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Water-Compatible Molecularly Imprinted Microspheres in Pipette Tip Solid-Phase Extraction for Simultaneous Determination of Five Fluoroquinolones in Eggs

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ABSTRACT: New water-compatible molecularly imprinted microspheres were synthesized by reversible addition−fragmentation chain transfer (RAFT) polymerization using 3-(2-carboxyethylsulfanylthiocarbonyl-sulfanyl) propionic acid as a hydrophilic chain-transfer agent, and employed as the sorbent of pipet tip molecularly imprinted solid-phase extraction (PT-MISPE) for rapid extraction and screening of ofloxacin, pefloxacin, norfloxacin, ciprofloxacin, and enrofloxacin in eggs. In comparison to conventional SPE methods, the presented PT-MISPE showed special selectivity, easy operation, and accessible device without expensive SPE apparatus. The presented PT-MISPE method combined advantages of dummy molecularly imprinted polymers and pipet tip solid-phase extraction. The presented method was linear over a calibration range of 25−2500 μg/kg with the limits of detections of 0.53−1.07 μg/kg. Good recoveries (89.1−102.5%) were achieved with relative standard deviations of 2.6−4.8%.

KEYWORDS: *dummy molecularly imprinted microspheres, RAFT polymerization, pipette tip solid-phase extraction, fluoroquinolones, egg*

INTRODUCTION

Fluoroquinolones (FQs), an important group of synthetic antibacterial agents, are effective in controlling a wide range of Gram-positive and Gram-negative organism bacteria.[1,2] FQs are used extensively in the prophylaxis and treatment of various diseases of food-producing animals.[3] However, the wide use of FQs would result in residues in edible parts of animals and enhance bacterial resistance, which is potentially harmful to consumers' health.[4,5] To minimize the risk of a potential health hazard for consumers, FQs have been forbidden in laying hens in many countries such as China and EU countries. However, some farmers still illegally use FQs for prophylactic effects. Thus, it is necessary to develop an efficient method to monitor FQs residues in egg products.

The analysis of FQs in eggs is a difficult task as they may bind to lipoproteins,[6−8] so egg samples must be cleaned-up prior to instrumental analysis in order to eliminate interfering compounds.[9] Several methods have been developed for extraction of FQs in eggs, such as liquid-phase extraction,[10] solid-phase extraction (SPE),[11] dispersive solid-phase extraction (DSPE),[4] matrix solid-phase dispersion (MSPD),[6] solid-phase microextraction (SPME),[12] pressurized liquid extraction,[13,14] and magnetic solid-phase extraction (MSPE).[15] Although each method above has its advantages, most of these procedures are tedious, time-consuming, and need a large amount of chemicals or regents.

Pipette tip solid-phase extraction (PT-SPE) is one of the most promising methods for miniaturized solid-phase extraction. It differs from common SPE in that a small amount of sorbent is inserted into a pipet tip, and it is relatively inexpensive without special auxiliary device for extraction. In general terms, an advantage of pipet tips for sample preparation is that extraction can be carried out faster and more facile than conventional SPE cartridges.[16−18] The small capacity of pipet tip with less sorbent would significantly reduce solvent consumption in washing and elution steps, shorten the operation time of SPE, and increase sample throughout.[17] The PT-SPE method is now an effective means for purification, concentration, and isolation of proteins and peptides in genomics, proteomics, metabolomics, etc. However, most sorbents of PT-SPE suffer from low selectivity, especially for trace levels of analytes in complex samples. Therefore, improving affinity and specific recognition of PT-SPE sorbents is of great significance.[19] The application of molecularly imprinted polymers (MIPs) for extraction and cleanup of various antibiotics from several matrices has demonstrated that MIPs are superior to traditional sorbents in its extraction affinity.[20,21]

Molecular imprinting as a new molecular recognition technology developed rapidly in recent years, which mimics the action of antibodies and enzymes.[22] Molecularly imprinted polymers (MIPs) can be readily tailored with special selectivity for a guest molecule, and it is accomplished by synthesizing a network polymer in the presence of template molecules.[23,24] Removal of the template from the polymeric matrix leaves cavities of complementary size, shape, and chemical functional groups to the template molecules.[25] Most of MIPs are frequently prepared by radical polymerization due to its properties (e.g., simple reaction procedure, low cost and good compatibility with various monomers).[26,27] However, a weakness of conventional radical polymerization is that the

