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Simultaneous analysis of fluoroquinolones and xanthine derivatives in serum by molecularly imprinted matrix solid-phase dispersion coupled with liquid chromatography

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ABSTRACT

A new molecularly imprinted polymer was synthesized using ofloxacin and theophylline as template and methacryclic acid as function monomer and it was employed as a special dispersant of matrix solid-phase dispersion for selective extraction of fluoroquinolones (ofloxacin, ciprofloxacin and enrofloxacin) and xanthine (caffeine and theophylline) from human serum samples. To eliminate the influences of template leaking on quantitative analysis, acetonitrile–trifluoracetic acid (99:1, v/v) was used as the template removing solution. By using water and acetonitrile–trifluoracetic acid (99.5:0.5, v/v) as the washing and elution solvent, respectively, satisfactory recoveries and clean enough chromatogram could obtained. Good linearity of all the analytes was observed in a range of 0.35–150 $\mu g\,g^{-1}$ with the correlation coefficient (r^2) $\geq$ 0.9991. The recoveries of spiked human serum samples were in a range of 89.5–104.0% for fluoroquinolones and xanthine derivatives with RSD less than of 5.0%.

1. Introduction

Quinolones are a family of synthetic broad-spectrum antibiotics and the majority of quinolones in clinical use belong to the subset fluoroquinolones, which show excellent activity against both pathogenic Gram-negative and Gram-positive bacteria, as well as anaerobes [1]. However, when fluoroquinolones are employed in clinical use, not only their bad reactivity to stomach and intestines, circle system and centrum neural system, but also the allergic interaction between quinolones with other medicaments should be paid more attention. Among them, the typical bad interaction is between quinolones and xanthine derivatives (caffeine and theophylline) when they coexist or administrate simultaneously [2,3]. Considering that caffeine was existed in many medicaments and theophylline was widely used for the treatment of bronchial disease, there were many potential opportunities causing interaction between quinolones and xanthine derivatives. To avoid the coexistence and the bad reactivity of quinolones and xanthine derivatives, so as to protect the health of people, a fast, simple and accurate method for the simultaneous determination of quinolones and xanthine derivatives in human serum was necessary.

At present, many methods had been proposed for quinolones or xanthine derivatives analysis [4–7], but the majority work was only focused on characters or analysis method of one kind (quinolones or xanthine derivatives) with less work on both. Generally, trace analysis of these compounds need a preliminary pretreatment procedure followed by further purification, such as liquid–liquid extraction (LLE) [8], solid-phase extraction (SPE) [9,10] and membrane separation, etc. [11]. These protocols for analysis of quinolones or xanthine derivatives in biological samples involve several purification and concentration steps, which make them suffer to complicate processes, time-consuming or large amount of organic solvent. Moreover, because the solvents or adsorbents are non-selective and therefore tend to coextract endogenous components that might interfere with the analytes. So high selective method of quinolones or xanthine derivatives (such as molecular imprinting techniques) was paid more attention by many researchers [12,13]. More efficient techniques to reduce the number of sample treatment steps and improve the selectivity are needed.

Matrix solid-phase dispersion (MSPD), that combined both sample homogenization and extraction in one step, could be regarded as a more effective sample preparation method, especially for solid, semi-solid and highly viscous samples [14]. It involved blending of the sample with appropriate sorbent (C_{18}, C_8, silica gel, florisil, etc.), packaging of the mixture into a cartridge, washing and elution with a small volume of solvent [15–20] and could eliminate most

