Comparison of Direct Injection and Online SPE for Quantification by LC/MS of Trace-Level Herbicides in Water

Application Note

Food Testing & Agriculture, Environmental

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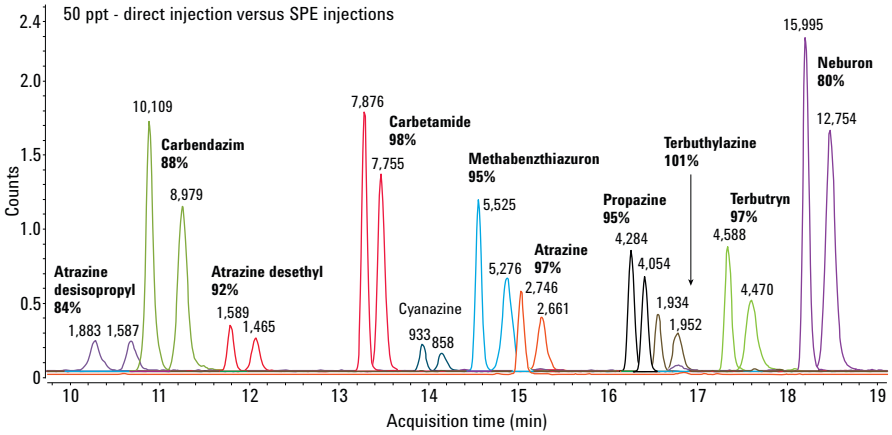
Abstract

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 herbicides at trace levels down to 1 part per trillion (ppt) in surface and drinking water. The analysis of a sequence of samples including calibration, quality controls and recovery control samples will be demonstrated. To perform the control of the trapping process, a valve solution for direct on-column injection and subsequent online SPE is described.



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Introduction

According to the requirements of the European Union drinking water directive 98/83/EC, pollutants like 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 either before the analysis or online by enriching the sample on a trapping column. This can be done with the Agilent 1200 Infinity Series Online-SPE solution which allows for LODs down to 0.5–1 ppt with excellent calibration linearity and precision values for retention times and peak areas. The recoveries of the trapping process are typically higher than 90%[2]. The trapping process is the critical part to achieve these low LODs. Due to ageing of the trapping columns, recovery may fall below a defined threshold. To maintain the quality of the trapping process during a large number of sample injections, it is necessary to check its performance. To achieve this requirement, a valve solution is introduced which allows subsequent direct injections on the analytical column and injections on the trapping column to monitor the performance of the solid phase extraction (SPE) process by checking the recoveries.

This Application Note describes the Agilent 1200 Infinity Series Online-SPE solution based on the Agilent 1290 Infinity Flexible Cube for the analysis of herbicides at trace levels down to 1ppt in surface and drinking water. The analysis of a sequence of samples including calibration, quality controls and recovery control samples will be demonstrated. To perform the quality control of the trapping process, a valve solution for direct on-column injection and subsequent online SPE will be described.

Experimental

Instrumentation

Agilent 1200 Infinity Series Online-SPE Solution (Figure 1 shows a system stack).

- 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 sample cooler (G1330B)
- Agilent 1290 Infinity Flexible Cube (G4227A, two valve drives) with online SPE starter set (G4742A) and online SPE direct injection kit (G4744A) providing two 2 position/10 port Quick-Change valve heads and respective capillaries.
- Agilent 1290 Infinity Thermostatted Column Compartment

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 (part of G4742A)

- 2 x 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

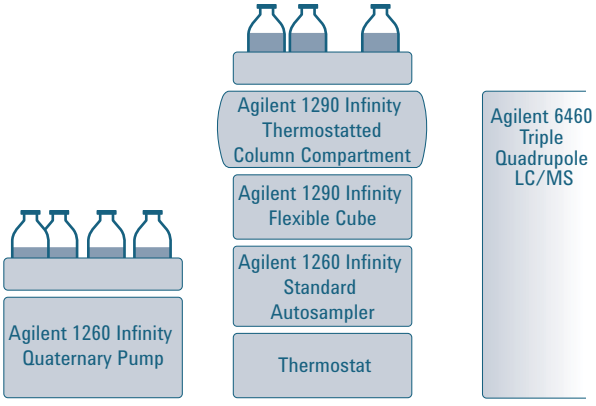

Figure 1. Setup of the Agilent 1200 Infinity Series Online-SPE solution with MS detector (the solvent bottles in the center are for SPE loading, rinsing and conditioning).

