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Preparation of molecularly imprinted hybrid monoliths for the selective detection of fluoroquinolones in infant formula powders

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ABSTRACT

A novel molecularly imprinted inorganic-organic hybrid monolith (MIP hybrid monolith) was fabricated through a facile single-step polymerization strategy with levofloxacin (LEV) as the template, 3-aminopropyltriethoxysilane-methacrylic acid as the hybrid functional monomer and ethylene glycol dimethacrylate as the crosslinker in a mixed porogen of methanol, toluene and dodecanol. The optimized LEV-MIP hybrid monolith was characterized using scanning electron microscopy and fourier transform-infrared spectroscopy. Uniform monolithic matrix with large through-pores in the network skeleton of LEV-MIP hybrid monolith was observed. The influence of polymerization conditions on the specific recognition behavior of the resulting monolith was systematically investigated. The LEV-MIP hybrid monolith exhibited much better adsorption (3.62 times) and selectivity towards LEV in comparison with non-imprinted hybrid monolith. Furthermore, the LEV-MIP hybrid monolith based solid-phase extraction combining with liquid chromatography-mass spectrometry was applied for the selective determination of fluoroquinolones (FQs) in infant formula powder. The average recoveries of six FQs in milk powders spiked at 20, 50 and 100 μg kg^{-1} were in the range of 82.91–102.00% with the precision of 1.04–7.39%. The limit of detection and limit of quantitation of the proposed method were in a range of 0.19–1.24 μg kg^{-1} and 0.63–4.13 μg kg^{-1}, respectively.

1. Introduction

Infant food safety has always received a special attention because infants are more vulnerable to food contaminants or residues (antibiotics, pesticides and pharmaceuticals, etc.) than adults [1]. Fluoroquinolones (FQs), an important family of antibiotics with a broad-spectrum activity against Gram-positive and Gram-negative bacteria, have been widely used as veterinary drugs to promote the growth of livestock and prevent bacterial infections [2]. However, due to the abuse of antibiotics, FQs residues have been detected in various infant foods and could provoke allergic reactions or induce pathogen resistance to antibiotics [3,4]. Therefore, monitoring the FQs residues in infant foods is crucial.

Several analytical technologies, including liquid chromatography [5], immunoassays [6], microbial inhibition assays [7], capillary electrophoresis [8] and liquid chromatography-mass spectrometry (LC–MS) [9], have been developed for the detection of FQs residues in different matrix. Due to the complexity of sample matrices and low analyte concentration present in infant foods, sample pre-treatments are usually required prior to the determination of these FQs residues. Liquid-liquid extraction [10], dispersive liquid-liquid micro-extraction [11], solid-phase extraction (SPE) [12] or matrix solid-phase dispersion [13] based on C_{18}, MAX, WCX, or HLB sorbents were often employed for the purification and concentration of FQs residues in infant foods. However, these approaches are often not satisfied owing to the lack of specific recognition for target analytes in complex biological samples [13,14]. Herein, it is of interest to develop novel sample pre-treatment methods with satisfactory specificity and selectivity.

Organic polymer-based molecularly imprinted monoliths (MIP monoliths) have attracted considerable interest owing to their high specific recognition ability, easy fabrication, good mechanical stability and reusability [15,16]. Moreover, molecularly imprinted

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solid-phase extraction (MISPE) methods have been successfully applied for the enrichment and detection of trace antibiotics in complex samples [17]. However, some organic polymer-based MIP monoliths could tend to shrink or swell when exposed to different organic solvents [18,19], thus result in the morphology change of polymeric skeleton and decreasing the recognition ability towards the target molecules [20,21]. In recent years, organic-inorganic hybrid molecularly imprinted monolith (MIP hybrid monolith) has been proved to be an effective alternative to organic polymer-based MIP monoliths for overcoming the above shortcomings [19]. However, the preparation of MIP hybrid monoliths through the conventional sol-gel method often requires curing and aging at a high temperature, which inevitably results in poor properties due to the cracking and shrinkage of MIP hybrid monoliths. This fabrication strategy is also tedious, time-consuming, and difficult to conduct [20,21]. Recently, a hybrid monomer 3-aminopropyltriethoxysilane-methacrylic acid (APTES-MAA) was introduced to prepare MIP particles through bulk polymerization because it could produce an independent control of silica skeleton. The resulting silica skeletons showed high permeability, excellent mechanical strength and good organic solvent resistance [22–24]. It might be a solution for improving the preparation and performance of MIP hybrid monoliths. However, to the best of our knowledge, the hybrid functional monomer APTES-MAA has not been reported for the preparation of MIP hybrid monoliths.

