Application Note

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Contaminants Screening Using High-Resolution GC/Q-TOF and an Expanded Accurate Mass Library of Pesticides and Environmental Pollutants

Authors

Sofia Nieto,
Anastasia Andrianova,
Jessica Westland, Kai Chen,
and Bruce Quimby
Agilent Technologies, Inc.

Abstract

The use of high-resolution, accurate mass GC/Q-TOF for broad scope screening of pesticides and other contaminants in complex food matrices has been increasing over the past few years. The complex high resolution data coming from GC/Q-TOF can increase confidence for both screening and quantitative workflows but up until now it has been time consuming to leverage it's full value. The software described in this application note, simplifies the review of such data whilst maximizing its value, to allow labs to quantitate priority targets and reliably screen for many more suspects, all achieved simultaneously in one environment. The workflow also uses a recently updated GC/Q-TOF accurate mass library of pesticides and environmental contaminants.

Introduction

Testing for pesticide residues in food is essential in ensuring food safety. Screening for contaminants in food matrices requires high sensitivity to meet strict regulatory requirements for maximum residue levels (MRLs), and a comprehensive scope. One advantage of a high-resolution GC/Q-TOF system is its capability to screen for a virtually unlimited number of compounds in a single run, without compromising sensitivity. However, traditionally, the most tedious and time-consuming part of this approach is processing complex high-resolution data. Ideally, data processing software, used for this purpose, should be able to automate the multifaceted assessments possible with this type of data, so that compounds previously missed with other technologies can be found (that is, less false negatives). The user should then be presented with confident but easy to review identifications in positive samples, as well as reliably flagging aspects of the data when potential false positives require review. Such capability should be reliable both for priority compounds that are calibrated during a batch but also for suspect compounds screened purely from a personal compound database and library (PCDL). Finally, such software should also minimize data processing time for these functions and crucially be sufficiently reliable that user intervention is rarely required.

This Application Note describes a streamlined workflow for pesticides screening that is designed to comply with SANTE/11945/2015 guidelines,[1] while offering a high degree of flexibility for the data review process.

The workflow was demonstrated using strawberry extracts, since the USDA considers strawberry one of the most commonly contaminated foods.[2]

Experimental

Strawberry samples were extracted using the EN QuEChERS method with the use of a dSPE cleanup for general fruits and vegetables (part numbers 5982-6650 and 5982-5056). For more information, see the Agilent Application Note *GC/MSD Pesticide Screening in Strawberries at Tolerance Levels Using Library Searching of Deconvoluted Spectra*.[3]

The samples were separated using an Agilent 8890 GC with a 40 minute retention time locked (RTL) method using a 15 m × 15 m midcolumn backflush configuration (Figure 1), locked to chlorpyrifos-methyl at an RT of 18.111 minutes. The samples were analyzed on an Agilent 7250 GC/Q-TOF as well as an Agilent 5977B GC/MSD in full spectrum acquisition mode. Table 1 describes the conditions for GC/Q-TOF. Backflush within the method helped maintain consistent RTs, avoid carryover, extend column lifetime, and reduce source contamination. The experimental conditions for the 5977 GC/MSD were as described elsewhere.[3]

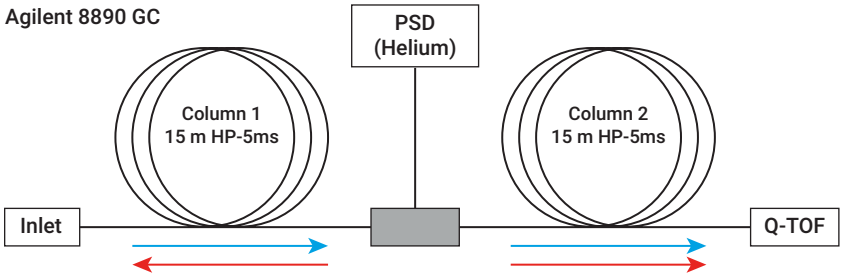


Figure 1. Midcolumn backflush configuration. The helium flowpath during the backflushing at the end of the run is depicted by red arrows. The pressure at the purged union is increased while the pressure at the inlet drops. This results in reversing the flow on the first column, and allows high boiling compounds to be removed through the split vent. The pneumatic switching device (PSD) is an Agilent 8890 GC pneumatic control module. The PSD provided backflush capability with significantly reduced carrier gas consumption due to the fixed purge flow.

