Ultra-Low Level Quantification of Pesticides in Baby Foods using an Advanced Triple Quadrupole GC-MS/MS

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ABSTRACT

Purpose: The maximum residue level (MRL) for the majority of pesticide-commodity combinations is set at the default level of 10 μg/kg. However, a small number of pesticides and their metabolites may allow infants and young children to exceed the acceptable daily intake values. The European Union (EU) has established LoD MRLs between 3-8 μg/kg for a number of specific pesticides prohibited in baby foods. In this study, the quantitative performance of the Thermo Scientific™ TSQ™ 9000 GC-MS/MS system was assessed for the analysis of ~200 pesticides in baby foods at very low concentrations (as low as 0.025 μg/kg). A complete evaluation of method performance included sample preparation, overall method suitability measured from pesticides recoveries, selectivity, sensitivity, linearity and long term robustness.

Methods: To test the performance of the TSQ 9000 GC-MS/MS system, carrot/potato and apple/pear baby food samples were used following an extraction method using the citrate buffered QuEChERS and the Thermo Scientific™ HyperSep™ Dispersive Solid Phase Extraction (SPE) products. The GC-MS/MS instrument was equipped with an advanced electron ionisation (AEI) source and coupled with a Thermo Scientific™ TRACE™ 1310 GC. Liquid injections of the sample extracts were performed using a Thermo Scientific™ TriPlus™ RSH autosampler and chromatographic separation was achieved by a Thermo Scientific™ TraceGOLD TG-5SilMS 30 m × 0.25 mm I.D. × 0.25 μm capillary column.

Results: The method performance was tested in accordance to the SANTE/10518/2017 guidance document. Pesticide recoveries were obtained from the QuEChERS extractions performed on the samples spiked before extraction. All detected compounds, at the three spiking levels in both matrices satisfied all SANTE requirements. More than 97% of the target pesticide residues had recoveries between 70 – 120% at the 1 μg/kg spiking level. Over 90% or the target compounds had an LOI (satisfying all SANTE requirements) below 0.5 μg/kg, and over 60% below 0.1 μg/kg – 100 times lower than the default MRL. Compound linearity was assessed by injecting matrix matched standards in the range of 0.025 to 250 μg/kg in duplicate for both carrot/potato and apple/pear/banana. Both sets of linearity data showed R2 > 0.990 and RF % RSDs of <20% for over 96% of component peaks indicating excellent linear response. Robustness displayed over ~400 consecutive injections of sample matrix (1 g/mL), with SANTE compliance at the default MRL throughout.

INTRODUCTION

The detection and subsequent quantification of pesticide residues and other chemical residues and contaminants is of paramount importance, especially when the food stuff is intended to be consumed by infants or young children.

In the EU, the maximum residue level (MRL) for pesticide-commodity combinations is set at the default level of 10 μg/kg. 1-3 However, a small number of pesticides and their metabolites may allow infants and young children (under worst-case intake conditions) to exceed the acceptable daily intake (ADI) values. The EU has therefore established LoD MRLs, between 3-8 μg/kg for a number of specific pesticides prohibited in baby foods.4 The use of GC-MS/MS for the detection and identification of residues of prohibited compounds, in compliance with the residue definitions, has proved challenging, especially when the diverse composition of multi-ingredient baby foods are taken into account. Also, the increased levels of selectivity and sensitivity provided by triple quadrupole instruments compared to single quadrupole instruments enabled analysts to adopt faster, less specific sample extraction procedures. The QuEChERS procedure has become the standard approach for sample preparation in many laboratories because of an improvement in productivity.5

In this study, the quantitative performance of the TSQ 9000 triple quadrupole GC-MS/MS system with AEI source was assessed for the analysis of >200 pesticides in baby foods at very low concentrations (as low as 0.025 μg/kg). A complete evaluation of method performance include: Sample preparation, overall method suitability measured from pesticides recoveries, selectivity, sensitivity, linearity and long term robustness.

