217

RECOMBINANT HUMAN GROWTH HORMONE: PRODUCTION AND QUALITY CONTROL

Bartolini, P; Arkaten, R;. Affonso, R; Bellini, M.H; Dalmora, S; Gimbo-Vianna, E; Morganti, L. E; Oliveira, J. E; Peroni, C.N; Ribela, M. T. C. P; Soares, C.R. J.
IPEN-CNEN/SP. São Paulo - Brasil.

The potency of recombinant human growth hormone (rec-hGH) secreted in bacterial periplasmic space was directly determined in the osmotic shock fluids by reversed-phase (RP)-HPLC. Considering that this assay does not identify size isomers, these were determined via a parellel run of the same osmotic shock on size-exclusion (SE)-HPLC, followed by radioimmunoassay (RIA) of eluted fractions. The methodology provides within 24 hours from the beginning of the fermentation process, a complete picture of the recombinant protein being produced with respect to its identity, yield, potency and hGH-related contaminants. These include sulfoxide, desamido derivatives dimer, and high molecular weight (HMW) forms. The purification process include several chromatographic steps, each one controlled by physico-chemical methods. The final preparation of rec-hGH was analysed according to recent pharmacopeial specifications: last edition of the European (1994) and of the Brazilian Pharmacopeia. For this purpose a standard of rec-hGH, calibrated against the 1st International Standard (WHO 88/624) was also set up and it is ready for distribution to other laboratories. Purity was tested by SDS-PAGE, SE and RP-HPLC. Potency was assessed by SE-HPLC and biological assays. Identity was confirmed by tryptic mapping. Sterility, bacterial endotoxins, contaminant E. coli proteins, DNA vector stability were also tested. Single dose toxicity study was carried out in dogs (beagles). The results of this study have shown that the recombinant human growth hormone produced at IPEN-CNEN/SP complies with the pharmacopeial requirements and manufacturing guidelines for this type of medicinal products.

Supported by IAEA (Vienna, Austria) and FAPESP (São Paulo, Brazil)