

Profiling of Botanical Samples to Identify Unknown Chemical Contaminants and the Authenticity of Natural Ingredients Using High Resolution and Accurate Mass MS/MS



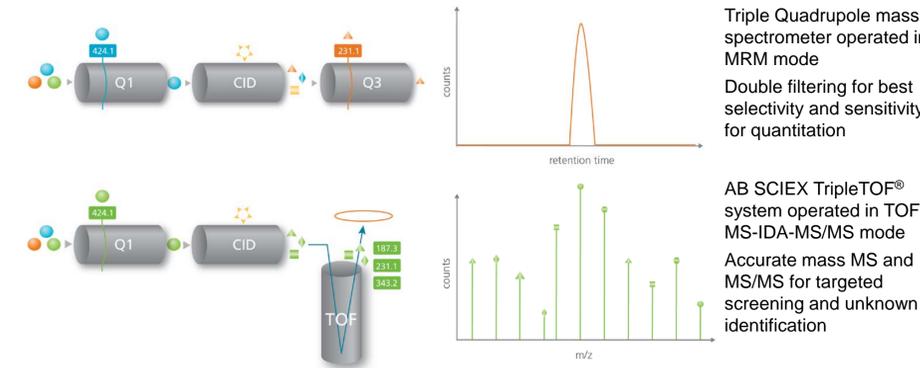
André Schreiber¹, Yun Yun Zou¹, and Jon Wong²

¹ AB SCIEX Concord, Ontario (Canada), ² FDA – CFSAN College Park, MD

INTRODUCTION

LC-MS/MS is routinely used to analyze food and botanical samples for hundreds of chemical contaminants in a single analysis. Triple quadrupole based mass analyzers are preferred for these analyses because of their high selectivity and sensitivity. However, in recent years there is a growing movement to analyze food beyond target compound lists, and a shift towards non-targeted or general unknown screening. This change can be explained by the recent high profile food residue scandals, such as the detection of melamine in infant formula, the identification of the non-registered pesticide isofenphos-methyl in strawberries, and the identification of counterfeit botanical products and herbal supplements. Because neither of these chemicals were targeted as potential contaminants or authenticity markers they did not show up in conventional analyses; further investigation of food samples was not required until people became ill.

Accurate mass LC-MS/MS using the AB SCIEX TripleTOF[®] 5600 system was used to acquire full scan MS and MS/MS data of extracts of a variety of botanical and food samples, including ginseng, hops, broccoli, cabbage. Possible data processing strategies for the challenging task of unknown screening were investigated. By performing statistical data analysis, unique contaminants and authenticity markers were detected. Empirical formula calculation, followed by searching of the internet for possible structures, and MS/MS fragment ion prediction was used to identify compounds and to characterize chemical structures.



MATERIALS AND METHODS

Different botanical samples were extracted using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe), acetonitrile extraction and partitioning, followed by dispersive SPE cleanup. Extracts were diluted 5x to minimize possible matrix effects, like ion suppression. LC separation was performed using different RP-LC phases with gradients of water/methanol or water/acetonitrile with volatile buffers, like ammonium formate or formic acid.

Sample were analyzed using an AB SCIEX TripleTOF[®] system with DuoSpray[™] source operated in electrospray ionization (ESI). The mass spectrometer was automatically and continuously calibrated between injections using the Calibrant Delivery System (CDS). Information Dependent Acquisition (IDA) methods were used containing a TOF-MS survey scan 100-1000 Da (100 ms) and up to 20 dependent TOF-MS/MS scans 50-1000 Da (50 ms) using Collision Energy Spread (CE = 35 V with CES = ± 15 V). CES was found to give more characteristic and reproducible MS/MS spectra for compound identification in contrast to single energy spectra.

RESULTS

TripleTOF[®] Performance Characteristics and use for Identification

High resolution and accurate mass LC-MS/MS chromatograms contain comprehensive information of all molecules present in the sample that are amenable to the ionization technique and polarity used. Narrow extracted ion chromatograms (XIC) can be generated to selectively screen for targeted compounds. 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.^{1,2}

Examples of TOF-MS and MS/MS spectra to highlight achieved resolution and mass accuracy are presented in Figures 1 to 4.

