Waters™

Applikationsbericht

Building a UNIFI Scientific Library for HRMS Screening Experiments

Lauren Mullin, Gareth E. Cleland, Kendon S. Graham, Dimple D. Shah, Jennifer A. Burgess

Waters Corporation

Abstract

The UNIFI Scientific Information System offers a streamlined approach for the management of data and

metadata associated with high-resolution accurate mass screening libraries.

Benefits

Using purchased standards for the compounds of interest, a comprehensive scientific library may be constructed by:

- Acquiring low and high energy MS data using MS^E in a single injection.
- Determining the theoretical mass, formulae, and structure of identified fragment ions.
- Ensuring the accuracy of product ion identification by obtaining a MS/MS reference spectrum.
- Uploading all detection results, such as retention time and theoretical accurate mass product ions into the scientific library for future analyses.

Information from previous targeted screening experiments may also be of use, such as known MRM transitions for the compounds of interest.

Introduction

The acquisition of information-rich datasets collected using techniques such as MS^E is placing a demand on the content of the scientific libraries used to screen for a large number of compounds in complex matrices. Using more criteria with wider tolerances controls the number of false detects while ensuring that false negatives are not introduced in a high-resolution mass spectrometry (HRMS) screening experiment. In order to capture and manage the wealth of analytical information that can be gained from powerful chromatographic separations and HRMS, Waters has created the UNIFI Scientific Library.

Software functionality within UNIFI aids in the creation of a scientific library that contains all the critical detection criteria, such as retention time and the theoretical masses of fragment ions generated using structural information.

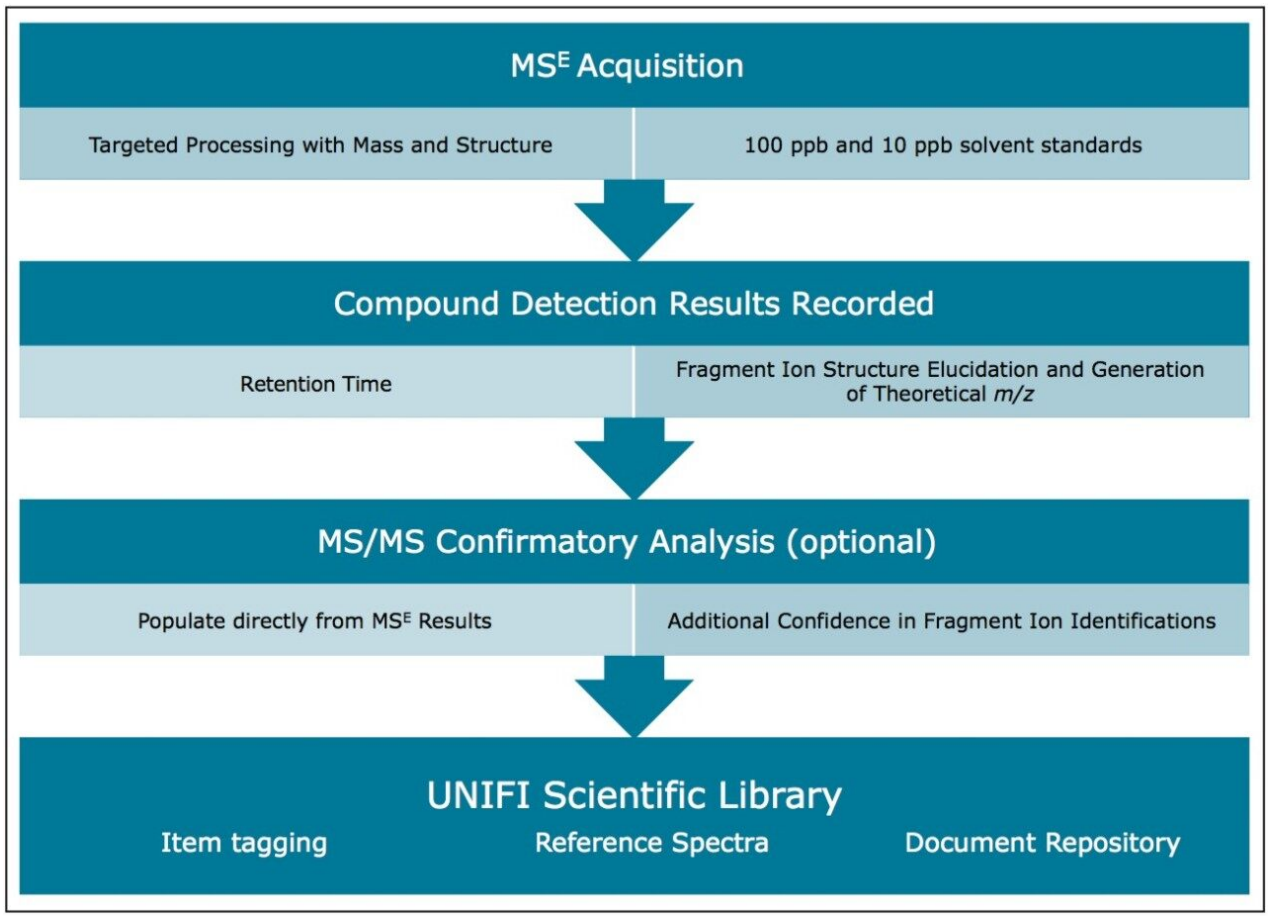
Here we demonstrate the ease with which a comprehensive scientific library can be built within UNIFI, considering two different scenarios:

1. Using purchased standards for the compounds of interest with no prior LC-MS knowledge about the compounds of interest.

2. Using purchased standards for the compounds of interest with prior LC-MS knowledge in the form of MRM transitions.

For both scenarios the following four steps were taken to build the new scientific library, as described in the workflow shown in Schematic 1.

