NBOMe Designer Drugs: GC-MS and LC-QTOF/MS Detection on Blotter Paper by Brazilian Federal Police (Rio Grande do Sul, Brazil)

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Abstract. The NBOMes are classified as new psychoactive substances and have recently become popular as drugs of abuse, being associated with several intoxication cases and even deaths, leading to its ban in several countries. Until now, the most widely used analytical instrument among forensic laboratories in Brazil is GC-MS. In this study, this instrumentation was employed for routine analyzes of twenty blotter paper seizure by Brazilian Federal Police (BFP) in the southernmost state of the country. However, to acquire more information about these samples, LC-QTOF/MS was used as a supplementary analysis to determinate degradation products, metabolites and unknown compounds. The GC-MS analysis detected only 25B-NBOMe, while the LC-QTOF/MS analysis detected 25B-NBOMe, 2C-B and MDMA. The compounds found in these analyzes are quite different from that found

http://dx.doi.org/10.17063/bjfs7(3)y2018193
in the national profile of seizures by BFP, suggesting that another route or supplier act in this region. For research and drug intelligence purposes the use of more versatile, sensitive and specific analytical tool provides a greater number of information that could be employed as a valuable strategy in the drug trafficking combat.

**Keywords.** Designer drugs; NBOMe; 25B-NBOMe; GC-MS; LC-QTOF/MS.

## 1. Introduction

New psychoactive substances (NPS), also known as designer drugs, has become an issue regarding public health in many parts of the world, demanding constant improvements on the chemistry, pharmacology and toxicology of such substances and on their legal regulation as well\(^1\)-\(^3\). As a consequence of globalization, when a NPS is discovered or synthesized it is rapidly revealed to the entire world by “dark net”\(^4\),\(^5\). In this context, United Nations’ world drug annual report 2017 indicate that the NPS market continues to be very dynamic and is characterized by the emergence of large numbers of new substances belonging to diverse chemical groups, like tryptamines, synthetic cathinones, synthetic cannabinoids, piperazines, phenethylamines, among others. Between 2009 and 2016, 106 countries and territories reported the emergence of 739 different NPS to UNODC\(^5\).

Marketed in many different ways and forms, like blotter papers, seals, tablets, crystals, among others, new substances often emerge quickly and disappear again, while some become used regularly among a small group of users. Over 80 NPS were reported every year during the period 2009-2015 and appear to have become established on the global market; a number of them have been placed under international control. On the other hand, about 60 NPS seem to have disappeared from the market since 2013. Problems in identifying them in a laboratory may be a factor, however, in the low level of reporting of these lesser-known substances\(^5\). Another factor is due to the versatility of the changes occurring in the molecules and the speed with which they appear in the market, being faster the development of new molecules than the tools for unequivocal identification and even faster than them have been placed under international control\(^6\). Among the wide variety of NPS, the NBOMe designer drugs (Figure 1) have been attracting attention from medical and legal issues, due to its association with several intoxication cases and even deaths, leading to its ban in several countries, including Brazil\(^1\),\(^6\)-\(^8\).
The most widely used analytical instrument among forensic laboratories by Brazilian Federal Police (BPF) is the gas chromatography-mass spectrometry (GC-MS). In this study, routine analyzes for drug determination in blotter paper seized by BFP in the State of Rio Grande do Sul, Brazil were done. However, to acquire more information about the samples blotter papers proceeding from Rio Grande do Sul BFP seizures performed in 2016, the liquid chromatography coupled to hybrid quadrupole time-of-flight mass spectrometer (LC-QTOF/MS) was also employed as a supplementary analysis to determinate degradation products, metabolites and unknown compounds for research and drug intelligence purposes, as a valuable strategy in the drug trafficking combat.

2. Material and methods

2.1 Samples

The sample set consisted of twenty blotter papers proceeding from a single BFP seizure performed in 2016.

2.2 GC-MS method

2.2.1 Sample preparation

For GC-MS analysis the entire samples of each blotter papers were extracted with 3 mL of methanol by sonication for 3 min in ultrasonic bath and after were vortexed for 1 min. 1 mL of the resulting supernatant solution was transferred to a GC vial ready for injection.

