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Development of a method for trace level determination of antibiotics in drinking water sources by high performance liquid chromatography-tandem mass spectrometry

Wenhuan Cheng[1,2], Lei jiang[3], Ning Lu[3], Lei Ma[1], Xiaoyan Sun[1,2], Yi Luo[4], Kuangfei Lin[2], Changzheng Cui*[2]

1. Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, Shanghai, China, 200237

2. State Environmental Protection Key Laboratory of Environmental Risk Assessment and Control on Chemical Process, School of Resources and Environmental Engineering, East China University of Science and Technology, Shanghai, China, 200237

3. National Engineering Research Center of Urban Water Resources, Shanghai, China, 200082

4. College of Environmental Science and Engineering, Ministry of Education Key Laboratory of Pollution Processes and Environmental Criteria, Tianjin Key Laboratory of Environmental Remediation and Pollution Control, Naikai University, Tianjin, China, 300071

*Corresponding author, Changzheng Cui, Tel: +86 21 64253988; Fax: +86 21 64253988; e-mail: cuichangzheng@ecust.edu.cn

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Abstract

The presence of antibiotics in drinking water sources is worthy of concern regarding their potentially harmful effects on drinking water quality. In this study, a sensitive and reliable method was developed for the detection of 14 antibiotics in drinking water sources based on solid phase extraction (SPE) and high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The primary parameters for the SPE procedure, including different SPE cartridges, pH value of the sample, extraction volume and washing conditions, were optimized to extract the analytes efficiently in a single step with improved recoveries. Chromatographic separation conditions and MS/MS parameters in multiple reaction monitoring (MRM) mode were optimized to improve the sensitivity and specificity of the method. The optimized method provided acceptable recoveries ranging from 60.5% to 103.3%. The validation study indicated that the method detection limits varied from 0.001 to 2.16 ng L^{-1}, and the method quantification limits varied from 0.003 to 6.74 ng L^{-1}. The precision of the method, expressed as relative standard deviation (RSD), ranged from 0.1% to 2.6% and from 0.3% to 3.8% for inter- and intra-day analysis, respectively. Assessment of matrix effects exhibited partial signal suppression from 1.2% to 28.7% for most analytes, but it indicated signal enhancement for tetracycline (15.2%) and oxytracycline (12.6%). The method was successfully applied to the determination of trace level of antibiotics in drinking water sources in East China. Up to 13 antibiotics were detected at concentration ranging from 0.16 to 147.05 ng L^{-1}, and the primary antibiotic residues belonged to the groups of fluoroquinolones and tetracyclines.

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48 **Keywords:** antibiotics; drinking water sources; solid phase extraction; HPLC-MS/MS

49 ## 1 Introduction

50 In recent years, antibiotics have been widely used for the treatment of human
51 infections and to promote growth at sub-therapeutic levels in livestock.[1-4] A
52 significant percentage of these administered antibiotics (30%-90%) is excreted
53 unchanged or in conjugated forms that can be readily converted back to the parent
54 compounds in the environment.[1,5,6] A recent study by Zhou et al.[7] reported the
55 occurrence of 50 antibiotics belonging to 11 classes in different water matrices.
56 Although their concentrations are usually below 1 μg L^{-1}, the long-term presence of
57 antibiotics in aquatic environments not only affects water quality but also accelerates
58 the development, maintenance and spread of (multi-) resistance of bacterial
59 pathogens,[2,8-10] which could eventually pose a serious threat to public health.

60 Concerns regarding the occurrence, transport and fate of antibiotics in aqueous
61 environments have been increasing in the past decade since detection of these
62 compounds has been reported in wastewater,[1,9,11-17] surface water,[13,14,18-20] ground
63 water[15], and even drinking water[21,22] and tap water[23] throughout the world. In China,
64 the average annual consumption of antibiotics is 25,000 tons,[24] and a variety of
65 antibiotics have been detected in certain surface waters, such as the Pearl River
66 (11-460 ng L^{-1}),[25] the Yellow River (3-300 ng L^{-1}),[26] the Huangpu River (0.17-313 ng
67 L^{-1}),[3,27] the Haihe River (26-210 ng L^{-1}) [4] and the Yangtze Estuary (0.03-219 ng L^{-1}).[10]
68 Furthermore, risk assessment of antibiotics by Yan et al.[10] demonstrated that
69 sulfapyridine and sulfamethoxazole could cause medium risk to daphnia in the

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70 Yangtze Estuary. Taking into account that certain surface waters are potential drinking
71 water sources, the possible presence of antibiotics in drinking water sources is of great
72 concern because of the unknown health effects of chronic low-level exposure to
73 antibiotics over a lifetime.

74 This work focused on the occurrence of 14 commonly used human and
75 veterinary antibiotics in the drinking water sources in East China, which is the most
76 developed and urbanized region in China. In this region, which has a population of
77 more than 400 million, antibiotics are being widely used for human infections and
78 livestock productions. Research by Jiang et al.[28] demonstrated that 11 antibiotics had
79 been detected in multiple wastewaters in Yangtze Delta. In addition, Yan et al.[10]
80 indicated the occurrence of 20 antibiotics in Yangtze Estuary. Given the
81 ineffectiveness of sewage treatment plants in eliminating the antibiotic medicines[8,29]
82 and the location of East China in the downstream portion of the Yangtze, wastewater
83 and surface water containing antibiotics may be released into the drinking water
84 sources of this region.

85 To the best of our knowledge, most of the studies on the fate of antibiotics in
86 aqueous environments focused on wastewater[1,9,11,14-16] and surface water.[13,18,19]
87 However, concentrations of the antibiotics in drinking water sources were rarely
88 determined. Furthermore, methods developed for the determination of antibiotics in
89 other matrix water bodies may be not appropriate for our study because of the
90 differences in the species of antibiotics analysed and the complicated matrix of
91 drinking water sources. Therefore, sensitive, reliable and selective methods for the

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determination of antibiotics in drinking water sources are urgently needed. Thus, the aims of the present study were (1) to develop a sensitive and reliable method for the determination of trace concentration levels of 14 selected antibiotics in drinking water sources; (2) to apply this method to determine the occurrence of these commonly used antibiotics in the drinking water sources in East China; (3) to provide a foundation for further studies of the occurrence, fate and potential health effects of antibiotics in drinking water sources.

