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Mass Spectrometry & Spectroscopy

New Tools to Quickly Identify Unknown Compounds in Complex Food Samples

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Here we present results using a novel data processing approach to quickly and automatically identify unknown and unexpected compounds, such as residues and contaminants, in complex food samples. The data analysis workflow combines non-target peak finding, sample-control-comparison, empirical formula finding, MS/MS library searching, and ChemSpider search combined with MS/MS interpretation – with most of these as automated steps - making data processing fast and easy for the user. Food samples were prepared using a simple solvent extraction or QuEChERS extraction and analysed with reversed phase LC. High resolution and accurate mass MS and MS/MS information was collected in a single injection on AB Sciex TripleTOF® 4600 and 5600 systems. Data were processed using the new MasterView™ software.

Introduction

LC-MS/MS is a powerful analytical tool for the analysis of food residues and contaminants. In particular, triple quadrupole-based mass analysers 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 quadruple linear ion trap (QTRAP®) and quadrupole-quadrupole Time-of-Flight (TripleTOF®), now provide the ability to perform both targeted and non-targeted screening in food samples on a routine basis. However, full scan chromatograms are very rich in information and easily contain thousands of ions from both chemical 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 to get answers and results from these complex data.

MasterView™ software utilises a powerful non-target peak finding algorithm to find chromatographic features. Sample-control-comparison was performed to separate matrix and sample-specific signals from true contaminations. Ions of interest were processed using automatic MS/MS library searching, empirical formula finding, and ChemSpider searching to tentatively identify the molecular formula and structure of the unknown compound. The accurate mass MS/MS information was utilised to reduce the number of potential formulae during the formula finding step and to compare the experimental MS/MS pattern with a theoretical fragmentation pattern of structures proposed by ChemSpider.

The new MasterView™ software offers an intuitive workflow to tentatively identify unexpected compounds in complex samples. The software allows quick processing, easy results review, and reporting.

Experimental

- Food samples were purchased from a local supermarket. Organic produce was used for sample-control-comparison.
- QuEChERS extraction of fruit and vegetable samples following guideline EN 15662/2007 using commercial QuEChERS kits and solvent extraction of cereal samples using acetonitrile/water (80/20) + 1% formic acid
- 5-20x dilution of sample extracts to minimise possible matrix effects
- UHPLC using a Shimadzu UFLC$_{XR}$ system with Restek Ultra Aqueous C18 (100 x 2.1mm) 3µm column
- Gradient of water and methanol with 10mM ammonium formate at a flow rate of 0.5 mL/min and injection volume of 10µL
- AB Sciex TripleTOF® 4600 and 5600 system with DuoSpray™ source operated in ESI mode
- Continuous recalibration between injections using the Calibrant Delivery System (CDS)

- Information Dependent Acquisition (IDA) using a TOF-MS survey scan 100-1000 Da (100 ms) and up to 10 or 20 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
- Dynamic background subtraction (DBS) was activated for best IDA coverage, no inclusion list was used to allow unknown identification without the need for a second injection to acquire MS/MS data

LC-MS/MS data were processed using the new MasterView™ software version 1.0.

Results and Discussion

TripleTOF® System Performance Characteristics

Resolution >20,000 (at full width half height) and mass accuracy <5 ppm are often sufficient to separate the analytes of interest from interfering matrices and, thus, are identified as the set requirements for compound identification in various guidelines [1, 2]. The TripleTOF® 4600 and 5600 systems provides resolving power of up to 35,000 and 40,000, respectively, dependent on the mass detected and stable mass accuracy of ~2 ppm at fast acquisition speed in MS and MS/MS mode [3, 4].

