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Application Note

Qualitative Pesticide Screening of a Dried Cherry Sample Using HRMS

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Abstract

In this application note we describe the ease of use and efficacy of a non-targeted, data independent, analysis type (MS^E and $HDMS^E$), coupled with UNIFI, a state-of-the-art scientific information system for multi-analyte screening in food and environmental samples. Here, we focus on introducing the novel way a user in a routine environment can customize data review within the scientific information system to establish a concise, rapid, facile, and consistent approach to reviewing HRMS data. This is demonstrated with a case study involving an authentic sample analyses. The aim of these case studies is to show how a user can get from injection of a sample to submission of an accurate report in a fast, efficient, systematic, and reproducible way using the workflows, views, and filters in UNIFI.

Benefits

Review complex high resolution, non-targeted MS^E or $HDMS^E$ datasets using workflows, filters, and views within an integrated scientific information system that allows:

- Screening for a theoretical unlimited number of compounds in a single injection.
- Simultaneous collection of qualitative and quantitative unbiased data for either targeted or non-targeted analysis.
- Interrogation of data for the presence of unknown compounds of interest via filtering, binary compare, and statistical analysis.
- Structural elucidation of isolated unknown compounds of interest.
- Historical data review performed using accurate mass precursor and fragment ion information.
- Utilization of Collision Cross Section (CCS) as an identification point for accurate mass screening.

Introduction

Multi-analyte screening methodologies are essential for monitoring food and environmental samples across the globe. The goal of these methods is to eliminate the compliant samples and identify the non-compliant samples for subsequent confirmation and quantification. Sensitivity must be in line with the relevant regulatory limits for residues in complex matrices. Also, a method must be validated in accordance with legislative requirements. This method would ideally be a rapid, cost effective, and streamlined process, from sample preparation to reporting results.

To date, LC-MS/MS or GC-MS/MS tandem quadrupole technologies meet the requirements above and

currently exist as the de-facto technique used to perform these analyses. However, with a constantly increasing number of analytes being added to monitoring and watch lists, the scope of a typical screening method is being extended. In addition, requests to screen for compounds beyond a target list are becoming increasingly common. As a result, many laboratories are progressing towards High Resolution Mass Spectrometry (HRMS) screening techniques that, in theory, can monitor for an unlimited number of targets, and at the same time, provide information to help discover unknown compounds or metabolites of interest.

In this application note we describe the ease of use and efficacy of a non-targeted, data independent, analysis type (MS^E and $HDMS^E$),[1] coupled with UNIFI, a state-of-the-art scientific information system for multi-analyte screening in food and environmental samples. Here, we focus on introducing the novel way a user in a routine environment can customize data review within the scientific information system to establish a concise, rapid, facile, and consistent approach to reviewing HRMS data. This is demonstrated with a case study involving an authentic sample analyses.

Experimental

UPLC conditions

LC system:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC BEH C_{18} 1.7 µm, 2.1 x 100 mm
Column temp.:	45 °C
Injection volume:	5 µL
Flow rate:	0.45 mL/min
Mobile phase A:	10 mM ammonium acetate (pH 5) in water
Mobile phase B:	10 mM ammonium acetate (pH 5) in methanol

Sample manager purge:	90/10 water/methanol
Sample manager wash:	50/50 water/methanol
Seal wash:	90/10 water/methanol

Gradient

Time (min)	Flow rate (mL /min)	%A	%B	Curve
Initial	0.45	98	2	6
0.25	0.45	98	2	6
12.25	0.45	1	99	6
13.00	0.45	1	99	6
13.01	0.45	98	2	6
17.00	0.45	98	2	6

MS conditions

MS system:	Xevo G2-XS QTof
Ionization mode:	ESI + and -
Capillary voltage:	0.9 kV
Desolvation temp.:	550 °C
Desolvation gas flow:	1000 L/Hr

Source temp.:	120 °C
Reference mass:	Leucine enkephalin $[M+H]^+$=556.2766
Acquisition range:	50 to 1200 *m/z*
Acquisition rate:	4 spectra/s
Low CE:	4 eV
High CE ramp:	10 to 45 eV

Results and Discussion

The aim of these case studies is to show how a user can get from injection of a sample to submission of an accurate report in a fast, efficient, systematic, and reproducible way using the workflows, views, and filters in UNIFI. This type of data collection, processing, and review is described in a previously published technical note[2] by the same authors.

