

Nota applicativa

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.

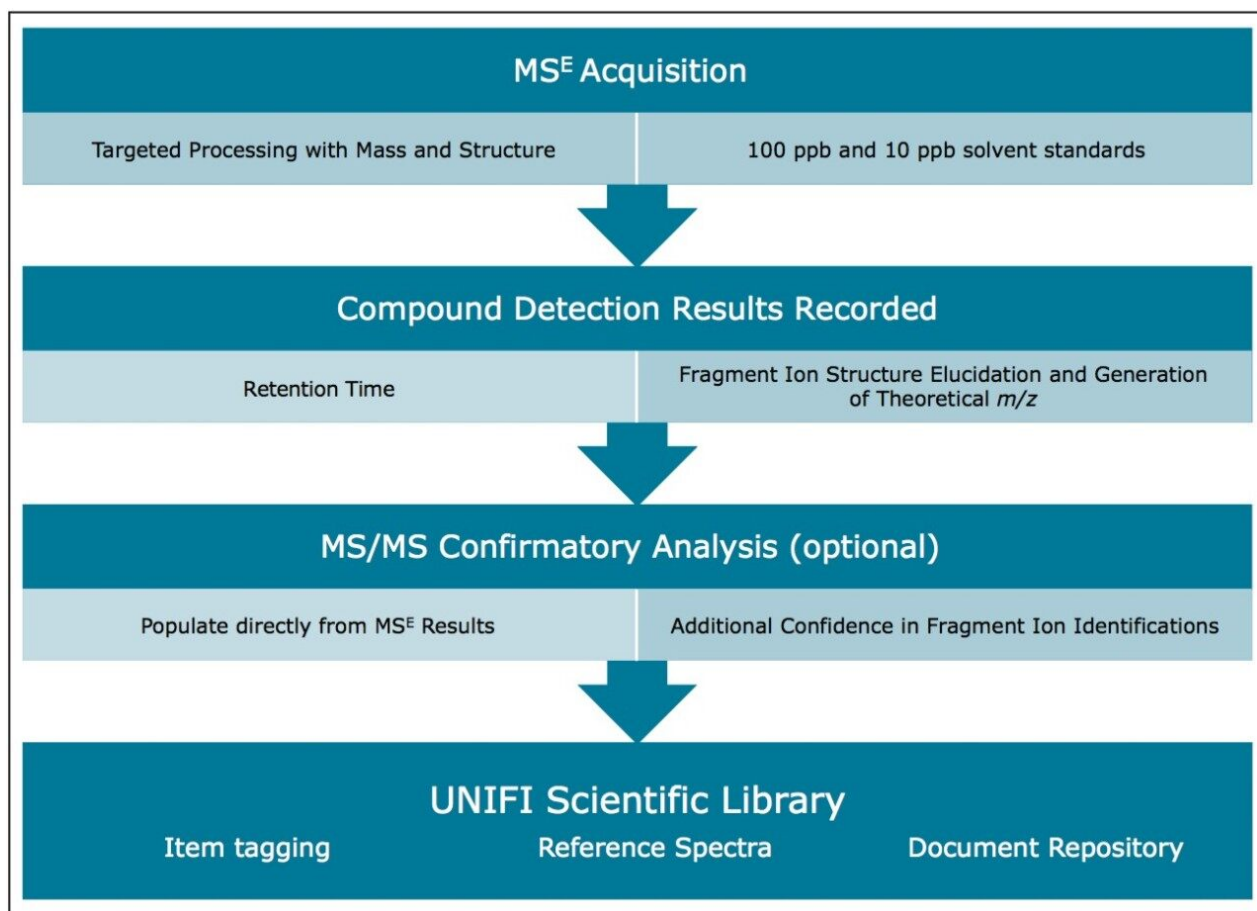
Here we demonstrate the ease with which a comprehensive scientific library can be built within UNIFI, considering two different scenarios:

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 C₁₈ 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:	<i>m/z</i> 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.

