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Chloramphenicol Extraction from Honey, Milk, and Eggs Using Polymer Monolith Microextraction Followed by Liquid Chromatography−Mass Spectrometry Determination

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A rapid confirmatory method for monitoring chloramphenicol (CAP) residues in honey, whole milk, and eggs is presented. This method is based on the polymer monolith microextraction (PMME) technique and high-performance liquid chromatography (HPLC)−electrospray ionization mass spectrometry (MS). A poly(methacrylic acid−ethylene glycol dimethacrylate) monolithic capillary column was selected as the extraction medium. To obtain optimum extraction efficiency, several parameters related to PMME were investigated. After dissolution in 20 mM phosphate solution at pH 4.0 and centrifugation, honey, eggs, or milk samples were directly passed through the extraction tube. The LC−MS instrument was equipped with an electrospray ion source and a single quadrupole. The eluates were analyzed by LC−MS in the negative-ion mode and by monitoring a pair of isotopic ions for the target compound. The in-source collision-induced dissociation process produced confirmatory ions. The recoveries of CAP from real samples spiked at 0.1−10 ng/g (honey), 0.2−10 ng/mL (milk), and 0.2−10 ng/g (egg) were in the range of 85−102%, with relative standard deviations ranging between 2.1% and 8.9%. The limits of detection (S/N = 3) were 0.02 ng/g, 0.04 ng/mL, and 0.04 ng/g in honey, milk, and eggs, respectively. The proposed method was proved to be robust in monitoring CAP residue in honey, milk, and eggs.

KEYWORDS: Polymer monolith microextraction; liquid chromatography-electrospray ionization mass spectrometry; chloramphenicol; honey; milk; egg

INTRODUCTION

Antibiotics are widely used in animals for the treatment of diseases and also as animal growth promoters. The use of antibiotics may lead to drug residues present in animal-derived foods, the side effects of which would threaten public health. To minimize possible exposure to antibiotics, an allowable level of antibiotics in food has been established by the U.S. Food and Drug Administration and European Union. Some antibiotics even were prohibited in food-producing animals in the U.S. and on the European Union's "group A" list of compounds (pharmacologically active substances for which a "zero tolerance residue limit" can be fixed in edible tissues) (*1*). One of these drugs is chloramphenicol (CAP), a broad-spectrum antibiotic obtained naturally from *Streptomyces venezuelae* or produced synthetically. It exhibits activities against both aerobic and anaerobic microorganisms. However, CAP can cause aplastic anemia and leukemia. CAP is still illegally used in animal farming because of its easy access and low cost. Therefore, effective detection techniques are required for a strict control of this compound at MRPLs (minimum required performance limits) of 0.3 ng/g (*2*).

Qualitative and quantitative analyses of CAP have been performed using rapid test kits (radio-immunoassays and enzyme immunoassays) (*3*, *4*), thin layer chromatography (*5*), gas chromatography (GC) with an electron capture detector after chemical derivatization (*6*, *7*), immunoaffinity chromatography (*8*), or high-performance liquid chromatography (HPLC) with ultraviolet detection (*9*, *10*). All of these methods suffer from low sensitivity and are considered unsuitable for the detection of CAP, because the confirmation of suspect positive samples must be carried out by mass spectrometry (MS) coupled to chromatographic separation such as GC and HPLC, according to Commission Decision 2002/657/EC (*11*). However, the derivatization in GC−MS (*12*) usually causes large variability, sample loss, and a long analytical time. In recent years, efficient interfaces between liquid chromatographs and mass spectrometers have been developed. The determination of CAP in animal-derived foods has been more practical and affordable by coupling LC to mass spectrometry detection, such as electrospray ionization (ESI) mass spectrometry (*13*) and atmospheric pressure photoionization (APPI) mass spectrometry (APPI) (*14*).

Although superior results were obtained from mass spectrometry detection after a reversed-phase chromatographic

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separation, conventional sample treatment procedures (*15*−*17*) for honey, milk, and egg usually involved tedious extraction steps, low extraction yields, or a prolonged elution time (*18*). Most of these methods required large amount of toxic organic solvents before concentration (*19*). Taking milk and egg samples, for example, necessary pretreatment steps include deproteinization with organic acids and further defatting with hexane (*20*). Other techniques, such as matrix solid-phase dispersion (*21*), solid-phase extraction employing a molecularly imprinted polymer (*22*), and the diphasic dialysis membrane procedure (*23*), have also been applied to the extraction and concentration of CAP in biological matrixes. All of these methods, however, still require an extensive cleanup step to meet the requirements of analysis as well as the specificity and sensitivity of the detection system. On the other hand, solid-phase microextraction (SPME) can overcome these disadvantages (*24*). The poly(methacrylic acid−ethylene glycol dimethacrylate) (poly(MAA−EGDMA)) monolithic material is a kind of polymer sorbent that possesses polar carboxylic acid groups in the hydrophobic bone structure and has been demonstrated to be biocompatible in dealing with complicated samples (*25*−*28*). A novel polymer monolith microextraction (PMME) using this monolith, developed by Feng's group, has been coupled to capillary electrophoresis (CE) and HPLC to analyze basic drugs and low aliphatic aldehydes in biological samples (*29*, *30*).

The objective of the present study was to extend the potential use of this novel PMME technique to the determination of CAP in honey, milk, and eggs. After PMME, the eluate could be directly injected into an LC−MS system. The LC−ESI-MS method was also optimized, and lower detection limits have been achieved compared to to those of previous methods (*15*−*23*). The CAP residues in 30 samples were detected and confirmed by this PMME−LC−MS method.

MATERIALS AND METHODS

Reagents and Solutions. EGDMA, methacrylic acid (MAA), 2,2′-azobis(2-methylpropionitrile) (AIBN), dodecanol, and toluene were obtained from Shanghai Chemical (Shanghai, China) and were of analytical reagent grade. CAP and thiamphenicol (THAP) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Thiamphenicol was selected as the internal standard (IS). The CAP and IS stocks were prepared as 50 μg/mL solutions in methanol and stored at 4 °C in the dark. Dilutions for fortification were prepared daily in 20 mM phosphate solution (pH 4.0).

