Screening and Quantitation of 240 Pesticides in Difficult Food Matrixes Using the Agilent 6545 Q-TOF LC/MS System

Application Note

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Abstract

This Application Note describes an UHPLC/Q-TOF/MS method for screening 240 pesticides and pesticide metabolites in difficult food matrixes. The method benefits from increased chromatographic resolution using the Agilent 1290 Infinity UHPLC System and improved ionization capabilities with an Agilent Jet Stream ionization source. More importantly, the successful screening of analytes is accomplished by the innate sensitivity improvement of the Agilent 6545 Q-TOF LC/MS System and ion transmission tuning to facilitate the optimal ion transmission of small, fragile organic molecules. Targeted MS/MS acquisition was used for analyte quantitation and confirmation. Black tea matrix was chosen for its complexity, and avocado matrix was chosen to represent food commodities with high lipid content.

Our results demonstrate that the improved detection of small organic molecules by the 6545 Q-TOF LC/MS System allows the screening and quantitation of most of the targeted pesticides below the maximum residue limits (MRLs) specified by the European Commission regulations.

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Introduction

Pesticide residue screening in food products is one of the most important and most demanding applications in food safety. There are more than 1,000 pesticides and pesticide metabolites which can be present in food. European Commission regulation (EC) 396/2005 and its annexes set maximum residue limits (MRLs) for more than 170,000 matrix-pesticide combinations[1]. Similar regulations are in place in other regions[2]. Accurate mass LC/MS in food safety applications permits the detection of a large number of analytes. This is especially important for some metabolites where chemical standards are hard to obtain, and the development of a triple quadrupole method is rendered difficult. Accurate mass LC/MS method setup is relatively easy and can be accomplished without knowing the retention time or fragmentations. This is especially important for a commercial testing lab to increase the scope of testing and throughput.

A typical workflow in accurate mass LC/MS includes the screening and quantitation of regulated pesticide residues by using MS domain data. Mass accuracy, isotopic abundance, isotopic spacing, and adduct pattern are used to verify positives. Quite often, retention time is also considered a critical factor for compound matching. Using a comprehensive personal compound database and library (PCDL) search can disclose a list of likely pesticide residues. The pesticide residues can be further confirmed by auto or targeted MS/MS with the application of appropriate collision energy for fragmentation, and searched against the PCDL. This is of critical importance to rule out potential false positives in the context of complex matrixes such as QuEChERS extracts. The MS/MS information gives a higher level of identification confidence.

Most pesticides are analyzed with multiresidue methods covering hundreds of compounds applied to various food commodities. Therefore, fast and reliable analytical methods are required for pesticide identification at low concentrations in a broad range of food matrixes. Criteria for the identification of pesticide residues and requirements for method validation and quality control procedures for quantitation are specified in guidance documents such as SANCO/12571/2013[3].

This Application Note describes the development of an UHPLC/QTOF/MS method for the screening and quantitation of hundreds of pesticides in food samples. The method was developed using the Pesticide Comprehensive Test Mix (p/n 5190-0551). An Agilent 1290 Infinity UHPLC System was coupled to the Agilent 6545 Q-TOF LC/MS System. The acquisition was carried out in positive ion mode. Several modifications associated with the 6545 Q-TOF LC/MS System have resulted in higher analytical performance. Hardware improvements include a new:

- Slicer design with the option to operate under high sensitivity or high resolution mode
- High performance high voltage power supply that improves the mass resolution for higher molecular weight entities
- Enhanced gain shifted detector that provides much longer detector lifetime

More importantly, for the first time, Particle Swarm Optimization technology is commerically used to optimize mass spectrometers, resulting in much faster (4x) and more robust tuning of the instrument. The improvements in ion transmission for small molecules also results in mass accuracy enhancement below 100 *m/z*. A substantial 4x increase in signal response compared with the previous generation of the Agilent 6540 Q-TOF LC/MS System is achieved.

Experimental

Reagents and chemicals

All reagents and solvents were HPLC or LC/MS grade. Acetonitrile and methanol were purchased from Honeywell (Morristown, NJ, USA). Ultrapure water was produced using a Milli-Q Integral system equipped with a LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid was from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA) and ammonium formate solution (5 M) was from Agilent (p/n G1946-85021). Pesticides were included in the Agilent Pesticide Comprehensive Test Mix (p/n 5190-0551). A 10 ppm amount of pesticides working solution was used for spiking the QuEChERS extracts and preparing the calibration samples.