MATERIALS AND METHODS

Sample Preparation

To test the linearity and dynamic range of the system, post-spiked carrot/potato and apple/pear baby food samples were prepared using the citrate buffered QuEChERS protocol using HyperSep Dispersive Solid Phase Extraction (dSPE) products. Immediately after dSPE clean up the final extracts (1 g sample/mL of acetonitrile) were acidified with 5% formic acid in acetonitrile and were spiked with a mixture of 211 pesticides (including internal standard) at 14 concentrations spanning a range of 0.025–250 μg/kg.

Robustness was tested using repeat injections of samples (carrot/potato) spiked at the 10 μg/kg level. For method evaluation, pre-spiked carrot/potato and apple/pear/banana baby food samples were each prepared at 1.0, 2.5 and 10.0 μg/kg (n = 6 for each concentration).

Test Method

The experiments described here evaluate the sample preparation procedure and the quantitative performance of the TSQ 9000 GC-MS/MS system with Advanced Electron Ionization (AEI) source for the analysis of pesticides in baby food matrices. Gas chromatograph and mass spectrometer instrument parameters are listed in Table 1.

Data Analysis

All data acquisition, processing and reporting was carried out using Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System (CDS) software.

Table 1. Gas Chromatograph and Mass Spectrometer instrument parameters.

Thermo Scientific™ TRACE™ 1310 GC System Parameters	
Injection Volume:	1 μL
Liner:	Thermo Scientific™ LinerGOLD™ PTV via 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	0.30	
Transfer	3.0	300	2.00	
Cleaning	14.5	320	5.00	75.0

Oven Temperature Program:				
	Ramp	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			

TSQ 9000 Mass Spectrometer Parameters	
Transfer line:	250°C
Ionization Type:	Electron Ionization (EI) using the Thermo Scientific™ Advanced Electron Ionization (AEI) source
Ion Source:	320°C
Acquisition Mode:	Timed SRM, peak width 3 seconds, 10 scans per peak
	Detector gain factor x7
Tuning parameters:	AEI SmartTune = 50eV
Collision gas and pressure:	Argon at 70 psi
Peak Width:	0.7 Da both (Q1 and Q3 @FWHM)

RESULTS

Sample recovery and precision

Pesticide recoveries were obtained from the extracts performed on the samples spiked before extraction and clean up. All detected compounds, at the three spiking levels in both matrices satisfied all SANTE requirements.3 For method validation, acceptable limits for the pesticides were: 70 – 120% for recovery and 20% RSD for precision. More than 98% of the target pesticide residues had recoveries between 70 – 120% at the 10 μg/kg spiking level with only one pesticide displaying a precision >20% (anthraquinone in carrot/potato). An example of the recovery and precision data for the 10 μg/kg apple/pear/banana extract is displayed in Figure 1, with a summary of the full results in Table 2.

Figure 1. Recovery and precision data for apple/pear/banana extractions (n=6) at a concentration of 10 μg/kg. Not all components are labelled.
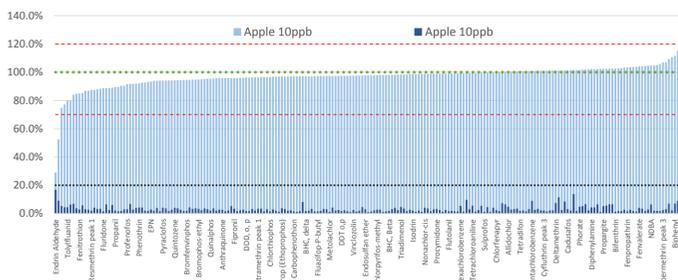

Table 2. Pesticide recoveries and precision values obtained from the QuEChERS extractions performed on the samples spiked before extraction. All detected compounds, at the three spiking levels in both matrices satisfied all SANTE identification requirements.

