B63 Rapid Analysis of Peptides and Proteins Utilizing Matrix-Assisted Inlet Ionization Mass Spectrometry

Kyle E. Vircks, MS, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; Jesse M. Zavala, MS, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; Yibin Wang, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; Robert B. Cody, PhD, JEOL USA, Inc, 11 Dearborn Road, Peabody, MA 01960; Warren C. Samms, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; and Roger Kahn, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will be aware of a recently developed mass spectrometric technique capable of analyzing a vast array of peptides and large biomolecules with minimal-to-no sample preparation prior to analysis.

This presentation will impact the forensic science community by demonstrating a simple method for the analysis of peptides and large biomolecules that can be easily implemented in drug identification laboratories, often utilizing pre-existing equipment.

The number of internet vendors selling performance-enhancing peptides and cosmetic peptides to the public is staggering. These vendors allow customers the convenience of purchasing peptides online at minimal cost with no questions asked. Received as lyophilized powders, these products are reconstituted in bacteriostatic water with the intention of being injected into the body to promote muscle growth or cosmetic augmentation. Though the sale of peptides may not necessarily be illegal, it is a major concern. Counterfeit peptide sales are known to be fairly common, especially those reportedly containing recombinant Human Growth Hormone (rHGH). This leads to questions regarding the authenticity of the peptides being sold and, more importantly, concerns regarding health and safety.

Many drug identification laboratories do not have protocols in place for successful identification of peptides and large biomolecules. Forensic drug analysis typically relies on Gas Chromatography/Mass Spectrometry (GC/MS) for identification; however, this technique is limited to relatively small molecules that are readily vaporized at the inlet. Due to the increasing presence of high-resolution ambient ionization mass spectrometers in crime laboratory settings, protocols can be easily implemented that utilize the analytical method discussed in this presentation. More importantly, this technique requires no external ion source or additional equipment aside from the mass spectrometer.

Various peptide standards and case samples were successfully analyzed utilizing matrix-assisted inlet ionization MS. To determine the molecular mass of the peptides, samples were dissolved in 1:1 acetonitrile:water with 1% formic acid. The matrix compound, 3-Nitrobenzonitrile (3-NBN), was added to the sample solutions until a noticeable amount of solid accumulated at the bottom of each sample vial. Approximately 5µL of liquid sample, including some of the solid 3-NBN, was drawn up into a microliter pipette. The sample mixtures were introduced directly to the mass spectrometer inlet to initiate ionization. In a matter of seconds, Electrospray Ionization (ESI) -like spectra were obtained. Molecular masses were calculated using a mass spectral interpretation software package.

To further characterize the peptides, a simple enzymatic protein digestion procedure was utilized. Matrix-assisted inlet ionization was utilized to analyze the resulting peptide fragments. Possible identifications for each peptide were assigned by comparing each digested spectrum against an online peptide database. Combined with the

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molecular mass attained from analysis of the intact peptides, identification of each peptide was made at a reasonable level of certainty.

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Peptides, Mass Spectrometry, Inlet Ionization

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