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New designer drug 4-iodo-2,5-dimethoxy-β-phenethylamine (2C-I): Studies on its metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric and capillary electrophoretic/mass spectrometric techniques†

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Studies are described on the metabolism and the toxicological analysis of the phenethylamine-derived designer drug 4-iodo-2,5-dimethoxy-β-phenethylamine (2C-I) in rat urine using gas chromatographic/mass spectrometric (GC/MS) techniques, and for a particular question, using capillary electrophoretic/mass spectrometric (CE/MS) techniques. The identified metabolites indicated that 2C-I was metabolized on the one hand by *O*-demethylation in position 2 and 5, respectively, followed either by *N*-acetylation or by deamination with subsequent oxidation to the corresponding acid or reduction to the corresponding alcohol, respectively. The latter metabolite was hydroxylated in β-position and further oxidized to the corresponding oxo metabolite. On the other hand, 2C-I was metabolized by deamination with subsequent oxidation to the corresponding acid or reduction to the corresponding alcohol, respectively. 2C-I and most of its metabolites were partially excreted in conjugated form. The authors' systematic toxicological analysis (STA) procedure using full-scan GC/MS after acid hydrolysis, liquid–liquid extraction and microwave-assisted acetylation allowed the detection of an intake of a dose of 2C-I in rat urine that corresponds to a common drug users' dose. Assuming similar metabolism, the described STA procedure should be suitable for proof of an intake of 2C-I in human urine. **Copyright © 2006 John Wiley & Sons, Ltd.**

KEYWORDS: 4-iodo-2,5-dimethoxy-β-phenethylamine; 2C-I; metabolism; GC/MS; CE/MS/MS; urinalysis

INTRODUCTION

A wide variety of β-phenethylamine derivatives are abused as illicit synthetic drugs. β-phenethylamine itself is not a common drug of abuse, because it is rapidly metabolized,[1] but derivatives with methoxy groups in positions 2 and 5 and a hydrophobic 4-substituent, so-called 2Cs, have obviously psychoactive properties and are abused. Typical 2Cs are 4-bromo-2,5-dimethoxy-β-phenethylamine (2C-B), 4-iodo-2,5-dimethoxy-β-phenethylamine (2C-I), 4-ethylthio-2,5-dimethoxy-β-phenethylamine (2C-T-2), or 2,5-dimethoxy-4-propylthio-β-phenethylamine (2C-T-7).[1–4] 2C-I was described in Shulgin's compilation 'PIHKAL' in 1991.[1] Later, it appeared on the illicit drug market as a designer drug in the form of tablets with the 'i' logo, powder, and liquid preparations.[3,5–7] Therefore, it was scheduled in the lists of controlled substances of several countries.[3,8]

Only little information is available on pharmacological and toxicological properties of 2C-I, but it is known that the compounds of the 2C series show affinity to 5-HT_2 receptors, acting as agonists or antagonists at different receptor subtypes.[9–17] For the related substance 2C-B, partial agonism at the α_1-adrenergic receptor was described.[18,19] Because of these properties, radioactive 2C-I was synthesized as a label for the 5-HT_2 receptor and as a potential brain scanning agent for nuclear medicine.[9,20] The chemical structure that is responsible for hallucinogen-like activity comprises a primary amine functionality separated from the phenyl ring by two carbon atoms ('2C'), the presence of methoxy groups in positions 2 and 5 of the aromatic ring, and a hydrophobic 4-substituent (alkyl, halogen, alkylthio, etc.).[21] Furthermore, several quantitative structure-activity relationships (QSAR) studies were published about hallucinogenic β-phenethylamines including 2C-I.[22–29] Using

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the results of these analyses, predictions of the hallucinogenic potency of new β-phenethylamines should be possible.

For some substances of the 2C-series, analytical data are available.[30–44] Screening for and validated quantification of 2C-I and others of the group in human blood plasma have recently been published using gas chromatography/mass spectrometry (GC/MS).[45] However, comprehensive screening for detection of thousands of drugs, poisons and/or their metabolites is performed in urine.[46] For developing such procedures, the analytical data of the metabolites of the compounds in question must be known, because they are the target analytes, if excreted in urine primarily or even exclusively. Furthermore, data on the metabolism are needed for toxicological risk assessment, because the metabolites may play a major role in the toxicity of a drug. Some studies have been published about the metabolism of psychoactive β-phenethylamines,[39,42,47–53] but metabolism of 2C-I has never been described before. Therefore, the aim of the study presented here was to identify the 2C-I metabolites in rat urine using GC/MS in the electron ionization (EI) and positive-ion chemical ionization (PICI) modes. For a particular question, capillary electrophoretic/tandem mass spectrometric techniques (CE/MS/MS) were used owing to their high separation efficiency and orthogonal selectivity with the added benefit of a high tolerance of very complex sample matrices.[54–56] In addition, the detectability of 2C-I and its metabolites within the authors' systematic toxicological analysis (STA) procedure in urine by GC/MS was studied.[57–61]

EXPERIMENTAL

Chemicals and reagents

2C-I HCl was provided by the Landeskriminalamt Baden-Württemberg (Stuttgart, Germany) for research purposes. *N*-Methyl-bis(trifluoroacetamide), ammonium formate and LC/MS-grade 2-propanol/water (50 : 50) were obtained from Fluka (Taufkirchen, Germany). All other chemicals and biochemicals were obtained from Merck (Darmstadt, Germany). All chemicals and biochemicals were of analytical grade.

Urine samples

The investigations were performed using urine of male rats (Wistar, Ch. River, Sulzfleck, Germany) for toxicological diagnostic reasons according to the corresponding German law. They were administered a single 20 mg/kg body mass (BM) dose for metabolism studies or a single 0.3 mg/kg BM dose for the STA study in aqueous suspension by gastric intubation ($n = 2$ for each dose). Urine was collected separately from the feces over a 24 h period. All samples were directly analyzed. Blank rat urine samples were collected before drug administration to check whether they were free of interfering compounds.

Sample preparation for identification of metabolites by GC/MS or CE/MS/MS

A 5-ml portion of urine was worked up as described for 2C-T-7.[39] After enzymatic cleavage of conjugates, the liquid–liquid extract was derivatized by acetylation or trifluoroacetylation, as described for 2C-T-7.[39] Aliquots (2 µl) of the derivatized or underivatized extracts were injected into the GC/MS. Another urine sample was worked up as described in the following. A 1-ml portion of urine was adjusted to pH 5.2 with acetic acid (1 M) and incubated at 50 °C for 1.5 h with 100 µl of a mixture (100 000 Fishman units per ml) of glucuronidase (EC no. 3.2.1.31) and arylsulfatase (EC no. 3.1.6.1). The sample was then diluted with 2 ml of water and loaded on a solid-phase extraction (SPE) cartridge (Isolute Confirm HCX, 130 mg, 3 ml), previously conditioned with 1 ml of methanol and 1 ml of water. After passage of the sample, the cartridge was conditioned with 1 ml of water and 1 ml of 0.01 M hydrochloric acid. The retained nonbasic compounds were eluted into a 1.5-ml reaction vial with 1 ml of methanol and gently evaporated under a stream of nitrogen at 56 °C. After evaporation, the residue was dissolved in 50 µl of methanol and derivatized for 10 min at room temperature with 100 µl of a solution of diazomethane in diethyl ether. After evaporation to dryness, the sample was derivatized by trifluoroacetylation as described for 2C-T-7[39] and finally redissolved in 50 µl of ethyl acetate. Again, 2 µl were 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. For CE/MS/MS analysis, 10 µl of the rat urine SPE extract derivatized by methylation and trifluoroacetylation were evaporated to dryness and redissolved with 20 µl of isopropanol/run buffer (1 : 1). A 1 µl aliquot of this mixture was diluted to 20 µl with run buffer. The run buffer consisted of ammonium formate (0.02 mol/l), adjusted to pH 10.0 with ammonia and containing 25% (v/v) isopropanol.

Sample preparation for STA by GC/MS

A 5-ml portion of urine was worked up as described for 2C-T-7.[39] After cleavage of conjugates by acid hydrolysis, the liquid–liquid extract was derivatized by acetylation. Aliquots (2 µl) of the derivatized extracts were injected into the GC/MS.

GC/MS apparatus for identification of metabolites

The extracts were analyzed using a Hewlett Packard (Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph combined with an HP 5989B MS Engine mass spectrometer and an HP MS ChemStation (DOS series) with HP G1034C software version C03.00. 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 100–310 °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.

GC/MS apparatus for STA

The extracts were analyzed using a Hewlett Packard (Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph

combined with a HP 5972A MSD mass spectrometer and a HP MS ChemStation (DOS series) with HP G1034C software version C03.00. 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 100–310 °C at 30°/min, initial time 3 min, final time 8 min. The MS conditions were as follows: full-scan mode, m/z 50–550 u; EI mode, ionization energy, 70 eV; ion source temperature, 220 °C; capillary direct interface, heated at 280 °C.

GC/MS procedure for recording the mass spectra of 2C-I and its GC artifacts formed in methanolic solution

A solution of 0.1 mg/ml 2C-I in ethyl acetate, methanol or methanol-d_4 was prepared and 2 µl of each solution were injected into the GC/MSD in the EI full-scan mode.

GC/MS procedure for identification of metabolites and for STA by GC/MS

2C-I and its metabolites were separated by GC and identified by MS in the corresponding urine extracts. For STA, mass chromatography was used extracting characteristic fragment ions from the total ion current. The following ions were used for this purpose: m/z 290, 349, 276, and 335. They were selected from the mass spectra recorded during this study. The identity of the peaks in the mass chromatograms was confirmed by computerized comparison[62] of the mass spectra underlying the peaks (after background subtraction) with reference spectra recorded during this study.

CE/MS/MS apparatus and procedure for identification of metabolites

The sample was introduced into a Beckman-Coulter P/ACE 5510 system by hydrodynamic injection with 3.45 kPa for 6 s. Separation was performed in a bare 75 µm I.D. (363 µm O.D.) fused silica capillary from Polymicro Technologies LLC (Phoenix, AZ, USA) with a length of 90 cm. For separation, the capillary inlet was put on a voltage of +30 kV, keeping the sprayer on the ground. For data acquisition the software Beckman P/ACE Station 1.2 was used. The CE was connected to an HCTplus ion-trap mass spectrometer (Bruker Daltonik, Bremen, Germany) via the Agilent coaxial sheath-liquid sprayer interface (Agilent Technologies, Palo Alto, CA, USA). Electrospray ionization (ESI) was performed at 4500 V. The sheath-liquid isopropanol : water (1 : 1) was adjusted to pH 11.3 with ammonia and supplied at a flow rate of 2 µl/min by a syringe pump (Cole-Parmer, Vernon Hill, IL, USA). Nebulizer gas pressure was set to 5 p.s.i. Flow and temperature of the dry gas (nitrogen) were 4.0 l/min and 250 °C. ESI-MS spectra were obtained in the negative-ion mode with a scan speed of 26 000 m/z per second in the mass range m/z 50–600 in scanning mode and automatic data-dependent switching between MS and MS/MS mode. Auto-MS/MS experiments were performed by isolation and subsequent fragmentation with ramped fragmentation amplitude. Postprocessing software DataAnalysis (version 3.2, Bruker Daltonik) was used for data processing.

