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Determination of β-lactam antibiotics in milk based on magnetic molecularly imprinted polymer extraction coupled with liquid chromatography–tandem mass spectrometry

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ABSTRACT

In this work, a rapid and selective method was successfully developed using the magnetic molecularly imprinted polymer (MMIP) as sorbent for the extraction of β-lactam antibiotics (BLAs) from milk samples. The MMIP has been prepared using penicillin V potassium (PENV) as template molecule, methacrylic acid as functional monomer, ethylene glycol dimethacrylate as crosslinking agent and Fe_3O_4 magnetite as magnetic component. The experimental results showed that the MMIP had high affinity and selectivity toward PENV and other structurally related BLAs. The extraction process was carried out in a single step by mixing the extraction solvent, MMIPs and milk samples under ultrasonic action. When the extraction was completed, the MMIPs adsorbing the analytes were separated from the sample matrix by an external magnet. The analytes eluted from the MMIP were analyzed by liquid chromatography–tandem mass spectrometry. For achieving optimal preconcentration and reducing non-specific interactions, various parameters affecting the extraction efficiency such as extraction mode, extraction solvent, the amount of MMIPs, extraction time, washing solution and eluting solution were comprehensively evaluated. Under the optimal conditions, the detection limits of BLAs are in the range of 1.6–2.8 ng mL^{-1}. The relative standard deviations of intra- and inter-day ranging from 3.2% to 8.3% and from 3.6% to 9.8% are obtained, respectively. The method was applied to determine BLAs including PENV, amoxicillin and oxacillin in five milk samples from different provenances. The recoveries of BLAs in these samples from 71.6% to 90.7% are obtained.

1. Introduction

Nowadays the widespread use of antibiotics in dairy cattle for the purpose of therapy has caused significant food safety issues since antibiotic resistance can be transferred to man by the ingestion of affected milk products [1]. β-Lactam antibiotics (BLAs) are used in the treatment of lactating dairy cattle for several contagious diseases [2]. BLAs are one of the most important groups of antibiotics and have been the most widely used as antimicrobial drugs for more than 80 years [3]. However, the BLAs residues which remain in milk can provoke allergic reactions in some hypersensitive individuals [2]. In recent years, BLAs are receiving increasing attention as injurants. In view of this situation, a rapid, selective and simple method for the analysis of the BLAs in milk to assure this natural product does not contain the antibiotics residues is required.

Various techniques have been used for the determination of BLAs including spectrophotometry [4], capillary electrophoresis [5] and liquid chromatography (LC) with ultraviolet detection [3,6], tandem mass spectrometry [7,8] or chemiluminescence detection [9]. Different sample matrixes including pharmaceutical dosage forms [4], environmental aqueous samples [5], wastewater [3], human plasma [6], bovine kidney [7], foodstuffs of animal [8] and blood serum [9], contaminated with BLAs can be analyzed by the methods. Among the methods, liquid chromatography–tandem mass spectrometry (LC–MS/MS) is the preferred choice, because it can provide more selective separation and lower limit of detection for the target analytes.

Sample preparation is a crucial and important step in the whole analytical procedure. The preparation procedure usually includes two necessary steps. One is the extraction procedure releasing the BLAs from sample matrix to extraction solvent, which could provided satisfactory recoveries for the analytes, the other is the clean-up one, which could removed some co-extracted compounds in order to reduce the interference of the following step [2]. In sample preparation procedures, the food samples are usually

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extracted with solvent under vortexing [2] or shaking [10] and then centrifugation. These methods may result in lower extraction yield, relatively complex sample preparation, and often need reextraction. Some technologies such as solid phase extraction (SPE) [3,11,12], liquid solvent extraction [13] or liquid membrane extraction [14] have been applied in the food analysis. However, these classic clean-up methods might retain not only the targeted analytes but also some impurities which would interfere with the detection of the targeted analytes. If the extraction and clean-up could be combined in one step, the sample pretreatment would become simpler.

