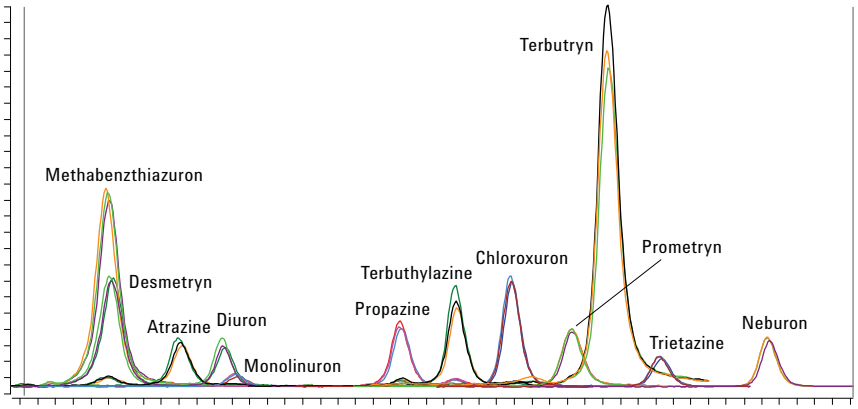
Quantification of trace-level herbicides in drinking water by online enrichment with the Agilent 1200 Infinity Series Online-SPE Solution and Triple Quadrupole MS Detection

Application Note

Environmental

Author

Edgar Naegele
Agilent Technologies, Inc.
Waldbronn, Germany



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 herbicides at trace levels down to 1 ppt in drinking water. Performance data of the online-SPE system for linearity, area and retention time precision, recovery and concentration precision, and accuracy in real samples is shown and discussed.

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Introduction

According to the requirements of the European Union drinking water directive 98/83/EC, pollutants such as neutral herbicides have to be monitored in drinking water[1]. The current regulation demands a limit of detection (LOD) of 25 ng/L (25 ppt) for all pesticides. To achieve this limit of detection with an entry level or mid-range triple quadrupole mass spectrometer, a larger volume of the water sample (typically > 1 mL) has to be enriched on a trapping column. Then, the compounds are eluted to the analytical HPLC column for separation.

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 herbicides and second, separating them from each other. The online-SPE system comprises an Agilent 1260 Infinity Quaternary pump because commercially available SPE cartridges work satisfactorily at 600 bar. The full functionality of the online-SPE system is achieved using only one LC pump. The performance of the system is demonstrated by the mass spectrometric detection of a suite of 28 neutral herbicides down to a LOD of less than 10 ppt. To enhance the throughput of the system, a valve solution for the parallel use of two alternating trapping columns is presented.

Experimental

Instrumentation

Agilent 1200 Infinity Series Online-SPE solution system comprising:

- 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 with 2-position/10-port valve G4232B
- Agilent 1290 Infinity Thermostatted Column Compartment G1316C

Figure 1 shows how the modules of the system are set up, and Table 1 lists the capillaries and accessories needed to run the online-SPE application. With the Agilent part number G5067-5708 for the Online SPE Starter Set, all necessary parts such capillaries and the 2-position/10-port valve head can be ordered in one package.

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

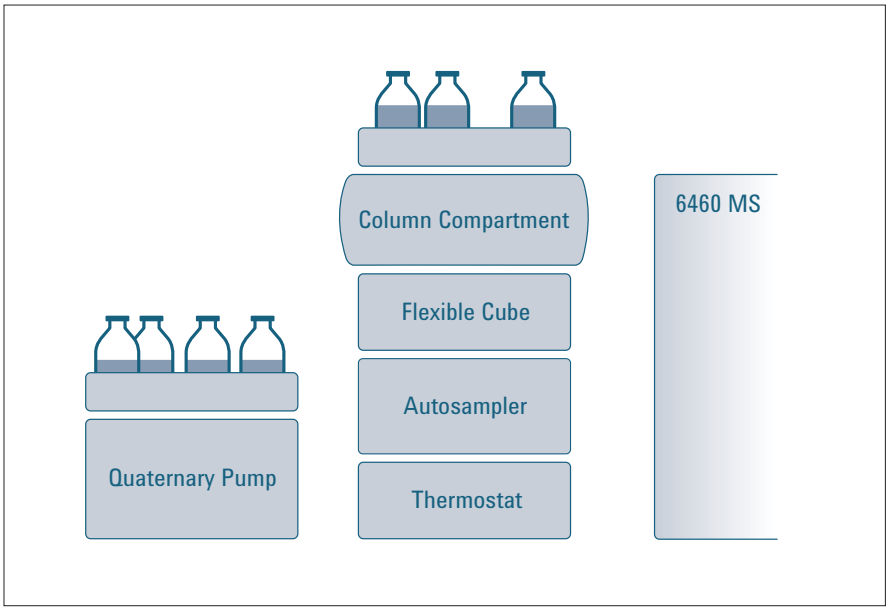

Figure 1
Setup of the Agilent 1200 Infinity Series Online-SPE solution with MS detector (the solvent bottles in the center are for SPE loading, rinsing and conditioning).