Case Study 1: Qualitative pesticide screening of a dried cherry sample using HRMS

A sample was submitted for analysis using the Waters Pesticide Screening Application Solution (PSAS) with UNIFI to evaluate the performance of the system for qualitative pesticide screening. Criteria used to assess the performance of the system were the false positive and false negative rates, as well as the ease and speed of data review. In addition to providing details of the sample preparation technique used, the collaborators provided a list of the compounds that were previously detected using a tandem quadrupole LC-MS/MS prior to sample submission to Waters. See Table 1 for details of those compounds.

Dried Cherries	LC-QQQ Results
Analyte	(ppb)
Acetamiprid	16
Boscalid	8
Fenhexamid	13
Malathion	8
Piperonyl Butoxide	8
Thiacloprid	81
Thiophanate methyl	15
Trichlorfon	188

Table 1. Pesticides and their observed concentrations in the characterized dried cherry extract as found by investigators using tandem quadrupole LC-MS/MS prior to submission for analysis using the Waters Pesticide Screening Application Solution with UNIFI.

Sample analysis and data processing

A vial containing 200 µL dried cherry extract at 2.5 g/mL matrix in 100% acetonitrile (ACN) prepared using QuEChERS was supplied by the collaborator. The sample was dried down and reconstituted in 500 µL of water:acetonitrile (75:25), resulting in a concentration of 1 g/mL matrix. A 10 µL injection was performed. A non-targeted, data independent analysis, $(MS^E)^1$ was collected and processed in UNIFI.

This analysis focuses on the qualitative accurate mass screening capabilities of the Waters Pesticide Screening Application Solution with UNIFI, attempting to answer the highlighted question in Figure 1.

The workflow used for this qualitative analysis is shown in Figure 2. Figure 3 shows the UNIFI report containing the list of pesticides that were confidently matched in the dried cherry sample using the Qualitative Screening Workflow in Figure 2. In total, 15 pesticides were reported to the collaborator, which was seven more than they had originally supplied with the sample submission. Following submission of the Waters report and demonstrating data review, the collaborator then provided the full list of pesticides

present in the characterized sample, as shown in Table 2.

By comparing the results shown in Figure 3 and Table 2, it can be seen that all pesticides detected by tandem quadrupole LC-MS/MS were also detected using the Waters Pesticide Screening Application Solution (PSAS). This demonstrates the efficacy of truly non-targeted data acquisition using MS^E in combination with data review via a UNIFI workflow.

