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Ultrasound-assisted dispersive liquid–liquid microextraction for determination of fluoroquinolones in pharmaceutical wastewater

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ABSTRACT

A simple and rapid ultrasound-assisted dispersive liquid–liquid microextraction (UA-DLLME) coupled with liquid chromatography-ultraviolet detection (LC-UV) was developed for the determination of four fluoroquinolones (ofloxacin, norfloxacin, enrofloxacin, and lomefloxacin) in pharmaceutical wastewater samples. Various parameters affecting the extraction efficiency including type and volume of extraction and dispersive solvents, sample pH, and extraction time were investigated. Good linear relationships were obtained for all analytes in a range of 0.01–2.0 μg/ml with LODs ranged from 0.14 to 0.81 μg/l. Average recoveries at three spiking levels were over the range of 82.7–110.9% with RSD less than 5.2% ($n = 3$). Under the optimized conditions the enrichment factors for the four fluoroquinolones were ranged from 32 to 134 folds. The presented method was applied for the determination of four fluoroquinolones in pharmaceutical wastewater samples.

1. Introduction

In the present years, awareness of residual antibiotics in animal-derived food and aquatic ecosystems is growing as their application increases in both human and veterinary medicine. Among them, fluoroquinolones (FQNs) have evolved to be a new potential pollutant in both food and environmental water samples due to their extensive use [1,2]. Their residues may persist in animal body and may result in the development of drug-resistant bacterial strains or allergies. The pharmaceutical wastewater without sufficient treatment is one of the important sources of FQN residues in aquatic ecosystems, which could pass into the human body by food chain or even drinking water. Therefore, a simple, rapid, and sensitive analytical method for determination of FQNs in pharmaceutical wastewater is desired.

Several methods have proposed for the analysis of FQNs in food and aqueous samples including LC [3], LC–MS/MS [4] and CE [5]. Owing to the complexity of sample matrices and the relatively low concentration of FQNs, it is very difficult to directly monitor the residue of FQNs in those samples. Hence, sample pretreatment and enrichment processes are crucial steps in the analytical procedure. Several pretreatment methods including solid-phase extraction (SPE) [3–6], liquid–liquid extraction [7,8], stir bar sorptive extraction (SBSE) [9], microwave-assisted extraction (MAE) [10], diphasic dialysis [11], and supercritical fluid extraction (SFE) [12] have been developed. The main limitations of these methods include time-consuming extraction procedures, low enrichment factors, tedious operation, and the large amounts of poisonous organic solvent.

Recently, Assadi and co-workers [13] developed a new microextraction technique termed dispersive liquid–liquid microextraction (DLLME), which is based on a ternary solvent system like homogeneous liquid–liquid extraction and cloud point extraction. In this method, the appropriate mixture of extraction solvent and dispersive solvent is injected rapidly into an aqueous solution, resulting in a cloudy state consisting of fine droplets of the extraction solvent dispersed in the aqueous phase, which markedly increases the contact surface between phases and reduces the extraction time with the increasing enrichment factors [14–17]. After extraction, the cloudy solution is centrifuged and the enriched analytes in the sediment phase are determined by chromatographic or spectrometric methods. The advantages of DLLME are simplicity, rapidity, low cost, good recovery and high enrichment factors. Until now, DLLME has been successfully applied for the determination of trace organic and inorganic compounds in water samples [18–25].

The aim of the present work is to develop a simple and sensitive UA-DLLME method coupled with LC-UV for the determination of four FQNs in pharmaceutical wastewater samples. Various parameters affecting the extraction efficiency including type and volume of extraction solvent and dispersive solvent, sample pH, and extraction time were investigated.

