Hindawi
Journal of Chemistry
Volume 2019, Article ID 4783432, 9 pages
https://doi.org/10.1155/2019/4783432

Research Article

Microextraction by Packed Molecularly Imprinted Polymer Combined Ultra-High-Performance Liquid Chromatography for the Determination of Levofloxacin in Human Plasma

Jia Meng[1] **and Xu Wang**[2]

[1]*Department of Kidney Disease and Blood Purification Center, The Second Hospital of Tianjin Medical University, Tianjin 300211, China*
[2]*Department of Urology, Tianjin Institute of Urology, The Second Hospital of Tianjin Medical University, Tianjin 300211, China*

Correspondence should be addressed to Xu Wang; johnwx2003@tmu.edu.cn

Received 22 June 2018; Revised 20 August 2018; Accepted 17 December 2018; Published 21 January 2019

Academic Editor: Victor David

Fluoroquinolones are considered as gold standard for the prevention of bacterial infections. To improve assessment of antibacterial efficacy, a novel method for determination of levofloxacin was developed and validated. Deep eutectic solvents (DESs) as only green solvent were used as a porogen for preparation of water-compatible molecularly imprinted polymers (MIPs) with a pseudotemplate. The DESs-MIPs were characterized in detail, including scanning electron microscope, nitrogen sorption porosimetry, and Fourier transform-infrared spectra. Clearly, the maximum binding capacity of levofloxacin on DESs-MIPs in water and methanol was 0.216 and 0.077 μmol g^{-1}, respectively. The DESs-MIPs as adsorbing materials were applied in microextraction by packed sorbent (MEPS), and the DESs-MIPs-MEPS conditions were optimized. The DESs-MIPs-MEPS coupled with ultra-high-performance liquid chromatography (UHPLC) was used to determine levofloxacin in human plasma. The method was found linear over 0.05–10 μg mL^{-1} with coefficient of correlation equal to 0.9988. The limit of detection and limit of quantification were 0.012 and 0.04 μg mL^{-1}, respectively. At three spiked levels, the precision of proposed method was between 95.3% and 99.7% with intraday and interday relative standard deviations ≤8.9%. Finally, the developed method was used to examine levofloxacin from human plasma of 20 hospitalized patients after transrectal ultrasound-guided prostate biopsy, and the average concentration (±SD) of levofloxacin was 2.35 ± 0.99 μg mL^{-1} in plasma.

1. Introduction

Bacterial resistance of antimicrobial agents has become a serious public health problem in the worldwide context for several decades. New drug-resistant bacterial strains are emerging every day, resulting in wide-spread of antibiotic resistance. Therefore, the World Health Organization (WHO) has proposed the possible strategic objectives for tackling antimicrobial drug resistance [1]. One of them emphasizes strengthening research of new antibacterial agents, whereas another relies on the optimization of the usage of antimicrobial medicines. To tackle the spread of irrational use, accurate evaluation of prescribed antimicrobial agents is necessary [2]. Levofloxacin, one of the commonly used third-generation quinines, has a broad spectrum of activity against Gram-positive and Gram-negative bacteria [3, 4]. Due to its low probability of adverse effects and outstanding antibacterial activity with oral administration, levofloxacin has been widely applied to the treatment of infectious diseases, including acute exacerbation of chronic bronchitis and community-acquired pneumonia [5, 6].

Recently, numerous chromatographic methods have been reported for the quantification of levofloxacin in body fluid samples, including traditional high-performance liquid chromatography with ultraviolet detection (HPLC-UV) [7–10], high-performance liquid chromatography with fluorescence detection (HPLC-FL) [11, 12], high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [13, 14], ultra-high-performance liquid chromatography with ultraviolet detection (UHPLC-UV) [2, 15], and ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) [16]. The mass

spectrometry technique could create a low detection limit and high resolution, but it is not widely available for all laboratories, resulting from the high cost of the equipment. Anyhow, a valid sample pretreatment is necessary prior to chromatographic analysis. The main purpose of sample pretreatment is to eliminate interferences, enrich target analytes, and create a reproducible method [17].

Microextraction by packed sorbent (MEPS), initially introduced by Abdel-Rehim et al. [18], is a miniaturization of conventional solid-phase extraction (SPE) with the advantages of easy-to-automate, cost-effective, eco-friendly, and time efficient. Thus, the new sample pretreatment technology has been widely used in food, environmental water, and biological sample analyses [17, 19–21]. However, the commercial MEPS sorbents, such as C2, C8, and C18, ionic exchange sorbent, and polystyrene polymer [19] lack selectivity, which usually results in decrease of separation and enrichment efficiency. Therefore, selective sorbent, for example, molecularly imprinted polymers (MIPs), is developed to meet the requirement of selective extraction of target analytes from complex matrix samples [22].

MIPs are the synthetic materials with the advantage of high predetermined recognition ability to target molecules, and they combined with MEPS have been used for the selective extraction of drugs from human plasma and urine sample [23–25]. In the preparation process of MIPs, porogen plays an important role, which should dissolve template and conduct synthetic reaction. Organic solvents (such as chloroform and toluene) are often employed for obtaining excellent imprinting efficiency. But the side effects of toxic reagents may involve serious risks to operators, as well as damage to the environment [26].

To overcome the toxicity of organic solvents, deep eutectic solvents (DESs) have emerged as a green solvent with the advantages of environmental friendliness, biodegradability, low cost, easy preparation and chemical inertness with water [26]. The DESs can be obtained simply by complexion of quaternary ammonium salts with hydrogen bond donors, which are able to form eutectic mixture. As a result, DESs as porogen have been successfully applied in the synthesis of MIPs, and DESs-MIPs have been used as SPE sorbents [27–31]. However, the application of DESs-MIPs combined with MEPS (DESs-MIPs-MEPS) for extracting levofloxacin from biological samples has not been reported.

In this work, DESs were used as pure porogens for the preparation of DESs-MIPs, and the DESs-MIPs were synthesized using gatifloxacin as pseudotemplate, which could avoid the problem of "template leakage" [32]. The microstructure, chemical composition, and selectivity of DESs-MIPs were characterized in detail. DESs-MIPs-MEPS procedure followed by UHPLC was used for selective enrichment and determination of levofloxacin in human plasma. The scientific novelty of this work is the use of the MEPS technique with DESs-MIPs as packing sorbents.

