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Rapid characterization of commercial polysorbate 80 by ultra-high performance supercritical fluid chromatography combined with quadrupole time-of-flight mass spectrometry

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ABSTRACT

Polysorbate 80, as a nonionic surfactant, is widely used in the food, personal care, and pharmaceutical industries due to the advantages of high surface activity, low toxicity, etc. In fact, the products of polysorbate 80 are complex mixtures of oligomers. In this work, a novel and fast method was developed to characterize the commercial polysorbate 80 by ultra-high performance supercritical fluid chromatography (UHPSFC) combined with quadrupole time-of-flight mass spectrometry (QTOF-MS). Some crucial parameters, such as temperature, back pressure and flow rate were optimized. UHPSFC could distinguish n-mer from (n – 1)-mer and (n + 1)-mer in the same series, which provided the high separation resolution needed for quantitative determination of each oligomer in same series. It was not achieved in previous studies. Furthermore, the characteristic ion fragments were found in MS/MS experiment and used to identify different series. The results revealed that main components of this nonionic surfactant comprise polyethylene oxide (PEO), PEO-monooleate, PEO-isosorbide, PEO-isosorbide monooleate, PEO-isosorbide dioleate, PEO-sorbitan, PEO-sorbitan monooleate, PEO-sorbitan dioleate and PEO-sorbitan trioleate, etc. The separation was performed using BEH stationary phase, so the relationship between molecular structure of these oligomers and chromatographic retention behavior in supercritical fluid chromatography were also investigated for first time. The whole analytical process only takes 8 min for one sample. Therefore, UHPSFC-QTOF-MS is a simple, novel and efficient tool to analyze polysorbate 80.

1. Introduction

Polysorbate 80 as a nonionic surfactant, is made from sorbitol, oleic acid, epoxy ethane, which is widely used as emulsifiers, solubilizers, and stabilizers in food [1], cosmetics [2], drugs [3] and biodegradation media [4] due to their high surface activity and low toxicity [3]. Commercial polysorbate 80 is also named Tween 80, the components of which are more complicated than its chemical name. Fig. 1 showed the synthesis route of the production of Tween 80. The products of these reactions are complex mixtures of PEO, PEO oleate(s), and PEO-sorbitan/isosorbide oleate(s) with varying numbers of EOs, varying degrees of esterification (none, mono-, di-, etc.) [5]. It was reported that the byproducts of polysorbate 80 may cause allergic reactions or other potential hazards [6]. So, it required that the components of polysorbate 80 should keep consistent when it was used in the pharmaceutical industry. So, it was necessary to develop an effective method to characterize the ingredients of polysorbate 80.

Recently, several methods had been used to analyze polysorbate 80. Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) as a convenient and powerful technique was used to make a quick analysis of polysorbate 80 [7]. However, it was difficult to identify some oligomers which have approximate mass weight only by MALDI-TOF-MS. Because the weight of oleate group (264 Da) is approximately equal to the weight of six EO units. For example, the ion at m/z 1327 may belongs to ammoniated PEO_{26}-sorbitan (calcd. mass 1326.7844 Da), PEO_{20}-sorbitan monooleate (calcd. mass 1326.8725 Da), PEO_{14}-sorbitan dioleate (calcd. mass 1326.9605) or PEO_8-sorbitan trioleate (calcd. mass 1327.0485 Da). The liquid chromatography (LC) tandem mass

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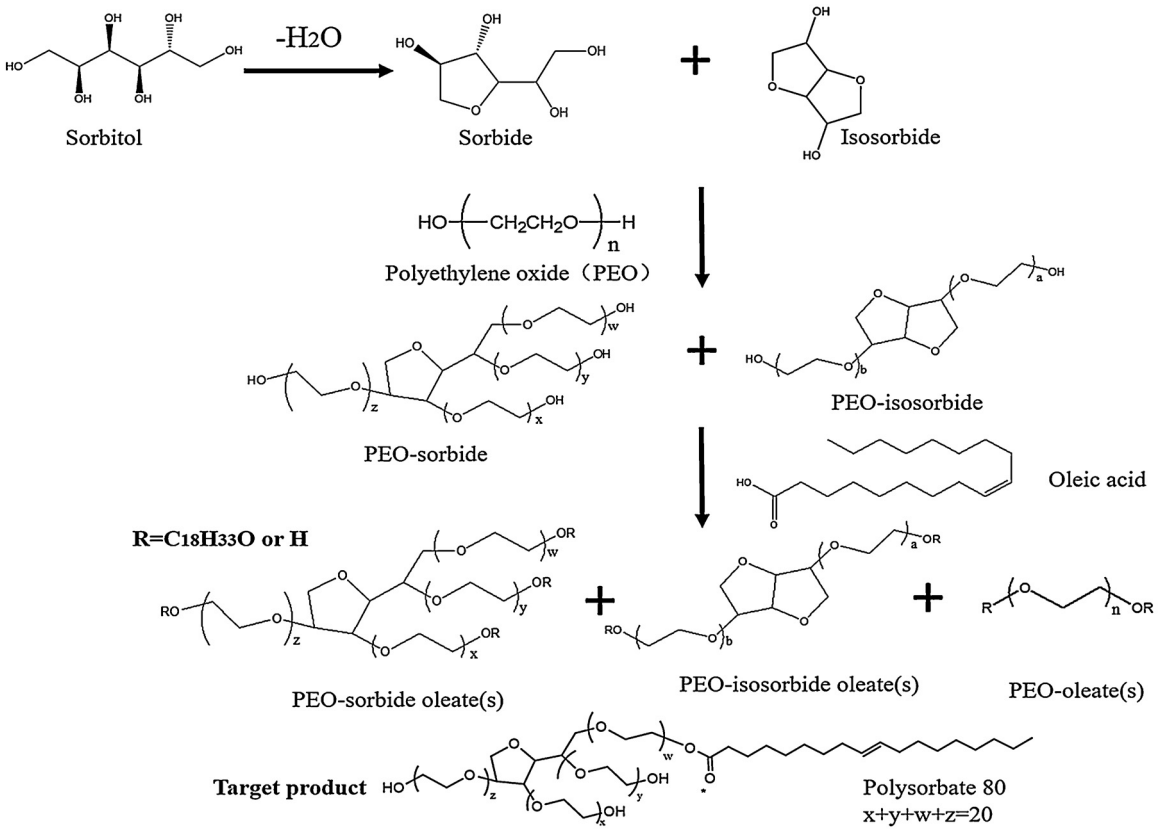

