proteomics

thermoscientific

Proof of performance

Orbitrap Exploris 240 mass spectrometer

Versatility–one system with leading performance for proteomics and metabolomics

Summary

The Thermo Scientific™ Orbitrap Exploris™ 240 MS delivers leading performance across a broad range of qualitative and quantitative applications with Orbitrap data quality and optimized methods for your life science research applications. Maximizing productivity and efficiency for your research and clients is more important than ever. Projects may include both proteomics and metabolomics. Leading performance in terms of sub-ppm mass accuracy and higher resolution benefits both applications as well as ease-of use with optimized methods.

This document describes the flexibility of the Orbitrap Exploris 240 mass spectrometer for metabolomics applications and how the core values of leading performance, versatility and ease of use are applied.

Best-in-class system for proteomics and metabolomics: When the highest quality data and the versatility for both protein and small molecule analysis is key to your research goals, you can rely on the Orbitrap Exploris 240 MS to deliver high quality reproducible results. For metabolomics, obtain valuable quantitative insights from small to large scale studies across a variety of applications from targeted, semi-targeted to untargeted metabolomics studies. Unique Thermo Scientific™ AcquireX™ intelligent data acquisition workflow produces more meaningful data, delivering the results you need for publications and actionable outcomes.

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Versatile metabolomics workflows

Obtain valuable insights from targeted, semi-targeted, untargeted and stable isotope labeling workflows (Figure 1), using a single mass spectrometer.

Figure 1. The Orbitrap Exploris 240 MS is perfectly suited for all metabolomics workflows – The Orbitrap Exploris 240 mass spectrometer utilizes high resolution, accurate mass (HRAM) to deliver high quality data for all metabolomics applications (targeted, semi-targeted, untargeted and stable isotope labeling).

To demonstrate the versatility of Orbitrap Exploris 240 MS and its benefits across all metabolomics workflows, we utilized a combination of neat standards and complex matrices.

Experimental conditions

Sample

- Neat standards obtained from Sigma Aldrich, including various amino acids and vitamins
- Cambridge Isotope Laboratories Credentialed E. Coli Cell Extract Kit
- NIST SRM1950, Metabolites in human plasma

LC method

- Thermo Scientific™ Hypersil GOLD™ column (15 cm × 2.1 mm ID, 1.9 µm particle size, CAT # 25002-152130)
- Flow rate 0.3 mL/min
- Mobile phase A: Water + 0.1% formic acid, Mobile phase B: Methanol + 0.1% formic acid

Time (min)	%B
-3	0
0	0
8	50
9	98
13	98
13.1	0
15	0

Instrumentation

- Thermo Scientific™ Vanquish™ UHPLC system
- Thermo Scientific™ OptaMax™ NG ion source
- Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer
- AcquireX intelligent data acquisition workflow

Software

- Thermo Scientific™ TraceFinder™ software
- Thermo Scientific™ Compound Discoverer™ software

Leading performance for metabolomics

Sub-ppm mass accuracy and very high resolution

HRAM Orbitrap technology allows accurate mass assignments, resolving near mass isobaric species from complex mixtures, thus enabling confident compound identification and quantitation. To demonstrate the excellent mass accuracy afforded by the Orbitrap Exploris 240 MS, we analyzed a mixture of 25 neat standards, including amino acids and vitamins, in positive and negative mode repeatedly across 5 days of acquisition (Figure 2). We repeated this experiment on 3 different units. The Thermo Scientific™ EASY-IC™ ion source was enabled during these measurements, resulting in sub-ppm mass measurements for all compounds independent of molecular weight or polarity.