2. Materials and Methods

2.1. Reagents. Levofloxacin, ciprofloxacin, and gatifloxacin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methacrylic acid (MAA), acetonitrile (HPLC grade), methanol (HPLC grade), acetic acid, ethylene glycol, choline chloride, and 2,2′-azobisisobutyronitrile (AIBN) were purchased from Kermel Chemical Reagents Development Center (Tianjin. China). Ethylene glycol dimethacrylate (EDMA) was obtained from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). All other reagents were of analytical grade and procured from Tianjin Chemical Reagent Ltd. Co. (Tianjin, China).

Stock solutions of levofloxacin and ciprofloxacin were prepared in methanol at a concentration of 100 μg mL^{-1} and stored at −20C°. The working solution of levofloxacin was freshly prepared by dilution of stock solution before use. Internal standard solution of ciprofloxacin was freshly prepared by mixing of stock solution and methanol to gain concentrations of 50 μg mL^{-1}.

2.2. Instrumentation. The morphology of the resultant polymers was measured with scanning electron microscope (SEM, Hitachi X-650, Tokyo, Japan). Brunauer–Emmett–Teller (BET) surface area and porosity of the polymers were measured on a V-Sorb 2800TP surface area and pore size analyzer (Gold APP Instruments, Beijing, China) at 77 K by nitrogen adsorption-desorption isotherms. Fourier transform-infrared (FT-IR) spectra of choline chloride and polymers were recorded on OPUS spectroscopy produced by Bruker Optics (Germany) in the 4000–400 cm^{-1} region. The chromatographic analysis was performed with an Acquity H-Class UHPLC system equipped with a binary high-pressure pump, an autosampler, a thermostated column compartment, and a diode array detector (Water, Milford, MA, USA). The chromatographic separation was performed with ACQUITY UPLC BEH C_{18} column (2.1 mm × 100 mm I.D., 1.7 μm, Water, Milford, MA, USA). Methanol : pH 3 phosphoric acid solution (28 : 72, v/v) was used as the mobile phase with a detection wavelength of 293 nm. The flow rate and the injection volume were 0.3 mL min^{-1} and 1.0 μL, respectively.

2.3. Preparation of DESs-MIPs by Precipitation Polymerization. For preparing DESs, choline chloride (0.1 mol) and ethylene glycol (0.2 mol) were mixed and heated at 80°C until a homogeneous liquid formed. Prepolymerization mixture was prepared by mixing pseudotemplate (gatifloxacin), MAA, EDMA, AIBN, and DESs (Table 1) and placed in dark for 24 h. The mixture was sparged with nitrogen for 10 min, sealed, and polymerized in a 60°C water bath for 24 h. The resultant polymer was crushed with a mortar and pestle, and the template was extracted with acetic acid/MeOH (1/9, v/v) using a Soxhlet apparatus. The nonimprinted polymers (NIPs) without template (DESs-NIPs) were synthesized in the same manner.

2.4. Equilibrium Rebinding Experiments. The dried particle (20.0 mg) was placed in 5 mL Ep tube containing 3 mL of levofloxacin solution (0.1 mmol/L). After incubation for 24 h at room temperature, the suspension was centrifuged and determined by UHPLC. The imprinting effect of the polymer was assessed with the imprinting factor (IF), which is defined as follows:

Table 1: Recipes of resultant polymers.

Names	Porogen (mL)	Gatifloxacin (mmol)	MAA (mmol)	EDMA (mmol)	AIBN (mg)
DESs-MIPs	15 (DESs)	0.2	1.6	6.0	20
DESs-NIPs	15 (DESs)	—	1.6	6.0	20
Methanol-MIPs	15 (methanol)	0.2	1.6	6.0	20
Methanol-NIPs	15 (methanol)	—	1.6	6.0	20

$$\mathrm{IF} = \frac{Q_{\mathrm{MIP}}}{Q_{\mathrm{NIP}}}, \quad (1)$$

where Q_{MIP} and Q_{NIP} are the amount of bound levofloxacin on the DESs-MIPs and DESs-NIPs, respectively.

To estimate the binding parameters of DESs-MIPs and DESs-NIPs, the data of static adsorption experiment were processed with the Langmuir model. The Langmuir model assumes that polymers possess only a single class of binding site and can be expressed as follow:

$$Q_{\mathrm{e}} = \frac{Q_{\max} b C_{\mathrm{e}}}{1 + b C_{\mathrm{e}}}, \quad (2)$$

where $Q_{\max}$ is the apparent maximum number of binding site for levofloxacin, b is the association constant, and C_{e} is the equilibrium template concentrations.

2.5. DESs-MIPs-MEPS Conditions. For the DESs-MIPs-MEPS procedure, the polyethylene frit was inserted into the insulin syringe (1 mL), and then 4.0 mg of DESs-MIPs was packed. After that the second polyethylene frit was inserted into the syringe on the top. Prior to each MEPS, the DESs-MIPs-MEPS syringe was activated with 1.0 mL of methanol and 1.0 mL of water. In a typical process, 400 μL of the sample solution (water-methanol, 25 : 75, v/v) plus 10 μL of internal standard was aspirated 20 times of draw-eject cycles at a speed of 40 μL s^{-1}. Then, the DESs-MIPs-MEPS syringe was washed with 200 μL of water-methanol (50 : 50, v/v) and eluted with 400 μL of acetonitrile/ammonia (95/5, v/v). The eluate was evaporated to dryness with nitrogen gas and quantified to 50 μL of volume with the mobile phase. Finally, the treated sample was injected into UHPLC for quantitative analysis.

2.6. Sample Pretreatment. Plasma samples were collected using the standard venipuncture technique from healthy volunteers and hospitalized patients with transrectal ultrasound-guided prostate biopsy (12 h after oral administration of levofloxacin at a dose of 500 mg, $n = 20$), who had signed the informed consent for the usage of their plasma. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Committee of The Second Hospital of Tianjin Medical University. 100 μL of plasma was added into 300 μL of methanol and 10 μL of internal standard, vortexed for 5 min, and then centrifuged at 12 000 rpm for 5 min. The supernatant was transferred and filtered through a 0.45 μm syringe filter before DESs-MIPs-MEPS.

