Orbitrap Exploris 240 Mass Spectrometer with Novel Data Acquisition Features Ensures Confident Metabolite Identification and Structure Elucidation

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INTRODUCTION

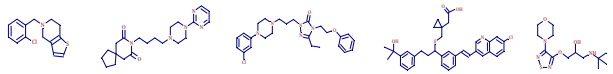
Metabolite profiling is an essential part of the drug development process. High resolution mass spectrometry (HRMS) is the gold standard for metabolite identification.

Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer with very high resolution and advanced data dependent acquisition was used for selected commercial drugs metabolite profiling. By utilizing AcquireX advanced data acquisition, low-abundant metabolites masked by matrix were triggered MS/MS, which facilitated confident metabolite profiling and structural elucidation. In addition, the fast scan speed, rapid positive/negative polarity switching, and EASY IC features ensured overall data quality and enhanced usability.

MATERIALS AND METHODS

Sample Preparation

Selected commercial drugs for this study (purchased from Sigma-Aldrich).



Ticlopidine Buspirone Nefazodone Montelukast Timolol

Drugs (10 μM) were incubated with human and rat liver microsomes (1 mg/mL) in the presence of NADPH (1 mM) and GSH (1 mM) as co-factors. Negative control, used for background subtraction, was prepared with incubations in the absence of drug substrates. The enzymatic reaction was stopped by the addition of three volume of acetonitrile at 0 and 30 minutes, followed by centrifugation for protein precipitation. The supernatants were concentrated to the original volume under a stream of nitrogen and spiked into human and rat plasma respectively in 1:5 (v/v) ratio. The mixtures were deproteinated with the same procedure described above.

Liquid Chromatography

Chromatographic separations were carried out on Vanquish Flex Binary UHPLC system with DAD detector with gradient at flow rate of 0.5 mL/min. The column: Hypersil GOLD C18 (2.1X50 mm μm).

Mobile phase A : H_2O/5mM ammonium formate/0.05% formic acid
Mobile phase B: Acetonitrile-H_2O 9:1/5mM ammonium formate/0.05% formic acid

Mass Spectrometry

The mass spectrometry analysis was carried out on Orbitrap Exploris™ 240 mass spectrometer using an electrospray source. The HRAM full scan was followed by top 5 DDA HCD MS^2 acquisition. The resolution settings of full-scan and MS/MS were 60,000 and 15,000 respectively.

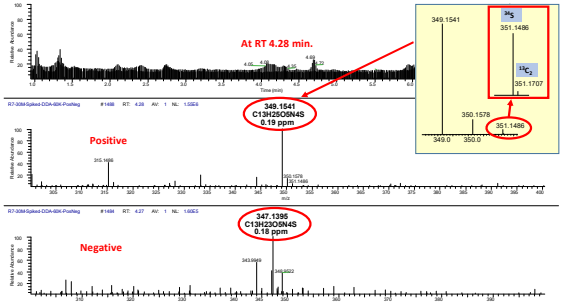
Source parameter:
Ion source: OptaMax TNG
Ionization mode: ESI positive
Scan Range (Full MS): 100-1000 amu
Spray voltage (KV): +3.5 pos/ -2.5 neg
Capillary temp (C): 320
S-lens RF level: 70.0
Heater temp (C): 400

INSTRUMENT AND METHODS

Exploris 240 MS – The improved instrumentation with very high resolution, fast scan speed, AcquireX advanced data acquisition, and internal calibration (EASY-IC) yielded enhanced performance and usability.

Figure 1. Exploris 240 MS Method Editor

Figure 2. HRAM with Polarity Switching and Isotope Fine Structure of Sulfur ^{34}S and $^{13}C_2$



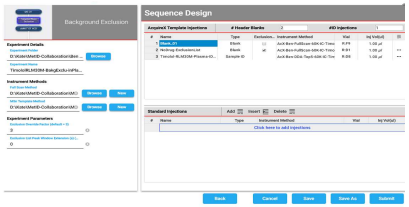
AcquireX Advanced Data Acquisition Workflows

AcquireX advanced data acquisition workflows generates background exclusion list and sample component inclusion list, it updates the acquisition methods in real time in automated fashion. It increases the triggering MS/MS of low abundant ions of interest and improves quality and efficiency of metabolite analysis. There are four AcquireX workflows which are designed to meet the needs of different applications, see Figure 2.

Compared with traditional DDA data acquisition, by eliminating the matrix interference, background exclusion workflows were able to trigger low abundant metabolite MS/MS, see Figure 4 and 5 for results using DDA and background exclusion workflow.

