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RESEARCH PAPER

Solid-phase extraction in combination with dispersive liquid-liquid microextraction and ultra-high performance liquid chromatography-tandem mass spectrometry analysis: the ultra-trace determination of 10 antibiotics in water samples

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Abstract A novel method, solid-phase extraction combined with dispersive liquid-liquid microextraction (SPE-DLLME), was developed for ultra-preconcentration of 10 antibiotics in different environmental water samples prior to ultra-high performance liquid chromatography-tandem mass spectrometry detection. The optimized results were obtained as follows: after being adjusted to pH 4.0, the water sample was firstly passed through PEP-2 column at 10 mL min^{-1}, and then methanol was used to elute the target analytes for the following steps. Dichloromethane was selected as extraction solvent, and methanol/acetonitrile (1:1, *v*/*v*) as dispersive solvent. Under optimal conditions, the calibration curves were linear in the range of 1–1000 ng mL^{-1} (sulfamethoxazole, cefuroxime axetil), 5–1000 ng mL^{-1} (tinidazole), 10–1000 ng mL^{-1} (chloramphenicol), 2-1000 ng mL^{-1} (levofloxacin oxytetracycline, doxycycline, tetracycline, and ciprofloxacin) and 1–400 ng mL^{-1} (sulfadiazine) with a good precision. The LOD and LOQ of the method were at very low levels, below 1.67 and 5.57 ng mL^{-1}, respectively. The relative recoveries of the target analytes were in the range from 64.16 % to 99.80 % with relative standard deviations between 0.7 and 8.4 %. The matrix effect of this method showed a great decrease compared with solid-phase extraction and a significant value of enrichment factor (EF) compared with dispersive liquid-liquid microextraction. The developed method was successfully applied to the extraction and analysis of antibiotics in different water samples with satisfactory results.

Keywords Antibiotics · Dispersive liquid-liquid microextraction · Solid-phase extraction · Ultra-high performance liquid chromatography-tandem mass spectrometry · Water samples

Introduction

Antibiotics, such as penicillins, quinolones, cephalosporins, tetracyclines, aminoglycosides, amphenicols, macrolides, have important effects on human health due to its strong antimicrobial properties. They are commonly used as antitumor agents, immunosuppressive agents, hypocholesterolemic agents, enzyme inhibitors, antimigraine agents, and antiparasitic agents [1], which are released in large amounts into natural ecosystems. Unfortunately, the half-life period of most antibiotics is relatively long, leading to a long existence in the environment. Furthermore, some antibiotics can be prone to bioconcentration in aquatic organisms, particularly in fish, and eventually absorbed by our body [2, 3]. Recent researches indicated that antibiotics can exert adverse influence on ecology and human health even at the low concentrations [4]. The obvious consequence of antibiotic release in natural environments is the selection of resistant bacteria, impacting the structure and activity of environmental microbiota [5]. Resistance genes have been also found at clinical settings, and they are propagating among pristine ecosystems without any record of antibiotic contamination [6]. It is significantly essential to detect and eliminate the antibiotics in our surroundings. Levofloxacin, ciprofloxacin, oxytetracycline, tetracycline, doxycycline, sulfadiazine, sulfamethoxazole, tinidazole,

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chloramphenicol, and cefuroxime axetil were determined by many researchers for the wide usage in the treatment of diseases [7–10], which belonged to the five common antibiotics types. In order to realize the status of antibiotics presented in water, those 10 target analytes were finally selected.

Antibiotics are found in aquatic environment at levels up to micrograms per liter, and they have been detected in surface waters as a result of their resistance to the wastewater treatment and (bio)degradation processes [11]. Fluorquinolones were detected at 3–87 μg L^{-1} in hospital wastewaters by Hartmann et al. [12]. Similarly, Gros et al. [13] found that the concentration of ofloxacin and ciprofloxacin in hospital wastewaters were up to 10.368 μg L^{-1}. The research studied by Wei et al. [14] in 2010 indicated that the maximum concentration of veterinary antibiotics in animal wastewater and surface water around farms (Jiangsu Province, China) were 3.67 and 39.5 μg L^{-1} for chlortetracycline and doxycycline, respectively. Furthermore, the residues of antibiotics in effluent wastewater were significantly detected at 8.26 μg L^{-1} as a result of incomplete treatment [15].

Recently, there have been an increasing number of the methods published for the simultaneous determination of several classes of antibiotics in water environment [16–18], among which, two of the common methods are solid-phase extraction (SPE) and dispersive liquid-liquid microextraction (DLLME).

SPE gained its popularity owing to its superiority, such as less organic solvents, time-saving, and high separation efficiency [19]. And it has been successfully applied to the preconcentration of several trace substances in water and environmental samples [20, 21]. However, because of the diversity and complexity of the environmental matrix, the SPE analysis of the trace substances in complex samples becomes very limited. DLLME is a novel liquid-liquid microextraction technique developed by Assadi and his co-workers [22] based on a ternary component solvent system after the extraction solvent and disperser solvent being rapidly injected into an aqueous sample. DLLME has gained increased prominence for its rapidity, simplicity of operation, low cost, environmental friendliness, and decreasing waste generation [23, 24]. In comparison with SPE, however, this method has low enrichment factor (EF) and so proves to be unsatisfactory to the ultra-trace residue analysis.

Thus, it is worthwhile to combine SPE and DLLME to take the advantages of both. The application has been reported for the analysis of organics in aqueous, meat, soil, plant, and other complex matrices, and it exhibited a strong point of high extraction factor and wide range of applications [25–28]. However, its potential applications in antibiotics have not been exploited in depth yet.

Therefore, the main objective of this study is to establish a method which can not only enrich the ultra-trace antibiotics, but also reduce the matrix effect effectively. For this purpose, we developed a simple and effective SPE-DLLME with the UHPLC-MS/MS technique for the simultaneous determination of 10 antibiotics in different water samples. SPE materials, elution solvent, pH, extraction solvent, and other parameters affecting extractive efficiency were investigated in our study. Finally, under the optimized conditions, the proposed method was validated for the analysis of five different water samples (drinking water, running water, river water, influent, and effluent wastewater).

Materials and methods

Reagent and standards

Levofloxacin, ciprofloxacin, oxytetracycline, tetracycline, tinidazole, sulfadiazine, doxycycline, sulfamethoxazole, chloramphenicol, cefuroxime axetil were purchased from the National Institute for Food and Drug Control (Beijing, China). The purity of these standard compounds was higher than 97 %. Stock solutions were prepared by dissolving each substance in chromatographic-grade methanol at a concentration of 1 mg mL^{-1} and stored at 4 °C in darkness.

Acetonitrile and formic acid of chromatographic grade were obtained from Fisher Scientific (Pittsburgh, PA, USA). Methanol of chromatographic grade was purchased from Yuwang Industrial Co., Ltd. (Shandong, China). Chromatographic-grade water was purified using a Milli-Q Reagent Water system (Millipore, Bedford, MA). The other chemicals and solvents in these experiments, such as dichloromethane (CH_2Cl_2), dichloroethane ($C_2H_4Cl_2$), trichloromethane ($CHCl_3$), carbon tetrachloride (CCl_4), chlorobenzene (C_6H_5Cl) were all of analytical grade.

