Optimized Workflow for Structure Elucidation of Pharmaceutically Relevant Extractables and Leachables

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ABSTRACT

Purpose: Optimized workflow for extractables and leachables (E&L) analysis.

Methods: Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer with AcquireX background exclusion data acquisition coupled with Thermo Scientific™ Compound Discoverer™ 3.0 software and Thermo Scientific™ Mass Frontier™ 8.0 software.

Results: Automatic background exclusion and MS^n data improve overall E&L analysis quality and confidence.

INTRODUCTION

The identification of unknown small molecules—such as impurities, metabolites, degradants, extractables, and leachables—remains one of the most challenging workflows. Mass spectrometry based chemical and structural characterization of small molecules greatly benefits from multistage fragmentation (MS^n) coupled with high resolution and high mass accuracy analysis. However, applying these sophisticated methods to complex samples is challenging, owing to the slow precursor interrogation rate and the large number of potential precursors. Herein we describe new data acquisition approaches for the characterization of small molecule extractables and leachables. Building upon sophisticated methods that employ multistage Orbitrap™ analysis ($FTMS^n$), we dynamically update inclusion and exclusion lists between LC analyses to enable efficient and deep interrogation of all the features in a complex sample.

MATERIALS AND METHODS

Sample Preparation

Sample 1. Additive standard mixture was prepared at 10 ppm each in 1:1 IPA/H_2O. The solution was diluted 10-fold using drug excipients (USP).

Sample 2. Three different types of medical grade O-rings, A, B, and C, were extracted using PH_3 H_2O, PH_8 H_2O, and IPA. The weight and volume ratio was 1/10: 2 g of O-ring material and 20 mL of solvent. The extraction vials were placed in oven at 50 °C for 7 days. The extracts solutions were analyzed directly by LC/MS.

Liquid Chromatography

Thermo Scientific™ Vanquish™ Flex UHPLC system consisting of: Vanquish Binary Pump, Vanquish Autosampler, Vanquish Column Compartment, Vanquish Diode Array Detector

Column:	Thermo Scientific™ Hypersil GOLD™ C18 100 x 2.1 mm, 1.9 µm						
Temperature:	45 °C						
Gradient:	Mobile phase A: H_2O/0.1% Formic acid; B: ACN/0.1% Formic acid						
Flow Rate:	400 µL/min						
Injection Volume:	5 µL						
LC Gradient:	Time (min)	0	1.0	4.0	15.0	20.0	20.1
	B%	5	5	30	95	95	5

Mass Spectrometry

The MS analyses were carried out on an Orbitrap ID-X Tribrid mass spectrometer using electrospray ionization in positive mode. The AcquireX data acquisition workflow was used for data acquisition. High-resolution full-scan MS and MS^n data, HCD MS^2 and CID MS^3, were collected in a data-dependent fashion at resolving powers of 120,000, 30,000, and 15,000 at FWHM *m/z* 200, respectively. Stepped HCD collision energy (%): 15, 35, 55 was used.

Source Parameters:

Positive Ion Spray Voltage:	3400 V
Sheath Gas (Arb):	40 Arb
Aux Gas (Arb):	5 Arb
Sweep Gas (Arb):	1 Arb
Ion Transfer Tube Temp:	300 °C
Vaporizer Temp:	400 °C

INSTRUMENTS AND ACQUISITION METHOD

Orbitrap ID-X MS

This study was conducted on an Orbitrap ID-X mass spectrometer, a dedicated Tribrid instrument for small molecule research. The MS methods were set up using predefined experimental method templates in the Orbitrap ID-X method editor, see Figure 1.

Figure 1. Orbitrap ID-X MS predefined experimental method templates for E&L analysis

AcquireX

The AcquireX acquisition workflow includes three ready-to-use workflow templates, see Figure 2:

1. BACKGROUND EXCLUSION. This workflow automatically creates and uses a background exclusion list to reduce background fragmentation in sample ID runs.
2. BACKGROUND EXCLUSION & COMPONENT INCLUSION. Combines exclusion and inclusion lists automatically and reliably acquires more relevant MS^n data in a single injection.
3. DEEP SCAN: Combines a single exclusion and inclusion list with multiple ID injections to increase fragments of ions of interest.

These three workflows are flexible and can be used for a variety of E&L analyses, such as different loads, batches of bio-production bags, tubing, filters, etc., or one subject under different extraction conditions, applications, and samples.

