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Porous molecularly imprinted monolithic capillary column for on-line extraction coupled to high-performance liquid chromatography for trace analysis of antimicrobials in food samples

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ABSTRACT

A novel porous molecularly imprinted monolithic capillary column (MIMCC) based on ternary porogen was synthesized by in situ technique with sulfaquinoxaline as the template molecule. The characteristics of the MIMCC were investigated by scanning electron microscopy, infrared spectrum, thermogravimetric analysis and solvent resistance test. The saturated adsorption amount of sulfaquinoxaline on MIMCC was 2.7 times over that on the non-imprinted monolithic capillary column (NIMCC). The MIMCC also exhibited good enrichment ability to its analogs and the enrichment factors were 46–211 for five antimicrobials. High permeability and imprinting factors as well as good stability, reproducibility and long lifetime were obtained. An on-line method based on MIMCC solid-phase microextraction coupled with high-performance liquid chromatography was developed for the determination of trace antimicrobials in complex samples. The good linearity for sulfametoxydiazine, sulamethoxazole and sulfaquinoxaline was 0.05–10 μg/L, the limits of detection (LODs) were 10.0–14.0 ng/L. The linear range for mequindox and quinocetone were 0.10–10.0 μg/L, the LODs were 20.0–27.0 ng/L respectively. The recoveries were 71.0–108.2% with relative standard deviation of 1.6–8.5%, correspondingly. The results showed that MIMCC could effectively enrich antimicrobials from complex matrices. The on-line method based on MIMCC and HPLC was selective, sensitive and convenient for trace determination of antimicrobials in complex samples.

1. Introduction

Developing novel selective extraction and clean-up techniques as well as rapid, sensitive analysis methods is urgently needed for the determination of trace or ultratrace analytes in complex matrices. Traditional off-line sample pretreatments generally suffer from drawbacks including time-consuming, solvent contamination and poor reproducibility [1], while on-line coupling techniques, that are based on efficient and selective sample preparation coupled to sensitive instruments, especially chromatographic techniques, have received much attention and interest in recent years. Performing better sensitivity with low limits of detection (LODs) and good reproducibility, on-line coupling techniques require less solvent and sample, and also could reduce analytical errors [2]. They have become popular alternatives for the analysis of organic and inorganic pollutants in food, environmental and biological samples [3]. The development trend of modern analytical chemistry is to exploit simple, miniature and solventless sample pretreatment techniques [4].

In-tube solid-phase microextraction (SPME) was developed from fiber-based SPME technique that uses a capillary column as an extraction device [5], including open tubular [6], fiber/adsorbent-packed technique [7,8] and monolithic capillary columns [9–11], which can significantly improve the mechanical resistance and extraction efficiency [12]. However, open tubular faced with low extraction phase volume due to its thin coating, fiber-packed format was still not free of the similar drawbacks of open tubular while absorbents-packed SPME's high backpressure restricted its uses. The monolithic capillary columns had a relatively lower backpressure and higher extraction phase ratio. The presence of micronized macropores ensured fast mass transport in application, allowing a high flow rate to shorten analytical time. Thus, monolithic capillary columns were quite preferable to improve the sensitivity of analytical method in many fields [13–15]. However, it is difficult for some monolithic capillary columns to achieve selective separation of trace or ultratrace analytes in complex sample matrices.

The molecular imprinting technique is one of the most attractive methods for selective separation, which involves the formation of

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recognition sites in a synthetic polymer matrix and favors the rebinding of template molecules to other compounds with similar structures [16,17]. Due to the advantages of selective recognition, facile synthesis and low cost, it had been widely utilized in sample pretreatment [18,19]. The molecularly imprinted monolithic capillary columns (MIMCC), which combined the advantages of molecular imprinting and monolithic capillary columns technique, are an attractive sample pretreatment form, with benefits of high permeability, fast mass transfer, and low backpressure [20–22]. Therefore, MIMCC is suitable for online sample preparation and trace analysis of complex samples [23].

Antimicrobials of quinoxalines and sulfonamides have been widely used in animal farming as a prevention or treatment of microbial infections. Quinoxalines are feed additives because of their growth-promoting effects to pig, chicken and fish [24,25]. Sulfonamides are a group of synthetic antimicrobials which play an important role in veterinary medicine. There has been growing attention for the residues in food products and their potential carcinogenicity. The maximum acceptable limit is 100 μg/kg for those antimicrobials in foods of animal origin adopted by the European Commission, America and China [26–29]. It is necessary to develop a simple, selective and sensitive analytical method for simultaneous determination of antimicrobials in complex sample. Up to now, several quantitative methods have been described for the determination of residues of antimicrobials, including capillary electrophoresis [30], gas chromatography mass spectrometry [31], high-performance liquid chromatography (HPLC) with varied detection [32–36]. Samples were often pretreated to remove protein, fat, and reduce potential interference from the sample matrix. In some previous reports, molecularly imprinted polymers had been used as solid-phase extraction (SPE) [32,33] and stir bar sorptive extraction (SBSE) [34] for extracting antimicrobials. Although these methods have been successfully applied for analysis of antimicrobials in various matrices, the tedious procedure, more solvents, high cost and not sensitivity of the methods are the main disadvantages.