HPLC Method for Online SPE

Agilent 1260 Infinity Quaternary pump

Solvent A: Water, 5 mM ammonium formate + 0.1% formic acid

Solvent B: ACN + 5% water, 5 mM ammonium formate + 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 Thermostatted Column Compartment

- Column temperature: 40 °C

Agilent 1290 Infinity Flexible Cube

- Right valve: 2-position/10-port QuickChange valve head, alternating with trapping columns for SPE
- Left valve: 2-position/10-port QuickChange valve head, Position for sample load on SPE trapping columns
- Pumping rate: 1.5 mL/min
- Solvent A1: Water, Solvent 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 900 µL, twice) applied for blanks, samples, calibration standards, calibration quality controls
- Injection volume: 900 µL, applied for injection on SPE column for recovery quality control of the trapping process
- 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)

HPLC Method for Direct Injection

Agilent 1260 Infinity Quaternary Pump

Solvent A and B: See method for online SPE

Flow rate: 0 minutes – 0.8 mL/min,
5 minutes – 0.8 mL/min,
5.1 minutes – 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, not used
- Left valve: 2-position/10-port QuickChange valve head, Position for direct injection on analytical column

Agilent 1260 Infinity Standard Autosampler

- Injection volume: 900 µL, applied for direct injection on analytical column for recovery quality control of the trapping process.
- Injector program:
  1) Draw 900 µL sample.
  2) Wash needle in vial.
  3) Inject.
  4) Wait 5 minutes.
  5) Valve bypass.
- Draw and eject speed: 1,000 µL/min
- Sample temperature: 10 °C
- Two trays with 15 positions each (see above)
- 6-mL screw cap vials (see above)

In the described setup of the online SPE LC system with direct injection capability, the 1290 Infinity Flexible Cube (Figure 2) is hosting two 2-position/10 port valves. The two trapping columns are located at the right valve. The left valve is plumbed to switch between direct on-column injection and injection on the trapping columns for SPE (Figure 3A). In addition, the Flexible Cube also contains the piston pump and the solvent selection valve for flushing the sample on the trapping columns and for the re-equilibration of those columns (Figure 3B and 3C). If the valve position for loading the sample on one of the SPE columns is selected, 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 pump and is eluted in backflush mode onto the analytical column (Figure 3B). After loading the trapping column with sample, the right 2-position/10-port valve is switched and, thus, the positions of the trapping columns are exchanged (Figure 3C). Now, the LC pump delivers the gradient to elute the enriched analytes from the trapping column (SPE 1) onto the analytical column. Simultaneously, the trapping column (SPE 2) which had been eluted in the previous run is further cleaned and re-conditioned by a purging procedure. This cleaning procedure is done by the piston pump of the Fexible Cube with the cleaning solvents selectable by the solvent selection valve.

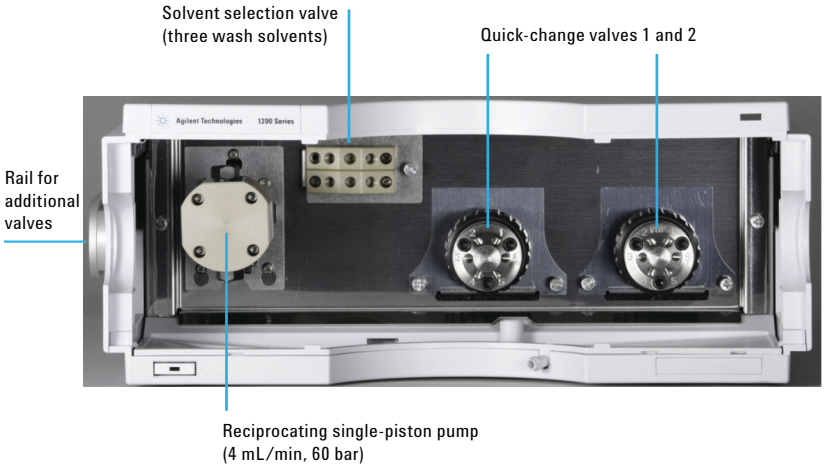

Figure 2. The Agilent 1290 Infinity Flexible Cube is an additional module for the Agilent 1290/1260 Infinity LC system, hosting up to two Agilent 1200 Infinity Series quick-change valves.

