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Improved separation of sucrose ester isomers using gradient high performance liquid chromatography with evaporative light scattering detection

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Abstract

A reversed-phase high performance liquid chromatography (HPLC) with evaporative light scattering detection is described for the analysis of commercial sucrose esters. The binary gradient consists initially of 75% methanol and 25% water. After 70 min, the ratio is changed to 95% methanol and 5% water. The gradient is then reverted back to the initial ratio after 120 min for another injection. This procedure provides a complete separation of monoesters and diesters with different acyl chain lengths (C_{16} and C_{18}). The monoester analyzed was resolved into 3–6 peaks and diesters into 14–18 peaks. The identities of monoesters with various acyl chain lengths were further established by liquid chromatography mass spectrometry (LCMS). This HPLC procedure has several advantages over other reported methods in terms of improved resolution and simultaneous separation of mono- and diesters. With this method it is, thus, possible to determine the approximate composition of mono- and diester of sucrose esters in one analysis.
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Keywords: HPLC; Sucrose ester; Isomers

1. Introduction

Sucrose esters (Fig. 1) are products of sugar and fats after interesterification. Since sucrose has eight hydroxyl groups, 255 different possible isomers from mono- to octaesters could theoretically be formed (Torres, Dean & Wagner, 1990). However, only the primary hydroxyl groups at C6 of the glucose unit, and C1' and C6' of the fructose unit of the sucrose molecule, being the most reactive sites. They can be prepared by (a) the direct esterification of fatty acid chloride (Kea & Walker, 1987), (b) interesterification with fatty acid esters (Feuge, Zeringue, Weiss & Brown, 1970) and (c) the enzymatic reaction between sucrose and fatty acid (Sarney, Barnard, McManus & Vulfson, 1996; Seino & Uchibori, 1984). It is one of the rapidly growing products for the food industry. Sucrose polyester (SPE), with six or more of the eight sucrose hydroxyl groups esterified with fatty acids, has physical and organoleptic properties similar to those of cooking and frying fats. However, due to its large molecular weight and resistance to lipolysis, SPE cannot be readily metabolized by the body (Mattson & Nolen, 1972; Mattson & Volpenhein, 1972). This product is currently permitted by the U.S. Food and Drug Administration as a fat substitute for use in snack foods (Jones, 1996). Sucrose esters with three or lesser fatty acids are surfactants, and are suitable as food additives because of their emulsifying, stabilizing, and conditioning properties. They are non-toxic, tasteless, odorless and non-irritating to skin. They are also available in a wide range of hydrophile-lipophile-balance (Nakamura, 1997).

The most common method for separating and identifying sucrose mono- (SME), di- (SDE) and higher esters (SPE) was based on thin layer chromatography (TLC) (Feuge et al., 1970; Mima & Kitamori, 1962; Weiss, Brown, Zeringue & Feugre, 1971). Gas chromatographic (GC) methods were used for quantification after derivatization (Gupta, James & Smith, 1983, Karrer and Herberg, 1992). Previous reports by Kaufman and Garti

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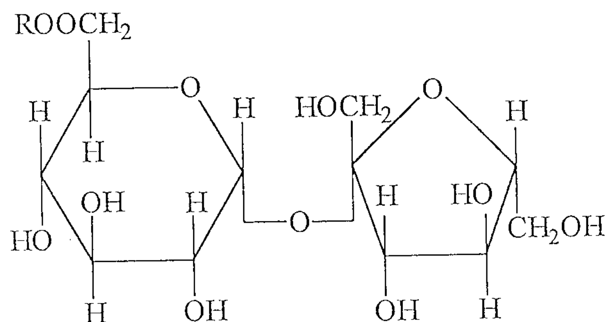

Fig. 1. Structure of sucrose monoester with only one ester linkage. R = alkyl group.