In this work, a novel porous MIMCC was originally prepared with sulfaquinoxaline as template molecule using a ternary porogen. It was characterized by scanning electron microscopy (SEM), infrared spectrum (IR) and thermogravimetric (TG) analysis. Moreover, an on-line MIMCC–HPLC method was developed for the determination of trace antimicrobials, including sulfametoxydiazine, sulamethoxazole, sulfaquinoxaline, mequindox and quinocetone in chicken, pork and egg samples.

2. Experimental

2.1. Chemicals and materials

Sulfaquinoxaline (SQX) was purchased from Kailun Chemical and New Material Technology Ltd. (Wuhan, China). Sulamethoxazole (SMZ), sulfametoxydiazine (SMD), mequindox (MEQ) and quinocetone (QCT) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The chemical structures of antimicrobials are shown in Fig. S1, Supplementary material. 3-(Methacryloxy) propyltrimethoxysilane (γ-MPS) was obtained from Shengda Fine Chemical Industry Corporation (Guangzhou, China). Methacrylic acid (MAA), N, N-dimethyl formamide (DMF), azo(bis)-isobutyronitrile (AIBN), paraxylene and isooctane were obtained from Damao Chemical Regent Company (Tianjin, China). Ethylene glycol dimethacrylate (EGDMA) was purchased from Corel Chemical Plant (Shanghai, China). Acetonitrile of HPLC grade used for mobile phase was obtained from LAB-SCAN (Bangkok, Thailand). Water used for HPLC was double distilled and filtered through a 0.45 μm nylon filter membrane. All other chemicals were of analytical grade. Fused silica capillaries (I.D. 320 μm) were supplied by Yongnian Optic Fiber Plant (Handan, China).

2.2. MIMCC preparation

Fused-silica capillaries (500 cm × I.D. 320 μm) were connected on the injection valve, then they were injected with 1 mol/L NaOH and 1 mol/L HCl by liquid pump and immersed for 4 h, respectively. After being rinsed with purified methanol, they were dried at 150 °C for 2 h. The activated capillaries were pretreated by γ-MPS and cut into pieces of 17 cm length. The template molecule, SQX (9.1 mg) was dissolved in DMF (250 μL), the functional monomers MAA (25 μL), EGDMA (135 μL), paraxylene (580 μL) and isooctane (250 μL) were mixed thoroughly and kept for 60 min at room temperature. Then AIBN (3.5 mg) was added, the solution was homogenized and degassed in an ultrasonic bath for 5 min and then it was filled into the pretreated capillary column. The capillary was sealed with silicone rubber at each end, subsequently, it was placed into 60.0 °C oven to initiate the polymerization reaction for 70 h. Finally, the capillary column was heated at 120 °C for 2 h. Non-imprinted monolithic capillary column (NIMCC) was prepared following the same procedure without adding SQX template. The obtained MIMCC was eluted by methanol/acetic acid (9:1, v/v) and acetonitrile/water (2:8, v/v) to remove the template until it could not be detected by HPLC-UV. The monolithic capillary column was cut by cutting knife to keep the final length to 12.0 cm.

2.3. MIMCC–HPLC on-line coupling system setup

The MIMCC on-line coupled to HPLC system is illustrated in Fig. 1. The whole system consisted of a six-port injection valve (valve **1**), two six-port valves (valve **2** and **3**), a sample loop and a MIMCC. The MIMCC was placed in the position where the sample loop normally resides on the six-port injection valve (valve **1**). The MIMCC–HPLC procedures could be divided into three steps. (A) Extraction. It was connected with a–b–c–d–e–f–n–m–j–i. The sample solution was

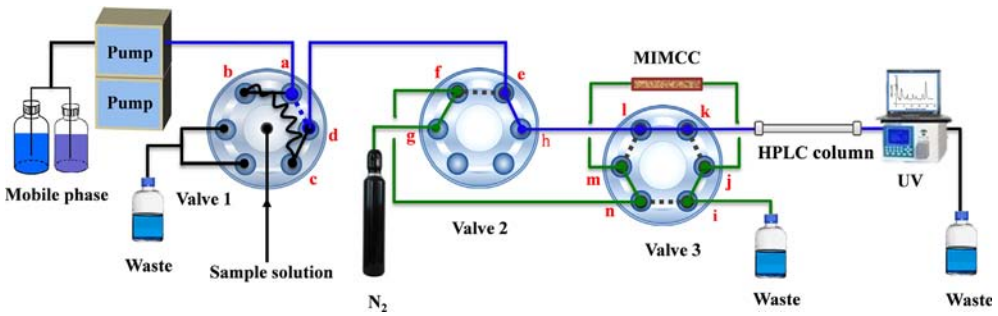

Fig. 1. The illustration of on-line MIMCC–HPLC system. (A) Extraction: a–b–c–d–e–f–n–m–j–i; (B) cleaned up: g–f–c–d–n–m–j–i; (C) desorption: a–d–e–h–i–m–j–k.

introduced into the MIMCC at a flow rate of 0.15 mL/min. (B) Clean up. It was connected with g–f–c–d–n–m–j–i. MIMCC was cleaned up with N_2 in order to eliminate the residual sample solution in the MIMCC and prevent sample contamination of the analytical column. (C) Desorption. It was connected with a–d–e–h–i–m–j–k. The extracted analytes were desorbed from the MIMCC to the analytical column with the mobile phase at a flow rate of 0.15 mL/min.

