INTRODUCTION: The cloning of the human erythropoietin gene led to the production of recombinant protein (EPOhr) for treatment of anemias associated with chronic renal therapeutic use of zidovudine (AZT), oncology treatments and reduction of blood transfusion. The quality control of recombinant proteins requires the combination of methodologies for complete identification, chemical characterization and assessment of biological potency. Among the methodologies advocated by the European Pharmacopoeia (E.P.) for the analysis of homogeneity stands the Size Exclusion Chromatography (SEC).

OBJECTIVES: To standardize and optimize the methodology SEC in order to determine the presence of aggregates and degradation products of EPOhr.

MATERIAL AND METHODS: For the analysis of the candidate reference material (cMR) was used analytical column TSK Gel G2500 with a flow of 1.0 µL/min, injection volume 100µL, pressure limit 10 mPa and detection at 220nm and 280nm. The analysis was performed with different concentrations of cMR and the results compared to BRP (Reference Preparation of Biological E.P.).

RESULTS AND DISCUSSION: The samples presented a single chromatographic peak with retention time and area equivalent between replicas of cMR and BRP. The area increases with the concentration of the sample, it is possible to set the limit of quantification. Analysis was performed in less time compared to what is described by E.P. CONCLUSION: The integration of chromatographic peaks of the samples demonstrates that the methodology is very reproducible and the samples have purity percentage specifications as the E.P.

Keywords: Recombinant Human Erythropoietin, Homogeneity and Exclusion Chromatography and Molecular Sieve test.

Thanks: FIOTEC, Centro Inmunología Molecular.