Pseudo-template molecularly imprinted polymer for selective screening of trace β-lactam antibiotics in river and tap water

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ABSTRACT

To assess the potential risks associated with the environmental exposure of β-lactam antibiotics (BLAs), the monitoring of the occurrence, distribution, and fate of these emerging contaminants in the environment is required. Herein, we demonstrate a molecularly imprinted solid-phase extraction (MISPE) method for selective and reliable screening of trace BLAs in river and tap water. By developing a low-temperature photopolymerization, highly selective molecularly imprinted polymers (MIPs) for five BLAs (penicillin G, amoxicillin, ampicillin, nafcillin and mezlocillin) were synthesized. Nafcillin was chosen as a so-called pseudo template to make the MIP sorbent (Nafc-MIP), which was used in pseudo-template MISPE for pre-concentration of the other four BLAs from river and tap water. The application of pseudo-template MISPE overcomes the template bleeding, which significantly elevates the sample background and restricts the application of MIP for detection of the target BLA below 2 μg/L. The average recoveries of BLAs are in the range of 60–90% when Nafc-MIP was adopted as the selective MISPE sorbent. The developed method was validated, and applied to the screening of trace β-lactam antibiotics in river and tap water. The linearity of the calibration curve for each BLA was observed over the range of 0.1–20 μg/L (r2 > 0.998). The β-lactam antibiotics were found within the range of 0–9.56 μg/L in river water at the downstream of antibiotics manufacturers, and none were detected in the tap water.

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1. Introduction

β-Lactam antibiotics (BLAs) are one of the most widely used anti-microbial drugs since 70 years ago, as they act through binding to certain antibiotic binding proteins (e.g. penicillin-binding proteins) of bacteria [1]. The wide use of β-lactam antibiotics increases the concerns on their environmental exposure [2]. Long-term exposure to antibiotics may be associated with an increased risk of development and spread of antibiotic resistance, posing a potential threat to public health [3]. To assess the potential risks associated with environmental exposure of BLAs, the monitoring of the occurrence, distribution, and fate of these emerging contaminants is strongly required.

The determination of BLAs in environment exposure is relatively limited in contrast to other antibiotics such as fluoroquinolones and sulfonamides [4,5]. The β-lactam ring of BLA is chemically unstable and highly labile to acidity, heat, and β-lactamase enzymes, and the carbonyl carbon can be attacked by nucleophilic agents and also become labile [1,4]. Moreover, the environmental exposure levels of BLAs are generally very low, typically in quantities of parts per billion (ppb) or even trillion (ppt) [6,7].

Because of their low exposure levels, BLAs in environmental samples are often required to be preconcentrated to a detectable level by solid-phase extraction (SPE) prior to HPLC analysis. For this purpose, ODS C18, hydrophilic lipophilic balance (HLB) copolymer and mixed phase SPE sorbents were extensively employed for BLAs extraction, and exhibited a merit of high recovery [8–10]. However, these traditional sorbents suffer from a major problem: lack of adequate selectivity to the target BLAs. Molecularly imprinted solid-phase extraction (MISPE) provides an alternative to achieve selective extraction of the target analytes, by offering both good selectivity and high cleanup efficiency [11–15].

MIPs for direct extraction of BLAs from aqueous samples were synthesized by both conventional protocols [16,17] and novel approaches including sol-gel imprinting [18] and the stoichiometric imprinting [19–21]. Since MIPs are synthesized by using the target molecule as the template, trace amount of the template bleaching from the resultant polymer may significantly contaminate the sample, thereby deteriorating the accuracy and precision of the analysis [22,23]. To overcome this problem, a structural analogue of the target analyte, so-called pseudo template (dummy template), can be used as an alternative in MIP preparation [22–24]. Thus, it does not matter if
template bleeding due to the interested analyte can be separated from the pseudo template in the subsequent analysis.