Instruments

The solid-phase extraction equipment was a 24-fold vacuum extraction manifold (Agilent, USA). The cartridges used for solid-phase extraction were Cleanert PEP-2 (60 mg, 3 mL) that was from Agela Technologies (Tianjin, China). Other tested cartridges were Strata C_{18}-E (500 mg, 6 mL) and Strata X (60 mg, 3 mL) that were obtained from Phenomenex (Torrance, CA, USA), Oasis HLB (30 mg, 1 mL) was obtained from Waters (Milford, MA, USA), and InertSep Pharma (60 mg, 3 mL) was obtained from Shimadzu (Tokyo, Japan).

Chromatographic analysis was performed on an ACQUITY™ UPLC system (Waters Corp., Milford, MA, USA), equipped with a binary pump solvent management system, micro degasser, an autosampler, and thermostatic column compartment, coupled to a Micromass Quattro micro™ API mass spectrometer (Waters Corp., Milford, MA, USA) equipped with an electrospray ionization (ESI) interface. Positive-negative ion fast switching techniques and multiple

reaction monitoring (MRM) were used. The optimal MS parameters for the analysis are shown in Table 1. Chromatographic separation was carried out on a Waters ACQUITY UPLC® BEH Phenyl (50 mm × 2.1 mm, 1.7 μm) with an in-line filter in front of the column. The column temperature was set at 35 °C and the flow rate was set at 0.2 mL min^{-1}. The optimal conditions were cone voltage, 30 V; capillary voltage, 3.0 kV; desolvation temperature and source temperature, 350 and 120 °C, respectively; desolvation gas flow, 450 L h^{-1}. Argon was used as the collision gas in all cases and nitrogen as the auxiliary and sheath gas in the ESI source. The mobile phase consisted of aqueous formic acid (0.1 %, *v*/*v*) (A) and acetonitrile (B), with a gradient elution as follows: 35–65 % B at 0–4 min, 65 % B at 4–5 min. The injection volume was 10 μL.

Samples

Influent (IWW) and effluent (EWW) wastewaters were collected at the urban wastewater treatment plant of Shenyang. River water (RW) samples were collected from the South Canal of Shenyang. The samples of running water were taken from the tap in the laboratory and drinking water from bottled water in Shenyang. All samples were collected in December 2014 and filtered by 0.45 μm nylon membrane and stored in amber glass bottles at −20 °C before analysis.

SPE-DLLME procedure

Prior to SPE, 0.1 g Na_2EDTA was added to each 100 mL aqueous samples to prevent tetracycline antibiotics from complexation with metal ions. The pH of the samples was adjusted to 4 with 1 M HCl. SPE cartridge (PEP-2) was conditioned with 5 mL methanol and 5 mL water. Volume of 500 mL (drinking water or running water) or 250 mL (river water or wastewater) was passed through the cartridge at a flow rate of 10 mL min^{-1}. The analytes were subsequently eluted with 5 mL methanol after being vacuum dried for 10 min. The elute solution was collected and concentrated under a gentle nitrogen flow. The residue was diluted by 5 mL aqueous phase (pH 4.0) in a 10-mL screw cap glass test tube with conical bottomed for the subsequent DLLME procedure.

For the DLLME, 800 μL dichloromethane mixed with 600 μL methanol and 600 μL acetonitrile was rapidly injected into 5 mL aqueous sample above. A cloudy solution was formed in the tube after ultrasound for 5 min. Then, the mixture was centrifuged for 10 min at 4000 rpm and the dispersed fine particles of extraction phase were deposited at the bottom of the test tube. The supernatant was removed by a microsyringe. The remaining sedimented phase was evaporated with a gentle nitrogen stream at 35 °C and the residue was reconstituted with 100 μL acetonitrile/water (1:1, *v*/*v*). Ten microliters of the above solution was injected into UHPLC-MS/MS for analysis.

Results and discussion

Optimized SPE-DLLME conditions

The combination of SPE-DLLME was proved to be a highly sensitive and selective method for the ultra-trace analysis. In order to obtain high extraction efficiency, the effects of several experimental parameters, such as SPE column, the pH of samples, the type and the volume of extraction and dispersive solvent were investigated. In this experiment, 500 mL Milli-Q water samples, which were free of target analytes and spiked at 1 ng mL^{-1}, were used to optimize the extraction conditions. Recovery acts as the indicator, and it was calculated by the ratio of the amount of analytes in after-extraction and before-extraction. All the experiments were performed in triplicate and the means of the results were used for optimization.

Effect of different SPE materials

SPE cartridge was the key factor to the isolation and purification efficiency of the target analytes. Five different commercially available extraction cartridges were tested regarding

Table 1 Mass spectrometric parameters of ten antibiotics

Antibiotics	t_R (min)	Precursor ion	Transitions (*m*/*z*)	Collision energy (eV)
Levofloxacin	1.49	$[M+H]^+$	362.4 → 318.3	20
Ciprofloxacin	1.50	$[M+H]^+$	332.2 → 288.3	18
Oxytetracycline	1.52	$[M+H]^+$	461.4 → 426.2	17
Tetracycline	1.57	$[M+H]^+$	445.4 → 410.2	18
Sulfadiazine	1.67	$[M+H]^+$	251.2 → 155.9	14
Tinidazole	1.79	$[M+H]^+$	248.2 → 128.0	20
Doxycycline	1.98	$[M+H]^+$	445.4 → 428.3	18
Sulfamethoxazole	2.27	$[M+H]^+$	254.3 → 155.9	15
Chloramphenicol	2.35	$[M-H]^+$	321.2 → 152.0	15
Cefuroxime axetil	2.96	$[M+Na]^+$	533.3 → 447.1	16

their analyte recovery. The performance of the cartridges test is summarized in Fig. 1. The RSDs for all measurements were between 0.1 and 16.1 % (data not shown). Cleanert PEP-2 cartridges were much more efficient, yielding recoveries for all target compounds. This sorbent, with the combination of the hydrophilic-lipophilic polymer, can extract acidic, neutral and basic analytes at a wide range of pHs, and because of the presence of ureido group, most of highly polar compounds can be adsorbed by this SPE cartridge. PEP-2 obtained the best recovery for all antibiotics, which was chosen for further study.

Effect of pH for SPE and DLLME

The pH of water samples is a significant parameter for both SPE and DLLME. The pK_a of the antibiotics is between 3.30 and 9.61. For most antibiotics, the sample solution should be rather acidic to effectively deionize the analytes and consequently reduce their solubility within sample solution. To investigate the influence of pH on extraction efficiency, the pH values of the sample solution were adjusted in a range of 2 and 6 by 1 M HCl or NaOH solution. As can be seen in Fig. 2, with increases in pH, the sorption amount of SPE increased before reaching a maximum at pH 4.0. Higher pHs weakened the extraction efficiency because the antibiotics were in ionic state which preferred to stay in water. The results of DLLME were consistent with that of SPE (Fig. 3); therefore, pH 4.0 was selected as optimum for the subsequent study.

Effect of the flow rate of the sample solution

The flow rate of the sample solution through SPE cartridge influences the effective retention and controls the analytical time. In our study, different flow rates (5, 10, 15, 20, 25 mL min^{-1}) were investigated. As can be seen from Fig. 4, the recovery of all antibiotics almost unchanged in the flow rate 5–10 mL min^{-1}, indicating that it was slow enough to perform an effective retention. But then along with the increase of the flow rate, the recoveries were decreased. Considering the flow rate must be high enough to shorten the analytical time, 10 mL min^{-1} was selected as the final flow rate.

