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# Metabolism of the designer drug 4-bromo-2,5-dimethoxyphenethylamine (2C-B) in mice, after acute administration

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**Abstract**

4-Bromo-2,5-dimethoxyphenethylamine (2C-B) is a psychoactive drug of abuse often sold under the general street name "Ecstasy". Recent reports on the abuse of 2C-B and analogues denote the lack of knowledge on this drug metabolism. In the present study, we investigated the metabolic profile of 2C-B in the mouse and found unchanged 2C-B and several metabolites, which could be identified by GC/MS in the mice urine. The identification of 2C-B metabolites may give important clues for the biological and toxicological effects of this drug of abuse and provides new important data for forensic analysis on samples taken from 2C-B abusers.


*Keywords:* 2C-B; Designer drugs of abuse; Metabolism; Mice

## 1. Introduction

4-Bromo-2,5-dimethoxyphenethylamine (2C-B), also known under the street names "Venus", "Bromo", "Erox", "XTC" or "Nexus", is a psychoactive drug related both with the amphetamine-like stimulants and the mescaline-like hallucinogens. It has been recently recommended for international control [1] and listed as a controlled substance in most European countries. Information about its use and related morbidity and mortality is scarce. However, the fact that 2C-B is often sold under the general street name "Ecstasy", and frequently taken together with 3,4-methylenedioxyamphetamine-like compounds, and also with other drugs such as ketamine, gives raise to concern regarding its abuse potential and the possibility of cumulative or even potentiation of toxic effects [2–4]. Recent reports on the abuse of 2C-B and analogues in the Netherlands and in Japan denote the lack of knowledge on the metabolism of such compounds, which may in turn lead to the inability to detect intoxications [5,6].

In humans, 2C-B is usually consumed at doses between 4 and 30 mg, which induce euphoria and increased receptiveness of the visual, auditory, olfactory and tactile sensations [4]. Doses between 5 and 10 mg induce amphetamine-like stimulating effects while doses between 10 and 20 mg are required to obtain the hallucinogenic effects of the drug [7]. Higher doses are known to cause frightening hallucinations and sympathomimetic effects such as tachicardia, hypertension and hyperthermia [2]. Accordingly, in a behavioral study performed in the newly hatched chicken, 2C-B not only induced some amphetamine-like effects but also produced an effect typical of mescaline [8].

The available pharmacological data on 2C-B indicate that its effects are probably mediated by the serotonergic pathways since it has been shown to have affinity to 5-$HT_1$ receptors and a high affinity for 5-$HT_2$ receptors, thus suggesting that it is a potential 5-$HT_2$ agonist [9]. Studies performed on isolated rat thoracic aorta also indicate that 2C-B behaves as

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a partial agonist for both $\alpha_1$-adrenergic receptors and 5-$HT_2$ serotonergic receptors [10].

Up to the present moment some analytical procedures for the identification and quantification of 2C-B in seized drug samples have already been implemented, namely: gas chromatography–mass spectrometry detection (GC/MS) [4] nuclear magnetic ressonance [4], ultraviolet spectrophotometry [11], high performance liquid chromatography–diode-array detection (DAD) [4], capillary electrophoresis–DAD [4], infrared spectroscopy [11], Raman spectroscopy [12] and Fourier-transform infrared spectroscopy [4].

In reported drug seizures in the Netherlands [7] and Switzerland [3,4] 2C-B was found in tablets in association with 3,4-methylenedioxymethamphetamine (MDMA) and *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB) and in the USA 2C-B was found alone in a powder used to spike a drink during a student's party [11].

Given its abuse potential it is of great toxicological and forensic interest that the metabolism of this drug is enlightened. Preliminary studies on the human metabolism of 2C-B reported the presence of unchanged 2C-B in the urine of a male subject abusing this drug [13]. Some metabolites were also found of which 4-bromo-2,5-dimethoxyphenylacetic acid, 4-bromo-2,5-dimethoxybenzoic acid and 4-bromo-2-hydroxy-5-methoxyphenethylamine could be identified by GC/MS [13]. An in vivo study performed in the rat reports the formation of 4-bromo-2,5-dimethoxyphenylacetic acid, 2-(4-bromo-2,5-dimethoxyphenyl)-ethanol, 4-bromo-2-hydroxy-5-methoxyphenethylamine, 4-bromo-2-hydroxy-5-methoxyphenethylamine and also the acetylated derivatives of metabolites 4-bromo-2-hydroxy-5-methoxyphenethylamine and 4-bromo-2-hydroxy-5-methoxyphenethylamine [14].

The aim of our study was to investigate the metabolic profile of 2C-B in the mouse, also in an attempt to find a good animal model for further toxicokinetic studies with this drug. GC/MS data of our findings are presented.

## 2. Experimental

### 2.1. Materials

All chemicals and reagents were of analytical grade. Trimethylsilylchloride (TMSCl), *N*-methylbis(trifluoroacetamide) (MBTFA) and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) were obtained from Macherey-Nagel (Düren, Germany). Trifluoroacetic anhydride (TFAA) was obtained from Janssen Chimica (Beerse, Belgium). Phenaceturic acid was obtained from Tokyo Kasei (Tokyo, Japan). Methyl orange solution was prepared at a concentration of 2 mg/mL by dissolving sufficient methyl orange (Koch-Light Laboratories, Colnbucks Buck, UK) in a mixture of acetonitrile and trifluoroacetic acid (6:4, v/v). 2C-B hydrochloride was generously provided by the United Nations – Scientific Section, PDAB/DOA/UNDCP (Vienna, Austria). 4-Bromo-2,5-dimethoxyphenylacetic acid was kindly provided by The Netherlands Institute of Drugs and Doping Research (Utrecht, The Netherlands).

### 2.2. Drug administration and sample collection

Male CD1 mice (Charles-River, Barcelona, Spain) weighing 30–35 g were used. The animals were housed individually in metabolic cages in a temperature-controlled room at 20 °C ± 1 °C with a 12/12 h lighting schedule. Water was available ad libitum but food was withdrawn after the administration of 2C-B. Urine samples were collected during 24 h after the i.p. administration of either 20 or 40 mg/kg 2C-B or saline. Five different animals were used for each 2C-B dose. All urine samples were centrifuged at 3000 × *g* for 5 min and then stored at −20 °C until GC/MS analysis.

