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Research Article

Selective determination of trace thiamphenicol in milk and honey by molecularly imprinted polymer monolith microextraction and high-performance liquid chromatography

A novel solid-phase microextraction (SPME) method based on molecularly imprinted polymer (MIP) monolith as the sorbent for the selective extraction of thiamphenicol (TAP) in milk and honey was developed. The newly developed MIP monolith was produced using TAP as the template molecule, 4-vinylpyridine (4-VP) as the functional monomer. The TAP-MIP monolith synthesized in a micropipette tip could be connected with syringes in different sizes simply to perform SPME process without any other treatment. The derivated MIP monolith showed high selectivity and enrichment ability for TAP. A simple, rapid and sensitive method for the determination of TAP in milk and honey using polymer monolith microextraction (PMME) based on the MIP monolith combined with high-performance liquid chromatography-photodiodes array detector was developed. Several parameters affecting MIP monolith microextraction were investigated, including the flow rate, volume, pH and salt concentration of sample, the type and volume of washing solution, the type and flow rate of eluent. The recovery of this method for TAP was investigated and high recoveries of 92.9–99.3% from milk and honey were obtained with relative standard deviations less than 4.9%.

Keywords: High-performance liquid chromatography / Milk / Molecularly imprinted polymer monolith / Polymer monolith microextraction / Thiamphenicol
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1 Introduction

Thiamphenicol (TAP), Fig. 1, is an analogue of chloramphenicol (CAP) in which the nitro group in the benzene ring is replaced with a methylsulfonic group. It has been reported that TAP showed particular therapeutic effect in respiratory infections, bacterial prostatitis and venereal diseases. But TAP also showed hematological toxicity [1]. For strict control of TAP, the maximum residue limit (MRL) is 50 ng/g for TAP in foodstuffs of animal origin in the European Union (EU) [2]. Therefore, it is important to develop a fast and effective analytical method for determination of TAP in food commodities.

Up to now, the main approaches for the detection of TAP residue included chromatography [3–10] and multiple techniques of chromatography are linked with mass spectrometry [11–17]. The complexity of food matrices and the presence of much potential interference require specific and selective methods for extracting and isolating analytes from food samples before detection. Solid-phase extraction (SPE) is routinely used for clean-up and preconcentration in the analysis of food samples. Compared with liquid–liquid extraction (LLE), SPE has the advantages of simplicity, rapidity and less consumption of organic solvents. However, generic sorbents usually lack selectivity, and are easily subjected to interference by non-target substances with similar characteristics. Although immunoaffinity chromatography (IAC) is capable of adsorbing target analytes differentially, it still has some disadvantages such as lack of stability and high costs of antibody preparation. Recent research has been oriented towards the development of efficient, economical and miniaturized sample preparation methods. As a result, solid-phase microextraction (SPME) [18, 19] and liquid-phase microextraction (LPME) [20]

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Abbreviations: CAP, chloramphenicol; **EGDMA**, ethylene dimethacrylate; **MIP**, molecularly imprinted polymer; **MIPMME**, molecularly imprinted polymer monolith microextraction; **NIP**, non-imprinted polymer; **NIPMME**, non-imprinted polymer monolith microextraction; **PMME**, polymer monolith microextraction; **SD**, sulfadiazine; **SPME**, solid-phase microextraction; **TAP**, thiamphenicol; **4-VP**, 4-vinylpyridine

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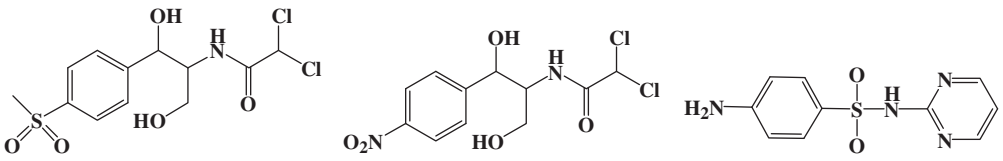

Thiamphenicol (TAP) Chloramphenicol (CAP) Sulfadiazine (SD)

Figure 1. The molecular structures of TAP, CAP and SD.

methods have been developed. Compared with LLE, SPME is a solvent-free process that includes simultaneous extraction and preconcentration of analytes from aqueous samples or the headspace of the samples. However, SPME is expensive, its fiber is fragile and has limited lifetime, and the sorbents usually lack selectivity. LPME is a solvent-minimized sample pretreatment procedure that is inexpensive, and since very little solvent is used, there is minimal exposure to toxic organic solvents. However, the method suffers from some disadvantages as follows: fast stirring would tend to form air bubbles, extraction is time-consuming and equilibrium cannot be attained after a long time in most cases [21].

