

# Studies on the metabolism and toxicological detection of the new designer drug *N*-benzylpiperazine in urine using gas chromatography–mass spectrometry<sup>☆</sup>

Roland F. Staack<sup>a</sup>, Giseller Fritschi<sup>b</sup>, Hans H. Maurer<sup>a,\*</sup>

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

<sup>b</sup>Hessisches Landeskriminalamt, D-65187 Wiesbaden, Germany

## Abstract

Studies are described on the metabolism and on the toxicological analysis of the piperazine-like designer drug *N*-benzylpiperazine (BZP, scene name “A2”) in rat and human urine using gas chromatography–mass spectrometry (GC–MS). The identified metabolites indicated that BZP was hydroxylated at the aromatic ring and that the piperazine moiety is metabolically degraded. Our systematic toxicological analysis (STA) procedure using full-scan GC–MS after acid hydrolysis, liquid–liquid extraction and microwave-assisted acetylation allowed the detection of the parent compound as well as of the above mentioned metabolites in rat urine after single administration of a dose calculated from the doses commonly taken by drug users. It has also proved to be applicable in authentic clinical or forensic cases. However, it should be considered that BZP is also a metabolite of the medicament piberaline. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** *N*-Benzylpiperazine (BZP); A2

## 1. Introduction

Piperazine-like compounds have been found on the illicit market as a new group of designer drugs. The best known and most widespread compound of this drug group is *N*-benzylpiperazine (BZP, scene name “A2”). BZP seems to become more and more popular in the scene of drug abusers as it could be seized by the police in different countries [2–5].

Organisations which check the “purity” of illegally sold tablets report its occurrence more and more often (<http://www.dancesafe.org>). Even a fatality after use of BZP and MDMA has already been reported [4]. Shulgin mentioned BZP in his book PIKHAL as a “pure stimulant” [6], so-called drug information web sites describe it as a psychoactive chemical (<http://www.erowid.org>, <http://www.lycaeum.org>, <http://www.eve-rave.ch>) and generally it is offered as an alternative to amphetamines.

Actually, BZP is not really a new compound. Originally, it was synthesized as a potential anthelmintic agent, but it was found to produce amphetamine-like effects in rats and (at higher dosage) in humans [7,8]. These studies suggested an approximate ratio of 10:1 (BZP:amphetamine) in the effect

<sup>☆</sup>Part of these results was reported at the 39th International TIAFT Meeting, Prague, August 26–30, 2001 [1].

\*Corresponding author. Tel.: +49-6841-16-26050; fax: +49-6841-16-26051.

E-mail address: [hans.maurer@uniklinik-saarland.de](mailto:hans.maurer@uniklinik-saarland.de) (H.H. Maurer).

potency. A double blind study with former amphetamine addicts showed that BZP and amphetamine produced indistinguishable subjective effects, so that Campbell et al. already predicted a possible abuse in 1973 and advised that it should be scheduled [8]. However, this advice has not yet been followed in any country.

Pharmacological studies of BZP showed a central serotoninomimetic action, which involves serotonin (5-HT) uptake inhibition and 5-HT<sub>1</sub> receptor agonistic effects [9]. In other publications its pharmacological profile was compared to that of 1-[3-chlorophenyl]piperazine (mCPP), an active metabolite of trazodone and nefazodone showing 5-HT agonistic and antagonistic activities [10]. Although an enhanced noradrenaline release by BZP has been reported, contrary effects of BZP on  $\alpha_2$ -adrenoceptors are described [11,12]. Furthermore, interactions with the dopamine system have been described [13].

The metabolism of BZP has not yet been studied. However, the knowledge about metabolic steps is a prerequisite for developing toxicological screening procedures and for toxicological risk assessment, as in both cases the metabolites may play a major role. So far, only a GC procedure for determination of BZP as metabolite of piberaline in plasma and urine has been described [14]. Some analytical data on BZP have been published recently [5]. Screening procedures for detection of BZP and its metabolites have not yet been published.

The aim of our study was to identify the BZP metabolites in rat and human urine using GC–MS in the electron impact (EI) and positive-ion chemical ionization (PCI) mode and to study the detectability of BZP within our systematic toxicological analysis (STA) procedure in urine by GC–MS in the EI mode [15,16].

## 2. Experimental

### 2.1. Chemicals and reagents

All chemicals and biochemicals used were obtained from E. Merck, Darmstadt (Germany) and were of analytical grade. BZP–HCl was provided from Hessisches Landeskriminalamt and the refer-

ence metabolites *N*-benzylethylenediamine and benzylamine were obtained from Lancaster Synthesis, Mühlheim (Germany).

### 2.2. Urine samples

The investigations were performed using urine of male rats (Wistar, Ch. River, Sulzfeld, Germany) which were administered a single 50 mg/kg body mass dose (for metabolism studies) or a 1-mg/kg body mass dose (for STA) in aqueous suspension by gastric intubation. Urine was collected separately from the faeces over a 24-h period. All samples were directly analyzed and then stored at  $-20^{\circ}\text{C}$  before further analysis. Blank urine samples were collected before drug administration to check whether the samples were free of interfering compounds. Authentic urine samples had been submitted to our laboratory for toxicological analysis.

### 2.3. Sample preparation for metabolism studies including enzymatic hydrolysis

A 5-ml portion of urine was adjusted to pH 5.2 with acetic acid (1 *M*) and incubated at  $37^{\circ}\text{C}$  for 12 h with 100  $\mu\text{l}$  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), then adjusted to pH 8–9 and extracted with 5 ml of a dichloromethane–isopropanol–ethylacetate mixture (1:1:3; v/v/v). After phase separation by centrifugation, the organic layer was transferred into pear-shaped flasks and evaporated to dryness and the residue was derivatized.

