Direct analysis of polar pesticides in water: a routine accredited method

Using the SCIEX QTRAP® 6500+ LC-MS/MS System

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"The Glyphosate Paradox" is that, although glyphosate is the most widely used agrochemical in the world, it is also one of the least often determined by analytical methods.[1] Monitoring a highly polar, small organic pesticide such as glyphosate in food and water from diverse sources presents a significant challenge. Polar pesticides are not amenable to standard extraction procedures, are frequently poorly ionized, and demonstrate poor chromatographic separation. These pesticides, therefore, have historically required complex, single-residue methods to make them amenable to analysis—usually involving time-consuming derivatization steps and considerable clean-up procedures.

In 2018, NofaLab, in collaboration with SCIEX, developed a robust and sensitive method for the direct analysis of polar pesticides in food and environmental samples without derivatization.[2] All analytes were well retained with very reproducible retention times and peak areas, and sufficient separation to allow unambiguous identification. The large injection volume used for water samples allowed the detection of a concentration of 20 ng/L in drinking water samples, which is easily within the requirements of the current European legislation (100 ng/L) and anticipated future legislation.

Since then, the polar pesticides method has been implemented in several water labs which, after having achieved accreditation, are now using the method for the routine analysis of glyphosate and other polar pesticides in water samples. In France, to become accredited by the COFRAC (Comité Français d'Accréditation), the laboratory must demonstrate their analytical method is fit for a routine use by achieving required specifications. It covers, in particular, the validity of the calibration, the determination of the limit of quantification (LOQ) and the trueness of the method on real samples.[3] The overall uncertainty of the final results can also be calculated.[4]

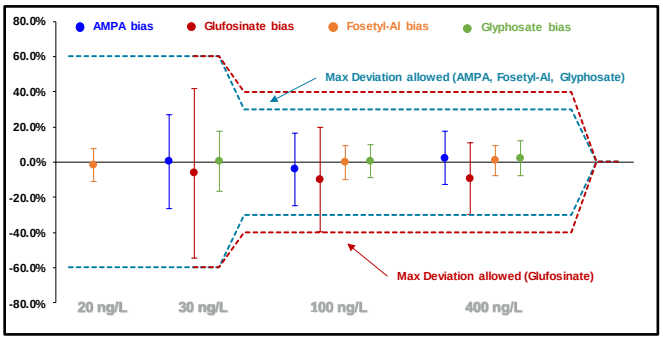


Figure 1. Trueness profiles of the method for AMPA, glufosinate, fosetyl-Al and glyphosate obtained for the accreditation at LDAR24. Solid circles show the bias of the mean value calculated from the 32 spiking water samples compared to the reference spiking value. The error bars are representative of the uncertainty of the measurement. The dotted lines depict the maximum allowed deviation, i.e 60 % at LOQ level and 30% for the rest of the calibration range (40 % for glufosinate).

The "Laboratoire Départemental d'Analyse et de Recherche de la Dordogne (LDAR24)" is dedicated to food safety, animal health and to the control of water and environment. LDAR24 is therefore involved in the control of pesticides and especially polar pesticides. LDAR24 was in need of a sensitive and robust method to quantify polar pesticides in water that is also flexible enough to allow them to easily switch to classical reverse-phase methods. The modified NofaLab / SCIEX method for polar pesticides was implemented on-site in October 2019 and LDAR24 performed the accreditation tests in November and December 2019. During that time, LDAR24 ran 8 analytical sequences containing 16 different samples spiked in duplicate at 20, 30, 100 and 400 ng/L. The results, kindly shared with SCIEX, demonstrate the method performance in the three main drinking water types: chlorinated, surface and underground water.

Key features

- Accredited method in drinking water with
  - ✓ LOQ = 20 ng/L for fosetyl-Al
  - ✓ LOQ = 30 ng/L for AMPA, glufosinate, glyphosate
- Flexibility and ease of use
- Long term stability and robustness

Methods

Sample preparation: Chlorinated water samples were stabilized with sodium thiosulfate to neutralize the presence of chlorine and then analyzed with the LC-MS/MS system. Surface water and underground water were directly injected. However, filtration or centrifugation is advised to remove suspended particles if a significant amount is present in the sample.

Chromatography: Separation was achieved using an ExionLC™ System fitted with a 500 µL loop and a CTO-20A column oven. A LC column of 150 x 4 mm with a guard column of the same material and a 0.5 µm filter was used for separation. This column allows the use of MS amenable mobile phases at around pH 9.

Table 1. List of analytes with MRMs transitions and parameters.