Table 1. GC/Q-TOF acquisition parameters.

Parameter	Value
GC/Q-TOF	Agilent 7250 Q-TOF
GC	Agilent 8890 GC
Column	2 × Agilent J&W HP-5ms Ultra Inert, 15 m, 0.25 mm, 0.25 µm
Inlet	MMI, 4 mm UI liner single taper with wool
Injection Volume	1 µL
Injection Mode	Pulsed splitless
Inlet Temperature	280 °C
Oven Temperature Program	60 °C for 1 minute; 40 °C/min to 120 °C; 5 °C/min to 310 °C
Carrier Gas	Helium
Column 1 Flow	~1.2 mL/min
Column 2 Flow	~ 1.4 mL/min
Backflushing Conditions	5 minutes (post run), 310 °C (oven), 50 psi (AUX EPC pressure), 2 psi (inlet pressure)
Transfer Line Temperature	280 °C
Quadrupole Temperature	150 °C
Source Temperature	280 °C
Electron Energy	70 eV
Spectral Acquisition Rate	5 Hz
Mass Range	*m/z* 45 to 650

The GC/Q-TOF data were processed using the GC/Q-TOF Screening workflow available in Agilent MassHunter Quantitative Analysis software 10.1, and the accurate mass pesticide personal compound database and library (PCDL) of pesticides and environmental contaminants, containing over 1,000 unique compounds (Figure 2A). Briefly, the GC/Q-TOF data were imported into MassHunter Quantitative Analysis software and converted to the SureMass format for enhanced downstream data analysis speed and quality. A combined screening and quantitation method was automatically created from the GC/Q-TOF accurate mass library (Figure 3). The updated GC/Q-TOF PCDL now contains increased numbers of compounds corresponding to the following classes: PAHs, amines, organophosphates, phthalates, nitroanilines, and chloronitrobenzenes (Figure 3).

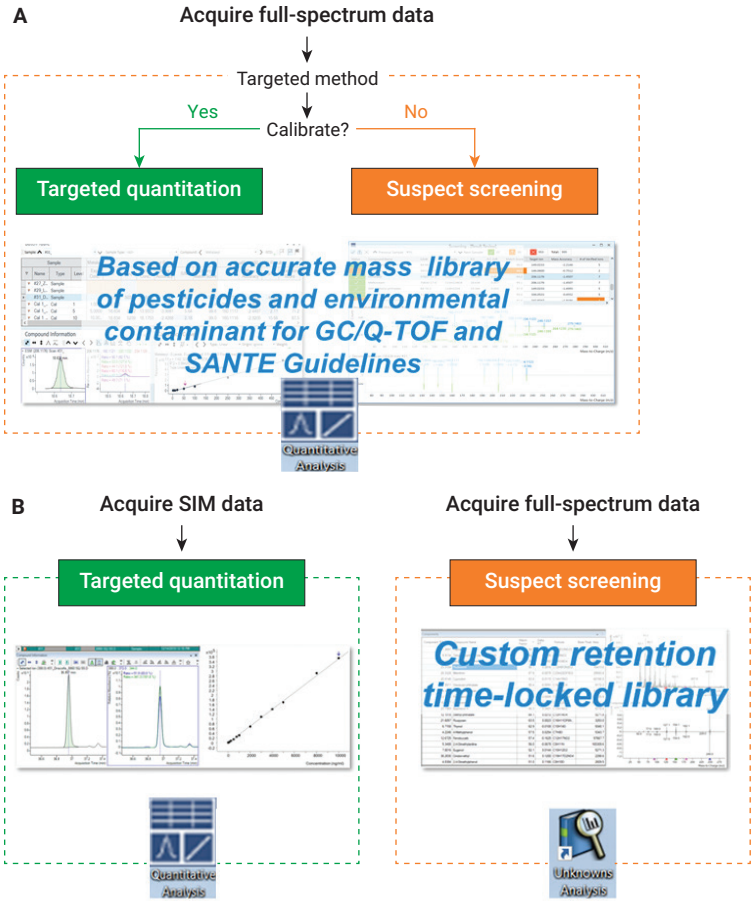


Figure 2. A) Combined contaminants screening and target quantitation workflow based on the pesticides and environmental contaminants PCDL for GC/Q-TOF. B) Screening and target quantitation workflows using custom retention time-locked unit mass libraries for GC/MSD.