Due to their high selectivity, reusability, inexpensiveness to prepare, physiochemical stability and applicability in harsh chemical media, molecularly imprinted polymers (MIPs) have been used as a sorbent in SPE and SPME to selectively extract analytes from complex matrices [22–25]. Traditionally, MIPs were synthesized in bulk polymerization followed by a grinding and sieving process to acquire the desired particles in shape and size, which limited the extraction efficiency. To overcome these disadvantages, the MIP monolithic columns were prepared by *in situ* polymerization directly inside appropriate columns or capillaries [26, 27]. This strategy could avoid the tedious grinding and sieving procedures as well as the problems of costly particle loss, particle inhomogeneity and molecularly imprinted spots loss, to easily obtain a MIP monolith with good resolution and low backpressure at a high flow rate. Polymer monolith microextraction (PMME) was a type of SPME in which the polymer monolith was used as the sorbent [28]. The combination of MIP technology with PMME could exhibit excellent extraction selectivity in dealing with biological samples [29, 30]. But, the MIP monolith synthesized in a capillary was fragile, and tedious post-preparation to connect it with syringes was needed. To the best of our knowledge, no TAP-MIPMME method has been reported in previous studies for the preconcentration of TAP in food.

In this work, a TAP-MIP monolith was synthesized in a micropipette tip using TAP as the template, 4-vinylpyridine (4-VP) as the functional monomer, ethylene dimethacrylate (EGDMA) as the cross-linker and the mixture of toluene–dodecanol as the porogenic solvent. The robust micromonolith could be connected with syringes in different sizes simply to perform PMME process without any other treatment. The derivated MIP monolith showed high selectivity and enrichment ability for TAP. Further, an MIPMME-HPLC procedure has been employed for the determination of TAP by using the MIP monolith for the clean-up and preconcentration of TAP. The results indicated that this method could be applied for the rapid and sensitive analysis of TAP in milk and honey samples.

2 Materials and methods

2.1 Reagents and materials

EGDMA purchased from Acros (New Jersey, USA) was extracted with 5% aqueous sodium hydroxide and water, then dried over anhydrous magnesium sulfate. 2,2-Azobisisobutyronitrile (AIBN) was obtained from Shanghai No. 4 Chemical Reagent (Shanghai, China) and recrystallized in anhydrous ethanol before use. 4-VP was obtained from Acros. Methacrylic acid (MAA), acrylic acid (AA), acrylamide (AM), toluene and dodecanol purchased from Fuchen Chemical Reagent Company (Tianjin, China) were distilled under vacuum prior to use. TAP, CAP, and sulfadiazine (SD) were purchased from Sigma (St. Louis, MO, USA). Methanol and acetonitrile (HPLC grade) were obtained from Tedia Company (Ohio, USA). Sodium chloride, phosphoric acid and other reagents used were all of analytical grade. The water used was purified on an Ultrapure Water System (Beijing, China).

2.2 Instrumentation

The chromatographic analysis was carried out on a Dionex Summit U3000 HPLC system equipped with a manual injector and a photodiode array detector (PAD) (Dionex Technologies, USA). A personal computer equipped with a Chromeleon ChemStation program for LC was used to process chromatographic data. An Amethyst-C18 column (4.6 mm × 250 mm, 5 μm) from Sepax Technologies (Newark, USA) was connected with a guard column (cartridge 2.1 mm × 12.5 mm, 5 μm, Agilent Technologies, Palo Alto, CA, USA) filled with the same packing material. The mobile phase was a mixture of methanol–water (45:55, v/v) and the flow rate was 1.0 mL/min. The column temperature was set at 25°C by a temperature controller for column oven (Nuohai Technologies, China). The UV detector was set at a wavelength of 225 nm for TAP, 278 nm

