TL12

A REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE ANALYSIS OF DIFFERENT PREPARATIONS OF HUMAN FOLLICLE STIMULATING HORMONE

Loureiro, RF; Oliveira, JE; Sousa, JM; Bartolini, P; Ribela, MTCP.
IPEN/CNEN-SP, São Paulo – Brasil - Biotechnology Department - CBM (mtribela@ipen.br)

A novel reversed-phase high performance liquid chromatography (RP-HPLC) method for the qualitative and quantitative analysis of human follicle stimulating hormone (hFSH), a dimeric glycoprotein hormone widely used as a diagnostic analyte and as a therapeutic product in reproductive medicine, has been set up and validated for accuracy, precision and sensitivity. The developed techinique preserves the protein integrity allowing, for the first time, the analysis of the intact heterodimeric form and not only of its subunits, as done by the majority of the RP-HPLC conditions currently employed. This RP-HPLC methodology was employed also for comparing the hydrophobicity of pituitary, urinary and CHO-derived hFSH preparations and also of other two glycoproteic hormones of the anterior pituitary: human thyrotropin (hTSH) and human luteotropin (hLH). A significant difference ($p<0.005$) in t_R between the pituitary and recombinant hFSH preparations, reflecting very fine structural differences of the carbohydrate moiety, was observed. In urinary hFSH, two main isoforms were detected, one of them also presenting a significant difference ($p<0.005$) with relation to the pituitary and recombinant preparations. The less hydrophobic of the three glycohormones analyzed was hFSH followed by hTSH and hLH. The linearity of the dose-response curve ($r = 0.9965$, $n=15$) for this RP-HPLC methodology as well as an inter-assay precision of less than 4% for the quantification of different hFSH preparations and a sensitivity of the order of 40 ng were attained. The present methodology represents an unsubstitutable tool for the physico-chemical characterization and quality control of this pharmaceutical, which has not been described yet by the main pharmacopoeias.

Supported by CNPq (PI 381970/01-9 and PQ 301520/91-7)