Journal of Chromatography A, 1190 (2008) 390–393

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

# Simultaneous analysis of 16 sulfonamide and trimethoprim antibiotics in environmental waters by liquid chromatography–electrospray tandem mass spectrometry

Hong Chang[a], Jianying Hu[a,*], Mari Asami[b], Shoichi Kunikane[b]

[a] College of Urban and Environmental Sciences, Peking University, Beijing 100871, China
[b] Department of Water Supply Engineering, National Institute of Public Health, Saitama 351-0197, Japan

ARTICLE INFO

*Article history:*
Received 28 November 2007
Received in revised form 19 February 2008
Accepted 11 March 2008
Available online 25 March 2008

*Keywords:*
Environmental analysis
Silica cartridge cleanup
Sulfonamides
LC–ESI-MS/MS

ABSTRACT

A sensitive liquid chromatography–electrospray tandem mass spectrometry method combined with solid-phase extraction and silica cartridge cleanup was established for 16 sulfonamides and trimethoprim in various water matrices. Signal suppression of all target analytes in sewage treatment plant influent, effluent and river water was improved by this method developed in this study. The method detection limits for 17 analytes were 20–200 pg/L for influent, 16–120 pg/L for effluent and 8.0–60 pg/L for river water with overall mean recoveries of 62–102% in all studied matrices. This method was used to analyze residual sulfonamides and trimethoprim in wastewater and river samples from Japan, and 8 analytes (0.08 (sulfadimethoxine)–161 ng/L (sulfapyridine) in wastewater and 10 (0.03 (sulfamethizol)–8.9 ng/L (sulfaquinoxaline) in river samples were detected.



## 1. Introduction

Sulfonamides (SAs) are broad-spectrum synthetic antibiotics, widely used to treat diseases and infections for both humans and livestock since their development in 1968 [1]. Due to the potential spread and maintenance of bacterial resistance, environmental scientists have paid an increasing concern for their occurrence and fate in the environment [2,3]. To assess the environmental risk of SAs, there is a need for sensitive identification of these compounds in various environmental waters.

High-performance liquid chromatography–electrospray tandem mass spectrometry (HPLC–ESI-MS/MS) combined with off-line solid-phase extraction (SPE) is increasingly used to quantify SAs in surface, drinking and sewage treatment waters [4–7]. In those reports, Oasis hydrophilic–lipophilic balanced (HLB) cartridge is generally used to enrich and extract of SAs from water samples with relatively low sample volume (e.g. 50 or 100 mL sewage treatment plant (STP) influents or primary effluents) [6,7]. Despite the low enrichment factor and the high specificity of MS/MS detection, a general and well-known problem of matrix effects (e.g. ionization suppression and isobaric interferences) still existed as exemplified by over 40 and 60% of sulfamethoxazole and trimethoprim signals being suppressed. To obtain low limits of detection, which are necessary for the environmental analysis of SAs, a high enrichment factor is desirable, and thus more matrixes will be introduced into the detection instrument. One study has used an anion-exchange cartridge stacked on top of HLB cartridge to purify two SAs and trimethoprim in effluents; however, no obvious improvement on significant matrix suppression was achieved [8].

Recently, an improved LC technology called ultra-performance liquid chromatography (UPLC) distinguishing from conventional HPLC can be expected to reduce co-extracted interferences and then reduce signal suppression due to the great improvement of chromatographic resolution by the use of 1.7 μm porous stationary phases [9]. In this paper, this improved LC system coupled to MS/MS was applied to the analysis of 16 SAs and trimethoprim in STP influents, effluents and river waters, where a silica cartridge was used to purify the water samples. The 16 SAs were selected based on the occurrence reported in previous studies [4–7] or the potential to be present in the environment, and trimethoprim, a synthetic antibiotic, is commonly used in combination with SAs and thus also included in this study.

## 2. Experimental

### 2.1. Reagents and materials

Sixteen SAs (sulfamethoxypyridazine (SMP), sulfamoxol (SMO), sulfaquinoxaline (SQX), sulfanitran (SNT), sulfisomidine (SIM),

* Corresponding author. Tel.: +86 10 62765520; fax: +86 10 62765520.
*E-mail address:* hujy@urban.pku.edu.cn (J. Hu).