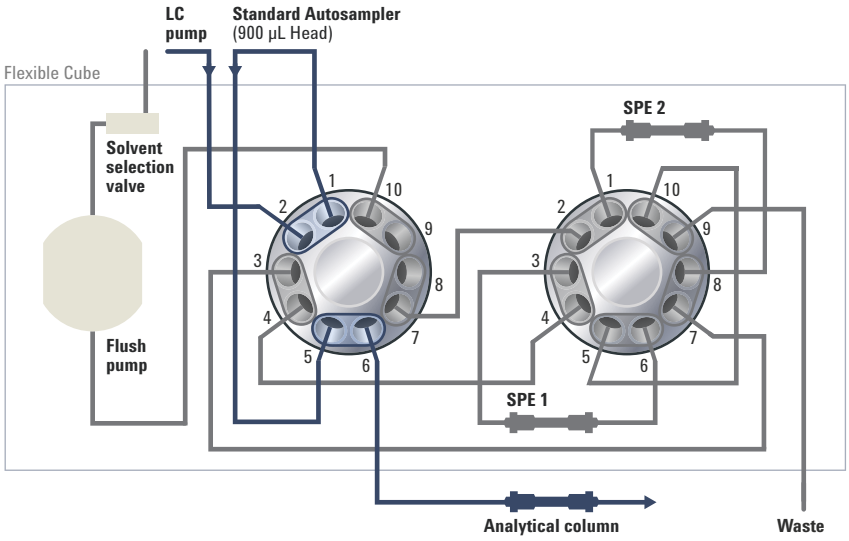

Figure 3A. Configuration of the modules with the Agilent 1290 Infinity Flexible Cube showing the plumbing for switching between direct on-column injection and injection on SPE trapping columns. The Agilent 1260 Infinity Quaternary Pump is connected with the Agilent 1260 Infinity Autosampler and the analytical column for direct injection (left valve position, blue flow path, grey lines are not used).

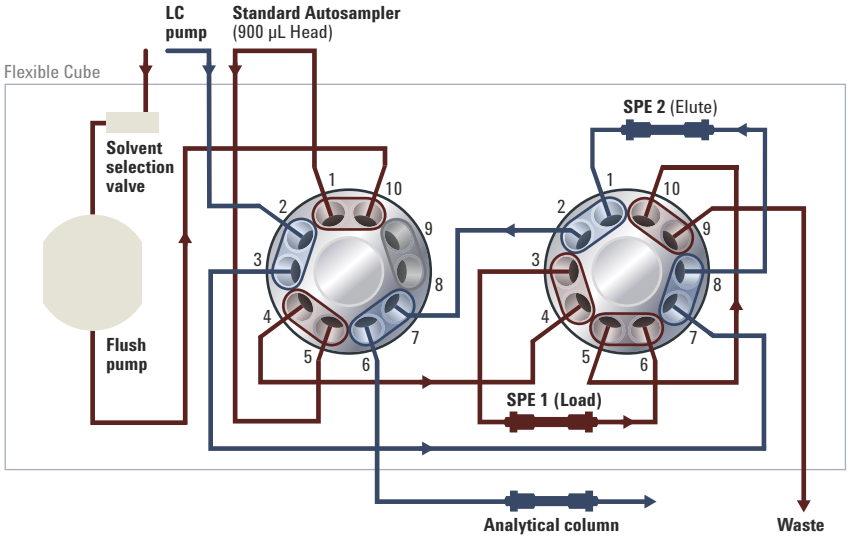

Figure 3B. Configuration of the modules with the Agilent 1290 Infinity Flexible Cube showing the plumbing for switching between direct on-column injection and injection on SPE trapping columns. The Agilent 1260 Infinity Quaternary Pump is connected with the left valve and the trapping columns (SPE2) towards the analytical column (left valve position, blue flow path). At the beginning of the analysis the piston pump is delivering the sample from the autosampler to the trapping column SPE1 (red flow path).