RESULTS AND DISCUSSION

Mass spectrum of 2C-I

The mass spectrum of 2C-I recorded in methanolic solution differed markedly from that recorded in ethyl acetate solution, as already described for 2C-T-7 and 2C-T-2.[39,42] In analogy to them, the mass spectrum of 2C-I dissolved in ethyl acetate showed a molecular ion of m/z 307 corresponding to the molecular mass of 2C-I (mass spectrum no. 1 in Fig. 1) and fragment ions that were interpreted as depicted. In methanolic solution, the mass spectrum (no. 2 in Fig. 1) corresponded to a methylene artifact, which was in accordance with that of the deuteromethylene artifact recorded in a solution prepared with methanol-d_4 (no. 3 in Fig. 1). Further discussion was conducted in Refs 39,42.

Identification of the metabolites

The urinary metabolites of 2C-I were separated by GC and identified by EI MS and PICI MS after gentle enzymatic hydrolysis, extraction, acetylation, trifluoroacetylation, methylation plus trifluoroacetylation, or without derivatization. Acetylation was chosen as main derivatization procedure, because of the authors' experiences it is considered as a versatile method for elucidation of the structures of metabolites.[39,42,57,59,63–68] Furthermore, acetylation is the standard derivatization procedure in the authors' STA, so for STA it was necessary to record the spectra of the acetylated metabolites. However, using acetylation as derivatization procedure, physiologically *N*-acetylated metabolites cannot be differentiated from acetyl derivatives. For this particular question, the presence of *N*-acetylated metabolites was confirmed in urine extracts without derivatization and after trifluoroacetylation. For detection of acidic metabolites, the urine samples were extracted by SPE, after enzymatic cleavage of conjugates, followed by methylation and trifluoroacetylation. SPE was chosen as extraction procedure of acidic metabolites, because it led to cleaner extracts than the LLE procedure used for similar metabolism studies in the authors' laboratory in the past.

The postulated structures of the (derivatized) metabolites were deduced from the fragments detected in the EI mode, which were interpreted in correlation to those of the parent compound according to the rules described by e.g. McLafferty and Turecek[69] and Smith and Busch.[70] To verify the molecular mass of the postulated metabolites, PICI mass spectra were recorded, because they contain abundant peaks of the protonated molecule $[M + H]^+$ with adduct ions typical for PICI using methane as reagent gas $[M + C_2H_5^+, M + C_3H_5^+]$. The EI and PICI mass spectra, the retention indices (RI), the structures and predominant fragmentation patterns of 2C-I and its metabolites after derivatization are shown in Fig. 2. In the acetylated urine extract, the following compounds could be identified. The numbers of EI and PICI spectra in Fig. 2 are given in brackets: *N*-acetyl-2C-I (1), *N*-acetyl-acetoxy-4-iodo-methoxy-β-phenethylamine isomer 1 (2), *N*-acetyl-acetoxy-4-iodo-methoxy-β-phenethylamine isomer 2 (3), 2-(4-iodo-2,5-dimethoxyphenyl)ethyl acetate

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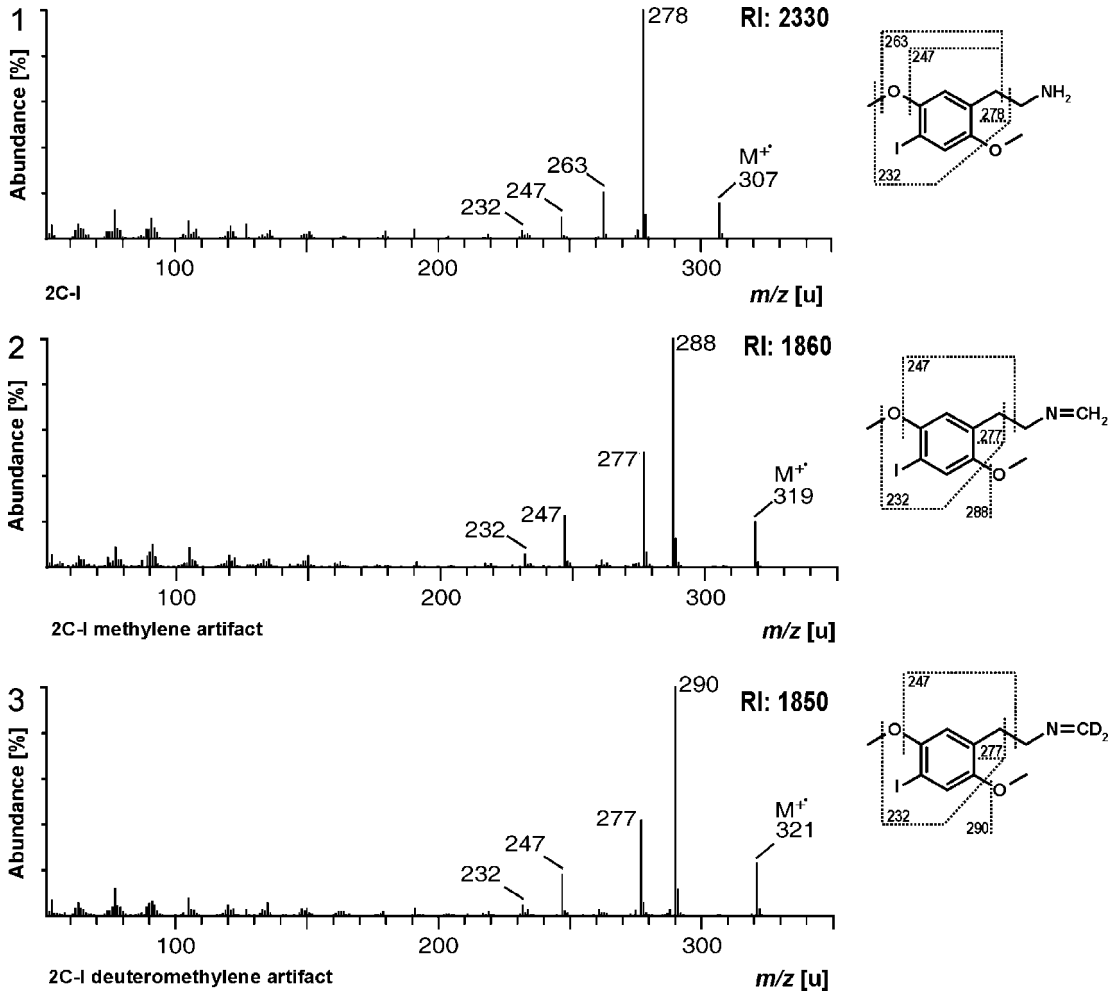

Figure 1. EI mass spectra, RIs, structures and predominant fragmentation patterns of 2C-I, its methylene and deuteromethylene artifacts.

(4), 2-(acetoxy-4-iodo-methoxyphenyl)ethyl acetate isomer 1 (5), 2-(acetoxy-4-iodo-methoxyphenyl)ethyl acetate isomer 2 (6), 2-acetoxy-2-(acetoxy-4-iodo-methoxyphenyl)ethyl acetate (7), and 2-(acetoxy-4-iodo-methoxyphenyl)-2-oxo-ethyl acetate (8).

For confirmation whether the *N*-acetyl derivatives were formed by metabolism or by derivatization, the urine extracts were analyzed without derivatization or after trifluoroacetylation. The following metabolites in question could be identified in an underivatized urine extract: *N*-acetyl-hydroxy-4-iodo-methoxy-β-phenethylamine isomer 1 (9), and *N*-acetyl-hydroxy-4-iodo-methoxy-β-phenethylamine isomer 2 (10) showing that both metabolites were excreted as *N*-acetyl conjugate. In the trifluoroacetylated urine extract, the following compounds could be identified: *N*-trifluoroacetyl-2C-I (11), *N*-trifluoroacetyl-trifluoroacetoxy-4-iodo-methoxy-β-phenethylamine isomer 1 (12), and *N*-trifluoroacetyl-trifluoroacetoxy-4-iodo-methoxy-β-phenethylamine isomer 2 (13). After enzymatic hydrolysis of urine, SPE followed by methylation and trifluoroacetylation, methyl 4-iodo-2,5-dimethoxy-β-phenyl acetate (14), methyl trifluoroacetoxy-4-iodo-methoxy-β-phenyl acetate (15) and 6-iodo-5-methoxy-1-benzofuran-2(3H)-one (16) could be identified in addition.

