

# Sensitive Monitoring of Fluoroquinolones in Milk and Honey Using Multiple Monolithic Fiber Solid-Phase Microextraction Coupled to Liquid Chromatography Tandem Mass Spectrometry

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## S Supporting Information

**ABSTRACT:** In the present study, a new multiple monolithic fiber solid-phase microextraction (MMF-SPME) based on poly(apronal-*co*-divinylbenzene/ethylenedimethacrylate) monolith (APDE) was synthesized. The effect of the preparation parameters of APED on extraction efficiency was studied thoroughly. The combination of APDE/MMF-SPME with high-performance liquid chromatography tandem mass spectrometry detection (HPLC/MS-MS) was developed for sensitive monitoring of ultratrace fluoroquinolones (FQs) in foodstuffs, including milk and honey samples. Under the optimized experimental conditions, the limits of detection (S/N = 3) for the targeted FQs ranged from 0.0019 to 0.018  $\mu\text{g}/\text{kg}$  in milk and 0.0010 to 0.0028  $\mu\text{g}/\text{kg}$  in honey. The relative standard deviations (RSDs) for method reproducibility were less than 9% in all samples. The established method was successfully applied for the monitoring of FQs residues in milk and honey samples with the recoveries between 74.5% and 116% (RSDs were in the range 0.9–9.5%). In comparison to previous methods, the developed APDE/MMF-SPME-HPLC/MS-MS showed some merits, including satisfactory sensitivity, simplicity, high cost-effectiveness, and low consumption of organic solvent.

**KEYWORDS:** fluoroquinolones, multiple monolithic fiber solid-phase microextraction, adsorbent, honey, milk

## INTRODUCTION

Fluoroquinolones (FQs) are an important and powerful group of synthetic antimicrobial agents.<sup>1</sup> Because of their effective antibacterial activities, FQs have been widely used to treat a variety of diseases and infections.<sup>2</sup> The widespread use of FQs has led to potential residues in foodstuffs of animal origin, such as milk and honey. The residues of FQs in milk and honey may do harm to human health.<sup>3</sup> To ensure the safety of customers, the maximum residue limits (MRLs) for some FQs in milk have been regulated by the European Union (EU), the Chinese government, and other organizations<sup>4,5</sup> For example, the EU has established the MRLs for enrofloxacin (ENR) and ciprofloxacin (CIP) in milk as 100  $\mu\text{g}/\text{kg}$  and 75  $\mu\text{g}/\text{kg}$ , respectively.<sup>4</sup> In honey, however, there is no related MRL regulation for FQs-based antibiotics. Even so, the use of FQs is strictly forbidden. Consequently, the presence of FQs residues and their metabolites in bee products will be deemed illegal beekeeping practices.<sup>6</sup> Based on the above-mentioned reasons, sensitive and effective methods for monitoring of trace FQs in milk and honey samples are required.

Up to now, several methodologies, such as immunoassays,<sup>6,7</sup> biosensors,<sup>8,9</sup> and chromatography,<sup>10,11</sup> have been used to analyze FQs. Because of simplicity, convenience, and effective power in qualification and quantification, the chromatographic method has become widespread for the determination of FQs in all kinds of complex samples. However, to extract the trace FQs from complex matrices, appropriate sample pretreatment steps are required. So far, a few sample preparation techniques, including liquid–liquid extraction (LLE),<sup>12</sup> solid-phase extraction (SPE),<sup>13–15</sup> stir bar sorptive extraction (SBSE),<sup>16</sup> stir cake

sorptive extraction (SCSE)<sup>17</sup> and magnetic solid-phase extraction (MSPE),<sup>18</sup> have been utilized to analyze FQs. The main shortcomings of these techniques include the use of a large amount of sample and toxic organic solvent, tedious and time-consuming extraction and cleanup procedures, unsatisfactory repeatability, and low extraction capacity.

Solid-phase microextraction (SPME) is another promising sample pretreatment technique for the analysis of FQs. SPME possesses some outstanding merits, including simplicity, flexibility, easy-of-operation, and environmental friendliness. Therefore, SPME has been utilized to extract trace FQs from real samples.<sup>19–21</sup> The advantages of SPME have been utilized in these studies. However, low extraction capacity resulting from low quantity of adsorbent (coating) limits the wide acceptance of SPME. So, in order to utilize SPME to extract FQs effectively, there is an urgent need to develop a new adsorbent and extraction format. Based on the principle of SPME, multiple monolithic fiber solid-phase microextraction (MMF-SPME) using porous monoliths as adsorbent were proposed in our previous studies.<sup>22,23</sup> The MMF-SPME possesses expected extraction capacity because there are four independent thin monolithic fibers in a MMF-SPME. Furthermore, MMF-SPME utilizes porous monoliths as adsorbent. There are many advantages for monoliths, including easy synthesis, fast mass-transfer, and various kinds of chemical

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Table 1. Extraction Performance of Different APDE/MMF-SPME for Seven FQs<sup>a</sup>

No.	Monomer mixture (% w/w)		Polymerization mixture (% w/w)		Peak area						
	Monomer	Cross-linker	Monomer mixture	Porogen solvent	MAR	NOR	CIP	LOM	ENR	SAR	SPA
1	20	80	40	60	22783	51993	30330	24180	72611	44263	52524
2	25	75	40	60	28260	52694	32585	25819	70020	33977	37673
3	30	70	40	60	28627	60923	41504	30865	89697	50437	63912
4	35	65	40	60	9685	17071	10996	9382	37136	20765	31303
5	40	60	40	60	9289	16622	12100	10508	37489	21628	33196
6	45	55	40	60	16830	22188	16158	13348	43059	22327	31840
7	50	50	40	60	7966	14849	9859	9043	33541	19630	29090
8	30	70	25	75	24378	67382	41739	27930	97389	44523	57720
9	30	70	30	70	64217	125334	79505	62454	157729	71017	79952
10	30	70	35	65	30723	53423	34846	29361	85777	40473	46976
11	30	70	45	55	29373	50002	32925	27210	98473	45752	55106
12	30	70	50	50	26094	48081	23265	20690	68475	33138	56039

<sup>a</sup>Note: ultrapure water was used as sample matrices, and the spiking concentration for each targeted analytes was 10.0 µg/L.

properties. Up to now, a few monolith-based adsorbents have been prepared and used to extract FQs with microextraction formats. Feng et al. fabricated a poly(methacrylic acid-co-ethylene glycol dimethacrylate) monolithic column and used it to extract FQs under in-tube solid-phase microextraction (IT-SPME) format.<sup>24</sup> In their succedent study, molecularly imprinted polymers (MIPs) were prepared and served as the adsorbent of polymer monolith microextraction (PMME) of FQs.<sup>25</sup> Feng's works indicate that monoliths may serve as the adsorbent for the enrichment of FQs from real samples. Nevertheless, the extraction capacity is not as high as expected. The reasons may be that, in the PMME and IT-SPME formats, the quantities of adsorbents (monoliths) are low. Furthermore, the prepared monoliths cannot produce multiply interactions with FQs molecules.