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 1a
Parts of the Online SPE Starter Set G4742A which is required for the Agilent 1200 Infinity Series online SPE solution.

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 1b
Capillaries and parts of the online SPE capillary kit (SST = stainless steel).

HPLC Method

Agilent 1260 Infinity Quaternary Pump:

- Solvent A: Water, 5 mM ammonium formiate + 0.1% formic acid
- Solvent B: ACN + 5% water, 5 mM ammonium formiate + 0.1% formic acid
- 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 Thermostated Column Compartment:

- Column temperature: 40 °C

Agilent 1290 Infinity Flexible Cube:

- Right valve: 2-position/10-port QuickChange valve head
- Pump: 1.5 mL/min
- Solvent: A1: Water, B1: ACN
  - 0 minutes – Pump 300 s, Solvent A1
  - 5 minutes – right valve change position
  - 7 minutes – Pump 180 s, Solvent B1
  - 11 minutes – Pump 300 s, Solvent A1

Agilent 1260 Infinity Standard Autosampler

- Injection volume: 1,800 µL (automated multidraw of 2 times 900 µL)
- Needle wash in vial (MeOH)
- Draw and eject speed: 1,000 µL/min.
- Sample temperature: 10 °C.
- 2 halftrays for 15 × 6 mL vials G1313-44513
- 6-mL screw cap vials (glass, p/n 9301-1377), screw caps (p/n 9031-1379), pre-slit 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) is hosting the 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 for 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) while the other trapping column (SPE 2) is eluted in front of the analytical column and connected to the LC pump (Figure 3A).

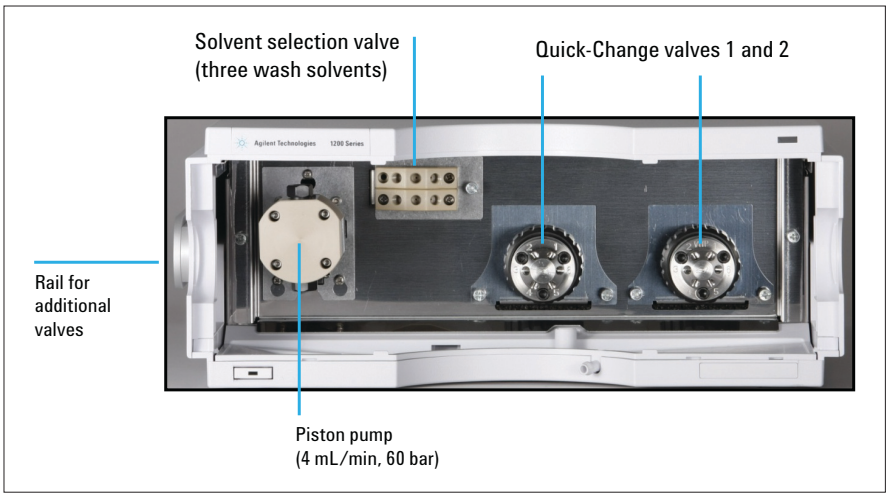

Figure 2
The Agilent 1290 Infinity Flexible Cube is an additional module for the Agilent 1290/1260 Infinity LC system, hosting up to two Agilent 1200 Infinity Series Quick-Change valves.

