THERAPEUTIC DRUG MONITORING OF ANTIRETROVIRAL AGENTS: DETERMINATION OF PLASMA CONCENTRATIONS BY MEANS OF A HIGH-PRESSURE LIQUID CHROMATOGRAPHIC TECHNIQUE

Pinetti Diego[i], Motta Alice[ii], Gallesi Daniela[i], Guaraldi Giovanni[ii]

i: Toxicology and Clinical Pharmacology, Department of Laboratory Services, University of Modena and Reggio Emilia
ii: Department of Medicine and Medicine Specialities, University of Modena and Reggio Emilia

Since 1996, the availability of Highly Active Anti-Retroviral Therapy (HAART) for HIV-infected patients has resulted in a remarkable decrease in morbidity and mortality due to AIDS-related complications. There is a consensus that current antiretroviral agents meet most of the characteristics of drugs that can be considered candidates for Therapeutic Drug Monitoring (TDM) strategy. This is based on the marked interindividual variability in drug concentrations in patients taking the same dose and data relating to plasma drug concentrations and efficacy or toxicities. The focus of TDM of antiretrovirals has been primarily on protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs).
We report the development and validation of a reversed-phase HPLC/UV method for the simultaneous quantitation of eight PIs (indinavir, nelfinavir, nelfinavir-M8, saquinavir, amprenavir, atazanavir, ritonavir and lopinavir) and one NNRTI (nevirapine) in human plasma. The method involves solid-phase extraction (SPE) on 0.5mL human plasma using C18 extraction columns. The chromatography is performed at 37°C on a reversed-phase C18 analytical column 150x4.6mm ID, 5µm ps using a mobile phase consisting of solvent A: 0.05M phosphate buffer pH 3.0 and solvent B:acetonitrile, delivered at 1mL/min with linear gradient elution beginning at 25% solvent B.
Validation of the analytical procedure was assessed by evaluating linearity, accuracy, precision, sensitivity, extraction efficiency and specificity for each analyte on its concentration range. All calibration curves were linear over the calibration range and calibration replicates' percentage relative standard deviations (RSD%) were <10%. Quality controls for three concentration levels for each analyte were used to evaluate intra- and interday accuracy (index: percentage inaccuracy) and precision (index: RSD%) of the assay. The extraction efficiency was evaluated using percentage recovery of analytes following SPE procedure and sensitivity was set on Lower Limit of Quantitation (LLOQ), defined as the minimum concentration level whose accuracy and precision indexes lie within 25%. The specificity was evaluated by monitoring the presence of interfering peaks in the chromatographic profile. The calculated parameters lied within generally adopted laboratory guidelines limits and this is confirmed by the successful participation of our laboratory to the Anti-HIV Quality Control Program KKGT.