Bioanalysis of Emixustat (ACU-4429) in Whole Blood Collected with Volumetric Absorptive Microsampling by LC–MS/MS

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Methods

Blood was spiked with selected concentrations of Emixustat (ACU-4429) and IS with and without Anticoagulant. The LLOQ was 50 ng/mL and the ULOQ was 500 ng/mL. Emixustat and IS were dissolved in MeOH and stored at −80 °C until analysis. Samples were vortex-mixed for 15 minutes before being centrifuged at 2,000 × g for 10 minutes at 4 °C.

Sample Preparation

500 μL of whole blood was centrifuged at 2,000 × g for 10 minutes and the supernatant was aspirated into a 2 mL amber vial. The samples were then vortex-mixed for 15 minutes and centrifuged at 2,000 × g for 10 minutes at 4 °C. The supernatant was aspirated into a 2 mL amber vial and stored at −80 °C.

Results

Precision and Accuracy

The accuracy and precision were evaluated for four concentration levels in the range of 0.100 ng/mL to 500 ng/mL. The intra-assay precision and accuracy were within 15% and 20% respectively, and the inter-assay precision and accuracy were within 20% and 25% respectively (Table 1).

Stability

The samples were stored at −80 °C, −20 °C, 4 °C, Room Temperature, and Refrigerated conditions for 28 days and then analyzed. The samples were also stored at 10 °C and 37 °C for 10 days and then analyzed. The samples were also stored at 25 °C and 40 °C for 24 hours and then analyzed. All samples were within ±10 % of the original concentration (Table 2).

Summary

The described method is robust and reliable for the quantitation of Emixustat in whole blood collected by Mitra™ volumetric absorptive microsampling devices. The validated method can be used for PK sample collection during late-stage clinical trials.

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