Plants, Food, Environment and Microbes

Profiling soybeans using UPLC-UV-CAD-MSn with UVPD to build and curate comprehensive spectral libraries for saponins and flavonoids

Eric D. Tague, Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA, 95134, USA

Abstract

Soybeans and their derivatives are widely used for livestock feed and protein sources in consumer foods. It's well known that two classes of compounds (flavonoids and saponins) are abundant in soy-based products. Flavonoids and saponins have been touted for their health benefits but some compounds in these classes could have negative side effects. With many people adopting a more plant-based diet, consumer products are beginning to utilize a wider variety of protein alternatives.
By leveraging a new inline LC universal detector, charged aerosol, and an advanced fragmentation technique, ultraviolet photo dissociation, a more comprehensive library focused on these two compound classes can be created with only a few injections and limited sample quantity. This library can then be applied to other plants to accelerate annotation of compounds in these classes during untargeted experiments.
A UPLC was used with a Hypersil GOLD™ aQ column to separate a wide variety of analytes. Following separation, the flow was split to 2 different detectors, a CAD and a VWD. Eluent was sent to the Thermo Scientific™ Orbitrap™ IQ-X™ Tribrid™ mass spectrometer where different data fragmentation techniques were performed including HCD, CID, and UVPD, within the same injection.
Using this plethora of data collected from the VWD, CAD, and different fragmentation types, a curated spectral library of 12 unique saponins, and unique 6 flavonoids was curated using Thermo Scientific™ Mass Frontier™ 8.1 software. This library was then used to identify the other related compounds within the soy sample. The library was used to search a sample of hops for related flavonoids or saponins.

Introduction

High resolution mass spectrometry is as great way to collect a wealth of information rapidly but it's only useful if it can be easily accessed and mined in later experiments. Library building and spectral curation are essential but can be very time consuming. By leveraging sophisticated tools aimed at optimizing time spent curating data, users can spend more time interrogating data.

Many times, just a full MS1 scan and single fragmentation mode isn't enough to confidently identify unknown compounds especially when working with plant-based compounds that have multiple isomers and isobars. This works adds 3 different fragmentation techniques (HCD, CID, and UVPD) to capture subtle differences in structure change in how compounds fragment.

Orthogonal to the MS data, the standard LC retention time was collected in addition to an inline UV detector and split flow to a Charged Aerosol Detector (CAD). This will help collect as much information as possible with a single injection.

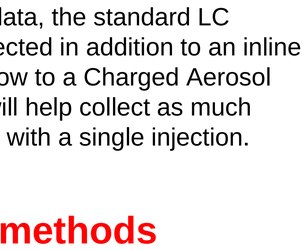
Materials and methods

Sample Preparation
Samples used in this project were received as defatted dry soymeal. In brief, preparation involved grinding the whole germ to a fine powder. Soxhlet extraction with hexane was allowed to proceed overnight. The resulting powder was allowed to thoroughly dry before metabolite extraction was completed[4].

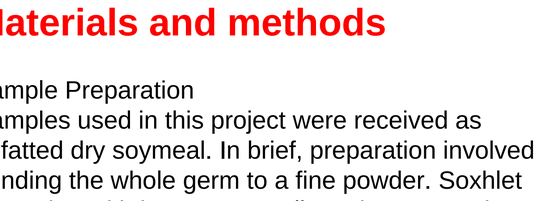
Metabolite Extraction
Approximately 150 mg of sample was aliquoted into 5 replicates samples. Each extracted with 4 mL of methanol. Samples were sonicated for 30 minutes and allow to extract overnight. After 10 minutes of centrifugation the supernatant was evaporated to dryness under a stream of nitrogen.
Samples were reconstituted in 300 µL of methanol.

Figure 1. A. whole soy meal B. processed and weighed sample

A.



B.



Learn more at thermofisher.com/

Instrumental design

Figure 2. A.- Thermo Scientific Vanquish™ Horizon UHPLC System. B.- Column used for separation.

