

Direct aqueous analysis of pesticides and PPCPs in drinking and bottled water at parts per trillion levels

Using the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready

Jianru Stahl-Zeng¹, Bertram Nieland⁴, Phil Taylor², Jack Steed², Ian Moore³, Ronny Bosch⁵, Bernard Bajema⁵

¹SCIEX, Germany, ²SCIEX, United Kingdom, ³SCIEX, Canada, ⁴SCIEX, Netherlands, ⁵Vitens, Netherlands

Drinking water analysis is subjected to extremely low and rigid prescribed consent values (PCV) for determinands. Water suppliers and utility companies need to ensure that the final water product they send out for bottling or into supply networks is safe and complies with state and country regulations.^{1,2,3} Water can be a challenging matrix, in particular if it is sourced from a river, lake or reservoir before entering the water treatment process. The laboratories which serve the water companies must have highly sensitive instrumentation to quantify beyond PCV limits with precision and confidence and also handle all the matrix challenges.

Here, a series of experiments was conducted in collaboration with Vitens Water Company, to test several types of water samples spiked with a mixed standard of 431 determinands, including pesticides, pharmaceuticals and personal care products. This work explores the sensitivity levels of the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready, the reproducibility of the acquired data as well as the simplicity of data processing using SCIEX OS Software.



Key features of the SCIEX 7500 System for drinking water analysis

- Very high sensitivity demonstrated using the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready for sub part per trillion detection limits for analytes in drinking water
- Includes improvements in the generation of ions with the OptiFlow® Pro Ion Source and the E Lens™ Technology and the capture of ions with the D Jet™ Ion Guide¹⁸
- Very simple sample preparation strategy of direct aqueous injection greatly streamlines the water analysis workflow
- SCIEX OS Software is a single platform for data acquisition, data processing and streamlined results review
- QTRAP Ready system future proofs the lab with an easy upgrade path to obtain the qualitative functionality of the linear ion trap scan features in addition to the quantitative triple quadrupole functionality

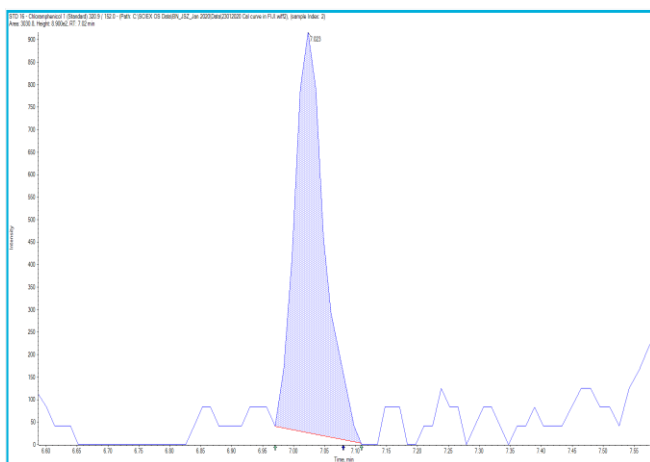


Figure 1. Chloramphenicol spiked in Fiji mineral water at 0.1ng/L. This direct aqueous injection analysis removes the need for laborious sample preparation.

Methods

Sample preparation: Direct aqueous injection of spiked water samples - MilliQ water, tap water (SCIEX Concord Laboratory), Evian bottled water and Fiji bottled water. The samples were spiked with a custom mix standard provided by Vitens, Netherlands, containing 431 analytes, include 46 internal standards. A series of 12 standards were made in the respective matrices at sequential concentrations from 0.1 to 500 ng/L. A collection of unknown blank samples was also included in this study.

Liquid chromatography: HPLC separation was performed on an ExionLC™ System using a Phenomenex Luna Omega C18 column (100 Å, 1.6 µm, 100 x 2.1 mm P/N 00F-4742-AN). The gradient used for the separation is outlined in Table 1. The column oven temperature was set to 40°C. For this experiment, the selected injection volume was 500 µL.

Table 1. Gradient profile and mobile phase composition.

Total Time (min)	Flow rate (µL/min)	% A	% B
0.00	500	99	1
0.50	500	99	1
16.00	500	1	99
21.00	500	1	99
21.10	500	99	1
25.00	500	99	1

Mobile phase A - Water + 0.1% formic acid + 5mM NH₄ Formate
Mobile phase B - Methanol + 0.1% formic acid + 5mM NH₄ Formate

Mass spectrometry: The SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready was equipped with the OptiFlow Pro Ion Source and operated in conventional high flow ESI mode. To increase the efficiency in the transfer of the sample into the mass spectrometer, the E Lens Technology was also used. Due to the nature of this acquisition method, the system was configured for positive and negative analysis with a fast polarity switching time of five milliseconds (ms).

The acquisition method with 431 determinands was created in SCIEX OS Software, using the Scheduled MRM™ Algorithm to maximize the number of analytes in a single injection. Each determinand had two MRM transitions. Standards and spiked samples were injected multiple times to conduct a reproducibility and accuracy study.

