Screening for More Than 740 Pesticide Residues in Food Using an Agilent GC/Q-TOF and an Exact Mass Pesticide Library

Application Note

Authors

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Abstract

Six organically grown fruit and vegetable samples were extracted using the EN QuEChERS method, and these extracts were spiked with 93 pesticides at the 10 and 100 ng/mL level. The spiked extracts were analyzed using the Agilent 7200A GC/Q-TOF operating in high resolution TOF mode with electron ionization. The data were analyzed using Agilent MassHunter Qualitative Analysis Software (B.07.00) with the new Agilent Exact Mass GC/Q-TOF Pesticides Personal Compound Database and Library. Two GC methods were tried:

- A 20-minute run using a 5 m column coupled to a 15 m column through a Purged Ultimate Union
- A 40.5-minute method in the same configuration but with two 15 m columns

In each case, the first column was backflushed at the end of the run. At the 10 ng/mL spiking level, the 20-minute and 40.5-minute methods were able to identify 97.3 % and 97.1 % of the pesticides, respectively. At the 100 ng/mL level, the two methods found 99.6 % and 99.8 % of the pesticides, respectively. The Q-TOF was able to measure molecular ion masses with better than 2 ppm accuracy when the signal/noise ratio was >10.

Agilent Technologies

Introduction

To ensure a safe food supply, pesticide maximum residue limits (MRLs) have been set by various governmental authorities [1-3] and by Codex Alimentarius [4]. These laws regulate which pesticides can be legally used on a given crop, and the maximum amount of the pesticide that can remain after harvest. Pesticides that are allowed for use on a crop in one country may be disallowed in another, and MRL values usually are not the same across the world for various pesticide/food combinations. Given that more than 900 different pesticides are in use around the world, and that international food trade is increasing, there is a need to screen for large numbers of pesticides.

Typical pesticide residue methods use gas and liquid chromatography with tandem mass spectral detection (GC/MS/MS [5,6] and LC/MS/MS [7]). While very sensitive and highly selective, these are approaches based on targeted acquisition, so they detect only pesticides that are included in the method. One of the most comprehensive GC/MS/MS methods included 375 target compounds, 349 of which were pesticides [8]. More often, GC/MS/MS methods include many fewer compounds because calibrating for hundreds of pesticides can be expensive and time-consuming.

A comprehensive method that does not require regular calibration could screen for a much larger number of pesticides. This approach could complement targeted methods by providing additional confidence that a sample does not contain any pesticides that are outside the normal scope of the quantitative method. Such a screening method does not have to be quantitative, but it should be sufficiently sensitive that it could detect most pesticides at the 10 ng/g level (10 ppb), which is the generally accepted default MRL for pesticides with no established tolerance. Pesticides found by the screening method could then be added to the GC/MS/MS targeted analysis.

One approach that is currently in use is to analyze samples on an Agilent 7890B Gas Chromatograph, coupled with an Agilent 5977A Series GC/MSD System in scan mode followed by analysis using the Agilent Deconvolution Reporting Software (DRS) with the Pesticides and Endocrine Disruptor database/library [9,10]. This is a relatively low-cost and comprehensive approach, but sensitivity is limited for some pesticides, and deconvolution may not be as effective in very complex matrices.

This application note discusses a new approach to pesticide screening using a 7890 GC with the 7200 Quadrupole Time-of-Flight Mass Spectrometer (GC/Q-TOF). Agilent MassHunter Qualitative Analysis Software uses a personal compound database and library (PCDL) to select characteristic exact mass ions for each compound, and then extracts them to determine if they are present at the correct retention time (RT) and if they coelute. Three pesticide PCDLs, containing more than 700 entries, were created using MassHunter Qualitative Analysis Software. The main difference between the three PCDLs is the GC method, so each one has locked retention times that correspond to the GC method used.

Experimental

Sample preparation

Equipment, instruments, and materials

- Agilent Bond Elut QuEChERS EN Extraction packets, p/n 5982-5650 (Agilent Technologies Inc., Folsom, CA, USA)
- Agilent Bond Elut QuEChERS EN Dispersive SPE kit for General Fruits and Vegetables, p/n 5982-5056 and EN Dispersive SPE kit for High Pigmented Fruits and Vegetables, p/n 5982-5356
- Agilent Bond Elut QuEChERS Ceramic Homogenizers, p/n 5982-9311
- Robot Coupe Blender (Robot Coupe USA, Inc., Ridgeland, MS, USA)
- 2010 Geno Grinder (SPEX Sample Prep, Metuchen, NJ, USA)
- VWR Signature Digital Multi-Tube Vortexer (VWR, Radnor, PA, USA)
- CentraCLR3R Centrifuge (Thermo-Fisher, Waltham, MA, USA)

Procedure

Preparation of the fruit and vegetable (carrot, broccoli, tomato, green beans, celery, and red apple) extracts was based on the European Standard (EN) version of the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method [11] using Agilent extraction salts and dispersive kits. Organically grown fruits and vegetables were finely chopped, frozen, then homogenized with dry ice in a Robot Coupe blender. The homogenized samples were then stored at –20 °C until extraction.

Extraction/partitioning

Ten grams of homogenized sample were weighed into a 50-mL centrifuge tube, and two ceramic homogenizers were added to the sample. Ten mL of ACN was added to the sample tube, which was capped and vortexed for 1 minute. A packet of Agilent EN QuEChERS salts (p/n 5982-5650) containing 4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate, and 0.5 g disodium citrate sesquihydrate was added directly to the tubes. Sample tubes were sealed tightly, and vigorously shaken on the Geno Grinder for 1 minute. Sample tubes were then centrifuged at 5,000 rpm for 5 minutes.

