Talanta 161 (2016) 748–754

Contents lists available at ScienceDirect

Talanta

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

Salting-out homogenous extraction followed by ionic liquid/ionic liquid liquid–liquid micro-extraction for determination of sulfonamides in blood by high performance liquid chromatography☆

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ARTICLE INFO

Keywords:
Salting-out homogenous extraction system
Ionic liquid/ionic liquid dispersive liquid-liquid micro-extraction system
Blood
Sulfonamides
High-performance liquid chromatography

ABSTRACT

Salting-out homogenous extraction followed by ionic liquid/ionic liquid dispersive liquid-liquid micro-extraction system was developed and applied to the extraction of sulfonamides in blood. High-performance liquid chromatography was applied to the determination of the analytes. The blood sample was centrifuged to obtain the serum. After the proteins in the serum were removed in the presence of acetonitrile, ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate, dipotassium hydrogen phosphate, ionic liquid 1-Hexyl-3-methylimidazolium hexafluorophosphate were added into the resulting solution. After the resulting mixture was ultrasonically shaken and centrifuged, the precipitate was separated. The acetonitrile was added in the precipitate and the analytes were extracted into the acetonitrile phase. The parameters affecting the extraction efficiency, such as volume of ionic liquid, amount of dipotassium hydrogen phosphate, volume of dispersant, extraction time and temperature were investigated. The limits of detection of sulfamethizole (STZ), sulfachlorpyridazine (SCP), sulfamethoxazole (SMX) and Sulfisoxazole (SSZ) were 4.78, 3.99, 5.21 and 3.77 μg L^{-1}, respectively. When the present method was applied to the analysis of real blood samples, the recoveries of analytes ranged from 90.0% to 113.0% and relative standard deviations were lower than 7.2%.

1. Introduction

Sulfonamides (SAs) are the first class of antimicrobial agents. They possess chemotherapeutic activity against infections caused by gram-positive and gram-negative bacteria as well as some protozoa [1]. For human medical purposes, they are often used to against digestive system inflammation, respiratory system inflammation, affections of the skin. However, long-term use of SAs can result in serious side effects, such as emiction and hemopoiesis turbulence. SAs are administered orally through pharmaceutical preparations in a concentration range of 250 to 500 mg L^{-1} [2]. A series of experiments have been studied to detect SAs in some tissues such as liver [3], muscle [4], blood [5], and urine [6]. In general, levels ranging between 50 and 150 mg/mL are therapeutically effective for most infections and levels between 120 and 150 mg/mL are optimal for serious infections.

Recently, a number of analytical methods have been developed for the analysis and determination of sulfonamide residues in different matrices, such as enzyme-linked immunosorbent assay (ELISA) [7], and microfluidic chip electrophoresis [8]. High performance liquid chromatography (HPLC) has been wildly used when coupled with different detectors. The ultraviolet (UV) [9] and mass spectrometric (MS) [10,12] detectors were widely used because of the high sensitivity, selectivity and the unambiguous identification capability offered by these detectors. Other detectors such as fluorescence [11], photodiode array [12], and diode array detector were also used [13]. An ideal sample preparation technique should able to isolate and enrich the analytes from sample matrices. Liquid–liquid extraction (LLE) [13] and solid phase extraction (SPE) [14] are the most commonly employed. Recently, some microextraction methods were developed, such as aqueous two-phase extraction [15], hollow fiber LPME [16], liquid phase microextraction (LPME) [17], dispersive liquid–liquid microextraction (DLLME) [18], ionic liquid/ionic liquid dispersive liquid-liquid microextraction [19], salting-out liquid–liquid extraction (SALLE) [20] and QuEChERS [21].

☆ This work was supported by Norman Bethune Program of Jilin University [2015435].
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http://dx.doi.org/10.1016/j.talanta.2016.09.006
Received 8 June 2016; Received in revised form 28 August 2016; Accepted 3 September 2016
Available online 04 September 2016
0039-9140/

In the aqueous two-phase systems (ATPs), two immiscible aqueous phases can be formed, such as $[C_4MIM]BF_4$ and trisodium citrate dehydrate [22]. However, salt-out would appear when some kinds of salts added in aqueous solution containing $[C_4MIM]BF_4$. This system first reported by Gutowski, they using 1-butyl-3-methylimidazolium chloride ($[C_4MIM]Cl$) and K_3PO_4 as the salting-out inducing inorganic salt [23]. When an inorganic salt is added to an aqueous solution containing ionic liquid, the two solutes compete for the solvent molecules. The competition is won by the inorganic ions and those of the ionic liquid lose. There is a migration of the analytes in the aqueous away from the ions of the ionic liquid towards those of the inorganic salt [24]. At the same time, there is some evidence that the salting-out of ionic liquid with inorganic salts from aqueous is driven by an entropic process resulting from the preferential formation of water-ion complexes [25]. The phosphate base inorganic salts were used as salting-out species which are frequently used in the biotechnological field [26].

In this study, salting-out homogenous extraction combined with ionic liquid/ionic liquid dispersive liquid-liquid micro-extraction (IL-ILDLLME) was frist developed and applied to the extraction of four SAs (sulfamethizole, sulfachlorpyridazine, sulfamethoxazole and Sulfisoxazole) in blood. Because the SAs were in clinical practice, the SAs were selected as the analytes in the work [3]. Several experimental conditions were studied and optimized. This method can be successfully applied to the extraction of SA residues.