The aim of this study is to develop a selective MISPE protocol to be applied to the environmental monitoring of β-lactam antibiotics in river and tap water. Two key technical issues are concerned: one is the chemical instability of template (BLAs) in MIP synthesis, and the other the template bleeding. These points have not been comprehensively considered in the previous studies [17–21]. Hence, in this work, we synthesized BLA-imprinted polymers by developing a low-temperature photopolymerization, and developed a pseudo-template MIP to overcome the template bleeding of MIP. Based on these improvements, a reliable MISPE coupled with liquid chromatography for the selective screening of trace BLAs in water samples was established.

2. Experimental

2.1. Chemicals and reagents

Penicillin G, amoxicillin, ampicillin, nafcillin and mezlocillin (>98%) were purchased from North China Pharmaceutical Co. (Shijiazhuang, China). Ethylene glycol dimethacrylate (EDMA) and methacrylic acid (MAA) were purchased from Acros (New Jersey, NJ, USA) and refined by distillation. The initiators 2,2'-azobisisobutyronitrile (AIBN) and 2,2’-azobis(2,4-dimethylvaleronitrile) (ABDV) were purchased from J&K Chemical Ltd. (Beijing, China) and recrystallized prior to use. HPLC grade acetonitrile and methanol were purchased from Fisher (Fair Lawn, NJ, USA). Tetrabutyl ammonium (TBA) was purchased from Sigma (St. Louis, MO, USA). Other chemicals of analytical grade were purchased from Beijing Chemical Reagent Co. (Beijing, China). Ultra pure water (resistivity 18.2 MΩ cm) was prepared from an ELGA system (Elga, UK) and all solutions were filtered through 0.45-μm membranes prior to use.

2.2. MIP preparation and characterization

2.2.1. Thermal polymerization

The BLA-imprinted polymers were prepared via thermal polymerization as described elsewhere [16]. Briefly, the template BLA (0.5 mmol), functional monomer MAA (2 mmol) and crosslinker EDMA (10 mmol) were dissolved in 8 ml methanol (or acetonitrile), and stood for 1 h at room temperature. Afterwards, 20 mg of the free radical initiator ABDV (for 40 °C) or AIBN (for 60 °C) was added into the above solution. The mixture was purged with nitrogen gas for 5 min, and then sealed in a conical flask, allowing the polymerization to be thermally initiated at 40 °C or 60 °C in a water bath for 24 h. As a reference, non-imprinted polymer (NIP) for control experiments was prepared similarly as MIP synthesis described above except that no BLA template was added in the polymerization process.

2.2.2. Photopolymerization

The prescription of the precursor for photopolymerization was similar to that in thermal polymerization, where ABDV was used as the UV initiator. The precursor, containing BLA template (0.5 mmol), MAA (2 mmol), EDMA (10 mmol) and the methanol (8 ml), was placed at 5 cm distance from the UV source (365 nm, 50 W) in a UV crosslinker (BINTA Instrument, Beijing, China) with defined ultraviolet energy for 36 h. NIP was also prepared in the same way as MIP described above except for no addition of BLA template in the precursor.

2.2.3. Characterization

After the polymerization, the resultant MIP bulk was ground into fine powders and sieved to 25–38 μm particles. The polymer particles were packed into a 100 × 4.6 mm ID steel column, washed on-line with methanol–acetic acid (4:1, v/v) and then methanol until no imprinted molecule was detectable in the rinses.

The selectivity of BLA-MIPs was evaluated by HPLC analysis. Retention factor (k) for BLA was calculated as $k = (t − t_0)/t_0$, where $t$ is the retention time of the analyte, $t_0$ is the void time determined by injection of acetone. The imprinting factor (IF) was used to evaluate the selectivity of MIP to an antibiotic, and defined as [21].

$$\text{IF} = \frac{k(\text{MIP})}{k(\text{NIP})}$$

where $k(\text{MIP})$ and $k(\text{NIP})$ are retention factors of the analyte on MIP and NIP, respectively.

