Journal of Chromatography A, 1217 (2010) 2075–2081

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Selective sample pretreatment by molecularly imprinted polymer monolith for the analysis of fluoroquinolones from milk samples

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ARTICLE INFO

Article history:
Received 19 January 2010
Received in revised form 2 February 2010
Accepted 3 February 2010
Available online 10 February 2010

Keywords:
Molecularly imprinted monolith
Polymer monolith microextraction
Fluoroquinolones
Milk
Water-compatible

ABSTRACT

Water-compatible pefloxacin-imprinted monoliths synthesized in a water-containing system were used for the selective extraction of fluoroquinolones (FQs). The MIP monolith was synthesized by using methacrylic acid as the functional monomer, di(ethylene glycol) dimethacrylate as a cross-linker and methanol–water (10:3, v/v) as the porogenic solvent. The ability of the derivated MIP for selective recognition of FQs (ciprofloxacin, difloxacin, danofloxacin and enrofloxacin) and quinolones (flumequine, and oxolinic acid) was evaluated. The derivated monolith showed high selectivity and was able to distinguish between FQs and quinolones. A simple rapid and sensitive method using polymer monolith microextraction (PMME) based on the MIP monolith combined with HPLC with fluorescence detection was developed for the determination of four FQs from milk samples. Owing to the unique porous structure and flow-through channels in the network skeleton of the MIP monolith, phosphate buffer diluted milk samples were directly supplied to PMME; allowing non-specific bound proteins and other biological matrix to be washed out, and FQs to be selectively enriched. The limit of detection of the method was 0.4–1.6 ng/mL and recovery was 92.4–98.2% with relative standard deviations less than 5.9%.

1. Introduction

Analysis of complex samples such as biological and food samples generally requires a pretreatment step aimed in reducing matrix content and the enrichment of the analyte that can be commonly achieved by solid-phase extraction (SPE). However, conventional SPE sorbents usually develop non-specific hydrophobic interactions that lead to the co-extraction of interfering compounds, thus preventing reliable quantification of the analyte. Recently, molecularly imprinted polymers (MIPs) have become increasingly attractive class-/compound-specific sorbents as they show antibody-like affinities toward the template analyte, and can therefore be used for highly selective SPE.

The most widely used technique for preparing MIP materials is by conventional free-radical solution polymerization. In order to acquire particles with the appropriate size suited for HPLC and SPE, the bulk MIPs have to be crushed, grounded and sieved [1]. The particles produced in this time-consuming process are irregular in size and shape, resulting in significant loss in chromatographic performance [2]. In addition, some interaction sites are destroyed during the grinding process leading to lower MIP loading capacity with respect to their theoretical values. These problems have been improved using different preparative processes such as bead polymerization techniques [3], including suspension [4], multistep swelling process [5] and precipitation polymerization [6] as well as surface imprinting on the spherical polymer or silica [7]. However, these methods are often complex and suffer from the use of special dispersing phases/surfactants.

The ideal porous structure of monolithic sorbents should be well defined and provide high mass transfer together with high permeability and good separation efficiency [8,9]. MIPs monolith prepared by *in situ* synthesis was first reported by Matsui et al. [10], and have been used for solid-phase extraction [11–16] and liquid chromatography [17]. Monoliths synthesized in capillaries offer a potential alternative to crushed monolith columns, polymer beads columns, or silica-based columns for producing molecularly imprinted polymers. Monoliths synthesized in capillaries can be synthesized quickly as crushing, sieving and packing is unnecessary. Furthermore, the amount of template molecule used during monolith preparation requires much less than that of other methods [8].

Fluoroquinolones (FQs) are piperazinyl derivates of quinolones, and are commonly used in human and veterinarian medicine as antibacterial agents against several diseases over the last decade [18,19]. The presence of FQs in edible animal products is a significant risk which can be directly toxic or cause of pathogen resistance in humans [20]. Therefore, sensitive and selective analyt-

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doi:10.1016/j.chroma.2010.02.011

