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Analysis of Plant Alkaloids Through Accurate Mass Screening and Discovery

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APPLICATION BENEFITS

Analyze plant alkaloids using the Forensic Toxicology Application Screening Solution with UNIFI[1] to demonstrate the simplicity of library creation and expansion. This application note also showcases the power of the latest suite of discovery tools within the UNIFI Scientific Information System v1.8.

WATERS SOLUTIONS

Forensic Toxicology Application Solution with UNIFI®

ACQUITY UPLC® I-Class System (FTN)

ACQUITY UPLC HSS Column

Xevo® G2-S QTof

Maximum Recovery Vials

KEY WORDS

Plant alkaloids, forensic toxicology, UPLC-QTof-MS^E, UNIFI, identification, discovery

INTRODUCTION

Over the last decade there has been a significant increase in the popularity of time-of-flight mass spectrometry (Tof-MS) for multi-residue analysis. Accurate mass imparts high specificity for substance identification and, together with the isotopic data, can provide the user with the opportunity to propose likely elemental compositions. The proposal of elemental formulae is often the starting point for a complex multi-stage process to elucidate chemical structures.

For screening, accurate mass instrumentation presents a significant, and key, advantage over its nominal mass counterpart, i.e., an ability to implement screening methodologies without the requirement of reference material. In this particular workflow the theoretical (expected) exact mass can be determined empirically from the elemental formula. In a toxicological setting this can provide a valuable means with which the analyst may 'prospectively' target novel psychoactive drugs, or new substances and metabolites where reference material may not yet, be available.

An on-going initiative to expand the UNIFI Toxicology Scientific Library led to the analysis of a series of plant alkaloids. These nitrogen-containing compounds are derived from plants and plant material. They are pharmacologically active and have been used for many centuries for both medicinal and recreational purposes. Consequently, their analysis is of forensic importance. Analysis of these substances provided an opportunity to evaluate the tools within the UNIFI Scientific Information System for both target assignment and structural elucidation.

EXPERIMENTAL

Materials

The following plant alkaloids were obtained from Sigma-Aldrich (Poole, UK) as solid material: amygdalin, berberine chloride, bufalin, coumarin, digitoxin, gitoxin, lanatocide C, neriifolin, and α-solanine.

Sample preparation

Individual stock solutions of the plant alkaloids were initially prepared, by dilution with methanol, to a concentration of 10 µg/mL; these solutions were stored at -20 °C until further use. Prior to Tof-MS analysis, the stock solutions were further diluted with mobile phase A to yield samples for injection at a concentration of 1 µg/mL.

LC-MS method conditions

ACQUITY UPLC conditions

System:	ACQUITY UPLC I-Class (FTN)
Column:	ACQUITY HSS C_{18}, 2.1 x 150 mm, 1.8 µm
Run time:	15 min
Vials:	Waters Maximum Recovery Vials
Column temp.:	50 °C
Sample temp.:	10 °C
Injection vol.:	10 µL
Flow rate:	0.4 mL/min
Mobile phase A:	5 mM aqueous ammonium formate, adjusted to pH 3.0
Mobile phase B:	Acetonitrile containing 0.1% formic acid

Gradient:

Time	%A	%B
0.00	87	13
0.50	87	13
10.00	50	50
10.75	5	95
12.25	5	95
12.50	87	13
15.00	87	13

MS^E conditions

MS system:	Xevo G2-S QTof
Ionization mode:	ESI+
Source temp.:	150 °C
Desolvation temp.:	400 °C
Desolvation gas:	800 L/h
Reference mass:	Leucine enkephalin $[M+H]^+$ = *m/z* 556.2766
Acquisition range	*m/z* 50–1000
Scan time:	0.1 s
Capillary voltage:	0.8 kV
Cone voltage:	25 V
Collision energy:	Function 1: 6 eV
	Function 2: Ramped 10 to 40 eV

Data management

Forensic Toxicology Screening Application Solution with UNIFI v1.8

RESULTS AND DISCUSSION

Prior to analysis, a new UNIFI Scientific Library was created specifically for plant alkaloids, by simply entering the names of the nine alkaloids. A MOL file describing the structure of each substance was added to each entry in the library (Figure 1). Individual solutions of the plant alkaloids were injected and data were acquired using the standard screening conditions supplied with the Forensic Toxicology Screening Application Solution with UNIFI.[1] These data were subsequently processed using the UNIFI Scientific Information System and screened against the new plant alkaloid library.

