Real-Time Library Search on Orbitrap IQ-X Tribrid MS enhances metabolite profiling

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Abstract

Purpose: To demonstrate a use case of the "Real-Time Library Search" intelligent data acquisition strategy on the Thermo Scientific™ Orbitrap IQ-X™ Tribrid™ Mass Spectrometer for enhanced small molecule structure identification.

Methods: Model compounds were incubated in human hepatocytes and metabolites identified using an Orbitrap IQ-X Tribrid mass spectrometer and the Met-IQ workflow via the new "Real-Time Library Search" (RTLS) filter.

Results: The Met-IQ workflow enhanced MS^2 sampling while simultaneously retaining generation of MS^3 scans for ions of interest to assist in the structural characterization of metabolites.

Introduction

Drug metabolite profiling is an integral part of drug discovery. High resolution mass spectrometry with effective data acquisition methodology plays an essential role in the identification and structural characterization of metabolites from compounds of interest.

Here, we present a case study using the Orbitrap IQ-X Tribrid MS, leveraging the real-time decision-making Met-IQ workflow via the new RTLS filter for metabolite profiling. By using RTLS with the parent compound MS^2 spectral library, we selectively trigger MS^3 for precursors which display fragmentation similarity with the parent drug, allowing for structural characterization of drug-related compounds, but simultaneously freeing the duty cycle to enhance MS^2 sampling of additional precursors.

Materials and methods

Sample preparation

The model compounds losartan and montelukast (obtained from Sigma Aldrich) were dissolved and diluted in catabolism buffer at a concentration of 2 µM and incubated at 37ºC for 0 and 4 hours in the presence of human hepatocytes. Samples were crashed with an equal volume of cold acetonitrile containing 2% formic acid, centrifuged for 10 minutes at 10ºC, and the supernatant transferred to sample vials for LC/MS analysis.

Liquid chromatography

Chromatographic separations were carried out with the Thermo Scientific™ Vanquish™ UHPLC system consisting of:

- Thermo Scientific™ Vanquish™ Binary pump
- Thermo Scientific™ Vanquish™ Autosampler
- Thermo Scientific™ Vanquish™ Column Compartment
- Thermo Scientific™ Vanquish™ Diode Array Detector

LC separations were performed using a Thermo Scientific™ Hypersil™ GOLD C18 column (2.1x100 mm, 1.9 µm) with mobile phases composed of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile and gradient elution as described below.

Column temp: 40 ºC.
Injection volume: 15 µl
Flow rate: 0.3 mL/min

Table 1. LC gradients

Time (min)	0	0.5	9.0	11.0	12.0	13.0	15.0
%B (montelukast)	1	1	75	95	99	1	1
%B (losartan)	1	1	50	95	99	1	1

Mass spectrometry

Mass spectrometric data were acquired on a Orbitrap IQ-X Tribrid Mass Spectrometer via electrospray ionization in positive mode. The HRAM full scan followed by data-dependent product ion scan were collected at resolution settings of 120,000 and 15,000 at FWHM m/z 200 respectively.

Normalized stepped HCD collision energy (%): 20, 40, 60.
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

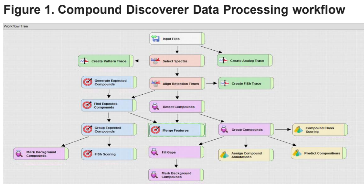
Data processing

The data was processed using Thermo Scientific™ Compound Discoverer™ 3.3 (CD 3.3) software, a small molecule structure analysis software which employs a flexible and customizable node-based processing workflow.

Compound Discoverer 3.3 node-based processing workflow enables the detection of targeted metabolites through biotransformation list, as well as detection of unexpected metabolites using "Compound Class Scoring" node.

In this study, the processing workflow was built using the pre-defined workflow template "MetID w Expected and Unknown w Background Removal", see Figure 1.

Figure 1. Compound Discoverer Data Processing workflow



Results

Mass spectrometer and novel data acquisition workflows

The Orbitrap IQ-X™ Tribrid™ mass spectrometer is dedicated to small molecule structure analysis. The tribrid architecture with ultra-high field orbitrap analyzer and state of the art hardware guarantee the highest performance. As illustrated by the instrument schematic in Figure 2, this system offers exceptional versatility for enhanced small molecule structural analysis through an array of novel data acquisition workflows, including Met-IQ workflow using real-time library search (RTLS) and AcquireX workflows for automatic background exclusion and in-depth MS^n triggering.

