Gas-Liquid Chromatographic Analysis of Mono- and Diglycerides

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Abstract

A method is described for the quantitative analysis of mono- and diglycerides by GLC as their trimethylsilyl derivatives. Glyceryl mono- and di-esters of myristic, palmitic, stearic, and oleic acids are separated by GLC on stainless steel columns, packed with 3% JXR on Gas Chrom Q. Relative response factors for monoglycerides and diglycerides have been calculated. Analyses of control and commercial mixtures with recoveries of 96 to 101% are reported.

Introduction

MONOGLYCERIDES ARE USED as emulsifiers and also occur naturally in foods. Commercial monoglycerides are usually mixtures of glyceryl mono- and di-esters of mixed, long-chain fatty acids. During the last few years several workers have reported on the analysis of monoglycerides by GLC. McInnes et al. (1) used allyl ethers and isopropylidine derivatives; Hubner et al. (2) separated monoglycerides as their acetylated derivatives; and more recently Wood et al. (3) reported on the analysis of monoglycerides as trimethyl-silyl ethers (TMS). The ease of preparation and the volatility of the TMS derivatives provide excellent criteria for the GLC applications for a number of compounds containing hydroxyl groups, such as sterols (4–6), phenols (7,8), carbohydrates (9), and polyglycerols (10).

This paper describes a procedure for the analysis of mono- and diglycerides of myristic, palmitic, stearic, and oleic acids.

Experimental Procedure

Materials

Mono- and diglycerides were synthesized in the laboratory by the procedure described by Hartman (11). Several commercial samples of distilled monoglycerides and mono- and diglyceride compositions were also obtained. All synthetic and commercial samples were purified by preparative thin-layer chromatography (TLC) on Silica Gel G which contained 4.0% boric acid (10). β-Monoglycerides were prepared from simple triglycerides by selective splitting with pancreatic lipase, followed with the separation of the lipid classes on a silicic acid column (12). In some instances, where more than one g of material was available, the chromatographic procedure of Quinlin and Weiser (13), as described by these authors (14), was employed.

The purified fractions obtained by column chromatography and TLC were analyzed for fatty acid distribution as identity checks. Fatty acids were analyzed as methyl esters by GLC on butane-diol-succinate (BDS) columns, as described in an earlier publication (15) from this laboratory.

Several control mixtures of known mono- and diglycerides were prepared from purified compounds.

Preparation of TMS Derivatives

A 30- to 50-mg sample was dissolved in 0.5 ml of pyridine in a 5-ml conical centrifuge tube and treated with 0.2 ml hexamethyldisilizane and 0.1 ml trimethylchlorosilane. The reaction mixture was shaken for 15 to 30 sec and allowed to stand for 5 min. About 0.2 μl of the supernatant was injected in the gas chromatograph.

Gas Chromatography

A Perkin-Elmer model 800 Gas Chromatograph, equipped with 3-ft, ⅛-in. stainless steel columns, packed with 3% JXR on Gas Chrom. Q (Applied Science Laboratories) and with dual flame ionization detectors, was used in this study. Column temperature was programmed from 125C to 325C at 10C/min. Helium flow was regulated at 33 ml/min at ambient temperature.

The percentage of distribution was calculated from the summation of peak areas, as measured by the Disc-integrator. Relative response factors (RRF) for individual compounds in each class were calculated from control mixtures by assigning the unit of one to the weight/peak area ratios of α-monomyristate for monoglycerides and α,α′-dimyristate for diglycerides.

TABLE I

Analysis of Control Mixtures and Commercial Samples of Mono- and Diglycerides

	RRF[a]		Control mixture		Commercial samples[b]			Shortening composition[c]	
	M	D	Added	Recovery %	1	2	3	Added	Found
α-Monoglycerides									
myristate	1.00		11.9	98.5	0.6	2.7	4.2	.....	0.1
palmitate	1.01		12.5	98.2	17.4	7.6	29.3	5.00	4.92
stearate	0.96		11.7	101.0	25.5	48.5	15.5	5.00	5.10
oleate	1.08		12.4	96.2	13.8	2.7	45.9	.....	.....
linoleate	1.15		.....						
linolenate	1.10		.....						
α,α′-Diglycerides									
dimyristate	1.81	1.00	9.4	100.5	0.1	3.6	.....	.....	.....
myristopalmitate	.....	1.01	.....	.....	.....	.....	.....	.....	.....
dipalmitate	1.79	0.98	13.7	101.2	2.6	8.6	.....	5.00	5.12
palmitostearate	.....	1.06	.....	.....	13.7	.....	.....	.....	.....
distearate	1.89	1.01	14.8	98.8		20.5	.....	5.00	5.15
oleostearate	1.89	1.10	.....	.....	14.1	.....	.....	.....	.....
dioleate	1.89	1.15	13.6	97.5	.....	.....	.....	.....	.....
			(100.0)[d]		(87.8)	(94.2)	(94.9)	(20.00)	(20.39)

[a] Relative response factors: M—relative to α-monomyristate; D—relative to α,α′-dimyristate.
[b] All values are percentage and averages of three determinations.
[c] Shortening composition was prepared from lard to contain 20% of mono- and diglycerides.
[d] Figures in parenthesis are totals for mono- and diglycerides. The difference from 100 accounts for triglycerides and other components.

Analysis of Unknown Mixtures

Commercial mono- and diglycerides and shortening compositions prepared in the laboratory, containing various proportions of mono- and diglycerides, were analyzed as follows. The sample was fractionated into lipid classes by silicic acid column chromatography as described earlier (14), and the percentage distribution of total mono- and diglycerides was calculated from actual weights in each fraction. Then fractions containing monoglycerides and diglycerides were converted to TMS derivatives and analyzed by GLC for individual ester distribution.

