Detection of Trace Level Pharmaceuticals in Drinking Water by Online SPE Enrichment with the Agilent 1200 Infinity Series Online-SPE Solution

Application Note

Environmental

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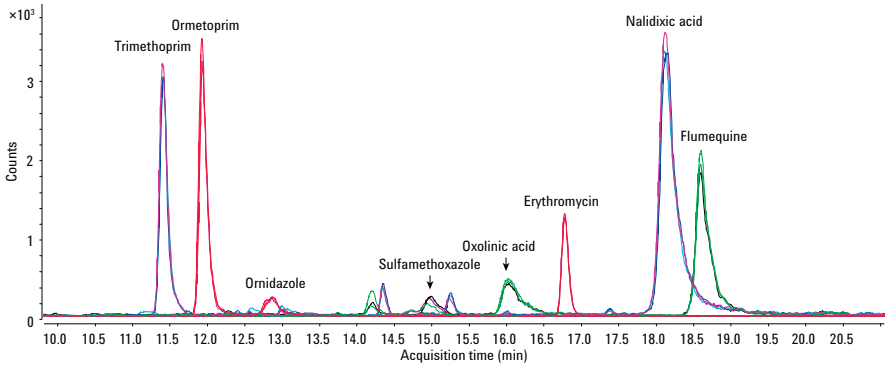
Abstract

This Application Note demonstrates the use of the Agilent 1200 Infinity Series Online-SPE solution combined with triple quadrupole mass spectrometric detection for the analysis of antibiotics at trace levels down to 1 ppt in drinking water. In addition, performance data of the online SPE system for linearity, area and retention time precision, recovery, and concentration precision and accuracy in real water samples from different sources are shown and discussed.



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Introduction

Antibiotics are widely used in human and animal treatment of bacterial infections. After passing through water treatment plants, they can be found in surface water and drinking water. It is important to detect those compounds, even at trace levels, down to the lower single digit ppt (ng/L) level. There are nine typical classes of antibiotics in medical use. Thirteen compounds from these classes were used in this Application Note (Table 1).

This Application Note describes the Agilent 1200 Infinity Series Online-SPE solution based on the Agilent 1290 Infinity Flexible Cube for first, enriching different trace level antibiotics and second, separating them from each other. The online-SPE system includes an Agilent 1260 Infinity Quaternary Pump because commercially available SPE cartridges do not support backpressures above 600 bar. The full functionality of the online-SPE system is achieved by using just one HPLC pump. The performance of the system is demonstrated by the mass spectrometric detection of a suite of 13 antibiotics down to a limit of detection (LOD) of less than 10 ppt. To enhance the throughput of the system, a valve solution for the parallel use of two alternating SPE cartridges is presented.

Experimental

Instrumentation

Agilent 1200 Infinity Series Online-SPE solution system consisting of:

- Agilent 1260 Infinity Quaternary Pump with internal degasser (G1311C) and LAN card (G1369C)
- Agilent 1260 Infinity Standard Autosampler (G1329B) with 900 µL head (G1313-60007), multidraw kit (G1313-68711) and thermostat (G1330B)
- Agilent 1290 Infinity Flexible Cube (G4227A) to mount the 2-position/10-port valve
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1200 Infinity Series online SPE starter set G4742A.

Figure 1 shows how the modules of the system are set up, and Table 2 lists the capillaries and accessories needed to run the online-SPE application. With the G4742A Online SPE starter set, all necessary parts such as capillaries, cartridges, and the 2-position/10-port valve head (600 bar) are ordered in one package.

Table 1. Classes of antibiotics and individual compounds used in this study.

Antibiotics class	Compounds	
Fluoroquinolones	Flumequine	
Quinolones	Nalidixic acid	Oxolinic acid
Sulfonamides	Sulfamethoxazole	
Imidazoles	Ornidazole	
Macrolides	Erythromycin	Tylosin
Tetracyclines	Tetracycline	Chlortetracycline
b-Lactams	Cefotaxim	
Diaminopyrimidines	Ormetoprim	Trimetoprim
Glycopeptides	Vancomycin	

