thermo scientific

Solutions for routinely measuring metabolic knowns and identifying unknowns

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METABOLOMICS APPROACHES FOR DIVERSE APPLICATIONS

Metabolomics measures all small molecules in a biological sample. These endogenous metabolites represent the biochemical phenotype for a given condition or state. The phenotype or profile of an organism is useful in understanding functional biology at the molecular level. Metabolomics experiments are often designed as comparative studies where two or multiple groups are used to determine differences between sample populations, such as control versus disease. These phenotypical differences can shed direct insight into the molecular underpinnings of the biological system. Lipidomics, or the complete profile of lipid species, is a subset of metabolomics. In turn, this information can be combined with other “omics” disciplines like genomics, proteomics and transcriptomics for a complementary readout.

Metabolomics is found in many areas of research. The need for understanding human health and disease has led to metabolomics studies involving disorders such as diabetes and cardiovascular disease, and its potential for determining diagnosis by signatures or biomarkers of disease. Metabolomics provides understanding of cancer biology and the potential to determine disease progression. Further, pharmacometabolomics studies can be useful in determining an individual’s response to drug therapies and the potential application of precision medicine. Other areas where metabolomics has been applied include studies of the microbiome, the exposome, diet and nutrition, and plant metabolomics. The use of metabolomics is diverse because it provides a snapshot of the biochemical network of small molecules.

Three common experimental approaches in metabolomics are targeted, targeted profiling, and untargeted analysis (Figure 1).

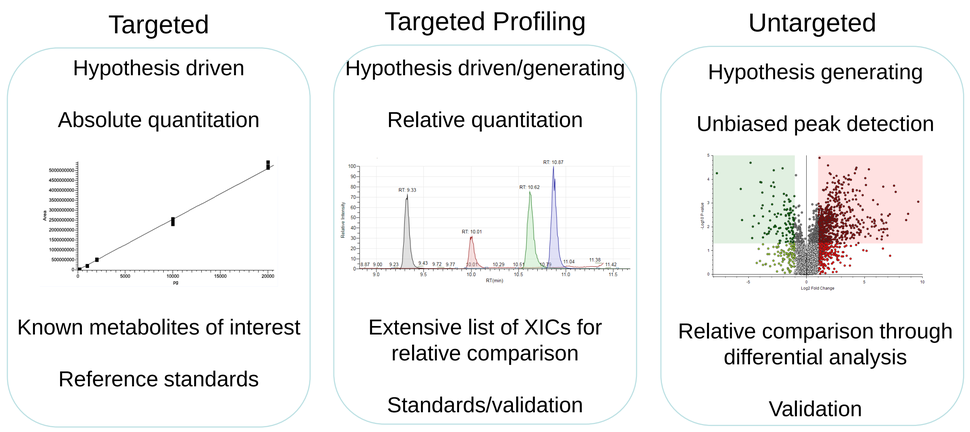


Figure 1. Experimental metabolomics approaches. Targeted analysis involves the determination and absolute quantitation of known metabolites. Conversely, untargeted analysis provides comprehensive coverage of the metabolome through unbiased detection of all compounds present. Targeted profiling incorporates both approaches generating a large set of compounds of both known and unknown identity. Which approach to choose depends on the goal of the experiment.

Biology is complex. To decipher these complexities, metabolomics analysis demands sophisticated analytical technologies and software solutions. Whichever the desired approach, our proven scientific network, publications and pioneering solutions driven by Thermo Scientific™ Orbitrap mass spectrometers provide the right tools for any metabolomics experiment, from quantifying known metabolites to identifying unknown compounds.

ANALYTICAL TECHNIQUES FOR EVERY APPROACH

Endogenous metabolites are diverse in their physico-chemical properties, as well as varying in abundance. A true comprehensive metabolomics study requires various orthogonal sample preparation and separation techniques. In reality, most experiments are not comprehensive as there is a bias towards certain classes of compounds based on the selected sample preparation method and separation technique. To accomplish a broader and deeper analysis of the metabolome, complementary chromatographic separation approaches are employed (Figure 2).

Breadth of Chromatographic Separations

HPLC: Liquid chromatography (LC)-MS offers the broadest coverage of metabolites by the ability to change column chemistries such as reversed phase, RP (for none to moderately polar metabolites) and hydrophobic interaction liquid chromatography, HILIC (ionic and polar compounds not retained by RP).

IC: Ion chromatography (IC)-MS is best suited for charged or very polar metabolites that are difficult to analyze by LC-MS such as sugar phosphates, amino acids, etc. IC is also high resolving, and many isomers can be separated prior to mass spectrometry analysis.