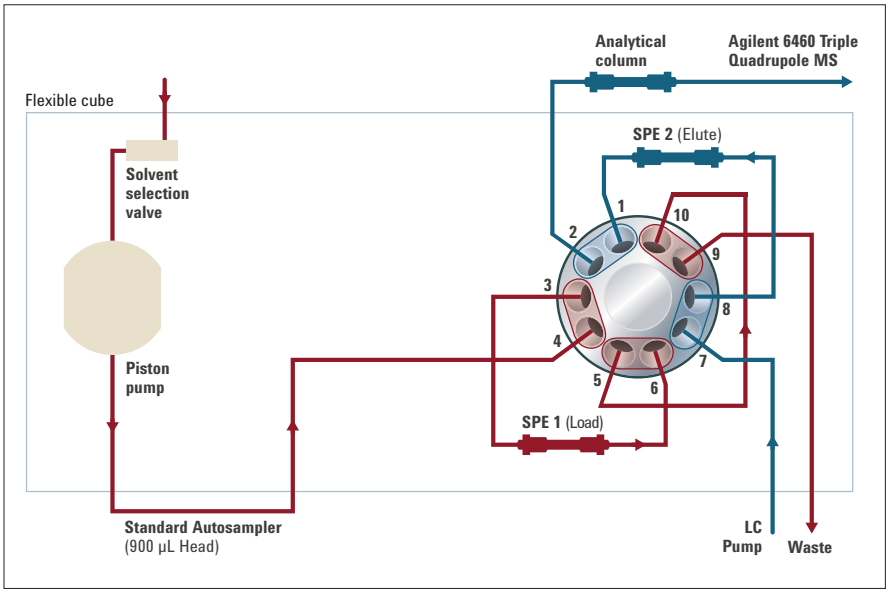

Figure 3A
Valve positions for loading the sample on trapping column SPE 1 while trapping column SPE 2 is being eluted.

After loading the trapping column with sample, the 2-position/10-port valve is switched and thus the positions of the trapping columns are 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 2 shows a summary of the LC method for the main modules.

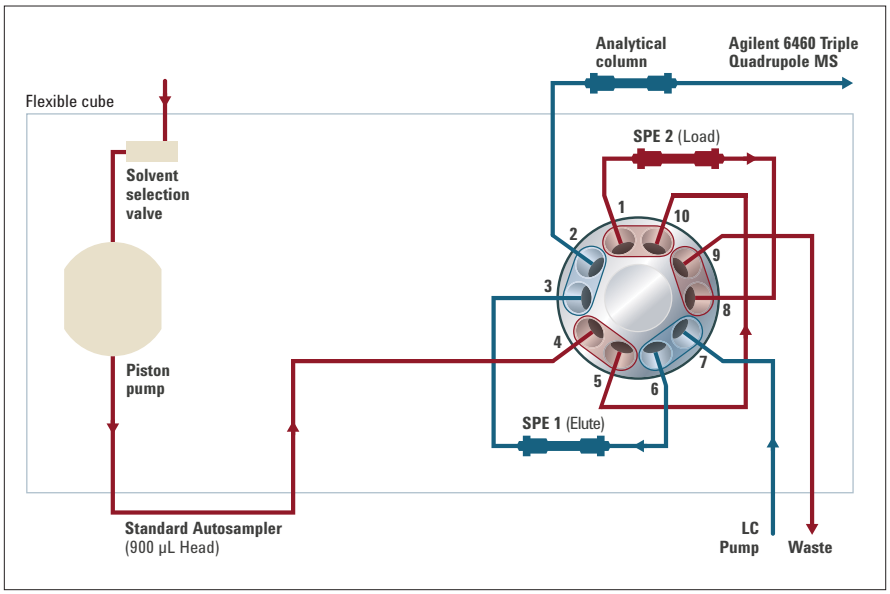

Figure 3B
Valve positions for loading the sample on trapping column SPE 2 while trapping column SPE 1 is being eluted.

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

Table 2
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.

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 Volt
- Nozzle: 0 Volt

The MRM and dynamic MRM triple quadrupole MS method was developed by means of the MassHunter optimizer software and direct injections of individual pesticide 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 3).

Name	RT	Precursor	Precursor ion [M+H]+	Fragmentor	Fragment ion (quantifier)	CE	Fragment ion (qualifier)	CE
Atrazine desisopropyl	10.52	173.05	174.1	105	96.1	16	104.0	24
Carbendazim	11.15	191.07	192.1	110	160.0	16	132.0	32
Metamitron	11.67	202.10	203.1	105	175.1	12	104.1	20
Fenuron	11.81	164.09	165.1	85	72.1	16	46.1	12
Atrazine desethyl	11.93	187.06	188.0	105	146.0	16	104.0	28
Chloridazon	11.96	221.04	222.0	125	104.0	20	92.1	24
Carbetamide	13.34	236.12	237.1	75	118.1	8	192.1	4
Metoxuron	13.55	228.07/230.07	229.1/231.1	110	72.1	20	72.1	20
Monuron	13.79	198.06/200.06	199.1/201.1	95	72.1	16	72.1	16
Simazine	13.80	201.08	202.1	120	132.0	16	124.0	16
Cyanazine	14.03	240.09	241.1	120	214.1	12	104.0	32
Methabenzthiazuron	14.83	221.06	222.1	95	165.0	12	150.0	36
Chlorotoluron	14.85	212.07/214.07	213.1/215.1	100	72.1	16	72.1	16
Desmetryn	14.92	213.10	214.1	115	172.1	12	82.1	32
Atrazine	15.31	215.09	216.1	125	174.0	12	104.0	28
Isoproturon	15.48	206.14	207.1	100	72.1	16	46.1	16
Diuron	15.64	232.02/234.02	233.02/235.02	100	72.1	20	72.1	20
Monolinuron	15.71	214.05	215.1	85	126.0	12	148.0	8
Propazine	16.62	229.11	230.1	120	146.0	20	188.0	12
Linuron	16.85	248.01	249.0	90	159.9	16	182.0	12
Terbuthylazine	16.92	229.11	230.1	110	174.0	15	104.0	32
Chloroxuron	17.21	290.08/292.08	291.1/293.1	120	72.1	20	72.1	20
Irgarol 1051	17.52	253.14	254.1	120	198.1	16	83.1	28
Prometryn	17.61	241.14	242.1	125	158.0	20	200.1	16
Diflubenzuron	17.76	310.03	311.0	90	158.0	8	141.0	32
Terbutryn	17.85	241.14	242.1	110	186.0	16	68.1	48
Trietazine	18.11	229.11	230.1	125	99.0	24	132.0	20
Neburon	18.71	274.06	275.1	120	88.1	12	57.1	24

