FOOD COMPOSITION AND ADDITIVES

Validated Method for Quantitation and Identification of 4,4-Desmethylsterols and Triterpene Diols in Plant Oils by Thin-Layer Chromatography–High Resolution Gas Chromatography–Mass Spectrometry

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Alkaline hydrolysis was performed on a series of different vegetable oils. The unsaponifiable lipid matter was extracted with ethyl ether, and the class of 4,4-desmethylsterols (sterols) plus the triterpene diols (diols) erythrodiol, uvaol, and betulinol were isolated by thin-layer chromatography. A validated method using the acetate derivatives of sterols instead of their silyl ethers is presented. The acetate derivatives were analyzed by high resolution gas chromatography (HRGC). Retention time, precision, recovery studies, and absolute response factors were calculated for these esters, and GC/mass spectrometric structure of the assigned retention times was confirmed for the sterols and triterpene diols.

Determining the authenticity of commercial products has become increasingly difficult due to the addition of adulterants and additives that mimic the advertised product. A number of techniques are available to analyze for fingerprint compounds in a commercial product, permitting confirmation of an adulterant (1–20). Sterols represent a small component of the total lipid fraction in plant oils; however, they comprise a major portion of the unsaponifiable fraction (21–23) and may serve as the fingerprint for specific oils, e.g., in differentiating olive oils as a class from sunflower oil (24–27).

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The 4,4-desmethylsterols and the triterpene diols erythrodiol, uvaol, and betulinol are sterol derivatives that occur in the unsaponifiable matter of plants (23–25, 28–31). Although the 4,4-desmethylsterols are the major tetracyclic compounds in this fraction, erythrodiol, uvaol, and betulinol are pentacyclic alcohols. These phytosterols may occur in the plant as the free alcohol or in an esterified form, differing from one another mainly in the composition of the side chain and in the number and location of double bonds in the sterol ring structure (23, 32).

In this study the accuracy, precision, and recovery of the sterols cholesterol and stigmasterol and the triterpene diols erythrodiol and uvaol were examined at various spiking levels. To quantitate the method, we chose a natural carrier medium, oil of evening primrose, which contains only trace levels of these sterols and no erythrodiol or uvaol (33). Accuracy, precision, and recovery of these compounds were then compared. The level of the analytes was arbitrarily chosen to equal 5–20% of the total sterol content of the oil of evening primrose used for spiking. Total identifiable sterol content of this oil was 1093 mg/100 g oil. The precision study was performed by determining the amount of each identifiable sterol-diol present in terms of both relative and absolute composition. High resolution gas chromatography (HRGC) was used to determine the identities of the various steryl and diol acetates by comparing their relative retention times to those of available standards, or, when standards were not available, by comparing them to their respective literature retention times versus β-sitosterol (1.00). Confirmation of their structure was obtained by electron impact mass spectrometry (EIMS).

Experimental

Apparatus

(a) *Gas chromatograph.*—Hewlett-Packard Model 5880A equipped with a flame ionization detector (FID) and a Level 4 GC Terminal (Hewlett-Packard, Palo Alto, CA). *GC column.*—WCOT fused silica, 25 m × 0.25 mm id × 0.12 μm D_f, CP-Sil 8 CB, (Chrompack, Inc., South Raritan, NJ) with one end of a 5 m × 0.25 mm id retention gap (Restek Corp., Bellefonte, PA) fitted to the GC column with a press tight connector (Restek) and the other end to the column injection port fitted with a 2 mm id untreated quartz glass liner (Hewlett-Packard). *Operating conditions.*—Temperature program as follows: Initial, 245°C; initial time, 16 min; program rate, 5°C/min; final, 280°C; final time, 15 min. Carrier gas linear velocity, H_2, 57.1 cm/s 248°C. Injector temperature, 280°C; injector mode, split; split vent flow rate, 100 cc/min; split ratio, 58.8. Septum purge, 3 cc/min. Chart speed, 1.0 cm/min. Attenuation, 16.2×10^{-12} AFS. Detector, 290°C; auxiliary makeup gas, N_2, 30 cc/min.

(b) *Syringe.*—5 μL fixed needle with guide assembly and 22° bevel needle point (Kloehn, CA).

(c) *Thin-layer chromatography (TLC).*—Tapered silica gel G preparative Uniplate-T (Analtech, Newark, DE).

Reagents

(a) *Solvents.*—Chloroform ($CHCl_3$), cyclohexane, ethyl acetate, ethyl ether, petroleum ether, (chromatographic grade). Acetic anhydride 99 + % (Aldrich Chemical Co., Milwaukee, WI), anhydrous pyridine 99 + % (Aldrich). ACS reagent grade anhydrous ethyl ether, methanol.

