GC/Q-TOF Screening of Pesticides in Food

Searching and Verification using Agilent MassHunter GC/Q-TOF Pesticide Personal Compound Database and Library

Application Note

Food Safety

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Abstract

The use of high-resolution accurate mass GC/Q-TOF mass spectrometry is of growing interest for the development of multiresidue pesticide screening methods. The electron ionization (EI) full spectrum acquisition mode of the Agilent 7200 Series high-resolution accurate mass GC/Q-TOF system provides rich accurate mass information on fragment ions of analytes. Used with Agilent MassHunter Software tools and the updated Agilent MassHunter GC/Q-TOF Pesticides Personal Compound Database and Library (PCDL), it enables suspect screening for pesticides in complex food matrices. Coelution of extracted ions, relative abundance ratio of fragment ions, and mass error of each qualified ion are used to verify compound identifications with highest confidence. Accurate mass spectra of new pesticides of interest can also be added into a customized PCDL directly from the Agilent MassHunter Qualitative Software, allowing continuous expansion of the surveillance scheme.

Agilent Technologies

Introduction

The screening of a broad scope of pesticides in food commodities is one of the most demanding applications in modern food safety laboratories. High-resolution quadrupole time of flight (Q-TOF) instruments enable the measurement of accurate mass trace contaminants for low screening detection limits and improved spectral confirmation. Using the Agilent 7200 series GC/Q-TOF system, spectra can be collected in full acquisition mode. The benefit of this untargeted approach is that it captures spectral information of all GC amenable components for more comprehensive data analysis, particularly in cases when unexpected or new contaminants emerge. Thus, the use of high-resolution GC/Q-TOF instrumentation is of growing interest for the development of multiresidue pesticide screening methods with high identification confidence [1].

The analytical method and workflow using the 7200 Series GC/Q-TOF system to screen pesticides in various foodstuffs has been previously discussed [2,3]. In that work, it was shown that a PCDL can be implemented to perform suspect screening for a large scope of pesticides using Agilent MassHunter Software. The Agilent workflow chooses the most specific ions from each compound with MS spectra in the PCDL, and extracts them from the total ion chromatogram. The qualification of hits is based on a coelution score, which determines the covariance of extracted ions in the reference retention time (RT) window. These studies demonstrated that more than 95% of a wide range of spiked pesticides at various concentrations can be identified in various food matrices.

This application note focuses on the review and verification of screening results of pesticides in food matrices using accurate mass spectral information. The enhanced Target/Suspect Screening workflow and the updated Agilent MassHunter GC/Q-TOF Pesticide Personal Compound Database and Library (PCDL) (p/n G3892AA), were used to perform suspect screening of a list of representative pesticides in a blend of food matrices. The RT difference, relative abundance ratio of characteristic fragment ions generated by electron ionization (EI), and the mass error of these ions were used to increase identification confidence. The GC/Q-TOF pesticide PCDL can be customized. This customization allows users to add new compounds into the PCDL, then add their own curated accurate mass spectra directly from Agilent MassHunter Qualitative Software.

Experimental

Instruments

This study was performed using an Agilent 7890B GC system coupled to an Agilent 7200B Q-TOF system. Figure 1 shows the instrument configuration, and Table 1 lists the instrument conditions. The GC operation enabled retention time locking (RTL) with a constant flow midcolumn backflushing full screening method.

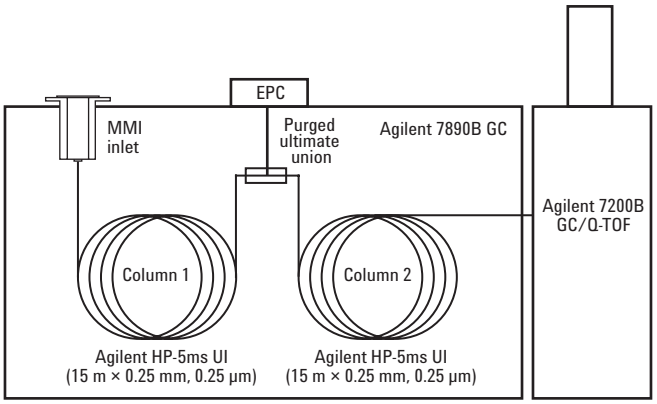

Figure 1. Agilent 7200 GC/Q-TOF System configuration depicting midcolumn backflush. The Agilent 7890B GC was coupled to an Agilent 7200B Q-TOF Mass Spectrometer.