(Fragmentor = Voltage [V]
RT = retention time [min]
CE = collision energy [eV])

Table 3

MRM and dynamic MRM MS method, showing the identified optimum fragmentor and collision energy values for the individual pesticides 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. For some chlorinated compounds, the transition from both chlorine isotopes to the same fragment were used when other transitions were of lower abundance.

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 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 (Figure 4).

Chemicals

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

Calibration standards

A stock solution containing all pesticides was prepared by dilution of 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, and 0.5 ppt.

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 25 ppt with a concentrated pesticide solution containing all 28 pesticides, vortexed, filtered with a syringe filter (0.45 µm) and injected without further sample prep.

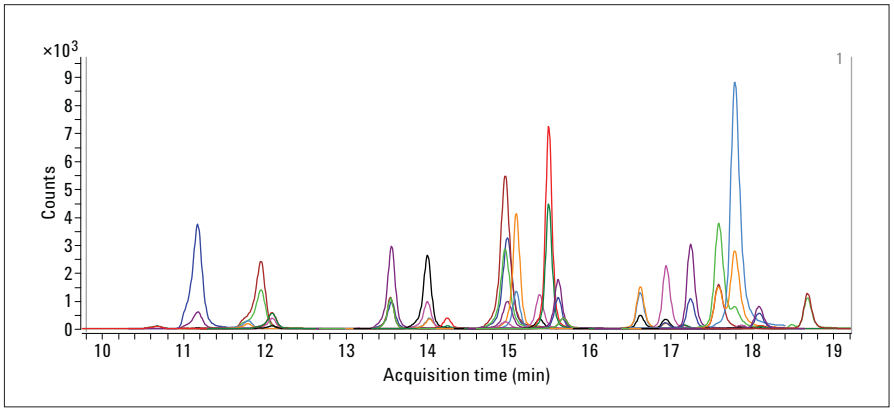

Figure 4
MRM chromatograms for a calibration standard with a concentration of 100 ppt (ng/L) each for all 28 pesticides measured by the final SPE LC dynamic MRM method with quantifier and qualifier ion.

Results and Discussion

Calibration curves for each individual compound were obtained by diluting the stock solution containing all 28 pesticides at a concentration of 100 ng/L (100 ppt) in a dilution series down to 0.5 ng/L (0.5 ppt). The pesticides were measured with the developed online-SPE LC method using dynamic MRM. Each calibration standard was injected four times with a volume of 1,800 µL and enriched on the SPE trapping column. The value at a signal-to-noise (S/N) ratio of 3 was used for the LOD and the value at a S/N ratio of 10 was used as LOQ. The calibration curve was calculated from LOQ up to 100 ng/L. Figures 5A and 5B show the quantifier transition (*m/z* 207.1 → *m/z* 72.1) of isoproturon for the concentration level of 100 ppt to 5 ppt (Figure 5A) and for 10 ppt to the LOQ of 1 ppt (Figure 5B) at a retention

