

ABSTRACT

The present study was aimed at developing a simple, sensitive, and specific liquid chromatography-tandem mass spectrometry method for the quantification of rifaximin in human plasma using rifaximin D6 as internal standard. Chromatographic separation was performed on Zorbax SB C18, 4.6×75 mm, 3.5 μm column with an isocratic mobile phase composed of 10 mM ammonium formate (pH 4.0) and acetonitrile in the ratio of (20:80 v/v), at a flow-rate of 0.3 mL/min. Rifaximin and rifaximin D6 were detected with proton adducts at m/z 786.4→754.4 and 792.5→760.5 in multiple reaction monitoring positive mode respectively. The acidified samples were subjected to liquid-liquid extraction using a mixture of methyl t-butyl ether-dichloromethane (75:25) followed by centrifugation, nitrogen-aided evaporation and reconstitution. The method was validated over a linear concentration range of 20-20000 pg/mL with correlation coefficient of more than 0.9995. This method demonstrated intra and inter-day precision within 0.6-2.6% and 2.2-5.6%, and accuracy within 95.7-104.2% and 95.8-105.0% for rifaximin, respectively. Rifaximin was found to be stable throughout freeze-thawing cycles, bench top and postoperative stability studies. This method was applied successfully for the analysis of blood samples following oral administration of rifaximin (200 mg) in 17 healthy Indian male human volunteers under fasting conditions.