2.2.2 GC-MS

Chromatographic separation was performed on an Agilent 6890 N gas chromatograph coupled to an Agilent 5973 mass selective detector (Agilent

![Chemical structures of NBOMe designer drugs.](image)

**Figure 1.** Chemical structures of NBOMe designer drugs.
Technologies, Palo Alto, CA, USA). A commercially available 25 m DB-1MS capillary column, with 0.20 mm inner diameter and a 0.33 μm film thickness was used as the stationary phase (DB-1MS, Agilent J&W Scientific, Folsom, CA, USA). Helium (99.9999% purity) was used as the carrier gas at a constant flow rate of 1.0 ml/min. Injection of 1.0 μl of sample solution was performed automatically in split mode with split ratio 5:1. The injector was maintained at 280°C. The column temperature program was as follows: starting at 100°C then holding for 2 min, followed by subsequent heating to 300°C at a heating rate of 12°C/min. The final temperature was held at 300°C for 10 min. The solvent delay was set to 1.8 min. The interface, ion source and quadrupole temperatures were 280°C, 230°C and 150°C, respectively. The MS system was operated in electron ionization mode at 70 eV and the positive ions were analyzed. Acquisition was carried out in scan mode from 40 to 550 amu. Under these conditions, the retention time (RT), the mass spectrum computerized databases (NIST02) and the data coming from literature was used for 25B-NBOMe identification.

2.3 LC-QTOF/MS method

2.3.1 Chemicals and reagents

Acetonitrile (ACN; HPLC grade) was purchased from Merck (Darmstadt, Germany), deionized ultra-pure water (<18.2 MΩ cm resistivity) was obtained from a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, US). Formic acid was supplied from J. T. Baker (Center Valley, PA, U.S).

2.3.2 Sample preparation

For LC-QTOF/MS, an aliquot of the methanolic solution of entire samples was diluted with a Methanol:Water solution (50:50). After dilution, the tubes were vortexed for 30 seconds, centrifuged at 2600 g for 7 minutes at 25°C. 1.5 mL of the supernatant from each sample was transferred to an amber vial with a capacity of 1.5 mL. An aliquot of 10 μL were diluted with 990 μL of water containing 0.1% of formic acid.

2.3.3 LC-QTOF/MS

LC-QTOF/MS was used to carry out qualitative analysis. The system was composed of a LC Agilent 1260 Infinity (Agilent Technologies, Palo Alto, CA, USA) and mass
spectrometer 5600 TripleTOF system (Sciex, Framingham, MA, US). Chromatographic separation was performed using an analytical column Waters X-Terra® C18 (100 x 2.1 mm, 3.5 mm) and a guard column (5 μm, 4.0 x 3.0 mm) (Phenomenex). The column was kept at 40°C. A binary mobile phase was used with a flow of 300 μL min⁻¹ in a total run time of 14 minutes. Mobile phase component A was an aqueous solution of 0.1% formic acid and component B was ACN with 0.1% formic acid. The gradient was optimized for the separation, and began with 95% A that decreased linearly to 10% A (4 min) and held at that concentration until 12 minutes. Finally, A was increased linearly over 1 minute back to 95% and kept at this concentration for 1 minute. An equilibrate time of 4 minutes was applied. The sample injection volume was 2 μL.

Mass spectrometer source dependent parameters were set as follows: collision gas (CAD) 4 psi, curtain gas (CUR) 20 psi, ion source gas 1 (GS1) 55 psi, dryer gas (GS2) 55 psi, interface heater temperature (IHT) 400°C, ion spray voltage floating (ISVF) 4500 V. Data acquisition was performed using AnalystTF® software. Data treatment applied PeakView® and MultiQuant® (Sciex, Framingham, MA, US), in a full scan TOF/MS mode in a mass range from 100 to 1000 Da with an IDA (Information Dependent Acquisition) criterion established as higher than 1000 counts, in a maximum of four candidates simultaneously. Selected ions were fragmented with collision energy of 35 ± 15 eV. Compounds were selected based on compound exact mass and searching window of ± 0.0030 Da.

3. Results and discussion

NBOMes have recently become popular as drugs of abuse⁹ and some compounds of this class of substances, like 25I-NBOMe, 25C-NBOMe, 25D-NBOMe, 25B-NBOMe, were considered illegal in Brazil since 2014¹⁰. The first identification of NBOMes in BFP was in 2012 and from that year until June 2016 the number of expert reports positive to NBOMes detection totaled 186 in the whole country. The main form of seized was blotter paper or micro blotter paper (more than 83,000 units)⁶. Until now, the most widely used analytical instrument among forensic laboratories in Brazil by BFP is GC-MS, so for this study, routine analyzes with this instrumentation were done. To acquire more information about the samples, LC-QTOF/MS was employed as a supplementary analysis to determinate degradation products, metabolites and unknown compounds.