All other reagents and solvents were of analytical-reagent grade and were supplied by Fisher Scientific. Purified water was obtained with a Milli-Q apparatus (Millipore, Bedford, MA).

Preparation of Honey, Milk, and Egg Samples. Honey, pasteurized whole milk, and eggs were purchased from local retail markets. These samples were homogenized and were stored at −20 °C before use. Preliminary analysis showed they were analyte-free.

IS solution (20 μL of 50 ng/mL THAP in 20 mM phosphate solution at pH 4.0) was added to 1.0 g of honey, egg, or 1.0 mL of milk samples, which were spiked with known variable amounts of CAP ranging from 0.1 to 10 ng. These samples were diluted with 20 mM phosphate solution (pH 4.0) to 5 mL for honey and 10 mL for milk and egg samples. After being mixed with a vortex mixer (XW-80A, Qilinbeier Corp., Shanghai, China) for 10 min, the samples were centrifuged for 5 min at 12000 rpm and 10 °C (MIKRO 22R, Hettich Zentrifugen, Germany). Then the supernatant was filtered through a 0.22 μm pore filter prior to PMME extraction. Blank samples were prepared in the same way as above but without the compound-spiking step.

PMME Apparatus and Procedures. The design of the PMME apparatus used in this work was described previously (*29*). The device configuration was composed of an extraction pinhead and a syringe barrel as shown in **Figure 1**. The metallic needle of the pinhead was replaced by a poly(MAA−EGDMA) monolith capillary column (2 cm × 530 μm i.d.).

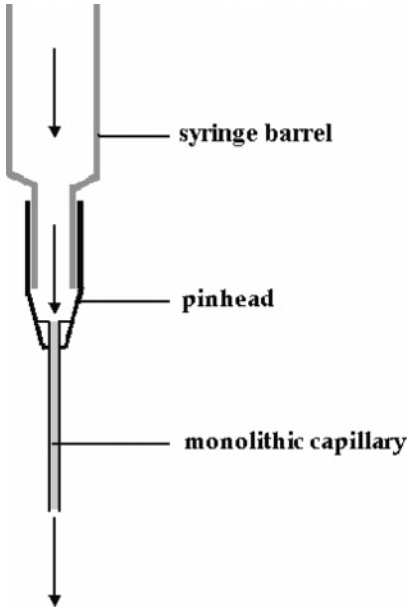

Figure 1. Scheme of the novel PMME device.

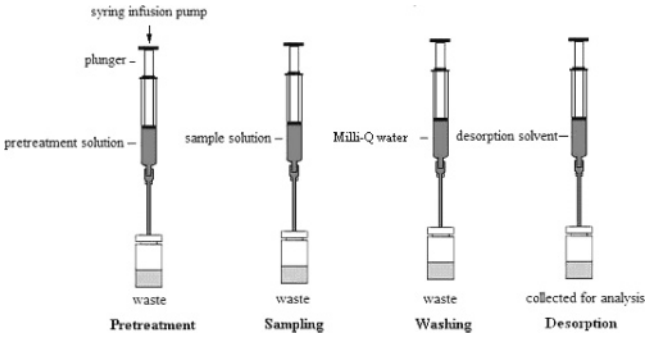

Figure 2. Scheme of the PMME process.

As shown in **Figure 2**, the whole procedure included preconditioning, sorption, washing, and desorption. A syringe infusion pump (CP 2000, Silugao High-Technology Development Corp., Beijing, China) was employed for the delivery of the sample, Milli-Q water (washing solution), and methanol−water (60:40, v/v) (desorption solvent). The extraction capillary column was preconditioned first with 0.2 mL of methanol and then 0.5 mL of phosphate solution at pH 4.0. The flow rates of preconditioning were 0.06 and 0.15 mL/min, respectively. For sorption, 2.0 mL of the sample solution was applied to and passed through the monolithic capillary at 0.15 mL/min, and then 0.2 mL of Milli-Q water was pumped through at 0.15 mL/min to remove the residual matrix in the capillary to reduce interference. Subsequently, the residual water in the pinhead and monolithic capillary was driven out with air using a clean syringe. For the desorption step, 0.05 mL of methanol−water (60:40, v/v) was injected into the monolithic capillary at 0.06 mL/min and the eluate was collected into a microvial followed by dilution with Milli-Q water to 0.1 mL for analysis by HPLC−MS. A 20 μL portion of the sample solution was injected into the LC−MS system.

LC−MS Equipment and Conditions. The LC−MS system consisted of a Waters 1525 pump and a Micromass ZQ4000 single-quadrupole mass spectrometer detector (Waters). Instrument control and data analysis were performed using MassLynx application software (MassLynx 4.0) from Micromass. The antibiotics were separated using a 2.1 mm × 150 mm, 3.5 μm XTerra MS C_{18} column at 45 °C. A binary gradient at a flow rate of 0.2 mL/min was used: mobile phase A was methanol−water (10:90, v/v), and mobile phase B was 100%

