Application Note: 435

Analysis of Sulfonamides in River Water using EQuan, an Online Concentration Analysis System

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Key Words

- Antibiotics
- EQuan™
- LC-MS/MS
- Online Concentration
- Sulfonamides

Introduction

Triple stage quadrupole LC-MS systems are often used for highly sensitive quantitative analyses of environmental pollutants. The performance of an LC-MS system is one important factor that determines if it will efficiently and accurately detect pollutants present at low concentrations in environmental samples. Another factor is the process by which the system pre-treats the samples.

In its sample pretreatment process, the EQuan system is able to quickly perform the time-consuming concentration process online, dramatically reducing the time required for analysis. Consequently, EQuan is capable of measuring multiple samples more efficiently than systems using off-line sample preparation, while reaching lower detection limits than are achievable with a conventional LC-MS/MS analysis.

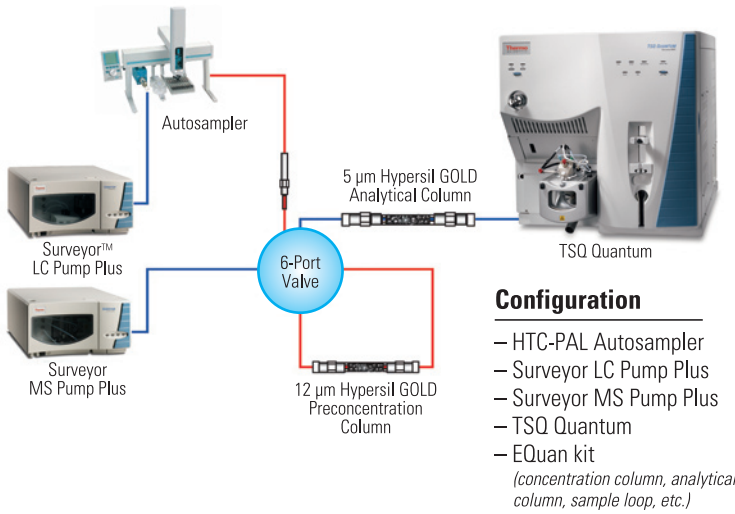

Figure 1: EQuan system schematic

The following application note presents a quantitative analysis of sulfonamide antibiotics using EQuan. These compounds are used widely as anti-inflammatory medications for humans and livestock, and have recently become compounds of interest to regulatory agencies worldwide. Figure 2 shows the library spectra and chemical structures for the nine sulfonamide antibiotics used in this experiment.

Experimental Conditions

Calibration standards were prepared using a mixed standard solution of the nine LC target sulfonamides (Kanto Chemical Co., Ltd.). For the test samples, river water collected in Kanagawa Prefecture was passed through a 0.4 µm filter prior to analysis.

HPLC

Analytical Column:	Hypersil GOLD™ C18 2.1 x 150 mm, 5 µm
Concentration Column:	Hypersil GOLD C18 2.1 x 20 mm, 12 µm
Mobile Phase A:	1 mM ammonium formate, 0.05% formic acid in water
Mobile Phase B:	1 mM ammonium formate, 0.05% formic acid-methanol
Gradient (for analysis):	5% B (1.5 min) to 90% B (in 10 min) to 90% B (5 min hold)
Injection Volume:	0.5 mL
Flow:	0.2 mL/min
Column Temperature:	40 °C

MS: Thermo Scientific TSQ Quantum

Ionization Mode:	Positive ESI
Spray Voltage:	4500 V
Sheath Gas:	50
AUX Gas:	20
Sweep Gas:	0
Capillary Temperature:	360 °C
Skimmer Offset:	7 V
Scan Time:	0.1 sec/SRM transition
Collision Gas Pressure:	Argon, 1.2 mTorr
Mass Resolution (FWHM):	Q1 & Q3 0.7 Da

