Fast Screening, Identification, and Quantification of Pesticide Residues in Baby Food Using GC Orbitrap MS Technology

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Baby Food, Exact Mass, Screening, Food Safety, GC Orbitrap, High Resolution GC-MS, Pesticide Analysis, Quantification, TraceFinder

Introduction

Pesticides are chemicals widely used to control a variety of pests, such as insects, plant pathogens, weeds, etc. The use of pesticides may result in residues in crops, therefore, strict regulations are in place to control the use of these chemicals and to ensure that concentrations do not exceed statutory maximum residue levels (MRLs).[1]

Pesticides are measured almost exclusively by liquid chromatography (LC) and gas chromatography (GC) analytical methodologies. GC coupled to a mass spectrometer (MS) as a detector is widely used in many pesticide residue laboratories, because many pesticides are not amenable to LC-MS or ionize poorly under soft ionization techniques. GC offers good separation efficiency and a choice of MS detectors, including single or triple quadrupoles. Quadrupole mass analyzers are selective, sensitive, and cost-effective instruments that operate at nominal mass resolution. When using quadrupole MS, the selectivity required to separate target pesticides from chemical background is achieved by by the use of either selected ion monitoring (SIM) or selected reaction monitoring (SRM). Both SIM and SRM are used in targeted experiments in which the mass spectrometer is pre-programmed using a list of preselected pesticides. How-ever, targeting specific compounds during acquisition limits the scope of analysis and can result in false negative results (non-detection) for both unknown and untargeted compounds, which may be of concern with respect to food safety.

This limitation has led to increased interest in developing methods using MS analyzers that can operate in full scan with a higher mass resolving power than triple quadrupoles, but provide similar levels of selectivity and quantitative performance. Until now, high-resolution, accurate-mass GC-MS instruments have not gained wide acceptance due to their limited ability to provide full scan selectivity and quantitative performance comparable to triple quadrupole instruments operated in SRM.

In this work, we demonstrate the use of GC coupled to Orbitrap™ MS technology for fast, high throughput pesticide residues analysis in baby food samples, with an almost unlimited scope in the analysis through full scan acquisition. Quantitative performance comparable to triple quadrupoles and compliance with SANCO guidelines[2] will also be demonstrated.

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Sample Preparation

Baby food samples were extracted using the a citrate buffered QuEChERS protocol, described previously.[4] The final extracts (1 g/mL in acetonitrile) were spiked with a mixture of 132 pesticides at concentrations corresponding to 0.5–100 ng/g (ppb) for the majority of analytes and 1.0–200 for some analytes.

Instrument and Method Setup

In all experiments a Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap mass spectrometer was used. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC and a Thermo Scientific™ TraceGOLD™ TG-5SilMS 15 m × 0.25 mm I.D. × 0.25 µm film capillary column (P/N: 26096-1301). Additional details of instrument parameters are shown.

GC and Injector Conditions	
TRACE 1310 GC Parameters	
Injection Volume (µL):	1.0
Liner:	asymmetric baffled (P/N: 45352062)
Inlet (°C):	75
Inlet Module and Mode:	PTV, cold splitless
Transfer delay (min):	1
Injection time (min):	0.1
Transfer rate (°C/sec):	2.5
Transfer temperature (°C):	300
Transfer time (min):	3
Cleaning rate (°C/sec):	330
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program	
Temperature 1 (°C):	40
Hold Time (min):	1.5
Temperature 2 (°C):	180
Rate (°C/min)	25
Temperature 3 (°C):	300
Rate (°C/min)	100
Hold Time (min):	3

The Q Exactive GC system was tuned and calibrated using peaks of known mass from a calibration solution (FC 43, CAS 311-89-7) to achieve mass accuracy of < 0.5 ppm RMS. The system was operated in electron ionization mode (EI) using full scan and 60,000 mass resolution (Full Width at Half Maxima, measured at *m/z* 200), meeting the recommended SANCO resolution criteria[2] for high resolution analytical instrumentation. Chromatographic data was acquired with a minimum of 12 points/peak to ensure consistent peak integration.

