

Detection of Trace-Level Herbicides in Drinking, Surface and Ground Water Using the Agilent 1200 Infinity Series Online SPE Solution

Application Note

Environmental

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Abstract

This Application Note demonstrates the performance of the Agilent 1200 Infinity Series Online SPE Solution, in combination with the Agilent 6460 Triple Quadrupole LC/MS System, for the analysis for herbicides at trace levels in water. The method conforms to the German DIN standard 38407-36 for the determination of selected neutral herbicides and herbicide metabolites relevant for surface and drinking water quality. The performance of the method has been successfully tested in an interlaboratory validation study. Linearity, area and retention time (RT) precision, as well as concentration precision in real water samples are shown.



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Introduction

Despite the use of modern, less persistent agrochemicals, herbicide residues and herbicide metabolites in water are a serious environmental problem. Even when used appropriately, water soluble herbicides can be found in surface water, ground water, and tap water. For this reason, the monitoring of herbicides and herbicide metabolites is important to ensure the quality of water.

The typical workflow for the analysis of herbicides in water includes the enrichment of the target analytes by solid phase extraction (SPE). With the development of more sensitive detection systems, there is a trend towards the use of direct injection or online-SPE methods for the analysis of water. Consequently, there are new standard methods available for the examination of water, waste water, and sludge that employ direct injection or online-SPE for the analysis of herbicides and herbicide metabolites. The method shown in this Application Note includes 27 herbicides and herbicide metabolites that are relevant for surface and drinking water, and are in agreement with the requirements of the German DIN standard 38407-36.

This method is based on the 1200 Infinity Series Online SPE Solution. Results are shown for different types of water samples such as ground water, tap water, and surface waters, spiked with 27 herbicides at relevant concentrations. The maximum residue limit in drinking water for all herbicides is 0.1 µg/L and analytical methods for the quantitation of herbicide residues should have a limit of quantitation below 0.025 µg/L^{1,2}.

Measurements down to low ng/L levels can be achieved by combining HPLC with a triple quadrupole mass spectrometer when large volumes of the water sample are injected. The 1200 Infinity Series Online SPE solution is based on an Agilent 1290 Infinity Flexible Cube for online enrichment and an Agilent 1260 Infinity Quaternary Pump, and allows the injection of large sample volumes of up to 1,800 µL with a multidraw kit. The combination of one HPLC pump and one low-pressure piston pump in the 1290 Infinity Flexible Cube is sufficient

to enrich the sample on the SPE trapping column and to run the analytical column in the same run.

A 2-position/10-port valve was used to operate two trapping columns alternately. This significantly enhanced the sample throughput. Using the Agilent 1200 Infinity Series Online SPE Solution saved time, solvent, and labor in the trace level detection of herbicides in water, compared to manual or automated offline SPE methods.

Experimental

Instrumentation

	Model no.
Agilent 1200 Infinity Series Online SPE Solution	
Agilent 1260 Infinity Quaternary Pump with internal degasser and LAN card (G1369C)	G1311B
Agilent 1260 Infinity Standard Autosampler with 900 µL head (G1313-60007)	G1329B
Agilent 1290 Infinity Thermostat	G1330B
Agilent 1290 Infinity Flexible Cube	G4227A
Agilent 1200 Infinity Series Online SPE starter kit (includes one 2-position/10-port valve)	G4742A
Agilent 6460 Triple Quadrupole LC/MS System with Agilent Jet Stream Technology	G6460A

Acquisition and Evaluation 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 05.02
- Agilent MassHunter Quantitative Software, Version 06.00

Settings for 1290 Infinity Flexible Cube

- Valve: 2-position/10-port Quick-Change valve
- Solvent selection valve:
Solvent A1: Water,
Solvent B1: Acetonitrile

System Configuration

The 1290 Infinity Flexible Cube for the online SPE solution contained one 2-position/10-port valve with two trapping columns as well as the piston pump and a solvent selection valve³. The SPE cartridges contain a PLRP-S phase (cross-linked styrene divinylbenzene polymer), which is a highly homogeneous material free from silanol groups and heavy metal ions. The SPE cartridges are re-useable (> 200 injections), low volumes of solvent were used for conditioning and elution.

