High Performance Liquid Chromatographic Separation of Sucrose Fatty Acid Esters

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The synthesis of sucrose fatty acid esters always results in complex mixtures. Two procedures for quantitative analysis of sucrose monoesters, respectively sucrose diesters, by means of high performance liquid chromatography on reversed-phase columns, are described. A mixture of methanol and water (85:15, v/v) was used for the separation of the monoesters, while methanol, ethyl acetate and water (65:25:10, v/v/v) was used for the separation of diesters. These methods gave information about the amount of monoesters and diesters in the product; the ratio between sucrose monopalmitate and sucrose monostearate, and the number of the most important structure isomers. A complete separation of all the possible diester products seemed to be impossible, due to the presence of more complex structure isomers. The described procedures can give important support during preparative work on sucrose fatty acid esters and also in the evaluation of these products for application purposes.

Of the many sucrochemicals, the sucrose fatty acid esters have attracted the most attention and are produced on an industrial scale in Japan. These products are especially used as emulsifiers in foods and cosmetics. Research into the synthesis of sucrose esters was started by the International Sugar Research Foundation. Osipow et al. (1) used solvents like dimethyl formamide and dimethylsulfoxide in which both reactants, sucrose and the methyl ester of a fatty acid, or sucrose and a fat, dissolve. For the use of these emulsifiers in foods and cosmetics, legal problems arose because of residual solvents. To avoid these problems, solvent-free transesterifications were developed, of which the micro-emulsion method (2) and the homogeneous melt (3) were the most successful. At Suiker Unie Research, we developed a new solvent-free process (4), which has technological advantages compared with the homogeneous melt. Independent of the method of synthesis, a complex product is formed because of the eight available hydroxyl groups. From these reaction sites, the three primary hydroxyl groups are the most reactive (5). Both for fundamental research in relation to the reactivity of the different hydroxyl groups of sucrose, and for research into the applications of sucrose esters (ratio of mono-, di- and higher substituted products), it is important to have at our disposal a good method of analysis for these complex mixtures. Until now most methods of analysis were based on thin layer chromatography (TLC), which gives qualitative ideas about the ratio of mono-, di- and higher substituted products (6-8). Gas chromatographic methods are used for more quantitative results, but these methods have as a major drawback the need to prepare volatile derivatives. Silylation is needed for a direct analysis of sucrose esters (9), and a quantitative saponification is needed for an analysis of present fatty acid chains (10). Particularly for non-volatile compounds high performance liquid chromatography (HPLC) is a fast and quantitative method of analysis. One of the first papers describing the analysis of sucrose fatty acid esters by HPLC was by Cormier et al. (11). The method they had developed, like the one reported later by Seino et al. (12), gave such a

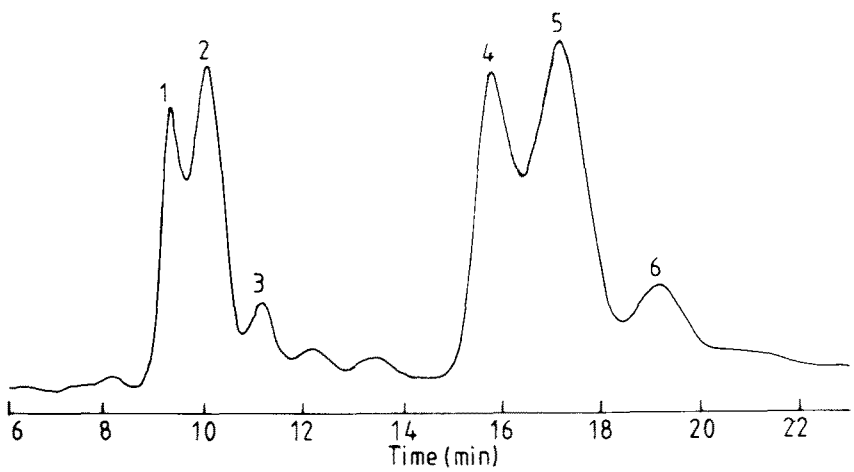

FIG. 1. HPLC separation of SURES 70 PS 37 sucrose esters (70% monoester, palmitoyl esters:stearoyl esters = 3:7) on 5 μm ODS column. Solvent, methanol and water (85:15, v/v); flow rate, 1.2 ml/min; sample concentration, 10% (w/v). Refractive index detection. Peaks: 1-3, sucrose palmitoyl monoesters; 4-6, sucrose stearoyl monoesters.

