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PHYSICO-CHEMICAL AND IMMUNOLOGICAL QUALITY CONTROL OF BACTERIALLY EXPRESSED RECOMBINANT PROTEINS, CARRIED OUT DIRECTLY ON OSMOTIC SHOCK FLUIDS.

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Recombinant DNA-derived proteins, when expressed in genetically modified bacteria, can be directly stored as cytoplasmic inclusion bodies or secreted in the periplasmic space thanks to the introduction, in the constructed vector, of a properly designed leader sequence. It is well known that the proteins obtained via this last mechanism and extracted in osmotic shock fluids, do not have an extra N-terminal methionine, are properly folded not needing denaturation and renaturation and, consequently, are practically identical to the natural product. Osmotic shock fluids, obtained right after, or even during, the bacterial fermentation process, are therefore an ideal medium for a precocious qualitative and quantitative control of the physico-chemical properties of the protein being biosynthetized. This way it may be possible to correctively act on the fermentation conditions, avoid a useless, time-consuming and expensive purification process and, what is more important, investigate the causes and moment of occurring alterations.

We applied this study to bacterially derived human growth hormone (hGH), setting up a reversed-phase (RP) HPLC methodology that can immediately determine the amount of recombinant protein being secreted, together with the percentage of undesirable hGH related forms, like sulfoxide and desamido-derivatives. For this purpose a standard of rec-hGH, calibrated against the 1st International Standard (WHO 88/624), was also set up. This secondary standard, which is ready for distribution to other laboratories, is suitable for the characterization of the recombinant product according to the requirements for "Somatropin"(Recombinant DNA-derived hGH) described in the last edition of the European (1994) and of the Brazilian Pharmacopoeia.

Concerning the direct determination of aggregates and dimeric forms of hGH, directly on osmotic shock fluids, a study was carried out applying size-exclusion (SE-HPLC) determination to a RP-HPLC purified sample. Unfortunately this approach did not work out due to the modifications introduced by the RP solvent on the same high molecular weight (MW) forms. Better results have been obtained by the introduction of a fast radioimmunoassay determination on the SE-HPLC eluted fractions of the same osmotic shock fluids.

With the complete set up of this methodology it will be possible to have within 24 hours from the start of the fermentation process, a complete picture of the recombinant protein being produced, with respect to its identity, yield, potency, related and two-chain forms, dimer and higher MW forms.

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