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Short communication

Studies on the toxicological detection of the designer drug 4-bromo-2,5-dimethoxy-β-phenethylamine (2C-B) in rat urine using gas chromatography–mass spectrometry

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Abstract

The phenethylamine-derived designer drug 4-bromo-2,5-dimethoxy-β-phenethylamine (2C-B) is known to be extensively metabolized in various species including humans. In rat urine, 2C-B was found to be excreted mainly via its metabolites. In the current study, the toxicological detection of these metabolites in the authors' systematic toxicological analysis (STA) procedure was examined. The STA procedure using full-scan GC–MS allowed proving an intake of a common drug abusers' dose of 2C-B by detection of the *O*-demethyl deaminohydroxy and two isomers of the *O*-demethyl metabolites in rat urine. Assuming similar metabolism, the described STA procedure should be suitable for proof of an intake of 2C-B in human urine.

Keywords: 4-Bromo-2,5-dimethoxy-β-phenethylamine; 2C-B; Designer drug; Metabolism; GC–MS

1. Introduction

4-Bromo-2,5-dimethoxy-β-phenethylamine (2C-B, Nexus, Venus, Bromo, Erox, Bees) is a hallucinogenic drug that was first synthesized in 1974 by Shulgin and Carter [1]. It belongs to the so-called 2C-series, which are phenethylamine derivatives having in common two methoxy groups in position 2 and 5 of the ring and one lipophilic substituent in position 4. The hallucinogenic properties of the 2C-drugs seem to be mediated by agonistic and/or antagonistic effects on various serotoninergic and α_1-adrenergic receptors [2–10]. In the mid 1980s, 2C-B appeared on the illicit drug market [11] and gained increasing popularity in the 1990s, when it was sold in so-called smart shops via the internet [12]. Since that time, 2C-B was identified in drugs seized in the illicit drug market all over the world [12–15]. Common drug abusers' doses for 2C-B ranged from 4 to 30 mg [14]. In 1998, it was the third most reported drug of ring substituted phenethylamines in England and Wales following MDMA and MDEA [11]. Consequently, 2C-B was scheduled in the lists of controlled substances in many countries [12]. Further evidence about its popularity among drug abusers can be found on internet web sites (http://www.erowid.org/, http://www.lycaeum.org/; June 2006) where experience reports and descriptions of 2C-B have been published.

The metabolism of 2C-B has been extensively studied in rats [16–18], mice [19], and in hepatocytes from various species including humans [20,21]. Preliminary data are also available on excretion of 2C-B metabolites in human urine [22]. All these metabolism studies showed that major metabolic steps of 2C-B were *O*-demethylation of the parent compound, *N*-acetylation, and deamination with oxidation to the corresponding acids or reduction to the corresponding alcohols and combinations of these steps. Theobald et al. could further identify a deaminohydroxy-side chain hydroxy, a *O*-demethyl deaminohydroxy-side chain hydroxy, and a *O*-demethyl deaminohydroxy-side chain oxo metabolite [23], which is in accordance with the data found for the iodo analogue 2C-I [16].

In clinical and forensic toxicology, such drugs of abuse must be analyzed for monitoring an abuse or a poisoning. Some stud-

