Journal of Chromatography B, 969 (2014) 205–212

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination of phenolic acids and flavonoids in raw propolis by silica-supported ionic liquid-based matrix solid phase dispersion extraction high performance liquid chromatography-diode array detection

Zhibing Wang[a,b], Rui Sun[a,c], Yuanpeng Wang[b], Na Li[b], Lei Lei[b], Xiao Yang[b], Aimin Yu[b], Fangping Qiu[a,*], Hanqi Zhang[b]

[a] College of Chemistry and Life Science, Changchun University of Technology, Changchun 130012, China
[b] College of Chemistry, Jilin University, Changchun 130012, China
[c] School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, China

ARTICLE INFO

Article history:
Received 19 June 2014
Accepted 17 August 2014
Available online 27 August 2014

Keywords:
Silica-supported ionic liquid-based matrix solid phase dispersion (S-SIL-based MSPD)
Phenolic acids and flavonoids
Raw propolis
High-performance liquid chromatography (HPLC)

ABSTRACT

The silica-supported ionic liquid (S-SIL) was prepared by impregnation and used as the dispersion adsorbent of matrix solid phase dispersion (MSPD) for the simultaneous extraction of eight phenolic acids and flavonoids, including caffeic acid, ferulic acid, morin, luteolin, quercetin, apigenin, chrysin, and kaempferide in raw propolis. High performance liquid chromatography with a Zorbax SB-C18 column (150 mm × 4.6 mm, 3.5 μm) was used for separation of the analytes. The mobile phase consisted of 0.2% phosphoric acid aqueous solution and acetonitrile and the flow rate of the mobile phase was 0.5 mL/min. The experimental conditions for silica-supported ionic liquid-based matrix solid phase dispersion (S-SIL-based MSPD) were optimized. S-SIL containing 10% [C_6MIM]Cl was used as dispersant, 20 mL of *n*-hexane as washing solvent and 15 mL of methanol as elution solvent. The ratio of S-SIL to sample was selected to be 4:1. The standard curves showed good linear relationship ($r > 0.9995$). The limits of detection and quantification were in the range of 5.8–22.2 ng mL^{-1} and 19.2–74.0 ng mL^{-1}, respectively. The relative standard deviations (RSDs) of intra-day and inter-day determination were lower than 8.80% and 11.19%, respectively. The recoveries were between 65.51% and 92.32% with RSDs lower than 8.95%. Compared with ultrasound-assisted extraction (UAE) and soxhlet extraction, the present method consumed less sample, organic solvent, and extraction time, although the extraction yields obtained by S-SIL-based MSPD are slightly lower than those obtained by UAE.

1. Introduction

Propolis is a resinous material collected by bees mainly from buds and exudates of various plants [1–8]. The resulting material is used by bees to seal cracks and holes of the hives and protect the hives against invaders and contamination [1–5,9]. It is reported that propolis is characterized by a series of biological and pharmacological properties, such as antibacterial, antiviral, antifungal, anti-inflammatory, antioxidantive, antitumoral, antiulcer, hepatoprotective, cardioprotective, and immunostimulating activities [1–16]. Due to its well-known characteristics, propolis is considered as a functional ingredient and widely used in food, beverages, cosmetics, and medicine to improve health and prevent diseases [8–10,12,13,16]. The composition of propolis varies with regional vegetation, season, climate, and honeybee races at the site of collection [8,12,15,16]. In the past few years, at least 150 constituents in propolis have been identified, including polyphenols (flavonoids, phenolic acids, and their esters), lignans, quinones, terpenoids, aromatic aldehydes, fatty acids, steroids, and amino acids [2–5,13,15]. However, among these constituents of propolis, phenolic acids [5,6,8,12,13] and flavonoids [5–8,12,13,17] are thought to be main pharmacologically active constituents and responsible for many biological and pharmacological activities.

In order to determine the chemical compounds in raw propolis, to ensure the reliability of pharmacological and clinical research, and to enhance product quality control, it is necessary to establish an appropriate analytical method for the determination of phenolic acids and flavonoids in raw propolis. Recently, several analytical

* Corresponding author. Tel.: +86 431 85168399; fax: +86 431 85112355.
E-mail address: analchem@jlu.edu.cn (F. Qiu).

http://dx.doi.org/10.1016/j.jchromb.2014.08.022
1570-0232/

methods were reported for the determination of phenolic acids and flavonoids in raw propolis, such as capillary zone electrophoresis (CZE) [8,12], micellar electrokinetic chromatography (MEKC) [14], gas chromatography–mass spectrometry (GC–MS) [15], cyclic voltammetry [18], and high performance liquid chromatography (HPLC) coupled with ultraviolet detection (UV) [4,9,20], diode array detection (DAD) [3,7,11], and mass spectrometric (MS) detection [6,11].

Owing to the complexity of sample matrices, sample pretreatment becomes the crucial step in analytical method. So far, the most widely used sample pretreatment methods, including ultrasound assisted extraction (UAE) [11,12,14,17], microwave assisted extraction (MAE) [11], solid-phase extraction (SPE) [21], solvent extraction (SE) [3,22], reflux extraction (RE) [11], pressurized liquid extraction (PLE) [23], high hydrostatic pressure extraction (HHPE) [13], hot-pressurized fluid extraction (HPFE) [4], and supercritical fluid extraction (SCFE) [24], have been employed in extracting phenolic acids and flavonoids. However, these methods are limited when the concentrations of target compounds are extremely low or there is interference of complex matrix in biological samples. A sequence of separation and purification process is required and the analytical procedures are constantly time-consuming, laborious, complicated, and solvent-depending. Matrix solid-phase dispersion (MSPD), first developed in 1989 by Barker et al. [25], was one of the most promising techniques for the simultaneous disruption, extraction, and purification of solid and semi-solid samples [26,27]. MSPD involves dispersion of the sample matrix with an appropriate dispersant, followed by a preliminary purification and the subsequent washing and elution of the analytes with a relatively small volume of solvent [28,29]. The dispersant is used as not only a blending solid support to disrupt and disperse the sample but also an adsorption separation material. However, due to the lack of special selectivity of the common dispersants (C18, silica gel, diatomite, alumina, and florisil.), MSPD was confronted with difficulty of extracting target analytes from complex samples [26]. Although MSPD can be applied to the extraction of flavonoids in biological samples [19], a large amount of organic solvents and clean-up adsorbent are required and its handling can be time-consuming and tedious. Thus, it is beneficial to extend the type of dispersant.

