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Preventing misidentification of 25I-NBOH as 2C-I on routine GC–MS analyses

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Abstract 25I-NBOH is a novel psychoactive substance (NPS) recently reported to have been found on blotter paper samples seized on the streets of Brazil, used as a replacement for the NBOMes now scheduled in many countries. The presence of this NPS on the street market may go undetected because the most widely and routinely utilised analytical technique for drug sample analyses is gas chromatography–mass spectrometry (GC–MS), which can misidentify 25I-NBOH (and indeed the other members of the NBOH series), because of its degradation into 2C-I (and corresponding 2C for the other members of the series) within the injector, unless a derivatization procedure is employed, which is often non-standard. While direct detection of 25I-NBOH under routine GC conditions is still to be achieved, slight adjustments in standard GC methods, including shortening of the solvent delay window, enabled the detection of an additional peak containing 25I-NBOH degradation product's fragmentation ions. Consequently, this secondary early chromatographic peak allowed for the distinction between 25I-NBOH and 2C-I using routine GC–MS without resorting to derivatization (or other analytical processes), thus preventing misidentification of 25I-NBOH as 2C-I.

Keywords 25I-NBOH · 2C-I · NPS · Misidentification · Thermal degradation · GC–MS

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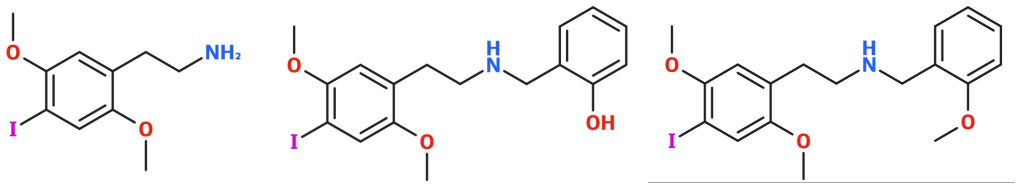


a 2C-I **b** 25I-NBOH **c** 25I-NBOMe

Fig. 1: Structures of **a** 2C-I, **b** 25I-NBOH and **c** 25I-NBOMe

Introduction

The challenge of novel psychoactive substances (NPSs) is a matter of interest to forensic laboratories and regulatory agencies all around the world, with several new compounds continuously entering the market to replace scheduled ones. According to the United Nations Office for Drugs and Crime (UNODC), a total of 644 NPSs have been reported between 2008 and 2015, with 75 in 2015 alone [1].

25I-NBOH is among the latest emerging NPSs and the first reported member of the NBOH series, a new branch of the 2C family of substituted psychedelic phenethylamines. NBOHs are structurally similar to the NBOMes, which are now widely controlled and involved in many intoxication and death cases [2–5], and are characterized by a hydroxy group replacing the asymmetric metoxy group on the secondary substituted phenyl ring (Fig. 1).

The emergence of NBOHs shortly after NBOMes became scheduled in a growing number of countries [6] seems to indicate that they are being used as a legal replacement for the latter, since both structures present very strong affinity and selectivity to 5-HT_{2A} receptors, thus inducing hallucinogenic effects comparable to lysergic acid diethylamide (LSD) [7–9]. Comparably to NBOMes, NBOHs were found impregnated on blotter papers seized by legal authorities in Brazil and are possibly being sold as LSD to unsuspecting users [10]. As of October 19, 2016, following reports from local forensic laboratories concerning the presence of this NPS on the streets, Brazilian authorities listed 25I-NBOH as a proscribed substance [11].

Correct identification of NBOHs in questioned samples by forensic laboratories relying on gas chromatography–mass spectrometry (GC–MS) may, however, present an increased difficulty when compared to NBOMes, because reported NBOHs are thermolabile under typical GC conditions and degrade into their corresponding 2C during injection [10, 12]. Therefore, if subjected to standard GC–MS alone, samples containing 25I-NBOH will produce a chromatographic peak with the same retention time (RT) and mass spectrum characteristic of 2C-I, leading to its misidentification, even though such analysis complies with Scientific Working Group for the Analysis of Seized Drugs' (SWGDRUG) recommendations [13]. According to the latest International Collaborative Exercises' (ICE) report from the UNODC [14], 91% of the forensic laboratories participating in the exercise rely on GC–MS for identification/confirmation of tested drugs and may be subject to misidentification when analysing samples putatively containing NBOHs. Consequently, should such misidentification occur, reliability and trustworthiness of forensic laboratories could be compromised. Additionally, legal implications are also to be considered, because 2C-I is a scheduled substance in many countries, while 25I-NBOH is not.

