Ultra-Sensitive Detection of Pesticides in Drinking Water with a Simple, Rapid, and High Quality Analysis

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APPLICATION BENEFITS

- Using direct injection on Xevo[™] TQ-S removes sample preparation and enables a simple, high-throughput analysis of pesticides in drinking water.
- Allows detection of pesticides to parts-perquadrillion (ppq), to enable real background concentrations in samples to be observed.
- Analyze over five samples per hour and over 80 samples in a typical overnight run.
- Collect spectral information on background components in the sample matrix while simultaneously collecting MRM data.

WATERS SOLUTIONS

ACQUITY UPLC Xevo TQ-S, Quanpedia, RADAR, TrendPlot

KEYWORDS

drinking water, fungicides, triazine and phenylurea herbicides, organophosphorous, and organothiophosphorous pesticides

INTRODUCTION

Rapid and highly sensitive analysis of drinking water is essential for protecting human health and well-being. The assurance of clean, safe drinking water has become more critical given the potential of accidental or intentional contamination, which have increased in recent years.

Monitoring for harmful substances in drinking water, or water used in products intended for consumption is required to ensure that exposure to the consumer is limited. Apart from the duty of care to consumers, organizations have regulatory testing imposed on them to ensure a safe product is delivered. The World Health Organization (WHO) publishes guidelines for drinking water quality¹ and these are used as the basis of much of the drinking water regulation across the globe.

Highly efficient water treatment processes remove a majority of pesticides that have entered water sources², but drinking water regulations still require testing to ultra-trace concentrations. In order to report results to regulators, testing must be of high quality to conform to international standards such as ISO17025.

This requirement has led to multiple approaches for enriching samples before instrumental analysis, and solid phase extraction prior to LC/MS/MS is a popular choice.³ In addition, online pre-concentration and large volume injection using specialized injection systems have been used to introduce samples to LC/MS/MS systems.⁴ These techniques can be very successful but add time, resources, and complexity to the analysis.

Cleaner aqueous samples, such as drinking water, are highly compatible for direct injection onto an LC/MS/MS system; however large multi-analyte determinations require extremely fast systems with ultra-sensitive detection.

This application note describes the use of direct injection ACQUITY UPLC® coupled with Xevo TQ-S for the rapid, high quality, and ultra-sensitive analysis of multiple pesticides in drinking water.

EXPERIMENTAL

Sample preparation

 $Na_2S_2O_3$ was added to drinking water samples to 200 mg/L to ensure dechlorination.

1.0 mL aliquots were transferred into Waters[®]
 LC/MS Certified Amber glass vials certified vials
 (Part No. 600000669CV) and presented for analysis.

LC conditions

| LC system: | ACQUITY UPLC |
|-------------------|--|
| Runtime: | 10 min |
| Column: | ACQUITY UPLC BEH C ₁₈ Column 1.7 μm, 2.1 x 100 mm |
| Mobile phase A: | 98:2 H ₂ 0 : MeOH + 0.1% HCOOH |
| Mobile phase B: | MeOH + 0.1% HCOOH |
| Flow rate: | 0.5 mL/min |
| Injection volume: | 100 μL full loop |
| Load ahead: | Enabled |

| | Flow rate | | | |
|------------|---------------|-----------|-----------|--|
| Time (min) | <u>mL/min</u> | <u>%A</u> | <u>%B</u> | |
| 0.00 | 0.5 | 90 | 10 | |
| 0.25 | 0.5 | 90 | 10 | |
| 7.75 | 0.5 | 2 | 98 | |
| 8.50 | 0.5 | 2 | 98 | |
| 8.51 | 0.5 | 90 | 10 | |

MS conditions

| MS system: | Xevo TQ-S | | | | |
|--------------------|---|--|--|--|--|
| lonization mode: | ESI positive | | | | |
| Capillary voltage: | 0.6 kV | | | | |
| Source temp: | 150 °C | | | | |
| Desolvation gas: | 1200 L/h | | | | |
| Desolvation temp: | 650 °C | | | | |
| Cone gas flow: | 150 L/h | | | | |
| Acquisition mode: | Multiple Reaction Monitoring (MRM) with RADAR™ enabled | | | | |

Quanpedia[™]- generated MRM parameters (full MRM list can be found in Appendix 1) were used as the basis of RADAR-enabled mass spectrometer acquisition method. RADAR is an information-rich acquisition approach that allows measurement of target analytes with precision in MRM mode, while simultaneously scanning the background for all other components.