Sample preparation

Organic black tea and organic avocado were obtained from a local grocery store. Samples were extracted according to the official citrate-buffered QuEChERS protocol using Agilent BondElut QuEChERS kits (p/n 5982-5650)[4]. Ten grams of homogenized avocado and 2 g of black tea were weighed into 50-mL polypropylene tubes and extracted with 10 mL acetonitrile for 1 minute while shaking vigorously. The tea samples were wetted with 8 mL ultrapure water for 2 hours prior to extraction. Raw extracts were cleaned up by dispersive SPE with lipid removal for avocado (p/n 5982-5158) and with graphitized carbon black (GCB) (p/n 5982-5356H) for black tea. Final extracts were spiked in six relevant concentrations with the pesticides at 1 ng/g, 5 ng/g, 10 ng/g, 20 ng/g, 50 ng/g, and 100 ng/g. The matrix-matched standards were prepared before injection and were measured with five technical replicates at lower concentration levels.

Equipment and software

Separation was carried out using an Agilent 1290 Infinity UHPLC System consisting of:

- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1290 Infinity High Performance Autosampler (G4226A) and sample cooler (G1330B)
- Agilent 1290 Infinity Thermostatted Column compartment (G1316C)

The UHPLC system was coupled to an Agilent 6545 Q-TOF LC/MS System equipped with an Agilent Jet Stream electrospray dual ionization source. Agilent MassHunter workstation software was used for data acquisition (B.06.01, build 6.01.6145), qualitative analysis (B.07.00, build 7.0.7024.0) and quantitative analysis (B.07.00, build 7.0.457.0).

Methods

The 1290 Infinity UHPLC conditions are summarized in Table 1. Analysis was carried out with positive ion mode. Three microliters of the final extract were injected. Source parameters are optimized with a subset of 14 pesticides that represents the cohort of 240. The summary of the 6545 Q-TOF LC/MS System parameters are listed in Table 2.

Data were evaluated using the MassHunter Qualitative and Quantitative Analysis software. Calibration curves were generated using quadratic fitting, 1/x weighting, and including the origin.

Table 1. Agilent 1290 UHPLC parameters.

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C18 2.1 × 150 mm, 1.8 µm (p/n 959759-902)
Column temperature	45 °C
Injection volume	3 µL
sampler temperature	5 °C
Needle wash	10 seconds (80 % MEOH/20 % water)
Mobile phase	MPA: Water, 5 mM NH_4formate + 0.1 % formic acid MPB: MeOH, 5 mM NH_4formate + 0.1 % formic acid
Flow rate	0.4 mL/min
Gradient program	Time % B 0 5 1 5 4 50 17 100 20 100 20.1 5 Stop time 22 minutes Post time 1 minute

Table 2. Agilent 6545 Q-TOF LC/MS System parameters.

Parameter	Value
Mode	Positive; 4 GHz High Resolution
Tune	50–250 *m/z*
Drying gas temperature	150 °C
Drying gas flow	10 L/min
Sheath gas temperature	375 °C
Sheath gas flow	11 L/min
Nebulizer pressure	35 psi
Capillary voltage	3,500 V
Nozzle voltage	200 V
Fragmentor	125 V
Skimmer	45 V
Oct1 RF Vpp	750 V
Acq mass range	100–1,000 *m/z* (MS only)
Acq rate	3 spectra/s
Ref mass ions	121.050873, 922.009798

Results and Discussion

New tuning algorithm: particle swarm optimization tune (SWARM tune)

An artificial intelligent optimization algorithm that simulates social learning behavior in a bird flock was used to optimize the mass spectrometer with different criteria. Unlike classic Auto Tune, that generates one-size-fits-all optimization, the SWARM tune opens a new chapter for customizable instrument optimization based on application needs. The algorithm provides the possibility to optimize the ion transmission for fragile smaller molecules (for example, 50–250 *m/z*, and 50–750 *m/z*) depending on the user selection. In conjunction with the hardware improvement, the optimization dramatically improves signal response for small molecules. Moreover, acetonitrile-sodium adducts can be used as an additional calibrant to improve the mass accuracy in the 50–100 *m/z* range under the fragile ions tune. In addition to sensitivity and mass accuracy improvements associated with the 6545 Q-TOF LC/MS System, a substantial increase in tuning and calibration speed can be achieved. Comparing with the classic auto tune on the 6540 Q-TOF LC/MS System, the system tuning time was reduced by a factor of four. The new algorithm and methodology also let the system tune TOF and Quad simultaneously under both polarities.

