerols and residual triacylglycerols. The sterol esters and acylglycerols of the free sterol fraction were transesterified to fatty acid methyl esters prior to silylation of sterols and the gas chromatographic sterol analysis.

3.2. Gas chromatographic analysis of free sterols and transesterified sterol esters in vegetable oils

During a set of experiments for fractionation of vegetable oils and analyzing their free and esterified sterols the recoveries were determined. The total recovery including fractionation and transesterification was 90.2% (RSD 3.4%) for sterol esters and 97.1% (RSD 3.7%) for free sterols obtained in duplicate analyses of six fractionated vegetable oil samples (Table 3). The RSD (%) determined for duplicate analyses of two samples of each vegetable oil varied between 0.3 and 7.6 for free sterols and between 2.6 and 15.2 for sterol esters (Table 3). The transesterification of sterol esters seems to increase the variation.

The ratio of free and esterified sterols varies between different oils (Table 3). In peanut oil, rapeseed oil (Fig. 1) and sunflower oil (Fig. 2), the molar ratios of total free and esterified sterols are 1:0.86, 1:2.16, 1:0.59, respectively. There is also variation in these ratios between different sterols, e.g. the molar ratios of free and esterified campesterol are nearly equal in peanut oil and sunflower oil, but in the ratio 1:2.85 in rapeseed oil. Stigmasterol is mainly as free sterol in all analyzed vegetable oils. The sum of different free and esterified sterols of analyzed oils is in agreement with the data presented

Table 3
Gas chromatographic determination of free sterols and sterol esters in vegetable oils

Sterol	Free sterols		Sterol esters		Sum of free sterols and sterol esters (μg/g oil)
	μmol/g oil	RSD (%) $n=4$	μmol/g oil	RSD (%) $n=4$	
Peanut oil					
Campesterol	0.43	5.6	0.53	7.0	385
Stigmasterol	0.33	6.8	0.11	6.8	182
Sitosterol	1.98	3.7	1.71	4.6	1530
Total	2.74		2.35		
Rapeseed oil					
Brassicasterol	0.76	1.7	0.67	15.2	570
Campesterol	2.06	1.5	5.87	6.1	3178
Stigmasterol	0.06	7.6			25
Sitosterol	3.35	1.8	6.91	5.2	4255
Total	6.23		13.45		
Sunflower oil					
Campesterol	0.64	1.5	0.51	2.2	461
Stigmasterol	0.57	1.1	0.09	6.2	272
Sitosterol	3.52	0.9	1.73	2.6	2177
Δ^7-Stigmastenol	0.27	0.3	0.60	16.7	359
Total	5.0		2.93		
Total recovery (%) $n=12$	97.1	3.7	90.2	3.4	

by Biedermann et al. [11]. The analysis of fatty acids esterified with sterols revealed also differences between analyzed oils. In Table 6 is shown the gas chromatographically determined proportions of those esterified fatty acids, which were detected by precursor ion MS–MS. Unlike peanut oil and rapeseed oil sterol esters, the fatty acid composition of sunflower oil sterol esters is very similar to the fatty acid composition of triacylglycerols. In peanut oil and rapeseed oil sterol esters, the proportion of linoleic acid was over 60% and, in addition, the proportion of linolenic acid was high in rapeseed oil sterol esters. These differences may reflect the different role of sterol esters in these plant species.

3.3. Precursor ion mass spectrometry of intact sterol esters of butterfats

Direct inlet electrospray mass spectra of cholesterol ester standard mixture, butterfat and vegetable oil samples in 10 m*M* ammonium acetate in chloroform–methanol 1:1 were recorded. The mass spectra showed abundant sterol ester ammonium adducts $[M+NH_4]^+$ and characteristic sterol fragment ions *m/z* 369, 381, 383, 395, 397, 399 (Table 4). The precursor ion ESI-MS–MS (Fig. 3) was recorded for the cholesterol fragment ion of cholesterol ester standard mixture (Table 2). The spectrum reveals that intensities of precursor ions are dependent on the chain-length and the unsaturation of the fatty acyl moiety. Molar correction factors were determined by calculating the concentration/intensity ratio and normalizing with respect to the internal standard (cholesteryl heptadecanoate). The molar correction factors for saturated esters not present in the standard mixture were calculated by non-linear regression and those for unsaturated esters from that of respective saturated ester reducing it in the ratio unsaturated/saturated C_{18}-esters. Precursor ion ESI-MS–MS were recorded for cholesterol fragment ions of

