DETECTING THE “UN-NATURAL” IN NATURAL PRODUCTS

Jimmy Yuk, Dhalavikumar Narendrabhai Patel, Giorgis Isaacs, Mark Wrona, Diego Rodriguez-Cabalero and Kate Yu

1. Waters Corporations, 34 Maple Street, Milford, MA 01757,
2. Waters Pacific Private Ltd, Singapore
3. Waters S.A.S. France

INTRODUCTION

There are an increasing number of herbal products being used around the world to treat an assortment of diseases 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 deliberate substitution of ingredients for economic benefit and pharmacological activity. 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 selectivity 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 infographics 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, embedded structure elucidation tools such as in-silico fragmentation also facilitated identification of unmatched unknown peaks. Here, in this post, 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 provided 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 uL of diluted sample was injected for analysis.

Instrumentation Platform

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

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: 30 % Acetonitrile in water: 70 % water:0.1% Formic Acid
- Gradient elution: 0.05-0.80 min, 98 to 2 % A then 0.80-1.10 min, 2 to 98 % A

- Column temp: 40°C
- Cone voltage: 40 V
- Cone: low CE: 5/3/29 CE: 15-40
- Source temp: 120°C, Desolvation temp: 500°C
- Gain setting: 1.5 kv (ESI+), 1.5 kv (ESI-)
- Collision energy (eV): low CE: 5/High CE: 15
- Capillary voltage: 1.5 kV (ESI+), 1.0 kV (ESI-)
- MS acquisition range: 50-1500 Da (0.1 Da scan rate)
- Acquisition mode: M5+, ESI+ and ESI- in resolution mode
- Captivity voltage: 1.5 kv (ESI+) 1.5 kv (ESI-)
- Retention time: 8.77

Results and Discussion

Streamline workflow using the Natural Products Application Solution with UNIFI:

1) 3A shows the 8 preset templates for the Adulterants Screening Workflow.
2) The System Suitability helps the routine system check prior to running the dataset.
3) The Good Match, Tentative Match, No Match High Intensity results quickly due to the complexity and large sizes of LC-MS datasets.
4) The Waters Natural Products Application Solution (NPAS) with UNIFI was used for the identification of ingredients and potential adulterants because of their high sensitivity and selectivity for samples with complex matrices.
5) In the M5 spectrum, the blue X mark indicates the fragment ion that matches from the expected fragment list in the library. For thioaildenafil, all four key diagnostic fragments were observed in the high energy spectrum. 448.1470 m/z was a characteristic fragment for thioaildenafil compared to the structural isomer, thiohomosildenafil.

CONCLUSIONS

- Two PDE-5 inhibitors, aldenafil and thioaildenafil were identified and confirmed from a single LC injection using the Waters Natural Product Application Solution with UNIFI®. Natural ingredients from Epimedi 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 In-silico fragmentation tools. This approach can significantly enhance efficiency and productivity for natural product researchers for a rapid comprehensive understanding of their complex natural product samples.