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New designer drug *p*-methoxymethamphetamine: studies on its metabolism and toxicological detection in urine using gas chromatography–mass spectrometry☆

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Abstract

Studies are described on the metabolism and the toxicological analysis of the new designer drug rac-*p*-methoxymethamphetamine (PMMA) in rat urine using gas chromatography–mass spectrometry (GC–MS). The identified metabolites indicated that PMMA was extensively metabolized mainly by O-demethylation to pholedrine and to a minor extent to *p*-methoxyamphetamine (PMA), 1-hydroxypholedrine diastereomers (one being oxilofrine), 4′-hydroxy-3′-methoxymethamphetamine and 4′-hydroxy-3′-methoxyamphetamine. 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 the main metabolites of PMMA in rat urine after a dose corresponding to that of drug users. Therefore, this procedure should be suitable for detection of PMMA intake in human urine via its metabolites. However, it must be considered that pholedrine and oxilofrine are also in therapeutic use. Differentiation of PMMA, PMA and/or pholedrine intake is discussed.

Keywords: Metabolism; Designer drug; Methoxymethamphetamine

1. Introduction

A wide variety of phenylisopropylamine derivatives are offered and consumed as drugs of abuse. One of these derivatives, *p*-methoxymethamphetamine [PMMA, *R,S-N*-methyl-1-(4-methoxyphenyl)-2-aminopropane], has surfaced on the illicit drug market in form of tablets containing PMMA only as well as mixtures with other designer drugs [1–5]. It is already scheduled in the German Act of Controlled substances and, according to a decision of the council of the EU, will be placed under control measures and criminal penalties in all EU member states [6]. PMMA is the *N*-methyl analogue of the common designer drug *p*-methoxyamphetamine [PMA, *R,S*-1-(4-methoxyphenyl)-2-aminopropane] which has been responsible for a number of fatalities in Europe, Australia and the USA [7–14]. Mixtures of both compounds have also been seized and

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fatalities after intake of these mixtures have been reported [2,3]. The PMMA isomer methoxyphenamine [*R,S-N*-methyl-1-(2-methoxyphenyl)-2-aminopropane, Orthoxine®] was in use as a bronchodilator [15]. Analytical differentiation of the two isomers was described by Dal Cason [4].

Pharmacological discrimination studies using rats showed that PMMA lacks amphetamine-like effects in contrast to PMA. It was shown to share considerable similarities with MDMA [*R,S-N*-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane], but without the amphetaminergic stimulant component of MDMA. It produced stimulant effects similar to MBDB [*R,S-N*-methyl-1-(3,4-methylenedioxyphenyl)-2-aminobutane] and DMA [1-(3,4-dimethoxyphenyl)-2-aminopropane] [16–20]. The only data available on the effects in humans has been published by Shulgin, who described its effects as somewhat different from those of MDMA [21]. Structurally related compounds which are widely abused have been shown to possess serotonergic neurotoxic potential. Likewise, Steel et al. showed long-term (possibly neurotoxic) effects on brain serotonin neurons for PMMA, however, being less potent compared to neurotoxic effects of MDMA [5]. Furthermore, they uttered concerns about the possibility of a narrow margin between the behaviorally active and lethal doses of PMMA as described for PMA. This was due to their observation of 43% lethality in male Spague–Dawley rats after the administration of 80 mg/kg body mass.

The metabolism of PMMA has not been studied yet. 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 few analytical data on PMMA itself have been published [4]. Screening procedures for detection of PMMA and its metabolites have not been published yet.

Therefore, the aim of the presented study was to identify the PMMA metabolites in rat urine using GC–MS in the electron impact (EI) and positive-ion chemical ionization (PICI) mode and to study the detectability of PMMA within the authors' systematic toxicological analysis (STA) procedure in urine by GC–MS in the EI mode [22,23].

2. Experimental

2.1. Chemicals and reagents

All chemicals and biochemicals used were obtained from Merck (Darmstadt, Germany) and were of analytical grade. *rac*-PMMA–HCl and *rac*-PMA–HCl as reference metabolites were provided by Bayerisches Landeskriminalamt (Munich, Germany) for research purposes before the drug had been scheduled in the German Controlled Substances Act. Pholedrine was obtained from Sigma (Taufkirchen, Germany) and oxilofrine from Aventis Pharma (Bad Soden, Germany).

2.2. Urine samples

The investigations were performed using urine of male rats (Wistar, Charles River, Sulzfleck, Germany) which were administered a single 10 mg/kg body mass (for metabolism studies) or a 0.5 mg/kg body mass dose (for STA) of *rac*-PMMA in aqueous suspension by gastric intubation. The low dose corresponds to the PMMA content of seized tablets. Furthermore, *rac*-PMA and *rac*-pholedrine were likewise administered to male rats in doses of 10 mg/kg body mass (for metabolism studies) and 0.5 and 1 mg/kg body mass of *rac*-PMA and *rac*-pholedrine, respectively (for STA) to study the differentiation of the compounds in a urine screening. These low doses likewise correspond to the content of PMA in seized tablets or to a common therapeutic dose of pholedrine. Urine was collected separately from faeces over a 24 h period. All samples were directly analyzed and then stored at −20 °C before further analysis. Blank urine samples were collected before drug administration to check whether the samples were free of interfering compounds.

2.3. Sample preparation for metabolism studies

A 5-ml portion of urine was adjusted to pH 5.2 with 1 *M* acetic acid 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), 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 100 μl of an acetic anhydride–pyridine mixture (3:2, v/v) for 5 min under microwave irradiation at about 440 W [24–26]. After evaporation, the residue was dissolved in 100 μl of methanol and 2 μl of this solution were injected into the GC–MS. Heptafluorobutyrylation was conducted with 50 μl heptafluorobutyric anhydride for 5 min under microwave irradiation at about 440 W [24,25]. After evaporation of the reagent the residue was dissolved in 50 μl alcohol- and water-free ethyl acetate and 1–2 μl were injected into the GC–MS. Methylation was conducted after reconstitution of the extraction residue in 50 μl of methanol with 50 μl of a solution of diazomethane in diethyl ether, synthesized according to the procedure of McKay et al. [27]. 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 μl of methanol and a 3 μl aliquot was injected into the GC–MS system [25]. The same procedure with the exception of enzymatic hydrolysis was used to study which metabolites of PMMA are excreted as glucuronides and/or sulfates.