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As expected, considering the high resolution and the high sensitivity of the LC-QTOF/MS detected some compounds that were not detected in GC-MS. The analysis performed by GC-MS detected only 25B-NBOMe (characteristic fragment m/z: 91, 121, 150, 346; RT 18.01 min). The analysis performed by LC-QTOF/MS detected 25B-NBOMe (mass range - m/z - 380.0829; RT 6.14 min), 2,5-dimethoxy-4-bromophenethylamine (2C-B) (mass range - m/z - 260.0256 - 260.0316 at RT 5.60 min) and 3,4-methylenedioxymethamphetamine (MDMA) (mass range - m/z - 194.1151 – 194.1211 at RT 5.02 min). The presence of other compounds were also investigated: caffeine (mass range - m/z - 195.0852 - 195.0912); ethylone (mass range - m/z - 222.1100 - 222.1160); ketamine (mass range - m/z - 238.0968 - 238.1028); amphetamine (mass range - m/z - 136.1096 - 136.1156); 3,4-methylenedioxymphetamine (mass range - m/z - 180.0994 - 180.1054); 4-fluoramphetamine (mass range - m/z - 154.1002 - 154.1062); and methamphetamine (mass range - m/z - 150.1252 - 150.1312). LC-QTOF/MS Compound Targets are describe on Table 1. The physical characteristic and the substances identified from the analysis of the twenty (20) blotter papers samples by GC-MS and LC-QTOF/MS analysis are describe on Table 2.

**Table 1.** LC-QTOF/MS Compound Targets.

| Compounds       | Molecular Formula | Molecular Weight | Exact Mass | [M+H]+ Mass | Mass range [M+H]+ |
|-----------------|-------------------|------------------|------------|-------------|-------------------|
| Caffeine        | C8H10N4O2         | 194.1900         | 194.0804   | 195.0882    | 195.0852 – 195.0912 |
| MDMA            | C11H15NO2         | 235.2500         | 235.1000   | 236.0982    | 236.0952 – 236.1002 |
| Ethylone        | C12H15NO3         | 225.2000         | 225.0500   | 226.0482    | 226.0452 – 226.0502 |
| Ketamine        | C13H16CINO        | 237.7200         | 237.5700   | 238.5682    | 238.5652 – 238.5702 |
| Anfetamine      | C9H13N            | 135.2000         | 135.0500   | 136.0482    | 136.0452 – 136.0502 |
| Tenamfetamina (MDA) | C10H13NO2       | 179.2100         | 179.0600   | 180.0582    | 180.0552 – 180.0602 |
| 4-Fluoroamphetamine | C9H12FN         | 153.2000         | 153.0500   | 154.0482    | 154.0452 – 154.0502 |
| Methamphetamine | C10H15N          | 149.2000         | 149.1000   | 150.0982    | 150.0952 – 150.1002 |
| 2-CB            | C10H14BrNO2       | 260.1300         | 259.9800   | 260.9782    | 260.9752 – 260.9802 |
| 25B-NBOMe       | C18H22BrNO3       | 379.0781         | 379.0281   | 380.0269    | 380.0239 – 380.0289 |

For legal and criminal purposes, the current Brazilian legislation determines that the sample identification of proscribed compounds is sufficient for law procedures, not being necessary its quantification. Considering the national norm this study focused only on the designer drug determination. Although, complementary studies about drug quantification are highly recommended.

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**Table 2.** Analysis of 20 samples of blotters paper seized by the Brazilian Federal Police in the State of Rio Grande do Sul. * Data represent Mean ± Standard Deviation. 2C-B : 2,5-dimethoxy-4-bromophenethylamine (Figure 2). MDMA: 3,4-methylenedioxymethamphetamine (Figure 3). 25B-NBOMe: 2-(4-bromo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl) ethanamine (Figure 4).

| Blotter Paper Samples | Design Exemple          | Weight (mg)* | Dimensions (mm)* | GC-MS identifications | LC-QTOF/MS identifications |
|-----------------------|-------------------------|--------------|------------------|-----------------------|--------------------------|
| 1 - 11                | “Blue Background”       | 12.22 ± 0.76 | 6.59 ± 0.27      | 25B-NBOMe             | 25B-NBOMe + 2C-B          |
| 12 - 18               | “Pink Panther”          | 16.86 ± 0.98 | 9.66 ± 0.16      | 25B-NBOMe             | 25B-NBOMe + 2C-B          |
| 19 - 20               | “Pink Panther”          | 16.70 ± 0.42 | 9.53 ± 0.18      | 25B-NBOMe             | 25B-NBOMe + 2C-B + MDMA   |

