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ABSTRACT

A method is presented to analyse 10 chlorothalonil metabolites in the low ng/L range using the SCIEX 7500 system. Due to the high sensitivity observed, a 20 µl injection volume was used with no sample concentration needed, therefore, minimizing matrix effects, and improving the robustness of the analysis.

INTRODUCTION

Chlorothalonil is a fungicide that was widely used around the world for grain and vegetable cultivation. In Switzerland it was one of the top 10 agrochemicals using during the last few years. ¹ Due to increasing concerns about the toxicity of chlorothalonil and the discovery of several metabolites in the environment it was banned in 2020. ²⁻³ The two main metabolites R471811 and R417888 were detected at high concentrations in ground and surface water. Therefore, there is a growing need to analyse and monitor these metabolites, to protect drinking water supplies, map the distribution, and to understand pathways and long-term behaviour. Different types of water such as ground water, surface water and drinking water should be analysed to assess potential exposure.

MATERIALS AND METHODS

Target analytes and internal standards: 10 chlorothalonil metabolites were analysed: R471811, R417888, SYN507900, R611968, chlorothalonil 4-hydroxy, SYN548580, SYN548581, R611965, R611553 and M7. Isotopically labelled internal standards (IS) were used for R471811 and R417888.

Standard preparation: A calibration curve from 0.1 to 1000 ng/L was prepared in mineral water. 10 µL of the IS solution was added to 1 mL of standard solution. The final IS mixture concentration in the water samples was 10 ng/L.

Sample preparation: The samples have been obtained from different labs using unique sample preparation techniques. These typically included filtering, centrifugation, or no treatment at all to avoid adsorption or losses. The internal standard mix solution was added prior to analysis.

Liquid chromatography: HPLC separation was performed on a Shimadzu LC40 system using a Phenomenex Kinetex Polar C18 column (100 × 4.6 mm, 2.6 µm, 00D-4759-E0). The flow rate was 0.9 mL/min. The injection volume was 20 µL and the total LC runtime was 17 minutes.

Mass spectrometry: The ionisation source was operated with electrospray ionisation (ESI) in negative mode using optimised settings. For each transition, voltages were optimised per analyte for compound-specific parameters such as collision energy (CE). A single acquisition method consisting of 24 MRM transitions (22 for the compounds and 2 for the internal standards) was created using the Scheduled MRM™ Algorithm in SCIEX OS Software. Two MRM transitions were monitored for each of the targeted analytes. As SYN507900 and R611968 are isobaric compounds which have the same main transitions, a unique third transition was added for both compounds.

Data processing: Data was processed using SCIEX OS Software.

METHOD DEVELOPMENT AND OPTIMISATION

Initial method development used a QTRAP 5500 system with an injection volume of 100 µL. To improve sensitivity, the method was transferred to the SCIEX 7500 system with a Shimadzu LC-40 HPLC. The retention windows were adjusted, and source parameters optimised. An intensity increase of up to 15x was observed as a result.

Due to the high sensitivity of the SCIEX 7500 system a decrease of the injection volume was investigated. Injection volumes between 10 and 100 µL were used. Volume overload was observed for early eluting compounds such as R471811, but not for late eluters such as R417888. Ultimately, an injection volume of 20 µL was selected as the optimal compromise between sensitivity and peak shape.

Figure 1 shows the elution profile of the 10 metabolites resulting from the optimized data acquisition method. The optimized LC and MS conditions ensured reliable integration, quantification, and confirmation of the peak for each chlorothalonil metabolite.