Molecularly imprinted polymers (MIPs) are synthetic crosslinked polymers which have highly specific recognition ability for target molecule or similar compounds [15–22]. MIPs are produced by growing a polymer around a molecule which is used as the template molecule [23,24]. After polymerization, the template molecule can be removed from the polymers, leaving cavities which can selectively rebind template molecule on size, shape and functionality [22]. In recent years, MIPs have attracted much attention due to their outstanding advantages, such as predetermined recognition ability, stability, relative ease, low cost of preparation and potential application to a wide range of target molecules [25]. The resulting MIPs can be used in a wide range of applications, such as chiral separation [26], chemical sensing [27], competitive drug assays [28] and chemical catalysis [29]. Among these applications, there is a special and interesting one which is the SPE method using MIPs as the sorbent. The MIPs have been prepared and applied in selective recognition and enrichment of β-lactam antibiotics [30–32], and they exhibited high recognition selectivity.

In recent years, the magnetic adsorbent for SPE has received increasing attention. Compared with conventional SPE method, the application of magnetic adsorbents in analysis not only has the advantages of conventional SPE, but also provides a relatively rapid and convenient way for the removal of magnetic adsorbents from complex sample matrix by applying an external magnetic field. Also, if some magnetic components are encapsulated into MIPs, the resulting composite polymer, magnetic MIPs (MMIPs) will have not only magnetically susceptible characteristic but also selectivity for the guest molecule [26,33]. This technology makes separation faster and easier, because the magnetic polymer adsorbing analytes can be collected by an external magnetic field without centrifugation or filtration. The preparation of MMIPs has been reported [22,34–38]. But the applications of the MMIPs in the analyzing complex sample were scarce [22,34]. To the best of our knowledge, the MMIPs technique for the extraction of BLAs in milk has not been reported yet.

In this work, the MMIP which was synthesized using PENV as template molecule was used as sorbent for the extraction of BLAs from milk samples and then followed by LC–MS/MS analysis. The technique of one-step extraction and clean-up was performed under the action of ultrasound. When the extraction was completed, the MMIPs adsorbing target analytes were separated from the sample matrix by an external magnet. Compared with conventional method, the application of MMIP in analytical technology can provide a relatively rapid and easy way for separation and clean-up. It could simplify the sample pretreatment procedure and avoided the time consuming use of column. Meanwhile, the high extraction recovery and the high sensitivity of this method showed great potential for the daily analysis work.

2. Experimental

2.1. Materials

2.1.1. Reagents and chemicals

The standards of penicillin V potassium (PENV), amoxicillin (AMOX) and oxacillin (OXA) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Their chemical structures are shown in Fig. 1. Chromatographic pure methanol was obtained from Fisher (Pittsburgh, PA, USA). Ethylene glycol dimethacrylate (EGDMA) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Methacrylic acid (MAA), iron (II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), oleic acid, polyvinylpyrrolidone (PVP), azobisisbutyronitrile (AIBN) and formic acid were obtained from Guangfu Fine Chemical Research Institute (Tianjin, China). Methanol, ethanol and acetic acid were purchased from Beijing Chemical (Beijing, China). High purity water was obtained from a Milli-Q water system (Millipore, Billerica, MA, USA).