Table 1
Approximate composition by degree of esterification (Walker, 1984)

DKS ester	Mono (%)	Di (%)	Tri (%)	Tetra (%)	Penta (%)	> Penta (%)
F160	71.0	24.0	5.0	–	–	–
F140	60.5	30.0	8.5	1.0	–	–
F110	50.0	36.0	12.0	2.0	–	–
F90	46.0	39.0	13.0	2.0	–	–
F70	41.5	42.5	14.0	2.0	–	–
F50	33.0	49.0	16.0	2.0	–	–
F20	11.0	21.0	15.0	15.0	15.0	23.0
F10	1.0	7.0	15.0	21.0	21.0	35.0

(1981) and Seino and Uchibori (1984) on the separation of SME from SDE and SPE using high performance liquid chromatography (HPLC) were inadequate for the separation of positional isomers. Jaspers, van Leewen, Nieuwenhuls and Vianen, (1987) managed to separate SME into sucrose monopalmitate and monostearate, each with three main peaks, using a solvent mixture of methanol-water (85:15, v/v). However, a separate analysis was necessary to elute SDE using a less polar mobile phase consisting of methanol-ethyl acetate-water (65:25:10, v/v/v). In the Jaspers's method, regeneration of the column was needed after each run. More recently, Torres et al. (1990) also reported a procedure for resolving SME with different acyl chain lengths (C_{14}, C_{16} and C_{18}). The isocratic mobile phase used was acetone:water (70:30, v/v). The chromatograms showed that the method was capable of resolving monomyristate, monopalmitate and monostearate into two, three and four peaks respectively, but the elution of SDE was not reported. Obviously, all these HPLC methods showed some limitations. As the refractive index detector is not suitable with gradient elution, and the UV detector is highly dependent on chemical composition, simultaneous separation of SME, SDE and SPE on a single analysis reported was difficult. In contrast, the evaporative light scattering detector (ELSD) has been recognized as an universal detector compatible with gradient analysis (Stolyhwo, Colin, Martin & Guiochon, 1984). The principle of the operation of this detector has been described in details by Macrae (1988) and Christie (1992). Several studies have also been reported for the analysis of SPE in food products (Chase, Akoh & Eitenmiller, 1995, 1995; Tallmadge & Lin, 1993). However, to our knowledge, simultaneous separation of SME and SDE on a single run using ELSD detector is yet to be reported. The present study describes an HPLC method that uses a reversed-phase column for the separation of some commercial sucrose esters. The SME and SDE were detected using ELSD on a single run and the identification of ester groups was carried out using LC-mass spectrometer (MS).

2. Materials and methods

2.1. Reagents and chemicals

All solvents were of either analytical or HPLC grade. Distilled demineralized water was used. Sucrose was purchased from Ajax Chemicals (N.S.W., Australia). Fatty acid and fatty acid methyl ester standards were obtained from Sigma Chemical Co., MO, USA. Commercial sucrose esters of various molecular weights were supplied by Dai-Ichi Kogyo Seiyaku Co. Ltd., Tokyo, Japan (DKS F160, F140, F110, F90, F70, F50, F20, F10 and F10E) and Mitsubishi-Kagaku Foods Corp., Tokyo, Japan (Ryoto S170, S370, S570, S1170, S1670, P170 and P1670).

2.2. Thin layer chromatography (TLC)

TLC was performed according to a modified procedure of Jaspers et al. (1987). Separation was achieved on a Whatman LK6DF silica gel plate (20×20 cm, and 250 μm thickness). The plate was developed for 45 min using chloroform:methanol:water:acetic acid (70:28:2:2, v/v/v/v). After being air dried for 15 min, visualization of various sucrose ester components on the plate was made possible by spraying with 5% sulfuric acid in ethanol, and then drying it in a fume hood for 30 min prior to heating in an oven at 100°C for 5 min.

2.3. High performance liquid chromatography (HPLC)

The Jasco (Tokyo, Japan) HPLC system model PU-980 consisting of a binary gradient pump, a model 851-AS autosampler, a model CO-965 column oven, and an evaporative light scattering detector (ELSD) (Sedex 55, SEDERE, Alfortville, France) was used. The instrument was controlled by a Wilson 486DX computer and run under Window-based Intuitive Software for Chromatography (Borwin, version 1.21) manufactured by JMBS Developpements (Le Fontanil, France). The separation was effected on a 150×4.6 mm id stainless steel column pre-packed with 5 μm C18-ODSA (Jasco,

Japan). The column was maintained at 40°C and the mobile phase used was a gradient mixture of methanol and water set at 1.2 ml/min. The detector was operated at 2.3 bar air pressure and at 60°C. Sucrose ester solutions (5%) in dichloromethane or tetrahydrofuran were passed through a 0.45-μm disc filter and 10-μl aliquots were injected. The DKS F110 sucrose ester mixture was used as standard solution.