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for CAP and SD. All injections were performed manually with a 20.0-μL sample loop. A DZF-6021 vacuum drying oven (Yiheng Instrument Factory, Shanghai, China) was used for polymerization. An LSP01-1A longer pump (Baoding Longer Precision Pump, China) was used for pumping. A 0.45-μm membrane was obtained from Xingya Scavenging Material Company (Shanghai, China). The microscopic morphology of the monolith was examined by a Model X-650 scanning electron microscope (Hitachi, Tokyo, Japan). The infrared spectrograms of MIP and non-imprinted polymer (NIP) monoliths were examined by a Fourier transform infrared spectrometer (Perkin Elmer, USA). The measurement of the surface area and mesopore size distribution of the MIP monolith was carried out with a Coulter SA 3100 plus surface area and pore size analyzer (Beckman, USA).

2.3 Preparation of molecularly imprinted monolith

For the preparation of the TAP-MIP monolith, the template molecule TAP (0.05 mmol) was dissolved in appropriate porogenic solvents (52 μL toluene, 0.3100 g dodecanol) in a clean PE tube and mixed with 4-VP (0.1 mmol) as the functional monomer. The mixture was surged ultrasonically for 5 h. Then, 1 mmol of cross-linker EGDMA and 8.5 mg of initiator AIBN were added and degassed by ultrasonication for about 10 min. Next, 50 μL of the homogeneous solution was filled into a micropipette tip which had been sealed at one end. Subsequently, the other end of the pipette tip was sealed with silicon rubber. After polymerization at 60°C for 24 h, the silicon rubber was removed. The resultant MIP monolith was washed with ample methanol to remove the template molecules using a syringe infusion pump again and again until no TAP was found in the chromatogram when the elution was analyzed by LC. A reference, NIP, was prepared simultaneously with the same procedure including washing, but in the absence of the template molecule.

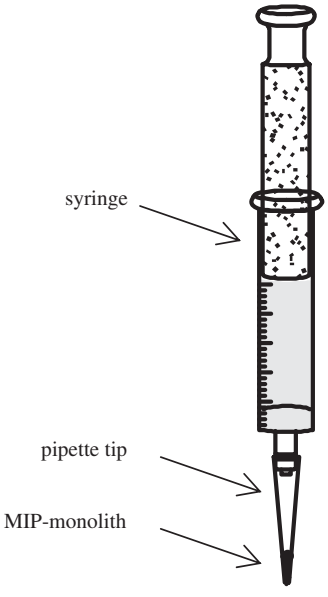

Figure 2. Scheme of the TAP-MIPMME device.

2.4 Preparation of the extraction device

As shown in Fig. 2, the MIP monolith could be connected with syringes in different sizes simply without any other treatment. A syringe infusion pump (Baoding Longer Precision Pump) was employed for the delivery of sample solution, washing solution and desorption solvent.

2.5 MIPMME procedure

The MIP monolith was washed with 5.0 mL of methanol and 2.0 mL of water, respectively. Then, an aliquot of 5.0 mL pretreated sample solution (1.5 g NaCl was added) was loaded at a flow rate of 0.2 mL/min with the aid of an infusion pump. The MIP monolith was washed with 0.5 mL water at a flow rate of 0.2 mL/min to remove the matrix interferences. Then, the analytes were eluted with 0.1 mL of methanol at a flow rate of 0.05 mL/min. The eluent solution in the PE tube was removed using a 100-μL HPLC microsyringe and injected into the HPLC system for analysis directly. All experiments were performed repeatedly and means of results were used in plotting of curves or in tables.

2.6 Standard solutions and samples

The stock standard solutions of TAP, CAP and SD were prepared in methanol at a concentration of 1 mg/mL and stored at 4°C in a refrigerator. Working standard solutions of analytes were prepared by appropriate dilution of the stock solution using purified water.