0021-9673/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2008.03.057

sulfamethoxazole (SMX), sulfamerazine (SMR), sulfathiazole (STZ), sulfadiazine (SDZ), sulfamethizol (SMT), sulfadimidine (SDMD), sulfadimethoxine (SDM), sulfapyridine (SPD), sulfisoxazole (SIA), sulfachloropyridiazine (SCP), sulfameter (SME)) and trimethoprim (TMP) were all obtained from Sigma (St Louis, MO, USA), and surrogate standard [$^{13}C_6$]sulfamethazine ($^{13}C_6$-SMA, 90%), was obtained from Cambridge Isotope Labs. (Andover, MA, USA). Methanol, dichloromethane, ethyl acetate, and hexane were all HPLC grade purchased from Fisher Chemical (Japan). SPE cartridges (Oasis HLB, 500 mg/6 mL and Sep-Pak silica, 500 mg/3 mL) were from Waters (Milford, MA, USA). Glass fiber pads (GF/F, 0.7 μm) were obtained from Whatman (Maidstone, UK). Stock solutions (1000 mg/L in methanol) for all standard substances were prepared, and stored at −20 °C.

### 2.2. Sample collection

Influent and final effluent samples were collected from two STPs in Saitama, Japan on 27 July 2007. Both STPs receive mainly domestic wastewater, and are operated with primary, aerobic biological and secondary treatments. Four river water samples were collected from Koyama river basin which is known as a major farming area in Saitama prefecture, Japan. All samples were extracted on the same day after being filtered on a glass microfiber filter GF/F 0.7 μm (Whatman).

### 2.3. Sample extraction and cleanup

To avoid SPE cartridge plugging, suspended materials were removed by filtration with glass fiber pad. After filtration, 250 mL of influent, 500 mL of effluent and 1 L of river water added with 0.5 g/L $Na_2EDTA$ and 50 ng/L of surrogate standard were extracted through an Oasis HLB cartridge, previously conditioned with 6 mL of dichloromethane, 6 mL of methanol and 12 mL of 50 mM $Na_2EDTA$. The cartridge was washed with 10 mL of distilled water, and then was dried under a flow of nitrogen. Dichloromethane/methanol (2:1, v/v; 6 mL) was used to elute the analytes from SPE cartridge, and the extracts were dried under a gentle nitrogen stream. The dry residues were redissolved in chloroform, and then 1.8 mL of hexane was added. The mixed solutions were applied to silica SPE cartridge, which had been preconditioned with 4 mL of hexane. After the cartridges were rinsed with 3 mL of hexane, 3 mL of hexane/ethyl acetate (90:10, v/v) and 3 mL of hexane/ethyl acetate (3:2, v/v), the analytes were eluted with 3 mL of methanol/acetone (1:1, v/v) followed by 3 mL of acetone. The solution was evaporated to dryness under a gentle stream of nitrogen, and reconstituted with 0.5 mL of methanol for LC–MS/MS analysis.

### 2.4. LC–MS/MS analysis

The LC apparatus was an Acquity Ultra Performance LC (Waters). All analytes were separated using a Waters Acquity UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μm particle size). The column was maintained at 40 °C at a flow rate of 0.3 mL/min, and the injection volume was 2 μL. Methanol (A) and water containing 0.1% formic acid were used as mobile phases. Gradient conditions were initiated with 3% A (hold for 0.5 min) followed by a linear increase to 65% A in 5.7 min, and then to 100% in 0.8 min (hold for 1.5 min).

Mass spectrometry was performed using a Waters TQ detector operated with an ESI interface in positive ionization mode. The capillary voltage was set at 3.0 kV. The flow rates of desolvation gas and cone gas were set to 900 and 50 L/h, respectively. The source temperature and desolvation gas temperature were held at 120 and 400 °C, respectively. Quantitative analysis was performed in multi-selected reaction monitoring (MRM), and the two most abundant MRM transitions and the relative ratios between them were listed in Table 1.