3. Results and Discussion

3.1. Preparation and Characterization of DESs-MIPs. In traditional MIPs studies, porogen is typically aprotic and weakly polar volatile organic compound, which will promote the formation of template-monomer complex. DESs consist of charged species and exhibit intraliquid structural properties. The DESs, including betaine-ethylene glycol [27], choline chloride-ethylene glycol [28, 31], choline chloride-caffeic acid-ethylene glycol [29], and choline chloride-glycerol [30], have been used as porogen to prepare MIPs. Still, it is necessary to further study DESs-MIPs. In this study, choline chloride-ethylene was selected as porogen for preparing DESs-MIPs. As a control, we also prepared MIPs using methanol as porogen (methanol-MIPs) and nonimprinted polymers using DESs (DESs-NIPs) and methanol (methanol-NIPs) as porogen, respectively.

The resulted polymers were characterized by scanning electron microscopy (SEM), nitrogen adsorption-desorption experiments, and Fourier transform-infrared (FT-IR) spectra. The SEM images (Figures 1(a) and 1(b)) showed that the polymers based on DESs possessed rough and irregular morphology, and a lot of small cavities which could be due to the DESs. But the polymers based on methanol were agglomerated by smaller microglobules with a particle size of near 2–3 μm (Figures 1(c) and 1(d)).

The FT-IR spectra of choline chloride and polymers (Figure 2) provided information to confirm the successful synthesis of DESs-MIPs. Choline chloride had the characteristic peaks of $\nu_{\mathrm{C\text{-}O}}$ (primary alcohol) near 1011 cm^{-1} and $\nu_{\mathrm{C\text{-}N}}$ (tertiary amine) near 1086 cm^{-1}. These characteristic peaks did not appear in the spectra of DESs-MIPs and DESs-NIPs, which indicates that choline chloride is not involved in polymerization and acted just as porogen. The characteristic bands of–COO- (1258 and 1156 cm^{-1}) and C=O (1730 cm^{-1}) in the spectrum of DESs-MIPs and DESs-NIPs demonstrated the existence of poly(EDMA). The significant peak at 3540 cm^{-1} was assigned to O-H stretching from MAA. There are no obvious differences between the spectra of DESs-MIPs and DESs-NIPs, which indicates that pseudotemplate has been washed out completely after elution.

The BET surface areas of DESs-MIPs and DESs-NIPs were 111.2 and 120.9 m^2/g, which are far higher than those of Me-MIP (6.9 m^2/g) and Me-MIP (10.6 m^2/g). In addition, the pore size of DES-based polymer is also far higher than that of methanol-based polymers (Table 2). This was similar to the SEM result, and this indicated that DESs as porogen could change the morphology and improve the surface area and adsorption site of the resultant DESs-MIPs.

3.2. Binding Characteristic and Selectivity. To demonstrate the superior property, the selectivity of resultant polymers was compared in different solvents. As shown in Figure 3, the binding capacity of levofloxacin adsorbed by DESs-MIPs was found to be significantly higher than that of DESs-NIPs,

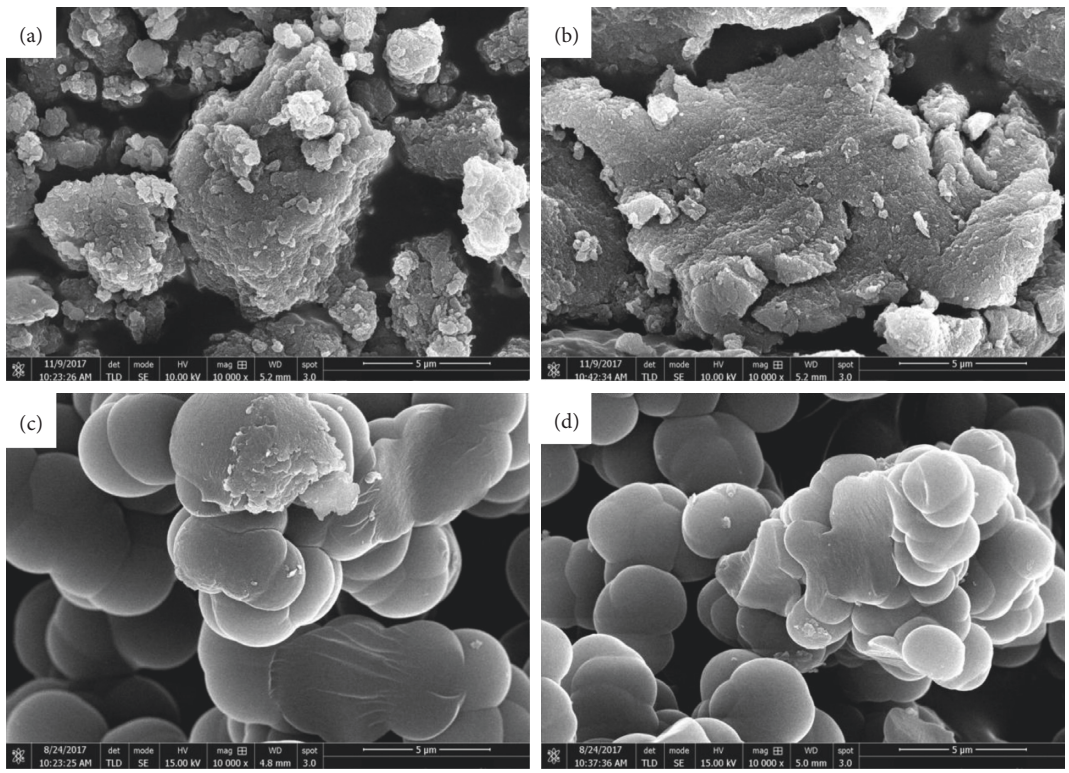

Figure 1: SEM micrographs of DESs-MIPs (a), DESs-NIPs (b), methanol-MIPs (c), and methanol-NIPs (d).