2.3. Rebinding assay

Rebinding experiment was carried out to evaluate the binding properties and the homogeneity of binding sites in BLA-MIP. Amount of 40 mg Nafc–MIP prepared via photopolymerization was immersed into 4.0 mL of a series of known concentration of nafcillin solutions (in methanol) in a container for 12 h. Afterwards, each solution was filtrated through a 0.45-μm filter from Milipore (MA, USA). The concentration of free nafcillin (C) in the filtrate was determined by HPLC. The amount of nafcillin bound to MIP (Q) was calculated by subtracting the amount of free nafcillin from the initial quantity. Binding isotherm was determined in a concentration range of 0.1–4.0 mM. Data were processed with Langmuir–Freundlich (LF) fitting [25],

$$Q = \frac{N_a a^m}{1 + a^m}$$

where $N_a$ is the total number of binding sites, a is related to the median binding affinity constant $K_a (K_a = a^{1/m})$, and $m$ is the heterogeneity index (0–1, which is equal to 1 for a homogeneous material).

2.4. MISPE protocols

BLAs imprinted polymers were prepared via photopolymerization at 15 °C, ground to 25–38 μm particles and then packed into a short stainless steel column (10 × 4.6 mm ID) for MISPE. The MISPE column was first preconditioned with 4 mL of deionized water by a peristaltic pump (2 mL/min) to generate a neutral and hydrophilic environment. Then aliquots of 250 mL water samples containing BLAs were pumped through the column at a flow-rate of 2.5 mL/min. The selective washing procedure was performed with 2 mL methanol/water (1:3, v/v) and 1 mL methanol/water (4:1, v/v) in sequence. Finally, BLAs retained on the MISPE column were eluted with 2 mL of 0.2% tetrabutyl ammonium (TBA) in methanol. The flow-rate for selective washing and analytes elution was 2 mL/min. All fractions were collected and evaporated to dryness (at 15 °C under N2 stream). The residues were reconstituted in 100 μL acetonitrile and an aliquot of 20 μL was subjected to HPLC.

2.5. Sample collection and preparation

River water samples were collected from three locations (Site-1, Site-2 and Site-3, from upstream to downstream) of a drainage river in May 2008. The river connects with a drainage ditch of the pharmaceutical factories located in this area, and receives the effluent from two sewage treatment plants (STPs). The drainages emitted by these industries undergo hydrolyzation, acidification, anaerobic and aerobic treatments in STPs before entering the river. Away from this area by 5 km, another river is regarded far beyond the influence of the BLA drainage by preventing the wastewater from
entering its stream. Therefore, the water samples collected in this river were used as the references.

The water samples were filtered through 0.45-μm filter from Millipore (MA, USA) and stored at 4 °C in a refrigerator. SPE pre-treatment and HPLC analysis were carried out within 72 h after the samples collection, avoiding the biodegradation and hydrolysis of the labile BLAs. Tap water samples were directly collected from the water pipe in Shijiazhuang and Beijing, the treatment steps were similar to the river samples described above.

2.6. HPLC analysis

The detection of BLAs was performed on a Hitachi L-2000 HPLC system (Tokyo, Japan), which consists of a L-2130 low-pressure gradient pump, a L-2200 autosampler and a L-2455 diode array detector (DAD). A Capcell Pak C18 column (150 × 4.6 mm ID, 5 μm) from Shiseido (Tokyo, Japan) was employed for BLAs separation. Two solvents (A and B) were prepared as the mobile phases for gradient elution. Solvent A consists of water/acetonitrile (90:10, v/v) containing 0.06% formic acid, and solvent B is acetonitrile with 0.06% formic acid. The gradient program was as: 0–42% B (5 min), 42–60% B (12 min), 60% B (8 min). The flow-rate was 1.2 mL/min and injection volume was 20 μL. UV detection was set at 235 nm.