Effect of the elution solvent type and its volume

Some researchers have reported that the elution solvent could be the next DLLME's extraction solvent, or the mixture of extraction and dispersive solvent [21]. And the elution solvent must be able to dissolve the analytes to ensure the recoveries. Several solvents were studied in our experiment, including methanol, methanol/acetonitrile (1:1, *v*/*v*), dichloromethane, acetonitrile-dichloromethane (1:1, *v*/*v*), and 0.5 % ammonia solution in methanol. From the obtained results (Fig. 5), methanol was chosen as the elution solvent used in all further experiments.

The volume of elution solvent was another important factor for SPE. In our study, 2, 3, 4, 5, 6 mL were investigated. Recoveries were increased from 2 mL to 5 mL, but there was no significant increase on recoveries of all antibiotics from 5 mL to 6 mL (Fig. 6). Consequently, 5 mL was employed as the amount of methanol in the following experiments.

Effect of the extraction solvent and its volume

In DLLME, the extraction solvent can significantly affect the extraction of the target analytes in this experiment. The extraction solvent should meet some requirements, such as higher density than water, low solubility in water, and high extraction capability for the analytes of interest. After

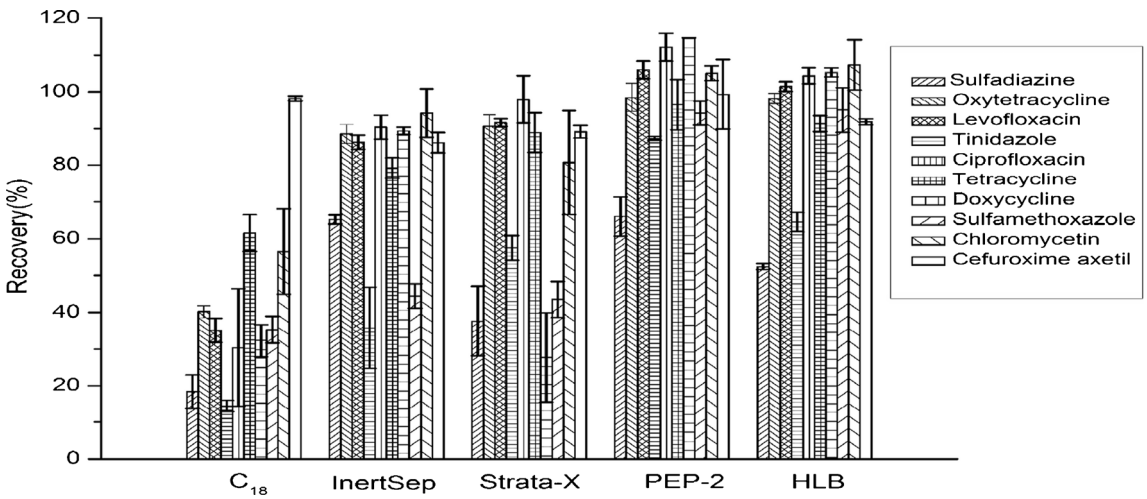

Fig. 1 Effect of SPE materials on the recoveries of the antibiotics. pH, 4.0; flow rate, 5~10 mL min^{-1}; elution solvent, methanol, 5 mL; extraction solvent, dichloromethane, 800 μL; dispersive solvent, acetonitrile, 1 mL; no salt

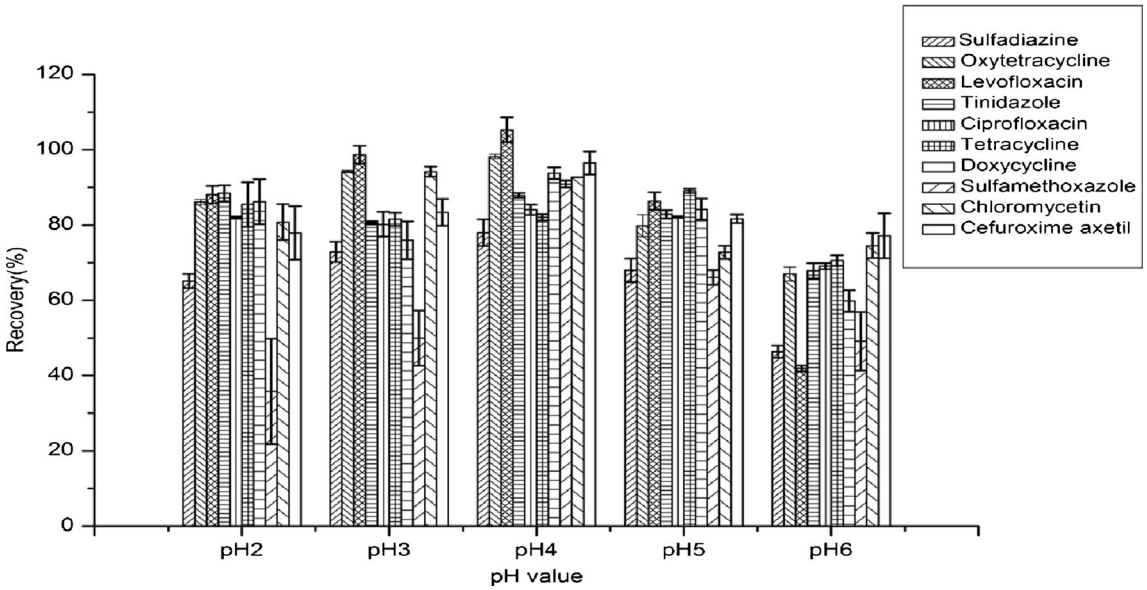

Fig. 2 Effect of pH on the recoveries of the antibiotics for SPE. SPE column, PEP-2; elution solvent, methanol, 5 mL; extraction solvent, dichloromethane, 800 μL; dispersive solvent, acetonitrile, 1 mL; no salt

extraction, the mixture must form a stable two-phase system to separate the extraction layer. Based on these criteria, $C_2H_4Cl_2$, C_6H_5Cl, $CHCl_3$, CH_2Cl_2, and CCl_4 were selected for this study (Fig. 7). CH_2Cl_2 was selected as the extraction solvent.

Figure 8 shows the variation of extraction recovery versus volume of CH_2Cl_2. As the volume of CH_2Cl_2 increased, the recovery was first increased until 800 μL, and then remained for almost target analytes but decreased for some analytes when more than 800 μL of CH_2Cl_2 was used. The results indicated that the best extraction efficient was obtained when 800 μL CH_2Cl_2 was used.

Effect of the dispersive solvent and its volume

The dispersive solvent has to be miscible with both water and CH_2Cl_2, and it also has to promote the dispersion of extraction solvent in water samples. Methanol, ethanol, acetonitrile, and acetone are the most widely used dispersive solvent. In addition, methanol/acetonitrile (1:1, *v*/*v*) was investigated. As a result (Fig. 9), methanol/acetonitrile (1:1, *v*/*v*) gave the best extraction efficiency, so methanol/acetonitrile (1:1, *v*/*v*) was selected as the dispersive solvent.

