

Confident analysis of ultra-trace pesticides residues in baby food using triple quadrupole GC

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ABSTRACT

Purpose: The application of pesticides in agriculture practices can result in residues at detectable concentrations in food posing consumers at risk.

Methods: Gas chromatography triple quadrupole mass spectrometry (GC-MS/MS) is the industry standard for analyzing GC amenable pesticides in food. In this study the Thermo Scientific™ TSQ™ 9610 GC-MS/MS was used to assess the method suitability for analysis of pesticide in baby food in compliance with SANTE/11312/2021 guidance document.

Results: The results obtained in these experiments demonstrate that the TSQ 9610 mass spectrometer equipped with the Thermo Scientific™ NeverVent™ AEI ion source delivers excellent analytical performance for multi-residue analysis of pesticides in baby food samples.

INTRODUCTION

Pesticides are commonly used in agriculture to control various pests during cultivation, storage, and transportation. To ensure food safety for consumers and protect the environment, many organizations and countries around the world have established maximum residue limits (MRLs), which for the majority of pesticide-commodity combinations are set at the default level of 10 µg/kg. However, the European Union (EU) has established LOD MRLs between 3–8 µg/kg for specific pesticides prohibited in baby foods. GC-MS/MS ensures high sensitivity and selectivity for confident detection, identification, and quantitation of pesticide residues, while using faster, generic, small-scale sample extraction procedures such as QuEChERS (quick, easy, cheap effective, rugged, and safe).

MATERIALS AND METHODS

Sample Preparation

Several baby food samples (blank and pre-spiked with some pesticides at 1 or 3 µg/kg) were extracted using the citrate-buffered QuEChERS protocol according to the procedure described in Figure 1.