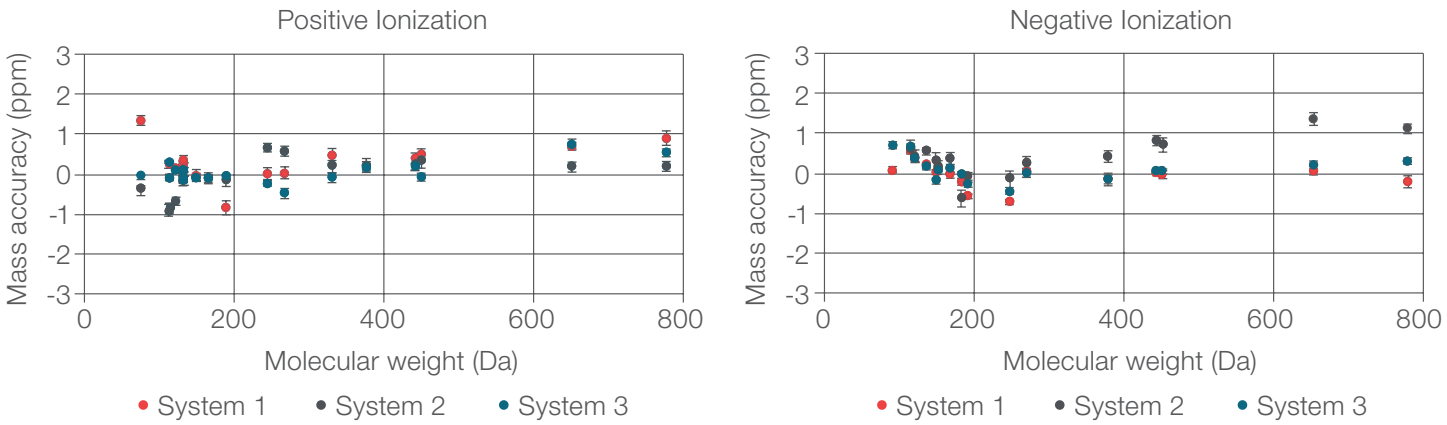


Figure 2. The Orbitrap Exploris 240 MS delivers unsurpassed mass accuracy for all small molecules. A mixture of 25 neat standards ranging in molecular weight from 75 Da to 777 Da, was analyzed repeatedly across 5 days. All standards were measured with excellent mass accuracy, often <1 ppm for the entirety of the experiment. The EASY-IC source was enabled during these measurements.

High resolution reveals fine isotope pattern (Figure 3) and the presence of heteroatoms in an unknown molecule, facilitating elemental formula prediction.