Housing and experimental treatment of the animals were conducted under the European Community guidelines for the use of experimental animals (European convention for the protection of vertebrate animals used for experimental and other scientific purposes, 1986, and Protocol of amendment to the European convention for the protection of vertebrate animals used for experimental and other scientific purposes, 1998).

### 2.3. Isolation of compounds of interest for GC/MS analysis

#### 2.3.1. Basic fraction

Aliquots of 2 mL of mice urine were transferred to a 25 mL tube and 200 μL of 5 M KOH solution were added in order to obtain a pH >13. The basic compounds were extracted by adding 2.5 mL of *tert*-butylmethyl ether. The tube was vortex mixed for a few seconds, shaken for 10 min and centrifuged for 5 min at 1500 × *g*. The organic phase was transferred to a clean tube and the water phase was kept. The basic extraction process was repeated twice and the organic phases were combined. The organic solvents were removed partially under a gentle nitrogen stream. An aliquot of 10 μL of MBTFA was added to prevent the loss of volatile compounds, after which the evaporation process was completed. The obtained residue was dried under reduced pressure over $P_2O_5$ and KOH for at least 1 h.

#### 2.3.2. Acidic fraction

To the remaining water phase obtained after the basic extraction, sufficient drops of 37% HCl were added to obtain a pH <3.4. The acidic compounds were extracted by adding 3 mL of a mixture of *n*-hexane and ethyl acetate (9:1, v/v). The tube was vortex mixed for a few seconds, shaken for 10 min and centrifuged for 5 min at 1500 × *g*. The organic phase was transferred to a clean tube and the water phase was kept. The acidic extraction process was repeated twice and the organic phases were combined. The organic solvents were removed in a rotating evaporator. The obtained residue

was dried under reduced pressure over $P_2O_5$ and KOH for at least 1 h.

*2.3.3. Conjugates fraction*

After the basic and acidic extraction the remaining water phase was kept and chemical hydrolysis was performed for the finding of possible conjugates metabolites. To the water phase 200 μL of 5 M KOH solution were added in order to obtain a pH >13. The tube was vortex mixed for a few seconds, and incubated at 60 °C for 15 min. Basic and acidic compounds were extracted according to the procedures described above for the extractions of the basic and acidic fractions.

*2.4. Derivatization procedures for GC/MS analysis*

*2.4.1. N-TFA-O-TMS derivatization of the basic fraction*

To the respective dry residue, 50 μL of a methyl orange solution were added. Sufficient MSTFA was added until a yellow colour appeared. The mixture was heated for 5 min at 80 °C. After cooling to room temperature 10 μL of MBTFA were added. The mixture was vortex mixed and heated for 10 min at 80 °C. If in one of the previous steps the colour of the mixture turned to red, sufficient drops of MSTFA were added to re-obtain a yellow colour. After cooling to room temperature the mixture was used without further preparation for GC/MS analysis.

*2.4.2. N,O-TFA derivatization of the basic fraction*

To the respective dry residue, 50 μL of ethyl acetate and 50 μL of TFAA were added. The mixture was heated for 30 min at 65 °C. After cooling to room temperature the excess of reagents was evaporated under a gentle stream of nitrogen. The obtained residue was dissolved in 100 μL of ethyl acetate and used without further preparation for GC/MS analysis.

*2.4.3. N,O-TMS derivatization of the acidic fraction*

To the respective dry residue, 50 μL of a mixture of MSTFA and TMSCl (95:5, v/v) were added. The mixture was heated for 30 min at 80 °C. After cooling to room temperature, the mixture was used without further preparation for GC/MS analysis.

*2.5. GC/MS analysis*

GC/MS analysis was performed on a Hewlett Packard 6890 gas chromatograph (Agilent Technologies, Waldbron, Germany) coupled to a Hewlett Packard 5971 mass selective detector (Agilent Technologies). The gas chromatograph was equipped with a Hewlett Packard capillary column HP-1 (Agilent Technologies), 18 m long, 0.20 mm i.d. and 0.33 μm film thickness. A two level oven program was applied: the temperature was maintained at 100 °C for 1 min, programmed up to 300 °C at 15 °/min and maintained at 300 °C for 20 min. The temperatures of the injection port and the transfer line were 250 and 310 °C, respectively. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. An aliquot of 2 μL was injected at a split ratio of 1:10. The mass selective detector was operated in the electron ionization mode according the manufacturer's recommendations in the full scan range of *m/z* 50/600.

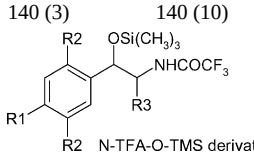
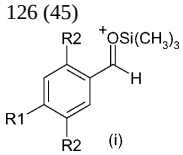
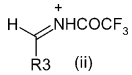
## 3. Results

*3.1. Basic extraction*

The *N*-TFA derivative of 2C-B, extracted at basic pH, was always present in the chromatograms obtained after the analysis of the urine samples. Its identification was based on the retention time and the mass spectrum relative to the analysis of the 2C-B standard. Besides the peak of the parent compound, several peaks were observed in the chromatograms that could potentially correspond to metabolites. These metabolites were searched taking into account that bromine containing compounds show a characteristic pattern of their mass spectrum isotope peaks (2 ions with a very similar abundance and with a difference of 2 Da).