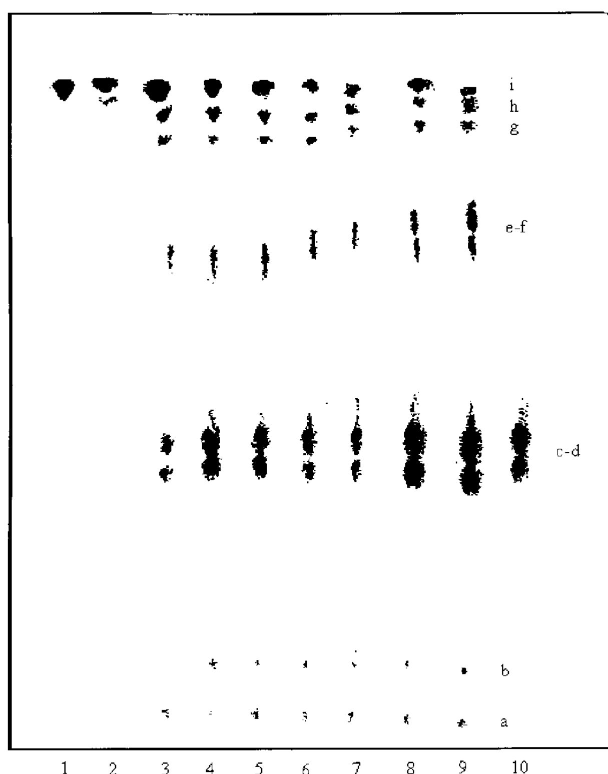

Fig. 2. TLC separation of commercial DKS esters. 1, F10E; 2, F10; 3, F20; 4, F50, 5, F70; 6, F90; 7, F110; 8, F140; 9, F160; 10, SME fraction recovered from TLC. Spot labels are: a, sucrose; b, fatty acid; c and d, monoester; e and f, diester; g, h and i, polyesters.

2.4. Atmospheric pressure ionization mass spectrometer (API-MS)

Identification of the components of sucrose ester was carried out with a single quadrupole LCMS system model PE SCIEX API 100 (Perkin–Elmer Sciex Instruments, CA) equipped with a heated nebulizer probe. The HPLC condition and column used were same of those described earlier. The mass ions (*m*/*z*) were recorded in a full scan mode using negative polarity; mass ranges of 100–1500 at a vaporizer temperature of 300°C.

3. Results and discussion

The approximate sucrose ester compositions of DKS esters manufactured by Dai-Chi Kogya Seiyaku Co., Japan are given in Table 1 (Walker, 1984). Fig. 2 illustrates an improved TLC separation of the DKS sucrose esters (labeled 1–9) based on their differences in polarity. At least seven well-resolved components can be seen in all the F160 to F20 esters, except for F10 and F10E esters where there was only one spot each. The spots labeled a and b (Fig. 2) are sucrose and fatty acids respectively, based on comparison with reference compounds. The triplets labeled c and d are SME, e and f are SDE, and g–i are SPE according to Jaspers et al. (1987). The ester at spot 10 is a pure SME fraction previously collected from DKS F160 using the preparative TLC. This fraction (labeled 10) was subjected to HPLC analysis using methanol:water (75:25, v/v) mixture as illustrated in Fig. 3. Two groups of peaks were observable, each with at least five peaks. By calculating the normalized areas under the peaks, the first group accounted for of 29.8%, while the second group is 70.2% of the total areas. Comparison with the compositional data given by the manufacturer (30% palmitate and 70% stearate), it can be inferred that peaks in the first group consist of isomers of monopalmitate whereas