Dispersive SPE cleanup

A 6-mL aliquot of the upper ACN layer from the extracts was transferred to an Agilent QuEChERS EN dispersive SPE 15-mL tube. For carrot, tomato, celery, and red apple extracts, QuEChERS EN dispersive SPE (p/n 5982-5056) containing 150 mg PSA and 900 mg $MgSO_4$ was used. For broccoli and green beans, QuEChERS EN dispersive SPE (p/n 5982-5356) containing 150 mg PSA, 45 mg GCB, and 855 mg $MgSO_4$ was used. The tubes were tightly capped and vortexed for 1 minute, then centrifuged at 5,000 rpm for 5 minutes.

Standard mix extracts

Custom pesticide standards were purchased at 100 ppm (100 µg/mL) in nine different mixes from AccuStandard (New Haven, CT). Combining 100 µL from each of the nine vials and an additional 100 µL of ACN resulted in a 1:10 dilution of the standard mix (10 µg/mL). A 1:10 dilution of the 10 µg/mL solution was made to yield a 1 µg/mL standard mix. One microliter of the 10 µg/mL standard mix was added to 99 µL of the fruit or vegetable extract to prepare a spiked solution at 100 ng/mL. One microliter of the 1 µg/mL standard mix was added to 99 µL of the fruit or vegetable extract to prepare a spike at 10 ng/mL. Figure 1 shows the workflow for the QuEChERS sample extraction procedure.

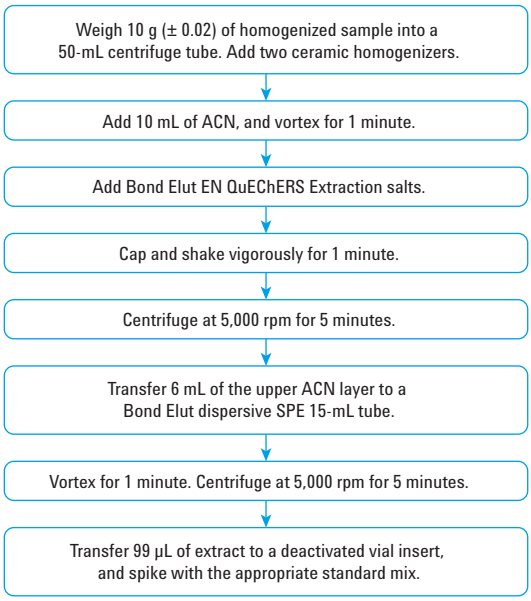

Figure 1. Workflow for the Agilent QuEChERS sample extraction procedure.

Instrumentation and analytical conditions

The instrumentation and analytical conditions are listed in Tables 1 and 2. Two midcolumn backflushing methods were used, each with its own GC column configuration, total run time, and analyte retention times. The first method used a 5 m × 0.25 mm, 0.25 µm DB-5 column connected between the multimode inlet (MMI) and a purged union with a second column (15 m × 0.25 mm, 0.25 µm DB-5) connected between the purged union and the Q-TOF transfer line (Figure 2). The second method configures the columns in the same way but uses two 15 m × 0.25 mm, 0.25 µm columns. These configurations, referred to as the 5×15 method and the 15×15 method, are run in the constant column flow mode and have run times of 20 and 40.5 minutes, respectively.

Table 1. Instrumentation and Conditions for Analysis Using the 5×15 Method

Parameter	Value
Gas chromatograph	Agilent 7890B Gas Chromatograph with 240 V power supply
Autosampler	Agilent 7693A Series Automatic Liquid Sampler injector and tray
Injection volume	2 µL cold splitless
Injection speed	Fast
Inlet liner	2 mm id Ultra Inert Dimpled (p/n 5190-2296)
Septum purge flow and mode	3 mL/min, switched
Column 1	Agilent DB-5, 5 m × 0.25 mm, 0.25 µm installed between MMI and purged union (cut from a 15 m column (p/n 122-5012)
Column 2	Agilent DB-5, 15 m × 0.25 mm, 0.25 µm (p/n 122-5012) installed between purged union and Q-TOF
Column 1 flow	He, nominally 1.0 mL/min
Column 2 flow	He, nominally 1.1 mL/min (Column 1 flow + 0.1 mL/min)
Backflushing (post run)	3 minutes at 290 °C Column 1 flow = –36.852 mL/min Column 2 flow = 12.672 mL/min
Retention time locking	Chlorpyrifos-methyl locked to 8.524 minutes
MMI temperature program	60 °C for 0.02 minutes, 600 °C/min to 300 °C, hold
MMI mode	Splitless (purge flow to split vent = 100 mL/min at 1.5 minutes) gas saver = 20 mL/min at 2.0 min
Oven temp program	60 °C for 1.5 minutes, 50 °C/min to 160 °C (0 minutes), 8 °C/min to 240 °C (0 minutes), 50 °C/min to 280 °C (2.5 minutes), 100 °C/min to 290 °C (3.1 minutes)
Mass spectrometer	Agilent 7200A Q-TOF
Mass spec mode	EI; TOF only in high resolution (4 GHz) mode
Collision gas	N_2 on at 1.5 mL/min
Stored mass range	*m/z* 35–550
Acquisition rate	5 Hz
Transfer line temperature	300 °C
Source and quadrupole temperature	300 °C and 180 °C

Table 2. Columns and Conditions for the 15×15 Method

Parameter	Value
Columns 1 and 2	Agilent DB-5, 15 m × 0.25 mm, 0.25 µm (p/n 122-5012) installed as shown in Table 1
Column 1 flow	He, nominally 1.5 mL/min
Column 2 flow	He, nominally 1.7 mL/min (Column 1 flow + 0.2 mL/min
Backflushing (post run)	5 minutes at 310 °C Column 1 flow = –11.536 mL/min Column 2 flow = 11.95 mL/min
Retention time locking	Chlorpyrifos-methyl locked to 18.111 minutes
Oven temperature program	60 °C for 1 minute, 40 °C/min to 120 °C (0 minutes), 5 °C/min to 310 °C (0 minutes)

Instrumentation and conditions not shown here are the same as shown in Table 1.