3. Results and discussion

3.1. Synthesis of BLA-imprinted polymers by photopolymerization

Imprinted polymers for five BLAs (penicillin G, amoxicillin, ampicillin, nafcillin and mezlocillin) were synthesized by UV-initiated photopolymerization at 15 °C or by thermally initiated polymerization at 40 °C and 60 °C. The imprinting factors (IF) for the photopolymerized BLA-MIPs are higher than that for the thermally polymerized BLA-MIPs with an exception of ampicillin-MIP (Fig. 1). The IF value is 2.86 for Nafc-MIP prepared by UV-initiated polymerization at 15 °C, whereas it declines to 1.84 and 1.72 when prepared by thermally initiated polymerization at 40 °C and 60 °C. Generally, polymerization at higher temperature would deteriorate the recognition selectivity of MIP polymers and bring about lower imprinting factors. This observation is consistent with the previous work [26]. The only exception is Ampi-MIP, in which an imprinting factor of 1.94 is obtained by thermally initiated polymerization at 40 °C, being slightly higher than those prepared by UV photopolymerization at 15 °C (1.81) and by thermal polymerization at 60 °C (1.28).

In additional to higher IF values, the BLA-MIP polymers prepared by photopolymerization at 15 °C also show less heterogeneity of their binding sites (Table 1). The heterogeneity of the binding sites in nafcillin-imprinted polymers was evaluated by the Langmuir–Freundlich (LF) model [25,27–29]. The heterogeneity index (m) is equal to 0.68 for Nafc-MIP prepared by photopolymerization at 15 °C, indicating the highest homogeneous distribution of the binding sites when compared with that of thermally polymerized Nafc-MIPs at 40 °C (m = 0.68) and 60 °C (m = 0.45).

The higher heterogeneity of thermally polymerized BLA-MIP is believed to be associated with the template degradation at higher temperature because these BLAs are not chemically stable at high temperature. We investigated the stability of the five β-lactam antibiotics at different temperature, corresponding to the selected temperature for photopolymerization (15 °C) and thermal polymerization (40 °C and 60 °C). It is evident that the stability of BLAs decreases with the increasing temperature (Fig. 2). The recovery for each BLA was in 92–98% at 15 °C (with UV irradiation), 74–89% at 40 °C, and 62–80% at 60 °C, respectively. The losses of initial BLAs at the elevated temperature (40 °C and 60 °C) should be attributed to their degradation. The degradation products formed during polymerization, including penicilloic and penilloic acids [4], may also serve as the templates, rendering multi-templates adapted recognition sites in the resultant MIP polymers and significantly tailing off the specificity of BLA-MIPs. Compared with the thermal polymerization at 40 °C or 60 °C, BLAs at 15 °C have the highest recovery (≥92%), which ensures the template remain nearly intact throughout the UV-initiated polymerization at 15 °C. Benefiting from the least degradation of BLAs under the reaction conditions of UV-initiated polymerization, photopolymerized BLA-MIPs exhibit both good homogeneity in recognition sites and good selectivity towards the imprinted BLA (Fig. 1 and Table 1).

BLA-MIPs and NIPs prepared by UV-initiated photopolymerization at 15 °C were used in followed investigations.

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**Table 1** Parameters for Langmuir–Freundlich fitting of nafcillin on nafcillin-MIP.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nafc-MIP(15)b</th>
<th>Nafc-MIP(40)b</th>
<th>Nafc-MIP(60)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (μmol/g)</td>
<td>32.64</td>
<td>41.25</td>
<td>27.82</td>
</tr>
<tr>
<td>a (μM)</td>
<td>0.69</td>
<td>0.45</td>
<td>0.58</td>
</tr>
<tr>
<td>m</td>
<td>0.87</td>
<td>0.68</td>
<td>0.45</td>
</tr>
</tbody>
</table>

*a N (μmol/g) is the total number of binding sites, a is related to the median binding affinity constant K0 (a1/m1), and m is the heterogeneity index (0–1, which is equal to 1 for a homogeneous material). b Nafc-MIP(15), Nafc-MIP(40) and Nafc-MIP(60) represent nafcillin-MIPs prepared at 15 °C (with UV), 40 °C and 65 °C.