- MS^E Acquisition – For Scenario 1 (above), the analysis is performed in positive and negative ion modes. For Scenario 2 (above), the analysis is performed in the same polarity mode as the MRM information.
- Identify the compound using accurate mass information and update the retention time in the library. Theoretical accurate masses for fragment ions can be generated using the Fragment Match algorithm in UNIFI.
- If dedicated MS/MS is desired, the Send to MS/MS function is deployed to automatically populate the MS/MS analysis method with the precursor ion information and retention time. Upon completion of the MS/MS acquisition, the MS/MS reference spectrum is available for library upload.
- Additional scientific library functionality can be used to provide further information. For example, items can be tagged with classification information, keywords, description, and reference information which can be uploaded to complete the repository.



Schematic 1. A streamlined approach to building a screening library for complex HRMS data using UNIFI Software.

The information stored in a scientific library is a powerful tool for ensuring confident identifications in future HRMS screening experiments. Once a compound is added to the library, relevant reference and classification information can be added within the software. Due to the emergence of novel pesticides, metabolites and other newly recognized contaminants, the ability to create additions to the existing scientific library is of great utility.

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:	10 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

Time (min)	Flow rate (mL/min)	%A	%B	Curve
13.01	0.45	98	2	6
17.00	0.45	98	2	6

Table 1. UPLC method for the Pesticide Screening Application Solution.

MS conditions

MS system:	Xevo G2-S QTof
Ionization mode:	ESI + and -
Capillary voltage:	1 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:	*m/z* 50 to 1200
Acquisition rate:	4 spectra/s
Low CE:	4 eV
High CE ramp:	10 to 45 eV

Results and Discussion

MS^E Acquisition of Standards

In order to include compound structures in the UNIFI Scientific Library, *.mol* files are required for each of the compounds of interest. These can be downloaded from ChemSpider(www.chemspider.com). An Excel spreadsheet containing the names of the compounds of interest, their molecular formulae, and the name of the corresponding .mol file were saved in the directory containing the .mol files. This spreadsheet was then imported into UNIFI as a scientific library container (ULC), to be used as the initial screening list, shown in Figure 1.

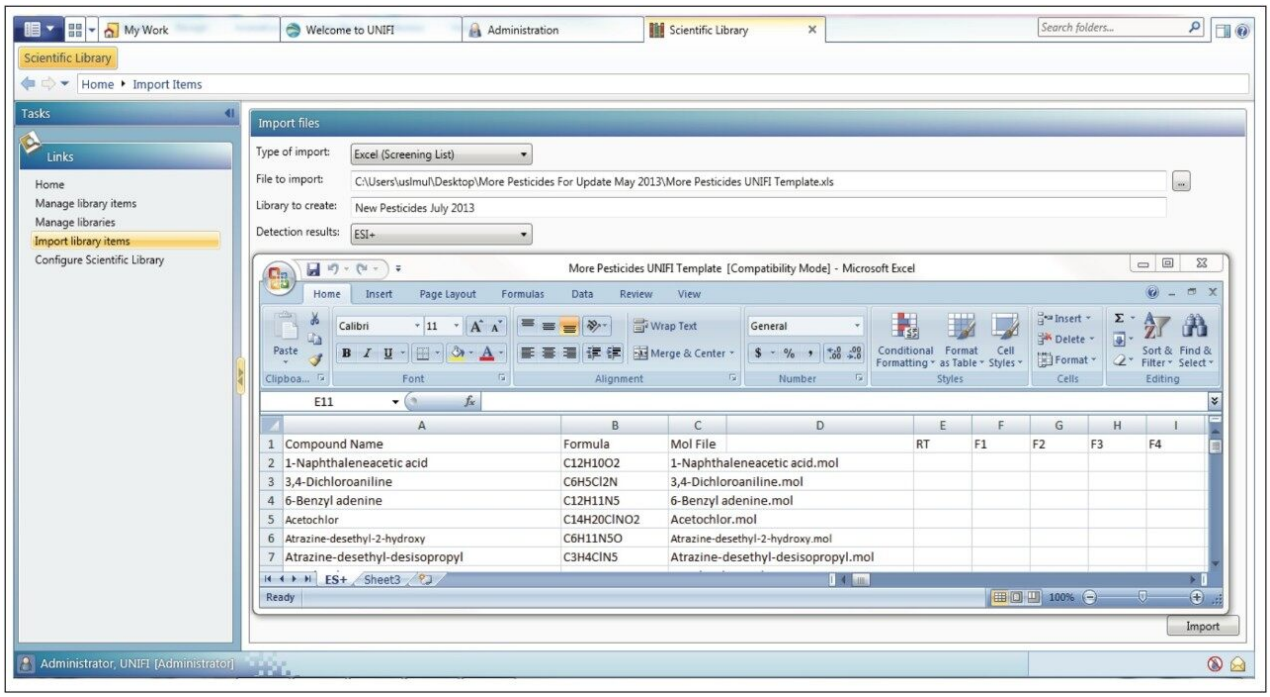
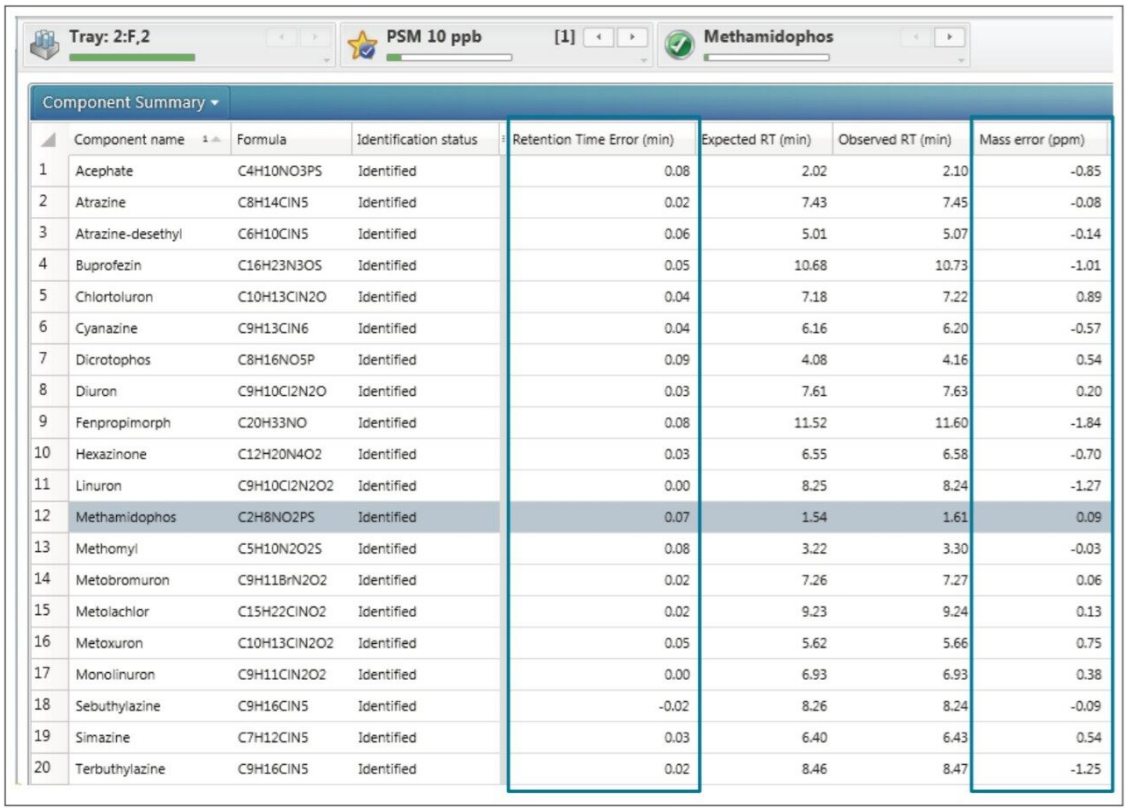


