LC-MS/MS Based Strategy for the Non-Targeted Screening of an Unlimited Number of Contaminants in Food Using the AB SCIEX TripleTOF™ 5600 System and Advanced Software Tools

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Overview

The following paper presents a procedure for non-targeted screening of an unlimited number of contaminants in food samples using high resolution and accurate mass Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) using the AB SCIEX TripleTOF™ 5600 system.

QuEChERS extracts of different fruit and vegetable samples were analyzed, and the data statistically processed to find any unexpected contaminants. High resolution and accurate mass MS and MS/MS spectra were further processed to empirically calculate molecular formulae and to identify and characterize chemical structures.

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. The most common LC-MS/MS routine to analyze food samples is the targeted quantitation of hundreds of food contaminants in a single analysis. Triple quadrupole based mass analyzers are preferred for these analyses, as they provide an extra degree of selectivity and sensitivity when operated in Multiple Reaction Monitoring (MRM) mode.

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 and the identification of the non-registered pesticide isofenphos-methyl in strawberries. Because neither of these compounds were targeted as potential contaminants they did not show up in conventional analyses; further investigation of food samples was not required until people became ill.[1-2]

The ability to perform non-targeted screening on a routine basis is made possible through advancements in LC-MS/MS technology, including hybrid systems like the triple quadrupole linear ion trap (QTRAP®) and tandem quadrupole Time-of-Flight (TripleTOF™). However, since this workflow does not use a target analyte list, compound detection is not based on any *a priori* knowledge, such as retention times and information on molecular and fragment ions. Nor is it limited to a specific database of spectra or masses. These full scan data files are very rich in information and can easily contain thousands of ions from any compounds present in the sample as well as from the sample matrix itself. In order to mine these chromatograms for useful information powerful software tools are needed.

Here we used MarkerView™ software to find unexpected contaminants in full scan chromatograms. By performing statistical data analysis using Principal Components Analysis (PCA) and Principal Components Variable Grouping (PCVG), unique contaminants in unknown samples were detected. PeakView™ software was used for empirical formula calculation, followed by searching of the internet for possible structure identities, and MS/MS fragment ion prediction to identify compounds and to characterize chemical structures.

Method Details

- QuEChERS extraction of different kohlrabi and tomato samples
- UHPLC using a Shimadzu UFLC$_{XR}$ system with a Restek Ultra Aqueous C18 (100 x 2.1 mm) 3 µm column and a gradient of water and methanol with 10 mM ammonium formate at a flow rate of 0.5 mL/min
- Injection volume of 10 µL
- AB SCIEX TripleTOF™ 5600 system with Turbo V™ source and ESI probe
- Continuous recalibration between injections using the Calibrant Delivery System (CDS)
- Information dependent acquisition using a TOF-MS survey scan 100-1000 Da (100 ms) and up to 10 dependent TOF-MS/MS scans 50-1000 Da (100 ms) using Collision Energy (CE) of 35 V with Collision Energy Spread (CES) of ± 15 V
- Statistical data processing using MarkerView™ software (minimum spectral peak width 0.01 Da, retention time tolerance 0.3 min, noise threshold 100 cps; Figure 1)
- Empirical formula calculation and fragment ion prediction using PeakView™ software

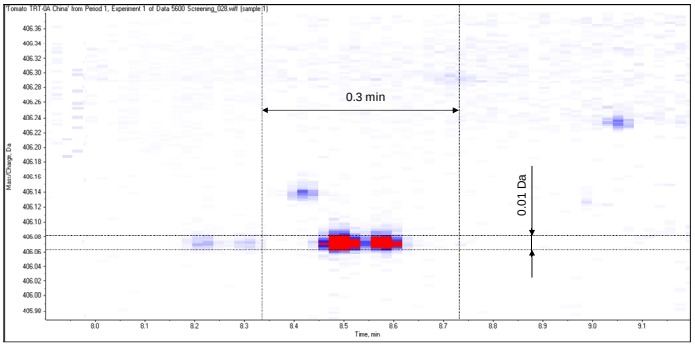

Figure 1. Non-target peak finding parameters applied in MarkerView™ software before PCA

Results and Discussion

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. A non-targeted peak finding algorithm, like that integrated into the XIC Manager application of PeakView™ software, allows screening for unexpected compounds.[3] Mass spectral library searching can be used to identify non-targeted compounds based on their molecular fingerprint; however, identification is limited to compounds present in the MS/MS

