GC/Q-TOF MS Surveillance of Pesticides in Food

A Combined Workflow for Quantitative and Qualitative Screening of Pesticides using the Agilent MassHunter GC/Q-TOF Pesticide Personal Compound Database and Library

Application Note

Food Safety

Authors

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Abstract

High resolution accurate mass GC/Q-TOF mass spectrometry has become an increasingly promising technique to routinely perform both quantitative and qualitative screening for a wide range of pesticide residues in food samples with a single injection. The Agilent 7200 Series high-resolution accurate mass GC/Q-TOF, together with Agilent MassHunter Software tools, and an updated Agilent MassHunter GC/Q-TOF Pesticides Personal Compound Database and Library (PCDL) offers pesticide surveillance laboratories a combined workflow to achieve:

- Quantitative screening for pesticides whose standards will be used for calibration of response when running the analysis to perform comprehensive multilevel calibration or fast quantitation.
- Qualitative (suspect) screening against the PCDL for those pesticides whose standards will not be used when running the analysis for reasons of availability, cost, or likelihood of occurrence.

Agilent Technologies

Six different organic food extracts were prepared using the QuEChERS methods, and spiked with a mixture of 120 pesticides at multiple concentration levels (ng/mL). A midcolumn backflushing GC configuration provided excellent stability and precision of results. Six levels of matrix-matched calibration was demonstrated, with the majority of pesticides yielding a linear calibration curve fitting coefficient (R^2) of ≥ 0.99 from 5 to 200 ng/mL. Fast quantitative screening of 10 ng/mL spiking levels permitted quantification of more than 117 pesticides within a variation range of ±20 % in all food extracts. The same pesticide mixture was also used to evaluate a qualitative screening approach in which over 116 pesticides at spiking level of 10 ng/mL were identified in all studied food matrices. The intention was to show that laboratories using GC/Q-TOF for pesticide surveillance in food can flexibly choose which pesticides to quantify, and which pesticides can be screened qualitatively with a view to subsequent precise quantitation based on need.

Introduction

Monitoring pesticide residues is crucial to ensure a safe food supply. More than 1,000 pesticides are in use today, and the number continues to increase. Thus, there is a strong demand to screen a broad scope of pesticides, and determine whether residual levels of those pesticides are in compliance with the regulated maximum residue limits (MRLs). There is also increasing global emphasis on reliable validation of methods for pesticide screening as reflected by the guideline advised in the European Union (EU) through SANTE/11945/2015 [1].

For pesticides amenable to gas chromatography, triple quadrupole mass spec detection has been shown to be an effective way to perform precise quantitative screening with a wide scope of up to 400 pesticides. However, with increasing demands for a broader scope, some laboratories are questioning whether precise quantitation is required for rarely occurring pesticides. Calibration of GC/MS methods with wide scope can be time-consuming and expensive, and it is often necessary to create different calibrations for different matrices or sample prep procedures. Qualitative screening without extensive in-batch calibration is an attractive way to increase scope without increasing time and cost. If this strategy is implemented with untargeted full-spectrum detection, it can allow laboratories to look for things they previously might not have considered, or to add further compounds to the targets without extensive additional method development.

For pesticides detected in this manner, a subsequent precise quantitation will be required, and in some cases additional confirmation of identity. Either way, it makes sense to use technology that can perform a simple screen with inherently high confidence in identifications. This way, only reliable results move forward for extra work, and laboratory efficiency is kept high.

Gas chromatography coupled to high-resolution accurate mass quadrupole time-of-flight (GC/Q-TOF) MS serves as a fit-for-purpose solution to address these challenges. Benefitting from the full scan accurate mass spectra acquired for all GC-amenable pesticides, GC/Q-TOF in electron ionization (EI) mode can screen pesticides with very high identification confidence. Furthermore, high resolution data enable the use of a narrow mass window to be extracted if the accurate masses of characteristic ions from target pesticides are known. The resulting extracted ion chromatograms (EICs) from high resolution data suffer significantly less from interference by complex food matrices, and lower screening detection limits can be achieved. Therefore, a library containing accurate mass spectra of target pesticides is also essential to streamline analysis of high resolution mass spectrometry data when it comes to qualitative screening workflows.

For those compounds that a lab might still wish to quantitate on first injection, it is extremely useful to have verification of results from full scan accurate mass spectra, particularly since (unlike with triple quadrupoles) quantitation with a Q-TOF is typically performed in MS domain for the selectivity reasons explained above.

The qualitative screening of pesticides in various foodstuffs by GC/Q-TOF MS has been studied previously [2,3]. Compound identification results can be reviewed comprehensively through enhanced software compound verification features [4]. This application note looks at performance (compound by compound) for both quantitative and qualitative pesticide screening, using the Agilent 7200 Series GC/Q-TOF system, and an updated Agilent MassHunter GC/Q-TOF pesticide PCDL.

Experimental

Reagents and standards

All pesticide standards were obtained as multiple mix stock solutions (100 mg/L of each pesticide in acetonitrile) from ULTRA Scientific (North Kingstown, RI, USA). The mixture of 120 pesticide standards contains diversified pesticide categories including carbamates, organochlorines, organophosphorus, triazoles, pyrethroids, and so forth. The standard mix solution was further diluted to appropriate concentrations in acetonitrile before being spiked into food extracts. Acetonitrile was obtained from Honeywell (Muskegon, MI, USA). Ultrapure water was produced using a Milli-Q Integral system equipped with an LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (EMD Milllipore, Billerica, MA, USA).

Sample preparation

Organic apple, avocado, cucumber, peach, tomato, and salmon were obtained from a local grocery store. Ten grams of homogenized food samples (except peach) were extracted based on the buffered EN 15662 method using an Agilent QuEChERS Extraction Kit (p/n 5982-5650CH). The extraction of peach sample (15 g) followed the buffered AOAC 2007.1 method using an Agilent QuEChERS Extraction Kit (p/n 5982-5755CH). The fruit and vegetable samples were cleaned up with a dedicated Agilent Bond Elut QuEChERS Dispersive Kit (p/n 5982-5058 for AOAC method, p/n 5982-5056 for EN method). To remove the high-lipid content in avocado and salmon, the extracts were cleaned up with Agilent Bond Elut EMR—Liquid tubes (p/n 5982-1010) and Polish Pouch (p/n 5982-0102) with dry steps. The final extracts of food matrices were spiked with the mix of standards (120 pesticides) at various concentrations in a range of 5–200 ng/mL. Sample solutions spiked with pesticides were subsequently analyzed by GC/Q-TOF.

Instrumental analysis

All samples were analyzed in EI full-spectrum acquisition mode using an Agilent 7890B GC system coupled to an Agilent 7200B high resolution accurate mass Q-TOF system. The instrument was configured with a midcolumn backflush setup (Figure 1). The constant flow acquisition method was retention time locked (RTL) with chlorpyrifos-methyl to 9.143 minutes. Table 1 lists the conditions and parameters of GC/Q-TOF operation.

