
Case Report

Detection of 25C-NBOMe in Three Related Cases

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Abstract

An accidental death associated with the use of the designer drug, 2-(4-chloro-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine (25C-NBOMe), is reported. A 23-year-old Caucasian male experienced severe respiratory distress and died after being subdued by military law enforcement. At autopsy, remarkable findings upon internal examination included mild to moderate coronary atherosclerosis, biventricular dilation, mild right ventricular hypertrophy and bilateral pulmonary edema and congestion. The decedent's blood contained no drugs, ethanol or other volatile compounds. Pseudoephedrine, nicotine and cotinine were detected in his urine. A LC-QTOF designer drug screen, employing a basic solid-phase extraction, was used to isolate 25C-NBOMe, 25C-NBOH and 2C-C from both blood and urine specimens. Quantitative analysis was performed by LC-MS-MS operating in multiple reaction monitoring mode. 25C-NBOMe and 2C-C were present in the blood (2.07 and 0.12 ng/mL) and in the urine (27.43 ng/mL and 0.38 ng/mL), respectively. 25C-NBOMe concentrations were determined by standard addition in the brain (19.10 ng/g), spleen (27.13 ng/g), lung (25.21 ng/g), liver (15.20 ng/g), kidney (25.06 ng/g) and gastric contents (30.24 µg total in 100 mL submitted). On the basis of decedent case history, autopsy and toxicological findings, the medical examiner ruled the cause of death as 25C-NBOMe toxicity temporally associated with excited delirium and forcible restraint. The manner of death was ruled accidental.

Introduction

The search for a legal high has occurred for decades, leading to a diverse and wide-ranging group of compounds simply referred to as 'designer drugs'. This class of compounds includes substituted phenethylamines, piperazines, tryptamines and most recently cannabinoids. The substituted phenethylamines contain a subset of compounds first synthesized in the 1970s by Alexander Shulgin, including the '2C' compounds characterized by methoxy groups at the 2 and 5 positions on the benzyl ring and commonly halogens at the 4 position (1). The 2C compounds serve as the chemical backbone for a newer class of designer drugs that substitutes a 2-methoxybenzyl group onto the amine nitrogen of the 2C structure. The result is a class of compounds known as 25-NBOMe or NBOMe (2), shown in Figure 1.

NBOMe compounds were first synthesized by researchers studying the activity of the 5-HT_{2A} serotonin receptor. These compounds have a high affinity for, and are potent agonists of, the 5-HT_{2A} receptor.

The activation of these receptors is responsible for the hallucinogenic effects caused by these drugs (3–5). It was also observed that the N-benzyl derivative exhibited a higher binding affinity to the receptor than its respective 2C analog (6, 7). As a result, NBOMe compounds are active at a sub-milligram dose, similar to lysergic acid diethylamine (LSD). Owing to the effect and dosing similarities, it is often illicitly sold as an alternative to LSD, as LSD itself, synthetic LSD or simply by the LSD's street name 'Acid' (8, 9).

A majority of NBOMe intoxication reports, both fatal and non-fatal, have involved one of the three compounds: 2-(4-iodo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine (25I-NBOMe), 2-(4-chloro-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine (25C-NBOMe) and 2-(4-bromo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine (25B-NBOMe) (9–12). These compounds are sold as either a powder or a blotter paper (8, 13–16). The dose can be administered sublingually/orally (17–19), or intranasally (14), as well

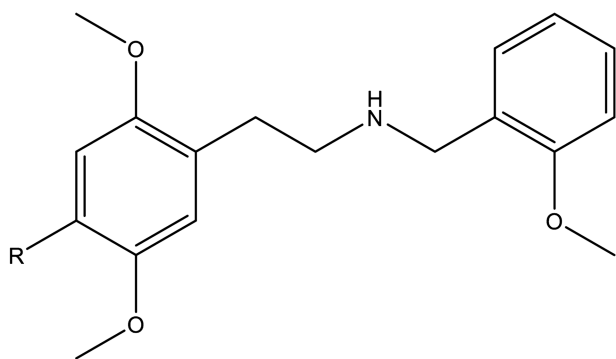


Figure 1. Chemical structure of 25C-NBOMe class of compounds. Substitution of halogen at the 4-position on the dimethoxyphenyl ring results in the specific compound: R = chlorine (25C-NBOMe), R = bromine (25B-NBOMe) and R = iodine (25I-NBOMe).

as smoked and intravenously injected. In regard to 25C-NBOMe specifically, sublingual and intranasal doses range from 200 to 1,000 µg, while only 50–500 µg when smoked as a free base (2). Users have reported a number of hallucinogenic effects, including extreme patterning, vibrant coloring, introspection, euphoria, acceleration of thought and time distortion (2, 20).

