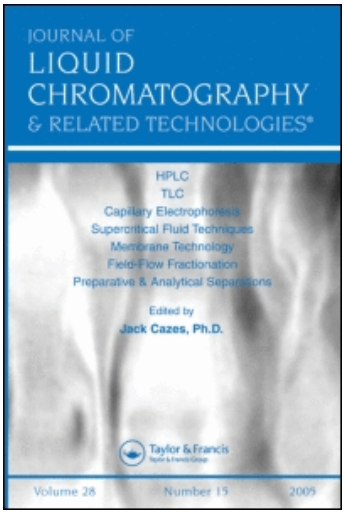

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Simultaneous Separation of Monoacylglycerols, Free Fatty Acids, and Fatty Acid Methyl and Ethyl Esters by Reversed-Phase HPLC

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ABSTRACT

We have developed a reversed-phase C_{18} high performance liquid chromatography (HPLC) method to separate molecular species of monoacylglycerols (MAG), fatty acids, fatty acid methyl esters, and fatty acid ethyl esters, simultaneously. This system also separates the regioisomers, 2-acyl-*sn*-glycerol and 1-acyl-*sn*-glycerol, with 2-acyl-*sn*-glycerol eluting earlier than 1-acyl-*sn*-glycerol. The elution order of the fatty acid and esters was ricinoleate, linolenate, linoleate, palmitate, oleate, and stearate.

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This method is also useful for the identification of radiolabeled metabolites using co-chromatography.

Key Words: HPLC; Monoacylglycerols; Molecular species; Lipids; Metabolism.

INTRODUCTION

Separation of the molecular species of monoacylglycerols (MAG) has rarely been reported. A reversed-phase C_{18} high-performance liquid chromatography (HPLC) system using isocratic elution with acetonitrile/water (67 : 33) separates a mixture of 11 molecular species of underivatized MAG standards.[1] The *sn*-2-isomers eluted slightly earlier than their respective *sn*-1-isomers, and were partially separated. The enantiomeric isomers (*sn*-1 and *sn*-3) were not separated,[2] but can be separated by chiral HPLC[3,4] as MAG derivatives.

We have previously reported a C_{18} HPLC method for the separation of the molecular species of triacylglycerols (TAG) and diacylglycerols (DAG) with their relative retention times (RRT).[5] Using this method, we have identified 61 molecular species of radiolabeled TAG and DAG produced by incorporation of six radiolabeled fatty acids (FA) in castor microsomal incubations.[6] DAG were minor metabolites in castor microsomal incubations,[6] and are intermediates in the biosynthetic pathway of TAG.[7] The C_{18} HPLC method for separation of TAG and DAG is not suitable for MAG, as these and free FA (FFA) eluted very early (before 3 min) with poor resolution. To identify these MAG we required an alternative HPLC method to resolve the MAG. We have previously reported a C_{18} HPLC method for the separation of FFA using a linear gradient of 85% methanol to 100% methanol containing 0.05% of acetic acid as ion suppressor.[8] Since MAG and FFA eluted closely in this system, the same HPLC method used for FFA could resolve FFA and MAG, simultaneously. Therefore, we tested the method developed for FFA in order to separate the molecular species of MAG. FFAs are acidic and their separation requires an ion suppressor to obtain sharp peaks, whereas MAG as well as methyl and ethyl esters of FA do not require an ion suppressor. The retention times of the esters are slightly shorter when eluent without acetic acid is used.

We have recently studied the incorporation of radiolabeled oleate and ricinoleate into acylglycerols in soybean microsomal incubations. Both radiolabeled methyl esters and ethyl esters of FA were also identified. The commercial radiolabeled oleate and ricinoleate were stored in ethanol, and radiolabeled ethyl esters were found together with commercial radiolabeled oleate and ricinoleate. During the lipid extraction, methanol was used and

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the radiolabeled methyl esters were also found. These esters are artifacts, not metabolites resulting from the radiolabeled FA in the soybean microsomal incubations. Because the polarities of these esters are similar to those of MAG and FA, we also included their separation here as part of this study.

The HPLC methods we have developed[4,8] use solvents of low toxicity only: methanol, 2-propanol, and water. In metabolic studies, these methods can be used for co-chromatography with standards to identify the radiolabeled metabolites, by matching the retention times from the absorbance detector at 205 nm and flow scintillation analyzer.[6,7] These solvents are among the least toxic and are also the least quenching in radioactivity scintillation counting. In order to save time and to obtain better resolution, we used underivatized TAG, DAG, and FA for HPLC systems.

