High sensitivity quantification of chlorothalonil metabolites in surface, ground and bottled drinking water

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SCIEX October 2022

RUO-MKT-11-15315-A

SCIEX
The Power of Precision

Outline

- Discovery of metabolites and ban of chlorothalonil
- Method development and optimization of chlorothalonil metabolites by LC/MS-MS
- Method performance and results: analysis of chlorothalonil metabolites in multiple water matrices

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Chlorothalonil

BAN AND DISCOVERY OF METABOLITES

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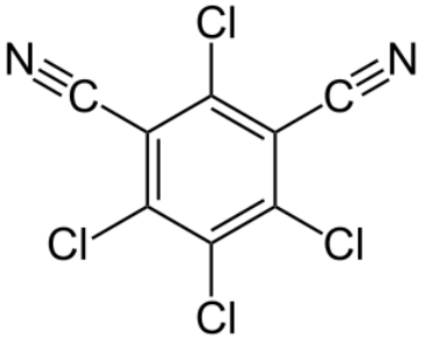
Chlorothalonil

- Broad-spectrum, non systemic fungicide
  - Since > 50 years
  - Treatment of potatoes, wheat, tomatoes and peanuts
  - Among top ten used agrochemicals in Switzerland: annual use 30 tonnes
  - Third most used fungicide in the US: 4500 tonnes in 2011



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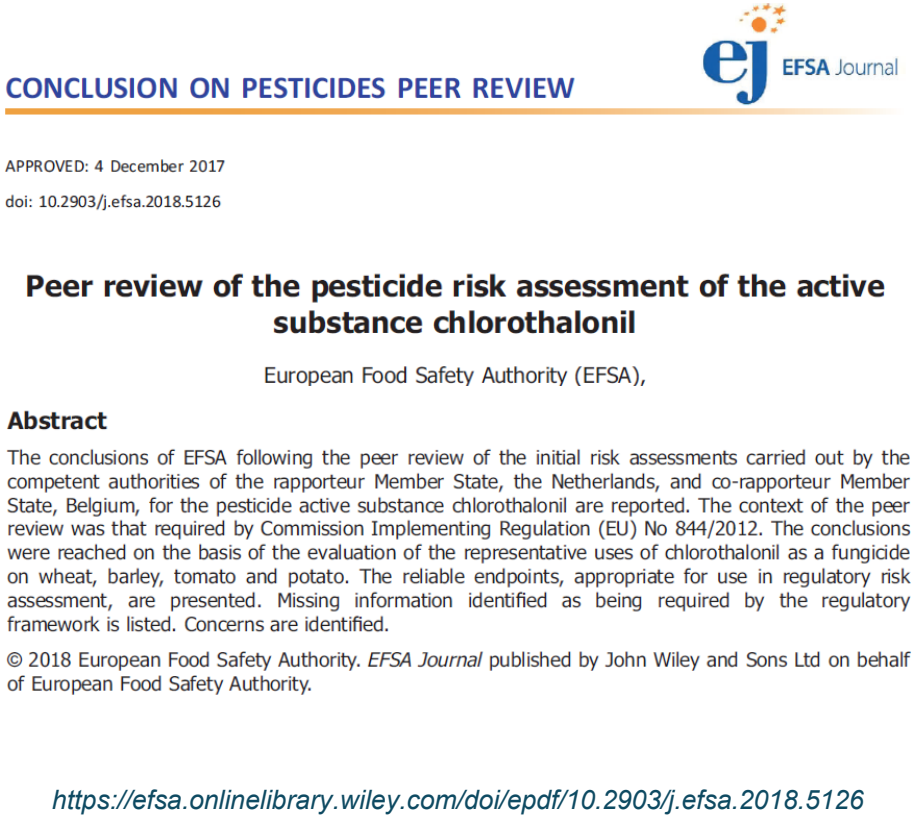
EFSA risk assessment 2018

- Concerns about toxicity of metabolites
- Data gap identified



CONCLUSION ON PESTICIDES PEER REVIEW

EFSA Journal

APPROVED: 4 December 2017

doi: 10.2903/j.efsa.2018.5126

Peer review of the pesticide risk assessment of the active substance chlorothalonil

European Food Safety Authority (EFSA),

Abstract

The conclusions of EFSA following the peer review of the initial risk assessments carried out by the competent authorities of the rapporteur Member State, the Netherlands, and co-rapporteur Member State, Belgium, for the pesticide active substance chlorothalonil are reported. The context of the peer review was that required by Commission Implementing Regulation (EU) No 844/2012. The conclusions were reached on the basis of the evaluation of the representative uses of chlorothalonil as a fungicide on wheat, barley, tomato and potato. The reliable endpoints, appropriate for use in regulatory risk assessment, are presented. Missing information identified as being required by the regulatory framework is listed. Concerns are identified.

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https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5126

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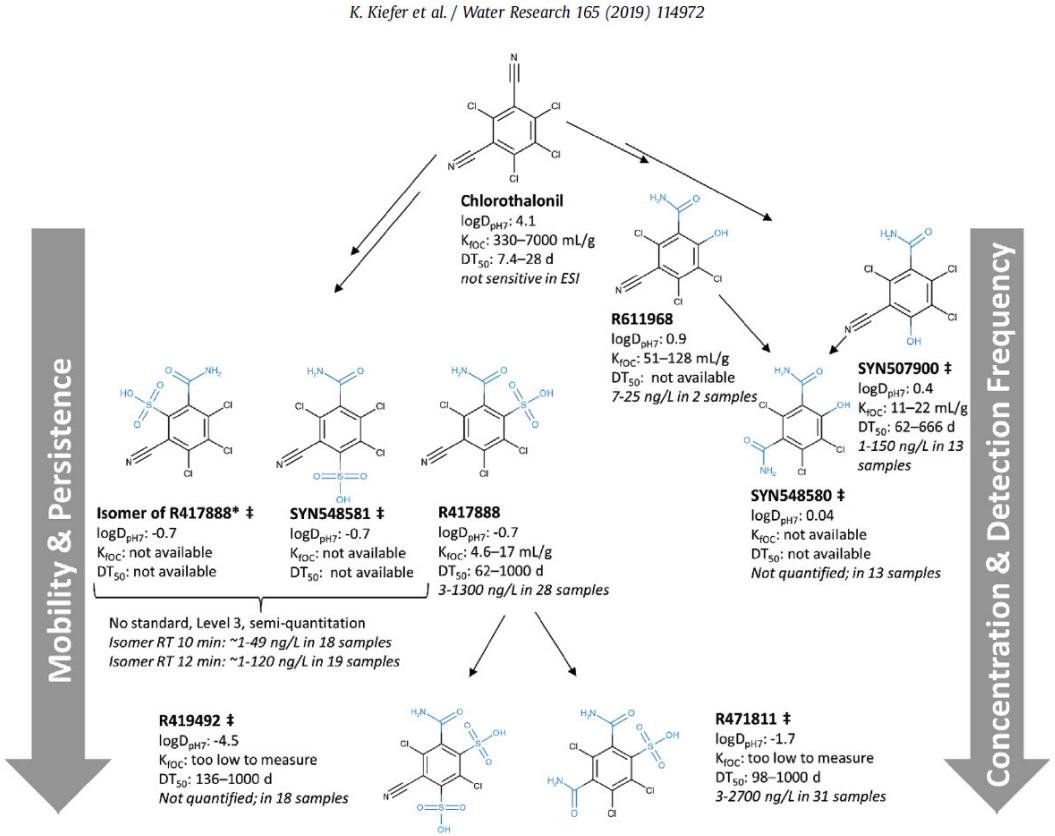
Switzerland: EAWAG Paper 2019

Discovery of several previously unknown metabolites by HRMS screening which have the potential to be genotoxic and prevalent in the environment



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“The 9/11 of Swiss drinking water supply”

BAN OF CHLOROTHALONIL

EU and Swiss ban - 2020

Zulassung für Chlorothalonil wird mit sofortiger Wirkung entzogen

Chlorothalonil banned immediately

Streit um Pestizide

Plötzlich gilt das Wasser als verschmutzt

Abbauprodukte von Chlorothalonil verunreinigen das Grundwasser in zwölf Kantonen, warnt das Bundesamt für Umwelt. Syngenta kritisiert die Behörde dafür scharf.

Our water is suddenly considered to be polluted

Auswirkung auf Trinkwasser

Grundwasser in 12 Kantonen stark belastet

Das Bundesamt für Umwelt hat erstmals die Belastung von Chlorothalonil-Abbauprodukten im Grundwasser gemessen. Die Konzentrationen mehrerer Metaboliten überschreiten den Grenzwert im Mittelland grossflächig.

Water in 12 regions is strongly polluted

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Method development and optimization

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Method development

BRIEF: TO DEVELOP A SIMPLE, TARGETED QUANTIFICATION METHOD FOR ROUTINE USE

- Cover as many metabolites as possible in one method
- Applicable for different water types such as drinking water, mineral water, surface water, river filtrate and ground water
- Use a simple method design for routine labs
  - Direct injection
  - No further sample concentration steps
  - Working range 1 ng/L (or lower) to 1000 ng/L
  - Possibility to quantify directly without individual internal standards when possible

~~60mL sample volume was filled into BÜCHI glass vials (0.3 mL appendix volume), spiked with 224 isotope labelled internal standards and evaporated at 20 mbar and 45 °C to 1e5mL using the back-flush unit…. Then, the samples were evaporated to ~0.3 mL…~~

- Check method performance with multiple sample types
- Check recovery using spiking experiments with/without ISTD for R471811 and R417888

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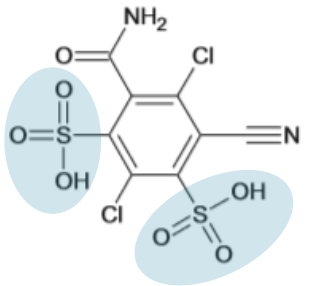
Method development

COMPOUND SELECTION

- Focus on main metabolites R471811 and R417888, and 8 other metabolites (ESI negative)
- Chlorothalonil quantification not included – analysis typically performed using GC-MS or LC-MS using APCI negative mode
- Compounds with two sulfonic acid groups (R419492, R418503) were not included as they do not elute using typical reversed phase chromatography

Zum Metaboliten R419492 existiert noch keine breit abgestützte Analysenmethode und auch die Standardsubstanz kann bislang durch keinen unabhängigen Hersteller bezogen werden. Aus diesen Gründen empfehlen wir, auf die Anagbe von Analyseresultate zum Metaboliten R419492 zu verzichten.