2 Materials and Methods

2.1 Chemicals and reagents

Antibiotic standards of sulfonamides (SAs) including trimethoprim (TMP), sulfadiazine (SD), sulfamethazine (SMZ), sulfamethoxazole (SMX), sulfachlororyidazine (SCP), fluoroquinolones (FQs) including enrofloxacin (ENR), ofloxacin (OFL), norfloxacin (NOR), ciprofloxacin (CIP), tetracyclines (TCs) including tetracycline (TC), oxytracycline (OTC), macrolides (MLs) including roxithromycin (ROX), and chloramphenicols (CPs) including chloramphenicol (CAP) and thiamphenicol (TAP), were all purchased from Dr. Ehrenstorfer (Augsburg, Germany). Isotopically labelled $^{13}C_3$-caffein solution (1 mg mL^{-1} in methanol, purity 99%), used as surrogate, was obtained from Cambridge Isotope Laboratories(Andover, USA). Simatone was purchased from Sigma-Aldrich (Steinheim, Germany) and used as internal standard. The physicochemical properties of these compounds were summarized in Table S1 (see supplementary information).

HPLC grade acetonitrile (ACN) and methanol (MeOH) were purchased from

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Fisher Scientific UK Limited. Ultrapure water was prepared using a Milli-Q water system (Millipore, USA). Analytical grade formic acid (98.5%), hydrochloric acid (99%), sodium hydroxide (98.5%), and fluoride disodium ethylene diaminetetraacetic (99%) were obtained from Sigma-Aldrich.

Individual stock solutions of 14 antibiotics (100 mg L^{-1}) were prepared by dissolving each compound in methanol, and 1% (v/v) acetic acid were added in NOR, OFL and SD solutions to increase their solubility in methanol. The antibiotic stock solutions were stored at -20°C and renewed monthly considering their stability. Working standard solutions at a concentration of 1 mg L^{-1} were prepared by diluting the stock solutions before use and stored at 4°C in the dark.

2.2 Sample preparation

Water samples were collected from drinking water sources using pre-cleaned 2.5 L amber glass bottles. Once in the laboratory, the samples were vacuum-filtered through 0.7 μm glass fibre filters (Whatman GF/F, UK). Next, the filtrate was kept in the dark at 4°C and extracted by solid phase extraction (SPE) within 24 h.

2.3 Solid phase extraction

In the present study, solid phase extraction was selected to complete enrichment of the drinking water source samples. To obtain the maximum extraction efficiency, four primary extraction parameters, i.e., SPE cartridges (Isolute C18, Cleanert PEP and Oasis HLB), pH value of the sample (3.2±0.2, not adjusted and 9.6±0.1), the extraction volume (500 mL, 1000 mL and 2000 mL) and the washing conditions (0%, 5%, 10%, 15% and 20%, v/v), were optimized using 1000 mL of drinking water

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4 136 sources spiked with target antibiotics at a concentration of 50 ng L^{-1}. All experiments
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6 137 were carried out in triplicate.
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8 138 The SPE is conducted as follows: Adjust the pH of the water sample (500 mL,
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10 139 1000 mL or 2000 mL) to the desired value (3.2±0.2 or 9.6±0.1) using 5% (v/v) HCl
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12 140 and 0.5 mol L^{-1} NaOH according to the pK_a range of the target antibiotics, while the
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14 141 pH values of the non-adjusted samples were 7.5±0.5; Add 0.2 g Na_2EDTA and 1mL of
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16 142 100 μg L^{-1} $^{13}C_3$-caffein to the sample; Precondition the SPE cartridges (Isolute C18,
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18 143 Cleanert PEP and Oasis HLB) sequentially with 6 mL of methanol, 6 mL of Ultrapure
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20 144 water and 6 mL of Ultrapure water (the same pH value as the sample); Load the
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22 145 sample with flow rate approximately 3 mL min^{-1}; Rinse the cartridge using 10 mL
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24 146 Ultrapure water containing various percentages of methanol (0%, 5%, 10%, 15% or
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26 147 20%, v/v) and dry it for 20 min under vacuum; Elute the cartridge with 2×3 mL of
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28 148 methanol; Concentrate the eluant to approximately 100 μL in a 35°C water bath under
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30 149 a gentle nitrogen stream; Spike the concentrated eluant with 10 μL of 2 mg L^{-1}
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32 150 simatone and reconstitute with methanol-water (1:1, v/v) to a final volume of 1 mL.
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34 151 Before HPLC-MS/MS, 0.22 μm PTFE filters were used to remove any solid particles
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36 152 from the SPE extract. The final extracts were stored at -20°C and analysed as soon as
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38 153 possible.
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41 154 **2.4 High performance liquid chromatography-tandem mass spectrometry**
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43 155 **2.4.1 High performance liquid chromatography**
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45 156 Chromatographic separation of the antibiotics was performed in an Agilent
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47 157 Technologies 1260 HPLC system consisting of binary solvent manager and sample
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manager. Separation of compounds was performed with Agilent Zorbax Eclipse Plus C18 column (1.8 μm, 2.1 mm×100 mm) (Agilent Technologies, USA). To obtain the chromatographic separation and the higher signal intensity, several variables were studied including mobile phase A (deionized water with different concentration of formic acid additive, 0%, 0.05%, 0.1% and 0.2%, v/v), mobile phase B (acetonitrile, methanol and acetonitrile-methanol (2:1, v/v), flow rate (0.1 mL min^{-1}, 0.2 mL min^{-1}, 0.3 mL min^{-1} and 0.4 mL min^{-1}) and injection volume (2 μL, 5 μL and 10 μL).

For SAs, FQs, TCs and MLs, the elution gradient started with 85% A, decreased to 50% in 15 min, then to 5% in 1 min and held for 4 min, and finally back to initial conditions in 2 min and maintained for 6 min until the next injection. For CPs, the elution gradient was as follows: held at 80% A for 8 min, and decreased to 10% in 5 min and then reset to the initial conditions for 7 min.

