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# Fast and Selective Extraction of Sulfonamides from Honey Based on Magnetic Molecularly Imprinted Polymer

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A fast and selective method was developed for the determination of sulfonamides (SAs) in honey based on magnetic molecularly imprinted polymer. The extraction was carried out by blending and stirring the sample, extraction solvent and polymers. When the extraction was complete, the polymers, along with the captured analytes, were easily separated from the sample matrix by an adscititious magnet. The analytes eluted from the polymers were determined by liquid chromatography−tandem mass spectrometry. Under the optimal conditions, the detection limits of SAs are in the range of 1.5−4.3 ng $g^{-1}$. The relative standard deviations of intra- and interday ranging from 3.7% to 7.9% and from 4.3% to 9.9% are obtained, respectively. The proposed method was successfully applied to determine SAs including sulfadiazine, sulfamerazine, sulfamethoxydiazine, sulfamonomethoxine, sulfadimethoxine, sulfamethoxazole and sulfaquinoxaline in different honey samples. The recoveries of SAs in these samples from 67.1% to 93.6% were obtained.

**KEYWORDS: Magnetic molecularly imprinted polymer; sulfonamides; honey; liquid chromatography−tandem mass spectrometry**

## INTRODUCTION

Sulfonamides (SAs) are broad-spectrum synthetic antibiotics that competitively inhibit conversion of *p*-aminobenzoic acid to dihydropteroate (*1*). They are used in an attempt to treat the European and American foulbrood diseases in bee-keeping (*2*). However, such drugs can produce adverse reactions, which may lead to disorder of the hemopoietic system and potential carcinogenic effects (*3*). Previous studies indicated that these compounds have been detected in several honey samples in some countries (*1*, *4*). Development of a fast method for the analysis of SAs in honey is very important to ensure that this natural product does not contain SA residues that could imply a risk to the consumer.

For monitoring SAs in honey, liquid chromatography (LC) coupled with ultraviolet (UV) detector (*4*), fluorimetric detector (*2*, *5*) or mass spectrometry (MS) (*6*−*9*) is the commonly used analytical method. Among them, MS detection is the most preferred choice, because it can provide sensitive and selective monitoring for the target analytes.

In the whole analytical procedure, sample preparation is also an important and crucial step (*10*). When determining SAs in honey, two sample preparation steps must be included, such as hydrolysis or extraction procedure that releases the bound SAs back to the free form and provides satisfactory recoveries for the analytes, and a cleanup step to remove some of the coextracted compounds, especially for sugars (*1*). Typically SAs are extracted from honey with either hydrochloric acid (*2*), phosphoric acid (*5*) or trichloroacetic acid (*6*) aqueous solutions under shaking, and then the extracts obtained are cleaned up by liquid−liquid extraction (LLE) with dichloromethane (*2*−*4*) or solid phase extraction (SPE) with florisil (*4*), hydrophilic−lipophilic balanced material (*9*) or C18 (*11*) as sorbent. Moreover, the strong cation exchange resin was also used as the SPE sorbent for adsorbing SAs when analyzing other animal products, such as tissue and egg (*12*).

The LLE procedure or classic SPE sorbents retain the targeted analytes but also other chemicals which can interfere with the detection of the compounds of interest (*13*). Molecularly imprinted polymers (MIPs) are synthetic materials which can selectively recognize a guest molecule or related analogous compounds and can be obtained simply and rapidly (*14*). They have become increasingly attractive in many fields, such as chiral separation (*15*), chemical sensors (*16*) or immunoassay-like analysis as synthetic antibody (*17*). However, one of the most interesting applications of this imprinted functionalized material is as sorbent for SPE (*18*−*22*).

Magnetic SPE (MSPE) is a new procedure of SPE based on the use of magnetic sorbents (*23*). In this procedure, magnetic sorbent is added to a solution or suspension containing the target analyte. The analyte is adsorbed onto the magnetic sorbent under stirring. Then the sorbent with captured analyte is recovered from the suspension using an appropriate magnetic separator. The analyte is consequently eluted from the sorbent and analyzed. The application of MSPE makes the sample pretreatment simple (*24*). The sorbent need not be packed into the SPE cartridge like the traditional SPE, and the phase separation could be conveniently realized by applying an external magnetic field. If some magnetic components are encapsulated into MIPs, the resulting composite polymer, magnetic MIPs (MMIPs) not only will have magnetically susceptible characteristic but also will have selectivity for the guest molecule (*25*, *26*).

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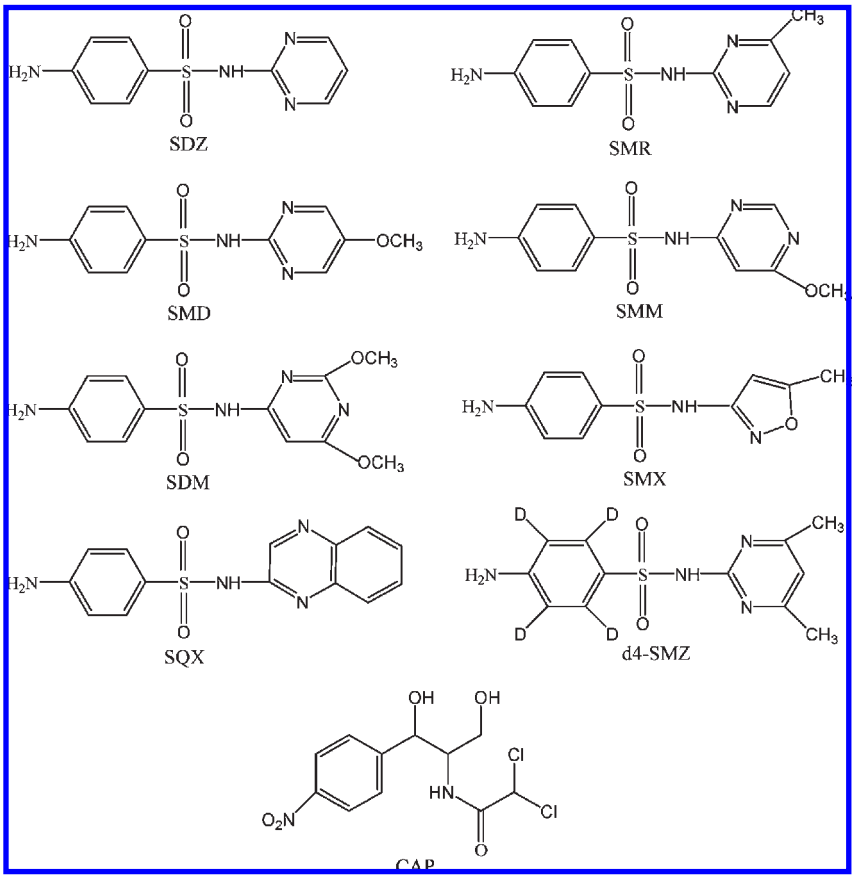


**Figure 1.** Chemical structures of the sulfonamides and chloramphenicol (CAP). SDZ, sulfadiazine; SMR, sulfamerazine; SMD, sulfamethoxydiazine; SMM, sulfamonomethoxine; SDM, sulfadimethoxine; SMX, sulfamethoxazole; SQX, sulfaquinoxaline; d4-SMZ, sulfamethazine-$d_4$.