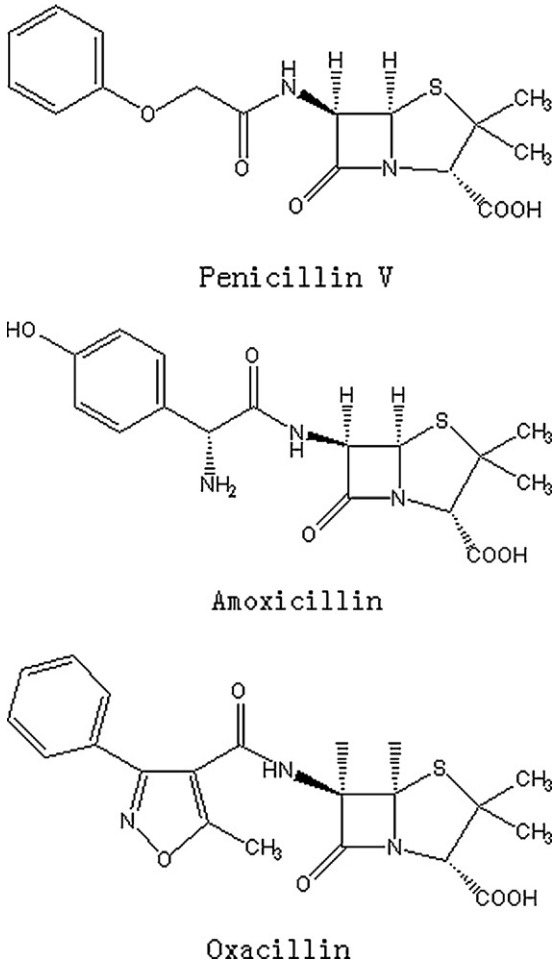

Fig. 1. Chemical structures of the penicillin V, amoxicillin and oxacillin.

Stock solutions of the standards (1 mg mL^{-1}) were prepared by dissolving each BLAs in high purity water. They were stored in a refrigerator at −18 °C and found to be stable for a week. Work standard solutions were freshly prepared by diluting the stock solutions with water.

Milk samples from different provenances were randomly purchased from the local market (Changchun, China). One sample was checked to be free of any of the targeted antibiotics and it was used as blank milk for calibration and validation purposes. The spiked milk samples were obtained by adding certain amounts of BLAs stock solution to the milk samples. Due to the instability of analytes, storage of milk samples could be stably stored for 6 days at 4 °C.

2.1.2. Apparatus

The MMIPs were characterized by the scanning electron microscopy (SEM; JEM-6700F, JEOL, Tokyo, Japan) and a vibrating

sample magnetometry (VSM; JDM-13, Jilin University, Changchun, China). Chromatographic analysis was performed on an Agilent 1100 liquid chromatograph (Palo Alto, CA, USA) which was equipped with a quaternary pump, a heated column compartment, a UV detector and a LC workstation. A Q-Trap mass spectrometer (Applied Biosystems/MDS Sciex, Concord, Canada) equipped with an electro-spray ionization (ESI) source was used.

2.2. Methods

2.2.1. Preparation of Fe_3O_4 magnetite

The Fe_3O_4 magnetite was synthesized by the coprecipitation method: 0.01 mol $FeCl_2{\cdot}4H_2O$ and 0.02 mol $FeCl_3{\cdot}6H_2O$ were dissolved in 100 mL of water in a three-necked reactor (250 mL). The mixture was stirred equably and purged with nitrogen gas. When the temperature increased to 80 °C, 40 mL sodium hydroxide solution (2.0 mol L^{-1}) was added into it. This reaction lasted 1 h and remained at 80 °C. When the temperature dropped to the room temperature, the magnetic precipitates obtained were isolated from the solution by an external magnetic field and washed with deionized water several times until it was neutral.

2.2.2. Preparation of MMIPs

The MMIPs were synthesized as follows: the PENV (1.0 mmol) dissolved in 10 mL water:ethanol (9:1, v/v) and 8.0 mmol MAA was stirred for 30 min for the preparation of the pre-assembly solution. The Fe_3O_4 magnetite (1.0 g) was mixed with 1.0 mL oleic acid and stirred for 10 min. Then 20 mmol EGDMA and the pre-assembly solution were added into the mixture of Fe_3O_4 and oleic acid. This mixture was subjected to ultrasound for 30 min for preparation of the prepolymerization solution. After that the PVP (0.4 g) was dissolved in 100 mL ethanol in a three-necked round-bottomed flask. The mixture was stirred at 300 rpm and purged with nitrogen gas while the temperature increased to 60 °C. The prepolymerization solution was added into the three-necked flask, and then 0.1 g AIBN was also added into it. The reaction was allowed to proceed at 60 °C for 24 h. After the polymerization, the polymers were separated by the external magnetic and washed with methanol:acetic acid (8:2, v/v) for several times, then washed with methanol until the template molecule could not be detected by LC–MS/MS. Then the polymers were washed with water three times again and dried at 60 °C. During the whole progress, PENV would not be decomposed. The magnetic nonimprinted polymers (MNIPs) were prepared and processed similarly as above, except that the template molecule PENV was not added.