Switzerland: statement that routine labs do not currently need to include R419492



R419492

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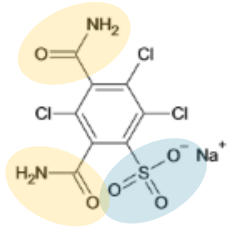
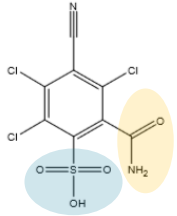
Method development

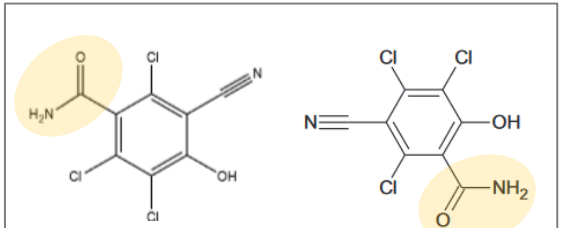
10 METABOLITES COVERED IN APP NOTE



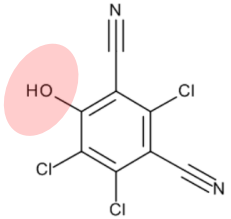
R471811
Main metabolite 1



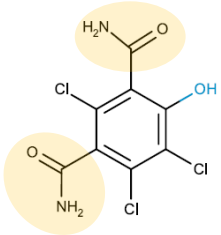
R417888
Main metabolite 2



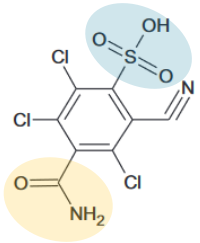
SYN507900 R611968
isobaric, chromatographic separation needed



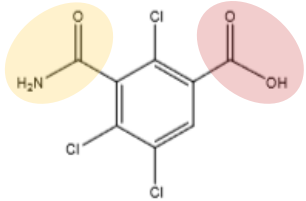
4-Hydroxy



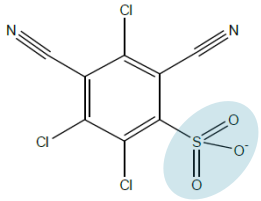
SYN548580



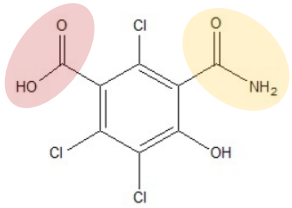
SYN548581



R611965



R611553



M7

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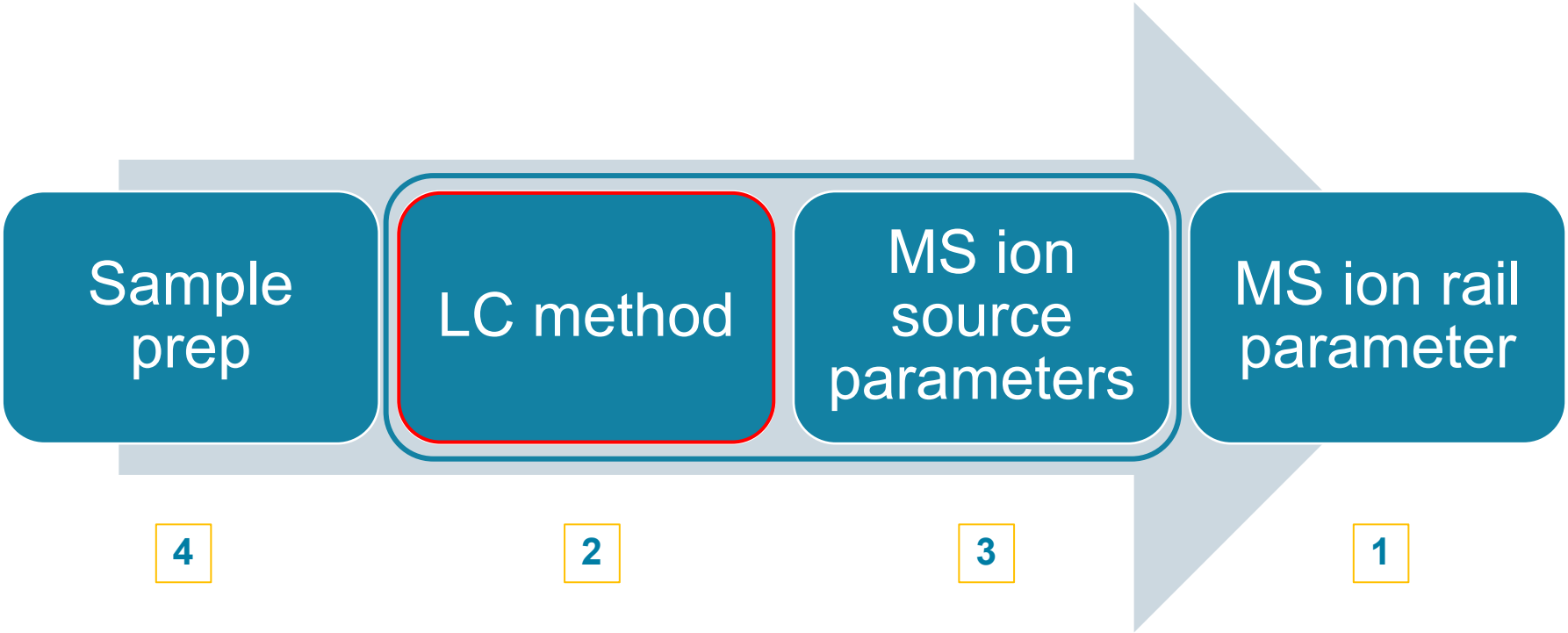
Method development



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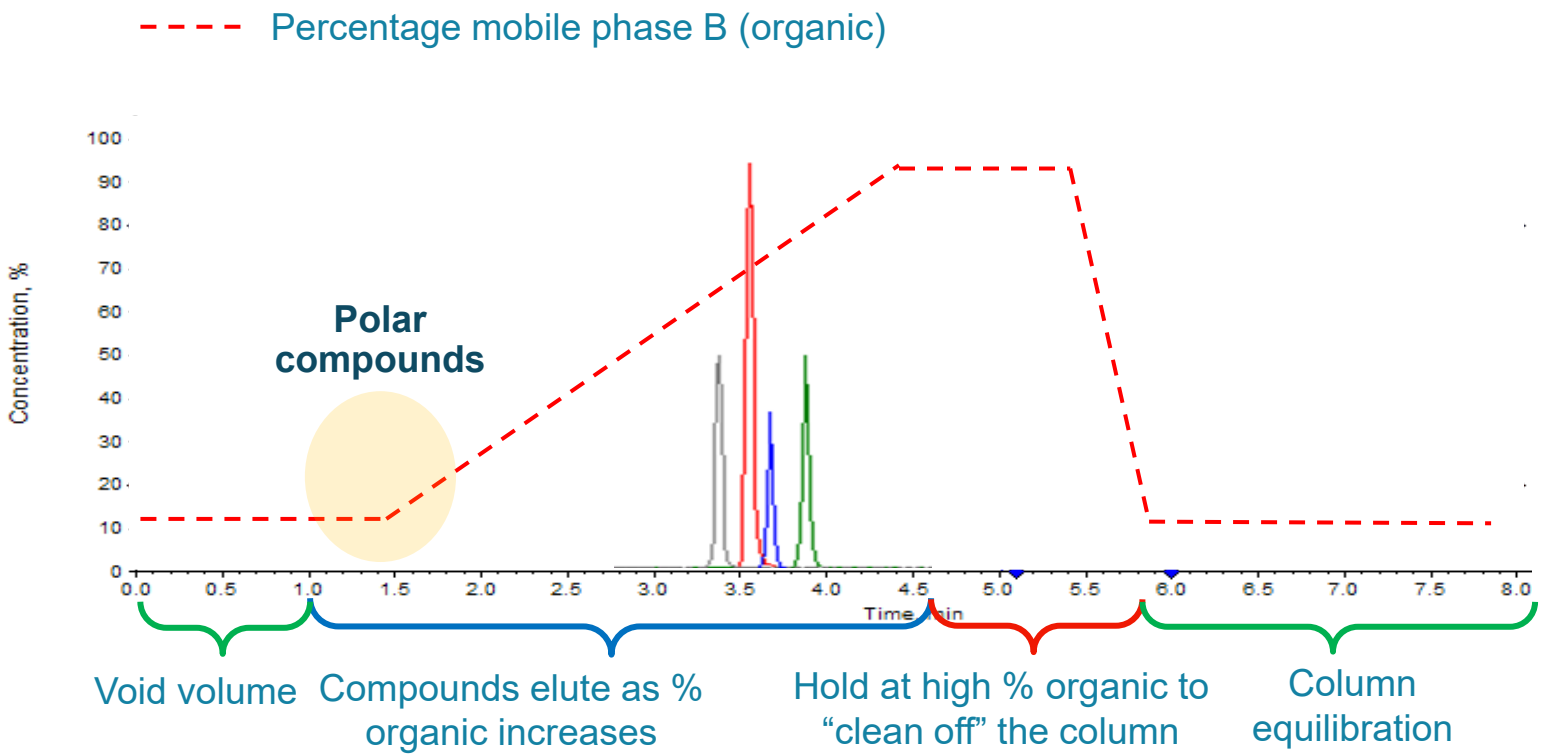
Method design



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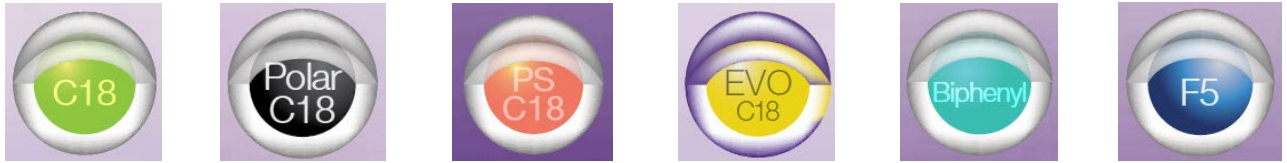
Method development

LC METHOD DESIGN

- LC column and mobile phase screening was performed for the 10 metabolites of interest using the SCIEX Triple Quad 5500 system
- 6 phases and 2 mobile phase systems were investigated



