Research Article

Journal of MASS SPECTROMETRY

Received: 13 October 2008 Accepted: 25 January 2009 Published online in Wiley Interscience: 5 March 2009

(www.interscience.com) DOI 10.1002/jms.1571

New designer drug α-pyrrolidinovalerophenone (PVP): studies on its metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques†

Christoph Sauer,[a] Frank T. Peters,[a] Claudia Haas,[a] Markus R. Meyer,[a] Giselher Fritschi[b] and Hans H. Maurer[a]*

The aim of the present study was to identify the metabolites of the new designer drug α-pyrrolidinovalerophenone (PVP) in rat urine using GC/MS techniques. Eleven metabolites of PVP could be identified suggesting the following metabolic steps: hydroxylation of the side chain followed by dehydrogenation to the corresponding ketone; hydroxylation of the 2″-position of the pyrrolidine ring followed by dehydrogenation to the corresponding lactam or followed by ring opening to the respective aliphatic aldehyde and further oxidation to the respective carboxylic acid; degradation of the pyrrolidine ring to the corresponding primary amine; and hydroxylation of the phenyl ring, most probably in the 4′-position. The authors' screening procedure for pyrrolidinophenones allowed the detection of PVP metabolites after application of a dose corresponding to a presumed user's dose. In addition, the involvement of nine different human cytochrome P450 (CYP) isoenzymes in the side chain hydroxylation of PVP was investigated and CYP 2B6, 2C19, 2D6, and 3A4 were found to catalyze this reaction.

Keywords: α-pyrrolidinovalerophenone (PVP); metabolism; GC/MS; urinalysis

Introduction

1-Phenyl-2-pyrrolidin-1-ylpentan-1-one [α-pyrrolidinovalerophenone (PVP)] is a new designer drug of the pyrrolidinophenone type.[1] After α-pyrrolidinophenone (PPP),[2,3] 4′-methyl-α-pyrrolidinopropiophenone (MPPP),[2,4] 4′-methoxy-α-pyrrolidinopropiophenone (MOPPP),[5] 3′4′-methylenedioxy-α-pyrrolidinopropiophenone (MDPPP),[6] 4′-methyl-α-pyrrolidinohexanophenone (MPHP),[7] and 4′-methyl-α-pyrrolidinobutyrophenone (MPBP),[8] PVP was the latest of this class of designer drugs to occur on the illicit drug market in Germany. PVP was seized as powder by the German police and is assumed to be taken orally as the other pyrrolidinophenones, which have been distributed among drug abusers as tablets, capsules, or powders.[9] A recent seizure of a powder containing PVP in the Netherlands indicates that this drug is also available on illicit drug markets outside Germany (personal communication, Jorrit D. J. van den Berg, Department Illicit Drugs, Netherlands Forensic Institute). As the pyrrolidinophenones cannot be detected with usual routine analysis procedures,[10,11] they might have been overlooked, and therefore statements on the frequency of their occurrence cannot be made. Nevertheless, PPP (1998), MPPP (1999), and MDPPP (1997) have been scheduled in the German Controlled Substances Act and possession of them is prohibited. So far, little information is available about the dosage and the pharmacological and toxicological effects of these pyrrolidinophenones. However, they may be expected to be very similar to those of pyrovalerone (4′-methyl-α-pyrrolidinovalerophenone) because of their close structural relation to this drug. Pyrovalerone is a psychostimulant that acts by releasing dopamine and norepinephrine from the respective nerve terminals.[12,13] In 2006, Meltzer *et al*.[14] reported that pyrovalerone and several structural analogs, among them PVP, are inhibitors of dopamine, serotonin, and norepinephrine transporters. In the early 1960s, pyrovalerone was first pharmacologically characterized in animal experiments by Stille *et al*.[15] These authors reported comparable central stimulatory effects but less influence on locomotor activity and autonomous function compared with amphetamine. The oral LD_{50} of pyrovalerone in mice was reported to be 350 mg/kg body mass. Comparable psychostimulatory effects[16,17] and smaller effects on motor function[16] of pyrovalerone in comparison with amphetamine were also found in controlled studies with humans.

* *Correspondence to: Hans H. Maurer, Department of Experimental and Clinical Toxicology, Saarland University, D-66421 Homburg (Saar), Germany. E-mail: hans.maurer@uks.eu*

† *Parts of these results were presented at the 15th GTFCh Symposium, April 18–21, 2007, Mosbach, Germany*

a *Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Saarland University, D-66421 Homburg (Saar), Germany*

b *Hessisches Landeskriminalamt, D-65187 Wiesbaden, Germany*

Journal of MASS SPECTROMETRY

Pyrovalerone was also evaluated as a therapeutic drug,[18–20] for example, for treatment of lethargy or chronic fatigue, where it was reported to reduce such symptoms.[18] However, it was withdrawn from the market and scheduled as a controlled substance after reports of its intravenous abuse by multidrug users.[21] Such pharmacological profiles are in line with the abuse of pyrrolidinophenones as stimulant designer drugs.

The knowledge about metabolic steps of a drug is a prerequisite for toxicological risk assessment and for developing screening procedures for toxicological detection, as in both cases the metabolites may play a major role. The qualitative metabolism of PPP, MPPP, MPHP, MPBP, MOPPP, MDPPP, and pyrovalerone has been studied in animals[3–8,22–25] and, in the case of pyrovalerone, also in humans.[23] So far, no data are available on the metabolism of PVP.

Screening for and determination of drugs of abuse are important tasks in clinical and forensic toxicology. General screening procedures allow detection of series of drug classes in one step.[10,11,26,27] However, the pyrrolidinophenones cannot be detected therein, because the majority of the metabolites showed amphoteric properties. Therefore, Springer *et al*. developed a special screening procedure for such drugs in urine using solid-phase extraction, trimethylsilylation, and full-scan EI GC/MS.[3–7] So far, no data are available on the toxicological analysis of PVP. The aim of the present study was to identify the metabolites of PVP in rat urine and to incorporate PVP into an existing toxicological screening procedure for pyrrolidinophenones.

Experimental

Chemicals and reagents

PVP–HNO_3 from a drug seizure was provided by Hessisches Landeskriminalamt, Wiesbaden (Germany) for research purposes. 4′-Hydroxymethyl-α-pyrrolidinobutyrophenone (HO-MPBP) was biotechnologically synthesized.[28] Bond Elut Certify cartridges (130 mg, 3 ml) were obtained from Varian (Darmstadt, Germany). *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA), trifluoroacetic anhydride (TFA), and heptafluorobutyric anhydride (HFBA) were obtained from Fluka (Steinheim, Germany). Diazomethane was synthesized according to the procedure of McKay *et al*.[29] $NADP^+$ was obtained from Biomol (Hamburg, Germany), isocitrate and isocitrate dehydrogenase from Sigma (Taufkirchen, Germany). All other chemicals and reagents were obtained from E. Merck, Darmstadt (Germany) and were of analytical grade. The following microsomes were from Gentest and delivered by NatuTec (Frankfurt/Main, Germany): baculovirus-infected insect cell microsomes (Supersomes), containing 1 nmol/ml of human cDNA-expressed CYP 1A2, CYP 2A6, CYP 2B6, CYP 2C8, CYP 2C9, CYP 2C19, CYP 2D6, CYP 2E1 (2 nmol/ml), or CYP 3A4, pooled human liver microsomes (pHLM, 20 mg microsomal protein/ml, 400 pmol total CYP/mg protein). After delivery, the microsomes were thawed at 37 °C, aliquoted, snap-frozen in liquid nitrogen, and stored at −80 °C until use.

Urine samples

The investigations were performed using male rats (Wistar, Charles River, Sulzfleck, Germany) for toxicological diagnostic reasons according to the corresponding German law. They were administered a single 20 mg/kg body mass dose of PVP for identification of the metabolites and a single of 1 mg/kg body mass dose of PVP–HNO_3 for toxicological analysis in an aqueous suspension by gastric intubation. The rats were housed in metabolism cages for 24 h, having water *ad libitum*. Urine was collected separately from the feces over a 24 h period. All samples were directly analyzed and then stored at −20 °C. Blank 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 0.5 ml portion of urine was adjusted to pH 5.2 with acetic acid (1 M, approximately 50 µl) and incubated at 56 °C for 1.5 h with 50 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. The urine sample was then diluted with 2.5 ml of water and loaded on a Bond Elut Certify cartridge, 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, 1 ml of 0.01 M hydrochloric acid, and again with 1 ml of water. The retained nonbasic compounds were first eluted into a 1.5 ml reaction vial with 1 ml of methanol (fraction 1), whereas the basic compounds were eluted in the second step into a different vial with 1 ml of a freshly prepared mixture of methanol/aqueous ammonia 32% (98 : 2 v/v, fraction 2). The eluates were gently evaporated to dryness under a stream of nitrogen at 56 °C and derivatized by methylation, acetylation, combined methylation/acetylation, or trimethylsilylation according to published procedures.[10] A 2 µl aliquot was injected into the GC/MS (with an alcohol-free and water-free syringe in the case of trimethylsilylated extracts). The same experiments were repeated without the use of enzymatic hydrolysis to study which metabolites of PVP were excreted as glucuronides/sulfates.

