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COMPARISON OF AN HPLC-UV METHOD WITH A RAPID AUTOMATED IMMUNOASSAY (EIA) TO MEASURE NEVIRAPINE CONCENTRATION IN HIV+ PATIENTS

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Quantification of nevirapine (NVP) in plasma is integral to clinical use of this antiretroviral drug. Available methods include liquid chromatography with UV and mass detection that are specific for NVP. A new antibody-based method using enzyme immunoassay technology (EIA) has been lately tested for use on automated platforms (VIVA-E, Dade Behring). This technique has several advantages over HPLC-based methods in terms of ease of use, availability of equipment and semi-automation.

The aim of this study was to compare a validated HPLC method using ultraviolet detection (UV) with an EIA (ARK NVP Test^{TM}) in plasma samples collected at steady-state, just before drug administration (C_{trough}), in 35 HIV+ patients.

The EIA is based on competitive binding to an antibody between NVP in the sample and the NVP-labeled enzyme. Reaction rate is measured spectrophotometrically (Dade-Behring, VIVA-E)

QC samples at 0.75, 3.0 and 7.5 μg/mL show intra-assay precision of 2.2, 1.8 and 2.6 CV%, respectively. Accuracy was within 10% for all three QCs assayed.

In the 35 samples collected from these patients the mean±SD blood concentration of NVP measured by HPLC-UV was 5.0±2.4 μg/mL. The corresponding value for NVP obtained by EIA was 4.9±2.3 μg/mL.

A Bland-Altman plot demonstrated that EIA gave similar results to HPLC-UV. The mean absolute difference between EIA and HPLC-UV was –0.1±0.5 μg/mL (range: -1.1 μg/mL to +1.4 μg/mL) for a concentration range (as determined by HPLC-UV) of 0 μg/mL to 9.0 μg/mL, which corresponds to a mean±SD relative difference of –0.7%±8.9%.

A good correlation between the two methods was found using linear regression

$$[\text{EIA}] = 0.25 + 0.94 \times ([\text{HPLC-UV}]) \text{ with } r^2 = 0.96$$

In conclusion, HPLC-UV and the ARK ATV Test^{TM} both gave satisfactory results in the analysis of clinical samples for therapeutic drug monitoring of NVP.