Data analysis: The Explorer and Analytics tools in SCIEX OS Software were used to review the acquired sample data.

Table 2. MS Method parameter settings

Parameter	Setting	Parameter	Setting
Acquisition mode	MRM	Curtain gas	32 psi
Ionization mode	ESI	Ion source gas 1	30 psi
Polarity	positive and negative	Ion source gas 2	70 psi
CAD gas	9	Source temperature	500°C

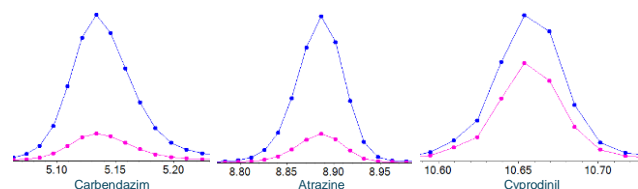
Identification and integration of the peaks were achieved with the Auto Peak Algorithm. Improvements in the recognition and baseline integration of peaks expedited data review and reduced the need for manual adjustments.

Method development and optimization

A custom mix of 431 pesticides, pharmaceuticals and personal care products in acetonitrile was prepared by Vitens water laboratory to prepare a sequential series of calibration standards in MilliQ water. The calibration standard concentrations were 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 and 500 ng/L. A calibration blank was also prepared.

Store-bought bottles of Fiji Water, Evian Water and tap water from SCIEX Concord Laboratory were spiked with varying concentrations within the calibration range of 0.1 to 500 ng/L. Chromatography gradients and conditions along the SCIEX 7500 System were optimized to enable adequate data points across all peaks (Table 1 and 2).

XIC Data Points – Positive Mode



XIC Data Points – Negative Mode

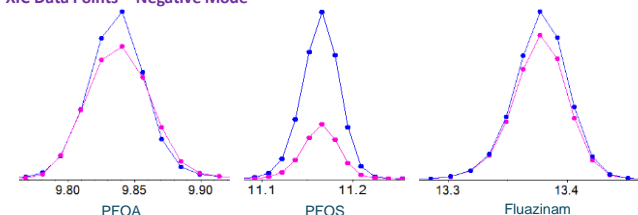


Figure 2. Fast polarity switching ensures high data quality. The method was optimized to ensure there were sufficient data points across the peak to quantify the determinands and meet regulatory requirements. This figure shows the extracted ion chromatogram (XIC) positive determinands of carbendazim, atrazine and cyprodinil at 50 ppt. The negative XIC determinands are PFOA, PFOS and fluzinam.

Due to the broad range of compounds analyzed in this study, polarity switching was used to maximize compound coverage. The speed of polarity switching ensured that very good peak sampling was obtained for all analytes, with sufficient data points across the LC peak (Figure 2, 50 ng/L). The two fungicides shown in the positive mode are carbendazim and cyprodinil. Carbendazim⁴ is a benzimidazole fungicide used across fruit, vegetable and cereals. Cyprodinil⁵ is for the treatment of rhynchosporium outbreaks on crops. Atrazine⁶ is a weed killer that has been used in corn farming.

Negative mode compounds perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are perfluoroalkyl and polyfluoroalkyl substances (PFAS).⁷ These compounds are used in industrial applications such as cookware manufacturing, the upholstery industry and firefighting. The fungicide fluazinam⁸, used in the farming of potatoes, peanuts and grapevines, was also selected.

Very good chromatographic separation was observed (Figure 3), which also helps ensure good quantitative coverage.

Calibration curves

A series of calibration curves were prepared in MilliQ water to evaluate the sensitivity of the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready and determine the lower limits of quantification. To enable maximum coverage of analytes in the samples, a calibration range from 0.1 ng/L to 500ng/L was spiked into MilliQ water. Using SCIEX OS Software for data processing, calibration curves were quickly generated and supporting statistical data was summarized for quick review (Figure 4 and 5). The ion ratio confidence column shows that all concentrations passed, with no non-conforming ratios flagged. Strong linearity was observed for most compounds. Furthermore, SCIEX OS Software will also flag any erroneous calibrators and automatically remove the standards for regression.

