



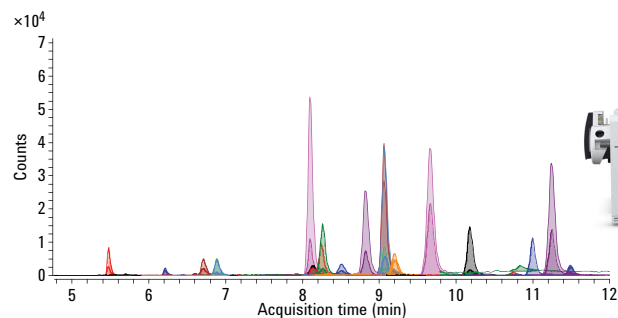
Analysis of Trace Level Herbicides from Different Water Samples with the Agilent 1200 Infinity Series Online SPE Solution

Application Note

Environmental

Author

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Abstract

This Application Note shows the analytical results of 25 herbicides and herbicide metabolites in different environmental water samples using the Agilent 1200 Infinity Series Online SPE Solution in combination with triple quadrupole mass spectrometric detection at trace levels down to 2 ng/L. The method used was developed and validated during an interlaboratory test as previously described³ and was applied to real water samples from different origins showing good precision, recovery, and linearity. All samples were measured automatically, in a cost-effective short time. The absence of sample preparation makes the system a very powerful analytical tool for monitoring residues in water.



Agilent Technologies

Introduction

Due to concerns about the contamination of water by herbicides, drinking water quality is monitored to meet the requirements of the European Union drinking water directive 98/83/EC¹. EU regulations for drinking water quality set a limit of 0.5 µg/L for the sum of all pesticides and 0.1 µg/L for each individual compound. These requirements, for example, have driven the development of more sensitive methods for the analysis of herbicides in water.

Generally, herbicide water analysis requires two different methods: a sample preparation method and a detection method². Agilent Technologies offers, with the Agilent 1200 Infinity Series Online SPE Solution, an automated SPE enrichment process with chromatography and MS detection. Sample preparation is not necessary to analyze water samples.

This Application Note describes the advantages and the sensitivity of the 1200 Infinity Series Online SPE Solution for three different environmental water samples. Real water samples were analyzed and performance data such as linearity, area and retention time precision, as well as limit of detection (LOD) and limit of quantitation (LOQ) are shown. This study discusses apparent recovery, area precision, and carryover.

Experimental

Instrumentation

Agilent 1200 Infinity Online SPE Solution

Agilent 1260 Infinity Binary Pump G1312B and LAN card G1369C

Agilent 1260 Infinity Standard Autosampler G1329B with 900 µL head (G1313-60007) and an Agilent 1290 Infinity Thermostat G1330B

Agilent 1290 Infinity Flexible Cube G4227A with two valve drives

Agilent 1200 Infinity Online SPE starter kit G4742A including one 2-position/10-port valve

Agilent 1290 Infinity Thermostatted Column Compartment G1316C

Agilent Triple Quadrupole LC/MS System with Agilent Jet Stream Technology G6460A

Analytical column

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

Trapping columns

2 × Guard Column Hardware Kit (p/n 820999-901)

PLRP-S Cartridges, 2.1 × 12.5 mm, 15-20 µm (p/n 5982-1271)

6 mL screw cap vials (glass p/n 9301-1377), screw caps (p/n 9301-1379), pre-slit septa (p/n 5188-2758)

Software

Agilent MassHunter data acquisition for triple quadrupole mass spectrometer, Version 06.00

Agilent MassHunter Optimizer software, Version 06.00

Agilent MassHunter Source and iFunnel Optimizer software, Version 06.00

Agilent MassHunter qualitative software, Version 06.00

Agilent MassHunter quantitative software, Version 05.02

HPLC method

Agilent 1260 Infinity Binary Pump

Solvent A	Water, 5 mM Ammonium Formate + 0.1 % formic acid	
Solvent B	Acetonitrile + 5 % Water, 5 mM ammonium formate + 0.1 % formic acid	
Flow rate	0.4 mL/min	
Gradient	Time (min)	% B
	0	2
	2	2
	2.5	25
	12	100
	22	100
	22.1	2
Stop time	22.1 minutes	
Post time	10 minutes	