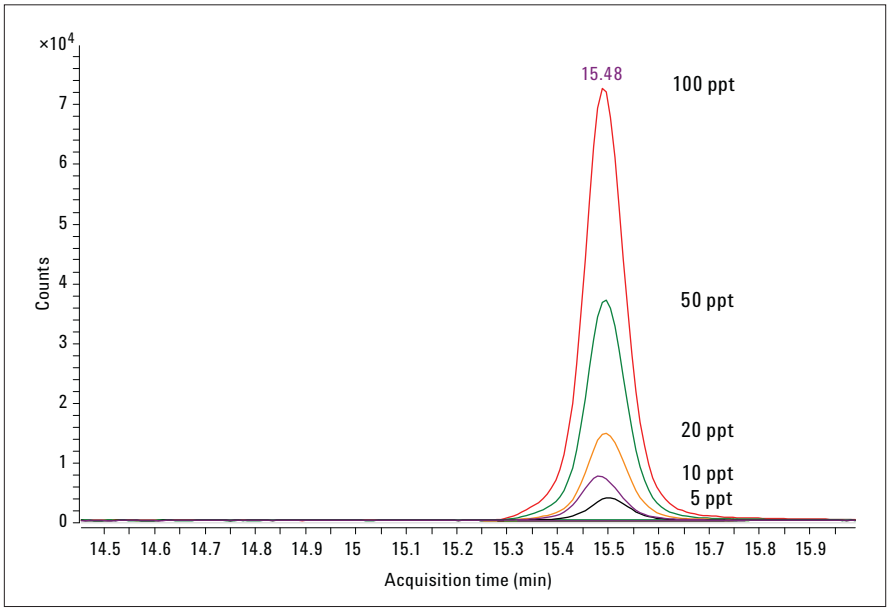

Figure 5A
Dynamic MRM chromatograms for the quantifier transition *m/z* 207.1 → 72.1 of Isoproturon, at a concentration of 5 to 100 ppt.

time of 15.48 minutes. The calibration curve of the seven levels from the LOQ of 1 ppt to 100 ppt was calculated including all 28 injections and resulted in a linear coefficient of 0.9986 (Figure 6).

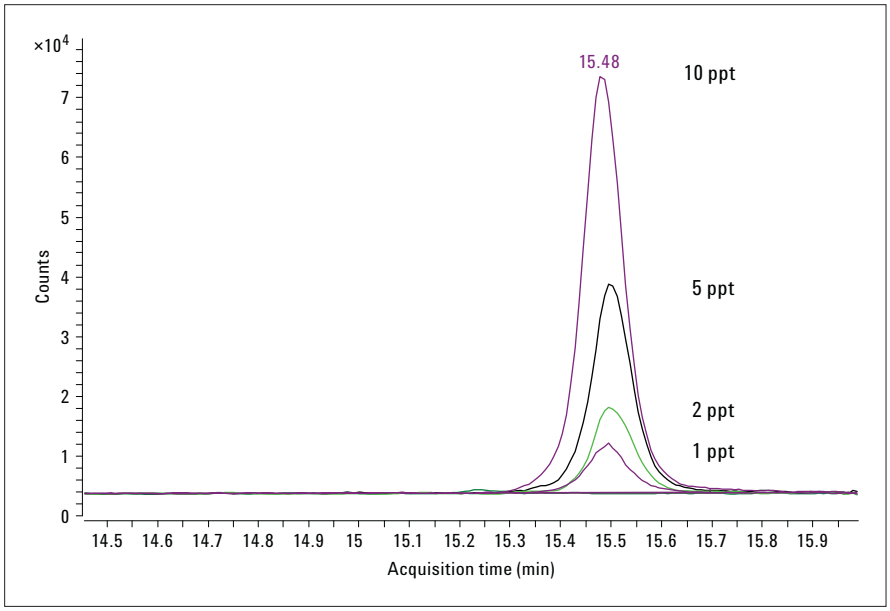

Figure 5B
Dynamic MRM chromatograms for the quantifier transition *m/z* 207.1 → 72.1 of isoproturon at a concentration of 1 to 10 ppt.

