Lowering Detection Limits for Routine Analysis of Pesticides Residues in Foods Using the Agilent 7000C Triple Quadrupole GC/MS

Application Note

Food Safety

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Abstract

An analytical method that is well established for the Agilent 7000 Series Triple Quadrupole GC/MS has been employed to demonstrate performance of the 7000C Triple Quadrupole GC/MS. When 110 pesticides were spiked into plum and winter squash matrix at a concentration of 1 ng/g, calculated %RSDs were ≤ 20 (n = 5) for 92 pesticides analyzed in difficult plum matrix and for 92 of the pesticides in winter squash as well. It was estimated that limits of quantitation (LOQ) ≤ 5 ng/g could be reached for 91% of the pesticides studied in either commodity. Thus, it was demonstrated that data collection at levels below the threshold MRL in the EU and in Japan, 0.01 mg/kg (10 ng/g), is achievable to monitor exposure.

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Introduction

Concerns regarding threats to the environment and human health from the use of pesticides have prompted government agencies worldwide to continually lower required detection limits for these compounds. These limits are necessary to ensure compliance to mandated maximum residue levels (MRLs).

The European Commission has been very active in establishing MRLs, described as safe limits that define the maximum expected levels of a pesticide on a food commodity after safe and authorized use of that pesticide (guidance document SANCO/3346/2001 rev 7). They serve to prevent illegal or excessive use of a pesticide, and protect the health of consumers. These MRLs are based upon residue levels from trials in which the pesticide was used on the crop at the correct application rate and waiting time. To check if this level is acceptable for consumer exposure, intake calculations for various consumer groups are made for acute and chronic intake. If the level is acceptable, the MRL is set by the Commission. If not, the limit of detection (LOD) is applied.

The default threshold MRL value is set at 0.01 mg/kg, or 10 ng/g, in the European Union (EU) and Japan. However, there is interest in collecting pesticide residue data at levels as low as possible in infant and baby food to assess exposure in this sensitive population [1,2].

Many foodstuffs are very complex, or "dirty", due to the presence of a large number of background compounds. Backflushing the GC column ensures that high-boiling compounds in the matrix are not passed through the column, reduces column bleed, eliminates ghost peaks, and minimizes contamination of the mass spectrometer [3]. In addition, tandem mass spectrometry (MS/MS) on a triple quadrupole platform is very useful for screening, confirming, and quantitating trace level target compounds in these complex matrices because it can minimize interferences. This application note describes a study using backflushing and the Agilent 7000C Triple Quadrupole GC/MS to measure pesticide residue levels well below the current threshold of 10 ng/g.

Experimental

Extraction and analytical methodologies have been fully validated in several state laboratories in the United States with the Agilent 7000 Series Triple Quadrupole GC/MS and are described in the Agilent GC/MS/MS Pesticide Residue Analysis Guide [4] and in Agilent Application Note 5990-1054EN [5]. The analysis guide is available from your Agilent sales representative or product specialist. A rugged core method for pesticide analysis is also discussed in an on-demand webinar: http://www.sepscience.com/Information/Events/Webinars/2344-/Introducing-a-rugged-core-method-for-GCMSMS-pesticide-residue-analysis---Offering-a-new-Reference-Guide-for-Pesticides-GCMSMS-Analysis.

Standards and solutions

A concentrated standard mix of 110 pesticide standards was a gift from the Florida Department of Agriculture and Consumer Services in Tallahassee, FL, USA. This mix was used to make working dilutions in acidified acetonitrile to spike blank matrix when preparing calibration standards. For analysis, ISTDs and analyte protectants were used as described in Application Note 5990-1054EN and the GC/MS/MS Pesticide Residue Analysis Guide [4,5].

Instruments

This study was performed on an Agilent 7890B GC coupled to a 7000C Triple Quadrupole GC/MS with an electron ionization (EI) source. The GC system was equipped with an Electronic Pneumatics Control (EPC), a Multi-Mode Inlet (MMI) with air cooling, an Agilent 7693A Automatic Liquid Sampler (ALS), and a backflushing system based on a purged ultimate union controlled by an AUX EPC module [6,7]. Agilent MassHunter Software was used for instrument control, and for qualitative and quantitative data analysis.