Proposed fragmentation patterns

N-acetyl-2C-I (1) showed a fragmentation pattern that was characteristic also for most of the detected metabolites. Loss of acetamide may lead to a fragment ion of *m/z* 290, additional loss of one methyl group may lead to a fragment ion of *m/z* 275. Benzyl cleavage may lead to fragment ion *m/z* 277 and additional loss of the methyl moieties of the two-methoxy groups may lead to ion *m/z* 247. Alternatively, the fragment with *m/z* 247 might also be explained by benzyl cleavage and neutral loss of CH_2O from one methoxy group. *N*-acetyl-acetoxy-4-iodo-methoxy-β-phenethylamine isomer 1 (2) showed a fragment ion of *m/z* 335, which may be explained by the loss of the acetyl moiety, additional loss of acetamide may form ion *m/z* 276. Benzyl cleavage and loss of the acetyl moiety may lead to fragment ion *m/z* 263 and additional loss of CH_2O from the methoxy group may produce *m/z* 233. The exact position of the metabolically formed hydroxy group could not be determined by means of GC/MS. This is indicated by the wavy binding lines in Fig. 2. However, since two isomers of this compound could be detected, it can be concluded that both methoxy groups are demethylated. *N*-acetyl-acetoxy-4-iodo-methoxy-β-phenethylamine isomer 2 (3) showed mass spectra similar to the other isomer. 2-(4-iodo-2,5-dimethoxyphenyl)ethyl

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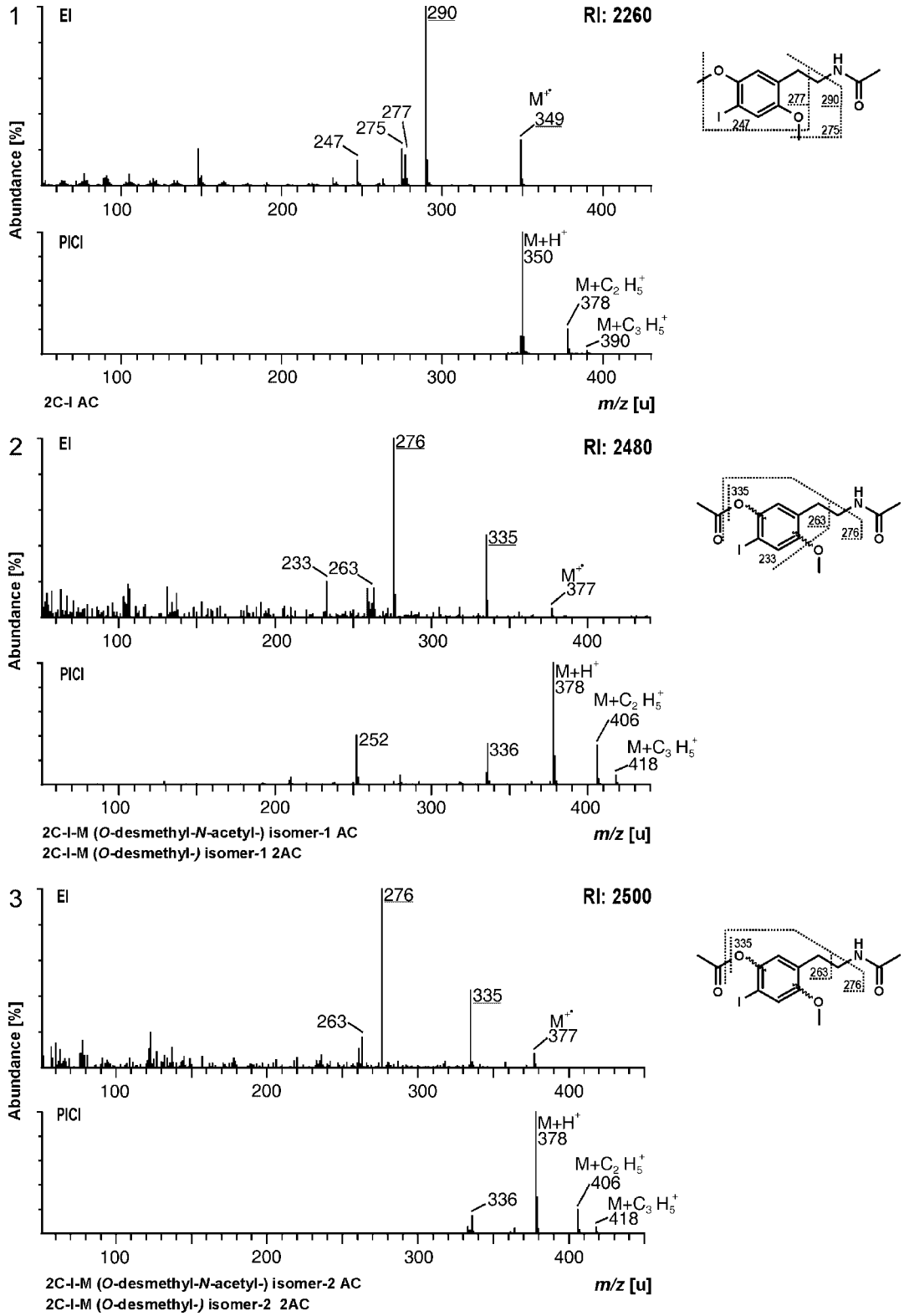

Figure 2. EI and PICI mass spectra, RIs, structures and predominant fragmentation patterns of 2C-I and its metabolites after acetylation, trifluoroacetylation, methylation and trifluoroacetylation, or underivatized. The numbers of the spectra correspond to those of the structures shown in Fig. 5. Ions selected for toxicological detection are underlined.

acetate (4) showed a molecular ion of m/z 350. Loss of acetic acid may lead to a fragment ion of m/z 290, additional loss of one methyl group may lead to a fragment ion of m/z 275. Benzyl cleavage may lead to fragment ion m/z 277 and additional loss of the methyl moieties of the two-methoxy groups may lead to ion m/z 247. Again, the fragment with m/z 247 might also be explained alternatively by benzyl cleavage and neutral loss of CH_2O from one methoxy group. 2-(acetoxy-4-iodo-methoxyphenyl)ethyl acetate isomer 1 (5) showed a molecular ion of m/z 378, loss

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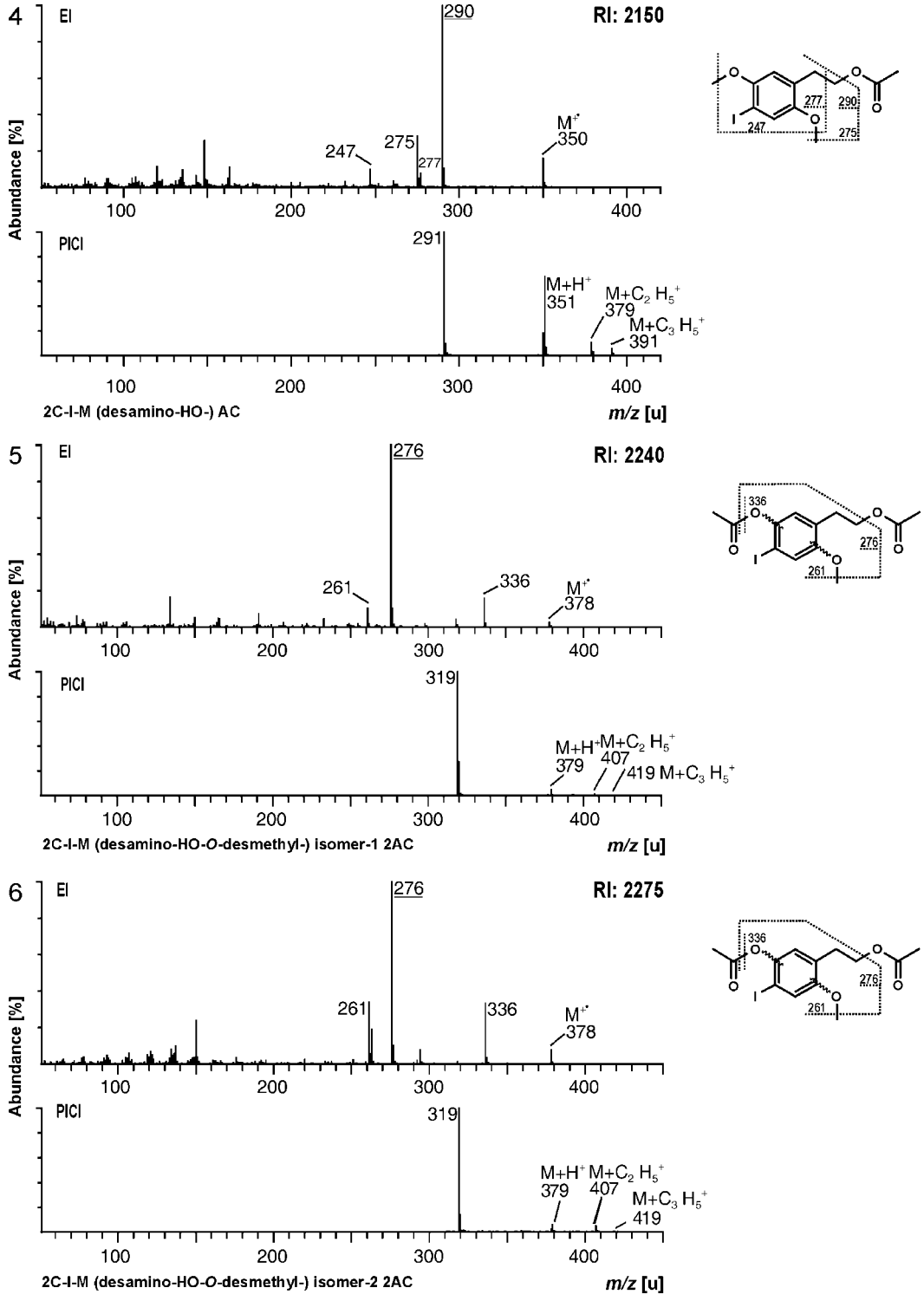

Figure 2. (*Continued*).

of the acetyl moiety may lead to fragment ion *m/z* 336. Additional loss of acetic acid may form *m/z* 276, further loss of one methyl group may lead to fragment ion *m/z* 261. 2-(acetoxy-4-iodo-methoxyphenyl)ethyl acetate isomer 2 (6) showed mass spectra similar to the other isomer. Again, the exact position of the metabolically formed hydroxy group could not be determined by means of GC/MS, but since two isomers of this compound could be detected it can be concluded that both methoxy groups are demethylated.

2-Acetoxy-2-(acetoxy-4-iodo-methoxyphenyl)ethylacetate (7) showed a molecular peak of *m/z* 436. Loss of one acetyl moiety may lead to fragment ion *m/z* 394, further loss of acetic acid may lead to fragment ion *m/z* 334. Further loss of another acetyl moiety may form *m/z* 292. Unfortunately, no PICI spectra of this compound could be recorded, probably due to its low concentration in the sample. 2-(acetoxy-4-iodo-methoxyphenyl)-2-oxoethyl acetate (8) showed a molecular ion of *m/z* 392, loss of one acetyl moiety might lead to fragment ion *m/z* 350, further