Agilent 1290 Infinity Thermostatted Column Compartment

Temperature 30 °C

Agilent 1290 Infinity Flexible Cube

Valve 2-position/10-port QuickChange valve head

Solvent selection valve Solvent A1: Water

Solvent B1: Acetonitrile

System configuration and principle of operation

This Application Note used the Agilent 1200 Infinity Series Online SPE Solution, which is based on the 1290 Infinity Flexible Cube. The Flexible Cube has one 2-position/10-port valve for the alternate use of two trapping columns to enhance the sample throughput. Up to three solvents can be used to load and clean the SPE cartridges (PLRP-S phase) which are reusable (> 200 injections). The full functionality of the online-SPE system is achieved with only one HPLC binary pump. More information about the principle of operation and the 2-position/10-port valve configuration is described in Application Note 5991-2405EN³.

The system was coupled to an Agilent 6460 Triple Quadrupole LC/MS System for the detection of herbicides at trace levels.

The Multiple Reaction Monitoring (MRM) triple quadrupole MS method was developed with MassHunter optimizer software. Direct injections (10 ng/ μ L) of all individual pesticide standards were used. For every compound, two MRM transitions were optimized regarding fragmentor voltage and collision energy. Table 2 shows the suite of 25 pesticides with the individual optimum of fragmentor voltage and collision energy for the product ions (quantifier and qualifier ions). The MRM method was developed to measure the retention times required to set up a Dynamic MRM (DMRM) method.

Agilent 1260 Infinity Standard Autosampler	
Injection volume	900 μ L
Needle wash in vial (ACN)	
Draw and eject speed	1,000 μ L/min.
Sample temperature	5 °C.

Agilent 1290 Infinity Inline Filter (0.3 μ m) (p/n 5067-4638)

Table 1. Piston pump timetable in the Flexible Cube for the online SPE method.

Time	Function	Parameter
0	Pump for time	Pump 60 s, flow: 1 mL/min Channel A1
2	Right valve change position	Increase valve position (switch valve)
2.1	Pump for time	Pump 180 s, flow: 1.5 mL/min Channel B1
6	Pump for time	Pump 300 s, flow: 1.5 mL/min Channel A1

MS method

The source parameters for the Agilent Jet Stream were optimized with the MassHunter Source and iFunnel Optimizer.

Agilent 6460 Triple Quadrupole LC/MS System with positive electrospray Agilent Jet Stream technology	
Gas temperature	260 °C
Gas flow	9 L/min
Nebulizer	45 psi
Sheath gas temperature	300 °C
Sheath gas flow	12 L/min
Capillary	+3,500 V
Nozzle	+500 V
Delta EMV	+400 V

Table 2. MRM and DMRM method. All 25 pesticides are listed with their precursors, product ions, optimum collision energies, and optimum fragmentor voltage.

