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Short communication

Nonfatal and fatal DOB (2,5-dimethoxy-4-bromamphetamine) overdose

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Abstract

2,5-Dimethoxy-4-bromoamphetamine (DOB) is a strongly acting hallucinogen with an estimated effective dose of 2 mg for an 80 kg man. The case of two men who ingested a new "hallucinogen LSD-like" designer drug is reported here. They experienced intense hallucinations (onset after 15 min) and vomiting. The men became unconscious and fell into deep coma lasting several days. After an unknown period of time elapsed they were admitted to a hospital in a comatose state. One subject (AX, body mass 113 kg) survived, while the second subject (BX, body mass 65 kg) experienced convulsions, metabolic acidosis and died 6 days later. Gastric, blood, and urine specimens collected on admission into the hospital were sent for toxicological examination. Ethanol concentrations in the blood samples were less than 0.2 g/kg. CEDIA urine screening indicated the presence of THCOOH in both cases, while cocaine and its metabolites were only indicated in the urine from AX. Immunoassay for amphetamines was negative in urine specimens collected from both subjects. GC–MS analysis for unknown drugs allowed for the discovery of the presence of DOB in the gastric and urine samples of both persons. DOB-targeted analysis for the acetylated analyte confirmed its presence in both subjects. Quantitative analysis yielded concentrations in serum of 13 ng/ml (AX) and 19 ng/ml (BX). This report on both nonfatal and fatal DOB overdose cases is based on clear toxicological evidence, and is the first documentation of DOB consumption in the Czech Republic.

Keywords: 2,5-Dimethoxy-4-bromamphetamine (DOB); Overdose; Fatality

1. Introduction

The popularity of synthetic drugs, sometimes referred to as designer or rave drugs, has been increasing. This is due to their reputation as being safe, having stimulating and empathic effects, and also causing unusual perceptions and experiences after ingestion. Synthetic drugs of this type are produced illegally with small variations in molecular structure generating new drugs with very different pharmacological properties. The true composition of an illicit drug is without any guarantee of its identity or purity. Even if a drug in tablet form is labelled with a well-known logo, it can contain other potentially lethal substances. The occurrence of modified phenalkylamines in the black market has been reported to be a serious social problem in many European countries (www.emcdda.org). Some compounds derived from amphetamines or phenethylamines and their metabolites are hardly detectable in urine with commercial immunoassays and little is known about their pharmacological properties, metabolism, and toxicity.

Here, we report on two cases of severe intoxication with 2,5-dimethoxy-4-bromamphetamine (brolamphetamine or DOB). DOB is a serotonin receptor agonist with strong hallucinogenic properties and the potential for tolerance in chronic users [1]. According to Shulgin and Shulgin [2], the effective dosage for a human is within 1–3 mg. Severe cramps with pain, flashes of depersonalisation, and hallucinations have been described with doses of 2.8 mg. Overdose cases involving convulsions, arterial vascular

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spasm, extended coma state, and even death are mentioned in Shulgin's monograph without any specified reference. It is reported that DOB distributes first and foremost to the human lungs where it accumulates before the level in brain builds up. According to EROWID, an online drug culture database (www.erowid.org), oral DOB dosages are: threshold 0.2 mg, common 0.75–1.75, and heavy 2.5–3.5 mg. Toennes et al. reported on an unclear, neurological syndrome that occurred after a nonfatal poisoning with DOB [3]. The symptoms of intoxication were vomiting and diarrhoea, tingling in the limbs, exogenous psychosis with restlessness, and optical hallucinations. All symptoms disappeared within a night.

No data about the fate of DOB in humans or animals have been found in literature. This report on two cases of DOB overdose and the tentative identification of excreted metabolites in human urine can contribute to fill this information gap. Details concerning these overdose cases have been included in the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) early warning system on risk assessment of new synthetic drugs (www.emcdda.eu.int).