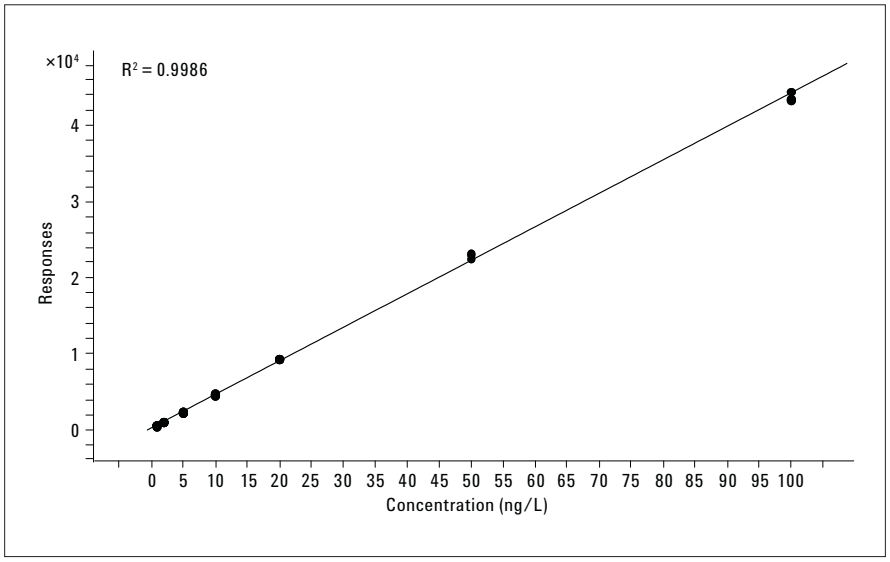

Figure 6
Calibration curve of Isoproturon at a concentration of 1 ppt–100 ppt (seven levels, seven levels used, 28 points, 28 points used), linear coefficient 0.9986, LOQ 1ppt.

Table 4 outlines the complete set of data for all 28 pesticides. Typically, the LOQs were between 5 ppt and 1 ppt and the respective LODs were between 2 ppt and 0.5 ppt. Linear coefficients were good for all compounds, with values typically better than 0.997. The relative standard deviation (RSD) of the retention timed was excellent with values typically below 0.1%. The RSD of the peak areas was typically between 5.0% and 7.6%. The recovery of the SPE trapping process was determined by comparing the peak areas of an injection onto the SPE column to a direct injection of the same concentration level and volume (900 µL of 50 ppt standard) onto the analytical column. The recoveries of 20 compounds were > 90%, the other compounds were between 80 and 90% (Table 4).

Name	r.t.	LOQ (ng/L) (S/N=10)	R^2	LOD (ng/L) (S/N=3)	Area RSD (%)	r.t. RSD (%)	Recovery (%)
Atrazine desisopropyl	10.52	5	0.9969	2.0	5.0	0.20	84.3
Carbendazim	11.15	1	0.9971	0.5	7.8	0.10	88.8
Metamitron	11.67	5	0.9988	2.0	5.2	0.30	87.8
Fenuron	11.81	2	0.9985	1.0	7.0	1.00	96.1
Atrazine desethyl	11.93	5	0.9971	2.0	7.0	0.10	92.2
Chloridazon	11.96	2	0.9977	1.0	6.9	0.10	96.8
Carbetamide	13.34	2	0.9981	1.0	6.9	0.70	98.5
Metoxuron	13.55	2	0.9982	1.0	7.6	0.05	96.8
Monuron	13.79	2	0.9981	1.0	6.8	0.03	97.0
Simazine	13.80	5	0.9986	2.0	7.5	0.04	97.9
Cyanazine	14.03	5	0.9965	2.0	7.6	0.06	92.0
Methabenzthiazuron	14.83	1	0.9982	0.5	3.7	0.03	95.5
Chlorotoluron	14.85	1	0.9982	0.5	5.1	0.03	94.9
Desmetryn	14.92	1	0.9986	0.5	4.7	0.10	95.6
Atrazine	15.31	2	0.9982	1.0	6.6	0.04	96.9
Isoproturon	15.48	1	0.9986	0.5	6.8	0.03	98.0
Diuron	15.64	2	0.9986	1.0	6.4	0.80	82.1
Monolinuron	15.71	5	0.9976	2.0	4.8	0.05	92.3
Propazine	16.62	2	0.9980	1.0	4.8	0.03	94.6
Linuron	16.85	5	0.9981	2.0	7.6	0.08	87.1
Terbuthylazine	16.92	1	0.9920	0.5	4.2	0.05	100.9
Chloroxuron	17.21	1	0.9983	0.5	5.2	0.02	105.5
Irgarol 1051	17.52	1	0.9965	0.5	8.7	0.07	89.8
Pormetryn	17.61	1	0.9988	2.0	4.2	0.10	94.3
Diflubenzuron	17.76	5	0.9954	2.0	6.0	0.06	78.0
Terbutryn	17.85	1	0.9988	0.5	5.6	0.80	97.4
Trietazine	18.11	5	0.9984	2.0	5.9	0.02	97.3

Table 4
Performance data for all pesticide compounds present in the study. (R^2 = linear coefficient, RSD = Relative standard deviation, r.t. = retention time, RSD (%) and recovery (%)).

The carryover was determined for three of the most intense compounds isoproturon (Figure 7A), terbutryn (Figure 7B) and metoxuron (Figure 7C). The carryover from a 100 ppt injection of isoproturon to a subsequent blank injection was determined to be at 0.11%. This was approximately 10% of the LOQ (Figure 7A). The carryover from a 100 ppt injection of terbutryn to a subsequent blank injection was determined to be 0.28%, which was approximately 26% of the LOQ (Figure 7B).

The carryover from a 100 ppt injection of Metoxuron to a subsequent blank injection was below the LOD (Figure 7C).

