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Quantitative analysis of flavanones from citrus fruits by using mesoporous molecular sieve-based miniaturized solid phase extraction coupled to ultrahigh-performance liquid chromatography and quadrupole time-of-flight mass spectrometry

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ABSTRACT

An analytical procedure based on miniaturized solid phase extraction (SPE) and ultrahigh-performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry was developed and validated for determination of six flavanones in Citrus fruits. The mesoporous molecular sieve SBA-15 as a solid sorbent was characterised by Fourier transform-infrared spectroscopy and scanning electron microscopy. Additionally, compared with reported extraction techniques, the mesoporous SBA-15 based SPE method possessed the advantages of shorter analysis time and higher sensitivity. Furthermore, considering the different nature of the tested compounds, all of the parameters, including the SBA-15 amount, solution pH, elution solvent, and the sorbent type, were investigated in detail. Under the optimum condition, the instrumental detection and quantitation limits calculated were less than 4.26 and 14.29 ng mL^{-1}, respectively. The recoveries obtained for all the analytes were ranging from 89.22% to 103.46%. The experimental results suggested that SBA-15 was a promising material for the purification and enrichment of target flavanones from complex citrus fruit samples.

1. Introduction

To date, solid phase extraction (SPE) has been developed as an acceptable alternative to liquid–liquid extraction (LLE) [1]. In SPE, the sample is passed through a packed column or a cartridge filled with a solid phase sorbent where the solutes are absorbed and then eluted with an organic solvent [2]. This process presents several advantages: particularly it can help decrease the use of toxic solvents, which have been regulated as priority pollutants; it consumes less time than LLE and offers the possibility of automation [3,4]. In addition, SPE with high enrichment factor and good recovery could preserve chemical species and avoid modifications in their distribution [3]. However, traditional SPE method requires large volumes of elution solvent and high consumption of sorbents, making the whole procedure expensive and time consuming [5]. Therefore, to establish a reliable miniaturized SPE approach for the extraction of tested compounds from complex samples is quite meaningful.

In recent years, mesoporous molecular sieves have attracted great attention in the application of separation [6], catalysis [7], and sensors [8] due to their highly ordered structures, large surface area, and tunable pore size [9]. Among the various classes of molecular sieves, SBA-15 (SBA = Santa Barbara Amorphous) is an interesting material which was synthesized and reported by Zhao et al. in 1998 [10]. It is well known that mesoporous SBA-15 possess not only hexagonal arrangement of uniform sized main mesopores, but also the highly ordered arrays of 1D mesoporous channels [11]. Moreover, SBA-15 has potential applications in sorption [12], sensor [13], heterogeneous catalysis [14], and drug delivery [15]. However, SBA-15 material has not been explored extensively as a sorbent for miniaturized SPE. In particular, its applications for the extraction of trace compounds from complex fruits are scarce, barring work from our group.

Some fruits of the *Citrus* family are important in many countries and are available on the market year-round, such as grapefruits, orange and lemon. In general, citrus fruits are rich sources of

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phenolic acids and flavonoids among which flavanones are predominant. Flavanones reportedly demonstrate many biological properties, such as lipidlowering, cardiovascular and anticancer activities [16]. The secondary metabolites exist in the form of flavanones mainly as naringenin, naringin, hesperidin and neohesperidin derivatives, and they possess anti-inflammatory, antioxidant, antimutagenic, antiatherogenic, anti-carcinogenic therapeutical activities [17,18]. Therefore, extraction and quantitative analysis of flavanone compounds in citrus fruits are important for physiological and pharmacological studies. Numerous separation techniques have been developed for the determination of flavanones in citrus samples, *i.e.* liquid chromatography–mass spectrometry (LC–MS) [19], high-performance liquid chromatography (HPLC) [20], and capillary electrophoresis (CE) [21]. Recently, modern ultrahigh-performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (UHPLC-Q-TOF/MS) with the advantages of accurate mass measurement and abundant fragmentation ion information has received increasing attention. However, no information is available in literature about the application of UHPLC-Q-TOF/MS in miniaturized SPE for analysis of target solutes from citrus fruits.

In the present work, a simple, rapid extraction process followed by miniaturized SPE for six flavanones, including narirutin, naringenin, hesperidin, neohesperidin, naringenin and hesperetin, prior to UHPLC-Q-TOF/MS analysis was proposed. Mesoporous molecular sieve as a powerful solid sorbent was used to quality evaluations of different citrus fruit juices. Moreover, various variables, such as sorbent type, amount of sorbent and diluent pH, were optimized in detail. Finally, the proposed method was used for determination of flavanones in several citrus fruits.

2. Experimental

2.1. Chemical reagents and materials

Triblock copolymer P123, tetraethyl orthosilicate (TEOS), chromatographic-grade methanol, hexane, acetonitrile and formic acid were supplied by Sigma–Aldrich (St. Louis, MO, USA). Poly (alkylene oxide)-based triblock copolymer Pluronic P123 ($EO_{20}PO_{70}EO_{20}$, where EO = ethyleneoxide, PO = propyleneoxide, BASF), anhydrous Na_2SO_4, and mesoporous carbon CMK-3 were purchased from Sigma–Aldrich Shanghai Trading Co., Ltd. (Shanghai, China). Analytically pure ethanol, sodium hydroxide and acetone were obtained from Hangzhou Chemical Reagent Co., Ltd. (Hangzhou, China). Purified water (Wahaha Group Ltd., Hangzhou, China) was employed throughout the experiment. The tested standards including narirutin, naringenin, hesperidin, neohesperidin, naringenin and hesperetin were collected from Shanghai Winherb Medical Technology Co., Ltd. (Shanghai, China). The purity of each standard was higher than 98%.