2. History and clinical data of the reported cases

Two men were provided with an Eppendorf capsule filled with white powder containing an unknown substance described as ''a new hallucinogenic LSD-like drug''. In order to test its effects, they shared the entire contents of the capsule consuming it orally. Subsequently, they experienced a rapid onset of intense hallucinations (within 15 min) followed by vomiting and unconsciousness. After an unknown period of time, both men were admitted to the hospital in a comatose state. The first subject (AX) was 28-year-old, weighed 113 kg. He survived but experienced serious convulsions, was restless and had to be treated with strong sedation. The second subject (BX) was 29-year-old and weighed 65 kg. BX was in a deep coma without response to pain, with attacks of generalized convulsions, and metabolic acidosis (pH 6.6). He died after 6 days. Immediately after admission to the hospital, gastric, blood, and urine specimens were collected and sent for toxicological examination with suspicion of intoxication with an unknown drug. Ethanol concentrations in both men's blood samples were not present in significant amount (below 0.2 g/kg). CEDIA urine drug screening indicated the presence of cannabinoids (THCOOH) in both men's urine. Cocaine and its metabolites were only detected in the urine from AX. Neither urine sample gave a response on the CEDIA amphetamines assay. Finally, a GC–MS analysis for unknown drugs disclosed the presence of DOB in gastric and urine samples from both subjects. GC–MS targeted analysis for acetylated DOB confirmed its presence in both persons and quantitative analysis in blood serum was performed.

3. Toxicological methods

3.1. Chemicals

Solvents and other chemicals were of analytical-grade purity. Bond Elut Certify LRC 130 mg columns were from AMEDIS Prague (Varian, USA). DOB.HBr reference standard was purchased from Sigma–Aldrich Prague (Prague, Czech Republic) and the internal standard brompheniramine. HCl was from the State Institute for Drug Control (Prague, Czech Republic).

3.2. Sample preparation procedures

3.2.1. GC–MS screening for unknown drugs in gastric content and urine

The biological fluid (2 ml each) was prepared for GC–MS by SPE on Bond Elut Certify LRC cartridges according to the published procedure [4] with slight modification (2 ml sample instead of 1 ml, modified elution solvent for bases consisted of dichloromethane/isopropanol/ammonia, 80/20/2, v/v/v). Two resulting extracts of acids and bases were evaporated to dryness and dissolved in 100 μl of ethyl acetate, from which 1 μl was analyzed.

3.2.2. GC–MS analysis of bases as acetyl derivatives in urine—confirmation of DOB

Urine (2 ml) was alkalinized (pH 11–12) and extracted with 4 ml ethyl acetate before back-extraction into acid media and re-extraction back into ethyl acetate. Once separated, 3 ml of the organic layer was acetylated with 500 μl acetic anhydride/pyridine (10/1) at 60 °C for 30 min. After cooling, the reaction mixture was evaporated to dryness, dissolved in 100 μl ethyl acetate, and 1 μl was analyzed.

3.2.3. GC–MS search for potential metabolites of DOB in urine

A separate 2 ml urine aliquot was hydrolyzed with 0.5 ml concentrated HCl at 110 °C for 30 min. After cooling, impurities were extracted into ethyl acetate. The aqueous layer was removed and alkalinized to pH 11–12 before extraction of DOB and metabolites with ethyl acetate and acetylation as described above.

3.2.4. GC–MS quantitative assay of DOB in serum

A 1 ml serum sample was made basic with 0.5 ml 1 M NaOH. A methanolic solution of the brompheniramine internal standard (500 ng) was added and the analytes were extracted with 4 ml ethyl acetate. This was followed with a back-extraction into 0.5 ml 1 M HCl and re-extraction into ethyl acetate with acetylation as above. Calibrators were prepared by spiking bovine serum with DOB at 0, 10, 20, 100, 500, and 1000 ng/ml. Validation of the quantitative method demonstrated that the calibration was linear in the range of 0–1000 ng/ml ($r^2 = 0.9970$). The assay precision at

the concentration of 20 ng/ml ($n = 5$) was 7.7% and the limit of detection was lower than 10 ng/ml.

