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Original Scientific Paper

verIDENTIFICATION AND DETERMINATION OF 2,5-DIMETOXY-4-BROMOPHENETHYLAMINE (2C-B) IN REAL SAMPLE BY GC-MS METHODS AND DERIVATIZATION AFTER SPE PREPARATION

VERA LUKIĆ[1], RUŽICA MICIĆ[2], ŽIVANA RADOSAVLJEVIĆ[2*]

[1]Institute of Forensic Medicine, Faculty of Medicine, University of Beograd, Beograd, Serbia
[2]Faculty of Sciences and Mathematics, University of Priština in Kosovska Mitrovica, Kosovska Mitrovica, Serbia

ABSTRACT

The abuse of new psychoactive substances is attracting a lot of attention from the world public. There is an increasing use among young people, who are not aware of the harmful effects of this substances. Some of these substances may have been around for years, but have reemerged in the market in altered chemical forms and launched as legal alternatives to common drugs of abuse. This paper describes application of gas chromatography coupled with mass spectrometry method (GC-MS) to identify 2,5-dimetoxy-4-bromophenethylamine (2C-B) compound in urine sample after solid phase extraxtion and derivatization with N-methyl-bis-trifluoroacetamide (MBTFA). Gas chromatographic separation of TFA derivative of 2C-B (2C-B TFA) was successfully performed using DB-5MS capillary column (5% diphenyl-95% dimethilsiloxane). Selected ion monitoring (SIM) technique was used for qvantitative analyses wich was performed using matrix mached calibratores, whereby good results were achieved. Urine sample wich contained 2C-B was obtained within International Quality Assurance Programme - International Collaborative Exercises (ICE) program organized by the scientific department of United Nations Office on Drugs and Crime (UNODC). The aim of this study was to develop a simple and sensitive method of gas chromatography mass spectrometry (GC-EI-MS) for the identification, extraction and quantitative analysis of 2C-B in the urine sample, which is near the blood remains a priority analyzed matrix in clinical and forensic toxicology.

Keywords: New psychoactive substances (NPS), Phenethylamines, 2-CB, GC-MS method.

INTRODUCTION

New psychoactive substances (NPS, or better known as "legal highs" products) denote synthetically modified natural substances or completely newly designed molecular structures, which lead to a number of harmful effects when consumed, often even ends in death. (National Forensic Service (2014) Special report; Wikström et al., 2013).

This group of compounds includes synthetic cannabinoids, cathinones, phenethylamines, piperazines and tryptamine.

Pheneteliamines are a group of compounds that contain chemical structures that are in fact molecular variants of basic compounds, ie. amphetamines, 3,4-methylenedioxymethamphetamine (MDMA), etc. A series of modifications in the basic structure, which are partially induced by natural products, can significantly change the pharmacological activity, and create a new compound, with completely different effects that occur when consumed. (Jiang et al., 2014; Mahmoud et al., 2014; Choi et al., 2013). 2C-B is a psychedelic drug of the 2C family. It is a group of compounds with a common phenethylamine backbone, which contains two methoxy groups on benzene, at positions 2 and 5, as well as substituents that are different, at position 4 and, very rarely, at position 3. The general structure of 2C compaund and structure of 2C-B are shown in Figure 1 and Figure 2.

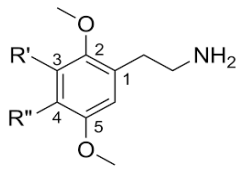

Figure 1. General structure of 2C compound.

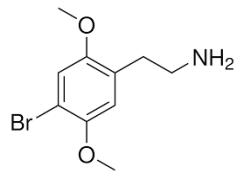

Figure 2. Structure of 2C-B compound.

2C-B (4-bromo-2,5-dimethoxy-b-phenethylamine) formed by benzene substitution, acts as an agonist at 5-HT2 (serotonin 2) receptors. (Cole et al., 2002; Hill et al., 2011).

