Application Note: 397

Determination of Sulfonamide Antibiotics in Wastewater by Liquid Chromatography–Tandem Mass Spectrometry

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Key Words

- TSQ Quantum™ Ultra
- Surveyor™ HPLC System
- Environmental Application
- Sensitivity
- Solid Phase Extraction (SPE)

Introduction

Antibiotics are widely used in human and veterinary medicine for the prevention and treatment of bacterial infectious diseases. An important but often disregarded aspect of antibiotic use is the fate of antibiotic residues entering the environment.[1] Pharmaceutical industry wastewater, improperly-disposed of unused antibiotics, and non-metabolized antibiotics excreted by humans can all enter the sewer system in low concentrations. Because sewage treatment plants are rarely equipped to filter these drugs from wastewater, antibiotics are released into the water system. Veterinary antibiotics used in livestock operations are another major source of antibiotics in the environment. Agricultural waste such as manure and water runoff can carry these antibiotics into the soil and groundwater.

The effects of antibiotics in the environment are still poorly understood. One major concern is the development of antibiotic resistant strains of bacteria that could critically disturb the natural bacteria ecosystems and lead to a serious threat to human health. There are also concerns that, exposure to environmental antibiotic residues might lead to carcinogenic or allergic reactions in humans and create hazards to aquatic and soil organisms.[2,3]

Sulfonamides (Figure 1) are a common class of synthetic antimicrobials that are widely used in human and in veterinary medicine and as feed additives to promote growth in concentrated animal feeding operations. They are regarded as emerging contaminants that are introduced into the environment predominantly in the USA and Europe. There is no regulation of the levels of these compounds in environmental matrices (water, sediment, soil). This is likely because of the limited knowledge of the input, fate, and effects of most pharmaceuticals in the environment. Therefore, sensitive and reliable analytical methods for detection of low concentrations (ng/L) of these compounds are needed.

Goal

To develop methods for the determination of sulfonamide antibiotics at trace levels in effluent wastewaters.

Experimental Conditions

Sample Preparation

Samples of secondary effluent were collected from sewage treatment plants in Greece and then vacuumed filtered. Each 50 mL sample was diluted with 200 mL deionized water. After acidification to pH 4, 5 ng of the internal standard d4-sulfamethoxazole (d4-SMX) was added before enrichment to assess possible losses during the analytical procedure. The effluent samples were enriched by solid phase extraction (SPE). The diluted wastewater samples were percolated through the cartridges at a flow rate of 5 mL/min. The cartridges were then washed with 5 mL deionized water. Wastewater organics were eluted with 2×4 mL methanol. The solvents were evaporated under a stream of nitrogen gas and then the extracts were redissolved in 0.5 mL mobile phase A (0.1% formic acid in water).

HPLC

HPLC analysis was performed using the Surveyor HPLC System (Thermo Fisher Scientific, San Jose, CA). Each 20 µL sample was injected directly onto a 150×2.1 mm, 3.5 µm, C_{18} HPLC column. A gradient LC method used mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) at a flow rate of 0.2 mL/min.

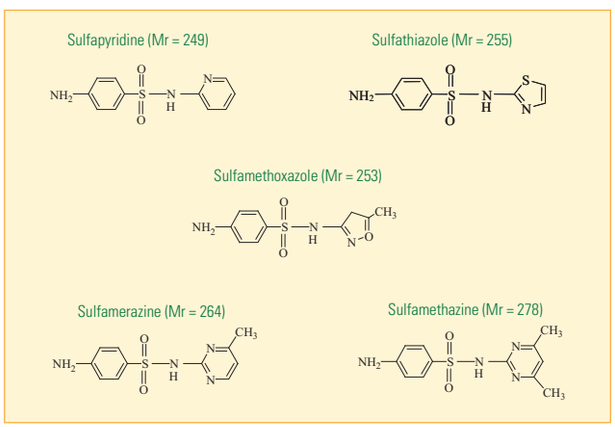

Figure 1: Chemical structures of some sulfonamide compounds

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MS

MS analysis was carried out on a TSQ Quantum Ultra triple stage quadrupole mass spectrometer with an electrospray ionization source (Thermo Fisher Scientific, San Jose, CA).

The MS conditions were as follows:

Ion source polarity: Positive ion mode
Sheath gas pressure (N_2): 40 units
Ion transfer tube temperature: 350 °C
Collision gas pressure (Ar): 1.0 mTorr
Q1 resolution: 0.2 FWHM, Q3 resolution: 0.7 FWHM
Dwell time: 0.2 s
Scan Type: SRM

Table 1 summarizes the SRM transitions that were monitored. MS detection of the target compounds was divided into three time segments on the basis of their retention times during chromatography. The protonated molecular ion of the compound $[M+H]^+$ was selected as the precursor ion. Detection was performed in the multiple reaction monitoring mode using, usually, the two most intense and characteristic precursor/product-ion transitions obtained from the MS/MS optimization procedure. Identification criteria for the target compounds were based on the LC retention time (t_R) and on the ratio of the two monitored transitions for each compound.

