Target and Non-Target Accurate Mass Screening for Pesticides using LC-MS/MS

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OVERVIEW
Here we present results of using a novel approach of accurate mass LC-MS/MS to identify and quantify chemical residues in food and simultaneously search for unknown and unexpected chemicals. Fruit and vegetable samples were extracted using a QUECHERS procedure and analyzed with 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 AB SCIEX TripleTOF 4600 and 5600 system. Data was processed using the new MasterView™ software.

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 extreme 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®) and quadrupole-tripole Time-of-Flight (TripletOP™), 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 residue results of using a novel approach of comparative multi-target 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 AB SCIEX TripleTOF 4600 and 5600 systems. Samples were also compared against control (blank) sample to further identify unexpected chemicals.

TOF-MS information was used to screen for and identify targeted food contaminants. Quantitative information was achieved by performing single concentration standard addition at the level of the Maximum Residue Level (MRL). Identification was based on retention time, accurate mass quasi-molecular ion, isotopic pattern and MS/MS fragmentation pattern (library searching). The molecular fingerprint saved into MS/MS spectra allowed to differentiate isomeric compounds and greatly reduced the number of potential false positive results. The MasterView™ software allows quick processing and easy result review and reporting capabilities.

EXPERIMENTAL
• Fruit and vegetable samples
• Quantitation using the EQuanti™ standards kit for pesticide analysis
• QUECHERS extraction following guideline EN 15662:2007 using commercial QUECHERS kits
• 5-20x dilution of sample extracts to minimize possible matrix effects
• Restek Ultra Aqueous C18 (100 x 2.1 mm) 3 µm column
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• Restek Ultra Aqueous C18 (100 x 2.1 mm) 3 µm column
• Linear gradient from 5% methanol in water to 100% methanol in water in 10 min
• Sample-control comparison was also used to screen for unknown and unexpected compounds

RESULTS

Targeted Data Analysis Workflow

Quantitative Data Analysis Workflow in MasterView™ Software

1) Open data file(s), build or open target XIC list, define concentrations and standard addition at the level of the Maximum Residue Level (MRL). Identification was based on retention time, accurate mass quasi-molecular ion, isotopic pattern and MS/MS fragmentation pattern (library searching).

2) Continuous recalibration between injections using the Calibrant Delivery System (CDS)
3) Results review using ‘traffic lights’, and MS and MS/MS spectra (Figure 2)

Figure 1. XIC are generated based on user input (formula and expected retention time of all target analytes) – MS and MS/MS information is automatically evaluated based on user defined intensity threshold or S/N, confidence in identification is ranked based on retention time matching, mass accuracy, isotopic fit, and MS/MS library searching.

Data Analysis Workflow in MasterView™ Software

1) Open data file(s), build or open target XIC list, define concentrations and threshold (ratio of unknown sample(s) and control) for quantitative comparison
2) Set confidence settings and start processing by clicking ‘Process’
3) Results review using ‘traffic lights’, and MS and MS/MS spectra (Figure 2)