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Short communication

Metabolism and toxicological detection of the designer drug 4-iodo-2,5-dimethoxy-amphetamine (DOI) in rat urine using gas chromatography–mass spectrometry

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Abstract

The amphetamine-derived designer drug 4-iodo-2,5-dimethoxy-amphetamine (DOI) is an upcoming substance on the illicit drug market. In the current study, the identification of its metabolites in rat urine and their toxicological detection in the authors' systematic toxicological analysis (STA) procedure were examined. DOI is extensively metabolized by *O*-demethylation and beside small amounts of parent compound it was found to be excreted mainly in form of metabolites. The STA procedure using full-scan GC–MS allowed proving an intake of a common drug users' dose of DOI by detection of the two *O*-demethyl metabolite isomers in rat urine. Assuming similar metabolism, the described STA procedure should be suitable for proof of an intake of DOI in human urine.

Keywords: 4-Iodo-2,5-dimethoxy-amphetamine; DOI; Designer drug; Metabolism; GC–MS

1. Introduction

4-Iodo-2,5-dimethoxy-amphetamine (DOI) is a hallucinogenic drug that was synthesized by A. Shulgin who also gave qualitative and quantitative comments on its effects [1,2]. DOI belongs to the 2,5-dimethoxy-phenylisopropylamines which are amphetamine derivatives having in common two methoxy groups in position 2 and 5 of the ring and a 4-substituent, for example iodine (DOI), bromine (4-bromo-2,5-dimethoxy-amphetamine, DOB; 4-bromo-2,5-dimethoxy-metamphetamine, MDOB), chlorine (4-chloro-2,5-dimethoxy-amphetamine, DOC), methoxy (4-methoxy-2,5-dimethoxy-amphetamine TMA-2), or methyl (4-methyl-2,5-dimethoxy-amphetamine, DOM). Structure–activity relationship studies revealed that the highest hallucinogen-like activity was caused by the primary amine functionality separated from the phenyl ring by two carbon atoms, the presence of methoxy groups in position 2 and 5, and a hydrophobic 4-substituent, especially a halogen [3,4]. The methyl moiety in α-position to the nitrogen is reported to be responsible for increased *in vivo* potency and duration of action compared to β-phenethylamines (so-called 2Cs) [3]. The hallucinogenic properties of DOI seem to be mediated by agonistic effects on serotoninergic receptors. DOI as a non-scheduled substance is widely used in many studies where a specific 5-HT_2 agonist is needed [5,6]. Nevertheless because of its hallucinogenic properties DOI has also appeared on the illicit drug market in some countries such as Germany or the USA [7,8]. Several seizures in clandestine laboratories in these countries lead to the assumption that DOI is abused as designer drug but so far no intoxications have been reported. Common drug abusers' doses for DOI ranged from 1 up to 3 mg [1]. Further evidence about its popularity among drug abusers can be found, with reservation, on internet web sites (http://www.erowid.org, http://www.lycaeum.org; March 2007) where experience reports and descriptions of DOI intake have been published.

The metabolism of DOI has not been studied systematically so far. Metabolism studies of other 2,5-dimethoxy-amphetamines such as DOB, MDOB or TMA-2 showed that

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these substances were mainly metabolized by *O*-demethylation [9,10]. In clinical and forensic toxicology, such drugs of abuse must be analyzed for monitoring an abuse or a poisoning. DOB, MDOB, and TMA-2 were excreted in urine mainly as their metabolites [9,10]. Therefore, the first aim of this study was to identify the main metabolites of DOI to be screened for, besides approximately 2000 other compounds, by the authors' systematic toxicological analysis (STA) procedure in urine by GC–MS [9–17]. The second aim was to investigate the detectability of DOI and its major metabolites as target analytes within the authors' STA procedure.

2. Experimental

2.1. Chemicals and reagents

DOI 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 1 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 5.0 or 0.05 mg/kg BM dose for metabolism studies or the STA study, respectively, 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 metabolism studies

The urine samples were worked up as described for DOB and MDOB [10]. A 5-ml portion of urine was adjusted to pH 5.2 with acetic acid (1 mol/l) and incubated at 50 °C for 1.5 h with 100 μl of a mixture (100000 Fishman units per ml) of glucuronidase (EC no. 3.2.1.31) and arylsulfatase (EC no. 3.1.6.1) from *Helix pomatia*, then adjusted to pH 8–9 with 1 ml of 37% hydrochloric acid, 2 ml of 2.3 mol/l aqueous ammonium sulfate and 1.5 ml of 10 mol/l aqueous sodium hydroxide and extracted with 5 ml of a dichloromethane–isopropanol–ethyl acetate mixture (1:1:3; v/v/v). After phase separation by centrifugation, the organic layer was evaporated to dryness under reduced pressure at 70 °C. The residue was either dissolved in 100 μl of methanol or it was derivatized by acetylation with 100 μl of an acetic anhydride–pyridine mixture (3:2; v/v) for 5 min under microwave irradiation at about 440 W. After careful evaporation, the residue was dissolved in 100 μl of methanol. Aliquots (2 μl) of the derivatized or underivatized extracts were injected into the GC–MS. Another urine sample was worked up after enzymatic cleavage of conjugates at pH 4–5 and derivatized by methylation followed by acetylation [10]. Again, 2 μl was injected into the GC–MS. The same procedures, with the exception of enzymatic hydrolysis, were used to study whether the metabolites were excreted as glucuronide and/or sulfate conjugates.