For maximum GC/MS sample path inertness, the following components were used:

- Agilent J&W HP-5ms Ultra Inert GC columns in dimensions of 5 m × 0.25 mm, 0.25 µm and 15 m × 0.25 mm, 0.25 µm (p/n G3903-61005 and p/n 19091S-431UI)
- Agilent Ultra Inert 2-mm dimpled liners (p/n 5190-2297)
- Agilent UltiMetal Plus Flexible Metal Ferrules at the Purged Ultimate Union used for column backflushing (p/n G3188-27501)

Sample preparation

Preparation of fruit and vegetable extracts was based on the AOAC version of the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method [8], using Agilent extraction and dispersive kits (p/n 5982-5755 and p/n 5982-5058). The homogenized commodities were gifts and had been processed using a Robot Coupe blender (Ridgeland, MS, USA) at the Center for Analytical Chemistry of the California Department of Food and Agriculture in Sacramento, CA. Blank matrix extracts at a concentration of 1 g/mL were used for preparation of matrix-matched calibration standards, which were used for quantification.

GC/MS/MS method parameters

GC conditions

Column 1	Agilent J&W HP-5ms UI; 5 m x 250 µm, 0.25 µm (p/n G3903-61005) – configured from the MMI to AUX EPC
Column 2	Agilent J&W HP-5ms UI; 15 m x 250 µm, 0.25 µm (p/n 19091S-431 UI) – configured from the AUX EPC to vacuum
Carrier gas	Helium
Injection mode	PTV solvent vent
Injection volume	2 µL (syringe size: 5 µL)
Solvent washes	**Pre-injection** 1x solvent A, methanol/water (4 µL) and 1x solvent B, acetonitrile (4 µL) **Post-injection** 7x solvent A, methanol/water, and 7x solvent B, acetonitrile (4 µL each)
Sample wash	1 × 2 µL
Sample pumps	5
Injection speed	Fast
MMI temperature program	60 °C for 0.35 minutes; then 900 °C/min to 280 °C (15 minutes hold); then 900 °C/min to 300 °C until the end of the analysis
Purge flow to split vent	50 mL/min at 1.5 minutes
Vent flow	25 mL/min
Vent pressure	5 psi until 0.3 minutes
Gas saver	Off
Septum purge flow	3 mL/min
Air cooling (cryo)	ON at 100 °C (MMI Liquid N_2 option selected on GC for air cooling)
Oven temperature program	60 °C for 1.5 minutes; then 50 °C/min to 160 °C; then 8 °C/min to 240 °C; then 50 °C/min to 280 °C (2.5 minutes hold); then 100 °C/min to 290 °C (1.1 minutes hold)
Column 1 flow program	1.1 mL/min for 15.2 minutes; then 100 mL/min to-2.283 mL/min (flow balanced with the Column 2 flow to achieve 2 psi inlet pressure) until the end of the analysis for concurrent column backflush Post run –10.683 mL/min
Column 2 flow program	1.2 mL/min until the end of the analysis Post run 4 mL/min
Retention time locking	Chlorpyrifos-methyl locked at 8.524 minutes
Run time	18 minutes
Post-run	0.5 minutes at 290 °C

MS conditions

MS source	EI, –70eV
Source temperature	280 °C
Quadrupole temperature	150 °C
Transfer line temperature	280 °C
Solvent delay	4.0 minutes
Helium quench gas	2.25 mL/min
Nitrogen collision gas	1.5 mL/min
Acquisition mode	Multiple Reaction Monitoring (MRM)
MS1/MS2 resolution	Wide
Time segments	Refer to page 94 of the Pesticide Analysis Reference Guide, available upon request from a sales representative [4].
Acquisition parameters	A full list of the MRM transitions used is provided on pages 95-105 of the Pesticide Analysis Reference Guide [4].

