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Application Note

Screening Synthetic Adulterants from Herbal and Dietary Supplements (HDS) Using the Natural Products Application Solution with UNIFI

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Abstract

In this application note demonstrates the utility of the Natural Products Application Solution with UNIFI Adulterant Screening Application and the Synthetic Adulterant Library with the analysis of a commercial herbal supplement product.

Benefits

- The Adulterant Screening Application within the Natural Products Application Solution with UNIFI consists of a set of dedicated workflows
- The Natural Products Application Solution with UNIFI offers a turn-key solution from sample to report that utilizes ultra performance liquid chromatography (UPLC), coupled with a quadrupole time of flight mass spectrometer (Q-Tof MS) for data acquisition
- Significant enhancement in efficiency and ease of use for the screening and identification of synthetic adulterants with minimal requirement for an operator's technical expertise

Introduction

In recent years, there has been a tremendous increase in the use of natural products in the form of herbal supplements for health benefits and therapeutic effects. Unfortunately, this rise in usage has also led to the increased chance of products being adulterated with synthetic compounds by unscrupulous manufacturers. There have been reports of severe to even fatal cases[1] related to adulteration in herbal products. This has raised global concerns within the natural products community and with various regulatory authorities since it poses a significant risk to consumers.

Liquid chromatography coupled with mass spectrometry (LC–MS) is widely-used for screening herbal supplement products due to their high sensitivity and selectivity, which is critical for analyzing complex matrices typical of natural product samples. However, a major challenge for LC-MS screening has been the lack of robust streamlined workflows that allow quick and easy processing from sample to report.

Here, we demonstrate the utility of the Natural Products Application Solution with UNIFI Adulterant Screening Application and the Synthetic Adulterant Library with the analysis of a commercial herbal supplement product. This product is a herbal capsule sold by street vendors to alleviate back and joint pain

(in rheumatoid arthritis). The label claim indicates that it contains various herbs such as *Picrorhiza kurroa* and *Strychnos nux-vomica Linn.* Using the Natural Products Application Solution with UNIFI Adulterant Screening Application, we were able to automatically detect the existence of any synthetic compounds, and then confirm the identified adulterants through an easy review process, to quickly draw a conclusion.

We showcase a turn-key solution with a streamlined process from sample to report using the Adulterant Screening Application within the Natural Products Application Solution with UNIFI. Figure 1 displays the entire analytical process which integrates all steps (data acquisition, peak picking, library searching, fragment ion confirmation, and report generation) into a streamlined process. Additionally, structure elucidation tools are available to allow further investigation on unknown peaks that did not show matches from the Synthetic Adulterants Library.