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

GC	
Columns	Agilent HP-5ms UI, 15 m × 0.25 mm, 0.25 µm (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	2 mm id Ultra Inert Dimpled (p/n 5190-2296)
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	5 minutes (post run)
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

Sample preparation

An extract of blended food matrices was used for the evaluation in this study. The food samples were obtained from local markets. Extract preparation was based on the European Standard (EN) version of the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method using Agilent extraction salts and disperse kits, with extraction and cleanup steps as previously outlined [2]. The extract was spiked with the Agilent Pesticide Checkout Standard Mix (p/n 5190-0494) at a concentration of 10 ng/mL, then analyzed using the GC/Q-TOF system.

Results and Discussion

Compound identification using the Find by Fragments tool

The suspect screening was performed with the latest Agilent MassHunter GC/Q-TOF Pesticide PCDL (p/n G3892AA). The PCDL contains more than 850 compounds, each with high-resolution accurate mass spectra and reference RT for the RT locked chromatographic method used in this study. Data analysis for suspect screening relies on the Find by Fragments algorithm in the MassHunter Qualitative Analysis Software (B.08.00). The software picks accurate mass ions of each compound from the PCDL EI MS spectra to extract ion chromatograms around each analyte's reference RT. One ion is chosen as the reference ion. The software then evaluates the covariance of each fragment ion by calculating a Coelution Score (value 0–100) based on the intensity ratio between the fragment ion and the reference ion across the elution time range of the reference ion. Any RT shift difference in peak width or peak symmetry (fronting, tailing) will negatively impact the coelution score. To identify each compound (indicated in software as Qualified) requires a user-defined number of other fragment ions to meet coelution criteria to the reference ion (for example, coelution score ≥70). The user can also set the maximum allowed RT deviation (for example, ≤0.1 minutes) on both sides of the reference RT in the PCDL. Table 2 shows that all 17 pesticides in the pesticide checkout standard mix could be successfully identified in the blend of food matrices, at 10 ng/mL, in all six replicate injections. The RTs of these pesticides obtained by the RTL acquisition method match the reference values well within a window of ±0.03 minutes. Thus demonstrating that, when an RTL method is used for acquisition, the reference RTs in the PCDL serve as valuable analyte identifiers, in particular for isobaric compounds that can be chromatographically separated. Each of the 17 pesticides has at least two qualified ions with mass error no greater than 5 ppm, as indicated in Table 2.

*Table 2. Summary of Compound Identification in the Blend of Food Extracts**

					Qual ion (I)		Qual ion (II)	
Name	**Formula**	**Ref RT (min)**	**ΔRT (min)**	**Qual ions**	***m/z***	**Mass diff**	***m/z***	**Mass diff**
Dichlorvos	$C_4H_7Cl_2O_4P$	4.679	0.013	6	219.9464	3.6	184.9744	2.2
Mevinphos	$C_7H_{13}O_6P$	5.610	0.005	4	192.0198	2.8	164.0233	2.8
Ethalfluralin	$C_{13}H_{14}F_3N_3O_4$	7.139	0.005	5	316.0911	1.8	292.0548	2.7
Trifluralin	$C_{13}H_{16}F_3N_3O_4$	7.247	0.003	6	306.0709	2.3	290.0755	3.3
Atrazine	$C_8H_{14}ClN_5$	7.887	0.000	6	215.0932	3.7	202.068	3.2
Chlorpyrifos-methyl	$C_7H_7Cl_3NO_3P_S$	9.143	0.002	6	287.9236	3.8	285.9267	2.3
Heptachlor	$C_{10}H_5Cl_7$	9.339	0.000	6	336.8496	2.8	271.8106	2.9
Malathion	$C_{10}H_{19}O_6PS_2$	9.729	0.003	5	124.9824	3.0	99.0077	3.6
DDE, *p,p′*-	$C_{14}H_8Cl_4$	11.612	0.006	6	317.9349	3.9	315.9375	1.9
Dieldrin	$C_{12}H_8Cl_6O$	11.717	0.005	6	276.8722	1.3	260.8595	1.1
Hexazinone	$C_{12}H_{20}N_4O_2$	13.195	0.012	5	172.0896	5.0	171.0877	1.7
Propargite	$C_{19}H_{26}O_4S$	13.318	–0.001	4	173.0955	3.9	136.0835	2.9
Mirex	$C_{10}Cl_{12}$	14.874	0.005	6	269.8127	2.1	236.8409	2.1
Fenarimol	$C_{17}H_{12}Cl_2N_2O$	15.084	0.009	5	219.0317	2.9	138.9941	2.6
Coumaphos	$C_{14}H_{16}ClO_5PS$	15.853	0.012	5	362.0139	2.6	333.9822	2.4
Etofenprox	$C_{25}H_{28}O_3$	16.777	0.019	3	164.1155	3.0	163.1124	2.0
Deltamethrin	$C_{22}H_{19}Br_2NO_3$	18.117	0.023	3	252.9053	3.8	171.9882	4.2