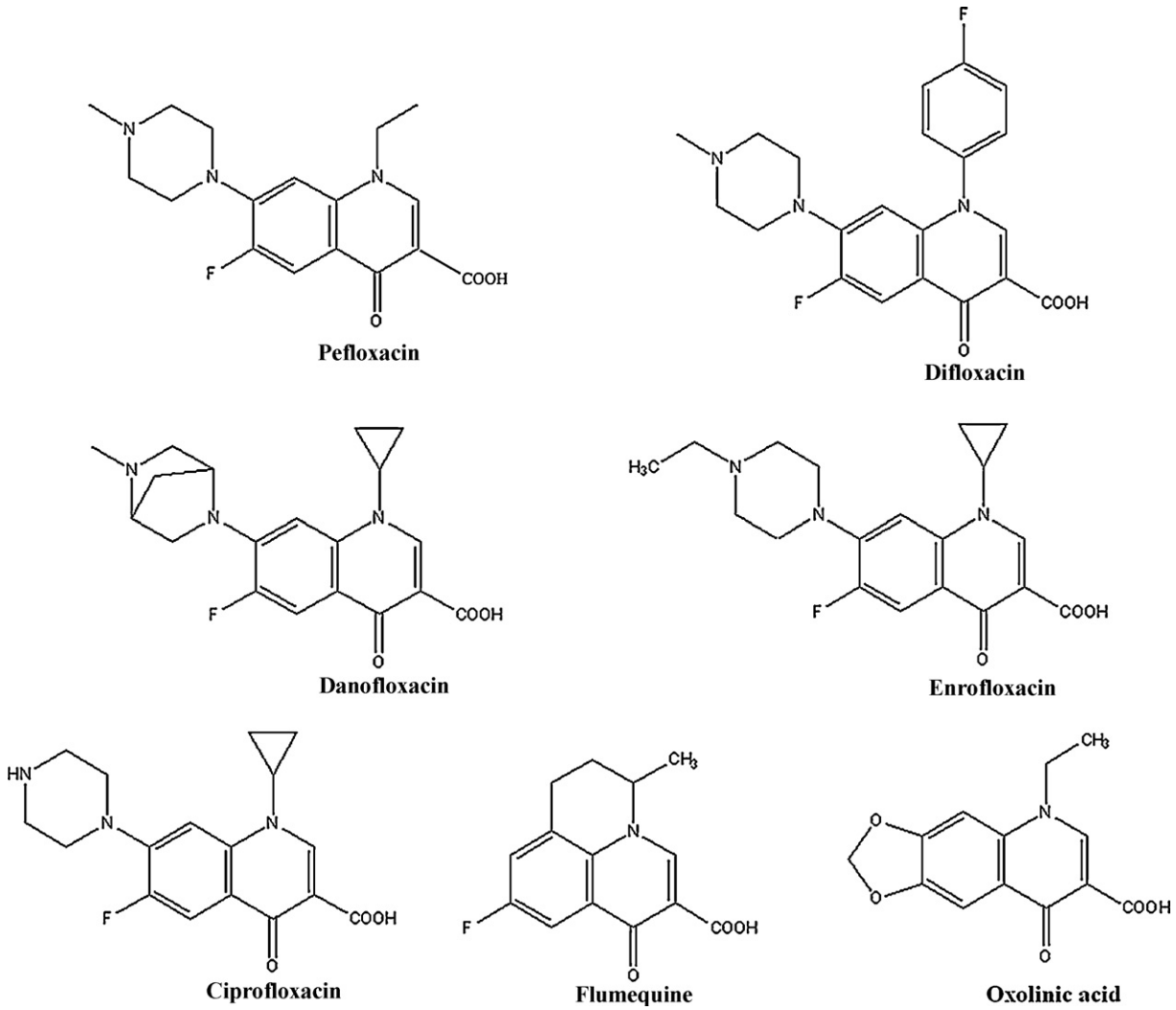

Fig. 1. The molecular structures of FQs and quinolones.

ical methods are required to monitor the residues possibly present in animal-producing food.

Due to the complexity of food samples, sample preparation is necessary prior to instrumental analysis. The commonly used SPE and liquid–liquid extraction (LLE) processes are complex, time consuming, have low selectivity, and use large amounts of organic solvents. Polymer monolith microextraction (PMME) is one type of solid-phase microextraction in which the polymer monolith is used as the sorbent [21–23]. Based on the poly(methacrylic acid-co-ethylene glycol dimethacrylate) (poly(MAA-co-EGDMA)) monolith, this technique combined with HPLC has high extraction efficiency during the determination of analytes in several kinds of edible animal-based products such as milk, egg, fish and chicken [24–28]. The polymer monolith is highly durable, showing stability over a large pH range, and exhibiting excellent biocompatibility in dealing with biological samples [21]. The combination of a polymer monolith with MIP technology can be used as an extraction medium for achieving high extraction efficiency and selectivity of the analyte from complex matrices.

Recently, several MIP sorbents using FQs as the templates have been reported [29–33]. However, little attention has been paid to take advantage of MIP monolith for selective extraction of FQs residues from food matrices. In this article, a pefloxacin-imprinted monolith was prepared in a water-containing system by *in situ* thermal-initiated polymerization using methacryclic acid as monomer, di(ethylene glycol) dimethacrylate as cross-linker and water–methanol as the porogenic solvent. By using this MIP monolith, FQs were selectively isolated from biological samples and the impurities were eliminated simultaneously. Our present methods showed high selectivity and sufficient accuracy to be used on trace levels of FQs analysis in biological samples.

