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Note

Simultaneous high-performance liquid chromatographic analysis of oleic acid and simple glycerides of oleic acid

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Food analysts have tended to follow selectively those relevant applications developed by analysts in the biochemical, clinical, pharmaceutical and oleochemical fields, where interest has included blood lipids[1,2], steroids[3,4], prostaglandins[5,6], carotenoids[7–10], vitamins[11–13] and technical emulsifiers[14–17].

In lipid analysis, ion-exchange high-performance liquid chromatography (HPLC) is seldom useful and high-performance gel permeation chromatography finds some applications. The most widely used mode of HPLC for lipid analysis, however, involves reversed-phase systems, where an hydrophobic stationary phase competes with an hydrophilic mobile phase for the lipid component.

Until recently, thin-layer chromatography (TLC) with flame ionization detection (FID) has been mainly used for quantification of fatty acids and their glycerides[18]. However, this technique has limitations such as poor resolution and artifact formation. On the other hand, the use of HPLC offers several advantages including high efficiency, selectivity and sensitivity. Although it was reported that oleic acid and its glycerides were analyzed by a refractive index detector[19] or a moving-wire detector[20], the more sensetive UV detection has not been reported and it is necessary to analyze oleic acid and simple glycerides of oleic acid for our study of enzymatic glycerolysis.

Therefore, the purpose of this study was to develop a simple, sensitive and rapid procedure for the analysis of oleic acid, 1(3)-monoolein, 1,2-diolein, 1,3-diolein and triolein which are major components of glycerolysis carried out using triolein and glycerol as substrates and aimed at mono- or diolein production.

EXPERIMENTAL

Reagents

1(3)-Monoolein, 1,2- and 1,3-diolein (99.9%) were obtained from Sigma (St. Louis, MO, U.S.A.), oleic acid and triolein (99.9%) from Supelco (Bellefonte, PA, U.S.A.). Acetonitrile, isopropanol, hexane and choloroform of HPLC grade (Burdick & Jackson, Muskegon, MI, U.S.A.) were stored over a Type 4A molecular sieve and filtered prior to use.

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TABLE I

GRADIENT PROGRAMME

Time (min)	*Solvent A (%)*	*Solvent B (%)*	*Elution mode*
0.00	100	0	Isocratic elution
14.00	100	0	Isocratic elution
17.00	40	60	Convex gradient
19.00	40	60	Isocratic elution
20.00	100	0	Linear gradient

Chromatography

The analysis of triolein, 1,3-diolein, 1,2-diolein, 1(3)-monoolein and oleic acid in chloroform was performed with a Waters Assoc. high-performance liquid chromatograph equipped with a Waters Model 510 pump and connected to a Waters 740 data module. The column used was a Z-module C_{18} and the operating conditions were as follows: elution solvent A, acetonitrile–isopropanol–hexane (50:3:2, v/v/v); elution solvent B, isopropanol–hexane (5:4 v/v), sample solvent, chloroform, flow-rate, 2.20 ml/min. The gradient programme is shown in Table I.

Absorption was measured at 214 nm by a Waters Model 441 absorbance detector. The quantitative analysis was performed with the single point calibration method using an external standard, the concentration of which was confirmed by a spectrophotometric measurement.

RESULTS AND DISCUSSION

TLC–FID analysis for the separation of lipids involves a time-consuming procedure of spotting, developing, drying and scanning. Compared to this method, on the HPLC column lipid mixtures were satisfactorily separated in less than 25 min, as shown in Fig. 1. Since a reversed-phase column (Z-module C_{18}) was used, the components were eluted in the order of decreasing polarity; 1(3)-monoolein, oleic acid, 1,3-diolein, 1,2-diolein, triolein. Each peak was identified by comparison of its retention time with that of the corresponding standard compound. The purity of each standard compound was greater than 95% as determined using the HPLC peak area. This typical chromatogram of lipids was reproducible. The reproducibility of the quantification of the components is presented in Table II.

1,2-Diolein has optical isomers which have been separated by TLC[21] but they were not detected in our system.

Although the separation of 1,3-diglyceride and 1,2-diglyceride was done using a moving-wire detector by Aitzetmüller and Koch[20], the specific separation of 1,3-diolein and 1,2-diolein in this study should be noted and is the key part of a study of the glycerolysis reaction mechanism.

In conclusion, reversed-phase HPLC provides an efficient method for the separation and quantification of oleic acid, 1(3)-monoolein, 1,2-diolein, 1,3-diolein and triolein. Furthermore, the method may be extended to the study of enzyme reaction mechanisms.

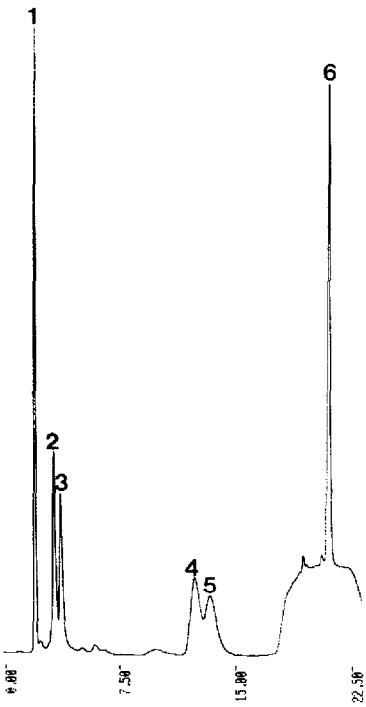

Fig. 1. HPLC analysis of lipid compounds. Concentrations (μmol) are as follows; 1(3)-monoolein, 1; oleic acid, 1; 1,2-diolein, 2; 1,3-diolein, 2; triolein, 1 in 5 μl of chloroform injected. Reaction conditions as described in Experimental. Peaks: 1 = chloroform; 2 = 1(3)-monoolein; 3 = oleic acid; 4 = 1,3-diolein; 5 = 1,2-diolein; 6 = triolein.

TABLE II

QUANTIFICATION OF THE PEAKS FOUND FOR TEN REPLICATE EXPERIMENTS
Conditions as described in Experimental.

Component	*Retention time*[a] *(min)*	*Amount (μmol)*	
		Actual	*Found*
1-Monoolein	3.24 ± 0.01	1.00	1.02 ± 0.03
Oleic acid	3.70 ± 0.02	1.00	1.04 ± 0.05
1,3-Diolein	12.29 ± 0.11	2.00	2.11 ± 0.10
1,2-Diolein	13.32 ± 0.09	2.00	2.07 ± 0.09
Triolein	21.04 ± 0.12	1.00	1.02 ± 0.04

[a] Mean value ± standard deviation based on ten samples.

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