

Expanding plasma metabolome coverage with enhanced dynamic range and iterative MS/MS acquisition on Orbitrap Excedion mass spectrometer

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Abstract

Purpose: Untargeted plasma metabolomics is challenged by the broad concentration range of plasma metabolites, which can bias traditional full-scan acquisition toward highly abundant ions. This work evaluated enhanced dynamic range (eDR) acquisition combined with AcquireX iterative DDA for improving plasma metabolome coverage and Thermo Scientific™ mzCloud™ Mass Spectral Library-based annotation in NIST SRM 1950.

Methods: SRM 1950 plasma extracts were analyzed using a Thermo Scientific™ Vanquish™ Horizon UHPLC System on a Thermo Scientific™ Hypersil GOLD™ VANQUISH™ C18 UHPLC Column coupled to Thermo Scientific™ Orbitrap™ Excedion™ Mass Spectrometer with matched eDR and conventional full-scan MS1 acquisition. Iterative AcquireX Deep Scan was used for DDA MS/MS acquisition, and the effects of plasma-equivalent load and MS2 isolation width on quality-filtered MS1 compounds and strict mzCloud Mass Spectral Library hits were evaluated.

Results: eDR increased quality-filtered compound entities and strictly filtered mzCloud Mass Spectral Library MS/MS matches across all tested plasma-equivalent loads. Representative cortisone traces showed that eDR redistributed ion injection time (IT) toward lower-abundance ions, increasing signal-to-noise, while narrower MS2 isolation improved MS2 purity for small molecules.

Introduction

Untargeted LC-HRAM-MS plasma metabolomics is challenged by the extreme dynamic range of plasma, where highly abundant ions can dominate ion accumulation and limit detection and fragmentation of lower-abundance metabolites. As a result, conventional full-scan acquisition may undersample biologically relevant low-intensity ions and reduce downstream MS/MS-based annotation depth. eDR acquisition on the Orbitrap Excedion Mass Spectrometer is designed to address this limitation through intelligent redistribution of ion injection times across segmented m/z windows during full-scan acquisition. In eDR mode, the mass range is divided into multiple windows with optimized ion accumulation across two Orbitrap subs cans, improving signal-to-noise for low- and medium-abundance ions while maintaining broad scan-range coverage. In this study, eDR-enabled full-scan acquisition and AcquireX iterative DDA were evaluated using NIST SRM 1950 plasma extracts to assess the effects of plasma-equivalent load, ion injection time redistribution, and MS2 isolation width on quality-filtered MS1 compounds and strict mzCloud Mass Spectral Library matches.

Materials and methods

NIST SRM 1950 plasma extracts were prepared by methanol protein precipitation followed by centrifugation, evaporation, and reconstitution prior to LC-MS analysis. Multiple extract dilution and injection-volume conditions were normalized to plasma-equivalent volume loaded on column. Samples were analyzed using a Vanquish Horizon UHPLC System with Hypersil GOLD VANQUISH C18 UHPLC Column (150 × 2.1 mm, 1.9 μm) coupled to an Orbitrap Excedion Mass Spectrometer. Full-scan MS1 data (m/z 67–1000, 120k resolution) were acquired with eDR enabled and disabled under matched conditions in positive ionisation, with eDR acquisition segmenting the MS1 scan range into 16 windows. AcquireX-assisted iterative Deep Scan DDA was used for ddMS2 acquisition with stepped normalised HCD collision energies of 10, 35, and 60% and MS2 isolation widths of 0,4–2,0 Da. Representative AcquireX sequences included blank-derived exclusion lists, one MS1 reference injection with two MS1 replicate injections at the end of the sequence, and 6 or 7 iterative ID injections for plasma-load and isolation-width studies, respectively. Raw data were processed using Thermo Scientific™ Compound Discoverer™ 3.5 Software and Thermo Scientific™ FreeStyle™ 1.8 SP2 software.

Results

eDR increases quality-filtered plasma coverage across the loading range, with the largest advantage at higher plasma-equivalent load

To evaluate whether enhanced dynamic range acquisition increases usable plasma coverage under different sample-load conditions, SRM 1950 plasma extracts were analyzed at three representative plasma-equivalent loads using matched eDR and conventional full-scan acquisition (without eDR). Extract dilution and injection volume were normalized to plasma-equivalent volume loaded on column. To avoid reporting raw feature inflation, results were evaluated using stringent MS1 and MS2 quality filters.