* ΔRT and mass error reflect average values of replicated injections (n = 6)

Compound verification provides added confidence

An EI full spectrum acquisition high-resolution accurate mass measurement provides rich fragment ion information for each analyte. To take full advantage of this rich information for pesticide identification, the Agilent MassHunter Find by Fragments algorithm can generate a fragment ratio score based on matching the relative abundance ratios of qualified ions for each identified analyte with the reference spectra in the library. The identification confidence of each compound can be further increased by verifying the fragment ratio score, mass error of qualified ions, and the RT difference to the expected RT in the PCDL. Figure 2 shows an example where the identification of the pesticide atrazine has been verified.

The measured RT of this pesticide was 7.887 minutes, identical to the reference RT in the PCDL. The EIC plots of the fragment ions show good coelution (score 98.64), and the mass errors of all qualified ions (including both molecular and fragment ions) were <5 ppm. The characteristic ions of atrazine can clearly be distinguished in the raw spectrum, even in the presence of a complex background originating from the blend of food matrices. The fragment ratio score of over 99.62 (out of 100) indicates that the abundance ratios of all six identified ions are similar to those in the MS spectral library. The high score can be confirmed by a comparison between the reconstructed ion plot shown as part of the screening results for the food sample and the library spectrum of atrazine.

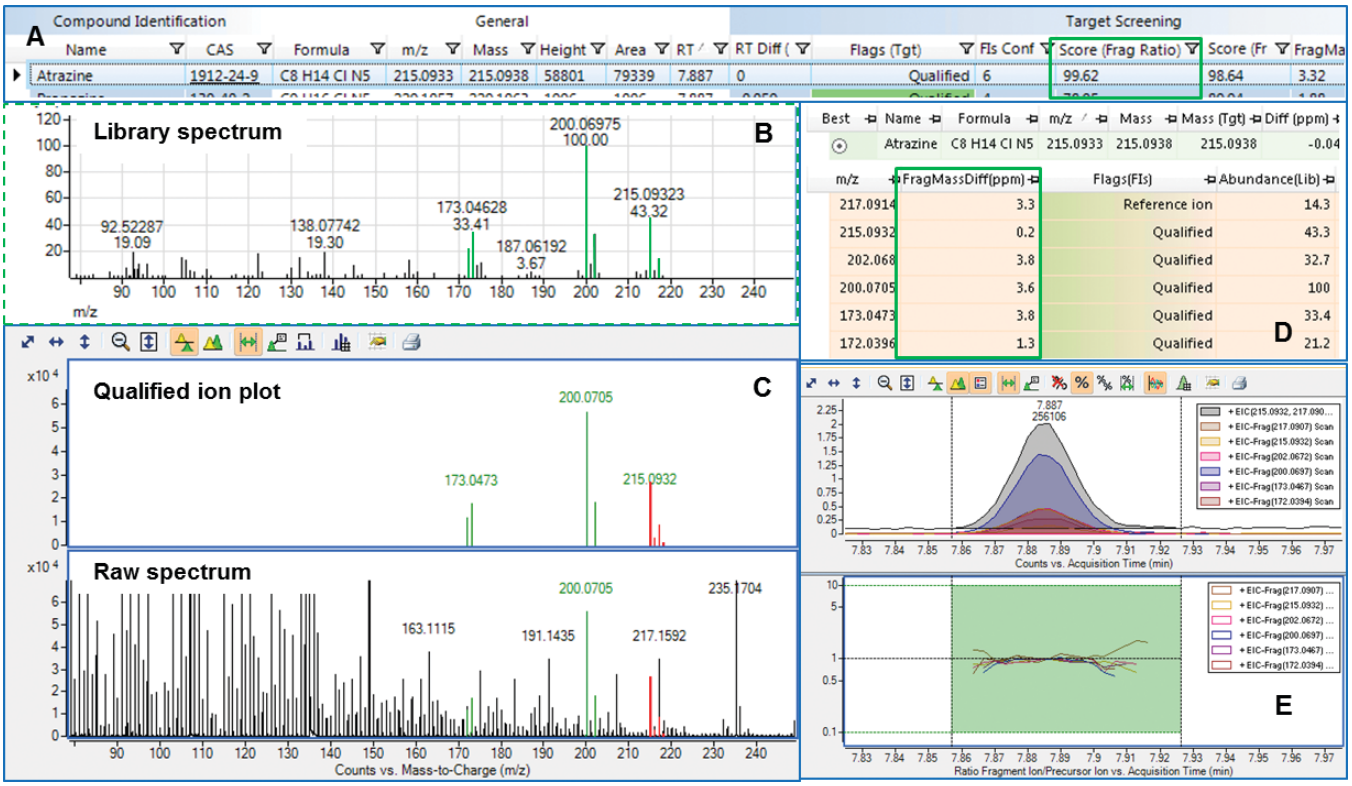

Figure 2. Identification results of atrazine were verified for increased identification confidence. The fragment ratio score of over 99 (A) reflects a similar relative abundance ratio of qualified ions between the library spectrum (B) and measured spectrum (C). The mass error of all qualified ions is <5 ppm (D), and EICs of these ions are well aligned (E).*