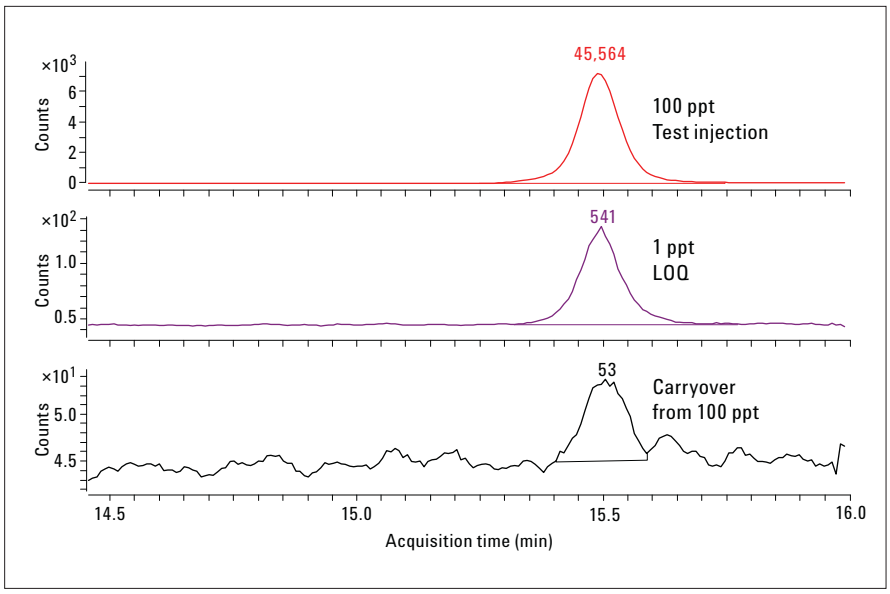

Figure 7A
Carryover from a 100 ppt injection of isoproturon to a following blank injection was determined to be 0.11%. This was approximately 10% of the LOQ.

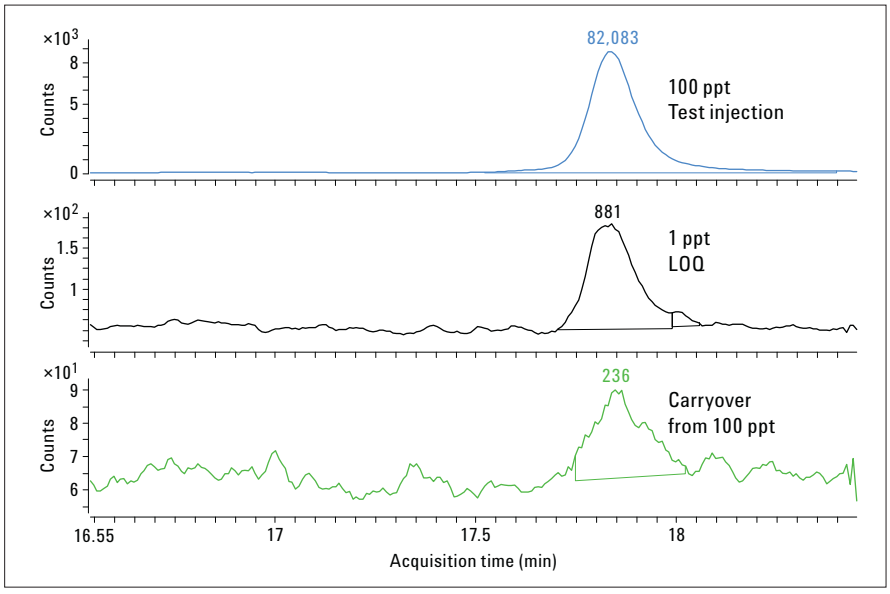

Figure 7B
Carryover from a 100 ppt injection of terbutryn to a following blank injection was determined to be 0.28%. This was approximately 26% of the LOQ.

Finally, water samples from the Rhine river, tap water, and spring water were spiked with all 28 pesticides to a final concentration of 25 ppt. Analysis of all samples yielded comparable intensities for a large number of the spiked herbicides independent from the source of the water sample (Figure 8). This indicates that residual salt contaminations from the water samples or other contaminants with high ion strength which might cause ion suppression were effectively flushed out of the SPE column. The spiked tap water and river water samples were rich in calcium hydrogen carbonate. The measured concentrations of all pesticides shown in Figure 8 were averaged dependent on the source of water. The calculated concentration precision was between 2.3% and 2.8%. The concentration accuracy was always above 90%.