Acetylation was conducted with 50  $\mu\text{l}$  of an acetic anhydride–pyridine mixture (3:2; v/v) for 10 min under microwave irradiation at about 440 W [17–19]. After evaporation, the residue was dissolved in 50  $\mu\text{l}$  of methanol and 2  $\mu\text{l}$  of this solution were injected into the GC–MS. Trifluoroacetylation was conducted with 50  $\mu\text{l}$  trifluoroacetic anhydride and 50  $\mu\text{l}$  ethyl acetate for 5 min under microwave irradiation at about 400 W [17,18]. After evaporation of the derivatization mixture the residue was dissolved in 40  $\mu\text{l}$  alcohol- and water-free ethyl acetate and 1–2  $\mu\text{l}$  were injected into the GC–MS. Heptafluorobutyrylation was conducted with 50  $\mu\text{l}$  hepta-

fluorobutyric anhydride for 5 min under microwave irradiation at about 400 W [17,18]. After evaporation of the reagent the residue was dissolved in 50  $\mu$ l alcohol- and water-free ethyl acetate and 1–2  $\mu$ l were injected into the GC–MS. Methylation was conducted after reconstitution of the extraction residue in 50  $\mu$ l of methanol with 50  $\mu$ l of a solution of diazomethane in diethyl ether, synthesized according to the procedure of McKay et al. [20]. The reaction vials were sealed and left at room temperature for 15 min. Thereafter, the mixture was once again gently evaporated to dryness under a stream of nitrogen and redissolved in 50  $\mu$ l of methanol and a 3- $\mu$ l aliquot was injected into the GC–MS system [18].

The same procedure with the exception of enzymatic hydrolysis was used to study whether metabolites of BZP are excreted unconjugated.

#### 2.4. Sample preparation for toxicological analysis including acid hydrolysis

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 basified with 1.5 ml of 10 mol/l aqueous sodium hydroxide and the resulting solution was mixed with 2 ml of 2.3 mol/l aqueous ammonium sulphate to obtain a pH value between 8 and 9. Before extraction, the other aliquot of native urine was added. 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 and evaporated to dryness. The residue was derivatized by acetylation with 50  $\mu$ l of an acetic anhydride–pyridine mixture (3:2; v/v) for 5 min under microwave irradiation at about 400 W [15]. After evaporation of the derivatization mixture, the residue was dissolved in 50  $\mu$ l of methanol and 2  $\mu$ l were injected into the gas chromatograph.

#### 2.5. Gas chromatography–mass spectrometry

BZP and its metabolites were separated and identified in acetylated, trifluoroacetylated, heptafluorobutyrylated, methylated plus acetylated or underivatized urine extracts using a Hewlett-Packard (HP, 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. The GC conditions were as follows: splitless injection mode; column, HP capillary (12 m $\times$ 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 to 310 °C at 30°/min, initial time 3 min, final time 8 min. The MS conditions were as follows: full scan mode; EI ionization mode; ionization energy, 70 eV; ion source temperature, 220 °C; capillary direct interface heated at 260 °C.

For toxicological detection of BZP and its metabolites, mass chromatography with the selected ions  $m/z$  91, 107, 137, 146, 191 and 204 was used. Generation of the mass chromatograms could be started by clicking the corresponding pull down menu which executes the user defined macros [21] (the macros can be obtained from the authors: e-mail: hans.maurer@uniklinik-saarland.de). The identity of the peaks in the mass chromatograms was confirmed by computerized comparison [22] of the mass spectra underlying the peaks (after background subtraction) with reference spectra (Fig. 1) recorded during this study.

#### 2.6. Fluorescence polarization immunoassays (FPIA)

Native urine samples from volunteers were used for immunological determination. The TDx system of Abbot (Irving, TX, USA) with the amphetamine/metamphetamine II assay (AM/MA II) was applied. The cut off value and the detection limit recommended by the manufacturers were 300 and 100 ng/ml, respectively. To determine the cross reactivities of BZP and its metabolite *N*-benzylethylenediamine with this assay, blank urine samples were spiked in concentrations of 100–1 000 000 ng/ml. Furthermore, authentic human urines containing BZP and its metabolites have been measured.

### 3. Results and discussion

#### 3.1. Sample preparation

Cleavage of conjugates by gentle enzymatic hydrolysis was necessary before extraction and GC–MS