Preliminary analyses showed the milk and honey samples purchased from the local retail market to be analyte free. Then, 5 g of milk and honey samples were spiked with known variable amounts of TAP, respectively. The spiked samples were extracted with 5 mL acetonitrile by using a vortex mixer (WH-3, Luxi Analysis Instrument Factory, Shanghai, China). Then, the samples were centrifuged at 7°C for 10 min at 10 000 rpm (Xiangzhi Centrifuge Instrument, Changsha, China). The supernatant was completely transferred to another 25 mL volumetric flask using a 10-mL syringe. After evaporation of the solvent under a gentle nitrogen flow, the residue was redissolved in 25 mL purified water. Finally, the reconstituted solutions were stored at 4°C and filtered through a 0.45-μm membrane filter prior to use. Blank samples were prepared in the same way as above but without the compound-spiking step.

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3 Results and discussion

In order to obtain the optimized extraction conditions, enrichment factor (EF) and extraction recovery (ER) were used to evaluate the extraction efficiency of MIP monolith under different conditions

$$EF = \frac{C_{elu}}{C_0},\ ER = \frac{n_{elu}}{n_0} \times 100 = \left[\frac{C_{elu} \times V_{elu}}{C_0 \times V_{aq}}\right] \times 100 = EF \times \left(\frac{V_{elu}}{V_{aq}}\right) \times 100 \quad (1)$$

where C_{elu}, n_{elu} and V_{elu} are the TAP concentration and numbers of moles in eluent, and the volume of eluent, respectively. C_0, n_0 and V_{aq} are the TAP concentration and numbers of moles in sample solution, and the volume of sample solution, respectively.

The imprinting factor (IF) and selective factor (SF) were used to evaluate the recognition abilities of the TAP-MIP monolith

$$IF = \frac{EF_{MIP}}{EF_{NIP}},\ SF_{TAP/CAP} = \frac{Q_{TAP}}{Q_{CAP}},\ SF_{TAP/SD} = \frac{Q_{TAP}}{Q_{SD}} \quad (2)$$

where EF_{MIP} and EF_{NIP} are the EFs of TAP extracted in MIP and NIP monoliths under the same conditions, respectively. Q_{TAP}, Q_{CAP} and Q_{SD} are the adsorption capacities of TAP, CAP and SD in MIP monolith, respectively.

3.1 Optimization of synthesis conditions

Several parameters were investigated to obtain better final characteristics, affinity and selectivity for the target analyte, such as the nature of functional monomer, the molar ratios of template to cross-linker and template to functional monomer, and the nature of porogenic solvent.

For investigation of the synthesis conditions, 1 mL of mixed 5 μg/mL TAP, CAP and SD standard solution was loaded on the MIP monolith at a flow rate of 0.2 mL/min. Then, the analytes were eluted with 0.1 mL of methanol at a flow rate of 0.05 mL/min. The elutent solution was analyzed by HPLC.

3.1.1 Selection of functional monomer

Different functional monomers will construct different binding sites with template. To improve the recognition and selectivity property of MIP, four different functional monomers, including AA, MAA, AM and 4-VP, were investigated.

The EFs of different TAP-MIPs were compared. The results showed that, compared with other functional monomers, 4-VP has the best imprinting recognition for TAP. A possible explanation for the result was that the nitrogen atom of the 4-VP body was a hydrogen-bond acceptor, and the hydroxyl group of TAP was a hydrogen-bond donor. Thus, hydrogen bonds were expected to be formed between TAP and 4-VP. So, in our further work, 4-VP was chosen as the functional monomer.

3.1.2 Effect of the molar ratio of the monomer and cross-linker

Increasing the amount of cross-linker can maintain the stability of the recognition sites and lead to high selectivity for the target. On the other hand, with increasing the amount of cross-linker, the difficulty of mass transfer of analytes in MIP monolith increased, and the backpressure of MIP monolith increased. In this study, the mol ratios of the monomer (4-VP) to cross-linker (EGDMA) 1:5, 1:10, 1:20, 1:30 and 1:40 were investigated, respectively. The experimental results showed that the MIP monolith has much better selectivity for TAP when the mol ratio of the monomer and cross-linker was 1:20.

