# Detection of Basic and Acidic Pesticides and Herbicides at Trace Levels by Online SPE LC/MS in Drinking Water

## Application Note

Environmental

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## Abstract

This Application Note demonstrates the use of the Agilent 1200 Infinity Series Online SPE Solution coupled to an Agilent 6460 Triple Quadrupole Mass Spectrometer System for the determination of basic and acidic pesticides and herbicides in positive and negative MS ionization modes at sub parts per trillion (ppt) levels in drinking water. The method is described in detail to provide a starting point for the analysis of basic and acidic pesticides and herbicides, which can be set up and verified with an available Agilent LC/MS Pesticide Application Kit. In addition, the system performance is demonstrated by linearity, limit of quantification (LOQ), limit of detection (LOD), relative standard deviation (RSD) of retention time, and area RSD, as well as recoveries.

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## Introduction

The presence of pesticides and herbicides in drinking water poses significant threats to human health and the environment. As a result, levels in municipal water supplies are regulated by several government agencies down to ppt levels[1]. The diverse range of analyte properties, low analyte concentrations, and variable water matrix composition provide significant analytical challenges.

We used a 1200 Infinity Series Online SPE Solution with a 6460 Triple Quadrupole MS for the simultaneous quantitation of basic and acidic pesticides and herbicides at ppt or sub-ppt levels in drinking water. This flexible system features an integrated loading pump and interchangeable loop capillaries to support large sample volume injections (up to 1,800 μL) with two alternating trapping cartridges for analytes enrichment[2].

In addition, a system that also allows direct on-column injections is advantageous because physical changes of plumbing and configuring are not necessary and can be done from the method side[3].

## Experimental

### Instrumentation

Agilent 1200 Infinity Series Online SPE Solution

- Agilent 1260 Infinity Binary Pump (G1312B), external degasser (G4225A) and LAN card (G1369C)

- Agilent 1260 Infinity Standard Autosampler (G1329B) with a 900 μL head (G1313-60007) 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 1200 Infinity Series Quick-Change valve heads (600 bar) and respective capillaries.

- Agilent 1260 Infinity Thermostatted Column Compartment (G1316C)

### 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 × 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, Version 06.00

- Agilent MassHunter Optimizer software, Version 06.00

- Agilent MassHunter Qualitative software, Version 06.00

- Agilent MassHunter Quantitative software, Version 05.02

### HPLC method for direct injection

| Agilent 1260 Infinity Binary Pump | |
| --- | --- |
| Solvent A and B | See method for online SPE |
| Flow rate | 0.4 mL/min |
| Gradient | 0 minutes – 2 % B, 5 minutes – 2 % B, 11 minutes – 95 % B, 12.50 minutes – 95 % B, 12.60 minutes – 2 % B Stop time: 17 minutes Post time: 6 minutes |
| Agilent 1290 Infinity Thermostated Column Compartment | |
| Column temperature | 40 °C |
| Agilent 1290 Infinity Flexible Cube | |
| Right valve | 2-position/10-port Quick-Change valve head, not used |
| Left valve | 2-position/10-port Quick-Change valve head, position for direct injection on analytical column |
| Agilent 1260 Infinity Standard Autosampler | |
| Injection volume | 900 μL |
| Needle wash in vial (MeOH) | |
| Draw and eject speed | 1,000 μL/min |
| Sample temperature | 8 °C |
| Two trays with 15 positions each (see HPLC method for online SPE) | |
| 6-mL screw cap vials (see HPLC method for online SPE) | |

## HPLC method for online SPE

| Agilent 1260 Infinity Binary Pump | |
| --- | --- |
| Solvent A | Water, 5 mM ammonium formate + 0.1 % formic acid (without ammonium formate for negative mode) |
| Solvent B | ACN + 0.1% formic acid |
| Flow rate | 0.4 mL/min |
| Gradient | 0 minutes – 2% B, 5 minutes – 2% B, 11 minutes – 95% B, 12.50 minutes – 95% B, 12.6 minutes – 2% B Stop time: 17 minutes Post time: 6 minutes |

| Agilent 1260 Infinity Thermostatted Column Compartment | |
| --- | --- |
| Column temperature | 40 °C |