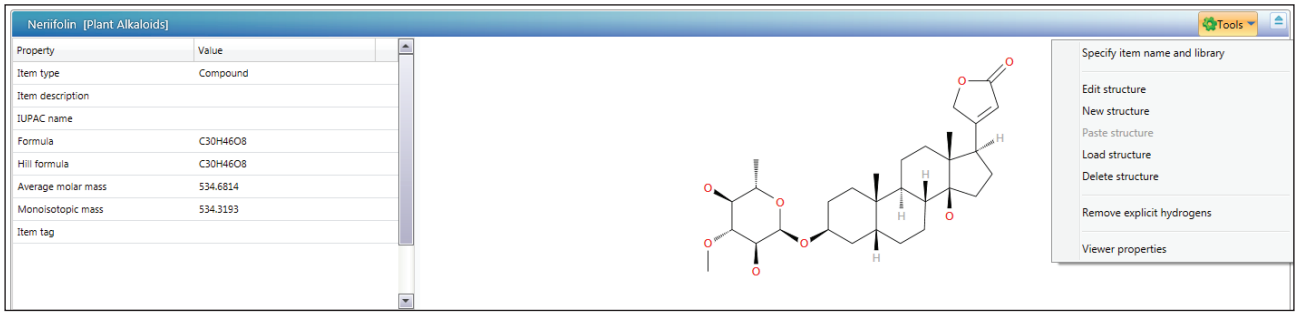

Figure 1. Creating a library entry for neriifolin. Existing MOL file structures can be appended (Load structure) or created by standard chemical drawing packages and subsequently appended (New structure).

Identification through the application of *in-silico* fragmentation techniques

The presence of each plant alkaloid was confirmed through the mass accuracy of the protonated precursor ion in combination with theoretical fragment ions that were automatically generated from the structure of each substance and matched to ions in the high-energy spectrum.

Figure 2 shows the identification of α-solanine as presented in UNIFI. The Component Summary table presents the information related to the identification of this alkaloid and includes; the observed *m/z* value together with the deviation from the expected *m/z* value, the difference between measured and theoretical isotope patterns in terms of both *m/z* and intensity distributions, the observed retention time, the number of theoretical fragment ions found, and the detector counts, which represents the abundance of all the low-energy ions associated with the detected compound.

