Applying Q Exactive Benchtop Orbitrap LC-MS/MS and SIEVE Software for Cutting Edge Metabolomics and Lipidomics Research

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Introduction

Application of metabolomics to disease phenotype analysis and identification of unique biomarkers to distinguish healthy individuals compared to those with a disease has renewed the promise of personalized medicine. We present here the application of a Thermo Scientific benchtop quadrupole-Orbitrap mass spectrometer coupled to a UHPLC and Thermo Scientific SIEVE software for several metabolomics and lipidomics studies. The performance of the entire analytical platform is illustrated with relevant examples including the metabolomic analysis of lean and obese Zucker rat serum and mitochondrial lipids in Yeast.

Methods

Sample Preparation

Metabolites were extracted from plasma with cold methanol and the supernatant was removed, dried under nitrogen, and reconstituted with 80:20 water/methanol for LC/MS. Mitochondrial lipids were isolated from wild-type (WT) yeast (*S. cerevisiae*) and a knockout (KO) strain that does not produce coenzyme Q (CoQ), by extraction using isopropanol (1).

Liquid Chromatography Mass Spectrometry

High-resolution accurate-mass full scan LC-MS and LC-MS/MS analyses were performed with short UHPLC gradient at 70,000 resolving power on the Thermo Scientific Q Exactive mass spectrometer in both positive and negative ion modes with electrospray ionization source. MS-MS data was obtained at 35,000 resolution.

Data Analysis

All of the datasets were analyzed with the SIEVE™ 2.0 software with the Component Extraction algorithm designed specifically for optimal data analysis in untargeted metabolomics experiments. Components that were significantly different between the sample groups were detected and identified via local or online database search. MS/MS data were then acquired and used to confirm the structure of these components. Thermo Scientific Mass Frontier 7.0 RS1 software provided spectral interpretation tools including MS/MS library search and assigning structures to the fragment ions automatically in the MS/MS spectrum. Thermo Scientific TraceFinder 2.1 software was then used for streamlined targeted quantitative analysis.

Results

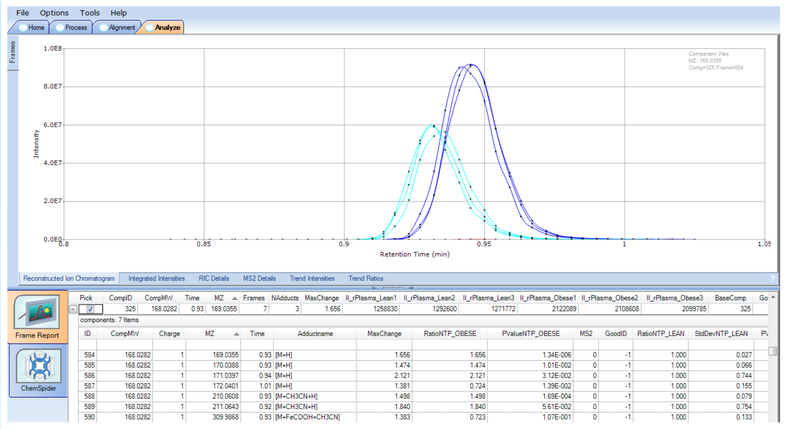
Analysis of the ZDF fatty rat serum compared to normal rat serum served as a benchmarking study to determine the merits of the technology platform. We observed an increase in acylcarnitines, branched chain amino acids (2), phospholipids, fatty acids and conjugated bile acids ($P < 0.001$) in fatty ZDF rat plasma relative to normal ZDF rats (Table 1).

Preliminary analysis of yeast mitochondrial lipid extracts demonstrates the ability to obtain statistically significant results with a single injection of each biological replicate. The expected change in CoQ6 levels was accompanied by changes in over 60 different components including amino acids, acylglycerols, sterols, phospholipids and sphingolipids.

Table 1. Compounds Identified via ChemSpider Database Search.
Metabolites that significantly decrease (red) or increase (green) in ZDF Obese vs. Normal Rats ($p < 0.001$).

