DETECTING THE "UN-NATURAL" IN NATURAL PRODUCTS

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HIGHLIGHTS

- **Overview of the Waters Natural Product Application Solution with UNIFI®, which combines UPLC® QTof/ MS technology with workflow-driven informatics.**
- **An easy to use workflow-driven automatic streamlined process incorporating a traditional medicine and a synthetic adulterants library to quickly screen and identify chemical constituents and potential adulterants from commercial herbal supplement products.**
- **Significant enhancement in efficiency and productivity with less demand for operator's technical expertise**

INTRODUCTION

There are an increasing number of herbal products being used around the world due to their perceived nature as a safe and cheaper alternative to prescription drugs. However, one major concern is the rising number of cases of adulterated herbal products containing undeclared synthetic compounds or delibrate substitution of ingredients for economic benefit and pharmacological action. Due to the diversity and complexity of chemical compounds in herbal natural products, it is imperative to develop analytical methods to confirm the natural constituents for proper quality control and authentication. LC–MS based methods are widely-used for the identification of ingredients and potential adulterants because of their high sensitivity and selectively for samples with complex matrices.

However, researchers have a large bottleneck in obtaining results quickly due to the complexity and large sizes of LC-MS datasets. In this study, a novel LC-MS informatics platform, UNIFI was used to screen an "all-natural" commercial herbal product for erectile dysfunction. A streamlined workflow in UNIFI allows researchers to rapidly extract LC-MS results utilizing a traditional medicine (TM) and a synthetic adulterant library (SA) to effectively identify and characterize ingredients in the herbal product. In addition, the embedded structure elucidation tools such as *in-silico* fragmentation also facilitated identification of unmatched unknown peaks. Here, In this poster, we will show results from a comprehensive analysis of the herbal product using a novel informatics platform based on a single LC/MS injection.

METHODS

Sample Information and Preparation

A private testing lab provided a suspicious unknown herbal product used for erectile dysfunction. The product label claims all natural ingredients but with no specific ingredient listed on the label. 1 g of powdered sample in 20 mL of LC-MS grade 100% methanol solution was sonicated for 30 minutes. The supernatant was diluted 800 times by methanol prior to injection. 1 µL of diluted sample was injected for analysis.

Informatics Platform

The Waters Natural Products Application Solution (NPAS) with UNIFI was used for: System control for data acquisition/Data processing/Result review/Reporting.

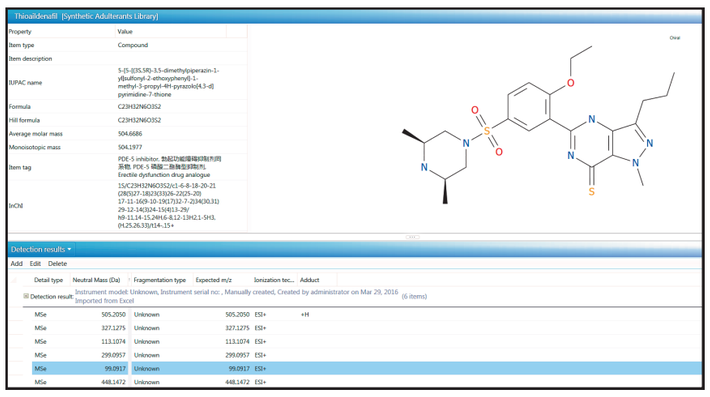


Figure 1. Screenshot of UNIFI Synthetic Adulterants (SA) library. Each compound entry in the library contains list of expected diagnostic fragment ions. Item tags containing pharmacological actions are also embedded.

