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Environmental testing

A robust and sensitive method for the determination of pesticides in surface and ground water by triple quadrupole GC-MS

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Goal
The aim of this study is to demonstrate the suitability of a robust and sensitive analytical method for the determination of pesticides in surface and ground water to meet the demands of analytical testing laboratories for increased productivity and return of investment.

Keywords
EU Directive, pesticide residues, contract testing laboratories (CTLs), TraceGOLD TG-Contaminants column, ThermoSpray injector (TSI), robustness, gas chromatography, triple quadrupole mass spectrometer

Challenges	Benefits
Fast run time to analyze around 350 pesticides residues[1]	Increase throughput of samples
	Increase the productivity
	Simplify method for an easier quality control process
	Decrease the turnaround time
Higher sensitivity	Applicable to most regulations
Higher robustness	Reduce system maintenance
	Ease of use of the instrument
	Increase productive instrument time
	Cost-efficiency per sample

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Introduction

Pesticides are widely used in agriculture—privately and commercially to protect crops. Therefore, their usage is continually expanding, posing risks for human health and the environment.[2] Pesticides have different chemical and physical properties that drive the need to develop a generic method that can be applied to a diverse analyte list in different sample types. Developing such a method requires a large amount of effort and requires a labor-intensive validation procedure.

A fast, sensitive, and robust method for multiclass analysis of pesticides residues by GC-MS/MS[1] was developed to address the challenges of analytical testing laboratories by optimizing four critical parameters including liner choice, injection parameters, effect of analytical protectants, and dwell-time prioritization mode for time-dependent selected reaction monitoring (*t*-SRM) acquisition.

Experimental

In the experiments described here, a Thermo Scientific™ TSQ™ 9610 triple quadrupole mass spectrometer equipped with the Thermo Scientific™ NeverVent™ Advanced Electron Ionization (AEI) ion source was coupled to a Thermo Scientific™ TRACE™ 1610 gas chromatograph. The GC system was equipped with a Thermo Scientific™ iConnect™ Thermospray SSL (iConnect TSI) injector. The NeverVent AEI ion source provides high sensitivity and improved robustness for trace and ultra-trace detection and quantitation of pesticides. Combined with the extended dynamic range and detector lifetime provided by the Thermo Scientific™ XLXR™ detector, the TSQ 9610 triple quadrupole GC-MS/MS system is the perfect choice for this analysis. The NeverVent technology allows the ionization source to be removed, column to be replaced, and filaments to be exchanged without breaking instrument vacuum, enabling minimal downtime. The unique Thermo Scientific™ EvoCell collision cell provides transition speeds of up to 800 SRMs/s, allowing users to schedule several thousand unique SRM transitions in a single run without compromising sensitivity.

Instrument and method performance were evaluated by assessing the sensitivity for approximately 350 compounds, the quantitative performance, and the robustness by injecting n=70 surface water extracts followed by quality controls (QCs). Optimized GC-MS/MS and injection parameters as well as a list of the target compounds are detailed in Tables 1 and 2, respectively.

Table 1. Optimized GC-MS/MS and injection parameters

TRACE 1610 GC parameters	
Injection volume (µL)	3
Solvent	DCM + PEG (analyte protectant)
Injection temperature (°C)	200
Liner	Thermo Scientific™ LinerGold™, 5 mm x 105 mm (**P/N 45350033-UI**)
Inlet module and mode	TSI, split with surge
Carrier gas, flow (mL/min)	He, 1.2
GC run time (min)	18.33
Chromatographic column	Thermo Scientific™ TraceGOLD™ TG-Contaminants 15 m, 0.25 mm, 0.10 µm (**P/N 26056-0350**)
TSQ 9610 mass spectrometer parameters	
Ion source type and temperature (°C)	NeverVent AEI, 250
Ionization type	EI
Emission current (µA)	50
Acquisition mode	*t*-SRM
Electron energy (eV)	35
Dwell time prioritization	Enabled, 10

Table 2. List of representative target compounds, current established LOQs, and calculated LOQs for sample extracts (concentration factor = 500)

