Trace level analysis of herbicides in drinking and surface water by online-SPE LC/triple quadruploe MS to the lower ppt range

Edgar Naegele, Agilent Technologies R&D and Marketing GmbH & Co.KG

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Introduction

According to the requirements of the European Union drinking water directive 98/83/EC pollutants like herbicides have to be monitored in drinking water. The currently demanded limit of detection (LOD) is 25 ng/L (25 ppt). To achieve this limit of detection with an entry level or mid-range triple quadrupole mass spectrometer a larger volume of the water sample has to be enriched on a trapping column. Then, the compounds are eluted to the analytical HPLC column for separation. This work describes the method to enrich different trace level herbicides and the separation from each other. The performance of the system is demonstrated by the mass spectrometric detection of a suite of 28 neutral typical herbicides down to a LOD of less than 10 ppt.

Experimental

Instrumentation

Agilent 1200 Infinity Series Online-SPE solution system comprising:

• Quaternary Pump with internal degasser, Standard Autosampler with sample thermostat, Flexible Cube with two 2-position/10-port valves G4232B, Thermostatted Column Compartment. Agilent 6460 Triple Quadrupole LC/MS with Agilent Jet Stream Technology

LC/MS Method (Table 1):

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

Thermostated Column Compartment:

• Column temperature: 40 °C.

Flexible Cube (Figure 1):

• Valves: 2-position/10-port QuickChange valve heads.
• Pump: 1.5 mL/min. Solvent: A1: Water. B1: CAN.
• 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

Standard Autosampler

• Injection volume: 1,800 µL (automated multi draw of 2 times 900 µL). Needle wash in vial (MeOH). Draw and eject speed: 1,000 µL/min. Sample temperature: 10 °C.

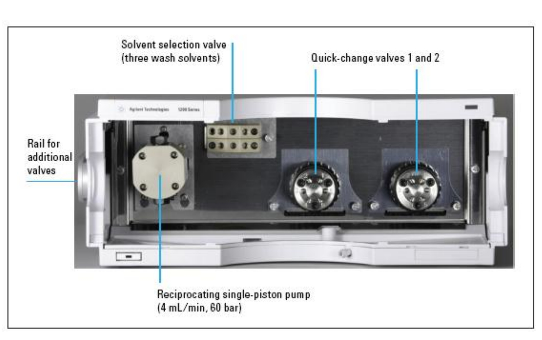

Figure 1: Flexible Cube LC module hosting up to two quick-change valves, a single piston pump and 3 solvent selection valve

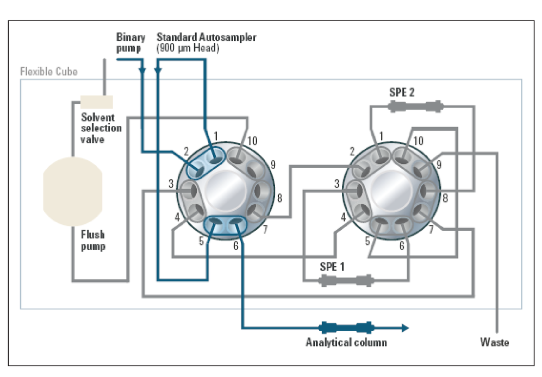

Figure 2A: Configuration showing the plumbing for direct on-column injection

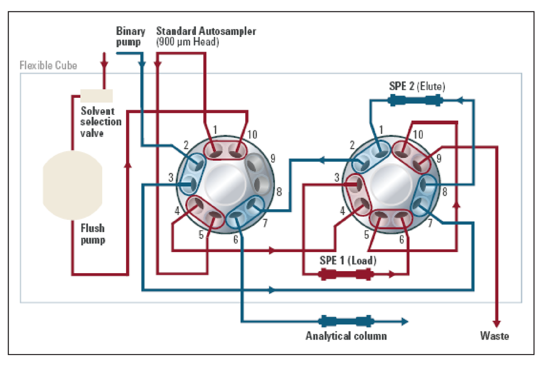

Figure 2B: Configuration showing the plumbing for injection on SPE trapping columns. The Quaternary Pump is connected with the left valve and the trapping columns (SPE2) towards the analytical column (left valve position, blue flow path). At the beginning of the analysis the piston pump is delivering the sample from the autosampler to the trapping column SPE1 (red flow path).

