# Elimination of Matrix Effects and Interferences when Performing High Sensitivity and High Selectivity LC-MS/MS Screening

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## OVERVIEW

Here we present a new multi-pesticide screening method using QuEChERS extraction followed by UHPLC-MS/MS analysis to identify and quantify pesticides in food and botanical samples. The new AB SCIEX QTRAP® 6500 system operated in Multiple Reaction Monitoring (MRM) mode using the *Scheduled* MRM™ algorithm provides highest selectivity, sensitivity, and data quality. QTRAP® MS/MS spectra were acquired to enable compound identification with high confidence based on mass spectral library searching. In addition, Differential Mobility Separation (DMS) using SelexION™ technology helped to reach required detection limits for polar and small molecular weight compounds, such as triazole derivative metabolites.

## 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 AB SCIEX QTRAP® 6500 system uses *Ion*Drive™ technology to address these challenges by improving ionization efficiency, increasing sensitivity, extending the linear dynamic range, QTRAP® scanning to increase confidence in identification, and SelexION™ separation to increase selectivity of detection.



## EXPERIMENTAL

- The iDQuant™ 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.
- 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.
- A flow rate of 350 µL/min was used.
- The injection volume was set to 10 µL.
- The AB SCIEX QTRAP® 6500 system was operated with IonDrive™ Turbo V ion source using the electrospray ionization (ESI) probe. The ion source temperature was optimized to 450 ° C.
- A total of 493 Multiple Reaction Monitoring (MRM) transitions (two transitions per pesticide plus one 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.
- QTRAP® full scan MS and MS/MS scanning was explored to monitor matrix effects and to increase confidence in identification by MS/MS library searching.
- DMS using SelexION™ technology was used to enhance selectivity of LC-MS/MS detection of triazole derivative metabolites.

## RESULTS

**Method Transfer and Increased Sensitivity**
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.

The new design of the ion source and the dual stage design of the IonDrive™ QJet ion guide result in increased sensitivity (Figure 1).

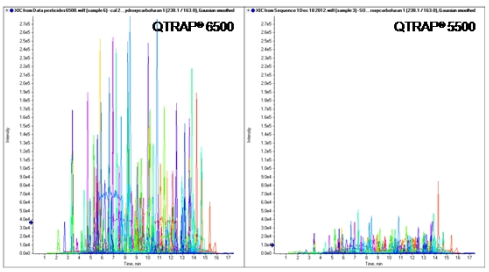


**Figure 1.** Sensitivity comparison of a 0.1 ng/mL standard analyzed using the QTRAP® 6500 system (left) and QTRAP® 5500 system (right)

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 2).

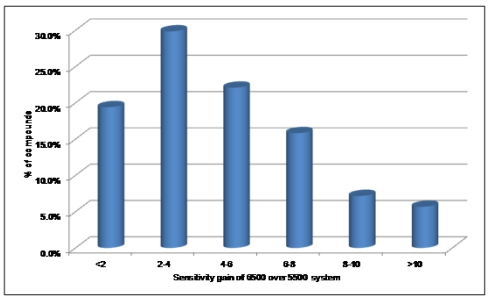


**Figure 2.** Distribution of sensitivity gain for multi-pesticide analysis with an average gain of 4.7.

**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 3 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%.

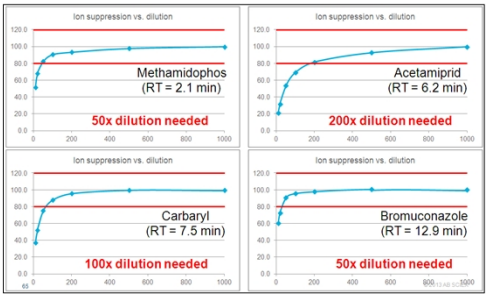


**Figure 3.** Ion suppression caused by peppermint tea was successfully reduced by dilution of 50 to 200x

**Monitoring of Matrix Effects using QTRAP® MS Scanning**
Acquiring full scan MS chromatograms is a valuable tool to monitor and understand matrix effects. Figure 4 shows an example of combining an MRM and Enhanced MS (EMS) scanning when analyzing a black tea extract.

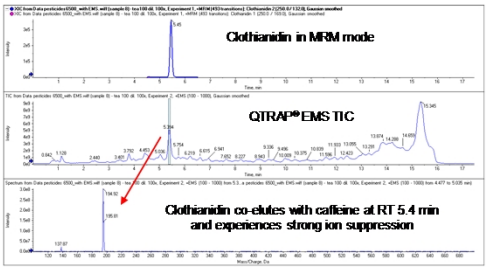


**Figure 4.** Monitoring of matrix effects by simultaneous acquisition of target MRM transitions and EMS scans, the ion suppression observed for Clothianidin can be explained by co-elution with highly concentrated caffeine.

**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 Figure 5. 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 5.** Quantitation of Benalaxyl, a high sensitivity pesticide, 0.05 to 50 ng/mL, no points had to be excluded due to extended pulse counting (left) and Nitenpyram, a low sensitivity pesticide, 0.05 to 50 ng/mL, no points had to be excluded due to increased sensitivity (right) of the QTRAP® 6500 system with HED IonDrive™ detector

**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 (see poster PA14).

Sample data was processed using MultiQuant™ software version 2.1 with the 'Multicomponent' query to automatically flag compounds above a user specified maximum residue level and to calculate MRM ratios for identification. 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. Library searching was performed in LibraryView™ software for easy data review and reporting (Figure 6).

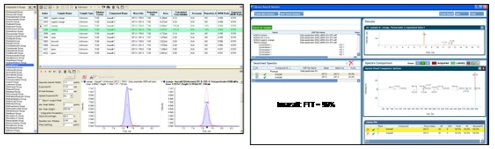

**Figure 6.** Identification based in MRM ratios and MS/MS library search

**Increased Selectivity using DMS with SelexION™ Technology**
Differential mobility separation can be used to remove matrix interferences. The example shown in Figure 7 compares the detection of 1,2,4-triazole (TRZ) and triazole acetic acid (TAA) in different matrices at 10 µg/kg.

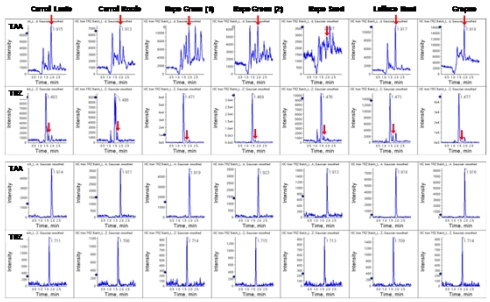


**Figure 7.** Detection TRZ and TAA without DMS (top) and with DMS (bottom)

## SUMMARY

The new AB 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, and SelexION™ separation to increase selectivity of detection.

## 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 AB SCIEX Triple Quad™ 6500 LC/MS/MS System' Application Note AB SCIEX (2012) 5730212-01
3 Document N ° SANCO/12495/2011 'Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed'

## TRADEMARKS/LICENSING

For Research Use Only. Not for use in diagnostic procedures.