The following case report presents the detection of 25C-NBOMe in a postmortem case and two related investigative impairment cases. These are the first cases where NBOMe compounds were identified by the Division of Forensic Toxicology, and the first case of 25C-NBOMe reported as a contributing factor to the cause of death of an individual by the Armed Forces Medical Examiner System.

Case report

Case 1

A 23-year-old Caucasian male became agitated and combative shortly after being detained by the military police. The individual continued to resist while he was handcuffed and placed prone on the ground despite the use of an electronic control device (ECD) and oleoresin capsicum (OC) spray. The individual began experiencing respiratory difficulties while in custody, necessitating life-saving measures to be initiated at the scene. He was transported to a hospital where he was pronounced deceased.

Upon external examination, the decedent displayed multiple abrasions and contusions, as well as puncture wounds consistent with the use of an ECD. Internally, remarkable findings included mild to moderate coronary atherosclerosis, biventricular dilatation, mild right ventricular hypertrophy and bilateral pulmonary edema and congestion. Postmortem specimens were submitted for a comprehensive toxicological examination including volatiles, drugs of abuse and therapeutic medications. Additional testing was assigned based on the pictures of plastic bags containing white powder labeled 'LSD' that was discovered on the decedent's phone.

Cases 2 and 3

Two adult males accompanied the decedent on the night of the incident, a 30-year-old Hispanic (Case 2) and a 24-year-old Caucasian (Case 3). As part of the investigation, blood and urine samples were also collected from these two individuals. Blood and urine samples from each individual were submitted for a comprehensive toxicological examination.

Experimental

Chemicals and materials

All solvents (HPLC-grade or better) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Hydrochloric acid, formic acid, ammonium hydroxide and ammonium formate were also purchased from Fisher Scientific. Glacial acetic acid, sodium acetate, sodium phosphate monobasic, sodium phosphate dibasic and β-glucuronidase type HP-2 from *Helix pomatia* were purchased from Sigma-Aldrich (St Louis, MO, USA). Clean Screen® mixed-mode silica-based solid-phase extraction columns (ZCDAU020) were purchased from United Chemical Technologies (Bristol, PA, USA). CEREX® Clin II polymeric solid-phase extraction columns (691-0506) were purchased from SPEware (Baldwin Park, CA, USA).

25C-NBOMe hydrochloride (2-(4-chloro-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine) and 25C-NBOH hydrochloride (2-[[[2-(4-chloro-2,5-dimethoxyphenyl)ethyl]amino]methyl]-phenol) were purchased from Cayman Chemical (Ann Arbor, MI, USA). 2C-C hydrochloride (4-chloro-2,5-dimethoxyphenethylamine) and internal standards MDPV-d₈ hydrochloride (3,4-methylenedioxypyrovalerone-d₈) and 25I-NBOMe-d₃ hydrochloride (4-iodo-2,5-dimethoxy-N-[[2-methoxyphenyl]methyl]-benzeneethanamine-d₃) were purchased from Cerilliant (Round Rock, TX, USA). Proadifen (SKF-525A) hydrochloride was purchased from Sigma-Aldrich.

Preparation of standards, calibrators and controls

Stock standards of 25C-NBOMe, 25C-NBOH and 2C-C were prepared at target concentrations of 100 µg/mL in methanol. For quantitative analysis, mixed calibrator working standards were prepared at 0.1 and 0.01 µg/mL by serial dilution with methanol. A mixed control working standard was also prepared at 0.1 µg/mL in methanol from separate stock solution. A working internal standard solution containing MDPV-d₈ and 25I-NBOMe-d₃ was prepared at 0.1 µg/mL in methanol. All solutions were stored in amber glass vials at less than or equal to –20°C.

Calibrators containing 25C-NBOMe, 25C-NBOH and 2C-C were prepared for quantitative analysis in 1.0 mL of in-house certified drug-free negative blood at concentrations of 0.05, 0.10, 0.25, 0.50, 1.0 and 10 ng/mL. A positive blood control was prepared at 0.75 ng/mL. Calibrators, a positive blood control and a negative blood control were analyzed with each batch of samples.

