Application Note: 354

On-line Enrichment HTLC/MS/MS Assay for Multiple Classes of Antibiotics in Environmental Water Sources

Kevin J. McHale,[1] Chris Esposito,[2] and Francois Espourteille[2]

[1]Thermo Fisher Scientific, Somerset, NJ, USA; [2]Thermo Fisher Scientific, Franklin, MA, USA

Key Words

- TSQ Quantum Ultra™
- Aria™ TLX-2
- H-SRM
- Turbulent-flow Extraction

Introduction

There is a growing concern over the presence of antibiotics in environmental sources of water. This has caused environmental and government labs to develop LC/MS methods to monitor water supplies for the presence of antibiotics.[1-4] However, the low-level concentration of antibiotics in environmental water sources often requires extensive sample preconcentration and cleanup. Preparation of water samples (100-1000 mL) prior to LC/MS analysis, even with an "unlimited" sample volume, is time consuming and reduces sample throughput. This report presents a method that significantly decreases sample preparation time by applying on-line preconcentration and extraction in conjunction with detection using the Thermo Scientific TSQ Quantum Ultra in highly-selective reaction monitoring (H-SRM) mode for assaying antibiotics at low pg/mL concentrations.

Goal

Develop a method to screen for antibiotics in surface water by applying on-line preconcentration and analyte extraction with LC/MS/MS detection.

Experimental Conditions

The antibiotics assayed in this method (Table 1) were purchased from Sigma (St. Louis, MO) and used without further purification. Stock solutions of the antibiotic standards were prepared at 1.0 mg/mL in methanol and stored in amber polypropylene vials at -20 °C until needed. Prior to High-Throughput HPLC (HTLC/MS/MS) analysis, water samples were prepared in 2 µg/mL Na_2EDTA (aq) to inhibit binding of the tetracycline antibiotics to glass surfaces and to metal ions in solution.[1] Using the Thermo Scientific Aria TLX-2 system, water samples in 1 mL volumes were injected onto a TurboFlow® column without any further sample preparation. Targeted antibiotics were focused and concentrated on the turbulent-flow extraction column, then transferred to the analytical column. Analyte separation was accomplished using a reverse-phase gradient prior to detection with the TSQ Quantum Ultra in highly-selective reaction monitoring (H-SRM) mode.

Compound	Precursor *m/z*	Product *m/z*	Resolution
Sulfamethoxazole	254	108, 156	H-SRM
Sulfamerazine	265	156, 172	H-SRM
Sulfamethizole	271	108, 156	H-SRM
Sulfamethazine	279	156, 186	H-SRM
Lincomycin	407	126, 359	H-SRM
Tetracycline	445	154, 410	H-SRM
Doxycycline	445	321, 428	H-SRM
Chlortetracycline	479	444, 462	H-SRM
Dehydroerthromycin	716	158, 558	H-SRM
Erythromycin	734	158, 576	Unit H-SRM
Roxithromycin	837	158, 679	Unit H-SRM
Tylosin	916	174, 772	Unit H-SRM

Table 1: List of antibiotics and SRM transitions for the HTLC/MS/MS assay

On-line TurboFlow Extraction

Aria TLX-2

- TurboFlow Column: 0.5 × 50 mm Cyclone® MAX
- Autosampler: CTC PAL (Leap Technologies)
- Injection Volume: 1.0 mL
- Loading Pump Mobile Phase:
  (A) 10 mM ammonium bicarbonate,
  (B) 0.5% HAc + 0.04% TFA,
  (C) ACN + 0.1% HCOOH
- Flow Rate: 2.0 mL/min

Liquid Chromatography

- Analytical Column: 4.6 × 100 mm, 3 µm Thermo Scientific Hypersil GOLD™
- Flow Rate: 1.2 mL/min
- Eluting Pump Mobile Phase:
  (A) 0.5% HAc + 0.04% TFA,
  (B) ACN + 0.5% HAc + 0.04% TFA
- Flow Split: post-column, 0.5 mL/min to ESI source

Mass Spectrometry

- TSQ Quantum Ultra
- Ionization mode: Positive ion ESI
- Ion Transfer Tube Temperature: 375 °C

Selective Reaction Monitoring (SRM) Parameters

- Q2 Pressure: 1.5 mTorr argon
- SRM Transitions: see Table 1
- SRM Scan Time: 40 ms per transition
- Q1 Resolution: Unit (0.7 Da FWHM) and H-SRM (0.15 Da FWHM)
- Q3 Resolution: Unit (0.7 Da FWHM)