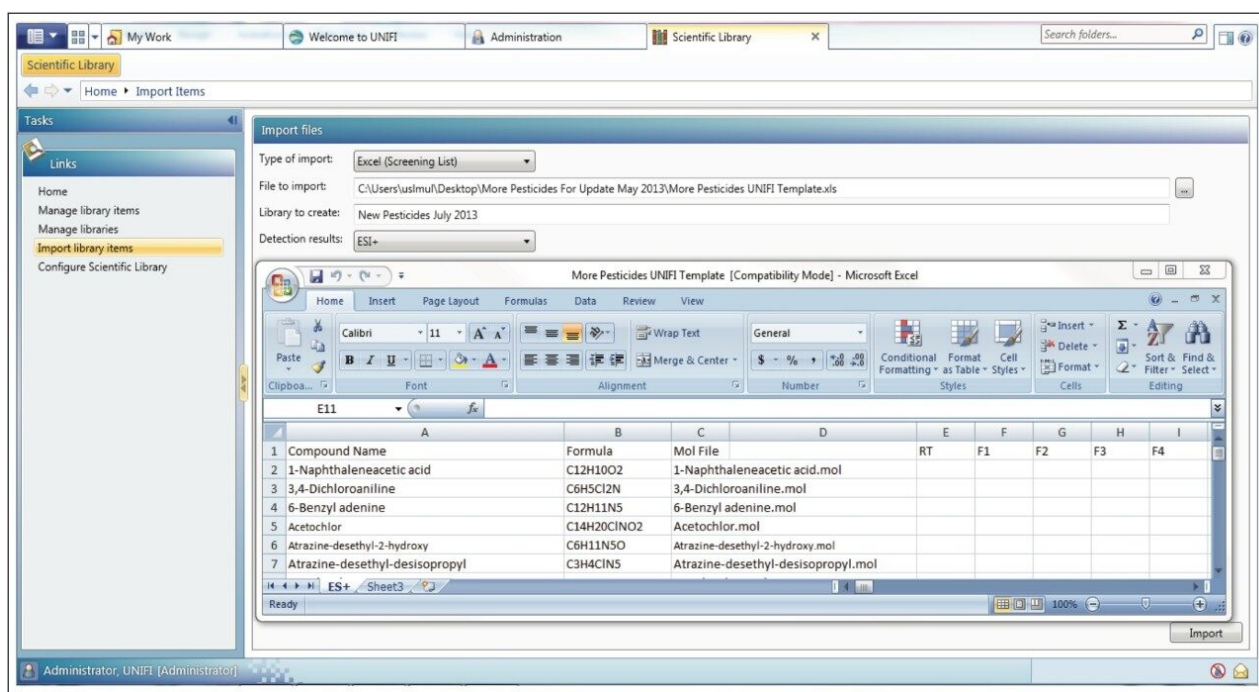


Figure 1. From Administration, select the Scientific Library tab, then the Import Library Items tab, and browse for the appropriate spreadsheet, formatted in the displayed layout. Importing will create the UNIFI Library Container (ULC) to be used for the analysis.

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	C8H14CN5	Identified	0.02	7.43	7.45	-0.08
3 Atrazine-desethyl	C6H10CN5	Identified	0.06	5.01	5.07	-0.14
4 Buprofezin	C16H23N3O5	Identified	0.05	10.68	10.73	-1.01
5 Chlortoluron	C10H13CN2O	Identified	0.04	7.18	7.22	0.89
6 Cyanazine	C9H13CN6	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	C10H13CN2O2	Identified	0.05	5.62	5.66	0.75
17 Monolinuron	C9H11CN2O2	Identified	0.00	6.93	6.93	0.38
18 Sebutylazine	C9H16CN5	Identified	-0.02	8.26	8.24	-0.09
19 Simazine	C7H12CN5	Identified	0.03	6.40	6.43	0.54
20 Terbutylazine	C9H16CN5	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	Imazosulfuron
12 Imazosulfuron 100 ppb	Unknown	1:A,6	17.00	10.00	3	Imazosulfuron
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