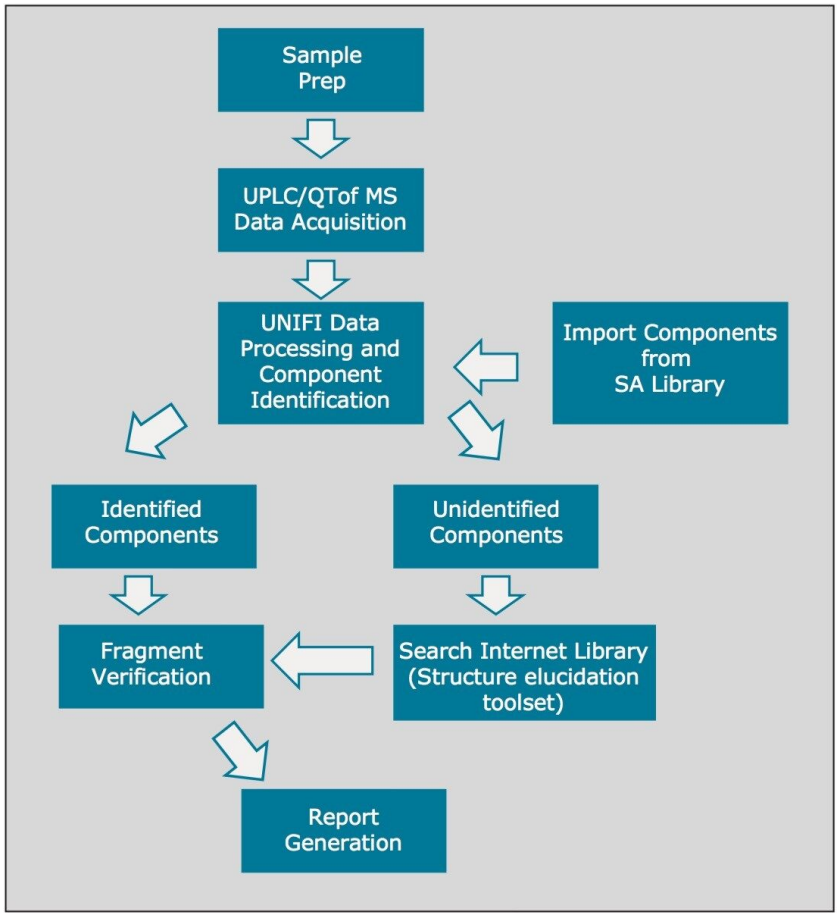

Figure 1. The step by step procedure of the Natural Products Application Solution with UNIFI Adulterant Screening Application.

Experimental

Sample preparation

1 g of powdered sample (Figure 2) in 10 mL of LC-MS grade methanol solution was sonicated for 20 minutes, followed by centrifugation at RCF of 4472 g for 5 minutes. The supernatant was diluted 250 times with methanol prior to injection (1 µL).

Catechin standard mixture (p/n 186007465) was used for the system suitability test. The purchased stock solution was diluted 10x with methanol with a final concentration of 10 µg/mL. Injection volume was 1 µL.

Figure 2. Powdered anti-inflammatory herbal supplement product obtained from a street vendor.

LC conditions

LC system:	ACQUITY UPLC I-Class with Flow Through Needle (FTN) Sample Manager
Column:	ACQUITY UPLC HSS T3, 1.8 µm, 2.1 x 100 mm, 40 °C
Sample temp.:	15 °C

Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Acetonitrile + 0.1% formic acid

Gradient table:

Time	Flow rate(mL/min)	Solvent A(%)	Solvent B (%)	Curves
0.0	0.6	98	2	Initial
0.5	0.6	98	2	6
1.0	0.6	95	5	6
5.0	0.6	70	30	6
9.0	0.6	40	60	6
11.0	0.6	40	60	6
12.0	0.6	30	70	6
12.5	0.6	30	70	6
14.5	0.6	0	100	1
15.0	0.6	98	2	1
17.0	0.6	98	2	1

MS conditions

MS system:	Xevo G2-XS QTof

Acquisition range:	50–1500 Da
Scan time:	0.1 s
Acquisition mode:	ESI+ and ESI-; MS^E; resolution mode
Lock mass:	Leucine enkephalin (LE) 0.2 ppm (scan for 0.3 s, interval: 30 s)
Capillary voltage:	1.5 KV (ESI+) 1.5 KV (ESI-)
Cone voltage:	40 V
Collision energy (eV):	Low CE:6 High CE: 10–40
Source temp.:	120 °C
Desolvation temp.:	500 °C
Cone gas flow:	50 L/h
Desolvation gas flow:	1000 L/h
Acquisition time:	17 min

Data management

Natural Products Application Solution with UNIFI for instrument control, data mining, and report generation.

Results and Discussion

The Natural Products Application Solution with UNIFI provides turn-key solutions for two types of

applications: ingredient profiling for natural product extracts, and adulterants screening for herbal supplements. Each application comes with its own set of workflows. For the Ingredient Profiling Application, a set of 14 preset templates (data filters linked to specified views) are provided in a workflow which incorporates the utility of the Traditional Medicine Library. Detailed utility of this ingredient profiling application had been elaborated in previous applications notes.[2-4]

For the Adulterant Screening Application, a dedicated set of acquisition method templates with generic conditions and parameters is provided for data acquisition. A dedicated workflow containing a set of 7 templates is also provided, which incorporates the utility of the Synthetic Adulterants Library for adulterant screening, reviewing, and confirmation. Finally, a specific report template is provided for automatic report generation. Similarly as with the Ingredient Profiling Application, catechin standard mixture and green tea extract are provided for system suitability testing, and to facilitate familiarization with the utility of the Natural Products Application Solution with UNIFI.

