SIMULTANEOUS QUANTIFICATION OF BENZNIDAZOLE, ATAZANAVIR AND EFAVIRENZ BY UHPLC-MS/MS IN PLASMA OF PATIENTS COINFECTED WITH CHAGAS' DISEASE AND HIV/AIDS

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Background: Patients with chronic Chagas disease (CD) who acquire HIV have a high probability of CD reactivation, manifested by severe cardiac and CNS involvement. Antiparasitic treatment with benznidazole (BNZ) is commonly used in an attempt to reduce the high mortality associated to these reactivations, but the pharmacokinetics of BNZ and its potential drug interactions with antiretroviral drugs are insofar unknown in the context of these patients. The objective of this study was to develop a method for rapid simultaneous therapeutic monitoring of BNZ and relevant antiretrovirals used in these patients, atazanavir (ATZ) and efavirenz (EFV). **Methods**: Standard solutions of BNZ, ATZ and EFV were prepared (500 ng/mL in ACN) and their ion fragmentation profile $[M+H]^+$ was studied by direct infusion in a *Sciex-QTRAP-6500* mass spectrometer. Optimization of ESI source parameters for each compound led to the quantifying MRM transitions: *m/z* 261→91 (BNZ), 705→335 (ATZ), 316→244 (EFV) and 147→103 (IS). An eight-point multi-drug calibration curve with three quality controls was constructed by adding 2 µL of a mixture of standards to 60 µL of human plasma from healthy donors. Coumarin was used as internal standard (IS). After extraction with 120 µL cold ACN, 2 µL of supernatant was injected into a *Restek-Force-C18* column (100 x 2.1 mm, 1.8 µm), and chromatography was done using a *Shimadzu-Nexera-X2* UHPLC with water (A) and ACN (B) plus formic acid 0.1%; The HPLC gradient was: 50-95% (0-0.5 min), 95% (0.5-2.6 min), 95-50% (2.6-2.7 min), 50% (2.7-3.0 min), run at 0.35 mL/min and 45 ºC. **Results**: Excellent linearity ($R^2 > 0.98$) was obtained in the therapeutic ranges for BNZ (from 15 to 15,000 ng/mL), ATZ and EFV (both from 10 to 10,000 ng/mL). The method was sensitive, with good precision (RSD% < 15%) and accuracy (RE% <15%). **Conclusions**: A rapid and sensitive method was developed capable of simulatenously quantifying BNZ and the antiretrovirals ATZ and EFV in plasma. This method could contribute to the knowledge of tolerability and possible drug-drug interactions through population-based pharmacokinetic studies, in order to improve the treatment of CD-HIV co-infected patients.

Keywords: Benznidazole; Atazanavir; Efavirenz; Chagas; HIV/AIDS; UHPLC-MS/MS.