SQUAD metabolomics

Extending dynamic range and enabling simultaneous quantitation and discovery in metabolomics using eDR

Alexander Harder[2], Bashar Amer[1], Michal Kaczmarek[2], Claire Dauly[2], Rahul Ravi Deshpande[1], Thomas Moehring[2], and Susan S. Bird[1]

[1]Thermo Fisher Scientific, San Jose, California, United States
[2]Thermo Fisher Scientific, Bremen, Germany

Abstract

Purpose: Metabolomics analyses are often limited by the broad concentration range of metabolites in biological samples, making it challenging to detect low-abundance compounds while maintaining quantitative performance. This study evaluates enhanced Dynamic Range (eDR) acquisition on the Thermo Scientific™ Orbitrap™ Excedion™ Hybrid Mass Spectrometer for simultaneous untargeted and quantitative metabolomics.

Methods: Plasma samples from a diet-induced obesity (DIO) mouse study and NIST SRM1950 plasma were analyzed using conventional full-scan and eDR acquisition. Metabolite detection, MS/MS acquisition, annotation, biological differentiation, and quantitative performance were assessed using untargeted and SQUAD workflows.

Results: eDR extended the effective MS1 dynamic range from ~5 to ~6 orders of magnitude, improving detection of low-abundance metabolites. In the DIO study, eDR detected over 16,000 compounds and generated more than 6,000 MS/MS spectra, resulting in increased metabolite annotations and high-confidence library matches. Enhanced metabolome coverage improved biological group separation and enabled detection of differential metabolites not observed with conventional acquisition. Quantitative analysis demonstrated a 10-fold improvement in the lower limit of quantitation while maintaining a six-order dynamic range.

Introduction

Comprehensive metabolomics requires the measurement of metabolites spanning several orders of magnitude in concentration. However, highly abundant ions can suppress signals from low-abundance species, limiting metabolite coverage and reducing confidence in biological interpretation. Traditional workflows often require separate experiments to balance quantitative accuracy and metabolome discovery.

The Orbitrap Excedion MS incorporates eDR acquisition to improve recovery of low-abundance metabolites while preserving the analytical performance required for quantitative measurements. This study investigates the impact of eDR on metabolite detection, biological interpretation, and quantitative sensitivity in metabolomics applications.

Figure 1. Orbitrap Excedion Mass Spectrometer.

Materials and methods

Samples

Diet-induced obesity (DIO) mouse plasma samples and NIST SRM1950 human plasma were analyzed.

LC-MS analysis

Instrument: Thermo Scientific Orbitrap Excedion Mass Spectrometer. Data were acquired using both conventional full MS1 and eDR acquisition modes.

eDR acquisition

eDR divides the MS1 mass range into multiple overlapping sub-scans. Sub-scans are automatically combined into a single reconstructed MS1 spectrum with extended dynamic range (Figure 2).

Data analysis

Data processing and metabolite annotation were performed using Thermo Scientific™ TraceFinder™ Software and Thermo Scientific™ Compound Discoverer™ Software.

