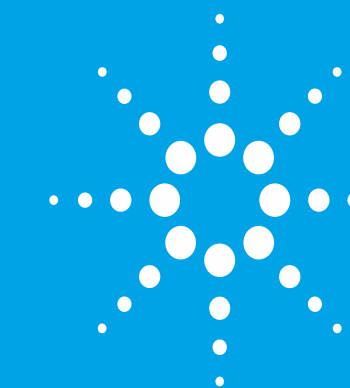


Detection of trace level herbicides from drinking water, surface water and ground water by automated online SPE-LC MS

Sonja Schneider, Bettina Schuhn, Edgar Nägele, Agilent Technologies, Waldbronn, Germany

HPLC 2013
SAMP30_WE



Agilent Technologies

Introduction

An automated online SPE-LC triple quadrupole MS/MS system with parallel column regeneration for high throughput trace level pesticides determination was used for the determination of 27 herbicides in environmental and drinking water. The method conforms to the German DIN standard 38407-36 for the determination of selected and neutral herbicides and herbicide metabolites relevant for surface and drinking water quality. Although the herbicides were at low concentrations, the automated online enrichment ensures sensitive and reproducible results, especially suited for the analysis for herbicides at trace levels. Linearity, accuracy, area and retention time precision in real water samples are shown.

Four different water samples (ground, tap and two surface waters), spiked with unknown concentrations of 27 pesticides, were measured during a validation interlaboratory test. The concentrations were in a range of 10 to 350 ng/l. To achieve successful quantification of these low concentrations with a routine triple quadrupole mass spectrometer, a large volume (900 µl) of water sample was enriched on a trapping column and subsequently analyzed on the analytical column. The method run time was only 22 min, including the trapping procedure and analysis.

This work presents a convenient, fast and very flexible online SPE setup with just one HPLC pump.



Figure 1 1200 Infinity Series Online SPE Solution and 6460 triple quadrupole MS

Experimental

Systems

1. Agilent 1260 Infinity Quaternary Pump with internal degasser
2. Agilent 1260 Infinity Autosampler with 900 µL head and sample cooler
3. Agilent 1290 Infinity Flexible Cube with 2-position/10-port valve
4. Agilent 1290 Infinity Thermostated Column Compartment
5. Agilent G6460A Triple Quad MS with Agilent Jet Stream Technology

Software

Agilent MassHunter for triple quadrupole mass spectrometer, Version B 06.00

Principle of Operating

The piston pump in the 1290 Infinity Flexible Cube is used to pump the sample from the autosampler onto the trapping columns but also to clean those columns after each run with up to three different solvents. With this setup the sample can be transferred directly onto one of the trapping columns (figure 2) while the other trapping column is connected to the analytical column. When the first trapping column was loaded with sample, the 2-position/10-port valve is switched to change the positions of the trapping columns (figure 3).

