Monoglyceride Analysis with Reversed Phase HPLC[1]

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A simple, convenient method for the simultaneous determination of the homologous distribution and ratio of positional isomers of monoglycerides in commercial products has been developed. Monoglycerides were analyzed with reversed phase HPLC using the glyceride-selective post-column reactor detector (GS-PCRD). The GS-PCRD indicated molar responsibility to all monoglycerides up to monostearin. It can, for example, detect 0.07 nmol of 1-monolaurin and give a linear working curve between 0.18-80.3 nmol of 1-monolaurin. Furthermore, monoglycerides in commercial margarines could be selectively detected and quantitatively determined without any pretreatment. Therefore, the proposed method should be applicable to the analysis of monoglycerides in various commercial products.

In a preceding paper (1), development of a new post-column reactor detector (PCRD) for the analysis of triglycerides with high sensitivity, high selectivity and molar responsibility was detailed. By using this glyceride-selective PCRD (GS-PCRD), triglycerides of natural fats and oils were sufficiently separated and quantitatively analyzed with non-aqueous reversed phase (NARP) chromatography with octadecyl chemically bonded silica (ODS) column. The combination of argentation HPLC using an infra-red detector with NARP chromatography using the GS-PCRD also was investigated for the full analysis of triglycerides of natural fats and oils (2).

On the other hand, monoglycerides are widely used as emulsifiers in the food, household and cosmetic industries. Analysis of monoglycerides has been done by means of gas chromatography (GC) (4), thin layer chromatography (5) and HPLC (6). However, there is no entirely convenient method for the simultaneous determination of the homologous distribution and the ratio of positional isomers of monoglycerides in commercial products. By using the GS-PCRD, the authors tried to develop a convenient and useful method for this purpose.

This paper deals with a simple, convenient method for the simultaneous determination of the homologous distribution and the ratio of positional isomers of monoglycerides in various commercial products with reversed phase HPLC using the GS-PCRD.

EXPERIMENTAL PROCEDURES

Apparatus. Apparatus are similar to those described previously (1,2).

Reagents. Hypersil MOS (Shandon, Runcorn, United Kingdom) was used as a stationary phase, which was octyl chemically bonded silica packings of an average 3 μm diameter. Authentic monoglycerides were purchased from Sigma Chemical Co. (St. Louis, Missouri) and P-L Biochemicals (Milwaukee, Wisconsin). Acetonitrile of HPLC analysis grade was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Other reagents were of analytical reagent grade.

Procedure for monoglyceride analysis. A stainless column (4.6 mm i.d., 150 mm long) packed with Hypersil MOS (3 μm) was used as a stationary phase and kept

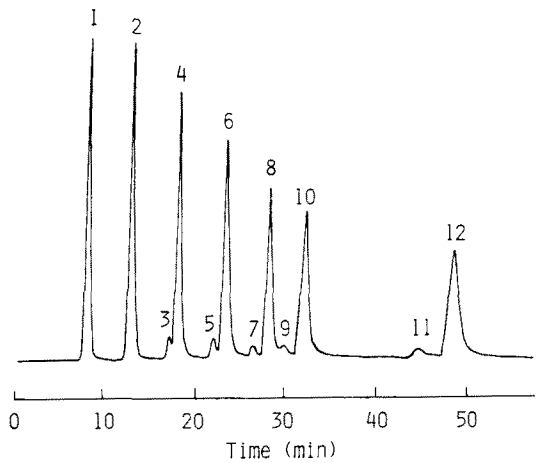

FIG. 1. Typical chromatogram of a standard mixture of monoglycerides using the post-column reactor detector. 1, glycerin; 2, 1-monolaurin; 3, 2-monomyristin; 4, 1-monomyristin; 5, 2-monolinolein; 6, 1-monolinolein; 7, 2-monopalmitin; 8, 1-monopalmitin; 9, 2-monoolein; 10, 1-monoolein; 11, 2-monostearin; 12, 1-monostearin.

TABLE 1

Response of the Post-Column Reactor Detector to Glycerin and Monoglycerides

Monoglyceride	Relative molar response to glycerin
Glycerin	1.00
1-Monolaurin	1.01
1-Monomyristin	0.97
1-Monopalmitin	1.03
2-Monopalmitin	1.00
1-Monostearin	0.98
1-Monoolein	1.00
1-Monolinolein	0.99

TABLE 2

Range of Calibration Curves and Reproducibilities

Monoglyceride	Range of calibration curves (nmol)	Coeff. of variation[a] (%)
Glycerin	0.22-77.2	0.35
1-Monolaurin	0.18-80.3	0.45
1-Monomyristin	0.20-82.8	0.67
1-Monopalmitin	0.21-84.8	0.58
1-Monostearin	0.21-55.9	2.53

[a]Each sample (2.5 μg) was injected and analyzed 5 times.

