# Orbitrap Fusion Tribrid Mass Spectrometer for Pharmaceutical Impurity Analysis

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## OVERVIEW

**Purpose:** Demonstrate a workflow for API impurity identification and structure elucidation using very high resolution MS with multiple analyzers and fragmentation techniques couple with a customizable data processing software.

## INTRODUCTION

Impurity analysis is an integral part of drug R&D, required by regulatory agencies[1]. LCMS is routinely used for API impurities analysis because of its speed and sensitivity. A very high resolution mass spectrometer with multiple analyzers and dissociation techniques provides two dimensional, in-depth structure information, which is essential for impurity identification and structure elucidation.

This study demonstrates a workflow for API impurity profiling using a Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer and Thermo Scientific™ Compound Discoverer™ 2.0 small molecule structure analysis software.

## MATERIALS AND METHODS

### Material

The commercial compound Fexofenadine (Sigma-Aldrich) was dissolved in 1:1 ACN/Water at a concentration of 0.3 µg/mL.

### Liquid Chromatography

The liquid chromatographically separations were conducted on a Thermo Scientific™ UltiMate™ 3000 RS UHPLC system consisting of: DGP-3000RS pump, WPS-3000RS sampler, TCC-3000RS column compartment and DAD-3000RS UV detector.
Column: Thermo Scientific Accucore™ C18 (150x2.1 mm 2.6 µm)
olumn temp: 25ºC
Mobile phase: (A) water, (B) acetonitrile, and (C) water/0.05% ammonium hydroxide.

| Gradient: | Time (min) | A% | B% | C% |
|---|---|---|---|---|
| | 0 | 80 | 10 | 10 |
| | 0.5 | 80 | 10 | 10 |
| | 15.0 | 30 | 60 | 10 |
| | 16.0 | 10 | 90 | 10 |
| | 17.0 | 10 | 90 | 10 |
| | 17.1 | 80 | 10 | 10 |

Flow rate (µl/min): 400
Injection volume (µl): 2

### Mass Spectrometry

Orbitrap FusionTribrid mass spectrometer
Ion source: Thermo Scientific™ EASY-Max™ NG
Ionization mode: ESI positive
Sheath gas flow rate: 45 units N2
Auxiliary gas flow rate: 15 units N2
Spray voltage (KV): +3.5
Ion transfer tube temp (ºC): 350
S-lens RF level: 60.0
Heater temp (ºC): 250

## RESULTS

### MS Method

**Multiple analyzers and fragmentation techniques, method editor, and internal calibration**

The full scan and multiple MS/MS method was quickly built using the method editor by selecting the small molecule ID template from the templates library. HRAM data were acquired at high-resolution full scan (60,000 FWHM @ *m/z* 200) followed by data-dependent HCD MS$^2$ fragmentation, then data-dependent CID MS$^3$. Because of the parallel acquisition of the Orbitrap and linear trap analyzers, this method allowed access to the in-depth structure information without additional time.

The “EASY-IC” internal calibration was used, which generates internal calibrant ions for real-time mass calibration on every spectrum, and assured the mass accuracy was <1 ppm throughout.

**FIGURE 1. Orbitrap Fusion MS Method for Full Scan, HCD, and CID Acquisition**

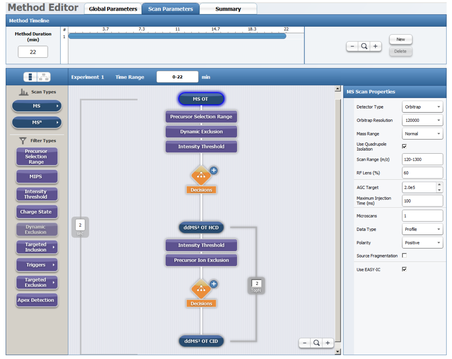



**Figure 2. Base Peak Chromatogram of Fexofenadine Impurity ID**

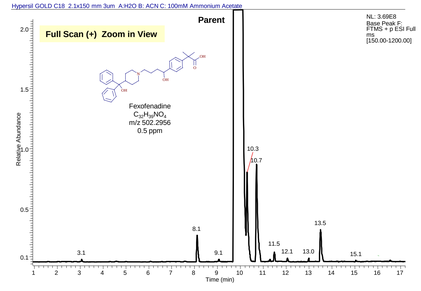
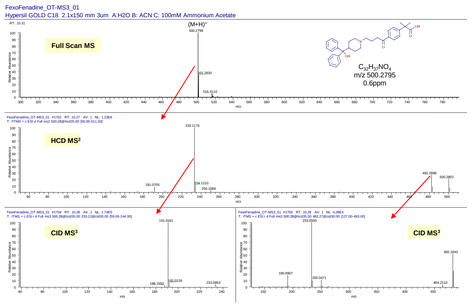