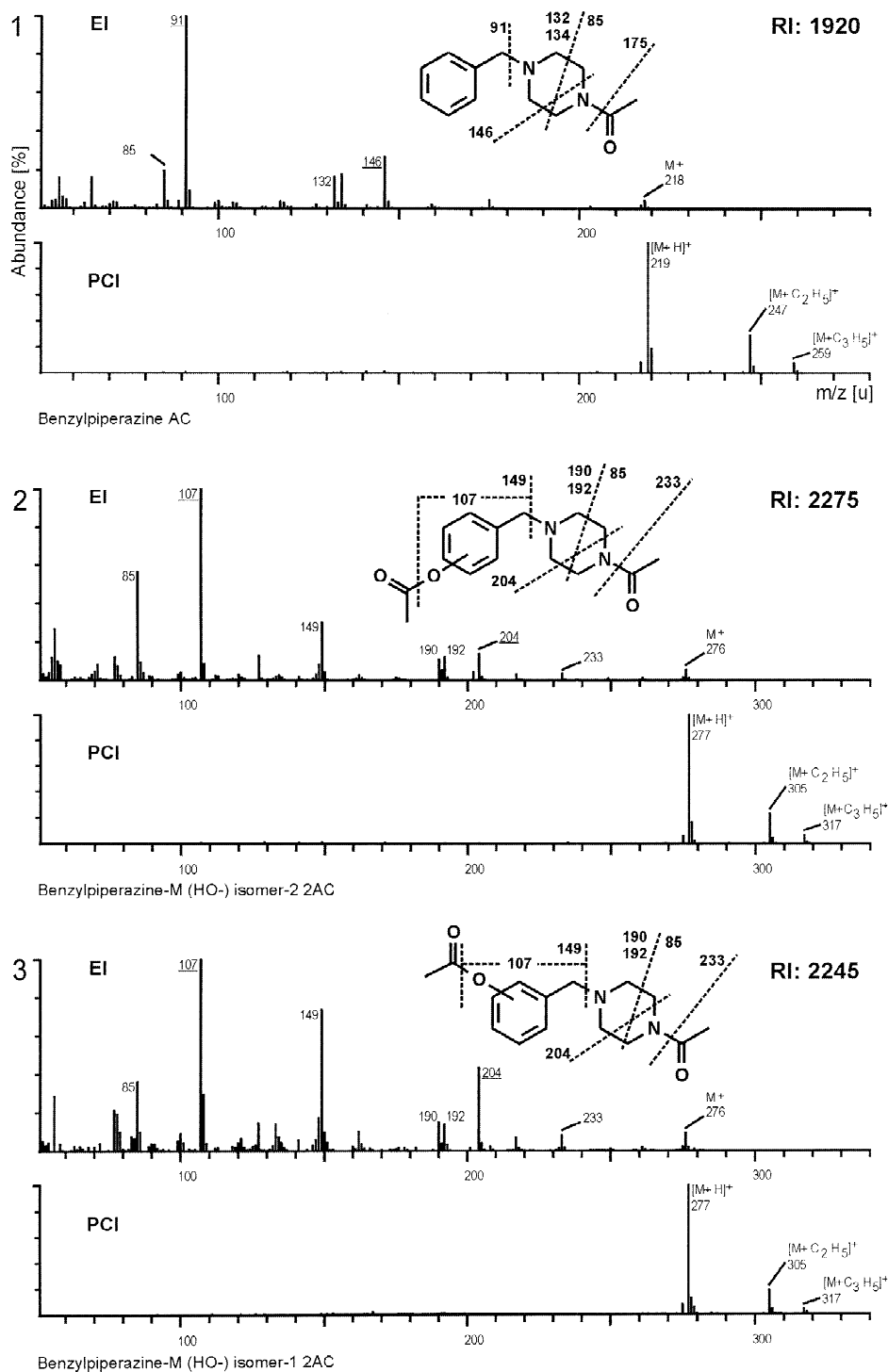


Fig. 1. EI and PCI mass spectra, the gas chromatographic retention indices (RI), structures and predominant fragmentation patterns of BZP and its metabolites after acetylation for precise identification. The numbers of the spectra correspond to those in Fig. 2. Ions selected for the toxicological detection are underlined. (The axes are only labelled for 1).

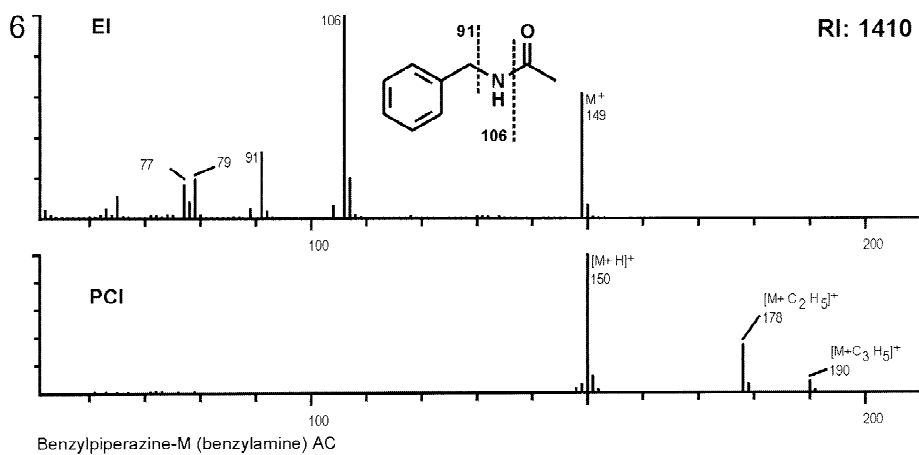
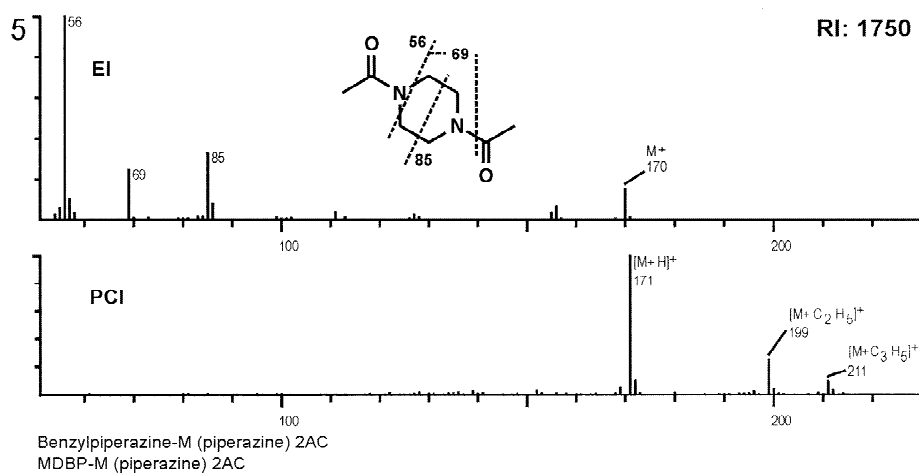
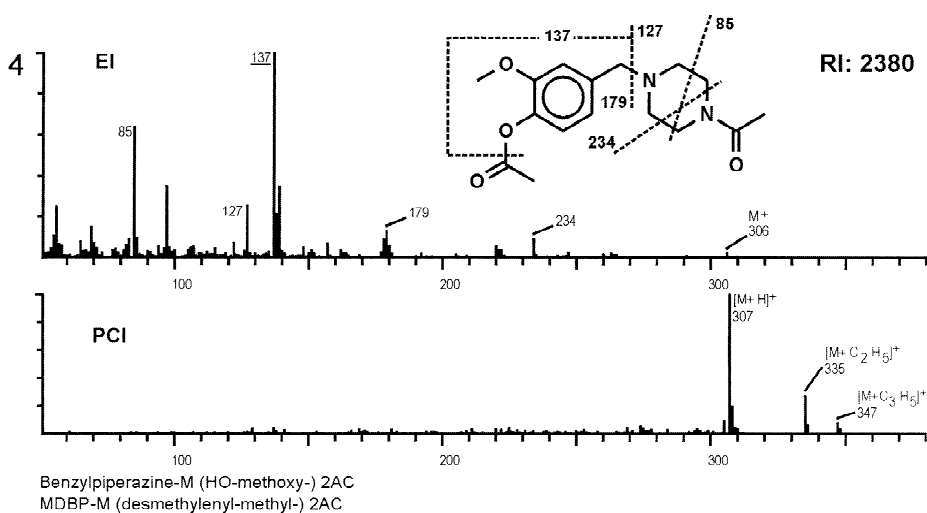