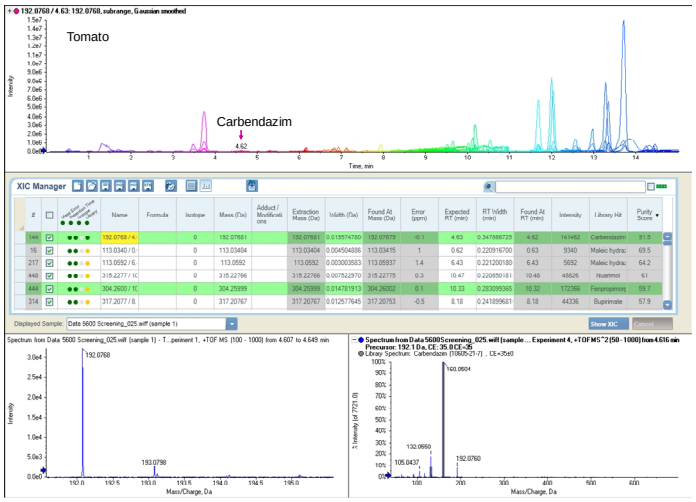

Figure 2. Tomato sample processed using the non-targeted peak finding algorithm of the XIC Manager application in the PeakView™ software, Carbendazim was identified based on MS/MS library searching against an existing library of pesticides

library (Figure 2). Different software tools are needed to identify compounds which are not present the library, or which are completely unknown.

As a first step of data processing a list of the peaks of interest need to be generated. Every non-targeted peak finding algorithm is capable of doing so. However, the peak list generated by such an algorithm can easily contain thousands of chromatographic and mass spectrometric signals. In depth investigation of these signals would be time consuming and inefficient, since most of these signals are derived from chemical background and matrix components. Thus the peak list generated by a non-targeted peak algorithm needs to be reduced to a list containing peaks of interest only, i.e. undesired chemical residue in food.

Statistical data processing is the most effective procedure to find peaks of interest in complex samples. Here 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). PCA is a mathematical algorithm of orthogonal linear transformation to reduce data complexity, while retaining most differentiating information. The information of greatest variance is saved into principal components (PC).

The result of this mathematical transformation is displayed in a coordinate system of two PC, the scores plot. Similar samples cluster in similar areas of the scores plot. Thus PCA visually differentiates between ‘clean’ and ‘contaminated’ samples.

As shown in the scores plots in Figures 3 and 4 (top left) PCA clearly differentiated between the contaminated kohlrabi and tomato sample, respectively, from both control samples and store bought samples.

The loadings plots in Figures 3 and 4 (top right) show all variables (m/z-retention time pairs in this case) that make the most difference in separating samples. The list of variables can be fairly long depending on the complexity of samples. Principle Component Variable Grouping (PCVG) was successfully used to

