Quantitative Determination of Monoglycerides and Diglycerides by High-Performance Liquid Chromatography and Evaporative Light-Scattering Detection

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Neutral lipid classes were separated with normal-phase high-performance liquid chromatography, and mono- and diglycerides were determined with an evaporative light-scattering detector (ELSD). The 1,3-diacylglycerols were resolved from the 1,2-diacylglycerol positional isomers, although some 1,3-diacylglycerols of low molecular weight interfered with the 1,2-diacylglycerols of high molecular weight. For monoglycerides, the separations between 1-(and 3-)acyl and 2-acylglycerols were optimized only between those pairs with identical fatty acyl groups. Samples were dissolved in a solvent mixture and analyzed without derivatization. The results (monoglyceride) obtained from this method agreed well with those derived from gas chromatographic and supercritical fluid chromatographic methods. The universal nature of the ELSD makes this method applicable to oils and emulsifiers containing both saturated and unsaturated fatty acyl moieties.

KEY WORDS: Diglyceride, emulsifier, evaporative light-scattering detector, HPLC, monoglyceride, positional isomer, universal detector.

Mono- and diglycerides are the major emulsifiers used in food products (1). Several methods are available for the determination of mono- and diglycerides. The periodic acid method is a commonly used method but it is time consuming (2). Another commonly used method is the gas chromatographic method with FID (flame-ionization detector), although the method requires derivatization (3–5). Mono- and diglycerides are usually derivatized to methyl or propyl esters prior to gas chromatography (GC) analysis. A supercritical fluid chromatography (SFC)-FID method has been developed and demonstrated as an alternative method for the analysis of mono- and diglycerides (6). Mono- and diglycerides can be analyzed by SFC with or without derivatization. A simple high-performance liquid chromatography-ultraviolet (HPLC-UV) method has been developed to separate 1,3-diacylglycerols, 1,2-diacylglycerols and 1-monoacylglycerols by normal-phase HPLC and measure their absorptions at 213 nm with a UV detector for quantitation. The method is applicable to saturated or fully hardened emulsifiers only (7). Unsaturated diglycerides (or monoglycerides) absorb more UV radiation at this wavelength than their saturated counterparts due to the double bond of unsaturated fatty acid moieties. Consequently, it would be difficult to choose the correct response factor for an HPLC peak that contains coeluting saturated and unsaturated lipids. The alternative would be to use a universal detector for quantitation under the same HPLC condition. Recently, the evaporative light-scattering detector (ELSD) has been used in the analyses of tocopherols and phytosterols (8), lipid classes (9,10) and triglycerides (11,12). The ELSD is a universal detector, and its response is a function of the mass of solute particles regardless of their chemical identities. In addition, the ELSD allows the use of gradient elution and some organic solvents, which are otherwise unsuitable for the refractive index (RI) or UV detector. This paper reports the application of an HPLC-ELSD method to the determination of mono- and diglycerides in vegetable oils and emulsifiers. In this study, samples were dissolved in a hexane/2-propanol solvent mixture and were analyzed without derivatization.

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MATERIALS AND METHODS

HPLC and ELSD parameters. A Hewlett-Packard 1050 HPLC system with the HP ChemStation (Palo Alto, CA) and a Varex ELSD II evaporative light-scattering detector (Varex Corp., Rockville, MD) were used. The drift tube temperature was set at 90°C. The flow of the carrier gas (nitrogen) was set at 30 mm on the flowmeter of the detector. For HPLC separations, a 150 × 4.6 mm, 10 μ Chromegasphere SI-60 (ES Industries, Marlton, NJ) column was used. The column temperature was maintained at 40°C with a column heater. HPLC-grade solvents *n*-hexane, 2-propanol and ethyl acetate were purchased from Burdick & Jackson (Muskegon, MI). A 10% (vol/vol) formic acid solution in 2-propanol was prepared from 88% reagent-grade formic acid (J.T. Baker, Phillipsburg, NJ). The mobile phase was: channel A, hexane; channel B, hexane/2-propanol/ethylacetate/10% formic acid (80/10/10/1). The mobile phase gradient program is shown in Table 1. The flow of the mobile phase was 2 mL/min with an initial pressure of 27 bars. The total run time was 19 min. When not in use, the column was rinsed with the solvent mixture of hexane/2-propanol/ethyl acetate (80/10/10) to remove formic acid.

