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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†

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Studies are described on the phase I and II metabolism and the toxicological analysis of the piperazine-derived designer drug 1-(3-trifluoromethylphenyl)piperazine (TFMPP) in rat urine using gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS). The identified metabolites indicated that TFMPP was extensively metabolized, mainly by hydroxylation of the aromatic ring and by degradation of the piperazine moiety to *N*-(3-trifluoromethylphenyl)ethylenediamine, *N*-(hydroxy-3-trifluoromethylphenyl)ethylenediamine, 3-trifluoromethylaniline, and hydroxy-3-trifluoromethylaniline. Phase II reactions included glucuronidation, sulfatation and acetylation of phase I metabolites. 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 TFMPP and its above-mentioned metabolites in rat urine after single administration of a dose calculated from the doses commonly taken by drug users. Assuming similar metabolism, the described STA procedure should be suitable for proof of an intake of TFMPP in human urine.

KEYWORDS: TFMPP; metabolism; urine; GC/MS; LC/MS

INTRODUCTION

Piperazine-derived compounds like *N*-benzylpiperazine (BZP), 1-(3,4-methylenedioxybenzyl)piperazine (MDBP), 1-(4-methoxyphenyl)piperazine (MeOPP), 1-(3-trifluoromethylphenyl)piperazine (TFMPP) and 1-(3-chlorophenyl) piperazine (mCPP) have entered the illicit drug market as a new group of designer drugs. Besides BZP (scene name ''A2''), TFMPP is the most abused compound of this drug group. It is mentioned as an active hallucinogen in scene books[2] and also on so-called drug information web sites (http://www.erowid.org, http://www.lycaeum.org). Seizures could be made throughout the world[3–11] and organizations that check the ''purity'' of illegally sold tablets report their occurrence more and more often (http://www.dancesafe.org). Even a fatality involving piperazine-derived compounds has been reported.[12] In 2002, the increasing abuse of BZP and TFMPP in the USA led to the temporary placement of these two compounds into schedule I of the Controlled Substance Act (CSA).[13] Generally, they are touted as capsules, tablets or pills but also in powder or liquid form and are consumed as an alternative to amphetamine-derived designer drugs.[13]

TFMPP, a 1-arylpiperazine, is known as a centrally active compound with serotoninergic properties.[14–20] It has widely been used as a pharmacological probe drug for drug discrimination procedures in animals for this reason.[21–30] On the internet, drug abusers describe its effects to be similar to those of the classical designer drugs methylenedioxymethamphetamine (MDMA, ecstasy) or methylenedioxyethylamphetamine (MDE), which could partly be supported by animal studies.[22,26,31] The reported anxiogenic effects and panic reactions could also be supported by animal studies[30,32,33] and human studies on structurally related compounds.[34,35] Further pharmacological effects of TFMPP and other arylpiperazines are extensively reviewed by Murphy *et al*.[34]

Metabolism and screening procedures for the detection of BZP and mCPP and their metabolites in urine have

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†Part of these results were reported at the XIIth GTFCh Symposium in Mosbach, April 26–28, 2001.[1]

already been described,[36,37] in contrast to those of TFMPP. However, knowledge about metabolic steps is a prerequisite for developing toxicological screening procedures, especially if the compounds are excreted in urine only as their metabolites,[38,39] and for toxicological risk assessment, where metabolites may also play a major role. Some GC/MS data on TFMPP itself have been published[4] as well as a screening and validated quantification procedure for TFMPP in human blood plasma using GC/MS.[40] However, in clinical and forensic toxicology as well as in doping control, screening procedures in urine are necessary, because the taken drugs or toxicants can be detected for several hours or even days after ingestion, in contrast to blood analysis which covers only a few hours.[41,42] Such urinalysis of TFMPP has not yet been published.

The aim of the study presented here was to identify the phase I and II metabolites of TFMPP in rat urine using GC/MS in the EI and PICI mode as well as LC/MS in the ESI mode. In addition, the detectability of TFMPP within the authors' systematic toxicological analysis (STA) procedure in urine by GC/MS was studied.[36,38,43,44]

EXPERIMENTAL

Chemicals and reagents

TFMPP-HCl was provided by Hessisches Landeskriminalamt and the reference metabolite 3-trifluoromethylaniline was obtained from Lancaster Synthesis (Mühlheim, Germany). All other chemicals and biochemicals were obtained from E. Merck (Darmstadt, Germany) and were of analytical grade.

Urine samples

The investigations were performed using urine of male rats (Wistar, Ch. River, Sulzfleck, Germany) which were administered a single 50 mg/kg body mass (BM) (for metabolism studies) or a 1 mg/kg BM dose (for STA) in aqueous solution by gastric intubation. Urine was collected separately from the faeces over a 24-h period. All samples were directly analyzed. Blank rat urine samples were collected before drug administration to check whether the samples were free of interfering compounds.

Sample preparation for identification of metabolites by GC/MS

A 5-ml portion of urine was adjusted to pH 5.2 with acetic acid (1 mol/l) and incubated at 37 °C for 12 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) from *Helix pomatia*, then adjusted to pH 8–9 and extracted with 5 ml of a dichloromethane/isopropanol/ethyl acetate mixture (1 : 1 : 3, v/v/v). After phase separation by centrifugation, the organic layer was transferred into pear-shaped flasks and carefully evaporated to dryness at 56 °C and the residue was derivatized.[45] Acetylation was conducted with 100 µl of an acetic anhydride/pyridine mixture (3 : 2, v/v) for 5 min under microwave irradiation at about 440 W.[46–48] After careful evaporation, the residue was dissolved in 100 µl of methanol and 2 µl of this solution were injected into the GC/MS system. Trifluoroacetylation was conducted with 50 µl of trifluoroacetic anhydride and 50 µl of ethyl acetate for 5 min under microwave irradiation at about 440 W.[47] After careful evaporation of the derivatization mixture the residue was dissolved in 50 µl of alcohol- and water-free ethyl acetate and 1–2 µl were injected into the GC/MS system.[47] The same procedure with the exception of enzymatic hydrolysis was used to study whether metabolites of TFMPP are excreted as conjugates.