**Table 1**
Two multi-selected reaction monitoring (MRM) transitions and their relative ratios

| Compound | MRM transition | Relative ratio |
|---|---|---|
| SCP | 285 > 156<br>285 > 207 | 0.78 |
| SDM | 311 > 156<br>311 > 92 | 1.39 |
| SDMD | 279 > 156<br>279 > 124 | 0.98 |
| SDZ | 251 > 156<br>251 > 92 | 0.96 |
| SIA | 268 > 156<br>268 > 113 | 1.36 |
| SIM | 279 > 124<br>279 > 186 | 2.53 |
| SME | 281 > 156<br>281 > 108 | 2.14 |
| SMO | 268 > 156<br>268 > 108 | 2.03 |
| SMP | 281 > 156<br>281 > 108 | 2.23 |
| SMR | 265 > 156<br>265 > 110 | 1.77 |
| SMT | 271 > 156<br>271 > 92 | 1.31 |
| SMX | 254 > 156<br>254 > 92 | 0.64 |
| SNT | 336 > 156<br>336 > 198 | 2.04 |
| SPD | 250 > 156<br>250 > 184 | 3.13 |
| SQX | 301 > 156<br>301 > 108 | 2.03 |
| STZ | 256 > 156<br>256 > 92 | 1.18 |
| TMP | 291 > 230<br>291 > 123 | 1.38 |
| $^{13}C_6$-SMA | 285 > 186 | |

### 2.5. Quantitation

Identification of the 17 analytes was accomplished by comparing the retention time (within 2%) and the ratio (within 20%) of the two selected precursor ion-production ion transitions with those of standards. To automatically correct the losses of analytes during sample preparation and the matrix-induced change in ionization, and to compensate for variations in instrument response from injection to injection, surrogate standard ($^{13}C_6$-SMA) were used in this study.

All equipment rinses were done with methanol to avoid sample contamination, and laboratory blanks were analyzed to assess potential sample contamination. Recovery experiments were done by spiking standard solutions to wastewater from a STP and a river water sample. Analyte addition was made with the criterion of at least three times the original concentration that was determined prior to the fortification experiment.

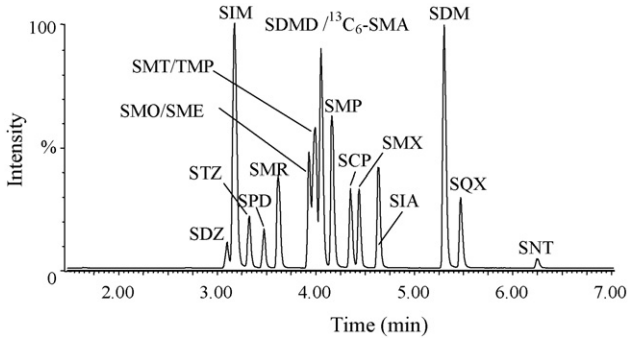


**Fig. 1.** LC–MS/MS total ion chromatogram showing the separation of the 17 target analytes (10 μg/L standard solution).

## 3. Results and discussion

### 3.1. Method development and validation

Fig. 1 shows the LC–MS–MS chromatogram of the 17 analytes when using the 1.7 μm, 100 mm × 2.1 mm Acquity C18 column at a flow rate of 0.3 mL/min. It could be found that very sharp peaks were obtained with peak width of 5–10 s at base, and the 17 analytes were eluted in less than 6.5 min which would provide short analysis time and a high sample throughput. Very low instrumental detection limits (IDLs) were obtained for the 17 analytes (0.02–0.2 pg). Calibration curves were constructed for the standard solutions between 50 and 70,000 ng/L, and the coefficients of determination ($r^2$) were typically greater than 0.99. In the recovery experiments, the overall mean recoveries of all target analytes in the influent, effluent and river water ranged from 62 to 102% with an RSD lower than 9.2%; simultaneously, the original concentrations of analytes in three separate samples were determined in duplicate on two different days and the RSD was <10% for analytes in various matrices, indicating good performance of the methodology developed in this work. The method detection limits (MDLs), defined as the amount of analyte that produced a signal-to-noise of 3 (peak to peak) reach from several to hundreds of pg/L in various water matrices. The MDLs of the 17 analytes are 20–200 pg/L for the influent, 16–120 pg/L for the effluent, and 8.0–60 pg/L for the river water, which are much lower than the previous reports for corresponding type of environmental matrices using HPLC–MS/MS analysis. Gros et al. [7] reported on the MDLs of SMX and TMP being 42 and 25 ng/L for wastewater influent, 20 and 10 ng/L for effluent, and 5 and 1 ng/L for river water; Botitsi et al. [5] reported on the MDLs of five SAs and TMP being 7.0–10 ng/L for wastewater effluent; and Ye and Weinberg [4] reported the MDLs for seven SAs and TMP in chlorinated drinking water ranged from 0.5 to 2.1 ng/L.