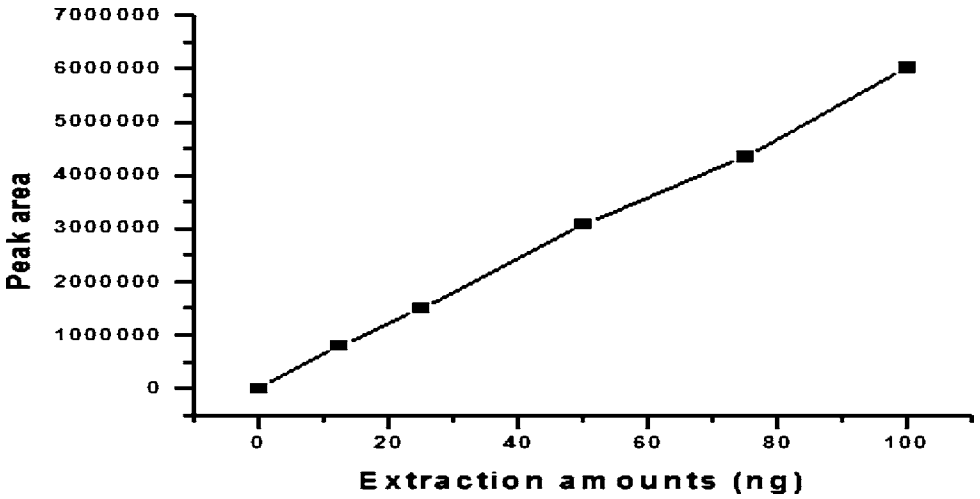

Figure 3. Extracted sample equilibrium profile of CAP for PMME. The sample solution was 20 mM phosphate solution at pH 4.0 spiked with CAP at 25 ng/mL. Operating conditions: extraction flow rate 0.15 mL/min, desorption flow rate 0.06 mL/min. The LC–ESI-MS conditions are outlined in the Materials and Methods. Detection mode: SIR, two channels, *m*/*z* 321, 323.

methanol. The mobile phase gradient profile (where *t* refers to time (min)) was as follows: t_0, 22% B; t_5, 67% B; t_8, 67% B; $t_{8.1}$, 22% B; t_{15}, 22% B. Negative mode electrospray ionization and selected ion recording (SIR) were used. The source parameters of the tune page were capillary voltage 3 kV, cone voltage 25, 30, 35, and 45 eV (depending on the monitored *m*/*z*), extractor voltage 3 V, block temperature 105 °C, desolvation temperature 350 °C, and the nitrogen gas flow rate for desolvation 700 L/h and for the cone 50 L/h. The MS method was an SIR of five channels (*m*/*z* 321, 323, 257, and 152 for CAP, *m*/*z* 354 for THAP (IS)) with different cone voltages for each ion: 25 eV for *m*/*z* 321 and 323, 35 eV for *m*/*z* 354, 35 eV for *m*/*z* 257, and 45 eV for *m*/*z* 152.

The diagnostic fragment ions were obtained by in-source CID of the deprotonated molecular ion $[M - H]^-$ by adjusting the voltage of the skimmer cone. The target compound was identified on the basis of the retention time, presence, and relative abundances of *m*/*z* 321, 323, 257, and 152 (*31*) and quantified by selecting the parent ion (*m*/*z* 321) and the corresponding isotopic ion of CAP (*m*/*z* 323).

RESULTS AND DISCUSSION

Optimization of the PMME Conditions. The parameters affecting the extraction efficiency such as pH, extraction equilibrium profiles, extraction flow rate, desorption flow rate, and volume were investigated. A deprotonated molecular ion (*m*/*z* 321) and its isotopic ion (*m*/*z* 323) were selected as target ions.

After the optimization of pH, the high and constant extraction efficiency of CAP in aqueous solution was achieved in a wide pH range of 2.5−9.0. This can be explained by the fact that the interaction between the analytes and the monolithic capillary was mainly based on the hydrophobic interaction. Finally, pH 4.0 was selected considering the effect of matrixes described in detail in Method Validation.

The extraction equilibrium profile was constructed by increasing the volume of the extracted sample from 0.5 to 4.0 mL (corresponding to 12.5−100 ng of CAP) at the same extraction flow rate. As shown in **Figure 3**, the amount of CAP (presented as the peak area) increased rapidly and the extraction equilibrium was not reached even after 4.0 mL of sample solution was fed. The sharp slopes of the profiles indicated that the monolithic capillary exhibited remarkable extraction capacity for CAP. To achieve sufficient sensitivity within a short time, a sample volume of 2.0 mL was selected for subsequent analysis.

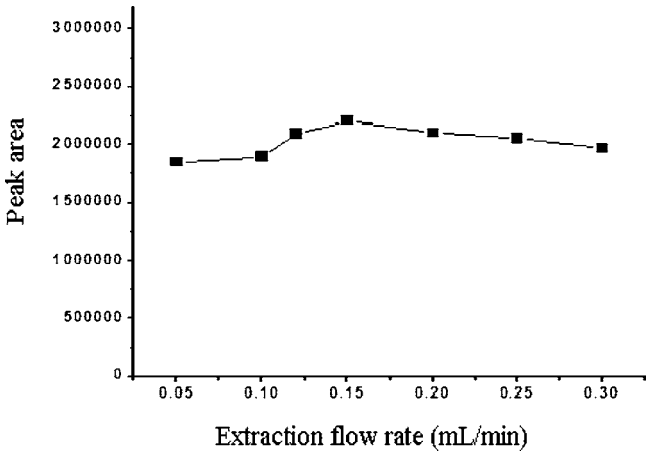

Figure 4. Extraction flow rate profile of CAP for PMME. The sample solution was 20 mM phosphate solution at pH 4.0 spiked with CAP at 25 ng/mL. Operating conditions: desorption flow rate 0.06 mL/min. The LC–ESI-MS conditions are outlined in the Materials and Methods. Detection mode: SIR, two channels, *m*/*z* 321, 323.

The flow rate of the extraction solution was optimized in the range of 0.05−0.3 mL/min by feeding 2.0 mL of sample solution. As shown in **Figure 4**, no significant change in the extraction efficiency was found. Thus, 0.15 mL/min was chosen due to the shorter extraction time and the acceptable back-pressure of the monolith capillary.

Methanol−water (60:40, v/v) was selected as the desorption solvent. The eluates can go directly to LC−MS analysis after dilution with Milli-Q-water. The procedure of desorption was optimized to achieve accurate quantification of the analytes. The result indicated that 0.05 mL of methanol−water (60:40, v/v) can elute completely the extracted CAP from the monolithic capillary. Moreover, we optimized the flow rate of the desorption solution in the range of 0.02−0.1 mL/min, and a flow rate of 0.06 mL/min was used suitably in light of the back-pressure caused by the flow rate.