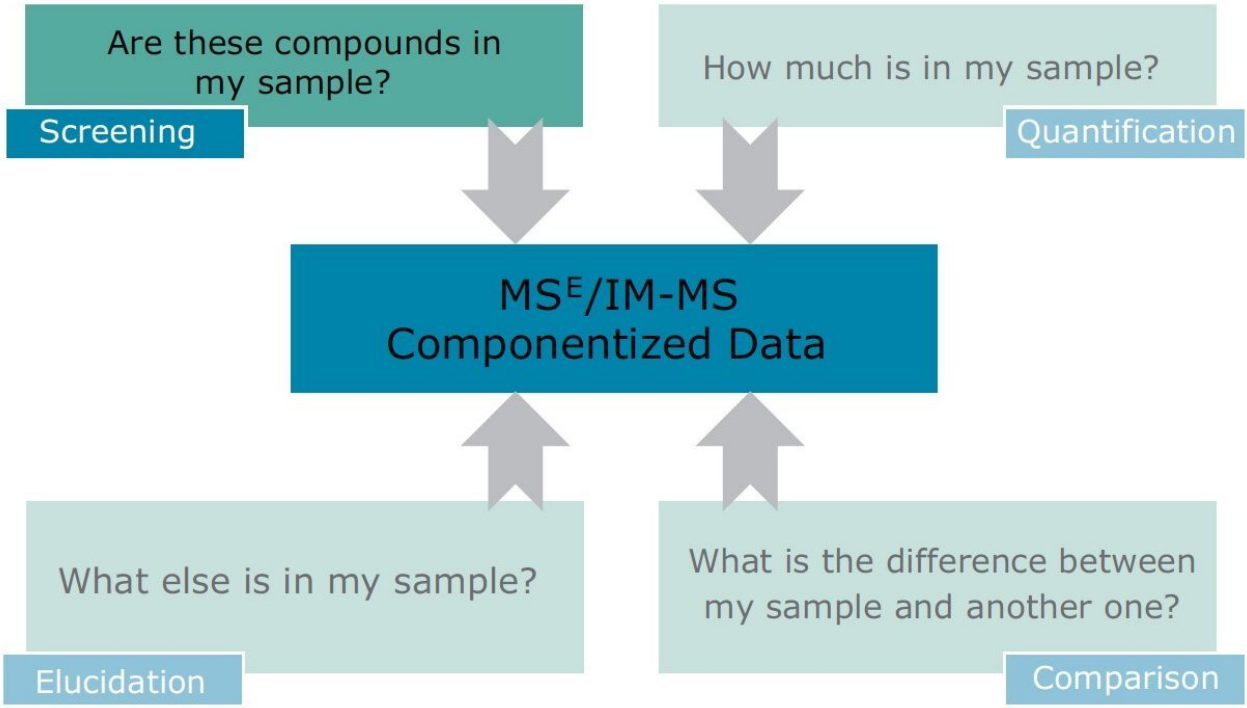


Figure 1. Fundamental questions for modern multi-residue