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Application Note

Using the Elucidation Tool in UNIFI Scientific Information System to Identify Unknown Compounds in Natural Products

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates, to identify components from a sample that either were not matched with scientific libraries within the UNIFI Scientific Information System, or were matched but were suspected to be false positive, the Elucidation Tool within UNIFI can be used. An example is used to demonstrate the process using the Natural Product Application Solution with UNIFI.

Benefits

The Elucidation Tool is a standard feature within the UNIFI Scientific Information System that facilitates the identification of unknown compounds. This feature combines compound identification, by searching online libraries based on elemental composition, with structural elucidation using MassFragment and its MS fragmentation data.

Introduction

Several scientific libraries are integrated within workflows in the UNIFI Scientific Information System, providing convenience and support for component identification of unknown samples. For components successfully matched with a library, researchers only need to verify the rationality of the fragments that have been classified by MassFragment Software to confirm the target components.

However, for components that cannot be matched with a UNIFI library, or that can be matched but false positives are suspected, UNIFI's Elucidation Tool can be used to manually identify the target compounds of interest through searching online libraries. Here, using the Natural Products Application Solution with UNIFI, we illustrate this process by investigating the identification of an unknown component in a natural product extract as an example.

Results and Discussion

The processes and steps for unknown compound identification using the Elucidation Tool are shown in Figures 1 and 2.

The first step is to determine the elemental composition of the unknown component. In UNIFI, the elemental composition of an unknown compound is determined by three combined factors: exact mass of the intact precursor ion, exact mass and abundance ratio of isotopic peaks, as well as confirming elemental composition of the secondary fragment ions that are correspond to precursor ions.

As shown in Figure 1, for the unknown component with an accurate mass of 577.1550, we can obtain the only possible elemental composition combining the above three factors: $C_{27}H_{30}O_{14}$ (assuming this natural product is only composed of C, H, and O).

Figure 1. The process of an unknown component's elemental composition determination. 1A: Low collision energy mass spectrum of the unknown component. 1B: High collision energy mass spectrum of the unknown component. 1C: Settings for elemental composition search. 1D: Search results of the unknown component's elemental composition. 1E: Isotope distribution plot for the unknown sample.

If the elemental composition corresponding to the secondary fragments is not taken into consideration, three possible molecular formulas could be obtained when searching the elemental composition of this unknown component, and manual evaluation is then required. However, because the fragmentation ions are taken into consideration, the false positives were excluded, leading to a single elemental composition as the accurate and reliable result.

The second step in this identification process is to search possible names and associated structures of chemical ingredients through online libraries. UNIFI Software links directly to ChemSpider,

enabling researchers to search online and obtain possible structures in a variety of ways. For example, one can select all 558 UNIFI default libraries, or simply select some associated libraries. Alternatively, one can conduct the online search by either elemental composition or by accurate mass. Figure 2 shows the process of searching the online libraries.

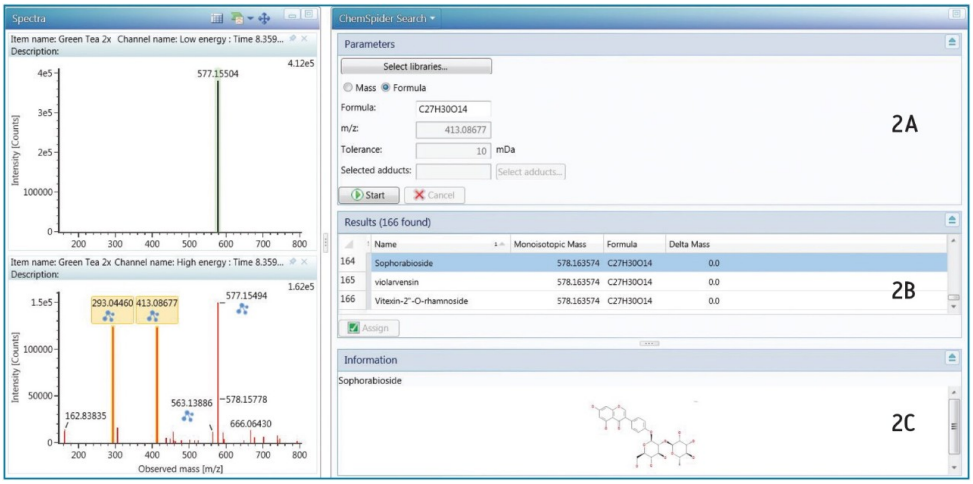

Figure 2. UNIFI directly links to ChemSpider for online library searches. 2A: Parameter settings for ChemSpider online search. 2B: Matching list as the result of the online search;. 2C: Chemical structures corresponding to the results list. This figure shows the preliminarily confirmed compound structures after screening.

After the confirmation of the name and structure of the target compound, clicking Assign will allow this result to be directly brought into the Component Summary List, which resides within the Review tab, and consequently changes the target compound's identification status from Unknown to Identified.

