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Analysis of sucrose acetates in a crude 6-*O*-acetyl sucrose product by on-line hydrolysis-high-performance liquid chromatography with pulsed amperometric detection

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ABSTRACT

A standard-free and sensitive method was developed for analysis of sucrose acetates in a crude 6-*O*-acetyl sucrose (S-6-a) product by on-line hydrolysis-high-performance liquid chromatography with pulsed amperometric detection (PAD). Sucrose, three regio-isomers of acetyl sucrose and five regio-isomers of diacetyl sucrose were separated on a C18 column using 3% (v/v) acetonitrile in water as eluent within 25 min. After purification with LC followed by semi-preparative HPLC, their chemical structures were identified by 1D, 2D NMR and LC–MS. Moreover, quantification of those regio-isomers was achieved by on-line alkaline hydrolysis to liberate sucrose using a post-column delivery system, and then detected by PAD for indirect estimation of the sucrose acetate content. Under optimal conditions, the linear ranges were from 0.03 to 150 μmol L^{-1} for sucrose corresponding to sucrose acetates with coefficient of determination as 0.9997 and detection limit as 0.01 μmol L^{-1} ($S/N = 3$). Good repeatability was obtained (RSD < 3%, $n = 6$). Furthermore, this method has been successfully applied to the analysis of sucrose and sucrose acetates in a crude S-6-a product during synthesis, purification and structure elucidation studies. The recoveries were from 94.89% to 102.31% for sucrose and sucrose acetates.

1. Introduction

Sucrose esters, an interesting group of compounds with biodegradable and non-toxic properties, have wide applications in agriculture, food, pharmaceuticals and cosmetics [1–3]. As a short-chain monoester, 6-*O*-acetyl sucrose (S-6-a) is a key intermediate in the preparation of sucralose which is a high-intensity sweetener and has replaced sucrose in many food and beverage products due to its properties regarding non-nutrition, high temperature stability and safety [4–6].

At present, S-6-a can be synthesized chemically [7–9] or enzymatically [10–12]. Many chemical methods have been reported for the regioselective synthesis of S-6-a in industry, such as the sucrose alkyl 4, 6-orthoacylates method [7], dibutyltin oxide method [8] and demethoxy-ethylene method [9]. However, the specificity of these methods is insufficient, resulting in a major product S-6-a and some other byproducts, such as mono-, di- or higher acetates with different positional isomers as shown in Fig. S1. Reaction conditions such as catalyst, solvent and temperature can greatly affect the kinds and yields of the esterified products, and usually need to be optimized to achieve maximum regioselectivity. Qualitative and quantitative analyses of positional isomers are indispensable in quality control of the synthesis and purification of S-6-a. Consequently, establishing a fast and simple method to detect the products of the synthesis process is important.

Although spectrophotometry [13], thin-layer chromatography [14,15] and GC [16] have been used to determine sucrose esters after preliminary treatments such as derivatization, they are time-consuming and laborious. HPLC with UV or refractive index (RI) detection for determination of sucrose esters has been reported [17,18]. However, UV detection is not sensitive enough because sucrose esters have weak absorbance in the UV range. In addition to its low sensitivity, RI detection is not compatible with gradient elution which might be required for a good separation of sucrose acetic acid esters. Liquid chromatography with electrospray ionization mass spectrometric detection (LC/ESI–MS) method has also been proposed for the determination of sucrose esters [19]. Unfortunately, this method suffers from comparably expensive

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instruments and operational cost. Recently, HPLC methods with evaporative light-scattering detection (ELSD) or charged aerosol detection (CAD) make detection of low UV-absorbing sucrose esters feasible without using complex or costly detection methods [20–23]. However, one disadvantage of this type of detector is that its mass-response relationships follow exponential functions, making direct linear regression inaccurate for fitting calibration curves [24]. Apparent deviation was expected when the contents of various ester regio-isomers were determined by the normalization method according to the HPLC peak area. In order to get a more precise content of a certain isomer with ELSD or CAD detection, it is necessary to prepare pure sucrose ester with a defined structure as external standard [21]. However, it is difficult to obtain appropriate amounts of pure regio-isomers because of the instability of some isomers and the complicated purification process. Such problem is also encountered in the determination of sucrose acetates in a crude S-6-a product.

Pulsed amperometric detection (PAD) in LC is of great interest due to its simplicity, high sensitivity and selectivity for the determination of various carbohydrates and related compounds [25–32]. For sensitive detection of carbohydrates, PAD is recommended at Au electrode under alkaline condition (pH > 12) in which sucrose acetates would hydrolyze, making direct detection of such compounds infeasible. On the other hand, sucrose acetates could hydrolyze completely at high pH to liberate the equal molar sucrose which could be used for indirect quantification of sucrose acetates. Therefore, combining HPLC-PAD with an on-line alkaline hydrolysis procedure might overcome the difficulty in quantification of sucrose acetates in the absence of standards for calibration. Furthermore, the relative molar percentage of each regio-isomer can also be obtained by the normalization method, since sucrose acetates have same molar response factor in the PAD detector upon alkaline hydrolysis.