Figure 1 shows a new user interface under the tune context.

Agilent 6545 Q-TOF LC/MS System performance

The changes in hardware and ion transmission tune result in a factor of ~4 signal response improvement by the 6545 Q-TOF LC/MS System compared to the 6540 Q-TOF LC/MS System when the high resolution slicer position is chosen. Figure 2 shows the overlay of 10 ppb thiabendazole chromatographs on a 6540 Q-TOF LC/MS System and a 6545 Q-TOF LC/MS System. Thiabendazole has a molecular weight of 201.3, and the optimized ion transmission of the *m/z* 50–250 range further improved the signal response. In addition to thiabendazole, the other 13 pesticides in this small study demonstrated a similar improvement trend.

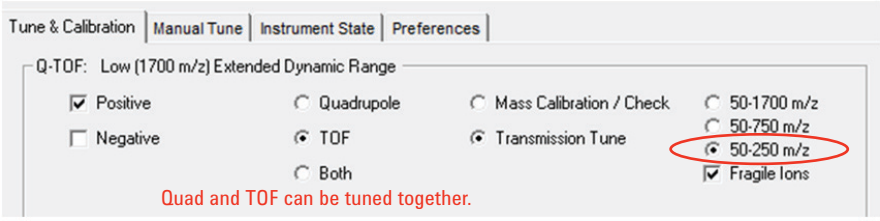

Figure 1. User interface under the tune context: the SWARM tune is implemented and defines optimal conditions for ion transmission based on application needs.

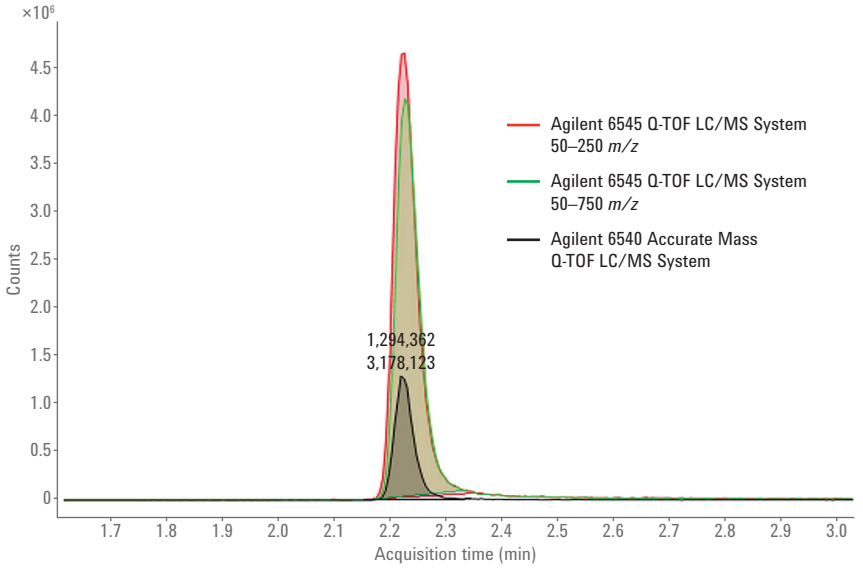

Figure 2. Exemplary chromatogram of signal response of 10 ppb thiabendazole in acetonitrile on an Agilent 6540 Q-TOF LC/MS System and an Agilent 6545 Q-TOF LC/MS System. The optimized ion transmission at 50–250 *m/z* provides further improvement compared with optimized ion transmission at 50–750 *m/z* for the analyte with *m/z* 202.3.

Similar improvement for a group of 240 pesticides has been observed in black tea and avocado matrixes at 10 ng/g spike concentration, corresponding to 2 ppb and 10 ppb respectively. For compounds that can be detected at these levels, the ratios are taken from the average of five replicates with each instrument. Histograms are shown in Figure 3. The improvement in sensitivity results in more compounds identified by the 6545 Q-TOF LC/MS System than by the 6540 Q-TOF LC/MS System. An example of 10 ng/g alanycarb spiked in black tea matrix is shown in Figure 4. The 6540 Q-TOF LC/MS System failed to detect the compound. The above results were obtained with a high resolution slicer position. It is expected that another two-fold signal response improvement can be achieved when a high sensitivity slicer position is chosen.