(b) *Chemicals.*—ACS reagent grade potassium hydroxide (KOH), anhydrous granular sodium sulfate (Na_2SO_4), phenolphthalein, 2,7-dichlorofluorescein. Neutral alumina (Al_2O_3), Brockman activity 1, 80–200 mesh (Fisher Scientific Co.); Plant Sterol Mixture, 25 mg/mL (β-sitosterol, stigmasterol, campesterol, brassicasterol; Matreya Inc., Pleasant Gap, PA); erythrodiol (Spectrum Chemical Mfg. Corp., Gardena, CA); uvaol (Spectrum); β-sitosterol (Sigma Chemical Co., St. Louis, MO); betulinol, (Atomergic Chemetals Corp., Farmingdale, NY); cholesterol (Applied Science, State College, PA); stigmasterol (Sigma); β-cholestanol (5α-cholestan-3β-ol; Stearloids, Inc., Wilton, NH). *2N methanolic KOH*: Dissolve 130 g in 200 mL distilled water. Dilute to volume with methanol. Store away from light.

(c) *Deactivated neutral Al_2O_3.*—Dry 100 g neutral alumina at 105°C for 3 h. Cool, add 6 mL water and shake vigorously to disperse any lumps. Allow to equilibrate at least 12 h before use.

(d) *TLC spray solution.*—Dissolve 0.2 g 2,7-dichlorofluorescein in 100 mL ethanol. Neutralize with a few drops of a dilute solution of alcoholic KOH.

(e) *Phenolphthalein test solution.*—Dissolve 1 g phenolphthalein in 100 mL 95% ethanol.

Preparation of Standards

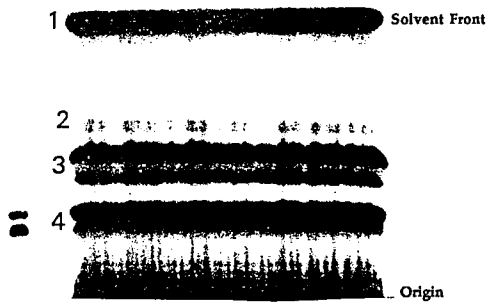
The qualitative reference standard mixture for TLC consisted of 10 mg/mL each of β-sitosterol and erythrodiol dissolved in ethyl acetate. Prepare qualitative reference standard for HRGC by placing contents of ampule containing Plant Sterol Mixture in 5 mL conical amber glass vial and evaporating to dryness at 60°C under N_2. Add 2 mg each erythrodiol, uvaol, and betulinol to vial. Acetylate with 2 mL acetic anhydride–pyridine solution (5 + 1). Heat on steam bath for 1 h and evaporate under N_2. Cool, dissolve esters in 1 mL cyclohexane, and reevaporate to dryness. Dissolve esters in 5 mL cyclohexane.

Prepare individual quantitative standards for GC as acetate derivatives by dissolving ca 2 mg each of cholesterol, stigmasterol, erythrodiol, and uvaol, derivatized as described above and dilute to 5 mL with cyclohexane. The internal standard consists of 400 mg β-cholestanol diluted to 100 mL with ethyl acetate.

Preparation of Spiked Samples

Approximately 5–20 mg of each standard was accurately weighed in 300 mL Erlenmeyer flask. Evening primrose oil (10 g ± 0.1) and 0.5 mL (2 mg) internal standard solution were added to the flask. A total of 100 mL 2N methanolic KOH was added and the mixture was refluxed for 1 h. The solution was cooled, transferred to a separatory funnel with 250 mL distilled water, and extracted with 200 mL ethyl ether. The aqueous layer was extracted with an additional 2 × 100 mL ethyl ether. Ether extracts were combined and gently washed with 80 mL portions of distilled water until neutral to phenolphthalein T.S. The combined ether extracts were added to a column containing a lower bed of 10 g anhydrous Na_2SO_4, followed by 10 g deactivated Al_2O_3, and finally 50 g Na_2SO_4 and eluted at maximum flow rate.