Qualitative analysis

On the basis of case history, a directed screening analysis targeting emerging drugs of abuse (including LSD, tryptamines, piperazines, cathinones, NBOMe compounds and other substituted phenethylamines) was ordered for all three cases. Urine aliquots of 0.5 mL were spiked with a 2.5 µg/mL proadifen (SKF-525A) working internal standard for a final concentration of 125 ng/mL. One milliliter of 0.1 M sodium acetate buffer (pH 4.5) and 25 µL of crude β-glucuronidase solution (≥100,000 units/mL) were added to each sample and vortexed briefly. Enzymatic hydrolysis was performed during a 30-min incubation at 70°C. Samples were removed from incubation and allowed to cool to room temperature. Sample pH was adjusted with 3 mL of 0.1 M phosphate buffer (pH 6.0) and the samples were briefly vortexed. The samples were centrifuged at 3,000 rpm for 5 min before application onto CEREX® Clin II polymeric solid-phase extraction columns (691-0506). No conditioning steps were necessary. Sample flow was assisted with 1–2 psi (N₂) positive pressure. Columns were subsequently washed with 1 mL of deionized water, 1.25 mL of 0.1 M acetic acid and 2 mL of methanol at 1–2 psi positive

pressure. Columns were dried at full positive pressure for 10 min. Target compounds were eluted by gravity with 2 mL of methylene chloride/isopropanol/ammonium hydroxide (78/20/2, v/v/v) into conical glass tubes. Ten microliters of 10% (v/v) hydrochloric acid in methanol was added to each extract prior to drying at 40°C under nitrogen. Samples were reconstituted with 100 μ L of initial mobile phase conditions, vortexed briefly and transferred to autosampler vials for analysis.

LC-QTOF screening was performed using a Waters Acquity ultra performance liquid chromatograph (UPLC) coupled with a Xevo G2 quadrupole time-of-flight mass spectrometer (Milford, MA, USA). Waters MassLynx (version 4.1), TargetLynx, ChromaLynx XS and TOF Toxicology Database (version 1, user-modified) software were used for data acquisition and processing.

Chromatographic separation was performed using a Waters HSS C18 column (150 mm $\times$ 2.1 mm $\times$ 1.8 μ m) maintained at 50°C. The sample injection volume was 4 μ L. Mobile phase A was 5 mM ammonium formate, pH 3.0 (adjusted with formic acid) and mobile phase B was 0.1% formic acid in acetonitrile. A constant flow rate of 0.4 mL/min was used. Initial gradient conditions 87% A and 13% B were held for 0.5 min. The gradient was increased to 50% B at 10 min, ramped to 95% B at 10.75 min and held until 12.25 min. The column was re-equilibrated to initial conditions (13% B) at 12.50 min and held for 2.5 min. The total run time was 15 min.

The QTOF mass spectrometer was operated in positive electrospray ionization (ESI) mode utilizing MS^E acquisition. Briefly, MS^E permits simultaneous acquisition of low collision energy (precursor ion spectra) and high collision energy (product ion spectra) functions (21). Source conditions were as follows: capillary voltage 0.8 kV, sample cone voltage 20 V, extraction cone voltage 4 V, source temperature 140°C, desolvation gas (nitrogen) temperature 500°C, desolvation gas flow 900 L/h and cone gas (nitrogen) flow 20 L/h. For the MS^E functions, collision energy was constant at 6 eV for low energy and ramped from 10 to 40 eV for high energy. Centroid data were acquired in resolution mode from m/z 50–1000 with an acquisition time of 0.1 s in both MS^E functions. A 0.5 μ g/mL solution of leucine enkephalin in 50/50 (v/v) deionized water/acetonitrile with 0.1% formic acid was infused as a reference compound during acquisition through a separate capillary. Reference ('LockSpray') conditions were as follows: flow rate 20 μ L/min, scan time 0.2 s and intervals 20 s. Mass correction using the reference compound was applied automatically during acquisition. Observed resolution was >20,000 for m/z 556.2771.