A second urine sample was worked up as described above, except that the extraction was carried out at pH 4–5. The corresponding extract was analyzed after methylation plus acetylation.

2.4. Sample preparation for toxicological analysis

The urine samples (5 ml) were divided into two aliquots. One aliquot was refluxed with 1 ml of 37% HCl for 15 min. Following hydrolysis, the sample was mixed with 2 ml of 2.3 *M* aqueous ammonium sulfate and 1.5 ml of 10 *M* aqueous sodium hydroxide to obtain a pH value of 8–9. Before extraction, the other aliquot of native urine was added. This mixture was extracted with 5 ml of dichloromethane–isopropanol–ethyl acetate (1:1:3, v/v/v). After phase separation by centrifugation, the organic layer was transferred into a pear-shaped flask and evaporated to dryness. The residue was derivatized by acetylation with 100 μl of acetic anhydride–pyridine (3:2, v/v) for 5 min under microwave irradiation at about 440 W [22]. After evaporation of the derivatization mixture, the residue was dissolved in 100 μl of methanol and 2 μl were injected into the gas chromatograph.

2.5. Gas chromatography–mass spectrometry

PMMA metabolites were separated and identified in acetylated, heptafluorobutyrylated, methylated plus acetylated or underivatized urine extracts 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. The GC conditions were as follows: splitless injection mode; column, HP-1 capillary (12 m ×0.2 mm I.D.), crosslinked 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; chemical ionization using methane, positive mode (PICI): ionization energy, 230 eV; ion source temperature, 220 °C; capillary direct interface heated at 260 °C.

For toxicological detection of PMMA and its metabolites, mass chromatography with the selected ions *m/z* 58, 100, 148, 164 and 176 was used. Generation of the mass chromatograms could be started by clicking the corresponding pull down menu which executes the user defined macros [28] (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 [29] of the mass spectra underlying the peaks (after background subtraction) with reference spectra (Fig. 1) recorded during this study.

2.6. Fluorescence polarization immunoassays

Native urine samples from volunteers were used

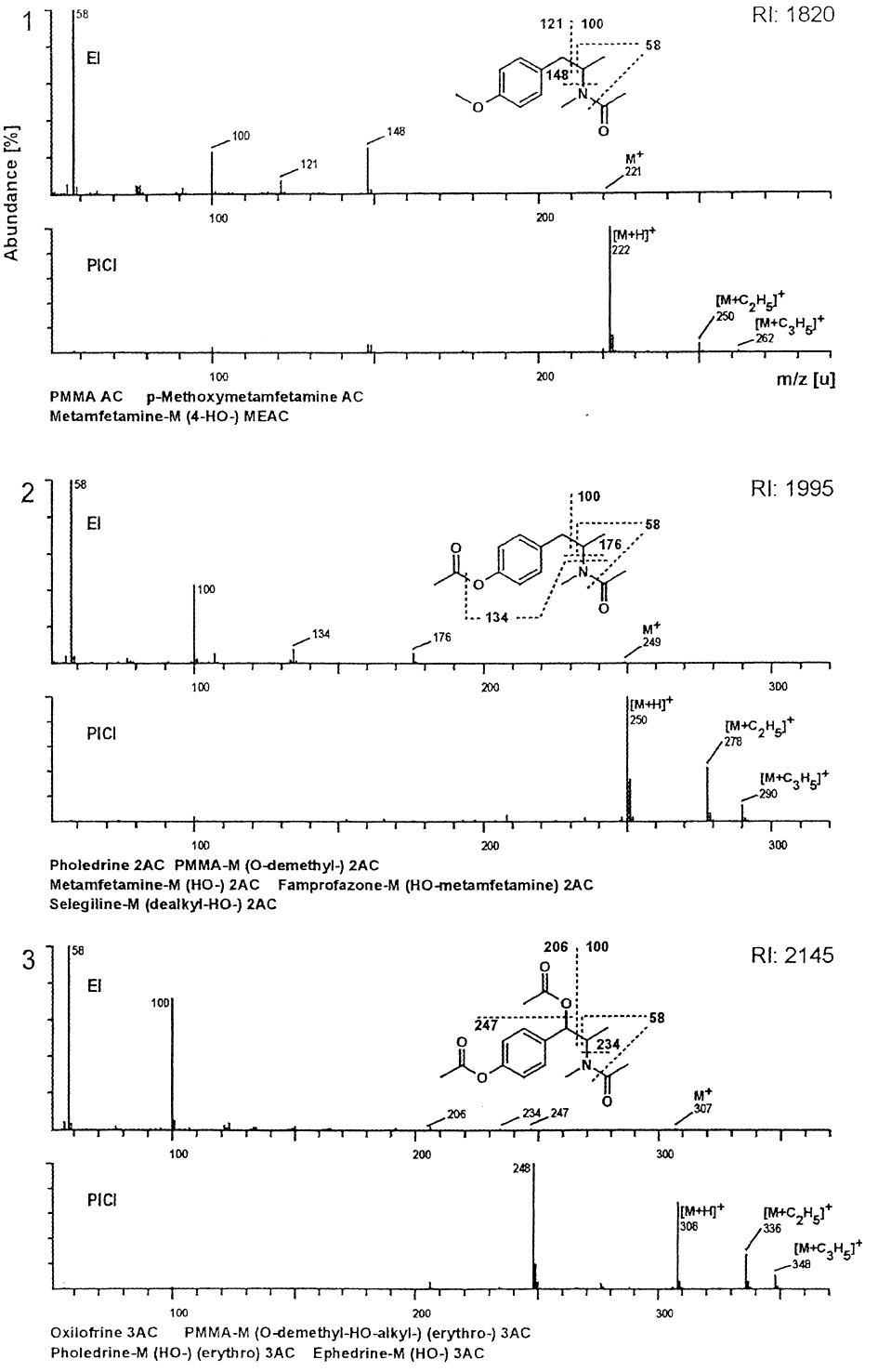
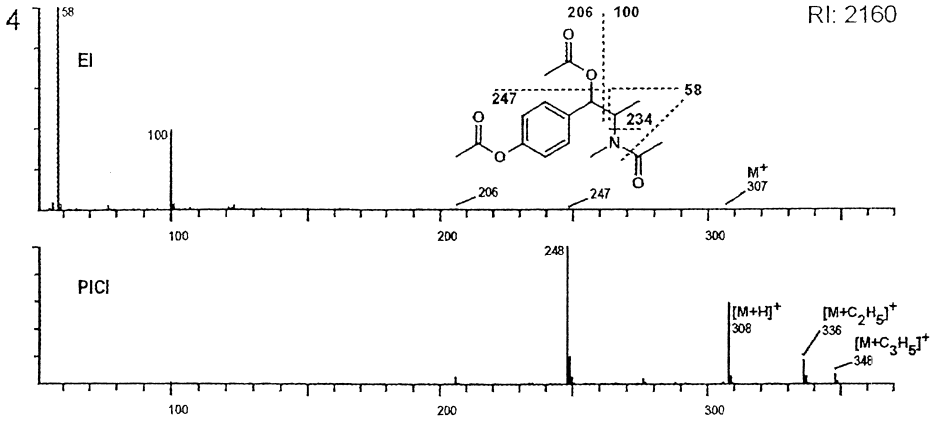
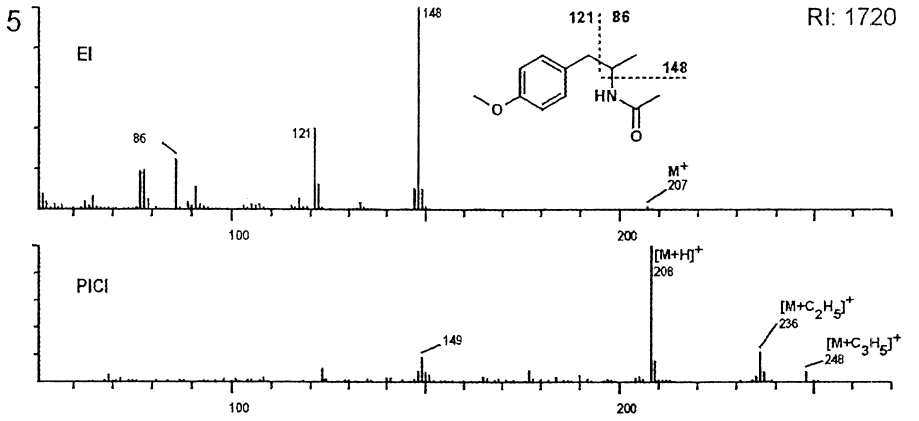