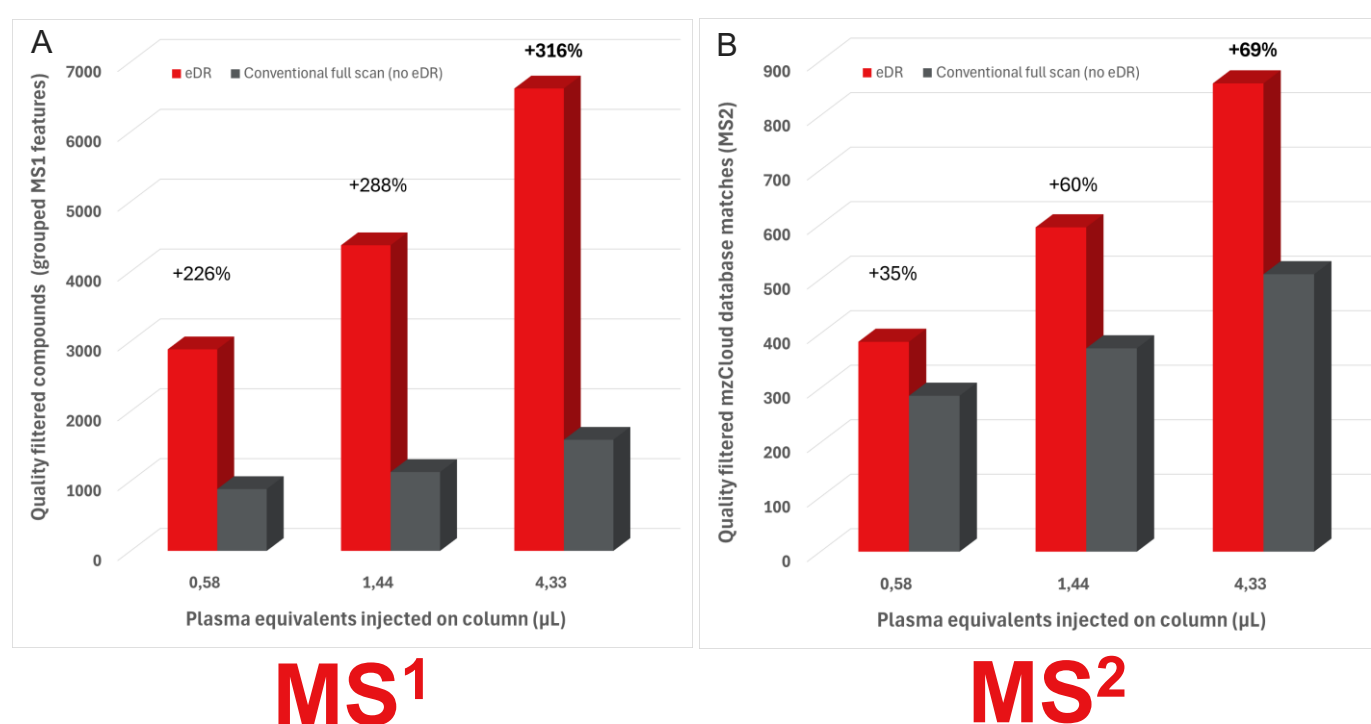


Figure 1. eDR increases plasma coverage across representative plasma-equivalent loads. SRM 1950 plasma extracts were analyzed employing AcquireX deep scan workflow at 0,58, 1,44, and 4,33 μL plasma equivalent loaded on column using matched eDR and conventional full-scan acquisition. (A) Quality-filtered MS1 compound entities with filters: CV <20%, formula assignment, and peak rating >6 in all three MS1 reference replicates. (B) Strict mzCloud MS/MS matches with filters: CV <20%, non-blank formula assignment, peak rating ≥6 in 3/3 MS1 reference replicates, best mzCloud Mass Spectral Library match score ≥60, and MS2 spectral purity >60.

eDR increased quality-filtered MS1 compound entities at every plasma-equivalent load tested. At the highest load tested, eDR produced 6,619 quality-filtered MS1 compound entities compared with 1,591 using conventional full scan, corresponding to a +316% increase. The eDR advantage also carried through to strict MS2-level outputs. At the highest load tested, eDR produced 859 strict mzCloud MS/MS matches compared with 509 using conventional full scan, corresponding to a +69% increase. eDR provided a consistent advantage at all tested plasma-equivalent loads, with the largest absolute gain and the largest relative gain observed at the highest load tested. The load-response behavior is consistent with improved, more balanced handling of higher ion loads under eDR.

