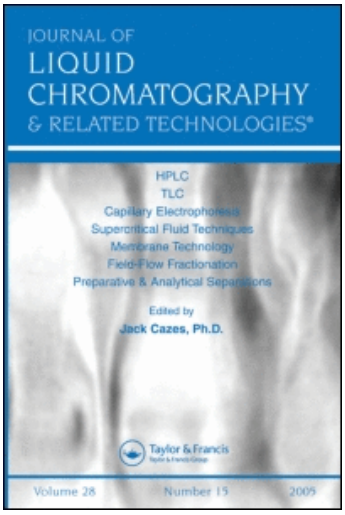

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HPLC Analysis of Sucrose Ester Analogs using Evaporative Light Scattering Detection

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Abstract: A high performance liquid chromatographic method has been developed for the separation and quantitation of sucrose ester analogs using an evaporative light scattering detector (ELSD). By varying the mobile phases and gradient conditions, the complex mixtures of synthetic or commercial sucrose esters were completely separated into several fractions, such as mono-, di- or higher esters, with different regioisomers in each one on a single run. A separation and quantitative method of ten pure monoesters and diesters was also described to evaluate the method's potential application in the quantitation of all sucrose ester analogs using external standards. The described method is found to be accurate, relatively inexpensive, straight forward, and reproducible, and in principle can be readily applied to the analysis of any sucrose ester analogs of interest with little variation, to meet all requirements of qualitative and quantitative analytical procedures.

Keywords: Sucrose ester, HPLC, ELSD, Separation, Quantitative analysis, External standards

INTRODUCTION

The use of sucrose as an alternative industrial bulk chemical to replace fossil resources offers advantages, since it is a readily available renewable commodity. Sucrose esters (SE), also known as sucrose fatty acid esters, have attracted continual interest in the past decades owing to their superior

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performance and compatibility in the health and environmental arenas compared to the petroleum derived products.[1,2] As the most developed derivatives of sucrose by far, they are currently being employed in the food, cosmetic, and detergent industries, and are being produced at about 4000 ton/year.[1–4] In addition, several types of synthetic sucrose esters have shown potent antimicrobial,[2] antitumoral, antibiotic activity,[1] and insecticidal activity against soft bodied arthropods.[5,6] Vesicles containing nonionic surfactants, such as SE, are able to encapsulate both hydrophilic and lipophilic drugs and protect them against acidic and enzymatic degradation in the gastrointestinal tract.[7,8]

However, sucrose is a complex molecule with a complex chemistry because of its polyfunctionality. The preparation of SE using the esterification or transesterification reaction yields a complex mixture of monoesters, diesters, and higher esters with fatty acyl groups of various chain lengths.[9] Even the acylation of sucrose with a single fatty acid can, theoretically, yield 255 different possible isomers from mono- to octa-esters.[10–13] They are used as mixtures of regioisomers as well as mono-, di-, and trimesters, with physicochemical properties depending on the average degree of substitution and on the length of the fatty chains. In addition, a lot of studies have aimed at preparing pure sucrose fatty acid esters of a defined structure (Figure 1) in order to get some precise structure activity relationships in this series of compounds,[14–17] especially for the functional performance of antimicrobial, antitumoral, and antibiotic applications. Thus, an effective method for the separation and analysis of SE during synthesis, purification, and studies of structure function relationships is essential and absolutely necessary.

Numerous techniques have been published for separation and quantitative analysis of these SE compounds. The most common method was based on

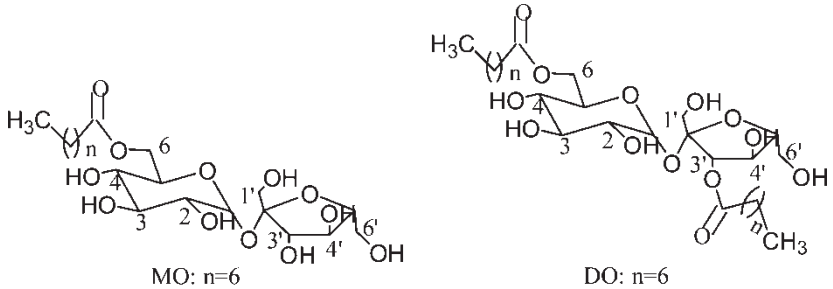

Figure 1. Structures of SE compounds.