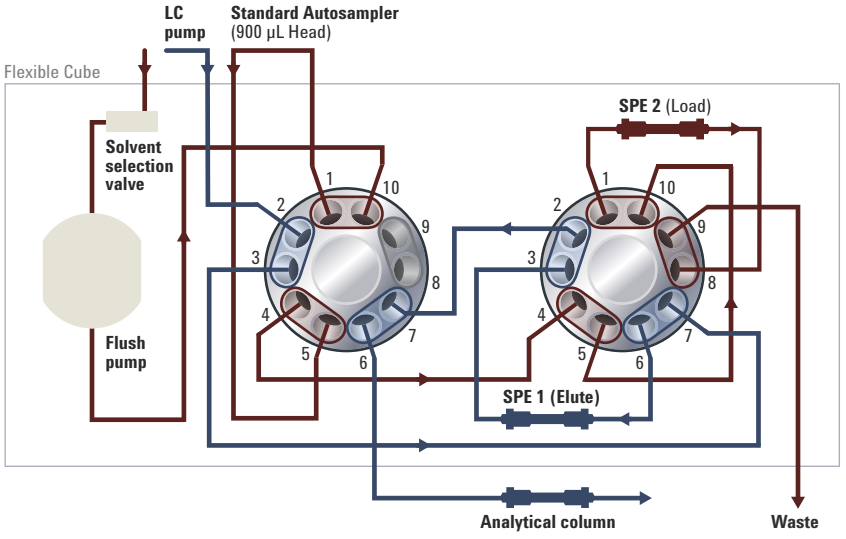

Figure 3C. Configuration of the modules with the Agilent 1290 Infinity Flexible Cube showing the plumbing for switching between direct on-column injection and injection on SPE trapping columns. The Agilent 1260 Infinity Quaternary Pump is connected with the left valve and the trapping columns towards the analytical column for SPE (left valve position, blue flow path). After loading the SPE1, the right valve is switched and moves SPE1 in front of the analytical column (blue flow path). The SPE 2 is cleaned and equilibrated for the following sample (red flow path).

Tables 1A and 1B summarize the content of the Agilent 1200 Infinity Series online SPE solution starter set G4742A and online SPE direct injection kit G4744A.

Table 1A. Content of the Agilent 1200 Infinity Series online SPE solution starter set G4742A and online SPE direct injection kit G4744A. G4744A in an upgrade to G4742A (SST = stainless steel).

parts	Qty	description	Order no.
2-position/10-port valve head	2	To be mounted in Flexible Cube	5067-4145
Guard column hardware kit	2	Hosting the trapping column cartridges (BondElut Online SPE cartridges, 5982-1271)	820999-901
Online SPE capillary kit	1	Contains required capillaries for starter set	5067-5708
Direct injection kit	1	Contains required capillaries for direct injection	5067-5710

Table 1B. Online SPE kits G4742A and G4744A contain all necessary accessories such as capillaries (SST = stainless steel).

Capillaries and parts	Qty	Description	Order no.
120 mm , 0.12 mm id, SST	5	Valve to cartridge, valve crossing	5067-4652
BondElut online PLRP-S 15-20 µm, 2.1 × 12.5 mm 3/pk	1	BondElut Online SPE cartridges	5982-1271
340 mm , 0.12 mm id, SST	2	valve to column, valve to ALS	5067-4647
Wasteline	2 m	Valve to waste	0890-1713
500 mm, 0.25 mm i.d., SST	1	Flexible Cube pump to ALS	5067-5713
700 mm,, 0.17 mm i.d, SST	1	LC pump to valve	5067-4648
Finger tight fittings	1	For waste line	0100-1516
280 mm, 0.12 mm i.d., SST	3	Valve to valve	5067-4687
250 mm, 0.25 mm i.d., SST	1	Flexible Cube pump directly to vlave	5067-5709
Plug long 10-32	2	Plug free valve position	5043-0277

Table 2 shows a summary of the LC methods for direct on-column injection and injection onto the trapping SPE columns for the main modules.

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.

A) Method for injection on SPE trapping columns																																
Agilent 1260 standard autosampler	multidraw 1,800/900 µL sample	Inject																														post run
Agilent 1260 Infintiy Quaternary Pump				5% Solvent B						Gradient 5% B to 98% B																			98% Solvent B				
Agilent 1290 Infintiy Flexible Cube	Left valve position for SPE			Pump 300 seconds solvent A1					Switch right valve to next position		Pump 180 seconds, solvent A2				Pump 300 seconds, solvent A1																	
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			
B) Method for direct injection on the analytical column																																
Agilent 1260 standard autosampler	Injector program	Inject		Wait 5 minutes					Valve bypass																							post run
Agilent 1260 Infintiy Quaternary Pump				5% Solvent B						Gradient 5% B to 98% B																			98% Solvent B				
Agilent 1290 Infintiy Flexible Cube	Left valve position for direct injection																															
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			

A) Method summary for sample injection on the trapping columns.
B) Method summary for direct injection of the sample on the analytical column.

