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Robust Metabolomics Workflows Using a Modified Benchtop Orbitrap Mass Spectrometer

Mark Schroeder[1], Ioanna Ntai[1], Siegrun Mohring[2], Markus Kellmann[2], Alexander Harder[2], Ralf Tautenhahn[1], Amanda Souza[1], and Andreas Huhmer[1]
[1]Thermo Fisher Scientific, San Jose, CA, USA, [2]Thermo Fisher Scientific, Bremen, Germany

ABSTRACT

Purpose: To develop robust metabolomics workflows using the new Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer.

Methods: Chemical standards and complex biological matrices were analyzed with Thermo Scientific™ Vanquish™ UHPLC system and Orbitrap Exploris 240 MS. Data were processed using Thermo Scientific™ Compound Discoverer™ software and Thermo Scientific™ TraceFinder™ software.

Results: Robust reproducible measurements were achieved over multiple days with sub-ppm mass accuracy and signal response between 5 – 10% CV. Confident elemental compositions were generated from accurate mass measurements and high-resolution settings while the AcquireX intelligent acquisition workflow resulted in more unique compounds with fragmentation spectra, thus delivering high quality data and versatility for all metabolomics workflows.

INTRODUCTION

Metabolomics, the investigation of small molecules present in a biological system, finds application in diverse biomedical and industrial research. However, for metabolomics to provide valuable biological insights, robust reproducible measurements and confident metabolite identifications are required. Fast-scanning high resolution accurate mass spectrometers allow accurate mass assignments, resolving near mass isobaric species from complex mixtures, thus enabling confident compound identification and quantitation. Intelligent data acquisition algorithm, AcquireX, maximizes the number of metabolites interrogated by MS/MS, while minimizing the acquisition of fragmentation spectra from background and redundant signals. Here, we describe the development of robust metabolomics workflows utilizing an Orbitrap Exploris 240 MS for the quantitation and confident annotation of polar metabolites and lipids in diverse biological matrices.

MATERIALS AND METHODS

Sample Preparation

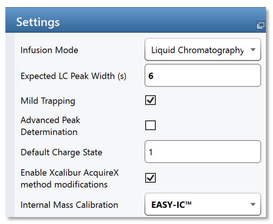
Twenty-five independent chemical standards were purchased from Millipore Sigma and suspended in water at a concentration of 1 ng/µL. Human plasma (NIST SRM 1950) was purchased from NIST. Metabolites were extracted by addition of methanol at a ratio of 3:1 (methanol:sample). After centrifugation, the supernatant containing the metabolites was evaporated. Dried metabolites were resuspended in water containing 0.1% formic acid.

Method

Two microliters of the resuspended metabolites were injected on a Thermo Scientific™ Hypersil GOLD™ column (15 cm × 2.1 mm ID, 1.9 µm particle size). The mobile phase consisted of solvent A (water with 0.1% formic acid) and solvent B (methanol with 0.1% formic acid). The flow rate was 0.3 ml/min. Instrumentation included a Vanquish UHPLC system, in-line with an Orbitrap Exploris 240 MS. LC and MS methods are illustrated in Figure 1.

Figure 1. LC-MS Method Parameters. A) general settings for MS. B) LC gradient. C) Ion source properties and full scan mode properties.

A)



B)

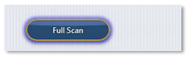
Time (min)	Flow [ml/min]	%B
-3.00	0.300	0.0
0.00	0.300	0.0
8.00	0.300	50.0
9.00	0.300	98.0
13.00	0.300	98.0
13.10	0.300	0.0
15.00	0.300	0.0

C)

Ion Source Properties	Orbitrap MS
Ion Source = H-ESI	**Method – 1 LC-MS**
Positive Ion = 3500V	MS R = 120K
Negative Ion = 3000V	AGC Target = 100%
Sheath Gas = 40	Max. Inj. Time = 100ms
Aux Gas = 8	**Method – 2 LC-MS/MS**
Sweep Gas = 1	MS R = 60K, MS/MS R = 30K
Ion Transfer Tube = 275ºC	Iso. Width = 1.5 Da
Vaporizer = 320ºC	AGC Target = 100%
RF Lens = 70%	Max. Inj. Time = 54ms
Method Time = 15min	Stepped HCD = 30, 50, 100%

Figure 2. Orbitrap Exploris 240 MS method setup in the acquisition software. A) full scan mode and B) data dependent MS/MS acquisition using the AcquireX Deep Scan workflow.

