

# Rapid Screening and Quantitation of 240 Pesticides in Difficult Food Matrixes Using the Agilent 6545 Q-TOF Mass Spectrometer with the All Ions MS/MS Technique

## Application Note

### Authors

Dan-Hui Dorothy Yang,  
Christian Klein, Crystal Cody,  
and Huy Bui  
Agilent Technologies, Inc.  
Santa Clara, CA 95051

### Abstract

This Application Note describes a UHPLC/Q-TOF/MS method for the detection of 240 pesticides and pesticide metabolites in difficult food matrixes using the Agilent All Ions MS/MS technique. The method benefits from increased chromatographic resolution using the Agilent 1290 Infinity UHPLC system and improved ionization capabilities with Agilent Jet Stream ionization source. More importantly, the successful screening and quantitation are 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. The All Ions MS/MS technique features the easy acquisition method setup and retrospective data interrogation with expanded library. 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 regulations.



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## Introduction

Pesticide residue screening in food products is one of the most demanding applications in food safety. There are more than 1,000 pesticides and pesticide metabolites that 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<sup>1</sup>. Similar regulations are in place in other regions<sup>2</sup>. Therefore, fast and reliable analytical methods are required to quantify pesticides at low concentrations in a broad range of food matrixes. Accurate mass LC/MS meets the challenge since it allows an easy method setup to detect a large number of analytes without preknowledge of fragmentation conditions. This is especially important for a commercial lab to increase the testing scope and throughput at manageable cost.

A typical workflow in accurate mass LC/MS includes the screening and quantitation of 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 compounds matching. The identified pesticide residues can be further confirmed by auto or targeted MS/MS with the application of appropriate collision energy and a comparison of the MS/MS spectra from Personal Compound Database and Library (PCDL). This is critically important to rule out potential false positives in the context of complex matrixes such as QuEChERS extracts.

However, it is often difficult to obtain meaningful auto MS/MS spectra for analytes at low concentrations in complex matrixes, such as black tea and avocado, when matrix compounds are often selected for fragmentation due to their high abundance. Targeted MS/MS can be used for pesticide screening and structure confirmation. To set up a targeted MS/MS method, knowledge of the retention time for a particular ion species is required. It is not possible to re-investigate data for new and unknown residues without reacquiring the data.

To overcome the limitation imposed by auto and targeted MS/MS in the presence of complex matrixes, Agilent has developed the All Ions MS/MS technique<sup>3</sup>.

The All Ions MS/MS technique features easy acquisition method set up without the preselection of precursor ions, where all precursors are fragmented in high energy channels. The product ions are then verified by the spectra from the MS/MS library. To eliminate false positives, chromatographic coelution of the precursor ion and product ions are required to qualify the fragmentation, the critical link to ensure that the product ions belong to the precursor ion. Furthermore, rapid MassHunter Qualitative/Quantitative method for data analysis can be streamlined into the workflow. Compound information in MassHunter Qualitative analysis is used to create a quantitative method with multiple product ions as qualifiers. This can meet the criteria for the identification of pesticide residues for method validation and quality control procedures specified in guidance documents such as SANCO/12571/2013<sup>4</sup>. Since data were acquired using TOF spectra with accurate mass, they can be re-investigated for additional compounds simply by expanding the library. Due to the sensitivity improvement associated with the optimal small molecule tune under MassHunter Acquisition B.06.01, hundreds of pesticides can be screened and quantified in a single run using the All Ions MS/MS technique, which can drastically improve the testing scale of laboratories.

This Application Note describes an All Ions MS/MS method for the compounds in the Agilent 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 resulted in higher analytical performance. Hardware improvements include a new slicer design with the option to operate under high sensitivity or high resolution mode, a new high

performance high voltage power supply that improves the mass resolution for higher molecular weight entities, and a new enhanced gain shifted detector that provides much longer detector lifetime. More importantly, Particle Swarm Optimization technology, for the first time, is commercially used to optimize the mass spectrometer resulting in a much faster and more robust tuning of the instrument. 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's selection. When compared with the classic autotune on a 6540 Q-TOF LC/MS, the system tuning speed is increased by a factor of four.