| Agilent 1290 Infinity Flexible Cube | |
| --- | --- |
| Right valve | 2-position/10-port Quick-Change valve head, alternating with trapping columns for SPE |
| Left valve | 2-position/10-port Quick-Change valve head, Position for sample load on SPE trapping columns |
| Pumping rate | 1.0 mL/min |
| Solvent A1 | Water with 0.1 % formic acid |
| Solvent B2 | ACN |
| Gradient | 0 minutes – Pump 4 mL, Solvent A1 4 minutes – Right valve change position 5 minutes – Pump 4 mL, Solvent B2 10 minutes – Pump 7 mL, Solvent A1 |

| Agilent 1260 Infinity Standard Autosampler | |
| --- | --- |
| Injection volume | 900 µL |
| Needle wash in vial (MeOH) | |
| Draw and eject speed | 1,000 µL/min |
| Sample temperature | 8 °C |
| Two trays with 15 positions each (G1313-44513) | |
| 6-mL screw cap vials (glass, p/n 9301-1377), screw caps (p/n 9301-1379), pre-slit septa for 6-mL screw cap vials (p/n 5188-2758) | |

## Triple Quadruple MS method in positive ionization mode with Agilent Jet Stream thermal gradient focusing technology

| | |
| --- | --- |
| Gas temperature | 250 °C |
| Gas flow | 7 L/min |
| Nebulizer | 40 psi |
| Sheath gas temperature | 325 °C |
| Sheath gas flow | 11 L/min |
| Capillary | 3,500 V |
| Nozzle | 0 V |
| MS2 Resolution | Unit |

## Plumbing and usage of the Agilent 1290 Infinity Flexible Cube[2,3]

In the setup of the 1200 Infinity Series Online SPE Solution with direct injection capability, the 1290 Infinity Flexible Cube hosts 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 1A).

## Triple Quadruple MS method in negative ionization mode with Agilent Jet Stream thermal gradient focusing technology

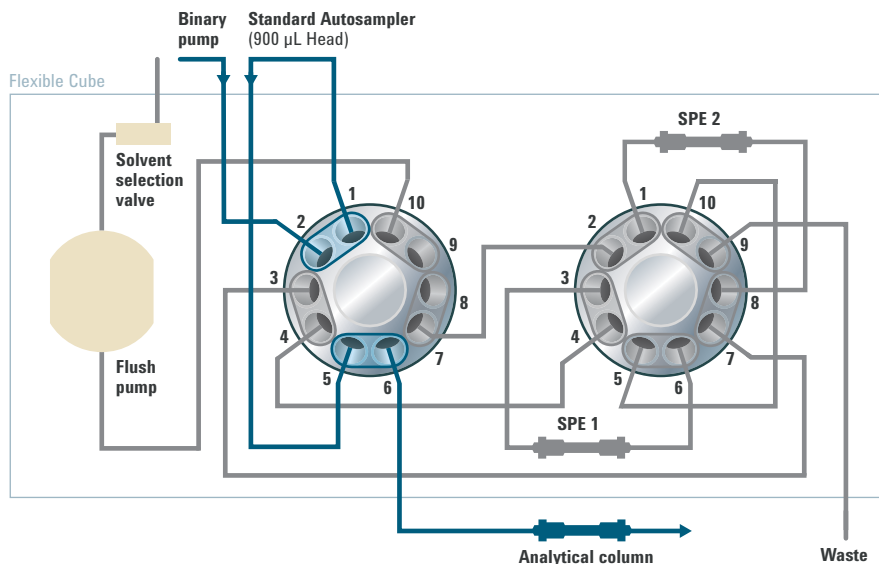| | |
| --- | --- |
| Gas temperature | 325 °C |
| Gas flow | 9 L/min |
| Nebulizer | 35 psi |
| Sheath gas temperature | 350 °C |
| Sheath gas flow | 12 L/min |
| Capillary | 4,000 V |
| Nozzle | 500 V |
| MS2 Resolution | wide |



Figure 1A. 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 Binary Pump is connected with the Agilent 1260 Infinity Autosampler and the analytical column for direct injection (left valve position, blue flow path, grey lines show the path that is not used).