A.

B.

Column: **Thermo Scientific™ Hypersil™ Gold AQ C18 selectivity**, 1.9 µm, 150 x 2.1 mm

Column Temperature: 45 °C

Autosampler Tray Temperature: 20 °C

Injection Volume: 2 µL

Mobile Phase: A = 0.1% Formic acid in Water
B = 0.1% Formic acid in Methanol

Flow rate: 0.3 ml / min

UV: 280 nm

CAD: 35 °C

Figure 3. LC gradient used in separation

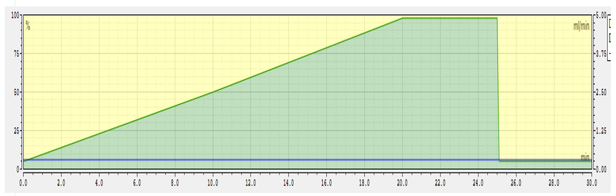
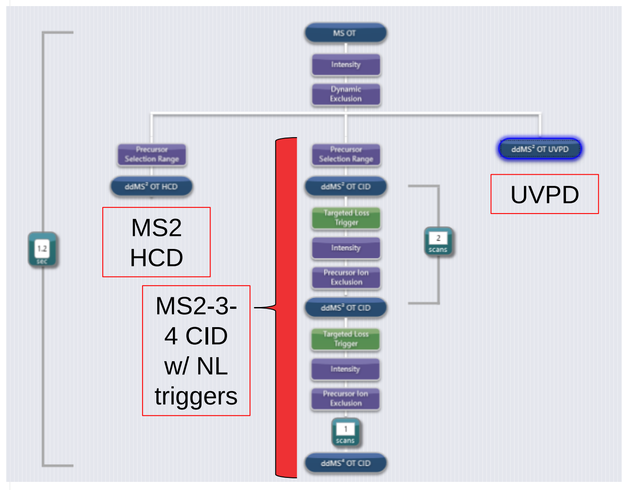


Figure 4. A.- Thermo Scientific Orbitrap™ IQ-X ™ Tribrid mass spectrometer. B.- Mass spectrometry method

A.

B.



A single injection was focused on the *m/z* range of 300-1250, HCD from 300- 500 *m/z*, CID from 550-1250 *m/z*, and UVPD fragmentation types for all MS2 data. MS3 and MS4 with CID fragmentation were acquired using NL triggers for 6 different sugars. This method was used for library searching and building using Mass Frontier 8.1 software. Data was collected in both positive and negative mode using separate injections.

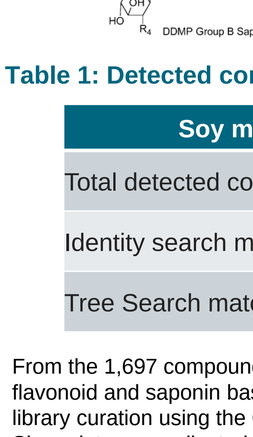
Data Processing

Preliminary data processing was performed in FreeStyle 1.8 SP2. This allowed for the comparison among the MS, CAD, and UV channels. Sample suitability and mass accuracy correction assessed before further processing.

Thermo Scientific Mass Frontier 8.1 software was used to for all other processing. This included component detection, library building, curation, fragment annotation, recalibration, spectral averaging, and searching.

Results

Figure 5. Different flavonoids of interest[1]



Mass Frontier 8.1 is capable to performing component detection for a sample with 3 built in detection algorithms each with user customizable parameters.
Results using Joint Component Detection and searches against mzCloud are shown in Table 1. This method was as semi targeted method with a focus on a list of known flavonoids and known saponins (Table 2).