To the best of our knowledge, direct identification of 25I-NBOH has not yet been achieved under standard routine GC-MS conditions, with 25I-NBOH always degrading into 2C-I during injection. To ensure correct identification, samples reported as containing 2C-I when analysed by GC–MS should be submitted to derivatization and re-analysed or subjected to additional alternative analytical techniques, such as liquid chromatography–mass spectrometry (LC–MS), infrared spectrometry or nuclear magnetic resonance. However, such procedures and techniques may not be routinely available to most forensic laboratories.

Here we propose an alternative procedure enabling indirect, nevertheless correct, identification of NBOHs using routine GC–MS methods, without resorting to derivatization or other analytical techniques. The procedure is based on the identification of the residual fragment ions from the degraded NBOH, together with the corresponding 2C component, when methanol is used as solvent.

Materials and methods

Chemicals and reagents

25I-NBOH certified standard was purchased from Cayman Chemical (Ann Arbor, MI, USA). 2C-I certified standard was kindly donated by UNODC. Gradient grade methanol, ethanol, isopropanol and acetonitrile were purchased from Merck KGaA (Darmstadt, Germany); tetradeuterated methanol from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA); analytical grade ethyl acetate and propanone from Labsynth (Diadema, SP, Brazil).

Samples

Blotter papers seized on the streets by legal authorities from Minas Gerais state and the Federal District during 2015 and 2016 were used as test samples.

Instrumentation

GC–MS analyses were performed on an Agilent 7890A gas chromatograph connected to a 5975C Mass Spectrometer (Agilent Technologies, Santa Clara, CA, USA.). The system was controlled by Agilent Chemstation GC/MS Software version E 02.02.1431. Hardware and software (Agilent Technologies).

Methods

Sample extraction and preparation were performed as follows: separated blotter paper samples were placed in 1.5 mL microcentrifuge tubes. Each tube was then completely filled with methanol and agitated on a vortex mixer for 60 s. The resulting solution was passed through 0.45 μm millipore filter and 1 mL was transferred to a GC vial, ready for injection. Similar extractions were performed using acetonitrile, acetone, ethyl acetate, ethanol, isopropanol and tetradeuterated methanol, in order to assess the 25I-NBOH's thermal stability and the proposed injector degradation reaction.

An Agilent J&W HP-1MS fused silica capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μm film thickness) was used. Sample injection volume was 1 μL with a 25:1 split ratio. Helium was used as carrier gas, with constant flow rate of 1 mL min^{-1}. Injector temperature was set to 280^oC. The oven program started at 150^oC with a hold for 1.5 min, ramped up at 30^oC min^{-1} to reach 250^oC with a hold for 1 min, and then ramped up at 50^oC min^{-1} to 300^oC with a hold for 3 min. The transfer line temperature was set at 300^oC. The solvent delay was set to 1.5 min. Mass scan range was m/z 35 – 550.

Results and discussion

When injected under the same chromatographic conditions, 25I-NBOH and 2C-I certified standards produced peaks with identical RT and the same mass spectra, matching 2C-I from SWGDRUG and Cayman

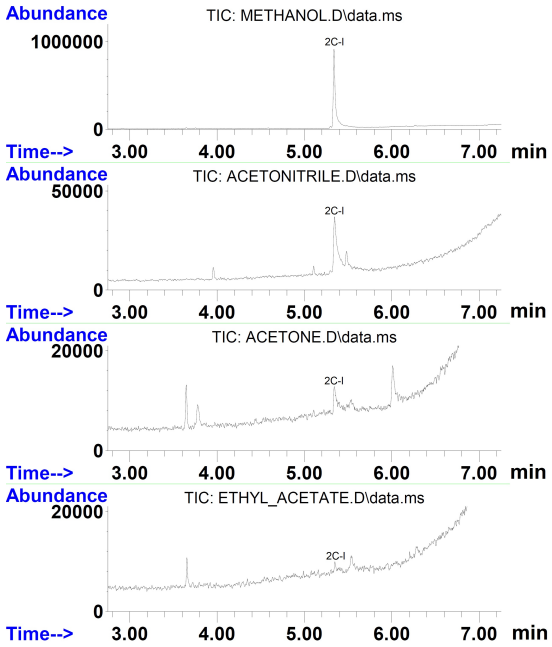


Fig. 2: Total ion current chromatograms (TICs) obtained from the extracts of seized blotter papers using different solvents. In all cases, the 2C-I peak was observed and no peak could be identified as 25I-NBOH. For solvents other than methanol, extraction efficiency was greatly decreased, as can be seen from the lower abundances obtained for 2C-I

Chemical MS Libraries [15, 16], thus reproducing and confirming the recently reported misidentification issue of 25I-NBOH as 2C-I [10, 12].