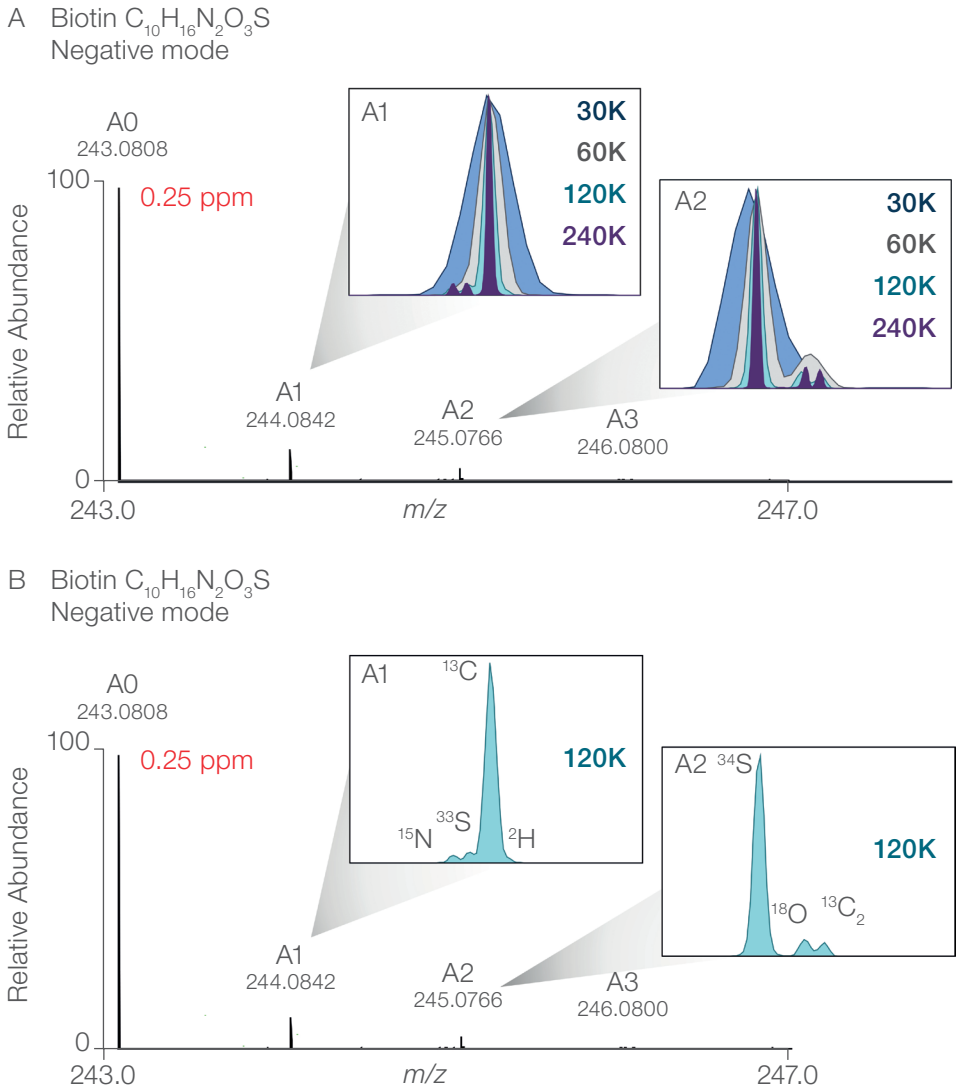


Figure 3. High resolution for fine isotope pattern determination is illustrated in the mass spectrum of biotin (negative mode). (A) Low resolution settings, such as 60K or lower, typically found on quadrupole time-of-flight (QTOF) analyzers, prevent detection of isotopologues in the A2 and A3 clusters. With 240K resolution of the Orbitrap Exploris 240 MS, fine isotope pattern emerges for the A1 and A2 isotope clusters (B) Even at 120K resolution, it is possible to distinguish the isotopologues due to heteroatoms, clearly supporting that this molecule contains N, S and O in addition to C and H.

The combination of high mass accuracy and resolution enabled by Thermo Scientific™ Orbitrap™ technology provides fine isotope structure in the mass spectrum of unknown compounds, narrows down elemental formulas and fast-tracks confident elemental composition determination, which is the first step in unknown identification (Figure 4).

Extended low mass range

Confident annotations require high resolution accurate mass measurements for elemental composition determination and high-quality fragmentation spectra for spectral matching against a library. With an extended low mass range down to *m/z* 40, the confident identification of small molecules is improved, Figure 5.