[1]For part II, see reference 2.

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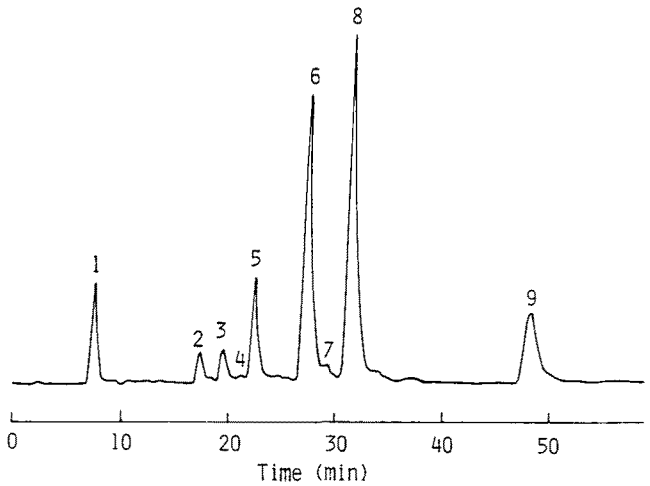

FIG. 2. Analysis of a commercial emulsifier. 1, glycerin; 2, 1-monomyristin; 3, 1-monopalmitolein; 4, 2-monolinolein; 5, 1-monolinolein; 6, 1-monopalmitin; 7, 2-monoolein; 8, 1-monoolein; 9, 1-monostearin.

at 30 C. Acetonitrile-water (67/33) or (58/42) was used as a mobile phase at a flow rate of 0.8 ml/min. The effluent was monitored with the GS-PCRD.

A sample, containing 0.2-200 μg of monoglycerides dissolved in 5-30 μl of ethanol or acetone, was injected into HPLC.

RESULTS AND DISCUSSION

Separation of monoglyceride. Many investigations on monoglyceride analysis by means of HPLC have been reported (3,6,7). However, none has presented an entirely useful method for the simultaneous analysis of the homologous distribution and the ratio of positional isomers of monoglycerides. Therefore, the complete separation of monoglycerides using the GS-PCRD was investigated.

On the other hand, only a few solvents can be used as a mobile phase because of the interference effects on the GS-PCRD. Among these solvents, acetonitrile and ethanol were compatible with the mobile phase (1). Preliminary investigations on the separation of homologs of monoglycerides with reversed phase chromatography demonstrated that acetonitrile-water was suitable for a mobile phase. Further, the separation of the saturated homologs could be made with six HPLC packings, Develosil C8-3 (3 μm), Develosil ODS-3 (3 μm), Hypersil MOS (3 μm), LiChrosorb RP-8 (5 μm), Unisil ODS-3 (3 μm) and Hitachi gel 3057 (ODS, 3 μm), using acetonitrile-water as a mobile phase. However, the separation of saturated and unsaturated homologs was accomplished with three octyl chemically bonded silica packings, Develosil C8-3, Hypersil MOS and LiChrosorb RP-8. Among these columns, LiChrosorb RP-8 gave poor resolution compared with other columns, and a little tailing of peaks was observed with Develosil C8-3. Therefore, Hypersil MOS was used in the following study.

Isomerization of 2-monoglyceride to 1-monoglyceride in an HPLC column was observed with increasing column temperature. Therefore, the column temperature was set at 30 C, where no isomerization was observed.

TABLE 3

Comparison of the Homologous Distributions of Monoglycerides Obtained by the Proposed Method with Those of Gas Chromatography[a]

Homolog	Proposed method Sample 1 (mol %)	Proposed method Sample 2 (mol %)	GC method[a] Sample 1 (mol %)	GC method[a] Sample 2 (mol %)
C14	2.3	2.6	2.3	2.7
C14:1	-	3.0	-	3.0
C16	29.0	3.1	28.3	3.9
C16:1	3.3	12.2	4.2	12.2
C18	12.4	-	11.5	-
C18:1	42.7	76.9	43.5	75.2
C18:2	10.3	2.2	10.2	3.1

[a]Fatty acid methyl esters were obtained from monoglycerides by interesterification, and their homolog distributions were analyzed by GC.