Quantitative analysis

Two milliliters of calibrators, controls and case samples (blood, urine or tissue homogenates) were used for extraction. Tissue homogenates were prepared with deionized water at appropriate dilutions. Tissue aliquots were analyzed by standard addition as described by Poklis *et al.* (17). Four aliquots were used for each tissue specimen. Mixed calibrator working standards containing 25C-NBOMe, 25C-NBOH and 2C-C were added to tissue aliquots at 0, 0.1, 0.5 and 1.0 ng.

Internal standard (MDPV-d₈ and 25I-NBOMe-d₃) was added yielding final concentrations of 1.0 ng/mL. Sample pH was adjusted with 2 mL of 0.1 M phosphate buffer (pH 6.0) followed by brief vortexing. Samples were centrifuged at 3,500 rpm for 5 min in preparation for solid-phase extraction using UCT mixed-mode silica-based columns (ZCDAU020). Columns were conditioned sequentially with 3 mL of methanol, 2 mL of deionized water and 2 mL of 0.1 M phosphate buffer (pH 6.0). The samples were loaded on the columns and allowed to flow by gravity. Columns were washed sequentially with 2 mL of deionized water, 2 mL of 0.1 M acetic acid and 3 mL of methanol. Columns

Table I. LC–MS–MS Compound-Dependent Parameters used for Quantitation

Analyte	RT (min)	MRM transition (m/z) ^a	DP	CE	CXP
2C-C	1.48	216.1 > 184.0	51	27	10
		216.1 > 168.9	51	37	16
MDPV-d ₈	1.70	284.1 > 134.1	86	35	10
		284.1 > 175.1	86	31	8
25C-NBOH	2.76	322.1 > 199.0	46	29	8
		322.1 > 107.0	46	43	10
25C-NBOMe	3.62	336.0 > 91.1	41	55	8
		336.0 > 121.1	41	29	10
25I-NBOMe-d ₃	3.84	431.0 > 124.0	71	25	12
		431.0 > 92.1	71	77	8

RT, retention time; DP, declustering potential; CE, collision energy; CXP, collision cell exit potential.

^aQuantitation ion transitions listed first, followed by qualifier ion transition.

were dried under full vacuum for 10 min. Target compounds were eluted by gravity with 3 mL of methylene chloride/isopropanol/ammonium hydroxide (78/20/2, v/v/v) into conical glass tubes. Ten microliters of 10% (v/v) hydrochloric acid in methanol was added to each extract prior to drying at 40°C under nitrogen. Samples were reconstituted with 100 μ L of mobile phase (50/50 A/B), vortexed briefly and transferred to autosampler vials for analysis.

LC–MS–MS confirmation was performed using a Shimadzu (Columbia, MD, USA) Nexera X2 UPLC coupled with an AB SCIEX (Framingham, MA, USA) 4500 QTRAP tandem mass spectrometer. AB SCIEX Analyst software 1.6.2 was used for data acquisition and quantitative processing.

Chromatographic separation was performed on a Restek (Bellefonte, PA, USA) Pinnacle DB PFPP column (100 mm $\times$ 2.1 mm $\times$ 3 μ m) maintained at 40°C. The sample injection volume was 10 μ L. Mobile phase A was 10 mM ammonium formate with 0.01% formic acid (pH 4.5) and mobile phase B was 50/50 (v/v) acetonitrile/methanol with 0.01% formic acid. A constant flow rate of 0.9 mL/min was used. Initial gradient conditions 60% A and 40% B were held for 0.5 min. The gradient was increased to 75% B at 4.0 min and held until 7.5 min. The column was re-equilibrated to initial conditions (40% B) at 7.6 min and held for 0.4 min. The total run time was 8.0 min.

The 4500 QTRAP mass spectrometer was operated in positive turbo spray (ESI) mode. Source parameters were as follows: ion spray voltage, 4,500 V; curtain gas, 25 psi; nebulizer gas (GS1), 50 psi; auxiliary gas (turbo, GS2), 60 psi and turbo gas temperature, 450°C. Collision gas was set to medium. Data were acquired in multiple reaction monitoring (MRM) mode using unit mass resolution. Optimized settings for compound-dependent parameters are listed in Table I, including retention times (RT), MRM ion transitions, declustering potentials, collision energies and collision cell exit potentials. Entrance potentials for all compounds were set to 10 V. Dwell times for all MRM transitions were 30 ms.

