Development of a Multi Analyte Method for the Screening of Products Marketed as Dietary Supplements

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Introduction

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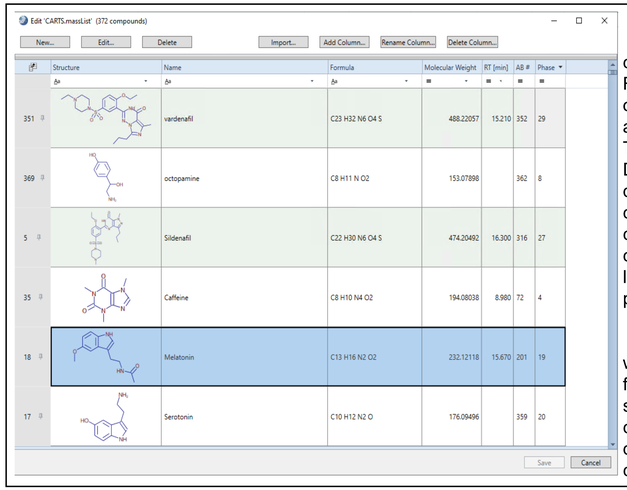
The dietary supplement market can be perceived by consumers as a safer and natural alternative to some conventional medicines to support bodily needs and functions. The market is diverse including a variety of dietary ingredients (e.g., vitamins, minerals, herbs, amino acids, and enzymes) which can be manufactured in many forms (e.g., tablets, capsules, softgels, powders, and liquids). Additionally, products marketed as dietary supplements may have unallowed ingredients, substitutions of an intended botanical ingredient, or the addition of adulterants such as active pharmaceutical ingredients. To assist in monitoring and to enforce regulations on the composition of products marketed as dietary supplements, this research will include the development and validation of a high-resolution mass spectrometry method for compound screening, identification, and quantification.

Database Curation

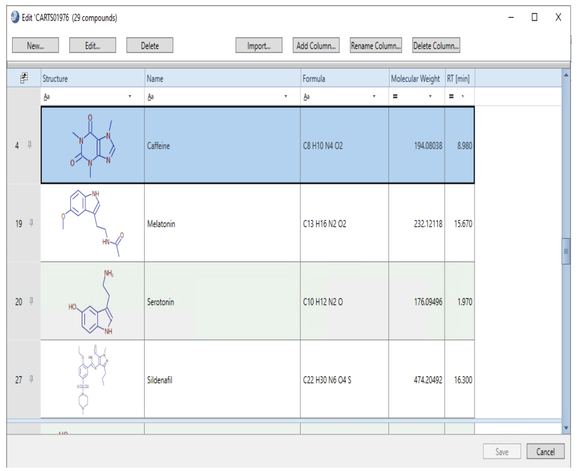


◄ Untargeted Method Development

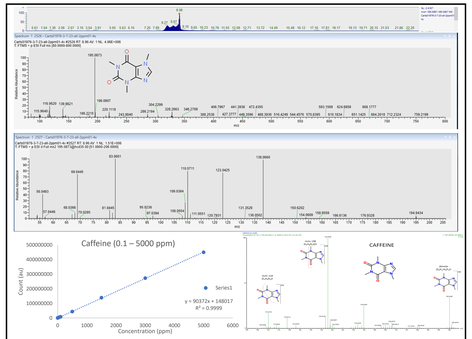
A compound library was curated through collaborations with the CFSAN Office of Regulatory Science (ORS), the CFSAN Office of Dietary Supplements Programs (ODSP), and the Office of Regulatory Affairs (ORA). The library was created in Compound Discoverer (Thermo) using the ChemSpider data available for each compound. After curation to remove redundancies in compound nomenclature, a total of 372 compounds remained including but not limited to dietary ingredients, adulterants, plant toxins and pharmaceuticals.

Targeted Method Development ►

A sub-set of library targets (Phase One) were selected for quantitation. Analytical figures of merit (e.g., accuracy, precision, selectivity) will be determined for 29 target compounds and compared for representative dietary supplement matrix types (tablet, capsule, softgel, powders, liquid, and gummy.



Targeted Data



For confirmation of identity, the measured exact mass (± 5 ppm) of at least two structurally specific ions (precursor ion and one or more fragment ions) at the retention time of a comparison standard is required. Quantification is performed using an external solvent standard calibration.