A second urine sample was worked up as described above, but pH was adjusted to 4–5. The corresponding extract was analyzed after methylation and subsequent acetylation. After reconstitution of the extraction residue in 50 µl of methanol, methylation was conducted with 50 µl of a solution of diazomethane in diethyl ether, synthesized according to the procedure of McKay *et al*. using 1-methyl-3-nitro-1-nitroso-3-nitroguanidine, KOH and diethyl ether.[49] The reaction vials were sealed and left at room temperature for 15 min. Thereafter, the mixture was once again carefully evaporated to dryness under a stream of nitrogen, acetylated as described above and finally redissolved in 50 µl of methanol and 3 µl of this sample were injected into the GC/MS system.[47]

Sample preparation for identification of glucuronides by GC/MS

A 1-ml aliquot of rat urine was loaded on solid-phase extraction (SPE) cartridges (Isolut C2, 100 mg, 1 ml), previously conditioned with 1 ml of acetonitrile and 1 ml of purified water. After extraction, the cartridges were washed with 1 ml of acetonitrile/purified water (1 : 10, v/v). Vacuum was applied until the cartridges were dry and the analytes were eluted with 1 ml of acetonitrile into autosampler vials. The eluate was carefully evaporated to dryness under a stream of nitrogen at 56 °C and then reconstituted in 50 µl of ethyl acetate and silylated after addition of 50 µl of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) for 5 min under microwave irradiation at 440 W. A 2-µl aliquot of this mixture was injected into the GC/MS system with an alcohol- and water-free syringe. The same procedure was conducted with urine after enzymatic conjugate cleavage as well as with blank rat urine.

Sample preparation for identification of glucuronides and sulfates by LC/MS

For the metabolism studies performed by LC/MS, rat urine samples were ultrafiltrated (Sartorius Minisart RC4, poresize 0.2 µm) and 1 µl of the filtrate was injected into the LC/MS system without further sample preparation steps. The same procedure was conducted with urine after enzymatic conjugate cleavage as well as with blank rat urine.

Sample preparation for STA by GC/MS

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 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 and this solution 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 pear-shaped flasks and carefully evaporated to dryness at 56 °C. 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 careful evaporation of the derivatization mixture, the residue was dissolved in 100 µl of methanol and 2 µl were injected into the GC/MS system.[43]

GC/MS system for identification of metabolites and STA

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 methylsilicone, 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 °C/min, initial time 3 min, final time 8 min. The MS conditions were as follows: full scan mode, m/z 50–550 u; EI ionization mode: ionization energy, 70 eV; chemical ionization using methane, positive mode (PICI): ionization energy, 230 eV; ion source temperature, 220 °C; capillary direct interface heated at 260 °C.

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

TFMPP and its metabolites were separated by GC and identified by MS in acetylated, trifluoroacetylated, methylated plus acetylated or underivatized urine extracts. For toxicological analysis of TFMPP and its metabolites, mass chromatography with the selected ions m/z 157, 161, 174, 200, 216, and 330 was used. These ions were selected from the mass spectra recorded during this study. The identity of the peaks in the mass chromatograms was confirmed by computerized comparison[50] of the mass spectra underlying the peaks (after background subtraction) with reference spectra (Fig. 1) recorded during this study.

LC/MS system for identification of glucuronides and sulfates

Studies for the identification of glucuronides were performed using an Agilent Technologies (AT, Waldbronn, Germany) AT series 1100 LC/MSD, SL version, using electrospray ionization (ESI) G1948A, including an AT 1100 Series HPLC system which consisted of a degasser, a binary pump, a column thermostat and an autosampler. Gradient elution was performed on a Merck LiChroCART column (125 × 2 mm i.d.) with Superspher60 RP Select B as stationary phase and a LiChroCART 10-2 Superspher60 RP Select B guard column. The mobile phase consisted of 5 mM aqueous ammonium formate adjusted to pH 3 with formic acid (eluent A) and acetonitrile (eluent B). Before use, the mobile phases were degassed for 30 min in an ultrasonic bath. During use, the mobile phase was degassed by the integrated AT 1100 series degasser. Until the beginning of the analysis, the HPLC system was flushed with a 95 : 5 mixture of the two eluents. The gradient and the flow rate were programmed as follows: 0–5 min 5% B (flow: 0.4 ml/min), 5–11 min gradient 5–90% B (flow: 0.4 ml/min), 11–13 min 90% B (flow: 0.6 ml/min), 13–14 min gradient 90–5% B (flow: 0.6 ml/min), 14–14.5 min 5% B (flow: gradient 0.6–0.4 ml/min), 14–18 min 5% B (flow: 0.4 ml/min). Subsequently, re-equilibration of the HPLC column was achieved and the autosampler could begin with the next injection. The following ESI inlet conditions were applied: drying gas, nitrogen (7 l/min, 300 °C) and nebulizer gas, nitrogen (103.5 kPa); capillary voltage, 4000 V; negative full scan mode (scan range m/z 50–550), fragmentor voltage, −200 V. Tuning of the mass spectrometer was performed with the help of the autotune feature of the LC/MS ChemStation software (rev. A.08.03) using the ESI acetonitrile solution tuning mix supplied with the apparatus.