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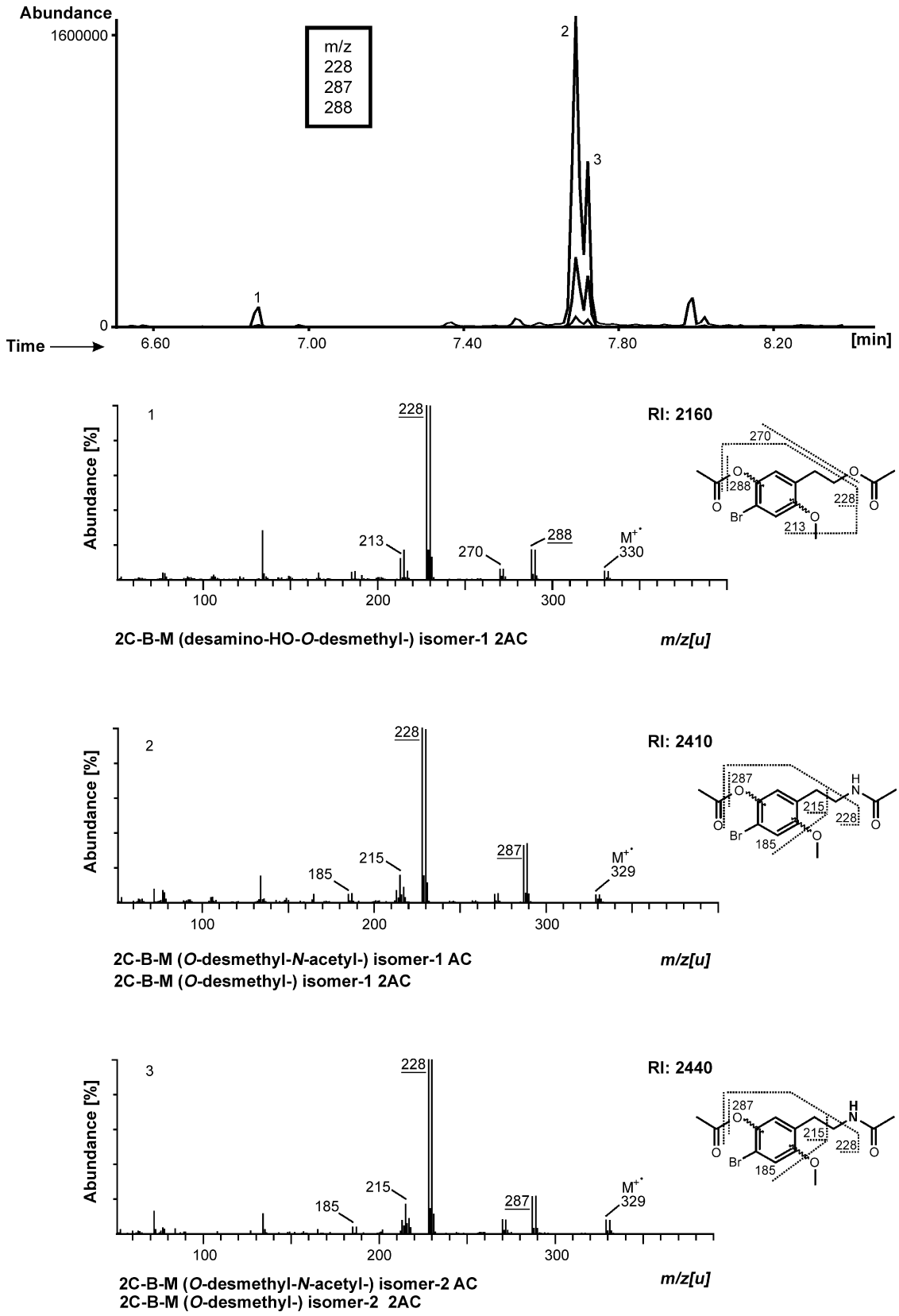

Fig. 1. Typical reconstructed mass chromatograms with the given ions of an acetylated extract of a rat urine sample collected over 24 h after intake of 0.3 mg/kg BM of 2C-B (upper part). They indicate the presence of 2C-B metabolites. EI mass spectra, RIs, structures and predominant fragmentation patterns of 2C-B metabolites included in the STA procedure after acetylation (lower part). The numbers of the spectra correspond to those of the peaks in the upper part. Selected ions are underlined.

ies have been published on the detection of 2C-B itself in blood and/or urine [24–27]. However, as shown by the metabolism studies, 2C-B itself was excreted into urine only to a very small extent, whereas the metabolites played the main role in the excretion process. Ingestion of other compounds of the 2C-series could be screened for by the authors' systematic toxicological analysis (STA) procedure in urine by GC–MS and this STA procedure allows simultaneous detection of about 2000 other drugs, poisons and/or their metabolites [16,23,28–33]. Therefore, the aim of this study was to investigate the detectability of these major 2C-B metabolites as target analytes within the authors' STA procedure.