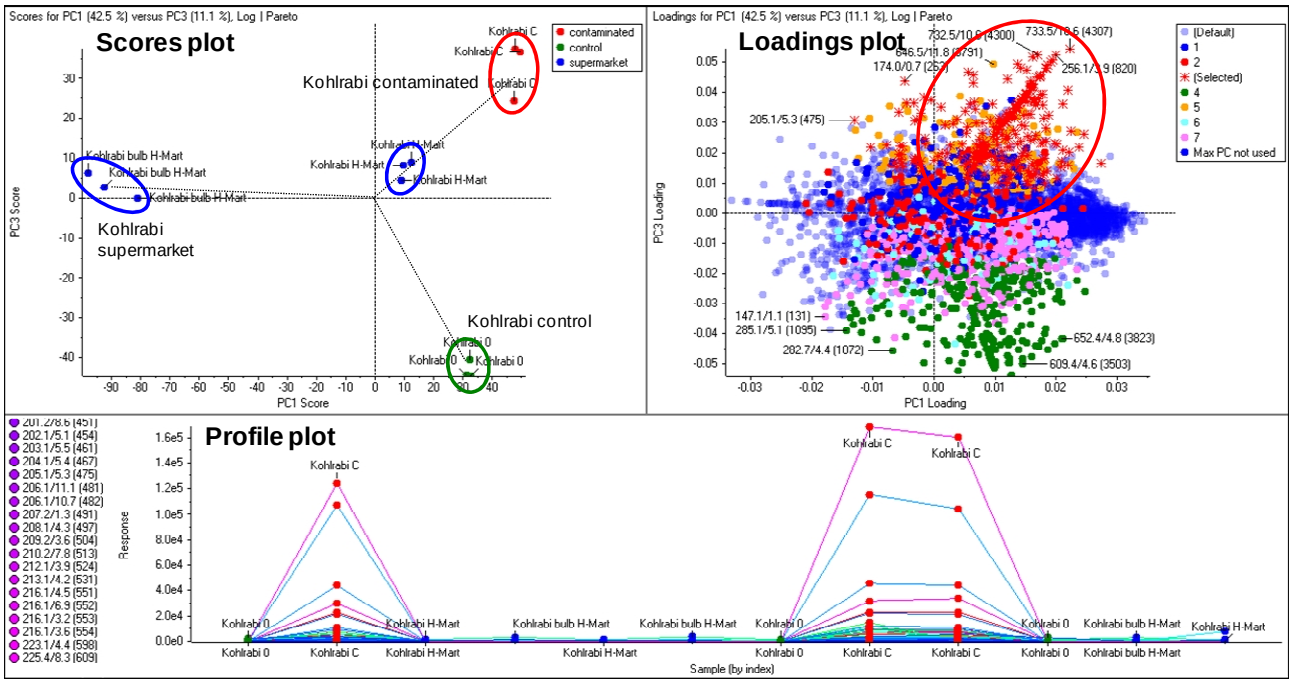

Figure 3. PCA and PCVG of kohlrabi samples, the scores plot was used to visualize the difference between samples (top left), the loadings plot with PCVG was used to identify characteristic m/z-retention time pairs (top right) which were verified in the profile plot (bottom)

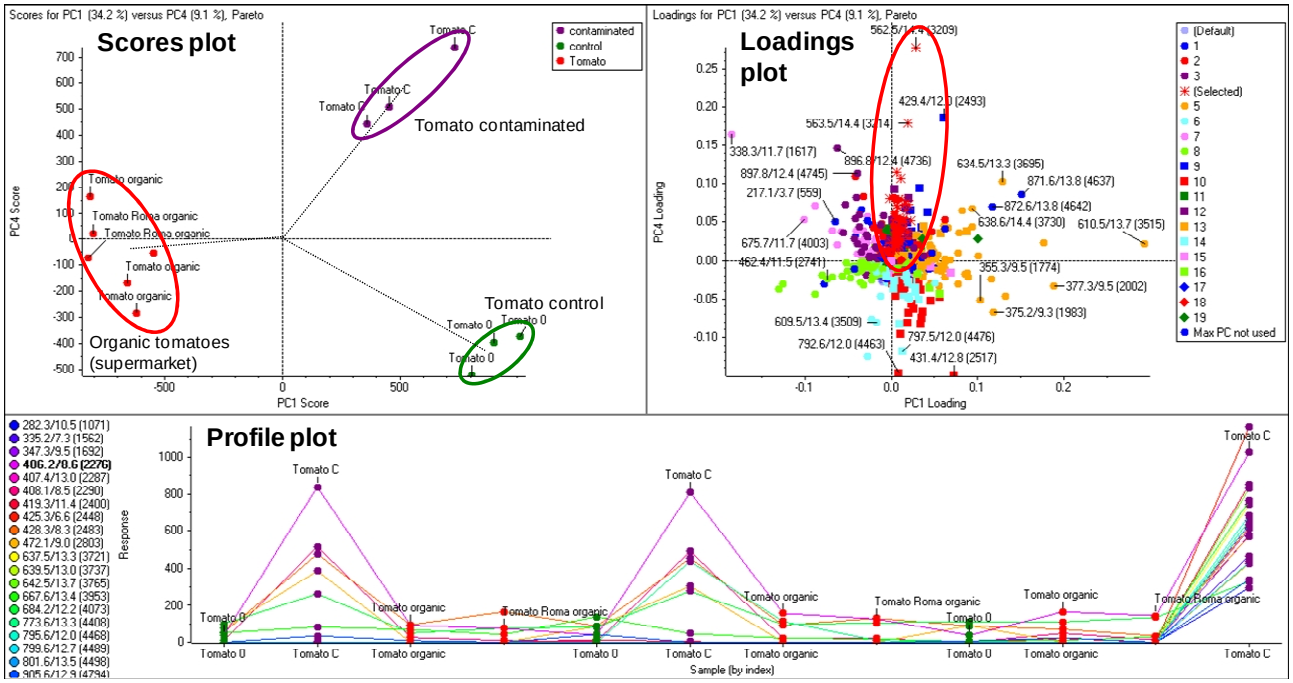

Figure 4. PCA and PCVG of tomato samples

reduce the number of variables selected for further data processing. The loadings plot of PCVG is a two-dimensional display using the information of more than two PC to color code the display in such a way that additional variation not otherwise visible can be seen.[4]

The profile plot in MarkerView™ software was used to verify that all variables of the selected group were characteristic for the contaminated sample (Figures 3 and 4, bottom).

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.

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 $C_{12}H_6N_2O_2F_2$ (Figure 5a).