2.4. Sample pretreatment

The chicken, pork and egg samples were purchased from the local supermarket. These samples were homogenized and stored at −20 °C before use. The spiked concentrations were achieved with three levels of 1.0, 3.0 and 5.0 μg/kg, respectively. The spiked samples were pretreated by the following procedures. Firstly, 5.0 g anhydrous sodium sulfate was added into 5.0 g samples and then the mixtures were ultrasonic-assisted extracted with 10.0 mL acetonitrile for 10 min. The sample was homogenized and centrifuged for 5.0 min at 8000 rpm and the supernatant was collected. The operation was repeated another two times and the combined extraction solution was dried. Then it was dissolved with 25.0 mL acetonitrile/toluene/n-hexane (1:4:45, v/v) for MIMCC extraction. The NIMCC extraction with the same procedure was used for comparison. All sample solutions were filtered through a 0.45 μm pore cellulose filter prior to on-line analysis.

2.5. Chromatographic conditions

All separations were carried out on a Diamonsil C_{18} column (250 mm × 4.6 mm i.d., 5 μm) from Dikma (Beijing, China), and a 7.5 mm C_{18} security guard column from Phenomenex (Torrance, Canada) was attached to the analytical column. The mobile phase was acetonitrile/water (0.2% acetic acid, v/v) at a flow rate of 1.0 mL/min. Acetonitrile concentration was increased from 20% to 70% during 20 min. The flow rate was 1.0 mL/min and ultraviolet detection wavelength was monitored at 265 nm.

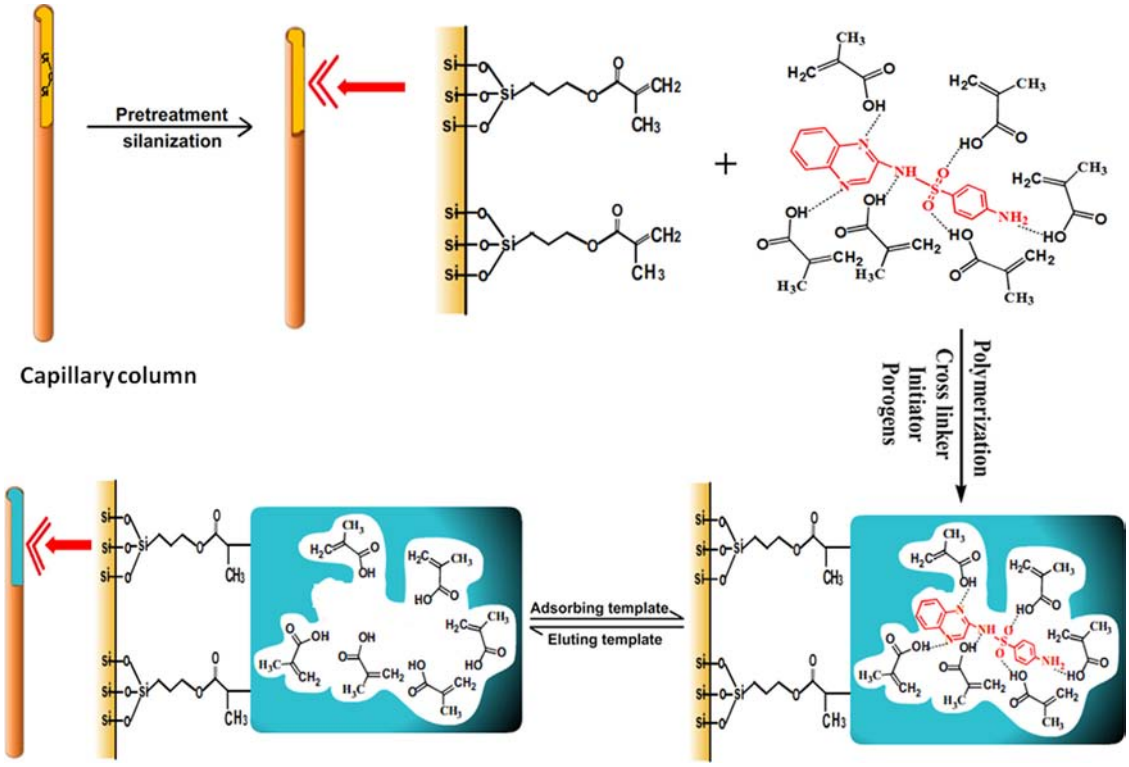

Fig. 2. Schematic diagrams of the MIMCC preparation.