Fig. 1. (continued)

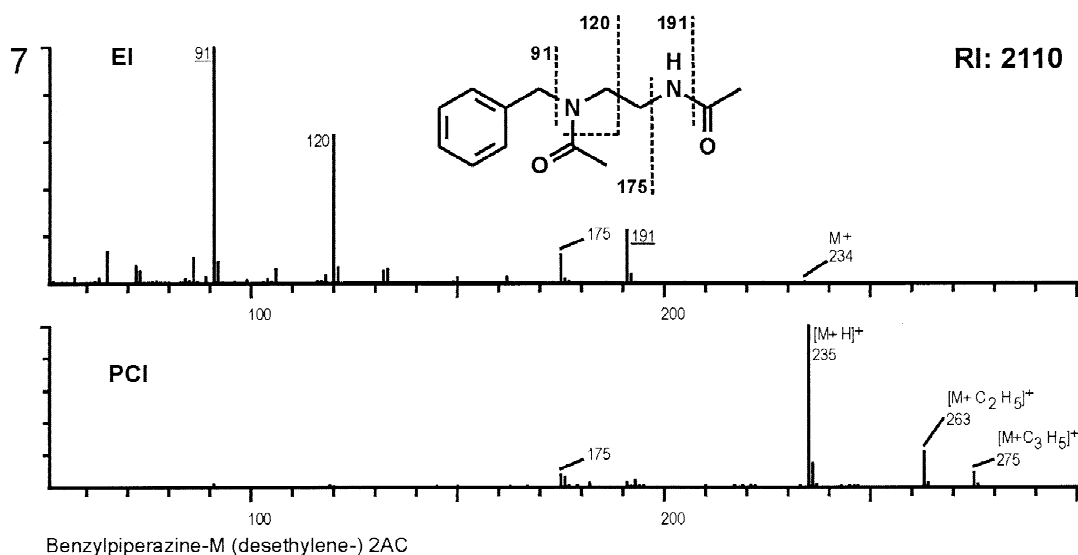


Fig. 1. (continued)

analysis of the suspected metabolites in order not to overlook 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 proved to be very efficient and fast for cleavage of conjugates [15,19,23–26]. However, some compounds covered by this STA were altered or destroyed during hydrolysis [15,16]. Therefore, one part of unhydrolyzed urine was added 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 apparatus [15,16].

The samples were extracted at pH 8–9. The (metabolic) introduction of an aromatic hydroxy group into BZP leads to phenolbases which are best extracted at pH 8–9. Using a more alkaline pH for extraction leads to the loss of such hydroxy metabolites which are often excreted for a longer time than the parent compounds [23,27–31]. Derivatization of the extracts was indispensable for sensitive detection. The extraction efficacy of BZP itself determined

after STA work up was  $45 \pm 8\%$ . The rather poor extraction efficacy may be due to the fact that the extraction at pH 8–9 is a compromise to extract also phenolbases and not best for extraction of pure bases such as BZP. Of course, the extraction yield of BZP could be improved by using a more alkaline pH, but other toxicologically relevant compounds with phenolbase structure may be lost. Careful sample preparation, especially gentle evaporation of the extraction and derivatization mixtures, should be performed due to the high volatility of BZP.

### 3.2. Identification of metabolites

The urinary metabolites of BZP were separated by GC and identified by EI and PCI MS after enzymatic hydrolysis, extraction, acetylation, trifluoroacetylation, heptabutyrylation, methylation plus acetylation or without derivatization. The GC and MS data of these derivatives will be included in the forthcoming update of our handbook and library [22,32]. The different derivatization procedures were used to gain more information on the structures of the metabolites. The EI mass spectra of the postulated metabolites were interpreted in correlation to those of the parent compound according to the rules described by McLafferty and Turecek [33]. The PCI mass spectra were additionally used to ensure the identity of the

metabolites, because they gave strong molecular peaks ( $M+H$ ) with adduct ions typical for PCI using methane as reagent gas. The identity of *N*-benzylethylenediamine and benzylamine could further be confirmed by comparing their mass spectra and the gas chromatographic retention indices (RI) with those of the corresponding reference substances. The EI and PCI mass spectra, the RIs, the structures and predominant fragmentation patterns of the acetylated metabolites are shown in Fig. 1. The spectra are arranged according to the numbers given in Fig. 2.

Besides BZP (mass spectrum no. 1 in Fig. 1), the following metabolites could be identified in rat and human urine: mainly 4'-hydroxy-BZP (mass spectrum no. 2 or 3), but also 3'-hydroxy-BZP (mass spectrum no. 3 or 2), 4'-hydroxy-3'-methoxy-BZP (mass spectrum no. 4), piperazine (mass spectrum no. 5), benzylamine (mass spectrum no. 6) and *N*-benzylethylenediamine (mass spectrum no. 7). Although we could not determine the exact position of the hydroxy groups by means of GC–MS, we can assume positions 3' and 4'. The corresponding hydroxy-methoxy-metabolite is identical with the hydroxy-methoxy metabolite formed from the 3,4-

methylenedioxy analogue 1-[3,4-methylenedioxy-benzyl]piperazine (MDBP) [1]. Both compounds showed identical RIs and the same mass spectrum. In general, metabolic hydroxylation in the para position is preferred, so that we assume that the 4'-hydroxy-BZP is the predominant hydroxy metabolite. As the methylation step of the dihydroxy metabolite to the corresponding hydroxy-methoxy-metabolite is catalyzed by catechol-*O*-methyl-transferase (COMT), we concluded that the 4'-hydroxy-3'-methoxy-BZP is predominantly formed due to the selectivity of COMT for the methylation of the hydroxy group in position 3. The involvement of COMT was confirmed by in vitro studies according to Maurer et al. [34].