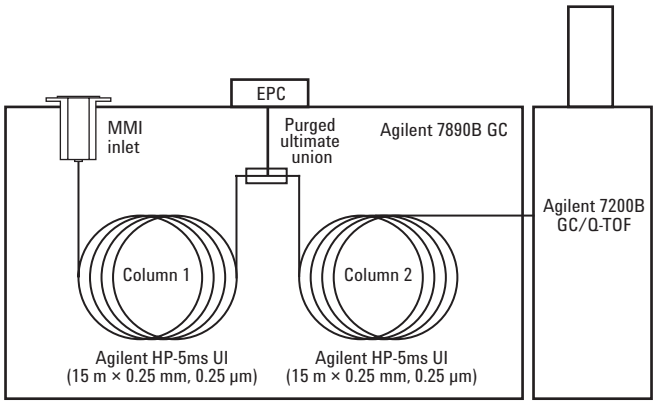

Figure 1. Agilent 7200 Series GC/Q-TOF System configuration depicting midcolumn backflush. The Agilent 7890B GC was coupled to an Agilent 7200B Q-TOF Mass Spectrometer.

Table 1. Agilent 7890B GC and Agilent 7200B GC/Q-TOF MS Conditions

GC	
Columns	Agilent HP-5ms UI, 15 m × 0.25 mm, 0.25 µm film (two each)
Carrier gas	Helium
Column 1 flow	1.0 mL/min
Column 2 flow	1.2 mL/min
Injection volume	2 µL cold splitless
Inlet liner	4 mm id Agilent Ultra Inert Liner Single Taper w wool (p/n 5190-2293)
MMI temperature program	60 °C for 0.2 minutes 600 °C/min to 300 °C, hold 330 °C, post run
Oven temperature program	60 °C for 1 minute 40 °C/min to 170 °C, 0 minutes 10 °C/min to 310 °C, 3 minutes
Run time	20.75 minutes
Backflush conditions	5 minutes (post run) 310 °C (oven temperature) 50 psi (aux EPC pressure), 2 psi (inlet pressure)
Retention time locking	Chlorpyrifos-methyl locked to 9.143 minutes
Transfer line temperature	280 °C
Q-TOF MS	
Ionization mode	EI
Source temperature	300 °C
Quadrupole temperature	180 °C
Mass range	45 to 550 *m/z*
Spectral acquisition rate	5 Hz, collecting both in centroid and profile modes
Acquisition mode	4 GHz high resolution

Data analysis

Data analysis relies on Agilent MassHunter software, Qualitative Analysis B.08 and Quantitative Analysis B.08. Agilent MassHunter GC/Q-TOF pesticide PCDL (p/n G3892AA) contains RTs, and full accurate mass EI spectra of 850+ compounds were used as input to set up data analysis. MassHunter offers an integrated workflow for pesticide screening from method development to routine implementation (Figure 2).

Results and Discussion

Quantitative screening

Evaluation of controlled sample data (for example, validation samples) helps to create quantitation methods with lowest interference. This is a necessary evaluation when developing a method to look at new food types, or when adding a new compound to a quant method, because it is difficult to predict appropriate quantifier and qualifier ions for all compounds of interest with no preknowledge of matrix background ion interferences [5]. In this study, food sample data (with pesticides spiked at 20 ng/mL) were used for this evaluation.

Quantitative screening methods developed in this manner can be used with comprehensive multiple-level calibration, or where desired with a one or two-level calibration if only a rapid estimation on whether a broad range of pesticides is in compliance with certain MRLs. Figure 3 shows matrix-matched calibration curves of three example pesticides in peach and avocado. The matrix-matched calibrations of peach and avocado samples with pesticides spiked at 5–200 ng/mL (triplicates) yielded excellent linearity ($R^2 \geq 0.99$) for over 105 pesticides in these two complex matrices.
To evaluate the accuracy of a two-level fast quantitative screening approach, we used sample data (triplicates) with each pesticide spiked at 5 and 20 ng/mL to set up calibration and quantitate pesticides at 10 ng/mL in food extracts. Figure 4 shows the accuracy of fast qualitative screening analyses. The number of pesticides quantified at 10 ng/mL within a deviation of ±20 % exceeds 117 in all matrices, with detailed results shown in Table 2.

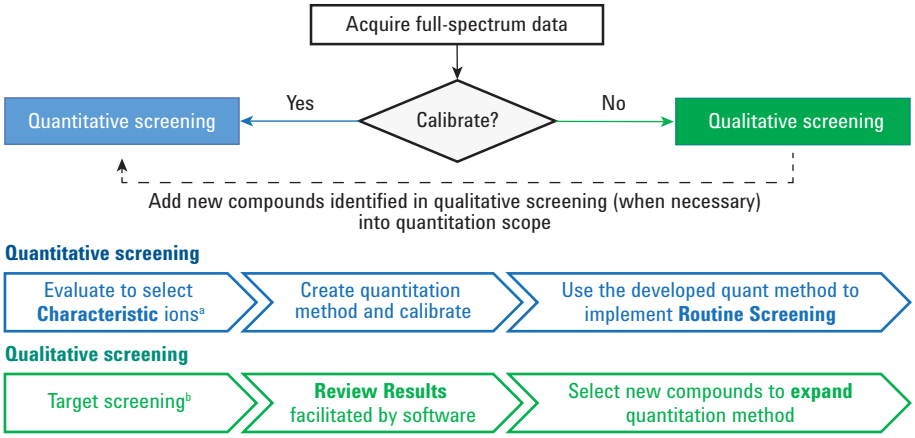

Figure 2. Workflow for quantitative and qualitative screening. [a]Evaluate is only applied to the method development stage with curated accurate mass spectra from the PCDL as an input for ion selection (a subset of compounds with standards for calibration). [b]Suspect screening against a PCDL subset including compounds without authentic standards.