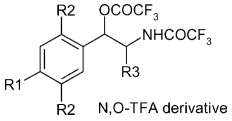
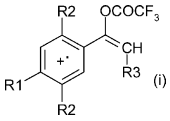
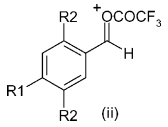
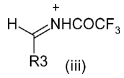
Using *N*-TFA-*O*-TMS derivatization, two peaks with identical mass spectra were observed in the retention time interval from 9.0 to 9.2 min. The mass spectrometric characteristics are given in Table 1. These characteristics are consistent with those of the *N*-TFA-*O*-TMS deriva-

Table 1
Partial mass spectrometric characteristics of the *N*-TFA-*O*-TMS derivatives of the two β-hydroxyamphetamine diastereomers (cathine and phenylpropanolamine) and of the β-hydroxylated 2C-B diastereomers (β-OH-2C-B)

| Ion structure | Mass-to-charge ratios of molecular ions and fragments (relative intensity) | | | | | |
|---|---|---|---|---|---|---|
| | $R_1$, $R_2$ = H; $R_3$ = $CH_3$ | | $R_1$ = Br; $R_2$ = $OCH_3$; $R_3$ = H | | | |
| | Cathine | Phenylpropanolamine | β-OH-2C-B (diastereomer no. 1) | | β-OH-2C-B (diastereomer no. 2) | |
| $M^{+\bullet}$ | 319 (<1) | 319 (<1) | 443 (3) | 445 (4) | 443 (3) | 445 (2) |
| (i) | 179 (100) | 179 (100) | 317 (88) | 319 (100) | 317 (98) | 319 (100) |
| (ii) | 140 (3) | 140 (10) | 126 (45) | | 126 (34) | |
| |  | |  |  | | |
| | N-TFA-O-TMS derivative | | (i) | (ii) | | |

General structure of the *N*-TFA-*O*-TMS derivative and proposed structures of relevant ions.

Table 2
Partial mass spectrometric characteristics of the *N*,*O*-TFA derivative of the two β-hydroxyamphetamine diastereomers (cathine and phenylpropanolamine) and β-hydroxylated 2C-B diastereomers (β-OH-2C-B)

| Ion structure | Mass-to-charge ratios of molecular ions and fragments (relative intensity) | | |
|---|---|---|---|
| | Cathine/phenylpropanolamine ($R_1$, $R_2$ = H; $R_3$ = $CH_3$) | β-OH-2C-B (diastereomers no. 1 and 2) ($R_1$ = Br; $R_2$ = $OCH_3$; $R_3$ = H) | |
| $M^{+\bullet}$ | 343 (<1) | 467 (56) | 469 (43) |
| (i) | 230 (15) | 354 (47) | 356 (61) |
| (ii) | 203 (7) | 341 (113) | 343 (111) |
| (iii) | 140 (100) | 126 (100) | |
|  |  |  |  |
| N,O-TFA derivative | (i) | (ii) | (iii) |

General structure of the *N*,*O*-TFA derivative and proposed structures of relevant ions.

tive of β-hydroxyamphetamine-like compounds, such as cathine (norpseudoephedrine) and phenylpropanolamine (norephedrine), also presented in Table 1. Both β-hydroxyphenethylamine-like compounds fragmentations are characterised by the cleavage between $C_\alpha$ and $C_\beta$ resulting in two ions at relatively high abundance. Furthermore, similarly to what was previously observed with the amphetamine metabolism in mice [15], the presence of one diastereomer was more dominant than the one of the other. Therefore, it could be suggested that these peaks correspond to the isomers of 4-bromo-2,5-dimethoxy-β-hydroxyphenethylamine metabolites. In order to confirm this proposal a second derivatization reaction was performed, namely *N*,*O*-TFA derivatization. This type of derivatization also resulted in typical mass spectra for the *N*-TFA-*O*-TFA derivatives of cathine and phenylpropanolamine (see Table 2). An additional characteristic was that the derivatives of cathine and phenylpropanolamine were not separated chromatographically under these conditions. Additionally, a fragment at *m*/*z* 287/289 and a fragment at *m*/*z* 311/313 resulting from the simultaneous β-cleavage and loss of a methoxyl group was also observed in the mass spectra obtained with both derivatization procedures (see fragment (A) in Figs. 1 and 2). Thus, it was confirmed that those peaks corresponded to the β-hydroxylated metabolites of 2C-B.

A possible metabolite, with a molecular ion at *m*/*z* 413/415 was observed at 8.9 min, which could correspond to a demethylated metabolite of 2C-B. The presence of the ion at *m*/*z* 126 and the absence of one at *m*/*z* 317/319 indicated that the hydroxyl group was not located at the $C_\beta$ in the amine side chain. Therefore, the hydroxyl group must be located at the phenyl ring. A comparison between the fragmentation pattern of this metabolite and that of the parent compound 2C-B is presented in Table 3. The same fragmentation pattern was found for both compounds and the fragments of the demethylated metabolite showed a shift of 58 Da corresponding to the difference between the mass of the *O*-TMS derivatized hydroxyl group and that of a methoxyl group. The α-cleavage that originates ions at *m*/*z* 242/244 and *m*/*z* 302/304 in the mass spectra of 2C-B and demethylated 2C-B, respectively, has already been reported [14]. It was thus concluded that the metabolite corresponded to either 4-bromo-2-hydroxy-5-methoxyphenethylamine or to its isomer 4-bromo-5-hydroxy-2-methoxyphenethylamine.

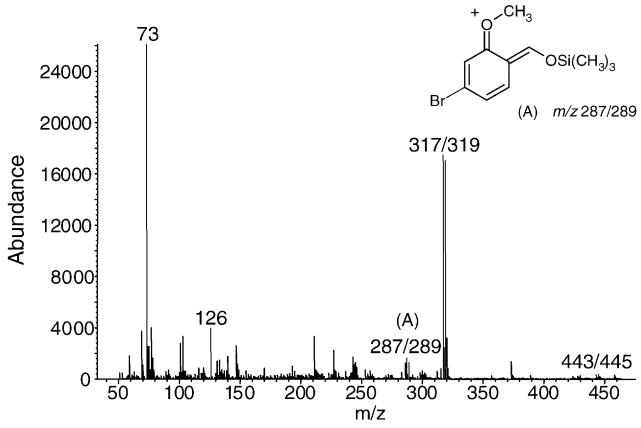



Fig. 1. Mass spectrum of the *N*-TFA-*O*-TMS derivative of the two peaks corresponding to the two β-hydroxylated 2C-B diastereomers (β-OH-2C-B) observed in the chromatograms obtained with the mice urine samples extracted at basic pH. (A) Characteristic fragment at *m*/*z* 287/289.

