Efficient separation of the two subunits of human glycoprotein hormones by high performance liquid chromatography

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This paper describes a method based on reversed-phase high-performance liquid chromatography (RP-HPLC) for the rapid and easy separation of the alpha and beta subunit of CHO- and pituitary-derived human thyrotropin (hTSH) and human follicle stimulating hormone (hFSH). Optimized conditions for an efficient dissociation of these heterodimeric glycoproteins into their subunits were set up. A complete dissociation was attained incubating hTSH and hFSH with respectively 0.4 M and 3M of acetic acid, at 37ºC, overnight. Dissociation yields for α -and β -hTSH were 47% and 53% and 48% and 52% for α -and β -hFSH respectively, in agreement with the theoretical yields based on mass determinations, carried out by us via MALDI-TOF mass spectrometry. The separation of the two subunits was achieved by chromatography on a C_4 RP-HPLC column using a linear gradient of acetonitrile. Isolated subunits, either recombinant or natural, were compared concerning their molecular mass, hidrophobicity and purity via size exclusion high performance liquid chromatography (HPSEC), RP-HPLC and SDS-PAGE. The described method is practical and flexible and it can be readily applied to the dissociation of any recombinant or native heterodimeric glycoprotein, allowing direct characterization and studies of each individual subunit. It can moreover detect the presence of undesired free subunits (product-related contaminants) in a pharmaceutical preparation intended for human use. We propose that this method be included in the monographs of these recombinant hormones, that have to be included in the Pharmacopoeias.

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