Under these optimal experimental conditions, the total ion chromatograms (TICs) of CAP and THAP (IS) obtained by PMME−LC−ESI-MS and direct LC−ESI-MS analysis are shown in **Figure 5**. In comparison with the chromatogram of

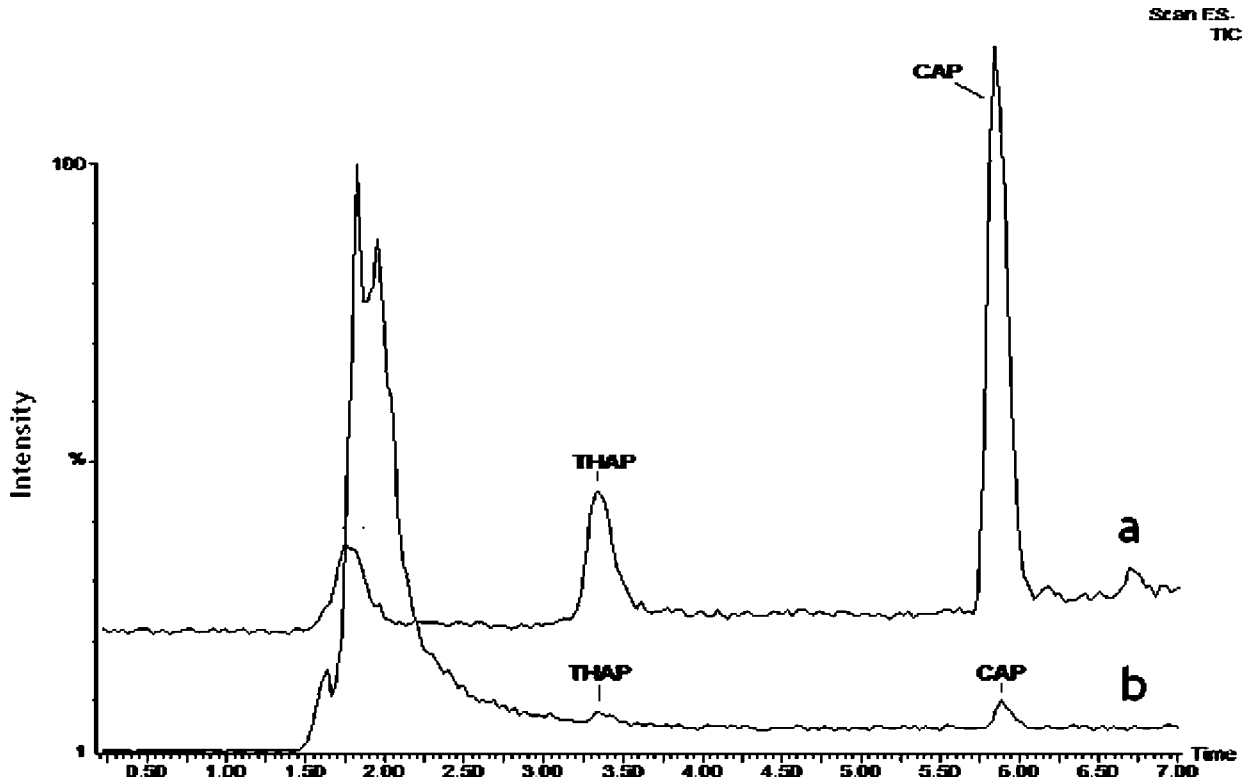

Figure 5. TICs of CAP and THAP standard samples obtained by PMME (**a**) and direct LC–MS analysis (**b**) (detection mode, TIC; scan range, *m*/*z* 100–500; cone voltage, 40 eV). The concentrations of CAP and THAP were 25 and 10 ng/mL, respectively. The PMME conditions and LC–ESI-MS conditions are outlined in the Materials and Methods.

direct injection, a dramatic enhancement of the peak height was observed, indicating the remarkable preconcentration ability of the monolithic capillary.

Optimization of HPLC−MS Conditions. At first, the analytical performance of the optimized LC−ESI-MS was determined by a standard solution of CAP in pure solvents. Deprotonated molecular ions *m*/*z* 321 and 323, corresponding to the characteristic isotopes of the cluster of the two chlorine atoms, were selected as target compound ions because of their high sensitivity and the fact that there was no interference peak in the two channels at a retention time near that of CAP. The separation of CAP from the sample matrix peaks was optimized using methanol, Milli-Q water, 0.1% formic acid, and 10 mM ammonium acetate. The addition of 0.1% formic acid or 10 mM ammonium acetate had no influence on the separation and sensitivity. THAP was used as the internal standard (*32*) considering its extraction by the PMME method in different matrixes and appropriate separation from CAP as well as matrix interference in the analysis. Typical chromatograms and spectra of blank and positive samples are presented in **Figure 6**.

Selection of Diagnostic Ions and Screening Ions. In each chromatographic run, ions *m*/*z* 321 ($[M - H]^-$ for the $^{35}Cl^{35}Cl$ isotope of CAP), 323 ($[M - H]^-$ for the $^{35}Cl^{37}Cl$ isotope of CAP), 257 ($[M - H - (HCOCl)]^-$), and 152 ($[O_2N - C_6H_4 - CHOH]^-$) were monitored. The presence of isotopic ions (*m*/*z* 321, 323) acted as screening ions. The quantification of CAP was based on the response ratio of CAP (*m*/*z* 321, 323)/THAP (*m*/*z* 354) and calculated according to the obtained external regression curve of the spiked samples.

The reproducibility of three ratios used for confirmatory purposes (*m*/*z* 323/321, *m*/*z* 257/321, and *m*/*z* 152/321) was studied in all sample matrixes. All of them presented good variation coefficients (CVs) over three CAP-spiked concentration levels: mean 0.65 for *m*/*z* 321/321 with CV = 5%, mean 0.18 for *m*/*z* 257/321 with CV = 16%, and mean 0.79 for *m*/*z* 152/321 with CV = 11% in the 0.1−10 ng/g CAP-spiked honey, 0.2−10 ng/mL CAP-spiked milk, and 0.2−10 ng/g CAP-spiked egg samples, respectively.