LC/MS procedure for identification of glucuronides and sulfates

The corresponding glucuronides were screened for in the full-scan mode by mass chromatography in the −100 V and −200 V (fragmentor voltage) traces of the same run with the ions m/z 245 and 421 for the hydroxy-TFMPP glucuronide and 245 and 325 for the hydroxy-TFMPP sulfate.

RESULTS AND DISCUSSION

Sample preparation

Cleavage of conjugates was necessary before extraction in order not to miss conjugated metabolites. For studies on the metabolism, gentle enzymatic hydrolysis was preferred. For studies on the toxicological detection, rapid acid hydrolysis was performed. Acid hydrolysis has been proven to be very efficient and fast for cleavage of conjugates.[41,43,48,51–53] However, some compounds were altered or destroyed during hydrolysis.[43,44] Therefore, one part 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 and metabolites 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 systems.[43,44]

The samples were extracted at pH 8–9, because metabolic formation of aromatic hydroxy groups may lead to phenol bases which 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.[51,54–58] Derivatization of the extracts was indispensable for sensitive detection. The extraction efficacy determined for TFMPP after STA workup was 61 ± 8% (n = 5) at 1000 ng/ml. Careful evaporation of the extraction and derivatization mixtures should be performed in as short a time as possible due to the rather high volatility of TFMPP.

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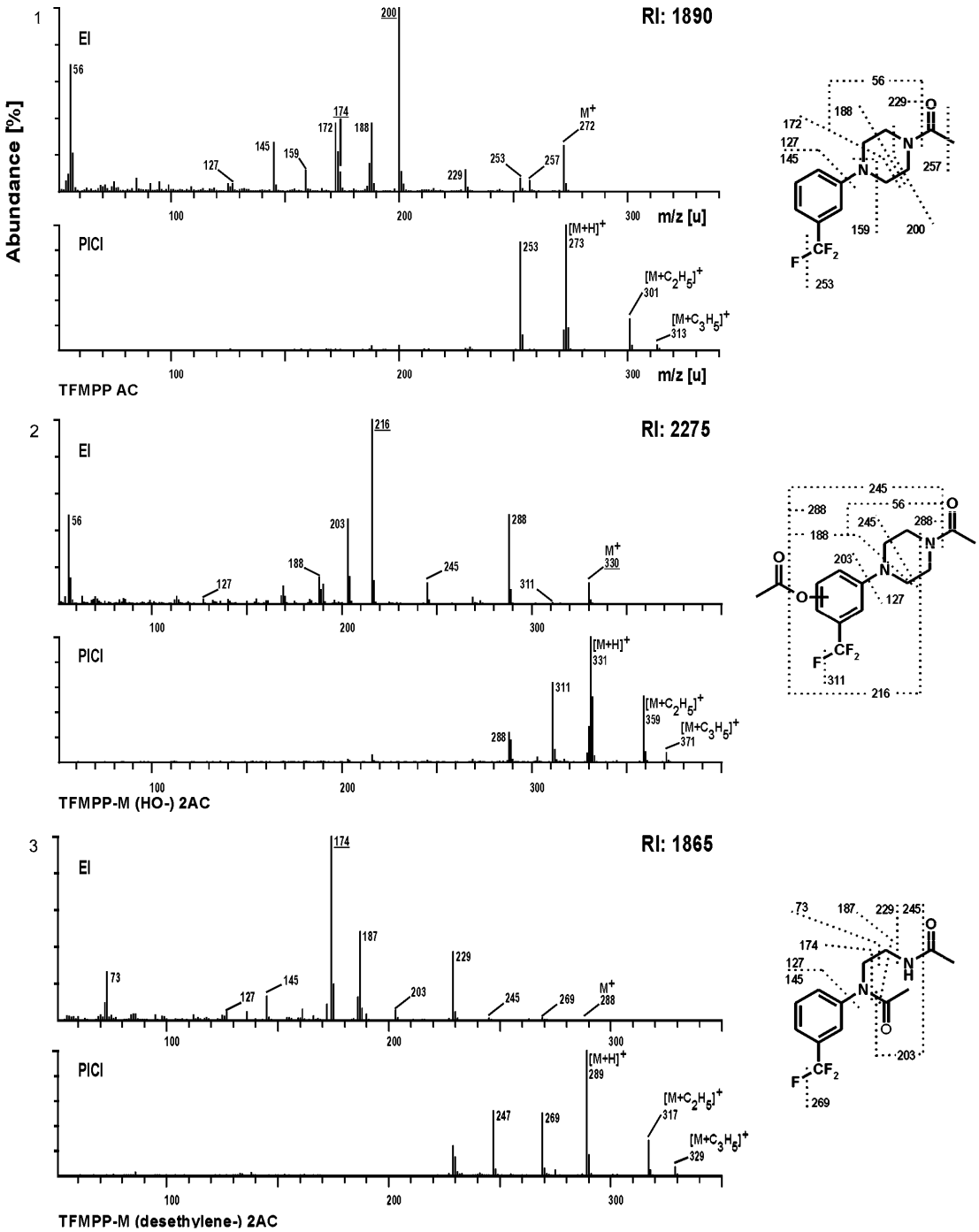

Figure 1. EI and PICI mass spectra, the RIs, structures and predominant fragmentation patterns of TFMPP and its metabolites after acetylation. The numbers of the spectra correspond to those in Figs 4 and 5. Ions selected for the toxicological detection are underlined (axes only labelled for spectra no. 1).

