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Studies on the metabolism and toxicological detection of the designer drug 4-ethyl-2,5-dimethoxy-β-phenethylamine (2C-E) in rat urine using gas chromatographic–mass spectrometric techniques☆

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Abstract

The phenethylamine-derived designer drug 4-ethyl-2,5-dimethoxy-β-phenethylamine (2C-E) was found to be mainly metabolized in rats by *O*-demethylation, *N*-acetylation, hydroxylation of the ethyl side chain at C2′ or at C1′ followed by oxidation at C1′ to the corresponding ketone, by deamination followed by reduction to the corresponding alcohols or by oxidation to the corresponding acids, and finally combinations of these steps. Most of the metabolites were excreted in conjugated form. The authors' systematic toxicological analysis (STA) procedure using full-scan GC–MS allowed the detection of an intake of a dose of 2C-E 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-E in human urine.

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

1. Introduction

The members of the so-called 2C-series [1] belong to a class of substances abused as designer drugs that are all phenethylamine derivatives. β-Phenethylamine itself is not a common drug of abuse, because it is rapidly metabolized [2], but other compounds of this type like 3,4,5-trimethoxy-β-phenethylamine (mescaline), 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) have obviously psychoactive properties and are often abused [1]. 2C-E was described in Alexander Shulgin's compilation "PIHKAL" as a hallucinogenic substance [2]. Further data are very limited, but descriptions and experience reports on internet web sites (http://www.erowid.org, http://www.lycaeum.org; February 2006) indicate that 2C-E plays a role among drug abusers. Furthermore, 2C-E was identified in several countries on the illicit drug market [3,4]. Lood and Eklund reported that they could identify 2C-E (parent drug) in urine samples of three males [5]. In most countries with exception of Sweden, 2C-E is not a controlled substance. This fact may enhance the spreading among drug abusers, because the more popular members of the 2C-series like 2C-B or 2C-T-7 are all scheduled now in many countries.

Only little information is available on pharmacological and toxicological properties of the members of the 2C-series, but it is known, that they show affinity to 5-HT_2 receptors, acting as agonists or antagonists at different receptor subtypes [6–12]. For 2C-B, partial agonism at α_1-adrenergic receptors was described [13,14]. The chemical structure 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.) [9]. For some of the substances that belong to the 2C-series, analytical data are available [15–23]. Screening for and

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validated quantification of 2C-E itself in human blood plasma has been published using gas chromatography–mass spectrometry (GC–MS) [24]. Furthermore, a GC–MS procedure was presented for detection of 2C-E parent compound in urine [5]. However, for developing toxicological screening procedures, especially in urine, it is a prerequisite to know the metabolism of the compounds in question, especially if they are excreted in urine primarily or even exclusively in form of metabolites. 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 phenethylamines [25–35]. The aim of the study presented here was to identify the 2C-E metabolites in rat urine using GC–MS in the electron ionization (EI) and positive-ion chemical ionization (PICI) modes. In addition, the detectability of 2C-E and its metabolites within the authors' systematic toxicological analysis (STA) procedure in urine by GC–MS was studied [25,26,36,37].

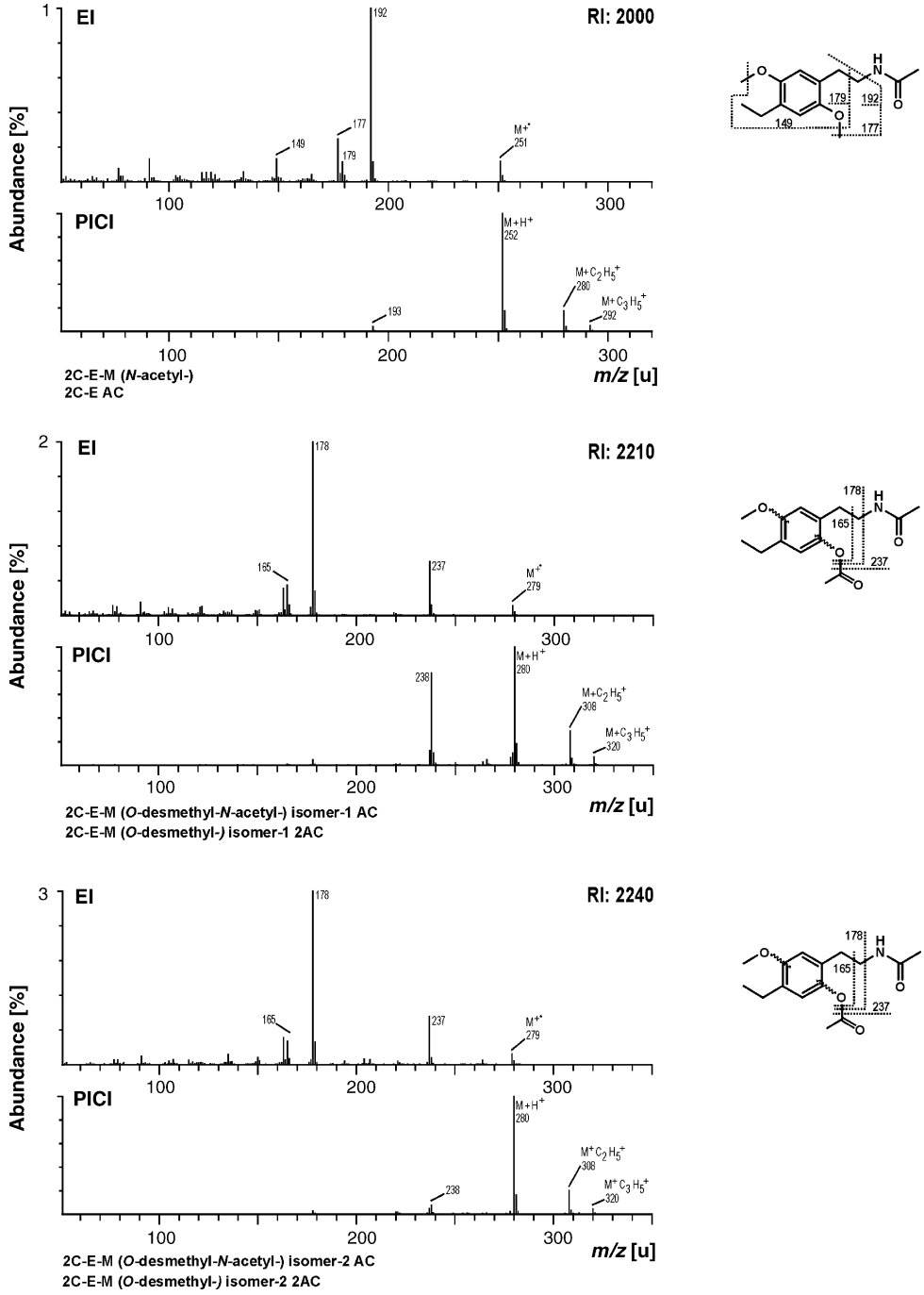


Fig. 1. EI and PICI mass spectra, RIs, structures and predominant fragmentation patterns of 2C-E and its metabolites after acetylation, methylation plus acetylation, trifluoroacetylation, or propionylation. The numbers of the spectra correspond to those of the structures and peaks shown in Figs. 2 and 3.