- Requirements
  - Retention of polar compounds
  - Elution of all peaks
  - Acceptable peak intensity and peak shape
- Best results: Kinetex Polar C18 100 x 4.6 mm, mobile phase system with acetic acid

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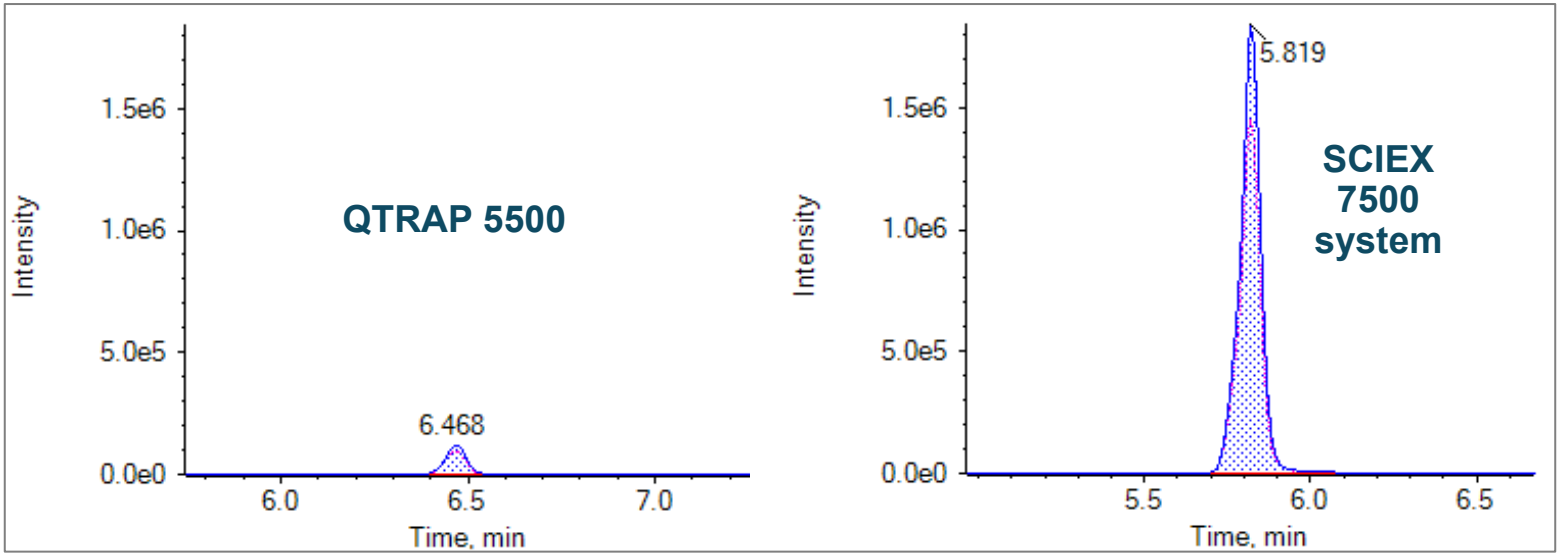
Method transfer

From the SCIEX Triple Quad 5500 system to the SCIEX 7500 system



R417888 metabolite at 100 ng/L in solution. Intensity increase of 15x

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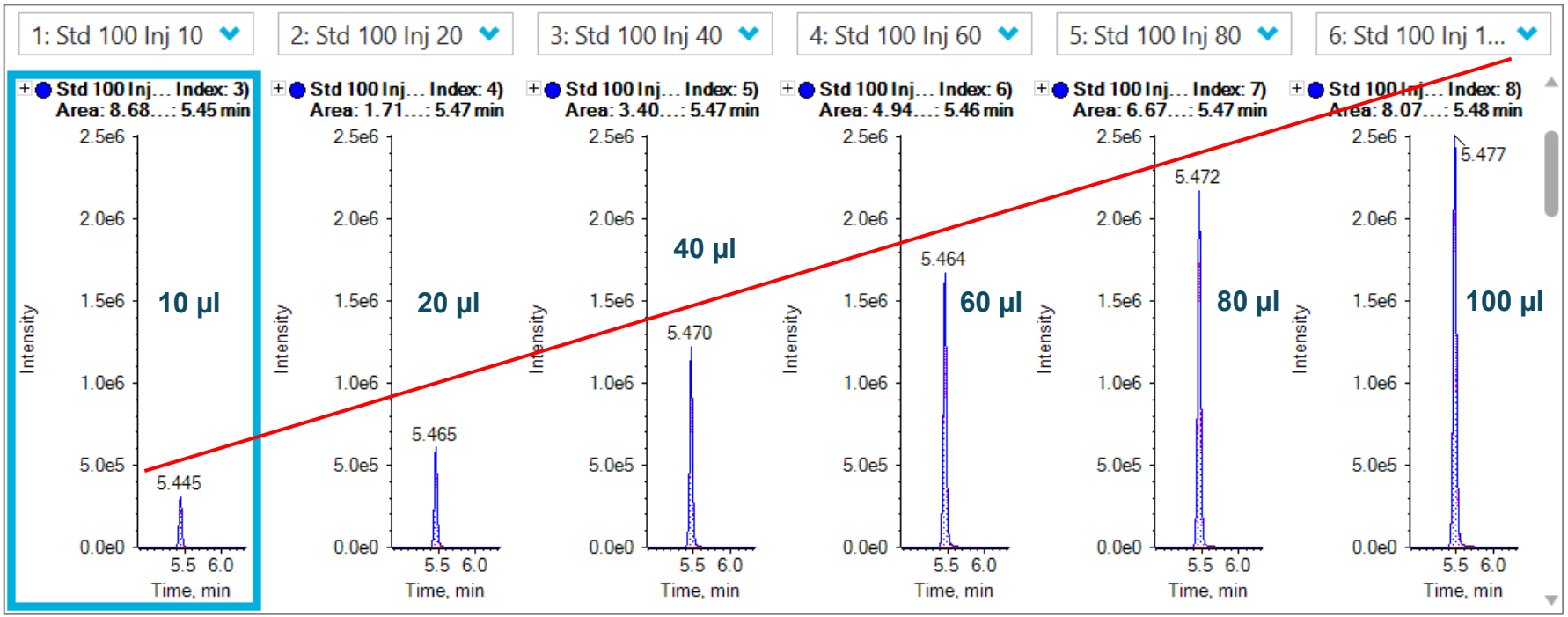
Loading optimization

R417888: LINEAR INCREASE WITH INJECTION VOLUME



Loading behavior of R417888 (non-polar metabolite)

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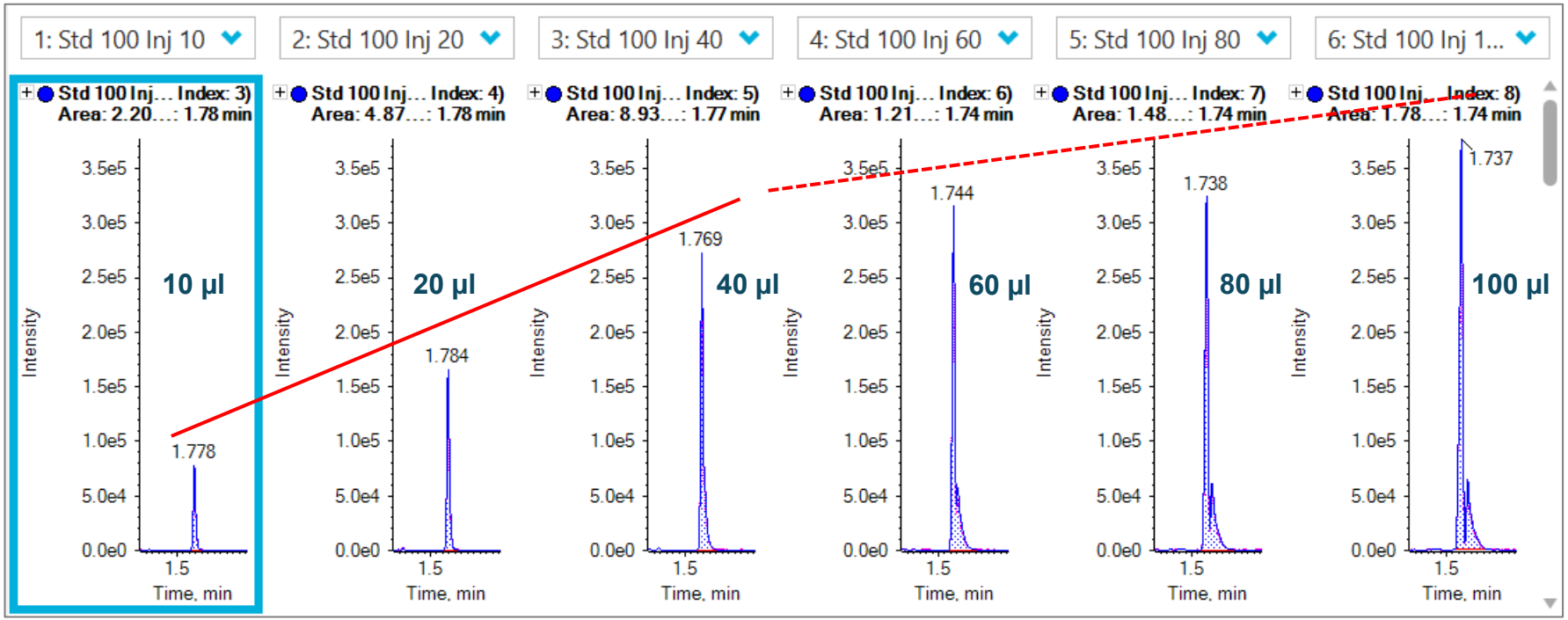
Loading optimization

R471811: VOLUME OVERLOADING, PEAK SPLITTING



Loading behavior of R471811 (polar metabolite)

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Method development

FINAL LC METHOD

- Column — Phenomenex Kinetex Polar C18 (2.6 µm, 100 Å, 100 x 4.6 mm)
- Mobile phase A — 0.05% acetic acid in water
- Mobile phase B — acetonitrile/methanol (2/1 ratio)
- Flow rate — 900 µl/min
- Oven temperature — 40 ºC
- Total run time — 17 min
- Injection volume — **20 µL**