Sample preparation. The weights of samples and standards were recorded to 0.0001 g throughout this study. For the monoglyceride emulsifier, approximately 0.1 g of sample was accurately weighed into a 50-mL volumetric flask, dissolved in hexane/2-propanol (90/10) solvent mixture and brought to volume. The solution was further

TABLE 1

The Mobile Phase Gradient Program Used for Neutral Lipids Separation on a 150 × 4.6 mm, 10 μ Chromegasphere SI-60 Column[a]

		% of each channel	
Time (min)	Flow (mL/min)	A	B
0	2	98	2
8	2	65	35
8.5	2	2	98
15	2	2	98
15.1	2	98	2
19	2	98	2

[a]Column temperature, 40°C. Channel A, hexane; Channel B, hexane/2-propanol/ethyl acetate/10% formic acid (80/10/10/1).

diluted to a final concentration of approximately 0.5 mg/mL prior to HPLC analysis. For vegetable oils, up to 4% (wt/vol) sample solution was prepared in the same solvent mixture. For the spike recovery study, approximately 2 g peanut oil and 0.1 g of a commercial monoglyceride emulsifier were accurately weighed into a 100-mL volumetric flask and brought to volume with the 90/10 solvent mixture. Twenty microliters of the prepared sample solution was injected into the HPLC.

Response curves and calibration. The responses of the ELSD to 1,3-distearin, 1,3-dipalmitin, 1,3-dilinolein, 1-monostearin, 1-monopalmitin and 1-monoolein were studied with a series of solutions prepared from the individual standards. All the standards were obtained from Nu-Chek-Prep (Elysian, MN) with 99%+ purity. Approximately 0.1 g each of the standard was accurately weighed into a 100-mL volumetric flask, dissolved with sonication and diluted in hexane/2-propanol (90/10) solvent mixture. This solution was then used to prepare the lower-concentration solutions. The log of the peak area (log [response]) was plotted against the log of the amount (log [mass], μg or mcg) of monoglyceride and diglyceride.

For the analysis of a commercial monoglyceride emulsifier, 1-monostearin was used as the calibration standard. Approximately 0.12 g of 1-monostearin was accurately weighed into a 100-mL volumetric flask, dissolved and diluted with the 90/10 solvent mixture to prepare the working solutions of approximately 1.2, 0.63 and 0.12 mg/mL. A linear log/log calibration curve was obtained with the three working standards.

RESULTS AND DISCUSSION

HPLC separation of lipids. As shown in Figure 1, standards of various lipid classes were well separated from each other. The least polar steryl ester (cholesteryl myristate) eluted first while the most polar 2-monoglyceride (2-monopalmitin) came last. The 1,3-dipalmitin eluted at approximately 5 min and its positional isomer 1,2-dipalmitin at 6 min. Cholesterol eluted between the two diglycerides. In our previous study (J. Liu, M. Guzman-Harty and C. Hastilow, unpublished) with a similar mobile phase without formic acid, phytosterols such as stigmasterol and β-sitosterol coeluted with cholesterol. In addition, 1,3-diglycerides with fatty acyl moieties of 14 or less carbons eluted later than 1,3-dipalmitin and 1,3-distearin. In general, diglycerides of lower molecular weight tended to elute later than those of higher molecular weight. The 1,3-dioctanoin eluted so late (at approximately 6 min) that it interfered with the 1,2-dipalmitin. This interference is probably not a concern for most vegetable oils except babassu, coconut and palm oils, which may contain this medium-chain diglyceride. However, the interference from phytosterols remains an issue in the determination of 1,3-diglycerides in vegetable oils.

For monoglycerides, the separation was optimized only between positional isomers with an identical fatty acyl moiety. The 1-monopalmitin was well separated from 2-monopalmitin; however, 1-monomyristin eluted later than 1-monopalmitin and overlapped with 2-monostearin and 2-monopalmitin peaks.