Figure 1. From Administration, select the Scientific Library tab, then the Import Library Items tab, and browse for the appropriate spreadsheet, formatted in the displayed layout. Importing will create the UNIFI Library Container (ULC) to be used for the analysis.

A new analysis method was created using the Accurate Mass MS^E Experiment type. The instrument settings of the Pesticide Screening Application Solution (PSAS) were used for this analysis. For QC purposes, the Pesticide Screening Mix (p/n 186006348) compounds were also added to the component list. Prior to analysis of the new compounds, a test injection of 10 ppb of the pesticide screening mix was performed. The

mass error and observed retention times of the pesticide screening mix components were checked against the expected values, according to our QC requirements, to provide consistency in the library updates (Figure 2). This step ensures uniform results between new library entries and those created in the past, by different users, or on a different system.



	Component name	Formula	Identification status	Retention Time Error (min)	Expected RT (min)	Observed RT (min)	Mass error (ppm)
1	Acephate	C4H10NO3PS	Identified	0.08	2.02	2.10	-0.85
2	Atrazine	C8H14ClN5	Identified	0.02	7.43	7.45	-0.08
3	Atrazine-desethyl	C6H10ClN5	Identified	0.06	5.01	5.07	-0.14
4	Buprofezin	C16H23N3OS	Identified	0.05	10.68	10.73	-1.01
5	Chlortoluron	C10H13ClN2O	Identified	0.04	7.18	7.22	0.89
6	Cyanazine	C9H13ClN6	Identified	0.04	6.16	6.20	-0.57
7	Dicrotophos	C8H16NO5P	Identified	0.09	4.08	4.16	0.54
8	Diuron	C9H10Cl2N2O	Identified	0.03	7.61	7.63	0.20
9	Fenpropimorph	C20H33NO	Identified	0.08	11.52	11.60	-1.84
10	Hexazinone	C12H20N4O2	Identified	0.03	6.55	6.58	-0.70
11	Linuron	C9H10Cl2N2O2	Identified	0.00	8.25	8.24	-1.27
12	Methamidophos	C2H8NO2PS	Identified	0.07	1.54	1.61	0.09
13	Methomyl	C5H10N2O2S	Identified	0.08	3.22	3.30	-0.03
14	Metobromuron	C9H11BrN2O2	Identified	0.02	7.26	7.27	0.06
15	Metolachlor	C15H22ClNO2	Identified	0.02	9.23	9.24	0.13
16	Metoxuron	C10H13ClN2O2	Identified	0.05	5.62	5.66	0.75
17	Monolinuron	C9H11ClN2O2	Identified	0.00	6.93	6.93	0.38
18	Sebuthylazine	C9H16ClN5	Identified	-0.02	8.26	8.24	-0.09
19	Simazine	C7H12ClN5	Identified	0.03	6.40	6.43	0.54
20	Terbuthylazine	C9H16ClN5	Identified	0.02	8.46	8.47	-1.25

Figure 2. Mass error and retention time differences were assessed prior to the analysis to ensure the system was running properly.

Standards of the new compounds in Milli-Q grade water were injected at two concentrations, 10 ppb and 100 ppb, to benchmark the sensitivity and response of each compound. For each of the standards injected, a target list of the .mol files for the compound(s) in that standard can be added directly to the sample list, as shown in Figure 3. For the pesticide Screening Mix, all of the compounds were added to the target list (first line, Figure 3 “20 targets specified”). Using a target list can be used as an alternative to the aforementioned Excel spreadsheet. Upon analysis, retention times were easily determined, based on identifications made using the exact mass, as can be seen for the rodenticide Coumachlor in Figure 4.