Different volumes of dispersive solvent (600, 800, 1000, 1100, 1200, 1300, and 1400 μL) were evaluated. It

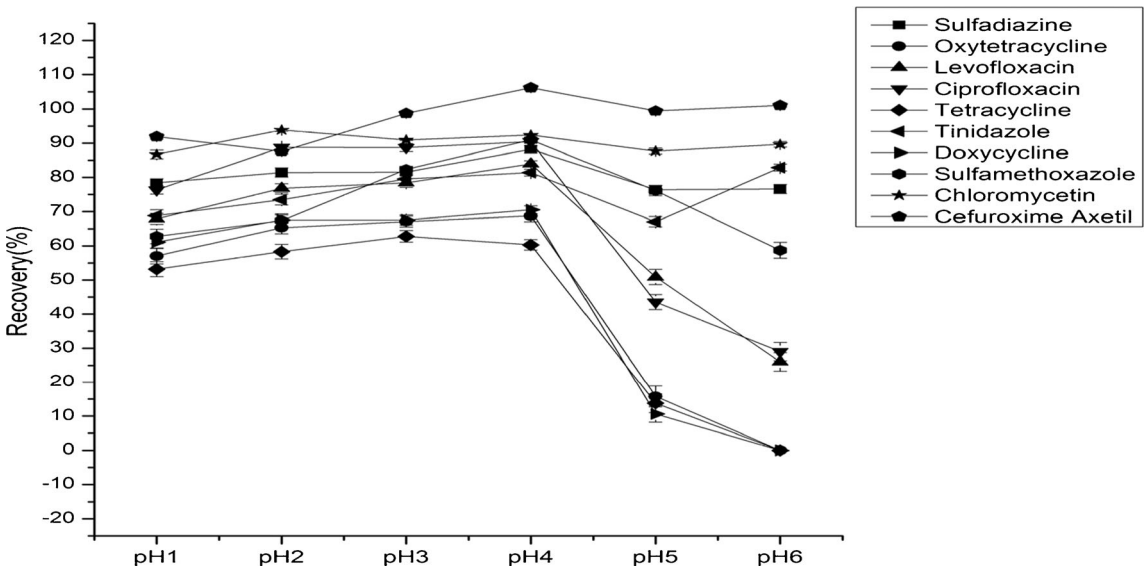

Fig. 3 Effect of pH on the recoveries of the antibiotics for DLLME. SPE column, PEP-2; flow rate, 10 mL min^{-1}; elution solvent, methanol, 5 mL; pH, 4.0; extraction solvent, dichloromethane, 800 μL; dispersive solvent, methanol/acetonitrile (1:1, *v*/*v*), 1 mL; no salt

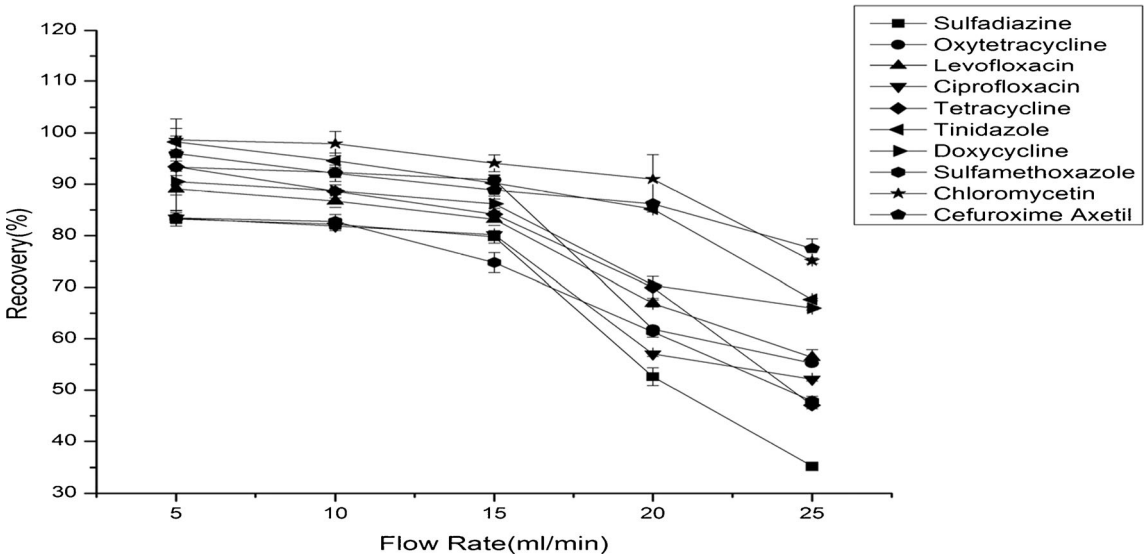

Fig. 4 Effect of flow rate on the recoveries of the antibiotics. SPE column, PEP-2; pH, 4.0; elution solvent, methanol, 5 mL; extraction solvent, dichloromethane, 800 μL; dispersive solvent, acetonitrile, 1 mL; no salt

was obvious that from Fig. 10 that 1200 μL dispersive solvent has slightly higher recovery than that of others. Therefore, 1200 μL was selected as the volume of dispersive solvent.

Effect of ionic strength

The effect of ionic strength on extraction was examined using different concentrations of NaCl (0–10 %, *w*/*v*) as electrolyte. Results showed the different salt concentrations had no significant effect on the recoveries of the antibiotics. Therefore, NaCl was not added in this method.

Application of SPE-DLLME in water samples

Matrix effect

The occurrence of matrix effect (ME) is mainly from the endogenous component of the samples. The ESI source is highly susceptible to the endogenous component in the matrix, such as natural organic matter, salts, and ion pair reagents and so on, which may typically result in a signal suppression or enhancement leading to erroneous results. Matrix effect has significant interference for the analysis of the targets and affects the accuracy and precision of the method. So it is necessary to investigate the matrix effect.

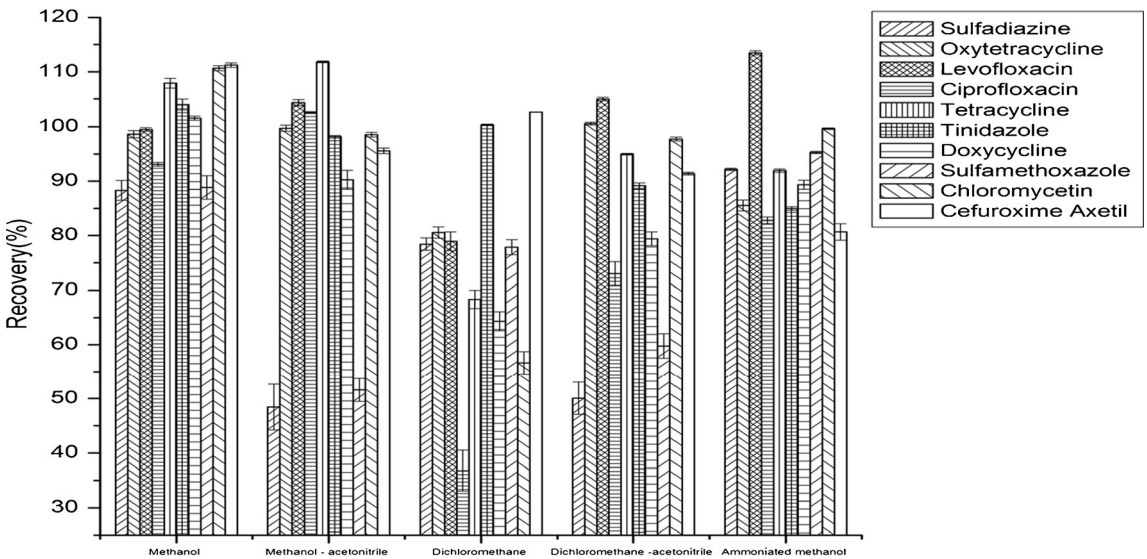

Fig. 5 Effect of the elution solvent type on the recoveries of the antibiotics. SPE column, PEP-2; flow rate, 10 mL min^{-1}; pH, 4.0; volume of elution solvent, 5 mL; extraction solvent, dichloromethane, 800 μL; dispersive solvent, acetonitrile, 1 mL; no salt

