High Resolution LC-MS for Screening and Quantitative Analysis of Antibiotics in Drinking Water Using an Orbitrap and Online Sample Preparation

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Overview

Purpose: To demonstrate online sample pre-concentration and extraction of water samples and analysis with high-resolution, accurate mass (HR/AM) detection, quantitation and confirmation.

Methods: Inject 1 mL water samples directly onto a trapping column. The trapped compounds are then backflushed onto an analytical HPLC column and detected using a Thermo Scientific Orbitrap mass analyzer.

Results: This poster describes a method to perform screening and quantitation of antibiotics at ppt and sub-ppt levels in drinking water using online pre-concentration together with HR/AM confirmations of the compounds.

Introduction

Most current methodologies for the quantitation of antibiotics in drinking water revolve around analysis using triple stage quadrupole platforms with offline sample preparation. While this is a proven technique for the analysis of many contaminants in drinking water, ground water and other environmental water samples, the offline sample preparation steps are time-consuming and prone to operator error and reproducibility problems. In addition, the need to transport large sample volumes from the collection site to the laboratory, typically 1 L samples, is laborious. This poster illustrates the ability to directly inject the water sample without any offline pre-concentration steps, while achieving the same sensitivities required for the experiment. Thus smaller sampling volumes can be used.

The method described here utilizes liquid chromatography-mass spectrometry (LC-MS) with a Thermo Scientific Exactive Plus Orbitrap™ mass spectrometer using HR/AM. While the triple stage quadrupole instrument is routinely used in these types of experiments, we demonstrate the ability to use a benchtop HR/AM instrument to quantitate and confirm the contaminants of interest. The advantages of HR/AM instruments includes high resolution to isolate contaminants of interest from interfering matrix peaks at similar masses as well as the ability to re-interrogate data at a later date for additional compounds. Furthermore, compared to the triple stage quadrupole instrument, method development time is greatly reduced as there is no need to individually optimize each analyte of interest.

Methods

Sample Preparation

Samples were prepared from a stock solution of pesticides in methanol. Calibration solutions were prepared from the stock solutions, resulting in 8 levels of antibiotics for positive analysis. Dilutions were made in laboratory water (HPLC-grade) to create eight different calibration levels. The antibiotic calibration samples were acidified with formic acid to a concentration of 0.1% formic acid. The concentration range varied for each compound, but were in the approximate range of 1 ppt to 10 ppb. This ensured compatibility with the mobile phase for chromatography. No further sample preparation was conducted. The antibiotics studied for this poster were: carbamazepine, erythromycin, ketoprofen, norethindrone, roxithromycin, sulfachloropyridazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfathiazole, trimethoprim and tylosin.

Liquid Chromatography

Liquid chromatography was performed using the Thermo Scientific EQuan MAX system. The EQuan MAX system consists of two high pressure liquid chromatography (HPLC) pumps, autosampler and switching valves. The first HPLC pump, a Thermo Scientific Accela 600 pump, is use to transfer the large volume sample from the autosampler loop to the loading column (Thermo Scientific Hypersil GOLD aQ column, 20 x 2.1 mm, 12µ) at a flow rate of 1.0 mL/min. After 1.2 minutes, a six-port valve is switched to back-flush the loading column onto the analytical column (Thermo Scientific Accucore aQ column, 100 x 2.1 mm, 2.6µ), and remains inline for 11 minutes. The analytes are eluted using an 11-minute reversed-phase gradient from the second HPLC pump, the Thermo Scientific Accela 1250 pump. The mobile phases were water (A) and methanol (B), both containing 0.1% formic acid and 4mM ammonium formate. The gradient program for both pumps is shown in Table 1. After 12 minutes of runtime, the loading column is returned to its original position, taking the analytical column offline from the loading column, and the system is re-equilibrated for the next injection. The total run time is 15 minutes. The flow diagram for the EQuan MAX system is shown in Figure 1.

Mass Spectrometry

The Exactive™ Plus Orbitrap mass spectrometer was used in this experiment. The Exactive Plus was operated in alternating full scan and all ion fragmentation (AIF) mode with positive electrospray ionization. One scan of full scan MS data was collected, and subsequently, all of the ions entering the MS were fragmented in the higher-energy C-trap dissociation (HCD) collision cell at a collision energy (CE) of 30 eV with a 20% stepped CE, and analyzed in the Orbitrap mass analyzer. The resolution for the full scan experiment was 70,000 and the resolution of the AIF experiment was 35,000. The mass range 150-1000 amu was monitored in full scan, and 80-1000 amu in the AIF experiments.