Results and Discussion

Accurate calibration

This study used two matrices to assess the method. Plum is known to be a difficult matrix from which to obtain reliable data. Working with winter squash is comparatively less difficult. Calibration standards for a mixture of 110 pesticides were prepared by spiking extracted blank matrix from both commodities at 0.1, 0.5, 1, 5, 10, 20, 50, and 100 ng/g. Sets of eight standards were injected consecutively five times, with calibration on the middle set, using a linear curve fit. The other four sets of standards were designated as QCs and appear as blue diamonds in Figure 1 as an indication of the precision of the method. Calibration sets yielded coefficient of correlation values (R^2) that were > 0.99 in all cases. One solvent blank was injected between each set of eight calibration standards.

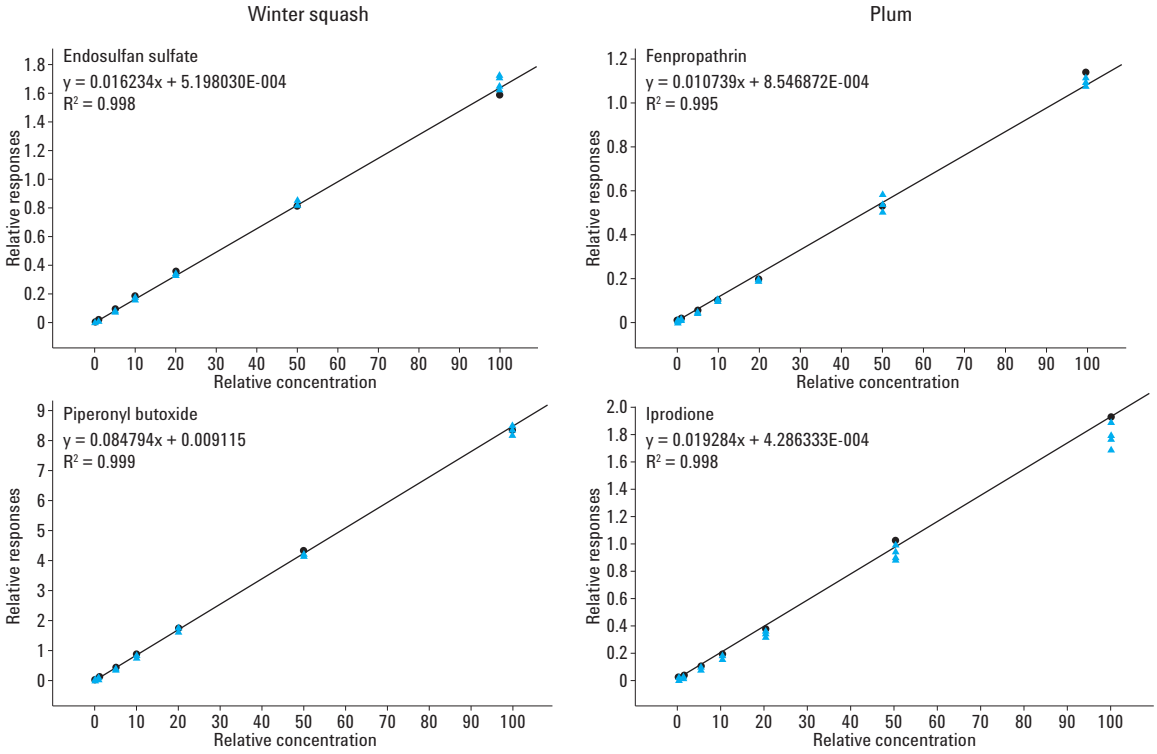

Figure 1. Example calibration curves for four of the 110 pesticides analyzed (eight levels used); n = 5. The endosulfan sulfate and piperonyl butoxide calibration curves were determined in winter squash matrix, while the fenopathrin and iprodione calibration curves represent plum matrix.

LOQs well below MRLs

The limits of quantitation (LOQs) that could be reached during this study were estimated based on criteria including a resultant percent relative standard deviation (%RSD) ≤ 20 (n = 5) for calculated amounts and S/N > 10. Figure 2 shows the average calculated amount and %RSD at the estimated LOQ for four commonly incurred (or representative) pesticides that were spiked into winter squash and plum.

A comparison of the estimated LOQ values for 110 pesticides and their EU MRLs is provided in Table 1. Reliable quantitation was achieved at well below EU MRLs for all residues with few exceptions. In winter squash, 84 pesticides were estimated to have LOQs ≤ 1ng/g and 100 had LOQs ≤ 5 ng/g.