Identification of metabolites by GC/MS and LC/MS

The urinary metabolites of TFMPP were separated by GC and identified by EI and PICI-MS after enzymatic hydrolysis, extraction, acetylation, trifluoroacetylation, methylation plus acetylation or without derivatization. Trifluoroacetylation was conducted in order to differentiate between acetyl-derivatized and acetyl-conjugated metabolites. The postulated structures of the 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[59] and Smith and Busch.[60] 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. The identity of 3-trifluoromethylaniline could further be confirmed by comparing its mass spectrum and the gas chromatographic retention index (RI) with those of the corresponding reference substance. The EI and PICI mass spectra, the RIs, the structures and predominant fragmentation patterns of

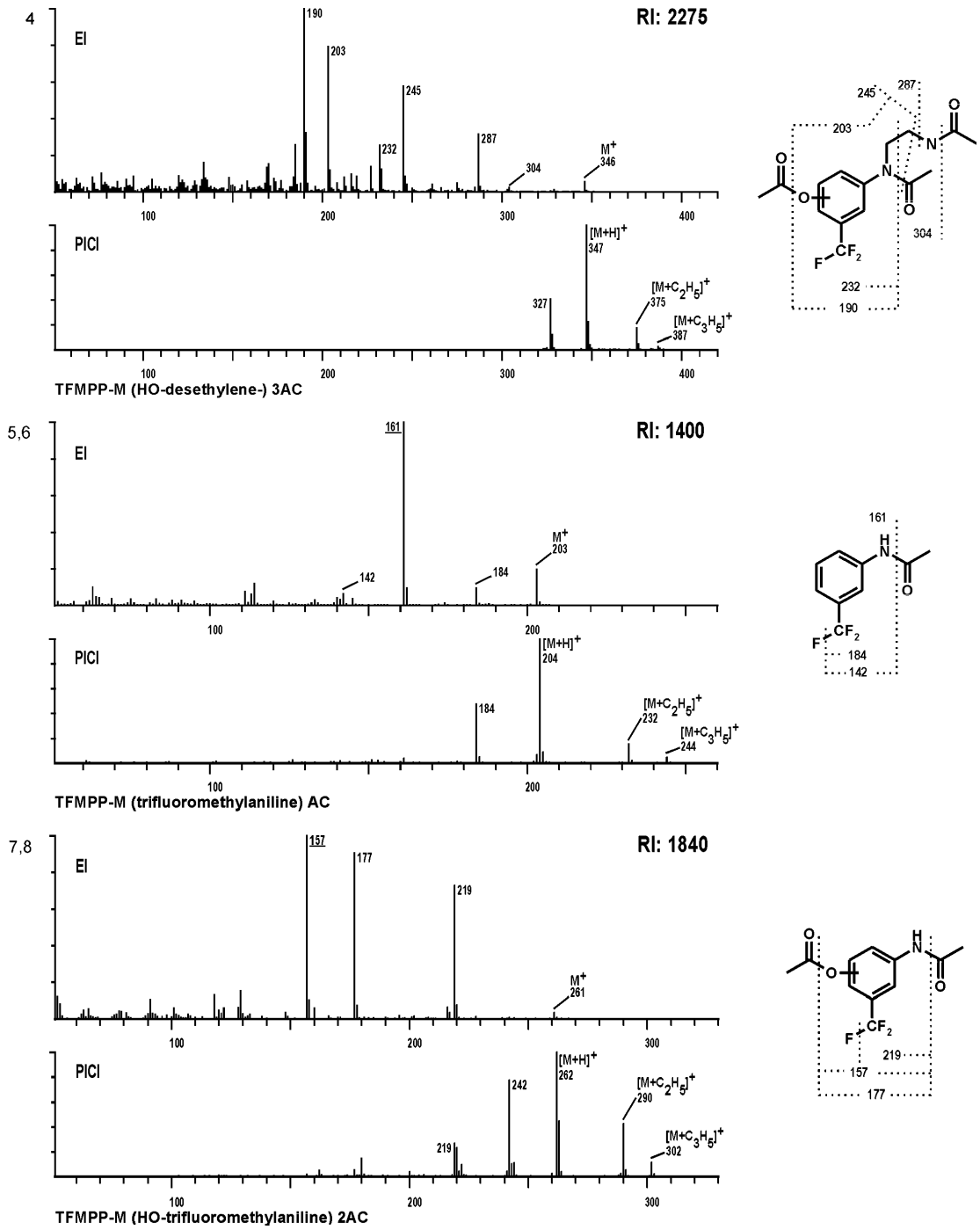

Figure 1. (*Continued*).

acetylated TFMPP and its acetylated metabolites are shown in Fig. 1. The spectra are arranged according to the numbers given in Fig. 4. In order to check for acidic metabolites, the urine samples were also extracted after cleavage of conjugates at acidic pH (4–5) and the corresponding extracts were analyzed after methylation followed by acetylation.[45] However, no acidic metabolites could be found.

