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# Ionic liquid-anionic surfactant based aqueous two-phase extraction for determination of antibiotics in honey by high-performance liquid chromatography



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## ABSTRACT

An ionic liquid-anionic surfactant based aqueous two-phase extraction was developed and applied for the extraction of tetracycline, oxytetracycline and chloramphenicol in honey. The honey sample was mixed with Na<sub>2</sub>EDTA aqueous solution. The sodium dodecyl sulfate, ionic liquid 1-octyl-3-methylimidazolium bromide and sodium chloride were added in the mixture. After the resulting mixture was ultrasonically shaken and centrifuged, the aqueous two phase system was formed and analytes were extracted into the upper phase. The parameters affecting the extraction efficiency, such as the volume of ionic liquid, the category and amount of salts, sample pH value, extraction time and temperature were investigated. The limits of detection of tetracycline, oxytetracycline and chloramphenicol were 5.8, 8.2 and 4.2 μg kg<sup>-1</sup>, respectively. When the present method was applied to the analysis of real honey samples, the recoveries of analytes ranged from 85.5 to 110.9% and relative standard deviations were lower than 6.9%.

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## 1. Introduction

Honey is a kind of directly consumed food. It is a conglomeration of several organic and inorganic constituents, with glucose and fructose contributing about 75% of the total. Other organic constituents include di/tri/oligo saccharides, aliphatic acids, vitamins, amino acids and proteins and inorganic constituents, including water, potassium, sodium, calcium, magnesium, copper, manganese, iron, chloride, sulfur, phosphorus and silica [1]. Antibiotics such as tetracycline (TC), oxytetracycline (OTC) and chloramphenicol (CAP) are used in beekeeping to fight against bacterial diseases, among which the worst ones are the American Foulbrood (AFB) and the European Foulbrood (EFB) which destroy honeybee larvae [2]. Consequently, such antibiotics may persist at trace levels in honey, being a potential risk to honey consumers in terms of possible allergic reactions, liver damage, yellowing of teeth, gastro-intestinal disturbance, aplastic anemia and the development of bacterial resistance [3]. Therefore, reliable

analytical techniques are increasingly demanded to detect antibiotic residues in honey.

Some countries have established maximum residue limits (MRLs) for tetracycline antibiotics in honey, while others do not tolerate any residue level. For instance, Codex Alimentarius and the European Union (EU) have not established MRL for veterinary medicine in honey [4,5]. In China, the MRL for the total tetracycline antibiotics in honey should not exceed 50 μg kg<sup>-1</sup> [6]. The Spanish plan for residue control and healthy food (Plan CRE HA) has established the MRL of 100 μg kg<sup>-1</sup> for TC, OTC and chlortetracycline (CTC) in honey [7]. In Brazil the National Program for Honey Residues Control established by the Ministry of Agriculture has provided that the MRL for TC, OTC and CTC should not exceed 200 μg kg<sup>-1</sup> [8]. China, the European Commission, the United States and some other countries have strictly banned the use of CAP in food-producing animals.

TC, OTC and CAP were successfully determined by high-performance liquid chromatography (HPLC) in the reversed-phase mode, with different detection modes, such as ultraviolet [9,10,11], fluorescence [12], chemiluminescence [13] and mass spectrometry [14,15]. Furthermore, extraction and preconcentration procedures are very important before instrumental analysis. The liquid-liquid extraction (LLE) [16] and solid-phase extraction

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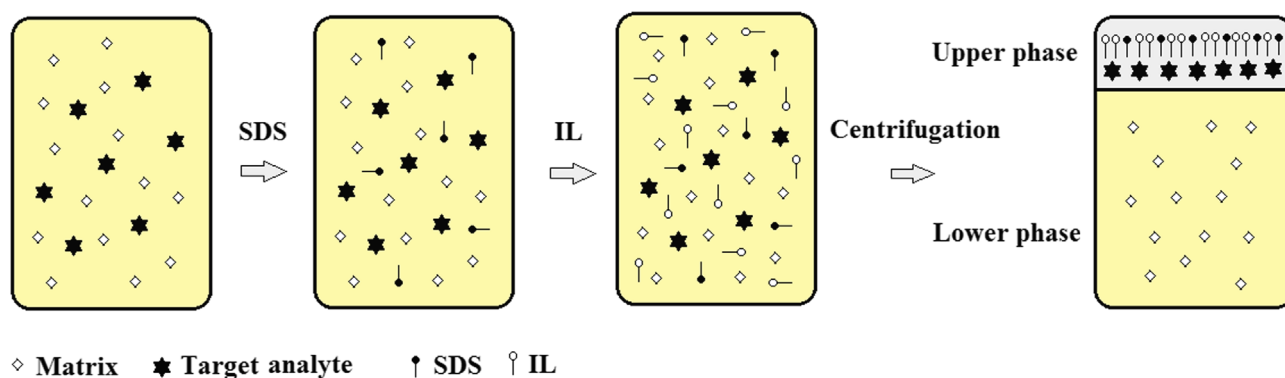


Fig. 1. Aqueous two-phase extraction.