** Library spectra can be viewed in Agilent MassHunter PCDL Manager Software.*

Detection of the unexpected

Another advantage of the full spectrum acquisition mode of GC/Q-TOF is that the untargeted acquisition enables the detection of unexpected compounds. The identification of unexpected compounds can be comprehensively evaluated through fragment ratio score, mass error, and RT difference for verification purposes. Figure 3 shows that the pesticide *o,p′*-DDE was identified in the food sample with a trace signal, however, it was not present in the Pesticide Checkout Standard Mix compound list. The fragment ratio score of >80, together with good EIC alignment and small mass errors of each qualified ion (< 5 ppm), confirm the presence of the pesticide *o,p′*-DDE. To understand the origin of this compound, comparison of EIC results (*m/z* ion 245.9998) from the blank matrix with samples spiked with two different concentrations of the Pesticide Checkout Standard Mix was performed. The ion at *m/z* 245.9998, when combined with the reference RT for each isomer, is a unique identifier for the presence of both *o,p′*-DDE and *p,p′*-DDE. The absence of a peak in the EIC for this characteristic ion at a RT of 11.1 and 11.6 minutes shows that both compounds, *o,p′*-DDE and *p,p′*-DDE, are not present in the blank matrix. The EIC signal at a RT of 11.6 minutes confirms that *p,p′*-DDE is, as expected, present in the pesticide standard mix. It can also be seen that the isomer *o,p′*-DDE is also present (RT of 11.1 minutes) at a much lower concentration. Furthermore, a similar response ratio (approximate 1.5%) of *o,p′*-DDE to *p,p′*-DDE was observed at both concentration levels of the Pesticide Checkout Standard Mix. This confirmed that *o,p′*-DDE is a trace impurity in the standard.

