RP-HPLC Qualitative and Quantitative Analysis of Glycoprotein Hormones in the Presence of High Amounts of Human Serum Albumin: hLH, hCG, hFSH and hTSH

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A precise qualitative and quantitative analysis of the human glycoprotein hormones lutropin (hLH), coriogonadotropin (hCG), follitropin (hFSH) and thyrotropin (hTSH) in the presence of large excess (>100:1) of a protecting/stabilizing protein e.g. human serum albumin (HSA) was set up by reversed-phase high performance liquid chromatography (RP-HPLC). For hLH, RP-HPLC elution was performed with a linear gradient of 27-42% of acetonitrile in 0.05M sodium phosphate buffer, pH 7.0, over 60 min, at a flow-rate of 0.5 mL/min. In these conditions, a relative retention time (t_{RR}) of 2.3 between hLH (30.82 min) and HSA (70.68 min) was attained. For hCG, an elution linear gradient of 25-50% of acetonitrile in 0.05M sodium phosphate buffer, pH 7.0, over 50 min, at a flow-rate of 0.5 mL/min was utilized. In these conditions, a t_{RR}=1.9 between hCG (21.65 min) and HSA (44.99 min) was attained. Concerning hTSH and hFSH, due to their lower hydrophobicity, the large amount of HSA did not elute under the running conditions set up. This study, showing complete resolution between the hormone and HSA peaks, is important considering that WHO International Standards, widely used as reference preparations, present such high amount of HSA, hampering their utilization for physico-chemical testing.

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