Establishment of Recombinant Human Erythropoietin reference material for 2-D gel electrophoresis


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Introduction: The advent of recombinant DNA technology led to an increasing number of biopharmaceuticals produced and marketed in Brazil and worldwide. The quality control of these drugs is a challenge for both industry and regulatory agencies. A critical step in the control of biopharmaceuticals is the need for reasonable amounts of standards for physicochemical properties determination of these products. In order to overcome this problem, producers and research institutions work together to establish reference materials (RMs) that allow this use. RMs are homogenous preparations of well-defined proprieties, for a method use or value attribute, with result consistency for product quality. Recombinant Human Erythropoietin (rh-EPO), a 34 kDa glycoprotein, is the most used biopharmaceutical in the world. Nowadays, the only rh-EPO RM is from European Pharmacopoeia.

Material and Methods: In the present work a National RM of rh-EPO for 2-D-gel electrophoresis was developed. In this technique, the first charge separation is based on protein denaturation and isoelectric focusing (IEF). The second separation by apparent molecular mass was performed using denaturing sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Results and Discussion: One batch of rh-EPO was submitted to 2D electrophoresis 6 times, in a period of 6 months. The profile of these gels were very similar to EP RM gels, used as controls. Six major spots were detected, with similar MW (34 kDa) and pl (4.5 to 6.5).

Conclusions: The results revealed bands with pl and MW compatible with the product and the presence of important isoforms responsible for maintaining the half-life of rh-EPO. No changes in electrophoretic mobility were observed and Relative Standard Deviations for MW and pl values were satisfactory. These results demonstrate that this material could be used as a RM for rh-EPO 2D analysis.

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