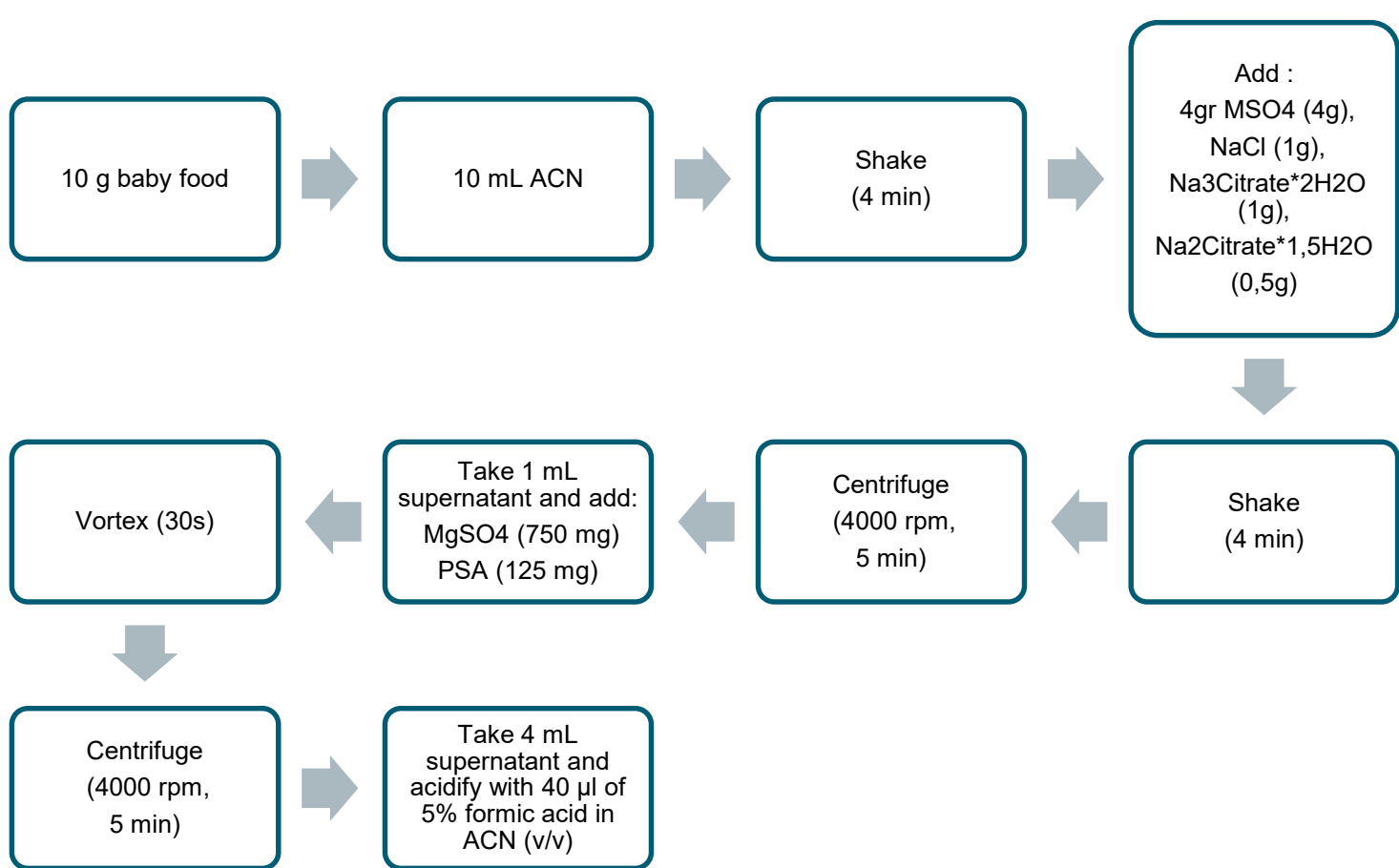
Test Method

Matrix-matched calibration standards were prepared by spiking the final extracts with a mixture of > 200 pesticides and used to assess linearity, detection limits, and repeatability. Pre-spiked samples were used to assess recovery and quantitative performance. Some aliquots of blank sample extracts were spiked at 10 µg/kg and used to assess the long-term instrument robustness.

Data Analysis

Data was acquired, processed, and reported using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.3.

Figure 1. Citrate-buffered QuEChERS protocol used for sample extraction



RESULTS

Chromatography

A timed-selected reaction monitoring (t-SRM) acquisition method allowed for simultaneous acquisition of multiple characteristic ions for each pesticide, maintaining high sensitivity combined with high selectivity to discriminate between the target compounds and the matrix thus providing a confident and selective identification and quantitation of analytes to ensure compliance with the maximum residue levels (MRLs) defined by the regulations. The identification of the target pesticides followed the criteria stated in the SANTE/11312/2021 guidance document: (i) a minimum of two product ions were detected for each pesticide with peak S/N >3, (ii) retention time tolerance of ±0.1 minutes compared with standards in the same sequence, (iii) ion ratio within ±30% (relative) of the average of calibration standards from the same sequence. As an example, the total ion chromatogram (TIC) acquired in EI, full-scan mode (*m/z* 50–500), and the t-SRM acquisition of an organically labeled baby food sample pre-spiked at 3 µg/kg are shown in Figure 2A, B. The use of the PTV injector allowed for efficient transfer of the analytes, reducing the risks of formation of breakdown products (Figure 3).

Figure 2. TIC (full scan: *m/z* 50–500, A-upper trace) and t-SRM acquisition (B-bottom trace) for organic food sample pre-spiked at 3 µg/kg

