An Executive Summary

Ultra-Low Level Pesticide Residues Analysis using a New State-of-the-Art Enhanced Sensitivity GC-MS/MS System

Richard Law
Senior Applications Specialist
Thermo Fisher Scientific

A fully integrated system of advanced hardware and software is shown to enable robust detection of extremely low levels of pesticides in baby foods.

Introduction

In the European Union (EU), the Health and Food Safety Commission (SANTE), through Directive 2006/125/EC, has established default maximum residue levels (MRLs) at 10 µg/kg for the majority of pesticide residues in processed baby foods. The limit is set at lower levels for pesticides, which are considered to present an unacceptable toxicological risk. SANTE's guidelines for analytical quality control and method validation procedures are stringent (see **Figure 1**) and the identification criteria must be satisfied to demonstrate the validity of the analytical results. The guidelines are intended for laboratories involved in the official control of pesticide residues in food across the EU, but are also used in many other countries. The monitoring of the food supply to ensure compliance with MRL regulations involves the analysis of high numbers of many different types of samples. Because of the perishable nature of many foods, samples must be analyzed quickly, typically within 48 hours. It is, therefore, essential that the analytical workflow is fast and the analytical system used is highly sensitive, selective, and robust in order to minimize downtime and enable high productivity and fast turnaround times.

The preferred methods for the detection and quantification of targeted pesticide residues in food involve tandem mass spectrometry (MS/MS) in combination with either liquid chromatography (LC-MS/MS) or gas chromatography (GC-MS/MS). This summary illustrates the advantages of using a complete workflow, with the Thermo Scientific™ HyperSep™ Dispersive Solid Phase Extraction (dSPE) for sample extraction and clean-up, the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system for data acquisition, and specialized software (Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System [CDS] software) for instrument control, data processing, and reporting. This article describes the results of experiments that demonstrate the system can deliver the limits of detection, identification, linearity, robustness, and repeatability to comply with SANTE criteria and EU MRL regulations.

Sample Preparation and Experimental Setup

Two different varieties of ready-prepared baby food (apple/pear/banana and carrot/potato) were used for the evaluation. Aliquots (10-g) were fortified with more than 200 pesticides, each at 1, 2.5, and 10 µg/kg. Samples were extracted using citrate-buffered QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) with dispersive solid phase extraction (dSPE) as per European Committee for Standardization CEN 15-662 (2017) protocols.

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Figure 1: SANTE/11813/2017 identification requirements.

Retention time ±0.1 min +

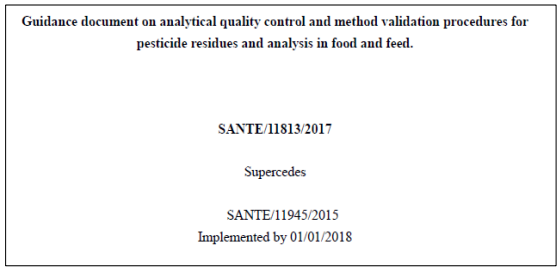

Table 4. Identification requirements for different MS techniques[2]

MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	Typical systems (examples)		minimum number of ions	other
Unit mass resolution	Single MS quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	S/N ≥ 3[d] Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap. Ion ratio from sample extracts should be within **±30% (relative)** of average of calibration standards from same sequence
	MS/MS triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm[a, b, c]	S/N ≥ 3[d] Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap. Ion ratio: see D12

[a] preferably including the molecular ion, (de)protonated molecule or adduct ion
[b] including at least one fragment ion
[c] < 1 mDa for m/z < 200
[d] in case noise is absent, a signal should be present in at least 5 subsequent scans

Primary secondary amine (PSA) was used to remove acidic co-extractives and graphitized carbon black (GCB) was used to remove pigments from the sample containing carrot. The cleaned-up extracts were acidified directly after dSPE to minimize the degradation of base-sensitive pesticides such as captan, folpet, and dichlofluanid. Final extracts were in acetonitrile at a crop concentration of 1 g/mL. Acetonitrile extracts were analyzed directly, avoiding the need for a solvent exchange, which prevents possible losses of pesticides and saves time.