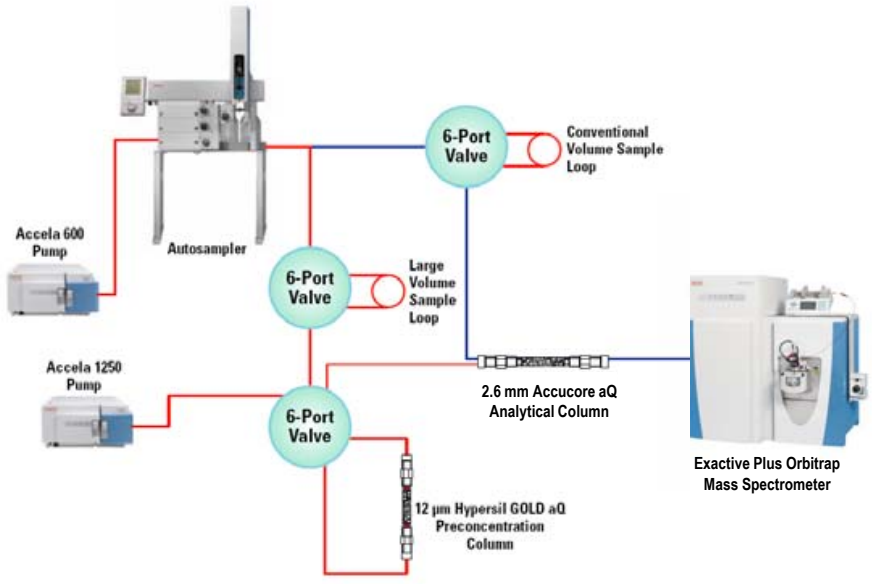
Data Analysis

Data was collected and analyzed using Thermo Scientific TraceFinder 2.1 software. Spectral confirmation was carried out with Thermo Scientific ExactFinder, 2.0 software.

TABLE 1. HPLC gradients for the loading and analytical pumps in the method

Time (min)	Loading Pump %A	Flow Rate (µL/min)	Time (min)	Analytical Pump %A	Analytical Pump %B	Flow Rate (µL/min)
0.0	100	1000	0.0	98	2	350
1.3	100	1000	1.5	98	2	350
1.5	100	100	3.0	70	30	350
12.0	100	100	8.0	2	98	350
12.1	100	1000	9.0	2	98	350
15.0	100	1000	9.1	98	2	350
			15.0	98	2	350

FIGURE 1. EQuan MAX system flow schematic



Results

Quantitation

Acquisition and quantitation was carried out using TraceFinder™ software. The theoretical mass of each protonated antibiotic compound was used as the mass for quantitation in this analysis. Calibration lines were created for each compound, and fit with either a linear or quadratic curve. Each calibration level was run in triplicate. Due to the large concentration range of the standards, some compounds exhibited non-linear calibration lines, and were fitted with a quadratic fit. All calibrators used a 1/X weighting. An example calibration line for the compound sulfamerazine is show in Figure 2. The chromatogram shown in Figure 2 corresponds to the second to lowest level, 3 pg/mL.

FIGURE 2. TraceFinder screen shot for the quantitation of the antibiotic sulfamerazine at 3 pg/mL

Limits of Quantitation

The limit of quantitation (LOQ) was determined by the lowest calibration standard group with a %RSD of less than 15%. The LOQ for this experiment is shown in Table 2. The %RSD for each compound at its LOQ is included in Table 2. In some cases, the LOQ was lower than the concentration of the lowest calibration standard.