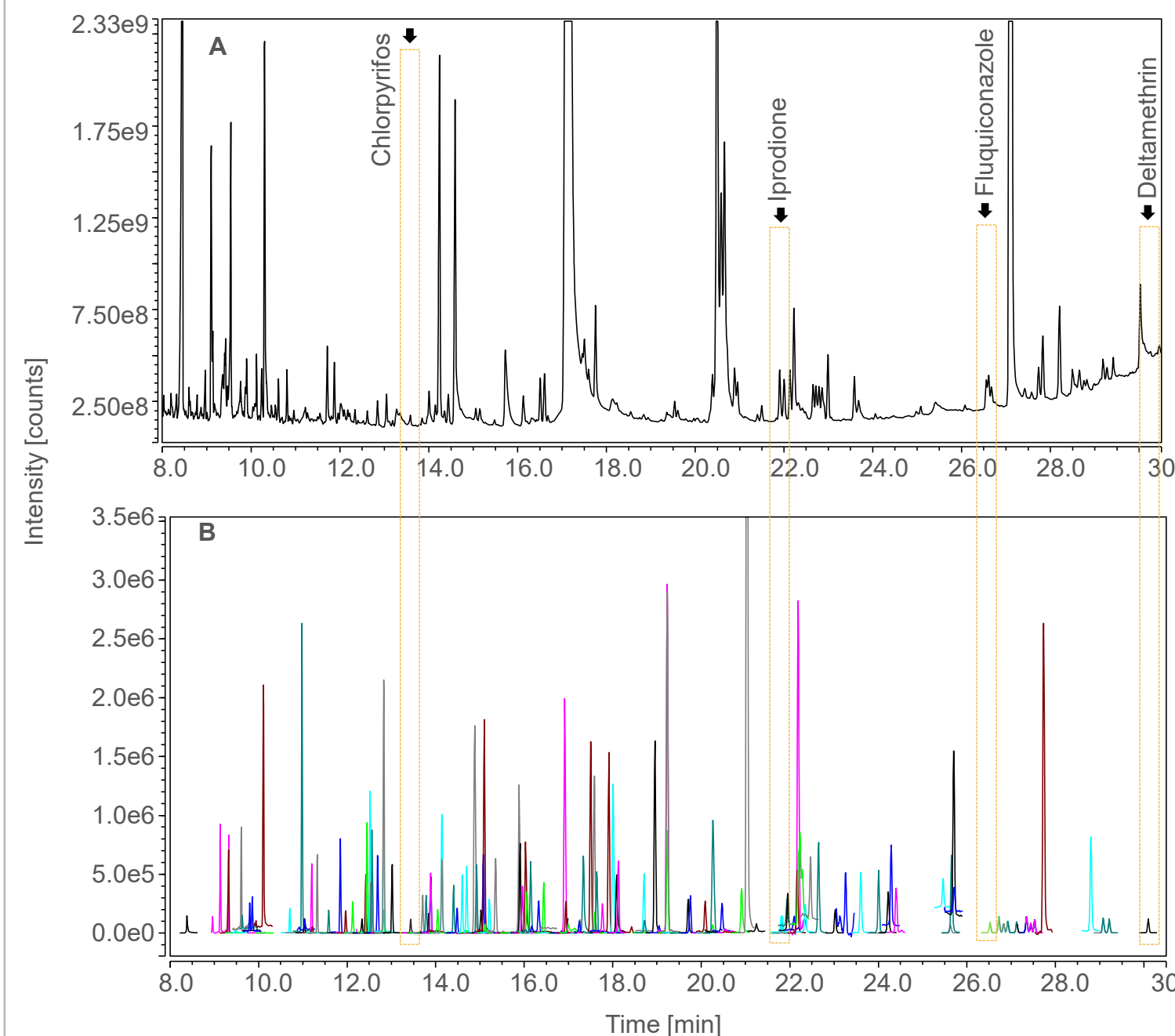
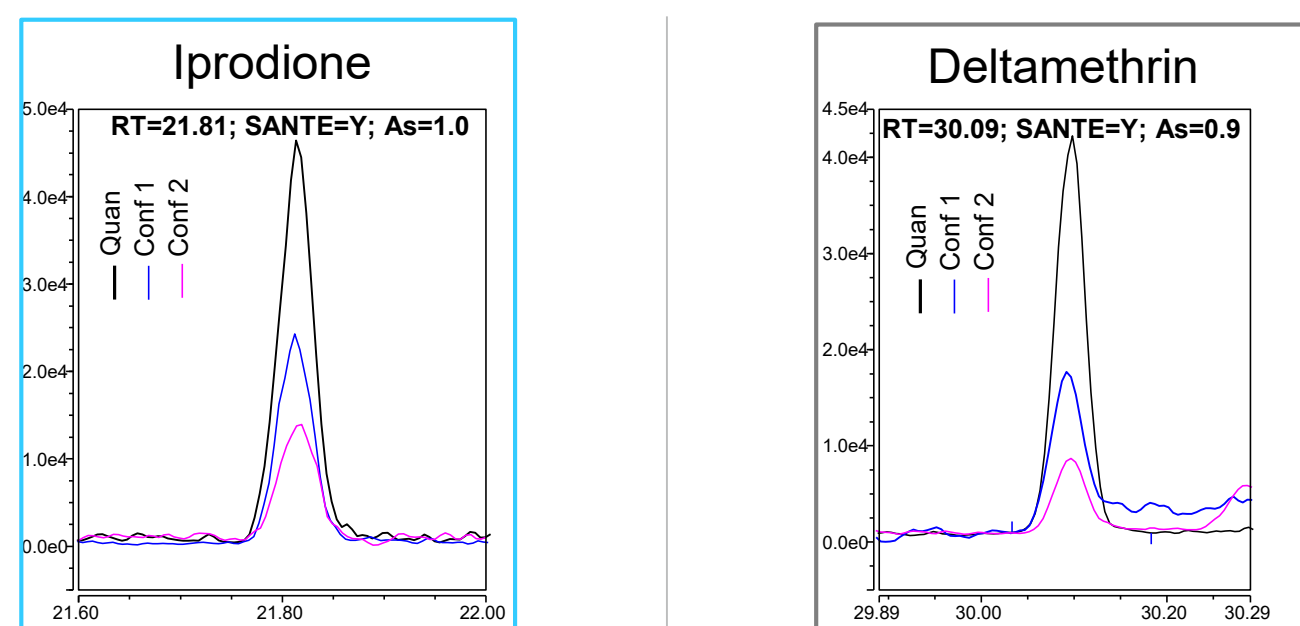


