Target and Non-Target Screening for Pesticide Residues in Food Samples using the AB SCIEX TripleTOF™ 5600 System

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Overview

The new AB SCIEX TripleTOF™ 5600 System was used to screen for pesticide residues in extracts of fruit and vegetable samples. High resolution and accurate mass MS was used to quantify pesticides with excellent accuracy and high reproducibility. A catalogue of accurate mass information and retention times was used for targeted data processing. Fast Information Dependent Acquisition (IDA) MS/MS spectra were used to additionally confirm the identity of detected pesticides based on mass spectral library searching. The acquired full scan MS and MS/MS data can further be used to retrospectively mine data to identify non-targeted and unexpected compounds.

Introduction

Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) using Electrospray Ionization (ESI) is a powerful analytical tool for the analysis of polar, semi-volatile, and thermally labile compounds of a wide molecular weight range, such as pesticides, veterinary drugs, mycotoxins and other food residues. Mass analyzers based on triple quadrupole technology operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results and are therefore well established for multi-target screening of food contaminants. Compound identification is typically performed by monitoring of two MRM transitions and calculating the area ratio of quantifier and qualifier ion. The potential risk of false positive and negative results can be further minimized by acquiring Enhanced Product Ion (EPI) spectra followed by library searching using a QTRAP® hybrid LC/MS/MS System. MS/MS spectra contain the complete molecular fingerprint of a molecule and thus providing an added degree of confidence for compound identification.

However, the use of triple quadrupole based mass analyzers is limited to targeted screening and quantitation. But there is an increasing demand for retrospective and non-targeted data analysis. The new AB SCIEX TripleTOF™ 5600 LC/MS/MS System using the Accelerator TOF™ Analyzer and continuous recalibration for EasyMass™ Accuracy combined with powerful software tools, like PeakView™ and MultiQuant™, is capable of performing targeted and non-targeted screening in a single LC-MS/MS run. Highly sensitive full scan MS spectra can be measured with unmatched speed, high resolution and mass accuracy allowing accurate and reproducible quantitation of targeted compounds at the lowest concentration levels on an UHPLC time scale. A catalogue containing accurate mass information of approximately 1700 pesticides and metabolites was used for data processing. Fast IDA of high resolution and accurate mass MS/MS spectra was used to additionally confirm the identity based on mass spectral library searching.

The acquired full scan MS data can further be used to retrospectively mine data for non-targeted compounds. The information of the accurate molecular ion, isotopic pattern, and detected fragment ions can be used to characterize the structure of unexpected compounds.

This paper describes the use of the new AB SCIEX TripleTOF™ 5600 system for the targeted screening and quantitation of food contaminants and discusses the possibility of retrospective data processing for the identification of non-targeted compounds.

Method Details

- QuEChERS extraction of food samples[1-2] and 10x dilution to minimize possible matrix effects
- Ultra High Pressure Liquid Chromatography using a Shimadzu UFLC$_{XR}$ system with a Restek Ultra II Aqueous C18 2.2µm (100x2.1mm) column and a gradient of water and methanol with ammonium formate buffer
- Flow rate of 0.5mL/min
- Injection volume of 10µL
- Total run time of 15min
- AB SCIEX TripleTOF™ 5600 System with Accelerator TOF™ Analyzer and Electrospray Ionization source
- Continuous recalibration for EasyMass™ Accuracy
- IDA experiment with a TOF-MS survey (accumulation time of 100ms) and up to 30 dependent MS/MS scans (accumulation time of 25ms)
- Standardized collision energy (CE) = 35V with collision energy spread (CES) = ±15V
- Data processing using PeakView™ Software and MultiQuant™ Software

Results and Discussion

The new AB SCIEX TripleTOF™ 5660 system with Accelerator TOF™ Analyzer provides high resolution of up to 40,000, dependent on the mass detected, and stable mass accuracy of ~1 ppm at fastest acquisition speed in MS and MS/MS mode. Examples of MS/MS resolution acquired using scan speeds between 1 and 100Hz at mass 315 and 86 Da are shown in Figure 1.