Pesticide	Q1 m/z	Q3 m/z	RT (min)
AMPA 1	*110*	*63*	*4.3*
AMPA 2	*110*	*79*	*4.3*
AMPA IS	*112*	*63*	*4.3*
Glufosinate 1	*180*	*63*	*4.4*
Glufosinate 2	*180*	*85*	*4.4*
Glufosinate 3	*180*	*95*	*4.4*
Glufosinate IS	*183*	*63*	*4.4*
Fosetyl-Al 1	*109*	*63*	*5.3*
Fosetyl-Al 2	*109*	*81*	*5.3*
Fosetyl-Al IS	*114*	*82*	*5.3*
Glyphosate 1	*168*	*63*	*8.4*
Glyphosate 2	*168*	*81*	*8.4*
Glyphosate 3	*168*	*150*	*8.4*
Glyphosate IS	*171*	*63*	*8.4*

Mass spectrometry: The SCIEX QTRAP 6500+ System was employed for its sensitivity and robustness. Optimized MRM transitions, detailed in Table 1, were selected and utilized for maximum sensitivity. Isotopically labelled target analytes (AMPA $^{13}C^{15}N$, glyphosate 1,2-$^{13}C2$ ^{15}N, fosetyl-aluminium D15) were utilized as internal standards for achieving the highest quality quantification. Note that AMPA $^{13}C^{15}ND_2$ was also used for the correction of glufosinate results. Details of ion source parameters can be found in Table 2.

Table 2. Ion source parameters. Electrospray ionization (ESI) conducted in negative ion mode.

Parameter	Setting
Curtain Gas (CUR)	*35 psi*
Collision Gas (CAD)	*9*
Ion Spray voltage (IS)	*-3500 V*
Temperature (TEM)	*700 °C*
Nebulizer Gas (GS1)	*55 psi*
Heater Gas (GS2)	*65 psi*

Configuration of the analytical sequences for accreditation: Following implementation in October 2019, LDAR24 performed the accreditation of the polar pesticides' method in November and December 2019 by running a total of 8 analytical sequences. These sequences were integrated with the routine use of the LC-MS/MS system by switching automatically from existing reverse phase conditions used for the other analytical methods.

As displayed in Figure 2, the 8 sequences were built in a similar way and all contained: a calibration curve from 20 to 500 ng/L in pure water, followed by a blank injection to verify that there is no carryover, a quality control at 100 ng/L, two water samples with a blank and four spiking levels (20, 30 ,100 and 400 ng/L) in duplicate, and finally QCs at LOQ values of 20 and 30 ng/L. Depending on the sequence, other injections corresponding to stability studies or other tests needed for the laboratory were

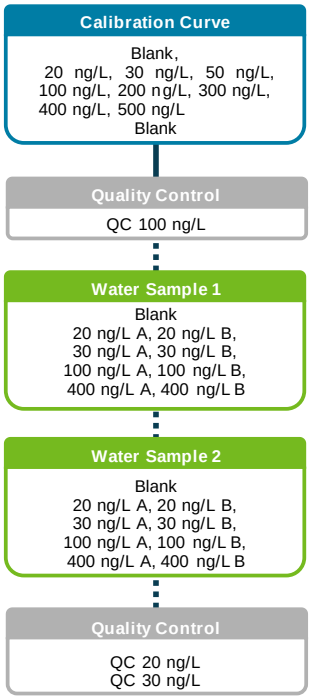


Figure 2. Common structure of the analytical series done for accreditation.

inserted between the different injection units. Using this procedure, the performance of the modified NofaLab / SCIEX method was evaluated on 16 different water samples representing 8 chlorinated waters, 6 surface waters and 2 underground waters, spiked in duplicates at 20, 30, 100 and 400 ng/L.

Accreditation results

Sensitivity and linearity

The calibration function was assessed over a range from 20 ng/L to 500 ng/L. Figure 3 displays the calibration curves obtained for the third accreditation sequence, together with the extracted ion chromatograms of the blank and calibration levels at 20 ng/L and 30 ng/L. The exceptional sensitivity of the SCIEX QTRAP 6500+ System allows the unequivocal detection of all four compounds with very good signal at 20 ng/L and 30 ng/L with negligible blank response. At 20 ng/L, both quantification and qualification MRMs show S/N > 20 for unsmoothed chromatograms (data not shown), except for qualification MRMs of glyphosate. Note that for fosetyl-Al, MRM 1 (*m/z* 109 → *m/z* 63) is less intense than MRM 2 (*m/z* 109 → *m/z* 81) but is also more specific and has a significantly better S/N. Therefore MRM 1 has been chosen for quantification purposes.