Results and Discussion

Quantitative separation of lipid classes into triglycerides, diglycerides, and monoglycerides by silicic acid column chromatography has been demonstrated by several workers (13,14,16). A recent collaborative study on the Quinlin and Weiser (13) procedure for the analysis of monoglycerides, carried out jointly by the Association of Official Analytical Chemists (AOAC) and AOCS, confirmed the quantitative nature of the separation (17). The same procedure was used in this study for the separation of mono- and diglycerides. No attempt was made to extract the derivatives in hydrocarbon solvents as suggested by Wood et al (3). The supernatant from the reaction mixture was injected directly into the chromatograph. A slight turbidity of the solution did not affect quantitation. Reproducibility of the percentage distribution of mixed monoglycerides and diglycerides estimated repeatedly over a period of weeks was within 2%. In the laboratory the same column for TMS analysis has been used continuously for more than one year without any perceptible decrease in sensitivity.

Figures 1 and 2 show typical separations of α-monoglycerides and α,α'-diglycerides of myristic, palmitic, stearic, and oleic acids. Two separate chromatograms for monoglycerides and diglycerides are superimposed in Figure 1. Figure 2 shows a pattern obtained with a mixture of mono- and diglycerides without prior separation into classes. The β-monoglycerides have relatively shorter retention times than the α-isomers and appear as shoulder peaks; α,α'- and α,β diglycerides showed the same retention

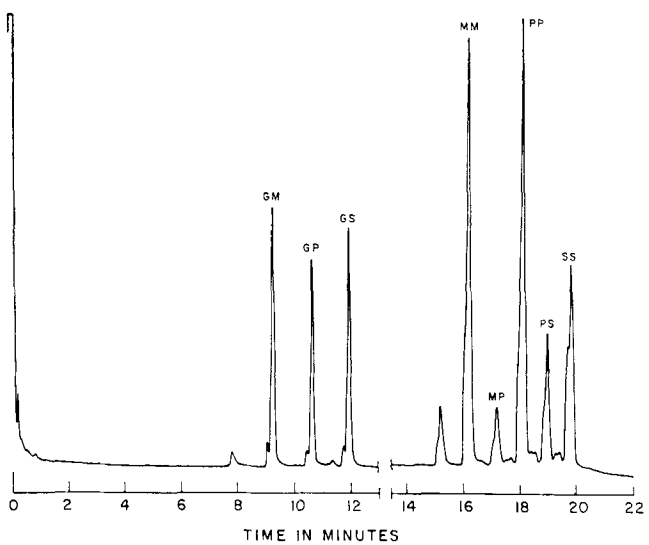

FIG. 1. GLC of mono- and diglycerides: GM, α-monomyristate; GP, α-monopalmitate; GS, α-monostearate; MM, α,α'-dimyristate; MP, α,α'-myristopalmitate; PP, α,α'-dipalmitate; PS, α,α'-palmitostearate; SS, α,α'-distearate.

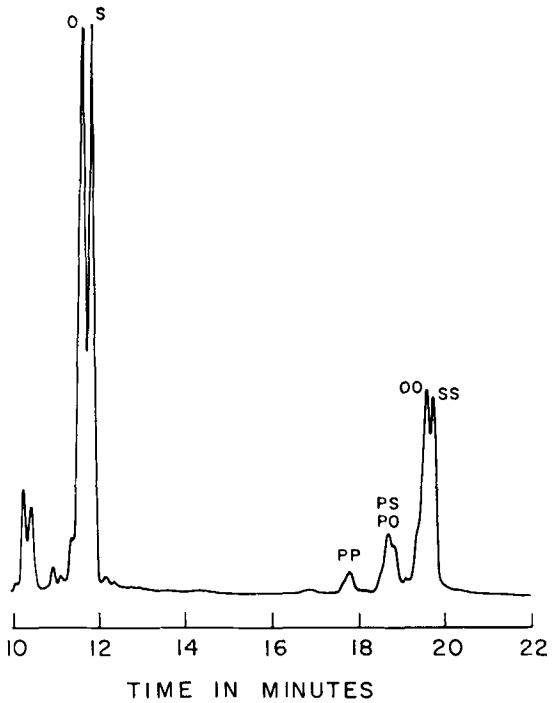

FIG. 2. GLC of mono- and diglycerides: O, α-monooleate; S, α-monostearate; PP, α,α'-dipalmitate; PS, α,α'-palmitostearate; PO, α,α'-palmitooleate; OO, α,α'-dioleate; SS, α,α'-distearate.

times and could not be separated.

Relative response factors (RRF) were calculated from control mixtures as weight-to-peak-area ratios, separately, for monoglycerides relative to α-monomyristate and for diglycerides relative to α,α'-dimyristate. These factors are used when mono- and diglycerides are separated by partition chromatography. Analysis of composite mixtures of mono- and diglycerides required the calculation of a separate set of RRF for diglycerides relative to monoglycerides. The RRF for compounds studied are shown in Table I along with the analytical data on control mixtures and commercial monoglyceride compositions. The analysis of the monoglycerides agrees with the fatty acid distribution, as determined by GLC. The RRF's are dependent upon the sensitivity of the instrument and the operative conditions. It is important that these factors be calculated under conditions of GLC used in each laboratory. The recoveries, as shown in Table I and also as determined in several shortening compositions containing 20% mono- and diglycerides, were in the range of 96 to 101%.

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