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**Fig. 1.** Imprinting factor for each BLA-MIP prepared by UV-initiated polymerization at 15 °C, or thermo-initiated polymerization at 40 °C or 60 °C.

**Fig. 2.** Recovery of β-lactam antibiotics at 15 °C (with UV irradiation), 40 °C and 60 °C for 36 h. The initial concentration for each BLA is 25 mg/mL, which approximates to the concentration of BLAs involved in MIP synthesis.
environmental samples are mostly blow this level [6, 7], and the dependent manner. Unfortunately, the concentrations of BLAs in such a level (e.g. 2 \text{μg/L}) are already acceptable for quantitative analysis.

Locally decrease to a reasonable level of 85–125%. MISPE recoveries of pumping 250 mL BLAs test standard (0.2 \text{μg/L}) in water) through the MISPE columns, the obtained recoveries are 676% for penicillin G on Pen G-MIP, 1224% for amoxicillin on Amox-MIP, 750% for ampicillin on Ampi-MIP, 478% for nafcillin on Nafc-MIP, and 437% for mezlocillin on Mezl-MIP, respectively. However, when BLA concentration increases to 20 \text{μg/L}, MISPE recoveries dramatically decrease to a reasonable level of 85–125%. MISPE recoveries of BLAs in this range are already acceptable for quantitative analysis.

These results suggest the template bleeding of BLA-MIPs remains at the level of μg/L (ppb). The concentrations of BLAs blow such a level (e.g. 2 \text{μg/L}) will be overestimated in a concentration-dependent manner. Unfortunately, the concentrations of BLAs in environmental samples are mostly blow this level [6, 7], and the template bleeding will significantly influence on the quantitative analysis of the templated BLAs in environmental samples.

To get rid of template bleeding, pseudo-template MISPE was further developed by using the photopolymerized BLA-MIPs. Here, nafcillin was selected as the pseudo template because of its good thermal stability, together with high recovery and high selectivity of Nafc-MIP towards other BLAs analogues (Table 2 and Fig. 3).

3.2. Template bleeding and pseudo-template MIP for BLAs

Since MIPs are synthesized by using the target BLA as the template, trace amount of the template bleeding from the synthesized polymer may contaminate the samples, thereby deteriorating the accuracy and precision of the analysis [30, 31]. To evaluate the template bleeding, we investigated the recovery of the five template BLAs at the concentrations of 0.2, 2.0, and 20 \text{μg/L}. The MISPE recoveries of BLAs on their own MIPs are always much higher than that of other analogues (Table 2), indicating the good selectivity of BLA-imprinted polymers towards the template molecules. However, all recoveries of BLAs on their own MIPs are higher than 200% at low concentrations of 0.2 and 2.0 μg/L. For instance, pumping 250 mL BLAs test standard (0.2 μg/L in water) through the MISPE columns, the obtained recoveries are 676% for penicillin G on Pen G-MIP, 1224% for amoxicillin on Amox-MIP, 750% for ampicillin on Ampi-MIP, 478% for nafcillin on Nafc-MIP, and 437% for mezlocillin on Mezl-MIP, respectively. However, when BLA concentration increases to 20 μg/L, MISPE recoveries dramatically decrease to a reasonable level of 85–125%. MISPE recoveries of BLAs in this range are already acceptable for quantitative analysis.