Compounds	CAS number	Class	LOQ (µg/L) in water	LOQ (µg/L) in extract
4,4-DDT	50-29-3	Organochlorine	0.003	1.5
Acetochlor	34256-82-1	Chloroacetanilides	0.005	2.5
Bifenox	42576-02-3	Diphenylether	0.01	5
Bromacil	314-40-9	Uracil	0.03	15
Chlordecone	143-50-0	Organochlorine	0.03	15
Chlorpyrifos	2921-88-2	Organophosphorus	0.01	5
Deltamethrin	52918-63-5	Pyrethroid	0.001	0.5
Diflufenican	83164-33-4	Carboximide	0.003	1.5
Endrin	72-20-8	Organochlorine	0.002	1
Ethofumesate	26225-79-6	Benzofuran	0.03	15
Flonicamid	36734-19-7	Pyridinecarboxamide	0.03	15
Iprodione	36734-19-7	Dicarboximide	0.01	5
Lenacil	2164-08-1	Uracil	0.03	15
Malathion	121-75-5	Organophosphorus	0.005	2.5
Pendimenthalin	40487-42-1	Dinitroaniline	0.01	5
Prochloraz	67747-09-5	Imidazole	0.01	5
Propiconazole	60207-90-1	Triazole	0.01	5
Pyrimethanil	53112-28-0	Anilinopyrimidine	0.03	15
Quinoxyfen	124495-18-7	Quinoline	0.03	15
Simazine	122-34-9	Triazine	0.01	5
Trifluralin	1582-09-8	Dinitroaniline	0.01	5

Results and discussion

Optimizing injection critical parameters

When developing a new method, especially for a high number of individual compounds, the selection of the proper liner is critical to ensure rapid, uniform vaporization of the sample and efficient transfer onto the head of the GC column as a tight band.[3] Pesticides are a diverse group of compounds with different chemical and physical properties. Some of them, such as deltamethrin and iprodione, are thermolabile, while others, such as 4,4-DDT and endrin, are prone to reaction with the active sites and the packing material in the liner.

The adoption of a highly inert liner without glass wool, such as the Thermo Scientific™ LinerGold™ GC Liner, combined with the application of a higher pressure during the injection, has proven to be effective in reducing sample degradation and interactions with the active sites in the liner. This allows for rapid transfer of the analytes to the analytical column, thus ensuring sample integrity. An analyte protectant such as PEG (polyethylene glycol) 300 (CAS number: 25322-68-3) can be useful to mask the active sites of the liner, minimizing the gradual build-up of non-volatile matrix components in the injection port, improving ruggedness and injection reproducibility, and reducing the need of frequent inlet maintenance.

