A Chromatographic Method for the Analysis of Propylene Glycol Fatty Acid Esters in Shortenings Containing Mono- and Diglycerides

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Abstract

A procedure is described for the separation, identification, and quantitative estimation of propylene glycol mono- and diesters of lauric, myristic, palmitic, stearic, and oleic acids in shortenings and lard containing mono- and diglycerides. Lipid classes are separated on a silicic acid column, and individual esters are estimated by gas chromatography. Analyses of several control mixtures and commercial samples are reported. Recoveries for individual components range from 92 to 105%, and total recoveries range from 96 to 100%.

Introduction

PROPYLENE GLYCOL (PG; 1,2 propanediol)—mono fatty acid esters are used as emulsifiers either alone or in combination with mono- and diglycerides in several foods, such as shortenings and cake mixes (1–5). Methods for the preparation of PG mono esters have been described by Martin and Lutton (6), also by Bradner and Birkmeier (7); several commercial products are available.

In the food industry the terms propylene glycol mono-stearate (PGMS) and propylene glycol stearate (PGS) are applied to mixtures of products which result from the esterification of PG with edible-grade stearic acid. The equilibrium mixture consists of free fatty acids, monoesters, diesters, and free PG. Edible-grade stearic acid invariably contains up to 51% palmitic acid and 2% to 8% of other fatty acids, principal among which is oleic acid. Several mixed esters of fatty acids derived from edible fats and oils are also available. Little published information is available on the methods of analysis of such mixtures alone or in combination with other emulsifiers, with the result that these compounds are seldom correctly identified.

In earlier publications from this laboratory (8,9) it has been shown that mono- and diglycerides can be quantitatively separated on silicic acid columns and analyzed by GLC. This paper describes the separation, identification, and quantitative estimation of PG esters of lauric, myristic, palmitic, stearic, and oleic acids along with mono- and diglycerides.

Several emulsifier compositions containing PG esters and mono- and diglycerides are used in foods. In order to analyze all the different types of classes it is necessary that a scheme be developed for the simultaneous analyses of all components. Therefore the solvents used for the column chromatographic analysis were the same as used for mono- and diglycerides, lactylated glycerides, and polyglycerol esters (8). GLC conditions were also the same as reported earlier for mono- and diglycerides (9).

Materials and Methods

The esters were prepared by three procedures with varying ratios of PG to fatty acid. As examples, methods for stearic acid esters are described.

Method A. (6) Stearic acid (0.015 mole) in xylene (200 ml) was refluxed with PG (0.15 mole) and toluenesulphonic acid (0.1 g) for three hours. The reaction mixture was then poured onto ice with stirring. The xylene layer was separated, washed with water three times, and dried over sodium sulphate. The filtered xylene solution was then diluted with 600 ml of n-hexane and stored over-night at −18C. Next morning the PGMS crystals were recovered and recrystallized from 600 ml of n-hexane (yield 53.0%).

Method B. (7) Stearic acid (0.0035 mole) was heated with PG (0.0130 mole) and NaOH (20 mg) in an oil bath at 140–160C for three hours. Nitrogen was bubbled through the mixture during the entire reaction period. The mixture was then dissolved in pentane-ethyl ether (1:1), washed with water till free of NaOH, and dried over sodium sulphate. The solvent was evaporated in a rotary evaporator at 40C (yield 51.6%).

Method C (10) The PG (0.1 mole) in dimethylformamide (40 ml) with dry pyridine (2 ml) and dry chloroform (30 ml) was cooled in an ice bath. Stearoyl chloride (24.1 g, 0.08 mole) in chloroform (30 ml) was added dropwise with constant stirring, and the reaction mixture was stored over-night at room temperature. Next morning the mixture was dissolved in 200 ml of ether and successively washed with water, 0.5M HCl, and water. The solvent was removed on a flash evaporator, and the esters were crystallized from n-hexane (yield 53.2%).

The fatty acid esters of lauric, myristic, palmitic, and oleic acid were prepared by Methods B and C. All fatty acids were of 96–99% purity as determined by GLC. Fatty acid chlorides were commercial technical grade.

