

Analysis of Macrolides and Three Other Antibiotic Classes at Low ppt Levels in WWTP Effluent and Surface Waters by LC-MS/MS

Ola Svahn,¹ Erland Björklund,¹ and Euan Ross.²

¹Kristianstad University, Kristianstad, Sweden. ²Waters Corporation, Wilmslow, United Kingdom



GOAL

To confirm and quantify the presence of macrolides and other important classes of antibiotics in waste water treatment plant (WWTP) effluent and surface waters at low ppt levels.

BACKGROUND

The presence of macrolides such as clarithromycin in WWTP effluent waters poses a potential ecotoxicological risk. Additionally the general occurrence of antibiotics in treated wastewater is concerning due to the probable development of antibiotic resistance among bacteria in nature. In the latest update from the EU Commission Implementing Decision (EU) 2015/495, a watch list of substances for EU-wide monitoring in the field of water policy was established.¹ Compounds of interest include the three macrolides azithromycin, clarithromycin, and erythromycin, requiring a maximum method detection limit of 90 ng/L by LC-MS/MS. These were included to gather additional data concerning their presence in aquatic environments and the possible risks associated thereby. In this technology brief, we describe the analysis of azithromycin,

This method has undergone full validation according to the method previously published by the U.S. EPA.

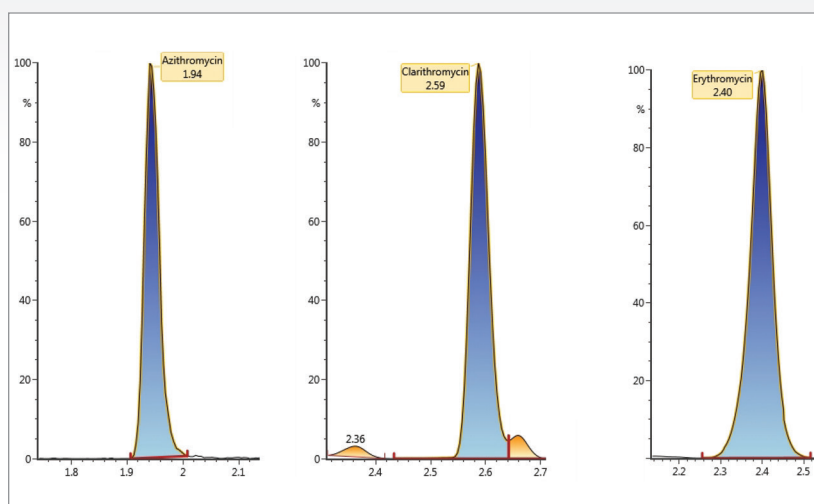


Figure 1. Chromatography of injected standard solutions of azithromycin, clarithromycin, and erythromycin on an ACQUITY UPLC BEH C₁₈ Column (2.1 mm x 50 mm, 1.7 μm).

clarithromycin, and erythromycin in WWTP effluent and surface waters, along with three additional important classes of antibiotics, utilizing off-line solid phase extraction followed by analysis on a Waters® ACQUITY® UPLC® H-Class System equipped with a column manager, coupled to a Xevo® TQ-S Mass Spectrometer.

THE SOLUTION

WWTP effluent and surface water samples were extracted by means of an optimized solid-phase extraction methodology using 200 mg Oasis® HLB Solid Phase Extraction (SPE) Cartridges.² Prior to extraction, the cartridges were conditioned with methanol (MeOH) and reagent water. Samples were passed through the SPE cartridges followed by air drying under a positive pressure, a step that proved crucial for both recovery results and evaporation time reproducibility. The extracts were eluted with MeOH into disposable borosilicate glass tubes and evaporated to complete dryness. The dry extracts were reconstituted to a total volume of 1 mL in

Table 1. Three macrolides and six other important antibiotics analyzed with off-line SPE and UPLC-ESI-MS/MS in positive mode at basic and acidic UPLC conditions, depending on the compound. In all cases positive mode electrospray was applied. Linearity R^2 injecting 1 μ L standard solution. Method Quantification Limit (MQL) for the complete SPE procedure using 50 mL of spiked natural humic rich pond water injecting 1 μ L of sample.

*Samples fortified in humic rich surface water at \approx 100 ng/l level.