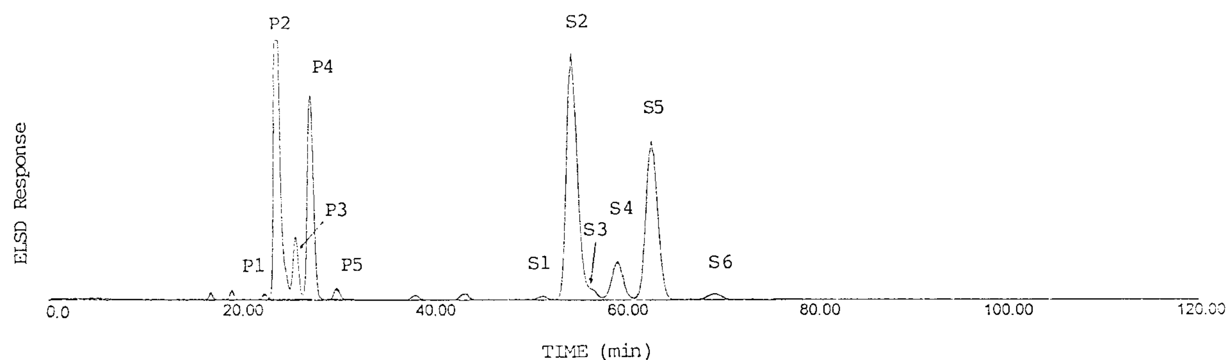

Fig. 3. Reversed-phase HPLC separation of TLC pure SME fraction. Isocratic mobile phase, methanol:water (75:25, v/v); flow rate, 1.2 ml/min. Peaks: P1–P5, sucrose monopalmitates; S1–S6, sucrose monostearates.

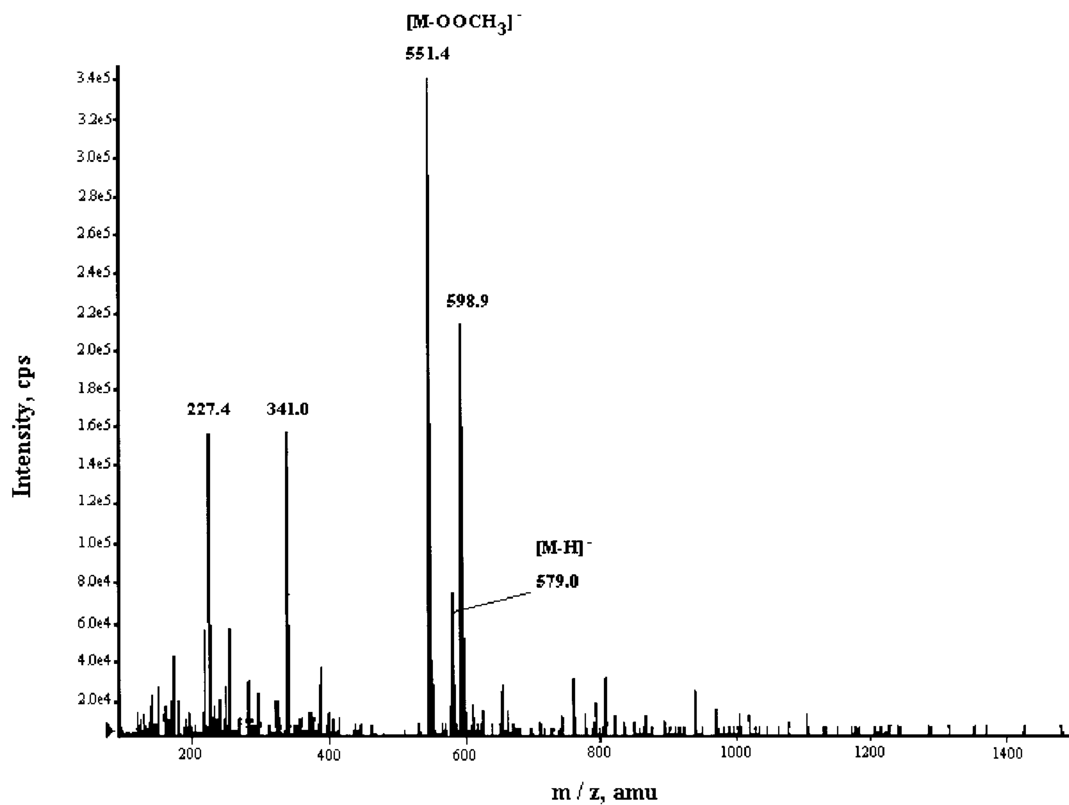

Fig. 4. Mass spectrum of sucrose monopalmitates.