2.4.2 Mass spectrometry

An Agilent 6430 triple quadruple mass spectrometer equipped with electrospray ionization (ESI) source was used for mass spectrometry analyses. SAs, FQs, TCs and MLs were analysed with positive ion mode electrospray ionization (ESI+), with the capillary voltage set to 4 kV, while CPs were analysed with negative ion mode electrospray ionization (ESI-), with the capillary voltage set to 3.5 kV. The ESI+ and ESI- were carried out by two separate procedures instead of one LC-MS/MS run using a polarity switch. Other instrument parameters for the analysis were set as follows: gas temperature, 350°C; gas flow, 11 L min^{-1}; nebulizer gas pressure, 15 psi. The analysis was performed in multiple reaction monitoring (MRM) mode and

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180 MS/MS parameters were optimized by infusing 2 mg L^{-1} of an individual standard
181 solution in the mobile phase (deionized water-acetonitrile, 1:1, v/v) directly into the
182 mass spectrometer under combined mode in a continuous-flow form. During the
183 infusion, the parameters (fragment, collision energy) were optimized for each
184 antibiotic to obtain the maximum sensitivity with the highest amount of product ions
185 available.[20] The two most sensitive product ions were selected, of which the most
186 abundant product ion was chosen for quantification (marked with "*") and the other
187 for further confirmation.[9] Dwell time for each transition was set to ensure the number
188 of cycles in one second were between 3 and 3.5.

189 **2.5 Matrix effects**

190 A significant barrier in quantitative analysis with ESI-MS is the matrix effect
191 because the ESI source is more susceptible to matrix components (i.e., humic and
192 fulvic acids), which may result in a signal enhancement or suppression leading to
193 quantitation unreliablility[2,7,14] In the present study, matrix effects for each antibiotic
194 were expressed as a percent decrease in peak area in a sample matrix versus in
195 standard solution based on the method of Vieno et al.[15] (see supplementary
196 information).

197 **2.6 Quantification and method validation**

198 Antibiotics were quantified by an internal standard method using the highest
199 intensity precursor ion/product ion transitions. $^{13}C_3$-caffein was added to each water
200 sample as surrogate to monitor the recovery. Simatone was applied as the internal
201 standard to enhance analytical precision. Considering the unavailability of certain

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202 isotope labelled compounds, the use of multiple internal standards and/or surrogates is
203 constrained, although it is preferred for the analysis of multiple compounds with
204 different physicochemical properties.[4,18]

205 Nine concentration levels of 1, 2, 5, 10, 20, 50, 100, 200 and 500 μg L^{-1} were
206 prepared by serial dilution of the working standard solutions (1 mg L^{-1}) with
207 methanol-water (1:1, v/v). Nine-point multi-compound internal standard calibration
208 was applied for quantification of antibiotics based on the ratio of the peak area of the
209 quantitative product ion to the peak area of the internal standard.

210 The method detection and method quantification limits (MDL and MQL,
211 respectively) were determined for Ultrapure water spiked with known concentrations
212 of antibiotics and extracted according to the procedure described in Section 2.3. No
213 antibiotics were present in extracts of Ultrapure water prior to their enrichment with
214 antibiotics. The MDL and MQL were calculated using a signal-to-noise ratio of 3 and
215 10, respectively.

216 Recoveries of antibiotics and the surrogate ($^{13}C_3$-caffein) were determined for
217 drinking water sources at three spiking concentration levels (10, 50 and 100 ng L^{-1})
218 with three replicates. Because these spiked samples contained target compounds, no
219 spiked water samples were analysed as the blanks.[16] All samples were subject to the
220 SPE extraction procedures described above. The recoveries were determined by
221 comparing the concentrations measured, calculated by subtracting the blanks from the
222 spiked samples, with the initial spiking levels.[14,16]

223 Precision was expressed as the relative standard deviation (RSD). Both intra- and

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224 inter-day precisions of the assay were evaluated. Precision was determined from
225 triplicate spiked drinking water source samples at three levels (10, 50 and 100 ng L^{-1})
226 during the same day (repeatability) and in 3 successive days (reproducibility).[9,18]

227 **2.7 Drinking water sources application**

228 The developed method was used to determine the levels of antibiotic residues in
229 two drinking water source sites located in East China in December 2013. The sample
230 collection and preparation procedures used were the same as described in Section 2.2.
231 All experiments were performed in duplicate.

232 **2.8 Statistical analysis**

233 Qualitative Analysis software (B.04.00) was used for instrumental control,
234 chromatograms acquisition and qualitative analysis, while Quantitative Analysis was
235 used for accurate quantification. All duplicate or triplicate data in this study were
236 expressed as the mean.

237 3 Results and Discussion

238 3.1 Optimization of solid phase extraction

239 3.1.1 Effect of SPE cartridges and sample pH

240 The selection of SPE cartridges and pH of the water sample proved to be crucial
241 for the simultaneous analysis because antibiotics are complex molecules which
242 possess different functions within a single molecule.[30] In this study, three different
243 SPE cartridges corresponding with three pH values were evaluated to obtain an
244 acceptable recovery for target antibiotics characterized by different physicochemical

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245 properties. The solid phase extraction materials tested were two polymeric sorbents
246 (Cleanert PEP and Oasis HLB) and a nonpolar sorbent (Isolute C18). Simultaneously,
247 three values of pH were studied. The SPE was performed according to Section 2.3.