GC: Samples which are volatile and amenable to chemical derivatization are well suited for gas chromatography (GC)-MS. GC offers high resolving power. Plant secondary metabolites are well suited to this technique.

Figure 2. Thermo Fisher’s leading breath in chromatography separations, GC, LC and IC, combined with proven Thermo Scientific™ Orbitrap™ MS technology delivers the greatest coverage of the metabolome. With high robustness and technical reproducibility, these solutions provide both novel yet stringent results for high impact discoveries.

LC-MS: Thermo Scientific™ Vanquish™ Flex Binary HPLC with Thermo Scientific™ Q Exactive™ HF MS

IC-MS: Thermo Scientific™ Dionex™ IC-5000+ Capillary HPIC™ with Thermo Scientific Q Exactive HF MS

GC-MS: Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC- MS/MS

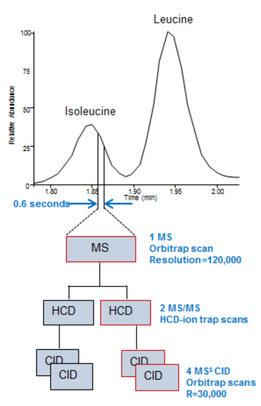
Orbitrap High Resolution Accurate Mass (HRAM) for Successful Metabolomics

Leading Orbitrap-based mass spectrometers provide HRAM measurements and sensitivity required to measure metabolites in complex matrices. When combined with advanced separations, high throughput and quantitative capabilities expanded the scope of what we know about metabolites and their role in several different areas of study.

Key attributes of Orbitrap HRAM include -

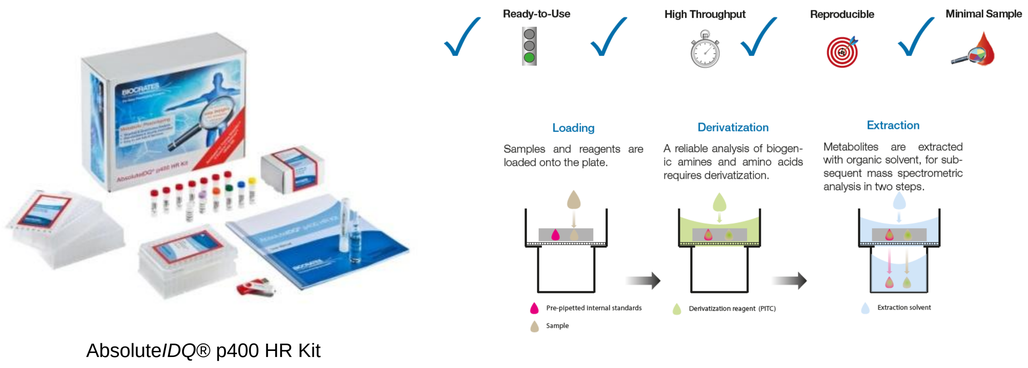
1. High Resolution Accurate Mass: With up to 1M resolution, Orbitrap analyzers provide high specificity delivering confident mass assignments, thus giving more confident identification and quantitation. Higher resolution distinguishes metabolites of similar mass, particularly of isobaric species and with isotope labeling studies.
2. Sensitivity: Metabolomics is and always has been a quantitative science. Levels of endogenous metabolites range in orders of magnitude. The mass spectrometer must detect a wide concentration range for a comprehensive and meaningful view of the phenotype. Orbitrap technology enables quantitation down to low fmol concentration, with 5 orders of linear dynamic range achieving tight coefficient of variation (CV).
3. Technical Reproducibility: Due to it’s sensitivity and selectivity, mass spectrometry techniques have become the method of choice for metabolomics research. To find meaningful answers with a large number of data sets, typically found with metabolomics, the mass spectrometer must provide accurate and reproducible data from run to run, without the need for internal calibration.
4. Fast Polarity Switching: Sample volume or instrument time should not be limited factors in obtaining a comprehensive analysis. Polarity switching enables data acquisition in both positive and negative ionization modes within the same analytical run. Orbitrap MS polarity switching operates on a chromatographic time scale increasing productivity.
5. MS^n Capabilities for Metabolite Structural Elucidation: MS^n offers extensive fragmentation capability for de novo identification and structural elucidation. MS^n is usually achieved using ion traps and tribrid mass spectrometers. This advanced technique requires high resolution, accurate mass, speed on an HPLC time scale, and a variety of dissociation modes such as HCD, CID and UVPD.