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polymerization processes are usually difficult to control chain propagation and termination, resulting in the formation of heterogeneous and highly cross-linked polymer networks,[28,29] and therefore could restrict imprinting capacity and extraction efficiency of the resultant MIPs.[30] Furthermore, low water-compatibility of MIPs is still an issue, due to poor molecular recognition of MIPs in aqueous environments.[31,32]

Recently, reversible addition−fragmentation chain transfer (RAFT) polymerization has been an ideal candidate for controlled/living free radical polymerization,[33] which could significantly improve the match in chain growth and chain relaxation rates and thus lead to the homogeneous networks of the polymer with a narrow distribution of the network chain length and a much lower cross-linking density.[34,35] Pan et al. revealed that MIPs prepared by the RAFT method possessed improved binding capacity and binding constant than traditional polymerization.[29,36] Moreover, water-compatible MIPs could be synthesized by the RAFT method since the functional groups of the RAFT agent could be grafted onto the polymer.[4,37]

In this work, an efficient one-pot approach to obtain water-compatible and narrowly dispersed molecularly imprinted microspheres (MIMs) was developed by using facile RAFT suspension polymerization mediated by a hydrophilic chain-transfer agent. The resultant MIMs were packed in self-assemble pipet tip devices to develop a new sample pretreatment method for simultaneous quantitative determination of five FQs in eggs. Various factors affecting extraction of the analytes were elucidated and the applicability of this method was also evaluated. Under the optimized conditions, FQs were selectively isolated from biological samples and matrix interferences were eliminated simultaneously.

MATERIALS AND METHODS

Chemicals and Reagents. Pazufloxacin (PAZ), ofloxacin (OFL), pefloxacin (PEF), norfloxacin (NOR), ciprofloxacin (CIP), enrofloxacin (ENR), and ethylene glycol dimethacrylate (EDMA) were obtained from Sigma (St Louis, MO). Methanol and acetonitrile were obtained from Xingke Biochem. (Shanghai, China). Chloroform acetone, polyvinylpyrrolidone, carbon disulfide, 3-mercaptopropionic acid, acetic acid, 3-bromopropionic acid, trifluoroacetic acid (TFA), methacryclic acid (MAA), tetrabutyl ammonium bromide (TBAB), and 2,2-azodiisobutyronitrile (AIBN) were purchased from Kermel Chem. (Tianjin, China). The reagents used in this study were analytical grade for materials synthesis and HPLC grade for high performance liquid chromatographic (HPLC) analysis. Double deionized water was filtered through a 0.45 μm fiber membrane before use.

Instrumentation and Conditions. FT-IR data were obtained by a Shimadzu Fourier transform infrared spectrometer IRAffinity-1 (Kyoto, Japan) in a range of 400−4000 cm^{-1}. Morphological evaluation was carried out by KYKY-2800B scanning electron microscopy (SEM, FBI Co. Hillsboro, OR). The chromatographic analysis was performed by a Shimadzu LC-20A HPLC system equipped with two LC-20AT Solvent Delivery Units, a SUS20A gradient controller, and a SPD-20A UV−vis Detector (Kyoto, Japan). An LC-solution workstation (Shimadzu, Kyoto, Japan) was used as the data acquisition system. The analytical columns (250 mm × 4.6 mm I.D., C_{18}, 5 μm) were purchased from YMC Co. Ltd. (Kyoto, Japan). The mobile phase was 0.02 mol/L TBAB−acetonitrile-TFA (87.2:12.8:0.060, v/v/v), and its flow rate was set at 1.0 mL/min. The injection volume was 20 μL and the detection wavelength of the detector was set at 280 nm.