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 3 shows the identified optimum fragmentor and collision energy values for the quantifier and qualifier ions of the individual pesticides. 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 (Fragmentor = voltage [V], r.t. = retention time [min], CE = collision energy [eV]).

The MRM and dynamicMRM triple quadrupole MS method was developed by means of the MassHunter optimizer software and flow injection of individual pesticide standard solutions (10 ng/µL) 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). 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[2].

Table 3. MRM and Dynamic MRM MS Method.

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

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

Samples

Water samples were taken directly from the river Rhine, from tap water and from a spring in the region of Karlsruhe, Germany. The water samples were filtered with a syringe filter (0.45 µm) and injected without further sample prep.

Sample Sequence

(all samples were injected in duplicate)

1. Blank
   (injection on SPE column 1 and 2)
2. Blank
   (injection on SPE column 1 and 2)
3. Water from river Rhine
   (injection on SPE columns)
4. Water from tap
   (injection on SPE columns)
5. Water from Spring
   (injection on SPE columns)
6. Water from river Rhine spiked with 25 ppt of all pesticides (injection on SPE columns)
7. Water from tap spiked with 25 ppt of all pesticides (injection on SPE columns)
8. Water from spring spiked with 25 ppt of all pesticides (injection on SPE columns)
9. Blank
   (injection on SPE column 1 and 2)
10. Quality control sample for the calibration, 20 ppt pesticide standard (injection on SPE columns)
11. Trapping recovery sample, ultrapure water spiked with 50 ppt of all pesticides (900 µL injection on SPE columns)
12. Direct injection sample, ultrapure water spiked with 50 ppt of all pesticides (900 µL injection on analytical column)
13. Blank
    (direct injection on analytical column)
14. Blank
    (injection on SPE column 1 and 2)

Results and Discussion

A sequence of water samples, blanks and controls samples was analyzed with the described method and the obtained data files were evaluated with a calibration for a suite of 28 herbicides[2]. To check the reliability for the current batch of samples a quality control for the calibration at a level of 20 ppt was measured with the samples applying the SPE trapping method. The 20 ppt level calibration quality control was included in the calibration and showing an excellent fit to the previously acquired data (Figure 4). This gives confidence for the following qualitative determination of herbicides in real water samples. The samples were taken from the river Rhine, tap water and spring water.

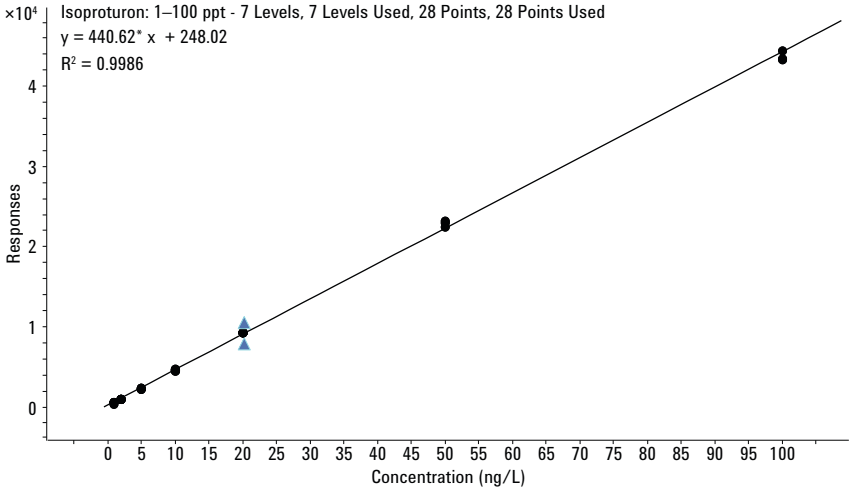

Figure 4. Calibration curve of isoproturon at a concentration of 1 ppt–100 ppt (7 levels, 7 levels used, 28 points, 28 points used), linearity coefficient 0.9986, limit of quantitation (LOQ) 1 ppt, blue triangle indicates calibration quality controls.