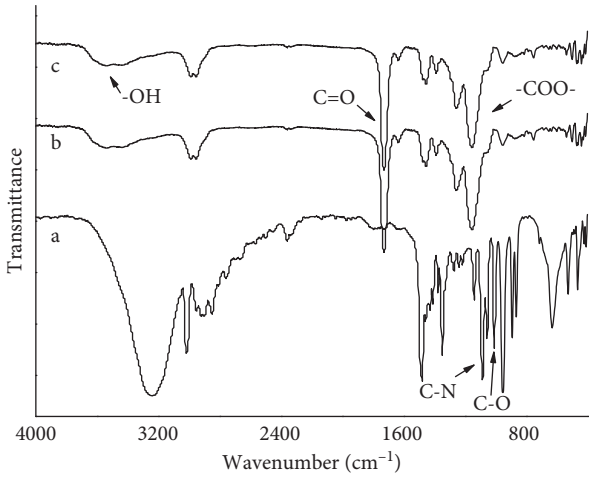

Figure 2: FT-IR spectra of choline chloride (ChCl) (a), DESs-NIPs (b), and DESs-MIPs (c).

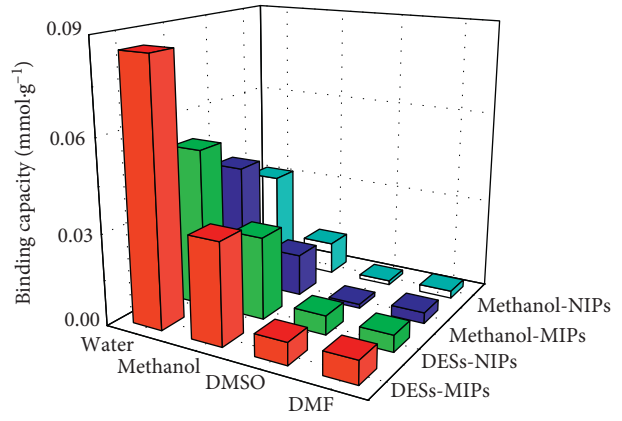

Figure 3: Binding capacities of DESs-MIPs, DESs-NIPs, methanol-MIPs, and methanol-NIPs in water, methanol, dimethyl sulfoxide (DMSO), and dimethyl formamide (DMF). 20.0 mg of dried particles was placed in a 5 mL Ep tube containing 3 mL of levofloxacin solution (1 μmol/L).

Table 2: The pore data and surface areas obtained from the DESs-MIPs, DESs-NIPs, methanol-MIPs, and methanol-NIPs.

Polymer	S_{BET} (m^2 g^{-1})[a]	S_I (m^2 g^{-1})[b]	V_P (cm^3 g^{-1})[c]	D_{mean} (nm)[d]
DESs-MIPs	111.2	91.6	0.99	35.6
DESs-NIPs	120.9	97.2	1.21	39.5
Methanol-MIPs	6.9	3.1	0.03	16.5
Methanol-NIPs	10.6	11.1	0.09	21.1

[a]BET surface area. [b]BJH adsorption cumulative surface area. [c]Single-point adsorption total pore volume. [d]Total adsorption average pore with (4 V/A by BET).

methanol-MIPs, and methanol-NIPs, as well as the binding capacity in water solution was significantly higher than that in methanol, dimethyl sulfoxide, and dimethyl formamide. These results indicated great water compatibility of DESs-MIPs, which resulted from the "solvent effects" that MIPs prepared in polar porogen show better recognition in similarly polar solvent [32].

Figure 4 shows the static adsorption curves in water and methanol. The binding capacities of the DESs-MIPs were much higher than that of DESs-NIPs, methanol-MIPs, and methanol-NIPs at various concentrations. The Q_{max} and b

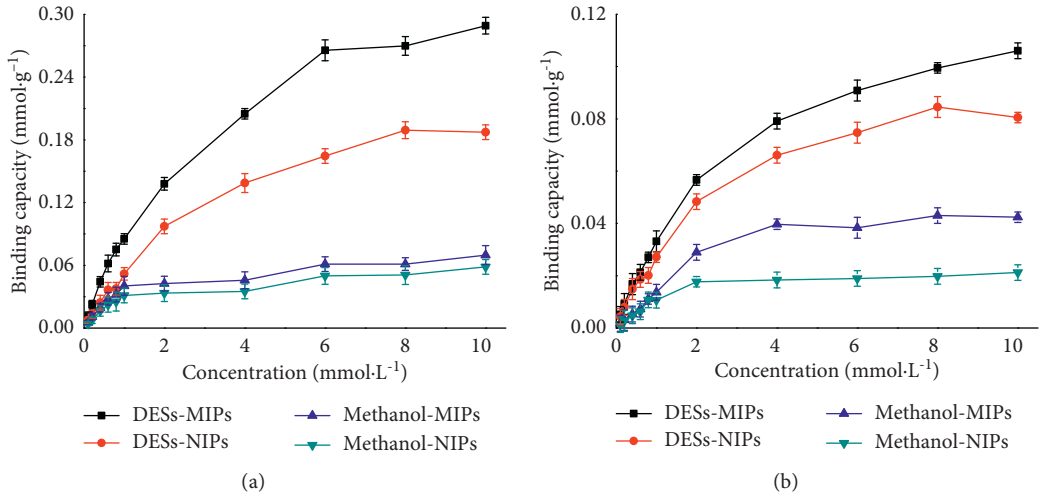

Figure 4: Static adsorption curves in water (a) and methanol (b) of the DESs-MIPs, DESs-NIPs, methanol-MIPs, and methanol-NIPs.

were calculated from the Langmuir model and are listed in Table 3. Clearly, in water, Q_{max} on DESs-MIPs (0.216 mmol/g) was considerably higher than that on DESs-NIPs (0.119 mmol/g), methanol-MIPs (0.097 mmol/g), and methanol-NIPs (0.062 mmol/g). In methanol, the DESs-MIPs likewise possessed higher Q_{max}. The IF of DESs-MIPs was 1.8 in water, while 1.2 in methanol. This result once again indicated the water compatibility of DESs-MIPs.

3.3. Investigation of DESs-MIPs-MEPS Conditions. To enhance the extraction efficiency of DESs-MIPs-MEPS, the relevant conditions were investigated, including the pH of loading sample, volume of loading sample, time of draw-eject cycles, washing solution, and elution solution.