EXPERIMENTAL

HPLC was carried out on a liquid chromatograph (Waters Associates, Milford, MA), using an absorbance detector (Waters 2487) at 205 nm. A C_{18} column (25 × 0.46 cm, 5 μm, Luna C_{18}, Phenomenex, Torrance, CA) was used. The eluent was a linear gradient from 85% aqueous methanol (containing 0.05% of acetic acid) to 100% methanol (containing 0.05% of acetic acid) in 40 min, followed by 100% methanol (containing 0.05% of acetic acid) isocratically for another 5 min. The eluent contained 0.05% glacial acetic acid as ion suppressor. The standards (about 5–50 μg each) in about 30 μL of methanol were chromatographed at a flow rate of 1 mL/min. Molecular species of MAG, FA, FA methyl esters, and FA ethyl esters standards were obtained from Sigma (St. Louis, MO) and Nu-Chek (Elysian, MN).

Diricinoleoylglycerols were synthesized from triricinolein using a lipase from *Penicillium roquefortii* in diisopropyl ether.[9] Monoricinoleoylglycerols were synthesized from triricinolein using a 1,3-specific lipase from *Rhizopus oryzae* in toluene (manuscript in preparation). Triricinolein was fraction collected from castor oil.

RESULTS AND DISCUSSION

The chromatogram of oleate and its derivatives is shown in Fig. 1. The elution order is 2-oleoyl-*sn*-glycerol < 1-oleoyl-*sn*-glycerol < oleic acid < oleic acid methyl ester < oleic acid ethyl ester, with baseline separation. In a previous study,[1] 2-oleoyl-*sn*-glycerol, and 1-oleoyl-*sn*-glycerol were only

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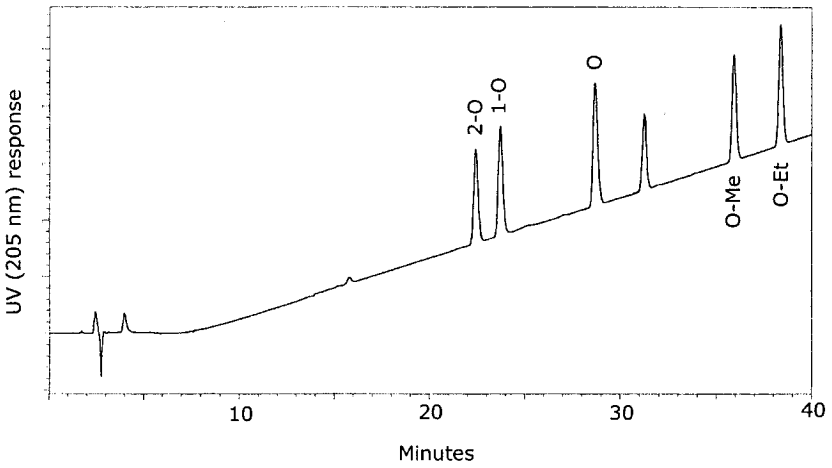

Figure 1. Reversed-phase C_{18} HPLC chromatogram separating oleic acid derivatives. *Abbreviation:* 2-O, 2-oleoyl-*sn*-glycerol; 1-O, 1-oleoyl-*sn*-glycerol; O, oleic acid; O-Me, oleic acid methyl ester; O-Et, oleic acid ethyl esters. The peak at 31.2 min is an unknown contaminant. For HPLC conditions, see Experimental.

partially separated. 2-Oleoyl-*sn*-glycerol elutes before 1-oleoyl-*sn*-glycerol, most likely because a primary alcohol is more polar than a secondary alcohol. Oleic acid methyl ester is less polar than oleic acid and, thus, elutes after oleic acid. The ethyl ester is even less polar and elutes later.

The RRT of several molecular species of MAG, FA, FA methyl esters, and FA ethyl esters are shown in Table 1. They include those of the derivatives of ricinoleate, linolenate, linoleate, palmitate, oleate, and stearate in order of elution. This elution order is the same as we observed previously for FFA,[8] FA methyl esters,[8] TAG, and DAG.[5] The order should be the same as that of many FAs in the C_{18} HPLC reported earlier.[8] The elution characteristics of different FA derivatives were similar to those of oleate, e.g., 2-acyl-*sn*-glycerol is more polar than 1-acyl-*sn*-glycerol. Diricinoleoylglycerols are also included in Table 1, because their polarity is within this range. 1,2-Diricinoleoyl-*sn*-glycerol elutes earlier than its 1,3-isomer. However, in the HPLC system separating the molecular species of TAG and DAG[5] using the gradient of methanol/2-propanol without water and ion suppressor, 1,3-diolein (16.19 min) eluted slightly earlier than 1,2-diolein (16.32 min). The difference may be due to the eluent and/or the hydroxyl group on the ricinoleate chain.