2. Experimental

2.1. Chemicals and reagents

2C-B tartrate was provided by Hessisches Landeskriminalamt (Wiesbaden, Germany) for research purposes. All chemicals and biochemicals were obtained from Merck (Darmstadt, Germany) and were of analytical grade.

2.2. Urine samples

The investigations were performed using urine of male Wistar rats (about one year old and 400 g body mass (BM), Ch. River, Sulzfleck, Germany) for toxicological diagnostic reasons according to the corresponding German law. They were administered a single 0.3 mg/kg BM dose for the STA study in aqueous suspension by gastric intubation ($n = 2$). Urine was collected separately from the faeces over a 24-h period. The samples were directly analyzed. Blank rat urine samples were collected before drug administration to check whether they were free of interfering compounds.

2.3. Sample preparation for toxicological analysis

A 5-ml portion of urine was worked-up as previously described for 2C-E [32]. After acidic hydrolysis, the liquid–liquid extract was derivatized by acetylation. Aliquots (2 μl) were injected into the GC/MS system.

2.4. GC–MS apparatus and method

A Hewlett Packard (Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph combined with a HP 5972A MSD mass spectrometer was used under the condition described for 2C-E [32]. The GC conditions were as follows: splitless injection mode; column, HP-1 capillary (12 m × 0.2 mm I.D.), cross linked methyl silicone, 330 nm film thickness; injection port temperature, 280 °C; carrier gas, helium; flow-rate 1 ml/min; column temperature, programmed from 100 to 310 °C at 30°/min, initial time 3 min, final time 8 min. The MS conditions were as follows: full-scan mode, *m/z* 50–800 u; EI mode, ionization energy, 70 eV; ion source temperature, 220 °C; capillary direct interface, heated at 280 °C.

For toxicological detection of acetylated 2C-B metabolites, mass chromatography with the selected ions *m/z* 228, 287, and 288 was used. The identity of the peaks in the mass chromatograms was confirmed by computerized comparison of the mass spectra underlying the peaks (after background subtraction) with reference spectra recorded during the previous metabolism study [23,34].

3. Results and discussion

Using the STA procedure, the most abundant isomer of bisacetylated deamino hydroxy *O*-demethyl 2C-B as well as the bisacetylated two isomers of *O*-demethyl 2C-B were found to be the target analytes. The latter could also be formed by monoacetylation of the corresponding *N*-acetyl conjugates [17,23]. They could be detected by mass chromatography with the ions *m/z* 228, 287, and 288. Fig. 1 (upper part) shows typical reconstructed mass chromatograms with the given ions of an acetylated extract of a rat urine sample collected over 24 h after application of 0.3 mg/kg BM of 2C-B which corresponded to a common abusers' dose of about 20 mg. The lower part of Fig. 1 shows the EI mass spectra, the retention indices (RI), the structures and the predominant fragmentation patterns of the three target analytes. In poisoning cases, 2C-B and most of the other metabolites should also be detectable using the reference spectra published elsewhere [23,34], because they were detected in rat urine after a 20-fold higher dose.

Although interferences by biomolecules or other drugs cannot be entirely excluded, they are rather unlikely, because their mass spectra and/or their RIs should be different. In addition, the characteristic bromine isotope clusters in the EI mass spectra of 2C-B facilitate its unambiguous identification.

For lack of authentic human urine samples, a comparison of the metabolites found in rat and human urine after administration of 2C-B was not yet possible. However, in other studies good correspondence has been reported for the metabolic pathways as well as for the detectability between rats and humans [35–39]. Finally, de Boer et al. [22] have reported detection of *O*-demethyl 2C-B in human urine.

4. Conclusions

The authors' STA procedure allowed proving an intake of a common drug abusers' dose of 2C-B in rat urine by detection of its major metabolites. Earlier studies and the authors' experience in metabolism and analytical studies on rats and humans support the assumption that the metabolites found in rat urine should also be present in human urine. Therefore, it can be concluded that the procedure should also be applicable for human urine screening for 2C-B in clinical or forensic toxicology.

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