Journal of Chromatography, 120 (1976) 411–417

CHROM. 8903

COMPARISON OF CHROMATOGRAPHIC METHODS FOR THE ANALYSIS OF GLYCEROL ESTERS

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(Received November 10th, 1975)

SUMMARY

Column, thin-layer and gel chromatography have been compared as methods for the analysis of glycerol esters. It was found that gel chromatography gave much easier and faster analyses of monoglyceride emulsifiers, while at the same time providing a satisfactory distribution of fractions and giving an accuracy of determination corresponding to that of the standard method for their analysis.

INTRODUCTION

The determination of the content of tri-, di- and monoacylglycerols in the presence of each other is important in the analysis of monoglyceride emulsifiers. Chromatography on a silica gel column is used as the standard method[1], in which triacylglycerols are eluted with benzene together with free fatty acids, diacylglycerols are eluted with benzene–diethyl ether (90:10) and monoacylglycerols and other polar solvents are eluted with diethyl ether. Satisfactory separation of more complicated mixtures is achieved by developing of the chromatogram with mixtures of light petroleum and diethyl ether in various proportions[2]. Many similar procedures have been suggested for the determination of glycerol esters by thin-layer chromatography[3]. A simple method of separation on a silica gel layer with a mixture of hexane, diethyl ether and methanol has been suggested by Sallee and Adams[4], and the separation of monoacylglycerols from di- and triacylglycerols by gas chromatography of the silyl derivatives has been described[5]. In all of the above methods, the presence of esters of substituted polar fatty acids is an interfering factor.

Owing to the considerably different dimensions of the molecules of tri-, di- and monoacylglycerols, mixtures of these compounds could be separated by gel

chromatography. Calderon and Baumann[6,7] separated glycol and glycerol esters and other neutral lipids on a Sephadex LH-20 column. This paper reports a comparison of the analyses of monoglyceride emulsifiers by using the standard method, thin-layer chromatography and gel chromatography with tetrahydrofuran as eluent.

EXPERIMENTAL

Materials

Samples of monoglyceride emulsifiers were prepared by glycerolysis of groundnut oil, sunflowerseed oil and beef tallow with an alkaline catalyst. Pilot-plant molecular distillation was used to separate them into fractions of volatile products containing a large amount of glycerol, a further monoacylglycerol fraction and the distillation residue in which diacylglycerols predominated together with a minor amount of mono- and triacylglycerols. Pure esters were prepared by column chromatography[1] and re-purification of the fractions by preparative thin-layer chromatography.

Analytical methods

A procedure suggested by Quinlin and Weiser[1] was used in the chromatography on a silica gel column. Glycerol was isolated before the chromatographic analysis by extraction with water from a diethyl ether solution of the sample and determined by the periodate method[8]; free fatty acids were determined titrimetrically and the result of the determination of the diacylglycerol fraction was corrected accordingly.

The analytical separation by thin-layer chromatography was carried out on macroporous silica gel on Silufol UV-254 layers bonded with starch (Kavalier Glass Works, Votice, Czechoslovakia) with light petroleum (b.p. 40–60°)–diethyl ether–acetone (80:19:1), for less polar components and (45:50:5), for more polar components.

In the thin-layer preparative chromatography, we used macroporous Silpearl silica gel (Kavalier Glass Works) bonded with 15% of gypsum. The layer was 0.8 mm thick, activation at 105° took 60 min, 10 mg of the sample in chloroform solution was placed on a 10-mm strip and a mixture of light petroleum (b.p. 40–60°), diethyl ether and acetone (80:18:2) was used for development. The glycerol esters were detected with molybdophosphoric acid and free glycerol was detected with a solution of potassium periodate plus potassium permanganate.

Fractions obtained by thin-layer chromatography were eluted with ethanol and the esters content in the fractions was determined spectrophotometrically after conversion into hydroxamic acids[9]. Free fatty acids were converted into copper(II) salts and determined with sodium cupralate[10]. Diglycerol esters were identified after the isolation of diglycerol (esters were eluted from a stain at the start, saponified, unsaponifiable compounds were removed by extraction with diethyl ether, the solution was acidified and fatty acids were removed; the solution was cautiously evaporated to dryness and the residue was extracted with diethyl ether[11]) by means of gas chromatography and mass spectrometry of the silylated derivatives with an LKB 9000 apparatus at an ionization voltage of 70 eV. The glycerol content was determined spectrophotometrically with chromotropic acid[12] after elution with water from the thin layer followed by oxidation with potassium periodate[13].

