Separation of Monoacylglycerols by High-Performance Liquid Chromatography on Nitrile-Bonded Phase[1]

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Monoacylglycerol molecular species, as their di-3,5-dinitrophenylurethane derivatives, were well separated by normal-phase high-performance liquid chromatography on nitrile-bonded phase. The peaks emerged in the order 20:0, 18:0, 16:0, 18:1, 16:1, 18:2 and 18:3. The peaks of 1- and 2-monoacylglycerols with the same acyl group showed complete overlapping. This method could be applied to get acyl compositions in the three positions of triacyl-*sn*-glycerols in their stereospecific analysis.

KEY WORDS: High-performance liquid chromatography, monoacylglycerols, nitrile-bonded phase, soybean oil, stereospecific analysis, triacyl-*sn*-glycerols.

High-performance liquid chromatography (HPLC) of monoacylglycerol (MG) homologous mixtures on chiral stationary phases, Sumichiral OA-2100 and OA-4100, as di-3,5-dinitrophenylurethane (di-3,5-DNPU) derivatives has been reported under various conditions, but no separation has been observed for a critical pair, 1-hexadecanoyl- and 1-octadecenoylglycerols (1–3). This paper presents clear separation of the critical pair of MGs as di-3,5-DNPUs by normal-phase HPLC on nitrile-bonded phase.

Some recent papers presented new methods for stereospecific analysis of triacyl-*sn*-glycerols (TGs) (3–6). The method presented by us was based on chiral HPLC resolution of MG di-3,5-DNPUs prepared by partial hydrolysis of TGs. Analyses of acyl groups in each enantiomeric or isomeric MG fraction were carried out by gas–liquid chromatography (GLC) after conversion to methyl esters. HPLC on nitrile-bonded phase presented in this paper could be used for direct analysis of the acyl groups of MG di-3,5-DNPUs instead of GLC of methyl esters.

MATERIALS AND METHODS

Preparation of MGs and conversion of them to di-3,5-DNPUs were carried out by the procedure described in previous papers (1–4). HPLC of MG di-3,5-DNPUs on nitrile-bonded phase was done by a Shimadzu LC-6A pump (Shimadzu, Kyoto, Japan), together with a Hitachi L 4000 UV detector (Hitachi, Tokyo, Japan) and a Shimadzu C-R6A integrator. A column of nitrile-bonded phase, LiChrospher 100 CN (50 cm × 4.6 mm i.d.; E. Merck, Darmstadt, Germany) was used with *n*-hexane/1,2-dichloroethane/ethanol (40:10:1, vol/vol/vol) as mobile phase at a flow rate of 1.2 mL/min. Detection was at 254 nm. Stereospecific analysis of TGs was carried out as described in a previous paper (7). Total products obtained from TGs by partial hydrolysis with ethyl magnesium bromide were converted to di-3,5-DNPUs and fractionated to each of 1- and 2-MG fractions by semi-preparative HPLC on silicic acid (7). Di-3,5-DNPU derivatives of 1-MGs were resolved into *sn*-1- and *sn*-3-MG fractions by HPLC on a Sumichiral OA-4100 chiral column (50 cm × 4 mm i.d.; Sumitomo Chemicals, Osaka, Japan) with *n*-hexane/1,2-dichloroethane/ethanol (40:12:3, vol/vol/vol) as mobile phase at a flow rate of 1.0 mL/min. Detection was at 254 nm (7).