Figure 3. AcquireX Workflows

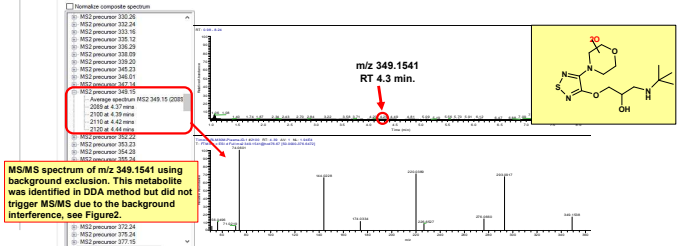
Figure 4. AcquireX Background Exclusion Acquisition Sequence for Timolol Metabolite ID



ANALYSIS RESULT

AcquireX Background Exclusion Workflow for Improved Metabolites Profiling

Figure 5. AcquireX Background Exclusion Triggered Low-level Timolol Metabolites in RLM



Data Analysis

The data was processed using "Compound Discoverer 3.3" software by Thermo Scientific. The flexible node-based process workflow detected expected and unexpected metabolites. see figure 6 and 7.

Figure 6. Compound Discoverer 3.3 Workflow Tree and Result

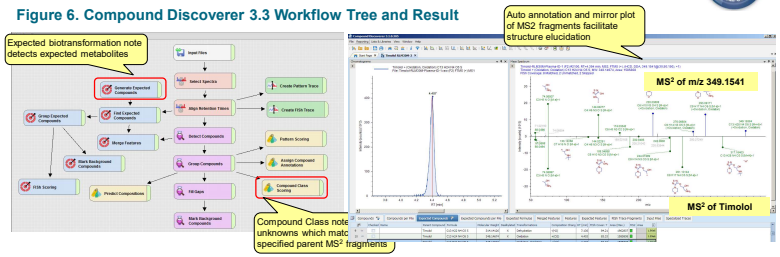


Figure 7. Unexpected Metabolites M2 and M3 were Identified by Class Coverage and Pattern Matches

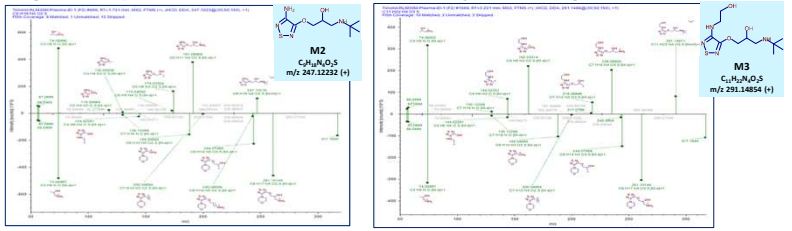


Figure 8. MS Chromatogram of Timolol Metabolites

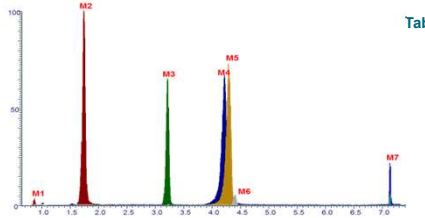
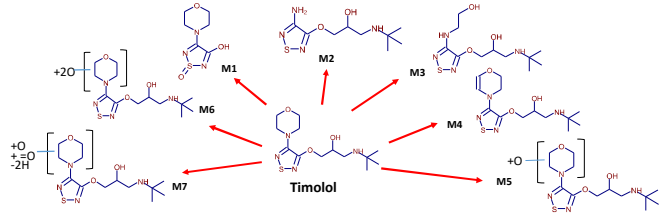


Table 1. Results of AAD and Background Exclusion

Metabolite	RT (min)	Formula Weight	Formula	DDA	AcquireX
M1	1.74	246.1151	C9H18N4O2S	√	√
M2	3.22	290.1413	C11H22N4O3S	√	√
M3	4.40	348.1467	C13H24N4O5S		√
M4	4.22	314.1413	C13H22N4O3S	√	√
M5	4.30	332.1518	C13H24N4O4S	√	√
M6	7.14	314.1413	C13H22N4O3S	√	√
M7	7.14	346.1311	C13H22N4O5S		√

Figure 9. Timolol RLM Metabolites Detected



CONCLUSIONS

The bench-top Orbitrap Exploris 240 MS, when used in conjunction with AcquireX (intelligent and automatic background removal tool designed specifically for DDA MS^2), provides clear improvement to metabolite identification with its much faster scan speed and significantly higher success rate for MS2 generation that drug-related metabolites.

Exploris 240 MS coupled with software suite Compound Discoverer 3.3, Mass Frontier, and FreeStyle, provide a comprehensive solution for confident metabolite identification.

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