Synthesis of MIMs. Trithiocarbonate [3-(2-carboxyethylsulfanylthiocarbonyl-sulfanyl) propionic acid (CPA) as a hydrophilic chain-transfer agent] was synthesized according to the protocol reported by Wang et al.[38] Then water-compatible MIMs were prepared by RAFT polymerization according to the following procedure: (a) PVP (2.5 g) was dissolved in 100 mL of water by stirring at 600 r/min at room temperature under nitrogen stream. (b) PAZ (2 mmol) and MAA (8 mmol) were dissolved in 2 mL of methanol and 15 mL of chloroform in a beaker and then allowed to self-assemble at 4 °C for 1 h. (c) CPA (150 mg), EDMA (50 mmol) and AIBN (1.5 mmol) were added into the beaker and sonicated for 5 min to make them fully dissolved. This solution was then added dropwise to solution a and then reacted at 60 °C for 2 h, 70 °C for 20 h. (d) After polymerization, the obtained MIMs were separated and washed thoroughly with methanol-acetic acid (9:1, v/v), water, and methanol, respectively (at least 50 mL solvent was sonicated for 15 min for five times, water and methanol were used to wash to neutrality). Finally, MIMs were dried in vacuum at 50 °C for 12 h. The nonimprinted microspheres (NIMs) were prepared by the same procedure as above, in the absence of template molecule.

Preparation of Egg Samples. Egg samples (10 g of each sample) purchased from local markets of Baoding was mixed with 3.0 mL of water. After blending, 2.0 mL of 16% (w/v) lead acetate solution was added. The mixture was centrifuged (1970 g) for 10 min, and then the supernatant was loaded into a PT-MISPE cartridge.

PT-MISPE Procedure. A schematic illustration of PT-MISPE procedure is shown in Figure 1. MIMs (50 mg) were packed into 1.0 mL pipet tip, and absorbent cotton was set as frits at each side to hold the sorbent bed (a bed of 1.2 cm height of MIMs). Prior to each extraction, the pipet tip was consecutively preconditioned with 1.0 mL of methanol and 1.0 mL of water, followed by loading 3.0 mL of egg samples. Then each sample was washed with 2.0 mL water to remove the matrix interferences and eluted by 2.0 mL of acetonitrile−acetic acid (95:5, v/v). The eluted fractions were collected and evaporated to dryness at 40 °C. The concentrated extracts were reconstituted in 0.5 mL of mobile phase before HPLC analysis.

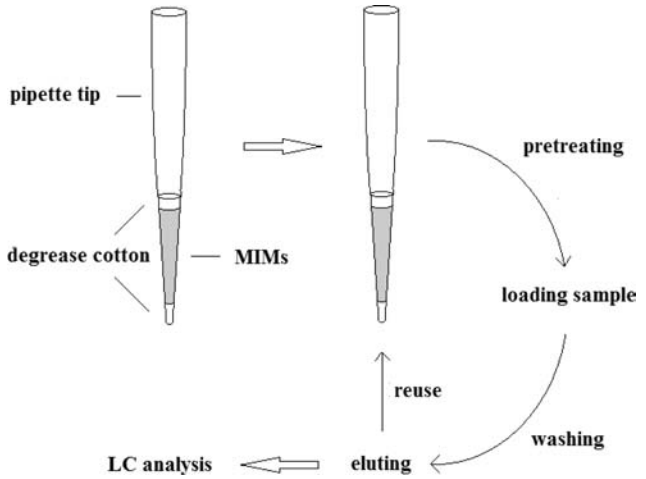

Figure 1. Schematic illustration of the PT-MISPE procedure.

RESULTS AND DISCUSSION

MIMs Synthesis and Morphological Characterization. To eliminate the effect of template leakage of MIMs on quantitative analysis, pazufloxacin (PAZ) was chosen as dummy template due to its similar molecular structure to target analytes. They have a 4-oxo-1,4-dihydroquinoline skeleton, in which the pharmacophore unit consists of a pyridine ring with a carboxyl group, a piperazinyl group, and a fluorine atom placed in positions 3, 7, and 6, respectively.[39] Therefore, the template molecules and analytes are expected to have several proton-accepting and hydrogen-bonding functional groups that are able to interact with the polar groups of monomers.