The contents of 1- and 2-monoacylglycerols in the monoglyceride fraction were determined by the periodate method after isomerization with perchloric acid[14].

Gel chromatography was carried out according to a method described earlier[15] on a gel chromatographic apparatus (Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague) provided with a system of five columns, size 1200 × 8 mm, packed with the styrene–divinylbenzene copolymer S-Gel 832 (Institute of Macromolecular Chemistry) with an exclusion limit of molecular weight of *ca.* 1000. Peroxide-free tetrahydrofuran was used as eluent at room temperature with a flow-rate of 35 ml/h. A system of series-connected detectors was used in the experiments (Waters R-4 differential refractometer and flow ultraviolet detector, Czechoslovak Academy of Sciences, Development Works, wavelength 254 nm); the differential refractometer was used for the quantitative evaluation of the content of components in the samples, while the flow ultraviolet spectrophotometer served as a supplementary detector.

A sample of an analyzed emulsifier was diluted with tetrahydrofuran to give an approximately 2% solution, and about 0.3 ml of the solution was injected into the first measuring column. The contents of the components of the mixture were calculated from the peak heights on the gel chromatograms by the usual procedure; the components were identified by comparing the elution volumes with those of the standards. One count on the photoelectric siphon flow meter corresponds to a volume of 2.7 ml.

RESULTS AND DISCUSSION

The following types of samples may appear in the analysis of monoglyceride emulsifiers: (1) mixtures in which the individual components are in the state of equilibrium (usually containing 30–50% of monoacyl derivatives) in which they arise in glycerolysis; (2) distillation fractions of monoacylglycerols (containing 80–95% of monoacylglycerols); (3) distillation fractions of volatile components with a high content of free glycerol (forming the first fraction in molecular distillation); and (4) distillation fractions of the starting product. The separation of components using the chromatographic techniques investigated in this work was examined for all four types of analyzed samples. With the standard method, a check on the fractions by thin-layer and gel chromatography showed that the components were well separated if free glycerol was removed before the analysis. When the samples were analyzed by thin-layer chromatography, the positional isomers could also be separated (2-monoacyl- from 1-monoacyl- and 1,2-diacyl- from 1,3-diacylglycerols), but the chromatogram had to be developed with two systems of different polarity (Fig. 1). The components of the unsaponifiable fraction did not interfere in the determination. Gel chromatography also provided a good separation of all types of samples (Fig. 2), 2-monoacylglycerols were determined in the presence of 1-monoacylglycerols, but 1,2-diacylglycerols could not be separated from 1,3-diacylglycerols on the gel used. Diglycerol esters were separated from glycerol esters. The reproducibility of the elution times was very good (Table I). The sterol fraction (0.2–0.8% of the sample) did not interfere in the determination. As can be seen from Table III, in some instances the differences between the two methods are larger than follows from the mean results summarized in Table II. Of course, Table III gives a comparison of the results

