Glycolisation Evaluation of Recombinant Human Erythropoietin in Drugs

Conceição, CM[1,2]; Silva, F.S.Q.[1,3]; Silva Junior, J.G[4];
1-INCQS Fiocruz; 2-Escola de Farmácia UBM, 3-Escola de Farmácia, Unigranrio 4-Bio-manguinhos Fiocruz

Guidelines for pharmaceutical recombinant glycoprotein are very rigid and isoform determination and glycan structure characterization in all batches is necessary. Carbohydrates are important for pharmacokinetics and stability of these products. The high sialic acid content isoforms have greater plasma half-life. Glycan structure is also considered as a batch consistency marker. For this purpose, the most used techniques are capillary zone electrophoresis (CZE). Comparing retention time of sample peaks with a standard, lose of sialic acid can be identified. In this work, we have developed a new method for isoform evaluation of recombinant human erithropoietin (rh-EPO) in formulations, using bi dimensional electrophoresis. rh-EPO was isolated from other formulation components by RP-HPLC. Colleted peaks were submitted to 2D electrophoresis under different first and second dimension conditions. Different isoforms could be separated, and using an internal standard the absolute pI could be determinate. In some samples, loose of sialic acid decreased the molecular weight and increase the isoelectric point. These results prove that 2D electrophoresis can be used in samples with 20 µg of rh-EPO for isoforms evaluation.

Acknowledgements: Fiocruz (PDTSP-SUS); Faperj and CNPq