Data Analysis Workflow for Non-Targeted Screening

Extracted Ion Chromatograms (XIC) are generated using a non-targeted peak finding algorithm. Sample-control-comparison is used to differentiate matrix and sample specific signals from true contaminations. High resolution and accurate mass MS and MS/MS data are used to automatically calculate matching molecular formulae. Formulae are searched against ChemSpider to find matching structures. The theoretical fragmentation pattern of these structures is automatically compared with the experimental MS/MS pattern to rank confidence in identification. In addition, all acquired MS/MS spectra are searched against available MS/MS spectral libraries to identify compounds present in these libraries. AB Sciex offers true MS/MS spectral libraries for over 2500 compounds, including pesticides, toxins, pharmaceuticals, veterinary drugs, and drugs of abuse.

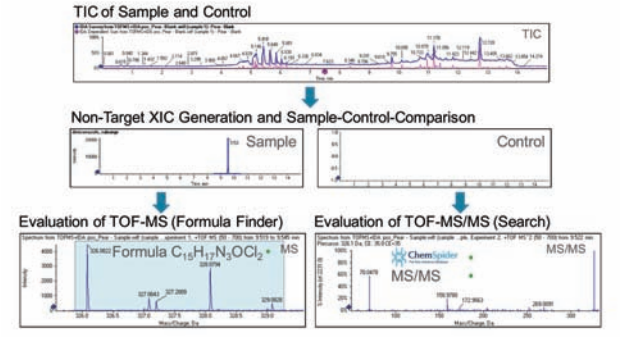
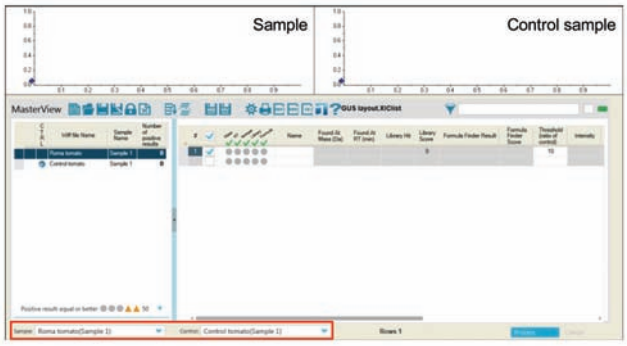


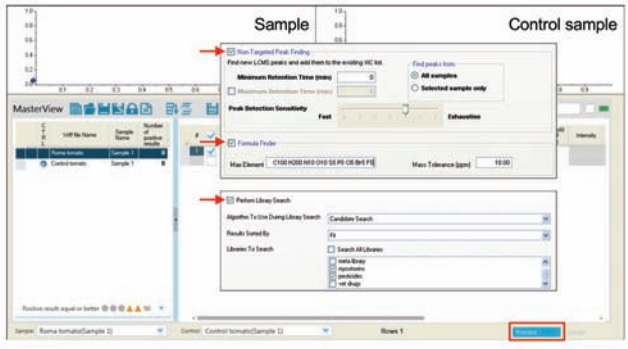
Figure 1. Data analysis workflow for non-targeted screening in MasterView™ software, XIC are generated by a non-targeted peak finding algorithms and sample-control-comparison is used to identify relevant chromatographic features, empirical formula finding, ChemSpider searching and MS/MS information is used to tentatively identify compounds.

Non-Targeted Compound Identification in MasterView™ Software

1.Open data files and select contaminated and control sample.