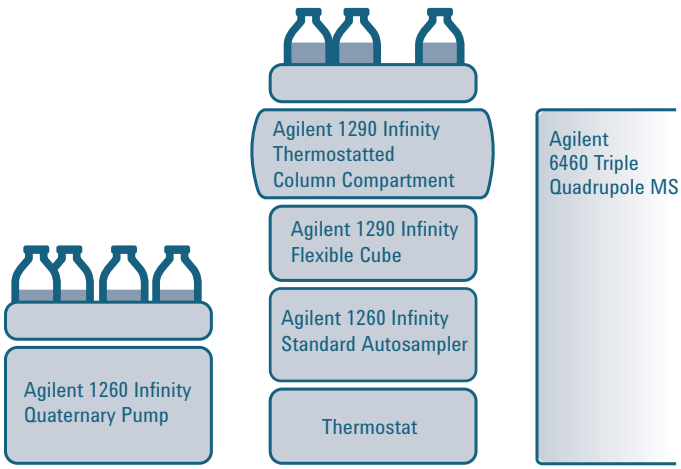

Figure 1. Setup of the Agilent 1200 Infinity Series Online-SPE solution with MS detector (the solvent bottles in the center are for SPE loading, rinsing and conditioning).

MS-Detection

- Agilent 6460 Triple Quadrupole LC/MS with Agilent Jet Stream Technology

Analytical Column

- Agilent ZORBAX Eclipse Plus C18, 2.1 × 150 mm, 3.5 µm (p/n 959763-902)

Trapping Columns

- 2x Guard Column Hardware Kit (p/n 820999-901)
- Agilent PLRP-S Cartridges, 2.1 × 12.5 mm, 15-20 µm (p/n 5982-1271)

Software

- Agilent MassHunter data acquisition for triple quadruple mass spectrometer, Version 06.00
- Agilent MassHunter Optimizer software, Version 06.00
- Agilent MassHunter Qualitative software, Version 06.00
- Agilent MassHunter Quantitative software, Version 05.02

Table 2A. Content of the Agilent 1200 Infinity Series online SPE solution starter set G4742A. (SST = stainless steel).

Parts	Qty	Description	Order No.
2-position/10-port valve head	1	Valve head to be mounted in Flexible Cube	5067-4145
Guard column hardware kit	2	To insert SPE cartridge	820999-901
Online SPE capillary kit	1	Contains required capillaries	5067-5708

Table 2B. Content of the online SPE capillary kit which is part of G4742A, Agilent 1200 Infinity Series online SPE solution starter set. (SST = stainless steel).

Parts and Capillaries	Qty	Description	Order No.
120 mm, 0.12 mm id, SST	5	Valve to guard column hardware and back to valve; and one valve crossing	5067-4652
BondElut online PLRP-S 15-20 µm 2.1 × 12.5 mm	1	SPE cartridges	5982-1271
340 mm, 0.12 mm id, SST	2	Valve to column, valve to autosampler	5067-4647
Waste line	2m	Valve to waste	0890-1713
500 mm, 0.25 mm id, SST	1	Flexible Cube pump to autosampler	5067-5713
700 mm, 0.17 mm id, SST	1	LC pump to valve	5067-4648
Finger tight fitting	1	For waste line	0100-1516

Table 3. Detailed HPLC method according to the individual modules.

HPLC Method	
Agilent 1260 Infinity Quaternary Pump	
Solvent A	Water, 5 mM ammonium formate
Solvent B	ACN +5 % water, 5 mM ammonium formate
Flow rate	0.4 mL/min
Gradient	0 minutes – 5% B, 5 minutes – 5% B, 20 minutes – 98% B
Stop time	25 minutes
Post time	10 minutes
Agilent 1290 Infinity Thermostatted Column Compartment	
Column temperature	40 °C
Agilent 1290 Infinity Flexible Cube	
Right valve	2-position/10-port Quick-Change valve head
Pump	1.5 mL/min
Solvents	A1: Water, B1: ACN 0 minutes – Pump 300 seconds, Solvent A1 5 minutes – right valve change position 7 minutes – Pump 180 seconds, Solvent B1 11 minutes – Pump 300 seconds, Solvent A1
Agilent 1260 Infinity Standard Autosampler	
Injection volume	1,800 µL (automated multidraw of two times 900 µL)
Needle wash	In vial (MeOH)
Draw and eject speed	1,000 µL/min
Sample temperature	10 °C
Two trays with 15 positions each (G1313-44513)	
6-mL screw cap vials (glass, p/n 9301-1377), screw caps (p/n 9031-1379), preslit septa for 6-mL screw cap vials (p/n 5188-2758)	

In the setup of the online-SPE LC system, the 1290 Infinity Flexible Cube (Figure 2) has a 2-position/10-port valve with two trapping columns next to the piston pump and the solvent selection valve for flushing the sample on the trapping columns and the re-equilibration of those columns (Figures 3A and 3B). The piston pump inside the Flexible Cube is connected to the autosampler to flush the sample directly onto one trapping column (SPE 1). The other trapping column (SPE 2) is connected to the analytical column and to the LC pump (Figure 3A).