In order to confirm the structure of the main metabolite, further derivatization procedures were used. Trifluoroacetylation was performed after reconstitution in 50 µl of ethyl acetate with 50 µl of TFA for 3 min under microwave irradiation at about 400 W. After evaporation, the residue was dissolved in 50 µl of ethyl acetate. For heptafluorobutyrylation, 50 µl of HFBA was added to the extracts previously reconstituted in 50 µl of ethyl acetate, and derivatization was carried out for 5 min under microwave irradiation at about 440 W. After evaporation, the residue was dissolved in 50 µl of ethyl acetate. A 2 µl aliquot each was injected into the GC/MS system. Moreover, methylation with methyl iodide was used. After reconstitution in 150 µl of a mixture of TMAH–DMSO (1 : 20, v/v), the sample was left for 2 min at room temperature. Then, 50 µl of methyl iodide was added, and the sample was left at room temperature for 5 min for derivatization. After addition of 1 ml of isooctane, the sample was shaken for 1 min. The organic phase was evaporated to dryness and then redissolved in 30 µl of methanol. A 2 µl aliquot was injected into the GC/MS system.

Sample preparation for toxicological analysis by GC/MS

The extraction procedure was the same as described above for identification of the metabolites, but only the second eluate (fraction 2) was used for further workup. It was gently evaporated to dryness under a stream of nitrogen at 56 °C and then reconstituted in 50 µl of ethyl acetate. After addition of 50 µl of MSTFA, the reconstituted extract was trimethylsilylated for 5 min under microwave irradiation at about 440 W. A 2 µl aliquot of this mixture was injected into the GC/MS with an alcohol-free and water-free syringe.

Microsomal incubations

In order to investigate the involvement of particular CYP isoenzymes in the side chain hydroxylation of PVP, incubations were performed at 37 °C with 250 μM PVP and 75 pmol/ml CYP 1A2, CYP 2A6, CYP 2B6, CYP 2C8, CYP 2C9, CYP 2C19, CYP 2D6, CYP 2E1, or CYP 3A4 for 45 min. Besides enzymes and substrate, incubation mixtures (final volume: 50 μl) consisted of 90 mM phosphate buffer (pH 7.4), 5 mM Mg^{2+}, 5 mM isocitrate, 1.2 mM $NADP^{+}$, 0.5 U/ml isocitrate dehydrogenase, and 200 U/ml superoxide dismutase. For incubations with CYP 2A6 or CYP 2C9, phosphate buffer was replaced with 45 and 90 mM Tris-buffer, respectively, according to the Gentest manual. Reactions were started by addition of the ice-cold microsomes and terminated with 5 μl of 60% (w/w) perchloric acid ($HClO_4$). After adding 5 μl of 0.01 mM HO-MPBP in methanol, the samples were centrifuged, and the supernatants were transferred to autosampler vials.

PVP, its metabolite hydroxy-alkyl-pyrrolidinovalerophenone (HO-PVP), and the internal standard HO-MPBP were analyzed using an Agilent Technologies (AT, Waldbronn, Germany) 1100 series atmospheric pressure chemical ionization (APCI) electrospray LC/MSD, SL version and an LC/MSD ChemStation using the A.08.03 software. Gradient elution was achieved on a Merck LiChroCART column (125 mm × 2 mm ID, 5 μm) with Superspher 60 RP Select B as stationary phase and a LiChroCART 10 2 Superspher 60 RP select B-4 μ guard column. The mobile phase consisted of 70% eluent A (50 mM ammonium formate adjusted to pH 3.5 with formic acid) and 30% eluent B (acetonitrile containing 1 ml/l formic acid) according to Maurer *et al*.[30] at a flow rate of 0.4 ml/min. The injection volume was 5 μl. The following APCI inlet conditions were applied: drying gas, nitrogen (7000 ml/min, 300 °C); nebulizer gas, nitrogen (25 psi, 172.3 kPa); vaporizer temperature set at 400 °C; capillary voltage, 4000 V; corona current, 5.0 μA; positive selected ion monitoring (SIM) mode, *m/z* 232 for PVP, and *m/z* 248 for HO-PVP and HO-MPBP; fragmentor voltage, 50 V.

GC/MS apparatus for identification of metabolites

The extracts were analyzed using a Hewlett Packard (HP, 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 ID), cross-linked methyl silicone, 330-nm film thickness; injection port temperature, 280 °C; carrier gas, helium; flow rate 1 ml/min; column temperature, programmed from 100 to 310 °C at 30°/min, initial time 3 min, final time 8 min. The MS conditions were as follows: full-scan mode, *m/z* 50–800 u; EI mode, ionization energy, 70 eV; PICI mode using methane: ionization energy, 230 eV; ion source temperature, 220 °C; capillary direct interface, heated at 260 °C.

GC/MS apparatus for toxicological detection

The extracts were analyzed using a HP 5890 Series II gas chromatograph combined with a HP 5972A MSD mass spectrometer and a HP MS ChemStation (DOS series) with HP G1034C software version C03.00. The GC conditions were as follows: splitless injection mode; column, HP-1 capillary (12 m × 0.2 mm ID), cross-linked methyl silicone, 330-nm film thickness; injection port temperature, 280 °C; carrier gas, helium; flow rate 1 ml/min; column temperature, programmed from 100 to 310 °C at 30°/min, initial time 3 min, final time 8 min. The MS conditions were as follows: full-scan mode, *m/z* 50–550 u; EI mode, ionization energy, 70 eV; ion source temperature, 220 °C; capillary direct interface, heated at 280 °C.

GC/MS procedure for identification of metabolites and toxicological detection by GC/MS

The apparatus, GC, and MS conditions were the same as described above except for the scan range, which was *m/z* 50–550 u to incorporate PVP into the special screening procedure for pyrrolidinophenones.[3–8]

For toxicological detection of PVP and its trimethylsilylated metabolites, mass chromatography with the selected ions *m/z* 126, 140, and 144 was used. Generation of the mass chromatograms could be started by clicking the corresponding pull down menu that executed the user-defined macros.[31] The identity of the peaks in the mass chromatograms was confirmed by computerized comparison[32] of the mass spectra underlying the peaks (after background subtraction) with reference spectra recorded during this study.

Results and Discussion

Identification of the metabolites

From the recorded mass spectra, the following metabolites (numbers in Fig. 1) could be deduced in the acetylated urine extract: PVP (1), *N,N*-bis-dealkyl-PVP (2), 2″-oxo-PVP (3), hydroxyalkyl-PVP (4), hydroxyphenyl-*N,N*-bis-dealkyl-PVP (5), hydroxyphenyl-PVP (7), hydroxyalkyl-2″-oxo-PVP (8), carboxy-4-oxo-PVP (9), hydroxyphenyl-2″-oxo-PVP (12), di-hydroxy-PVP (13), and hydroxyphenyl-carboxy-4-oxo-PVP (14). Additionally in a methylated urine extract, the methyl derivatives of carboxy-4-oxo-PVP (11) and of hydroxyphenyl-carboxy-4-oxo-PVP (15) could be identified. In the trifluoroacetylated urine extract, the trifluoroacetyl derivative of carboxy-4-oxo-PVP (10) could be identified. In the trimethylsilylated urine extract, the trimethylsilylated derivatives of *N,N*-bis-dealkyl-PVP (6) could be identified.

Cleavage of conjugates was necessary before extraction and GC/MS analysis of the suspected metabolites in order not to overlook conjugated metabolites. Gentle enzymatic hydrolysis was used because most of the analytes were destroyed during acid hydrolysis. The described cleavage procedure at elevated temperature (56 °C) for a short period of time (1.5 h) had been successfully used in routine screening procedures for other pyrrolidinophenones.[3,5–8] In the present study, this short procedure was also used for metabolism studies, because it had proven to yield similar results (i.e. same compounds detected at similar abundances) as the conditions used in previous metabolism studies (37 °C for 12 h).[3–8]

The use of common liquid–liquid extraction under alkaline or acidic conditions followed by acetylation and methylation, respectively,[26,33–35] was not appropriate, because the majority of the metabolites showed amphoteric properties. In addition, volatility of the free bases and the instability of the analytes under alkaline and high temperature conditions had caused difficulties with structurally related compounds.[36,37] On the contrary, mixed-mode solid-phase extraction (SPE) had been used successfully for extraction of amphoteric metabolites of other pyrrolidinophenones[3–8] and also proved applicable for extraction of PVP and its metabolites.