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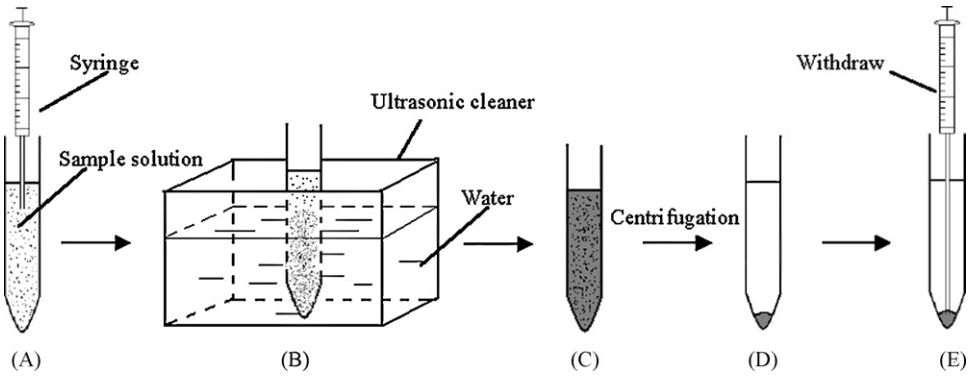

Fig. 1. Schematic procedure of UA-DLLME. (A) Injection of extractant and dispersant into aqueous sample; (B) ultrasound-assisted formation of emulsion; (C) emulsion of ternary mixture; (D) phase separation by centrifugation; (E) collection of high-density extractant.

2. Experimental

2.1. Chemicals and reagents

Ofloxacin (OFL), norfloxacin (NOR), enrofloxacin (ENR), and lomefloxacin (LOM) were obtained from Sigma (St Louis, MO, USA). Dichloromethane, 1,2-dichloroethane, chloroform and tetrachloroethane were purchased from Huaxin Chemical Reagent Co. (Baoding, China). Methanol, acetone, tetrahydrofuran (THF), acetonitrile, acetic acid, ammonia water, trifluoroacetic acid (TFA) and sodium chloride were purchased from Kermel Chemical Co. Ltd. (Tianjin, China). All the other reagents used in the experiment were of the highest grade commercially available. Double deionized water was filtered with 0.45 μm filter membrane before use.

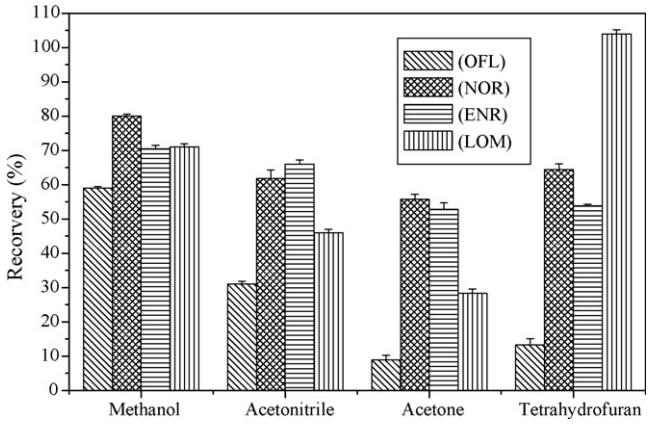

Fig. 2. Effect of dispersive solvent on the recovery of FQNs.

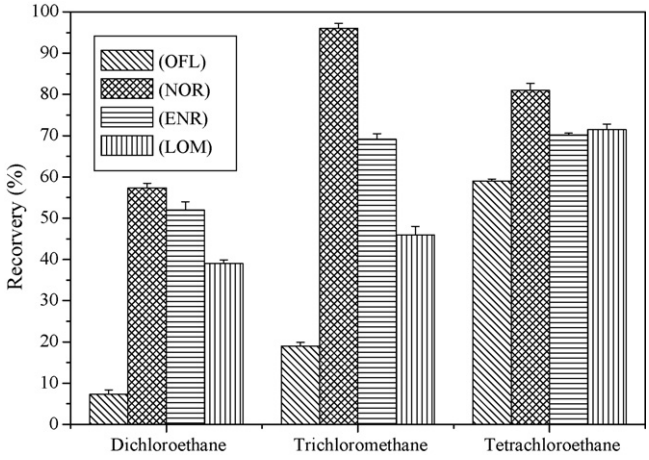

Fig. 3. Effect of extraction solvent on the recovery of FQNs.