2. Experimental

2.1. Reagents and chemicals

Sulfamethizole (STZ), sulfachlorpyridazine (SCP), sulfamethoxazole (SMX) and Sulfisoxazole (SSZ) were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). The structures of these SAs are shown in Fig. 1. The purity of all the compounds is higher than 98%. The structures and properties of these SAs are shown in Fig. 1. Standard stock solutions for the SAs at the concentration level of 500 μg mL^{-1} were prepared in acetonitrile containing 5.24 mmol L^{-1} acetic acid. The working solutions were obtained by diluting the stock solutions with pure water. 1-butyl-3-methylimidazolium tetrafluoroborate ($[C_4MIM]BF_4$)and 1-hexyl-3-methylimidazolium hexafluorophosphate ($[C_6MIM]PF_6$) were purchased from Shanghai Chengjie Chemical Reagent Co. Ltd. Formic acid was of analytical-reagent grade and purchased from Beijing Chemical Factory (Beijing, China). Anhydrous di-potassium hydrogen phosphate was purchased from Aladdin Chemistry Co. Ltd (Nanqiao Town, Fengxian District, Shanghai). Chromatographic grade acetonitrile was purchased from Fisher Scientific Company (UK) and pure water was obtained with a Milli-Q water purification system (Millipore Co., USA).

2.2. Instruments

The 1100 series liquid chromatograph (Agilent Technologies Inc., USA) equipped with UV detector and quaternary gradient pump was used. Zorbax Eclipse Plus-C_{18} column (150 mm×4.6 mm, 3.5 μm,162 Agilent, USA) and a C_{18} guard column (7.5 mm×2.1 mm I.D., 5 μm) were used. The KQ-100DE ultrasonic cleaner was purchased from Kunshan Ultrasonic Instrument Co., Ltd. (Kunshan, China). The frequency and output power of the ultrasonic cleaner are 40 kHz and 100 W, respectively. The SH-39 oscillator was purchased from Shanghai Zhenghui Instrument Co., Ltd. (Shanghai, China). The phase separation was performed on high-speed centrifuge (Anhui USTC Zonkia Scientific Instruments Co. Ltd. HC-2066. China).

2.3. Samples

In this study, six kinds of samples were used, The fresh human blood sample (Sample 1) was obtained from local hospital. Rabbit blood sample (Sample 2) was obtained from local animal experiment center. Pig (Sample 3), cow (Sample 4) and chicken (Sample 5) blood samples were obtained from local market respectively, urine (Sample 6) obtained from 12 years old children after fasting 12 h. The samples were stored at −20 °C before use. Except for the experiments mentioned in Section 3.3, which were performed with all six samples, all other results were obtained with Sample 1. The spiked samples containing SAs were prepared by spiking the working solutions into blood samples and stored at 4 °C for one week.

2.4. HPLC-UV conditions

The mobile phase consists of acetonitrile (2.67 mmol L^{-1} formic acid) (A) and aqueous solution (2.67 mmol L^{-1} formic acid) (B). The gradient program is as follows: 0–10 min, 10–30% A; 10–20 min, 30–34% A; 20–25 min, 34–10% A. The flow rate of mobile phase was kept at 0.5 mL min^{-1} and column temperature was kept at 40 °C. The injection volume of analytical solution was 10 μl. The monitoring wavelength was 270 nm.

2.5. Extraction procedure

The extraction procedure is shown in Fig. 2. 4.0 mL of sample was centrifuged and the upper layer of serum was placed into 10 mL centrifuge tube, 2 mL of acetonitrile was added into the serum and the mixture was centrifuged to remove protein impurities. The upper liquid was placed into 10 mL centrifuge tube. 110 μl of $[C_4MIM]BF_4$ and 1.0 g of K_2HPO_4 were added in the solution. The homogeneous precipitation can be observed. 70 μl of $[C_6MIM]PF_6$ were added into the tube. The mixture was ultrasonically shaken for 3 min and centrifuged for 5 min

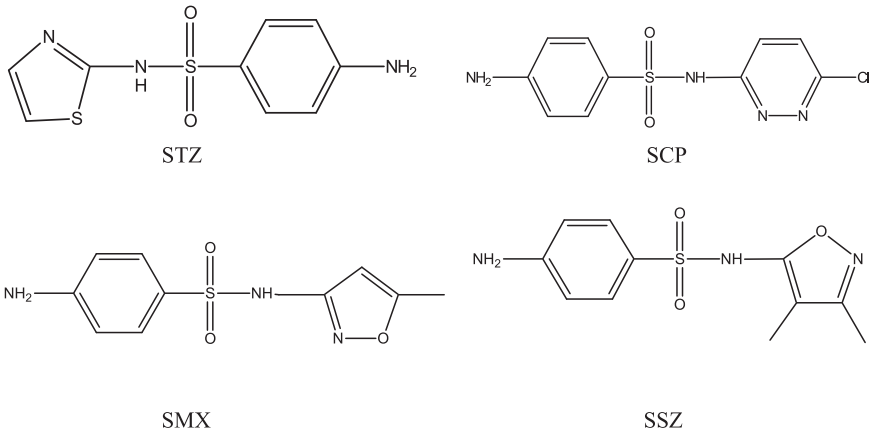

Fig. 1. Chemical structures of SAs.