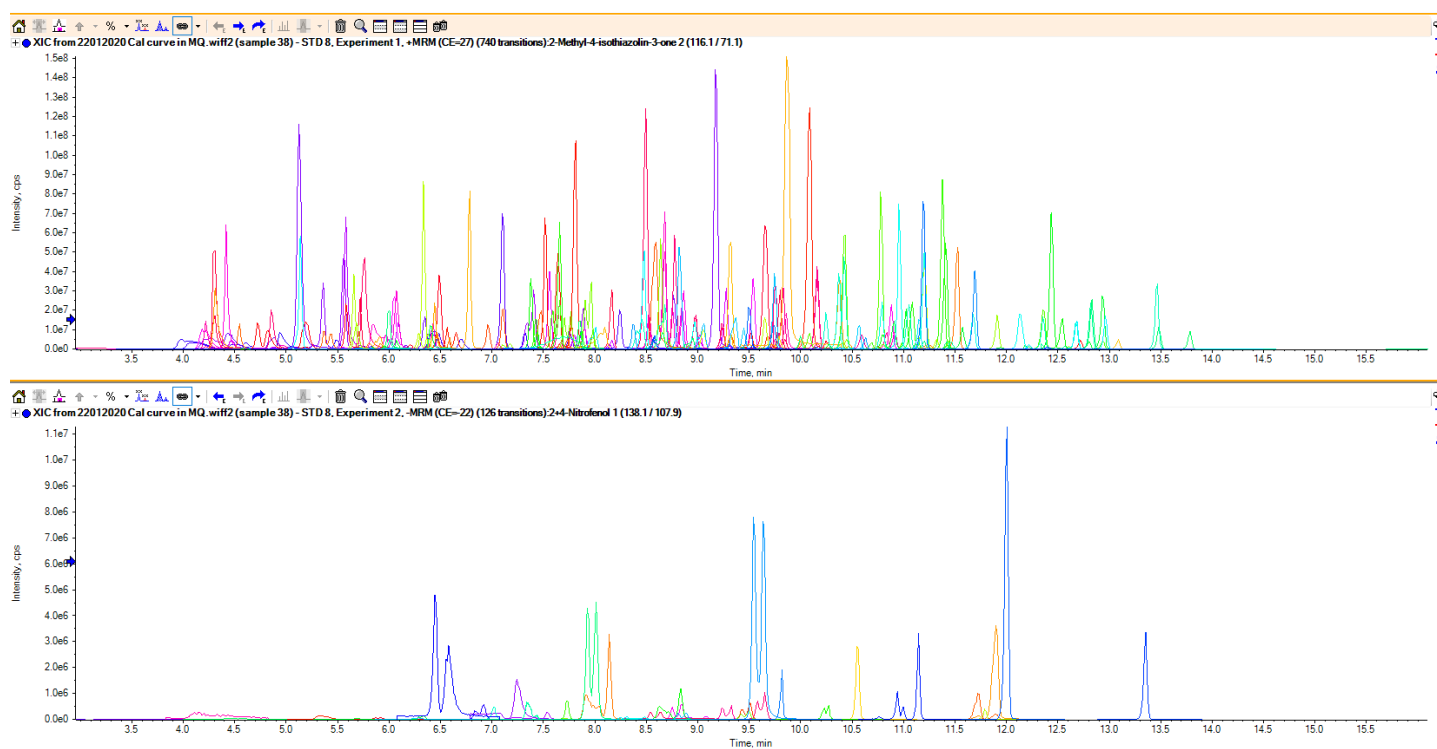
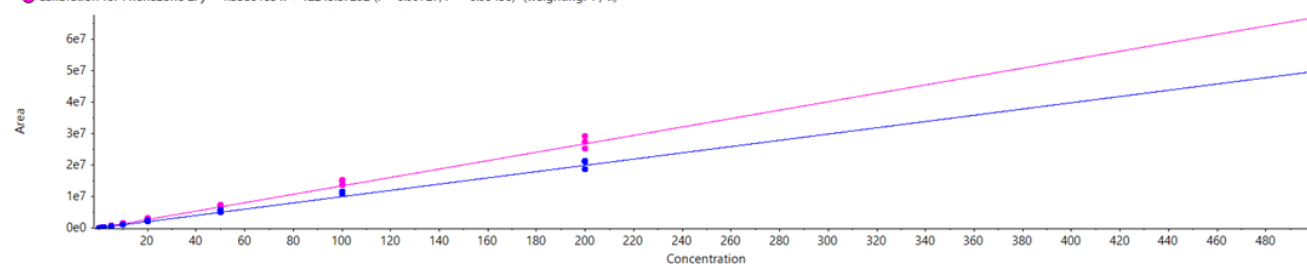


Figure 3. All 431 compounds optimized for analysis with fast positive and negative switching. Extracted ion chromatograms (XIC) show the positive mode compounds (top) and the negative mode compounds (bottom). Excellent separation was achieved.