Results and discussion

All three cases were screened for volatiles, therapeutic drugs and drugs of abuse. No ethanol or other volatile compounds were detected at a 20 mg/dL cutoff. Enzyme immunoassay urine screens for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates, oxycodone, phencyclidine, 6-acetylmorphine and LSD were negative for all cases. Further testing for postmortem Case 1 included a full-scan GC–MS alkaline drug screen and color tests for acetaminophen

and salicylates. Pseudoephedrine, nicotine and cotinine were detected in the urine. Pseudoephedrine was subsequently confirmed in urine by GC-MS, but was not detected in the decedent's blood at a 0.05 mg/L cutoff.

Tryptase testing was requested by the medical examiner for Case 1. Testing was performed externally by Viracor-IBT Laboratories, Inc. (Lee's Summit, MO, USA). Tryptase was reported as 10.1 ng/mL in serum, which is within the normal laboratory reference range.

On the basis of case history, urine specimens from all three cases were screened by LC-QTOF. Data deconvolution was performed with the Waters ChromaLynx XS software using the TOF Toxicology database (version 1), modified in-house to contain entries for over 1,100 total compounds. Acceptance criteria for positive findings included observed mass error ≤ 5 ppm for both expected precursor (low energy) and product (high energy) ions. RT limits for data processing were set as ± 0.3 min of the expected RT defined in the database. Retention times for positive findings were also required to be within $\pm 2\%$ of the batch positive controls. Target ions and retention times used for ChromaLynx XS data processing are listed in Table II. 25C-NBOMe and 25C-NBOH were identified in all three urine case samples. 2C-C was also detected in Case 1 urine. Observed mass error for 25C-NBOMe, 25C-NBOH and 2C-C was ≤ 2.1 ppm in all control and case specimens.

25C-NBOMe, 25C-NBOH and 2C-C were quantitated by LC-MS-MS for all three cases. Internal standardization was performed by pairing 25I-NBOMe- d_3 with 25C-NBOMe and 25C-NBOH. Internal standard MDPV- d_8 was paired with 2C-C. Six-point linear regression curves with $1/x$ weighing were used to calibrate each compound from 0.05 to 10 ng/mL. Compound confirmation requirements included a retention time $\pm 3\%$ and qualifier MRM ion transition ratios $\pm 20\%$ of the average observed in the calibrators. As described in previous sections, standard addition was used for the quantitation of tissue specimens to account for matrix effects (22). Linear regressions with $r^2 \geq 0.980$ were obtained for each tissue matrix. 25C-NBOMe was confirmed in all analyzed case specimens. Complete quantitative LC-MS-MS results are listed in Table III.

2C-C was confirmed in Case 1 and was also detected in urine samples from Cases 2 and 3, although not detected in these later cases by the less sensitive LC-QTOF screen. 2C-C was also not detected in any case tissue homogenates, likely due to the dilution factors used for this quantitation method. 25C-NBOH was not detected in any case specimens using LC-MS-MS confirmation due to the absence of the hydrolysis step. Poklis *et al.* (23) recently reported that 25I-NBOMe desmethyl metabolites, including 25I-NBOH, underwent significant phase II glucuronidation in mouse hepatic microsome preparations and two human urine case samples. This may be true for 25C-NBOMe and its potential desmethyl metabolite 25C-NBOH, although further study is required. 2C-C and 25C-NBOH are both available as designer drugs and cannot be ruled out as components of the illicit preparations used in any of the

three analyzed cases. 2C-C may also be present as a clandestine synthesis impurity of 25C-NBOMe. However, 2C-C may also be a legitimate metabolite of 25C-NBOMe, as has been suggested for 2C-I of 25I-NBOMe (18, 23). The same correlation may be drawn for 25C-NBOH as an *O*-desmethyl metabolite of 25C-NBOMe using 25I-NBOMe metabolism data reported by Poklis *et al.* (23).

