Using the SCIEX QTRAP® 6500 System to Quantify and Identify Pesticides in Complex Food Samples

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Introduction

Recent regulations on food analysis require screening for pesticides using confirmatory techniques, such as GC-MS and LC-MS/MS. More than 1000 pesticides are used worldwide and, along with their metabolites and degradation products, are present in food. There is a demand for powerful and rapid analytical methods that can identify pesticides with high confidence in a broad range of food matrices and quantify them at low concentrations with good accuracy and reproducibility.

Challenges for pesticide residue laboratories at the moment are the request to test for more compounds, in a wider range of samples, all without sacrificing data quality.

The SCIEX QTRAP 6500 LC-MS/MS system uses multi-component IonDrive™ technology to:

- Improve ionization efficiency using the new IonDrive™ Turbo V ion source
- Increase robustness using a reengineered curtain gas interface acting as a better barrier against neutrals and micro droplets
- Increase sensitivity using the new IonDrive™ QJet ion guide with dual stage design
- Extend the linear dynamic range for quantitation using the HED IonDrive™ detector.

In addition, the SCIEX QTRAP 6500 System uses the patented and proven Linear Accelerator™ trap technologology to:

- Acquire full scan MS and MS/MS spectra with high selectivity, sensitivity, and speed.

A new method for the quantitation and identification of hundreds of pesticides in food samples was developed and successfully applied to the analysis of complex food samples using the SCIEX QTRAP 6500 System. Results are compared to QTRAP 5500 data. The increased sensitivity was used to extensively dilute sample extracts to eliminate ion suppression caused by matrix components and the extended linear dynamic range allowed quantifying more pesticides across a wider range

of chemical properties. QTRAP scanning was used to investigate the presence of matrix components and to identify targets with high confidence through library searching. Quantitative and qualitative results were generated using MultiQuant™ 2.1 and LibraryView™ 1.0 software.

Experimental

Standards and Sample Preparation

- The iD*Quant*™ standard kit for pesticide analysis containing 204 pesticides was used for method setup and analysis. A few more pesticides of interest were added.
- A QuEChERS protocol was used for sample extraction followed by extensive dilution to eliminate ion suppression.

UHPLC

- Separation was achieved on a Shimadzu UFLC$_{XR}$ system with a RESTEK Ultra Aqueous C18 (100 x 2.1 mm) 3 µm and a gradient of water/methanol containing 10 mM ammonium formate and 0.1% formic acid (Table 1).
- A flow rate of 350 µL/min was used.
- The injection volume was set to 10 µL.

MS/MS Detection

- The SCIEX QTRAP® 6500 System was operated with IonDrive™ Turbo V ion source using the electrospray ionization probe.
- The ion source temperature was optimized to 450°C.
- A total of 493 Multiple Reaction Monitoring (MRM) transitions (2 transitions per pesticide plus 1 transition for the internal standard D_{10}-Diazinon) were detected to allow quantitation and identification of all target pesticides using the MRM ratio.
- The *Scheduled* MRM™ algorithm was activated to achieve highest data quality. The MRM detection window was set to 120 sec and a target scan time of 0.7 sec was used.
- In addition, Enhanced MS (EMS) and Enhanced Product Ion (EPI) scanning features were explored to monitor matrix effects and to increase confidence in identification by MS/MS library searching.

Table 1. Gradient conditions used for separation

Time	Flow (mL/min)	A (%)	B (%)
0	0.35	95	5
5	0.35	40	60
12.5	0.35	5	95
14.5	0.35	5	95
14.6	0.35	95	5
17.5	0.35	95	5

Results and Discussion

Method Setup

An existing pesticide screening method optimized for use on a QTRAP 5500 system was transferred to the QTRAP 6500 system without adjusting compound dependent parameters, such as Declustering Potential (DP) and Collision Energy (DP) values.