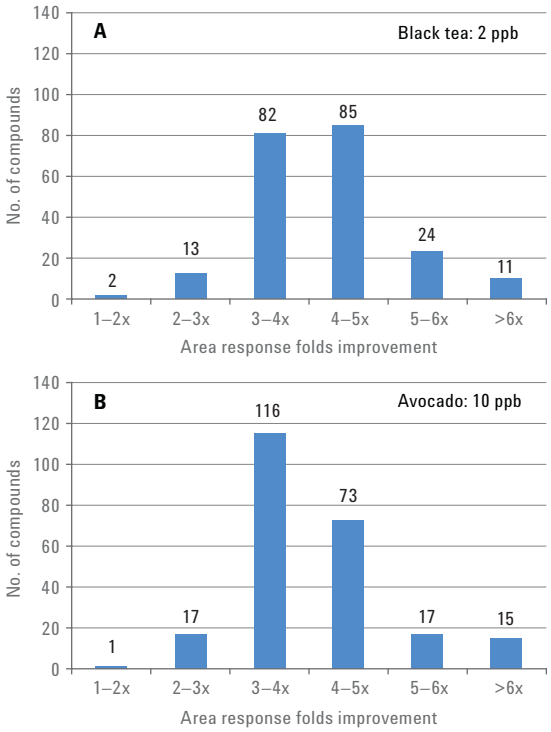

Figure 3. Signal response improvements of ~240 pesticides in black tea and avocado matrixes. The area response ratio of Agilent 6545 Q-TOF LC/MS System/Agilent 6540 Q-TOF LC/MS System was taking from the average of five replicates for each analyte. Another two-fold signal response improvement is expected when the high sensitivity slicer position is chosen.

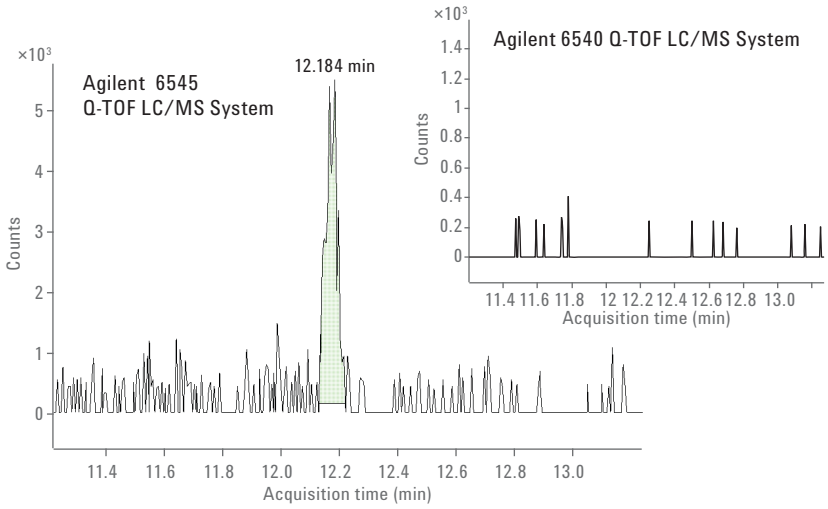

Figure 4. The Agilent 6545 Q-TOF LC/MS System sensitivity improvement benefits the analyte at the borderline of detection. Alanycarb could be detected by the Agilent 6545 Q-TOF LC/MS System, but not by the Agilent 6540 Q-TOF LC/MS System in black tea matrix at 10 ng/g spike level.

Compounds identification using Find by Formula (FbF)

The PCDL containing all spiked pesticides was generated from Agilent Pesticide PCDL (G3878-60003 MassHunter Personal Pesticide Library). The FbF search of analytes in matrixes against the PCDL was conducted, and some parameters settings of FbF algorithm are listed in Table 3. Software automatically generated an extracted ion chromatogram for the expected ion species of the target compounds in the accurate mass database. Peak spectra were extracted and the measured results were compared with calculated results to generate a matching score based on mass accuracy, isotopic abundance, and isotopic spacing[5].