Peak areas, corrected with IS, show a perfectly linear response (with 1/X weighting) over the acquired range. Excellent coefficients of determination $R^2 > 0.995$ were obtained for all the curves and calculated concentration accuracies were within 20%. Of the 8 calibration curves performed for the accreditation, the maximum biases observed were 11% for AMPA (S2, 200 ng/L), 7% for fosetyl-Al (S2, 20 ng/L), 15% for glufosinate (S1, 20 ng/L) and 10% for glyphosate (S3, 20 ng/L). Please note that calibration curve without IS correction show even better results with $R^2 > 0.998$ for all the curves (data not shown).

Trueness study

The trueness study is designed to assess the intermediate precision and the bias of the measurement by comparison against reference values. For this purpose, 16 water samples were spiked at 20 and 30 ng/L (LOQ levels), 100 ng/L (20% of the linearity range) and 400 ng/L (80% of the linearity range).[3] To test repeatability and intra-lab reproducibility, duplicates were with various conditions (i.e. operators, calibration, equipment, environment, time between measurement) and injected within the 8 analytical sequences in November and December 2019.

Figure 4 displays chromatograms obtained at LOQ levels in 6 water samples, representative of the three water types analyzed. The four compounds show very good signal in the three water types, allowing their detection, quantification and confirmation at levels of 20 and 30 ng/L. Although analyzed in different sequences (S4, S5, S6 and S7) the 6 samples show stable signal intensities. They are even comparable to the calibration levels (Figure 3) showing few or no matrix effects. One should note that signals of AMPA and glyphosate in surface water samples are more intense due to an initial presence of these compounds in the blank (insert of Figure 4). Chromatograms also allow visual verification that the ion ratio tolerance of 20% is met since the apex of the confirmation MRM (in pink) is seen between the two dotted blue lines in all samples, thereby increasing the degree of confidence of the results.

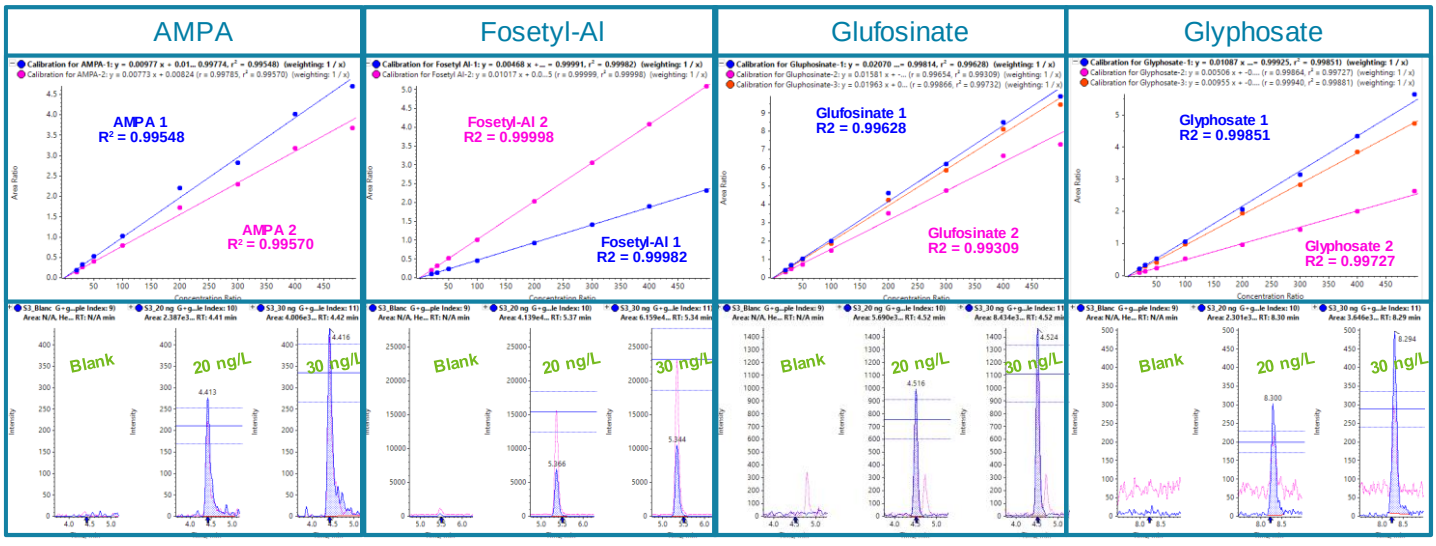


Figure 3. Calibration curves obtained for the third accreditation sequence. (Top) Calibration curves of quantification and qualification MRMs of AMPA, fosetyl-Al, glufosinate and glyphosate corrected with internal standards for S3. (Bottom) Extracted ion chromatogram (XIC) of MRM 1 and MRM 2 for the blank, 20 ng/L and 30 ng/L samples for the four compounds. The solid blue line shows the mean ion ratio calculated from the standards and the dotted lines the tolerance of 20%.