Journal of MASS SPECTROMETRY

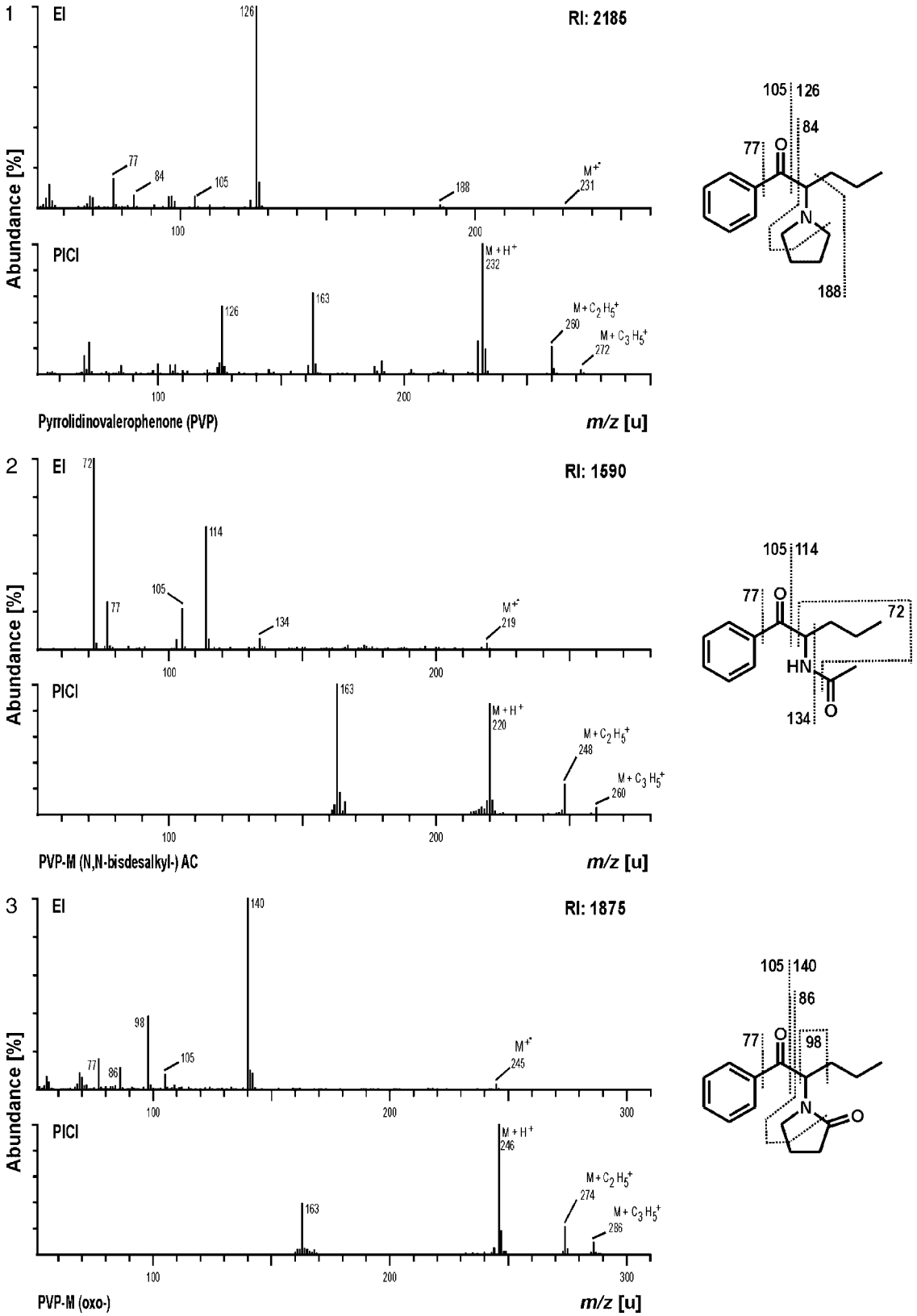

Figure 1a. EI and PICI mass spectra, RI, structures, and predominant fragmentation patterns of PVP and its metabolites after acetylation (AC), methylation (ME), trifluoroacetylation (TFA), and trimethylsilylation (TMS). The numbers of the mass spectra correspond to those of the respective metabolites in Figs 3 and 4.

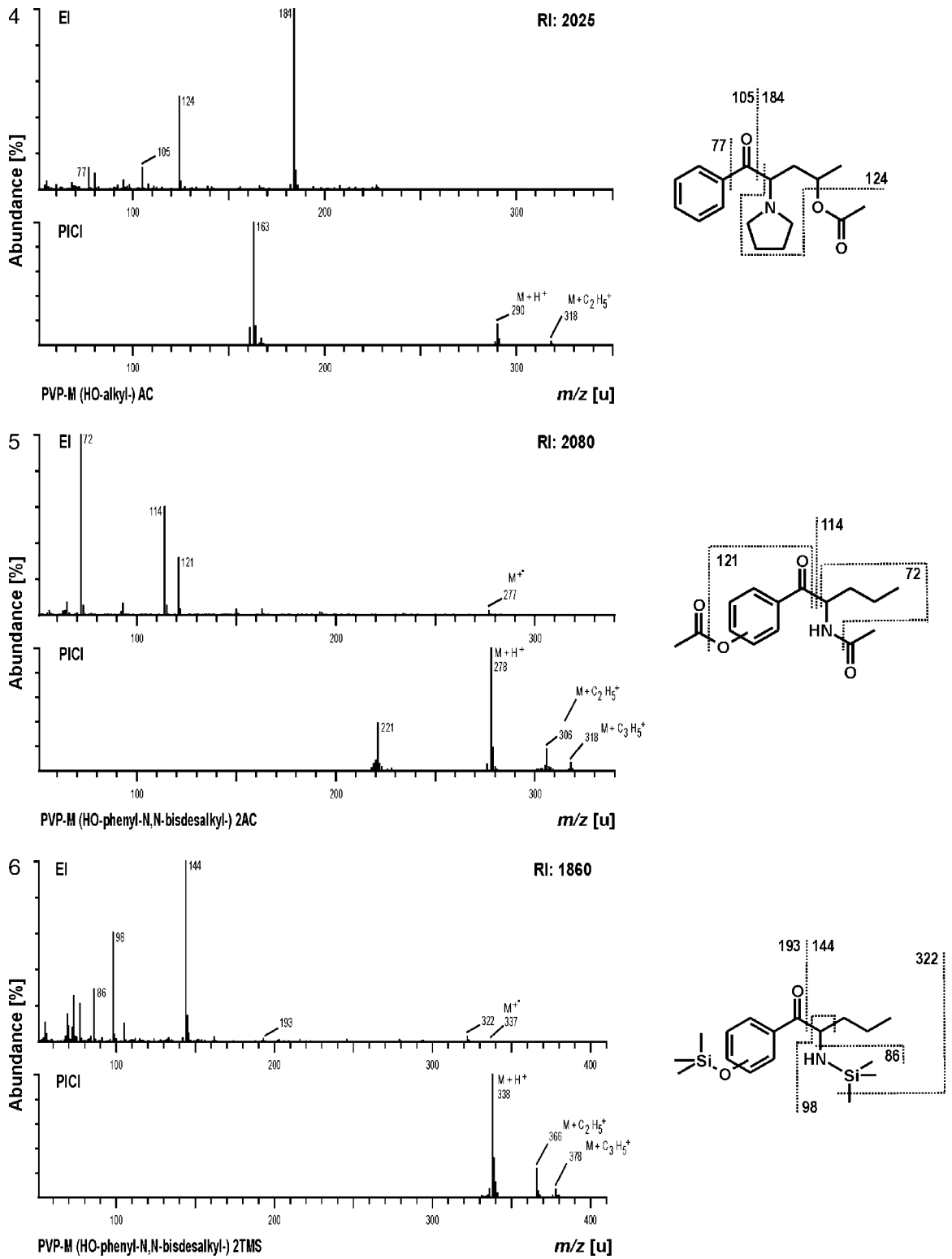

Figure 1b. (*Continued*).

Derivatization was needed to improve the GC properties of these relatively polar metabolites, thus increasing the sensitivity of their detection. Methylation with diazomethane is well known to be versatile for derivatization of metabolites with phenolic hydroxy or carboxy groups. On the contrary, aliphatic hydroxy groups remain unaffected, thus allowing their distinction from phenolic hydroxy groups. Moreover, the resulting derivatives often show favorable fragmentation properties in the EI mode facilitating elucidation of metabolite structures. In the present study, methylation was performed to check for hydroxylation at the phenyl ring and carboxylic acids. Acetylation also proved to be useful in metabolism studies.[10,33–35] It can be used for derivatization of primary and

Journal of MASS SPECTROMETRY

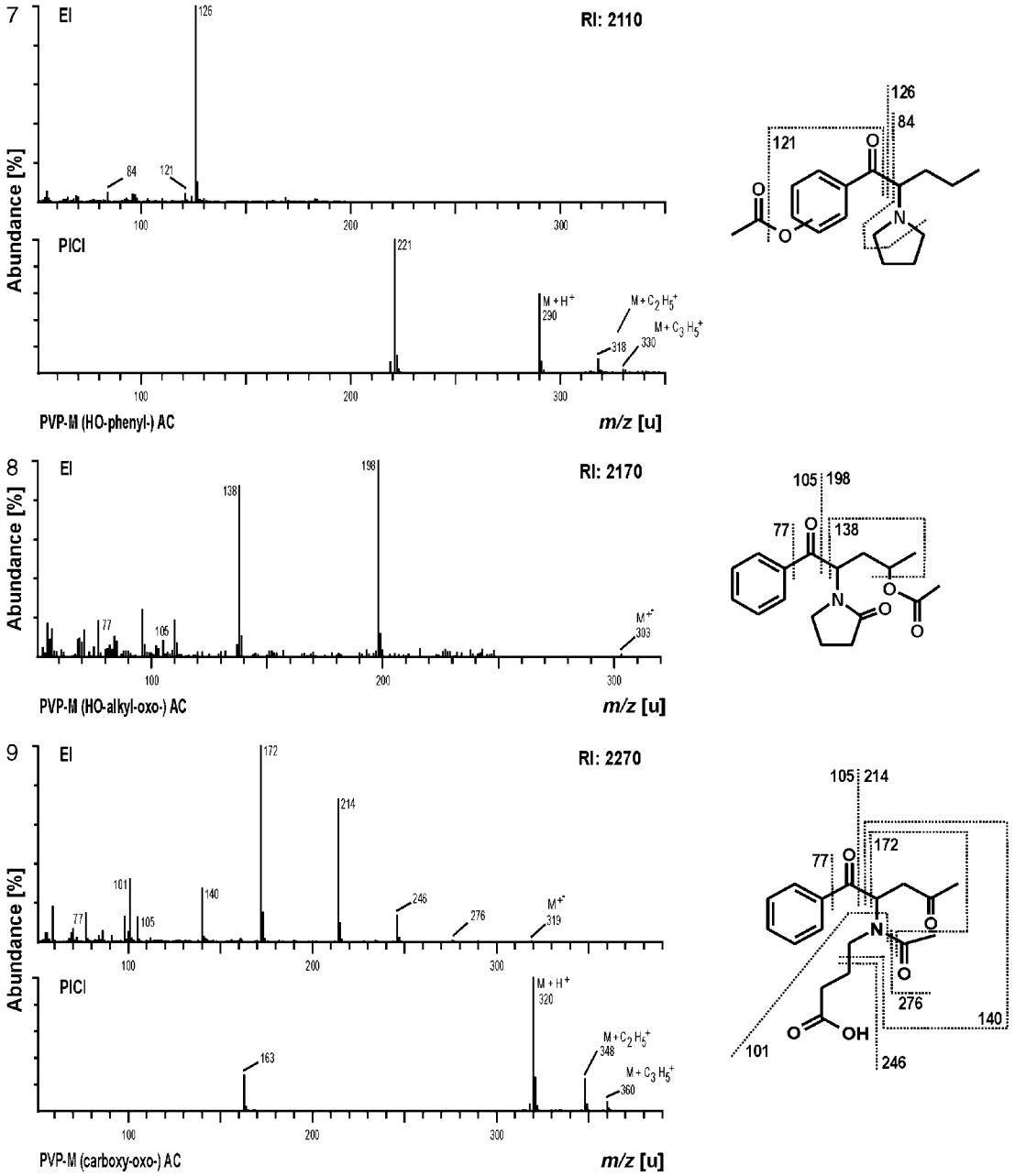

Figure 1c. (*Continued*).