When performing the screening and identification of synthetic adulterants in herbal supplements by LC-MS, one of the most difficult challenges comes from the fact that a fit for purpose library is not available. To solve this challenge, and to help enhance the speed of the entire analytical process, the Synthetic Adulterants Library was created and incorporated into the Adulterants Screening Application as a critical component.

Figure 3 shows the detailed screen capture of the Synthetic Adulterants Library. The library contains a list of synthetic compounds that are frequently found in herbal supplement products as adulterants. These compound names are listed both in English and in simplified Mandarin, and grouped according to their known pharmacological actions. Other relevant information such as chemical structure, molecular formula, average molecular and mono-isotopic exact molecular mass, CAS number, Chemspider ID, and PubChem ID are also included for each compound. In addition, for each of the compound entry, key diagnostic fragment ions are included.

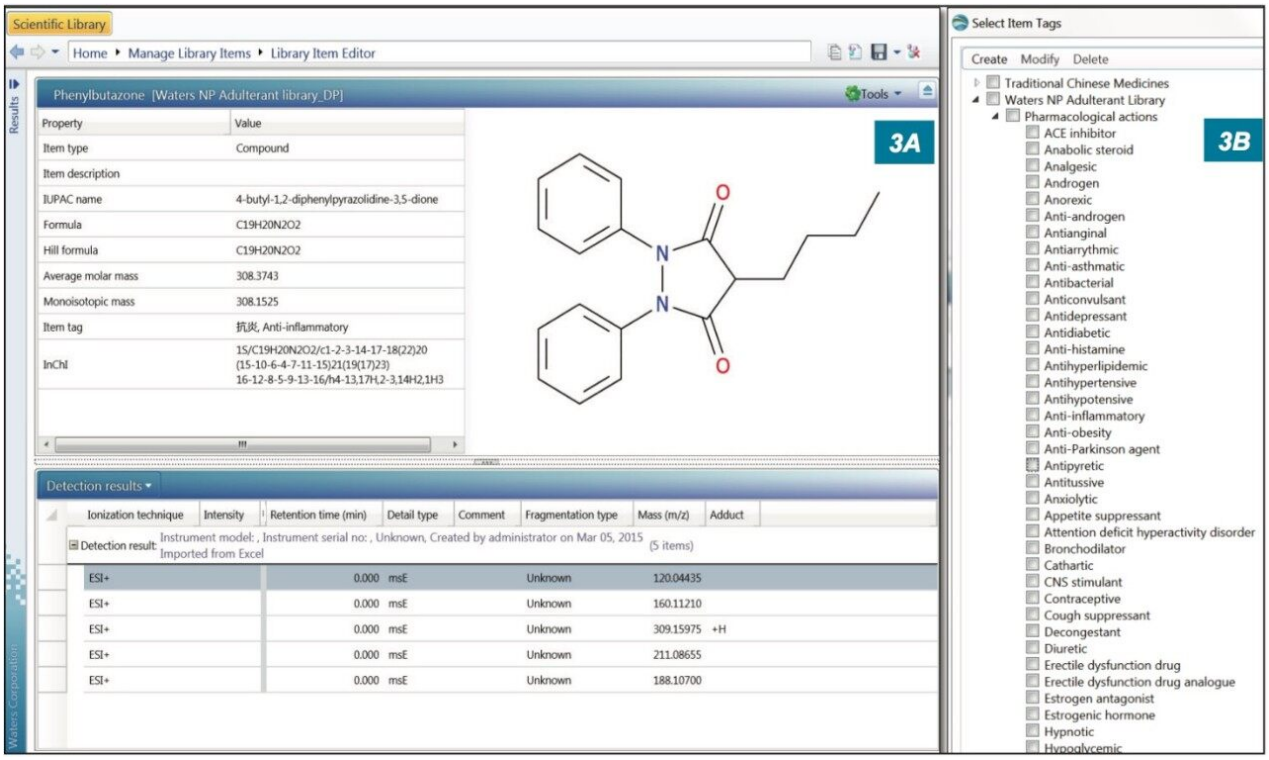

Figure 3. This screen shows the infra-structure of the Synthetic Adulterants Library. A) Example screen capture for each of the compound entries. B) List of the pharmacological actions from the item tags.

