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Analysis of free phytosterols by gas chromatography using liquid phase OV-101

The most often used stationary phase for the analysis of phytosterols by gas–liquid chromatography (GLC) is the dimethyl silicone polymer SE-30[1–6] but this stationary phase gives an excessive amount of tailing with free sterols[3] and poor component resolution even when an acid-washed, silanized support in a glass column is used[6]. Therefore, the GLC determination of free phytosterols on SE-30 is not considered suitable and derivatives such as esters[3,5,7] and ethers[8] including trimethylsilyl ethers[5–7] are generally used. A number of new silicone stationary phase for GLC have become available and of special interest for the analysis of free phytosterols is OV-101, a liquid dimethylsiloxane polymer with 0% phenyl subsitution. Used in this investigation were the biologically important phytosterols: cholesterol (cholest-5-en-3 β-ol), campesterol (24 α-methylcholest-5-en-3 β-ol), β-sitosterol (24 α-ethylcholest-5-en-3 β-ol) and stigmasterol (24 β-ethylcholest-5, 22-dien-3 β-ol) (Applied Science Laboratory, State College, Pa., U.S.A.).

All GLC work was carried out on a F & M Model 402 equipped with a flame ionization detector. The columns were 1.80 m U-shaped glass with a 6 mm I.D. and packed with Anakrom ABS 80/90 mesh (Analabs, Hamden, Conn., U.S.A.) coated with 3% OV-17, OV-22, and OV-101 (Supelco, Bellefonte, Pa., U.S.A.) and Anakrom ABS 60/80 mesh coated with 5% SE-30. The column temperature was 250° and the flash heater temperature was kept at least 25° above that of the column. The flame detector temperature was 300°. Helium was the carrier gas at a flow rate of 100 ml/min. Ethyl acetate was chosen as the solvent because it showed minimum tailing. Samples of 2–10 μg of sterol either alone or in a mixture dissolved in ethyl acetate were injected with a 10 μl Hamilton syringe. Cholestane was used as the internal standard. The trimethylsilyl (TMS) ether derivatives were formed as described by KLEBE *et al.*[9].

The GLC results for the four phytosterols and their TMS ethers on three liquid phases are presented in Table I. Stationary phase OV-17, phenylmethyl silicone, which has a 50% phenyl substitution was also tested but the results were very similar to those obtained with OV-22 and, therefore, are not shown in Table I. All relative retention values (r) are given with respect to cholestane, and their effective plate values (N) are calculated according to ETTRE[10]. The order of sterol elution was the same for all liquid phases: cholesterol, campesterol, stigmasterol and β-sitosterol. Liquid phase OV-101 at the 5% level gave the best over-all results and higher or lower column loadings did not improve peak separation.

For the four phytosterols SE-30 had N-values of 796–1490, OV-22 had N-values of 641–780, and OV-101 had N-values of 1810–2200. The TMS ether derivatives of the four sterols gave higher N-values on SE-30, somewhat lower N-values on OV-22, and about the same N-values on OV-101. Liquid phase OV-101 gave superior N-values in all cases even if the TMS ether derivatives were compared.

For completeness of separation, or resolution (R), peak width at the base must be taken into account. Resolution was calculated according to ETTRE[10]. If $R = 1$, the two peaks are 95% resolved and if $R = 1.5$, the two peaks are better than 99% resolved from each other. The three liquid phases can be compared directly if it is assumed that $R = 1$ is an acceptable resolution. OV-22 is undesirable for the analysis

TABLE I

COMPARISON OF RELATIVE RETENTION (r), EFFECTIVE PLATE VALUE (N), AND RESOLUTION (R) OF VARIOUS PHYTOSTEROLS AND THEIR TMS ETHER DERIVATIVES ON THREE GAS CHROMATOGRAPHIC COLUMN SUBSTRATES

Column characteristics: column temperature 250°, detector temperature 300°, carrier gas, helium at a flow rate of 100 ml/min, column support Anakrom ABS (for details see text).

