B149 Proteomics-Based Method for the Identification of Human Growth Hormone

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Upon the completion of the presentation, participants will be provided with a new methodology for the analysis and identification of human growth hormone.

This presentation will impact the forensic science community encouraging the analysis and identification of suspected human growth hormone submissions to provide the intelligence community with more complete information regarding the frequency that HGH is encountered.

Recent publicity surrounding the purported use of performance- enhancing drugs such as steroids and Human Growth Hormone (HGH) by professional athletes has focused on the need for more stringent anti-doping testing protocols and the ability to identify unusual substances in laboratories. HGH is often seized and submitted to forensic drug laboratories in conjunction with steroids. The analysis of steroids is relatively straightforward; however, most forensic laboratories are not accustomed to analyzing exhibits of HGH and qualitative methods are needed to perform these analyses. Addressing this issue will enable accurate and consistent reporting of HGH and provide valuable intelligence as to the frequency in which it is encountered by law enforcement personnel. HGH is considered a small protein, but it is still approximately 50 times larger than regularly encountered drug substances. Since routine analysis is not amenable to these large biomolecules, HGH cannot be identified using the instrumentation and methodologies typically utilized in a common forensic drug laboratory.

In a standard forensic drug analysis laboratory, gas chromatography-mass spectrometry (GC/MS) is the confirmatory instrument of choice. Most controlled substances of interest have molecular weights that are less than 400 amu and are easily vaporized for multi-component separation and isolation by gas chromatography. The isolated drug is then usually fragmented by electron impact ionization and the results are compared to a standard for identification. Proteins present a unique problem for a typical forensic drug analysis laboratory. The size of proteins (> 1000 amu) and their thermal instability make them poor choices for analysis by GC/MS. Therefore, a biochemical, proteomics-based high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC/MS) approach to identifying proteins is necessary for the analysis of HGH. One aspect of proteomics analysis is analogous to using electron impact ionization in GC/MS. While energetic electrons are used to fragment gaseous molecules in GC/MS to yield a unique pattern, an enzyme can be used to cleave a protein at specific and reproducible locations to also yield a unique pattern.

HGH is a relatively small protein consisting of 191 amino acids and has an average mass of 22,125 amu. The forensic analysis of proteins such as HGH must meet the analytical sufficiency requirements for the laboratory and must consist of a binary approach. A suspected sample is analyzed as the whole protein for retention time and mass determination using HPLC equipped with a photodiode array and LC/MS. Further fragmentation of the protein using a proteolytic enzyme adds another dimension to the specificity of the analysis. Porcine trypsin digests proteins in a very predictable manner and yields peptide fragments of the original protein that can be used as a means for fingerprinting the larger biomolecule. *In silico*, or theoretical, digestion of HGH by trypsin yields 21 peptides ranging in size from 1-23 amino acids in length. Of these 21 peptides, 17 sequences are 5 amino acids or longer. The larger fragments containing higher numbers of amino acids give more specificity to identifying a protein based on a fragment produced by the digestion of trypsin.

Herein, the analysis of HGH using a proteomics approach is presented that meets the SWGDRUG recommendations for the identification of unknown substances.

HGH, Proteomics, Steroids

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