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DETERMINATION OF QUINOLONE ANTIBIOTICS BY BIOMASS WASTE-MONOLITHIC SPIN COLUMNS-SOLID PHASE EXTRACTION COUPLED WITH LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple, green and rapid method for the simultaneous determination of three kinds of quinolone antibiotics (QNs) from environmental water samples and biological samples was established by monolithic spin columns-solid phase extraction coupled with high performance liquid chromatography (MSC-SPE-HPLC). The QNs was completely adsorbed by 300 mg spent coffee grounds as the sorbents of MSC-SPE at 1000 rpm, and eluted by 2500 µL acetonitrile and 4% acetic acid (60:40, V:V). The efficient and rapid elution was obtained under the speed of 500 rpm. After separation on a Pursuit 5 C18 column (250 mm×4.6 µm, 5 µm), the samples were determined. The three QNs showed good liner relationship in the range of 0.1-10 µg/mL with the correlation coefficients greater than 0.998. The limit of detection (LODs, S/N=3) for ciprofloxacin, norfloxacin and enrofloxacin was 33.3 ng/mL.Moreover, the recoveries were 70.3-112.6% at three spiked levels (0.5, 1 and 2 µg/mL) with the relative standard deviation 1.02-9.78%. The obtained results revealed that the method was simple to operate, green and environment-friendly, and could be used for the determination of antibiotics in environmental samples.

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INTRODUCTION

Quinolone antibiotics (QNs) have been widely used in human and veterinarian medicine. However, the excessive use of quinolones and discharged antibiotics can be transferred to and accumulated in water, soil and other media. Unfortunately, it can enter the human body through the food chain and drinking water, endangering human health (Moreno-Bondi *et al.*2009). Several studies have reported the QNs remain in many media, especially in some animal derived foods and water (Pan *et al.* 2021; Lu *et al.* 2019; Huang *et al.* 2020; Zhang *et al.* 2018). Furthermore, antibiotics can lead to the emergence of antibiotic resistance bacterial. Thus, it is necessary to develop reliable analytical methods for quinolone in environment. Because the concentration of antibiotic residues in various environmental media is low and the sample matrix of environmental media is complex, sample preparation is necessary. Solid-phase extraction (SPE), QuEChERSand liquid-liquid extraction techniques are usually used to cleanup and preconcentration of QNs (Zhang *et al.* 2019; Wen *et al.* 2014; Dawadi*et al.* 2021; Hu *et al.* 2021; Bitas*et al.* 2018; Dugheri*et al.* 2019).

At present, some materials such as C18, HLB, graphene oxide and nano materials are mostly used in SPE (Naing *et al.* 2015; Shirkhanloo *et al.* 2016). However, these materials are commercial materials and need to be purchased. In addition, some of them should be synthesized in lab, the preparation process is cumbersome. Furthermore, the consumption of organic reagent is large and the operation time is long in the whole process. Fortunately, a new SPE column, developed by Tsunoda and Saito (Tsunoda *et al.* 2011; Saito *et al.* 2011), solved some of these problems. They highly extracted catecholamines and organophosphorus compounds from biological samples by spin integrated column solid phase extraction. The new monolithic spin columns-solid phase extraction (MSC-SPE) method can integrate the process of adsorbent activation, cleaning, loading and elution through ultracentrifuge, which is simple and fast. The batch processing of biological samples can be processed by using ultracentrifuge, which is environment friendly. In this work, MSC-SPE method coupled with high performance liquid chromatography (HPLC) for the determination of threeQNs in environmental water samples and biological samples was developed. The spent coffee grounds were used as an effective MSC-SPE adsorbent for extraction of norfloxacin (NOR), enrofloxacin (ENR) and ciprofloxacin (CIP).

The SPE operation was carried out with a self-made MSC device made by two 1.5 mL centrifuge tubes. HPLC was used to determine the three QNs. The extraction parameters were accurately optimized, such as the pH, the amount of spent coffee grounds, the type and volume ratio of eluent, sample loading speed, elution speed and the volume of eluent. Under optimal conditions, the concentrations of the three QNs were determined. This MSC-SPE-HPLC method has the advantages of environmental protection, low cost, simple and convenient preparation.

