K48 Sample Mining: The Identification of Emerging Novel Psychoactive Substances (NPS) Through Reanalysis of Biological Extracts From Forensic Toxicology Casework

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Learning Overview: After attending this presentation, attendees will understand the value of real-time sample analysis and retrospective data analysis for the identification of emerging NPS present in toxicological extracts, but not within the initial scope of analytical testing.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by describing novel workflows and approaches for the identification of emerging NPS and providing information regarding NPS currently being detected, or undetected, in forensic casework.

NPS continue to pose health and safety threats to drug users and various clinical, forensic, public health, and public safety communities within the United States. Medical and forensic toxicologists are now tasked with the difficulty of interpretation of clinical and analytical findings, but this interpretation is dependent on the scope of testing, the availability of standard reference material, and the ability to characterize novel substances. This study sought to evaluate innovative methodologies for the identification of emerging NPS timely to first incident or detection using an NPS-rich dataset.

Discarded sample vial extracts from a large forensic toxicology laboratory (NMS Labs) were acquired for re-analysis against an extensive in-house library database. Sample extracts were collected from initial testing procedures for the directed analysis of select novel stimulants, novel opioids, and designer benzodiazepines, as well as other drug classes. Sample extracts were de-identified prior to inclusion in this study.

In-house re-analysis of sample extracts was performed via Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) using a SCIEX™ TripleTOF™ 5600+ coupled with a Shimadzu® Nexera® XR UHPLC. Chromatographic separation was achieved by a standard reverse phase gradient using ammonium formate (10mM, pH 3), methanol/acetonitrile (50:50), and a Phenomenex® Kinetex C18 analytical column (50mm x 3.0mm, 2.6µm). The total runtime was 15.5 minutes. Precursor ion mass acquisition was achieved by TOF/MS scan from 100-510 Da. Product ion mass acquisition was achieved following isolation using SWATH™ acquisition. Fragmentation occurred using a rolling collision energy of 35eV±15eV for the generation of mass-window specific fragment ion spectra. Resulting datafiles were compared against an extensive in-house library database containing precursor ion exact mass, retention time, fragment ion accurate masses (n=5), and library mass spectra for more than 700 drugs, including a clear majority of NPS.

To date, 2,136 sample extracts have been re-analyzed using the described workflow. Comprehensive data processing has resulted in the identification of a wide-variety of NPS across several categories, some of which were incorporated into initial testing procedures. The most common novel opioids detected included cyclopropylfentanyl, methoxyacetylfentanyl, and fluoro-isobutyrylfentanyl; the most common novel stimulant detected was N-ethyl pentylone; and the most common designer benzodiazepines detected were flubromazelam and etizolam.

This novel workflow has resulted in the identification of NPS for the first time in toxicological specimens, including isopropyl-U-47700 and 3,4-methylenedioxy-U-47700. In addition, novel opioid precursors have been identified in the extracts, including N-methylnorfentanyl, benzylfentanyl, despropionyl-ortho-methylfentanyl, despropionyl-3-methylfentanyl, and benzylfuranylfentanyl. While many of these fentanyl analogue precursors are believed to be inactive, their presence in toxicological extracts can provide useful information to pinpoint a likely route of manufacture or possibly novel opioid active agent.

NPS continue to emerge on the drug market. Based on the results of this study, biological extracts for sample mining and archived datafiles for data mining proved to be a rich dataset for the identification and discovery of emerging NPS. Forensic laboratories not currently utilizing updated broad-based screening methodologies or retrospective data analysis workflows should be aware that NPS in toxicological specimens could go undetected.

NPS, Novel Opioids, Isopropyl-U-47700