Based on the identified metabolites of BZP the following, partially overlapping, metabolic pathways shown in Fig. 2 could be postulated: alteration of the phenyl ring by single or double aromatic hydroxylation followed by catechol-*O*-methyl-transferase (COMT) catalyzed methylation to 4'-hydroxy-3'-methoxy-BZP. The piperazine moiety was degraded by double *N*-dealkylation either to *N*-benzylethylenediamine or to benzylamine. Metabolic dealkylation reaction at the benzyl carbon leads to

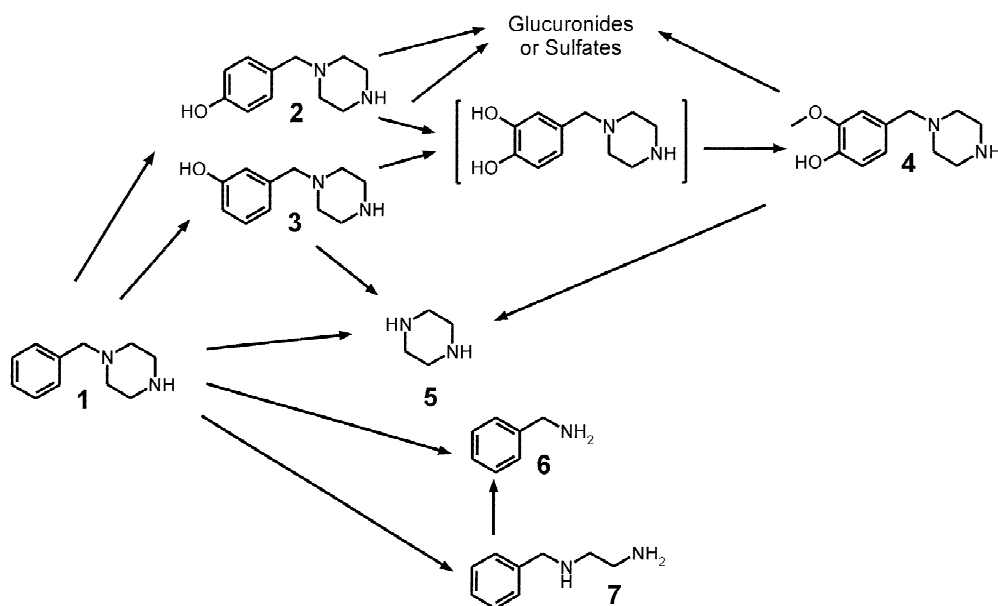


Fig. 2. Proposed scheme for the metabolism of BZP in rats and humans. The metabolites 2–4 were also excreted as glucuronic and/or sulfuric acid conjugates in urine.

the liberation of piperazine. Besides piperazine, benzaldehyde should be formed by this metabolic step which can further be oxidized to benzoic acid. To check for such acidic metabolites, the urine samples had also been extracted after cleavage of conjugates at acidic pH (4–5) and the corresponding extracts had been analyzed after methylation plus acetylation. Benzoic acid was found. However, as benzoic acid is ubiquitous in rat urine, the formation of this metabolite could only be postulated. Nevertheless this metabolic step has already been described elsewhere for a BZP derivative [35]. No further acidic metabolites were found. The phenolic compounds are partially excreted as conjugates, since the peak areas were greater after cleavage of conjugates.

### 3.3. Detection by GC–MS within the STA

BZP and its metabolites were separated by GC and identified by EI MS after acid hydrolysis, extraction and acetylation within our standard STA. Mass chromatography with the following ions  $m/z$  91, 107, 137, 146, 191 and 204 was used to indicate the presence of BZP and/or its metabolites. Generation of the mass chromatograms could be started by clicking the corresponding pull down menu which executes the user defined macros. Fig. 3 shows reconstructed mass chromatograms indicating the presence of BZP and its metabolites in an acetylated extract of a rat urine sample after administration of 1 mg/kg body mass of BZP, a dose that corresponds to the common dose of abusers (upper part) and of a human urine sample after intake of an unknown BZP dose. 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 [22]. The limit of detection of BZP in urine was 100 ng/ml ( $S/N$  3) under routine MS conditions.

The ion  $m/z$  91 was used for indicating the presence of BZP and metabolites with unchanged aromatic ring structure forming a benzyl ion. The selected ions  $m/z$  91 and 146 were used for indication of BZP itself, the ions  $m/z$  91 and 191 were used for indication of *N*-benzylethylenediamine and the ions  $m/z$  107 and 204 were used for indication of hydroxy-BZP isomers, the main metabolites. The ion

$m/z$  137 was used for indicating the presence of the 4'-hydroxy-3'-methoxy-metabolite. Screening for piperazine and benzylamine was not useful, since they were excreted only in minor amounts and are not unique metabolites of BZP or structural related designer drugs. However, they could be detected in the rat urine samples as well as in authentic human urine samples. As shown in Fig. 4, the identity of peaks in the mass chromatograms was confirmed by computerized comparison of the underlying mass spectrum with reference spectra (Fig. 1) recorded during this study. In our experience, the gas chromatographic retention indices (RI) 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 (Section 2.5) and calculated in correlation with the Kovats' indices [36] of the components of a standard solution of typical drugs which is measured daily for testing the GC–MS performance [37,38]. The reproducibility of retention indices measured on capillary columns 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.

It should be noted that BZP is also a metabolite of the antidepressant piberaline (1-(benzyl)-4-(2-pyridinylcarbonyl)-piperazine) [9,35,39]. Therefore, intake of piberaline has to be taken into consideration if BZP and/or its metabolites can be found in a urine screening. It has therefore to be checked whether piberaline or specific metabolites are present. As piberaline is only excreted to a small extent (3–4%) as BZP in urine [35], this differentiation should be possible. However, further studies have to be conducted. Furthermore, some of the metabolites of BZP are not unique. 1-[3,4-methylenedioxy-benzyl]piperazine (MDBP) is also metabolized to 4'-hydroxy-3'-methoxy-BZP (demethylene-methyl-MDBP) and to piperazine, which again is in use as an anthelmintic.