The influence of the pH of the loading sample was studied over the range 2.0–12.0 (adjusted with 1 M of hydrochloric acid and sodium hydroxide). 300 μL of sample containing 0.5 μg mL^{-1} of levofloxacin was loaded. As shown in Figure 5, the recoveries of levofloxacin increased from pH 2.0 to 6.0, remained at the best value with the pH values from 6.0 to 8.0, and decreased from pH 8.0 to 12.0. At a low pH (pH = 2.0), most of these carboxyl groups from the imprinted cavity in polymer skeleton were in the neutral form, whereas levofloxacin was positively charged and interacted mainly with ionized carboxyl groups. This would explain the very low recoveries of levofloxacin. At the pH range 6.0–8.0, most levofloxacins were present in the neutral form, which was beneficial to electrostatic interactions between levofloxacin and DESs-MIPs. At higher a pH (pH = 12.0), the deprotonation of carboxyl groups was enhanced and the protonation of levofloxacin was decreased. This would dramatically decrease ionic interaction between levofloxacin and DESs-MIPs. Therefore, the optimal pH of loading sample was between 6.0 and 8.0, which included the pH of human plasma.

In MEPS, only a few milligrams of sorbent were packed as absorbent material. This resulted in that the extraction capacity was less than the traditional SPE. Fortunately, the increase of draw-eject cycles could enhance the binding amount of target molecules. Therefore, the impact of sample volume and draw-eject cycles on the recovery of levofloxacin was optimized when the concentration of levofloxacin was 0.5 μg mL^{-1}. As shown in Figure 6(a), the recovery of levofloxacin increased with increase of draw-eject cycles and reached the maximum value after 20 of draw-eject cycles. When the time of draw-eject cycles was 20, the recovery of levofloxacin varied from 99.4% to 99.6% with the sample volume increasing from 300 μL to 400 μL and slightly

Table 3: Langmuir fitting parameters for DESs-MIPs, DESs-NIPs, methanol-MIPs, and methanol-NIPs.

Polymer	Solution	b (L mmol^{-1})	Q_{max} (mmol g^{-1})	R^2
DESs-MIPs	Water	2.29	0.216	0.997
	Methanol	1.07	0.077	0.999
DESs-NIPs	Water	1.32	0.119	0.982
	Methanol	0.99	0.066	0.992
Methanol-MIPs	Water	1.11	0.079	0.991
	Methanol	0.48	0.042	0.990
Methanol-NIPs	Water	1.00	0.062	0.992
	Methanol	0.44	0.034	0.991

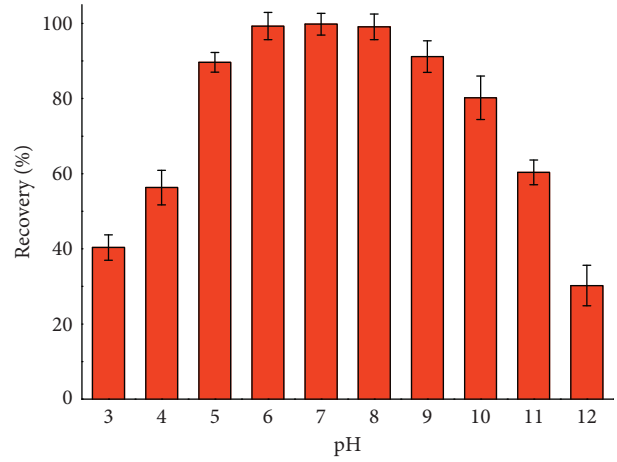

Figure 5: Effect of pH on the recovery of levofloxacin.