Compound	Precursor	Precursor (M+H) ⁺	Product ion 1	CE (V)	Product ion 2	CE (V)	Fragmentor (V)
2,6-Dichlorbenzamid	190,0	191,0	109,0	40	172,9	16	100
Atrazin	215,71	216,1	174,0	16	104,0	28	105
Atrazin-desethyl	187,63	188,1	104,0	28	68,1	36	105
Atrazin-desisopropyl	173,6	174,1	104,2	24	68,1	32	95
Boscalid	342,2	343,0	307,0	16	271,0	32	130
Bromacil	260,1	261,0	205,0	8	188	28	105
Carbamazepin	236,3	237,1	194,1	16	192	20	135
Chlorfenvinphos	358,6	359,0	155,0	8	99,0	28	70
Chloridazon	221,6	222,0	92,2	24	104,2	20	125
Chloridazon-methyl-desphenyl	159,57	160,0	130,1	24	88	36	120
Chlorpyrifos	350,6	350,0	97,0	32	198,0	16	95
Chlortoluron	212,7	213,1/215	72,1	20	72,1	20	100
Diuron	233,1	233,1/235	72,0	16	72,0	16	100
Isoproturon	206,3	207,1	72,1	20	165,0	8	85
Lenacil	234,3	235,1	153,1	12	136,1	36	70
Metalaxyl	279,3	280,2	220,0	8	192,1	16	70
Metamitron	202,2	203,1	175,1	12	104,1	24	105
Metazachlor	277,8	278,1	210,1	4	134,1	20	85
Metolachlor	283,8	284,1	252,1	12	176,1	24	90
Metribuzin	214,3	215,1	187,2	25	84,0	29	105
Pendimethalin	281,3	282,2	212,2	4	194	16	125
Quinoxifen	307,1	308,0	196,9	36	262,0	52	150
Simazin	201,7	202,1	104	24	124,0	16	120
Terbuthylazin	229,7	230,1	174,0	12	104,0	32	105
Terbuthylazin-desethyl	201,66	202,1	146,0	12	104,0	28	80

Chemicals

All solvents used were LC/MS grade. Acetonitrile was purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22- μ m membrane point-of-use cartridge (Millipak). Formic acid was from Agilent (p/n G2453-85060) as well as ammonium formate (p/n G1946-85021).

All herbicide standards were purchased from Dr. Ehrenstorfer GmbH, Germany at a concentration of 100 mg/L in acetonitrile.

Samples

Three different water samples were collected in the region of Karlsruhe, Germany.

- Sample 1: ground water
- Sample 2: river water
- Sample 3: stream water

To protect the column and the system from small sample particles, an inline filter was installed in the autosampler between the injection port and the switching valve.

The water samples were centrifuged for 5 minutes at 15,000 rpm. Seven calibration standards of mixed herbicides, ranging from 2 to 500 ng/L (2, 10, 20, 50, 100, 200, and 500 ng/L) each, were prepared in tap water by appropriate dilution of the stock solution (2.8 μ g/L).

Results and Discussion

Three different environmental water samples were measured with the 1200 Infinity Series Online SPE Solution.

For a seven level calibration curve, dilutions in tap water were prepared between 2 and 500 ng/L. The calibration standards and the samples were measured with the 1200 Infinity Series Online SPE Solution and a DMRM method. The calibration standards were measured twice and the water samples were measured 10 times. Quality control (QC) standards were in a concentration of 10 and 50 ng/L and were measured between the samples. All QCs showed good recoveries.

Figure 1 shows a calibration curve of atrazine desisopropyl (RT = 6.21). The curve contains two replicates of each standard as well as eight QC. The weighting is $1/SD^2$ and the curve is not forced to origin.

Table 3 shows the complete set of 25 herbicides in three environmental water samples with the average herbicide concentration, the area relative standard deviation (RSD), linear coefficient, and retention time (RT). The linear coefficients were good for all compounds, with values typically better than 0.997. The RSD of the peak areas was typically < 5 %. Some compounds, which were found to be present in low concentrations, showed an area RSD of > 10 %.