Several commercial samples of PG esters were also used. All samples in control mixtures were purified by preparative TLC on Silica Gel-G (500 μ), impregnated with 4% boric acid or 11.7% sodium arsenite. Benzene-methanol (8:3) was found to be the most suitable solvent system.

The 1- and 2-isomers of PG mono stearate were prepared as described by Martin and Lutton (6). Purified samples obtained from Lutton were used for comparison. Several mixtures of vegetable shortening and lard with purified PG esters and mono- and diglycerides were prepared. Mono- and diglycerides in the mixtures were prepared and purified as described earlier (9). The identity of each fraction was confirmed by quantitative fatty acid analysis by GLC (11).

TABLE I
Analyses of Propylene Glycol Stearates

	Sample Numbers[a]							
	1	2	3	4	5	6	7	8
PG diesters:								
PGPP[b]	0.3	1.0	9.6	15.1	4.6	20.1	1.5	1.3
PGPS	2.0	1.9	13.5	24.5	16.9	34.5	4.8	4.0
PGSS	22.8	73.1	22.3	6.9	18.5	13.1	21.2	18.4
Other[c]	.....	.....	.....	2.1(3)	3.0(4)	2.8(3)	0.1(2)	+
	24.1	76.0	45.4	48.6	43.0	70.5	27.6	23.7
PG monoesters:								
PGP	2.8	+	16.9	27.8	17.2	14.3	7.3	6.2
PGS	73.1	20.5	31.6	19.5	35.3	11.1	62.9	54.4
Other[c]	.....	+	.....	4.1(4)	1.5(3)	3.1(3)	2.3(2)	2.0(3)
	79.9	20.5	48.5	51.4	54.0	29.5	72.2	62.6
Total	100.0	96.5[d]	93.9	100.0	97.0	100.0	100.0	86.3[e]

[a] Samples 1, 2, and 3 are products prepared in the laboratory by methods A, B, and C as described in the text. Samples 4 to 8 are commercial products. All values are percentage of total.
[b] P, palmitic acid; S, stearic acid.
[c] Includes myristates and oleates. Figures in parentheses represent numbers of peaks.
[d] Difference from 100 in Samples 2, 3, and 5 accounts for unreacted fatty acids.
[e] Sample 8 is a mixture of PG esters with mono- and diglycerides (10.4%) and triglycerides (3.3%).

Column Chromatography. Samples (1 g) were eluted from silicic acid columns as described earlier (9). The lipid material was eluted successively with 300 ml each of benzene (I), benzene with 10% ethyl ether (II), and ethyl ether (III). The lipids fractionated as follows: Fraction I, triglycerides and PG diesters; Fraction II, diglycerides and PG monoesters; Fraction III, monoglycerides.

Gas-Liquid Chromatography. Trimethylsilyl ether (TMS) derivatives were prepared as described earlier (9) and analyzed by GLC. A Perkin-Elmer model 800 gas chromatograph, equipped with dual ⅛-in. stainless steel columns packed with 3% JXR on Gaschrom-Q with dual ceramic tip flame ionization detectors, was used. Helium flow was regulated at 37 ml/min at ambient temperature. Columns were programmed from 120 to 325C at 10C per minute. Percentage distribution was calculated as area under each peak by a disc integrator.

Results and Discussion

Separation of lipid classes on silicic acid columns is based on the differences in polarity because of free hydroxyl groups. Mono fatty acid esters of PG are eluted with diglycerides whereas diesters of PG, with both hydroxyl groups esterified, are eluted with triglycerides. Recoveries of known mixtures were in the range of 96 to 100%.

Table I shows the analyses of PGS prepared in the laboratory by the three methods. In choosing these three methods for the synthesis of the esters, it was not the intention to study the comparative yields of mono- and diesters but to obtain PG esters from various sources. Fraction I along with PG diesters also included some of the unreacted fatty acids. All diester fractions in this study were purified by preparative thin-layer chromatography. Unreacted fatty acid derivatives have a lower retention-time than PG esters and can be estimated quantitatively by GLC. Commercial samples contained up to 3% of unreacted material. Analyses of five commercial samples are shown also in Table I.