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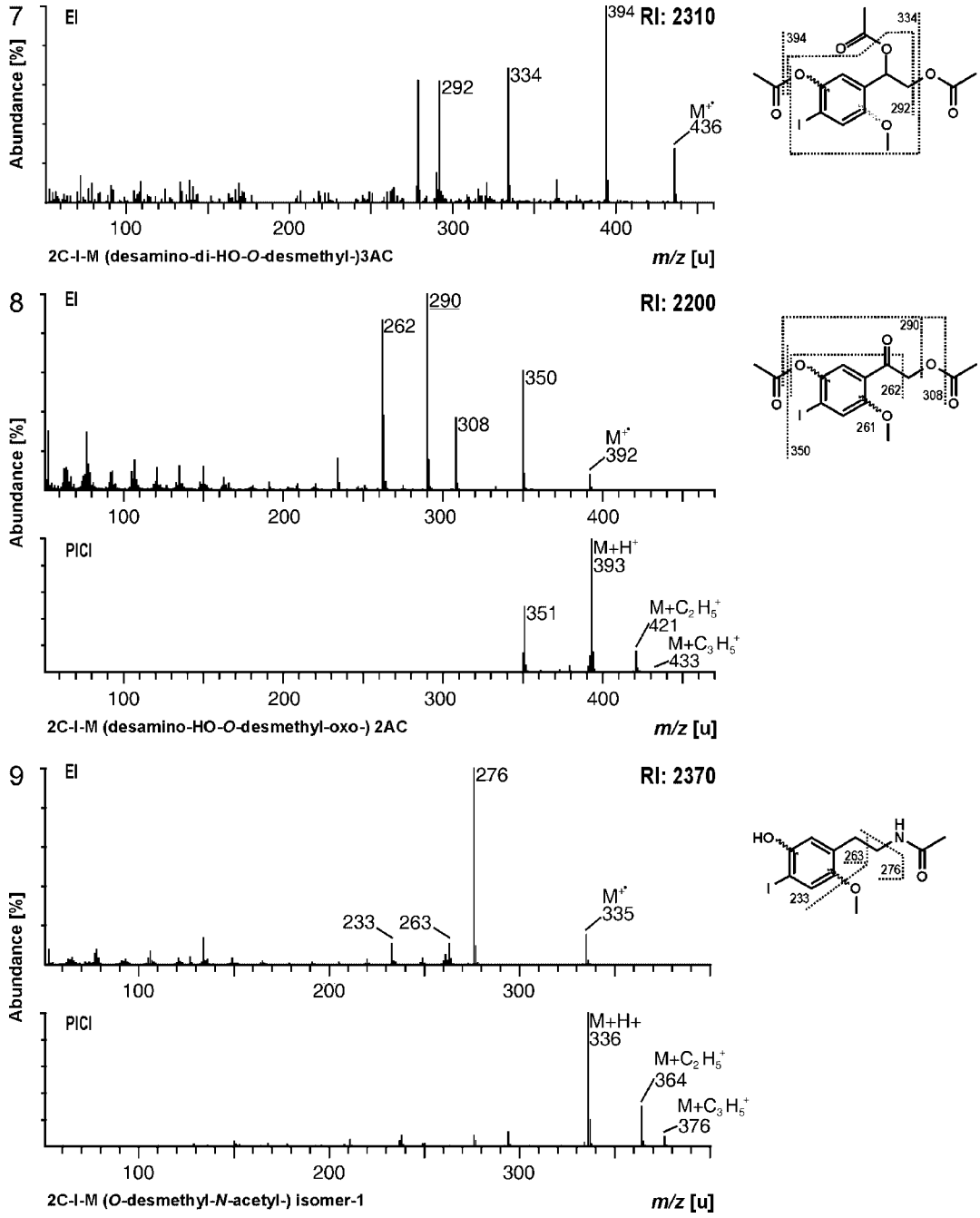

Figure 2. (*Continued*).

loss of another acetyl moiety to *m*/*z* 308. Loss of one acetyl moiety and of acetic acid may form *m*/*z* 290, loss of the acetyl moiety and cleavage in benzylic position with loss of the oxygen moiety may produce *m*/*z* 262. Another hint for the correctness of the deduced structures of the latter two metabolites was the authors' metabolism study with the related halogen compound 2C-B. In this study similar to the presented one here, the corresponding metabolites to 2C-I were detected with corresponding fragmentation patterns.[71] The two *N*-acetyl conjugates (9 and 10) showed fragmentation patterns similar to the corresponding derivatized *O*-demethyl-*N*-acetyl compounds (2 and 3) with benzyl cleavage and loss of acetamide. *N*-trifluoroacetyl-2C-I (11) showed a fragmentation pattern that was similar to that one of the acetylated parent compound (1). *N*-trifluoroacetyl-trifluoroacetoxy-4-iodo-methoxy-*β*-phenethyl amine isomer 1 (12) showed a fragment ion *m*/*z* of 372, which may be explained by loss of trifluoroacetamide, additional loss of one trifluoromethyl moiety may lead to fragment ion *m*/*z* 303. Furthermore, benzyl cleavage may lead to fragment ion *m*/*z* 359. *N*-trifluoroacetyl-trifluoroacetoxy-4-iodo-methoxy-*β*-phenethylamine isomer 2 (13) showed mass spectra similar to the other isomer. Methyl 4-iodo-2,5-dimethoxy-*β*-phenylacetate (14) showed a molecular ion of *m*/*z* 336. Loss of one methyl group may lead to *m*/*z* 321. Benzyl cleavage may produce fragment ion *m*/*z* 277 and additional loss of two methyl moieties may form fragment ion *m*/*z* 247. Alternatively, the fragment with *m*/*z* 247 might also be explained by

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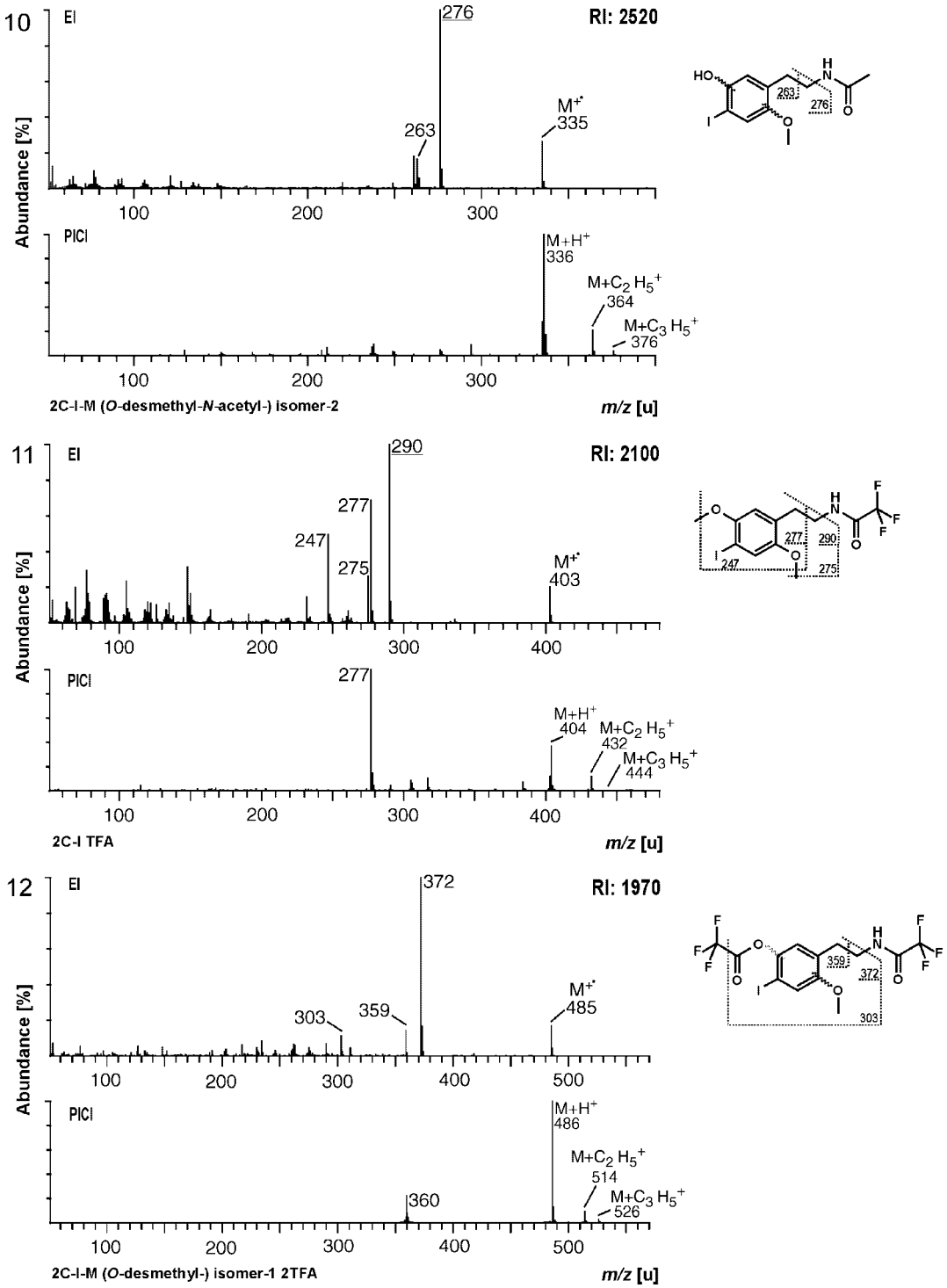

Figure 2. (*Continued*).

benzyl cleavage, and neutral loss of CH_2O from one methoxy group.

Methyl trifluoroacetoxy-4-iodo-methoxy-β-phenylacetate (15) showed a molecular ion of m/z 418. Benzyl cleavage may produce fragment ion m/z 359, additional loss of the trifluoromethyl moiety may form fragment ion m/z 289. Furthermore, loss of the trifluoroacetyl moiety may produce fragment ion m/z 321. Loss of trifluoroacetic acid may lead to fragment ion m/z 305. Only one isomer of this derivative could be detected, but the detected lactone (16) could be formed probably during GC by dehydration of the other isomer ((2-hydroxy-4-iodo-5-methoxyphenyl)acetic acid). This lactone (16) showed a molecular ion of m/z 290, loss of carbon monoxide may lead to fragment ion m/z 262, loss of the furanone ring may produce fragment ion m/z 234. A further hint for a ring structure is the fact that the molecular ion is the base peak. The question arose, why (2-hydroxy-4-iodo-5-methoxyphenyl)acetic acid was not derivatized in contrast to the other isomer. One of the most probable reasons could be formation of an intramolecular hydrogen bond between the 2-hydroxy group and the carboxy group. If this assumption was correct, the free acid should be detectable by