A)



B)

Data Analysis

TraceFinder software was used for the analysis of target metabolites while Compound Discoverer software provided untargeted analysis generating elemental composition, database searching and fragmentation matching.

RESULTS

Figure 3. The average mass accuracy of 25 neat standard compounds analyzed from three Orbitrap Exploris 240 instruments across five days of acquisition time. Accurate mass measurements with <1 ppm were observed in both polarities.

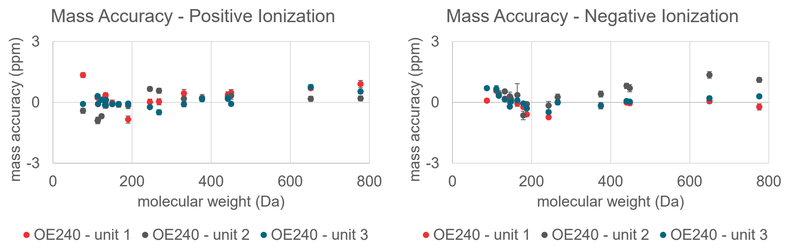


Figure 4. Signal response remains stable across five days of acquisition time for positive, negative, and polarity switching mode as evidenced by peak areas of standards with %CV between 5 – 10%. Data were collected using 120k resolution.

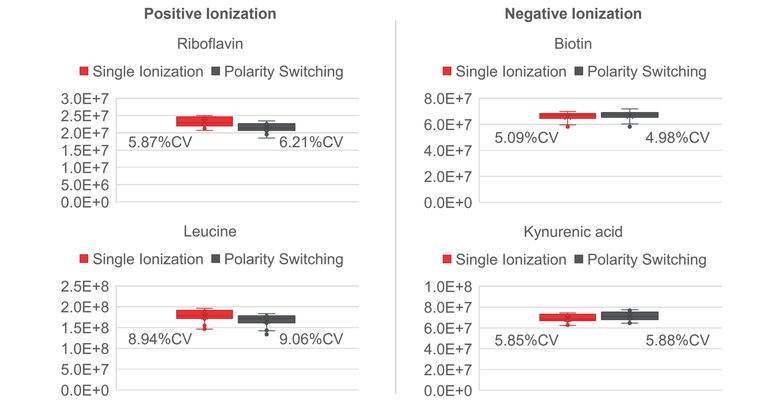
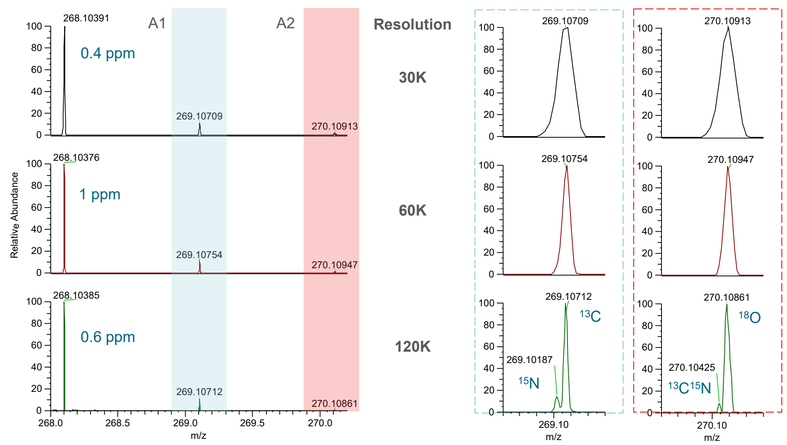


Figure 5. Detection of isotope pattern and fine structure in *E. coli* extracts are achieved with very high resolution. Combined with ≤1 ppm accurate mass measurements, confident elemental compositions are determined for the molecular species. Adenosine is putatively assigned with $[M+H]^+$ *m/z* 268.10385 and formula $C_{10}H_{13}N_5O_4$.



