APPLICATION NOTE

No. 10509

Routine Quantitative Method of Analysis for Pesticides using GC Orbitrap Mass Spectrometry in accordance with SANTE/11945/2015 Guidelines

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Introduction

The international trade in food commodities has enabled a wide variety of fruits and vegetables to be made available year round. However, this also creates a challenge for food safety regulators who seek to ensure a safe food supply chain, particularly with regard to the potentially hundreds of different pesticides in use across the globe. The European Union (EU) has some of the most stringent pesticide residue regulations. In 2008, it implemented regulation EC No. 396/20051, which sets default maximum residue levels (MRLs) at 10 µg/Kg for all pesticide/commodity combinations for which no substantive MRL had been set. Further to this, in 2009, the pesticide safety review EU 91/414/EEC2 led to the approval of approximately 250 pesticides and effectively set the permissible level for all other pesticides to the default limit (10 µg/Kg). Recently, at the beginning of 2016, the latest version of the SANTE/11945/2015 guidance document on analytical quality control and validation procedures for pesticide residues in food and feed took effect.[3] This document describes the method validation and analytical quality control (AQC) requirements to support the validity of data reported within the framework of official controls on pesticide residues and used for checking compliance with maximum residue levels (MRLs), enforcement actions, or assessment of consumer exposure. It is intended for use by Official control laboratories in Europe, but in practice it is used by pesticide laboratories worldwide. Implementation of the stringent requirements present a major challenge to testing laboratories who seek to provide an accurate and cost competitive services.

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Pesticide residue testing requires detection using both liquid and gas chromatographic techniques typically coupled with triple quadrupole mass spectrometers. These analytical techniques can cover the range of compounds that need to be monitored with the required sensitivity and selectivity. However, they are limited to detecting pesticides that are measured at the time of acquisition and require careful method optimization and management to ensure selected ion monitoring windows remain viable. In recent years, high-resolution Orbitrap mass spectrometry has provided an alternative to MS/MS techniques with additional analytical advantages.[4] With high-resolution mass spectrometry (HRMS), the default acquisition mode is untargeted (full-scan) making it simple to manage and potentially allows for an unlimited number of pesticides to be monitored in a single injection. In addition to this, full-scan data analysis provides access to supplementary identification points such as spectral matching and enables retrospective interrogation of samples to additionally search for emerging pesticides or other contaminants that were not considered at the time of acquisition.

In this study, the quantitative performance of the Thermo Scientific™ Exactive GC Orbitrap™ mass spectrometer was evaluated for the routine analysis of GC-amenable pesticides in fruits and vegetables following SANTE/11945/2015 guidelines using full scan acquisition. The Exactive GC-MS system provides routine high-mass resolving power up to 60,000 (*m/z* 200) full width at half maximum (FWHM) with scan speeds suitable for GC peaks to facilitate the detection of trace compounds in the presence of high matrix components.

Experimental Conditions

Sample Preparation

Tomato, leek and orange were purchased from a local supermarket and extracted following a citrate buffered QuEChERS procedure. Briefly, 10 mL of acetonitrile was added to 10 g of homogenized sample and shaken for 4 minutes. A mixture of salts was added and the centrifuge tube shaken for 4 minutes and centrifuged for 5 minutes at 3700 rpm. Supernatant (5 mL) was transferred to a 15 mL PTFE centrifuge tube containing magnesium sulphate and 125 mg of PSA. The extract was shaken in a vortex mixer and centrifuged as above. The final acetonitrile extracts (1g/mL) were used as blank matrix. The calibration series was prepared by taking 100 µl of acetronitrile blank matrix and drying under a stream of nitrogen to complete dryness. The sample was reconstituted in 100 µl ethyl acetate containing the appropriate concentration of pesticides.

Three calibration series of 51 pesticides were prepared in tomato, leek and orange at concentrations equivalent to 0.5, 1, 2, 5, 10, 20, 50, 100, 200 and 500 µg/Kg. The 51 pesticides included in the study cover a wide range of chemical classes and, with the three matrices, it generated a total of 153 pesticide/matrix combinations. To assess compound linearity, the matrix matched calibration series were analyzed first, followed by ten replicate injections of the 10 µg/Kg sample for each matrix. To assess repeatability over an extended period of time, the 10 µg/Kg tomato standard was further injected 100 times from the same vial.

Instrument and Method Setup

In all experiments, an Exactive™ GC Orbitrap™ mass spectrometer was used. Automatic sample injection was performed using a Thermo Scientific™ TriPlus™ RSH™ autosampler, and chromatographic separation was obtained using a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph and a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m x 0.25 mm I.D. x 0.25 µm film capillary column with a 5 m integrated guard (P/N:26096-1425). Additional details of instrument parameters are given in Table 1 and Table 2.

Table 1. GC and Split/Splitless injector conditions.

TRACE 1310 GC Parameters	
Injection Volume (µL):	**1**
Liner:	LinerGOLD™ single taper (P/N: 453A1345-UI)
Inlet (°C):	280
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program:	
Temperature 1 (°C):	40
Hold Time (min):	1.5
Temperature 2 (°C):	90
Rate (°C/min):	25
Hold Time (min):	1.5
Temperature 3 (°C):	280
Rate (°C/min):	5
Hold Time (min):	0
Temperature 3 (°C):	300
Rate (°C/min):	10
Hold Time (min):	5

Table 2. Mass spectrometer conditions

Exactive GC Mass Spectrometer Parameters	
Transfer line (°C):	**280**
Ionization type:	EI
Ion source(°C):	250
Electron energy (eV):	70
Acquisition Mode:	Full-scan
Mass range (Da):	50-550
Resolving power (FWHM at *m/z* 200):	60,000
Lockmass, column bleed (*m/z*):	207.03235

Data Processing

Data were acquired using the Thermo Scientific™ TraceFinder™ software. This single platform software package integrates instrument control, method development functionality, and qualitative and quantitation-focused workflows. For target analysis a compound database for the 51 pesticides was prepared using the Thermo Scientific™ Orbitrap GC-MS Contaminants Library containing compound name, quantification ion and identification ions, accurate masses, retention times and elemental compositions of molecular ion and fragment masses. For the generation of extracted ion chromatograms an mass extraction window of 5 ppm was used.