The new IonDrive Turbo V ion source has larger heaters (11 mm) and an optimized geometry transfers heat more efficiently resulting in improved ionization. The source temperature was optimized from 350 to 700°C with steps of 50 K to investigate best settings for a multi-pesticide screening method. Most compounds gave best Signal-to-Noise (S/N) at 450 or 500°C. A temperature of 450°C was used in the final method not to compromise sensitivity of low stability analytes. To achieve similar ionization 550°C were used in the original method of the QTRAP 5500 system.

Figure 1 shows temperature maps of the spray region at different temperature settings visualizing the efficiency of heat transfer and the wider 'sweet' spot making probe optimization less crucial to gain maximum sensitivity and reproducibility.

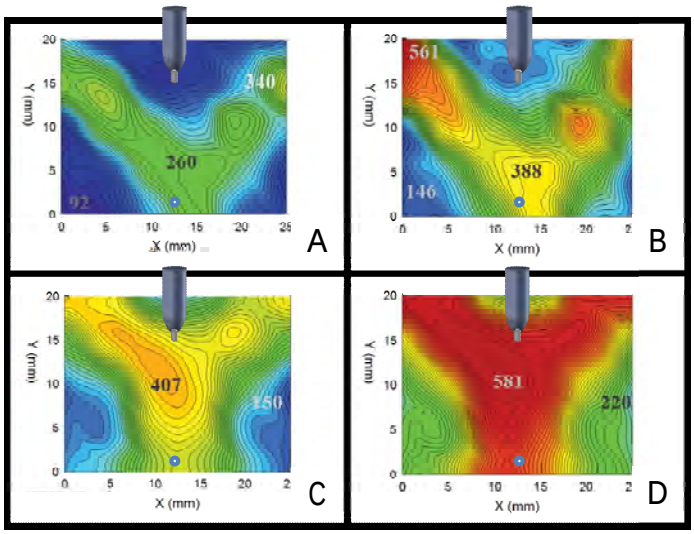

Figure 1. Temperature maps of the spray region of the traditional Turbo V™ source (top) and the new IonDrive™ Turbo V source (bottom). A and C show the source operated at 500°C and B and D at 700°C with Gas 2 set to 70 psi.

The increased heat transfer and wider 'sweet' spot for ionization of the IonDrive™ Turbo V source is clearly depicted in the maps C and D.

Increased Sensitivity

The new design of the ion source and the dual stage design of the IonDrive™ QJet ion guide result in increased sensitivity.

