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Studies on the metabolism and toxicological detection of the new designer drug 4′-methyl-α-pyrrolidinopropiophenone in urine using gas chromatography–mass spectrometry

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Abstract

4′-Methyl-α-pyrrolidinopropiophenone (MPPP) is a new designer drug which has appeared on the illicit drug market. The aim of our study was to identify the MPPP metabolites and to develop a toxicological detection procedure in urine using solid-phase extraction, ethylation and GC–MS. In urine samples of rats treated with MPPP, MPPP was found to be completely metabolized by oxidative desamination, hydroxylation of the 4′-methyl group followed by oxidation finally to the corresponding carboxy compound and/or by hydroxylation of the pyrrolidine ring followed by dehydrogenation to the corresponding lactam. The carboxy groups were found to be partly conjugated. Based on these data, MPPP could be detected in urine via its metabolites by GC–MS using mass chromatography for screening and library search for identification.

Keywords: 4′-Methyl-α-pyrrolidinopropiophenone; Designer drug; Metabolism

1. Introduction

α-Pyrrolidinopropiophenone derivatives like α-pyrrolidinopropiophenone (PPP) itself, 4′-methyl-α-pyrrolidinopropiophenone (MPPP), 4′-methoxy-α-pyrrolidinopropiophenone (MOPPP) and 3′,4′-methylenedioxy-α-pyrrolidinopropiophenone (MDPPP) are new designer drugs which have appeared on the illicit drug market [2]. These substances are scheduled in the German Controlled Substances Act and strictly prohibited to be dealt with. So far, little information about their pharmacological and toxicological effects is available. Their chemical structures are closely related to α-aminopropiophenone anorectics like amfepramone, drugs of abuse like cathinone/methcathinone and antidepressants like bupropion, and might therefore evoke similar effects including dopamine release and sympathomimetic properties [3–6]. The metabolism of MPPP has not been studied before. However, 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, procedures for determination of or screening for MPPP and/or its metabolites have not yet been published.

The aim of our study was first to identify the

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MPPP metabolites in rat urine using GC–MS in the electron impact (EI) and chemical ionization (CI) mode. Rat urine samples after defined drug dosage were used, since authentic human urine samples were not available. In addition, a toxicological screening procedure by EI-GC–MS based on the identified metabolites was developed.

2. Experimental

2.1. Chemicals and reagents

All chemicals used were obtained from Merck (Darmstadt, Germany) and were of analytical grade. MPPP-HCl was provided from Hessisches Landeskriminalamt for research purposes before the drug had entered the German Controlled Substances Act.

2.2. Urine samples

The investigations were performed using urine of male rats (Wistar, Ch. River, Sulzfleck, Germany) which were administered a single 40 mg/kg body mass dose (for metabolism studies) or a 1 mg/kg body mass dose (for development of the screening procedure) of MPPP 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 °C until 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 0.5 ml portion of urine was adjusted to pH 5.2 with acetic acid (1 *M*) and incubated at 37 °C for 12 h with 50 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). The urine sample was then diluted with 2.5 ml of water and loaded on an Isolute Confirm HCX cartridge (130 mg, 3 ml) previously conditioned with 1 ml of methanol and 1 ml of water. After passage of the sample, the cartridge was washed with 1 ml of water, 1 ml of 0.01 *M* hydrochloric acid and 2 ml of methanol. The retained compounds were eluted into 1.5 ml reaction vials with 1 ml of a freshly prepared mixture of methanol–aqueous ammonia (98:2, v/v) and the eluate was gently evaporated to dryness under a stream of nitrogen at 56 °C. After reconstitution in 50 μl of methanol, 50 μl of a solution of diazoethane in diethyl ether, synthesized according to the procedure of McKay et al. [7], were added, the reaction vials were sealed and left at room temperature for 8 h. Thereafter, the mixture was once again gently evaporated to dryness under a stream of nitrogen, redissolved in 100 μl of methanol and a 3 μl aliquot was injected into the GC–MS system.

2.4. Sample preparation for toxicological analysis

The working-up was the same as described in Section 2.3, but the enzymatic hydrolysis was performed at 56 °C and stopped after 1 h and the ethylation was stopped after 15 min.

2.5. Gas chromatography–mass spectrometry

MPPP and its metabolites were separated and identified in ethylated 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×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, m/z 50–550 u; EI ionization mode: ionization energy, 70 eV; ion source temperature, 220 °C; capillary direct interface heated at 260 °C.

