Determination of Quinolones in Human Urine and Water Samples by Ultrasound Assisted Dispersive Liquid-Liquid Microextraction Based on Solidification of Floating Organic Droplet Followed High Performance Liquid Chromatography

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Abstract: *A ultrasound-assisted dispersive liquid–liquid microextraction based on solidification of floating organic droplet (UDLLME-SFO) combined with high performance liquid chromatography (HPLC) method was developed for the determination of quinolones (QNs) in human urine and water samples. The parameters of UDLLME-SFO procedure including sample pH, type and volume of disperser solvent, type and volume of extraction solvent and ultrasound time were optimized. The optimal extraction conditions of UDLLME-SFO were: sample pH 5; extraction solution, 300 µL 1-dodecanol; dispersion solution, 200 µL methanol; ultrasound time, 10 min. Under the optimum extraction and determination conditions, good responses for QNs were obtained in a range of 1–15 µg/mL, with linear coefficients greater than 0.97. The recoveries of the quinolones ranged from 66% to 102%. The limits of detection based on a signal-to-noise ratio of 3 was 0.03 µg/mL. The method was applied to the determination of QNs in human urine, tap and lake water samples with a satisfactory result. The results demonstrated that this UDLLME-SFO-HPLC method was successfully applied to determine QNs in urine and water samples.*

Keywords: *Dispersive liquid–liquid microextraction based on solidification of floating organic droplet; Quinlonones; Human urine; Water samples;*

Abbreviations:

ACN: acetonitrile; CIP: ciprofloxacin; HPLC-UV: high performance liquid chromotagraphy-ultraviolet; LOD: limit of detection; MeOH: methanol; MOX: moxifloxacin; NOR: norfloxacin; QNs: quinolones; UDLLME-SFO: ultrasound assisted dispersive liquid–liquid microextraction based on solidification of floating organic droplet.

1. INTRODUCTION

Quinolones (QNs) are the most important class of synthetic antibiotics in human and veterinary medicines. They are effective to control a wide range of bacteria, both gram positive and negative as well as other bacteria and often used in the treatment of a range of illnesses. Due to the fact that its resistance in the environment can cause undesirable effects on aquatic ecosystems and human health, QNs have obtained growing attention [1,2]. Therefore, the analysis of the occurrence, distribution and risks of quinolone antibiotics became crucial to environment monitoring.

In recent years, several extraction methods have been developed for DES including solid phase extraction [1], dispersive liquid-liquid microextraction [3], liquid-liquid extraction [4], QuEChERS[5], ultrasound assisted extraction[6], stir bar sorptive extraction [7] and so on. In these extraction methods, DLLME

method is a novel miniaturized sample pre-treatment technique which requires smaller amounts of organic solvents and reduces the analysis cost more compared with other methods, such as LLE and SPE. The principle of DLLME was already described in our previous works[8-10]. Another mode of DLLME called dispersive liquid–liquid microextraction based on solidification of floating organic droplet (DLLME-SFO) has also been applied in various samples[10].

In this study, we propose the use of ultrasound assisted dispersive liquid–liquid microextraction based on solidification of floating organic droplet (UDLLME-SFO) for extraction of QNs from human urine and two water samples, and high performance liquid chromotagraphy-ultraviolet (HPLC-UV) for determination of QNs. To the best of our knowledge, this is the first demonstration for UDLLME-SFO-HPLC-UV for QNs analysis.

2. EXPERIMENTAL

2.1. Chemicals and Materials

QNs including norfloxacin (NOR), ciprofloxacin (CIP) and moxifloxacin (MOX) were purchased from Sigma-Aldrich (Steinheim, Germany), 1-undecanol, and 1-dodecanol were also from Sigma-Aldrich (Steinheim, Germany), high performance liquid chromatography-grade methanol (MeOH) and acetonitrile (ACN) were provided by J&K Chemical (Beijing, China). NaH2PO4, H3PO4, NaOH, and other affiliated chemicals were all obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All solvents and chemicals were of analytical grade and used without further purification unless otherwise specified. HPLC-grade water was obtained by purifying demineralized water in a Milli-Q system (Millipore, Bedford, MA, USA), and was used throughout the work.