In this study, a novel levofloxacin-MIP hybrid monolith was fabricated via a facile single-step polymerization strategy using levofloxacin (LEV) as the template, APTES-MAA as the hybrid functional monomer, ethylene glycol dimethacrylate (EDMA) as the cross-linker and γ-methacryloxypropyltrimethoxysilane (γ-MAPS) as the silane coupler in a mixed porogenic system of methanol, toluene and dodecanol. The optimized LEV-MIP hybrid monolith was characterized by fourier transform-infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). The selectivity of the LEV-MIP hybrid monolith was evaluated using 2-naphthalenecarboxylic acid as the reference and compared with non-imprinted hybrid monolith (NIP hybrid monolith). Finally, the proposed MISPE method was developed and applied for the selective extraction and determination of FQs residues in infant powders.

2. Material and methods

2.1. Reagents

LEV, fleroxacin (FLE), norfloxacin (NOR), ciprofloxacin (CIP), lomefloxacin (LOM), gatifloxacin (GAT), MAA, APTES, 2, 2-azodiisobutyronitrile (AIBN), γ-MAPS, EDMA and formic acid were all purchased from Aladdin Chemicals (Shanghai, China). Toluene, dodecanol, ammonium hydroxide, trichloroacetic acid and acetic acid (HAc) were obtained from Tianjin Kermel Chemical Reagent Co. Ltd. (Tianjin, China). HPLC grade methanol (MeOH) and acetonitrile (ACN) were bought from Merck (Shanghai, China), while potassium bromide (KBr) was purchased from Concord Chemical Research Institute (Tianjin, China). Ultrapure water obtained from a Milli-Q water purification system (Millipore Corporation, Bedford, MA, USA) was used throughout all experiments. All sample solutions were subjected to a filtration through a 0.22 μm filter before HPLC injection.

2.2. Instrumentation

A Jinghong DKS22 water bath (Shanghai, China) was used for thermally initiated copolymerization. FT-IR spectra were obtained on a JASCO FT/IR-4600 spectrophotometer (Tokyo, Japan) within the wavenumber range of 4000–400 cm^{-1} using KBr pellets. The morphology of the MIP hybrid monolith was measured on a ZEISS ULTRA 55 field emission scanning electron microscope (Oberkochen, Germany). All SPE experiments were performed on a SPE apparatus (Ruisen Biotechnology Co., Ltd, Guangzhou, China). HPLC experiments for investigating the recognition and adsorption properties of the LEV-MIP monolith were performed on a Dionex UltiMate 3000 HPLC system equipped with an UltiMate 3000 RS pump, an UltiMate 3000 RS autosampler, an UltiMate 3000 RS column compartment and Chromeleon version 6.8 software (Thermo Scientific, former Dionex, Bannockburn, IL). The quantitative analysis of the extracted analytes from the infant formulas was carried out using a Dionex Ultimate 3000 HPLC system coupled to a Triple Quad 3500 mass spectrometer equipped with a Turbo V ion source (AB SCIEX, Singapore).

2.3. Preparation of the MIP and NIP hybrid monoliths

To fabricate the MIP hybrid monoliths, a hybrid monomer APTES-MAA was synthesized according to literatures [22–24]. The template molecule LEV (0.05 mmol) and APTES-MAA (0.15 mmol) were first dissolved in 1.0 mL of mixed porogenic solvents (toluene:dodecanol:MeOH, 1:2:2, v/v/v). The mixture was poured into a 2.0-mL syringe which was sealed at the tip end, and then sonicated for 20 min. After self-assembling for 60 min at room temperature, γ-MAPS (0.2 mmol), EDMA (1 mmol) and AIBN (20 mg) were added into the above solution. The resulting solution was then deoxidized with bubbling nitrogen for 5 min and the syringe was sealed with a silicon rubber at the other end. After thermally initiated polymerization at 60 °C for 24 h, the silicon rubber was discarded and the sealed end of the syringe was cut off. Finally, the prepared LEV-MIP hybrid monolith was installed on the SPE apparatus and flushed with MeOH-HAc (80:20, v/v) to remove the template (Fig. S1). A NIP hybrid monolith was also prepared through the same procedure as that for the LEV-MIP hybrid monolith without adding LEV.

2.4. Sample preparation of infant formula powder

Infant formula powder was purchased from a local supermarket in Guangzhou. Prior to the extraction, 1.0 g of milk powder spiked with known variable amounts of FQs standard solution was vortexed with 10 mL of 10% (w/w) trichloroacetic acid-water for 2 min and then sonicated for 20 min. After being equilibrated at room temperature for 10 min, the mixture was centrifuged at 10,000 rpm for 10 min. The supernatant was transferred into a new centrifuge tube and adjusted to pH 7.0 with 10% ammonium hydroxide. Finally, the supernatant was filtered through a 0.22-μm syringe filter and then loaded on the LEV-MIP hybrid monolith for extraction.