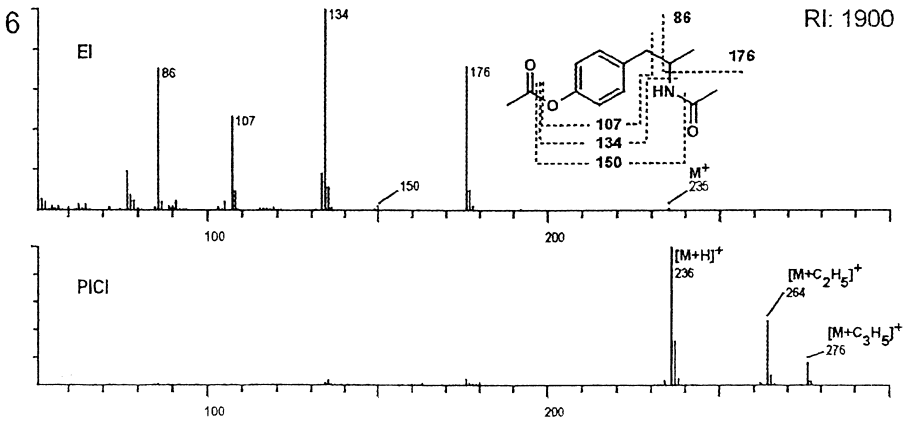
Fig. 1. EI and PICI mass spectra, the gas chromatographic retention indices (RI), structures and predominant fragmentation patterns of PMMA and its metabolites after acetylation. The numbers of the spectra correspond to those in Fig. 2. The axes are only labelled for spectrum no. 1.