2.2. Apparatus and Software

For chromatographic separation, an Agilent 1260 HPLC system (Agilent Technologies, CA, USA), equipped with a quaternary pump, a degasser, a column compartment, and a UV detector was used. Separation was performed on a Pursuit 5 C18, 5 µm, 4.6 mm × 250 mm column. The injection volume was 20 µL and the ultraviolet (UV) detector was set at 290 nm. The mobile phase consisted of (A) 0.2% acetic acid and (B) ACN with binary gradient elution at a flow rate of 1.0 mL/min. The gradient elution started with 80% (A) for 3 min, linearly decreased to 50% (A) in 7.0 min, then was brought back to 80% (A) in 2.0 min and maintained for 2.0 min with a total running time of 14 min. All the samples were passed through microporous nylon filters of 0.45 µm pore sizes in diameter (Pall Corporation, USA). An Ion 510 pH meter (Ayer Rajah Crescent, Singapore) was used to monitor pH adjustment. A centrifuge (Xiangyi, Hunan, China) was used for sample preparation.

2.3. Preparation of Standard and Sample

Standard stock solution containing 1000 µg/mL of QNs was prepared by dissolving the required amounts of the standard in MeOH. It was stored in a refrigerator at 4 °C. Working solutions were prepared from the stock solutions by dilution with appropriate amounts of Milli-Q water.

Midstream urine sample was collected from a volunteer (22-year-old male) who had not taken QNs at all. Lake water was collected from an artificial lake located in Longhua District of Haikou City (China). Tap water was obtained in the laboratory when needed. All the water samples were passed through microporous nylon filters with the pore sizes of 0.45 µm in diameter. The samples were kept under refrigeration at 4°C in the dark. Several aliquots from 10 mL filtered samples were spiked with QNs standard with different concentrations and followed by the DLLME-SFO procedure.

2.4. UDLLME-SFO Procedure

Briefly, 10.00 mL of water sample was placed in a 15 mL of screw-cap glass tube with conic bottom and spiked with QNs standard at 10 µg/mL. Two hundred microliter (200 µL) of MeOH (as disperser solvent) containing 300 µL 1-dodecanol (as extraction solvent) were rapidly injected into the sample solution with a 1.00 mL glass syringe and the mixture was gently shaken then ultrasonicated for 10 min. In this step, a

cloudy solution was formed and the QNs in the water samples was extracted into fine droplets. Then, the mixture was centrifuged for 10 min at 2000 rpm. After centrifugation, the fine 1-dodecanol droplets floating on the top of the tube were cooled in an ice bath. The organic solvent was solidified and transferred into a conical vial by a small medicine spoon and then melted quickly at ambient conditions. Finally, the extractant was redissolved in 1.5 mL MeOH and analyzed by HPLC-UV. The peak area (A) of each QNs was used as the index of extraction efficiency.