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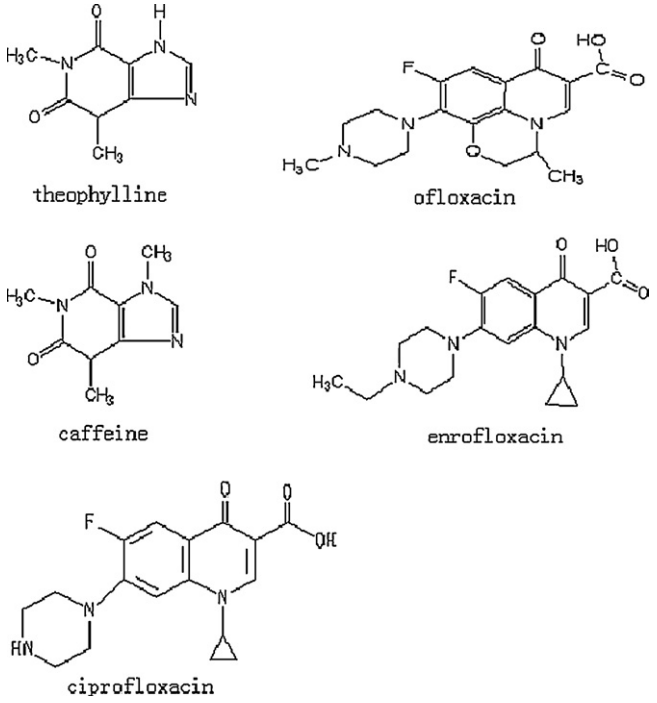

Fig. 1. The molecular structure of the five target analytes.

of the complications of classical LLE and SPE for solid matrixes. However, for the lack of special selectivity of the common sorbents, a further clean up step was often required [14]. Recently a new selective sorbent-molecularly imprinted polymer (MIP) as was employed in MSPD to extract organic compounds from complex materials [21,22]. The combination of MIP and MSPD techniques made the sample pretreatment rapid and selective and would lead to a higher accuracy and lower detection limit in subsequent analysis [21]. Owing to the special selectivity and stability of MIP, the technique of MIP–MSPD would have a broad application in the field of sample pretreatment.

In this work, a new MIP was synthesized by using ofloxacin and theophylline as mixed template with methacryclic acid as functional monomer, and it was used as the special sorbent of MSPD to selectively extract fluoroquinolones (ofloxacin, ciprofloxacin and enrofloxacin) and xanthine derivatives (caffeine and theophylline) from human serum samples. By using water and acetonitrile–trifluoracetic acid as washing and elution solvent, respectively, the sample extract was clean enough for further high performance liquid chromatographic (HPLC) analysis with satisfactory results.

2. Experimental

2.1. Reagents

Ofloxacin (OFL), ciprofloxacin (CIP) and enrofloxacin (ENR) were obtained from National Institute for Control of Pharmaceutical and Biological Products (Shandong, China), and caffeine (CAF) and theophylline (THEP) were obtained from Sigma (ST Louis, MO, USA) (Fig. 1). Methacryclic acid (MAA) from Tianjin No. 1 Chemical Reagent Factory (Tianjin, China) was purified by distillation to remove inhibitor. Ethylene glycol dimethacrylate (EDMA) was obtained from Shanghai Trading Co., Ltd. (Shanghai, China). All the other reagents used in the experiment were of the highest grade commercially available.

2.2. HPLC analysis

HPLC analysis was performed on a Shimadzu HPLC system equipped with a LC-10A Multisolvent Delivery System, a DGU-12A on-line-degasser, a SCL-10Avp gradient controller and a SPD-M10Avp diode array detector (Shimadzu, Kyoto, Japan). A CLASS-VP workstation (Shimadzu, Kyoto, Japan) with an OP-C_{18} stationary phase (250 mm × 4.6 mm I.D., particle size 5 μm). The mobile phase was acetonitrile–0.02 mol L^{-1} tetrabutyl ammonium bromide aqueous solution (9:91, v/v; pH: 2.50) with flow rate 1.0 mL min^{-1} (30 °C). The detection wavelength for OFL, CIP, ENR, CAF and THEP was 293 nm, 277 nm, 277 nm, 272 nm, 272 nm, respectively.

2.3. Preparation of molecularly imprinted polymers

2.0 mmol OFL, 2.0 mmol THEP, 4.30 mL MAA, 25.0 mL EDMA, and 0.12 g α,α′-Azobis(isobutyronitrile) were dissolved in appropriate solvent (methanol:acetonitrile = 8:1, v/v) and sonicated for 10 min, then purged with nitrogen gas for 5 min before being sealed under a nitrogen stream. Polymerization was performed at 53 °C in water bath for 24 h and the obtained MIP was grinded and passed through a 32 μm sieve, then it was suspended in acetone and washed by tetrahydrofuran and acetonitrile–trifluoracetic acid (99:1, v/v) in a chromatographic column at the rate of 0.5 mL min^{-1} for 24 h, respectively, and then dried in a vacuum drying oven at 40 °C. Non-imprinted polymer (NIP, in the absence of a template) was prepared and treated in an identical manner.