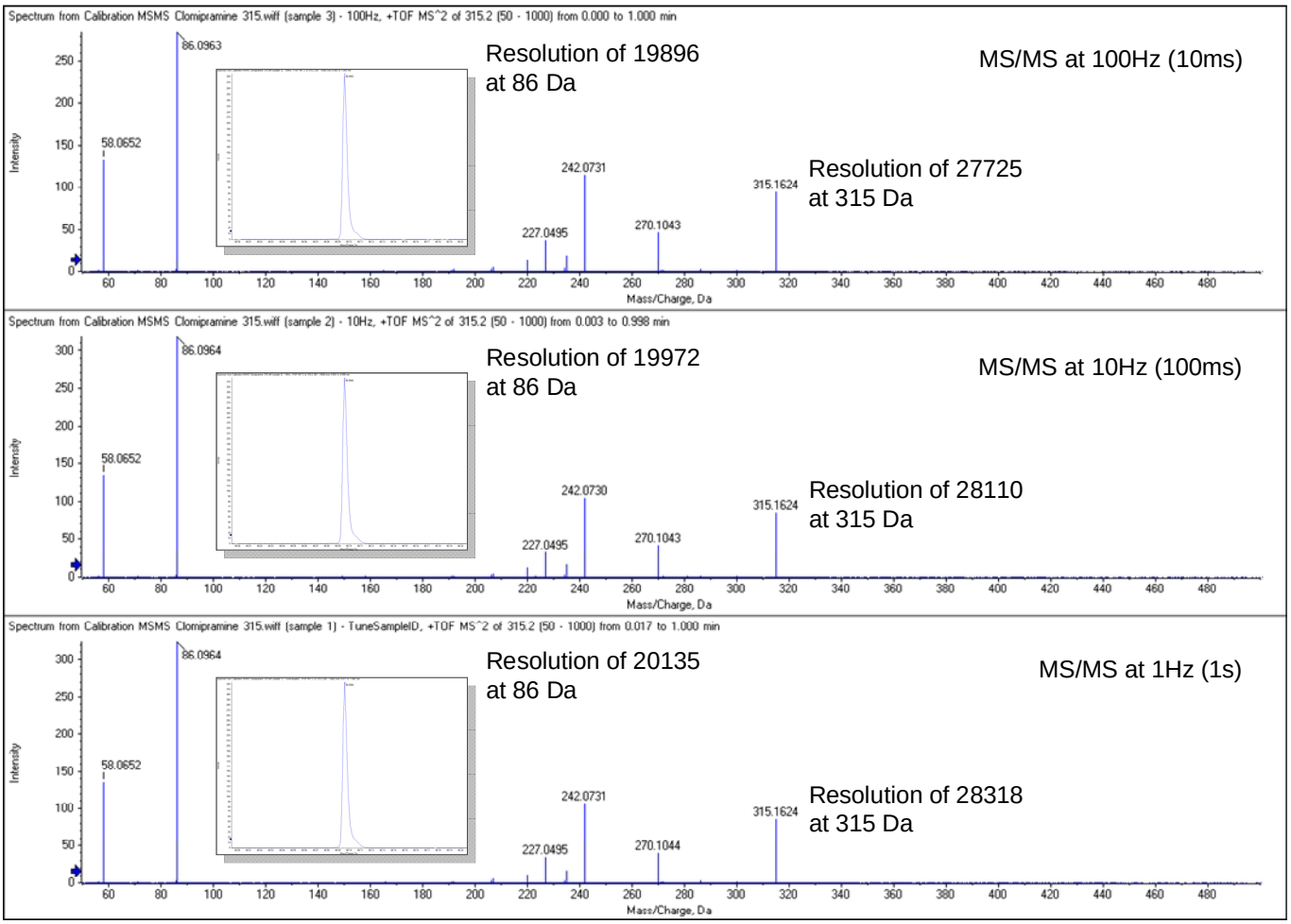

Figure 1. Highest resolution at every scan speed over the entire mass range with stable mass accuracy of ~1 ppm

A variety of fruit and vegetable samples were extracted using a QuEChERS procedure, diluted 10 times to minimize possible matrix effects, such as ion suppression or enhancement, and analyzed by high resolution and accurate mass LC-MS/MS using the developed method for pesticide residues. An IDA cycle consisting of a 100ms TOF-MS survey and up to 30 dependent MS/MS experiments of 25ms accumulation time was sufficient to acquire enough data points across the LC peak to allow reproducible and accurate quantitation. Example calibration lines of two selected pesticides and statistical results of repeat injections at different concentration levels are presented in Figure 2. The sensitivity, linearity and reproducibility of the developed method easily meets regulatory requirements for multi-pesticide screening.

Selected samples were reanalyzed with a targeted MRM screening method on a QTRAP® 5500 System operated in MRM mode using the Scheduled MRM™ Algorithm. This high end triple quadrupole instrument is known to provide sufficient sensitivity to measure pesticides at concentrations of 1µg/kg (1ppb) in food samples. A 10x dilution was used on samples resulting in an extract concentration of 0.1ppb.

