# Quantitative analysis of different preparations of human thyrotropin (hTSH): a comparison between RP-HPLC and the in vivo bioassay based on thyroxine stimulation in mice

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With the intention of setting up physico-chemical methods that can reduce animal use, the hTSH content of different preparations of native, pituitary and of recombinant hormone was determined by reversed-phase high performance liquid chromatography (RP-HPLC) and compared with the data obtained by the classical mouse in vivo bioassay. Recombinant hTSH produced by Genzyme (Thyrogen$^{R}$) was used as a reference preparation in both assays. A linear relation was obtained between the two methods, with a significant coefficient of correlation: $r = 0.8359$; $p<0.001$; $n=16$. The corresponding equation was: BAµg =0.9177 RP-HPLCµg + 0.4097. Considering, though, that this correlation should be improved and that a specific individual sample could be eventually classified as an outlier, we decided to improve the reliability of protein determination based on the BCA assay. We are thus in the process of setting up an in-house calibrator of hTSH (possibly a WHO International Standard), whose molar content is strictly determined by amino acid composition analysis, and of re-determining all protein content of each sample analyzed. The pharmacokinetics properties and N-glycan structures of a typical pituitary and recombinant hTSH preparation have also been determined and will be studied in the light of the relative potencies obtained with the present comparative work.