

# Non-Target and Unknown Screening of Food Samples using High Resolution LC-MS/MS



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## OVERVIEW

Here we present results of using a novel approach of comparative unknown screening to identify unexpected substances, such as residues and contaminants, in food. Fruit and vegetable samples were extracted using a QuEChERS procedure and analyzed with core-shell particle reversed phase LC. High resolution and accurate mass MS and MS/MS information was collected in a single run using information dependent acquisition on the SCIEX X500R QTOF system. Data was processed using the new SCIEX OS software utilizing sample-control-comparison and automated formula finding, library searching, and ChemSpider searching.

## INTRODUCTION

LC-MS/MS using Electrospray Ionization (ESI) is a powerful analytical tool for the analysis of a wide molecular weight range of polar, semi-volatile and thermally labile compounds. Especially triple quadrupole based mass analyzers are popular for targeted quantitation of hundreds of food contaminants in a single analysis because of their extra degree of selectivity and sensitivity when operated in Multiple Reaction Monitoring (MRM) mode. Advancements in LC-MS/MS technology, including hybrid systems like triple quadrupole linear ion trap (QTRAP<sup>®</sup>) and quadrupole-quadrupole Time-of-Flight (QTOF), now provide the ability to perform targeted and non-targeted screening on a routine basis. However, full scan chromatograms are very rich in information and contain easily thousands of ions from both any compounds present in the sample as well as from the sample matrix itself. Thus, powerful software tools are needed to explore the high resolution and accurate mass data generated.

Here we present screening results of using a novel approach of comparative unknown screening using a generic extraction and LC separation procedure followed by high resolution and accurate mass MS/MS detection. TOF-MS and MS/MS data were acquired using the SCIEX X500R QTOF system.

TOF-MS and MS/MS information was used to screen for unexpected chemicals, such as residues and contaminants, in food. Sample-control-comparison was performed to separate matrix and sample specific signals from true contaminations. Ions of interest were automatically searched against libraries and processed using formula finding and ChemSpider searching for identification. The new SCIEX OS software offers an easy to use and intuitive workflow to tentatively identify unexpected chemicals in food.

## EXPERIMENTAL

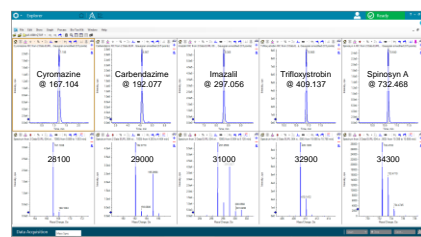
- Fruit and vegetable samples from a local supermarket
- Quantitation using a standard provided by the EURL
- QuEChERS extraction using Phenomenex roQ QuEChERS kit buffer-salt mix and dSPE kits following the European standard method 15662
- 10-20x dilution of sample extracts to minimize possible matrix effects
- UHPLC using a SCIEX ExionLC<sup>™</sup> AC system with a Phenomenex Kinetex Biphenyl column (50 x 2.1 mm, 2.6 μm)
- Gradient of water and methanol with 5 mM ammonium formate
- Flow rate of 0.5 mL/min
- Injection volume of 5 μL



- Detection using a SCIEX X500R 500 QTOF system with Turbo V<sup>™</sup> source operated in Electrospray Ionization (ESI)
- Continuous recalibration between injections using the Calibrant Delivery System (CDS) using a TwinSpray setup
- Information Dependent Acquisition (IDA):
  - TOF-MS survey scan 100-1000 Da (100 ms)
  - 10 dependent TOF-MS/MS scans 50-1000 Da (50 ms) using Collision Energy (CE) of 35 V with Collision Energy Spread (CES) of ±15 V
- Dynamic background subtraction (DBS) was activated for best IDA coverage, no inclusion list was used to allow retrospective unknown identification without the need for a second injection to acquire MS/MS data
- Non-target data processing using SCIEX OS software

## RESULTS

**SCIEX X500R QTOF System Performance Characteristics**  
Resolution > 20,000 (at full width half height) and mass accuracy <5 ppm is often sufficient to separate the analytes of interest from interfering matrices and, thus, is a requirement for compound identification in various guidelines.<sup>1,2</sup> The X500R QTOF system, using an N-optic design and a 4 mm orifice leading into the accelerator, provides resolving power of 25000 to 35000 for small molecular weight compounds and mass accuracy <2 ppm. The sensitivity and linear dynamic range of the X500R QTOF system is comparable to a QTRAP<sup>®</sup> 5500 system operated in MRM mode, allowing extract dilution to minimize ion suppression while detecting easily at 10 μg/kg levels (Figures 1 and 2).