PMMA-M (O-demethyl-HO-alkyl-) (threo-) 3AC
Pholedrine-M (HO-) (threo-) 3AC

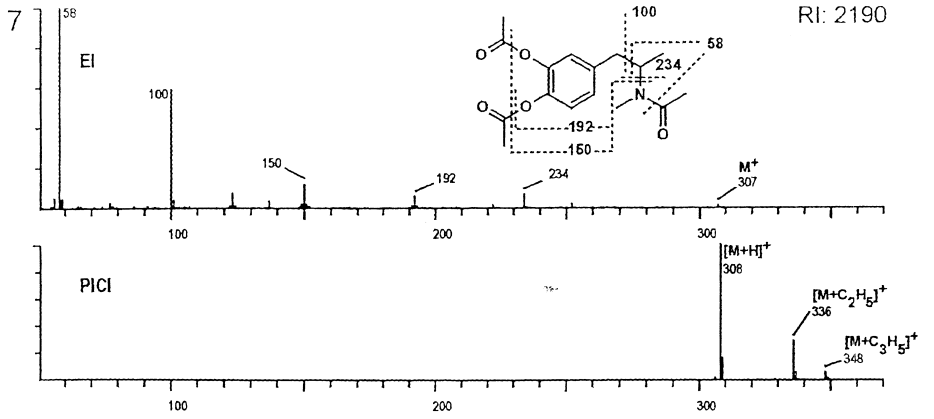


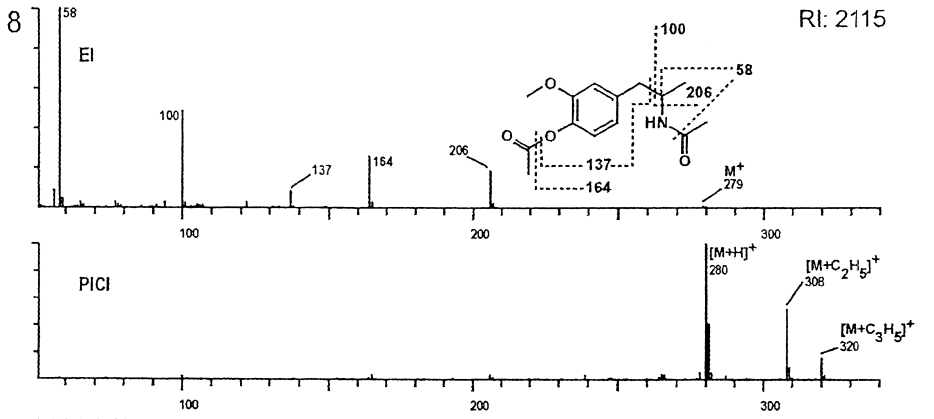
PMA AC PMMA-M (nor-) AC
Mebeverine-M (N-dealkyl-N-deethyl-) AC Formoterol HYAC



Amfetamine-M (4-HO-) 2AC PMMA-M (bis-demethyl-) 2AC PMA-M (O-demethyl-) 2AC
Etilamfetamine-M (AM-4-HO-) 2AC Fenproporex-M (N-dealkyl-4-HO-) 2AC
Metamfetamine-M (nor-4-HO-) 2AC Clobenzorex-M 2AC Selegiline-M 2AC

Fig. 1. (*continued*)





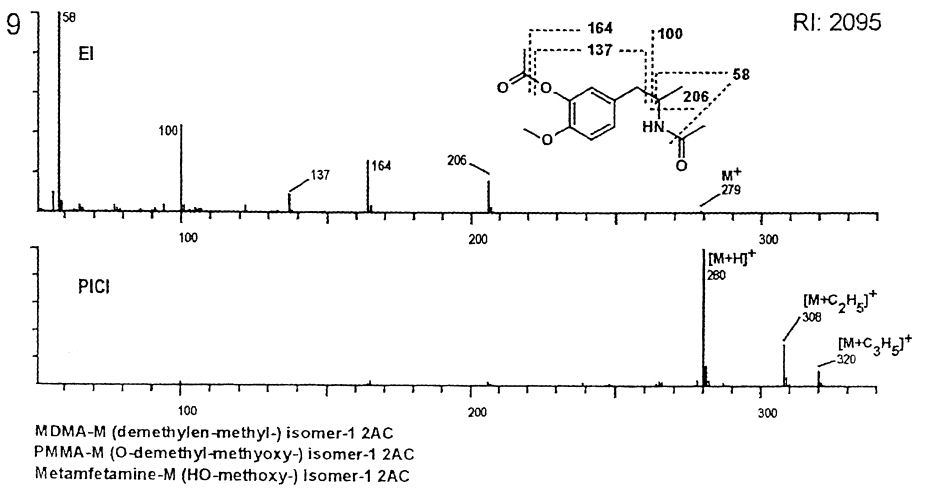


Fig. 1. (*continued*)

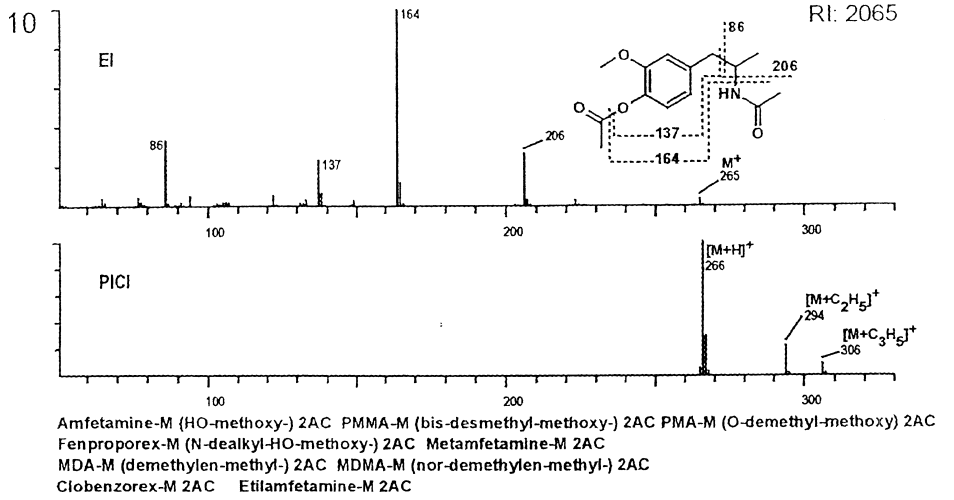


Fig. 1. (*continued*)

for crossreactivity studies. The TDx system of Abbot (Irving, TX, USA) with the amphetamine/methamphetamine 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 crossreactivity of PMMA with this assay, blank urine samples were spiked at concentrations of 500–10 000 ng/ml. Furthermore, native rat urine samples after administration of commonly used doses and urine from untreated rats were used as a negative control for immunoassay determination.

3. Results and discussion

3.1. Sample preparation

Cleavage of conjugates was necessary before extraction and GC–MS analysis of the suspected metabolites in order not to overlook conjugated metabolites. For studies on the metabolism, gentle enzymatic hydrolysis was preferred, whereas 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 [22,26,30–33]. However, some compounds covered by this STA are altered or destroyed during hydrolysis [22,23]. 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 [22,23].

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 value. Using a more alkaline pH value for extraction leads to decreased extraction efficiencies of such hydroxy metabolites which are, however, often excreted for a longer time than the parent compounds [30,34–38]. Derivatization of the extracts was indispensable for sensitive detection.

The extraction efficiency of PMMA was 83±19% ($n=5$). However, as PMMA is almost completely metabolized, the determination of the extraction efficiency is of little use. Therefore, the extraction efficiency of its main metabolite pholedrine and of some other metabolites were determined and were as follows: pholedrine 73±11.7%, PMA 89±12% oxilofrine 51±20.4% ($n=5$ each). Careful sample preparation, especially gentle evaporation of the extraction and derivatization mixtures, should be

performed due to the rather high volatility of PMMA and its metabolites.

