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A PRACTICAL RP-HPLC METHOD FOR RECOMBINANT HUMAN THYROTROPIN (hTSH) LABORATORY SCALE PURIFICATION

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A purification strategy consisting of an ion exchange chromatographic step with a subsequent reversed-phase high performance liquid chromatography was sucessfully utilized for a laboratory scale purification of CHO-derived human thyrotropin, synthetized in our laboratory and resulting in a very pure protein. A 120 X concentration of CHO cells conditioned medium (from ~ 10L) with low hTSH concentration (0.58 μg hTSH/mL, mass fraction 0.35) and a purification factor of 43 X, were obtained after the first chromatographic step carried out on a SP-Sepharose Fast Flow cation exchanger (2.6 X 12 cm). In the resultant hTSH pool, contaminants of higher molecular size and lower hydrophobicity than hTSH were observed. Utilizing a small semi-preparative C_4-Vydac RP-HPLC column (10 mm ID x 250 mm), practically all contaminants were efficiently eliminated, a concentrated (1.2 mg/mL) and pure (~100%) product being generated in an extremely short chromatographic time (~70 min). The overall recovery of this purification scheme was of the order of 40%. The final product was then lyophilized and tested by a high-precision single dose (n=6 replicates of 10 μg/animal) in vivo bioassay. This same methodology can be easily adapted to the laboratorial purification and production of other biopharmaceuticals, even when obtained at extremaly low concentrations and mass fractions.

Key words: hTSH, purification, reversed-phase HPLC

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