The aim of the study is to develop a new method to improve and simplify the analysis of SAs including sulfadiazine (SDZ), sulfamerazine (SMR), sulfamethoxydiazine (SMD), sulfamonomethoxine (SMM), sulfadimethoxine (SDM), sulfamethoxazole (SMX) and sulfaquinoxaline (SQX) in honey. These seven SAs were selected as target analytes, because they are frequently used in bee-keeping in China (*5*, *27*−*29*). In the work presented here, a new MMIP was synthesized using SMD as template molecule. The characteristics of the MMIP and binding experiment were investigated. The polymers were used as sorbents for the extraction of SAs from honey samples, followed by LC−MS/MS analysis. The SAs were selectively isolated and the matrix interferences were eliminated using these polymers in a short time, which simplifies the sample procedure.

## MATERIALS AND METHODS

**Reagents and Chemicals.** The standards of SDZ, SMR, SMD, SMM, SDM, SMX, SQX, sulfamethazine-$d_4$ (d4-SMZ) and chloramphenicol (CAP) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Their chemical structures are shown in **Figure 1**. Chromatographic grade acetonitrile (ACN) was obtained from Fisher (Pittsburgh, PA). Ethylene glycol dimethacrylate (EGDMA) was purchased from Sigma−Aldrich (St. Louis, MO). 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 dimethyl sulfoxide (DMSO) were obtained from Guangfu Fine Chemical Research Institute (Tianjin, China). Methanol, ethanol, acetic acid and phosphoric acid were purchased from Beijing Chemical (Beijing, China). High purity water with a resistivity of 18.2 MΩ $cm^{-1}$ was obtained from a Milli-Q water system (Millipore, Billerica, MA).

Stock solutions of the standards (1 mg $mL^{-1}$) were prepared by dissolving each SA in methanol. They were stored in a refrigerator at 4 °C and found to be stable for two months. Work standard solutions were daily prepared by diluting the stock solutions with water.

**Honey Samples.** Some honey samples were randomly obtained from the local market in Changchun (China), and one raw floral honey was obtained from the bee keeper living in Baishan (China). The samples were stored at room temperature in the dark. One sample was checked to be free of any of the selected antibiotics, and it was used as blank honey for calibration and validation purposes.

**Preparation of $Fe_3O_4$ Magnetite.** The $Fe_3O_4$ magnetite was prepared by the coprecipitation method, as follows: 0.01 mol of $FeCl_2 \cdot 4H_2O$ and 0.02 mol of $FeCl_3 \cdot 6H_2O$ were dissolved in 100 mL of water. The mixture was stirred vigorously and purged with nitrogen gas while the temperature increased to 80 °C, and then 40 mL of sodium hydroxide solution (2.0 mol $L^{-1}$) was added into it. After 1.0 h, the magnetic precipitates were isolated from the solvent by a permanent magnet and washed several times with water.

**Preparation of MMIPs.** The MMIPs were prepared as follows: the SMD (1.0 mmol) was dissolved in 10 mL of DMSO, and then 4.0 mmol of MAA was added into it. This mixture was stirred for 30 min for preparation of the preassembly solution. The $Fe_3O_4$ magnetite (1.0 g) was mixed with 1.0 mL of oleic acid and stirred for 10 min. Then 20 mmol of EGDMA and the preassembly 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. The PVP (0.4 g) used as dispersant was dissolved into 100 mL of DMSO:water (9:1, v/v) in a three-necked round-bottomed flask. The mixture was stirred at 300 rpm and purged with nitrogen gas to displace oxygen while the temperature increased to 60 °C. The prepolymerization solution was added into the three-necked flask, and then 0.1 g of 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, and washed with methanol:acetic acid (8:2, v/v) several times under ultrasound, until the template molecule could not be detected by LC−MS/MS. The polymers were washed with water three times again and dried at 60 °C.

The magnetic nonimprinted polymers (MNIPs) were prepared and processed similarly as above, except that the template molecule SMD was not added.

The MMIPs and MNIPs did not need to be ground before they were used. The MMIPs were characterized by scanning electron microscopy (SEM; JEM-6700F, JEOL, Tokyo, Japan) and vibrating sample magnetometry (VSM; JDM-13, Jilin University, Changchun, China).

**Binding Experiment.** The binding experiment was carried out by adding 20.0 mg of MMIPs or MNIPs in a glass tube containing 2.0 mL of SMD standard solution which was prepared in water varied in the concentration of 0.1−2.0 mmol $L^{-1}$. The solution was incubated for 24 h at room temperature, and then the suspension was separated and analyzed by LC with UV detection at 270 nm. The amount of SMD bound on the polymers was obtained by subtracting the free concentration from initial concentration of SMD added to the mixture.

**Selectivity of MMIPs.** The selectivity of the MMIPs was investigated with SDZ, SMR, SMM, SDM, SMX and SQX as the structural analogues of SMD template, and CAP as reference compounds. The experiment was carried out by adding 20.0 mg of MMIPs or MNIPs in a glass tube containing 2.00 mL of each standard solution at the concentration of 0.1 mmol $L^{-1}$. The solution was incubated for 24 h at room temperature, and then the supernatant was separated and analyzed by LC with UV detection at 270 nm. The SAs were separated by an XTerra C18 column (250 mm × 4.6 mm i.d., 5 $\mu$m, Waters, Milford, MA). The mobile phase was a mixture of 0.5% acetic acid aqueous solution and methanol. The gradient elution was carried out starting from 20% to 54% methanol in 17 min, and then back to 20% methanol in 1 min, held for 7 min to equilibrate the column. The flow rate was 0.9 mL $min^{-1}$.

**Extraction Procedure.** An amount of 50 mg of MMIPs was put into a conical flask. The polymers were conditioned in sequence with 3.0 mL of methanol and 3.0 mL of phosphoric acid aqueous solution (pH = 2.0). The supernatant was separated from the polymers with a magnet and discarded. Then 2.0 g of honey sample was added into the conical flask and fortified with 0.1 mL of the internal standard solution, d4-SMZ, giving a final internal standard concentration of 100 ng $g^{-1}$. Twenty milliliters of phosphoric acid aqueous solution (pH = 2.0) as the extraction solvent was also added into the flask. The mixture was stirred for 5 min. Then the MMIP captured SAs were separated rapidly from the solution under a strong external magnetic field. After the supernatant solution was discarded, the MMIPs were washed with 2 × 3.0 mL of water (3.0 mL every time and washed two times). Finally, the SAs were eluted from the MMIPs with 3 × 1.5 mL of methanol solution containing 5.0% acetic acid (1.5 mL every time and eluted three times). In order to improve the recoveries, the MMIP captured SAs 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 20% aqueous ACN for further LC−MS/MS analysis.