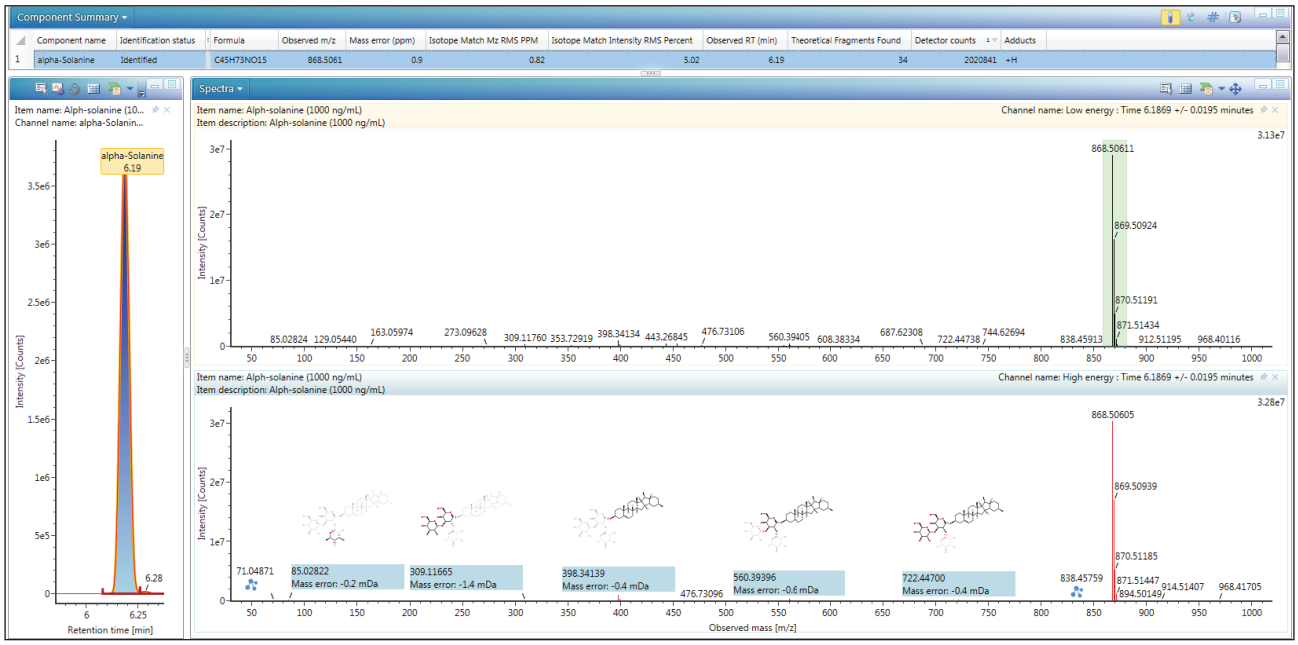

Figure 2. Identification of α-solanine in the UNIFI Scientific Information System.

Updating library entries

All of the alkaloids were identified on the basis of the mass accuracy of the precursor ion and theoretical fragment ions generated during processing. Upon identification, a retention time was associated with each substance. With UNIFI, the library entries can be updated directly from the analysis such that they contain the expected retention time and the expected *m/z* value for each assigned adduct and fragment ion. Following the update, a typical library entry has information similar to that shown for neriifolin in Figure 3. This additional information can be used to target the substance in subsequent analyses.