Method accuracy and precision were evaluated by recovery studies, using deionized water spiked with appropriate amounts of the sulfonamides at three concentrations (2 ng/L, 20 ng/L, and 200 ng/L). Calibration plots were obtained by analysis of standard solutions at eight concentrations in the range 0.1 µg/L–100 µg/L (2 pg–2000 pg injected).

Results and Discussion

The method validation data are summarized in Table 2. Linearity of the method was assumed because the r^2 values were greater than 0.99 for the linear regression equations (1/x weighted) and the residuals were less than 20% for each calibration point in the concentration range 0.1 µg/L–100 µg/L. Quantification was performed on the basis of external calibration plots using the peak area of the most intense transition of the analyte. For SMX, quantification was performed using the ratios of the peak areas of the most abundant monitored ion of the analyte to that of the respective ion of the surrogate standard d4-SMX.

The accuracy of the method was determined by recovery studies conducted on spiked deionized water samples at three concentration levels: 2 ng/L, 20 ng/L, and 200 ng/L. The precision of the method was determined by repeated intra-day (n=3) and inter-day analysis (n=6) of samples spiked at the three concentrations. High rates of recovery were achieved, usually greater than 72%, and relative standard deviations for inter-day analysis (n=6) ranged between 3.1% and 19.0%. The SRM chromatograms obtained from deionized water spiked at a concentration of 2 ng/L are shown in Figure 2.

The method was applied to the wastewater samples to investigate the occurrence of sulfonamide antibiotics. Sulfamethoxazole was detected in all of the samples. The median concentration was 150 ng/L. The SRM chromatograms of SMX in the wastewater effluent extract are shown in Figure 3.

Compound	Retention time (min)	Precursor ion $[M+H]^+$ *m/z*	Product ions *m/z*	CE (V)
Sulfapyridine, SPY	10.1	250	108, 156	20
Sulfamethoxazole, SMX	26.2	254	108, 156	25
Sulfathiazole, STZ	10.3	256	108, 156	17
Sulfamerazine, SMR	12.0	265	108, 156	17
Sulfamethazine, SMZ	18.6	279	186, 204	24
D4-Sulfamethoxazole, D4-SMX	26.0	258	112, 160	25
D4-Sulfathiazole, D4-STZ	10.0	260	112, 160	20

Table 1: Diagnostic ions of sulfonamide antibiotics

Compound	R^2* concentration range 0.1–100 µg/L	LOD (µg/L)	Mean Recovery (± %RSD) 2 ng/L H_2O (n=6)	20 ng/L H_2O (n=6)	200 ng/L H_2O (n=6)
Sulfapyridine, SPY	0.997	0.053	77 (±7.8)	83 (±5.1)	120 (±8.4)
Sulfamethoxazole, SMX	0.999	0.055	102 (±7.5)	99 (±5.5)	110 (±7.2)
Sulfathiazole, STZ	0.999	0.054	79 (±14.0)	83 (±17.0)	106 (±4.2)
Sulfamerazine, SMR	0.998	0.110	80 (±15.3)	87 (±3.1)	120 (±7.2)
Sulfamethazine, SMZ	0.999	0.110	72 (±19.0)	77 (±14.0)	116 (±8.4)

*linear fit calibration curves with 1/x weighting, (n=5 replicates)

Table 2: Validation data (linearity, accuracy, precision)