secondary amino groups, as well as alcoholic and/or phenolic hydroxy groups. Generally, the fragmentation patterns of the resulting derivatives in the EI mode can also easily be interpreted. In the present study, acetylation was used to detect possible primary or secondary amine metabolites resulting from degradation of the pyrrolidine ring as it has been described for a structural analogue.[3] A disadvantage of using acetylation in metabolism studies is the problem that physiologically acetylated metabolites, for example, described for phenethylamine-type designer drugs,[35,38–42] cannot be differentiated from acetyl derivatives. The presence of such physiologically acetylated metabolites was checked in urine extracts after trifluoroacetylation. Trifluoroacetylation and methylation with methyl iodide was necessary for derivatization and structural elucidation of metabolites carrying carboxylic acid groups.

Finally, trimethylsilylation was performed to record the mass spectra of the trimethylsilyl derivatives of the PVP metabolites needed to incorporate PVP into an existing screening procedure for pyrrolidinophenones.[3,5–8] Trimethylsilylation, which is a common derivatization procedure in many routine methods, is safer and easier to handle than methylation with diazomethane and the reagent is commercially available. Although the resulting mass spectra are generally less useful for elucidation of metabolite structures, the trimethylsilylated extracts were checked for the presence of additional metabolites.

The urinary metabolites of PVP were identified by full-scan EI and PICI MS after GC separation. The postulated structures of the metabolites were deduced from the fragments detected in the EI mode, which were interpreted in correlation to those of

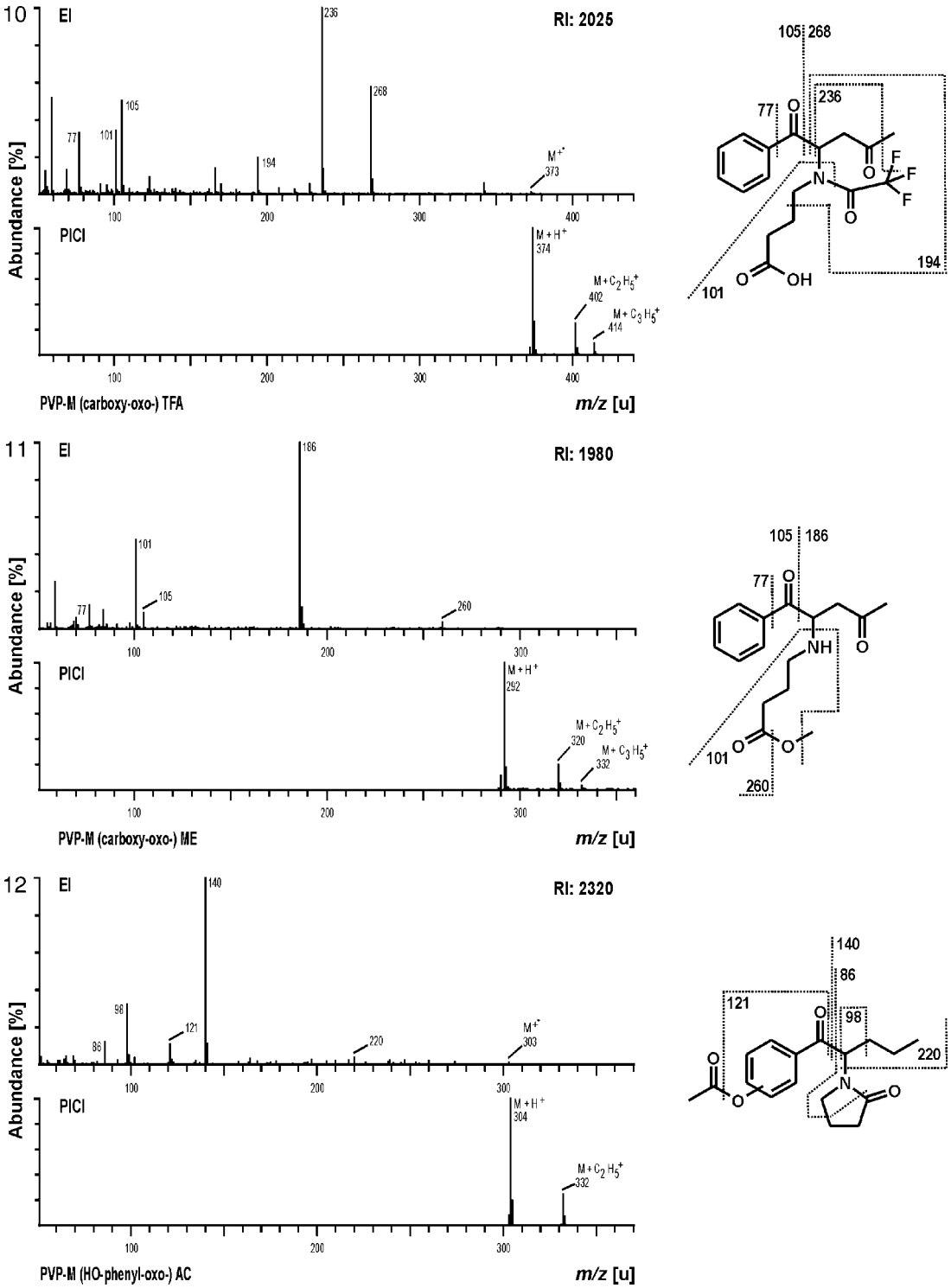

Figure 1d. (*Continued*).

the parent compound according to the fragmentation pattern of other pyrrolidinophenones[9] and the general fragmentation rules described by, for example, McLafferty and Turecek[43] and Smith and Busch.[44] In order to verify the molecular mass of the postulated metabolites, PICI mass spectra were recorded because they contain strong molecular peaks ($M + H^+$), in contrast to EI spectra. In addition, adduct ions typical for PICI with methane are produced ($M + C_2H_5^+$, $M + C_3H_5^+$).

EI and PICI mass spectra, the gas chromatographic retention indices (RI) determined according to de-Zeeuw *et al.*,[45] structures and predominant fragmentation patterns of PVP (mass spectrum no. 1), as well as of its acetylated, methylated, trifluoroacetylated, and trimethylsilylated metabolites are shown in Fig. 1. Although all the recorded PICI mass spectra contained the respective protonated molecular ions at considerable abundances, all the typical adduct ions could not be observed in case of mass spectra