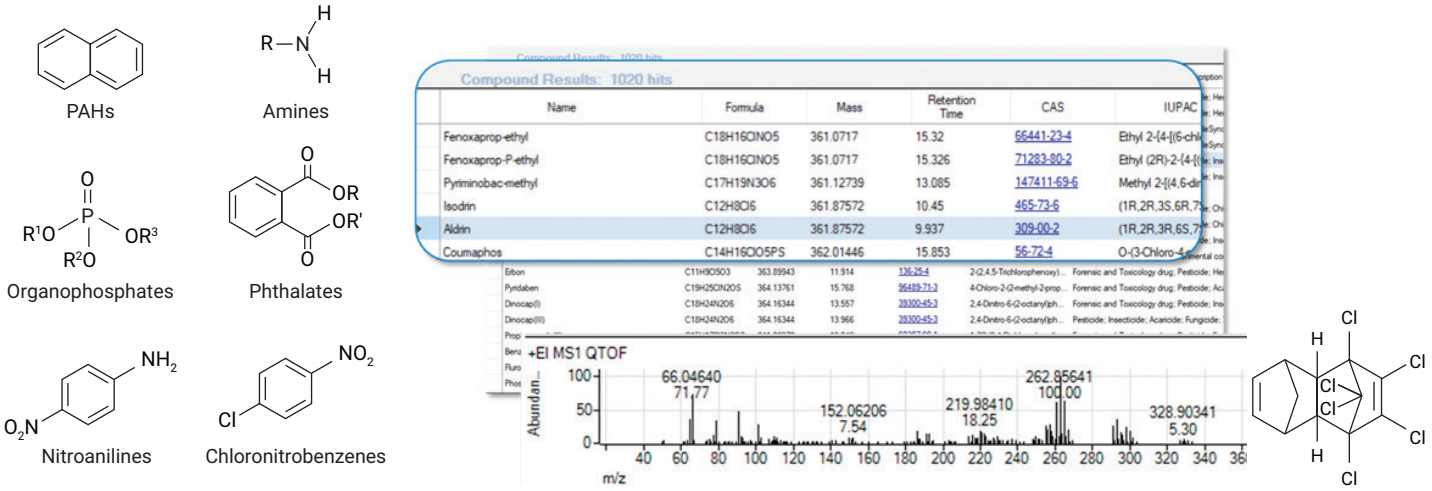


Figure 3. Updated GC/Q-TOF accurate mass library of pesticides and environmental contaminants containing accurate mass spectra for over 1,000 compounds.

Screening method parameters were set according to the SANTE guidelines, and further optimized to reduce the number of false positives and false negatives. The parameters included RT window, mass accuracy, coelution score, and library match score, among others. For example, mass accuracy was set to 5 ppm (in agreement with SANTE guidelines), and the RT window was set to 0.05 minutes. RT locking with backflush provides excellent RT precision and repeatability, and this setting can help reduce false positives. The library match score was set to 75. The latter setting has been optimized for this application, and appears to be one of the key parameters in eliminating false positives. For most of the confirmed compounds, the library match score was above 90. After applying the combined screening method, only a few marginal cases had to be reviewed manually to decide whether the compound was a true hit or not. These compounds are automatically highlighted in orange in the screener window.