Met-IQ workflow using the RTLS filter for metabolite profiling

For small molecule analysis, the Real-Time Library Search (RTLS) filter compares experimental MS^2 to a spectral library and generates a score based on similarity. Compounds that meet the scoring criteria set in the method can trigger additional scan behaviors, including MS^3 or alternate fragmentation. By selectively triggering MS^3 only on precursors with spectral similarity to the parent or compound(s) of interest, additional structural information is gained on the most important compounds while maintaining a high MS^2 scan rate.

In this study, the model compounds' HCD/CID spectra at multiple fragmentation energies were collected using the Library Builder template. The spectral libraries were constructed through mzVault™ spectral library curation software. Existing MassBank record spectral libraries can be converted to the .db mzVault format if applicable. The Met-IQ workflow methods were set up using the RTLS template in method editor, as shown in Figures 3 and 4.

Figure 3. Method setup for mzVault library generation

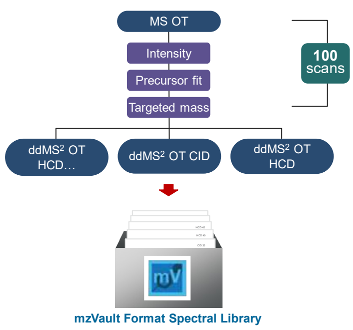


Figure 4. Met-IQ workflow MS method

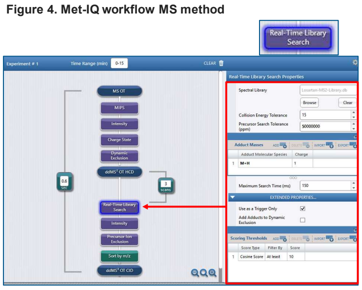
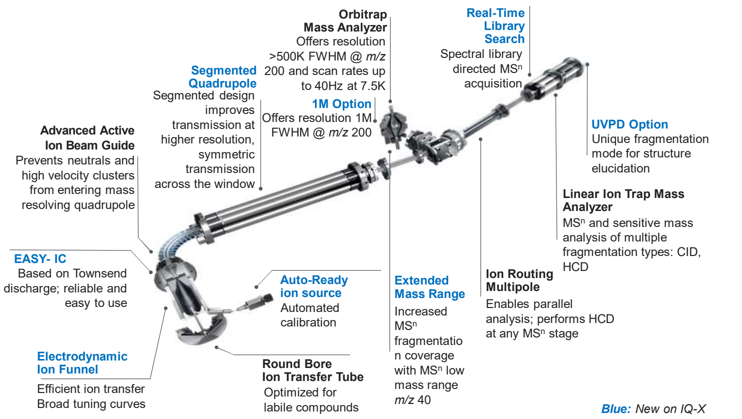


Figure 2. Schematic of the Orbitrap IQ-X Tribrid Mass Spectrometer


Orbitrap Mass Analyzer
Offers resolution >500K FWHM @ m/z 200 and scan rates up to 40Hz at 7.5K
Real-Time Library Search
Spectral library directed MSⁿ acquisition
Segmented Quadrupole
Segmented design improves transmission at higher resolution, symmetric transmission across the window
1M Option
Offers resolution 1M FWHM @ m/z 200
Advanced Active Ion Beam Guide
Prevents neutrals and high velocity clusters from entering mass resolving quadrupole
UVPD Option
Unique fragmentation mode for structure elucidation
Linear Ion Trap Mass Analyzer
MSⁿ and sensitive mass analysis of multiple fragmentation types: CID, HCD
EASY- IC
Based on Townsend discharge; reliable and easy to use
Auto-Ready ion source
Automated calibration
Extended Mass Range
Increased MSⁿ fragmentation coverage with MSⁿ low mass range m/z 40
Ion Routing Multipole
Enables parallel analysis; performs HCD at any MSⁿ stage
Electrodynamic Ion Funnel
Efficient ion transfer Broad tuning curves
Round Bore Ion Transfer Tube
Optimized for labile compounds
Blue: New on IQ-X