Instrumental conditions

LC conditions:

LC system: ACQUITY UPLC I-Class with FTN Sample Manager

Column: ACQUITY UPLC HSS T3 2.1 x 100 mm, 1.8 µm, 40°C

Sample temp: 15°C

Mobile Phase: Gradient elution

A: Water (0.1% formic acid)

& B: acetonitrile

Time(min.)	Flow rate mL/min	Solvent A (%)	Solvent B (%)	Curves
0	0.6	98	2	6
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	0.6	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 MS

Acquisition range: 50-1500 Da (0.1s scan rate)

Acquisition mode: MS^E, ESI^- and ESI^+ in resolution mode

Capillary voltage: 1.5 kV (ESI^+)/1.5 kV (ESI^-)

Cone voltage: 40 V

Collision energy (eV): low CE: 5/High CE: 15-40

Source temp: 120°C, Desolvation temp: 500°C

Gases: Nitrogen, Dissolvation: 1000 L/hr, Cone gas: 50 L/hr

RESULTS AND DISCUSSION

UPLC-QTOF-MS chromatogram

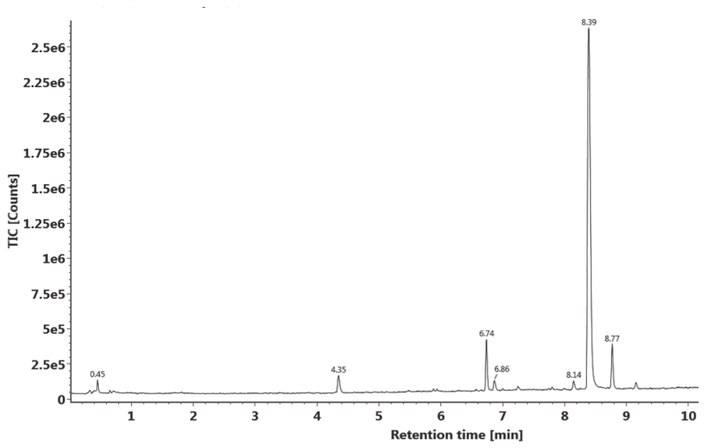


Figure 2. The UPLC-Qtof MS base peak ion (BPI) chromatogram obtained from the adulterated herbal product.

Streamline workflow using the Natural Products Application Solution with UNIFI:

1) 3A shows the 8 preset templates for the Adulterants Screening Workflow.
   - The System Suitability helps the routine system check prior to analysis to ensure data quality.
   - The Good Match, Tentative Match, No Match High Intensity and All Identified templates lists components that matched with the UNIFI SA library based on respective settings for parameters such as the specified intensity threshold, mass error tolerance, diagnostic fragment ion matching etc.
   - The Confirmed Table and Confirmed Plot are provided for quick review of all components that have been reviewed and confirmed by user.
2) 3B displays the list of components that correlates to each of the workflow step. For the herbal supplement, **aildenafil and thioaidenafil** were found under the Good Match list. Both **aildenafil** and **thioaidenafil** are listed as **PDE-5 inhibitor** drugs from the SA library.
3) 3C displays the extracted ion chromatogram (XIC) of thioaidenafil and correlates to what is selected in section 3B.
4) 3D displays the MS spectra that correlates to the component listed in 3B and displayed in 3C. Here, the top spectrum is the MS full scan spectrum and the bottom spectrum is the MS^E spectrum, both correlate to the 8.39 min peak from the XIC of m/z 504.1977.
5) In the MS^E spectrum, the blue " " mark indicates the fragment ion that matches from the expected fragment list in the library. For **thioaidenafil**, all four key diagnostic fragments were observed in the high energy spectrum. 448.1470 m/z was a characteristic fragment for **thioaidenafil** compared to the structural isomer, **thiohomosildenafil**.