The results were similar in the case of plum: 83 pesticides were able to be quantitated as low as ≤ 1 ng/g, and 100 had LOQs ≤ 5 ng/g.

MRLs were not met for three pesticides in winter squash and two in plum. Results for etridiazole, which is difficult to analyze in some matrices, did not consistently meet the MRL of 0.05 mg/kg (50 ng/g) in either winter squash or plum, therefore, the LOQ > MRL. The European Food Safety Authority (EFSA) is of the opinion that this residue in plants should be redefined to include metabolites and their conjugates [9].

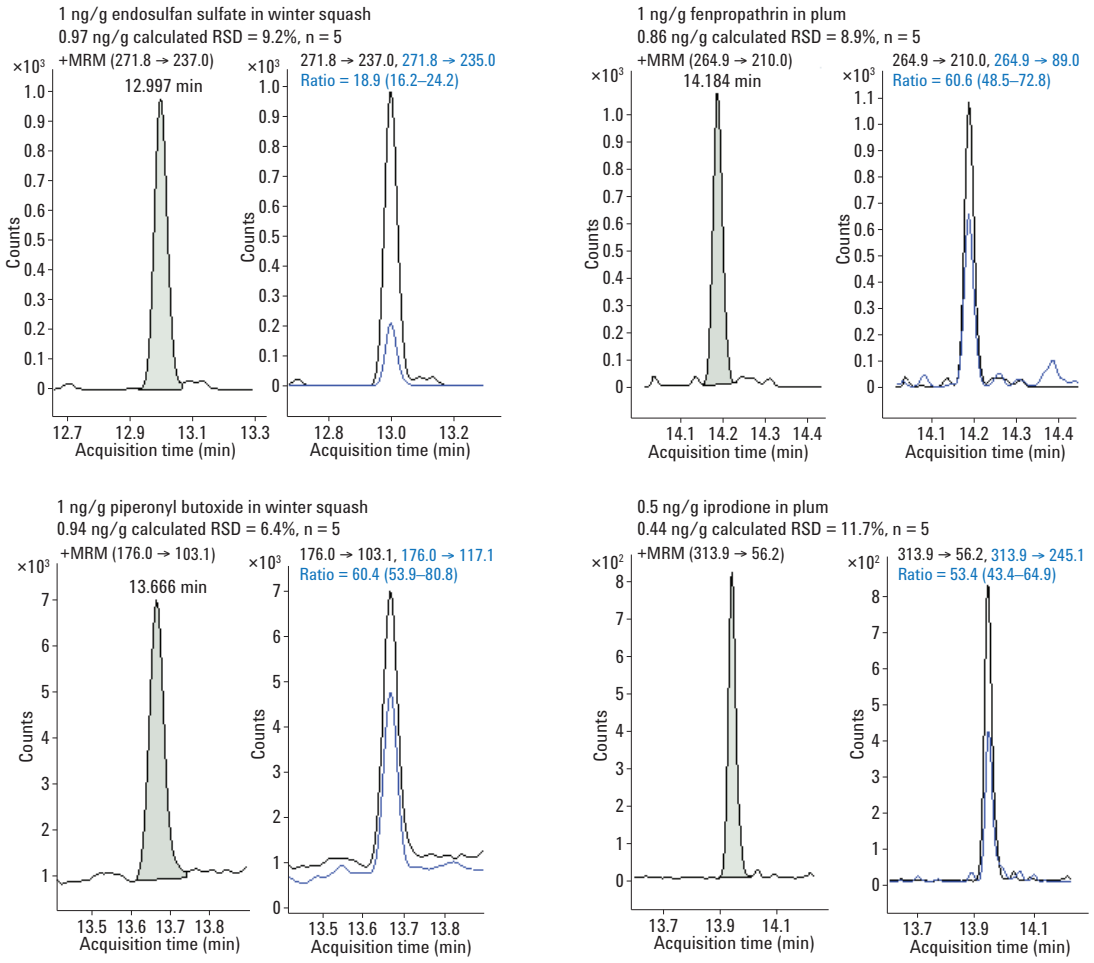

Figure 2. An example of quantitative results for four of the pesticides at their estimated LOQs, showing the average calculated amount, %RSD and number of replicates.