Attempts to avoid or at least significantly suppress thermal degradation of 25I-NBOH during GC–MS analysis were performed by lowering injector temperature (280^oC to 150^oC), changing the solvents used for preparing samples (methanol, acetonitrile, ethyl acetate and acetone) and testing different injection split ratios (10:1, 25:1, 50:1 and 100:1).

Injector temperature was decreased from 280^oC to 250^oC, 200^oC and 150^oC, with no effect over degradation, but with negative effect in peak areas. At 150^oC, no more peaks appeared on the chromatograms (data not shown), probably due to the high molecular weight or incomplete sample vaporization of 25I-NBOH.

When the standard solvent (methanol) was replaced with acetonitrile, the 2C-I peak was clearly observed, but with decreased abundance (about 25 times lower) when compared to methanol. For ethyl acetate and acetone, the 2C-I peak was barely visible, showing very low abundances (about 100 times lower) when compared to methanol (Fig. 2). In all cases, no 25I-NBOH peak was evidenced. These results led to the conclusion that, besides not being able to prevent degradation, extraction efficiency, a crucial factor in forensic analysis of questioned samples, was decreased when solvents other than methanol were employed.

Injections with different split ratios of 10:1, 25:1, 50:1 and 100:1 were also tested. Larger split ratios had strong impact in peak ratios, lowering peak areas, but presented no improving effects over the degradation (Fig. 3).

Considering the degradation of 25I-NBOH into 2C-I during standard routine GC injection as a fact, it was noticed that other by-products formed by degradation would enter the GC column, possibly gen-

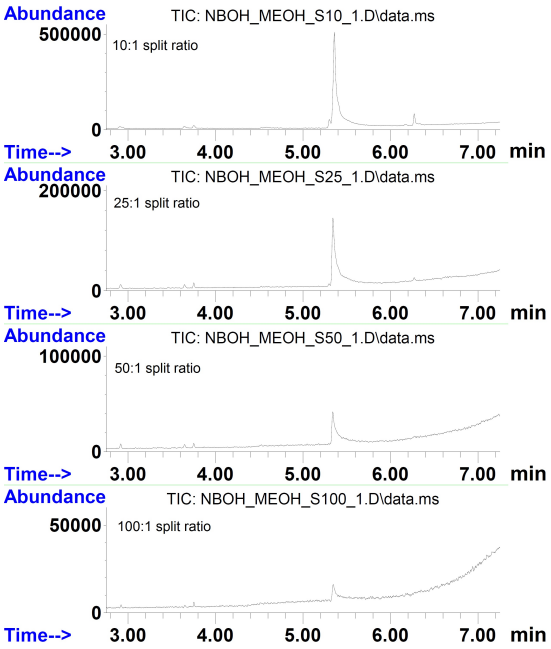


Fig. 3: TICs obtained from a methanol extract of one single blotter paper using different injection split ratios

erating other peaks in a total ion current chromatogram (TIC). Consequently, the GC oven program was adjusted, and the solvent delay shortened in order to monitor potentially fast eluting compounds. This enabled the observation of a secondary peak (RT = 2.17 min) in addition to the primary peak (RT = 5.34 min, identified as 2C-I) when analysing both the 25I-NBOH certified standard and extracts from recently seized blotter samples (Fig. 4). This secondary peak was not observed for the 2C-I certified standard and presented major fragment ions at *m/z* 138, 106 and 78, when methanol was used as solvent (Fig. 5a).

The mass spectrum for the secondary peak (RT = 2.17 min) had no reasonable match on any MS library available and could not be associated with any other molecules possibly present in the sample. Analysis of the fragment ions present suggested that, in the presence of an alcohol and under high temperature (GC injector temperature set to $280^{o}C$), 25I-NBOH degraded and generated 2C-I and 2-(methoxymethyl)phenol by means of nucleophilic substitution, where the alcohol acts as a nucleophile group and the 2C-I as a leaving group (Fig. 6).