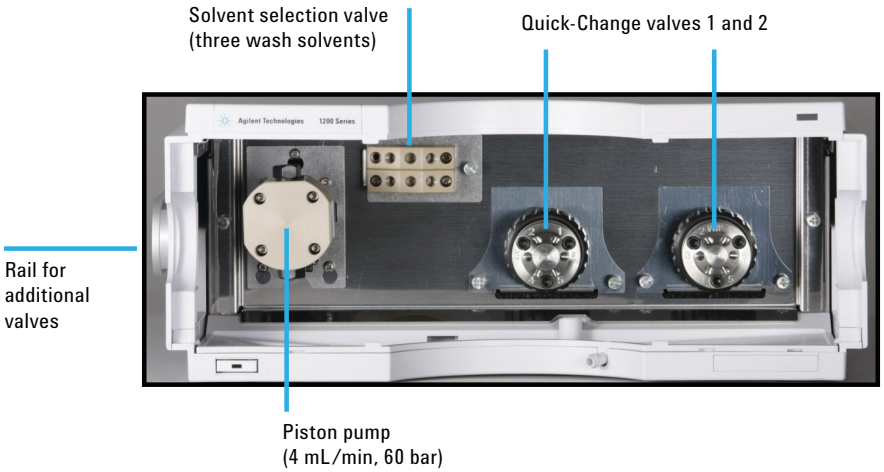

Figure 2. The Agilent 1290 Infinity Flexible Cube is an additional module for the Agilent 1290/1260 Infinity LC system, using one or two Agilent 1200 Infinity Series Quick-Change valves.

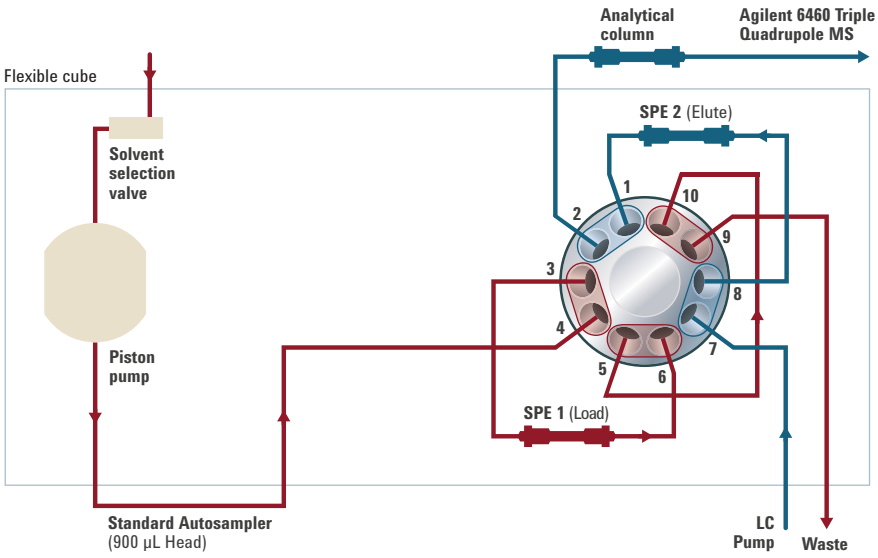

Figure 3A. Valve positions for loading the sample on trapping column SPE 1 while trapping column SPE 2 is being eluted.

After loading the trapping column with sample, the 2-position/10-port valve was switched and thus the positions of the trapping columns were exchanged (Figure 3B). Now, the LC pump delivers the gradient to elute the enriched analytes in backflush mode from the trapping column (SPE 1) onto the analytical column. Simultaneously, the trapping column (SPE 2), which had been loaded with sample in the previous run, is cleaned and reconditioned by a purging procedure. This cleaning procedure is done by the piston pump with the cleaning solvents selected by the solvent selection valve. Table 3 shows a summary of the LC method for the main modules.

