LC-MS/MS Method of Fingolimod in Human Whole Blood: Is Sensitivity and Required Blood Volume an Issue?

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Purpose

The current LC-MS/MS method outline the process for highly sensitive assay of fingolimod from human whole blood in K2-EDTA. Currently available methods within the industry are sensitive enough to achieve the LLOQ of 50 pg/mL only, using high sample volume ranges between 0.7mL to 1mL, which leads to high volume of blood drawn from the volunteers during clinical trials. Our method has been successfully developed to achieve a high sensitivity at 5pg/mL and using 0.35mL sample volume only. Selectivity, matrix factor, matrix effect, recovery, robustness and stabilities were evaluated during vigorous approach of validation.

Methods

Blood volume of 0.35ml of sample spiked with 50µl of WIS and 0.6ml of buffer. The mixture buffer was loaded on Solid supported liquid-liquid extraction plate. Analyte and Internal Standard (IS) were extracted using Methyl-t-Butyl Ether. After evaporation to dryness, the samples were reconstituted with mobile phase. Extracted samples were injected on LC-MS/MS 5500 AB Sciex platform.

Results

The new bioanalytical method for fingolimod was validated and the results are as discussed below in tabular format. The method was also tested for accuracy, precision, and robustness.

Recovery of Analyte/IS	40%/38.4%
Matrix factor IS normalized	1 with %CV of 1.0
Freeze Thaw stability -70°C/4°C	3 cycles
Bench Top stability at 4°C	72 hours
Long Term stability at -70°C	88 days
Post Preparative stability at RT and 4°C	114 hours
Solution stability at RT	21 hours

Conclusion

Highly sensitive and selective assay of fingolimod from whole blood was developed and validated to achieve a limit of quantitation of 5pg/mL and using only 0.35ml of blood per assay. The method was proved to be accurate, precise and robust during the method validation. It can be reliably used in clinical trials using very low dose of fingolimod such as the daily dose of 5 mg.