K5 A Validated Method for the Quantitative Determination of Isotonitazene in Hair by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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Learning Overview: After attending this presentation, attendees will better understand a validated method for the detection and quantification of isotonitazene and its metabolites in hair by LC/MS/MS.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by describing a method validation to rapidly and simultaneously confirm isotonitazene and its metabolites in hair.

Introduction: Isotonitazene is considered as an emerging New Psychoactive Substance (NPS) being sold as a designer drug. Preclinical pharmacology studies show that isotonitazene has the pharmacological profile similar to that of the potent synthetic opioid etonitazene, a Schedule I controlled substance. Because of the pharmacological similarities of isotonitazene to etonitazene, the use of isotonitazene presents a high risk of abuse and may negatively affect users and communities. According to the Federal Register (2020), abuse potential of isotonitazene is very high and no treatment is available in the United States. Pharmacological data suggest that isotonitazene has a potency similar to or greater than fentanyl and many fentanyl analogs. It is considered to be 2.5 times more potent than hydromorphone. Detection times of drugs and metabolites in hair are much longer than in urine, blood, and oral fluid. Blood and urine are utilized mainly in acute ingestions of isotonitazene, while hair is ideal for detection in chronic abuse. Therefore, hair is an ideal matrix for the detection of isotonitazene and metabolites if the tests are performed weeks after intake.

Methods: Recently, an LC/MS/MS method was developed for the detection, identification, and quantification of isotonitazene and its metabolites, 5 aminoisotonitazene and 4- hydroxy nitazene, in hair samples. Briefly, the method involved extraction of drugs and metabolites from hair using acidic methanol. The dried extracts, after reconstitution with organic/aqueous solvents mixture, were injected onto an Agilent® 6460 Triple Quadrupole (QQQ) LC/MS/MS in positive ionization mode. Separation was achieved on an Agilent® ZORBAX® Eclipse XDB-C18 column (4.6 x 50mm, 1.8µm) with a flow rate of 0.4mL/min of 5mM ammonium formate:methanol (9:1) (A) and 0.1% formic acid in methanol (B) mobile phase under gradient conditions. Sample preparation involves washing of hair with aqueous and organic solvents to remove the external contamination followed by incubation with 2mL of acidic methanol at 60°C.

Results: Good linearity and reproducibility were obtained for isotonitazene and metabolites with a coefficient of determination R^2>0.99. The linear range of the assay was 100–2,000pg/mg. Limits Of Quantification (LOQ) ranged from 1pg/mg to 5pg/mg for isotonitazene and its metabolites. Excellent imprecision and accuracy were achieved (CV <15%) for all compounds.

Isotonitazene, LC/MS/MS, Drug Testing

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