3.1.3 Effect of the molar ratio of the template and monomer

The mol ratios of the template (TAP) to monomer (4-VP) 1:1, 1:2, 1:4, 1:6 and 1:8 were investigated, respectively. The results illustrated that 0.05 mmol of the template in the presence of 0.1 mmol of functional monomer (template to functional monomer ratio of 1:2) resulted in a monolith with maximum extraction and separation ability.

Based on the results, dual hydrogen bonds were expected to be formed between TAP and 4-VP as a key interaction necessary for binding site construction. The hydroxyl group of TAP acted as a hydrogen bond donor. And, the nitrogen atom of the 4-VP body acted as a hydrogen bond acceptor. The schematic representation of molecular recognition on the TAP-MIP monolith is shown in Fig. 3.

3.1.4 Selection of porogenic solvent

The selection of the porogenic solvent is significant for the preparation of the molecularly imprinted monolith. Porogenic solvent can make all components into one phase in the polymerization process, and played an important role in the morphology of the MIP in terms of specific surface area and pore size. In the selection of porogenic solvent, some properties must be considered such as: (i) The porogenic solvent must be able to dissolve the template molecule, monomer, initiator and cross-linker. (ii) The porogenic solvent should form large pores to ensure good

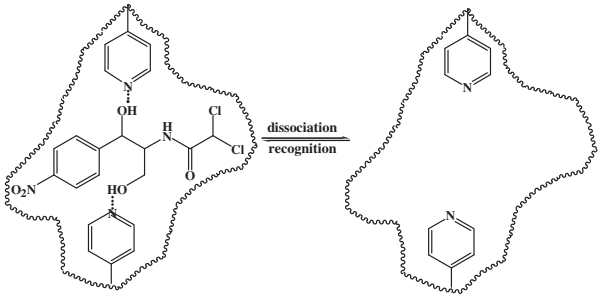

Figure 3. Schematic representation of molecular recognition on the TAP-MIP monolith.

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flow-through properties of the monolith. (iii) The polarity of the porogenic solvent should be lower in order to reduce the interferences between the template molecule and the monomer during complex formation. In this study, methanol, ACN, carbon tetrachloride, chloroform, toluene, dodecanol and their mixed solutions as the porogenic solvent were tested. The experimental results indicated that the low polar mixture of toluene and dodecanol displayed better extraction efficiency for TAP. Finally, the mixture of toluene and dodecanol was selected as the appropriate porogenic solvent.

3.2 The characterization and specificity evaluation of the MIP monolith

The MIP monolith morphological structure was investigated by scanning electron microscope. As can be seen in Fig. 4, there are many macropores and flow-through channels inlaid in the network skeleton of TAP imprinted monolith which provided flow paths through the column. Due to the size and density of the macropore network, the monolith had a high external porosity and, consequently, a large permeability and low-column hydraulic resistance. These pores allowed the mobile phase to flow through with low flow resistance.

The total surface area and the mesopore size distribution of the TAP-MIP monolithic material were both determined by N_2 sorption method. The mesopore size distribution [Barrett–Joyner–Halenda (BJH) plot] showed that a wide mesopore size distribution was obtained and mainly distributed in the range of 3–40 nm. The total surface area was 6.03 m^2/g through a BET plot.

Characteristic of the FT-IR spectra were obtained from the TAP-MIP and NIP monoliths, respectively. As shown in Fig. 5, the strong peak at 1729 cm^{-1} should be attributed to C═O stretching bands from the ester groups (in the network skeleton of monolith originated from EGDMA). Two weak peaks around 1600 cm^{-1} resulted from C═C stretching vibrations (in the network skeleton of monolith originated from 4-VP body). The observed features at 1300–1150 cm^{-1} indicated C–O–C stretching vibrations. The band at 2962 cm^{1} was the characteristic of ═CH stretching frequency from 4-VP or stretching vibration of C–H bonds on methyl groups from EGDMA. And, the NIP and MIP monoliths showed similar locations and appearances of the major bands. These results showed that the polymers have been successfully synthesized.