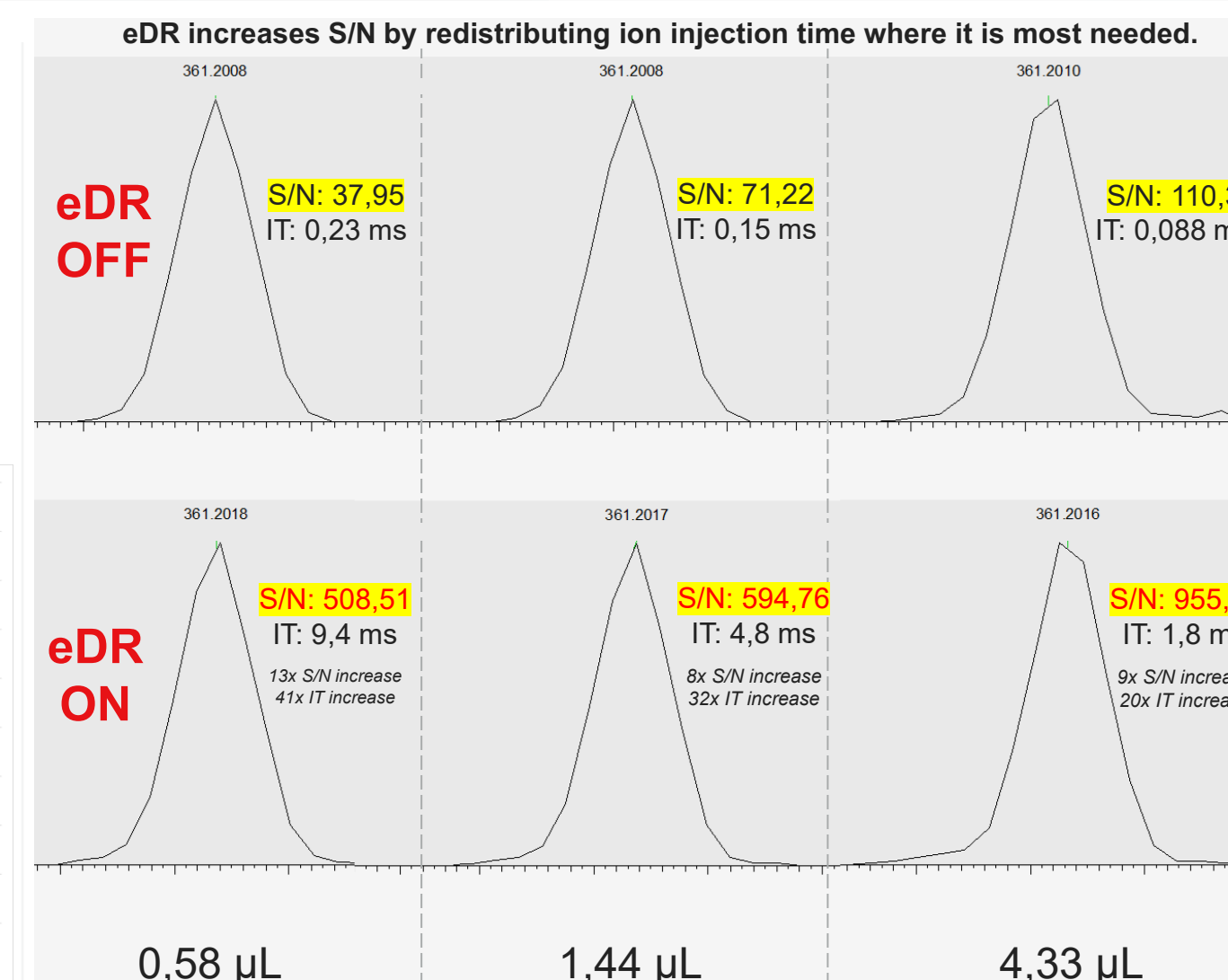


Figure 2. eDR improves cortisone MS peak S/N through ion injection-time redistribution.

Cortisone (361.201 m/z), a strict mzCloud Mass Spectral Library/MS2-filtered annotation, illustrates the mechanism behind the global eDR gain. eDR increased MS peak signal-to-noise ratio (S/N) at all loads by increasing ion injection time relative to conventional full scan. The largest IT gain occurred at the lowest load, where cortisone was least abundant. At higher loads, less IT was needed for registering satisfactory signal of cortisone, which is consistent with redistribution of acquisition time toward lower-abundance ions.

