K39 Fully Automated Detection and Quantification 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 informed about the current state of insulin analysis and the challenges surrounding its detection 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 and by describing its application in a series of forensic death investigations.

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, insulin may be implicated or suspected in the cause of a death; however, toxicological analysis is challenging due to complexities associated with immunoassay screening (cross-reactivity between endogenous and pharmaceutical analogs), challenges regarding multistage sample preparation (protein precipitation coupled to solid phase extraction or antibody immunopurification), as well as difficulties with mass analysis (poor fragmentation, low specificity transitions, analog/isotope coelution, and a reliance on low-flow microbore or nanobore chromatography). As a consequence, the determination of insulin in postmortem cases is not routinely performed. The work described here enables unambiguous differentiation of human insulin as well as five pharmaceutical analogs, including insulin glargine, glulisine, lispro, aspart, human, and detemir, through the use of robotic immuno-microchromatography coupled with insulin β-chain detection by LC/MS/MS.

Insulin extraction was performed on the Agilent® AssayMAP Bravo robotic platform using protein-G cartridges. Before extraction, 150µL of human vitreous humor is diluted 1:1 with Phosphate Buffer Saline (PBS) and fortified with porcine insulin as an internal standard. Cartridges are primed and conditioned with PBS prior to loading two mouse anti-insulin monoclonal antibodies (Santa Cruz SC-377071 and BioRad 5329-3806) to generate anti-insulin immunoaffinity microchromatography cartridges. Diluted vitreous humor is then loaded onto the immunoaffinity cartridges, washed sequentially with 4xPBS, 1xPBS, and 20% acetonitrile in 50mM ammonium bicarbonate, and eluted with 2% acetic acid into an existing volume of 40mM Tris(2-Carboxyethyl)Phosphine Hydrochloride (TCEP-HCL) in 30% acetonitrile. Following a brief incubation, insulin beta chains are analyzed in positive Multiple Reaction Monitoring (MRM) mode on an Agilent® 6495 triple quadrupole mass spectrometer coupled with a 1290 series Ultra High-Performance Liquid Chromatography (UHPLC). Chromatographic separation is performed using an Agilent® RRHD 300Å SB-C18 1.8µm, 2.1mm x 50mm analytical column with a stepwise gradient at 0.4mL/min over nine minutes.

Method validation was performed in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for Standard Practices for Method Validation in Forensic Toxicology. All analogs performed within criteria for acceptable performance. Parameters evaluated included linear range (500pg/mL–25,000pg/mL), limit of quantitation (500pg/mL), limit of detection (500pg/mL for insulin detemir and 125pg/mL for all other analytes), accuracy and precision (within and between run Coefficient of Variation (CV) <20%), interference, carryover, and stability (4°C and -20°C up to 30 days). In addition to the validation results, samples from five cases involving a suspected death by insulin have been analyzed. Of these, one case was positive for insulin aspart (743pg/mL) and one for insulin lispro (2,003pg/mL). A summary of the case history as well as an interpretation of findings will be discussed.

Insulin, LC/MS/MS, Overdose

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