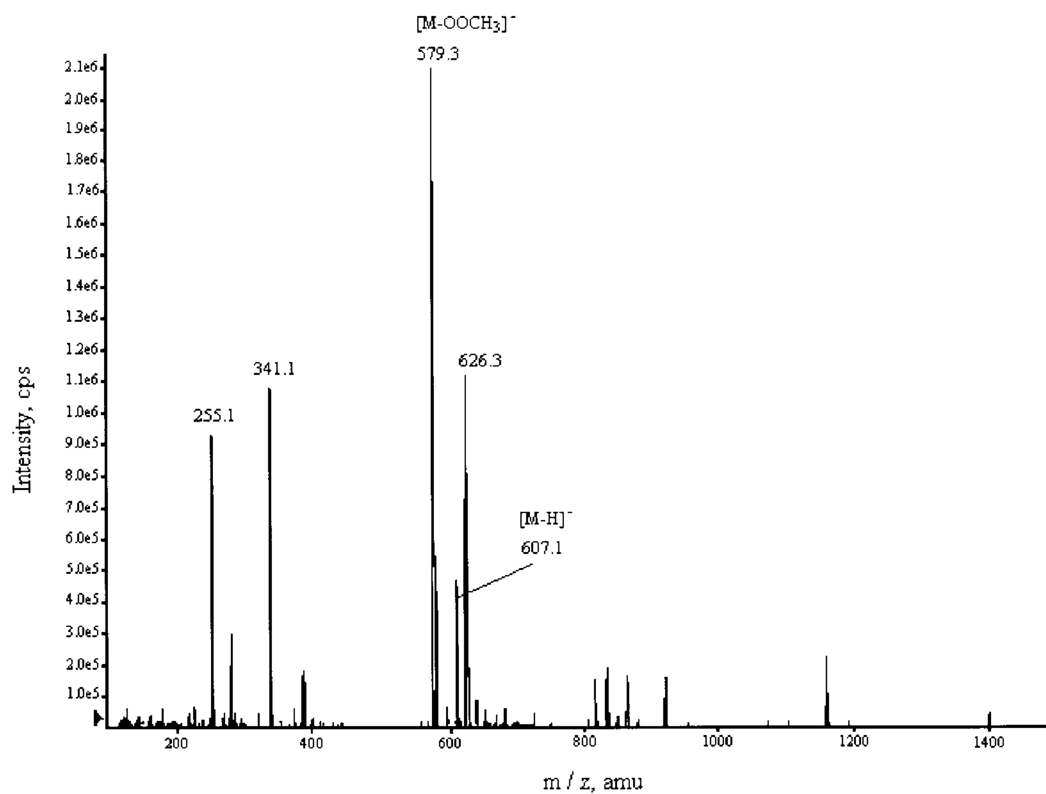

Fig. 5. Mass spectrum of sucrose monostearates.

those in the second group are those of monostearate. Confirmation of their identities were established by LC–MS. The mass spectrum obtained from the first group of SME is shown in Fig. 4, where the medium intensity peak at *m/z* 579 represents the molecular weight of the sucrose monopalmitate $[M\text{-}H]^-$ in the negative ionization mode. The ions at *m/z* 551 and 227 are the sucrose monopalmitate and palmitic acid moieties of the fragmented molecules respectively, whereas the sucrose moiety is present at *m/z* 341. A similar fragmentation pattern is observed in sucrose monostearate (Fig. 5). The fragmentation ions at *m/z* 579, 341 and 255 are attributed to sucrose monostearate, sucrose and stearic acid moieties of the fragmented molecules respectively. In addition, interestingly, these two esters exhibit a rather strong peak at *m/z* 598 and 626 which are not readily interpretable. The result also shows that monopalmitate and monostearate could not be well-resolved using TLC (Jaspers et al., 1987; Torres et al., 1990). However, when analyzing the F160 alone, using HPLC, no additional peaks, other than SME were detectable.

Higher esters are less polar due to a greater substitution of hydroxyl groups of the sucrose molecule. By changing the methanol:water ratio from 75:25 (v/v) to 95:5 (v/v) after SMEs eluted, thus reducing the polarity of the mobile phase, an additional group of peaks belonging to F160 was detected by ELSD. Those peaks are similar to those reported by Kaufman and Garti (1981) as SME using the methanol:water (95:5, v/v) mixture as the mobile phase. However, we interprete the identity of peaks differently. Based on the TLC (Fig. 2, labeled e and f), this group of peaks should be identified as SDE. Besides, by varying the concentration of DKS esters, we also managed to sub-divide the group into dipalmitates and distearates, respectively. The identification of SDE is similar to that reported by Jaspers et al. (1987) except that a better resolution and a more stable baseline were achieved.

The optimum gradient elution program for the separation of SME and SDE of DKS sucrose esters in a single analysis is summarized in Table 2. The validity of the method developed was also verified using the Ryoto series of sucrose esters from Mitsubishi-Kagaku Foods Corp. (Tokyo, Japan). The HPLC chromatograms of the various sucrose esters using the same condition described in Table 2 are shown in Fig. 6. Besides monopalmitates, monostearates and diesters, the sucrose ester samples also contain a small amount of monomyristate (Torres et al., 1990) detected at 10 min elution. The single peak eluted at 1.5 min could be attributed to sucrose, fatty acids or fatty acid methyl esters, or their combinations as these compounds have the same retention time as indicated by using known standards.