The GC-MS/MS system used in this application was the TSQ 9000 GC triple quadrupole mass spectrometer equipped with an advanced electron ionization (AEI) source, coupled to a Thermo Scientific™ TRACE™ 1310 GC system with a TriPlus RSH™ Autosampler. The system is equipped with user-exchangeable Instant Connect injector modules, which require no special tools, service engineers, or special training to service, maintain, or replace. The advanced electron ionization (AEI) source produces significantly more ions than classical EI designs, generating a more tightly focused ion beam, which, in turn, leads to simplified tuning and a high level of robustness. **Figure 2** shows the GC and injector conditions used in this work, including a carefully optimized programmed temperature vaporizer (PTV) injection method to account for the use of acetonitrile as the injection solvent.

Figure 2: Gas chromatography and injector conditions used for the analysis of pesticides in baby food samples.

Thermo Scientific™ TRACE™ 1310 GC System Parameters

Injection Volume:	1 µL
Liner:	Thermo Scientific™ LinerGOLD™ PTV six baffle liner (Siltek) (P/N 453T2120)
Injector temp:	70 °C
Carrier Gas:	He, 1.2 mL/min
Inlet and injection mode:	Programmable Temperature Vaporizing (PTV) Splitless (split flow 50mL/min after 2 min)
Column:	Thermo Scientific™ TraceGOLD™ TG-5SilMS GC column with SafeGuard (30m x 0.25mm, 0.25µm- with 5m integrated guard column – P/N: 26096-1425)

PTV Parameters:	Rate (°C/s)	Temperature (°C)	Time (min)	Flow (mL/min)
Injection	-	70	0.10	-
Transfer	5.0	300	2.00	-
Cleaning	14.5	320	5.00	75.0

Oven Temperature Program:

Ramp	RT (min)	Rate (°C/min)	Target Temperature (°C)	Hold Time (min)
Initial	0	-	40	1.50
1	1.5	25.0	90	1.50
2	5.0	25.0	180	0.00
3	8.6	5.0	280	0.00
Final	28.6	10.0	300	5.00
Run time	35.6	-	-	-

Recovery, Precision, and Sensitivity

The total ion chromatogram (TIC) overlay shown in **Figure 3** for the sample of carrot/potato baby food spiked at 10 µg/kg is typical of the results observed with this setup. The blue line shows the massive reduction in background signal using the selected reaction monitoring (SRM) mode on an absolute scale. All 211 individual peaks (210 pesticides and one internal standard, triphenyl phosphate) are eluted from the column in about 30 minutes. SRM transitions can be easily be removed, added, swapped, or further optimized using the AutoSRM software. Average recovery rates for pesticides across the concentration range spiked in both types of baby food exceeded 95%, with all 210 pesticides detected at the default MRL. Average precision values in terms of % relative standard deviation (%RSD) were between 3.1% and 6.1% at each concentration level.