3.2. Identification of metabolites

The urinary metabolites of PMMA were separated by GC and identified by EI and PICI MS after enzymatic hydrolysis, extraction, acetylation, heptafluorobutyrylation, methylation plus acetylation or without derivatization. The GC and MS data of these derivatives will be included in the forthcoming update of the authors' handbook and library [29,39]. The different derivatization procedures were used to gain more information on the structures of the 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 McLafferty and Turecek [40]. In order to verify the molecular mass of the postulated metabolites, PICI mass spectra were recorded, because they contain strong molecular peaks (M+H) with adduct ions typical for PICI using methane as reagent gas. The identity of pholedrine, PMA and oxilofrine could further be confirmed by comparing their mass spectra and the GC retention indices (RI) with those of the corresponding reference substances. The EI and PICI mass spectra, the RIs, the structures and postulated predominant fragmentation patterns of the acetylated metabolites are shown in Fig. 1. The spectra are arranged according to the numbers given in the metabolic scheme shown in Fig. 2. PMMA (mass spectrum no. 1 in Fig. 1) was extensively metabolized and could only be detected in very small amounts after the administration of 10 mg/kg body mass. The following metabolites could be identified in rat urine: mainly *p*-hydroxymethamphetamine (pholedrine, 4-HO-MA, mass spectrum no. 2), but also two 1-hydroxypholedrine diastereomers (mass spectra nos. 3 and 4), *p*-methoxyamphetamine (PMA, mass spectrum no. 5), *p*-hydroxyamphetamine (4-HO-AM, mass spectrum no. 6),

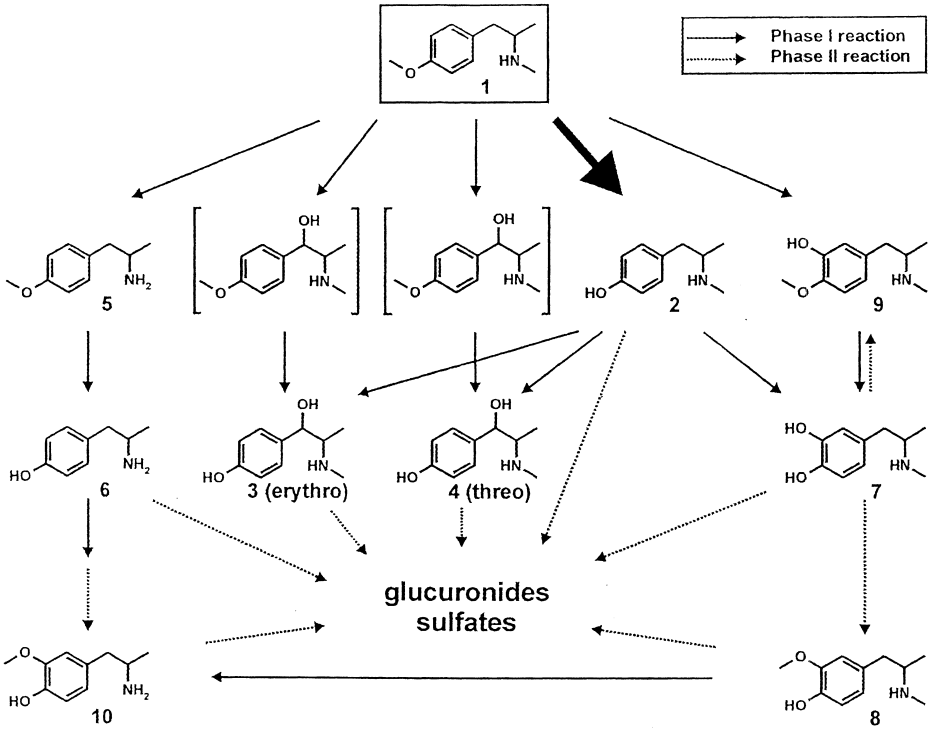


Fig. 2. Proposed scheme for the metabolism of PMMA in rats (metabolites in brackets not detected). The metabolites 2–4, 6–8 and 10 were also excreted as glucuronic and/or sulfuric acid conjugates in urine.

3′,4′-dihydroxymethamphetamine (di-HO-MA, mass spectrum no. 7), 4′-hydroxy-3′-methoxymethamphetamine (mass spectrum no. 8), 3′-hydroxy-4′-methoxymethamphetamine (mass spectrum no. 9) and 4′-hydroxy-3′-methoxyamphetamine (mass spectrum no. 10). Although the exact position of the second aromatic hydroxy group could not be elucidated by means of GC–MS, position 3′ could be assumed, as the corresponding dihydroxy compound is identical with the dihydroxy compounds formed from MDMA [41,42]. This identity was confirmed by cochromatography of acetylated extracts of PMMA urines and of urine samples containing the corresponding MDMA metabolite. Both compounds showed identical RIs and the same mass spectrum. There are two possible isomers of hydroxy-methoxy metabolites. Aromatic hydroxylation of PMMA in position 3′ would lead to the 3′-hydroxy-4′-methoxy metabolite, but only traces could be found. As PMMA was extensively metabolized to pholedrine (4-HO-MA) and further to Di-HO-MA followed by methylation at the 3′-position, the 4′-hydroxy-3′-methoxy isomer was the more abundant one. This methylation step was catalyzed by catechol-*O*-methyltransferase (COMT), as confirmed by in vitro studies according to Maurer et al. [42]. Therefore, it could be concluded that the 4′-hydroxy-3′-methoxy metabolite is predominantly formed due to the selectivity of COMT for the methylation of the hydroxy group in position 3′ [42]. As described above for the dihydroxy compound, this assumption could be confirmed by cochromatography of the 4′-hydroxy-3′-methoxy compound formed from MDMA. The identified PMMA metabolite hydroxymethoxyamphetamine should be the 4′-hydroxy-3′-methoxyamphetamine for the same reasons. Only this isomer could be detected, as this pathway is only a minor one.