Response curves and calibration. Figures 2 and 3 show the response curves of three diglycerides (1,3-dipalmitin, 1,3-distearin and 1,3-dilinolein) and three monoglycerides (1-monostearin, 1-monopalmitin and 1-monoolein). The responses of the ELSD to the three diglycerides were similar with each other from 0.25 to 25 mcg. This was also true for the three monoglycerides. As described in the literature (12), the ELSD is a universal detector whose

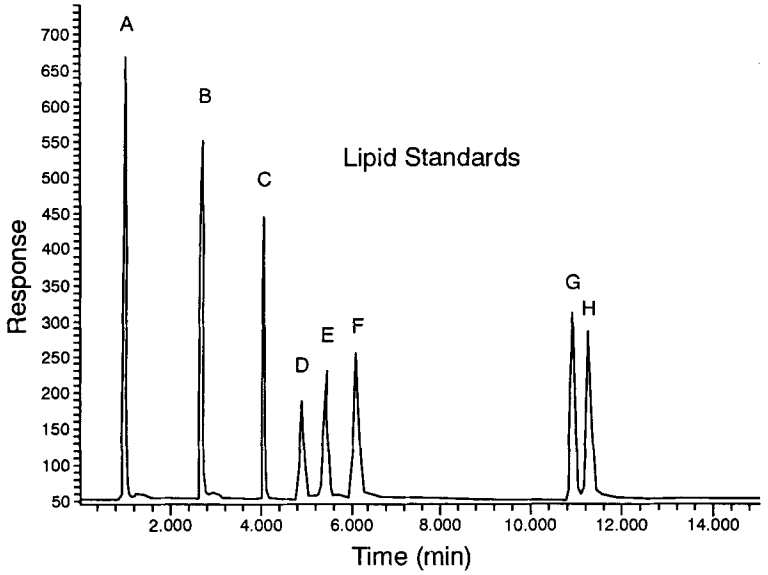

FIG. 1. The chromatogram of various lipid standards: (A) cholesteryl myristate, (B) tripalmitin, (C) palmitic acid, (D) 1,3-dipalmitin, (E) cholesterol, (F) 1,2-dipalmitin, (G) 1-monopalmitin and (H) 2-monopalmitin. The concentration of each standard is in the neighborhood of 1 mg/mL. The high-performance liquid chromatography parameters: Table 1. The Varex II evaporative light-scattering detector parameters: drift tube temperature, 90°C; nitrogen gas flow, 30 mm.

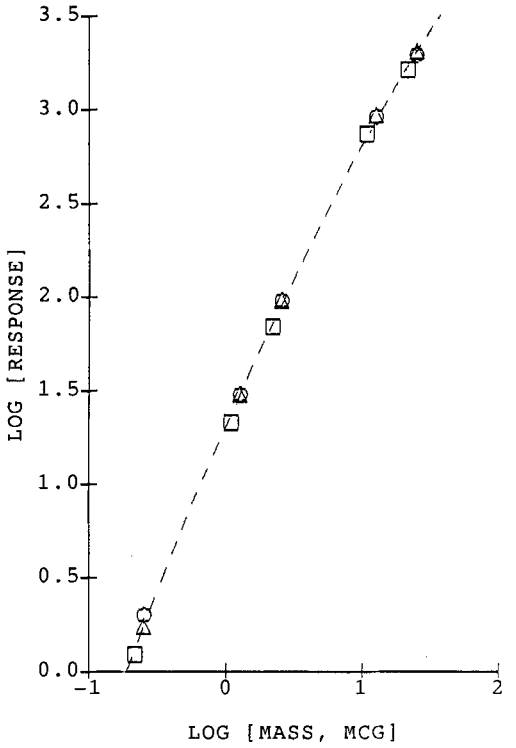

FIG. 2. The response curves of 1,3-dipalmitin (circle), 1,3-stearin (triangle) and 1,3-dilinolein (square) with a Varex ELSD; the HPLC and the ELSD parameters are as described in Figure 1. See Figure 1 caption for abbreviations.