Fig. 1. Schematic of polysorbate 80 synthesis.

spectrometry (MS) is a common tool for the characterization of polysorbates [8–10]. Abrar developed a 2D-chromatographic method for the separation of polysorbates [11]. Hvattum studied the oxidation products of thermally oxidized polysorbate 80 by HPLC–MS [12]. Through hydrolysis, Zhang quantified the oleic acid and different types of polyethers by HPLC [13]. The HPLC–MS method were used to analyze the polysorbate, which only gave the ambiguous shape of molecular weight distributions of polymers and cost a lot of time. In addition, the ion mobility mass spectrometry (IM-MS) was also utilized by Snelling to categorize different ingredients of polysorbate formulations [14], and the features of LC–MS and IM-MS were compared in characterization of polysorbate 85 by Erdem [15].

Compared to HPLC, UHPSFC has advantages of low-cost, green, faster separations and better resolution [16]. Several researches [17–19] revealed that SFC is an eminent technique of measuring molecular weight distributions of polymers, which can separate oligomers from same series with different extent of polymerization index (n). It can provide the exact molecular weight distribution which may help to optimize production process and to interpret the measured results of physicochemical properties. The surfactant's physicochemical behavior of polysorbate 80, such as the HLB value (hydrophilic lipophilic balance) [20], is an important parameter of surfactants, which is used to classify the function of surfactants.

In this study, we developed a novel and quick method to analyze polysorbate 80 by the UHPSFC-QTOF-MS with simple conditions. The experiment results showed that the method was applied successfully in the characterization of polysorbate 80 without pretreatment. The retention behavior of ingredient of polysorbate 80 in the supercritical fluid chromatography were studied, which also validated the analysis result on the other hand.

2. Experimental

2.1. Chemicals

Three polysorbate 80 samples: Sample 1 was obtained from the Tianjin chemical Factory (Tianjin, CHN), while sample 2 and sample 3 were obtained from the Nanjing Well (Nanjing, CHN), which were the same in brand but different in batch. The samples were dissolved in methanol and diluted to 100 μg/mL.

Methanol was purchased from Fisher Scientific (Pittsburgh, PA, USA). Ammonium formate was purchased from Sigma–Aldrich (St. Louis, MO, USA)

2.2. Mass spectra and chromatographic conditions

UHPSFC-QTOF-MS analysis was performed by using an ACQUITY UPC^2 system (Waters, Milford, USA) with a Xevo G2-S QTOF mass spectrometer (Waters, Milford, USA). The UHPSFC system consists of binary solvent manager, sample manager, column manager and convergence manager. The mass spectrometer is equipped with the ESI source. The separation was carried out on the Acquity UPC^2 BEH (Ethylene Bridged Hybrid) column (100 mm × 3 mm, 1.7 μm) at 50 °C, the mobile phase were CO_2 and modifier of methanol at flow rate of 1.6 mL/min, the modifier gradient was: 2%–30% in 5 min, 30%–40% in 1 min, held at 40% for 1 min, 40%–2% in 0.5 min, held at 2% for 0.5 min. The back pressure was set up at 1600 psi. The sample volume injected was 1 μL. Additional make-up solvent was brought by a Waters 515 HPLC Pump. Methanol with 10 mM ammonium formate was selected as the make-up solvent which helped to ionize and delivered at 0.3 mL/min. The parameters used for the mass spectrometer with ESI$^+$ mode were as follows: capillary voltage of 3 kV, cone voltage of 25 V, collision energy of 15–25 V, source