Time (min)	%A	%B
0.0	98	2
1.0	98	2
11.0	0	100
13.0	0	100
13.1	98	2
17.0	98	2

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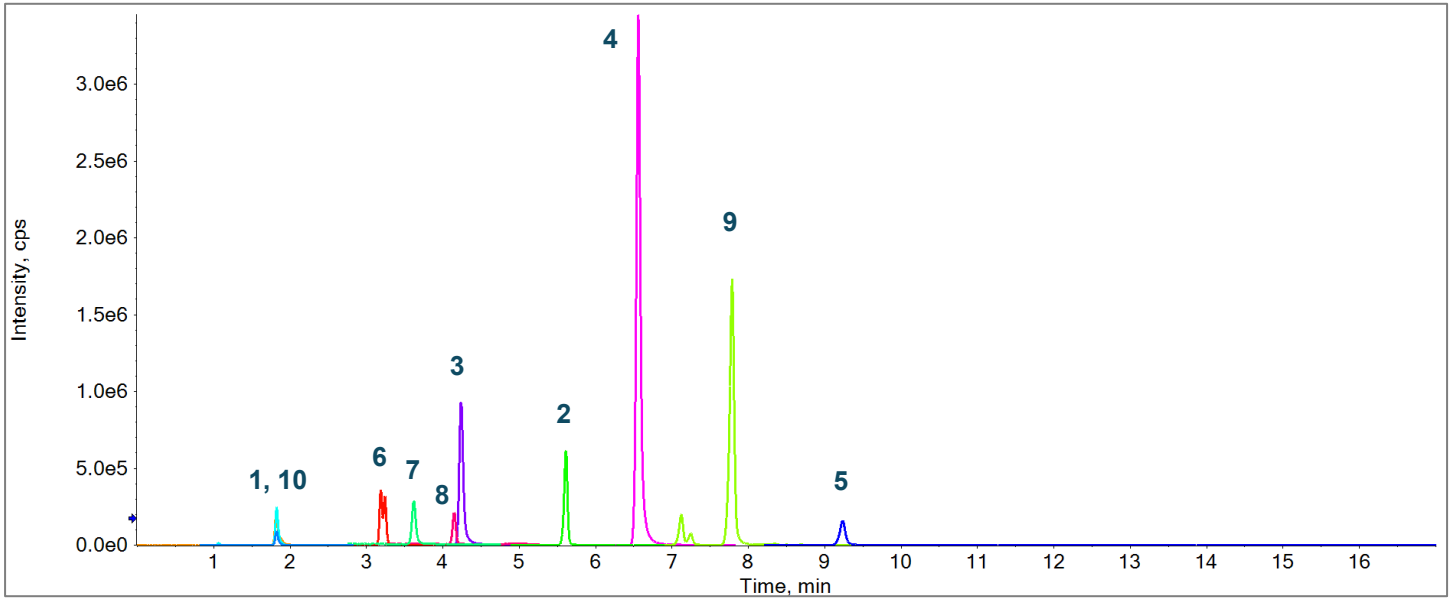
Final chromatography method

XIC OVERLAY OF 10 METABOLITES. INJECTION VOLUME: 20 µL



Chromatographic profile of the 10 Chlorothalonil metabolites: 100 ng/L neat standard mixture.

1. R471811, 2. R417888, 3. SYN507900, 4. R611968, 5. Chlorothalonil-4OH, 6. SYN548580, 7. SYN548581, 8. R611965, 9. R611553, 10. M7

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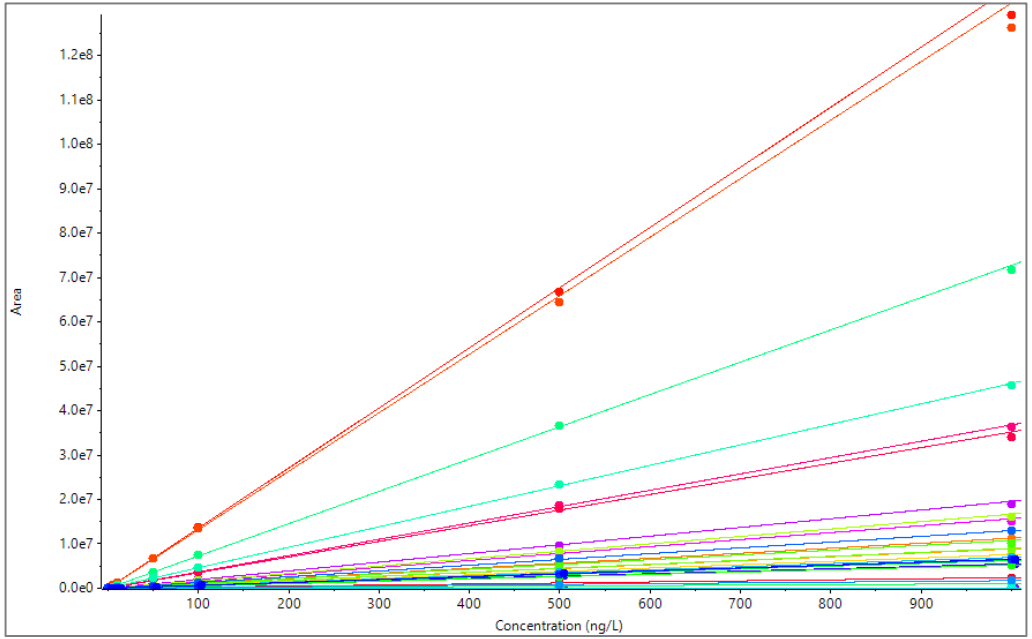
Calibration lines

10 METABOLITES, 2 TO 3 MRM PER COMPOUND



Linearity for 10 metabolites in mineral water. Linear regression curves (weighting: $1/x^2$) resulting from the calibration series of 0.1 to 1000 ng/L

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Method data

10 METABOLITES, FIRST MRM (QUANTIFIER) SHOWN

Compound name	Retention time [min]	Working range [ng/L]	Correlation coefficient [r^2]	RSD [%] 10 ng/L
R471811	1.82	0.5 – 1000 *	0.9988	3.6
R417888	5.60	0.1 – 1000	0.9996	1.1
SYN507900	4.23	0.5 – 1000	0.9990	0.9
R611968	6.54	0.1 – 1000	0.9994	0.5
Chlorthanolnil-4-OH	9.21	0.5 – 1000	0.9997	0.4
SYN548580	3.21	0.5 – 1000	0.9989	1.7
SYN548581	3.61	1 – 1000	0.9985	2.2
R611965	4.14	5 – 1000	0.9959	3.2
R611553	7.77	0.5 – 1000	0.9991	0.5
M7	1.81	1 – 1000	0.9994	2.3

** lower LOQ values have not been assessed due to amount in bottled drinking water used for calibration*

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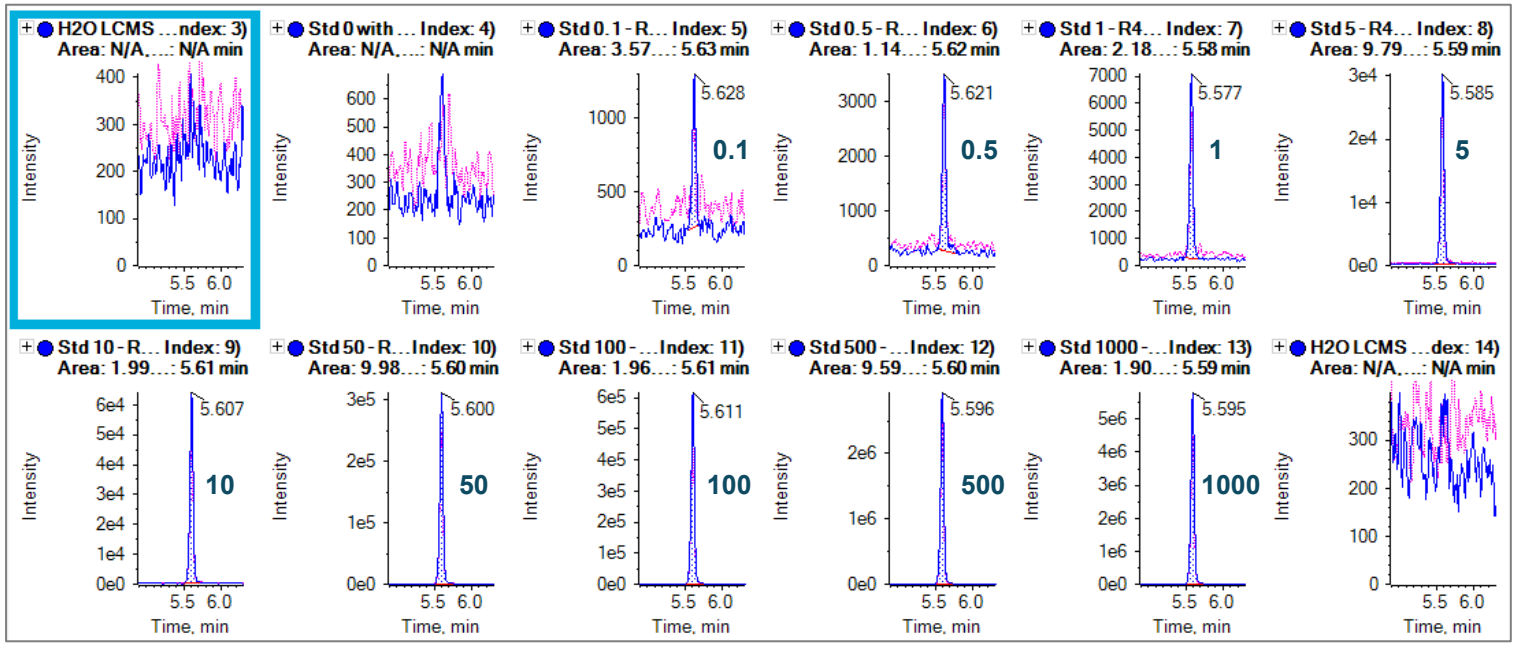
Calibration curve: R417888

CONCENTRATION IN ng/L



Injected concentrations of 0.1 to 1000 ng/L. Extracted ion chromatogram (XIC) overlays of the quantifier and qualifier ions for blank injections and for standards

