A simplified LC-MS/MS method for determination of isoniazid and pyrazinamide in human plasma using protein precipitation technique and its application to a pharmacokinetic study

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A sensitive and rapid liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed to simultaneous plasma drugs level measurement of Isoniazid (INH) and Pyrazinamide (PZA) first line anti-tuberculosis. Samples with the spiked drug standards were extracted using protein precipitation and then separated on ACQUITY UPLC BEH amide column (2.1 x 150 mm 1.7 μ m) under isocratic elution of 10 mM ammonium acetate in water and acetonitrile (60:40 v/v) at flow rate of 0.400 ml/min. Detection of all analytes was performed under positive ionization mode by multiple reaction monitoring (MRM). The analytical method was achieved within a run time of 10 minutes. The inter-assay calibrated variability data were obtained through concentrations of 1-100 μ g/ml for INH and PZA. The calibration curves were tested a linear 100-fold concentration range (r^2 > 0.995). Within-run and between-run precision (%CV) at concentration corresponding to LLOQ medium and high levels for two drugs were 1.45-17.83 % and 2.03-12.37 % respectively at concentration representing low medium and high levels for two drugs. The performance of our MS/MS detection technique was generally acceptable. The method was applied to a pharmacokinetic study in 30 tuberculosis Thai patients.