Figure 1 shows a RADAR enabled mass spectrometer acquisition method with time scheduled MRMs for target pesticides and a simultaneous full scan (MS2) acquisition.

MRM table in Appendix



Figure 1. RADAR-enabled mass spectrometer acquisition method with time scheduled MRMs for target pesticides and a simultaneous full scan (MS2) acquisition.

Data processing

MassLynx[™] Software v.4.1 was used for instrument control, and data acquisition. Data were processed using various MassLynx tools, and quantitation was performed using TargetLynx[™] Application Manager.

RESULTS AND DISCUSSION

Sample throughput

Direct injection of drinking water samples onto the Xevo TQ-S eliminated the need for sample preparation prior to analysis. Direct injection was performed using a standard ACQUITY UPLC System with standard 2-mL ACQUITY UPLC autosampler vials.

Rapid ACQUITY UPLC separations allowed a high-throughput analysis with all analytes of interest eluting before 7.5 min and a total runtime of 10 min for each sample. Separations of 81 typically analyzed pesticides were performed and an overlaid MRM total ion chromatogram is shown in Figure 2. The ACQUITY UPLC System was operated with load ahead enabled. This allows for the next sample to be ready to inject immediately after the previous sample has completed, which helps optimize instrument efficiency.

The simplicity of this approach enabled the full analysis of just over five samples per hour and over 80 samples in a typical overnight run.



Figure 2. Overlaid MRM chromatograms of 81 pesticides analyzed using direct injection of a drinking water sample fortified at 100 ng/L.

Ultra-sensitive pesticide detection

The EU drinking water directive (98/83/EC) lists quality standards for drinking water that are among the strictest in the world. This directive sets total pesticide contamination at a maximum of 500 ng/L and 100 ng/L for individual pesticides.⁵

Detection of pesticides to extremely low concentrations was achieved using direct injection Xevo TQ-S. This level of sensitivity allows detection of pesticides to parts-per-quadrillion (ppq) or pg/L. Figure 3 shows detection of a selection of pesticides in a water sample spiked at 200 ppq (pg/L). This is 500 times below the EU requirement.

The capability to measure to this level allows real background concentrations of contaminants to be observed and monitored. This can facilitate trending in sample points and batches and allows a better understanding of final product quality.

[APPLICATION NOTE]





In addition, increased sensitivity allows high confidence when reporting data around the regulatory limits. Increased signal (peak) sizes allow more comfortable measurements at lower concentrations, which translate to higher quality analyses.

Linearity and precision

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External calibration (7 point with replicates at each point) of target analytes was performed at concentrations around the common regulatory level for pesticides (100 ng/L). Good linearity was achieved for all compounds analyzed with typical coefficient of determinations (r2) of > 0.995. Calibration curves for diuron, desmetryn, imidacloprid and dicrotphos are shown in Figure 4.



Figure 4. TargetLynxgenerated calibration curves for diuron, imidacloprid, desmetryn, and dicrotophos.

The ability to measure with high precision at ultra-trace levels is a critical requirement for making high-quality determinations, as well as for observing trends in samples and sample batches. It also instills a higher degree of confidence in reported results.

The removal of variables that were introduced during sample preparation, combined with the precision of the ACQUITY UPLC coupled with Xevo TQ-S allowed very precise measurements in drinking water. Peak area precision was tested at 100 ng/L fortified QC samples over 32 injections. Table 2 shows peak area precision for different compounds from a variety of pesticide types in these QC samples. Figure 5 shows a TrendPlot[™]- generated chart showing peak area precision of fenuron, simazine, and flumeturon from the same set of QC samples. TrendPlot is a data analysis tool that can be used to examine TargetLynx datasets to show trends and outliers in and between batches.