2.2.3. Binding experiment

The binding experiment was carried out by adding 20.0 mg MMIPs or MNIPs in a glass tube containing 2.0 mL of PENV standard solution which was prepared in water varied in the concentrations of 0.1 to 2.0 mmol L^{-1}. The solution was incubated for 24 h at room temperature, and then the suspension was separated and analyzed by LC–MS/MS. PENV standard solution would not be decomposed during 24 h at room temperature. The amount of PENV bound on the polymers was obtained by subtracting the free concentration from initial concentration of PENV added to the mixture.

2.2.4. Extraction procedure

An amount of 100 mg MMIPs were put into a conical flask and conditioned in sequence with 3.0 mL methanol and 3.0 mL water. The supernatant was separated from the polymers with a magnet and discarded. Then 2.0 mL milk sample was added into the conical flask, and 18.0 mL hydrochloric acid aqueous solution (pH = 5) as the extraction solution was also added. The mixed solution was subjected to ultrasound for 5 min. After the extraction was completed, the MMIPs captured BLAs were separated rapidly from the solution under a strong external magnet. After discarding the supernatant solution, the MMIPs were washed with 3.0 mL water in order to reduce or eliminate the co-extracted impurities. Then the BLAs were eluted from the MMIPs with 3× 1.0 mL of methanol solution which contained 5.0% acetic acid (1.0 mL every time and eluted three times). To improve the recoveries, the MMIPs captured BLAs were subjected to ultrasound for 30 s during each elution process. The eluate was combined and evaporated to dryness under nitrogen gas at 40 °C, and the residue was reconstituted with 1.0 mL of 0.1% formic acid methanol solution and filtered through a 0.45 μm membrane for further LC–MS/MS analysis. All samples were prepared in triplicate.

2.2.5. LC–MS/MS analysis

The BLAs were separated by a Symmetry C_{18} column (150 mm × 4.6 mm I.D., 5 μm, Waters, Milford, MA, USA). The mobile phase was the mixture of 0.1% formic acid aqueous solution and methanol (40:60, v/v). The flow rate of the mobile phase was maintained at 1.0 mL min^{-1}. The eluate was split and introduced into MS detector at the flow rate of 0.2 mL min^{-1}. The column temperature was kept at 30 °C and the injection volume was 20 μL.

The ESI-MS/MS detection was performed in the positive mode and the source dependent parameters were as follows: curtain gas, N_2 (50 psi); collision gas, N_2 (medium); gas 1, N_2 (45 psi); gas 2, N_2 (55 psi); ion spray voltage, 5000 V; temperature, 480 °C.

The data acquisition was carried out in the multiple reaction monitoring (MRM) mode which records the transitions between the precursor ion and the two most abundant product ions for each target analyte. MRM transitions as well as the corresponding declustering potential (DP), entrance potential (EP), collision cell entrance potential (CEP), collision energy (CE) and collision cell exit potential (CXP) are chosen at their suitable values. The transitions were recorded in one single retention time window with a dwell time of 100 ms. Data processing was carried out on the Applied Biosystems Analyst software (Version 1.4.1).

3. Results and discussion

3.1. Binding study

The binding isotherms plotted in Fig. 2a indicated that the amount of PENV bound to the MMIPs and MNIPs at binding equilibrium increased with the increasing of initial concentration of PENV. However, the amount of PENV bound to the MMIPs was higher than that bound to the MNIPs.

The Scatchard analysis was also used for evaluation of the absorption of MMIPs and MNIPs according to the equation:

$$\frac{Q}{[\mathrm{PENV}]} = \frac{Q_{\max} - Q}{K_d}$$

where Q is the amount of PENV bound to the polymers at equilibrium; [PENV] is the free PENV concentration at equilibrium; K_d is the dissociation constant and Q_{max} is the apparent maximum binding amount. The values of K_d and the Q_{max} can be calculated from the slope and intercept of the linear line plotted in Q/[PENV] versus Q.

