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Development and validation of anti-retroviral drug levels for therapeutic drug monitoring using dried blood spots

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Abstract

Background: Plasma concentrations of antiretroviral (ARV) drugs are frequently used for therapeutic drug monitoring of antiretroviral drugs. Dried blood spot (DBS) and dried plasma spots (DBS) sampling offers a patient-friendly and easy alternative to plasma sampling. Therefore the aim was to develop and validate a method for the measurement of ARVs in DBS and DPS from whole blood spotted onto filter paper and onto plasma separation cards (PSC) using ultra-pressure liquid chromatography in tandem with mass spectrometry (UHPLC-MS/MS).

Method: DBS and DPS were prepared by spotting whole blood onto the collection cards and PSC respectively. The cards were extracted and subsequently analysed using UHPLC-MS/MS. The method for measurement of ARVs from DBS and DPS was validated by determining linearity, accuracy, precision, recovery and limit of detection (LOD), limit of quantification (LOQ) and stability for each of the drugs. ARV Drugs in DBS (Lamivudine (3TC), Abacavir (ABC), Efavirenz (EFV), Ritonavir (RTV) and Lopinavir (LPV)) ARVs were compared plasma concentrations. Effect of Haematocrit on DBS was also investigated. ARVs in DPS (3TC, ABC, EFV, RTV, LPV, Nevirapine (NVP), Emtracitabine (FTC), Raltegravir (RAL), Atazanivir (ATV), and Darunavir (TMC114)) were compared for plasma and DPS.

Results: The assay had an acceptable linear regression over the concentration ranges tested in DBS for all the analytes, while in DPS it was acceptable for ranges tested for all the analytes except for 3TC, NVP, ATV. Accuracy was within acceptable deviation of 15% for all the analytes in both DBS and DPS. Inter- and intra-day precision gave a coefficient of variation that was within acceptable deviation of 15%, except for 3TC and LPV, while in DPS coefficient of variation was within acceptable deviation of 15%, except for EFV, RTV, LPV, ATV and TMC114. All drugs had acceptable recoveries in both DBS and DPS. In DBS, The LOD ranged from 0.006 µg/ml for RTV to 0.110 µg/ml for LPV and the LOQ ranged from 0.021 µg/ml for RTV to 0.367 µg/ml for LPV. In DPS the LOD ranged from 0.002 µg/ml for 3TC to 0.423 µg/ml for LPV and the LOQ ranged from 0.008 µg/ml for 3TC to 1.409 µg/ml for LPV. All the drugs in DBS were stable at bench top for 30days, while in DPS only ABC and RTV were stable at bench-top at day 7. Correction for haematocrit in patients with low haematocrit improved the agreement between plasma and DBS.

Conclusion: A method for the measurement of several ARVs from DBS and DPS was developed and validated. Accurate quantification of drugs in DBS was not satisfactory. However, the results suggest that DBS can be used to determine patient's adherence.