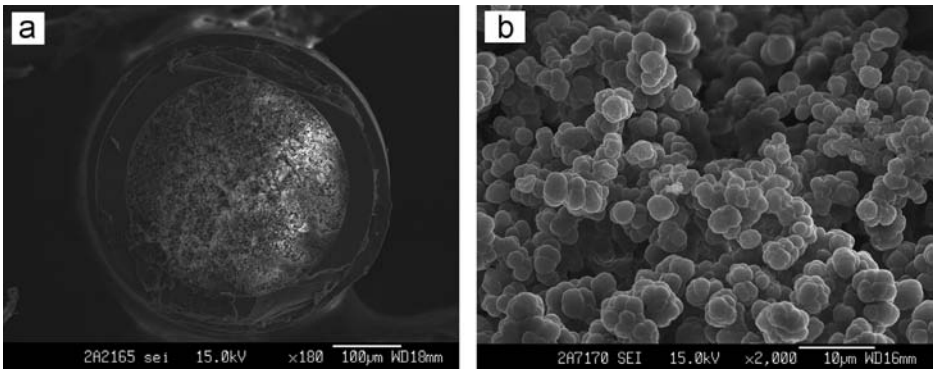

Fig. 3. SEM images of the MIMCC 180× (a) and 2000× (b).

3. Results and discussion

3.1. MIMCC preparation

In the preparation of MIMCC, good permeability and high imprinted factor are basic requirements. The porogen, polymerization solvent, functional monomer, cross-linking and reaction temperature were key parameters. Fig. 2 illustrates the preparation scheme of the SQX MIMCC.

The type of porogen is very important for the permeability and the recognition sites. Paraxylene, isooctane, toluene, dodecanol, chloroform, octane, n-heptane, polyethylene glycol 1000 and polyethylene glycol 6000 were used for the optimization of porogens. The results showed that the monolithic column was uniform morphology and good permeability when isooctane and paraxylene were used. Moreover, isooctane and paraxylene combined with DMF were used as the mixed porogens for in situ preparation of monolithic framework. In order to examine the permeability of the monolithic column, the backpressure was investigated when the flow rate was set at 100 μL/min. The values of permeability (K_F) were estimated by the following equation [37].

$$K_F = F\eta L/(S\Delta P) \quad (1)$$

where K_F is the permeability, F is the flow rate of the pump, η is the solvent viscosity, L is the column length, S is the inner cross sectional area of the column, ΔP is the backpressure. Water was used as mobile phase and the corresponding value of viscosity was 1×10^{-3} Pa s. The permeability of the MIMCC was 0.029, which was lower than that of 0.067 in previous work [14]. In addition, the flow rate can be increased to 200 μL/min in the MIMCC, which can shorten analytical time. These results indicated that DMF, isooctane and paraxylene were suitable to be used as porogens. To the best of our knowledge, this ternary porogen is the first attempt for preparing the MIMCC.

The polymerization solvent, functional monomer, cross-linker and reaction temperature were very important to the imprinted factor and they were also investigated. Both DMF and dimethylsulfoxide (DMSO) can satisfy the demanding solubility of SQX template. However, the polymerization was non-homogeneous and also changed from colorless to scarlet after several hours in DMSO solvent, while uniform polymerization was obtained in DMF solvent. As a result, DMF was selected as the polymerization solvent. MAA, 4-vinylphenylboronic acid, acrylamide and 4-vinylpyridine (4-VP) were used as functional monomer, while trimethylolpropane, trimethacrylate and EGDMA were used as cross-linker in preparation, the volume ratio of functional monomer to cross-linker from 1:20 to 1:2 were investigated. The imprinted factor (IF) was calculated by the following equation [38,39].

$$IF = Q_{MIP}/Q_{NIP} \quad (2)$$

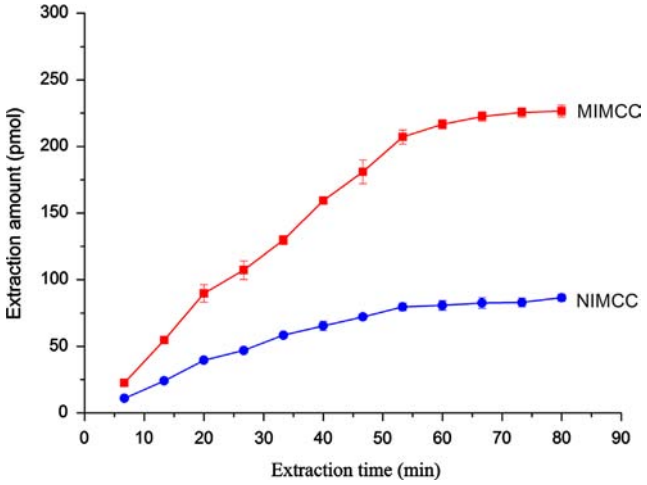

Fig. 5. Comparison of extraction amounts of 10.0 μg/L SQX on MIMCC and NIMCC.

