Impurity Profiling Using Orbitrap Exploris 120 Mass Spectrometer and Vanquish UHPLC Coupled with Compound Discoverer Software

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ABSTRACT

Purpose: Combine the power of high-resolution MS with advanced processing software to tackle the small molecule impurity structure characterization bottleneck.

Methods: API impurity profiling using Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer and Vanquish™ UHPLC coupled with Compound Discoverer™ software 3.2 for data interpretation and structure elucidation.

Results: Orbitrap Exploris 120 and Vanquish UHPLC coupled with Compound Discoverer 3.2 software, offers significant improvements in quality, speed, and overall efficiency for routine impurity identification and structure characterization.

INTRODUCTION

Small molecule drug API impurity and degradation product profiling and structure characterization are essential for drug R&D and regulatory approval.

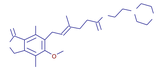
High resolution MS has been routinely used for small molecule impurity's structure analysis to obtain critical elemental composition and structure insights. However, data interpretation remains a time consuming and challenging task.

Here we present a case study for Mycophenolate Mofetil API impurity profiling using Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer and Thermo Scientific™ Vanquish™ UHPLC coupled with Thermo Scientific™ Compound Discoverer™ software 3.2, an advanced data processing software for data interpretation and structure elucidation.

MATERIALS AND METHODS

Sample Preparation

Mycophenolate Mofetil, CAS # 128794-94-5 (Sigma-Aldrich P/N: SML0284-10MG) stock solution at 1.0 mg/ml in Acetonitrile was prepared by dissolving 1.0 mg in 1 mL Acetonitrile. The working solution for LCMS analysis was 0.25mg/mL in Water with 25% ACN.



Liquid Chromatography

Chromatographic separations were carried out on the Thermo Scientific™ Vanquish™ UHPLC system consisting of the following modules:
- Vanquish Binary Pump H
- Vanquish Split Sampler FT
- Vanquish Column Compartment
- Vanquish Diode Array Detector FG

A Thermo Scientific™ Hypersil GOLD™ VANQUISH™ C18 UHPLC column (2.1X100 mm, 1.9 µm, P/N: 25002-102130-V) was used with the gradients specified below at a flow rate of 0.4 mL/min and column temperature of 50° C. Mobile phases were: (A) H2O/0.1% formic acid/10 mM ammonium formate and (B) Acetonitrile/0.1% formic acid.

LC gradients:

Time (min.)	0	1.0	3.0	12.0	15.0	18.5	18.6	20.0
B%	10.0	10.0	20.0	40.0	95.0	95.0	10	10

Mass Spectrometry

The mass spectrometry analysis was carried out on a Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer equipped with a Thermo Scientific™ OptaMax™ NG ion source.

Source parameters:
Ion source: OptaMax NG electrospray ion source
Ionization mode: ESI positive/negative
Scan range (Full MS) (m/z): 125-1500
Spray voltage positive (KV): +3.5
Spray voltage negative (KV): -2.5
Capillary temp (°C): 320
S-lens RF level: 70.0
Heater temp (ºC): 400
Sheath gas (units/N_2): 40
Aux gas (units/N_2): 10
Sweep gas (units/N_2): 2

The data was acquired using full scan MS followed by Top-4 ddMS^2 with polarity switching. An EASY-IC internal calibration was employed to ensure high mass accuracy throughout.

INSTRUMENT AND METHOD

Orbitrap Exploris 120 mass spectrometer

To capture impurities with different ionization preferences, data acquisition using rapid polarity switching is necessary for drug impurity profiling. In this study, data was acquired using full MS followed by top 4 DDA MS2 at resolution 60,000 (full MS) and 15,000 (MS2) respectively with polarity switching. The high scan speed of Orbitrap Exploris 120 enabled rapid data acquisition with duty cycle of ~1 second for total of 10 scan events, see Figure 1. As a result, information-rich HRAM full scan and MS/MS fragments of both polarities were obtained in a single run.

Figure 1. Fast Scan Speed for High Resolution Full Scan-DDA MS2 with Polarity Switching

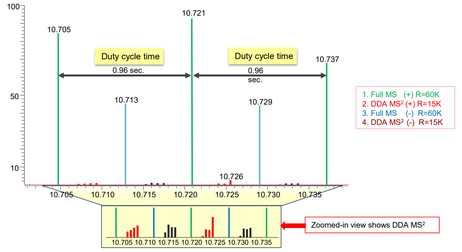


Figure 2. Full scan followed by 4 DDA with polarity switching and internal calibration method was set up using method template in method editor.