Figure 6. Different saponins of interest[2]

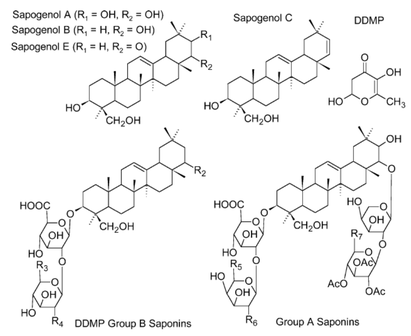


Table 1: Detected compound summary

Soy meal Compounds	
Total detected compounds	1,697
Identity search matches (MS2)	488
Tree Search matches (MSn)	512

From the 1,697 compounds that had at least 1 MS2, 22 flavonoid and saponin based compounds were chosen for library curation using the Curator module in Mass Frontier 8.1. Since data was collected for both ionization modes, the library will include data for these along with all three fragmentation types.

Table 2: Specific flavonoids and saponins chosen for library curation.

Soymeal Comounds of Interest					
Compound Class	Name	RT (max)	M-H	RT (max)	M+H
Isofalvones	Daidzin	8.19	415.1035	8.17	417.118
Isofalvones	Genistein	9.37	269.0457	9.36	271.0601
Isofalvones	6"-O-malonyl-genistin	10.98	517.193	11.05	519.1133
Isofalvones	6"-O-acetyl-genistin	12.05	473.1092	12.09	475.1235
Saponins Group A	A2	13.9	1105.503	13.91	1107.515
Saponins Group A	A1 w/ 4 Ac (22-gluc)	15.41	1435.638	15.41	1437.654
Saponins Group A	A3 w/ 4 Ac (22-gluc)	16.1	1243.577	16.08	1245.59
Saponins Group E	Bd	17.39	955.492	17.39	957.5063
Saponins Group B	III (Bb')	18.34	795.4553	18.39	797.4691
Saponins DMPP soy	betta a	18.99	1037.534	19	1039.546
Isofalvones	Daidzein	8.19	253.0507	8.19	255.0652
Isofalvones	Genistin	9.37	431.0985	9.36	433.1129
Misc	Isoflavone realated 2	12.26	467.2135	12.26	469.2807
Saponins Group A	A2 w/ 4 Ac (22-gluc)	15.92	1273.591	15.95	1275.602
Saponins Group B	V (Ba)	17.96	957.5076	17.99	959.5222
Saponins Group B	I (Bb)	18.39	941.5162	18.41	943.5273
Saponins Group E	Be	18.8	939.4986	18.81	941.5108
Saponins Group B	II (Be)	18.56	911.5029	18.56	913.516
Saponins Group B	IV	18.47	765.4437	18.41	767.4948
Saponins DMPP soy	betta g	18.84	1067.545	18.89	1069.558
Misc	Isoflavone realated 1	8.19	461.1091		ND
Misc	Isoflavone realated 3	9.37	477.1047		ND

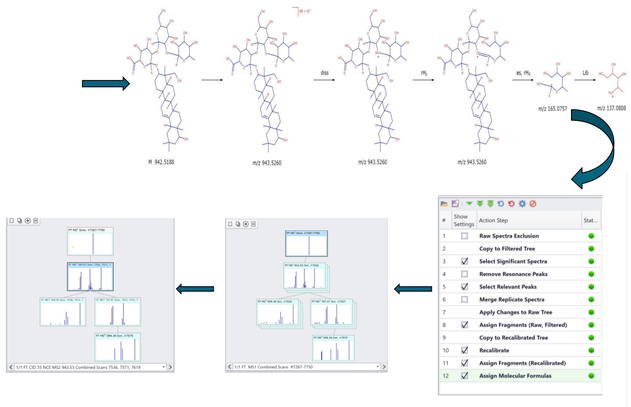
Several of the compounds had a match to mzCloud but matched multiple saponins with similar structures. By leveraging additional UVPD fragmentation, targeted NL, and advanced curation a highly annotated library can be created.

Mass Frontier 8.1 provides several ways to expedite library generation.