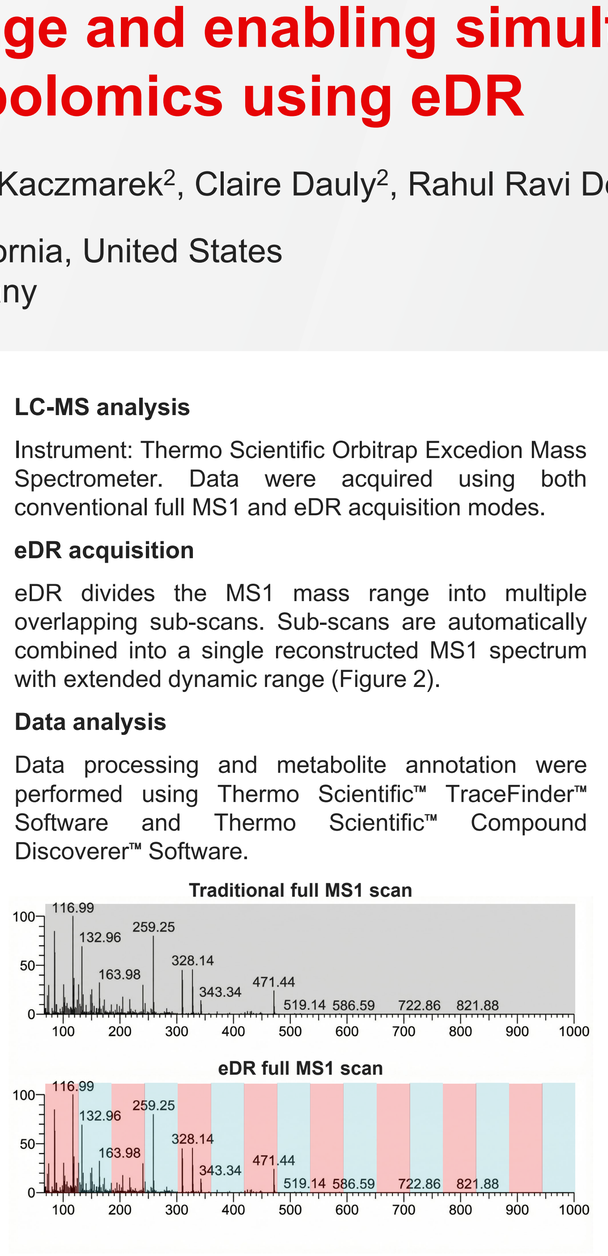

Figure 2. Traditional full MS1 scan vs. eDR full MS1 scan, which is based on two sub-scans.

Results

eDR extends dynamic range and improves metabolite detection

eDR acquisition increased the effective MS1 dynamic range from approximately 5 to 6 orders of magnitude, improving recovery of low-abundance metabolites in complex biological samples (Figure 3). Compared to traditional full-scan acquisition, eDR enabled detection of substantially more metabolites across the concentration range.

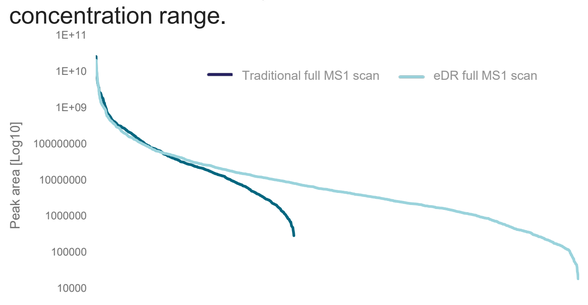

Figure 3. eDR extends MS1 dynamic range. eDR acquisition improves detection of low-abundance metabolites by extending the effective dynamic range from ~5 to ~6 orders of magnitude.

eDR expands metabolome coverage

In the DIO mouse study, eDR detected more than 16,000 compounds and acquired over 6,000 MS/MS spectra, representing a greater than five-fold increase in detectable features compared to conventional full-scan acquisition. The increased coverage resulted in more metabolite annotations and high-confidence mzCloud matches (Figure 4).

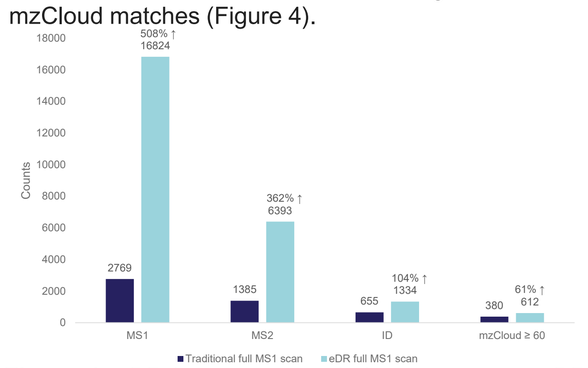

Figure 4. eDR increases metabolome coverage. eDR detected >5× more compounds and generated more MS/MS spectra, resulting in increased metabolite annotations and library matches.

Improved biological interpretation

The deeper metabolome coverage provided by eDR improved separation of biological groups in multivariate analyses (Figure 5) and enabled detection of differential metabolites that were not observed using traditional acquisition methods. Enhanced sensitivity revealed biologically relevant metabolic differences associated with diet and sex (Figure 6).

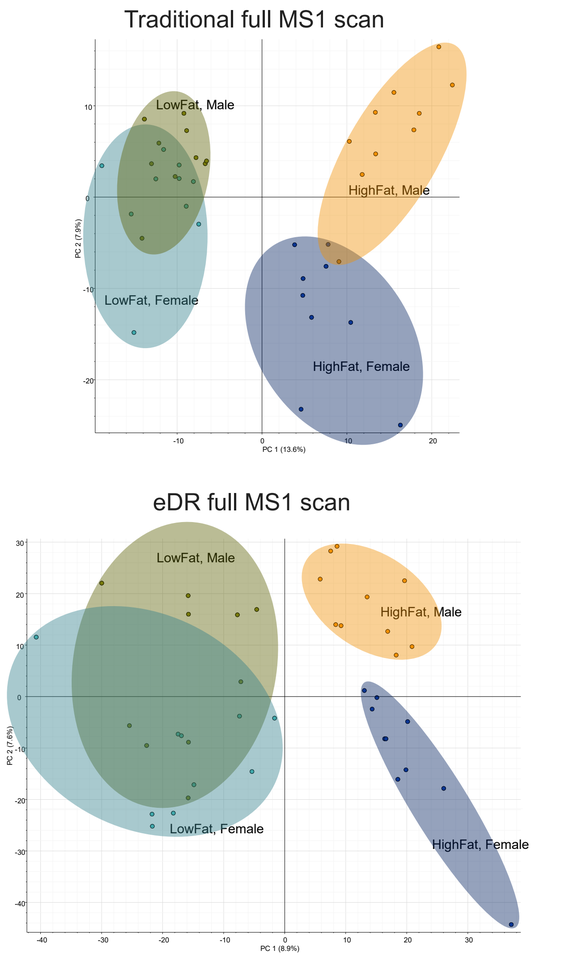

Figure 5. eDR improves biological separation. PCA analysis shows improved separation of diet- and sex-specific metabolic phenotypes using eDR acquisition.