**Figure 2.** Chemical structure of 2 C-B.

**Figure 3.** Chemical structure of MDMA.
The results present here are surprising considering the data from the survey done by Wayhs et al. 2016, when the 25I-NBOMe and the 25C-NBOMe were present in 70.27% of the positive expert reports for NBOMes. The 25B-NBOMe represented only 19.41% of the total. Also, the most prevalent banned substance found within NBOMes analysis was 3,4-methylenedioxymethamphetamine (MDMA)⁶. Different from the Brazilian’s standard seizures, in the State of Rio Grande do Sul, the analytical results shown that 25B-NBOMe was present in 100% of samples, 2,5-dimethoxy-4-bromophenethylamine (2C-B) non-target compound was detected in all samples and MDMA was present in only two samples. It is noteworthy that a possibility of contamination is discarded, in view of the MDMA’s chromatographic peak signal intensity. These data strongly demonstrate that the extreme south of Brazil is supplied by drug trafficking routes different from those commonly found in other states of the country (coming from different illegal location and/or suppliers). This analytical finding suggest that another routs of synthesis and a bigger range of raw material, excipients and active ingredients must be monitored, following the trends of drug dealers, once it is known that MDMA can be synthesized from different synthesis routes. In this context, chemical profile studies have been determined different contaminants or synthetic impurities, such as co-products, for example, may point to the use of one or the other pathway to obtain MDMA¹¹,¹². The 2C-B seems to be the most important substances within the substituted phenethylamines class of designer drugs and 25B-NBOMe appears as its novel and highly potent derivative¹³. In these samples, the presence of 2C-B may be seen as a synthesis residue, probably being an excess of precursor that has not been consumed during the reaction of 25B-NBOMe production.

2C-B is a synthetic psychedelic drug that is structurally related to mescaline and was first synthesized in the mid-1970s¹⁴, gained certain popularity as a legal substitute for MDMA after its prohibition in 1985¹⁵. 2C-B was legal in most countries until the mid-1990s, when it became a controlled drug in most countries, including Brazil¹⁰. It is, moreover, commonly employed in combination with other illegal drugs, particularly MDMA¹⁶, and is considered to be one of the favorites on the global drug market¹⁷. In addition, 2C-B is among the chemical compounds most often found as an adulterant when analyzing MDMA seizures¹⁸,¹⁹ which signifies that many individuals have consumed it unknowingly¹⁶.
These designer drugs are commonly produced in clandestine laboratories and are often synthesized from chemicals of unknown origin. The composition of these illicit preparations can vary significantly depending on the mode of synthesis, purification methods, source materials, and the presence of cutting agents and excipients used. The understanding of the NPS dynamic market can support more effectively the requires efforts on different fronts to provide countries to improve their forensic analytical capacity relating to NPS detection; to develop or upgrade their monitoring systems of drug trafficking or drug abuse; to provide medical clinics with real data on street drugs and their health consequences.

To date, only few and very recently papers about blotter paper samples seizures in Brazil were published. The present study is a small start in the sense of to know the actual scenario of the blotter paper drug market. It is necessary to perform studies with large range of samples and, although the quantification it is not mandatory for legal purposes, it is recommended that researches in this field have incentives. From forensic intelligence point of view these information brings a soft but valuable assistance for the investigation, prevention and combating of drugs trafficking.

4. Conclusions

In this study GC-MS and LC-QTOF/MS were used to identification of NPS in blotter samples seized by BFP in the state of Rio Grande do Sul. The analysis performed under routine GC–MS conditions detected 25B-NBOMe in all samples and complementary analysis by LC-QTOF/MS detected 25B-NBOMe and 2C-B in all samples and MDMA in two samples. The detected compounds are quite different from the national profile seizures by BFP, which may be associated with another rout or supplier act in this region. Although, to be able to affirm more accurately about the different traffic routes and their origins, it is necessary to carry out a more comprehensive chemical profile study and, preferably, use multivariate analysis techniques. In this context, for research and drug intelligence purposes the use of more versatile, sensitive and specific analytical tool provides a greater number of information that could be employed as a valuable strategy in the drug trafficking combat.
Acknowledgements

The Federal Police Department of Rio Grande do Sul (Porto Alegre / RS) for providing the data for this work. Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). This work was supported by CAPES, the Forensic Sciences Announcement Nº 25/2014 - "PRO-FORENSIC 3357/2014", Proceeding Nº. 23038.006845/2014-9, entitled "Scientific Exchange and Training Human Resources between Federal University of Espírito Santo (UFES), Goiás (UFG) and Rio Grande do Sul (UFRGS) with the Federal Police of Rio Grande do Sul."

Conflict of interest

The authors declare that there are no conflicts of interest.

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