Data processing and performance evaluation

The CD processing results showed that with Met-IQ workflow, more metabolites were detected and triggered MS^3 than using conventional DDA acquisition without RTLS. For instance, RTLS data acquisition accurately identified fragmentation level similarity within a low-abundant dealkylated metabolite of losartan. Despite being masked by a background ion's isotope peak, the RTLS filter observed sufficient MS^2 similarity to trigger MS^3 acquisition on the fragment *m/z* 171.06802. This metabolite was confidently identified based on MS^3, HRAM and fine isotope pattern, as shown in Figures 5 and 6.

Figure 5. Dealkylated metabolite of Losartan identified by MS^2 spectral similarity in t=4h RTLS Run

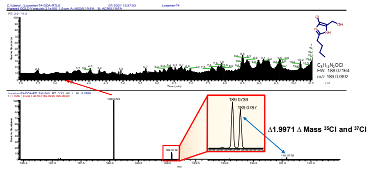
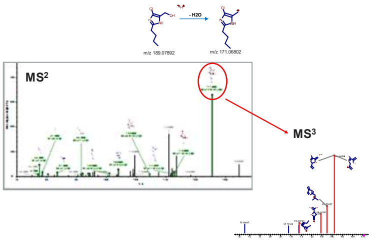


Figure 6. The auto-annotated MS^2 spectrum in the result view shows the same fragment at *m/z* 171.06802 as the parent compound. The MS^2 spectral similarity triggered acquisition of MS^3 spectra for the Top3 fragment ions.



By comparison, fewer metabolite MS^3 were triggered in DDA runs for compounds related to losartan and montelukast due to the un-targeted triggering, especially in the presence of complex biological matrices, as shown in Tables 2 and 3 below. By leveraging RTLS, selective MS^3 data acquisition resulted in freed duty cycle time and increased MS^2 triggering.

Table 2. Overview of 10 identified "Expected Compounds" metabolites of Losartan by DDA

Peak	RT [min]	Formula	Transformations	Composition Ch	DeltaMass [ppm]	Calc. MW	m/z	FISh Coverage	MS Depth
1	6.71	C22 H23 Cl N6 O2	Oxidation	+(O)	0.05	438.15713	439.16440	30.8	3
2	6.97	C22 H21 Cl N6 O3	Desaturation, Oxidation, Oxidation	-(H2) +(O2)	-0.01	452.13636	453.14365	48.5	3
3	6.98	C22 H23 Cl N6 O2	Oxidation	+(O)	0.3	438.15723	439.16452	29.3	3
4	7.44	C22 H21 Cl N6 O3	Desaturation, Oxidation, Oxidation	-(H2) +(O2)	0.07	452.1364	453.14368	36.4	3
5	7.79	C22 H23 Cl N6 O2	Oxidation	+(O)	0.18	438.15718	439.16446	23.9	3
6	7.96	C22 H23 Cl N6 O2	Oxidation	+(O)	0.18	438.15718	439.16446	27.2	3
7	8.04	C28 H31 Cl N6 O8	Oxidation, Glucuronide Conjugation	+(C6 H8 O7)	0.13	614.18927	615.19653	62.5	3
8	8.51	C28 H31 Cl N6 O7	Glucuronide Conjugation	+(C6 H8 O6)	0.26	598.19443	599.20172	19.8	3
	8.64	C22 H23 Cl N6 O	Losartan Parent Compound		0.54	422.16241	423.16970	18.2	3
9	9.28	C22 H21 Cl N6 O2	Desaturation, Oxidation	-(H2) +(O)	0.16	436.14152	437.14880	34.8	3
10	9.61	C22 H21 Cl N6 O2	Desaturation, Oxidation	-(H2) +(O)	0.17	436.14153	437.14880	36.5	3

Table 3. Overview of 24 identified "Expected Compounds" metabolites of Losartan with RTLS