Results and Discussion

Details on HTLC/MS/MS Method

The 13 antibiotics studied (Table 1) were preconcentrated on a new mixed-mode TurboFlow extraction column, the Cyclone MAX, which has reverse-phase and anion exchange characteristics. After sample loading and flushing the TurboFlow column at 2.0 mL/min to remove matrix interferences (see Figure 1), valve 1 is switched to allow the extraction solvent plug (50% ACN + 0.1% HCOOH) to transfer the antibiotics to the analytical column. The organic content of the extraction solvent plug, which contains the antibiotic analytes, is diluted by the highly aqueous mobile phase of the eluting pumps at 1.2 mL/min. This dynamic mixing occurs before the analytical column so that the antibiotics can be effectively refocused prior to the reverse-phase separation step. During the LC/MS/MS data acquisition, the TurboFlow column is reconditioned for the next sample injection.

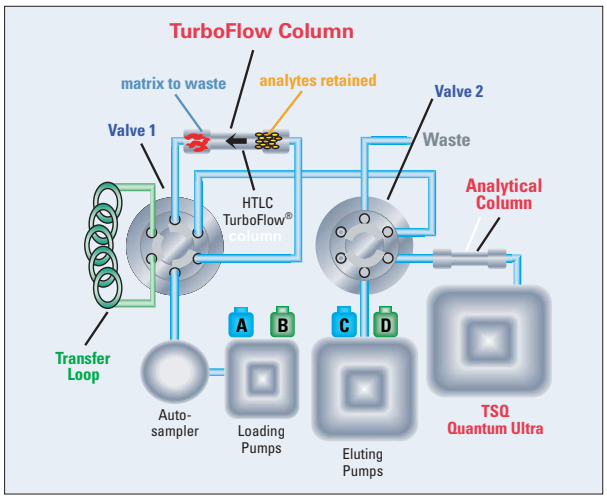

Figure 1: Schematic of the Aria TLX-2 system coupled to the TSQ Quantum Ultra

For unit SRM and H-SRM detection on the TSQ Quantum Ultra, data acquisition was sectioned into five time segments, whereby eight SRM transitions per segment were employed. Two SRM transitions were monitored for each antibiotic compound (see Table 1) for confirmation purposes and to improve ion statistics.

Sensitivity and Calibration Data for HTLC/MS/MS Assay

Figure 2 presents the HTLC/MS/MS chromatograms of the 13 antibiotic standards and dehydroerythromycin at their limits of quantitation (LOQs), which ranged from 0.5-5 pg/mL, using 1 mL injections. Calibration data were generated from the LOQ to 100 pg/mL for the 13 antibiotic standards in deionized water. Linear fit calibration curves with 1/x weighting were used for 11 of 13 antibiotics. Sulfamethoxazole and sulfamethizole provided the best results by employing quadratic fit calibration curves. All sample standard regressions yielded $R^2 \geq 0.990$ (n = 4 replicates).

Spike of Antibiotics into Surface Water Sample

Figure 3 shows the results for the HTLC/MS/MS assay for a surface water sample that was spiked at 25 pg/mL with the antibiotic standards. Prior to this experiment, the surface water sample was screened using the described method, and it was found to be devoid of the target antibiotics. Figure 4 presents a comparative HTLC/MS/MS assay for a neat standard solution at 25 pg/mL. The sulfonamide class showed a minor signal suppression in the surface water sample, while the response for the macrolides were slightly enhanced. The tetracyclines, however, showed a significant difference in response in the surface water sample vis-à-vis the neat standard. This difference may be attributed to binding of these tetracycline antibiotics to residual metals in the water sample.