In this work, we try to combine the advantages of MMF-SPME and porous monoliths, developing a convenient and effective sample preparation for the monitoring of FQs. In our previous studies, MMF-SPME using poly(vinylimidazole-co-ethylene dimethacrylate) monolith (VIED) and polymeric ionic liquid-based monolith (PILM) as adsorbents were prepared.<sup>22,23</sup> The VIED/MMF-SPME and PILM/MMF-SPME showed good extraction performance for chlorophenols and steroid sex hormones, respectively. However, our preliminary experiments indicated that there was no obvious enrichment of FQs on VIED/MMF-SPME and PILM/MMF-SPME. Therefore, to explore MMF-SPME to extract FQs effectively, new adsorbents should be designed and prepared according to the characteristics of FQs. There are abundant polar piperazine and carboxyl groups and nonpolar phenyl groups in the molecular structures of FQs (Table S1). Based on the principle of "like dissolves like", apronal (AP), which contains ample polar groups, was selected as monomer to copolymerize with dual cross-linkers (divinylbenzene and ethylenedimethacrylate) (DE) to synthesize the monolithic fibers. Because there are abundant functional groups including amino and phenyl groups in the prepared adsorbent, multi-interactions, including  $\pi$ - $\pi$ , hydrogen-bond dipole-dipole, and hydrophobic interactions, will involve the extraction. Hereby, it is reasonable to believe that the developed APDE/MMF-SPME possesses expected extract performance for FQs. After optimizing the preparation and extraction conditions, an efficient and sensitive methodology combining the APDE/MMF-SPME and high performance liquid chromatography tandem mass spectrometry

detection (APDE/MMF-SPME-HPLC/MS-MS) for the determination of ultratrace FQs in foodstuffs of animal origin, including milk and honey, was developed.

## MATERIALS AND METHODS

**Chemicals.** AP (98%) was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Divinylbenzene (DB) (80%) and ethylenedimethacrylate (EDMA) (97%) were obtained from Alfa Aesar Ltd. (Tianjin, China). Azobis(isobutyronitrile) (AIBN) (97%), dimethyl sulfoxide (DMSO), formic acid (FA), and trifluoroacetic acid (TFA) were supplied by Xilong Chemical Co. (Guangzhou, China). Methanol and acetonitrile (ACN) were HPLC-grade, and they were obtained from Tedia (Fairfield, Ohio, USA). Ultrapure water used in the study was obtained from a Milli-Q water purification system (Millipore Corp, Billerica, MA, USA). Targeted FQs, including CIP, ENR, marbofloxacin (MAR), norfloxacin (NOR), lomefloxacin (LOM), sparfloxacin (SPA), and sarafloxacin (SAR), were purchased from the Chinese Institute for the Control of Pharmaceutical and Biological Products. Table S1 shows the structures and relevant properties of the targeted FQs.

Targeted FQs were weighed accurately and dissolved in methanol respectively to obtain the individual standard stock solutions (10.0 mg/L), using the individual standard stock solution and methanol to prepare the mixed standard solution (10.0 µg/L) which was used to validate the method. All samples were preserved in a refrigerator at -4 °C before use.

**Equipment.** All analyses of FQs were performed using an Agilent 1290 Infinity LC system equipped with an auto sampler and coupled to an Agilent 6490 triple quadrupole mass spectrometer (MS/MS). The Agilent Masshunter Workstation Software (Foster City, CA, USA) was used to control the 1290 Infinity LC system.

The HPLC column used for the separation of FQs was a Phenomenex Kinetex C<sub>18</sub> (100 mm × 3.0 mm, 2.6 µm particle size) with guard from Phenomenex (Aschaffenburg, Germany). The mobile phase consisted of ultrapure water containing 0.5% (v/v) FA (solvent A) and ACN with 0.5% formic acid (v/v) (solvent B). To separate the analytes effectively, a gradient elution program was used, and the detailed program was as follows: 0–2 min = 12% B, 2–4 min = 12%–55% B and kept for 5 min, 5–7 min = 55% B–12% B and kept for 9 min. At the same time, the flow rate, column temperature, injection volume, and auto sampler temperature were set at 0.25 mL/min, 30 °C, 10 µL, and 10 °C, respectively.

The prepared monolith was characterized with elemental analysis (EA), Fourier transform infrared (FT-IR), scanning electron microscopy (SEM), and pore size distribution (PSD). The detailed information about the instruments used for the characterization can be found in the Supporting Information.

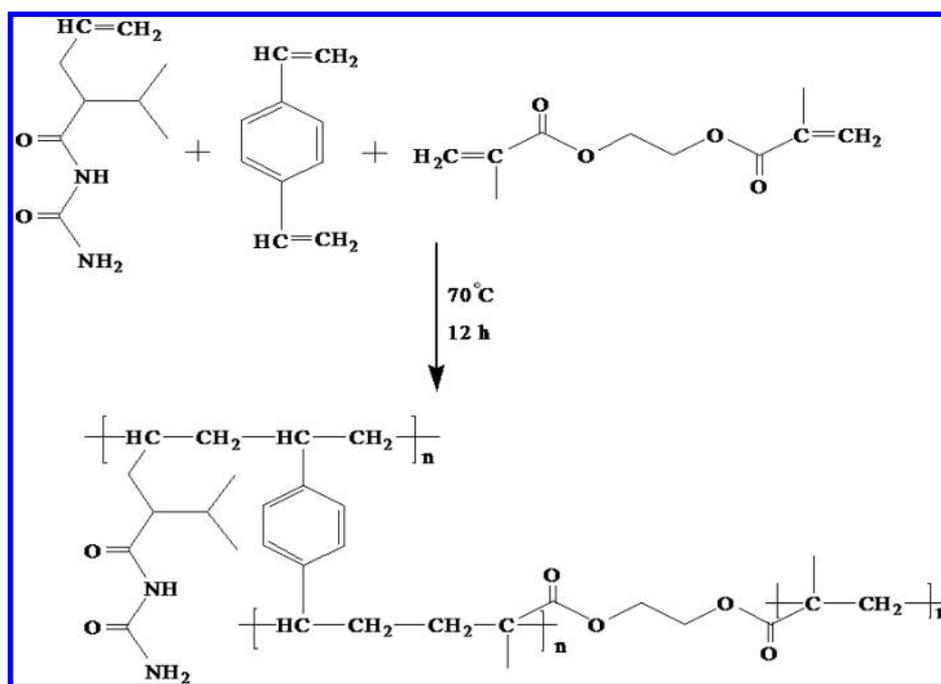


Figure 1. Reaction sketch of the poly(AP-co-DB/ED) monolith.