As an example, the overlaid extracted ion chromatograms (XIC) for iprodione (3 µg/L), deltamethrin (0.5 µg/L), 4,4'-DDT (1 µg/L), and endrin (1 µg/L) after 70 injections of surface water extracts is shown in Figure 1. The use of the LinerGold liner and optimized injection conditions, combined with the use of analyte protectant, ensured efficient transfer of the analytes even at such low concentrations, minimizing the risk of interactions or analyte degradation into the injector port.
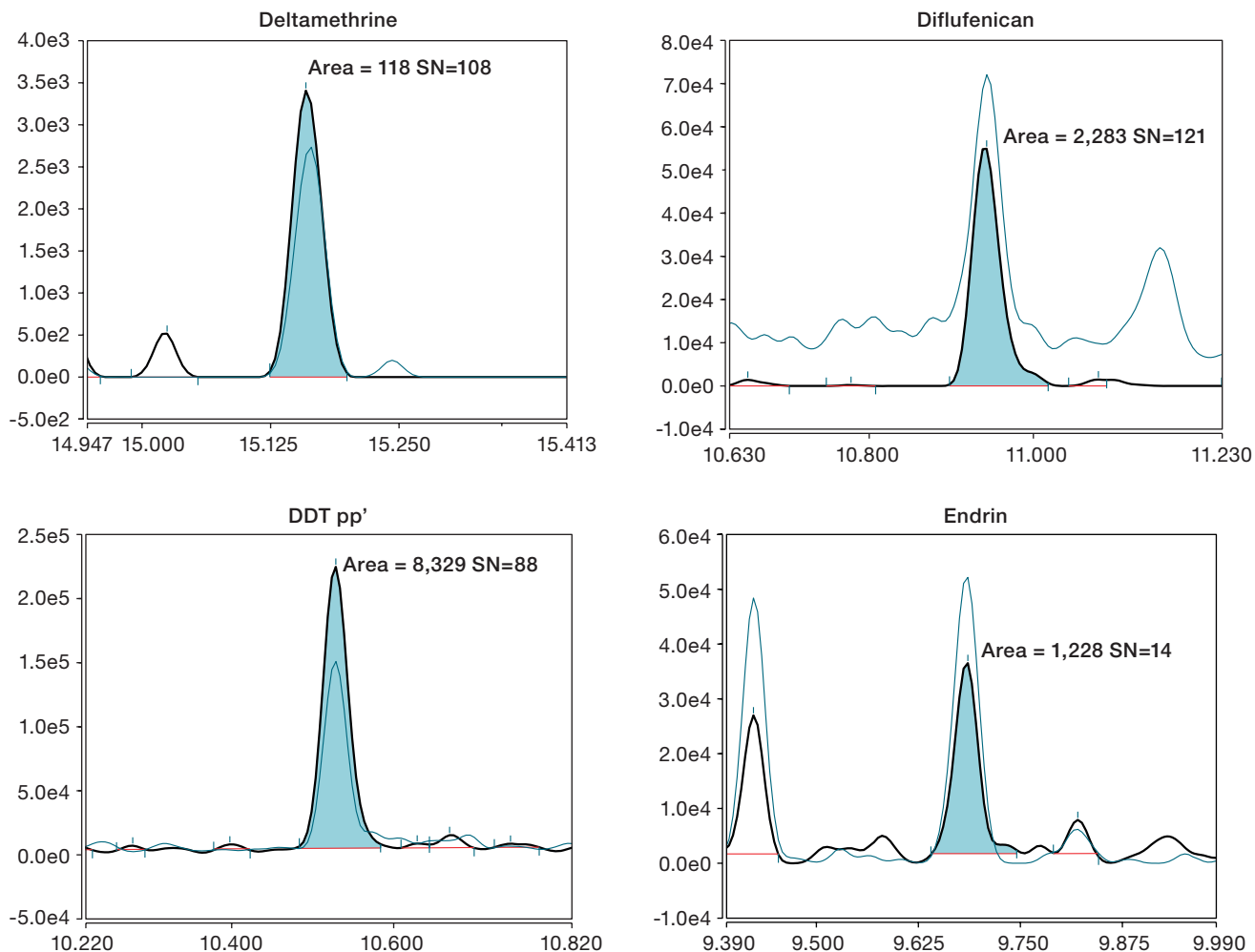
Figure 1. Overlaid XICs (quantifier (black trace) and qualifier ions) for iprodione (3 µg/L), deltamethrin (0.5 µg/L), 4,4'-DDT (1 µg/L), and endrin (1 µg/L) after the injection of 70 water extracts

Sensitivity for ultra-trace level pesticide detection and quantitation

To be compliant with the limits established by ever-evolving regulations, the analysis of pesticides requires sensitive and selective analytical methods to ensure confident detection and identification of target compounds as well as accurate quantitation at trace and ultra-trace concentrations. The use of an iConnect TSI injector enables larger sample volume injections due to its large spray chamber and unique design and helps to improve the sensitivity of the method.

Tips for success include:

- Utilization of an online solvent calculator[4] to verify the possibility of injecting 3 µL of dichloromethane on the TSI. Figure 2 shows the calculation of solvent expansion using the online tool.

- Use of a low energy mass spectrometer tune at 35 eV and prioritization of the dwell times during t-SRM acquisition to ensure enhanced sensitivity and improve signal-to-noise ratio (S/N), allowing for lower limits of detection (LOD) with values up to 3 times less the current established limits.[5]

- Use of dwell time prioritization in the instrumental method. This option allows for target complex compounds in terms of detection to achieve a low LOQ. In fact, in a multiresidue method, this parameter makes it possible to gain a better signal for some targeted compounds and CTLs can avoid creating a specific analytical method for these compounds. Figure 3 shows an example for a pyrethroid compound at 1 µg/L in solvent with and without dwell time prioritization.