2. Experimental

2.1. Chemicals and materials

Di(ethylene glycol) dimethacrylate (DEGDMA) (95% purity) was purchased from Acros (NJ, USA). Methacrylic acid (MAA), azobisisobutyronitrile (AIBN), disodium hydrogenphosphate (Na_2HPO_4) and N,N-dimethylformamide (DMF) were obtained from Shanghai Chemical Reagent Corp. (Shanghai, China). All reagents were of analytical grade. Methanol and acetonitrile (ACN) (HPLC grade) were obtained from Concord Technology (Tianjin, China). Purified water was obtained using an Aike water purification system (Chengdu, China).

Pefloxacin methanesulphonate (PEF), ciprofloxacin (CIP), danofloxacin methanesulphonate (DAN), enrofloxacin (ENR), difloxacin (DIF), oxolinic acid (OXO) and flumequine (FLU) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Individual stock solutions of CIP, ENR, DIF, FLU and OFL were prepared

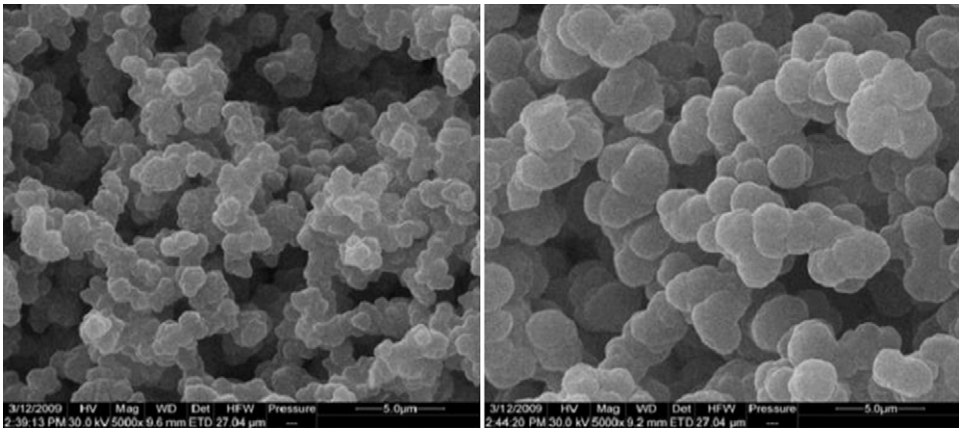

Fig. 2. SEM images of a monolith cross-section (magnification = 5000×) for MIP (left) and NIP (right).

as 500 μg/mL stock solutions in acetonitrile. Individual stock solution of OXO was prepared in acetonitrile at a concentration of 200 μg/mL and stock solutions of DAN and PEF were prepared in water at a concentration of 500 μg/mL. The working standard solution was diluted to the desired concentration for experiments. All of the above solutions were maintained at 4 °C. Chemical structures of the FQs and quinolones included in this study are shown in Fig. 1.

2.2. Preparation of the molecular imprinted monoliths

Fused-silica capillaries (530 μm I.D.) were purchased from Yongnian Optic Fiber Plant (Hebei, China), and were activated with 1 M NaOH followed by washing in 1 M HCl. After rinsing with pure water, the capillary was dried at 160 °C under N_2 flow for 6 h. The activated capillary was filled with a solution containing 3-(triethoxysilyl)propyl methacrylate in DMF (50%, v/v), and the two ends were sealed with silicon rubber. The inner-wall modification was performed at 70 °C for 12 h. The residual solution was removed and the capillary was washed thoroughly with methanol. Finally, the capillary was dried under nitrogen flow at room temperature before use.

The polymerization mixture composed of 24 mg (0.05 mM) PEF, 40 mg (0.47 mM) MAA, 240 mg (1.21 mM) DEGDMA and 5 mg AIBN, all dissolved in 800 μL methanol–water (10:3, v/v). The solution was sonicated for 10 min, and was used to fill the capillary. After polymerization at 65 °C for 16 h, the column was connected with an HPLC pump and washed with methanol–trifluoroacetic acid (98:2, v/v) to remove the porogenic solvents and the template molecules for 48 h. A non-imprinted blank polymer (NIP) was prepared in an identical manner but in the absence of the template molecule.

2.3. Instrumentation and analytical conditions

The analytical segment was performed with an Agilent 1100 series HPLC system (Agilent Technologies, CA, USA), which included a quaternary pump, a micro-vacuum degasser, a six-port valve, a photodiode detection (DAD) and a fluorescence detector (FLD). The analytical column was a Luna ODS column (150 mm × 4.6 mm I.D.; 5 μm), and was purchased from Phenomenex (CA, USA). The optimized mobile phase consisted of 25 mM phosphate buffer (pH 2.5)/acetonitrile/methanol (77:10:13, v/v/v), and the flow rate was maintained at 1 mL/min. The FLD condition was as follows: the excitation (EX) wavelength was 280 nm, and the emission (EM) wavelength was 450 nm. The microscopic morphology of the monolith was examined by a Model X-650 scanning electron microscope (Hitachi, Tokyo, Japan). Nitrogen sorption measurements were carried out at 77 K using a JW-BK surface area and pore size analyzer (JWGB Sci. & Tech., Beijing, China). Macroscopic monolithic materials were prepared by the same polymerization process in large amount of the corresponding mixtures in a 150 mm × 2.0 mm I.D. stainless steel column. After the polymerization was completed, the rod was thoroughly washed with ethanol for several times to remove unreacted species and dodecylamine completely, then vacuum dried at 80 °C over night. Prior to measurements, 250–300 mg of the samples was heated at 100 °C under high vacuum (10^{-5} Pa) for 12 h. The specific surface areas were calculated according to the BET (Brunauer–Emmett–Teller) equation at P/P_o between 0.05 and 0.35. The pore volumes were evaluated from the desorption branches of isotherms based on BJH (Barrett–Joyner–Halenda) model. The pore size distribution was measured by an Autopore IV 9500 mercury porosimeter (Micromeritics, Norcross, USA).