**Mass Spectrometry.** In the present work, LC-MS/MS analyses were operated in positive electrospray ionization mode (ESI) with the ion source temperature of 250 °C and capillary voltage of 3.5 kV. For each molecule of targeted analyte, the two most sensitive multiple reaction monitoring (MRM) transitions were determined. The desolvation temperature was set at 350 °C. The flow rates of the cone gas and desolvation gas were 840 and 50 L/h, respectively. The other MS parameters, including precursor ion (PI), fragmentor voltage (FV), daughter ions (DI), and collision energy (CE) for targeted FQs, can be seen in Table S1.

**Preparation of APDE/MMF-SPME.** The whole preparation procedure for APDE/MMF-SPME includes two steps. First, single thin fibers based on the poly(AP-co-DB/ED) monolith were fabricated utilizing the in situ polymerization technique of the porous monolith. In this work, AIBN was employed as initiator (1% (w/w) of the total amount). The cross-linker consisted of DB and ED (w/w = 2/1), and DMSO was used as porogen. To achieve the optimal preparation conditions, different concentrations of monomer, cross-linker, and porogen were used to synthesize different monolithic fibers. According to the corresponding proportions shown in Table 1, monomer, cross-linker, porogen, and AIBN were weighed accurately and mixed ultrasonically into a homogeneous solution. After that, the polymerization solution was purged with nitrogen for 3.0 min to remove the air in the solution. Subsequently, the solution was carefully infused into a glass capillary (0.5 mm in diameter and 10 cm in length). After the injection, silicon rubbers were used to seal both ends of the capillary immediately. The polymerization was realized in an oven at 70 °C for 12 h. After polymerization, 2 cm length of glass capillary was removed carefully by use of a grindstone to obtain white, elastic, and thin fibers (the dimension was 2 cm in length and 0.5 mm in diameter). After the preparation of thin monolithic fibers, four monolithic fibers were cautiously bound to form a fiber bunch. Subsequently, the fiber bunch was dipped in methanol for 24 h to remove the unreacted AP, DB/ED, and porogen in the monolith. Finally, the fiber bunch was dried in air for 0.5 h to obtain the APDE/MMF-SPME. The Figure 1 shows the reaction sketch of the poly(AP-co-DB/ED) porous monolith. The photos of prepared the single monolithic fiber and the final APDE/MMF-SPME can be seen in Figures S1a and S1b, respectively.

**APDE/MMF-SPME Procedure.** Stirring extraction and liquid desorption (LD) modes were used in the present study, before extraction, using methanol (2.0 mL) and water (2.0 mL) to precondition the APDE/MMF-SPME consecutively. Twenty milliliters

of sample solution (the pH value was adjusted to 8.0) was added into a 25 mL vial. The part of the monoliths in APDE/MMF-SPME was directly immersed into the sample solution to extract the analytes for 50 min under stirring mode. After extraction, the adsorptive analytes were eluted from the APDE/MMF-SPME with 400  $\mu$ L of desorption solvent (methanol/0.5% FA aqueous solution, v/v = 85/15) by stirring for 25 min. After that, the stripping solvent was evaporated to dryness with nitrogen. Methanol (0.1 mL) was used to dissolve the dried residue, and the solution was used for HPLC/MS-MS analysis. To avoid analyte carryover, the used APDE/MMF-SPME was reconditioned in turn with methanol and ultrapure water, respectively.

**Preparation of Honey and Milk Samples.** Different brands of honey and milk samples were bought from local retail markets. The contents of protein and fat in milk are 30 g/kg and 13 g/kg, respectively. The preparation procedure of honey and milk was as follows. For honey samples, the targeted FQs were directly spiked into 2.0 g of analyte-free honey samples. After 10 min for equilibration, the spiking samples were diluted with ultrapure water to 20 mL (the spiking concentration of each analyte was in the range 0.005–50.0  $\mu$ g/kg). The pH value of honey was adjusted to 8.0. Subsequently, the above-mentioned APDE/MMF-SPME procedure was used to extract targeted analytes.

For milk samples, the seven analytes were directly spiked into 2.0 g of analyte-free milk samples over the range 0.005–50.0  $\mu$ g/kg. To precipitate the protein and remove the fat in milk, 0.5 mL of TFA was added. The spiking sample solution was vortexed for 2.0 min and subsequently centrifuged at 4000 r/min for 10 min. The supernatant was transferred into a 25 mL vial and diluted to 20 mL with ultrapure water. The pH value of the solution was adjusted to 8.0, and the extraction procedure was performed with APDE/MMF-SPME.

**Method Validation.** Validation of the method was carried out in terms of linear dynamic ranges, correlation coefficients, LODs (S/N = 3), LOQs (S/N = 10), and reproducibility. The detailed investigation procedure for these parameters can be seen in the Supporting Information.

## RESULTS AND DISCUSSION

**Optimization of Preparation Conditions for APDE/MMF-SPME.** To achieve the best extraction performance and satisfactory longevity of the APDE/MMF-SPME, the proportions of the functional monomer (AP), cross-linker (the

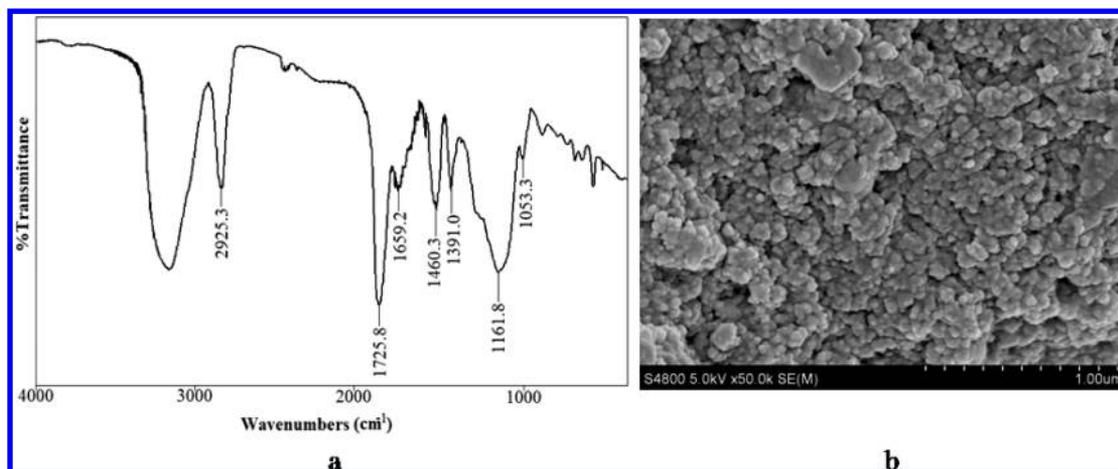


Figure 2. FT-IR spectrum (a) and SEM image (b) of the poly(AP-co-DB/ED) monolith.