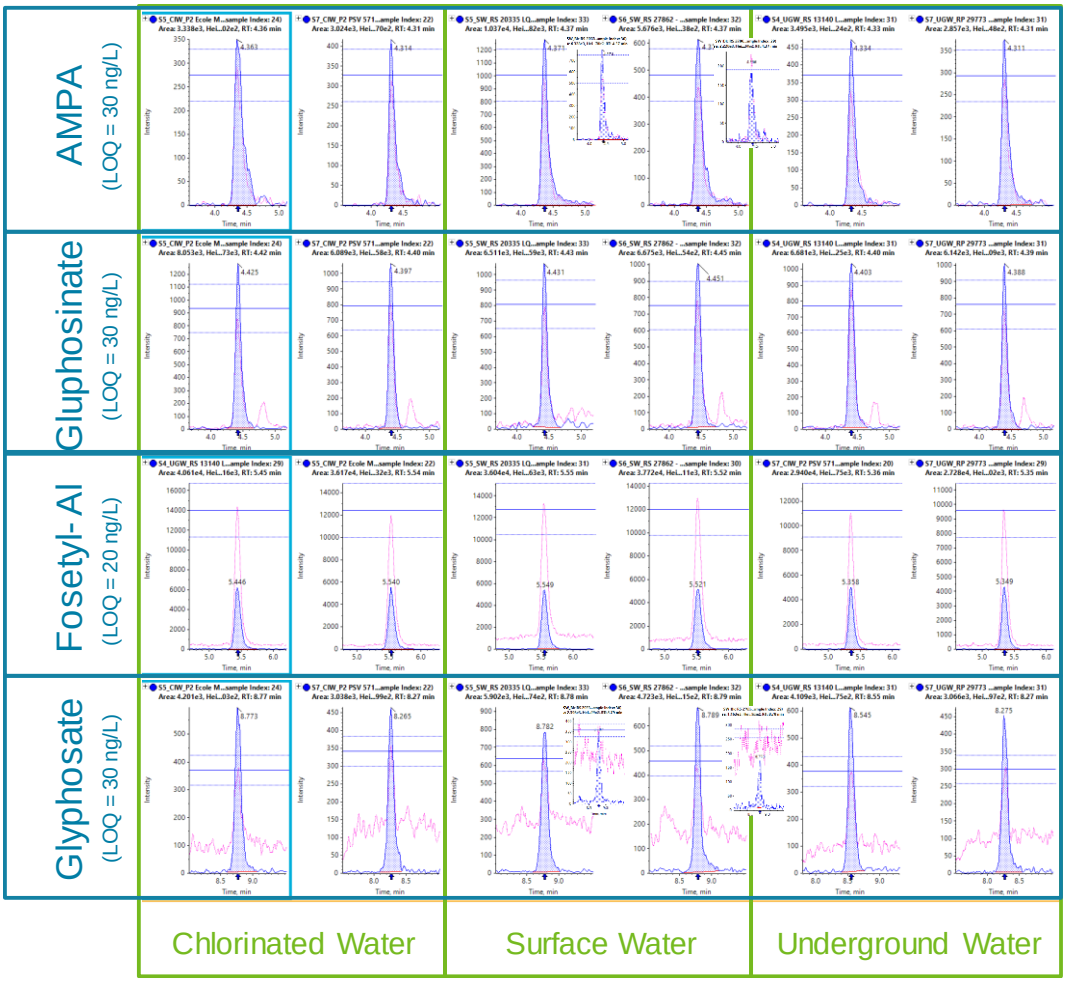


Figure 4. Chromatograms obtained at LOQ levels for the 3 water types analyzed. XICs of AMPA, fosetyl-Al, glufosinate and glyphosate at LOQ level in 2 chlorinated water, 2 surface water and 2 underground water samples. The solid blue line shows the mean ion ratio calculated among the standards and the dotted lines a tolerance of 20%.

Detecting and confirming pesticides at low levels is important but quantifying them with good accuracy is also essential. Figure 5 displays the calculated concentration in 32 water samples spiked with 20, 30 and 100 ng/L of glyphosate. With very good accuracy (mean values are respectively 20.1, 30.1 and 100.4 ng/L) and reproducibility (CV < 12%), these results demonstrate the high quality of the quantification of glyphosate in different water types.