screening methods.

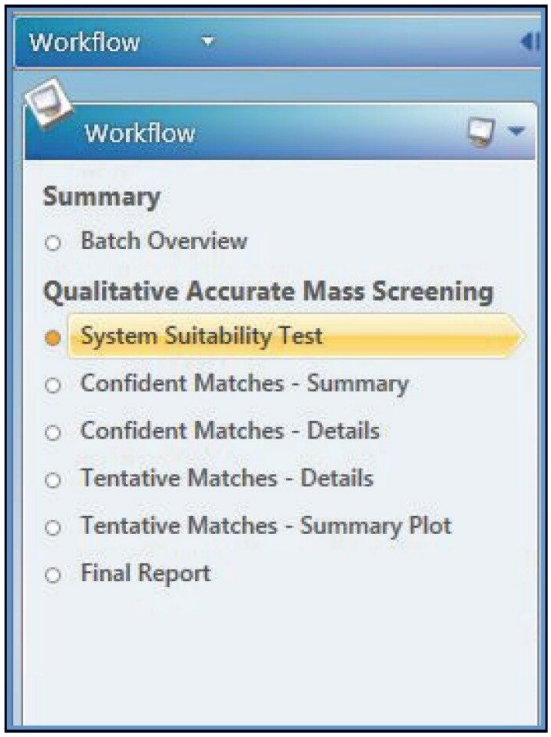
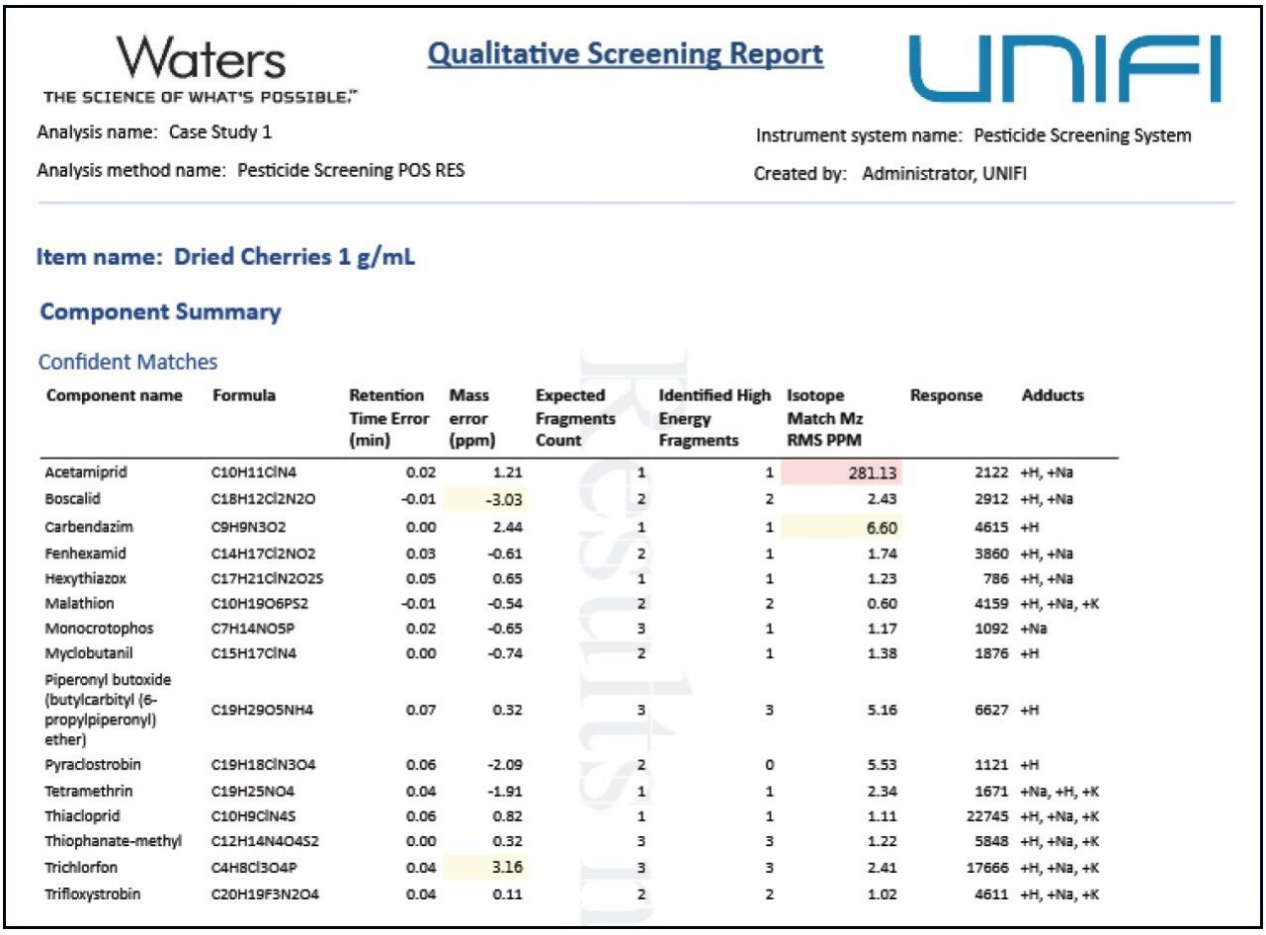