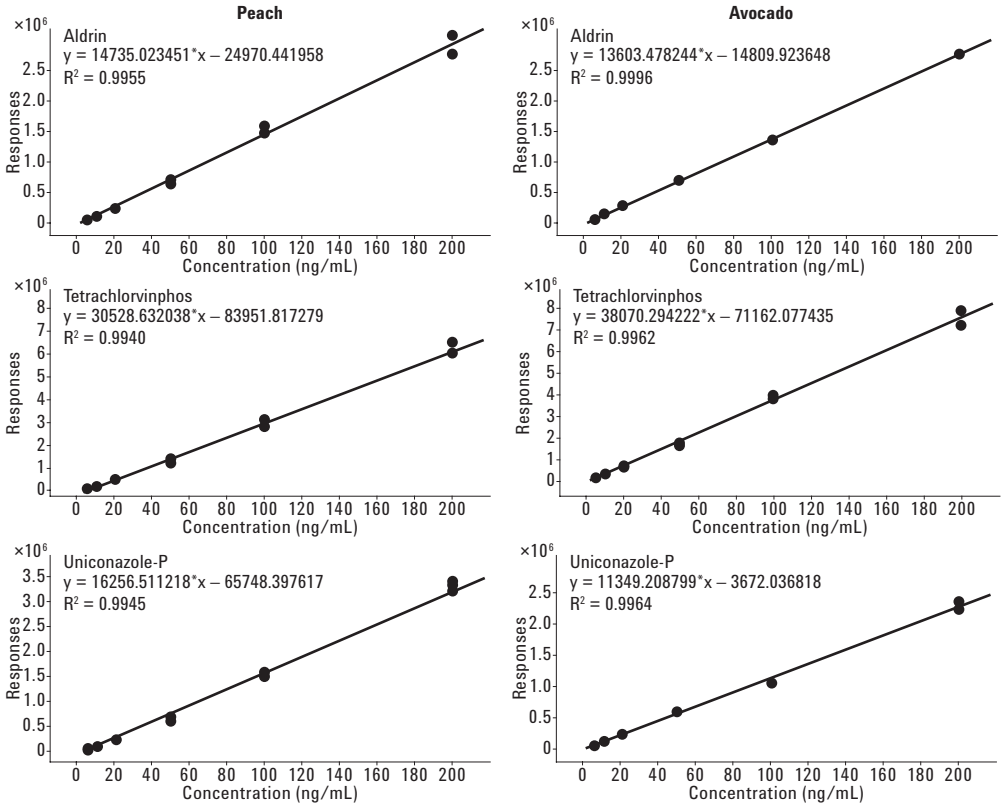

Figure 3. Matrix-matched calibration with concentrations of 5–200 ng/mL in peach and avocado.

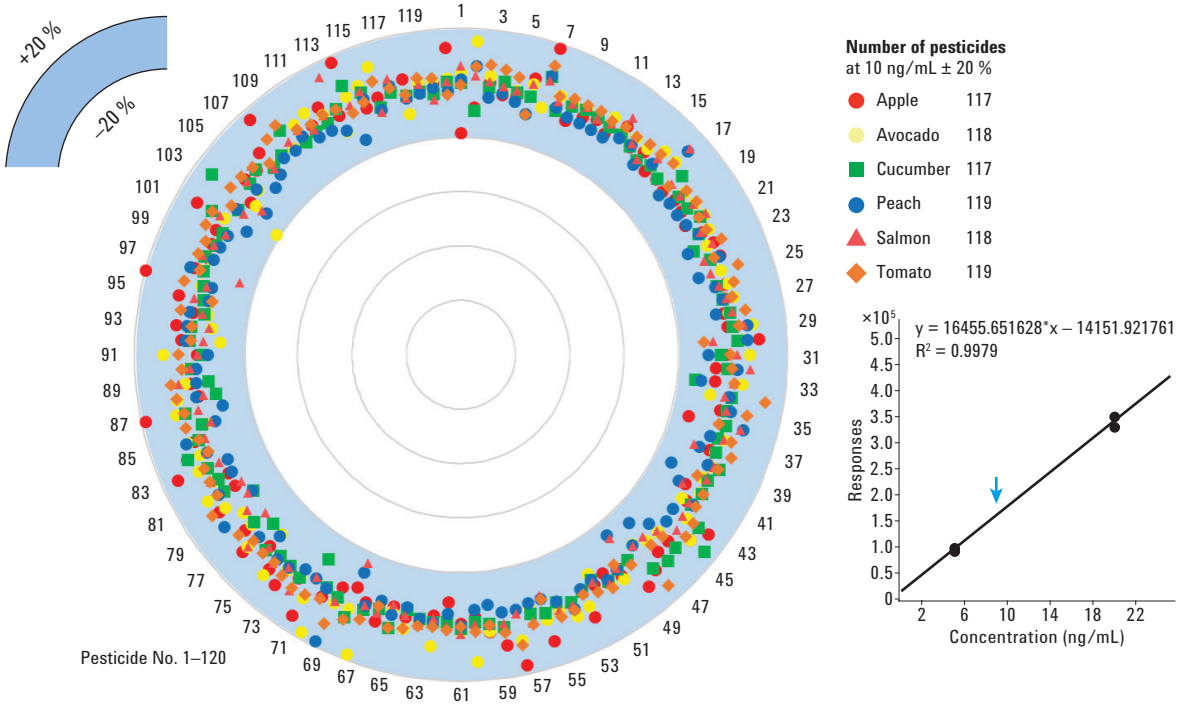

Figure 4. Fast quantitation of 10 ng/mL pesticides spiked in all food matrices. The inserted example plot shows quantitation result of cis-Permethrin in salmon based on a 2-level calibration.

Table 2. Results of Fast Quantitative Screening and Detectability of Qualitative Screening in Food Matrices