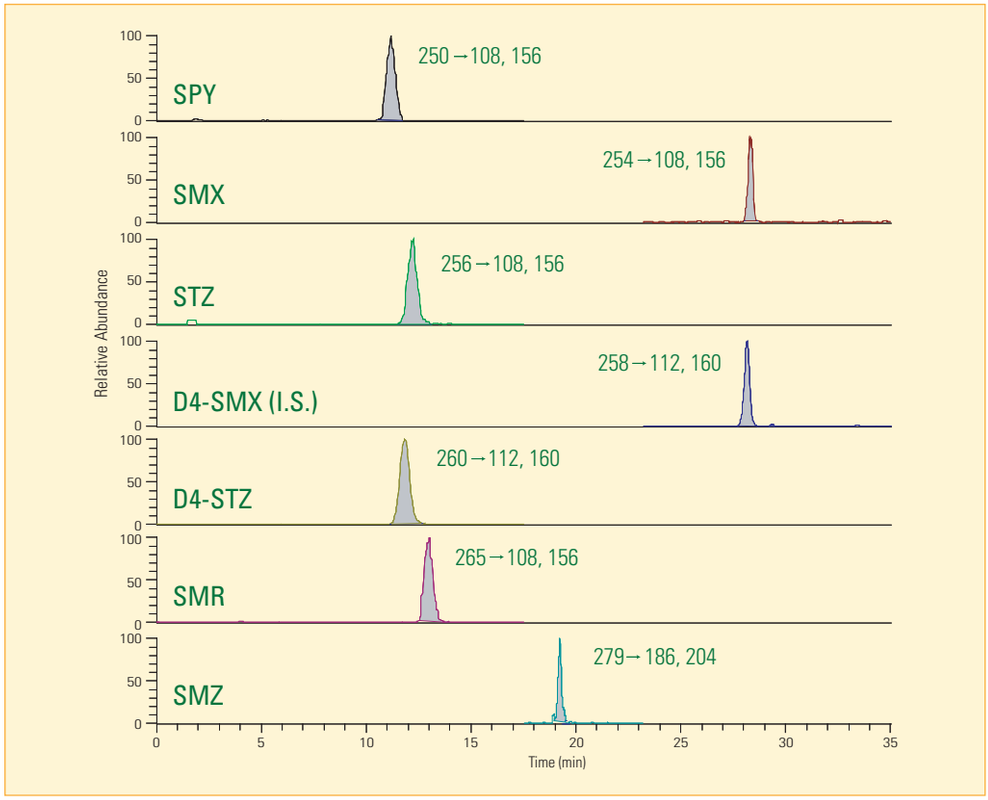

Figure 2: LC-ESI(+)-MS/MS SRM chromatograms of a spiked (2 ng/L) deionized water extract

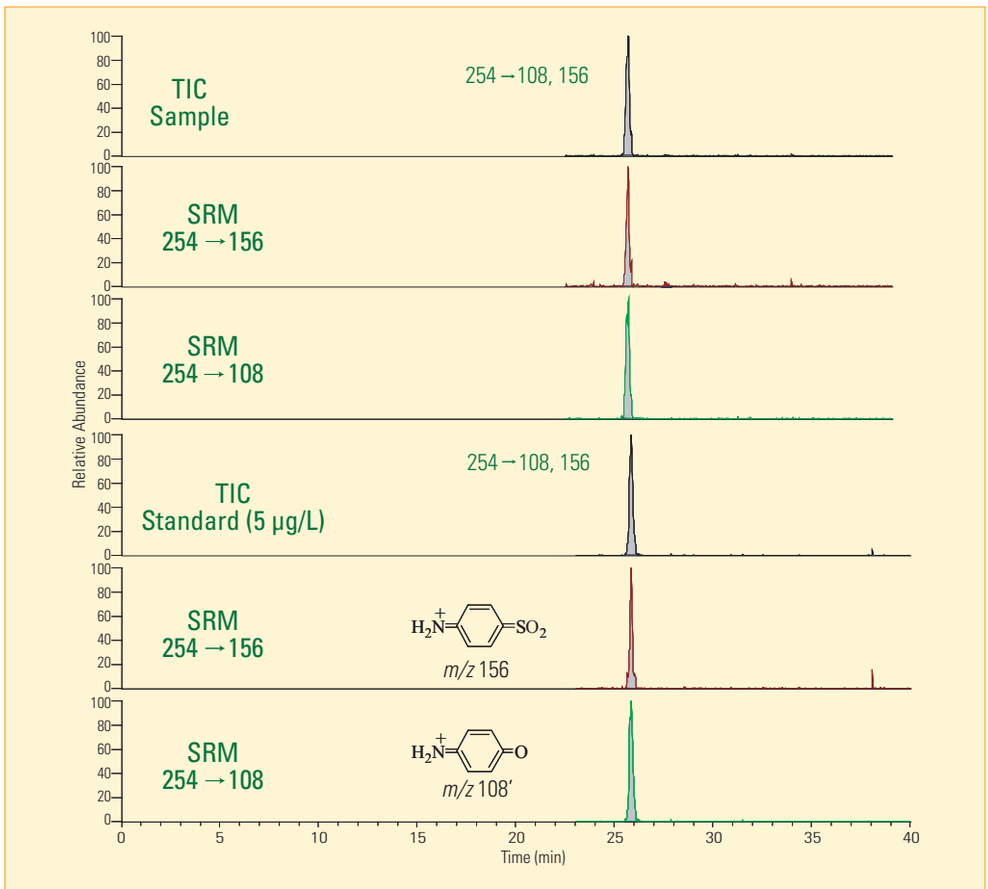

Figure 3: LC-ESI(+)-MS/MS chromatograms of sulfamethoxazole in wastewater sample extract and of a standard solution of sulfamethoxazole

Conclusion

LC-ESI-MS/MS is a powerful analytical method for the sensitive determination of sulfonamide antibiotics in municipal wastewater at low ppt levels (ng/L). The solid phase extraction scheme for trace enrichment and separation of sulfonamide compounds from wastewater samples yielded high recovery rates and enabled their accurate quantification. Sulfamethoxazole (median concentration 150 ng/L) was detected in the wastewater effluent samples, which indicated that it was not completely eliminated in the sewage treatment plants. The described method proved to be a valuable tool for the detection of pharmaceuticals in wastewater effluents before they reached the aquatic environment.

References

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