K13 The Detection and Quantitation of Insulin Analogs by Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS) in Postmortem Vitreous Humor

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After attending this presentation, attendees will be able to describe the chemistry of insulin and the challenges surrounding its analysis by LC/MS/MS in forensic samples. In addition, attendees will be able to implement a forensically validated LC/MS/MS method.

This presentation will impact the forensic science community by providing a novel approach for the simultaneous detection and quantification of human insulin and five pharmaceutical analogs as well as describing this approach's application in a series of forensic death investigations.

Insulin is a 51-amino acid peptide hormone produced and released by beta cells in the pancreas in response to rising blood glucose levels. Insulin plays a vital role in glucose metabolism, allowing glucose to enter cells where it can be used to drive cellular activity. The analysis of biological specimens for the presence of exogenous insulin is of special interest in select postmortem investigations. Like other drugs and chemical agents, these compounds may be implicated or suspected in the cause of a death. Toxicological analysis, however, is challenging due to the limited stability of insulin in whole blood and complexities associated with sample preparation and instrumental testing. As a consequence, the determination of insulin in postmortem cases is not routinely performed or offered by forensic laboratories.

Several novel LC/MS/MS based methodologies have been published combining low flow chromatography (microbore or nanobore), anti-insulin antibody immunopurification, as well as ion mobility mass spectrometry. While these approaches have seen some success, no single approach has successfully integrated a straightforward, high throughput, preparation method with clear, unambiguous, discrimination between insulin and the recombinant analogs. The work described here enables unambiguous differentiation of human insulin as well as five pharmaceutical analogs including insulin aspart, glulisine, glargine, lispro, and detemir through the use of high sensitivity liquid chromatography tandem mass spectrometry.

Analysis was performed from 500µL of human vitreous humor with porcine insulin as an internal standard. Insulins were extracted from 500µL of human vitreous via a protein crash (1:1 acetonitrile) followed by solid phase extraction. Purified extracts were evaporated to dryness and re-solubilized in Tris (2-carboxyethyl)phosphine hydrochloride to liberate the alpha and beta chains. Analysis was carried out on an Agilent 6495 triple quadrupole mass spectrometer coupled with a 1290 infinity UHPLC. Separation was performed on a stepwise gradient over 15 minutes on an AdvanceBio Peptide Mapping 2.1mm x 100mm superficially porous column. Validation has been performed in accordance with SWGTOX guidelines including an assessment of within and between day accuracy and precision, limits of detection (60pg/mL) and quantitation (500pg/mL), interference, and stability. Finally, the approach has been successfully applied to an authentic postmortem case.

Insulin, LC/MS/MS, Intact Protein