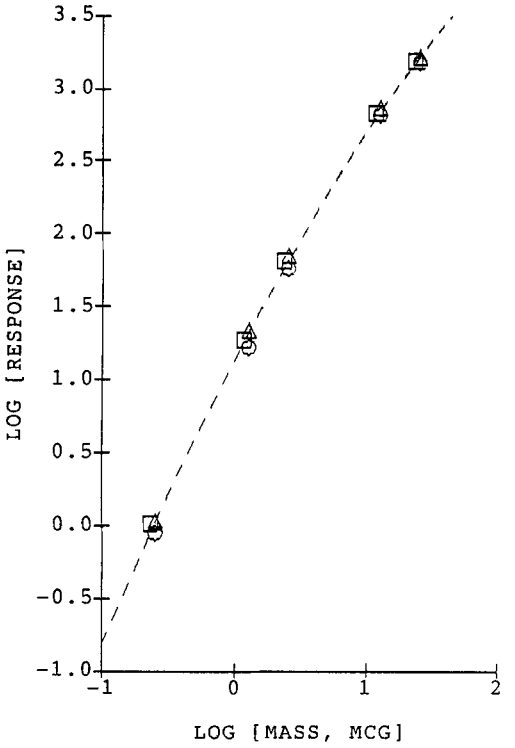

FIG. 3. The response curves of 1-monopalmitin (circle), 1-monostearin (triangle) and 1-monoolein (square) with a Varex ELSD; the HPLC and the ELSD parameters are as described in Figure 1. See Figure 1 caption for abbreviations.

response is a function of the mass of the analyte. When diglycerides (or monoglycerides) containing saturated, unsaturated and/or mixed fatty acyl moieties are coeluted as a single peak, it is necessary to use a universal detector for accurate quantitation.

The calibration curve in this study was established with

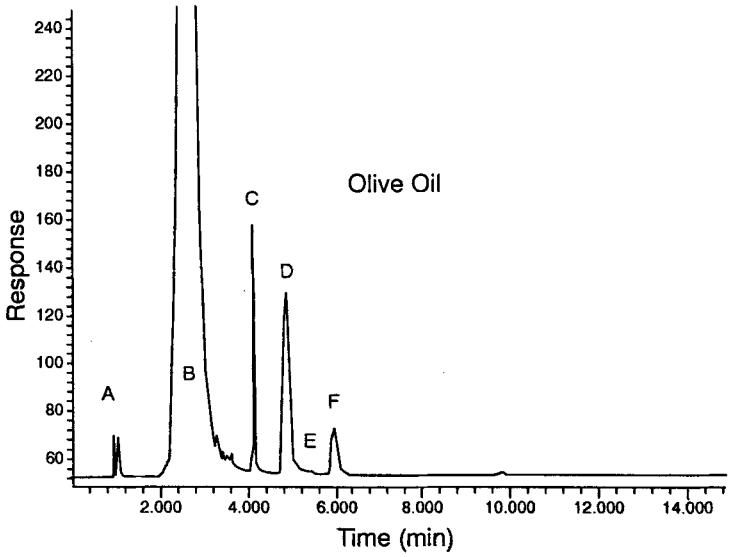

FIG. 4. The chromatogram of an olive oil obtained with the HPLC and the ELSD parameters as described in Figure 1. See Figure 1 caption for abbreviations. Peaks are identified by retention time only: (A) steryl esters, (B) triglycerides, (C) free fatty acids, (D) 1,3-diacylglycerols, (E) minor 1,3-diacyl glycerols and/or phytosterols and (F) 1,2-diacylglycerols.