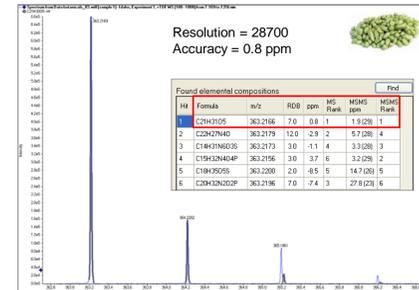


Figure 1. TOF-MS resolution and mass accuracy of humulone detected in hop extract, overlay of the acquired spectrum (blue) and theoretical isotopic pattern (grey)

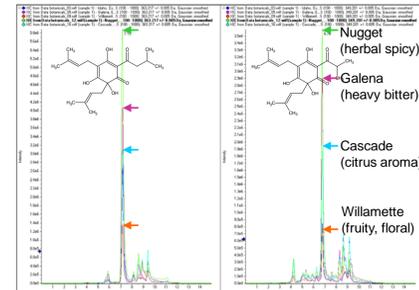


Figure 2. Extracted ion chromatograms of humulone and cohumulone in different hops (XIC of 363.2166 Da and 349.20010 Da ± 5 mDa, respectively)

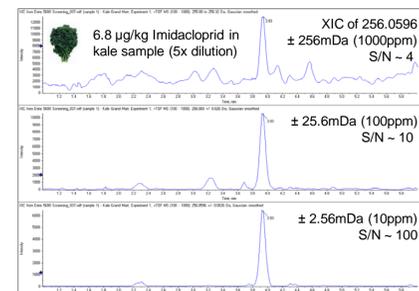


Figure 3. Quantitation of imidacloprid in a kale extract, TOF-MS resolution can be used to extract narrow ion chromatograms (XIC) to increase selectivity for best S/N

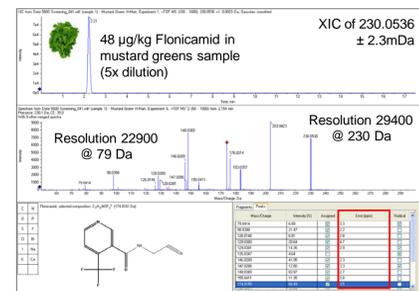


Figure 4. Identification of fonicamid in a mustard greens extract, TOF-MS/MS spectra can be used to identify target compounds based on mass spectral library searching or unknowns using fragment interpretation tools

Using TripleTOF[®] for Quantitative Screening and Identification with Highest Confidence

The XIC Manager software was used to screen for and identify pesticides in sample extracts. The XIC Manager consists of a table for defining a list of masses or formulae to generate XIC, and the ability to review the results for the identification of the detected compounds. High confidence in results is based upon retention times, accurate mass, isotopic pattern and MS/MS library searching. Confidence data of compound identification is visualized using 'traffic lights'.³

Examples of automatic identification of pesticides in clementine and raspberry extracts are shown in Figures 5 to 6.

The XIC manager can also quantitatively compare samples to highlight identified compounds above a target concentration. The software compares the sample data with a standard injection and automatically highlights all findings above a user defined threshold. Figure 7 shows an example of identification of imidacloprid, metalaxyl, and spirotramat in an Chinese broccoli extract at a concentration greater than 5 µg/kg.

After identification, the accurate mass information was exported into MultiQuant[™] software for external calibration (comparison to calibration line based on solvent standards). Figure 8 shows method performance criteria of the quantitation of imidacloprid. 23 µg/kg of imidacloprid were found in Chinese broccoli.

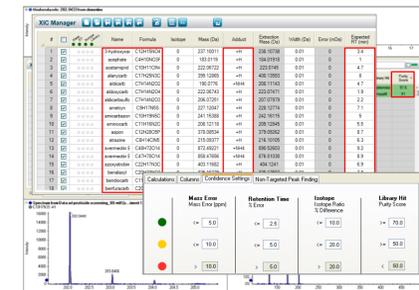


Figure 5. Processing options in the XIC Manager, XICs are automatically calculated after providing molecular formula and adduct, additionally retention times can be used for screening

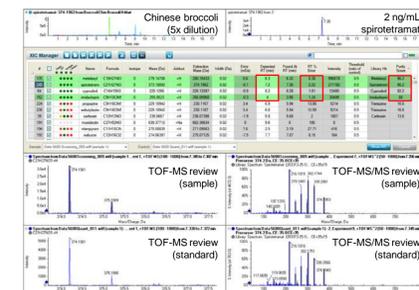


Figure 7. The XIC Manager was used to quantitatively compare 2 samples (vegetable extract and pesticide standard), compounds highlighted in green are present in the sample at a higher concentration than the standard

Using TripleTOF[®] for Identification of Unexpected Residues and Authenticity Markers

Statistical data processing is the most effective procedure to find peaks of interest in complex samples. A controlled set of samples is mathematically compared to remove background and to differentiate between signals characteristic for the sample matrix and true chemical residues. A popular tool for the processing of LC-MS/MS data is Principal Components Analysis (PCA). The result of this mathematical transformation is displayed in a coordinate system of two PC, the scores plot and loadings plot. Similar samples cluster in similar areas of the scores plot. Thus PCA visually differentiates between 'clean' and 'contaminated' samples. The combination of PCA and PCVG was able to reduce the list of peaks of interest found by the non-target peak finding algorithm from thousands to only a few dozen signals (Figure 9).

