

HUMAN FOLLICLE-STIMULATING HORMONE

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Recombinant human follicle-stimulating hormone (hFSH) is one of the most important pharmaceutical products, applied to the treatment of human infertility. It is extremely expensive and only two companies in the world are producing it: Serono (Switzerland) and Organon (Netherlands). Considering that its structure, formed by an alpha and beta subunit, is very similar to that of hTSH, our laboratory holds all the technology that is necessary for its synthesis in CHO cells, its purification and characterization. As already done for hTSH, before starting its synthesis we wanted to be able to analyze this hormone from a qualitative and quantitative point of view, directly in the culture medium and at the same time compare the quality of the products that are commercially available. For this reason we set up a novel RP-HPLC methodology, directed to the analysis of heterodimeric hFSH, which was already applied to the comparative analysis of a pituitary hFSH (Torjesen, Norway), two recombinant (Gonal, Serono and Puregon, Organon) and a urinary (Serono) preparation of hFSH.

The mentioned RP-HPLC methodology is now ready to be published and will be our seventh paper on this topic in the last 7 years. For this reason and considering our expertise in this field we have been invited by the journal "Current Pharmaceutical Analysis" to write a review article on "HPLC Analysis of Human Pituitary Hormones for Pharmaceutical Applications". This review analyzes 115 papers related to this subject and considers that to determine the identity, purity, potency, safety and stability of recombinant proteins, a variety of analytical techniques have been recommended, which are mostly based on physico-chemical properties of the proteins. Among these techniques High Performance Liquid Chromatography (HPLC), exploiting differences in protein properties such as size, hydrophobicity and charge, has become increasingly useful. Its accuracy, sensitivity, resolving power, speed and versatility have not only been established in research, but also in the quality and process control of industrial protein synthesis. In the case of biologically active proteins such as hormones, it has become more and more evident that HPLC is now strongly complementing, if not substituting, the classical biological and immunological assays whose accuracy limitations are well known. Moreover, it has been shown that the potential for early detection of impurities and molecular alterations provided by this technique allows more efficient and effective control both at the beginning and along the manufacturing process, leading to improved and more economical end products. This review article describes and analyzes recent developments of the main HPLC modalities, applied in the analysis and characterization of the above mentioned hormones, especially as they are relevant to the biopharmaceutical industry.

HUMAN GROWTH HORMONE

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This hormone is mainly used in treatment of dwarfism, Turner syndrome, chronic renal failure, severe debilitations as AIDS and post surgery conditions, several other applications being under study.

After the laboratorial preparation of pharmaceutical grade hGH, a pilot production plant was set up in collaboration with the company Hormogen, at the "Centro Incubador de Empresas Tecnológicas" (CIETEC). An improvement of fermentation, purification and expression yields was attained, having successfully concluded the two phases of the FAPESP Small Business Program (PIPE). Powerful expression vectors were prepared which enhanced the periplasmic expression of the human growth hormone gene from approximately 1.5 µg/mL/A₆₀₀ to more than 4 µg/mL/A₆₀₀. Thus the industrial production of one of the first recombinant protein obtained in Brazil is being developed, two patents having been deposited. For this purpose a joint-venture with one of the main pharmaceutical companies of our country has been set up.

Another work on growth hormone secretagogues (GHSs) is being carried out in collaboration with the University of São Paulo School of Medicine (FMUSP). GHSs are synthetic compounds that stimulate GH secretion in vitro and in vivo. It was recently reported a small but significant GH response to GH releasing peptide-2 (GHRP-2) administration in short individuals with GH deficiency due to an inactivating mutation in the growth hormone releasing hormone receptor.

Literature data, however, report a lack of GH release in response to GHS administration in the Lit/Lit mouse, an animal model presenting a spontaneous mutation in the GHRHR gene.

Our studies in normal C57BL mice have demonstrated a serum GH increase of 18X after intraperitoneal administration of GHRP-2, with a peak at 10-30 min after injection. GHRP-2 was then injected in Little/+ and Lit/Lit mice and a GH increment of 6X was seen in the Little/+ mice and of 9X in the Lit/Lit mice. These data indicate for the first time that acute GHRP-2 administration was able to release significant amounts of GH in the Lit/Lit mice, as in humans with GHRHR mutation. Thus, GHRP-2 can be a promising candidate for the treatment of absolute or relative GH deficiency.

A study is also being developed in collaboration with Prof. Mattanovich from the University of Vienna (Austria) in order to obtain even higher biomasses in our industrial fermentations of genetically modified *E. Coli*. More than 20 fermentations have been carried out in our "pilot bioreactor" using different culture media and three different steps: a batch, a fed batch and an activation phase. Up to now we could improve our biomasses from ~20 to >70 absorbance units.