Additional peaks related to 25C-NBOMe were observed in the LC-QTOF screen. A prominent peak was observed at 6.22 min in the urine of all three cases. The area for this peak was significantly more abundant than 25C-NBOMe, 25C-NBOH or 2C-C in all three cases. A second, smaller unknown peak at 5.06 min was observed only in Case 1. LC-QTOF product ion mass spectra and proposed fragmentation for both unknowns are shown in Figure 2. 25C-NBOMe and related compounds detected in Case 1 are overlaid in Figure 3 using extracted precursor ion chromatograms. A precursor $[M+H]^+$ mass 322.1216 Da was observed for the first unknown peak, which is <2 ppm of the theoretical 322.1210 Da expected for an *O*-desmethyl-25C-NBOMe metabolite $[C_{17}H_{20}ClNO_3+H]^+$. This proposed metabolite can be differentiated by retention time from its positional isomer 25C-NBOH (6.66 min). Furthermore, the characteristic product ion 121.0653 Da is shared with a 25C-NBOMe reference spectrum but absent from 25C-NBOH, indicating an intact *N*-methoxybenzyl group. Therefore, *O*-demethylation occurs at either the 2 or 5 position on the 4-chloro-2,5-dimethoxyphenyl ring as shown in Figure 2. This peak at 6.22 min is consistent with the metabolite proposed by Soh and Elliott (24) and is a similar metabolic pathway to 25I-NBOMe metabolism (23). The second peak at 5.06 min has a measured precursor $[M+H]^+$ mass 308.1049 Da, which is also <2 ppm of the theoretical 308.1053 Da expected for a di-desmethyl-25C-NBOMe metabolite $[C_{16}H_{18}ClNO_3+H]^+$. The absence of product ion 121.0653 Da suggests *O*-demethylation at the *N*-methoxybenzyl group, similar to 25C-NBOH. The second peak at 5.06 min is proposed as a 2- or 5-*O*-demethylation of 25C-NBOH. A similar di-desmethyl metabolite was previously observed by Soh and Elliott (24).

Various NBOMe compounds have been associated with intoxications, hospitalizations and several deaths in recent literature. There are limited data reported for deaths associated with 25C-NBOMe. A 22-year-old male died in the hospital after nasal ingestion of 25C-NBOMe with the following reported concentrations: 0.81 ng/g antemortem blood (drawn 2–4 h after ingestion), 0.60 ng/g postmortem

Table II. LC-QTOF Compound Targets for Processing Data with Waters ChromaLynx XS

Analyte	RT (min)	Formula	Precursor ion (m/z) ^a	Product ion (m/z)
2C-C	4.01	$C_{10}H_{14}ClNO_2$	216.0791	199.0526
25C-NBOH	6.66	$C_{17}H_{20}ClNO_3$	322.1210	199.0526
25C-NBOMe	7.86	$C_{18}H_{22}ClNO_3$	336.1366	121.0653
Proadifen (SKF-525A)	10.29	$C_{23}H_{31}NO_2$	354.2433	167.0861

^aPrecursor mass for $[M+H]^+$ is calculated by ChromaLynx XS software using a hydrogen atom instead of a proton. Therefore, the calculated mass is ~ 0.5 mDa greater than the theoretical mass target.

Table III. LC-MS-MS Quantitative Results for Three Case Specimens

ID	Matrix	25C-NBOMe (ng/mL or ng/g)	2C-C (ng/mL or ng/g)	Dilution for standard addition
Case 1	Blood (heart)	2.07	0.12	–
	Urine	27.43	0.38	–
	Vitreous	0.50	–	–
	Adipose	2.35	–	1 : 3
	Brain	19.10	–	1 : 10
	Spleen	27.13	–	1 : 10
	Lung	25.21	–	1 : 10
	Liver	15.20	–	1 : 20
	Kidney	25.06	–	1 : 50
Case 2	Gastric	30.24 μ g total (100 mL submitted)	–	1 : 200
	Blood	0.48	–	–
Case 3	Urine	1.73	0.20	–
	Blood	1.04	–	–
Case 3	Urine	4.06	0.11	–

