Legal Medicine 17 (2015) 56–59

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Brief Communication

Identification and quantitation of 4-bromo-2,5-dimethoxyamphetamine in seized blotters

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ARTICLE INFO

Article history:
Received 11 April 2014
Received in revised form 1 September 2014
Accepted 2 September 2014
Available online 11 September 2014

Keywords:
Amphetamine designer drugs
4-Bromo-2,5-dimethoxyamphetamine (DOB)
2,5-Dimethoxyamphetamine (DMA)
Blotter
LC–MS–MS

ABSTRACT

Blotters are usually impregnated with hallucinogens such as lysergic acid diethylamide (LSD); only rarely other psychoactive substances are detected. In this work we identified 4-bromo-2,5-dimethoxyamphetamine (DOB) and 2,5-dimethoxyamphetamine (DMA) in illicit blotters seized in Italy. This report describes a rapid method for the simultaneous identification and quantitation of DOB and its precursor (DMA) by liquid chromatography tandem mass spectrometry (LC–MS–MS), using 2,3-dimethoxyphenethylamine-d_3 as internal standard. Regression equations were linear over the tested concentration range with good correlation coefficients. The achieved levels of sensitivity may be suitable to confirm the possible presence of DOB and DMA also in low concentration or in traces in seized material for forensic analysis. The developed method showed good reproducibility and sensitivity, and could be used for similar routine analysis. To our knowledge, this is the first report describing the detection of DOB and DMA from blotters.

1. Introduction

In our laboratories, we synthesized a number of methoxy-substituted and methylenedioxy-substituted amphetamine designer drugs, which are available and still unknown in the illegal drug market, and reported their cross-reactivities to some immunoassay tests [1,2]. We also established a liquid chromatography–tandem mass spectrometry (LC–MS–MS) method for trimethoxyamphetamine drugs in rat plasma [3], and a liquid chromatography mass spectrometry (LC–MS) for *N*-substituted derivatives of 3,4-methylenedioxy amphetamine in rat urine [4]. In addition, Zaitsu et al. [5] reported the differentiation of isomers of methoxy-substituted amphetamines by gas chromatography–tandem mass spectrometry (GC–MS–MS).

In October 2013, the Forensic Laboratory of the University of Sassari received a blotting paper, commonly known as "blotters", from the Criminal Court of Sassari. The blotting paper strip (19.2 × 4.8 cm) consists of 100 small squares, which is pooled into 4 sectors of 25 blotters each. Every sector has a drawing shown in Fig. 1. According to the "Finance Guard" of Sassari, the seized blotters were suspected to be in the solution of LSD. Unexpectedly, a preliminary GC–MS analysis lead to the identification of 4-bromo-2,5-dimethoxyamphetamine (DOB) in the seized blotters, using the reference standard DOB synthesized in our laboratories. DOB is a psychoactive designer drug with hallucinogen-like activity comparable to that of LSD, a high *in vivo* potency, and a long duration of action [6–8]. DOB can be easily synthesized from 2,5-dimethoxyamphetamine (DMA) [9], another designer drug (Fig. 2). Both DOB and DMA are illicit by Italian Legislation [10,11]. Considering that DOB does not have the known LSD profile, supplying DOB as LSD could be dangerous for users. Indeed, unlike LSD, DOB can have physically harmful, sometimes fatal, side effects which could induce overdose [12]. The usual DOB dosage is about 0.75–1.75 mg; for chronic users, it is increased to 2.5–3.5 mg [13]. Dosages of 2.8 mg can lead to adverse effects such as cramps with muscular pain and flashes of hallucination [12]. Overdoses, caused by dosages of 3.5 mg or higher, are characterized by memory loss, irrational and sometimes violent behavior. The problem is that users do not realize the effect of DOB until a fairly long time (up to 3 h) from the oral intake. This could lead users to take additional DOB which consequently causes severe intoxication [9].