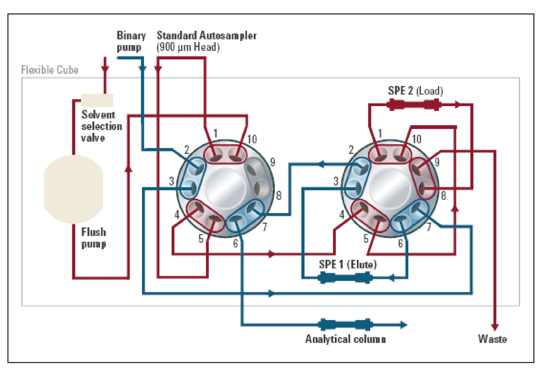

Figure 2C: Configuration showing the plumbing for injection on SPE trapping columns. The Quaternary Pump is connected with the left valve and the trapping columns towards the analytical column for SPE (left valve position, blue flow path). After loading the SPE1 the left valve is switched and moves SPE1 in front of the analytical column (red flow path). The SPE 2 is cleaned and equilibrated for the following sample (red flow path).

Experimental

Triple Quadrupole MS method with 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

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

Table 2:
MRM and dynamic MRM MS method, showing the identified optimum fragmentor [v] and collision energy [eV] 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.

<table>
<tr><td>Agilent 1260 Infinity Standard Autosampler</td><td>multidraw 1800 µL sample</td><td rowspan="3">Inject</td><td colspan="8"></td><td rowspan="3">post-run</td></tr>
<tr><td>Agilent 1260 Infinity Quaternary Pump</td><td></td><td colspan="2">5% Solvent B</td><td colspan="5">Gradient 5% B to 98% B</td><td>98% Solvent B</td></tr>
<tr><td>Agilent 1290 Infinity Flexible Cube</td><td></td><td>Pump 300 seconds</td><td>Switch valve to next position</td><td></td><td>pump 180 seconds</td><td></td><td>pump 300 seconds solvent</td><td colspan="2"></td></tr>
<tr><td colspan="3">Minutes</td><td>0 1 2 3 4</td><td>5</td><td>6</td><td>7 8 9</td><td>10</td><td>11 12 13 14 15</td><td>16 17 18 19 20</td><td>21 22 23 24 25</td><td>10</td></tr>
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Table 1: Summary of the LC method for the Autosampler, the Quaternary Pump and the Flexible Cube.

Results and Discussion

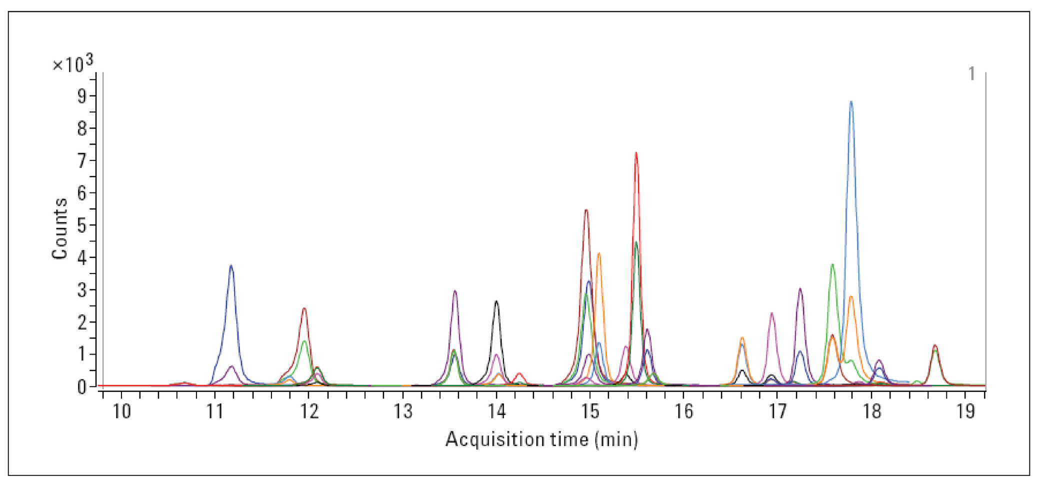

Figure 3: Chromatograms of a calibration standard with a concentration of 100 ppt (ng/L) each for all 28 pesticides measured by the final SPE-LC dynamic MRM method with quantifier and qualifier ion.

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 (Figure 4).