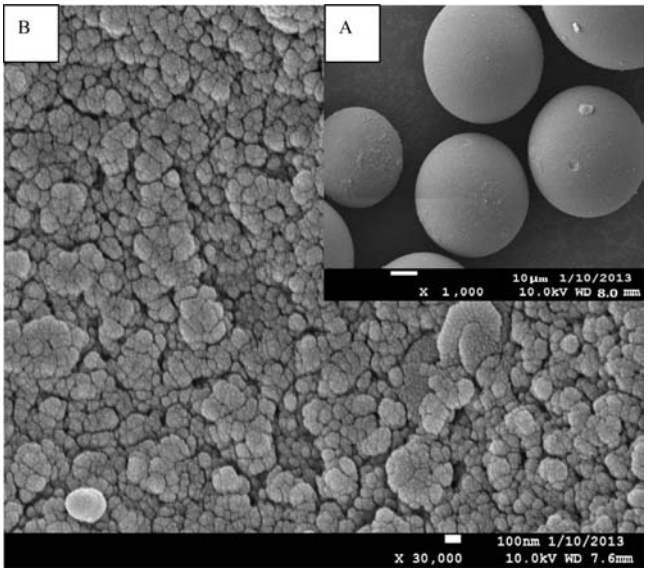

Figure 2. (A) Scanning electron micrograph of MIMs and (B) the surface of MIMs.

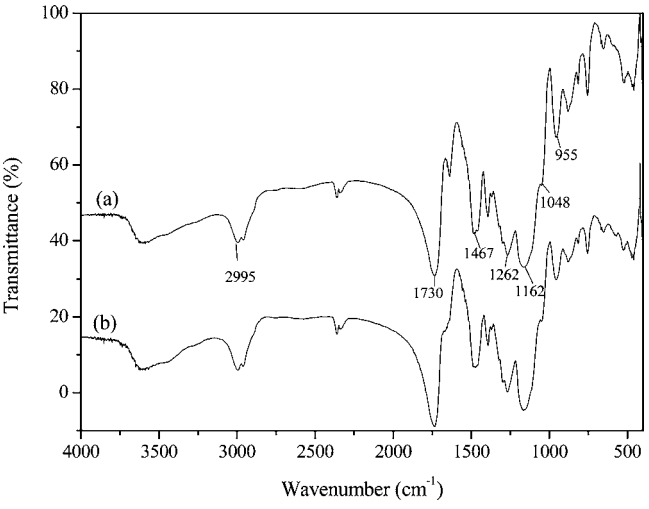

Figure 3. FT-IR spectra of the (a) MIMs and (b) NIMs.

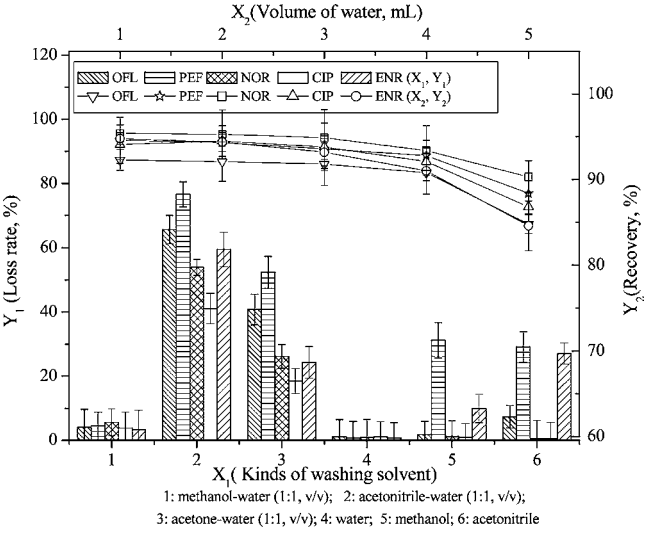

Figure 4. Effect of washing solvents on extraction efficiency of FQs.

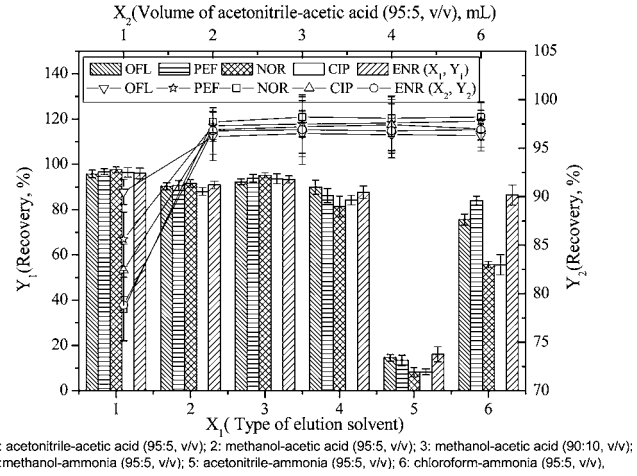

Figure 5. Effect of elution solvents on the recovery of FQs.