Chromatographic conditions – LC method

Parameter	Setting
Analytical column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 150 mm, 3.5 μm (p/n 959763-902) Agilent 1290 Infinity Inline Filter (0.3 μm) (p/n 5067-4638)
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)
Mobile phases	A = Water, 5 mM ammonium formate + 0.1% formic acid B = Acetonitrile + 5% water, 5 mM ammonium formate + 0.1% formic acid
Gradient	2% B at 0 minutes 2% B at 2 minutes 25% B at 2.5 minutes 100% B at 12 minutes 2% B at 22.1 minutes
Flow rate	0.4 mL/min
Stop time	22.1 minutes
Post time	10 minutes
Injection volume	900 μL Needle wash in vial (MeOH)
Draw and eject speed	1,000 μL/min
Sample temperature	5 °C
Column temperature	30 °C

Table 1. Piston pump timetable in the Agilent 1290 Infinity Flexible Cube.

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

Principle of Operation

The piston pump in the 1290 Infinity Flexible Cube was used to pump the sample from the autosampler onto the trapping columns and to clean those columns, with up to three different solvents, after each run. The autosampler was connected to the piston pump, which loads the sample directly onto one of the trapping columns (SPE 1) while the other trapping column (SPE 2) is in front of the analytical column and connected to the LC pump (Figure 1). When the first trapping column was loaded with sample, the 2-position/10-port valve was switched to change the positions of the trapping columns (Figure 2).

After changing the positions of the trapping columns, the HPLC pump delivered the gradient to backflush the sample from the trapping column (SPE 1) onto the analytical column. On the other side, the trapping column (SPE 2), which was used in the previous run, was cleaned and re-equilibrated. Using the solvent selection valve in the 1290 Infinity Flexible Cube, the operator can clean and re-equilibrate the trapping columns with up to three solvents.

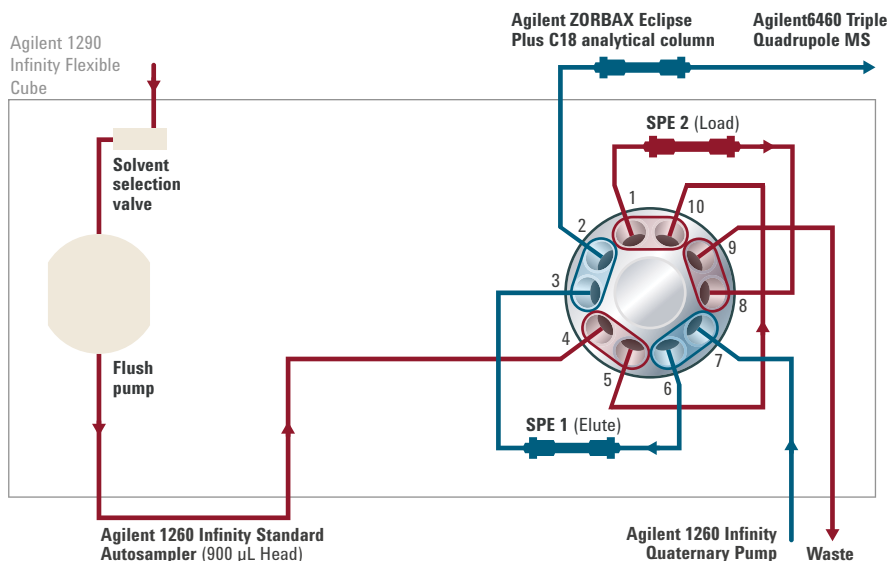


Figure 1. Agilent 1290 Infinity Flexible Cube – The piston pump is loading the first SPE trapping column (red) while the second SPE (blue) is in front of the analytical column in the flow path.