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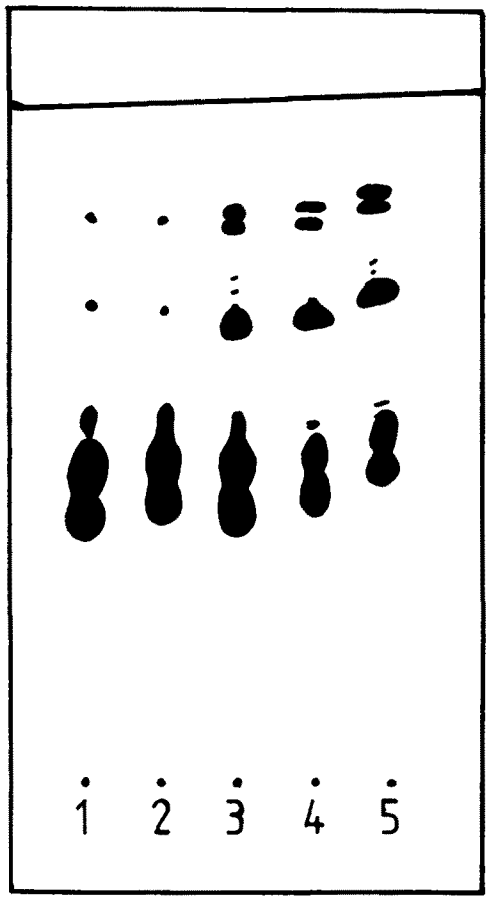

FIG. 2. TLC separation of some sucrose esters on silica gel 60 F 254. Solvent, chloroform, methanol, water and acetic acid (70:20:2:2, v/v/v/v). 1, SURES 100 P; 2, SURES 100 S; 3, DK F-160 (70% monoester); 4, DK F-110 (50% monoester); 5, DK F-50 (30% monoester).

separation that no distinction could be made between esters with different fatty acid chains and isomers. For our research into the synthesis of sucrose esters we needed a fast method of analysis, to be able to characterize intermediates and end-products. Checking the right quantitative ratio of palmitoyl to stearoyl sucrose esters can be important for the application of the esters, because a higher hydrophyl-lypophyl balance value is attached to palmitoyl esters than to stearoyl esters by Griffin (13). Kaufman and Garti (14) tried to analyze the sucrose fatty acid esters by means of HPLC, but their method seemed to be inadequate for the separation of the monoester isomers. We developed two procedures for analysis by HPLC which give quantitative results on mono- and disubstituted products.

EXPERIMENTAL

Reagents and chemicals. Methanol, 2-propanol, ethyl acetate, acetonitrile and chloroform were HPLC solvents (Rathburn, Walkerburn, United Kingdom or Merck, Darmstadt, Federal Republic of Germany). Acetic acid (99.5%) was obtained from J.T. Baker Chemical Co., Phillipsburg, New Jersey. Water was obtained by distillation of demineralized water. Palmitic acid, stearic acid and their methyl esters were obtained from the Sigma Chemical Co. (St. Louis, Missouri) (Purity ca. 99%). Sucrose was obtained from BDH Chemicals LTD. (Poole, United Kingdom). Sucrose esters were obtained from two Japanese companies, Dai-Ichi Kogyo Seiyaku Co. Ltd., Tokyo, Japan (DK esters F-160, F-140, F-110, F-90, F-70, F-50, F-20 and F-10), and Ryoto Co. Ltd., Tokyo, Japan (P-1570 and S-570). The SURES sucrose esters were produced by Suiker Unie Research (Roosendaal, The Netherlands) using the newly developed process (4). The SURES esters were purified by the procedures used by Osipow et al. (2). TLC-pure sucrose monoesters (SURES 100P and 100S) were obtained by reaction of methyl palmitate or methyl stearate (purity more than 96%) with sucrose and recrystallization from methanol. Silica gel 60 F 254 plates and silica gel 60 HPTLC plates with concentrating zone from E. Merck (Darmstadt, Federal Republic of Germany) were used for TLC.