SRM Conditions

m/z 251.07 → 156.0 at 17 eV (sulfadiazine)
m/z 254.07 → 156.0 at 16 eV (sulfamethoxazole)
m/z 265.08 → 156.0 at 18 eV (sulfamerazine)
m/z 268.08 → 156.0 at 14 eV (sulfisoxazole)
m/z 279.10 → 186.0 at 19 eV (sulfadimidine)
m/z 281.08 → 156.0 at 18 eV (sulfamethoxypyridazine)
m/z 281.08 → 156.0 at 18 eV (sulfamonomethoxine)
m/z 301.08 → 156.0 at 17 eV (sulfaquinoxaline)
m/z 311.09 → 156.0 at 19 eV (sulfadimethoxine)

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Results and Discussion

Standard Sample Results

It was possible to detect all of the target compounds at a concentration of 1.0 ppt using the EQuan system (Figure 3). Furthermore, linearity was obtained over the range of 0.5 to 100 ppt (Figure 4).

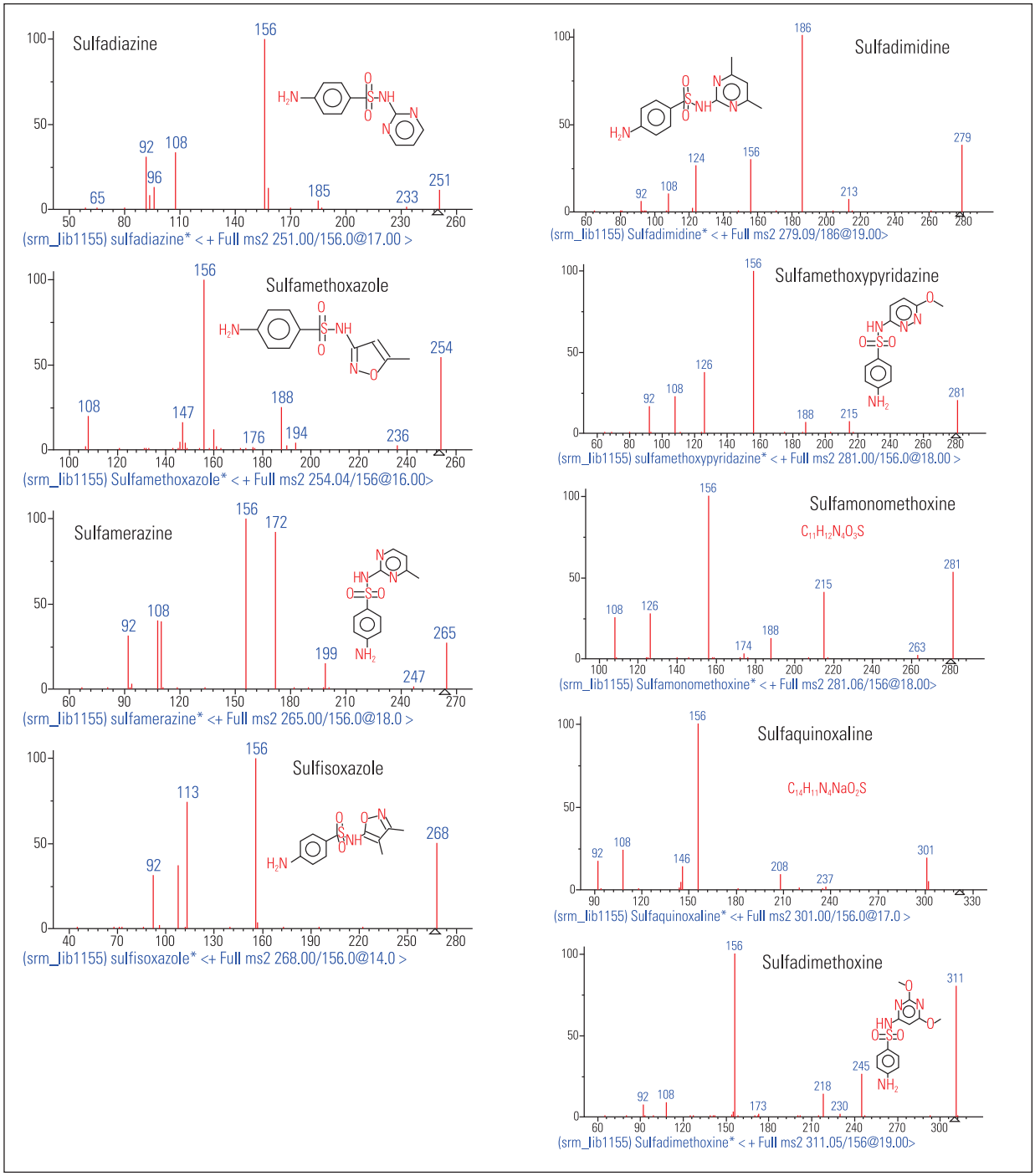

Figure 2: Product ion spectra (from MS/MS library)

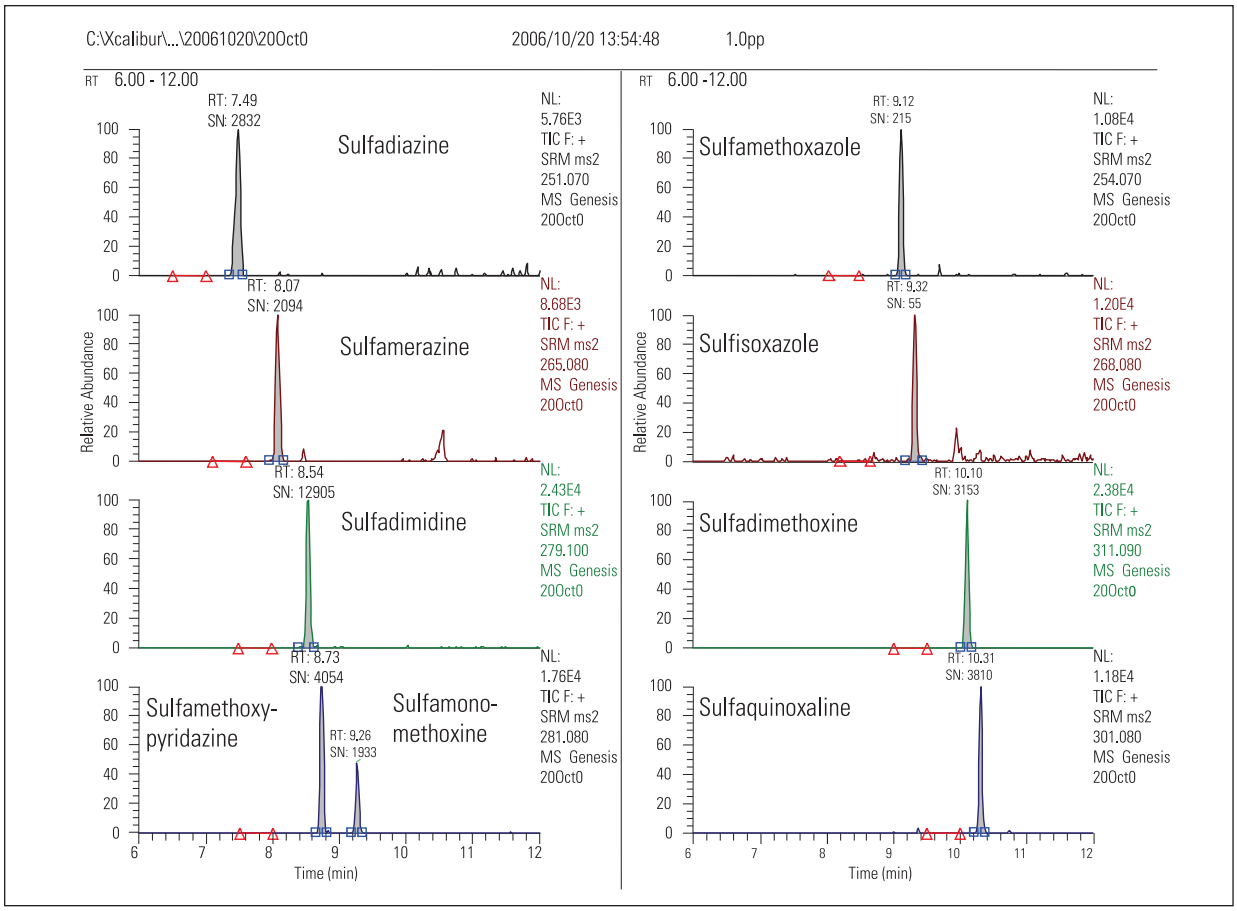

Figure 3: SRM chromatogram of 1.0 ppt standard samples. Signal-to-noise (S/N) value shown above each peak. △–△ on each chromatogram shows noise range for calculating S/N.