GLC separation of PG monoesters and diglycerides, which are eluted together in Fraction II, is shown in Fig. 1. In earlier studies on the application of GLC to quantitative analysis (9) it was emphasized that relative flame-response factors (RRF) should be established for the different classes of compounds. RRF for diesters in relation to PG monomyristate were as follows: PG-laurate 1.05; PG-myristate 1.00; PG-palmitate 1.03; PG-oleate 1.10; PG-stearate 1.00. RRF were established on the basis of five to six individual analyses. Under the conditions of GLC used in this study the two isomers of mono fatty acid esters were not resolved.

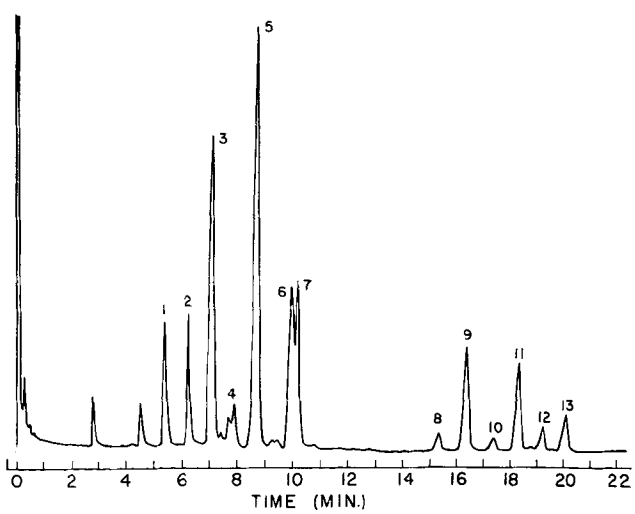

FIG. 1. Separation of PG monoesters and diglycerides. PG esters: 1, monolaurate; 3, monomyristate; 5, monopalmitate; 6, mono-oleate; 7, monostearate. Diglycerides: 8, myristolaurate; 9, dimyristate; 10, myristopalmitate; 11, dipalmitate; 12, palmitostearate; 13, distearate.

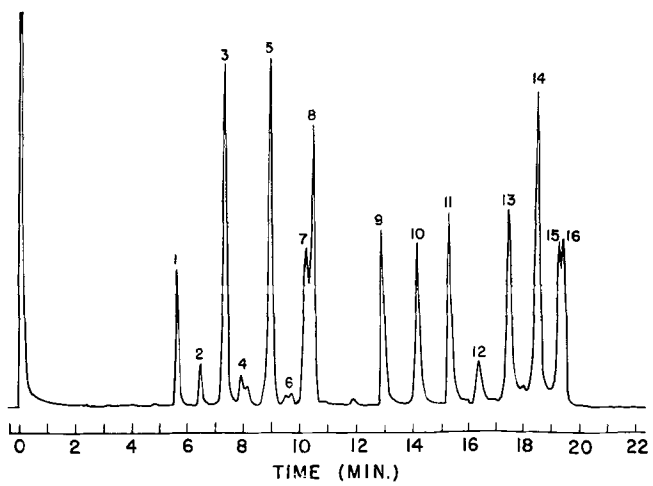

FIG. 2. Separation of PG mono- and difatty acid esters. 1, PG monolaurate; 2, unknown; 3, PG monomyristate; 4, unknown; 5, PG monopalmitate; 6, unknown; 7, PG monooleate; 8, PG monostearate; 9, PG dilaurate; 10, PG myristolaurate; 11, PG dimyristate; 12, PG myristopalmitate; 13, PG dipalmitate; 14, PG palmitostearate; 15, PG dioleate; 16, PG distearate.