RESULTS AND DISCUSSION

In HPLC of fatty acid derivatives, few applications have been reported with normal-phase HPLC (8). In this study, the MG 3,5-DNPU derivatives were resolved by normal phase-HPLC on nitrile-bonded phase (Fig. 1). It is noteworthy that a critical pair, 1-hexadecanoyl- and 1-octadecenoylglycerols, was clearly resolved into two peaks on a LiChrospher 100 CN column. HPLC of 1- and 2-MG mixtures showed complete overlapping of the isomers. Table 1 shows the retention data. Recently, saturated and unsaturated fatty acids were separated as 4-bromoethyl-7-methoxycoumarin derivatives by reversed-phase HPLC with a high-efficiency packed capillary column (9). Either of the following equations could be used to calculated the equivalent chainlength (ECL) values:

$$\mathrm{ECL} = N - yn \quad [1]$$

$$\mathrm{ECL} = xN - yn + z \quad [2]$$

where N and n are numbers of carbon atoms and olefin bonds in an unsaturated acid, and x, y and z are empirically derived coefficients. Calculations of x, y and z from the ECLs for 18:1, 16:1 and 18:2 in Table 1 show that Equation 1 is applicable in this case, and x, y and z are 1.0, 3.2 and 0, respectively. ECL for 18:3, calculated from this factor, agreed with that obtained by experiment. It suggests that the equation can be used for prediction of numbers of carbon atoms and olefinic bonds of the unidentified peak component.

Table 2 shows results of stereospecific analysis of soybean oil TGs by HPLC on the nitrile-bonded phase. The tendency of the results was not different from those reported in previous papers (3,10). The presented normal-phase HPLC method could offer a fast and simple way

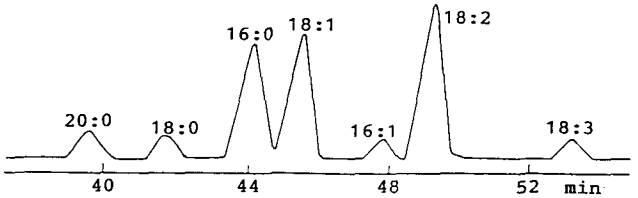

FIG. 1. Normal-phase high-performance liquid chromatography separation of a 1-monoacylglycerol mixture as its di-3,5-dinitrophenylurethane derivative on a LiChrospher 100 CN column (50 cm × 4.6 mm i.d.; E. Merck, Darmstadt, Germany). Mobile phase, *n*-hexane/1,2-dichloroethane/ethanol (40:10:1, vol/vol/vol). Flow rate, 1.2 mL/min. Detection, 254 nm.

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TABLE 1

Retention Data of MG Derivatives

MG homologue	Retention time (min)	ECL found	ECL calculated[a]
20:0	39.6	—	—
18:0	41.7	—	—
16:0	44.0	—	—
18:1	45.2	14.9	14.8
16:1	47.8	12.9	12.8
18:2	49.0	11.7	11.6
18:3	53.3	8.4	8.4

[a]Calculated from ECL = $N - 3.2n$ (N and n = numbers of carbon atoms and olefinic bonds, respectively). MG, monoacylglycerols; ECL, equivalent chainlength.

TABLE 2

Stereospecific Analysis of Soybean Triacylglycerols (mole %)

Fatty acids	Total[a]	*sn*-1[b]	*sn*-2[b]	*sn*-3[b]
16:0	11.7	16.9	7.5	11.6
18:0	3.4	6.3	—	4.5
18:1	24.2	22.5	22.4	27.9
18:2	54.2	45.7	64.3	50.8
18:3	6.4	8.7	5.8	5.1

[a]Obtained by gas–liquid chromatography analysis of fatty acid methyl esters.
[b]Calculated from the results of high-performance liquid chromatography on nitrile-bonded phase.

to carry out stereospecific analyses of TGs and facilitate the analysis of minor amounts of TGs. The data shown in Table 2 was obtained from 0.1 mg of TG sample mixed with 1 mg of trieicosan. Direct analysis of MG di-3,5-DNPUs can eliminate selective loss of polyunsaturated fatty acids in the steps of preparation of methyl esters and their GLC analysis. Present HPLC on nitrile-bonded phase is applicable to the analysis of ordinary vegetable oil TGs. This method will also be applicable to the analysis of TGs in animal and marine sources if the column resolution can be improved to separate highly unsaturated and other MGs obtained from them.

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