In addition, the 1290 Infinity 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 1B and 1C). Three solvents can be selected for loading, cleaning, and equilibration. If the valve position for loading the sample on one of the SPE columns is selected, the piston pump inside the 1290 Infinity Flexible Cube is connected to the 1260 Infinity Standard Autosampler. The sample is flushed 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 1B). 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 1C). 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 reconditioned. This cleaning procedure is done by the piston pump of the 1290 Infinity Flexible Cube with the cleaning solvents selectable by the solvent selection valve.
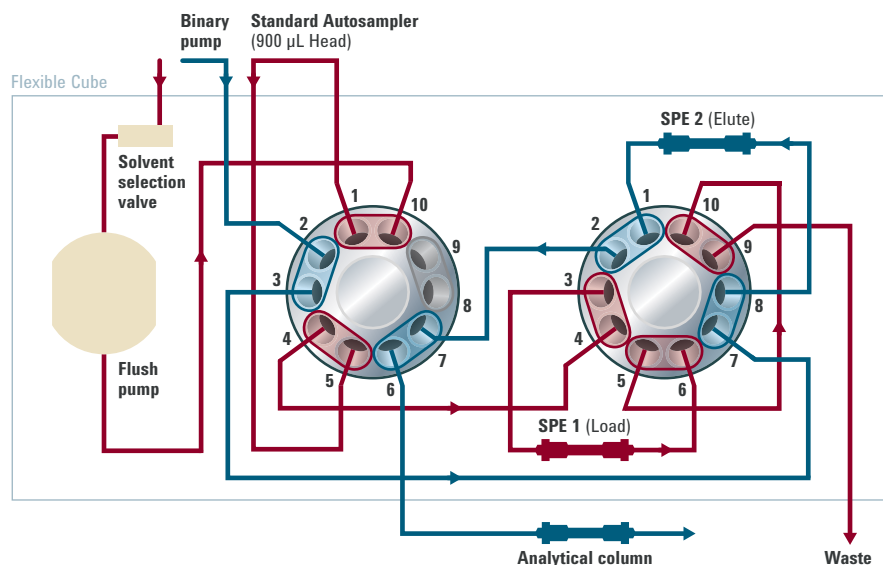


Figure 1B. Configuration of the modules with the Agilent 1290 Infinity Flexible Cube showing the plumbing for switching between direct on-column injection and injection on SPE trapping columns. The Agilent 1260 Infinity Binary Pump is connected with the left valve and the trapping columns (SPE2) towards 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).
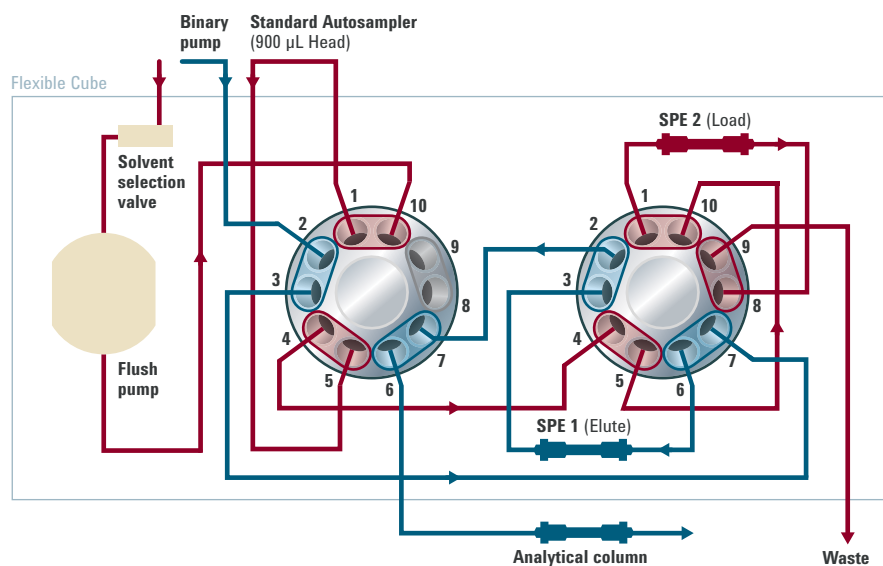


Figure 1C. Configuration of the modules with the Agilent 1290 Infinity Flexible Cube showing the plumbing for switching between direct on-column injection and injection on SPE trapping columns. The Agilent 1260 Infinity Binary Pump is connected with the left valve and the trapping columns towards the 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).