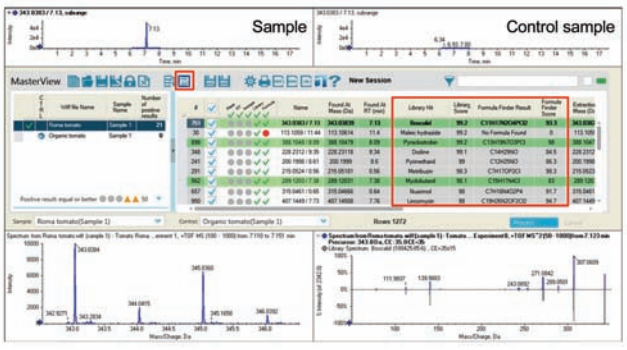


2. Define parameters for peak finding, formula finding, and library searching. Start processing by clicking 'Process'.

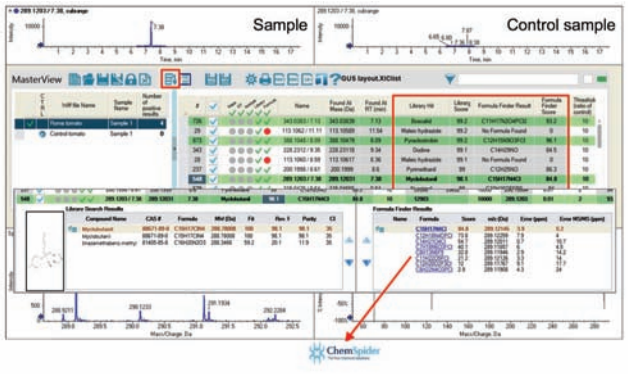


MasterView™ software will now automatically find peaks, compare signal intensity and perform formula finding and library searching. This can take several minutes depending on samples' complexity and processing settings. For example, the complete processing of two QuEChERS extracts took ~8:30 min (using a Win7, 64 bit, 4 core, 8 GB memory computer).

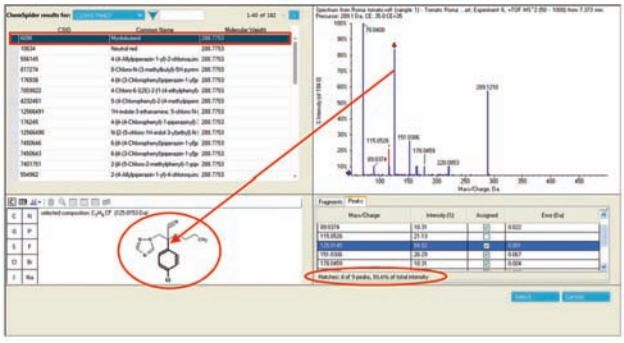
3. Review results using 'traffic lights' and display of MS and MS/MS spectra.



4. Review details of formula finding and MS/MS library searching. The hyperlink of the molecular formula starts a ChemSpider search and MS/MS interpretation.



5. Review ChemSpider search results. The theoretical MS/MS pattern is automatically compared with the experimental MS/MS spectrum and ranked by a confidence score. The predicted structure of a selected product ion is highlighted in the structure display to allow result evaluation.



Empirical Formula Finding Algorithm

MasterView™ software automatically uses the accurate mass MS and MS/MS to automatically predict possible molecular formulae. Details on the formula finding algorithm are illustrated in *Figure 2*.

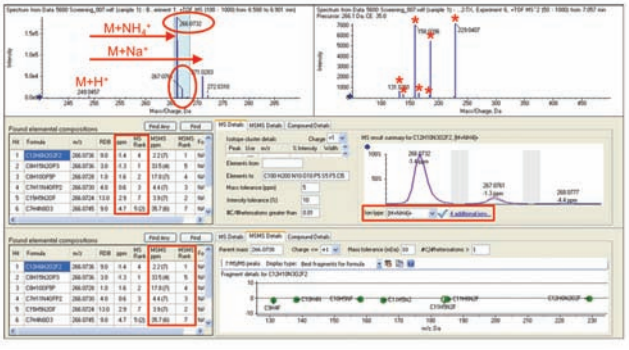


Figure 2. Empirical formula finding – mass accuracy and isotope pattern are used to calculate possible molecular formulae, theoretical MS/MS fragments are calculated and compared against to TOF-MS/MS spectrum to reduce the list of matching formulae, finally the software algorithm finds other adducts to predict the correct molecular formula of the uncharged species, $C_{12}H_6N_2O_2F_2$ ranked highest in the combined MS and MS/MS score.

Combining all available accurate mass MS and MS/MS information is crucial to minimise the list of potential formulae. *Figure 3* and *Table 1* illustrate that the number of formulae can be reduced from over 100 to a single match by not only using the accurate mass of the molecular ion but also including isotope pattern matching and MS/MS evaluation. The formula finding algorithm also checks for possible adducts to predict the correct molecular formula of the uncharged species.