Results and Discussion

The objective of this study was to evaluate the analytical performance of the Exactive GC system for the routine analysis of pesticides in three different sample matrices following SANTE requirements. The sample types chosen (tomato, leek and orange) provided both easy and difficult matrices that are typically encountered in routine testing. To illustrate, the varying sample complexity total ion chromatograms with fixed Y-axis are shown in Figure 1. The leek matrix is clearly the most complex matrix and this is where high-mass resolution is required to extract target analytes from background chemical noise. The QuEChERS generic sample extraction technique employed in routine testing produces complex extracts containing high and variable concentrations of matrix components depending on the sample type. The lack of selectivity during sample preparation needs to be compensated for by a selective instrumental analysis. This was achieved using high-mass resolving power of the Exactive GC system (60k @*m/z* 200). This capability in combination with a full-scan acquisition increases the scope of the analysis without the need for optimization of acquisition parameters, as is the case with targeted analyses.

For routine pesticide screening, the HRMS processing software needs to be fast, accurate and customizable. TraceFinder meets all of these requirements and was used to process each batch of calibration standards and ten replicates in less than five minutes. In TraceFinder, the results are presented to the user in a table format and data flags are used to quickly identify which pesticides are positive and which criteria are satisfied. Flexible reporting options means that data can be either exported to other software packages or reported directly from within TraceFinder.

Identification to Guideline Requirements

One aim of the analysis was to determine the limit of detection (LOD), limit of identification (LOI), linearity and peak area repeatability for all of the pesticides in all three matrices. Although the LOD is not discussed in the SANTE guidelines, it is useful to know the limit of detection of the quantifier ion as it is used in forming the calibration series that will ultimately be used in determining the concentration of a detected pesticide in a sample. This assessment was made by evaluating the matrix matched calibration series and the repeat injections at 10 µg/Kg for each matrix. The LOD was defined as the presence of a peak with S/N (peak to peak) >3 in the extracted ion chromatogram (XIC) of the main quantifier ion of a pesticide. For the determination of the LOI the SANTE/11945/2015 guidance document was followed. This requires that the following criteria are satisfied for a positive identification:

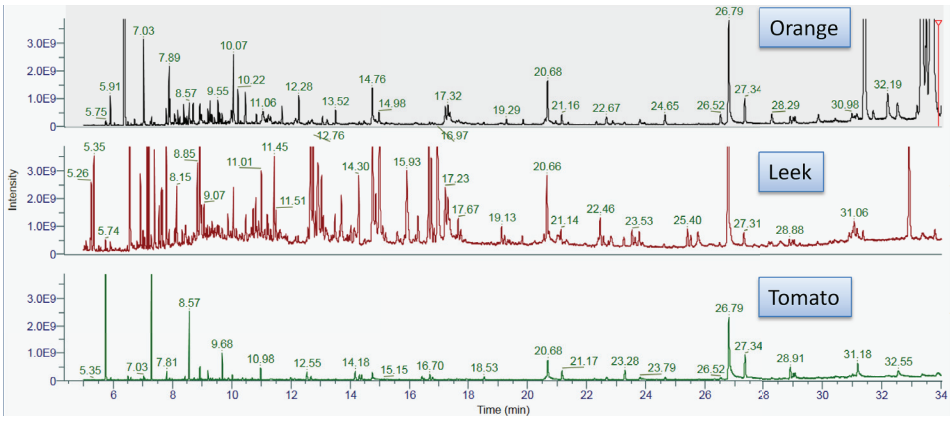

Figure 1. Full scan Total Ion Chromatogram (TIC) of orange, leek and tomato extracts with y axis fixed at4.0 e9 showing the complexity of the sample matrices used in this study.

(i) Two ions are detected for each pesticide with mass accuracy <5 ppm and peak S/N > 3

(ii) Retention time tolerance of ± 0.1 minutes compared with standards in the same sequence

(iii) Ion ratio within ± 30% of the average of calibration standards from the same sequence

(iv) Optional: For higher confidence in identification additional criteria can be used such as full-scan spectra, isotope pattern matching and additional fragment ions

All of the pesticides were identified following the regulatory criteria (LOI) in all of the matrices at or below 5 µg/Kg (Tables 3-5) with the exception of chlorothalonil in leek, which is known to suffer losses due to interaction with sulphur compounds in the leek matrix.5 The majority of the 153 pesticide/matrix combinations (79%) had an LOI ≤2 µg/Kg. The calculated LODs are summarized in Figure 2 which shows that the LOD for 93% of the pesticide/matrix combinations was ≤1 µg/Kg. Having multiple identification points and limits of detection well below the MRL increases the confidence in identifications and minimizes false negative and positive results. Using highly efficient electron ionisation (EI) in combination with full-scan acquisition provides the opportunity to use multiple diagnostic ions for the identification of pesticides. The

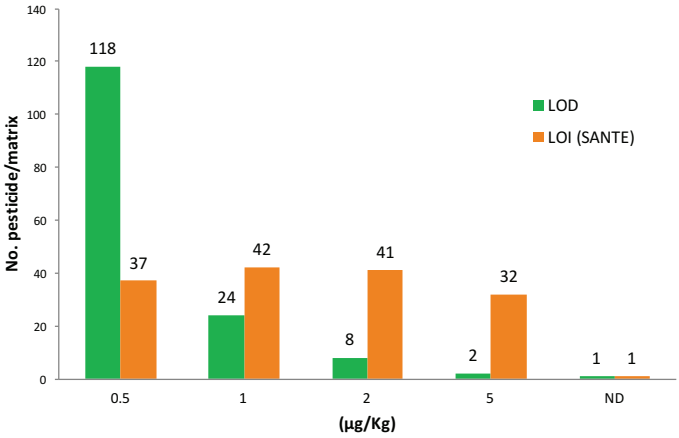

Figure 2. The limit of detection (LOD) and limit of identification (LOI) for pesticides/matrix combinations.