Although the details of the degradation reaction mechanism are beyond the scope of the present work and will be subsequently addressed in a future communication, several other GC injections were conducted using other alcohols (ethanol, isopropanol and tetradeuterated methanol), in order to investigate the potential solvent effects on the secondary peak's RT and ion fragment composition. In all cases, corresponding secondary peaks appeared. These peaks presented increasingly longer RT and smaller abundances according to the increase of the alcohol size (methanol RT = 2.17 min; ethanol RT = 2.40 min; isopropanol RT = 2.60 min). These changes follow the expected trend of a series of alkyl esters when analysed by GC. The resulting secondary peak, when tetradeuterated methanol was used, also showed expected results with similar abundance but slightly shorter RT (methanol-d_4 RT = 2.16 min) when compared to the methanol run. Indeed, deuterated versions of molecules typically present a slight volatility increase compared to their monoisotopic versions. Furthermore, all resulting mass spectra (Fig. 5) fully

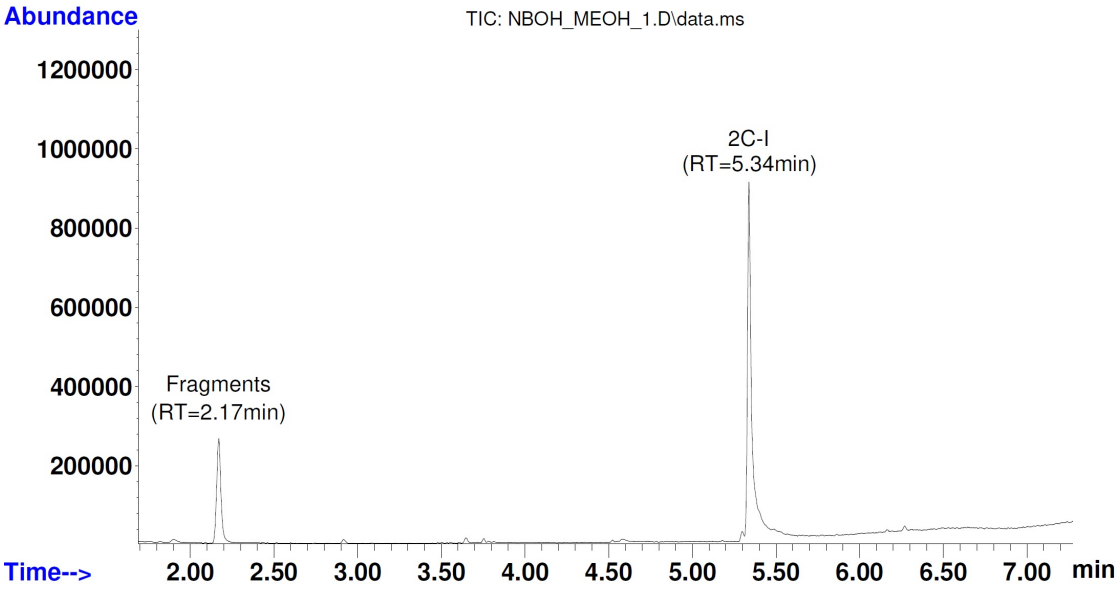


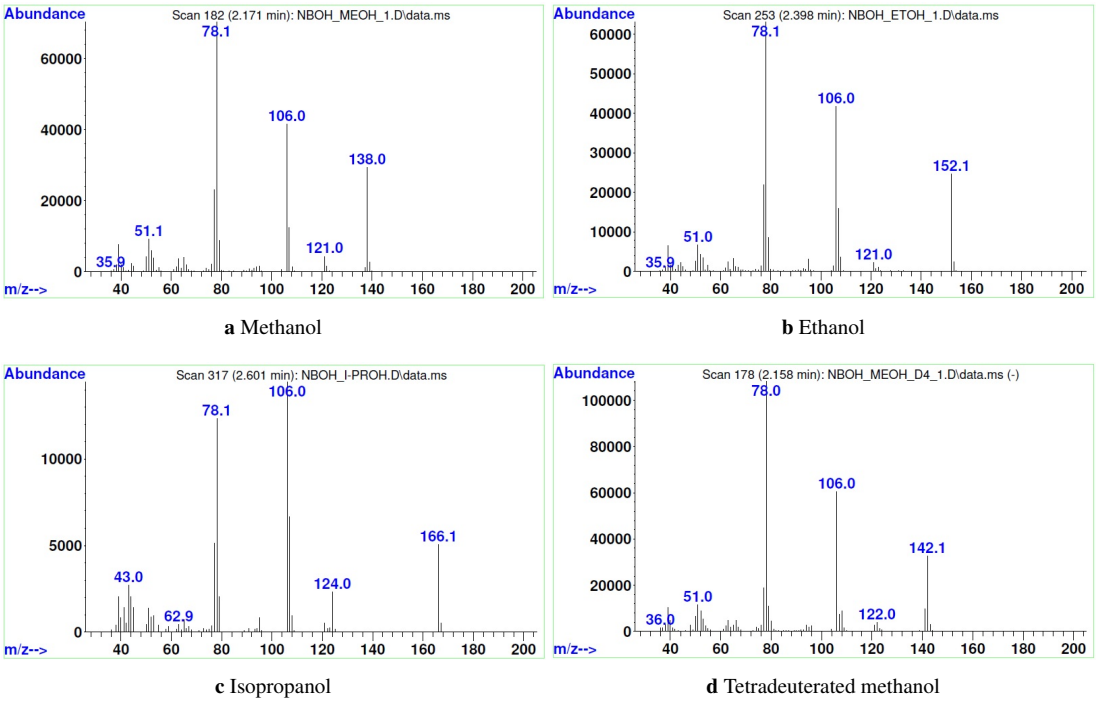
Fig. 4: Chromatogram obtained from the methanol extract of a street sample blotter paper seized by local authorities. The primary peak [retention time (RT) = 5.34 min] matched the RT from 25I-NBOH and 2C-I certified reference standards injected under the same conditions. The mass spectrum from this peak also matched 2C-I from SWGDRUG and Cayman Chemical MS Libraries. The secondary peak (RT = 2.17 min) was detected only for the 25I-NBOH certified standard and the seized blotter paper.