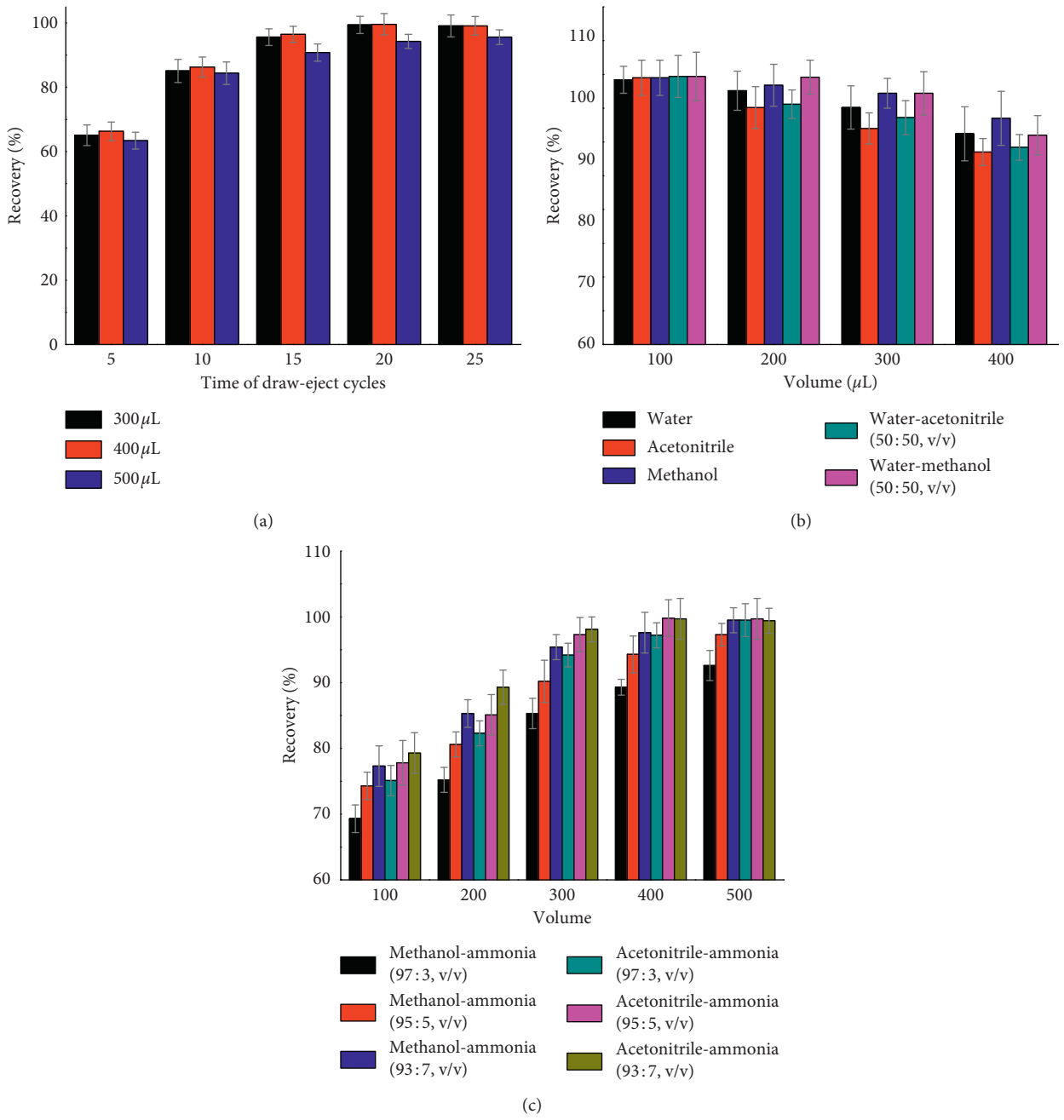

Figure 6: Effect of sample volume and draw-eject cycles (a) washing solution (b), and eluent solution (c) on the recovery of levofloxacin.

decreased to 94.3% with 500 μL of sample volume. Therefore, 400 μL of sample volume and 20 of draw-eject cycles were selected for further MEPS.

The aim of washing is to remove nonspecifically interfering substances. Figure 6(b) exhibits the effect of washing solution on the recovery of levofloxacin, including water, acetonitrile, methanol, water-acetonitrile (50 : 50, v/v), and water-methanol (50 : 50, v/v). 300 μL of sample containing 0.5 μg mL^{-1} of levofloxacin was drawn into and expelled from the syringe for 20 cycles. No significant differences of recovery were observed when the volume of washing solution was at 100 μL. Further increase of volume led to a slight decrease in recovery. At different volumes of the washing solution, the highest recovery of levofloxacin was observed with water-methanol (50 : 50, v/v) as the washing solution. To achieve excellent recovery and remove as much interfering substances as possible, 200 μL of water-methanol (50 : 50, v/v) was employed as the washing solution for further experiments.

To optimize the smallest volume of eluent that can sufficiently wash the levofloxacin adsorbed on DESs-MIPs, methanol and acetonitrile containing aqueous ammonia solution (3%, 5% and 7%, v/v) were evaluated for eluting levofloxacin, as shown in Figure 6(c). Acetonitrile-ammonia offered the acceptable recovery of levofloxacin. The recovery of levofloxacin increased with the volume of eluent

increasing from 100 μL to 400 μL and then barely changed from 400 μL to 500 μL, when the content of ammonia ranging was at 5% and 7%. Therefore, 400 μL of acetonitrile-ammonia (95 : 5, v/v) was selected for elution in subsequent experiments.

3.4. Method Validation. The DESs-MIPs-MEPS coupled with UHPLC was validated in accordance with specificity, linearity, sensitivity, accuracy, and precision. The specificity of the proposed method was characterized as non-interference at retention time of levofloxacin, which derived from the endogenous plasma. The specificity was evaluated through analysis of blank samples (n = 15). No interfering peaks from endogenous compounds were observed at the retention time of levofloxacin (2.13 min), and the DESs-MIPs-MEPS could remove potential interferences. Typical chromatogram for blank plasma is shown in Figure 7. Linear calibration curve of levofloxacin was constructed by plotting the peak areas versus nominal concentrations over the range from 0.05 to 10 μg mL^{-1} for plasma. The calibration curve was prepared at seven concentrations, which covered the range of expected concentrations in the tested plasma. An excellent linearity was obtained with a correlation coefficient equal to 0.9988. The limit of detection (LOD) and the limit of quantification (LOQ) determined as signal-to-noise ration of 3 : 1 and 10 : 1 were 0.012 and 0.04 μg mL^{-1}, respectively. The level of sensitivity could meet the requirement of trace residue detection in the target biological sample.

The precision and accuracy of the proposed method were determined by using spiked samples at three concentrations, including 0.05, 0.5, and 4.0 μg mL^{-1} for the blank plasma. The intraday and interday accuracies were tested by analysis of five sets on the same day and five separate days, respectively. No levofloxacin was determined in the blank plasma. As shown in Table 4, the precision values ranged from 95.3% to 99.7%, with intraday precision less than 5.1% and interday precision less than 8.9%. In addition, the sensitivity and the selectivity of the UHPLC method without DESs-MIPs-MEPS were performed. The sensitivity of the UHPLC method reduced to an LOD of 0.024 μg mL^{-1} and an LOQ of 0.08 μg mL^{-1}, respectively. The selectivity of the UHPLC method was also evaluated through analysis of blank samples. As shown in Figure 8, no interfering peaks were observed at the retention time of levofloxacin (2.13 min). However, the interfering peaks at 2.64 min could affect quantification of ciprofloxacin (internal standard at 2.71 min). This results in the difficulty of selecting appropriate internal standard because the internal standard must exist in biopharmaceutical analysis. These results indicated that the developed method based on DESs-MIPs-MEPS coupled with UHPLC could be used for the analysis of levofloxacin in plasma samples because the DESs-MIPs-MEPS could selectively enrich and separate levofloxacin and ciprofloxacin (internal standard) from complex biological samples.

3.5. Real Sample Analysis. To verify the practical applicability of the developed method, 20 plasma samples obtained from hospitalized patients after transrectal ultrasound-guided prostate biopsy, who had been taking oral levofloxacin at a dose of 500 mg for 12 hours, were analyzed by the

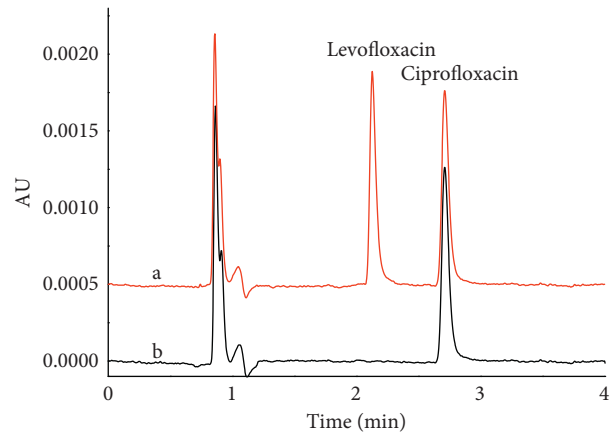

Figure 7: Representative chromatograms of patient plasma sample (a) and blank plasma (b).