2.4. Determination of binding capacity

20 mg imprinted particles and 3.00 mL mixed standards solution of the five target analytes were placed into a 10 mL flask. After being placed in dark at room temperature for 24 h, the solutions were centrifuged and 20 μL of the upper solution was used for HPLC analysis. The binding capacity (*B*%) was calculated by the following formulation [23].

$$B\%_{(\text{analyte})} = \frac{W_{\text{Total}} - W_{\text{upper}}}{W_{\text{Total}}} \times 100\%$$

where W_{Total} was the total analyte quantity that added in the 10 mL flask, W_{upper} was the total analyte quantity in upper solution.

2.5. Procedure of matrix solid-phase dispersion

200 mg serum sample and 200 mg MIP particles were gently blended by a mortar in porcelain for 5 min to get a homogeneous mixture by water as carried reagent, the mixture was transferred into a cartridge (65 mm × 10 mm) which was pre-packed with 50 mg of MIP. The cartridge was washed with 2.0 mL water and eluted with 4.0 mL acetonitrile–trifluoracetic acid (99.5:0.5, v/v). The eluent was evaporated at 30 °C to dryness under vacuum condition and the residue was re-dissolved in 0.30 mL of 0.02 mol L^{-1} tetrabutyl ammonium bromide aqueous solution for further HPLC analysis.

3. Results and discussion

3.1. HPLC analytical conditions

According to the previous work of quinolones analysis [13], acetonitrile–tetrabutyl ammonium bromide system provided a suitable mobile phase system for the baseline separation of the five analytes on C_{18} column and the results indicated that the five target analytes could be separated completely at pH 2.50. The percent of acetonitrile in mobile phase had great effect on separation

efficiency: lower percent meant long analysis time, while higher percent led to decreasing separation efficiency. If the percent of acetonitrile was more than 13%, the peak of CAF and OFL, CIP and ENR would be overlapped. Considering the analysis time and resolution, acetonitrile–0.02 mol L^{-1} tetrabutyl ammonium bromide aqueous solution (9:91, v/v) was used for further work.

3.2. Preparation of MIPs

The function monomer MAA could interact with OFL and THEP, for in the structure of OFL, there were 6-fluoro, 7-piperazinyl and 3-carboxyl groups, which would assist MAA in self-organization combination with OFL. For the structure of THEP, there were three kinds of sites that strong interact with MAA: amino group, contiguous O and N, which could also form hydrogen bond with MAA. So MAA was employed as the functional monomer to meet OFL and THEP templates. Using EDMA as crosslinker, the molecular imprinting polymerization was successfully held in methanol–acetonitrile (8:1, v/v) system. When the polymerization was held in methanol–water (9:1), the affinity between the obtained MIP and xanthine derivatives could be destroyed by methanol, while the affinity of that to quinolones without any influence. Additionally, quinolones would be decomposed under the illumination, so heat induced polymerization (53 °C water bath) was employed in this work.

3.3. Characters of the MIP

The obtained MIP and NIP were packed into a 200 mm × 4.6 mm steel column for chromatographic evaluation with acetonitrile, dichloromethane and methanol as mobile phase at 1.0 mL min^{-1}. The results showed that acetonitrile had the strongest elution ability among the selected three mobile phases. CAF and THEP could be eluted out from MIP column by acetonitrile with retention time of 16.0 min and 30.7 min, respectively, while for the selected quinolones, they could not be completely eluted out within 60 min. Dichloromethane as mobile phase only eluted CAF out from MIP column, while methanol showed lower elution ability than dichloromethane. After the addition of trifluoracetic acid into the mobile phase, the elution ability would be fortified and the results indicated that the mobile phase of acetonitrile–trifluoracetic acid (99.5:0.5, v/v) could elute all the analytes out from MIP column in 5 min. While for NIP column, the three kinds of mobile phase could elute out all the analytes within 10 min. All above indicated that the MIP particles had high affinity to fluoroquinolones and xanthine derivatives and it was a good sorbent for the selective adsorption of the target analytes.