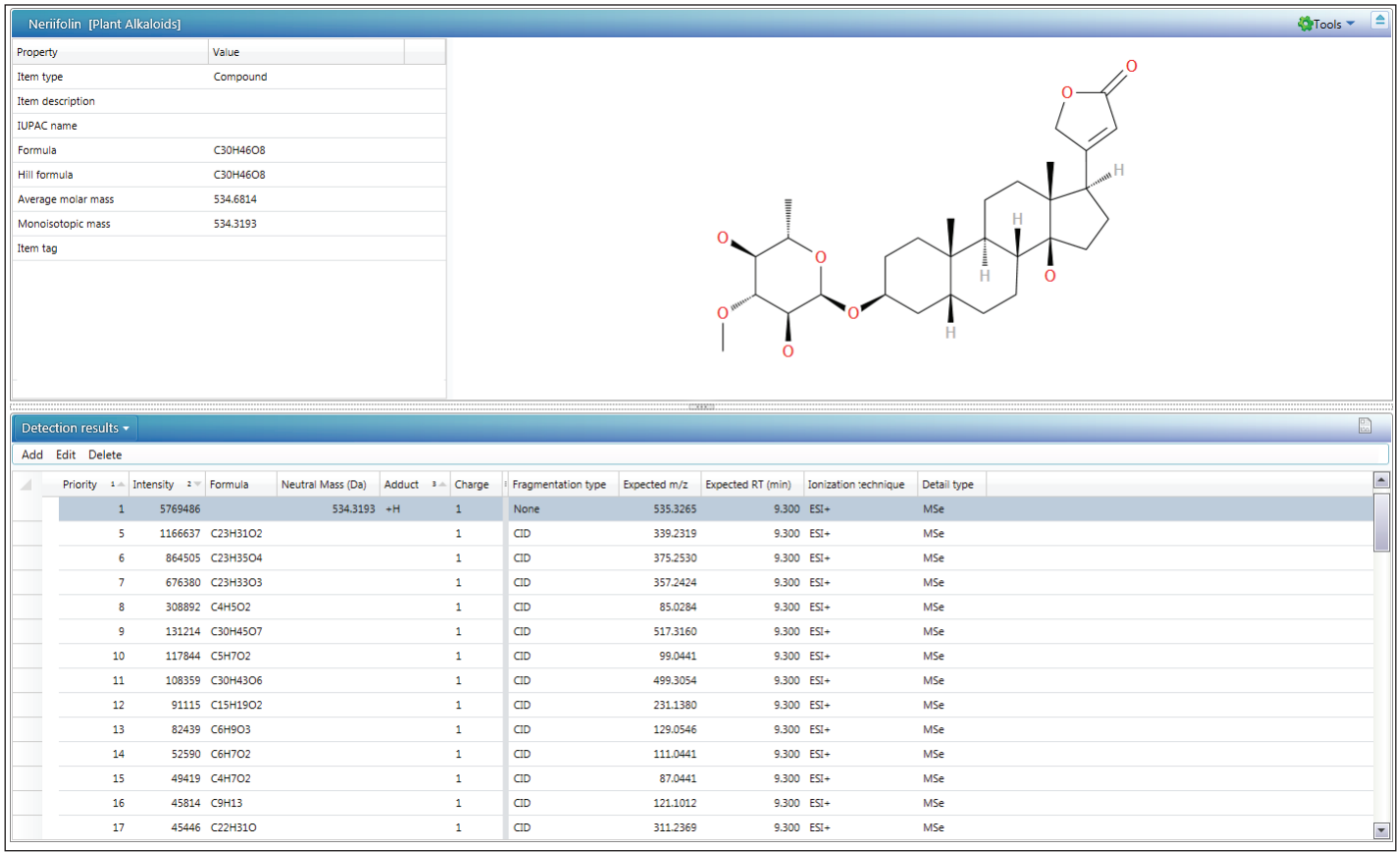

Figure 3. Library entry for neriifolin. The lower section of the composite is now populated with expected retention time and the expected m/z values of precursor and fragment ions.

Multiple adducts

Data for gitoxin, one of the other alkaloids investigated in this study, is shown in Figure 4. The low-energy ions assigned to this substance are highlighted in green within the spectrum and correspond to the protonated isotope cluster. The detector counts determined for the protonated isotope cluster of gitoxin is 568. The high-energy spectrum is annotated with sub-structures of gitoxin, as determined automatically by UNIFI and associated to the high-energy spectral peaks as fragment ions.