Basic UPLC	Class	R2	MQL (ng/L)	*Absolute SPE recovery (%)	*Method recovery (%)	WWTP effluent (ng/L)	Surface water (ng/L)
Clarithromycin	Macrolide	0.997	1.1	56	93	24	2.7
Erythromycin	Macrolide	0.999	0.5	91	110	215	23
Penicillin G	β -lactam	1.000	10.0	84	78	-	-
Penicillin V	β -lactam	1.000	20.0	82	103	-	-
Trimethoprim	-	1.000	0.6	76	99	30	2.6
Acidic UPLC							
Azithromycin	Macrolide	0.998	1.1	49	90	21	<1.1
Doxycycline	Tetracycline	0.997	1.1	74	101	-	-
Ciprofloxacin	Fluoroquinolone	0.998	32.0	55	71	-	-
Norfloxacin	Fluoroquinolone	0.998	21.0	48	62	-	-
Sulfamethoxazole	Sulfonamide	0.999	1.3	62	96	208	32

TruView™ LC-MS Certified Vials. Finally 1 μ L of this sample was injected onto the ACQUITY UPLC H-Class System equipped with a column manager, enabling fast column switching between two ACQUITY UPLC BEH C₁₈ Columns (2.1 mm x 50 mm, 1.7 μ m) in parallel. The columns were run using identical simple linear gradients, one column ran under basic conditions and the other acidic conditions. It has been shown that substantial selectivity and sensitivity gains can be achieved by placing each compound at a suitable column pH without prejudices regarding historical residence in terms of chromatographic conditions and ESI mode.³ This increases the method's flexibility so it can be implemented in routine analysis in response to new requests as well as for contaminants yet to be discovered.

The antibiotics analyzed and the UPLC® conditions are described in Table 1. The samples were determined using electrospray in positive ion mode with two MRM transitions per compound. Figure 1 shows the chromatograms obtained for the three macrolides, while Figure 2 demonstrates linearity showing the calibration curve for clarithromycin. The linearity, method quantification limit (MQL), absolute SPE recovery, and method recovery for all of the investigated antibiotics are detailed in

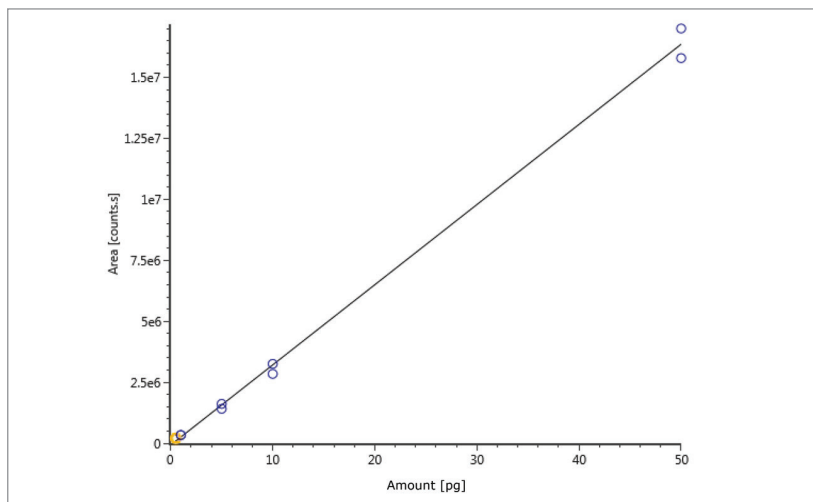


Figure 2. Example of calibration curve for clarithromycin in the range of 0.5 to 50 pg injected on column using standard solutions.