2.5. MISPE method

The LEV-MIP or NIP hybrid monolith installed on the SPE apparatus was first activated with 2.0 mL of MeOH and 1.0 mL of water at a flow rate of 0.5 mL min^{-1}, successively. Thereafter, the sample solution was loaded on the MIP/NIP hybrid monolith at a flow rate of 0.1 mL min^{-1}. Finally, the MIP/NIP hybrid monolith was washed with 2 mL of 5% ACN in water (v/v) to remove the matrix interferences and eluted with 6 mL of MeOH-HAc (80:20, v/v) solution at a flow rate of 0.1 mL min^{-1}. The elution fraction was collected and evaporated to dryness under nitrogen and the residue was redissolved in 1 mL of initial HPLC mobile phase (15% MeOH-0.1% formic acid in water) for HPLC analysis.

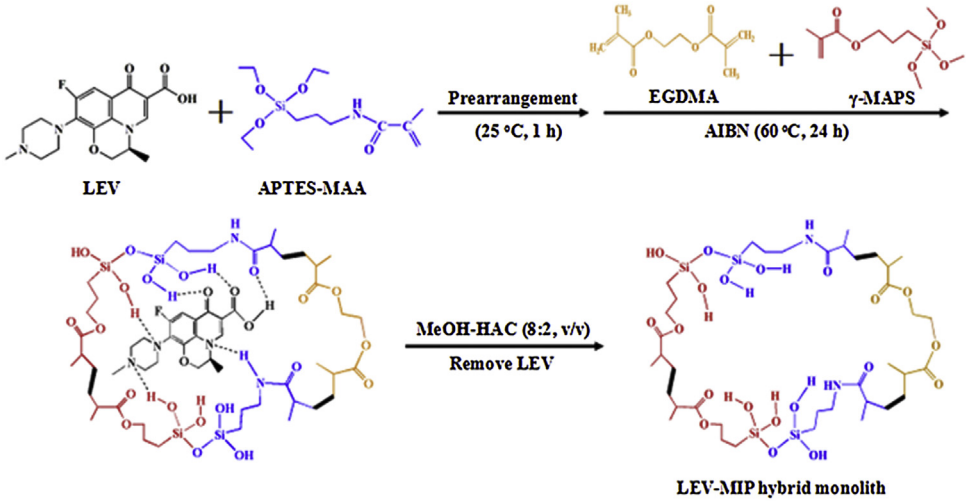

Fig. 1. Synthetic route of the LEV-MIP hybrid monolith.

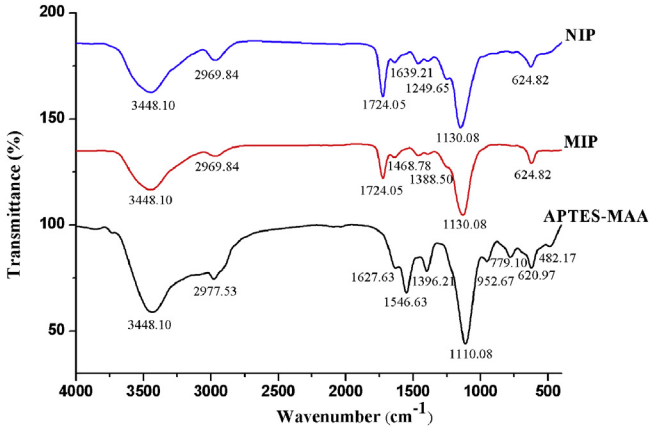

Fig. 2. FT-IR results of APTES-MAA, MIP hybrid monolith after removing LEV and NIP hybrid monolith.

2.6. LC–MS/MS conditions

LC–MS/MS experiments were performed using a Aenusil MP C_{18} column (100 Å 4.6 × 250 mm, 5 μm) from Bonna-Agela Technologies (Tianjin, China). The column temperature was maintained at 25 °C. The mobile phase was comprised of 0.1% formic acid in water (A) and MeOH (B), and the gradient program was as follows: 0–18.0 min, 15%–40% (v/v) B; 18.0–23.0 min, 40% B ; 23.0–25.0 min, 40%-15% B ; 25.0–30.0 min, 15% B. The injection volume and the flow rate were 10 μL and 0.6 mL min^{-1}, respectively.

MS was conducted in positive electrospray ionization (ESI) with multiple reaction monitoring mode. The major ESI parameters were as follows: ion spray voltage, 5500 V; ion source temperature, 500 °C; collision gas (CAD), 6 psi; nebulizer gas 1 (GS1), 45 psi; auxiliary gas 2 (GS2), 50 psi; curtain gas (CUR) 30 psi. The other optimal mass spectrometric parameters for each analyte were listed in Table S1.