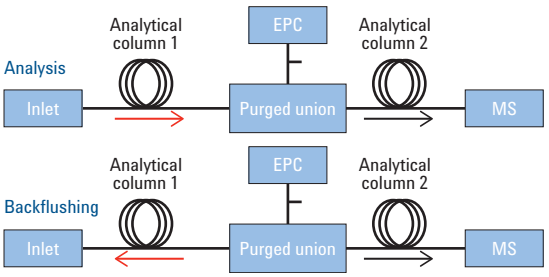

Figure 2. GC column configuration for backflushing column 1 at the end of the run. For the 5×15 method, Column 1 is 5 m long and Column 2 is 15 m. For the 15×15 method, both columns are 15 m long. During backflushing, the pressure is increased at the purged union and reduced in the inlet causing the flow in Column 1 to reverse direction. The column flow along with any retained compounds exit through the inlet's split vent.

GC columns

For these analyses, DB-5 columns were used.
The 5 m × 0.25 mm, 0.25 µm column was cut from a 15-m column. These DB-5 columns give retention times that are virtually identical to those obtained with the Agilent HP-5MS UI columns that were used to create the pesticide PCDLs. Note that the DB-5MS column has a different phase and will not give the same retention times.

Software for acquisition and data analysis

MassHunter GC/MS Acquisition Software (version B.07.00 SP2) was used for instrument control and data acquisition. MassHunter Workstation Qualitative Analysis Software (Qual, version B.07.00) was used for data analysis. In particular, the All Ions workflow embedded in the Find-by-Formula portion of Qual was employed to determine if the analyzed pesticides could be identified in the various matrices by the GC/Q-TOF method. Data analysis was performed on centroid spectra.

Tuning the Q-TOF

A 4 GHz EI autotune was used for all analyses. The source and quadrupole temperatures were set to 300 °C and 180 °C, respectively. Immediately before each sample was analyzed, the TOF mass assignments were calibrated automatically as part of the sample sequence. TOF mass calibration is automated, and takes about 90 seconds each time it is run.

Results and Discussion

The data analysis method discussed here was only for qualitative analysis. The goal was to identify pesticide contaminants, but not to quantify them. One could calibrate for a set of target compounds, but it is unlikely that anyone would calibrate for all 700+ pesticides in the PCDL. For this approach, the assumption was made that the GC/Q-TOF method would be used for screening, and any pesticides found could be added to a GC/MS/MS target compound method.

The fruit and vegetable extracts were spiked at the 10 ng/mL level because this is generally agreed upon as the default MRL when none exists for a pesticide/commodity combination. When screening, one does not need to quantify at this level, but only show that the pesticide is likely to be present. For pesticides that do have an established MRL, it is often much higher than 10 ng/mL. For this reason, samples were also spiked at the 100 ng/mL level. It would then be possible to compare the All Ions method performance on two relevant pesticide concentrations. Chromatograms of the spiked QuEChERS extracts are shown in Figure 3. QuEChERS extractions usually provide just enough cleanup for pesticide residue analysis by GC/MS/MS. As a result, the matrix response is generally many orders of magnitude greater than that of the target pesticides, and very high selectivity is required to detect the analytes in the presence of the matrix. One objective of this work was to determine if GC, with high-resolution accurate-mass (HRAM) TOF detection, could provide sufficient selectivity for low level screening.

Backflushing configuration

As seen in Figure 3, the extracts contained high concentrations of co-extracted endogenous compounds. Ordinarily, the GC column would need to be baked out at a high temperature after each run to ensure that it is clean for the next analysis [5]. However, the first column retains the less volatile compounds and can be backflushed at the end of the run by increasing the pressure at the purged union and reducing the pressure in the inlet (Figure 2). For the 5×15 method, Column 1 was backflushed at 290 °C for 3 or 4 minutes with a flow rate of –36.9 mL/min. For the 15×15 method, Column 1 was backflushed at 310 °C for 5 minutes with a flow rate of –11.5 mL/min. These backflushing times are 1 or 2 minutes longer than are usually needed because these methods were also used for the analysis of black pepper extracts, which are notoriously difficult samples.

