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# High Throughput Target and Suspect Pesticide Analysis using a new LC/Q-TOF Screener Software

Karen E Yannell and Kai Chen

Agilent Technologies, Inc., Santa Clara, CA

## Introduction

### Pesticide quantitation and screening is streamlined with the Agilent 6546 LC/Q-TOF and MassHunter Quantitative Analysis 10.0 LC/Q-TOF Screener tool.

Pesticide testing laboratories are facing an increasing list of analytes to be included in their testing panels. The length of the list has become economically challenging to perform routine target quantitation of each pesticide because of budget and time costs for all the standards. Instead, labs are looking for new analytical methods that improve their lab's efficiency while still maintaining an awareness for pesticides that violate the maximum residual levels (MRLs).

Non-targeted data acquisition using a high-resolution quadrupole time-of-flight (Q-TOF) collects all the data in the sample allowing for a panel of a thousand pesticides to be screened. However, this data is complex and analysis has historically been slow and difficult to perform routinely.

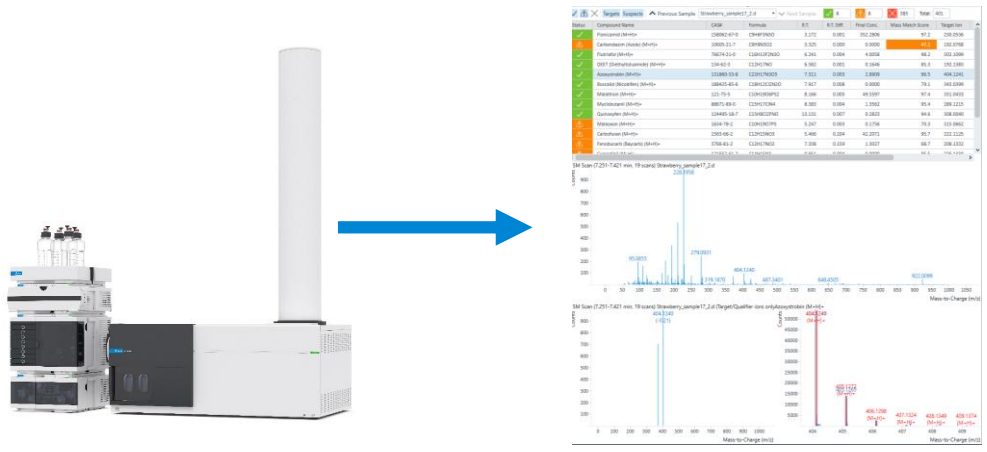

Figure 1: Agilent 6546 LC/Q-TOF (left) and the MassHunter Quantitative Analysis 10.0 LC/Q-TOF Screener tool (right).

Described here is a high throughput analysis workflow for target and suspect screening of hundreds of pesticides using the new 6546 LC/Q-TOF (Figure 1). This new hardware gives high-resolution data (30,000 at *m/z* 118) with large in-spectrum dynamic range ($10^5$) while using high acquisition rates (up to 50 Hz).

The analysis workflow is greatly improved by combining quantitation of target analytes and suspect screening into one software platform, MassHunter Quantitative Analysis 10.0. This software also has a new novel LC/Q-TOF Screener tool, which makes processing and reviewing data fast and easy.

This hardware and software allow one method to contain hundreds of target and suspect analytes without increasing the review burden for the scientist.

## Experimental

### A complete workflow allows for nearly 400 pesticides to be quantified and screened.

Organic black tea, strawberry, avocado, and broccoli matrices were prepared with QuEChERS EN sample preparation kits. The supernatant from these matrices were spiked with over 200 pesticides at five different levels from 5 ppb to 100 ppb. For each matrix, a positive control was prepared by spiking a small subset of analytes at 10 and 100 ppb. Additionally, conventional and organic strawberry samples purchased from various US supermarkets were prepared individually and treated as unknowns. Samples were analyzed using reverse phase chromatography with the 1290 Infinity II and All Ions non-targeted acquisition at 6 Hz with the Agilent 6546 LC/Q-TOF (Tables 1 and 2).