Since the improved chromatographic separation by the improved LC system could be expected to reduce co-extracted interferences, we evaluated the extent of signal suppression/enhancement in LC–ESI/MS/MS detection by spiking the influent, effluent and river water extracts obtained with high enrichment factors (500, 1000 and 2000, respectively). The signal suppression observed with each analyte was calculated using the percentage of signal intensity in a sample matrix versus the signal of the same concentration in the pure solvent (methanol). And results showed that generally less than 30, 20, and 10% of signal suppression for all target analytes were found in the influent, effluent and river water, respectively.

### 3.2. Environmental samples

Two influent, two effluent and four river samples were duplicate analyzed by this method. The mean concentrations of detected analytes are listed in Table 2. The precision of the method was good as determined by RSD being <10%. Of the 17 analytes, 12 (SDZ, SPD, STZ, SIM, SMR, SMT, SDMD, TMP, SMX, SDM, SQX and SIA) were detected in water samples; 8 analytes except for STZ, SMT, SDMD and SQX, were detected in wastewater samples with the concentrations ranging from 0.08 ng/L (SDM) to 161 ng/L (SPD) and 10 analytes except for SMR and SIA were found in river water samples with the concentrations ranging from 0.03 ng/L (SMT) to 8.9 ng/L (SQX). Fig. 2 shows the MRM LC–MS/MS chromatograms of the extracts from a river water sample with and without cleanup. It can be found that the signal/noise (S/N) ratios for all detected analytes with silica cartridge cleanup were largely improved by removing several isobaric interferences. Especially while a distinguishable peak of STZ was found with the cleanup procedure, no detectable signal in the chromatogram was obtained without cleanup.

It should be noted that SIM, SMT, SDM, and SQX were reported for the first time in the environmental waters. In the wastewaters, SPD, SMX and TMP were detected at higher concentrations than other analytes, possibly due to the fact that SPD or SMX was often used to treat human diseases together with TMP. Comparing the influent and effluent concentrations of detected analytes, we could find that SPD and SMX in effluents were detected higher than in influents, and SIM, SMR and SIA was detected in effluents but not detected in influents. The very likely reason may be due to the retransformation of *N*(4)-acetylated SAs (the main form of human metabolites) to the active parent SAs during the wastewater treatment as exemplified by the retransformation of *N*(4)-acetylsulfamethazine to the active parent compound sulfamethazine during the storage of manure [10] and the retrans-

**Table 2**
Concentration (ng/L) of target analytes detected in wastewater and river samples of Japan

| | STP1 | | STP2 | | River 1 | River 2 | River 3 | River 4 |
|---|---|---|---|---|---|---|---|---|
| | Influent | Effluent | Influent | Effluent | | | | |
| SDZ | –[a] | 3.8 | 3.7 | 1.9 | 0.04 | 0.05 | – | – |
| SPD | 127 | 98 | 154 | 161 | 3.0 | 3.0 | 1.5 | 0.62 |
| STZ | – | – | – | – | 1.7 | 6.6 | 4.4 | 0.08 |
| SIM | 3.1 | 0.62 | – | 0.06 | 0.05 | 0.48 | 0.08 | 0.17 |
| SMR | – | – | – | 0.21 | – | – | – | – |
| SMT | – | – | – | – | 0.03 | 0.07 | 0.04 | 0.03 |
| SDMD | – | – | – | – | 0.14 | 0.13 | – | – |
| TMP | 42 | 26 | 14 | 11 | 0.24 | 0.13 | 0.34 | 0.09 |
| SMX | 6.9 | 28 | 27 | 24 | 0.40 | 0.38 | 0.37 | 0.56 |
| SDM | 2.0 | 0.08 | 4.1 | 2.4 | 0.17 | 0.07 | 0.05 | 0.11 |
| SQX | – | – | – | – | – | – | 8.9 | – |
| SIA | – | – | – | 0.13 | – | – | – | – |

[a] Under the method detection limit.