- New SledgeHammer fragmentation algorithms
- Importing SMILES or InChI strings for structure drawing
- Improved curation speeds
- Quick filtering and searching of user generated libraires
- Improved negative mode fragmentation

Using semi automated curation workflow, a library of 22 unique compounds was created in a single afternoon. An example of the curation workflow is shown in Figures 7 and 8.

Figure 7. Example of curation process in Mass Frontier 8.1



Results

Figure 8. Additional views of Mass Frontier 8.1

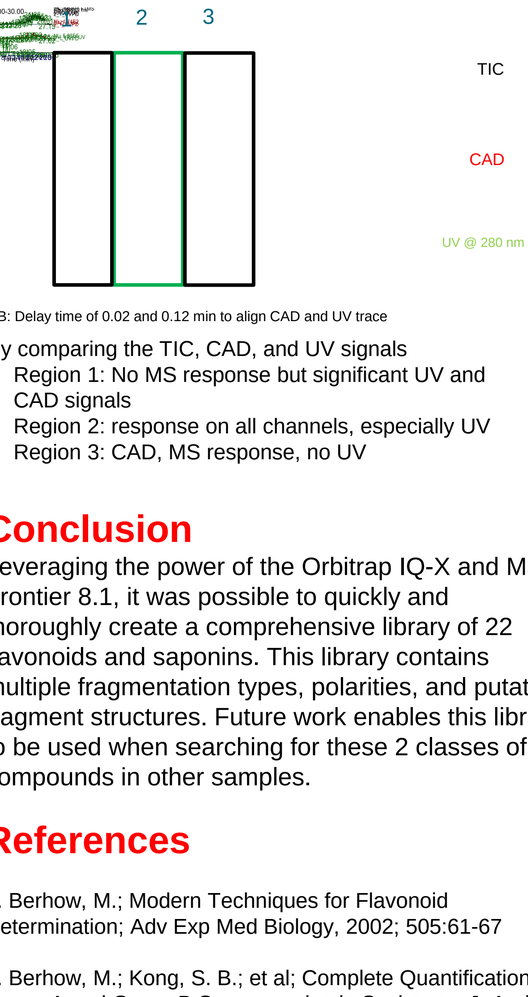


Figure 9. Comparison of chromatogram for TIC, CAD, and UV traces

NB: Delay time of 0.02 and 0.12 min to align CAD and UV trace

By comparing the TIC, CAD, and UV signals

- Region 1: No MS response but significant UV and CAD signals
- Region 2: response on all channels, especially UV
- Region 3: CAD, MS response, no UV

Conclusion

Leveraging the power of the Orbitrap IQ-X and Mass Frontier 8.1, it was possible to quickly and thoroughly create a comprehensive library of 22 flavonoids and saponins. This library contains multiple fragmentation types, polarities, and putative fragment structures. Future work enables this library to be used when searching for these 2 classes of compounds in other samples.

References

1. Berhow, M.; Modern Techniques for Flavonoid Determination; Adv Exp Med Biology, 2002; 505:61-67

2. Berhow, M.; Kong, S. B.; et al; Complete Quantification of Group A and Group B Soyasaponins in Soybeans; J. Agric Chem.; 2006; 54, 2035-2044

3. Kiyomani, Reiko; Ding, Caroline; Sharma, Seema; Huhmer, Andreas; Increased throughput and coverage for the annotation of saponins using a structure-based MSn approach on an Orbitrap Tribrid mass spectrometer. Thermo Fisher Scientific App note AN73236 2019.

4. Griffith, Aaron P.; Collison, Mark W.; Improved methods for the extraction and analysis of isoflavones form soy-containing foods and nutritional supplements by reverse phase…., Journal of Chromatography A; 913, 2021, 397-413.

Acknowledgements

I would like to acknowledge the support of Thermo Fisher Scientific's LSMS Center of Excellence Center and the metabolomics vertical marketing group for use for workspace and instrument time. I would like to thank Mark Berhow for providing the defatted soy meal samples.

Trademarks/licensing

Science at a scan
Scan the QR code on the right with your mobile device to download this and many more scientific posters.