Table 1: Chromatographic acquisition details.

| Parameter | 1290 Infinity II LC System |
|---|---|
| Analytical Column | Agilent ZORBAX Eclipse Plus C18 3.0 × 150 mm, 1.8 µm (p/n 959759-302) |
| Guard Column | ZORBAX Eclipse Plus C18, 2.1 mm, 1.8 µm, UHPLC guard column (p/n 821725-901) |
| Column Temperature | 45 °C |
| Injection Volume | 2 µL |
| Autosampler Temperature | 7 °C |
| Needle Wash | 10 seconds, standard (50:50 methanol:isopropanol) |
| Mobile Phase A | Water + 4.5 mM ammonium formate + 0.5 mM ammonium fluoride + 0.1 % formic acid |
| Mobile Phase B | Methanol + 4.5 mM ammonium formate + 0.5 mM ammonium fluoride + 0.1 % formic acid |
| Flow Rate | 0.45 mL/min |
| Gradient | Time(min) %B<br>0.00 2<br>0.50 2<br>1.00 50<br>4.00 65<br>16.00 100<br>18.00 100<br>18.10 2<br>20.00 2 |
| Post Time | 4 minutes |

Table 2: 6546 LC/Q-TOF acquisition parameters.

| Parameter | Value |
|---|---|
| Sheath Gas Temperature | 400 °C |
| Sheath Gas Flow | 12 psi |
| Gas Temperature | 325 °C |
| Gas Flow | 10 psi |
| Nebulizer | 20 psi |
| Capillary Voltage | 4,000 V |
| MS Tune | *m/z* 750, fragile |
| MS Mode | Positive |
| Acquisition | MS only with 0, 20, and 40 CE segments |
| MS Range | *m/z* 50 to 1,000 |
| Reference Mass | 121.0509 ($M+H^+$ for purine)<br>922.0098 ($M+H^+$ for HP-921) |

The acquired data was converted with SureMass which is a profile file based conversion algorithm to reduce data analysis time and provide improved accuracy across the concentration range. The data was analyzed with a MassHunter Quantitative Analysis method that contained targets and suspect compounds. Each target had one qualifier ion while the suspects had four qualifiers for greater confidence.

## High quality data within SANTE guidelines was acquired using the 6546 LC/Q-TOF.

The data had high mass accuracy across the chromatogram regardless of sample concentration (Figure 2). The majority of the analytes had less than 2 ppm error while all were less than 5 ppm, which follows SANTE guidelines[1].

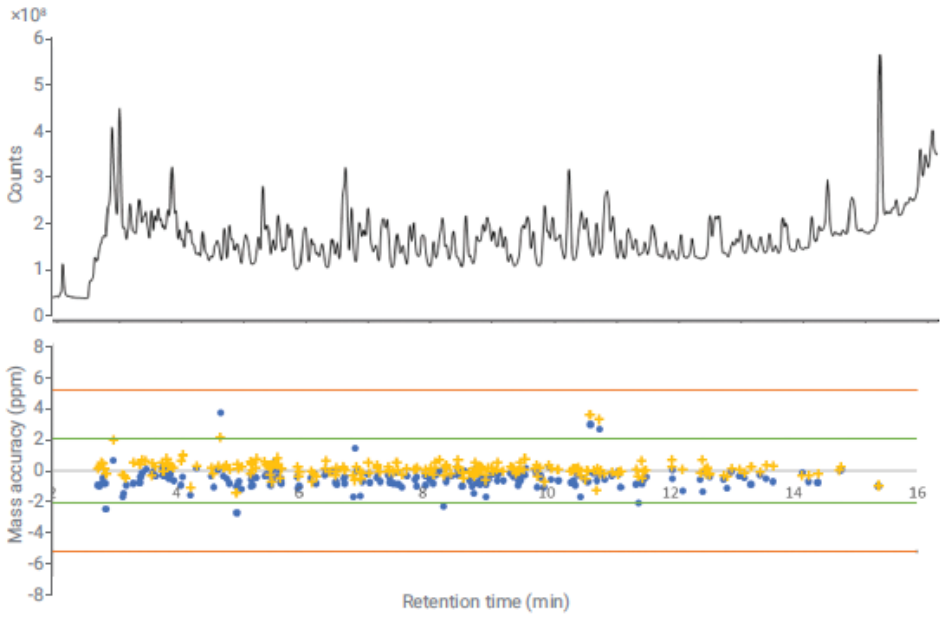


Figure 2: The TIC chromatogram for the strawberry matrix (top) had peaks eluting throughout the gradient. Target compounds mass error are plotted for the 10 ppb (blue) and 100 ppb (yellow) in strawberry matrix.