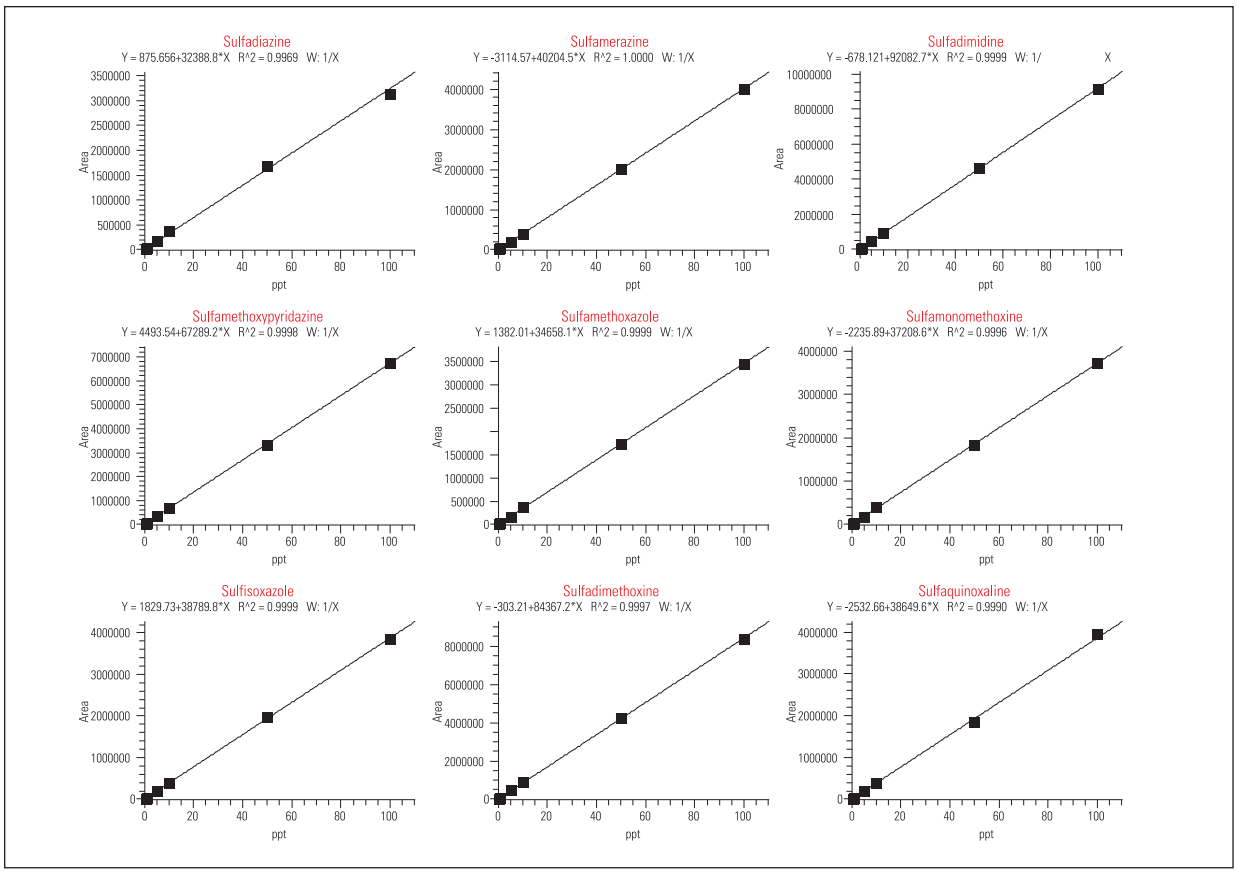

Figure 4: Calibration curves

River Sample Assay Results

In the measurement of the river water, sulfamethoxazole was detected at a concentration of 12.3 ppt and four other components were detected at concentrations of about 1 ppt or less (Figure 5).