Instrumentation. HPLC separations were obtained with a Spectra Physics 8700 HPLC, or Spectra Physics 8770 HPLC system, including a Rheodyne 7126 injector or a Rheodyne 7125 injector, respectively, both with 20 µl loops. An Erma ERC 7510 refractive index detector was used. All columns used were self-packed columns (300-4.6 mm i.d.) filled with 5 micron Spherisorb Octadecyl-1 (ODS1), or Spherisorb Octadecyl-2 (ODS2), or Spherisorb aminopropyl (Phase Separations Ltd., Queensferry, United Kingdom). A Jones column oven with built-in Rheodyne 7125 injector was used. Retention times and peak areas were measured with the aid of a Minichrom computing integrator (VG Laboratory Systems Ltd., Altrincham, United Kingdom/Digital Equipment Corp., Maynard, Massachusetts).

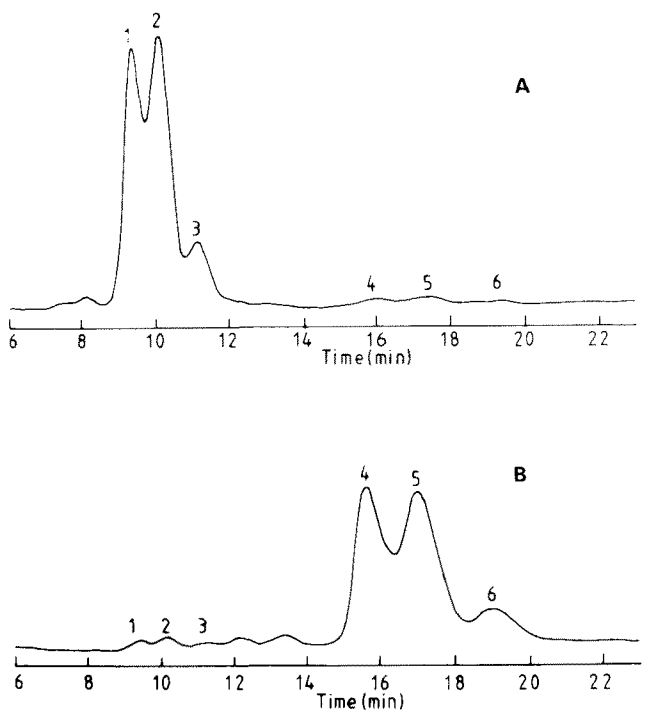

FIG. 3. HPLC separation of A, SURES 100 P, and B, SURES 100 S sucrose esters. Chromatographic conditions and peak numbers as in Fig. 1.

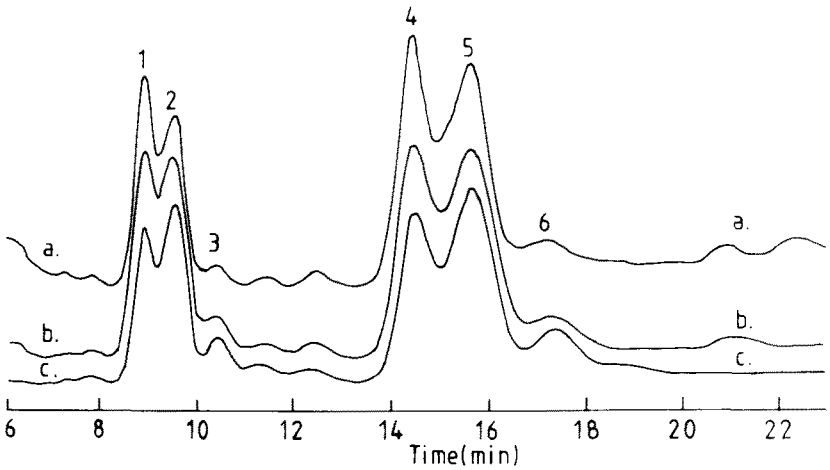

FIG. 4. HPLC separation of a, Ryoto S-570; b, DK F-160, and c, SURES 70 PS 37. Chromatographic conditions and peak numbers as in Fig. 1.

PROCEDURE

The HPLC separations of sucrose esters, fatty acids and methyl esters of the fatty acids were performed on reversed-phase columns. For the separation of sucrose monoesters the eluent consisted of a mixture of methanol and water (85:15, v/v). For the analysis of fatty acids and methyl esters a mixture of methanol and water (94:6, v/v) was used. The separation of sucrose diesters was performed with a mixture of methanol, ethyl acetate and water (65:25:10, v/v/v) as eluent.