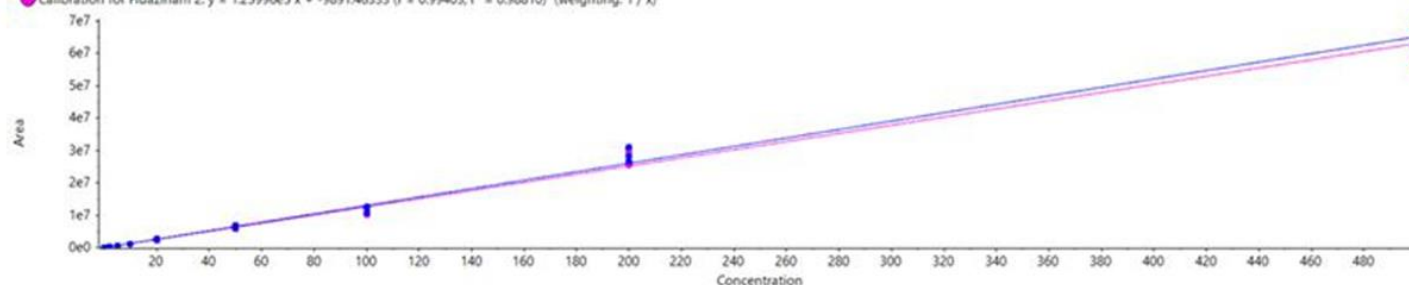
● Calibration for Phenazone 1: $y = 9.95424e4 x + 8444.26460$ ($r = 0.99688$, $r^2 = 0.99376$) (weighting: $1/x$)
● Calibration for Phenazone 2: $y = 1.33801e5 x + 12245.87292$ ($r = 0.99727$, $r^2 = 0.99456$) (weighting: $1/x$)



Index	Sample Name	Sample Type	Component Name	Component Type	Component Group Name	Actual Concentration	Area	Retention Time	Signal / Noise	Used	Calculated Concentration	Ion Ratio	Ion Ratio Confidence
923	STD 16	Standard	Phenazone 1	Quantifiers	Phenazone	0.10	21275.8	6.16	11.3	<input checked="" type="checkbox"/>	4.565e-2	0.89	✓
17...	STD 15	Standard	Phenazone 1	Quantifiers	Phenazone	0.20	37733.9	6.15	28.2	<input checked="" type="checkbox"/>	1.687e-1	0.67	✓
26...	STD 14	Standard	Phenazone 1	Quantifiers	Phenazone	0.50	67716.0	6.17	27.3	<input checked="" type="checkbox"/>	3.928e-1	0.88	✓
35...	STD 13	Standard	Phenazone 1	Quantifiers	Phenazone	1.00	154528.1	6.17	44.9	<input checked="" type="checkbox"/>	1.042e0	0.77	✓
43...	STD 12	Standard	Phenazone 1	Quantifiers	Phenazone	2.00	335476.6	6.17	118.5	<input checked="" type="checkbox"/>	2.395e0	0.67	✓
52...	STD 11	Standard	Phenazone 1	Quantifiers	Phenazone	5.00	725149.5	6.17	438.0	<input checked="" type="checkbox"/>	5.308e0	0.73	✓
61...	STD 10	Standard	Phenazone 1	Quantifiers	Phenazone	10.00	1573538.9	6.17	295.3	<input checked="" type="checkbox"/>	1.165e1	0.71	✓
69...	STD9	Standard	Phenazone 1	Quantifiers	Phenazone	20.00	2957920.5	6.18	627.1	<input checked="" type="checkbox"/>	2.200e1	0.70	✓
78...	STD 8	Standard	Phenazone 1	Quantifiers	Phenazone	50.00	7441689.9	6.15	1533.3	<input checked="" type="checkbox"/>	5.552e1	0.76	✓
87...	STD 7	Standard	Phenazone 1	Quantifiers	Phenazone	100.00	14790475.2	6.18	2688.3	<input checked="" type="checkbox"/>	1.105e2	0.72	✓
95...	STD 6	Standard	Phenazone 1	Quantifiers	Phenazone	200.00	27399055.8	6.17	5610.4	<input checked="" type="checkbox"/>	2.047e2	0.77	✓
10...	STD 5	Standard	Phenazone 1	Quantifiers	Phenazone	500.00	64296556.3	6.16	10642.9	<input checked="" type="checkbox"/>	4.805e2	0.73	✓

Figure 4. Example of calibration curve and ion ratio confirmation for phenazone in positive ion mode. SCIEX OS Software displays all the data involved in the creation of the calibration curve. The user can review and quickly identify any issues using the flagging tools. In this instance, the ion ratio confidence scores meet the satisfactory level for typical water regulatory analysis.

● Calibration for Fluazinam 1: $y = 1.30089e5 x + -9811.98288$ ($r = 0.99511$, $r^2 = 0.99025$) (weighting: $1/x$)
● Calibration for Fluazinam 2: $y = 1.25996e5 x + -9091.46333$ ($r = 0.99403$, $r^2 = 0.98810$) (weighting: $1/x$)