As can be seen from Fig. 2b, the Scatchard plot for MMIPs was not a single linear curve, but consisted of two linear parts with different slopes. The linear regression equation for the left part of the curve was $Q/[\mathrm{PENV}] = -4.02Q + 0.2842$. The K_d and Q_{max} were calculated to be 248.8 μmol L^{-1} and 70.71 μmol g^{-1} of dry polymer, respectively. The linear regression equation for the right part of this curve was $Q/[\mathrm{PENV}] = -0.8922Q + 0.1248$. The K_d and Q_{max} were calculated to be 1120.8 μmol L^{-1} and 139.88 μmol g^{-1} of dry polymer, respectively. The binding of PENV to the MNIPs was also analyzed by Scatchard method (Fig. 2c). It revealed homoge-

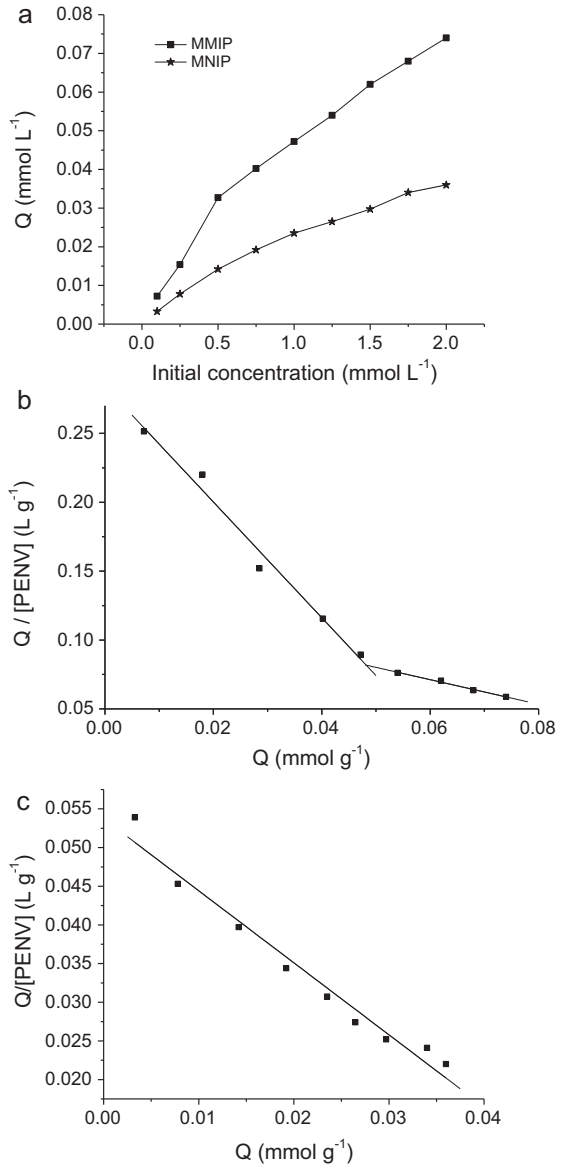

Fig. 2. Binding isotherms (a) and Scatchard plot analysis of the binding of BLAs onto the MMIPs (b) and MNIPs (c).

neous binding sites with K_d and Q_{max} values of 1073.9 μmol L^{-1} and 57.7 μmol g^{-1}, respectively. This demonstrates that the effect which MMIPs have on template molecule is not identical, and it can be divided into two ways. The possible reason is that there are two kinds of interactions between functional monomer and template molecule. It can form two different composites which have different forms. The composites can enter the holes during the different stages of polymerization. These holes have the function of memory for PENV. However, NMIPs do not have this kind of characteristic, so they have weak ability of absorption.

3.2. Selection of the extraction conditions

There were various parameters affecting the performance of the extraction including extraction mode, extraction solvent, MMIPs amount, extraction time, washing solution and elution solution. These conditions were investigated in the work carefully. The extraction conditions were optimized by analyzing spiked milk samples (100 ng mL^{-1}). When one parameter was changed, the other parameters were fixed at their optimized values.

a. *Extraction mode*. The extraction mode used for extracting BLAs from milk and then rebinding to the MMIPs was firstly tested. The modes of stirring and ultrasound were both examined under the same conditions. The higher recoveries ranging from 78.5% to 89.9% were obtained when the extraction process was performed under the action of ultrasound. But when the stirring was used for the extraction, the recoveries were decreased to 40.2–67.1%. So the ultrasound was selected as a necessary condition for completing the extraction process effectively.