248 As shown in Fig. 1, significantly different extraction efficiencies were observed
249 among the different solid phase extraction materials. The lowest recoveries were
250 obtained with Isolute C18 cartridges. The recoveries for most antibiotics were lower
251 than 40% (except for TMP, ENR and OFL) under basic conditions and less than 20%
252 when extracted under acidic or not adjusted conditions (except for TMP and ROX).
253 For Cleanert PEP and Oasis HLB, there were no significant differences in the
254 recoveries for most analytes under not adjusted conditions except those for ENR, OFL
255 and ROX. Under acidic and basic conditions, however, recoveries with Oasis HLB
256 cartridges were more than 3% to 45% for the majority of analytes compared with
257 Cleanert PEP cartridges. Isolute C18 is an octadecyl (uncapped) functionalized silica
258 sorbent; it is suitable for the retention of hydrophobic compounds. However, for types
259 of antibiotics with larger polarity differences, Isolute C18 was found not to be a good
260 choice in this study. Cleanert PEP and Oasis HLB are both polymeric sorbents and
261 provide good conditions for the simultaneous extraction of hydrophilic and
262 hydrophobic compounds from water. However, compared to Cleanert PEP, Oasis HLB
263 had been shown to be much more efficient, yielding higher recoveries for most
264 analytes. This could be attributed to the fact that Oasis HLB cartridges are composed
265 of hydrophilic N-vinyl pyrrolidone and lipophilic divinylbenzene in a specific ratio
266 and are able to improve the retention of polar compounds by a “special capturing

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group". Therefore, based on the special structure of this sorbent, Oasis HLB has been shown to provide excellent retention of acidic, neutral and basic compounds at a wide range of pHs.[9,15]

Because of the amphoteric properties of most of the analytes, the recoveries could be strongly effected by different pH conditions.[16] It can be observed that at pH 9.6±0.1, recoveries of FQs were more than 70%, whereas those of TCs, MLs, SD and SMX were less than 40%. By contrast, under not adjusted conditions, the recoveries of SAs (except for TMP) and TCs were higher than 80%, while those of FQs were lower than 30% (except for ENR). For CPs, no significant differences were observed between Cleanert PEP and Oasis HLB whether under acidic, not adjusted or basic conditions with approximately 100% recoveries. The results that CPs were hardly influenced by the pH sample values was consistent with those of Tong et al.[16] The recoveries of all analytes were within acceptable ranges from 62.8% to 102.2% when using Oasis HLB cartridges under acidic conditions at pH 3.2±0.2, which meets the demand to obtain an acceptable recovery for all target analytes simultaneously.

3.1.2 Effect of extraction volume

An appropriate extraction volume allows the enrichment of the maximum amount of target analytes without the occurrence of breakthrough. Generally, extraction volumes of 100 mL[9,14,15] and 250 mL[2,15] were selected for wastewater influent and effluent, respectively; while 500 mL[4,15] or 1000 mL[3,15,18,25] was selected for surface water and ground water. In this study, 500 mL, 1000 mL and 2000 mL of drinking water source samples spiked at 50 ng L^{-1} were evaluated. As shown in Fig. 2,

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289 the recoveries of analytes ranged from 60.2% to 103.2%, 63.9% to 102.0 and 49.4%
290 to 91.8% with extraction volumes of 500 mL, 1000 mL and 2000 mL, respectively.
291 Antibiotic recoveries were not improved with extraction volume increasing; on the
292 contrary, a large degree of analyte loss occurred in 2000 mL conditions. This may be
293 due to the breakthrough that occurred when extracted with 2000 mL of water samples
294 and only some of the target compounds in the sample were adsorbed or the matrix
295 components that increased with the analytes being enriched, resulting in the decrease
296 of recoveries. Though there was only a small difference in the range of recovery
297 between the extraction volumes of 500 mL and 1000 mL, the number of antibiotics
298 whose recoveries were more than 80% was greater with 1000 mL extraction volumes.
299 Hence, extraction volume of 1000 mL was selected.

300 **3.1.3 Effect of washing conditions**

301 Prior to the elution step, the cartridge was washed with a certain percentage of
302 methanol aqueous to reduce matrix effects. Matrix effects are known to cause
303 suppression of the analyte signals during electrospray ionization and also shorten the
304 lifetime of the chromatographic column.[15] The results obtained were shown in Fig. 3.
305 It can be observed that the presence of methanol in the washing solvent helped to
306 reduce the effect of matrix components but also reduced the recovery of analytes to a
307 great extent when the percentage was higher than 10%. Therefore, a concentration of
308 5% (v/v) methanol was selected because this could effectively remove some of the
309 matrix components without causing significant analyte losses.

310 **3.1.4 Breakthrough determination for HLB cartridge**

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Either high sample loads or high analyte concentration may result in the breakthrough of analytes, which would seriously decrease the recovery.[2] In the present study, breakthrough was assessed by extracting spiked drinking water source samples using two stacked cartridges. After the two stacked cartridges were eluted separately, the amount of analyte in the second cartridge eluent indicated the extent of breakthrough.[2,9]

For breakthrough studies, 1000 mL of water sample spiked to a relatively high concentration of 100 ng L^{-1}, which may hardly occur in drinking water sources, was loaded through two stacked cartridges. No antibiotics were detected in the second cartridge eluent for drinking water source samples at the spiked concentration. Therefore, all the analytes were well-enriched by the first HLB cartridge, and no breakthrough was observed in this study.

3.2 LC-MS/MS analysis

Chromatographic separation was crucial for obtaining higher sensitivity and selectivity of MS/MS detection. Several main factors affecting chromatographic resolution and signal intensity were studied using a standard mixture of 5 μg L^{-1}. The following optimization procedures were conducted for antibiotics ionized in positive ionization mode because only two antibiotics were analysed in negative ionization mode. Representative chromatograms of a 100 μg L^{-1} standard mixture of the analytes analysed in positive ion mode and negative ion mode are illustrated in Fig. 4.

In this study, acetonitrile, methanol and methanol-acetonitrile (2:1, v/v) were evaluated as options of organic mobile phase (mobile phase B). A sharp

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333 chromatographic separation with respect to resolution and peak shapes was obtained
334 using acetonitrile as organic mobile phase for almost all the analytes (Figure S1, see
335 supplementary information). Deionized water with different concentrations of formic
336 acid additive was studied as aqueous mobile phase (mobile phase A). Formic acid
337 concentrations of 0%, 0.05%, 0.1% and 0.2% (v/v) were evaluated for the
338 optimization of chromatographic separation (Figure S2, see supplementary
339 information). Previous studies have demonstrated that the addition of formic acid into
340 mobile phase improves the chromatographic separation and ionization efficiency,
341 especially in positive ESI mode.[2,9] Without formic acid addition, the ESI signal for
342 TCs and FQs were seriously enhanced because amphoteric antibiotics occur mainly in
343 the cationic forms at acid pH values.[9] However, at higher concentrations of formic
344 acid, the chromatographic separation showed poor peak shapes and decreased
345 ionization efficiencies. Therefore, formic acid at the concentration of 0.1% (v/v) was
346 chosen as the optimal results.