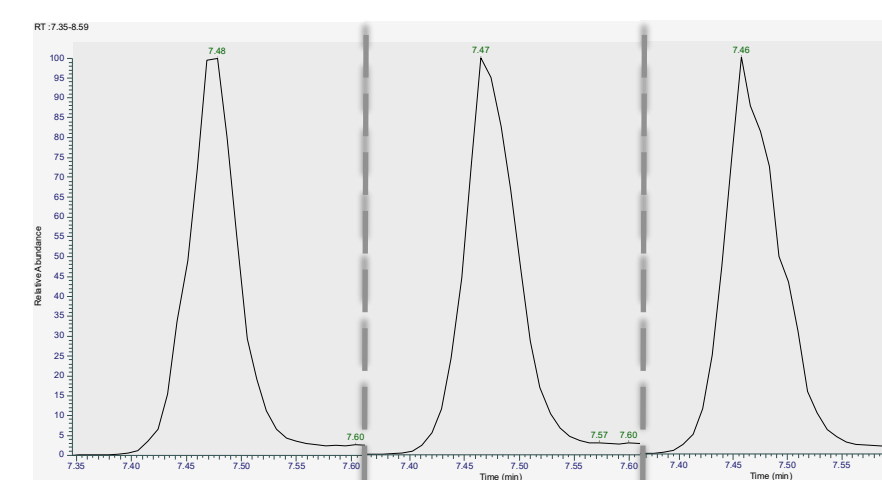


Figure 3. Extracted chromatograms of phenylalanine for three plasma-equivalent loads.

Phenylalanine was used to assess chromatographic performance at increasing plasma load. Across 0,58–4,33 μL plasma equivalent, peak shape and retention time remained stable, indicating no major chromatographic distortion at the highest load. This supports that eDR converts higher plasma load into additional usable signal without saturating the mass spectrometer, while the practical upper limit is still governed by chromatographic quality.

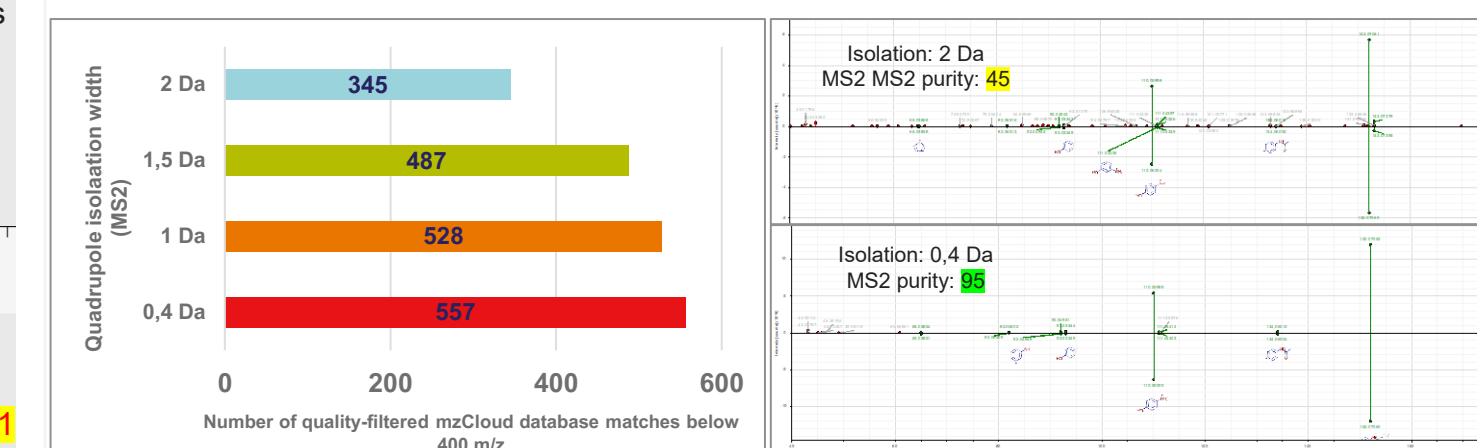


Figure 4. Narrower MS2 isolation width increases quality-filtered mzCloud Mass Spectral Library matches below 400 m/z. Filters: CV <20%, non-blank formula assignment, peak rating ≥6 in 3/3 MS1 reference replicates, best mzCloud match score ≥60, and MS2 purity >60.

Figure 5. Improved precursor selectivity of paracetamol at 0,4 Da isolation increased MS2 spectral purity score from 45 to 95, resulting in cleaner MS/MS spectra.

In order to maximize the benefits of eDR we optimized the method further. Reducing the MS2 isolation width increased the number of quality-filtered mzCloud matches under identical AcquireX conditions. Consistent with the quadrupole transmission specification for this optimization, MS2 match comparison was restricted to precursor ions with m/z below 400. Narrower isolation improved precursor selectivity and reduced co-isolation, resulting in cleaner MS/MS spectra and higher-quality library matches. The spectral example in Figure 5 illustrates the corresponding increase in MS2 purity.

Conclusions

eDR substantially increased quality-filtered plasma coverage and strict mzCloud Mass Spectral Library MS/MS matches compared with conventional full-scan acquisition. The results are consistent with redistribution of ion injection time toward lower-abundance ions, while optimized MS2 isolation further improved precursor selectivity and mzCloud annotation quality.

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