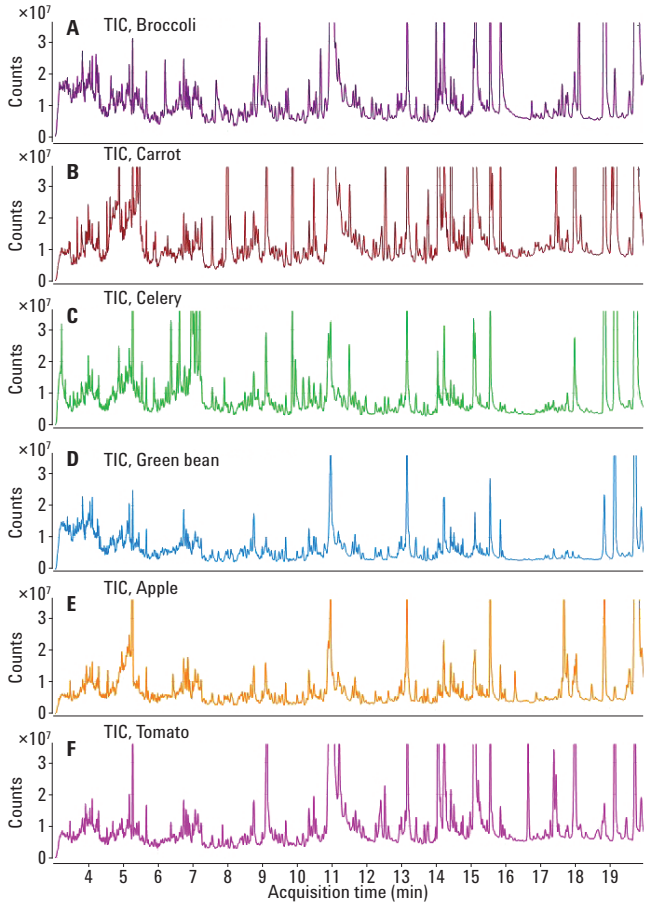

Figure 3. Chromatograms of QuEChERS extracts spiked at 100 ng/mL with the pesticide standard described in Table 3. A) broccoli, B) carrot, C) celery, D) green bean, E) red apple, and F) tomato. All chromatograms are on the same scale and have been enlarged along the Y axis to show the inherent complexity of each extract.

All Ions screening process

Exact mass ions selected from the PCDL are extracted from the chromatogram at the analyte's known retention time and the extracted ion chromatograms (EICs) are overlaid, as shown in Figure 4C for quinoxyfen in the green bean extract. The software chooses one EIC as the reference ion and determines if the peak shape and RT of the other selected ions match the reference ion. For each spectral acquisition across the peak, the response of each ion is compared to that of the reference ion. Ideally, this ratio will remain the same across the whole peak. The normalized values of these ratios are plotted, as shown in Figure 4D. If the ion peak shapes and RT were perfect, the plot in Figure 4D would be a horizontal straight line at 1.

If the target compound has a measurable molecular ion, its isotope pattern is plotted (Figure 4E) with the theoretical ion ratios and spacing shown by red boxes. The window shown in Figure 4B shows which ions are qualified (coelution score is above user-set requirement). The upper bar shows the compound name, its formula, *m/z* of the molecular ion, the difference in measured and theoretical mass of the M^{+*} in ppm, and the difference between the database and actual retention times. The top window in Figure 4F shows the extracted ions and molecular ion isotopes (if present), while the lower window shows the averaged total ion chromatogram (TIC) across the extracted peak. The table in Figure 4A summarizes all the information for each compound that was identified.

The TOF always acquires full spectra, so it is possible to go back to the data and reanalyze it long after it was acquired. Data acquisition is not dependent on the number of compounds in the PCDL so, theoretically, an unlimited number of compounds could be added to the PCDL to expand the scope of the screening method.

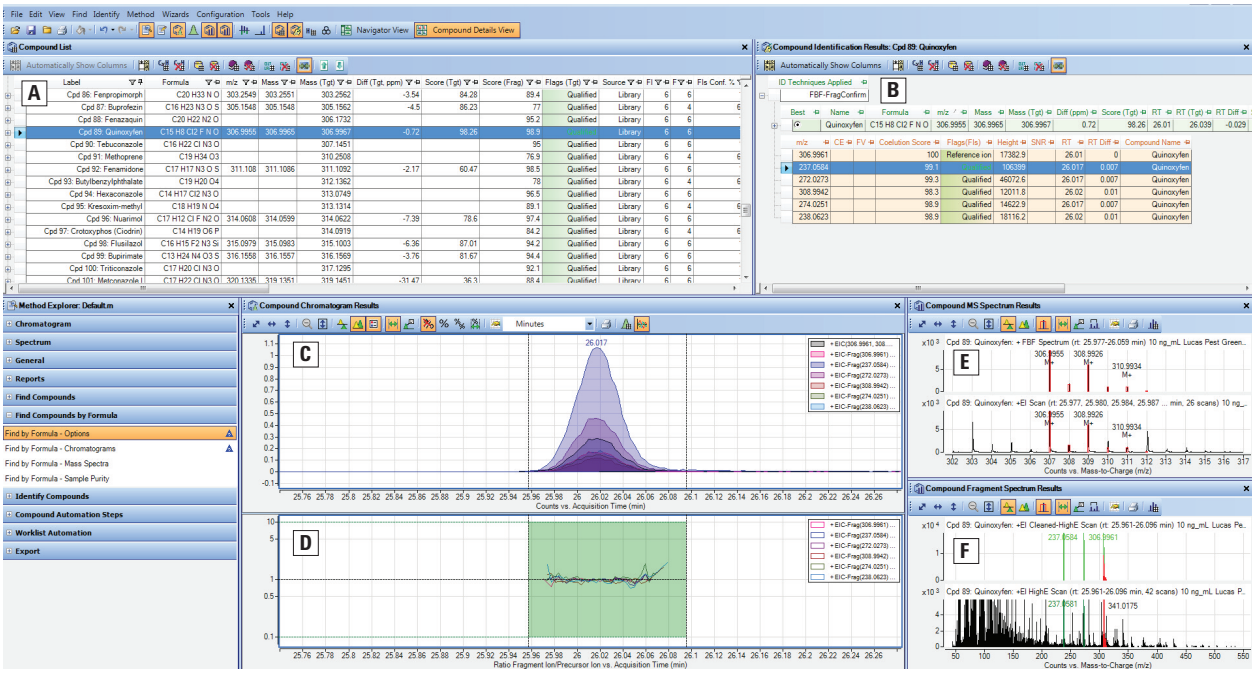

Figure 4. Results for the analysis of pesticides in a green bean extract with quinoxyfen highlighted. A) Compound list showing hits, B) Compound identification results for quinoxyfen, C) Extracted ion chromatograms for the most significant quinoxyfen ions, D) Coelution plot, E) Molecular ion isotope ratio plot, and F) Extracted ions (top) and TIC averaged across the chromatographic peak (bottom).