All separations were performed at a flow of 1.2 ml/min. Because of the poor solubility of the sucrose esters in these eluents, the analysis had to be performed at an elevated temperature (65 C). By using a heat exchanger in the solvent delivery tube, just in front of the injection valve, the eluent was heated to 85 C or, alternatively, a column oven was used. After each injection the sample loop was purged with chloroform. The DK F-110 sucrose fatty acid ester mixture from Dai-Ichi Kogyo Seiyaku, as described in Table 2, was used as standard solution. The standard sample was dissolved in a mixture of 2-propanol and ethyl acetate (90:10, v/v) at a concentration of 10% (w/v). Sucrose was analyzed after extraction according to Bligh and Dyer (15) on an amino column with acetonitrile and water (85:15, v/v) as eluent. As a detection system during all HPLC separations a refractive index detector was used. For TLC and HPTLC separations the solvent consisted of a mixture of chloroform, methanol, water and acetic acid (70:20:2:2, v/v/v/v). The spots were visualized by

TABLE 1

Approximate Sucrose Ester Composition of Commercially Available DK Esters

DK Ester	Sucrose ester composition (16)		
	Monoesters (%)	Diesters (%)	Higher esters (%)
F-10	1.0	7.0	92.0
F-20	11.0	21.0	68.0
F-50	33.0	49.0	18.0
F-70	41.5	42.5	16.0
F-90	46.0	39.0	15.0
F-110	50.0	36.0	14.0
F-140	60.5	30.0	9.5
F-160	71.0	24.0	5.0

TABLE 2

Composition of Sucrose Fatty Acid Ester Mixtures

Sample	Analyzed percentage			Corrected percentage			Calculated percentage	
	Sucrose and volatiles	Total fatty acids	Total methyl esters	Monoesters	Diesters	Higher esters	Monoesters	Diesters
DK F-20	0.9	1.8	6.7	10.0	19.0	61.6	8.2	—
DK F-50	1.9	1.0	0.5	31.9	47.3	17.4	31.5	47.9
DK F-70	1.9	1.5	1.0	39.7	40.6	15.3	38.2	41.0
DK F-90	1.8	1.6	1.2	43.9	37.2	14.3	41.4	37.2
DK F-110	2.3	1.9	1.4	47.2	34.0	13.2	47.5	33.4
DK F-140	2.4	2.2	2.1	56.5	28.0	8.8	56.8	28.1
DK F-160	2.3	2.5	2.7	65.7	22.2	4.6	66.9	20.9
SURES 100 S	0.5	1.0	0	98.4	0.1	0	98.5	—
SURES 100 P	0.5	0	0	99.0	0.5	0	99.7	—

TABLE 3

Ratio Between Palmitoyl and Stearoyl Monoesters of Sucrose

Sample	Ratio of mono:higher esters (17,18)	% Monoesters, analyzed	Ratio of P:S[a] (17,18)	Ratio of P:S[a] in monoesters, analyzed
DK F-160	70:30	67	30:70	35:65
DK F-50	30:70	32	30:70	20:80
Ryoto P-1570	70:30	70	70:30	80:20
Ryoto S-570	35:65	25	30:70	30:70

[a]P, Palmitoyl; S, stearoyl.

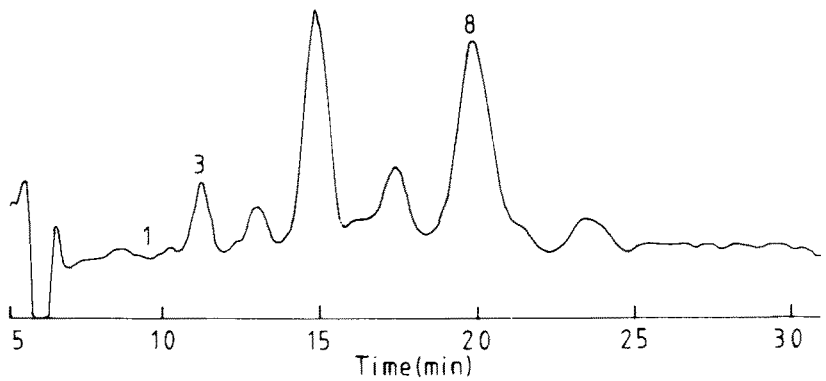

FIG. 5. HPLC separation of the sucrose diesters in DK F-50 on 5 μm ODS column. Solvent methanol, ethyl acetate and water (65:25:10, v/v/v); flow rate, 1.2 ml/min; sample concentration 10% (w/v). Refractive index detection. Peaks: 1, methyl stearate; 3, dipalmitoyl sucrose main peak; 8, distearoyl sucrose main peak.

spraying with sulfuric acid (50%) and charring at about 120 C.