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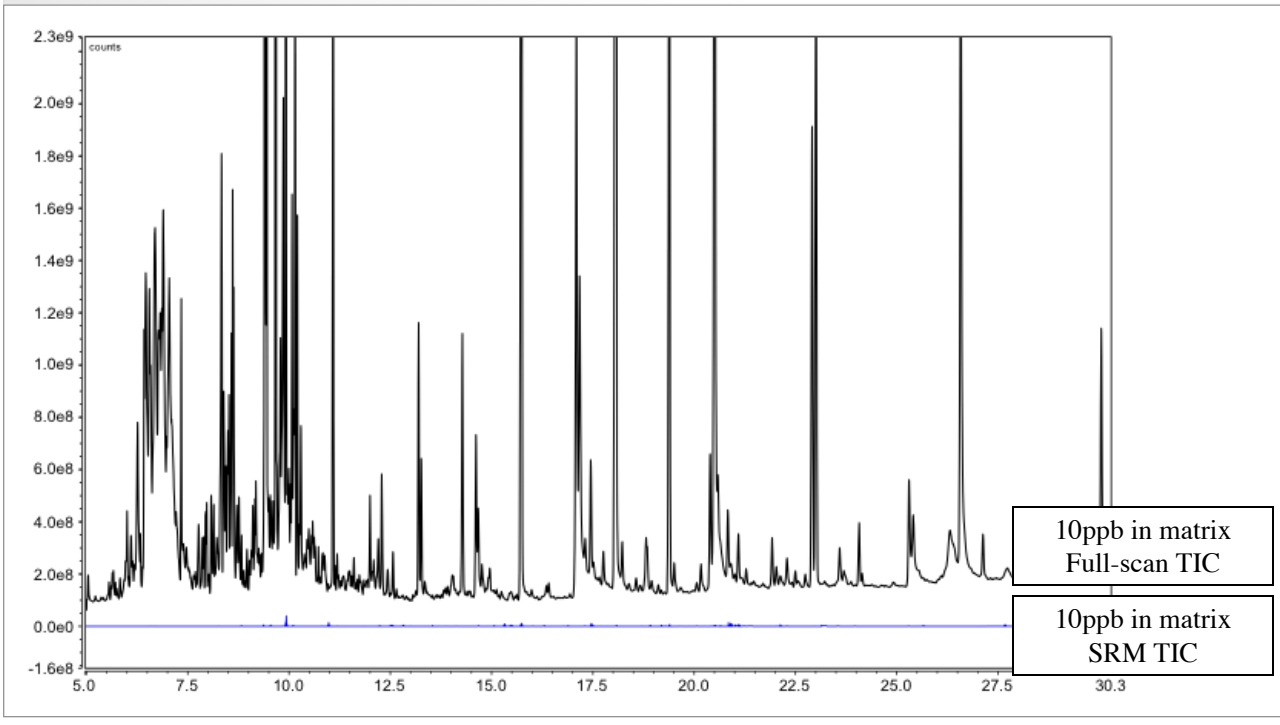
For method validation, almost all pesticides, spiked at 10 µg/kg, were in excellent agreement with the SANTE precision acceptance criteria (recovery values are between 70% and 120%, and with associated RSDs less than 20%): for endrin aldehyde and chlorothalonil, recovery rates were less than 70%.

To determine the response of the system, 14 matrix-matched calibrated standards at levels ranging from 0.025 µg/kg to 250 µg/kg in both carrot/potato and apple/pear/banana matrices were prepared. Results for the pesticide dichlobenil show that even at levels as low as

Figure 3: Total Ion chromatogram overlay for a sample of carrot/potato baby foor spike at 10 µg/kg.



25 femtograms on column (0.025 µg/kg in matrix), stable ion ratios were observed (**Figure 4**). The dichlobenil peak has a Gaussian shape and contains two additional SRM transitions, one more than the SANTE guidelines require. Additional data obtained for endosulfan sulfate at 0.05 µg/kg and hexachlorobenzene at 0.025 µg/kg again show excellent linearity, and can be quantified at lower levels than the EU MRLs of 10 µg/kg and 3 µg/kg, respectively (**Figure 5**).

A more stringent MRL of 4 µg/kg is imposed by the European Commission Directive 2006/125/EC for the combined level of fipronil and fipronil-desulfinyl because of their high toxicity. Data was collected demonstrating compliance with SANTE requirements at a combined level

Figure 4: Dichlobenil at 25 fg on column (0.025 µg/kg) in matrix-matched standard (carrot/potato).