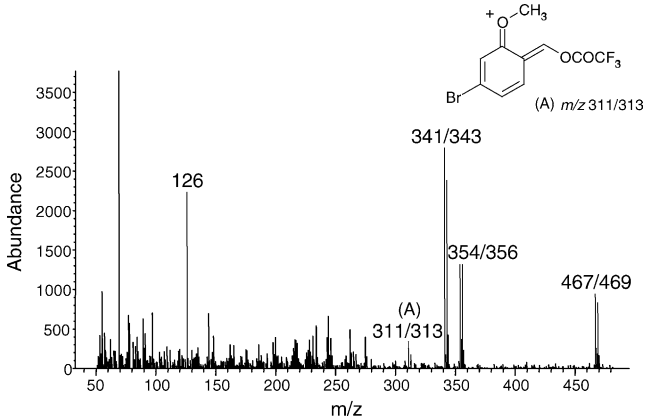
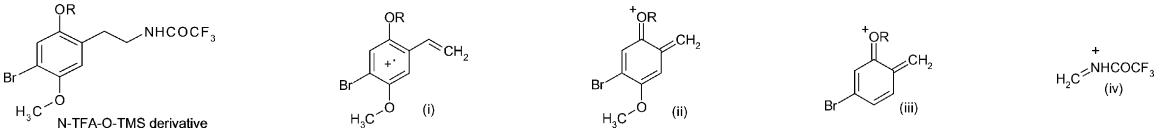



Fig. 2. Mass spectrum of the *N*,*O*-TFA derivative of the peak corresponding to the two β-hydroxylated 2C-B diastereomers (β-OH-2C-B) observed in the chromatograms obtained with the mice urine samples extracted at basic pH. (A) Characteristic fragment at *m*/*z* 311/313.

Table 3
Partial mass spectrometric characteristics of the *N*-TFA-*O*-TMS derivative of 2C-B and 4-bromo-2-hydroxy-5-methoxyphenethylamine (B-2-HMPEA)

| Ion structure | Mass-to-charge ratios of molecular ions and fragments (relative intensity) | | | |
|---|---|---|---|---|
| | 2C-B (R = $CH_3$) | | B-2-HMPEA (R = $Si(CH_3)_3$) | |
| $M^{+\bullet}$ | 355 (49) | 357 (49) | 413 (75) | 415 (88) |
| (i) | 242 (99) | 244 (100) | 300 (82) | 302 (100) |
| (ii) | 229 (76) | 231 (61) | 287 (59) | 289 (50) |
| (iii) | 199 (39) | 201 (39) | 257 (10) | 259 (16) |
| (iv) | 126 (56) | | 126 (62) | |




General structure of the *N*-TFA-*O*-TMS derivative and proposed structures of relevant ions.

Theoretically, it can be predicted that the 2-hydroxy-5-methoxy metabolite was observed rather than the 5-hydroxy-2-methoxy metabolite. This is based on the assumption that fragment (iii) in Table 3 can be explained by the formation of a stable quinonoid-like ion, which can only be formed in the case of the 2-hydroxy-5-methoxy metabolite [16].

Another peak showing the typical bromine isotope pattern was observed at 8.2 min. The molecular ion at *m*/*z* 332/334 and the presence of an ion at *m*/*z* 103, which is characteristic of *O*-TMS derivatives of primary alcohols [17] suggested that this compound could correspond to the neutral ethanol derivative of 2C-B. A comparison between the mass spectrometric characteristics of the spectra obtained with *O*-TMS derivatization and those of the mass spectra obtained with *O*-TFA derivatization is made in Table 4. The characteristic β-cleavage resulting in ions at *m*/*z* 229/231 at relatively high abundance has already been reported [14]. Additionally, a fragment at *m*/*z* 242/244 resulting from the α-cleavage and a fragment at *m*/*z* 199/201 resulting from the simultaneous β-cleavage and loss of the methoxyl group were also observed in the mass spectra obtained with *N*,*O*-TFA derivatization (see fragments (A) and (B) in Fig. 3). It was concluded that the 2-(4-bromo-2,5-dimethoxyphenyl)-ethanol was the fourth metabolite observed in the chromatograms of the mouse urines extracted at basic pH.

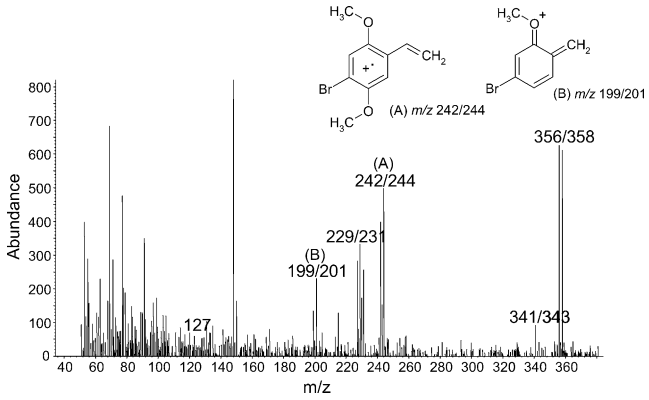


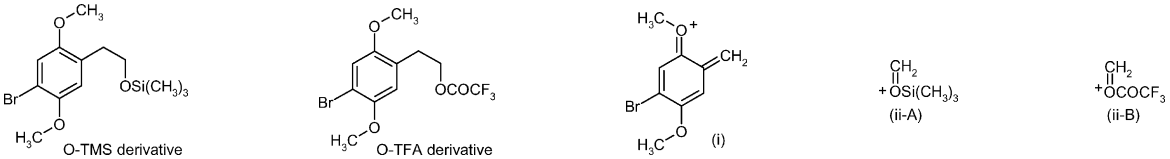

Fig. 3. Mass spectrum of the *O*-TFA derivative of the peak corresponding to the 2-(4-bromo-2,5-dimethoxyphenyl)-ethanol (BDMPE) metabolite observed in the chromatograms obtained with the mice urine samples extracted at basic pH. (A) Characteristic fragment at *m*/*z* 242/244; (B) characteristic fragment at *m*/*z* 199/201.