2.7. Method validation of the MISPE-LC–MS method

The method validation was performed in terms of specificity, linearity range, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision according to US Food and Drug Administration guidelines [25] and Commission Decision 2002/657/EC [26]. The calibration curves were established by measuring the blank milk extract spiked with seven different concentrations (5–400 μg kg^{-1}) of test samples, including LEV, FLE, NOR, CIP, LOM or GAT. Each sample was prepared in triplicate and injected three times. The LODs and LOQs were calculated by injecting a series of dilute solutions with known concentration and defined as concentrations with a peak height corresponding to 3 and 10 times the baseline noise, respectively. To assess the intra-day precision, five spiked samples at each concentration levels (20,

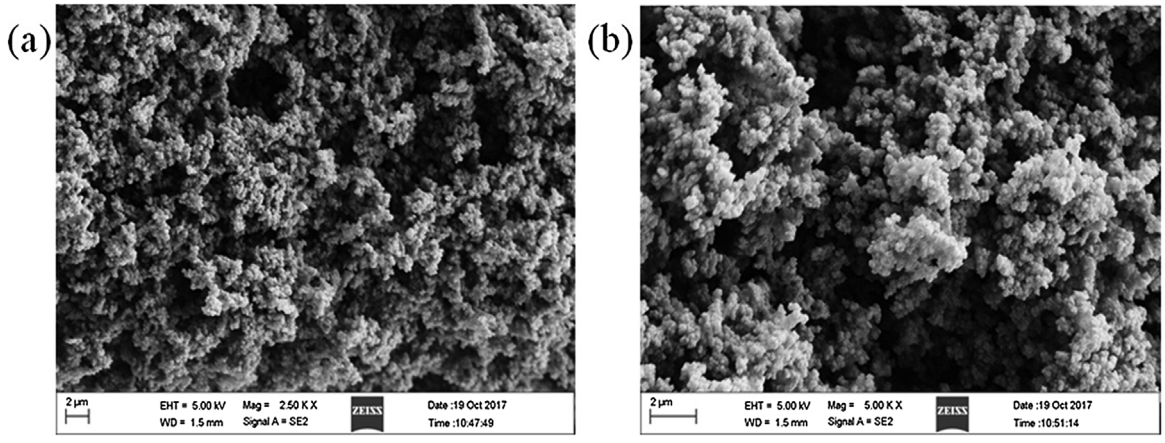

Fig. 3. SEM images of the LEV-MIP hybrid monolith. (a) SEM image magnified 2500-fold; (b) SEM image magnified 5000-fold.

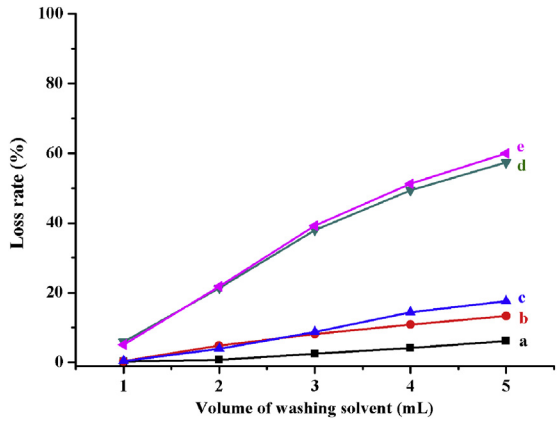

Fig. 4. Influence of the type and volume of washing solvents on the loss rate of LEV from the LEV-MIP hybrid monolith. (a) water, (b) 5% MeOH-H_2O, (c) 5% ACN-H_2O, (d) MeOH and (e) ACN.

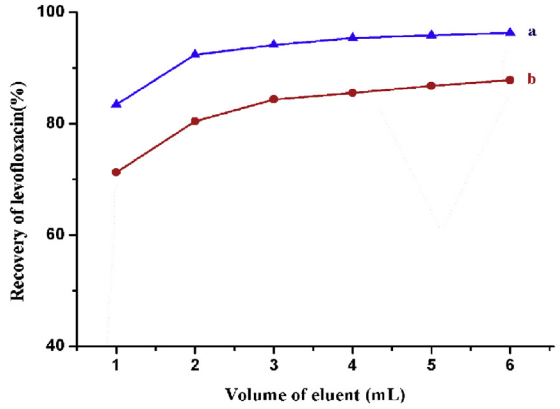

Fig. 5. Influence of the composition and volume of eluent on the recovery of LEV. (a) MeOH-HAc (80:20, v/v); (b) MeOH-HAc (90:10, v/v).