thin layer chromatography (TLC),[18] which gave qualitative and quantitative ideals about the ratio of mono-, di-, and higher esters. Gas chromatographic (GC) and GC-mass spectrometric methods were used for quantitative and qualitative results after derivatization.[19] Many attempts have been made for the separation and quantitative analysis of SE by high performance liquid chromatography (HPLC) in past 20 to 30 years.[10–12,20–22] Most of the published HPLC methods used UV detection by ultraviolet absorbance at low wave length or refractive index detection. UV detection, however, has one essential disadvantage: the absorbance of SE arises primarily from double bonds in the fatty acid moieties, which accounts for the extremely poor response of fully saturated species. Refractive index detection can satisfy the quantitative and qualitative analyses of some pure sucrose monoesters or diesters only in the isocratic elution mode. None of these methods enable a complete separation of SE with different degrees of substitution, positional isomers, and chain lengths of fatty acids to meet all requirements of a qualitative analytical procedure. Accurate quantitative analysis is, therefore, extremely difficult.

Some of these problems could be overcome with the evaporative light scattering detector (ELSD). Analytical methods using ELSD have previously been developed in conjunction with liquid chromatography and supercritical fluid chromatography for analytes difficult to detect by traditional methods.[23–26] It has been reported that ELSD is a mass detection method, which is based on LC column effluent nebulization into droplets by the nebulizing gas; the resulting vapor enters a temperature controlled evaporator tube, which causes the evaporation of mobile phase. The resulting nonvolatile analyte particles are then directed towards a narrow light beam. The amount of light scattered is measured by a photomultiplier tube and is directly proportional to the amount of analyte in the column effluent. The signal is indicative of molecular size and shape, but not the chemical identity of the residual particles passing through the light beam. ELSD can be used with most solvents, including water, and is able to detect all types of analytes regardless of their molecular construction. In addition, ELSD is insensitive to the composition of the mobile phase, creating flat baselines with solvent gradient programs that cover a wide range of solvent polarities, thus, rendering it a suitable method for the analysis of sucrose ester analogs.

The purpose of the present investigation was to develop an effective method for the separation and analysis of sucrose ester analogs with different degree of substitution, as well as the different lengths of fatty chain using reversed-phase HPLC coupled to an ELSD. This paper also describes a separation and accurate quantitative method of ten synthetic pure sucrose monoesters and diesters with the different lengths of fatty chain, to evaluate the method's potential application in the quantitation of all SE analogs, to meet all requirements stated previously. To the best of our knowledge, this is the first report on the separation and accurate quantitative analysis of pure SE analogs.

EXPERIMENTAL

Reagents and Chemicals

All solvents were of either analytical or HPLC grade. Water was purified by means of an in house Mill-Q Academic Ultrapure Water System (Millipore, Eschborn, Germany).

An octanoylsucrose sample (OS) was synthesized according to a method previously reported.[5,12] Synthesis of lauroylsucrose (LS) and palmitoylsucrose (PS) samples were performed using a similar procedure.

Synthesis of 6-O-Lauroylsucrose (ML) and 6,3′-Di- Lauroylsucrose (DL) was carried out according to a method developed in our laboratory recently.[17] 6-O-Octanoylsucrose (MO), 6,3′-Di-Octanoylsucrose (DO), 6-O-Decanoylsucrose (MD), 6,3′-Di-Decanoylsucrose (DD), 6-O-Myristoylsucrose (MM), 6,3′-Di-Myristoylsucrose (DM), 6-O-Stearoylsucrose (MS) and 6,3′-Di-Stearoylsucrose (DS) were synthesized using the similar procedure. These ten pure sucrose monoesters and diesters were isolated by column chromatography and fully characterized by chromatography and spectroscopic techniques (HPLC-ELSD, NMR). In all cases, HPLC and NMR analyses showed a degree of purity over 99%. Structures of the compounds are represented in Figure 1. Sample ASE was a mixture of the ten crude products during the synthesis before chromatographic purification.