Besides TFMPP (mass spectra no. 1 in Fig. 1), the following metabolites could be identified in rat urine: hydroxy-TFMPP (mass spectra no. 2), *N*-(3-trifluoromethylphenyl) ethylenediamine (mass spectra no. 3), *N*-(hydroxy-3-trifluoromethylphenyl)ethylenediamine (mass spectra no. 4), 3-trifluoromethylaniline (after acetylation, mass spectra no. 5), *N*-acetyl-3-trifluoromethylaniline (mass spectra no. 6), hydroxy-3-trifluoromethylaniline (after acetylation, mass spectra no. 7) and *N*-acetylhydroxy-3-trifluoromethylaniline (mass spectra no. 8). The *N*-acetyl derivatives were detected of course in the acetylated extracts, but also in underivatized extracts as well as in trifluoroacetylated extracts. In the latter, hydroxy-3-trifluoromethylaniline could be detected in acetylated plus trifluoroacetylated form (spectra no. 9 in Fig. 2). Therefore, it could be concluded that the aniline derivatives were metabolically acetylated probably by *N*-acetyltransferase, which is known to be responsible for phase II metabolism of aniline derivatives.[61] Although the exact position of the hydroxy groups could not be determined

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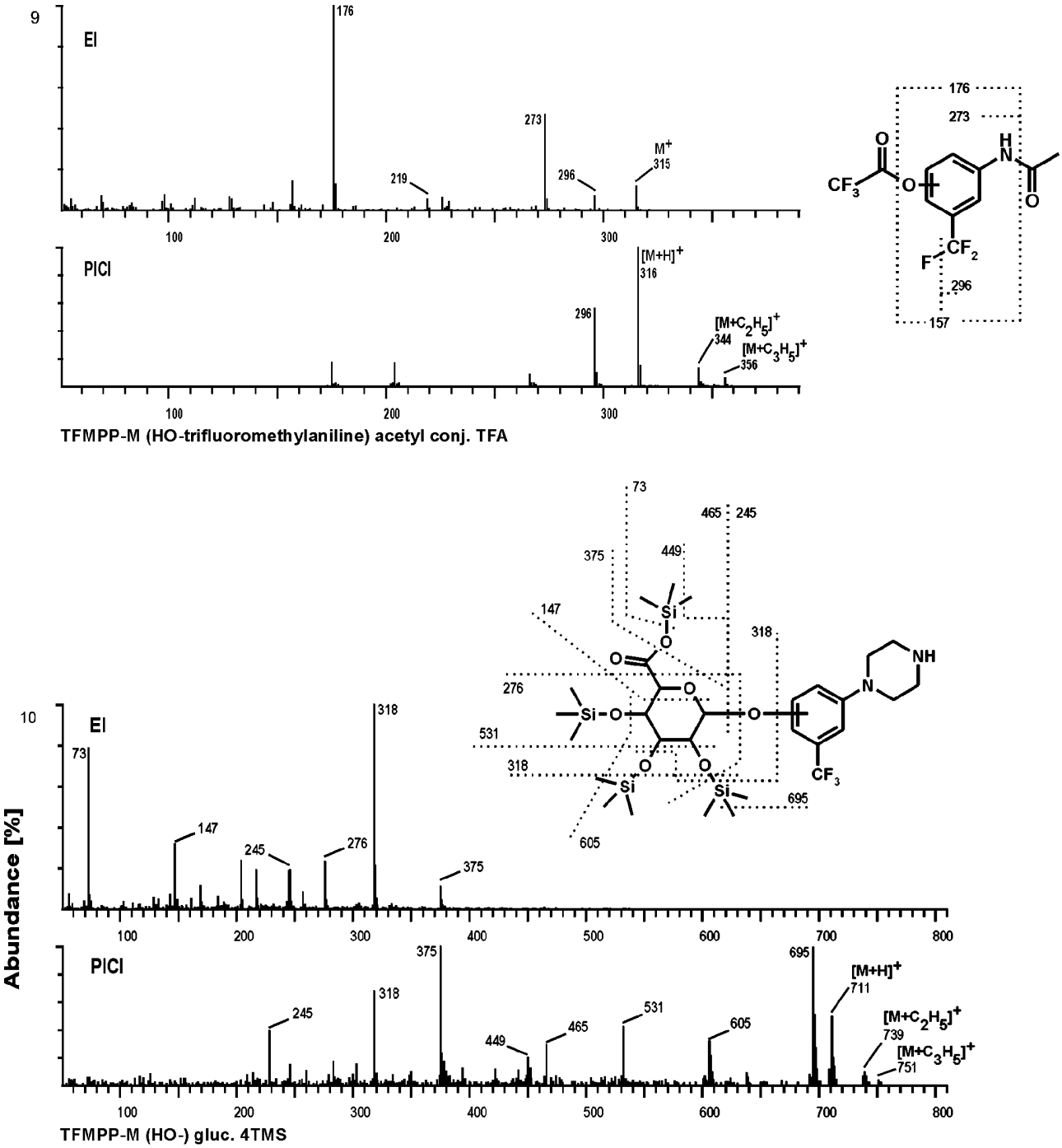

Figure 2. EI and PICI mass spectra, structure and predominant fragmentation patterns of hydroxy-3-trifluoromethylaniline *N*-acetyl conjugate after trifluoroacetylation (upper part) and of hydroxy-TFMPP glucuronide after trimethylsilylation (lower part). The numbers of the spectra correspond to those in Fig. 4.

by means of GC/MS, para position can be assumed to be the main hydroxylation position. This is in accordance with findings reported for structurally related compounds such as mCPP.[62]

The peaks of the hydroxy metabolites were more abundant after glucuronidase and sulfatase hydrolysis and so it could be concluded that they were partly excreted as glucuronides and/or sulfates. For further elucidation of the conjugates of hydroxy-TFMPP, GC/MS and LC/MS procedures were applied. The corresponding glucuronide could be identified after solid-phase extraction and trimethylsilylation by GC/MS. Its EI and PICI spectra, the postulated structure and predominant fragmentation patterns are shown in Fig. 2 (spectra no. 10). The PICI spectrum shows the peak of the protonated molecule $[M + H]^+$ with adduct ions typical for PICI using methane as reagent gas and an ion with m/z 375, characteristic for trimethylsilylated glucuronides.[48] After enzymatic conjugate cleavage, these spectra could no longer be found after the same sample preparation. The identity of the hydroxy-TFMPP glucuronide could further be confirmed by LC/MS after direct injection of rat urine. The corresponding mass spectrum (spectrum no. 11 in Fig. 3) shows the