Peak	RT [min]	Formula	Transformations	Composition Change	Anno. [ppm]	Calc. MW	m/z	FISh Coverage	MS Depth
1	3.36	C8 H13 Cl N2 O		-(C14 H10 N4)	-1.19	188.07142	189.07870	39.5	3
2	6.39	C22 H23 Cl N6 O3	Oxidation, Oxidation	+(O2)	0.19	454.15210	455.15930	45.5	3
3	6.72	C22 H23 Cl N6 O2	Oxidation	+(O)	0.06	438.15713	439.16440	34.4	3
4	6.72	C22 H23 Cl N6 O3	Oxidation, Oxidation	+(O2)	0.04	454.15204	455.15933	40.0	3
5	6.98	C22 H23 Cl N6 O2	Oxidation	+(O)	-0.04	438.15709	439.16437	28.1	3
6	6.98	C22 H21 Cl N6 O3	Desaturation, Oxidation, Oxidation	-(H2) +(O2)	-0.14	452.13630	453.14359	42.9	3
7	7.09	C28 H31 Cl N6 O8	Oxidation, Glucuronide Conjugation	+(C6 H8 O7)	-0.16	614.18909	615.19635	100.0	3
8	7.26	C22 H21 Cl N6 O2	Desaturation, Oxidation	-(H2) +(O)	-0.18	436.14137	437.14865	41.7	3
9	7.44	C22 H21 Cl N6 O3	Desaturation, Oxidation, Oxidation	-(H2) +(O2)	-0.16	452.13629	453.14359	39.0	3
10	7.81	C22 H23 Cl N6 O2	Oxidation	+(O)	0.05	438.15713	439.16440	26.9	3
11	7.90	C28 H31 Cl N6 O8	Oxidation, Glucuronide Conjugation	+(C6 H8 O7)	0.19	614.18931	615.19659	66.7	3
12	7.93	C22 H21 Cl N6 O3	Desaturation, Oxidation, Oxidation	-(H2) +(O2)	0.16	452.13644	453.14374	48.0	3
13	7.95	C22 H23 Cl N6 O2	Oxidation	+(O)	0.16	438.15717	439.16446	23.1	3
14	7.96	C22 H19 Cl N6	Dehydration, Desaturation	-(H4 O)	0.08	402.13600	403.14328	57.1	3
15	8.04	C28 H31 Cl N6 O8	Oxidation, Glucuronide Conjugation	+(C6 H8 O7)	0.02	614.18920	615.19647	64.3	3
16	8.33	C28 H33 Cl N6 O6	Glucoside Conjugation	+(C6 H10 O5)	0.21	584.21513	585.22241	100.0	3
17	8.37	C22 H21 Cl N6 O3	Desaturation, Oxidation, Oxidation	-(H2) +(O2)	-0.03	452.13635	453.14365	37.0	3
18	8.51	C28 H31 Cl N6 O7	Glucuronide Conjugation	+(C6 H8 O6)	0.06	598.19431	599.20160	20.8	3
19	8.54	C22 H21 Cl N6 O2	Desaturation, Oxidation	-(H2) +(O)	0.04	436.14147	437.14874	43.4	3
	8.64	C22 H23 Cl N6 O	Losartan Parent Compound		0.46	422.16238	423.16967	20.0	3
20	8.76	C22 H21 Cl N6 O3	Desaturation, Oxidation, Oxidation	-(H2) +(O2)	-0.05	452.13634	453.14362	34.9	3
21	9.28	C22 H21 Cl N6 O2	Desaturation, Oxidation	-(H2) +(O)	0.22	436.14155	437.14883	26.9	3
22	9.46	C28 H29 Cl N6 O8	Desaturation, Oxidation, Glucuronide Con	+(C6 H6 O7)	0.27	612.17371	613.18098		3
23	9.61	C22 H21 Cl N6 O2	Desaturation, Oxidation	-(H2) +(O)	0.10	436.14150	437.14877	27.3	3
24	10.57	C22 H21 Cl N6 O	Desaturation	-(H2)	0.13	420.14659	421.15387	46.6	3