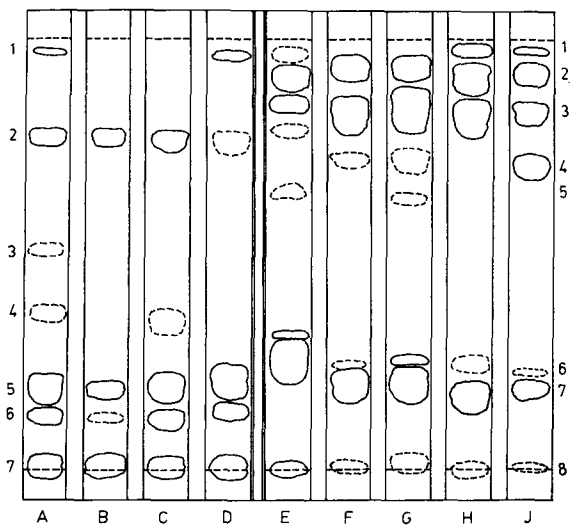

Fig. 1. Chromatographic separation of monoglyceride emulsifiers on a thin layer of silica gel. Layer, Silufol-UV_{254}; activation, 30 min at 105°. Sample, 20 g. Solvent system, light petroleum (b.p. 40–60°)–diethyl ether–acetone in the following ratios: A, B, C and D, 80:19:1; E, F, G, H and J, 45:50:5. Front distance, 140 mm. Detection, molybdophosphoric acid. Samples: A and E, original monoglyceride emulsifier; B and F, first volatile fraction obtained by molecular distillation; C and G, monoacylglycerol fraction obtained by molecular distillation; D and H, distillation residue from molecular distillation rich in diacylglycerols; J, original product with cholesterol added. Fractions in A–D: 1 = triacylglycerols; 2 = free fatty acids; 3 and 4 = unidentified trace fractions; 5 = 1,3-diacylglycerols; 6 = 1,2-diacylglycerols; 7 = monoacylglycerols and other polar components. Fractions in E–J: 1 = triacylglycerols; 2 = 1,3-diacylglycerols; 3 = 1,2-diacylglycerols; 4 = sterols; 5 = unidentified trace components; 6 = 2-monoacylglycerols; 7 = 1-monoacylglycerols; 8 = high-molecular-weight and polymeric fractions.

obtained with a single determination; reliable results by using both methods can therefore be provided only by carrying out three or more parallel analyses.

The suitability of the above three chromatographic methods for the quantitative analysis of monoglyceride emulsifiers was compared by taking the results of the analyses of 16 samples containing 20–95% of monoacylglycerols (an example of four similar samples is given in Table II). The results obtained for the individual samples agree well with each other and the differences do not exceed the limits of errors of observation. The content of 2-monoacylglycerols was $10.1 \pm 1.6\%$ of the total content of monoacylglycerols in the gel chromatographic determination, compared with $9.3 \pm 0.8\%$ by the chemical method, in good agreement with the literature data (11.2%)[16].

The contents of components determined by gel chromatography were calculated from the peak heights without correction factors, but for samples that contained more than 5% of glycerol a correction factor of 1.8 had to be used. The literature data for the refractive indices of glycerol and monoglycerols could not be used in re-calculations, probably because of the solvation of polar components in tetrahydrofuran. Owing to the small differences between the refractive indices of glycerol esters with higher fatty acids, the corrections did not have a substantial influence on

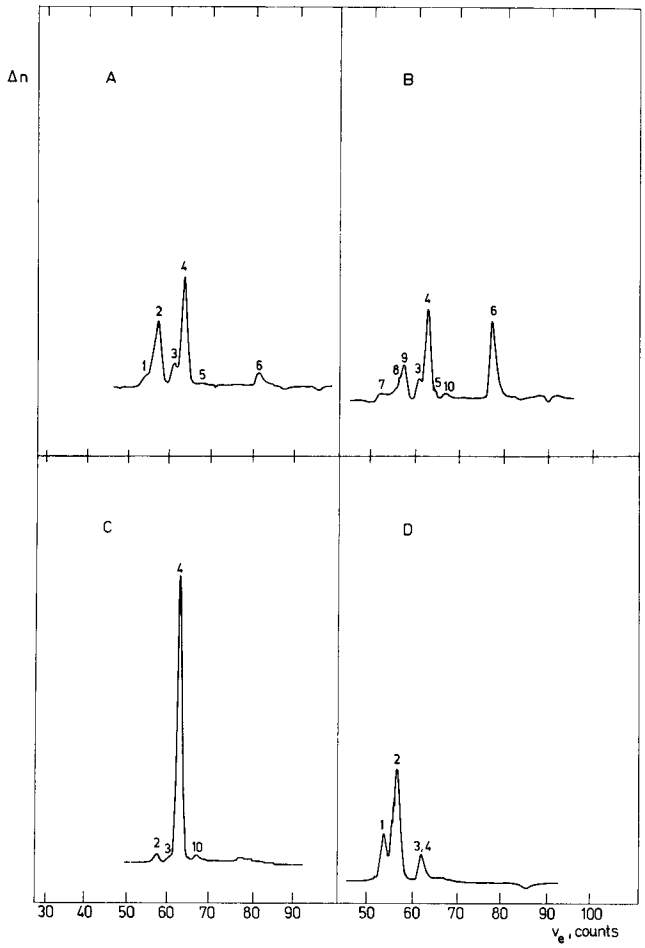

Fig. 2. Separation of monoglyceride emulsifiers by gel permeation chromatography. Injection: 0.35 ml of *ca.* 2% solution. A, original monoglyceride emulsifier; B, first volatile fraction obtained by molecular distillation; C, monoglyceride fraction obtained by molecular distillation; D, distillation residue from molecular distillation rich in diacylglycerols. Composition of samples is given in Table III. Peaks: 1 = triacylglycerols; 2 = diacylglycerols; 3 = 2-monoacylglycerols; 4 = 1-monoacylglycerols; 5 = sterols; 6 = glycerol; 7 = diglycerol esters; 8 = 1,2-diacylglycerols; 9 = 1,3-diacylglycerols; 10 = free fatty acids.

the result, as is also suggested by the good agreement between the uncorrected results and those obtained by the standard method.