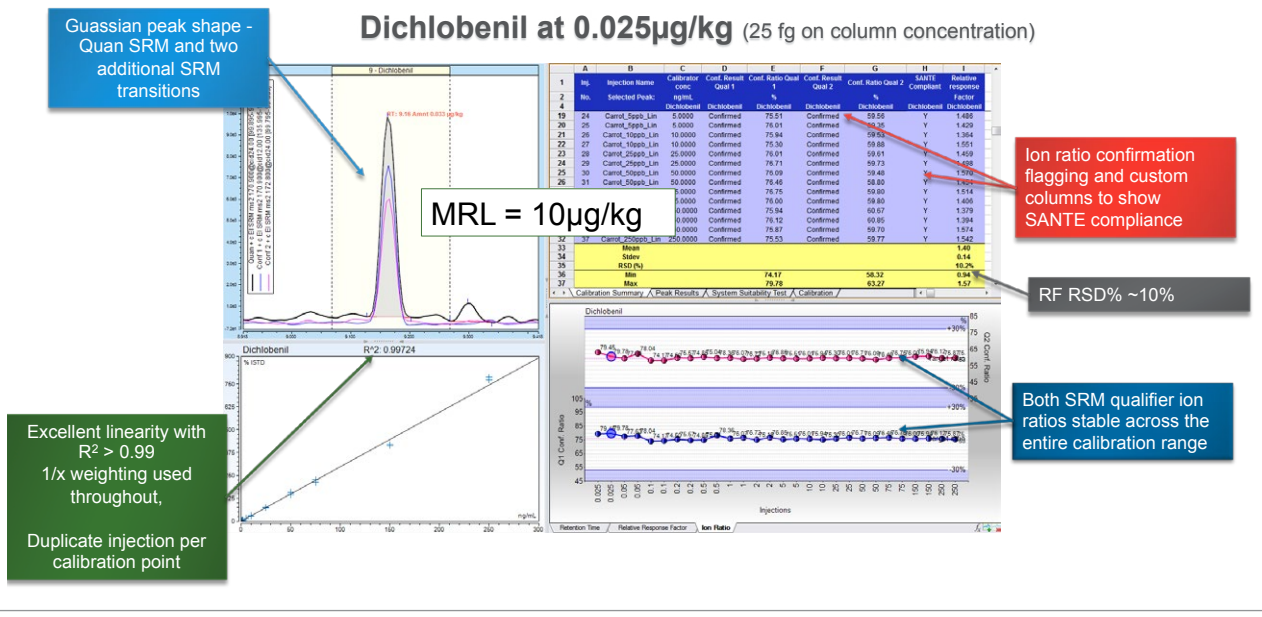
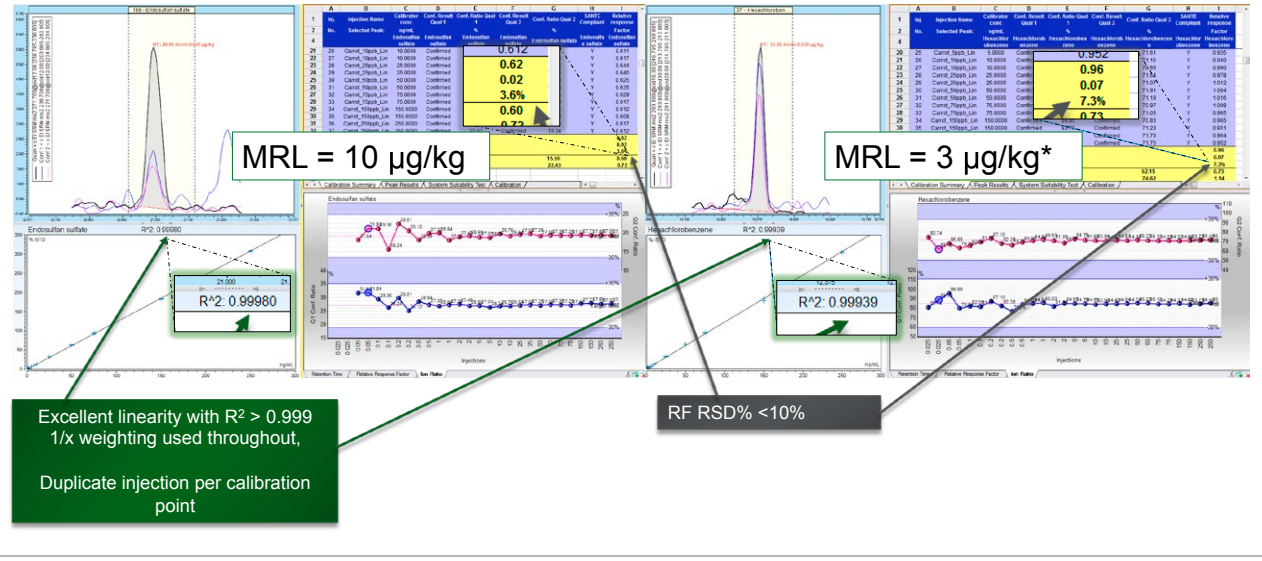