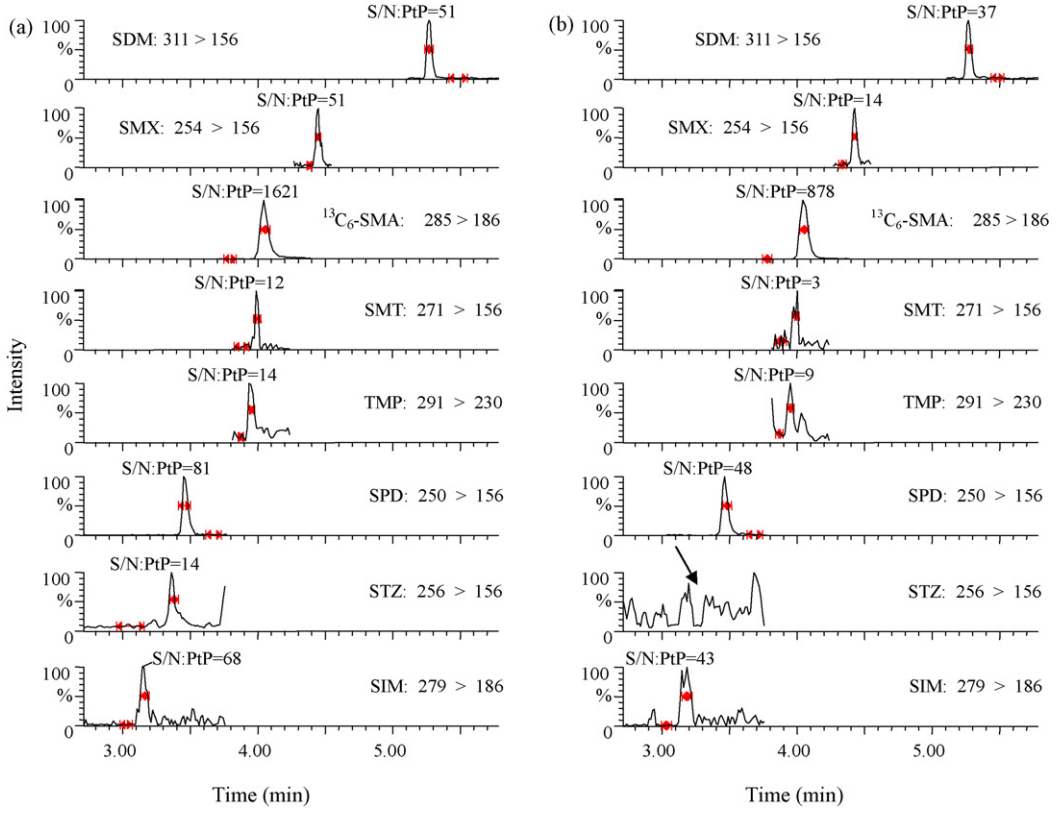


**Fig. 2.** LC–MS/MS MRM chromatograms of analytes detected in a river sample: (a) with cleanup and (b) without cleanup.

formation of *N*(4)-acetyl-SMX to SMX during wastewater treatment [11]. In addition, STZ, SMT, SDMD and SQX, not detected in the wastewaters, were found in the river waters possibly due to the fact that the four SAs would be used to treat animals as these river water samples were taken from the downstream of animal feeding operation sites.

## 4. Conclusions

A LC–MS/MS method with higher sensitivity and separation efficiency was established for analyzing 16 SAs and TMP in various water matrices by adapting a cleanup method on silica cartridge. This technique significantly improved the sensitivity for analyzing these compounds in environmental waters compared with the previous methods.

## Acknowledgements

Financial support from the National Natural Science Foundation of China (20610103 and 40471116) and the National Basic Research Program of China (2006DFA91130) is gratefully acknowledged.

## References

[1] N.A. Littlefeild, W.G. Sheldon, R. Allen, D.W. Gaylor, Food Chem. Toxicol. 28 (1990) 157.
[2] C.G. Daughton, T.A. Ternes, Environ. Health Perspect. 107 (1999) 907.
[3] L. Guardabassi, D.M.A.L.F. Wong, A. Dalsgaard, Water Res. 36 (2002) 1955.
[4] Z.Q. Ye, H.S. Weinberg, Anal. Chem. 79 (2007) 1135.
[5] E. Botitsi, C. Frosyni, D. Tsipi, Anal. Bioanal. Chem. 387 (2007) 1317.
[6] A. Göbel, C.S. Mcardell, M.J.F. Suter, W. Giger, Anal. Chem. 76 (2004) 4756.
[7] M. Gros, M. Petrović, D. Barceló, Talanta 70 (2006) 678.
[8] J.E. Renew, C.H. Huang, J. Chromatogr. A 1042 (2004) 113.
[9] M.E. Swartz, Ultra Performance Liquid Chromatography (UPLC): An Introduction, Separation Science Redefined (Special Issues of LC–GC N. Am.), May 2005.
[10] K. Berger, B. Petersen, H. Büning-Pfaue, Arch. Lebensmittelhyg. 37 (1986) 85.
[11] A. Göbel, A. Thomsen, C.S. Mcardell, A. Joss, W. Giger, Environ. Sci. Technol. 38 (2005) 3981.