Poster Note 64483

Effective Workflow for Pharmaceutical API Impurity Analysis using HR-LCMS and Compound Discoverer

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Overview

Purpose: Demonstrate an effective workflow for pharmaceutical impurity identification using Thermo Scientific™ Orbitrap Elite™ mass spectrometer and novel node-based small molecule structure ID software Thermo Scientific ™ Compound Discoverer™ software.

Methods: LC-HRMS and Compound Discoverer software for Fexofenadine API impurity analysis.

Results: The Fexofenadine API impurity profile was quickly obtained.

Introduction

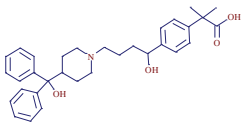
Pharmaceutical impurity analysis is crucial for drug R&D, production, and post-marketing surveillance. LCMS is routinely used for impurity analysis because of its speed and sensitivity. For rapid, accurate, and confident impurity ID, very high resolution mass spectrometer and effective data processing software are essential.

This study demonstrates an effective workflow for pharmaceutical impurity identification using very high resolution mass spectrometer and node-based small molecule structure ID software: Compound Discoverer software.

Methods

Sample Preparation

The commercial compound Fexofenadine (Sigma-Aldrich F9427-10MG, cas# 83799-24-0) was dissolved in 1:1 ACN/Water at a concentration of 0.3 µg/mL.



C32H39NO4
FW: 501.28791

Liquid Chromatography

HPLC system: Thermo Scientific™ Accela™ 1250 pump, Open Accela Autosampler and PDA

Column: Thermo Scientific™ Accucore™ C18 2.1x 150 columns, 2.6 µm. Injection volume: 5 µl

Mobile phases:
A - H_2O
B - Acetonitrile
C - H_2O with 0.05% Ammonium Hydroxide pH 9

Gradient :

Time (min.)	A%	B%	C%	ul/min
0	60	15	25	400
0.5	60	15	25	400
14.0	25	50	25	400
19.0	5.0	70	25	400
19.1	60	15	25	400
24.0	60	15	25	400

Mass Spectrometry

The high resolution accurate mass (HRAM) analysis was conducted on an Orbitrap Elite mass spectrometer equipped with a HESI II ion source. Full scan MS and top3 data-dependent MS/MS data were collected at resolutions of 120,000 and 15,000 respectively.

Ionization mode: ESI positive
Scan range: 160-1500 amu
Sheath gas flow rate (N_2): 45
Auxiliary gas flow rate (N_2): 10
Spray voltage (KV): +4.0 for positive
Capillary temp (°C): 300
S-lens RF level: 60.0
Heater temp (°C): 450

Thermo Scientific™ Orbitrap Elite™ mass spectrometer

Thermo SCIENTIFIC

Data Processing

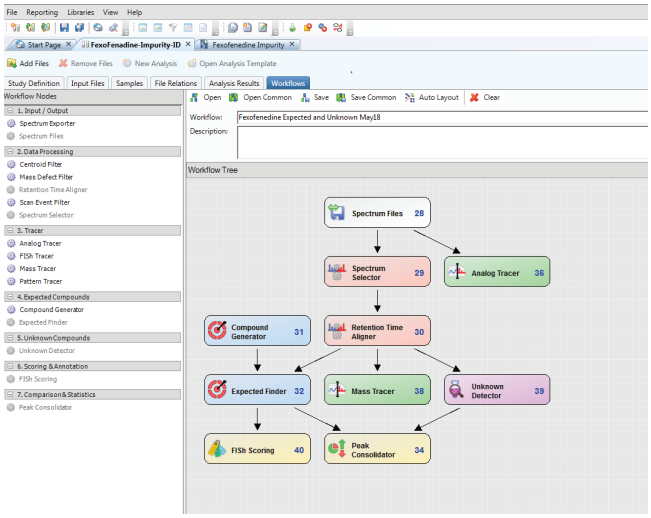
Node Based Processing Workflow in Compound Discoverer

The HRAM full scan and HCD ms/ms data acquired on an Orbitrap Elite MS was processed using Compound Discoverer (CD) software for Fexofenadine API impurity profiling.