Figure 5 shows, as an example, the methidathion chromatogram and peak spectrum in black tea matrix at 10 ng/g spike level, which corresponds to 2 ppb. At such a low concentration, and in one of the most complex matrixes, outstanding signal-to-noise (S/N) ratio can be obtained. Both proton adduct and ammonium adduct give excellent matching scores, with up to 400–500x coeluting background ions from the matrix. Consequently, the overall score for H^+ adduct is 99.66 out of 100 (mass accuracy 99.81 out of 100; isotopic abundance 99.74 out of 100; isotopic spacing 99.26 out of 100), and the overall score for ammonium adduct is 97.36 out of 100 (mass accuracy 99.29 out of 100, isotopic abundance 94 out of 100, isotopic spacing 97.52 out of 100). The mass error for major ion species is generally low, resulting in a matching score for most of the analytes > 90 out of 100.

Table 3. Parameter Settings for FbF Data Mining

Parameter	Value
Extraction data file	Profile for chromatographic and spectral extraction
Charge state	1
Isotopic model	Common organic molecule
PCDL	Subset of G3878CA
Adduct	$[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$
Mass tolerance	6 ppm
RT window	0.5 minutes
Mass accuracy weighting	100
Isotopic abundance weighting	60
Isotopic spacing weighting	50

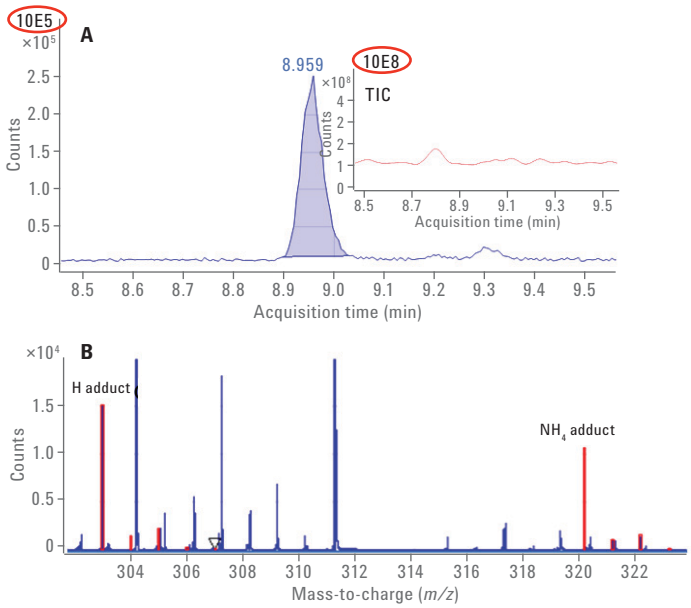

Figure 5. Compound chromatograph and peak spectrum by FbF algorithm for methidathion spiked into a QuEChERS extract of black tea at 10 ng/g. The identification of the compound with high confidence can be achieved even with 400–500x coeluting background ions.

The MS domain data can be used to obtain quantitative information for the spiked pesticides. The best ions for the quantitative method are derived from the compound results extracted from the MassHunter Qualitative Analysis Software using 20 ppb pesticides in neat solvent. The results are exported as a compound exchange file (.cef) that is used in quantitative analysis. Quantifier and qualifier are automatically selected from the observed adduct species and isotopic signals based on their relative abundance. All pesticides could be detected at 10 ng/g spike concentration in avocado matrix except propagite, which could not be detected in neat solvent at higher concentration, possibly due to degradation. We did not detect 20 compounds at 10 ng/g in black tea matrix due to the combination of 5x lower concentration and severe matrix effect. However, the improved sensitivity of the 6545 Q-TOF LC/MS System still achieved ~70 % analyte detection at a 1 ng/g spike level in black tea matrix, corresponding to 0.2 ppb. The number of the compounds detected at different spike concentrations is shown in Figure 6.

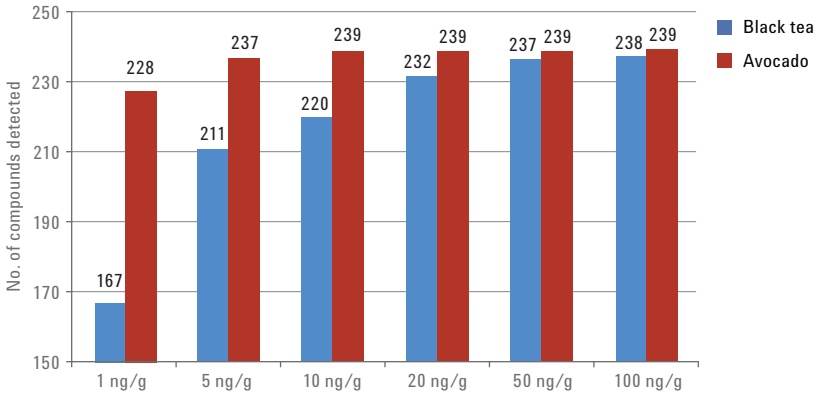

Figure 6. Number of the compound detected at each spike concentration in black tea and avocado matrixes. The improvement in sensitivity of the Agilent 6545 Q-TOF LC/MS System results in more compounds to be detected.