Figure 5: Examples of sensitivity and linearity.



equivalent to 0.2 µg/kg, which is equivalent to 20 times less than the EU MRL requirement (**Figure 6**). From the analysis of matrix-matched standards at concentrations prepared at 0.05 µg/kg to 5 µg/kg in a carrot/potato matrix, the limits of identification (LOI) were less than 0.5 µg/kg for 90% of the pesticides and below 0.1 µg/kg for 60% of the pesticides; equivalent to 100 times lower than the default MRL. In addition, the instrumental detection limit (IDL) of the analytical system were calculated to be 5 fg on-column for chlorobenzilate and 7 fg for cadusafos. Even more challenging compounds, such as folpet and captan, were detected below one picogram on column. These ultra-low detection limits present clear opportunities for dilution of sample extracts to minimize matrix effects and to further improve system performance.

Linearity and Robustness

The results shown in **Figure 7** demonstrate the excellent linearity obtained in apple/pear/banana matrices spiked at concentrations of 0.025 µg/kg to 250 µg/kg. Out of the more than 200 pesticides tested, all but three (captan, folpet, and azinphos-methyl) displayed correlation coefficients (R^2) greater than 0.990 µg/kg. In terms of the relative response factor RSD%, all but three pesticides (folpet, captan, and propisochlor) met the <20% requirement.

Figure 6: European Commission directive 2006/125/EC for combined fipronil.