MATERIALS AND METHODS

Chemicals and materials: The certified QNs standards of NOR, CIP, ENR with purity>99% were purchased from Sigma Aldich (Steinheim, Germany). HPLC-grade methanol (MeOH) and acetonitrile (ACN) were obtained from J&K Chemical (Beijing, China). Ethanol, hydrochloric acid, sodium hydroxide and acetic acid (AA) of analytical grade were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). The experimental water was obtained by Milli-Q system (Millipore, Bedford, MA, USA). The spent coffee grounds used in the experiment were the grounds of coffee beans purchasedfrom Yunnan Province after grinding and mixing.

Instrument: Agilent 1260 infinity II HPLC with diode array detector (DAD) were supplied by Agilent company (USA). Separation was performed on a Pursuit 5 C18, 4 μm, 4.6 mm ×250 mm column. The injection volume was 20 μL and DAD was set at 278 nm for CIP and ENR, 280 nm for NOR, respectively. The mobile phase consisted of (A) 0.2% AA and (B) ACN with binary gradient elution at a flow rate of 1.0 mL/min. The gradient elution started with 85% (A) for 3 min, linearly decreased to 50% (A) in 1.0 min, increased to 60% (A) in 1 min and maintained for 2 min, then was brought back to 85% (A) in 3.0 min and maintained for 5.0 min with a total running time of 15 min. All the samples were passed through microporous nylon filters of 0.22μm pore sizes in diameter (Pall Corporation, USA). S10P ultrasonic cleaner was supplied by ZEALWAY company (USA), TG16-WS desktop high-speed centrifuge was obtained by Xiangyi company (China), pH meter was purchased by Mettler Toledo company (Switzerland), MTN-2800W nitrogen blowing concentrator was supplied by Auto Science company (China).

Preparation of standard and samples: Preparation of standard stock solution accurately weigh 10.00 mg of the QNs standard in 10 mL MeOH. QNs standard were stored at -20 ℃. Midstream urine sample was collected from a volunteer (22-year-old male) who had not taken QNs at all. Seawater was collected from the coastal zone areas of the Haikou City of China. Lake water was collected from a lake in Haikou City of 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 0.22 μm in diameter.

Adsorptive performance experiment of sample pH and coffee spent grounds amount: Each desired QNs concentration used was prepared by appropriately diluting the stock solutions with 10 mmol/L Na_2HPO_4 and successive dilutions. Spent coffee grounds were added into 5 mL QNs solution with a fixed concentration. All the adsorption experiments were performed in conical flasks under ultrasonic bath for 30 min. After adsorption, all solutions were filtered through 0.22 μm membrane filters and analyzed by HPLC. The adsorption capacity (Q, mg/g) of spent coffee grounds for QNs was calculated by the following formula:

$$Q = \frac{c_0 - c_t}{m} \times V \quad (1)$$

where Q is the adsorption capacity (mg/g), C_0 is the concentration of the solution before adsorption（μg/mL）, C_t is the concentration of the solution after adsorption（μg/mL）, V is the volume of QNs solution (mL), m is the mass of spent coffee grounds (mg).

The impact of sample solution pH on QNs adsorption efficiency were conducted by addingspent coffee grounds into each QNs solution with ultrasonic bath assisting for 30 min. The pH value was adjusted by NaOH orH_3PO_4 solution (0.1 M) ranged from 3.0 to 9.0. The impact of spent coffee grounds usage amount on the adsorption efficiency was tested by adding different amount of spent coffee grounds to each QNs solutions with ultrasonic bath assisting for 30 min.