Prediction by Mass Alone

m/z 252.10930

Mass tolerance (ppm)	# of candidate formulas
30	29
20	18
10	8
5	5
3	3
1	1

Elements C, H, N, O, S, Cl, P

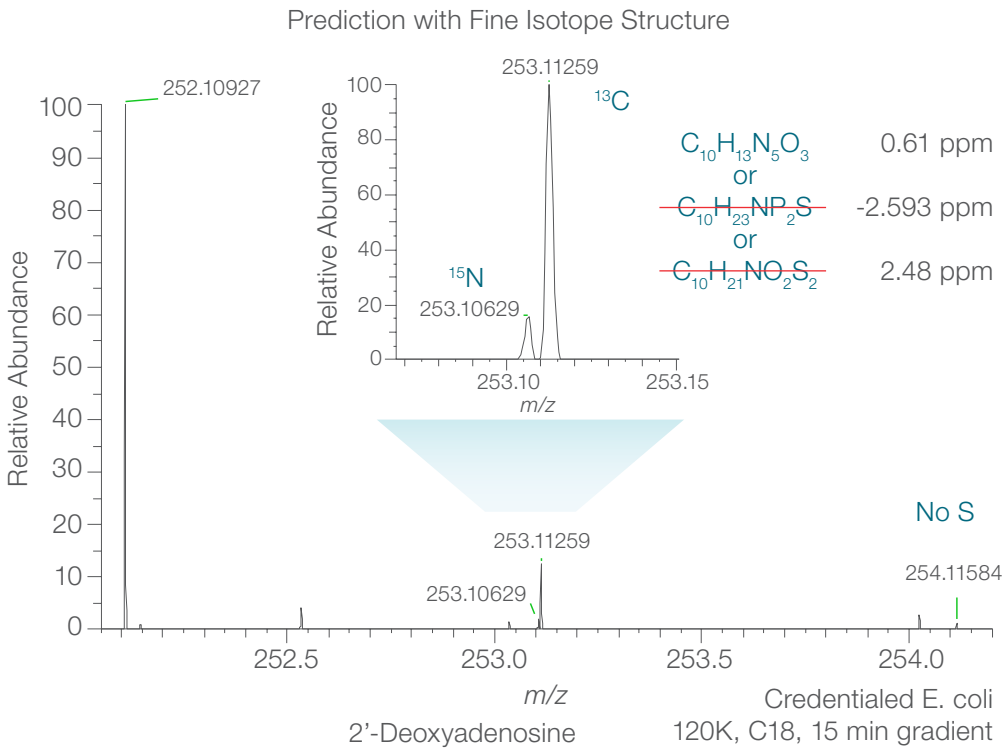


Figure 4. High Resolution and mass accuracy narrow down the candidate chemical formulas for this unknown compound expediting its putative annotation as 2-deoxyadenosine.

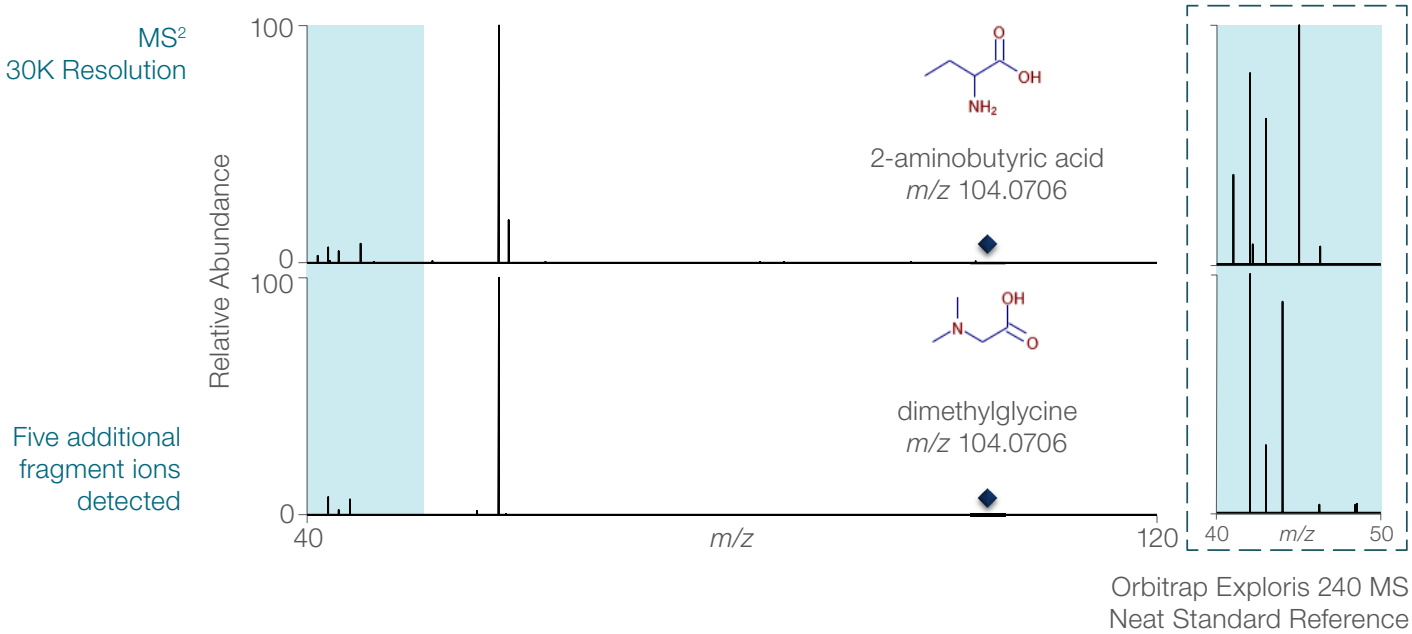
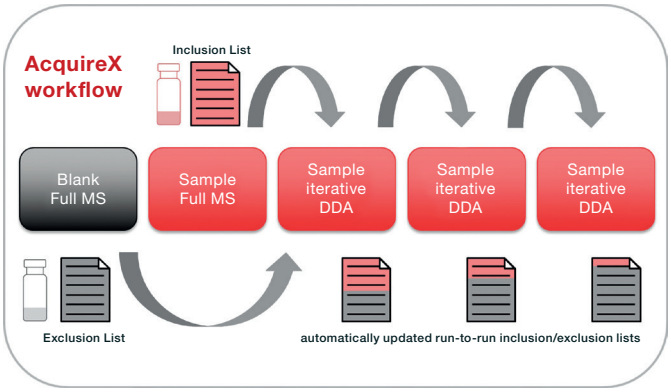