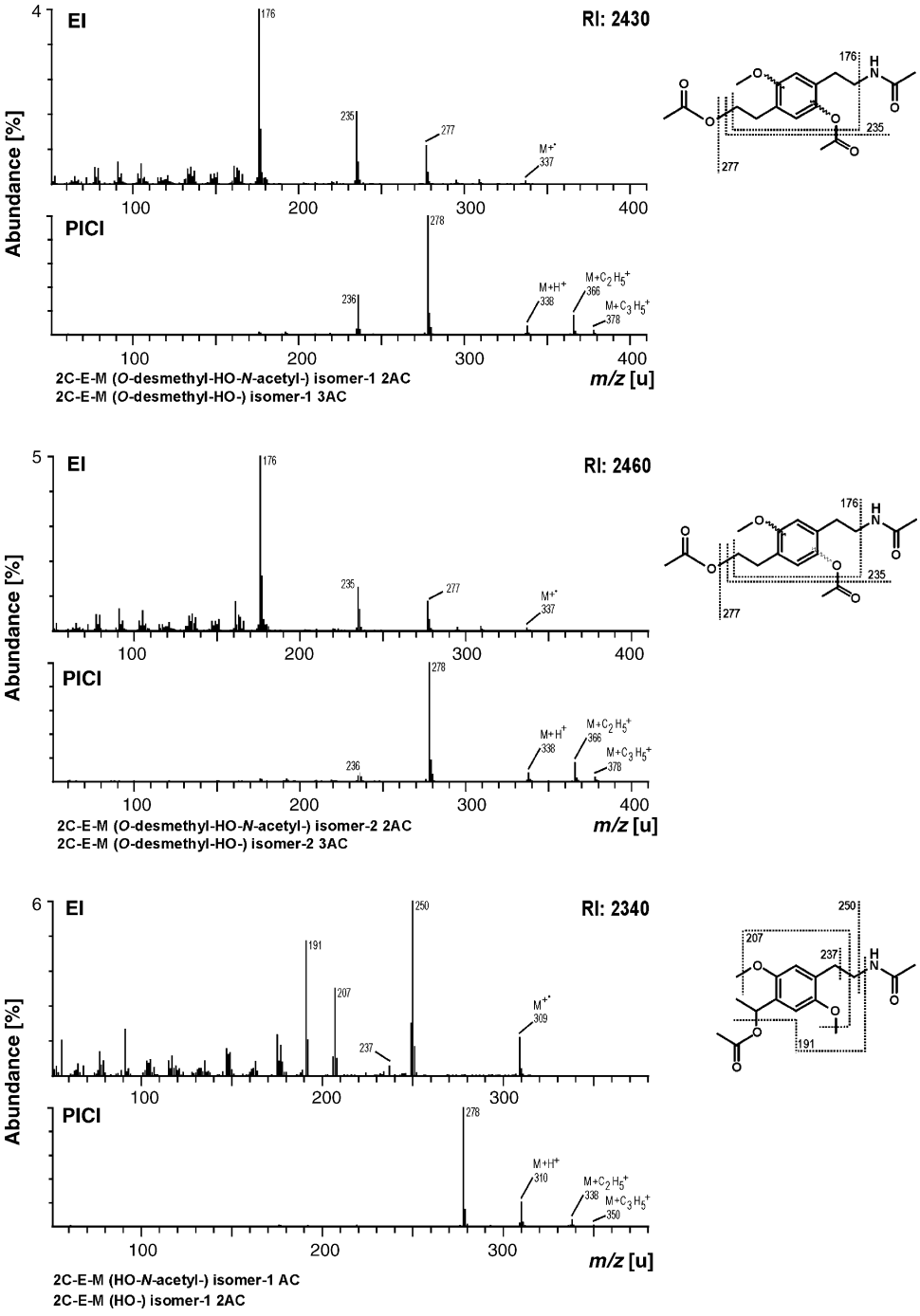


Fig. 1. (*Continued*)

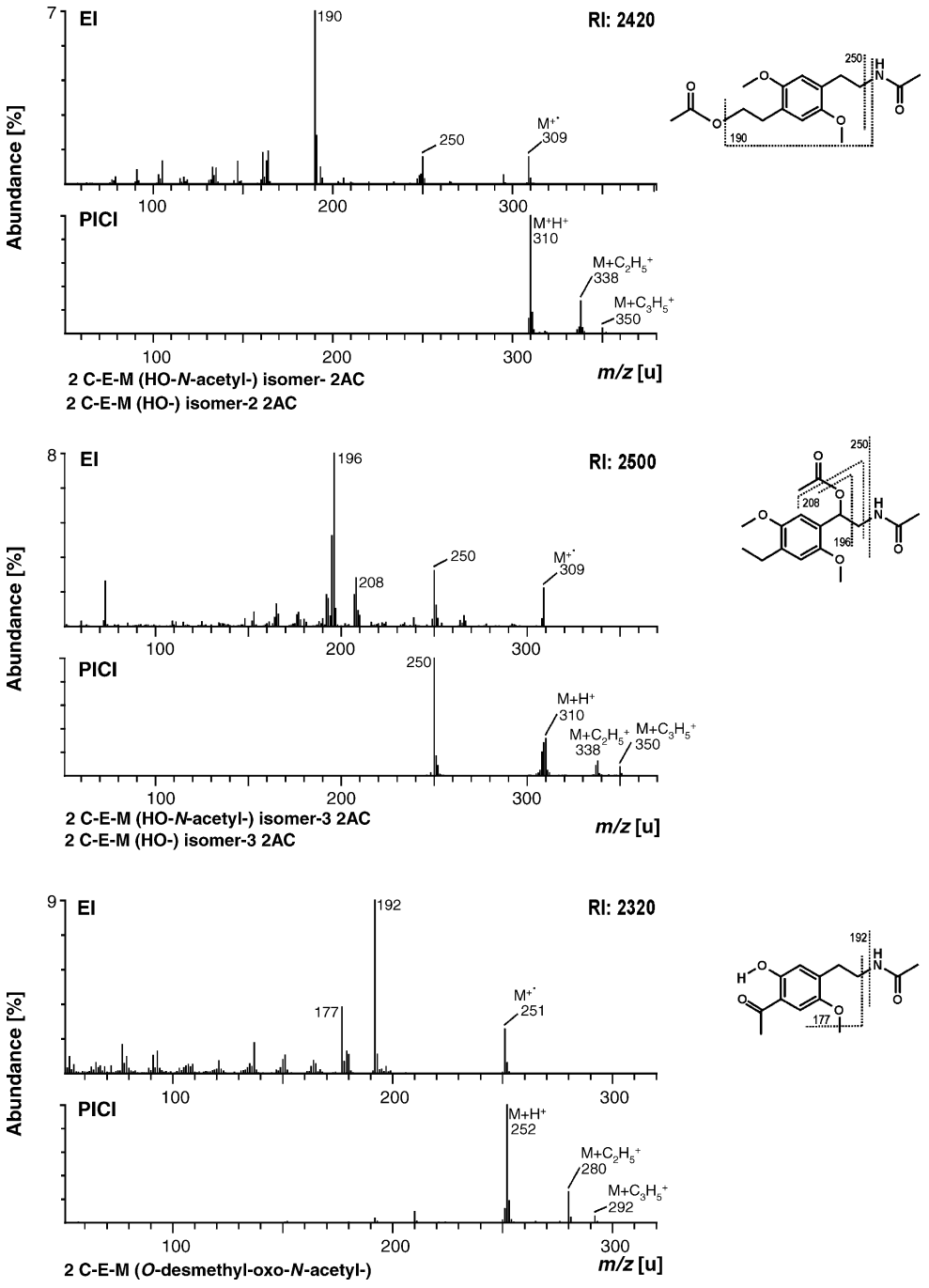
2. Experimental

2.1. Chemicals and reagents

2C-E HCl was provided by Dejachem (Schwendi, Germany) for research purposes. *N*-Methyl-bis(trifluoroacetamide) was obtained from Fluka (Taufkirchen, Germany). All other chemicals and biochemicals were obtained from Merck (Darmstadt, Germany). All chemicals and biochemicals were of analytical grade.

2.2. Urine samples

The investigations were performed using urine of male Wistar rats (about one year old and 400 g body mass (BM), Ch. River, Sulzfleck, Germany) for toxicological diagnostic reasons according to the corresponding German law. They were administered a single 20 mg/kg BM dose for metabolism studies or a 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 faeces over a 24 h period. All samples were



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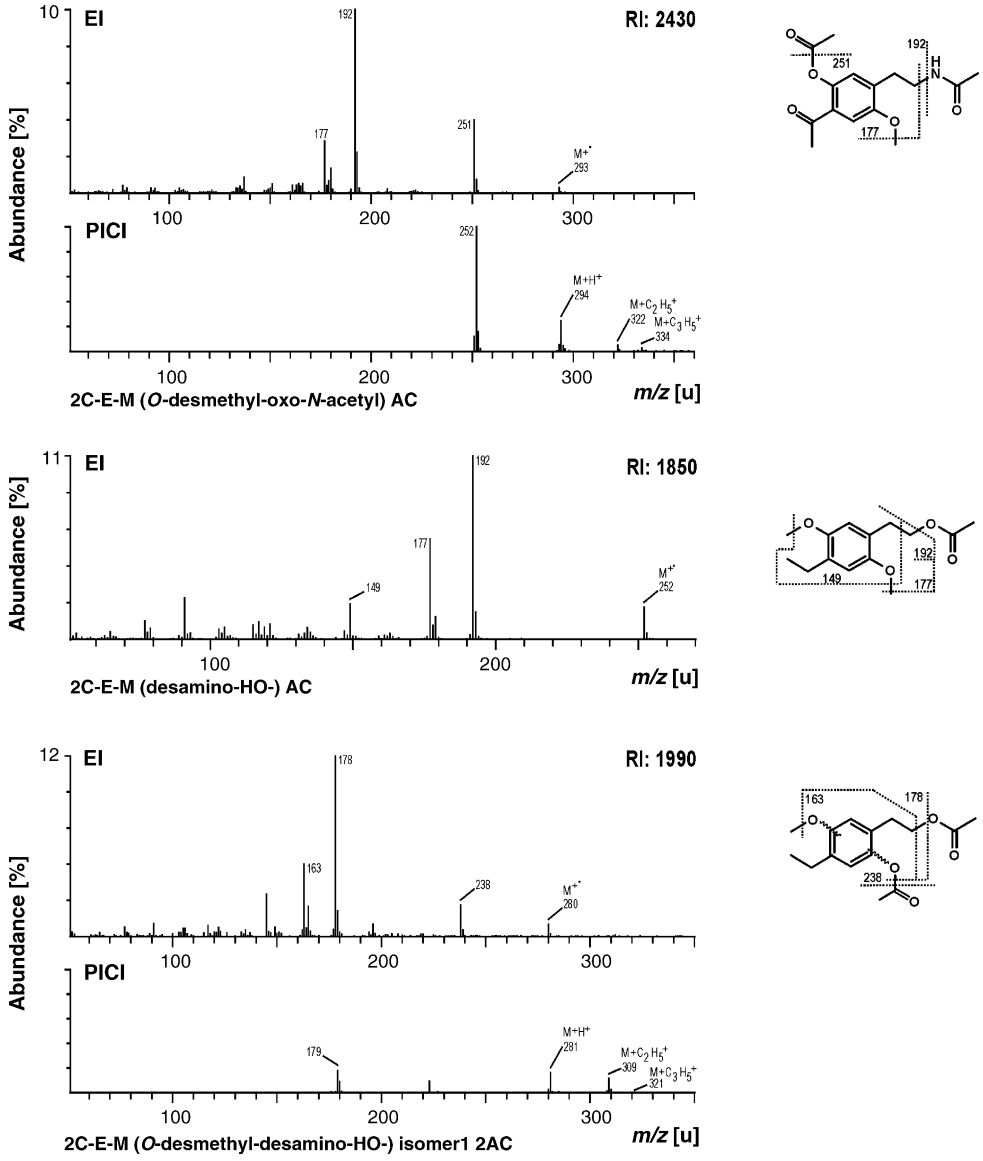
directly analyzed. Blank rat urine samples were collected before drug administration to check whether they were free of interfering compounds.

