metabolomics

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Features or Compounds? A Data Reduction Strategy for Untargeted Metabolomics Analysis to Generate Meaningful Data

Webinar Q&A Document | March 29, 2018 | Link to Webinar

Q: What is the recommended resolution for a metabolomics experiment?

A: This will depend on which Thermo Scientific™ Orbitrap-based LC-MS instrument is used; we suggest a range of 60,000 to 140,000 resolving power for untargeted metabolomics analysis.

Q: For unknown peak detection, a list of different ions were shown that can be considered for feature assembly. What if a target ion is not on the list? Can this be added?

A: Yes, ions can easily be added to the Ion Definitions list in Thermo Scientific™ Compound Discoverer software. Additionally, users can select which ions from this list should be considered for data processing for each analysis.

Q: I'm new to untargeted metabolomics, so I'm not sure where to start with data processing in Compound Discoverer. Are there any reference materials available to get started with?

A: Yes, Compound Discoverer software comes with a step-by-step tutorial for untargeted metabolomics along with sample data to practice with. The sake data set discussed in the webinar presentation is available to process on your own run. Similarly, there is a User Manual that goes into greater detail if needed in addition to the blog website https://mycompounddiscoverer.com

Q: What about exogenous metabolites? Are these metabolites considered in anyway?

A: Yes, if you perform an untargeted metabolomics experiment then these metabolites are typically included in the result.

Q: How to realize study under Compound Discoverer software when we work with reverse phase mode and HILIC mode. Do we have to create 2 studies for each mode?

A: You would have to create two separate studies as two different chromatography modes are used. There is currently no way to merge the results automatically.

Q: How do you know that a feature is the M+H and not another adduct? And how do you easily combine all adducts belonging to one compound?

A: The software takes into consideration all possible mass differences, the retention time as well as the peak shape. This procedure known as feature assembly, is a pretty complex algorithm and also tries to resolve certain ambiguities. In some case when ambiguities cannot be resolved then the software will report that.

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Q: From the exported compound list, we get raw peak area values. Do we assume the areas are integrated from the most abundant adducts (say there are many adducts associated) for a single compound?

A: Yes, a compound includes all associated adducts. Therefore the peak areas are summed together. You can easily find this information in the related table within Compound Discoverer software. When you select a specific compound, choose the related table, and there will be a features tab via the Compound per File tab. You can see the peak area of individual features that were associated.

Q: What databases do you use?

A: Compound Discoverer software gives you the option to use ChemSpider (which allows access to ~500 databases including KEGG, BioCyc, HMDB, EPA), as well as KEGG and BioCyc as pathway databases, mzCloud (https://www.mzcloud.org/) as a comprehensive spectral reference library. It also comes with a local version of mzCloud and you can create custom libraries and databases. Certain freely available databases and libraries can also easily be imported.

Q: How can you be confident with an annotation when there could be several compounds with the same molecular formula, especially in a sample with a wide range of chemical classes?

A: It's important to think about confidence when you're thinking about compound annotation. Molecular formula is one piece of information used in compound annotation. We can be confident when using fine isotope structure, which will narrow the candidate list. Similarly, accurate mass plays a role in this. In thinking about confident annotation there are additional measures we can consider like fragmentation spectral searching. In Compound Discoverer software, there is an option for identity searching as well as similarity searching for substructure information.

Q: How long does it take to process a data set like the sake experiment?

A: It takes about 2-3 hours to process this dataset depending on the PC's processing speed and parameters settings.

Q: Can Compound Discoverer software handle data acquired by polarity switching? Or does positive and negative data have to be acquired separately?

A: Yes, Compound Discoverer software can automatically handle polarity switching data. It automatically merges the data and shows the combined result.

Q: Does this also take into account in-source fragments? this is one of the main issues in peak grouping.

A: Compound Discoverer software can handle all in-source fragments that are defined in the ion list. This list can also be edited and additional adducts or in-source fragments can be added so that these will be annotated in the result.

Q: One of the common techniques used in metabolomics is stable isotope amino acids labelling, once a potential compound is identified and if it has a peptide precursor. While this is more of a targeted metabolomics approach, is this something Compound Discover software is able to analyze and how?

A: Currently Compound Discoverer 2.1 software does not support stable isotope labeling studies. We are looking into this capability.

Q: What file formats does the software support?

A: Compound Discoverer software currently only supports Thermo .raw files. The reason is certain information that is required for processing like resolution and accuracy is missing in file formats like mzML and mzXML.

Q: This is LCMS-based metabolomics, which cannot detect every peak, specially volatile compounds. How can we solve this issue in metabolomics? Is NMR based metabolomics complementary to this LCMS based method?

A: You are right, LC-MS does not detect all possible small molecules. We may need to use other available technologies to detect compounds like those that are highly volatile. We are finding that GC-MS provides complimentary capabilities to provide greater coverage of the metabolome.

Q: Can Compound Discoverer software work for medium size or high molecular weight molecules like protein for metabolomics/proteomics?

A: Thermo Scientific™ Proteome Discoverer™ software should be used to analyze the results from proteomics experiments.

For more information, visit: **thermofisher.com/metabolomics**

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