Intelligent Acquisition for Comprehensive Metabolome Coverage in Plants, Mammals, and Bacteria

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ABSTRACT

Purpose: Demonstration of an intelligent data acquisition workflow used for comprehensive coverage regardless of sample type and complexity using real-time data reduction.

Methods: Multiple types of samples with varying complexity (human plasma, urine, *E. coli*, yeast and green tea extract) were analyzed with a Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer using Thermo Scientific™ AcquireX intelligent data acquisition software. Data were evaluated using Thermo Scientific™ Compound Discoverer™ software with the mzCloud™ spectral library.

Results: By excluding background and degenerate signals, the total number of fragmentation targets was reduced without compromising metabolite coverage thus allowing for acquisition to focus on biologically relevant compounds and demonstrably increase the depth of MS/MS coverage.

INTRODUCTION

Unknown identification remains a challenge in untargeted metabolomics. In LC/MS based metabolomics, thousands of features from a single sample are routinely detected. However, not all features represent metabolites of biological origin and fragmentation spectra of background ions and degenerate signals detract from metabolite identification efforts. Data-dependent acquisition (DDA) often provides information for the most abundant ions. Data-independent acquisition can obtain fragmentation data for all features, but results in convoluted MS/MS spectra that complicate identification. Recently developed AcquireX intelligent data acquisition software minimizes fragmentation spectra from background and redundant signals. Using the AcquireX acquisition strategy for several samples, including plant, mammals, and bacteria, delivered a more efficient workflow to deeper metabolome coverage and increased metabolite annotation enhancing biological knowledge.

MATERIALS AND METHODS

Sample Preparation

Standard Reference Materials SRM1950 (human plasma), SRM3673 (non-smoker's urine), and SRM3225 (green tea extract) were purchased from NIST. Yeast and *E. coli* cell extracts were purchased from Cambridge Isotope Laboratories, Inc. Metabolites were extracted with methanol at a ratio of 3:1 (methanol:sample). Extracted metabolites were centrifuged and metabolite-containing supernatants were evaporated. Dried samples were resuspended in water containing 0.1% formic acid.

Instrumentation

Two microliters of resuspended metabolites were injected on a Thermo Scientific™ Hypersil GOLD™ column (15cm x 2.1mm ID). Chromatographic separation was achieved on a Thermo Scientific™ Vanquish™ UHPLC system in-line with an Orbitrap ID-X Tribrid mass spectrometer using AcquireX intelligent data acquisition software.

Data Analysis

For metabolite identification, traditional DDA was compared to the intelligent acquisition approach, AcquireX using Compound Discoverer software.

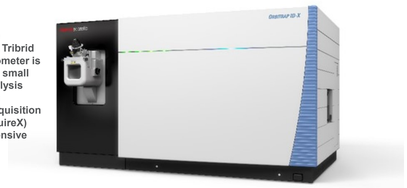


Figure 1. The Orbitrap ID-X Tribrid mass spectrometer is optimized for small molecule analysis and offers an intelligent acquisition method (AcquireX) for comprehensive metabolome coverage.

RESULTS

Background Exclusion and Its Implications Towards Relevant Compound Fragmentation

In LC/MS based untargeted metabolomics experiments, the detection of thousands of features in a single sample is routinely accomplished. However, this should not be equated to "global" metabolome coverage, as only a small percentage of those analytes are of biological origin. Recently developed AcquireX acquisition software can determine on-the-fly features corresponding to background contaminants and compound degeneracy, such as isotopes, adducts, and dimers, enabling efficient MS/MS and MS^n sampling of unique biologically relevant metabolites (figure 2). Unlike traditional DDA, during which fragmentation of background ions dominate the duty cycle, the AcquireX DeepScan workflow selects precursors intelligently by excluding background ions and targeting unique metabolites of biological relevance for fragmentation (figure 3).

Figure 2. Sampling of varying complexity of sample types by excluding background ions and degenerate signals, allows for reduction of the total number of fragmentation targets without losing metabolite coverage.

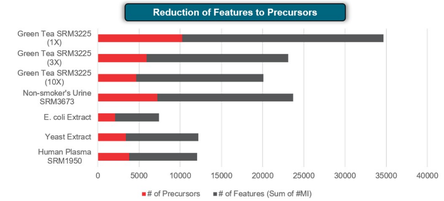
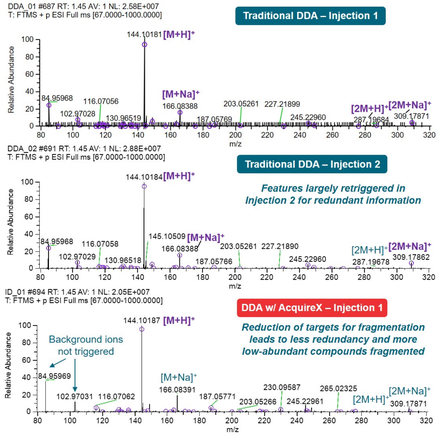


Figure 3. The AcquireX DeepScan acquisition excludes background ions and redundant features. For example, for Stachyrdine ([M+H]+ *m/z* 144.1018) only the [M+H]$^+$ feature is fragmented, with other adducts and isotopes omitted (purple circle denotes features triggered for MS2 within ±6 sec of the MS1 spectrum shown).



