Highly-sensitive pesticide analysis in baby food

Using the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready, powered by SCIEX OS Software

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Detecting and quantifying pesticide residues in food remains a moving target. Analytical technologies continue to evolve, but regulations combined with increased community awareness and public perception of pesticides in foods drive the pursuit of lower limits of quantification. Food testing represents a challenge not only because of the complexity and diversity of relevant matrices but also the need for low-level pesticide detection.

The technology advancements in the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready provide significant gains in the generation, capture and transmission of ions into the mass spectrometer.[2] The impact of this improved sensitivity was tested with a pesticide panel to assess the absolute sensitivity and other quantitative performance metrics in food matrices. Pesticide levels in baby foods are specifically regulated by regional food safety law, such as the EU Commission Directives of 2006, which set MRL levels for pesticides in baby foods explicitly. In addition to this consideration, commercially available baby foods are an excellent test matrix, as these are homogenous fruit and vegetable blends containing mixes of leafy greens such as kale, high water content fruits such as apples, and high starch fruits such as bananas. This allows for assessment of method performance in a matrix that covers a variety of different types of interferences and backgrounds. This work demonstrated the ability of the high sensitivity SCIEX 7500 System to achieve low level detection limits with a very small injection volume.

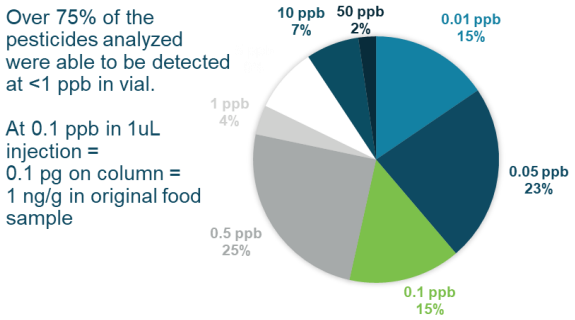


Figure 1. Extremely low concentration detection of pesticides. The pie chart shows the percentage of the analytes in the pesticide panel that were measured at each LLOQ value. The SCIEX 7500 System enables the detection of commonly analyzed pesticide residues at very low levels, allowing the method to leverage very low injection volumes of 1 µL. The translation to detection limits in an original food sample is significant to laboratories testing a wide variety of food matrices for regulated residues.

Key features of the SCIEX 7500 System for pesticides analysis

- Limits of detection well below 1 ppb in neat standards for most of the pesticide panel were observed, with plenty of room for the methods to achieve low-level detection with large dilution factors
- Quantitative performance — including raw sensitivity, linear dynamic range, reproducibility, and instrument robustness were also illustrated
- Low-level quantification in complex food matrices — fast data collection also allows for tightening chromatographic peak widths without compromising data quality. Chromatographic run was shortened to 10 minutes for 400 MRM transitions using the Scheduled MRM™ Algorithm.
- The modular design of the OptiFlow® Pro Ion Source provides flexibility with easy switching between ESI and APCI and between LC flow regimes, while maintaining high robustness.

Methods

Sample preparation: Analytical standards in iDQuant™ Standards Kit for Pesticide Analysis (SCIEX)[4] represent a well-characterized mixture of 209 pesticides with various chemical properties and spanning different chemical classes.

Three different baby foods were selected, which will be referred to as A, B, and C. Each brand was analyzed as an unspiked matrix, spiked at 1 ppb of pesticide mix, and spiked at 10 ppb pesticide mix. Each sample and treatment combination was prepared in triplicate (Table 1).

Table 1. Sample preparation for matrix test.

Baby food blend	Triplicate of each treatment:		
A	*unspiked*	*1 ppb spiked*	*10 ppb spiked*
B	*unspiked*	*1 ppb spiked*	*10 ppb spiked*
C	*unspiked*	*1 ppb spiked*	*10 ppb spiked*

The baby foods were extracted using acidic ACN (1% acetic acid in acetonitrile). For each, 15 g of homogenous food blend was added to a tube with 15 mL of the acidic ACN solvent. These were shaken vigorously for 1 minute, then centrifuged. The supernatant was then sampled for 1:10 dilution in mobile phase and LC-MS/MS analysis. This procedure is a “dilute-and-shoot” approach, adapted from the AOAC QuEChERS method but intended to produce a sample which has gone through very little matrix cleanup, in order to assess method performance in challenging matrix conditions.

Chromatography: Chromatography was performed using a Phenomenex Luna Omega Polar C18 (2.6 µm x 100 mm) at a flow rate of 0.4 mL/min, and an injection volume of 1 µL (Table 2).