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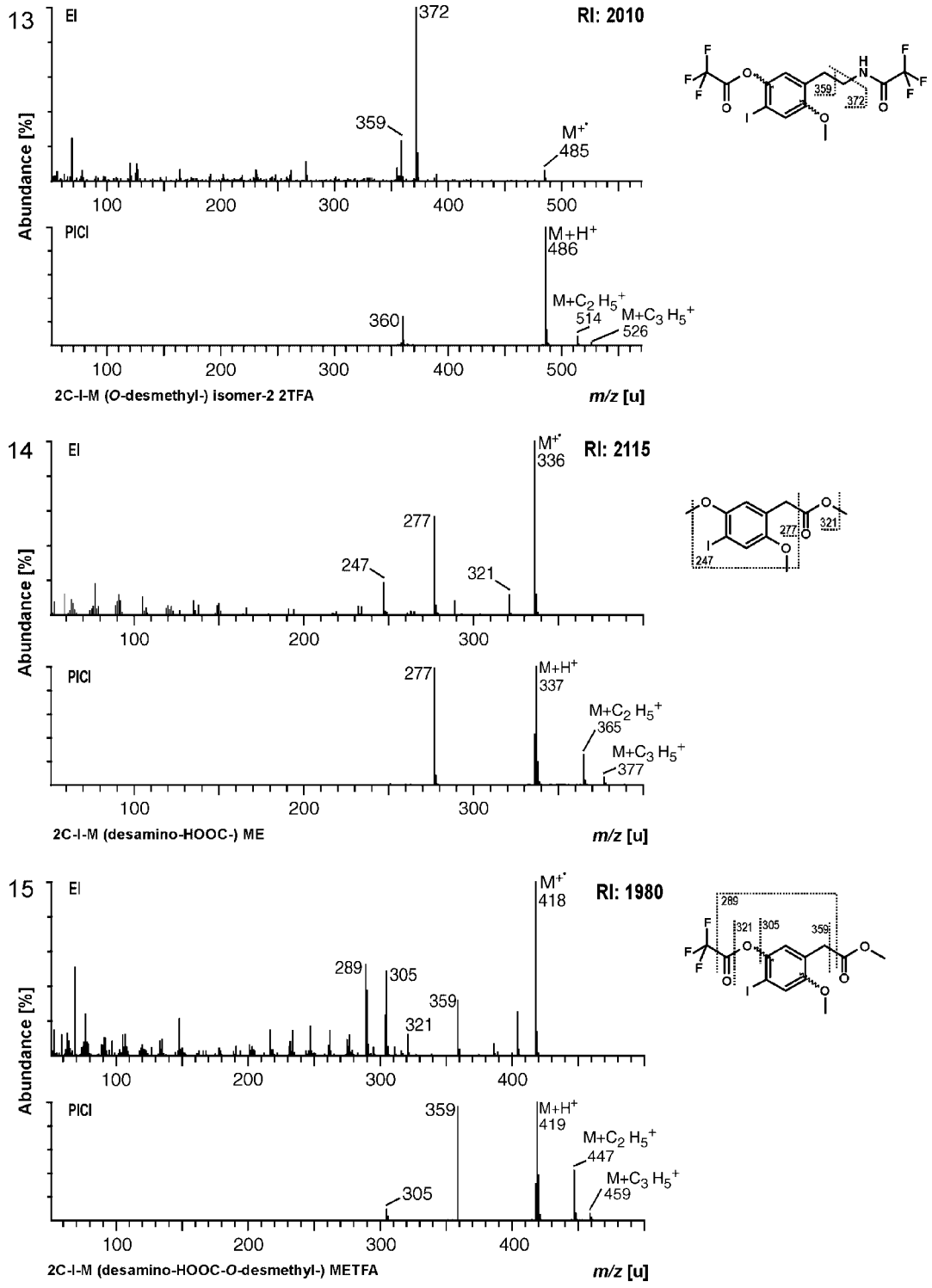

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an alternative analytical method without the extreme conditions of the GC injection port. Capillary electrophoretic/mass spectrometry (CE/MS) was considered to be such an appropriate method, under which conditions, the above described loss of water should not occur, and therefore, (2-hydroxy-4-iodo-5-methoxyphenyl)acetic acid should be detected, whereas the lactone should not be detectable. The pronounced suitability of capillary electrophoresis for separation of positively or negatively charged analyte ions ideally matches the high flexibility of modern ESI ion-trap MS instruments in both polarity modes that even permit the alternating detection of positively and negatively charged molecule ions within a single run. Together with the mild ESI conditions, a coelectroosmotic CE procedure with a high pH run buffer and ESI-MS in negative-ion mode permits the direct analysis and identification of unstable free carboxylic acid metabolites. Their anions migrate against the direction of the electroosmotic flow (EOF) and are, thus, separated from the big surplus of uncharged metabolites. The detected $[M - H]^-$ deprotonated molecules can be identified by subsequent

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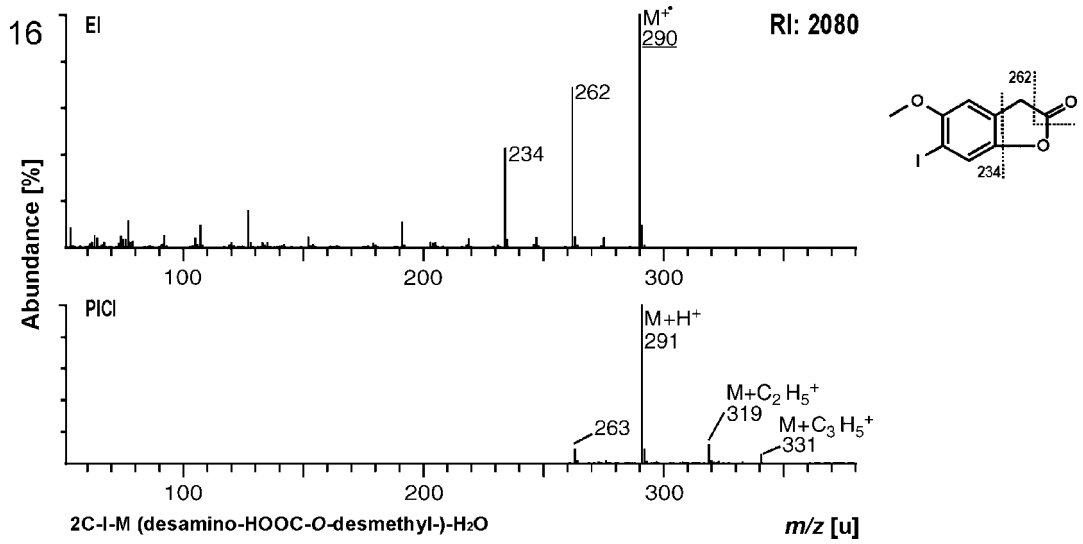

Figure 2. (*Continued*).

fragmentation in the auto-MS/MS mode. Figure 3 shows the extracted ion electropherograms (EIE, m/z 307) indicating the anion of (hydroxy-4-iodo-methoxyphenyl)acetic acid and the EIE (m/z 334) indicating the deprotonated molecule ion of the two isomers of *N*-acetyl-hydroxy-4-iodo-methoxy-β-phenethylamine, uncharged comigrating with the EOF. Figure 4 shows the corresponding MS/MS spectra (negative ion and neutral loss) for the parent ion m/z 307. The fragment anions correspond to decarboxylation (m/z 263) or loss of acetic acid (m/z 248) and to the cleaved iodide anion (m/z 127). Together with the migration time being typical for a monocharged aryl carboxylic acid, the fragmentation pattern and the molecule anion mass proved the presence of the questioned 2-*O*-demethyl deamino carboxy metabolite, which could not be derivatized as discussed before.

Proposed metabolic pathways

On the basis of these identified compounds, the following metabolic pathways, shown in Fig. 5, could be postulated: *O*-demethylation of the parent compound in position 2 and 5 of the aromatic ring, respectively, followed either by partial glucuronidation/sulfation or by *N*-acetylation with subsequent partial glucuronidation/sulfation or by deamination to the corresponding aldehyde, which was not detected, followed by oxidation to the corresponding acid or reduction to the corresponding alcohol, followed by partial glucuronidation/sulfation. The latter alcohol was further hydroxylated in β-position and further oxidized to the corresponding oxo metabolite.

Another metabolic pathway was the deamination of the parent compound to the corresponding aldehyde, which was not detected, followed by oxidation to the corresponding acid or reduction to the corresponding alcohol with subsequent partial glucuronidation/sulfation. Such conjugation was concluded because the peak areas of these metabolites were more abundant after glucuronidase and sulfatase hydrolysis. In the same way, *N*-glucuronidation of 2C-I parent compound was concluded.

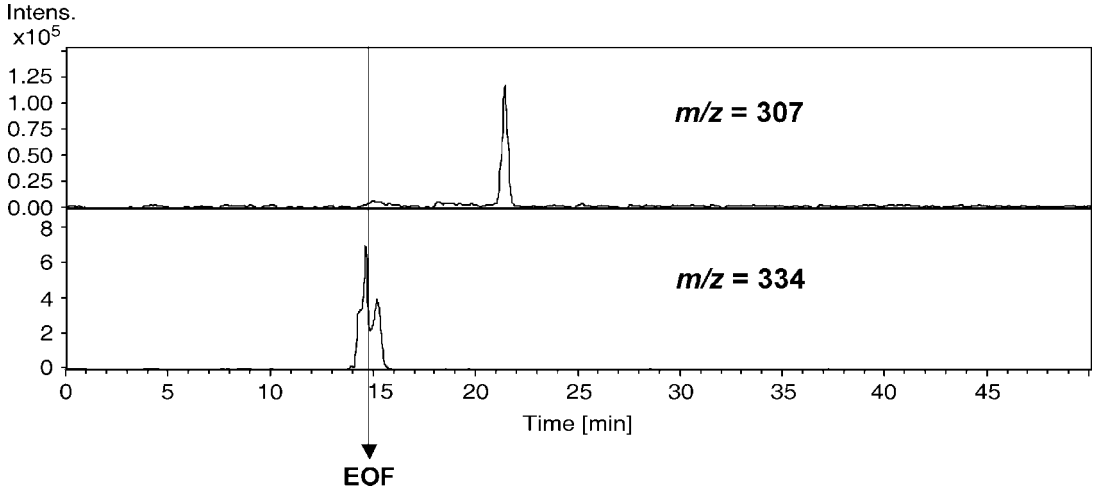

Figure 3. Extracted ion electropherograms (deprotonated molecule ions) of a CE/MS/MS analysis of a trifluoroacetylated rat urine extract. EIE (m/z 307) indicating the anion of an *O*-demethyl deamino carboxy metabolite (upper part). EIE (m/z 334) indicating the deprotonated molecule ion of the two isomeric *N*-acetyl *O*-demethyl metabolites, uncharged comigrating with the EOF (lower part).