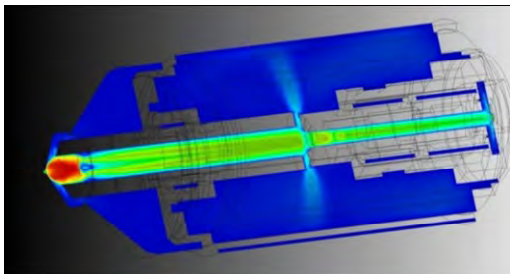

Figure 2. Computed gas flow model of the dual stage QJet™ ion guide

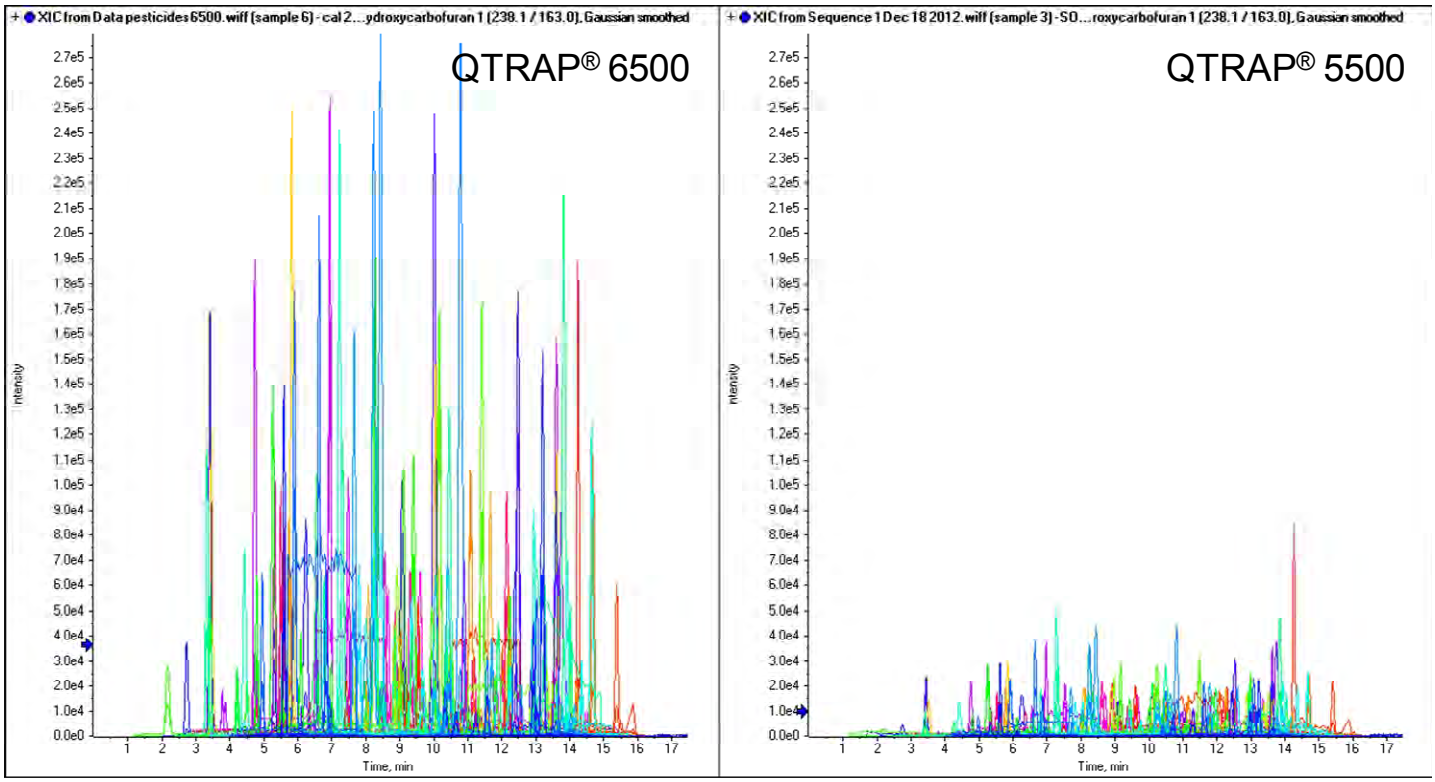

Figure 3. Sensitivity comparison of a 0.1 ng/mL standard analyzed using the QTRAP 6500 system (left) and QTRAP 5500 system (right)

The injection of a 0.1 ng/mL (100 parts-per-trillion) standard into the QTRAP 6500 and QTRAP 5500 systems is shown in Figure 3. An average gain in sensitivity by a factor of 4.7 was observed. Over 51% of all detected pesticides showed a sensitivity gain larger than 4x (Figure 4).

Results for selected pesticides spanning the entire range of chemical properties are presented in Figure 5. The sensitivity gain for specific compounds can be fine-tuned and mostly depends on ion source temperature.