Table 2
Gradient elution program

Time (min)	Methanol (%)	Water (%)
0	75	25
70	75	25
71	95	5
120	95	5

The results from this analysis give rough estimate of the ratio between monopalmitate and monostearate, as shown in Table 3. The percentage of SME in the DKS's analyzed is about 5–7% different compared to those indicated by the manufacturer as shown in Table 1. Similarly, slight variations are observed (±4%) in Ryoto SME.

The HPLC procedure described above has several advantages over previously reported methods. In contrast

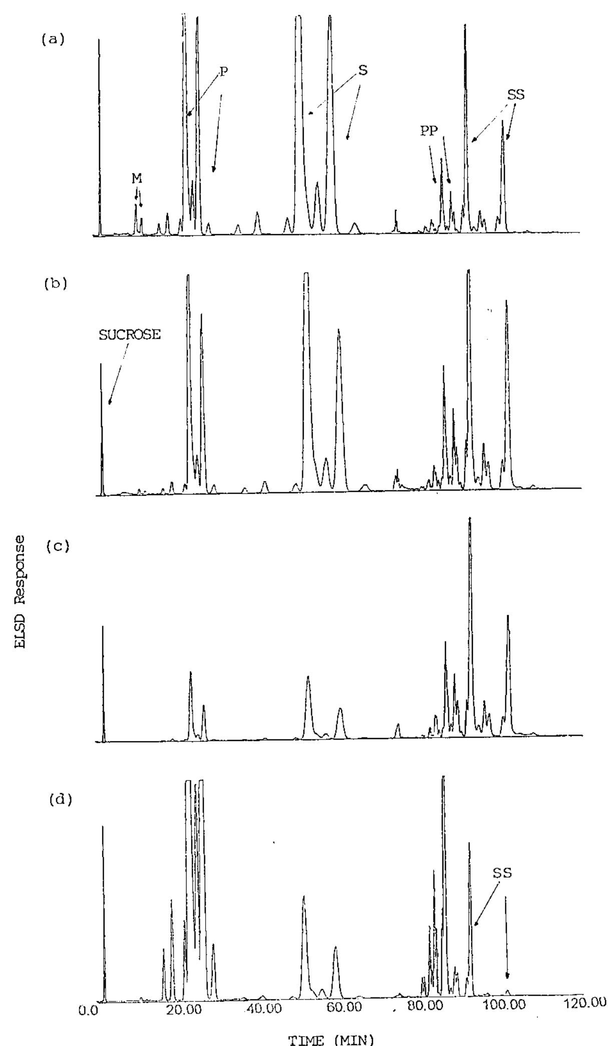

Fig. 6. Reversed-phase HPLC separation of commercial Ryoto esters showing different compositions of SME and SDE. Chromatograms: Gradient program is as shown in Table 2. (a), S1670; (b), S370; (c), S1170; and (d), P1670. Peak groups: M, monomyristates; P, monopalmitates; S, monostearates; PP, dipalmitates; and SS, distearates.

Table 3
Ratio of monopalmitate to monostearate

Sample	Ratio of P:S[a] in monoesters analyzed	Monoesters analyzed (%)	Diesters analyzed (%)	Polyesters estimated (%)
F160	34.4:65.6	67.9	23.4	8.7
F110	28.4:71.6	57.1	32.8	10.1
F90	14.4:85.6	46.7	33.5	19.8
F70	21.1:78.9	38.6	40.2	21.2
F50	19.7:80.3	31.5	47.5	21
F20	16.7:83.3	7.3	18.5	74.2
F10	–	–	5.1	94.9
F10E	–	–	–	100
P1670	87.2:12.8	76.9	18.2	4.9
S1670	34.2:65.8	81.2	17.4	1.4
S170	–	–	2.1	97.9
S370	32.6:67.4	16.7	35.7	47.6
S1170	35.2:64.8	53.4	38.9	7.7

[a] P-palmitate; S-stearate.

to the GC methods, our method allows simultaneous detection of SME and SDE in a single analysis with no prior derivatization of sucrose ester required. With a suitable gradient program, a good resolution of positional isomers of different acyl groups is also possible.

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