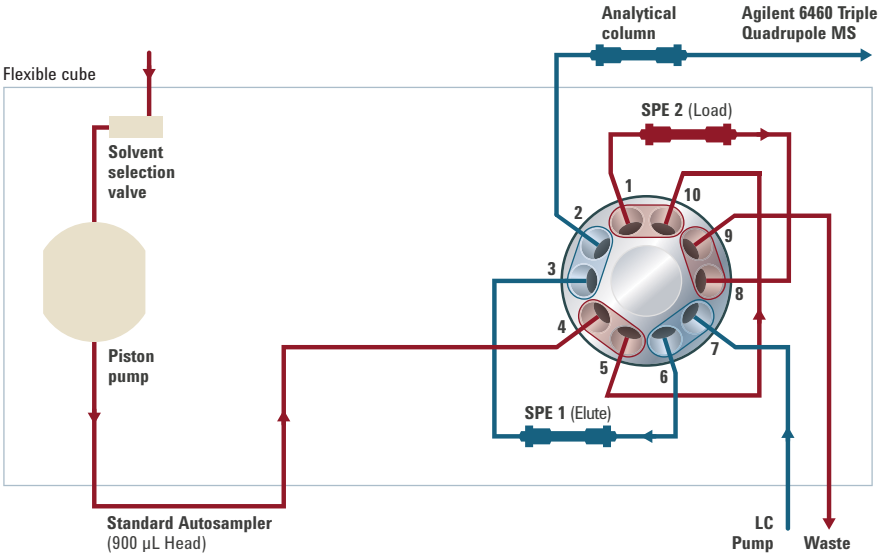

Figure 3B. Valve positions for loading the sample on trapping column SPE 2 while trapping column SPE 1 is being eluted.

Table 3. Summary of the LC method for the Agilent 1260 Infinity Standard Autosampler, the Agilent 1260 Infinity Quaternary Pump and the Agilent 1290 Infinity Flexible Cube.

Agilent 1260 Infinity Standard Autosampler	Multidraw 1800 µL sample	Inject																																post run
Agilent 1260 Infinity Quaternary Pump			5% Solvent B						Gradient 5% B to 98% B																				98% Solvent B					
Agilent 1290 Infinity Flexible Cube			Pump 300 seconds					Switch valve to next position		Pump 180 seconds					Pump 300 seconds solvent																			
Minutes			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25							10

The MRM and dynamic MRM triple quadrupole MS method was developed by using the MassHunter optimizer software and direct injections of individual antibiotic standards (10 ng/µL) by flow injection into the mass spectrometer. The optimization was done to find the optimum fragmentor voltage for each individual compound, and the optimum collision energies for the fragmentation to the quantifier and qualifier ions (Table 4).

The developed MRM method was applied to a 100 ng/L (100 ppt) mixture of all standards to identify the retention time of the individual compounds in the final online-SPE LC method. From the resulting data file, the dynamic MRM method was developed with a retention time window of ± 3 times the measured peak width around the retention time of each compound.

Chemicals

All solvents used were LC/MS grade. Acetonitrile was purchased from J.T. Baker, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22 µm membrane point-of-use cartridge (Millipak). All antibiotics standards were purchased from Dr. Ehrenstorfer GmbH, Germany.

Calibration Standards

A stock solution containing all antibiotics was prepared from the purchased standards to 100 ng/L (100 ppt) each in water. The dilution series for determination of the LOD, LOQ and the calibration curve was 100, 50, 20, 10, 5, 2, 1, 0.5 ppt.

Table 5. Detailed optimized MS parameter for Agilent Jet Stream thermal gradient focusing technology.

Triple Quadruple MS method	
Agilent Jet Stream thermal gradient focusing technology	
Gas temperature	325 °C
Gas flow	9 L/min
Nebulizer	35 psi
Sheath gas temperature	350 °C
Sheath gas flow	12 L
Capillary	4,000 V
Nozzle	0 V

Table 4. MRM and Dynamic MRM MS method showing the identified optimum fragmentor and collision energy values for the individual antibiotics as well as for the quantifier and qualifier ions. The retention time was used to develop the dynamic MRM method with a window of ± 3 times the peak width around the compound retention time.

