K5 The "I's" Have It: A High-Performance Liquid Chromatography Tandem Mass Spectrometry Method for the Determination of 25I-NBOH, 25I-NBOMe, and 2C-I in Urine

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After attending this presentation, attendees will understand how to analyze for the three 4-iodo-2,5-dimethoxy-phenethylamines designer hallucinogens.

This presentation will impact the forensic science community by offering a method for the identification and quantitation of three designer hallucinogens in human urine which may be used in clinical and forensic toxicology laboratories.

Introduction: In 2010, a novel class of designer hallucinogens, the N-methoxybenzyl-methoxyphenylethylamine (NBOMe) derivatives of Alexander Shulgin's 2,5-dimethoxy-phenethylamines, became readily available on the internet. These derivatives are potent serotonin 2A (5-HT2A) receptor agonists. Currently, NBOMe derivatives are sold as powders or on blotter paper with 25I-NBOMe (4-iodo-2,5-dimethoxy-N-(2-methoxybenzyl)-phenylethylamine) being the most commonly reported. Clinical presentations of severe NBOMe intoxication include tachycardia, agitation, hypertension, aggressive/violent behavior, hallucinations, and continuous agitation and seizures which can persist for as long as three days. In November of 2013, three designer phenethylamines, 25I-NBOMe, 25C-NBOMe, and 25B-NBOMe (the 4-chloro- and 4-bromo- 2,5-dimethoxy-N-(2-methoxybenzyl)-phenylethylamine), were temporarily declared Schedule I drugs. Recently, unscheduled NBOMe type derivatives have become available, with anecdotal evidence suggesting that 2-((2-(4-iodo-2,5-dimethoxyphenyl)ethylamino)methyl)phenol (25I-NBOH) is the most prevalent. Shulgin's designer hallucinogen, 2,5-Dimethoxy-4-iodophenethylamine (2C-I) is a precursor of both 25I-NBOMe and 25I-NBOH, and may be present in specimens as an impurity. Evidence also suggests 2C-I is a metabolite, formed by N-debenzylation, of both 25I-NBOMe and 25I-NBOH.

Objective: To develop a method for the detection and quantification of 25I-NBOMe, 25I-NBOH, and 2C-I in human urine as part of a dose response, disposition, metabolism, and behavioral studies concerning these designer hallucinogens.

Methods: An Ultra Performance Liquid Chromatography/Tandem Mass Spectrometry (UPLC/MS/MS) method was developed for the detection and quantification of 25I-NBOMe, 25I-NBOH, and 2C-I in human urine. Following the addition of the deuterated internal standard (25I-NBOMe-d3), the hallucinogens were isolated by a previously published solid phase extraction method.[1] Chromatographic separation was performed on a Selectra® PFPP column, 10cm x 2.1mm, 3.0µm. The mobile phase consisted of A: water with 10mM ammonium formate, and B: methanol with 10mM ammonium formate. The following gradient was used: 0.0-3.0min starting at 60% B, with a linear gradient to 95% B, and then returning at 4.5min to 60% B. An injection volume of 5µL was used with a mobile phase flow rate of 0.4mL/min and a total run time of 4.5min. The following transition ions (m/z) were monitored for 25I-NBOMe: 428>121, 428>91, 428>272; 25I-NBOH: 414>107, 414>291, 414>308; 2C-I; 308>91, 308>276, 308>291; and, 25I-NBOMe-d3: 431>124, 428>92, 428>275. The method was evaluated for absolute recovery, ion suppression, accuracy/bias, inter-day and intra-day precision, interferences, bench top stability, freeze/thaw, and post-preparative stability.

Results: Duplicate calibration curves were determined to be within 20% of the nominal value for each analyte. The linear regression correlation coefficients for each analyte's calibration r^2 were 0.99 or greater. 25I-NBOMe and 25I-NBOH were linear from 10pg/mL to 500pg/mL, while 2C-I was linear from 50pg/mL to 500pg/mL. The Limit Of Detection (LOD) was administratively set at 10pg/mL for 25I-NBOMe, 25I-NBOH, and 2C-I. Assay performance was evaluated using a set of five quality-control specimens. Accuracy/bias of the assay was determined to be within +/-20% of the target value for each analyte in each quality control specimen. The CV for inter-day and intra-day precision samples did not exceed 15%, except for the Limit of Quantitation (LOQ) samples which did not exceed 20%. Two urine specimens were analyzed; one contained all three analytes, while the other specimen contained only 25I-NBOH.

Conclusion: This validated method was found to be robust and reliable for the detection and quantification of 25I-NBOMe, 25I-NBOH, and 2C-I in human urine.

Reference:

1. Poklis JL, Charles J, Wolf CE, Poklis A. High-performance liquid chromatography tandem mass spectrometry method for the determination of 2CC-NBOMe and 25I-NBOMe in human serum. *Biomed Chromatogr*. 2013, 12:1794-800.

Designer Drugs, UPLC/MS/MS, 25I-NBOMe