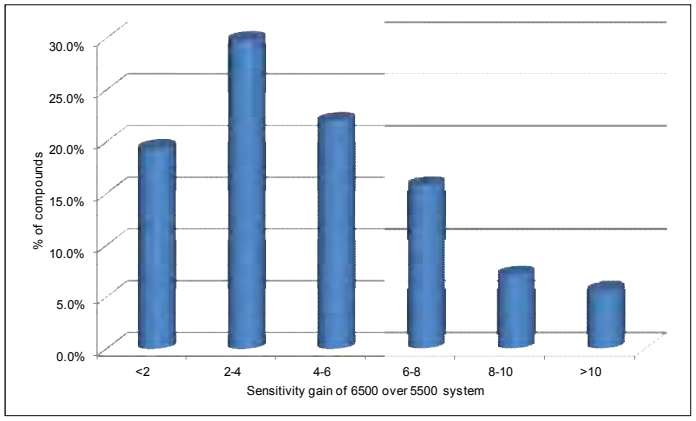

Figure 4. Distribution of sensitivity gain for multi-pesticide analysis with an average gain of 4.7

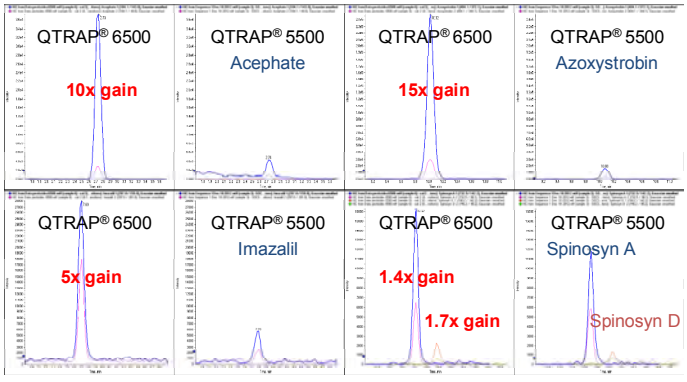

Figure 5. Compound specific sensitivity gains for selected pesticides, including Acephate, Azoxystrobin, Imazalil, and Spinosad, sensitivity gains are compound dependent and can be influenced by ion source temperature

Extensive Extract Dilution to Eliminate Matrix Effects

Matrix effects, like ion suppression and ion enhancement, are caused by co-elution of target analytes with matrix components. While matrix effects can be compensated with co-eluting internal standards or by standard addition, both techniques have limited use for multi-residue analysis since they are expensive and time consuming, respectively. In addition, compensating matrix effects using these techniques has the risk of false negative findings in case the analyte signal is completely suppressed when analyzing complex matrices. Dilution has been shown to be a valuable tool to overcome the problem of matrix effects.[1]

Here we used the increased sensitivity of the QTRAP® 6500 system to dilute QuEChERS extract extensively (up to 1000x) to eliminate matrix effects even in the most challenging matrices.

Figure 6 shows an example of reduced ion suppression of Acetamiprid spiked into a peppermint tea at 100 µg/kg. The 20 and 50x dilution did not result in the expected signal decrease by a factor of 2x or 5x indicating a successful reduction of matrix effects.

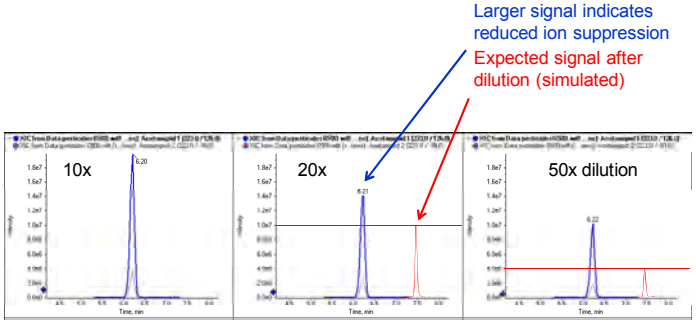

Figure 6. Dilution of peppermint tea extract spiked with Acetamiprid, the increase in sensitivity over the expected (simulated) peak demonstrates successful dilution of matrix effects

Figure 7 shows results of dilution experiments for 4 selected pesticides spiked into peppermint tea. It can be seen that matrix effects are different for each analyte due to different matrix components eluting at the respective retention time. Also different dilution factors are needed to eliminate matrix effects for each compound. A dilution factor of 50 to 200 was required to reduce ion suppression for the selected pesticides to less than 20%.