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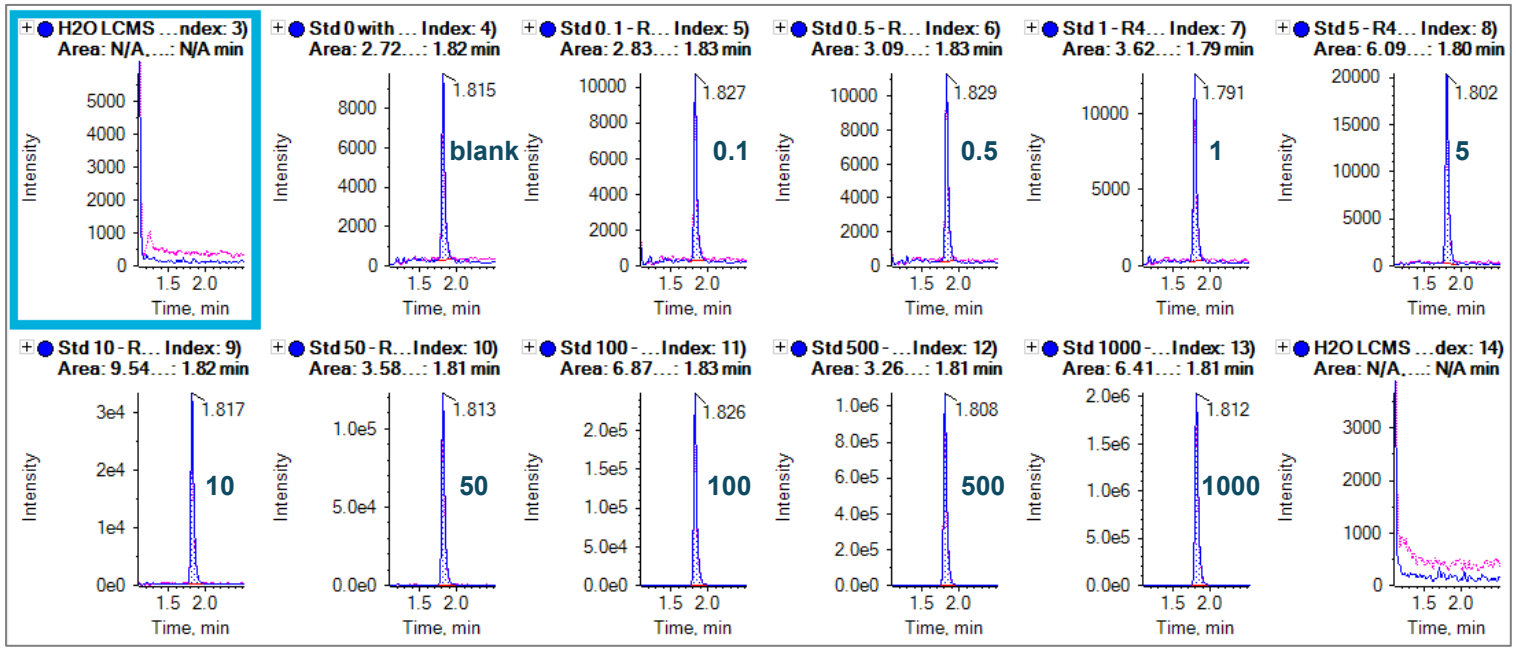
Calibration curve: R471811

CONCENTRATION IN ng/L. BLANK CONCENTRATION IN WATER OBSERVED



Injected concentrations of 0.1 to 1000 ng/L. Extracted ion chromatogram (XIC) overlays of the quantifier and qualifier ions for blank injections and for standards. There is a peak in the un-spiked water sample (std 0)

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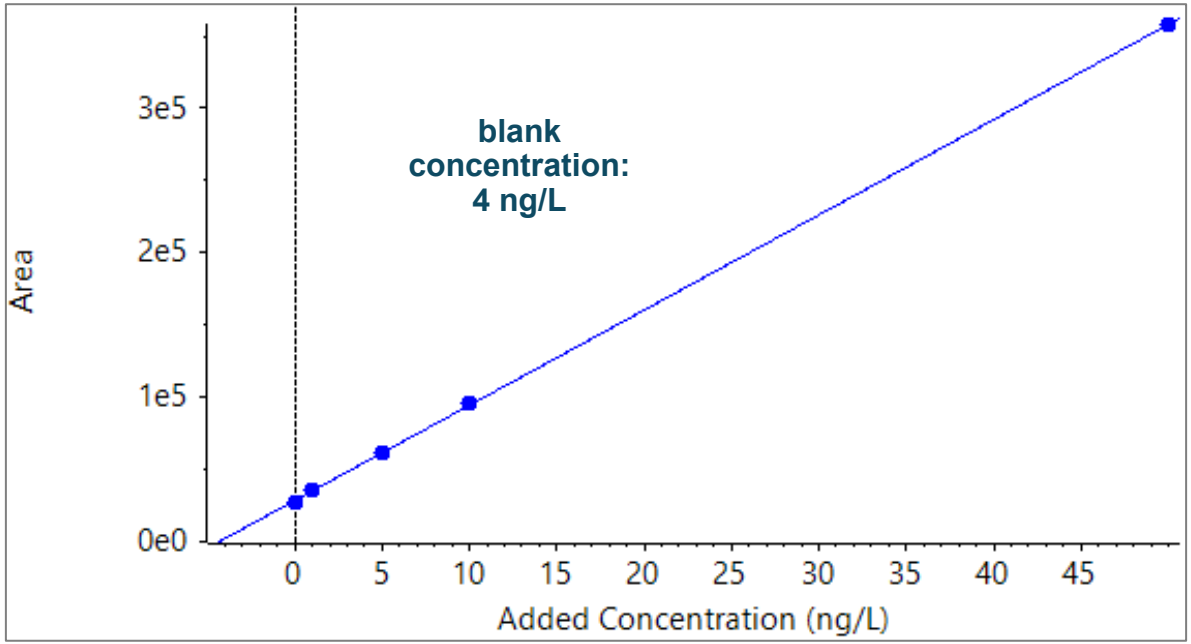
Blank concentration: R471811

QUANTIFICATION USING EMBEDDED STANDARD ADDITION



Injections from the calibration line calculated as standard addition. Concentrations: 1, 5, 10 and 50 ng/L. An r^2 value of 0.9994 was obtained.

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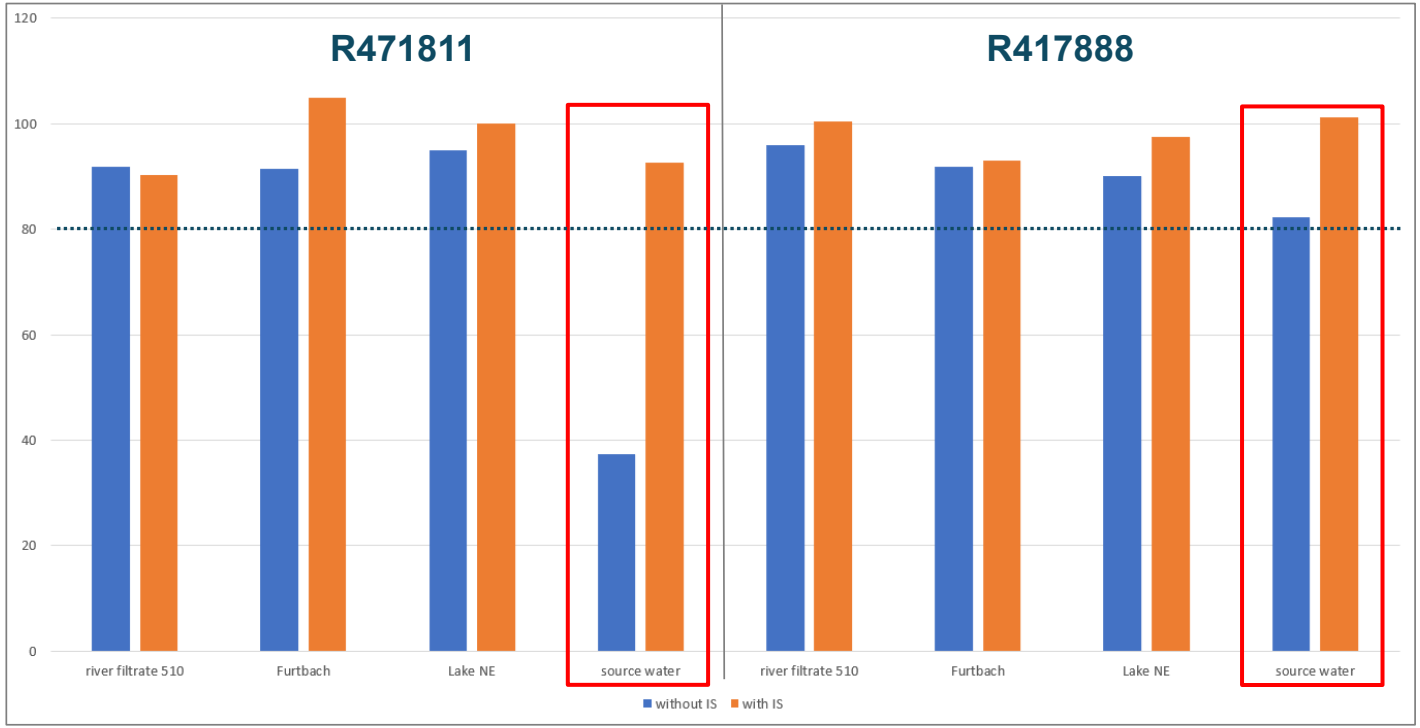
Recovery with and without ISTD



Calculation without internal standard

Calculation with internal standard

R471811 (left) and R417888 (right) in 4 spiked samples

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Method performance and results