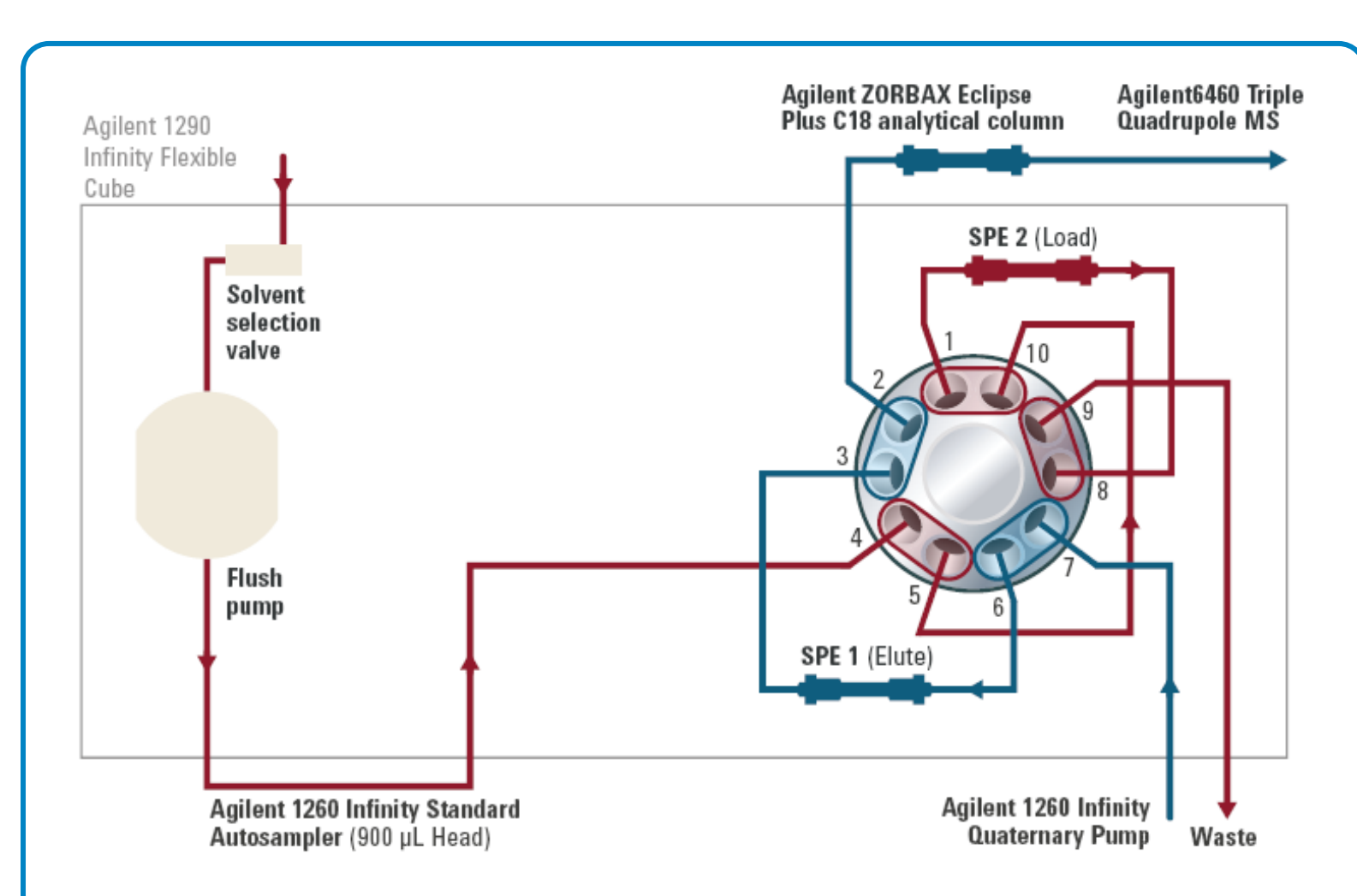


Figure 2 Agilent 1290 Infinity Flexible Cube – the piston pump is loading the first trapping column (red SPE) while the second SPE (blue) is in front of the analytical pump and in the flow path.