**LC−MS/MS Analysis.** LC−MS/MS analysis was performed using an Agilent 1100 liquid chromatograph (Palo Alto, CA) which was coupled to a Q Trap MS (Applied Biosystems/MDS Sciex, Concord, ON, Canada) equipped with an electrospray ionization (ESI) source. The SAs were separated by an XTerra C18 column. The mobile phase was a mixture of 0.5% acetic acid aqueous solution and ACN. The gradient elution was carried out starting from 20% to 30% ACN in 2 min, then to 50% ACN in 2 min, held for 2 min, and then back to 20% ACN in 2 min, held for 7 min to equilibrate the column. The flow rate of the mobile phase was maintained at 0.9 mL $min^{-1}$. The eluate was split and introduced into the MS detector at a flow rate of 0.2 mL $min^{-1}$. The column temperature was kept at 30 °C, and the injection volume was 20 $\mu$L.

ESI-MS/MS detection was performed in the positive mode, and the source dependent parameters were as follows: curtain gas, $N_2$ (30 psi); collision gas, $N_2$ (medium); gas 1, $N_2$ (55 psi); gas 2, $N_2$ (50 psi); ion spray voltage, 5000 V; temperature, 500 °C. Data acquisition was performed 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 ent. potential (CEP), collision energy (CE) and collision cell exit potential (CXP) are shown in **Table 1**. All transitions were recorded in one single retention time window with a dwell time of 100 ms. Data processing was performed using the Applied Biosystems Analyst software (version 1.4.1). Moreover, in order to obtain extra confirmation in the identification of the analytes, an enhanced product ion (EPI) scan with scan range *m*/*z* 50−500 was recorded simultaneously in the same chromatographic run (CE = 30 eV). This was performed by operating the system in the information dependent acquisition (IDA) mode.

**Table 1.** Precursor Ion, Product Ion, Corresponding Declustering Potential, Entrance Potential, Collision Cell Entrance Potential, Collision Energy and Collision Cell Exit Potential for the Sulfonamides

| | *m*/*z* | | | | | | |
|---|---|---|---|---|---|---|---|
| analytes | precursor ion | product ion | DP (V) | EP (V) | CEP (V) | CE (eV) | CXP (V) |
| SDZ | 251.1 | 156.1 | 48 | 12 | 42 | 25 | 5 |
| | | 92.1[a] | 48 | 5 | 40 | 36 | 5 |
| SMR | 265.1 | 156.1 | 50 | 11 | 30 | 20 | 4 |
| | | 92.1[a] | 45 | 11 | 30 | 36 | 4 |
| SMD | 281.1 | 156.1[a] | 48 | 10 | 44 | 26 | 5 |
| | | 108.1 | 46 | 10 | 40 | 34 | 5 |
| SMM | 281.1 | 156.1[a] | 48 | 10 | 44 | 26 | 5 |
| | | 108.1 | 46 | 10 | 40 | 34 | 5 |
| SDM | 311.1 | 156.1[a] | 45 | 11 | 30 | 26 | 4 |
| | | 92.1 | 45 | 11 | 30 | 36 | 4 |
| SMX | 254.1 | 156.1 | 45 | 11 | 30 | 20 | 4 |
| | | 92.1[a] | 50 | 11 | 30 | 36 | 4 |
| SQX | 301.1 | 156.1[a] | 46 | 5 | 42 | 27 | 5 |
| | | 108.1 | 48 | 10 | 44 | 32 | 5 |
| d4-SMZ | 283.2 | 160.1[a] | 45 | 8 | 25 | 25 | 4 |
| | | 96.1 | 46 | 8 | 30 | 30 | 4 |

[a] The product ion used for quantification.

**Calibration.** Matrix-based standards with the analyzed SAs over a concentration range from 10 to 1000 ng $g^{-1}$ and the internal standard (d4-SMZ) at the concentration of 100 ng $g^{-1}$ were prepared by spiking SAs into blank honey extract. Quantification was performed using internal standardization. Relative response factors were calculated by comparing the peak area response for the most intense MRM transition of each SA to that obtained for the d4-SMZ. Calibration curves were generated based on a linear least-squares regression analysis.

## RESULTS AND DISCUSSION

**Characterizations of the MMIPs.** Considering the structure of the seven SAs analyzed by this method, the SMD was selected as template molecule for preparation of the MMIPs, and the satisfactory SA recovery and selectivity were obtained by using SMD-MMIPs for the extraction of seven SAs. The MMIPs were prepared repeatedly several times, and the relative standard deviations (RSDs) of recoveries obtained by the proposed method for the same SAs did not exceed 10%.

The SEM image of the MMIPs shown in **Figure 2a** illustrated that the polymers were porous, with small cavities between larger ones. It is well-known that the cavities are of benefit because they can increase the adsorption capacity of polymers and improve the mass transfer rate for releasing and rebinding the analytes (*30*). The amplified SEM image of the MMIPs is shown in **Figure 2b**. The particle sizes of MMIPs used in our experiments were nonuniform.

The magnetic properties of the prepared MMIPs were investigated with a VSM. The magnetization curve (**Figure 3**) showed their superparamagnetic property. The magnetic saturation value of MMIPs is 4.0 emu $g^{-1}$.

**Binding Study.** The binding isotherms plotted in **Figure 4a** indicated that the amount of SMD bound to the MMIPs and MNIPs at binding equilibrium increased with the increasing of initial concentration of SMD. However, the amount of SMD bound to the MMIPs was higher than that bound to the MNIPs.