Table 2. LC Gradient.

Time (min)	B (%)
0.75	*5*
8	*100*
8.5	*100*
9	*5*
10	*End*

Mobile Phase A - 0.1% formic acid in water
Mobile Phase B - 0.1% formic acid in acetonitrile

Table 3. OptiFlow Pro Ion Source parameters.

Parameter	Value
CAD	*10*
CUR	*32 psi*
GS1	*40 psi*
GS2	*70 psi*
IHT	*200*
IS	*1500 V*
TEM	*350 ºC*

Mass spectrometry: The SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready was used to analyze the samples, to assess trace level quantitative performance of the analyte panel in both neat standards and the baby food blends.

Multiple reaction monitoring (MRM), employing two precursor to product ion transitions for each analyte in the panel, represents the most important analytical technique for trace level pesticide quantification. For each transition, optimized voltages are defined for compound-specific parameters such as Collision Energy (CE), but ion source parameters are also set which apply to all analytes in the method. The OptiFlow Pro Ion Source parameters were retuned as there are some key differences in design to the earlier Turbo V™ Ion Source. For example, the source temperature can be set lower than precedent methods, which can benefit the performance for thermally sensitive analytes. The source parameters can be found below (Table 3).

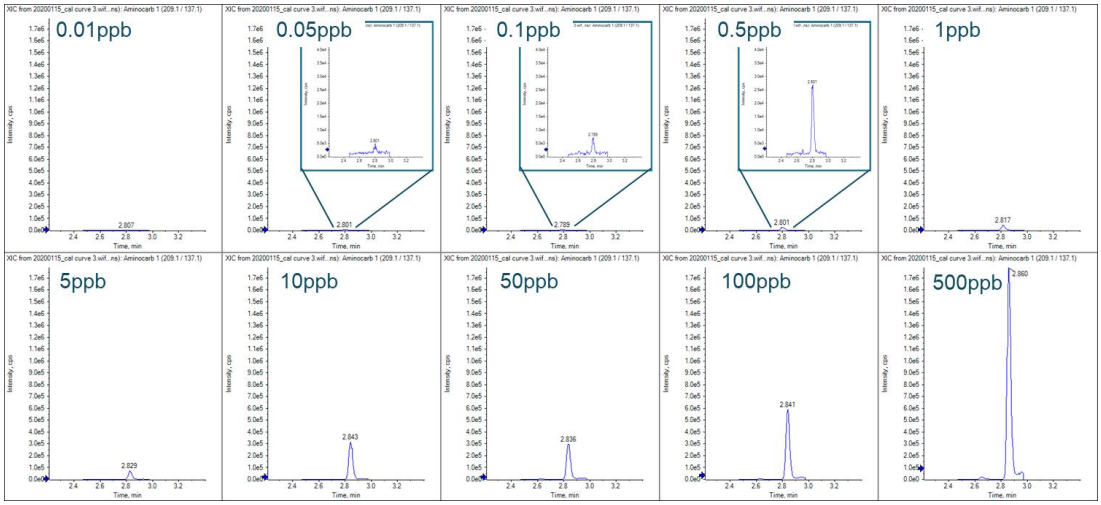
Data processing: All data were processed using the SCIEX OS Software. SCIEX OS Software, now available on SCIEX triple quadrupole platforms, integrates acquisition and processing into a single software platform to be used from sample analysis through to results reporting.

Results

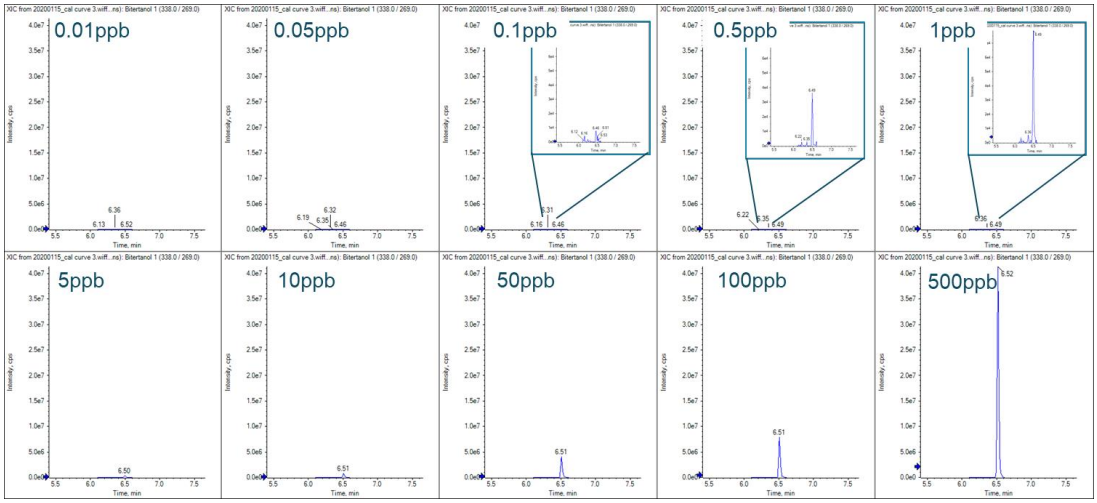
Low-level quantification: Most pesticides in the 209-compound panel were detected at levels below 1 ppb in the vial. Roughly half of them were measured at a lower limit of 0.1 ppb or lower (Figure 1). This is particularly notable as these values were achieved using a dilute-and-shoot method with a small (1 µL) injection volume.

