Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 793 (2003) 377–388
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Metabolism and toxicological detection of the new designer drug 3′,4′-methylenedioxy-α-pyrrolidinopropiophenone studied in urine using gas chromatography–mass spectrometry

Dietmar Springer[a], Giselher Fritschi[b], Hans H. Maurer[a,*]

[a]*Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, University of Saarland, D-66421 Homburg (Saar), Germany*
[b]*Hessisches Landeskriminalamt, D-65187 Wiesbaden, Germany*

Received 20 February 2003; received in revised form 14 April 2003; accepted 16 April 2003

Abstract

R,S-3′,4′-Methylenedioxy-α-pyrrolidinopropiophenone (MDPPP) is a new designer drug with assumed amphetamine-like effects, which has appeared on the illicit drug market. The aim of this study was to identify the MDPPP metabolites using solid-phase extraction, ethylation or acetylation as well as to develop a toxicological detection procedure in urine using solid-phase extraction, trimethylsilylation and GC–MS. Analysis of urine samples of rats treated with MDPPP revealed that MDPPP was completely metabolized by demethylenation of the methylenedioxy group followed by partial 3′-methylation of the resulting catechol, oxidative desamination to the corresponding diketo compounds and/or hydroxylation of the pyrrolidine ring with subsequent dehydrogenation to the corresponding lactam. The hydroxy groups were found to be partly conjugated. Based on these data, MDPPP could be detected in urine via its metabolites by full-scan GC–MS using mass chromatography for screening and library search for identification by comparison of the spectra with reference spectra.

Keywords: Designer drug; Metabolism; Toxicological detection; 3′,4′-Methylenedioxy-α-pyrrolidinopropiophenone

1. Introduction

α-Pyrrolidinophenone derivatives like *R,S*-α-pyrrolidinopropiophenone (PPP), *R,S*-4′-methyl-α-pyrrolidinopropiophenone (MPPP), *R,S*-4′-methyl-α-pyrrolidinohexanophenone (MPHP), *R,S*-4′-methoxy-α-pyrrolidinopropiophenone (MOPPP) and *R,S*-3′,4′-methylenedioxy-α-pyrrolidinopropiophenone (MDPPP) are new designer drugs which have appeared on the illicit drug market [1–4] and are distributed among drug abusers in tablet form. As the pyrrolidinophenones cannot be detected with usual routine analysis procedures, statements on the frequency of their occurrence cannot be made. Meanwhile, most of these substances including MDPPP are scheduled in the German Controlled Substances Act and possession is strictly prohibited. So far, little information about the dosage as well as the pharmacological and toxicological effects of the pyrrolidinophenones is available. The chemical structures of all the α-pyrrolidinophenones are close-

*Corresponding author. Tel.: +49-6841-162-6050; fax: +49-6841-162-6051.
E-mail address: hans.maurer@uniklinik-saarland.de (H.H. Maurer).

1570-0232/03/$ – see front matter
doi:10.1016/S1570-0232(03)00350-7

ly 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 [5–8]. The metabolism and toxicological detection of MPPP and MPHP have already been described [4,9]. The metabolism of MDPPP has not yet been studied. However, the knowledge about metabolic steps 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. So far, determination or screening procedures for MDPPP and/or its metabolites have not yet been published, although such procedures are necessary for confirmation of the diagnosis of an intoxication or drug abuse.

The aim of the presented study was firstly to identify the MDPPP metabolites in rat urine using gas chromatography–mass spectrometry (GC–MS) in the electron impact (EI) and positive-ion chemical ionization (PICI) modes and secondly to develop a toxicological screening procedure based on the identified metabolites using EI GC–MS.

2. Experimental

2.1. Chemicals and reagents

All chemicals used were obtained from E. Merck (Darmstadt, Germany) and were of analytical grade. *R,S*-MDPPP-HCl was provided by Hessisches Landeskriminalamt (Wiesbaden, Germany) for research purposes before it became a controlled substance.