To further confirm the authenticity and reliability of this matched compound, one can utilize MassFragment and manually match fragment ions based on the proposed matching structure for the compound. In this example, we use the fragment ions from the high collision energy scan along with the fragment matching function in the Elucidation Tool. In Figure 3, a fragment ion is used to demonstrate this process and to show how fragmentation pathway can be determined. The most reasonable choice can be manually confirmed from all possible structures matched with this fragment. When clicking the Assign button, this fragment structure is linked to the associated fragment peak in high collision energy scan spectrum, and displayed by a blue icon.

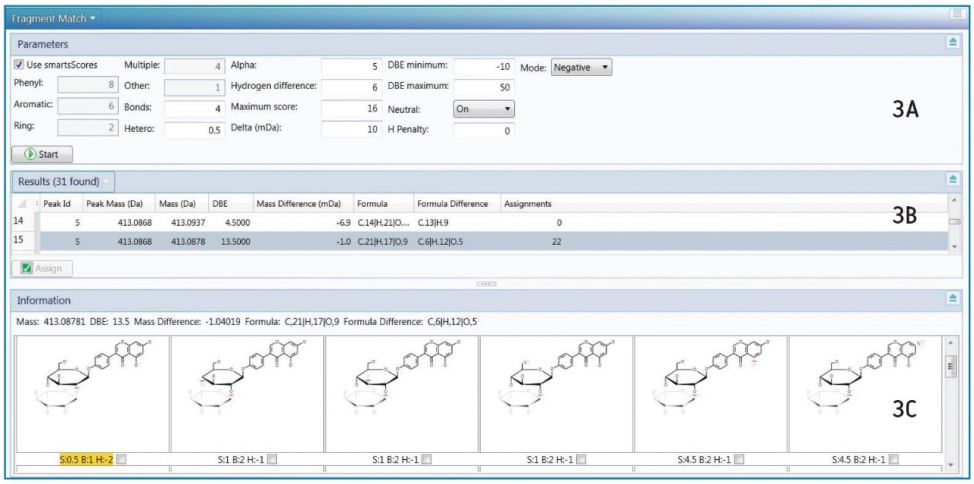

Figure 3. Manual fragment matching interface. 3A. Parameter settings for fragment matching; 3B. List of fragment matching results; 3C. Fragment structure diagram.

Upon confirmation of the key fragment ions, one can further label the target compound as Confirmed from the Component Summary List that resides within the Review tab. If required, one can indicate its database source in the Comment column, as seen in Figure 4. Finally, all Confirmed results are shown in the Components-Confirmed Table workflow.

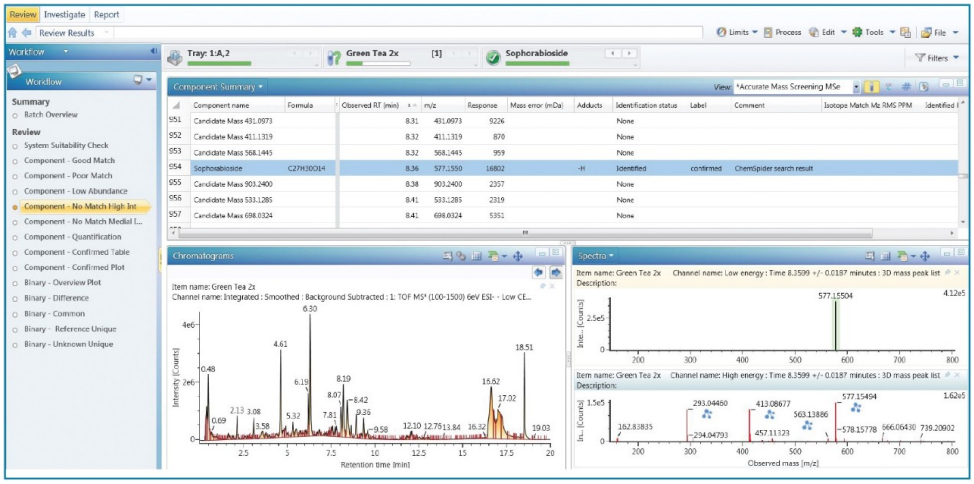

Figure 4. The final identification result of the unknown sample is shown in Compound Summary List in the Review interface.

Conclusion

This work describes how to use the Elucidation Tool within the UNIFI Scientific Information System to manually identify unmatched or matched but suspected false-positive components. This process has a rational design, the elemental composition search is reliable and accurate, and the ability to use online library searches is simple and intuitive. The target compound can be further confirmed utilizing MassFragment Software. In this example, by using the Natural Product Application Solution with UNIFI, the unknown compound that elutes at 8.35 min with $[M-H]^-$ of 577.1550 is confirmed to be Sophrabioside.

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