Commercial sucrose esters (S1670, S1170) were purchased from Ryoto Co. Ltd. Tokyo, Japan.

HPLC System

HPLC was performed with a Hewlett-Packard Model 1050 Series fitted with a solvent delivery system PV5 (proportioning value for gradient elution), autosampler, Alltech 2000 evaporative light scattering detector (ELSD), and HP personal computer with Agilent HPLC ChemStation software. An interface module HP-35900 converts the analogue signal from the ELSD to digital data, which are transmitted to the computer.

Chromatographic Conditions

All chromatographic separation was performed using gradient elution. The solvents used for separation of OS, LS, ASE, were water, solvent A; and methanol, solvent B. The solvents used for separation of PS, S1670, S1170, were water, solvent A; and methanol solution containing 10% tetrahydrofuran (v/v), solvent C. All injections were 10 μL in volume. The mobile phase was delivered at a total flow rate of 1.0 mL/min. All separations were carried out at 40°C, on a reversed-phase C18-ODSA column (150 × 4.6 mm, 5 μm

particle size) purchased from Elite Analytical Instruments Co., Ltd (Dalian, China). The column effluent was directed to ELSD. Nebulization of the ELSD was provided by a stream of dried air using an air compressor and the nebulization was performed at room temperature.

The mobile phase A and B were prepared by filtering water or HPLC grade methanol through a 0.2 μm nylon filter using a vacuum filtration assembly. Filtered solvents were degassed using a water vacuum assembly with gentle stirring using a magnetic stirrer for about 5 min. C was prepared by mixing HPLC grade tetrahydrofuran and methanol in a predetermined ratio, then filtering it as the procedure for preparing A and B.

Prior to each run, the HPLC-ELSD system was allowed to warm up for 20–30 min and the pumps were primed using the protocol suggested by the manufacturer. Using freshly prepared mobile phase, the baseline was monitored until stable and the samples run.

ELSD Parameters

In order to obtain minimum noise and maximum detection signal in ELSD, three basic parameters, nebulizer gas flow rate, evaporating temperature, and gain were varied to optimize the detection of samples.[23–26] The performance of the detector was periodically evaluated by executing the electronic noise, solvent noise, column noise, and signal stability tests, using the procedures recommended by the manufacturer. Dried air was used as the driving gas for nebulization and nitrogen was used as the carrier gas for analyte transport. The carrier gas was passed through filter frits prior to entering the detector to assure the absence of stray particles introduced by the gas.

Preparation of Sample Solutions

Individual solutions of OS, LS, PS, S1670, S1170, and ASE were prepared in HPLC grade methanol. The sample was dissolved and transferred with methanol to a 10 mL volumetric flask and made up to volume with methanol. The sample solution was filtered through a 0.2 μm hydrophilic membrane filter into an HPLC sample vial just before HPLC-ELSD analysis.

Preparation of Calibration Standards

In a clean, dry 10 mL volumetrick flask, a sample (approximately 50 mg) of MO was accurately weighed and dissolved in HPLC grade methanol to make a stock solution. It should be noted that this stock solution was found to be stable at −20°C. Calibration standards were prepared by diluting the stock solution with methanol in appropriate quantities. Eight calibration

standards were made at concentrations between the detection limit of MO and the concentration at which saturation of the detector occurred. Calibration standards of MD, ML, MM, MS, DO, DD, DL, DM, and DS were prepared following the above procedure.

RESULTS AND DISCUSSION

Separation

As sucrose has eight free hydroxyl groups, esterification or transesterification could result in the formation of a mixture of different degrees of substitution (such as monoester, diester, etc.), as well as the different regioisomers. Gradient elution was needed in order to obtain a complete separation of the complex mixtures. We have used a gradient elution program with A and B as the mobile phase to give a complete separation of mono- to octa-esters with different positional isomers in each fraction of an octanoylsucrose sample on a single run.[12] Similarly, complete separations of octanoylsucrose sample OS (Figure 2) and lauroylsucrose sample LS (Figure 3) prepared in this work were obtained by varying the gradient elution conditions with A and B as the mobile phase.