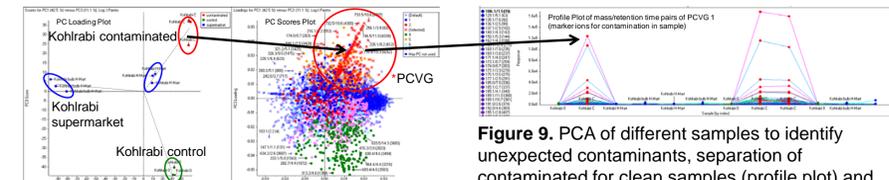


Figure 9. PCA of different samples to identify unexpected contaminants, separation of contaminated for clean samples (profile plot) and peaks of interest (scores plot and profile plot)

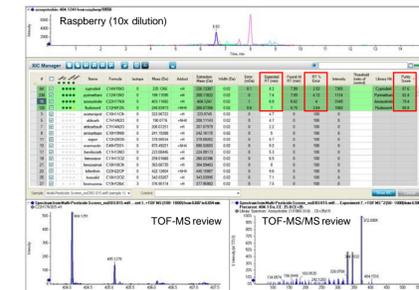


Figure 6. Result display in the XIC Manager, 4 pesticides were identified in a raspberry sample, the confidence score is based on mass accuracy, isotopic pattern, retention time match, and MS/MS library fit

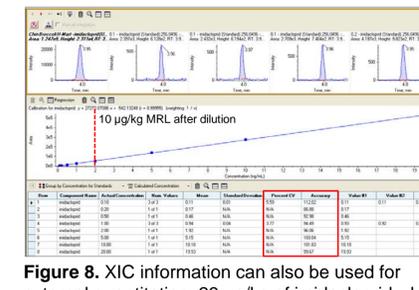


Figure 8. XIC information can also be used for external quantitation, 23 µg/kg of imidacloprid where found in Chinese broccoli (see figure 7), the calibration line had excellent linearity, reproducibility, and accuracy

In a second step found markers can be tentatively identified by combining empirical formula calculation, online database searching, and MS/MS fragment ion interpretation.

The highest signal responsible for the contamination in kohlrabi corresponded to m/z 266.0735 at 7.0 min. The Formula Finder in PeakView[®] software was used to empirically calculate the molecular formula with information available on the accurate mass quasi-molecular ion, isotopic pattern, cluster ions, and MS/MS fragment ions resulting in C₁₂H₆N₂O₂F₂. This formula was subsequently searched against online databases to find possible structures. The pesticide Fludioxonil was found by ChemSpider with the highest probability (Figure 10).

The suspected structure was then compared to the MS/MS spectrum using the fragment ion prediction tool in PeakView[®] software. All accurate mass fragment ions matched the suspected structure (Figure 5c). Thus, Fludioxonil was identified as a major contaminant in the investigated kohlrabi sample.

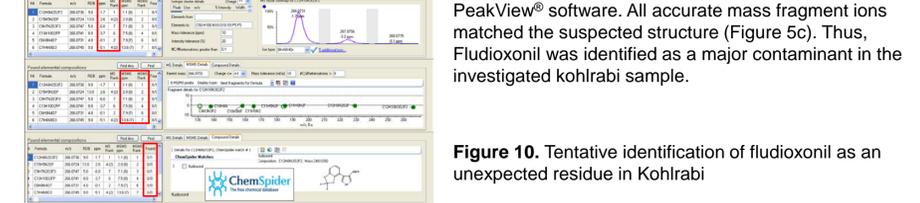


Figure 10. Tentative identification of fludioxonil as an unexpected residue in Kohlrabi

In a similar procedure different cabbage and seed samples* were compared to identify characteristic markers for broccoli to differentiate raw material mode of broccoli from other cabbages

The marker ion of 420.0463 at 14.1 min was tentatively identified as glucoserucin

* Samples for analysis were provided by Sandra Stanley (FutureCeuticals, Momence, IL)

SUMMARY

In this poster we presented the application of accurate mass LC-MS/MS using the AB SCIEX TripleTOF[®] 5600 system for targeted and non-targeted screening of botanical samples. The MS and MS/MS information was to automatically identify pesticide residues in QuEChERS extracts with high confidence based upon retention times, accurate mass, isotopic pattern and MS/MS library searching. Statistical data processing tools followed by empirical formula calculation, automatic ChemSpider search, and MS/MS interpretation was also used to find unexpected residues and to identify characteristic markers of authenticity.

REFERENCES

- 1 EU Commission Decision 'concerning the performance of analytical methods and the interpretation of results' #2002/657/EC
- 2 SANCO Document: 'Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed' #SANCO/10684/2009
- 3 A. Schreiber and D. Cox: 'Using PeakView[®] Software with the XIC Manager for Screening and Identification with HighConfidence based on High Resolution and Accurate Mass LC-MS/MS' Application Note AB SCIEX (2011) #2170811-03

TRADEMARKS/LICENSING

For Research Use Only. Not for use in diagnostic procedures. The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners. AB SCIEX[™] is being used under license. © 2012 AB SCIEX.