



Measurement of Antibiotics in Environmental Waters using LC-QQQ and fully automated Online Enrichment





Trace Level Measurement – LC-MS

Measurement of Antibiotics in Environmental Waters using LC-MS and Online Enrichment

Antibiotics are widely used for the treatment of bacterial infections in humans. After excretion they ultimately enter waste water treatment plants (WwTP) with subsequent discharge into surface water and the aquatic environment.

Their potential impact on the environment and human health (e.g. antibiotic resistance) is of interest and therefore their presence needs to be closely monitored.

This flyer outlines a method to measure 23 antibiotics from nine different drug classes in environmental waters using an Agilent 1200/6400 LC-MS system incorporating 'front end' automated online solid phase enrichment.

Linearity

was observed within 0.005 - 50 ng (on column), except for amoxicilline (0.050 - 50 ng) and ornidazole as well as for chlortetracycline (0.005 to 30 ng).

Accuracy and Precision were within $\pm 20\%$ of the nominal value and $< 8.5\%$.

Recovery and Precision were $> 65\%$ (for most analytes) and $< 15\%$.

The method was successfully applied to measure the antibiotics in two different waste water treatment plant inflows (hospital and domestic water), the WwTP influent and the WwTP effluent.

- Fully automated online enrichment
- Fast and reliable results
- Trace level analysis

Compounds

23 compounds 9 drug families
wide mass and chemical properties range

Fluoroquinolones

Ciprofloxacin
Enoxacin
Enrofloxacin
Flumequine
Lomefloxacin
Norfloxacin
Ofloxacin
Pipemidic Acid
Sarafloxacin