RESULTS AND DISCUSSION

High-Quality Full Scan/DDA MS2 Data with Polarity Switching

The MS total ion chromatogram with polarity switching and UV spectrum of Mycophenolate Mofetil (MMF) are shown in Figure 3. The HRAM positive/negative data provided confirmative information for formula mass and elemental composition of impurity at RT 5.97 min. In addition, the complementary polarity unique fragments aided in the definitive structure identification of this impurity, see Figure 4.

Figure 3. LCMS Chromatogram of Mycophenolate Mofetil (zoomed-in view)

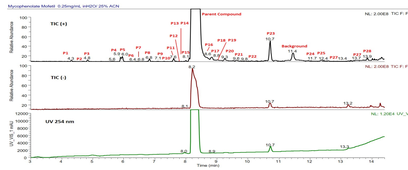
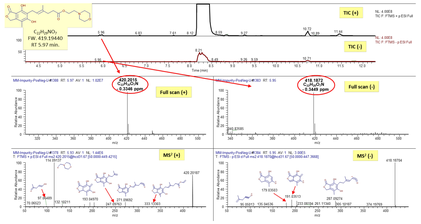


Figure 4. HRAM Full Scan – HCD MS2 with Polarity Switching in Single Run



DATA PROCESSING

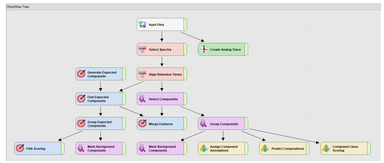
Compound Discoverer 3.2 Software

With HRAM full scan and HCD DDA MS2 data acquisition, data processing software with an effective data mining tool plays an important role for impurity identification and structure characterization.

In this study, the HRAM full scan and HCD DDA MS2 data was processed using Compound Discoverer 3.2 (CD 3.2), a small molecule structure analysis software which employs a flexible and customizable node-based processing workflow, see Figure 5.

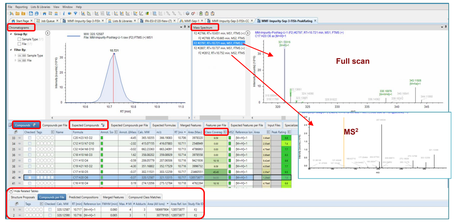
CD 3.2. utilizes accurate mass and isotope pattern for component extraction and elemental composition prediction. Based on the predicted formula, accurate mass, and the MS^n fragment spectra, CD 3.2 node-based workflow conducts targeted and untargeted compound identification through database search and user-defined approaches utilizing various application-specific nodes.

Figure 5. Compound Discoverer Impurity ID Processing Workflow Tree



This processing workflow captures expected and unexpected impurities using targeted and user-defined approaches, as well as unknown impurities based on relative abundance vs. blank, see Figure 6 for CD processing result view.

Figure 6. Compound Discoverer Result View



The known structure verification and unknown structure proposal are carried out using "Structure Proposal" and "FISh Scoring" (FISH = Fragment Ion Search) features. The validity of known compound and proposed unknown structures then is evaluated by its "FISh Coverage" score based on the number of matched and unmatched fragment ions.

Figure 7. Full Scan and Annotated MS2 Spectra of Identified Expected Compound

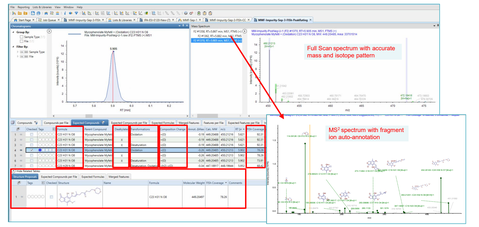
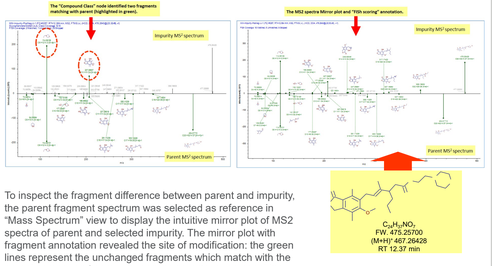


Figure 8. Unexpected Compound Impurity Identified Through Compound Class Feature



To inspect the fragment difference between parent and impurity, the parent fragment spectrum was selected as reference in "Mass Spectrum" view to display the intuitive mirror plot of MS2 spectra of parent and selected impurity. The mirror plot with fragment annotation revealed the site of modification: the green lines represent the unchanged fragments which match with the parent compound fragments, and the blue lines represent the fragments with modification. The impurity structure was proposed based on the fragment difference, see Figures 8 and 9.