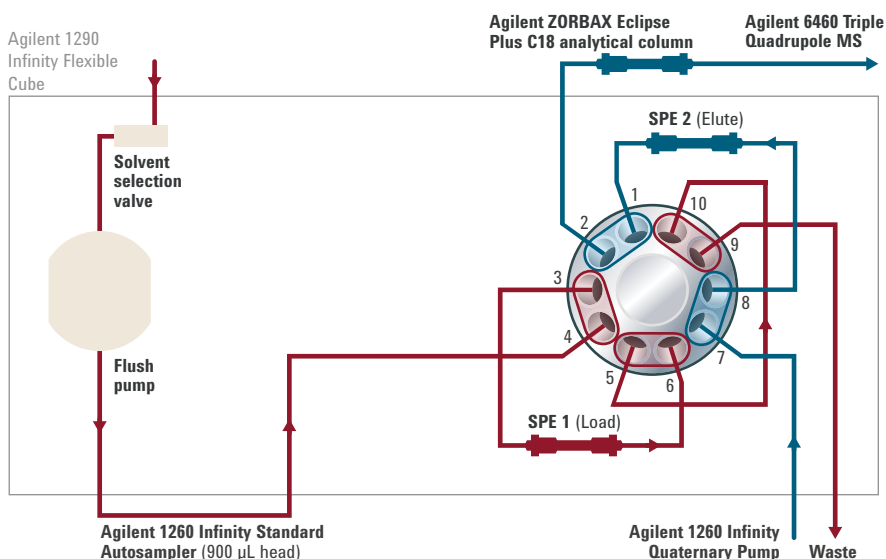


Figure 2. Agilent 1290 Infinity Flexible Cube – The alternating use of the SPE columns, the SPE 1 is now in front of the analytical column while SPE 2 is first cleaned and then loaded with new samples.

The source parameters for the Agilent Jet Stream were optimized with the Agilent MassHunter Source and iFunnel Optimizer. The following shows the source conditions of the 6460 Triple Quadrupole LC/MS System with positive electrospray Agilent Jet Stream technology:

Chromatographic conditions – MS method

Parameter	Setting
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

The Multiple Reaction Monitoring (MRM) mode of the 6460 Triple Quadrupole LC/MS System was used for the detection of the herbicides and herbicide metabolites. MRM transitions for all target compounds were optimized using the MassHunter Optimizer software. Individual herbicide standards were injected with flow injection for the

optimization of fragmentor voltage and collision energy. Two MRM transitions were used for every compound in the final Dynamic MRM (DMRM) method. Table 2 shows the suite of 27 herbicides with the individual optimum of fragmentor voltage and collision energy for the product ions (quantifier and qualifier ions). Retention times (RTs) for all target compounds were identified by analyzing a comprehensive herbicide standard with normal MRM mode. The DMRM method was subsequently automatically produced using the Update MRM method in the MassHunter Acquisition Software. With a cycle time of 500 ms, the minimum dwell time of the DMRM method was 17.33 ms and the maximum dwell time was 146.5 ms, with a maximum number of 24 concurrent MRMs.

Chemicals

All solvents 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 µm membrane point-of-use cartridge (Millipak). Formic acid (p/n G2453-85060) and ammonium formate (p/n G1946-85021) were from Agilent Technologies.

All herbicide standards were purchased from Dr. Ehrenstorfer GmbH, Germany at a concentration of 100 µg/mL in acetonitrile. A quality control (QC) standard with a concentration of 1 µg/mL in acetonitrile was prepared from different individual stock solutions. The QC was diluted to 0.1 µg/L with tap water.

Samples

Four different water samples were spiked with individual stock solutions at different concentrations. The samples were:

- **Sample 1:** tap water (residual water disinfectant was quenched with 50 mg/L sodiumthiosulfate)
- **Sample 2:** ground water
- **Sample 3:** surface water
- **Sample 4:** surface water (high waste water content).

The water samples were centrifuged for 5 minutes at 15,000 rpm, and afterwards injected directly.

Table 2 MRM and DMRM method. All 27 herbicides are listed with their precursors, product ions, the optimum collision energies, the optimum fragmentor voltage, and the RT.