Mass Spectrometer Conditions	
Q Exactive GC Mass Spectrometer Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source (°C):	230
Electron energy (eV):	70
Acquisition Mode:	Full scan
Mass range (*m/z*):	50–500
Mass resolution (FWHM at *m/z* 200):	60,000
Lockmass (*m/z*):	207.03235

Data Processing

Data was acquired and processed using Thermo Scientific™ TraceFinder™ software. TraceFinder software allows the analyst to build acquisition and processing methods for high throughput screening and quantitative analysis and incorporates library searching capabilities as well as easy data reviewing and data reporting.

Results and Discussion

The objective of this study was to evaluate the utility of Orbitrap-based GC-MS technology for fast pesticides screening and quantification to increase sample throughput and laboratory productivity. Various analytical parameters were assessed and the results of these experiments are described.

Chromatography

Good chromatographic separation was obtained using the GC conditions described. An example of chromatography for the matrix-matched standard (corresponding to 100/200 ng/g) is given in Figure 1. The total ion chromatogram, as well as the extracted ion chromatograms (XIC, ±2 ppm extraction mass window) of the first (dichlorvos, *m/z* 184.97650, RT = 4.46 min) and last (deltamethrin, *m/z* 252.90451, RT = 10.33 min) eluting pesticides, are shown. The fast separation allowed for a high sample throughput as described elsewhere.[4]

MS Acquisition Speed

When using short GC run times, the analyte chromatographic peak widths are narrow, typically 2.5 seconds. This narrow peak width necessitates fast MS acquisition rates in order to obtain enough scans/chromatographic peak. When the number of points per peak is not sufficient to define a Gaussian shape, the peaks of interest can be integrated inaccurately, which in turn affects the reproducibility, peak integration, and ultimately, the accuracy of target compound quantification. An example of typical number of scans acquired using the Q Exactive GC system operated at 60,000 resolution for EPTC in baby food is shown in Figure 2. Aside from producing an adequate number of scans/peak (17), excellent mass accuracy (0.5 ppm RMS) was obtained for every scan across the peak.

Pesticides Targeted Screening

A simple, targeted screening experiment was set up as a first test to screen for pesticides that were spiked into the baby food matrix. This was performed using the TraceFinder software against an in-house compound database containing 183 pesticides. The database contains the compound name, theoretical exact masses for at least three fragment ions, and expected retention time information for the GC conditions used for the sample analysis.

Compound detection and identification was based on retention time (±0.1 min window), accurate mass information (±2 ppm window), isotopic pattern similarity (measured versus theoretical), and library search hit (NIST14). Using these criteria, all 132 pesticides were positively detected and confirmed in the 10/20 ng/g baby food sample.

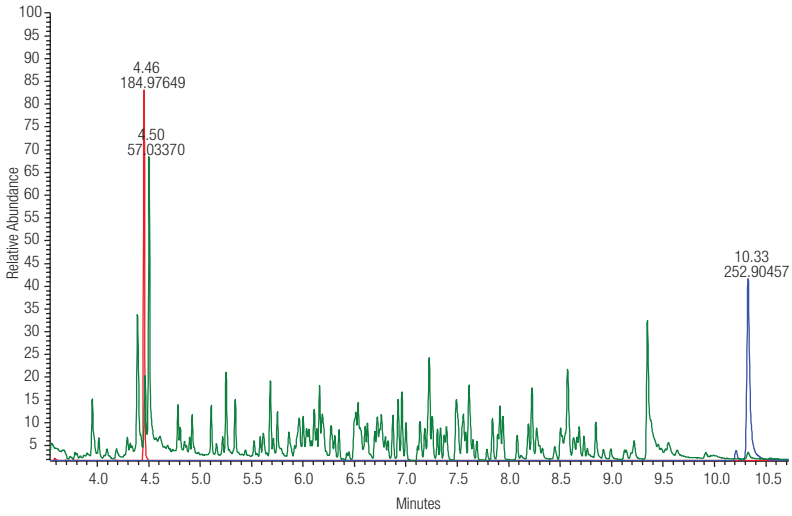

Figure 1. Overlay of the total ion chromatogram (EI full scan) and the extracted ion chromatograms (XIC) of the first (dichlorvos, RT = 4.46 min) and the last (deltamethrin, RT = 10.33 min) eluting pesticides. Relative abundance (Y axis) adjusted to emphasize XIC for dichlorvos and deltamethrin.