Figures 4A and 4B 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 4A) and for 10 ppt to the LOQ of 1 ppt (Figure 4B) at a retention 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 5C).

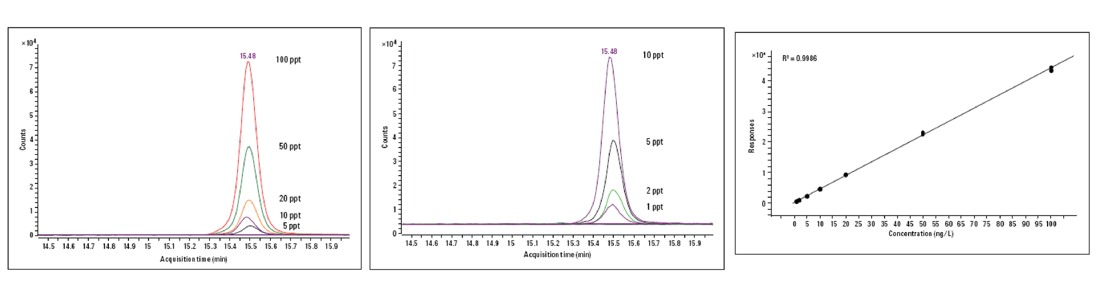

Figure 4: A) Dynamic MRM chromatograms for the quantifier transition m/z 207.1 & m/z 72.1 of Isoproturon, at a concentration of 5 to 100 ppt. B) 1 to 10 ppt. C) Calibration curve of Isoproturon at a concentration of 1 ppt–100 ppt (seven levels, seven levels used, 28 points, 28 points used), linear coefficient 0.9986, LOQ 1ppt.

Results and Discussion

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 3: Performance data for all pesticide compounds present in the study. (R2 = linear coefficient, RSD = Relative standard deviation, r.t. = retention time, RSD (%) and recovery (%)). 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

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 5). 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 5 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%.

	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

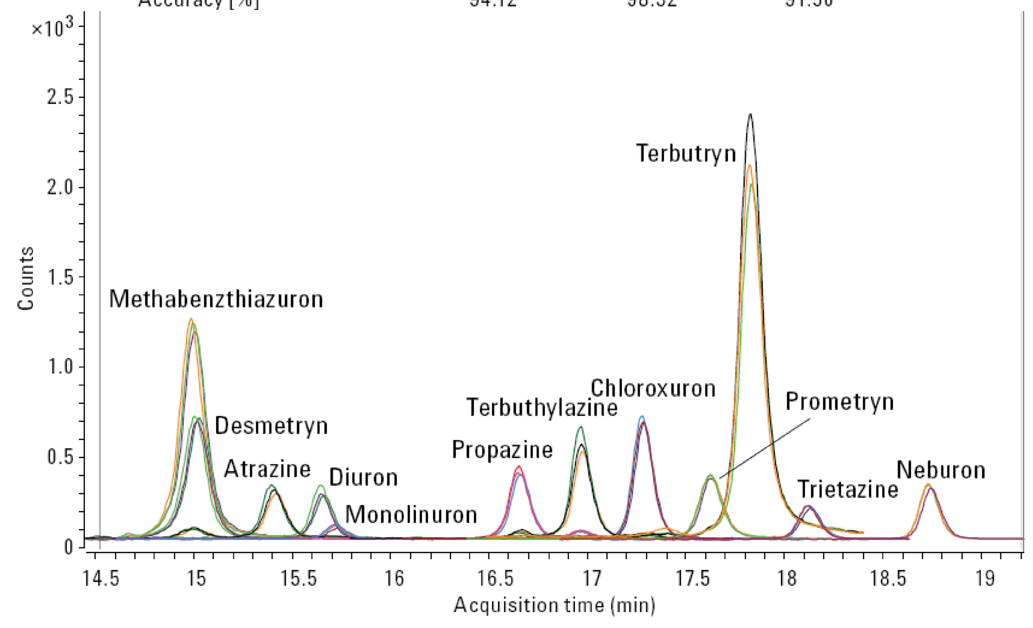

Figure 5: Water samples from a tap, Rhine river, and a spring spiked with 25 ppt of all 28 pesticides. The retention time window from 14.5 minutes to 19.1 minutes is shown. The table shows the average measured concentrations of all pesticides (n=12) within the retention time window dependent on the sources of water together with precision RSD and accuracy.

Conclusions

- This work demonstrates the use of the 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.