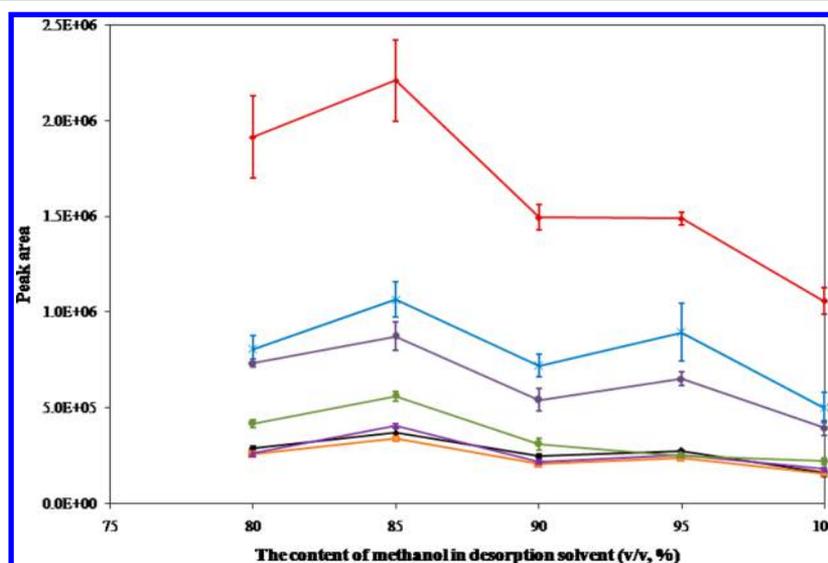
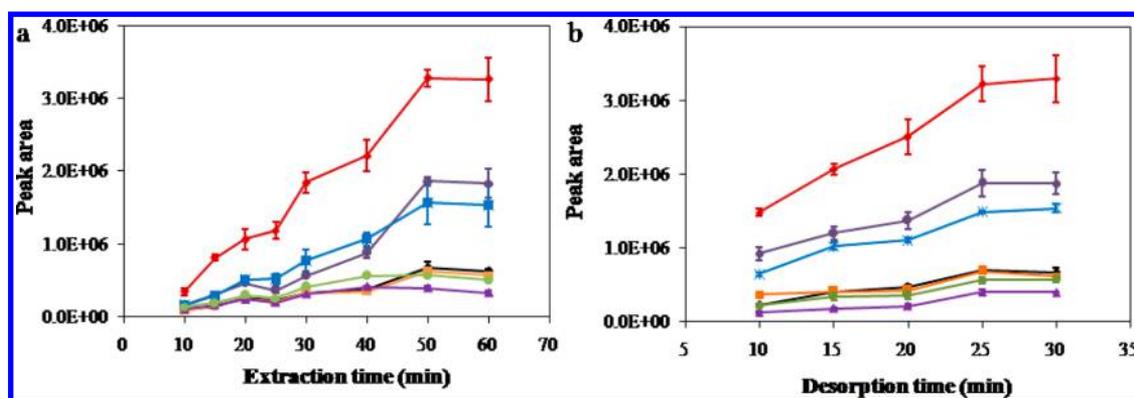


Figure 3. Effect of desorption solvent on extraction performance. Conditions: the extraction and desorption times were 40 and 30 min, respectively; no salt was added in the sample, and the pH value of the sample matrix was not adjusted. The spiked concentration was  $10.0 \mu\text{g L}^{-1}$  for each analyte. Symbols: black diamonds, MAR; orange squares, NOR; purple triangles, CIP; gray circles, LOM; blue stars, ENR; green circles, SRA; red diamonds, SPA.

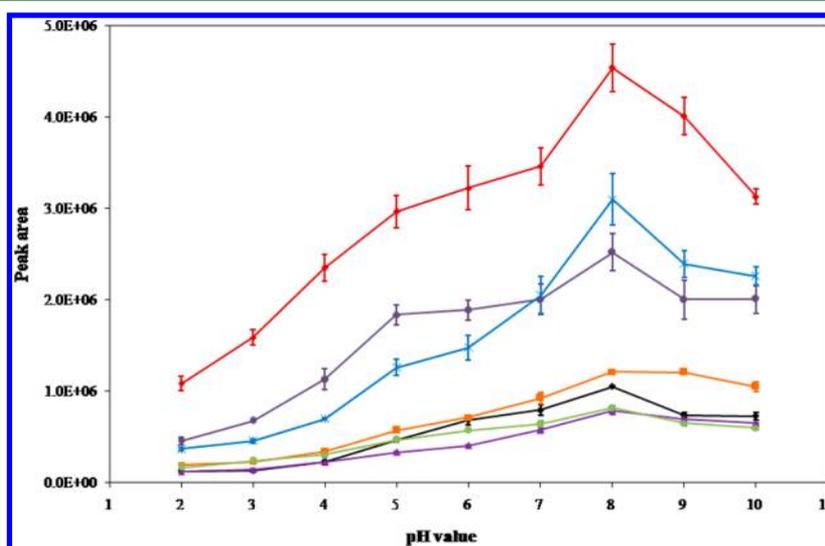
mixture of DB and ED, w/w = 1/2), and porogen solvent (DMSO) in the polymerization solution were investigated thoroughly. It can be seen from the data shown in Table 1 that the extraction capability and longevity of APDE/MMF-SPME were affected obviously by the amount of AP, DB/ED, and DMSO. Increasing the amount of AP could increase the extraction capability but at the expense of the stability and longevity of the fibers. Contrarily, enhancing the content of the cross-linker could improve the degree of cross-linking of the monolith with increased stability and longevity but less extraction performance. According to the results, the optimal preparation ratios of the polymerization solution for APDE/MMF-SPME were 30% AP (w/w) in the monomer mixture, and 70% (w/w) DMSO was used in the whole polymerization solution (APDE/MMF-SPME-9). Under the optimal proportion, white, integrated, and elastic monolithic fibers were obtained (Figure S1). At the same time, the fiber-to-fiber reproducibility was evaluated. The RSDs ( $n = 4$ ) of extraction efficiencies for targeted MAR, NOR, CIP, LOM, ENR, SAR, and SPA were 7.1%, 9.7%, 7.7%, 8.6%, 8.9%, 8.4% and 6.5%,

respectively. It is worth mentioning that the APDE/MMF-SPME prepared under optimized parameters possessed satisfactory longevity, and it could be reused to extract FQs in real samples more than 150 times. No loss in extraction performance and no fracture of the monolithic fibers were found during continuous tests.