All μHPLC experiments were carried out on a TrisepTM2010GV CEC system (Unimicro Technologies, Shanghai, China). The TrisepTM2010GV CEC system which could be applied to both μHPLC and pressure-assisted capillary electrochromatography (CEC) comprised of a UV–vis detector (190–800 nm), two microflow pumps, a microfluid manipulation module (including a splitter and a six-port nanoinjection valve), and a data acquisition module. Analyses were performed at a flow rate of 10 μL/min in isocratic mode. The mobile phase was made up of 15% ACN and 85% PBS (pH 6.0) and was used as the optimized washing solution. A sample volume of 0.5 μL was injected into the monolith (8 cm × 530 μm I.D.).

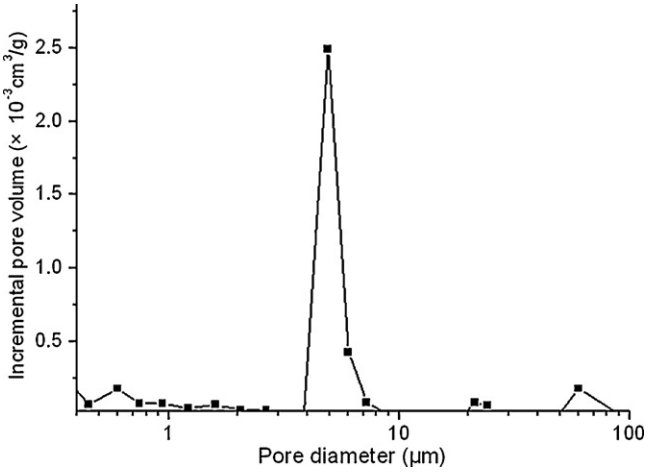

Fig. 3. Incremental pore size distribution profile of MIP monolith. The pore size distribution was measured by mercury porosimetry.

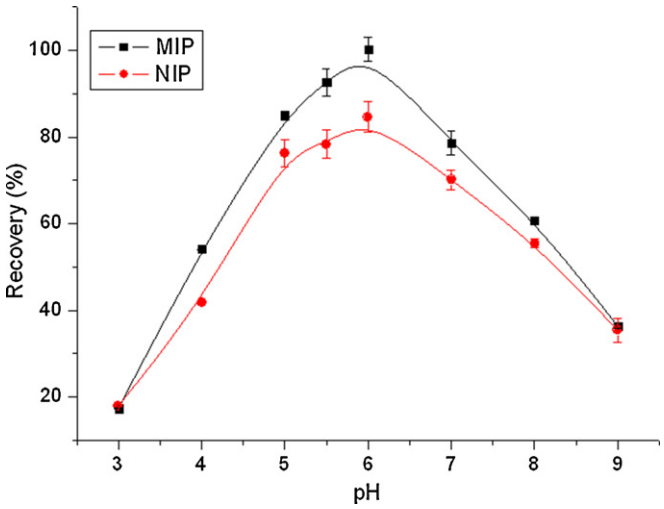

Fig. 4. Influence of pH on the recoveries of PEF on MIP and NIP monolith. Sample solutions were spiked with PEF at 20 ng/mL and were prepared in 25 mM phosphate solution at pH 3.0–9.0. The washing solution was the same as the sample solution.

The detection wavelength was set at 280 nm. All experiments were performed at 25 °C.

2.4. Preparation of milk samples

Pasteurized homogenized whole milk was purchased from the local retail market. Preliminary analyses showed the milk samples to be analyte-free. 0.5 mL of milk samples were spiked with known variable amounts of FQs, incubated for 10 min for equilibration at room temperature, before being mixed with a vortex mixer. These samples were diluted in 25 mM phosphate solution (pH 6.0) to 5.0 mL. After being mixed with a vortex mixer for a further 2 min, the samples were centrifuged at 4 °C for 5 min at 10,000 rpm (Anting Scientific Instrument Co., Shanghai, China). The supernatant was filtered through a 0.22 μm pore filter prior to MIP-PMME. Blank samples were prepared in the same way as above but without the compound-spiking step.