A targeted analysis method in MassHunter Quantitative Analysis 10.0 could be built directly from a Personal Compound Database and Library (PCDL) of pesticides. The software inputted the precursor and fragments from library spectra as well as the retention time for each compound. A isotope pattern and adduct pattern was also made from this data for greater confidence in the identification. The analysis method editor allowed for outlier thresholds to be set for key parameters such as retention time, mass accuracy, and co-elution score. The entire method development workflow and outliers are described in Figure 3.

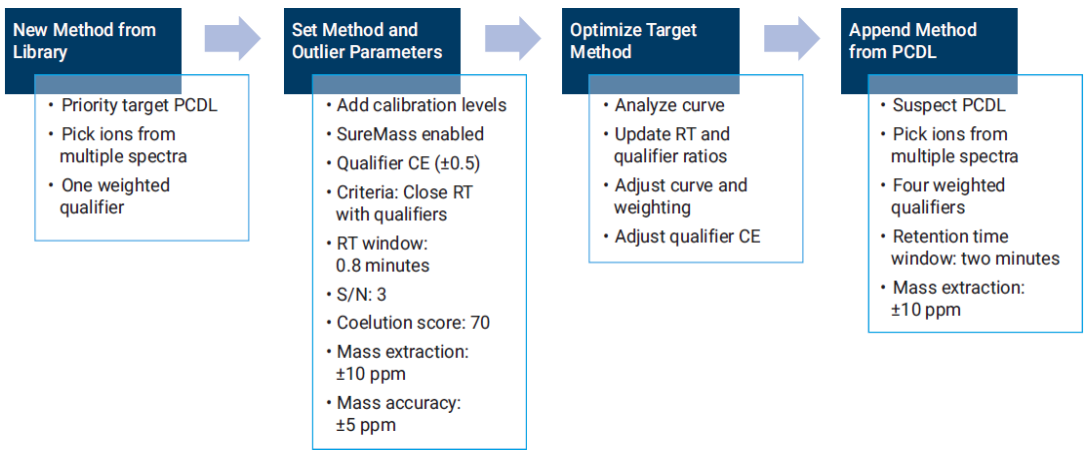


Figure 3: Method development workflow for making a target and suspect method in MassHunter Quantitative Analysis 10.0 from a PCDL. Parameters used for outlier identification in the LC/Q-TOF Screener tool are listed.

## Quantitation is performed on the first injection for target analytes.

The data showed good linearity and sensitivity even at low concentrations in each matrix. The qualifier ion was found at a stable ratio (within 20% variation) at all detectable levels. The results for a selected compound, paclobutrazol, is shown at 5 ppb in each matrix tested in Figure 4.