347 The effect of flow rate and injection volume was also studied. Flow rates from
348 0.1 to 0.4 mL min^{-1} were assayed (Figure S3, see supplementary information).
349 Compared to 0.2 mL min^{-1}, the chromatogram of 0.1 mL min^{-1} showed poor peak
350 shape and a smaller number of separated peaks (total 7 peaks), while the first three
351 peaks were slightly overlapping when the flow rate > 0.3 mL min^{-1}. Considering the
352 resolution, peak shape, intensity of the response and retention times, 0.2 mL min^{-1} was
353 selected as the optimal flow rate. Injection volumes of 2 μL, 5 μL and 10 μL were
354 tested, and 5 μL was chosen as the optimal results because severe tailing was

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355 observed for the peaks of most analytes under 10 μL of injection volume (Figure S4,
356 see supplementary information).

357 For mass spectrometry, the optimized MS/MS parameters and retention times are
358 summarized in Table 1. Among the 14 target antibiotics, most analytes were analysed
359 in positive ion mode (ESI+) except for CAP and TAP, which were more sensitive in
360 negative ion mode (ESI-).

361 **3.3 Matrix effects**

362 Matrix components in water samples could decrease the real concentration of the
363 analytes by adsorbing freely dissolved antibiotics, mask the analyte peaks by raising
364 the chromatogram baseline or reduce ionization efficiency of the analytes by
365 competing for the limited charged sites on electrospray droplets so that the signal
366 intensity of antibiotics is suppressed to some extent.[1,18,31-34] In this study, the signal
367 suppression (or enhancement) value of each antibiotic was calculated by Eq. (1) (see
368 supplementary information) and the results were summarized in Table S2 (see
369 supplementary information). It can be concluded that the signal intensity of antibiotics
370 belonging to the same class were generally suppressed or enhanced to a similar degree.
371 No significant matrix effects were found for SAs, MLs and CPs, while more severe
372 signal suppression was observed for FQs, especially NOR and CIP, for which
373 approximately 30% of signal intensity was lost during the analyses. Therefore, the
374 lower SPE recoveries for NOR and CIP are probably due to the suppression of the
375 signal during electrospray ionization. The conclusion that FQs are more susceptible to
376 signal suppression than other antibiotics was consistent with that of Renew et al.[1] and

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377 Dorival-García et al.[9]. However, obvious signal enhancement was observed for TCs as
378 the signal enhancement values were 15.2% and 12.6% for TC and OTC, respectively.
379 The matrix enhancement effect for TCs was also reported by Zhou et al.[7] This
380 phenomenon can be explained by the fact that signal suppression for the internal
381 standard is higher than for the analyte.[7,18]

382 **3.4 Method validation**

383 Linearity, sensitivity, trueness and precision, as well as the study of matrix
384 effects, were considered as criteria for the validation of the analytical methodology
385 developed.[14] This provided a more accurate estimation of the loss of sensitivity,
386 difficulties during sample treatment and interference and is a way of evaluating the
387 real potential of the analytical method.[9]

388 Linearity was evaluated with the linear correlation coefficient (R^2). Good
389 linearity of the method was observed over the established concentration range (1-500
390 $\mu g\ L^{-1}$) with R^2 higher than 0.99 for all analytes.

391 The MDL varied from 0.001 to 2.16 ng L^{-1}, while the MQL ranged from 0.003 to
392 6.74 ng L^{-1}. The low concentration levels of MDL and MQL makes the method useful
393 for the determination of trace levels of antibiotics in relatively clean aqueous
394 environments such as drinking water sources.

395 The recoveries achieved for all analytes ranged from 60.5% to 103.3%. The
396 lower recovery rates for NOR, CIP and ROX (60.5%-64.7%, 62.8%-70.7% and
397 64.6%-67.2%, respectively) was not considered to be an obstacle for their reliable
398 determination because the acceptable repeatability and reproducibility levels made

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them still applicable.[14]

For precision of the method, The intra- and inter-day variabilities were below 2.6% and 3.8%, respectively, indicating that the method is highly reproducible and reliable. The validation results were summarised in Table 2.

3.5 Occurrence of antibiotics in drinking water sources

The antibiotic concentrations measured using the developed method were presented in Table 3. In total, 13 antibiotics were detected in Site 1 and 11 antibiotics were detected in Site 2. Significant differences in the distribution of target antibiotics were observed between the two sites. Considerably higher concentrations were found in Site 2 compared with Site 1. This may be attributed to a lesser degree of contamination from terrestrial sewage, especially from wastewater treatment plants, for Site 1. FQs were the predominant antibiotic class detected in Site 1, while for Site 2, the main antibiotic residues were TCs, although the abundant concentration of 147.1 ng L^{-1} for NOR. Antibiotics of SAs, TCs and MLs were the most frequently detected antibiotics in 100% of the samples, with the highest concentration for TCs, followed by SAs and MLs sequentially in both sites. For CPs, TAP was found at the concentration of 11.8 ng L^{-1} and 29.7 ng L^{-1} for Site 1 and Site 2, respectively, while CAP was not detected in any site. Overall, the data indicate that the developed method is suitable for environmental monitoring of the trace concentration antibiotics in drinking water sources.

4 Conclusions

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A sensitive and reliable method was developed for trace analysis of 14 antibiotics belonging to five classes in drinking water sources based on SPE procedure and HPLC-MS/MS analysis. Several important parameters affecting the SPE procedure and HPLC-MS/MS analysis were optimized. Method validation results indicated that the whole method was reliable with acceptable recoveries and high sensitivities for all targeted antibiotics. The method had been demonstrated to be successful for the determination of trace level of multiple antibiotics in two drinking water source sites in East China. In addition, the analytical method may be used for more in-depth studies of the fate and potential health effects of antibiotics in drinking water source environments.