Figures 2 and 3 illustrate the sensitivity and performance of this method on the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready for some example pesticides. The chromatograms shown represent increasing concentrations of standard calibrators in neat solvent solution.

Aminocarb



Bitertanol



Bifenezate

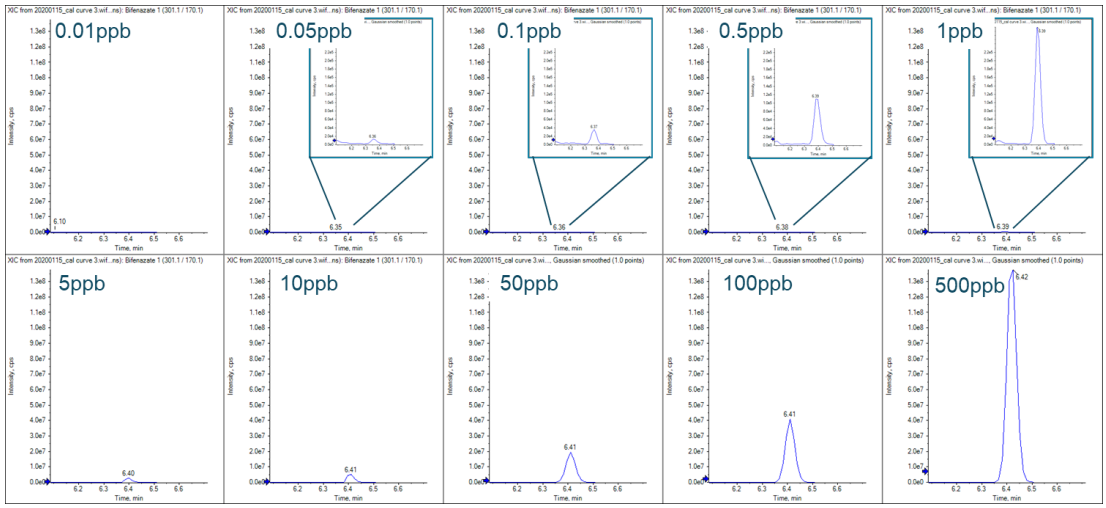
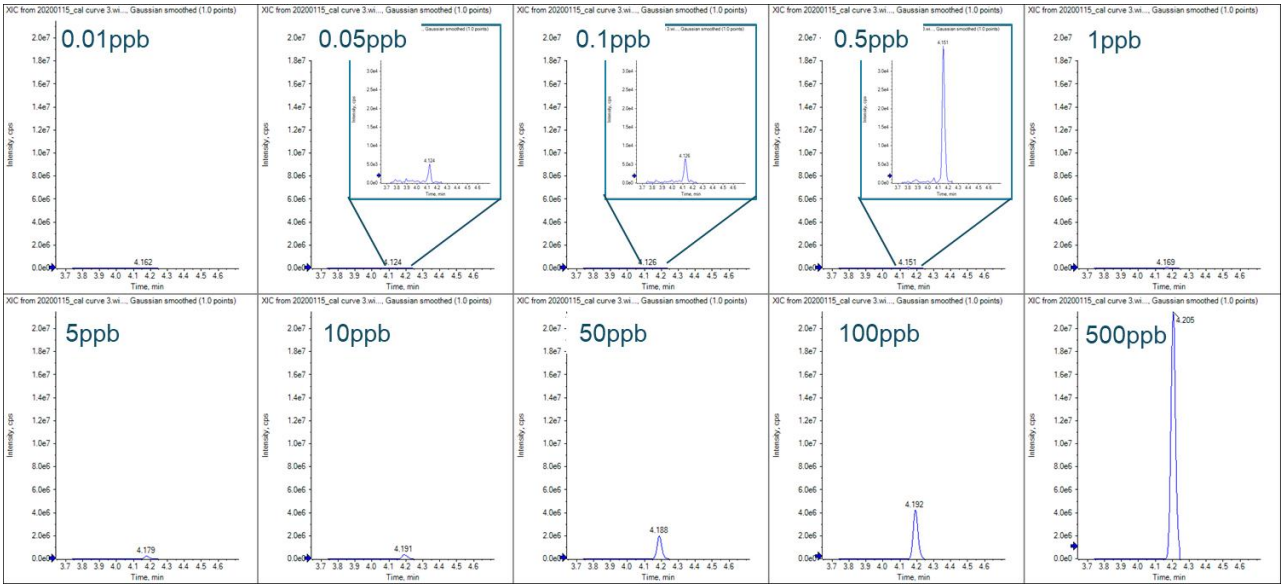