| Class | Compound | %RSD (n=32) | |
|----------------------------------|-------------------|-------------|--|
| | Ametryn | 1.39 | |
| | Terbutryn | 1.96 | |
| Triazine herbicides | Cyanazine | 1.26 | |
| | Atrazine | 1.46 | |
| | Simetryn | 1.78 | |
| | Spiroxamine | 1.85 | |
| | Kresoxim Methyl | 4.29 | |
| Fungicides | Azoxystrobin | 2.19 | |
| | Dimethomorph | 4.14 | |
| | Pyraclostrobin | 4.18 | |
| | Chlortoluron | 0.59 | |
| | Siduron | 1.24 | |
| Phenylurea herbicides | Monuron | 1.56 | |
| i nengarea nerbietaes | Monolinuron | 1.09 | |
| | Diuron | 1.24 | |
| | Dicrotophos | 0.94 | |
| | Heptenophos | 1.47 | |
| Organophosphorous pesticides | Mevinphos | 2.34 | |
| | Tetrachlorvinphos | 2.47 | |
| | Chlorfenvinphos | 3.67 | |
| | Omethoate | 1.22 | |
| | Demeton S Methyl | 1.50 | |
| Organothiophosphorous pesticides | Azinphos Methyl | 2.48 | |
| | Dimethoate | 2.27 | |
| | Ethoprophos | 2.31 | |
| L | Mean | 2.04 | |

Table 2. Peak area precision data for 32 injections of 100 ng/L QC samples showing different compound from a variety of pesticide classes.



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Figure 5. TrendPlot generated chart showing peak area precision of fenuron, simazine, and flumeturon for 100 ng/L QC samples.

Background matrix monitoring using RADAR-enabled MS methods

The simultaneously acquired full scan data (using RADAR-enabled MRM method) allowed observation of the matrix challenge for every individual sample injected. This information can help identify areas of potential ion suppression, observe untargeted contaminants, and aid in the development of further cleanup and matrix reduction strategies. It can also help track method cleanup efficiency, as well as any changes in sample matrix that may occur as different batches of samples are analyzed.

With simultaneous full scan capability matrix components that co-elute with MRM target analytes can be investigated by interrogating the "always available" spectral data. Figure 6 shows RADAR-enabled MS acquisition of drinking water sample spiked at 100 ng/L. Light blue colored chromatogram is MS2 base peak intensity (BPI) full scan chromatogram. Also shown overlaid is a selection of simultaneously acquired pesticide MRMs. The mass spectrum (Figure 6 inset) shows intense back-ground ions of a component that elutes over a broad region in the chromatogram. This co-elutes with a target analyte and highlights a component that may cause some matrix effect.



Figure 6. RADAR-enabled MS acquisition of drinking water sample spiked at 100 ng/L. Light blue colored chromatogram is MS2 fullscan BPI with spectrum from a region of co-elution (inset). Also shown overlaid is a selection of simultaneously acquired pesticide MRMs.

CONCLUSIONS

Using direct injection on Xevo TQ-S removes sample preparation and enables a simple, high-throughput analysis of pesticides in drinking water.

This is possible with ultra-sensitive detection down to ppq or pg/L concentrations to enable real background concentrations in samples to be observed.

Ultra-sensitivity facilitates a high-quality analysis with high precision and comfortable quantitation around the regulatory concentrations. This in turn instills confidence in the data reported.

The RADAR mode of acquisition enables the collection of spectral information on background components in the sample matrix while simultaneously collecting MRM data. This can help identify areas of potential ion suppression, observe untargeted contaminants, and aid in the development of matrix reduction strategies.

The capabilities and performance characteristics of the Xevo TQ-S provide an efficient, high-quality output for laboratories that perform pesticide analysis in drinking water. With less time required for sample preparation and troubleshooting extraction procedures more time can be devoted to reporting higher quality data. This can ultimately lead to an improvement in laboratory performance which in turn translates to more success with business activities that are reliant on the laboratory.

References

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APPENDIX 1 PESTICIDE MRM PARAMETERS