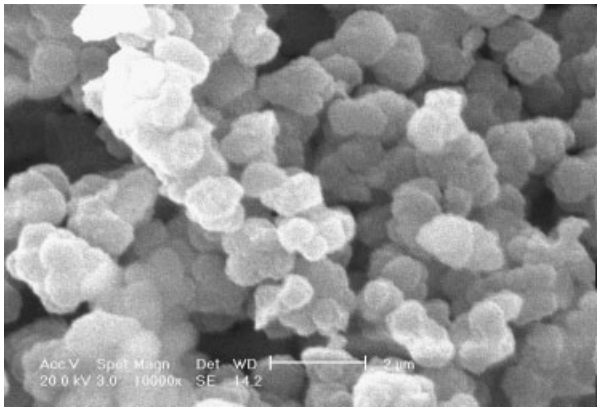

Figure 4. SEM image of the TAP-MIP monolith (magnification = 10 000×).

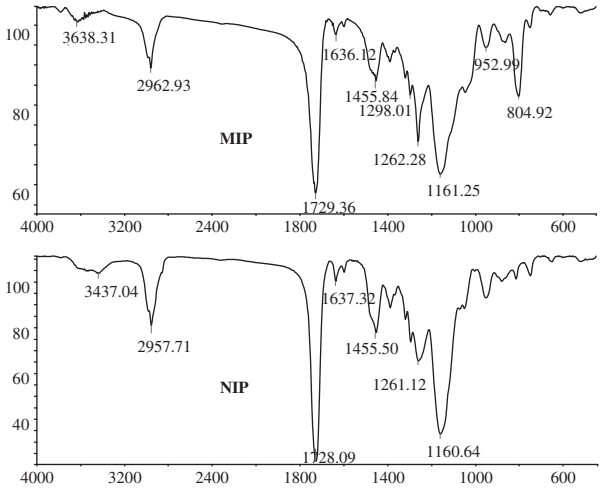

Figure 5. FT-IR spectra of TAP, 4-VP, MIP and NIP.

In order to evaluate the selectivity of the MIP monolith, CAP with similar structure to TAP as the analogue and SD as non-analogue (Fig. 1) were tested. For sampling, 5.0 mL of mixed solution, including 5 μg/mL TAP, CAP and SD, respectively, was loaded on the MIP and NIP monoliths at a flow rate of 0.2 mL/min. About 0.1 mL of methanol was used to elute analytes. The eluent was analyzed by HPLC directly. The results indicated that the MIP had a higher affinity for TAP than NIP, where IF was 7.50. And, the MIPMME possessed extraction efficiency higher than that of the non-imprinted polymer monolith microextraction (NIPMME) for CAP. This is due to the similar structure between CAP and TAP. The data also showed that the retention of SD on MIP monolith was similar to that of the NIP monolith.

To estimate the adsorption capacity of TAP, CAP and SD on the MIP monolith, an adsorption experiment was carried out under optimized conditions. In this experiment, 5 μg/mL TAP standard solutions were continuously passed through the MIP monolith at 0.05 mL/min until the peak area of TAP in eluent was equal to that of standard solution. Then, the adsorption capacity of TAP was calculated on the base of the TAP concentration, the volume of standard solution and eluent. The adsorption capacities of CAP and SD were determined and calculated in the same way. The experimental results showed that Q_{TAP}, Q_{CAP} and Q_{SD} were 183.1, 19.3 and 2.4 μg, respectively, and $SF_{TAP/CAP}$, $SF_{TAP/SD}$ were 9.5 and 76.3, respectively. These results demonstrated the good selectivity of the synthesized MIP monolith for TAP. And, the TAP-MIP monolith could be used for clean-up and enrichment of TAP.

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3.3 Optimization of MIPMME conditions

Several parameters associated with the MIPMME efficiency, such as the flow rate, volume, pH and salt concentration of sample, the type and volume of washing solution, the type, volume and flow rate of eluent were optimized in this study. Sample solutions were spiked with TAP at 0.2 μg/mL to perform the experiments.

3.3.1 Effect of sample flow rate

The flow rate of the sample solution was optimized in the range of 0.05–0.50 mL/min. The extraction efficiency decreased with the increase in the flow rate of sample solution from 0.2 to 0.5 mL/min. EF and ER increased slightly while changing the flow rate from 0.2 to 0.05mL/min. This may be due to the plenitudinous mass transfer of the analyte from sample solution to MIP monolith at low flow rate. To achieve high extraction efficiency within a short time, 0.2 mL/min was chosen as the optimized flow rate of sample solution in the following experiments.

