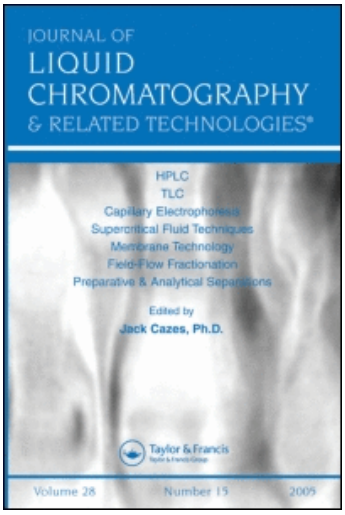

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LIQUID CHROMATOGRAPHIC METHOD FOR THE CONCURRENT ANALYSIS OF SUCROSE POLYESTER, VITAMIN A PALMITATE, AND β-CAROTENE IN MARGARINE

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ABSTRACT

A liquid chromatographic method is described for the concurrent analysis of sucrose polyester (SPE), vitamin A and ß-carotene in margarine. The SPE and vitamins are separated from the acylglycerol by gel permeation chromatography (GPC). The GPC system is equipped with a refractive index and ultraviolet (UV) detector (313 nm) to monitor the peak elution. Following collection, the vitamins and SPE are analyzed by C-18 reverse phase chromatography. A ternary gradient of acetonitrile, methylene chloride and isopropanol with evaporative light scattering detection is used for SPE analysis. Vitamin A and ß-carotene are quantitated after isocratic elution with acetonitrile/methylene chloride/methanol (700/300/2, v/v/v) as mobile phase and detection at 313 nm and 436 nm, respectively. Well resolved, interference free chromatograms were obtained for each analyte.

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INTRODUCTION

Considerable interest has been shown recently in fat substitutes. One such substitute, olestra or sucrose polyester (SPE) has been the target of considerable analytical chromatography work by Tallmadge and Lin (1) and Chase et al. (2,3). Tallmadge and Lin (1) developed a liquid chromatography (LC) method based upon reverse phase nonaqueous chromatography and evaporative light scattering detection (ELSD) to quantitate olestra from olestra-lipid blends. Chase et al. (2) used gel permeation chromatography (GPC) to separate and collect SPE from the acylglycerol. The collected SPE fraction was then analyzed on a C-18 column with a ternary gradient, evaporative light scattering detection (ELSD), and quantitated in the presence of sucrose octaacetate as an internal standard.

The Chase et al. (2) procedure provided interference-free chromatograms with improved resolution between SPE and acylglycerols compared to the Tallmadge and Lin (1) procedure. Chase et al. applied their procedure to the analysis of SPE in salad dressings (2). Three different salad dressings containing SPE were studied. An extraction procedure developed by Landen (4,5) for fat soluble vitamins in infant formula was modified and applied to the salad dressings. The mean percent recovery (n=3) and standard deviation were 92.4 ± 2.6. GPC as a clean-up step is a valuable asset for studies with more complex matrices such as salad dressings. Pending approval of SPE as a fat substitute, a likely food to contain SPE would be margarine or a spread. This study applies the earlier work of Chase et al. (2,3) to a higher degree of matrix complexity since margarine tends to contain hydrogenated vegetable oils, liquid vegetable oil, lecithin, mono- and diacylglycerols, ß-carotene and vitamin A palmitate. The SPE, vitamin A palmitate and ß-carotene were isolated from the margarine by GPC and quantitated on a non-aqueous reverse phase LC system.

MATERIALS AND METHODS

Chemicals and Standards

The solvents, methylene chloride, acetonitrile and isopropanol were HPLC grade and obtained from J. T. Baker Inc. (Philipsburg, NJ, USA). Anhydrous magnesium sulfate was reagent grade and from J. T. Baker Inc.

Internal standard. A 500 mg portion of sucrose octaacetate (Aldrich Chemical Co., Milwaukee, WI, USA) was diluted to 250 mL with methylene chloride to give a concentration of 2000 µg/mL.

SPE standard. The SPE was synthesized with sucrose and the fatty acid methyl esters of soybean oil using the method of Boutte (6). A 125 mg portion of SPE was accurately weighed and diluted to 25 mL with the internal standard solution. A 3.0 mL aliquot was diluted to 10.0 mL with the internal standard to give a concentration of 1500 µg/mL.