**FIGURE 3. HRAM Full Scan, HCD MS$^2$, and CID MS$^3$ for Impurity ID**

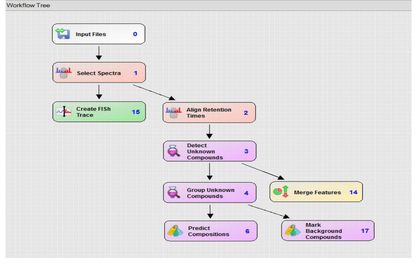



### Data Analysis

The HRAM full scan, HCD MS$^2$, CID MS$^3$ data acquired on the Orbitrap Fusion MS were processed using Compound Discoverer 2.0 (CD 2.0), a node-based small molecular structure analysis software, for Fexofenadine impurity identification and structure elucidation.

The HRAM full scan and MS/MS data, isotope pattern matching, as well as the MS/MS fragments were used for compound identification and structure elucidation. The node-based processing workflow was built by following the “New Study and Analysis Wizard”, which includes common small molecule analysis workflow templates. In this study, the workflow template “unknown compounds identification” was chosen (see Figure 4). In “Create FISh Trace” node, the Fexofenadine structure was selected, so its HCD MS/MS fragments would be used for reference to identify structurally related impurities.

**FIGURE 4. CD 2.0 Node Based Workflow for Fexofenadine API Impurity ID**




The comprehensive processing results are shown in Figure 5. For each identified compound, the predicted formula, mass accuracy, isotope pattern, and related information are listed in the table and sub-table. The results were filtered using the flexible “Result Filter”. The compounds with high matching fragments with parent compound, high mass accuracy, high spectral fit (Sfit) score, and high isotope matching (#MI) were added to the custom explanations table for structure elucidation.

The putative structure was proposed in the “Custom Explanation Editor”. The FISh Scoring feature (FISh stands for Fragment Ion Search) searched the embedded fragmentation library, and any matching fragment structures were auto-annotated on the spectra, see Figure 6.

**FIGURE 5. Result View Displays**

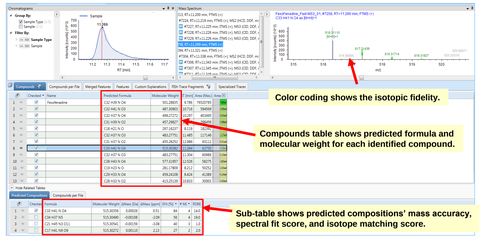



**FIGURE 6. Auto-Annotation**

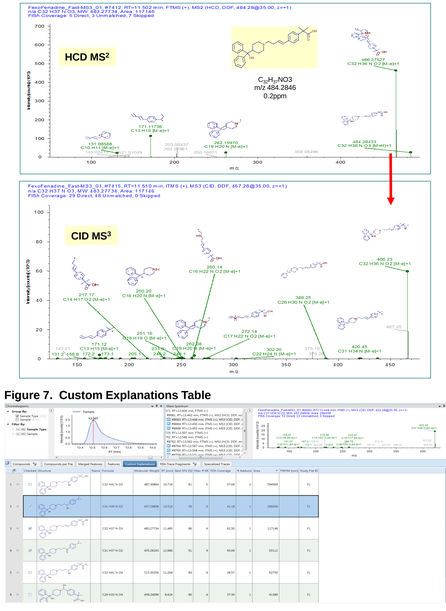



**Figure 7. Custom Explanations Table**



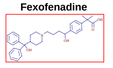
**TABLE 1. Impurities Identified Using Simple Unknown Workflow in CD 2.0**

