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VALIDATED LC-MS/MS METHOD FOR THE DETECTION AND CHARACTERIZATION OF IN-VITRO, IN-VIVO AND REACTIVE METABOLITES OF PONATINIB WITH METABOLIC STABILITY AND BIO-ACTIVATION PATHWAY ELUCIDATION

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Ponatinib (PNT) is new oral tyrosine kinase inhibitor (TKIs) that acts mainly by inhibiting vascular endothelial growth factor receptor (VEGFR). PNT is registered in the US and EU under the trade name of Iclusig®. A sensitive gradient LC–MS/MS method was established for Identification and characterization of in-vitro, in-vivo and reactive metabolites of PNT. In-vitro, reversed phase liquid chromatography resolved seven PNT metabolites. Since bio-activation is often speculated to be responsible for observed toxicities including hepatotoxicity, incubation of PNT with RLMs was carried out in the presence of 1.0 mM KCN to check its reactive metabolites. Four cyano adduct metabolites were determined and their structures were proposed. In-vivo, metabolism of PNT was done using Sprague Dawley rats kept inside metabolic cages. Single oral dose of ponatinib (4.7 mg/Kg) was given to each rat using oral gavage. Urine was collected and filtered at 0, 6, 12, 18, 24, 48, 72, 96 and 120 hours from PNT dosing. 13 in-vivo phase I and one in-vivo phase II metabolites of ponatinib were characterized. A rapid, sensitive and validated isocratic LC–MS/MS method was developed for the quantification of ponatinib in rat liver microsomes (RLMs) with its application to metabolic stability. PNT disappeared rapidly in the 1st 10 minutes of RLM incubation and the disappearance plateaued out for the rest of the incubation. In-vitro half-life (t1/2) was 6.26 min and intrinsic clearance (CLint) was 15.2. Chromatographic separation of PNT and vandetanib (IS) were accomplished on Agilent eclipse plus C18 analytical column (50 mm×2.1 mm, 1.8 µm particle size) maintained at 21±2°C. Flow rate was 0.25 mL/min with run time of 4 min. Mobile phase consisted of solvent A (10 mM ammonium formate, pH 4.1) and solvent B (acetonitrile).

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