Vitamin A standard. Approximately 38 mg of vitamin A palmitate (Fluka Bio Chemika, Switzerland) was dissolved in 50 mL of methylene chloride. The exact concentration was determined from the $E^{1\%}$ value of 975 (7) for vitamin A palmitate. Appropriate dilutions were made to give working standards with concentrations of 0.4, 0.8 and 1.5 µg/mL.

ß-Carotene standard. Approximately 20 mg of ß-carotene (Fluka Bio Chemika) was dissolved in 50 mL of methylene chloride. After determining the exact concentration from the $E^{1\%}$ value of 2620 (8) for ß-carotene, appropriate dilutions were made to give concentrations of 0.2, 0.4, and 0.7 µg/mL.

Instrumentation

Clean-up LC. The SPE was isolated from the acylglycerols in each sample by using four µStyragel 100 Å GPC columns (Waters Corp., Milford MA, USA) connected in series with a mobile phase of methylene chloride, injection volume of 250 µL, and a flow rate of 1.0 mL/min. The LC consisted of a Waters 510 pump, U6K injector, 440 detector (λ 313 nm), a 410 refractive index detector and a Kipp and Zonen (Delft, Holland) BD 41 dual pen strip chart recorder.

Analytical LC for SPE. The SPE was quantitated by injecting 50 µL on a Zorbax 4.6 mm X 25 cm, C-18, 5 µm column (Mac Mod Analytical, Chadds Ford, PA, USA). The LC consisted of a Constametric 4100 LC (Thermo Separations Products, Riviera Beach, FL, USA), a Waters 717 Plus autosampler, a Sedex 55 ELSD (Richard Scientific, Novato, CA., USA) and a Hewlett Packard (Avondale, PA, USA) model 3390 integrator. A flow rate of 1.0 to 1.5 mL/min was used with a gradient of methylene chloride, acetonitrile and isopropanol (2). The ELSD was set at 40°C, with a nebulizer pressure of 2.0 bar and a gain of 5.

Analytical LC for vitamins. Vitamin A palmitate and ß-carotene were quantitated by injecting 100 µL on a Zorbax 4.6 mm X 25 cm, C-18, 5 µm column (Mac Mod Analytical). The LC consisted of a Waters 510 pump, U6K injector, 440 dual channel detector (λ = 313 and 436 nm) and two Hewlett Packard model 3390 integrators. A flow rate of 1.0 mL/min was used with a mobile phase of acetonitrile/methylene chloride/methanol (700/300/2, v/v/v).

Analysis

A commercially available margarine was obtained at a local supermarket. Five portions of margarine were weighed out, four of which were blended with SPE to give a 5, 10, 20 and 30% level of SPE in the respective margarine. A fifth portion was used as a control sample.

Approximately, 10 g from each of the five portions of margarine was accurately weighed and dissolved in 50 mL of methylene chloride. After the margarine melted and dissipated in the methylene chloride at room temperature, 3 g of $MgSO_4$ was added and allowed to stand for two hours with frequent agitation. The mixture was filtered through a coarse porosity 60 mL fritted glass filter. The filtered solution was diluted to 100 mL with methylene chloride.

After establishing collection timeframes with standards of SPE, vitamin A palmitate and ß-carotene, the samples were injected onto the GPC system. At the appropriate times two fractions were collected for further analysis with one being for the SPE and the other for the vitamins. Each collected fraction was evaporated to dryness. The SPE fraction was reconstituted in the internal standard solution while the vitamin fraction was reconstituted in the mobile phase used for the analytical LC for vitamin A palmitate and ß-carotene. The amounts of each vitamin were reported as International Units/kg and were corrected for the amount of SPE added to each.