Index	Sample Name	Sample Type	Component Name	Component Type	Component Group Name	Actual Concentration	Area	Retention Time	Signal / Noise	Used	Calculated Concentration	Ion Ratio	Ion Ratio Confidence
17...	STD 16	Standard	Fluazinam 1	Quantifiers	Fluazinam	0.10	10828.6	13.35	60.8	<input checked="" type="checkbox"/>	1.587e-1	0.93	✓
25...	STD 15	Standard	Fluazinam 1	Quantifiers	Fluazinam	0.20	24399.6	13.34	98.8	<input checked="" type="checkbox"/>	2.630e-1	1.01	✓
34...	STD 14	Standard	Fluazinam 1	Quantifiers	Fluazinam	0.50	50224.7	13.35	406.8	<input checked="" type="checkbox"/>	4.615e-1	0.99	✓
43...	STD 13	Standard	Fluazinam 1	Quantifiers	Fluazinam	1.00	97459.0	13.36	1174.9	<input checked="" type="checkbox"/>	8.246e-1	0.92	✓
51...	STD 12	Standard	Fluazinam 1	Quantifiers	Fluazinam	2.00	244137.1	13.37	2714.3	<input checked="" type="checkbox"/>	1.952e0	0.90	✓
60...	STD 11	Standard	Fluazinam 1	Quantifiers	Fluazinam	5.00	572460.2	13.36	7434.7	<input checked="" type="checkbox"/>	4.476e0	0.89	✓
69...	STD 10	Standard	Fluazinam 1	Quantifiers	Fluazinam	10.00	1202552.8	13.35	9328.0	<input checked="" type="checkbox"/>	9.320e0	0.91	✓
77...	STD9	Standard	Fluazinam 1	Quantifiers	Fluazinam	20.00	2785341.8	13.36	14319.1	<input checked="" type="checkbox"/>	2.149e1	0.94	✓
86...	STD 8	Standard	Fluazinam 1	Quantifiers	Fluazinam	50.00	6745866.4	13.34	23155.2	<input checked="" type="checkbox"/>	5.193e1	0.99	✓
95...	STD 7	Standard	Fluazinam 1	Quantifiers	Fluazinam	100.00	12697634.5	13.37	46072.0	<input checked="" type="checkbox"/>	9.768e1	0.97	✓
10...	STD 6	Standard	Fluazinam 1	Quantifiers	Fluazinam	200.00	31008866.0	13.37	49666.8	<input checked="" type="checkbox"/>	2.384e2	0.98	✓
11...	STD 5	Standard	Fluazinam 1	Quantifiers	Fluazinam	500.00	70438403.0	13.36	145466.6	<input checked="" type="checkbox"/>	5.415e2	0.95	✓

Figure 5 Calibration curve and ion ratio for fluazinam analyzed in negative mode. SCIEX OS Software displays all the data involved in the creation of the calibration curve. The operator can review and quickly identify any issues through the flagging tool. Here, the ion ratio confidence meets the satisfactory level for typical water regulatory analysis.