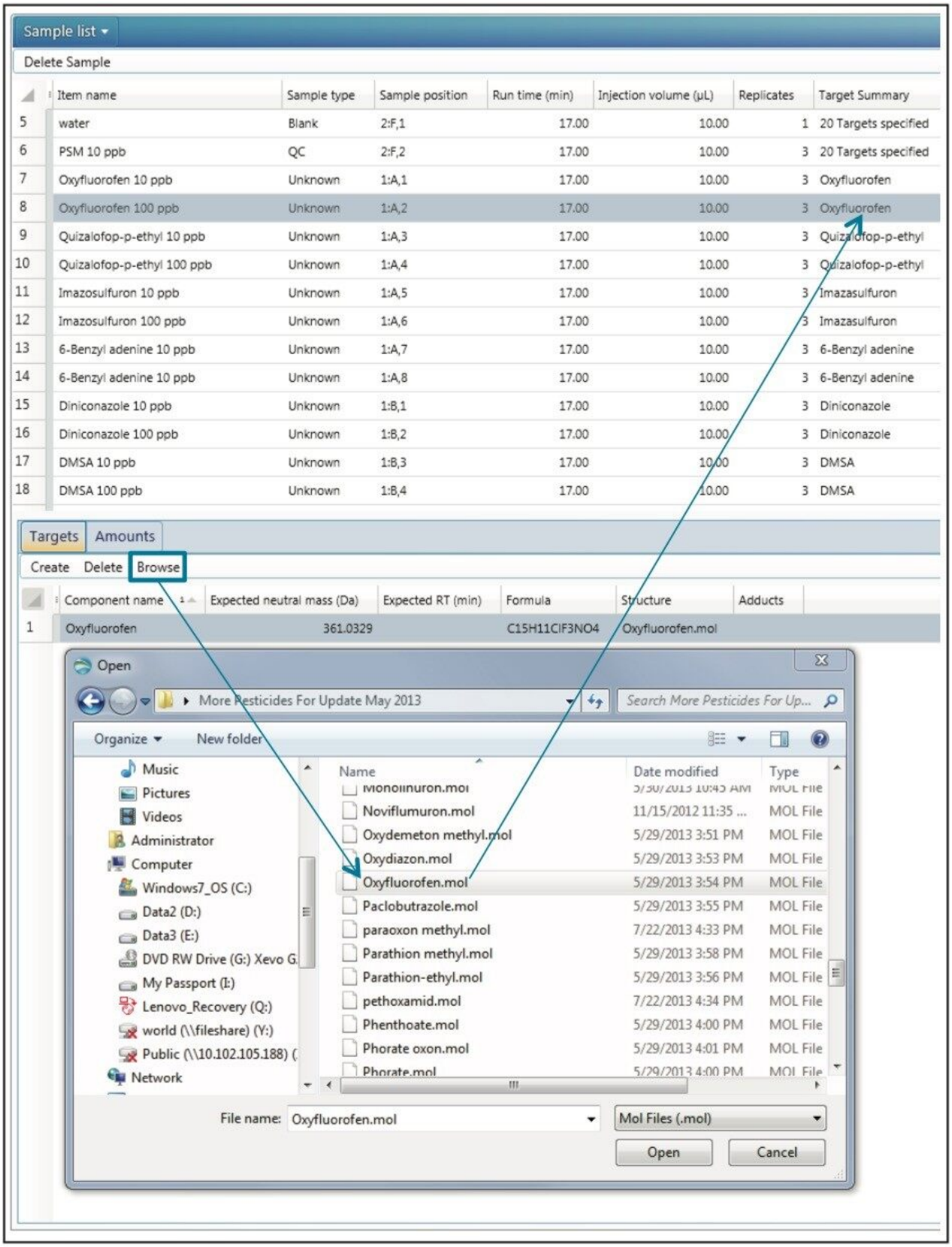

Sample list
Delete Sample
Item name | Sample type | Sample position | Run time (min) | Injection volume (µL) | Replicates | Target Summary
5 water Blank 2:F,1 17.00 10.00 1 20 Targets specified
6 PSM 10 ppb QC 2:F,2 17.00 10.00 3 20 Targets specified
7 Oxyfluorofen 10 ppb Unknown 1:A,1 17.00 10.00 3 Oxyfluorofen
8 Oxyfluorofen 100 ppb Unknown 1:A,2 17.00 10.00 3 Oxyfluorofen
9 Quizalofop-p-ethyl 10 ppb Unknown 1:A,3 17.00 10.00 3 Quizalofop-p-ethyl
10 Quizalofop-p-ethyl 100 ppb Unknown 1:A,4 17.00 10.00 3 Quizalofop-p-ethyl
11 Imazosulfuron 10 ppb Unknown 1:A,5 17.00 10.00 3 Imazasulfuron
12 Imazosulfuron 100 ppb Unknown 1:A,6 17.00 10.00 3 Imazasulfuron
13 6-Benzyl adenine 10 ppb Unknown 1:A,7 17.00 10.00 3 6-Benzyl adenine
14 6-Benzyl adenine 10 ppb Unknown 1:A,8 17.00 10.00 3 6-Benzyl adenine
15 Diniconazole 10 ppb Unknown 1:B,1 17.00 10.00 3 Diniconazole
16 Diniconazole 100 ppb Unknown 1:B,2 17.00 10.00 3 Diniconazole
17 DMSA 10 ppb Unknown 1:B,3 17.00 10.00 3 DMSA
18 DMSA 100 ppb Unknown 1:B,4 17.00 10.00 3 DMSA
Targets
Amounts
Create
Delete
Browse
Component name | Expected neutral mass (Da) | Expected RT (min) | Formula | Structure | Adducts
1 Oxyfluorofen 361.0329 C15H11ClF3NO4 Oxyfluorofen.mol
Open
More Pesticides For Update May 2013
Search More Pesticides For Up...
Organize
New folder
Music
Pictures
Videos
Administrator
Computer
Windows7_OS (C:)
Data2 (D:)
Data3 (E:)
DVD RW Drive (G:) Xevo G
My Passport (I:)
Lenovo_Recovery (Q:)
world (\\fileshare) (Y:)
Public (\\10.102.105.188) (
Network
Name
Date modified
Type
Monolinuron.mol
Noviflumuron.mol 11/15/2012 11:35 ... MOL File
Oxydemeton methyl.mol 5/29/2013 3:51 PM MOL File
Oxydiazon.mol 5/29/2013 3:53 PM MOL File
Oxyfluorofen.mol 5/29/2013 3:54 PM MOL File
Paclobutrazole.mol 5/29/2013 3:55 PM MOL File
paraoxon methyl.mol 7/22/2013 4:33 PM MOL File
Parathion methyl.mol 5/29/2013 3:58 PM MOL File
Parathion-ethyl.mol 5/29/2013 3:56 PM MOL File
pethoxamid.mol 7/22/2013 4:34 PM MOL File
Phenthoate.mol 5/29/2013 4:00 PM MOL File
Phorate oxon.mol 5/29/2013 4:01 PM MOL File
Phorate.mol
File name: Oxyfluorofen.mol
Mol Files (.mol)
Open
Cancel

Figure 3. Sample list with easily added .mol files of new pesticide standards, which are included for use in fragment ion structural elucidation during processing.

