

# Quantitative analysis of synthetic hallucinogens: 25I-NBOMe, 25C-NBOMe, and 25B-NBOMe in blood and urine by LC-MS/MS

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## BACKGROUND

Among the drug using population, the use of synthetic compounds has increased over the past decade. Popular synthetic compounds such as cathinones (bath salts) and synthetic cannabinoids (K2 and Spice) have been joined by the emerging synthetic LSD compounds, NBOMe. Recently, the DEA classified three NBOMe drugs 25I-NBOMe, 25B-NBOMe, and 25C-NBOMe as schedule I drugs [1].

## INTRODUCTION

The NBOMe drugs have been encountered as powders, liquid solutions, laced on edible items and soaked into blotter paper [1]. In November 2013, the US Drug Enforcement Administration (DEA) placed the three NBOMe drugs (25I-, 25C-, and 25B-NBOMe) on the schedule I list for two years citing lack of medical use or human consumption [2]. In postmortem cases, the concentrations of NBOMe drugs encountered average less than 0.5 ng/mL in both blood and urine specimens [2]. Concentrations below 1 ng/mL require selective extraction and sensitive instrumentation for accurate analysis.

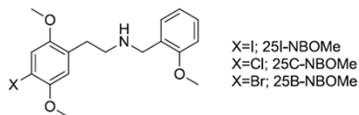


Figure 1: NBOMe class drugs (X represents halogen groups)

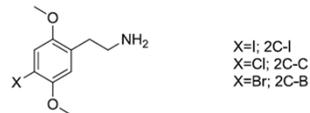


Figure 2: 2C class drugs (X represents halogen groups)

## MATERIALS AND METHODS

### Extraction

Quantitative analysis of 25I-NBOMe, 25C-NBOMe, and 25B-NBOMe was achieved using the following procedure. Calibrators and QCs, ranging from 5 pg/mL to 500 pg/mL, were prepared in 12 X 75 mm glass borosilicate tubes using negative urine for all three NBOMe drugs. These calibrators were spiked with 100 µL of 10 ng/mL D3-25I-NBOMe for a final ISTD concentration of 1 ng/mL. To adjust the pH, 200 µL of ammonium hydroxide were added to each calibrator and QC. Extraction was performed by adding 2.4 mL of 50%TO/30%EA/ 15% HE/ 5% IA mixture to each tube followed by rocking for 10 minutes and centrifugation at 2500 RPM for 10 minutes. From the upper organic layer, 2 mL were transferred to clean 12 X 75 glass borosilicate tubes and evaporated, reconstituted and transferred to autosampler tubes for LC-MS/MS analysis. The analysis was achieved using an Agilent 6460 LC-MS/MS with jet stream technology equipped with an Infinity 1200 LC and a 1260 autosampler.

### LC/MS/MS Analysis

Chromatographic separation was achieved on an Agilent Poroshell 120 C-18 column with gradient elution. Mobile phases of water:methanol (90:10 v/v) with 5mM ammonium formate (solvent A) and acetonitrile with 0.1% formic acid (solvent B) were used in gradient elution program; 30% B to 70% B over in 3 mins, returning to initial 30% of B over in 0.5 mins and held for 0.5 min for a total run time of 4 min. The transitions monitored (+MRM) were 25I-NBOMe (428-121/91 m/z); 25B-NBOMe (382- 121/91 m/z); and 25C-NBOMe (336- 121/91 m/z).

Compound	Precursor	Products	RT	Frag	CE
25I-NBOMe	428	121/91	2.19	25	30
25B-NBOMe	382	121/91	2.08	25	20
25C-NBOMe	336	121/91	2.02	25	30
D3-25I-NBOMe	431	121.1/91.5	2.18	25	40

Table 1: Acquisition parameters for 25I-, 25B-, 25C-, and D3-25I-NBOMe

## RESULTS Cont.

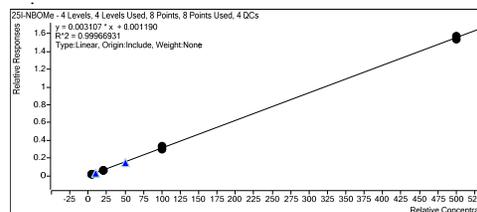


Figure 3: Linear plot of 25I-NBOMe (5 – 500 pg/mL)

Ion transitions for deuterated 25I-NBOMe (IS) were identified (431 – 121.1/ 91.5) and used in the quantification of all three compounds. All three compounds demonstrated good linearity over a concentration range of 5 pg/mL to 500 pg/mL for both blood ( $r^2 \geq 0.997$ ) and urine ( $r^2 \geq 0.999$ ); LOD and LOQ were established as 5 pg/mL for all three compounds.

	25I-NBOMe		25B-NBOMe		25C-NBOMe	
	Mean (pg/mL)	%CV	Mean (pg/mL)	%CV	Mean (pg/mL)	%CV
5 pg/mL	5.05	12.6	5.03	17.3	4.43	2.2
50 pg/mL	47.35	4.1	46.38	0.3	47.46	2.6
500 pg/mL	499.9	1.6	499.9	0.2	499.6	1.1

Table 2: Mean and %CV at 5, 50 and 500 pg/mL calibrators in urine

	25I-NBOMe		25B-NBOMe		25C-NBOMe	
	Mean (pg/mL)	%CV	Mean (pg/mL)	%CV	Mean (pg/mL)	%CV
5 pg/mL	5.13	6.2	5.71	7.0	5.15	6.7
50 pg/mL	47.57	7.1	48.29	2.1	50.28	6.8
500 pg/mL	500.2	1.6	500.2	4.2	500.0	6.9

Table 3: Mean and %CV at 5, 50 and 500 pg/mL calibrators in blood

## RESULTS

Concentrations of NBOMe class drugs were quantitated at six concentrations including four calibrators (5, 20, 100 and 500 pg/mL) and two QCs (10 and 50 pg/mL). The quantification method was evaluated for precision (%CV) and accuracy.

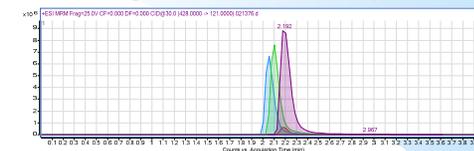


Figure 4: TIC of 25I-, 25B-, 25C-, and D3-25I-NBOMe

## CONCLUSIONS

The method and the calibration curves reconcile well with forensic toxicology criteria. The extraction and LC/MS/MS method developed for analysis of blood and urine for 25I-, 25B-, and 25C-NBOMe is precise, sensitive and reproducible at forensically relevant concentrations. The establishment of a deuterated internal standard (D3-25I-NBOMe) allowed for a precise analysis of the NBOMe drugs at low concentrations. The accuracy, precision, and  $R^2$  values of both the blood and urine calibration curves met SWGTOX validation criteria [3].

## REFERENCES

- [1] Poklis J, Devers K, Arbefeville E, Pearson J, Houston E. Postmortem detection of 25I-NBOMe [2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine] in fluids and tissues determined by high performance liquid chromatography with tandem mass spectrometry from a traumatic death. Forensic Science International. 2014;234:e14-e20.
- [2] Johnson R, Botch-Jones S, Flowers T, Lewis C. An Evaluation of 25B-, 25C-, 25D-, 25H-, 25I-, and 25T2-NBOMe via LC-MS-MS: Method Validation and Analyte Stability. Journal of Analytical Toxicology. 2014;38(8):479-84.
- [3] SWGTOX DOC 003, <http://www.swgtox.org/documents/Validation3.pdf>. May 2013