精密質量スクリーニングと次世代インフォマティクスプラットホームによる植物アルカロイドの分析

Analysis of Plant Alkaloids Through Accurate Mass Screening and Next Generation Informatics Platform

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INTRODUCTION

過去10年にわたり、化合物のスクリーニングに対する飛行時間型質量分析（TOF）の使用が増加している。精密質量と同位体パターンから可能性の高い組成式を推定することが可能なため、標準試料をすぐには入手できない化合物が多いアプリケーションにおいてこの分析法は有用となっている。

本発表では次世代インフォマティクスプラットホームUNIFIスクリーニングソリューションを用いて植物アルカロイドの分析を行った。これらの窒素含有化合物は植物から誘導され、薬理学的に活性であり、薬効やレクリエーションの両方の目的で何世紀にもわたって使用されてきたため、重要な化合物である。

植物アルカロイド標準試料をACQUITY UPLC I-ClassとXevo G2-S QTofを用いてLC/MS分析し、UNIFIトキシコロジースクリーニングソリューションVersion 1.8を用いたデータ解析によって化合物の同定を行った。

METHODS

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 C18, 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.

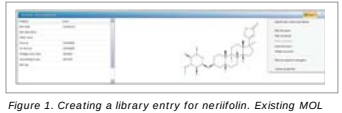

Figure 1. Creating a library entry for neriifolin. Existing MOL file structures can be appended (Load structure) or created by standard chemical drawing packages and subsequently appended (New structure).

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

Figure 2. Identification of a-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.

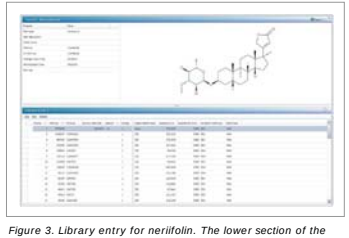

Figure 3. Library entry for neriifolin. The lower section of the composite is now populated with expected retention time and the expected m/z values of precursor and fragment ions.

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.

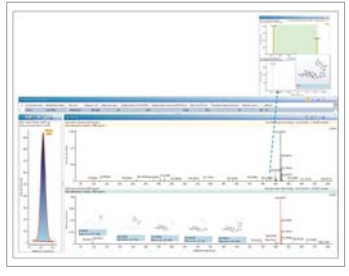

Figure 4. Identification of gitoxin in the UNIFI Scientific Information System.

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 [NH4]+, [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.

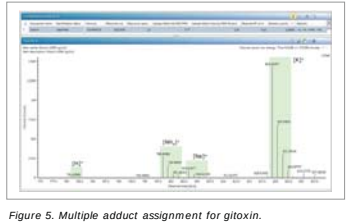

Figure 5. Multiple adduct assignment for gitoxin.

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.

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

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, C20H27NO11, 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.

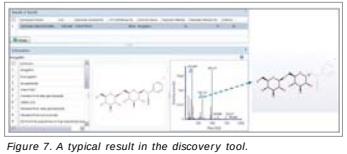

Figure 7. A typical result in the discovery tool.

CONCLUSION

本発表ではUNIFIトキシコロジースクリーニングソリューションを植物アルカロイドに適用した。

サイエンスライブラリーが容易に作成、更新が可能なことを示し、MS^Eデータを解析し、植物アルカノイドの複数のアダクトイオンを検出した。フラグメントマッチ機能によって、高コリジョンエネルギーデータのフラグメントイオンと部分構造を割り当て、構造の確認を行った。

さらに新規のディスカバーツールによって、未知化合物の構造解明が容易になった。

References

1. Forensic Toxicology Screening Application Solution. Waters Brochure (P/N 720004830EN).
2. Analysis of Plant Alkaloids Through Accurate Mass Screening and Discovery. Waters Application Note (720005461EN)

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