Exactive GC system generates standard EI spectra that are highly reproducible and library searchable (nominal or high resolution MS libraries). This facilitates detection and identification of pesticides based on spectral matching. Additional compounds can be quickly added to the compound database as chemical formulas can be easily assigned to accurate mass fragment ions due to the high mass accuracy of the Orbitrap analyzer.

Reliable Quantitation

Quantitative linearity was assessed using matrix matched standards across a concentration of 0.5-500 µg/Kg. In all cases, the coefficient of determination (R^2) was >0.99 for each pesticide from its LOD to 500 µg/Kg in the three matrices, an example of the TraceFinder browser showing propazine is given in Figure 3. One exception to this, possibly due to analyte adsorption, was fenpropidin which was linear up to 200 µg/Kg. Accurate quantitation is reliant upon a number of factors, one of which is an acquisition speed fast enough to provide at least 12 points across chromatographic peak. At a resolution of 60,000 the Exactive GC system has a scan speed of approximately 7 Hz. An example is shown in Figure 4 for the peak of chlorobenzilate which has 38 points across the 6 second peak.

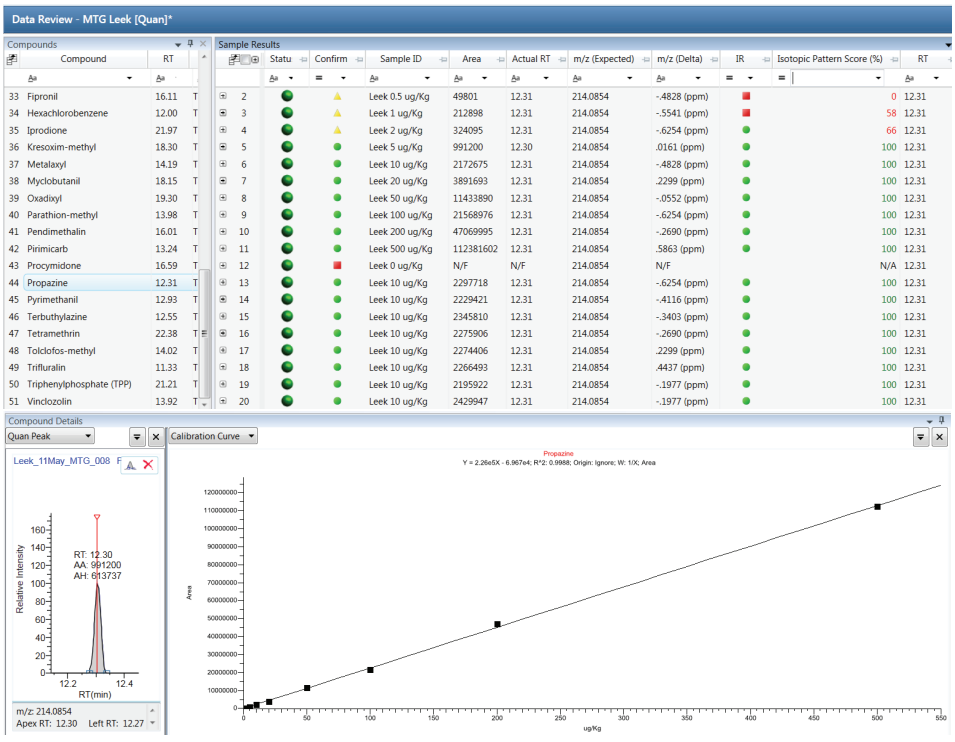

Figure 3. TraceFinder browser showing positively identified pesticides, extracted ion chromatogram and calibration graph (propazine as an example). Sub-ppm mass accuracy for propazine across the calibration range and in replicates of 10 µg/Kg. Identification criteria information is available and flagged when out of tolerance.

Table 3. Summary of method performance results for pesticides in leek. * Chlorothalonil known to degrade in leek.