Our STA procedure allowed the unambiguous identification of BZP and its metabolites in rat urine after administration of a dose corresponding to a common drug users' dose to rats and also in authentic human urine samples. It is therefore advantageous



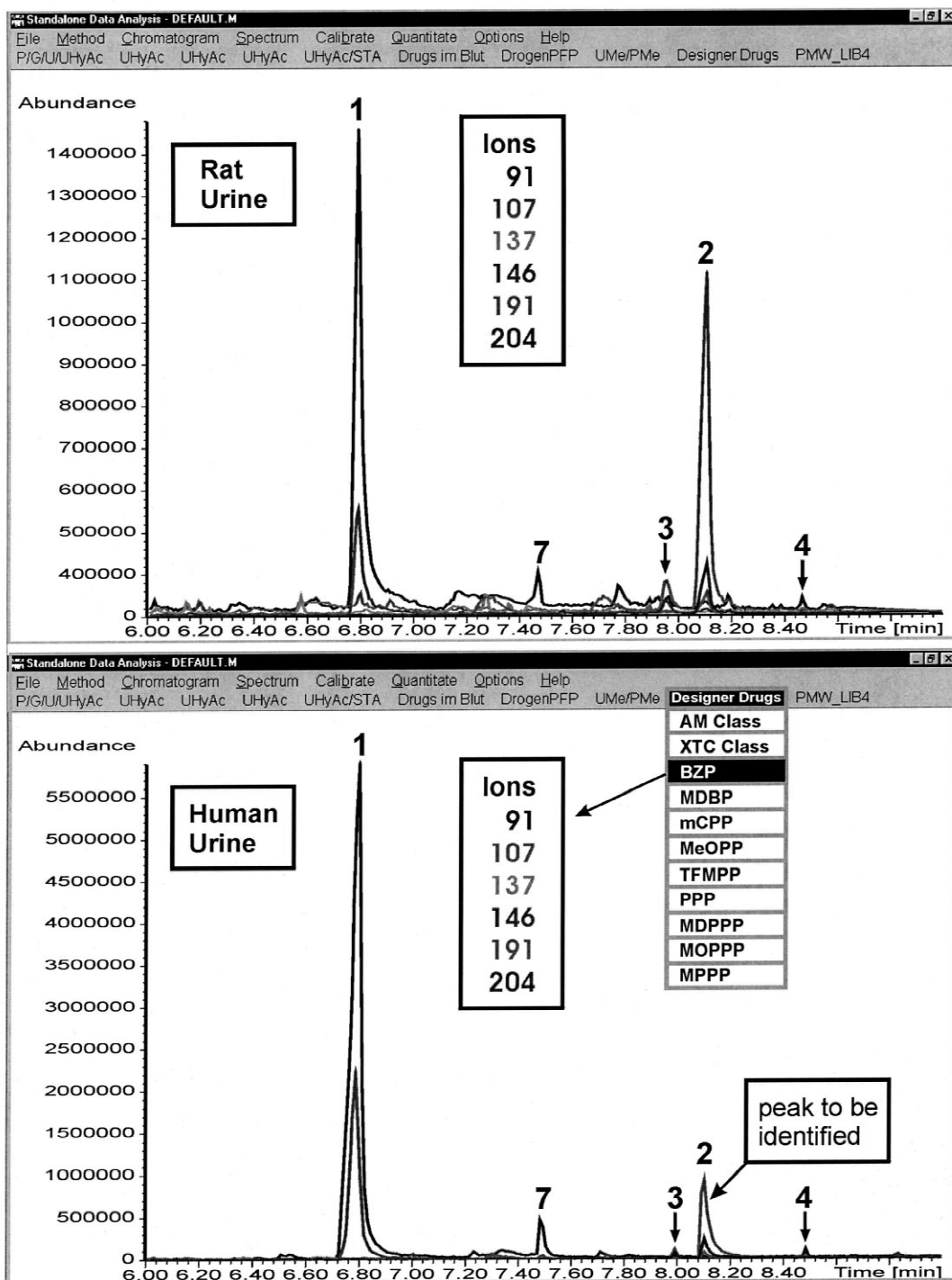


Fig. 3. Typical mass chromatograms with the ions  $m/z$  91, 107, 137, 146, 191 and 204. They indicate the presence of BZP and its metabolites in an acetylated extract of a rat urine sample after application of a common abuser's dose of 1 mg/kg body mass (upper part) and of a human urine sample after intake of an unknown dose. The numbers of the peaks correspond to those in Figs. 1 and 2. The merged chromatograms can be differentiated by their colors on a color screen.