Compound	*Substrates*								
	5% SE-30[a]			*5% OV-22*[b]			*5% OV-101*[c]		
	r	N	R	r	N	R	r	N	R
Free sterol									
Cholestane	1.00	—		1.00	—		1.00	—	
			5.58			6.32			6.62
Cholesterol	1.93	1490		2.69	752		1.90	1853	
			2.47			1.96			2.68
Campesterol	2.56	1201		3.64	641		2.46	1810	
			0.71			0.87			1.31
Stigmasterol	2.80	796		4.15	752		2.77	2130	
			1.16			0.95			1.87
β-sitosterol	3.24	1241		4.56	780		3.10	2200	
Sterol, TMS ether									
Cholestane	1.00	—		1.00	—		1.00	—	
			7.80			4.42			5.73
Cholesterol	2.35	1599		2.08	577		2.33	1934	
			2.74			1.70			2.27
Campesterol	3.09	1644		2.75	570		3.11	1947	
			0.85			0.59			1.07
Stigmasterol	3.37	1547		3.03	638		3.42	2113	
			1.56			0.91			1.02
β-sitosterol	3.94	1648		3.49	653		3.95	2140	

[a] Retention time cholestane 11.6 min.
[b] Retention time cholestane 6.8 min.
[c] Retention time cholestane 12.7 min.

of free phytosterols or their TMS ethers. SE-30 gave a resolution of 95% for three of the four free sterols and a 99% resolution for two of the four components. OV-101 had the best resolution—at least 95% for all components and a 99% resolution for three of the four free sterols. The TMS ether derivatives gave somewhat poorer resolution on OV-101 than did the free sterols, but all components were resolved at the 95% level. SE-30 resolved three of the four TMS ethers at the 99% level, however, campesterol and stigmasterol could not be resolved at the 95% level.

Highly symmetrical peaks were obtained with OV-101 and it was found that this liquid phase was superior to SE-30 in every respect for the analysis of free phytosterols and their TMS ethers. But no claim can be made that complete optimum conditions were obtained for each liquid phase but the results presented in this note are representative of our experience with these liquid phases which were tested under comparable conditions and which permitted evaluation of relative merits. This GLC procedure has been employed in the analysis of biological samples[11].

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epartment of Agronomy,
niversity of Kentucky,
exington, Ky. 40506 (U.S.A.)

C. GRUNWALD

1 P. ENEROTH, K. HELLSTROM AND R. RYHAGE, *J. Lipid Res.*, 5 (1964) 245.
2 A. G. KALLIANOS, F. A. SHELBURNE, R. E. MEANS, R. K. STEVENS, R. E. LAX AND J. D. MOLD, *Biochem. J.*, 87 (1963) 596.
3 C. J. W. BROOKS, E. C. HORNING AND J. S. YOUNG, *Lipids*, 3 (1968) 391.
4 W. J. A. VANDENHEUVEL AND K. L. K. BRALY, *J. Chromatog.*, 31 (1967) 9.
5 A. ROZANSKI, *Anal. Chem.*, 38 (1966) 36.
6 J. E. VAN LIER AND L. L. SMITH, *Anal. Biochem.*, 24 (1968) 419.
7 B. A. KNIGHT, *J. Gas Chromatog.*, 2 (1964) 160.
8 R. B. CLAYTON, *Biochem.*, 1 (1962) 357.
9 J. F. KLEBE, H. FINKBEINER AND D. M. WHITE, *J. Am. Chem. Soc.*, 88 (1966) 3390.
0 L. S. ETTRE, *J. Gas Chromatog.*, 1 (1963) 36.
1 C. J. KELLER, L. P. BUSH AND C. GRUNWALD, *J. Agr. Food Chem.*, 17 (1969) 331.

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Silylation and gas–liquid chromatographic analysis of an aqueous polyhydric alcohol mixture

As surveyed elsewhere[1,2] catalytic high pressure hydrogenolysis of sucrose has been studied in several countries. The process, in general, is carried out in water or a water–alcohol solution. The reaction product is an aqueous polyhydric alcohol mixture and it is impossible to evaporate the water completely. This fact explains why the well-known silylation method of SWEELEY *et al.*[3], so often applied for the analysis of polyhydric alcohols[4], has not been used, as far as we know, for the quantitative analysis of the mentioned hydrogenolysis products.

Recently Pierce Chem. Co. announced Tri-sil 'Z' (a mixture of trimethylsilylimidazoles in dry pyridine) as a silylation reagent for hydroxy and polyhydric compounds in either dry or aqueous solution.

This note reports on the use of Tri-sil 'Z' for the quantitative GLC analysis of the reaction product formed during catalytic high pressure hydrogenolysis of sucrose.

It is emphasized that the purpose of this study was not to develop optimum GLC conditions, but to investigate the applicability of the silylation method to our problem.

Materials and methods

Catalytic hydrogenolysis of sucrose[2]. A 250 ml autoclave, equipped with a