Figure 3. Examples of thermolabile pesticides pre-spiked in matrix at 1 µg/kg

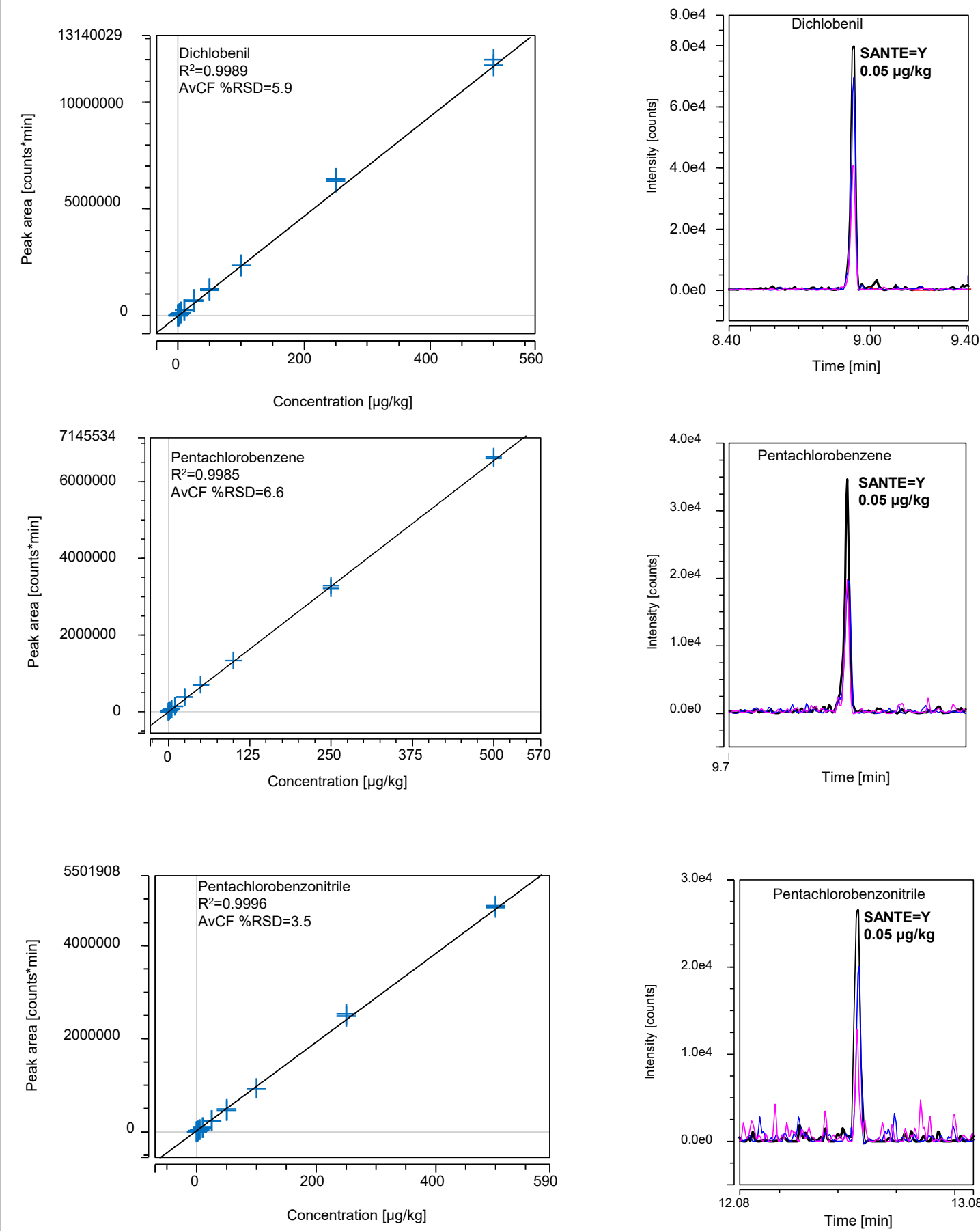


Compound identification as per SANTE guidance with compliance automatically flagged (SANTE=Y) in the CDS and peak asymmetry (A_s) are annotated in the peak label.

Linearity

The TSQ 9610 mass spectrometer with the NeverVent AEI ion source is equipped with the Thermo Scientific™ XLXR™ detector, which is an electron multiplier that offers extended detector lifetime and dynamic range. To test the performance of the detector, matrix-matched calibration curves were prepared by spiking the QuEChERS extracts with pesticide mix ranging from 0.05 to 500 µg/kg. Each calibration level was injected in duplicate. All target analytes showed a linear trend with coefficients of determination (R²) > 0.990 and residual values (measured as %RSD of average response factors, AvCF %RSD) <20, thus confirming a wider linear range can be easily achieved. All target compounds satisfied the SANTE criteria at the default MRL of 10 µg/kg and more than 95% were confirmed following the SANTE criteria at concentrations <1 µg/kg. Full range calibration curves (0.05–500 µg/kg) for dichlobenil, pentachlorobenzene, and pentachlorobenzonitrile and the quantifier and qualifier ions at 0.05 µg/kg, are reported as an example in Figure 4.

Figure 4. Example of matrix-matched calibration curves (full range: 0.05–500 µg/kg) for dichlobenil, pentachlorobenzene, and pentachlorobenzonitrile, as well as quantifier and qualifier ions at 0.05 µg/kg, confirmed as per SANTE criteria



Instrument detection limit (IDL), and limit of detection (LOD)

The instrument detection limit was determined for all the target compounds by spiking n=11 matrix extracts at 0.08, 0.8, 1.5, and 3.0 µg/kg. IDLs were calculated taking into account the one-tailed Student's t-test values for the corresponding n-1 degrees of freedom at 99% confidence, the concentration, and the absolute peak area %RSD (<15%) for each analyte. Calculated IDLs ranged from 6 to 650 fg on column (OC), corresponding to 0.006 to 0.65 µg/kg as reported in Figure 5.

Recovery and precision

Analytes recovery was assessed by spiking an organic baby food sample with some of the investigated pesticides at 3 µg/kg before extracting with QuEChERS. Calculated recoveries for the spiked compounds were between 70% and 120% with calculated precision ≤10%. Calculated recoveries for some of the spiked compounds are reported as an example in Figure 6.