2.5. MIP-PMME procedure

The design of the PMME apparatus used in this work was set up as described previously [21]. The metallic needle of the pinhead of a syringe was replaced by a polymer monolith (8 cm × 530 μm I.D.), and the outside wall was coated uniformly with adhesive. After the adhesive was dried, the extraction device was set up for use. A syringe infusion pump (TS2-60, Baoding Longer Precision Pump Co., Ltd., Hebei, China) was employed for the whole sample pretreatment procedure. The whole procedure included preconditioning, sorption, washing, and desorption, and are described in detail in [21]. Methanol containing 2% trifluoroacetic acid (v/v) was employed as the desorption solvent. The extraction monolith was first preconditioned with 0.2 mL of desorption solvent followed by 0.4 mL of 25 mM phosphate solution. For sorption, 1.0 mL of the sample solution was applied to and passed through the monolith at 80 μL/min and 0.2 mL washing solution (PBS solution containing *X*% ACN) was pumped through at 80 μL/min to remove residual matrix in the monolith. The residual solution in the pinhead and monolith was driven out with air using a clean syringe. For the desorption step, 400 μL of desorption solvent was injected into the monolith at 50 μL/min and the eluate was collected into a vial. The eluent was evaporated to dryness under a mild nitrogen stream at 50 °C and the residues were re-dissolved in 100 μL of mobile phase. A 20 μL portion of the sample solution was used for injecting into the analytical column.

For comparison, C18-SPE (200 mg) was also used for extracting FQs from milk using the following steps. The SPE condition was optimized using standard mixture solution. The SPE cartridge was initially preconditioned with 1 mL methanol containing 0.3% NH_3 and then 1 mL 25 mM phosphate solution (pH 7.0). Next, the milk was diluted to 10-fold with 25 mM phosphate solution pH 7.0, loaded onto the cartridge, and washed with 1 mL pure water. The analytes were eluted with 1 mL methanol containing 0.3% NH_3. The eluent was evaporated to dryness under a mild nitrogen stream at 50 °C and the residues were re-dissolved in 100 μL of mobile phase. A 20 μL portion of the sample solution was used for injection into the analytical column.

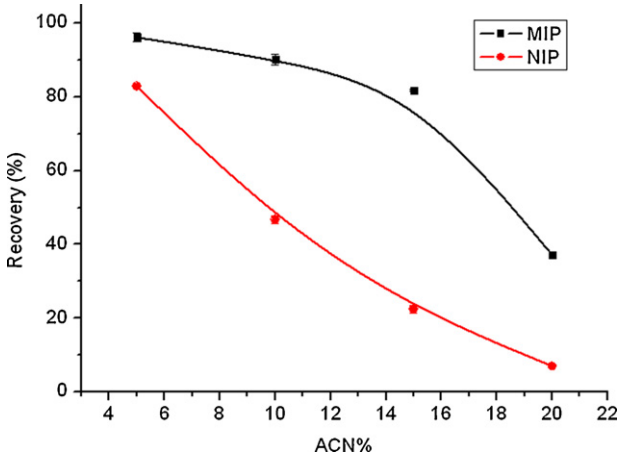

Fig. 5. Influence of washing solution ACN concentration on the recoveries of PEF from a MIP and NIP monolith. Sample solutions were spiked with PEF at 20 ng/mL and were prepared in 25 mM phosphate solution at pH 6.0.

Table 1
Retention and imprint factors of PEF and FLU on MIP and NIP monolith based on the gravity center of the peaks when injecting 10 μg/mL solution in a 500 nL loop.

Compound	k_{NIP}	k_{MIP}	IF
PEF	5.6	17.3	3.1
FLU	5.5	5.3	1.0

3. Results and discussion

3.1. Preparation and characterization of the MIP monolith

In order to make the MIP monolith capable of purification in aqueous solution, the MIP monolith was prepared in a water-based system and investigated by using a methanol and water mixture as the porogenic solvent [17]. The percentage of water and methanol in the polymerization mixtures was critically important as the porogenic solvent not only brings the template, functional monomers, cross-linkers and initiators into a single phase but also creates macropore structures in the MIP monolith. Methanol–water (10:3, v/v) was selected as the optimized porogenic solvent as it provided the necessary surface properties and mechanical strength for the polymer monolith. If the water content during preparation is further increased, the monolith would become flexible and provide poor mechanical strength. In contrast, lowering water content can not only result in poor solubility of the template but also lead to a monolith with small flow-through pore size and decreased ability of the solvent to flow through. Moreover, pre-polymerization complexes using DEGDMA as a cross-linker showed higher stability than MIP prepared with EGDMA as DEGDMA showed better solubility with the template.