(SPE) [17,18] have been extensively applied to the pretreatment of liquid food samples. However, most of these methods are time consuming and usually toxic due to the use of organic solvents.

When cationic and anionic surfactants in aqueous solution are mixed at an appropriate molar ratio, two immiscible aqueous phases with a clear interfacial boundary can be formed [19]. Aqueous two-phase (ATP) was first discovered by Karler, et al. in 1989 [20]. They reported the spontaneous formation of vesicles by mixing a cationic surfactant cetyltrimethylammonium tosylate (CTAT) and an anionic surfactant sodium dodecylbenzene sulfonate (SDBS) in aqueous solution. The hydrophobic and electrostatic interactions are regarded as the main driving forces for the spontaneous formation of vesicles [21]. Aqueous mixtures of anionic and cationic surfactants exhibit rich microstructured phase behavior and many unique properties that arise from stronger electrostatic interactions than single pure surfactant does and are often more surface active than either pure surfactant [22]. There are a wide variety of microstructures, such as spherical and rodlike micelles, vesicles, lamellar phase and flat discs, etc [23]. The formation of ATP can be attributed to the coexistence of different sized micelles in the upper and lower phase [24].

In the study, the ATP was formed in the presence of anionic surfactant sodium dodecyl sulfate (SDS) and ionic liquid (IL) 1-octyl-3-methylimidazolium bromide (O[MIM]Br) which is surface active according to a hydrophobic tail and a hydrophilic head-group. When the molar ratio of the two surfactants changes, two kinds of ATPs are observed. One is SDS-rich ATP which means that the concentration of SDS is higher than that of IL and the other one is IL-rich which means that the concentration of IL is higher than that of SDS. When the ATP is formed, the upper phase is surfactant-rich which forms lamellar micelles and the lower phase is surfactant-dilute with spherical micelles. Analytes are extracted into the surfactant-rich phase according to the different distribution coefficients of them in the two phases, as can be seen in Fig. 1. The ionic liquid-anionic surfactants based aqueous two-phase extraction (IL/AS-based ATPE) is an easy and rapid sample pretreatment method. The present method is considered to be environmentally friendly because of the avoidance of traditional volatile solvents. Compared with traditional IL/salt ATPE, the amount of ionic liquid and salts used to form ATP is smaller and the phase separation time is shorter.

## 2. Experimental

### 2.1. Instrumentations

The 1100 series liquid chromatography (Agilent Technologies, Palo Alto, USA) equipped with UV detector and quaternary gradient pump was used. Zorbax Eclipse XDB-C18 column (4.6 mm × 150 mm,

3.5 μm) and a C18 guard column (7.5 mm × 2.1 mm, 5 μm) were used. Ultrasonic mixing was performed with a 100 W ultrasonic cleaner (model KQ-100DE, Kunshan Ultrasonic Instrument, Kunshan, China). The phase separation was performed on HC-2066 high-speed centrifuge (Anhui USTC Zonkia Scientific Instruments, Hefei, China).

### 2.2. Reagents and chemicals

TC, OTC and CAP were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The chemical structures of the compounds are shown in Fig. 2. O[MIM]Br was purchased from Shanghai Chengjie Chemical Reagent Co. Ltd. SDS was purchased from Sinopharm Chemical Reagent (Shanghai, China). Chromatographic grade methanol was purchased from Fisher Scientific Company (Loughborough, UK) and pure water was obtained with a Milli-Q water purification system (Millipore, Billerica, USA). All other chemicals used, such as formic acid, hydrochloric acid and Na<sub>2</sub>EDTA•2H<sub>2</sub>O, were of analytical grade.

### 2.3. Standard solutions

The individual stock solution of each analyte (1000 μg mL<sup>-1</sup>) was prepared by dissolving 10 mg of the analyte in 10 mL of methanol and stored in the refrigerator at 4 °C. The mixed stock solution containing all analytes (100 μg mL<sup>-1</sup>) was prepared with individual stock solutions by diluting with methanol and stored under dark condition at 4 °C. The mixed working standard solution was prepared by diluting the mixed stock solution with methanol.

### 2.4. HPLC-UV conditions

The mobile phase consisted of acetonitrile (A) and 0.8% formic acid aqueous solution (B). The gradient elution was carried out, starting from 10% to 24% A in 8 min, held for 2 min, then to 40% A in 3 min and held for 5 min. The flow rate of the mobile phase was 0.5 mL min<sup>-1</sup>. The column temperature of 25 °C was maintained. The injection volume of analytical solution was 20 μL. The detection wavelength was 270 nm for TC and OTC and 275 nm for CAP.

### 2.5. Samples

Honey samples, including the jujube blossom honey (Sample 1), lime tree honey (Sample 2 and 3), motherwort honey (Sample 4) and snow honey (Sample 5 and 6), were purchased from a local market. Except for the experiments mentioned in Section 3.3, which were performed with Samples 1–5, all other experiments were performed with Sample 6.

The freshly-spiked samples containing TC, OTC and CAP were prepared by spiking the mixed working standard solutions into

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