**Characterization of APDE/MMF-SPME.** The APDE/MMF-SPME prepared under the optimal proportion was characterized with several techniques, including EA, FT-IR, MIP, and SEM. The carbon, nitrogen, and hydrogen contents obtained from EA results were 69.2%, 4.68%, and 8.78% (w/w), respectively. Figure 2a shows the FT-IR spectrum of the adsorbent. The adsorption band around  $2959.3 \text{ cm}^{-1}$  belongs to the vibrations of  $\text{CH}_3$  and  $\text{CH}_2$  groups. The strong absorption band at  $1725.8 \text{ cm}^{-1}$  is ascribed to the vibrations of carbonyl groups. The bands at 1659.2, 1460.3, and  $1391.0 \text{ cm}^{-1}$  are indicative of phenyl groups. The strong absorption band at  $1161.8 \text{ cm}^{-1}$  corresponds to the C–N bond bending vibration of uramino groups. The weak absorption band at  $1053.3 \text{ cm}^{-1}$  belongs to amino groups. The above results well



**Figure 4.** Effect of extraction (a) and desorption (b) time on extraction performance. Conditions: (a) methanol/0.5% FA aqueous (85/15, v/v) was used as desorption solvent; desorption time was 20 min; (b) methanol/0.5% FA aqueous (85/15, v/v) was used as desorption solvent; extraction time was 50 min. The other conditions and symbols were the same as in Figure 3.



**Figure 5.** Effect of sample pH value on extraction performance. Conditions: extraction and desorption time were 50 and 25 min, respectively; the sample pH values were adjusted by 0.1 mol/L HCl or 0.1 mol/L NaOH. The other conditions and symbols were the same as in Figure 4.

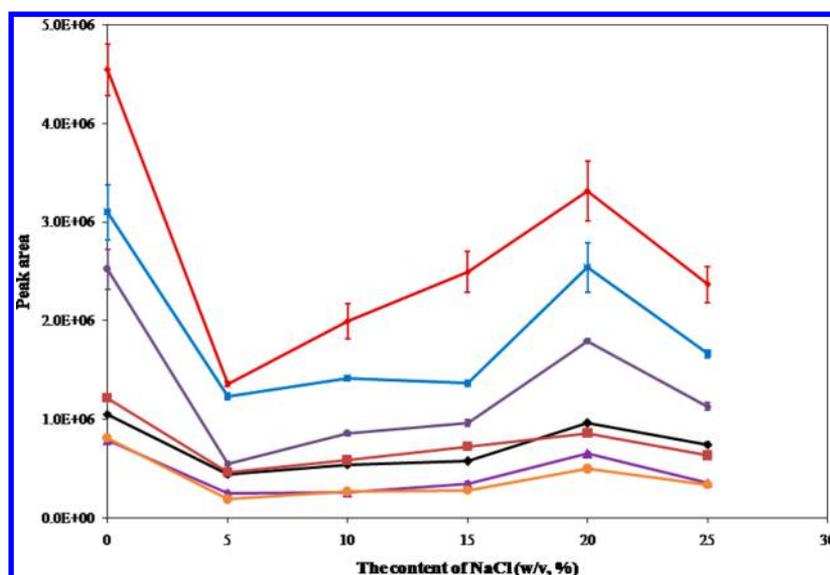
evidence that AP and DB/ED were polymerized successfully. The morphology of the new monolithic fiber was observed by SEM and MIP. It can be seen from the SEM image that there is a homogeneous, porous, and wrinkled structure, which ensures the monolithic fiber has favorable mass transfer during extraction (Figure 2b). Figure S2 shows the PSD of the monolith. It can be seen that the PSD is well uniform. The pore sizes are mainly around 200 nm. At the same time, it can be calculated from the Brunauer–Emmett–Teller plot that the total surface area (TSA) was 54.2 m<sup>2</sup>/g for the prepared adsorbent. The relatively large TSA indicates that there are ample active sites which can contact with targeted FQs.

**Optimization of APDE/MMF-SPME.** Some experimental variables, including desorption solvent, extraction, and desorption time, pH value, and ionic strength, can influence the extraction performance of APDE/MMF-SPME. These parameters were investigated and optimized thoroughly in this study. The peak area of the targeted analytes was used to evaluate the extraction efficiency under different conditions.

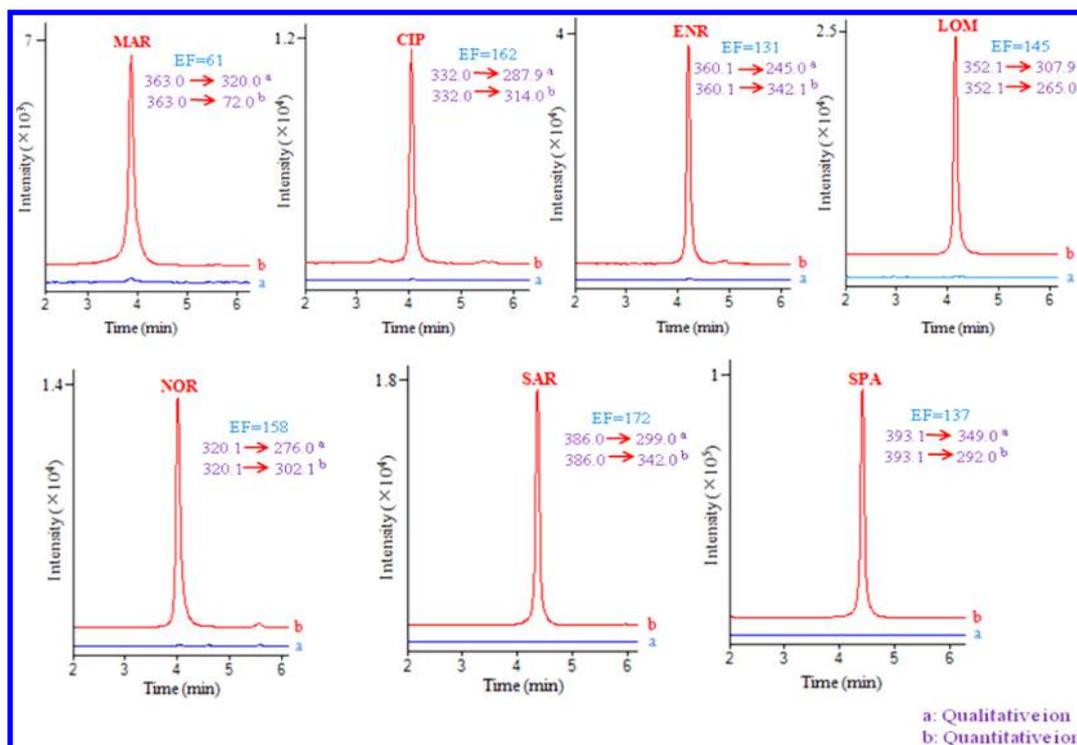
In our preliminary experiment, the adsorptive FQs could not elute from the monolith completely when using pure methanol as desorption solvent. Considering that the hydrogen-bond and dipole–dipole interactions may also involve the extraction, 0.5% FA aqueous solution was added in the methanol. Figure 3