RESULTS AND DISCUSSION

When we tried to reproduce the HPLC method for the separation of sucrose monoesters and diesters as described by Kaufman and Garti (14) with a mixture of methanol and water (95:5, v/v), we were not able to obtain a similar chromatogram. Because we used a reversed phase column with a high octadecyl coverage (ODS2), we also tried a regular ODS column of the type Kaufman used, but both columns gave similar chromatograms. By collecting different fractions during the analysis, and analyzing those by means of TLC, we came to the conclusion that the sucrose monoesters eluted together with the solvent. Further, this could be confirmed with our pure (more than 98%) sucrose monoesters. By changing the composition of the eluent (methanol:water 85:15, v/v) we were able to determine the monoesters. As we had pure sucrose monoesters at our disposal, we were able to prove, in addition, that the fatty acids and the fatty acid methyl esters eluted after sucrose monoesters instead of before the monoesters as Kaufman assumed.

Sucrose monoesters. The chromatogram which was obtained under the conditions set out for the determination of the sucrose monoesters showed for mixed stearoyl/palmitoyl esters, two groups of peaks (Fig. 1).

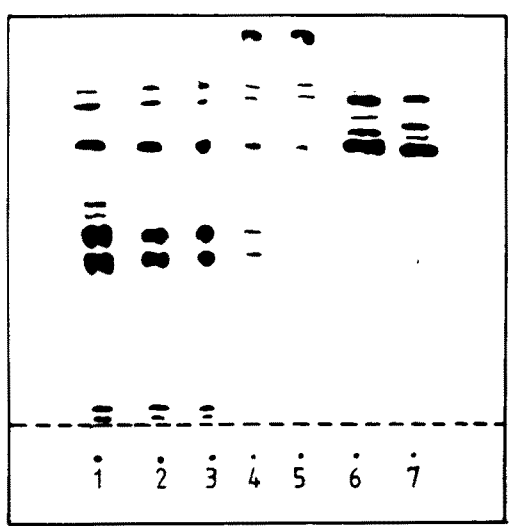

FIG. 6. HPTLC separation of some commercial sucrose esters and some HPLC fractions on silicagel 60. Solvent as in Fig. 2. 1, DK F-160; 2, DK F-110; 3, DK F-50; 4, DK F-20; 5, DK F-10 (1% monoester); 6, HPLC Fraction of SURES 15 P; 7, HPLC Fraction of SURES 15 S.

With our TLC-pure monopalmitoyl sucrose esters and monostearoyl sucrose esters, we could prove that the triplet, which eluted first, was palmitoyl esters, while the second triplet was the stearoyl esters (Figures 2 and

3). In accordance with the work of Lemieux and McInnis (5), it is plausible that the triplet consists of the 6, 6′ and 1′ places substituted sucrose monoesters. Characteristic differences exist between the sucrose esters from different manufacturers with regard to the ratio of the peak heights of the three main peaks within each of the two groups of peaks (Fig. 4). It is not clear yet how important these differences, that might be caused by the applied method of synthesis, are for the application of the sucrose esters. The linearity of the response was confirmed with the aid of a sample which was composed of pure palmitoyl and pure stearoyl monoesters (1:1, w/w), and which was injected at different concentrations. This showed that the areas of the peaks of the sucrose monoesters were directly proportional to the injected concentrations, up to at least 15% (w/v), and that the response factors for stearoyl and palmitoyl sucrose esters were equal. As pure, well defined sucrose ester standards were not commercially available, we defined the composition of the DK F110 sucrose ester mixture in the following manner. The approximate sucrose ester composition of the commercially available DK esters is described by Walker (16) (Table 1). Based on these data from the literature, we calculated the absolute percentages of mono-, di- and higher sucrose esters after determination of impurities.

Correction has been made for the amounts of sucrose, free fatty acids, and methyl esters of fatty acids, as analyzed by us using HPLC, while volatiles were calculated from the loss of weight after drying to constant weight in a vacuum oven at 70 C. From the corrected monoester values of the DK esters (F-20 up to F-160), combined with those values of our pure palmitoyl- and stearoyl-sucrose esters (SURES 100 P and 100 S), the average response factor for monoesters was determined with the help of a linear regression analysis. With this factor the monoester concentrations were calculated (Table 2). The described HPLC method gives clear information about the ratio between palmitoyl and stearoyl monoesters of sucrose (Table 3), which might be of importance to the application of these esters. Especially during analysis of intermediates of the synthesis of sucrose esters, one should bear in mind that free fatty acids and methyl esters of fatty acids elute later than the monoesters, and these peaks might interfere with the next chromatogram. The described method for the analysis of monoesters was not applicable to the analysis of the formed monoesters during the transesterification of fats with sucrose, because of the similar retention times of glycerol monostearate and the formed monostearoyl sucrose esters. Other HPLC methods were developed for the analysis of fatty acids and methyl esters of fatty acids, and for sucrose diesters, because of the lower polarity of these components.