Figure 4. Coumachlor identified with accurate mass alone. Observed retention time was then added to the library. Blue icons in the high energy spectrum indicate an accurate mass product ion, automatically matched by the Fragment Match algorithm. Green area in the low energy spectrum highlight all adducts and their isotopes observed.

Product Ion Assignment

Continuing with the Coumachlor example, the Fragment Match results (Figure 5) were displayed by right clicking on the name of the identified compound and selecting Elucidate. The most ideal fragment ions to include in the scientific library were assigned based on their intensity and match scores. Common Fragment Match functionality was used to bring up an XIC of the proposed fragment mass, shown in Figure 6. The chromatogram provides more confidence in correct fragment ion assignment and also gives an indication of the selectivity of that particular fragment ion. If prior knowledge of MRM transitions for a specific compound is available, referring to those would offer additional data for fragment ion selection. Using this approach, theoretical fragment masses were determined and were used to populate the new library, either by adding to the Excel spreadsheet, or entering them directly into the UNIFI Scientific Library. Once the information for all

compounds was added, entries from the newly created scientific library were replaced in the original analysis and the data re-processed to ensure that all compounds were identified using the new library.

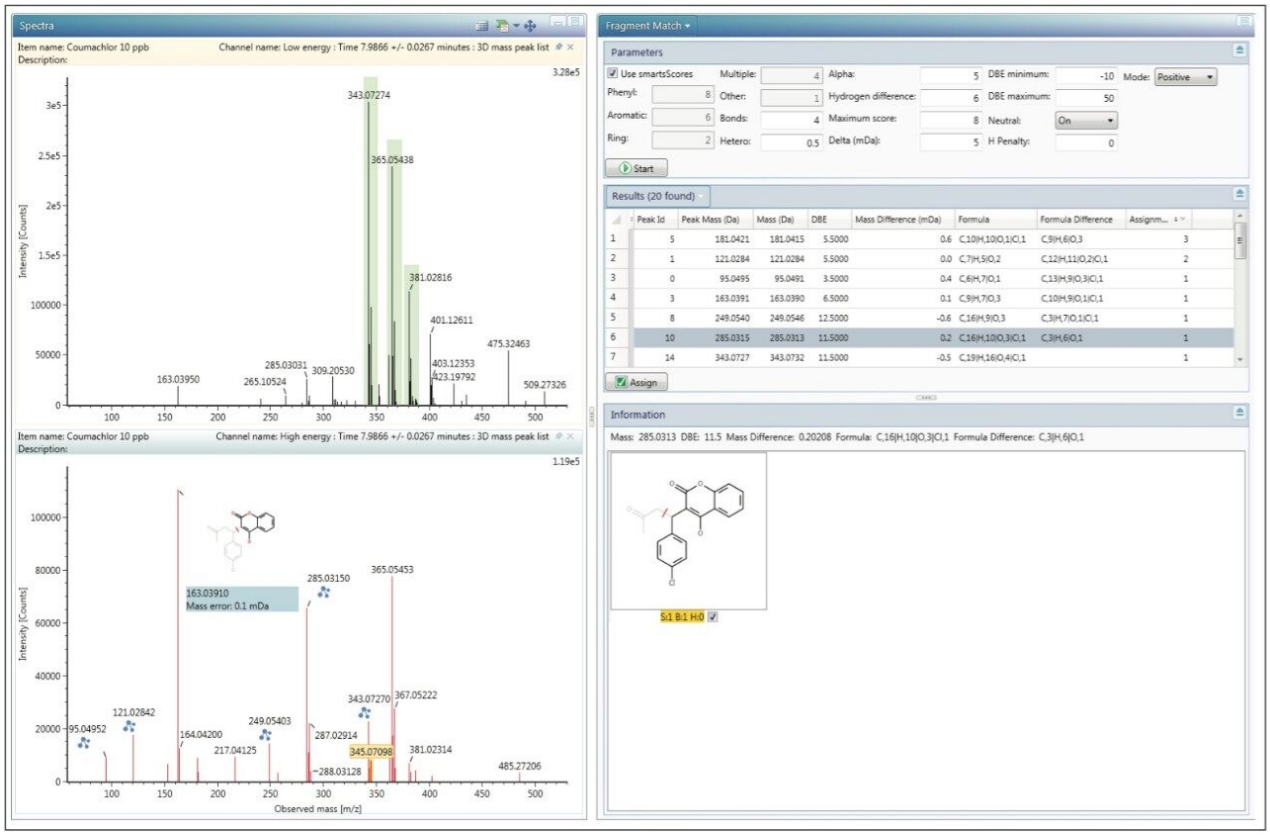


Figure 5. Fragment Match functionality is easy to use and comprehensive for determining product ions.