3.3.2 Effect of type and volume of washing solution

The washing solution was adjusted by optimizing the proportion of CH_3OH in water. The experimental results indicated that EF and ER of TAP decreased obviously by increasing CH_3OH content in the washing solution. And, there was no observed difference in EF and ER of TAP after washing with 0.5 and 1 mL of purified water. So, 0.5 mL of purified water was selected as the optimized washing solution.

3.3.3 Effect of the type, volume and flow rate of eluent

The selection of an appropriate eluent is of high importance for the PMME process. Considering the consistency to the mobile phase used in liquid chromatography, the eluent is limited to solvents such as methanol, acetonitrile and purified water. Different proportions of methanol with water, acetonitrile with water as the eluent were tested. The results indicated that the addition of high polarity solvent (water) in eluent was disadvantage for eluting TAP. And, methanol as the eluent was better than acetonitrile. The experimental results showed that both the EF and ER decreased when the amount of acetic acid in the eluent increased. So, methanol was selected as the eluent in the following experiments.

In order to study the effect of eluent volume on the extraction efficiency, different volumes of eluent (methanol) were tested. The results showed that 0.1 mL eluent was sufficient to elute analyte from the monolith. And, further increasing the volume of the eluent was not preferred because EF decreased with increasing eluent volume. Thus, 0.1 mL of methanol was selected for subsequent work.

The flow rate of the eluent was optimized in the range of 0.01–0.3 mL/min. The results showed that no significant change in the extraction efficiency was found when the flow rate of eluent was in the range of 0.01–0.05 mL/min. Then, the extraction efficiency decreased with increasing flow rate. So, 0.05 mL/min was selected as the optimized flow rate of eluent in the following experiments.

3.3.4 Effect of sample volume

The effect of sample volume was monitored by loading sample solution (containing 0.2 μg/mL of the analyte) from 2.0 to 10.0 mL at a constant flow rate. The eluent volume (methanol) was 0.1 mL. The results indicated that EF of TAP increased with increasing sample volume from 2.0 to 10.0 mL. This indicated that the extraction capacity was not reached even when 10.0 mL of sample solution was loaded. However, ER began to decrease when the sample volume increased. To achieve sufficient sensitivity within a short time, 5.0 mL of sample solution was selected in the PMME procedure.

3.3.5 Effect of sample pH

The sample pH is a significant factor, which may affect the molecule form of the analyte and closely relate to the interaction between analytes and the MIP monolith. Considering that TAP is unstable when pH >7, the effect of the sample pH on the extraction efficiency for TAP was investigated using several buffer solutions with pH 2–7. The experimental results showed that EF and ER decreased slightly when sample pH decreased from 7 to 2. This can be explained by the fact that the interaction between the analyte and the monolith was mainly based on the hydrogen binding. TAP was likely to exist in positively charged form at low pH, resulting in the weakening of interaction between TAP and the polymer and thus poor extraction performance. Finally, no buffer solution was needed to adjust the sample pH in the subsequent experiments.

3.3.6 Effect of salt concentration

The effect of salt concentration of the sample on the extraction efficiency was also investigated. The results indicated that EF and ER increased (from 28 to 49.4 and from 55.8 to 98.9, respectively) as the concentration of NaCl increased from 0 to 30% w/v. Addition of salt into the sample solutions could lead to the salting-out effect, and more analyte molecules would be extracted onto the MIP monolith. To obtain high extraction efficiency, 30% NaCl w/v was added in the sample solution in the following experiments.

3.4 Evaluation of the method

Under the optimized conditions, the method was applied for the determination of TAP in milk and honey samples. Blank

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milk and honey samples were spiked in the range of 0.01–100 μg/g with TAP. Then, the spiked samples were analyzed by the proposed MIPMME-HPLC method. The regression coefficients (r) were 0.9983 and 0.9991, respectively. The limits of detection (LODs), based on signal-to-noise ratios (S/N) of 3, were 0.003 μg/mL and 0.002 μg/g for TAP in milk and honey, respectively.