Method Validation. *Elimination of the Sample Matrix Effect in PMME−LC−MS.* The target compounds were adsorbed onto the extraction phase, and the proteins and fats did not affect the extraction efficiency of the target compounds because the poly(MAA−EGDMA) monolith behaved with good biocompatibility (*25*, *27*, *28*). Thus, the present sample preparation required no additional steps to remove the proteins and fats of the milk and egg samples prior to extraction.

Due to the highly viscous property, the honey, milk, and egg samples should be diluted with phosphate solution prior to extraction. The CAP standard and THAP (IS) solutions were added to 1.00 g or 1.0 mL samples, and then the samples were diluted at ratios (weight or volume of the sample:volume of the phosphate solution) of 1:1, 1:3, 1:5, and 1:10. The acceptable pressure of the monolith capillary was achieved when the honey sample was diluted at a ratio of 1:5 and the milk and the egg samples were diluted at a ratio of 1:10. Furthermore, since the PMME method was coupled off-line to LC−ESI-MS, the washing procedure using 0.2 mL of water can expel the residuals such as protein and fat in the monolith and pinhead and eliminate the phosphate solution, which is unsuitable for MS detection.

One of the problems frequently encountered in the extraction of antibiotic contaminants from complicated biological samples is matrix interference. Although the high and consistent extraction efficiency of CAP in aqueous solution was obtained in the pH range of 2.5−9.0, the matrix pH significantly influenced the extraction efficiency of analytes in real samples. As shown in **Figure 7**, for CAP in honey, milk, and egg matrixes, the extraction efficiencies are consistent with those

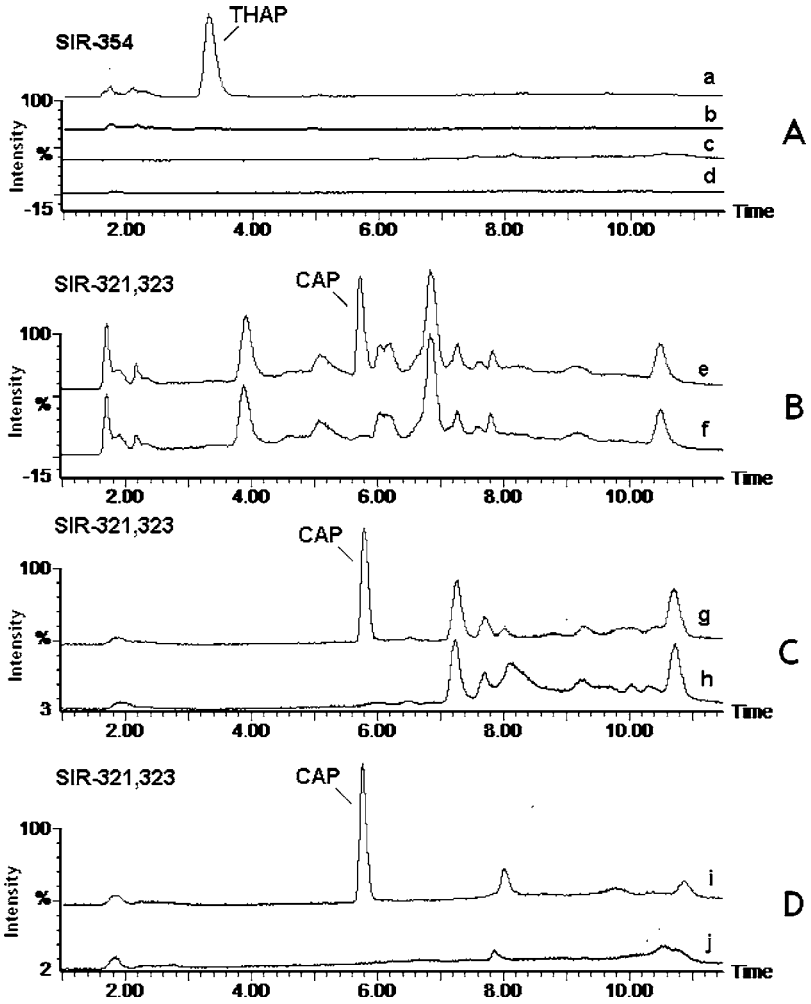

Figure 6. Typical PMME–LC–MS chromatograms for honey, milk, and egg samples spiked with CAP. (**A**) Chromatogram of THAP and a blank sample (SIR, *m*/*e* 354; cone voltage, 35 eV): (**a**) 1.0 ng/mL THAP (IS), (**b**) blank honey sample, (**c**) blank milk sample, (**d**) blank egg sample. (**B**) Chromatogram for a blank honey sample spiked with 0.1 ng/g CAP (SIR, *m*/*e* 321, 323; cone voltage, 25 eV): (**e**) spiked honey sample, (**f**) blank honey sample. (**C**) Chromatogram for a blank milk sample spiked with 0.2 ng/mL CAP (SIR, *m*/*e* 321, 323; cone voltage, 25 eV): (**g**) spiked milk sample, (**h**) blank milk sample. (**D**) Chromatogram for a blank egg sample spiked with 0.2 ng/g CAP (SIR, *m*/*e* 321, 323; cone voltage, 25 eV): (**i**) spiked egg sample, (**j**) blank egg sample. The PMME conditions and LC–ESI-MS conditions are described in the Materials and Methods.

in aqueous solutions in the pH range 2.5−5.0. However, in the pH range 6.0−9.0, the extraction efficiencies of CAP in real samples were lower than those in aqueous samples although no obvious interferences appeared in the chromatograms. Therefore, the phosphate solution at pH 4.0 was used in this study.

