Enhanced Metabolite Identification Using Orbitrap Tribrid Mass Spectrometer

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OVERVIEW

Purpose: AcquireX acquisition workflow for improved MS^n data quality for metabolite identification.

Methods: Orbitrap Tribrid ID-X with AcquireX acquisition workflow

Results: AcquireX background exclusion workflow improves metabolite ID

INTRODUCTION

Metabolite Identification is a critical component through all stages of drug discovery and development. High resolution mass spectrometry (HRMS) is an essential tool for metabolite identification. HRMS with sophisticated data acquisition features can provide the critical information for metabolite structure elucidation and alleviate the difficulties of matrix complexity.

Here we present a study for metabolite ID using Orbitrap Tribrid ID-X MS with AcquireX data acquisition workflow which includes automatic background ions subtraction, inclusion/exclusion lists generation, and real-time decision-making to optimize MS^n data acquisition quality and speed.

The MS^n data generated using the AcquireX data acquisition workflow was processed using structure analysis and data mining software "Compound Discoverer 3.0" and "Mass Frontier 8.0".

The results of using Data Dependent Acquisition (DDA) and AcquireX Background Exclusion workflow were compared.

MATERIALS AND METHODS

Sample Preparation

Sample: Test compounds (5 µM): Amprenavir, Bosentan, Lopinavir, Tipranavir, Ritonavir
Control: Human liver microsomes (1 mg/mL) incubation in the presence of GSH and UDPGA without test article at 37 degree for 1 hr

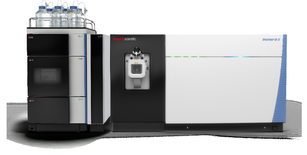
Liquid Chromatography

Thermo Vanquish Flex UHPLC system consisting of:
Vanquish Binary pump
Vanquish Autosampler
Vanquish Column Compartment
Vanquish Diode Array Detector
Column: Thermo Hypersil C18 100 x 2.1 mm, 1.9 µm Temperature: 45ºC
Gradient: Mobile A: H_2O/0.1% Formic Acid
B: ACN/0.1% Formic Acid
Flow rate: 400µl/min Injection Volume: 10 µl
LC gradient:

Time(min)	0	1.0	2.0	14.0	14.1	16.0	16.1	19
B%	5	5	15	70	95	95	5	5

Mass Spectrometry

The MS analyses were carried out on Thermo scientific Orbitrap ID-X™ Tribrid™ mass spectrometer using electrospray ionization in positive mode. High resolution full-scan MS and top3 MS/MS data were collected in a data-dependent fashion at resolving power of 120,000 and 30,000 at FWHM m/z200 respectively; stepped HCD Collision Energy (%): 20, 40, 60. AcquireX data acquisition
Source parameters:
Positive Ion Spray Voltage (V): 3400
Sheath Gas (Arb): 40
Aux Gas (Arb): 5
Sweep Gas (Arb): 1
Ion Transfer Tube Temp (ºC): 300
Vaporizer Temp (ºC): 400



INSTRUMENT and METHOD

Orbitrap ID-X MS and AcquireX Acquisition

This study was conducted on the Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer which is a Tribrid instrument dedicated for small molecule research only. Orbitrap ID-X ready-made method templates were used to generate the acquisition method, see Figure 1.

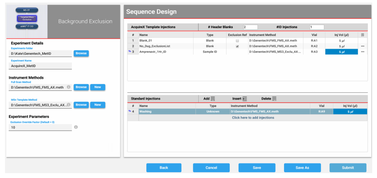
Figure 1. Orbitrap ID-X Ready-Made Method Template

AcquireX Data Acquisition workflow

AcquireX is a data acquisition workflow which can generate background exclusion list and sample component inclusion list in an automated fashion to improve quality and efficiency of analysis. There are three AcquireX workflows with ready-to-use acquisition templates, see Figure 2. The Background Exclusion workflow was used for this study to reduce the biologic matrices, see Figure 3.

