APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Method Validation for Atrazine Analysis in Fresh, Atypical Mineral and Carbonated Waters

Introduction

European Union water polices are continuously being reevaluated to determine safe levels of many contaminants in water sources, resulting in the lowering of detection limits by various governments to meet more stringent regulatory requirements. To adapt to the new detection limits, scientists are embracing more sensitive technologies, such as triple quad LC/MS/MS, to ensure compliance. Direct-injection LC/MS/MS techniques are often identified as the best option for the detection of contaminants at ultra-low levels owing to the fast, reliable, repeatable and easy to implement nature of the technique. Analytical results need to be compliant with multiple regulations across Europe, which adds increased complexity. In France, for example, results need to meet the NF EN T90-210 (EU reference ISO/TS 13530:2009) protocol, which includes statistical tests on the calibration and accuracy of the method, while referring to the determination of uncertainties based on the NF-ISO-11352 regulatory standard.

In this application note, a method for the determination of 214 contaminants in various water sources is presented. To achieve the ultra-low detection limits required throughout the European Union, a PerkinElmer QSight® 420 LC/MS/MS with dual (APCI and ESI) ionization sources was utilized. A simple filtration and direct injection process was followed, resulting in excellent recoveries and accuracy for all analytes, demonstrating compliance with ISO standards. In addition, an inter-laboratory study was included to demonstrate the ease of method transferability to other QSight LC/MS/MS systems. The method was validated by detection of atrazine at La Drôme Laboratories, and subsequently submitted for accreditation.

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Experimental

Hardware/Software

Among the 214 existing contaminants analyzed by LC/MS/MS, atrazine, an herbicide banned in the European Union since 2003 (but still present in the environment), was utilized for this method validation study. The 2018 French regulation on water analysis stipulated that accredited labs should be able to quantify atrazine at levels less than or equal to 0.010 µg/L.

Chromatographic separation of the compounds was conducted utilizing a PerkinElmer LX50 UHPLC system. Subsequent analyte determination was achieved with a PerkinElmer QSight 420 triple quadrupole mass detector, utilizing a dual ionization (ESI and APCI) source. All instrument controls, data acquisition and data processing have been performed by using Simplicity 3Q™ Software.

Instrumental Parameters

The UHPLC method and MS ion source parameters are shown in Tables 1 and 2, respectively. The multiple reaction monitoring mode (MRM) transitions of the studied pesticides are shown in Table 3, including both positive and negative analytes. The collision energy of every transition was determined by injection of the solution standard. To facilitate method development, the MS analysis parameters were automatically generated by selecting the pesticides of interest from the built-in, customizable "compound library" in the time-managed-MRM module of the Simplicity 3Q software. Depending on expected peak width, the cycle time of the instrument is set, and dwell times are optimized accordingly by the built-in algorithm.

Standard Preparation

Standards were obtained from Cluzeau (Bordeaux, France). The standard solutions were prepared by dilution in Evian water and acidified with 0.1% formic acid. The calibration line was obtained with 11 points distributed between 0 and 1000 ng/L. QC standard solutions were obtained from Carlo Erba (Peypin, France).

Sample Preparation

Samples were acidified with 0.1% formic acid, and then filtered through a membrane of PTFE 0.45 µm (Whatman, Syringeless filter device) before being injected into the QSight LC/MS/MS instrument. For this analysis validation, the following samples have been used: Valence fresh water, La Véore source water, and groundwater from the Valence region. For the atypical mineral waters, Hépar and Contrex were utilized, and for carbonated waters, Vals, Perrier and Saint-Yorre samples were used.

Note: According to protocol NF EN T-90-210, using a standard addition method, laboratories have to develop methods on several types of water samples. The results indicate that the accuracy of the method is perfectly fitting within the tolerance window.

Table 1. LX 50 UHPLC Parameters.