b. *Extraction solution*. A series of extraction solution used for rebinding BLAs to MMIPs were investigated in this step, which included hydrochloric acid aqueous solution of different pH values ranging from 2 to 10, and 5%, 10%, 20% and 50% ACN or methanol aqueous solution, ACN and methanol. Eighteen milliliters of these solutions were tested for rebinding BLAs. The hydrochloric acid solution of pH = 5 which had the most satisfactory recoveries was chosen and the recoveries of the three kinds of BLAs ranged from 76.3% to 90.4%. A sharp decrease in the recovery was observed when the pH values were higher than 7. This is because of the deprotonation of the acidic functional groups of the polymer in the neutral or basic solution, which interferes with the formation of hydrogen bonds between the functional groups of the polymers and the template molecule, and then decreases the rebinding of template molecule [39]. With the increase in the proportion of ACN or methanol in water, there was a gradual decline in recovery. It is because the organic solvent such as ACN or methanol would decrease the hydrophobic interaction in the rebinding process [40]. In this work, the hydrochloric acid aqueous solution of pH = 5 was the most appropriate choice.

c. *The amount of MMIPs*. Different amounts of MMIPs ranging from 20 to 150 mg were evaluated to extract the analytes from 2 mL milk sample diluted with 18 mL the hydrochloric acid solution of pH = 5. The recoveries increased with the increasing of the amount of the polymers from 20 to 100 mg. When the amount was more than 100 mg, it gave no obvious improvement for recovery. The results showed that 100 mg polymers were well enough in the extract process and satisfactory recoveries ranged from 74.5% to 91.8% were obtained.

d. *Extraction time*. The effects of the extraction time from 1 to 10 min on the recoveries were examined respectively. When the extraction time is in the range of 1–5 min, the recoveries of BLAs increased from 25.6–38.5% to 72.0–92.4% with the increase of the extraction time. But the recoveries had no obvious change after 5 min. So the extraction time of 5 min was chosen in the work.

e. *Washing solution*. Because the milk samples contain complex matrix components, the step of washing which could remove the remained potentially interfering compounds from sample matrices without eluting out the target analytes became important. In the washing progress, 3.0 mL water, methanol, ACN, 10% or 50% methanol aqueous solution, and 10% or 50% ACN aqueous solution were evaluated as washing solution. The satisfying recoveries of the BLAs (76.2–90.3%) were obtained using 3.0 mL water as washing solution.

f. *Elution solution*. In order to obtain satisfactory recoveries of BLAs, a series of elution solutions, methanol, ACN, methanol–acetic acid (95:5, v/v), ACN–acetic acid (95:5, v/v) were evaluated. The satisfactory recoveries (81.3–90.1%) were obtained when using 3.0 mL (1.0 mL every time and eluted three times) methanol–acetic acid (95:5, v/v) as eluting solution. In order to improve the recoveries, the MMIPs captured BLAs were subjected to ultrasound for 30 s during each elution process.

3.3. Qualitative and quantitative analysis

The structural information of the BLAs was also achieved from their enhanced product ion (EPI) full-scan mass spectra. The only molecular-ion species formed in the acidic mobile phase are protonated molecules. Under the given conditions, no sodium or potassium adducts were observed. Thus, the protonated molecular ion $[M+H]^+$ was selected as precursor ion. Fragmentation of penicillins (PENV, AMOX and OXA) was the result of the opening and cleavage of the β-lactam ring. Therefore, fragmentation of penicillins produced the class-specific product ion, $[C_6H_9NO_2S+H]^+$ at *m*/*z* 160 representative of penicillin compounds. Due to the loss of HCOOH from the product ion $[C_6H_9NO_2S+H]^+$ at *m*/*z* 160, fragmentation of PENV produced the production at *m*/*z* 114. AMOX, OXA and PENV exhibited the product ions (*m*/*z* 243.1, 208.0, or 176.1) corresponding to the loss of $C_6H_9NO_2S$ from the precursor ion [41].