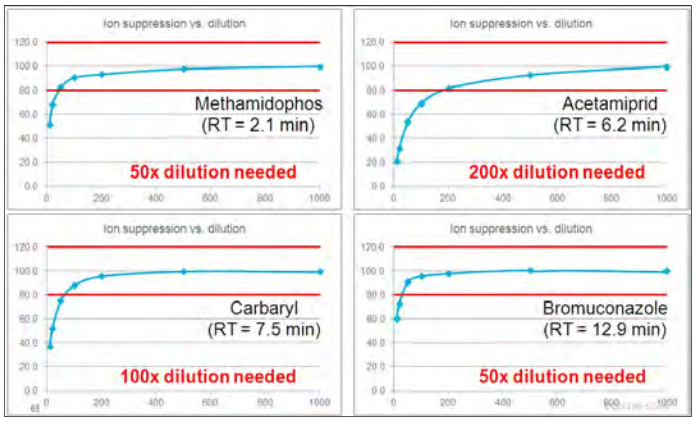

Figure 7. Ion suppression caused by peppermint tea was successfully reduced by dilution of 50 to 200x

Monitoring of Matrix Effects using QTRAP EMS Scanning

Acquiring full scan MS chromatograms is a valuable tool to monitor and understand matrix effects. Figure 8 shows an example of combining an MRM experiment and Enhanced MS (EMS) scanning when analyzing a black tea extract.

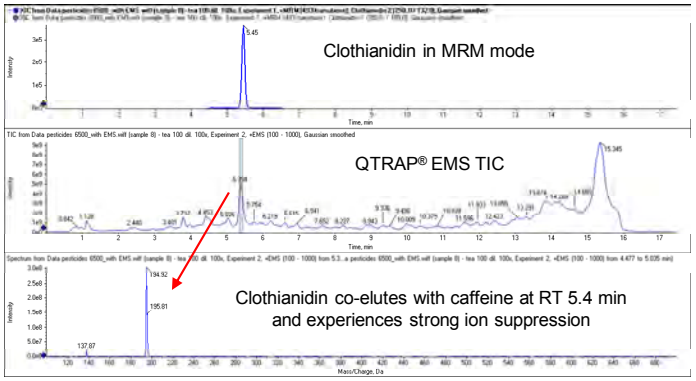

Figure 8. Monitoring of matrix effects by simultaneous acquisition of target MRM transitions and EMS scans, the strong ion suppression observed for Clothianidin can be explained by co-elution with caffeine at a much higher concentration

Extended Linear Dynamic Range

The new HED IonDrive™ detector allows taking advantage of sensitivity gains not at the expense of the dynamic range for quantitation. The detector enables ultra-fast pulse counting up to 10^8 cps without compromising data quality of low sensitivity ions. Up to 6 orders of magnitude linear dynamic range were reported.[2]

The extended linear dynamic range of the QTRAP® 6500 system can also be beneficial when quantifying larger panels of compounds covering a wide range of chemical properties (low sensitivity analytes to high sensitivity analytes). Examples of calibration lines obtained from the QTRAP 6500 and QTRAP 5500 systems are presented in Figures 9 a-c.

The extended linear dynamic range allowed easier and faster data processing and review since fewer points had to be excluded from the calibration line.