Figure 5 shows another example where carboxin was identified at 10 ng/mL in the carrot extract. The six EICs show good peak shape and retention alignment (Figure 5A) which is supported by the high coelution scores shown in Figure 5B. In addition, the measured mass of the molecular ion was just 1.1 ppm away from the calculated monoisotopic mass.

Furthermore, the measured retention time of carboxin was only 0.013 minutes (0.78 seconds) different from the 5x15 database value. As can be seen in the library spectrum (Figure 5C) three of the six extracted ions were relatively weak, but these were still found.

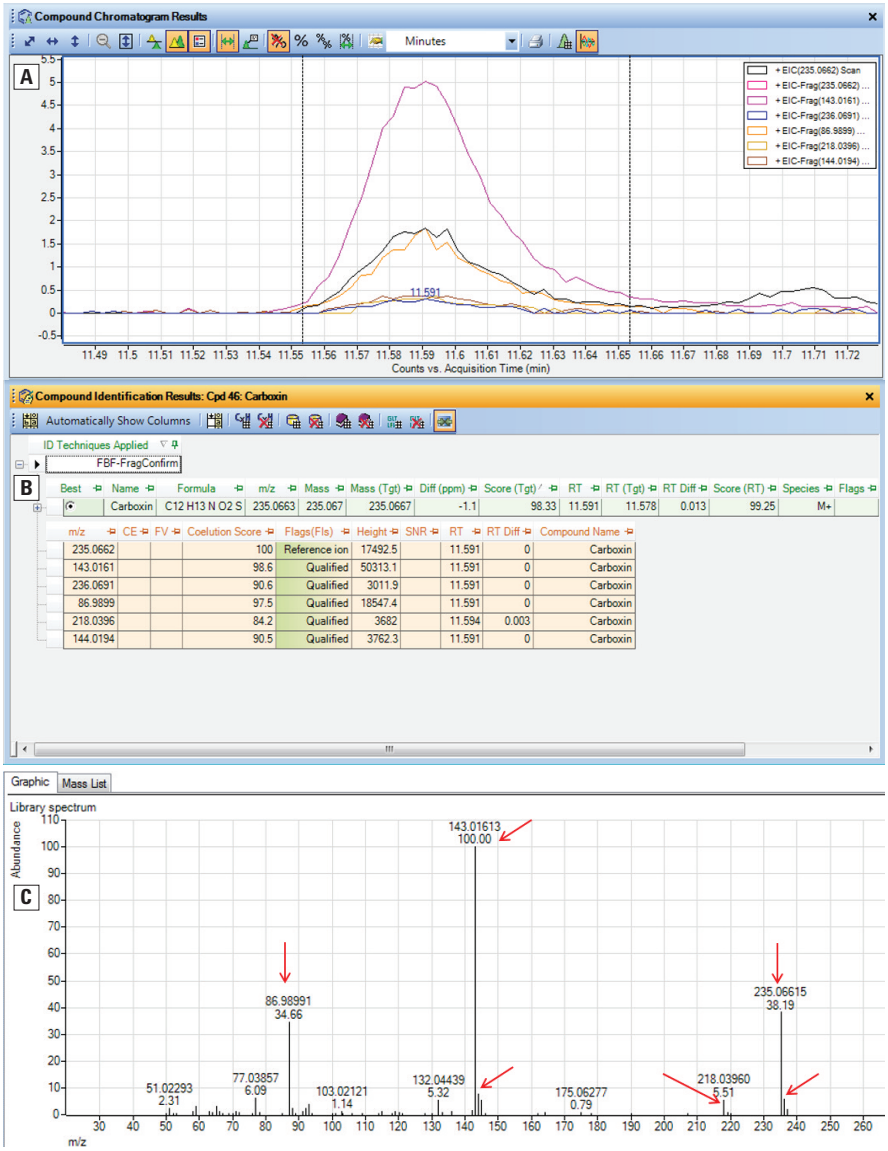

Figure 5. Carboxin in carrot extract at 10 ppb. A) EICs showing the reference ion and five additional ions characteristic of carboxin. B) Compound identification results showing that all six ions were found. The mass accuracy of the M^{++} and the retention time accuracy are shown to the right of the compound name. C) PCDL spectrum of carboxin showing the six ions that were used for identification.

Results for spiked fruits and vegetables

Tables 3 and 4 summarize the results obtained for the two different GC/Q-TOF methods: the 5×15 20-minute method and the 15×15 40-minute method, respectively. All of the pesticides listed in Table 3 are included in the Agilent GC HRAM Pesticide PCDL for the 5×15 method. Seven of them are not currently included in the 15×15 PCDL, and those are not included in Table 4. In all, 93 pesticides were targeted by the 5×15 method and 86 pesticides by the 15×15 method. The locked retention times and exact mass spectra for all of the pesticides listed can be found in the Agilent GC/Q-TOF Pesticides PCDL (p/n G3892AA).

Table 3 also lists the preferred analysis method for each pesticide. All of the pesticides listed are amenable to LC/MS analysis and for some of those listed LC/MS is preferred even though it is possible, under optimum circumstances, to analyze them by GC/MS. Most of the compounds can be analyzed by either technique.

Using the 5×15 method, an average of 97.3 % (10 ng/mL spike) and 99.6 % (100 ng/mL spike) of the pesticides were identified in the six extracts. The 15×15 method takes twice as long to run (40.5 minutes rather than 20 minutes for the 5×15 method), and there is a possibility that it could provide better separation that would result in more identifications. However, the results were virtually the same, with 97.1 % of the compounds found at the 10 ng/mL level and 99.8 % for the 100 ng/mL spikes. These results are summarized at the bottom of Tables 3 and 4.