Figure 2. Calculation of expansion of DCM using a 3 µL injection
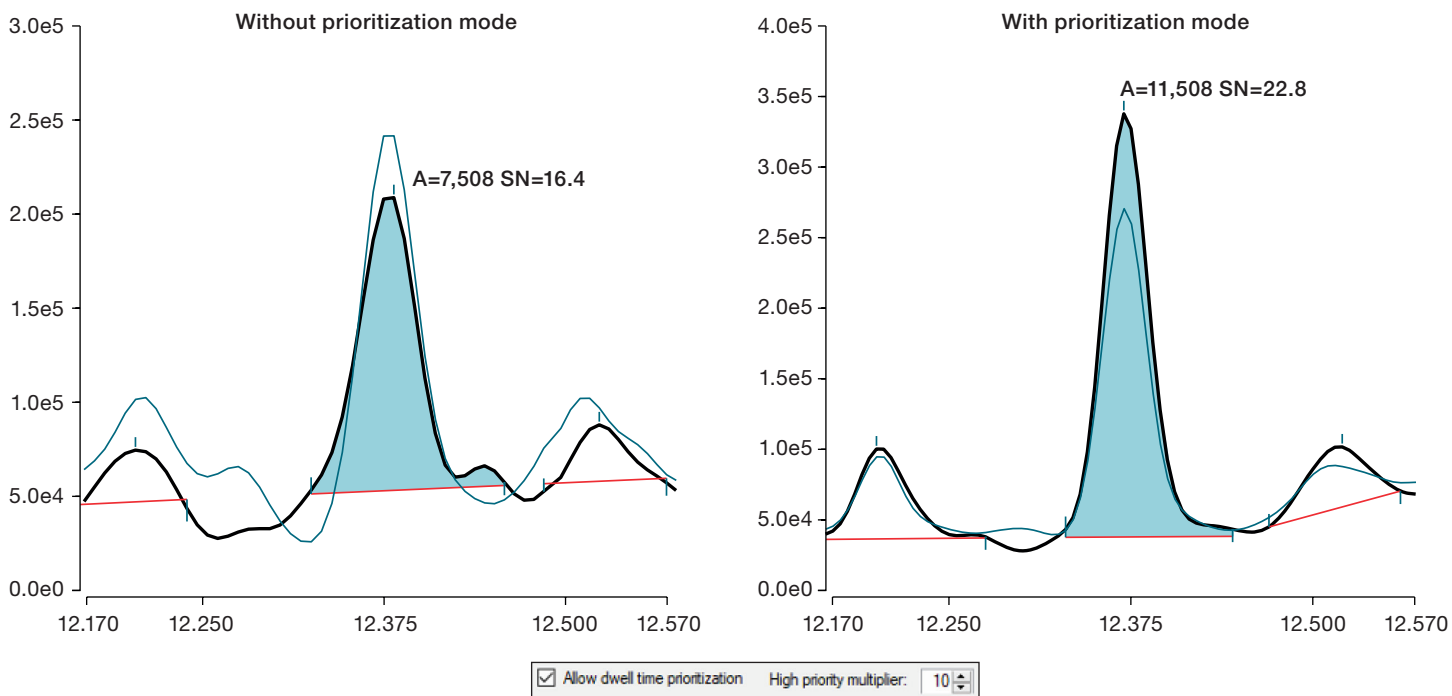

Figure 3. Pyrethroid compound at 1 µg/L in solvent without and with dwell time prioritization

Proof of concept

The following chromatograms present all selected compounds injected at **LOQ values or below after 70 injections of surface water.** Applicable LOQ levels were established following the applicable regulations: LOQs in vial were calculated according to the process of sample preparation (volume of water and concentration factor) corresponding to a concentration factor of 500 in this study (Table 2).

To highlight the performance of this method, chromatograms for a few representative compounds are shown in Figures 2 to 5. Apart from a classification via the different substance classes, the compounds could be classified using the targeted LOQs. Figure 4 shows representative chromatogram for diflufenican, a compound that is typically analyzed with a targeted LOQ of