The reproducibility of the method was determined by the within-day and between-day precisions at the concentration of 0.05, 1, 10 μg/g for TAP in spiked milk and honey samples. The results showed that the within-day precision (RSD, $n = 5$) was less than 4.9%, while the between-day precision (RSD, $n = 5$) was less than 6.6%.

The chromatograms of spiking milk samples before and after treatment by MIPMME and NIPMME are shown in Fig. 6. It can be seen that after treatment by MIPMME, a majority of interfering substances in milk sample were eliminated, thus quantification of TAP can be successfully achieved. And, in comparison with the chromatogram of direct injection and treatment by NIPMME, a dramatic enrichment of the peak height was observed. This result indicated that the TAP-MIP monolith had remarkable preconcentration ability and selectivity for TAP from complex matrices.

The MIP monolith showed high stability since no significant changes in the backpressure and extraction efficiency of the monolith were found in the experiment.

3.5 Real samples analysis

The developed MIPMME-HPLC technique was applied for the determination of TAP in milk and honey to further elucidate the applicability and reliability of this method. Three batches of milk sample and five batches of honey sample were collected from local supermarkets. The results showed that these milk and honey samples were free of TAP

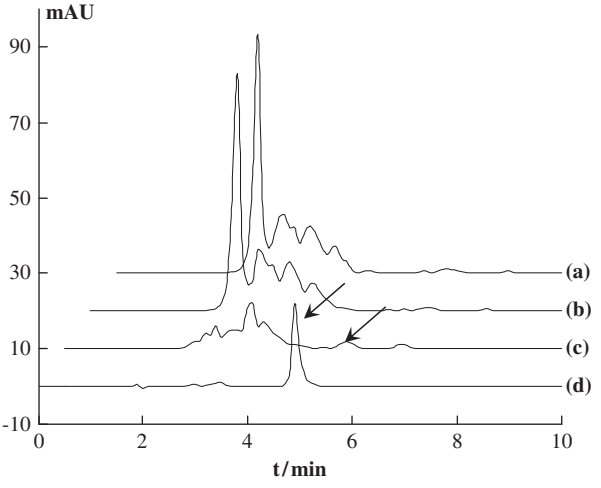

Figure 6. HPLC chromatograms of (a) blank milk; (b) blank milk spiked with 0.2 μg/g TAP; (c) blank milk spiked with 0.2 μg/g TAP after NIPMME; (d) blank milk spiked with 0.2 μg/g TAP after MIPMME.

Table 1. Extraction recoveries (%) and RSDs obtained for the TAP-MIPMME of milk and honey samples spiked with TAP

Test sample	Spiked level (ng/g)[a)]	Recovery (%)	RSD (%) $n = 3$
Milk	25	92.9	4.3
	50	97.5	3.7
	100	99.3	1.9
Honey	25	97.1	2.6
	50	98.0	3.1
	100	95.6	4.9

a) The MRL of TAP in milk is 50 ng/g. So, the recovery test was designed at three levels: 1/2 MRL, MRL and 2 MRL.

residue. To test the performance of this established method, the extraction recoveries were performed by spiking fresh milk and honey samples with TAP standard solution. For each concentration level, three replicate experiments with the whole analysis process were made. Recoveries ranging between 92.9 and 99.3% were obtained (Table 1). Thus, the developed method is robust and reliable for routine analysis of TAP in complex milk sample.

4 Concluding remarks

A novel, durable TAP-MIP monolith was synthesized in a micropipette tip for the first time. The micro-monolith could be connected with syringes in different sizes simply without any other treatment to perform PMME process. The derivated MIP monolith showed high selectivity and enrichment ability for TAP. MIPMME followed by HPLC and PAD detection was developed as an analytical method for the sensitive, selective determination of TAP in milk and honey. The optimum conditions of synthesis and extraction performance have been obtained. The experimental results revealed that this method provided high selectivity, lower solvent consumption, higher extraction efficiency and good linearity over the investigated concentration range. The performance of this procedure in the analysis of TAP in milk and honey samples was satisfactory.

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