The accreditation results are summarized both in Table 3, which displays the detailed statistics for the four compounds and in Figure 1, which depicts the corresponding trueness profiles of the four compounds. With most of the observed biases below 2%, the estimated mean values are very close to the reference values and show very good accuracy of the method in all water types. The intermediate precision is also very satisfying with values below 15%, except for glufosinate, which can show higher values. Although in agreement with the accreditation specifications, the results for glufosinate are not as good as the other 3 compounds as illustrated in Figure 1. This behavior could

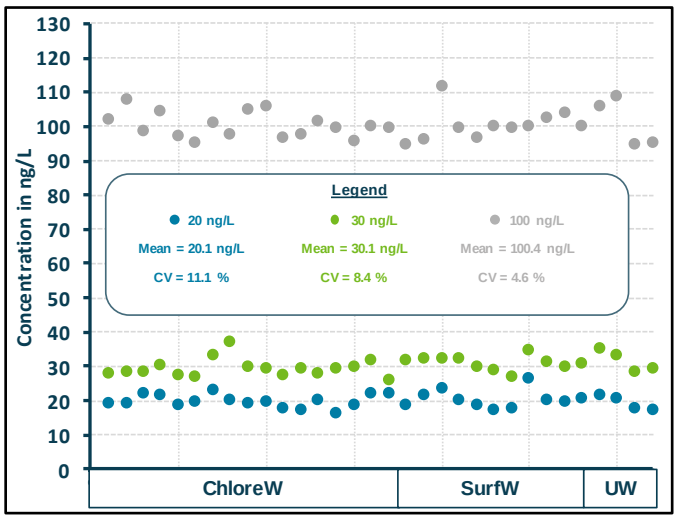


Figure 5. Calculated concentration of glyphosate in 32 water samples Calculated concentration of glyphosate from 16 water samples (8 chlorinated, 6 surface and 2 underground waters) in duplicate spiked with 20, 30 and 100 ng/L of glyphosate. Results for 400 ng/L spiking are not shown for scale reasons (Mean = 408.4 ng/L; CV = 4.7 %).

be explained by the use of AMPA-IS to correct peak areas, and therefore should be improved by the use of an isotopically labelled glufosinate standard instead.

Conclusions

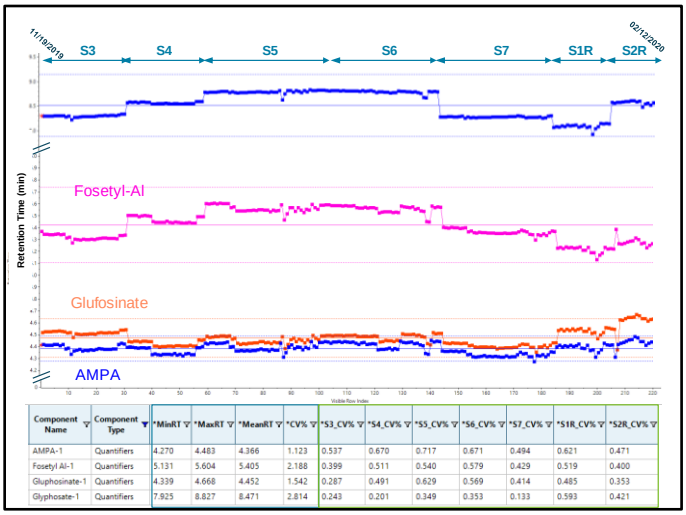
The validation process and results led to the authorization of LDAR24 to perform the routine analysis and gave accredited results with the following LOQs: AMPA 30 ng/L, glufosinate 30 ng/L, fosetyl-Al 20 ng/L, glyphosate 30 ng/L. By employing the qualification MRM for verification, AMPA, Glufosinate and Glyphosate could also have been validated at 20 ng/L. However, LDAR24 decided to apply stricter criteria and used the qualification MRM to determine the validated LOQ.

Discussing further the stability and robustness of the method

Data from the accreditation study as well as two routine sequences (S1R and S2R) acquired at the end of December and mid-February 2020 allow assessment of the stability and robustness of the chromatographic method, i.e retention time and peak shape, and signal intensities over a period of 3 months.

Retention time stability

Figure 6 displays the retention times and statistics observed for calibration levels, QC, spiked matrix samples and routine samples where a peak could be quantified for sequences S3, S4, S5, S6, S7, S1R and S2R. Retention times are extremely stable since the maximum CV observed is 0.7 % within a sequence (S5, AMPA) and 2.8% across all sequences (glyphosate) with a total of 225 samples considered.