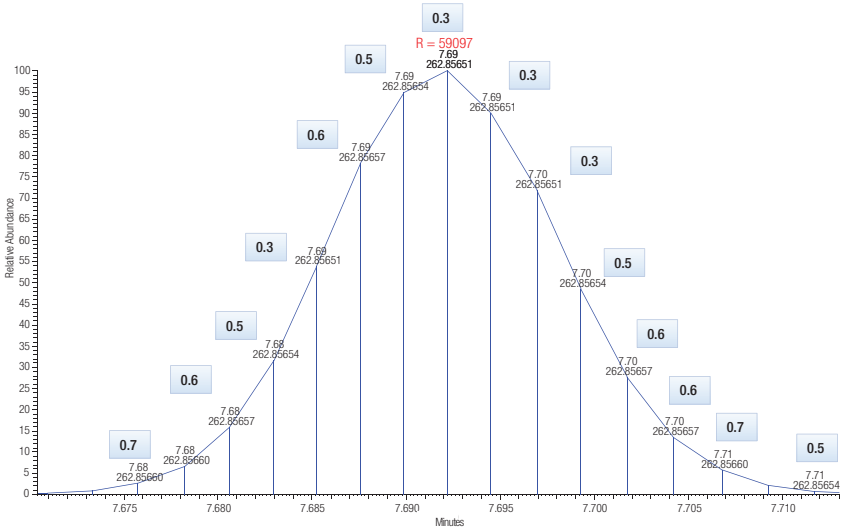

Figure 2. Extracted ion chromatogram (XIC) of dieldrin (*m/z* 262.85642, ±2 ppm mass window) showing 17 scans/peak (peak width 2.4 sec). Data acquired in full scan at 60,000 FWHM resolution (the exact resolution used is annotated in red). Measured accurate mass for each scan is shown as well as mass difference (ppm).

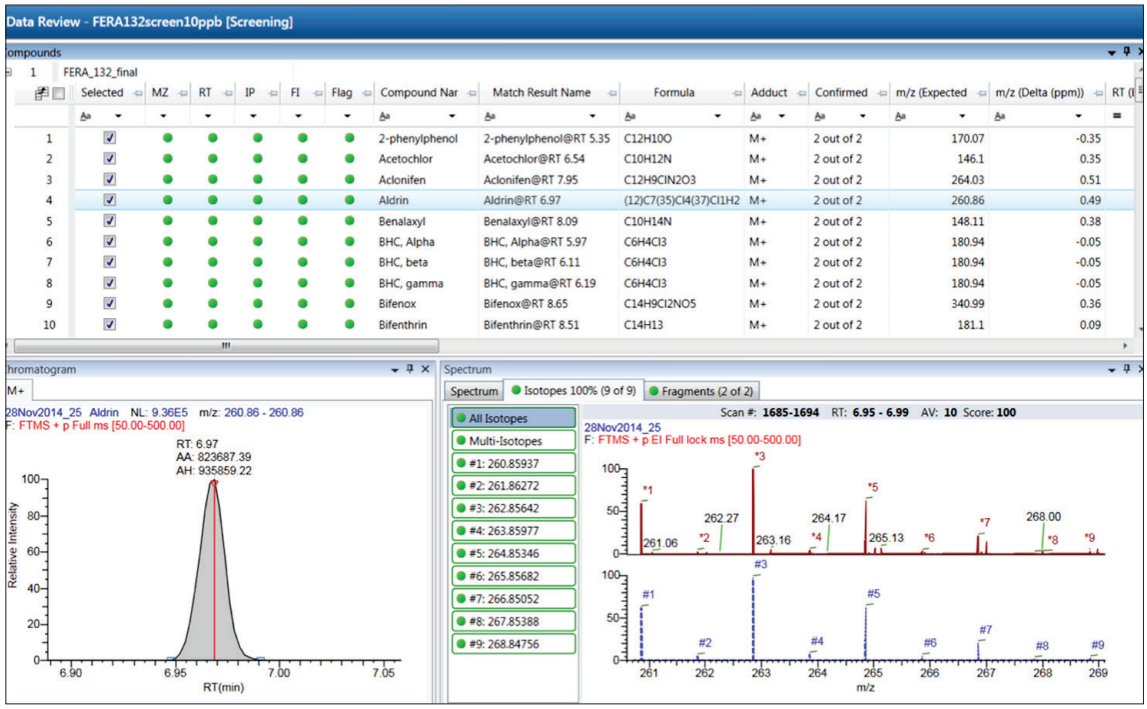

Figure 3. TraceFinder software screening result browser showing positively identified pesticides in the 10 ng/g sample. Compound identification and confirmation (aldrin showed as an example) was based on accurate mass identification (±2 ppm mass window), retention time (RT), isotopic pattern (IP), and fragment ions (FI). Measured and theoretical isotopic clusters are shown.