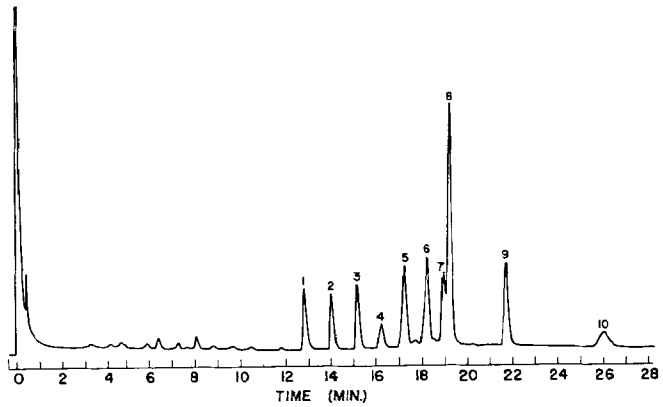

FIG. 3. Separation of PG difatty acid esters and triglycerides. 1, PG dilaurate; 2, PG myristolaurate; 3, PG dimyristate; 4, PG palmitomyristate; 5, PG dipalmitate; 6, PG palmitostearate; 7, PG dioleate; 8, PG distearate + trilaurin (C_{36}); 9, trimyristin (C_{42}); 10, tripalmitin (C_{48}).

Fig. 2 shows the separation of PG mono- and diesters. As the diesters of PG are eluted with triglycerides in Fraction I, it was necessary to study the GLC separation of mixtures of PG diesters with triglycerides (Fig. 3). Triglycerides have been analyzed by GLC by several workers (12–13). In the author's laboratory the GLC conditions described have yielded quantitatively reproducible results. The diesters of PG can be quantitatively estimated in mixtures with triglycerides either from the percentage distribution, as calculated from the RRF for triglycerides in relation to PG esters, or by use of an internal standard.

Unsaturated triglycerides encountered in shortenings require the calculation of RRF for several individual triglycerides. Under the conditions of this study triolein had an RRF of 6.4. On hydrogenation of the sample the RRF for all triglycerides were in the range of 1.25 to 2.8. As this involves an additional step in the procedure and because triglyceride analysis requires exacting conditions, the use of an internal standard was preferred. Fig. 4 is a chromatogram of such a mixture (Fraction I), derived from lard and PG esters. Trimyristin used as an internal standard had an RRF of 1.05.

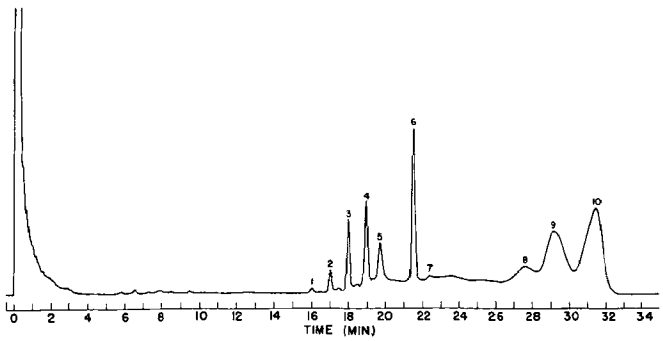

FIG. 4. Separation of PG diesters from triglycerides (lard). 1, unidentified; 2, PG dipalmitate; 3, PG palmitostearate; 4, PG distearate; 5, unidentified; 6, trimyristin; 7, unidentified; 8, triglyceride type C_{50}; 9, triglyceride type C_{52}; 10, triglyceride type C_{54}.

In GLC, triglyceride separation is based on molecular weights, and peaks are assigned total carbon numbers of the constituent fatty acids. In this study, trilaurin ($C_{12+12+12}$) could not be separated from PG-distearate (C_{18+18}). It is assumed that, except for butter fat, palm oils, and coconut oil, there is little possibility that shorter chain-length triglycerides will occur in commercial shortenings. Shortening compositions in this study were prepared from hydrogenated soybean oil, hydrogenated marine oil, and lard.