Calibration Curves and Detection Limits. The application of the LC−ESI-MS method for the determination of CAP was verified using an internal standard for quantification. The internal calibration in honey, milk, and egg samples was performed by plotting peak area ratios (CAP/IS) versus CAP concentrations. Calibration graphs were obtained for honey samples between 0.1 and 10 ng/g, for milk samples between 0.2 and 10 ng/mL, and for egg samples between 0.2 and 10 ng/g. The SIR mode was employed to achieve suitable sensitivity. The calibration curves were linear in all cases with correlation coefficients of $R^2 > 0.99$. Detection and quantification limits were calculated as the concentration corresponding to a signal 3 and 10 times the standard deviation of the baseline noise, respectively. The detection limits of CAP in honey, milk, and egg samples were 0.02 ng/g, 0.04 ng/mL, and 0.04 ng/g, respectively. The quantification limits were 0.07 ng/g, 0.14 ng/mL, and 0.14 ng/g, respectively. Thus, the developed method is robust for routine analysis of CAP in these complex samples. Quality parameters for each sample are summarized in **Table 1**.

Recoveries, Precisions and Stability. To evaluate the extraction recoveries, the proposed method was applied to the analysis of the spiked CAP-free samples at different concentrations. The spiking levels range from 0.1 to 10 ng/g for honey samples, from 0.2 to 10 ng/mL for milk, and from 0.2 to 10 ng/g for egg samples. The recovery was also calculated by comparing the extracted amounts of CAP from the samples with the total spiking amounts. The recoveries and relative standard deviations (RSDs) are summarized in **Table 2**. Mean recoveries are in the range of 85.2−102.3%.

The reproducibility of the developed method was evaluated by the interday and intraday precisions. As shown in **Table 3**, the data of intraday precisions were based on the analysis of honey, milk, or egg samples spiked at three levels of concentration. Interday precision data were obtained by analysis of the samples extracted on five consecutive days. The intraday precisions of the relative peak areas were below 5.6% and the interday precisions below 8.9%. Both were calculated as RSDs for five measurements.

The monolithic capillary showed high stability since no significant changes in the back-pressure and extraction efficiency

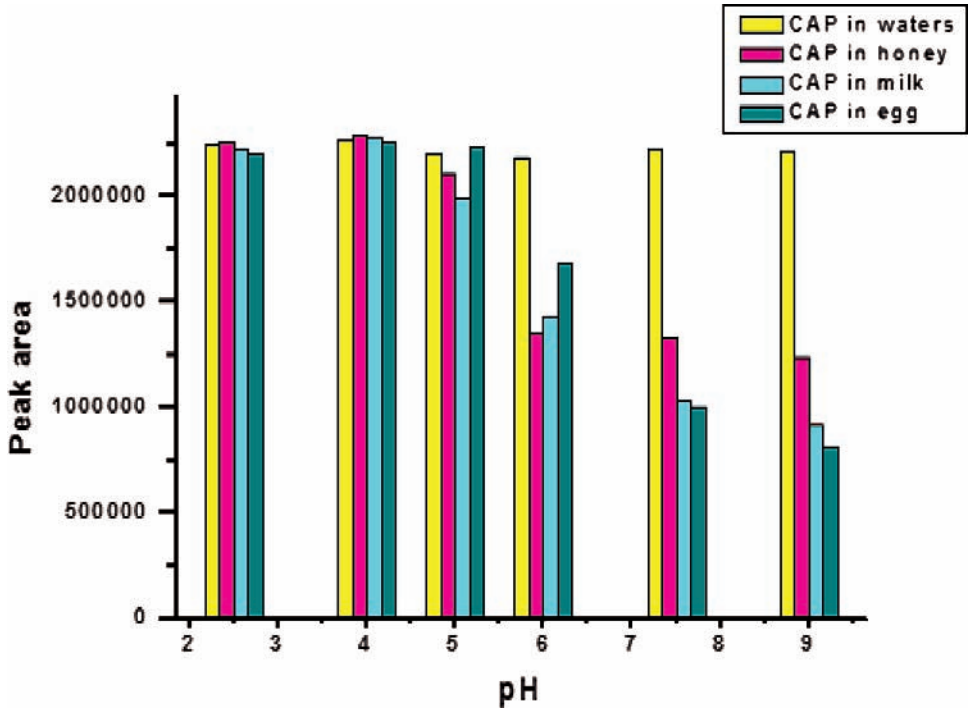

Figure 7. Effect of the sample matrix in PMME and optimization of pH on the extraction efficiency. The sample solution was 20 mM phosphate solution at pH 4.0 spiked with CAP at 25 ng/mL. Operating conditions: extraction flow rate 0.15 mL/min, desorption flow rate 0.06 mL/min. The LC–ESI-MS conditions are outlined in the Materials and Methods. Detection mode: SIR, two channels, *m*/*z* 321, 323.

Table 1. Linear Regression Data for PMME of CAP from Honey, Milk, and Egg Samples[a]

		regression line				
matrix	linear dynamic range (ng/g)	slope	intercept	R^2	LOD (ng/g)	LOQ (ng/g)
honey	0.1–10	10.023	6.325	0.999	0.02	0.07
milk	0.2–10[b]	11.023	5.023	0.998	0.04[b]	0.14[b]
egg	0.2–10	10.664	5.306	0.999	0.04	0.14

[a] The number of data points is six, with three repetitions per point. The PMME and LC–ESI-MS conditions are outlined in the Materials and Methods. [b] The units are nanograms per milliliter.

Table 2. Extraction Recoveries[a] (%) Obtained for PMME[b] of Honey, Milk, and Egg Samples Spiked with CAP

	recovery[a] (%, $n = 3$)		
matrix	low, 0.2 ng/g	medium, 1.0 ng/g	high, 10 ng/g
honey[c]	85.2	95.5	102.3
milk[d]	93.4	100.0	99.5
egg	90.2	89.9	100.2

[a] The percentage of extracted amounts of CAP over the total amounts loaded. [b] The PMME and LC–ESI-MS conditions are outlined in the Materials and Methods. [c] The low concentration of CPA spiked into the honey sample is 0.1 ng/g. [d] The units of the spiked CPA concentration are nanograms per milliliter.

of the capillary column were found in the experiments. The repetition of the extraction performance of monolith capillaries prepared in three batches was also investigated. The interbatch precisions of the relative peak areas were 7.2% for 0.2 ng/mL spiked CAP solutions and 5.8% for 2 ng/mL spiked CAP solutions.