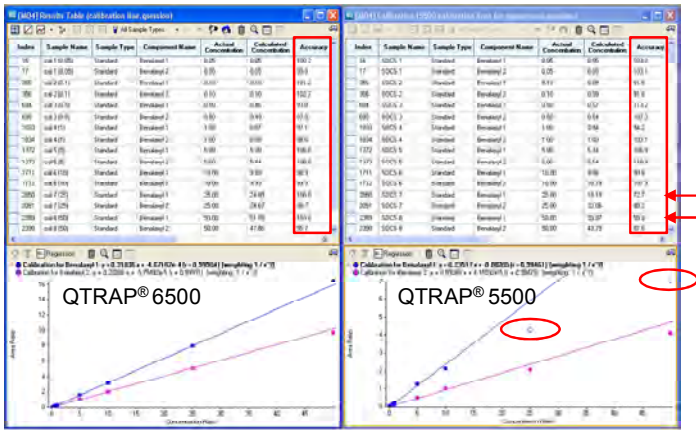

Figure 9a. Quantitation of Benalaxyl, a high sensitivity pesticide, 0.05 to 50 ng/mL, no points had to be excluded when the HED IonDrive™ detector was used due to extended pulse counting

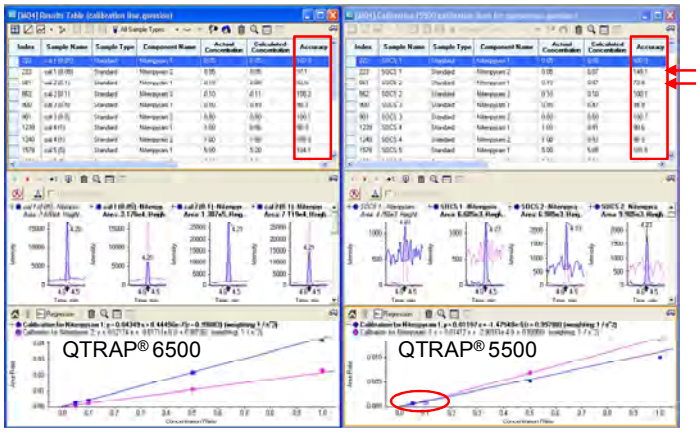

Figure 9b. Quantitation of Nitenpyram, a low sensitivity pesticide, 0.05 to 50 ng/mL, no points had to be excluded when the HED IonDrive™ detector was used due to increased sensitivity

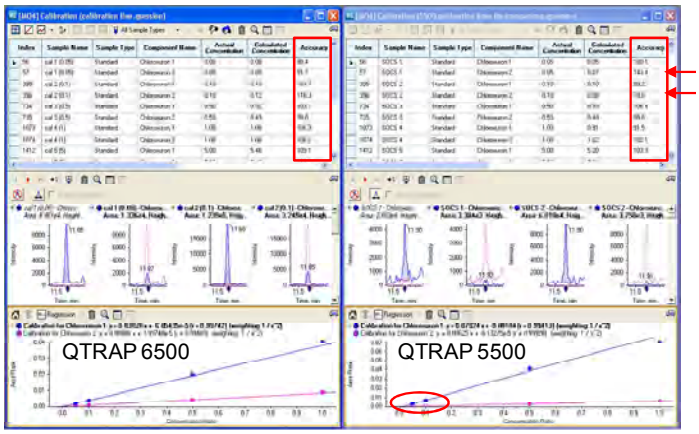

Figure 9c. Quantitation of Chloroxuron, a pesticide with a weak qualifier ion, 0.05 to 50 ng/mL, no points had to be excluded when the HED IonDrive™ detector was used to extended pulse counting and increased sensitivity

Automatic Compound Identification using MRM Ratios and Full Scan MS/MS Library Searching

Guidelines for food residue analysis require the identification of MRL exceeding compounds and unusual residues.[3]

LC-MS/MS can be used in different ways to acquire the mass spectrometric information needed to identify compounds with high confidence, including ratio of quantifier and qualifier MRM transition, full scan product ion spectra (i.e. Enhanced Product Ion (EPI) scanning using QTRAP functionality), or accurate mass measurements.[4]

Sample data was processed using MultiQuant™ software version 2.1 with the 'Multicomponent' query. Query files are customizable commands to perform custom querying of the result table. The 'Multicomponent' query automatically calculates and compares MRM ratios for compound identification and highlights concentrations above a user specified maximum residue level. An example of the results and peak review after running the query file is shown in Figure 10.