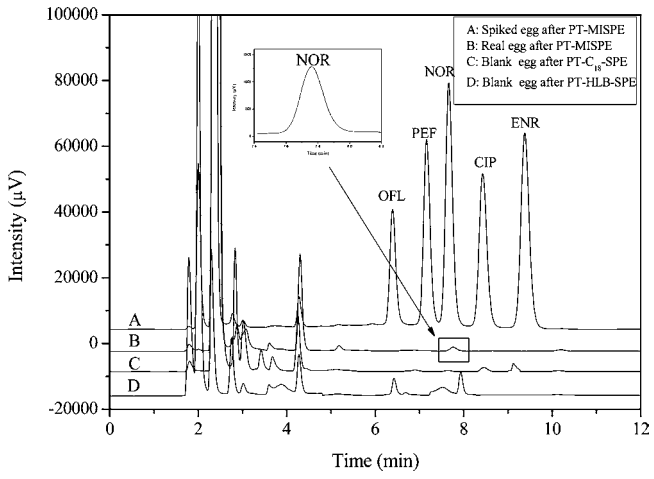

Figure 6. Chromatograms of (A) spiked sample after PT-MISPE, (B) real sample after PT-MISPE, (C) blank sample after PT-C_{18}−SPE, and (D) PT-HLB-SPE.

Additionally, PAZ can be completely separated from analytes in the following HPLC analysis.

The morphological properties of the MIMs are related to the reactants, reaction temperature, and stirring speed. The kinds of functional monomers and the ratio of template and monomer are essential to maintain stable monomer-template complexes and control the imprint binding site during the imprinting process, respectively.[40] The amount of cross-linker could impact mechanical stability of the polymer. Therefore, a common cross-linker (EDMA) and different functional monomers (4-VP and MAA) were evaluated for the synthesis of MIMs and NIMs, respectively. The polymers using MAA as monomers showed higher binding affinity for the analytes in aqueous solution. This may be attributed to that MAA is a hydrophilic monomer, which would be propitious to form strong interaction and stable host−guest complexes with template.[41]

RAFT polymerization provides molecular weights that are predetermined by reagent concentrations and conversion, and the polymerization process is easily controlled by remaining the equilibrium between active and dormant; this makes very narrow polydispersities possible.[42] Adding a different RAFT agent could act on the nucleation stage and influence the size distribution of the polymer. A RAFT agent can combine with

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Table 1. Parameters of the PT-MISPE-HPLC Method

analytes	linearity (μg/kg)	linear equation	r^2	LOD (μg/kg)	LOQ (μg/kg)
OFL	25−2500	$y = 403.2x - 5.19 \times 10^3$	0.9997	1.07	3.57
PEF	25−2500	$y = 615.3x - 9.60 \times 10^3$	0.9994	0.68	2.27
NOR	25−2500	$y = 820.2x - 1.12 \times 10^4$	0.9998	0.53	1.77
CIP	25−2500	$y = 613.8x - 9.73 \times 10^3$	0.9996	0.79	2.63
ENR	25−2500	$y = 809.9x + 0.94 \times 10^3$	0.9994	0.65	2.17

Table 2. Comparison of the Present Method with Previously Reported Methods

sample matrix	treatment method	detection method	recovery (%)	LOD (ng/g)	LOQ (ng/g)	RSD (%)	ref.
egg	DSPE	HPLC-FLD[a]	85−93	3−10	10−30	5−9	4
egg, tissue	MSPD	HPLC-FLD[a]	85.7−104.6	0.05−0.09	0.17−0.3	1.9−7.0	6
egg	MISPE	LC-ESI-MS/MS[b]	90−106	0.12−0.85	0.36−2.59	3−8	7
urine	MISPE	HPLC-FLD[a]	53−88	1.9−34	6.3−113	1−10	8
egg	SPME	HPLC	89−106	0.1−2.6	0.4−8.6	1−7	12
egg	MSPE	HPLC-DAD[c]	95.2−100.7	0.25−0.40	0.80−1.25	2.8−4.5	15
egg	MISPE	HPLC-UV	94.4−96.9	5.0	16−17	2.4−4.7	21
egg	PT-MISPE	HPLC-UV	89.1−102.5	0.53−1.07	1.77−3.57	2.6−4.8	Present

[a]Fluorescence detection. [b]Liquid chromatography−electrospray ionization−tandem mass spectrometry. [c]Diode array detector.