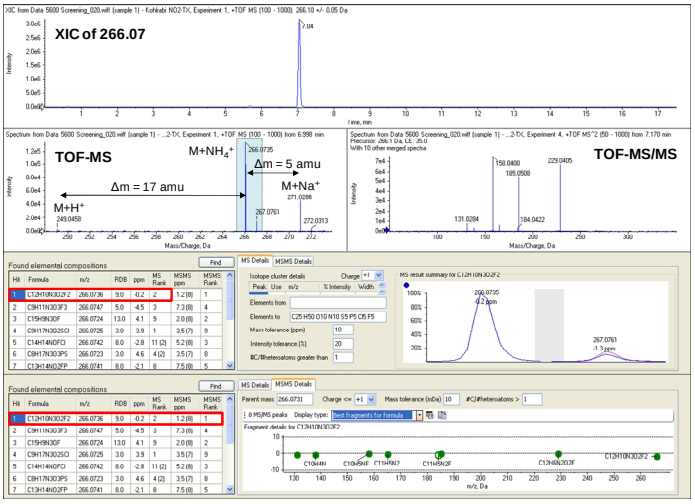

Figure 5a. Empirical calculation of the molecular formula of the signal 266.07 Da at 7.0 min resulted in a molecular formula of $C_{12}H_6N_2O_2F_2$

This formula was subsequently searched against online databases to find possible structures. The pesticide Fludioxonil was found by ChemSpider with the highest probability (Figure 5b).

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.

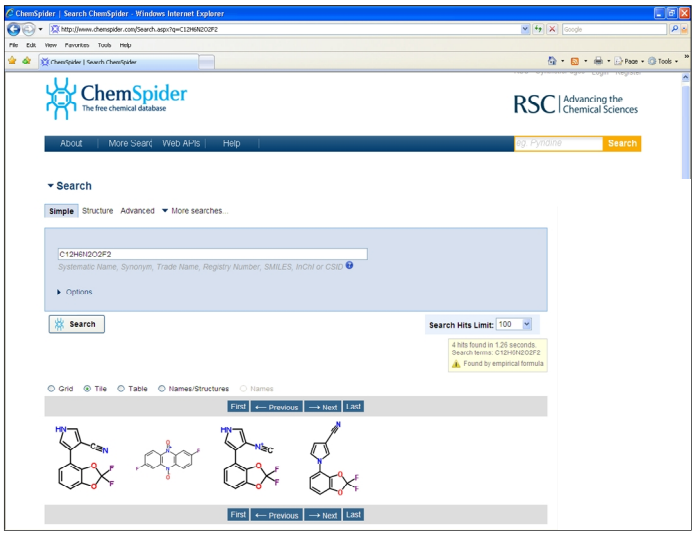

Figure 5b. Online search with ChemSpider to find possible structures matching the empirically calculated formula $C_{12}H_6N_2O_2F_2$. The highest probability match was found to be the pesticide Fludioxonil.

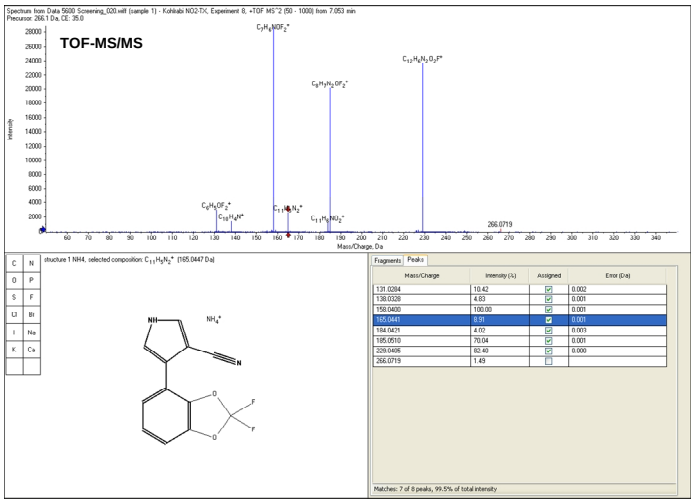

Figure 5c. With all MS/MS fragment ions matching the suspected structure, the detected compound was consequently identified as Fludioxonil.

The same workflow was applied to the determination of the major contaminant of the tomato sample, m/z 406.0712. This mass revealed two chromatographic peaks at 8.4 and 8.6 min. The pesticide Difenoconazole was identified as the major residue in the contaminated tomato sample using empirical formula calculation, online database search, and fragment ion prediction (Figure 6a-c).