2.3. Sample preparation for metabolism studies

A 5 ml portion of urine was adjusted to pH 5.2 with acetic acid (1 mol/l) and incubated at 50 °C for 1.5 h with 100 μl of a mixture (100,000 Fishman units/ml) of glucuronidase (EC no. 3.2.1.31) and arylsulfatase (EC no. 3.1.6.1) from *Helix Pomatia*, L. Then pH was adjusted to 8–9 with 1 ml of 37% hydrochloric acid, 2 ml of 2.3 mol/l aqueous ammonium sulfate and 1.5 ml of 10 mol/l aqueous sodium hydroxide and the sample was extracted with 5 ml of a dichloromethane–isopropanol–ethyl acetate mixture (1:1:3; v/v/v). After phase separation by centrifugation, the organic layer was carefully evaporated to dryness at 56 °C under a stream of nitrogen. The residue was derivatized by one of the following three procedures. Acetylation was conducted with 100 μl of an acetic anhydride–pyridine mixture (3:2; v/v), propionylation with 100 μl of a propionic anhydride–pyridine mixture (3:2; v/v), or trifluoroacetylation with 50 μl of *N*-methyl-bis(trifluoroacetamide) for 5 min under



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microwave irradiation at approximately 440 W. After careful evaporation, the corresponding residue was dissolved in 100 μl of methanol (acetylation and propionylation) or 50 μl of ethyl acetate (trifluoroacetylation). Aliquots (2 μl) of the derivatized extracts each 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 mol/l) and incubated at 50 °C for 1.5 h with 100 μl of a mixture (100,000 Fishman units/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 washed with 1 ml of water and 1 ml of 0.01 mol/l hydrochloric acid. The retained non-basic 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 with 100 μl of a solution of diazomethane in diethyl ether, synthesized according to the procedure of McKay et al. [38]. The reaction vial was sealed and left at room temperature for 30 min. Thereafter, the mixture was gently evaporated to dryness under a stream of nitrogen at 56 °C. After evaporation to dryness, the sample was acetylated as described above. The final residue was dissolved in 50 μl of methanol and 2 μl were injected into the GC–MS. All work-up procedures were additionally performed without enzymatic hydrolysis to study which metabolites of 2C-E were excreted as glucuronides/sulfates.

2.4. Sample preparation for toxicological analysis

The urine samples (5 ml) were divided into two aliquots. One aliquot was refluxed with 1 ml of 37% hydrochloric acid for 15 min. Following hydrolysis, the sample was mixed with 2 ml of 2.3 mol/l aqueous ammonium sulfate and

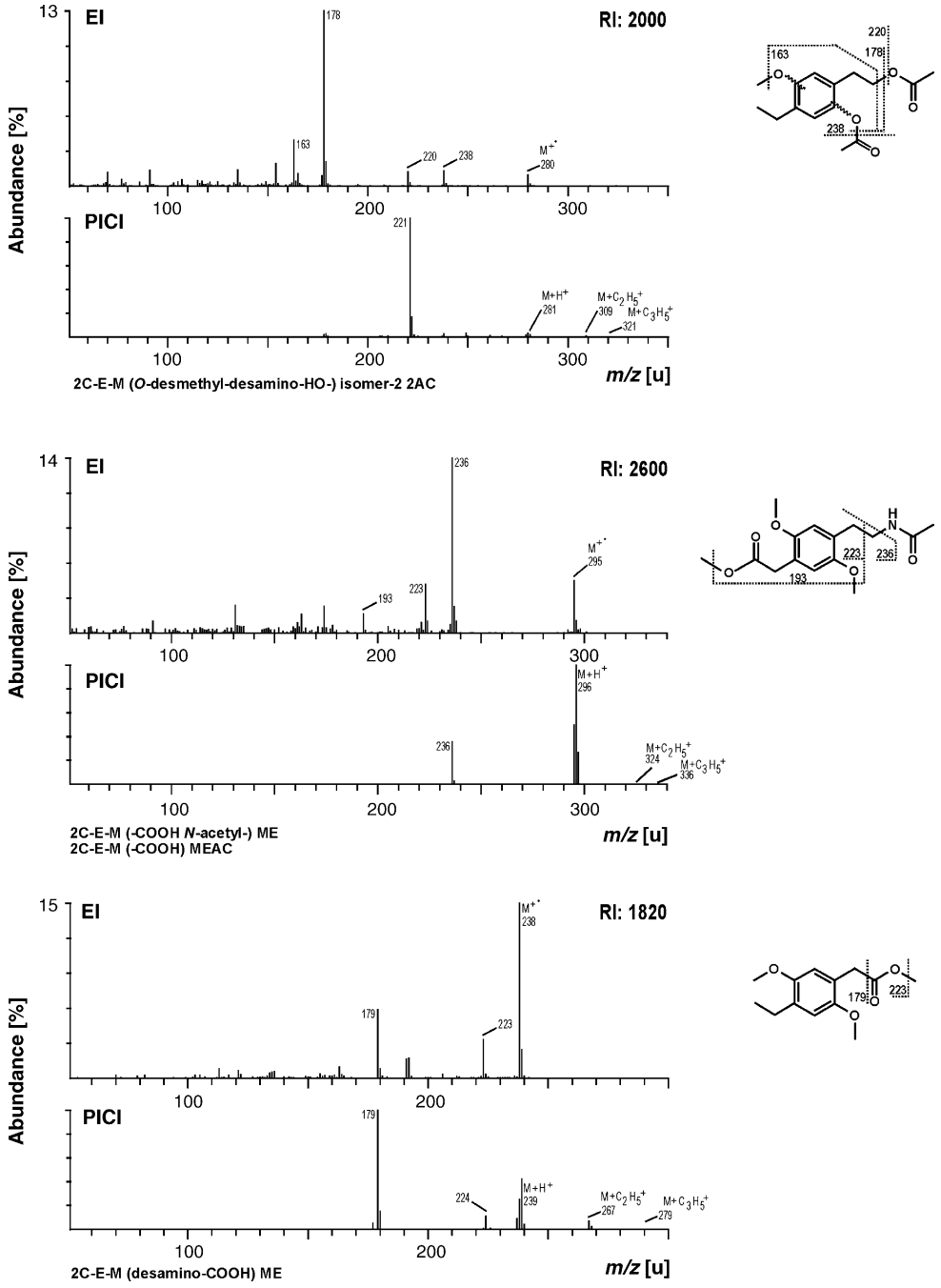


Fig. 1. (*Continued*)

1.5 ml of 10 mol/l aqueous sodium hydroxide to obtain a pH value of 8–9. Before extraction, the other aliquot of native urine was added. This mixture was extracted with 5 ml of a dichloromethane–isopropanol–ethyl acetate mixture (1:1:3; v/v/v). After phase separation by centrifugation, the organic layer was transferred into a glass flask and evaporated to dryness. The residue was derivatized by acetylation with 100 μl of an acetic anhydride–pyridine mixture (3:2; v/v) for 5 min under microwave irradiation at about 440 W. After evaporation of the derivatization mixture, the residue was dissolved in 100 μl of methanol and 2 μl were injected into the GC–MS system.