Figure 2. UNIFI Data Review workflow used for a qualitative accurate mass screening experiment.

With the exception of pyraclostrobin, at least one fragment ion was detected for each pesticide using the Waters PSAS. The detection of accurate mass fragments provides confirmatory evidence for presence of a pesticide residue and greatly reduces the number of false positives reported compared to detection by precursor mass accuracy alone. The specificity afforded by the use of fragment ion presence far exceeds any other identification parameter used during accurate mass screening. The use of UNIFI workflows, like the example shown in Figure 2, allows rapid review of all supporting evidence for compound identification including mass accuracy of precursor and fragment ions, retention time, the presence of multiple adducts and isotope scores.



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Qualitative Screening Report

UNIFI

Analysis name: Case Study 1

Instrument system name: Pesticide Screening System

Analysis method name: Pesticide Screening POS RES

Created by: Administrator, UNIFI

Item name: Dried Cherries 1 g/mL

Component Summary

Confident Matches

Component name	Formula	Retention Time Error (min)	Mass error (ppm)	Expected Fragments Count	Identified High Energy Fragments	Isotope Match Mz RMS PPM	Response	Adducts
Acetamiprid	C10H11ClN4	0.02	1.21	1	1	281.13	2122	+H, +Na
Boscalid	C18H12Cl2N2O	-0.01	-3.03	2	2	2.43	2912	+H, +Na
Carbendazim	C9H9N3O2	0.00	2.44	1	1	6.60	4615	+H
Fenhexamid	C14H17Cl2NO2	0.03	-0.61	2	1	1.74	3860	+H, +Na
Hexythiazox	C17H21ClN2O2S	0.05	0.65	1	1	1.23	786	+H, +Na
Malathion	C10H19O6PS2	-0.01	-0.54	2	2	0.60	4159	+H, +Na, +K
Monocrotophos	C7H14NO5P	0.02	-0.65	3	1	1.17	1092	+Na
Myclobutanil	C15H17ClN4	0.00	-0.74	2	1	1.38	1876	+H
Piperonyl butoxide (butylcarbityl (6-propylpiperonyl) ether)	C19H29O5NH4	0.07	0.32	3	3	5.16	6627	+H
Pyraclostrobin	C19H18ClN3O4	0.06	-2.09	2	0	5.53	1121	+H
Tetramethrin	C19H25NO4	0.04	-1.91	1	1	2.34	1671	+Na, +H, +K
Thiacloprid	C10H9ClN4S	0.06	0.82	1	1	1.11	22745	+H, +Na, +K
Thiophanate-methyl	C12H14N4O4S2	0.00	0.32	3	3	1.22	5848	+H, +Na, +K
Trichlorfon	C4H8Cl3O4P	0.04	3.16	3	3	2.41	17666	+H, +Na, +K
Trifloxystrobin	C20H19F3N2O4	0.04	0.11	2	2	1.02	4611	+H, +Na, +K

Figure 3. UNIFI report showing confident matches obtained following data review via the Qualitative UNIFI workflow in Figure 2.

Dried cherries	LCQQQ results (ppb)	Detected by Waters PSAS
Acetamiprid	16	Yes
Boscalid	8	Yes
Carbendazim	14	Yes
Fenhexamid	13	Yes
Hexythiazox	4	Yes
Malathion	8	Yes
Monocrotophos	4	Yes
Myclobutanil	4	Yes
Piperonyl Butoxide	8	Yes
Pyraclostrobin	1	Yes
Tetramethrin	4	Yes
Thiacloprid	81	Yes
Thiophanate methyl	15	Yes
Trichlorfon	188	Yes
Trifloxystrobin	2.5	Yes

Table 2. The full list of pesticides and observed concentrations detected in the dried cherry sample by the collaborator compared to results obtained via the Waters PSAS.

Conclusion

- The results obtained here demonstrate the efficacy of non-targeted HRMS data acquisition in combination with a workflow-driven approach to data review for pesticide screening.
- The use of filters, workflows and views present a consistent, concise and comprehensive review of large data sets
- The use of MS^E provides unbiased, non-targeted datasets with sufficient sensitivity to detect precursors and product ions for pesticides at concentrations below their MRL
- Componentization increases specificity and enables interrogation of data for targeted, non-targeted, and unknown masses of interest in a complex sample without additional processing of raw data.

References

1. An Overview of the Principles of MS^E, The Engine that Drives MS Performance. Waters white paper No. 720004036. October, 2011.
2. G Cleland, K Graham, K Rosnack, and J Burgess. Simple HRMS Data Review Using Workflows, Views and Filters Within a Novel Integrated Scientific Information System. Waters technical note No. 720005436en. June 2015.
3. Waters UNIFI Scientific Information System Componentization. Waters white paper No. 720004587en. April 2013.

Appendix: Application note focus

Workflow Step 3: Confident matches – details

This section highlights a specific workflow step to discuss in more detail.

The “Confident Matches-Details” workflow step is designed to filter and review confident matches made on any of the 529 compounds in the target list.

Immediately after collection, a data file is processed and componentized.[3] An identified status is achieved for targets that satisfy the wide criteria set for retention time (1.5 minute window) and mass accuracy (10 ppm) in the analysis method. Making this identification tolerance wide in the analysis method results in never having to reprocess raw data during a routine screening analysis. Within data review we can narrow the tolerances, and add additional criteria, using filters. An example filter for the third workflow step used in this application

note, is shown in Figure 4.