Compound Discoverer (CD) software provides flexible processing workflows which are assembled from a suite of advanced algorithms (nodes). The drag-and-drop workflow editor allows greater control and visibility in terms of how data should be processed.

Most API impurities are structurally related to the API, but unrelated unknowns do occur. In this study, the CD processing workflow included the following nodes to ensure complete impurity identification: Using “Expected Finder” to get an expected ions list from “Compound Generator” node and detect expected compounds. Using “FISh Scoring” node for fragment ion matching and fragment structure annotations on spectra. “Unknown Detector” node was added to detect structurally unrelated impurities. “Peak Consolidator” node grouped the peaks detected from both expected and unknown mechanisms for quick comparison and more confident identification. See Figure 1.

FIGURE 1. Node Based Processing Workflow



Results

FIGURE 2. Base Peak Chromatogram of Fexofenadine in CD “Specialized Traces”

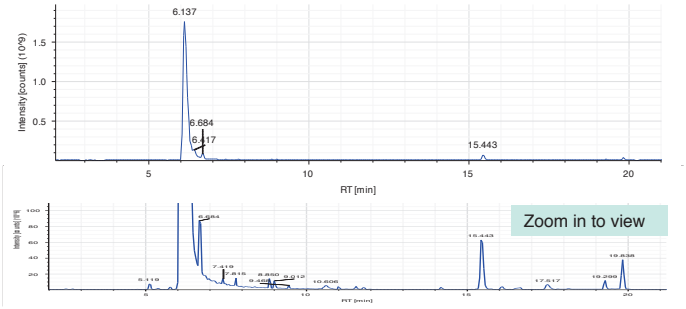
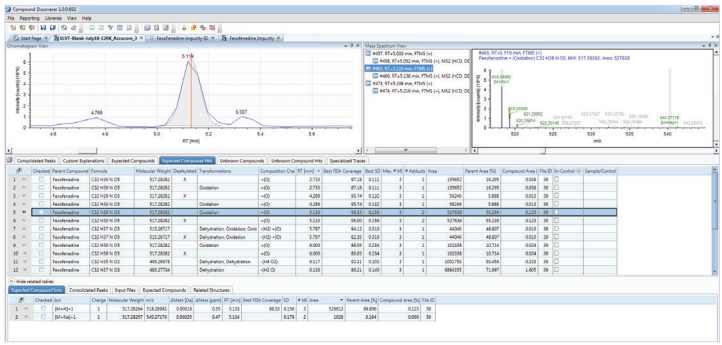


