Application of HRMS and Advanced Data Processing in Metabolite Profiling of Ibrutinib

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Overview

Identification and characterization of oxidative metabolites and reactive metabolites trapped with labeled glutathione (GSH) using high resolution mass spectrometry (HRMS) and novel data analysis software.

Introduction

Residue metabolite trapping using glutathione (GSH) and analysis using HRMS have enabled screening for reactive metabolite and oxidative metabolite occurrence by drug discovery. Testing quality assay and analyze relevant drug information from large HRMS datasets, efficient data processing is essential.

In this study, a workflow consisting of information data collection using HRMS and novel data mining and structure analysis software for comprehensive data analysis was developed for confident and rapid active metabolite identification. The principal aim of the present work is to demonstrate the metabolite profiling workflow because of its extensive metabolite and bioactivation.

Materials and Methods

Sample Preparation

Tailing compound bratlibel, ca. #1966000 Wt., was purchased from Selleck-Chemicals (p/n PC-07265).

Elemental (HR) MS/MS was used. Column (HPLC): hexafluorobenzene-treated 1% acetonitrile post-run was the presence of NO 300 °C (1.0 µm) GRACE 1.0 µm of various chiral GSH and stable isotopic labelled GSH (for C, C, Cl, and TMS, at 0.75 °C). The samples were separated by ionization of the target groups, followed by centrifugation at 20,000 g for 15 minutes. The supernatant was concentrated to the original volume under a stream of nitrogen and analyzed by LC/HRMS.

Liquid Chromatography

Chromatographic separations were carried out on Shimadzu Xevo TQ system and an ICAP autosampler.

Column: Phenomenex Kinetex® C18 (1.7 µm, 2.1 x 150 mm).

Mobile phase: Isocratic 0.1% formic acid/0.1% H3PO4/hexane/ACN (90:10:9).

Flow rate: 0.4 mL/min.

Result and Discussion

Comprehensive HRMS Data acquisition to identify oxidative metabolites and GSH adducts

The combination of comprehensive HRMS with advanced data processing has been demonstrated to be an effective method to identify and characterize oxidative metabolites in the rat and human liver samples from the treatment of Ibrutinib (77-184-1).

Figure 1. Compound Discoverer 2.0 Node-based Workflow

Characterization of Oxidative Metabolism

For stable oxidative metabolite, the compound specific UV absorption at 380 nm was used to identify the ablation drug metabolism components in isolation standard. The percentage of UV drug peaks (%UV) was calculated for semi-quantification of these metabolites (Table 1). The HRMS extracted on a chromatogram of oxidative metabolites and negative oxidation were automatically summed and matched with the UV absorption of the identified compound.

The analysis resulted in comprehensive characterization for the identified compounds with matched formula. The UV-based MS/MS signal displays the color-coded isoform ratio pattern for confirmation. The ‘Metabolite Finder’ software allows very high identity between the spectral ions of identified GSH adducts and their derived ions.

Table 1. Oxidative Metabolites of Ibrutinib Formed in Liver Micromes

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<tr>
<th>Metabolite</th>
<th>RT (min)</th>
<th>Molecular Weight</th>
<th>M/Z (Da)</th>
<th>ID</th>
<th>% UV</th>
<th>10% Abs.</th>
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Conclusions

This work demonstrates a workflow that uses highly efficient HRMS data acquisition and in-depth data mining software. It significantly enhances speed and confidence in complex metabolite identification and structure characterization in drug discovery and development, especially for the metabolites bearing unique isotopic patterns.

As a result, eleven oxidative metabolites of Ibrutinib were detected and characterized by data mining of the HRMS and MS/MS spectra in both positive and negative mode. Moreover, fifteen lower abundant GSH and CysGly adducts were identified by a highly sensitive and selective filtering tool based on the unique isotopic pattern of stable isotopic labeled GSH. This approach successfully characterized two unique CysGly adducts formed via biodegradation of the prodrug moiety, which is not yet reported in the literature.

References