

Direct measurement of active thiol metabolite levels of clopidogrel in human plasma using tris (2-carboxyethyl) phosphine as a reducing agent by LC-MS/MS

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A simple, robust, and rapid liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for the simultaneous quantitation of clopidogrel and its active metabolite (AM) in human plasma. Tris (2-carboxyethyl) phosphine (TCEP) was used as a reducing agent to detect the AM as a disulfide bonded complex with plasma proteins. Mixtures of TCEP and human plasma were deproteinized with acetonitrile containing 10 ng/mL of clopidogrel-d4 as an internal standard (IS). The mixtures were separated on a C₁₈ reversed-phase column with an isocratic mobile phase consisting of 0.1% formic acid in acetonitrile and water (90:10, v/v) at a flow rate of 0.3 mL/min. Detection and quantification were performed using a mass spectrometer with a positive electrospray ionization source. The detector was operating in selected reaction-monitoring mode at m/z 322.0→211.9 for clopidogrel, m/z 356.1→155.2 for the AM, and m/z 326.0→216.0 for the IS. The linear dynamic range for clopidogrel and its AM were 0.05–20 and 0.5–200 ng/mL, respectively, with correlation coefficients (r) greater than 0.9976. Precision, both intra- and inter-day, was less than 8.26% with an accuracy of 87.6–106%. The validated method was successfully applied to simultaneously analyze clinical samples for clopidogrel and its AM.

Biography

Jung Bae Park is a Ph.D. candidate in drug metabolism and pharmacokinetics in the Catholic University of Korea. He graduated from the Catholic University of Korea in 2013, where he majored in pharmacokinetics.

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