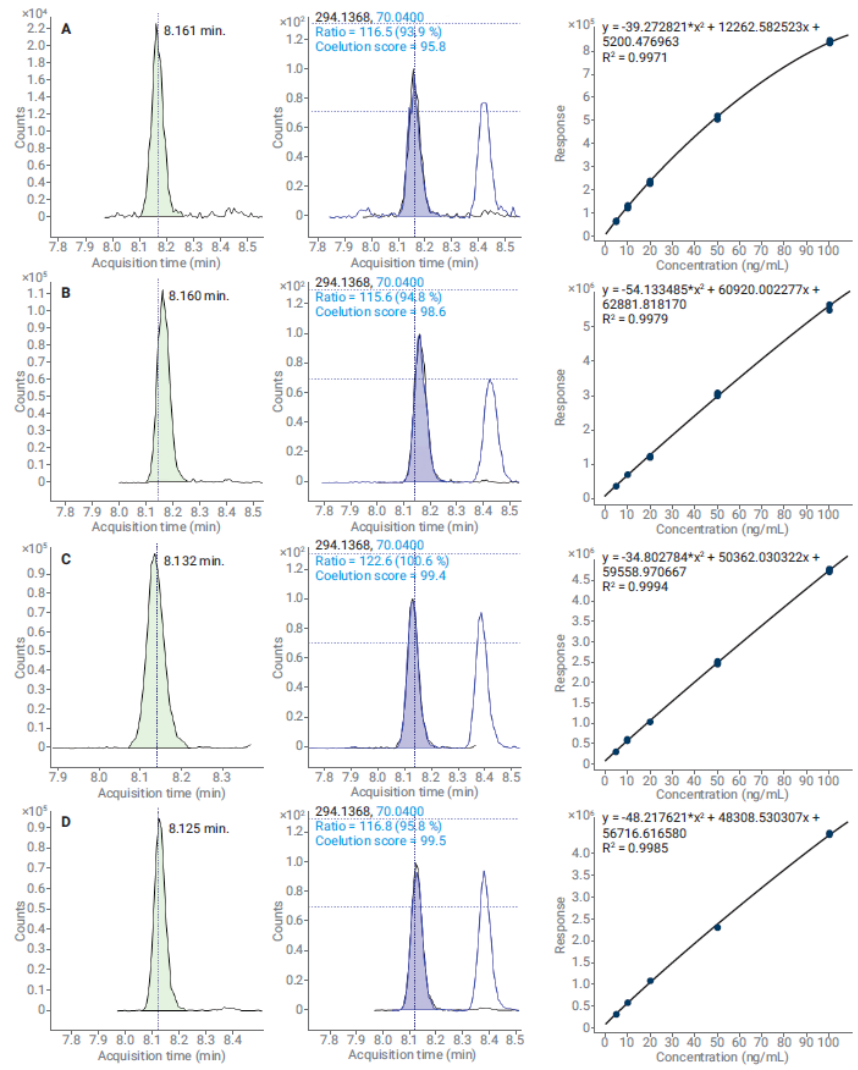


Figure 4: Target results for one compound, paclobutrazol, in four different matrices: A) black tea, (B) broccoli, (C) avocado, and (D) strawberry.

Every target analyte had an $R^2$> 0.99 and a relative standard deviation (RSD) <20% at the limit of quantitation, 5 ppb. A summary of the target results is in Table 3.

Due to a further dilution of the black tea matrix, to account for the matrix complexity, some analytes were not detected at 5 ppb and removed from the preferred target list. Every analyte in the positive controls were detected in the different matrices except for two analytes which did not reach 5 ppb in the black tea matrix.

Table 3: All target analyte results in different matrices.

| | Strawberry | Avocado | Broccoli | Black Tea |
|---|---|---|---|---|
| Number of Targeted Compounds | 195 | 195 | 195 | 145 |
| Number of Targets with S/N >3 at 5 ppb | 195 | 195 | 195 | 145 |
| Number of Targets with RSD <20 % at 5 ppb | 195 | 195 | 195 | 145 |
| Number of Targets with $R^2$ >0.99 | 195 | 194 | 195 | 145 |
| Number of Screened Compounds | 182 | 182 | 182 | 182 |

## Results and Discussion

### High throughput data analysis of non-targeted Q-TOF data is possible with the LC/Q-TOF Screener tool.

To analyze hundreds of pesticides quickly in a whole batch of samples a new tool, LC/Q-TOF Screener , allows a user to quickly navigate through confirmed or suspect analytes in a sample and also make a more detailed review if needed. This reduces the review burden to only analytes in a sample that are suspected or confirmed.

