Poster: Immunosuppression/Other

A SIMPLE HPLC-UV METHOD FOR 6-THIOGUANINE DETERMINATION IN RED BLOOD CELLS DURING PHARMACOTHERAPY WITH AZATHIOPRINE – APPLICATION TO THERAPEUTIC DRUG MONITORING IN CLINICAL PRACTICE

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Background: To reduce gastrointestinal inflammation, Azathioprine (AZT) is widely used to immunosuppress inflammatory bowel diseases (IBD), including Crohn's disease. After biotransformation, 6-thioguanine nucleotide (6-TG), the main active metabolite of AZT, may cause a serious bone marrow toxic effect. To appropriately control therapy (mainly to avoid toxicity), the therapeutic drug monitoring (TDM) of 6-TG should be performed routinely. The 6-TG should be determined in red blood cells (RBC) despite the distribution in that blood compartment. The therapeutic window oscillates around 200–400 pmol/8 x 10^8 RBC. Therefore, high-performance liquid chromatography with spectrophotometric detection (HPLC-UV) is the golden standard in the presented methodology. The study aimed to validate a simple HPLC-UV method for 6-TG determination in RBC obtained from patients with IBD diseases.

Methods: The 6-TG levels were determined in the RBC pellet obtained after centrifugation. The 100µL of RBS were purified and lysed using perchloric acid at 100 under dithiothreitol protection. After this stage, the supernatant was diluted with water and injected into the HPLC-UV system. The method was developed using Varian 230 ProStar HPLC with UV detection set at 340 nm. 6-mercaptopurine (6-MP) was used as the internal standard. An Ascentis C_{18} column (250 × 4.60 mm, 5µm) with complementary guarded precolumn was used for chromatographic separation with mobile phase contained water, acetonitrile and phosphate buffer as well (25:430:45, v/v/v) with 1.1 mL/min flow rate during 7 minutes.

Results: The linearity ranged from 20-10000 pmol/8 × 10^8 RBC with an average R^2=0.999. The calculation factor (F) was set as 17032/10^8 RBC. The accuracy and precision fulfilled the EMA/FDA acceptance criteria. The carry-over and matrix effects were not observed during validation. The stability of 6-TG at room temperature was set at 36h while at 4ºC for 7-10 days. A validated method has been used to determine 6-TG levels in 300 whole blood samples obtained from adults and children treated with AZT for IBD.

Conclusions: The method was successfully validated according to EMA and FDA guidelines. Additionally, the method was implemented in clinical practice for routine TDM of azathioprine therapy in adults and children.

Key Words: Crohn's disease, azathioprine, 6-thioguanine, TDM, HPLC-UV