2.4. Sample preparation for STA

A 5-ml portion of urine was worked-up as previously described [13,15]. The urine samples were divided into two aliquots. One aliquot was refluxed with 1 ml of 37% hydrochloric acid for 15 min. Following hydrolysis, the sample was mixed with 2 ml of 2.3 M aqueous ammonium sulfate and 1.5 ml of 10 M aqueous sodium hydroxide to obtain a pH value of 8–9. Before extraction, the other aliquot of native urine was added. This mixture was extracted with 5 ml of a dichloromethane–isopropanol–ethyl acetate mixture (1:1:3; v/v/v). After phase separation by centrifugation, the organic layer was transferred into a glass flask and evaporated under reduced pressure at 70 °C to dryness. The residue was derivatized by acetylation with 100 μl of an acetic anhydride–pyridine mixture (3:2; v/v) for 5 min under microwave irradiation at about 440 W. After evaporation of the derivatization mixture under reduced pressure at 70 °C, the residue was dissolved in 100 μl of methanol and 2 μl was injected into the GC–MS system.

2.5. GC–MS apparatus for metabolism studies

A Hewlett-Packard (Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph combined with a HP 5989B MS Engine mass spectrometer was used under the conditions described for DOB and MDOB [10]. 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

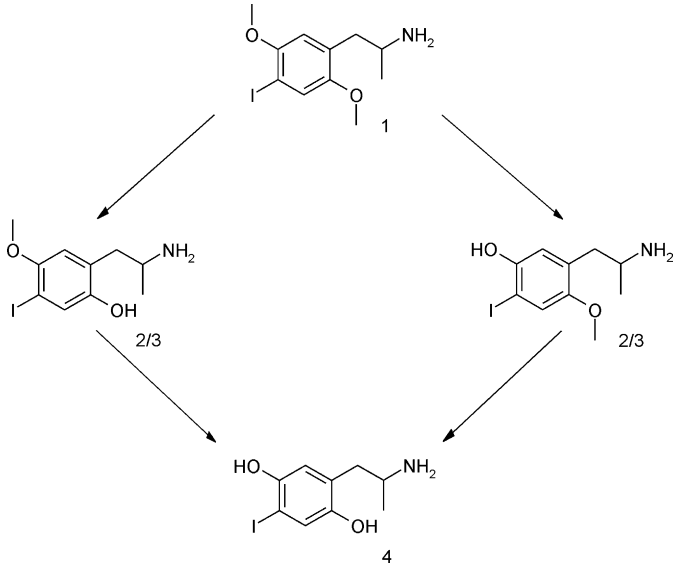

Fig. 1. Proposed scheme for the metabolism of DOI in rats. The numbers correspond to those of the spectra and peak given in Fig. 2.