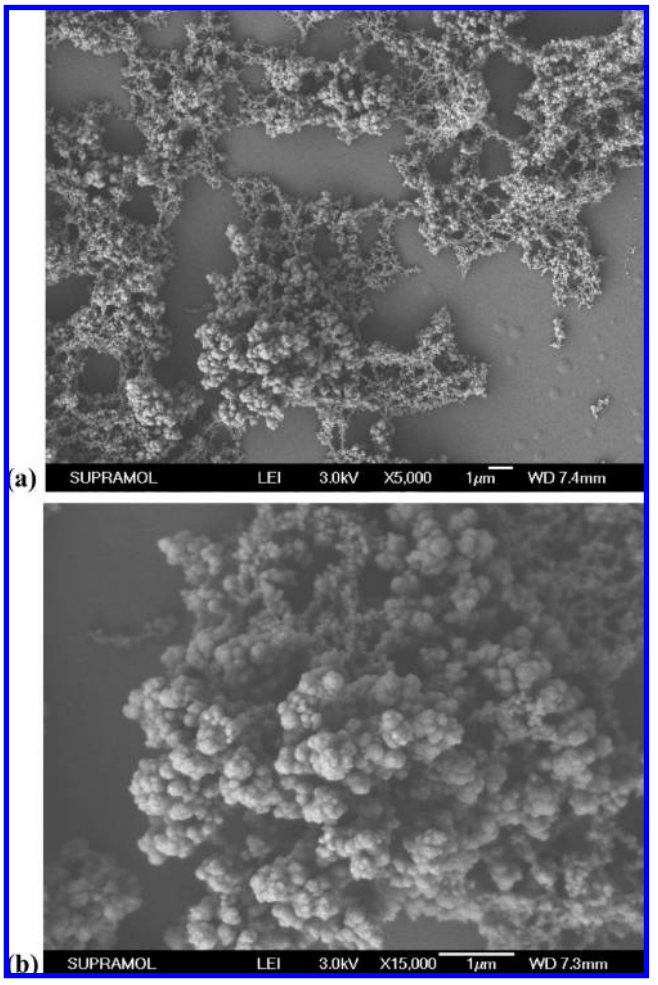


**Figure 2.** The scanning electron microscopy image of magnetic molecularly imprinted polymers.

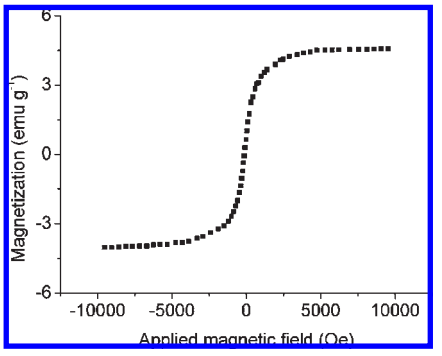


**Figure 3.** The magnetization curve of magnetic molecularly imprinted polymers.

Scatchard analysis was also used for evaluation of the adsorption of MMIPs and MNIPs according to the equation

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

where $Q$ is the amount of SMD bound to the polymers at equilibrium; [SMD] is the free SMD 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 $Q$/[SMD] versus $Q$.

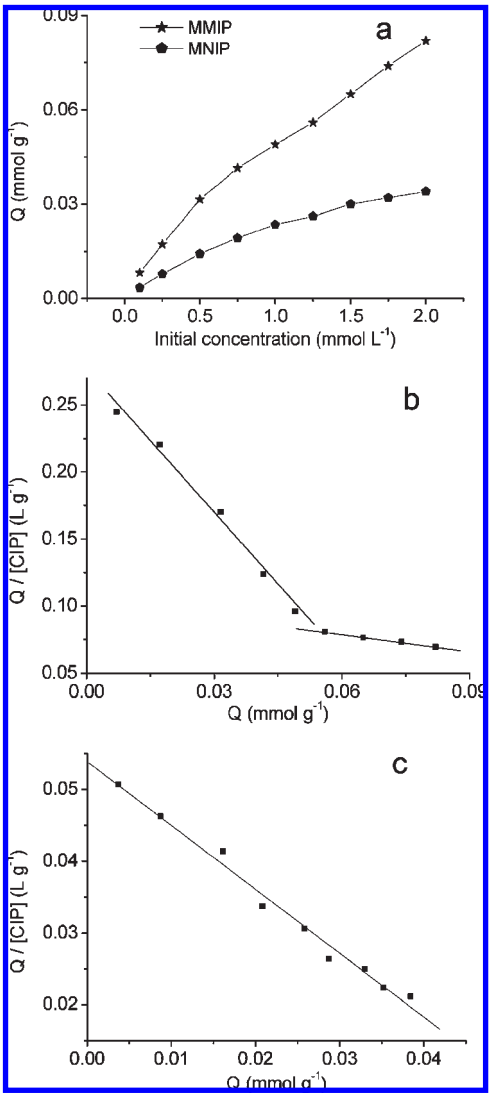


**Figure 4.** Binding isotherms (**a**) and Scatchard plot analysis of the binding of sulfamethoxydiazine onto the magnetic molecularly imprinted polymers (**b**) and magnetic nonimprinted polymers (**c**).

As can be seen from **Figure 4b**, 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 $Q/[\mathrm{SMD}] = -3.6329Q + 0.2774$. The $K_d$ and $Q_{max}$ were calculated to be 275.3 $\mu$mol L$^{-1}$ and 76.4 $\mu$mol g$^{-1}$ of dry polymer, respectively. The linear regression equation for the right part of this curve was $Q/[\mathrm{SMD}] = -0.4391Q + 0.1055$. The $K_d$ and $Q_{max}$ were calculated to be 2277.4 $\mu$mol L$^{-1}$ and 240.3 $\mu$mol g$^{-1}$ of dry polymer, respectively. The binding of SMD to the MNIPs was also analyzed by Scatchard method (**Figure 4c**). It revealed homogeneous binding sites with $K_d$ and $Q_{max}$ values of 1124.9 $\mu$mol L$^{-1}$ and 60.5 $\mu$mol g$^{-1}$, respectively.

**Selectivity of MMIPs.** The selectivity of the MMIPs was investigated with SDZ, SMR, SMM, SDM, SMX and SQX as the structural analogues of SMD template, and CAP as reference compounds (**Table 2**). Obviously, the adsorption amounts of SMD and its analogues on the MMIPs were higher than those on the MNIPs. There was no obvious difference between the MMIPs and MNIPs to adsorb CAP.

The relative selectivity coefficients of SAs toward CAP were in the range of 1.61−2.46. This might result from the imprinting effect, the difference of the molecular interactions and their structures. During the preparation of the MMIPs, the template

**Table 2.** Selectivity of Magnetic Molecularly Imprinted Polymers[a]

| | binding amount ($\mu$mol g$^{-1}$) | | distribution coeff ($K_d$, mL g$^{-1}$) | | selectivity coeff ($K$) | | |
|---|---|---|---|---|---|---|---|
| analytes | MMIP | MNIP | MMIP | MNIP | MMIP | MNIP | rel selectivity coeff ($K'$) |
| SDZ | 6.1 | 2.6 | 61 | 26 | 2.18 | 0.96 | 2.27 |
| SMR | 6.4 | 2.8 | 64 | 28 | 2.29 | 1.04 | 2.20 |
| SMD | 8.2 | 3.2 | 82 | 32 | 2.93 | 1.19 | 2.46 |
| SMM | 7.6 | 3.1 | 76 | 31 | 2.71 | 1.15 | 2.36 |
| SDM | 8.3 | 3.7 | 83 | 37 | 2.96 | 1.37 | 2.16 |
| SMX | 7.9 | 4.6 | 79 | 46 | 2.82 | 1.70 | 1.66 |
| SQX | 8.0 | 4.8 | 80 | 48 | 2.86 | 1.78 | 1.61 |
| CAP | 2.8 | 2.7 | 28 | 27 | | | |

[a] $K_d$ = binding amount/initial concentration; $K$ = $K_d$ (SAs)/$K_d$ (CAP); $K'$ = $K_{MMIP}/K_{MNIP}$.

of SMD was incorporated into inorganic−organic networks. After removal of SMD, the imprinted cavities and specific binding sites in a predetermined orientation were formed, whereas the MNIPs had no such imprinted cavities and specific binding sites.