An example of the compound detection and identification workflow for aldrin is shown in Figure 3. Data acquired in full scan is deconvoluted and retention time and accurate mass information are then used to identify the compound. Aldrin was identified based on the RT, and the presence of an accurate mass quantification ion (<0.5 ppm mass error) and the characteristic fragment ions. Moreover, the elemental composition of the quantification ion ($C_7C_{15}H_2$) was used to check the isotopic pattern fit against the measured isotopic pattern. As shown in Figure 3, a 100% isotopic fit was obtained for aldrin, adding to the confidence in compound identification.

Pesticide Residue Quantification

The quantitative performance of the Q Exactive GC system for compound quantification was tested for all 132 pesticides. To assess quantitative performance, a matrix-match calibration curve was constructed over a concentration range of 0.5–100 ng/g (or 1.0–200 ng/g). System sensitivity, linearity, and peak area reproducibility were evaluated. Additionally, mass accuracy of the target pesticides was assessed across the concentration levels.

Sensitivity

Almost all pesticides (95%) were detected in the lowest calibration matrix-matched standard 0.5 (or 1.0) ng/g. Examples of chromatography at this concentration level are shown in Figure 4. At the 5 ng/g level, all of the compounds detected had ion ratios valued within a 15% limit of the average ion ratio values derived from the calibration curve across all concentrations.

Estimation of Instrument Detection Limit (IDL) and Peak Area Repeatability

System sensitivity was assessed by calculating the IDL for each pesticide. The IDL of the target pesticides represents the smallest signal above background noise that an instrument can consistently and reliably detect. This signal was determined empirically by repeatedly injecting (n=10) the 5 ng/g (and 10 ng/g) matrix-matched standard and taking into account the Student's-*t* critical values for the corresponding degrees of freedom (99% confidence). The results of this experiment showed an average %RSD for the peak area reproducibility of 6 % (Figure 5).