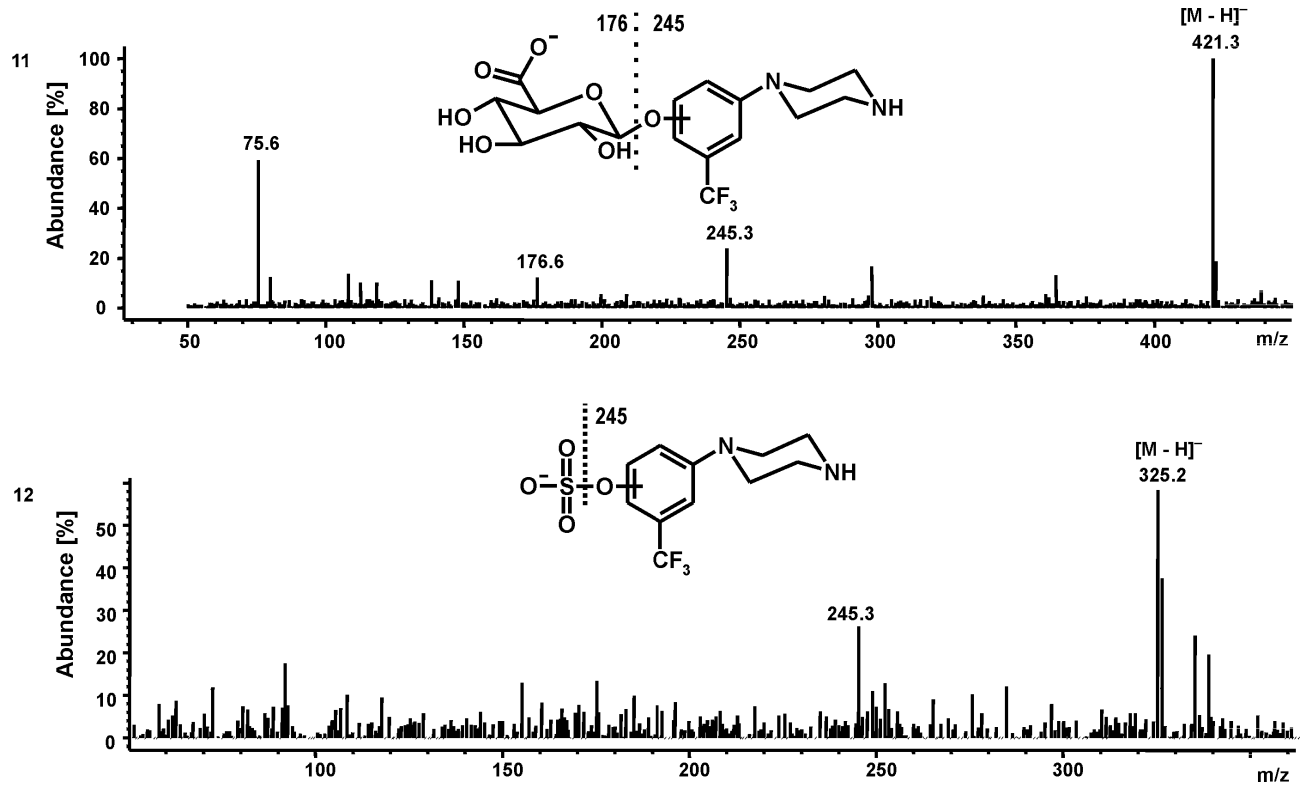

Figure 3. ESI mass spectra (negative mode, −200 V fragmentor voltage), structure and predominant fragmentation patterns of hydroxy-TFMPP glucuronide (upper part) and sulfate (lower part). The numbers of the spectra correspond to those in Fig. 4.

characteristic peak of the deprotonated molecule $[M - H]^-$ and a characteristic loss of m/z 176 for glucuronides. The corresponding hydroxy-TFMPP sulfate could only be detected to a minor extent. The mass spectrum (spectrum no. 12 in Fig. 3) shows the characteristic peak of the deprotonated molecule $[M - H]^-$ and a characteristic loss of m/z 80, typical for the sulfate moiety. Again, enzymatic conjugate cleavage led to the disappearance of the glucuronide and sulfate peaks. These findings are supported by the reported glucuronidation and sulfatation of the structurally related compound para-hydroxy-mCPP.[62]

Based on these identified metabolites, the following metabolic pathways, shown in Fig. 4, could be postulated: alteration of the phenyl ring by hydroxylation to hydroxy-TFMPP followed by partial glucuronidation or sulfation; degradation of the piperazine heterocycle by double N-dealkylation of TFMPP to *N*-(3-trifluoromethylphenyl)ethylenediamine or to 3-trifluoromethylaniline and of hydroxy-TFMPP to *N*-(hydroxy-3-trifluoromethylphenyl)ethylenediamine or to hydroxy-3-trifluoromethylaniline followed by partial N-acetylation of the anilines. In summary, hydroxylation of TFMPP is the main metabolic step which is in accordance with findings reported for structurally related compounds.[36,37,62]