**Optimization of the Extraction Conditions.** In order to evaluate the applicability of the MMIPs for separation and enrichment of SAs in honey samples, the parameters affecting the performance of the extraction, such as extraction solvent, MMIPs amount, extraction time, washing and elution conditions, were investigated. The extraction conditions were optimized by analyzing spiked honey samples (1000 ng g$^{-1}$). When one parameter was changed, the other parameters were fixed at their optimized values.

(*a*) *Extraction solvent*. The solvent used for rebinding SAs to MMIPs was first tested before extracting honey sample. Different solvents, such as 5%, 10%, 20% and 50% ACN or methanol aqueous solution, ACN, methanol, water with different pH (1−10) were employed to prepare 100 ng mL$^{-1}$ SAs standard solutions. Twenty milliliters of each of these solutions was used for rebinding SAs. The SAs were strongly retained by MMIPs with recoveries in the range of 78.5%−97.9% when water with pH ranging from 2 to 7 was employed. A sharp decrease in the recovery was observed for pH values higher than 7. The results can be attributed to the deprotonation of the acidic functional groups of the polymer in the basic solution, which interferes with the formation of hydrogen bonds between functional groups of the polymers and the template molecule, and then decreasing the rebinding of template molecule (*31*). When the acidity was too strong (pH = 1), the hydrogen bond strength decreased. With the increase in the proportion of ACN or methanol in water, there was a gradual decline in recovery. This is because water was contained in the polymerization solvent when preparing the MMIPs, and thus increased hydrophobic interaction between polymers and analytes due to the high polarity of water (*32*). Organic solvents such as ACN or methanol would decrease the hydrophobic interaction in the rebinding process. This observation suggested that the retention of SAs on MMIPs was by hydrogen bonding and hydrophobic interaction.

Honey is a complex matrix and mainly composed of sugars, which can form a very stable bond with the SAs, the N-glycosidic bond (*33*). This bond makes the extraction of SAs in honey difficult, and it must be broken by a solvent such as 2 mol L$^{-1}$ hydrochloric acid (*2*), phosphoric acid (pH = 2) (*5*), 10% trichloroacetic acid (*6*) aqueous solutions or methanol (*34*). In order to break the N-glycosidic bond as well as rebind SAs to MMIPs, 20 mL of phosphoric acid aqueous solution (pH = 2) was used as the extraction solvent for extracting a 2.0 g honey

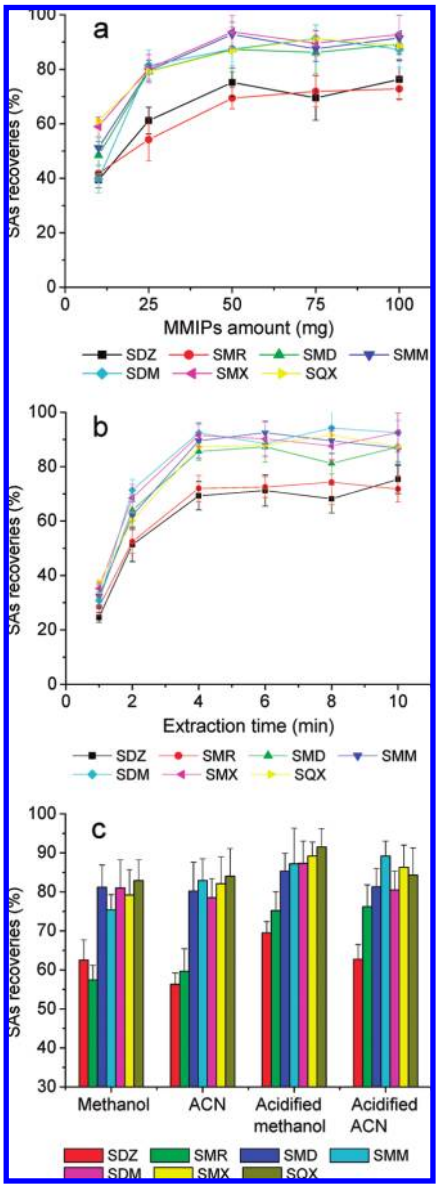


**Figure 5.** The effects of magnetic molecularly imprinted polymer amount (**a**), extraction time (**b**) and elution solution (**c**) on the recoveries of sulfonamides ($n$ = 3).

sample which obtained the recoveries of SAs in the range of 71.2%−92.8%. If pure water (pH = 7) was used for the extraction, the recoveries decreased to 62.5%−83.9%.

(*b*) *MMIP Amount*. Different amounts of MMIPs ranging from 10 to 100 mg in 20 mL of extraction solvent were applied to extract the SAs from a 2.0 g honey sample (**Figure 5a**). The results indicated that 50 mg polymers were enough, and satisfactory recoveries ranging from 69.4% to 93.7% were obtained. Further increasing the amount of MMIPs gave no improvement for recoveries of SAs.

(*c*) *Extraction Time*. The effect of the extraction time from 1 to 20 min on the recoveries of SAs was investigated (**Figure 5b**). The results indicated that the SAs recoveries increased from 24.6%−37.5% to 72.0%−92.4% with the increasing of the extraction time from 1 to 4 min, and then did not significantly change from 4 to 20 min. In this work, the extraction time of 5 min was chosen for obtaining the complete extraction.

(*d*) *Washing Conditions*. The retained potentially interfering compounds can be removed during the wash procedure. In honey samples, the high sugar content presents unique challenges (*1*).

The sugars can contribute to ion suppression of SAs during LC−MS/MS analysis (*1*). In the study, the MMIPs capturing SAs were washed with 2 × 3.0 mL water. The satisfactory recoveries of SAs ranging from 71.3% to 95.1% were obtained in this way.

(*e*) *Elution Conditions*. In order to obtain the highest recoveries of SAs, a series of elution solutions, methanol, ACN, acidified methanol and acidified ACN were used to optimize the elution condition (**Figure 5c**). In order to improve the recoveries, the MMIPs captured with SAs were subjected to ultrasound for 30 s during each elution process. The poor recoveries (57.4%−84.0%) were found by using methanol and ACN. The best recoveries (69.5%−91.5%) were obtained using 4.5 mL (1.5 mL every time and eluted three times) of methanol−acetic acid (95:5, v/v) as eluting solution. When the ultrasound was not used, the SA recoveries decreased to 49.4%−80.7%.