Compound	Precursor species	Precursor	Precursor + Adduct	Product ion 1	CE (V)	Product ion 2	CE (V)	Fragmentor (V)	Retention time (min)
2,6-Dichlorbenzamide	[M+H] ⁺	190.0	191.0	109.0	40	172.9	16	100	6.5
Alachlor	[M+H] ⁺	269.8	270.1	238.1	4	45.12	28	75	11.2
Atrazine	[M+H] ⁺	215.71	216.1	174.0	16	104.0	28	105	8.89
Atrazine-desethyl	[M+H] ⁺	187.63	188.1	104.0	28	68.1	36	105	6.73
Atrazine-desisopropyl	[M+H] ⁺	173.6	174.1	104.2	24	68.1	32	95	6.12
Boscalid	[M+H] ⁺	342.2	343.0	307.0	16	271.0	32	130	10.66
Bromacil	[M+H] ⁺	260.1	261.0	205.0	8	188	28	105	7.74
Carbamazepine	[M+H] ⁺	236.3	237.1	194.1	16	192	20	135	7.93
Chlorfenvinphos	[M+H] ⁺	358.6	359.0	155.0	8	99.0	28	70	11.41
Chloridazone	[M+H] ⁺	221.6	222.0	92.2	24	104.2	20	125	6.73
Chloridazone-methyl-desphenyl	[M+H] ⁺	159.57	160.0	130.1	24	88	36	120	5.51
Chlorpyrifos	[M+H] ⁺	350.6	350.0	97.0	32	198.0	16	95	13.53
Chlortolurone	[M+H] ⁺	212.7	213.1/215	72.1	20	72.1	20	100	8.66
Diuron	[M+H] ⁺	233.1	233.1/235	72.0	16	72.0	16	100	9.05
Ethofumesate	[M+NH ₄] ⁺	286.3	304.1	121.1	27	161.2	31	85	10.84
Isoproturone	[M+H] ⁺	206.3	207.1	72.1	20	165.0	8	85	8.92
Lenacil	[M+H] ⁺	234.3	235.1	153.1	12	136.1	36	70	8.09
Metalaxyl	[M+H] ⁺	279.3	280.2	220.0	8	192.1	16	70	8.89
Metamitrone	[M+H] ⁺	202.2	203.1	175.1	12	104.1	24	105	6.57
Metazachlor	[M+H] ⁺	277.8	278.1	210.1	4	134.1	20	85	9.54
Metolachlor	[M+H] ⁺	283.8	284.1	252.1	12	176.1	24	90	11.14
Metribuzine	[M+H] ⁺	214.3	215.1	187.2	25	84.0	29	105	8.32
Pendimethalin	[M+H] ⁺	281.3	282.2	212.2	4	194	16	125	13.57
Quinoxifen	[M+H] ⁺	307.1	308.0	196.9	36	262.0	52	150	13.19
Simazine	[M+H] ⁺	201.7	202.1	104	24	124.0	16	120	7.94
Terbutylazine	[M+H] ⁺	229.7	230.1	174.0	12	104.0	32	105	10.05
Terbutylazine-desethyl	[M+H] ⁺	201.66	202.1	146.0	12	104.0	28	80	8.06

Results and Discussion

A suite of 27 herbicides was measured in four water samples including tap water, ground water, and two different surface waters. Figure 3 shows a chromatogram of all compounds (standard solution in tap water, 50 ng/L).

A 1,000 ng/L stock solution, containing all 27 herbicides, was made. A dilution series in tap water down to 10 ng/L was prepared for a six level calibration between 10 to 500 ng/L. The QC (100 ng/L) was measured during the calibration and between the samples to ensure the precision of the measurement over time. For all experiments, the recoveries of the QC samples were in acceptable ranges for most of the 27 target compounds.

The majority of the compounds delivered calibration curves with excellent linearity coefficients of > 0.999 for the six level calibration curve. No weighting was used and the curve was forced through the origin. For Ethofumesate, higher limits of quantitation (LOQ) were observed and the calibration curve had a lower linear correlation coefficient of > 0.973.

Figure 4 shows the calibration curves of Metolachlor, a nonpolar herbicide, with a RT of 11 minutes. The calibration curve demonstrates an excellent linearity with a coefficient of > 0.999. The chromatogram shows the overlay of the quantifier and the qualifier trace for spiked surface water (sample 3). With an estimated concentration of 50 ng/L, the measurement matched the spiked reference value.

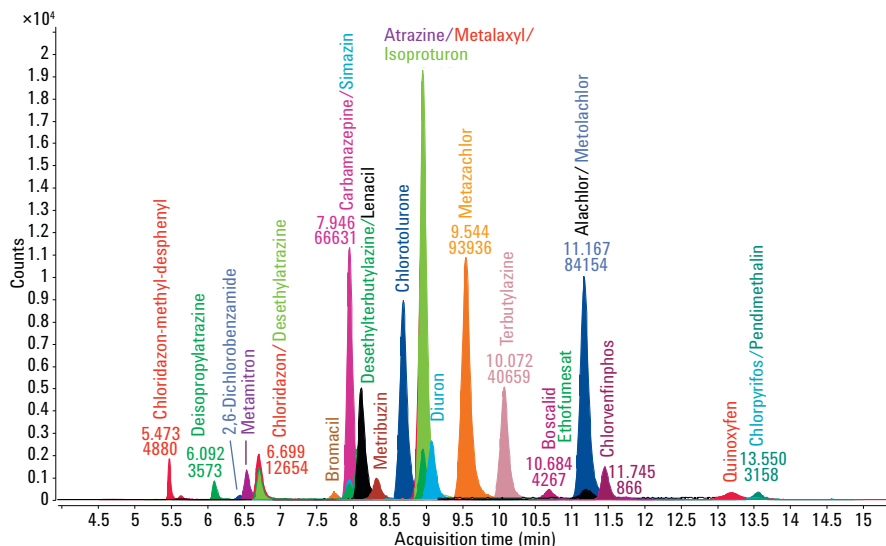


Figure 3. DMRM Chromatogram of a calibration standard with a concentration of 50 ng/L for all 27 herbicides.