Table 3. Pesticides Identified in Fruit and Vegetable Extracts Spiked at 10 and 100 ppb Using the 5x15 GC/Q-TOF Method and the 5x15 GC/Q-TOF Pesticide PCDL (continued on next page)

The number of pesticides found (out of 93) and the percentage found are summarized at the bottom of the table.

		Green bean		Tomato		Carrot		Red apple		Celery		Broccoli	
Pesticides analyzed by the 5×15 method	**Preferred analytical method**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**
1-Naphthol	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Acibenzolar-S-methyl	L	X	X	X	X	X	X	X	X	X	X	X	X
Ametryn	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Azoxystrobin	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Benalaxyl	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Boscalid	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Bromuconazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Bupirimate	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Buprofezin	G or L	X	X	X	X		X	X	X		X	X	X
Carboxin	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Carfentrazone-ethyl	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Clethodim	L	X	X	X	X	X	X	X	X	X	X	X	X
Cycluron	L	X	X	X	X	X	X	X	X	X	X	X	X
Cyproconazole	G or L	X	X	X	X	X	X	X	X		X	X	X
Cyprodinil	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Diclobutrazol	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Diethofencarb	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Difenconazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Dimethoate	G or L	X	X	X	X	X	X	X	X	X	X		X
Dimethomorph	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Diniconazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X

X = pesticide identified
M = Metabolite identified
G = GC/MS
L = LC/MS

Table 3. Pesticides Identified in Fruit and Vegetable Extracts Spiked at 10 and 100 ppb Using the 5x15 GC/Q-TOF Method and the 5x15 GC/Q-TOF Pesticide PCDL (continued on next page)

		Green bean		Tomato		Carrot		Red apple		Celery		Broccoli	
Pesticides analyzed by the 5×15 method	**Preferred analytical method**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**
Diuron	L	M	M	M	M	M	M	M	M	M	M	M	M
Epoxiconazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Etaconazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Ethofumesate	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Etoxazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Famoxadone	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Fenamidone	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Fenarimol	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Fenazaquin	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Fenbuconazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Fenhexamid	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Fenoxycarb	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Fenpropimorph	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Fipronil	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Fludioxonil	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Flufenacet	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Fluoxastrobin	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Fluquinconazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Flusilazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Flutriafol	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Furalaxyl	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Furathiocarb	L	X	X	X	X	X	X	X	X	X	X	X	X
Hexaconazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Imazalil	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Ipconazole	G or L		X	X	X	X	X	X	X	X	X	X	X
Isoxaflutole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Kresoxim-methyl	G or L	X	X		X	X	X	X	X	X	X		X
Lufenuron	L	X	X	X	X	X	X	X	X	X		X	
Mefenacet	G or L	X	X	X	X	X	X	X	X		X	X	X
Mepanipyrim	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Mepronil	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Metalaxyl	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Metconazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Methoprotryne	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Metobromuron	G or L		X	X	X	X	X	X	X	X	X	X	X
Metribuzin	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Mexacarbate	L	X	X	X	X	X	X	X	X	X	X	X	X

X = pesticide identified
M = Metabolite identified
G = GC/MS
L = LC/MS

Table 3. Pesticides Identified in Fruit and Vegetable Extracts Spiked at 10 and 100 ppb Using the 5x15 GC/Q-TOF Method and the 5x15 GC/Q-TOF Pesticide PCDL (continued on next page)

		Green bean		Tomato		Carrot		Red apple		Celery		Broccoli	
Pesticides analyzed by the 5×15 method	**Preferred analytical method**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**
Myclobutanil	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Novaluron	L	X	X	X	X	X	X	X	X	X	X	X	X
Nuarimol	G or L	X	X	X	X	X	X	X	X	X	X	X	X
o-Phenylphenol	G or L	X	X	X	X		X	X	X	X	X	X	X
Oxadixyl	G or L	X	X		X	X	X	X	X	X	X	X	X
Paclobutrazol	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Penconazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Picoxystrobin	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Piperonyl butoxide	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Pirimicarb	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Prochloraz	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Prometon	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Prometryn	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Propargite	G	X	X	X	X	X	X	X	X	X	X		X
Propiconazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Pyracarbolid	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Pyridaben	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Pyrimethanil	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Pyriproxyfen	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Quinoxyfen	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Secbumeton	G or L	X	X	X	X	X	X	X	X		X	X	X
Spirodiclofen	G or L	X	X		X	X	X	X	X	X	X	X	X
Spiromesifen	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Spiroxamine	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Sulfentrazone	G or L	X	X	X	X	X	X	X	X		X	X	X
Tebuconazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Tebufenpyrad	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Terbumeton	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Terbutryn	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Tetraconazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Thiamethoxam	L	X	X	X	X	X	X	X	X	X	X	X	X
Triadimefon	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Triadimenol	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Trifloxystrobin	G or L	X	X	X	X	X	X	X	X	X	X	X	X
Triticonazole	G or L	X	X	X	X	X	X	X	X	X	X	X	X
No. Found by 5x15 20-min method (out of 93)		91	93	90	93	91	93	93	93	88	92	90	92
% Found by 5x15 method		97.8	100	96.7	100	97.8	100	100	100	94.6	98.9	96.7	98.9

X = pesticide identified
M = Metabolite identified
G = GC/MS
L = LC/MS

Table 4. Pesticides Identified in Fruit and Vegetable Extracts Spiked at 10 and 100 ppb Using the 15x15 GC/Q-TOF Method and the 15x15 Pesticide PCDL (continued on next page)

The number of pesticides found (out of 86) and the percentage found are summarized at the bottom of the table.