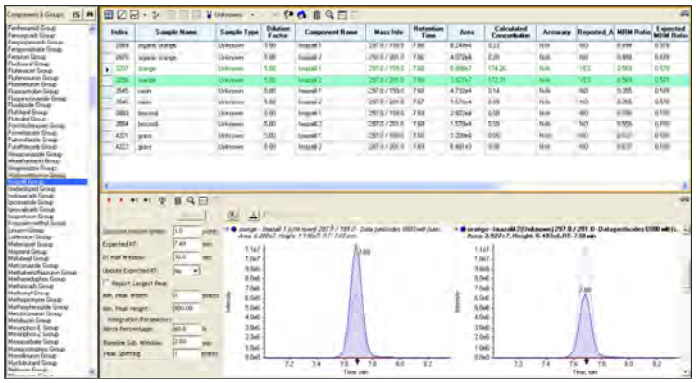

Figure 10. Automatic reporting of pesticides using the 'Multicomponent' query in MultiQuant™ Software: Imazalil was flagged in the result table because of a concentration above a user defined threshold and positive identification using the MRM ratio

Despite the high selectivity of MRM detection, there is always a risk of false positive findings due to interfering matrix signals. Identification based on full scan MS/MS data searched against mass spectral libraries significantly increases confidence in identification. Here MS/MS spectra acquired in the EPI mode of the QTRAP® 6500 system were searched against the iMethod™ pesticide library (version 1.0 for LibraryView™ Software). Library searching was performed in LibraryView™ Software for easy data review and reporting (Figure 11).

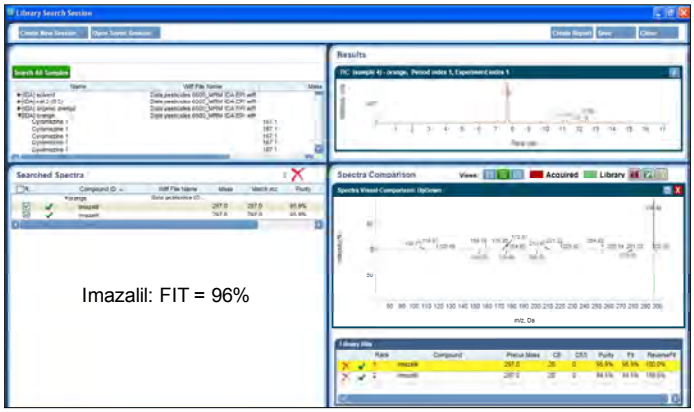

Figure 11. Review of MS/MS search results in LibraryView™ Software

Summary

The new SCIEX QTRAP 6500 system was used for multi-pesticide quantitation and identification in complex food samples. The increased sensitivity was used to extend the scope of the method and to dilute matrix extracts extensively to eliminate matrix effects. The extended linear dynamic range allowed easier and faster data processing and review while monitoring high sensitivity and low sensitivity pesticides in a single method. QTRAP scanning was used to investigate the presence of matrix components and to identify targets with high confidence through library searching.

Acknowledgement

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References

1 Carmen Ferrer et al.: 'Overcoming Matrix Effects Using the Dilution Approach in Multiresidue Methods for Fruits and Vegetables' J. Chromatogr. A 1218 (2011) 7634-7639

2 Michael Jarvis: 'Ultra-Sensitive Analysis of Aldosterone in Serum Using the SCIEX Triple Quad™ 6500 LC-MS/MS System' Application Note SCIEX (2012) 5730212-01

3 Document N° SANCO/12495/2011 'Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed'

4 André Schreiber et al.: 'Target and Non-Target Screening for Chemical Residues in Food Samples using the SCIEX TripleTOF® 4600 System and Intuitive Data Processing Tools' Application Note SCIEX (2012) 5680212-01

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