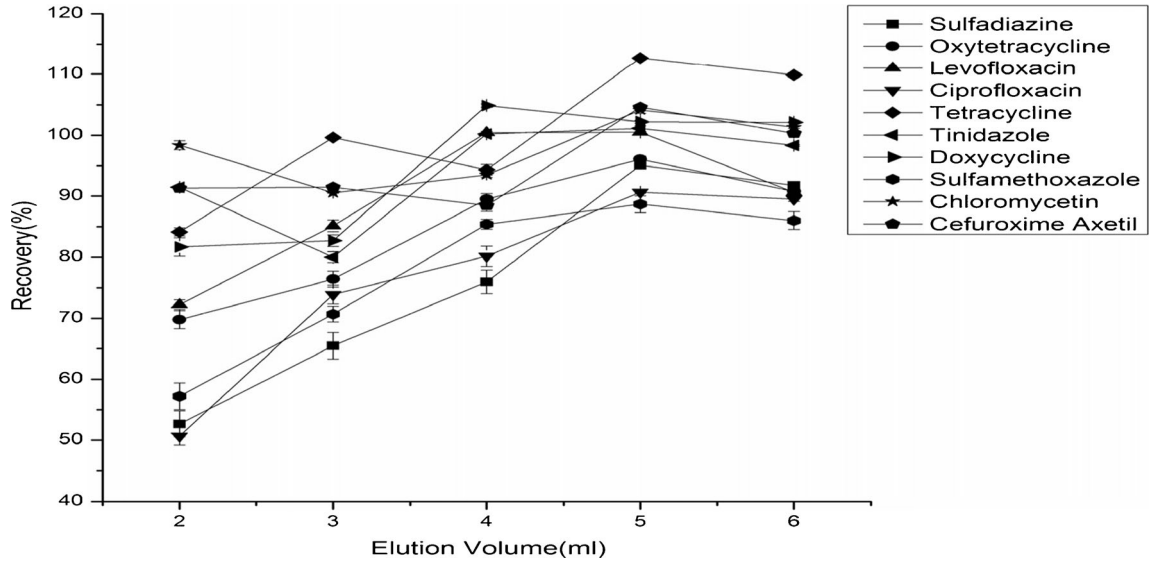

Fig. 6 Effect of the volume of elution solvent on the recoveries of the antibiotics. SPE column, PEP-2; flow rate, 10 mL min^{-1}; pH, 4.0; elution solvent, methanol; extraction solvent, dichloromethane, 800 μL; dispersive solvent, acetonitrile, 1 mL; no salt

ME, recovery of sample preparation procedure (R), and overall process efficiency (PE) was established according to Niessen et al. [29]. To investigate the ME in different environment, a series of responses were set as follows. A was set as the responses of each real samples (drinking water, running water, river water, or wastewater), B was standard solution, C was pre-extraction spiked samples with concentration that was same as B, and D was the post-extraction. And then, the ME, recovery of extraction procedure (RE) and overall PE was calculated from the following equation. ME, overall PE, and their precision (RSD) of the proposed method in different environmental water matrices spiked at the 100 ng mL^{-1} level are indicated in Table 2.

$$\mathrm{ME}(\%) = (D{-}A)/B \times 100$$

$$\mathrm{RE}(\%) = (C{-}A)/(D{-}A) \times 100$$

$$\mathrm{PE}(\%) = \mathrm{ME} \times \mathrm{RE}/100$$

Under the premise that the chromatographic condition is good, the method to eliminate or reduce the matrix effect is the appropriate sample pretreatment. So the matrix effect of SPE was also considered to compare with the method used in this study, whose results were exhibited in Fig. 11. Significant matrix effect for many of the antibiotics were observed for SPE in effluent wastewater (34.47–87.80 %), while SPE-DLLME (65.50–95.90 %) had an obvious superiority over

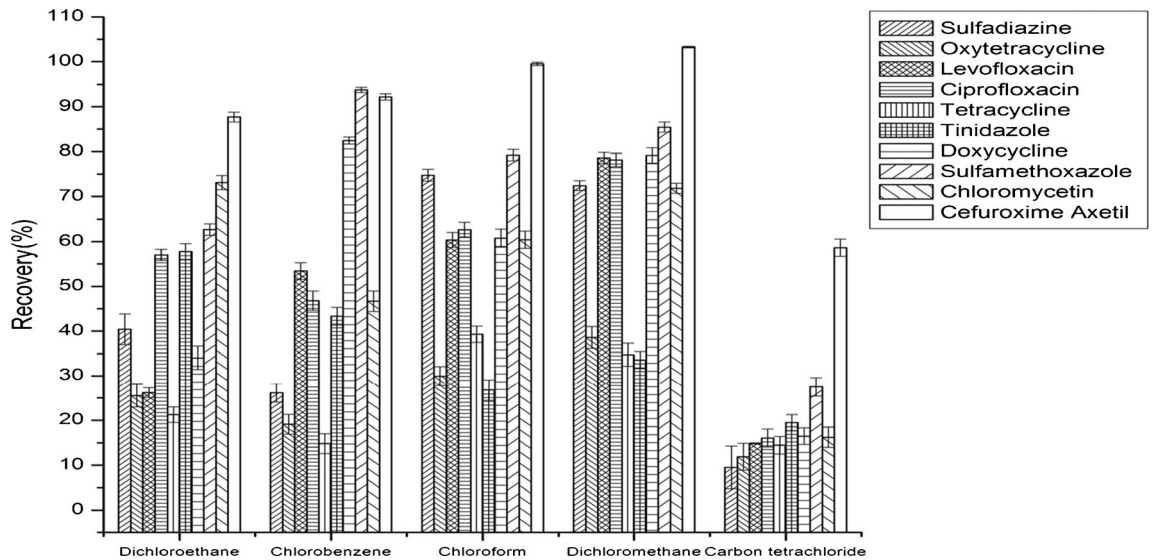

Fig. 7 Effect of the extraction solvent on the recoveries of the antibiotics. SPE column, PEP-2; flow rate, 10 mL min^{-1}; pH, 4.0; elution solvent, methanol, 5 mL; volume of extraction solvent, 800 μL; dispersive solvent, acetonitrile, 1 mL; no salt