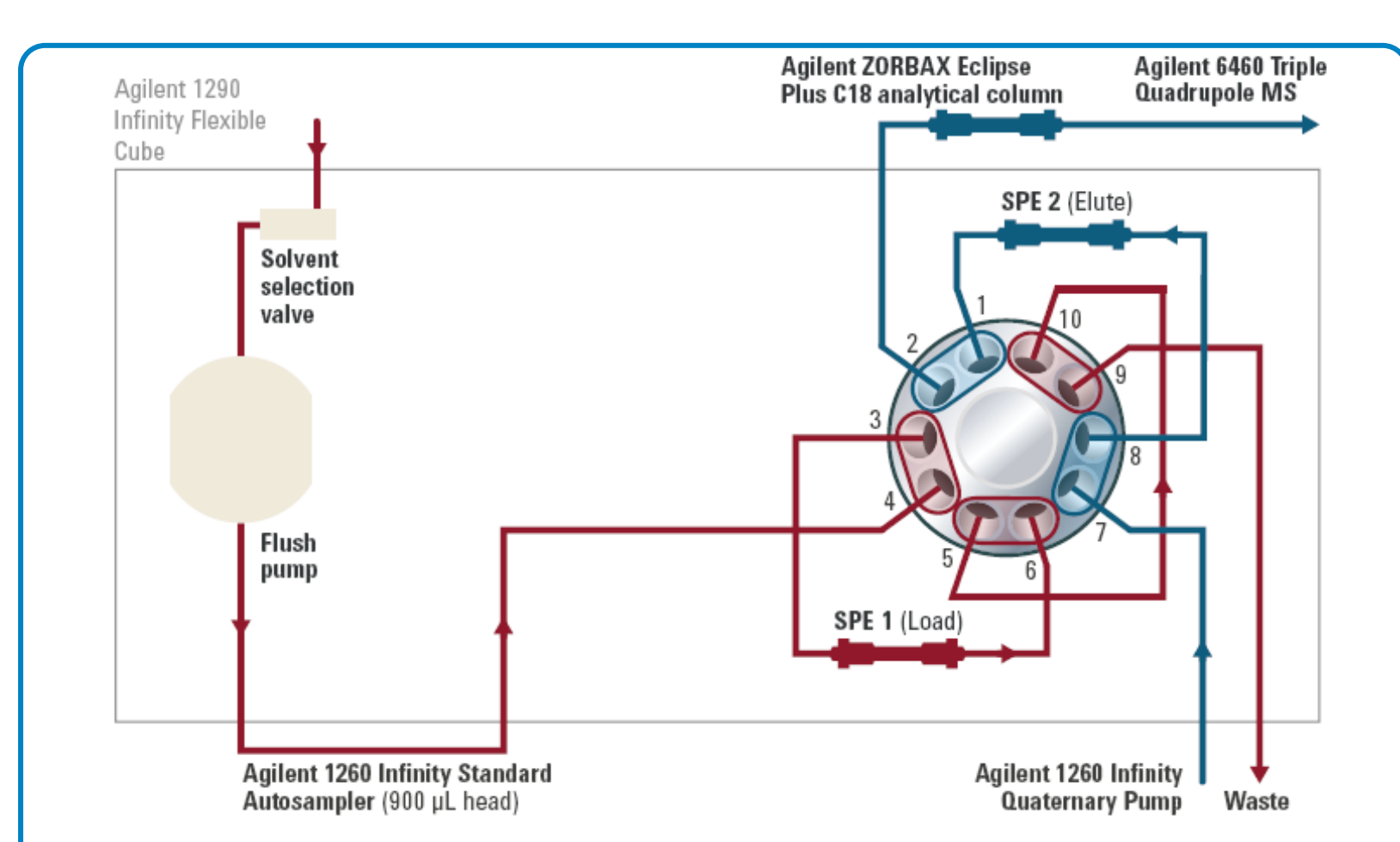


Figure 3 Agilent 1290 Infinity Flexible Cube – The 2-position/10-port valve is switched and the column which has been loaded in the previous run is now eluted and connected to the analytical pump while the second SPE is cleaned.

Experimental

After changing the positions of the trapping columns the HPLC pump delivers the gradient to back flush the sample from the trapping column (SPE 1) onto the analytical column. On the other side the trapping column (SPE 2), which has been used in the previous run, is cleaned and re-equilibrated.

HPLC Method:

1260 Infinity Quaternary Pump:

- Solvent A: Water, 5mM Ammonium Formate + 0.1% formic acid
- Solvent B: Acetonitrile + 5% Water, 5mM Ammonium Formate +0.1% formic acid
- Flow rate: 0.4 mL/min @ 30°C
- Gradient: 0 - 2 min – 2 %B, 2.5 min – 25 %B, 12 – 22 min – 100 % B, Post time 10 min

1290 Infinity Flexible Cube:

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

Table 1 Piston pump timetable in the 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

MS Source conditions of the 6460 Triple Quadrupole LC/MS System with positive electrospray Agilent Jet Stream technology: Gas Temp.: 260°C, Gas Flow: 9 l/min, Nebulizer: 45 psi, Sheath Gas Temp.: 300°C, Sheath Gas Flow: 12 l/min, Capillary: + 3500 Volt, Nozzle: +500 Volt, Delta EMV: + 400 Volt

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 4 shows a chromatogram of all compounds (standard solution in tap water, 50 ng/L).