For UPLC/Q-Tof MS experiments, MS^E data acquisition mode was used for sample analysis, which in essence is a data independent acquisition strategy. From a single LC injection, the mass spectrometer is collecting data in two independent functions, one with collision energy set to low so that the MS full scan precursor ion information is collected, and another with collision energy set to high, so that corresponding MS/MS fragment ion information is collected. The two sets of data are connected and correlated by the LC retention time, as a result for each of the analytes in the sample, both MS and MS/MS information is obtained. Figure 4 shows the two UPLC/Q-Tof MS base peak ion (BPI) chromatograms obtained from the single injection on the test sample. Figure 4A is the BPI from the low CE scan, and Figure 4B is the BPI from the high CE scan.

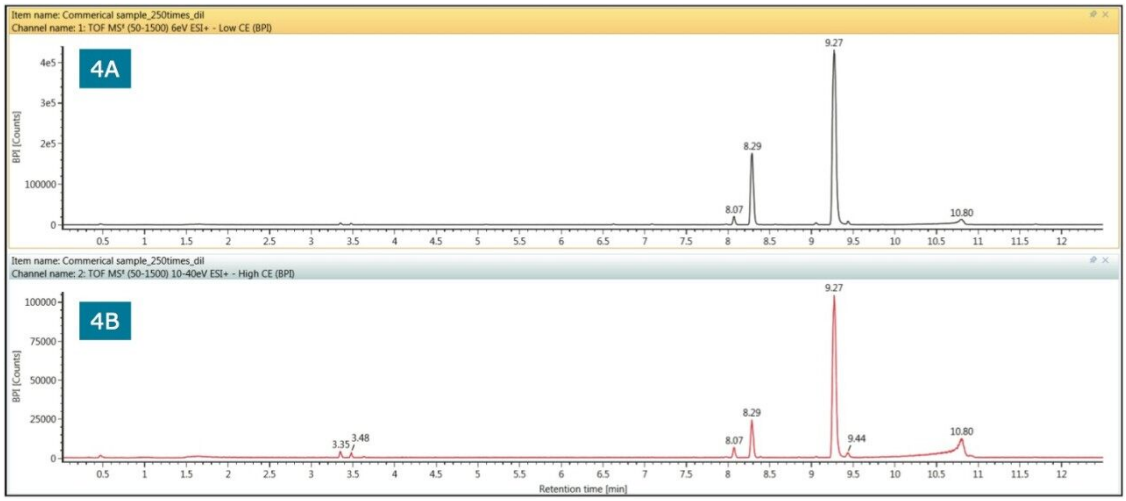

Figure 4. The UPLC/Q-Tof MS BPI chromatograms of an anti-inflammatory capsule. 4A) BPI from a low CE scan; 4B) BPI from a high CE scan.

Data processing is automatic and requires minor parameter setting as templates are provided in the Natural Products Application Solution with UNIFI Adulterant Screening Application. The only user intervention required is to enable sample specific processing, to ensure that the correct analysis method is chosen, the peak processing time window matches the specific analysis, and to ensure that the Synthetic Adulterants Library is connected to the analysis method. The next step is for the user to go to the review page, click the Process tab, and wait for the result to display while all process steps take place automatically.