Figure 5. Calculated IDLs for all investigated pesticides. IDLs ranged from 6 to 650 fg OC corresponding to 0.006 to 0.65 µg/kg in the sample extract

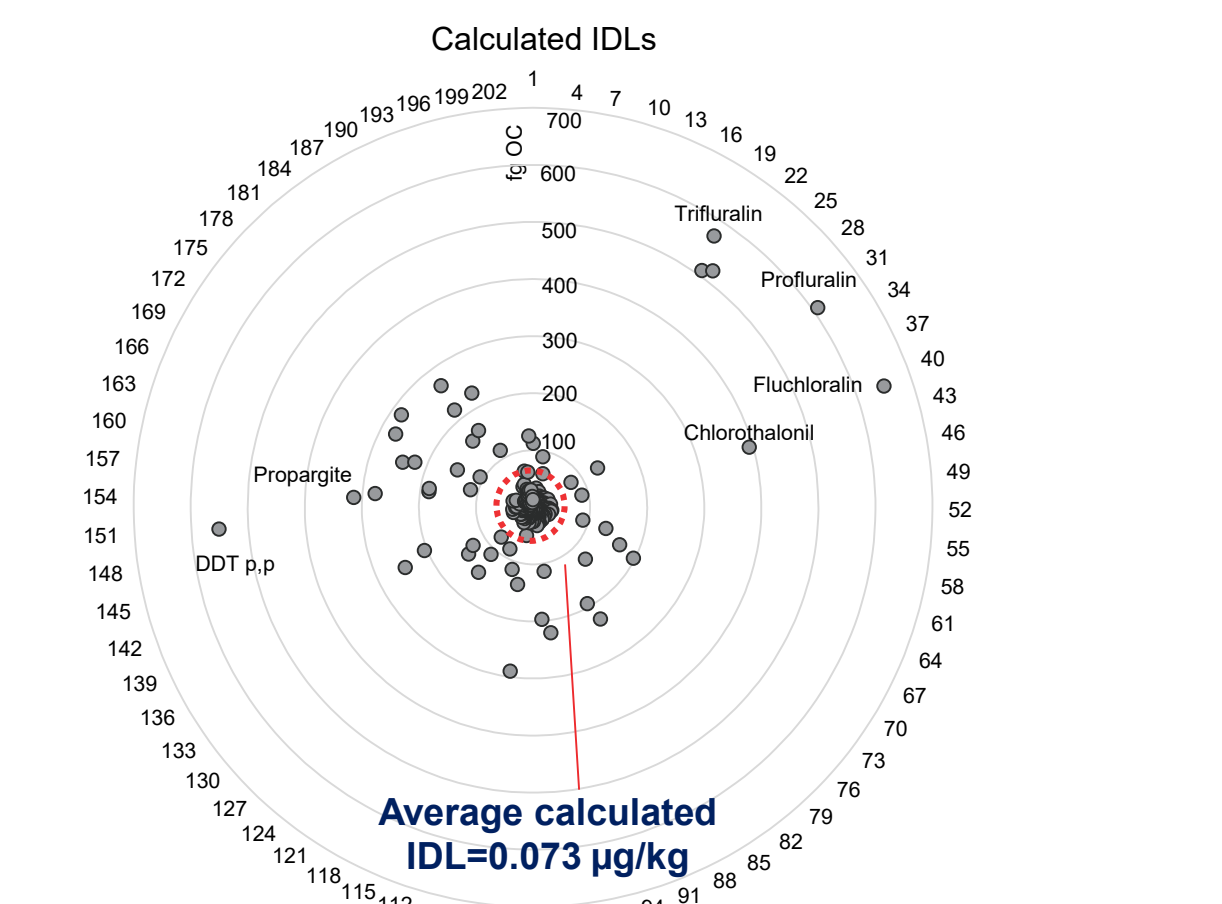
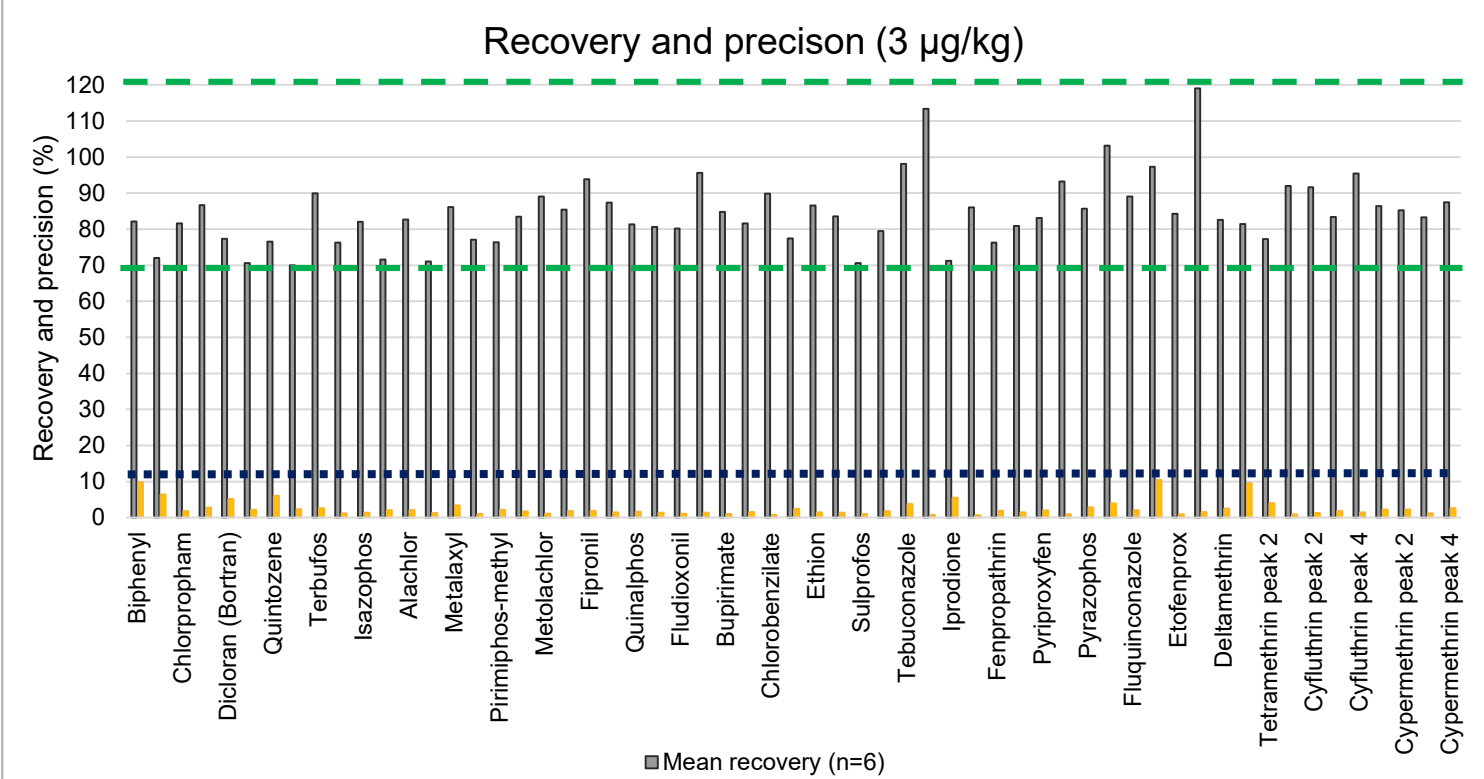