### 3.2. Acidic extraction

The *O*-TMS derivative of the 4-bromo-2,5-dimethoxyphenylacetic acid, extracted at acidic pH, was always a dominant peak in the chromatograms obtained after the analysis of the urine samples (see Fig. 4;

Table 4
Partial mass spectrometric characteristics of the *O*-TMS and *O*-TFA derivatives of 2-(4-bromo-2,5-dimethoxyphenyl)-ethanol (BDMPE)

| Ion structure | Mass-to-charge ratios of molecular ions and fragments (relative intensity) | | | |
|---|---|---|---|---|
| | BDMPE (*O*-TMS derivative) | | BDMPE (*O*-TFA derivative) | |
| $M^{+\bullet}$ | 332 (108) | 334 (100) | 356 (102) | 358 (100) |
| $[M\text{-}CH_3^{\bullet}]^+$ | 317 (29) | 319 (21) | 341 (16) | 343 (10) |
| (i) | 229 (68) | 231 (61) | 229 (52) | 231 (39) |
| (ii) | 103 (156) | | 127 (14) | |




General structure of the *O*-TMS and *O*-TFA derivatives and proposed structures of relevant ions.

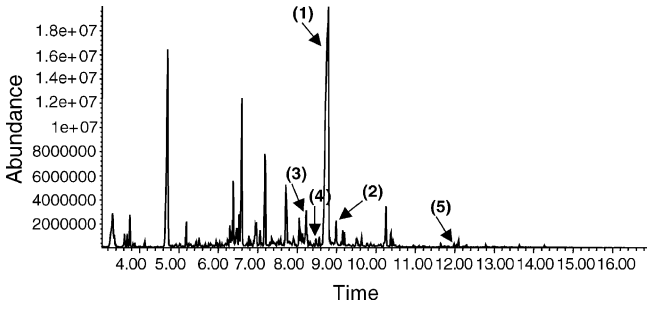



Fig. 4. Typical chromatogram obtained with the mice urine samples extracted at acidic pH. Peak identification: 1, *O*-TMS derivative of 4-bromo-2,5-dimethoxyphenylacetic acid; 2, *O*-TMS derivative of 4-bromo-2-hydroxy-5-methoxyphenylacetic acid; 3, *O*-TMS derivative of 4-bromo-2,5-dimethoxybenzoic acid; 4, *O*-TMS derivative of 2-(4-bromo-2-hydroxy-5-methoxyphenyl)-ethanol; and 5, *O*-TMS derivative of 4-bromo-2,5-dimethoxyphenaceturic acid.

peak 1). Its identification was based on the retention time and the mass spectrum relative to the analysis of the 4-bromo-2,5-dimethoxyphenylacetic acid standard.

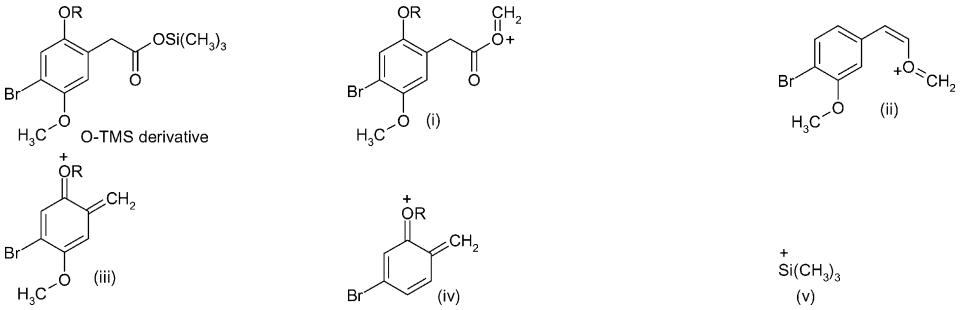
A possible metabolite, with a molecular ion at *m/z* 404/406 was observed at 9 min, which could correspond to the demethylated 4-bromo-2,5-dimethoxyphenylacetic acid. A comparison between the fragmentation pattern of this metabolite and that of the 4-bromo-2,5-dimethoxyphenylacetic acid is presented in Table 5. The same fragmentation pattern was found for both compounds and the fragments of the demethylated metabolite showed a shift of 58 Da corresponding to the difference between the mass of the O-TMS derivatized hydroxyl group and that of a methoxyl group. It was thus concluded that the metabolite corresponded to either the 4-bromo-2-hydroxy-5-methoxyphenylacetic acid or to the 4-bromo-5-hydroxy-2-methoxyphenylacetic acid. Similarly to what was discussed above for the demethylated metabolite of 2C-B it is theoretically predictable that the observed metabolite corresponds to the 2-hydroxy-5-methoxy metabolite rather than to the 5-hydroxy-2-methoxy metabolite [16].

A small peak with a molecular ion at *m/z* 332/334 was observed at 8.3 min, which could correspond to the 4-bromo-2,5-dimethoxybenzoic acid. A comparison of the fragmentation pattern of this metabolite with that of the 2,5-dimethoxybenzoic acid is presented in Table 6. The matching fragmentation pattern of both compounds and the shift of 78/80 Da corresponding to the difference between the mass of the bromine atom and that of a hydrogen atom, confirmed that this brominated metabolite corresponded to the 4-bromo-2,5-dimethoxybenzoic acid.

Another relatively small peak showing the characteristic brominated pattern was found at 12 min. The molecular ion at *m/z* 403/405 suggested that this peak could correspond to the conjugation product of the phenylacetic acid-like metabolite with glycine, i.e. to the 4-bromo-2,5-dimethoxyphenaceturic acid metabolite. In order to confirm this hypothesis a comparison with the fragmentation pattern of the ring unsubstituted phenaceturic acid was performed and a matching profile of both fragmentations was found, as can be seen in Table 7. Additionally a fragment at *m/z* 199/201 resulting from the simultaneous β-cleavage and loss of the methoxyl group and also a fragment at *m/z* 241/243 resulting from the cleavage at the amide were found (see fragments (A) and (B) in Fig. 5).