## 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- $\mu$ m 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 for 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)<sup>5</sup>. Ten grams of homogenized avocado and 2 g of black tea were weighed in 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.

### Equipment and software

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

- 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 Accurate Mass 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 in positive ion mode. Three microliters of the final extract were injected. Source parameters were optimized with a subset of 14 pesticides that represents the cohort of 240. The summary of the 6545 Q-TOF LC/MS parameters are listed in Table 2.

Data were evaluated using 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 temp	5 °C														
Needle wash	10 seconds (80 % MeOH/20 % water)														
Mobile phase	A) Water, 5 mM NH <sub>4</sub> formate + 0.1 % formic acid B) MeOH, 5 mM NH <sub>4</sub> formate + 0.1 % formic acid														
Flow rate	0.4 mL/min														
Gradient program	<table border="1"> <thead> <tr> <th>Time</th> <th>%B</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>5</td> </tr> <tr> <td>1</td> <td>5</td> </tr> <tr> <td>4</td> <td>50</td> </tr> <tr> <td>17</td> <td>100</td> </tr> <tr> <td>20</td> <td>100</td> </tr> <tr> <td>20.1</td> <td>5</td> </tr> </tbody> </table>	Time	%B	0	5	1	5	4	50	17	100	20	100	20.1	5
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 <i>m/z</i>
Drying gas temperature	150 °C
Drying gas flow	10 L/min
Sheath gas temperature	375 °C
Sheath gas flow	12 L/min
Nebulizer pressure	35 psi
Capillary voltage	3,500 V
Nozzle voltage	200 V
Fragmentor	125 V
Skimmer	45 V
Oct1 RF V <sub>pp</sub>	750 V
Acq mass range	50–1,000 <i>m/z</i>
Acq rate	3 spectra/s
Collision energy	0 V, 20 V, 40 V
Ref mass ions	121.050873, 922.009798

## Results and Discussion

### Data processing

The data file was processed against a customized PCDL containing all the pesticides in this study using the Find by Formula (FbF) algorithm. FbF has been updated to support the All Ions MS/MS technique. MassHunter Qualitative Analysis B.07 has implemented a very useful function under the **fragment confirmation** tab for All Ions MS/MS technique. When the **molecular ion optional** check box is selected, saturating precursor ion or very weak precursor ion exerts no effect on the matching score in All Ions MS/MS applications. This is of great importance for multiple analyte identification (for example, hundreds of pesticides screen), since mass response and fragility of compounds can vary

significantly. The MS peak in the low energy channel was first searched against PCDL for the compound with the same  $m/z$  value. For the identified compounds, the fragment ions in MS/MS spectra from PCDL were compared with the ions in the high energy channel to confirm the presence of correct fragments.

For a majority of the compounds (224 out of 240), MS/MS spectra are available in PCDL and used to extract the EIC of the most abundant product ions. If no MS/MS spectra were available in the PCDL, the MS spectrum is used for compound identification. For each fragment ion, chromatographic evaluation is performed against a precursor ion in the time domain to ensure the relevance of the product ions.

Under MassHunter Qualitative Analysis, the compound detail view window allows users to browse quickly through the compounds for matching quality, removing nonqualified fragments. The general layout is illustrated in Figure 1, using 5 ppb linuron as an example. Compounds are listed, and can be quickly searched. The precursor ion and product ion chromatograms are overlaid, and the coelution score is presented. The coelution score is generated using the entire peak data, not just the retention time, as shown on the left side of Figure 1. Library matching results are listed on the right side with intensity, signal-to-noise (S/N) ratio, as well as collision energy applied. MS and fragment spectra are also shown, and fragment ions matched from the library are color-coded from the background ions.