Acknowledgement

This research was supported by the National Water Pollution Control and Management Technology Major Projects (No. 2012ZX07403-002) and the National Environmental Protection Public Welfare Science and Technology Research Program of China (No. 201309031) and Shanghai Rising-Star Program, China (No. 14QB1400800).

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502 **Table 2** Recoveries, precisions, detection limits and quantification limits of the
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508 **Fig. 2.** Influence of different extraction volumes on the recoveries of selected
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511 recoveries of selected antibiotics in 1000 mL of drinking water source spiked at 50 ng
512 L^{-1}.

513 **Fig. 4.** Example of a HPLC-MS/MS chromatogram of a standard mixture at 100 μg
514 L^{-1} for target compounds analyzed by (A) positive ion mode and (B) negative ion
515 mode.

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524 **Table 1** Optimized MS/MS parameters for the target antibiotics by MRM mode.

Compounds	Retention time (min)	Precursor Ion (m/z)	Product Ion (m/z)	Fragment (V)	Collision Energy (eV)	Polority
Sulfonamides (SAs)						
TMP	5.34	291.2	230.1* 123.1	135	23	+
SD	5.48	251.2	156.0* 92.1	100	15	+
SMZ	7.61	279.1	186.0* 156.0	105	15	+
SMX	10.12	254.2	156.1* 108.1	100	15	+
SCP	9.43	285.1	156.1* 92.2	95	15	+
Fluoroquinolones (FQs)						
ENR	6.75	360.2	342.2* 316.3	125	20	+
OFL	5.77	362.2	318.3* 261.2	125	18	+
NOR	5.70	320.2	302.2* 233.2	115	20	+
CIP	6.01	332.2	314.2* 288.2	125	18	+
Tetracyclines (TCs)						
TC	6.70	445.2	410.2* 154.2	120	18	+
OTC	5.96	461.2	426.2* 443.1	115	18	+
Macrolides (MLs)						
ROX	12.40	837.5	158.2* 679.5	155	35	+
Chloramphenicols (CPs)						
CAP	7.72	321.1	152.2* 257.0	105	11	-
TAP	6.13	354.0	184.9* 289.9	125	15	-
Surrogate and internal standard						
Simatone	6.77	198.2	128.1* 170.1	125	20	+

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caffeine-$^{13}C_3$	5.02	198.1	140.1* 112.1	105	20	+

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553 **Table 2** Recoveries, precisions, detection limits and quantification limits of the method.

Compounds	Spiked	Recovery	Precision			
	($ng\ L^{-1}$)		repeatability	reproducibility	method detection limits	method quantification limits
		(%) (n=9)	Intra-day(%)[a] (n=3)	Inter-day(%)[a] (n=9)	MDL $ng\ L^{-1}$	MQL $ng\ L^{-1}$
	10	70.3	0.1	1.2		
TMP	50	83.5	0.9	2.2	0.005	0.019
	100	77.5	0.1	0.3		
	10	84.2	1.6	2.1		
SD	50	91.8	2.0	2.5	0.018	0.057
	100	86.6	0.7	2.2		
	10	79.5	1.2	2.7		
SMZ	50	82.1	2.6	3.2	0.006	0.020
	100	85.4	0.5	1.2		
	10	85.5	0.7	2.6		
SMX	50	87.0	1.9	2.0	0.003	0.011
	100	97.2	0.3	3.8		
	10	84.9	2.1	3.4		
SCP	50	79.6	1.4	1.9	0.001	0.004
	100	81.6	0.8	1.3		
	10	73.6	0.3	2.7		
ENR	50	74.3	1.8	2.9	0.26	0.83
	100	87.9	1.0	3.5		
OFL	10	79.2	0.9	1.1	0.15	0.52

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	50	89.1	1.8	2.4		
	100	80.6	0.7	2.1		
	10	64.7	2.3	3.2		
NOR	50	60.5	0.7	3.1	0.82	2.67
	100	63.5	0.5	1.6		
	10	62.8	0.8	2.8		
CIP	50	70.7	0.2	1.5	1.21	3.85
	100	69.9	0.4	1.6		
	10	79.2	2.5	3.7		
TC	50	73.2	1.2	2.6	1.74	5.60
	100	81.6	1.6	1.9		
	10	84.3	0.9	2.5		
OTC	50	78.9	0.8	1.9	2.16	6.74
	100	86.2	0.2	2.3		
	10	64.6	1.7	1.9		
ROX	50	66.1	0.4	2.3	0.001	0.003
	100	67.2	0.3	1.5		
	10	103.3	0.2	1.3		
CAP	50	92.7	0.7	0.4	0.25	0.83
	100	102.5	1.1	0.6		
	10	101.2	0.3	1.1		
TAP	50	98.4	0.9	0.8	0.58	1.91
	100	100.6	0.1	0.7		
	10	88.0	0.2	0.3	0.003	0.010
caffeine-$^{13}C_3$	50	87.8	0.1	0.5		

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	100	94.7	0.9	1.5

[a] Relative standard deviation (RSD, %)

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3 571 **Table 3** Concentration of antibiotics in two drinking water source sites in East China.

	[a] Concentration (ng L^{-1})						
	TMP	SD	SMZ	SMX	SCP	ENR	OFL
Site 1	0.16±0.04	1.80±0.02	1.25±0.15	6.26±0.06	1.68±0.10	10.62±0.07	8.51±0.06
Site 2	1.78±0.34	17.53±0.97	24.06±0.50	17.94±0.54	1.77±0.38	nd[b]	16.92±0.99
	NOR	CIP	TC	OTC	ROX	CAP	TAP
Site 1	27.36±0.18	41.11±0.21	14.60±0.42	21.56±0.56	0.36±0.03	nd	11.84±0.08
Site 2	147.05±1.38	nd	53.62±0.13	129.33±0.74	0.63±0.15	nd	29.66±0.10

572 [a] Concentration were expressed as Average±Standard deviation.

573 [b] Not detected.