Name	RT [min]	Molecular mass	Molecular ion	Fragmentor	Quantfier ion	CE	Qualifier ion	CE
Vancomycin	10.25	1,447.43	724.80	120	144.0	12	100.1	40
Trimethoprim	11.37	290.13	291.14	150	230.1	20	123.0	24
Ormetoprim	11.88	274.14	275.15	150	259.1	24	123.0	24
Cefotaxime	12.27	455.05	456.06	110	125.0	52	126.0	44
Tetracycline	12.44	444.15	445.16	90	410.1	16	154.0	28
Ornidazole	13.01	219.04	220.04	100	128.0	12	82.1	32
Chlorotetracycline	14.31	478.11	479.12	125	444.1	20	462.1	12
Sulfamethoxazole	15.11	253.95	254.06	95	92.1	28	108.0	20
Oxolinic acid	16.08	261.06	262.07	85	244.0	16	216.0	28
Erythromycin	16.79	733.46	734.50	120	158.1	32	576.0	16
Tylosin	17.55	915.51	916.52	155	174.1	40	772.4	28
Nalidixic acid	18.19	232.08	233.09	85	215.1	8	187.0	24
Flumequine	18.66	261.08	262.08	75	244.0	12	202.0	32

The molecular ion of vancomycin is $[M+H]^{2+}$, all others are $[M+H]^{+}$
Fragmentor = voltage (V)
RT = retention time (min)
CE = collision energy (eV)

Samples

Water samples were taken directly from the Rhine river, from tap water, and from a spring in the region of Karlsruhe, Germany. The water samples were spiked to a final concentration of 20 ppt with a concentrated solution of the antibiotics, vortexed, filtered with Captiva Premium Syringe Filter (0.45 µm, 15 mm, p/n 5190-5109), and injected without further sample prep.

Results and Discussion

At first, a calibration from 100 ppt to 1 ppt was done for all compounds in the mixture. Within the used group of antibiotics, there is large molecular variety from very polar to nonpolar, small molecules to very large macrocyclic, and polypetidic molecules. For the molecules with very low limits of quantification (LOQ) down to 1 ppt, all levels in the calibration curves were used. For compounds with higher LOQs, fewer levels were used for the calibration curves (Figure 4).