| | Precursor ion | Product ion | Collison (V) | | Precursor ion | Product ion | Collison (V) |
|--------------------------|---------------|-------------|--------------|-------------------|---------------|-------------|--------------|
| Acephate | 206 206 | 64 117 | 10 12 | Imazapyr | 262 262 | 69 86 | 24 24 |
| Acetamiprid | 223 223 | 56 126 | 28 12 | Imazaquin | 312 312 | 86 267 | 26 18 |
| Aldicarb | 213 | 89 | 14 | Imidacloprid | 256 | 175 | 18 |
| Ametryn | 213 | 68 | 15 | Isoproturon | 207 | 46 | 14 |
| Atrazine | 228 | 96 | 34 | Isoxaben | 333 | 107 | 20 56 |
| | 216 188 | 174 79 | 16 21 | Karanda Matal | 333 336 | 165 229 | 16 15 |
| Atrazine-desemyi | 188 | 146 79 | 17 | Kresoxim Metnyl | 336 | 246 | 15 15 |
| Atrazine-desisopropyl | 174 | 96 | 15 | Linuron | 249 | 182 | 15 |
| Azamethiphos | 325 325 | 112 | 16 | Malaoxon | 315 315 | 127 | 11 |
| Azinphos Ethyl | 368 368 | 132 160 | 22 35 | Metalaxyl | 280 280 | 192 220 | 16 12 |
| Azinphos Methyl | 340 340 | 132 160 | 15 10 | Metamitron | 203 203 | 104 175 | 20 15 |
| Azoxystrobin | 404 | 329 372 | 15 10 | Methamidophos | 142 | 94 125 | 12 |
| Buturon | 237 | 84 | 28 | Metobromuron | 259 | 148 | 14 |
| Cadusafos | 237 | 126 | 14 | Metosulam | 418 | 140 | 50 |
| | 271 202 | 159 117 | 28 20 | Mantakar | 418 225 | 175 127 | 26 |
| Carbary | 202 | 145 182 | 15 22 | Mevinphos | 225 | 193 99 | 9 |
| Chlorbromuron | 293 | 204 | 12 | Monolinuron | 215 | 126 | 20 |
| Chlorpyrifos | 350 350 | 97 198 | 20 | Monuron | 199 | 126 | 23 |
| Chlorpyrifos Methyl | 322 322 | 125 290 | 25 15 | Omethoate | 214 214 | 125 183 | 20 10 |
| Chlortoluron | 213 213 | 46 72 | 15 15 | Parathion | 292 292 | 236 264 | 12 10 |
| Clodinafop-propargyl | 350 350 | 91 266 | 15 16 | Phoxim | 299 299 | 129 | 15 7 |
| Coumaphos | 363 | 289 | 30 | Pirimiphos-ethyl | 334 | 182 | 23 |
| Cyanazine | 241 | 96 214 | 22 | Pirimiphos-methyl | 306 306 | 108 | 30 |
| Cyromazine | 167 | 60 108 | 23 | Prometon | 226 | 86 184 | 26 |
| Demeton S Methyl | 253 253 | 61 | 17 | Propaquizafop | 444 | 100 | 15 |
| Demeton S methyl sulfone | 263 263 | 121 | 28 | Pymetrozine | 218 218 | 79 | 28 |
| Desmetryn | 214 214 | 82 172 | 28 19 | Pyraclostrobin | 388 388 | 163 194 | 23 11 |
| Dicrotophos | 238 | 112 | 10 | Pyrazophos | 374 | 194 | 30 20 |
| Difenoxuron | 238 | 72 | 18 | Quinmerac | 222 | 141 | 28 |
| Diflubenzuron | 311 | 123 | 30 | Ouizalofop-ethul | 373 | 91 | 30 |
| Dimefuron | 311 339 | 158 72 | 15 24 | Siduron | 373 233 | 299 94 | 16 23 |
| | 339 230 | 167 125 | 18 | | 233 202 | 137 96 | 15 22 |
| | 230 388 | 199 165 | 10 28 | Simazine | 202 | 124 96 | 16 23 |
| Dimethomorph | 388 | 301 | 18 | Simetryn | 214 | 124 | 18 |
| Disulfoton | 297 | 89 | 12 | Spiroxamine | 298 | 144 | 19 |
| Diuron | 233 233 | 46 72 | 13 | Sulfotep | 323 323 | 97 171 | 30 14 |
| Ethoprophos | 243 243 | 97 131 | 29 18 | Tebuthiuron | 229 229 | 116 172 | 24 16 |
| Fenuron | 165 165 | 46 72 | 13 15 | Terbuthylazine | 230 230 | 96 174 | 26 15 |
| Flamprop-methyl | 336 336 | 77 105 | 46 15 | Terbutryn | 242 242 | 186 200 | 15 15 |
| Fluazafop-P-butyl | 384 | 282 | 20 | Tetrachlorvinphos | 365 | 127 | 15 |
| Flufenacet | 364 364 | 152 | 18 | Thiabendazole | 202 202 | 131 | 26 |
| Fluomethuron | 233 | 46 | 16 | Trietazine | 230 | 71 | 28 |
| Heptenophos | 251 | 125 | 13 | Zoxamide | 336 | 159 | 36 |
| Hexazinone | 251 | 71 | 28 | | 330 | 181 | 23 |
| | 253 | 171 | 15 | | | | |