Improved Orbitrap Tribrid MS for Pharmaceutical Impurity Identification

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OVERVIEW

Purpose: Improving pharmaceutical impurity identification with an intelligent FTMS^n data acquisition method on an Orbitrap Tribrid MS.

Methods: High resolution LC/MS with AcquireX data acquisition.

Results: The AcquireX data acquisition algorithm allows automatic background exclusion which enables trace impurities ID in complex formulation.

INTRODUCTION

Pharmaceutical impurity profiling is critical for drug R&D because it directly impacts drug quality and patient safety. Identifying drug product impurities is especially challenging due to the presence of excipients. Hybrid mass spectrometers with Orbitrap mass analyzers are routinely used for pharmaceutical impurity ID due to their speed, sensitivity, selectivity, mass accuracy, and their ability to collect higher order MS^n scans.

Herein, we present a study of drug product impurities ID for an HIV-1 drug product, Tipranavir, using AcquireX, a new data acquisition algorithm, that is optimized for small molecule structure analysis on a Thermo scientific Orbitrap ID-X™ Tribrid™ mass spectrometer. This workflow includes automatic background subtraction through automatic exclusion list generation, and MS^n data acquisition of multiple dissociation HCD and CID method designed to increase the speed and confidence of small molecule impurity identification. Coupled with data processing software Compound Discoverer 3.0 and Mass Frontier 8.0, confident impurity ID was obtained.

MATERIALS AND METHODS

Sample Preparation

HIV drug Tipranavir product was selected for this study. The sample was collected from the capsule and was diluted 100-fold using EtOH:IPA (1:1) and used as stock solution. The stock solution was further diluted 10 times using H_2O:IPA (1:3). The excipients in the formulation include Cremophor® EL, propylene glycol, Propyl gallate, Tris, and Capmul® MCM. A simulated placebo was prepared by combining all excipients at the ratio recommended by USP, and water and EtOH were added for mixing purposes. The simulated placebo was further diluted 1000-fold using H_2O:IPA (1:3).

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: 1 µl
LC gradient:

Time(min)	0	1.0	2.0	14.0	14.1	16.0	16.1	18
B%	5	60	80	100	80	5	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. The AcquireX data acquisition feature was used to automatically generate background exclusion list and increase identification efficiency, accuracy, and confidence. High resolution full-scan MS and MS^n 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 was used.

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 ACQUISITION USING AcquireX

Orbitrap ID-X MS

Orbitrap ID-X MS is a dedicated Tribrid MS optimized for small molecule structure analysis. With its high resolution, sensitivity, and high scan speed, it assured confident impurity detection. The MS^n capability and the multiple dissociation HCD, CID techniques provided ample information for structure elucidation.

AcquireX – The Fully Automated All-inclusive Data Acquisition Workflow

The AcquireX feature of Xcalibur is an automated data acquisition workflow designed for small molecule analysis, see Figure1. AcquireX helps quickly and efficiently obtain more relevant and deeper MS^n data. It automatically generates the background exclusion list, and inclusion list when desired, and subsequently incorporates the lists into the MS method for intelligent data acquisition: excluding the background ion from triggering MS^n, and only triggering the ions of interest.

Figure 1. AcquireX Acquisition Workflows

In this study, samples included very complex excipients background. The AcquireX "Background Exclusion" workflow was employed, see Figure 2. It effectively excluded the background ions from MS^n triggering. As a result, it triggered MS^n of low abundant impurity ions, which increased the impurity analysis accuracy and confidence, see Figure 3.

Figure 2. AcquireX Background Exclusion Workflow Acquisition Sequence

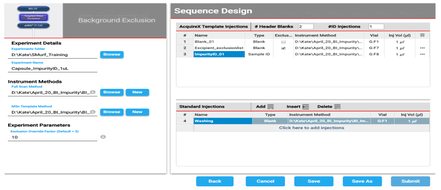
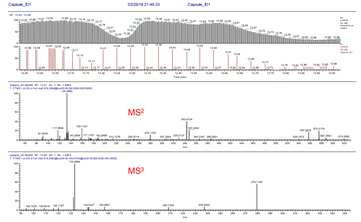
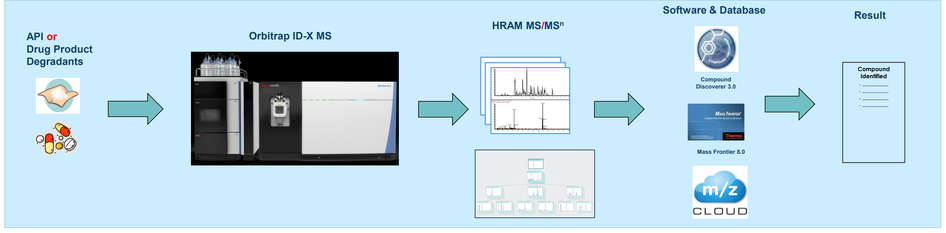