		Apple		Avocado		Cucumber		Peach		Salmon		Tomato	
No.	**Name**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**
1	1,2-Dibromo-3-chloropropane	8.1	○ ●	10.4	○ ●	10.0	○ ●	10.1	○ ●	10.6	○ ●	9.9	○ ●
2	Acephate	9.1	○ ●	11.5	○ ●	9.0	○ ●	10.6	○ ●	10.3	○ ●	10.6	○ ●
3	Acibenzolar-S-methyl (BTH)	10.1	○ ●	10.3	○ ●	9.9	○ ●	9.5	○ ●	10.1	○ ●	10.7	○ ●
4	Alachlor	9.7	○ ●	9.8	○ ●	10.2	○ ●	9.7	○ ●	10.0	○ ●	10.6	○ ●
5	Aldrin	9.7	○ ●	9.9	○ ●	9.9	○ ●	9.5	○ ●	9.9	○ ●	10.4	○ ●
6	Azoxystrobin	10.5	○ ●	10.3	○ ●	9.8	○ ●	9.2	○ ●	10.3	○ ●	9.1	●
7	Benalaxyl	11.8	○ ●	9.6	○ ●	10.8	○ ●	10.8	○ ●	10.6		11.2	○ ●
8	Benfluralin	9.6	○ ●	10.2	○ ●	9.6	○ ●	9.7	○ ●	10.0	○ ●	10.2	○ ●
9	BHC-*alpha*	9.4	○ ●	10.1	○ ●	10.0	○ ●	9.6	○ ●	10.1	○ ●	10.3	○ ●
10	BHC-*beta*	9.8	○ ●	10.2	○ ●	10.1	○ ●	9.6	○ ●	10.2	○ ●	10.3	○ ●
11	BHC-*delta*	10.0	○ ●	10.1	○ ●	10.1	○ ●	9.6	○ ●	10.0	○ ●	10.3	○ ●
12	Lindane	9.7	○ ●	10.4	○ ●	10.0	○ ●	9.5	○ ●	10.1	○ ●	10.2	○ ●
13	Bromacil	10.4	○ ●	10.1	○ ●	10.0	○ ●	9.9	○ ●	10.8	○ ●	10.3	○ ●
14	Bromophos	9.8	○ ●	10.2	○ ●	9.9	○ ●	10.0	○ ●	10.0	○ ●	10.3	○ ●
15	Butralin	10.0	○ ●	10.2	○ ●	9.6	○ ●	9.4	○ ●	9.7	○ ●	10.4	○ ●
16	Cadusafos	9.6	○ ●	10.6	○ ●	9.8	○ ●	9.9	○ ●	10.2	○ ●	10.4	○ ●
17	Carbofuran	9.7	○ ●	10.6	○	9.7	○ ●	11.2	○ ●	11.3	○ ●	10.5	○ ●
18	Chlorantraniliprole	9.5	○ ●	9.3	○ ●	10.4	○ ●	9.3	○ ●	10.0	●	9.8	○ ●
19	Chlordane-*cis*	9.7	○ ●	10.2	○ ●	9.9	○ ●	9.5	○ ●	10.0	○ ●	10.3	○ ●
20	Chlordane-*trans*	9.6	○ ●	10.2	○ ●	9.9	○ ●	9.4	○ ●	10.2	○ ●	10.3	○ ●
21	Chlordimeform	9.5	○ ●	10.3	○ ●	10.2	○ ●	9.6	○ ●	10.4	○ ●	10.1	○ ●
22	Chlorfenvinphos	9.9	○ ●	10.2	○ ●	9.8	○ ●	9.8	○ ●	9.9	○ ●	10.1	○ ●
23	Chlornitofen	10.2	○ ●	9.9	○ ●	9.4	○ ●	9.1	○ ●	10.1	○ ●	10.5	○ ●
24	Chlorobenzilate	10.2	○ ●	9.8	○ ●	9.8	○ ●	10.2	○ ●	9.6	○ ●	10.0	○ ●
25	Chlorothalonil	>12.0	○ ●	10.0	●	<8.0	○ ●	9.2	○ ●	9.7	●	10.7	○ ●
26	Chlorpyrifos	10.0	○ ●	10.2	○ ●	9.7	○ ●	9.7	○ ●	9.8	○ ●	10.4	○ ●
27	Chlorpyrifos-methyl	9.9	○ ●	10.2	○ ●	9.8	○ ●	9.8	○ ●	10.0	○ ●	10.2	○ ●
28	DCPA	9.8	○ ●	9.9	○ ●	10.0	○ ●	9.5	○ ●	10.1	○ ●	10.6	○ ●
29	Clomazone	9.8	○ ●	10.8	○ ●	10.0	○ ●	10.6	○ ●	10.5	○ ●	10.3	○ ●
30	Deltamethrin	11.0	○ ●	10.3	○ ●	10.2	●	10.5	○ ●	9.3	○ ●	9.0	●
31	Demeton-O	9.7	○ ●	10.6	○ ●	9.6	○ ●	9.1	○ ●	10.2	○ ●	9.9	○ ●
32	Demeton-S	9.4	○ ●	9.9	○ ●	9.8	○ ●	10.2	○ ●	10.7	○ ●	10.0	○ ●
33	Demeton-S-methyl	9.4	○ ●	10.4	○ ●	8.7	○ ●	8.9	○ ●	10.1	○ ●	10.1	○ ●

Quant – fast quantitation result of each pesticide at 10 (ng/mL). Average of triplicate injections is presented.

Qual – detectability by automated compound identification in qualitative screening.

○ = pesticide identified at 5 (ng/mL) spiking level

● = pesticide identified at 10 (ng/mL) spiking level

Blank cell = not detected

Table 2. Results of Fast Quantitative Screening and Detectability of Qualitative Screening in Food Matrices (Continued)

		Apple		Avocado		Cucumber		Peach		Salmon		Tomato	
No.	**Name**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**
34	Demeton-S-methylsulfone	>12.0	○ ●	9.3	●	10.1	○	10.0	○ ●	9.8	○ ●	11.3	○ ●
35	Diazinon	9.7	○ ●	10.1	○ ●	10.0	○ ●	10.1	○ ●	10.0	○ ●	10.8	○ ●
36	Dichlorvos	8.7	○ ●	10.1	○ ●	10.1	○ ●	10.7	○ ●	10.6	○ ●	9.8	○ ●
37	Dicloran (Dichloran)	9.9	○ ●	10.1	○ ●	9.9	○ ●	9.6	○ ●	10.2	○ ●	10.5	○ ●
38	Dieldrin	9.8	○ ●	10.0	○ ●	10.0	○ ●	9.4	○ ●	9.2	○ ●	10.6	○ ●
39	Dimethoate	9.9	○ ●	10.1	○ ●	10.4	○ ●	9.9	○ ●	10.2	○ ●	10.4	○ ●
40	Dimethomorph (E)	10.1	○ ●	9.9	○ ●	10.2	○ ●	8.7	○ ●	10.1	○ ●	9.8	○ ●
41	Diphenamid	9.5	○ ●	10.1	○ ●	10.2	○ ●	9.2	○ ●	9.4	○ ●	10.3	○ ●
42	Disulfoton	9.8	○ ●	9.7	○ ●	10.1	○ ●	10.2	○ ●	10.0	○ ●	10.3	○ ●
43	Disulfoton-sulfone	11.2	○ ●	10.3	○ ●	10.8	○ ●	9.6	○ ●	10.1	○ ●	10.6	○ ●
44	Endosulfan (*alpha* isomer)	10.8	○ ●	10.8	○ ●	11.5	○ ●	9.7	○ ●	10.2	○ ●	10.6	○ ●
45	Endosulfan (*beta* isomer)	10.2	○ ●	9.7	○ ●	10.6	○ ●	9.3	○ ●	9.7	○ ●	10.0	○ ●
46	Endosulfan sulfate	10.2	○ ●	9.7	○ ●	10.7	○ ●	8.7	○ ●	9.4	○ ●	9.9	○ ●
47	Endrin	10.7	○ ●	9.4	○ ●	10.6	○ ●	9.4	○ ●	9.5	○ ●	11.4	○ ●
48	EPN (Tsumaphos)	11.0	○ ●	9.7	○ ●	9.7	○ ●	8.5	○ ●	8.7	○ ●	10.0	○ ●
49	Ethion	10.0	○ ●	10.2	○ ●	9.7	○ ●	9.7	○ ●	9.7	○ ●	9.5	○ ●
50	Ethoprophos (Ethoprop)	9.6	○ ●	10.1	○ ●	9.9	○ ●	9.9	○ ●	10.0	○ ●	10.2	○ ●
51	Fenamiphos	9.8	○ ●	9.3	○ ●	9.5	○ ●	9.6	○ ●	9.7	○ ●	10.1	○ ●
52	Fenamiphos-sulfone	10.8	○ ●	10.4	○ ●	9.8	○ ●	9.6	○ ●			9.9	○ ●
53	Fenchlorphos (Ronnel)	10.0	○ ●	10.5	○ ●	9.9	○ ●	10.2	○ ●	10.1	○ ●	10.3	○ ●
54	Fenitrothion	10.1	○ ●	10.1	○ ●	9.7	○ ●	9.7	○ ●	10.1	○ ●	10.3	○ ●
55	Fenvalerate	11.1	●	10.2	○ ●	10.0	○ ●	9.4	●	9.3	●	9.5	○ ●
56	Fonofos	9.7	○ ●	9.6	○ ●	9.8	○ ●	9.4	○ ●	10.2	○ ●	10.3	○ ●
57	Formothion	11.7	○ ●	10.7	○ ●	<8.0	○ ●	9.6	○ ●	9.7	○ ●	10.9	○ ●
58	Heptachlor	10.8	○ ●	10.1	○ ●	10.1	○ ●	9.6	○ ●	10.0	○ ●	10.4	○ ●
59	heptachlor endo-epoxide isomer A	10.2	○ ●	10.2	○ ●	9.9	○ ●	9.2	○ ●	10.0	○ ●	10.0	○ ●
60	Heptachlor exo-epoxide isomer B	9.6	○ ●	11.3	○ ●	9.8	○ ●	9.4	○ ●	9.9	○ ●	10.2	○ ●
61	Heptenophos	9.9	○ ●	10.2	○ ●	10.1	○ ●	10.0	○ ●	10.3	○ ●	10.0	○ ●
62	HCB	9.1	○ ●	9.8	○ ●	9.8	○ ●	9.5	○ ●	10.0	○ ●	10.1	○ ●
63	Iprobenfos	9.6	○ ●	10.8	○ ●	9.9	○ ●	9.9	○ ●	9.7	○ ●	10.1	○ ●
64	Isazofos (Miral)	10.0	○ ●	10.0	○ ●	9.8	○ ●	9.8	○ ●	10.1	○ ●	10.1	○ ●
65	Isopropalin	9.9	○ ●	10.3	○ ●	9.6	○ ●	9.5	○ ●	9.8	○ ●	10.3	○ ●