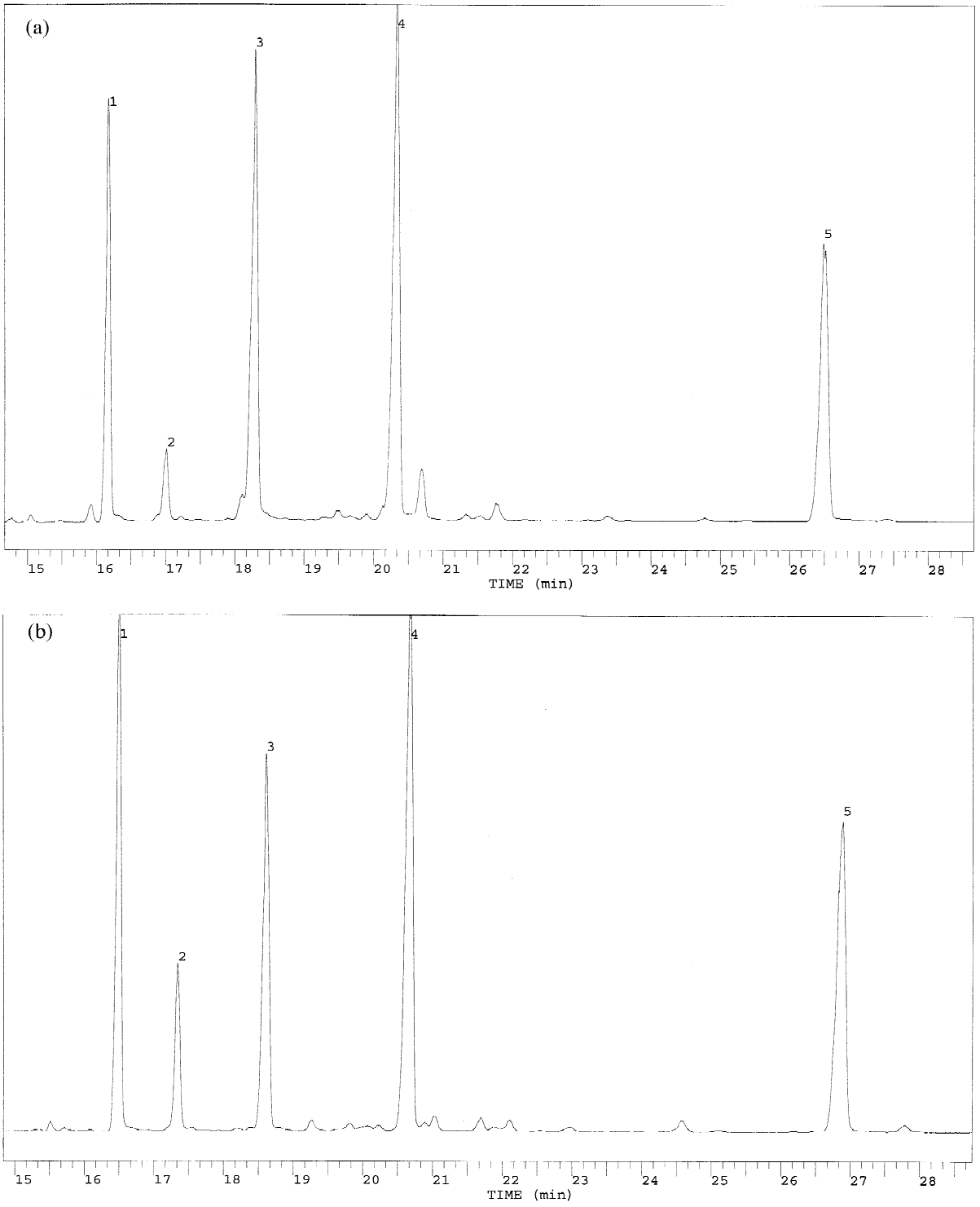

Fig. 1. Gas chromatograms of rapeseed oil sterols. (a) Sterol esters. (b) Free sterols. Peak assignment: 1, cholesterol (internal standard); 2, brassicasterol; 3, campesterol; 4, sitosterol; 5, betulin (secondary internal standard).