shows the effect of the proportion of 0.5% FA aqueous solution in the desorption solvent on the extraction performance. Results indicated that addition of suitable FA in methanol favored the elution of targeted FQs from monolithic fibers. The extraction performance for all analytes reached its maximum when 15% FA aqueous solution was added in the desorption solvent. Based on the results, a methanol/0.5% FA aqueous solution (85/15, v/v) was selected as the optimal eluent.

MMF-SPME is an equilibrium-based technique; the extraction efficiency depends on extraction time. In the present work, the effect of extraction time on extraction performance was studied by varying the time from 10 to 60 min. As shown in Figure 4a, the extraction performance of the targeted FQs increased rapidly from 10 to 50 min and no obvious change was found after 50 min. Thus, the extraction time of 50 min was chosen in the following experiments. The effect of desorption time on desorption performance was also optimized by changing time from 10 to 30 min (Figure 4b). Results indicated that 25 min of desorption time could ensure the complete elution of targeted FQs from APDE/MMF-SPME. Consequently, 50 and 25 min were selected as the preferred extraction and desorption times, respectively, in the following research.



**Figure 6.** Effect of ionic strength on extraction performance. Conditions: the pH value of the sample matrix was adjusted to 8.0. The other conditions and symbols were the same as in Figure 5.



**Figure 7.** TIC chromatograms of seven FQs. (a) Direct injection of spiking water sample with each analyte at 10.0  $\mu\text{g/L}$ ; (b) Spiked water sample with each analyte at 10.0  $\mu\text{g/L}$  and treated with APDE/MMF-SPME. Conditions: methanol/0.5% FA aqueous solution (85/15, v/v) was selected as the desorption solvent; extraction and desorption time were 50 and 25 min, respectively; the pH value of the matrix was 8.0, and no salt was added to the samples.

Because there are ionizable carboxylic and piperazine groups in the molecules of FQs, thus, the sample pH value will affect the molecular forms of FQs. At the same time, the protonation situation of amino groups in the monolith will also be affected by sample pH values. So, the sample pH value plays a key role in the extraction efficiency of APDE/MMF-SPME for FQs. In this work, the sample pH was set between 2.0 and 10.0 with an interval of 1.0. The results are shown in Figure 5 and indicate that the extraction efficiency increased with the increase of pH values from 2.0 to 8.0. Further increase in sample pH values led

to the decrease of extraction performance. The different molecular status of FQs and adsorbent at different pH values is responsible for the changed trend. The piperazine groups of FQs were protonized at low pH values, and only  $\pi$ - $\pi$  interaction is involved in the extraction. With the enhancement of pH values, the deprotonized procedure happening on FQs resulted in the increase of hydrophobic interaction. Furthermore, dipole-dipole and hydrogen-bonding interactions engendered by the polar groups between the monolithic fibers and the analytes also took part in the extraction. Under the

Table 2. Analytical Performance of APDE/MMF-SPME-HPLC/MS-MS for FQs

Sample	Compd	Linear range <sup>a</sup> ( $\mu\text{g kg}^{-1}$ )	$R^2$	LOD <sup>b</sup> ( $\mu\text{g kg}^{-1}$ )	LOQ <sup>c</sup> ( $\mu\text{g kg}^{-1}$ )	Repeatability (RSD %; $n = 4$ )		Intermediate precision (RSD%; $n = 4$ )	
						Spiked concentration ( $\mu\text{g kg}^{-1}$ )		Spiked concentration ( $\mu\text{g kg}^{-1}$ )	
						0.5	20.0	0.5	20.0
Honey	MAR	0.01–50.0	0.9965	0.0028	0.0092	5.8	7.5	2.1	5.7
	NOR	0.005–50.0	0.9986	0.0011	0.0035	4.8	6.7	6.4	3.5
	CIP	0.005–50.0	0.9985	0.0014	0.0048	7.9	4.7	3.3	7.8
	LOM	0.005–50.0	0.9984	0.0013	0.0044	7.0	8.7	3.6	7.1
	ENR	0.005–50.0	0.9991	0.0011	0.0035	4.6	5.4	8.9	3.4
	SAR	0.005–50.0	0.9979	0.0037	0.0038	7.1	3.2	3.8	3.5
	SPA	0.005–50.0	0.9972	0.0010	0.0033	4.5	4.9	2.2	3.0
Milk	MAR	0.01–50.0	0.9969	0.0028	0.0093	5.8	7.5	2.1	5.7
	NOR	0.01–50.0	0.9984	0.0029	0.0098	4.8	6.7	6.4	3.5
	CIP	0.10–50.0	0.9991	0.018	0.059	7.9	4.7	3.3	7.8
	LOM	0.01–50.0	0.9987	0.0023	0.0076	7.0	8.7	3.6	7.1
	ENR	0.01–50.0	0.9946	0.0019	0.0062	4.6	5.4	8.7	3.4
	SAR	0.01–50.0	0.9993	0.0029	0.0096	7.1	3.2	3.8	3.5
	SPA	0.01–50.0	0.9976	0.0022	0.0073	4.5	4.9	2.2	3.0

<sup>a</sup>Spiking level included 0.005, 0.01, 0.50, 1.00, 5.00, 10.0, 20.0, and 50.0  $\mu\text{g L}^{-1}$ , respectively. <sup>b</sup> $S/N = 3$ ; <sup>c</sup> $S/N = 10$ .

above-mentioned interactions, the extraction efficiency increased obviously with the increase of sample pH values. When the pH value was higher than 8.0 and increased continuously, dissociation happened on the carboxylic groups of FQs and led to the decline of hydrophobic interaction. Furthermore, the favorable hydrogen-bonding and dipole-dipole interactions between fibers and FQs were weakened by excess hydroxyl groups in solution. Therefore, the extraction efficiency declined at high pH values. Therefore, pH 8.0 was selected as the optimum sample pH value for further experiments.