The GC-MS method can be used to identify and quantify psychoactive compounds, such as cannabinoids, amphetamines, cocaine, antidepressants, antipsychotics, etc. Samples were analyzed by this method after derivatization. (Pujades et al., 2007).

* Corresponding author: zivana.radosavljevic30@gmail.com

This paper presents the application of the GC-MS method for the identification of a newly designed drug, using the SPE for sample preparation and with special emphasis on the derivatization procedure with MBTFA for qualitative and quantitative analysis of the newly designed drug 2C-B in a urine sample. Urine sample was obtained as a part of International Quality Assurance Programme, International Collaborative Exercises program organized by the scientific department of United Nations Office on Drugs and Crime.

EXPERIMENTAL

Materials and methods

Chemical and reagents

All chemicals were purchased in the highest possible purity and used without any further purification. A reference standard of 4-Bromo-2,5-dimetoxyphenethylamine.hydrochloride (2C-B.HCI) with chemical purity declared 99,3 % was purchased from Lipomed AG (Arlesheim, Switzerland). Methanol, ethyl acetate, n-hexane 2-propanol (HPLC grade, 99,9%), ammonium hydroxide (25%), hydrochloric acid (36%) were purchased from Fisher Scientific (Pittsburgh, PA). The derivatization reagent used for the acylation reaction was N-methyl-bis (trifluoroacetamide) (MBTFA), 98%, which was purchased from Macherey–Nagel GmbH & Co. (Düren, Germany). In this study, Strata X-C, 33 µm particle size, Polymeric Strong Cation, 60.0 mg / 3.0 mL, solid-phase extraction (SPE) columns were used and were obtained from Phenomenex (Torrance). Blank urine samples, collected from volunteer laboratory personnel, were used for the development of the method. They were firstly screened by GC/MS to confirm absence of drugs.

Calibration standards

Stock standard solution of 2C-B **were** prepared in methanol at a concentration of 1.0 mg/mL and stored at **−20◦C**. Five working standard solutions containing 2C-B at the following concentrations 5.0, 8.0, 10.0, 12.0, and 15.0 µg/mL, were prepared by mixing the appropriate volumes of the corresponding stock solution and then by diluting with methanol (stroed at -20°C) .

Spiked urine samples for calibration curves (calibrators) were prepared by spiking 1.8 mL of blank human urine with 200.0 µl of working standard solutions. The five calibrators contained 2C-B at concentrations of 0.5, 0.8, 1.0, 1.2 and 1.5 µg/mL.

Sample preparation

Strata-X-C cc (60 mg) columns (Phenomenex) were used for SPE extraction of 2C-B from urine samples (spiked urine samples and urine sample from ICE program). 3 mL of urine sample was acidified with 30.0 µL of 5.0 M HCl. The SPE column was conditionered with 2.0 mL of 0.1 M MeOH and 2.0 mL of deionized water at a rate of 2.0 mL/min. Then 2.0 mL urine sample was loaded at a rate of 2.0 mL/min. Rinsing was made by 2.0 mL of 0.1 M NaOH at a rate of 2 mL/min, 2.0 mL of deionized water at a rate of 8 mL/min and 4.0 mL of hexane at a rate of 8 mL/min. After washing the column, elution was performed with 3.0 mL of 2-propanol/methylene chloride/ammonium hydroxide (80:20:2, v/v/v), at a rate of 2 mL/min. The procedure is followed by acidification and evaporation by adding 100.0 µL of 1% HCl to each tube, before evaporation under N_2. After evaporation of the extract (eluate) samples were derivatized with 50.0 µL of MBTFA (N-methyl-bis-trifluoroacetamide). The reaction was performed at 80°C for 15 min. Reconstitution in 200.0 µL of ethyl acetate is then performed. Gas-chromatographic separation TFA derivative of 2C-B was successfully performed using DB-5MS capillary column (5% diphenyl-95% dimethilsiloxane). External standards method was used for quantification. The calibration curve is constructed using five calibrators, samples of spiked urine in which standard solutions were added to achieve 2-CB concentrations of 0.5, 0.8, 1.0, 1.2 and 1.5 µg/mL respectively (matrix-matched calibrator samples). Urine sample for ICE control program was analyzed after extraction and derivatization in the same way as the spiked samples.