Pesticide	LOD (µg/Kg)	LOI (µg/Kg)	R^2 LOD-500 (µg/Kg)	Mass Accuracy at LOI (ppm)	Leek 10 µg/Kg (%RSD) n=10
2-phenylphenol	0.5	1	0.9986	-0.53	2.5
Acrinathrin	2	5	0.9975	-0.68	6
Azoxystrobin	1	5	0.9961	0.1	6.3
BHC, Alpha	0.5	1	0.9993	-0.6	4.4
BHC, beta	0.5	1	0.9992	0.8	4.4
BHC, gamma	0.5	2	0.9986	-0.8	4.5
Bifenthrin	0.5	0.5	0.9989	-0.5	4
Biphenyl	0.5	0.5	0.9986	-0.9	3.3
Bromopropylate	0.5	1	0.9973	0.3	6.4
Bupirimate	0.5	1	0.9979	-0.4	5.1
Chlorobenzilate	0.5	2	0.9979	1.04	3.8
Chlorothalonil	ND*	ND*	-	-	-
Chlorpropham	0.5	2	0.9991	0.7	2.9
Chlorpyrifos	1	5	0.999	0.1	4.6
Chlorpyrifos-methyl	0.5	2	0.9988	0.5	4.1
Cyhalothrin	1	2	0.9954	-0.6	6.9
Cypermethrin I-IV	5	5	0.9962	0.5	7.9
DDD p,p'	0.5	2	0.9982	0.7	4.7
DDE p,p'	0.5	1	0.9988	0.41	3.5
DDT o,p	0.5	2	0.9982	0.7	4.4
DDT p,p'	0.5	5	0.9962	0.1	4.2
Diazinon	1	2	0.9983	-0.34	3.5
Dichlorvos	0.5	1	0.9991	-0.5	4.1
Dieldrin	2	5	0.992	0.3	3.6
Endosulfan sulfate	1	5	0.999	-0.2	5.9
Endosulphan alpha	2	5	0.994	-0.2	9.1
Endosulphan beta	2	5	0.9982	-0.4	7.5
Etofenprox	2	5	0.9978	-0.1	6.2
Fenitrothion	2	2	0.9968	0.1	6.6
Fenpropidin	0.5	5	0.9986	-0.3	4.1
Fenpropimorph	0.5	5	0.9977	-1.1	2.8
Fenvalerate SS,RR	0.5	2	0.9954	0.6	6.5
Fipronil	0.5	1	0.9979	0.2	6.3
Hexachlorobenzene	0.5	1	0.9985	1.1	3
Iprodione	0.5	5	0.9975	0.4	7.5
Kresoxim-methyl	0.5	2	0.9989	0.36	4.3
Metalaxyl	2	5	0.9989	-0.91	4.9
Myclobutanil	0.5	5	0.9987	-0.96	5
Oxadixyl	1	2	0.9983	0.34	6
Parathion-methyl	1	5	0.9985	0.61	4.8
Pendimethalin	2	5	0.9989	0.98	6.5
Pirimicarb	0.5	2	0.9991	-0.28	3.1
Procymidone	1	1	0.9988	0.26	5.9
Propazine	0.5	2	0.9988	-0.62	2.9
Pyrimethanil	0.5	1	0.9984	-0.31	3.6
Terbuthylazine	0.5	1	0.9985	-0.19	4
Tetramethrin	1	5	0.9991	-0.23	5.4
Tolclofos-methyl	0.5	1	0.9991	0.55	2.5
Trifluralin	1	1	0.9963	-0.52	3.9
Triphenylphosphate	1	2	0.9979	0	6
Vinclozolin	0.5	2	0.9987	-0.6	4.6

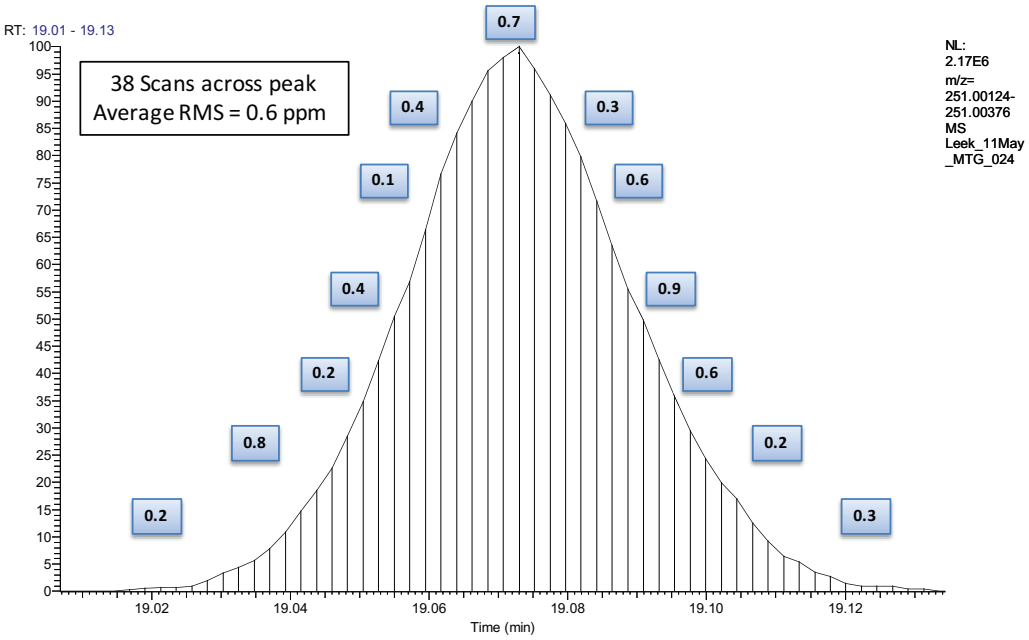

Figure 4. Extracted ion chromatogram of chlorobenzilate (m/z 251.0025 ±5 ppm mass window) acquired at 60,000 resolution (FWHM at m/z 200) in leek spiked at 10 µg/Kg showing ~38 scans/peak (peak width 6 sec). Sub 1 ppm accurate mass is achieved for each individual scan (every third scan labelled). Average RMS mass difference of 0.6 ppm across the peak.

The results of the 10 replicate injections at 10 µg/Kg in all three matrices are presented in Figure 5. All detected pesticides had RSD% of less than 10%, well below the 20% threshold requirement in the SANTE guidance document. This shows that the Exactive GC system operated in full-scan at 60k resolution has the selectivity and sensitivity required to analyse pesticides in a robust manner well below the respective MRLs.

