The purpose of this work was to develop a chirally selective method for the quantitative analysis of MLN9708 and its enantiomer from 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 LLOQ 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 peaks, achieving the target LLOQ could be challenging even if a compatible extraction method was successfully applied to the analysis of MLN9708 and its enantiomer from human plasma samples.

Sample: plasma samples.

Column: Chiralpak IC™, 2.0×150 mm, 5 µm

Mobile Phases: A: Heptane, B: Isopropanol/ Ethanol @ 80:20 (V:V)

Sample: MLN9708/MLN9708 enantiomer, 500/500 ng/mL in Acetonitrile:Water:Formic Acid @ 20:80:0.1 (V:V:V)

Plate Activated w/ MeOH Equilibrated with Water

Sample Loading

Plate Washed w/ H2O Sample Eluted w/ ACN: NH4OH@100:1 (V:V)

Adding Derivatization Agent 37 °C, 1 hr

Dry Down @ 40 °C

Reconstitution in ACN:H2O at 20:80 (V:V)

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**NOVEL ASPECT**

Identified an excellent agent for the derivatization of the boronic acid moiety to overcome the challenges on chromatography, chiral separation and needed sensitivity at the same time.

**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.

**INTRODUCTION**

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

**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 MeOH:NH4OH@100:1 (V:V)] after dry-down.

**MATERIALS AND METHODS**

**Sample:** ULOQ level human plasma sample extracted by LLE (vialdiluted by the achiral assay) and re-dissolved in IPA.

**Sample:** ULOQ level human plasma sample extracted by SFE (WAX) and redissolved in IPA.

**Plate Activated w/ MeOH Equilibrated with Water**

**Sample Loading**

**Plate Washed w/ H2O Sample Eluted w/ ACN:NH4OH@100:1 (V:V)**

**Adding Derivatization Agent 37 °C, 1 hr**

**Dry Down @ 40 °C**

**Reconstitution in ACN:H2O at 20:80 (V:V)**

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**LOW ANTELO Signal (<10% of Theoretical)!**

- Low recovery (~5%) with ACN:NH4OH@100:1 (V:V) elution, but with unstable derivatization product
- Can not be re-dissolved in ACN:NH4OH@100:1 (V:V) after dry-down
- Best reconstitution solvent (ACN:Water:FA) does not appear to be favored by the derivatization reaction per literatures

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**WILL THE REACTION HAPPEN UNDER ACIDIC CONDITION?**

**Derivatize in ACN : Water : FA @ 20:80:1 (V:V:V), 37 °C, 1 hr**

Chromatogram of an Extracted LLOQ Level Sample