

# QUANTIFICATION OF PYRAZINAMIDE IN HUMAN PLASMA: DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD

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## ABSTRACT

A simple and efficient liquid chromatography-tandem mass spectrometry method was developed to quantify pyrazinamide in human plasma, using pyrazinamide D3 as the internal standard. Plasma samples were extracted with Hi-purity MCX cartridges via solid phase extraction. Chromatographic separation of pyrazinamide and the internal standard was performed on a Hypurity C8 column (100×4.6mm, 5μ) using isocratic conditions at 30 °C. The mobile phase consisted of an organic mixture and 10 mM ammonium acetate (50:50 V/V) at a flow rate of 1 mL min<sup>-1</sup>. Pyrazinamide and the internal standard eluted at 1.44 and 1.43 minutes, respectively, within a 3-minute runtime. Detection employed the use of a Hypurity HPLC column coupled with an AB Sciex API 4000 tandem mass spectrometer with electrospray ionization. Pyrazinamide was detected via ion transition 124.2 to 81, while the IS used ion transition 127.2 to 84. The method was validated as per European Medicine Agency guidelines, meeting acceptance criteria.