Table 3. Intraday and Interday Precision of Relative Peak Areas at Three Different Concentrations for PMME[a] of CAP from Honey, Milk, and Egg Samples

	intraday precision (%, $n = 5$)			interday precision (%, $n = 5$)		
matrix	low, 0.2 ng/g	medium, 1.0 ng/g	high, 10 ng/g	low, 0.2 ng/g	medium, 1.0 ng/g	high, 10 ng/g
honey[b]	5.6	5.2	3.1	8.9	7.2	7.2
milk[c]	4.6	2.3	2.1	6.4	5.2	4.9
Egg	5.6	4.6	3.2	6.1	3.5	3.7

[a] The PMME and LC–ESI-MS conditions are outlined in the Materials and Methods. [b] The low concentration of CPA spiked into the honey sample is 0.1 ng/g. [c] The units of the spiked CPA concentration are nanograms per milliliter.

Table 4. Repeatability of Real Positive Samples[a]

sample	sample A	sample B	sample C	sample D	sample E	sample F	sample G
mean found concn (ng/g)	2.3	0.2	0.8	0.6[b]	0.9[b]	1.9	0.4
precision (RSD, %)	3.4	5.6	5.7	2.1	1.8	0.9	0.6

[a] Samples A–C are honey samples, samples D and E are milk samples, and samples F and G are egg samples. [b] The units are nanograms per milliliter.

Quantitative Analysis of CAP in Honey, Milk, and Egg from the Market. The PMME−LC−ESI-MS method was applied to the analysis of the CAP in 10 brands of honey samples, 10 brands of milk samples, and 10 eggs from local supermarkets. The screening test showed that about 30% of the honey samples, 20% of the milk samples and 20% of the eggs were positive samples. By further confirmation, they were found to be real positive samples. The contamination concentrations of CAP were in the range of 0.2−2.3 ng/mL (or ng/g), and the

precision of the real positive sample was also investigated. The result is shown in **Table 4**. The RSD ($n = 3$) of the mean concentration of each sample is lower than 7.9%.

Conclusion. PMME using a poly(MAA−EGDMA) monolithic capillary coupled to LC−ESI-MS provides a simple, fast, sensitive, and selective procedure for the identification and determination of CAP residues in complex sample matrixes such as honey, milk, and eggs. The proposed PMME advocated an environmentally friendly, inexpensive, and rapid sample pretreatment technique compared with other CAP pretreatment methods reported previously. The mass spectra provide adequate information for confirmation that is difficult to obtain by other methods.

LITERATURE CITED

(1) Council Directive 96/23/EC of 29 April 1996. *Off. J. Eur. Commun.* **1996**, *L125*, 10.

(2) Commission Decision 2003/181/CE of 13 March 2003. Amending decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin. *Off. J. Eur. Commun.* **2003**, *L71*, 17−18.

(3) Lynas, L.; Currie, D.; Elliott, C. T.; McEvoy, J. D. G.; Hewitt, S. A. Screening for chloramphenicol residues in the tissues and fluids of treated cattle by the four plate test, Charm II radioimmunoassay and Ridascreen CAP-Glucuronid enzyme immunoassay. *Analyst* **1998**, *123*, 2773−2777.

(4) Valerie, G.; Nathalie, C.; Maris, P. Inter-laboratory studies for the evaluation of ELISA kits for the detection of chloramphenicol residues in milk and muscle. *Food Agric. Immunol.* **2004**, *15*, 143−157.

(5) Gallo, P.; Nasi, A.; Vinci, Fl.; Guadagnuolo, G.; Brambilla, G.; Fiori, M.; Serpe, L. Vovk, I.; Simonovska, B. Development and validation of a thin-layer chromatographic method for determination of chloramphenicol residues on pharmaceutical equipment surfaces. *J. AOAC Int.* **2005**, *88*, 1555−1561.

(6) Pengov, A.; Flajs, V. C.; Zadnik, T.; Marinsek, J.; Pogacnik, M. Distribution of chloramphenicol residues in lactating cows following an external application. *Anal. Chim. Acta* **2005**, *529*, 347−351.

(7) Ding, S. Y.; Shen, J. Z.; Zhang, S. X.; Jiang, H. Y.; Sun, Z. W. Determination of chloramphenicol residue in fish and shrimp tissues by gas chromatography with a microcell electron capture detector *J. AOAC Int.* **2005**, *88*, 57−60.

(8) Gallo, P.; Nasi, A.; Vinci, F.; Guadagnuolo, G.; Brambilla, G.; Fiori, M.; Serpe, L. Development of a liquid chromatography/electrospray tandem mass spectrometry method for confirmation of chloramphenicol residues in milk after alfa-1-acid glycoprotein affinity chromatography. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 574−579.

(9) Posyniak, A.; Zmudzki, J.; Niedzielska, J. Evaluation of sample preparation for control of chloramphenicol residues in porcine tissues by enzyme-linked immunosorbent assay and liquid chromatography. *Anal. Chim. Acta* **2003**, *483*, 307−311.

(10) Tyrpenou, A. E.; Rigos, G. G.; Athanassopoulou, F. Determination of chloramphenicol residues in gilthead sea bream (Sparus aurata L.) tissues by HPLC-PDA. *J. Liq. Chromatogr. Relat. Technol.* **2002**, *25*, 655−663.

(11) Commission Decision 2002/657/EC of 12 August 2002. Implementing Council Directive 96/23/EC concerning the performance analytical methods and the interpretation of results. *Off. J. Eur. Commun.* **2002**, *L221*, 8C36.

(12) Santos, L.; Barbosa, J.; Castilho, M. C.; Ramos, F.; Ribeiro, C. A. F.; da Silveira, M. I. N. Determination of chloramphenicol residues in rainbow trouts by gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta* **2005**, *529*, 249−256.