	Green beans		Tomato		Carrot		Red apple		Celery		Broccoli	
Pesticides analyzed by the 15×15 method	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**
1-Naphthol	X	X	X	X	X	X	X	X	X	X	X	X
Acibenzolar-S-methyl	X	X	X	X	X	X	X	X	X	X	X	X
Ametryn	X	X	X	X	X	X	X	X	X	X	X	X
Azoxystrobin	X	X	X	X	X	X	X	X	X	X	X	X
Benalaxyl	X	X	X	X	X	X	X	X	X	X	X	X
Boscalid	X	X	X	X	X	X	X	X	X	X	X	X
Bromuconazole	X	X	X	X	X	X	X	X	X	X	X	X
Bupirimate	X	X	X	X	X	X	X	X	X	X	X	X
Buprofezin	X	X		X		X		X	X	X	X	X
Carboxin	X	X	X	X	X	X	X	X	X	X	X	X
Carfentrazone-ethyl	X	X	X	X	X	X	X	X	X	X	X	X
Cycluron	X	X	X	X	X	X	X	X	X	X	X	X
Cyproconazole	X	X	X	X	X	X	X	X	X	X	X	X
Cyprodinil	X	X	X	X	X	X	X	X	X	X	X	X
Diclobutrazol	X	X	X	X	X	X	X	X	X	X	X	X
Diethofencarb	X	X	X	X	X	X	X	X	X	X	X	X
Difenconazole		X	X	X	X	X	X	X		X	X	X
Dimethoate	X	X	X	X	X	X	X	X	X	X	X	X
Dimethomorph	X	X	X	X	X	X	X	X	X	X	X	X
Diniconazole	X	X	X	X	X	X	X	X	X	X	X	X
Diuron	M	M	M	M	M	M	M	M		M	M	M
Etaconazole	X	X	X	X	X	X	X	X	X	X	X	X
Ethofumesate	X	X	X	X	X	X	X	X	X	X	X	X
Etoxazole	X	X	X	X	X	X	X	X	X	X	X	X
Famoxadone	X	X	X	X	X	X	X	X		X	X	X
Fenamidone	X	X	X	X	X	X	X	X	X	X	X	X
Fenarimol	X	X	X	X	X	X	X	X	X	X	X	X
Fenazaquin	X	X	X	X	X	X	X	X	X	X	X	X
Fenhexamid	X	X	X	X	X	X	X	X	X	X	X	X
Fenoxycarb	X	X	X	X	X	X	X	X	X	X	X	X
Fenpropimorph	X	X	X	X	X	X	X	X	X	X	X	X
Fipronil	X	X	X	X	X	X	X	X	X	X	X	X
Fludioxonil	X	X	X	X	X	X	X	X	X	X	X	X
Flufenacet	X	X	X	X	X	X	X	X	X	X	X	X
Fluquinconazole	X	X	X	X	X	X	X	X	X	X	X	X
Flusilazole	X	X	X	X	X	X	X	X	X	X	X	X
Flutriafol	X	X	X	X	X	X	X	X	X	X	X	X
Furalaxyl	X	X	X	X	X	X	X	X	X	X	X	X

X = pesticide identified
M = Metabolite identified

Table 4. Pesticides Identified in Fruit and Vegetable Extracts Spiked at 10 and 100 ppb Using the 15x15 GC/Q-TOF Method and the 15x15 Pesticide PCDL (continued on next page)

	Green beans		Tomato		Carrot		Red apple		Celery		Broccoli	
Pesticides analyzed by the 15×15 method	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**
Furathiocarb	X	X	X	X	X	X	X	X	X	X	X	X
Hexaconazole	X	X	X	X	X	X	X	X	X	X	X	X
Imazalil	X	X	X	X	X	X	X	X	X	X	X	X
Ipconazole	X	X	X	X	X	X	X	X	X	X	X	X
Isoprocarb	X	X	X	X	X	X	X	X	X	X	X	X
Kresoxim-methyl	X	X	X	X	X	X	X	X	X	X	X	X
Mefenacet	X	X	X	X	X	X	X	X	X	X	X	X
Mepanipyrim	X	X	X	X	X	X	X	X	X	X	X	X
Mepronil	X	X	X	X	X	X	X	X	X	X	X	X
Metalaxyl	X	X	X	X	X	X	X	X	X	X	X	X
Metconazole	X	X	X	X	X	X	X	X	X	X	X	X
Methoprotryne	X	X	X	X	X	X	X	X	X	X	X	X
Metobromuron	X	X	X	X	X	X	X	X	X	X	X	X
Metribuzin	X	X	X	X	X	X	X	X	X	X	X	X
Mexacarbate	X	X	X	X	X	X	X	X	X	X	X	X
Myclobutanil	X	X	X	X	X	X	X	X	X	X	X	X
Nuarimol	X	X	X	X	X	X	X	X	X	X	X	X
o-Phenylphenol	X	X	X	X	X	X	X	X	X	X	X	X
Oxadixyl	X	X	X	X	X	X	X	X	X	X	X	X
Paclobutrazol	X	X	X	X	X	X	X	X	X	X	X	X
Penconazole	X	X	X	X	X	X	X	X	X	X	X	X
Picoxystrobin	X	X	X	X	X	X	X	X	X	X	X	X
Piperonyl butoxide	X	X	X	X	X	X	X	X	X	X	X	X
Pirimicarb	X	X	X	X	X	X	X	X	X	X	X	X
Prochloraz	X	X	X	X	X	X	X	X	X	X	X	X
Prometon	X	X	X	X	X	X	X	X	X	X	X	X
Prometryn	X	X	X	X	X	X	X	X	X	X	X	X
Propargite		X		X			X	X		X		X
Propiconazole	X	X	X	X	X	X	X	X	X	X	X	X
Pyracarbolid	X	X	X	X	X	X	X	X	X	X	X	X
Pyridaben	X	X	X	X	X	X	X	X	X	X	X	X
Pyrimethanil	X	X	X	X	X	X	X	X	X	X	X	X
Pyriproxyfen	X	X	X	X	X	X	X	X	X	X	X	X
Quinoxyfen	X	X	X	X	X	X	X	X	X	X	X	X
Secbumeton	X	X	X	X	X	X	X	X	X	X	X	X
Spirodiclofen	X	X		X	X	X	X	X	X	X		X
Spiromesifen	X	X	X	X	X	X	X	X	X	X	X	X
Spiroxamine	X	X	X	X	X	X	X	X	X	X	X	X
Sulfentrazone	X	X	X	X	X	X	X	X		X	X	X