Typically, the ionic strength in the sample matrix is adjusted by the addition of salt. When salt is added, the change of extraction efficiency is related to the properties of analytes, type of adsorbent, and salt concentration.<sup>26</sup> In this study, the ionic strength of the sample matrix was adjusted by addition of different concentrations of NaCl. It can be seen from Figure 6 that the extraction performance of APDE/MMF-SPME for all analytes decreased rapidly when NaCl concentration increased from 0 to 5% (w/v). The extraction efficiencies improved from 5 to 20% (w/v), and decreased again when NaCl concentration was higher than 20% (w/v). Considering the extraction performance and experimental convenience, no addition of salt was recommended when using APDE/MMF-SPME to extract FQs.

Under the optimized extraction conditions, the developed APDE/MMF-SPME showed satisfactory extraction performance for targeted analytes. Figure 7 shows the total ion chromatograms (TIC) of FQs before (a) and after (b) treatment with APDE/MMF-SPME. It could be seen that the peak heights for all targeted FQs increased obviously after the extraction. The enriched factors (the ratio of concentration of FQs in elution to spiking concentration) for MAR, CIP, ENR, LOM, NOR, SAR, and SPA were 61, 162, 131, 145, 158, 172, and 137, respectively. The results well demonstrate that the targeted FQs can be enriched effectively by the developed APDE/MMF-SPME. Figure 7 also shows that the targeted FQs can be well identified and quantified because without interferences they can be found after APDE/MMF-SPME. At

the same time, complete resolution is not achieved for some of the targeted FQs. However, the tandem mass spectrometry (MS/MS) used in present study allows the selective determination of these FQs because unique transitions are monitored.

**Validation of APDE/MMF-SPME-HPLC/MS-MS.** To validate the proposed method of APDE/MMF-SPME-HPLC/MS-MS, some key parameters, including linear dynamic ranges, correlation coefficients, LODs ( $S/N = 3$ ), LOQs ( $S/N = 10$ ), and reproducibility, were investigated under the optimized working conditions. Relevant results are summarized in Table 3. The linearity of the method was investigated by varying the concentration from 0.005 to 50.0  $\mu\text{g/kg}$  and analyzed at each concentration level in duplicate. Results showed that, in honey samples, the linear ranges were 0.01–50.0  $\mu\text{g/kg}$  for MAR and 0.005–50.0  $\mu\text{g/kg}$  for CIP, ENR, NOR, LOM, SPA, and SAR. The linear range of CIP was 0.1–50.0  $\mu\text{g/kg}$ , and 0.01–50.0  $\mu\text{g/kg}$  for other FQs in milk samples. All linear dynamic ranges have good coefficients of correlation ( $R^2 > 0.99$ ). The LOD and LOQ values achieved in honey samples were in the ranges 0.0010–0.0028  $\mu\text{g/kg}$  and 0.0033–0.0092  $\mu\text{g/kg}$ , respectively. For milk samples, The LOD and LOQ values were 0.0019–0.018  $\mu\text{g/kg}$  and 0.0062–0.059  $\mu\text{g/kg}$ , respectively. The LOD values are far below the related MRL values set by EU and other governments. It also can be seen from the data that satisfactory intraday precision and interday reproducibility were achieved, and the RSDs for all compounds were below 9.0% in all cases.

To further evaluate the procedure of APDE/MMF-SPME, the matrix effect (ME) was also investigated. Matrix standard solutions and reagent standard solutions of 0.5, 5.0, and 50  $\mu\text{g/kg}$  were prepared. According to Kim and Thomaidis' studies,<sup>27,28</sup> the following equation was used to calculate the %ME.

$$\%ME = (\text{peak area in matrix} - \text{peak area in solvent}) / \text{peak area in solvent} \times 100.$$

If the %ME values are in the range  $-20$  to  $20$ , indicating there is no obvious matrix effect, the signal is enhanced if the %ME value is above  $20$ , whereas the signal is suppressed if the

Table 3. Analytical Results and Recoveries of Real Samples

Samples	Spiked ( $\mu\text{g}/\text{kg}$ )	Recoveries/RSDs (% , $n = 3$ )						
		MAR	NOR	CIP	LOM	ENR	SAR	SPA
Honey 1	0.1	79.8/9.2	96.0/8.3	116/8.1	105/7.6	116/8.9	103/7.0	89.0/4.5
	1.0	111/4.6	98.0/5.3	80.0/5.7	84.3/7.8	86.2/4.5	85.4/9.1	103/6.2
	10.0	96.6/8.8	109/3.9	95.0/4.6	102/3.4	85.5/7.8	83.3/2.0	108/9.5
Honey 2	0.1	94.8/5.0	101/7.0	93.0/9.2	108/6.7	106/2.8	87.4/8.1	103/4.8
	1.0	90.8/2.7	79.7/9.8	99.3/8.1	86.1/4.7	76.2/3.2	99.8/3.1	74.5/3.0
	10.0	97.9/9.8	99.7/7.6	106/3.0	74.8/3.1	84.0/8.1	103/7.6	109/5.7
Milk 1	0.1	94.5/2.1	88.5/8.8	87.4/6.1	111/8.7	93.4/7.1	106/8.5	88.0/2.5
	1.0	110/3.1	82.7/3.9	93.7/8.2	92.3/6.4	106/4.0	108/9.5	92.2/6.6
	10.0	93.2/7.1	99.8/5.6	99.8/9.2	106/3.2	103/8.9	104/8.9	97.0/1.5
Milk 2	0.1	87.0/7.8	87.9/5.1	88.4/5.6	106/5.0	86.3/2.2	104/3.2	80.2/8.6
	1.0	109/9.1	82.9/3.9	92.3/4.9	105/6.9	102/3.4	106/5.2	91.9/5.3
	10.0	86.4/4.5	83.9/4.4	97.9/6.8	83.0/1.6	86.6/5.1	81.4/3.7	88.9/0.9

Table 4. Comparison of the Volume of Extraction Solvent, LODs, and Recoveries of the Developed Method with Previous Methods