We even tried to reproduce the HPLC-ELSD method for the separation of commercial SE samples S1670 and S1170 as described by Moh,[11] but we were not able to obtain anticipatory results. This method provides a separation of monoesters and diesters using a gradient elution program with A and B as the mobile phase. However, we found it is difficult to obtain the replicate results for two injections of one sample under the same condition, especially, for the analysis of S1170. After sucrose mono- and diesters were eluted, we used the elute C to elute the column; several groups of peaks with high

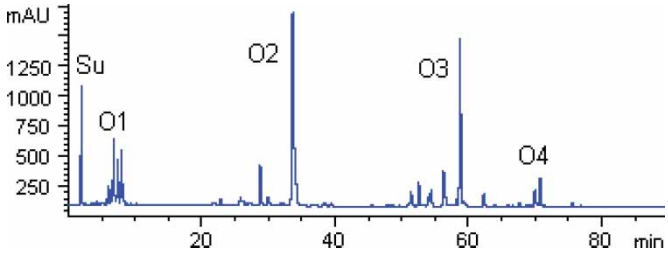

Figure 2. Chromatogram of OS. Su = sucrose, O1 = monooctanoates, O2 = dioctanoates, O3 = trioctanoates, O4 = tetraoctanoates. Mobile phase: A and B. The following linear gradient was used: 60% B from 0 to 10 min and to 70% B at 11 min; 70 to 75% B from 11 to 30 min; 75 to 100% B from 30 to 80 min; 100% B from 80 to 100 min. Evaporating temperature in ELSD: 90°C. Nebulizer gas flow rate: 2.4 L/min. Signal gain: 12.

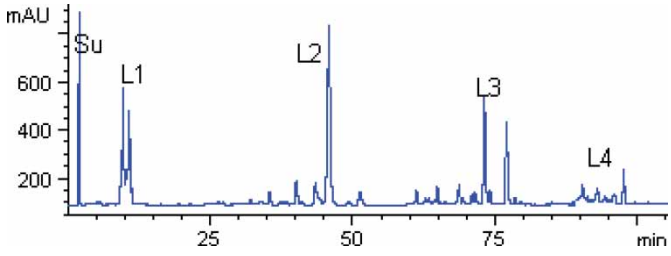

Figure 3. Chromatogram of LS. Su = sucrose, O1 = monolaurates, O2 = dilaurates, O3 = trilaurates, O4 = tetralaurates. Mobile phase: A and B. The following linear gradient was used: 70% B from 0 to 20 min; 70 to 75% B from 20 to 40 min; 75 to 100% B from 40 to 80 min; 100% B from 80 to 120 min. Evaporating temperature in ELSD: 85°C. Nebulizer gas flow rate: 2.2 L/min. Signal gain: 12.

intensity were detected. Consequently, it is believed that partial sucrose diesters, and all higher esters, are reserved in the column. In order to avoid the reservation of sucrose diesters and higher esters with long lengths of fatty chain (palmitoylsucrose and stearoylsucrose), we changed the mobile phase from A and B to A and C. It was found, that apart from the two groups of sucrose monoesters and diesters that were similar to those reported by Moh, an additional group of peaks belonging to S1170 was detected by HPLC-ELSD. Similarly, palmitoylsucrose sample PS was completely separated using the same chromatographic conditions (Figure 4).

Separation of sample ASE, which contained ten structure determined pure SE compounds with various degrees of substitution from mono- to di- and length of fatty chain from C_8-C_{18}, was a more attractive and challenging work. These ten SE compounds cover a wide range of polarities and their separation also needs a gradient elution with a wide range of solvent polarities. Separation of up to ten pure compounds in ASE on a single run was accomplished by the described HPLC-ELSD method shown in Figure 5. Table 1 summarized our results concerning retention times of the ten SE compounds.

The retention times of most SE compounds were sufficiently reproducible with relative standard deviations of 0.3 to 1.1. Only MO and MD showed retention times with relative standard deviations of 3.9 and 2.3%, respectively.