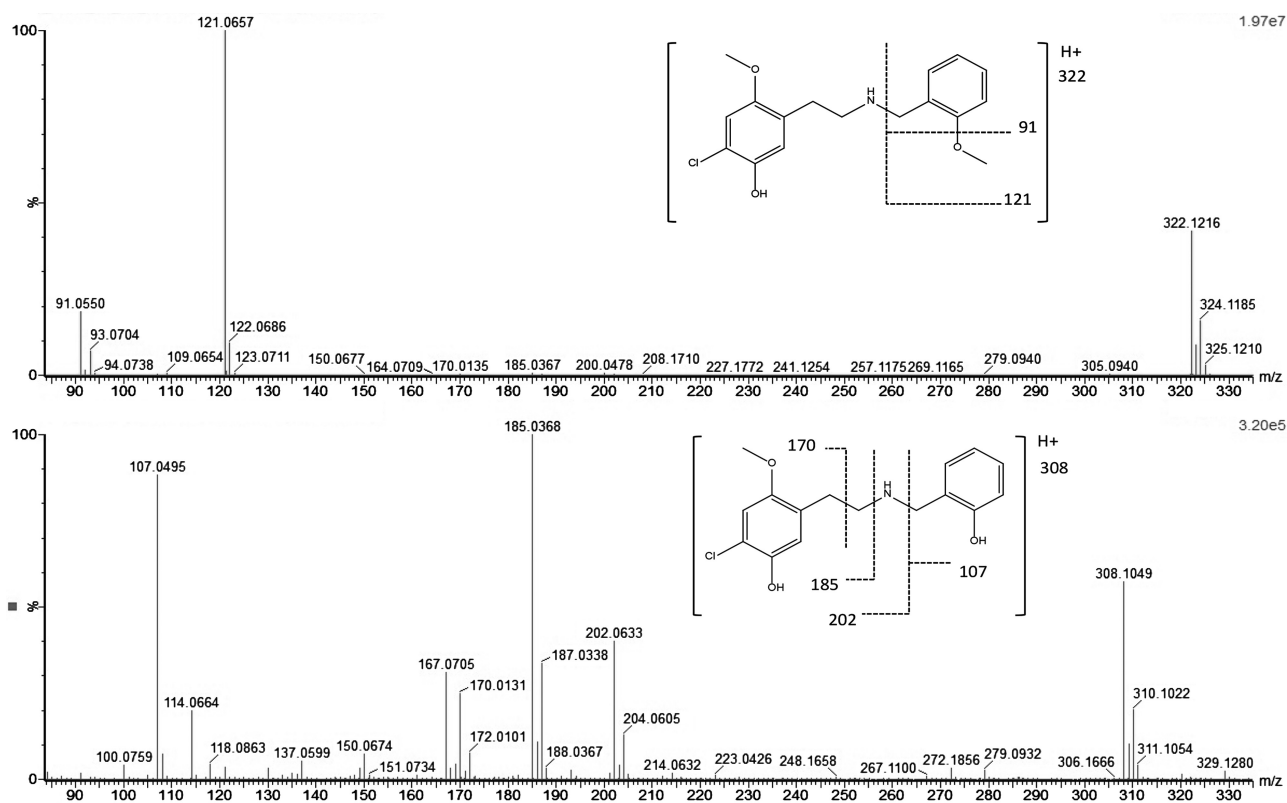


Figure 2. LC-QTOF product ion spectra for two unknown peaks observed in the decedent's urine. Proposed metabolites are *O*-desmethyl-25C-NBOMe (top, 6.22 min) and *O*-desmethyl-25C-NBOH (bottom, 5.06 min). Structures are shown with demethylation at the 5-dimethoxyphenyl position. However, it cannot be determined from these data if demethylation occurs at the 2- or 5-position.