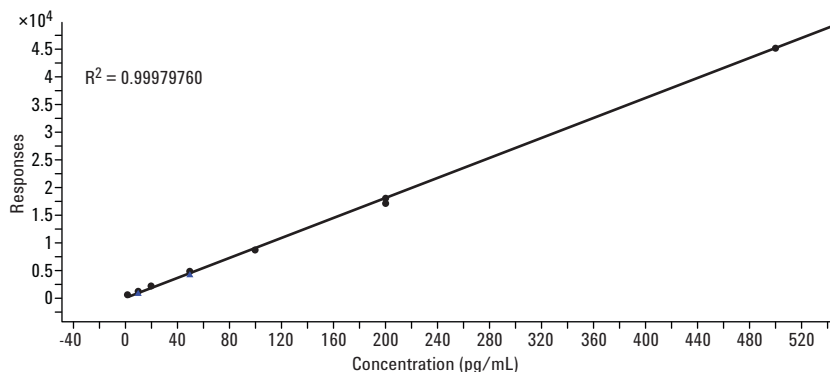


Figure 1. A seven level calibration curve of Atrazine desisopropyl (2 – 500 ng/L). The triangle symbolizes the QCs at a concentration of 10 and 50 ng/L. The correlation coefficient is 0.9997.

Table 3. The average herbicide concentration (n = 10) of three environmental samples are listed with their average concentration, area RSD, correlation coefficient, and RT.

Compound	Ground water		River water		Stream water		R ²	RT (min)
	Average concentration (n = 10) (ng/L)	Area RSD (%)	Average concentration (n = 10) (ng/L)	Area RSD (%)	Average concentration (n = 10) (ng/L)	Area RSD (%)		
Chloridazon-methyl-desphenyl			30.99	6.3	90.67	5.6	0.9966	5.49
Atrazine desisopropyl	5.57	19.0	10.38	19.8			0.9999	6.24
2,6 Dichlorbenzamid					34.29	12.1	0.9934	6.61
Metamitron							0.9981	6.67
Atrazine desethyl	49.79	6.1	26.10	10.2	7.54	17.1	0.9989	6.89
Chloridazon					2.41	11.5	0.9991	6.92
Bromacil					20.35	13.6	0.9997	7.94
Carbamazepin	61.63	1.4	193.48	2.1	185.84	2.1	0.9993	8.21
Simazine	7.63	11.2	7.80	21.5			0.9993	8.16
Terbuthylazin-desethyl			12.76	6.5			0.9975	8.28
Lenacil							0.9923	8.36
Metribuzin							0.9967	8.57
Chlorotoluron			21.69	1.8			0.9959	8.82
Metaxalyl			3.85	4.4	2.70	4.5	0.999	9.00
Atrazine	29.19	4.5	18.04	4.1	6.06	9.4	0.9971	9.09
Isoproturon			22.63	1.5	23.75	1.8	0.9983	9.10
Diuron	66.35	3.3	35.59	3.2	19.53	7.1	0.9981	9.24
Metazachlor			2.51	4.8	11.28	1.1	0.999	9.69
Terbuthylazine			14.94	3.1	4.68	4.8	0.9967	10.13
Boscalid					37.76	8.4	0.9999	10.67
Metolachlor			35.67	1.2	2.02	13.1	0.9986	11.27
Chlorphenvinphos							0.9978	11.67
Quinoxifen							0.9893	13.17
Chlorpyrifos							0.9974	13.58
Pendimethalin							0.9924	13.54

In the ground water, river water, and stream water samples, different herbicides were found in relevant concentrations but still below 100 ng/L. This is the limit for drinking water in the European Union¹. Carbamazepine was the only compound which had a concentration of 185 ng/L in stream water and 193 ng/L in river water. All other herbicides were below the maximum value in drinking water.

The RSD of the RT was calculated for the standards and samples and was found to be excellent, with < 0.1 % for 10 replicates and two alternating SPE cartridges. The value at a signal-to-noise (S/N) = 3 was used for the determination of the LOD and a value of S/N = 10 was used for the determination of the LOQ. Typically, the LOD was 2 ng/L and the LOQ 10 ng/L for most compounds. These results are in agreement with Jansson and Kreuger².

Carryover was determined with a blank injection of tap water after the highest herbicide standard (500 ng/L). A carryover was observed in only two herbicides, chloridazon and metolachlor. Figure 3 shows the carryover of metolachlor and, for comparison, a standard of 2 ng/L. A 2 ng/L standard has an S/N = 30 compared to the observed carryover with an S/N = 5, which is below the LOQ. The blank injection was determined to be 0.17 % of a 500 ng/L standard.