An additional 50 mL ethyl ether was added to the column and collected. The solution was taken to dryness under vacuum; the residue was dissolved in 1 mL $CHCl_3$ and subsequently used for TLC fractionation. A portion of 200 mL was streaked onto the preadsorbent layer of an activated TLC plate (1 h at 110°C) and developed by using a petroleum ether–anhydrous ethyl ether (70 + 30) solvent system. The plate was removed, air-dried, lightly sprayed with 2,7-dichlorofluorescein solution, and visualized under longwave UV light. Sterol and triterpene diol bands were located with the aid of reference standard spots. The area enclosing both bands was scored and the silica gel was collected and then



igure 1. The bands correspond to 1: hydrocarbons, 2: -tocopherol + impurities, 3: fatty alcohols + methyl and imethysterols, 4: desmethylsterols + triterpene iols.

xtracted with 3 × 30 mL hot $CHCl_3$–ethyl ether (80 - 20). The combined filtrates were evaporated to dryess in a vial under N_2. Freshly prepared acetic nhydride–pyridine solution (1 mL/25 mg) was added; ıe vial was tightly capped; immersed in a water bath ıaintained at 75°–80°C, heated for 1 h, and then vaporated to dryness under N_2. Reaction products 'ere dissolved in 500 μL cyclohexane and reevapoıted to dryness. The vial was cooled, and the acetate sters were redissolved in 300 μL cyclohexane for IRGC analysis.

To determine the relative percentage composition, each sterol was calculated as ratio of each peak area to total area of identifiable sterol peaks in the oil. Similarly, percentage of each triterpene diol was calculated as ratio of individual peak area to total identifiable sterol-triterpene diol peak areas. Each sterol or triterpene diol is related to total sterol-triterpene diol areas versus internal standard in mg/100 g oil.

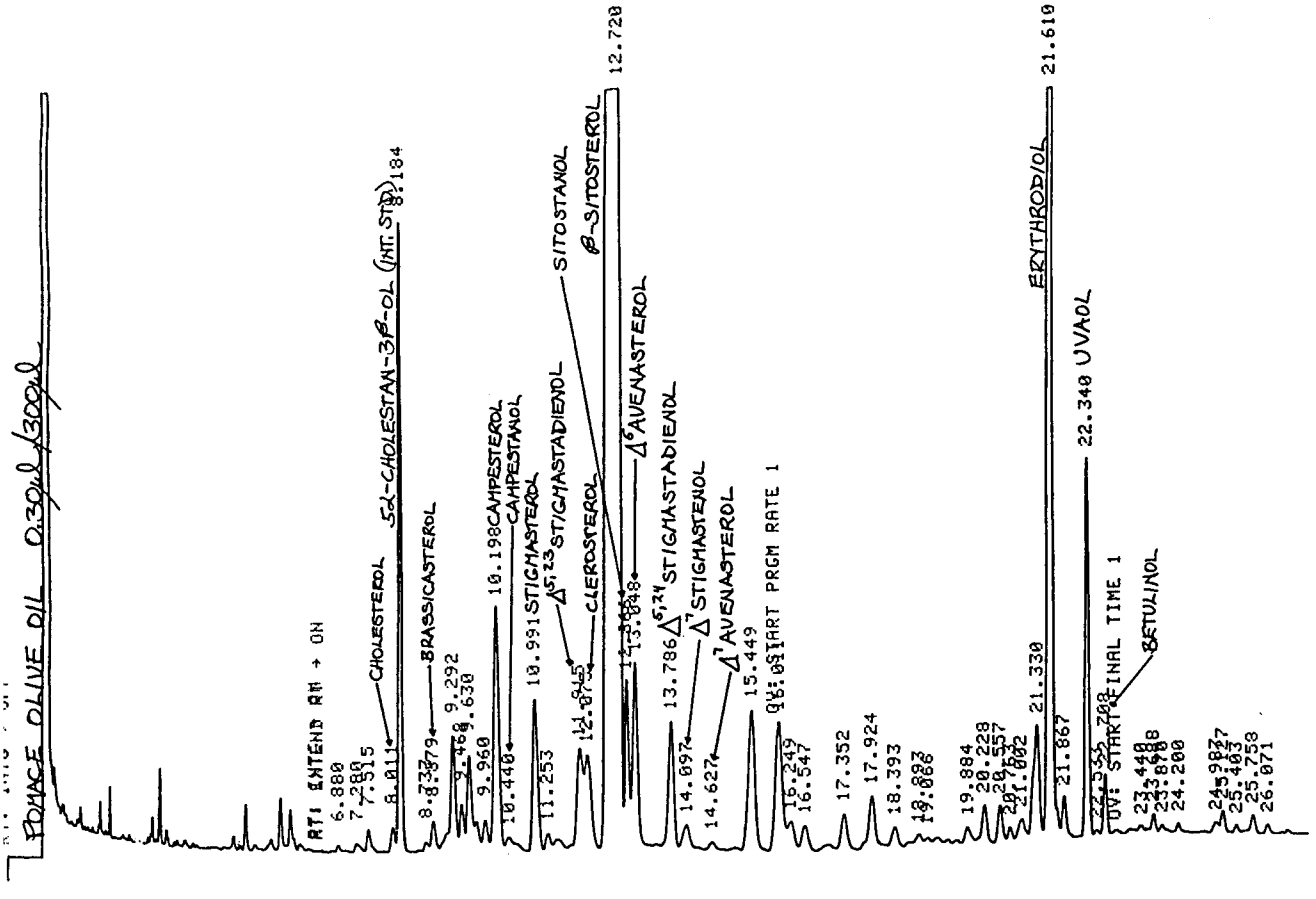
The standard calculation is:

$$\text{Sterol (triterpene diol)} = (A_z \times m_{is} \times 100)/(A_{is} \times m)$$

where A_z is sterol (triterpene diol) peak area, A_{is} is area of internal standard, m_{is} is weight (mg) of internal standard, and m is weight (g) of oil, taken for analysis. Response ratios were calculated in absolute terms of ng/unit area. The compounds were purchased as their acetate esters or, if not available, as the free alcohol and acetylated in the laboratory. Each standard solution was chromatographed 4 times at various injection volumes, and the peak area was determined in triplicate for each chromatogram.

Relative Percent Composition

The percentage of each sterol is calculated as the ratio of each peak area to the sum of the area of all



igure 2. Saponified TLC extract analyzed by HRGC. The relevant peaks are identified on the chromatogram.

identifiable sterol peaks present:

$$\text{Sterol, \%} = (A_x/A_s) \times 100$$

where A_x = area of sterol peak and A_s = sum of sterol areas.

The percentage of each triterpene diol present is calculated as the ratio of the area of each peak to the sum of the area of all identifiable triterpene diol plus sterol peaks present:

$$\text{Triterpene diol, \%} = (A_y/A_{tds}) \times 100$$

where A_y = area of triterpene diol peak; A_{tds} = sum of triterpene diol plus sterol peak areas.

Absolute Composition

The level of each identifiable sterol and triterpene diol present is calculated as mg/100 g oil:

Table 1. Steryl and triterpene diol acetate retention times relative to β-sitosteryl acetate

Peak	Sterol or triterpene diol	Relative retention time, min
1	Cholesterol Δ^5 cholesten-3β-ol	0.62
2	Brassicasterol [24S]-24-methyl-$\Delta^{5,22}$-cholestadien-3β-ol	0.69
3	24-Methylenecholesterol 24-methylene-$\Delta^{5,24}$-cholestadien-3β-ol	0.79
4	Campesterol [24R]-24-methyl-Δ^5-cholesten-3β-ol	0.80
5	Campestanol [24R]-24-methyl-cholestan-3β-ol	0.84
6	Stigmasterol [24S]-24-ethyl-$\Delta^{5,22}$-cholestadien-3β-ol	0.86
7	Δ^7 Campesterol [24R]-24-methyl-Δ^7-cholestan-3β-ol	0.90
8	$\Delta^{5,23}$ Stigmastadienol [24R,S]-24-ethyl-$\Delta^{5,23}$-cholestadien-3β-ol	0.93
9	Clerosterol [24S]-24-ethyl-$\Delta^{5,25}$-cholestadien-3β-ol	0.94
10	β-Sitosterol [24R]-24-ethyl-Δ^5-cholesten-3β-ol	1.00
11	Sitostanol 24-ethyl-cholestan-3β-ol	1.01
12	Δ^5 Avenasterol [24Z]-24-ethyliden-Δ^5-cholesten-3β-ol	1.02
13	$\Delta^{5,24}$ Stigmastadienol [24R,S]-24-ethyl-$\Delta^{5,24}$-cholestadien-3β-ol	1.07
14	Δ^7 Stigmastenol [24R,S]-24-ethyl-Δ^7-cholesten-3β-ol	1.11
15	Δ^7 Avenasterol [24Z]-24-ethyliden-Δ^7-cholesten-3β-ol	1.15
16	Erythrodiol olean-12-en-3β, 28-diol	1.73
17	Uvaol urs-12-en-3β, 28-diol	1.80
18	Betulinol lup-20(29)-en-3β, 28-diol	1.83

$$\text{Sterol (triterpene diol)} = \frac{(A_z \times m_{is} \times 100)}{(A_{is} \times m)}$$

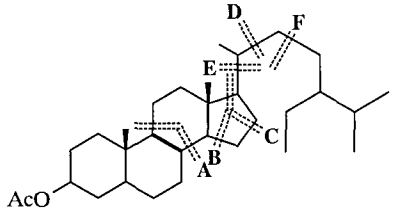
where A_z = sterol (triterpene diol) peak area; A_{is} = internal standard peak area; m_{is} = weight (mg) of internal standard; m = weight (g) of oil taken for analysis.