1 µg/L (Table 2). Other compounds in this classification are endrin and 4,4'-DDT, which were already presented in Figure 1. Figure 5 shows the chromatograms obtained for malathion and acetochlor, two compounds that are normally analyzed with a targeted LOQ of 2 µg/L (see Table 2 for more compounds in this class). Figure 6 contains chromatograms for compounds that are targeted for LOQ levels of 5 µg/L, such as chlorpyriphos, simazine, trifluralin, and propiconazole 1 and 2. Finally, Figure 7 contains chromatograms for the compounds chlordecone, flonicamid, ethofumesate, and bromacil, each typically analyzed with a target LOQ of 15 µg/L. For these compounds, a concentration of only 5 µg/L was injected, which is 3 times lower than the regulated LOQ. Again, additional representative compounds for the individual substance classes are listed in Table 2.
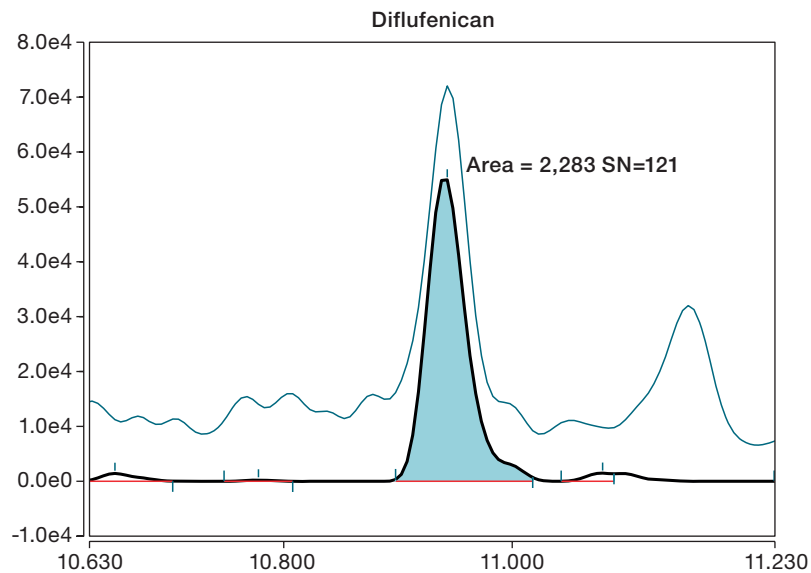

Figure 4. Overlaid XICs (quantifier (black trace) and qualifier ions) for diflufenican (1 µg/L) after 70 injection of surface water extracts
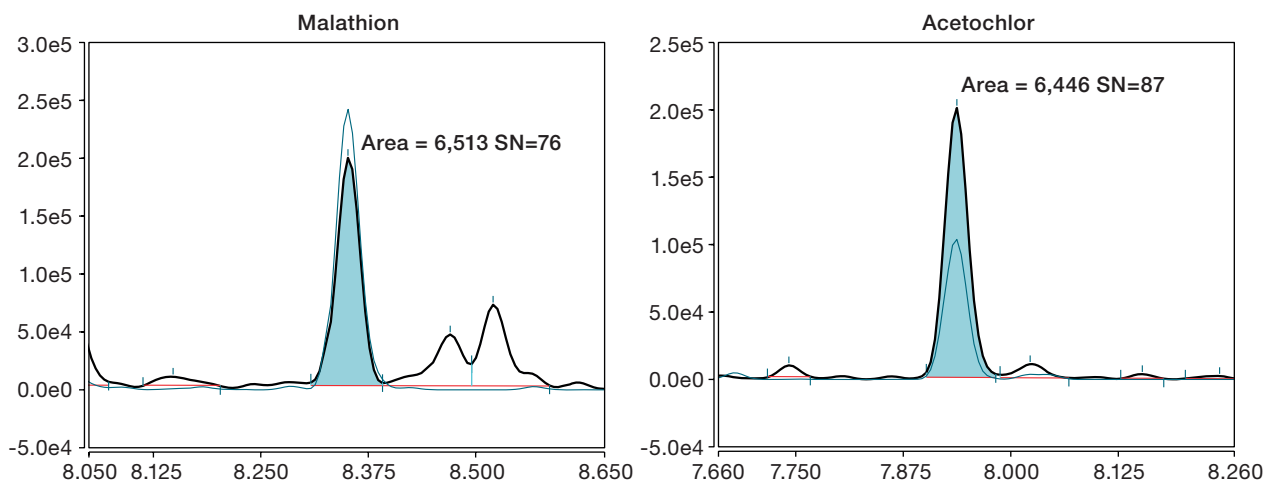