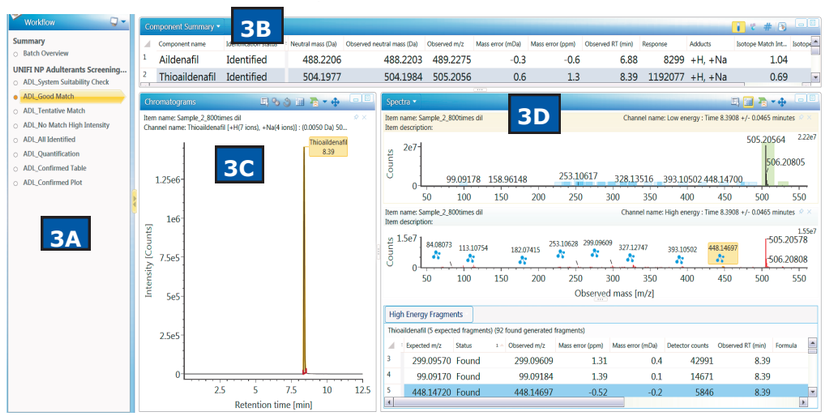


Figure 3. Results displayed in Review tab within the UNIFI after processing. 3A) List of the available workflow templates. Here, "ADL" refers to adulterant. 3B) list of the component associated with each of the workflow. Here shows the list associated with the "Good Match" filter; 3C) Extracted ion chromatograms (XIC) associated to 3B 3D) MS spectra correlate to what's on display in 3C, both low energy MS full scan and high energy MS^E scan.

Structural isomer determination: Thioaildenafil or Thiohomosildenafil?

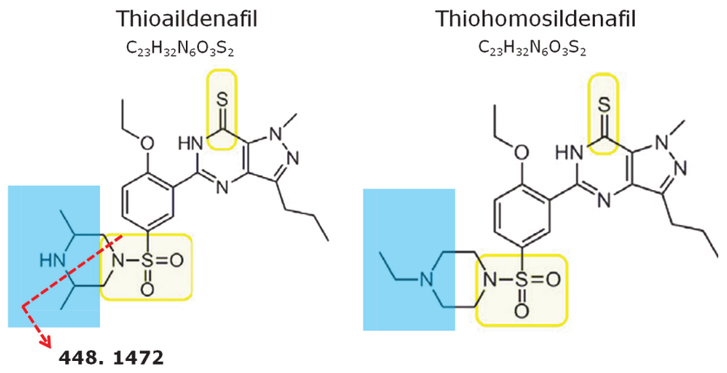


Figure 4. Two structural isomers of thiohomosildenafil and thiohomosildenafil. UNIFI identified thioaildenafil correctly for the peak at 8.39 mins due to the diagnostic fragment detected at 448.1472.

Are There Any Natural Ingredients?

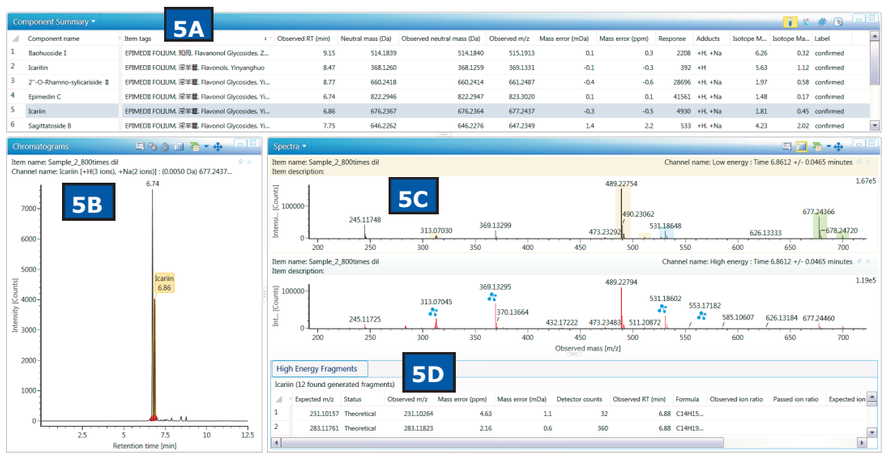
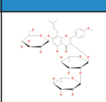
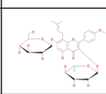
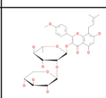
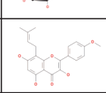
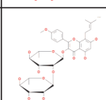
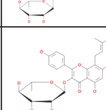


Figure 5. Results automatically generated after unknown peaks were searched against the UNIFI Traditional Medicine Library.