## MRM method

Tables 1 and 2 show the optimum fragmentor voltage, collision energy values, dwell time, and cell accelerator voltage for the quantifier and qualifier ions of the individual pesticides and herbicides in positive and negative mode.

The MRM triple quadrupole MS method was developed by means of the MassHunter optimizer software and flow injection of the herbicide standard solutions (1 mg/L, 1 ppm) 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.

## Chemicals

All solvents used were LC/MS grade. Acetonitrile was purchased from Honeywell, USA. 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).

## Standards

The Agilent Pesticide Test Mix (p/n 5190-0469) contains 14 basic and six acidic herbicides in a 100 ppm stock solution for each compound. They were diluted in acetonitrile to a 1 ppm working solution, and finally diluted in tap water to defined concentration.

Table 1. Optimum fragmentor voltage, collision energy (CE) values, dwell time, and cell accelerator voltage for the quantifier and qualifier ions of the individual pesticides and herbicides in positive mode.

| Compound name | Precursor ion | Product ion | Fragmentor (V) | CE (V) | Dwell (msec) | Cell acc (V) |
|---|---|---|---|---|---|---|
| Metosulam | 418.02 | 175 | 140 | 32 | 5 | 3 |
| Metosulam | 418.02 | 140 | 140 | 60 | 5 | 3 |
| Pyraclostrobin | 388.11 | 193.8 | 95 | 8 | 5 | 2 |
| Pyraclostrobin | 388.11 | 163.1 | 95 | 20 | 5 | 2 |
| Malathion | 331 | 126.9 | 80 | 5 | 5 | 2 |
| Malathion | 331 | 99 | 80 | 10 | 5 | 2 |
| Diazinon (Dimpylate) | 305.1 | 169.1 | 105 | 32 | 5 | 2 |
| Diazinon (Dimpylate) | 305.1 | 97 | 105 | 40 | 5 | 2 |
| Imazalil (Enilconazole) | 297.1 | 201 | 115 | 15 | 5 | 2 |
| Imazalil (Enilconazole) | 297.1 | 159 | 115 | 20 | 5 | 2 |
| Metazachlor | 278.1 | 210.1 | 70 | 4 | 5 | 5 |
| Metazachlor | 278.1 | 134.2 | 70 | 15 | 5 | 5 |
| Imazapyr | 262.1 | 217.1 | 120 | 20 | 5 | 3 |
| Imazapyr | 262.1 | 69.1 | 120 | 40 | 5 | 3 |
| Dimethoate | 230 | 198.8 | 70 | 0 | 5 | 5 |
| Dimethoate | 230 | 125 | 70 | 16 | 5 | 5 |
| Metoxuron | 229 | 72.1 | 95 | 16 | 5 | 3 |
| Metoxuron | 229 | 46.1 | 95 | 12 | 5 | 3 |
| Carbofuran | 222.1 | 165.1 | 80 | 20 | 5 | 2 |
| Carbofuran | 222.1 | 123.1 | 80 | 30 | 5 | 2 |
| Atrazine | 216.1 | 174.1 | 125 | 16 | 5 | 3 |
| Atrazine | 216.1 | 68 | 125 | 40 | 5 | 3 |
| Aminocarb | 209.1 | 152.2 | 105 | 12 | 5 | 2 |
| Aminocarb | 209.1 | 137.2 | 105 | 24 | 5 | 2 |
| Thiabendazole | 202 | 175 | 130 | 24 | 5 | 2 |
| Thiabendazole | 202 | 131 | 130 | 36 | 5 | 2 |
| Molinate | 188 | 126.1 | 90 | 25 | 5 | 2 |
| Molinate | 188 | 83.2 | 90 | 16 | 5 | 2 |

Table 2. Optimum fragmentor voltage, collision energy (CE) values, dwell time, and cell accelerator voltage for the quantifier and qualifier ions of the individual pesticides and herbicides in negative mode.