A comparison of the Signal-to-Noise ratios (S/N) of Cyprodinil and Thiabendazole detected in cucumber and spinach extracts is presented in Figure 3. The S/N using both analytical procedures is comparable. However, a direct comparison of both analytical procedures is very challenging since selectivity is gained through two different mechanisms. While full scan MS' sensitivity is solely based on the molecular ion and high resolution is used to remove interferences, in MRM mode selectivity is achieved by double mass filtering in Q1 and Q3 of the triple quadruple system and additionally depends on the fragmentation efficiency of the detected analyte. For example, Cyprodinil, a pesticide with a dominant molecular ion, shows high sensitivity in full scan MS mode and the efficient MS/MS fragmentation of Thiabendazole results in higher S/N in MRM mode.

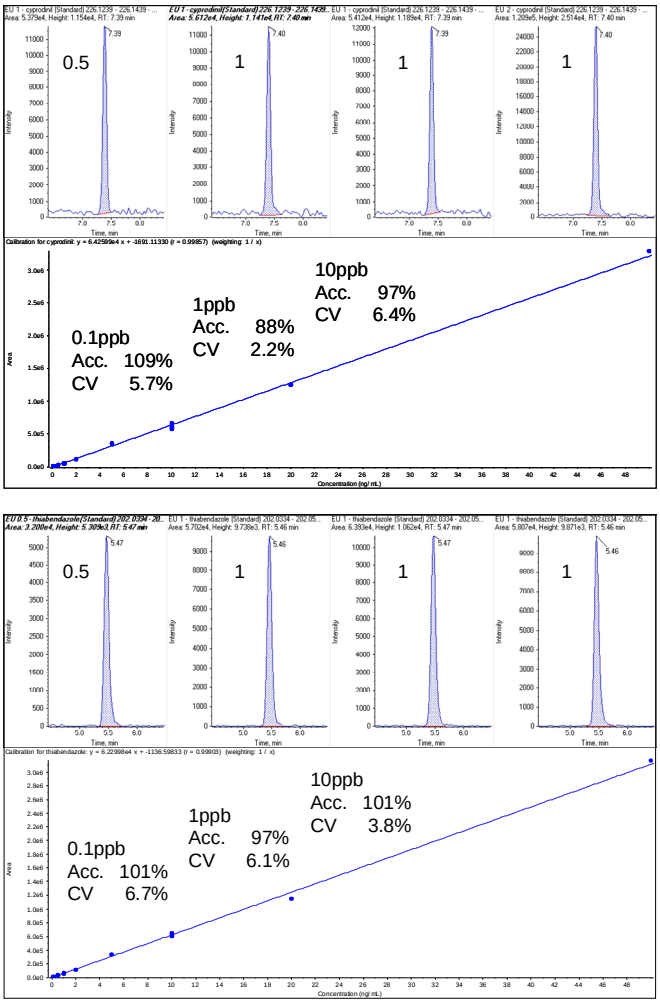

Figure 2. Calibration lines (0.05 to 50ppb) and statistical results (triplicates) at different concentrations for the two selected pesticides Cyprodinil and Thiabendazole

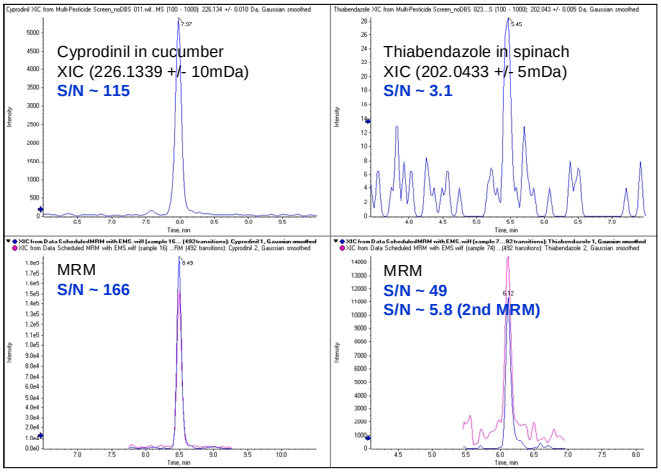

Figure 3. Comparison of Signal-to-Noise (S/N) of pesticides detected in fruit extracts using the AB SCIEX TripleTOF™ 5600 System in TOF-MS mode (100ms accumulation time) vs. the QTRAP® 5500 System using Scheduled MRM™; the difference in S/N depends of the fragmentation efficiency of the detected analyte

A catalogue of accurate molecular ions was generated containing the information of ~ 1700 pesticides using an online database of pesticides to screen for pesticides in food samples.[3]

The example data presented in Figure 4, 5 and 6 were processed using the XIC Manager in PeakView™ Software. Extracted Ion Chromatograms (XIC) were automatically generated for each targeted analyte and compared against a user defined threshold. Pesticides with an XIC above the threshold were highlighted in the result table. The information of the isotopic pattern of the detected molecular ion and the MS/MS spectra searched against an LC/MS/MS library was further used to confirm the identity of the detected pesticide.