3. RESULTS AND DISCUSSION

3.1. Udllme-Sfo Condition Optimization

3.1.1. Effect of Sample Ph

It is well known that the pH of the solution can affect the extraction of acidic/basic analytes due to their dissociation equilibrium. According to the literature, the acid dissociation constants of QNs were lower than 5[11]. The ionizable functional groups carboxylic ($pKa,1$) and nitrogen ($pKa,2$) are responsible for cationic, anionic, neutral and zwitterionic species according to pH. The pH of sample solution was studied within the range 2.0–6.0. As shown in Fig. 1a, the peak area of NOR, CIP and MOX was the biggest when the sample pH was 5. This was because the neutral species of QNs were the dominant form when the sample pH was 5, therefore QNs was easily extracted to the 1-dodecanol phase. Finally, pH 5 was selected as the optimum pH value of sample solution.
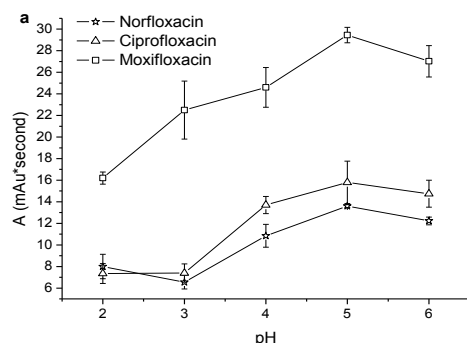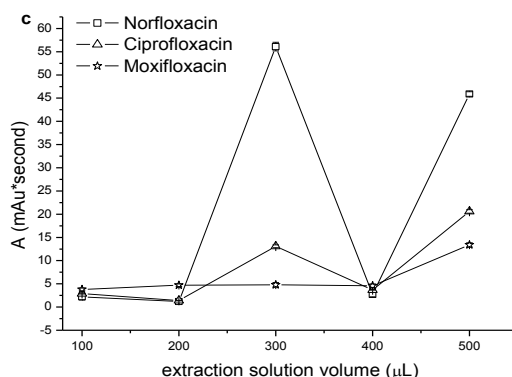

3.1.2. Effect of Extraction Solution Type and Volume

In UDLLME, it is very important to select an appropriate organic solvent for obtaining a satisfactory extraction. In this work, 1-undecanol and 1-dodecanol were optimized. The result showed that the extraction efficiency of 1-dodecanol was higher than the extraction efficiency of 1-undecanol, see Fig. 1b.
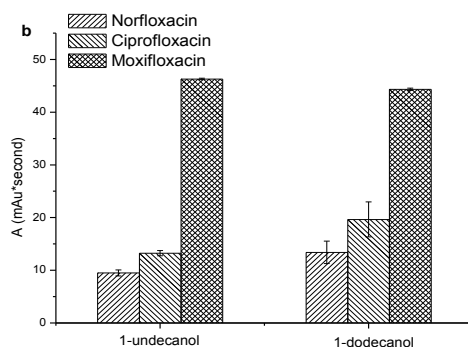

Initially, the amount of the extraction solution is also one of the important factors which affected the peak area of the QNs. Increasing the volume of extraction solution increased the recovery of QNs; however, the large volume of extraction solution decreased the concentration of QNs and therefore resulted in low extraction efficiency. The result was shown in Fig. 1c. When the extraction solution volume was 300 μL, the A values of QNs were the biggest. Therefore, three hundred microliter 1-dodecanol was selected as the extraction solution.

3.1.3. *Effect of Dispersion Solution Type and Volume*

Different dispersion solution will have different extraction efficiency. In this work, ACN and MeOH were optimized. The result showed that the extraction efficiency of MeOH was higher than the extraction efficiency of ACN, see Fig. 1d.
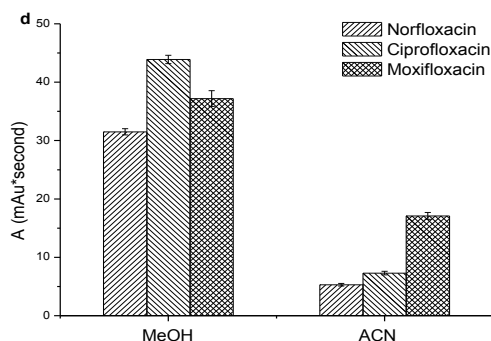
Increasing the volume of extraction solution increased the solubility of QNs, this may help QNs be extracted from sample to extraction solution; however, the large volume of dispersion solution decreased the concentration of QNs and therefore resulted in low extraction efficiency. The result was shown in Fig. 1e. When the extraction solution volume was 200 μL, the A values of QNs were the biggest. Therefore, two hundred microliter 1-dodecanol was selected as the extraction solution.
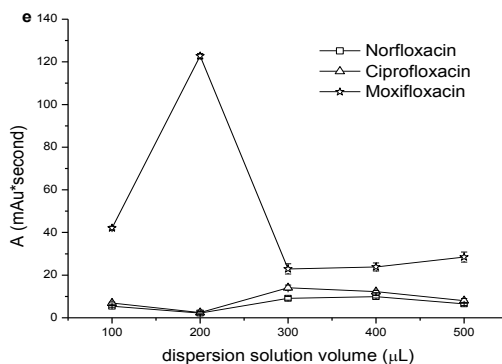
3.1.4. Effect of ultrasound time