Figure 2. Examples of low level detection of pesticides. Data is shown for three selected pesticides at increasing concentrations of standard calibrators in neat solvent solution, for aminocarb (top), bitertanol (middle), and bifenezate (bottom).

Imidicloprid



Spiromesifen

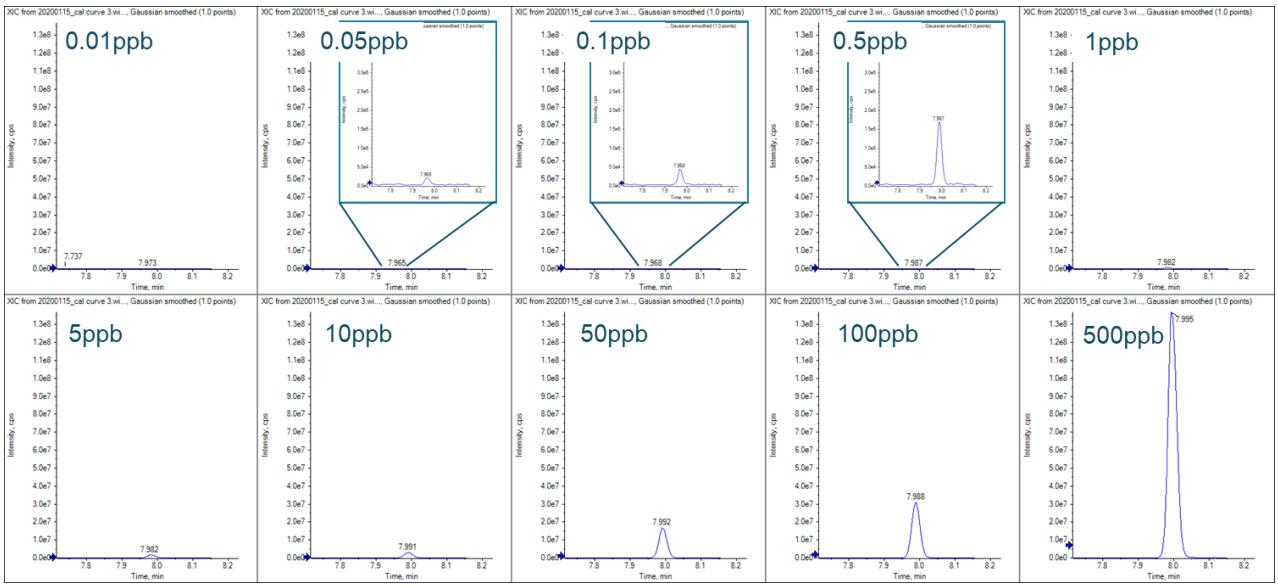
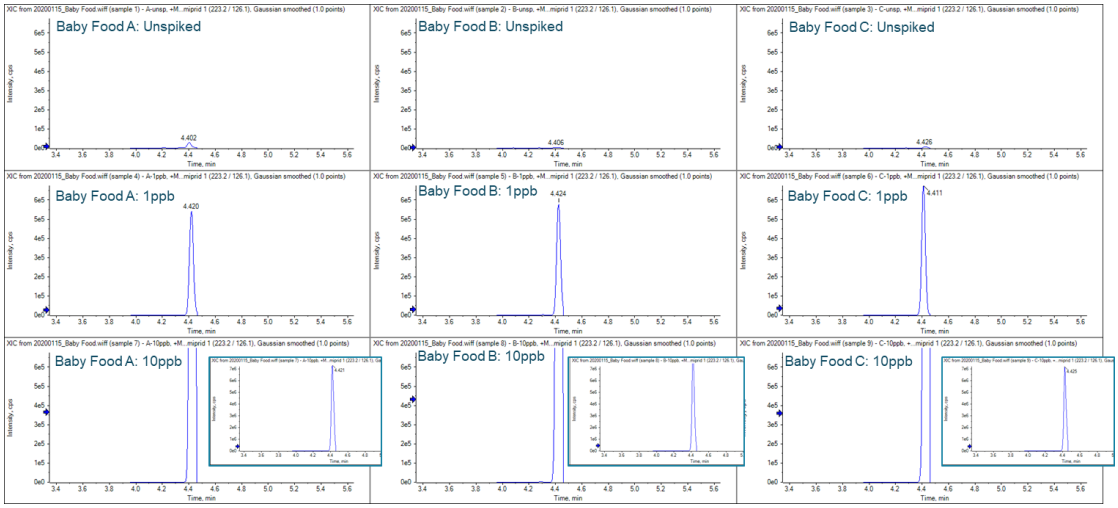
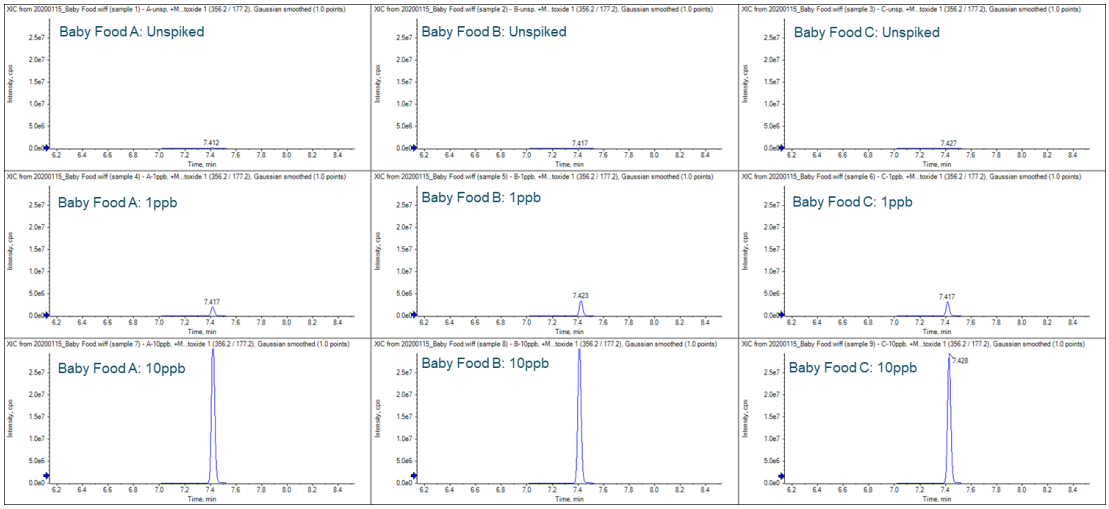