Organize New folder

Name	Date modified	Type
<input type="checkbox"/> monoinuron.mol	2/29/2013 10:45 AM	MOL File
<input type="checkbox"/> Noviflumuron.mol	11/15/2012 11:35 ...	MOL File
<input type="checkbox"/> Oxydemeton methyl.mol	5/29/2013 3:51 PM	MOL File
<input type="checkbox"/> Oxydiazon.mol	5/29/2013 3:53 PM	MOL File
<input checked="" type="checkbox"/> Oxyfluorofen.mol	5/29/2013 3:54 PM	MOL File
<input type="checkbox"/> Paclobutrazole.mol	5/29/2013 3:55 PM	MOL File
<input type="checkbox"/> paraoxon methyl.mol	7/22/2013 4:33 PM	MOL File
<input type="checkbox"/> Parathion methyl.mol	5/29/2013 3:58 PM	MOL File
<input type="checkbox"/> Parathion-ethyl.mol	5/29/2013 3:56 PM	MOL File
<input type="checkbox"/> pethoxamid.mol	7/22/2013 4:34 PM	MOL File
<input type="checkbox"/> Phenthoate.mol	5/29/2013 4:00 PM	MOL File
<input type="checkbox"/> Phorate oxon.mol	5/29/2013 4:01 PM	MOL File
<input type="checkbox"/> Phorate.mol	5/29/2013 4:00 PM	MOL File

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.

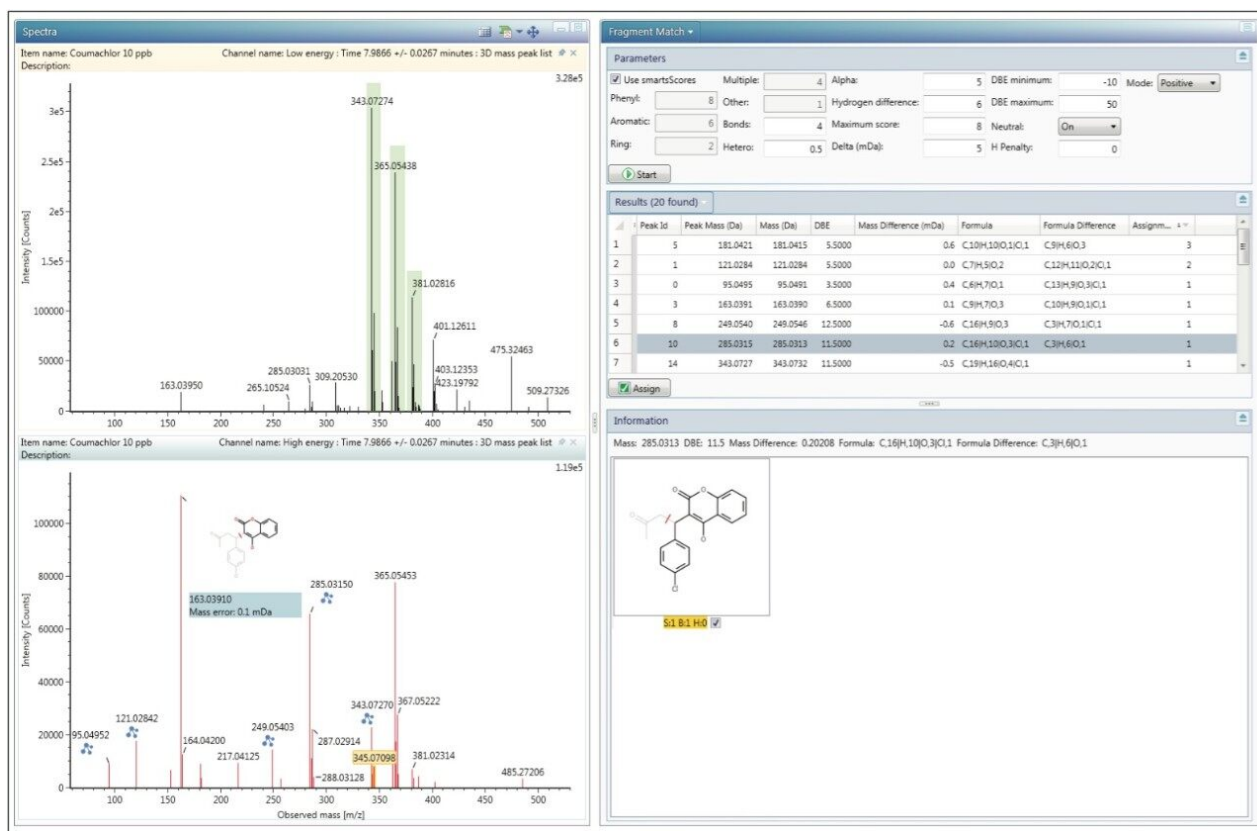


Figure 5. Fragment Match functionality is easy to use and comprehensive for determining product ions.