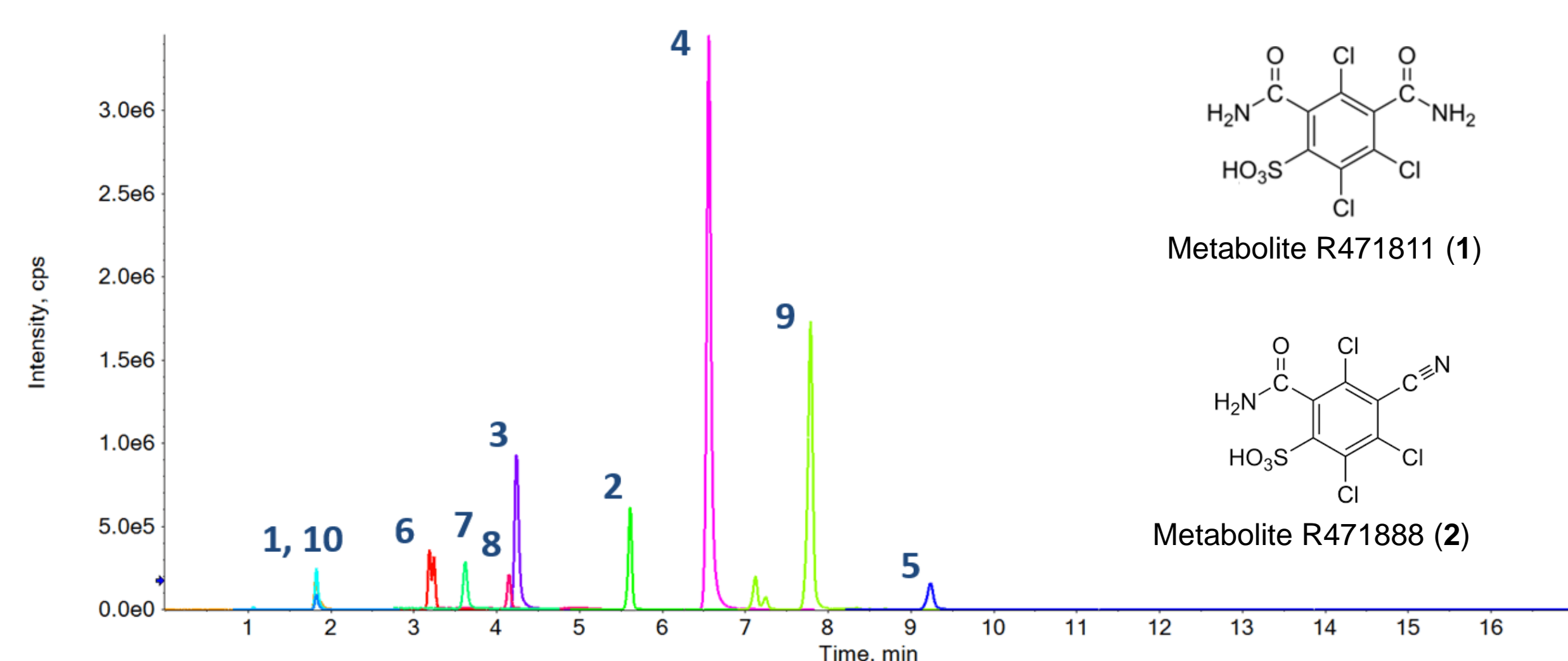


Figure 1. Chromatographic profile of the 10 chlorothalonil metabolites analyzed on the SCIEX 7500 system using 20 µL injection volume. Shown is an extracted ion chromatogram (XIC) resulting from the optimized LC and MS method using a 100 ng/L neat standard mixture. The numbered peaks are assigned as follows: 1. R471811 (structure shown), 2. R417888 (structure shown), 3. SYN507900, 4. R611968, 5. chlorothalonil-4OH, 6. SYN548580, 7. SYN548581, 8. R611965, 9. R611553, 10. M7.

METHOD PERFORMANCE

A series of calibration solutions between 0.1 and 1000 ng/L were prepared in mineral water before being injected to evaluate the quantitative performance of the system. Calibration curves were generated for each of the analytes. Table 1 summarises the linearity and calibration curve information including precision results.

Table 3. Method performance for the analyte suite. The table contains retention times, correlation coefficients, working range in mineral water, and %RSD of 6 injections at 10 ng/L. Injection volume 20 µL.