Results and Discussion

A variety of HRGC methods exist for analysis of derivatized sterols, including trimethylsilyl derivatives and butyrate esters (11,12). The initial work on this project was with trimethylsilyl ethers of known sterols and the sterol extracts from natural oils. However, precision and resolution of triterpene diols as trimethylsilyl derivatives lacked reproducibility, perhaps because of decomposition in the injector port. The obvious modifications to the injector and instrumentation did not enhance the analysis, and examination of other derivatization techniques led to the study and acceptance of steryl acetate esters as suitable alternatives (34). The oil samples were hydrolyzed, extracted with ether and dried, and polar compounds were removed by passage through neutral alumina. The resulting eluate was evaporated, the residue was dissolved in CHCl and the various classes of compounds therein separated by silica gel TLC (Figure 1). The sterol and triterpene diol fractions were removed as one, and extracted and acetylated as described. A typical chromatogram for pomace olive oil obtained by HRGC is shown in Figure 2.

A summary of retention times for each of the identifiable sterols and triterpene diols is listed in Table 1. Retention times for the acetylated compounds are re

Table 2. Fragmentation patterns of selected sterols



Steryl Acetate	A, %	B, %	C, %	D, %	E, %	F, %
Sitostanyl acetate	100	52	14	—	5	2
β-Sitosteryl acetate	62	25	21	3	16	—
Δ^7Stigmastanyl acetate	51	19	14	9	—	1
Stigmasteryl acetate	32	11	9	—	32	2
$\Delta^{5,23}$Stigmastadienyl acetate	—	3	4	29	51	7
$\Delta^{5,24}$Stigmastadienyl acetate	13	12	9	7	20	86
Δ^5Avenasteryl acetate	21	13	9	25	87	100
Δ^7Avenasteryl acetate	3	4	—	100	37	20
Clerosteryl acetate	58	14	11	20	4	—

Table 3. Relative percentage of compositions of sterols and diols in pomace olive oil

Sterol-diol	Replicate analyses					
	1	2	3	4	5	6
Cholesterol	0.16	0.16	0.15	0.15	0.10	0.15
Brassicasterol	0.13	0.17	0.16	0.13	0.13	0.17
Campesterol	3.21	3.36	3.34	3.23	3.27	3.30
Campestanol	0.17	0.37	0.33	0.87	0.22	1.72
Stigmasterol	1.75	1.75	1.74	1.75	1.73	1.72
$\Delta^{5,23}$ Stigmastadienol	1.23	1.29	1.23	1.17	1.20	1.78
Clerosterol	1.24	1.20	1.22	1.20	1.17	1.16
β-Sitosterol	86.4	85.9	86.4	86.2	86.7	85.2
Sitostanol	2.21	2.26	2.08	2.34	2.33	2.34
Δ^{5} Avenasterol	1.29	1.29	1.24	1.14	1.16	1.17
$\Delta^{5,24}$ Stigmastadienol	1.67	1.57	1.68	1.48	1.59	1.49
Δ^{7} Stigmastenol	0.39	0.40	0.39	0.37	0.38	0.38
Δ^{7} Avenasterol	0.12	0.26	N.I.[a]	N.I	N.I.	N.I.
Erythrodiol	14.8	14.9	14.7	14.4	14.4	14.6
Uvaol	2.46	2.44	2.40	2.35	2.36	2.39
Betulinol	0.46	0.45	0.48	0.45	0.42	0.43

[a] N.I., not integrated.

ported as relative to β-sitosteryl acetate. The plant oils were further characterized by GC/MS. EIMS analyses confirmed assignments of sterols and triterpene diols based on retention times relative to β-sitosteryl acetate. EIMS data were compared with mass spectral libraries for confirmation of structure based on retention time. Major fragments were examined and interpreted. It was of interest that fragmentation patterns differed significantly depending on placement of the side chain double bond. Table 2 shows that sitostanyl and stigmasteryl derivatives with no double bonds in the side chain cleave mainly along patterns designated by fragments originating from A, B, or C, whereas those steryl acetates containing side chain double bonds primarily fragment along D, E, or F. This could prove useful in identifying unknown peaks.