Figure 5. Overlaid XICs (quantifier (black trace) and qualifier ions) for malathion (2 µg/L) and acetochlor (2 µg/L) after 70 injection of surface water extracts
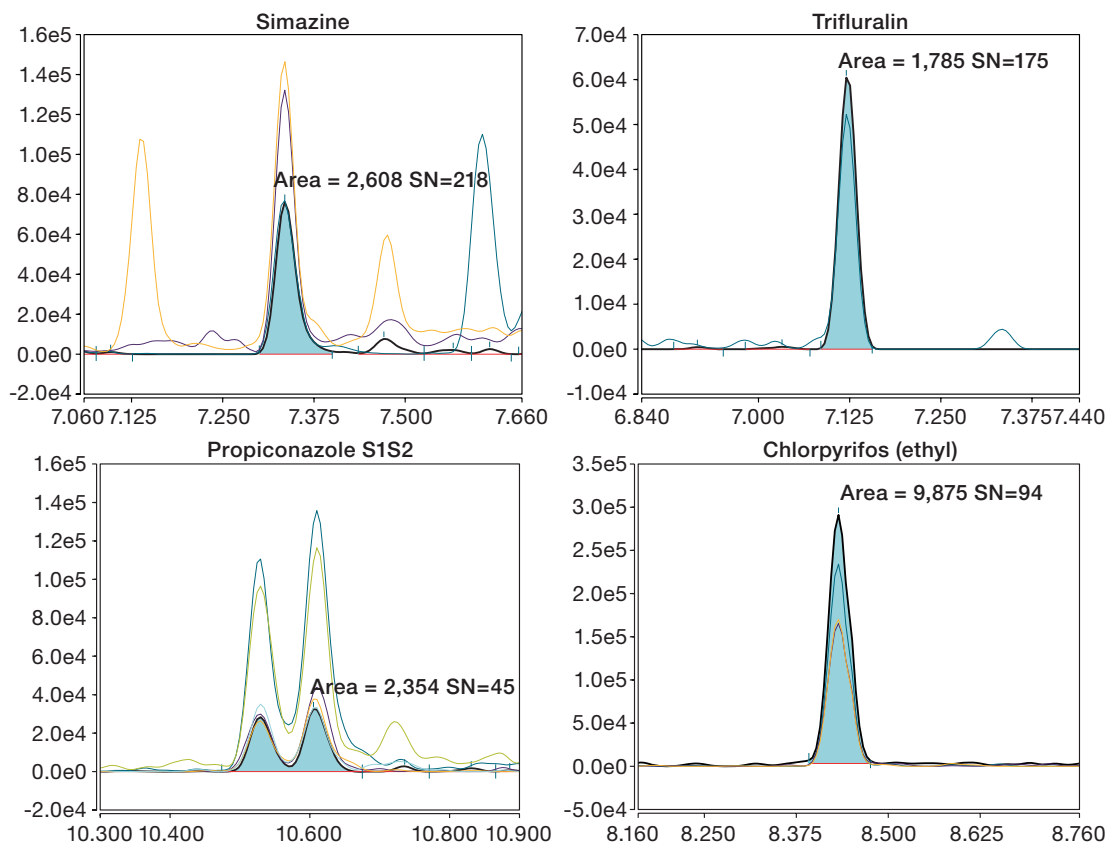
Figure 6. Overlaid XICs (quantifier (black trace) and qualifier ions) for simazine (3 µg/L), trifluralin (3 µg/L), propiconazoles (3 µg/L), and chlorpyrifos (3 µg/L) after 70 injection of surface water extracts