50 and 100 μg kg^{-1}) were prepared and analyzed three times by LC–MS/MS. The procedure was repeated on three different days to determine inter-day precision. The extraction recoveries were determined as the peak area ratio of traget FQ spiked into the infant milk powder before or after MISPE [27,28].

3. Results and discussion

3.1. Preparation and optimization of the LEV-MIP hybrid monolith

In this study, the LEV-MIP hybrid monolith was prepared via a facile single-step polymerization approach (Fig. 1), which may significantly simplify the preparation process of MIP hybrid monolith in comparison with the conventional sol-gel approach. Several crucial factors affecting the preparation and imprinting capacity of the resulting MIP hybrid monolith were systemically investigated, including the types of functional monomer, porogenic solvents and cross-linker, the molar ratio of the template molecule to functional monomer, the amount of cross-linker.

Hybrid functional monomer plays a prominent role in forming stable host-guest complexes prior to the polymerization, and thus affects the affinity and selectivity of the resulting MIPs [29]. In this study, APTES-MAA was selected as the hybrid functional monomer because of its rich functionalities including vinyl, silicate ester and acylamino groups. As shown in Fig. 1, the vinyl groups can be cross-linked with EDMA through the thermally initiated free radical polymerization in the presence of initiator

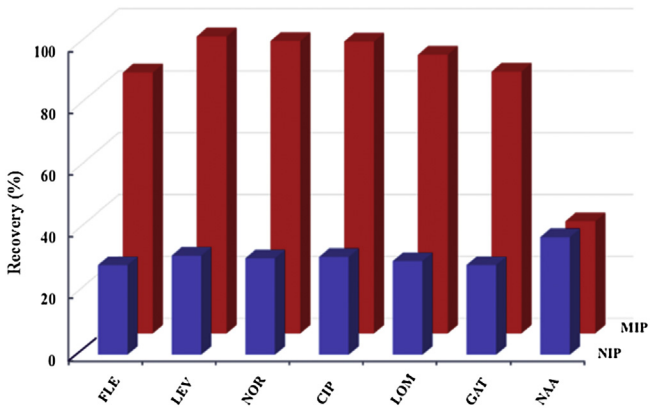

Fig. 6. Recoveries of all test analytes after MISPE and NISPE under the optimized conditions.

(AIBN). After alcoholysis. the silicate ester from APTES becomes silicon hydroxyl, which will react with γ-MAPS through condensation to produce the polymeric skeleton. Moreover, these silicon hydroxyl groups and the acylamino groups could also form plentiful hydrogen bonds with the carboxyl and tertiary amine groups of LEV. These interactions are beneficial for the selective capture of LEV.

The selection of porogenic solvents was another important step in the preparation of MIP hybrid monoliths since the type and amount of porogens could affect the pore size distribution, mechanical strength and permeability of the resulting MIP hybrid monoliths [30,31]. In order to choose an appropriate porogenic system, the following properties were considered: (a) a good dissolution ability for the template, hybrid monomer, initiator and crosslinker [32]; (b) to produce a suitable distribution between macropores and micropores, guaranteeing a good permeability and large surface area for the resulting MIP hybrid monoliths [33]; (c) a relatively low polarity in order to reduce the interferences during complex formation between the template molecule and the monomer [32]. Based on these considerations, dodecanol and toluene were initially selected as porogenic solvents for the preparation of LEV-MIP hybrid monoliths. However, it was found that LEV is difficult to be dissolved in these nonpolar solvents, and thus MeOH was added to improve the solubility of LEV. The proportion of the porogenic mixture (toluene: dodecanol: MeOH) was then systematically optimized. The results showed that a soft gel-like form appeared when the content of toluene in the porogenic mixture was less than 10%, while the imprinted monolith was too dense to allow mobile phase passing through if the toluene content was higher than 30%. Hence, the volume ratio of toluene: dodecanol: MeOH (1:2:2, v/v/v) was finally selected as the optimum porogenic mixture because it yields a MIP hybrid monolith with sufficient rigidity and permeability.