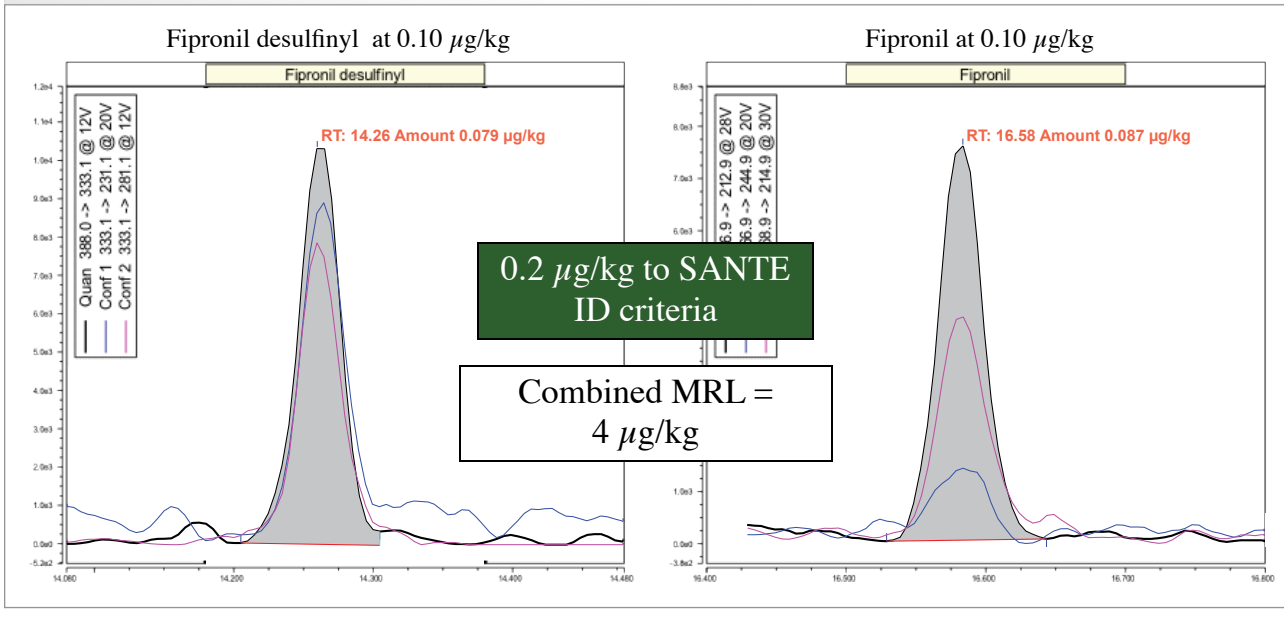
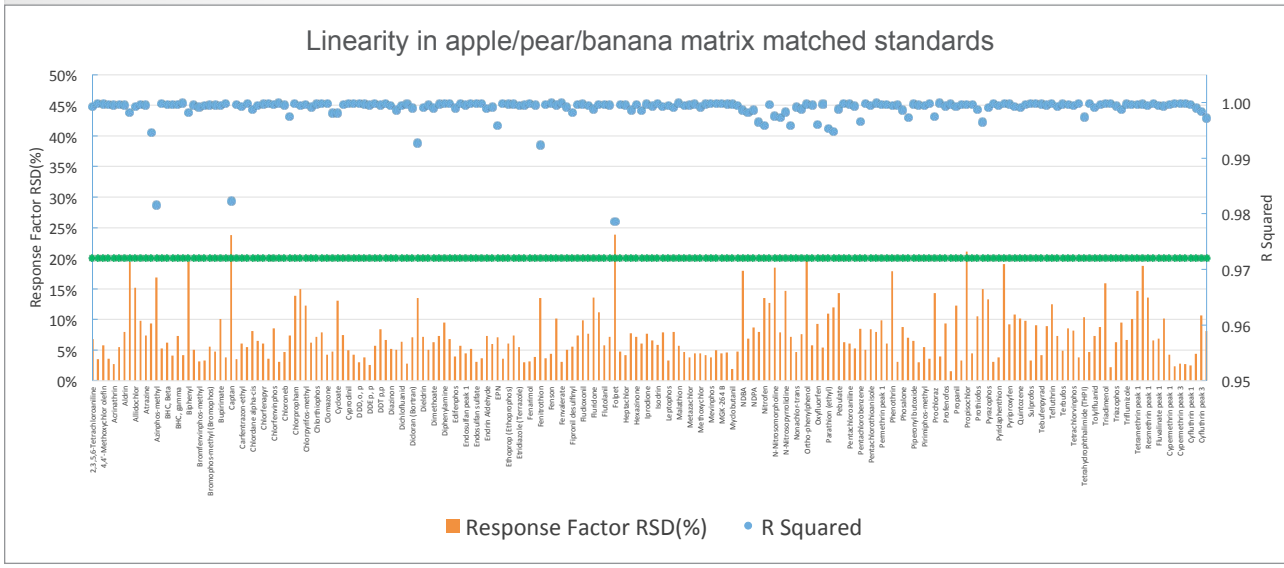


Figure 7: Linearity in apple/pear/banana matrices spiked at concentrations of 0.025 µg/kg to 250 µg/kg.



A measure of the excellent robustness of this system can be seen in terms of the repeatability of the results. In a study comprising approximately 900 sequential injections of sample extracts spiked at 10 µg/kg for each of 200 pesticides, the ion flux (absolute peak area divided by the multiplier gain factor) remained essentially constant (**Figure 8**). There was no need for AEI source cleaning in this case, only liner replacement, and re-tuning using the software's intuitive SmartTune function every 100 injections.

A further measure of the inherent repeatability of this system is shown in **Figure 9**, which compares the ion ratio (IR) for the primary qualifier, and peak shapes, of the first and 395th injections of four pesticides. There was essentially no change in the results even after close to 400 injections. The results are fully compliant with SANTE criteria of ±30% ion ratio and at least two product ions with fully overlapping peaks.

Figure 8: Robustness/repeatability demonstrated in a study involving 900 sequential injections of sample extracts spiked at 10 µg/kg for each of 200 pesticides.