Table 2

| MISPE recovery of β-lactam antibiotic on BLA-imprinted polymers. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| MIPs            | C (μg/L)        | Pen G-MIP       | Amox-MIP        | Ampi-MIP        | Nafc-MIP        | Mezl-MIP        |
| Penicillin G    | 0.2             | 676 ± 59        | n.d. a          | n.d.            | 72 ± 11         | n.d.            |
|                 | 2.0             | 279 ± 24        | 60 ± 23         | 55 ± 15         | 60 ± 10         | 37 ± 13         |
|                 | 20              | 106 ± 15        | 56 ± 27         | 63 ± 14         | 79 ± 3          | 56 ± 7          |
| Amoxicillin     | 0.2             | n.d.            | 1224 ± 542 b    | –               | 64 ± 9          | 72 ± 26         |
|                 | 2.0             | 57 ± 9          | 420 ± 116       | –               | 77 ± 10         | 60 ± 15         |
|                 | 20              | 67 ± 8          | 85 ± 34         | –               | 76 ± 4          | 68 ± 10         |
| Ampicillin      | 0.2             | 61 ± 10         | 58 ± 13         | 750 ± 318       | 79 ± 12         | n.d.            |
|                 | 2.0             | 53 ± 6          | 77 ± 31         | 219 ± 135       | 65 ± 7          | 53 ± 14         |
|                 | 20              | 69 ± 13         | 59 ± 8          | 95 ± 39         | 81 ± 10         | 69 ± 17         |
| Nafcillin       | 0.2             | 74 ± 6          | –               | 478 ± 103       | 102 ± 19        | n.d.            |
|                 | 2.0             | 84 ± 16         | –               | 216 ± 34        | 72 ± 20         | 55 ± 16         |
|                 | 20              | 49 ± 7          | –               | 124 ± 21        | 82 ± 14         | 72 ± 13         |
| Mezlocillin     | 0.2             | n.d.            | –               | 89 ± 16         | 437 ± 191       | n.d.            |
|                 | 2.0             | 66 ± 18         | –               | 77 ± 9          | 217 ± 85        | 57 ± 15         |
|                 | 20              | 44 ± 7          | –               | 91 ± 14         | 106 ± 39        | 84 ± 9          |

*a* Not detected (concentration < LOD).

*b* Not test in this experiment.

3.3. Pseudo-template MISPE

Fig. 3 shows the imprinting factors for 7 antibiotics on the five BLA-MIPs. All BLA-MIPs were synthesized by UV-initiated polymerization at 15 °C. As expected, each BLA obtained the largest IF on its own MIP, for instance, 2.43 for penicillin G, 1.86 for amoxicillin, 1.85 for ampicillin, 2.89 for nafcillin and 2.14 for mezlocillin. Furthermore, we found that most of BLAs had acceptable IF on the Nafc-MIP; they were 1.92 for penicillin, 1.87 for amoxicillin, 1.63 for ampicillin, 2.89 for nafcillin, and 2.02 for mezlocillin, respectively. In contrast, two structurally related cephalosporins antibiotics, cefradine and cefalexin, were not strongly retained on either MIP or NIP, exhibiting low IF values of 1.00–1.37 for cefradine, and 1.02–1.48 for cefalexin. This confirms the specificity of BLA-MIPs towards the antibiotics of penicillin’s family. These data suggest that the Nafc-MIP can selectively recognize penicillin, amoxicillin, ampicillin, and mezlocillin besides the template of nafcillin.

Based on above observations, nafcillin was chosen as the pseudo template for MIP preparation, and Nafc-MIP as the sorbent for MISPE. It was expected that, by using pseudo-template MIP, the selective detection of penicillin G, amoxicillin, ampicillin, and mezlocillin in the river and tap water samples can be achieved.

