Development of a UPLC-MS/MS Method for the Determination of MLN9708 and its Enantiomer from Human Plasma

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INTRODUCTION

The purpose of this work was to develop a chirally selective method for the quantitative analysis of MLN9708 (with a boronic acid moiety at its chiral center) and its enantiomer from EDTA human plasma. Due to the inadequate retention of MLN9708 and its enantiomer on reverse phase columns, the chiral separation was achieved on a Chiralpak IC™ column with a normal phase retention mechanism in neat analyte solutions. However, the condition was not suitable for the extracts from human plasma samples due to the observation of highly noisy chromatography baselines and shifted retention time. This issue associated with the extracted samples cannot be resolved by modifying the method despite several attempts. Besides, due to the broad analyte peaks, achieving the target LOD could be challenging even if a compatible extraction method could be developed. To overcome the challenges, the approach is to convert MLN9708 and its enantiomer with a chiral derivatization reagent (1S,2S,3R,5S)-(+)-pinanediol into diastereomers and have them chromatographically separated by UPLC achiral column. Indeed, an LC condition with Acquity UPLC® BEH Phenyl column gave not only baseline separation but also clean baseline. In addition, better sensitivity was achieved in this approach due to much narrower peaks. The method was successfully applied to the analysis of MLN9708 and its enantiomer from human plasma samples.

METHODS

The quantification utilized 13C4-MLN9708 as the internal standard. The plasma samples were extracted by solid-phase extraction (SPE) with Waters Oasis WAX 96-Well SPE Plates (30 mg). The extracted samples were re-dissolved in (1S,2S,3R,5S)-(+)-pinanediol solution [2 mg/mL in ACN, and FA at a flowrate of 0.4 mL/min,. The pinanediol derivatized MLN9708 and its enantiomer were eluted with a mixture of 10 mM ammonium formate (pH 3.0), A: Acetonitrile:Water:Formic Acid @ 20:80:0.1, V:V:V] and incubated at 37 °C for approximately 1 hour. The samples were then injected onto an Acquity UPLC® BEH Phenyl column. MLN9708 and its enantiomer were eluted with a mixture of 30 mM ammonium formate (pH 3.0), ACN, and FA at a flow rate of 0.4 mL/min. The pinanediol derivatized MLN9708 and its enantiomer were detected by an AB Sciex API 6500 mass spectrometer.

RESULTS

For both analytes, the method is capable of achieving a lower limit of quantification (LLOQ) of 0.5 ng/mL, the same LLOQ achieved by an achiral assay of MLN9708. The calibration range of the method was 0.5-500 ng/mL for both analytes. The linearity, accuracy, and precision were demonstrated by a successful qualification run. The methods also demonstrated good selectivity and minimum matrix effect. The qualified method was utilized for the analysis of non-GLP samples, examining the existence of MLN9708 enantiomer from subjects dosed with MLN9708.

Chromatogram of an Extracted LLOQ Level Sample