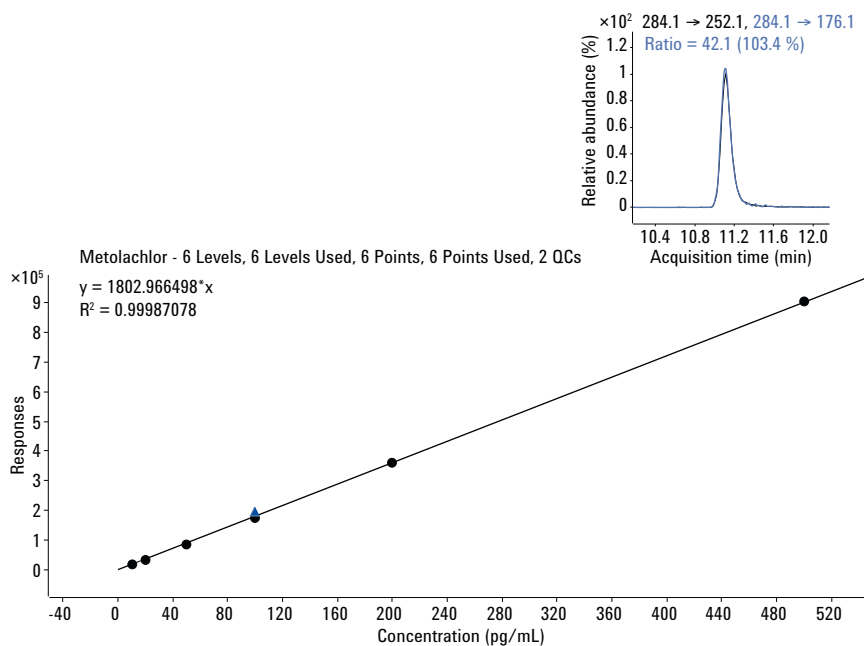


Figure 4. Calibration curve of Metolachlor with a six level calibration (10–500 ng/L). The triangle symbolizes the QC at a concentration of 100 ng/L. The chromatogram shows Metolachlor (RT 11 minutes) in a spiked surface water sample with a measured concentration of 50 ng/L (reference value: 50 ng/L).

Tables 3 and 4 summarize the results of the interlaboratory validation study. Table 3 shows the quantitation results for spiked tap water (sample 1) and spiked ground water (sample 2) and Table 4 summarizes the results for the two spiked surface water samples (samples 3 and 4) based on an external calibration.

Four runs for every sample were averaged and the relative standard deviation (% RSD) of the area was calculated. The reference value is given and the apparent recoveries were calculated. Deviations from the reference value of more than 20% are highlighted in orange and red, respectively.

For sample 1, most compounds showed apparent recoveries with less than 20% deviation from the reference value. Most compounds had higher apparent recoveries and, for one compound (Chlorpyrifos), the reference value in tap water was higher than the measured concentration with the Online SPE.

The area RSD of four runs was, for most compounds, < 5%. For four herbicides, the RSD was < 9%.

Table 3 also shows the Online SPE results of spiked ground water (sample 2). The RSD of the area for all compounds, except Ethofumesate, was < 4%.

Most compounds showed good apparent recoveries. Compared to sample 1, some compounds showed a slightly higher deviation to the reference value. The estimated concentration for Terbutylazine and Atrazine in samples 1 and 2 were slightly higher than the reference value.

The surface water (samples 3 and 4) had a higher matrix and some particulate matter. To protect the analytical column from small particles in the sample, an in-line filter was installed in front of the column.

Table 3. Results of an interlaboratory validation study for spiked tap water (sample 1) and a spiked ground water sample (sample 2).