3.3. GC–MS condition

Analysis of the extracts was with a HP6890-5973 GC–MS equipped with a splitless injector, a capillary HP5-MS 30 m × 0.25 mm × 0.25 μm column in standard EI mode. The analyses of extracts of gastric and urine were performed in the full scan mode in the range 35–550 (*m/z*) with 70 eV. The quantitative analyses of serum extracts were performed in SIM mode by monitoring ions for DOB: (44, 86, 162, 256, 258, 315) and for the brompheniramine internal standard: (58, 167, 247, 249). The underlined ions were used for quantification. The GC was temperature programmed for the screening of unknowns in the range 65–300 °C (65 °C held for 1.5 min before a temperature ramp at 20 °C/min to 300 °C and held at 300 °C for 11.75 min) and for the analysis of acetyl derivatives in the range 85–250 °C (85 °C for 2 min then a temperature change at 30 °C/min to 150 °C followed by 15 °C/min to 250 °C and held for 10 min).

4. Results

Fig. 1 shows the TIC chromatograms of gastric and urine samples which were screened by GC–MS for unknown bases. Due to the acceptable concordance of the mass spectrum (*m/z* 44, 77, 105, 230, 232, and 273) and the retention time of the peak detected in the sample and the ones of the reference standard, it was possible to assign the searched toxic agent to DOB. However, the idea to verify the presence of DOB in the samples came from the availability of its spectrum in spectral library databases (Wiley275 and PMW_TOX3).

Fig. 2 demonstrates the confirmation of DOB in one of the urine samples after acetylation. A good match was made based on the combination of retention times and MS characteristics (*m/z* 44, 86, 162, 256, and 315) of suspected substance and the reference standard in the acetylated form.

DOB serum level in the surviving patient (AX) was 13 ng/ml. The concentration in the serum of the deceased patient (BX) was 19 ng/ml. Apparently, the difference in body masses between the two men played a significant role

