ABSTRACT

Background:
Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that is widely prescribed in resource limited settings as part of first line antiretroviral therapy (ART). The quantification of antiretroviral drugs in plasma is a valuable pharmacological tool since the NNRTI are known to exhibit pharmacokinetic/pharmacodynamic (PK/PD) and pharmacokinetic/toxicity relationships. Thus characterization of the relationship between nevirapine plasma concentrations and drug response is key to the optimization of ART. Pharmacokinetic studies require accurate and precise analytical methods for the measurement of antiretroviral drug concentrations to ensure that correct and meaningful data are fed back into clinical care.

Objectives:
The main objective was to validate a simple, sensitive and rapid method for the determination of nevirapine concentrations in human plasma using a high performance liquid chromatographic (HPLC) method.

Methods:
Nevirapine and the internal standard (carbamazepine) were extracted from plasma into ethyl acetate under basic conditions and evaporated to dryness. The dried sample was reconstituted with methanol and 90 μL injected into the chromatograph. Separation of the analytes was achieved on a C18 reversed phase analytical column and detection was done at 282 nm. The mobile phase consisted of acetonitrile and phosphate with a flow rate of 0.8 mL/min under isocratic conditions. Method validation followed FDA guidelines.

Results:
The method was of good selectivity and specificity for nevirapine and the internal standard (IS) with no interference from endogenous substances and concurrent drugs. Calibration curves were linear over the range of 0.645 μg/mL to 17.2 μg/mL (R2 = 0.93 – 0.997). Intra and inter-day precision (%CV) were less than 10%. The absolute recoveries for the analytes (>96%) were consistent and reproducible. The carryover effects as well as the effects of hemolysis and freeze-thaw cycles were all within acceptable limits.

Discussion:
The method employed a single step extraction procedure that made it simple and rapid. The use of carbamazepine as the internal standard was an advantage because it is readily available making the method suitable for resource limited laboratories. The method has been employed in the field for the determination of nevirapine plasma levels in HIV patients on various HAART regimens containing lamivudine, stavudine, zidovudine and tenofovir. The successful field application of this method is a testimony of its reliability and future expansion is envisaged.

Key Words:
High Performance Liquid Chromatographic method, nevirapine, carbamazepine, human plasma, HIV patients.