Mobile Phase	**Solvent A:** Water (UltraPure MilliQ)+ 0.1% formic acid (Biosolve ULC-MS) **Solvent B:** Methanol (Biosolve ULC-MS) + 0.1% formic acid

Step	Time (min)	Flow Rate (mL/min)	%A	%B	Curve
1	Initial	0.5	99.0	1.0	Equil
2	0.5	0.5	99.0	1.0	Step
3	18.0	0.5	30.0	70.0	Linear
4	21.0	0.5	1.0	99.0	Linear
5	23.0	0.5	1.0	99.0	Step
6	23.1	0.5	99.0	1.0	Linear
7	25.0	0.5	99.0	1.0	Step

Analysis Time	Injection to injection: 25 min
Pressure	8800psi Initial
Oven Temp	40 °C
Injection Volume	100 µL

Table 2. QSight ESI Source Parameters.

Ionization Mode	ElectroSpray with Polarity Switching
Drying Gas	120
Nebulizing Gas	300
HSID Temp	400 °C
Voltage	+4000V/-4000V
Source Temp.	400 °C
Mode	Time Managed MRM with 105 experiments

Table 3. Collision parameters for the targeted pesticides.

Polarity	Compound Name	Q1	Q2	Tr (min)	Delta (min)	CE	EV	CC L2
Positive	Atrazine – 1	216.1	174.1	14.67	0.8	-25	13	-56
Positive	Atrazine – 2	216.1	132.1	14.67	0.8	-34	7	-92
Positive	Atrazine D5	221.0	179.0	14.58	0.8	-18	24	-60
Positive	DEDIA 13C3	149.0	70.0	2.38	0.8	-29	13	-40
Positive	DEDIA – 2	145.8	104.0	2.38	0.8	-28	17	-32
Positive	DEDIA – 1	145.8	79.0	2.38	0.8	-24	10	-44
Positive	Metolachlore – 1	284.1	176.0	18.62	0.8	-29	8	-116
Positive	Metolachlore – 2	284.1	252.0	18.62	0.8	-19	20	-124
Negative	Fosethyl – 3	108.2	62.0	0.84	0.8	29	-9	60
Negative	Fosethyl – 4	108.2	80.0	0.84	0.8	18	-15	24
Negative	Fosethyl D5	113.3	62.0	0.84	0.8	45	-18	64

Results

Analytical Challenges for Multi-residue Pesticides

The analysis of multi-residue pesticides presents a number of challenges, including:

- Simultaneous analysis in both positive and negative mode on 214 pesticides with an extended polarity range.
- Ensuring analytical accuracy. Check the non-deterioration of the analytical performances during the sequences with the use of the "direct injection" technique.
- No manual input of MS parameters – automated timed MRM algorithm for optimal dwell times and 8-12 data points across the peak for reliable quantification.
- Easy transfer for MS parameters from one QSight LC/MS/MS system to another (i.e. demo laboratory vs customer site or customer to customer), by simply sharing a list of optimized parameters and copying it into the timed-MRM window.
- Matrix effects tested and evaluated based on fortified matrix calibration curves versus standards in neat solution.
- The need to achieve ultra-low LOQs for compounds of interest, including 10 ng/L for atrazine, 30 ng/L for atrazine aesethyl aisopropyl (DEDIA), 10 ng/L for metolachlor and 30 ng/L for fosethyl.
- To approve healthy drinking water, the uncertainty must be <50% at the LOQ.

Method Performance

Wide Range of Polarity of Targeted Pesticides

230 pesticides were analyzed, including metabolites with polarities ranging from log Kow = -2.7 for fosetyl-Al, to log Kow = 4.05 for prothioconazole. During the method development, several columns were tested. A silica-based bonded phase with C18 functionality was chosen, offering compatibility with aqueous mobile phases, and superior retention and separation for a wider pH range. The method and column described herein demonstrates acceptable performance in the ability to separate both polar and non-polar compounds.