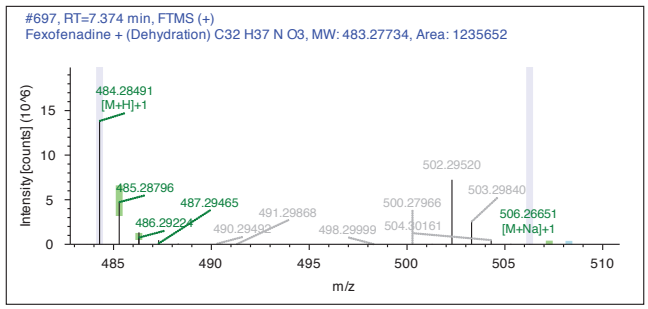
FIGURE 3. Results Review



Structure Characterization for Expected Compound Hits

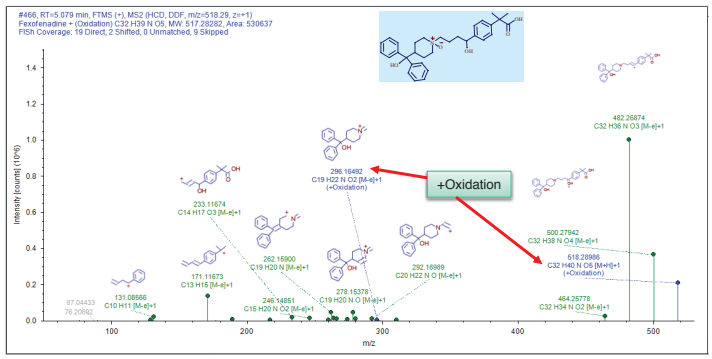
The detailed and comprehensive processing results are shown in Figure 3. It includes “Expected Compound Hits” and “Unknown Compound Hits” from Expected Finder node and Unknown Detector node respectively. An example of fine isotopic pattern confirmation of elemental formula assignment for “Expected Compound Hits” is shown in Figure 4. Color coding of isotopic fidelity gave greater confidence in elemental composition assignment from CD. Automatic adduct grouping reduced false positive hits.

FIGURE 4. Isotope Pattern Fidelity for Assigned Elemental Composition.



For each expected impurity hit, FISh Scoring automatically searched the fragmentation spectra, and annotated matching fragment structures directly on the spectra. The annotations are color-coded to visually indicate the transformation shifted ones for transformation localization, see Figure 5.

FIGURE 5. Expected Compound Hit with Automatically FISh Fragment Annotations



Structure Characterization for Unknown Compound Hits

For unknown compound hits, "Mass Spectrum View" showed the HRAM mass and corresponding ms/ms spectrum. The interested unknown compound s were added to a custom explanation table. Based on the HRAM fragmentation data, putative structures were propose in "Custom Explanation Editor" (Figure 7), followed by "FISh Scoring" on the fly, the unknown component ms/ms spectra were automatically annotated with matching fragment structures (Figure 6). "FISh Coverage" score indicated the percentage of fragment ion matching between experimental data and theoretical predictions from Mass Frontier™ Fragmentation Libraries™.

FIGURE 6. Unknown Compound Structure Elucidation

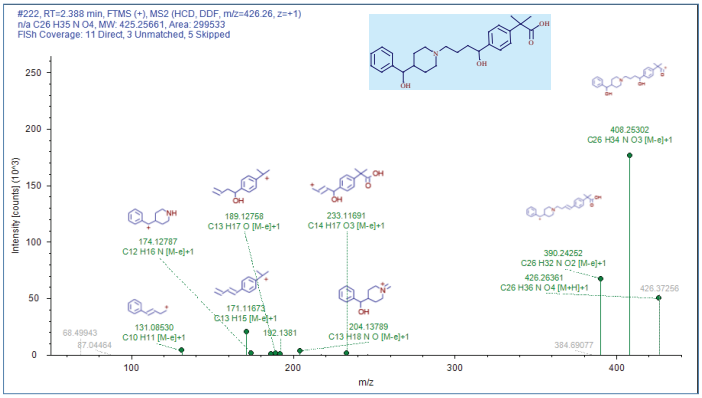
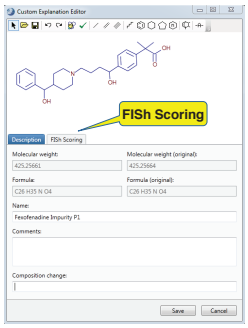
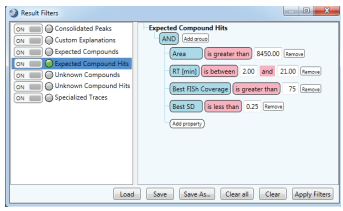


FIGURE 7. Custom Explanations Editor



The versatile and flexible "Result Filters" was used for quick data manipulation by selecting the criteria and options.

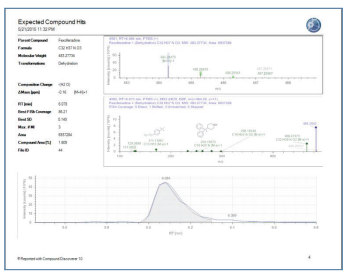
FIGURE 8. Result Filters



Data Reporting

The result was reported in the Expected and Custom explanation formats. For each identified impurity, it's isotope pattern, annotated ms/ms spectrum, transformation, Fish coverage, spectral distance, and others were included in the report, see Figure 9.