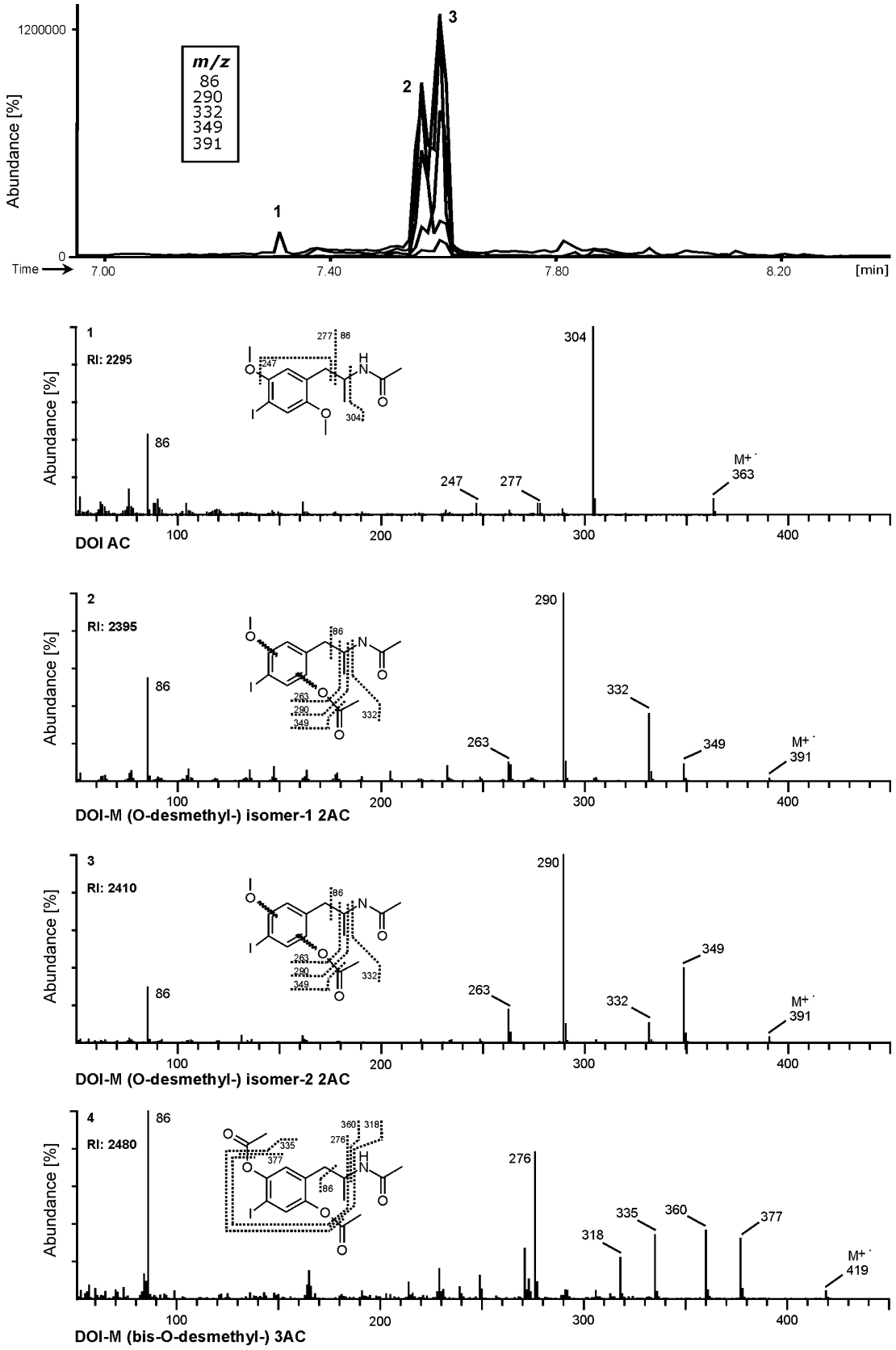

Fig. 2. Typical reconstructed mass chromatograms with the given ions of an acetylated extract of a rat urine sample collected over 24 h after intake of 0.05 mg/kg BM of DOI (upper part). They indicate the presence of DOI metabolites. EI mass spectra, RIs, structures and predominant fragmentation patterns of DOI and its metabolites after acetylation (lower part). The numbers of the spectra correspond to those of the peaks in the upper part and in Fig. 1.

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; PICI mode using methane: ionization energy, 230 eV; ion source temperature, 220 °C; capillary direct interface, heated at 260 °C.

2.6. GC–MS apparatus for STA

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 DOB and MDOB [10]. 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.

2.7. GC–MS procedure for identification of metabolites and STA

DOI and its metabolites were separated by GC and identified by MS in acetylated urine extracts. For toxicological detection of DOI and its metabolites, mass chromatography with the selected ions *m/z* 86, 290, 332, 349, and 391 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 this study.

3. Results and discussion

The postulated structures of the (derivatized) metabolites of DOI were deduced from the fragments detected in the EI mass spectra which were interpreted in correlation to those of the parent compound according to the rules described by, e.g. McLafferty and Turecek [18] and Smith and Busch [19]. As depicted in Fig. 1, besides DOI (1), two *O*-demethyl metabolites (2, 3) and the *O*,*O*-bisdemethyl metabolite (4) could be identified. All metabolites were partly excreted as glucuronides or sulfates. Such conjugation was concluded because the peak areas of these metabolites were more abundant after glucuronidase and sulfatase hydrolysis. Only *O*-demethylation was observed for DOI in contrast to the other 2,5-dimethoxy-amphetamines DOB [10] or TMA-2 [9] which were additionally metabolized by oxidative deamination followed by reduction of the ketone. This may be explained by the fact that DOI was described to be a potent monamineoxidase (MAO) A inhibitor [20], so that the oxidative deamination was impossible.

Using the STA procedure, the two isomers of *O*-demethyl DOI were found to be suitable as target analytes. They could be detected by mass chromatography with the ions *m/z* 86, 290, 332, 349, and 391. Fig. 2 (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 0.05 mg/kg BM of DOI which corresponds to a common users' dose. The lower part of Fig. 2 shows the EI mass spectra, the retention indices (RI), the structures and the predominant fragmentation patterns of DOI and its metabolites. Spectrum no. 4 is depicted to confirm the structure of metabolite 4 and to allow its detection in case of severe poisoning. 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 [12,21].

For lack of authentic human urine samples, a comparison of the metabolites found in rat and human urine after administration of DOI was not yet possible. However, in other studies on DOB [10,22], *N*-benzylpiperazine [23], nutmeg ingredients [24], antidepressants neuroleptics, and hypnotics [25], or various other drugs or poisons [12], good agreement has been reported for the metabolic pathways as well as for the detectability between rats and humans.

4. Conclusions

DOI undergoes single and double *O*-demethylation to three metabolites. The authors' STA procedure allowed proving an intake of a common drug users' dose of DOI 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 DOI in clinical or forensic toxicology.

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