(13) Penney, L.; Smith, A.; Coates, B.; Wijewickreme, A. Determination, of chloramphenicol residues in milk, eggs, and tissues by liquid chromatography/mass spectrometry. *J. AOAC Int.* **2005**, *88*, 645−653.

(14) Takino, M.; Daishima, S.; Nakahara, T. Determination of chloramphenicol residues in fish meats by liquid chromatography-atmospheric pressure photoionization mass spectrometry *J. Chromatogr., A* **2003**, *1011*, 67−75.

(15) Shen, H. Y.; Jiang. H. L. Screening, determination and confirmation of chloramphenicol in seafood, meat and honey using ELISA, HPLC−UVD, GC−ECD, GC−MS−EI−SIM and GC-MS−NCI−SIM methods *Anal. Chim. Acta* **2005**, *535*, 33−41.

(16) Penney, L.; Smith, A.; Coates, B.; Wijewickrem, A. Determination of chloramphenicol residues in milk, eggs, and tissues by liquid chromatography/mass spectrometry. *J. AOAC Int.* **2005**, *88*, 645−653.

(17) Ortelli, D.; Edder, P.; Corvi, C. Analysis of chloramphenicol residues in honey by liquid chromatography-tandem mass spectrometry. *Chromatographia* **2004**, *59*, 61−64.

(18) Humayoun M.; Claude danis;Andre Sauve Gas chromatographic determination of incurred chloramphenicol residues in eggs following optimal extraction *J. Chromatogr., A* **1995**, *696*, 123−130.

(19) Forti, A. F.; Campana, G.; Simonella, A.; Mulatari, M.; Scotichini, G. Determination of chloramphenicol in honey by liquid chloramography-tandm mass spectrometry. *Anal. Chim. Acta* **2005**, *529*, 257−263.

(20) Guy, P. A.; Royer, D.; Mottier, P.; Gremaud, E.; Perisset, A.; Stadler, R. H. Quantitative determination of chloramphenicol in milk powders by isotope dilution liquid chromatography coupled to tandem mass spectrometry *J. Chromatogr., A* **2004**, *1054*, 365−371.

(21) Kubala-Drincic, H.; Bazulic, D.; Sapunar-Postruznik, J.; Grubelic, M.; Stuhne, G. Matrix solid-phase dispersion extraction and gas chromatographic determination of chloramphenicol in muscle tissue *J. Agric. Food Chem.* **2003**, *51* (4), 871−875.

(22) Mena, M. L.; Agüí, L.; Martinez-Ruiz, P.; Yáñez-Sedeño, P.; Reviejo, A. J.; Pingarrón, J. M. Molecularly imprinted polymers for on-line clean up and preconcentration of chloramphenicol prior to its voltammetric determination. *Anal. Bioanal. Chem.* **2003**, *376*, 18−25.

(23) Bayo, J.; Moreno, M. A.; Prieta, J.; Díaz, S.; Suárez, G.; Domínguez, L. Chloramphenicol Extraction From Milk by Using the Dysphasic Dialysis Method Followed by Liquid Chromatographic Determination *J. AOAC Int.* **1994**, *77*, 854−856.

(24) Xie, F. S.; Svec, F.; Frechet, J. M. J. Porous polymer monoliths: Preparation of sorbent materials with high-surface areas and controlled surface chemistry for high-throughput, online, solid-phase extraction of polar organic compounds. *Chem. Mater.* **1998**, *10*, 4072−4078.

(25) Fan, Y.; Feng, Y. Q.; Da, S. L.; Shi, Z. G. Poly (methacrylic acid-ethylene glycol dimethacrylate) monolithic capillary for intube solid-phase microextraction coupled to high performance liquid chromatography and its application to determination of basic drugs in human serum. *Anal. Chim. Acta* **2004**, *523*, 251−258.

(26) Nie, J.; Zhao, Q.; Huang, J. F.; Xiang, B. R.; Feng, Y. Q. Determination of telmisartan in rat tissues by in-tube solid-phase microextraction coupled to high performance liquid chromatography. *J. Sep. Sci.* **2006**, *29*, 650−655.

(27) Huang, J. F.; Lin, B.; Yu, Q. W.; Feng, Y. Q. Determination of fluoroquinolones in eggs using in-tube solid-phase microextraction coupled to high-performance liquid chromatography. *Anal. Bioanal. Chem.* **2006**, *384*, 1228−1235.

(28) Wen, Y.; Zhang, M.; Zhao, Q.; Feng, Y. Q. Monitoring of five sulfonamide antibacterial residues in milk by in-tube solid-phase microextraction coupled to high-performance liquid chromatography. *J. Agric. Food Chem.* **2005**, *53*, 8468−8473.

(29) Zhang, M.; Fang, W.; Zhang, Y. F.; Nie, J.; Feng, Y. Q. Novel polymer monolith microextraction using a poly (methacrylic acid-ethylene glycol dimethacrylate) monolith and its application to simultaneous analysis of several angiotensin II receptor antagonists in human urine by capillary zone electrophoresis. *J. Chromatogr., A* **2006**, *1102*, 294−301.

(30) Zhang, H. J.; Huang, J. F.; Wang, H.; Feng, Y. Q. Determination of low-aliphatic aldehyde derivatizatives in human saliva using polymer monolith microextraction coupled to high -performance liquid chromatography. *Anal. Chim. Acta* **2006**, *565*, 129−135.

(31) Ramos, M.; Munoz, P.; Aranda, A.; Rodriguez, I.; Diaz, R.; Blanca, J. Determination of chloramphenicol residues in shrimps by liquid chromatography-mass spectrometry. *J. Chromatogr., B* **2003**, *791*, 31−38.

(32) Gikas, E.; Kormali, P.; Tsipi, D.; Tsarbopoulos, A. Development of a Rapid and Sensitive SPE-LC-ESI MS/MS Method for the Determination of Chloramphenicol in Seafood. *J. Agric. Food Chem.* **2004**, *52*, 1025−1030.

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