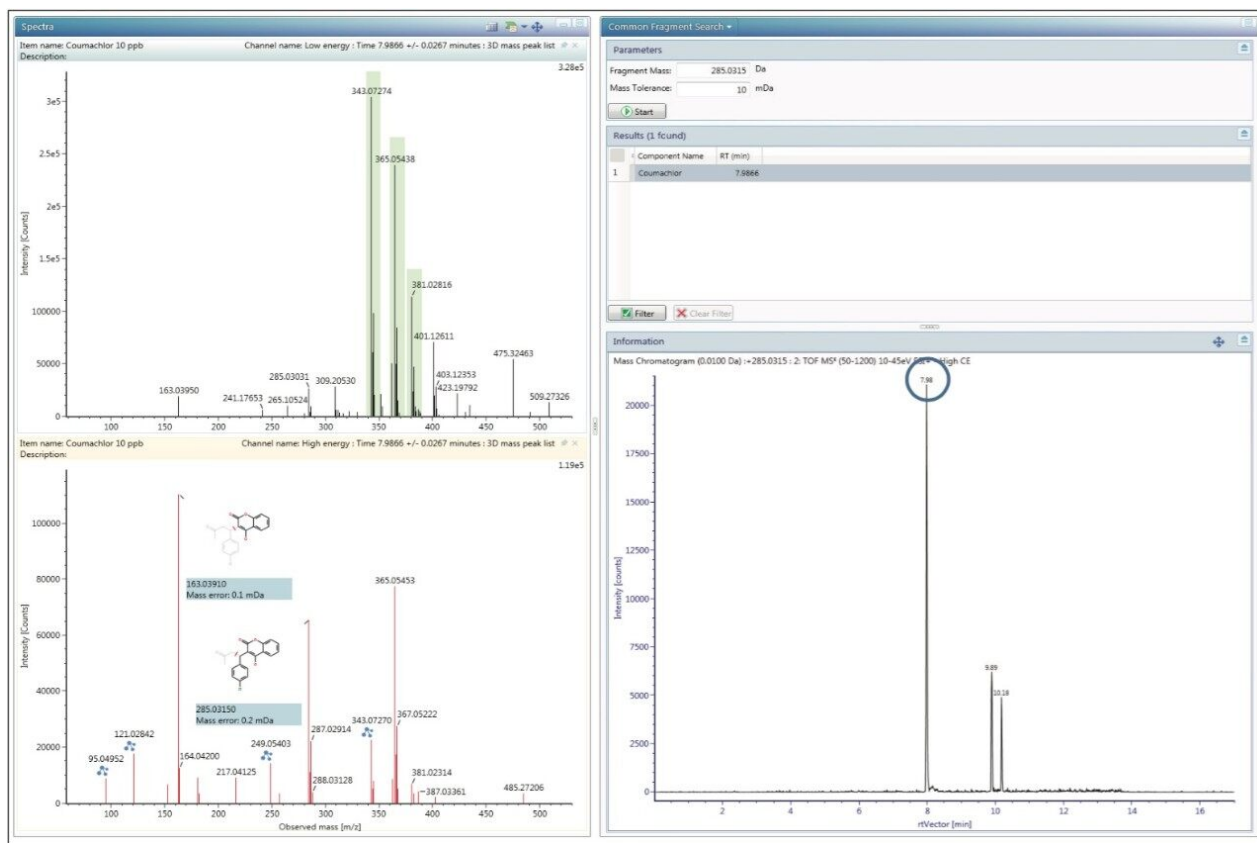


Figure 6. Using Common Fragment search, extracted ion chromatograms were generated for the selected product ion, while also searching for all compounds in the current analysis for the same retention time as the chosen product.

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).