MSC-SPE procedure: MSC is made of two 1.5 mL centrifuge tubes including outside tube and inside tube, one end of the tube body on the outside is open and the other end is closed. Both ends of the pipe body on the inner side are open, the two pipe bodies are sleeved together, and the pipe body on the inner side is communicated with the pipe body on the outer side. The inner tube body is filled with degreasing cotton, 300 mg spent coffee grounds and degreasing cotton from bottom to top. After preparing MSC, 3 mL MeOH and 3 mL water were successively added to activate the extraction column. 0.5 mL sample was loaded at 1000 rpm. Then 2500 μL mixture of ACN and 4% AA (60:40, V/V) was eluted at the speed of 500 rpm. Subsequently, the eluent was blown to dry with nitrogen. Next, it was redissolved with 0.5 mL mixture of ACN and 0.2% AA (15:85, V:V). After votexed for 1 min, the solution was filtered through a 0.22 μm filter membrane and then analyzed by HPLC. The extraction efficiency results were calculated by recovery (R), as shown in formula (2):

$$R = \frac{A_1}{A_0} \times 100\% \quad (2)$$

Where A_1 is the peak area value of the sample after extraction, A_0 is the peak area value of standard solution.

RESULTS AND DISCUSSION

Effect of pH of the sample solution: The pH of the sample solution plays an important role for the extraction of QNs in MSC-SPE procedure. Therefore, the effect of the solution on the extraction efficiency of the three QNs was studied at acidic, neutral and basic conditions. In this experiment, the pH in the range of 3~9 was investigated. The results were shown in Figure 1. It can be seen that the Q value was biggest when pH was 6. Therefore, pH = 6 was selected.

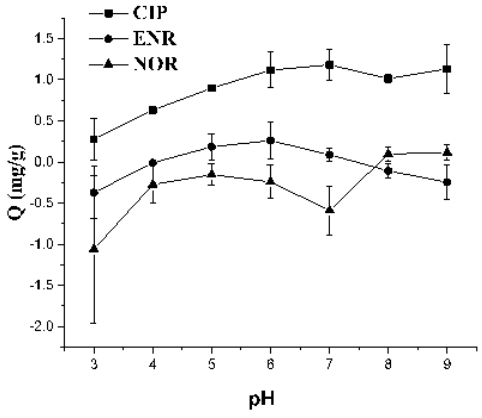

Figure 1. Influence of different pH values on adsorption capacity

Effect of spent coffee grounds usage amount: As shown in Figure 2, the Q value decreased as the usage amount of spent coffee grounds increasing from 10 mg to 80 mg for CIP; while the Q value decreased as increasing from 10 mg to 50 mg. Therefore, 80 mg was selected for CIP, 50 mg was selected for NOR and ENR. In order to extract all three QNs together, 300 mg was selected for the MSC-SPE procedure.

Effect of the eluent types and volume ratio: The selection of elution solvent was also very important for MSC-SPE procedure.

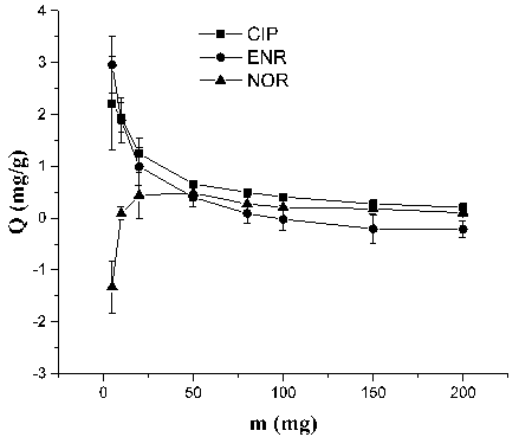

Figure 2. Effect of spent coffee grounds usage amount

In this study, several types of elution solvent including MeOH, ACN, 4% AA, MeOH+4% AA and ACN+4% AA were selected. The results were shown in Figure 3a, which indicated that ACN+4% AA was the best eluent. Moreover, the volume ratio of eluent ACN+4% AA (90:10, 80:20, 70:30, 60:40, 50:50, 40:60, V:V) was further investigated. As shown in Figure 3b, the recovery of CIP and NOR reached the maximum recovery when the eluent was ACN+4% AA (60:40, V:V), while ENR reached the maximum when the eluent was ACN+4% AA (50:50, V:V). However, the recovery values of ENR were more or less when the ratio of the elute were 60:40 and 50:50. In order to extract three QNs simultaneously, ACN+4% AA (60:40, V:V) was adjusted for subsequent experiments.