CSID	Compound Name	MW	MZ	Time	Ratio	p-Value
63798	Imidazole propionic acid	140.0585	141.0658	0.83	0.418	2.45E-04
757	Homocysteine	135.0352	136.0425	0.68	0.485	8.32E-04
6050	Valine	117.0792	118.0865	0.61	0.655	5.44E-06
718	Glutamine	146.0690	147.0762	0.59	0.688	1.58E-04
96904	Propionyl carnitine	217.1314	218.1387	1.90	0.768	8.31E-04
2006614	Carnitine	161.1050	162.1123	0.62	1.203	5.44E-05
969	Phenylalanine	165.0789	166.0861	2.65	1.273	1.69E-04
834	Leucine	131.0946	132.1019	1.54	1.448	1.40E-04
1142	Uric acid	168.0282	169.0355	0.93	1.656	1.34E-06
24766528	C18:3 Lyso PC	517.3170	518.3243	9.78	1.980	2.18E-04
1141	Uracil	112.0275	113.0348	0.86	2.015	2.49E-06
5858	Adenonsine monophosphate	347.0625	348.0698	0.83	2.377	1.30E-05
388877	Butyl carnitine	231.1469	232.1541	3.54	2.967	2.73E-06
9931788	C20:5 Lyso PC	541.3168	542.3240	9.69	4.368	7.88E-04

FIGURE 1. Grouping of Adducts and Isotopes, m/z 169.0355, Uric Acid.
Uric acid, identified by ChemSpider search, increased 3-fold in Obese vs. Normal rats.



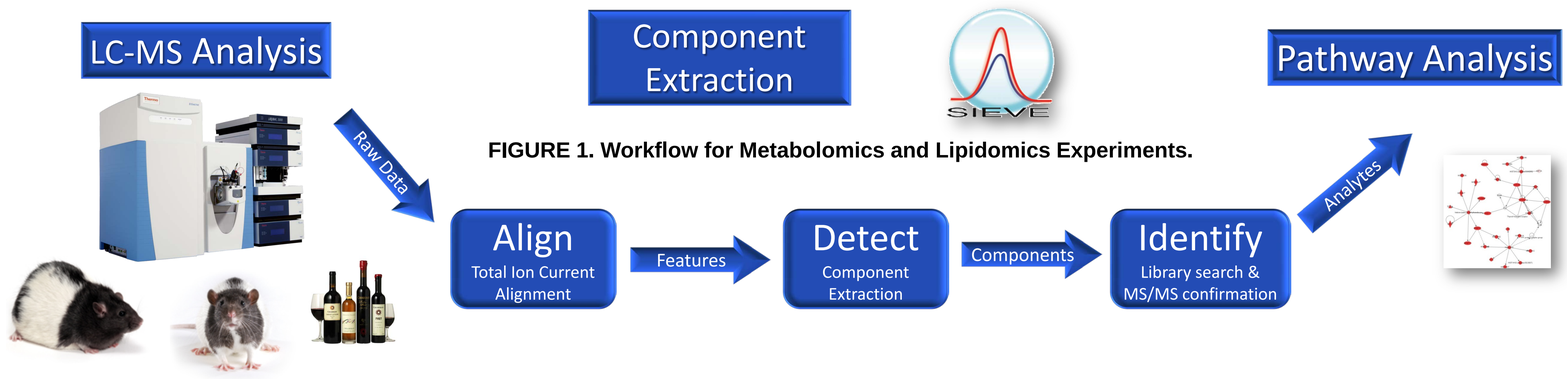


FIGURE 1. Workflow for Metabolomics and Lipidomics Experiments.

FIGURE 2. Principal Components Analysis of Lean vs. Obese ZDF rat serum. The Principle Components Analysis (PCA) results shows that the LCMS analysis of the two ZDF rat serum groups are clearly different.