The influence of the molar ratio of the template molecule, functional monomer and cross-linker (T/M/C) on the selectivity and capacity of the LEV-MIP hybrid monolith was also investigated. The NIP hybrid monolith was employed as the comparison. The effect of the amount of cross-linker was first studied by varying T/M/C from 1:3:10 (column **M1**) to 1:3:40 (column **M4**). The monolith **M1** exhibited bad mechanical stability and the bulk polymer was detached and flushed out from the syringe using MeOH-HAc (80:20, v/v) at 0.5 mL/min. For the rest three LEV-MIP hybrid monoliths, their recovery for LEV decreased with increasing the content of cross-linker (Table S2). Therefore, T/M/C (1:3:20) was selected for further experiments. The influence of the template-monomer ratio on the selectivity was also investigated by varying the molar ratio

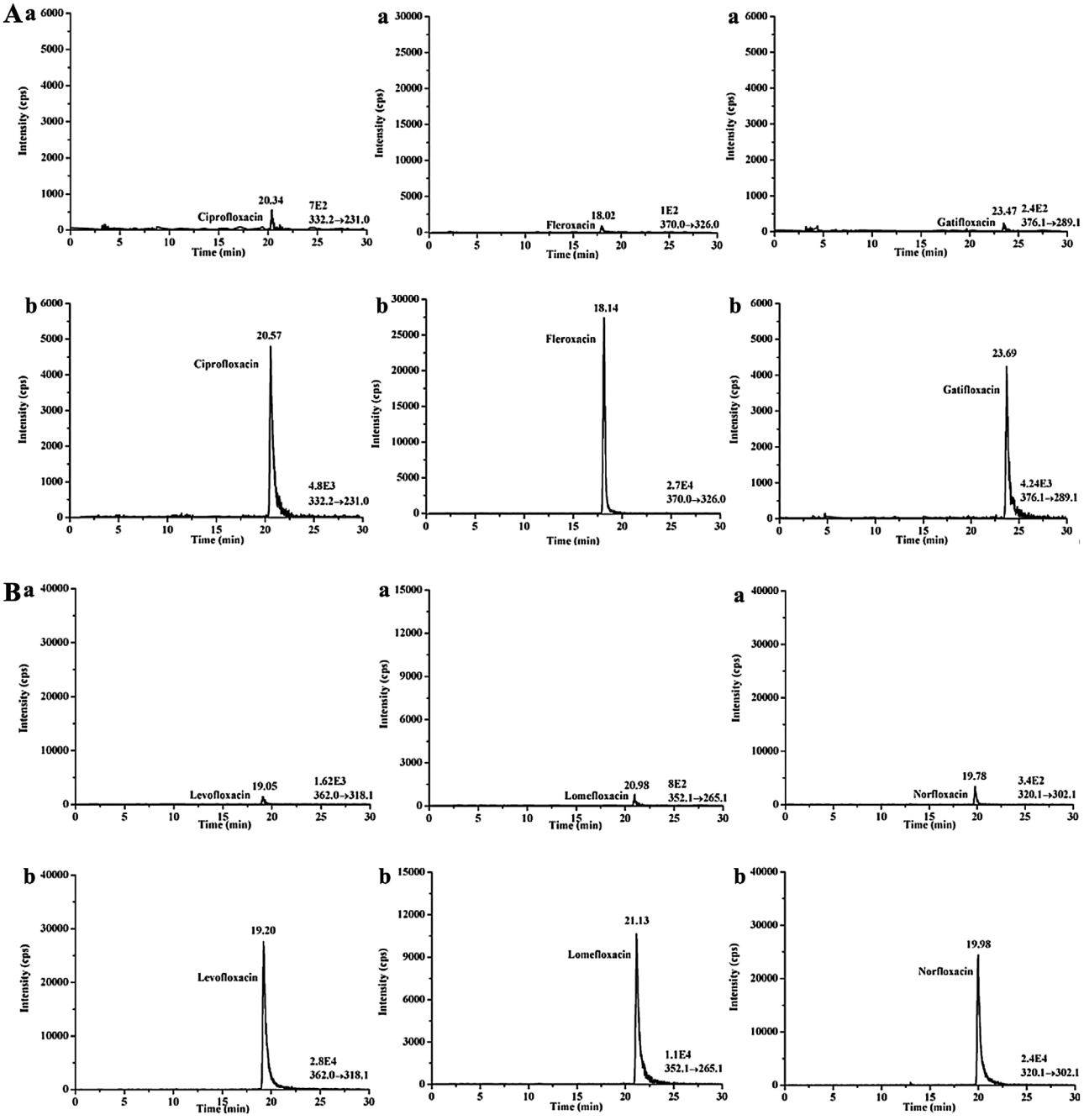

Fig. 7. Typical MRM chromatograms of the infant formula powder samples spiked with FQs. (A) before the MISPE treatment (a), (B) after the MISPE treatment (b). Milk powder samples were spiked at 50 μg kg^{-1} with FLE, LEV, NOR, CIP, LOM and GAT, separately.

of T/M from 1:2 to 1:6, while keeping the other components constant. Compared with column **M2,** either the decrease (column **M5**) or increase (column **M6**, **M7** and **M8**) of the amount of the functional monomer resulted in a significant reduction in recovery. Therefore, the optimized molar ratio of T:M:C (1:3:20) was chosen for further studies.