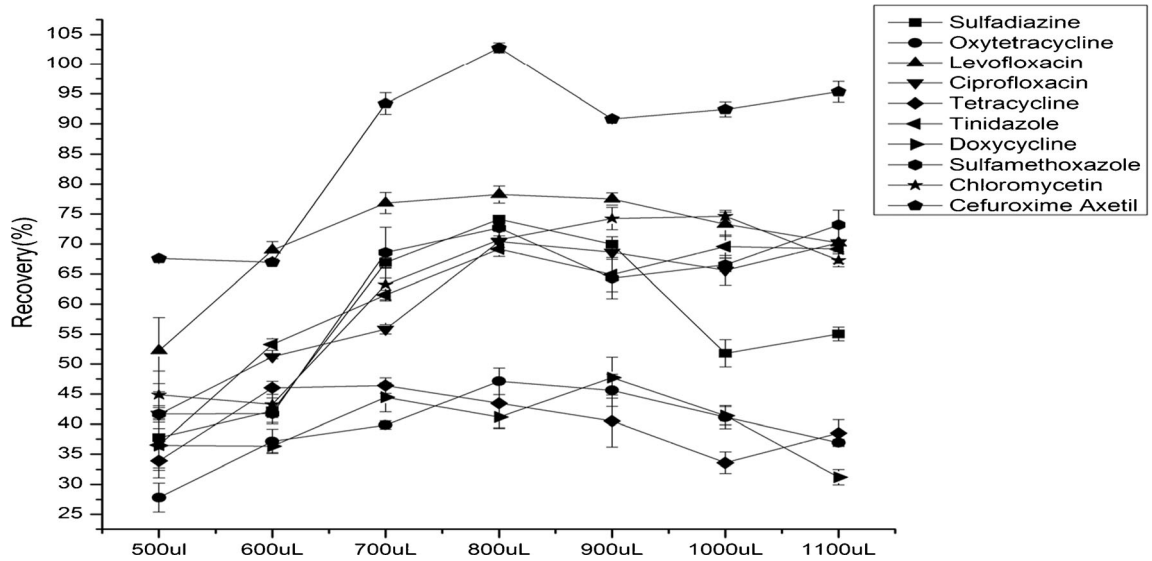

Fig. 8 Effect of the volume of extraction solvent on the recoveries of the antibiotics. SPE column, PEP-2; flow rate, 10 mL min^{-1}; pH, 4.0; elution solvent, methanol, 5 mL; extraction solvent, dichloromethane; dispersive solvent, acetonitrile, 1 mL; no salt

SPE only, which meant that the results obtained using SPE-DLLME were closer to the actual contents of samples. The possible reason is that the process of DLLME has further purified the matrix on the basis of SPE process as the extraction solvent has similar polarity with our target analytes. Thus, there would be an obvious decreasing in the endogenous component of the samples to reduce the influence of co-existing matrix constituents.

Evaluation of the method

Using the optimized conditions, the analytical characteristics of the proposed method were determined in terms of linearity, repeatability, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). The obtained results are listed in Tables 3 and 4.

Linearity of the method was estimated in the working range of 5–1000 ng mL^{-1} for tinidazole at eight concentration levels; 10–1000 ng mL^{-1} for chloramphenicol at seven concentration levels; 2–1000 ng mL^{-1} for levofloxacin, oxytetracycline, doxycycline, tetracycline, and ciprofloxacin at nine concentration levels; 1–1000 ng mL^{-1} for sulfamethoxazole and cefuroxime axetil at ten concentration levels; and 1–400 ng mL^{-1} for sulfadiazine at nine concentration levels. For each level, three replicate extractions were performed. All the experiments were carried out by a series of solutions

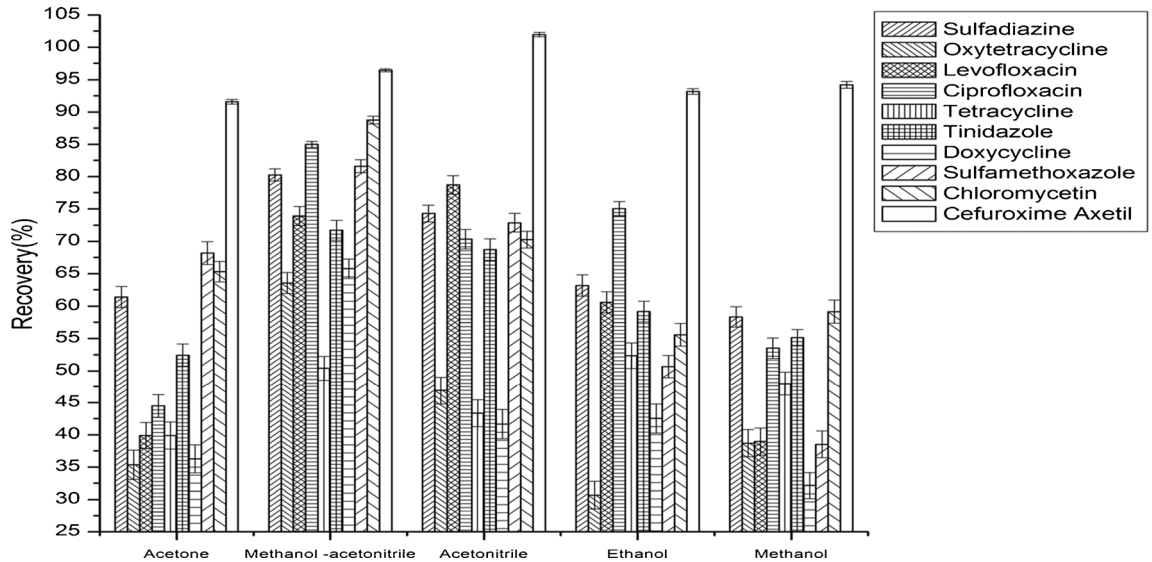

Fig. 9 Effect of the dispersive solvent on the recoveries of the antibiotics. SPE column, PEP-2; flow rate, 10 mL min^{-1}; pH, 4.0; elution solvent, methanol, 5 mL; extraction solvent, dichloromethane, 800 μL; volume of dispersive solvent, 1 mL; no salt

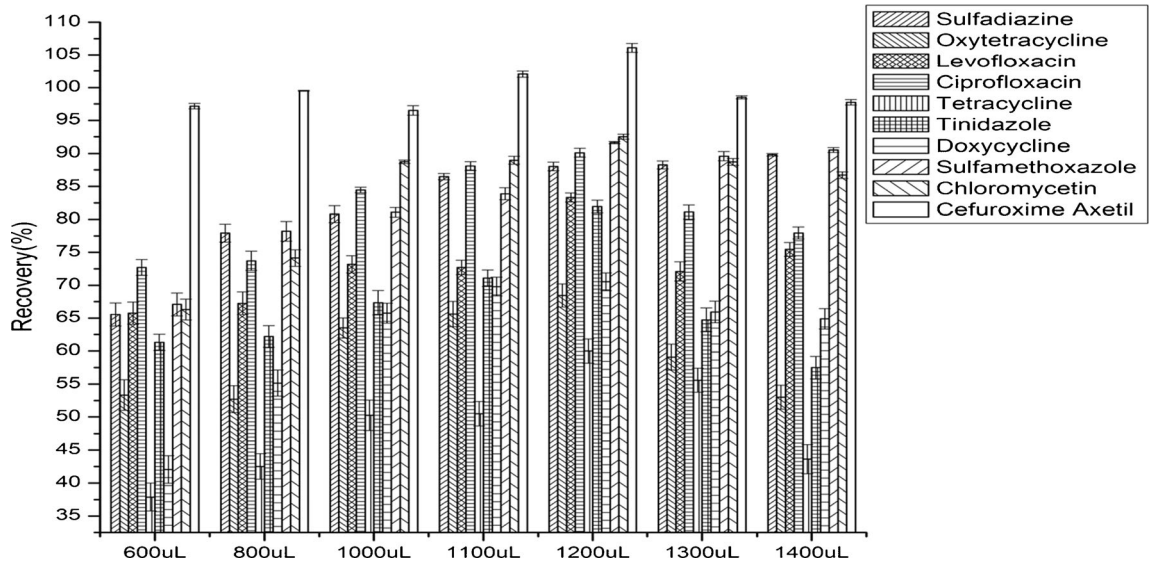

Fig. 10 Effect of the volume of dispersive solvent on the recoveries of the antibiotics. SPE column, PEP-2; flow rate, 10 mL min^{-1}; pH, 4.0; elution solvent, methanol, 5 mL; extraction solvent, dichloromethane, 800 μL; dispersive solvent, methanol/acetonitrile (1:1, *v*/*v*); no salt

containing standards through a whole extraction procedure. The linearity of calibration was tested by the analysis of variance (ANOVA). Full calibration curves of the 10 analytes calculated by least squares regression and the performance characteristics are presented in Table 3. The satisfactory correlation coefficients, the F values and t values ($\alpha = 0.05$, $p < 0.001$) of ANOVA confirmed that all of the 10 analytes responses were linear over the studied range.