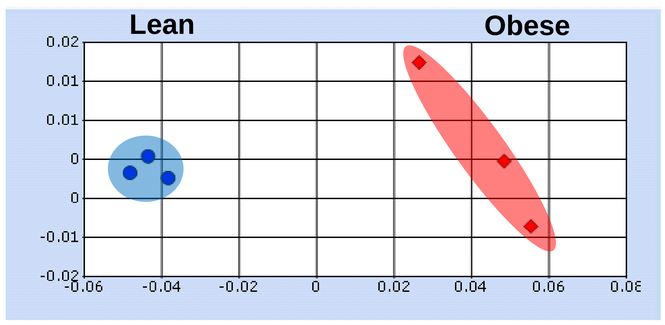


FIGURE 3. LC-MS/MS of Kynurenine, m/z 209.1 from Rat Serum.
Mass Frontier software was used to annotate the accurately-measured fragment ions giving excellent confirmation of the known structure from a single MS-MS scan.

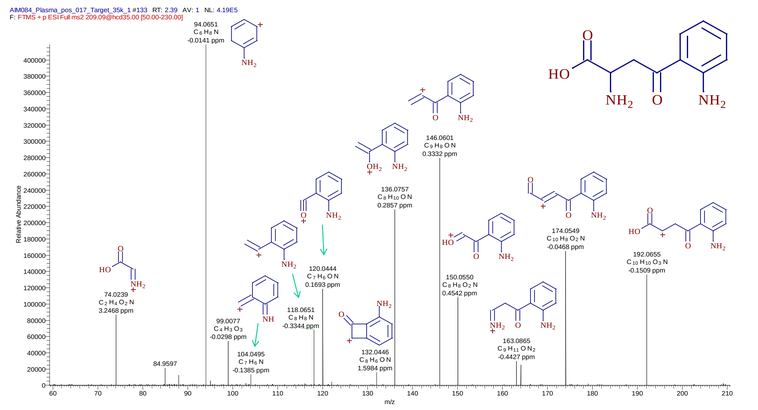


FIGURE 4. ZDF Rat Metabolomics Data – Pathway Analysis (4)
Annotated metabolites in Red were found from MW search using ChemSpider.

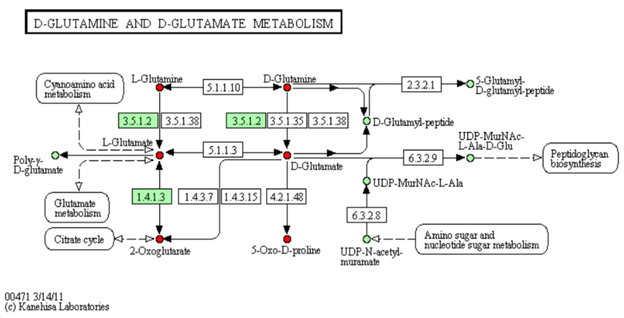


FIGURE 5. Chromatographic Alignment of Yeast Lipid Extract LC-MS Data

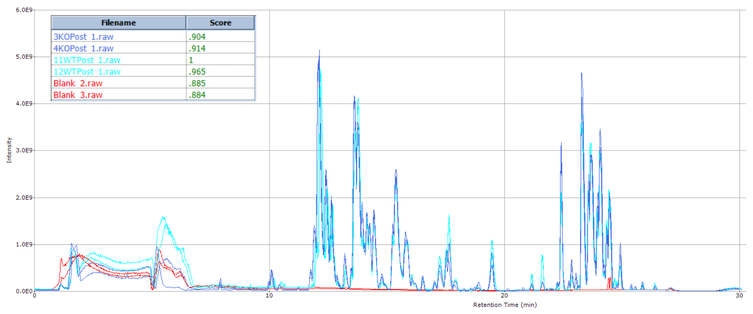


FIGURE 6. MS-MS Identification of PE 34:2, m/z 714.51
Excellent mass measurement and ID from a single MS-MS scan.

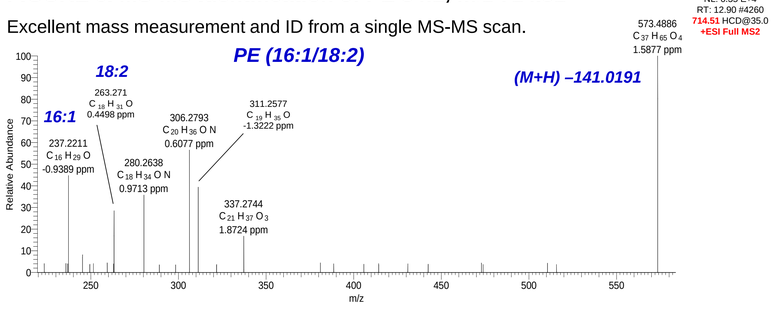


FIGURE 7. Significant Metabolite Differences Found in WT vs. KO Yeast.
Relative amounts of metabolites from KO Yeast (Dark Blue) vs. WT Yeast (Light Blue) increased for sterols, ceramides and Co-Q9 whereas histidine, sphinganine and CoQ6 decreased in the KO yeast.

