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

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Quantification of atazanavir (ATV) 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 ATV. 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 ATV Test™) in plasma samples collected at steady-state, just before drug administration (C_{trough}), in 40 HIV+ patients.

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

QC samples at 0.2, 0.75, and 3.0 μg/mL show intra-assay precision of 4.8, 5.1 and 9.0 CV%, respectively. Accuracy was within 10% for all three QCs assayed.

In the 40 samples collected from these patients the mean±SD plasma concentration of ATV measured by HPLC-UV was 1.4±1.3 μg/mL. The corresponding value for ATV obtained by EIA was 1.2±1.0 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 –0.2±0.5 μg/mL (range: -2.5 μg/mL to +0.2 μg/mL) for a concentration range (as determined by HPLC-UV) of 0 μg/mL to 3.8 μg/mL, which corresponds to a mean±SD relative difference of –4.5%±2.1%.

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

$$[\text{EIA}] = 0.00 + 0.98 \times ([\text{HPLC-UV}]) \text{ with } r^2 = 0.82$$

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