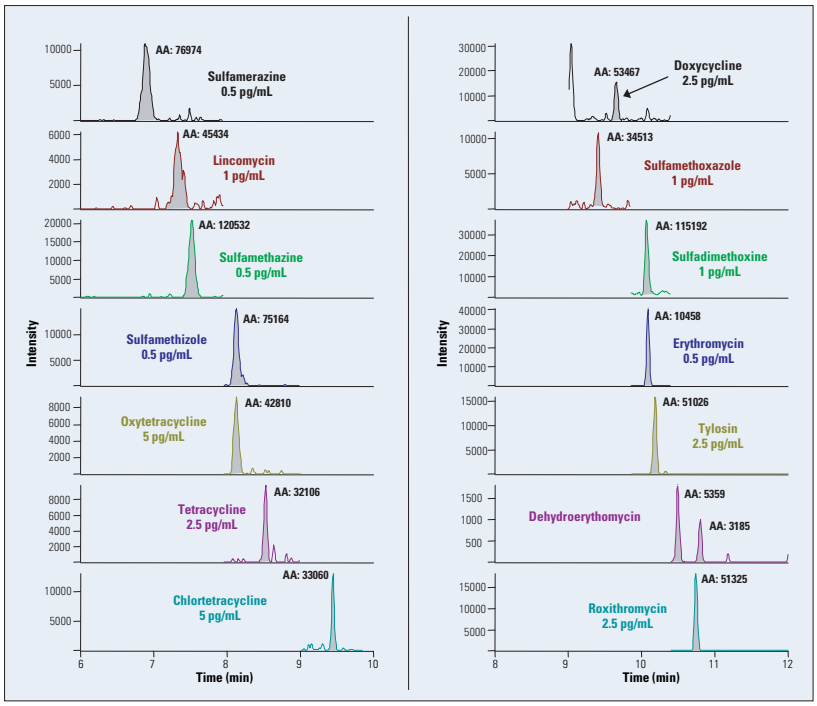

Figure 2: Chromatograms for antibiotics at their LOQs using HTLC/MS/MS

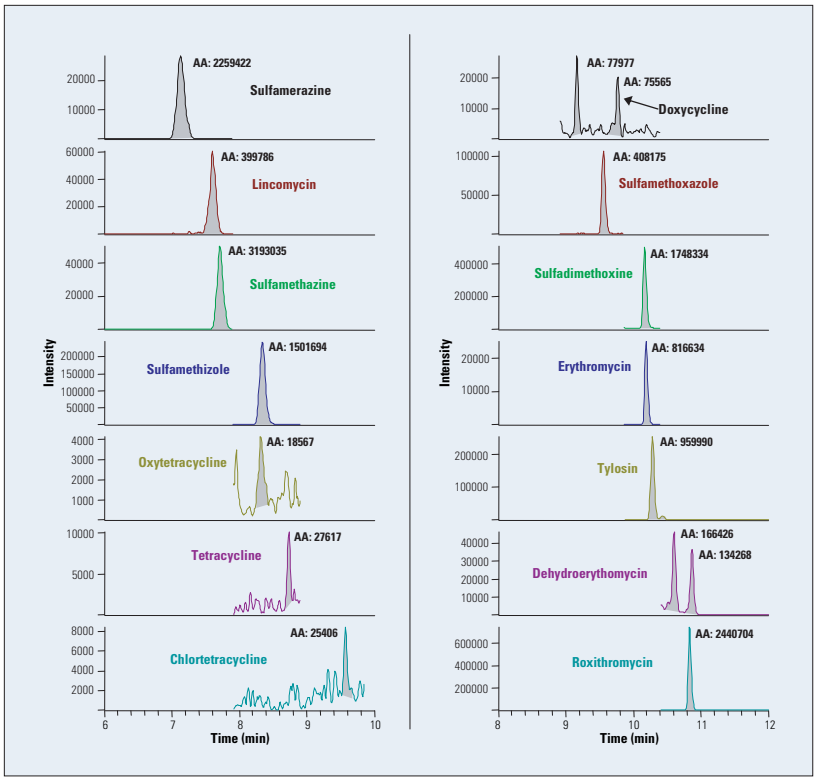

Figure 3: 25 pg/mL antibiotics spiked into a surface water sample

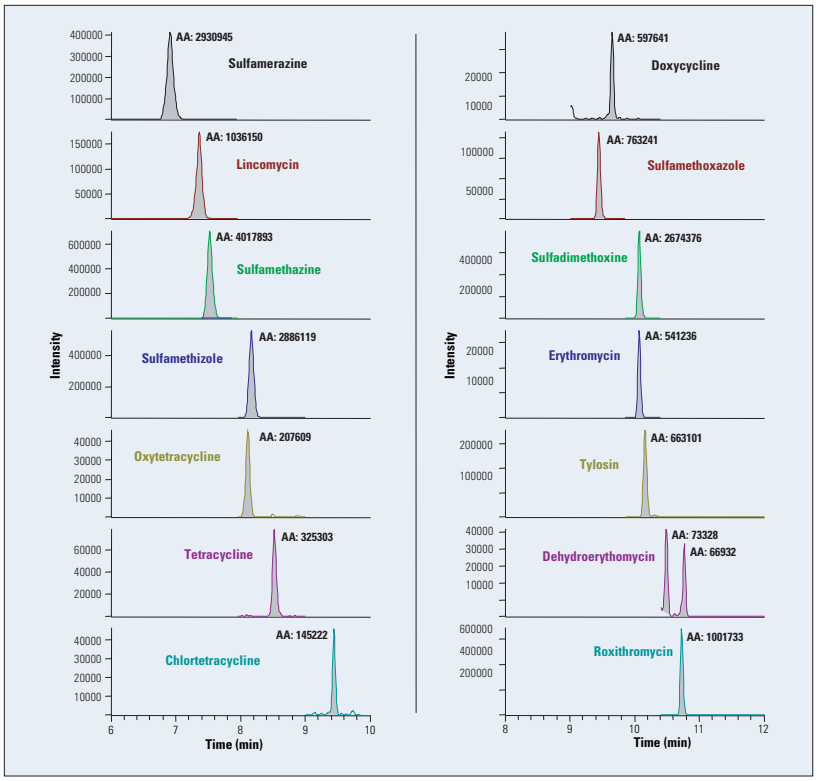

Figure 4: Neat 25 pg/mL antibiotics standard

HTLC/MS/MS Screening for Antibiotics in Environmental Water Samples

Surface water samples from multiple locations in California, Florida and Ontario were screened for antibiotics using the described HTLC/MS/MS method. Of the water samples screened only the Lake Ontario sample showed measurable levels of the targeted antibiotics (Figure 5). Insets in Figure 5 show chromatographic traces for the two monitored SRM transitions for the observed antibiotics, providing additional confirmation and higher confidence in these results.