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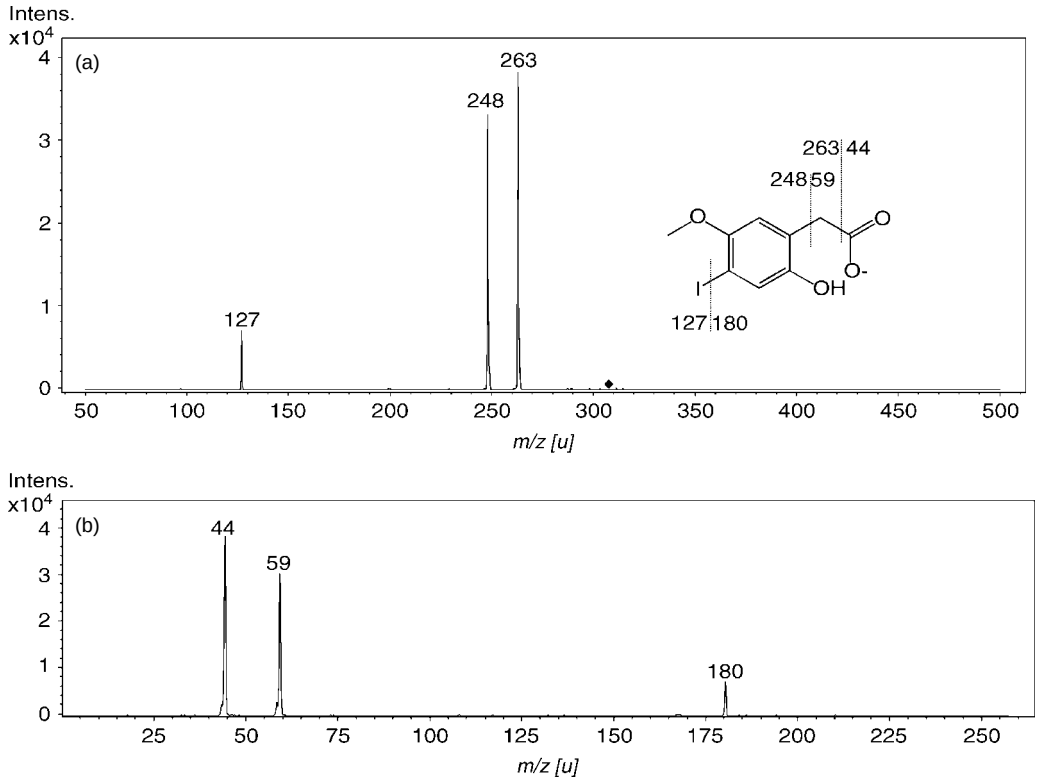

Figure 4. MS/MS spectra (negative ion (a) and neutral loss (b)) for the parent ion m/z 307 (underlying the peak at a migration time of 21.6 min in Fig. 3) from an auto-MS/MS CE analysis of a rat urine extract.

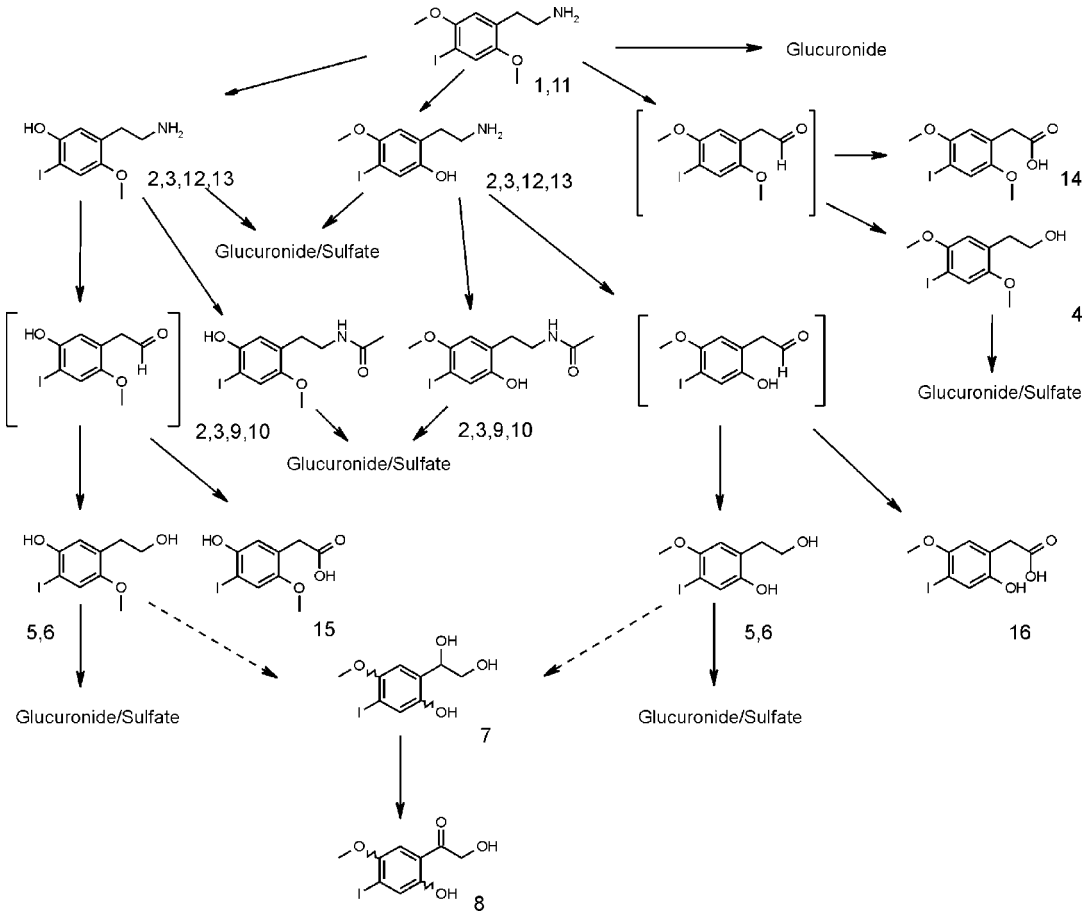

Figure 5. Proposed scheme for the metabolism of 2C-I in rats. The compounds in brackets are assumed intermediates. The numbers of the compounds correspond to those of the mass spectra in Fig. 2.

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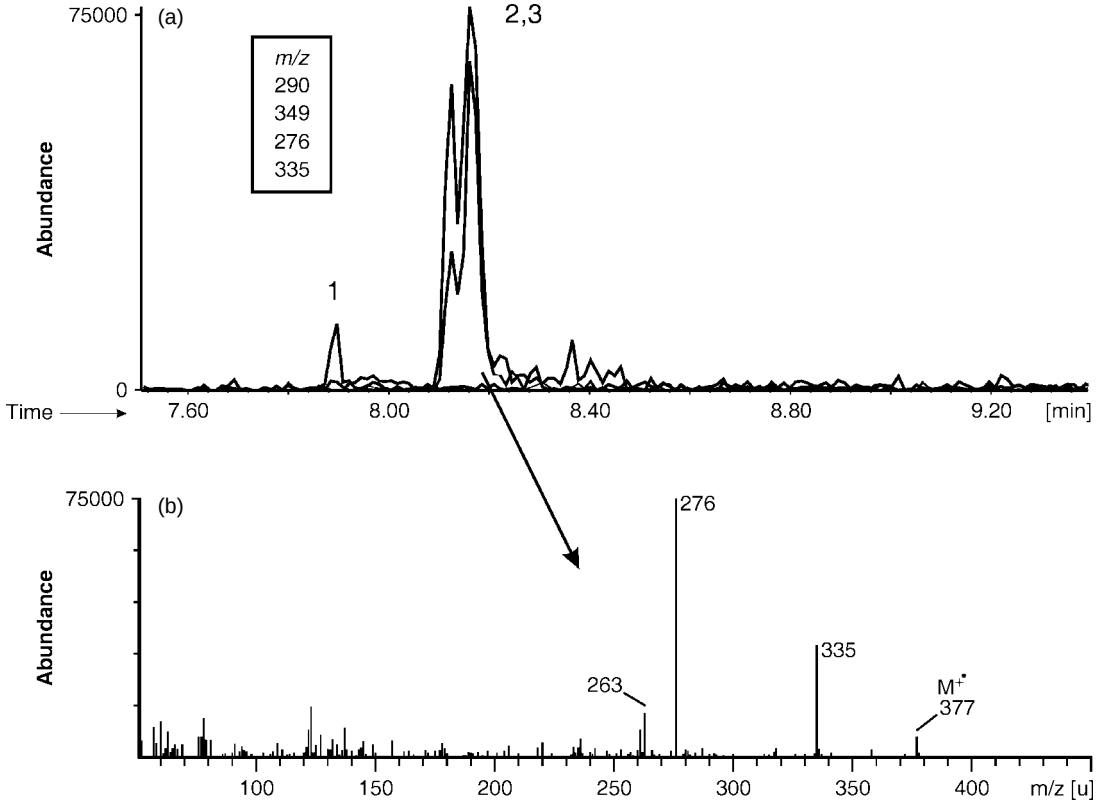

Figure 6. Typical reconstructed mass chromatograms with the given ions of an acetylated extract of a rat urine sample collected over 24 h after intake of 0.3 mg/kg BM of 2C-I (a). They indicate the presence of 2C-I and its metabolites. Mass spectrum underlying the peak at retention time 8.16 min (b). The peak numbers correspond to those of the mass spectra in Fig. 2.

The above mentioned *O*-demethylation has also been observed for the related compounds 2C-B,[48,51,71] 2C-T-2,[42,49] *para*-methoxyamphetamine (PMA),[72,73] or *para*-methoxymethamphetamine (PMMA).[59,73,74] *N*-Acetylation seems to be a common metabolic step for β-phenethylamines and was described, e.g. for 2C-T-2,[42,49] 2C-T-7,[39] for 2C-B,[48,71] or for mescaline.[75] The metabolic pathways of 2C-I in rats described here are similar to those of the related halogen 2C compound 2C-B in rats or mice.[48,51,71]

Detection by GC/MS within the STA

2C-I and its metabolites were separated by GC and identified by full-scan MS. Mass chromatography with the ions m/z 290, 349, 276, and 335 was used to indicate the presence of 2C-I and its main metabolites. Figure 6 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-I that corresponded to a common users' dose of about 20 mg. The identity of peaks in the mass chromatograms was confirmed by computerized comparison of the underlying mass spectrum with the reference spectra.[62] The selected ions m/z 290 and 349 were used for indication of acetylated 2C-I itself, the ions m/z 276 and 335 were used for indicating the presence of *N*-acetyl-2-acetoxy-5-methoxy-β-phenethylamine as well as *N*-acetyl-5-acetoxy-2-methoxy-β-phenethylamine. The ions m/z 290 and 276 are characteristic fragments resulting from loss of acetamide, ion m/z 335 results from loss of the acetyl moiety. Although interferences by biomolecules or further drugs cannot be entirely excluded, they are rather unlikely, because their mass spectra and/or their RIs should be different. The RIs were recorded during the GC/MS procedure and calculated in correlation with the Kovats' indices[76] of the components of a standard solution of typical drugs which is measured daily for testing the GC/MS performance.[77] The extraction efficacy determined for 2C-I after STA working-up was $60 \pm 17\%$ (mean $\pm$ standard deviation, $n = 5$) at 1000 ng/ml and the limit of detection was 50 ng/ml (signal-to-noise S/N > 3) for 2C-I. For lack of authentic human urine samples, a comparison of the metabolites found in rat and human urine after administration of 2C-I was not yet possible. However, other studies showed a good correspondence between the rat model and humans.[58,61,63,78,79]

CONCLUSIONS

The metabolism studies presented here showed that the β-phenethylamine-derived designer drug 2C-I was mainly metabolized by *O*-demethylation of the parent compound in position 2 and 5 of the aromatic ring, respectively.