Journal of MASS SPECTROMETRY

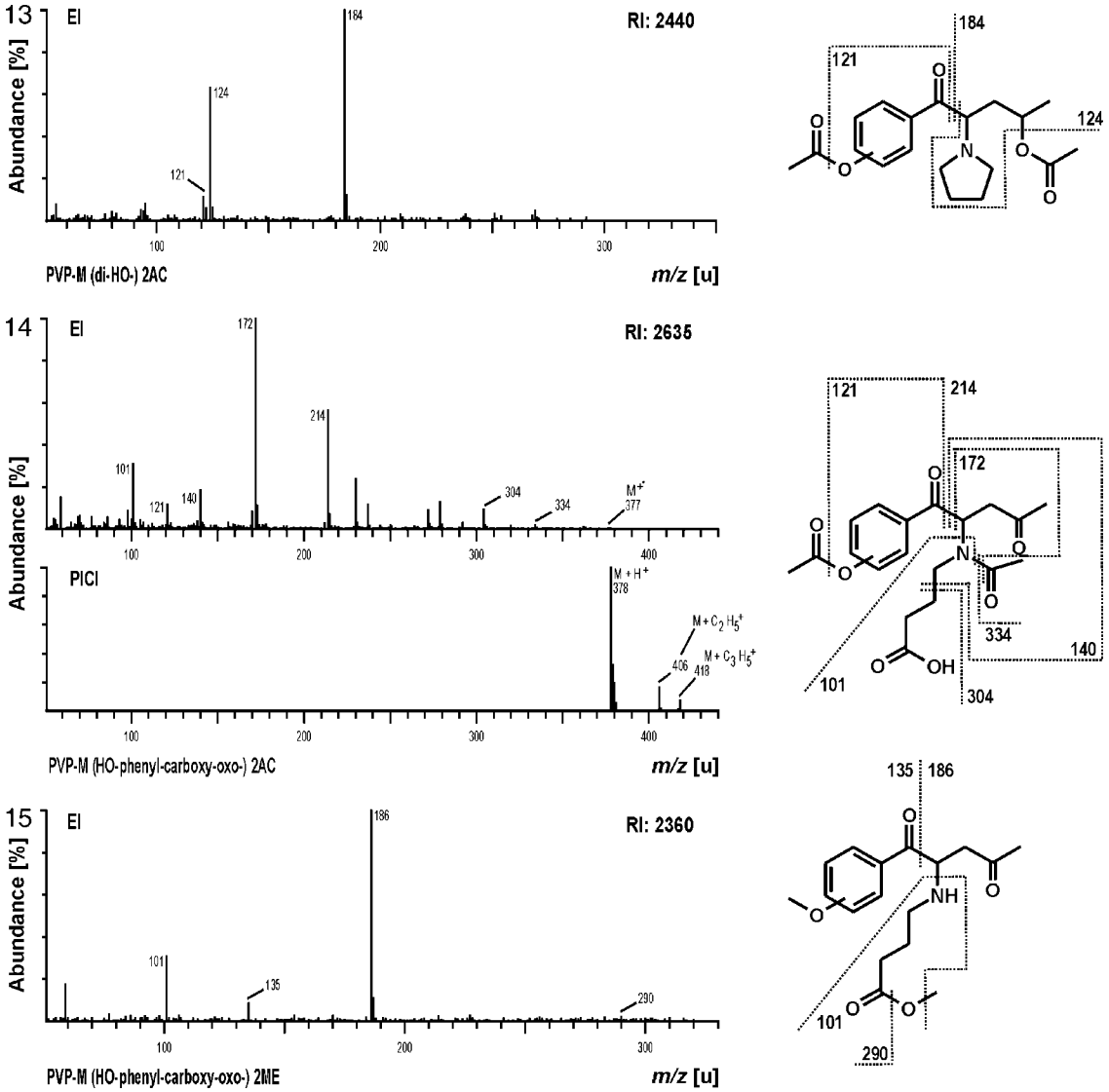

Figure 1e. (*Continued*).

numbers 4 and 12. No PICI mass spectra could be observed in case of mass spectra numbers 8, 13, and 15. This could be explained by the fact that these metabolites were formed to a minor extent not sufficient for detection in PICI mode.

Proposed fragmentation patterns

In this section, possible fragmentation patterns of the EI mass spectra of PVP and its derivatized metabolites will be discussed in relation to the postulated metabolite structures depicted in Fig. 1. The numbers of the corresponding mass spectra in Fig. 1 are given in parenthesis. PVP (1) showed a β-cleavage between position 1 and position 2 as depicted in Fig. 2A, cleavage I. This resulted in an ammonium ion *m/z* 126 representing the base peak of the spectrum. The alternative cleavage at this position led to ion *m/z* 105 (Fig. 2A, II) stabilized both by mesomerism of the phenyl ring and by mesomerism of the carbonyl moiety. On the other side of the molecule, β-cleavage is also possible between position 2 and position 3 leading to ion *m/z* 188 (Fig. 2A, III). Furthermore, β-cleavage between position 2 and position 3 followed by a second β-cleavage between the position 4″ and position 5″ followed by a third cleavage between the nitrogen and the position 2″ of the pyrrolidine ring led to ion *m/z* 84 (Fig. 2A, IV). Finally, cleavage between position 1 and position 1′ led to ion *m/z* 77 (Fig. 2A, V).

In analogy to the parent compound, for all derivatized metabolites, a β-cleavage between position 1 and position 2 could be observed leading to *m/z* 114 (2, 5), 140 (3, 12), 184 (4, 13), 126 (7), 198 (8), 214 (9, 14), 268 (10), and 186 (11, 15). All metabolites with an unchanged benzophenone system showed a benzyl-like cleavage between position 1 and position 2 resulting in *m/z* 105 (2–4 and 8–11). For acetylated metabolites with a hydroxylated phenyl ring, benzyl-like cleavage between position 1 and position 2 followed by a neutral loss of an acetyl moiety corresponding to 42 u led to *m/z* 121 (5, 7, and 12–14). For methylated hydroxyphenyl metabolites, a fragment ion *m/z* 135 (15) could be observed. For trimethylsilylated hydroxyphenyl metabolites, a fragment ion *m/z* 193 (6) could be observed. Cleavage between position 1 and position 1″ led to ion *m/z* 77 in case of (2–4 and 8–11). A β-cleavage between position 1 and position 2 followed by a neutral loss of an acetyl moiety corresponding to 42 u led to ion *m/z* 72 (2, 5) and *m/z* 172 (9, 14). A cleavage between position 2 and position

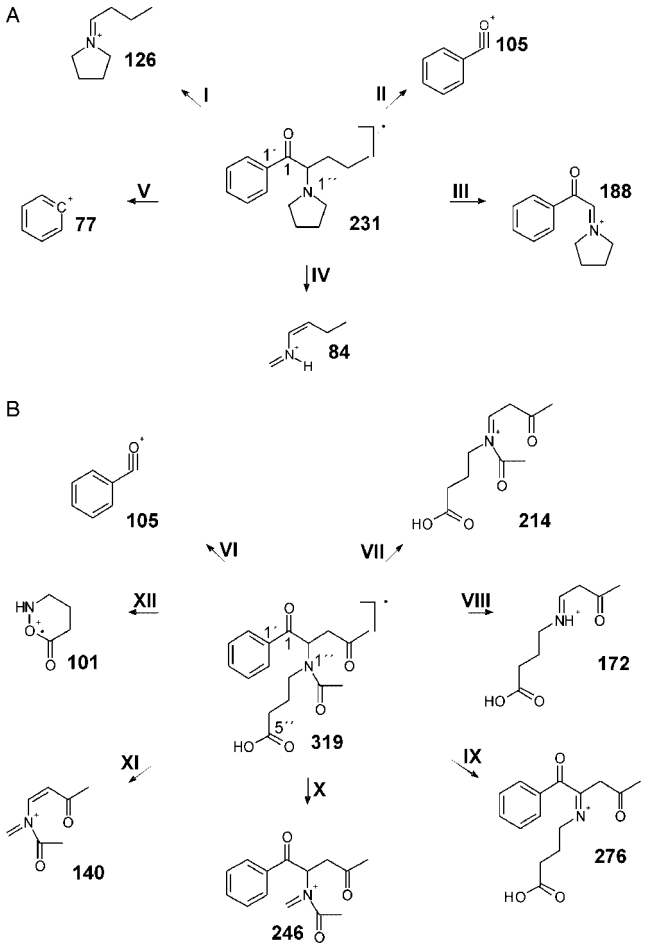

Figure 2. Proposed fragment ions characterizing PVP and the acetylated carboxy-oxo-metabolite of PVP.

3 followed by a neutral loss of an acetyl moiety corresponding to 42 u led to ion *m/z* 134 (2). A β-cleavage between position 1 and position 2 followed by a second β-cleavage between position 4″ and position 5″ followed by a third cleavage between nitrogen and position 2″ of the pyrrolidine ring led to ion *m/z* 86 (3, 12). Furthermore, a β-cleavage between position 1 and 2 followed by a cleavage between position 2 and 3 led to ion *m/z* 98 (3, 12). A β-cleavage between position 1 and 2 followed by a neutral loss of acetic acid corresponding to 60 u led to ion *m/z* 138 (8) and *m/z* 124 (13). A cleavage between position 2 and position 1″ led to ion *m/z* 220 (12). In case of the trimethylsilylated metabolites, a loss of 15 u corresponding to a methyl moiety from the molecular ion could be observed for (15).

The fragmentation pattern of acetylated carboxy-4-oxo-PVP (9) was more complex than that of the other metabolites and will therefore be discussed in detail. It also showed the characteristic cleavages between position 1 and position 2 leading to ions *m/z* 105 (Fig. 2B, VI) and *m/z* 214 (Fig. 2B, VII). A further neutral loss of the acetyl moiety (42 u) from *m/z* 214 led to *m/z* 172 (Fig. 2B, VIII). Neutral loss of 43 of the acetyl moiety (43 u) from the molecular ion led to fragment ion *m/z* 276 (Fig. 2B, IX). A similar loss was observed in the case of hydroxyphenyl-carboxy-4-oxo-PVP (14) leading to fragment ion *m/z* 334. A β-cleavage between position 2″ and position 3″ led to ion *m/z* 246 (Fig. 2B, X) for carboxy-4-oxo-PVP (9) and *m/z* 304 for hydroxyphenyl-carboxy-4-oxo-PVP (14). The same β-cleavage followed by a β-cleavage between position 1 and position 2 led to ion *m/z* 140 (Fig. 2B, XI) in both metabolites. A cleavage between position 2 and position 1″ followed by a loss of an acetyl moiety corresponding to 42 u led to ion *m/z* 101 (Fig. 2B, XII), which could also be observed in spectra numbers 10 and 14. In case of the methylated carboxylic acids, an additional loss of a methoxy moiety was necessary to form ion *m/z* 101 (11, 15). For trifluoroacetylated derivatives, *m/z* values of fragment ions carrying the trifluoroacetyl moiety should show a shift of 54 u compared with the respective acetyl derivatives. This was indeed observed for the trifluoroacetyl derivative of carboxy-4-oxo-PVP (10) that showed this characteristic shift from *m/z* 140 to *m/z* 194. A β-cleavage between position 1 and position 2 of trifluoroacetylated carboxy-4-oxo-PVP (10) followed by a loss of 18 u corresponding to a fluorine atom and a loss of a methyl moiety led to ion *m/z* 236 (10). Methylation was performed to confirm the structure of the carboxylic acids. In comparison with underivatized carboxylic acids, a shift of 14 u would be expected after methylation. Carboxy-4-oxo-PVP (11) showed this shift for both molecular ion (*m/z* 277 to *m/z* 291) and base peak (*m/z* 172–*m/z* 186). A loss of a methoxy moiety from the molecular ions of the methylated carboxylic acids (11, 15) led to ions *m/z* 260 and *m/z* 290, respectively. Trimethylsilylated hydroxyphenyl-*N*,*N*-bis-dealkyl-PVP (6) showed analog fragmentation patterns as the acetylated metabolite. Benzyl-like cleavage between position 1 and position 2 led to ion *m/z* 193. β-Cleavage between position 1 and position 2 led to ion *m/z* 144. The loss of a methyl moiety led to *m/z* 322.