Figure 9. Fragment Spectra Mirror Plot and Auto Annotation

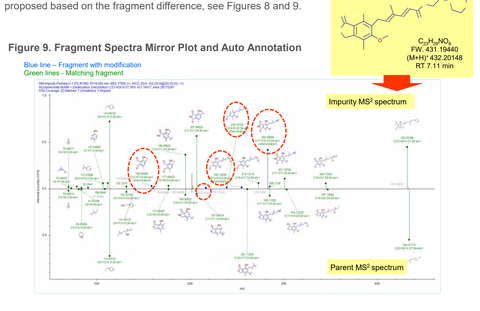
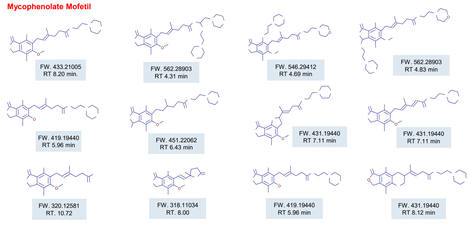


Table 1. Identified Impurities

Peak #	Tags	RT [min]	Formula	Calc. MW	m/z	DeltaMass [ppm]	By Compound Class	By Expected Compounds
1	A	4.31	C29 H42 N2 O9	562.28880	563.29608	-0.40	A	
2	A	4.69	C29 H42 N2 O8	546.29393	547.30121	-0.34	A	
3	A	4.84	C29 H42 N2 O9	562.28874	563.29602	-0.51	A	
4	D	5.90	C23 H31 N O8	449.20485	450.21213	-0.26		D
5	D	5.96	C22 H29 N O7	419.19428	420.20154	-0.34		D
6	D	6.43	C23 H33 N O8	451.22051	452.22778	-0.24		D
7	A	6.73	C23 H29 N O8	447.18923	448.19650	-0.20	A	
8	D	6.83	C23 H31 N O8	449.20485	450.21213	-0.26		D
9	D	7.11	C23 H29 N O7	431.19420	432.20148	-0.47		D
10	D	7.30	C23 H29 N O8	447.18920	448.19647	-0.27		D
11	D	7.61	C22 H29 N O7	419.19426	420.20154	-0.34		D
12	D	7.69	C17 H18 O6	318.11022	319.11749	-0.38		D
13	D	8.00	C17 H18 O6	318.11024	336.14407	-0.32		D
14	D	8.09	C23 H31 N O8	449.20485	450.21213	-0.26		D
15	D	8.12	C23 H29 N O7	431.19417	432.20145	-0.54		D
	MMF	8.20	C23 H31 N O7	433.21733	434.21460	-0.53		Parent Compound
16	D	8.61	C24 H34 N2 O6	446.24147	447.24875	-0.48		D
17	D	8.83	C24 H33 N O7	447.22554	448.23282	-0.36		D
18	D	8.85	C23 H31 N O8	449.20482	450.21210	-0.33		D
19	A	9.04	C27 H38 N2 O8	518.26262	519.26990	-0.38	A	
20	D	9.26	C25 H35 N O8	477.23598	478.24326	-0.60		D
21	A	9.52	C26 H37 N O8	491.25176	492.25903	-0.33	A	
22	D	10.72	C17 H20 O6	320.12587	321.13315	-0.37		D
23	D	10.88	C19 H22 O6	346.14153	347.14880	-0.32		D
24	D	11.72	C23 H33 N O6	419.23061	420.23788	-0.43		D
25	A	12.37	C26 H37 N O7	475.25673	476.26401	-0.57	A	
26	D	12.59	C23 H29 N O8	447.18923	448.19650	-0.20		D
27	A	13.72	C28 H39 N O7	501.27254	502.27982	-0.23	A	
28	D	13.85	C18 H22 O6	334.14149	335.14877	-0.44		D

Table 2. Proposed Structures of Identified Impurities (partial, see Table 1 for detail)

Mycophenolate Mofetil



CONCLUSIONS

Impurity analysis of Mycophenolate Mofetil was achieved using a workflow consisting of bench-top Orbitrap Exploris 120 mass spectrometer and Vanquish UHPLC system coupled with Compound Discoverer 3.2 software.

Exploris 120 MS high sensitivity, high dynamic range, and high mass accuracy enabled confident impurity profiling, especially for accurate identification of low abundant, trace level impurities in the presence of excessive amount of parent compound.

HRAM full scan and HCD MS2 spectra with polarity switching data acquisition generate a high quality dataset in a single run.

Compound Discoverer 3.2 software's advanced algorithm and versatile features enabled confident impurity ID and structure elucidation.

TRADEMARKS/LICENSING

PO66132 EN0921S