For toxicological detection of MPPP and its metabolites, mass chromatography with the selected ions m/z 98, 104, 112, 121, 177 and 222 was used. Generation of the mass chromatograms could be started by clicking the corresponding pull down menu which executes the user-defined macros [8] (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 [9] of the mass spectra underlying the peaks (after background subtraction) with reference spectra (Fig. 1) recorded during this study.

3. Results and discussion

3.1. Sample preparation

Cleavage of conjugates by gentle enzymatic hydrolysis was necessary before extraction and GC–MS analysis of the suspected metabolites in order not to overlook conjugated metabolites. Our common liquid–liquid extraction under alkaline conditions followed by acetylation [10–14] was not appropriate, because of the volatility of the free bases of the pyrrolidinopropiophenones, and the instability of the analytes under alkaline and high-temperature conditions [15,16]. In addition, part of the metabolites showed bipolar properties. In contrast, solid-phase extraction (SPE) showed good results because of more volatile solvents, thus avoiding temperature stress. In addition, mixed-mode SPE is suitable for extraction of bipolar compounds.

Derivatization was needed for sensitive detection of carboxy metabolites after lower drug doses. The carboxy groups could easily be ethylated with diazoethane, and in contrast to methylation, this allowed to distinguish between metabolic and artificial (GC artifact in methanol solution) methylation. Derivatization by diazoethane has the advantage over that by ethyl iodide that the reagent could be completely evaporated without a further extraction step and that quarternary amines are not formed. Other derivatization procedures (e.g., trifluoroacetylation) were used in order to detect further metabolites (e.g., with primary or secondary amino or hydroxy groups) which might not have been detected after ethylation.

3.2. Identification of metabolites

The urinary metabolites of MPPP were identified by EI- and PCI-MS after GC separation. 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 [17]. 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. EI and PCI mass spectra, the gas chromatographic retention indices (RI), structures and predominant fragmentation patterns of the ethylated metabolites are shown in Fig. 1. The spectra are arranged according to the numbers given in Fig. 2.

The following metabolites of MPPP could be identified after high dose application: 2-oxo-4′-carboxy propiophenone (mass spectrum no. 3 in Fig. 1), 4′-carboxybenzoic acid (mass spectrum no. 4), 2″-oxo-MPPP (mass spectrum no. 5), 2″-oxo-4′-carboxy-PPP (mass spectrum no. 6), 4′-carboxy-PPP (mass spectrum no. 2). The mass spectra nos. 5 and 6 did not allow to identify the position of the carbonyl group in the pyrrolidine ring. However, from a metabolic point of view, if the hydroxylation preceding the dehydrogenation took place in position 3″, the metabolite would have been excreted most probably in its conjugated hydroxy form. Furthermore, other compounds carrying a pyrrolidine ring are also excreted as their lactam metabolite (e.g., nicotine to cotinine). The GC and MS data of MPPP and its metabolites will be included in the forthcoming update of our handbook and library [9,18]. The parent compound MPPP could not be found, although the limit of detection was as low as 100 ng/ml and the extraction efficacy for MPPP was approximately 70%.

Based on the identified metabolites of MPPP, the following partly overlapping metabolic pathways could be postulated: hydroxylation of the α-carbon followed by desamination and further biotransformation to the corresponding benzoic acid in analogy to the structurally related amfepramone [19], hydroxylation of the 4′-methyl group followed by oxidation to the corresponding carboxylic acid and/or hydroxylation of the 2″-position of the pyrrolidine ring followed by dehydrogenation to the corresponding lactam (Fig. 2). Since the peaks of the metabolites 2–4 and 6 were markedly higher after enzymatic hydrolysis, it can be concluded that they are excreted as acyl glucuronides.