2.5. GC–MS apparatus

The extracts were analyzed using a Hewlett Packard (Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph combined with a HP 5989B MS Engine mass spectrometer for the metabolism studies or using a Hewlett Packard (Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph combined with a HP 5972A MSD mass spectrometer for STA. For both apparatus, a HP MS ChemStation (DOS series) was used 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

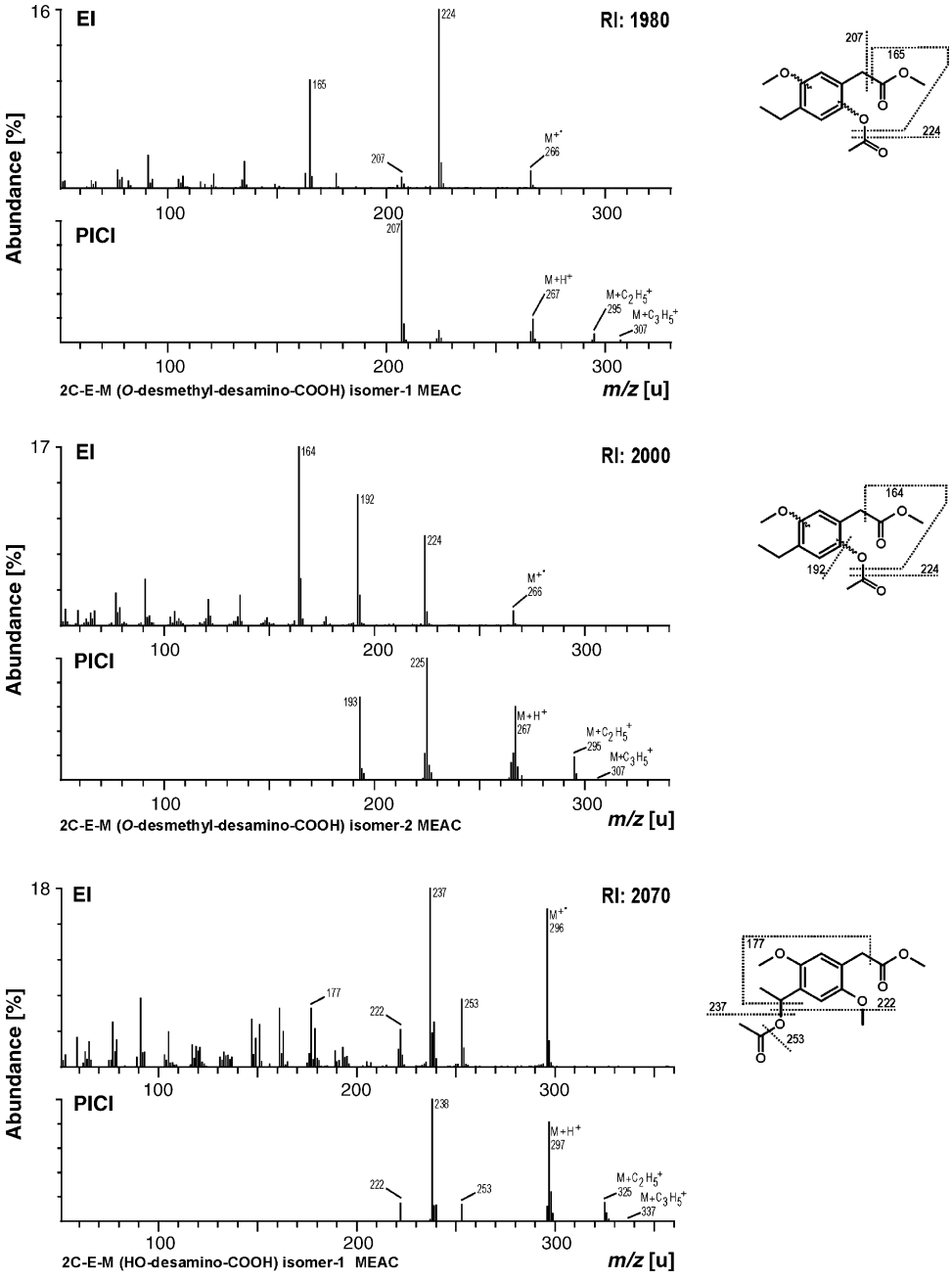


Fig. 1. (*Continued*)

film thickness; injection port temperature, 280 °C; carrier gas, helium; flow-rate 1 ml/min; column temperature, programmed from 100 to 310 °C at 30°/min, initial time 3 min, final time 8 min. The MS conditions for both apparatus were as follows: full-scan mode, *m/z* 50–800 u; EI mode, ionization energy, 70 eV, and for the HP 5989B MS Engine in the PICI mode using methane: ionization energy, 230 eV; ion source temperature, 220 °C; capillary direct interface, heated at 280 °C.

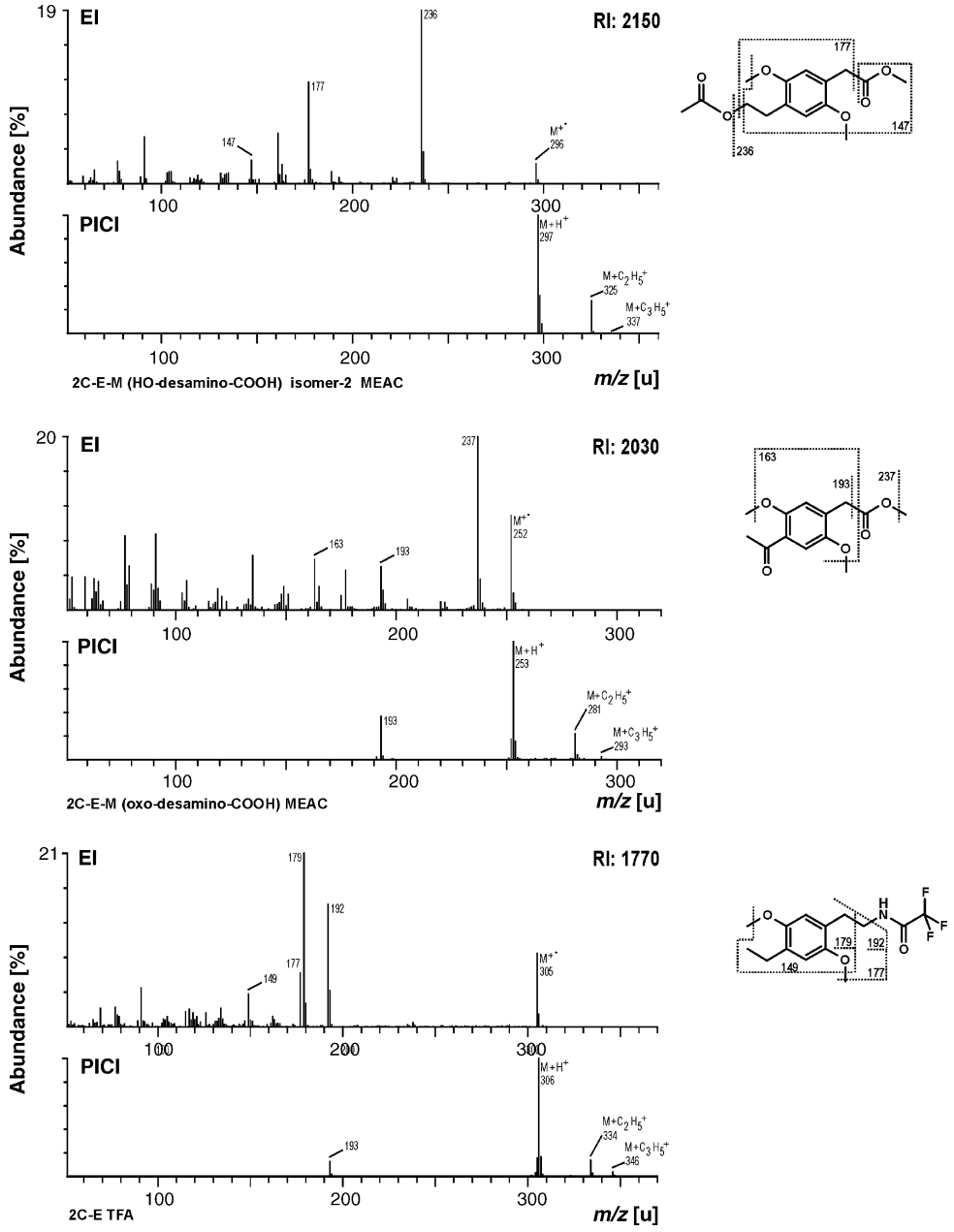
2.6. GC–MS method for STA

For toxicological detection of acetylated 2C-E and its metabolites, mass chromatography with the selected ions *m/z* 192, 251, 178 and 237 was used. The identity of the peaks in the mass chromatograms was confirmed by computerized comparison of the mass spectra underlying the peaks (after background subtraction) with reference spectra recorded during this study.

3. Results and discussion

3.1. Identification of metabolites

The urinary metabolites of 2C-E were separated by GC and identified by EI and PICI MS after gentle enzymatic hydrolysis, extraction, acetylation, trifluoroacetylation, propionylation or methylation plus acetylation. Acetylation was chosen as



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derivatization step, because due to the authors' experiences it is considered as a versatile method for elucidation of the structures of metabolites [25,26,37]. Furthermore, acetylation is the standard derivatization step in the authors' STA. However, using acetylation as derivatization procedure, metabolically *N*-acetylated metabolites cannot be differentiated from acetyl derivatives. For this particular question, the presence of *N*-acetylated metabolites was confirmed in urine extracts after trifluoroacetylation. Unfortunately, the trifluoroacetyl derivatives of some compounds in question could not be detected, probably because of incomplete derivatization or hydrolysis of the trifluoroacetyl esters. Therefore, propionylation was performed to detect these compounds. For detection of acidic metabolites, the urine samples were extracted by SPE, after enzymatic cleavage of conjugates, followed by methylation and acetylation.