Figure 2. AcquireX Workflows

Figure 3. AcquireX Acquisition Sequence for Metabolite ID



ANALYSIS RESULTS

Low-levels Model Compounds in Complex Matrices were Triggered MS^n

A mixture of 5 model compounds was spiked into bile and human plasma at 0.1 uM level. The samples were analyzed using data dependent acquisition (DDA) and AcquireX Background Exclusion workflow. The results showed that with AcquireX Background Exclusion, the model compound was detected with high mass accuracy and triggered MS^n, see Figure 4, while DDA was not able to trigger MS^n for all five compounds due to the presence of complex biological matrices.

Figure 4. Model Compound Amprenavir in Bile

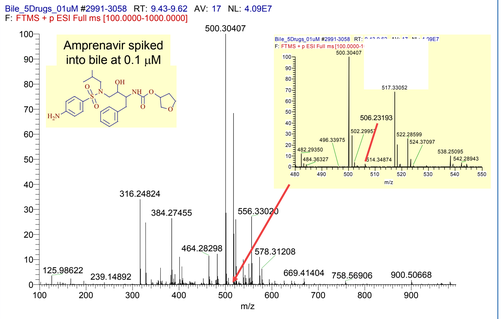
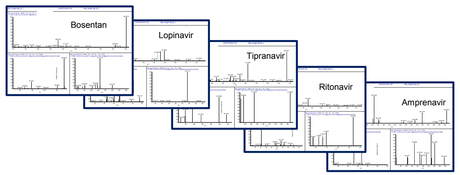


Figure 5. AcquireX Workflow Triggered MS^n of All 5 Model Compounds in Bile



DATA ACQUISITION

Model Compounds HLM Metabolite ID Using AcquireX

The model compounds HLM digest were analyzed on Orbitrap ID-X™ Tribrid™ mass spectrometer using AcquireX Background Extraction workflow. The results showed that AcquireX acquisition identified more metabolites by effectively triggering more MS^n compared with data-dependent acquisition (DDA), see Figure 8. The MS^n spectra were critical for metabolites structure elucidation, which is especially useful for identifying structures for isomeric metabolites.

DATA PROCESS

Data Processing Using Compound Discoverer 3.0 and Mass Frontier 8.0

The data was processed using "Compound Discoverer 3.0" and "Mass Frontier 8.0" software. Compound Discoverer 3.0 (CD 3.0) features automatic dealkylation prediction, and nodes enable the detection of targeted metabolites through biotransformation list, as well as detection of unexpected using "Compound Class Scoring" node. Parent compound fragments were used to generate "Class Coverage" list, and user can also add other fragments based on parent compound structure. This feature effectively enhances the detection of unknown or unexpected metabolites other than common phase I and II biotransformation. See Figure 6.

CD 3.0 Fragment Ion Search (FISh) features automatically annotated fragment ions. The blue color-coded peaks indicate the mass shift of the fragment, which facilitates localizing the site of biotransformation, see Figure 7.

Figure 6. Detect Expected and Unexpected Metabolites Using Biotransformation List and Compound Class

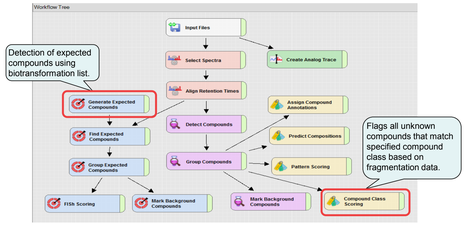


Figure 7. Auto Annotations of Fragment Ion Search (FISh) – Localize the Site of Biotransformation