The formation of the above mentioned 1-hydroxypholedrine isomers is in keeping with metabolism studies on PMA [43] and can be interpreted as follows: 1-hydroxylation of pholedrine introduces a second chiral center into the molecules leading theoretically to four stereoisomeric metabolites, namely the *erythro* and *threo* diastereomers which each consist of a pair of enantiomers (*erythro* 1*R*,2*S* and 1*S*,2*R*; *threo* 1*R*,2*R* and 1*S*,2*S*). Two peaks detected in urine could be related to the two diastereomers. Using cochromatography with oxilofrine reference substance, the more abundant peak could be attributed to oxilofrine, the *erythro*-isomers (mass spectrum no. 3). However, as an achiral GC column was used, it could not be clarified whether these two peaks consisted of one or two enantiomers each. This will be clarified within the authors' future studies on the stereoselective disposition of PMMA.

Based on the identified metabolites of PMMA, the following partially overlapping metabolic pathways shown in Fig. 2 could be postulated: mainly O-demethylation to pholedrine (no. 2) and to a minor extent *N*-demethylation to PMA (no. 5). Pholedrine could theoretically further be metabolized by *N*-demethylation to 4-HO-AM (no. 6). However, according to Hutchaleelaha et al. [44], pholedrine was not *N*-demethylated, which could be confirmed by the authors' metabolism studies of pholedrine in rats. Therefore, 4-HO-AM should be formed predominantly via PMMA *N*-demethylation to PMA followed by O-demethylation. This postulation is further supported by the fact, that 4-HO-AM was described as the main metabolite of PMA [43,45]. Pholedrine is further metabolized by aromatic hydroxylation to dihydroxymethamphetamine (no. 7) followed by COMT catalyzed methylation to 4′-hydroxy-3′-methoxymethamphetamine (no. 8). The isomer 3′-hydroxy-4′-methoxymethamphetamine (no. 9) is formed only to a very small extent by COMT methylation of the 4′-position [42] or by direct hydroxylation of PMMA. The 4′-hydroxy-3′-methoxymethamphetamine should further be *N*-demethylated to 4′-hydroxy-3′-methoxyamphetamine (no. 10), which should also be formed from 4-HO-AM by hydroxylation and COMT catalyzed methylation. Furthermore, the O-demethyl metabolite pholedrine was additionally hydroxylated at the alkyl side chain in position 1 (nos. 3 and 4). The resulting metabolite should theroretically also be formed by PMMA 1-hydroxylation followed by O-demethylation. However, the latter pathway is not very likely, as 1-hydroxy-PMMA could not be detected.

All the phenolic compounds are partially excreted as conjugates, since the peaks were more abundant after cleavage of conjugates. As a mixture of glucuronidase and arylsulfatase were used for conjugate cleavage, it was not possible to determine whether the metabolites were excreted as glucuro-

nides or sulfates. Pholedrine itself has been described to be excreted as a sulfate conjugate [46–50].

In order to check for acidic metabolites, the urine samples were also extracted after cleavage of conjugates at acidic pH and the corresponding extracts were analyzed after methylation plus acetylation. However, no acidic metabolites were found.

3.3. Detection of PMMA metabolites by GC–MS within the STA

PMMA 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 following ions m/z 58, 100, 148, 164 and 176 was used to indicate the presence of PMMA and 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 a PMMA metabolite in an acetylated extract of a rat urine sample after administration of 0.5 mg/kg body mass of PMMA, a dose that corresponds to the common dose of abusers. 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 [29]. Although the limit of detection of PMMA in urine was as low as 50 ng/ml (S/N 3) under routine MS conditions, no more parent compound could be detected, which means that PMMA is excreted almost completely metabolized; it could only be detected after administration of the higher dose. The limits of detection (S/N 3) determined under routine MS conditions were as follows: for pholedrine 10 ng/ml and for PMA and oxilofrine 50 ng/ml.

The m/z 58 and 100 ions were used for indicating the presence of the methamphetamine derivatives PMMA, pholedrine, 1-hydroxypholedrine diastereomers, dihydroxymethamphetamine and the hydroxymethoxymethamphetamine metabolites. The selected ion m/z 148 was used for indication of PMA, the ion m/z 164 was used for indication of hydroxymethoxyamphetamine and the ion m/z 176 for 4-hydroxyamphetamine.

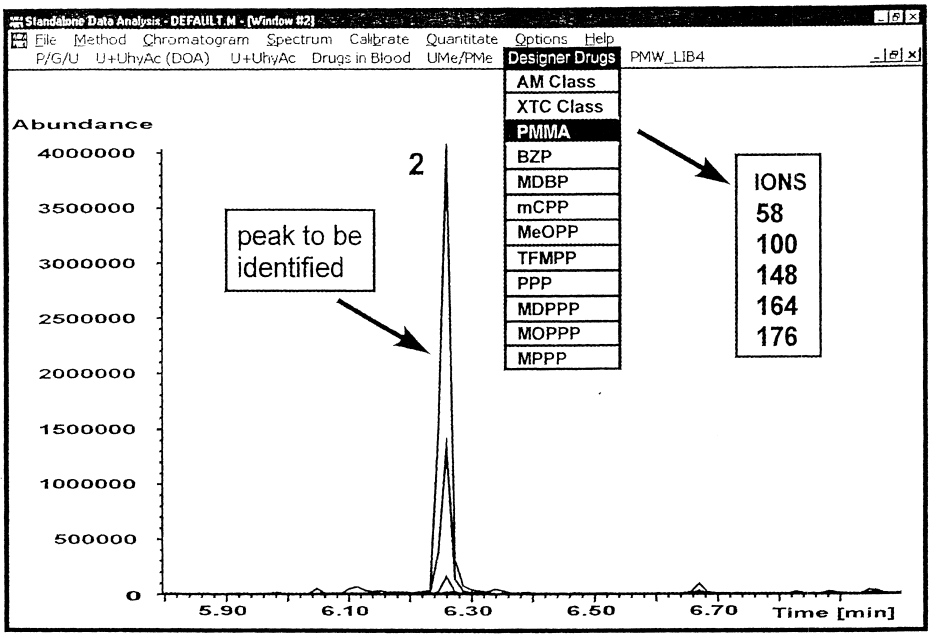


Fig. 3. Typical mass chromatograms with the ions m/z 58, 100, 148, 164 and 176. They indicate the presence of a PMMA metabolite in an acetylated extract of a rat urine sample after application of a common abuser's dose of 0.5 mg/kg body mass. The number of the peak corresponds to that in Figs. 1 and 2. The merged chromatograms can be differentiated by their colors on a color screen.