Gas chromatography–mass spectrometry analysis

The GC/MS analysis was performed using a Shimadzu GC-2010 Plus equipped with a Shimadzu AOC-5000 auto sampler system and interfaced with a Shimadzu QP 2010 Ultra mass spectrometer (Shimadzu, Tokyo, Japan).

The GC was equipped with a split /splitless injection port operated at splitless mode. **1.0 µL** of extract injecting into the GC–MS. The separation of analytes was carried out using a cross-linked DB-5MS capillary column (30 m × 0.25 mm i.d.,0.25 um film thickness) supplied by Agilent Technologies (Illinois, IL, USA). Helium was employed as the carrier gas and used at a flow rate of 1.32 mL / min. The temperatures of injection port, ion source and interface were 250, 200 and 280ºC, respectively. Initial oven temperature of 60ºC was held for 2 min, followed by an increase to 280ºC at a rate of 20ºC/min and a final hold time of 4 min, resulting in a total run time of 17 min per sample with a solvent delay of 4.0 min.

Ionization voltage of 70 eV was used to obtained full scan and selected ion monitoring of analytes in the m/z range 50–450 at a scan rate of 3.62 scan/s. The mass spectrometer (MS) was operated in full scan and selected ion monitoring (EI/Scan/SIM and SIM) mode.

RESULTS AND DISCUSSION

Z-score of 0.6 for the our resultat of 1.38 µg/mL of 2C-B in the urine sample, was obtained after evaluation of results by ICE program organizers for round 2013/2 in wich 74 countries was participated.

Figure 3 shows ful scan mass spectrum TFA derivative of 2C-B obtained from urine sample.

For Selected ion monitoring we used tree ions m/z 242, m/z 229 and m/z 148, Figure 4.

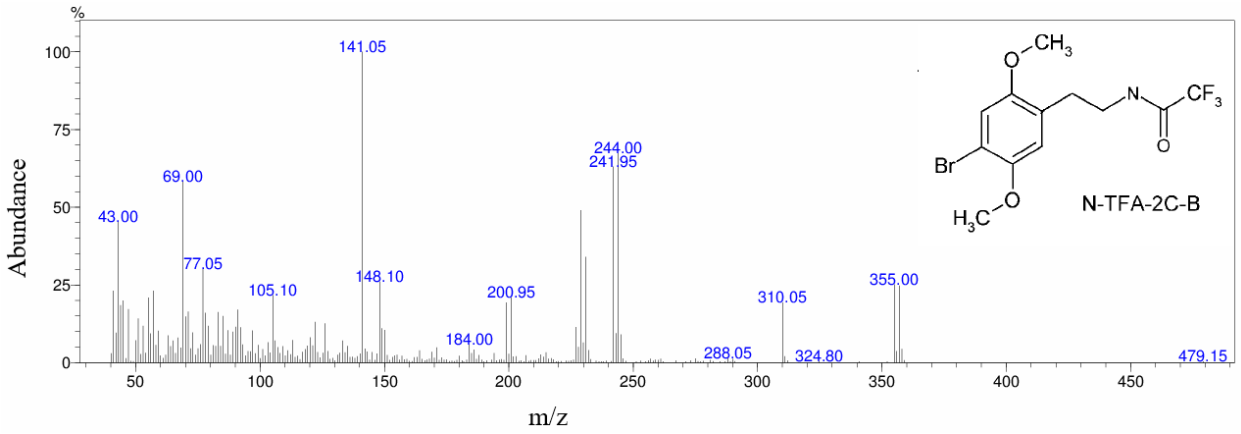

Figure 3. EI GC-MS mass spectrum of TFA derivative of 2C-B.