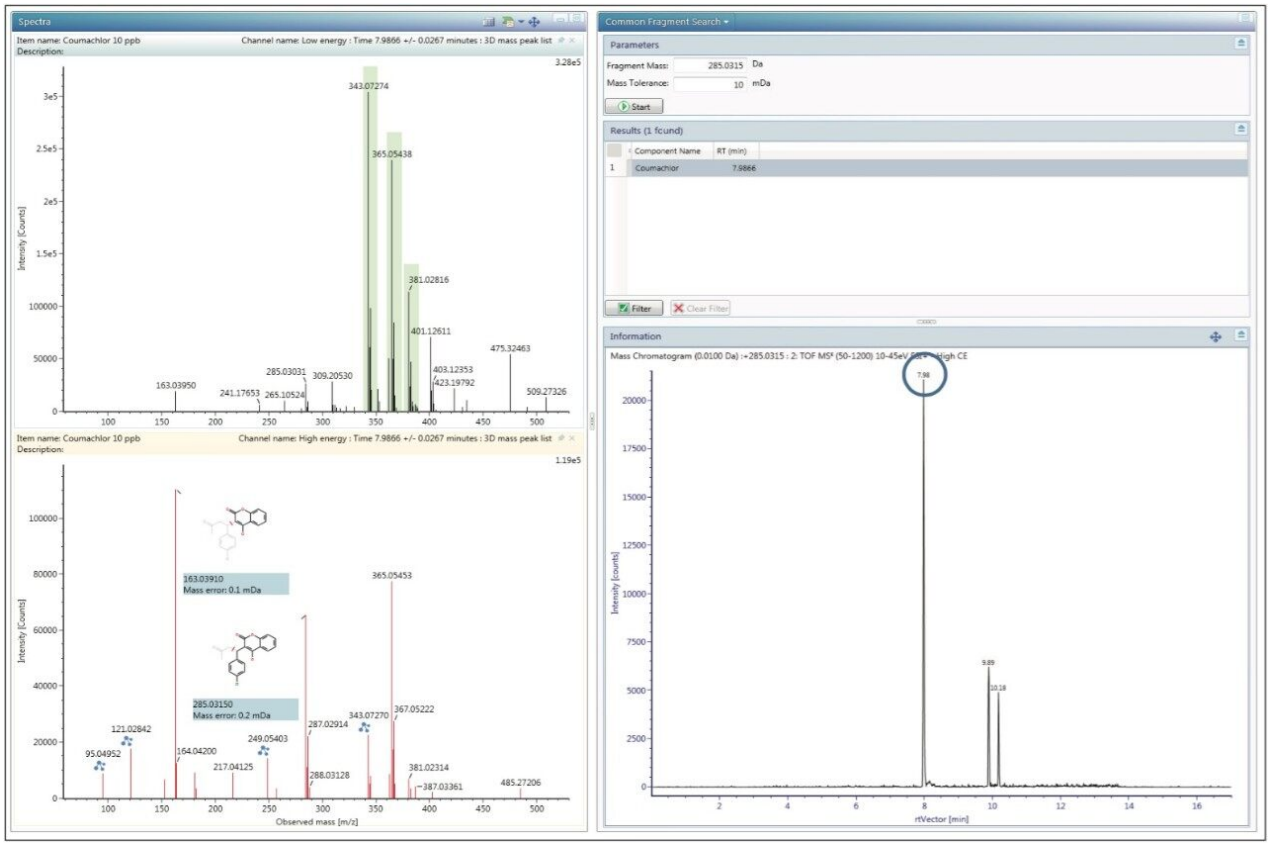


Figure 6. Using Common Fragment search, extracted ion chromatograms were generated for the selected product ion, while also searching for all compounds in the current analysis for the same retention time as the chosen product.

MS/MS Acquisition

As an additional means of confirmation of the selected fragment ions, MS/MS analysis can be performed on the standards following MS^E identification. In the past, separate MS/MS experiments had to be set up for each compound of interest, which was laborious and time consuming. UNIFI allows the user to directly import the compound information and detection results from the MS^E analysis by clicking Send to MS/MS. With a generic MS/MS method open, compound specific information from the MS^E analysis are sent to the MS/MS analysis by right clicking the identification, then select the Send To (Figure 7). As with the MS^E analysis, system performance was first verified using the Pesticide Screening Mix to ensure acceptable retention times and mass accuracies. From these acquisitions, MS/MS spectra of compounds can be added to the scientific library for future reference (Figure 8).

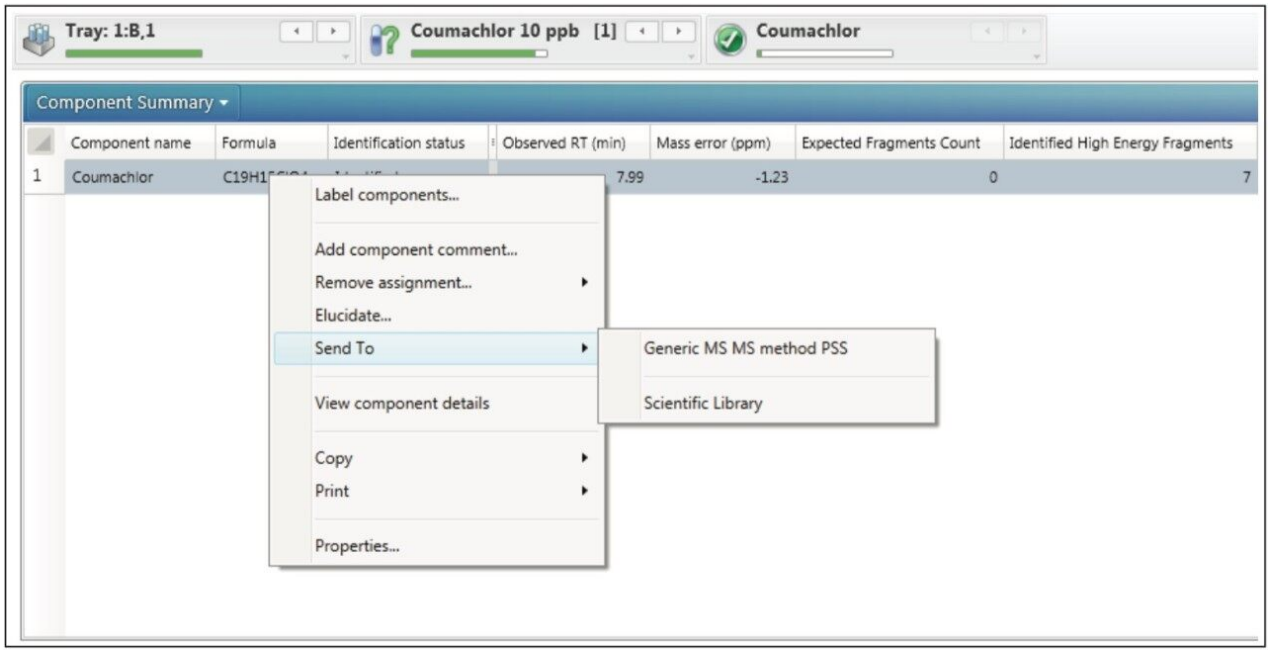


Figure 7. Sending compounds to MS/MS analysis is a time-saving measure that will import all of the targeted compounds with retention time in a given injection into the new analysis.