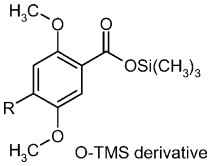
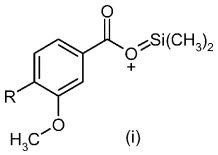
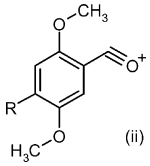
Table 5
Partial mass spectrometric characteristics of the *O*-TMS derivative of 4-bromo-2,5-dimethoxyphenylacetic acid (BDMPAA) and 4-bromo-2-hydroxy-5-methoxyphenylacetic acid (B-2-HMPAA)

| Ion structure | Mass-to-charge ratios of molecular ions and fragments (relative intensity) | | | |
|---|---|---|---|---|
| | BDMPAA (R = $CH_3$) | | B-2-HMPAA (R = $Si(CH_3)_3$) | |
| $M^{+\bullet}$ | 346 (21) | 348 (21) | 404 (12) | 406 (13) |
| $[M\text{-}CH_3^{\bullet}]^+$ | 331 (6) | 333 (6) | 389 (2) | 391 (2) |
| $[M\text{-}CH_2O^{\bullet}]^+$ | 316 (5) | 318 (5) | 374 (1) | 376 (1) |
| (i) | 287 (9) | 289 (9) | 345 (2) | 347 (1) |
| (ii) | 241 (5) | 243 (5) | 241 (1) | 243 (1) |
| (iii) | 229 (4) | 231 (4) | 287 (3) | 289 (2) |
| (iv) | 199 (4) | 201 (4) | 257 (5) | 259 (5) |
| (v) | 73 (100) | | 73 (100) | |



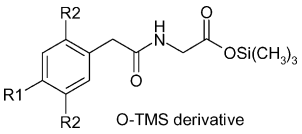
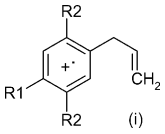
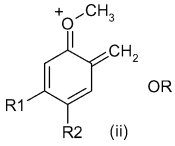
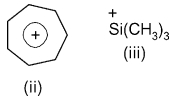

General structure of the *O*-TMS derivative and proposed structures of relevant ions.

Table 6
Partial mass spectrometric characteristics of the *O*-TMS derivative of 4-bromo-2,5-dimethoxybenzoic acid (BDMBA) and 2,5-dimethoxybenzoic acid (DMBA)

| Ion structure | Mass-to-charge ratios of molecular ions and fragments (relative intensity) | | |
|---|---|---|---|
| | BDMBA (R = Br) | | DMBA (R = H) |
| $M^{+\bullet}$ | 332 (66) | 334 (51) | 254 (47) |
| $[M\text{-}CH_3^{\bullet}]^+$ | 317 (46) | 319 (43) | 239 (19) |
| $[M\text{-}CH_2O^{\bullet}]^+$ | 302 (100) | 304 (100) | 224 (100) |
| (i) | 287 (29) | 289 (32) | 209 (30) |
| (ii) | 243 (102) | 245 (97) | 165 (49) |
|  O-TMS derivative | |  (i) |  (ii) |

General structure of the *O*-TMS derivative and proposed structures of relevant ions.

Table 7
Partial mass spectrometric characteristics of the *O*-TMS derivative of 4-bromo-2,5-dimethoxyphenaceturic acid (BDMPA) and phenaceturic acid (PA)

| Ion structure | Mass-to-charge ratios of molecular ions and fragments (relative intensity) | | |
|---|---|---|---|
| | BDMPA ($R_1$ = Br; $R_2$ = $OCH_3$) | | PA ($R_1$, $R_2$ = H) |
| $M^{+\bullet}$ | 403 (16) | 405 (17) | 265 (31) |
| $[M\text{-}CH_3^{\bullet}]^+$ | 388 (7) | 390 (9) | 250 (40) |
| (i) | 256 (57) | 258 (53) | 118 (26) |
| (ii) | 229 (55) | 231 (51) | 91 (160) |
| (iii) | 73 (100) | | 73 (100) |
|  O-TMS derivative |  (i) |  OR (ii) |  (ii) (iii) |

General structure of the *O*-TMS derivative and proposed structures of relevant ions.

At 8.5 min a peak was observed with a mass spectrum showing a molecular ion at *m/z* 390/392 and also the presence of an ion at *m/z* 103, which, as mentioned above is characteristic of the *O*-TMS derivatives of primary alcohols [17]. It was thus hypothesised that the corresponding metabolite could be the demethylated ethanol derivative of 2C-B. The fragmentation pattern observed in the mass spectra was consistent with that of the *O*-TMS derivative of the 2-(4-bromo-2,5-dimethoxyphenyl)-ethanol, which was found in the mouse urines extracted at basic pH (see Table 8). Additionally, a fragment at *m/z* 257/259 resulting from the β-cleavage and loss of methoxyl group was identified in the mass spectrum (see fragment (A) in Fig. 6). Concerning the position of the hydroxyl group in the phenyl ring it was assumed once more, that it is theoretically predictable that the observed metabolite corresponds to the 2-hydroxy-5-methoxy metabolite rather than to the 5-hydroxy-2-methoxy metabolite. After all, fragment (i) in Table 8 can only be formed if this is the case [16]. Based on these consistent findings it was concluded that the corresponding metabolite is the 2-(4-bromo-2-hydroxy-5-methoxyphenyl)-ethanol.

A summary of the 2C-B metabolites found in this study is presented in Table 9.

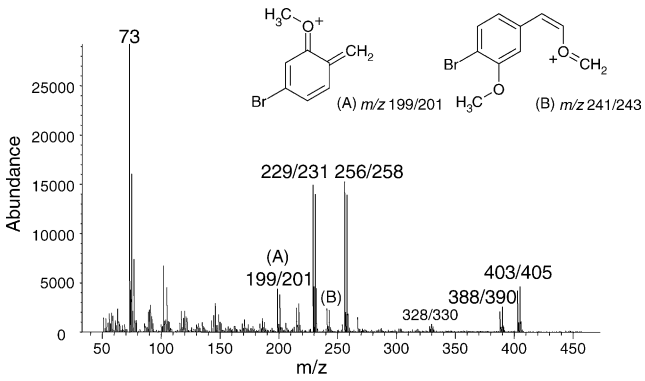
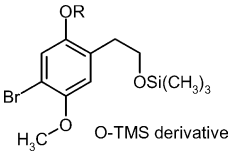
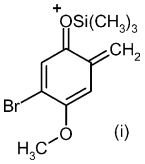



Fig. 5. Mass spectrum of the *O*-TMS derivative of the peak corresponding to the 4-bromo-2,5-dimethoxyphenaceturic acid (BDMPA) metabolite observed in the chromatograms obtained with the mice urine samples extracted at acidic pH. (A) Characteristic fragment at *m/z* 199/201; (B) characteristic fragment at *m/z* 241/243.