Two characteristic MRM transitions were chosen for each compound. The transition ions for PENV were 335.1/160.1 and 335.1/176.1. The transition ions for OXA were 402.1/243.1 and 402.1/160.1. The transition ions for AMXO were 366.1/208.0 and 366.1/114.1. The product ions used for quantification were 335.1/160.1, 402.1/160.1 and 366.1/114.1, respectively. LC–MS/MS extracted ion chromatograms obtained by the analysis of spiked milk sample (10 ng mL^{-1}) which were determined by MRM are illustrated in Fig. 3.

3.4. Matrix effect

The calibration curves were constructed in aqueous solvent and in blank milk extract at a concentration range of 10–1000 ng mL^{-1}. The calibration curves were built by plotting the areas against the concentrations of the analytes. The calibration curves constructed in aqueous solvent and the matrix-matched standard calibration curves constructed in blank milk extract were built at the same time for evaluation the matrix effect. The concentrations of the eluate were obtained according to the matrix-matched standard calibration curves and this could avoid matrix effects effectively. The two kinds of calibration curves are compared to determine if the ionization of BLAs at the MS source was enhanced or suppressed by the matrix. The results showed that the slopes of calibration curves of AMOX and PENV obtained for matrix-matched standard were lower than the aqueous solvent standard, which indicated the signal suppression of the two kinds of BLAs. However, the OXA had the opposite result that indicated the matrix had signal enhancement for the OXA. The values of the suppression of AMOX and PENV were −21.6% and −16.0% and the value of the enhancement of OXA was 13.4%.

3.5. Linearity and limit of detection

The method validation can be done according to the European Commission Decision 2002/657/EC. The correlation coefficients (*r*) ranging from 0.9997 to 0.9999 are obtained for the analytes in the concentration range of 10–1000 ng mL^{-1}. Limit of detection (LOD) is considered as the analyte minimum concentrations that can be confidently identified by the method. The conventional measurement of the signal-to-noise ratio was adopted in the work. It allowed the use of peak area rather than peak height because the peak area is the measure which is more relevant to sample quantization. The way to satisfy the analyst that the system can measure low concentration samples is to put a limit on the signal-to-noise ratio as a measure of system performance independent of the instrument. According to this method, the LODs estimated based on the analytes concentration producing signal/noise ratio of 3:1 are 1.6, 2.8 and 1.9 ng/mL for AMOX, PENV and OXA, respectively.