The mixed standard sample was spiked in the river water samples at the concentration of 1.0 ppt (except for Sulfamethoxazole, which was spiked at 10 ppt) and the samples were analyzed. A good recovery rate of 70% to 98% was obtained for each of the compounds. Furthermore, reproducibility for all replicates was 11% or less for the spiked samples (see Table 1).

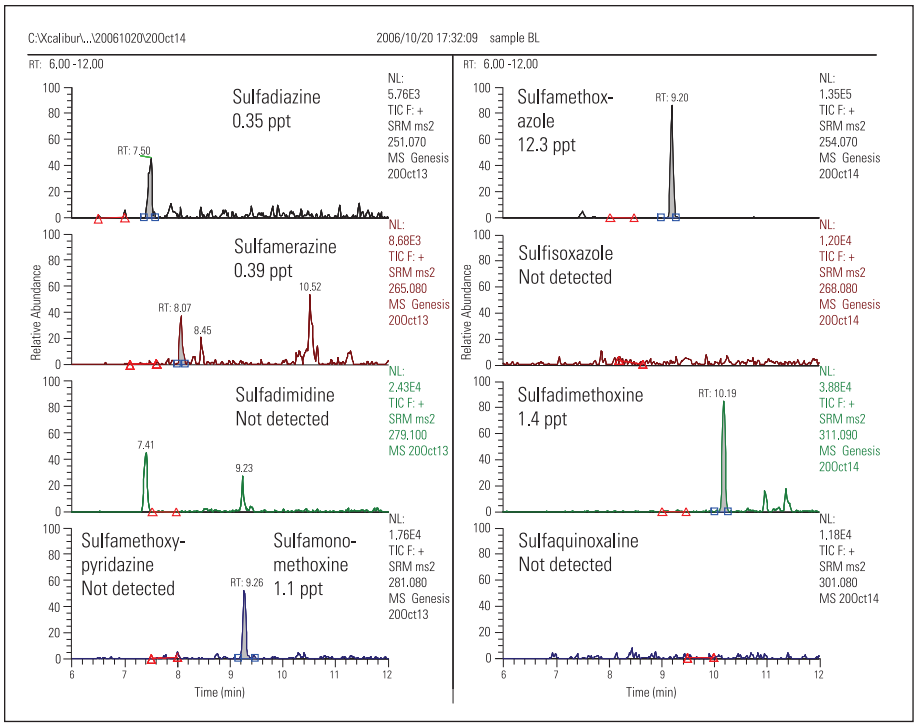

Figure 5: SRM chromatogram of river samples. Signal-to-noise (S/N) value shown above each peak. △–△ on each chromatogram shows noise range for calculating S/N.

Conclusion

With the EQuan online concentration analysis system, it was possible to measure the sulfonamide antibiotics that were present in the river water samples at low concentrations quickly and accurately.

		1.0 ppt spiked samples (n = 4)		
Compound	Concentration in river water sample (ppt)	Concentration in spiked samples (ppt)	Recovery rate (%)	CV (%)
Sulfadiazine	0.35	1.19	84	10.4
Sulfamerazine	0.39	1.13	73	5.1
Sulfadimidine	NF	0.98	98	11.3
Sulfamethoxypyridazine	NF	0.87	87	7.1
Sulfamethoxazole	12.35	19.35*	70	2.8
Sulfamonomethoxine	1.11	1.85	74	3.3
Sulfisoxazole	NF	0.95	95	8.5
Sulfadimethoxine	1.42	2.19	77	1.4
Sulfaquinoxaline	NF	0.85	85	3.3

** The spiking concentration was set at 10 ppt because sulfamethoxazole was detected in the river water samples at concentrations higher than 10 ppt.*

Table 1: River water and spiked sample assay results and reproducibility

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