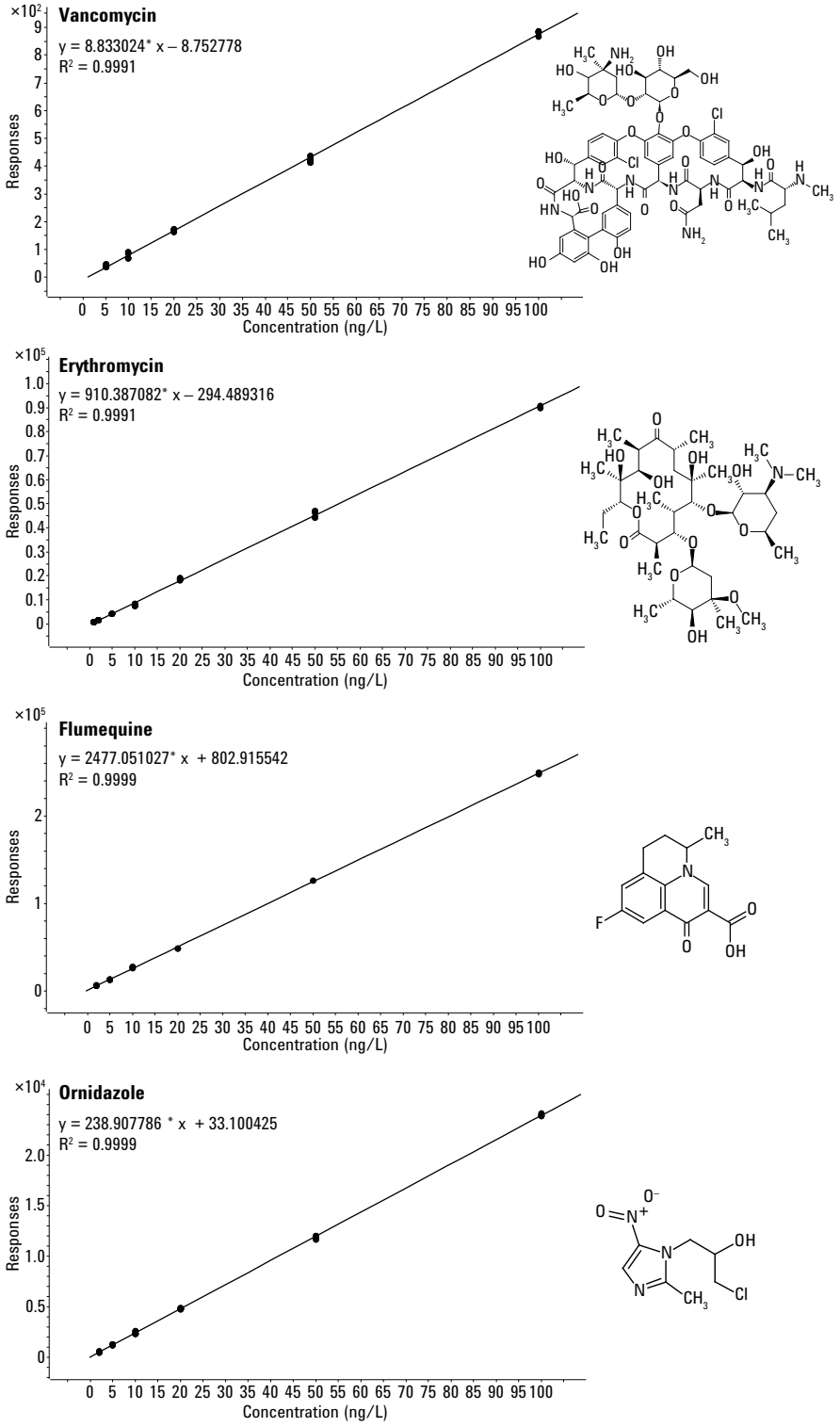

Figure 4. Calibration curves for selected antibiotics. All compounds were measure from 100 ppt to 1 ppt with seven levels. Depending on the molecule, the LOQ was taken at S/N = 10 and levels adjusted accordingly.

As LOQ, a signal-to-noise (S/N) ratio of 10 (S/N=10) was used, and as LOD, S/N=3 was used. Typically, the LOD was found one level below the LOQ. For instance, flumequine had a LOQ of 2 ppt and a LOD of 1 ppt. All compounds delivered calibration curves with good linearity coefficients (Table 5). For a statistical evaluation, multiple injections of the 50 ppt level were done. The relative standard deviations (RSD) of the retention time were all below 0.15%. The RSD of the peak areas were typically below 6%. The recovery of the trapping process was measured for a 50 ppt level injection of the trapping columns in comparison to a 50 ppt injection going directly onto the analytical column. The recovery was typically above 70%, except for the tetracycline compounds. They have lower recoveries, which might be due to weaker bonding on the trapping column material but deliver enough signal intensity for LOQ at 5 ppt each.

Table 6. Overview of calibration data (LOD and LOQ), recovery of the trapping process as well as statistical evaluation (retention time RSD and peak area RSD) of antibiotics used in this study.

Compound	RT (min)	RT RSD (%)	Area RSD (%)	LOD (ppt)	LOQ (ppt)	Linearity	Recovery (%)
Vancomycin	10.25	0.10	4.15	2.00	5.00	0.9991	76
Trimethoprim	11.37	0.05	2.31	0.50	1.00	0.9995	97
Ormetoprim	11.88	0.03	2.95	0.50	1.00	0.9991	95
Cefotaxime	12.27	0.14	5.66	2.00	5.00	0.9994	99
Tetracycline	12.44	0.07	5.64	2.00	5.00	0.9993	41
Ornidazole	13.01	0.07	1.45	1.00	2.00	0.9998	94
Chlorotetracycline	14.31	0.10	12.60	2.00	5.00	0.9950	45
Sulfamethoxazole	15.11	0.07	1.33	2.00	5.00	0.9994	99
Oxolinic acid	16.08	0.02	4.42	2.00	5.00	0.9992	81
Erythromycin	16.79	0.07	1.66	0.50	1.00	0.9991	106
Tylosin	17.55	0.09	7.96	5.00	10.00	0.9990	69
Nalidixic acid	18.19	0.03	2.99	0.50	1.00	0.9994	109
Flumequine	18.66	0.04	3.31	1.00	2.00	0.9998	103

Finally, an experiment for the determination of carryover of the most intense polar (hydrophilic) and nonpolar (hydrophobic) compounds was done. An injection of the 100 ppt level of all compounds was done, followed by a blank injection (Figure 5).