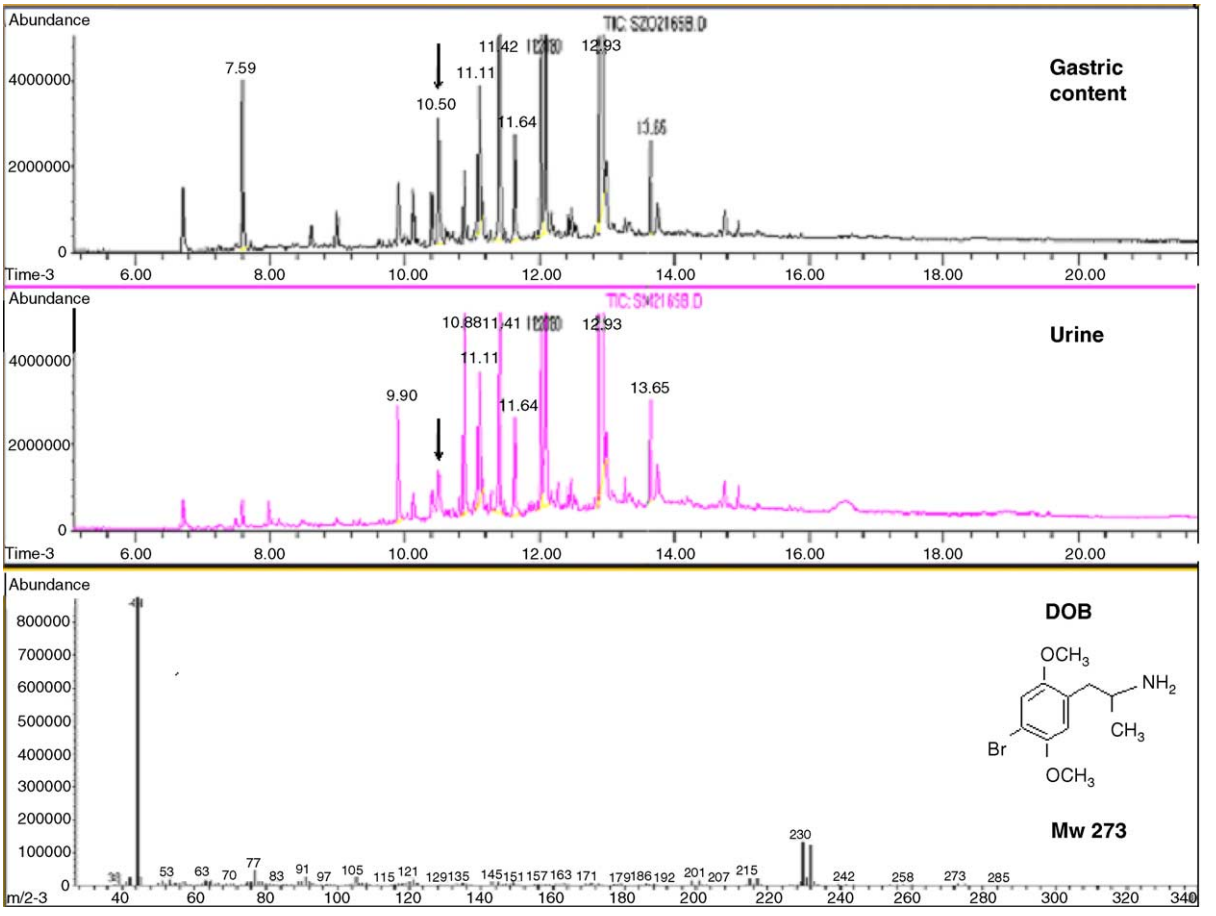


Fig. 1. GC–MS screening for unknown drugs in extracts of bases of gastric content and urine samples.

in the different course and final effects of poisoning. The time elapsed between drug ingestion and blood collection remained unknown. Literature references on the half-life of DOB were not found; therefore, it is difficult to fully evaluate the DOB serum levels in the subjects.

Scientific references to the urinary metabolites of DOB have not been found. Further, no reference standards are commercially available. A preliminary investigation on metabolism of DOB in the examined subjects was performed. A urine sample was hydrolyzed as described and in the hydrolyzed sample, in addition to DOB, another significant peak of a substance containing a bromine atom was detected with these MS characteristics: *m/z* 44, 86, 242, 284, 301, and 343. The ions at *m/z* 242, 284, 301, and 343 in the spectrum have been followed with isotopic ions *m/z* 244, 286, 303, and 345 with the same relative intensities. Therefore, the compound detected appears to be a human metabolite of DOB (Fig. 2). An attempt has been made to compare the GC–MS findings in hydrolyzed and nonhydrolyzed urine samples after acetylation. A comparison of the peak areas in chromatograms extracted for ion 242 is presented in Fig. 3. The free form of the assumed metabolite was less than 1% of the conjugated one excreted in human urine.