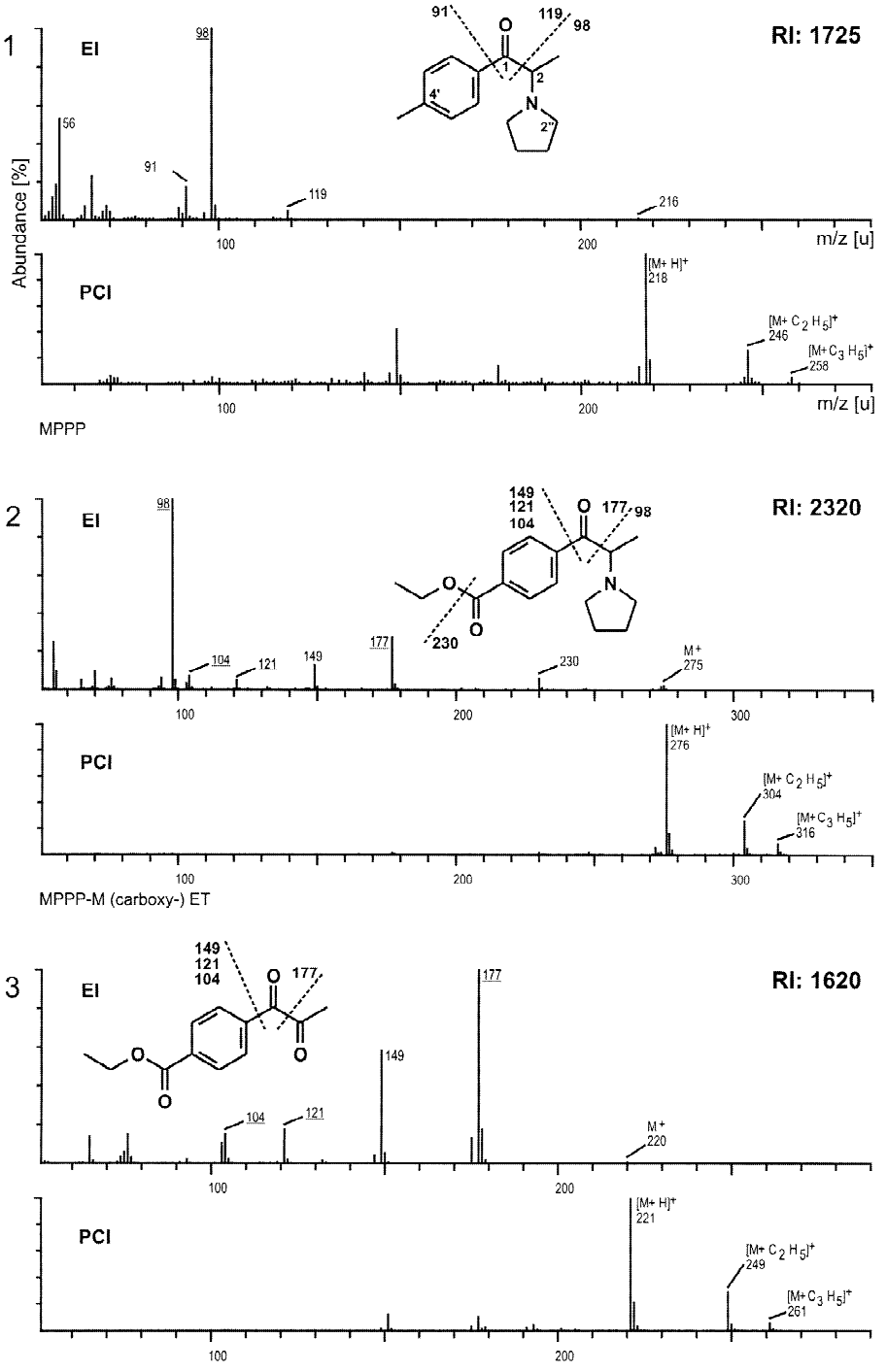
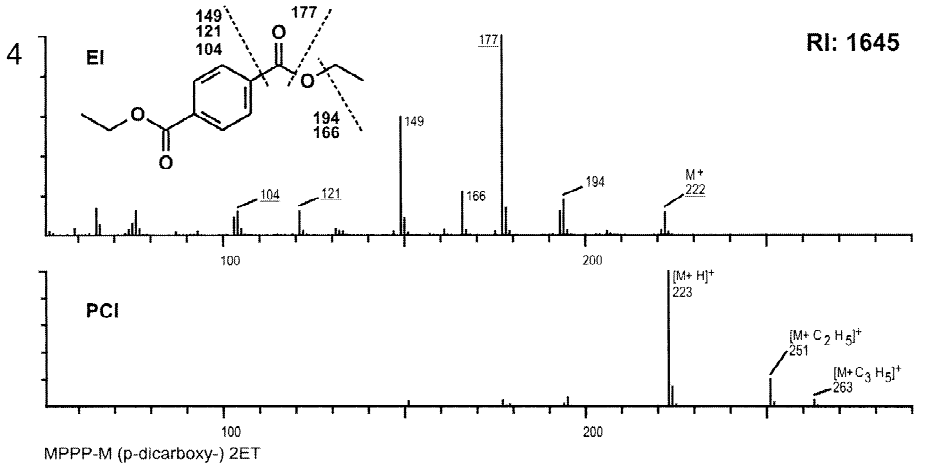
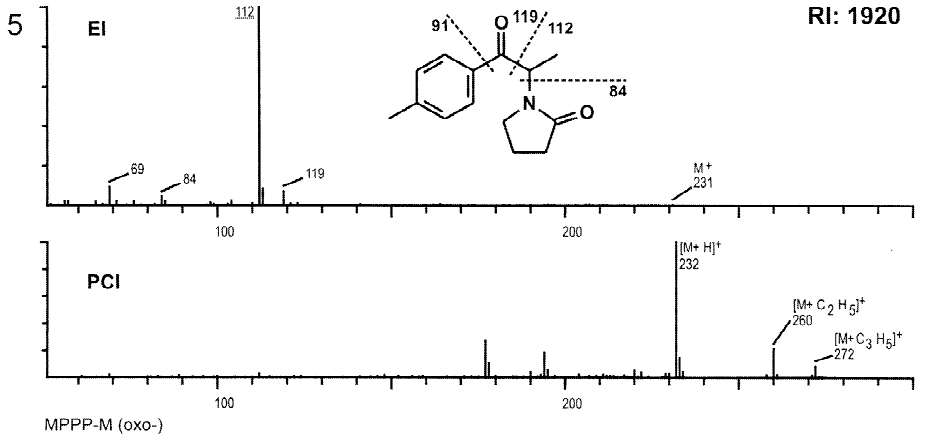


