E25 A Qualitative Analysis of Human Growth Hormone (HGH) by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) and Complementary Techniques

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Learning Overview: The goal of this presentation is to introduce attendees to the analysis and identification of protein-based molecules used in conjunction with controlled steroids, such as HGH. Liquid Chromatography/Mass Spectrometry (LC/MS) and LC/MS/MS methods on intact and digested proteins will be discussed, as well as the theory and protocol for commercial Enzyme-Linked Immuno-Sorbent Assay (ELISA) kits specific to the protein of interest.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by presenting a new series of techniques for highly specific identification of a protein-based hormone, such as HGH.

The United States Food and Drug Administration states that HGH and other related proteins are only approved as a prescription for a limited number of conditions and are prohibited for use as an anti-aging product, body-building supplement, or for muscle enhancement. Independently, United States Army Regulation 600-85 prohibits the use of prescription medications for anything other than their intended purpose or possessing a medication without a prescription. Military investigating agents often submit for analysis HGH and related proteins along with other controlled steroids. When submitted substances are suspected to be testosterone-related steroids, there are routine small molecule assays suitable for their identification. However, due to their complexity and size, proteins such as HGH must be analyzed using alternative methodologies.

In this study, HGH was analyzed using two LC/MS/MS-based methods, as well as sandwich ELISAs. The intact HGH protein was analyzed via LC/MS using a reverse phase, highly porous column. The intact protein demonstrated a repeatable retention time and characteristic multiply-charged ions from which the whole molecular weight of intact HGH was calculable. The ELISA-based assay successfully bound the folded HGH protein as indicated by the colorimetric response, with no observed cross-reactivity. Last, the trypsin digested peptide sequencing assay provided the greatest specificity. Predictable peptide fragments, unique to HGH, were obtained via a classic trypsin digestion of the protein, then were sequenced using LC/MS/MS fragmentation. An average of 85% coverage of the protein's expected peptides was observed. The ten specific HGH peptides that were further fragmented to acquire amino acid sequencing data resulted in the unique identification of HGH when compared to more than 139 million sequences in the National Center for Biotechnology Information (NCBI) protein database. Each of the three experimental methods resulted in complementary and orthogonal proteomic data, thereby making the identification of HGH highly specific.

Once an identification protocol was developed, the stability of HGH was investigated, since submitted samples can be in various states of degradation, potentially resulting in the misidentification of the protein. Numerous storage conditions were probed, including as a lyophilized powder or in assorted diluents, at different temperature ranges (up to 37°C), and over various time scales (up to three months).

For up to one month, no samples, regardless of storage conditions, showed degradation in the whole molecule LC/MS method, the trypsin digested LC/MS/MS method, or the ELISA method.

While the pros and cons of each method should be considered when creating an overall method for protein identification, the complementary information gained from each technique creates an overall highly specific method for the identification of HGH. The introduction of probabilistic language into forensic chemistry chemical identification reports will be discussed.

Human Growth Hormone, Protein Identification, LC/MS Method Development