MS^n Capabilities enabled by Thermo Scientific™ Orbitrap Fusion™ Lumos Tribrid™ MS.



STANDARDIZED TARGETED METABOLOMICS AND LIPIDOMICS

Reliable results are a must for targeted analysis. The Biocrates Absolute*IDQ*® p400 HR Kit , developed in partnership with Biocrates, offers a standardized and quantitative assay measures up to 408 metabolites spanning 11 compound classes including amino acids, biogenic amines, acylcarnitines, triglycerides, diglycerides, ceramides, cholesterol esters, sphingomyelins, phophatidylcholines, lysophophatidylcholines, and hexoses (Figure 3). Consisting of LC-MS and FIA-MS runs, the kit is an established tool for standardizing routine targeted metabolic phenotyping and now relies on Orbitrap-based HRAM mass spectrometry. Either new to metabolomics or a seasoned veteran, this quick start kit offers direct access to targeted metabolomics with quantification, reproducibility, and high throughput within one day. Coupled with the Thermo Scientific™ Q Exactive™ MS family of instruments, precise quantitative measurements of important metabolites provide consistency across studies and between labs. The kit is fully validated for human plasma and tested for mouse and rat plasma, and rat brain homogenate samples.



Absolute*IDQ*® p400 HR Kit

Figure 3. The Biocrates Absolute*IDQ*® p400 HR Kit. A ready-to-use targeted metabolomics kit for standardized, routine analysis requiring only 10 µL of biofluid. The entire kit workflow is supported by the Biocrates Met*IDQ*™ software ensuring reproducible results.

UNTARGETED METABOLOMICS

An unknown or non-targeted approach captures a wide space of chemical entities. Unbiased peak detection collects a considerable number of compounds in often complex samples ranging from biofluids to tissue to cells. This hypothesis generating approach aims to determine differences between condition or states. For instance, patient cohorts are compared between those with disease and matched controls (Figure 4). Statistical analysis of the relative abundances aids in determining discriminating compounds. Beyond that, sophisticated identification tools are needed to determine putative identification of unknowns, and ultimately confirmed with purified standards. One advantage of obtaining data acquired in an unknown fashion is the ability to retrospectively mine the data, which can answering future questions without reprocessing samples.

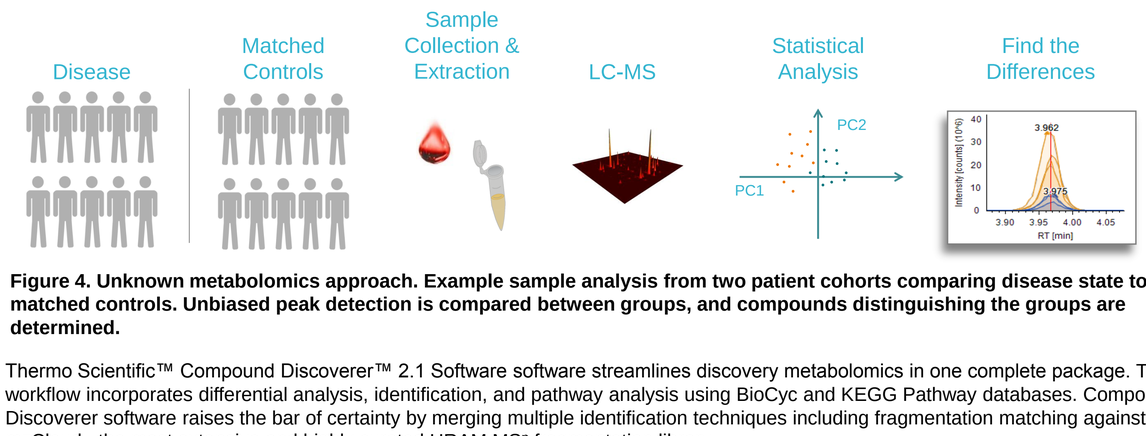
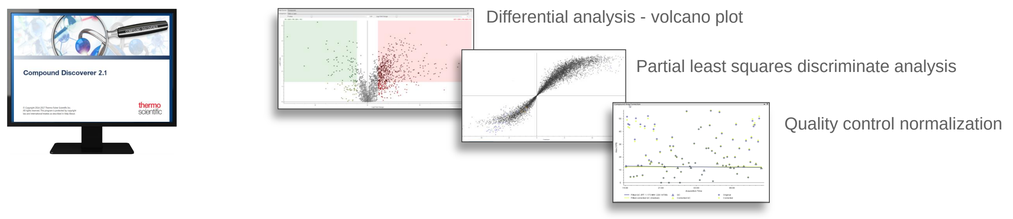


Figure 4. Unknown metabolomics approach. Example sample analysis from two patient cohorts comparing disease state to matched controls. Unbiased peak detection is compared between groups, and compounds distinguishing the groups are determined.