The relative percentage for compositions for 6 replicate analyses of a pomace olive oil are reported in Table 3. The absolute compositions are presented in Table 4. There is excellent agreement among all 6 analyses except that of campestanol. Figure 1 shows that the TLC separation of the desmethylsterol from

Table 4. Absolute compositions of sterols and diols (mg/100 g) in pomace olive oil

Sterol-diol	Replicate analyses						Avg.	CV, %
	1	2	3	4	5	6		
Cholesterol	0.45	0.46	0.44	0.41	0.40	0.43	0.43	5.0
Brassicasterol	0.36	0.49	0.45	0.36	0.36	0.46	0.41	14
Campesterol	9.09	9.49	9.51	9.01	9.00	9.19	9.21	2.5
Campestanol	0.48	1.03	0.95	2.43	0.60	4.79	—	—
Stigmasterol	4.95	4.94	4.96	4.86	4.78	4.78	4.88	1.7
$\Delta^{5,23}$ Stigmastadienol	3.47	3.65	3.50	3.27	3.32	3.28	3.42	4.4
Clerosterol	3.51	3.39	3.48	3.36	3.23	3.22	3.36	3.6
β-Sitosterol	245	242	246	240	239	237	242	1.4
Sitostanol	6.27	6.37	5.94	6.53	6.42	6.50	6.34	3.4
Δ^{5}Avenasterol	3.66	3.63	3.53	3.19	3.19	3.26	3.41	6.5
$\Delta^{5,24}$Stigmastadienol	4.74	4.44	4.78	4.12	4.39	4.14	4.44	6.4
Δ^{7}Stigmastenol	1.09	1.13	1.11	1.03	1.04	1.05	1.08	3.8
Δ^{7}Avenasterol	0.34	0.74	N.I.[a]	N.I	N.I.	N.I.	—	—
Erythrodiol	51.1	51.3	50.8	48.5	47.9	49.4	49.8	2.9
Uvaol	8.48	8.39	8.29	7.91	7.87	8.06	8.17	3.1
Betulinol	1.58	1.55	1.65	1.52	1.40	1.44	1.52	6.0

[a] N.I., not integrated.

the lipid alcohol band immediately preceding it is not baseline. Quantitation of campestanol, therefore, may sometimes be in error due to a coeluting interference during HRGC analysis.

A statistical examination of 8 sterols and 2 triterpene diols is presented in Table 5. The results indicate that in general the methodology is successful not only for the relative percentage composition of the sterol-diol components but also for the absolute composition of these compounds.

To demonstrate that acetate esters did not interfere and were not selectively discriminated against during

Table 5. Statistical data on sterols and 2 triterpene diols[a]

Sterol-diol	$\bar{x}$	SD	V	CV, %	Range
Campesterol	9.21	0.23	0.053	2.5	9.00–9.49
Stigmasterol	4.88	0.08	0.007	1.6	4.78–4.96
$\Delta^{5,23}$ Stigmastadienol	3.41	0.15	0.024	4.4	3.26–3.65
Clerosterol	3.36	0.12	0.015	3.6	3.22–3.51
β-Sitosterol	241	3.51	12.6	1.5	237–246
Sitostanol	6.34	0.24	0.047	3.8	5.94–6.53
Δ^{5} Avenasterol	3.41	0.22	0.048	6.4	3.19–3.66
$\Delta^{5,24}$ Stigmastadienol	4.43	0.28	0.80	6.3	4.12–4.78
Erythrodiol	49.8	1.44	2.08	2.9	47.9–51.3
Uvaol	8.17	0.26	0.066	3.2	7.87–8.48

[a] $\bar{x}$, arithmetic mean, mg; SD, standard deviation, mg; V, variance, mg^2; CV, coefficient of variation, %.

