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Integration of phase separation with ultrasound-assisted salt-induced liquid–liquid microextraction for analyzing the fluoroquinones in human body fluids by liquid chromatography

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ABSTRACT

Herein, we developed a novel integrated device to perform phase separation based on ultrasound-assisted salt-induced liquid–liquid microextraction for determination of five fluoroquinones (FQs) in human body fluids. The integrated device consisted of three simple HDPE components used to separate the extraction solvent from the aqueous phase prior to retrieving the extractant. A series of extraction parameters were optimized using the response surface method based on central composite design. Optimal conditions consisted of 945 μL acetone extraction solvent, pH 2.1, 4.1 min stir time, 5.9 g Na_2SO_4, and 4.0 min centrifugation. Under optimized conditions, the limits of detection (at S/N = 3) were 0.12–0.66 μg L^{-1}, the linear range was 0.5–500 μg L^{-1} and recoveries were 92.6–110.9% for the five FQs extracted from plasma and urine. The proposed method has several advantages, such as easy construction from inexpensive materials, high extraction efficiency, short extraction time, and compatibility with HPLC analysis. Thus, this method shows excellent prospects for sample pretreatment and analysis of FQs in human body fluids.

1. Introduction

The widespread application of fluoroquinolones (FQs) in human and food-producing animals has increased human health concerns because of induced pathogen resistance and possible allergic hypersensitivities in humans [1–6]. Previous research has demonstrated that low amounts of FQs were often found in plasma and their main excretion pathway in urine [5,6]. Because of the possible resistance of human pathogens to antibiotics, there is currently concern about low level exposure to these compounds [7]. Therefore, sensitive and selective analytical methods for the determination of FQs in plasma and urine or other biological fluids are urgently required. To date, many methods have been developed for the determination of FQs in many matrices, such as spectroscopy [8], capillary electrophoresis [9], spectrofluorometry [10–12], potentiometric titration [13] and high performance liquid chromatography (HPLC) [14,15] coupled with mass spectrometry (MS) [16]. Because of interference from complex matrices in biological fluids, these analytical methods often require extensive sample preparation. Accordingly, there is considerable interest in developing a cost-effective, efficient and reliable extraction and quantification method for FQs in complex matrices.

Abbreviations: PS-USLM, phase separation based on ultrasound-assisted, salt-induced, liquid–liquid microextraction; FQs, fluoroquinones; HPLC-FLD, high performance liquid chromatography equipped with fluorescence detector; PPCPs, pharmaceuticals and personal care products; HPLC, high performance liquid chromatography; MS, mass spectrometry; SPE, solid-phase extraction; LLE, liquid–liquid extraction; LPME, liquid-phase microextraction; DLLME, dispersive liquid-liquid microextraction; SALLME, salting-out assisted liquid–liquid microextraction; RSM, response surface method; CCD, central composite design; LOD, limits of detection; ER, extraction recovery; FLE, fleroxacin; OFL, ofloxacin; NOR, norfloxacin; CIP, ciprofloxacin; ENR, enrofloxacin; $MgSO_4$, magnesium sulfate; Na_2SO_4, sodium sulfate; CH_3COONa, sodium acetate; $(NH_4)_2SO_4$, ammonium sulfate; CH_3COONH_4, ammonium acetate; HDPE, high-density polyethylene; LDR, linear dynamic range; RSDs, relative standard deviations; RR, relative recovery; LLE-HPLC, liquid-liquid extraction combined with high performance liquid chromatography; SPE-HPLC, solid phase extraction combined with high performance liquid chromatography; SOMC-HPLC, second-order multivariate calibration combined with high performance liquid chromatography; SSPC-HPLC, single-step precipitation cleanup method combined with high performance liquid chromatography; UFLC, ultra-fast liquid chromatography; HF-LPME-HPLC, hollow fiber-based liquid phase microextraction combined with high performance liquid chromatography.

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Salting-out assisted liquid–liquid microextraction (SALLME) is based on phase separation of water-miscible organic solvents from the aqueous phase at high salt concentration [17]. In the SALLME procedure, the collection and measurement of microliter volumes of the separated organic phase are difficult because the wide diameter glass tube makes the thin layer of extractant difficult to retrieve and requires relatively long extraction times. A few studies have introduced extraction devices or vessels to classical dispersive liquid–liquid microextraction (DLLME) that allow for the use of lower density organic extraction solvents, either by using a narrow-necked glass tube [18], or by using a glass vial [19]. Hashemi et al. [20] introduced a home-made, narrow-necked glass tube for the effective collection of extractant, and inserted it into a centrifuge tube for centrifugation after extraction. Zhang et al. [21] designed a special flask equipped with two narrow open necks with one having a capillary tip to facilitate the DLLME process. However, these glass-based devices are fragile and require special design, therefore their cost is relatively high and their commercial availability is limited [22].

Recently, a cheap, flexible and disposable polyethylene Pasteur pipette was introduced as an extraction device for low-density solvent-based DLLME [23,24]. Wang et al. [19] developed a new device, which consisted of a dropper and a sample vial, to perform extraction, separation and concentration of trace pesticides from solvents. The bulb end of the cut polyethylene dropper was inserted into the neck of the sample vial and the tip end of the polyethylene dropper was cut to an appropriate length [22]. The plastic pipette afforded advantages of low cost, use of easily available materials and ease of operation. However, the major drawback of this device is that the extracted organic phase was difficult to completely retrieve because the organic and aqueous phases were not separated prior to collection of the extractant. Thus, repartitioning of extractant into the aqueous phase can occur over the relatively long retrieval time, which will result in a low extraction recovery.