Concerning the structures of the metabolites, it must be mentioned that the position of the oxo group in the pyrrolidino-oxo metabolites of PVP could not be deduced from the fragmentation patterns, but there is a strong evidence for position 2″ of the pyrrolidine ring. In previous studies on the metabolism of other pyrrolidinophenones,[3–8,25] introduction of an oxygen atom in position 2″ leading to the respective lactams had been considered most likely. This assumption was based on the fact that lactam formation is common in the metabolism of other pyrrolidino compounds such as prolintane and nicotine.[46] The present study is the first in which carboxy metabolites corresponding to the hydrolyzed lactam ring were detected. Formation of such a metabolite could be explained by hydroxylation in position 2″ leading to a tertiary half aminal, followed by ring opening to the respective aliphatic aldehyde that is further oxidized to the respective carboxylic acid. This is in accordance with a similar metabolic pathway of phencyclidine described by Holsztynska *et al*.[47] Because such metabolites can only be considered as either precursors or hydrolysis products of respective lactams, their existence provides very strong evidence that the oxo group of the oxo metabolites is indeed in position 2″. Identification of the exact position of the hydroxy group in the side chain could not be deduced from fragmentation patterns. A similar problem had previously been described for the side chain homologue MPHP.[7] However, Adas *et al*. have shown that short alkyl chains are preferentially hydroxylated in position $\omega - 1$, that is, at the penultimate carbon atom. Assuming that this was also the case for PVP side chain hydroxylation, the oxo groups resulting from the side chain hydroxy metabolites would also be in position $\omega - 1$. In this position, they would be able to form a hydrogen bond with the nitrogen atom in the carboxy-4-oxo metabolites leading to a six-membered ring. The stability of such rings could explain why such carboxy metabolites were only observed in the case of PVP. In the related pyrrolidinophenones MPBP and MPHP, a carbonyl group in position $\omega - 1$ would either be too close to or

Journal of MASS SPECTROMETRY

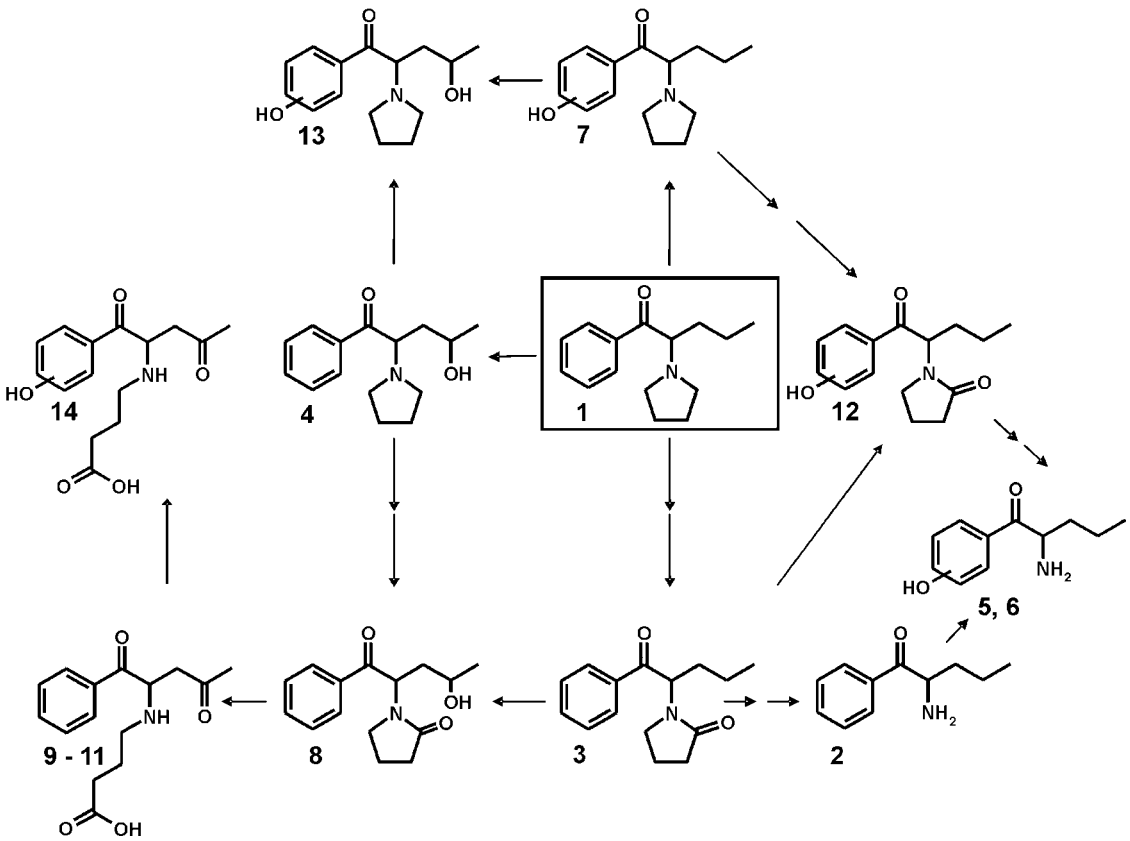

Figure 3. Proposed scheme for the metabolism of PVP in rats. The numbering of the compounds corresponds to that of the mass spectra of the corresponding compound in Fig. 1. Metabolites 4, 5, 12, and 13 are partly excreted as glucuronides and/or sulfates. The numbers correspond to those of the respective spectra in Fig. 1.

be too far away from the nitrogen to form such a stable nitrogen bond. In our opinion, these findings clearly indicate hydroxylation of the PVP side chain indeed occurs in position $\omega-1$, although they are of course not definitive proof. Only one peak was detected for the diastereomeric side chain hydroxy metabolite. However, for di-HO-PVP, two isomers could be identified in the trimethylsilylated extracts. This implies that more than one diastereomer of the side chain hydroxy metabolite had been formed, which were, however, not separated under the applied chromatographic conditions. No other diastereomers could be identified. This might either be explained by enantioselective formation of only one diastereomer, or, as already mentioned above, by formation of diastereomers that were not separated under the applied conditions.

Proposed metabolic pathways

On the basis of the identified metabolites of PVP, the following partly overlapping metabolic pathways could be postulated (Fig. 3): hydroxylation of the side chain (numbers 4, 8, and 13) followed by dehydrogenation to the corresponding ketone (numbers 9 and 14); hydroxylation of the 2″-position of the pyrrolidine ring followed by dehydrogenation to the corresponding lactams (numbers 3, 8, and 12) or followed by ring opening to the respective aliphatic aldehyde and further oxidation to the respective carboxylic acid (numbers 9 and 14); degradation of the pyrrolidine ring to the corresponding primary amines (numbers 2 and 5); hydroxylation of the phenyl ring, most probably in the 4′-position resulting in a phenylogous carboxylic acid (numbers 5, 6, and 12–14). As the peaks of HO-phenyl-PVP (7), hydroxyphenyl-2″ oxo-PVP (12), di-HO-PVP (13), HO-phenyl-amino-PVP (5), and HO-alkyl-PVP (4) were more abundant after glucuronidase and sulfatase hydrolysis, it can be concluded that they were partly excreted as glucuronides and/or sulfates.

CYPs involved in the main metabolic step

The initial screening studies with the nine most abundant human hepatic CYPs were performed to identify their possible role in PVP side chain hydroxylation. According to the supplier's advice, the incubation conditions chosen were adequate to make a statement on the general involvement of a particular CYP enzyme. The results showed that CYP2B6, CYP2C19, CYP2D6, and CYP1A2 were markedly capable of side chain hydroxylation of PVP.

GC/MS screening and identification

PVP and its metabolites were separated by GC and identified by full-scan EI MS after fast enzymatic hydrolysis, SPE, and trimethylsilylation according to Springer *et al.*[3–8] The main metabolites detected in the screening procedure are the oxo (3) and the hydroxyphenyl-*N*,*N*-bis-dealkyl metabolite (6). Mass chromatography with the following ions was used to detect the presence of PVP and these metabolites: *m/z* 126, 140, and 144. The selected ion *m/z* 126 was used for monitoring the presence of compounds with unchanged pyrrolidine ring, *m/z* 140 for compounds with 2″-oxo structure and *m/z* 144 for the amino-metabolites.

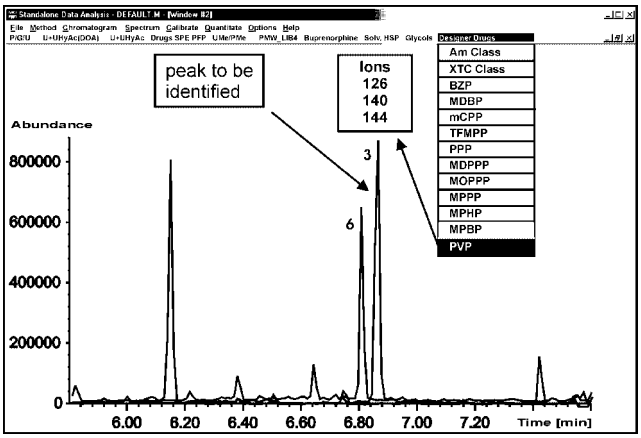

Figure 4. Typical mass chromatograms with the ions *m/z* 126, 140, and 144. They indicate the presence of PVP metabolites in a trimethylsilylated extract of a rat urine sample collected over 24 h after ingestion of 1 mg/kg body mass of PVP–HNO_3. The numbering of the peaks corresponds to that of the mass spectra of the corresponding derivative in Fig. 1. The merged chromatograms can be differentiated by their colors on a color screen.