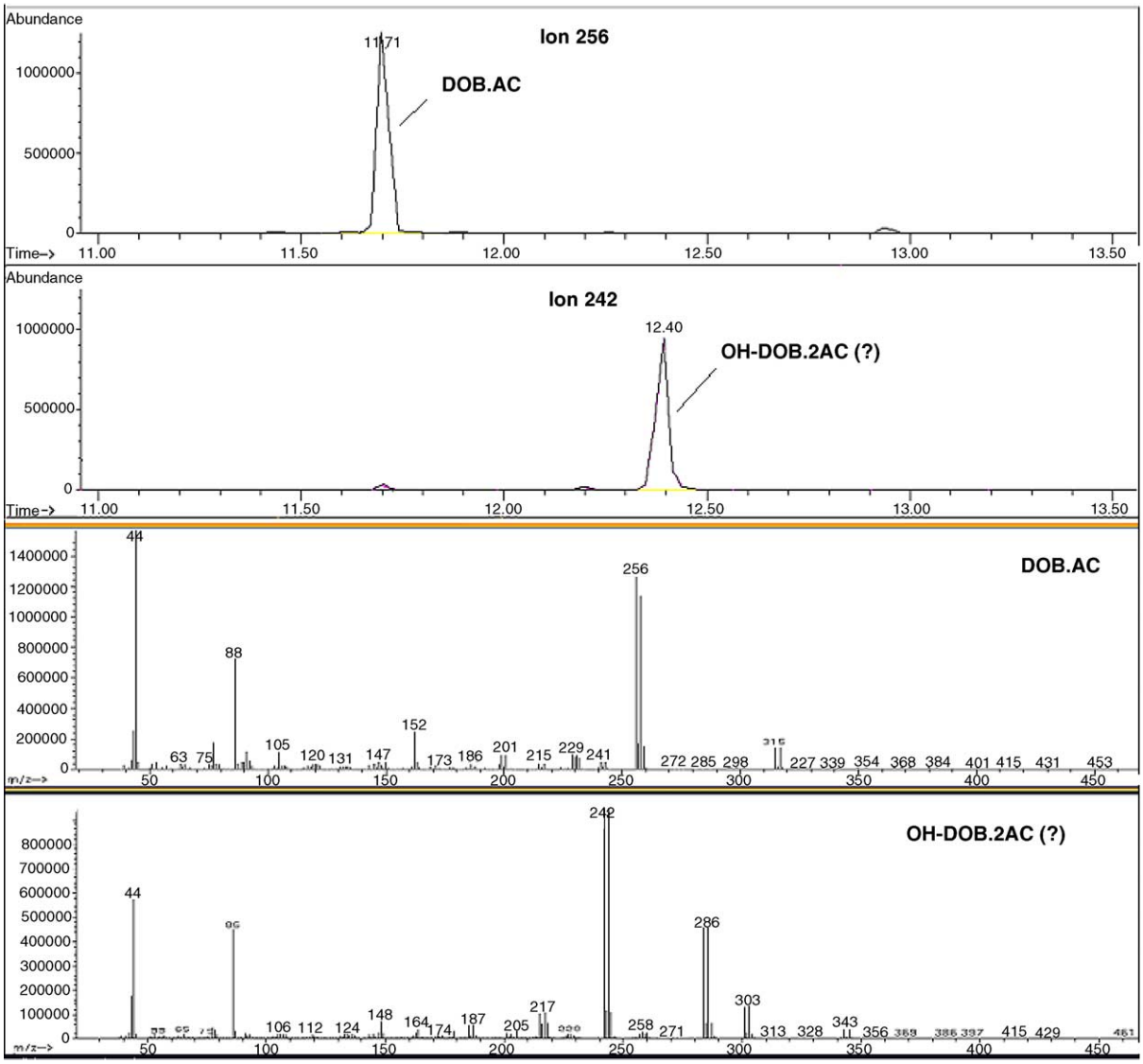


Fig. 2. GC–MS confirmation of suspected DOB in hydrolyzed urine sample after acetylation. Additional peak of a substance with bromine atom was detected as a potential metabolite.