3.2. Characterization of the LEV-MIP hybrid monolith

FT-IR experiments were performed to characterize the prepared LEV-MIP hybrid monoliths. As shown in Fig. 2, the characteristic peaks of APTES-MAA are around 3448, 1627, 1396 and 1110 cm^{-1}, corresponding to the stretching vibration of N—H, C=O, C=C and Si-O, respectively [24,34]. Almost the same FT-IR spectra were observed for LEV-MIP and NIP hybrid monoliths. The sharp peaks at 1724 cm^{-1} could be ascribed to C=O stretching vibration [24]. The bands at 1130 cm^{-1} were assigned to the Si-O-Si stretching vibration [24]. The broad peak at around 3448 cm^{-1} (N—H from APTES-MAA) was also observed, which indicated that APTES-MAA participated in the copolymerized and embedded into the LEV-MIP and NIP hybrid monoliths.

The morphology of the optimized LEV-MIP hybrid monolith was inspected using SEM. As depicted in Fig. 3, large clusters of interconnected uniform microglobules were formed, which is favorable for the mass transfer of target molecules during the extraction of target molecules from complex matrix [14]. All these results con-

Table 1
Recoveries, precisions, LODs and LOQs of the MISPE-LC–MS method for six FQs in infant formula powder.

Compounds	Linear equation	r	Added ($\mu g\,kg^{-1}$)	Recovery (%)	RSD (%)		LOD ($\mu g\,kg^{-1}$)	LQD ($\mu g\,kg^{-1}$)
					Intra-day (n = 5)	Inter-day (n = 5)		
			20	84.22	3.77	6.35		
FLE	A = 8674.5C-12120	0.9995	50	85.45	3.04	6.58	0.53	1.76
			100	83.57	5.86	6.45		
			20	96.64	3.91	5.93		
LEV	A = 12189C + 58,946	0.9988	50	95.32	2.65	6.75	0.19	0.63
			100	90.70	1.04	5.51		
			20	88.64	2.35	5.17		
NOR	A = 11527C-28667	0.9998	50	87.30	2.43	7.07	0.46	1.53
			100	93.50	1.95	4.62		
			20	98.69	5.97	6.86		
CIP	A = 2071.8C-11917	0.9994	50	84.17	2.93	5.32	0.90	3.00
			100	89.94	2.86	6.40		
			20	94.68	3.26	6.25		
LOM	A = 5467.3C-10555	0.9998	50	82.91	3.02	5.27	0.85	2.83
			100	96.78	6.86	7.35		
			20	102.00	5.20	7.39		
GAT	A = 2123.4C-7943.5	0.9998	50	89.04	4.85	5.21	1.24	4.13
			100	98.44	4.58	7.00		

firmed the LEV-MIP hybrid monolith was successfully prepared via a facile single-step polymerization.

3.3. Optimization of MISPE method

The washing step is to remove the interferences from the sample matrix without sacrificing the specific interactions between analytes and MIP hybrid monolith. Therefore, different solvents were investigated to obtain the optimal washing solvent for purifying the FQs from milk samples. Fig. 4 showed the loss rate (loss rate = 100% – recovery of LEV) of LEV from the LEV-MIP hybrid monolith after washing with five different solvents separately, i.e. H_2O, 5% MeOH-H_2O, 5% ACN-H_2O, MeOH and ACN. From the results, it can be found that the loss rate of LEV caused by 2.0 mL of H_2O (0.76%) was lower than those caused by 2.0 mL of 5% ACN-H_2O (3.87%), 5% MeOH-H_2O (4.86%), MeOH (21.39%) and ACN (21.83%), respectively. Because the elution ability of H_2O for organic impurity is poor, 5% ACN-H_2O was selected for the further investigation. The volume of washing solvents was also investigated by varying the amount of 5% ACN-H_2O (1.0, 2.0, 3.0, 4.0, and 5.0 mL). It was found that when the volume of washing solvent is less than 2.0 mL, the removal efficiency of impurities is relatively poor although the loss rate of LEV is negligible (only 0.32%). When washing solvent is higher than 2.0 mL, the loss rate for LEV increased from 3.87% to 8.74%. In addition, the hydrophilic impurities in infant formula powder samples could be mostly cleaned up. Therefore, 2 mL of 5% ACN-H_2O was selected as the washing solvent.