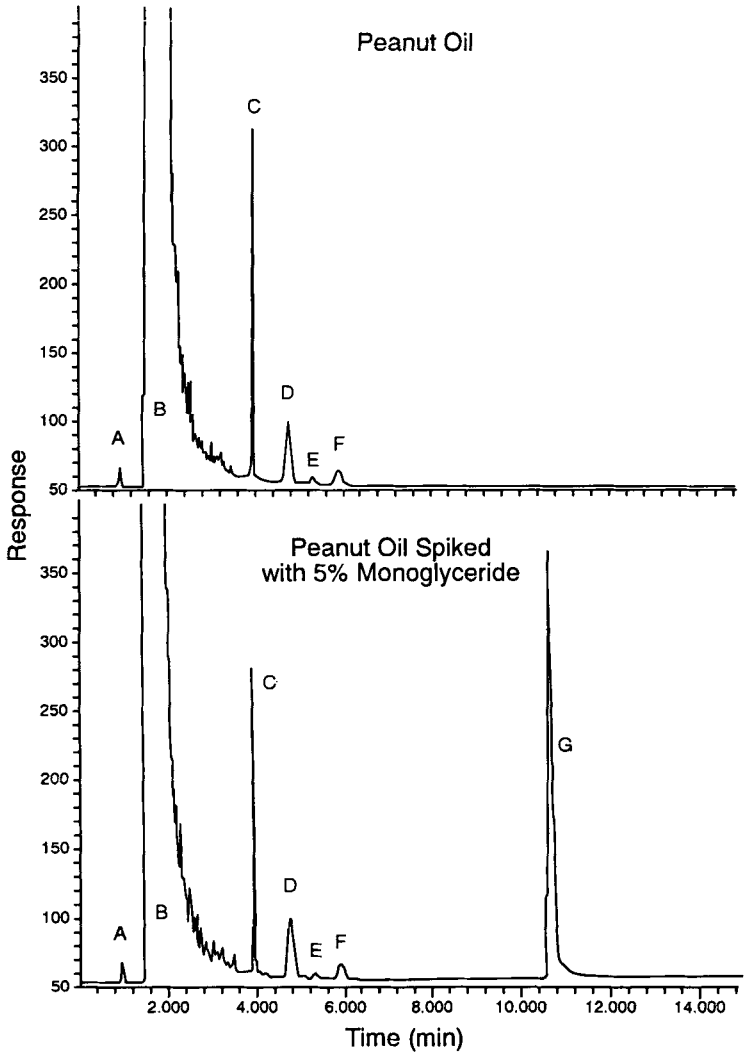

FIG. 5. The HPLC-ELSD chromatogram of a peanut oil with and without the addition of a monoglyceride emulsifier. Peaks are identified by retention time only: (A) steryl esters, (B) triglycerides, (C) free fatty acids, (D) 1,3-diacylglycerols, (E) minor 1,3-diacylglycerols and/or phytosterols, (F) 1,2-diacylglycerols and (G) monoglycerides. Refer to Figure 1 for the HPLC and the ELSD parameters. See Figure 1 caption for abbreviations.

TABLE 2

The Comparison of Monoglyceride Results of a Commercial Emulsifier as Determined by the HPLC-ELSD, GC and SFC Methods[a]

	Total monoglycerides, g/100 g		
	HPLC-ELSD	GC/derivatized[b]	SFC/underivatized[b]
Lot 1840			
Mean	92.5	92.3	93.4
%RSD (n)	1.1 (4)	1.3 (4)	3.5 (11)
Lot 6022			
Mean	94.7	94.0	95.9
%RSD (n)	1.5 (4)	1.8 (4)	3.4 (12)

[a]Abbreviations: HPLC-ELSD, high-performance liquid chromatography-evaporative light-scattering detector; GC, gas chromatography; SFC, supercritical fluid chromatography; RSD, relative standard deviation.
[b]Data are from Reference 6.

three working standards. Because the response of the ELSD was not linear, a linear log/log calibration curve was established with $r^2 = 0.996$. The method quantitation and detection limits (S/N = 2) were approximately 0.25 and 0.06 μg, respectively.

Determination of monoglycerides. By the HPLC-ELSD method, the total monoglyceride content of a commercial emulsifier was found to be 92.5 and 94.7 g/100 g for two separate lots. The method is precise as indicated by the small (<2%) relative standard deviation between duplicates and between days. To confirm the accuracy of this method, the monoglyceride results from this HPLC-ELSD method were compared with those obtained from GC and SFC methods (Table 2). The results from the three methods were in agreement. This method recovered an average of 93.9% of a monoglyceride emulsifier spiked in a peanut oil.

Analysis of vegetable oils. This HPLC-ELSD method may be useful in monitoring the quality of vegetable oils by analyzing their mono- and diglycerides. Figures 4 and 5 show the chromatograms of olive and peanut oils. The oils tested in this study were about two years old from the date of purchase to the time of analysis. The olive and peanut oils appeared to contain free fatty acids (peak C), 1,3-diglycerides (peak D) and 1,2-diglycerides (peak F) but no detectable monoglycerides. Figure 5 also shows the chromatogram of the peanut oil spiked with 5% (w/w) of a commercial monoglyceride emulsifier. This study demonstrates the feasibility of using this method for the analysis of mono- and diglycerides in selected vegetable oils. However, as previously mentioned, phytosterols may interfere with the determination of 1,3-diacylglycerols in vegetable oils.

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