Linearity of Calibration Curves (1-1000 ng/L)

The quantification is based on the usage of an isotopically labelled internal standard, positive and negative. Calibration accuracy was evaluated using a quadratic regression model with 1/x as the weighting factor, which provides the best results in terms of "sample accuracies." The calibration was repeated on several days, and the QC solutions were analyzed every 20 samples to verify the calibration accuracy. At least two MRM transitions, one for the quantifier ion (quant) and one for the qualifier ion (qual), were monitored for each pesticide. LOQs (limits of quantification) were calculated based on a minimum S/N of three for both transitions.

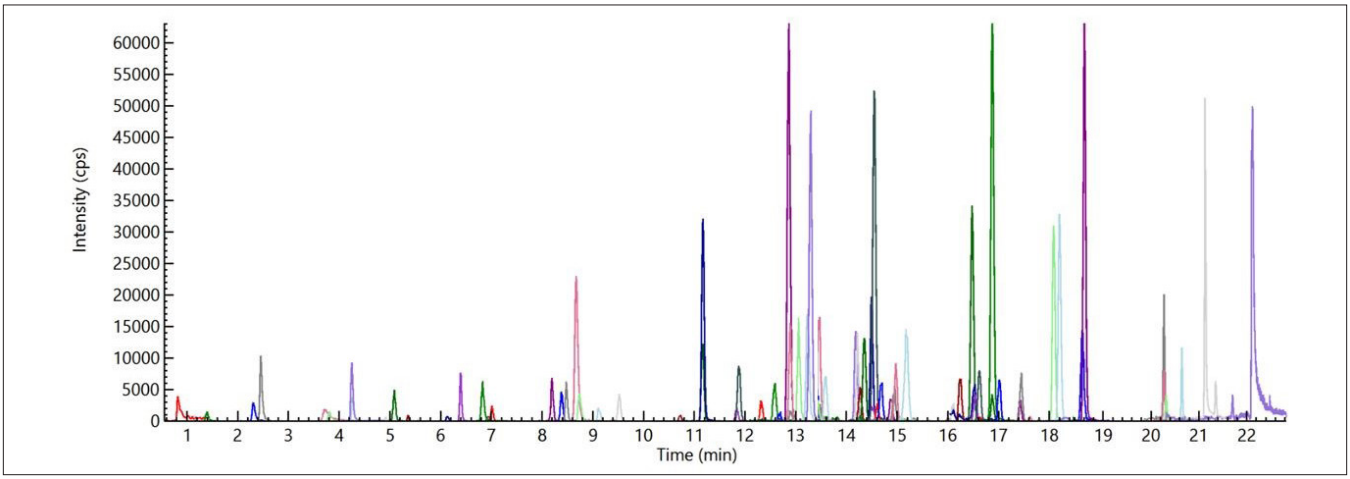
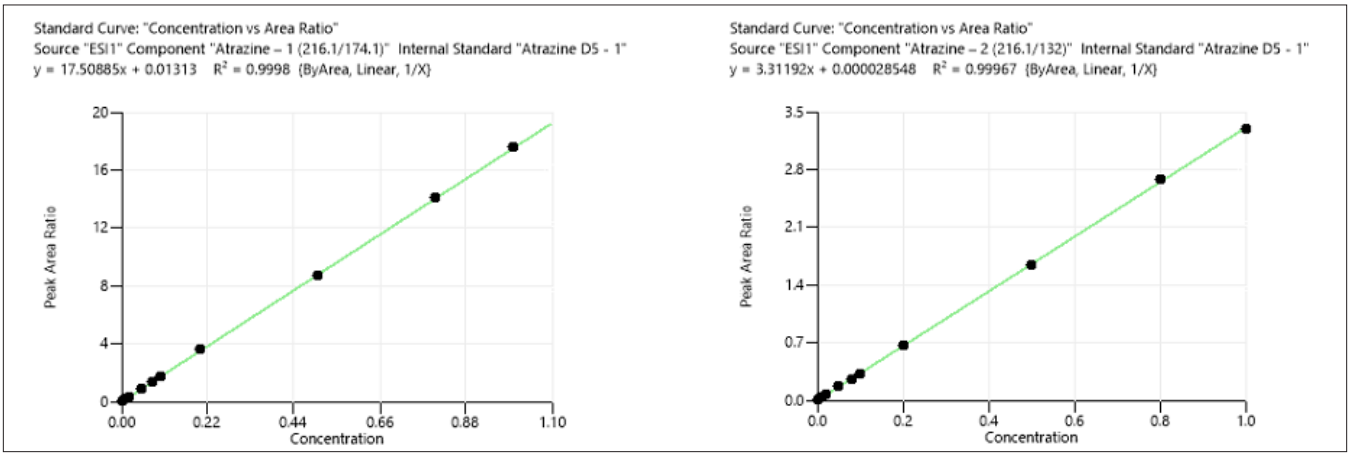