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 [39] and Smith and Busch [40]. In order 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 ($[M+C_2H_5]^+$ and $[M+C_3H_5]^+$). The EI and PICI mass spectra of the parent compound, the retention indices (RI), the structures and predominant fragmentation patterns of 2C-E and its metabolites after derivatization are

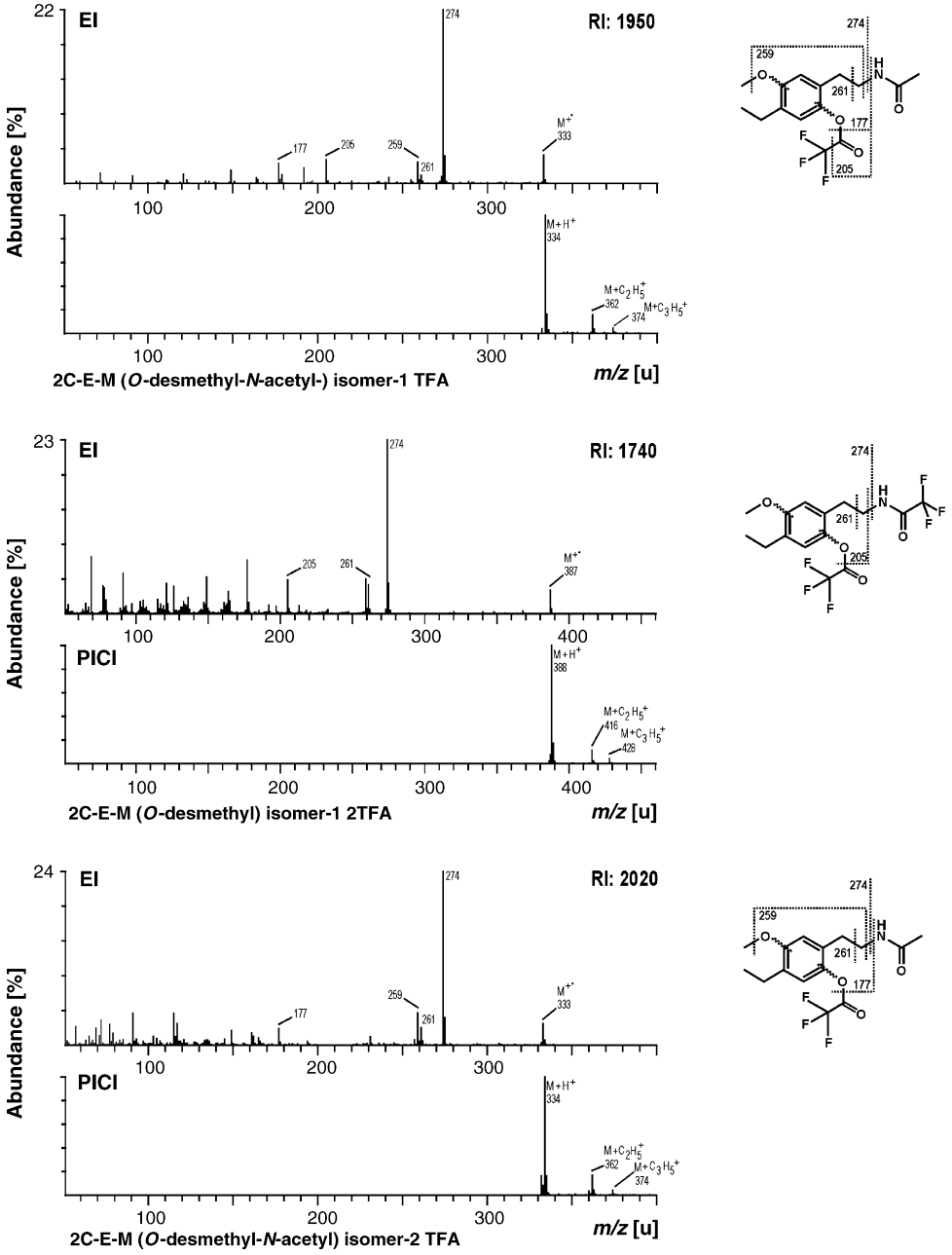


Fig. 1. (*Continued*)

shown in Fig. 1. In the following, the mass spectra numbers in Fig. 1 are given in parentheses. Besides acetylated 2C-E (1), the following metabolites could be identified in the acetylated urine sample: *N*-acetyl-acetoxy-4-ethyl-methoxy-β-phenethylamine isomer 1 (2), *N*-acetyl-acetoxy-4-ethyl-methoxy-β-phenethylamine isomer 2 (3), *N*-acetyl-4-(2′-acetoxyethyl-)-acetoxy-methoxy-β-phenethylamine isomer 1 (4), *N*-acetyl-4-(2′-acetoxyethyl-)-acetoxy-methoxy-β-phenethylamine isomer 2 (5), *N*-acetyl-4-(1′-acetoxyethyl-)-2,5-dimethoxy-β-phenethylamine (6), *N*-acetyl-4-(2′-acetoxyethyl)-2,5-dimethoxy-β-phenethylamine (7), *N*-acetyl-β-acetoxy-4-ethyl-2,5-dimethoxy-β-phenethylamine (8), *N*-acetyl-5-hydroxy-2-methoxy-4-(2′-oxoethyl)-β-phenethylamine (9), *N*-acetyl-5-acetoxy-2-methoxy-4-(2′-oxoethyl)-β-phenethylamine (10), 4-ethyl-2,5-dimethoxy-β-phenylethyl acetate (11), acetoxy-4-ethyl-methoxy-β-phenylethyl acetate isomer 1 (12), and acetoxy-4-ethyl-methoxy-β-phenylethyl acetate isomer 2 (13). In the sample worked-up by SPE, methylation and acetylation, the following compounds could be detected: *N*-acetyl-2,5-dimethoxy-4-methylcarboxymethyl-β-phenethylamine (14), methyl (4-ethyl-2,5-dimethoxyphenyl)acetate (15), methyl (acetoxy-4-ethyl-methoxyphenyl)acetate isomer 1 (16), methyl (acetoxy-4-ethyl-methoxyphenyl)acetate isomer 2 (17), methyl [4-(1′-acetoxyethyl)-2,5-dimethoxyphenyl]acetate (18), methyl [4-(2′-acetoxyethyl)-2,5-dimethoxyphenyl]acetate (19), and methyl [4-(1′-oxoethyl)-2,5-dimethoxyphenyl]acetate (20).

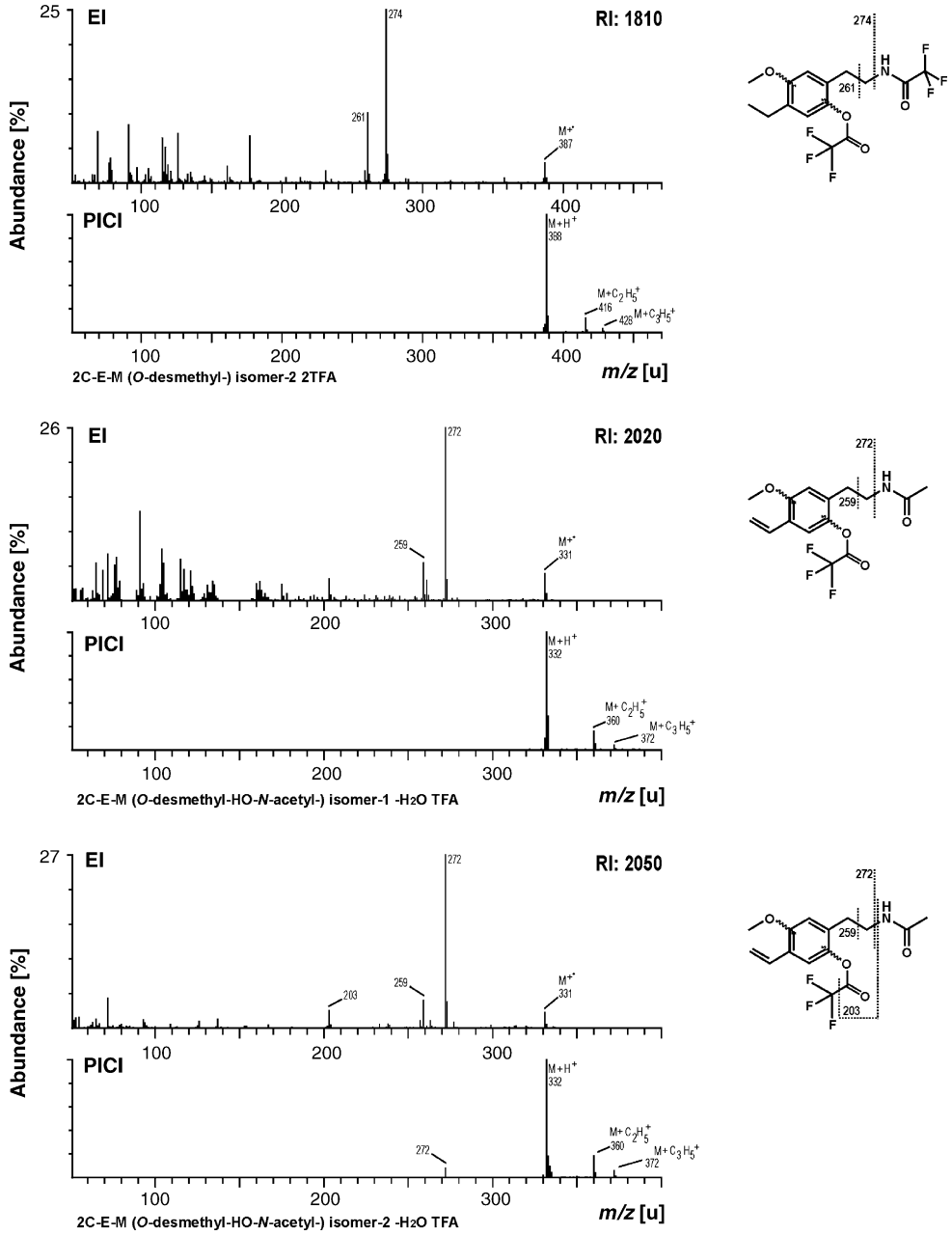


Fig. 1. (*Continued*)