Component Name	Component Type	*MinRT	*MaxRT	*MeanRT	*CV%	*S3_CV%	*S4_CV%	*S5_CV%	*S6_CV%	*S7_CV%	*S1R_CV%	*S2R_CV%
AMPA-1	Quantifiers	4.270	4.483	4.366	1.123	0.537	0.670	0.717	0.671	0.494	0.621	0.471
Fosetyl Al-1	Quantifiers	5.131	5.604	5.405	2.188	0.399	0.511	0.540	0.579	0.429	0.519	0.400
Glufosinate-1	Quantifiers	4.339	4.668	4.452	1.542	0.287	0.491	0.629	0.569	0.414	0.485	0.353
Glyphosate-1	Quantifiers	7.925	8.827	8.471	2.814	0.243	0.201	0.349	0.353	0.133	0.593	0.421

Figure 6. Retention times and statistics observed. (Top) plots of the retention times for AMPA, glufosinate, fosetyl-Al and glyphosate for series S3, S4, S5, S6, S7, S1R and S2R. (Bottom) In the table, blue squares correspond to statistics for all sequences and green squares for CV within a sequence.

Signal intensity stability

The stability of signal intensities is illustrated in Table 4 with the example of glyphosate. Table 4 shows peak areas for glyphosate at all the calibration levels from sequences S3, S4, S5, S6, S7, S1R and S2R, together with the CV across the seven values for both quantifier and qualifier MRMs. Over 3 months, the peak area of glyphosate showed CVs below 11.85 % (100 ng/L) for MRM 1 and 14.1 % (100 ng/L) for MRM 2 which demonstrates very good signal stability.

Table 3. Summary of the accreditation results for the quantitative MRM of AMPA, glufosinate, fosetyl-Al and glyphosate.

Parameters	AMPA 1			Glufosinate 1			Fosetyl-Al 1			Glyphosate 1		
Reference Value	*30*	*100*	*400*	*30*	*100*	*400*	*20*	*100*	*400*	*30*	*100*	*400*
Number of Series (n)	*14*	*14*	*14*	*14*	*14*	*14*	*16*	*16*	*16*	*16*	*16*	*16*
Number of repetition (r)	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*
Mean value estimated	*30.09*	*95.80*	*409.14*	*28.14*	*90.00*	*362.36*	*19.66*	*99.66*	*403.41*	*30.13*	*100.38*	*408.41*
Bias on Mean value (%)	*0.3*	*-4.2*	*2.3*	*-6.2*	*-10.0*	*-9.4*	*-1.7*	*-0.3*	*0.9*	*0.4*	*0.4*	*2.1*
Repeatability (%)	*7.1*	*5.0*	*4.9*	*9.2*	*4.9*	*6.5*	*4.3*	*3.2*	*2.4*	*5.4*	*4.3*	*3.7*
Intermediate Precision (%)	*13.3*	*10.7*	*7.5*	*25.6*	*16.5*	*11.4*	*4.9*	*4.8*	*4.2*	*8.4*	*4.6*	*4.7*
Low Tolerance Limit (ng/L)	*22.1*	*75.3*	*348.1*	*13.7*	*60.3*	*280.0*	*17.7*	*90.1*	*369.3*	*25.1*	*91.1*	*369.7*
High Tolerance Limit (ng/L)	*38.1*	*116.3*	*470.1*	*42.6*	*119.7*	*444.7*	*21.6*	*109.2*	*437.6*	*35.2*	*109.7*	*447.1*
Expended Relative Uncertainty k=2 (%)	*27.5%*	*23.6%*	*16.0%*	*54.4%*	*39.5%*	*30.0%*	*10.5%*	*9.9%*	*8.9%*	*17.3%*	*9.5%*	*10.6%*

