



### **K35 Analysis of N-Bombs: Quantitation of NBOMe-type Novel Psychoactive Substances (NPS) in Biological Fluids by Liquid Chromatography With Tandem Mass Spectrometry (LC/MS/MS)**

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After attending this presentation, attendees will be able to describe the characteristics and effects of NBOMe-class compounds, implement methods for their analysis in biological fluids using LC/MS/MS, and learn about the concentrations of these analytes detected in biological fluids in forensic cases.

This presentation will impact the forensic science community by describing a comprehensive assay for the detection of this emerging class of NPS with known adverse effects. The detection and quantitation of these drugs in toxicology casework will also be discussed.

NBOMes, known as N-Bombs by the drug-using community, are a group of psychedelic substituted phenethylamines being seen with increasing frequency in recreational drug products being sold online and through underground suppliers in the United States. They are derivatives of the 2C family of phenethylamine stimulants first described by Alexander Shulgin throughout the 1980s. 25I-NBOMe is the N-benzyl methoxy derivative of 2C-I; the substitution is considered to increase the potency and psychoactivity of the compound. Substitutions of the other members of the 2C class result in additional members of the NBOMe series. These compounds have gained popularity among recreational drug users as an alternative to LSD and are often impregnated onto blotter papers or administered in powder and liquid formulations. Members of the NBOMe series have been linked to fatal intoxications, characterized by violent behavior, psychosis, and extreme sympathetic stimulation. Case reports of NBOMe use have included hospitalizations and deaths. Due to the substantial risk of toxicity from this group of compounds, and the need to confirm their use, it was necessary to develop testing for the detection of NBOMes for forensic toxicology applications.

A method for the detection of four members of the NBOMe class — 25I-NBOMe, 25B-NBOMe, 25C-NBOMe, and 25H-NBOMe — is described. Since the NBOMes have been reported to be active in sub-milligram doses, a positive ion mode LC/MS/MS procedure was developed for the determination of these compounds in serum, plasma, and whole blood.

Samples were prepared for analysis by a simple protein precipitation process using acetonitrile. Ultra Performance Liquid Chromatography (UPLC) conditions for the LC/MS/MS method included 0.1% formic acid in water vs 0.1% formic acid in methanol, at 0.400mL/min on a BEH C18, 2.1mm x 50mm column. The test is routinely performed qualitatively; however, quantitative values were achieved using the standard addition approach. The calibration range for the standard addition method used a calibration curve from 0.5ng/mL-50ng/mL of each drug. A qualitative urine extraction for the detection of NBOMe samples was developed separately. The NBOMes were extracted from urine using a solid-phase extraction after pH adjustment. Conditions for analysis by UPLC are similar to the blood method.

Since September of 2013, this study has encountered 25 positive cases for the NBOMe drugs, including 22 whole blood samples, one serum/plasma sample, and three urines. Of the 25 cases, 14 screened positive for 25I-NBOMe, three for 25C-NBOMe, five for 25B-NBOMe, and three cases contained more than one of the target drugs (25I-NBOMe plus 25B-NBOMe; 25I-NBOMe plus 25H-NBOMe; and, 25C-NBOMe plus 25H-NBOMe). Of the cases with demographic information available (n=18), the median age was 18 years and included 11 males and seven females.

Available samples were subsequently subjected to quantitative analysis using the standard addition method. Of 12 blood cases tested, 25I-NBOMe was detected in seven of these cases with mean and median concentrations of 2.63ng/mL and 1.82ng/mL, respectively (range 0.79ng/mL-6.3ng/mL). 25C-NBOMe was detected in three of the blood samples, with mean and median results of 2.96ng/mL and 2.18ng/mL (range 2.16ng/mL-4.53ng/mL). In one sample, 25H-NBOMe was present at a concentration of 2.10ng/mL in conjunction with 4.53ng/mL of 25C-NBOMe.



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Although quantitation of NBOMe drugs have been previously reported in isolated cases, this is the first documented attempt to develop a comprehensive panel for the most popular NBOMe drugs which can be easily revalidated for new members of the drug class when they appear. The method can be used qualitatively as a screen or with standard addition for confirmation and quantitation. This LC/MS/MS approach provides the sensitivity and specificity necessary for the detection and quantitation of NBOMes in forensic toxicology casework.

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## **NBOMe, Novel Psychoactive Substances, Toxicology**