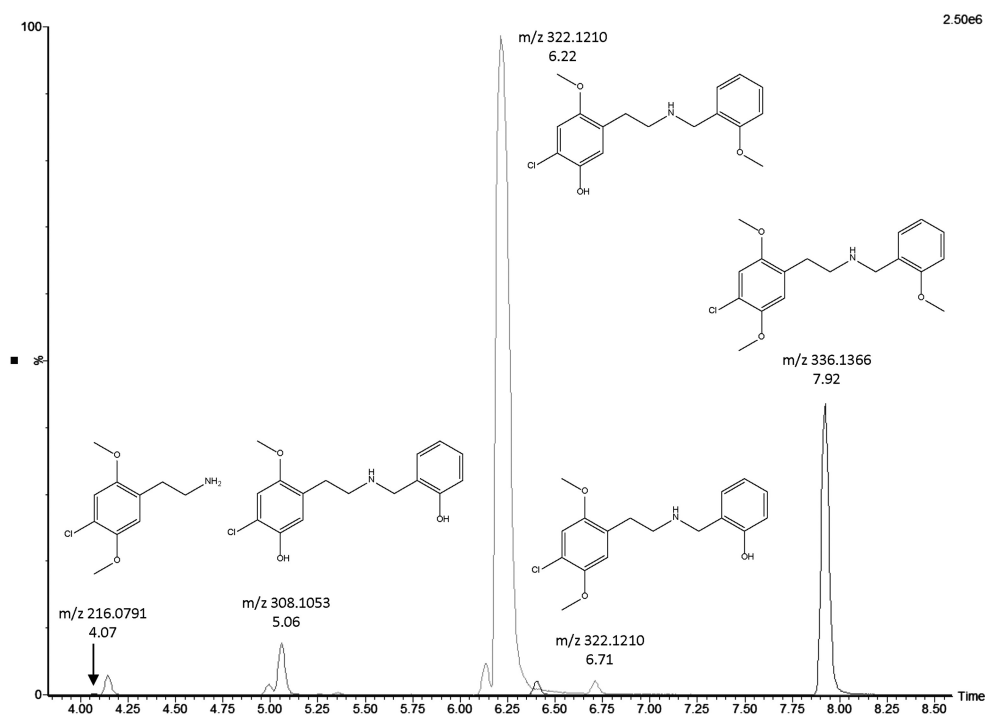


Figure 3. Overlay of LC-QTOF extracted ion chromatograms for 25C-NBOMe and metabolites detected in the decedent's urine. Structures, retention times and target precursor ions are shown. Peaks are identified in retention time order: 2C-C (4.07 min), *O*-desmethyl-25C-NBOH (5.06 min), *O*-desmethyl-25C-NBOMe (6.22 min), 25C-NBOH (6.71 min) and 25C-NBOMe (7.92 min).

blood, 2.93 ng/g urine, 0.33 ng/g vitreous, 0.82 ng/g liver and 0.32 µg total in submitted gastric contents. The cause of death was determined as combined 25C-NBOMe and amphetamine intoxication (14). Another case involved a deceased adult male with observed uncontrollable body movements after recent ingestion of yellow pills. Blood and urine had detectable amounts of 25C-NBOMe and 25I-NBOMe (>5 ng/mL). No quantitative results were reported (25). Postmortem detection of 25I-NBOMe appears most often in the literature. A 19-year-old male with no prior history of drug abuse died after 25I-NBOMe ingestion. The following concentrations were reported: 0.41 ng/mL heart blood, 0.405 ng/mL peripheral blood, 2.86 ng/mL urine, 0.099 ng/mL vitreous, 2.54 ng/g brain, 7.20 ng/g liver, 10.9 ng/g bile and 7.1 µg total submitted gastric contents (17). In another case, a 23-year-old woman died of combined drug toxicity with postmortem aortic (non-preserved) blood concentrations of 28 ng/mL 25I-NBOMe, 0.7 ng/mL 25C-NBOMe and 1 ng/mL 25H-NBOMe (26). Heart blood from a 16-year-old male decedent was positive for 25I-NBOMe at 19.8 ng/mL; the urine was reported qualitatively positive (27). Hospital blood (admission) from a 15-year-old male who died 3 days after drug ingestion was positive for 25I-NBOMe at 0.76 ng/mL (19). 25B-NBOMe has also been detected in several fatalities, although there are limited quantitative data. 25B-NBOMe was detected in an 18-year-old-male decedent in heart blood at 1.59 ng/mL and reported qualitatively positive in urine (27).

Postmortem ranges published for 25C-, 25I- and 25B-NBOMe appear to be comparable between all three compounds. The postmortem blood results for Case 1 described in this report appear to coincide with values found in the literature. However, the tissue values determined for Case 1 are notably higher than 25C- and 25I-NBOMe tissue values reported in two published cases (14, 17). We also observed similar concentrations between postmortem 25C-NBOMe values in Case 1 and antemortem values found in Cases 2 and 3. Hospital specimen values reported from NBOMe intoxications show similar overlap with postmortem values. 25I-NBOMe was reported in serum in three cases at 0.25, 2.78 (28) and 0.76 ng/mL (29). 25B-NBOMe was detected in serum of a severe intoxication at 0.18 ng/mL (30).

On the basis of decedent case history, autopsy and toxicological findings, the medical examiner ruled the cause of death as 25C-NBOMe toxicity temporally associated with excited delirium and forcible restraint. The manner of death was ruled accidental.

Funding

This work was funded in part by the ARP Sciences, LLC.

Conflict of interest statement

The opinions or assertions presented hereafter are the private views of the authors and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the US Army Medical Research and Material Command or that of the Armed Forces Medical Examiner System.

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