2.2. Instrumentation

Chromatographic analysis was carried out on a LC-20A system equipped with two LC-20AT Solvent Delivery Units, a SUS20A gradient controller, and a SPD-20A UV-VIS Detector (Shimadzu, Kyoto, Japan). A N-2000 data workstation (Zheda Zhineng Co. Ltd., Hangzhou, China) was used as the data acquisition system. The analytical column was a Zorbax Eclipse XDB-C18 column (4.6 mm × 150 mm, 5 μm) from Agilent Company (Wilmington, DE, USA). The mobile phase was a mixture of methanol–water–TFA (70/30/0.05, v/v/v) with a flow rate of 1.0 ml/min. The injection volume was 10 μl for all the solutions and the UV detector was set to 280 nm. An ultrasonic cleaner (KQ3200E, Kunshan Ultrasonic Instrument, Jiangsu, China) set at 40 kHz (equivalent to the wavelength of 37.5 mm) was used to emulsify the solutions and a centrifuge (0406-1, Medical Devices, Shanghai, China) was used to accelerate the separation of sediment phases.

2.3. Ultrasound-assisted dispersive liquid–liquid microextraction

The schematic procedure of the UA-DLLME is shown in Fig. 1. 8.0 ml of water sample (basified using 1.0% aqueous ammonia to pH 8.0) was placed in a 10.0 ml conic tube and then 0.5 ml of methanol (as disperser solvent) containing 110.0 μl of tetrachloroethane (as extraction solvent) was rapidly injected into the sample solution by syringe. The mixture solution was gently shaken and ultrasonicated for 2.0 min to form a homogeneous cloudy solution. The phase separation was performed by a rapid centrifugation at 4000 rpm for 5.0 min, and the sediment phase was evaporated to dryness and re-dissolved in 50 μl of the mobile phase for further HPLC analysis. All operations were carried out at room temperature (25 °C).

3. Results and discussion

3.1. Selection of dispersive solvent

For DLLME method, the dispersive solvent should be miscible with the organic extraction solvent as well as the aqueous phase. Appropriate dispersive solvent can disperse the extraction solvent to fine droplets in water sample and increases the surface area for transferring the target compounds from sample matrix to extraction solvent. Several solvents, such as methanol, acetonitrile, acetone, tetrahydrofuran were used as dispersive solvents to investigate their effect on extraction efficiency. After centrifugation of the cloudy solution, a small amount of white floccus was observed

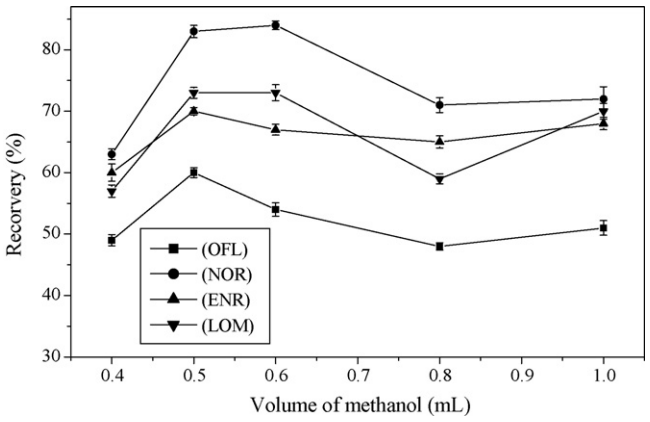

Fig. 4. Effect of volume of methanol on the recovery of FQNs.