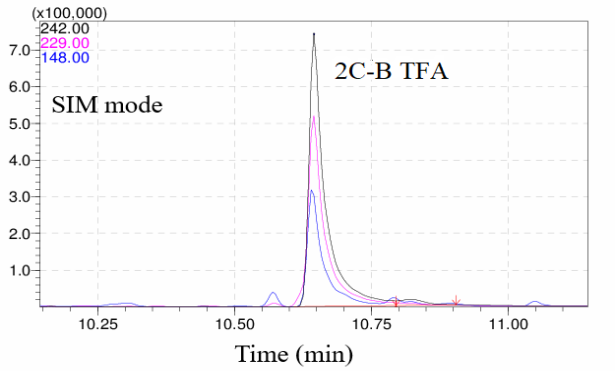

Figure 4. Chromatogram of three ions m/z 242, m/z 229 and m/z 148 using for SIM mode.

Calibration curve, which was constructed using five calibrators, matrix-matched calibrator samples is shown on Figure 5. Obtained correlation coefficient was r 2>0,999. SIM mode was used for quantitative analyses.

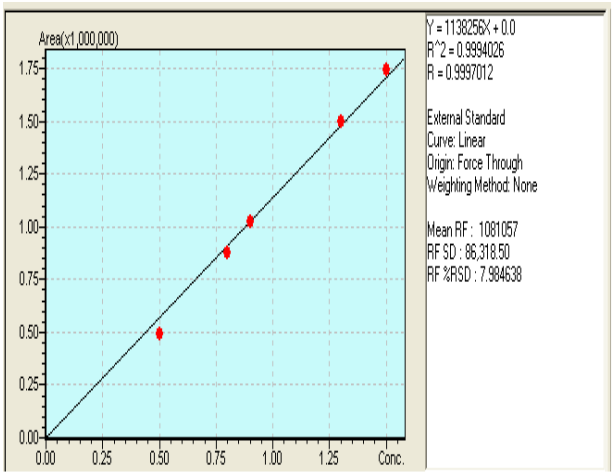

Figure 5. Calibration curve obtained from matrix matched calibrators of 2C-B TFA.

In modern chemical analysis two specific trends can be noticed. One is the requirement for more sensitive and accurate analytical methods and the other for simpler methods that require as little as possible human intervention. Derivatization procedure use specific chemical changes to make analytical methods more sensitive and accurate.

That usualy involve more human intervention than the direct use of advanced instrumentation. For this reason, derivatization is not the first choice when selecting an analytical method. But, the benefits of derivatization in many cases, are more important than the disadvantage of requiring human intervention (Serban, 2018).

The advantages of its use are reflected on changes chemical properties derivatized analytes (higher volatility, better thermal stability) and therefore better separation, selectivity, sensitivity and identification of compounds.

If combined with the GC-MS method, as shown within this study, derivatization can significantly increase the ability to identify new designed drug from phenethylamine group, 2-CB.

Because compound from phenethylamine group (e.g methamphetamine, amphetamine and methylenedioxyphenylalkylamine derivatives, such as 3,4-methylenedioxymethamphetamine (MDMA)) have relatively low molecular weights, high polarity, and volatility, derivatization is necessary when using gas chromatography for analysis biological samples in clinical and forensic toxicology.

There are basically three types of derivatization reactions, such as alkylation (which is the main esterification process), then acylation and silylation. (Orata, 2012).

In this study we were successfully applied acylation as one of the most popular derivatization reactions for primary and secondary amines to 2C-B and obtained stabile 2C-B TFA derivative that gave an enhanced response in GC compared with the parent compound.

CONCLUSIONS

Identification of 2-CB compounds in samples of biological material is very important. With the help of GC-MS method, which is a very reliable and sensitive method, thanks to the library of mass spectra, this is the method of choice, together with derivatization of compounds, a successful analysis was achieved, and the presence of 2-CB compound in urine sample confirmed.

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