Figure 1. Overlay of 80 pesticides (Quantifier MRM) at 100 ng/L in neat solution.



Figures 2 and 3. Calibration curves for the 2 MRM transitions from Atrazine.

Targeted LOQs Based on the Current Regulation – Reproducibility Tests

A superficial water sample was spiked with 10 ng/L of atrazine. Using the LC method described earlier, we focused on the atrazine peak.

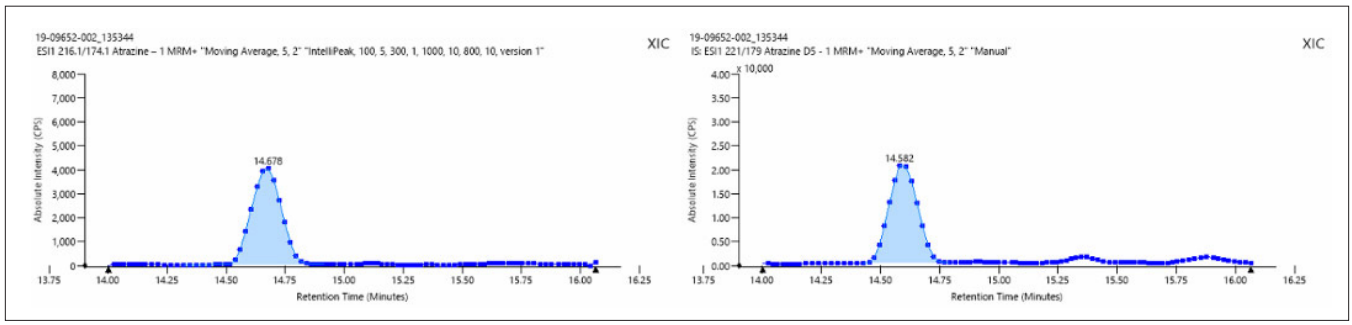


Figure 4. Chromatograms from spiked surface water samples injections.

With an automatic dwell time of 17.5 ms, the peaks show 12 data points, which is more than the number of data points set by the XP T90-214 regulation, which states a minimum of seven raw data points must be used.

Reproducibility tests have been applied at the LOQ level using replicates on blank and spiked samples injections.

Figure 5 demonstrates that the calculated amount reaches the regulation limit of 60% at the LOQ (0.004 to 0.016ng/L), and that the RSD of 8.8% is far below the regulation of 50%.

Targeted LOQs Based on the Current Regulation – Achievable LOQ on Atrazine

Based on mass spectrometry analysis requirements set forth by rule XP-T90-214, a S/N ratio of 2-3 should be obtained for the qualitative MRM transition, using the peak height calculation.

An Evian water sample spiked at 1 ng/L is then injected into the QSight UHPLC/MS/MS.