Fig. 4 shows recoveries of the four β-lactam antibiotics, phenol and naphthalene in each step during MISPE when percolating 250 mL water standards (spiked with 40 μg/L of each analyte and 0.4% acetonitrile) through the Nafc-MIP packed MISPE column. In this experiment, phenol was added as a model for the hydrophilic compounds, and naphthalene as a model for the hydrophobic compounds. The strong hydrophilic compound (phenol) in water samples could be cleaned up very well after the first two washing steps, while the four BLAs were still retained on the MISPE sorbent. The hydrophobic compound (naphthalene) was mainly eliminated in the second washing step. The hydrophobic naphthalene could
be well cleaned up by using 1 mL of methanol/water (4:1), and the loss of the antibiotics was not obvious. However, when more than 2.0 mL of such solvents or another 0.5 mL methanol was applied after the second washing step, larger losses of BLAs were found (20–50%). Therefore, 2 mL methanol/water (1:3, v/v) and 1 mL methanol/water (4:1, v/v) were adopted for selective removal of hydrophilic and hydrophobic interferers.

To fast elute the four BLAs form MISPE column, neutral or alkaline conditions were often required to avoid the undesirable hydrolysis of the β-lactam ring. For this purpose, an ion pair agent TBA was selected as additive to methanol, as it can enhance the elutropic strength of methanol by formation of ion pairs with the BLAs [19,20]. In this experiment, 2 mL of 0.2% TBA in methanol was adequate for analytes elution, and the recoveries of BLAs were over 78.8%. These optimal conditions for MISPE were selected in the followed experiments.

3.4. Method validation

The developed pseudo-template MISPE method for BLAs analysis was then validated using BLAs standard solutions. Aliquots of 250 mL of blank water were spiked with 1.0 mL BLAs stock solutions (in acetonitrile) to yield BLAs spiked standards with concentrations corresponding to 0.1, 0.2, 1.0, 2.0, 5.0, 10, and 20 µg/L. If not used immediately, these calibration standards and river samples were stored at −20°C in a fridge.

The linearity of the calibration curve was evaluated for each BLA over the range of 0.1–20 µg/L in river water and tap water with a correlation coefficient (r) ≥ 0.998. The limit of detection (LOD, S/N = 3) in river water was calculated to be 40 ng/L for penicillin G and mezlocillin, 25 ng/L for amoxicillin and ampicillin, respectively. LODs of BLAs in tap water were lower than that in river water, being 20 ng/L for penicillin G and mezlocillin, 12.5 ng/L for amoxicillin and ampicillin, respectively. The intra-day precision was evaluated by five repeated injections of each spiked standard (0.2, 2 and 20 µg/L) prepared in blank river water and tap water in the same day. Similarly, the inter-day precision was examined by performing the assays in three consecutive days. The intra- and inter-day precisions are well consistent with the limit of 20% required for method validation (Table 3).

The recovery of BLAs for the MISPE-HPLC analysis was also investigated (Table 3). All recoveries for the proposed method are more than 85% at the two typical concentration levels (0.2 and 2 µg/L), which are sufficient for quantitative determination of these BLAs in water samples.

3.5. Selective screening of trace β-lactam antibiotics in river and tap water

The developed method was applied to the screening of BLAs in river and tap water. It was found that the dominant BLAs in the drainage river were penicillin G and amoxicillin, whereas ampicillin and mezlocillin were present at very low levels, and even not detectable in some river water samples (Table 4). The result is consistent with the known fact that penicillin G and amoxicillin are the largest output antibiotics in the antibiotics factories nearby.

From the upstream to the downstream of the drainage river, the concentration of the BLAs in the river rapidly decreases with the