RESULTS AND DISCUSSION

This study combines the early work of Landen and Eitenmiller (9) with vitamin A palmitate and ß-carotene in margarines with the recent work of Chase et al. (2,3)

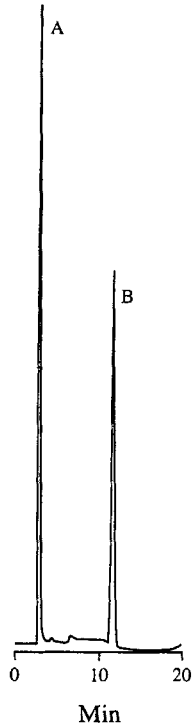

FIGURE 1: Reverse phase liquid chromatography chromatogram (C-18 column) of sucrose polyester (SPE) and the internal standard, sucrose octaacetate using ELSD with a pressure of 2.0 bar, temperature of 40°C and a gain of 5. A ternary gradient of methylene chloride, acetonitrile and isopropanol was used. The chromatogram was obtained after GPC clean-up step. A is the internal standard and B is the SPE peak.

with SPE. The procedure provides methodology for the concurrent analysis of SPE and vitamin A palmitate and ß-carotene from margarine type matrices.

Figure 1 illustrates the analytical LC chromatogram of the SPE fraction collected from the GPC column. Without GPC clean-up the SPE is poorly resolved as illustrated in Figure 2. In addition, without the use of GPC, we have observed the column pressure to gradually increase until the upper pressure limit is reached and the system shuts down. Excessive fat on the column will ultimately shorten the life of the column. Isolation of the SPE from the acylglycerol prior to determinative chromatography prevents column problems related to lipid injection.

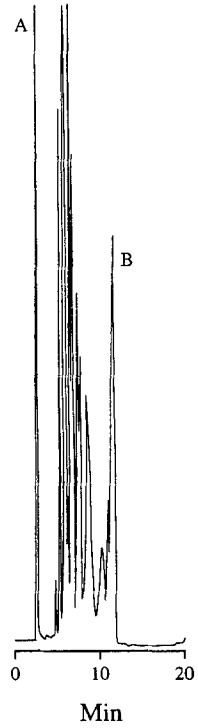

FIGURE 2: LC chromatogram of SPE and the internal standard. The conditions are the same as Fig. 1 except there was no GPC clean-up step. A is the internal standard and B is the SPE peak. Between A and B are the acylglycerols.

The chromatograms obtained for vitamin A palmitate and ß-carotene are illustrated in Figure 3. These chromatograms resemble classic examples of the method developed earlier by Landen (4, 5) and Landen and Eitenmiller (9). Concentrations of the vitamins were calculated by linear regression analysis. Since different wavelengths were used for vitamin A (313 nm) and ß-carotene (436 nm), a suitable internal standard that absorbs at both wavelengths and elutes at a time that does not interfere with either vitamin was not available. The peak purity of vitamin A palmitate and ß-carotene was confirmed by diode array comparison of the spectra of standard and sample peaks.

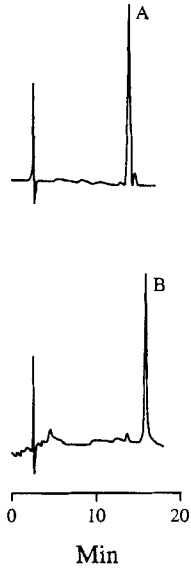

FIGURE 3: Reverse phase C-18 chromatogram of ß-carotene (A) and vitamin A palmitate (B). A mobile phase consisting of acetonitrile/methylene chloride/methanol (700/300/2, v/v/v) was used with λ 313 nm for vitamin A and λ 436 nm for ß-carotene.

Figure 4 shows the chromatogram used to identify and monitor the collection timeframes. One trace is from the UV detector (λ 313 nm) and the other is from the RI detector. The SPE peak eluted first, followed by the fat and finally vitamin A palmitate and ß-carotene. Therefore, the SPE and vitamin fractions are easily and efficiently isolated from the acylglycerols.

The amount of vitamin A palmitate and ß-carotene found is consistent from sample to sample as shown in Table 1. The average total vitamin A found was 36,934 IU/kg ± 1305 (cv 3.5), which was 92.3% of the manufacturers declared value. The amounts of vitamins found as listed in Table 1 were corrected for the SPE that was added. Table 1 also shows the percent recoveries for SPE of each sample, with an average of 104%. The linearity and detection limits have been previously calculated and discussed in the earlier work of Landen (4, 5), Landen and Eitenmiller (9) and Chase et al. (2,3).