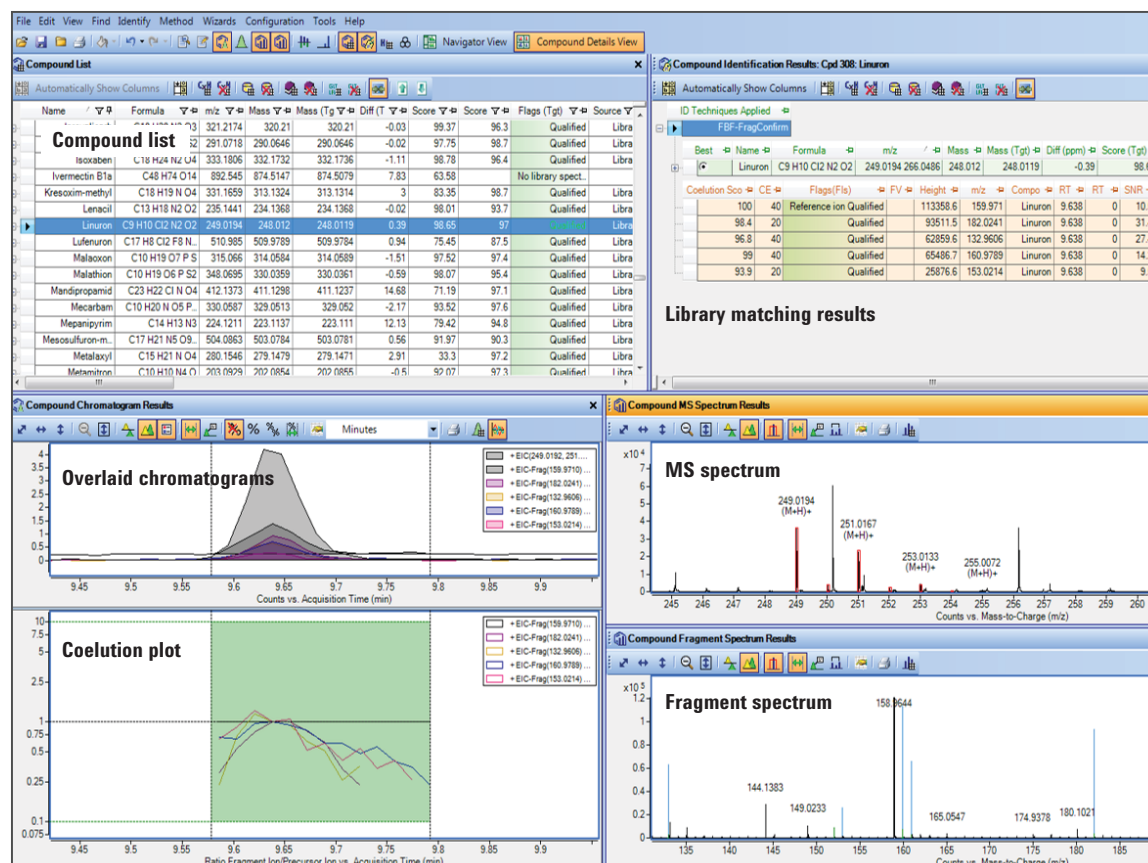


Figure 1. Data browsing window in Agilent MassHunter Qualitative Analysis for the All Ions MS/MS technique. Users can evaluate data quality for coelution score, library matching, and mass spectra for each compound easily.

The compound exchange file (.cef), which contains relevant compound information (name, RT, precursor ion  $m/z$  value, and product ions  $m/z$  values with associated collision energy values) can be generated and exported into MassHunter Quantitative Analysis. A batch analysis method can then be generated simply by adding calibration levels. Using this workflow, the quantitation of hundreds of pesticides can be done in batch mode.

### Detectability of 240 pesticides in complex matrixes

Several of the compounds were disqualified due to low S/N on product ions, low coelution score or retention time outside the retention time window ( $\pm 0.2$  minutes), or the combination thereof. The disqualified compounds were not part of the .cef file for quantitative analysis. Figure 2 shows the exemplary calibration curves of benalaxyl in black tea matrix and amidosulfuron in avocado matrix. Two of the most abundant product ions were selected as qualifiers. At 10 ng/g spike level, corresponding to 2 ppb in black tea matrix, and 10 ppb in avocado matrix respectively, 72 % of the pesticides can be quantified in black tea matrix and 80 % of the compounds can be quantified in avocado matrix.