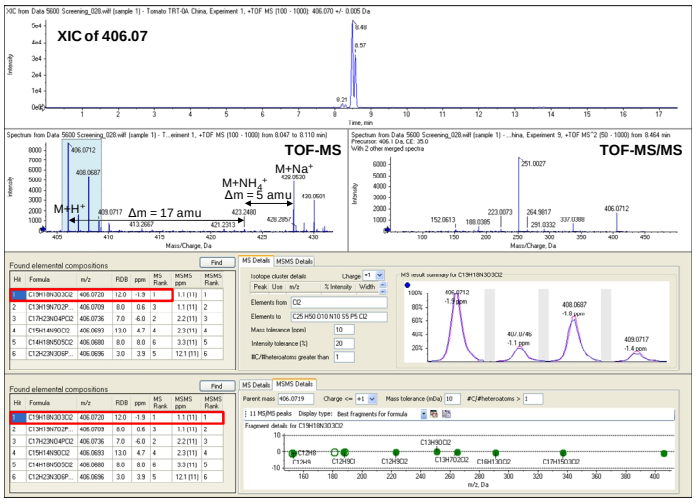

Figure 6a. Empirical calculation of the molecular formula of the isomeric signals 406.07 Da at 8.4 and 8.6 min resulted in a molecular formula of $C_{19}H_{17}N_3O_3Cl_2$

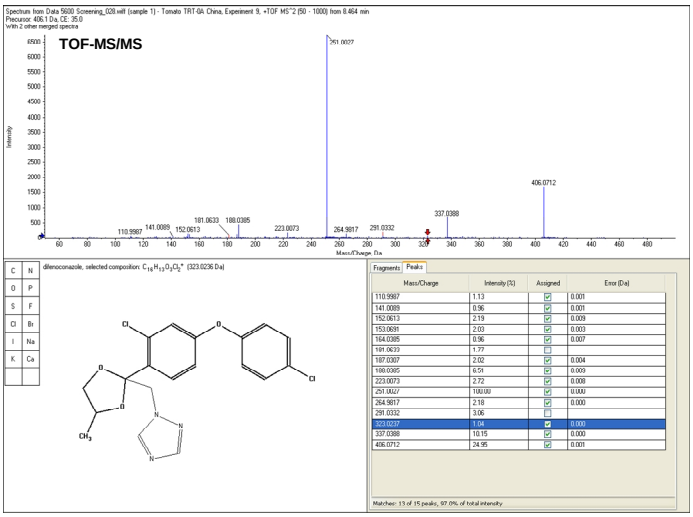

Figure 6c. With all MS/MS fragment ions matching the suspected structure, the detected compound was consequently identified as Difenoconazole

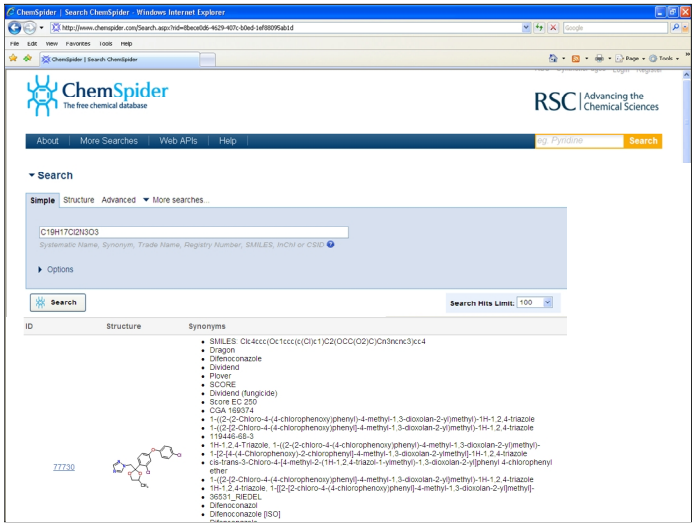

Figure 6b. Online search to find possible structures matching the empirically calculated formula $C_{19}H_{17}N_3O_3Cl_2$. The highest probability match was found by ChemSpider to be the pesticide Difenoconazole

Summary

A straightforward procedure using generic QuEChERS extraction and high resolution accurate mass LC-MS/MS was used to screen for and identify non-targeted chemical residues in food samples using the AB SCIEX TripleTOF™ 5600 system. Here we combined statistical data analysis using PCA and PCVG followed by empirical formula calculation, online database searching, and MS/MS fragment ion interpretation to successfully detect unknown contaminants.

Utilizing minimal sample preparation, maximum data collection, intelligent signal filtering and an internet search of molecular formula; this workflow can provide the user with the ability to screen a set of samples for virtually every known chemical compound and identify undesirable contaminants quickly and easily.

Acknowledgements

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