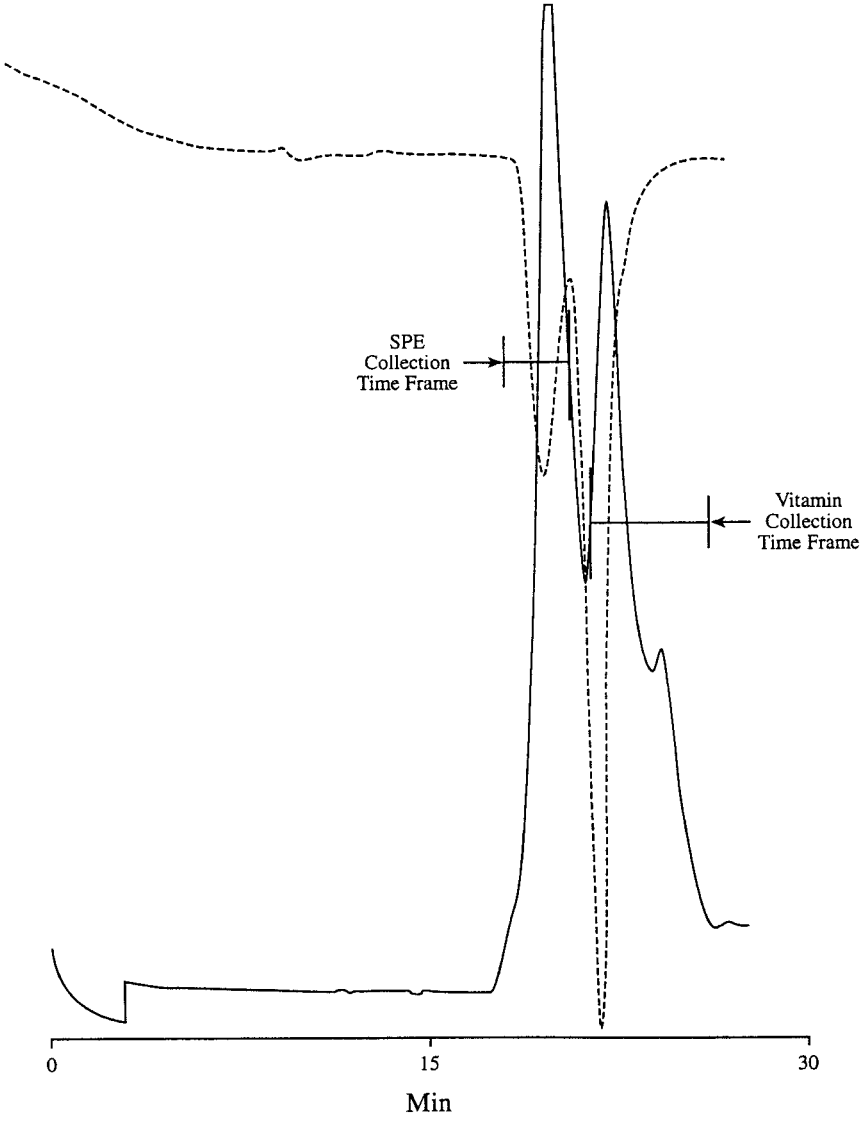

FIGURE 4: GPC chromatogram of the extract of SPE-margarine blend eluted with a mobile phase of methylene chloride. The solid trace is UV detection at λ 313 nm for vitamins and the broken trace is RI detection for SPE. Horizontal lines on the chromatograms indicate the collection points for the SPE and vitamins.

TABLE 1

Analysis of Vitamin A Palmitate, ß-Carotene and Sucrose Polyester (SPE) from SPE-Margarine Blends

	SAMPLE									
	Control		5% SPE		10% SPE		20% SPE		30% SPE	
Vit A (IU/kg):	25906	23763	25313	25509	25006	24943	25942	26715	26940	26521
ß-Carotene (IU/kg):	11446	10461	11873	11489	11491	10940	11572	11333	11777	11779
Total (IU/kg):	37352	34224	37186	36999	36496	35883	37514	38044	38716	38301
SPE Found (g/100g):	-	-	5.22	5.09	10.10	10.10	21.80	21.80	32.70	32.90
SPE Added (g/100g):	0	0	5.27	4.99	10.10	10.10	20.70	20.60	30.20	30.50
% SPE Recovered:	-	-	99.1	102	100	100	105	106	108	108

Note: The values found for the vitamins were corrected for the SPE that was added

This study provides an accurate method to assay not only the SPE content in a highly complex food matrix such as margarine but also the concurrent assay of vitamin A palmitate and ß-carotene.

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