The resultant repeatabilities were investigated by six running water samples spiked with 100 ng mL^{-1}, and expressed as RSDs varied from 2.0 to 9.6 %, which showed an acceptable repeatability. The inter-day precision was measured in 3 days with six repetitions of working standard solutions of 100 ng mL^{-1} each day, with RSDs in the range of 0.2–3.8 %. All the data show that the proposed method has a satisfied precision. The LOD and LOQ, determined by the serial dilution of working standard solutions at signal-to-noise ratios (S/N) of 3 and 10, ranged from 0.08–1.67 ng mL^{-1} and 0.27–5.57 ng mL^{-1}, respectively, which confirm a sensitive detection of the proposed method. The stability of the target analytes in the final extraction solution stored at −3 °C ~ −5 °C was tested by replicate assays of the solution at 0, 2, 4, 6, 8, 12, and 24 h. And the sample solution was found to be stable from 0 to 24 h with RSD values was lower than 9.62 % ($n = 3$), indicating a good stability of all target analytes.

The spiking recoveries of the target analytes in influent wastewater at different concentration levels are summarized in Table 4. For each concentration level, three replicate

Table 2 Matrix effect (ME), overall process efficiency (PE) and their precision (RSD%) of the proposed method in different environmental water matrices spiked at the 100 ng mL^{-1} level

Antibiotics	Drinking water		Running water		River water		Influent water		Effluent water	
	ME (RSD%)	PE (RSD%)	ME (RSD%)	PE (RSD%)	ME (RSD%)	PE (RSD%)	ME (RSD%)	PE (RSD%)	ME (RSD%)	PE (RSD%)
Levofloxacin	48 (5)	45 (3)	80 (4)	78 (5)	73 (6)	75 (8)	73 (4)	73 (1)	68 (2)	65 (3)
Ciprofloxacin	98 (3)	97 (1)	90 (7)	95 (1)	77 (7)	75 (6)	79 (5)	75 (4)	80 (5)	82 (3)
Oxytetracycline	110 (1)	78 (5)	82 (2)	60 (6)	86 (3)	61 (3)	80 (3)	57 (2)	86 (1)	64 (5)
Tetracycline	107 (2)	84 (3)	94 (7)	70 (4)	73 (5)	55 (4)	79 (6)	58 (3)	78 (8)	56 (6)
Sulfadiazine	92 (3)	93 (5)	98 (1)	95 (5)	84 (2)	89 (7)	88 (1)	91 (2)	96 (7)	94 (2)
Tinidazole	98 (5)	97 (1)	104 (1)	95 (2)	87 (5)	91 (2)	82 (6)	81 (3)	82 (3)	87 (2)
Doxycycline	103 (4)	77 (6)	90 (3)	65 (6)	88 (3)	62 (2)	80 (2)	57 (3)	90 (2)	63 (6)
Sulfamethoxazole	104 (2)	94 (5)	79 (5)	81 (1)	83 (2)	71 (1)	76 (1)	65 (3)	81 (2)	79 (4)
Chloromycetin	97 (2)	90 (5)	72 (1)	74 (5)	67 (3)	62 (2)	58 (4)	52 (4)	65 (5)	64 (4)
Cefuroxime axetil	92 (3)	90 (3)	97 (3)	97 (3)	92 (2)	96 (3)	100 (5)	94 (7)	91 (6)	95 (6)

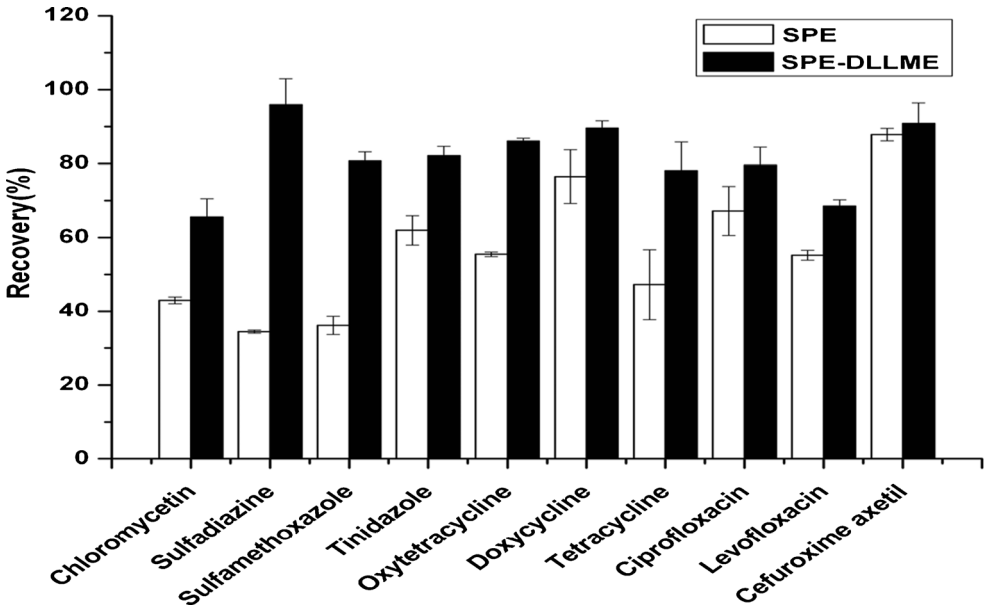

Fig. 11 Matrix effect (ME) for SPE and SPE-DLLME in effluent wastewater spiked at the 100 ng mL^{-1} level

experiments with the whole analysis process were performed. The recoveries of the method for the analytes were in the range between 64.16 and 99.80 % with relative standard deviations between 0.7 and 8.4 %, indicating a good performance of the SPE-DLLME method for the determination of the ten antibiotics.

These results showed that the proposed method has a high sensitivity and repeatability.

Real samples analysis

To evaluate the performance of the presented method and the content of antibiotics in water environment, the proposed analytical procedure was applied to the analysis of real samples. As can be seen in Table 5, all of the target 10 compounds were detected above the LOD in drinking water and influent wastewater; 9 antibiotics in running water and river water and 8 antibiotics in effluent wastewater. Antibiotics have been detected in drinking, running, and river water mainly due to their wide consumption and continuous release into the water and incomplete treatment. Comparing with the content of antibiotics in influent and effluent wastewater, we can see there was only a small decrease in effluent wastewater in general, which indicated that antibiotics cannot be effectively removed by water treatment process in wastewater treatment plant [30].