| Compound name | Precursor ion | Product ion | Fragmentor (V) | CE (V) | Dwell (msec) | Cell acc (V) |
|---|---|---|---|---|---|---|
| Hexaflumuron | 461 | 158 | 120 | 15 | 15 | 2 |
| Hexaflumuron | 461 | 141 | 120 | 45 | 15 | 2 |
| Acifluorfen | 359.99 | 316 | 60 | 5 | 15 | 2 |
| Acifluorfen | 359.99 | 286 | 60 | 5 | 15 | 2 |
| 2,4,5-TP (Silvex) | 266.94 | 194.9 | 80 | 12 | 15 | 2 |
| 2,4,5-TP (Silvex) | 266.94 | 158.9 | 80 | 30 | 15 | 2 |
| 2,4,5-T | 254.9 | 196.9 | 70 | 15 | 15 | 2 |
| 2,4,5-T | 252.9 | 194.9 | 70 | 10 | 15 | 2 |
| Bentazone | 239.1 | 197 | 75 | 20 | 15 | 5 |
| Bentazone | 239.1 | 132 | 75 | 24 | 15 | 5 |
| Dinoseb | 239.07 | 209 | 120 | 25 | 15 | 2 |
| Dinoseb | 239.07 | 163 | 120 | 25 | 15 | 2 |

## Results and Discussion

### Basic pesticides and herbicides

For the mixture of basic pesticides and herbicides, the stock solution was diluted to 100 ppt in tap water to simulate the drinking water matrix. From this solution, 10 calibration levels were created in tap water (100, 50, 20, 10, 5, 2, 1, 0.5, 0.2, and 0.1 ppt). They were measured under the given conditions and calibration curves were created. The $R^2$ values of all calibration curves were typically better than 0.9990 (Table 3). An overlay of all MRM signals at the 50 ppt level is shown in Figure 2. The LOQ was calculated from the lowest calibration point with a signal-to-noise (S/N) ratio above 10. The LOQs for all basic pesticides and herbicides are in the single digit ppt range and for the majority of the 14 compounds in the sub-ppt range. The LOD was calculated for a S/N ratio of 3. All LODs, with the exception of imazalil and molinate, were in the sub-ppt range. To determine the recovery of the trapping process on the SPE cartridges, the same volume of the 10 ppt solution was injected directly on the analytical column and onto the SPE trapping column. Typically, the recoveries were better than 80 %. For the basic pesticides and herbicides, possible carryover was determined by an injection of 100 ppt on the trapping column directly followed by a blank injection. The highest carryover was determined for atrazine at a level of 0.005 % (Figure 3).

Table 3. Results for the 14 compounds inherent in the basic pesticides and herbicides test mixture. Values for $R^2$, LOD, LOQ, retention time RSD, peak area RSD, and percentage of recovery measured by the described online SPE LC/MS method are shown.

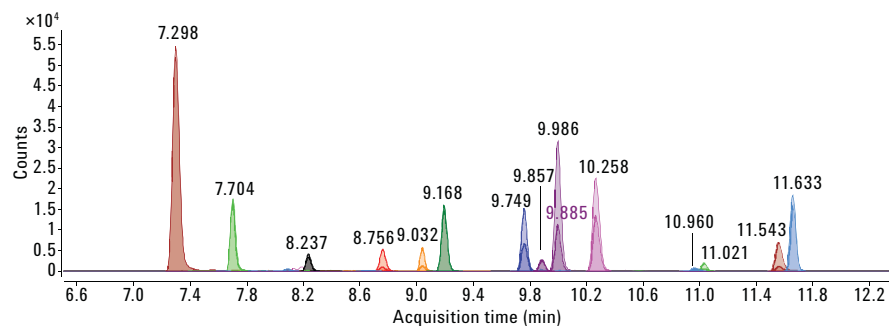| Compound name | Retention time (min) | Retention time RSD (%) | Peak area RSD (%) | $R^2$ | LOQ (ppt) | LOD (ppt) | Recovery (%) |
|---|---|---|---|---|---|---|---|
| Aminocarb | 7.298 | 0.03 | 8.42 | 0.9996 | 0.125 | 0.038 | 96 |
| Thiabendazole | 7.704 | 0.02 | 15.40 | 0.9970 | 0.273 | 0.081 | 98 |
| Imazapyr | 8.237 | 0.05 | 7.18 | 0.9990 | 0.666 | 0.200 | 67 |
| Dimethoate | 8.756 | 0.02 | 7.88 | 0.9992 | 0.666 | 0.200 | 84 |
| Imazalil (Enilconazole) | 9.032 | 0.02 | 7.83 | 0.9990 | 5.000 | 2.000 | 82 |
| Metoxuron | 9.186 | 0.04 | 8.30 | 0.9998 | 0.150 | 0.053 | 93 |
| Carbofuran | 9.749 | 0.03 | 6.62 | 0.9995 | 0.200 | 0.050 | 71 |
| Metosulam | 9.857 | 0.03 | 5.78 | 0.9990 | 0.500 | 0.200 | 81 |
| Atrazine | 9.986 | 0.04 | 8.31 | 0.9995 | 0.050 | 0.015 | 75 |
| Metazachlor | 10.258 | 0.05 | 5.94 | 0.9990 | 0.410 | 0.046 | 79 |
| Molinate | 10.960 | 0.05 | 10.72 | 0.9980 | 5.000 | 2.000 | 56 |
| Malathion | 11.021 | 0.04 | 9.53 | 0.9990 | 1.750 | 0.400 | 69 |
| Pyraclostrobin | 11.543 | 0.05 | 8.83 | 0.9960 | 1.000 | 0.500 | 88 |
| Diazinon (Dimpylate) | 11.633 | 0.05 | 6.33 | 0.9975 | 4.166 | 0.125 | 89 |