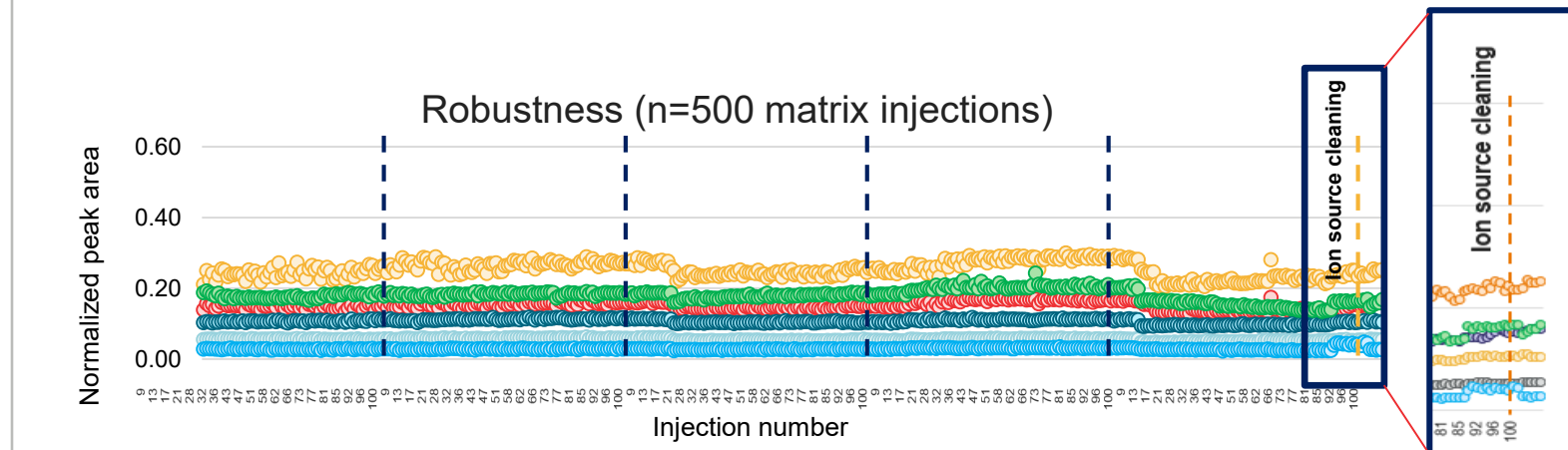
Figure 6. Calculated recoveries for a selection of the spiked compounds