FIGURE 9. Reports of Fexofenadine Impurity: Expected and Custom Explanations



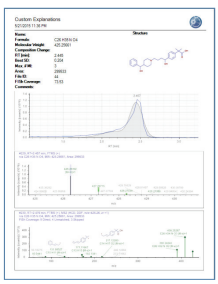
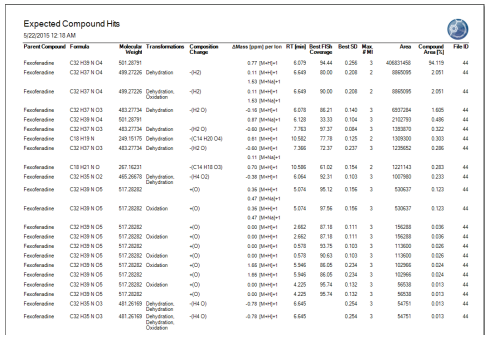


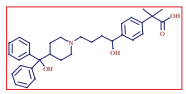
TABLE 1 Expected Compound Hits without Graphs (partial list)

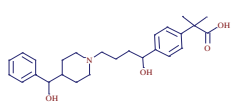

Expected Compound Hits

Parent Compound	Formula	Molecular Weight	Transformations	Composition Change	ΔMass (ppm) per Ion	RT [min]	Best FISh Coverage	Best SD	Max. # MI	Area	Compound Area [%]	File ID
Fexofenadine	C32 H39 N O4	501.28791			0.77 [M+H]+1	6.079	94.44	0.256	3	406831458	54.119	44
Fexofenadine	C32 H37 N O4	499.27226	Dehydration	-(H2)	0.11 [M+H]+1 1.63 [M+Na]+1	6.649	90.00	0.208	2	8865095	2.051	44
Fexofenadine	C32 H37 N O4	499.27226	Dehydration, Oxidation	-(H2)	0.11 [M+H]+1 1.63 [M+Na]+1	6.649	90.00	0.208	2	8865095	2.051	44
Fexofenadine	C32 H37 N O3	483.27734	Dehydration	-(H2 O)	-0.16 [M+H]+1	6.078	86.21	0.140	3	6937264	1.605	44
Fexofenadine	C32 H39 N O4	501.28791			0.87 [M+Na]+1	6.128	33.33	0.104	3	2102793	0.486	44
Fexofenadine	C32 H37 N O3	483.27734	Dehydration	-(H2 O)	-0.60 [M+H]+1	7.763	97.37	0.084	3	1383870	0.322	44
Fexofenadine	C16 H19 N	249.15175	Dehydration	-(C14 H20 O4)	0.81 [M+H]+1	10.582	77.78	0.125	2	1309300	0.303	44
Fexofenadine	C32 H37 N O3	483.27734	Dehydration	-(H2 O)	-0.60 [M+H]+1 0.11 [M+Na]+1	7.366	72.37	0.237	3	1235652	0.286	44
Fexofenadine	C16 H21 N O	267.16231		-(C14 H18 O3)	0.70 [M+H]+1	10.586	61.02	0.154	2	1221143	0.283	44
Fexofenadine	C32 H35 N O2	465.26678	Dehydration, Dehydration	-(H4 O2)	-0.39 [M+H]+1	6.064	92.31	0.103	3	1007660	0.233	44
Fexofenadine	C32 H39 N O5	517.28282		+(O)	0.56 [M+H]+1 0.47 [M+Na]+1	5.074	95.12	0.156	3	530637	0.123	44
Fexofenadine	C32 H39 N O5	517.28282		+(O)	0.56 [M+H]+1 0.47 [M+Na]+1	5.074	97.56	0.156	3	530637	0.123	44
Fexofenadine	C32 H39 N O5	517.28282		+(O)	0.00 [M+H]+1	2.662	87.18	0.111	3	156288	0.036	44
Fexofenadine	C32 H39 N O5	517.28282	Oxidation	+(O)	0.00 [M+H]+1	2.662	87.18	0.111	3	156288	0.036	44
Fexofenadine	C32 H39 N O5	517.28282		+(O)	0.00 [M+H]+1	0.578	53.75	0.103	3	113600	0.026	44
Fexofenadine	C32 H39 N O5	517.28282	Oxidation	+(O)	0.00 [M+H]+1	0.578	90.63	0.103	3	113600	0.026	44
Fexofenadine	C32 H39 N O5	517.28282	Oxidation	+(O)	1.66 [M+H]+1	5.946	86.05	0.234	3	102966	0.024	44
Fexofenadine	C32 H39 N O5	517.28282		+(O)	1.66 [M+H]+1	5.946	86.05	0.234	3	102966	0.024	44
Fexofenadine	C32 H39 N O5	517.28282	Oxidation	+(O)	0.00 [M+H]+1	4.225	95.74	0.132	3	56538	0.013	44
Fexofenadine	C32 H39 N O5	517.28282		+(O)	0.00 [M+H]+1	4.225	95.74	0.132	3	56538	0.013	44
Fexofenadine	C32 H35 N O3	481.26169	Dehydration, Dehydration	-(H4 O)	-0.78 [M+H]+1	6.645		0.254	3	54751	0.013	44
Fexofenadine	C32 H35 N O3	481.26169	Dehydration, Dehydration, Oxidation	-(H4 O)	-0.78 [M+H]+1	6.645		0.254	3	54751	0.013	44