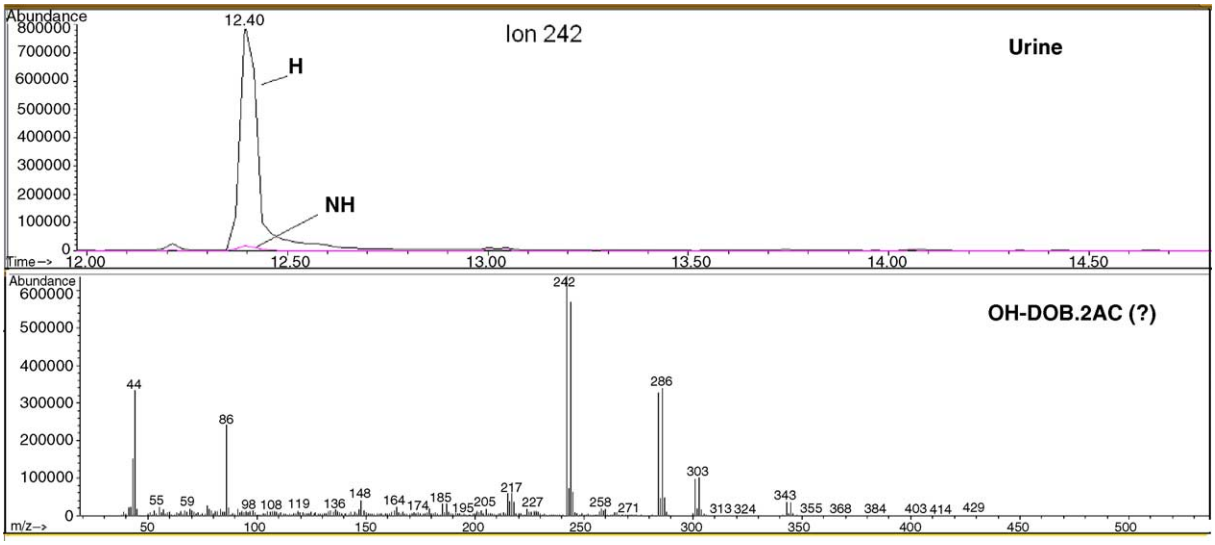


Fig. 3. The presence of assumed phenolic metabolite of DOB in hydrolyzed and nonhydrolyzed urine according to the GC–MS results.

5. Discussion

DOB metabolites have not been identified in animals or humans to our knowledge. Kanamori et al. [5] published a study on the metabolism of the analogous synthetic drug 2,5-dimethoxy-4-bromphenethylamine (2C-B) in rats. Another biotransformation study with 2,5-dimethoxy-4-ethylthiophenethylamine in rats has been published by Lin et al. [6]. In both studies, metabolic pathways are proposed involving excretion of mono-phenolic metabolites into urine created by *O*-demethylation from methoxygroups in position 2 or 5 of the appropriate parent molecule. These phenolic metabolites could be further *N*-acetylated to form the final excreted metabolites.

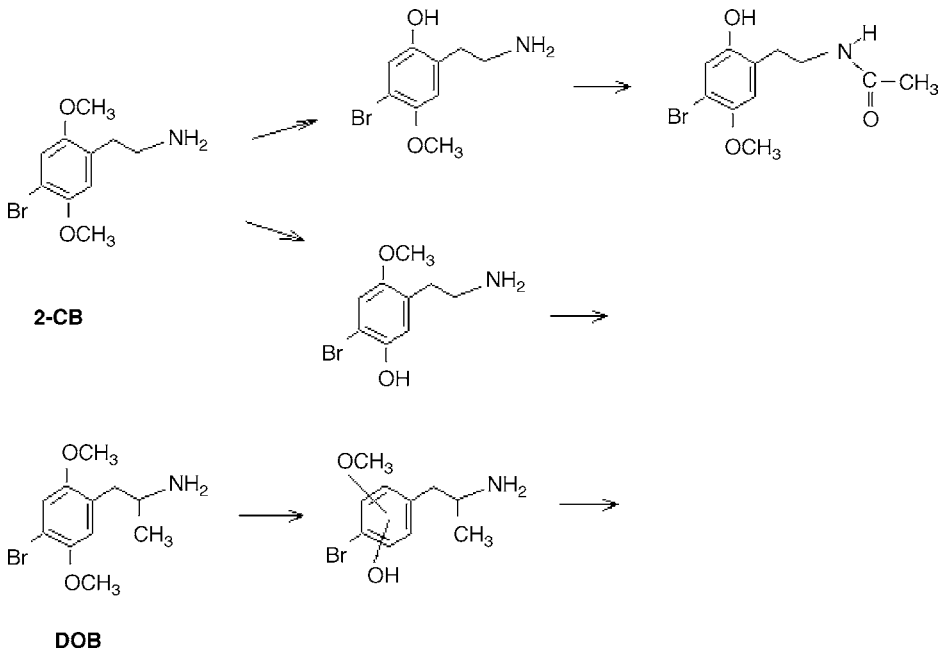


Fig. 4. Proposed partial metabolic pathway of DOB in analogy to the one of 2C-B published by Kanamori et al. [5].