Table 1. The MQL for all macrolides is at the very low ppt level for the complete SPE methodology. The developed procedure has undergone full validation according to the method previously published by the U.S. Environmental Protection Agency.⁴

Example chromatography for antibiotics, fortified at approximately 100 ng/L in humic rich surface water using the basic conditions chromatography method, is shown in Figure 3. Chromatograms showing the detection of azithromycin, clarithromycin, and erythromycin in WWTP effluent samples from the City of Kristianstad Sweden is shown in Figure 4. Analysis of the WWTP effluent water, as well as a surface water from Lake Hammarsjön,

which is downstream of the WWTP, revealed the presence of all of the macrolides on the EU watch list in the range of 2.6 to 215 ng/L. Penicillin G and V were not detected, most likely since they belong to the β -lactams which are easily degraded. Ciprofloxacin and norfloxacin were also not detected, as these have been shown to bind to the solid sludge phase in the WWTPs. The presented methodology is flexible and allows for a simple introduction of new antibiotics with minimal time spent on method development which is demanding and costly.²

References

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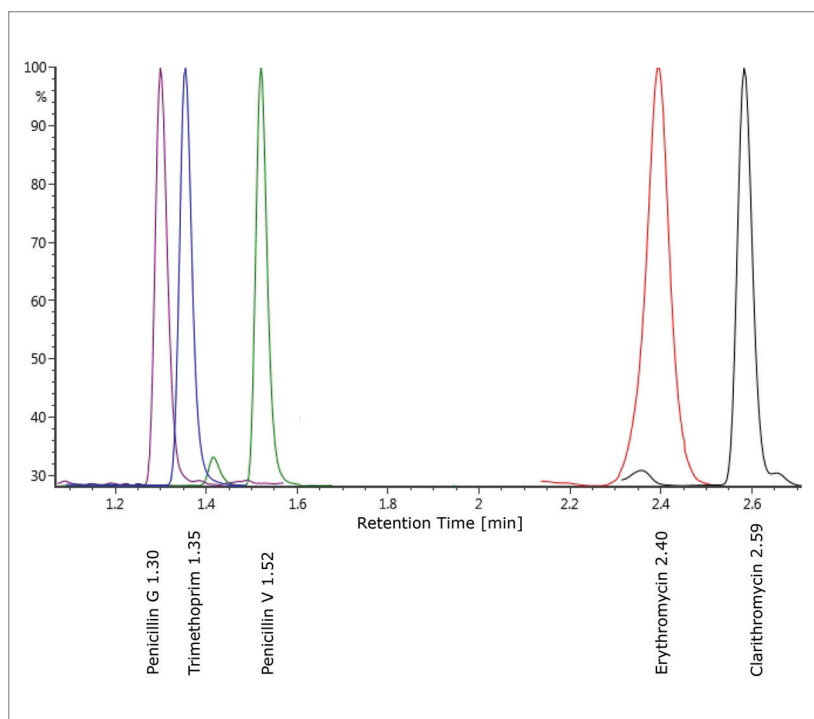


Figure 3. Example of chromatography using basic UPLC conditions at 100 ng/L fortified in surface water.

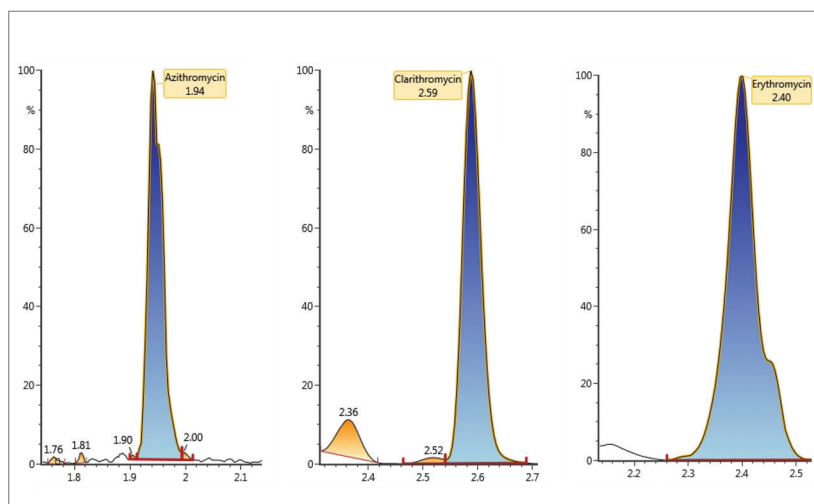


Figure 4. Example of the detection of azithromycin, clarithromycin, and erythromycin at low ppt levels in WWTP effluent samples from the City of Kristianstad, Sweden.

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Waters Corporation
 34 Maple Street
 Milford, MA 01757 U.S.A.
 T: 1 508 478 2000
 F: 1 508 872 1990
www.waters.com