The GC/MSD data were also processed using MassHunter Quantitative Analysis software 10.1 and MassHunter Unknowns Analysis with a customized unit mass pesticide library (Figure 2B).[3]

Results and discussion

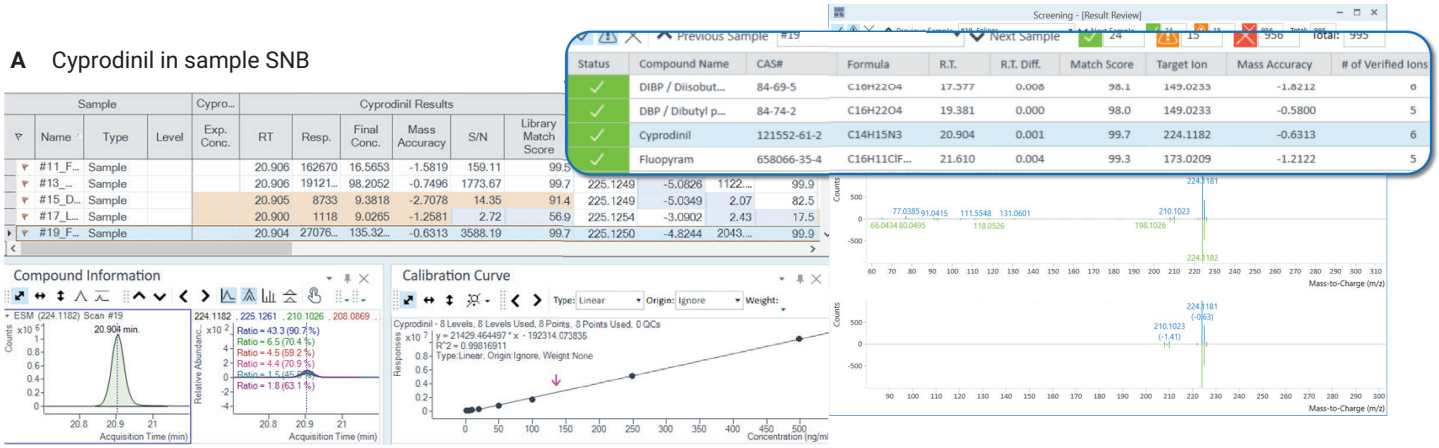
Suspect screening using the GC/Q-TOF

To test the GC/Q-TOF accurate mass screening workflow, 14 organic and nonorganic strawberry samples were obtained from different retail stores and farmer's markets in Northern California, and extracts were prepared as described above. A pooled sample of organic strawberry extracts, in which no pesticides were detected by a GC single quadrupole instrument, was spiked with 1 to 500 ppb of 40 priority pesticides typically applied when growing nonorganic strawberries.[4] This workflow (Figure 2A) was used simultaneously for quantitative analysis of our selected priority pesticides and for the quick suspect screening of the many other pesticides and environmental pollutants in the Agilent PCDL.

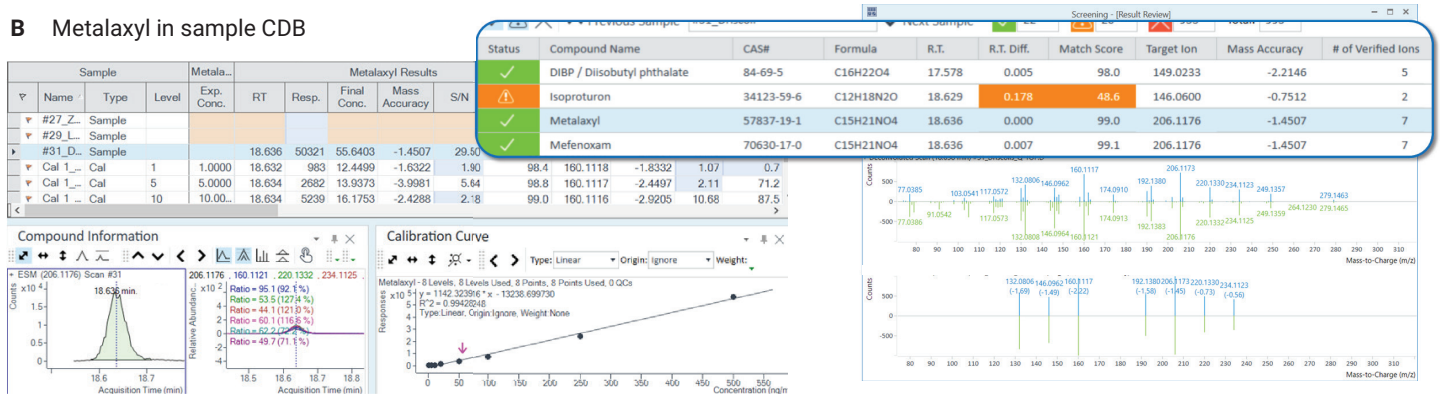
The suspect screening method was applied for all compounds present in the PCDL used to create the data analysis method. Whenever the calibration standard was present for an identified compound, the concentration was reported. This workflow for contaminants screening is significantly more efficient and streamlined compared to the previous workflow,[5] and combines a range of features in a single tool that covers target quantitation and suspect screening.