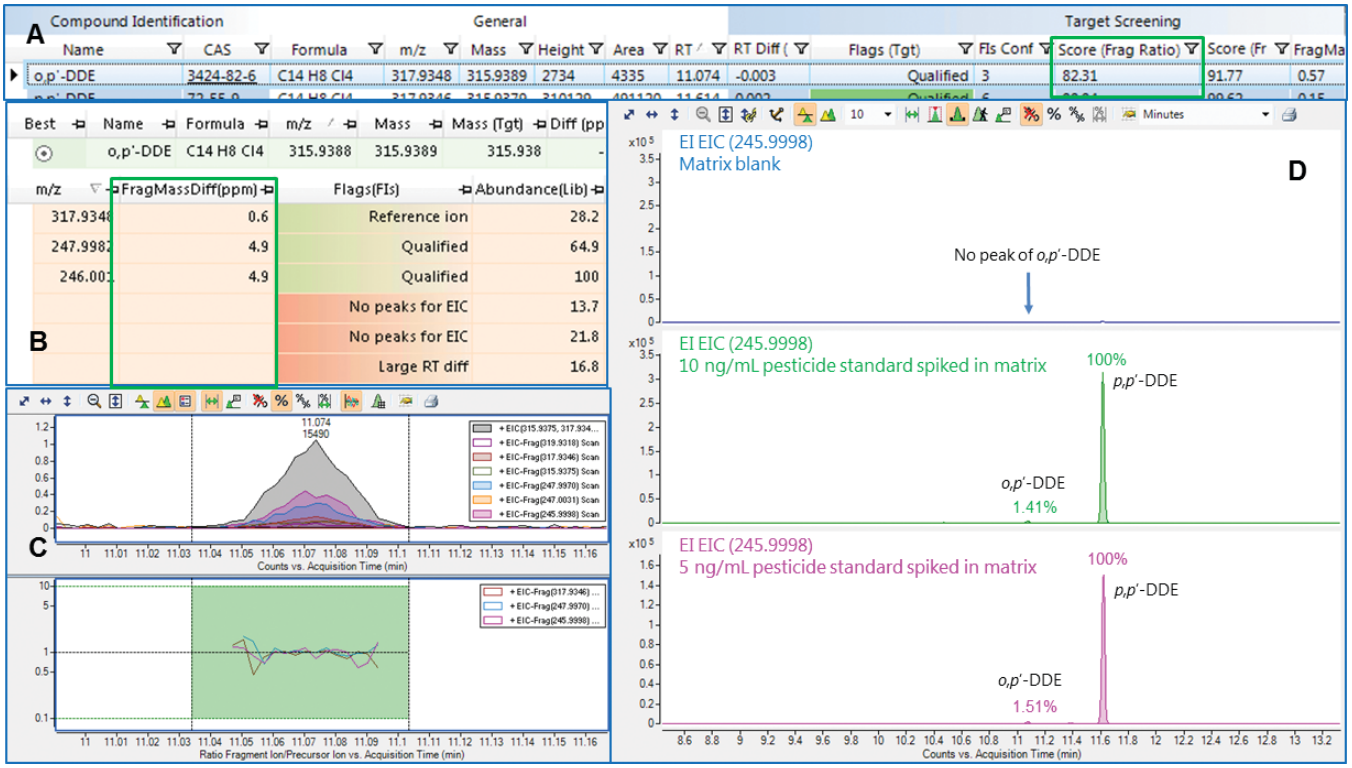

Figure 3. The unexpected peak of o,p′-DDE can be verified by fragment ratio score (A), mass error of qualified fragment ions (B) and alignment of EICs (C). No signal of o,p′-DDE was detected in a matrix blank (D top). Samples with the Pesticide Checkout Standard Mix spiked at two concentrations (D middle and bottom panels) show similar response ratios of o,p′-DDE compared to reference peak of p,p′-DDE, which confirms that the unexpected peak is a trace impurity of spiking standard.

The scalable PCDL

In full spectrum acquisition mode, the GC/Q-TOF system records the mass spectra of all GC amenable compounds present in each sample. The resulting data can be archived, then reanalyzed at a future date for any new analytes of interest. Large numbers of highly diverse pesticides are applied globally each year. Therefore, some pesticides may not be included in the current surveillance scheme or commercially available libraries. The MassHunter software provides a workflow that allows users to add curated EI GC/Q-TOF accurate mass spectra of new compounds into a custom PCDL. These new PCDL entries can also be used for retrospective analysis of historic data. During the curation process, the software determines formulas of fragment masses, and verifies if they are valid subformulas of the neutral formula of any given compound. The software then corrects the measured accurate mass values to the corresponding theoretical values. Ions for which no valid subformula can be determined will be considered as not originating from the compound, and eliminated. The curated spectra can then be sent to the designated PCDL using the Send Spectra to PCDL function as new spectral entries for the identified compound. Figure 4 illustrates the curation process of a pesticide EI GC/Q-TOF spectrum using an acquired accurate mass spectrum of cyprazine ($C_9H_{14}ClN_5$). The fragment ion mass peaks in the measured mass spectrum were automatically annotated by the software.