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Table 1. RRT of the molecular species of MAG, free fatty acids, fatty acid methyl esters, fatty acid ethyl esters, and diricinoleoylglycerol.[a]

Standards[b]	RRT[c]	Standards	RRT	Standards	RRT	Standards	RRT
2-R	7.3	1-L	18.1	Ln-Me	25.5	L-Et	31.9
1-R	7.8	Ln	19.0	2-S	26.1	S	32.2
R	10.5	2-P	19.5	O	27.1	P-Me	32.5
2-Ln	13.9	1-P	20.8	1-S	27.2	O-Me	33.8
1-Ln	14.9	2-O	21.2	Ln-Et	28.1	P-Et	34.7
R-Me	15.0	1-O	22.2	1,2-RR	29.1	O-Et	36.0
2-L	16.0	L	22.7	1,3-RR	29.5	S-Me	38.7
R-Et	17.0	P	25.3	L-Me	29.6	S-Et	40.9

[a]For HPLC conditions, see Experimental.
[b]*Abbreviation:* R, ricinoleate; S, stearate; O, oleate; L, linoleate; Ln, linolenate; P, palmitate; Me, methyl ester; Et, ethyl ester; stereospecific number given are for acylglycerols. Enatiomers cannot be separated. The abbreviations using single letters are free fatty acids.
[c]RRT given here are normalized to RT (27.1 min) of free oleic acid reported previously.[8]

We have used the HPLC system developed earlier for FA to separate the molecular species of MAG, FA, FA methyl esters, and FA ethyl esters, simultaneously. This is particularly useful in the identification of the molecular species of these radiolabeled lipid classes in metabolic studies using co-chromatography with standards.

REFERENCES

1. Takano, S.; Kondoh, Y. Monoglyceride analysis with reversed phase HPLC. J. Am. Oil Chem. Soc. **1987**, *64*, 1001–1003.
2. Sempore, B.G.; Bezard, J.A. Separation of monoacylglycerols by reversed-phase high-performance liquid chromatography. J. Chromatogr. **1992**, *596*, 185–195.
3. Takagi, T.; Ando, Y. Enantiomer separation of mixtures of monoacylglycerol derivatives by HPLC on a chiral column. Lipids **1990**, *25*, 398–400.
4. Kim, J.-H.; Nishida, Y.; Ohrui, H.; Meguro, H. Highly sensitive high-performance liquid chromatographic method to discriminate enantiomeric monoacylglycerols based on fluorescent chiral derivatization with (*S*)-(+)-2-*tert*-butyl-2-methyl-1,3-benzodioxole-4-carboxylic acid. J. Chromatogr. A **1995**, *709*, 375–380.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

5. Lin, J.T.; Woodruff, C.L.; McKeon, T.A. Non-aqueous reversed-phase high-performance liquid chromatography of synthetic triacylglycerols and diacylglycerols. J. Chromatogr. A **1997**, *782*, 41–48.
6. Lin, J.T.; Chen, J.M.; Liao, L.P.; McKeon, T.A. Molecular species of acylglycerols incorporating radiolabeled fatty acids in castor (*Ricinus communis* L.) microsomal incubations. J. Agric. Food Chem. **2002**, *50*, 5077–5081.
7. Lin, J.T.; Woodruff, C.L.; Lagouche, O.J.; McKeon, T.A.; Stafford, A.E.; Goodrich-Tanrikulu, M.; Singleton, J.A.; Haney, C.A. Biosynthesis of triacylglycerols containing ricinoleate in castor microsomes using 1-acyl-2-oleoyl-*sn*-glycero-3-phosphocholine as the substrate of oleoyl-12-hydroxylase. Lipids **1998**, *33*, 59–69.
8. Lin, J.T.; McKeon, T.A.; Stafford, A.E. Gradient reversed-phase high-performance liquid chromatography of saturated, unsaturated, and oxygenated free fatty acids and their methyl esters. J. Chromatogr. A **1995**, *699*, 85–91.
9. Turner, C.; He, X.; Nguyen, T.; Lin, J.T.; Wong, R.; Lundin, R.; Harden, L.; McKeon, T. Lipase-catalyzed methanolysis of triricinolein in organic solvent to produce 1,2(2,3)-diricinolein. Lipids **2003**, *38*, 1197–1206.

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