This report describes a rapid method for the simultaneous identification and quantitation of DOB and its precursor (DMA) by LC–MS–MS, using 2,3-dimethoxyphenethylamine-d_3 as internal standard. The developed method shows high reproducibility and sensitivity, and could be used for similar routine analysis.

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http://dx.doi.org/10.1016/j.legalmed.2014.09.001
1344-6223/

Fig. 1. The seized blotters analyzed in this study.

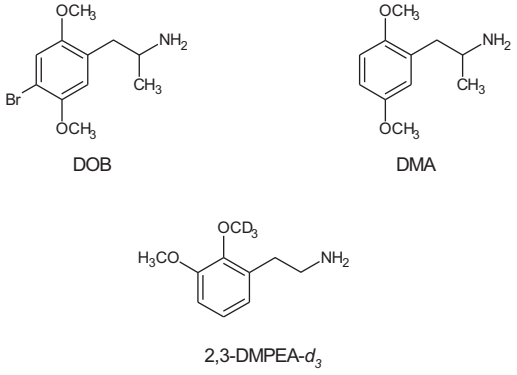

Fig. 2. Molecular structures of target analytes and IS.

2. Materials and methods

2.1. Reagents

MS grade methanol, acetonitrile, and formic acid were purchased from Sigma (Sigma–Aldrich, Milano, Italy). Deionised and distilled water was purified through a Milli Q water system (Millipore, Billerica, MA, USA). Other reagents and solvents used were of the highest commercial quality. Blotter samples ($n = 100$) were provided by the Criminal Court of Sassari (Italy) and they were seized by the Guardia di Finanza of Sassari. Standard DOB and DMA were synthesized in our laboratory at their maximum level of purity according to the method of Shulgin and Shulgin [13]. The product characterization by ^{1}H-NMR spectrometry was carried out using a Bruker AMX 400 (Bruker, Bremen, Germany). Melting points were determined with a Kofler hot stage microscope. 2,3-dimethoxyphenethylamine-d_3 (internal standard, IS) was synthesized in our laboratory at its maximum level of purity and was used as internal standard.

2.2. Instrumentation: GC–MS and LC–MS–MS

The GC–MS analysis was performed using a Hewlett Packard 5890 Series II GC (Hewlett Packard, Wilmington, DE, USA) equipped with a Hewlett Packard 5971 MS selective detector operating in the EI mode at 70 eV, using an HP-5 capillary column (30 m × 0.25 mm, film thickness 0.17 μm). The column temperature was kept at 190 °C for 1 min, then programmed to 300 °C at

Table 1
Tandem mass spectrometric conditions used for DOB, DMA, and IS.

Compound	Parent ion (m/z)	Fragment ions (m/z)	CE[c] (V)	CXP[d] (V)	DP[e] (V)	FP[f] (V)	EP[g] (V)
DOB	274.0	257.0[a] 229.0[b]	22 33	5.9 4.3	15	300	5
DMA	196.2	179.2[a] 151.2[b]	16 24	3.2 6.0	15	200	8
IS	185.0	168.0	20	7	25	400	10

[a] Fragment ion used for quantitation.
[b] Fragment ion used for confirmation.
[c] CE, collision energy.
[d] CXP, collision cell exit potential.
[e] DP, declustering potential.
[f] FP, focusing potential.
[g] EP, entrance potential.

Table 2
Parameters for calibration curves for the present analytical method for the target analytes.

Compound	t_R[a] (min)	r[b] ± SD[c] ($n = 5$)	Slope ± SD[c] ($n = 5$)	Intercept ± SD[c] ($n = 5$)	LOD[d] (ng mL^{-1})	LOQ[e] (ng mL^{-1})
DOB	4.33	0.9976 ± 0.0189	0.2890 ± 0.0001	0.0159 ± 0.0069	3.66	8.49
DMA	3.17	0.9964 ± 0.0913	1.148 ± 0.0003	0.0783 ± 0.0333	17.8	41.1

[a] t_R, retention time.
[b] r, correlation coefficient.
[c] SD, standard deviation.
[d] LOD, limit of determination.
[e] LOQ, limit of quantitation.