Figures 4A to 4C show a few examples of contaminants identified in the strawberry extract by GC/Q-TOF using the suspect screening workflow. Compounds with rich EI spectra present in an extract above trace levels are typically identified easily, with over 70% of selective ions verified, mass error within ~2 ppm, library match score in the high 90s, and a negligibly small RT difference (Figure 4A). A combination of poor library match score and high RT difference is presented to the reviewer to allow for possible isomers of a given library pesticide. However, usually, after manual examination, it was clear that this combination was very likely to be a false positive (Figures 4B and 4C). In fact, a lower library match score (a threshold for this score is a user-adjustable parameter) was usually a good indicator of a false positive. This power to differentiate good from bad identifications, even when there is no standard run to compare to, reflects the power of the 7250's high resolution and accurate mass performance allowing it to maintain accurate fragment ion ratios even in a complex matrix.

A Cyprodinil in sample SNB



B Metalaxyl in sample CDB



C Pyrimethanil in sample HSD

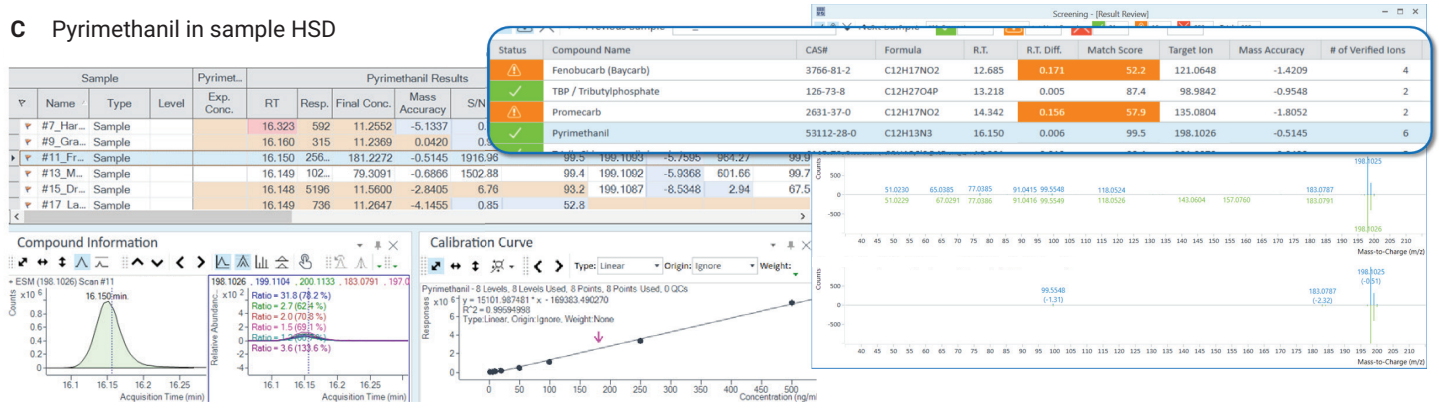


Figure 4. Screening results review.

Typically, 10 to 20 pesticides were identified in each nonorganic extract (Table 2). Flonicamid, pyrimethanil, cyprodinil, fluopyram, fludioxonil, captan, and bifenthrin were among the pesticides most frequently detected. Most organic extracts contained only few trace-level pesticides, including some legacy contaminants. The lowest pesticide concentrations detected in strawberry extracts were 1 ppb for pyrimethanil and 1.2 ppb for cyprodinil.