ANALYSIS OF CHLOROTHALONIL METABOLITES IN MULTIPLE WATER MATRICES

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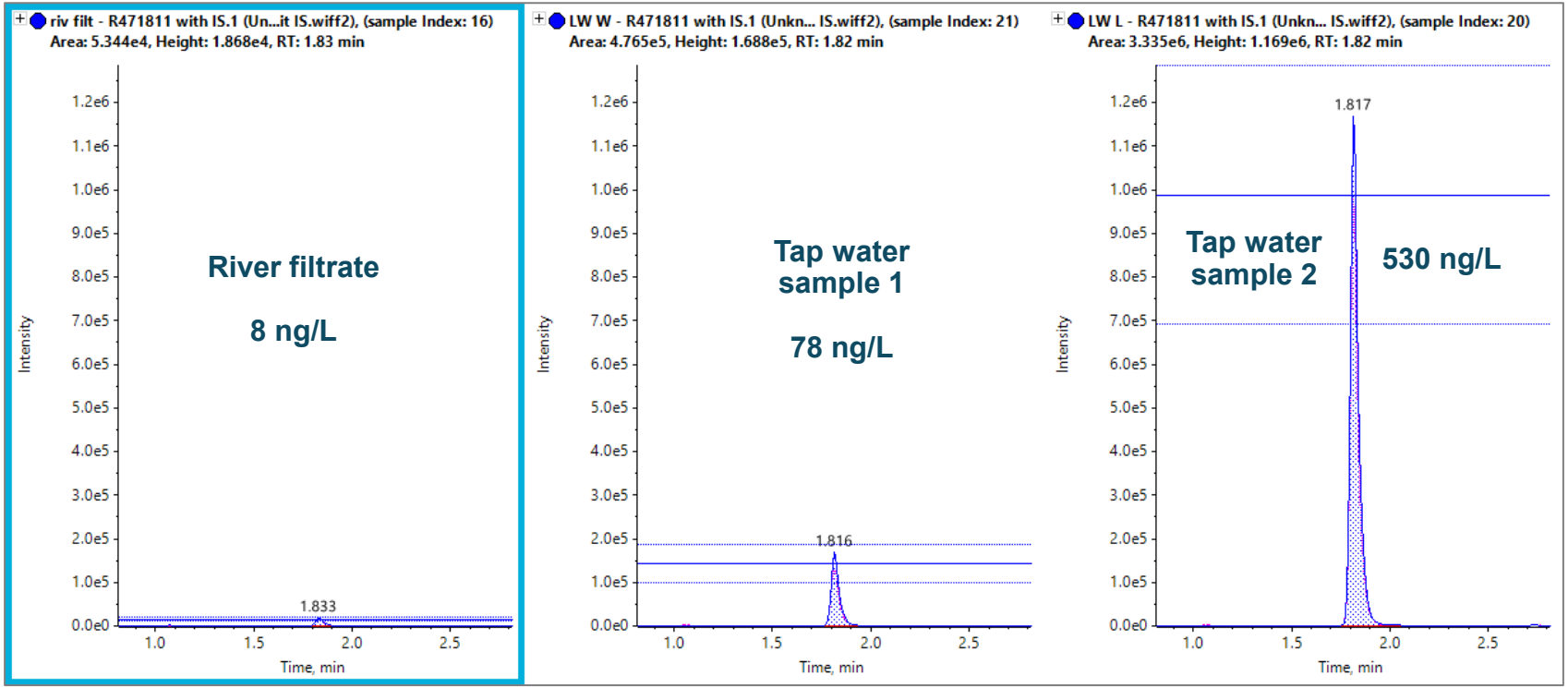
Quantification of R4171811 in tap water and river filtrate



R471811 in tap water and river filtrate. Concentrations between 8 and 530 ng/L were detected

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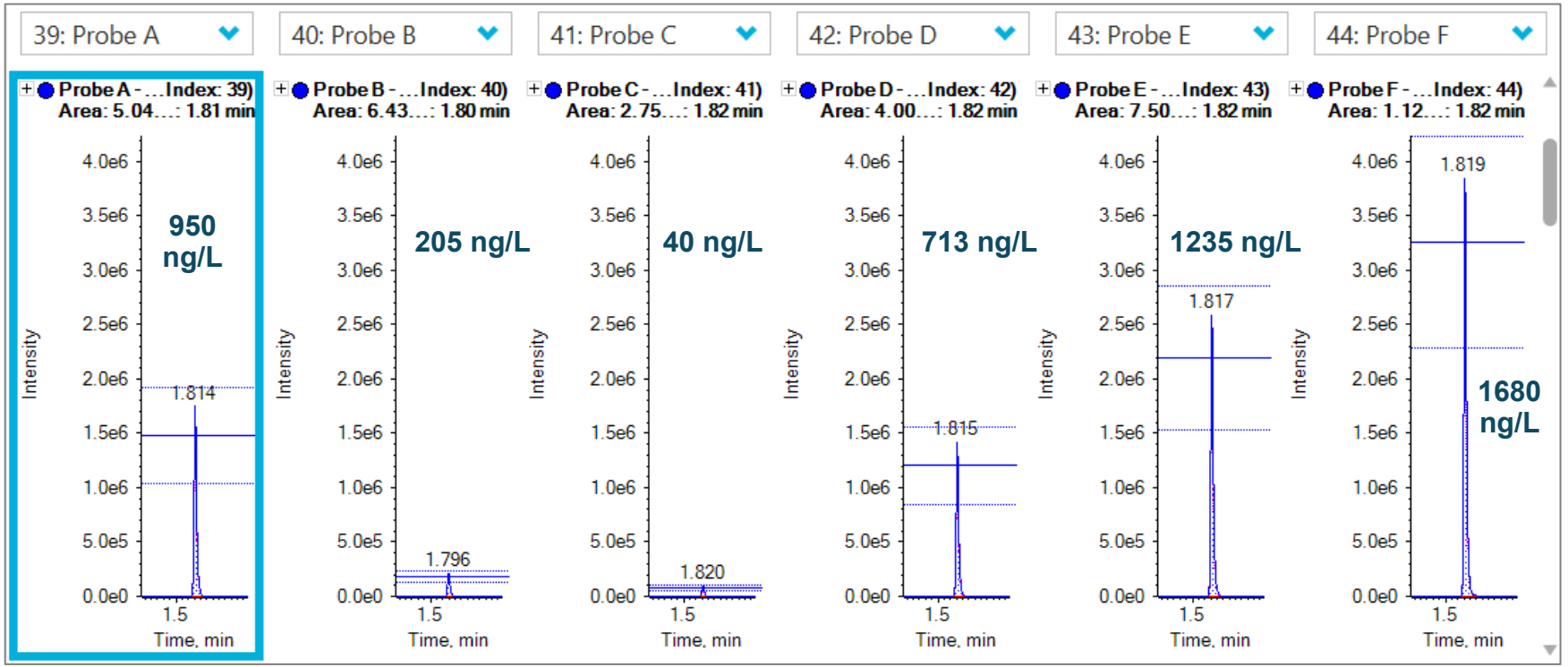
Quantification of R471811 in source and ground water



R471811 in 6 water samples from the midland region. Concentrations between 40 and 1680 ng/L were detected.

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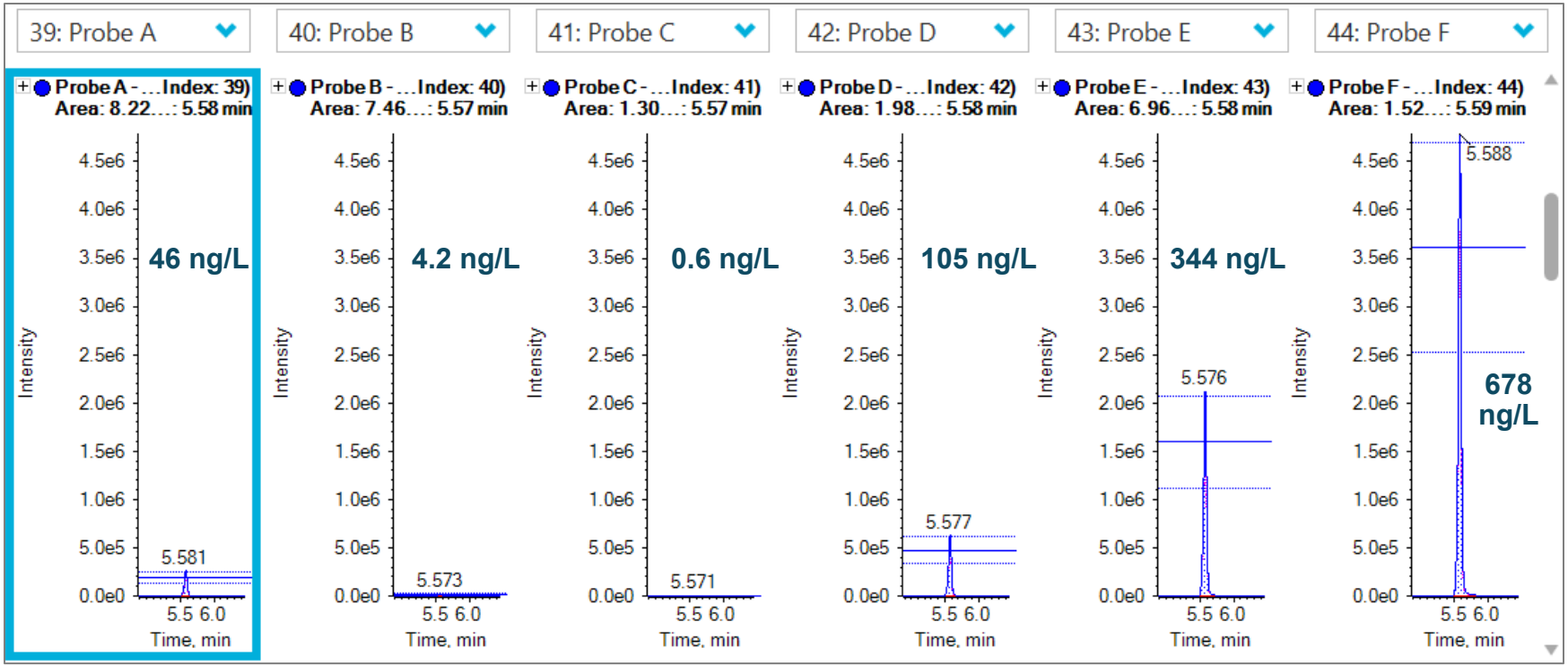
Quantification of R417888 in source and ground water



R417888 in 6 water samples from the midland region. Concentrations between 1 and 678 ng/L were detected.