The steps involved after the Process button is clicked are as follows: 1. Peak picking based on 3D Apex Peak Detection, so a complete peak list is obtained for this sample. The peak picking happens simultaneously for both low CE MS full scan and high CE MS full scan mode. 2. Matching of the component list obtained from the Low CE MS full scan with the parent ion information that was obtained from the library. 3. Fragment ion confirmation by either matching with known key diagnostic fragments ions from the library, or matching with predicted fragment ions generated with MassFragment in high CE MS^E full scan. MassFragment is an in-silico informatics tool which utilizes mol file structure of a given compound to predict possible fragmentation patterns based on the soft spot of that molecule. This is significantly helpful to confirm the correct match and to eliminate false positives.

Figure 5 shows a screen capture of the review window that displays results obtained at the end of process. This review window is consistent across UNIFI Scientific Information System. Results are organized in multiple segments within this window and discussed in detail below.

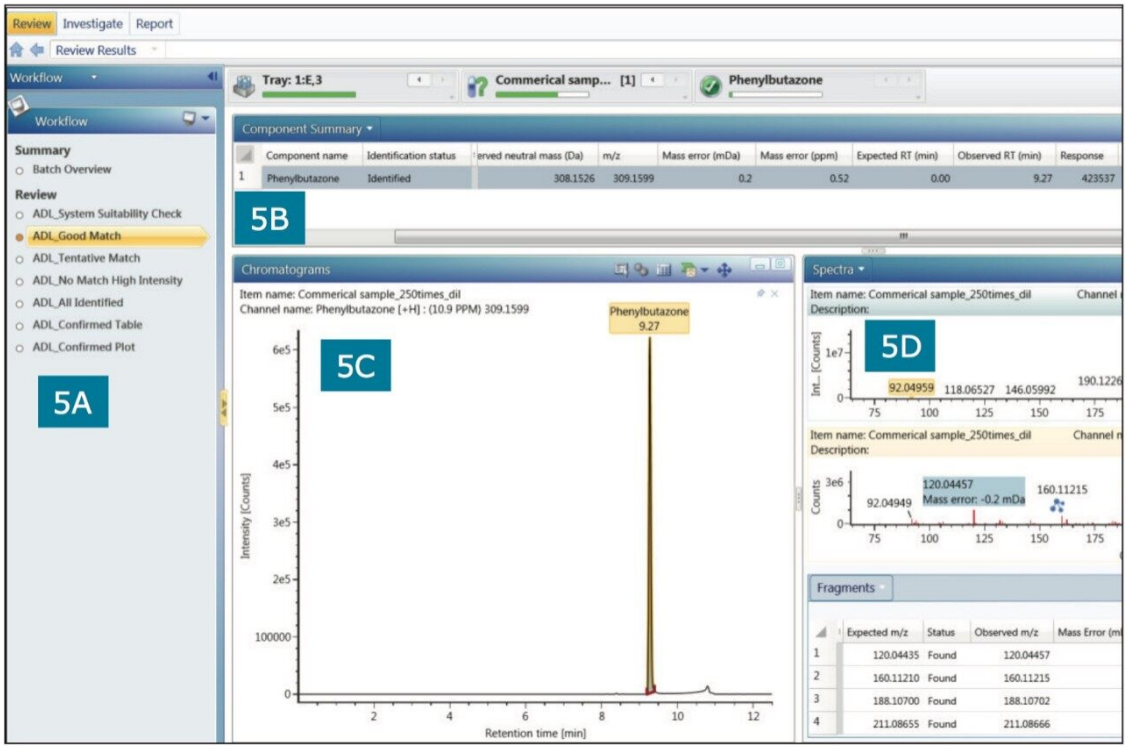

Figure 5. Results displayed in the Review tab within UNIFI Scientific Information System after processing. 5A) List of the available workflows. The Adulterant Screening Application provides a total of seven preset templates. Here, ADL refers to adulterant. 5B) Shows a list of components associated with the Good Match filter. 5C) XICs of m/z 309.1599 of the adulterant. 5D) MS spectra correlate to the XICs, both in low energy MS full scan and high energy MS^E scan

Figure 5A shows the available preset templates in the Adulterant Screening Application. As mentioned previously, for the Natural Products Application Solution with UNIFI, two complete sets of workflows are provided: the Ingredient Profiling Application[2-4] and the Adulterant Screening Application.