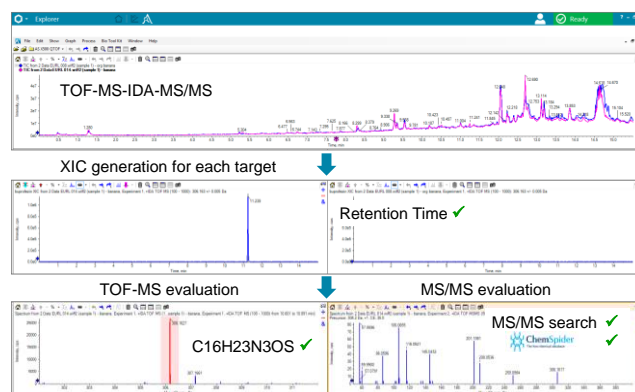


**Figure 1.** Sensitivity and resolving power for pesticide screening, compounds spiked at 20 ng/mL into a QuEChERS fruit extract



**Figure 2.** Linearity and mass accuracy for Paclobutrazol (0.1 to 10,000 ng/mL), linearity was achieved over 4 orders of magnitude, mass accuracy of less than 1 ppm was maintained even above the upper limit of quantitation ULOQ

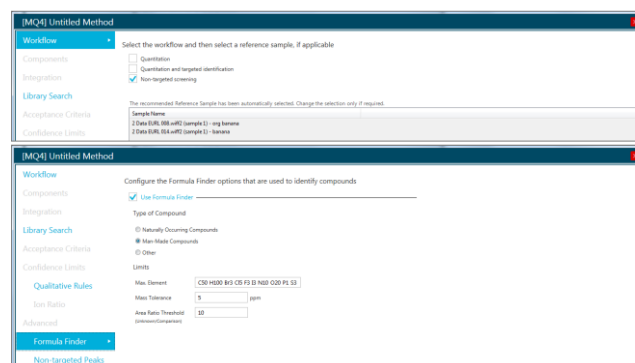
## Non-Target Data Analysis Workflow



**Figure 3.** XIC are generated using a non-target peak finding algorithm, XIC of sample and control are compared to differentiate relevant signals of contaminants from matrix signals, the MS and MS/MS information is automatically used for formula finding, MS/MS library searching, and ChemSpider searching, structures returned by ChemSpider are automatically compared to the HR-MS/MS spectrum to tentatively identify the compound

## Data Analysis Workflow in SCIEX OS Software

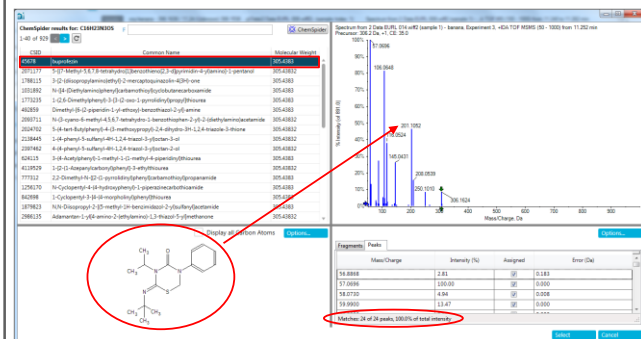
1) Define non-target peak finding and identification parameters



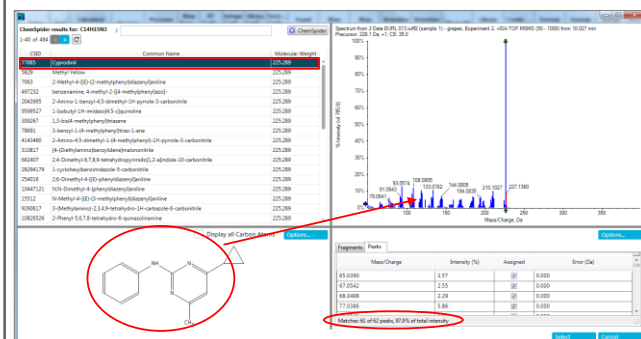
2) Automatic sample-control comparison and identification



3) ChemSpider Searching and Structure Elucidation



Tentative identification of buprofezin in banana after comparison to an extract of organic banana



Tentative identification of cyprodinil in grapes after comparison to an extract of organic grapes

## SUMMARY

A novel approach of comparative non-target screening to identify unexpected and unknown chemicals in food samples was developed and successfully applied to food samples. Fruit and vegetable samples were extracted using a QuEChERS procedure and analyzed with core-shell particle reversed phase LC. High resolution and accurate mass MS and MS/MS information was collected in a single run using information dependent acquisition on the SCIEX X500R QTOF system. Data was processed using the new SCIEX OS software.

Sample-control-comparison was performed to differentiate matrix and sample specific signals from true contaminations. Ions of interest were automatically searched against mass spectral libraries and processed using automatic formula finding and ChemSpider searching for identification. Structures returned by ChemSpider are automatically compared against the HR-MS/MS spectrum for tentative identification. The new SCIEX OS software offers an easy to use and intuitive workflow to tentatively identify unexpected chemicals in food.

## REFERENCES

- EU Commission Decision 'concerning the performance of analytical methods and the interpretation of results' #2002/657/EC
- SANCO Document: 'Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed' #SANCO/12495/2011

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