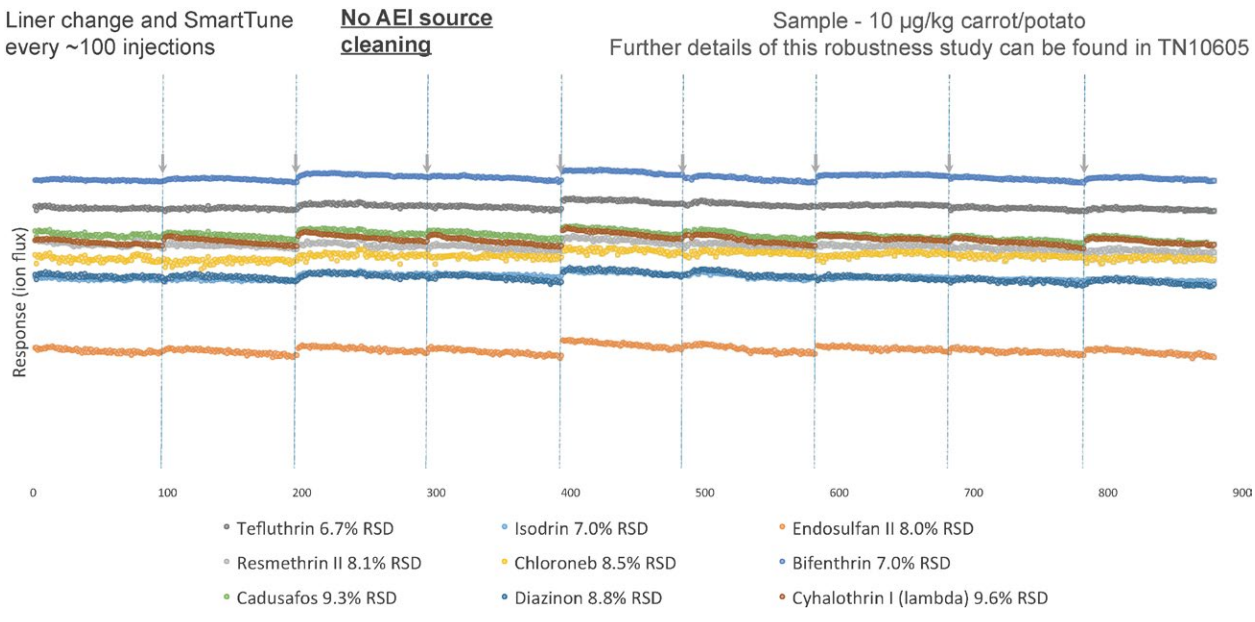
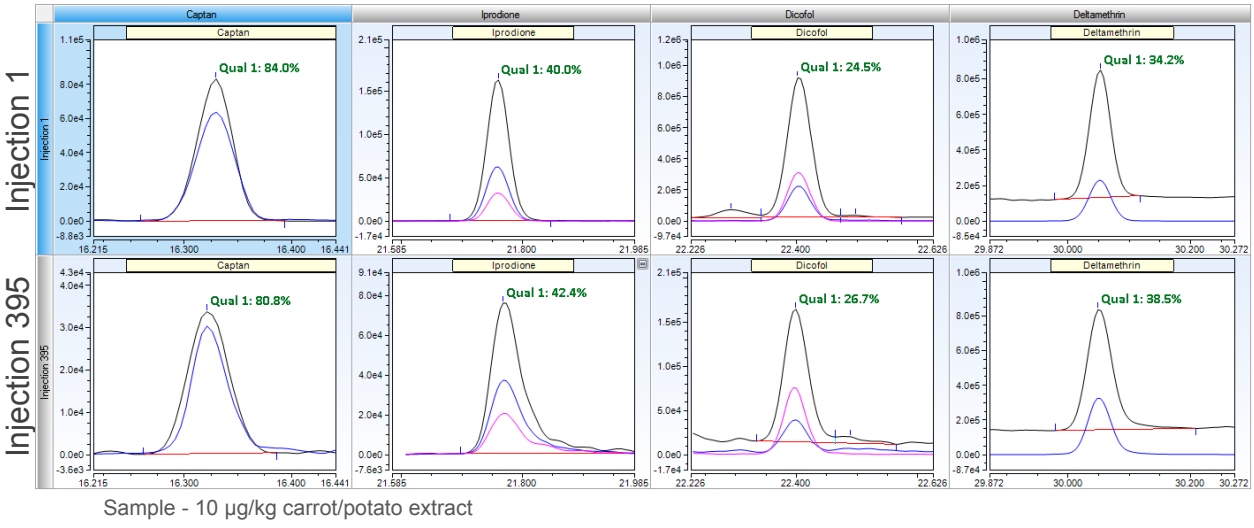


Figure 9: Repeatability demonstrated in a study comparing the ion ratio for the primary qualifier and peak shapes of the first and 395th injections of four pesticides.