Compound Name	RT (min)	r2	Working range (ng/L)	RSD (%)
R471811	1.82	≥0.9988	0.5* - 1000	≤3.6
R417888	5.60	≥0.9994	0.1 - 1000	≤1.1
SYN507900	4.23	≥0.9985	0.5 - 1000	≤2.1
R611968	6.54	≥0.9994	0.1 - 1000	≤1.2
Chlorothalonil-4OH	9.21	≥0.9991	0.5 - 1000	≤0.7
SYN548580	3.21	≥0.9973	0.5 - 1000	≤1.7
SYN548581	3.61	≥0.9985	1 - 1000	≤2.7
R611965	4.14	≥0.9959	5 - 1000	≤3.9
R611553	7.77	≥0.9979	0.5 - 1000	≤1.1
M7	1.81	≥0.9994	1** - 1000	≤4.3
R471811 13C2 15N2	N/A	N/A	N/A	N/A
R417888 13C2 15N2	N/A	N/A	N/A	N/A

*= Lower LOQ values have not been assessed due to amount observed in blank bottled drinking water.

**= Qualifier has working range 5 – 1000 ng/L

SPIKING EXPERIMENTS AND RECOVERY

Trace levels of chlorothalonil metabolite R471811 in mineral water ^{4,5} makes the calibration of analytical methods difficult at low ng/L levels while maintaining high levels of accuracy and precision, and mineral salts can affect method performance.

To assess the direct injection method used in this experiment, 23 water samples were injected, with 4 of them spiked at 3 concentration levels (10, 50 and 100 ng/L). The recovery of metabolites R471811 and R417888 was calculated with and without the use of internal standards (Figure 2). In river filtrate (sample 510), river Furtbach and Lake Neuchatel samples there was only minor differences in the calculated concentrations for both analytes, with and without use of internal standard. In a source water sample with high mineral content a significant suppression effect was observed for R471811. This led to low recovery, which can be corrected for with the use of an internal standard.