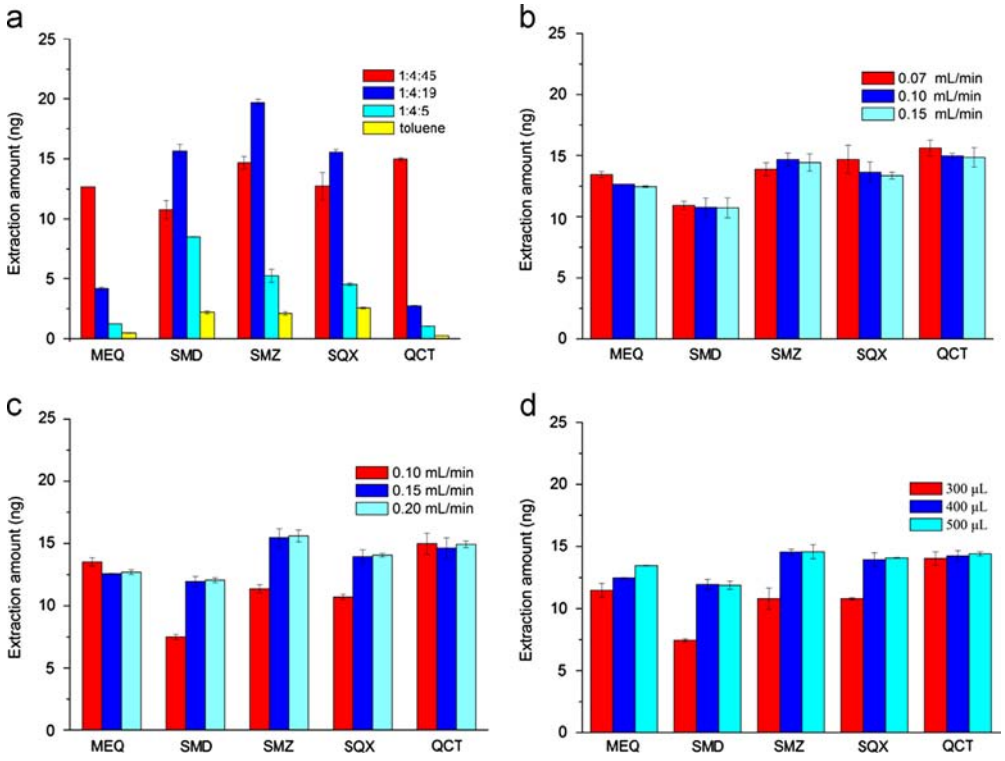

Fig. 4. Optimization of the extraction condition of the MIMCC. (a) effect of volume ratio of acetonitrile/toluene/hexane (v/v) and toluene; (b) the extraction flow rate; (c) the desorption flow rate; (d) the desorption volume.

where Q is the extraction amount of MIMCC or NIMCC. The highest IF value (2.3) was obtained when the volume ratio of EGDMA and MAA was 5.4. The results indicated that the MIMCC prepared with MAA and EGDMA had better imprinted factor, uniformity and solvent resistance. Reaction temperature can be influential to the surface morphology; 54.0 °C, 57.0 °C, 60.0 °C, 63.0 °C, 66.0 °C and 70.0 °C were studied for the temperature optimization. The results as shown in Table.S1 clearly show that MIMCC had no flaw and good uniformity at 60.0 °C. At the same time, aging was very important for recognition lifetime, it influenced imprinted sites strength of the polymer.

3.2. Characteristics of the MIMCC

The morphological structure of MIMCC was investigated by SEM and the results are shown in Fig. 3. It was obvious that the MIMCC was loose and microporous. The morphology was essential to ensure fast mass transport and low pressure, being advantageous to the enhancement of extraction performance.

The infrared spectrum of the MIMCC was also investigated (Fig. S2, Supplementary material). It showed that the broad absorption band at 3565 and 3553 cm^{-1} corresponding to the stretching vibration of O–H bonds attributed to hydroxyl groups of MAA (monomer). The band observed at 2990 cm^{-1} is indicative of C–H stretching while that at 1730 and 1728 cm^{-1} can be to C=O stretching. The absorption peak around 1663 cm^{-1} was attributed to the stretching vibration of residual C=C bonds. The peak intensity of C=C in the MIP was weaker than in the non-imprinted polymer (NIP).

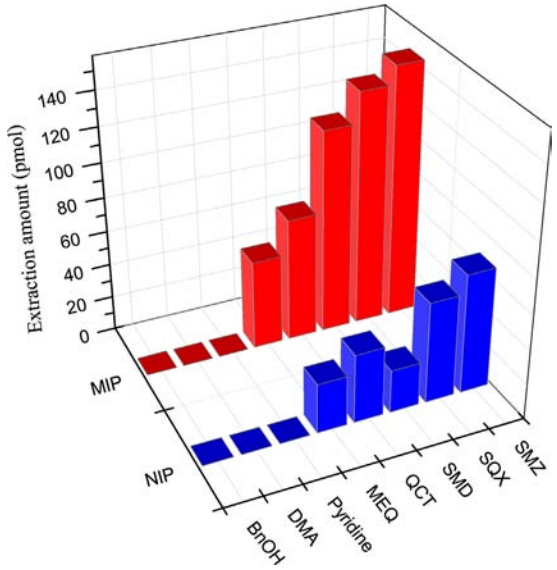

Fig. 6. Extraction selectivity of antimicrobials and reference compounds on MIMCC and NIMCC.