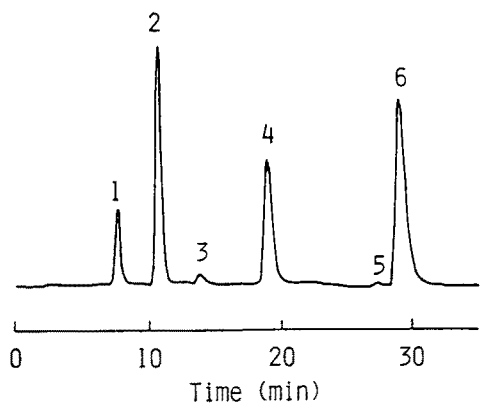

FIG. 3. Analysis of commercial margarine with the glyceride-selective detector. 1, glycerin; 2, 1-monolaurin (internal standard); 3, 1-monomyristin; 4, 1-monopalmitin; 5, 2-monostearin; 6, 1-monostearin.

The best isocratic separation was obtained with acetonitrile-water (58/42) as a mobile phase as shown in Figure 1, where 1- and 2-monolaurin could not be separated. Since monolaurin was rarely used as an emulsifier, the quantitative determination of monoglycerides in commercial products was carried out by an internal standard method using 1-monolaurin as an internal standard.

Response and reproducibility of the GS-PCRD. Table 1 shows the response of the GS-PCRD to glycerin, 1-monolaurin, 1-monomyristin, 1-monopalmitin, 2-monopalmitin, 1-monostearin, 1-monoolein and 1-monolinolein. Since the relative responses of various monoglycerides to glycerin are between 0.97 and 1.03, it is demonstrated that the GS-PCRD has molar responses to all monoglycerides up to monostearin. The linear range of calibration curves of glycerin, 1-monolaurin, 1-monomyristin, 1-monopalmitin and 1-monostearin are shown in Table 2, and their detection limits are 0.07-0.1 nmol. Reproducibilities of the analyses of 2.5 μg of each monoglyceride were within 2.5% relative.

Applications. Figure 2 shows the chromatogram of a commercial emulsifier by the proposed method, and the comparison of the determination of homolog distribu-

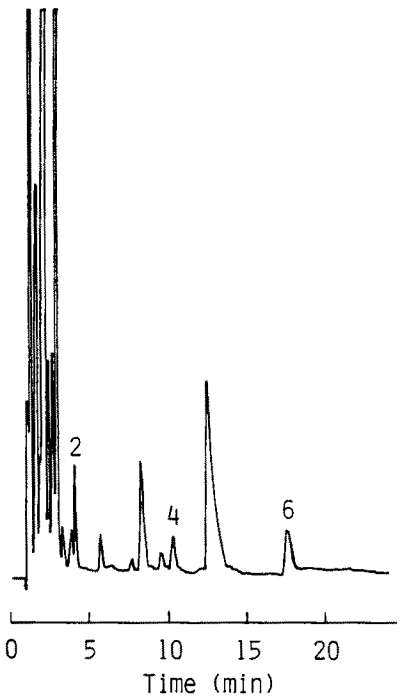

FIG. 4. Analysis of a commercial margarine with a UV detector (210 nm). 1, glycerin; 2, 1-monolaurin (internal standard); 3, 1-monomyristin; 4, 1-monopalmitin; 5, 2-monostearin; 6, 1-monostearin.

tion with that of the GC method is shown in Table 3. The agreement of both distributions is satisfactory.

The GS-PCRD detected only monoglycerides in a commercial margarine without any pretreatment, as shown in Figure 3, while a UV detector (210 nm) detected unknown UV active substances in the margarine except monoglycerides as shown in Figure 4. In these analyses, acetonitrile-water (67/33) was used as a mobile phase because of the absence of unsaturated monoglycerides. The determination was carried out directly with an internal standard method using 1-monolaurin as an internal standard.

The results of the determination of the added monoglyceride in a margarine are shown in Table 4, and satisfactory recoveries were obtained. Table 5 shows the results of the determination of monoglycerides in commercial margarines. Their contents were from 0.05% to 0.25%, and no interference was observed. Therefore, the proposed method should conveniently be applicable to the analysis of monoglycerides in various commercial products.

TABLE 4

Recoveries of Added Monoglycerides to Margarines

Added (%)	Found (%)	Recovery (%)
0.201	0.203	101.0
0.502	0.496	98.8
2.00	2.03	101.5

TABLE 5

Analysis of Monoglycerides in Commercial Margarines

Margarine	Homolog distribution (mol %) C_{14}	C_{16}	C_{18}	Content (%)
A	-	35.8	64.2	0.06
B	1.5	32.0	66.5	0.18
C	-	43.6	56.4	0.06
D	1.7	32.3	66.0	0.25
E	-	29.2	70.8	0.05

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