**Qualitative and Quantitative Analysis.** MS data acquisition was performed in the MRM mode, recording the transitions between the precursor ion and the two most abundant product ions for each target analyte. It achieved the requirements set by the European Union regulation for the confirmation and identification of pharmaceuticals (*35*). The regulation determines that the instrumental technique used should provide a minimum of three identification points (IP) to ensure a correct detection of target analytes. When monitoring two transitions in MRM mode, 4 IP are obtained (1 for the precursor ion and 1.5 for each transition product), which is sufficient to confirm the identity of a compound in a sample. Additionally, the inclusion of the EPI scan in the same experiment when operating in the IDA mode is an extra tool for unequivocal confirmation of target compounds in the complex sample (*36*). LC−MS/MS extracted ion chromatograms obtained by the analysis of spiked honey sample (10 ng $g^{-1}$) which were determined by MRM are illustrated in **Figure 6**.

The structural information of the SAs was also achieved from their 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 for all compounds. Two characteristic MRM transitions were chosen for each compound (**Table 1**). Quantification was done on the most intense MRM transition. After fragmentation, all of the SAs exhibited the ions at *m*/*z* 156, 108, and 92 corresponding to $[M - RNH_2]^+$, $[M - RNH_2 - SO]^+$ and $[M - RNH_2 - SO_2]^+$ (*37*). R in product ions represents N-substituted derivatives of SAs (**Figure 1**). In addition, ions corresponding to $[M + H - 93]^+$ and $[M + H - 66]^+$ are also observed in some SA mass spectra, and most probably correspond to $[RNHSO_2]^+$ and $[M + H - H_2SO_2]^+$ (*6*).

The calibration curves were constructed in solvent and in blank honey extract at a concentration range of 10−1000 ng $g^{-1}$ (100 ng $g^{-1}$ for the internal standard). Initially, the calibration curves were built by plotting the ratio of the area against the concentration of the analytes for evaluation the matrix effect. The results showed that the slopes of calibration curves obtained for matrix-matched standard were lower than obtained for solvent standard, which indicated the signal suppression of the SAs. However, the signal suppression was not very large with values in the range of 8%−23%, which indicated that the selectivity of the method is good. Then, the calibration curves were built by plotting the area ratio of analyte versus internal standard against the concentration ratio. There are no significant differences of the calibration curve slopes obtained both for in solvent and for in matrix. The differences are less than 7% and 6% when analyzing the honey sample obtained from the market and obtained from the bee keeper, respectively. In this work, matrix-matched calibration curves with internal standardization were used for reliable quantification. The correlation coefficients (*r*) ranging from 0.997 to 0.999 are obtained for all the analytes in the concentration range of 10−1000 ng $g^{-1}$.

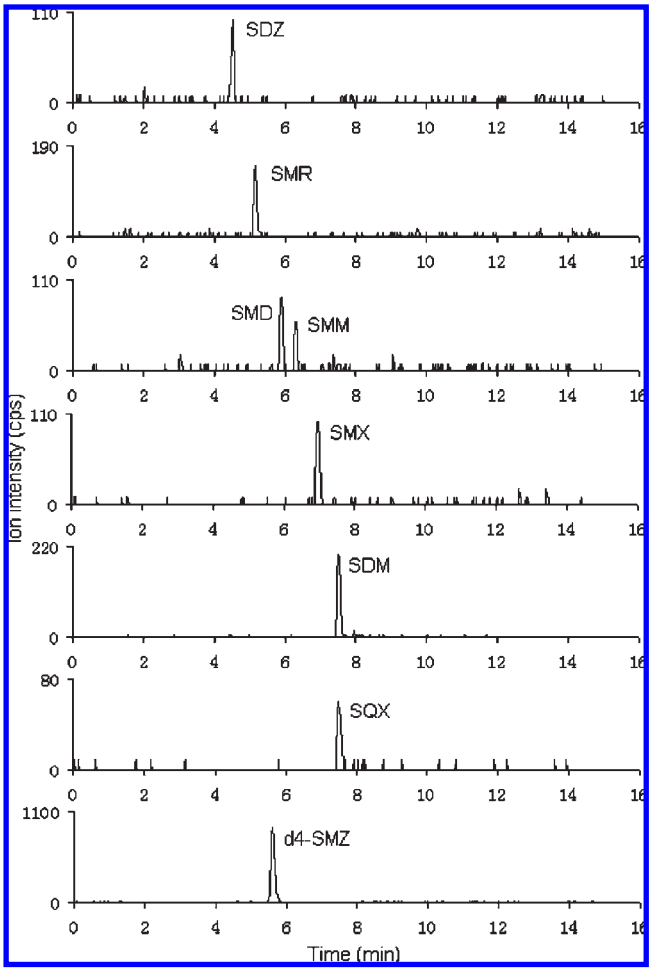


**Figure 6.** Liquid chromatography−tandem mass spectrometry extracted ion chromatograms obtained by the analysis of spiked honey sample (10 ng $g^{-1}$). The internal standard sulfamethazine-$d_4$ concentration is 100 ng $g^{-1}$. The MRM transitions used for quantification were recorded in the chromatograms.

**Validation of the Method.** The method validation was done according to the European Commission Decision 2002/657/EC (*35*).

The specificity of the method was checked by analyzing different blank honey samples. No interfering peaks and false positive results were observed in the blank chromatograms, which indicated that the selectivity of the method is good.

The stability of analytes in standard solution and extract was also checked. Stock solutions of the analyte standards (1 mg $mL^{-1}$) were stored in a refrigerator at 4 °C and found to be stable for two months. Work standard solutions were daily prepared by diluting the stock solutions with water. The analytes in the extract were found to be stable at room temperature for 24 h.