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		Apple		Avocado		Cucumber		Peach		Salmon		Tomato	
No.	**Name**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**
66	Isoprothiolane	9.6	○ ●	9.8	○ ●	10.4	○ ●	9.9	○ ●	9.5	○ ●	10.4	○ ●
67	Leptophos	10.3	○ ●	9.5	○ ●	9.8	○ ●	9.5	○ ●	10.0	○ ●	9.7	○ ●
68	Malathion	9.7	○ ●	11.8	○ ●	10.0	○ ●	9.9	○ ●	9.9	○ ●	10.4	○ ●
69	Metalaxyl	9.4	○ ●	10.2	○ ●	10.6	○ ●	8.8	○ ●	8.4	○ ●	10.6	○ ●
70	Methamidophos	9.6	○ ●	10.2	○ ●	10.0	○ ●	11.8	○ ●	10.2	○ ●	11.1	○ ●
71	Methidathion	10.2	○ ●	11.8	○ ●	9.9	○ ●	9.9	○ ●	10.2	○ ●	10.4	○ ●
72	Methiocarb	11.4	○ ●	10.7	○ ●	8.9	○ ●	9.8	○ ●	9.7	○ ●	10.5	○ ●
73	Metolachlor	10.1	○ ●	10.1	○ ●	10.1	○ ●	9.7	○ ●	10.7	○ ●	10.6	○ ●
74	Mevinphos	10.9	○ ●	10.2	○ ●	10.0	○ ●	10.1	○ ●	10.1	○ ●	10.5	○ ●
75	Mexacarbate	10.6	○ ●	10.9	○ ●	9.9	○ ●	10.1	○ ●	10.3	○ ●	10.3	○ ●
76	Mirex	9.9	○ ●	9.3	○ ●	10.2	○ ●	9.4	○ ●	10.0	○ ●	10.3	○ ●
77	Monocrotophos	10.8	○ ●	10.6	○ ●	9.3	○ ●	10.0	○ ●	10.5	○ ●	10.2	○ ●
78	Myclobutanil	10.2	○ ●	9.9	○ ●	9.8	○ ●	>12.0	○ ●	9.2	○ ●	10.5	○ ●
79	Naled	>12.0	○ ●	9.9	○ ●			10.8		9.7	●		
80	Nitrofen	10.6	○ ●	10.3	○ ●	9.1	○ ●	9.2	○ ●	9.3	○ ●	10.9	○ ●
81	*o,p′*-DDD	9.6	○ ●	10.8	○ ●	10.1	○ ●	10.3	○ ●	9.3	○ ●	10.3	○ ●
82	*o,p′*-DDE	9.6	○ ●	10.2	○ ●	10.0	○ ●	9.5	○ ●	10.3	○ ●	10.3	○ ●
83	*o,p′*-DDT	11.4	○ ●	10.6	○ ●	10.2	○ ●	9.4	○ ●	9.7	○ ●	10.3	○ ●
84	Omethoate	10.8	○ ●	10.3	○ ●	10.8	○ ●	10.0	○ ●	10.0	○ ●	9.9	○ ●
85	*p,p′*-DDD	10.3	○ ●	10.6	○ ●	10.2	○ ●	10.6	○ ●	9.9	○ ●	10.1	○ ●
86	*p,p′*-DDE	9.8	○ ●	9.8	○ ●	9.9	○ ●	9.4	○ ●	9.5	○ ●	10.5	○ ●
87	*p,p′*-DDT	11.9	○ ●	10.7	○ ●	10.4	○ ●	9.0	○ ●	9.9	○ ●	10.4	○ ●
88	Parathion	9.8	○ ●	10.6	○ ●	9.1	○ ●	9.9	○ ●	9.6	○ ●	10.6	○ ●
89	Parathion-methyl	10.0	○ ●	10.2	○ ●	9.3	○ ●	9.8	○ ●	10.4	○ ●	10.7	○ ●
90	Penconazole	9.8	○ ●	10.0	○ ●	9.9	○ ●	9.8	○ ●	10.0	○ ●	10.3	○ ●
91	Pendimethalin	9.7	○ ●	11.0		9.6	○ ●	9.3	○ ●	9.7	○ ●	10.4	○ ●
92	Permethrin, *cis-*	10.3	○ ●	8.9	●	10.0	○ ●	10.1	○ ●	9.5	●	9.9	○ ●
93	Permethrin, *trans-*	10.5	○ ●	9.2	●	10.0	○ ●	10.1	○ ●	9.3	○ ●	9.9	○ ●
94	Phorate	10.0	○ ●	9.4	○ ●	9.6	○ ●	10.1	○ ●	9.6	○ ●	10.4	○ ●
95	Phosalone	10.6	○ ●	9.4	○ ●	9.7	○ ●	9.5	○ ●	10.0	○ ●	9.3	○ ●
96	Phosphamidon	12.0	○ ●	9.9	●	9.8	○ ●	9.5	○ ●	9.7	○ ●	10.5	○ ●
97	Piperonyl butoxide	10.3	○ ●	10.0	○ ●	10.0	○ ●	10.5	○ ●	8.6		10.1	○ ●
98	Pirimicarb	9.9	○ ●	9.8	○ ●	10.1	○ ●	9.7	○ ●	10.2	○ ●	10.3	○ ●

Quant – fast quantitation result of each pesticide at 10 (ng/mL). Average of triplicate injections is presented.