The molecular ion shown in *Figure 2* was identified as an $M+NH_4^+$ ion based on the presence of 4 additional ions.

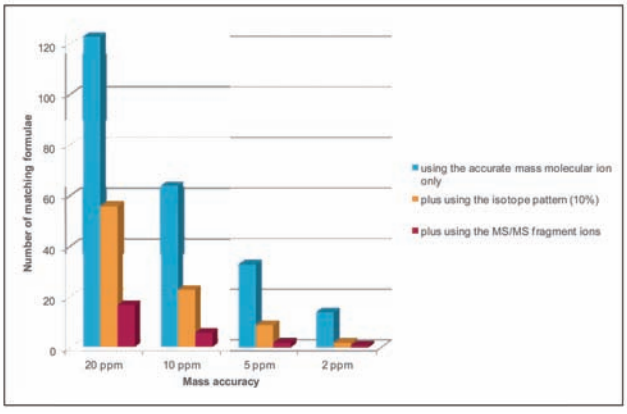


Figure 3. Number of matching molecular formulae depending on the information used for empirical formula finding (elements allowed $C_{100}H_{200}N_{10}O_{10}S_5F_5Cl_5Br_5$).

Table 1. Ranking of found formulae using MS and MS/MS information, the MS rank combines mass accuracy and isotope pattern matching and the MS/MS rank combines mass accuracy and number of ions (n).

Hit	Formula	MS Rank	ppm	MS/MS Rank	ppm (n)
1	$C_{12}H_6N_2O_2F_2$	4	-1.4	1	2.2 (7)
2	$C_8H_{15}N_2OP_3$	1	-1.3	5	33.5 (4)
3	$C_8H_{10}OF_5P$	2	16	4	17.8 (7)
3	$C_7H_{11}N_4OFP_2$	3	0.6	3	3.9 (7)

This formula finding process is completely automated in the MasterView™ software.

Tentative Identification based on ChemSpider Searching and MS/MS Interpretation

MasterView™ software allows searching of molecular formulae against ChemSpider to find matching structures. ChemSpider can rank the found structures based on the number of references making it easy to identify the most likely structure [5].

The structure is automatically compared against the measured accurate mass MS/MS spectrum using common knowledge on breaking and closing bonds, and re-arrangement. A match factor is calculated to describe the matching of experimental MS/MS pattern with a theoretical fragmentation pattern using the proposed structures. Fragment ions which can be explained by theoretical fragmentation are displayed in blue and fragment ions which cannot be explained are displayed in red in the MS/MS spectrum. The predicted structure of a selected product ion is highlighted in the structure display to allow result evaluation. *Figures 4 and 5* show examples of identification of Pyraclostrobin and Boscalid with an MS/MS match of 99.4% and 100%, respectively.

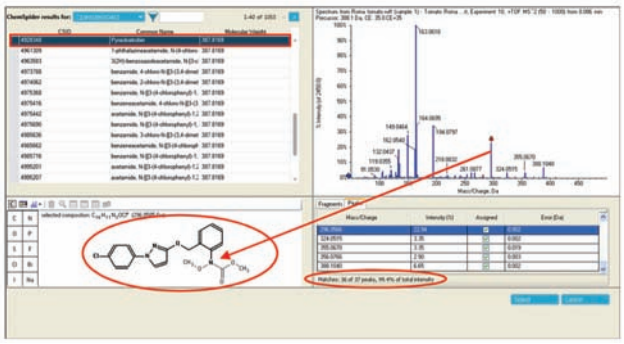


Figure 4. ChemSpider search results performed in MasterView™ software suggesting Pyraclostrobin being present in a tomato sample, the MS/MS match of 99.4% further confirms the finding of the pesticide.