It is well known that UAE utilized acoustic vibrations to cause cavitation in the liquid, and cavitation enhances the removal of analytes from the matrix to the extraction solution. Therefore, ultrasound can accelerate the QNs transformation process from matrix to extraction solvent. In this work, 5–25 min was optimized; the results were shown in Fig. 1f. It can be seen that the extraction efficiency was best when the time was set at 10 min. So, we selected 10 min as the optimized ultrasound time.
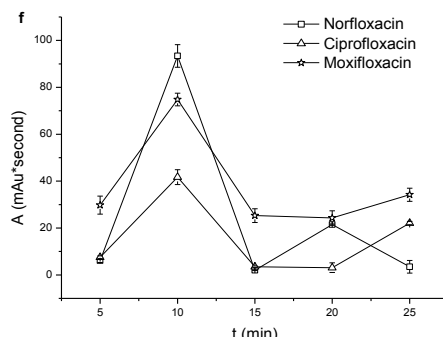
Fig1. *Effect of each parameter on extraction efficiency*

Therefore, the optimized conditions for UDLLME-SFO extraction of QNs were attained as follows: sample pH 5; extraction solution, 300 µL 1-dodecanol; dispersion solution, 200 µL MeOH; ultrasound time, 10 min.

3.2. Analytical Figures of Merit of the UDLLME-SFO-UV Method

The performance of the UDLLME-SFO-UV method under optimal conditions was investigated. Linear correlation coefficients (r) assessed at six different concentrations were obtained between A and the corresponding concentrations of QNs in the range of 1–15 µg/mL, as shown in Table 1. Limit of detection (LOD) for the QNs, calculated as the analyte concentration for which the value of A was three times the background noise (3S/N), was 0.03 µg/mL for the HPLC-UV analysis.

Table1. *Linear range, slope, intercept, correlation coefficients, and LOD for QNs*

QNs	Linear range (µg/mL)	Slope±SD (%)	Intercept±SD (%)	R^2	LOD (µg/mL)
CIP	1–15	2.4128±0.09	0.0849±0.01	0.9840	0.03
NOR	1–15	2.507±0.10	-0.2961±0.01	0.9740	0.03
MOX	1–15	2.593±0.08	-0.7372±0.00	0.9996	0.03

3.3. Determination of QNs in Human Urine and Water Samples

The developed UDLLME-SFO-HPLC-UV method was further applied to human urine, tap and lake water samples in order to check its practicality. The samples were free with QNs. Recoveries were calculated for the spiked water samples with 2, 5 and 10 µg/mL standards, respectively. The results are listed in Table 2. Satisfactory recoveries were obtained, ranged from 66% to 102%. This validated the UDLLME-SFO-HPLC-UV greatly applicable for the selective extraction, and accurate quantitation of trace QNs in human urine and water samples.

Table2. *Recovery of DES from tap and lake water samples*

QNs	Added(µg/mL)	Recovery±SD (%)		
		Human urine	Lake water	Tap water
CIP	2	80±3.9	73±8.1	79±2.0
	5	95±2.0	96±5.3	97±4.6
	10	102±2.8	92±6.2	99±2.9
NOR	2	70±7.8	74±9.5	100±1.0
	5	85±5.4	88±8.0	96±3.8
	10	92±5.0	96±6.1	84±8.2
MOX	2	66±9.8	85±8.7	72±8.7
	5	86±6.2	80±4.8	78±6.0
	10	94±4.6	82±5.0	73±7.1

4. CONCLUSION

In conclusion, a good, easy, and efficient method for the determination of QNs in human urine and water samples was developed. The developed UDLLME-SFO-HPLC-UV offered wide linear range, good

quantitative ability, and high precision. Also, this method used microliter extraction and dispersion solutions, was demonstrated to be a cost effective, and eco-friendly option for determination of QNs in water samples.

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