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**Table 3**

<table>
<thead>
<tr>
<th>BLAs</th>
<th>Concentration (µg/L)</th>
<th>Precision (%) (RSD) Intra-day (n=5)</th>
<th>Inter-day (n=3)</th>
<th>Recovery (%) (n=3)</th>
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<tr>
<td>Penicillin G</td>
<td>0.2</td>
<td>13.6 (9.4)</td>
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<td>2.0</td>
<td>8.8 (3.3)</td>
<td>12.1 (6.5)</td>
<td>88 ± 4 (79 ± 6)</td>
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<td>20</td>
<td>4.5 (-)</td>
<td>7.7 (-)</td>
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<td>11.4 (5.6)</td>
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<td>2.3 (-)</td>
<td>4.6 (-)</td>
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<tr>
<td>Ampicillin</td>
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<td>86 ± 7 (99 ± 8)</td>
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<td>8.3 (6.9)</td>
<td>91 ± 4 (94 ± 6)</td>
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<td>20</td>
<td>4.3 (-)</td>
<td>4.6 (-)</td>
<td>–</td>
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<tr>
<td>Mezlocillin</td>
<td>0.2</td>
<td>10.6 (4.2)</td>
<td>12.1 (9.2)</td>
<td>90 ± 3 (97 ± 4)</td>
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<td>98 ± 6 (102 ± 8)</td>
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<td>7.4 (-)</td>
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</tbody>
</table>

* Data outside the parentheses are for river water samples and inside the parentheses are for tap water samples.

* Recovery for MISPE-HPLC analysis of river (outside the parentheses) and tap (inside the parentheses) water standards spiked with 0.2 and 2 µg/L of BLAs.

* Not test.
incubating distance from the discharging source. The concentration of Penicillin G on Site-1 (near the discharging points) is 9.56 μg/L, which is approximately 40 times higher than that (0.29 μg/L) on Site-3 (10 km away from Site-1). Similarly, the concentrations of amoxicillin and ampicillin decline by 8 and 5 folds from Site-1 to Site-3, respectively. The decline in BLAs concentrations should be attributed to three causes, one being the dilution by freshwater from the STPs, the second the adsorption of BLAs to the river sediments, and the third the degradation of themselves into other related chemicals. Among the BLAs of interest, penicillin G is the most unstable one that can be easily degraded into high levels of penicilloyl and penilloic acids, penicilloylaldehyde, and isopenilaldehyde at high temperature or under an acidic condition [4, 32]. It should be stressed that the concentrations of these degradation compounds, typically at a level of hundreds of ppb, are much higher than those of the intact BLAs in this river, and most of them are generated from the degradation of BLAs during the wastewater treatment in STPs [4].

The matrix of the drainage river water cannot be completely cleaned up by the use of C18 based SPE (Fig. 5, trace a). The peak of amoxicillin is overlapped in the former peaks due to large amount of interferers. To remove these interferers, more washing steps in C18 SPE protocol are usually required [33], where almost 30–50% BLAs will be eluted from C18 sorbent during these steps. This trouble could be easily gotten rid of by the use of a simple MISPE strategy, in which all peaks in the chromatogram are well distinguished and integrated, and more than 74% of each BLA was collected from Shijiazhuang and Beijing (Table 4).

Another merit of the MIP sorbent is their good robustness, which enables their remarkable reusability. The same cartridge could be reused more than 60 times without losing its capability in MISPE (decline in recovery less than 10%).

4. Conclusions

This work describes a pseudo-template MISPE-HPLC method for simultaneous screening of five β-lactam antibiotics in river and tap water. BLA-imprinted polymers prepared by UV-initiated photopolymerization at 15 °C exhibit higher selectivity than those prepared by thermal polymerization at 40 °C and 60 °C. It was found that the template bleeding generally occurred when the concentration of the target BLA was blow 2 μg/L. This problem could be avoided by the use of pseudo-template MIP as the SPE sorbent. Relying on its good cross-reactivity and selectivity towards the other BLAs, nafcillin-imprinted polymer was finally selected as the pseudo-template MISPE sorbent for selective preconcentration of these penicillins. High recovery, precision and sensitivity were achieved for the pseudo-template MISPE-HPLC method despite the low concentration of BLAs in water samples. Those properties enabled the applications of pseudo-template MISPE for selective extraction and sensitive screening of BLAs in real environmental samples. The proposed method also provides an effective tool for monitoring the occurrence, distribution and fate of BLAs in the surface water.

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