Calibration

Calibration curves of ELSD were non-linear. Calibration functions were obtained by curve fitting using the equation:[23]

$$a = Km^E \tag{1}$$

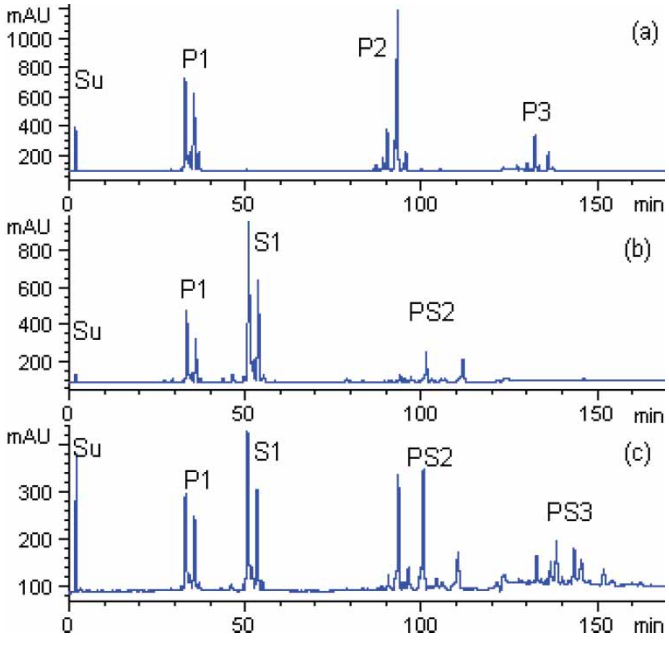

Figure 4. Chromatogram of PS (a), S1670 (b) and S1170 (c). Su = sucrose, P1 = monopalmitates, P2 = dipalmitates, P3 = tripalmitates, S1 = monostearates, PS2 = mixtures of dipalmitates and distearates, PS3 = mixtures of tripalmitates and tristearates. Mobile phase: A and C. The following linear gradient was used: 70 to 85% C from 0 to 70 min; 85 to 95% C from 70 to 80 min; 95% C from 80 to 115 min; 95 to 100% C from 115 to 120 min; 100% C from 120 to 180 min. Evaporating temperature in ELSD: 85 °C. Nebulizer gas flow rate: 2.2 L/min. Signal gain: 12.

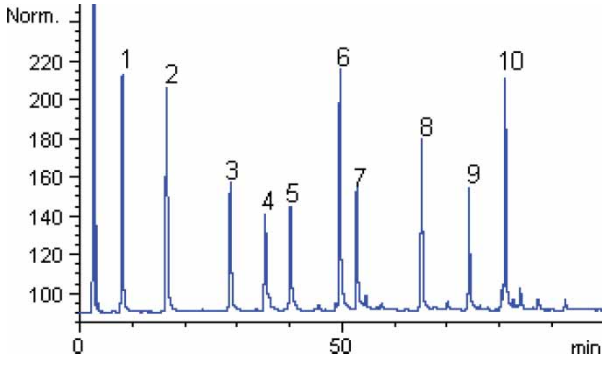

Figure 5. Chromatogram of ASE. 1 = MO, 2 = MD, 3 = ML, 4 = DO, 5 = MM, 6 = MS, 7 = DD, 8 = DL, 9 = DM, 10 = DS (for abbreviations see Table 1). Mobile phase: A and B. The following linear gradient was used: 0 to 100% B from 0 to 100 min. Evaporating temperature in ELSD: 100°C. Nebulizer gas flow rate: 2.8 L/min. Signal gain: 12.

Table 1. Retention times of ten synthetic pure SE compounds (mean of n = 8 with standard deviation and relative standard deviation)