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Value #1	Value #2	Value #3	Value #4	Value #5	Value #6	Value #7
Glyphosate-1	20.0000	7 of 7	2.219e3	2.075e2	9.35	2.357e3	1.790e3	2.382e3	2.306e3	2.228e3	2.134e3	2.337e3
Glyphosate-1	30.0000	7 of 7	3.431e3	4.055e2	11.82	3.717e3	3.025e3	3.893e3	3.818e3	3.500e3	2.869e3	3.193e3
Glyphosate-1	50.0000	7 of 7	5.610e3	6.016e2	10.72	5.972e3	5.096e3	6.574e3	5.988e3	5.511e3	4.841e3	5.290e3
Glyphosate-1	100.0000	7 of 7	1.123e4	1.331e3	11.85	1.178e4	1.077e4	1.329e4	1.208e4	1.136e4	1.010e4	9.268e3
Glyphosate-1	200.0000	7 of 7	2.193e4	2.084e3	9.50	2.290e4	2.168e4	2.500e4	2.334e4	2.189e4	1.988e4	1.886e4
Glyphosate-1	300.0000	7 of 7	3.308e4	3.237e3	9.79	3.615e4	3.297e4	3.779e4	3.358e4	3.250e4	3.027e4	2.831e4
Glyphosate-1	400.0000	7 of 7	4.440e4	4.684e3	10.55	4.774e4	4.346e4	5.011e4	4.748e4	4.540e4	3.934e4	3.726e4
Glyphosate-1	500.0000	7 of 7	5.527e4	4.705e3	8.51	5.983e4	4.798e4	6.123e4	5.671e4	5.617e4	5.088e4	5.411e4
Glyphosate-2	20.0000	7 of 7	1.163e3	1.605e2	13.80	1.149e3	9.259e2	1.346e3	1.237e3	1.004e3	1.344e3	1.135e3
Glyphosate-2	30.0000	7 of 7	1.556e3	1.714e2	11.01	1.489e3	1.679e3	1.571e3	1.824e3	1.563e3	1.498e3	1.271e3
Glyphosate-2	50.0000	7 of 7	2.503e3	2.757e2	11.01	2.543e3	2.024e3	2.819e3	2.473e3	2.394e3	2.831e3	2.434e3
Glyphosate-2	100.0000	7 of 7	5.269e3	7.417e2	14.08	5.862e3	5.036e3	6.387e3	5.414e3	5.422e3	4.479e3	4.282e3
Glyphosate-2	200.0000	7 of 7	1.024e4	1.280e3	12.50	1.073e4	1.012e4	1.217e4	1.048e4	1.081e4	9.262e3	8.119e3
Glyphosate-2	300.0000	7 of 7	1.532e4	1.807e3	11.80	1.641e4	1.563e4	1.785e4	1.572e4	1.572e4	1.334e4	1.255e4
Glyphosate-2	400.0000	7 of 7	2.066e4	2.020e3	9.78	2.198e4	1.957e4	2.187e4	2.225e4	2.261e4	1.895e4	1.737e4
Glyphosate-2	500.0000	7 of 7	2.574e4	2.383e3	9.26	2.798e4	2.318e4	2.831e4	2.745e4	2.640e4	2.248e4	2.438e4

Table 4. Peak area stability. Peak areas for Glyphosate (MRM 1 and 2) at calibration levels of S3, S4, S5, S6, S7, S1R, S2R and corresponding CVs showing the very good signal stability over 3 months.

Possible extension of the validity of a calibration curve

Today, the more common way to analyze and quantify unknown samples routinely with good accuracy and confidence is to use a new calibration curve for each new sample sequence, especially when different analytical methods are used on the same instrument. However, considering the excellent signal stability of the method in combination with the use of IS, the question of using the same calibration curve for an extended period of one week, one month or several months is raised. To assess this possibility, the calibration curve from sequence S3 was used as a unique calibration curve to quantify the other calibration levels and spiked water samples from both the accreditation and routine sample sequences. The boxplots displayed in Figure 7 show the distribution of the calculated concentrations for both calibration levels (on the left) and spiked water samples (on the right) at 30 and 100 ng/L using the usual quantification (one calibration curve per sequence) and the single curve quantification (one calibration curve for all sequences).

Figure 7 shows that the two strategies give very satisfying results both in accuracy and reproducibility. This demonstrates that using only one calibration curve to quantify samples from multiple sequences acquired over one or several months is perfectly conceivable. However, although the results are very good for glyphosate and AMPA, there is room for improvement regarding glufosinate and fosetyl-Al. Indeed, as already