Figure 2. Chromatogram of the 50 ppt level of all compounds inherent in the basic herbicide mixture showing an overlay of the quantifier and qualifier MRM signals, the compound names, and retention times are shown in Table 3.
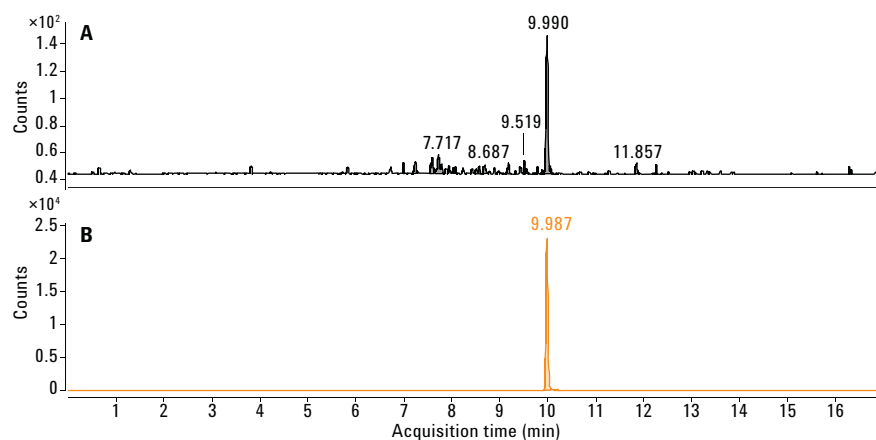
Figure 3. Determination of carryover from a 100 ppt injection (B) followed by a blank injection (A). For atrazine a carryover of 0.005 % was identified, all other compounds were at less carryover or carryover was not detectable.

## Acidic pesticides and herbicides

For the acidic pesticides and herbicides, a calibration curve was created starting with 2 ppb and diluted down to a concentration of 10 ppt with the same dilution pattern as used for the basic pesticides and herbicides. The calculated LOQs as well as the LODs were typically in the single digit ppt range, for example, bentazone showed a LOQ of 0.25 ppt and a LOD of 0.08 ppt (Table 4). The highly fluorinated compound hexaflumuron was an exception with a LOQ of 100 ppt and a LOD of 50 ppt. All lower concentrations showed no or non-linear response. This was due to adhesion to the walls of the glass vials. This problem could be solved by adding organic solvent to the dilution solvent or by using silanized glass vials (not part of this work). The $R^2$ values of the calibration curves are typically better than 0.9990. Figure 4 shows a chromatogram overlay of quantifier and qualifier ions at 1 ppb and at 50 ppt.

The peak area and retention time RSDs were calculated from 10 replicate injections of the 1 ppb level. The area RSDs are typically below 5 % and retention time RSDs were always below 0.03 %. (Table 4).