Figure 5. Extended low *m/z* range enables confident identification of isomers. Additional fragment ions are detected, and thus structural information, generated from this *m/z* range extension. In the case of 2-aminobutyric acid and dimethylglycine, there are 5 additional fragment ions detected that allow the confident differentiation of these 2 isomers.

Built in intelligent acquisition strategies

Small-molecule analysis demands reliable acquisition of high-quality MS and MS^2 data to facilitate successful compound identification and characterization. The recently developed AcquireX data acquisition algorithm can determine on-the-fly the features corresponding to background contaminants and compound degeneracy, such as isotopes, adducts and dimers, enabling efficient MS/MS sampling of unique biologically relevant metabolites (Figure 6).

A



B

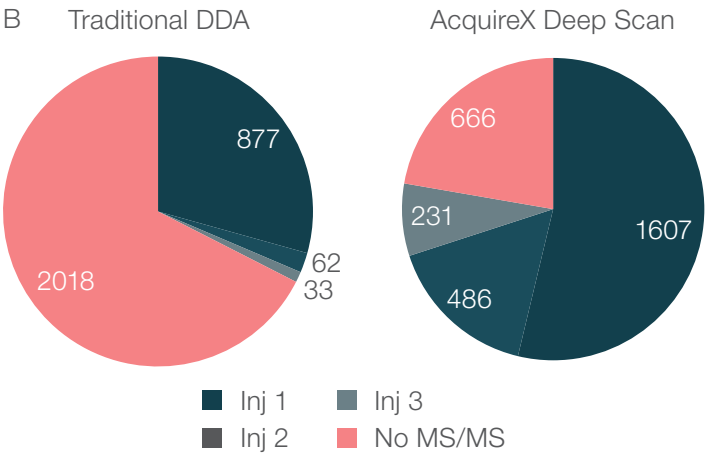


Figure 6. AcquireX workflow represents a new acquisition paradigm. (A) First, an exclusion list is generated from a blank run. Then, an injection of the sample followed by feature detection and component assembly populates the inclusion list with compounds detected in the sample. A series of iterative DDA injections follow. Each injection is informed from the previous one, minimizing redundant fragmentation spectra and maximizing relevant spectra and metabolite annotations. (B) AcquireX intelligent data acquisition significantly increased the number of compounds with fragmentation data in human plasma (NIST SRM1950) over traditional DDA after three injections. The circle represents the total number of compounds detected in human plasma and in pink is the percentage of compounds without corresponding MS/MS spectra.

Results

- The Orbitrap Exploris 240 mass spectrometer offers robust performance with high resolution up to 240,000 and unmatched mass accuracy, making it the ideal mass spectrometer for all metabolomics applications.
- Internal calibration, provided by the inclusion of the EASY-IC ion source, enables stable scan-to-scan and run-to-run mass accuracy at sub-ppm levels for multiple days.
- Extended low mass range down to *m/z* 40 enables the detection of additional fragment ions resulting in the confident differentiation of isomers.
- AcquireX workflow deep scan delivers improved MS/MS sampling by automatically excluding background ions and focusing acquisition on true sample components. This data-informed workflow minimizes irrelevant spectra and provides deeper metabolome coverage.

Outlook

The Orbitrap Exploris 240 MS redefines versatility for research and core labs: expanding capabilities to include both proteomics and metabolomics on one system, all with expectational data quality and robustness that leads to publishable results and actionable outcomes.

Conclusion

The Orbitrap Exploris 240 MS with leading high resolution, accurate mass performance is the ideal system for metabolomics, delivering high confidence results that empower your research day after day. With unique built-in intelligence, such as the AcquireX data acquisition strategy, the Orbitrap Exploris 240 MS delivers reliable acquisition of high-quality MS and MS^2 data to facilitate successful compound identification and characterization.

Find out more at
thermofisher.com/OrbitrapExploris240Proof

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