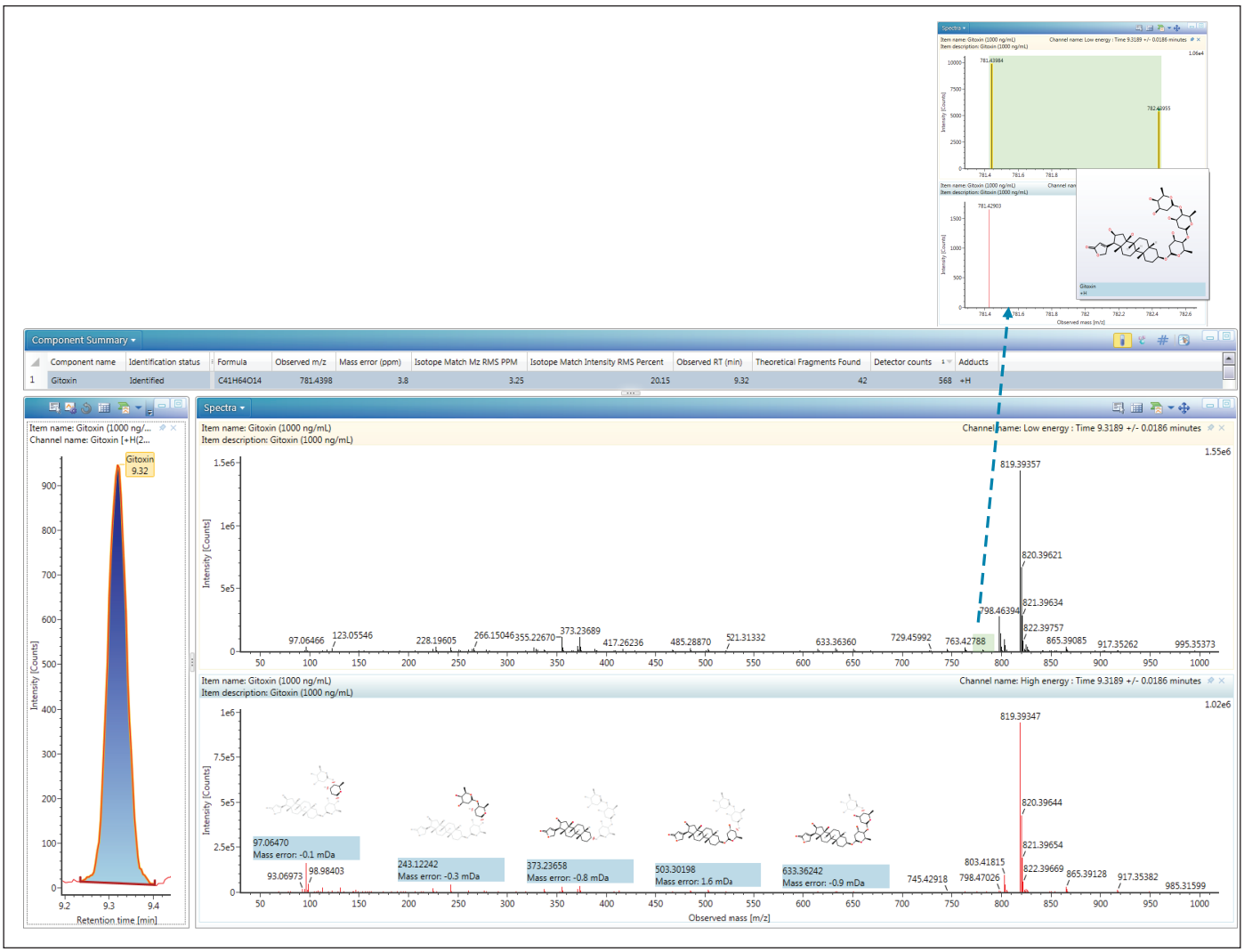

Figure 4. Identification of gitoxin in the UNIFI Scientific Information System.

Further examination of the low-energy spectrum for this substance revealed that some of the ions may correspond to other adducts of gitoxin. Consequently the data was reprocessed to target the $[NH_4]^+$, $[Na]^+$, and $[K]^+$ adducts in addition to the protonated species. Figure 5 details the isotope clusters in the low-energy data assigned to each adduct following reprocessing. The assignment of the additional adducts to gitoxin has been reflected in the detector counts which has increased from 568, determined from the isotope cluster of the protonated adduct, to 118680. Similar results were obtained for the other substances in this analysis.