Figure 3. Examples of additional low-level detection of pesticides. Data is shown for three selected pesticides at increasing concentrations of standard calibrators in neat solvent solution, for imidicloprid (top), and spiromesifen (bottom).

Amidicloprid



Piperonyl-butoxide



Hexythiazox

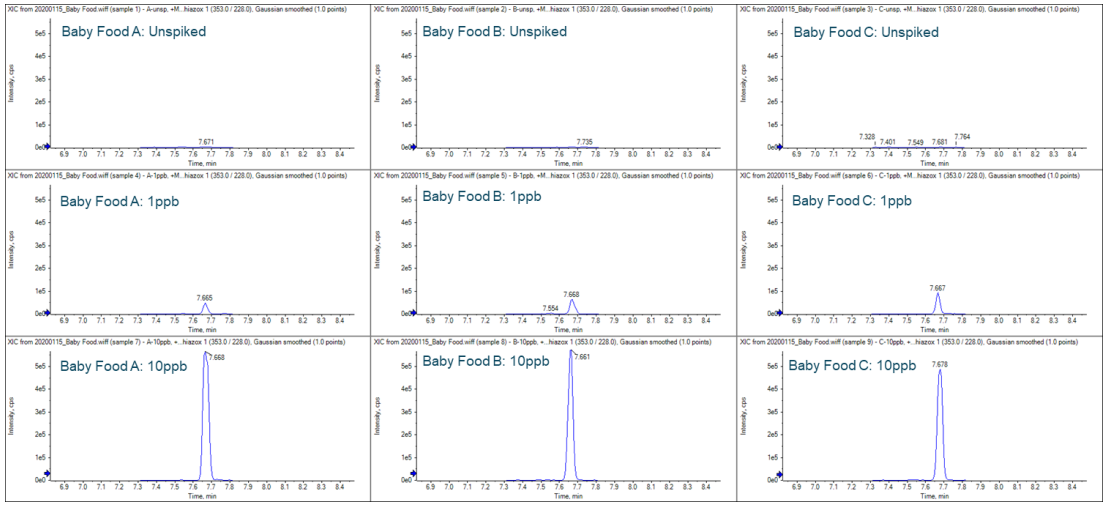


Figure 4. Examples of pesticide detection in three baby food blends. Each was measured without the pesticide standards spiked in, as well as with low and higher level additions of pesticide standards. Data is shown for three example pesticides, amidicloprid (top), piperonyl-butoxide (middle) and hexythiazox (bottom).

Analysis in baby food matrix: Each baby food blend was measured without fortification, and with a low- and high-level fortification. Figure 4 illustrates three example pesticides in the three matrix treatments. The EU Commission Directives outlines limits for pesticides in baby foods specifically.[1] The measures, intended to protect vulnerable populations such as infants, set generic tolerance limits of 0.01 mg/kg in the food sample. Table 4 shows the direct correlation between the LOQs determined with this method and the mass on column and LOQ in sample. The mass on column value is critical for comparing LOQs across acquisition methods that differ by platform, injection volume, or separation strategy. The LOQ in sample is the value on which most regulatory limits are based, and its relationship to the LOQ in vial is dependent on sample extraction and preparation as well as injection volume.

Considering the EU MRL of 0.01 ng/g in sample, the calculated LOQ required to achieve this with the presented method would be:

$$\left(0.01\frac{ng}{g}\right) x\ (15g\ of\ sample) = 0.15\ ng$$

$$\frac{0.15ng}{15\ mL\ extraction\ solvent} = \frac{0.01ng}{mL}(ppb) in\ extract$$

In this method, the extract was then diluted 1:10.

$$\frac{\frac{0.01ng}{mL}}{10} = 0.001\frac{ng}{mL}(ppb) LOQ\ in\ vial\ required\ for\ EU\ MRL$$

$$OR: 0.001\ pg\ mass\ on\ column$$

With the 1:10 dilution, 1 µL injection volume, and absence of matrix cleanup steps, the achievement of LOQs as low as 0.01 represents exemplary instrument performance in challenging matrix conditions, and suggest that sample preparation adjustments to meet the 0.001 pg mass on column required to assess food products for EU compliance is readily achievable.

Qualitative confirmation of pesticide detection in complex matrix is achieved using ion ratios of two MRM transitions per compound and matching these to the compound-dependent ratios observed in calibration standards. This ion ratio confirmation can be automatically visualized, flagged, and filtered in the SCIEX OS Software results table (Figure 5).

Component Name	Sample Name	Sample T...	Expected Ion Ratio	Ion Ratio	Ion Ratio...
Etaconazole 1	A-1ppb	Unknown	0.4426	0.3902	✓
Etaconazole 1	A-10ppb	Unknown	0.4426	0.3766	✓
Etaconazole 1	0.5ppb	Standard	0.4426	0.3947	✓

Figure 5. Confirmation using ion ratios. Ion ratios shown for a standard (left), then confirmed in fortified matrix samples (1 ppb center, 10 ppb right). The expected ion ratio is represented as the line across the chromatogram, with ±20% tolerance limits on the ion ratio also drawn as upper and lower bound lines. The results table will display a filterable green check mark for rapid assessment of samples meeting ion ratio criteria.

Linear response: For some compounds increased instrument sensitivity provides lower detection limits, however this added sensitivity can result in the upper range of the calibration curve becoming limited by detector saturation. In Figure 6, two examples of calibration curves are shown. For chlorfluazuron, the response across the concentration range remains linear. For desmedipham, a very good detection limit was observed (0.05 ppb) however, the signal saturation is apparent at the highest concentrations, as seen by a plateau in the calibration curve. The linear range extends from 0.05 ppb to 100 ppb.

Identifying the true linear dynamic range for calibration curves generated on a large panel of pesticides can be time consuming, as variable analyte responses are common, leading to different LLOQs and ULOQs across the analyte panel. One SCIEX OS Software feature that makes this aspect of data processing more streamlined is the Automatic Outlier feature. Here, the user defines the criteria the calibration standards must meet in order to achieve optimal quantitative method performance. In Figure 8, a screenshot from SCIEX OS Software shows the functionality of the outlier removal tool, along with a calibration curve where the high-end points which have been automatically excluded from the curve and model fit.