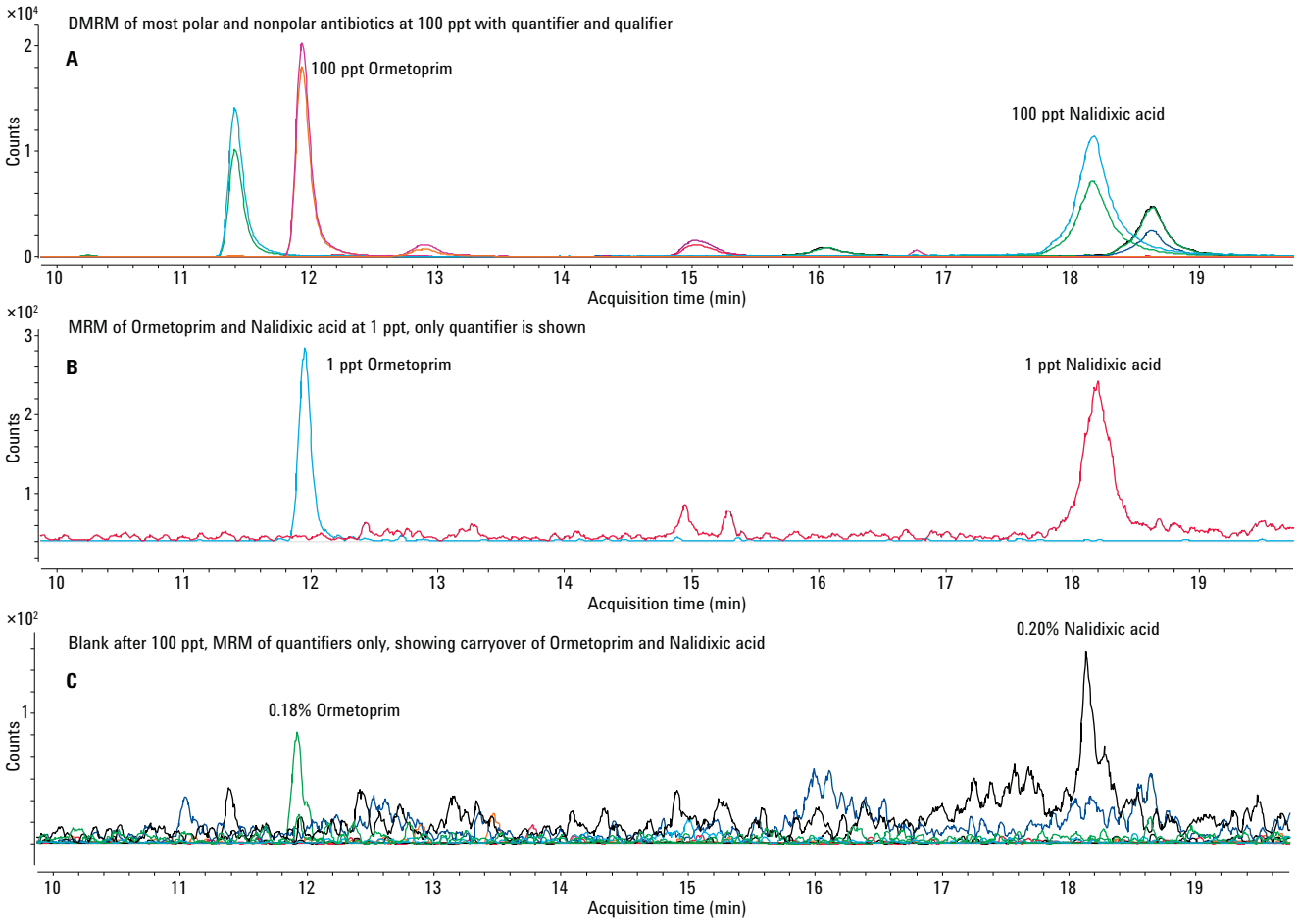

Figure 5. Determination of carryover of the two most intense hydrophilic and hydrophobic compounds. The carryover was determined from an injection of 100 ppt by a following blank. The 1 ppt injection is shown for comparisons.

The most intense polar compound was ormethoprim, and the most intense nonpolar compound was nalidixic acid. In the following blank, ormethoprim showed 0.18% carryover and nalidixic acid showed 0.2%. Both compounds had an LOQ of 1 ppt and the carryover was approximately 25% of that (Figure 6).

