Intelligent MSn Workflow for Improved Metabolome Coverage and Increased Confidence in Unknown Identification

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1. Introduction

Compound identification is a bottleneck in untargeted metabolomics, hindering biological interpretation of results. Here, we describe a data-informed workflow that maximizes the number of metabolites interrogated by MS/MS and MSn, while minimizing the acquisition of uninformative spectra. This workflow was used to analyze human plasma resulting in high confidence identifications, deeper metabolome coverage and enhanced biological knowledge generation.

2. Approach

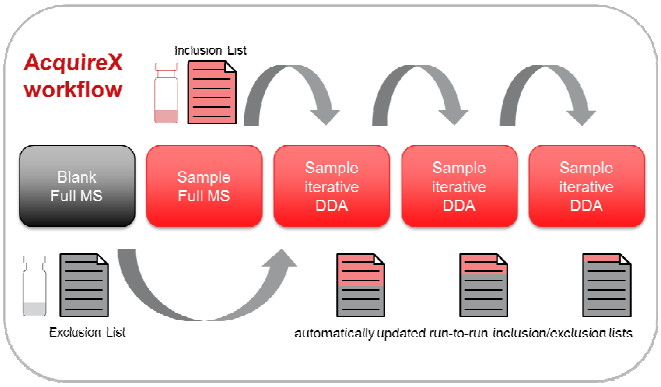
Human plasma was purchased from NIST. Metabolites were extracted with methanol and injected on a Thermo Scientific™ Hypersil GOLD™ column. Instrumentation included a Thermo Scientific™ Vanquish™ UHPLC system and a Thermo Scientific™ Orbitrap Tribrid™ Mass Spectrometer with modified instrument control and data acquisition software. Data were analyzed using Thermo Scientific™ Mass Frontier software and Thermo Scientific™ Compound Discoverer™ software.

3. Results

During data-dependent MS/MS, ions are selected based on abundance, without any knowledge of biological relevance or type of ion. In a typical DDA experiment, we determined, that >40% of MS/MS spectra could be attributed to background ions. By enabling the automatic generation and implementation of a background exclusion list based on real-time feature detection in LC-MS data, background ion MS2 spectra were practically eliminated (<0.1%), allowing for the analysis of more true sample components.

Small molecules form different types of adducts and cluster ions during electrospray ionization. Highly abundant compounds may prevent the fragmentation of metabolites of lower abundance. By populating the inclusion list with the preferred ion for each metabolite, more compounds can be sampled by MS/MS and MSn in a single run. Additionally, by automatically updating inter-run inclusion and exclusion lists during analysis, we can ensure that compounds not selected for MS/MS and MSn will be prioritized during a subsequent injection.

Figure 1. AcquireX represents a new acquisition paradigm. 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.



4. Conclusions

The combination of MSn and automatically generated inter-run inclusion and exclusion lists resulted in fragmentation of more unique metabolites and a greater number of metabolites confidently annotated. Application of this innovative workflow addresses the identification bottleneck of untargeted metabolomics studies and enables confident biological interpretation of the results.

5. Novel Aspect

Automated workflow for information-rich fragmentation data acquisition, designed to minimize irrelevant spectra and maximize metabolome coverage.