The result of binding capacity (Fig. 2) indicated that the ordinal absorption quantity of the analytes from high to low was OFL, ENR, THEP, CIP and CAF. For the same kind of analytes, the MIP showed higher affinity to template molecules.

3.4. Optimization of MIP–MSPD procedures

In order to obtain the optimum selectivity and recovery, the main parameters of MIP–MSPD including the ratio of sample/dispersant, washing and elution solvent were optimized.

Commonly, the quantity of dispersant depends on the sample type, a suitable ratio of sample/dispersant should make the dispersant adsorb the serum components completely and facilitate the mixture of serum matrix and dispersant to transfer into the cartridge. After optimization, the sample/dispersant ratio of 1:1 (m/m) was employed in this work.

Washing solvent should leave the target analytes absorbed on the cartridge and remove the sample matrix interferences as much as possible. Methanol, dichloromethane, water, acetonitrile–water

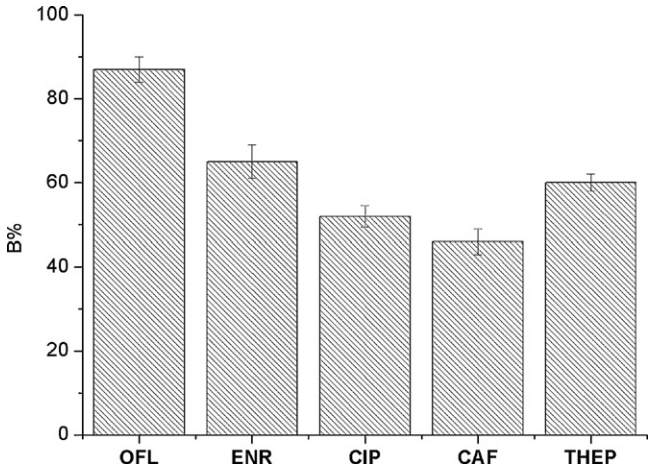

Fig. 2. The average binding capacity of the target analytes (n = 5, 0.5 mmol L^{-1} of the mixed standard solution).

(1:9, v/v) and methanol–water (1:1, v/v) as washing solvents were investigated. The result (Fig. 3) indicated that the best recoveries of the target analytes were obtained when water was employed as the washing solvent. For the purpose of the minimum losing of analytes and better purification efficiency, different volumes of water were investigated from 1.0 to 5.0 mL and the result indicated that the volume lower than 2.0 mL was not sufficient to purification, while the volume more than 3.0 mL was led to lower recovery, especially for CAF (≤71.4%). 2.0 mL was used as the optimum volume of rinsing solvent.

The elution solvent should have enough elution ability to desorb the analytes and facilitate the further sample treatments. According to the foregoing chromatographic evaluation, it could be concluded that the addition of acid into the elution solvent would fortified the elution ability. So methanol–acidic acid (9:1, v/v), acetonitrile–acidic acid (9:1, v/v), methanol–trifluoracetic acid (99.5:0.5, v/v) and acetonitrile–trifluoracetic acid (99.5:0.5, v/v) as elution solvents were investigated and the result (Fig. 4) indicated that acetonitrile–trifluoracetic acid (99.5:0.5, v/v) as elution solution provided the best recoveries for the target analytes. The quantity of acetonitrile–trifluoracetic acid (99.5:0.5, v/v) that loaded on the cartridge had great effect on the recovery

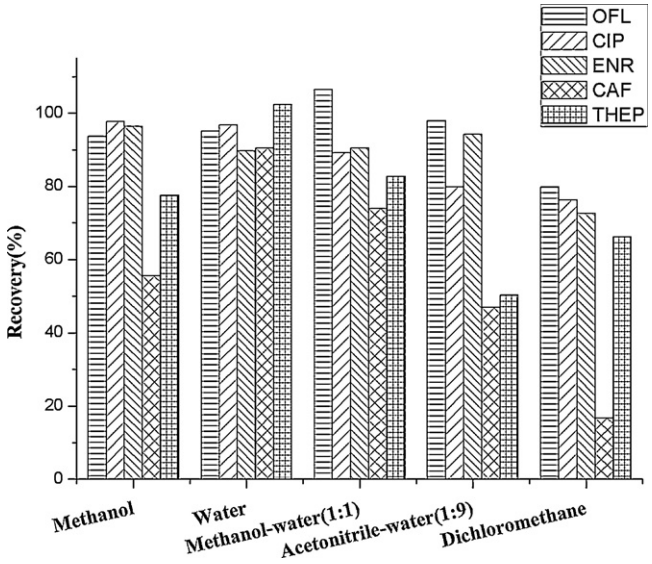

Fig. 3. The washing solvent on recovery of each analyte.