The recovery was determined by comparing a direct injection and an injection on an SPE cartridge at the 1 ppb level. The recoveries were typically at or slightly above 100 %. The apparent recoveries were high because these acidic compounds had poor affinity toward C18 column, but had better affinity toward PLRP-S cartridge. Analyte loss on the analytical C18 column was possible with 900 µL direct injection. This can be avoided by acidifying the samples prior to analysis or using a column that has better affinity toward these acidic compounds (not part of this work).

The carryover was determined by an injection of a 10 ppb concentration, followed by a blank injection. In this experiment, bentazone showed the highest carryover, with about 0.003 %.

Table 4. Results for the six compounds inherent in the negative pesticides and herbicides test mixture. Values for $R^2$, LOD, LOQ, retention time RSD, peak area RSD, and percentage of recovery measured by the described online SPE LC/MS method are shown.

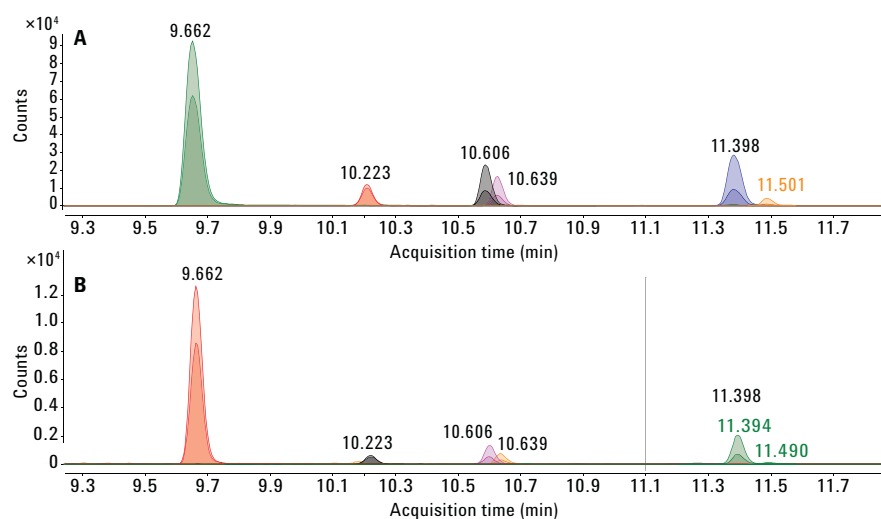| Compound name | Retention time (min) | Retention time RSD (%) | Peak area RSD (%) | $R^2$ | LOQ (ppt) | LOD (ppt) | Recovery (%) |
|---|---|---|---|---|---|---|---|
| Bentazone | 9.662 | 0.03 | 1.04 | 0.9959 | 0.25 | 0.08 | 194 |
| 2,4,5-T | 10.223 | 0.03 | 2.20 | 0.9995 | 6.8 | 2.0 | 106 |
| 2,4,5-TP (Silvex) | 10.606 | 0.02 | 4.85 | 0.9996 | 5.13 | 1.54 | 115 |
| Acifluorfen | 10.639 | 0.00 | 5.58 | 0.9993 | 10.3 | 3.1 | 109 |
| Dinoseb | 11.398 | 0.02 | 1.41 | 0.9991 | 3.84 | 1.15 | 135 |
| Hexaflumuron | 11.501 | 0.02 | 7.04 | 0.9995 | 100 | 50 | 96 |



Figure 4. Chromatogram of the A) 1 ppb and B) 50 ppt level of acidic herbicide mixture showing an overlay of the quantifier and qualifier MRM signals, the compound names and retention times are shown in Table 4.

## Conclusion

This Application Note describes the analysis of basic and acidic pesticides and herbicides by online SPE LC/triple quadrupole MS and delivers a starting method applicable for an available kit containing compounds of both classes. The performance for compounds of both classes is demonstrated. All compounds show excellent linearity, LOQs typically in the single digit ppt level, and LODs typically in the sub-ppt range. Peak area RSDs and retention time RSDs as well as recoveries are excellent for the applied online SPE method.

## References

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

2. Naegele, E., 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*, **2013**, Publication Number 5991-1738EN.

3. Naegele, E., Comparison of Direct Injection and Online SPE for Quantification by LC/MS of Trace-Level Herbicides in Water, *Agilent Technical Note*, **2013**, Publication Number 5991-2140EN.

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