Qual – detectability by automated compound identification in qualitative screening.

○ = pesticide identified at 5 (ng/mL) spiking level

● = pesticide identified at 10 (ng/mL) spiking level

Blank cell = not detected

Table 2. Results of Fast Quantitative Screening and Detectability of Qualitative Screening in Food Matrices (Continued)

		Apple		Avocado		Cucumber		Peach		Salmon		Tomato	
No.	**Name**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**
99	Pirimiphos-methyl	10.0	○ ●	9.7	○ ●	9.9	○ ●	9.7	○ ●	9.9	○ ●	10.3	○ ●
100	Profenofos	10.1	○ ●	9.7	○ ●	9.8	○ ●	9.6	○ ●	9.7	○ ●	10.5	○ ●
101	Propoxur	11.2	○ ●	10.1	●	10.6	○ ●	9.1		10.2	○ ●	10.5	○ ●
102	Prothiofos	9.8	○ ●	8.1	○ ●	9.8	○ ●	9.8	○ ●	9.5	○ ●	10.1	○ ●
103	Pyrazophos	9.6	○ ●	9.3	○ ●	11.3	○ ●	8.9	○ ●	9.0	●	10.5	○ ●
104	Quinalphos	10.2	○ ●	9.6	○ ●	10.0	○ ●	9.7	○ ●	10.1	○ ●	10.4	○ ●
105	Quinomethionate	10.1	○ ●	>12	○ ●	10.2	○ ●	9.2	○ ●	10.4	○ ●	10.3	○ ●
106	Quizalofop-ethyl	10.5	○ ●	9.8	○ ●	9.7	○ ●	9.4	○ ●	9.7	○ ●	10.0	○ ●
107	Schradan (OMPA)	11.6	○ ●			9.9	○ ●	9.8	○ ●			10.6	○ ●
108	Tefluthrin	9.8	○ ●	9.8	○ ●	10.6	○ ●	9.7	○ ●	10.1	○ ●	10.9	○ ●
109	Terbufos	9.8	○ ●	10.5	○ ●	9.7	○ ●	9.9	○ ●	10.2	○ ●	10.2	○ ●
110	Terbufos sulfone	10.1	○ ●	10.6	○ ●	9.8	○ ●	9.5	○ ●	9.9	○ ●	10.1	○ ●
111	Tetrachlorvinphos	10.5	○ ●	10.2	○ ●	9.8	○ ●	9.5	○ ●	10.1	○ ●	10.2	○ ●
112	Tetradifon	9.9	○ ●	9.2	○ ●	10.2	○ ●	9.3	○ ●	11.5	○ ●	10.1	○ ●
113	Thiamethoxam	11.8	○ ●	10.4	○ ●	10.8	○ ●	8.7	○ ●	10.0	○ ●	9.7	○ ●
114	Thionazine	9.7	○ ●	10.6	○ ●	10.3	○ ●	10.1	○ ●	10.4	○ ●	9.9	○ ●
115	Triadimefon	10.0	○ ●	11.1	○ ●	9.6	○ ●	9.5	○ ●	9.4	○ ●	10.6	○ ●
116	Triadimenol	10.0	○ ●	10.2	○ ●	10.0	○ ●	10.2	○ ●	10.5	○ ●	10.1	○ ●
117	Triazophos	10.4	○ ●	9.1	○ ●	9.8	○ ●	9.7	○ ●	9.6	○ ●	9.8	○ ●
118	Trifluralin	9.7	○ ●	10.1	○ ●	9.8	○ ●	9.8	○ ●	10.1	○ ●	10.3	○ ●
119	Uniconazole-P	10.0	○ ●	10.1	○ ●	9.6	○ ●	9.9	○ ●	9.4	○ ●	10.2	○ ●
120	Vamidothion	11.3	●	10.2	○ ●	10.0		9.7	○ ●	10.0	○ ●	9.6	

Quant – fast quantitation result of each pesticide at 10 (ng/mL). Average of triplicate injections is presented.

Qual – detectability by automated compound identification in qualitative screening.

○ = pesticide identified at 5 (ng/mL) spiking level

● = pesticide identified at 10 (ng/mL) spiking level

Blank cell = not detected

Qualitative screening

Qualitative screening was set up to automatically extract up to six ions per pesticide from the PCDL, and to require at least two of these to produce EICs with a coelution score ≥70 and an S/N ≥3. If a compound passing these requirements had an RT within ±0.15 minutes, it was considered identified. The same mixture of 120 pesticides used in the quantitative assessment was used to evaluate the effectiveness of this approach.

Over 110 spiked pesticides at 5 ng/mL, and 116 in 10 ng/mL were identified in all investigated food matrices. Table 2 lists the detailed results for each pesticide. The latest Qualitative Analysis (Workflows) offers a comprehensive review of qualitative screening results, assisted by delta RT, EIC coelution, fragment ratio score, and mass accuracy to verify the compound identification with enhanced confidence. The methodology using software to review and verify the automated identification results on target analytes and unexpected compounds has been discussed elsewhere [4].

Retention time and response repeatability

The RTL backflushing capability ensured the retention time and response repeatability of the method. Six replicate injections of peach, avocado, and salmon samples spiked with 5 and 10 ng/mL pesticides were used to evaluate RT and response repeatability. The standard deviation (SD) of RT was less than 0.01 minutes for every identified pesticide. The response repeatability was demonstrated by the percentage relative standard deviation (%RSD) of identified pesticides at these low spike levels, as shown in Figure 5. Most of the pesticides yielded single digit %RSD. EICs are shown for two example compounds (Figure 5).