Table 3. Recoveries of Analytes in Spiked Egg after PT-MISPE (n = 3)

	25 μg/kg		125 μg/kg		725 μg/kg	
analyte	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)
OFL	90.6−101.6	4.6	90.5−102.2	4.6	89.1−97.9	3.6
PEF	92.5−102.5	4.4	92.8−100.3	3.0	91.3−98.2	3.3
NOR	92.9−102.3	3.7	91.1−100.4	3.6	93.4−101.6	3.2
CIP	93.9−102.0	3.6	90.5−99.1	3.2	89.5−97.5	3.1
ENR	90.0−102.5	4.8	90.0−100.1	4.0	90.8−97.7	2.6

the initiated radicals and keep the dynamic equilibrium between the active and dormant species so that the reaction is uniform. In RAFT polymerization, the functional groups could be easily introduced into the chain ends of polymers.[32] CPA, with two carboxyl groups in a molecule, was selected as the RAFT agent. The hydrophilic groups presented in CAP are prone to form hydrogen binding with more monomers and template molecules. The polymers generated via RAFT polymerization were endowed with carboxyl end groups which constituted hydrophilic layers and could impart the polymers with water compatibility. Furthermore, the polymerization is well controlled with RAFT agent, which resulted in a narrow size distribution of the polymer. Additionally, rotating speed, volume of porogenic solvent, and bath temperature could also mainly affect the size distribution of the obtained polymer. Appropriated volume of porogenic solvent leads to a microsphere surface with good pore structure and large pore size.[43,44] The morphology of MIMs was evaluated with a scanning electron microscope (SEM). It can be seen from Figure 2 that the spherical particles (diameter is about 40−60 μm) with well-developed pore structures and a relatively narrow distribution of particle size were obtained. The macroporous and rough surface of the MIMs were favorable for adsorption and release target molecules from MIMs.

Characterization of MIMs and NIMs by FT-IR Spectra. To further ensure that MIMs was synthesized, FT-IR analysis was performed. In Figure 3, the characteristic peaks around at 2995 cm^{-1} and 955 cm^{-1} were attributed to the stretching and bending vibrations of −OH. The peak band observed at 1162 cm^{-1} is indicative of C−O−C stretching bending of EDMA. The feature peak at 1730 cm^{-1} resulted from C═O group, and the relatively strong peak at 1467 cm^{-1} corresponded to C−H bonds bending vibration of methylene groups. In addition, the bands at 1262 cm^{-1} (C−S−C bond stretching vibration) and 1048 cm^{-1} (C═S bond stretching vibration) were observed, which suggested that MIMs had been converted with the RAFT agent successfully.[34] All the FT-IR features indicated that MIMs had been prepared successfully. The FT-IR spectra of MIMs (Figure 3a) and NIMs (Figure 3b) were similar, which indicated that templates were removed from the imprinted polymer completely.

Evaluation of Adsorption Capacity of MIMs. To evaluate the recognition ability of MIMs in different solvents, binding experiments were conducted by loading different solvents (1.0 mL of water, methanol, and acetonitrile) containing the five FQs (4.0 μg/mL) into PT-MISPE. The results showed that MIMs exhibited excellent retention ability in these three solvents. When water was used as loading solvent, the loss rate of the five analytes was less than 1.2%, and it proved that the MIMs were equipped with excellent recognition properties in aqueous media.