### 3.3. Conjugates fraction

Under our experimental conditions we could not detect the formation of conjugates.

Table 8
Partial mass spectrometric characteristics of the *O*-TMS derivative of 2-(4-bromo-2-hydroxy-5-methoxyphenyl)-ethanol (B-2-HMPE) and 2-(4-bromo-2,5-dimethoxyphenyl)-ethanol (BDMPE)

| Ion structure | Mass-to-charge ratios of molecular ions and fragments (relative intensity) | | | |
|---|---|---|---|---|
| | B-2-HMPE (R = $Si(CH_3)_3$) | | BDMPE (R = $CH_3$) | |
| $M^{+\bullet}$ | 390 (11) | 392 (12) | 332 (23) | 334 (22) |
| $[M\text{-}CH_3^{\bullet}]^+$ | 375 (1) | 377 (1) | 317 (6) | 319 (5) |
| $[M\text{-}CH_2O^{\bullet}]^+$ | 360 (1) | 362 (2) | 302 (13) | 304 (9) |
| (i) | 287 (13) | 289 (13) | 229 (15) | 231 (13) |
| (ii) | 103 (21) | | 103 (34) | |
| (iii) | 73 (100) | | 73 (100) | |
|  |  | |  |  |
| O-TMS derivative | (i) | | (ii) | (iii) |

General structure of the *O*-TMS derivative and proposed structures of relevant ions.

Table 9
Excretion of 2C-B metabolites identified in the mice urines extracted at basic and acidic pH after 24 h of the administration of the drug

| 2C-B metabolites extracted at basic pH | | | |
|---|---|---|---|
| 2C-B | BDMPE | B-2-HMPEA | β-OH-2C-B |
| +++ | + | + | ++ |

| 2C-B metabolites extracted at acidic pH | | | | |
|---|---|---|---|---|
| BDMPAA | B-2-HMPAA | BDMPA | BDMBA | B-2-HMPE |
| ++++ | + | + | + | + |

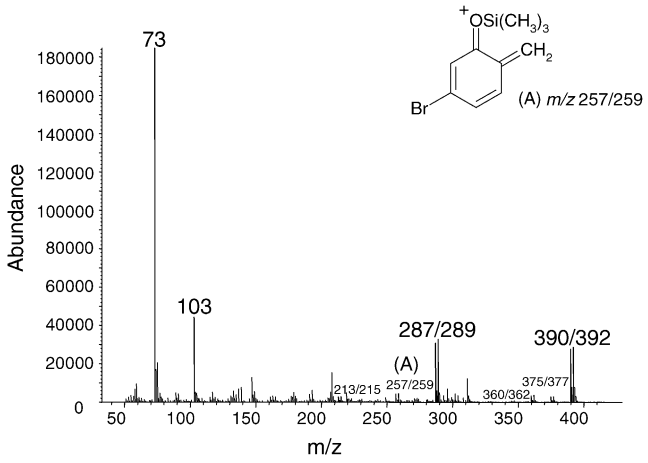



Fig. 6. Mass spectrum of the *O*-TMS derivative of the peak corresponding to the 2-(4-bromo-2-hydroxy-5-methoxyphenyl)-ethanol (B-2-HMPE) metabolite observed in the chromatograms obtained with the mice urine samples extracted at acidic pH. (A) Characteristic fragment at *m/z* 257/259.

## 4. Discussion

The increasing number of reports on the abuse of 2C-B in several countries has raised concern due to its similarity to both amphetamine-like stimulants and to the hallucinogen mescaline [1]. Although its use seems to be less common than that of other more popular amphetamine derivatives such as MDMA, it has been noted that abusers of this type of compounds are often polydrug users and frequently ingest different compounds under the general name "Ecstasy" even though convinced that they are ingesting amphetamine or MDMA [4]. The limited number of available studies on 2C-B makes it more likely that its use is undetected and therefore underestimated, as well as the toxicological consequences of its abuse left undetermined. The present study was conducted in order to study the in vivo metabolism of this drug using the mouse as the animal model. We have identified several metabolites excreted in the mouse urine. With the exception of 2C-B and the dominant acidic metabolite 4-bromo-2,5-dimethoxyphenylacetic acid, for which reference standards were available, all other metabolites found in the present study were identified based on the characteristic fragmentations shown in their mass spectra. The metabolic pathways for 2C-B in mice as constructed by our findings are presented in Fig. 7. In mice, the main metabolite found in our experimental model was the 4-bromo-2,5-dimethoxyphenylacetic acid. The oxidative deamination leading to the formation of both the phenylacetic acid metabolite and the 2-(4-bromo-2,5-dimethoxyphenyl)-ethanol seems to be the most important pathway for 2C-B metabolism. This is also the case for the related hallucinogenic compound mescaline [18]. It is well known that monoamine oxidase (MAO) catalyses this metabolic transformation of mescaline in vivo [19,20]. It can be expected that an aldehyde intermediate is produced and further oxidized to the phenylacetic acid metabolite or reduced to the ethanol metabolite. Although the aldehyde intermediate and the enzymes responsible for its formation have not been identified in our study, this mechanism is likely to