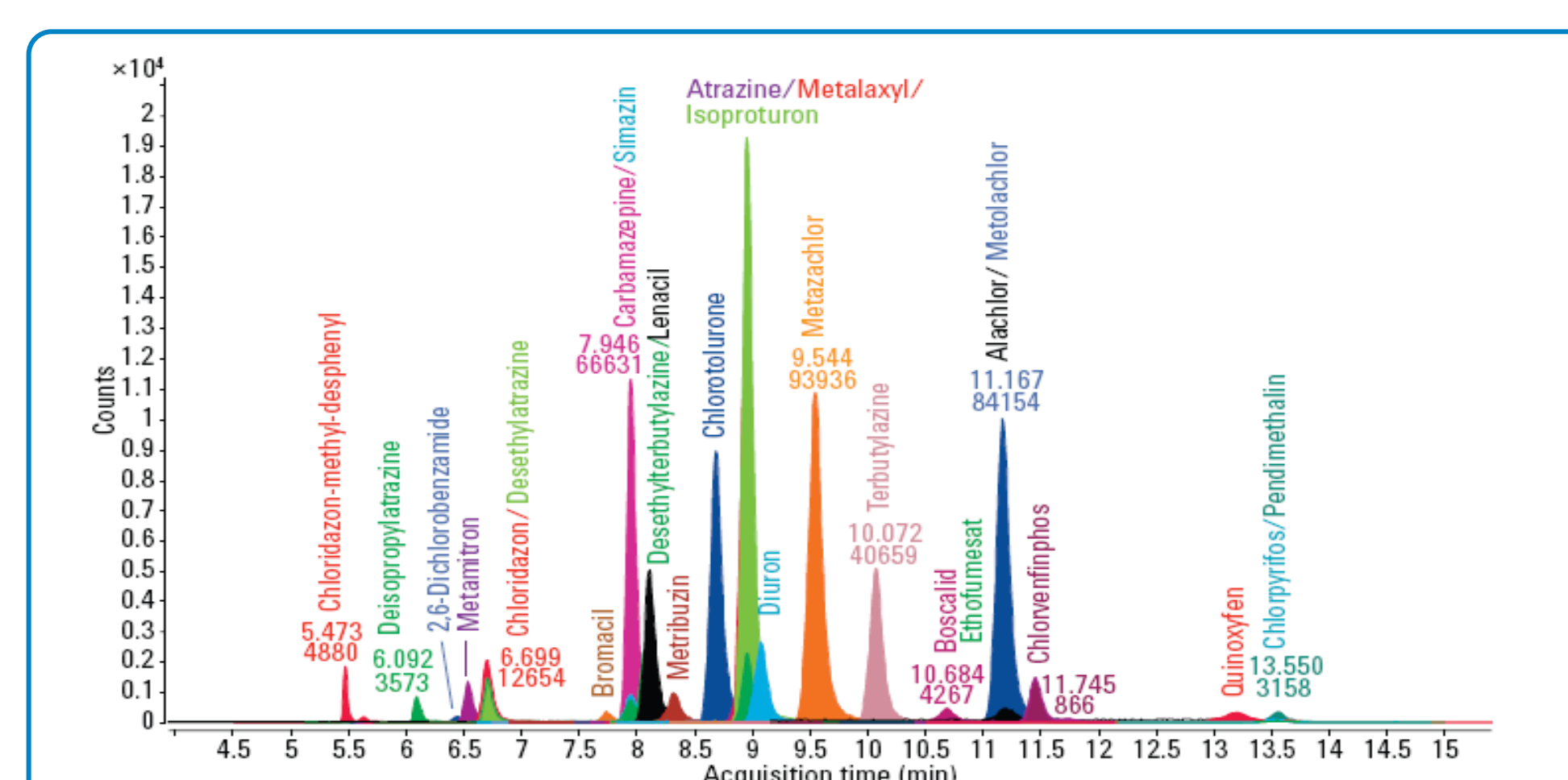


Figure 4 Dynamic MRM chromatogram of a calibration standard in tap water at 50 ng/L.