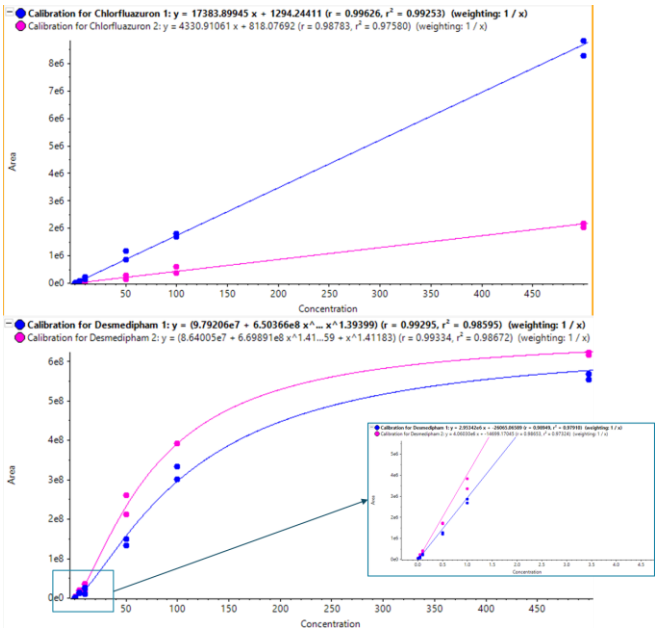


Figure 6. Two calibration curves demonstrate the difference between compounds in the increased sensitivity and its impact to the linear range. (Top) chlorfluazuron maintains a linear response on the **SCIEX** 7500 System across the concentration range studied here. (Bottom) desmedipham calibration curve plateaus at the high concentration end due to detector saturation. However, the low end of the concentration range still behaves linearly.

Reproducibility and robustness: It is readily apparent that method reproducibility as well as hardware robustness are critical for routine analysis of low-level residues in food. Two evaluations were performed in order to demonstrate reproducibility and robustness. First, triplicate analyses of the pesticide panel at a 10 ppb concentration in the baby food matrix were used to assess and report %CV values across the panel of compounds. Figure 7 shows a breakdown of where the majority of these %CV values fall.

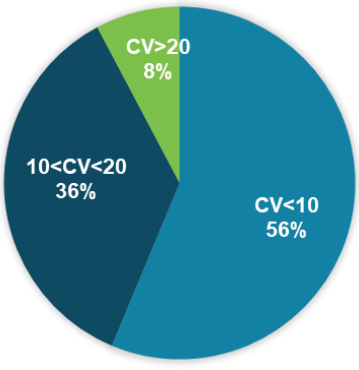


Figure 7. Reproducibility of pesticides in baby food matrix. The vast majority of pesticides demonstrated %CV values in baby food matrix of < 20%CV. Over half of these demonstrated %CV values of <10%. Precision in complex matrices was assessed based on integrated peak area.