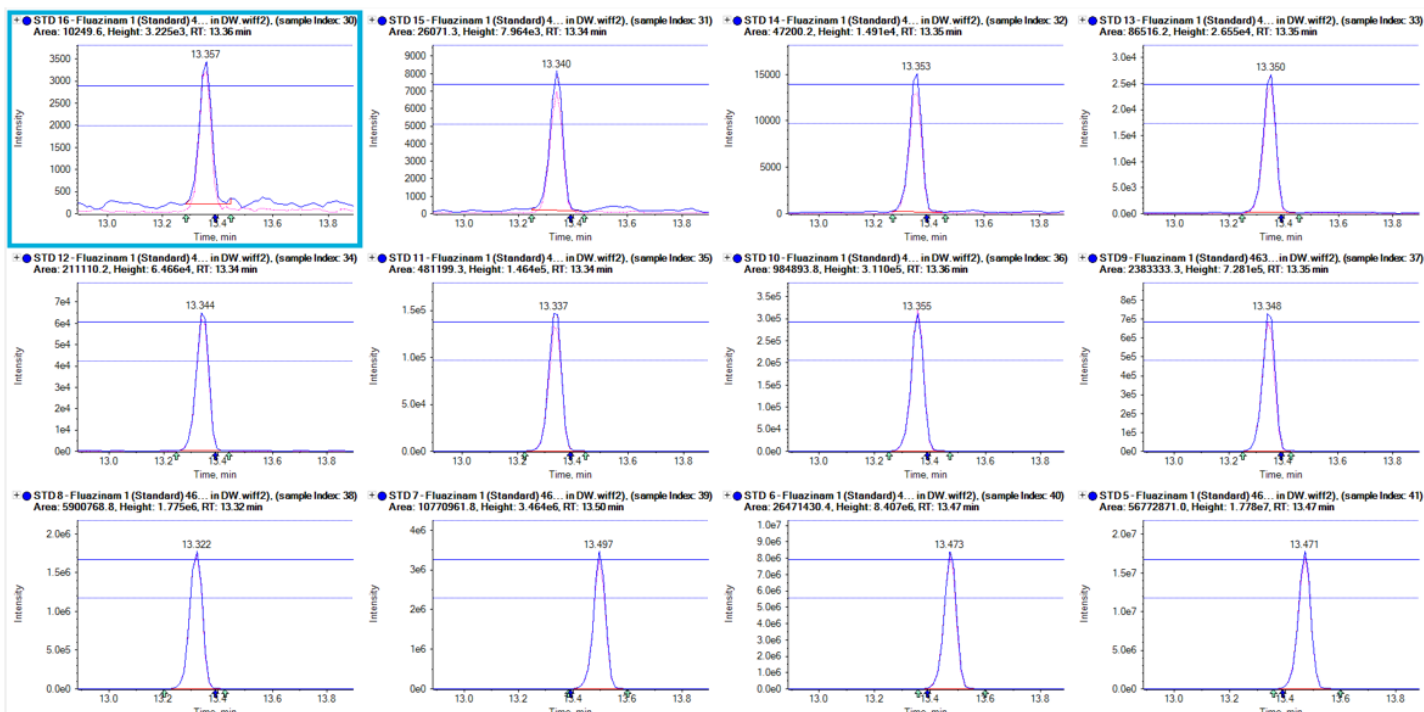


Figure 6. Extracted ion chromatography (XIC) for fluazinam in negative ion mode from 0.1 to 500 ng/L. As can be observed in the above figure, SCIEX OS Software is capable of easily and clearly displaying overlays of both quantifier ion chromatography as well as showing the tolerance in the form of ion ratio lines to visually depict a pass or failed chromatogram.

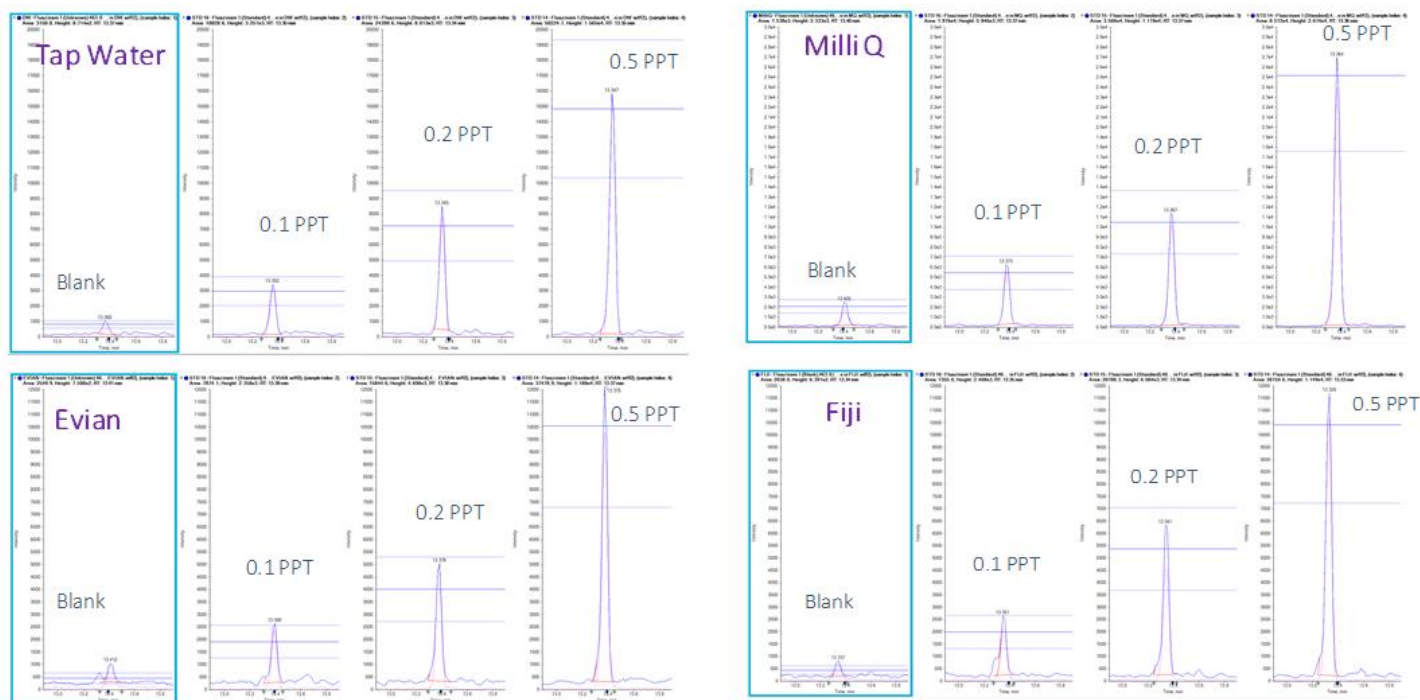


Figure 7. Fluazinam spiked into tap water, MilliQ water, and both Evian and Fiji bottled waters at low concentrations. MRM peak areas for the blanks as well as concentrations from 0.1 to 0.5 ppt are shown using Analytics in SCIEX OS Software, showing both the quantifiers and qualifiers for ion ratio confirmation. Very small peak in the blank indicates contamination of this compound and also indicates the sensitivity level of the system.

