Utilizing LC-HRMS for the Identification of Protonitazene and Its Metabolites in Urine

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Background:
New synthetic opioids (NSOs) are a leading cause of drug overdose deaths, with the emergence of "nitazenes" as a subclass within this category. Protonitazene, a nitazene analog first detected in Canada and the USA in 2020, is more potent than fentanyl and is not approved for human or veterinary use. To date, protonitazene abuse and deaths have been reported globally. Forensic and clinical laboratories need to be ready for the ongoing emergence of nitazene analogs. Among the challenges they face is the availability of analytical standards. ACFT has successfully developed a liquid chromatography high resolution mass spectrometry (LC-HRMS) method using accurate mass to detect protonitazene and its metabolites in urine.

Methods:
Urine samples were hydrolyzed using IMCSzyme RT (IMCS) at room temperature and then cleaned up by dilution and filtration. The analysis was conducted on an Ultimate3000 HPLC coupled with a Q-Exactive® Orbitrap high resolution mass spectrometer (HRMS) (ThermoFisher) operated in full-MS mode at resolving power of 140,000. Separation was achieved on an Agilent Poroshell column (120 EC-C18, 100x2.1mm, 2.7µm) using gradient elution of 0.1% acetic acid and acetonitrile at 45°C with flow rate at 400 µL/min.

Results
Protonitazene metabolism is based on reported isotonitazene data as the information is limited. Separation of protonitazene and its metabolites from interferences in urine was achieved either by accurate mass measurement using the highest resolving-power of Orbitrap MS or chromatographically. Separation of the structural isomers, isotonitazene and protonitazene, was accomplished on the column as the Orbitrap MS is unable to differentiate them. Detection was based on accurate mass and relative retention time (RRT) to available reference standards. The method was highly selective and specific. Using this method, we effectively identified protonitazene and three metabolites in patient urine, relying on isotonitazene as the sole available standard. Detected analytes were confirmed after standards became available. Protonizene, N-desethyl protonitazene, 4-OH nitazene and N-desethyl O-desalkyl nitazene were detected in patient urine, while 5-amimo-protonitazene, another possible metabolite, was not found. N-Desethyl O-desalkyl nitazine and 4-OH nitazene exhibited higher concentrations and a longer detection window.

Conclusions
A highly selective LC-HRMS method was developed for the detection of protonitazene in human urine. Accurate mass measurement serves as a valuable tool for identifying emerging drugs in the absence of standards.

Key words:
protonitazene, LC-HRMS, accurate mass