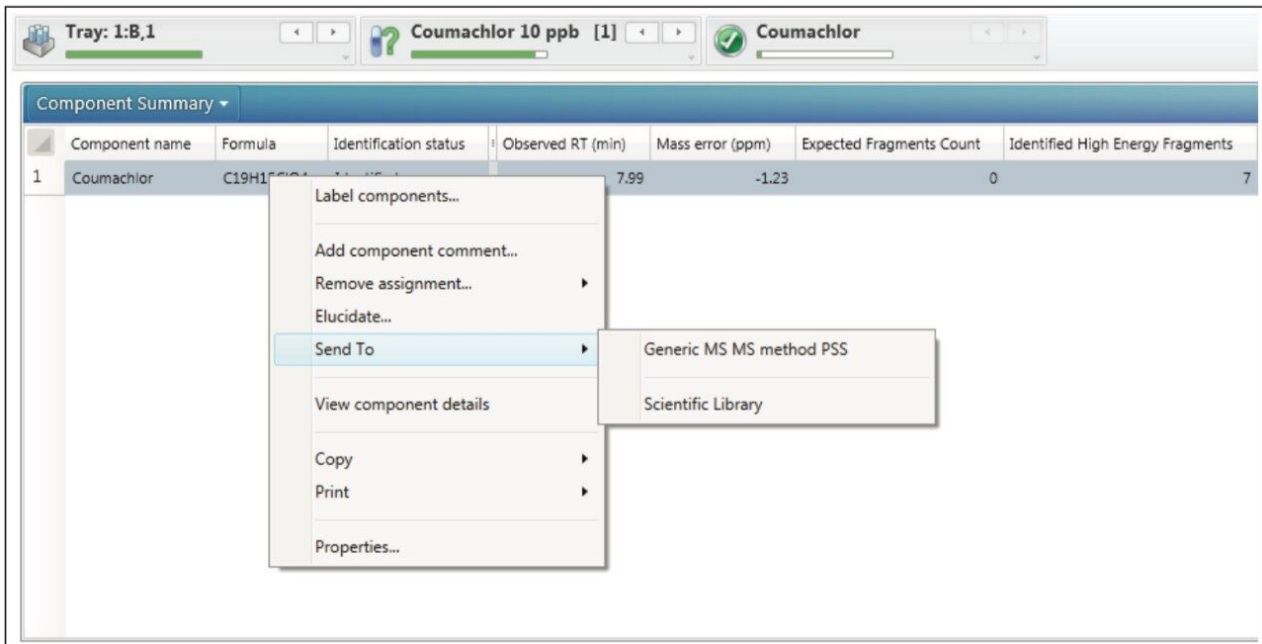


Figure 7. Sending compounds to MS/MS analysis is a time-saving measure that will import all of the targeted compounds with retention time in a given injection into the new analysis.

