



Criminalistics Section - 2016

B199 Characterization of Performance-Enhancing Peptides Via Ambient Ionization Time-of-Flight/Mass Spectrometry (TOF/MS)

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After attending this presentation, attendees will better understand how preliminary identification of proteins and other large biomolecules can be easily and readily attained using a high-resolution ambient ionization mass spectrometer, instrumentation that is becoming more common in forensic laboratories.

This presentation will impact the forensic science community by demonstrating methods for the analysis of various macromolecules in drug identification laboratories without the need for the specialized protein-sequencing systems typically required.

The increasing presence of recombinant Human Growth Hormone (rHGH) and related performance-enhancing peptides has been observed as internet vendors sell these substances not only to professional competitors but also to the general public. Most drug identification laboratories do not have protocols useful for the analysis and identification of such large molecules. The primary technique of Gas Chromatography/Mass Spectrometry (GC/MS) is limited to relatively small molecules that are readily vaporized at the inlet. This study investigated the feasibility of analyzing macromolecules by using a high-resolution TOF/MS coupled with an ambient ionization source.

Several methods for ionizing proteins prior to entry into the MS were investigated. Electrospray Ionization (ESI), which is common for ionization of such species, as well as Paper Spray Ionization (PSI) and, for simplicity, simple inlet ionization were tested. Mass determinations of various high molecular-weight peptides were successfully attained with all three ionization methods.

Proteins less than 10kDa were amenable to inlet ionization. This involves introducing a small aliquot of dissolved protein directly into the inlet of the MS with a glass capillary. Multiple charge states of the ionized protein were observed, similar to ESI, even in the absence of an externally applied voltage. The mass of the intact protein was calculated by deconvolution of the spectrum either manually or with spectral analysis software. The simplicity of this ionization method makes it especially well-suited for the analysis of small peptides in a typical forensic setting. In contrast, PSI involves spotting small aliquots of dissolved proteins on a triangular piece of filter paper. The tip of the filter paper is then positioned near the MS inlet while high voltage from the onboard voltage supply of the MS is applied to the opposite edge of the paper. Results of inlet ionization, PSI, and ESI approaches will be discussed.

To make it possible for large biomolecules to be further characterized beyond a parent mass, enzymatic digestion of larger peptides is also being investigated.

This project was supported by an award from the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this exhibition are those of the authors and do not necessarily reflect those of the Department of Justice.

Peptides, Mass Spectrometry, Ambient Ionization