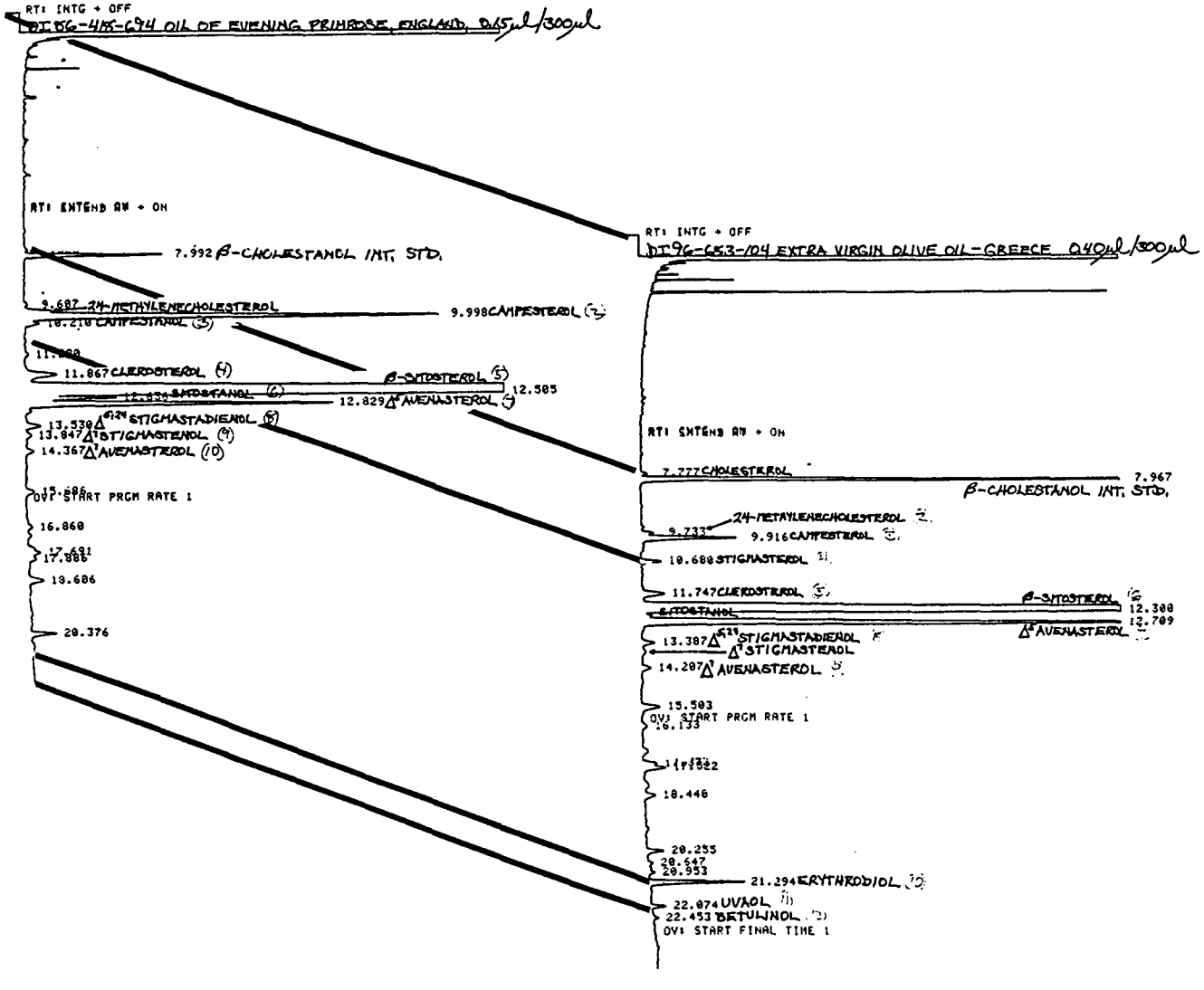


Figure 3. Comparison of a virgin olive oil with oil of evening primrose, which was used as a natural carrier medium for quantitation. It contains trace levels of cholesterol but not stigmasterol and the triterpene diols erthrodiol and uvaol.

Table 6. Recovery of 4 sterols and triterpene diols added (5–20 mg) to oil of evening primrose (10 g)

Sterol-diol		Added, mg	Found, mg	Recovery, %	Average recovery, %	CV, %
Cholesterol					77.0	4.9
	1a	5.132	4.286	83.5		
	2a	5.016	3.687	73.5		
	3a	5.158	3.859	74.8		
	4a	19.964	15.880	79.5		
	5a	19.427	14.865	76.5		
	6a	19.585	14.580	74.4		
Stigmasterol					77.9	8.4
	1b	5.044	4.290	85.0		
	2b	5.042	4.245	84.2		
	3b	5.313	4.005	75.4		
	4b	16.935	13.715	81.0		
	5b	19.816	14.445	72.9		
	6b	19.428	13.395	68.9		
Erythrodiol					79.3	8.4
	1c	19.954	16.455	82.5		
	2c	10.056	8.140	80.9		
	3c	8.221	6.055	73.6		
	4c	10.320	7.880	76.4		
	5c	11.111	9.720	87.5		
	6c	19.412	14.515	74.8		
Uvaol					78.7	6.74
	1d	5.393	4.996	78.1		
	2d	5.530	4.465	80.7		
	3d	4.989	3.583	71.8		
	4d	7.213	5.655	78.4		
	5d	5.771	5.050	87.5		
	6d	17.348	13.080	75.4		

esterification or chromatography, a recovery study was performed using cholesterol, stigmasterol, erythrodiol, and uvaol as known additives to the oil of evening primrose (Figure 3). The results are listed in Table 6. In general, recoveries ranged from 70 to 85% and were calculated using external standards. All areas used in the calculations were the average of triplicate determinations. Response ratios were calculated in absolute terms of ng/unit area. The compounds were purchased as their acetate esters or as the free alcohol and acetylated in the laboratory. Each standard solution was chromatographed 4 times at various injection volumes and the peak area was determined in triplicate for each chromatogram. The amount of each compound entering the GC column ranged from 2 to 15 ng. Each absolute response ratio was an average of 4 determinations (Table 7). FID response was linear in this range for these compounds. No significant variation was observed in absolute detector response among these structurally similar compounds.