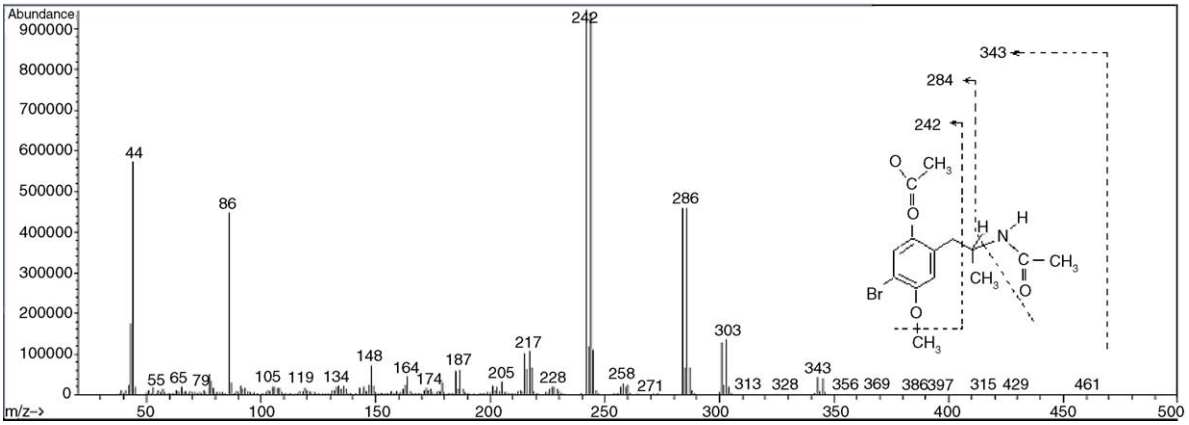


Fig. 5. Possible fragmentation (EI 70 eV) of potential phenolic *O*-desmethylmetabolite of DOB and its mass spectrum.

However, Lin et al. [6] did not identify the phenolic metabolites proposed in experimental rat urine. Kanamori et al. [5] indicated that *O*-desmethyl-*N*-acetyl metabolites of 2C-B are major metabolites excreted into rat urine. It is also known from other studies that the methoxy-substituted phenylalkylamines (*p*-methoxyamphetamine, mescaline, etc.) produce the *O*-desmethylmetabolites by oxidation in the organism [7–9].

Our limited investigation so far does not claim to present the entire extent of biotransformation of DOB in humans. First, by GC–MS itself it is not possible to determine the position of *O*-demethylation of the DOB molecule. Second, the excretion form of the major human metabolite detected could have been in the *N*-acetylated form, which would not have been distinguished using the acetylation method in our sample preparation step. Third, the potential acidic metabolites have not yet been investigated. Nevertheless, from our investigation it can be concluded that one of the assumed phenolic metabolites of DOB has been detected in urine of overdosed persons (Figs. 4 and 5). The mass spectral characteristic of this metabolite is in accordance with possible molecular fragmentation of this compound in standard EI conditions (Fig. 5).

Poisoning with DOB is probably rare and it is not included in a routine differential diagnosis unless there is a special suspicion. In the event described, the early warning system just being built by the initiative of EMCDDA, has proved to attract more toxicological attention to unusual cases of unknown drug overdoses. Various GC–MS screening and confirmation methods in effective combination can solve such a diagnostic task. However, it is of dominant importance and endless need to devote resources to expand the databases of spectral libraries for new drugs, their metabolites, and appropriate chemical derivatives. The certified reference standards of new substances are also needed in this context and biotransformation studies are an integral part of this endeavour. Hopefully, this case report on two cases of DOB overdose can contribute to elucidation of DOB fate in human and to toxicological diagnostics.

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