A new era of sensitivity

Very good sensitivity was observed across the series of experiments conducted in this study. Using the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready, which includes improvements in both ion generation and ion sampling, detection limits of 0.1 ng/L were achieved for many of the analytes. Figure 8 shows the data for bottled water spiked with very low levels of concentrations of six different compounds compared with blank bottled water. All data were acquired through direct aqueous injection.

Figure 8 demonstrates sub-part per trillion limits of detection with direct aqueous injection. Directly testing a water sample reduces the costs and time demands associated with having to perform more laborious sample preparation approaches such as concentrating the sample with solid phase extraction (SPE). Furthermore, by removing the sample preparation step, there is a reduced risk of cross-contamination, leading to false-positive results.

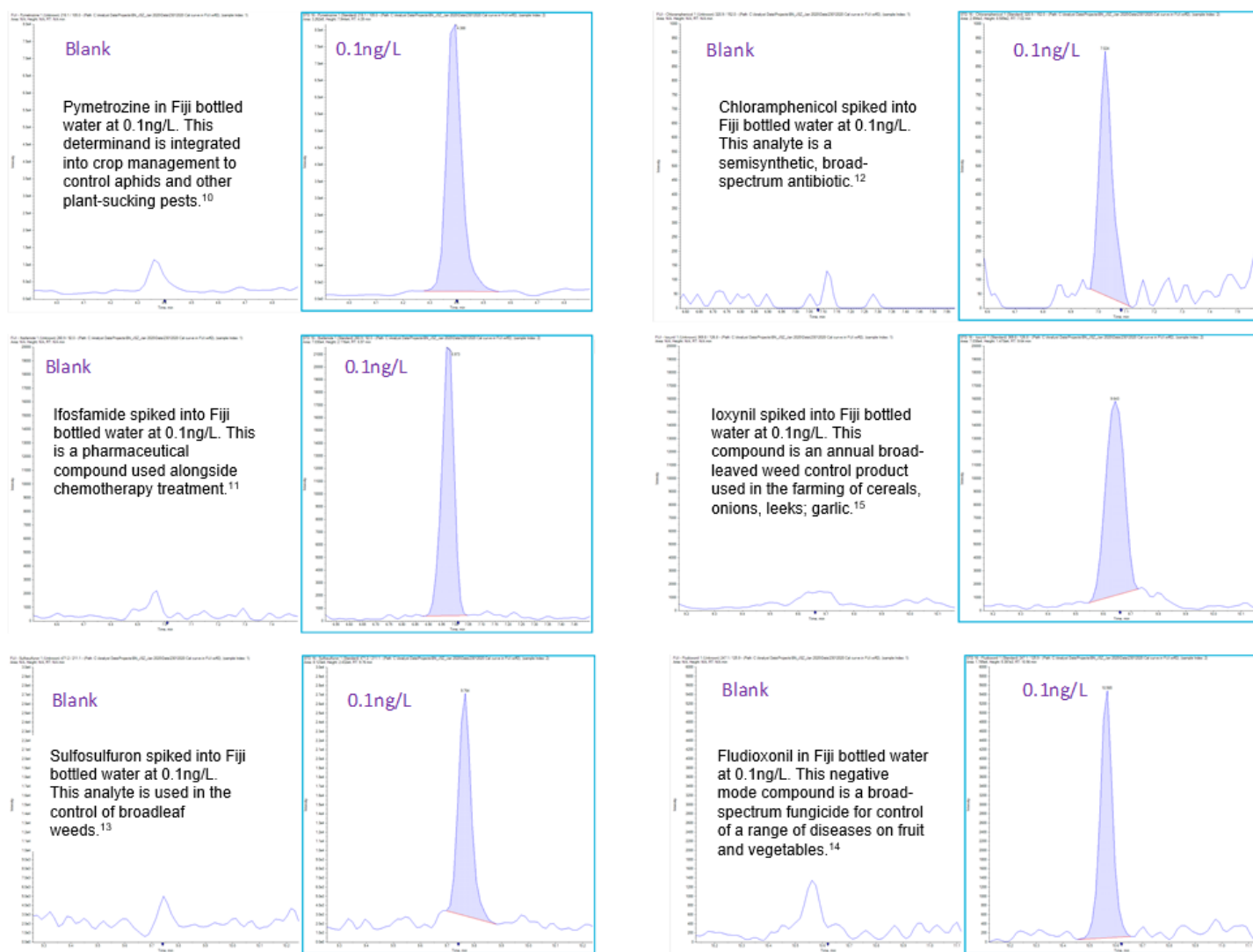


Figure 8. XIC for different compounds spiked into bottled water at 0.1 ng/L. (Left) Analytes analyzed using positive ionization. (Right) Analytes analyzed using negative ionization.