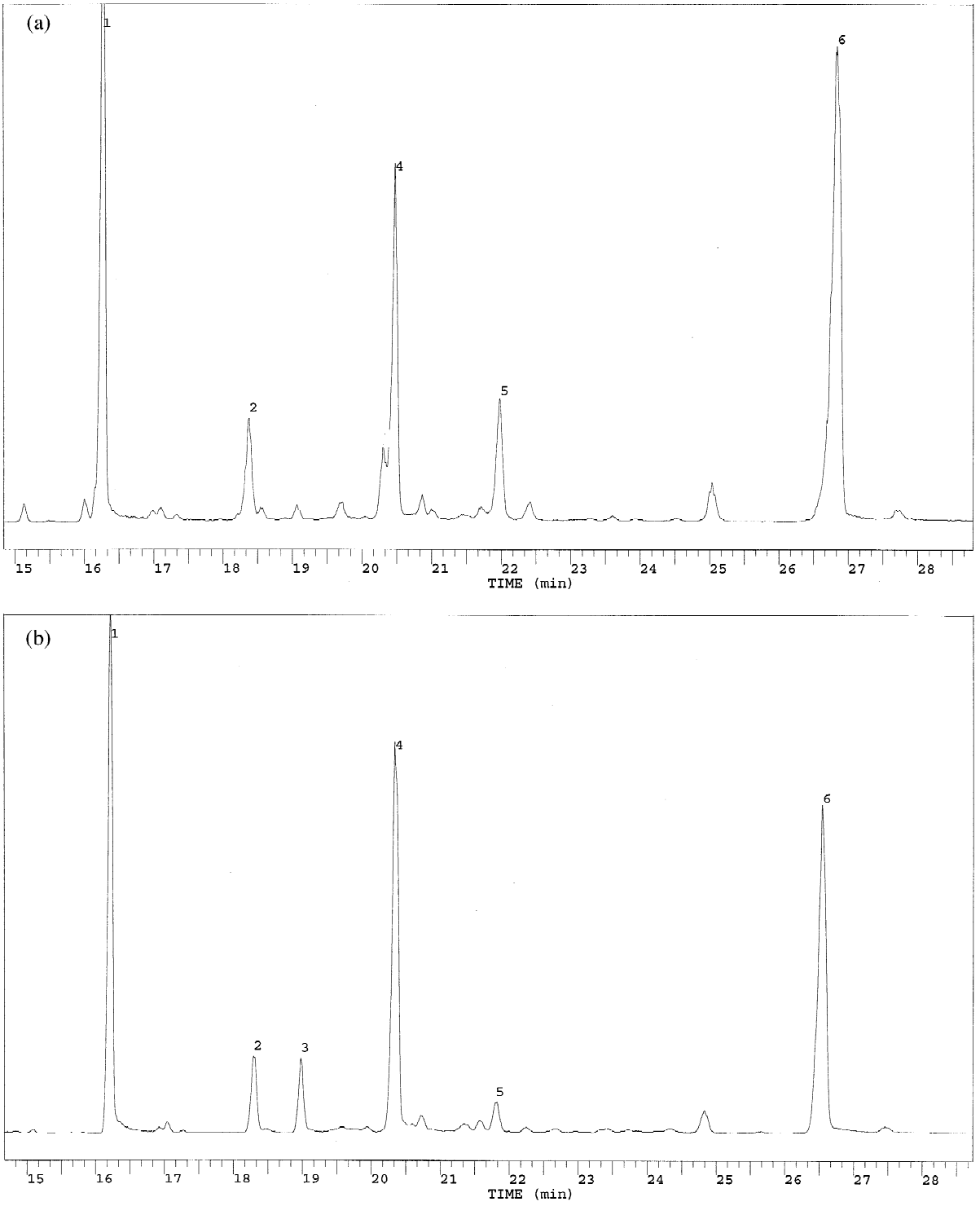

Fig. 2. Gas chromatograms of sunflower oil sterols. (a) Sterol esters. (b) Free sterols. Peak assignment: 1, cholesterol (internal standard); 2, campesterol; 4, sitosterol; 5, Δ^7-stigmastenol; 6, betulin (secondary internal standard).

Table 4
Precursor and fragment ions of various sterol esters

Sterol ester	Precursor ion	Fragment ion (m/z)
Cholesterol ester	$[M+NH_4]^+$	369
Brassicasterol ester	$[M+NH_4]^+$	381
Campesterol ester	$[M+NH_4]^+$	383
Stigmasterol ester	$[M+NH_4]^+$	395
Δ^7-Stigmastenol ester	$[M+NH_4]^+$	395
Sitosterol ester	$[M+NH_4]^+$	397

lipase-modified butterfat (Fig. 4) and intact butterfat samples. The molar proportions of cholesterol esters of lipase-modified butterfat are shown in Table 5. The similarity of the compositions determined by different methods is obvious. The molar proportions determined by precursor ion ESI-MS–MS and by GLC of fatty acid methyl esters of transesterified sterol ester fraction are very similar. Precursor ion ESI-MS–MS data revealed the presence of cholesterol esters 19:0, 19:1, 19:2, 21:0, 21:1, and 21:2, which were not detected in the previous study using high-temperature GLC. Due to lack of suitable fatty acid methyl ester standards, only cholesterol ester 19:0 could be tentatively identified in this GLC study by relative retention times. The proportion of palmitic acid is much higher in sterol esters than in triacylglycerols of butterfat. This may be due to high specificity of *Pseudomonas fluorescens* lipase for palmitic acid [21]. The proportion of palmitic acid seems to be higher and that of oleic acid lower in the interesterified butterfat analyzed in this study than in the previous study [8]. This may be due to more