For confirmation whether the *N*-acetyl derivatives were formed by metabolism or by derivatization, the urine extracts were analyzed after trifluoroacetylation. The following metabolites in question could be identified: *N*-acetyl-2C-E (1), trifluoroacetylated 2C-E (21), *N*-acetyl-4-ethyl-trifluoroacetoxy-methoxy-β-phenethylamine isomer 1 (22), *N*-trifluoroacetyl-4-ethyl-trifluoroacetoxy-methoxy-β-phenethylamine isomer 1 (23), *N*-acetyl-4-ethyl-trifluoroacetoxy-methoxy-β-phenethylamine isomer 2 (24), *N*-trifluoroacetyl-4-ethyl-trifluoroacetoxy-methoxy-β-phenethylamine isomer 2 (25), *N*-acetyl-trifluoroacetoxy-methoxy-4-vinyl-β-phenethylamine isomer 1 (26), *N*-acetyl-trifluoroacetoxy-methoxy-4-vinyl-β-phenethylamine isomer 2 (27), *N*-trifluoroacetyl-4-(2′-trifluoroacetoxyethyl-)-trifluoroacetoxy-methoxy-β-phenethylamine (28), *N*-acetyl-*N*-trifluoroacetyl-4-(2′-trifluoracetoxyethyl-)-2,5-dimethoxy-β-phenethylamine (29), *N*-trifluoroacetyl-4-(2′-trifluoroacetoxyethyl)-2,5-dimethoxy-β-phenethylamine (30), and *N*-acetyl-5-trifluoroacetoxy-2-methoxy-4-(2′-oxoethyl)-β-phenethylamine (31). In the propionylated samples, the following compounds could be detected: *N*-acetyl-2,5-dimethoxy-4-(1′-propionyloxyethyl)-β-phenethylamine (32) and *N*-acetyl-4-ethyl-2,5-dimethoxy-β-propionyloxy-β-phenethylamine (33). Only those compounds are shown, that allowed differentiation between metabolically *N*-acetylated metabolites and the free amines.

Unfortunately, PICI mass spectra of certain compound could not be recorded due to their low concentrations in the sample (nos. 11, 28 and 32). Characteristic fragmentation patterns in the

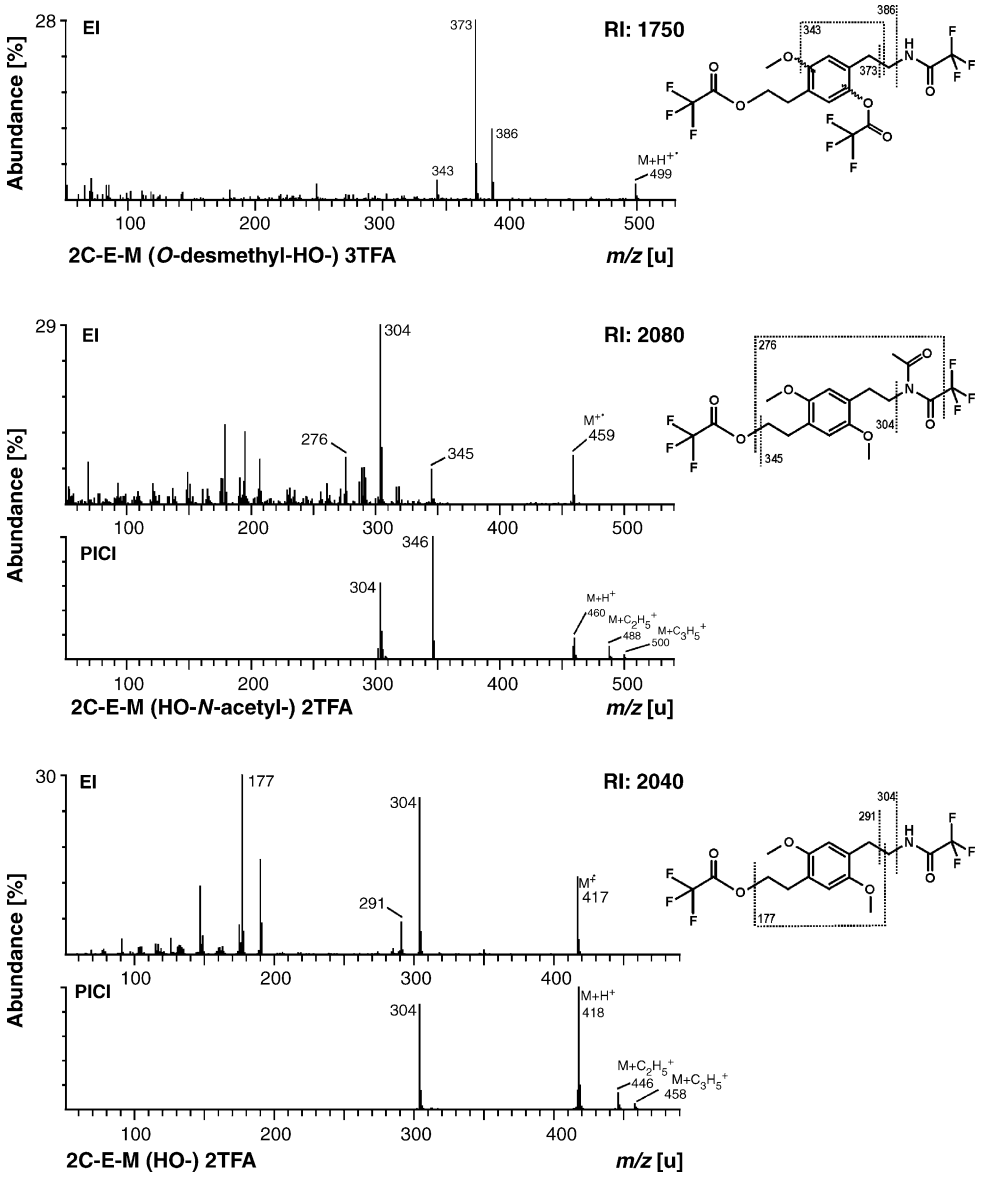
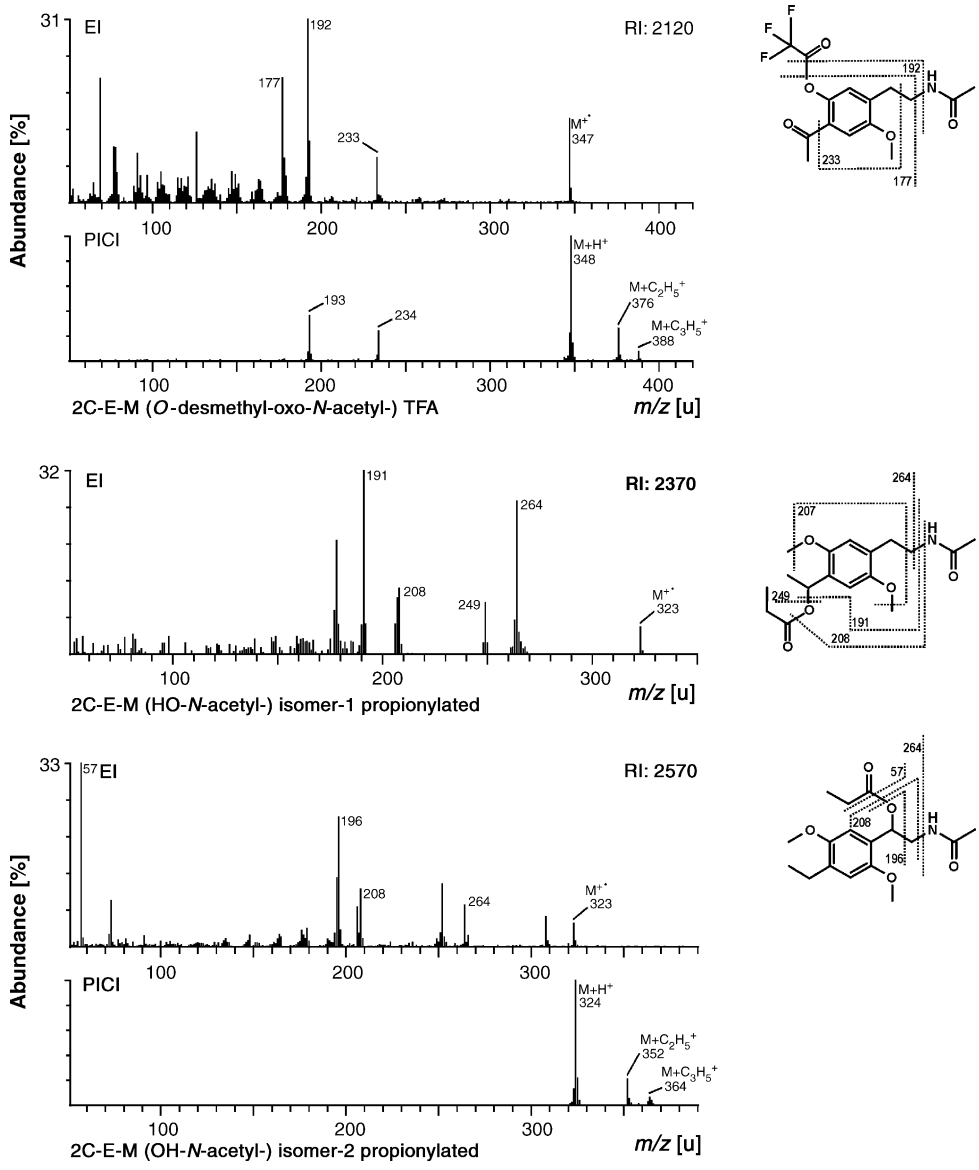