	Carrot 1ppb		Apple 1ppb		Carrot 2.5ppb		Apple 2.5ppb		Carrot 10ppb		Apple 10ppb	
	Mean recovery (n=6)	Precision (% RSD)	Mean recovery (n=6)	Precision (% RSD)	Mean recovery (n=6)	Precision (% RSD)	Mean recovery (n=3)	Precision (% RSD)	Mean recovery (n=6)	Precision (% RSD)	Mean recovery (n=6)	Precision (% RSD)
Average	100.3%	6.1%	95.2%	6.3%	95.2%	4.4%	98.7%	3.6%	96.1%	3.2%	96.9%	3.3%
No. of Pesticides detectable	202		202		202		202		208		208		209		209		210		210		210		210
No. of Pesticides within limits	197		201		197		201		203		207		206		208		206		209		208		210

Sensitivity and linearity

Figure 2 shows the lowest detectable standard for dichlobenil, dieldrin and deltamethrin which satisfies SANTE requirements. The MRLs are 10 μg/kg, 3 μg/kg* and 10 μg/kg respectively. Calibration curves show duplicate injection at 14 discrete levels ranging from 0.025 to 250 μg on column. *Dieldrin is classed as a prohibited pesticide and 3 μg/kg considered to be the current limit of quantification, but is subject to regular review.4

Figure 2. Example (A – dichlobenil, B - dieldrin and C – deltamethrin) chromatographic peaks showing the lowest detectable matrix matched carrot/potato standard which meets SANTE requirements.
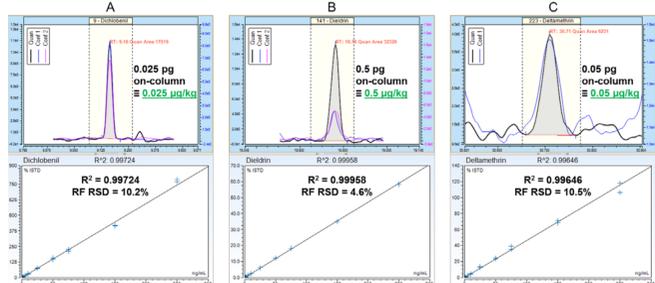
Compound linearity was assessed by injecting matrix matched standards in the range of 0.025 to 250 μg/kg in duplicate for both carrot/potato and apple/pear/banana. Both sets of linearity data showed R2 > 0.990 and RF % RSDs of <20% for over 96% of component peaks indicating excellent linear response.

Figure 3. Calibration data for Apple, pear and banana baby food matrix with calibrations ranging from 0.025 – 250 μg/kg to 10 – 250 μg/kg. Not all components are labelled.

System sensitivity, defined as instrumental detection limits (IDLs) was determined experimentally by performing n=10 replicates of the lowest matrix matched standard of carrot and potato that met all SANTE criteria. Calculations of IDLs were then made using one-tailed student t-test at the 99% confidence interval for the corresponding degrees of freedom and taking into account the concentration and absolute peak area %RSD for each compound (Figures 3 & 4).

Figure 3. Example of overlaid SRM transition (quantification and confirmation) of cadusafos and chlorbenzilate injected at the lowest level that met all SANTE criteria. Annotations indicate on column concentration, %RSD derived from absolute peak area response and calculated IDLs.
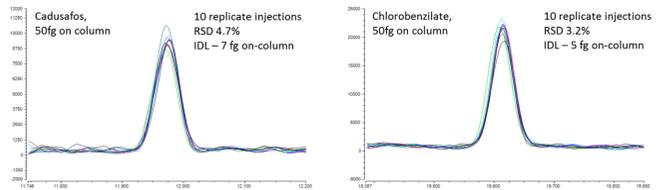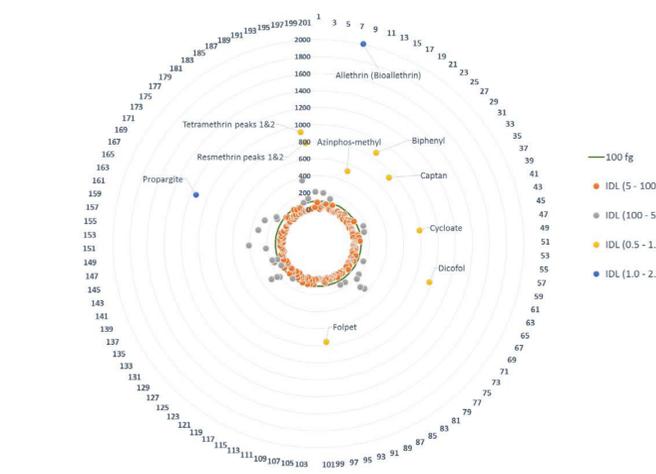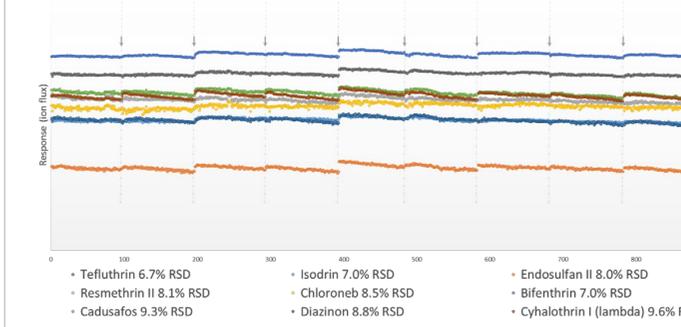