Figure 4 shows reconstructed mass chromatograms indicating the presence of PVP metabolites in a trimethylsilylated extract of rat urine after administration of 1 mg/kg body mass dose of PVP–HNO_3. For the structural homologue pyrovalerone, single doses of 20–75 mg of pyrovalerone had been used in studies on pharmacological and therapeutic effects in humans,[12,21,24,48] whereas intravenous drug abusers reportedly injected single doses of 60–140 mg of pyrovalerone. Assuming a similar dosage for PVP, the above-mentioned 1 mg/kg body mass dose of PVP should therefore approximately correspond to a dose ingested by abusers. This assumption is further supported by the fact that seized tablets of the related designer drug PPP contained approximately 40 mg.

The identity of the peaks in the mass chromatogram was confirmed by computerized comparison of the underlying mass spectrum with reference spectra recorded during this study.[32] Fig. 5 illustrates the mass spectrum underlying the marked peak in Fig. 4, the reference spectrum (number 3 in Fig. 1), the structure, and the hit list found by computer library search.

Unfortunately, no authentic human urine samples after intake of PVP were available. However, experience from other metabolism and analytical studies in rats and humans support the assumption that the metabolites found in rat urine should also be present in human urine.[10,33,49–53] Therefore, it can be concluded that the procedure should also be applicable for human urine screening for PVP in clinical and forensic cases.

Conclusions

The presented findings show that the new designer drug PVP is extensively metabolized by rats via several pathways. Therefore, the urine screening procedure should be focused on the metabolites. Assuming similar metabolism and dosages in humans, an intake of PVP should be detectable via its metabolites in urine, although the proportion of the different metabolites may vary between species. As different CYP isoenzymes were found to be involved in the main metabolic step of PVP, genetic polymorphisms and/or interactions should be of minor relevance.

Acknowledgements

The authors like to thank Andrea E. Schwaninger, Gabriele Ulrich, and Armin A. Weber for their support.

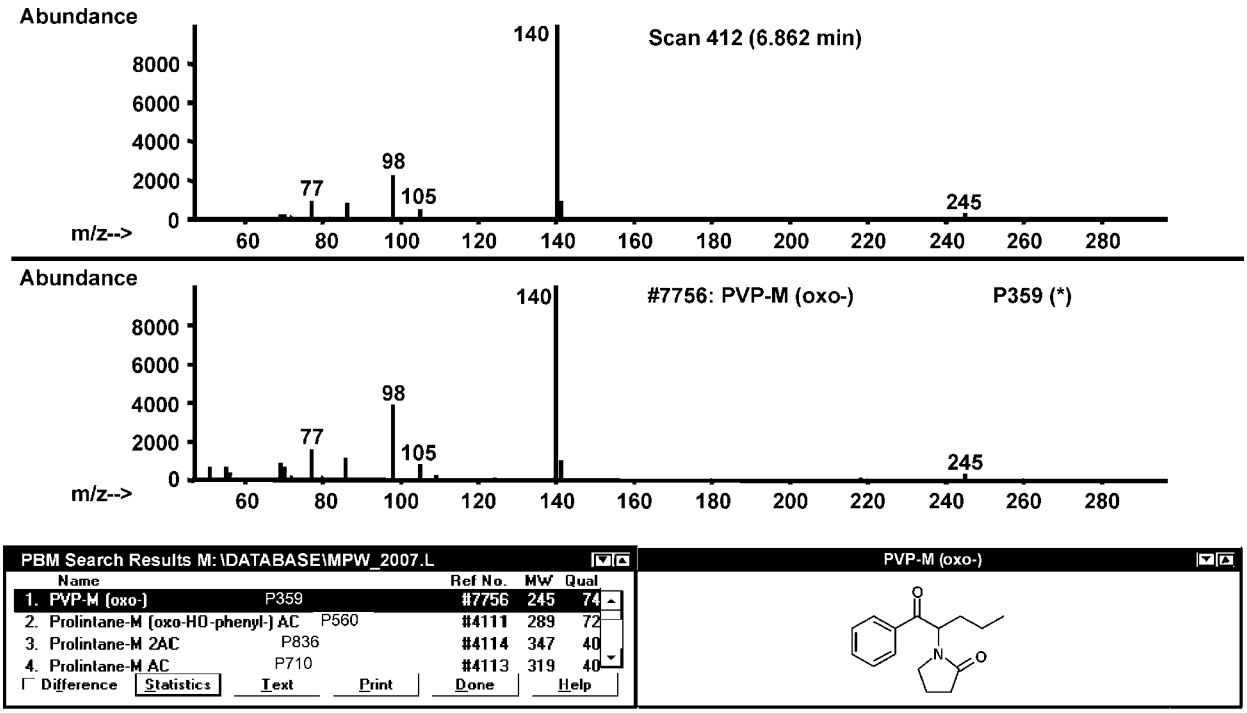

Figure 5. Mass spectrum underlying the marked peak in Fig. 4, the reference spectrum, the structure, and the hit list found by computer library search.

Journal of MASS SPECTROMETRY

References

[1] C. Haas, C. Sauer, M. R. Meyer, G. Fritschi, H. H. Maurer, F. T. Peters. Studies on the metabolism and toxicological analysis of the new designer drug alpha-Pyrrolidino-valerophenone (PVP) in rat urine using GC-MS techniques. In *Current Contributions to Forensic and Clinical Toxicology*, F. Pragst, R. Aderjan (eds). GTFCH: Bad Vilbel **2008**, 269.

[2] P. Roesner, T. Junge, G. Fritschi, B. Klein, K. Thielert, M. Kozlowski. Neue synthetische drogen: piperazin-, procyclidin- und alpha-aminopropiophenonderivate. *Toxichem und Krimtech* **1999**, *66*, 81.

[3] D. Springer, G. Fritschi, H. H. Maurer. Metabolism of the new designer drug alpha-pyrrolidinopropiophenone (PPP) and the toxicological detection of PPP and 4′methyl-alpha-pyrrolidinopropiophenone (MPPP) studied in urine using gas chromatography-mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* **2003**, *796*, 253.

[4] D. Springer, F. T. Peters, G. Fritschi, H. H. Maurer. Studies on the metabolism and toxicological detection of the new designer drug 4′-methyl-alpha-pyrrolidinopropiophenone in urine using gas chromatography-mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* **2002**, *773*, 25.

[5] D. Springer, G. Fritschi, H. H. Maurer. Metabolism and toxicological detection of the new designer drug 4′-methoxy-α-pyrrolidinopropiophenone studied in rat urine using gas chromatography-mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* **2003**, *793*, 331.

[6] D. Springer, G. Fritschi, H. H. Maurer. Metabolism and toxicological detection of the new designer drug 3′, 4′-methylenedioxy-alpha-pyrrolidinopropiophenone studied in urine using gas chromatography-mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* **2003**, *793*, 377.

[7] D. Springer, F. T. Peters, G. Fritschi, H. H. Maurer. New designer drug 4′-methyl-alpha-pyrrolidinohexanophenone: Studies on its metabolism and toxicological detection in urine using gas chromatography-mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* **2003**, *789*, 79.

[8] F. T. Peters, M. R. Meyer, G. Fritschi, H. H. Maurer. Studies on the metabolism and toxicological detection of the new designer drug 4′-methyl-alpha-pyrrolidinobutyrophenone (MPBP) in urine using gas chromatography-mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* **2005**, *824*, 81.

[9] F. Westphal, T. Junge, P. Rosner, G. Fritschi, B. Klein, U. Girreser. Mass spectral and NMR spectral data of two new designer drugs with an alpha-aminophenone structure: 4′-Methyl-alpha-pyrrolidinohexanophenone and 4′-methyl-alpha-pyrrolidinobutyrophenone. *Forensic Science International* **2007**, *169*, 32.

[10] H. H. Maurer, K. Pfleger, A. A. Weber. In *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and their Metabolites*, 3rd ed. Wiley-VCH: Weinheim **2007**.

[11] H. H. Maurer. Forensic screening with GC-MS. In *Handbook of Analytical Separation Sciences: Forensic Sciences*, 2nd ed, M. Bogusz (ed). Elsevier Science: Amsterdam **2007**, 429.

[12] J. P. Fauquet, E. Morel, C. Demarty, J. R. Rapin. Role of central catecholamines in the psychostimulant activity of pyrovalerone. *Archives Internationales De Pharmacodynamie Et De Thérapie* **1976**, *224*, 325.

[13] A. Servin, J. P. Fauquet, C. Jacquot, J. R. Rapin. Effects of pyrovalerone on peripheral noradrenergic mechanisms. *Biochemical Pharmacology* **1978**, *27*, 1693.

[14] P. C. Meltzer, D. Butler, J. R. Deschamps, B. K. Madras. 1-(4-Methylphenyl)-2-pyrrolidin-1-yl-pentan-1-one (pyrovalerone) analogues: a promising class of monoamine uptake inhibitors. *Journal of Medicinal Chemistry* **2006**, *49*, 1420.

[15] G. Stille, H. Ackermann, E. Eichenberger, H. Lauener. Comparative pharmacological study of a new central stimulant, 1-p-tolyl-1-oxo-2-pyrrolidino-n-pentane HCl. *Arzneimittel-Forschung* **1963**, *13*, 871.