Table 2. Target quantitation and suspect screening results summary. Whenever a standard was available, the concentration of the contaminant in the strawberry extract is shown in the table, otherwise, a cell is labeled N/A. The first six samples are organic.

Compound	RT	Sample/concentration in extract, ppb													
		CVQ	ZTV	BRV	RST	RCH	NMT	CDB	NMP	DPR	NMJ	RSN	HSD	RTP	SNB
Isophorone	4.83	N/A		N/A				N/A							
Novaluron	8.28					117.8		119	122.8	101	159.8	182	17.2		
Diphenyl ether (Diphenyl oxide)	8.61									N/A	N/A				
Tetrahydrophthalimide, *cis*-1,2,3,6-	9.90			55.3		197.9		615*	893*	37.8	520*	54.9	715*	347.8	
Flonicamid	12.42			48.8		18		62.8	519.7	157	70.8	83.2		50.2	40.7
Pyrimethanil	16.16			1.2	11.4			<LOQ	79.3		233.5	1	181.2		
Diazinon (Dimpylate)	16.42					14.71									
Pentachloroaniline	17.33				N/A										
Chlorpyrifos-methyl	18.11				N/A									N/A	
Carbaryl	18.23					34.8									
Metalaxyl	18.64							55.6					28.9		<LOQ
Anthraquinone	19.56										N/A				
Malathion	19.64					36.2				44	<LOQ	39.7	3.5		
Tetraconazole	20.37							68.1	36.2		27.9				
Fthalide (Tetrachlorophthalide)	20.45							N/A							
Cyprodinil	20.91			1.6	1.2				111.8		179.6	1.2	11.2	20.6	153.7
Captan	21.43					151		3,294*	16,598*	58.7	5,188*	92.9	105.3	3,600*	
Fluopyram	21.62								N/A	N/A	N/A		N/A	N/A	N/A
Folpet	21.67								N/A						
Hexythiazox	21.98										46				
Flutriafol	22.75									17.3		18.2	21.6		
Fludioxonil	23.41			28.2					101.5		200.2		28.7	36.2	147.9
p,p'-DDE	23.44		<LOQ	<LOQ			<LOQ	<LOQ						<LOQ	<LOQ
Myclobutanil	23.73							2	1.6		18.2	18		1.6	127.6
Quinoxyfen	26.05							30.6				14.3	<LOQ		
Fenhexamid	26.20								90.2		242.5				41.8
Trifloxystrobin	26.50										20.1		21.2		52
Piperonyl butoxide	27.22					273.9	19.7								
Acetamiprid	27.99								<LOQ						
Fluxapyroxad	28.32														N/A
Bifenthrin	28.34								229.2	220		96.5	36.8	40.7	230.7
Bifenazate	28.35								52		44				
Etoxazole	28.62														45.7
Boscalid (Nicobifen)	33.36			N/A											
Azoxystrobin	37.00							<LOQ							

Verified automatically

Verified after review

* Calculated concentration value outside of calibration

Reducing false negatives

Using the accurate mass screening approach, GC/Q-TOF was generally able to identify a higher number of pesticides in each sample compared to the GC/MSD (Figure 5). The purple bars correspond to the number of pesticides detected in each sample by GC/MSD; the green and orange bars are those confirmed in the GC/Q-TOF screening. Note that, in organic strawberry extracts, where the levels of the detected pesticides were substantially lower compared to nonorganic extracts, the difference between the number of pesticides reported by GC/MSD and GC/Q-TOF was particularly evident.