The MIP and NIP monolith structural morphological properties were examined by microscopy (Fig. 2). Both monoliths were observed to possess particle agglomeration with interconnecting macropores. The microglobules are interconnected to produce large clusters that yield a continuous skeleton. The clusters of uniform-sized MIP skeletons were smaller than that of the NIP monolith. The flow-through pores size distribution was determined by mercury porosimeter (Fig. 3). MIP monoliths possess larger flow-through pores (around 5 μm) and a narrow size distribution, which can lead to higher permeability and favorable mass transfer during extraction. The specific surface areas and pore volumes from nitrogen adsorption–desorption experiments were 8.7 m^2/g and 0.013 cm^3/g for MIP, and 7.4 m^2/g and 0.013 cm^3/g for NIP, respectively. The similar surface areas and pore volumes of MIP and NIP indicated that selectivity was due to the imprinted recognition properties of MIP.

3.2. Optimization of MIP-PMME conditions

Specific recognition of PEF molecules in aqueous-rich media on MIP monolith depends on selective interactions, such as hydrogen bonding, ionic interactions, and hydrophobic effects.

To determine the optimum conditions of MIP-PMME, the influence of pH on PEF retention was investigated. PEF possess a piperazine substituent, which can be present as cationic, anionic, or intermediate forms in aqueous solution due to the presence of their carboxylic group and charged amino groups. PEF extraction behavior is therefore pH-dependent, with the pKa_1 and pKa_2 values reported to be 6.2 and 7.9, respectively [34]. The pH optimization was conducted in 25 mM phosphate buffer over a pH range of 3.0–9.0 with the highest extraction efficiency obtained at pH 6.0 (Fig. 4). As the carboxyl groups (pKa = 5.5) of the poly(MAA-co-EGDMA) monolith are ionized at pH 6.0, the PEF exists in cationic form ($HBAH^+$) from pH 4.0 to 6.0, resulting in strong ion-exchange interactions and thus higher extraction efficiency. When the pH is above 6.0, PEF is converted into the intermediate form (HB^+A^-), which can result in weakened ion-exchange interactions and lower extraction efficiency. Due to extraction efficiency, pH 6.0 was selected as the pH for sample solution.

In order to reduce non-specific adsorption and improve selective binding of PEF, the washing solution was adjusted by optimizing the proportion of ACN in PBS. There was no observed difference in the recoveries of MIP and NIP monoliths after washing with PBS solution, as non-specific binding could not be disrupted between the MIP monolith and PEF (Fig. 4). By increasing ACN content in the washing solution, the recoveries of PEF decreased precipitously in the NIP monolith. When washing with 15% ACN, the recovery of PEF in the NIP monolith was reduced to 22.5% while the recovery of the MIP monolith was 81.8%, indicating stronger retention for PEF on the MIP than the NIP monolith. The results confirm that MIP has higher specificity for PEF than the NIP monolith and showed that hydrophobic interactions is one of the main factors for retaining PEF on the MIP monolith. A washing step consisting of 0.2 mL of 15% ACN and 85% PBS (pH 6.0) was selected as a compromise for reducing non-specific interactions without sacrificing the recovery of PEF on the MIP monolith (Fig. 5).

3.3. Specificity evaluation of the MIP monolith

The imprinting factor (IF) proposed for the evaluation of recognition ability was applied to examine the recognition abilities of the prepared PEF monolith. The retention factor:

$$k = \frac{t_R - t_0}{t_0},$$

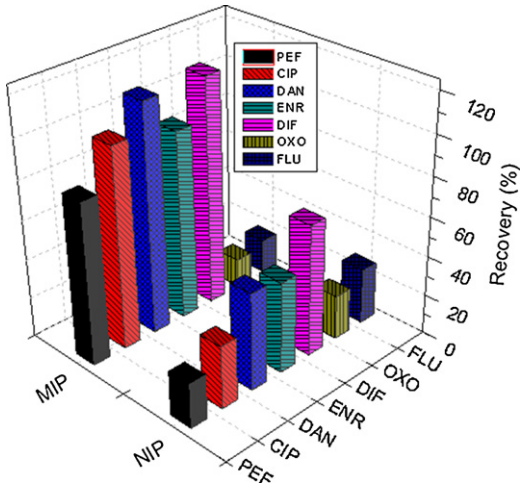

Fig. 6. Recoveries obtained for FQs and quinolones after MIP-PMME and NIP-PMME in optimized conditions.

where t_R is the retention time of analyte, t_0 is the retention time of the void volume marker (acetone), and the imprinting factor:

$$IF = \frac{k_{MIP}}{k_{NIP}},$$

where k_{MIP} is the retention factor of the MIP and k_{NIP} is the retention factor of the NIP were calculated. Table 1 shows the retention factors and the imprinting factors (IFs) of PEF and FLU (quinolone) on MIP and NIP monoliths. The highest IF was obtained for PEF and this is due to the MIP monolith possessing binding sites that are complementary to the spatial structure of the PEF. The retention factor of FLU on MIP monolith was similar to that of the NIP monolith, where IF is 1.0. The results demonstrate that MIP has a higher affinity for PEF than NIP.