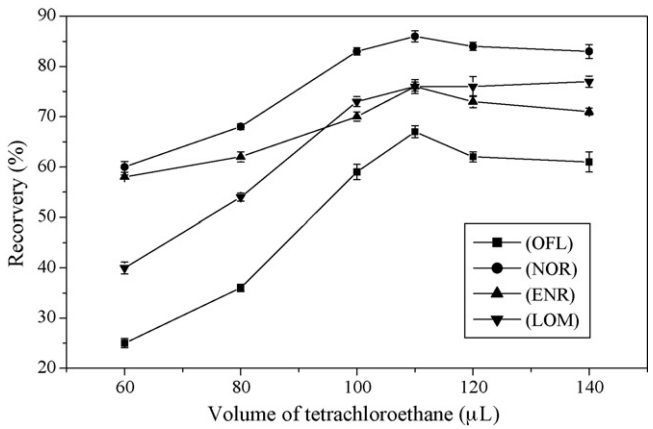

Fig. 5. Effect of tetrachloroethane volume on the recovery of FQNs.

on the interface of the two phases when acetone and tetrahydrofuran were used, which made inconvenient to transfer the organic sediment phase. The best enrichment recovery (ER) was obtained by using methanol as dispersive solvent (Fig. 2). Thereby, methanol was selected as dispersive solvent for further work.

3.2. Selection of extraction solvent

The extraction solvent of DLLME should be of high density, low water solubility, and should have extraction capability of the analytes. Dichloroethane, trichloromethane, tetrachloroethane and dichloromethane were evaluated by applying 100.0 μl of each extraction solvent to the DLLME process. No sediment phase was observed when 100.0 μl dichloromethane was used as extraction solvent, which was due to its higher solubility in aqueous solution. The results of Fig. 3 indicated that the best ERs were achieved by using tetrachloroethane as extraction solvent, hence tetrachloroethane was employed in the subsequent studies.

3.3. Effect of dispersive solvent volume

The volume of dispersive solvent is a crucial parameter that has an important effect on extraction efficiency. Commonly, at low dispersive solvent volume, the tiny droplet of extraction solvent may not be effective formation, thereby lowering the extraction efficiency. At the same time, at higher volumes of dispersive solvent the solubility of the analytes in sample solution increased, which lowered the partitioning of the analytes into the droplets of extraction solvent leading to decreased extraction efficiency. Therefore, different volumes of dispersive solvent (0.4, 0.5, 0.6, 0.8, and 1.0 ml) were tried. The results (Fig. 4) indicated that the ERs increased with the increase of methanol volume from 0.4 to 0.5 ml and then slightly decreased. At the same time, the volume of sediment phases decreased with the increasing of methanol. No phase separation was observed when the volume of methanol was higher than 2.0 ml, which was due to the higher solubility of tetrachloroethane in sample-disperser solution. Considering the ERs and the volume of sediment phase, 0.5 ml of methanol was chosen for further work.

3.4. Effect of extraction solvent volume

The volume of extraction solvent is a crucial parameter that has an important effect on the extraction efficiency. To study the effect of extraction solvents, different volumes of tetrachloroethane (60, 80, 100, 110, 120, and 140 μl) were subjected to the DLLME procedure. The results show that the ERs and EFs increased with the volume of extraction solvent increasing from 60 to 110 μl (Fig. 5). When the volume of tetrachloroethane was further increased, nearly constant EFs and ERs were observed, which was due to the completed extraction equilibrium. Considering the EFs, ERs, droplet volume, and reproducibility, 110 μl of tetrachloroethane was used in subsequent experiments.

3.5. Effect of sample pH

FQNs contain both amino (piperazinyl) groups and carboxylic groups, thus, their forms vary between cationic, anionic and intermediate state with the changing of sample pH, which has obvious influences on extraction efficiency. The effect of sample pH was investigated in the range of 2.5–9.0 (adjusted by acetic acid and ammonia water, the original solution of pH 6.2). The highest ERs were achieved at pH 8.0. Therefore, this pH was selected for further investigation.