### System robustness

Hardware changes associated with the 6545 Q-TOF LC/MS make the system tolerate the complex matrixes better. In approximately 1 week of food safety testing with injections of black tea and avocado matrixes, 20 ppb of 240 pesticides in acetonitrile were measured in MS domain as system QC runs. Seven QC runs were performed in duplicate (a total 14 data files). After the removal of saturated/split peaks, > 90 % compounds gave a CV of less than 10 %. Even for a slightly larger CV, there were generally no clear descending trends, but rather one or two random outliers. The average CV was about 7.6 % from seven QC runs, indicating good system stability with the continuous injections of complex matrixes.

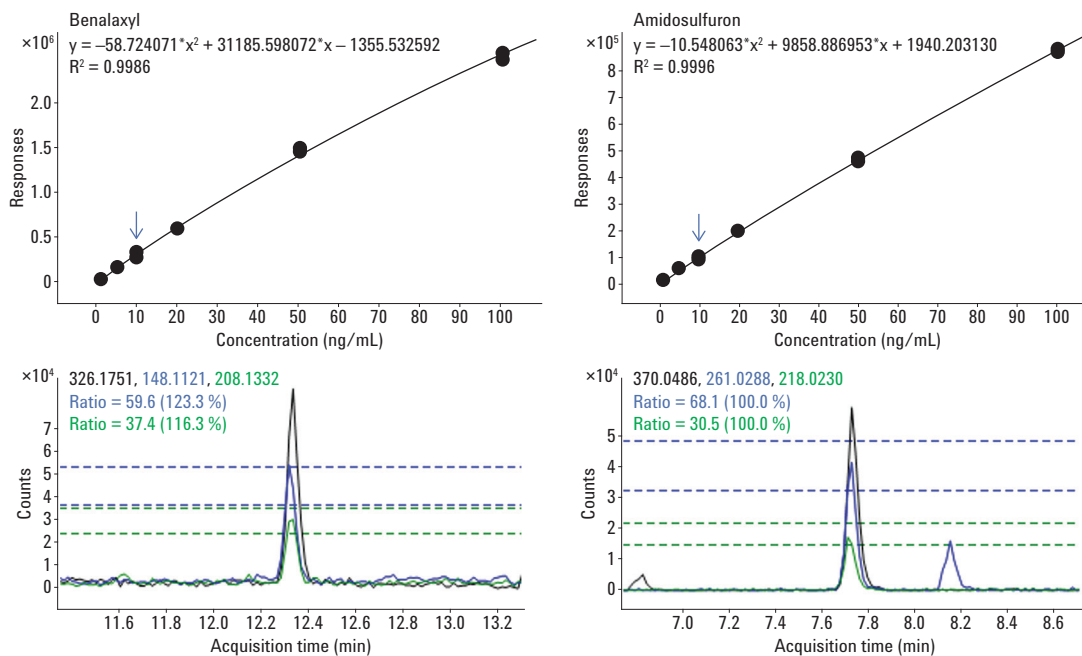


Figure 2. Exemplary calibration curves for benalaxyl in black tea matrix (left) and amidosulfuron in avocado matrix (right). 72 % and 80 % of the pesticides can be detected at 10 ng/g spike level in black tea matrix and in avocado matrix, respectively.

## Conclusions

The All Ions MS/MS technique is very valuable for detecting hundreds of analytes in complex matrixes such as foods, plasma, or urine, where targeted MS/MS or auto MS/MS has limitations. The technique features easy method setup without knowledge of precursor ion selection and fragmentation conditions. It also allows re-investigation of data files for new analytes without re-running the sample. The scoring system is visualized easily in MassHunter Qualitative Analysis for each compound on fragment library matching and chromatographic coelution of precursor and product ions. We have also provided a streamlined MassHunter Qualitative/Quantitative method for batch analysis under MassHunter Quantitative Analysis, enabling users to analyze hundreds of pesticides in a batch by simply inputting calibration levels. Moreover, the Agilent 6545 Q-TOF LC/MS system is a valuable addition to the product family for applied markets with its improvement in sensitivity and optimized ion transmission for fragile organic molecules. We have demonstrated that most pesticides and pesticide metabolites can be detected at or below maximum residue limits (MRLs) set by regulations in complex matrixes. The Agilent total solution, from comprehensive reagent kits, UHPLC/MS, PCDLs, to MassHunter Qualitative/Quantitative Analysis software, has allowed us to facilitate method development and validation for end users.

## References

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