Fig. 1. EI and PCI mass spectra, the gas chromatographic retention indices (RI), structures and predominant fragmentation patterns of MPPP and its metabolites after ethylation. The numbers of the spectra correspond to those in Fig. 2. Ions selected for the toxicological detection are underlined. The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





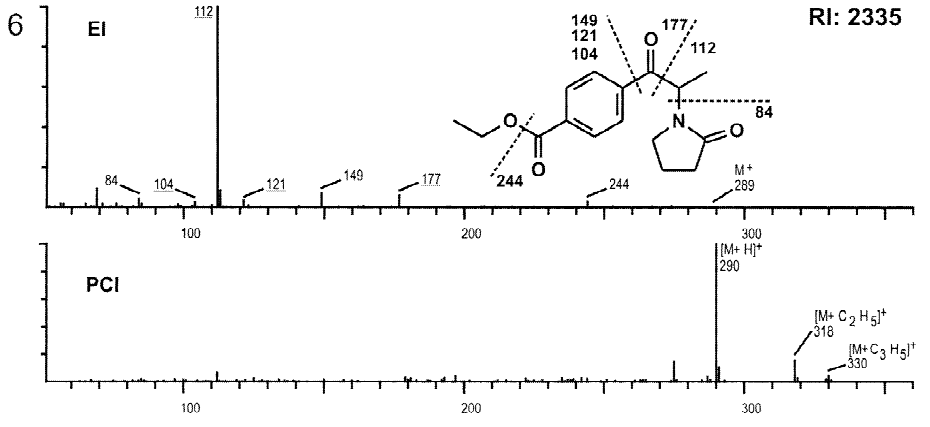


Fig. 1. (*continued*)