Table 1. Comparison of Estimated LOQs with the EU MRLs

Pesticide	EU MRL* in winter squash (ng/g)	LOQ(s) (ng/g)	EU MRL* in plum (ng/g)	LOQ(s) (ng/g)	Pesticide	EU MRL* in winter squash (ng/g)	LOQ(s) (ng/g)	EU MRL* in plum (ng/g)	LOQ(s) (ng/g)
Aldrin and dieldrin	30	1,10	10	0.5, 5	HCB	10	0.5	10	1
Allethrin I and II (summed)	10[c]	5	10[c]	5	Heptachlor and heptachlor epoxide	10	0.5, 0.5	10	0.5, 0.1
Amitraz and metabolites (2,4-dimethylaniline moiety)	50	> 50[f]	50	> 50[g]	Iprodione	1,000	0.5	3,000	0.5
Anthraquinone	10[c]	0.5	10[c]	1	Lenacil	100	0.1	100	0.5
Atrazine	50	0.5	50	0.5	Lindane (*gamma*-BHC)	10	5	10	1
Azinphos-methyl	50	0.5	50	5	Linuron	50	> 50	50	1
BHC, sum of isomers except gamma (*alpha-, beta-*)[a]	10	0.5, 0.5	10	1,1	Metalaxyl, sum of isomers including metalaxyl-M	50	0.5	50	0.5
Bifenthrin	50	1	200	0.5	Methoxychlor-p,p	10	0.5	10	0.5
Bromopropylate	10	0.1	10	0.1	Metolachlor (sum of isomers including S-metolachlor)	50	0.1	50	0.1
Bupirimate	200	0.5	50	0.1	Mevinphos (sum of isomers)	10	0.5	10	0.5
Captan	20	1	7,000	5[e]	MPCPS	10[c]	1	10[c]	0.5
Carfentrazone-ethyl	10	0.5	10	0.5	Myclobutanil	200	1	500	0.5
Chlordane, *cis-* and *trans-*	10	0.5, 0.5	10	0.5, 0.5	Oxyfluorfen	50	1	50	1
Chlorfenapyr	10	5	10	10	Paclobutrazol	20	0.5	500	0.5
Chlorothalonil	1,000	0.5	10	1	Parathion methyl (and paraoxon-methyl)[b]	10	5	10	5
Chlorpropham (and 3-chloroaniline)[b]	50	0.5	50	0.5	Parathion-ethyl	50	0.5	50	0.5
Chlorpyrifos	50	0.5	200	0.5	PCNB (quintozene) and pentachloroaniline	20	0.5, 0.5	20	0.5, 0.5
Chlorpyrifos methyl	50	0.5	50	1	Pebulate	10[c]	5	10[c]	0.5
Clomazone	10	0.5	10	1	Penconazole	100	0.5	50	0.5
Coumaphos	10[c]	0.1	10[c]	0.1	Pendimethalin	50	1	50	5
Cyfluthrin I-IV	20	1	200	1	Pentachlorobenzene (PCB)	10[c]	0.1	10[c]	1
Cyhalothrin, *lambda*-I and II (summed)	10[c]	5	10[c]	5	Permethrin I and II	50	0.5, 5	50	0.5, 10
Cypermethrin I-IV	200	20	2,000	20	Phenothrin I and II (summed)	50	5	50	10
Cyprodinil	50	0.5	2,000	1	Phorate (including oxygen analog and sulfones)[b]	10	0.5	10	0.5
DCPA (Dacthal, Chlorthal-dimethyl)	10	0.5	10	0.5	Phosalone	10	5	2,000	0.5
DDD-p,p'	—	0.5	—	0.5	Phosmet and phosmet oxon[b]	50	1	600	5
DDE-p,p'	—	0.5	—	1	Piperonyl Butoxide	10[c]	1	10[c]	5
DDT-p,p' (o,p, p,p', p,p'-DDE, p,p'-DDD)[b]	50	0.5[b]	50	0.5[b]	Pirimiphos-methyl	50	1	50	0.5
Deltamethrin, *cis-*	200	1	100	5	Prochloraz (sum of metabolites containing 2,4,6-trichlorophenol moiety)[b]	50	10	50	5

