

18th Annual Pharmaceutical and Chemical Analysis Congress

November 05-06, 2018 | Madrid, Spain

Growth hormone: Chromatographic separations coupled with mass spectrometry towards a novel method for anti-doping tests

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Statement of the Problem: The recombinant human growth hormone (rhGH) is now widely manufactured by biotech companies. The rhGH is used to treat growth hormone deficiency diseases. On the other side, the availability of rhGH in the black-market has continuously increased because of doping in sports. To date, the detection of GH doping is still very challenging. In fact, the natural and the biosynthetic hGH have identical peptidic sequences. So far, the valid human growth hormone anti-doping tests are based on immunological recognition. However, immunoassays have their own limitations. The human growth hormone concentration in blood depends on multiple factors. The secretion of GH is increased by physical activity and thus athletes produce more GH. In addition, the secretion of GH is pulsatile and influenced by nictemeral variations. These variations may lead to non-representative GH measurements. Therefore, the next generation analysis of GH has to be more specific and accurate. Mass spectrometry coupled with separative methods such as electrophoresis and chromatography could represent a more advanced instrumental set-up to find GH doping practices.

Methodology & Theoretical Orientation: Mass spectrometry coupled to reversed phase chromatography was used to find chemical differences between the pituitary hGH and the rhGH. Intact GH proteins were separated by C8 or C18 columns prior to mass analysis.

Findings: The pituitary extracted hGH is glycosylated whereas the biotech product is sugar free. Moreover, by chance this glycosylation is bounded to a tryptic fragment that is proteospecific of the GH protein. Thus we expect to be able to measure the concentration ratio between the wild type and the synthetic one.

Conclusion & Significance: The present work represents the first building blocks towards a novel methodology for a novel hGH anti-doping test. In addition, the collected data from the analysis of different hGH preparations should lead to other practical analytical applications for quality control. The availability of a test that quantifies the natural and the rhGH will also be beneficial for GH deficiency diagnosis and treatment follow-up.

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