The chromatograms clearly show that the LOQ of 1 ng/L , which is 10 times lower than expected regulation requirements, is achievable.

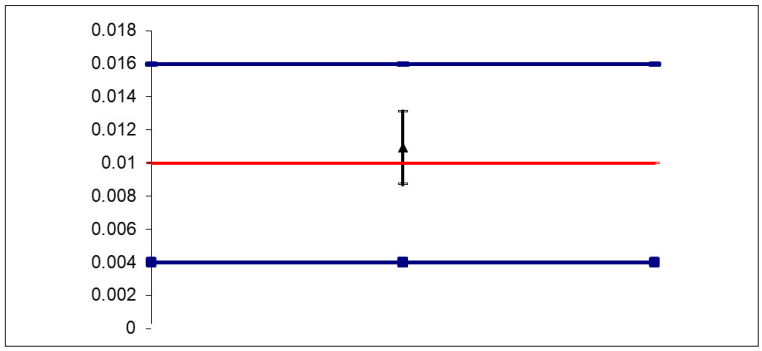


Figure 5. Reproducibility tests with regulation limits.

Table 4. Reproducibility tests on atypic or carbogas waters.

Atypical Waters or Carbonated Waters	Batch #1	Batch #2
Hépar	0.009	0.010
Contrex	0.009	0.010
Hépar	0.012	0.011
Contrex	0.011	0.011
Perrier	0.012	0.011
St-Yorre	0.012	0.012
Vals	0.011	0.012
Global Average (ppb)	*0.011*	
RSD (%)	***10.08***	

Table 5. Reproducibility tests on fresh waters.

Water Sample	Batch #1	Batch #2
Drinking water + thiosulfate Na	0.011	0.010
Surface waters	0.013	0.011
Underground waters	0.012	0.011
Volvic	0.012	0.011
Evian	0.010	0.010
Drinking water	0.010	0.011
Global Average (ppb)	*0.011*	
RSD (%)	***8.80***	

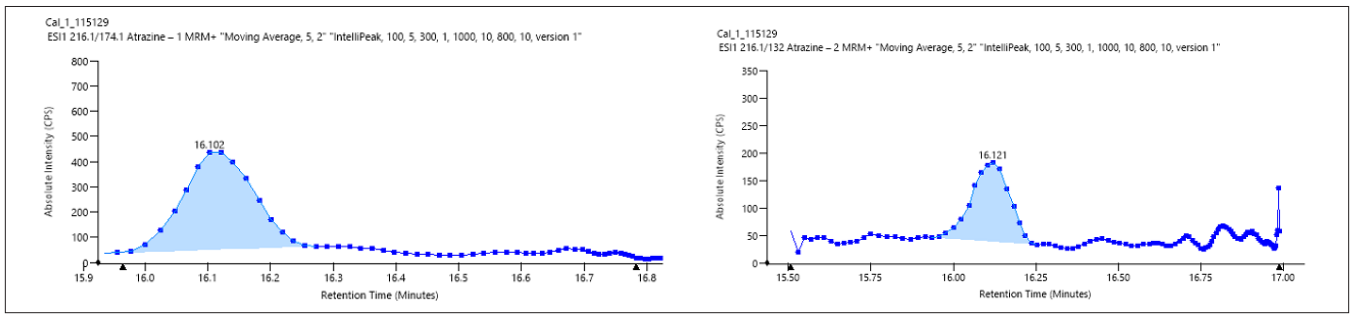


Figure 6. 1 ng/L Atrazine on Evian water for Quant and Qual MRM transitions.

Targeted LOQs Based on the Current Regulation – Achievable LOQ on DEDIA, Metolachlore and Fosethyl-Al

Since August 2019, French water regulations state that accredited laboratories should be able to quantify DEDIA at 0.030 µg/L, Metolachlore at 0.01 0 µg/L, and Fosethyl-Al at 0.1 µg/L in 2019 and 0.030 µg/L in 2021. Using our multi-component method, we evaluated the method sensitivity with these difficult compounds, as shown in Figures 7 to 9.

