Simultaneous Quantitation of Delamanid (OPC-67683) and its Eight Metabolites in Human Plasma Using UHPLC-MS/MS

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Introduction

Delamanid (OPC-67683) is a novel nitro- dihydroimidazo-oxazole derivative that is being developed by Otsuka Pharmaceutical Co. Ltd., Japan, for the treatment of multi-drug resistant tuberculosis (TB). An ultra-high performance liquid chromatographic-mass spectrometry (UHPLC-MS/MS) method has been developed and validated for the determination of OPC-67683 and its eight metabolites, DM-6704, DM-6705, DM-6706, DM-6717, DM-6718, DM-6720, DM-6721 and DM-6722 in human plasma to support regulatory trials. This method was fully validated over the calibration curve range 1.00 to 500 ng/mL for over ten thousand samples have been analyzed using this bioanalytical method.

Methodology

Sample Preparation and Extraction

1. Aliquot 50 µL of samples to corresponding wells of a 96-well deep well plate.
2. Add 50.0 µL of working internal standard.
3. Add 300 µL of MeCN
4. Transfer 200 µL of supernatant using Hamilton® Microlab® NIMBUS® 96 channel liquid handling robot

Chromatographic Conditions

UHPLC system: Acquity UPLC BEH C18 1.7 µm, 2.1 x 50 mm, was selected. Ionization mode: Turboionspray, positive ion mode. AS Temperature: 5°C. Injection volume: 5μL. LC program: Gradient. DM-6721 483.1→305.2 DM-6722 482.1→352.2 DM-6717 481.1→303.2 DM-6718 480.1→352.2 OPC-67683 535.1→352.2 DM-6704 467.1→289.2

Results and Discussion

Chromatography Development

The LC development was very challenging: (1) there are nine analytes, instability and conversion, (2) there are robust peak shapes, (3) there are high recoveries, (4) there is a strong matrix effect. During initial LC development, endogenous phospholipid (PLs) eluted randomly. SRM transitions were monitored. It was found that with a very shallow gradient 65-75% B, the PLs peaks elute out randomly. Therefore, a forward flush was incorporated. Using new shallow gradient 65-75% B, the PLs peaks elute out randomly. During initial LC development, a Waters Xterra C18 column, Waters BEH C18 1.7 µm, 2.1 x 50 mm, was selected. During initial LC development, endogenous phospholipid (PLs) were observed (Figure 3). Therefore, a forward flush was incorporated. Using new shallow gradient 65-75% B, the PLs peaks elute out randomly. The LC development was very challenging: (1) there are nine analytes, instability and conversion, (2) there are robust peak shapes, (3) there are high recoveries, (4) there is a strong matrix effect.

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Assay Validation

Accuracy/precision: Demonstrated at n=6 at LLOQ, Low, Medium, High concentrations over 3 validation runs. (Tables 1-2) Additional MV tests were conducted and data not shown: are: LLOQ reproducibility (8 b lots), Selectivity (8 b lots), Ability to dilute, Extraction recovery, Matrix factor, Carryover, Stock solution stability, Hemolysis test, Stability in matrix, Matrix interference, etc.

Table 1. Methodology and Assay Validation

<table>
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<th>Parameter</th>
<th>Low QC</th>
<th>Medium QC</th>
<th>High QC</th>
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Sample Analysis

This method has been used to support regulatory trials during the last decade, and over ten thousand samples have been analyzed using this bioanalytical method.

Representative chromatograms: Control blanks (Figure 4), LLOQ (Figure 5)

Conclusion

A bioanalytical method was developed to simultaneously quantify Delamanid (OPC-67683) and eight of its metabolites in human plasma using UHPLC-MS/MS.