The thermogravimetric analysis (Fig. S3, Supplementary material) of the MIP and NIP indicated that the prepared columns were thermo-stable; notable mass loss occurred at around 170 °C for both monolithic material, and the fastest mass loss occurred at 350 °C for the MIP or NIP, respectively. As observed in Fig. S3, it is stable for the MIMCC was aged at 120 °C and applied at normal temperature.

The solvent resistance was important to the MIMCC used for on-line sample pretreatment in HPLC analysis. DMF, methanol, acetonitrile, acetone, ethyl acetate, toluene, paraxylene, 10% acetic acid in methanol (v/v) and n-hexane were selected for the investigation of the MIMCC and NIMCC. After these solvents flowed through the capillary column at a flow rate of 0.2 mL/min for 60 min, no obvious change was observed for the backpressure, permeability and morphology. The results indicated that MIMCC and NIMCC exhibited good solvent resistance.

3.3. Study of extraction conditions

In MIMCC extraction experiments, the extraction efficiencies depend on the different variables such as the extraction solvent, flow rate desorption volume, etc.

The extraction solvent usually plays an important role in the extraction performance of MIMCC. In order to investigate the effect of extraction solvents, the concentration of SQX in each solvent was fixed at 10.0 μg/L and the adsorption amount of SQX on the column was measured. The results are illustrated in Fig. S4, Supplementary material. It was found that the MIMCC had larger adsorption amount than the NIMCC in each solvent. Moreover, larger amount of adsorption was achieved in the solvents of mixed-solvent of acetonitrile/toluene/n-hexane. Since the polarity and solubility of the selected five antimicrobials were significantly different, the extraction efficiency of affinity compounds such as MEQ had been low in toluene solvent. Then, the mixed-solvent of acetonitrile/toluene/n-hexane was selected and the volume ratio was further investigated. A strong molecular recognition to SQX and good imprinted factor were obtained when volume ratio of mixed-solvent was 1:4:45, which is shown in Fig. 4a.

The effects of extraction flow rate in the range of 0.07–0.15 mL/min are also investigated (Fig. 4b). The experimental results showed that the increase of flow rate had no obvious effect on the total extraction amounts of five antimicrobials. It might be attributed to the fact that the mass transfer of analytes from solution to MIMCC is a fast mass process. A flow rate of 0.15 mL/min was selected.

The desorption solvent was also studied and the mobile phase of HPLC showed good desorption efficiency. Moreover, using the mobile phase as desorption solvent can simplify the manipulation of microextraction, so the analytes were desorbed directly by the mobile phase. The desorption flow rate was optimized in the range

Table 1
Linear range, LOD, LOQ, enrichment factor, repeatability and reproducibility.

Analytes	Linear range (μg/L)	R	LOD[a] (μg/L)	LOQ[a] (μg/L)	RSDs (%, n=5)	Enrichment Factor[b]	Column-to-column RSDs (%, n=5)	
							Intra-batch	Batch-to-batch
MEQ	0.1–10.0	0.9939	0.027	0.096	1.4	46	4.6	4.2
SMD	0.05–10.0	0.9971	0.014	0.047	5.0	189	6.4	6.7
SMZ	0.05–10.0	0.9974	0.014	0.047	5.2	211	6.5	3.6
SQX	0.05–10.0	0.9991	0.010	0.039	3.3	207	8.3	7.5
QCT	0.1–10.0	0.9947	0.020	0.068	2.5	86	5.8	6.7

The injection volume was 20 μL for direct injection. The concentration of sample solution was 5.0 μg/L for each antibacterial.

[a] LOD and LOQ were estimated on the basis of 3:1 and 10:1 signal to noise ratios, respectively.

[b] Enrichment factor is calculated by comparing the peak areas obtained with MIMCC extraction and without preconcentration.

of 0.10–0.20 mL/min and the results are shown in Fig. 4c. No significant changes in the desorbed amounts of the analytes were observed, and moderate backpressure was adopted at desorption flow rate of 0.15 mL/min. Moreover, desorption volume was also investigated from 300 μL to 500 μL. Fig. 4d showed that antimicrobials concentrated in the MIMCC can be completely transferred to the analytical column by 400 μL of desorption solvent without peak broadening.

3.4. Investigation of the extraction performance

3.4.1. Extraction capability, enrichment factor, reproducibility and stability

The amounts of extraction for MIMCC and NIMCC were compared with SQX standard solutions. Under the optimum conditions, the extraction was equilibrated at 67 min; the adsorption amount of MIMCC is much higher than that of NIMCC as shown in Fig. 5. The saturated adsorption amounts of SQX were 222.4 and 82.6 pmol for the MIMCC and NIMCC, respectively. It was 2.7 times of the MIMCC over the NIMCC. The MIMCC exhibited higher capacity to the template molecule than the NIMCC.