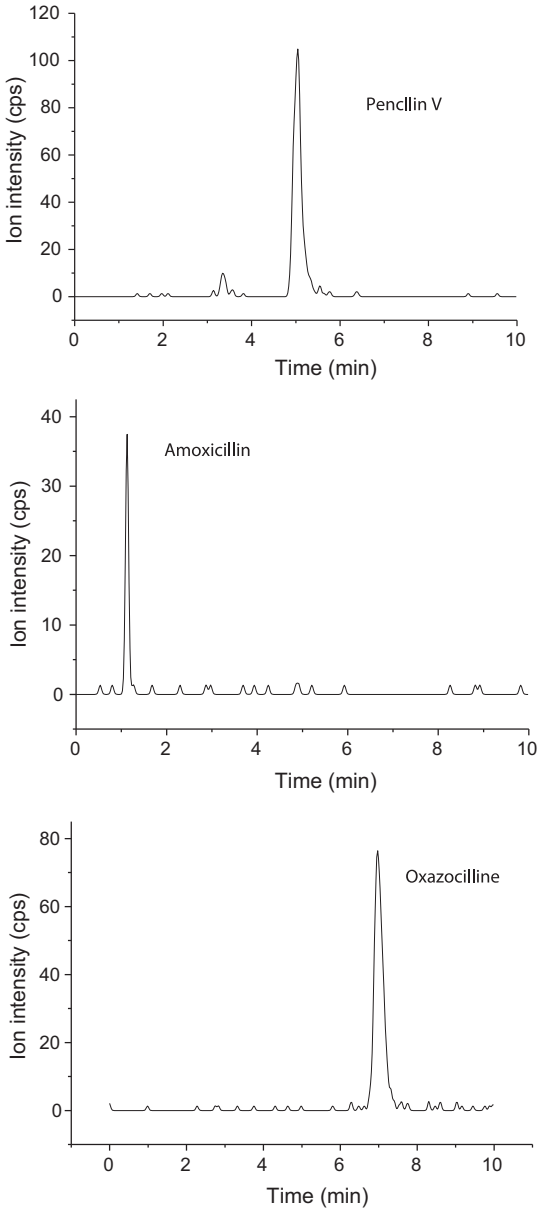

Fig. 3. Liquid chromatography–tandem mass spectrometry extracted ion chromatograms obtained by the analysis of spiked milk sample (10 ng mL^{-1}). Extraction conditions: fortified concentration, 2.0 mL spiked milk with BLAs (100 ng mL^{-1}); extraction solution, 18 mL hydrochloric acid aqueous solution of pH = 5; MMIP amount, 100 mg; extraction mode, ultrasound; extraction time, 5 min; washing solution, 3.0 mL water; elution condition, 3× 1.0 mL methanol–acetic acid (95:5, v/v) with ultrasound for 30 s.

3.6. Precision and recovery

Precision was evaluated by measuring intra- and inter-day relative standard deviations (RSDs) in the work. The intra-day precision was carried out by analyzing milk sample six times in one day at three different fortified concentrations of 10, 50 and 250 ng mL^{-1}. The inter-day precision was carried out over six days by analyzing milk samples at three different fortified concentrations of 10, 50 and 250 ng mL^{-1}.

After the extraction, washing and elution, the obtained 3.0 mL eluate was evaporated to dryness under nitrogen gas at 40 °C, and the residue was reconstituted with 1.0 mL methanol solution and filtered through a 0.45 μm membrane for further LC–MS/MS analysis. According to the matrix-matched standard calibration curves,

Table 1
The intra- and inter-day precisions and recoveries of the assay ($n = 6$).

Analytes	Intra-day precision						Inter-day precision					
	10 ng L^{-1}		50 ng L^{-1}		250 ng L^{-1}		10 ng L^{-1}		50 ng L^{-1}		250 ng L^{-1}	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
AMOX	70.3	4.5	77.7	3.8	87.7	3.2	71.1	4.3	78.9	3.6	88.9	4.8
OXA	72.3	5.7	76.7	6.2	90.1	6.7	72.0	8.8	79.9	8.6	91.2	9.8
PENV	76.5	7.6	78.6	7.2	84.6	8.3	75.9	7.8	78.8	7.4	82.1	7.2

Table 2
Recoveries of BLAs in five milk samples (%) ($n = 3$).

Samples		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
AMOX	10	71.6	72.3	72.6	73.2	72.3
(ng/mL)	50	77.5	75.9	76.5	76.3	77.4
	250	86.3	88.6	86.2	88.2	87.3
OXA	10	72.1	73.5	72.2	73.2	72.1
(ng/mL)	50	75.0	76.3	75.9	76.3	77.9
	250	89.9	88.3	90.2	89.1	90.7
PENV	10	74.9	75.0	75.2	76.0	74.3
(ng/mL)	50	77.0	76.9	75.8	75.7	76.2
	250	85.2	85.9	87.0	86.2	85.9

the concentrations of the recovered BLAs can be got and then in order to get the recovery values, they were compared with the fortified concentrations. The results are shown in Table 1. The RSDs of intra- and inter-day ranging from 3.2 to 8.3% and from 3.6 to 9.8% were obtained. In the three fortified levels, recoveries of the three kinds of BLAs are in the range of 70.3–91.2%.

3.7. Application of the method

In order to demonstrate the feasibility of the proposed method, five milk samples from different provenances collected from different market located in Changchun (China) were analyzed. No BLAs residues at detectable levels were found in these samples. The recovery study was then carried out by spiking the five milk samples with BLAs standards at the level of 10, 50 and 250 ng mL^{-1}. The recoveries of BLAs were obtained in Table 2.

4. Conclusions

During this work, MMIPs which have strong magnetic responsiveness and selective character were prepared and applied for the extraction of BLAs from milk samples successfully, followed by LC–MS/MS. The MMIPs adsorbing the analytes can be collected by an external magnetic field easily. The proposed magnetic method possesses a lot of advantages which included that it avoided the time consuming column passing and filtration operation compared with traditional SPE. So it can be considered that this method is promising and may be a good alternative to the traditional techniques.

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