The authors' STA procedure allowed the detection of an intake of a dose of 2C-I that corresponds to a common drug users' dose in urine. The target analytes were the corresponding *O*-demethyl metabolites. 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.[58,61,63,78,79]

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Therefore, it can be concluded that the procedure should also be applicable for human urine screening for 2C-I in clinical or forensic cases.

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REFERENCES

1. Shulgin A. In *Pihkal, A Chemical Love Story*, Joy Dan (ed). Transform Press: Berkley, CA, 1991; 815.
2. De Boer D, Bosman I. A new trend in drugs-of-abuse; the 2C-series of phenethylamine designer drugs. *Pharm. World Sci.* 2004; **26**: 110.
3. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Report on the risk assessment of 2C-I, 2C-T-2 and 2C-T-7 in the framework of the joint action on new synthetic drugs. http://www. emcdda. eu. int/multimedia/publications/risk_assessments/2Cs_2003_1636.PDF, 2004.
4. Schifano F, Deluca P, Agosti L, Martinotti G, Corkery JM, Alex B, Caterina B, Heikki B, Raffaella B, Anna C, Lucia DF, Dorte DR, Magi F, Susana F, Irene F, Claude G, Lisbet H, Lene SJ, Mauro L, Christopher L, Aino M, Teuvo P, Milena P, Salman R, Damien R, Angela RM, Francesco R, Norbert S, Holger S, Josep T, Marta T, Francesco Z. New trends in the cyber and street market of recreational drugs? The case of 2C-T-7 ('Blue Mystic'). *J. Psychopharmacol.* 2005; **19**: 675.
5. Drug Enforcement Administration – Office of Forensic Sciences. Dipropyltryptamine and 2C-I in Portland, Oregon. *Microgram J.* 2004; **37**: 113.
6. Drug Enforcement Administration – Office of Forensic Sciences. Tryptamines and phenethylamines. *Microgram J.* 2004; **37**: 149.
7. Drug Enforcement Administration – Office of Forensic Sciences. 2C-I tablets in the Balearic islands (Spain). *Microgram J.* 2004; **37**: 48.
8. European Communities. Council decision 2003/847/JHA of 27 November 2003 concerning control measures and criminal sanctions in respect of the new synthetic drugs 2C-I, 2C-T-2, 2C-T-7 and TMA-2. *Off. J. Eur. Commun.* 2003; **L321**: 64.
9. Johnson MP, Mathis CA, Shulgin AT, Hoffman AJ, Nichols DE. [125I]-2-(2,5-dimethoxy-4-iodophenyl)aminoethane ([125I]-2C-I) as a label for the 5-HT2 receptor in rat frontal cortex. *Pharmacol., Biochem. Behav.* 1990; **35**: 211.
10. Glennon RA, Raghupathi R, Bartyzel P, Teitler M, Leonhardt S. Binding of phenylalkylamine derivatives at 5-HT1C and 5-HT2 serotonin receptors: evidence for a lack of selectivity. *J. Med. Chem.* 1992; **35**: 734.
11. Glennon RA, Titeler M, Lyon RA. A preliminary investigation of the psychoactive agent 4-bromo-2,5-dimethoxyphenethylamine: a potential drug of abuse. *Pharmacol., Biochem. Behav.* 1988; **30**: 597.
12. Nichols DE, Frescas S, Marona-Lewicka D, Huang X, Roth BL, Gudelsky GA, Nash JF. 1-(2,5-Dimethoxy-4-(trifluoromethyl) phenyl)-2-aminopropane: a potent serotonin 5-HT2A/2C agonist. *J. Med. Chem.* 1994; **37**: 4346.
13. Cozzi NV, Nichols DE. 5-HT2A receptor antagonists inhibit potassium-stimulated gamma-aminobutyric acid release in rat frontal cortex. *Eur. J. Pharmacol.* 1996; **309**: 25.
14. Acuna-Castillo C, Villalobos C, Moya PR, Saez P, Cassels BK, Huidobro-Toro JP. Differences in potency and efficacy of a series of phenylisopropylamine/phenylethylamine pairs at 5-HT(2A) and 5-HT(2C) receptors. *Br. J. Pharmacol.* 2002; **136**: 510.
15. Villalobos CA, Bull P, Saez P, Cassels BK, Huidobro-Toro JP. 4-Bromo-2,5-dimethoxyphenethylamine (2C-B) and structurally related phenylethylamines are potent 5-HT2A receptor antagonists in Xenopus laevis oocytes. *Br. J. Pharmacol.* 2004; **141**: 1167.
16. Khorana N, Pullagurla MR, Dukat M, Young R, Glennon RA. Stimulus effects of three sulfur-containing psychoactive agents. *Pharmacol., Biochem. Behav.* 2004; **78**: 821.
17. Fantegrossi WE, Harrington AW, Eckler JR, Arshad S, Rabin RA, Winter JC, Coop A, Rice KC, Woods JH. Hallucinogen-like actions of 2,5-dimethoxy-4-(n)-propylthiophenethylamine (2C-T-7) in mice and rats. *Psychopharmacology (Berlin)* 2005; **181**: 496.
18. Lobos M, Borges Y, Gonzalez E, Cassels BK. The action of the psychoactive drug 2C-B on isolated rat thoracic aorta. *Gen. Pharmacol.* 1992; **23**: 1139.
19. Saez P, Borges Y, Gonzalez E, Cassels BK. Alpha-adrenergic and 5-HT2-serotonergic effects of some beta-phenylethylamines on isolated rat thoracic aorta. *Gen. Pharmacol.* 1994; **25**: 211.
20. Braun U, Shulgin AT, Braun G, Sargent T III. Synthesis and body distribution of several iodine-131 labeled centrally acting drugs. *J. Med. Chem.* 1977; **20**: 1543.
21. Monte AP, Marona-Lewicka D, Parker MA, Wainscott DB, Nelson DL, Nichols DE. Dihydrobenzofuran analogues of hallucinogens. 3. Models of 4-substituted (2,5-dimethoxyphenyl)alkylamine derivatives with rigidified methoxy groups. *J. Med. Chem.* 1996; **39**: 2953.
22. Glennon RA, Kier LB, Shulgin AT. Molecular connectivity analysis of hallucinogenic mescaline analogs. *J. Pharm. Sci.* 1979; **68**: 906.
23. Gupta SP, Bindal MC, Singh P. Quantitative structure-activity studies on hallucinogenic mescaline analogs using modified first order valence connectivity. *Arzneimittelforschung* 1982; **32**: 1223.
24. Kier LB, Glennon RA. Progress with several models for the study of the SAR of hallucinogenic agents. *NIDA Res. Monogr.* 1978; **22**: 159.
25. Beuerle G, Kovar KA, Schulze-Alexandru M. Three-dimensional quantitative structure-activity relationships of hallucinogenic phenylalkanamine and tryptamine derivatives. Studies using comparative molecular field analysis (CoMFA). *Quant. Struct.-Act. Relat.* 1997; **16**: 447.
26. Bienfait B. Applications of high-resolution self-organizing maps to retrosynthetic and QSAR analysis. *J. Chem. Inf. Comput. Sci.* 1994; **34**: 890.
27. Clare BW. The frontier orbital phase angles: novel QSAR descriptors for benzene derivatives, applied to phenylalkylamine hallucinogens. *J. Med. Chem.* 1998; **41**: 3845.
28. Klopman G, Macina OT. Use of the computer automated structure evaluation program in determining quantitative structure-activity relationships within hallucinogenic phenylalkylamines. *J. Theor. Biol.* 1985; **113**: 637.
29. Mracec M, Mracec M, Kurunczi L, Nusser T, Simon Z, Naray-Szabo G. QSAR study with steric (MTD), electronic and hydrophobicity parameters on psychotomimetic phenylalkylamines. *THEOCHEM* 1996; **367**: 139.
30. Giroud C, Augsburger M, Rivier L, Mangin P, Sadeghipour F, Varesio E, Veuthey JL, Kamalaprija P. 2C-B: a new psychoactive phenylethylamine recently discovered in Ecstasy tablets sold on the Swiss black market. *J. Anal. Toxicol.* 1998; **22**: 345.
31. Cole MD, Lea C, Oxley N. 4-Bromo-2,5-dimethoxyphenethylamine (2C-B): a review of the public domain literature. *Sci. Justice* 2002; **42**: 223.
32. De Boer D, Gijzels MJ, Bosman IJ, Maes RA. More data about the new psychoactive drug 2C-B [letter; comment]. *J. Anal. Toxicol.* 1999; **23**: 227.
33. Bosman IJ, De Boer D, Siderius EB, Lesseps JALDR, Maes RAA. Mass spectrometric identification of some sulphur containing phenalkylamine designer drugs. In *Proceedings of the 1998 Joint SOFT/TIAFT Meeting*, Spiehler V (ed). TIAFT98: Albuquerque, NM, 1999.
34. DeRuiter J, Clark CR, Noggle FT. LC and GC-MS analysis of 4-bromo-2,5-dimethoxyphenethylamine (Nexus) and 2-propanamine and 2-butanamine analogs. *J. Chromatogr. Sci.* 1995; **33**: 583.

