DEVELOPMENT OF A NEW METHOD TO QUANTIFY GEMIFLOXACIN MESYLATE (GFM) AND SYNTHETIC IMPURITY BY CAPILLARY ZONE ELECTROPHORESIS

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Introduction: Gemifloxacin mesylate (GFM) is a synthetic broad-spectrum antibacterial agent for oral administration, showing enhanced potency against Gram-positive bacterial pathogens, as *Streptococcus pneumoniae*, exceeding the efficacy of the third generation agents[1]. The literature shows few studies of quantitative determinations of GFM in tablets[2,3] and there is no described method for the determination of GFM using the capillary zone electrophoresis (CZE).
Objective: The aim of this study was to develop and validate a simple, fast, inexpensive and reliable CZE method for the determination of GFM and the main synthetic impurity (1-cyclopropyl-6-fluoro-7-chloride-4-oxo-1,4-dihydro-1,8-napthyridine-3-carboxylic acid) in coated tablets and compare the results obtained in previous studies done by the authors with LC stability-indicating and microbiological assay.
Materials and Methods: CE experiments have been conducted in a capillary electrophoresis system (Agilent Technologies, model HP 3D CE, Palo Alto, CA, USA), equipped with a diode array and a temperature control device. Data acquisition and treatment software was supplied by the manufacturer (HP ChemStation, rev A.06.01). The capillary has been thermostated between 20 and 35 °C. Different sizes of the uncoated fused-silica capillary of 50 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA) have been used (40, 50 and 72 cm effective length) to improve the separation of the drug and the synthetic impurity. Samples are being injected hydrodynamically at the anodic side by pressure of 50mbar for 6 s. Constant voltages between 15 and 30 kV are being tested. Different wavelengths of detection are being tested: 220, 231, 265 and 340 nm. New capillaries were conditioned with a solution of 1 M sodium hydroxide (NaOH) for 40min, water for 15 min and finally buffer solution for 15 min. At the beginning of each working day, the capillary are being rinsed with 0.1 M NaOH for 15min, ultrapure water for 15 min and finally with running buffer for 15 min. Between injections, the capillary is rinsed with 0.1 M sodium hydroxide (NaOH) for 2 min, water for 2 min and finally buffer solution for 2 min. The influence of the applied voltage (15-30 KV), type of electrolyte (phosphate, citrate, and borate) molar concentration (25 -100 mM) and pH of the solutions buffer (3.0-10.0), diluents of the reference standard and solution samples (methanol or solution buffer) also are being evaluated.
Results and Discussion: The preliminary results showed that the borate buffer solution (pH 10 and 25 mM), uncoated fused-silica capillary of 50 μm with 40 cm of effective length at 30 KV of applied voltage presented good separation of the GFM and the synthetic impurity, but is necessary to establish an internal standard and proceed with the forced degradation studies. The methodology will be validated according to the International Conference on Harmonization (ICH, 2005) and USP 32 (2009) by determination of the following operational characteristics: specificity, linearity, precision, accuracy and robustness. GFM will be submitted to accelerated conditions to validation of the stability-indicating method. In order to compare the results with well characterized procedures (LC method and microbiological assay previously validate), the precision results of the methods will be statistically analyzed using analysis of variance ($\alpha = 0.05$).
Conclusions: The experimental conditions obtained are adequate for validation of the analytical method according to the Official Guides.

References

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