To the best of our knowledge, no work has been performed on the determination of sucrose acetates by HPLC-PAD. In this paper, we demonstrate an HPLC-PAD analytical method coupled with an on-line alkaline hydrolysis procedure. The proposed method would be applied to simultaneous determination of sucrose and sucrose acetates in a crude S-6-a product.

2. Experimental

2.1. Chemicals

Sucrose and 50% sodium hydroxide solution for HPLC were obtained from Sigma (St. Louis, MO, USA), and dibutyltin oxide was purchased from Aladdin Co. (Shanghai, China). HPLC-grade Acetonitrile was obtained from Merck (Darmstadt, Germany). Acetic anhydride was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All other chemicals used in this study were analytical grade. Deionized water employed in all experiments was obtained from a Millipore-Milli-Q system (Bedford, MA, USA). Stock solution of sucrose was prepared at 3.0 mmol L^{-1} with deionized water and stored at 4 °C. Further dilution of the stock solution was performed with the chromatographic eluent to obtain a series of standard solutions with concentrations in the range of 0.03–150 μmol L^{-1}. Calibration standard solutions were freshly prepared daily.

2.2. Apparatus

The HPLC-ELSD analysis and semi-preparative HPLC separation were performed using a Thermo Scientific Ultimate 3000HPLC system (Sunnyvale, CA, USA) equipped with a gradient pump (LPG-3400SD), column oven (TCC-3000RS), and autosampler (WPS-3000), coupled with an Agilent 1260 infinity ELSD (Santa Clara, CA, USA). The HPLC-PAD analysis was performed with a Thermo Scientific ICS 3000 (Sunnyvale, CA, USA) chromatograph consisting of two gradient pumps, a DC compartment, an AS autosampler and an ICS-3000 Electrochemical Detector whichequipped with a gold working electrode, an Ag/AgCl reference electrode and a stainless steel counter electrode. Suppression was achieved with a Thermo Scientific ASRS 300 4-mm suppressor. Chromeleon 6.8 chromatography data management software (Thermo Scientific, Sunnyvale, CA, USA) was used for system control and data processing.

The mass spectrum analysis was performed using a Sciex API 2000 LC/MS/MS system (Applied Biosystems, Stockholm, Sweden) with an atmospheric pressure chemical ionization (APCI) ion source. The ^{1}H and ^{13}C NMR data were obtained on a Bruker Ultrashield 600 Plus (^{1}H NMR at 600 MHz, ^{13}C NMR at 150 MHz) spectrometer (Karlsruhe, Germany).

2.3. Synthesis and purification of sucrose acetates

S-6-a was synthesized by sucrose (10 g) in *N*, *N*-dimethylformamide (DMF) using dibutyltin oxide as catalyst as described by Neiditch et al. [8]. Water generated in the reaction of sucrose with dibutyltin oxide was removed by codistilling with cyclohexane. Acetic anhydride was used as the acylating agent. After terminating the reaction, the crude product was then treated with water and extracted with cyclohexane to remove the organotin. Then the DMF solution was subjected to rotary evaporation under high vacuum to remove entrained cyclohexane, water and a portion of the DMF to afford a crude S-6-a product (25 g).

The crude S-6-a product was initially analyzed on a Phenomenex Luna C18 column, (250 mm × 4.6 mm i.d., 5 μm, Torrance, CA, USA) and detected by ELSD (the drift tube temperature: 65 °C, nitrogen nebulizer gas: 1.6 L min^{-1}), with 3% (v/v) acetonitrile/water as mobile phase at a flow rate of 1.0 mL min^{-1}, then loaded (5 g) onto a glass column (300 mm × 60 mm i.d.) packed with Amberlite XAD-16 Macroporous Resin (Sigma, St. Louis, MO, USA) equilibrated with water. Next, the crude S-6-a mixture was eluted by a stepwise gradient of methanol in water at 5% (v/v, 1.2 L) and 10% (v/v, 1.5 L), respectively, at ambient temperature with a gravitational flow. The obtained acetyl sucrose and diacetyl sucrose fractions were dried by lyophilization and subjected to further purification by semi-preparative HPLC.

For semi-preparative purification, a Phenomenex Luna C18 column (250 mm × 10 mm i.d., 5 μm, Torrance, CA, USA) was used for separation and detected by ELSD (the drift tube temperature: 65 °C, nebulizer gas flow rate: 1.6 L min^{-1}), with 3% (v/v) acetonitrile/water as mobile phase at a flow rate of 3.0 mL min^{-1} for isocratic elution. The dried acetyl sucrose (300 mg) and diacetyl sucrose fractions (200 mg) were separately resolubilized in water (5 mL) and filtered through a 0.2 μm nylon membrane (Nylaflo, Aldrich, St. Louis, MO, USA) and 20 μL of filtrate aliquots were injected. As shown in Fig. 1A, the stream after column was split into two directions by a three port union, one for detection, and the other one for sample collection. The ratio of flow rate was adjusted by changing the length of PEEK tubes (7:1). Based on the retention times, fractions were automatically collected using a Rheodyne 7 port, 6 position switching valve (Cotati, CA, USA). The semi-preparative HPLC procedure was run repeatedly, and identical isomer fractions were pooled and the solvent was then removed by lyophilization.