Method	Sample	Type and volume of extraction solvent	LOD ( $\mu\text{g}/\text{L}$ or $\mu\text{g}/\text{kg}$ )							Recovery (%)	ref
			MAR	NOR	CIP	LOM	ENR	SAR	SPA		
DLLME <sup>a</sup> -HPLC/MS-MS	milk	2.07 mL ACN and 0.57 mL TCM <sup>b</sup>	1.1	0.3	2.0	0.5	1.8	0.5		72.3–91.0	[29]
SPE-UPLC/MS-MS	milk	6.0 mL ACN and 15.0 mL methanol	0.09	0.28	0.14	0.09	0.17	0.25		87–117	[30]
SPE-HPLC/MS-MS	milk	2.0 mL methanol		0.2	0.2		0.2			85–115	[31]
SOSLE-UHPLC/HRMS	milk	10.0 mL ACN	2.0	0.2	2.0	0.6	2.0	0.2	0.2	45–92	[32]
Online SPE-LC/MS-MS	milk	1.0 mL ACN	0.5	0.5	0.5		0.5	0.5	0.5	70.2–117	[33]
UAE <sup>c</sup> -UPLC/MS-MS	milk	8.4 mL ACN and 3.6 mL methanol	0.2	0.4	0.4	0.6	0.3	0.2		96.8–102	[34]
CED <sup>d</sup> -HPLC/MS-MS	milk	0.6 mL ACN		1.5	0.5		5.0	2.0		72–80	[35]
SPE-HPLC/MS-MS	honey	5.4 mL methanol		0.2	0.2				0.2	86.9–138	[36]
SPE-UHPLC/MS-MS	honey	9.5 mL ACN	0.7		1.7		1.1	1.6		61.3–86.8	[37]
SPE-UPLC/MS-MS	honey	5.0 mL ACN and 6.0 mL methanol	0.09				0.60	0.21		81–111	[38]
SPE-HPLC/MS-MS	honey	5.0 mL hexane and 10.0 ACN	0.3	0.3	1.5		0.6	0.6		91–102	[39]
SPE-HPLC/MS-MS	honey	5.0 mL hexane	0.06	0.13	0.17		0.04	0.07	0.02	88.2–114	[40]
MMF-SPME-	milk	0.34 mL methanol	0.0028	0.0029	0.018	0.0023	0.0019	0.0029	0.0022	80.2–113	present
HPLC/MS-MS	honey	0.34 mL methanol	0.0028	0.0011	0.0014	0.0013	0.0011	0.0037	0.0010	74.5–116	method

<sup>a</sup>Dispersive liquid–liquid: microextraction. <sup>b</sup>TCM: trichloromethane. <sup>c</sup>Ultrasound-assisted extraction. <sup>d</sup>Centrifugation, evaporation, and dilution.

value is below  $-20$ . As shown in Table S2, the ME values for most of the FQs in milk and honey samples were in the range  $-20$  to  $20$ , which indicated that there was no obvious matrix residual after APDE/MMF-SPME. The data in Table 2 and the investigation of ME well demonstrate that the proposed APDE/MMF-SPME can remove interferences and enrich FQs effectively. The above-mentioned results also evidence that the developed APDE/MMF-SPME-HPLC/MS-MS possesses satisfactory reproducibility and enough sensitivity for the monitoring of ultratrace FQs in honey and milk samples.

**Assay of Real Samples.** To evaluate the practicability of the developed APDE/MMF-SPME-HPLC/MS-MS, real honey and milk samples were extracted and analyzed with the proposed method. In honey samples, trace levels of LOM, SAR, and SPA were detected; in milk samples, low contents of MAR

and NOR were found. All the content was below the related MRL values. To investigate the effect of the sample matrix on the analytical performance of the developed method, the recovery study was tested by spiking three different concentration levels (0.1, 1.0, and 10  $\mu\text{g}/\text{kg}$ ) of FQs in honey and milk samples. The related results are presented in Table 3. The results show that the recoveries of the seven FQs were in the range 74.5%–116% with the RSDs less than 10% (Table 3) in all cases. Moreover, three powdered milk samples including infant, follow-on, and growth powdered milks were analyzed with the developed APDE/MMF-SPME-HPLC/MS-MS method. No targeted FQs were founded in the powdered milks. The recoveries for FQs spiked with three different levels (0.1, 1.0, and 10  $\mu\text{g}/\text{kg}$ ) in the samples varied from 80.4% to 113%, and the RSD values were in the range 1.1%–10.2%

(Table S3). The results well demonstrate that the developed APDE/MMF-SPME-HPLC/MS-MS is reliable in the monitoring of FQs residues in honey, milk, and other dairy samples.

**Comparison with Previous Methods.** Table 4 shows the comprehensive comparison results of the type and volume of extraction solvent and the other characteristic data obtained in present work and reported techniques for the determination of FQs in milk and honey samples. In comparison to reported studies,<sup>27–38</sup> less amount of organic solvent (only 0.32 mL of methanol) was used in the developed APDE/MMF-SPME. Furthermore, the proposed method showed the best sensitivity compared to previous techniques.<sup>27–38</sup> The reason might be that the prepared APDE/MMF-SPME could extract and concentrate the FQs effectively. The comparison also indicated that the spiking recoveries obtained in the developed method were superior to that achieved in salting out supported liquid extraction (SOSLE)-UPLC/high resolution mass spectrometry (HRMS)<sup>28</sup> and were comparable with other work.<sup>27–29,31–38</sup>

## CONCLUSIONS

A new MMF-SPME based on a poly(AP-co-DB/EDMA) monolith was synthesized and successfully utilized to extract trace FQs in foodstuffs of animal origin, including honey and milk samples. Because there were abundant functional groups in the new monolith, the APDE/MMF-SPME could extract FQs and eliminate the interferences of sample matrices effectively. Under optimum working conditions, the proposed APDE/MMF-SPME-HPLC/MS-MS possessed high sensitivity and good reproducibility. The effective analysis of trace FQs in honey and milk samples well evidenced the usability of the proposed method. Compared to the reported sample preparation methods for the determination of FQs, the developed APDE/MMF-SPME was convenient, feasible, inexpensive, and environmentally friendly. Based on these remarkable merits, the proposed APDE/MMF-SPME-HPLC/MS-MS may serve as a practical method in the monitoring of trace FQs in honey, milk, and other complicated samples.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.6b03965.

Chemical properties of seven FQs and their MS conditions (Table S1); matrix effect (%ME) of FQs at the concentrations 0.5, 5.0, and 50  $\mu\text{g}/\text{kg}$  ( $n = 3$ , respectively) in honey and milk samples (Table S2); analytical results of the proposed method for the determination of FQs in real powered milk (Table S3); photos of a signal monolithic fiber (a) and APDE/MMF-SPME (b) (Figure S1); PSD of the poly(AP-co-DB/ED) monolith (Figure S2); detailed information about the instruments used for the characterization; detailed investigation procedure for the validation parameters (PDF)

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### Notes

The authors declare no competing financial interest.

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