3.6. Effect of salt concentration

Different amounts of sodium chloride in a range of 0–10% (m/v) were added to investigate the influence of ionic strength on extraction performance. There was a slight increase of the volume of sediment phase along with the increasing salt concentration, which was due to the decreased solubility of extraction solvent by salting out effect. The presence of sodium chloride increased the ionic strength of sample solution and decreased the solubility of extraction solvent in water, which increased the volume of sediment phase. However, there was no significant variation on the extraction efficiency for any of the target analytes. Therefore, salting out was not performed for further DLLME procedure.

3.7. Effect of extraction time

In DLLME, extraction time was defined as time interval between the formation of homogeneous cloudy solution and phase separation by centrifugation. The results show the EFs and ERs increased in the range of 5–10 min (Fig. 6). When the extraction time was further increased from 10 to 45 min, the EFs and ERs were maintained constant or slightly decreased. Due to the large contact surface between extraction solvent and aqueous phase in the emulsion system, the extraction equilibrium can be easily achieved within a short time. However, the emulsion solution was unstable and it would delaminate in the course of over-extension of extraction time, which could break the equilibrium and lead to lower extraction efficiency. Consequently, 10 min was chosen as the optimized extraction time.

Table 1
Features of the UA-DLLME-HPLC-UV method.

Analyte	Linear equation	R^2	EF	LOD (μg/l)	RSD (%)
OFL	$Y = 1.94 \times 10^3 X - 2.00 \times 10^3$	0.9994	100	0.34	2.2
NOR	$Y = 1.06 \times 10^3 X - 2.87 \times 10^4$	0.9994	32	0.81	3.7
ENR	$Y = 9.56 \times 10^3 X - 2.25 \times 10^5$	0.9990	134	0.14	2.1
LOM	$Y = 2.26 \times 10^3 X - 2.26 \times 10^4$	0.9999	59	0.37	1.9

3.8. Optimization of method to form emulsion

The formation of the ternary emulsion is a key step in DLLME, which influences the area of contact between the extraction solvent and aqueous phase and finally influences the extraction efficiency. Shaking was commonly used to produce the ternary emulsion. However, in practice the small volume of extraction solvent (few microlitres) made it hardly dispersed into the aqueous phase by shaking, accompanied with the danger of sample loss from the tube edge. So in this work, an ultrasound-assisted process was adopted to accelerate the formation of homogeneous cloudy solution. This results in finer droplets of extraction solvent and emulsifies the ternary mixture uniformly and rapidly. Therefore, the stability and reproducibility of the emulsion solution using ultrasound were improved, and 2 min was enough to form a stable cloudy solution.

3.9. Validation of the proposed method

In order to validate the developed UA-DLLME method, linearity, correlation coefficient, detection limits, enrichment factors and repeatability were tested using spiked samples under the optimum UA-DLLME condition. Good linear relationships were obtained over the concentration of 0.01–2.0 μg/ml with the correlation coefficient (r^2) ≥ 0.9990 for FQNs (Table 1). The limits of detection (LODs) based on signal to noise of 3 were ranged from 0.14 to 0.81 μg/l, which were significantly better than in previous LC-UV methods [26,27]. The sensitivity could be further improved by using MS or fluorescence detector. Comparing with the direct injection, the EFs for OFL, NOR, ENR, and LOM were 100, 32, 134 and 59 folds, respectively. Intra-assay and inter-assay precision expressed as the relative standard deviation (RSD) of concentrations calculated from the quality control samples were in the range of 1.9–3.7% for all analytes.