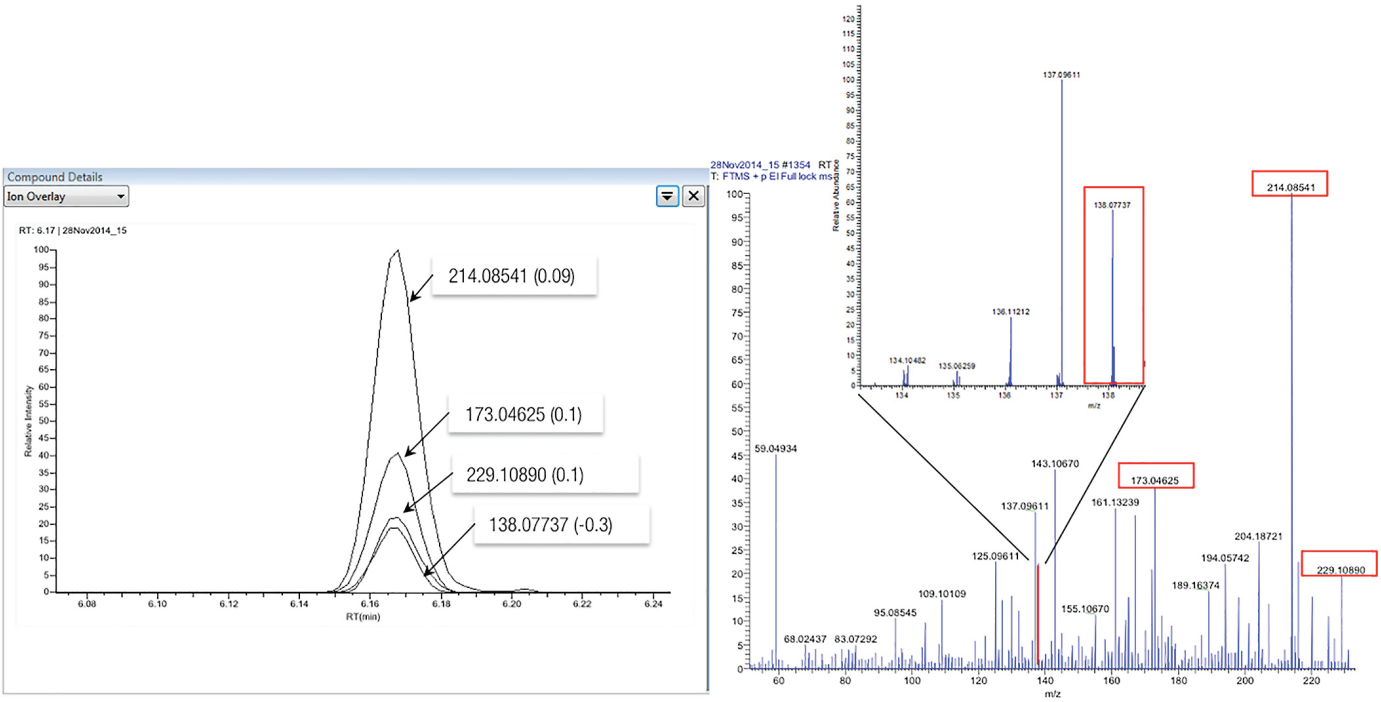

Figure 4. Terbuthylazine at 0.5 pg (on column concentration) showing an XIC overlay for the quantification ion and three additional confirmation fragment ions (left). The measured mass for each ion and mass error (in ppm) are annotated. Mass spectrum (right) highlighting the ions used for quantification and confirmation; the zoomed area shows the least intense fragment (*m/z* 138.07737) measured with a mass accuracy of 0.3 ppm.

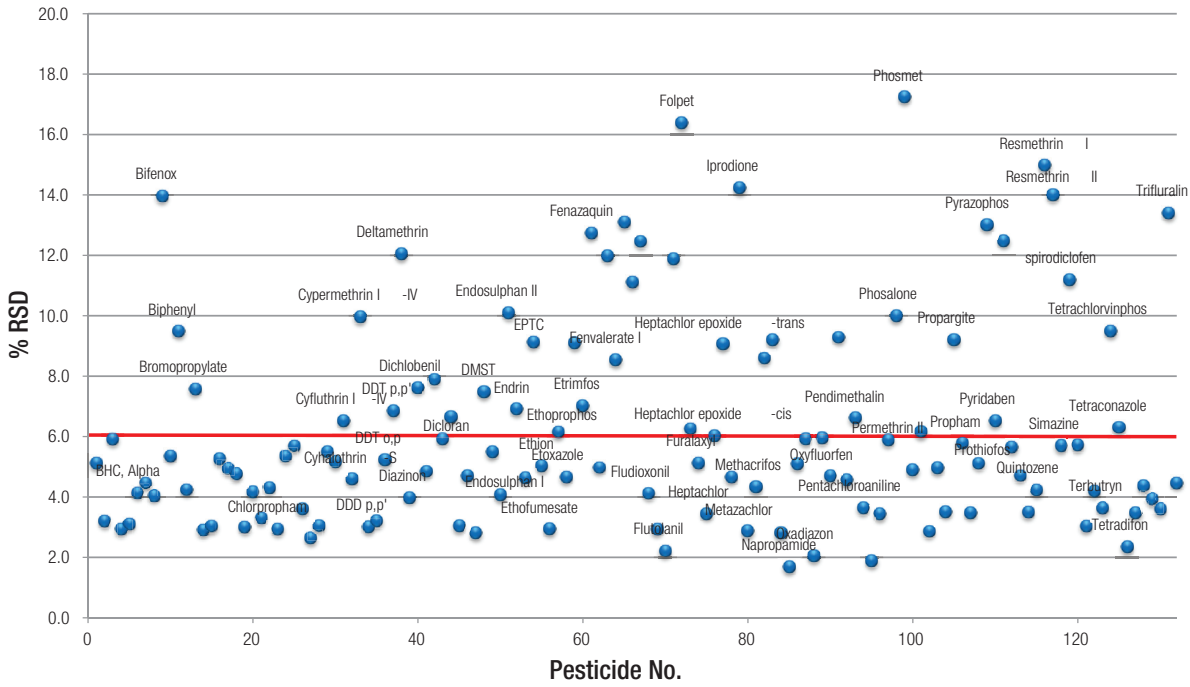

Figure 5. Absolute peak area repeatability (% RSD, n=10) at 5 or 10 pg injected on column for all 132 pesticides measured. The average %RSD value (solid line) is shown.

