

K11 Suicide After 25C-N-Methoxybenzyl (25C-NBOMe) and 25H-N-Methoxybenzyl (25H-NBOMe) Consumption

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After attending this presentation, attendees will understand the importance of developing new methods for the determination of New Psychoactive Substances (NPS) in biological matrices as the use of these drugs of abuse may lead to tragic consequences.

This presentation will impact the forensic science community by providing information on NBOMes and on NPS intoxication in general that represents an emerging problem; as such, forensic toxicology laboratories should improve routine methods for the detection of these new drugs of abuse. NPS in biological matrices should be routinely determined in forensic toxicology investigations, since the use of these drugs of abuse may lead to tragic consequences.

A 16-year-old male was found dead in a waterway after he was seen jumping into the water stream. The boy was seen deeply agitated and confused after having attended a party with friends. At the party location, policemen seized several doses of marijuana as well as pieces of blotter paper. A complete autopsy and a histological evaluation of the main tissues were performed; though the death occurred by drowning, toxicological exams were requested by the prosecutor in order to evaluate the potential role of drugs of abuse in this death. Blood (peripheral and central) and urine samples as well as blotter papers were collected and analyzed as follows.

The blotter paper was soaked into $500\mu L$ methanol and sonicated up to five minutes. An aliquot of the solution was directly injected into a gas chromatograph coupled with mass spectrometer (GC/MS), while $50\mu L$ of the same solution were taken to dryness and, after derivatization with pentafluoropropionic anhydride (PFPA), reconstituted in ethylacetate and injected into the GC/MS. The two analyses of the blotter paper revealed the presence of substances belonging to the 25-NBOMe family. The N-methoxybenzyl (NBOMe) derivatives are emerging psychedelic drugs, with severe hallucinogenic effects that can occur even after an intake of $50\mu g$ - $250\mu g$ of the substance.

A liquid chromatography tandem mass spectrometric (LC/MS/MS) system was used to identify and quantify five different 25-NBOMes (namely: 25B-NBOMe, 25C-NBOMe, 25D-NBOMe, 25H-NBOMe, and 25I-NBOMe) in blood and urine. 25E-NBOMe was used as internal standard, since at the time of the death, this NBOMe was not yet available. 1mL of urine and 1mL of blood (peripheral and cardiac) were diluted in 2mL phosphate buffer containing IS and purified on a solid phase extraction (SPE) cartridge. The elution solvent was dried under nitrogen

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stream and reconstituted in 200µL mobile phase. The chromatographic separation was performed on a C18 column in gradient elution. Two Multiple Reaction Monitoring (MRM) transitions were monitored for each compound, in positive polarization. Calibration curves ranged from 0.2-5.0ng/mL with LOD and LOQ for the five 25-NBOMes calculated at 0.05ng/mL and 0.1ng/mL respectively. Linearity, accuracy, precision, ion suppression, carry over, and recovery were tested and fulfilled the laboratory's acceptance criteria.

Blood and urine provided positive results for 25C-NBOMe and 25H-NBOMe are as follows: 2.80ng/mL and 0.29ng/mL in peripheral blood; 1.43ng/mL and 0.13ng/mL in central blood; and finally 0.94ng/mL and 0.14ng/mL in urine respectively. Eventually, the seized blotter papers were tested with LC-MS/MS and the presence of both 25C- and 25H-NBOMe was confirmed. THC and THCCOOH were also found in the biological fluids: 15.5ng/mL and 56.0ng/mL in peripheral blood, 9.9ng/mL and 8.5ng/mL in central blood respectively, and urine THCCOOH (9.5ng/mL).

Though the boy was certainly under the influence of THC at the time of his death, the concentration of cannabinoids in the biological fluids is not consistent with the symptoms (agitation, hallucinations, and confusion) just before the incident. On the contrary, NBOMes can produce severe hallucinations even at very low doses, and 25C-NBOMe is considered potentially toxic at the levels measured in this boy's blood.

This presentation will impact the forensic science community by improving information on NBOMes and generally on NPS intoxication that represent an emerging problem. Therefore, forensic toxicology laboratories should improve routine methods for the detection of these new drugs of abuse.

Forensic Toxicology, 25C-NBOMe, 25H-NBOMe