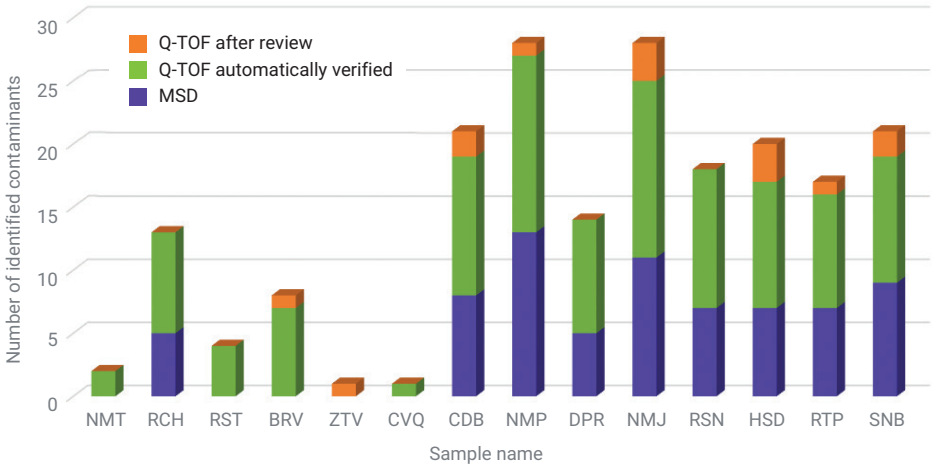


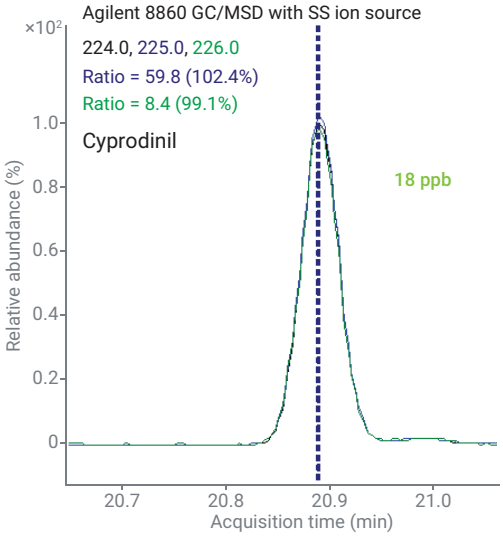
Figure 5. The number of identified contaminants in strawberry extracts, comparison between Agilent 7250 GC/Q-TOF and Agilent 5977B GC/MSD.

Eliminating false positives

The GC/Q-TOF screening workflow was also found to be less likely to report false positives, due to both the high-resolution, accurate mass capability of the instrument as well as multiple parameters of the screening software with easy-to-review capabilities for verification.

Often, both GC/MSD and GC/Q-TOF provided consistent identification as well as close concentration values. Figure 6 shows one of the typical examples of such a case, where cyprodinil was quantified by GC/MSD at concentrations of 18 ppb (stainless steel source) and 23 ppb (extractor source), while GC/Q-TOF reported 21 ppb for the same compound.

A



B Sample: RTP

Technique	Measured (ppb)
Agilent 8860 GC/MSD with SS ion source	18
Agilent 8890 GC/MSD with Extractor ion source	23
Agilent 8890 GC/Q-TOF	21

Figure 6. A) Overlay of quantifier and qualifier ions of cyprodinil (GC/MSD) and B) its calculated concentrations in sample RTP by GC/MSD and GC/Q-TOF.

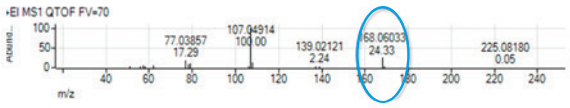
However, not all cases reported by a low resolution GC/MSD instrument were confirmed by the GC/Q-TOF. Figures 7 and 8 show one such example. Ethiofencarb was reported as a hit by GC/MSD but was not detected by the GC/Q-TOF screening workflow (Figure 7A). When accurate mass EIC (168.0603 ±20 ppm, Figure 7B) was extracted from the GC/Q-TOF data, no peak was detected either. When a Q-TOF spectrum was extracted from the chromatographic region where ethiofencarb was expected to elute, two accurate mass ions matching the *m/z* 168 unit were observed (Figure 7C), but neither ion's accurate *m/z* matched the theoretical *m/z* of ethiofencarb fragment 168.0603.

As shown in the screener window (Figure 8), the ion ratio of the compound accurate mass spectrum deviates noticeably from that of the accurate mass library spectrum of ethiofencarb. Such a discrepancy is also reflected in the low library match score of 20. This example provides clear evidence of how GC/Q-TOF is capable of reducing false positives that might be reported by other unit mass resolution techniques.