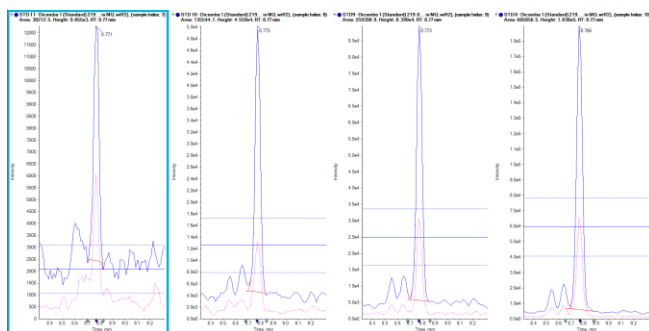


Figure 9. Sensitivity for dicamba analysis. The peak areas observed for dicamba at concentrations of 5, 10, 20 and 50 ng/L with a 500 μ L direct aqueous injection is shown.

Dicamba analysis

One of the more problematic determinands to analyze is dicamba. This herbicide is used in the commercial farming of crops and grains, its purpose is to kill perennial broadleaf weeds. Dicamba is applied to control brush and bracken in grazing pastures as well as legumes and cacti.¹⁷ Dicamba has been found to be highly soluble in water (500 g/L) and is therefore a determinand of concern for water laboratories. Direct aqueous analysis of this analyte is sometimes problematic and reaching low-level parts per trillion detection limits is very difficult.

In this series of experiments dicamba was one of the 431 compounds in the custom mix. Here, a limit of detection (LOD) of 5 ng/L (5 ppt) was observed with one transition of the dicamba MRM transitions, and a limit of quantification (LLOQ) of 10 ng/L (10 ppt) was obtained with 2 transitions with correct ion ratio was achieved. Figure 9 shows the chromatograms for dicamba spiked into MilliQ water at 50 ppt down to 5 ppt. The acquired peaks clearly display the confirmatory ion ratios between the two transitions for confident reporting of results.

Reproducible results

The ability to quantify to a low-level concentration is important, but the ability to consistently deliver this high-quality data time and time again is crucial. Figure 10 provides an example of the reproducibility of a determinand (phenazone) at low levels of concentration, which has been spiked into the sample. Even at the lowest concentrations at a sub-part per trillion, the percentage coefficient variation (%CV) is consistently low and shows a high degree of accuracy and precision. At the LOD for phenazone, the %CV is 6.76% in this example.

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	Phenazone 1	0.10	3 of 3	8.151e-2	5.509e-3	6.76	81.51
2	Phenazone 1	0.20	3 of 3	2.059e-1	3.638e-3	1.77	102.94
3	Phenazone 1	0.50	3 of 3	5.255e-1	3.191e-3	0.61	105.09
4	Phenazone 1	1.00	3 of 3	8.489e-1	5.448e-3	6.42	84.89
5	Phenazone 1	2.00	3 of 3	2.140e0	5.628e-3	2.63	106.99
6	Phenazone 1	5.00	3 of 3	4.345e0	5.723e-3	13.17	86.91
7	Phenazone 1	10.00	3 of 3	1.160e1	9.351e-3	8.06	116.01
8	Phenazone 1	20.00	3 of 3	2.215e1	7.684e-3	3.47	110.76
9	Phenazone 1	50.00	3 of 3	5.484e1	3.838e0	7.00	109.67
10	Phenazone 1	100.00	3 of 3	9.837e1	9.631e0	9.79	98.37
11	Phenazone 1	200.00	3 of 3	1.937e2	7.592e0	3.92	96.85

Figure 10. Reproducibility of the SCIEX 7500 System at a low-level concentration. At sub-parts per trillion, accuracy and precision are satisfactory for regulatory requirements.

Conclusions

In this work, the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready has demonstrated its potential for low-level quantification (parts per trillion detection limits) across a relatively large suite of 431 determinands. The ability to analyze this many compounds without the need for laborious sample preparation improves operational efficiencies. The direct aqueous injection approach enables water testing laboratories to go beyond their current limits of sensitivity. Combined with the ease of processing and flexibility of SCIEX OS Software, the time between sampling and reporting results was expedited through this workflow which can positively impact how the data produced by the labs that monitor the health and purity of the water network.

There is scope to expand the number of analytes tested and apply this system to other individual workflows which demand very low limits of quantification. The innovations of the SCIEX 7500 System can open the water industry up to an era of analysis where the impacts of the matrix studied is reduced while the levels of sensitivity are increased.

References

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18. Enabling new levels of quantification. [SCIEX technical note RUO-MKT-02-11886-A](#).

Acknowledgements

Vitens is a drinking water company based in the Netherlands that wants water to work for people. They try to achieve this by providing outstanding service, useful products and services, and reliable information. The state-of-the-art Vitens laboratory in Leeuwarden carefully monitors the quality of drinking water from the source to their customers. Every day, the laboratory processes hundreds of samples and the analyses are largely automated. Its logistics resources and advanced measuring equipment ensure that high efficiency and high-quality data is generated. The laboratory has implemented a quality management system in compliance with ISO/IEC 17025.

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