Conclusions

A method for assaying antibiotics in water samples at the low pg/mL level using on-line sample clean-up and pre-concentration has been demonstrated. The capability of on-line turbulent-flow extraction of large sample volumes (1 mL) significantly reduces sample analysis time from a matter of hours to a matter of minutes. Detection using highly-selective reaction monitoring (H-SRM) provides an additional level of selectivity and confidence over unit resolution SRM. This HTLC/MS/MS method, when applied to screening surface water samples, was able to detect and quantitate the observed antibiotics at the low pg/mL level.

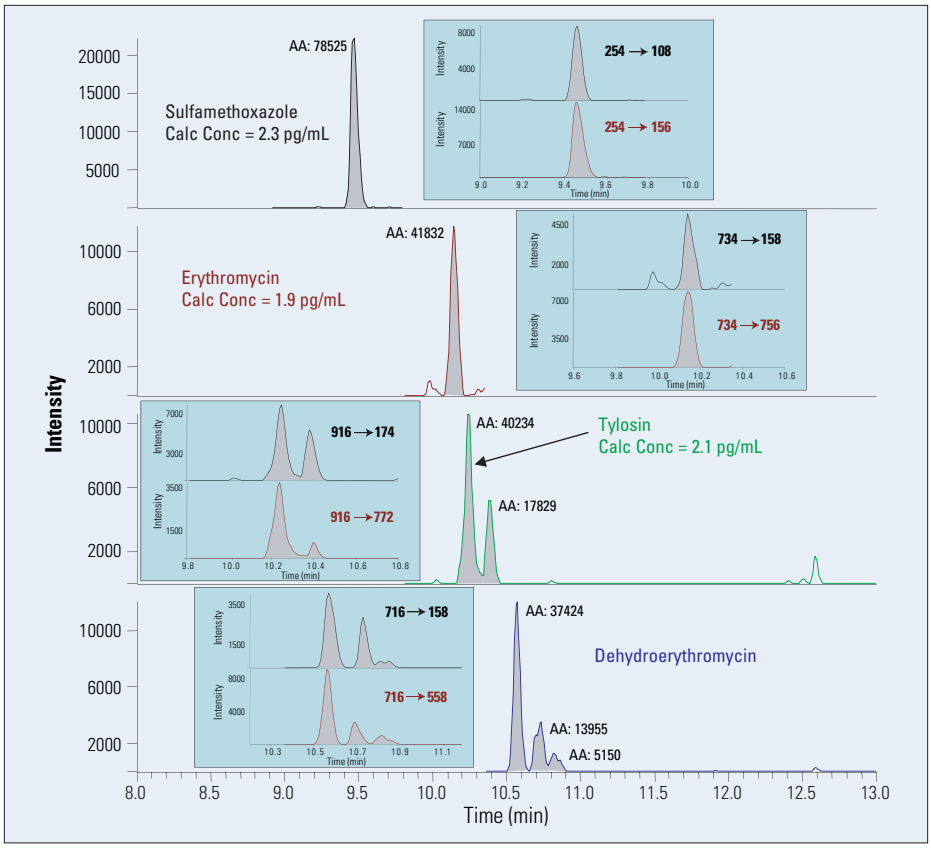

Figure 5: Chromatograms of the detected antibiotics in a Lake Ontario water sample

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