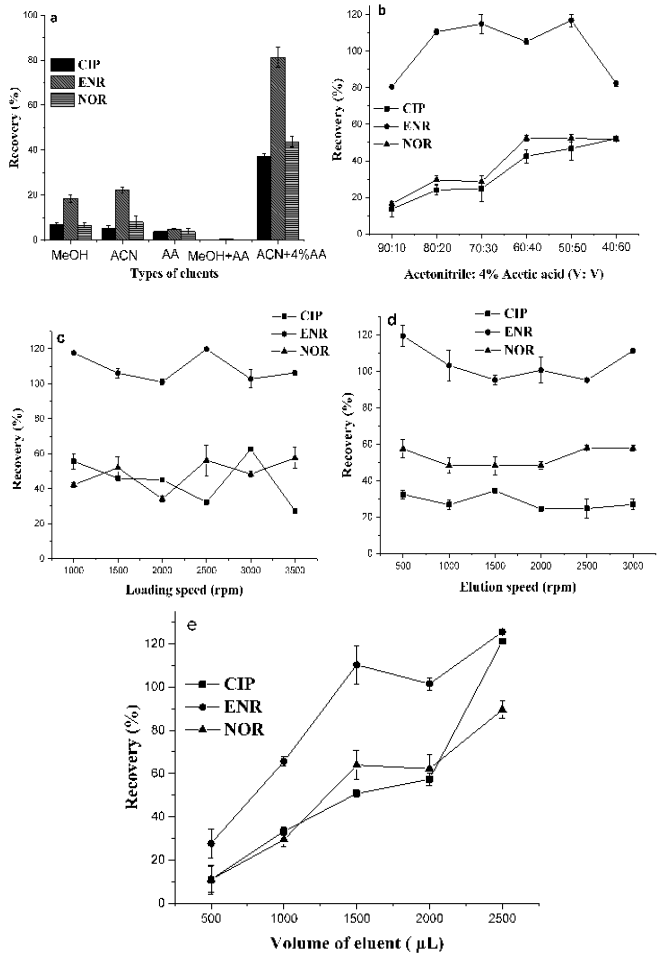

Figure 3. (a) Type of eluent (b) Volume ratio of eluent (c) Loading speed (d) Elution speed (e) Effect of eluent volume on extraction recovery

Table 1. Linear range and detection limit of QNs

QNs	Regression equation	Liner range/ (μg/mL)	r	LOD/ (ng/mL)
CIP	A=59.747C-14.001	0.1～10.0	0.9986	33.3
ENR	A=37.706C-12.478	0.1～10.0	0.9996	33.3
NOR	A=155.29C-50.079	0.1～10.0	0.9981	33.3

* A： peak area, C： concentration of QNs, μg/mL

Table 2. Precision of this method (n=6)

QNs	RSD (Intra-day, %)		RSD (Inter-day, %)	
	t	A	t	A
CIP	0.29	1.58	1.73	6.02
ENR	0.58	1.17	2.46	7.89
NOR	0.15	0.63	2.91	4.98

Effect of sample loading speed and elution speed: The effect of sample loading speed (1000, 1500, 2000, 2500, 3000, 3500 rpm) on extraction recovery was investigated. As shown in Figure 3c, the recovery of ENR reached a maximum value when the speed was 1000 rpm, while the recovery of CIP and NOR were not affected significantly by the sample loading speed. Thus, 1000 rpm was selected as the sample loading speed. The effect of elution speed (500, 1000, 1500, 2000, 2500, 3000 rpm) on the recovery was also investigated (Figure 3d). Similarly, the recovery of ENR reached a maximum value when the speed was 500 rpm, while the recovery of CIP and NOR was not affected significantly by elution speed. Therefore, 500 rpm was finally selected as the elution speed.