The non-covalent hydrogen bonds are responsible for the capture of LEV on the LEV-MIP hybrid monolith and these interactions can be destroyed by highly polar solvents [35]. The mobile phase containing HAc was usually employed as eluent to wash out the captured molecules from MIPs [14,36]. Therefore, MeOH-HAc system was selected as the eluent in this research. In order to achieve a high recovery for FQs, the composition and volume of the eluent were also investigated. As depicted in Fig. 5, higher recoveries were obtained using MeOH-HAc (80:20, v/v) as the eluent in comparison with those using MeOH-HAc (90:10, v/v). It was also found that the recoveries of LEV clearly increased with increasing the eluent volume from 1 to 2 mL, and then increased continuously and slowly. Using 6 mL of MeOH-HAc (80:20, v/v) as the eluent, the recovery reached 96.7%.

3.4. Specificity and adsorption capacity of the LEV-MIP hybrid monolith

In order to evaluate the selectivity of the LEV-MIP hybrid monolith, a mixture containing FLE, LEV, NOR, CIP, LOM and GAT and the reference 2-naphthalenecarboxylic acid (NAA) was employed as test sample (1 $\mu g\,mL^{-1}$ of spiking level for each compound). As shown in Fig. 6, the LEV-MIP hybrid monolith showed good capture ability for the above six FQs with good recoveries ranged from 84.3% to 96.0%, while the recoveries on the NIP monolith is only around 30%. For NAA, very similar recoveries (around 36.2%) were observed on both LEV-MIP and NIP hybrid monoliths. The higher recovery for FQs obtained on the LEV-MIP hybrid monolith could be attributed to the specific recognition of the imprinted LEV cavities.

In order to evaluate the absorption capacity of the LEV-MIP hybrid monolith for FQs, an adsorption experiment was performed as follows: 10 $\mu g\,mL^{-1}$ LEV standard solution was continuously pumped through the LEV-MIP hybrid monolith at a flow rate of 0.1 mL min^{-1} until the collected LEV concentration in eluents did not change any more (tested by HPLC).

The maximum adsorption capacity (Q) of the LEV-MIP hybrid monolith was calculated according to Eq. (1) [37]:

$$Q = \frac{CV}{m} \quad (1)$$

where V is the breakthrough volume of solution (mL); m is the amount of the LEV-MIP hybrid monolithic material; C is the initial concentration of LEV, respectively. The calculated Q value of the LEV-MIP hybrid monolith is 152.6 $\mu g\,g^{-1}$. This result suggested that the proposed LEV-MIP hybrid monolith has a good potential as a SPE material for the efficient extraction of FQs.

3.5. Determination of FQs in infant formula powder samples by MISPE-LC–MS

In order to evaluate the applicability and reliability of the LEV-MIP hybrid monolith, it was then employed as MISPE for LC–MS determination of the six FQs in a milk powder sample bought from local market. The results showed that the sample is free of FLE, LEV, NOR, CIP, LOM or GAT contaminations. Therefore, the six FQs were added into the milk powder sample at 50 $\mu g\,kg^{-1}$ separately, and the spiked infant formula powder samples were applied in

following studies. Fig. 7 showed the MRM chromatograms of the infant formula powder samples spiked with 50 μg kg^{-1} of each FQs separately. Significant differences in peak intensity were observed before and after treating by the MISPE, which further demonstrated that the LEV-MIP hybrid monolith has a good purification and enrichment ability for FQs.

The linearity, limit of detection (LOD, S/N = 3), limit of quantitation (LOQ, S/N = 10), accuracy and precision of the proposed MISPE-LC–MS method were investigated for the six FQs (FLE, LEV, NOR, CIP, LOM and GAT) under the optimized conditions. Seven-point matrix-matched calibration curves were established using the blank milk extract spiking with the FQs over the range of 5–400 μg kg^{-1}. As shown in Table 1, good linearities were obtained with correlation coefficients (r) ranged from 0.9988 to 0.9998, and the LODs and LOQs were in the range of 0.19–1.24 μg kg^{-1} and 0.63–4.13 μg kg^{-1}, respectively. The repeatability of the method was determined by the inter-day and intra-day precisions at three different spiking concentrations (20, 50, 100 μg kg^{-1}). Good repeatabilities were evidenced by the intra-day and inter-day relative standard deviation (RSD) values ranged from 1.04 to 7.39%. The recoveries for the six FQs in the spiked milk powder samples were in the range of 82.91–102.00%.

4. Conclusion

In this study, a novel LEV imprinted hybrid monolith was designed and synthesized for the sample pretreatment of FQs residue in complex matrices via a facile single-step polymerization method. The developed approach avoids the tedious curing and aging steps of the conventional sol-gel method. The resulting LEV-MIP hybrid monolith showed high selectivity, specificity, and absorption capacity for FQs. The optimized MISPE-LC/MS-MS method exhibited good potential for the selective purification and determination of FLE, LEV, NOR, CIP, LOM and GAT in milk powder matrices.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.chroma.2018.12.038.

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