INTELLIGENT ACQUISITION FOR IMPROVED COVERAGE

Figure 6. AcquireX represents a new acquisition paradigm for greater MS/MS coverage. A) The AcquireX Deep Scan workflow was used in the analysis of SRM 1950 human plasma. B) When compared to traditional DDA, AcquireX resulted in more than 75% of compounds with MS/MS spectra through iterative injections. C) Subsequent iterative injections contributed to an overall increase in compounds with fragmentation spectra.

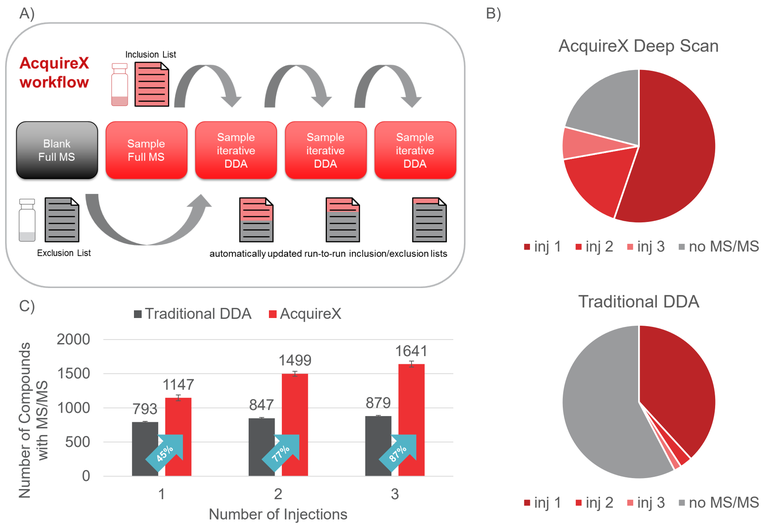
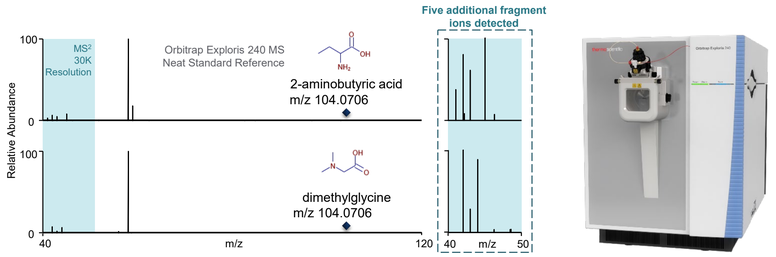


Figure 7. Fragmentation can be used to differentiate otherwise indistinguishable isomers, but mass range limitations limit the number of fragments detected particularly for smaller metabolites (<150 Da). Isomers 2-amiobutyric acid and dimethylglycine were differentiated by five additional MS/MS fragments with an extended mass range of 10 Da using the Orbitrap Exploris 240 MS (right).



CONCLUSIONS

Orbitrap Exploris 240 MS is a robust benchtop MS for metabolomics workflows as demonstrated by:

- Robust instrument performance with stable mass accuracy and signal response needed for data collection across multiple days for metabolomic data sets.
- Confident prediction of elemental compositions were generated with detection of isotope pattern and fine structure using higher resolution settings.
- The AcquireX Deep Scan workflow generated MS/MS spectra for more unique metabolites resulting in improved metabolome annotation.

TRADEMARKS/LICENSING

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