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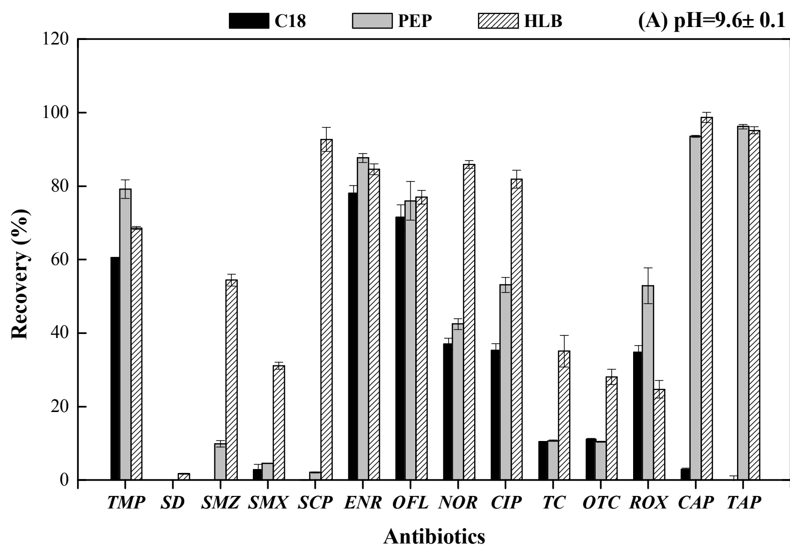

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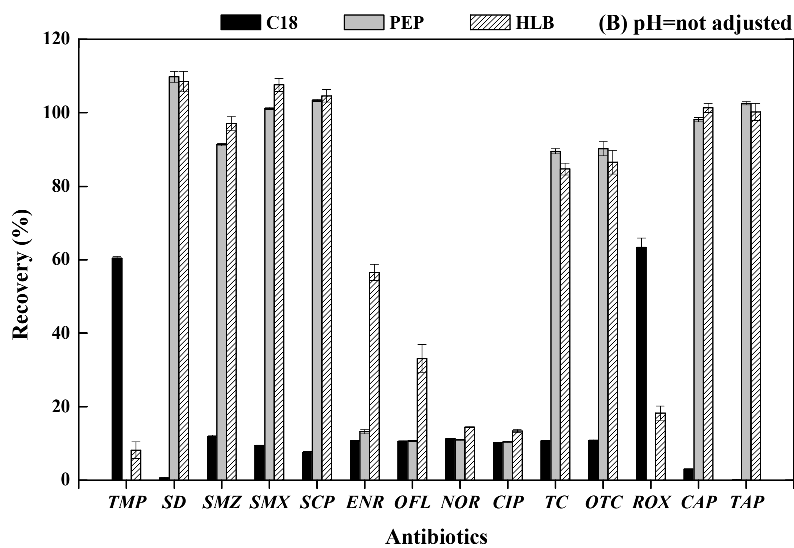

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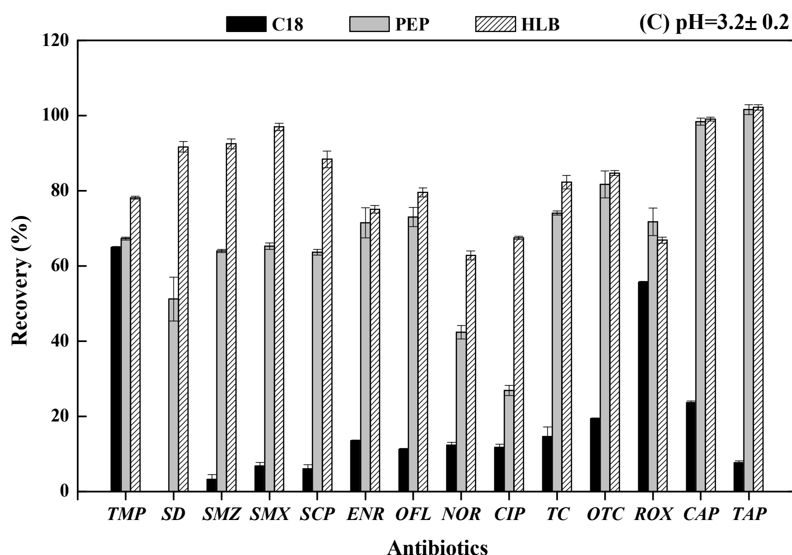

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22 604 **Fig. 1.** Influence of SPE materials and pH on the recoveries of selected antibiotics in
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24 605 1000 mL of drinking water sources spiked at 50 ng L^{-1} (A) Influence of SPE materials
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26 606 on the recoveries of selected antibiotics when water sample was adjusted to pH 9.6±
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28 607 0.1; (B) Influence of SPE materials on the recoveries of selected antibiotics when
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30 608 water sample was not adjusted; (C) Influence of SPE materials on the recoveries of
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32 609 selected antibiotics when water sample was adjusted to pH 3.2±0.2.
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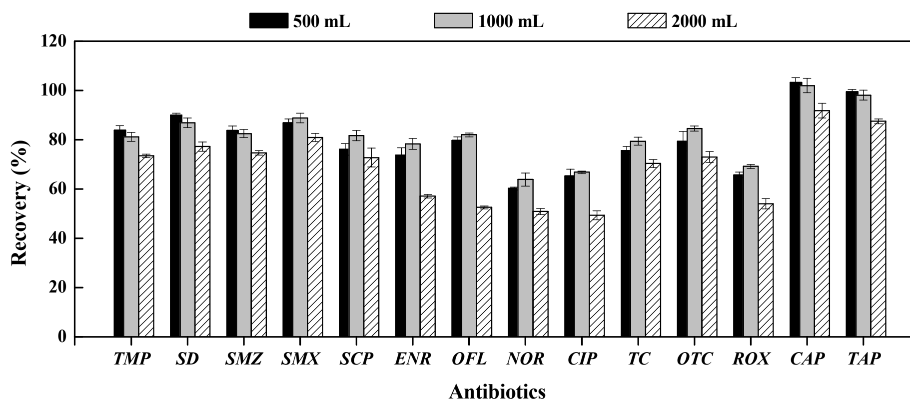