3.10. Real sample analysis

Wastewater from pharmaceutical factories is the main source of FQNs in environmental water: it contains higher levels of FQN residues than household/municipal wastewaters. Five wastewater samples collected from a pharmaceutical factory (Baoding, China) were served as real samples to validate the proposed UA-DLLME method. All the water samples were centrifuged at 4000 rpm for 5.0 min and the supernatants were filtered by 0.45 μm filter membrane to eliminate the particulate matters and then extracted with the UA-DLLME procedure. No FQN residues were measures in any of the samples, which demonstrated that the pharmaceutical wastewater had been appropriately treated before discharge from the factory. Recovery experiments were carried out to investigate the effect of sample matrix by spiking three different concentrations of standard analytes into the water samples. A representative chromatogram of the spiked pharmaceutical wastewater samples is shown in Fig. 7. As seen in Table 2, the average recoveries for all analytes were in the range of 82.7–110.9% with RSD less than 5.2%, which indicated that the UA-DLLME-HPLC method was reliable and could be used for the trace analysis of FQNs in aqueous samples.

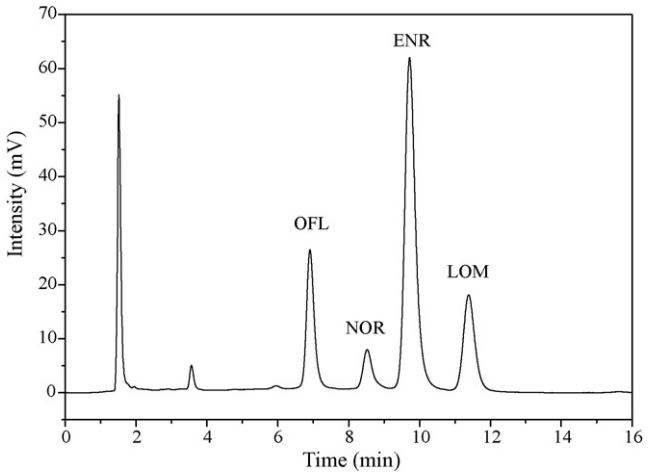

Fig. 7. Chromatograms of spiked pharmaceutical wastewater sample. *Extraction conditions*: sample volume, 8.0 ml; dispersive solvent (methanol) volume, 0.5 ml; extraction solvent (tetrachloroethane) volume, 110 μl; solution pH: 8.0; spiked level of 200 μg/l; injection volume: 10 μl.

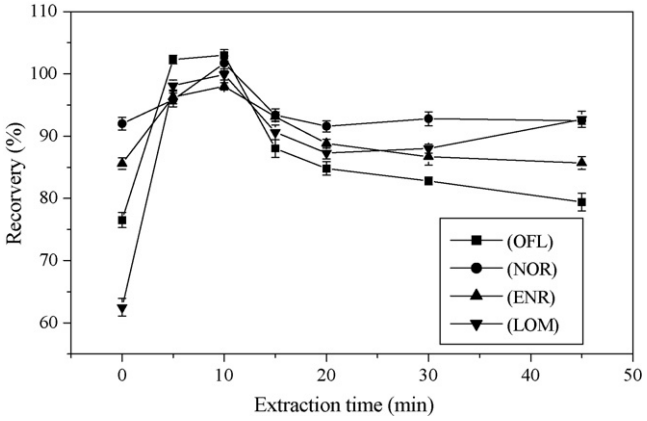

Fig. 6. Effect of extraction time on the recovery of FQNs.

Table 2
Recoveries of the UA-DLLME method for spiked water samples.

Analytes	Added (μg/l)	Found (μg/l)	Recovery (%)	RSD (%)
OFL	50.0	41.4	82.7	1.5
	100.0	103.9	103.9	2.0
	200.0	203.4	101.7	1.8
NOR	50.0	47.9	95.8	0.8
	100.0	89.2	89.2	2.8
	200.0	175.6	87.8	5.2
ENR	50.0	55.5	110.9	0.9
	100.0	88.3	88.3	4.0
	200.0	176.0	88.0	4.3
LOM	50.0	45.4	90.9	4.1
	100.0	104.8	104.8	2.5
	200.0	183.5	91.8	2.9

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