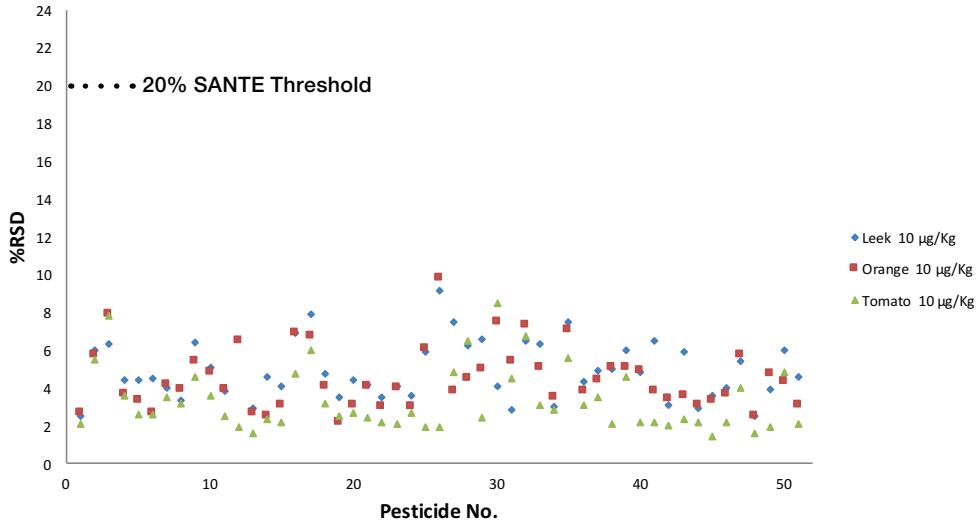

Figure 5. Peak area repeatability (%RSD) for 10 µg/Kg (n=10) for each pesticide in the three matrices studied. SANTE guideline of 20% threshold shown is also indicated.

Table 4. Summary of method performance results for pesticides in orange. *LOD-200 µg/Kg

Pesticide	LOD (µg/Kg)	LOI (µg/Kg)	R^2 LOD-500 (µg/Kg)	Mass Accuracy at LOI (ppm)	Leek 10 µg/Kg (%RSD) n=10
2-phenylphenol	0.5	0.5	0.997	-0.1	2.7
Acrinathrin	1	5	0.9956	-0.42	5.7
Azoxystrobin	1	5	0.9977	-0.1	7.9
BHC, Alpha	0.5	0.5	0.9984	-0.6	3.7
BHC, beta	0.5	1	0.9985	-0.6	3.3
BHC, gamma	0.5	0.5	0.9989	-0.21	2.7
Bifenthrin	0.5	0.5	0.9972	-0.7	4.2
Biphenyl	0.5	0.5	0.998	-0.37	3.9
Bromopropylate	0.5	1	0.9985	-0.16	5.4
Bupirimate	0.5	0.5	0.9987	0.36	4.8
Chlorobenzilate	0.5	0.5	0.9982	0.37	3.9
Chlorothalonil	0.5	0.5	0.9987	0.42	6.5
Chlorpropham	0.5	2	0.9981	-0.13	2.7
Chlorpyrifos	0.5	1	0.9982	0.1	2.5
Chlorpyrifos-methyl	0.5	1	0.9989	0.38	3.1
Cyhalothrin	1	5	0.9963	-0.6	6.9
Cypermethrin I-IV	5	5	0.9986	-0.5	6.7
DDD p,p'	0.5	2	0.9986	-0.1	4.1
DDE p,p'	0.5	0.5	0.9989	0	2.2
DDT o,p	0.5	2	0.9988	0.14	3.1
DDT p,p'	0.5	5	0.9967	-0.11	4.1
Diazinon	0.5	0.5	0.999	0.51	3
Dichlorvos	0.5	0.5	0.9983	0.29	4
Dieldrin	0.5	2	0.9989	0.5	3
Endosulfan sulfate	1	2	0.9986	1.2	6.1
Endosulphan alpha	1	5	0.9987	-1.2	9.8
Endosulphan beta	1	2	0.9988	0.4	3.8
Etofenprox	0.5	2	0.9937	0.4	4.5
Fenitrothion	0.5	2	0.998	0.1	5
Fenpropidin	1	5	0.993*	1	7.5
Fenpropimorph	0.5	2	0.9924	-0.44	5.4
Fenvalerate SS,RR	0.5	2	0.9919	0.37	7.3
Fipronil	0.5	0.5	0.9983	-0.8	5.1
Hexachlorobenzene	0.5	1	0.999	-0.17	3.5
Iprodione	0.5	1	0.9983	-0.5	7.1
Kresoxim-methyl	0.5	1	0.9984	0.43	3.8
Metalaxyl	0.5	1	0.9991	-0.8	4.4
Myclobutanil	0.5	2	0.9977	-0.2	5.1
Oxadixyl	0.5	2	0.9983	0.46	5.1
Parathion-methyl	0.5	2	0.9988	-0.3	4.9
Pendimethalin	0.5	2	0.9978	1	3.8
Pirimicarb	0.5	1	0.9976	-0.65	3.4
Procymidone	0.5	2	0.9977	0.1	3.6
Propazine	0.5	1	0.9981	0.3	0.3
Pyrimethanil	0.5	1	0.9935	-0.3	3.3
Terbuthylazine	0.5	1	0.999	-0.2	3.7
Tetramethrin	0.5	5	0.9979	-0.41	5.7
Tolclofos-methyl	0.5	1	0.9986	0.78	2.5
Trifluralin	0.5	1	0.9974	0.56	4.7
Tri-phenylphosphate	0.5	1	0.9977	0.28	4.3
Vinclozolin	0.5	1	0.999	0.5	3.1

Robust Mass Accuracy

Acquiring reliable accurate mass measurements is critical when detecting low level pesticides in complex sample matrices. Low mass errors, allow selectivity to be maintained through the use of narrow mass extraction windows during data processing and help ensure positive detections are robust. The low mass errors observed with the Exactive GC system are enabled through the high-mass resolving power that is able to discriminate between matrix interferences and target analyte ions. When the resolution is insufficient, the mass profile of two ions overlap, which results in the incorrect assignment of the mass of the target compound. This is demonstrated in Figure 6 where the leek 10 µg/Kg matrix standard was analysed at resolving powers of 15K, 30K and 60K. The zoomed mass spectra show the quantifier ion for pyrimethanil and a matrix ion of a similar mass causing interference. At 15K and 30K, the pyrimethanil ion is not resolved resulting in poor mass accuracy of 10.1 and 6.3 ppm respectively. However, the ions are sufficiently resolved at 60K resulting in the expected sub 1 ppm mass accuracy. Without this level of mass resolution this pesticide would have failed the SANTE identification criteria of <5 ppm and would have been a false negative (reported as not detected). This supports previous a report that a resolving power of 60k (at 200 *m/z*) is required in some cases to ensure the highest selectivity.[6]