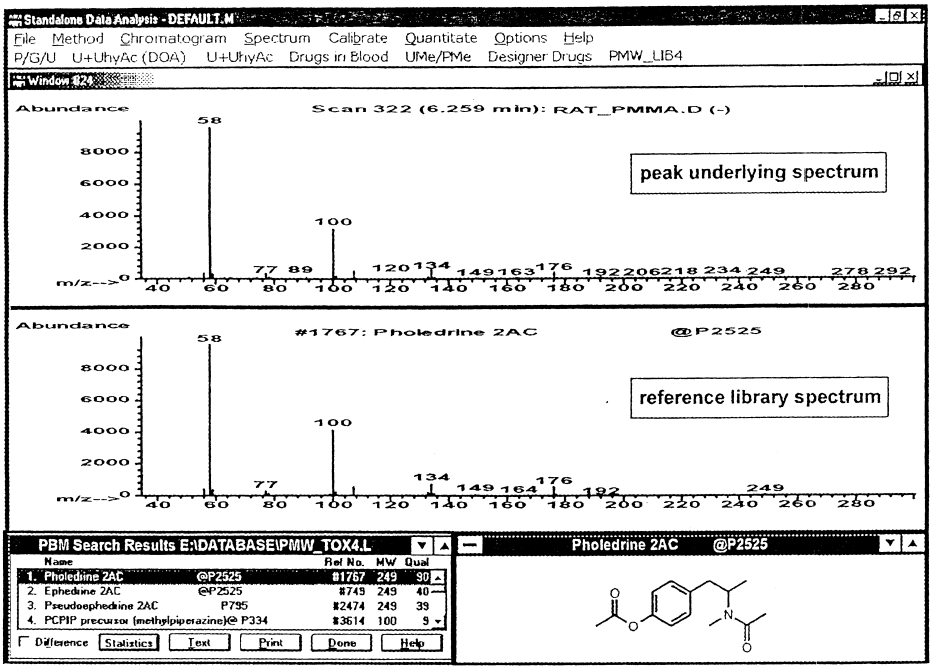


Fig. 4. Mass spectrum underlying the marked peak in Fig. 3, the reference spectrum, the structure, and the hit list found by a computer library search.

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 the authors' experience, the gas chromatographic RIs provide preliminary indications and may be useful to gas chromatographers without a GC–MS facility. In addition, they allow differentiation of isomers. Therefore, the RIs are also given in Fig. 1. They were recorded during the GC–MS procedure (Section 2.5) and calculated in correlation with the Kovats' indices [51] of the components of a standard solution of typical drugs which is measured daily for testing the GC–MS performance [52,53]. 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.

The PMMA main metabolite pholedrine itself is in use as an indirectly acting sympathomimetic, so differentiation between the illegal intake of PMMA and the legal intake of pholedrine is necessary. As described above, PMA and 4-HO-AM are metabolites of PMMA, but they were not formed from pholedrine. If these compounds can be found besides pholedrine, this is an indication of a PMMA and/or a PMA intake.

In Fig. 5, typical mass chromatograms of acetylated extracts of rat urines collected over 24 h after administration of PMMA (A), pholedrine (B) or PMA (C) are presented. After administration of a dose of PMMA corresponding to a common drug user's dose, the main metabolite pholedrine was predominantly found. After administration of a therapeutic dose of pholedrine, again, only pholedrine could be detected, so that differentiation of an intake of PMMA and/or pholedrine was hardly possible by the described STA in urine. Therefore, in cases of doubt, the toxicologist should particularly screen for the parent compound PMMA, for the unique metabolites PMA and/or 4-HO-AM in urine, blood or alternative matrices. As the presented metabolism studies showed, this differentiation should be possible at least after higher doses. Of course, in case of