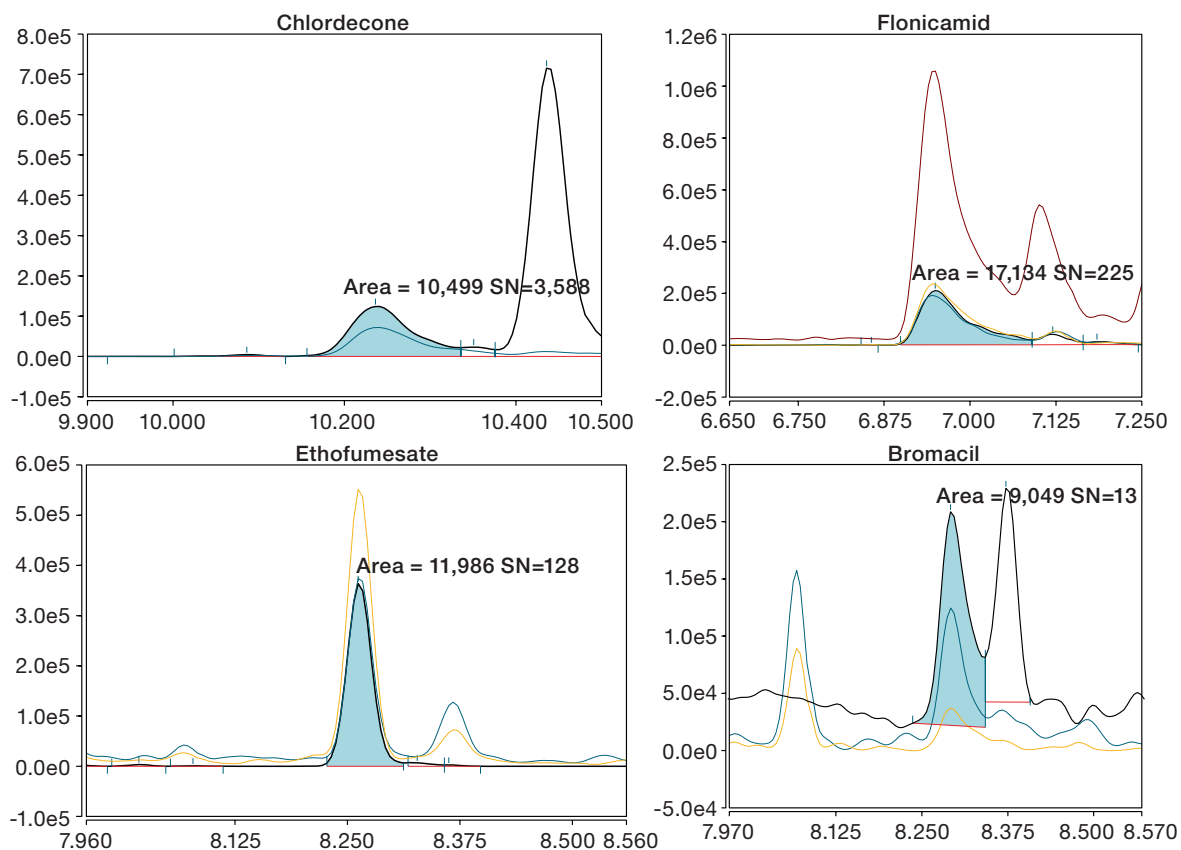**Figure 7.** Overlaid XICs (quantifier (black trace) and qualifier ions) for chlordecone (5 µg/L),flonicamid (5 µg/L), ethofumesate (5 µg/L), and bromacil (5 µg/L) after 70 injection of surface water extracts

Robustness for unstoppable confidence

Analytical testing laboratories need to process a high number of samples every day; therefore, it is critical that the instrument performs consistently. Instrument robustness was evaluated by injecting n=70 surface water extracts bracketed by two quality control standards (QCs) at 5 and 10 µg/L. The enhanced robustness and reliability of the TSQ 9610 triple quadrupole GC-MS/MS system, combined with the proper liner choice and the efficient transfer of the analyte through the iConnect-TSI injector, allowed for confident analyte quantitation with peak area ratio %RSD for the QC <10% as reported in Figure 8. No maintenance of the inlet or the mass spectrometer needed to be performed. Also, no re-tuning of the ion source parameters was required during the robustness evaluation, which covered over two days of continuous operations.

Robustness and stability of the area ratio at 5 µg/L

- QC5µg/L-1 AREA RATIO
- QC5µg/L-2 AREA RATIO
- QC5µg/L-3 AREA RATIO
- QC5µg/L-4 AREA RATIO
- QC5µg/L-5 AREA RATIO
- QC5µg/L-6 AREA RATIO
- QC5µg/L-7 AREA RATIO