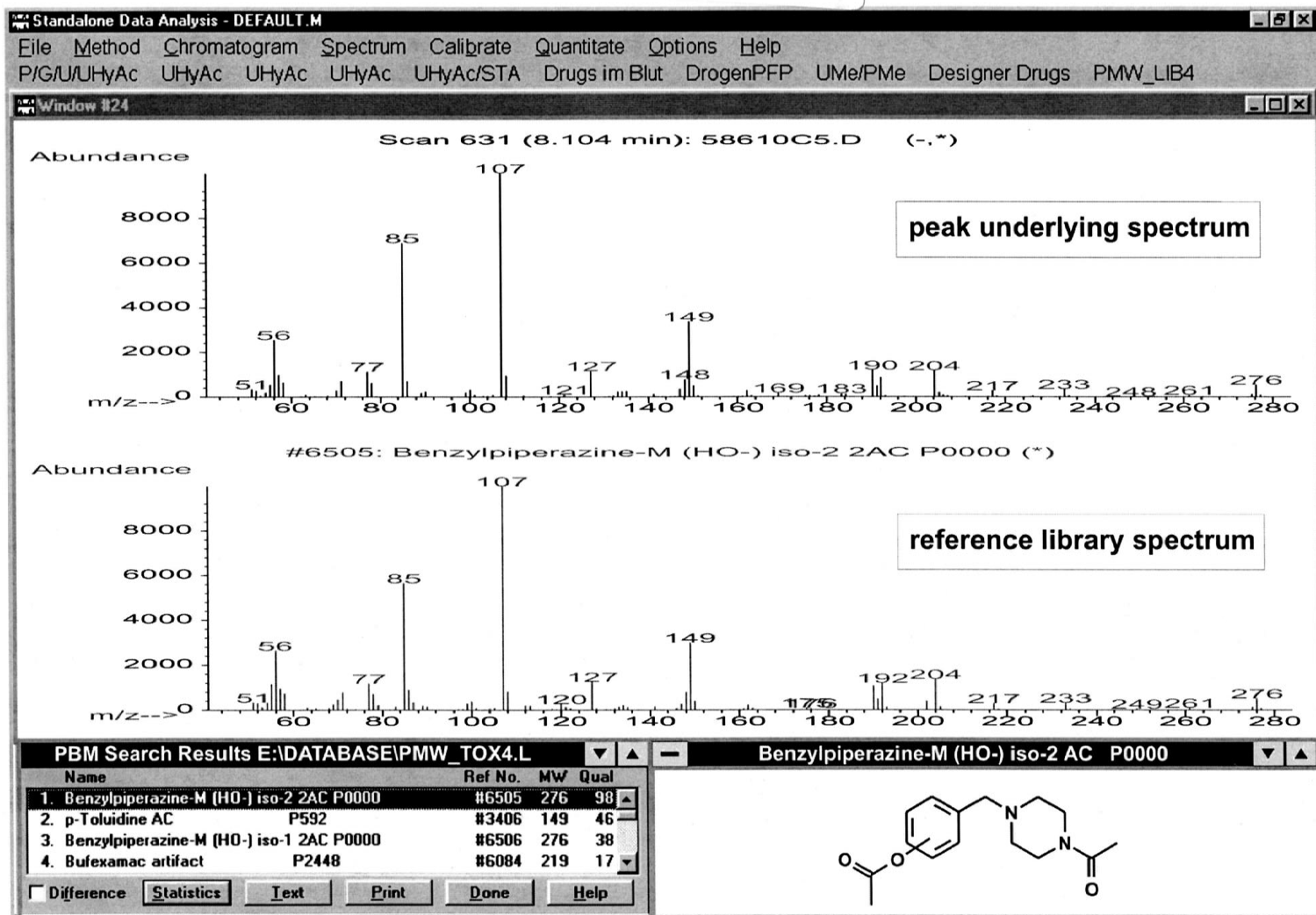


Fig. 4. Mass spectrum underlying the marked peak in Fig. 3, the reference spectrum, the structure, and the hit list found by computer library search.

as compared to other procedures. Studies by de Boer et al., conducted only with the parent compound BZP, showed that the REMEDI™ HS Drug profiling System could not directly identify BZP and GC analysis using nitrogen-selective detection has to be confirmed by GC–MS. Our studies showed that, in case of an acute intoxication, the parent compound as well as the hydroxy metabolites should serve as target analytes. Furthermore, it has to be kept in mind that hydroxy metabolites are often excreted for a longer time than the parent compounds [23,27–31]. If this is also the case for BZP, our STA procedure allows the detection of an BZP intake via its metabolites even if the parent compound is no longer excreted. Though, further studies on the pharmacokinetic properties of BZP have to be conducted.

### 3.4. Detection by FPIA

In case of a suspected intake of unknown “designer drugs” one has to think of amphetamine derivatives. Therefore, we investigated whether the AM/MA II assay could be used for detection. As we could show in our metabolism studies BZP is metabolized to *N*-benzylethylenediamine, a compound which side chain structure is somewhat closer to amphetamine as BZP itself. Both compounds showed only small cross reactivity values (<1%) and only at very high concentrations (>100 000 ng/ml). However it is unlikely that such urine concentrations can be reached. As shown by de Boer et al., the parent compound showed also only small cross reactivity in the EMIT® d.a.u.® Amphetamines test. Therefore, it has to be concluded that these assays are not suitable for detection. This conclusion is in accordance with our finding, that the authentic BZP urine samples from intoxication cases showed negative TDx results.

## 4. Conclusions

Our studies showed that the new piperazine-like designer drug BZP is metabolized to a considerable extent. A clinical or forensic analysis in body samples, especially in urine, should also be focussed on these metabolites. Our STA procedure allowed the detection of an intake of a dose of BZP, that

corresponds to a common drug users’ dose in rat urine and proved to be applicable in authentic human clinical or forensic cases. Besides detection of BZP and its metabolites, the detection and differentiation of most of the toxicologically relevant drugs like other designer drugs, barbiturates, benzodiazepines, opioids, analgesics, antidepressants, neuroleptics, antiparkinsonians, anticonvulsants, antihistamines,  $\beta$ -blockers, antiarrhythmics, and laxatives within the same procedure by clicking the corresponding pull down menu (e.g. “designer drugs”) executing user defined macros followed by library search of the spectra underlying the peaks [21,24,40]. The tested immunoassay was not suitable for detection of BZP intake.

## 5. Nomenclature

A2	<i>N</i> -benzylpiperazine (street name)
AM/MA II	Amphetamine/metamphetamine II assay for the Abbott TDx system
BZP	<i>N</i> -benzylpiperazine
COMT	Catechol- <i>O</i> -methyl-transferase
mCPP	1-[3-chlorophenyl]piperazine
EI	Electron impact ionization
EMIT	Enzyme-multiplied immuno technique
FPIA	Fluorescence polarization immunoassay
GC	Gas chromatography
GC–MS	Gas chromatography–mass spectrometry
5-HT	5-Hydroxy tryptamine (serotonin)
MDBP	1-[3,4-methylenedioxybenzyl]piperazine
MDMA	3,4-Methylenedioxymethamphetamine
<i>m/z</i>	Mass-to-charge ratio
PCI	Positive-ion chemical ionization
RI	Retention index
<i>S/N</i>	Signal-to-noise ratio
STA	Systematic toxicological analysis

## Acknowledgements

The authors thank Cornelia Brehmer, Peter X. Iten and Katharina Rentsch, Zürich, Thomas Kraemer, Frank T. Peters, Gabriele Ulrich and Armin A. Weber for their support.