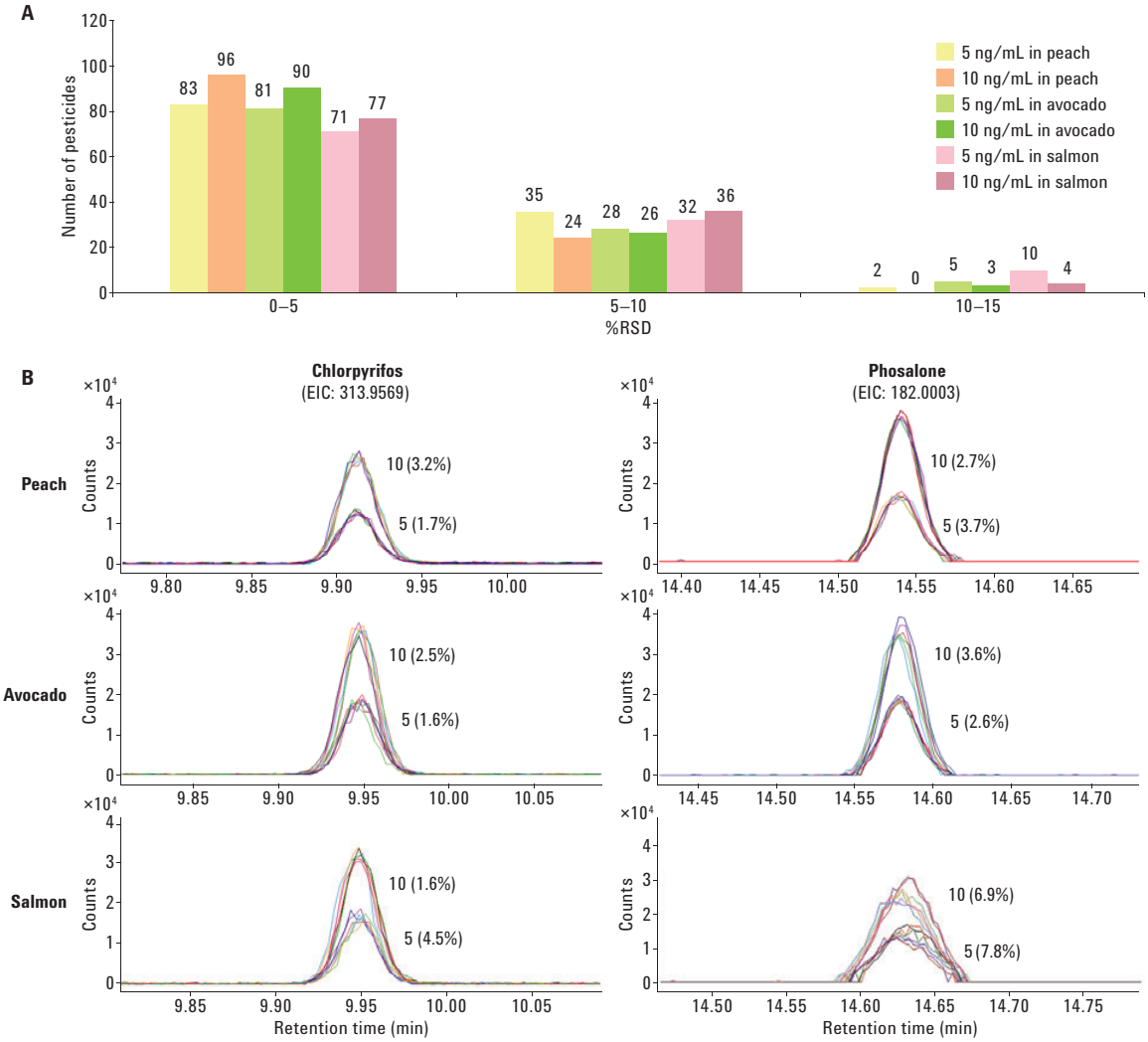

Figure 5. Response RSD% of pesticides in food matrices (A) and EICs of example compounds (B) from six replicate injections. EICs were extracted with a window of ±25 ppm. Numbers inserted in example EICs follow the format: concentration with the unit of ng/mL and (%RSD).

Backflushing also ensures long term system stability, and this was evaluated by a sequence of alternate injections of 5 and 10 ng/mL pesticides spiked in avocado, with 36 injections. Figure 6 shows the long term response stability of five example pesticides of various categories. These compounds also span a wide RT range, from mevinphos, which eluted at 5.6 minutes to deltamethrin at 18.12 minutes.

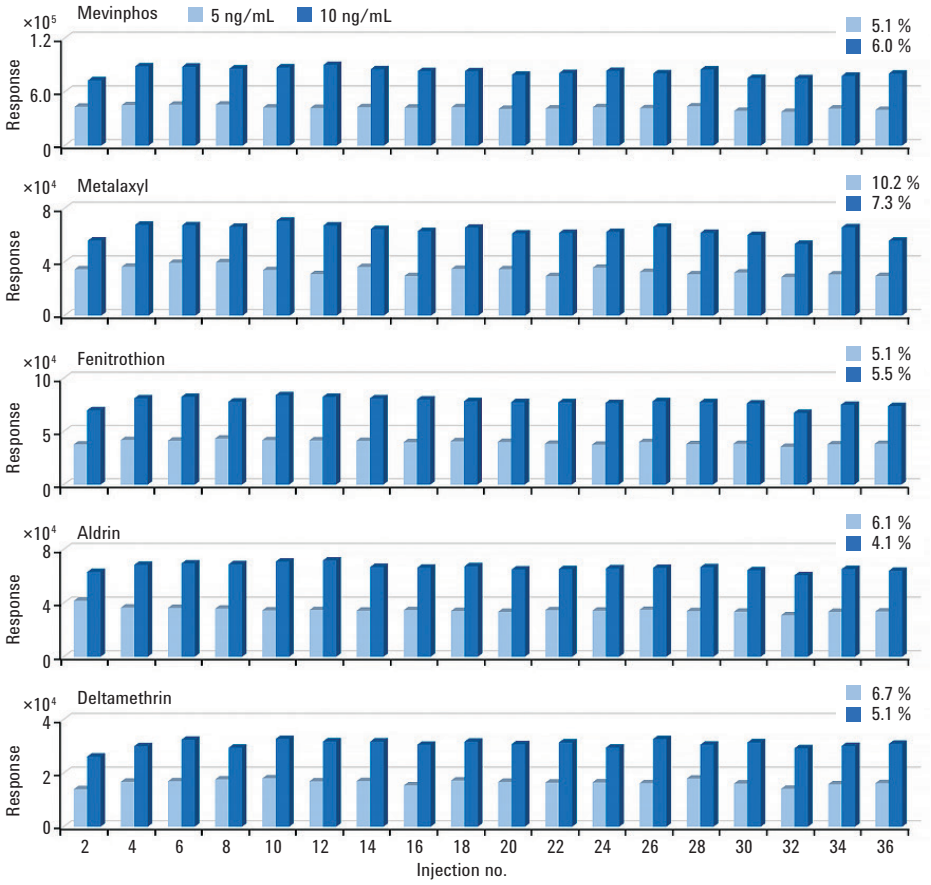

Figure 6. Long term response stability in avocado, with %RSD indicated in each plot.