The enrichment factor of MIMCC was evaluated using the peak area obtained before and after on-line microextraction. Compared with the chromatogram of direct injection, a significant enhancement of peak area was observed after extracted by the MIMCC, indicating the remarkable preconcentration ability of the column. The enrichment factors were measured from 46 to 211 for five antimicrobials while the enrichment factors of MEQ and QCT were relatively lower than those of the others.

The column-to-column reproducibility was assessed by calculating the relative standard deviation (RSD) for extraction of five antimicrobials (Table 1). Results revealed that satisfactory reproducibility was obtained both for intra-batches with RSD 4.6–8.3% and batch-to-batch with RSD 3.6–7.5%. Moreover, the MIMCC showed high stability and it could be used for extraction more than 100 times with no significant changes in column backpressure and extraction efficiency.

3.4.2. Selectivity of MIMCC

Quantitative analysis of trace antimicrobials in biological samples is difficult owing to the complicated matrix. The extraction selectivity is a key factor for MIMCC, which can greatly influence the specific recognition to target compounds with similar structures such as SQX.

Extraction selectivity was investigated based on the comparison of extraction yields of mixed five standard solutions by the SQX MIMCC and the NIMCC. All the concentrations of MEQ, SMD, SMZ, SQX and QCT were 10.0 μg/L. The results are shown in Fig. 6. The MIMCC had higher adsorption amounts than the NIMCC, the quotients of their adsorption amounts for MEQ, SMD, SMZ, SQX and QCT were 1.8, 4.3, 2.1, 2.3 and 1.8, respectively. SMD have more similar molecular structure to SQX; the better extraction selectivity showed that the strong interaction between SQX MIMCC and target compounds was based on the specific molecular recognition. The structures and hydrogen bonding site of MEQ and QCT are slightly different from the template molecule; SQX, the extraction mechanism of MEQ and QCT is more likely to depend on spatial structure, resulting in the lower extraction yields and lower imprinting factor. Moreover, the three reference compounds have less similarity to template and they could not be extracted by both monolithic columns. These results suggested that SQX MIMCC possessed good extraction selectivity to SQX and its analogs.

3.5. Application of MIMCC

3.5.1. Analytical method

An on-line method for the analysis of five antimicrobials by MIMCC extraction coupled with HPLC was developed. As shown in Table 1, the linear ranges for sulfametoxydiazine, sulamethoxazole and sulfaquinoxaline were 0.05–10 μg/L, and the limits of detection (LODs) were 10.0–14.0 ng/L with RSD 3.3–5.2%, respectively. The linear ranges for mequindox and quinocetone were 0.10–10 μg/L, the LODs were 20.0–27.0 ng/L and the RSDs were 1.4–2.5%, respectively.

Table 2
The analysis of spiked chicken, pork and egg samples using in-tube SPME-HPLC method with MIMCC as extraction unit (n=3).

Sample	Analytes	1.0 (μg/Kg)		3.0 (μg/Kg)		5.0 (μg/Kg)	
		Recovery (%)	RSDs (%)	Recovery (%)	RSDs (%)	Recovery (%)	RSDs (%)
Chicken	MEQ	71.0	7.0	71.6	5.0	80.9	2.4
	SMD	96.7	2.4	93.4	7.1	94.0	3.6
	SMZ	99.6	2.8	94.9	5.4	96.0	3.4
	SQX	84.2	3.6	99.4	6.1	84.1	4.5
	QCT	71.1	5.2	74.8	8.5	91.9	5.2
Pork	MEQ	79.2	2.7	82.8	1.6	97.4	3.3
	SMD	103.3	4.0	103.3	2.5	91.8	2.9
	SMZ	101.7	3.0	108.2	2.6	98.6	2.3
	SQX	94.8	2.4	92.2	1.9	87.0	2.4
	QCT	72.9	7.1	87.5	2.6	86.3	2.3
Egg	MEQ	70.2	5.8	71.2	3.7	85.7	4.3
	SMD	98.9	8.4	94.4	3.6	92.5	4.2
	SMZ	101.4	4.4	98.6	4.0	94.3	4.6
	SQX	80.1	7.4	83.3	3.4	83.1	3.6
	QCT	71.3	4.3	74.7	8.1	86.6	4.0