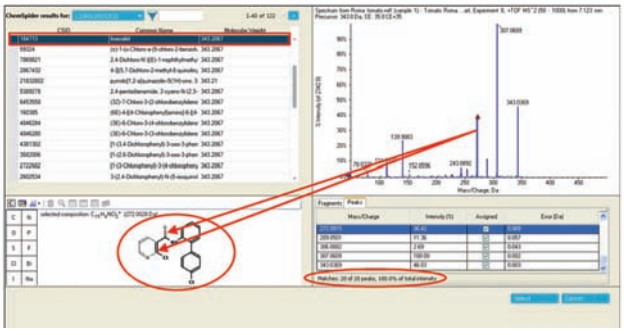


Figure 5. ChemSpider search results performed in MasterView™ software suggesting Boscalid being present in a tomato, the MS/MS match of 100.0% further confirms the finding of the pesticide.

Additional Results of Non-target Screening

Figures 6, 7, and 8 show additional results of non-targeted data processing on a series of different food samples.

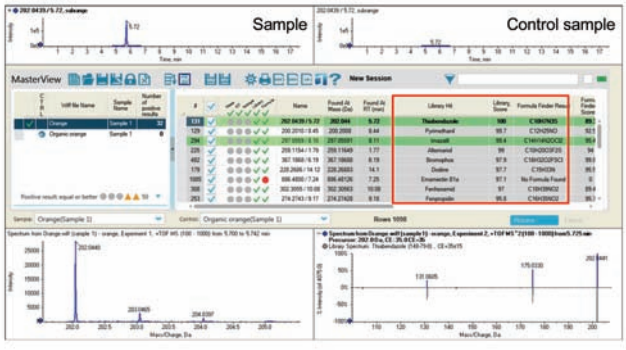


Figure 6. Automatic non-target screening of orange samples, sample-control-comparison reduce the number of chromatographic signals from 1098 to 32 relevant signals, Thiabendazole and Imazalil were identify with high confidence.

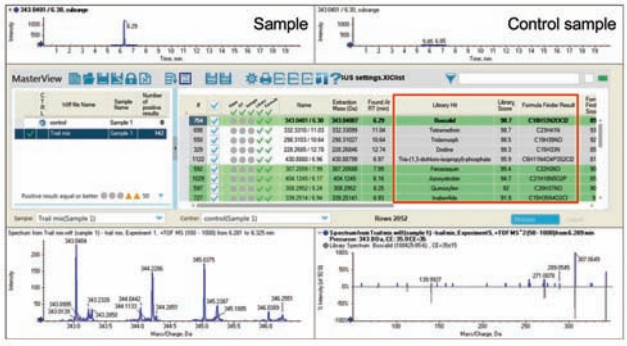


Figure 7. Automatic non-target screening of trail mix samples, samplecontrol-comparison reduce the number of chromatographic signals from 2052 to 142 relevant signals, several pesticides were identify with high confidence.

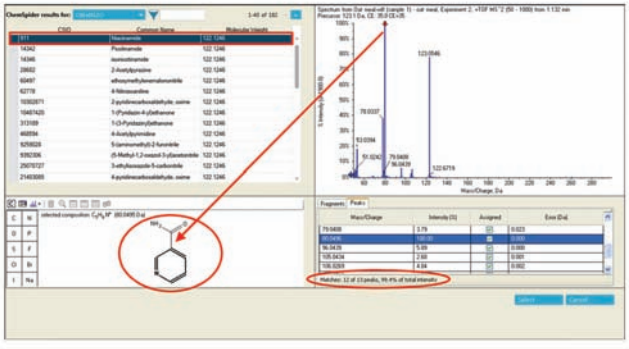


Figure 8. Identification of Niacinamide (vitamin B3) with 99.4% score after sample-control-comparison, formula finding, ChemSpider searching and automatic interpretation of the MS/MS pattern.

Summary

A novel approach of comparative non-target screening to identify unexpected and unknown chemicals in food samples was developed and successfully applied to a wide variety of samples. Samples were extracted using a QuEChERS procedure and analysed 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 systems. Data were processed using MasterView™ 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. The new MasterView™ software offers an easy to use and intuitive workflow to tentatively identify unexpected chemicals in food.