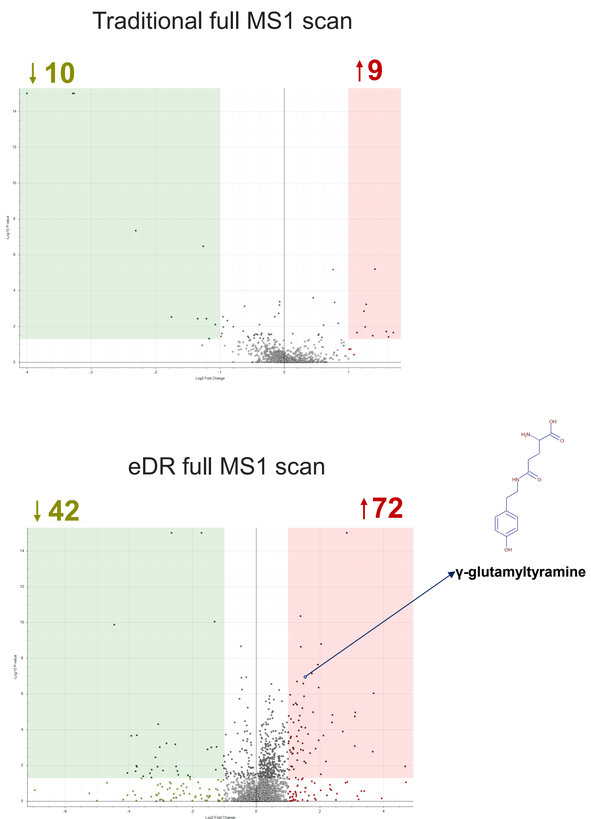

Figure 6. eDR enhances detection of differential metabolites. eDR acquisition identified substantially more significantly changing metabolites between biological groups, including γ-glutamyltyramine, which was not detected using traditional full MS1 acquisition.

Simultaneous quantitation and discovery

Combining eDR with the SQUAD workflow enabled quantitative and untargeted metabolomics analysis from a single injection. Quantitative experiments demonstrated a ten-fold improvement in lower limit of quantitation (LLOQ) for isotope-labeled phenylalanine while maintaining a six-order linear dynamic range (Figure 7).

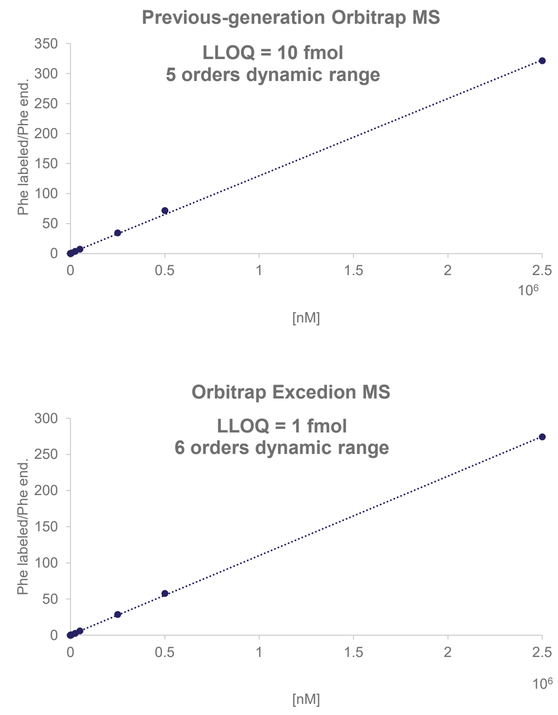

Figure 7. eDR enables simultaneous quantitation and discovery. The SQUAD workflow achieved a 10× lower LLOQ while maintaining a six-order quantitative dynamic range.

Conclusions

- eDR acquisition on the Orbitrap Excedion MS extends effective MS1 dynamic range and improves detection of low-abundance metabolites.
- eDR significantly increases metabolome coverage, MS/MS acquisition, metabolite annotation, and high-confidence spectral library matches.
- Improved metabolite recovery enhances biological interpretation by enabling stronger group separation and identification of differential metabolites.
- Combined with the SQUAD workflow, eDR supports simultaneous quantitative and untargeted metabolomics in a single injection, delivering both deeper coverage and improved quantitative sensitivity.

Trademarks/licensing

Learn more at thermofisher.com/orbitrapexcedion