Table 4: Recoveries and precision (n = 5).

Spike (μg mL^{-1})	Intraday (%)		Interday (%)	
	Recovery	Precision (RSD)	Recovery	Precision (RSD)
0.05	96.8	3.6	95.3	8.9
0.5	98.9	4.3	99.7	8.2
4.0	97.2	5.1	96.8	7.4

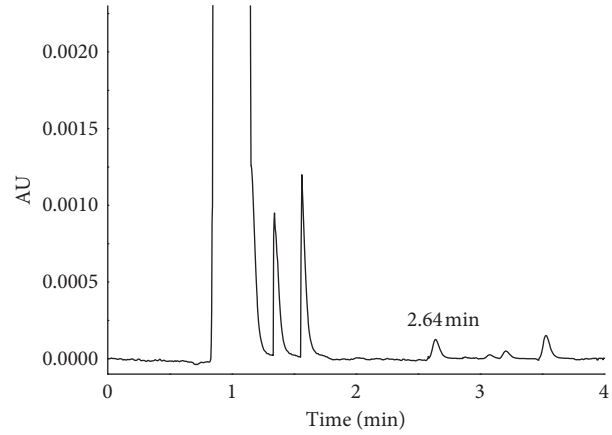

Figure 8: Representative chromatograms of blank plasma without DESs-MIPs-MEPS.

DESs-MIPs-MEPS-UHPLC method. The mean ± SD (n = 20) concentration of levofloxacin was 2.35 ± 0.99 μg mL^{-1} in plasma. Figure 7 showed the chromatographic separation of levofloxacin. The average concentration of levofloxacin obtained from plasma samples in this work agreed with that in the previous report [2, 33].

4. Conclusion

In this work, DESs were selected as porogen for successful preparation of pseudotemplate DESs-MIPs. The pseudotemplate DESs-MIPs possesses high adsorption capacity for levofloxacin in water and methanol. The DESs-MIPs used

for MEPS could avoid the problem of "template leakage" during application. The optimized DESs-MIPs-MEPS followed by UHPLC analysis was successfully applied to the determination of levofloxacin in plasma sample and provided excellent recoveries and reproducibility. Result of practical application demonstrated that the proposed DESs-MIPs-MEPS-UHPLC method was efficient, economic, and eco-friendly for the determination of levofloxacin in the biological sample.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors have declared no conflicts of interest.

Acknowledgments

The authors gratefully appreciate the financial support by Tianjin Health and Family Planning Commission Technology Fund—Key Projects (16KG117).

References

[1] P. Morency-Potvin, D. N. Schwartz, and R. A. Weinstein, "Antimicrobial stewardship: how the microbiology laboratory can right the ship," *Clinical Microbiology Reviews*, vol. 30, no. 1, pp. 381–407, 2016.

[2] O. Szerkus, J. Jacyna, P. Wiczling et al., "Ultra-high performance liquid chromatographic determination of levofloxacin in human plasma and prostate tissue with use of experimental design optimization procedures," *Journal of Chromatography B*, vol. 1029-1030, pp. 48–59, 2016.

[3] F. M. Wagenlehner, O. Umeh, J. Steenbergen, G. Yuan, and R. O. Darouiche, "Ceftolozane-tazobactam compared with levofloxacin in the treatment of complicated urinary-tract infections, including pyelonephritis: a randomised, double-blind, phase 3 trial (ASPECT-cUTI)," *The Lancet*, vol. 385, no. 9981, pp. 1949–1956, 2015.

[4] S. A. El-Zahaby, A. A. Kassem, and A. H. El-Kamel, "Formulation and *in vitro* evaluation of size expanding gastroretentive systems of levofloxacin hemihydrate," *International Journal of Pharmaceutics*, vol. 464, no. 1-2, pp. 10–18, 2014.

[5] T. Zhao, L.-A. Chen, P. Wang et al., "A randomized, open, multicenter clinical study on the short course of intravenous infusion of 750°mg of levofloxacin and the sequential standard course of intravenous infusion/oral administration of 500°mg of levofloxacin for treatment of community-acquired pneumonia," *Journal of Thoracic Disease*, vol. 8, no. 9, pp. 2473–2484, 2016.

[6] M. E. Falagas and M. Georgiou, "Early investigational antibiotics for the treatment of acute exacerbations of chronic bronchitis," *Expert Opinion on Investigational Drugs*, vol. 26, no. 3, pp. 313–317, 2017.

[7] L. Baietto, A. D'Avolio, F. G. De Rosa et al., "Simultaneous quantification of linezolid, rifampicin, levofloxacin, and moxifloxacin in human plasma using high-performance liquid chromatography with UV," *Therapeutic Drug Monitoring*, vol. 31, no. 1, pp. 104–109, 2009.

[8] E. Nemutlu, S. Kır, Ö. Özyüncü, and M. S. Beksaç, "Simultaneous separation and determination of seven quinolones using HPLC: analysis of levofloxacin and moxifloxacin in plasma and amniotic fluid," *Chromatographia*, vol. 66, no. S1, pp. 15–24, 2007.

[9] J. C. Aguilar-Carrasco, J. Hernández-Pineda, J. M. Jiménez-Andrade, F. J. Flores-Murrieta, M. d. C. Carrasco-Portugal, and J. S. López-Canales, "Rapid and sensitive determination of levofloxacin in microsamples of human plasma by high-performance liquid chromatography and its application in a pharmacokinetic study," *Biomedical Chromatography*, vol. 29, no. 3, pp. 341–345, 2014.

[10] S. A. Helmy, "Simultaneous quantification of linezolid, tinidazole, norfloxacin, moxifloxacin, levofloxacin, and gatifloxacin in human plasma for therapeutic drug monitoring and pharmacokinetic studies in human volunteers," *Therapeutic Drug Monitoring*, vol. 35, no. 6, pp. 770–777, 2013.

[11] J. Sousa, G. Alves, G. Campos, A. Fortuna, and A. Falcão, "First liquid chromatography method for the simultaneous determination of levofloxacin, pazufloxacin, gatifloxacin, moxifloxacin and trovafloxacin in human plasma," *Journal of Chromatography B*, vol. 930, pp. 104–111, 2013.