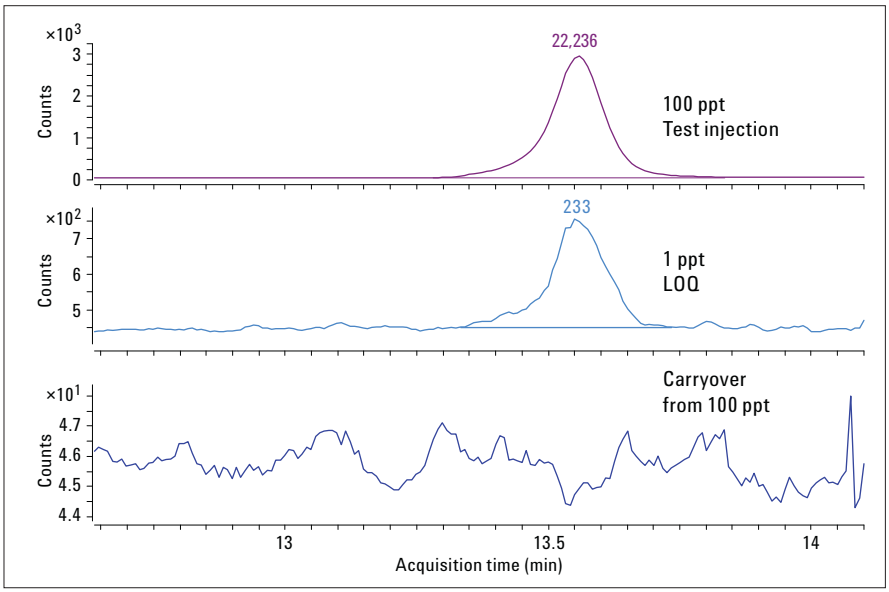

Figure 7C
Carryover from a 100 ppt injection of Metoxuron to a following blank injection could not be determined.

Conclusion

This Application Note demonstrates the use of the Agilent 1200 Infinity Series Online-SPE solution for enrichment, separation, and detection in trace level analysis of pesticide residues in water samples by HPLC with triple quadrupole MS detection. It was demonstrated that lowest LOD of 0.5 ppt and LOQ as low as 1 ppt could be achieved. The methodology shows a high sample-to-sample reproducibility with area deviation of less than 7%. The efficient online-SPE trapping process allows pesticide detection in real drinking water samples well below the regulatory limits with high precision and accuracy.

Reference

1.
European Union Drinking Water Directive 98/83/EC http://ec.europa.eu/environment/water/water-drink/index_en.html

	Rhine river water	Tap water	Spring water
Average concentration (n=12) [ng/L]	23.53	24.58	22.88
SD	0.76	0.87	0.90
Precision RSD [%]	2.37	2.72	2.82
Accuracy [%]	94.12	98.32	91.50

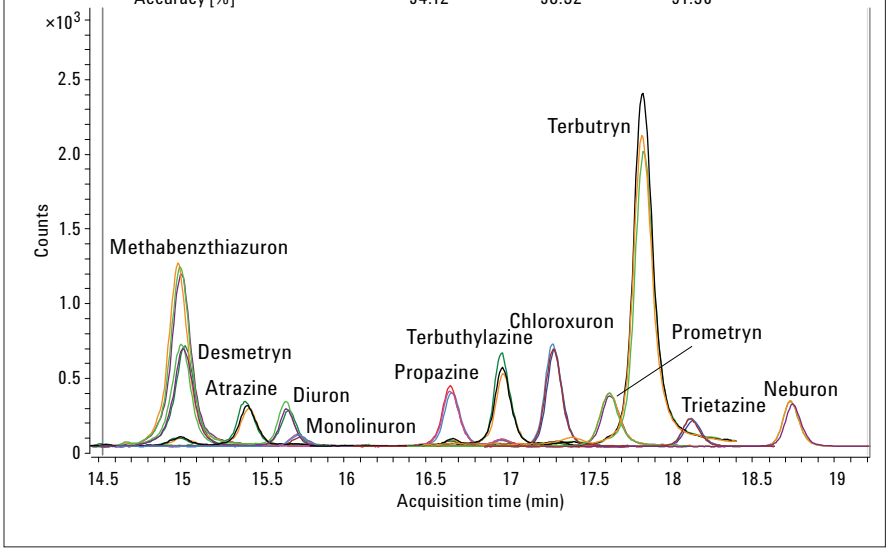

Figure 8
Water samples from a tap, Rhine river, and a spring spiked with 25 ppt of all 28 pesticides. The retention time window from 14.5 minutes to 19.1 minutes is shown. The table shows the average measured concentrations of all pesticides (n=12) within the retention time window dependent on the sources of water together with precision RSD and accuracy.

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