Quinolones

Nalidixic acid
Oxolinic acid

Sulfonamides

Sulfadimidine
Sulfamethoxazole

Imidazoles

Ornidazole

Macrolides

Erythromycin
Tylosin

Tetracyclines

Tetracycline
Chlortetracycline

β -lactams

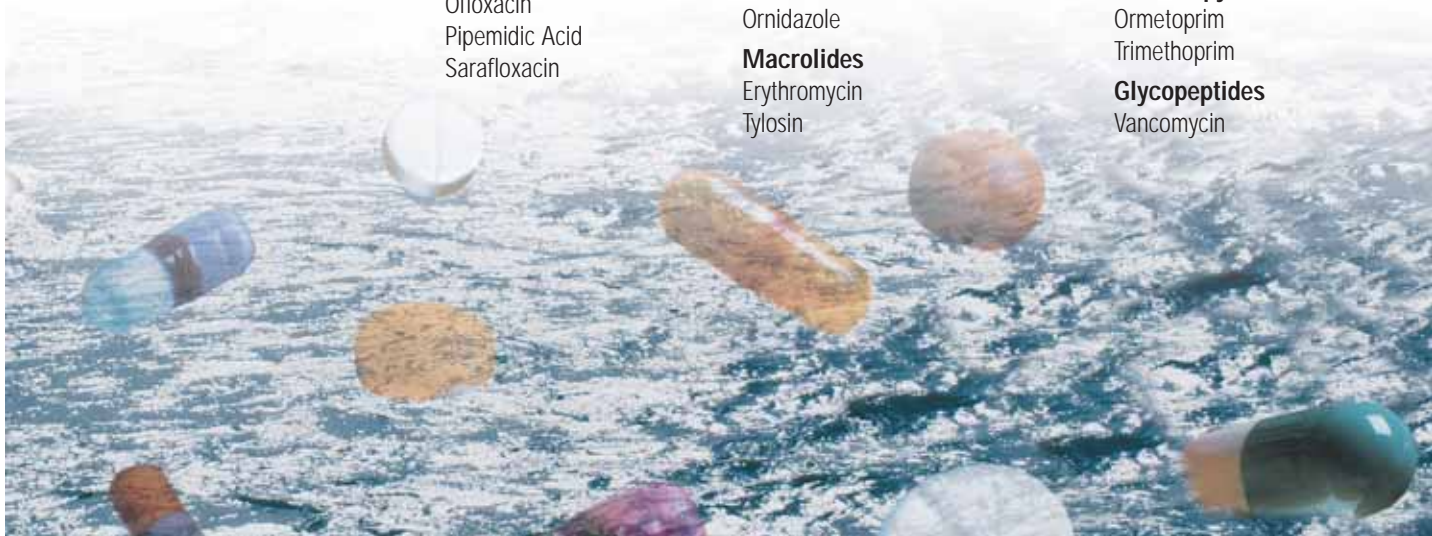
Amoxicillin
Cefotaxim

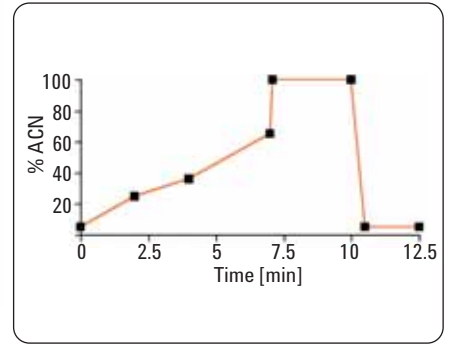
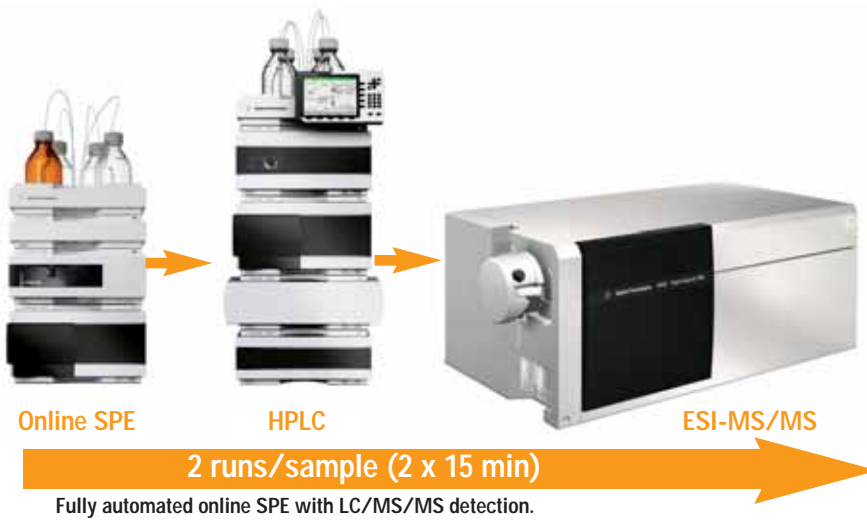
Diaminopyrimidines

Ormetoprim
Trimethoprim

Glycopeptides

Vancomycin





Step Gradient Profile

Materials and Methods

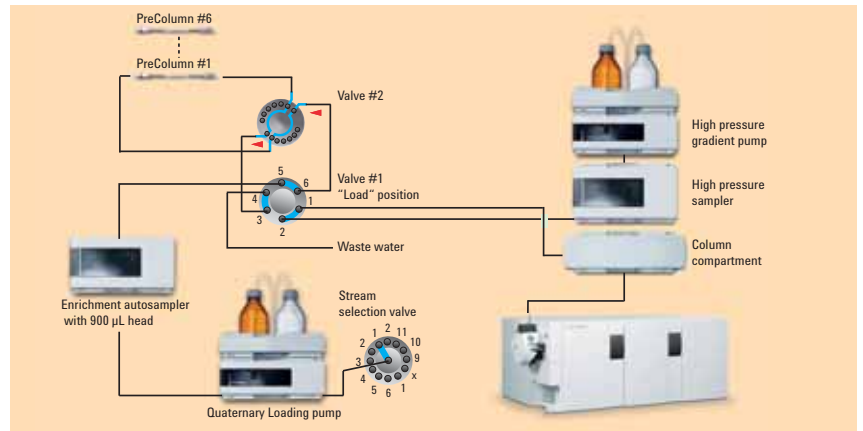
- Analytical Column:** ZORBAX Eclipse Plus C18 (3.5 μm , 2.1 mm I.D. x 150 mm)
- Mobile Phase:** H₂O/ACN + 0.1% formic acid
- Flow rate:** 0.5 mL/min (step gradient)
- Deuterated internal standards:** Norfloxacin-d4, amoxicillin-d4, sulfamethoxazole-d4



Enrichment valves

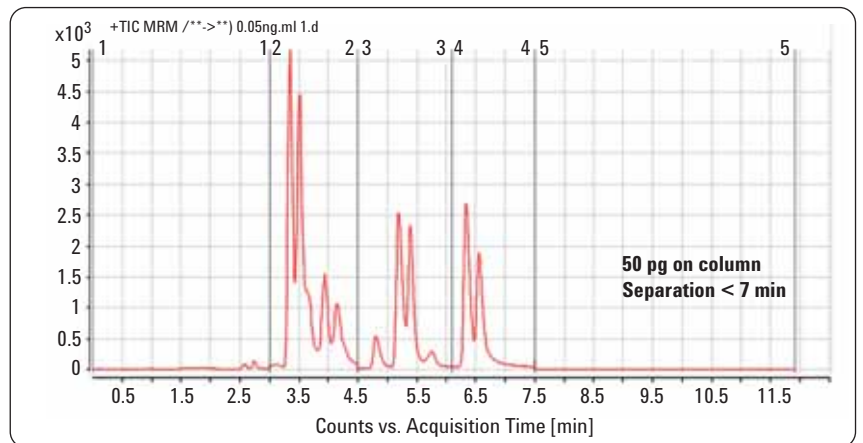
Optimization of the online enrichment for:

