QUALITY CONTROL OF RECOMBINANT HUMAN GROWTH HORMONE DIRECTLY IN OSMOTIC SHOCK FLUIDS

S. Dalmora, J.E. de Oliveira, R. Affonso, M.T.C.P. Ribela, R.R. Arkaten and P. Bartolini
Department of Application of Nuclear Techniques in Biological Sciences, IPEN-CNEN/São Paulo - Brazil

An isocratic reversed-phase high performance liquid chromatography (RP-HPLC) method for the determination of human growth hormone (hGH) directly in osmotic shock fluids is described. This methodology allows an initial rapid evaluation of the quality and quantity of hGH being secreted in the bacterial periplasmic space right after, or even during fermentation. Considering that RP-HPLC does not identify size isomers, these were determined via a paralell run of the same osmotic shock fluid on size-exclusion (SE)HPLC, coupled with radioimmunoassay (RIA) of the eluted fractions. The methodology provides a complete picture, within 24 h from the beginning of the fermentation process, of the recombinant protein being produced with respect to its activity, identity, yield, and hGH-related contaminants. These latter include sulfoxide and desamido derivatives, dimer and high molecular weight (HMW) forms.

One of applications of the described methodology was in following as closely as possible the fermentation and activation process. Under our conditions, 6 h seemed to be the best activation time, combining the highest hGH secretion with a low amount (6.6%) of altered derivatives. SE-HPLC/RIA analysis of this same osmotic shock fluid also confirmed the presence of minimal amounts of dimer and HMW forms (about 7%).

Supported by CNPq (Brasilia), IAEA (Vienna) and FAPESP (São Paulo)