Fig. 1. (*Continued*)

EI spectra consisted of, e.g. the loss of acetamide or trifluoroacetamide, loss of the methyl moieties or the benzyl cleavage, as described for other members of the 2C-series elsewhere [25,26]. The loss of two methyl moieties can alternatively be seen as a neutral loss of CH_2O from one methoxy group [26]. To differentiate between the positions of the hydroxy group in the molecule, the fragmentation patterns were interpreted as follows. Hydroxy moiety at the position 2' of the ethyl group (mass spectra nos. 4, 5, 7, 19, 29 and 30 in Fig. 1) may lead to neutral loss of water or, due to derivatization, to loss of acetic acid (loss of *m/z* 60) or trifluoroacetic acid (loss of *m/z* 114). Location at the 1' position (mass spectra nos. 6, 18 and 32) may lead to radical loss of acetic acid (loss of *m/z* 59), trifluoroacetic acid (loss of *m/z* 113) or propionic acid (loss of *m/z* 73), because the remaining positive charge at C1′ can be stabilized by the ring system, which is not the case if the charge is located at C2′. Location of the hydroxy group at the β-position (mass spectra nos. 8 and 33) may lead to a loss of *m/z* 42 or 56, respectively, because after the loss of acetamide, a conjugated double bond system is formed, which enables the neutral or radical loss of acetic acid or propionic acid, respectively. Therefore, only the loss of an acetyl or propionyl moiety can be seen.

Under the conditions of the GC injection port, the formation of artifacts could be observed, namely the hydroxy metabolites showed the loss of acetic acid or trifluoroacetic acid, respectively (data/mass spectra not shown). Unfortunately, in the case of the metabolites nos. 26 and 27 in Fig. 1, only these artifacts could be detected, but their origin of the corresponding hydroxy metabolites is obvious.

Based on these identified metabolites, the following metabolic pathways could be postulated (Fig. 2): *O*-dealkylation of the parent compound in position 2 or 5, followed by *N*-acetylation and hydroxylation at C2′, or by deamination with oxidation to the corresponding acid or reduction to the



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corresponding alcohol. Second pathway was *N*-acetylation of the parent compound followed on the one hand by hydroxylation at position C1′ of the ethyl side chain with subsequent dealkylation and oxidation to the corresponding ketone and followed on the other hand by β-hydroxylation. A third pathway was the hydroxylation of the parent compound at position C2′ of the ethyl side chain followed by *N*-acetylation and oxidation of the hydroxy group to the corresponding acid. A further pathway was the deamination of the parent compound followed by reduction to the corresponding alcohol or by oxidation to the corresponding acid. The latter was hydroxylated at position C2′ or C1′ followed by oxidation to the corresponding ketone.

A common metabolic step was the *O*-demethylation in position 2 or 5 of the aromatic ring. However, although in the most cases two isomers were detected, the exact position of the resulting hydroxy group could not be determined by means of GC–MS. An exception was the *O*-demethyl-oxo-*N*-acetyl metabolite (mass spectra nos. 9, 10 and 31). It was obvious that this metabolite showed good chromatographic properties in the underivatized form, furthermore it showed incomplete derivatization in the acetylated and trifluoroacetylated sample. Based on these findings and the fragmentation pattern, the structure shown in the mass spectra no. 9 in Fig. 1 was postulated. This structure contains the hydroxy group in position 5 that is able to form a hydrogen bond to the oxo function forming a stable six ring. This hydroxy group should be less polar than a hydroxy function in position 2 leading to the observed chromatographic properties. Furthermore, it should not be as reactive as a hydroxy function in position 2 due to the hydrogen bond. Therefore, the underivatized metabolite was detected.

Most of the metabolites were excreted in conjugated form. Such conjugation was concluded because the peak areas of these metabolites were more abundant after glucuronidase and sulfatase hydrolysis. The metabolites that were conjugated by

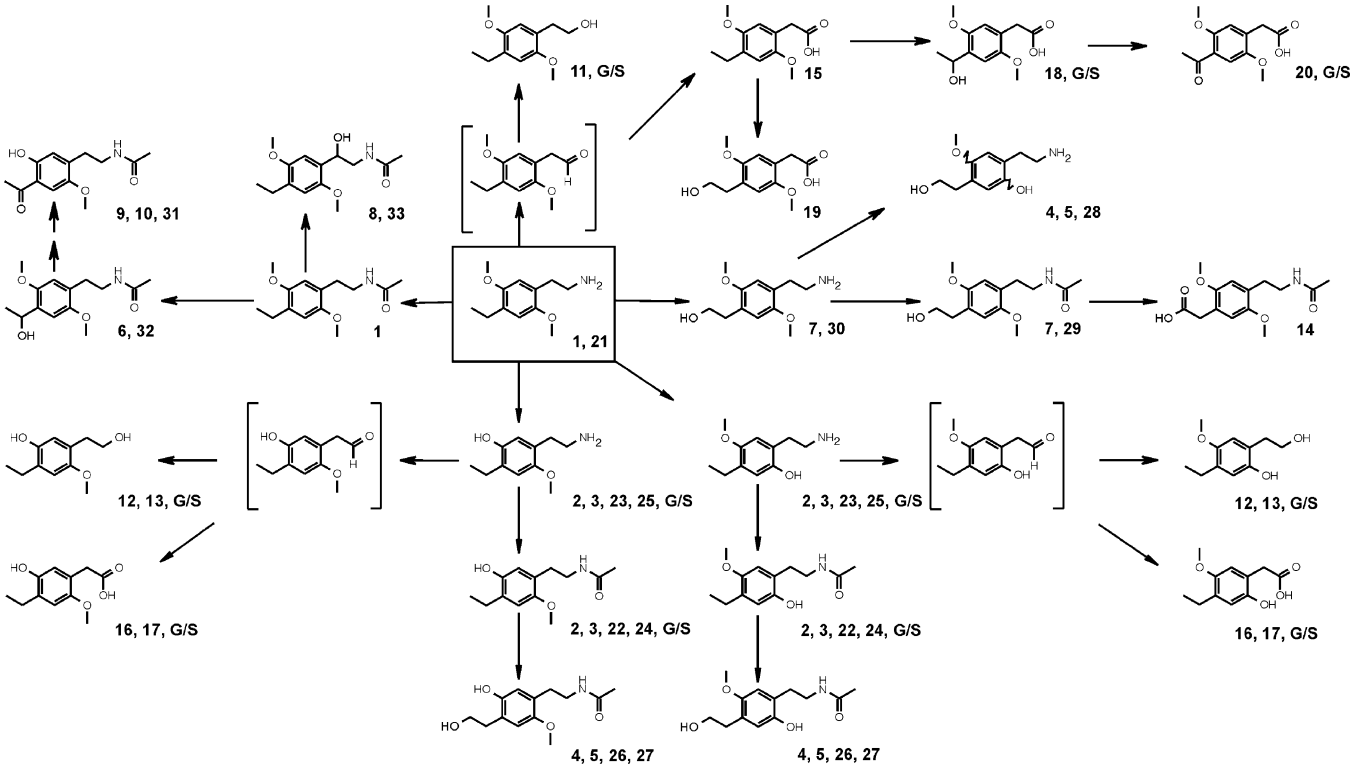

Fig. 2. Proposed scheme for metabolism of 2C-E in rats. The numbering of the compounds corresponds to that of the mass spectra in Fig. 1. The compound in parentheses are assumed intermediates. Compounds excreted as glucuronides/sulfates are marked by G/S.

glucuronidation/sulfation are indicated in Fig. 2. However, conjugation could not be examined for all metabolites, because many metabolites with an aliphatic hydroxy group formed artifacts, as mentioned above.