1) 5A displays the list of components that were identified in addition to the synthetic adulterants detected. The Waters traditional medicine library was searched for initial identification of the additional compounds. The item tags (table below) from the library allows easy identification of the botanical species, ***Epimedii* folium (a natural aphrodisiac plant)** and also the class of compounds that were detected, **flavonols**.

Compound name	Structure	Item Tag	Observed RT (Mins)	Neutral Mass (Da)
Epimedin C		Epimedii folium, Flavonol Glycosides	6.74	822.2946
Icariin		Epimedii folium, Flavonol Glycosides	6.86	676.2364
Sagittatoside B		Epimedii folium, Flavonol Glycosides	7.75	646.2262
Icaritin		Epimedii folium, Flavonol	8.47	368.1260
2''-O-Rhamno-sylicariside II		Epimedii folium, Flavonol Glycosides	8.77	660.2418
Baohuoside I		Epimedii folium, Flavonol Glycosides	9.15	514.1839

2) 5B displays the extracted ion chromatogram (XIC) of the peak at 6.86 mins for the selected compound in 5A, Icariin.

3) 5C displays the MS spectra that correlate to the component listed in 3B and displayed in 3C. Here, the top spectrum is the MS full scan spectrum and the bottom spectrum is the MS^E spectrum for Icariin.

4) In the MS^E spectrum (5C), the blue " " mark indicates the theoretical fragment ions that UNIFI identified (5D). Since a standard was not available, UNIFI can predict the possible fragments *in-silico* using the structure of the compound to confirm the presence of icariin. For icariin, 12 theoretical fragments were detected for the peak at 6.86 mins.

In-silico fragmentation of Icariin

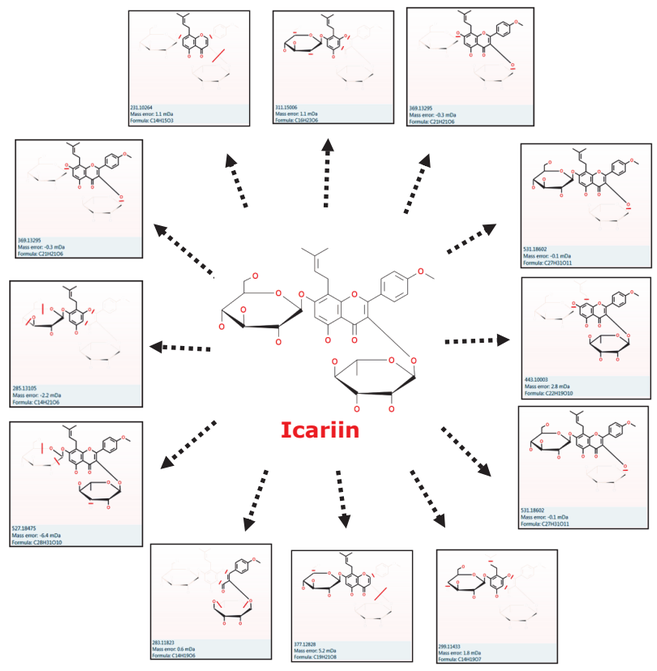


Figure 6. The 12 theoretical fragments for Icariin detected in the MS^E spectrum. UNIFI predicts the fragments *in-silico* using the structure of the original compound. The exact mass error and structure of each of the fragments are shown. This allows researchers to have higher confidence in their identification.

CONCLUSIONS

- **Two PDE-5 inhibitors, aildenafil and thioaidenafil were identified and confirmed from a single LC injection using the Waters Natural Product Application Solution with UNIFI® . Natural ingredients from *Epimedii* folium were identified by matching with the UNIFI traditional medicine library with compounds confirmed using the embedded in-silico fragmentation tool.**
- **Reliable answers about the ingredients in natural products can be obtained quickly with a streamlined screening workflow made possible by UNIFI Informatics Platform. This approach can significantly enhance efficiency and productivity for natural product researchers for a rapid comprehensive understanding of their complex natural product samples.**

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