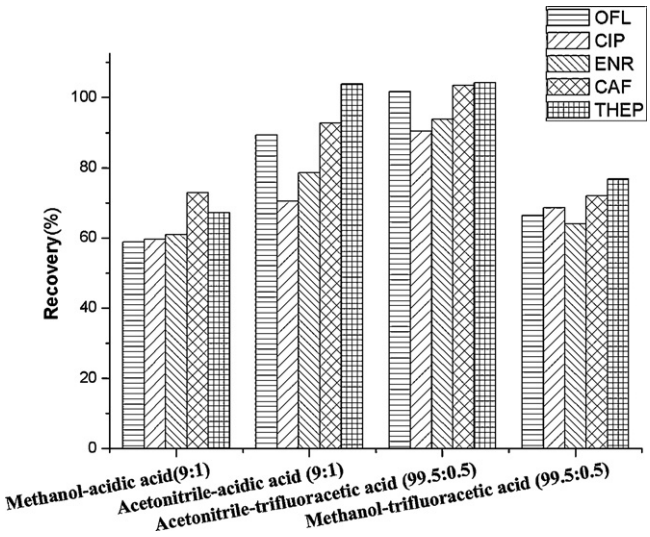

Fig. 4. The elution solvents on the recovery of each analyte.

of analytes, insufficiency volume meant uncompleted elution, excessive volume would lead to long time for the next dryness steps. After optimization, 4.0 mL of the elution solvent was found to be the optimum volume.

3.5. Validation of the methodology

Calibration curves were constructed using blank serum samples fortified in the range of 0.35–150 $\mu g\,g^{-1}$ and the results (Table 1) showed good linearity of all the analytes were obtained with $r^2 \geq 0.9991$. The limit of detections (LODs) based on S/N = 3 were ranged from 0.04 to 0.09 $\mu g\,g^{-1}$. Intra-assay and inter-assay precision were less than 5.6% and 7.0%. Five blank samples were used for the evaluation of the potential interferences of the serum matrix and no interfering peaks were observed at the retention time of the interest compounds, which demonstrated the good practicability of the proposed MIP–MSPD–HPLC method.

3.6. Samples analysis

In order to validate the MIP–MSPD–HPLC method, twenty human serum samples that obtained from the local hospitals were pretreated and determined by the proposed method and OFL was detected at the level of 0.86 $\mu g\,g^{-1}$ in one of the serum sample (Fig. 5A). The spiked serum samples were equilibrated at 37 °C in darkness for 60 min before analyzed by the proposed MIP–MSPD–HPLC method (Fig. 5B). The clean chromatogram indicated that the interferences originating from the