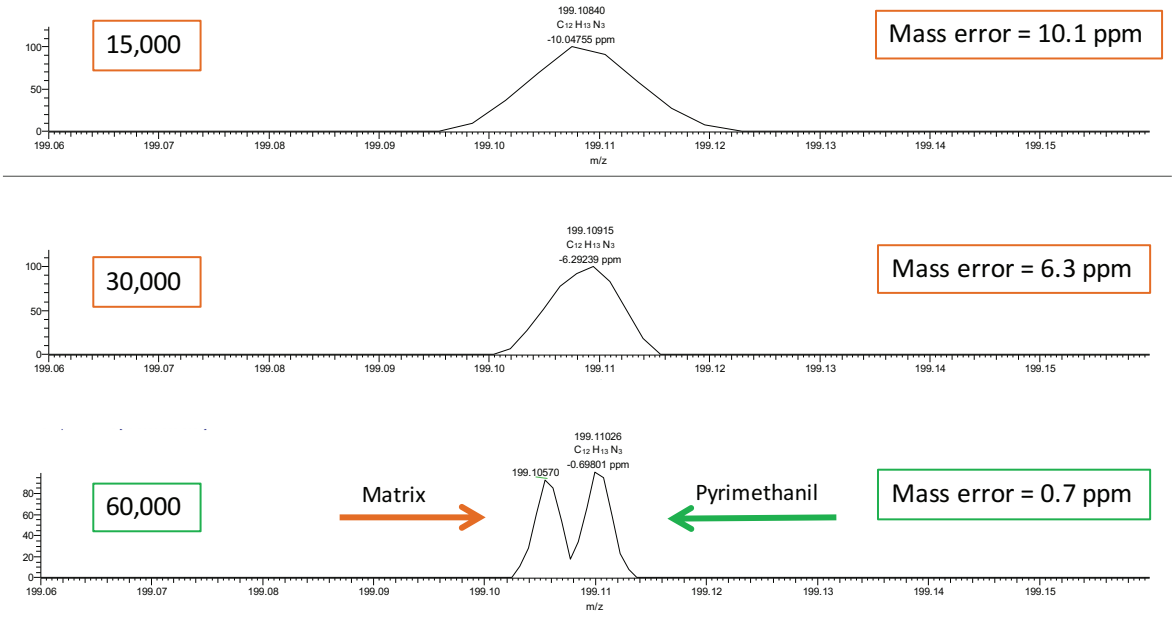

Figure 6. Effect of resolving power on mass accuracy of the diagnostic ion of Pyrimethanil at 10 µg/Kg in leek acquired at different resolutions of 15K, 30K and 60K. At 15K and 30K the Pyrimethanil ion is not resolved from the interfering matrix ion resulting in poor mass accuracy assignment.

Table 5. Summary of method performance results for pesticides in tomato.

Pesticide	LOD (µg/Kg)	LOI (µg/Kg)	R^2 LOD-500 (µg/Kg)	Mass Accuracy at LOI (ppm)	Leek 10 µg/Kg (%RSD) n=10
2-phenylphenol	0.5	0.5	0.9999	-0.71	2.1
Acrinathrin	1	5	0.9915	-0.34	5.5
Azoxystrobin	1	2	0.9938	-0.1	7.8
BHC, Alpha	0.5	0.5	0.9984	-0.46	3.6
BHC, beta	0.5	0.5	0.9984	-0.21	2.6
BHC, gamma	0.5	0.5	0.9984	-0.63	2.6
Bifenthrin	0.5	0.5	0.9981	-0.75	3.5
Biphenyl	0.5	0.5	0.9977	-0.37	3.2
Bromopropylate	0.5	1	0.9939	0.37	4.6
Bupirimate	0.5	0.5	0.9969	-0.51	3.6
Chlorobenzilate	0.5	0.5	0.9982	0.43	2.5
Chlorothalonil	0.5	0.5	0.9985	1	1.9
Chlorpropham	0.5	0.5	0.999	0.7	1.6
Chlorpyrifos	0.5	0.5	0.999	0.14	2.3
Chlorpyrifos-methyl	0.5	0.5	0.999	0.81	2.2
Cyhalothrin	0.5	1	0.999	-0.76	4.7
Cypermethrin I-IV	5	5	0.997	-0.5	6
DDD p,p'	0.5	1	0.9974	0.1	3.2
DDE p,p'	0.5	0.5	0.9995	0.35	2.5
DDT o,p	0.5	1	0.997	0.34	2.7
DDT p,p'	0.5	5	0.9923	-0.17	2.4
Diazinon	0.5	0.5	0.9991	-0.68	2.2
Dichlorvos	0.5	0.5	0.9987	-0.11	2.1
Dieldrin	0.5	2	0.9988	0.21	2.7
Endosulfan sulfate	1	2	0.9975	0.15	1.9
Endosulphan alpha	1	2	0.9993	0.19	1.9
Endosulphan beta	1	2	0.9981	-0.64	4.8
Etofenprox	1	5	0.9982	-0.37	6.5
Fenitrothion	0.5	2	0.9943	0.49	2.4
Fenpropidin	0.5	2	0.999	0.36	8.5
Fenpropimorph	0.5	5	0.999	0.51	4.5
Fenvalerate SS,RR	0.5	2	0.991	0.31	6.7
Fipronil	0.5	0.5	0.9949	0.36	3.1
Hexachlorobenzene	0.5	1	0.9993	0.54	2.8
Iprodione	0.5	1	0.9964	0.39	5.6
Kresoxim-methyl	0.5	0.5	0.9984	0.36	3.1
Metalaxyl	0.5	1	0.9993	-0.53	3.5
Myclobutanil	0.5	2	0.9984	0.4	2.1
Oxadixyl	0.5	1	0.9985	0.46	4.6
Parathion-methyl	0.5	1	0.9974	0.73	2.2
Pendimethalin	0.5	2	0.9936	0.62	2.2
Pirimicarb	0.5	0.5	0.9992	-0.37	2
Procymidone	0.5	1	0.9984	0.58	2.3
Propazine	0.5	0.5	0.9989	-0.12	2.2
Pyrimethanil	0.5	1	0.998	0.13	1.4
Terbuthylazine	0.5	0.5	0.9989	-0.12	2.2
Tetramethrin	0.5	5	0.9948	-0.41	4
Tolclofos-methyl	0.5	1	0.9992	0.78	1.6
Trifluralin	0.5	0.5	0.9947	0.76	1.9
Tri-phenylphosphate	0.5	0.5	0.9968	-0.1	4.8
Vinclozolin	0.5	0.5	0.9991	0.9	2.1