Quantitation and confirmation with targeted MS/MS

Targeted MS/MS is an important feature in data acquisition for analyte quantitation as well as for structure confirmation by comparing the fragmentation pattern against the database and library. Knowledge of the retention time of a particular ion species is required to set up the acquisition method. The retention times were retrieved from MS domain data that could be extracted from Agilent MassHunter Qualitative Analysis Software. Retention time window (delta RT) was set to 1 minute. Reference ions were excluded for the MS/MS spectrum. Acquisition rates were 15 spectra/s for MS and 12 spectra/s for MS/MS. Collision energy was set as a linear regression of 4 + 6 × mass/100, depending on the molecular weight of the target ions. As a result, a search filter on collision energy of ± 20 eV was applied to focus comparison of measured spectra to those library entries of similar collision energy. Most compounds were confirmed and quantified at or below the MRL in black tea matrix with reverse matching. Most of the compounds detected on MS domain could be detected by targeted MS/MS. Figure 7 shows a calibration curve and library match at 10 ng/g spike level of metobromuron in black tea matrix (top) and dimethoate in avocado matrix (bottom). It is expected that the measure MS/MS spectrum in the presence of matrix can be noisier than the library spectrum often acquired in neat solvent at relatively higher concentration. In MassHunter Quantitative Analysis, users can either set up the reference library with PCDL, or use the standards in neat solvent under the same LC/MS conditions through the compound exchange file.

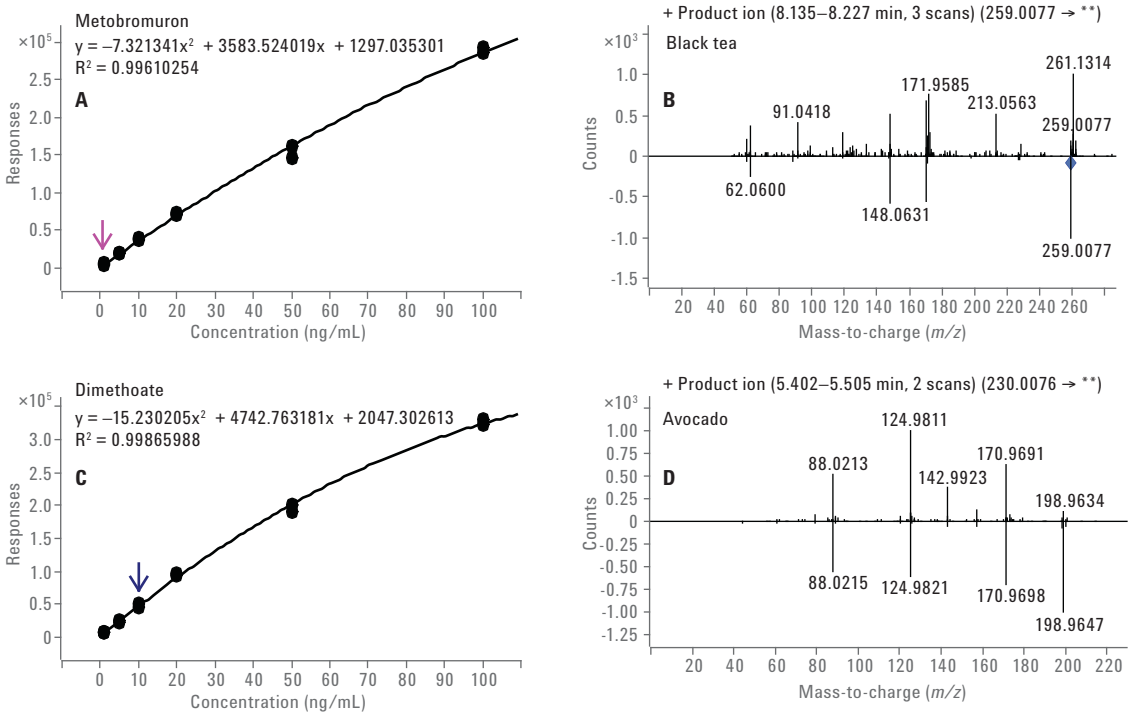

Figure 7. Targeted MS/MS can be used to quantify pesticides as well as confirm its structure by fragments comparing with PCDL spectrum. Most of the compounds detected on MS domain could be quantified by targeted MS/MS. A 10 ng/g spike of metobromuron in black tea (top) and dimethoate in avocado (bottom) are shown as examples.