The acquisition of full scan MS data enables retrospective data mining of initially non-targeted analytes. For example, the red bell pepper extract presented in Figure 7 was tested positive for 12 pesticide residues: Carbaryl, Clothianidin, Dimethoate, Imidacloprid, Methamidophos, Metalaxyl, Myclobutanil, Omethoate, Propamocarb, Spinosyn A, Spinosyn D, and Thiamethoxam. 6 of these pesticides were confirmed using GCxGC-TOFMS screening. But also 6 additional compounds were identified using the orthogonal technique, including the pesticide Acephate which was not included in the original targeted screening using LC-MS/MS.[4] The XIC manager was used to retrospectively screen for Acephate and the presence of this residue in the red bell pepper extract was confirmed based on the presence of an accurate mass molecular ion (0.1ppm) and the characteristic fragment ions in MS/MS mode.

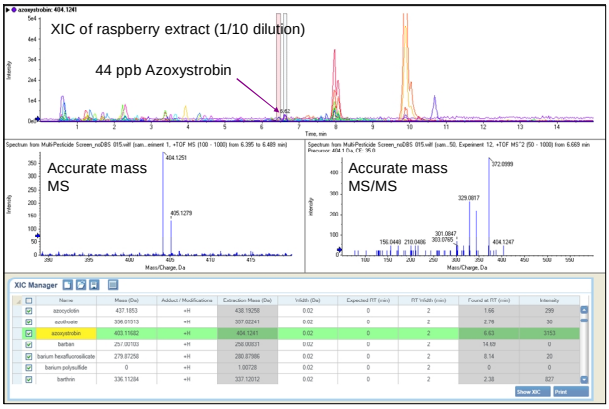

Figure 4: The pesticides Azoxystrobin (and Cyprodinil, spectra not shown) were identified and quantified using accurate mass MS and MS/MS information in a raspberry extract using the XIC Manager of PeakView™ Software

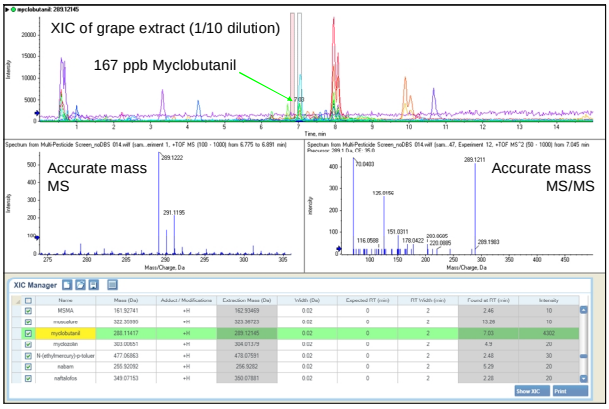

Figure 5: The pesticides Myclobutanil (Iprodion, Pyrimethanil, and Spinosyn A and D, spectra not shown) were identified and quantified using accurate mass MS and MS/MS information in a grape extract using the XIC Manager of PeakView™ Software

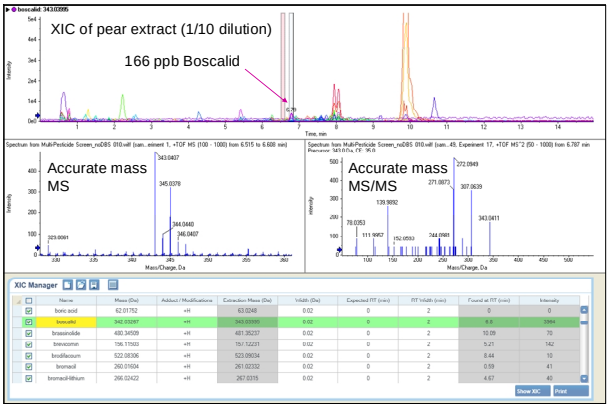

Figure 6: The pesticides Boscalid (Pyraclostrobin, Spinosyn A and D, and Trifloxystrobin spectra not shown) were identified and quantified using accurate mass MS and MS/MS information in a pear extract using the XIC Manager of PeakView™ Software