[12] G. Cao, J. Zhang, X. Wu et al., "Pharmacokinetics and pharmacodynamics of levofloxacin injection in healthy Chinese volunteers and dosing regimen optimization," *Journal of Clinical Pharmacy and Therapeutics*, vol. 38, no. 5, pp. 394–400, 2013.

[13] O. Szerkus, J. Jacyna, A. Gibas et al., "Robust HPLC-MS/MS method for levofloxacin and ciprofloxacin determination in human prostate tissue," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 132, pp. 173–183, 2017.

[14] J.-F. Jourdil, J. Tonini, and F. Stanke-Labesque, "Simultaneous quantitation of azole antifungals, antibiotics, imatinib, and raltegravir in human plasma by two-dimensional high-performance liquid chromatography-tandem mass spectrometry," *Journal of Chromatography B*, vol. 919-920, pp. 1–9, 2013.

[15] D.-J. Park, P. B. Phapale, I.-J. Jang et al., "An improved UPLC method for rapid analysis of levofloxacin in human plasma," *Chromatographia*, vol. 68, no. 3-4, pp. 187–192, 2008.

[16] R. Cazorla-Reyes, R. Romero-González, A. G. Frenich, M. A. Rodríguez Maresca, and J. L. Martínez Vidal, "Simultaneous analysis of antibiotics in biological samples by ultra high performance liquid chromatography-tandem mass spectrometry," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 89, pp. 203–212, 2014.

[17] M. M. Moein, R. Said, and M. Abdel-Rehim, "Microextraction by packed sorbent," *Bioanalysis*, vol. 7, no. 17, pp. 2155–2161, 2015.

[18] M. Abdel-Rehim, "New trend in sample preparation: on-line microextraction in packed syringe for liquid and gas chromatography applications," *Journal of Chromatography B*, vol. 801, no. 2, pp. 317–321, 2004.

[19] L. Yang, R. Said, and M. Abdel-Rehim, "Sorbent, device, matrix and application in microextraction by packed sorbent (MEPS): a review," *Journal of Chromatography B*, vol. 1043, pp. 33–43, 2017.

[20] M. Abdel-Rehim, "Microextraction by packed sorbent (MEPS): a tutorial," *Analytica Chimica Acta*, vol. 701, no. 2, pp. 119–128, 2011.

[21] M. M. Moein, A. Abdel-Rehim, and M. Abdel-Rehim, "Microextraction by packed sorbent (MEPS)," *TrAC Trends in Analytical Chemistry*, vol. 67, pp. 34–44, 2015.

[22] W. Du, C. Lei, S. Zhang et al., "Determination of clenbuterol from pork samples using surface molecularly imprinted

polymers as the selective sorbents for microextraction in packed syringe," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 91, pp. 160–168, 2014.

[23] M. M. Moein, A. Abdel-Rehim, and M. Abdel-Rehim, "On-line determination of sarcosine in biological fluids utilizing dummy molecularly imprinted polymers in microextraction by packed sorbent," *Journal of Separation Science*, vol. 38, no. 5, pp. 788–795, 2015.

[24] M. M. Moein, A. El-Beqqali, M. Javanbakht, M. Karimi, B. Akbari-adergani, and M. Abdel-Rehim, "On-line detection of hippuric acid by microextraction with a molecularly-imprinted polysulfone membrane sorbent and liquid chromatography-tandem mass spectrometry," *Journal of Chromatography A*, vol. 1372, pp. 55–62, 2014.

[25] S. M. Daryanavard, A. Jeppsson-Dadoun, L. I. Andersson, M. Hashemi, A. Colmsjö, and M. Abdel-Rehim, "Molecularly imprinted polymer in microextraction by packed sorbent for the simultaneous determination of local anesthetics: lidocaine, ropivacaine, mepivacaine and bupivacaine in plasma and urine samples," *Biomedical Chromatography*, vol. 27, no. 11, pp. 1481–1488, 2013.

[26] E. L. Smith, A. P. Abbott, and K. S. Ryder, "Deep eutectic solvents (DESs) and their applications," *Chemical Reviews*, vol. 114, no. 21, pp. 11060–11082, 2014.

[27] X. Li and K. H. Row, "Purification of antibiotics from the millet extract using hybrid molecularly imprinted polymers based on deep eutectic solvents," *RSC Advances*, vol. 7, no. 28, pp. 16997–17004, 2017.

[28] S. Liang, H. Yan, J. Cao, Y. Han, S. Shen, and L. Bai, "Molecularly imprinted phloroglucinol-formaldehyde-melamine resin prepared in a deep eutectic solvent for selective recognition of clorprenaline and bambuterol in urine," *Analytica Chimica Acta*, vol. 951, pp. 68–77, 2017.

[29] N. Fu, X. Liu, L. Li, B. Tang, and K. H. Row, "Ternary choline chloride/caffeic acid/ethylene glycol deep eutectic solvent as both a monomer and template in a molecularly imprinted polymer," *Journal of Separation Science*, vol. 40, no. 10, pp. 2286–2291, 2017.

[30] G. Li, W. Wang, Q. Wang, and T. Zhu, "Deep eutectic solvents modified molecular imprinted polymers for optimized purification of chlorogenic acid from honeysuckle," *Journal of Chromatographic Science*, vol. 54, no. 2, pp. 271–279, 2016.

[31] W. Tang, F. Gao, Y. Duan, T. Zhu, and K. Ho Row, "Exploration of deep eutectic solvent-based molecularly imprinted polymers as solid-phase extraction sorbents for screening chloramphenicol in milk," *Journal of Chromatographic Science*, vol. 55, no. 6, pp. 654–661, 2017.

[32] X.-H. Wang, J. Zhang, C. Peng, Q. Dong, Y.-P. Huang, and Z.-S. Liu, "Comparison of multi-recognition molecularly imprinted polymers for recognition of melamine, cyromazine, triamterene, and trimethoprim," *Analytical and Bioanalytical Chemistry*, vol. 407, no. 23, pp. 7145–7155, 2015.

[33] G. L. Drusano, S. L. Preston, M. Van Guilder et al., "A population pharmacokinetic analysis of the penetration of the prostate by levofloxacin," *Antimicrobial Agents and Chemotherapy*, vol. 44, no. 8, pp. 2046–2051, 2000.