Objective

Developing a Desirable Method

1. **Verify Ingredients**
2. **Detect Contaminants**

- **Simple and Direct Sample Preparation**
  Tablets, Capsules, Softgels, Liquids, Gummies, Powders, and Bars.
- **Analytical Methodology**
  Liquid Chromatography - High Resolution Tandem Mass Spectrometry (LC-HRMS/MS)
- **Untargeted Method**
  Support Market Surveillance
  Suspect/Non-targeted screening
- **Targeted Method**
  Analyte confirmation and quantitation

Surveillance Methods

Market Survey

Screening many products marketed as different supplement for a variety of know ingredients and the absence of contaminants and adulterants

For Cause

Screening of a specific product in response to a consumer complaint or adverse event report

Target Assignments

Targeting a specific class of products to support claimed ingredients or absence of adulterants

Method Parameters

Experimental Sample Extraction

Aggregate sampling based on serving size

Add 20 mL ACN

Centrifuge

Add 20 mL warm water

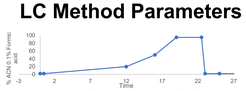
Shake for 30 mins

Shake for 30 mins

40 µL of supernatant diluted to 50 mL ACN:H_2O 1:1

2 µL injection

LC Method Parameters



Vanquish Conditions:

- Sample diluent: Acetonitrile (ACN)/H_2O (50:50)
- Column: ACQUITY PREMIER BEH Shield RP18, 1.7 µm VanGuard FIT 2.1 x 100 mm
- Column Temperature: 30°C
- Mobile Phase A: 0.1% formic acid in H_2O
- Mobile Phase B: 0.1% formic acid in acetonitrile
- Flow rate: 300 µL/min
- 2 µL injection

MS Method Parameters

Full MS → ddMS² (Inclusion List)
→ ddMS² (Top N)

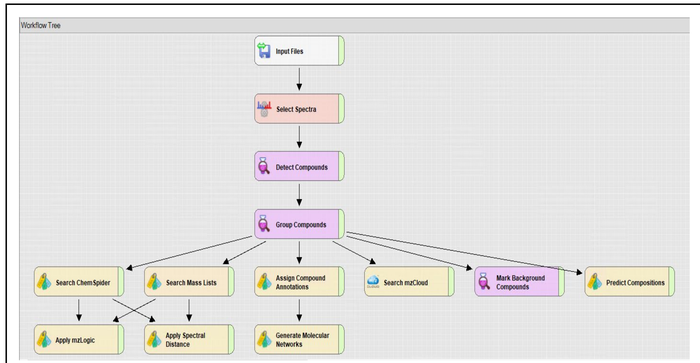
Orbitrap ID-X MS Conditions:

Full MS
- ESI
- Positive mode
- Resolution 60,000
- AGC Target: 4e6
- Scan Range:80 to 800 m/z

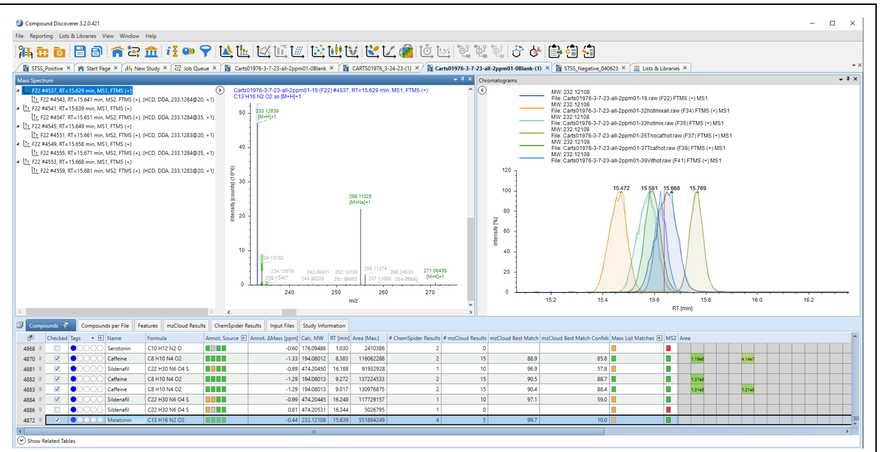
ddMS² (top intensity)
- Resolution: 30,000
- AGC Target: 5e5
- Scan Range: 80 to 800 m/z
- Collision Energy mode: Assisted (20, 35, 60)

ddMS² (Inclusion List)
- Resolution: 30,000
- AGC Target: 5e5
- Scan Range: 80 to 800 m/z
- Collision Energy mode: Assisted (20, 35, 60)

Untargeted Data Processing



Compound Discoverer Software (Thermo) was used to process the data for untargeted screening. A representative processing workflow is displayed, including an illustration for the identification of a melatonin in as standard solution and in different fortified matrix samples.



Conclusions

Current Progress

- Compound database of 372 compounds for untargeted screening has been curated
- 29 (Phase One) compounds have been selected and tested by targeted analysis and will undergo single laboratory validation
- A multi-phase liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS) method has been developed to screen for known ingredients and contaminants in products marketed as dietary supplements and quantify positive compound identifications.
- Current efforts are exploring improved or adaptive extraction method based on serving size to reduce the effects of dilution

Future Directions

- Validate data processing method for compound identification
- Extend targeted method to phase 2 compounds of interest. Single laboratory validate.
- Evaluate method performance in blinded test samples.

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  - Douglas Monroe
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