All the IDLs derived from the Q Exactive GC system data were lower than the typical MRLs established by the European Union for baby food samples. For most pesticides, these MRLs are currently set at <0.01 mg/kg (10 ng/g).[3] Calculated IDLs were compared to the IDL values obtained for the same pesticides using the Thermo Scientific™ TSQ™ 8000 Evo triple quadrupole GC-MS/MS system.[4] The results of this experiment demonstrated that the sensitivity of the Q Exactive GC system is comparable to that of the TSQ 8000 Evo GC-MS/MS system, with 91% of pesticides having an IDL < 2 ng/g (Figure 6).

Mass Accuracy

Obtaining accurate mass information in a consistent manner is critical for determining the identity of a pesticide as well as maintaining a high degree of discrimination through the resolving power of the instrument, against matrix interference.[5] The mass accuracy for all 132 pesticides was assessed at the 5 ng/g (or 10 ng/g, depending on compound) level from a series of n = 10 repeat injections. The mass deviation values did not exceed 1 ppm for any of the analytes and the overall mass accuracy average value was 0.4 ppm, providing the highest confidence in accurate and selective detection (Figure 7).

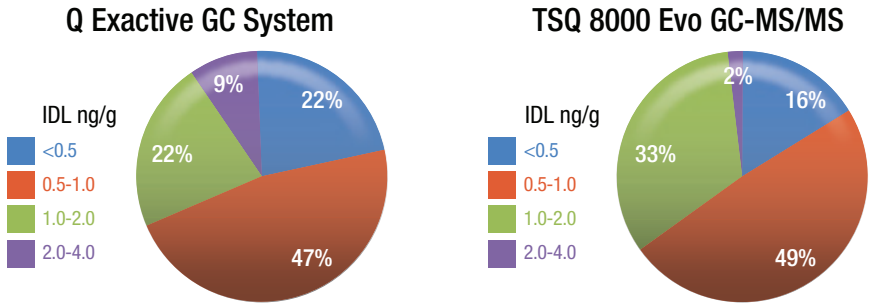

Figure 6. Comparison of the IDL99 (ng/g) calculated for 132 pesticides from a 5 ng/g matrix-matched standard from the Q Exactive GC System (left) and TSQ 8000 Evo GC-MS/MS system (right). The percentage of pesticides and corresponding IDL interval, relative to the total number of target compounds (132), is indicated.