Sucrose diesters. Under the conditions set out for the determination of the sucrose diesters, a complex chromatogram was obtained (Fig. 5). We could tentatively identify the peaks with retention times between 9 and 25 min as being mainly sucrose diesters by collecting the HPLC fractions of SURES 15 P (15% mono and 85% higher sucrose palmitoyl esters) between 9 and 16 min, and of SURES 15 S (15% mono and 85%

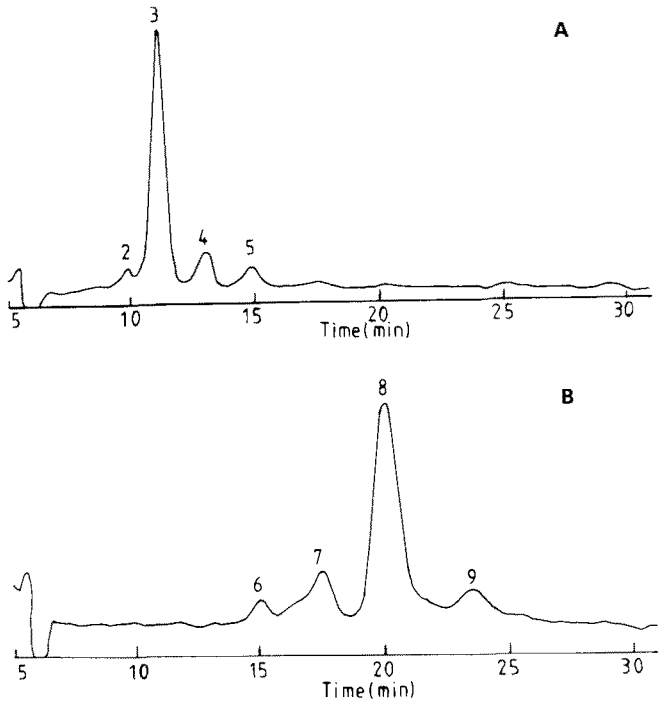

FIG. 7. HPLC separation of A, SURES 15 P, and B, SURES 15 S sucrose esters. Chromatographic conditions as in Fig. 5. Peaks: 2-5, dipalmitoyl sucrose; 6-9, distearoyl sucrose.

higher sucrose stearoyl esters) between 14 and 25 min, and analyzing those by HPTLC (Figs. 6 and 7). From the chromatograms of dipalmitoyl and distearoyl sucrose esters (Figs. 7a and 7b) we could deduce that the chromatogram of a reaction product between sucrose and a mixture of methylpalmitate and methylstearate could be divided into three groups of peaks. The first group (Fig. 5), with a main peak at about 11 min, consisted mainly of dipalmitoyl sucrose esters. The last group, with a main peak at about 20 min, consisted mainly of distearoyl esters. The intermediate part is formed by an overlap of dipalmitoyl-, distearoyl- and palmitoyl/stearoyl-sucrose esters. For this reason it is not possible to determine the ratio between the palmitoyl- and stearoyl-groups. An additional complication is the overlap of the methyl stearate peak and the dipalmitoyl sucrose ester part of the chromatogram (Fig. 5; retention time 10.2 min).

It is noteworthy that our HPLC method for diesters gives for the F-50 ester from Dai-Ichi Kogyo Seiyaku Co. Ltd. a chromatogram almost identical (Fig. 5) to the one reported by Kaufman and Garti (14) when they used their second procedure, in which the eluent consisted of methanol and water (95:5, v/v). However, in our chromatogram the identification of the peaks is quite different. The linearity of the response at higher concentrations was confirmed by means of the DK F-50 sucrose ester mixture with 47% diesters, and which was injected at different concentrations. This showed that the analysis of the sucrose diesters can be used for concentrations up to at least 8% of diesters (w/v). The average response factor for diesters was determined with the aid of some commercial sucrose esters from Dai-Ichi Kogyo in a way similar to that

described for the monoesters. With this factor the diester concentrations were calculated (Table 2).

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