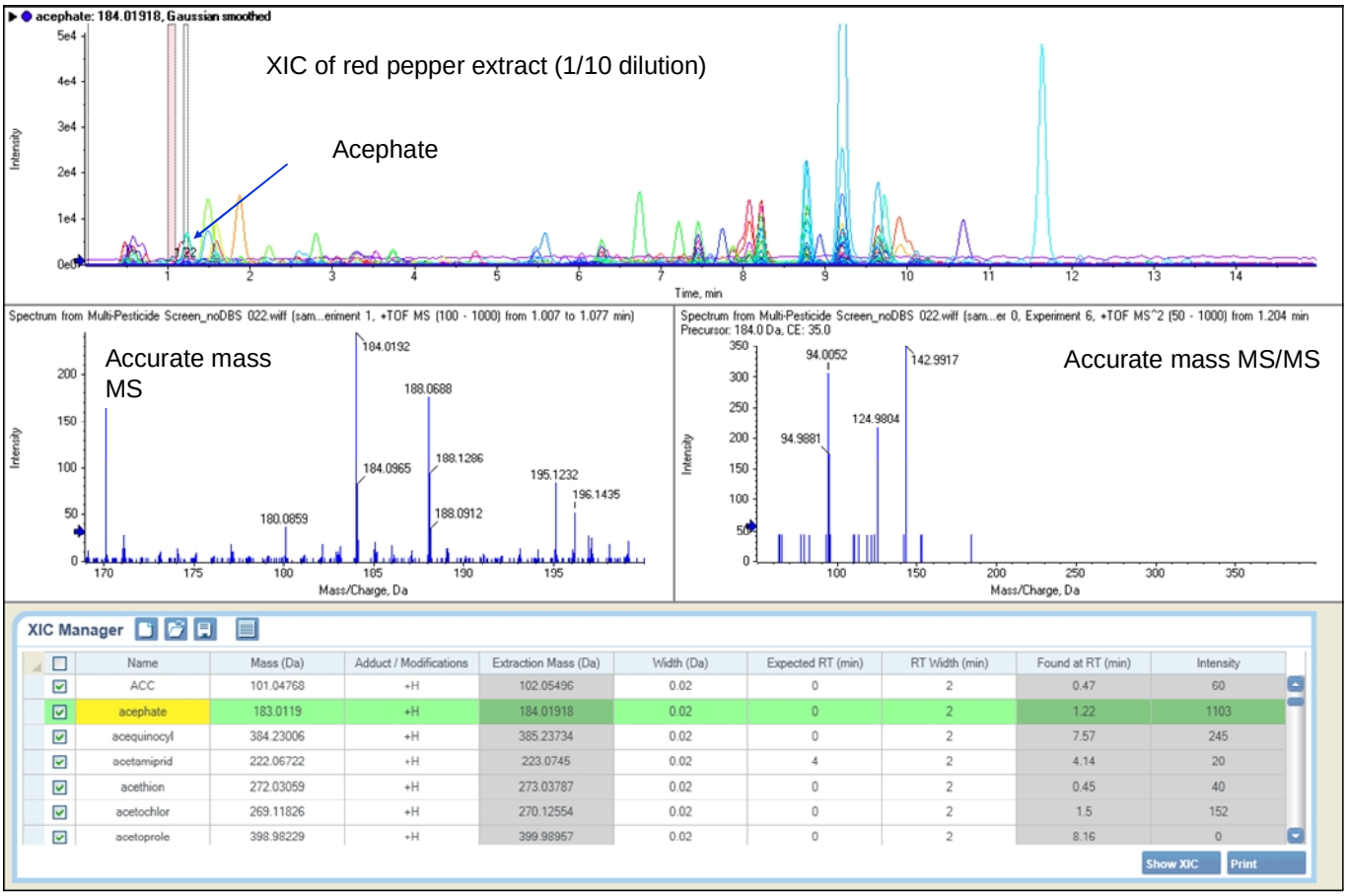

Figure 6: The pesticide Acephate was retrospectively confirmed in an extract of red bell pepper after previous GCxGC-TOFMS screening based on accurate mass MS and MS/MS information

Conclusion

The new AB SCIEX TripleTOF™ 5600 LC/MS/MS System was used to screen for, quantify and identify pesticide residues in food extracts. The high sensitivity, resolution and unmatched scan speed of Accelerator TOF™ Analyzer coupled to UHPLC enables reproducible and accurate quantitation at regulated maximum residue levels. Extract dilution is possible to minimize possible matrix effects. Accurate mass MS/MS spectra were searched against an existing LC-MS/MS library of pesticides to confirm the identity of quantified analytes. In addition, the acquired MS and MS/MS data can be used to retrospectively identify unexpected and non-targeted food residues.

Acknowledgements

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