Robustness and stability of the area ratio at 10 µg/L

- QC10µg/L-1 AREA RATIO
- QC10µg/L-2 AREA RATIO
- QC10µg/L-3 AREA RATIO
- QC10µg/L-4 AREA RATIO
- QC10µg/L-5 AREA RATIO
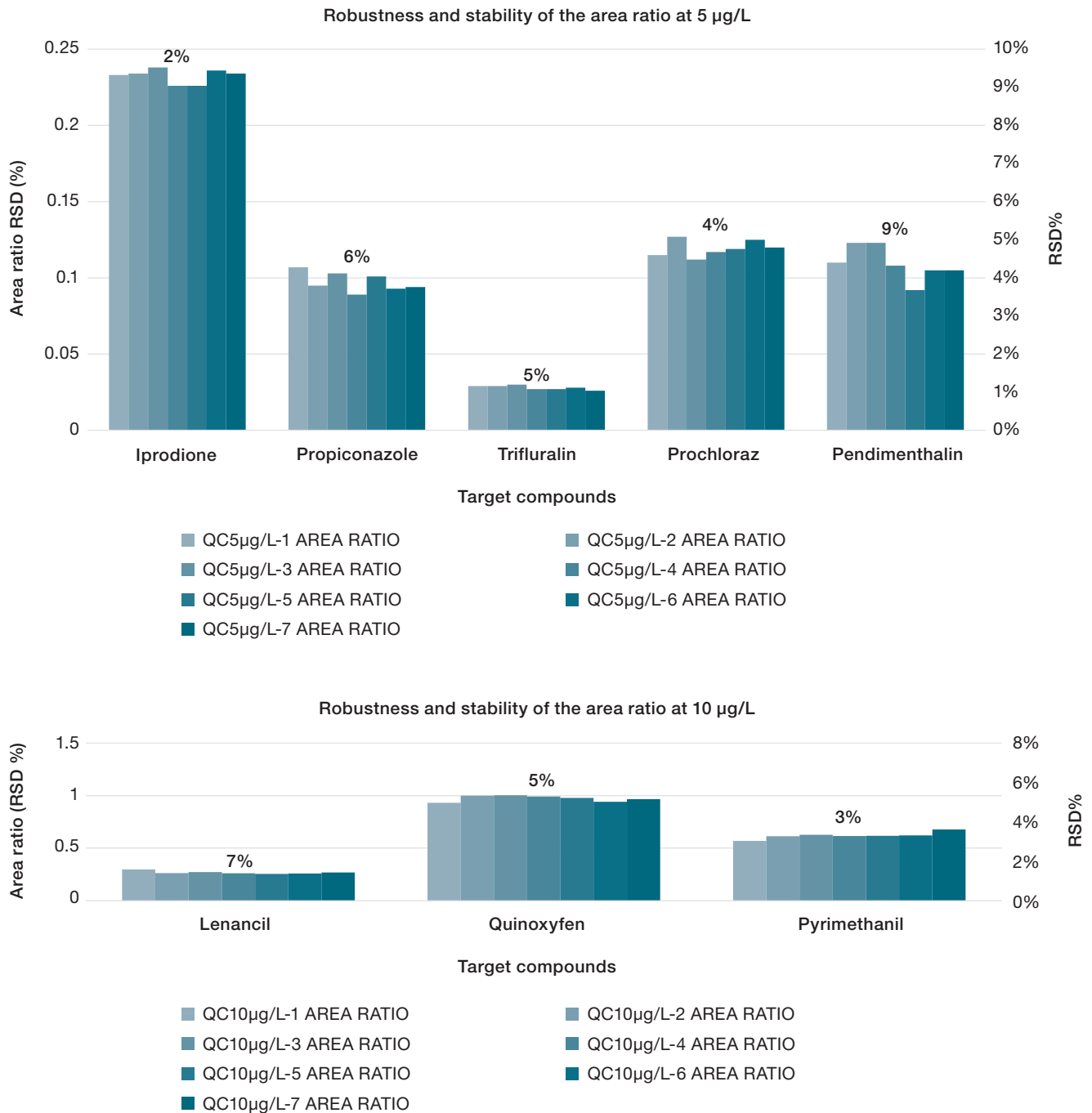- QC10µg/L-6 AREA RATIO
- QC10µg/L-7 AREA RATIO

Figure 8. Peak area ratio %RSD obtained for the QC checks at 5 and 10 µg/L, analyzed before and after the repeated injection of matrix samples (n=70). No inlet or MS tuning was performed during the robustness evaluation.

Conclusions

The results of these experiments demonstrate that the optimized method allowed for the determination of 350 pesticides, providing a suitable analytical solution for contract testing laboratories looking for improved productivity and confident results.

- The proper liner choice and the optimization of the injection parameters ensured rapid and efficient transfer of the analytes onto the column, minimizing the risk of cross reactions and the thermal degradation of the compounds that can occur into the injection port. The addition of PEG as an analytical protectant helped to improve resolution and sensitivity for critical compounds.

- The larger injection volume achievable with the iConnect-TSI injector, combined with the optimization of the mass spectrometer parameters, such as a lower electron energy and the prioritization of the dwell times ensured better signal-to-noise ratio and enhanced sensitivity. The method developed can achieve all LOQs corresponding to the regulations.

- The enhanced robustness, the inertness of the flow path, and the stability of the NeverVent AEI ion source allowed for multiple injections of water extracts over two days (around 150 injections), providing continuous operations with QC absolute peak areas <10%.

- After 70 injections of surface water, there was no maintenance required on any of the critical system components, such as the liner or the head of the chromatographic column. No tuning of the mass spectrometer was required either, so that the analysis could be continued without downtime for days at a time.

References

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