Limit of detection (LOD) and limit of quantification (LOQ) are considered as the analyte minimum concentrations that can be confidently identified and quantified by the method, respectively. The LODs estimated as the analyte concentration producing signal/noise ratio of 3:1 are 3.1, 1.8, 3.2, 3.9, 3.1, 1.5, and 4.3 ng $g^{-1}$ for SDZ, SMR, SMD, SMM, SDM, SMX and SQX, respectively. The LOQs estimated as the analyte concentration producing signal/noise ratio of 10:1 are 8.3, 6.3, 9.8, 8.5, 9.0, 5.2,

**Table 3.** Intra- and Interday Precisions and Recoveries of the Assay ($n = 6$)

| | intraday precision (%) | | | | | | interday precision (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 ng $g^{-1}$ | | 50 ng $g^{-1}$ | | 500 ng $g^{-1}$ | | 10 ng $g^{-1}$ | | 50 ng $g^{-1}$ | | 500 ng $g^{-1}$ | |
| analytes | recovery | RSD | recovery | RSD | recovery | RSD | recovery | RSD | recovery | RSD | recovery | RSD |
| SDZ | 69.4 | 5.2 | 71.0 | 4.2 | 76.2 | 4.2 | 68.2 | 5.2 | 72.6 | 6.2 | 74.3 | 4.8 |
| SMR | 72.8 | 7.9 | 70.9 | 6.9 | 79.0 | 4.9 | 70.1 | 8.7 | 74.0 | 7.4 | 79.0 | 5.2 |
| SMD | 82.5 | 4.8 | 81.3 | 6.5 | 87.2 | 5.2 | 78.4 | 5.4 | 79.3 | 5.3 | 82.3 | 5.4 |
| SMM | 79.8 | 7.2 | 84.2 | 7.8 | 91.4 | 4.7 | 76.0 | 4.3 | 82.4 | 5.9 | 89.5 | 5.0 |
| SDM | 84.3 | 5.9 | 76.2 | 5.2 | 87.5 | 6.0 | 82.1 | 8.2 | 88.5 | 6.5 | 87.2 | 6.8 |
| SMX | 85.0 | 5.7 | 82.0 | 6.0 | 90.3 | 4.3 | 77.2 | 9.9 | 84.9 | 4.7 | 85.0 | 7.0 |
| SQX | 79.2 | 4.7 | 81.4 | 5.8 | 82.6 | 3.7 | 80.3 | 7.4 | 90.3 | 6.3 | 91.2 | 4.9 |

and 9.7 ng $g^{-1}$ for SDZ, SMR, SMD, SMM, SDM, SMX and SQX, respectively.

Precision was evaluated by measuring intra- and interday RSDs. The intraday precision was performed by analyzing spiked honey sample six times in one day at three different fortified concentrations of 10, 50, and 500 ng $g^{-1}$. The interday precision was performed over six days by analyzing spiked honey samples at three different fortified concentrations of 10, 50, and 500 ng $g^{-1}$. The results obtained are shown in **Table 3**. RSDs of intra- and interday ranging from 3.7% to 7.9% and from 4.3% to 9.9% are obtained, respectively. In all three fortified levels, recoveries of the six SAs were in the range of 68.2%−91.4%.

To demonstrate the applicability of the method, five honey samples collected from different markets located in Changchun (China) and one raw floral honey obtained from the bee keeper living in Baishan (China) were analyzed. No SA residues at detectable levels were found in these samples. The recovery study was then carried out by spiking the honey samples with 50 ng $g^{-1}$ of SAs. SA recoveries from 67.1% to 93.6% and from 69.4% to 92.2% were obtained for the honeys collected from the market and the honey obtained from the bee keeper, respectively.

In conclusion, MMIPs were prepared in this study and they have strong magnetic responsiveness and selective character. The polymers were applied for the extraction of SAs from honey sample. The analytes were separated and detected by LC−MS/MS. The magnetic extraction method avoided the time-consuming column passing and filtration operation compared with traditional SPE. The whole extraction and cleanup procedure including condition of the sorbent, transferring of SAs from sample matrix to MMIPs, washing of the sorbent and elution of SAs from the sorbent can be completed within 15 min. It can be considered that this method is promising and may be a good alternative to the traditional techniques.

## LITERATURE CITED


(1) Sheridan, R.; Policastro, B.; Thomas, S.; Rice, D. Analysis and occurrence of 14 sulfonamide antibacterials and chloramphenicol in honey by solid-phase extraction followed by LC/MS/MS analysis. *J. Agric. Food Chem.* **2008**, 56, 3509–3516.

(2) Maudens, K. E.; Zhang, G. F.; Lambert, W. E. Quantitative analysis of twelve sulfonamides in honey after acidic hydrolysis by high-performance liquid chromatography with post-column derivatization and fluorescence detection. *J. Chromatogr., A* **2004**, 1047, 85–92.

(3) Mohamed, R.; Hammel, Y. A.; LeBreton, M. H.; Tabet, J. C.; Jullien, L.; Guy, P. A. Evaluation of atmospheric pressure ionization interfaces for quantitative measurement of sulfonamides in honey using isotope dilution liquid chromatography coupled with tandem mass spectrometry techniques. *J. Chromatogr., A* **2007**, 1160, 194–205.

(4) Granja, R. H. M. M.; Niño, A. M. M.; Rabone, F.; Salerno, A. G. A reliable high-performance liquid chromatography with ultraviolet detection for the determination of sulfonamides in honey. *Anal. Chim. Acta* **2008**, 613, 116–119.

(5) Pang, G. F.; Cao, Y. Z.; Fan, C. L.; Zhang, J. J.; Li, X. M.; Li, Z. Y.; Jia, G. Q. Liquid chromatography−fluorescence detection for simultaneous analysis of sulfonamide residues in honey. *Anal. Bioanal. Chem.* **2003**, 376, 534–541.

(6) Verzegnassi, L.; Savoy-Perroud, M. C.; Stadler, R. H. Application of liquid chromatography−electrospray ionization tandem mass spectrometry to the detection of 10 sulfonamides in honey. *J. Chromatogr., A* **2002**, 977, 77–87.

(7) Thompson, T. S.; Noot, D. K. Determination of sulfonamides in honey by liquid chromatography−tandem mass spectrometry. *Anal. Chim. Acta* **2005**, 551, 168–176.

(8) Hammel, Y. A.; Mohamed, R.; Gremaud, E.; LeBreton, M. H.; Guy, P. A. Multi-screening approach to monitor and quantify 42 antibiotic residues in honey by liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2008**, 1177, 58–76.

(9) Vidal, J. L. M.; Aguilera-Luiz, M. M.; Romero-Gonzlez, R.; Frenich, A. G. Multiclass analysis of antibiotic residues in honey by ultraperformance liquid chromatography-tandem mass spectrometry. *J. Agric. Food Chem.* **2009**, 57, 1760–1767.

(10) Chen, L. G.; Song, D. Q.; Tian, Y.; Ding, L.; Yu, A. M.; Zhang, H. Q. Application of on-line microwave sample-preparation techniques. *Trends Anal. Chem.* **2008**, 27, 151–159.

(11) Krivohlavek, A.; Šmit, Z.; Baštinac, M.; Žuntar, I.; Plavšic-Plavšic, F. The determination of sulfonamides in honey by high performance liquid chromatography−mass spectrometry method. *J. Sep. Sci.* **2005**, 28, 1434–1439.

(12) Stubbings, G.; Tarbin, J.; Cooper, A.; Sharman, M.; Bigwood, T.; Robb, P. A multi-residue cation-exchange clean up procedure for basic drugs in produce of animal origin. *Anal. Chim. Acta* **2005**, 547, 262–268.