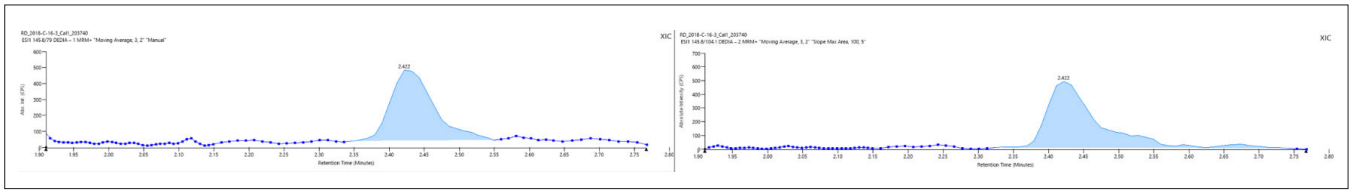


Figure 7. 10 ng/L DEDIA on Evian water for Quant and Qual MRM transitions.

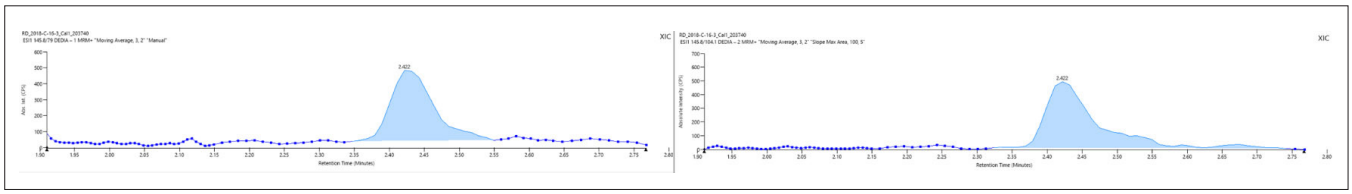


Figure 8. 10 ng/L Metolachlore on Evian water for Quant and Qual MRM transitions.

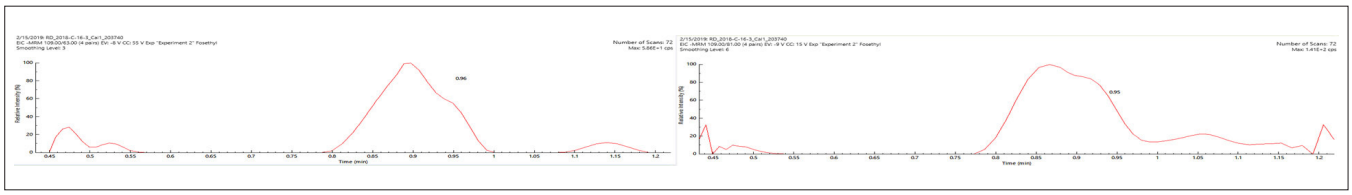


Figure 9. 10 ng/L Fosethyl-Al on Evian water for Quant and Qual MRM transitions.

For each compound, the QSIght 420 performed extremely well by reaching the newest and future LOQs.

Analytical Method Accuracy from a Statistical Point of View: Precision Study

Robustness was evaluated from several tests including averages, reproducibility (two repeated batches), and repeatability with different matrices, on different days. The RSD was then calculated to evaluate intermediate precision.

Three levels of calibration were also tested on all samples, regardless of the source of the water.

Figures 10 and 11 display, in red, the maximum La Drôme laboratory admittable deviations (confidential).

The obtained accuracies meet the requirements of standard NF EN T90-210 .

Method Transferability: Reproducibility Between Two Instruments

Using the same collision parameters, but two different LC conditions (two different columns), the same atrazine concentration was injected on two different QSight 420 systems - one located in Paris-Les Ulis, and the other one in La Drôme Laboratories. A chromatogram overlay is shown in Figure 12. Comparison of the peak area of the two atrazine peaks gave only 5% difference.