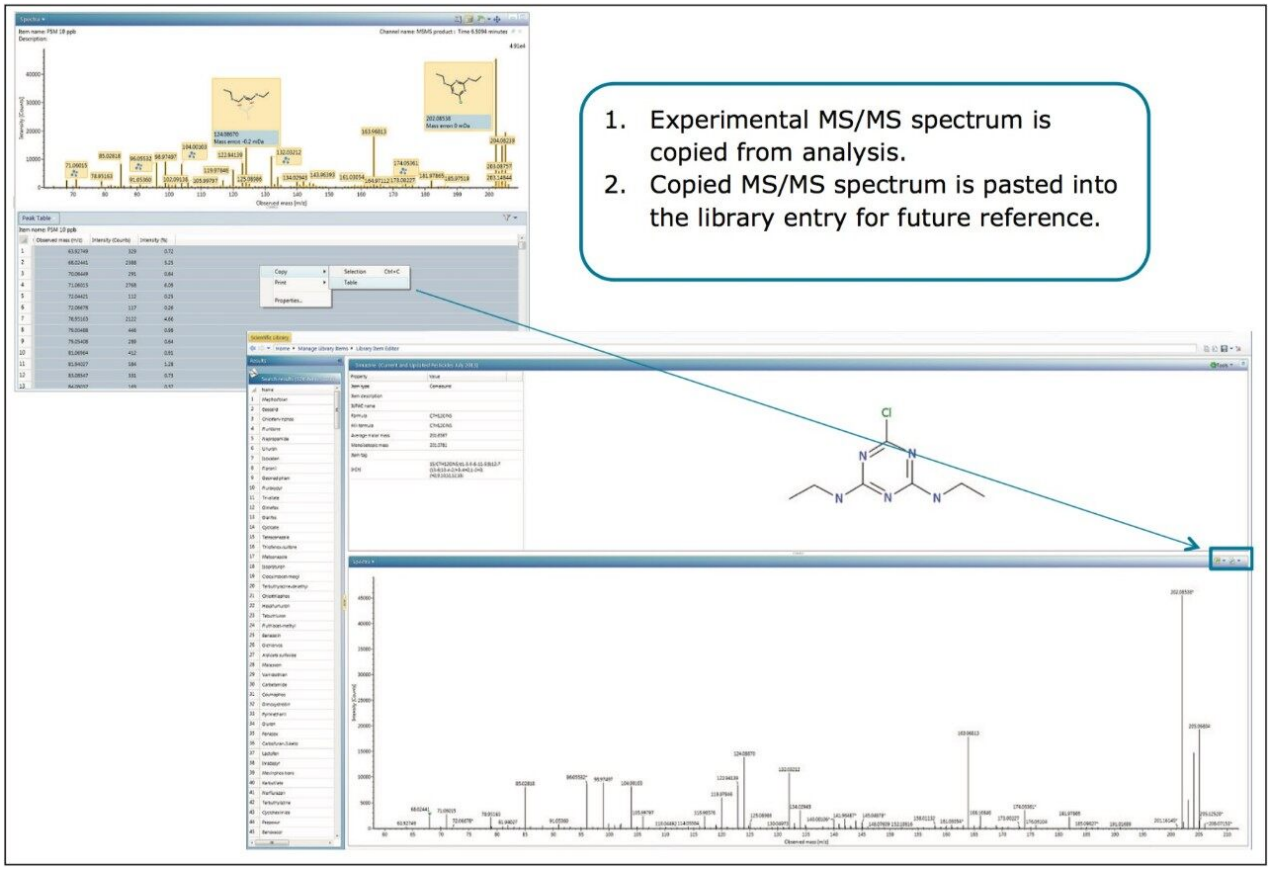


Figure 8. MS/MS spectrum for the compound Simazine saved in the scientific library for future reference.

Database Management and Tagging

In order to maximize the available information and facilitate future searches the UNIFI Scientific Library can associate user-definable tags, references, or other documents with library entries. In the example presented here, individual pesticides were tagged with their pesticide class and sub class, if available (Figure 9). References were added and categorized (Figure 10). Any edits can be made through the Manage Library Items section of the scientific library.