The adsorption capacity reflects the extraction and affinity of MIMs toward the analytes. Therefore, to evaluate the adsorption capacity of MIMs, 20 mg of MIMs and NIMs were introduced into two series of glass flasks, respectively. Water solutions (5 mL) containing spiked FQs concentrations of 5, 10, 20, 50, 100, 120, and 150 μg/mL were added to each series of glass flasks. The glass flasks were shaken for 4 h at room temperature with a horizontal shaker and then separated by centrifugation (1970 g) for 10 min. The FQs content of supernatant was analyzed by HPLC. With an increasing concentration of FQs, the adsorption amounts of MIMs and

NIMs also increased. However, the adsorption amounts of MIMs were larger than those of NIMs (1.3−2.1 μg/mg, 100 μg/mL), which indicated that the specific recognition sites of MIMs interacted efficiently with FQs. When the concentration of FQs increased to 100 μg/mL, the adsorption capacity of MIMs reached saturation (2.4−3.9 μg/mg).

Effect of the Amount of MIMs in Pipette Tip. A suitable amount of PT-MISPE sorbents could allow complete adsorption of analytes, facilitate the transfer of samples and reduce solvent consumption. In this study, different amounts of MIMs sorbents ranging from 20 to 200 mg were investigated. The results indicated that 50 mg of sorbents provided a complete retention for the five FQs, and good recoveries ranging from 96.8 to 98.3% were obtained. Further increasing the amount of MIMs resulted in no improvement for recoveries of FQs. However, the sorbent amounts lower than 30 mg resulted in decreased recoveries and high standard deviations. This was due to the fact that a small amount of sorbents led to inadequate adsorption toward analytes in the pipet tip. In comparison, when 50 mg of sorbents were packed in the pipet tip, a bed of 1.2 cm height of MIMs was obtained, which achieved a sufficient interaction between the analyte and the sorbents. With a further increase in the amount of sorbents, the bed volume in the pipet became larger, which restricted sample flow rate and required more elution solvent to elute out the analytes. Besides, the small amount of MIMs in the pipet tip significantly reduced solvent consumption and operation time and the process could be carried out faster and more easily. Taking this all into consideration, 50 mg of sorbents were used in the pipet tip for further analysis.

Optimization of PT-MISPE. In this work, a self-assembly pipet tip using 50 mg of MIMs as sorbents was employed to reduce experimental consumption (such as solvent and sorbents). In order to achieve high selectivity and recovery on the analytes and eliminate interferences originated from egg matrix, the parameters of PT-MISPE method including loading volumes, types, and volumes of washing and eluting solvent were investigated.

First, the loading volumes of samples in a range of 2.0−6.0 mL were evaluated, and almost no loss of analytes was observed in the range of 2.0−4.0 mL. With further increasing of sample volume, a gradually increased loss rate of analytes was observed. Moreover, when the loading volume was higher than 3.0 mL, the flow rate of sample solution became slower since egg matrix was a viscous liquid. Moreover, with further increasing of sample volume, a gradually increased loss rate of analytes was observed. Therefore, 3.0 mL of sample solution was applied to further work.

In order to reduce nonspecific adsorption and eliminate interferences of sample matrix, a washing step prior to elution step was necessary.[45] Thus, different washing solvents (3.0 mL) such as water, methanol, acetonitrile, methanol−water (1:1, v/v), acetonitrile−water (1:1, v/v), and acetone−water (1:1, v/v) were investigated (Figure 4). The results showed that acetonitrile and acetonitrile−water (1:1, v/v) were sufficient to remove the impurities from sample matrix, but most of the analytes were also eluted out simultaneously. Although similar purification efficiency of water and methanol−water was obtained, the recoveries for the five FQs were slightly higher with water than that of methanol−water. With the purpose of minimum volume of washing solution to efficiently rinse interferences as well as the minimum losing of analytes during the washing step, volumes of water ranging from 1.0 to 5.0 mL were investigated (line graph in Figure 4). The recoveries of the FQs remained constant with increasing the volume of water from 1.0 to 2.0 mL, and then decreased obviously with further increasing to 5.0 mL. Therefore, 2.0 mL of water was chosen as the washing solvent.

The optimization of elution step was performed using a series of elution solvents (3.0 mL) including acetonitrile−acetic acid (95:5, v/v), acetonitrile−ammonia (95:5, v/v), methanol−ammonia (95:5, v/v), methanol−acetic acid (95:5, v/v), methanol−acetic acid (90:10, v/v) and chloroform−acetic acid (95:5, v/v). Acetonitrile−acetic acid (95:5, v/v) offered the best recovery with the least interferences. Subsequently, various volumes (1.0−6.0 mL) of acetonitrile−acetic acid (95:5, v/v) were investigated. The results in Figure 5 show that the best recovery was achieved at 2.0 mL acetonitrile−acetic acid (95:5, v/v), and larger volumes just provided almost constant recovery. Therefore, 2 mL of acetonitrile−acetic acid (95:5, v/v) was adopted as the elution solvent.