Detection by GC/MS within the STA

TFMPP and its metabolites were separated by GC and identified by EI-MS after acid hydrolysis, extraction and acetylation within the authors' standard STA. Mass chromatography with the ions m/z 157, 161, 174, 200, 216 and 330 was used to indicate the presence of TFMPP and/or its metabolites. Figure 5 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 a common abuser's dose of 1 mg/kg BM of TFMPP. They indicate the presence of TFMPP and its metabolites. 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.[50] The selected ions m/z 174 and 200 were used for indication of TFMPP itself, the ions m/z 216 and 330 were used to indicate the presence of its main metabolite hydroxy-TFMPP. The ion at m/z 174 was selected to monitor the *N*-(3-trifluoromethylphenyl)ethylenediamine and the ions at m/z 161 and 157 were used for indication of the aniline metabolites 3-trifluoromethylaniline and hydroxy-3-trifluoromethylaniline, respectively. Screening for *N*-(hydroxy-3-trifluoromethylphenyl)ethylenediamine was not useful since it was only excreted in minor amounts and could only be detected in urine after application of higher doses of TFMPP.

As illustrated in Fig. 6, the identity of the marked peak in the mass chromatograms was confirmed by computerized comparison of the underlying mass spectrum with reference spectra recorded during this study. In the authors' experience, the gas chromatographic RIs provide preliminary indications and may be useful to gas chromatographers without a GC/MS facility. Therefore, they are also given in Fig. 1. The RIs were recorded during the GC/MS procedure and calculated in correlation with the Kovats' indices[63] of the components of a standard solution of typical drugs which is measured daily for testing the GC/MS performance.[64,65] The reproducibility of RIs measured on capillary columns

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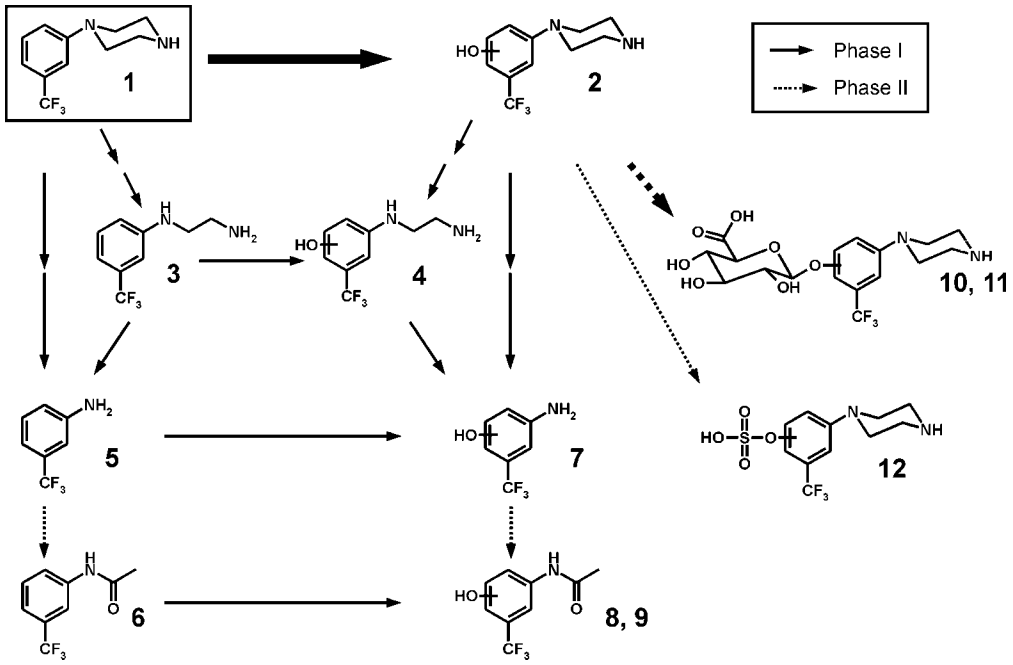

Figure 4. Proposed scheme for the phase I and II metabolism of TFMPP in rats.

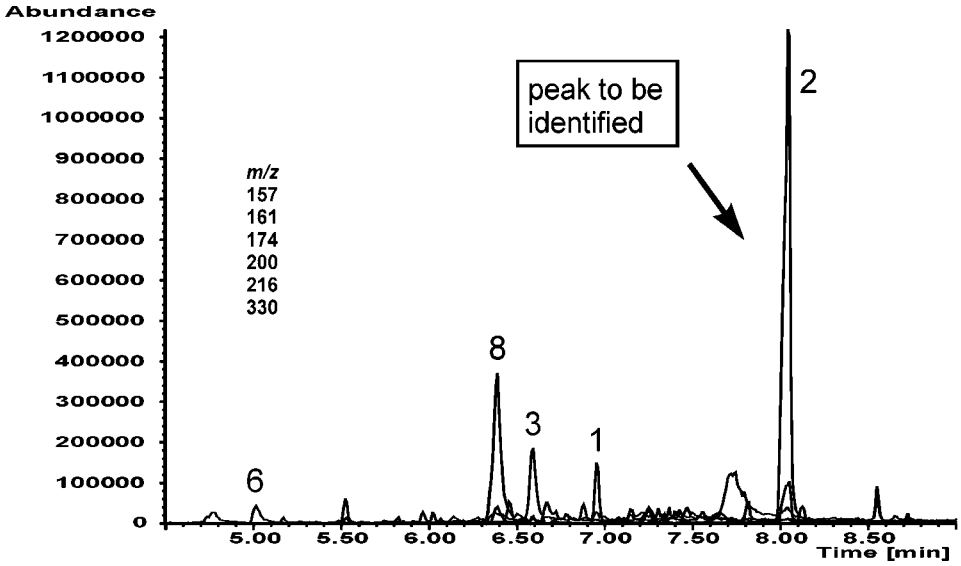

Figure 5. Typical reconstructed mass chromatograms with the given ions of an acetylated extract of a rat urine sample collected over 24 h after application of a common abuser's dose of 1 mg/kg BM of TFMPP. They indicate the presence of TFMPP and its metabolites. The peak numbers correspond to those in Figs 1 and 4. The merged fragmentograms can be differentiated by their colors on a color screen.