35. DeRuiter J, Clark CR, Noggle FT. Gas chromatographic-mass spectrometric and high-performance liquid chromatographic analyses of the bromination products of the regioisomeric dimethoxyphenethylamines: differentiation of Nexus from five positional isomers. *J. Chromatogr. Sci.* 1998; **36**: 23.
36. Ragan FA Jr, Hite SA, Samuels MS, Garey RE. 4-Bromo-2,5-dimethoxyphenethylamine: identification of a new street drug. *J. Anal. Toxicol.* 1985; **9**: 91.
37. Chiu YC, Chou SH, Liu JT, Lin CH. The bioactivity of 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2) and its detection in rat urine by capillary electrophoresis combined with an on-line sample concentration technique. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2004; **811**: 127.
38. Soares ME, Carvalho M, Carmo H, Remiao F, Carvalho F, Bastos ML. Simultaneous determination of amphetamine derivatives in human urine after SPE extraction and HPLC-UV analysis. *Biomed. Chromatogr.* 2004; **18**: 125.
39. Theobald DS, Fehn S, Maurer HH. New designer drug 2,5-dimethoxy-4-propylthiophenethylamine (2C-T-7): studies on its metabolism and toxicological detection in rat urine using gas chromatography/mass spectrometry. *J. Mass Spectrom.* 2005; **40**: 105.
40. Boatto G, Nieddu M, Carta A, Pau A, Palomba M, Asproni B, Cerri R. Determination of amphetamine-derived designer drugs in human urine by SPE extraction and capillary electrophoresis with mass spectrometry detection. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2005; **814**: 93.
41. Laks S, Pelander A, Vuori E, Ali-Tolppa E, Sippola E, Ojanpera I. Analysis of street drugs in seized material without primary reference standards. *Anal. Chem.* 2004; **76**: 7375.
42. Theobald DS, Staack RF, Puetz M, Maurer HH. New designer drug 2,5-dimethoxy-4-ethylthio-beta-phenethylamine (2C-T-2): studies on its metabolism and toxicological detection in rat urine using gas chromatography/mass spectrometry. *J. Mass Spectrom.* 2005; **40**: 1157.
43. Kikura-Hanajiri R, Hayashi M, Saisho K, Goda Y. Simultaneous determination of nineteen hallucinogenic tryptamines/beta-calbolines and phenethylamines using gas chromatography-mass spectrometry and liquid chromatography-electrospray ionisation-mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2005; **825**: 29.
44. Tsai CC, Liu JT, Shu YR, Chan PH, Lin CH. Optimization of the separation and on-line sample concentration of phenethylamine designer drugs with capillary electrophoresis-fluorescence detection. *J. Chromatogr., A* 2006; **1101**: 319.
45. Habrdova V, Peters FT, Theobald DS, Maurer HH. Screening for and validated quantification of phenethylamine-type designer drugs and mescaline in human blood plasma by gas chromatography/mass spectrometry. *J. Mass Spectrom.* 2005; **40**: 785.
46. Maurer HH. Position of chromatographic techniques in screening for detection of drugs or poisons in clinical and forensic toxicology and/or doping control [review]. *Clin. Chem. Lab. Med.* 2004; **42**: 1310.
47. De Boer D, Lesseps JALDR, Gijzels M, Pilon N, Bosman IJ, Maes RAA. Studies on the metabolism of 2C-B. In *Proceedings of the 1998 Joint SOFT/TIAFT Meeting*, Spiehler V (ed). TIAFT98: Albuquerque, NM, 1999.
48. Kanamori T, Inoue H, Iwata Y, Ohmae Y, Kishi T. In vivo metabolism of 4-bromo-2,5-dimethoxyphenethylamine (2C-B) in the rat: identification of urinary metabolites. *J. Anal. Toxicol.* 2002; **26**: 61.
49. Lin LC, Liu JT, Chou SH, Lin CH. Identification of 2,5-dimethoxy-4-ethylthiophenethylamine and its metabolites in the urine of rats by gas chromatography-mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2003; **798**: 241.
50. Kanamori T, Tsujikawa K, Ohmae Y, Iwata Y, Inoue H, Inouye Y, Kishi T. Excretory profile of 4-bromo-2,5-dimethoxyphenethylamine (2C-B) in rat. *J. Health Sci.* 2003; **49**: 166.
51. Carmo H, Boer D, Remiao F, Carvalho F, Reys LA, Bastos ML. Metabolism of the designer drug 4-bromo-2,5-dimethoxyphenethylamine (2C-B) in mice, after acute administration. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2004; **811**: 143.
52. Kanamori T, Tsujikawa K, Ohmae Y, Iwata YT, Inoue H, Kishi T, Nakahama T, Inouye Y. A study of the metabolism of methamphetamine and 4-bromo-2,5-dimethoxyphenethylamine (2C-B) in isolated rat hepatocytes. *Forensic Sci. Int.* 2005; **148**: 131.
53. Carmo H, Hengstler JG, De Boer D, Ringel M, Remiao F, Carvalho F, Fernandes E, Dos Reys LA, Oesch F, De Lourdes BM. Metabolic pathways of 4-bromo-2,5-dimethoxyphenethylamine (2C-B): analysis of phase I metabolism with hepatocytes of six species including human. *Toxicology* 2005; **206**: 75.
54. Smyth WF. Recent applications of capillary electrophoresis-electrospray ionisation-mass spectrometry in drug analysis. *Electrophoresis* 2005; **26**: 1334.
55. Pelzing M, Neususs C. Separation techniques hyphenated to electrospray-tandem mass spectrometry in proteomics: capillary electrophoresis versus nanoliquid chromatography. *Electrophoresis* 2005; **26**: 2717.
56. Huhn C, Neususs C, Pelzing M, Pyell U, Mannhardt J, Putz M. Capillary electrophoresis-laser induced fluorescence-electrospray ionization-mass spectrometry: a case study. *Electrophoresis* 2005; **26**: 1389.
57. Staack RF, Maurer HH. New designer drug 1-(3,4-methylenedioxybenzyl) piperazine (MDBP): studies on its metabolism and toxicological detection in rat urine using gas chromatography/mass spectrometry. *J. Mass Spectrom.* 2004; **39**: 255.
58. Staack RF, Fritschi G, Maurer HH. Studies on the metabolism and the toxicological analysis of the new piperazine-like designer drug N-benzylpiperazine in urine using gas chromatography-mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2002; **773**: 35.
59. Staack RF, Fehn J, Maurer HH. New designer drug para-methoxymethamphetamine: studies on its metabolism and toxicological detection in urine using gas chromatography-mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2003; **789**: 27.
60. Maurer HH, Bickeboeller-Friedrich J. Screening procedure for detection of antidepressants of the selective serotonin reuptake inhibitor type and their metabolites in urine as part of a modified systematic toxicological analysis procedure using gas chromatography-mass spectrometry. *J. Anal. Toxicol.* 2000; **24**: 340.
61. Bickeboeller-Friedrich J, Maurer HH. Screening for detection of new antidepressants, neuroleptics, hypnotics, and their metabolites in urine by GC-MS developed using rat liver microsomes. *Ther. Drug Monit.* 2001; **23**: 61.
62. Pfleger K, Maurer HH, Weber A. *Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites*, 4th ed. Agilent Technologies: Palo Alto, CA, 2006; in preparation.
63. Ewald AH, Fritschi G, Bork WR, Maurer HH. Designer drugs 2,5dimethoxy4bromoamphetamine (DOB) and 2,5dimethoxy4 bromomethamphetamine (MDOB): Studies on their metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques. *J. Mass Spectrom.* 2006; **41**: 487.
64. Staack RF, Theobald DS, Maurer HH. Studies on the human metabolism and the toxicologic detection of the cough suppressant dropropizine in urine using gas chromatography-mass spectrometry. *Ther. Drug Monit.* 2004; **26**: 441.
65. Staack RF, Maurer HH. Studies on the metabolism and the toxicological analysis of the nootropic drug fipexide in rat urine using gas chromatography-mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2004; **804**: 337.
66. Paul LD, Maurer HH. Studies on the metabolism and toxicological detection of the eschscholtzia californica alkaloids

californine and protopine in urine using gas chromatography-mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2003; **789**: 43.
67. Staack RF, Fritschi G, Maurer HH. New designer drug 1-(3-trifluoromethylphenyl)piperazine (TFMPP): gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry studies on its phase I and II metabolism and on its toxicological detection in rat urine. *J. Mass Spectrom.* 2003; **38**: 971.
68. Staack RF, Maurer HH. Piperazine-derived designer drug 1-(3-chlorophenyl)piperazine (mCPP): GC-MS studies on its metabolism and its toxicological detection in urine including analytical differentiation from its precursor drugs trazodone and nefazodone. *J. Anal. Toxicol.* 2003; **27**: 560.
69. McLafferty FW, Turecek F. *Interpretation of Mass Spectra*, 4th ed. University Science Books: Mill Valley, CA, 1993.
70. Smith RM, Busch KL. *Understanding Mass Spectra – A Basic Approach*. Wiley: New York, 1999.
71. Theobald DS, Fritschi G, Maurer HH. Studies on the metabolism and toxicological detection of the designer drug 4-bromo-2,5-dimethoxy-b-phenethylamine (2C-B) in rat urine using gas chromatography/mass spectrometry. In preparation.
72. Hubbard JW, Bailey K, Midha KK, Cooper JK. 3-O-methyl-alpha-methyldopamine, a urinary metabolite of p-methoxy amphetamine in dog and monkey. *Drug Metab. Dispos.* 1981; **9**: 250.
73. Staack RF, Maurer HH. Metabolism of designer drugs of abuse [review]. *Curr. Drug Metab.* 2005; **6**: 259.
74. Staack RF, Theobald DS, Paul LD, Springer D, Kraemer T, Maurer HH. Identification of human cytochrome p450 2D6 as major enzyme involved in the o-demethylation of the designer drug p-methoxymethamphetamine. *Drug Metab. Dispos.* 2004; **32**: 379.
75. Charalampous KD, Walker KE, Kinross-Wright J. Metabolic fate of mescaline in man. *Psychopharmacologia* 1966; **9**: 48.
76. Kovats E. Gaschromatographische charakterisierung organischer verbindungen. Teil 1. Retentionsindices aliphatischer halogenide, alkohole, aldehyde und ketone. *Helv. Chim. Acta* 1958; **41**: 1915.
77. De-Zeeuw RA, Franke JP, Maurer HH, Pfleger K. *Gas Chromatographic Retention Indices of Toxicologically Relevant Substances and their Metabolites (Report of the DFG Commission for Clinical Toxicological Analysis, Special Issue of the TIAFT Bulletin)*, 3rd ed. VCH publishers: Weinheim, New York, Basle, 1992.
78. Beyer J, Ehlers D, Maurer HH. Abuse of Nutmeg (Myristica fragrans Houtt.): studies on the metabolism and the toxicological detection of its ingredients elemicin, myristicin and safrole in rat and human urine using gas chromatography/mass spectrometry. *Ther. Drug Monit.* 2006; **28**: In press.
79. Balikova M. Nonfatal and fatal DOB (2,5-dimethoxy-4-bromoamphetamine) overdose. *Forensic Sci. Int.* 2005; **153**: 85.