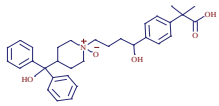
TABLE 2. Impurity Structures (Partial List)

Fexofenadine





$C_{32}H_{39}NO_4$
MF 501.2879
m/z 426.26389



$C_{26}H_{35}NO_4$
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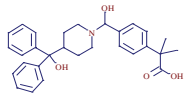
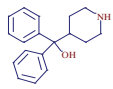
m/z 426.26389



$C_{28}H_{39}NO_5$
MF 517.2877
m/z 518.2901

$C_{26}H_{33}NO_4$
MF 459.2409
m/z 460.2482



$C_{19}H_{21}NO$
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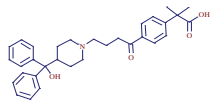
m/z 268.16959



$C_{32}H_{37}NO_4$
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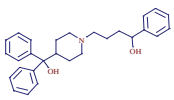

$C_{28}H_{33}NO_2$
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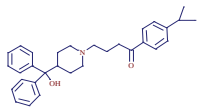
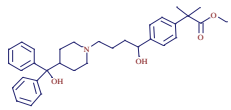
m/z 416.2584



$C_{31}H_{37}NO_2$
MF 455.2824
m/z 456.2897



$C_{33}H_{41}NO_4$
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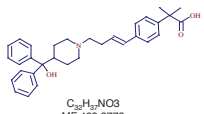
m/z 516.3108



$C_{32}H_{37}NO3$
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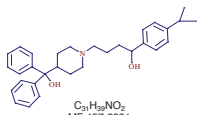
m/z 484.2846



$C_{31}H_{39}NO_2$
MF 457.2981
m/z 458.3054

$C_{28}H_{41}NO_2$
MF 487.3064
m/z 488.3159

Conclusion

- Effective and confident impurity analysis was achieved using very high resolution LCMS from the Orbitrap Elite mass spectrometer and Compound Discoverer software.
- Powerful workflow options in Compound Discoverer software detect components with targeted and untargeted mechanisms, and utilize very high resolution to quickly perform fine isotope searches. The determination of the structures of impurities is simplified with automatic FISh (fragment ion search) annotations.

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Netherlands +31 76 579 55 55
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