X = pesticide identified
M = Metabolite identified

Table 4. Pesticides Identified in Fruit and Vegetable Extracts Spiked at 10 and 100 ppb Using the 40.5-Minnute 15×15 GC/Q-TOF Method and the 15×15 Pesticide PCDL (continued from previous page)

	Green beans		Tomato		Carrot		Red apple		Celery		Broccoli	
Pesticides analyzed by the 15×15 method	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**	**10 ng/mL**	**100 ng/mL**
Tebuconazole	X	X	X	X	X	X	X	X	X	X	X	X
Tebufenpyrad	X	X	X	X	X	X	X	X	X	X	X	X
Terbumeton	X	X	X	X	X	X	X	X	X	X	X	X
Terbutryn	X	X	X	X	X	X	X	X	X	X	X	X
Tetraconazole	X	X	X	X	X	X	X	X	X	X	X	X
Triadimefon	X	X	X	X	X	X	X	X	X	X	X	X
Triadimenol	X	X	X	X	X	X	X	X	X	X	X	X
Trifloxystrobin	X	X	X	X	X	X	X	X	X	X	X	X
Triticonzaole	X	X	X	X	X	X	X	X	X	X	X	X
No. found by 15x15 40-min method (out of 86)	84	86	83	86	84	85	85	86	81	86	84	86
% found by 15x15 method	97.7	100	96.5	100	97.7	98.8	98.8	100	94.2	100	97.7	100

X = pesticide identified
M = Metabolite identified

There are several advantages to using electron ionization (EI) at 70 eV for this screening approach. EI provides a rich selection of diagnostic ions, increasing the confidence of identifications. The TOF MS produces standard EI spectra that are reproducible and library searchable. This means that data files can also be evaluated using Agilent's MassHunter Unknowns Analysis Software which first deconvolutes the chromatogram and then applies standard mass spectral library searching to identify the components. Indeed, the exact mass Pesticide PCDL can be used for this purpose as well as commercially available nominal mass libraries such as the one from NIST. When a compound has been tentatively identified, MassHunter Qual can be used to assign formulas to the accurate mass fragments in the spectrum to see if these are consistent with the assigned structure.

Mass accuracy

The mass accuracy of the TOF depends on the abundance of the ion being measured and the accuracy of the mass assignment calibrations, among other things. If the signal is too small to have good ion statistics, or if it is so large that it is approaching detector saturation, shifts in mass assignments can occur. To illustrate the ability of the Agilent 7200 GC/Q-TOF to measure *m/z* values accurately, the signal-to-noise (S/N) ratio was measured for the pesticides molecular ions (when present) in the broccoli extract (spiked at 100 ng/mL. In cases where the molecular ion was weak (S/N < 10), the average difference between the measured *m/z* value and the calculated mono-isotopic mass averaged 5.69 ppm (Table 5). When the S/N was between 10 and 100, the mass accuracy was better than 2 ppm, and when the S/N exceeded 100, the mass accuracy was 1.25 ppm. These values were obtained when the TOF mass assignments were automatically calibrated just before the sample was analyzed. One can also use the internal reference mass to do continuous mass adjustments throughout the run, but that was not tried for these experiments.

Table 5. Mass Accuracy of the Molecular Ion (for Pesticides Whose Spectrum Displayed One) as a Function of the S/N Ratio of that Ion in the Extracted Ion Chromatogram

Number of pesticides with $M^{+\bullet}$ in range	S/N range	Average mass error (ppm)[a]
10	< 10	5.69
34	> 10 but < 100	1.92
27[b]	> 100	1.25

[a] Average of the absolute values of the mass errors
[b] After removing one outlier which had a saturated molecular ion

Determinations were made on the broccoli extract that was spiked at 100 ng/mL and analyzed using the 5×15 method.

Conclusions

The Agilent 7200 GC/Q-TOF was used to screen QuEChERS extracts of six fruit and vegetable samples that were spiked with 93 pesticides at two different levels, 10 ng/mL and 100 ng/mL. The screening procedure used Agilent MassHunter Qualitative Analysis Software (B.07.00) with the All Ions workflow together with the new Agilent GC/Q-TOF Pesticides PCDL. A 20-minute GC method using a 5×15 mid-column backflushing configuration was compared to a 40-minute 15×15 midcolumn backflushing method to see if the longer run time would result in more pesticide identifications. There was virtually no difference between the two methods in the number of pesticides that were identifiable by the MassHunter All Ions approach. Both methods identified more than 97 % of the spiked pesticides at the 10 ng/mL level and more than 99 % at the 100 ng/mL level. Mass accuracy of the TOF was better than 2 ppm when the S/N of the molecular ion was > 10. Retrospective data analysis is possible because the TOF acquires full spectra all of the time.

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