Robustness

Instrument robustness for everyday analysis was evaluated over almost four weeks of continuous operation by repeatedly injecting various matrix extracts spiked at 10 µg/kg (total number of matrix injections = 500) in randomized order. A quality control standard (QC) was spiked at a concentration corresponding to 3 µg/kg and injected in duplicate every 12 samples to monitor the system stability. After every 100 injections, the PTV liner and septum were replaced, approximately 6 cm of the head of the column were trimmed, and the Thermo Scientific™ SmartTune tool was used to check the instrument status. Even though the instrument was still performing well, an evaluation of how long ion source maintenance would take was performed. The ion source was cleaned after n=500 injections and the consistency of the data assessed after the cleaning. The Thermo Scientific™ NeverVent™ technology allowed for ion source removal without breaking the vacuum so that the instrument was producing useable data with a cleaned ion source in less than two hours, thus ensuring minimal instrument downtime. Normalized peak area response (analyte peak area / ISTD peak area) as well as the ion ratios were stable at the default MRL of 10 µg/kg (Figure 7).

Figure 7. Normalized peak area response (analyte peak area / ISTD peak area) obtained for n=500 consecutive injections of matrix samples spiked at 10 µg/kg



CONCLUSIONS

The results obtained in these experiments demonstrate that the TSQ 9610 mass spectrometer equipped with a NeverVent AEI ion source in combination with the Thermo Scientific™ TRACE™ 1610 GC and the Thermo Scientific™ AI/AS 1610 liquid autosampler delivers excellent analytical performance for multi-residue analysis of pesticides in baby food samples.

- Wide linear response and accurate quantitative performance for the investigated pesticides was obtained with a coefficient of determination of R² >0.99 and AvCF %RSDs <20 in spiked matrix over a concentration range of 0.05 to 500 µg/kg.
- High recovery (70–120%) and precision (RSD ≤10%) were demonstrated for pre-spiked QuEChERS extracts of at 3 µg/kg.
- Low instrument detection limits ranging from 6 to 650 fg on column corresponding to 0.006 to 0.65 µg/kg were achieved. The average calculated IDL for all compounds was 0.073 µg/kg.
- The enhanced robustness and reliability of the AI/AS 1610 liquid autosampler combined with the efficient transfer of the analyte through the PTV injector, the inertness of the flow path, and the stability of the NeverVent AEI ion source allowed for n=500 injections of acetonitrile QuEChERS extracts over four weeks continuous operations with minimal maintenance, keeping compliance with SANTE guidelines.

REFERENCES

- Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. SANTE 11312/2021, implemented by 01/01/2022.

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSES

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