3.2. Detection by GC–MS within the STA

Acid hydrolysis has proven to be very efficient and fast for cleavage of conjugates [36]. However, some compounds were found to be altered or destroyed during hydrolysis [36]. Therefore, an aliquot of unhydrolyzed urine was added to the hydrolyzed aliquot before extraction. This modified sample preparation was a compromise between the necessity of a quick cleavage of conjugates and the detectability of compounds destroyed during acid hydrolysis. Although the modification of the STA procedure led to lower extract concentrations of compounds excreted in conjugated form, this modified procedure was sufficient, because of the high sensitivity of modern GC–MS apparatus [36].

The samples were extracted at pH 8–9, because metabolic formation of aromatic hydroxy groups may lead to phenolbases that are best extracted at this pH. Using a more alkaline pH for extraction leads to decreased extraction efficacies of such hydroxy metabolites which are often excreted for a longer period of time than the parent compounds [36]. Derivatization of the extracts was indispensable for sensitive detection.

The extraction efficacy determined for 2C-E after STA working-up was 108 ± 19.6% (mean ± S.D., $n = 5$) at 1000 ng/ml of urine.

2C-E and its metabolites were separated by GC and identified by full-scan MS. Mass chromatography with the ions *m/z* 192, 251, 178 and 237 was used to indicate the presence of the *N*-acetyl-*O*-demethyl and *N*-acetyl *O*-demethyl-oxo metabolite of 2C-E, as well as, to a minor extent, to indicate the presence of acetylated 2C-E itself. Fig. 3 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

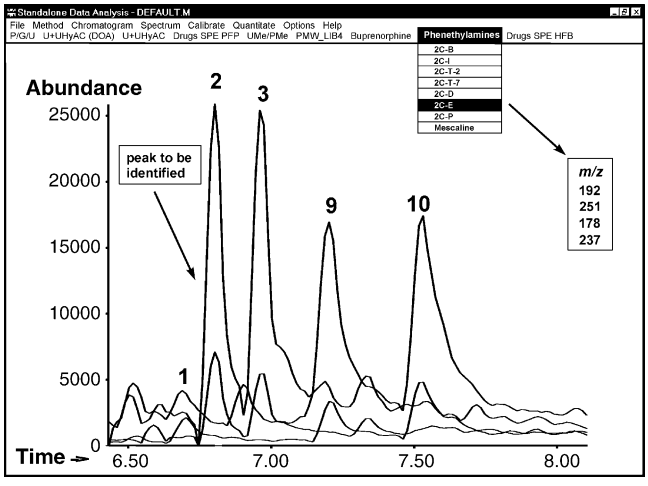

Fig. 3. Typical reconstructed mass chromatograms with the given ions of an acetylated extract of a rat urine sample collected over 24 h after intake of 0.3 mg/kg BM of 2C-E. They indicate the presence of 2C-E and its metabolites. The merged ion chromatograms can be differentiated by their colors on a color screen.

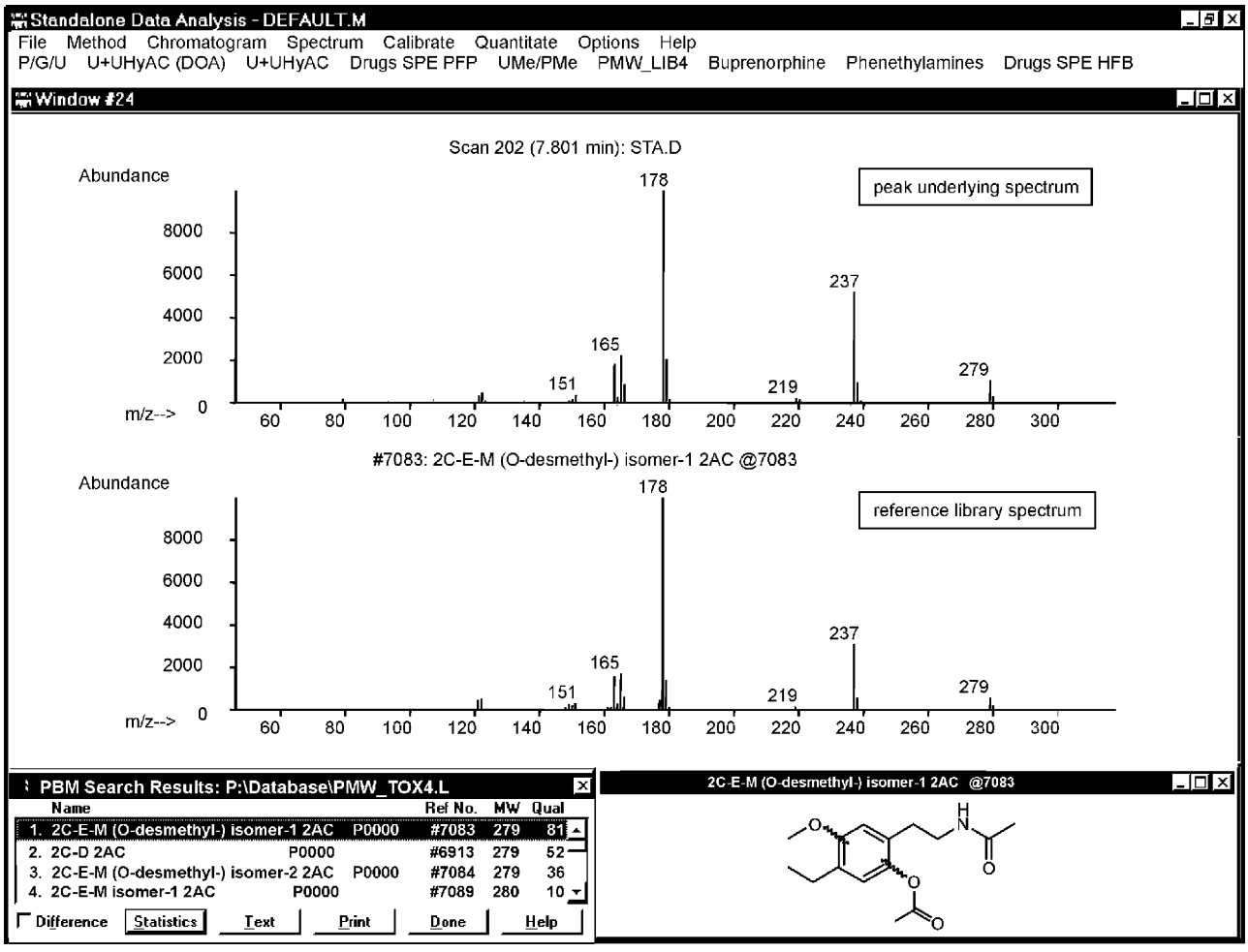

Fig. 4. Mass spectrum underlying the marked peak in Fig. 3, the reference spectrum, the structure, and the hit list found by computer library search.

0.3 mg/kg BM of 2C-E which corresponded to a common users' dose of about 10–25 mg. As exemplified in Fig. 4 for peak 2 in Fig. 3, the identity of peaks in the mass chromatograms was confirmed by computerized comparison of the underlying mass spectrum with reference spectra recorded during this study (Fig. 1). The selected ions *m/z* 192 and 251 were used for indication of acetylated 2C-E itself and detection of its *N*-acetyl-*O*-desalkyl-oxo metabolite, the ions *m/z* 178 and 237 were used for indicating the presence of the *N*-acetyl-*O*-desalkyl metabolites. Ion *m/z* 192 is a characteristic fragment resulting from loss of acetamide, ion *m/z* 251 is the molecular ion of this compound. The fragment ions *m/z* 178 and 237 result from loss of the acetyl moiety and from additional loss of acetamide, respectively. Although interferences by biomolecules or other drugs cannot be entirely excluded, they are rather unlikely, because their mass spectra and/or their RIs should be different. The RIs were recorded during the GC–MS procedure and calculated in correlation with the Kovats' indices [41] of the components of a standard solution of typical drugs which is measured daily for testing the GC–MS performance [42]. The limit of detection of 2C-E was 10 ng/ml of urine (signal-to-noise S/N > 3). For lack of authentic human urine samples, a comparison of the metabolites found in rat and human urine after administration of 2C-E was not yet possible. However, according the earlier studies [43–47] good agreement has been reported for the metabolic pathways between rat and human.

4. Conclusions

The metabolism studies presented here showed that 2C-E was extensively metabolized. The suggested metabolic pathways were similar to those of other members of the 2C-series. *O*-Demethylation and *N*-acetylation were also main metabolic steps for 2C-B, 2C-I, 2C-T-2 and mescaline. Deamination was detected also for 2C-B, 2C-I, 2C-T-2, 2C-T-7 and mescaline. Hydroxylation of the side chain in position 4 was also detected for 2C-T-2 and 2C-T-7. The authors' STA procedure allowed the detection of an intake of a dose of 2C-E in rat urine that corresponds to a common drug users' dose. The target analytes were found to be the *N*-acetyl-*O*-demethyl metabolites of 2C-E, the *N*-acetyl-*O*-demethyl-oxo metabolite as well as acetylated 2C-E itself. As shown for many lipophilic compounds, their metabolites should become the major analytes in late phase of excretion. The authors' experience in metabolism and analytical studies on rats and humans support the assumption that the metabolites found in rat urine should also be present in human

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

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