The recognition properties of the MIP monolith against other FQs (pefloxacin, ciprofloxacin, danofloxacin, enrofloxacin, difloxacin) and quinolones (flumequine and oxolinic acid) were evaluated. 1 mL of solution containing FQs and quinolones were mixed at a final concentration of 20 ng/mL and extracted onto the MIP and NIP monoliths under the optimized conditions (Fig. 6). MIP was able to distinguish FQs from quinolones, all FQs were recognized by MIP with recoveries above 81.6%, whereas the recoveries of quinolones were less than 17.6%; however FQs and quinolones were not discriminated on the NIP monolith. The recoveries of FQs ranged from 22.5% to 66.2%, which was significantly less than that of the MIP monolith. According to the structures of these compounds (Fig. 1), the piperazinyl ring on FQs, which is their main structural difference with quinolones, plays an important role in the recognition mechanism of the PEF imprinted monolith [19]. The MIP monoliths produced using the protocol designed in this study showed high selectivity and could be used for clean-up and enrichment of FQs.

3.4. Measurement of adsorption capacity

To estimate the adsorption capacity of PEF on the MIP and NIP monolith, an adsorption experiment was carried out under optimized conditions by comparing the extraction of different concentrations of PEF on the MIP and NIP monoliths. The adsorption capacities of PEF by the MIP and NIP monoliths are represented in Fig. 7. For the NIP monolith, when the concentration of loading solution reached 0.035 mmol/L, the adsorption capacity was close to saturation. When the concentration of loading solution reached 0.45 mmol/L, the maximum adsorption capacity of the MIP mono-

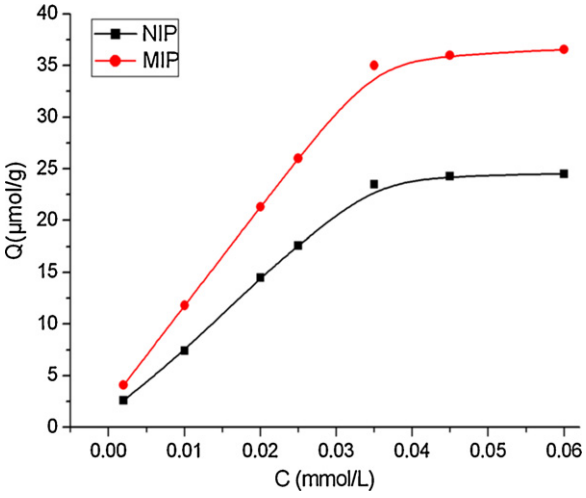

Fig. 7. Adsorption isotherm analysis of the MIP and NIP monolith.

Table 2
Column-to-column reproducibility of MIP monoliths.

Precision (RSD %)	CIP	DAN	ENR	FLU
Intra-batch (n = 5)	4.8	4.7	2.0	1.8
Batch-to-batch (n = 4)	5.4	5.5	2.4	2.4

lith was 36.0 μmol/g, which was 12.5 μmol/g higher than the NIP monolith under the same conditions.

3.5. Reproducibility and stability of MIP monolith

As an extraction media, preparation reproducibility is an important factor for ensuring the robustness and practicability of MIP monolith. The column-to-column reproducibility was assessed by calculating the relative standard deviation (RSD) for four FQs during extraction. The intra-batch and inter-batch RSDs were in the range of 1.8–4.8% and 2.4–5.5%, respectively (Table 2). Moreover, the MIP monolith showed high stability and could be used for extraction more than 50 times with no significant changes in column back-pressure and extraction efficiency.

3.6. Analysis of FQs in milk samples

The chromatograms of spiked milk samples after extraction with MIP monolith and C18-SPE are shown in Fig. 8. No interference from the biological matrix was observed after the MIP-PMME (Fig. 8b), demonstrating the high selectivity and affinity of the synthesized MIP monolith in a hydrous environment. In contrast, multiple interfering peaks from the milk sample were observed after C18-SPE separation, and these are due to non-specific interactions such as the hydrophobic interactions between the various components of the sample matrix and the C18 sorbent (Fig. 8a).

The recoveries were determined by comparing the peak area of FQs spiked in the samples after MIP-PMME with the peak areas of the standard solution. The recoveries for FQs spiked in milk sample are summarized in Table 3. The recoveries ranged from 92.4% to