The majority of the compounds delivered calibration curves with excellent linearity coefficients of > 0.999 for a six level calibration curve. Figure 5 shows a calibration curve of Metolachlor with R2 of 0.9998.

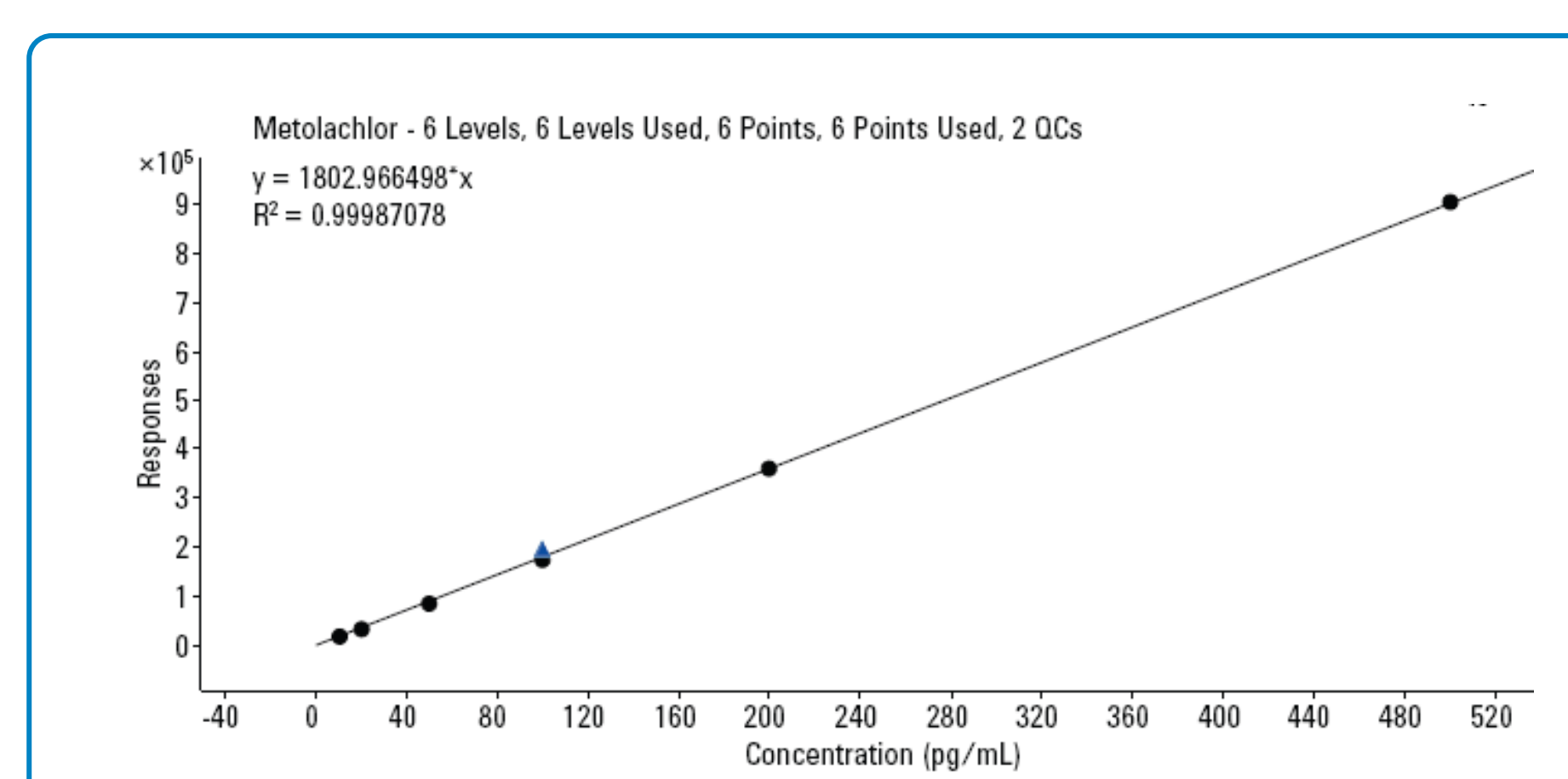


Figure 5 Calibration curve of Metolachlor with a six level calibration (10-500 ng/L).