Compound	Sample 1 (tap water)				Sample 2 (ground water)			
	Average (n=4) (µg/L)	Area % RSD	reference (µg/L)	Apparent recovery (±20%)	Average (n=4) (µg/L)	Area % RSD	Reference (µg/L)	Apparent recovery (±20%)
Chloridazone-methyl-desphenyl	0.089	4.3	0.080	111	0.064	3.8	0.050	128
2,6-Dichlorbenzamid	0.050	5.0	0.050	100	0.197	3.1	0.160	123
Atrazine-desisopropyl	0.112	1.7	0.100	112	0.099	1.7	0.080	123
Metamitron	0.092	1.9	0.080	115	0.120	1.4	0.100	120
Chloridazone	0.098	1.2	0.100	98	0.093	1.0	0.120	77
Atrazine-desethyl	0.053	1.0	0.050	106	0.142	1.7	0.160	89
Metribuzine	0.092	1.9	0.080	115	0.110	1.2	0.100	110
Bromacil	0.129	2.0	0.100	129	0.095	2.6	0.080	118
Simazine	0.090	1.5	0.080	113	0.102	2.4	0.100	102
Carbamazepine	0.106	0.4	0.100	106	0.053	1.4	0.050	107
Terbutylazine-desethyl	0.058	0.9	0.050	116	0.177	0.3	0.160	111
Chlortoluron	0.091	0.9	0.080	113	0.114	0.3	0.100	114
Metazachlor	0.095	0.3	0.080	119	0.062	1.3	0.050	124
Metalaxyl	0.056	1.0	0.050	112	0.093	0.9	0.080	116
Atrazine	0.104	1.6	0.080	130	0.080	2.2	0.050	161
Lenacil	0.055	1.1	0.050	111	0.129	2.1	0.120	107
Isoproturon	0.139	0.9	0.120	116	0.057	1.5	0.050	114
Diuron	0.095	0.9	0.080	119	0.057	1.0	0.050	114
Ethofumesate	0.104	6.8	0.100	104	0.140	12.3	0.120	117
Boscalid	0.097	1.1	0.080	122	0.119	2.2	0.100	119
Terbutylazine	0.127	0.6	0.100	127	0.154	0.6	0.120	128
Alachlor	0.096	0.5	0.080	120	0.129	1.3	0.100	129
Metolachlor	0.092	0.3	0.080	115	0.189	0.9	0.160	118
Chlorfenvinphos	0.062	1.2	0.050	124	0.146	2.1	0.120	121
Chlorpyrifos	0.042	6.9	0.120	35	0.054	0.8	0.050	108
Quinoxifen	0.077	8.9	0.080	96	0.122	3.8	0.160	76
Pendimethalin	0.139	8.6	0.100	139	0.124	1.5	0.120	103

Table 4 summarizes the results for spiked surface water (sample 3). The area RSD was < 5% for all compounds, except for Ethofumesate, which had an RSD of 12%. Apparent recoveries for most analytes were within the acceptable range of 80 to 120% of the reference value. For atrazine desethyl, metazachlor, terbutylazine, and alachlor, the estimated concentrations were more than 20 % above the reference value. In contrast, quinoxifen had an apparent recovery < 60%.

In the second spiked surface water sample (sample 4), which had a high sewage water content, area RSD values for most compounds were < 5%, and the

estimated concentrations were close to the expected concentrations. For six herbicides, the estimated concentrations were below 80% of the reference value.

In summary, the Online SPE results showed a good apparent recovery for all analyzed water samples. In some water samples, calculated concentrations were approximately 30% above the reference values. In sample 4, the three most hydrophobic compounds showed recoveries below 80% of the reference value. But variation in recovery did not correlate to polarity of the compounds.

For a statistical evaluation, eight injections of sample 1 (tap water) and sample 2 (ground water) were done for the estimation of repeatability and area and RT precision while using the trapping columns alternately in the system. The area precision and repeatabilities between the trapping columns and runs were excellent. The evaluation of the data showed that the area precision RSD is typically below 3%, with some exceptions like very polar herbicides and nonpolar herbicides having a higher area RSD (> 5%).

Table 4 Results of an interlaboratory validation study for two spiked surface water samples (samples 3 and 4).