Figure 2. AcquireX Workflows

RESULTS

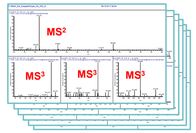
1. Additives Standard Spiked into Drug Excipients

Leachable studies investigate the possible leachables generated from the interaction of a single-use system with a bio-production medium, or drug container/packaging with a formulated drug. Leachable analysis is challenging because of the presence of complex biologic matrices.

In this study, a mixture of common additive standards was spiked into the drug formulation. Results obtained using traditional data-dependent acquisition (DDA) and AcquireX were compared. The results indicated that using AcquireX background exclusion yielded selection and fragmentation of more ions of compounds of interest compared with the DDA method, as shown by Figure 3.

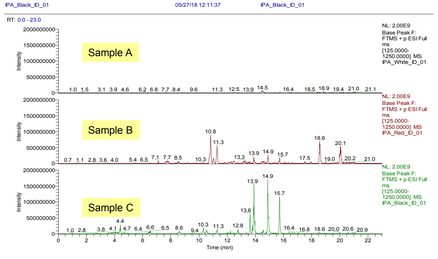
Figure 3. Additives spiked into drug formulation

Name	Structure	DDA Triggered MS^n	Acquire X Triggered MS^n
Irganox 1076		X	√
1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)-benzen		√	√
Irgafos 168		√	√
TINUVIN 328		√	√
Octabenzone		X	√
Ethylene bis[3,3-bis[3-(1,1-dimethylethyl)-4-hydroxyphenyl]		X	X
Irganox 1010		X	√



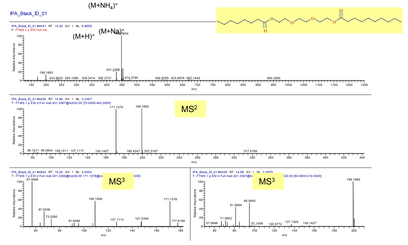
- Seven common additives spiked into complex drug formulation
- Traditional DDA only triggered MS^n for three additives
- AcquireX with background exclusion triggered MS^n for six additives
- **AcquireX triggered MS^n for twice as many additives, producing a two-fold increase in leachable IDs**

Figure 4. Positive ion chromatogram of IPA extraction of samples A, B, and C



The constant presence of solvent background often masks the low-abundant additives in the extracts solution. By using the AcquireX background exclusion workflow, several sulfur-containing additives were readily identified, which are substances of concern.

Figure 5. MS^n Spectra of identified additive



DATA PROCESSING

Compound Discoverer 3.0 software and Mass Frontier 8.0 software were used for data processing and structure characterization.

Figure 6. Compound Discoverer 3.0 software processing workflow

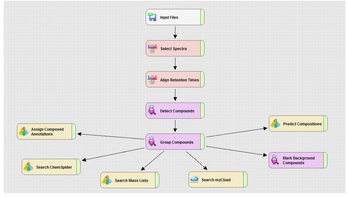


Figure 7. Compound Discoverer 3.0 software processing result

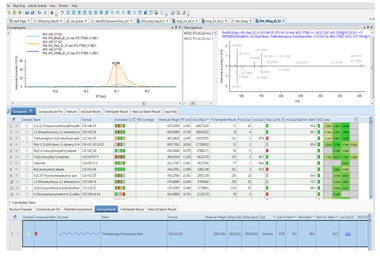


Figure 8. Mass Frontier 8.0 software – Ion tree for structure elucidation and library generation

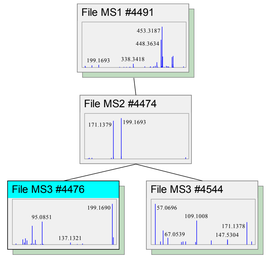
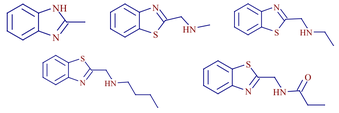


Figure 9. Partial list of compounds identified containing sulfur



CONCLUSIONS

The results of this study demonstrate how the Orbitrap ID-X MS, equipped with the AcquireX acquisition workflow, facilitates E&L structure elucidation. Coupled with the data processing suite consisting of Compound Discoverer 3.0 and Mass Frontier 8.0 software, the Orbitrap ID-X instrument improves the efficiency and increases the reliability of E&L identifications, and streamlines the E&L analysis from data acquisition to data processing. This workflow is also well-suited to other small molecule structure applications, such as Met ID, API impurity ID, degradants ID, and metabolomics research.

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