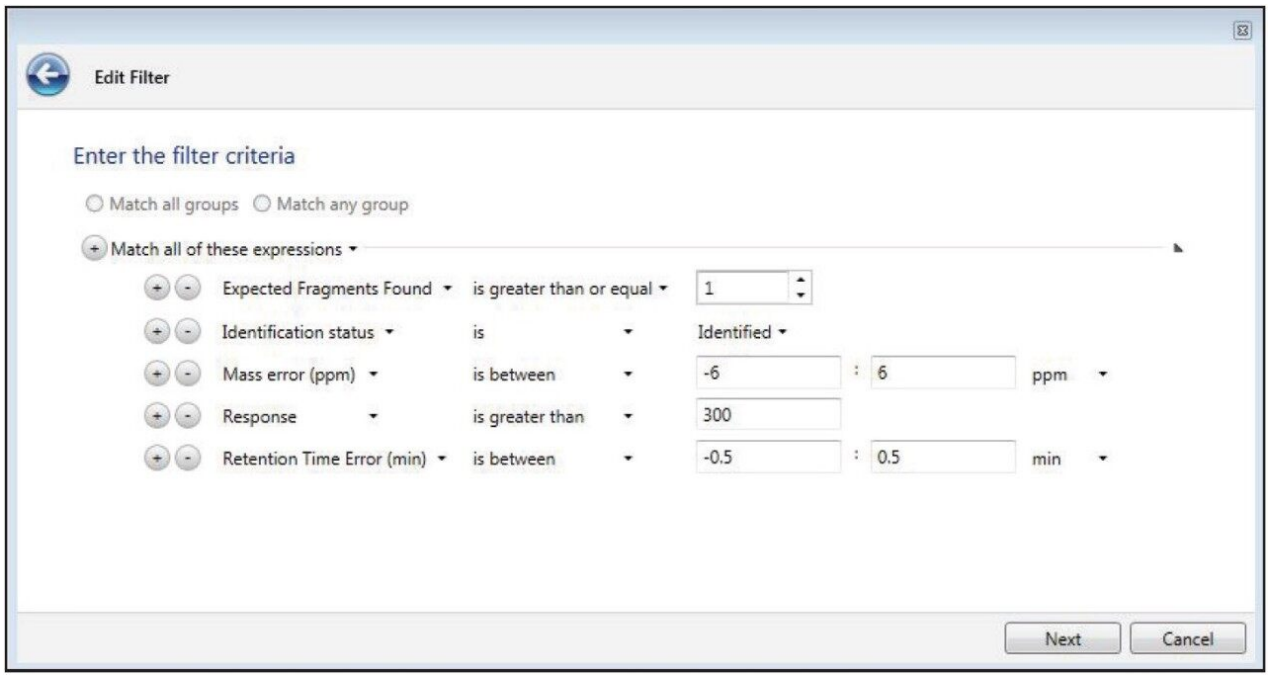

Figure 4. A filter used to interrogate componentized data in UNIFI for "confident" target list matches.

A filter represents a question and the customized view is designed to display information required to make a rapid yes/no answer to violations in the selected sample. The filter in Figure 4 ensures that only confident matches are displayed. This filter restricts the viewed target list matches to those that are within a retention time tolerance of ±0.5 minutes, that have a mass accuracy within ±6ppm, that have a response over 300 and that match at least one target fragment ion. False detections are avoided due to the specificity of requiring an accurate mass fragment ion in addition to matching the tighter criteria set for retention time, mass accuracy and intensity in the filter compared to the analysis method.

A "view" containing the component summary, mass spectra and extracted ion chromatograms was designed to display all of the information required to rapidly verify the confident matches in this workflow step. From Figure 5 we can see the wealth of information available for the match of thiacloprid in the dried cherry sample. In the component summary (Figure 5A) we see that the compound has been matched within 0.06 minutes of the expected retention time, a mass error of 0.82 ppm and an expected accurate mass fragment was matched from the target list. In addition, four accurate mass fragments have been identified by the Fragment Match tool, which automatically interrogates the high energy data once a target match is made. A low isotope score for m/z and intensity gives confidence that the component isotope pattern closely matches

that of the compounds theoretical isotopic pattern.

Data componentization is key to the success of UNIFI data review since all of the mass spectral data displayed for a match are from a tight retention time band at the apex of the chromatographic peak. This also results in cleaner looking spectra compared to a conventional background subtracted spectra from an extracted ion chromatogram, as can be seen in figure 5B. The isotope scores in the component summary are based on the entire cluster in the green shaded area for the most intense adduct (H+ in this case).