The accuracy of the determination of the composition of monoglyceride emulsifiers by gel chromatography was identical, on average, with that of the standard method[1]; the accuracy of thin-layer chromatography was distinctly lower. A disadvantage of thin-layer chromatography is also that it is very laborious, although densitometric evaluation would reduce this disadvantage[17]. Gel chromatography is also less labour-consuming than the standard method of chromatography on a silica gel column, because the analysis is carried out on an automatic device with virtually

TABLE I

CHROMATOGRAPHIC BEHAVIOUR OF GLYCEROL ESTERS IN GEL PERMEATION CHROMATOGRAPHY ON S-832 GEL

Five columns (1200 × 8 mm); flow-rate of tetrahydrofuran, 35 ml/h; 1 count = 2.7 ml.

Type of compound	*Elution volume (counts)*	*Standard deviation**
Diglycerol esters	50.3	0.4
Triacylglycerols	54.1	0.5
Diacylglycerols	56.9	0.5
2-Monoacylglycerols	60.8	0.3
1-Monoacylglycerols	62.8	0.4
Free fatty acids	67.0	0.2
Free glycerol	79.3	0.1
Sterols	68.2	0.2

* Calculated from duplicate determinations on 16 samples.

TABLE II

COMPARISON OF CHROMATOGRAPHIC METHODS FOR THE ANALYSIS OF MONOGLYCERIDE EMULSIFIERS

Analytical data obtained with four samples of a similar type were used to give mean values (%) and average mean deviations from the mean value.

Component analyzed	*Column chromatography*	*Thin-layer chromatography*	*Gel chromatography*
Triacylglycerols	3.4 + 0.3	4.6 ± 1.4	4.0 ± 0.5
Diacylglycerols	27.3 + 0.9	26.2 ± 2.8	26.2 ± 1.2
Monoacylglycerols	65.3 + 1.2	65.8 ± 3.0	66.9 ± 1.3
Free fatty acids	1.8 + 0.2	1.3 ± 0.2	1.1 ± 0.4
Free glycerol	2.0 + 0.3	2.1 ± 0.7	1.7 ± 0.2

TABLE III

COMPOSITION OF SAMPLES OBTAINED BY THE STANDARD METHOD AND BY GEL PERMEATION CHROMATOGRAPHY

For gel chromatograms of samples, see Fig. 2.

Sample	*Method*	*Content (wt.-%)**				
		TG	*DG*	*MG*	*FA*	*G*
A	Standard	1.1	37.6	53.0	1.4	6.9
	GPC	2.0	37.4	53.4	1.0	5.7
B**	Standard	0.5	28.5	65.6	5.4	—***
	GPC	0.3	27.4§	67.6	3.4	—§§
C	Standard	0.8	4.5	90.8	1.8	2.1
	GPC	0.3	3.5	93.5	1.6	1.1
D	Standard	22.4	60.2	17.0	—§§§	—§§§
	GPC	25.3	60.8	13.6†	—§§§	—§§§

* TG = triacylglycerols; DG = diacylglycerols and 2-monoacylglycerols; MG = 1-monoacylglycerols; FA = free fatty acids; G = glycerols.

** Calculated per sample after removal of glycerol.

*** 57.3% glycerol found in original sample.

§ Of which 4.4% = diglycerol esters.

§§ In original sample, 55.4% glycerol (corrected value).

§§§ Undetectable or in trace amounts.

† Broad band; band area corresponds to a higher value.

no requirements for skilled labour. Thus, it can be stated that gel chromatography proved to be useful for the analysis of monoglyceride emulsifiers because, for a satisfactory distribution of fractions and an accuracy of determination corresponding to that of the standard method, the determination is much less labour- and time-consuming.

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