Ionic liquids (ILs) are semi-organic molten salts at or close to room temperature (below 100 °C, which consist of organic cations and organic or inorganic anions [30,31]. The ILs are recognized as greener alternative than the conventional organic solvents in analytical chemistry [32] for their unique chemico-physical properties [30], such as miscibility with water and organic solvents, good solubility for organic and inorganic compounds, high thermal stability, and environmental friendliness [30,33]. ILs can be immobilized in the micropores on the surface of silica gel because of large surface area, high adsorption activity and good mechanical stability of silica gel. Once immobilized on the surface of silica gel, the ILs would loss its liquid state. But the advantages of ILs would be still remained. Because silica-supported ionic liquid (S-SIL) has many micropores filled with ILs, the S-SIL-based extraction offers a number of important benefits, such as reducing the amount of ILs, retaining properties of ILs, improving mass transfer rate and achieving high recoveries. At present, the S-SIL has been mainly applied as catalyst in organic synthesis [34]. No literature has been reported to apply S-SIL as dispersion adsorbent of MSPD for the extraction of active constituents in medicinal animals and plants.

In this work, S-SIL was prepared and applied as the dispersion adsorbent of MSPD for the simultaneous extraction of eight phenolic acids and flavonoids in raw propolis. The effects of several parameters were investigated to find the optimal MSPD conditions. For the comparison, the ultrasound assisted extraction (UAE) and soxhlet extraction (SE) were also applied. The SE is a standard method for extracting flavonoids recommended in Chinese Pharmacopoeia [35]. The UAE is widely accepted and applied for a long time.

2. Experimental

2.1. Chemicals and materials

The standards of caffeic acid, ferulic acid, morin, luteolin, quercetin, apigenin, chrysin, and kaempferide were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The chemical structures of the compounds are shown in Fig. 1. The standard stock solution for each analyte at the concentration level of 500 μg mL^{-1} was prepared by dissolving the analyte in methanol, and stored at 4 °C in the dark. The working solutions were obtained by diluting the stock solutions with methanol.

Chromatographic grade methanol and acetonitrile were purchased from Fisher Corporation (Pittsburgh, PA, USA). Analytical grade methanol, ethanol, acetonitrile, acetone, petroleum ether, ethyl acetate, chloroform, dichloromethane, *n*-hexane, diethyl ether, and phosphoric acid were purchased from Beijing Chemical Factory (Beijing, China). 1-Butyl-3-methylimidazolium chloride ([C_4MIM]Cl), 1-hexyl-3-methylimidazolium chloride ([C_6MIM]Cl), and 1-octyl-3-methylimidazolium chloride ([C_8MIM]Cl) were purchased from Chengjie Chemical Co., Ltd. (Shanghai, China). Pure water was obtained with a Milli-Q water purification system (Millipore Co., USA) and passed through a 0.45 μm nylon filter (Jinteng Instrument Co., Tianjin, China) before use. Silica gel (200–300 mesh), activated carbon (80–110 mesh), diatomite (200–300 mesh), florisil (60–100 mesh), acidic alumina (100–200 mesh), neutral alumina (200–300 mesh), and basic alumina (200–300 mesh) were obtained from Chinese Medical and Biological Products Institute (Beijing, China).

2.2. Apparatus

The 1100 series liquid chromatograph (Agilent Technologies Inc., USA) equipped with photodiode-array detector (DAD) and quaternary gradient pump was used. Chromatographic separation of target analytes was performed on a Zorbax SB-C18 column (150 mm × 4.6 mm I.D., 3.5 μm, Agilent, USA) with a C18 guard column (7.5 mm × 2.1 mm I.D., 5 μm). The scanning electron micrography was obtained with a JSM-5600LV scanning electron microscope (JEOL, Japan). The SBC-12 ion sputtering apparatus (KYKY, China) was used to prepare sample in scanning electron microscopy. The infrared absorption spectrum of S-SIL was obtained with a Nicolet FT-IR 360 spectrometer (Nicolet, USA). The KQ3200E ultrasonic generator was purchased from Kunshan Ultrasonic Instrument Co., Ltd. (Kunshan, China). The frequency and output power of the ultrasonic generator were 40 kHz and 150 W, respectively. RE-52AA vacuum rotatory evaporator (Yarong, Shanghai, China) and SH-36 magnetic stirring apparatus (Zhenghui, Shanghai, China) were employed.

2.3. Preparation of S-SIL

The IL used in this study was 1-hexyl-3-methylimidazolium chloride ([C_6MIM]Cl). According to the previous reports, the IL was immobilized on the surface of silica gel by direct impregnation method [36]. Before use, silica gel was dried at 150 °C for 3 h. To immobilize the [C_6MIM]Cl on the surface of the porous silica gel, the silica gel was immersed into methanol solution containing [C_6MIM]Cl. After stirring at room temperature for 12 h, the