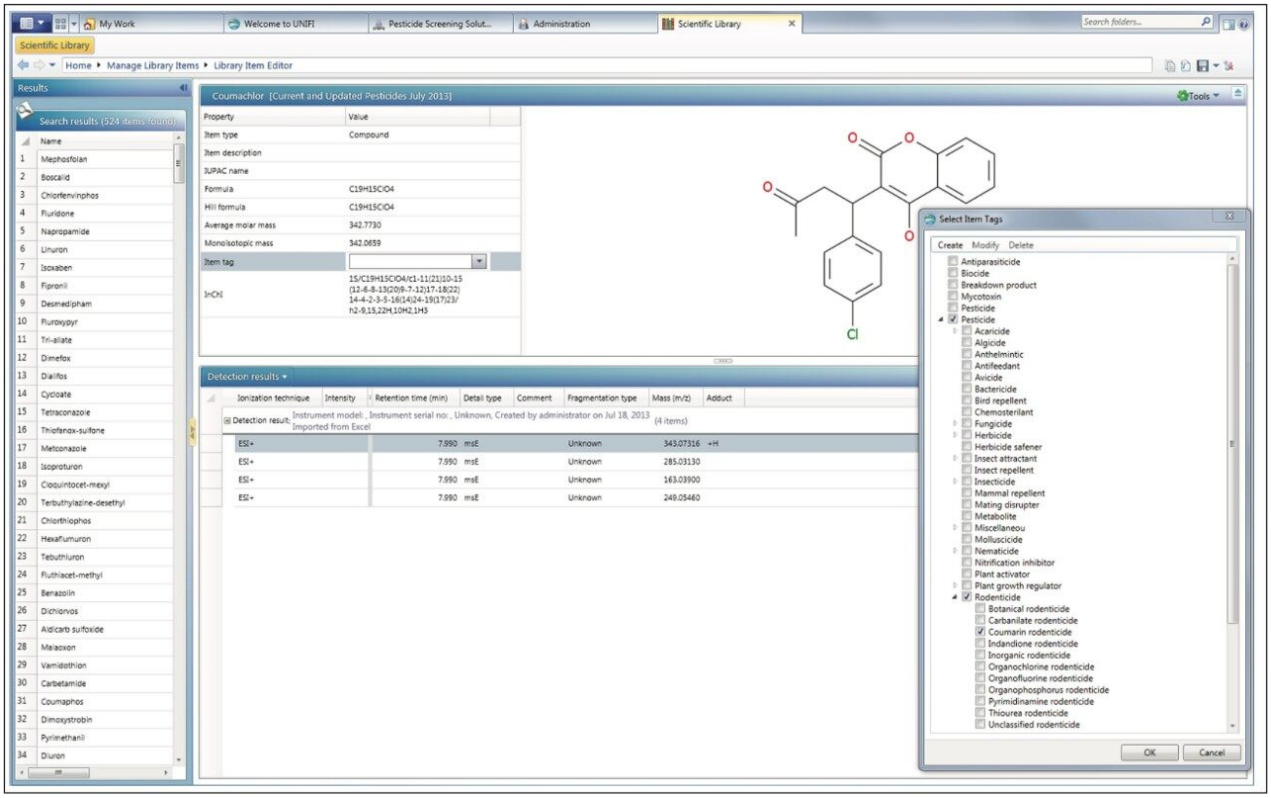


Figure 9. Tagging of compounds, in this case Coumachlor, is a powerful way to organize library updates for future use. Other useful information such as CAS number, or other names for the compound can also be added here.

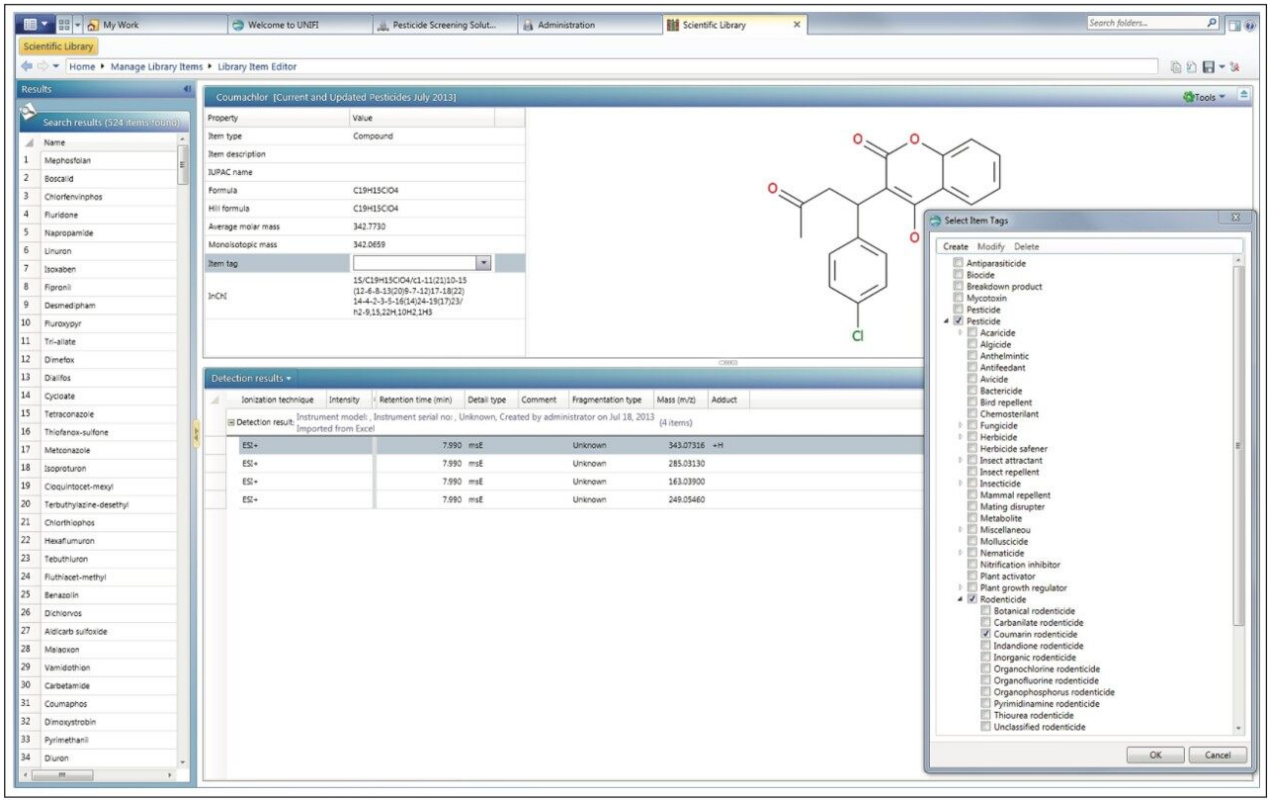


Figure 10. Literature references are easily assigned to compounds; other documentation can also be added here and categorized via the drop-down menu.

Conclusion

- The UNIFI Scientific Information System offers a streamlined approach for the management of data and metadata associated with high-resolution accurate mass screening libraries.
- Functionalities within the UNIFI Elucidation Toolset provide an efficient and reliable way to determine fragment ion masses, structures, and formulae.
- The ACQUITY UPLC I-Class System with Xevo G2-S QTof is a powerful solution for HRMS screening of a wide variety of compounds, providing more information and increased confidence from using accurate mass of both molecular ions and their fragments, along with retention times, isotope patterns, and adduct information.
- UNIFI provides an unparalleled platform for the compilation of valuable experimental and theoretical

information for use in future screening analyses in a wide range of matrices.

Featured Products

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

UNIFI Scientific Information System <https://www.waters.com/134801648>

Pesticide Screening Application Solution with UNIFI <https://www.waters.com/134682906>

720004927, January 2014