Complete RP-HPLC Analysis of WHO International Standards

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An important issue in biopharmaceutical drug quality control is the availability of physical-chemical tools that permit the accurate analysis of the active principle without interference from other components of the final drug formulation. In this work, analyses of WHO standards of thyrotropin (hTSH), follitropin (hFSH), choriogonadotropin (hCG) and lutropin (hLH) containing a substantial excess of human serum albumin (HSA) were successfully carried out by reversed-phase high-performance liquid chromatography (RP-HPLC), using a C4 column and specific elution strategies for each hormone. Parameters useful for the evaluation of the separation efficiency were determined: tailing factors (Tf) 1<Tf<1.6; resolution factor (Rf) >2. All peaks presented retention times with a relative standard deviation (RSD) <1%. The lowest resolution between hormone and HSA was found for hLH (Rf=2.72) and the highest (Rf=5.02) for hTSH. A correct quantification of HSA and of each hormone presented an inter-day precision of 0.95% - 4.5%. The minimal detectable amount was of the order of 6-20ng. The mean percent recovery of HSA relative to the nominal content was 97.6±9.1. Concerning hormone contents, the mean percent recovery was 103.4±5.5 for recombinant hFSH and hLH, while a recovery of only 56% and 53% was observed for native preparations of hTSH and hCG, respectively. In conclusion, this work demonstrates the potential of a physical-chemical method for the rapid quantitative analysis of reference preparations and biopharmaceutical in general in the presence of a large amount of an extraneous protein, allowing also a direct panorama of the quality and heterogeneity of the protein of interest.

Word Keys: RP-HPLC; HSA; glycoprotein hormones; excipient; WHO standards

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