A

Technique	Measured (ppb)
Agilent 8860 GC/MSD with SS ion source	77
Agilent 8890 GC/MSD with Extractor ion source	470
Agilent 8890 GC/Q-TOF	ND

B



C

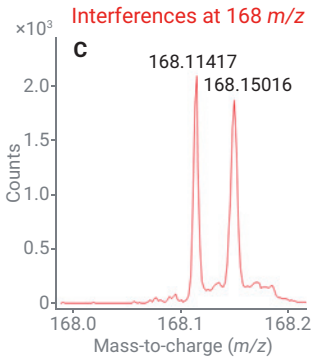


Figure 7. GC/Q-TOF, unlike GC/MSD, did not report a false positive ethiofencarb. A) Measured concentrations of ethiofencarb. B) The accurate mass ethiofencarb spectrum from the GC/Q-TOF PCDL. C) A fragment of the GC/Q-TOF spectrum from the chromatographic region corresponding to the ethiofencarb RT.

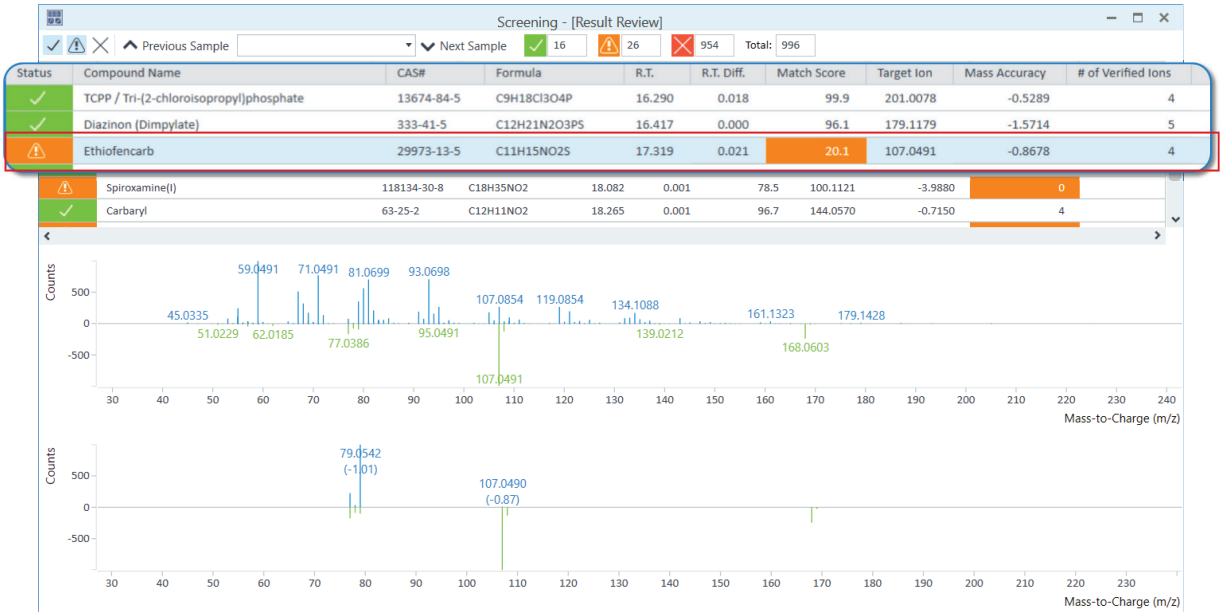


Figure 8. GC/Q-TOF correctly recognizes ethiofencarb as a false positive using a suspect screening workflow, which is evident from the low library match score as well as poor spectra matching.

Conclusion

A streamlined workflow for screening and quantitation of pesticides and environmental contaminants with high-resolution GC/Q-TOF and an accurate mass library has been demonstrated using organic and nonorganic strawberry extracts. Both quantitation and screening were performed with a single software, Agilent MassHunter Quantitative Analysis 10.1. This means that far more compounds than would be practical to calibrate for, were assessed.

The comparison of GC/Q-TOF and GC/MSD screening results demonstrated that the GC/Q-TOF screening workflow is less likely to generate false negatives and false positives compared to the unit mass resolution instrument, GC/MSD.

References

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