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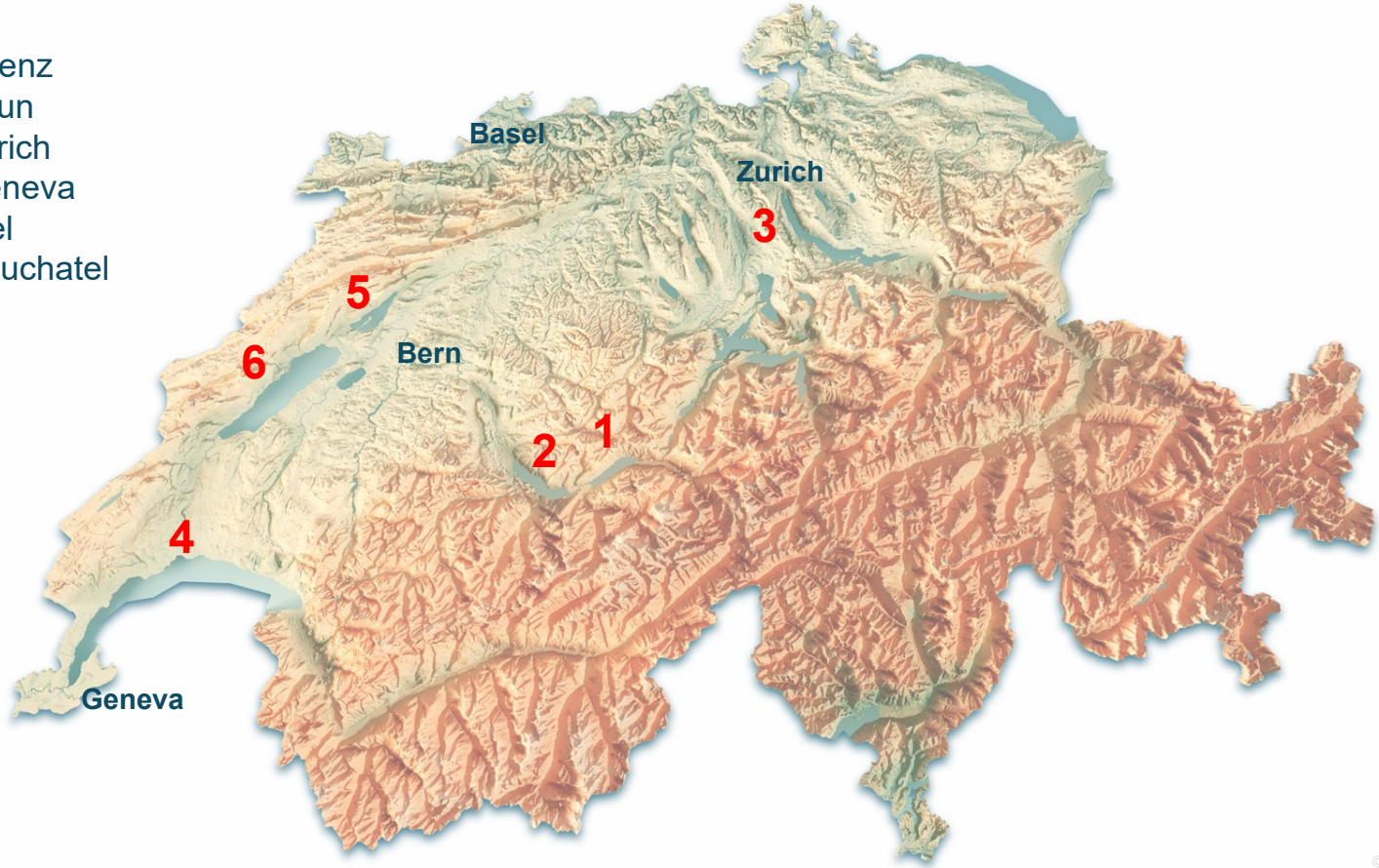
Swiss lakes

1. Lake Brienz
2. Lake Thun
3. Lake Zurich
4. Lake Geneva
5. Lake Biel
6. Lake Neuchatel



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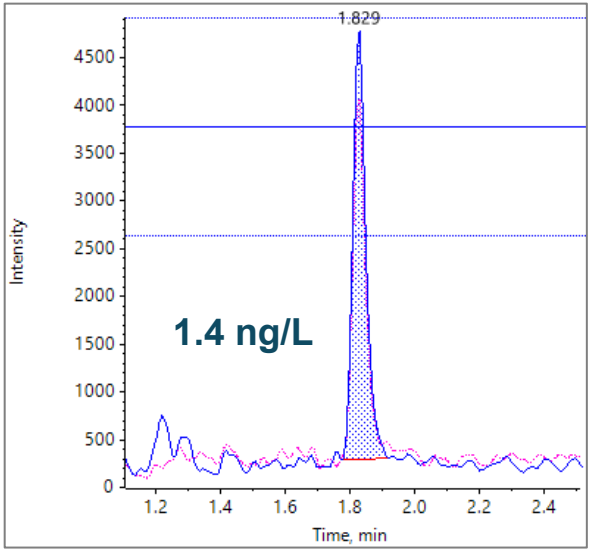
Swiss lakes

LAKE BRIENZ



Chlorothalonil R471811 in lake Brienz water analyzed on the SCIEX 7500 system

https://unsplash.com/photos/pm7SlYMMbZg

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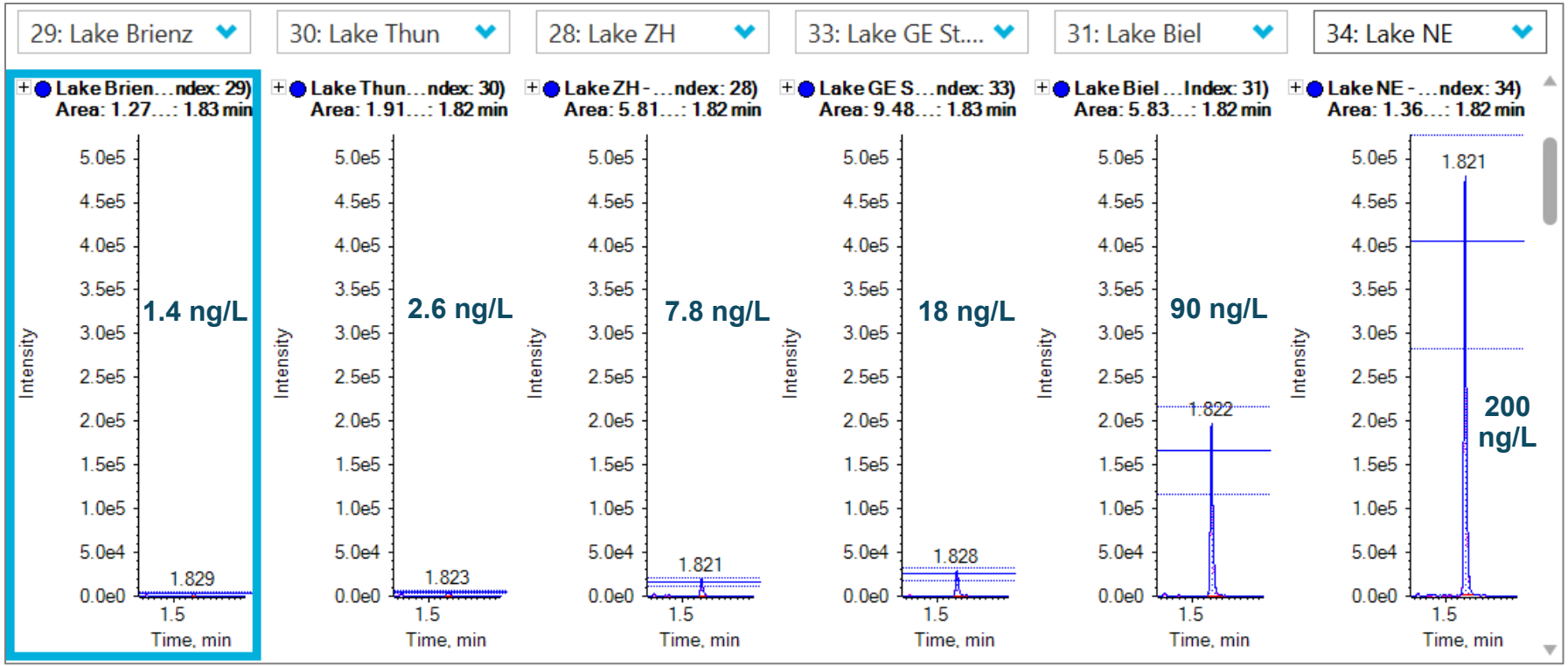
Quantification of R471811 in Swiss lake water



Lake Brienz, Lake Thun, Lake Zurich, Lake Geneva, Lake Biel and Lake Neuchatel. Concentration in ng/L.

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Overall metabolite occurrence

- R471811 and R417888 often found and at high concentrations
- If these two metabolites are present at high concentrations: SYN507900, chlorothalonil- 4OH, R611968, SYN548580 and SYN548581 were also detected, but at much lower levels
- Metabolites M7, R611553 and R611965 not observed in any of the samples analyzed