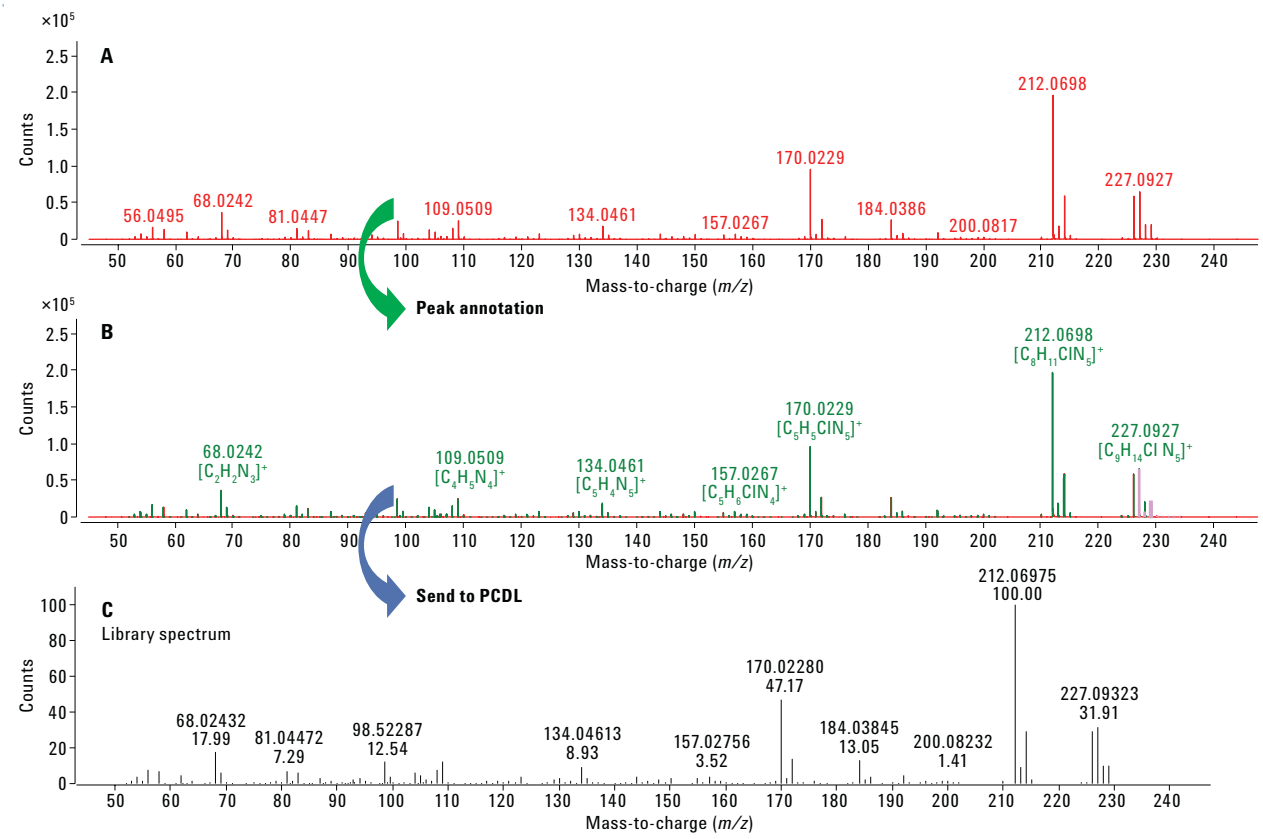

Figure 4. The MS spectrum for the pesticide cyprazine was curated and added to the PCDL using the Send Spectra to PCDL function in Agilent MassHunter Qualitative Analysis. Measured spectrum with background subtracted (A), fragment peak annotation based on its formula $C_9H_{15}ClN_5$ (B), and curated spectrum in PCDL (C) are depicted.

Conclusion

Pesticide residues at concentrations typically specified by regulatory agencies down to 10 ng/mL equivalent in food matrix extracts can be screened using the Agilent 7200 Series GC/Q-TOF system. This shows that this is a fit-for-purpose screening solution with the EI full spectrum acquisition accurate mass Agilent MassHunter GC/Q-TOF Pesticide PCDL and Agilent MassHunter Qualitative Analysis Software. The confidence in identification of pesticides can be greatly enhanced by reviewing the fragment ratio score, mass error, and RT difference. These are available in the compound results of the Find by Fragments algorithm. The PCDL can be customized, and spectra for new compounds of interest can be curated and added to the PCDL directly from the MassHunter Qualitative Analysis Software. These new PCDL entries can be used for the expansion of the surveillance scheme. While MassHunter Qualitative Analysis can be used to build the method and conduct a qualitative screening, the compound information can also easily be transferred to Quantitative Analysis for combined calibrated quantitation and confirmation.

References

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