## References

- [1] R.F. Staack, G. Fritschi, H.H. Maurer, in: M. Balikova (Ed.), Proceedings of the 39th International TIAFT Meeting in Prague, Prague, 2001.
- [2] P. Roesner, T. Junge, G. Fritschi, B. Klein, K. Thielert, M. Kozlowski, *Toxichem. Krimtech.* 66 (1999) 81.
- [3] M. Wikstroem, P. Holmgren, J. Ahlner, in: I. Rasanen (Ed.), Abstracts of the 38th International TIAFT Meeting in Helsinki, Helsinki, 2000, p. 88.
- [4] C. Balmelli, H. Kupferschmidt, K. Rentsch, M. Schneemann, *Dtsch. Med. Wochenschr.* 126 (2001) 809.
- [5] D. de Boer, I.J. Bosman, E. Hidvegi, C. Manzoni, A.A. Benko, R.L. dos, R.A. Maes, *Forensic Sci. Int.* 121 (2001) 47.
- [6] A. Shulgin, in: D. Joy (Ed.), Pihkal, A Chemical Love Story, Transform Press, Berkley, CA, 1991, p. 815.
- [7] C. Bye, F.A. Munro, A.W. Peck, P.A. Young, *Eur. J. Clin. Pharmacol.* 6 (1973) 163.
- [8] H. Campbell, W. Cline, M. Evans, J. Lloyd, A.W. Peck, *Eur. J. Clin. Pharmacol.* 6 (1973) 170.
- [9] K. Tekes, L. Tothfalusi, B. Malomvolgyi, F. Herman, K. Magyar, *Pol. J. Pharmacol. Pharm.* 39 (1987) 203.
- [10] B. Malomvolgyi, L. Tothfalusi, K. Tekes, K. Magyar, *Acta Physiol. Hung.* 78 (1991) 201.
- [11] Z. Szucks, T. Szentendrei, M.I. Fekete, *Pol. J. Pharmacol. Pharm.* 39 (1987) 185.
- [12] K. Magyar, M.I. Fekete, K. Tekes, T.L. Torok, *Eur. J. Pharmacol.* 130 (1986) 219.
- [13] C. Oberlander, C. Euvrard, C. Dumont, J.R. Boissier, *Eur. J. Pharmacol.* 60 (1979) 163.
- [14] S. Olajos, D. Sztaniszlav, *J. Chromatogr.* 378 (1986) 155.
- [15] H.H. Maurer, J. Bickeboeller-Friedrich, *J. Anal. Toxicol.* 24 (2000) 340.
- [16] J. Bickeboeller-Friedrich, H.H. Maurer, *Ther. Drug Monit.* 23 (2001) 61.
- [17] T. Kraemer, A.A. Weber, H.H. Maurer, in: F. Pragst (Ed.), Proceedings of the Xth GTFCh Symposium in Mosbach, Helm-Verlag, Heppenheim, ISBN 3-923032-10-2, 1997, p. 200.
- [18] H.H. Maurer, in: K. Pflieger, H.H. Maurer, A. Weber (Eds.), Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and their Metabolites, Wiley-VCH, Weinheim, 2000, p. 3.
- [19] H.H. Maurer, J. Bickeboeller-Friedrich, T. Kraemer, F.T. Peters, *Toxicol. Lett.* 112–113 (2000) 133.
- [20] A.F. McKay, W.L. Ott, G.W. Taylor, M.N. Buchanan, J.F. Crooker, *Can. J. Res.* 28 (1950) 683, Sec. B.
- [21] H.H. Maurer, *Spectroscopy Europe* 6 (1994) 21.
- [22] K. Pflieger, H.H. Maurer, A. Weber, Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants and their Metabolites (4th ed.), Agilent Technologies, Palo Alto (CA), 2002, in preparation.
- [23] H.H. Maurer, *Ther. Drug Monit.* 18 (1996) 465.
- [24] H.H. Maurer, *J. Chromatogr.* 580 (1992) 3.
- [25] H.H. Maurer, *J. Chromatogr. B* 733 (1999) 3.
- [26] H.H. Maurer, *Comb. Chem. High Throughput Screen.* 3 (2000) 461.
- [27] H.H. Maurer, T. Kraemer, *Arch. Toxicol.* 66 (1992) 675.
- [28] H.K. Ensslin, K.A. Kovar, H.H. Maurer, *J. Chromatogr. B* 683 (1996) 189.
- [29] H.K. Ensslin, H.H. Maurer, E. Gouzoulis, L. Hermle, K.A. Kovar, *Drug Metab. Dispos.* 24 (1996) 813.
- [30] T. Kraemer, I. Vernaleken, H.H. Maurer, *J. Chromatogr. B* 702 (1997) 93.
- [31] T. Kraemer, H.H. Maurer, *J. Chromatogr. B* 713 (1998) 163.
- [32] K. Pflieger, H.H. Maurer, A. Weber, Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and their Metabolites, part 5, Wiley-VCH, Weinheim, 2002, in preparation.
- [33] F.W. McLafferty, F. Turecek, Interpretation of Mass Spectra, University Science Books, Mill Valley, CA, 1993.
- [34] H.H. Maurer, J. Bickeboeller-Friedrich, T. Kraemer, *J. Chromatogr. B* 739 (2000) 325.
- [35] K. Magyar, *Pol. J. Pharmacol. Pharm.* 39 (1987) 107.
- [36] E. Kovats, *Helv. Chim. Acta* 41 (1958) 1915.
- [37] R.A. de-Zeeuw, J.P. Franke, H.H. Maurer, K. Pflieger, 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), VCH Publishers, Weinheim, New York, Basle, 1992.
- [38] DFG Senatskommission für Klinisch-toxikologische Analytik, *J. Clin. Chem. Clin. Biochem.* 20 (1982) 699.
- [39] A. Guttman, J. Nagy, K. Magyar, *Pol. J. Pharmacol. Pharm.* 39 (1987) 123.
- [40] H.H. Maurer, J.W. Arlt, T. Kraemer, C.J. Schmitt, A.A. Weber, *Arch. Toxicol.* 19 (Suppl.) (1997) 189.