| RT (min) | Peak ID | Molecular Formula | Calculated (M+H) | Measure (M+H) | Error (ppm)* |
|---|---|---|---|---|---|
| 9.8 | Parent | $C_{32}H_{39}NO_4$ | 502.2952 | 502.2954 | 0.5 |
| 8.1 | Impurity E | $C_{18}H_{21}NO$ | 268.1696 | 268.1696 | 0 |
| 8.2 | New | $C_{18}H_{23}NO$ | 282.1852 | 282.1854 | 0.6 |
| 8.4 | New | $C_{29}H_{33}NO_4$ | 460.2482 | 460.2483 | 0.2 |
| 10.3 | Impurity A | $C_{32}H_{37}NO_4$ | 500.2795 | 500.2798 | 0.6 |
| 11.3 | Impurity D | $C_{33}H_{41}NO_4$ | 516.3108 | 516.3111 | 0.5 |
| 11.6 | New | $C_{32}H_{37}NO_3$ | 484.2846 | 484.2847 | 0.2 |
| 13.0 | New | $C_{31}H_{37}NO_2$ | 456.2897 | 456.2898 | 0.2 |
| 6.9 | New | $C_{30}H_{35}NO_4$ | 426.2639 | 426.2640 | 0.3 |
| 10.6 | New | $C_{31}H_{37}NO_2$ | 488.3159 | 488.3162 | 0.6 |
| 13.5 | Impurity C | $C_{31}H_{39}NO_2$ | 458.3054 | 458.3055 | 0.4 |
| 12.5 | New | $C_{38}H_{43}NO_4$ | 578.3265 | 578.3268 | 0.6 |

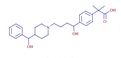
* Sub-ppm mass accuracy throughout.

**Table 2. Proposed Structures Using Custom Explanation and FISh Scoring Feature**

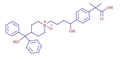
**Fexofenadine**



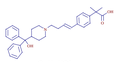
$C_{32}H_{39}NO_4$
MF 501.2879
m/z 502.2956



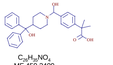
$C_{31}H_{37}NO_4$
MF 425.2566
m/z 426.26389



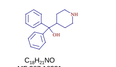
$C_{31}H_{37}NO_3$
MF 517.2871
m/z 518.2901



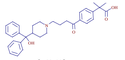
$C_{31}H_{37}NO3$
MF 483.2773
m/z 484.2846



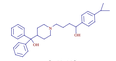
$C_{29}H_{33}NO_4$
MF 459.2409
m/z 460.2482



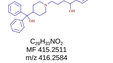
$C_{18}H_{21}NO$
MF 267.16231
m/z 268.16959



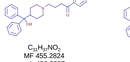
$C_{32}H_{37}NO_4$
MF 499.2723
m/z 500.2795



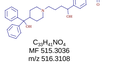
$C_{32}H_{39}NO_4$
MF 457.2981
m/z 458.3054



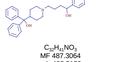
$C_{30}H_{33}NO_4$
MF 415.2511
m/z 416.2584



$C_{31}H_{37}NO_4$
MF 455.2824
m/z 456.2897



$C_{30}H_{33}NO_4$
MF 515.3036
m/z 516.3108



$C_{31}H_{37}NO_4$
MF 487.2981
m/z 488.3159

## CONCLUSIONS

This study demonstrates a workflow for API impurity identification and structure elucidation using the very high resolution Orbitrpa Fusion Tribrid MS coupled with data processing by Compound Discoverer 2.0 small molecule structure analysis software.

The results shown that:
1.The very high resolution data acquisition not only generated high mass accuracy, it also allowed access to isotope fine structure information for accurate elemental composition determination.
2.The multiple analyzers and fragmentation techniques enable flexible data acquisition: parallel, tandem, and any stages of CID/HCD MS$^n$ fragmentations, which generated in-depth structural information for confident structure characterization.
3.The Easy IC internal calibration feature ensures sub-ppm mass accuracy throughout.
4.Compound Discoverer 2.0 software advanced algorithms fully utilize the HRAM full scan and ms/ms data, resulting in confident impurity ID and structure elucidation.

## REFERENCES

1.FDA Guidance for Industry Q3A Impurities in New Drug Substances June 2008 ICH

## TRADEMARKS/LICENSING