2.2. Apparatus

The sample morphologies were recorded using scanning electron microscopy (SEM) (Hitachi HT7700, Tokyo, Japan). Fourier transform infrared (FT-IR) spectra of potassium bromide (KBr) powder-pressed pellets were determined on a Thermo scientific Nicolet iS5 spectrometer (Madison, USA).

Agilent 1290 series UHPLC system was applied in this experiment. The chromatographic process was performed on an Agilent SB-C_{18} (1.8 μm, 50 mm × 4.6 mm i.d.) at a column temperature of 40 °C. The mobile phase consisted of solvent A (1% of formic acid water) and solvent B (methanol) with a flow rate of 0.4 mL min^{-1}. The gradient elution carried out was: 0–2 min, 45–45% B; 2–3 min, 45–55% B; 3–5 min, 55–75% B; 5–6 min, 75–100% B. The injection volume was set at 2 μL.

A 6530 tandem quadrupole-time-of-flight (Q-TOF) mass spectrometry (Agilent Technologies Inc., CA, USA) equipped with a dual electrospray ionization (Dual ESI) source was used to quantify six flavanones. The analyses were carried out with a capillary voltage of 3500 V, a drying gas with the temperature of 350 °C and flow rate of 12 L min^{-1}. The nebulizer gas was nitrogen (N_2) at 45 psig pressure. The skimmer voltage, fragment voltage, and octopole RF Vpp were set at 65 V, 175 V, and 750 V, respectively. The MassHunter Acquisition and Qualitative Analysis B.05.00 (Agilent Technologies Inc., CA, USA) software was employed to record and analyze the data obtained, and the mass range was in the range m/z 100–700.

2.3. Synthesis of mesoporous materials

The material was synthesized according to a previously reported article [10] with a minor modification. Mesoporous molecular sieve SBA-15 was prepared using the triblock copolymer P123 ($EO_{20}PO_{70}EO_{20}$) as a structure-directing agent. In the synthesis procedure, 2 g of P123 was dispersed into 60 mL (2 M) hydrochloric acid. Next, 4.5 g of TEOS was gradually added at 35 °C. To prepare this material, the aqueous solution of P123 and TEOS was activated hydrothermally at 100 °C for 48 h after stirring at 40 °C for 20 h. The final product was filtered off, and dried at 80 °C for 10 h.

To prepare the KIT-6 mesoporous silica sorbent, 6 g of P123 was dispersed into 136.4 g distilled water and 17 g of 35 wt% HCl in a flask of total volume 100 mL, and then 6.8 g of n-butanol was added with continuous stirring. Then, about 89.4 g of sodium silicate solution composed of 1.6 wt% Na_2O, 5.0 wt% SiO_2, and 93.4 wt% H_2O was added dropwise into the P123-butanol-HCl solution. The mixture was reacted under magnetic stirring at 35 °C for 24 h. Subsequently, the resulting reaction mixture was aged at 100 °C for 24 h under static conditions. The white precipitated product was hot-filtered and dried at 100 °C for 24 h in air. Finally, the as-synthesized sample was washed with a small volume of ethanol–HCl mixture and calcined in air at 550 °C for 2 h before use [22]. Permanent confined micell arrays-60 (PCMA-60) was synthesized according to our previous reported procedure [23].

2.4. Extraction and SPE methodology

The citrus fruits (orange, citrus reticulata blanco, sand sugar oranges, lemon, grapefruit, and nectarine) were purchased from the local supermarket (Hangzhou, China). To prepare fruit samples, the edible parts of samples were cut into 1-cm cubes and blended using a food mixer. Then the extract was filtered, and 50 μL of each fruit supernatant was diluted to 5 mL by distilled water, without adjusting pH. The resulting solution was subjected to the following SPE procedure.

Before extraction, 25 mg of SBA-15 was accurately weighed and transferred into an empty 1-mL cartridge by weighing paper. The upper and lower sieve plates remained at each end of the column to hold the packing in place and then the solid was compacted by vacuum pump. Before loading sample solutions, the cartridge was preconditioned with 1 mL of methanol and 1 mL of water. Next, 5 mL of the model sample (10 μL of standard solution containing each analyte at a concentration of 500 μg mL^{-1} (1 μg mL^{-1} in aqueous mixture), 20 μL of fresh orange juice and 4.97 mL of water, pH 7) or 5 mL of each citrus fruit (previously diluted with water) was passed through the columns. The extraction process was controlled by keeping the flow rate at 1 mL min^{-1}, and the solid phase in the SPE cartridge was not allowed to dry during the preconditioning and sample loading steps. When the sample was completely passed, the cartridge was washed with 1 mL of water to remove interfering compounds. Subsequently, 1 mL of methanol