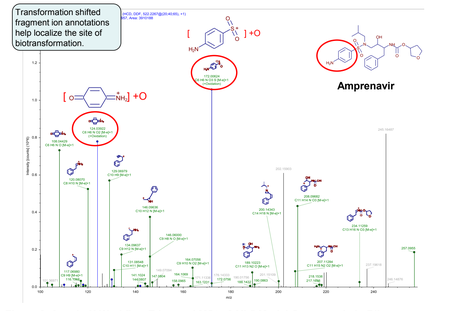


Figure 8. AcquireX Workflow Triggered MS^n of All 5 Model Compounds in Bile

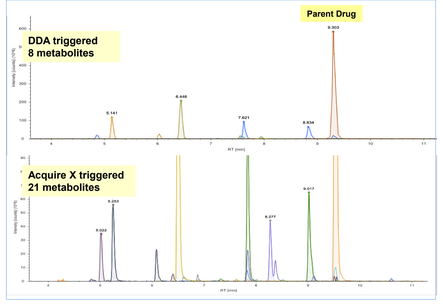
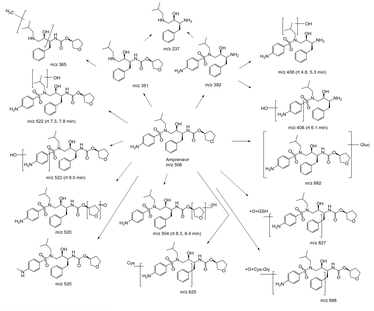


Table 1. Metabolites Identified by DDA and AcquireX

RT [min]	Molecular Weight	Formula	Transformations	DDA	AcquireX
2.29	236.1889	C14H24N2O	Sulfonamide hydrolysis + amide hydrolysis		Y
4.84	407.18788	C20H29N3O4S	Amide hydrolysis + oxidation		Y
5.02	350.22056	C19H30N2O4	Sulfonamide hydrolysis	Y	Y
5.14	364.23621	C20H32N2O4	Sulfonamide hydrolysis + methylation		Y
5.25	407.18788	C20H29N3O4S	Amide hydrolysis + oxidation	Y	Y
6.09	407.18788	C20H29N3O4S	Amide hydrolysis + oxidation	Y	Y
6.41	697.24513	C30H43N5O10S2	Oxidation + glucuronidation		Y
6.50	391.19296	C20H29N3O3S	Amide hydrolysis	Y	Y
6.61	519.20392	C25H33N3O7S	Oxidation (+O-2H)	Y	Y
6.88	826.28773	C35H50N6O13S2	Oxidation + GSH Conjugation		Y
7.19	624.22876	C28H40N4O8S2	Cysteine Conjugation		Y
7.32	521.21957	C25H35N3O7S	Oxidation	Y	Y
7.62	405.20861	C21H31N3O3S	Amide hydrolysis + methylation		Y
7.81	681.25674	C31H43N3O12S	Glucuronidation	Y	Y
7.84	521.21957	C25H35N3O7S	Oxidation	Y	Y
8.01	537.21449	C25H35N3O8S	Di-oxidation		Y
8.28	503.20901	C25H33N3O6S	Dehydration		Y
8.37	503.20901	C25H33N3O6S	Dehydration		Y
9.02	521.21957	C25H35N3O7S	Oxidation		Y
9.11	503.20901	C25H33N3O6S	Dehydration		Y
9.52	505.2246	C25H35N3O6S	Amprenavir Parent	Y	Y
10.61	519.24031	C26H37N3O6S	Methylation		Y

Figure 9. Amprenavir Metabolites Detected



CONCLUSIONS

- Orbitrap ID-X Tribrid MS delivers high quality data.
- AcquireX data acquisition workflow overcomes the matrices interference in automated fashion and enhances metabolites ID through increasing triggering MS^n.
- Compound Discoverer 3.0 and Mass Frontier software suite effective at data mining and generates confident ID and structure characterization.

Orbitrap ID-X tribrid MS with the AcquireX data acquisition feature, coupled with Compound Discoverer 3.0 and Mass Frontier software suite, provide a comprehensive workflow for confident metabolite identification.

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