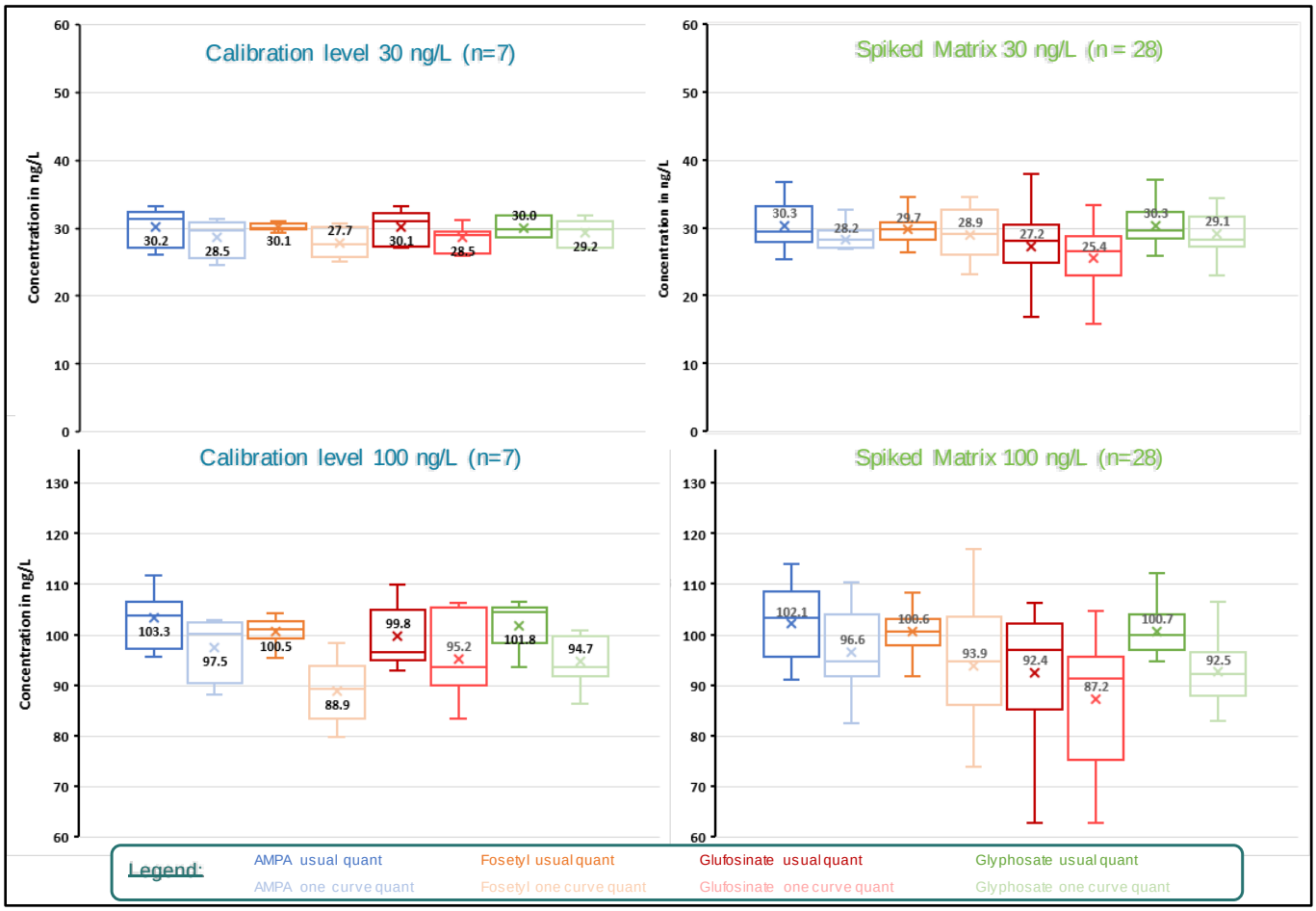


Figure 7. Distribution of the calculated concentrations. Boxplots showing the distribution of the calculated concentrations obtained for AMPA, fosetyl-Al, glufosinate and glyphosate using the usual quantification method (i.e one calibration curve/sequence) and the single curve quantification (i.e. one calibration curve for all the samples from different sequences). On the left, calibration levels at 30 and 100 ng/L (n=7). On the right, spiked water samples at 30 and 100 ng/L (n=28). Mean concentration of each boxplot is illustrated by a cross and the value is specified.

mentioned for glufosinate, the use of a dedicated internal standard should improve its overall results. Regarding Fosetyl-Al, one can note that the boxplots widened, and the mean concentration decreased when using the single calibration curve strategy. This behavior could be explained by a stability issue of the fosetyl-Al stock solution that was observed during the tests.

Conclusions

These results from the accreditation process at LDAR24 demonstrate that the modified NofaLab / SCIEX method for polar pesticides running on the SCIEX QTRAP 6500+ System is perfectly fit for routine quantification of AMPA, fosetyl-Al, glufosinate and glyphosate in different types of water samples. It delivers very good accuracy and reproducibility which allow high-quality quantification and confirmation at accredited LOQs of 20 ng/L for fosetyl-Al and 30 ng/L for AMPA, glufosinate and glyphosate. Positive samples can be confirmed with confidence by the use of ion ratio since all standards and matrix samples fall within a 20 % tolerance.

The very good stability of retention time and signal intensity observed during these 3 months of analyses in a non-dedicated SCIEX QTRAP 6500+ System demonstrates the robustness of both the method and instrument and also the ease of use and flexibility of the modified NofaLab / SCIEX method for polar pesticides in a routine laboratory.

The robustness of the column, demonstrated by more than 2000 samples injected since the installation of the method, is another key advantage of the method.

Finally, the modified NofaLab / SCIEX method for polar pesticides shows very good performance for the four priority polar pesticides in water - AMPA, glufosinate, fosetyl-Al and glyphosate However, further work is to be carried out to include other polar pesticides in the method.

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