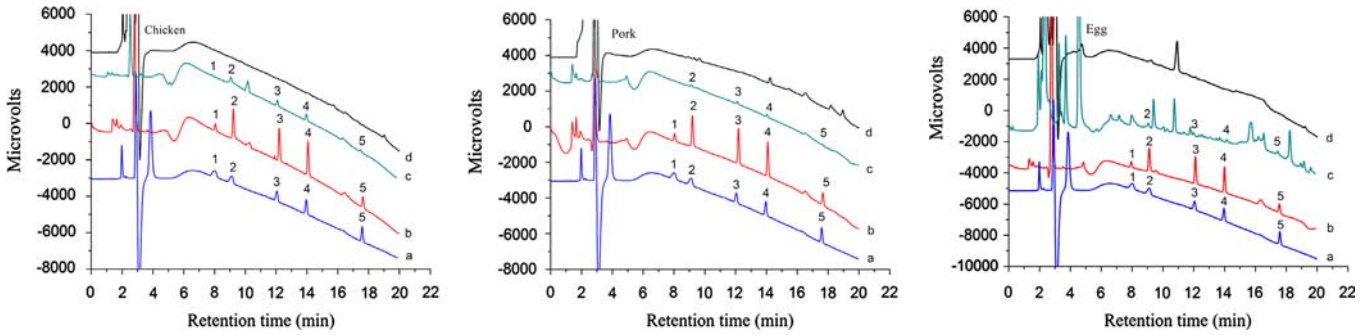

Fig. 7. HPLC chromatograms of antimicrobials in chicken, pork and egg samples. (a) 50 μg/L mixed standards solution. (b) MIMCC extraction of 3.0 μg/kg spiked sample. (c) NIMCC extraction of 3.0 μg/kg spiked sample. (d) Direct injection of the extract solution of 3.0 μg/kg spiked sample. Peaks identity: (1) MEQ, (2) SMD, (3) SMZ, (4) SQX, and (5) QCT.

Table 3
Comparison of different methods for the analysis of antimicrobials in complex samples.

Analytes	Sample	Pretreatment[a]	Mode	Method	Linear range	LODs	Recovery (%)	RSDs (%)	Ref.
MEQ, QCT	Feed	SPE	Off-line	LC/UV	No mention	0.15–0.20 mg/Kg	75.2–94.7	< 10	[33]
SMZ	Chicken, pork	SBSE	Off-line	LC/UV	2.0–100 μg/L	0.66 μg/L	75.6	8.3	[34]
SMD, SMZ, SQX	Egg	SPE	Off-line	LC/MS	10–1000 μg/Kg	7.9–9.2 μg/L	73.8–96.2	3.2–8.3	[40]
SMZ,	Chicken	Micro-SPE	Off-line	LC/UV	10–1000 μg/L	0.38 μg/L	57.79	7.1	[41]
SMZ	Sludge	PLE-SPE	Off-line	LC/MS/MS	31.6–500 μg/L	4.2 μg/Kg	95.7	0.7	[42]
SMZ, SQX	Grass carp	MSPD	On-line	LC/MS/MS	0.1–100 μg/L	0.75–1.25 μg/Kg	91.1–94.0	4.3–6.3	[43]
QCT	Chicken	SPE	Off-line	LC/MS/MS	1.0–100 μg/Kg	0.37 μg/Kg	81.4	9.8	[44]
MEQ, SMD, SMZ, SQX, QCT	Chicken, Pork, Egg	In-tube SPME	On-line	LC/UV	0.05–10.0 μg/L 0.10–10.0 μg/L	0.010–0.030 μg/L	71.0 –108.2	1.6–8.5	This work

[a] SPE: solid-phase extraction; SBSE: stir bar sorptive extraction; PLE: pressurized liquid extraction; MSPD: matrix solid phase dispersion; In-tube SPME: in-tube solid-phase microextraction.

3.5.2. *Sample analysis*

To validate the new established method in real samples with complex matrix, chicken, pork and egg sample were selected for the spiking analysis at three levels of 1.0, 3.0 and 5.0 μg/kg with MEQ, SMD, SMZ, SQX and QCT standards. The analysis of spiked sample with 3.0 μg/kg standards is shown in Fig. 7. The five antimicrobials could be accurately analyzed after MIMCC on-line extraction. The recoveries for MEQ, SMD, SMZ, SQX and QCT in different samples were 71.0–97.4%, 91.8–103.0%, 94.3–108.2%, 83.1–99.4% and 71.1–91.9% with the RSDs of 1.6–8.5%, respectively (Table 2). The results indicated that this method could be applied to the trace analysis of antimicrobials in complex biological samples. As the comparison in Table 3 shows, it also outperformed different methods for the determination of antimicrobials in complex samples. The proposed method provides excellent sensitivity, high accuracy, more convenience and is environmentally friendly.

4. Conclusion

In this work, a novel porous, chemical stable and long-lifetime MIMCC for on-line extraction of antimicrobials was in situ fabricated with sulfaquinoxaline as the template molecule. An on-line method for trace analysis of five antimicrobials in food samples was developed by MIMCC extraction coupled to HPLC. The limits of detection were in the range of 10.0–27.0 ng/L, which was much lower than the strict maximum residue level in milk of 10 μg/L in USA. The method was successfully applied to analysis of five antimicrobials in chicken, pork and egg samples. The recoveries were 71.0–108.2% with the RSDs of 1.6–8.5%, respectively. The on-line method was proven to be selective, sensitive and convenient for trace determination of antimicrobials in complex samples.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.02.010.

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