625
626 **Fig. 2.** Influence of different extraction volumes on the recoveries of selected
627 antibiotics in 1000 mL of drinking water sources spiked at 50 ng L^{-1}.
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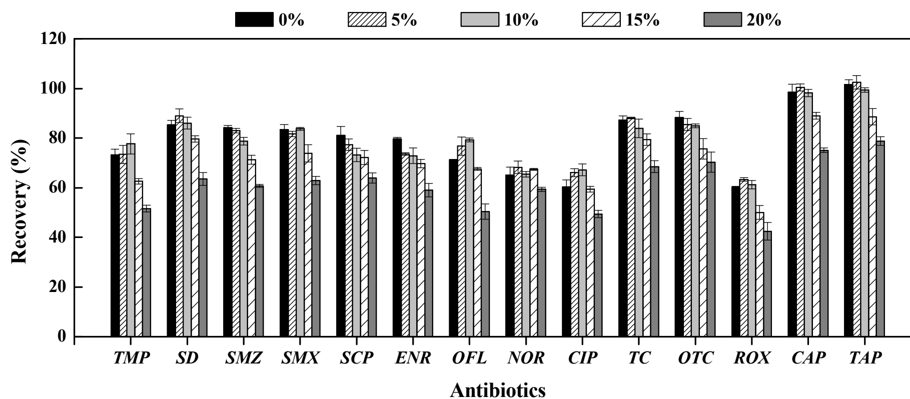

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653 **Fig. 3.** Influence of different percentages of methanol in washing solvent on the
654 recoveries of selected antibiotics in 1000 mL of drinking water sources spiked at 50
655 ng L^{-1}.

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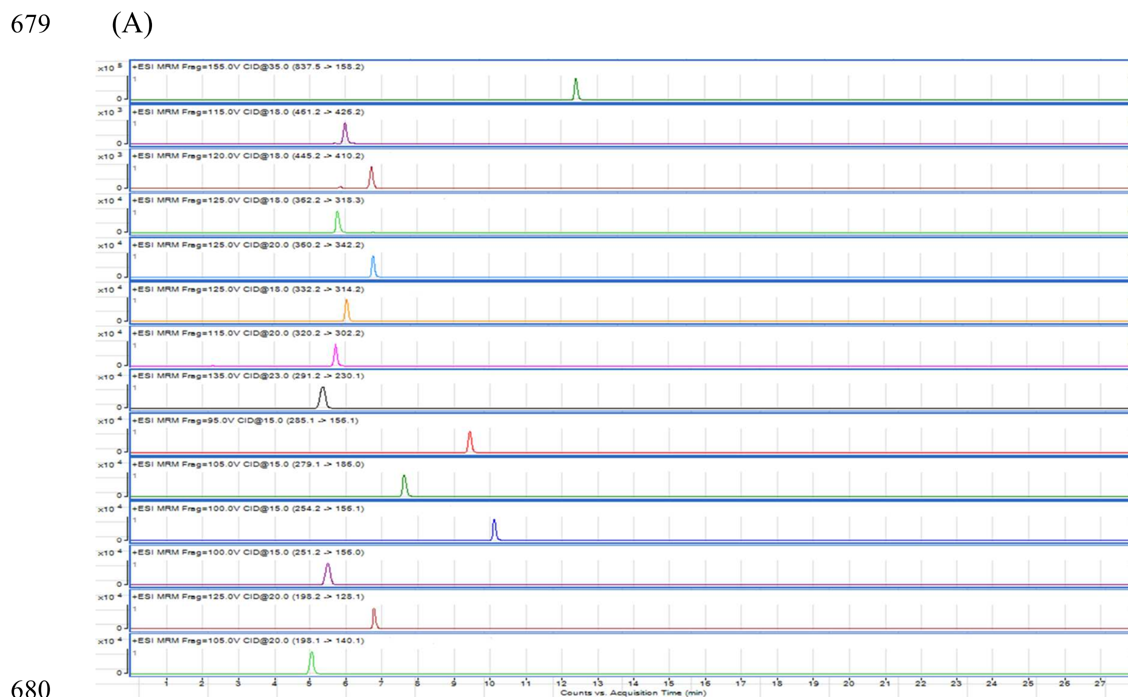

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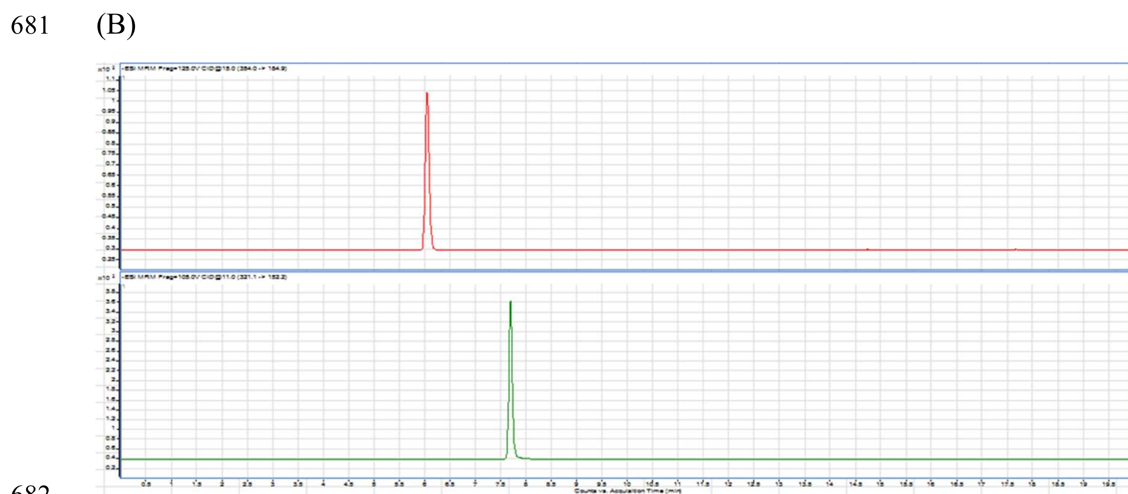

Fig. 4. Example of a HPLC-MS/MS chromatogram of a standard mixture at 100 µg L^{-1} for target compounds analyzed by (A) positive ion mode and (B) negative ion mode.

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