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Quantification of lopinavir and ritonavir in dried blood spots using liquid chromatography-triple quadrupole mass spectrometry

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Lopinavir/ritonavir is widely used as part of combination antiretroviral treatment in HIV-infected adults and children in Thailand. Lopinavir is a HIV protease inhibitor and is administered with low dose ritonavir to enhance its bioavailability. Antiretroviral drug measurement can be useful for the clinical management of patients with drug toxicities, drug-drug interactions, as well as optimization of dosing for pregnant women and young children. Drug measurements are performed using plasma samples and they require storage and shipping under frozen conditions. Dried blood spots (DBS) is an alternative sample matrix for drug measurement as they can be stored at room temperature and shipped in the normal post. We developed and validated a liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS) assay to measure lopinavir and ritonavir from DBS. Sample preparation involved a liquid-liquid extraction. Chromatographic separation was performed on a Gemini Polar Reversed Phase C18 column (150 x 2.0 mm ID, 5µm) using a stepwise gradient. The calibration curve was linear over the range 0.05 to 20 µg/mL. The lower limit of quantification was 0.05 µg/mL. The assay average accuracy was 102-112% for lopinavir and 90-112% for ritonavir. The assay precision (inter- and intra assay) expressed as coefficient of variation (%CV) was <5% for lopinavir and <8.0% for ritonavir. The recoveries for lopinavir and ritonavir were 82.1% and 102.6%, respectively. Both drugs were stable in DBS stored at room temperature for at least 3 months. No effect from the sample hematocrit (30-60%) was observed. Concentrations of lopinavir and ritonavir in paired plasma and DBS samples collected from 50 HIV-infected patients (median age 19 years) during 0.1-12.3 hours after the last doses were measured using the developed method. Plasma and DBS concentrations for lopinavir and ritonavir were highly correlated (Pearson correlation r = 0.913 and r =0.952, respectively). The Bland-Almond plot indicated no proportional bias between the DBS and plasma assays (p>0.05). However, the lopinavir and ritonavir concentrations were 31.6% and 16.4% lower in DBS than in plasma, respectively. In conclusion, the LC-MS/MS assay validated for the quantification of lopinavir and ritonavir in DBS is robust, accurate and precise. Lopinavir and ritonavir concentrations in DBS are lower than plasma. Current target drug concentrations are based on plasma concentration thresholds therefore drug concentrations determined from DBS need to be adjusted to estimate the plasma concentrations before interpretation. Further analysis of paired DBS-plasma samples is needed to establish an equation to predict plasma concentrations from DBS concentrations.