supported the proposed degradation reaction product [2-(alkoxymethyl)phenol)] (Fig. 6) with all molecular ions obtained (methanol *m/z* 138; ethanol *m/z* 152; isopropanol *m/z* 166; methanol-d_4 *m/z* 142).

Consequently, the detection of a secondary chromatographic peak presenting such a mass spectral profile (Fig. 5a) in questioned samples, where a 2C series peak was identified, gives sufficient evidence for analyte degradation during GC injection (considering that methanol is the typical solvent to be employed for such analyses). By detecting a secondary peak showing such a mass spectrum, together with a primary peak due to a 2C component, the correct identity of a NBOH series substance can be established without the necessity of submitting all samples to derivatization or other analytical techniques [such as LC–MS(/MS)], which are usually unavailable to many forensic laboratories. Although it may be argued that the use of methanol or other alcohol as solvent enhances thermal degradation, such enhancement likely benefits the identification of the original molecule, because the degradation now occurs through a specific reaction (Fig. 6).

Conclusions

GC–MS is by far the most used analytical technique employed by forensic laboratories for the analysis of drugs all over the world [14]. With the active and continuous emergence of NPSs over the last years, most of them derived from previously known ones, correct identification of questioned samples by GC–MS analysis becomes an increasingly subtler task, because some species of compounds undergo chemical changes during analysis, potentially leading to serious misidentification. In this context, forensic scientists dealing with routine GC–MS testing of seized samples should be mindful of this issue and be ready to recognize the possibility that such a change may take place during analysis, specially when solely relying on GC–MS.



a Methanol **b** Ethanol

c Isopropanol **d** Tetradeuterated methanol

Fig. 5: Mass spectra of the secondary peak observed for samples containing 25I-NBOH in the presence of different alcohols. **a** Methanol, **b** ethanol, **c** isopropanol and **d** tetradeuterated methanol as injection solvents. The fragments and molecular ions observed are in full accordance with the proposed degradation product [2-(alkoxymethyl)phenol)] for 25I-NBOH in the presence of an alcohol and high temperature (280^oC)

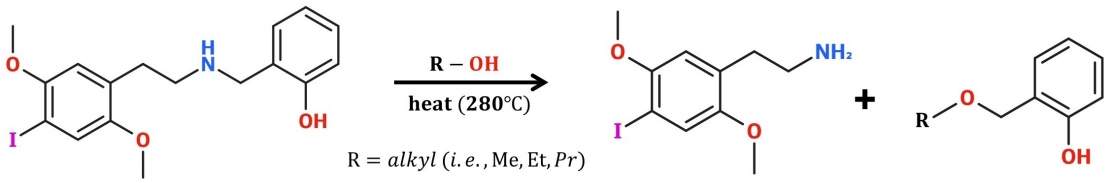


Fig. 6: Proposed degradation reaction of 25I-NBOH in the presence of an alcohol during GC injection.

Here we have provided an example of this type of GC–MS misidentification (25I-NBOH being wrongly identified as 2C-I) and, more importantly, a way to catch out such misidentification by looking for a residual fragment peak due to 25I-NBOH degradation appearing at the early stages of the TIC chromatogram. It is almost certain that similar misidentification cases are now occurring for other members of the NBOH series, such as 25B-NBOH and 25C-NBOH, which would be wrongly identified as 2C-B and 2C-C, respectively. Indeed, the presence of the 2-(methoxy methyl)phenol peak should be observed in the TIC chromatogram and be used to prevent misidentification in such cases.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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