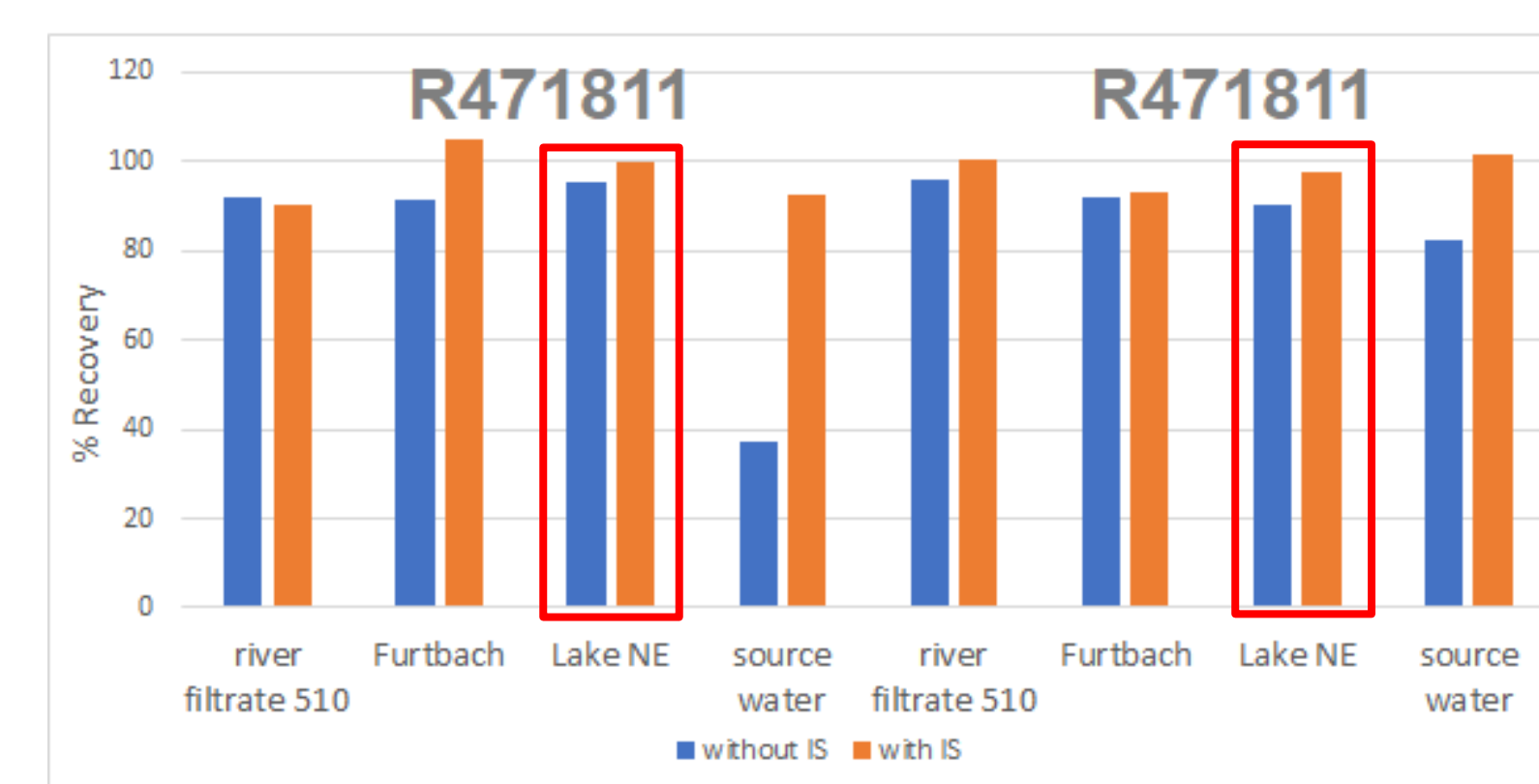


Figure 2. Accuracy of R471811 (left) and R417888 (right) in 4 spiked samples. Blue: external calibration without internal standard. Orange: external calibration using isotopically labelled internal standards. Only the source water sample showed significantly better recovery if internal standards are used (highlighted in red).

Water samples were taken from Swiss lakes showed an observed range of R471811 between 1 and 200 ng/L depending on the agricultural activities around the lakes and their watershed (Figure 3).

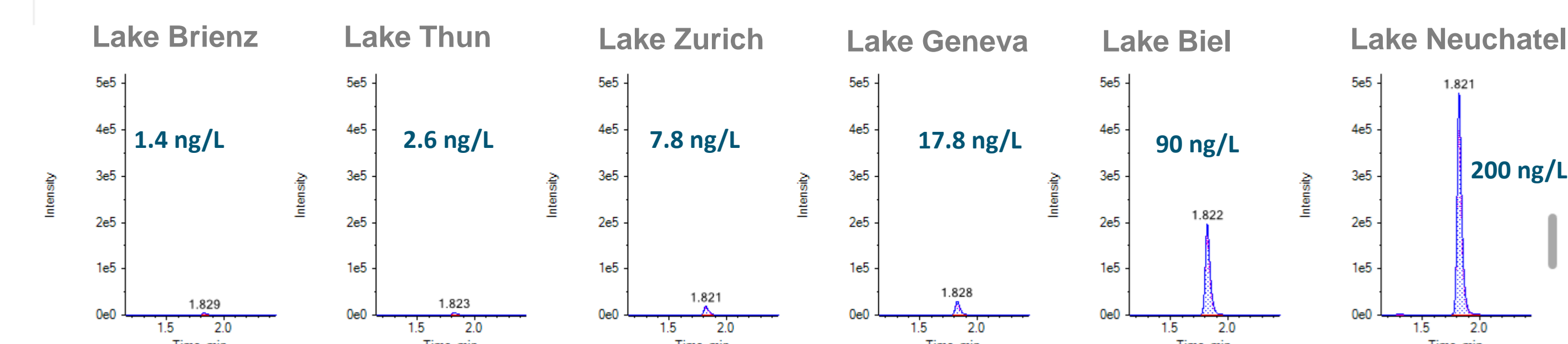


Figure 3. R471811 in Swiss lake water. Left to right: Lake Brienz, Lake Thun, Lake Zurich, Lake Geneva, Lake Biel and Lake Neuchatel, concentration in ng/L. Samples were quantified with external standard calibration in bottled drinking water. For confirmation of specificity both ion ratios and retention time was assessed and deemed acceptable.