Tables 2 and 3 summarize the results of the interlaboratory validation study.

Table 2 shows the quantitation results for spiked tap water (sample 1) and spiked ground water (sample 2) and Table 3 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 yellow (<80%) and red (>120%).

In table 2, sample 1 (spiked tap water), showed apparent recoveries for most compounds with less than 20% deviation from the reference value. The area RSD of four runs was, for the majority, < 5%.

Results and Discussion

Table 2 Results of an interlaboratory validation study for spiked tap water (sample 1) and spiked ground water (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-methyldephenyl	0.089	4.3	0.080	111	0.064	3.8	0.050	128
2,6-Dichlorobenzamid	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
Metribuzin	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	0.096	1.5	0.080	113	0.102	2.4	0.100	102
Carbamazepin	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
Chlorotoluron	0.091	0.9	0.080	113	0.114	0.3	0.100	114
Metazachlor	0.095	0.3	0.080	119	0.082	1.3	0.050	124
Metakyl	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
Duron	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
Chlorfeniphos	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	8.8	0.100	139	0.124	1.5	0.120	103

Table 2 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 surface water (samples 3 and 4) had a higher matrix and some particulate matter. Table 3 summarizes the results for spiked surface water (sample 3). The area RSD was < 5% for all compounds, except for ethofumesate. Apparent recoveries for most analytes were within the acceptable range of 80 to 120% of the reference value.

In the second spiked surface water sample (sample 4), which had a high sewage water content, area RSD values for the majority were < 5%, and the estimated concentrations were close to the expected concentrations.

Table 3 Results of an interlaboratory validation study for two different spiked surface waters (sample 3 and 4).

Compound	Sample 3 (surface water)			Sample 4 (surface water)				
	Average (n=4) (µg/L)	Area % RSD	Reference (µg/L)	Average (n=4) (µg/L)	Area % RSD	Reference (µg/L)		
Chloridazone-methyldephenyl	0.277	3.7	0.319	87	0.168	12.2	0.297	57
2,6-Dichlorobenzamid	0.169	3.3	0.100	169	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
Metribuzin	0.184	1.5	0.180	102	0.061	2.4	0.082	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
Carbamazepin	0.165	1.2	0.137	121	0.237	0.8	0.274	123
Terbutylazine-desethyl	0.092	2.1	0.080	115	0.111	0.8	0.100	111
Chlorotoluron	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
Metakyl	0.115	1.2	0.100	115	0.398	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
Isoproturon	0.130	0.8	0.117	111	0.188	1.4	0.182	103
Duron	0.260	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
Chlorfeniphos	0.196	1.5	0.160	122	0.109	3.4	0.100	109
Chlorpyrifos	0.094	2.0	0.100	94	0.125	3.6	0.140	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

In summary, the Online SPE results showed a good apparent recovery for all analyzed water samples.

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. Data for RT precision are shown as excellent in ground water (sample 2) with an RSD of < 0.1.

Conclusions

In this work the results of the Agilent 1290 Infinity Flexible Cube and online SPE enrichment coupled with a triple quadrupole MS system are summarized. It was demonstrated that the system achieved high accuracy and highly reproducible results in different kind of spiked waters. RSD area precision was for the most compounds <5% and the retention time precision showed an RSD of < 0.1%. Linearity coefficients are very good for all compounds with R2 0.999. The accuracy of 27 compounds in four different waters is mostly in the tolerance range of 20%.

The enrichment process makes it possible to accomplish high sensitive results with a routine MS and to run fast analysis of water samples without time-consuming sample preparation or offline enrichment with cartridges.

References:

1. Detection of Trace-Level Herbicides in Drinking, Surface and Ground Water Using the Agilent 1200 Infinity Series Online SPE Solution, Application Note, Agilent Publication Number 5991-2405EN.
2. DIN 38407-36 (Draft): German standard methods for the examination of water, waste water and sludge (HPLC-MS/MS) (F 36).