- Cartridge Sorbent (C18)
- pH-value (fluoroquinolones pH 4, other compounds pH 7)
- Sample volume (1800 μL , 2 x 900 μL)
- Sample loading flow rate (1 mL/min)
- Sample elution (Backflush with LC gradient)

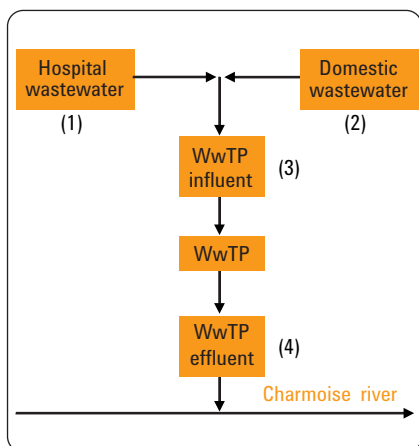


System schematic

Performance Examples



TIC chromatogram



Pilot Study
Fontenay WwTP, (1) - (4) sample collection points.

- Qualifying and Quantifying 23 antibiotics in river water
- Fully automated SPE
- Easy to use and suited for routine analysis

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www.agilent.com/chem/QQQ

Information, descriptions, and specifications in this publication are subject to change without notice.

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Drug class	Analyte	IDL (pg on column)	MDL (ng/L)
Macrolides	Tylosin	0.8	6.0
	Erythromycin	0.9	7.5
Tetracyclines	Chlortetracycline	1.1	9.8
	Tetracycline	1.5	1.8
Beta-lactams	Amoxicilline	16	15
	Cefotaxime	3.5	3.6
Diaminopyrimidines	Trimethoprim	2.4	1.4
	Ormetoprim	1.6	1.9
Sulfonamides	Sulfadimidine	0.5	1.4
	Sulfamethoxazole	2.8	3.1
Quinolones	Oxolinic acid	2.2	1.7
	Nalidixic acid	2.1	3.3
Fluoroquinolones	Flumequine	2.3	2.1
	Pipemidic acid	4.6	15
	Enrofloxacin	2.6	3.3
	Enoxacin	3.3	4.6
	Lomefloxacin	2.8	3.3
	Sarafloxacin	0.6	1.1
	Norfloxacin	5.5	5.0
	Ciprofloxacin	2.6	4.3
Ofloxacin	1.3	2.5	
Imidazoles	Ornidazole	3.6	4.3
Glycopeptides	Vancomycin	2.2	5.0

Instrument (pg) and method detection limit (ng/L).

IDL: Instrument Detection Limit, MDL: Method Detection Limit.

Drug Class	Analyte	Hospital Wastewater	Domestic Wastewater	WwTP Influent	WwTP Effluent
Macrolides	Tylosin	< LOD	< LOD	< LOD	< LOD
	Erythromycin	1871 ± 45	5.2 ± 1.1	1440 ± 302	498 ± 35
Tetracyclines	Chlorotetracycline	< LOD	< LOD	< LOD	< LOD
	Tetracycline	< LOD	< LOD	< LOD	< LOD
Beta-lactams	Amoxicilline	151 ± 47	< LOD	20.2 ± 4.8	17.3 ± 2.1
	Cefotaxime	< LOD	< LOD	< LOD	< LOD
Diaminopyrimidines	Trimethoprim	649 ± 33	1.9 ± 0.2	296 ± 72	491 ± 31
	Ormetoprim	14 ± 5	4.5 ± 0.3	15.0 ± 1.1	32.0 ± 1.9
Sulfonamides	Sulfadimidine	< LOD	< LOD	< LOD	< LOD
	Sulfamethoxazole	1298 ± 150	< LOD	1015 ± 95	4084 ± 93
Quinolones	Oxolinic acid	< LOD	< LOD	< LOD	< LOD
	Nalidixic acid	< LOD	< LOD	< LOD	< LOD
Fluoroquinolones	Flumequine	< LOD	< LOD	< LOD	< LOD
	Pipemidic acid	< LOD	< LOD	< LOD	< LOD
	Enrofloxacin	< LOD	< LOD	< LOD	< LOD
	Enoxacin	1425 ± 13	606 ± 36	842 ± 90	32 ± 9
	Lomefloxacin	1120 ± 90	< LOD	59 ± 4	8.6 ± 0.5
	Sarafloxacin	< LOD	< LOD	< LOD	< LOD
	Norfloxacin	10899 ± 52	49 ± 8	6658 ± 146	217 ± 8
	Ciprofloxacin	11105 ± 37	< LOD	6730 ± 328	1101 ± 96
Ofloxacin	13002 ± 527	79 ± 8	8115 ± 180	17086 ± 79	
Imidazoles	Ornidazole	< LOD	< LOD	< LOD	< LOD
Glycopeptides	Vancomycin	2204 ± 150	< LOD	1784 ± 116	1297 ± 29

Measured concentrations (ng/L) in hospital wastewater, domestic wastewater, WwTP influent and WwTP effluent.



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