Table II shows the analysis of control mixtures. Mixtures were prepared from individual purified mono- and diglycerides and PG esters. Triglycerides were obtained by fractionation from lard, hydrogenated soybean oil, and hydrogenated herring oil. Several combinations of PG esters and mono- and diglycerides were used in this study, and data from

TABLE II

Analysis of Shortening Compositions

	1			2			3		
	A[a]	B[a]		A	B		A	B	
Triglycerides	76.50	76.20	(99)[b]	70.24	70.13	(99)	80.0	79.2	(99)
PG diesters									
MM[c]	.....	.....		1.52	1.60	(105)	.....	.....	
PP	0.57	0.53	(92)	1.10	1.15	(105)	.....	.....	
PS	2.34	2.40	(102)	.....	.....		.....	.....	
SS	2.28	2.25	(98)	.....	.....		4.8	4.9	(102)
OO	.....	.....		2.00	1.92	(96)	.....	.....	
Total	5.19	5.18	(99)	4.62	4.67	(101)			
PG monoesters									
L	0.05	0.06	(120)	1.56	1.51	(96)	.....	.....	
M	0.10	0.09	(90)	1.18	1.20	(101)	.....	.....	
P	2.06	2.10	(102)	2.36	2.40	(101)	.....	.....	
S	4.34	4.42	(102)	3.08	2.95	(95)	5.0	5.0	(100)
O	0.05	0.06	(120)	2.05	2.15	(104)	.....	.....	
Total	6.60	6.77	(102)	10.23	10.21	(99)			
1,3 diglycerides									
MM	.....	.....		2.00	2.00	(100)	.....	.....	
PP	2.14	2.08	(97)	.....	.....		.....	.....	
SS	2.32	2.16	(93)	1.46	1.41	(95)	3.0	3.4	(105)
OO	.....	.....		1.84	1.90	(103)	.....	.....	
Total	4.46	4.24	(95)	5.30	5.30	(100)			
1-monoglycerides									
P	2.87	2.91	(101)	3.46	3.50	(101)	.....	.....	
S	4.37	4.42	(101)	3.63	3.72	(102)	7.2	7.4	(105)
O	.....	+	.....	2.52	2.34	(92)	.....	.....	
Total	7.24	7.33	(101)	9.61	9.56	(99)			

[a] A = known composition.
B = as estimated by the method; all values are averages of two or three determinations.
[b] Figures in parentheses are calculated percentage recoveries.
[c] L, lauric; M, myristic; P, palmitic; S, stearic; O, oleic.
Samples 1, 2, and 3 are compositions prepared with lard, hydrogenated soybean oil, and hydrogenated marine oil respectively. All values are percentage of total.

three such analyses are shown. Propylene glycol difatty acid esters were calculated from GLC curves with trimyristin as an internal standard. Recoveries of the individual components were satisfactory, in accordance with the quantities used in the mixtures.

The monoesters are the functional emulsifiers; however commercial samples contain varying amounts of diesters. Although it is not necessary that the food additive be 100% pure monoester, it is important for regulatory agencies to know the exact composition of the food additive. The procedure described herein makes this possible.

ACKNOWLEDGMENTS

Commercial samples of propylene glycol esters and mono- and diglycerides, also samples of fats used in the manufacture of shortenings were generously supplied by the industry.

REFERENCES

1. Howard, N. B., U.S. Patent 3,145,107 (August 18, 1964).
2. Abbott, C. J., Jr., U.S. Patent 3,145,110 (August 18, 1964).
3. Kuhrt, N. H., and R. A. Broxholm, U.S. Patent 3,034,897 (May 15, 1962).
4. Kuhrt, N. H., and R. A. Broxholm, U.S. Patent 3,034,898 (May 15, 1962).
5. Kuhrt, N. H., and R. A. Broxholm, JAOCS *40*, 730 (1963).
6. Martin, J. B., and E. S. Lutton, JAOCS *42*, 529 (1965).
7. Bradner, J. D., and R. L. Birkmeier, JAOCS *41*, 367 (1964).
8. Sahasrabudhe, M. R., J. J. Legari and W. P. McKinley, J. Assoc. Official Anal. Chem. *49*, 337 (1966).
9. Sahasrabudhe, M. R., JAOCS *44*, 379 (1967).
10. Hartman, L., J. Chem. Soc. London 1957, 3572.
11. Sahasrabudhe, M. R., JAOCS *42*, 862 (1965).
12. Kuksis, A., and M. J. McCarthy, Can. J. Biochem. Physiol. *40*, 679 (1962).
13. Litchfield C., R. D. Harlow and R. Reiser, JAOCS *42*, 849 (1965).

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