To evaluate the performance in real samples, the antibiotics were spiked in water samples from tap, the Rhine river, and spring water at a concentration of 20 ppt (Figure 6). The responses of the individual signal were in the same order for each compound in the different matrices. The evaluation of the data showed that the area precision RSD is typically below 2% for all compounds in all matrices (Table 6). The accuracy was typically above 70%. Only one compound, erythromycin, was a little below 50%.

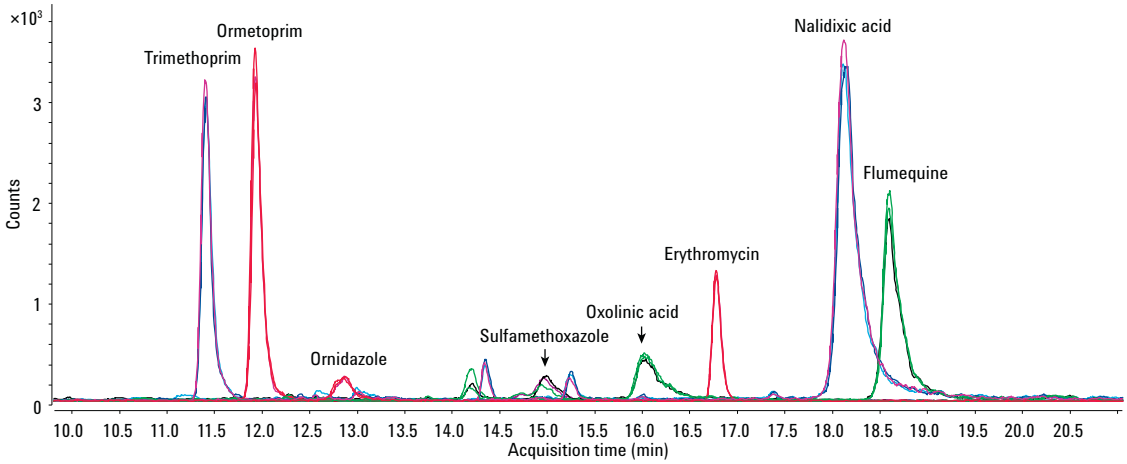

Figure 6. Selected antibiotics from a spiking experiment in Rhine river water, tap water, and spring water. The spiked concentration level of all antibiotics was 20 ppt.

Table 7. Statistical evaluation of compounds selected from the spiking experiment shown in Figure 6.

	Rhine river water				Tap water				Spring water			
Compound	Average (ppt)	SD	RSD (%)	Accuracy (%)	Average (ppt)	SD	RSD (%)	Accuracy (%)	Average (ppt)	SD	RSD (%)	Accuracy (%)
Trimethoprim	18.91	0.0636	0.34	94.53	19.40	0.6435	3.32	96.98	17.61	0.0283	0.16	88.05
Ormetoprim	14.91	0.1485	1.00	74.53	15.92	0.3041	1.91	79.58	14.71	0.4667	3.17	73.55
Ornidazole	13.00	0.1414	1.09	65.00	14.45	0.0707	0.49	72.25	13.45	0.0707	0.53	67.25
Sulfamethoxazole	18.75	0.0071	0.04	93.73	11.85	0.5515	4.65	59.25	18.36	0.2263	1.23	91.80
Oxolinic acid	17.65	0.2121	1.20	88.25	19.30	0.1414	0.73	96.50	18.25	0.0707	0.39	91.25
Erythromycin	9.46	0.4950	5.23	47.30	9.78	0.1485	1.52	48.88	9.51	0.0990	1.04	47.55
Nalidixic acid	15.57	0.0141	0.09	77.85	16.83	0.4596	2.73	84.13	15.83	0.2121	1.34	79.15
Flumequine	19.03	0.1768	0.93	95.13	20.86	0.1485	0.71	104.28	19.62	0.0495	0.25	98.08

Conclusion

This Application Note demonstrates the use of the Agilent 1200 Infinity Series Online-SPE solution for enrichment, separation, and detection of antibiotic residues in trace level analysis of water samples by HPLC using triple quadrupole MS detection. It was demonstrated that LOQs as low as 1 ppt could be achieved. The methodology showed a high sample to sample reproducibility with area deviation of less than 6%. The efficient online SPE trapping process allowed detection in real drinking water samples with high precision and accuracy.

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