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/12495/2011

3. A. Schreiber and C. Seto: 'Target and Non-Target Screening for Chemical Residues in Food Samples using the AB SCIEX TripleTOF® 4600 System and Intuitive Data Processing Tools' Application Note AB SCIEX (2012) #5680212-01

4. A. Schreiber and C. Borton: 'Target and Non-Target Screening for Pesticide Residues in Food Samples using the AB SCIEX TripleTOF® 5600 System' Application Note AB SCIEX (2010) #0460110-02

5. J. Little et al.: 'Identification of 'known unknowns' utilizing accurate mass data and ChemSpider' J Am Soc Mass Spectrom 23 (2012) 179-185

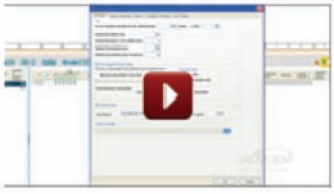
The Complete Series of Master View™ Software Workflow Demos:

Video 1:

Screen for targeted compounds in your unknown samples
Click www.absciex.com/MasterVideo1 to watch the video

Video 2:

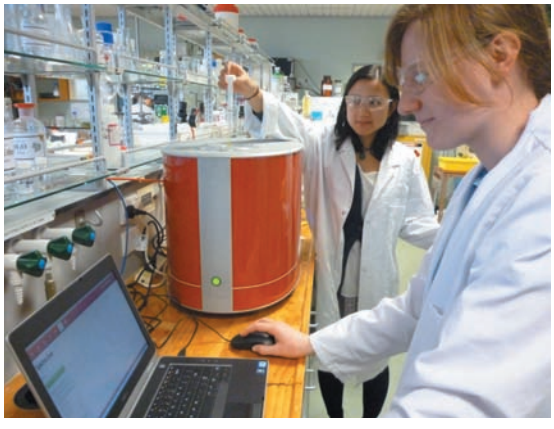
Easy set-up of data processing and data review parameters
Click www.absciex.com/MasterVideo2 to watch the video.



Video 3:

Screen for non-targeted compounds in your unknown samples
Click www.absciex.com/MasterVideo3 to watch the video

Benchtop NMR System used at Temple University for Chemical Education and Advanced Research



As part of the Temple University School of Pharmacy, the Moulder Center has recently acquired a **Magritek** Spinsolve™ NMR Spectrometer; a cryogen-free benchtop NMR system ideally suited for chemical education and advanced research. "The addition of this system will greatly enhance our capacity to train future generation of drug discovery scientists," said Professor Magid Abou-Gharbia, Director of the Moulder Center and Associate Dean of Research of Temple School of Pharmacy. "In order to ensure that our students are fully prepared to tackle the most pressing research problems of the next generation, we must ensure that they have the most advanced technology possible. The arrival of the Magritek Spinsolvein our institution is yet another example of Temple's commitment to its students and high quality research."

Continuing, Professor Abou-Gharbia said: "the sleek benchtop design of this instrument will greatly simplify its integration into the existing laboratory space of the school of pharmacy. Its ease of use is expected to significantly enhance productivity of students and faculty, providing them with greater opportunity to pursue advanced research on a highly competitive level. Students, post-doctoral scientists, and visiting faculty will all have access to the benchtop SpinsolveNMR Spectrometer, allowing rapid, hands-on acquisition of critical program data, enabling them to solve complex research problems in a more efficient manner. The system will be a centre piece of the Moulder Center for Drug Discovery Research's analytical technology suite."

The Spinsolve compact NMR spectrometer is controlled by easy-to-use software designed to enable users to operate the system with very little training. Traditional NMR complexities are hidden and automated. The system uses a fast automatic lock, giving the user the choice of whether or not to use deuterated solvents. Once the spectrum is acquired, it can be viewed immediately, printed, or quickly sent via email or network to another location for further analysis. For example, data is easily opened in third party NMR software with a license for the Mestrelab Mnova NMR package included with each purchase.