In mineral water, the concentration was calculated with an internal standard, to correct for any matrix effects caused by the combined salt content, which was up to 2.2 g/L. Only in one mineral water sample was R471811 not detected. In the other samples, the content was between 2 and 7 ng/L.

In the midland region of Switzerland, it was reported that R471811 and R417888 are the two most frequently observed metabolites. ^{4, 5, 6} This was confirmed for both metabolites (R471811 shown in Figure 4). If these two metabolites are present at high concentrations, SYN507900, chlorothalonil-4OH, R611968, SYN548580 and SYN548581 are also typically detected also. The metabolites M7, R611553 and R611965 were not observed in any of the samples analysed.

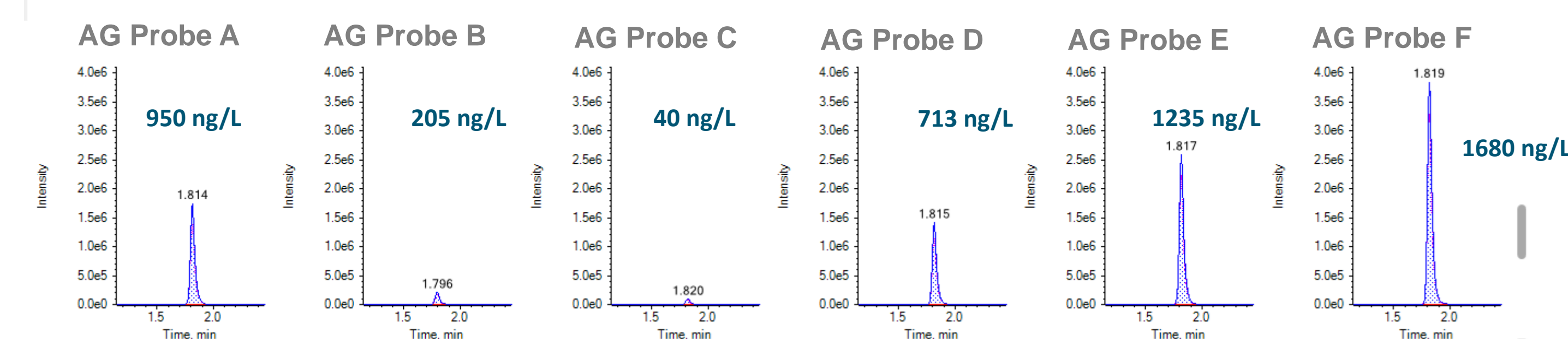


Figure 4. R471811 in 6 water samples from the midland region. Concentrations between 40 and 1680 ng/L were observed. Samples were quantified with external standard calibration in bottled drinking water. For confirmation of specificity both ion ratios and retention time was assessed and deemed acceptable.

CONCLUSIONS

- A highly sensitive method was developed that can quantify 10 chlorothalonil metabolites down to 0.1ng/L.
- The high sensitivity of the SCIEX 7500 system means that a lower injection volume (20µL) is possible without the need for sample preparation to concentrate the sample and reduced matrix effects and without the need for IS in low salt samples.
- For water samples with high salt content such as mineral water, the use of an IS gave recoveries were between 90% and 105% for all water matrices analysed (typical specifications: 80% to 120%).
- R471811 and R417888 were observed frequently, and sometimes at very high concentrations. 5 other metabolites were detected at lower concentrations, and 3 metabolites were not present in any sample investigated here.
- Excellent reproducibility was observed at 10 ng/L with RSD values between 0.4% and 4.3%.

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