(13) Mohamed, R.; Richoz-Payot, J.; Gremaud, E.; Mottier, P.; Yilmaz, E.; Tabet, J. C.; Guy, P. A. Advantages of molecularly imprinted polymers LC-ESI-MS/MS for the selective extraction and quantification of chloramphenicol in milk-Based matrixes, comparison with a classical sample preparation. *Anal. Chem.* **2007**, 79, 9557–9565.

(14) He, J.; Wang, S.; Fang, G.; Zhu, H.; Zhang, Y. Molecularly imprinted polymer online solid-phase extraction coupled with high-performance liquid chromatography-UV for the determination of three sulfonamides in pork and chicken. *J. Agric. Food Chem.* **2008**, 56, 2919–2925.

(15) Matthew, P. D.; Vern, D. B.; David, P. Approaches to the rational design of molecularly imprinted polymers. *Anal. Chim. Acta* **2004**, 504, 7–14.

(16) Vivek, B. K.; Hunagxian, J. Molecular imprinting: a dynamic technique for diverse applications in analytical chemistry. *Anal. Bioanal. Chem.* **2004**, 380, 587–605.

(17) Wulff, G. Molecular imprinting in cross-linked materials with the aid of molecular templates-away towards artificial antibodies. *Angew. Chem., Int. Ed. Engl.* **1995**, 34, 1812–1832.

(18) Francesco, P.; Carmelo, G.; Francesca, I.; Rita, M.; Umile, G. S.; Nevio, P. Molecularly imprinted solid phase extraction for detection of Sudan I in food matrices. *Food Chem.* **2005**, 93, 349–353.

(19) Christina, S.; Hans, M. Synthesis of a molecularly imprinted polymer for the selective solid-phase extraction of chloramphenicol from honey. *J. Chromatogr., A* **2006**, 1132, 325–328.

(20) Molinelli, A.; Weiss, R.; Mizaikoff, B. Advanced solid phase extraction using molecularly imprinted polymers for the

determination of quercetin in red wine. *J. Agric. Food Chem.* **2002**, 50, 1804–1808.

(21) Ramström, O.; Skudar, K.; Haines, J.; Patel, P.; Brüggemann, O. Food analyses using molecularly imprinted polymers. *J. Agric. Food Chem.* **2001**, 49, 2015–2114.

(22) Martin, S.; Anderson, L. I.; Mosbach, K. Selective recognition of the herbicide atrazine by noncovalent molecularly imprinted polymers. *J. Agric. Food Chem.* **1996**, 44, 141–145.

(23) Liu, Y.; Li, H.; Lin, J. M. Magnetic solid-phase extraction based on octadecyl functionalization of monodisperse magnetic ferrite microspheres for the determination of polycyclic aromatic hydrocarbons in aqueous samples coupled with gas chromatography−mass spectrometry. *Talanta* **2009**, 77, 1037–1042.

(24) Liu, Y.; Jia, L. Analysis of estrogens in water by magnetic octadecylsilane particles extraction and sweeping micellar electrokinetic chromatography. *Microchem. J.* **2008**, 89, 72–76.

(25) Chen, L. G.; Liu, J.; Zeng, Q. L.; Wang, H.; Yu, A. M.; Zhang, H. Q.; Ding, L. Preparation of magnetic molecularly imprinted polymer for the separation of tetracycline antibiotics from egg and tissue samples. *J. Chromatogr., A* **2009**, 1216, 3710–3719.

(26) Zhang, Y.; Liu, R.; Hu, Y.; Li, G. Microwave heating in preparation of magnetic molecularly imprinted polymer beads for trace triazines analysis in complicated samples. *Anal. Chem.* **2009**, 81, 967–976.

(27) Li, X.; Chen, X.; Jin, M.; Yao, X. Determination of sulfanilamide residues in honey by high-performance liquid chromatography with fluorescence detection and solid-phase extraction. *Chin. J. Health Lab. Technol.* **2007**, 17, 468–500.

(28) Wang, X.; Li, H.; Liu, W.; Xu, J.; Yan, F. Study on multiresidue determination of six sulfonamides in honey by high-performance liquid chromatograph. *Chin. J. Health Lab. Technol.* **2009**, 19, 810–812.

(29) Tang, Y.; He, H.; Wu, B.; Chen, H.; Shen, C.; Jiang, Y. Multiresidue determination of ten sulfonamides in honey by solid phase extraction and high performance Liquid Chromatography. *Chin. J. Instrum. Anal.* **2005**, 24, 96–99.

(30) Lu, S.; Cheng, G.; Pang, X. Preparation of molecularly imprinted $Fe_3O_4$/P(St-DVB) composite beads with magnetic susceptibility and their characteristics of molecular recognition for amino acid. *J. Appl. Polym. Sci.* **2003**, 89, 3790–3796.

(31) Guzmán-Vázquez de Prada, A.; Martínez-Ruiz, P.; Reviejo, A. J.; Pingarrón, J. M. Solid-phase molecularly imprinted on-line preconcentration and voltammetric determination of sulfamethazine in milk. *Anal. Chim. Acta* **2005**, 539, 125–132.

(32) Sun, H. W.; Qiao, F. X. Recognition mechanism of water-compatible molecularly imprinted solid-phase extraction and determination of nine quinolones in urine by high performance liquid chromatography. *J. Chromatogr., A* **2008**, 1212, 1–9.

(33) Kujawski, M. W.; Namieśnik, J. Challenges in preparing honey samples for chromatographic determination of contaminants and trace residues. *Trends Anal. Chem.* **2008**, 27, 785–793.

(34) Bernal, J.; Nozal, M. J.; Jiménez, J. J.; Martín, M. T.; Sanz, E. A new and simple method to determine trace levels of sulfonamides in honey by high performance liquid chromatography with fluorescence detection. *J. Chromatogr., A*, in press. doi: 10.1016/j.chroma.2009.05.046.

(35) Gros, M.; Pizzolato, T. M.; Petrović, M.; López de Alda, M. J.; Barceló, D. Trace level determination of $\beta$-blockers in waste waters by highly selective molecularly imprinted polymers extraction followed by liquid chromatography−quadrupole-linear ion trap mass spectrometry. *J. Chromatogr., A* **2008**, 1189, 374–384.

(36) Commision Decision (2002/657/EC) of 12 August 2002, Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Off. J. Eur. Union* **2002**, *L211*, 8−36.

(37) Chen, L.; Zeng, Q.; Wang, H.; Su, R.; Xu, Y.; Zhang, X.; Yu, A.; Zhang, H.; Ding, L. On-line coupling of dynamic microwave-assisted extraction to solid-phase extraction for the determination of sulfonamide antibiotics in soil. *Anal. Chim. Acta* **2009**, 648, 200–206.

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