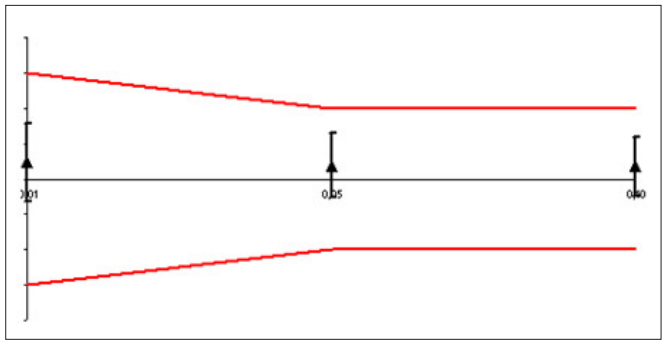


Figure 10. Atrazine 1 in fresh water: average (triangle) and SD (Bar) on additions at 0.01, 0.05 and 0.1µg /L for Transition 1 (216.1>174.1).

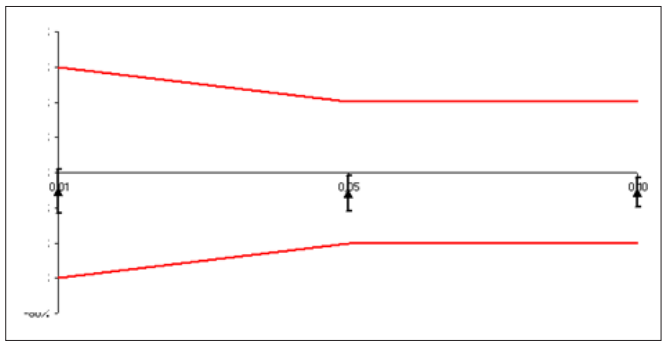


Figure 11. Atrazine two in fresh water: average (triangle) and SD (bar) on addition 0.01, 0.05 and 1µg/L for Transition 2 (216.1>132.1).

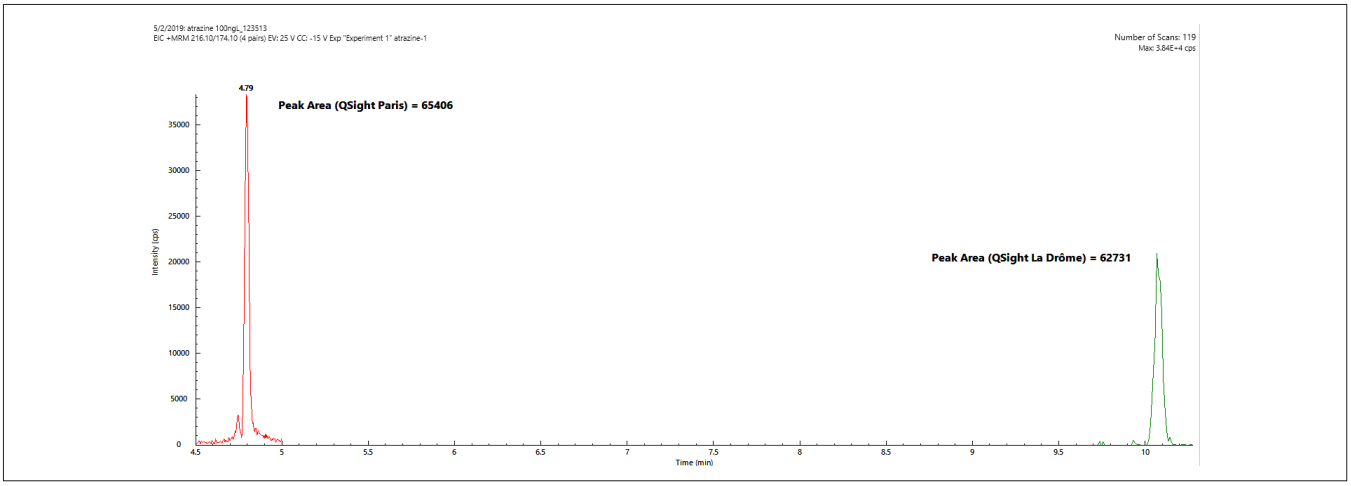


Figure 12. Comparison 100 ng/L between QSight 420 #1 and QSight 420 #2 applying the same collision parameters.