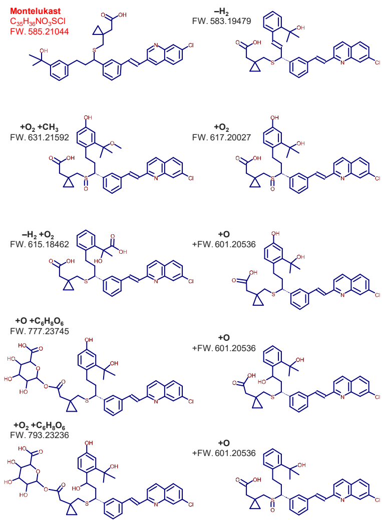
Table 4. Overview of 2 identified "Expected Compounds" metabolites of Montelukast by DDA

Peak	RT [min]	Formula	Transformations	Composit	DeltaMass [ppm]	Calc. MW	m/z	FISh Coverage	MS Depth
1	6.88	C35 H36 Cl N O5 S	Oxidation, Oxidation	+(O2)	0.25	617.20043	618.2077	16.7	3
2	7.93	C35 H36 Cl N O5 S	Oxidation, Oxidation	+(O2)	0.15	617.20036	618.20764	53.1	3
	10.17	C35 H36 Cl N O3 S	Montelukast Parent Compound		0.82	585.21092	586.2182	42.2	3

Table 5. Table 2. Overview of 2 identified "Expected Compounds" metabolites of Montelukast with RTLS

Peak	RT [min]	Formula	Transformations	Composition Change	DeltaMa	Calc. MW	m/z	FISh Covera	MS Depth
1	7.26	C41 H44 Cl N O11 S	Oxidation, Oxidation, Glucuronide	+(C6 H8 O8)	-0.09	793.23229	794.23956	100.0	3
2	7.63	C41 H44 Cl N O11 S	Oxidation, Oxidation, Glucuronide	+(C6 H8 O8)	-0.18	793.23221	794.23950	75.0	3
3	7.93	C35 H36 Cl N O5 S	Oxidation, Oxidation	+(O2)	0.17	617.20038	618.20764	56.4	3
4	8.04	C41 H44 Cl N O10 S	Oxidation, Glucuronide Conjugatio	+(C6 H8 O7)	-0.09	777.23737	778.24463	57.1	3
5	8.44	C41 H44 Cl N O10 S	Oxidation, Glucuronide Conjugatio	+(C6 H8 O7)	0.05	777.23748	778.24475	63.5	3
6	8.79	C35 H36 Cl N O4 S	Oxidation	+(O)	0.19	601.20547	602.21277	61.9	3
7	8.95	C35 H34 Cl N O3 S	Desaturation	-(H2)	0.35	583.19499	584.20227	54.8	3
8	9.03	C36 H38 Cl N O5 S	Oxidation, Oxidation, Methylation	+(C H2 O2)	-0.18	631.21581	632.22308	30.8	2
9	9.15	C35 H34 Cl N O5 S	Desaturation, Oxidation, Oxidation	-(H2) +(O2)	0.09	615.18468	616.19196	75.9	3
10	9.34	C35 H36 Cl N O4 S	Oxidation	+(O)	0.41	601.20560	602.21289	48.6	3
	10.17	C35 H36 Cl N O3 S	Montelukast Parent Compound		0.65	585.21082	586.21808	44.4	3

Figure 7. Overview of Montelukast Expected Metabolite structures identified by DDA with RTLS



Conclusions

The study results show that the Orbitrap IQ-X Tribrid MS Met-IQ workflow uses an intelligent data acquisition strategy "Real-Time Library Search" to guide data acquisition based on structural similarity and improves efficiency for metabolite identification.

RTLS can also be used for other small molecule structure ID applications, such as impurity and degradation profiling.

The Real-Time Library Search-based intelligent MS^3 triggering can be combined with AcquireX workflows for automated background exclusion to further improve efficiency of analysis.

Compound Discoverer 3.3 software facilitates effective data mining and generates confident metabolite ID and structure characterization.

References

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2. Hackbusch, S. *et al.*, Increased confidence in drug metabolite identification through intelligent data acquisition strategies and multiple fragmentation techniques on the Orbitrap Tribrid MS platform. Thermo Fisher Scientific Whitepaper 002154, **2023**. https://assets.thermofisher.com/TFS-Assets/CMD/Reference-Materials/wp-002154-lsms-orbitrap-iqx-dia-wp002154-na-en.pdf

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