Effect of eluent volume: The volume of eluent was an important factor in MSC-SPE procedure. It should guarantee the antibiotics adsorbed on spent coffee grounds could be completely washed off. However, too much eluent will lead to matrix interference in the analysis solution, which will affect the recovery and change the subsequent nitrogen blowing enrichment. Different eluent volumes (500 μL、1000 μL、1500 μL、2000 μL、2500 μL) were investigated. As shown in Figure 3e, the recovery of QNs basically showed an upward trend with the increase of volume. The recoveries of CIP and ENR attained about 121% and 125% respectively when the elute volume reached 2500 μL. If the volume of eluent continued to increase, a large matrix effect would be investigated. Therefore, the optimized volume was found to be 2500 μL.

Method validation: The analytical parameters were determined using the optimized conditions identified above to evaluate the performance of MSC-SPE-HPLC. Linear regression analysis based on QNs peak area (A) and three QNs concentrations(C) was assessed in the range of 0.1-10 μg/mL. The correlation coefficient (r) of calibration curves for all the three QNs were above 0.998, showing a good linear relationship (Table 1). The limits of detection (LODs) obtained as the QNs concentration for which the peak height was three times the background noise (3S/N) was 33.3 ng/mL (Table 1). The precision results were listed in Table 2 and it indicated that the intra-day relative standard deviation (RSD, %) varied from 0.63% to 1.58% and inter-day RSD ranged from 1.73% to 2.91%. These results displayed good reproducibility of the method.

Analysis of samples: In order to verify the accuracy and precision of the method, it was applied to the analysis of QNs in various samples. Before the spiking procedure, the samples were analyzed and found to be free of QNs in seawater, lake water, tap water, human urine. The recoveries of the QNs, which were studied by spiking the QNs standard solution into different samples at three concentrations (0.5, 1 and 2 μg/mL), varied from 70.3~112.6% with satisfactory analytical features (RSD<10%, n=3) which were shown in Table 3. The data indicated that the accuracy and precision of this method are good and can meet the analysis requirements.

Table 3. Recovery and precision of three QNs

QNs	Added /(μg/mL)	Sea water		Lake water		Tap water		Urine	
		Recovery/%	RSD/ %	Recovery/%	RSD/ %	Recovery/%	RSD/ %	Recovery/ %	RSD/%
CIP	0.5	82.6	5.21	88.4	8.70	92.6	1.89	72.8	6.78
	1	90.7	2.35	93.6	5.30	96.8	2.36	80.6	4.88
	2	97.8	3.08	99.9	3.28	100.5	2.46	91.4	5.96
ENR	0.5	79.8	7.09	83.2	6.36	89.9	4.68	70.9	9.03
	1	88.9	4.86	90.7	4.35	97.8	3.89	82.5	7.99
	2	95.2	3.90	98.8	3.21	101.3	1.02	89.9	5.09
NOR	0.5	96.2	6.33	99.3	6.06	111.2	5.66	78.0	2.16
	1	99.3	2.39	100.5	2.56	99.8	2.64	82.4	3.84
	2	101.5	3.45	112.6	3.14	100.0	3.68	90.1	4.45

CONCLUSION

The present work proposes a micro-solid phase extraction device. Likewise, the adsorption capacity of spent coffee grounds for QNs was evaluated. MSC-SPE-HPLC method was established for the simultaneous determination of three QNs in various water samples and biological samples. In summary, the contribution of this work not only demonstrates the development of more simple, less sample dosage, economical and environmentally friendly method, but also can be used for the efficient and accurate determination of antibiotics in environmental samples. As a kind of biomass solid waste, spent coffee grounds will have great application potential in the field of sample preparation in the future. It will facilitate further research on the occurrence, contamination pathways, fate and risk assessment of this important class of antibiotics in the environment.

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