TABLE 2. List of antibiotics analyzed with their theoretical masses, LOQs and reproducibility

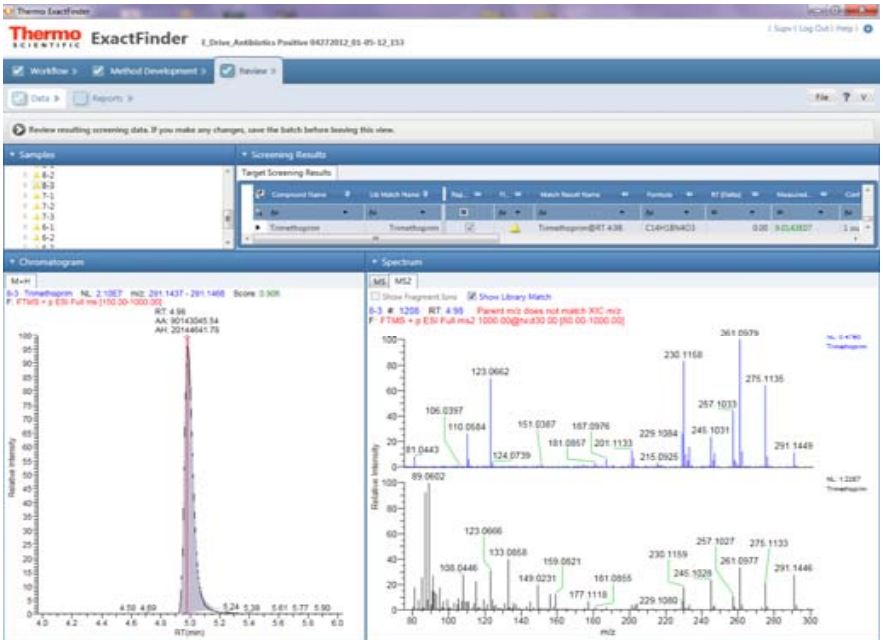
Compound	Theoretical Mass (*m/z*)	LOQ (pg/mL)	% RSD at LOQ
Carbamazepine	332.14050	0.2	8.90
Erythromycin	734.46852	40.0	14.30
Ketoprofen	255.10157	1.0	9.90
Norethindrone	299.20056	1.0	13.50
Roxithromycin	837.53185	9.9	4.20
Sulfachloropyridazine	285.02075	1.0	7.30
Sulfadimethoxine	311.08085	0.4	4.80
Sulfamerazine	265.07537	0.6	4.90
Sulfamethazine	279.09102	1.0	3.45
Sulfamethizole	256.02089	1.0	6.30
Sulfamethoxazole	254.05939	1.0	6.60
Sulfathiazole	271.03179	0.6	3.60
Trimethoprim	291.14517	1.6	13.10

Spectral Confirmation

To add additional confirmation to the antibiotics detected in the samples, spectral confirmation of the MS^2 spectrum collected in the HCD cell was performed using ExactFinder™ software. Samples were submitted to the software after acquisition. The MS^2 spectra were searched against the built-in Environmental and Food Safety and Clinical Research spectral libraries. These libraries contain MS^2 spectra collected on Orbitrap instruments. Because all Orbitrap platform mass spectrometers are compatible, they provide identical spectra.

The spectral match for the antibiotic trimethoprim is shown in Figure 4. This comparison is from the HCD MS^2 spectrum of the calibration standard corresponding to a concentration of 80 pg/mL. The top spectrum is the library reference spectrum. The bottom spectrum is the collected sample spectrum. The library reference spectrum is cleaner, because it was collected using a Thermo Scientific LTQ Velos Pro Orbitrap mass spectrometer by direct infusion. Thus, there is much less background, no co-eluting peaks, or matrix to generate extraneous ions. Nevertheless, the two spectra match in the main fragment peaks, as well as the protonated molecular ion at *m/z* = 291.1446 amu. This spectral confirmation helps to eliminate the possibility of false-positives, and can be used for identification point scoring systems.

FIGURE 4. Spectral comparision of the MS² spectrum of the antibiotic trimethoprim obtained at a concentration of 80 pg/mL. The library reference spectrum is the top spectrum, the lower spectrum is from the sample. The comparison was performed with ExactFinder software.



Conclusion

This poster demonstrates:

- Online pre-concentration and extraction for 1mL injections of antibiotics at the ppt level.
- The quantitation of HR/AM data using TraceFinder software from the Exactive Plus Orbitrap instrument.
- Spectral confirmation of MS² spectrum collected in the same data file as the quantitation data using ExactFinder software.
- Ability to quantitate and confirm samples in the same analytical run for antibiotics in water samples.

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