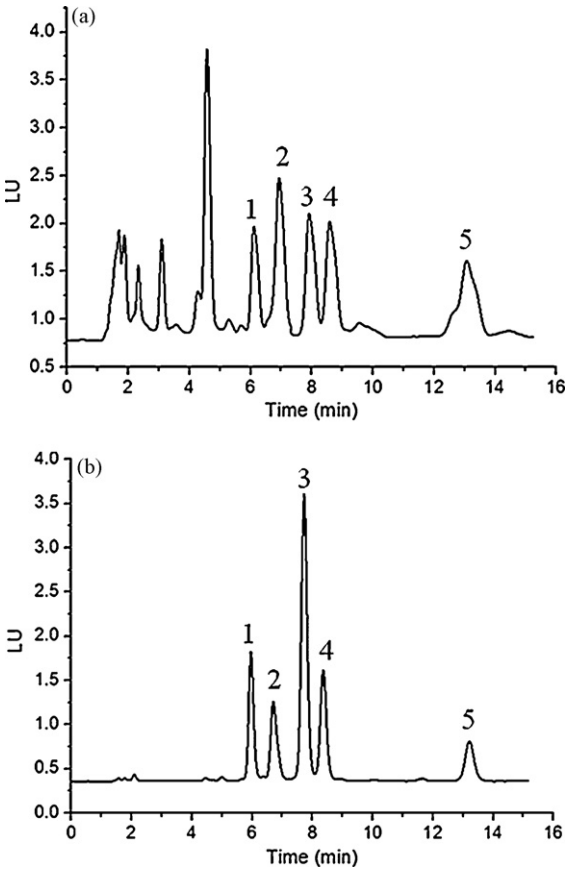

Fig. 8. Chromatogram of spiked milk samples by (a) C18-SPE and (b) MIP-PMME. Sample solutions of PEF were spiked at 20 ng/mL. Peaks: 1. PEF, 2. CIP, 3. DAN, 4. ENR, 5. DIF.

Table 3
Absolute recoveries (n = 3) of four FQs spiked in milk sample.

Analyte	Recovery (%)		
	5 ng/mL	50 ng/mL	200 ng/mL
CIP	93.3	95.0	92.8
DAN	94.4	95.3	92.4
ENRO	95.1	96.5	94.5
DIF	95.6	92.4	98.2

98.2% for all analytes investigated. The results show that FQs purification was not affected by the impurities from milk samples using the MIP-PMME/HPLC-FLD method. Therefore, the standard calibration curves were used as a reference to provide reliable results throughout this study.

In order to validate the linearity of the MIP-PMME/HPLC-FLD method, standard calibration curves were constructed by using the four FQs from the working standard solution. Linear regression analysis was performed by measuring the peak areas against their respective analyte concentrations. The calibration curves were established with R above 0.9986. Detection limits (LODs) and quantification limits (LOQs) were calculated as the concentration

Table 4
Linear regression and LOD, LOQ data for HPLC-FLD of the four FQs.

Analyst	Linear range (ng/mL)	Slope	Intercept	R	LOD (ng/mL)	LOQ (ng/mL)
CIP	2.0–200	0.53	1.03	0.9992	0.7	2.3
DAN	1.5–200	5.22	12.58	0.9988	0.4	1.3
ENRO	2.5–200	1.21	2.61	0.9988	0.8	2.7
DIF	5.0–200	0.34	0.74	0.9986	1.6	4.7

Table 5
The method precisions at three different concentrations for MIP-PMME/HPLC-FLD of the four FQs from milk sample.

Analyte	Intra-day (RSD %)			Inter-day (RSD %)		
	5 ng/mL	50 ng/mL	200 ng/mL	5 ng/mL	50 ng/mL	200 ng/mL
CIP	2.7	2.3	1.5	4.2	5.1	1.9
DAN	2.4	1.0	1.3	3.8	2.5	2.6
ENRO	2.7	1.7	2.2	4.1	3.7	4.9
DIF	2.6	2.7	2.2	5.9	5.7	2.5

corresponding to a signal 3 and 10 times the standard deviation of the baseline noise, respectively. As listed in Table 4, the LODs for the four FQs ranged from 0.4 to 1.6 ng/mL. The LOQs ranged from 1.3 to 4.7 ng/mL.

The precision of the method was assessed by determining the intra- and inter-day RSDs. Both intra- and inter-day RSDs were calculated with four FQs spiked at three different concentrations in the milk sample. The RSD data for FQs spiked in milk samples are summarized in Table 5. The intra- and inter-day precision of the four FQs was evaluated with RSDs less than 5.9%.

4. Conclusion

A novel pefloxacin-imprinted polymer monolith, prepared in a water–methanol system, showed high affinity for FQs in an aqueous environment. MIP-PMME followed by HPLC and fluorescence detection was developed as an analytical method for the simultaneous determination of four FQs in milk. By using water-compatible MIPs as a specific PMME sorbents, FQs in milk samples were selectively isolated and matrix interferences eliminated, which significantly enhanced PMME selectivity. The proposed MIP-PMME has been advocated as an environmentally friendly, inexpensive, and rapid sample preparation technique, which can be used as an alternative tool for extracting FQs from biological samples.

Acknowledgements

This work was supported by grants from the National Science Fund for Distinguished Young Scholars (No. 20625516) and the Science Fund for Creative Research Groups (No. 20921062). We would like to thank Dr. Barry Wong (Wuhan University) for helpful comments on the manuscript.

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