Comparison of MIMs, NIMs, C_{18} and HLB Sorbent. To evaluate extraction efficiency and potential interfering compounds originating from egg matrix, blank samples, spiked samples, and recovery experiments by PT-SPE method using MIMs, NIMs, C_{18}, and HLB as sorbent were investigated, respectively. The results showed that no interference peak was observed in the chromatogram of blank samples at the retention time of the target analytes in spiked samples by the proposed PT-MISPE method using MIMs as sorbent. The chromatogram of blank samples using NIMs, HLB, and C_{18} as sorbents revealed small interferences at the retention time of some individual FQs (Figure 6) (caused by the nonselective adsorption of NIMs, HLB, and C_{18}). The results were further confirmed by HPLC coupled with fluorescence detection and mass spectroscopy detection. Moreover, to further study the effect of sample matrix and the accuracy of the method, recovery experiments were carried out by the spiked samples at three different levels of the FQs. The results showed that PT-MISPE performed higher recovery than PT-NISPE, C_{18}−SPE, and HLB-SPE (recoveries were 63.9−78.1% for NIMs, 83.4−91.6% for C_{18}, and 69.9−73.9% for HLB). The recovery and chromatogram of the PT-MISPE method proved that the synthesized MIMs had better affinity and purification effect to the FQs, and the FQs in eggs were selectively isolated and interferences originating from egg matrix were eliminated simultaneously. The higher recovery of MIMs than NIMs demonstrated that the complementary cavities created by template molecule played an important role in specific adsorption. Besides, the relatively lower recovery from C_{18}−SPE and HLB-SPE may be ascribed to the insufficient elution or the loss in washing and loading procedure.

Validation of the PT-MISPE-HPLC Method. The presented method was validated for linearity, precision, repeatability, recovery, limit of detection, and interassay and intra-assay deviation under the optimum condition. The calibration curves were constructed using the peak areas of the analytes measured at nine increasing spiked levels ranging from 25 to 2500 μg/kg. Good linearity was observed for all analytes throughout the concentration range, and the regression equations are shown in Table 1. The limits of detection (LOD) and limits of quantification (LOQ) based on signal-to-noise ratios of 3.0 and 10.0 were 0.53−1.07 and 1.77−3.57 μg/kg. Accuracy and precision of the PT-MISPE method was assessed by performing replicate analyses of spiked samples in triplicate in a same day and consecutive three days. The intraday

precision of the method evaluated as relative standard deviation (RSD) were less than 4.8% and the interday reproducibility was less than 5.9% in all cases. The negative control test revealed that there are no peaks of interferences from sample matrix observed at the retention time of analytes in the chromatogram of blank egg samples, which further demonstrated the practicality and purification effect of the proposed method. A comparison of the present method with previously reported methods is shown in Table 2. The presented PT-MISPE-HPLC method is applicable to analyze low FQs multiresidues in eggs with similar or higher recoveries, satisfactory detection limits, and good repeatability when compared with other methods. Given the advantages, further research focusing on PT-MISPE-HPLC method will be promising for routine monitoring of trace FQs in food samples.

Assay of FQs in Eggs. To demonstrate the suitability and application of the PT-MISPE-HPLC method, five eggs from different origins (collected from various local markets of Baoding) were pretreated under the optimized condition. It was found that one of the samples contained NOR with a concentration of 37.7 μg/kg (Figure 6B). Moreover, the recovery study was performed to investigate the effect of sample matrix by spiking three different levels of FQs in eggs and the results were shown in Table 3. The average recoveries of all FQs were in the range of 89.1−102.5% with RSD $\leq$ 4.8%. As seen in Figure 6A, the chromatograms of the elution fractions revealed that the spiked egg samples were significantly clean after being pretreated with the PT-MISPE protocol. Based on the results proposed above, the method was reliable and could be potentially applied to monitoring FQs residues in eggs.

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Notes

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