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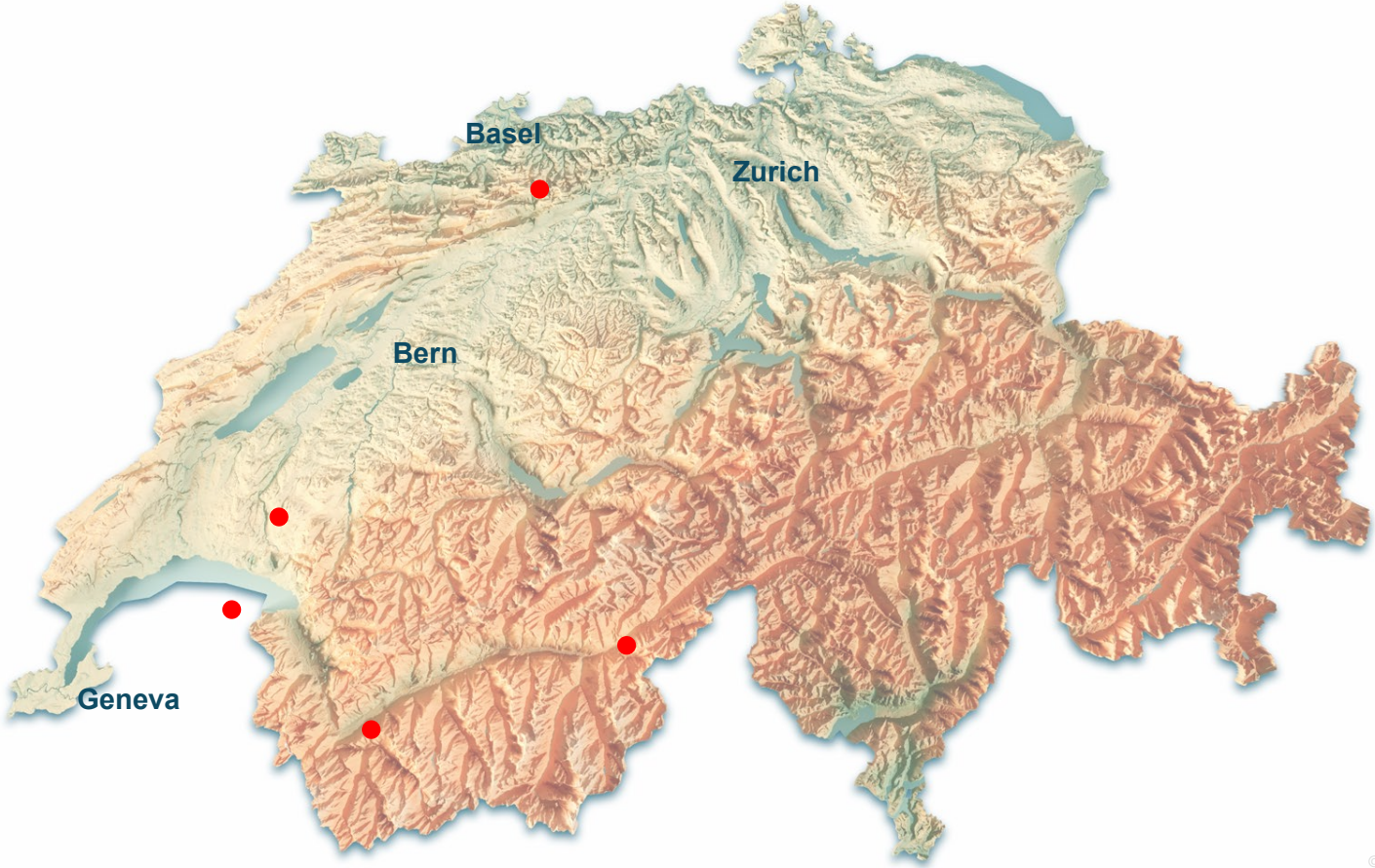
Bottled drinking water (mineral water)



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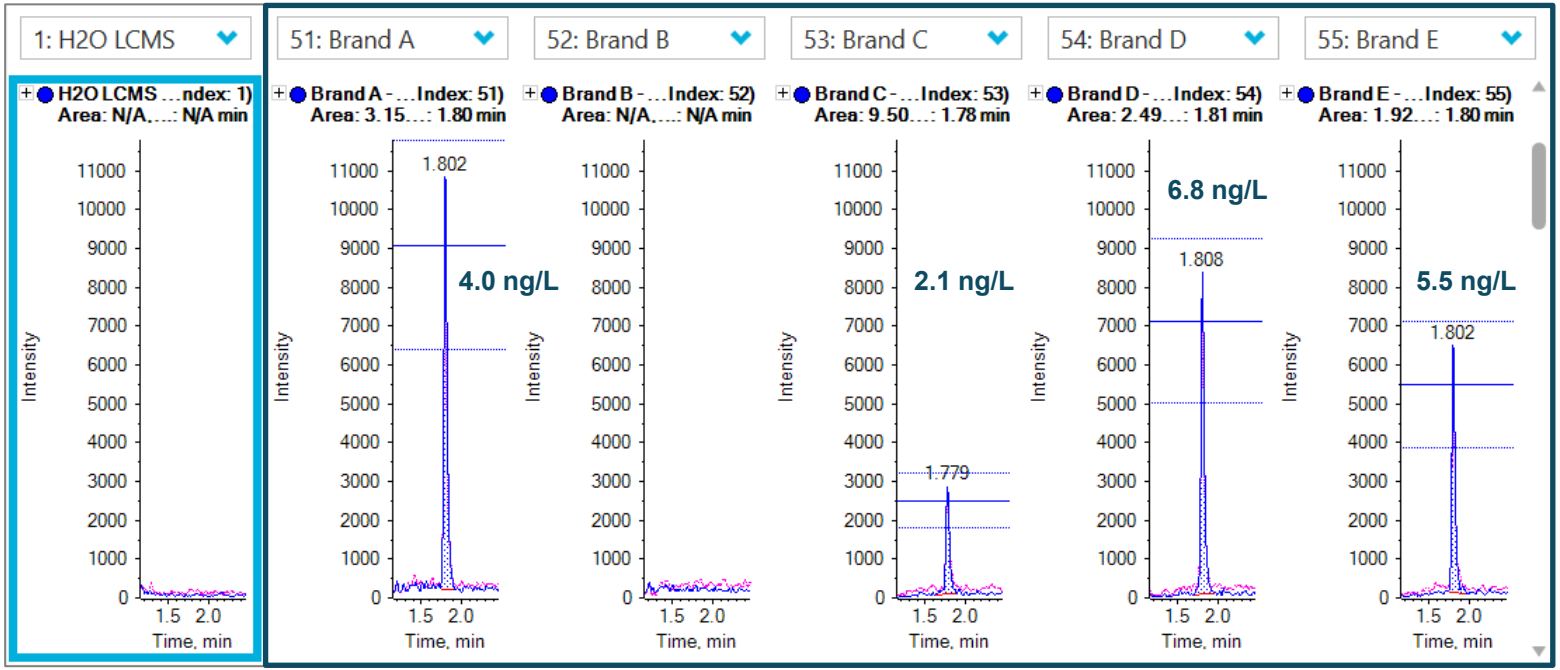
Quantification of R471811 in bottled drinking water



total mineral content [mg/L]	N/A (LCMS water)	345	571	909	1650	2222

Bottled drinking water, with the corresponding mineral content beneath. Left to right: LC-MS water, and A-B-C-D-E = 5 commercial mineral water samples bought in the supermarket.

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Summary

- Method design
  - A simple direct injection method has been developed, without need of further sample concentration steps
- Sensitivity
  - Chlorothalonil metabolites can be analyzed with ultra-high sensitivity down to 0.1 ng/L with only a 20 µL injection volume using the SCIEX 7500 system
- Ion suppression and recovery
  - Ion suppression effects are minimized by using a low injection volume, compared to traditional 100 µL
  - If high mineral content is present it is recommended to use an IS for R471811
- Versatility for a large variety of water samples
  - Excellent recovery in drinking water, mineral water, surface water, ground water and river filtrate
- The difficulty of finding a clean bottled water source for calibration
  - R471811 can be considered as a new “forever” chemical. Typical bottled water brand used was seen to be contaminated. Therefore, an alternative brand needs to be found.

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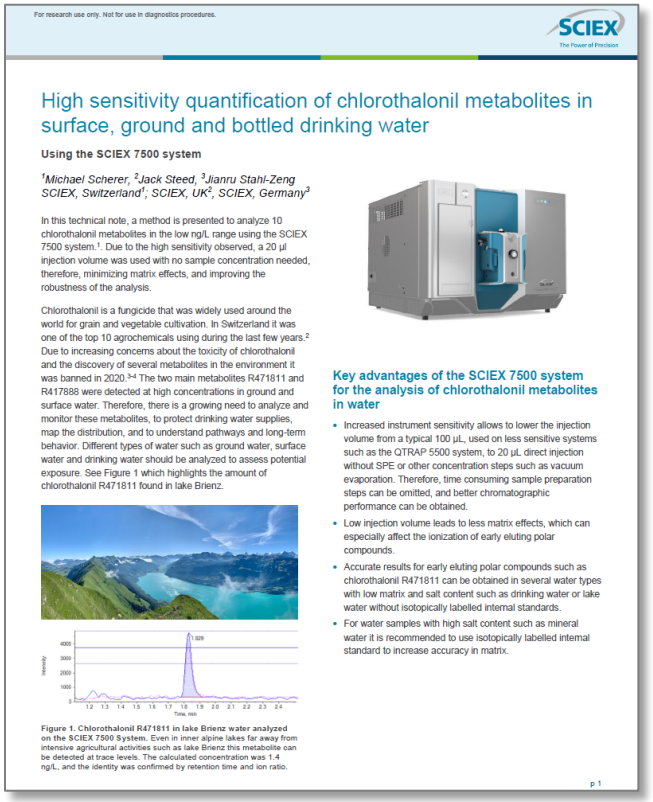
Application note



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SCIEX
The Power of Precision

High sensitivity quantification of chlorothalonil metabolites in surface, ground and bottled drinking water

Using the SCIEX 7500 system

[1]Michael Scherer, [2]Jack Steed, [3]Jianru Stahl-Zeng
SCIEX, Switzerland[1]; SCIEX, UK[2]; SCIEX, Germany[3]

In this technical note, a method is presented to analyze 10 chlorothalonil metabolites in the low ng/L range using the SCIEX 7500 system.[1]. 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.

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.[2] Due to increasing concerns about the toxicity of chlorothalonil and the discovery of several metabolites in the environment it was banned in 2020.[3,4] The two main metabolites R471811 and R417888 were detected at high concentrations in ground and surface water. Therefore, there is a growing need to analyze and monitor these metabolites, to protect drinking water supplies, map the distribution, and to understand pathways and long-term behavior. Different types of water such as ground water, surface water and drinking water should be analyzed to assess potential exposure. See Figure 1 which highlights the amount of chlorothalonil R471811 found in lake Brienz.

Figure 1. Chlorothalonil R471811 in lake Brienz water analyzed on the SCIEX 7500 System. Even in inner alpine lakes far away from intensive agricultural activities such as lake Brienz this metabolite can be detected at trace levels. The calculated concentration was 1.4 ng/L, and the identity was confirmed by retention time and ion ratio.

Key advantages of the SCIEX 7500 system for the analysis of chlorothalonil metabolites in water

- Increased instrument sensitivity allows to lower the injection volume from a typical 100 µL, used on less sensitive systems such as the QTRAP 5500 system, to 20 µL direct injection without SPE or other concentration steps such as vacuum evaporation. Therefore, time consuming sample preparation steps can be omitted, and better chromatographic performance can be obtained.
- Low injection volume leads to less matrix effects, which can especially affect the ionization of early eluting polar compounds.
- Accurate results for early eluting polar compounds such as chlorothalonil R471811 can be obtained in several water types with low matrix and salt content such as drinking water or lake water without isotopically labelled internal standards.
- For water samples with high salt content such as mineral water it is recommended to use isotopically labelled internal standard to increase accuracy in matrix.

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Application note which includes more details

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  - Service de l'Energie et de l'environnement, Neuchatel

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