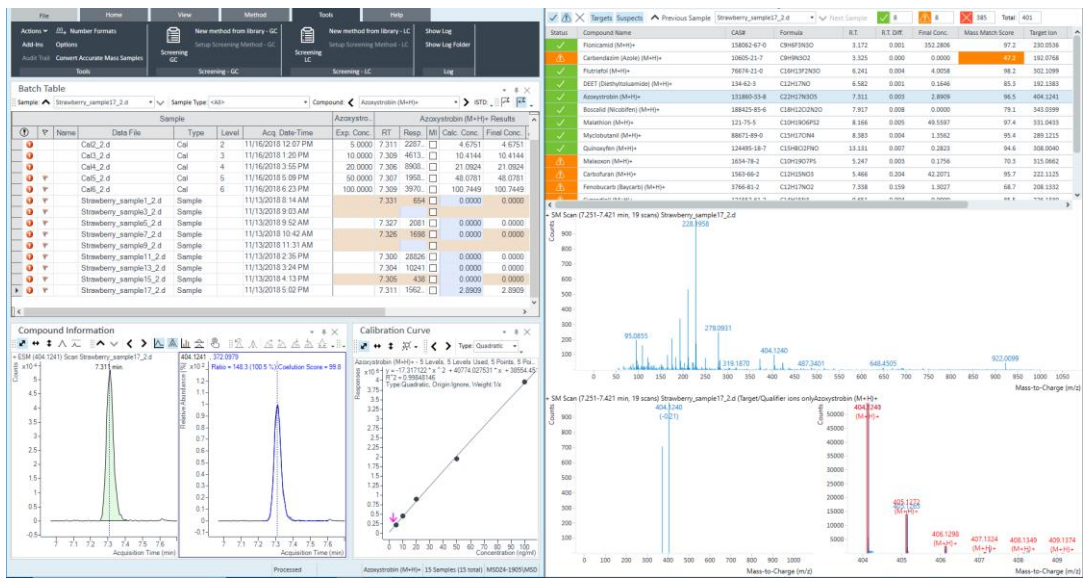

Figure 5: Screenshot of the MassHunter Quantitative Analysis 10.0 LC/Q-TOF Screener tool used for fast analysis of target and suspect analytes. The Screener UI on the right filters for the analytes confirmed or suspected and when a compound is selected the information for that analyte appears in the classic UI on the right.

The Screener determines if an analyte is confirmed (green) or suspect (orange) based on method outliers such as the mass accuracy, retention time, presence of qualifier ions, and isotope pattern (Figure 3). If one of these parameters is an outlier, the software flags the compounds for additional review (suspect). The Screener user interface also shows the isotope pattern of the precursor ion and the extracted fragments data, if present, for fast inspection and review.

In the unknown strawberry samples (n=16) a range of pesticides were detected (Table 4). Most were target analytes so a concentration is reported. A small number were suspects. These can be quantitated by borrowing a calibration curve or by a second injection with it's own standard, if needed.

Finally, since the analysis is targeted but the data acquisition on the Q-TOF is non-targeted, a retrospective data analysis is possible if a new analyte is suspected to be present in the data. No re-injection is needed.

### Unknown strawberry samples contained mostly target pesticides in conventional produce.

Table 4: Results for the sixteen strawberry samples collected in California and Delaware and tested on this method. If an analyte was a target then a concentration is reported in parenthesis.