CONFIDENCE WITH COMPOUND FRAGMENTATION

Improved Spectral Matches with MS/MS Sampling

During electrospray ionization of small molecules, different types of adducts and clusters can form creating an abundant parent ion or additional features such as isotopes and adducts. Because of this, fragmentation of low abundant metabolites might be passed over during acquisition. By using inclusion lists for preferred ions, more compounds can be sampled for MS/MS analysis (figure 5). With the real-time generation of inclusion and exclusion lists with AcquireX DeepScan workflow, compounds of low abundance will have opportunity to be sampled in subsequent injections thus providing more compound annotation (figure 6).

Figure 4. Using the AcquireX DeepScan workflow, three re-injections were acquired for human plasma (SRM1950), while six re-injections for *E. coli* extract, yeast extract, non-smoker's urine (SRM3673) and green tea extract (SRM3225) were attained. Each re-injection shows an improvement of the number of compounds with MS/MS fragmentation spectra for [M+H]+ by excluding background ions and focusing on achieving acquisition of real sample components. (*note: only four reinjections are displayed due to diminished improvements beyond fourth injection).*

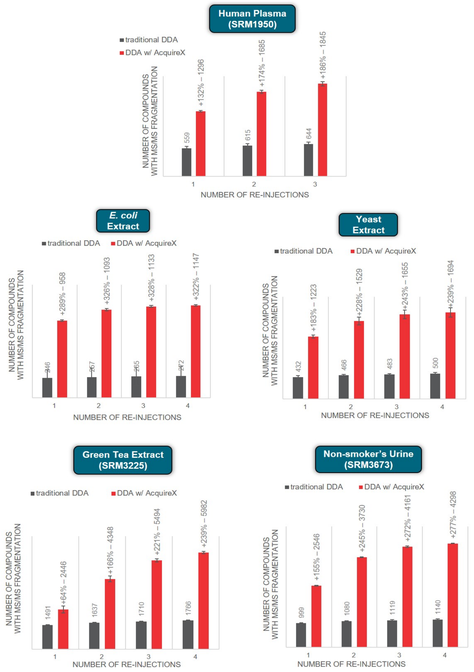


Figure 5. As exemplified on the non-smoker's urine sample, the comparison of traditional DDA and AcquireX DeepScan shows increased depth of MS/MS fragmentation coverage with the AcquireX acquisitions, as illustrated after four injections (Compounds triggered for MS/MS in red).

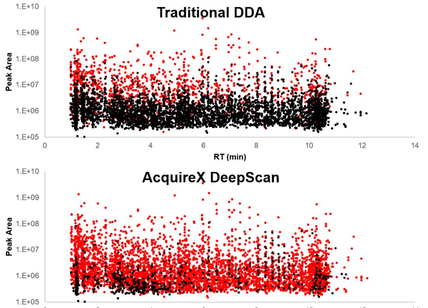
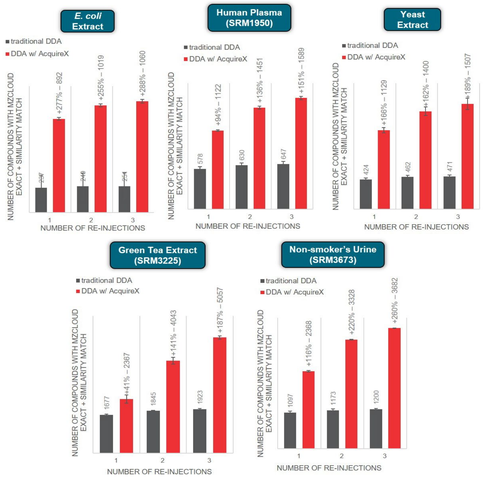
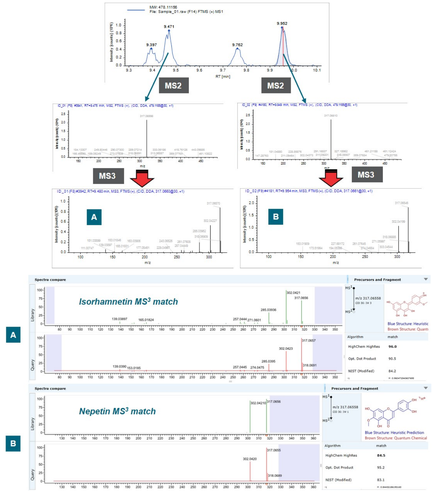


Figure 6. AcquireX DeepScan workflow results in more number of compounds with mzCloud Exact and Similarity Match searching, by number of cumulative re-injections.



Using the MS^n capabilities of the Orbitrap ID-X platform, the confidence in annotation of structural isomers can be increased in combination with the mzCloud spectral library. In Figure 7, two isomeric compounds (flavonoids) in the green tea extract (SRM3225) show different MS^3 from the same MS^2 precursor. Searching the sub-tree spectra against mzCloud identifies different flavones on the basis of the MS^3 spectra (figure 7 a/b).

Figure 7. An example of compound annotation with MS^n and AcquireX acquisition.



CONCLUSIONS

- Utilization of the AcquireX DeepScan workflow on the Orbitrap ID-X platform has shown to deliver improved MS/MS acquisition with real-time generation of exclusion and inclusion lists allowing for the focus to be on acquisition of true sample components.
- Sequential injections show an increased depth of MS/MS fragmentation coverage with the AcquireX acquisitions. Leading to less redundant acquisitions and more on low-abundant compound fragmentation.
- Additionally, implementing MS^n capabilities, we gain confidence in compound identification of isomeric species with searching mzCloud spectral library.

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TRADEMARKS/LICENSING

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PO65507-EN0519S

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