Software

A key element of the system is the Chromeleon 7.2 CDS that was used to control the instrument modules, collect data, and perform data analysis and reporting. It is fully compliant with FDA 21 *CFR* Part 11 regulations, enabling data integrity from sample to result. It features one-click e-workflows for simplified method sequence creation and reporting. Re-tuning can be done in a semi-automatic manner using the SmartTune feature. An Advanced Auto-Tune feature is also available. The software offers electronic integration with a wide variety of laboratory information management systems (LIMS) to manage the complete laboratory workflow. A large library of SRM data that includes over 1,000 compounds and more than 3,000 transitions is available. SRM transitions can be easily added or changed, and optimized by powerful AutoSRM software.

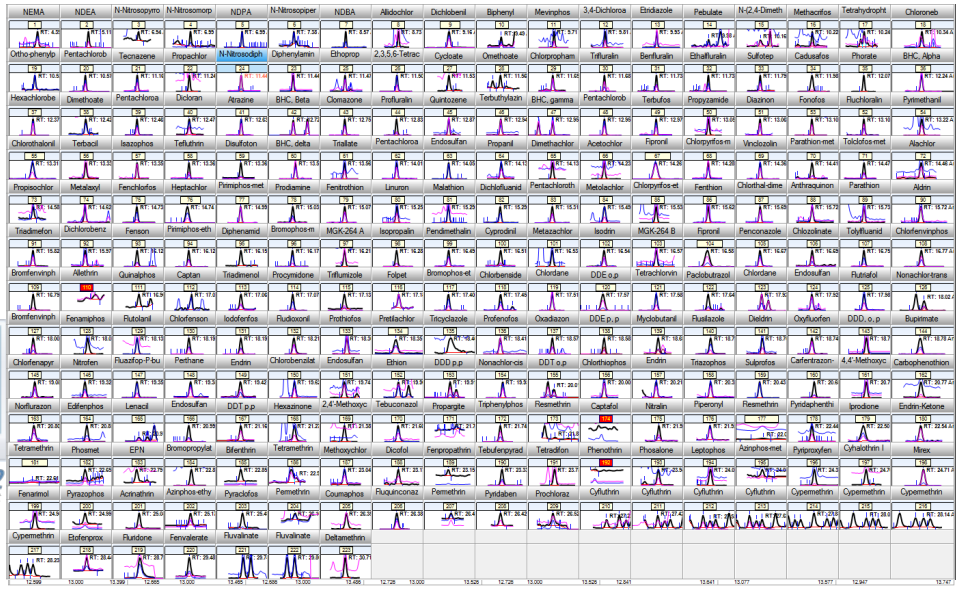
Calibration data and results are updated in real time and can be reviewed prior to the reporting stage. Powerful database query functions allow for rapid review of numerous components on a single band, with the possibility of displaying all the peaks at once (**Figure 10**) (limited only by monitor resolution). Analysts can switch between standards and samples easily and rapidly, as all the information (e.g., raw files, methods, and reports) is saved in one secure location.

Conclusion

The TSQ 9000 GC-MS/MS system is very robust, capable of analysis of over 900 injections of QuEChERS extracts with minimum downtime. It delivers excellent accuracy, recovery, and precision for more than 200 pesticides at very low concentrations, over five orders of magnitude. The GC-MS/MS system is able to meet stringent AQC SANTE requirements at the default EU MRL. In addition, this system offers the possibility of using even more dilute samples, which would further increase productivity and reduce matrix effects. The software package handles data natively in real time, allowing for easy and fast review of large numbers of compounds with huge flexibility in reporting formats.

Figure 10: Powerful database query functions allow for rapid review of numerous components.

Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System (CDS) software was used for instrument control, data acquisition, processing and reporting.



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