Carryover from a 500 ng/L injection of chloridazon to a following blank injection was determined to be 0.47 % with an S/N = 1.3. No other compound showed any carryover.

The environmental water samples showed no carryover for any compound.

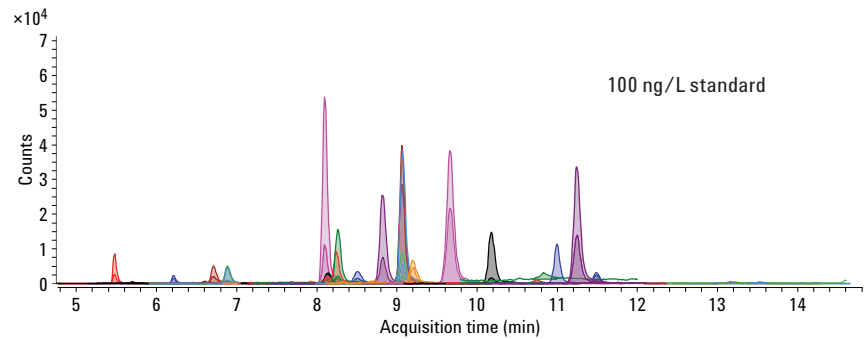


Figure 2. Chromatogram of an herbicide standard in tap water at 100 ng/L.

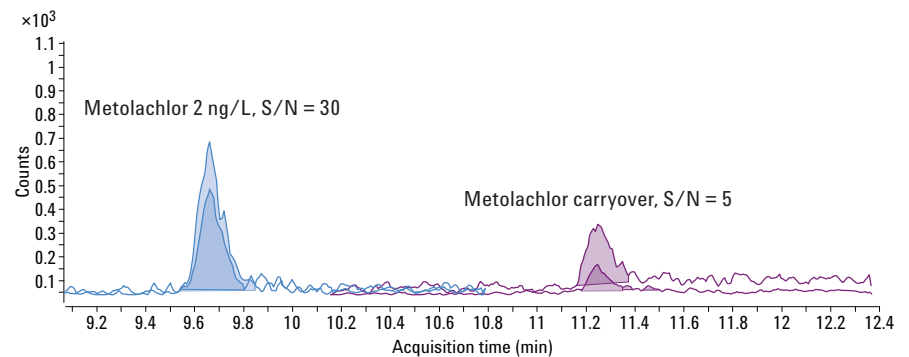


Figure 3. A 2 ng/L spike compared to a carryover from a 500 ng/L standard of metolachlor to a following blank injection.

Conclusion

This Application Note summarizes the results of the Agilent 1200 Infinity Series Online SPE Solution for the analysis of three different water samples. It demonstrates that, for all analyzed environmental waters, the herbicide concentration was below 0.1 µL/L. For a seven level calibration curve, the linear coefficients were good for all compounds (> 0.997). Area precision in real water samples was < 5 % (RSD) for the majority of compounds. No carryover was observed for real water samples.

This Application Note applies the method which was developed during a validation interlaboratory test³ of real water samples and shows excellent data regarding sensitivity, recovery, and repeatability.

References

1. EU Council, Directive on the Quality of Water Intended for Human Consumption, 98/83/EC, **1998**.
2. C. Jansson and J. Kreuger. Multiresidue Analysis of 95 Pesticides at Low Nanogram/Liter Levels in Surface Waters Using Online Preconcentration and High Performance Liquid Chromatography/Tandem Mass Spectrometry. *Journal of AOAC International*, Volume 93, No.6, **2010**.
3. Bettina Schuhn, "Detection of Trace-Level Herbicides in Drinking, Surface and Ground Water Using the Agilent Online SPE Solution", Agilent Technologies Application Note, **2013**, Publication Number 5991-2405EN

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