Table 1. Comparison of Estimated LOQs with the EU MRLs

Pesticide	EU MRL* in winter squash (ng/g)	LOQ(s) (ng/g)	EU MRL* in plum (ng/g)	LOQ(s) (ng/g)	Pesticide	EU MRL* in winter squash (ng/g)	LOQ(s) (ng/g)	EU MRL* in plum (ng/g)	LOQ(s) (ng/g)
Dichlobenil	10	0.1	10	0.5	Procymidone	10	0.1	10	0.1
Dicloran	300	1	100	1	Pronamide (propyzamide)	20	0.5	20	0.5
Dicofol degradation product (4,4'-dichlorobenzophenone)	10[c]	0.1	10[c]	0.1	Propargite	10	5	4,000	0.5
Diphenamid	10[c]	0.5	10[c]	0.5	Prothiofos	10[c]	0.5	10[c]	0.5
Diphenylamine	50	0.5	50	1	Pyridaben	50	1	500	10
Disulfoton (including disulfoton sulfoxide and disulfoton sulfone)[b]	10	1	10	1	Pyriproxyfen	50	10	50	0.5
Endosulfan, *alpha*-	50[d]	5	50[d]	10	Quinalphos	50	10	50	1
Endosulfan, *beta*-	50[d]	5	50[d]	5	Resmethrin I and II (summed)	100	10	100	5
Endosulfan Sulfate	50[d]	1	50[d]	0.5	Tebuconazole	200	0.5	1,000	0.5
Endrin	10	5	10	5	Tebufenpyrad	50	0.1	500	0.5
Etridiazole	50	> 50	50	> 50	Tecnazene (TCNB)	50	0.5	50	1
Fenarimol	50	0.5	20	0.5	Tefluthrin	50	0.5	50	0.5
Fenpropathrin	10	1	10	1	Terbacil	10c	0.5	10[c]	1
Fenthion (and its oxygen analogs, sulfoxides and sulfone)[b]	10	0.5	10	0.5	Terbuthylazine	50	1	50	0.5
Fenvalerate and esfenvalerate (sum of RS and SR)	20	0.5	20	0.1	Tetradifon	10	5	10	1
Fenvalerate and esfenvalerate (sum of SS and RR)	20	5	20	0.5	Tetramethrin I and II (summed)	10[c]	10	10c	5
Fipronil (and sulfone metabolite)[b]	5	0.5	5	0.5	THPI	10[c]	1	10c	5
Fludioxonil	300	0.5	500	1	Triadimefon and triadimenol	200	5, 0.5	100	5, 0.5
Flusilazole	20	0.1	100	0.5	Triallate	100	0.5	100	0.5
Fluvalinate, tau- I and II	10	1	300	10	Triazophos	10	0.5	10	0.5
Folpet	1,000	0.5	20	5[e]	Trifluralin	10	0.5	10	0.5
Fonofos	10[c]	0.5	10[c]	0.1	Vinclozolin and metabolites containing the 3,5-dichloraniline moiety[b]	50	0.5	50	0.5

* MRLs from Regulation (EC) No 1107/2009, updated 8/10/2013 (http://ec.europa.eu/food/plant/protection/pesticides/database_pesticide_en.htm)

[a] Delta isomer not measured

[b] Measured as the parent or first named compound only

[c] Not listed (MRL = 0.01 mg/kg)

[d] MRL for sum of alpha, beta isomers and endosulfan sulfate

[e] Estimation based on n = 3 (three consecutive calibration sets; equivalent to batch of < 30 injections)

[f] The estimated LOQ for DMF is 1 ng/g and that for 2,4-DMA is ≥50 (the latter was elevated due to the S/N requirement)

[g] The estimated LOQs for DMF and 2,4-DMA are 5 ng/g and 20 ng/g, respectively

The LOQ for total amitraz, which also has an MRL of 0.05 mg/kg in each matrix, was estimated to be greater than this value in both matrices. Amitraz is acid sensitive, accounting for its loss during this analysis and the inability to quantitate it at the MRL. It has a common moiety residue definition in the EU and should be monitored as its main metabolites N-2,4-dimethylphenyl-N-methylformamidine (DMPF) and 2,4-dimethylformanilide (DMF, also known as 2,4-dimethylphenylformamide). Both of these degrade to 2,4-dimethylaniline (2,4-DMA), which was also monitored in this study. The MRL for linuron in winter squash of 0.05 mg/kg was not met. However, the preferred technique for this pesticide is LC/MS/MS [4].