The mass accuracy was assessed for all 51 pesticides at their LOI and the results are shown graphically in Figure 7. The mass error values did not exceed 1.2 ppm for any of the analytes, well below the guideline limit of 5 ppm delivering the highest confidence in accurate and selective detection.

In pesticide analysis, it is also essential that the instrument is able to maintain mass accuracy across the complete range of possible analyte concentrations encountered. It would not be acceptable if a high concentration pesticide violation was missed due to detector saturation. On the Exactive GC system, the Orbitrap analyzer is protected from saturation through the use of automatic gain control (AGC) which regulates the number of ions entering. This ensures that, no matter what concentration is encountered, the mass accuracy performance is preserved. This is demonstrated in Figure 8 that shows the mass accuracy for four pesticides at concentrations ranging from 0.5 to 500 µg/Kg in leek matrix is always < 1 ppm.

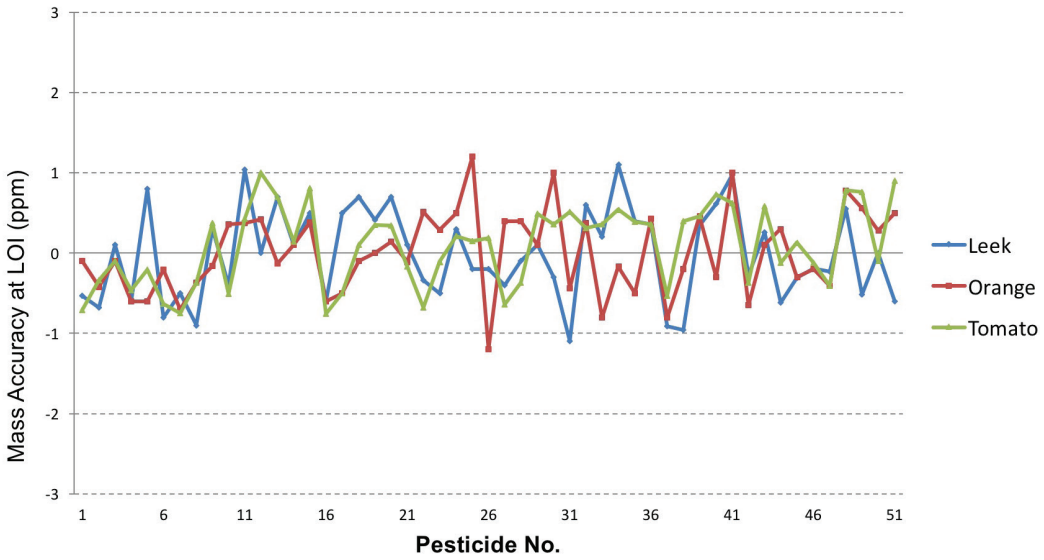

Figure 7. Mass difference measurements at the LOI level for each pesticide across the three matrices.

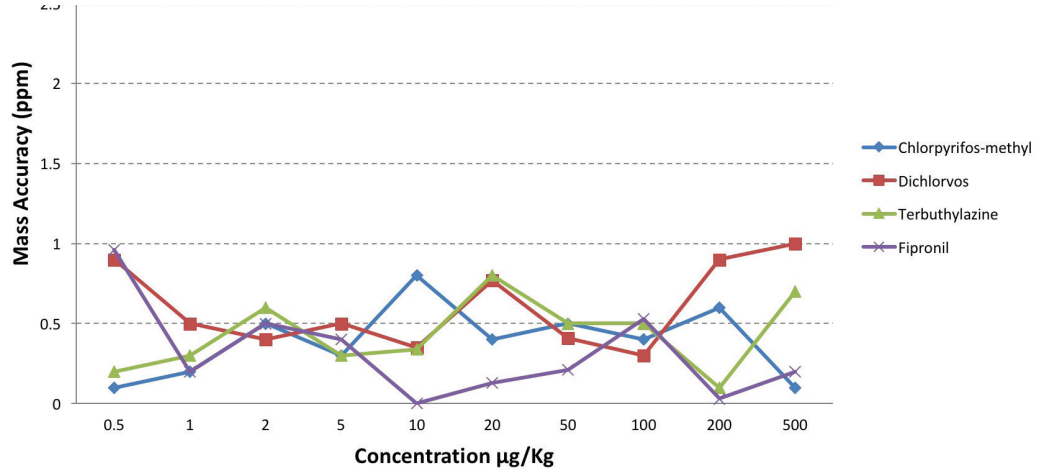

Figure 8. Mass accuracy measurements across the concentration range (0.5-500 µg/mL) for four pesticides in leek. Mass accuracy is maintained at sub 1 ppm level.