A set of 7 workflow templates is provided for the Adulterant Screening Application with the purpose of facilitating easy result review and confirmation. The System Suitability ensures a routine system check prior to analysis to ensure data quality. The Good Match lists all components (ion count above 5,000) that matched with the Synthetic Adulterants Library. Both parent ion (mass error <2mDa) and minimum of one diagnostic fragment ion must be present. The Tentative Match lists all components (ion count above 5,000) that match with the Synthetic Adulterants Library, parent ion only. The No Match High Intensity lists all components (ion count above 20,000) that did not match with the Synthetic Adulterants Library. The All Identified lists all components that matched with the Synthetic Adulterants Library with ion count above 5,000. The Confirmed Table and Confirmed Plot are provided for quick review of all components that have

been reviewed and confirmed by the user.

Figure 5B displays the list of components that correlate to each of the designated preset workflow templates. In this section, all critical information of the detected compound is listed such as expected neutral mass (Da), observed neutral mass (Da), mass error (ppm), observed retention time (minutes), response factor, and adduct ions. In this application example, for the herbal supplement we were analyzing, phenylbutazone is the only component listed under Good Match. Phenylbutazone is a nonsteroidal anti-inflammatory drug (NSAID) and has been banned for human use in US and UK as it can cause severe adverse effects such as suppression of white blood cell production and aplastic anemia.[5]

Figure 5C displays the extracted ion chromatogram (XIC) that correlates to the component displayed in Figure 5B. In this case, phenylbutazone from the Good Match template is selected in Figure 5B, therefore, the XIC associated with this component is displayed.

Figure 5D shows the MS spectra that correlate to the component listed in Figure 5B and the XIC displayed in Figure 5C. Here, the top spectrum is the MS full scan spectrum, and the bottom spectrum is the MS^E spectrum, both correlate to the 9.27 min peak from the XIC of *m/z* 309.1599. In the MSE spectrum, the blue mark indicates the fragment ion that matches the expected fragment list in the Synthetic Adulteration Library. For easy review, users can also choose to have all matched fragment ions listed as a table below the high energy spectrum, as shown in Figure 5D. For phenylbutazone, all expected fragments were observed in the high energy spectrum.

With the increasing trend in adulteration in herbal supplements, new or analogues of common synthetic adulterants are continually being created, as such, regardless of the size of the library, there will always be components that will not match. Any unmatched peaks that are in high abundance warrant further investigation. These peaks will be listed under the No Match High Intensity (Figure 6A and 6B). To further investigate unmatched high intensity components, the Structural Elucidation Tool6 can be used. The structural elucidation tool is easily accessible by right clicking and selecting Elucidate from the drop down list (Figure 6E). For this specific sample analysis, one component was listed under the No Match High Intensity at the retention time of 8.29 minutes with high intensity (Figure 6C).

Figure 6. High intensity component (Red Box in Figure 6C) from the No Match High Intensity preset template (Figure 6A) that did not match with the Synthetic Adulteration Library. The structure elucidation tools can be used by selecting the Elucidate option (Figure 6E). 6B Reveals the list of the components associated with each of the workflows. 6D) The low and high energy scan of the component selected from Figure 6B.

The key steps of elucidation are displayed in sequence in Figure 7. Step 1 is to determine the elemental composition of the component based on exact mass measurement. A unique feature in the UNIFI Scientific Information System for determining elemental composition is the consideration of the MS/MS fragment ions, in addition to the precursor ion exact mass and isotope distribution in the parameter settings (as displayed in Figure 7 Step 1).