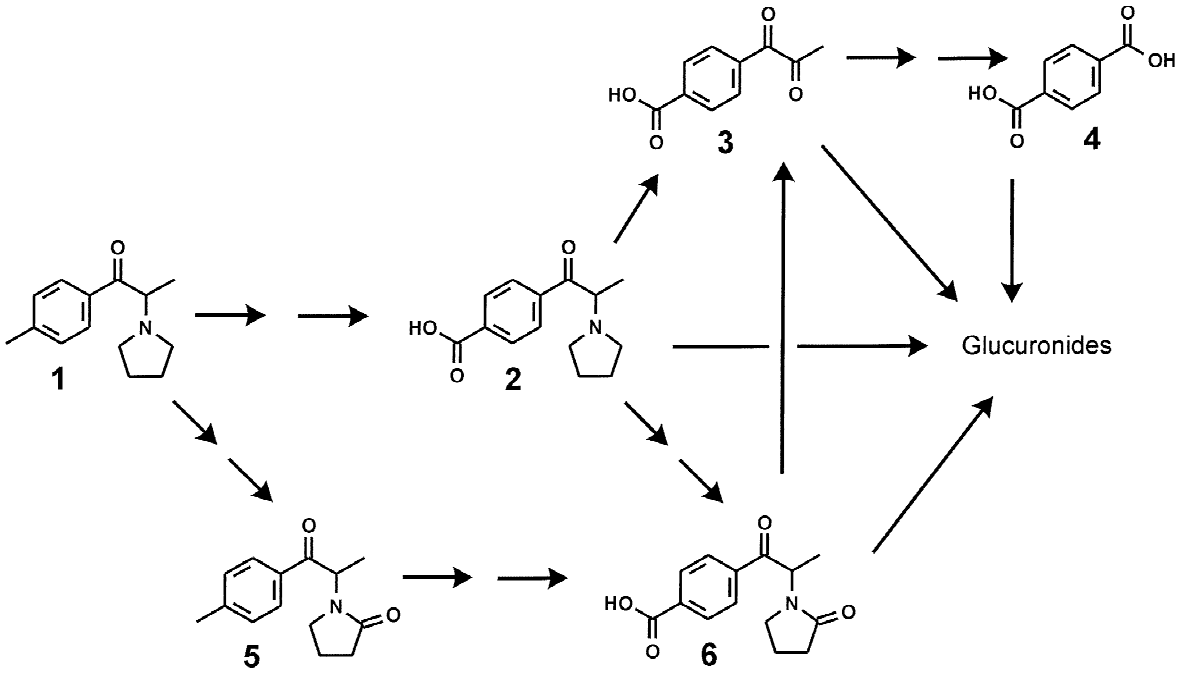


Fig. 2. Proposed scheme for the metabolism of MPPP in rats. The metabolites 2–4 and 6 were also excreted as acyl glucuronides.

In contrast to the metabolism of PPP [20], cathinone [15], amfepramone [19] and metamfepramone [21], the corresponding norephedrines formed by reduction of the keto group, could not be detected after suitable derivatization.

3.3. Toxicological detection by GC–MS

MPPP metabolites were separated by GC and identified by EI-MS after enzymatic hydrolysis, SPE and ethylation. Mass chromatography with the following ions (m/z) was used to indicate the presence of MPPP and/or its metabolites: m/z 98, 104, 112, 121, 177 and 222. Generation of the mass chromatograms could be started by clicking the corresponding pull down menu which executes the user-defined macros.

The selected ion m/z 98 was used for the identification of the compounds with intact pyrrolidine ring (mass spectra nos. 1 and 2 in Fig. 1), 112 for the lactam metabolites (mass spectra nos. 5 and 6 in Fig. 1), 104, 121 and 177 for the ethylated 4′-carboxy metabolites (mass spectra nos. 2–4 and 6 in Fig. 1) and additionally, 222 for the ethylated di-carboxy metabolite (mass spectrum no. 4 in Fig. 1).

Fig. 3 shows reconstructed mass chromatograms indicating the presence of MPPP metabolites in an ethylated extract of rat urine after administration of 1 mg/kg body mass of MPPP, a dose that might correspond to the common dose of abusers, if the ‘‘effective’’ dosage was similar to that of amfepramone. 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 [9]. Fig. 4 shows the mass spectrum underlying the marked peak in Fig. 3, the reference spectrum (Fig. 1), the structure, and the hit list found by computer library search. In our experience, the gas chromatographic retention indices (RI) provide preliminary indications and may be useful to gas chromatographers without a GC–MS