Real World Performance

In a high-throughput routine pesticide analysis laboratory, mass spectrometry instruments are in near constant operation and it is essential that they provide the same level of performance over an extended period of time. To evaluate the performance of the Exactive GC system over a longer period, a tomato extract at 10 µg/Kg was repeatedly injected (n=100) from a single vial. Prior to commencing analysis, a new injector liner was installed, the source tuned and the MS calibrated. No further interventions were made during the 66 hours of continual operation. The results showed that the system, from injector to MS, provided outstanding performance. Figure 9 shows the peak area response of hexachlorobenzene, vinclozolin and trifluralin at 10 µg/Kg in tomato over the 100 injections, with RSD% of 5.3, 4.6 and 3.8%, respectively. Furthermore, the mass accuracy stability remained <1.2 ppm (99% ≤1ppm) for the duration of the analysis without further mass calibration (Figure 10).

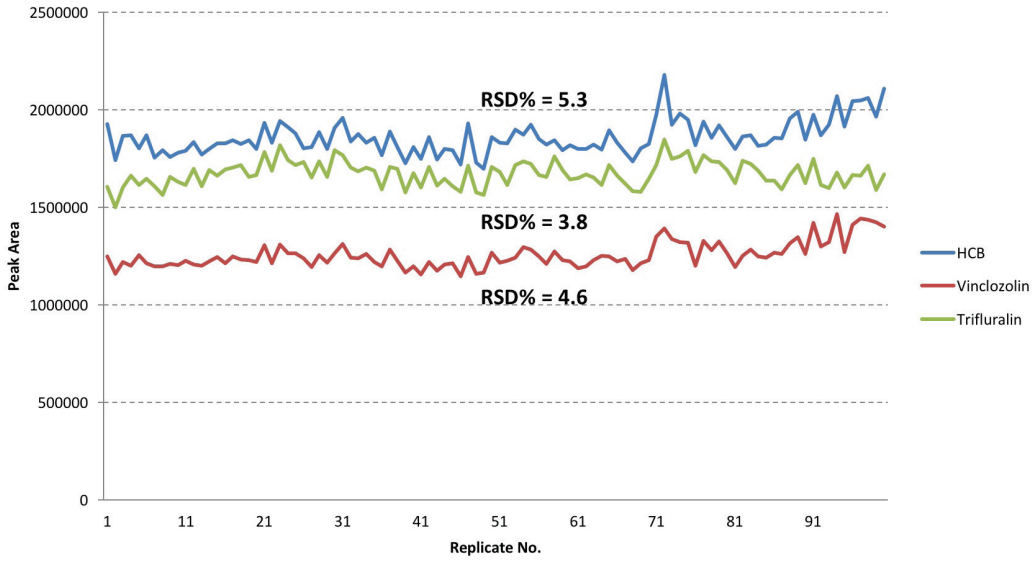

Figure 9. Repeat injections (n=100) of a tomato extract spiked at 10 µg/Kg showing that the sensitivity is maintained over the 66 hours of continual operation.

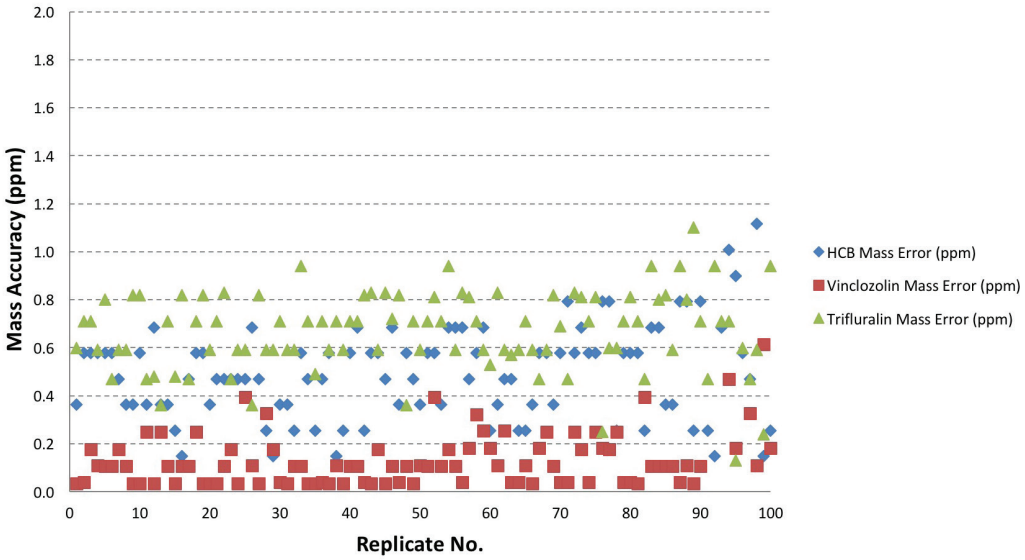

Figure 10. Mass accuracy (ppm) over 100 injections for hexachlorobenzene, vinclozolin and trifluralin in tomato extract at 10 µg/Kg. Data was acquired with same liner and without further calibration of the mass spectrometer or tuning of the source.

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Conclusions

The results of this study demonstrate that the Thermo Scientific Exactive GC Orbitrap high-resolution mass spectrometer, in combination with TraceFinder software, is a high performance analytical system that delivers robust and sensitive performance for routine pesticide analysis in fruits and vegetables in complete accordance with SANTE guidance document.

– 99.3% of the pesticide/matrix combinations were detected below the MRL with excellent linearity and meeting the required performance criteria. Importantly, the scope of the analysis is increased by acquisition in full-scan with targeted data processing with a compound database.

– Acquisition at 60,000 FWHM resolution dramatically reduces matrix interferences and increases confidence in results when screening for pesticides in complex sample matrices. Consistent sub ppm mass accuracy was achieved for all compounds over a wide concentration range ensuring that compounds are detected with confidence at low and high concentration levels.

– Repeated injections of a tomato matrix at 10 µg/Kg showed that the system is able to maintain a consistent level of performance over an extended period of time as is demanded by a routine testing laboratory.

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