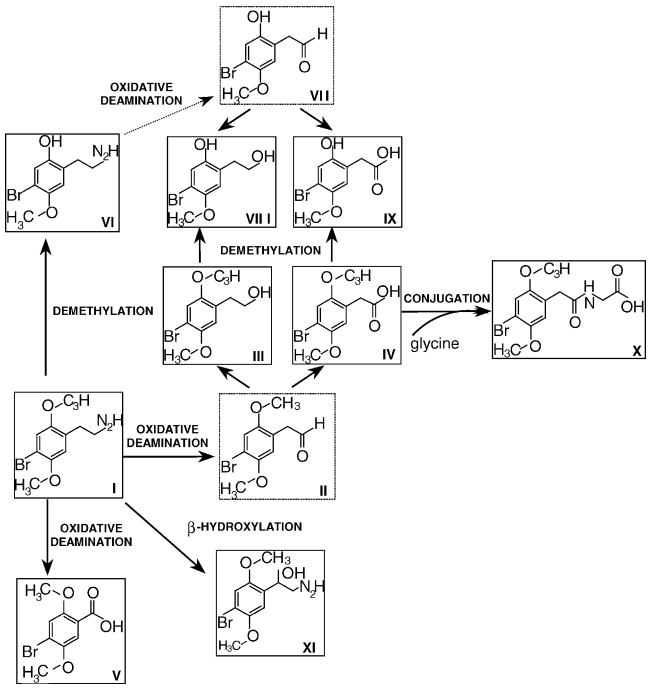



Fig. 7. Metabolic pathways found for 2C-B in mice after 24 h of acute drug administration. (I) 2C-B; (II) 4-bromo-2,5-dimethoxyphenylacetaldehyde; (III) BDMPE; (IV) BDMPAA; (V) BDMBA; (VI) B-2-HMPEA; (VII) 4-bromo-2-hydroxy-5-methoxyphenethyacetaldehyde; (VIII) B-2-HMPE; (IX) B-2-HMPAA; (X) BDMPA; and (XI) β-OH-2C-B. Structures outlined with full-line rectangles correspond to metabolites identified in the present study; structures outlined with discontinuous-line rectangles correspond to possible intermediate metabolites not identified in the present study.

occur since a similar pathway has already been described for mescaline in vitro [21].

Another observed pathway is the oxidative deamination with side chain degradation of the parent compound resulting in the formation of the respective benzoic acid metabolite, 4-bromo-2,5-dimethoxybenzoic acid. It can be expected that this reaction is catalysed by a cytochrome P450 dependent reaction similarly to what has been described for other amphetamines [22–25] and also for mescaline [26].

Demethylation of 2C-B was also found to be of relevance resulting in the production of 4-bromo-2-hydroxy-5-methoxyphenethylamine. Although the involved isoforms were not identified in the present study, it is expected that demethylation is catalysed by a cytochrome P450 dependent reaction. This metabolic pathway has been previously observed with other methoxylated amphetamine derivatives both in vitro [27–29] and in vivo [30,31]. 4-Bromo-2-hydroxy-5-methoxyphenethylamine can subsequently undergo oxidative deamination to the respective demethylated phenylacetic acid metabolite and ethanol metabolites, which were also identified in the present study. Alternatively, the later metabolites may be generated by direct demethylation of the 4-bromo-2,5-dimethoxyphenylacetic acid and 2-(4-bromo-2,5-dimethoxyphenyl)-ethanol metabolites, respectively.

A metabolic pathway also observed in mice was the hydroxylation of the aliphatic side chain of 2C-B originating two β-hydroxylated isomers. β-Hydroxylation is a minor metabolic pathway for other amphetamines, including *d*-amphetamine, but it is of considerable interest since it occurs in the sympathetic nerve terminals, and its products, norephedrine and *p*-hydroxynorephedrine, have relevant pharmacological activities ([32] and references therein [33,34]).

We have also identified, in the mice urine, a conjugate of the 4-bromo-2,5-dimethoxyphenylacetic acid with glycine, the 4-bromo-2,5-dimethoxyphenaceturic acid metabolite. Conjugation of amphetaminic compounds with glycine is well documented for several species, including mice and human [25,33].

Although extensively metabolised, it must be stress out that 2C-B was also excreted without biotransformation in the mice urine. This is a feature common to other amphetamine derivatives, including *d*-amphetamine [33] and it is of high relevance for forensic control of these substances, namely in human intoxications, since its presence in the urine can unequivocally identify the ingestion of the drug. The data collected in the present in vivo study indicates that 2C-B shares the same metabolic pathways common to other amphetamine-like compounds.

A recently published in vivo study with 2C-B performed in the rat also indicates the formation of the phenylacetic acid and ethanol metabolites indicating that oxidative deamination also occurs in that species [14]. However, according to the same study some discrepancies between the metabolism of 2C-B in the rat and in the mouse can be pointed out. Namely, the rat seems to demethylate both methoxy groups of 2C-B at positions 2 and 5 of the aromatic ring while in mice urine only the 2-*O*-desmethyl-metabolite was found. Additionally, the conjugates found in the rat urine were the *N*-acetylated metabolites of both the 2-*O*-desmethyl- and 5-*O*-desmethyl-metabolites, which were formed to a great extent [14]. In contrast, no *N*-acetylation seems to occur in mice. The later species produces the conjugate of the phenylacetic acid metabolite with glycine, yielding the phenaceturic metabolite. In fact, *N*-acetylation is known to be only a minor reaction in the metabolism of amphetamine and apparently limited to the rat and rabbit [33]. Additionally, there is no evidence for β-hydroxylation of 2C-B in the rat as well as for the oxidative deamination with side chain degradation to produce the benzoic acid metabolite. It therefore seems that more metabolic pathways for 2C-B are active in the mouse when compared to the rat.

In humans, only a preliminary study performed with the urine of a male subject abusing 2C-B is available. In that study unchanged 2C-B, the phenylacetic and benzoic acid metabolites and also the demethylated metabolite, 4-bromo-2-hydroxy-5-methoxyphenethylamine, were identified in urine, thus indicating that oxidative deamination and demethylation are probably important metabolic pathways for 2C-B operating in mice and humans [13]. Although a

more complete pattern of metabolites could be observed in mice urine, it must be considered that human in vivo studies with intoxicated individuals have the limitation that an ideal sampling after optimal exposure periods cannot be established and therefore some metabolites may be missed.

In conclusion, we have identified some metabolic pathways operative for 2C-B metabolism in the mouse. Additionally to the oxidative deamination and demethylation metabolic pathways that have been previously described for 2C-B in man and in the rat, both β-hydroxylation and conjugation of the phenylacetic acid metabolite with glycine seem to occur in the mouse. The identification of 2C-B metabolites may give extremely important clues for the biological and toxicological effects of this drug of abuse and provides new important data for forensic analysis on samples taken from 2C-B abusers. The finding of similar metabolic pathways operating in man and mouse for the metabolism of 2C-B seems to indicate that the mouse is a good animal model for further toxicokinetic and toxicity studies with this drug.

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