Table 3 The performance characteristics of SPE-DLLME combined UHPLC-MS/MS

Antibiotics	Linear range (ng mL^{-1})	r	F	T	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Precision RSD % ($n=18$)	Repeatability RSD % ($n=6$)
Levofloxacin	2~1000	0.9956	1599[a]	39.992[a]	0.29	0.97	0.5	3.7
Ciprofloxacin	2~1000	0.9997	22,520[a]	150.007[a]	0.21	0.70	1.5	9.6
Oxytetracycline	2~1000	0.9963	1960[a]	43.669[a]	0.36	1.20	0.2	3.6
Tetracycline	2~1000	0.9927	956[a]	30.916[a]	0.27	0.90	0.4	3.4
Sulfadiazine	1~400	0.9944	1240[a]	35.210[a]	0.11	0.37	1.7	2.0
Tinidazole	5~1000	0.9979	11,527[a]	107.364[a]	1.17	3.90	0.5	5.5
Doxycycline	2~1000	0.9920	868[a]	29.459[a]	0.08	0.27	2.0	3.1
Sulfamethoxazole	1~1000	0.9989	7464[a]	86.397[a]	0.17	0.57	1.2	4.3
Chloramphenicol	10~1000	0.9957	1867[a]	43.209[a]	1.67	5.57	3.8	3.0
Cefuroxime axetil	1~1000	0.9876	629[a]	25.073[a]	0.23	0.77	2.5	2.7

[a] Significant coefficient ($s < 0.001$)

Table 4 Spiked recoveries of the influent wastewater samples

Antibiotics	Spiked (ng mL^{-1})	Found (ng mL^{-1})	*R* (%)	RSD (%)
Levofloxacin	0	58.40		
	20	75.32	96.07	3.9
	100	154.82	97.74	1.3
	500	542.93	97.23	1.0
Ciprofloxacin	0	15.46		
	20	30.18	85.10	2.6
	100	111.70	96.74	6.7
	500	502.21	97.43	5.3
Oxytetracycline	0	11.33		
	20	21.60	68.94	3.5
	100	80.98	72.74	1.3
	500	357.01	69.82	3.5
Tetracycline	0	12.90		
	20	23.58	71.67	8.4
	100	76.67	67.91	5.8
	500	329.08	64.16	3.2
Sulfadiazine	0	14.89		
	8	20.65	90.23	5.5
	40	48.92	89.13	2.4
	200	182.18	84.78	3.6
Tinidazole	0	25.78		
	20	43.84	90.28	2.4
	100	121.62	95.84	5.1
	500	515.58	97.96	1.0
Doxycycline	0	12.31		
	20	22.79	70.54	5.4
	100	84.52	75.26	5.5
	500	381.62	74.49	1.8
Sulfamethoxazole	0	78.96		
	20	94.40	95.39	3.5
	100	172.62	96.46	8.2
	500	556.21	96.07	1.2
Chloramphenicol	0	2.60		
	20	21.53	95.26	6.6
	100	96.40	93.96	4.0
	500	468.57	93.23	4.4
Cefuroxime axetil	0	11.94		
	20	31.88	99.80	3.9
	100	108.11	96.58	0.7
	500	483.12	94.37	2.5

Comparison of SPE-DLLME with other methods

To highlight the robust application of the presented SPE-DLLME method, it was compared to several published methods for the determination of antibiotics such as SPE-UHPLC-MS/MS, DLLME-UHPLC etc. [14, 29, 31, 32]. As listed in Table 6, the proposed method has higher EF value between 1763 to 4990. Good sensitivity is also obtained by the present method as the LOD was 0.08~1.67 ng mL^{-1}. The recoveries of the antibiotics obtained by four other methods range from 58.7 to 100.9 %, 78 to 117 %, 72.3 to 104.4 %, and 70 to 120 %, respectively. By comparison, the recoveries obtained by the present method range from 64.16 to 99.80 %. In addition, the consumption of overall time was acceptable because of low volume of water sample and simple experimental procedure.

Table 5 Determination of the 10 antibiotics in real water samples (ng mL^{-1})

Antibiotics	Drinking water	Running water	River water	Influent wastewater	Effluent wastewater
Levofloxacin	3.3	7.7	100.4	58.4	12.2
Ciprofloxacin	3.9	6.4	19.4	15.5	6.2
Oxytetracycline	4.3	3.8	13.2	11.3	33.6
Tetracycline	5.2	5.5	9.7	12.9	–
Sulfadiazine	1.4	1.5	3.9	14.9	3.9
Tinidazole	12.1	6.1	13.4	25.8	89.6
Doxycycline	6.3	8.1	10.9	12.3	9.7
Sulfamethoxazole	1.96	2.3	22.8	79.0	23.1
Chloramphenicol	< LOQ	–	–	< LOQ	–
Cefuroxime axetil	6.2	4.9	9.9	11.9	9.6

–Not detected

Conclusion

In this study, SPE-DLLME with UHPLC-MS/MS has been developed as a novel method for the extraction of 10 antibiotics in different water samples. The method showed numerous advantages, such as high enrichment factor, low limits of quantification, high recovery for most analytes, and broad application in complex environmental samples. Furthermore, compared with conventional sample preparation methods, the analytical technique developed in this work was characterized by lower matrix effect and higher accuracy.

The whole method has been successfully applied to the extraction and analysis of antibiotics in different water samples (drinking water, running water, river water, and wastewater) with satisfactory results. Accordingly, it shows great potential in the analysis of ultra-trace compounds in different water samples.

Table 6 Comparison of SPE-DLLME-UHPLC-MS/MS with other methods for the determination of antibiotics

Methods	Analytes	Analytes in common with this report	Matrix	LOD (ng mL^{-1})	Precision (RSD%)	EF	Extraction time (min)	Recovery (%)	References
SPE HPLC/DAD	Trimethoprim, sulfamethoxazole, ciprofloxacin, dexamethasone, febantel	Sulfamethoxazole, ciprofloxacin	Production wastewater	0.7–3.9	7.5–17.0	<100	–	58.7–100.9	[31]
DLLME-UHPLC/DAD	11 sulfonamides, 14 quinolones	Levofloxacin, ciprofloxacin, sulfadiazine, sulfamethoxazole	Mineral water; Run-off water	0.35–10.5	1–20	26–39	25	78–117	[18]
DLLME-UPLC-MS/MS	17 quinolones, 14 β-lactams	Ciprofloxacin	Raw cow milk	0.1–2.0	0.1–9.3	3.6–5.2	>25	72.3–104.4	[24]
SPE-UHPLC-MS/MS	47 pharmaceuticals (including 26 antibiotics)	Ciprofloxacin, sulfadiazine, sulfamethoxazole, chloramphenicol	Surface water; Effluent wastewater	0.005–4.3	<20	–	>35	70–120	[32]
SPE-DLLME-UHPLC-MS/MS	Levofloxacin, ciprofloxacin, oxytetracycline, tetracycline, tinidazole, sulfadiazine, doxycycline, sulfamethoxazole, chloramphenicol, cefuroxime axetil	–	Drinking water; Running water; Influent wastewater; Effluent wastewater; River water	0.08–1.67	2.0–9.6	1763–4990	95	64.16–99.80	Represented method

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Compliance with ethical standards

Conflict of interest The authors have declared that there are no conflicts of interest.

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