Compound	Sample 3 (surface water)				Sample 4 (surface water)			
	Average (n=4) (µg/L)	Area % RSD	Reference (µg/L)	Apparent recovery (±20%)	Average (n=4) (µg/L)	Area % RSD	Reference (µg/L)	Apparent recovery (±20%)
Chloridazone-methyldephenyl	0.277	3.7	0.319	87	0.168	13.2	0.297	57
2,6-Dichlorbenzamid	0.109	3.3	0.100	109	0.077	9.9	0.092	84
Atrazine-desisopropyl	0.167	0.9	0.160	105	0.043	2.5	0.050	85
Metamitron	0.051	2.1	0.050	102	0.143	2.5	0.180	79
Chloridazone	0.047	1.8	0.050	94	0.117	7.4	0.160	73
Atrazine-desethyl	0.132	1.8	0.100	132	0.162	4.1	0.140	116
Metribuzine	0.184	1.5	0.180	102	0.061	2.4	0.062	98
Bromacil	0.055	5.3	0.050	110	0.277	5.2	0.300	92
Simazine	0.170	2.0	0.160	107	0.050	2.2	0.050	99
Carbamazepine	0.165	1.2	0.137	121	0.337	0.8	0.274	123
Terbutylazine-desethyl	0.092	2.1	0.080	115	0.111	0.8	0.100	111
Chlortoluron	0.075	0.6	0.068	110	0.166	0.4	0.160	103
Metazachlor	0.211	1.1	0.160	132	0.118	0.2	0.100	118
Metalaxyl	0.115	1.2	0.100	115	0.399	1.0	0.353	113
Atrazine	0.351	1.0	0.300	117	0.107	1.7	0.100	107
Lenacil	0.179	1.1	0.180	100	0.092	2.6	0.100	92
Isoproturone	0.130	0.8	0.117	111	0.188	1.4	0.182	103
Diuron	0.360	1.0	0.300	120	0.121	1.5	0.110	110
Ethofumesate	0.056	11.9	0.050	112	0.283	3.8	0.300	94
Boscalid	0.191	1.5	0.160	120	0.116	1.2	0.108	107
Terbutylazine	0.064	0.8	0.050	127	0.180	1.4	0.160	113
Alachlor	0.373	1.5	0.300	124	0.054	2.7	0.050	109
Metolachlor	0.060	1.2	0.050	120	0.106	1.7	0.100	106
Chlorfenvinphos	0.196	1.5	0.160	122	0.109	3.4	0.100	109
Chlorpyrifos	0.094	2.0	0.100	94	0.126	3.6	0.160	79
Quinoxifen	0.059	4.7	0.100	59	0.022	6.0	0.050	44
Pendimethalin	0.139	2.2	0.160	87	0.032	7.5	0.050	64

Data for RT precision are shown as excellent in ground water (sample 2). The RT precision for all measured compounds was excellent with an average standard deviation of ± 0.9 seconds and an RSD $< 0.1\%$.

Conclusion

Since ground and surface water are used as a basis for drinking water production, it is important to monitor the concentration of herbicides.

This Application Note summarizes a method for determination of relevant herbicides and herbicides metabolites in water using the Agilent 1200 Infinity Series Online SPE Solution coupled to the Agilent 6460 Triple Quadrupole LC/MS System. Method performance is shown for samples which were measured within an interlaboratory validation study. It was demonstrated that the system achieved

high apparent recoveries $> 95\%$ for most target compounds in the spiked tap water used for calibration, and that the method generated highly reproducible results in different types of water samples. Area precision, for most of the target compounds, was $< 5\%$. The RT precision showed an RSD of $< 0.1\%$. Linearity coefficients, for most compounds, were very good with $R^2 0.999$. The apparent recoveries in four different water samples tested during an interlaboratory validation study for most of the compounds was within the acceptable tolerance range of 20%.

The automated enrichment process makes it possible to accomplish low detection limits with a routine triple quadrupole MS and to run fast analysis of water samples without time-consuming sample preparation or offline enrichment with SPE cartridges.

References

1. DIN 38407-36 (Draft): German standard methods for the examination of water, waste water and sludge - Jointly determinable substances (group F) - Part 35: Determination of selected neutral and alkaline plant treatment agents using high performance liquid chromatography and mass spectrometric detection (HPLC-MS/MS) (F 36).
2. EU Council, Directive on the Quality of Water Intended for Human Consumption, 98/83/EC, **1998**.
3. E. Naegele, "Quantification of trace-level herbicides in drinking water by online enrichment with the Agilent 1290 Infinity Series Online-SPE Solution and Triple Quadrupole MS Detection", Agilent Technologies Application Note, **2013**, Publication Number 5991-1738EN.

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