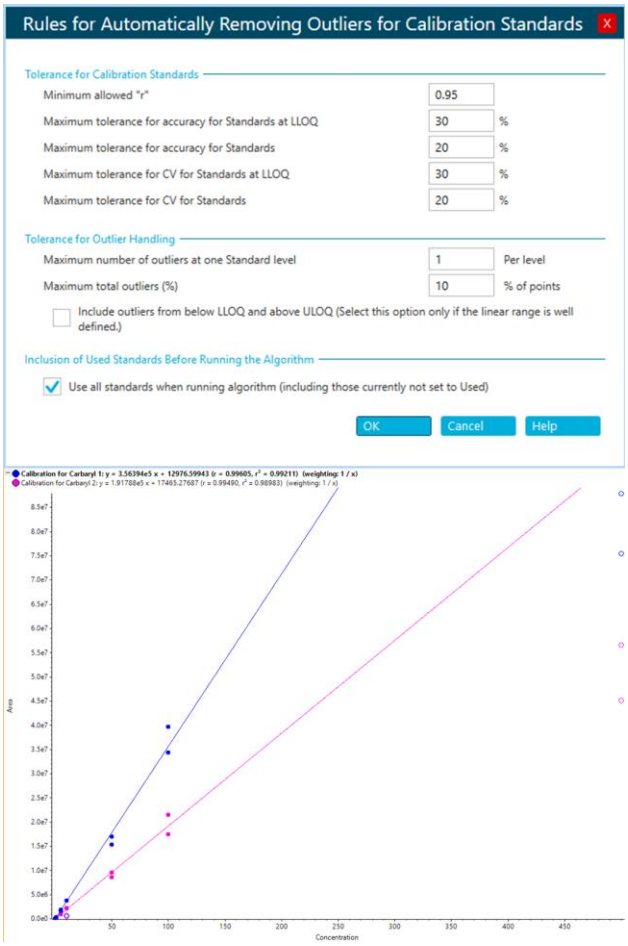


Figure 8. User-defined rules which dictate the exclusion of calibrator points from the regression model. (Top) This processing method has defined a minimum r value that each calibration curve must attain using a linear regression. Restrictions were placed on how many points would be excluded as well as tolerance limits for accuracy and %CV. These can be defined for all standards or have unique criteria for the lowest calibrator. (Bottom) In the example calibration curve, the outlier removal tool has excluded calibrators at the high concentration end (open circles) in order to achieve the best linear fit for the low end of the curve and meet the set criteria.

Second, a test was executed in order to demonstrate extreme robustness and reproducibility across a great number of injections in a complex matrix. This test injected over 2000 samples of pesticide mixture in a black tea matrix used exclusively for this purpose. Figure 9 shows exemplary consistency from the first to the last injections with no cleaning or maintenance having taken place over the course of the test.

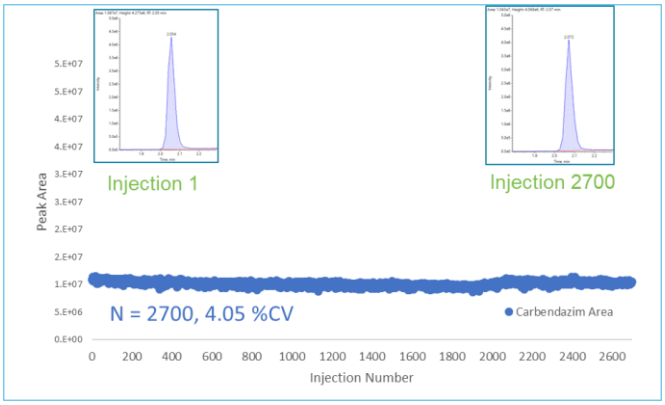


Figure 9. Excellent reproducibility and robustness in matrix. A short LC-MS run was performed (4 min gradient at 400 µL/min flow rate) for this specific test. The raw peak area was plotted across 2700 injections. The S/N also remained constant across injections.

Table 4. Translation of in-vial LOQ values to on-column analyte mass and original concentration in baby food sample. The mass on column value is critical for comparing LOQs across acquisition methods that differ by platform, injection volume, or separation strategy. The LOQ in sample value is that on which most regulatory limits are based, and its relationship to the LOQ in vial is dependent on sample extraction and preparation as well as injection volume.

LOQ in vial (ppb)	Mass on column for 1 µL injection (pg)	LOQ in sample (ng/g)
0.01	*0.01*	*0.1*
0.05	*.0.05*	*0.5*
0.1	*0.1*	*1*

Conclusions

The SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready was used to analyze a panel of 209 pesticides in both neat solvent and baby food matrices. The primary objective was to evaluate the absolute sensitivity of the system and investigate how the method would perform in a baby food matrix composed of fruits and vegetables. To this end, it was found that sub-ppb levels were easily achievable with high-quality quantitative metrics (reproducibility, peak quality, linear response) even with a dilute sample and small injection volumes.

This promising new generation of analytical technology allows for the continued pursuit of low-level residues for food safety testing while addressing historical challenges of food analysis by LC-MS/MS. Larger dilutions and smaller injection volumes will help laboratories maintain maximum uptime and reduce the need for cleaning and decontamination.

The SCIEX OS Software platform for acquisition and processing makes the process seamless from sample to report, and advanced features accelerate and streamline the quantification process. Having access to greater sensitivity than what might be currently required is a growing trend in laboratory preparedness for future changes in the regulatory and analytical landscape.

References

1. EU Commission Directives 2006/125/**EC** and 2006/141/**EC.**
2. Enabling new levels of quantification. SCIEX technical note RUO-MKT-02-11886-A.
3. Nougadère, *et al.*,Dietary exposure to pesticide residues and associated health risks in infants and young children – Results of the French infant total diet study, *Environment Int.***137.**
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5. M. Anastassiades, *et al.* (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *J. AOAC Int.* **86(2)** 412-431.

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