Figure 3. AcquireX – Trigger MS^n the Ions of Interest

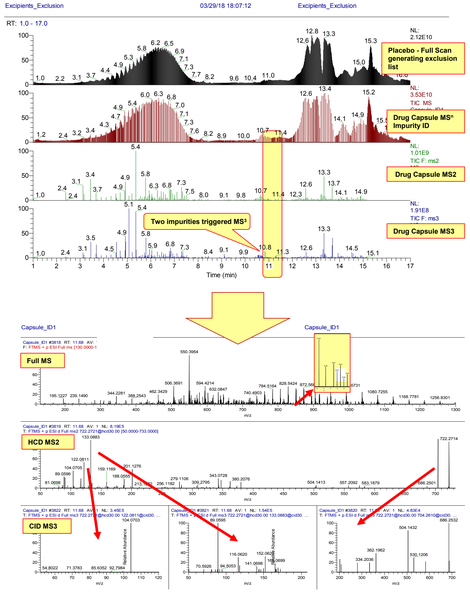


Complete Small Molecule Structure Analysis Workflow



AcquireX RESULTS

Figure 4. AcquireX Background Exclusion Workflow – Only Trigger the Ions of Interest

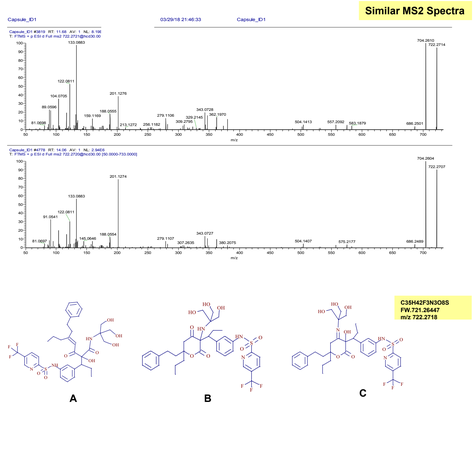


DATA PROCESSING

The HRAM LCMS data was processed with small molecule structure analysis software Compound Discoverer 3.0 and Mass Frontier 8.0. There were multiple peaks for the same m/z at different retention times. The MS2 and MS3 spectra with Mass Frontier "Fragments & Mechanisms" feature facilitated structure elucidation.

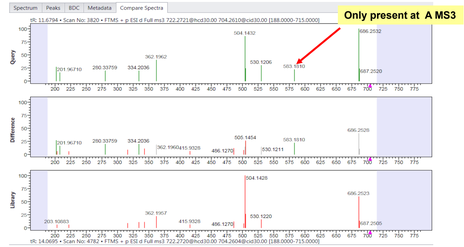
Two impurities at RT 11.68 min (A) and 14.06 min (B) were identified. They have the same m/z and similar MS^2 spectra. Based on the accurate mass and fragmentation, three possible structures were proposed.

Figure 5. MS2 Fragmentation Facilitate Structure Elucidation



Mass Frontier "Compare Spectra" shows MS^3 m/z 583.1873 only present at impurity A. Based on Mass Frontier "Fragments and Mechanisms", only structure A generates m/z 583.1873 in MS3 and structure B's m/z 583.1873 comes from a different precursor. Which explained why no m/z 583.1873 was observed from structure B's MS3 spectrum. The third possible structure does not generate the right fragment. Based on MS3 fragment, the possible structures were assigned.

Figure 5. MS3 Fragmentation Facilitate Structure Elucidation



CONCLUSIONS

This study demonstrates a workflow using AcquireX for drug product impurity analysis. The results show that AcquireX can overcome the impediment of matrix interference. By excluding the background ions from triggering data dependent MS^n, AcquireX increases the detectability of trace level impurities from overwhelming background interference, and thus significantly improves MS^n efficiency and reduces acquisition time. In addition, HCD, CID fragmentation techniques provide in-depth and complementary structure information, assisting unknown structure elucidation.

The data processing software "Mass Frontier 8.0" and "Compound Discoverer 3.0" (CD) are essential. The database and library searching assisted in structure characterization. Mass Frontier 8.0 "Ion Tree" made it easy to see the structure linkage for structure elucidation.

This workflow is applicable to other small molecule structure analysis applications.

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING

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