B8 - STANDARDIZATION AND OPTIMIZATION OF ELECTROPHORETIC METHODS FOR THE ANALYSIS OF HOMOGENEITY AND PHYSICOCHEMICAL CHARACTERIZATION OF RECOMBINANT HUMAN ERYTHROPOIETIN

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Objectives

Like other glycosylated proteins, the rhEPO comprises a mixture of isoforms related to the degree of glycosylation and the presence of sialic acid, influencing the electrophoretic mobility and isoelectric point (pI) of the molecule. The aim of this study was to standardize the distribution of rhEPO isoforms and analyzing the homogeneity by isoelectric focusing polyacrylamide gel (IEF-PAGE), zone capillary electrophoresis (ZCE) and electrophoresis denaturing polyacrylamide gel (SDS-PAGE) seeking the quality control of biopharmaceuticals in Bio-Manguinhos/Fiocruz.

Methods

For this study, we used rhEPO's Reference Material candidate (cMR), and Biological Reference Preparation (BRP) of the European Pharmacopoeia (E.P.). It was used for IEF-PAGE the equipment PhastSystem with PhastGel dry with a combination of ampholytes which generates an acid pH gradient, using 4µg of desalted rhEPO and staining with silver nitrate. The CZE analysis was performed using the parameters described in the methodology proposed by the E.P. and certain parameters were adjusted such as temperature, pressure, injection time and concentration of the sample to obtain an electropherogram with high resolution. SDS-PAGE was used to identify and confirm

homogeneity of rhEPO by its molecular weight (MW)using polyacrylamide gel 12.5% and coomassie blue staining.

Results

In IEF-PAGE it has been evidenced the presence of eight major isoforms, and the estimated pI indicated mean values: 6.43 and 4.12. The percentage of each isoform of the samples was calculated and compared to the limit set by E.P. with concordance of results between the replicas. Since established the conditions for CZE analysis results were reproducible and the technique showed sensitivity and reproducibility for the analysis of isoforms of rhEPO demonstrated by high resolution electropherograms with 8 isoforms. The percentage of each isoform is within specifications and the results were homogeneous among the samples analyzed. The SDS-PAGE showed as a robust methodology with excellent reproducibility. The samples are homogeneous and the average MW 34kDa was found according to specifications of the E.P. and the literature.

Conclusion

Methods based on separation by difference in liquid load and molecular weight standard and optimized in this study will enable a proper analysis of the concentrated solution of rhEPO as its homogeneity and physicochemical characterization by Quality Control in Bio-Manguinhos/Fiocruz.