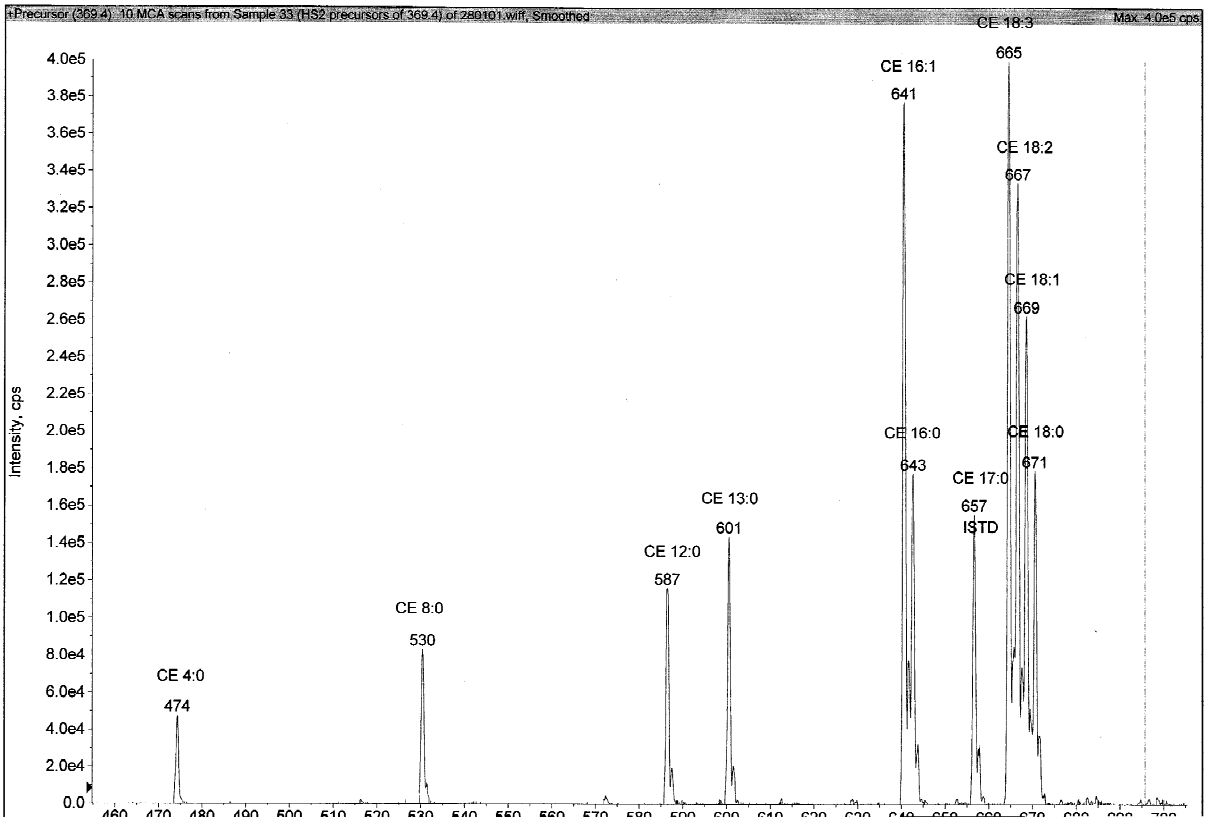

Fig. 3. Precursor ion tandem mass spectrum of cholesterol ester (CE) standard mixture. Precursors of cholesterol fragment ion.

Table 5
Molar proportions of cholesterol esters in intact and lipase-modified butterfat

Cholesterol ester	Cholesterol ester composition in intact butterfat (mol%)		Cholesterol ester composition in lipase-modified butterfat (mol%)		
	Calculated from precursor ion ESI-MS−MS data	Determined by high-temperature GLC[a]	Calculated from precursor ion ESI-MS−MS data	Calculated from fatty acid methyl ester composition of transesterified cholesterol ester fraction	Determined by high-temperature GLC[a]
4:0		1.46	0.84	N.D.[b]	2.68
6:0		0.40	0.85	1.74	0.92
8:0		2.06	0.00	1.58	0.77
10:0	1.34	1.33	0.88	1.98	1.55
12:0	1.92	1.93	1.92	2.11	1.26
14:0	7.32	3.46	11.73	10.91	9.47
14:1	1.40				
15:0	1.27		2.21	2.11	
16:0	10.60	14.44	46.74	42.48	40.05
16:1	5.50	1.80	1.91	0.79	0.87
17:0					0.24
17:1			0.71	0.00	
18:0	4.20	8.78	9.21	8.70	14.21
18:1	16.36	13.51	16.67	12.48	23.58
18:2	22.28	41.85	3.12	2.46	2.61
19:0			1.05	6.76	
18:3	27.81	8.98	1.25	0.84	0.81
19:1			0.22		
19:2			0.13		
21:0			0.19		
21:1			0.08		
21:2			0.29		
Other				5.08	

[a] Ref. [8].
[b] Not determined due to interference by impurities.

6, the precursor ion MS–MS data and gas chromatographic data are compared. The total esters of different sterols show a high degree of accordance in rapeseed oil. In peanut oil the proportion of campesterol ester and in sunflower oil those of campesterol and stigmasterol esters differ markedly. Table 6 also shows the molar proportions of all esterified fatty acids detected by precursor ion MS–MS in three vegetable oils. The gas chromatographic data also contained minor fatty acid components, but when proportions of those detected by MS/MS were calculated, a high similarity for all fats was observed. This similarity gave reason to present the fatty acid compositions of different sterols (Table 7). In rapeseed oil, sitosterol is esterified only with unsaturated fatty acids, but campesterol and brassicasterol esters also contain saturated fatty acid esters. In peanut oil, the major saturated sitosterol ester is palmitate and the major campesterol ester is stearate. In sunflower oil the major saturated sitosterol ester is stearate and major saturated campesterol ester is palmitate.