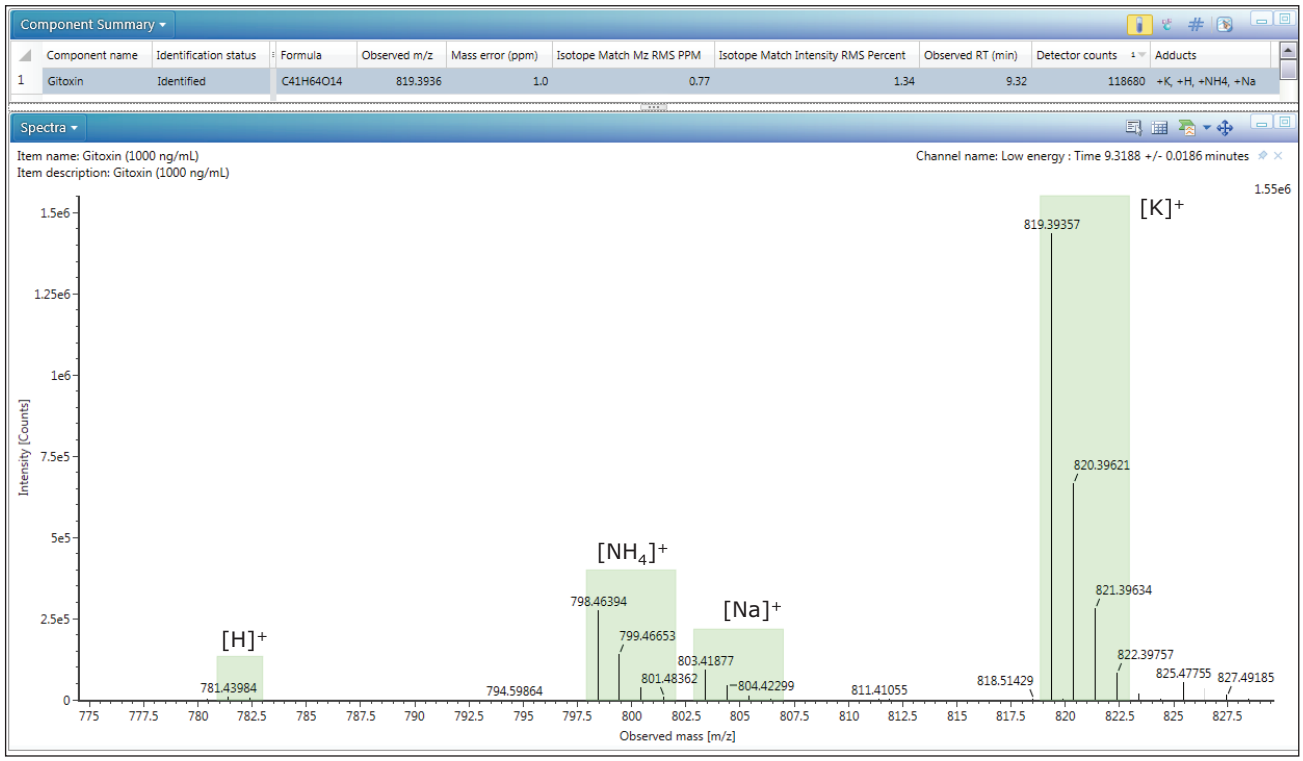

Figure 5. Multiple adduct assignment for gitoxin.

The discovery tool

Another new feature in the UNIFI Scientific Information System v1.8 is the discovery tool, which chains together elemental composition, library searching and fragment match functionality into a single step process making it easier to obtain the identity of unexpected substances within a sample. The parameters used to run the discovery tool are detailed in Figure 6A–D.

The first set of parameters, displayed in Figure 6A, control the maximum number of elemental compositions returned for each component, and the number of library hits returned for each elemental composition. For each component selected, the measured *m/z* value is submitted to the elemental composition application, the parameters of which are displayed in Figure 6B. Each scientific formula returned by the elemental composition application is then automatically submitted to the list of selected libraries. The libraries can either belong to the UNIFI Scientific Library or, if connected to the internet, ChemSpider. The dialog showing the selection of ChemSpider libraries is presented in Figure 6C.

Every hit for each scientific formula that is returned from the library search is then automatically submitted to the fragment match application, provided the library hit has an associated structure in the form of a MOL file.

The fragment match application performs a systematic bond disconnection of each structure, applying the parameters selected through the dialog displayed in Figure 6D, and matches the *m/z* values of theoretical sub-structures to measured high-energy fragment ions. The number of fragment ions matched and the percentage of the intensity of the high-energy spectrum accounted for by those matches are both determined.

For the purposes of illustration, the candidate component identified as amygdalin in the targeted analysis was submitted to the discovery tool. The results, upon running the application with respect to the parameters shown in Figure 6A–D, are presented in Figure 7.

The component submitted to the discovery tool was Candidate Mass *m/z* 458.1649. The results show that one elemental composition, $C_{20}H_{27}NO_{11}$, with an i-FIT™ confidence of 89% was determined for *m/z* 458.1649. This elemental composition, was automatically submitted to the FDA UNII – NLM library within ChemSpider and a hit for amygdalin was returned with a list of synonyms, a structure and the number of citations. The structure was used automatically in conjunction with fragment match and appropriate sub-structures were assigned to the high-energy spectrum associated with Candidate Mass *m/z* 458.1649, as shown in Figure 7. The number of high energy fragments matched by sub-structures and the percentage of the intensity of the high energy spectrum accounted for by those fragment matches are displayed for the library hit.

Access to this information for a range of components, elemental compositions, and library hits enables the analyst to make an informed decision with respect to the identity of unexpected substances in their samples.