In addition to the measurement of the pure water samples, the same samples were spiked with herbicides at a level of 25 ppt (Figure 5) and measured using the online-SPE method. In the tap water (and spring water), no pollution by herbicides was detected. In the spiked tap water sample, the concentration, for example, for the herbicide compound isoproturon was determined to be 23 ppt. In the water sample from the river Rhine, isoproturon was detected as a herbicide residue at a concentration of 16 ppt. Consequently, the spiked river Rhine sample showed a total amount of 40 ppt of isoproturon.

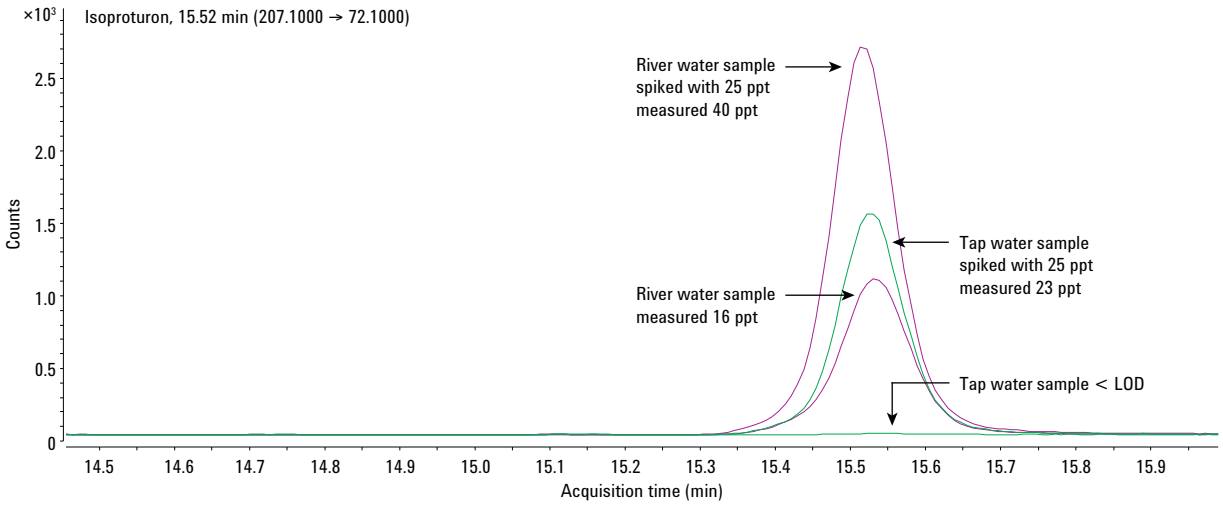

Figure 5. Measured concentrations of the herbicide isoproturon in water from river Rhine and tap. Additional measurements were made with these samples spiked with 25 ppt of the herbicides.

In the instrumental set-up described above, it is possible to inject a sample either onto a trapping column or directly onto the analytical column depending on the position of the left 10-port valve in the Flexible Cube. Since the trapping process is a critical part of the method, it can be controlled by a comparison of an injection of the same sample directly onto the analytical column and onto the SPE trapping columns. The comparison of both injections provides the recovery of the trapping process as a control criterion. This was done with an ultrapure water sample spiked with all 28 herbicides at a concentration of 50 ppt. 900 µL of this sample were injected onto the trapping column and then directly onto the analytical column. The comparison of the peak areas of each compound can be used to calculate the recoveries for the trapping column and to monitor the quality of trapping process (Figure 6). If recovery is falling below a defined threshold due to ageing of the trapping columns, they need to be exchanged. Figure 7 presents the recovery data for all herbicides present in the study. Besides recovery experiments for trapping column performance control the direct injection capability also enables the possibility to calibrate and analyze samples of higher concentrations where lower injection volumes are require. The used autosampler is able to provide the necessary precision to inject samples in the single digit µL range[3].