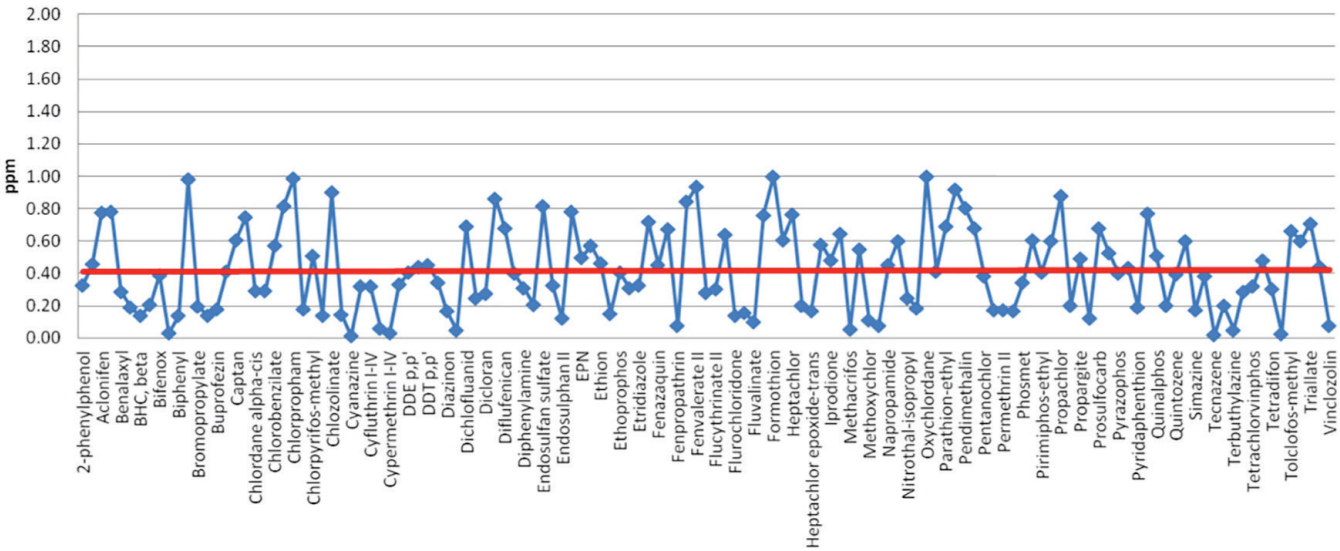

Figure 7. Accurate mass measurements (average value of n = 10) for the pesticides identified in the baby food sample at the 5 (or 10) ng/g level.

Linearity of Response

Quantitative linearity was assessed across a concentration range of 0.5–100 ng/g (or 1–200 ng/g for some analytes) using matrix-matched calibration standards injected in triplicate at each level. In all cases, the coefficient of determination (R^2) was >0.99 with an average value of R^2 = 0.997 and with residual values from the regression line of <25%. Examples of compound linearity are shown in Figure 8.

Conclusions

- The Q Exactive GC system provides high performance quantitative analysis in full scan for broad-scope pesticide residue testing, even with fast GC separations.
- The fast scan speed, high resolution, and outstanding mass accuracy, together with full scan sensitivity allow reproducible and accurate pesticide quantification at very low levels.
- Acquisition with a routine mass resolution of 60,000 FWHM at *m/z* 200 eliminates isobaric interferences, increasing confidence in results when screening pesticides in complex matrices. The consistent sub-ppm mass accuracy achieved for all compounds ensures confident compound identification.
- The Q Exactive GC system provides quantitative performance that is highly comparable to that of GC triple quadrupole MS instruments.
- Thermo Scientific TraceFinder software enables analysts to develop high throughput screening and quantitative analyses quickly and accurately.

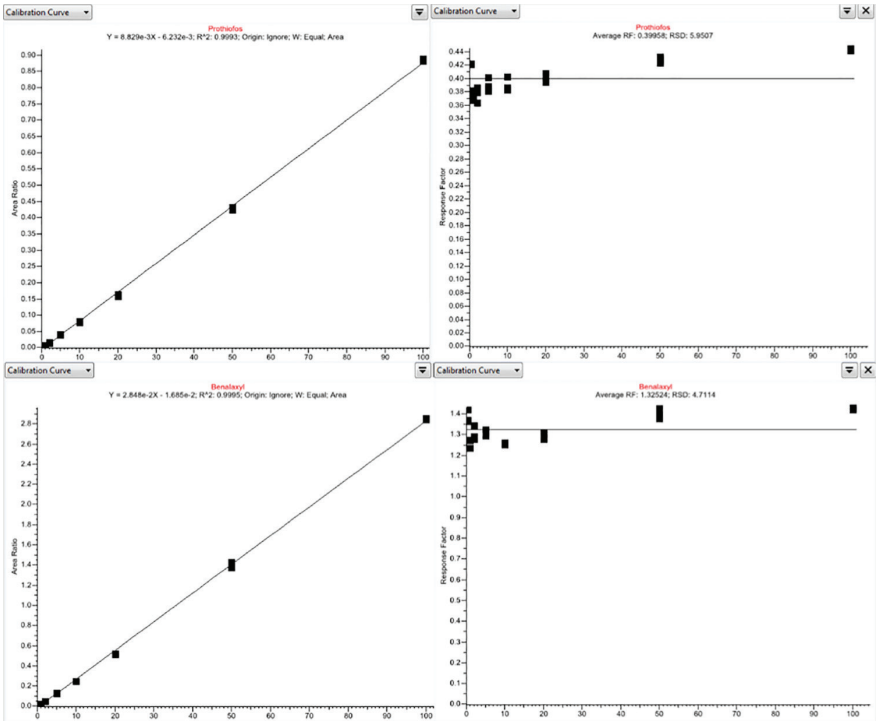

Figure 8. Coefficient of determination (left) and residuals values (%RSD) for prothiofos and benalaxyl calculated for a linear range of 0.5–100 ng/g.

References

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