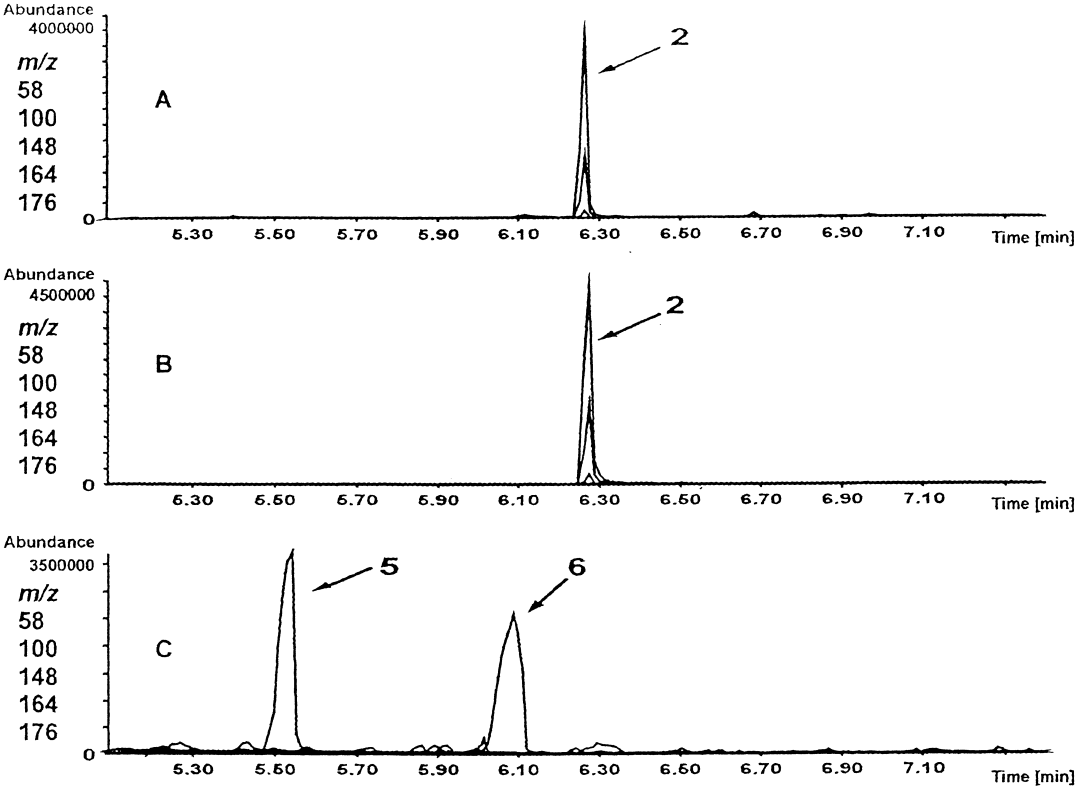


Fig. 5. Typical mass chromatograms with the given ions of acetylated extracts of rat urines collected over 24 h after administration of 0.5 mg/kg body mass of PMMA (A), 1 mg/kg body mass of pholedrine (B) or 0.5 mg/kg body mass of PMA (C). The numbers of the peaks correspond to those in Figs. 1 and 2.

an intake of a mixture of PMMA and PMA, PMA and its main metabolite 4-HO-AM cannot serve for differentiation between a PMMA or pholedrine intake, so that an unambiguous differentiation is only possible via PMMA itself.

A differentiation in urine between the intake of the designer drugs PMA and PMMA is possible via pholedrine which is not formed from PMA, assuming that pholedrine was not taken additionally. However, after PMA intake, PMA itself and 4-HO-AM should be found at higher abundances compared to the abundances after PMMA intake, as they are only minor metabolites of PMMA (Fig. 5A and C).

Oxilofrine, another PMMA metabolite, is in therapeutic use as a sympathomimetic. However, being only a minor PMMA metabolite, a differentiation of its intake from an intake of PMMA or pholedrine should be possible via the parent compounds or unique metabolites. Moreover, the metabolic formation of PMA from the antispasmodic drug mebeverine [54] can be differentiated via unique mebeverine metabolites. The differentiation of a PMMA intake from an intake of other drugs of abuse (e.g. MDMA) or medicaments (e.g. selegiline), which may also form common metabolites (cf. legends to the mass spectra in Fig. 1) may be possible via detection of unique metabolites [55].

Finally, the bronchodilator methoxyphenamine is the 2-methoxy isomer of PMMA. Unfortunately, the corresponding reference substance was not available for differentiation studies. However, assuming similar metabolism, its intake should be detectable via its O-demethyl metabolite in urine by the authors' STA procedure. Assuming similar mass spectra of PMMA and its metabolites and of methoxyphenamine and its metabolites, differentiation by mass spectral identification alone would not be successful. However, the

isomers should be distinguishable by their different retention times or indices, as described for the acetylated parent compounds under similar GC conditions by Dal Cason [4]. Extrapolating the RIs from the retention times given by Dal Cason, the RI of acetylated methoxyphenamine would be about 1750. In cases, in which PMMA (RI after acetylation 1820) or methoxyphenamine themselves are detectable, they should be distinguishable by determination of their RI values. In cases in which a corresponding O-demethyl metabolite is detected, a suitable amount of acetylated pholedrine should be added to the extract and the mixture should be reanalyzed. Assuming that the acetylated O-demethyl metabolites are also separated under the described GC conditions, both isomers should be distinguishable. If only one peak for the O-demethyl compound appears, PMMA or pholedrine have been consumed. If two peaks appear, the O-demethyl metabolite should be that of methoxyphenamine. Finally, interferences with biomolecules or further drugs can be excluded due to different mass spectra and/or retention indices.

Due to lack of authentic human urine samples after intake of PMMA only, a comparison of the metabolites found in rat and human urine was not possible. However, the analysis of a human urine sample after ingestion of PMMA, PMA and MDMA confirmed at least the formation of pholedrine from PMMA. The further detected metabolites could also be formed from PMA and/or MDMA. Nevertheless, many studies in the authors' laboratory demonstrated a high degree of qualitative correspondence of rat and human metabolism [23,54–59].

3.4. Detection by fluorescence polarization immunoassays

In case of a suspected intake of unknown 'designer drugs' one has to think of amphetamine derivatives. Therefore, whether the AM/MA II assay could be used for detection was investigated. PMMA showed crossreactivity ranging from 120 to 30% depending on the concentration. However, as the metabolism studies showed, PMMA is extensively metabolized and excreted in only very low amounts even after administration of higher doses.

However, the tested native rat urine after administration of a commonly used dose showed a positive result considering a cut-off of 300 ng/ml, whereas blank rat urine samples showed no response. This result can be explained by the metabolism studies showing that PMMA is mainly excreted as pholedrine, which has been shown by Felscher and Schulz to exhibit the same crossreactivity as *S*-(+)-amphetamine [60]. The less abundant metabolite PMA also shows crossreactivity with this assay [60]. As described above, differentiation which crossreacting compound had been administered was possible by GC–MS.

4. Conclusions

The presented studies showed that PMMA is extensively metabolized. Urine screening must be focused on the main metabolite pholedrine. For differentiation of an illegal intake of PMMA from a legal intake of pholedrine, it should be screened for the parent compound and the minor but more unique metabolites PMA and 4-HO-AM. The authors' STA procedure allowed the detection of an intake of a dose of PMMA, that corresponds to a common drug users' dose in rat urine via its metabolites. Therefore, it could be concluded that it should be detectable in human urine in a clinical or forensic case. According to the authors' experience in metabolism and analytical studies on rats and humans, it should be possible to detect in human urine samples the metabolites found in rat urine.

Besides detection of PMMA 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, β-blockers, antiarrhythmics, and laxatives were possible within the same procedure by clicking the corresponding pull down menu (e.g. 'designer drugs') executing user defined macros followed by a library search of the spectra underlying the peaks. The tested immunoassay was suitable for screening, but not for unambiguous detection of a PMMA intake. This study demonstrates the necessity for metabolism studies as a prerequisite for developing a toxicological screening procedure.

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