| Sample | Type | Pesticides Detected |
|---|---|---|
| Sample 1 | Organic | Malaoxon (2.6 ppb), Carbaryl (29.4 ppb), Malathion (31.8 ppb), Methoxyfenozide (Intrepid) (4.7 ppb), Diazinon (Dimpylate) (2.5 ppb) |
| Sample 2 | Organic | Spinosyn A (14.3 ppb), Spinosyn D (3.6 ppb) |
| Sample 3 | Organic | None found |
| Sample 4 | Organic | Prometryn |
| Sample 5 | Organic | None found |
| Sample 6 | Conventional | Flutriafol (12.0 ppb), Metalaxyl (28.6 ppb), Azoxystrobin, Pyrimethanil (218 ppb), Malathion (<5 ppb), Fenhexamid (5.6 ppb), Cyprodinil (26.6 ppb), Trifloxystrobin (6.2 ppb), DEET (Diethyltoluamide) (<5 ppb) |
| Sample 7 | Conventional | Thiamethoxam (<5 ppb), Carbendazim (Azole)(163 ppb), Thiophanate-methyl, Chlorantraniliprole (5.6 ppb), Pyrimethanil (105 ppb), Boscalid (Nicobifen) (23.5 ppb), Methoxyfenozide (Intrepid) (6.8 ppb), Myclobutanil (<5 ppb), Bifenazate (D 2341), Tetraconazole (33.2 ppb), Fenhexamid, Cyprodinil (248 ppb), Pyraclostrobin (<5 ppb) |
| Sample 8 | Organic | Boscalid (Nicobifen) (2.4 ppb) |
| Sample 9 | Conventional | Flonicamid, Malaoxon (<5 ppb), Flutriafol (<5 ppb), Azoxystrobin (<5 ppb), Malathion (48.2 ppb), Myclobutanil (<5 ppb), Quinoxyfen (<5 ppb) |
| Sample 10 | Conventional | Acetamiprid (<5 ppb), Metalaxyl (<5 ppb), Azoxystrobin (8.4 ppb), Myclobutanil, Bifenazate (D 2341) (<5 ppb), Fenhexamid (32.4 ppb), Cyprodinil (283 ppb), Pyraclostrobin (10.8 ppb), Trifloxystrobin (46.9 ppb), Etoxazole |
| Sample 11 | Conventional | Thiamethoxam (13.1 ppb), Flonicamid (97 ppb), Carbendazim (Azole), Imidacloprid, Pyrimethanil (278 ppb), Methoxyfenozide (Intrepid) (14.8 ppb), Myclobutanil (<5 ppb), Bifenazate (D 2341), Tetraconazole (12.3 ppb), Cyprodinil (312 ppb), Trifloxystrobin (<5 ppb), Hexythiazox (22.6 ppb), Thiophanate-methyl |
| Sample 12 | Conventional | Flonicamid (153 ppb), Acetamiprid (<5 ppb), Chlorantraniliprole (69 ppb), Methoxyfenozide (Intrepid) (<5 ppb), Cyprodinil (146 ppb), Trifloxystrobin (78 ppb), Quinoxyfen (34 ppb), Hexythiazox (<5 ppb) |
| Stample 13 | Conventional | Flonicamid (64 ppb), Azoxystrobin, Boscalid (Nicobifen)(14.7 ppb), Cyprodinil (<5 ppb), Pyraclostrobin (19.4 ppb), Cyflufenamid |
| Sample 14 | Conventional | Thiamethoxam (<5 ppb) , Flonicamid (29 ppb), Chlorantraniliprole (<5 ppb), Myclobutanil (<5 ppb), Cyprodinil (64 ppb), Trifloxystrobin (<5 ppb) |
| Sample 15 | Conventional | Flonicamid (77 ppb) , Malaoxon (<5 ppb), Flutriafol (8.1 ppb), Azoxystrobin (<5 ppb), Malathion (47.5 ppb), Myclobutanil (5.9 ppb), Quinoxyfen, Cyflufenamid |
| Sample 16 | Conventional | Imidacloprid (48.4 ppb), Metalaxyl (107 ppb), Chlorantraniliprole, Azoxystrobin (457 ppb), Boscalid (Nicobifen) (<5 ppb), Myclobutanil (<5 ppb), Tetraconazole (138 ppb), Quinoxyfen (21.4 ppb), Flonicamid (35.4 ppb) |

## Conclusions

### Simultaneous quantitation and screening analysis of hundreds of pesticides is routinely performed using the 6546 LC/Q-TOF and LC/Q-TOF Screener Tool.

- 6546 LC/Q-TOF provides accurate non-targeted data analysis of pesticides with fragment information.
- LC/Q-TOF Screener tool allows for fast review and reporting of large analyte and sample sets.
- This is an efficient workflow for labs with large sets of analytes to be tested but not all are commonly detected.

## References

[1]Guidance document on analytical quality control and method validation procedures for pesticides residues and analysis in food and feed, SANTE/11813/2017, 21–22 November 2017.


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