Thermo Scientific™ Compound Discoverer™ 2.1 Software software streamlines discovery metabolomics in one complete package. The workflow incorporates differential analysis, identification, and pathway analysis using BioCyc and KEGG Pathway databases. Compound Discoverer software raises the bar of certainty by merging multiple identification techniques including fragmentation matching against mzCloud , the most extensive and highly curated HRAM MS^n fragmentation library.

Powerful visualization tools help quickly find real statistical challenges – the differences that matter – between sample sets. Interactive linked displays enable the ability to see trends across studies and readily determine discriminators. Modeling after the community accepted practice[1], the software enables compound normalization to quality control samples, typically a pooled sample reflecting all experimental samples.



Confidently identify more unknown compounds, the toughest challenge in metabolomics (Figure 5). Compound Discoverer software is fully integrated and automated to search mzCloud, returning not only precursor ion accurate mass matches, but also similarity matches without precursor ion mass match which provides valuable sub-structure information for the unknown compound of interest. The most confident elemental composition predictions are made utilizing fine isotopic structures from high resolution Orbitrap full MS, as well as MS/MS information to improve predictions. Structurally annotate fragmentation spectra using the HighChem Fragmentation Library.

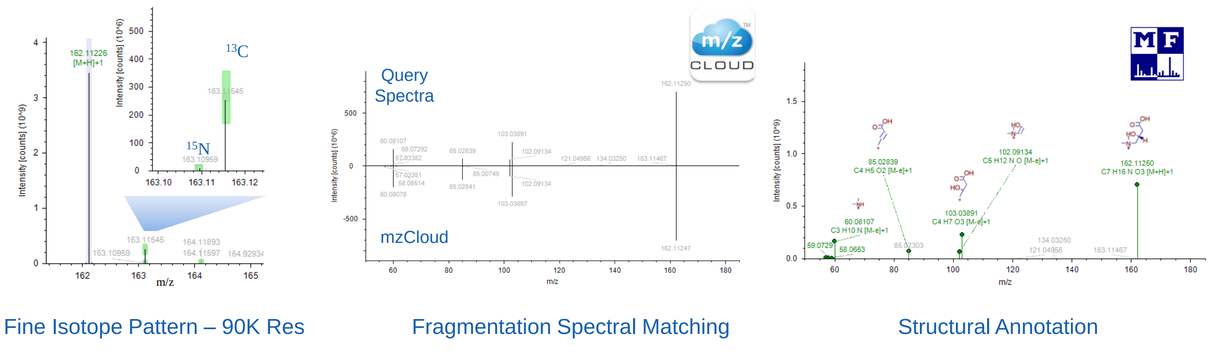


Figure 5. Putative identification of carnitine using Compound Discoverer 2.1 software. HRAM provides fine isotope patterning for increased confidence of elemental composition prediction. MS/MS fragmentation spectrum of observed data matched with mzCloud for carnitine. Structural annotation explains all fragment ions.

CONCLUSIONS

Metabolomics is in many different disciplines. With any experimental approach, from targeted to untargeted, sophisticated analytical technologies and software solutions are necessary to address the complexities of biology and the diversity of metabolites.

- Thermo Scientific™ chromatographic solutions enable the complementary coverage of the metabolome for a broader, deeper analysis
- Orbitrap mass spectrometry delivers HRAM measurements with both speed and sensitivity for confident identification and quantitation
- Obtain reliable quantitative results of targeted metabolites using the standardized Biocrates Absolute*IDQ*® p400 HR Kit
- Confidently identify unknowns using Compound Discoverer software and mzCloud MS^n fragmentation library
- Thermo Scientific™ Orbitrap™ GC-MS HRAM Metabolomics Library for electron ionization (EI) GC-MS

REFERENCES

1. Dunn, W.B., Broadhurst, D., Begley P., Zelena E., Francis-McIntyre S., Anderson N., Brown M., Knowles J.D., Halsall A., Haselden J.N., Nicholls A., Wilson I. D., Kell D.B., Goodacre R., Human Serum Metabolome C. 2011. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Nature Protocols, 6, 1060-1083.

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