The high energy data shows fragment ion information and two example fragment ion identifications are highlighted in figure 5C. The fragment ions matched from those in the Scientific Library are automatically annotated with the mass error. Fragment ions matched by the Fragment Match tool are automatically annotated with the proposed structure and mass error from the theoretically calculated mass. All isotopic information is obtained for fragments in addition to precursors, which is especially useful for atoms with characteristic isotopic patterns, such as the halogens. In the thiacloprid example, the expected fragment formula contains a chlorine atom. The measured isotopic pattern of the high energy fragment agrees with the theoretical distribution for the presence of a single chorine atom. This increases confidence in the compound match.

The final piece of evidence pointing to a confident match lies in the Chromatogram window. Extracted ion chromatograms (Figure 5D) are displayed for precursor ions and matched fragment ions. The peak shape and apex retention time should be identical for a precursor and its fragments, as can be seen for thiacloprid in Figure 5D.

Using the workflow step for confident matches, a user can verify these matches within seconds. Componentization of data makes all of this possible. Any false detects matched using a wide tolerance of retention time and mass error are automatically filtered out from this view, vastly improving efficiency of data interpretation without the risk of introducing false negatives in the data processing step.

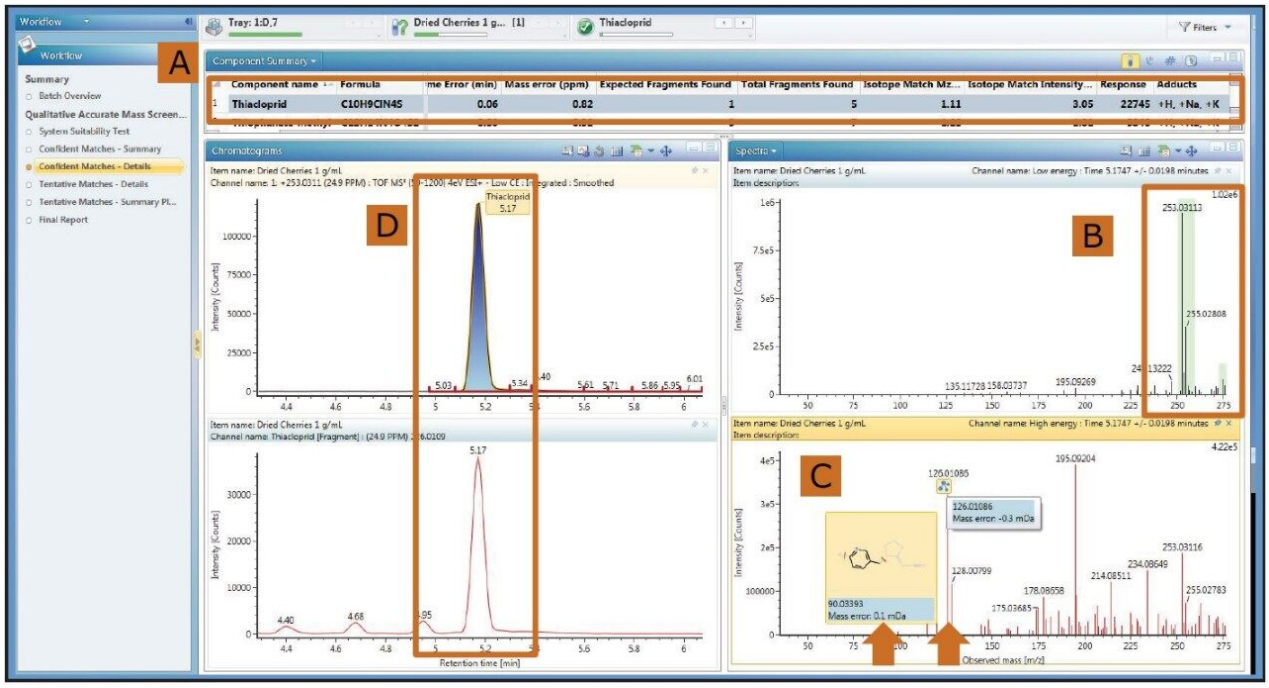

Figure 5. A customized view containing all the information required to make a rapid yes/no visual verification on compound matches.

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