To overcome the above-mentioned limitations of current methods, this study developed and optimized a novel integrated device and methodology for extracting and isolating FQs for HPLC quantification using phase separation based on ultrasound assisted, salt-induced, liquid–liquid microextraction (PS-USLM). The proposed PS-USLM method was optimized for major operational factors (stirring time, pH, salt type and volume, solvent type and volume, and centrifugation time) using a response surface method (RSM) based on central composite design (CCD). The optimized method was compared with other commonly used LPME methods to evaluate its advantages and feasibility for determining trace levels of FQs in plasma and urine samples. To the best of our knowledge, this integrated device, designed to completely and rapidly separate the organic and aqueous phases prior to collection of the extractant, is the first reported use of this approach for determination of FQs in biological fluids.

2. Experimental

2.1. Reagents and materials

Analytical standards for fleroxacin (FLE), ofloxacin (OFL), norfloxacin (NOR), ciprofloxacin (CIP) and enrofloxacin (ENR) were purchased from J&K Chemical, China and used without further purification. HPLC-grade ethanol, methanol, ethyl acetate, acetonitrile and acetone were sourced from Merck (www.merck.com.cn). Salts (magnesium sulfate ($MgSO_4$), sodium sulfate (Na_2SO4), sodium acetate (CH_3COONa), ammonium sulfate ($(NH_4)_2SO_4$) and ammonium acetate (CH_3COONH_4)) with purities ≥99% were obtained from Aladdin Industrial Co. Ltd.

Stock standard solutions (1000 μg mL^{-1}) for each FQ were prepared by dissolving each compound in methanol and stored at 4 °C. Stock solutions were diluted with methanol to prepare a secondary mixed stock solution of 10 μg mL^{-1}. Mixtures of standard working solutions for extraction at different concentrations were prepared daily by dilution with Milli-Q ultrapure water (Millipore, Bedford, USA).

2.2. Preparation of plasma and urine samples

Drug-free whole blood plasma and urine samples from male and female volunteers were collected from healthy individuals at Wenzhou Medical University, Wenzhou, China. The plasma samples were taken intravenously in the presence of EDTA-2Na as an anticoagulant and were centrifuged at 12,000 rpm for 10 min at 4 °C (the partial impurities in the blood settled at the bottom of the centrifuge tube). Then, the above treated blood samples or the collected urine samples were transferred into individual 50 mL polypropylene centrifuge tubes immediately after filtration using a 0.45 μm membrane filter and stored at −20 °C until analysis or validation of the analytical method. Before use, the samples were thawed at ambient temperature. Ethical approval for this study was obtained from the Ethics Committee at Wenzhou Medical University.

2.3. Instrumentation

FQs were analyzed with an Agilent 1260 HPLC equipped with a fluorescence detector (FLD). A Zorbax Eclipse XDB-C_{18} column (150 mm × 4.6 mm, 5 μm particle size) was used and injections were performed manually using a 20.0-μL sample loop. The operating conditions were as follows: mobile phase, methanol–acetonitrile–water (14:7:79, v/v; water consisting of 3.4 mL orthophosphoric acid and 6.0 mL triethylamine per liter); flow rate, 0.8 mL min^{-1}; column temperature, 40 ± 1 °C; and excitation and emission wavelengths of 290 and 455 nm, respectively. Solutions were heated and ultrasonicated using a model KS-600EI ultrasonic washing unit from Ningbo Kesheng Ultrasonic Equipment Factory, China. Centrifugation used a model TDL-50C centrifuge from Anting Instrument Factory, China and TGL-20M centrifuge from Xiangli Instrument Factory, China.

2.4. PS-USLM procedure

A schematic of the integrated PS-USLM procedure is shown in Fig. 1. This novel integrated device consists of three components: (1) a high-density polyethylene (HDPE) centrifuge tube with 12.0-cm height, 1.6-cm external diameter and 1.4-cm internal diameter (Fig. 1A); (2) an inverted cut HDPE dropper with 1.0-cm height and 1.4-cm external diameter joined to a 3.0-cm length of capillary tube (Fig. 1I); and (3) a "V" HDPE capillary tube with a 10.0-cm total length and 0.5 cm internal diameter (Fig. 1I). The inverted cut disposable HDPE dropper was inserted into the centrifuge tube, and the "V" tube was easily attached/detached from the inverted HDPE dropper (Fig. 1F and G). Advantages of this integrated device are simple design, low cost, practicability, HDPE material is not easily broken, repeatable application and ease of operation.

In operation, the sample solution was first added to the centrifuge tube followed by the extraction solvent, which was water-miscible and lower density than water. After centrifugation, the sedimented proteins and other interfering compounds were discarded (Fig. 1A). Then, an appropriate amount of salt was added to the remaining solution (Fig. 1B). After salting-out, the extraction solvent will float on the top of the sample solution following ultrasound treatment and centrifugation (Fig. 1C and D; extraction solvent, Fig. 1D-1; sample solution, Fig. 1D-2; undissolved salt, Fig. 1D-3)). The inverted HDPE dropper was then placed into