was better using a mixture of drugs than that of the homologous hydrocarbons recommended by Kovats. Because of the mass spectral identification, interferences by biomolecules or further drugs could be excluded, as their mass spectra are included in the reference library.[50]

As possible pitfalls in the toxicological interpretation, it should be mentioned that TFMPP has been identified as a metabolite of antrafenine.[66,67] Furthermore, the TFMPP moiety can be found in other different drugs such as terciprazine and fluprazine.[34] However, no data on the possible TFMPP formation from these two compounds is available. Leflunomide, an immunosuppressant for antirheumatoid use, is known to be metabolized to 4-trifluoromethylaniline.[68] Furthermore, the acid hydrolysis used led to the liberation of this moiety and has been found to be the target analyte using the authors' STA in a urine screening after intake of leflunomide.[69] Its spectrum is very similar to that of the TFMPP metabolite 3-trifluoromethylaniline, which may lead to misinterpretation. However, unequivocal identification of a TFMPP intake was possible via unique metabolites.

The authors' STA procedure allowed the unambiguous identification of TFMPP and its metabolites in rat urine after administration of a dose corresponding to a common drug users' dose to rats. Due to lack of authentic urine samples,

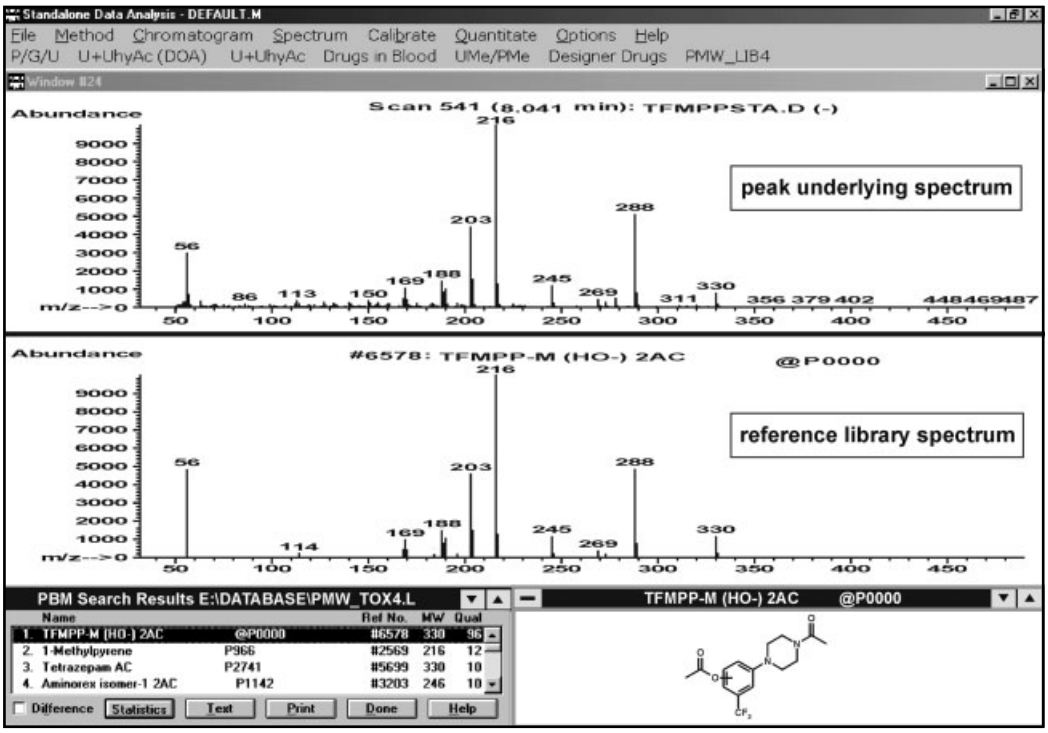

Figure 6. Mass spectrum underlying the marked peak in Fig. 5, the reference spectrum, the structure, and the hit list found by computer library search.

a comparison of the metabolites found in rat and human urine after administration of TFMPP was not yet possible. However, it is very likely that the results obtained from rat urine can be transferred to human urine, as studies on the structurally related compounds showed.[36]

The studies showed that TFMPP was extensively metabolized and, therefore, a urine screening should be focused on the metabolites with hydroxy-TFMPP as target analyte. Due to that fact, information on the extraction efficacy and on the limit of detection of the parent compound (10 ng/ml, S/N 3, measured under routine GC/MS conditions) is of less relevance. Both analytical data could not be determined for hydroxy-TFMPP, because a reference substance was not available. However, it was shown that an intake of a dose of TFMPP that corresponds to a common drug users' dose could be detected.

CONCLUSIONS

The studies presented here shows that the new piperazine-derived designer drug TFMPP is extensively metabolized, mainly by hydroxylation of the aromatic ring and by degradation of the piperazine moiety.

The authors' STA procedure allowed the detection of an intake of a dose of TFMPP that corresponds to a common drug users' dose in rat urine. The target analyte was found to be hydroxy-TFMPP. The authors' experiences 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.[36,44,45] Therefore, it can be concluded that the procedure should be applicable also for human urine screening in clinical or forensic cases.

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