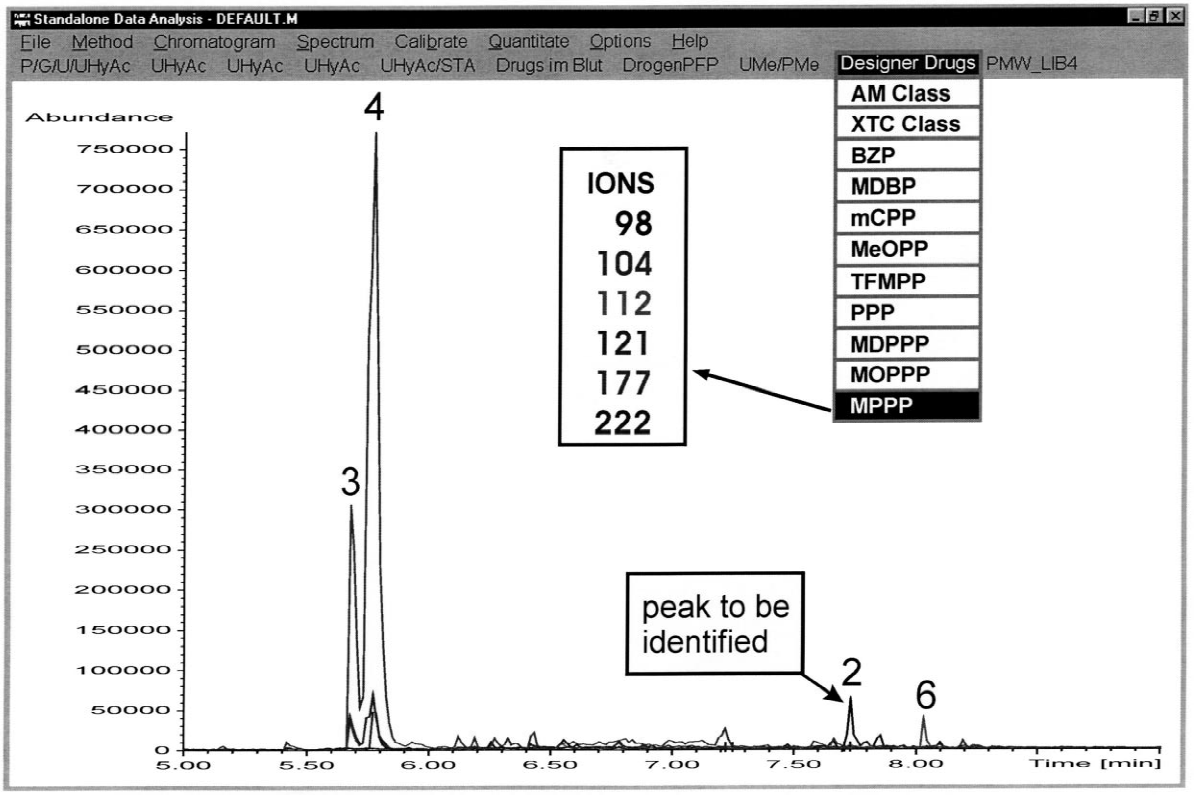


Fig. 3. Typical mass chromatograms with the ions m/z 98, 104, 112, 121, 177 and 222. They indicate the presence of MPPP metabolites in an ethylated extract of a rat urine sample taken 24 h after ingestion of 1 mg/kg body mass of MPPP. The numbers of the peaks correspond to those in Figs. 1 and 2. The merged chromatograms can be differentiated by their colors on a color screen.

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 [22] of the components of a standard solution of typical drugs which is measured daily for testing the GC–MS performance [23,24]. 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.

Unfortunately, no authentic human urine samples after intake of MPPP were available. However, according to our experience in metabolism and analytical studies on rats and humans [12,25,26], it should be possible to detect the metabolites found in rat urine also in human urine samples.

The presented screening procedure was suitable also for other designer drugs of the α-pyrrolidinopropiophenone type [1,20].

4. Conclusions

Our studies revealed that the new designer drug MPPP was extensively metabolized. Screening must be focussed on these metabolites. The new screening procedures should also be suitable for detection of MPPP and/or its metabolites in human urine in clinical or forensic cases.

Further studies will show, which cytochrome-P450 isoenzymes are involved in the different metabolic

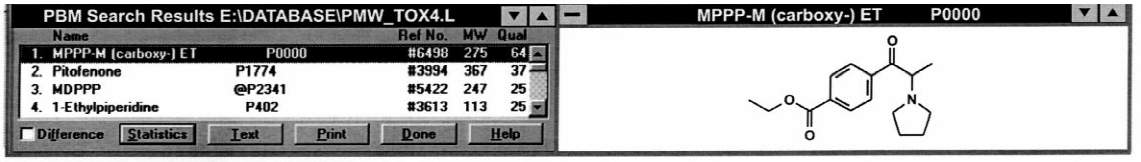


Fig. 4. Mass spectrum underlying the marked peak (No. 2) in Fig. 3, the reference spectrum, the structure, and the hit list found by computer library search.

steps. This knowledge may allow to predict possible drug–drug interactions and/or toxic risks.

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