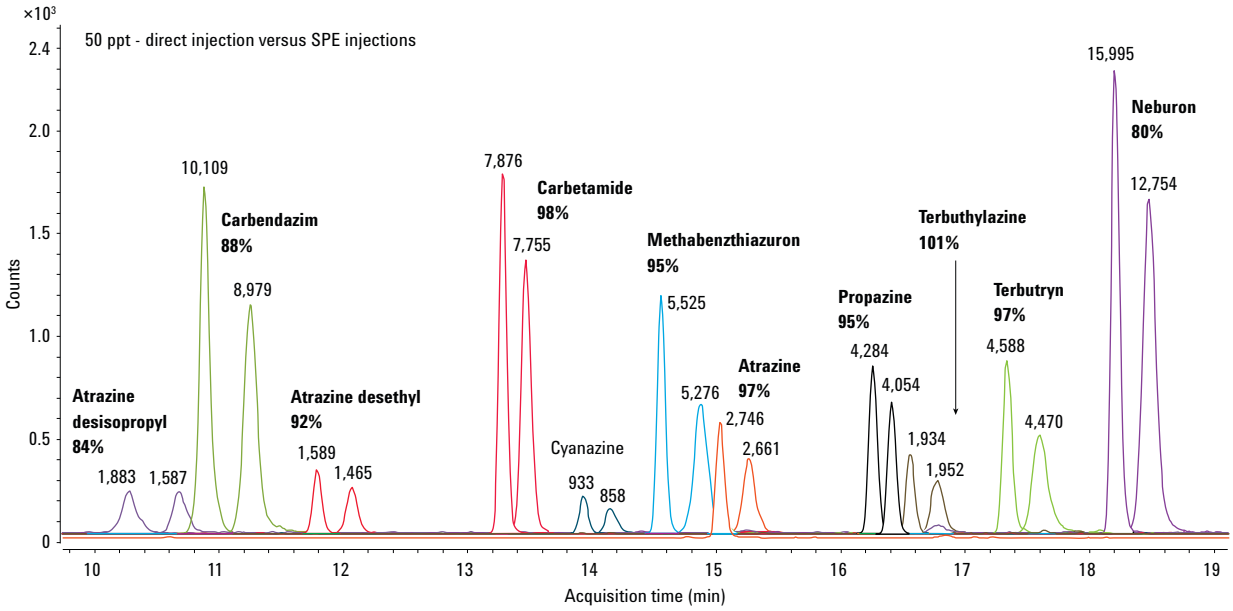

Figure 6. Determination of the recovery [%] of herbicide compounds for the SPE method by comparison to a direct injection of the same sample with the same injection volume (direct injection has earlier retention time).

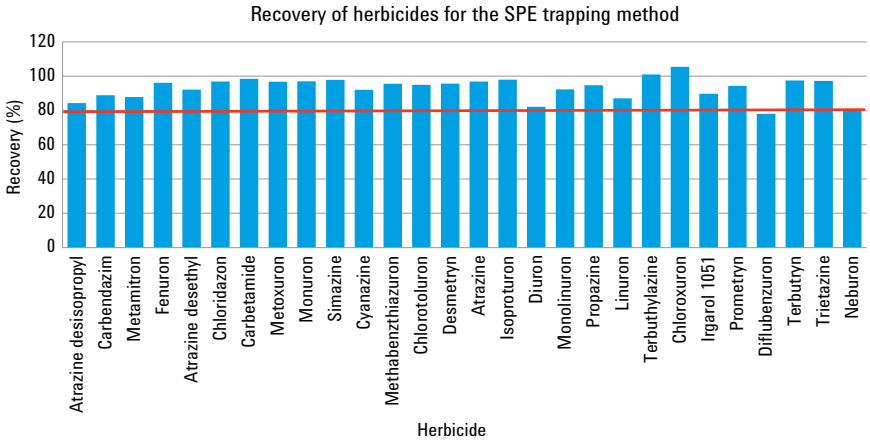

Figure 7. Recovery data for all pesticide compounds present in the study.

Conclusion

This Application Note describes the measurement of herbicides at a low ppt level in surface, spring, and drinking water. The instrument that was used had the ability to switch between the injection of samples onto SPE trapping columns or directly onto the analytical column. This was used to control the performance of the trapping process by comparison of an injection of the same sample onto the trapping columns and onto the analytical column. Observed recoveries were typically above 90%. Both injection modes can be used easily with the described set-up just by switching of a 10-port valve.

References

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

2. "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," Agilent Application Note, Publication Number 5991-1738EN, **2013**

3. "Injecting large sample volumes with the Agilent 1100 Series using the multi-draw and 900-µL injection upgrade kits," Agilent Technical Note, Publication Number 5968-8101E, **1999**

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