Finally, a series of plant oils was examined for sterol and triterpene diol content to evaluate applicability of this method for identification of plant oils. A comparison of the composition of pomace and virgin olive oils showed that pomace contains significant amounts of the triterpene diols erythrodiol and uvaol plus the sterols campestanol, $\Delta^{5,23}$ stigmastadienol, sitostanol, and $\Delta^{5,24}$ stigmastadienol, whereas virgin olive contains 24-methylenecholesterol and significantly more Δ^5 avenasterol. Table 8 compares the relative and absolute compositions of extra virgin olive, pomace olive, cottonseed, and evening primrose oils, demonstrating the

Table 7. FID absolute response ratios[a]

Steryl-diol acetate	Number of carbon atoms	Number of double bonds	Absolute response ratio, ng/unit area
Cholesterol	29	2	0.1122
β-cholestanol	29	1	0.1126
Brassicasterol	30	3	0.1025
Campesterol	30	2	0.1324
Stigmasterol	31	3	0.1480
β-sitosterol	31	2	0.1113
Erythrodiol	34	2	0.1152
Uvaol	34	2	0.1156
Betulinol	34	2	0.1050

[a] Average of 4 determinations for each compound.

Table 8. Plant oil composition[a]

Sterol	Extra virgin		Pomace olive		Cottonseed		Evening primrose	
	Abs. comp.	Rel. % comp.	Abs. comp.	Rel. % comp.	Abs. comp.	Rel. % comp.	Abs. comp.	Rel. % comp.
Cholesterol	0.22	0.16	1.39	0.42	1.19	−0.31	0.52	0.05
Brassicasterol	—	—	0.70	0.22	—	—	—	—
24-Methylenecholesterol	0.39	0.29	—	—	—	—	—	—
Campesterol	4.95	3.48	9.73	3.39	29.3	7.80	91.8	8.84
Campestanol	—	—	0.87	0.32	0.70	0.17	3.99	0.38
Stigmasterol	1.03	0.74	5.64	1.95	3.68	0.98	0.97	0.10
$\Delta^{5,23}$Stigmastadienol	—	—	2.87	0.99	—	—	—	—
Clerosterol	1.22	0.82	3.77	1.31	3.20	0.85	7.22	0.70
β-Sitosterol	118	81.1	236	83.9	325	86.7	864	83.3
Sitostanol	0.68	0.48	7.58	2.83	2.15	0.57	13.2	1.27
Δ^{5}Avenasterol	16.7	12.1	8.05	2.68	7.21	1.91	48.7	4.68
$\Delta^{5,24}$Stigmastadienol	0.46	0.31	4.02	1.35	0.41	0.10	3.98	0.38
Δ^{7}Stigmastenol	0.29	0.19	1.16	0.43	1.64	0.44	2.15	0.21
Δ^{7}Avenasterol	0.69	0.45	0.59	0.20	0.97	0.25	1.88	0.18
Erythrodiol	3.09	2.20	56.5	16.8	4.47	1.18	—	—
Uvaol	0.34	0.23	8.71	2.41	—	—	—	—
Betulinol	0.13	0.10	1.57	0.44	—	—	—	—

[a] Each result represents average of 3 oils tested.

ability of this technique to distinguish among these plant oils.

A comparison of individual sterol values for relative percentage of composition versus absolute composition in extra virgin olive oils indicates that although total plant production of sterols may change in terms of absolute composition, relative percentage of composition does not vary significantly (35). This suggests that the biosynthetic pathway for sterol production is closely controlled (36, 37). This observation is to be expected, because sterol levels of virgin oils from olives produced in different geographical areas and under different ecological conditions may vary significantly when calculated on an absolute basis. However, this reasoning may be applied only to virgin oils, because refining may alter the sterol composition, by degradation, intramolecular rearrangement, or decreasing the total sterol content, depending on the procedure, the type of materials used, and the severity of the process (14, 38, 39, 40). Evidence of this may be seen in the results listed for pomace olive oil. For example, $\Delta^{5,23}$ stigmastadienol was not found in any extra virgin olive oils examined, but was present in all the pomace olive oils analyzed in this study. This compound is formed as a consequence of the refining process along with a general increase in stanol levels (39).

Conclusion

The data from this study suggest that the use of acetate derivatives for identification of plant oils is a viable alternative to the use of either the trimethylsilyl or butyrate derivatives. In addition, these esters yield characteristic mass spectra that may be used to characterize individual members of the sterol and triterpene diol fraction isolated from plant oils. A preliminary comparison of 7 edible plant oils showed significant variations in the contents of sterol and triterpene diol, and, thus, may be useful in identifying adulterated commercial products.

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