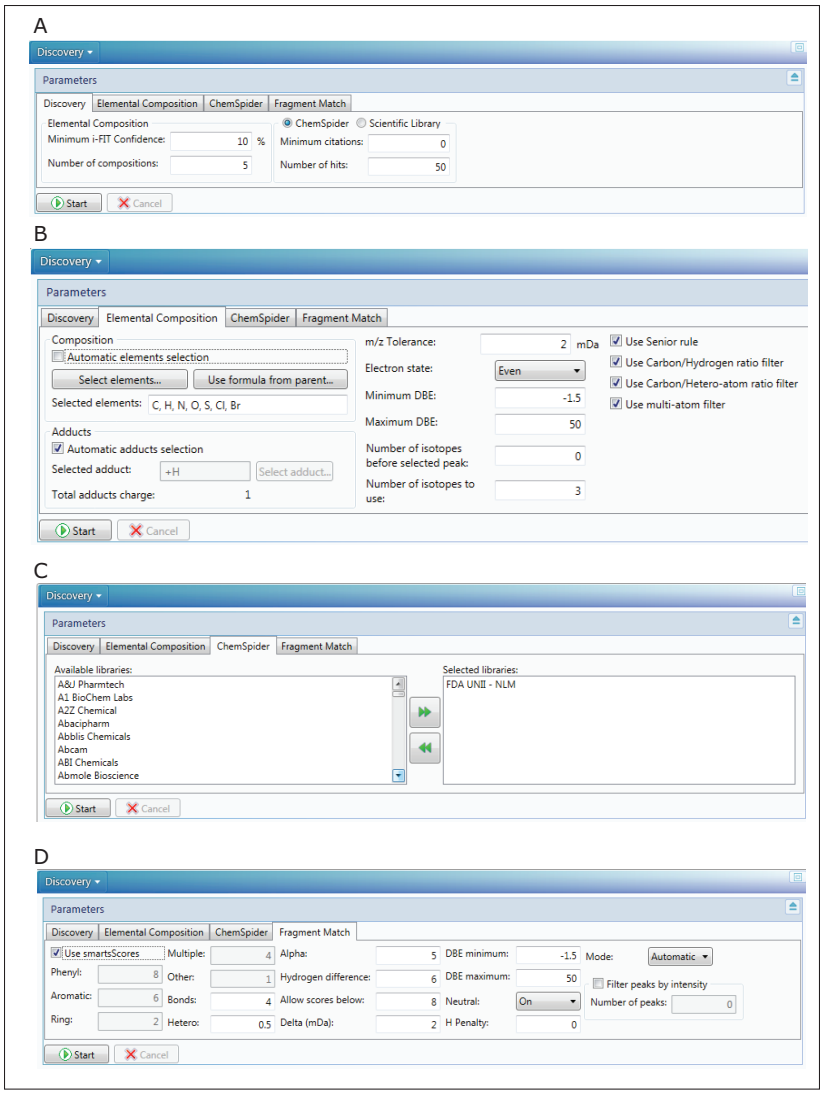

Figure 6. Discovery tool in UNIFI. A) General discovery tool parameters. B) Elemental composition parameters. C) ChemSpider parameters. D) Fragment match parameters.

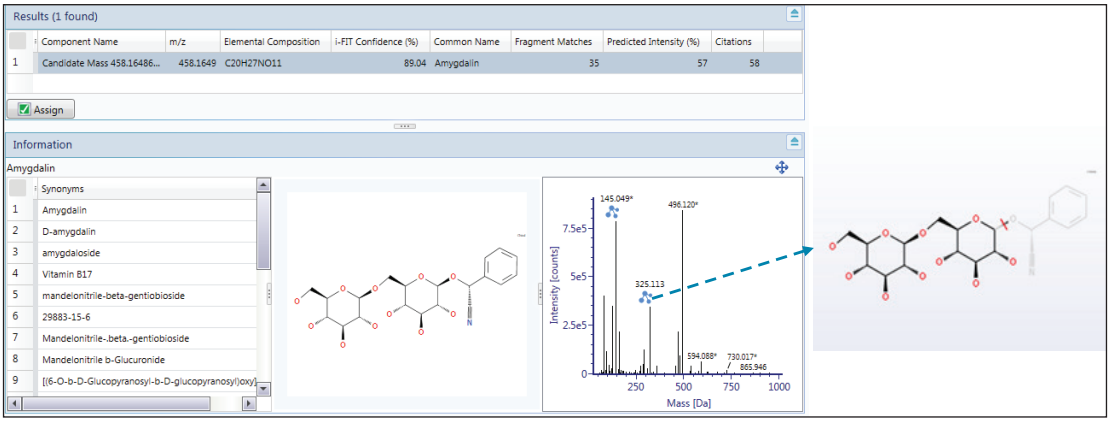

Figure 7. A typical result in the discovery tool.

CONCLUSIONS

In this study, the Forensic Toxicology Application Screening Solution with UNIFI[1] was applied to a selection of plant alkaloids. The ease by which the scientific library items can be created and updated has been clearly demonstrated. The UNIFI Scientific Information System v1.8 was used to process the MS^E data and for these plant alkaloids multiple adducts were detected. The fragment match functionality was also able to assign sub-structures to high-energy ions. Additionally, the new discovery tool has been shown to enhance the elucidation of unknown components.

Reference

1. Forensic Toxicology Screening Application Solution. Waters Brochure (P/N 720004830EN).

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