Approval of Healthy Drinking Water: Uncertainty Study

By applying the NF ISO 11352 standard, the uncertainties of the method are determined at three points - the LOQ level (0.010 µg/L), a specified level, and a high level (0.12 µg/L). Uncertainties should be below 50%.

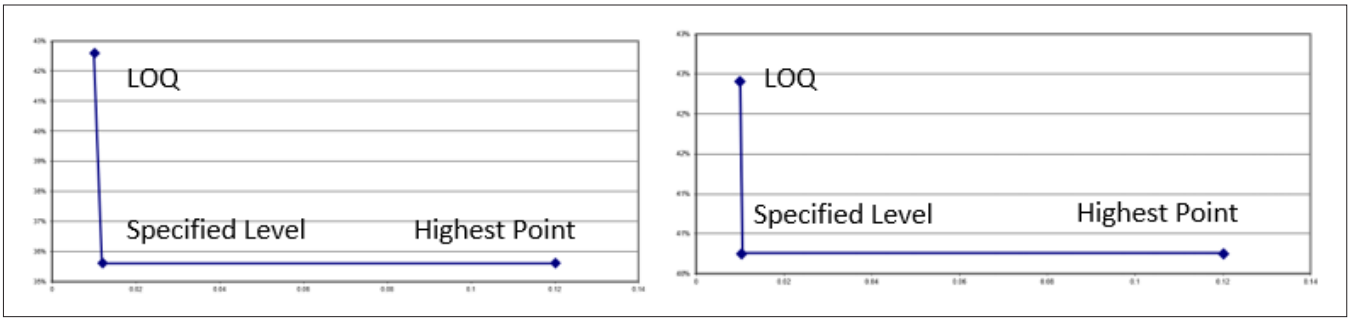


Figure 13. Uncertainty for Atrazine 2 in fresh waters: and for atypical mineral waters (right).

For each point, the calculated uncertainty is below the regulation limit of 50%. These calculations allow for set uncertainty values depending on the estimated amount in water samples.

Conclusion

A validated method for the determination of atrazine at 0.01µg/L levels in different water types ,with the help of the QSight 420, is presented in this work. The excellent sensitivity, stability and reproducibility, even at ultra-trace levels, helped reach the stringent French environmental regulations. Owing to the sensitivity and robustness of the QSight 420 LC/MS/MS, it is possible to inject the filtered samples directly into the system, allowing for a significant increase in productivity, without extra maintenance.

Following development of the method described herein, "La Drôme Laboratoires" has been positively audited on the atrazine method and received, from French Waters Authorities, the full accreditation for Atrazine determination in waters.

References

1. Drinking Water Directive (Council Directive 98/83/EC) (oct 2015).
2. 171205 Journal Officiel de la république Française N°36 Texte 138 LQ dans les eaux (nov 2017).
3. NF EN T90-210 Qualité de l'eau — Protocole d'évaluation initiale des performances d'une méthode dans un laboratoire (nov 2018).
4. XP T90-214 Qualité de l'eau — Caractérisation d'une méthode — Critères pour l'évaluation d'une méthode d'analyse pour la détermination de composés organiques multi-classes par spectrométrie de masse (june 2018).
5. NF-ISO-11352 Qualité de l'eau - Estimation de l'incertitude de mesure basée sur des données de validation et de contrôle qualité (feb 2013).

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