Ion ratio

The relative intensity or ratio of selective ions is an important aspect for compound identification. The EI accurate mass GC/Q-TOF spectrum of each pesticide in the PCDL offers relative abundances of ion peaks to serve as an initial reference value of ion ratio. Over 90 % of identified pesticides possessed at least one pair of identified ions with a relative ion ratio within 30 % variance to that in the corresponding library spectrum. The relative ion ratio of almost all identified pesticides deviates <30 % when it is compared to the measured spectrum using matrix-matched calibration solutions. Figure 7 illustrates the stability of ion ratio by examples from different pesticide categories.

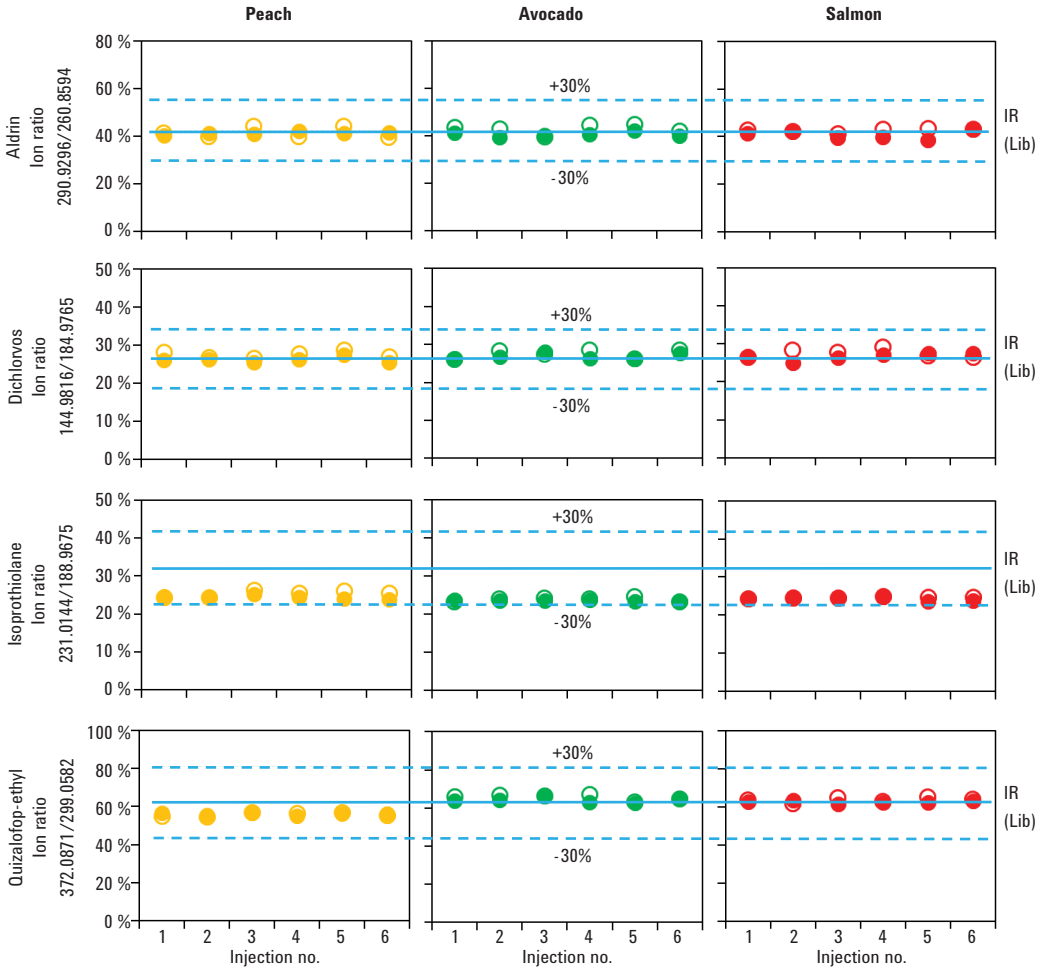

Figure 7. Ion ratio (IR) stability in food matrices.

Mass accuracy

The analysis of these pesticides by GC/Q-TOF provided excellent mass accuracy for all the investigated matrices (Table 3). The mass accuracy of each pesticide was calculated using the average spectrum extracted over its entire chromatographic peak. For those pesticides with mass accuracy >5 ppm, the majority had at least three ions identified with an S/N ≥ 3 for the corresponding EICs, and had relative ion ratio variance < 30 % compared to their reference spectra, thus meeting identification criteria in major guidelines.

Table 3. Summary of Mass Accuracy at 10 ng/mL in Food Matrices

Matrix	Number of pesticides (mass accuracy <5 ppm)
Apple	120
Avocado	108
Peach	117
Salmon	107
Tomato	118

Conclusion

Workflows for both quantitative and qualitative screening by high resolution accurate mass GC/Q-TOF has successfully been applied to screen pesticides in diverse food matrices. This illustrates that laboratories can use flexible strategies when performing wide scope screening, mixing both quantitative and qualitative approaches depending on need.

The confidence in identification of pesticides is enhanced by stable RT, repeatable response, and excellent mass accuracy as a result of using an RTL backflush method and high resolution accurate mass measurement. An increased calibration linearity range was also achieved with a new data processing algorithm in the latest Agilent MassHunter Quantitative software (with the SureMass feature). The GC/Q-TOF system and workflow together serve as a promising fit-for-purpose solution to routinely screen for a wide scope of pesticide residues in food samples.

References

1. SANTE/11945/2015. Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed (2015).
2. P. L. Wylie, J. Stevens, S. Nieto, Screening for More Than 740 Pesticide Residues in Food Using an Agilent GC/Q-TOF and an Exact Mass Pesticide Library, *Agilent Technologies Application Note*, publication number 5991-5633EN (**2015**).
3. A.R. Fernández-Alba, S. Uclés, J. Riener, Screening for Hundreds of Pesticide Residues Using a GC/Q-TOF with an Exact Mass Pesticide Database in Food, *Agilent Technologies Application Note*, publication number 5991-5894EN (**2015**).
4. K. Chen, J. Stevens, S. Nieto, GC/Q-TOF Screening of Pesticides in Food - Searching and Verification using Agilent MassHunter GC/Q-TOF Pesticide Personal Compound Database and Library, *Agilent Technologies Application Note*, publication number 5991-6884EN (**2016**).
5. S. J. Lehotay, Y. Sapozhnikova, H. G. J. Mol. "Current issues involving screening and identification of chemical contaminants in foods by mass spectrometry" *Trends Anal. Chem*. **60**, 62-75 (2015).

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