Captan and folpet are base-sensitive and often present issues in terms of recovery from matrix and precision during analysis. Although not used in this study, the evaluation of captan-d6 and folpet-d4 ISTDs is recommended to control recovery and assure reliable results, especially for longer batches in which the number of injections exceeds 40 [10]. For example, in this study, the estimated LOQ for folpet in plum exceeded the MRL of 0.02 mg/kg, or 20 ng/g, when five consecutive calibration sets of eight standards were injected. However, even without the use of labeled ISTD, when the number of injections was less than 30, the LOQ was estimated to be 5 ng/g (n = 3). In winter squash, the precision for folpet was not adversely affected by a larger number of injections, and the estimated LOQ of 0.5 ng/g (MRL = 1 mg/kg, or 1,000 ng/g) is based on consecutive injection of five calibration sets, or 40 injections (Table 1).

Excellent RSDs

Figure 3 shows the number of pesticides in winter squash and plum with given %RSD values based on calculated amounts at three concentrations: 0.5, 1, and 10 ng/g. RSD values were obtained from five consecutive injections of a set of eight calibration standards. Of 110 pesticides tested, 92 pesticides in winter squash and 92 in plum yielded %RSDs ≤ 20 at a concentration of 1 ng/g (84%).

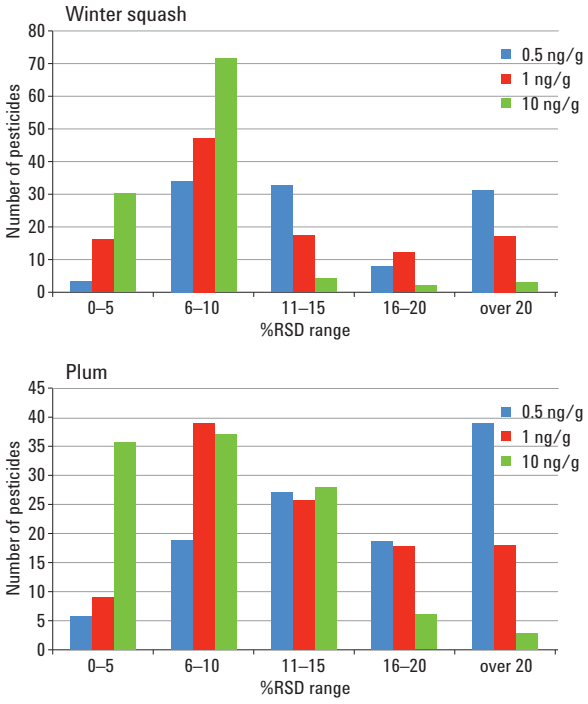

Figure 3. Distribution of %RSDs (n = 5) at 0.5, 1, and 10 ng/g in winter squash and plum.

Conclusions

The design of the Agilent 7000C Triple Quadrupole GC/MS enables lower detection limits for pesticides when combined with an inert sample path and GC column backflushing. The high sensitivity EI Extractor Ion Source with improved thermal characteristics delivers confident trace analysis even in complex matrices, and the Triple-Axis HED-EM Detector reduces neutral noise by the doubly off-axis position of the HED-EM.

These features enabled LOQs ≤ 1 ng/g for 75% of the 110 pesticides analyzed in plum, a matrix known for its difficulty in obtaining low detection limits, and 76% of the pesticides had LOQs ≤ 1 ng/g when analyzed in winter squash. A full 91% of the pesticides were able to be quantitated at levels ≤ 5 ng/g either in plum or winter squash, which is well below the EU MRLs for the most of these pesticides. The results demonstrate that data may be collected at levels below the current threshold MRL of 0.01 mg/kg (10 ng/g) for the majority of residues studied.

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