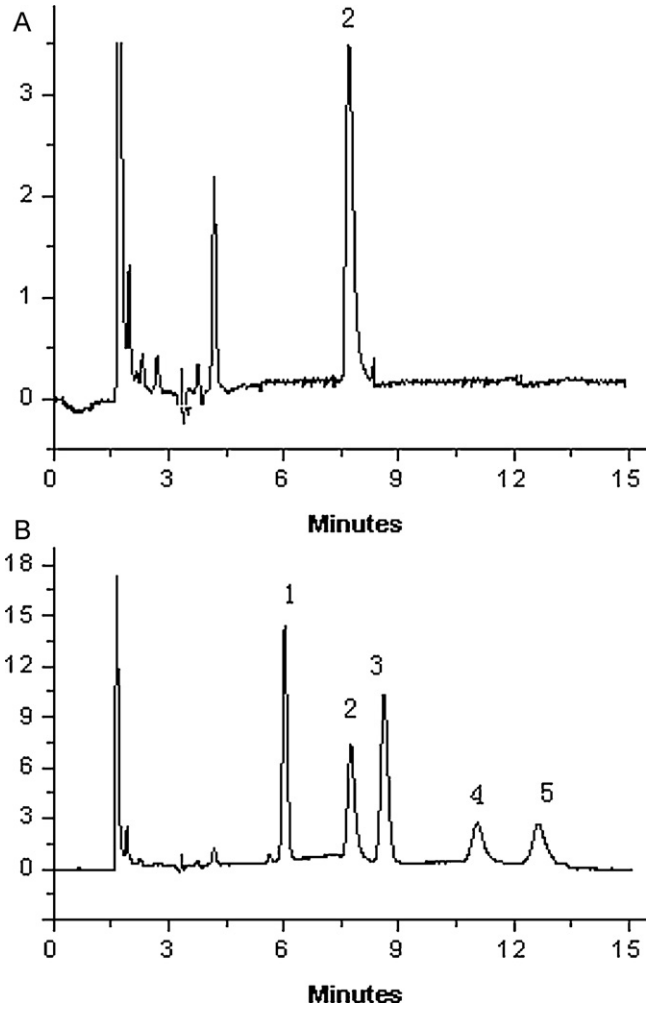

Fig. 5. The chromatogram of serum sample and spiked serum sample. A: serum sample, B: spiked serum sample; Peak: 1:theophylline, 2: ofloxacin, 3: caffeine, 4: ciprofloxacin, 5: enrofloxacin; mobile phase: acetonitrile–0.02 mol L^{-1} tetrabutyl ammonium bromide aqueous solution (9:91, v/v); flow rate: 1.0 mL min^{-1}; injection volume: 20 μL.

Table 1
Features of the MIP–MSPD–HPLC method ($n = 3$).

Analytes	Regression equation	r^2	LOD ($\mu g\,g^{-1}$)	RSD (%)
Theophylline	$Y = 2.41 \times 10^5 X - 0.13 \times 10^4$	0.9992	0.04	2.6
Ofloxacin	$Y = 0.68 \times 10^5 X + 3.4 \times 10^4$	0.9998	0.07	2.9
Caffeine	$Y = 1.92 \times 10^5 X + 2.4 \times 10^4$	0.9991	0.05	3.4
Ciprofloxacin	$Y = 1.14 \times 10^5 X + 7.6 \times 10^4$	0.9992	0.09	4.1
Enrofloxacin	$Y = 1.47 \times 10^5 X + 9.4 \times 10^4$	0.9996	0.08	4.0

Table 2
The recovery of the spiked serum samples ($n = 3$).

Spiked level of analytes	0.5 ($\mu g\,g^{-1}$)		1.0 ($\mu g\,g^{-1}$)		2.0 ($\mu g\,g^{-1}$)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Theophylline	104.0	3.9	101.2	4.0	98.9	2.5
Ofloxacin	101.2	3.9	96.2	4.5	97.2	3.3
Caffeine	95.6	3.1	98.7	3.6	96.1	4.8
Ciprofloxacin	90.7	5.0	96.0	4.2	101.2	4.1
Enrofloxacin	89.5	4.2	96.9	3.7	93.8	3.7

human serum matrixes could be efficiently eliminated by the proposed MIP–MSPD. The recoveries at three spiked levels (0.5, 1.0 and 2.0 $\mu g\,g^{-1}$) were ranged from 89.5% to 104.0% with the relative standard deviation (RSD) less than 5.0% (Table 2), which indicated that the method was reliable and could be used for the determination of trace fluoroquinolones and xanthine derivatives in serum samples.

4. Conclusions

A new MIP was synthesized using OFL and THEP as mixed template and employed as a special dispersant of matrix solid-phase dispersion for selective extraction of OFL, CIP, ENR, CAF and THEP from human serum samples. By using water as washing solution and acetonitrile–trifluoracetic acid as elution solution, the extract was clean enough for further chromatographic analysis and all matrix interferences were eliminated effectively. The recoveries of the spiked serum samples were ranged from 89.5% to 104.0% with RSD less than 5.0%. The proposed MIP–MSPD–HPLC method was a good alternative for routine analysis due to its simplicity, sensitivity and rapidity.

Acknowledgements

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