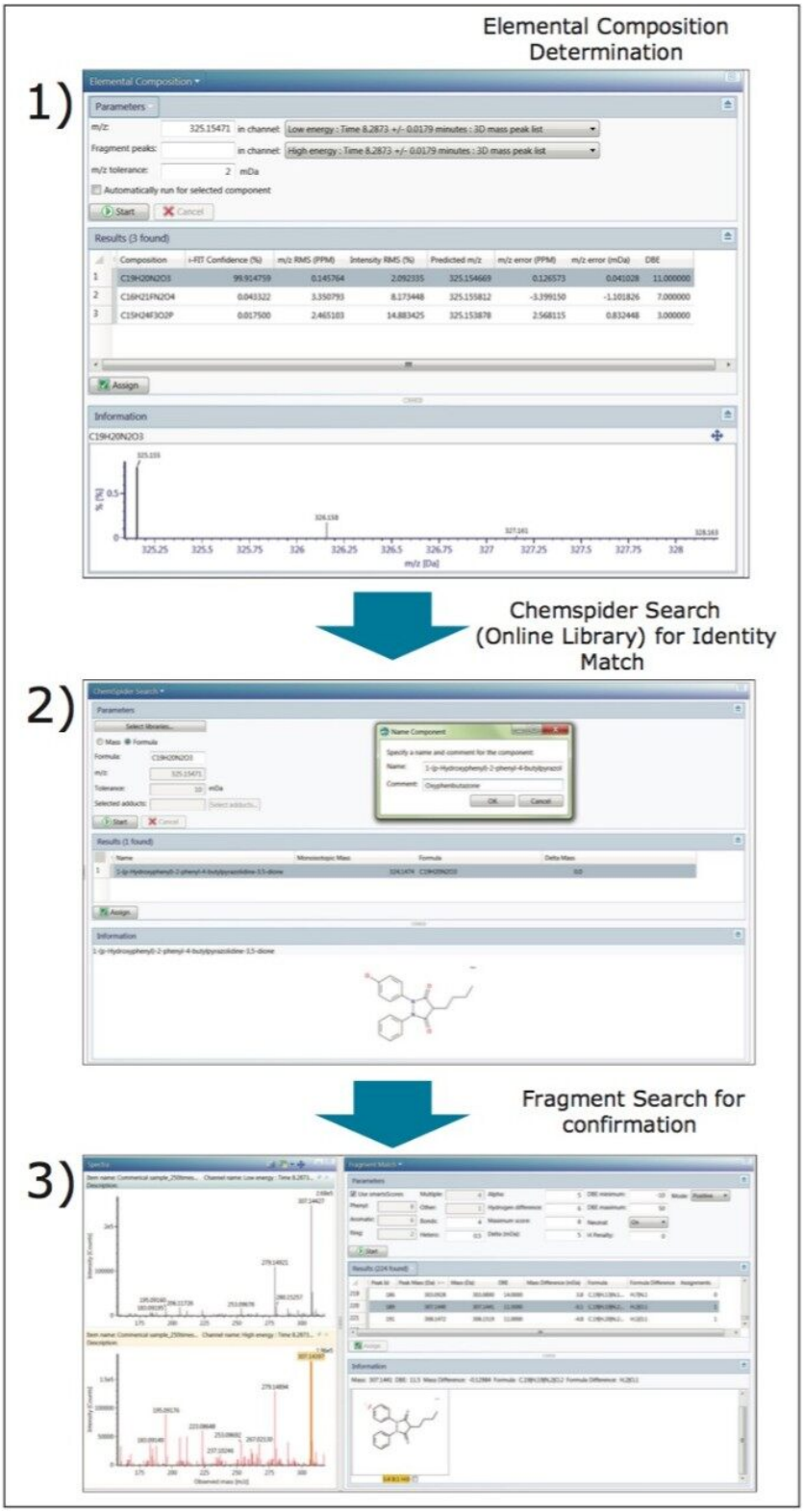

Figure 7. Step by step process of the structure elucidation tool for the identification and confirmation of the unknown component eluted at

8.29 min.

This significantly eliminates a large number of false positives. In addition, an i-FIT confidence (%) is generated to show which formulas have a higher probability of being correct. In our sample analysis, we obtained the elemental composition match of $C_{19}H_{20}N_2O_3$ with the highest i-FIT score of 99.86%.