Structure confirmation with Auto MS/MS

Auto MS/MS is a feature frequently used for untargeted analysis. In pesticide screening, to eliminate false positives in complex matrixes such as black tea and avocado, further structure confirmation is needed. The confirmation is conducted by comparing the measured MS/MS spectrum to the library spectrum. Caution should be taken when seting up auto MS/MS methods. Sometimes, a preferred ion list is required to overcome matrix interference and obtain meaningful MS/MS spectra. Ion species and associated retention times can be imported from MassHunter Qualitative Analysis on MS domain data. The advantage of auto MS/MS over targeted MS/MS is that the data mining can be performed retrospectively should the library be updated with more analytes for their presence in the samples. The acquisition typically lasts only 2–3 cycles for more compound coverage. Thus, auto MS/MS is not recommended for analyte quantitation. Two examples are shown in Figure 8 on the stucture confirmation for chlorfevinphos (II) in black tea and diethofencarb in avocado at 10 ng/g spike concentration.

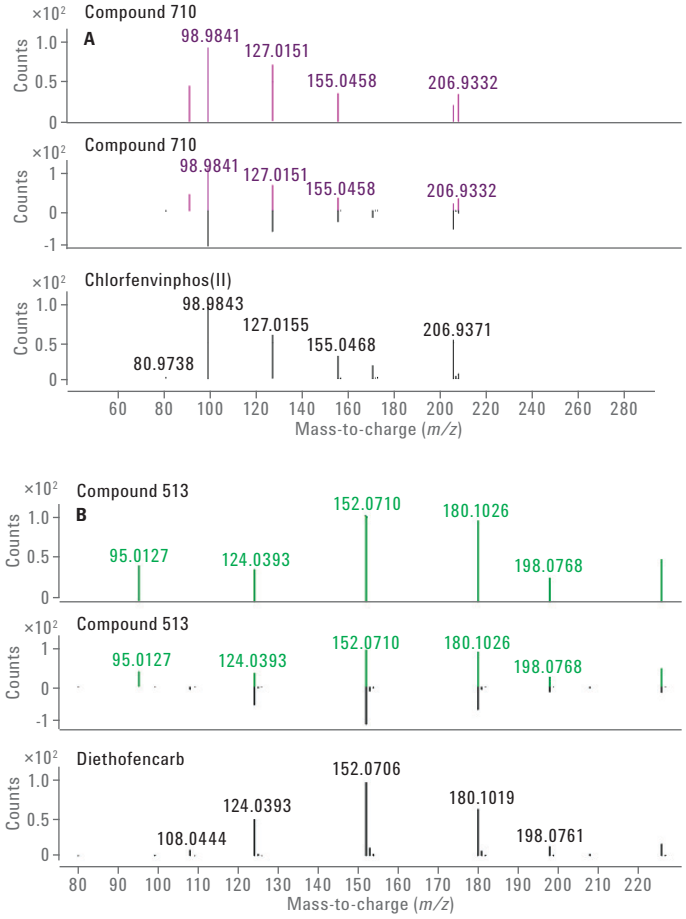

Figure 8. Auto MS/MS is useful for structure confirmation by the comparison of the measured spectrum and library spectrum. The examples show 10 ng/g chlorfevinphos (II) in black tea (A) and 10 ng/g diethofencarb in avocado (B).

Conclusions

The Agilent 6545 Q-TOF LC/MS System is a valuable addition to the product family for the applied market with its improvement in sensitivity and ion transmission tune for fragile organic molecules. We have demonstrated that most of the pesticides and pesticide metabolites can be detected well below the MRL in complex matrixes. The method can easily be extended for more analyte screening and quantitation. This can potentially allow laboratories to increase their testing scale and throughput. The Agilent total solution, from comprehensive reagent kit, UHPLC/MS, PCDL, and MassHunter Qualitative/Quantitative Analysis Software, has allowed us to facilitate method development and validation for the end users.

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