[16] H. Heimann, G. Lukacs. Experimental psychological differentiation of the effect of 2 psychostimulants (F-1983 and amphetamine) in humans. *Psychopharmacologia* **1965**, *8*, 79.

[17] A. R. Holliday, R. B. Morris, R. P. Sharpley. Compound 84/F 1983 compared with D-amphetamine and placebo in regard to effects on human performance. *Psychopharmacologia* **1964**, *6*, 192.

[18] J. Goldberg, G. Gardos, J. O. Cole. A controlled evaluation of pyrovalerone in chronically fatigued volunteers. *International Pharmacopsychiatry* **1973**, *8*, 60.

[19] H. Heinmann, K. Vetter. Clinical studies of a new psychostimulant (F-1983). *Schweizerische Medizinische Wochenschrift* **1965**, *95*, 306.

[20] S. Soderholm, N. M. Viukari, R. Rimon. The psychotropic effect of the Pyrovalerone (F-1983), placebo, the milieu therapy and mental condition of the patients in forensic psychiatry. *Nervenarzt* **1976**, *47*, 88.

[21] P. Deniker, H. Loo, H. Cuche, J. M. Roux. Abuse of pyrovalerone by drug addicts. *Annales Médico-Psychologiques* **1975**, *2*, 745.

[22] D. S. Lho, J. Lee, S. Kim, J. Park, H. S. Shin. Identification of a pyrovalerone metabolite in the rat by gas chromatography-mass spectrometry and determination of pyrovalerone by gas chromatography-nitrogen-phosphorus detection. *Journal of Chromatography. B, Biomedical Sciences and Applications* **1996**, *687*, 253.

[23] W. Michaelis, J. H. Russel, O. Schindler. The metabolism of pyrovalerone hydrochloride. *Journal of Medicinal Chemistry* **1970**, *13*, 497.

[24] H. S. Shin, Y. S. Shin, S. Lee, B. B. Park. Detection and identification of pyrovalerone and its hydroxylated metabolite in the rat. *Journal of Analytical Toxicology* **1996**, *20*, 568.

[25] H. Shin, J. Park. Identification of the new metabolites of pyrovalerone by various derivatization methods in the rat urine. *Korean Biochemical Journal* **1994**, *27*, 357.

[26] H. H. Maurer. Position of chromatographic techniques in screening for detection of drugs or poisons in clinical and forensic toxicology and/or doping control [review]. *Clinical Chemistry and Laboratory Medicine* **2004**, *42*, 1310.

[27] H. H. Maurer. Hyphenated mass spectrometric techniques – indispensable tools in clinical and forensic toxicology and in doping control [review]. *Journal of Mass Spectrometry* **2006**, *41*, 1399.

[28] F. T. Peters, C. A. Dragan, D. R. Wilde, M. R. Meyer, M. Bureik, H. H. Maurer. Biotechnological synthesis of drug metabolites using human cytochrome P450 2D6 heterologously expressed in fission yeast exemplified for the designer drug metabolite 4′-hydroxymethyl-alpha-pyrrolidinobutyrophenone. *Biochemical Pharmacology* **2007**, *74*, 511.

[29] A. F. McKay, W. L. Ott, G. W. Taylor, M. N. Buchanan, J. F. Crooker. Diazohydrocarbons. *Canadian Journal of Research* **1950**, *28*, 683.

[30] H. H. Maurer, C. Kratzsch, A. A. Weber, F. T. Peters, T. Kraemer. Validated assay for quantification of oxcarbazepine and its active dihydro metabolite 10-hydroxy carbazepine in plasma by atmospheric pressure chemical ionization liquid chromatography/mass spectrometry. *Journal of Mass Spectrometry* **2002**, *37*, 687.

[31] H. H. Maurer. Toxicological analysis of drugs and poisons by GC-MS [review]. *Spectroscopy Europe* **1994**, *6*, 21.

[32] H. H. Maurer, K. Pfleger, A. A. Weber. In *Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants and their Metabolites*, 4th Revision, Wiley-VCH: Weinheim **2007**.

[33] R. F. Staack, G. Fritschi, H. H. Maurer. Studies on the metabolism and the toxicological analysis of the new piperazine-like designer drug N-benzylpiperazine in urine using gas chromatography-mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* **2002**, *773*, 35.

[34] R. F. Staack, D. S. Theobald, L. D. Paul, D. Springer, T. Kraemer, H. H. Maurer. *In vivo* metabolism of the new designer drug 1-(4-methoxyphenyl)piperazine (MeOPP) in rat and identification of the human cytochrome P450 enzymes responsible for the major metabolic step. *Xenobiotica* **2004**, *34*, 179.

[35] D. S. Theobald, S. Fehn, H. H. Maurer. New designer drug 2,5-dimethoxy-4-propylthiophenethylamine (2C-T-7): studies on its metabolism and toxicological detection in rat urine using gas chromatography/mass spectrometry. *Journal of Mass Spectrometry* **2005**, *40*, 105.

[36] R. Brenneisen, S. Geisshusler. Psychotropic drugs. III. Analytical and chemical aspects of Catha edulis Forsk. *Pharmaceutica Acta Helvetiae* **1985**, *60*, 290.
[37] M. Pokrajac, B. Miljkovic, B. Misailovic. Mass spectrometric investigation of 2-aminopropiophenones and some of their metabolites. *Rapid Communications in Mass Spectrometry* **1991**, *5*, 59.
[38] D. S. Theobald, H. H. Maurer. Studies on the metabolism and toxicological detection of the designer drug 4-ethyl-2,5-dimethoxy-beta-phenethylamine (2C-E) in rat urine using gas chromatographic-mass spectrometric techniques. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* **2006**, *842*, 76.
[39] D. S. Theobald, G. Fritschi, H. H. Maurer. Studies on the toxicological detection of the designer drug 4-bromo-2,5-dimethoxy-beta-phenethylamine (2C-B) in rat urine using gas chromatography-mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* **2007**, *846*, 374.
[40] D. S. Theobald, E. Schneider, H. H. Maurer. New designer drug 4-iodo-2,5-dimethoxy-phenethylamine (2CI): Studies on its metabolism and toxicological detection in rat urine using GC-MS techniques. In *Proceedings of the XIVth GTFCh Symposium in Mosbach 2005*, F. Pragst, R. Aderjan (eds). Helm-Verlag: Heppenheim (Germany) **2006**, 248.
[41] D. S. Theobald, H. H. Maurer. Studies on the metabolism and toxicological detection of the designer drug 2,5-dimethoxy-4-methyl-beta-phenethylamine (2C-D) in rat urine using gas chromatographic-mass spectrometric techniques. *Journal of Mass Spectrometry* **2006**, *41*, 1509.
[42] D. S. Theobald, R. F. Staack, M. Puetz, H. H. Maurer. New designer drug 2,5-dimethoxy-4-ethylthio-beta-phenethylamine (2C-T-2): studies on its metabolism and toxicological detection in rat urine using gas chromatography/mass spectrometry. *Journal of Mass Spectrometry* **2005**, *40*, 1157.
[43] F. W. McLafferty, F. Turecek. In *Interpretation of Mass Spectra*, 4th ed. University Science Books: Mill Valley, **1993**.
[44] R. M. Smith, K. L. Busch. *Understanding Mass Spectra – A Basic Approach*. Wiley: New York (NY), **1999**.
[45] R. A. de-Zeeuw, J. P. Franke, H. H. Maurer, K. Pfleger. In *Gas Chromatographic Retention Indices of Toxicologically Relevant Substances and their Metabolites (Report of the DFG commission for clinical toxicological analysis, special issue of the TIAFT bulletin)*, 3rd ed. Wiley-VCH publishers: Weinheim **1992**.
[46] S. Vickers, S. L. Polsky. The biotransformation of nitrogen containing xenobiotics to lactams. *Current Drug Metabolism* **2000**, *1*, 357.
[47] E. J. Holsztynska, E. F. Domino. Biotransformation of phencyclidine. *Drug Metabolism Reviews* **1986**, *16*, 285.
[48] R. A. Glennon, M. Yousif, N. Naiman, P. Kalix. Methcathinone: a new and potent amphetamine-like agent. *Pharmacology, Biochemistry, and Behavior* **1987**, *26*, 547.
[49] J. Bickeboeller-Friedrich, H. H. Maurer. Screening for detection of new antidepressants, neuroleptics, hypnotics, and their metabolites in urine by GC-MS developed using rat liver microsomes. *Therapeutic Drug Monitoring* **2001**, *23*, 61.
[50] R. F. Staack, H. H. Maurer. Toxicological detection of the new designer drug 1-(4-methoxyphenyl)piperazine and its metabolites in urine and differentiation from an intake of structurally related medicaments using gas chromatography-mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* **2003**, *798*, 333.
[51] A. H. Ewald, F. T. Peters, M. Weise, H. H. Maurer. Studies on the metabolism and toxicological detection of the designer drug 4-methylthioamphetamine (4-MTA) in human urine using gas chromatography-mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* **2005**, *824*, 123.
[52] J. Beyer, D. Ehlers, H. H. Maurer. Abuse of nutmeg (*Myristica fragrans* houtt.): studies on the metabolism and the toxicological detection of its ingredients elemicin, myristicin and safrole in rat and human urine using gas chromatography/mass spectrometry. *Therapeutic Drug Monitoring* **2006**, *28*, 568.
[53] A. H. Ewald, G. Fritschi, W. R. Bork, H. H. Maurer. Designer drugs 2,5-dimethoxy-4-bromoamphetamine (DOB) and 2,5-dimethoxy-4-bromomethamphetamine (MDOB): studies on their metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques. *Journal of Mass Spectrometry* **2006**, *41*, 487.