Step 2 is to perform a library search from the on-line public database ChemSpider, which has compound entries from over 500 libraries. UNIFI Scientific Information System allows users to select searching parameters, and provides a direct link into ChemSpider. In the analysis, the ChemSpider search resulted in an initial match of oxyphenbutazone, which is the metabolite of phenylbutazone and is also an NSAID.

Step 3 is to validate and confirm the initial match with structural elucidation of fragment ions. This takes the advantage of the availability of the MS/MS fragment ions from the MS^E scan and utilizes MassFragment. MassFragment is one of the structural elucidation tools that conducts calculations on any mol file structure in silico and predicts possible fragmentation patterns based on the weak bonds of the molecule. The predicted fragment ion is then compared with the fragment ions obtained from the MSE scan. This significantly helps to confirm the right match, and to eliminate false positives. Shown in Figure 7 Step 3, the possible fragments of oxyphenbutazone predicted by MassFragment were checked and matched with fragment ions obtained from the high collision energy scan of the same sample. The results show that key predicted fragments of oxyphenbutazone were found in a high CE MS^E spectrum, which confirms the identified unknown peak as oxyphenbutazone.

As a result, two synthetic adulterants, phenylbutazone and oxyphenbutazone, were detected and identified in the herbal supplement sample. They both have pharmacological actions as NSAIDs. The final report of the analysis was then automatically generated using the report template, and is shown in Figure 8.

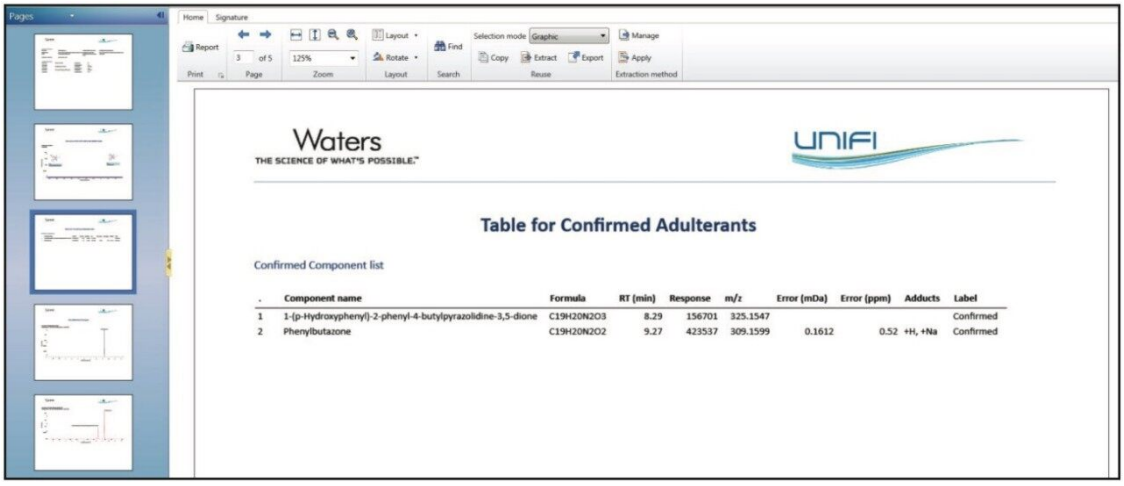

Figure 8. Summary report for the status of component identification, which is easily obtained by importing the natural products adulteration summary template

Conclusion

The utility of the Natural Products Application Solution with UNIFI Adulterant Screening Application is demonstrated through the analysis of a herbal supplement product obtained from a street vendor. Two synthetic adulterants, phenylbutazone and oxyphenbutazone (both NSAIDS), were identified and confirmed from a single LC injection. The entire process from sample to report was completed in a couple of hours.

The Natural Products Application Solution with UNIFI Adulterant Screening Application contains a set of acquisition method templates, processing method template, Synthetic Adulterants Library, a set of dedicated workflows, and a report template, that allow a streamlined process from sample to report. Reliable answers about commercial products can be obtained quickly, without demands for a chemist's expertise or extensive previous related experience. This results in significantly enhanced efficiency and productivity for contract testing labs and regulatory agencies.

References

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