SE compounds	Abbreviation	Retention time (min)	S.D. (min)	R.S.D. (%)
6-O-Octanoylsucrose	MO	6.89	0.27	3.9
6-O-Decanoylsucrose	MD	14.06	0.32	2.3
6-O-Lauroylsucrose	ML	25.36	0.28	1.1
6,3′-Di-Octanoylsucrose	DO	32.39	0.31	1.0
6-O-Myristoylsucrose	MM	37.36	0.22	0.6
6-O-Stearoylsucrose	MS	47.89	0.12	0.3
6,3′-Di-Decanoylsucrose	DD	51.58	0.23	0.4
6,3′-Di-Lauroylsucrose	DL	64.66	0.27	0.4
6,3′-Di-Myristoylsucrose	DM	73.97	0.27	0.4
6,3′-Di-Stearoylsucrose	DS	80.93	0.27	0.3

with peak area units a, mass of a component injected m, a constant K, and an exponent E. When the calibration functions are plotted on a logarithmic scale they are linear, following the equation:

$$\lg a = E \lg m + \lg K \tag{2}$$

with E being the slope and lg K the intercept. The limit of detection was determined using the criteria of the lowest detectable amount above three-times the peak-to-peak baseline noise (3 × p-p noise).

It is noted that the exponents E for the ten investigated SE classes in the mass range 0.5–25.0 μg were very similar (Table 2), with a mean value of

Table 2. Detection limits, calibration curve fitting Results according to Eq. (2), and recoveries of ten synthetic pure SE standards (for abbreviations see Table 1)

SE compounds	Detection limit (μg)	Curve fitting		
		Constant (K)	Exponent (E)	Correlation (r)
MO	0.11	13.2	1.73	0.9936
MD	0.09	15.4	1.62	0.9959
ML	0.07	20.2	1.60	0.9951
DO	0.05	29.6	1.56	0.9914
MM	0.08	17.6	1.59	0.9950
MS	0.11	16.8	1.60	0.9983
DD	0.05	27.6	1.54	0.9984
DL	0.07	20.5	1.57	0.9993
DM	0.06	19.4	1.52	0.9992
DS	0.08	18.2	1.60	0.9989

Table 3. Mass content quantitation of SE compounds in the ASE with standard deviation

SE compounds	Content (mean ± S.D.) (%)	R.S.D. (%)
MO	9.3 ± 0.2	2.1
MD	10.5 ± 0.2	1.9
ML	6.6 ± 0.2	3.0
DO	4.7 ± 0.2	4.3
MM	7.0 ± 0.3	4.3
MS	16.7 ± 0.4	2.4
DD	6.2 ± 0.2	3.2
DL	8.8 ± 0.3	3.4
DM	8.1 ± 0.3	3.7
DS	10.2 ± 0.2	2.0

1.59 and a standard deviation of 0.05. Constants *K* for six of the ten compounds except MO, MD, DO, and DD were also very similar. The detection limits of the ten SE compounds are comparable with a range from 0.05 μg for DO and DD to 0.11 μg for MO and MS. This may be due to their structural similarity as they differ only by the length of the side chain linkage and the degree of substitute. It is especially noted that exponents *E*, Constants *K*, and detection limits for MS and DS, which have the same chain length of fatty acid moiety and different degree of substitute were all very similar in this work, as well as for ML and DL. The results indicate that rough estimate contents of these compounds, based on the area percentage of ELSD response with a correction coefficient, sometimes would be very close to the accurate values. This would be very important for the quantitative analysis of SE, as the pure external standards were difficult to obtain currently.

Quantitation

The precise mass contents of the ten SE compounds in ASE were analyzed according to the calibration curves of these compounds (Table 3). The results of the quantitative analysis for a complex sample were adequately reproducible with low standard deviations *****(≦4.3%).

CONCLUSIONS

A high performance liquid chromatographic method has been developed for the separation and quantitation of SE analogues using an evaporative light

scattering detector. With this method, the complex mixture of synthetic or commercial SE were completely separated into several fractions, such as mono-, di-, or higher esters with different regioisomers in each one, on a single run by varying the mobile phases and gradient conditions. A separation and quantitative method of ten pure SE monoesters and diesters was also described to evaluate the method's potential application in the quantitation of all SE analogs. The results indicate that rough estimate contents of these compounds based on the area percentage of ELSD response sometimes would be very close to the accurate values when using a correction coefficient.

The HPLC-ELSD method described in this paper was found to be an accurate, relatively inexpensive, straight forward and reproducible procedure for the separation and quantitation of SE classes in complex mixtures. In principle, it can be used for the analysis of any SE analogue of interest with little variation and, thus, meet all requirements of qualitative and quantitative analytical procedures.

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