Figure 4. Plot showing the calculated IDLs for all pesticides. IDLs values ranged from ~5 fg (chlorbenzilate) to ~2.0 pg (bioallethrin) with >95% of compounds showing IDL<500 fg on column (equivalent to 0.5 μg/kg in sample extract – carrot/potato.)

The TSQ 9000 AEI was set up as described in Table 1. After an initial source cleaning, repeat injections of a sample extract (1g/mL carrot and potato) spiked at the default MRL (10 μg/kg) were made. Extracts resulting from the QuEChERS methodology contain a lot of undesirable matrix co-extracted components which can easily contaminate the GC inlet, the chromatographic column and the MS ion source. In order to test the robustness of the AEI ion source only, after every ~100 sample injections, the PTV liner was replaced along with the injector septum, approximately 10 cm trimmed from the guard column followed by automatic tuning of the system using the Smart Tune feature. Smart Tune uses the MS parameters established during the initial tuning on a clean source and intelligently assess the performance of the system, only re-tuning when MS performance has been compromised. No additional maintenance was performed.

Figure 5. Robustness data showing almost 900 sequential injections of carrot/potato matrix sample extract spiked at 10 ppb. Injector maintenance and tuning intervals are marked with an arrow. No source cleaning was performed during the sequence.

- Tefluthrin 6.7% RSD
- Resmethrin II 8.1% RSD
- Cadusafos 9.3% RSD
- Isodrin 7.0% RSD
- Chloroneb 8.5% RSD
- Diazinon 8.8% RSD
- Endosulfan II 8.0% RSD
- Bifenthrin 7.0% RSD
- Cyhalothrin I (lambda) 9.6% RSD

CONCLUSIONS

The experiments described here demonstrate that by using QuEChERS with HyperSep dispersive solid phase extraction (dSPE) and a direct injection of acetonitrile extracts, the TSQ 9000 AEI system delivers outstanding quantitative performance for low level pesticide residue analysis in baby food. Routine quantification of pesticide residue in food commodities such as baby food using this approach offers the following advatnages:

- Direct analysis of acetonitrile extracts using an optimised PTV injection.

- QuEChERS extraction and subsequent clean-up of over 200 pesticides from replicate analysis (n=6 each at three concentrations) of each of two sample matrices, demonstrating excellent accuracy (recovery) and precision.

- Accurate, quantitative analysis of over 200 pesticides over up to 5 orders of magnitude (0.025 – 250 μg/kg), showing outstanding LODs and linear responses.

- Source robustness displayed over ~900 consecutive injections of sample matrix (1 g/mL).

- High sensitivity providing the real possibility to dilute the sample extract, thus limiting matrix contamination and system maintenance, leading to a potential increase in laboratory productivity.

REFERENCES

1. http://ec.europa.eu/food/plant/docs/plant_pesticides_mrl_guidelines_wrkdoc_11945_en.pdf
2. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=URISERV:l13002a
3. SANTE/11813/2017. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. Supersedes SANTE/11945/2015 implemented by 01/01/2018.
4. http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32006L0125
5. http://quechers.cvua-stuttgart.de/

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