VALIDATION AND APPLICATION OF BIOANALYTICAL METHOD FOR TISSUE QUANTIFICATION OF BENZNIDAZOLE

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Chagas disease is caused by the protozoan *Trypanosoma cruzi*. Benznidazole (BNZ) is the only drug available for its treatment in Brazil, and with low therapeutic efficacy, especially in the chronic phase of the disease. The variability of the therapeutic response in the different phases of the disease may be related to the unfavorable pharmacokinetic properties of this compound, and studies to quantify BNZ in biological samples are essential to better understand this correlation. This study aimed to validate an analytical method for quantifying BNZ according to ANVISA recommendations for bioanalytical methods, applicable in the study of biodistribution of Chagas disease. The specific objectives were: (i) to evaluate the parameters linearity, residual effect, matrix effect, selectivity and quantification limit of the method; (ii) to perform a stability study after freezing/thawing cycles, short/long term under refrigeration and in the freezer. (iii) To apply the validated methodology to quantify benznidazole in heart and colon samples from dogs infected with the Berenice-78 strain of *T. cruzi* during the chronic phase of infection. The method developed was linear in the concentration range of 0.1 to 100.0 µg/mL for the colon and heart, and presented sensitivity with precise and accurate results. In addition, the stability of BNZ was verified under different handling conditions required by the method. Tissue concentrations of BNZ in the heart ranged from 4.73 µg/g to 6.42 µg/g; in the colon, the concentrations found were lower, with values of 0.18 to 0.65 µg/g. Studies involving the heart and colon are essential to understanding Chagas disease, since *T. cruzi* directly affects these organs, leading to serious complications such as chronic Chagas cardiomyopathy and megacolon, respectively. Such changes may interfere with the pharmacokinetics and biodistribution, not only of BNZ, but of other drugs used concomitantly in the pharmacotherapy of Chagas disease.

Keywords: chagas disease; biodistribution; bioanalythical method.

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