4. Conclusion

The proposed method for the analysis of free and

Table 6
Molar proportions of major sterols in free sterols and sterol esters and molar proportions of fatty acids in sterol esters determined by gas liquid chromatography and precursor ion tandem mass spectrometry

Sterol	Mol% of sterol esters		Mol% of free sterols	Mol% of major esterified fatty acids in sterol esters		
	Precursor ion MS/MS of sterol ester	GLC of sterols	GLC of free sterols	Esterified fatty acids	Precursor ion MS/MS of sterol ester	GLC of fatty acid methyl esters
Peanut oil						
Campesterol	6.51	22.55		16:0	6.96	10.53
Stigmasterol	6.62	4.73	12.02	18:0	1.82	2.14
Sitosterol	86.87	72.72	72.25	18:1	27.91	29.50
				18:2	60.67	55.30
				18:3	2.64	2.53
Rapeseed oil						
Brassicasterol	3.49	4.96	12.19	12:0	0.03	
Campesterol	44.61	43.67	32.98	16:0	0.76	2.73
Stigmasterol	2.31		1.00	18:0	0.43	0.15
Sitosterol	49.14	51.38	53.82	18:1	9.99	12.91
N.I.[a]	0.45			18:2	63.23	65.64
				18:3	25.56	18.57
Sunflower oil						
Campesterol	30.56	17.84	12.82	16:0	0.98	5.86
Stigmasterol	6.59	1.57	11.39	18:0	1.08	3.41
Sitosterol	51.41	59.95	70.48	18:1	23.12	23.81
Δ^7-Stigmastenol		20.64	5.32	18:2	68.46	63.98
N.I.	11.44			18:3	6.36	2.95

[a] Not identified.

esterified sterols is based on separation of sterol ester, triacylglycerol, and free sterol fractions on a short silica gel column. Sterol esters and acylglycerols co-eluted with free sterols were trans-esterified to fatty acid methyl esters. The esterified fatty acids of sterol ester fraction and sterols of sterol ester and free sterol fractions were analyzed by GLC. The total recovery, including fractionation and trans-esterification, for sterol esters was good and for free sterols very good. Intact butterfat, lipase-modified butterfat, and vegetable oils were analyzed by the method developed. Precursor ion ESI-MS–MS were recorded for a standard mixture of cholesterol esters. Molar correction factors were calculated and used in the determination of the relative proportions of the sterols esterified with different fatty acids and those of different sterols from the recorded precursor ion ESI-MS–MS data of butterfat, lipase-modified butterfat, and vegetable oil samples. The gas chromatographic and precursor ion ESI-MS–MS data were compared. The proportions of different sterols in the sterol ester fraction of vegetable oils showed a medium degree of accordance, and those of sterol esters of different fatty acids a high degree of accordance. The fatty acid compositions of different sterols could be calculated from ESI-MS–MS data.

Acknowledgements

Financial support from the Jenny and Antti Wihuri Foundation and the University Pharmacy is gratefully acknowledged.

Table 7
Molar proportions of esters of different sterols in vegetable oils

Esterified fatty acid	Mol% of esters of different sterols calculated from the precursor ion MS–MS data			
	Brassicasterol esters	Campesterol esters	Stigmasterol esters[a]	Sitosterol esters
Peanut oil				
16:0				8.02
18:0		9.05		1.42
18:1		35.46	29.98	27.18
18:2		55.04	70.02	60.38
18:3		0.44		3.00
Rapeseed oil				
12:0				
16:0	2.05	1.38		
18:0		0.85		
18:1	11.51	10.96		8.79
18:2	68.14	60.59		65.36
18:3	18.29	26.23		25.85
Sunflower oil				
16:0		3.19		
18:0		0.19	0.95	1.73
18:1		20.55	38.55	20.44
18:2		63.26	60.50	73.06
18:3		12.80		4.77

[a] Sunflower oil: stigmasterol+Δ^7-stigmastenol.

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