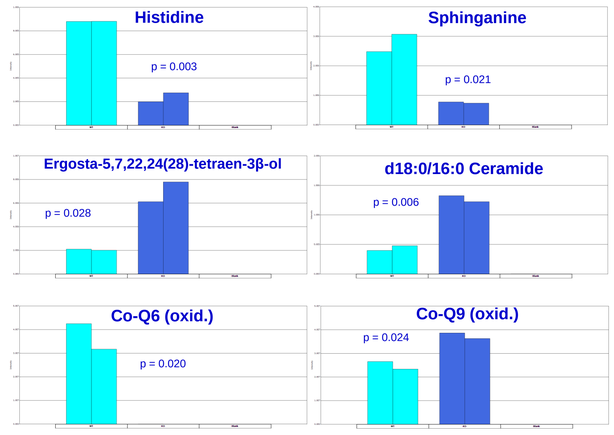
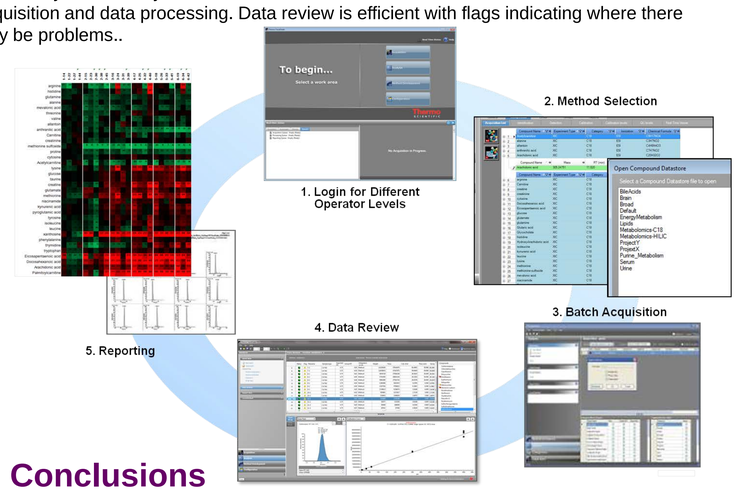


FIGURE 8. Thermo Scientific TraceFinder Software for Targeted Analysis . A compound database is selected dependant on the compound class, tissue or pathway under study . The customizable database contains all the information for batch acquisition and data processing. Data review is efficient with flags indicating where there may be problems..



Conclusions

- Ultra-high resolution accurate mass data from Orbitrap™ detectors provides the most reliable analysis of endogenous metabolites and lipids
- Component workflow decreases false positives due to chemical background and redundant signals
- Find the real differences in components across multiple sample groups with SIEVE 2.0
- MS^n on hybrid Orbitraps and Mass Frontier 7.0 provide a complete set of tools for structure elucidation

References

1. D. A. Peake, J. Wang, P. Huang, A. Jochem, A. Higbee, D. J. Pagliarini, Lipid Maps Annual Meeting, May 7-8, 2012, La Jolla, California, USA
2. J. R. Bain, R. D. Stevens, B. R. Wenner, O. Ilkayeva, D. M. Muoio and C. B. Newgard, Diabetes, 2009, 58, 2429-2443.
3. M. Dreyer, P. Tarr, M. Shahgholi, M. Athanas, T. Second, 60th ASMS Conference on Mass Spectrometry and Allied Topics, May 20-24, 2012, Vancouver, Canada
4. K Suhre and P Schmidt, MassTrix: Mass Translator Into Pathways, Nucleic Acids Res, Vol. 36, Web Server Issue, W481-W484, 2008

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