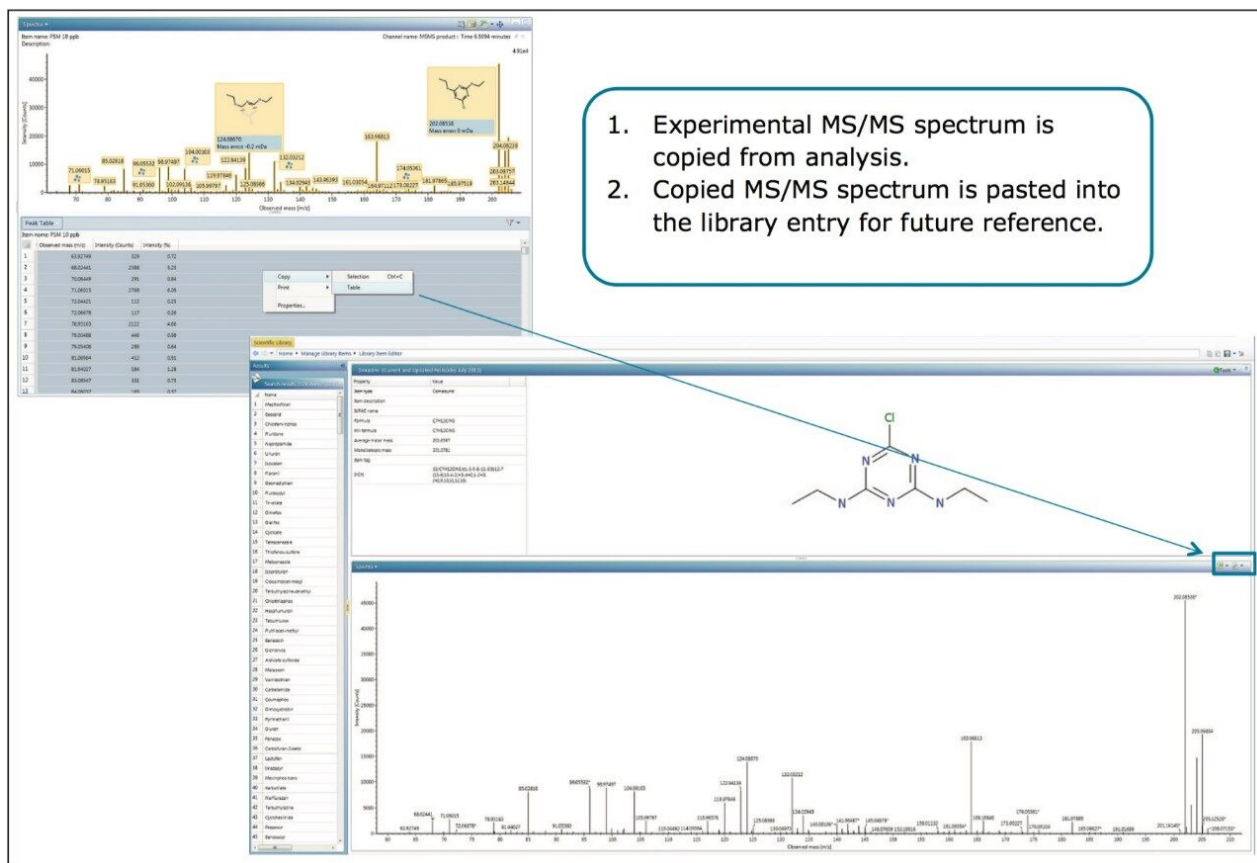


Figure 8. MS/MS spectrum for the compound Simazine saved in the scientific library for future reference.

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.

The screenshot displays the 'Scientific Library' application window. On the left, a search results list shows 324 items, with 'Coumachlor' selected. The main area shows the 'Library Item Editor' for 'Coumachlor', displaying its chemical structure and various properties. A 'Select Item Tags' dialog box is open, showing a tree view of pesticide categories with 'Pesticide' and 'Rodenticide' selected.

Property	Value
Item type	Compound
Item description	
IUPAC name	
Formula	C ₁₉ H ₁₅ O ₄
Hill formula	C ₁₉ H ₁₅ O ₄
Average molar mass	342.7730
Monoisotopic mass	342.0659
Item tag	
InChI	15/C19H15O4/C1-11/2100-15 13-A-8-33009-7-12017-18320 14-4-2-3-5-1815424-1917023/ n2-615,22H12H2,1H3

Ionization technique	Intensity	Retention time (min)	Detail type	Comment	Fragmentation type	Mass (m/z)	Adduct
ESI+		7.990	msE	Unknown		343.07316	+H
ESI+		7.990	msE	Unknown		285.03130	
ESI+		7.990	msE	Unknown		163.03900	
ESI+		7.990	msE	Unknown		249.05460	

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.

The screenshot displays the UNIFI Scientific Library interface. On the left, a search results list shows various pesticides. The main window displays the details for 'Coumachlor [Current and Updated Pesticides July 2013]'. The 'Property Value' table includes:

Property	Value
Item type	Compound
Item description	
IUPAC name	
Formula	C ₁₉ H ₁₅ ClO ₄
Hill formula	C ₁₉ H ₁₅ ClO ₄
Average molar mass	342.7733
Monoisotopic mass	342.0659
Item tag	15/C19H15ClO4/c1-11/2110-15 (11-4-8-13/209-7-1217-18/22) 14-4-2-3-5-16/14/24-19/17/23/ n2-9-13,22H10H2,1H3
INCX	

The chemical structure of Coumachlor is shown as a 2,3-dimethyl-4-(4-chlorophenyl)-5-hydroxy-2H-benzofuran derivative. Below the structure is a 'Detection results' table:

Ionization technique	Intensity	Retention time (min)	Detail type	Comment	Fragmentation type	Mass (m/z)	Adduct
ESI+		7.990	msE	Unknown		343.07316	+H
ESI+		7.990	msE	Unknown		285.03130	
ESI+		7.990	msE	Unknown		163.03900	
ESI+		7.990	msE	Unknown		249.05460	

A 'Select Item Tags' dialog box is open on the right, showing a hierarchical list of tags. The 'Pesticide' category is selected, and the 'Rodenticide' sub-category is expanded, with 'Botanical rodenticide' and 'Coumarin rodenticide' checked.

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>](https://www.waters.com/134613317)

[UNIFI Scientific Information System <https://www.waters.com/134801648>](https://www.waters.com/134801648)

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

720004927, January 2014