2.2. Urine samples

The investigations were performed using 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 MDPPP in an aqueous suspension by gastric intubation. Urine was collected separately from the feces 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 and 1 ml of 0.01 *M* hydrochloric acid. The retained non-basic 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 a second step into a different vial with 1 ml of a freshly prepared mixture of methanol–aqueous ammonia (98:2, v/v, fraction 2). The eluates were gently evaporated to dryness under a stream of nitrogen at 56 °C and derivatized by ethylation or acetylation according to published procedures (Refs. [4] or [10]). Briefly, ethylation was performed after reconstitution in 50 μl of methanol with 50 μl of a solution of diazoethane in diethyl ether, synthesized according to the procedure of McKay et al. [11], 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. Acetylation was conducted with 100 μl of acetic anhydride–pyridine (3:2, v/v) for 5 min under microwave irradiation at about 440 W [12–14]. After evaporation, the residue was dissolved in 100 μl of methanol. A 3-μl aliquot was injected into the GC–MS system. The same procedure was repeated without the use of enzymatic hydrolysis to study which metabolites of MDPPP are excreted as glucuronides and/or sulfates.

2.4. Sample preparation for toxicological analysis

A 0.5-ml portion of urine was adjusted to pH 5.2 with acetic acid (1 *M*) and incubated at 56 °C for 1 h with 50 μl of a mixture of glucuronidase and arylsulfatase (same as used in Section 2.3). This

sample was 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 1 ml of methanol. The retained compounds were then eluted into a 1.5-ml reaction vial with 1 ml of a freshly prepared mixture of methanol–aqueous ammonia (98:2, v/v). The eluate was gently evaporated to dryness under a stream of nitrogen at 56 °C and then reconstituted in 50 μl of ethyl acetate and trimethylsilylated after addition of 50 μl *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) for 5 min under microwave irradiation at 440 W. A 2-μl aliquot of this mixture was injected into the GC–MS system with an alcohol- and water-free syringe.

2.5. Gas chromatography–mass spectrometry

The MDPPP metabolites were separated and identified in derivatized urine extracts using a Hewlett-Packard (Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph combined with a HP 5989B MS Engine mass spectrometer and a 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 °C/min, initial time 3 min, final time 8 min. The MS conditions were as follows: full scan mode, *m/z* 50–550 u; EI ionization mode: ionization energy, 70 eV; chemical ionization using methane, positive mode (PICI): ionization energy, 230 eV; ion source temperature, 220 °C; capillary direct interface heated at 260 °C.

For toxicological detection of MDPPP and its trimethylsilylated metabolites, MS with the selected ions *m/z* 98, 112, 121 and 306 was used. Generation of the mass chromatograms could be started by clicking the corresponding pull down menu which executes the user defined macros [15]. The identity of the peaks in the mass chromatograms was confirmed by computerized comparison [16] of the mass spectra underlying the peaks (after background subtraction) with reference spectra (Fig. 1, mass spectra 1, 4, 7, 10, and 11 and Ref. [16]) 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. As usual for routine screening procedures, incubation was performed at a higher temperature and for a shorter time in contrast to metabolism studies, in which an almost complete cleavage can be achieved and temperature stress can be avoided. The use of common liquid–liquid extraction under alkaline conditions followed by acetylation [10,13,17–19] was not appropriate, because the majority of the metabolites showed amphoteric properties. Even MDPPP itself was only poorly extractable under the liquid–liquid extraction procedure applied (pH 8–9, ethyl acetate–isopropanol–dichloromethane, 3:1:1, v/v). In addition, volatility of the free bases and the instability of the analytes under alkaline and high temperature conditions caused difficulties [20,21]. In contrast, solid-phase extraction (SPE) demonstrated acceptable results due to mixed-mode SPE's ability to extract amphoteric compounds [9].

Derivatization was needed for sensitive detection of metabolites after administration of lower drug doses. For metabolism studies, acetylation has been shown to be preferred for derivatization of metabolites with primary and secondary amino groups as well as of alcoholic and/or phenolic hydroxy groups [17,22,23]. Ethylation was preferred for derivatization of metabolites with phenolic hydroxy and/or vinylogous carboxy groups [4]. In addition, ethylation by diazoethane has the further advantage that phenolic hydroxy groups can be derivatized without affecting alcoholic groups, thereby allowing a distinction between both types of hydroxy groups. Moreover, ethylation is favored over diazomethane methylation, allowing a distinction between metabolic methylation and derivatization. These procedures for sample preparation, extraction and derivatization have proven to be appropriate for metabolism studies

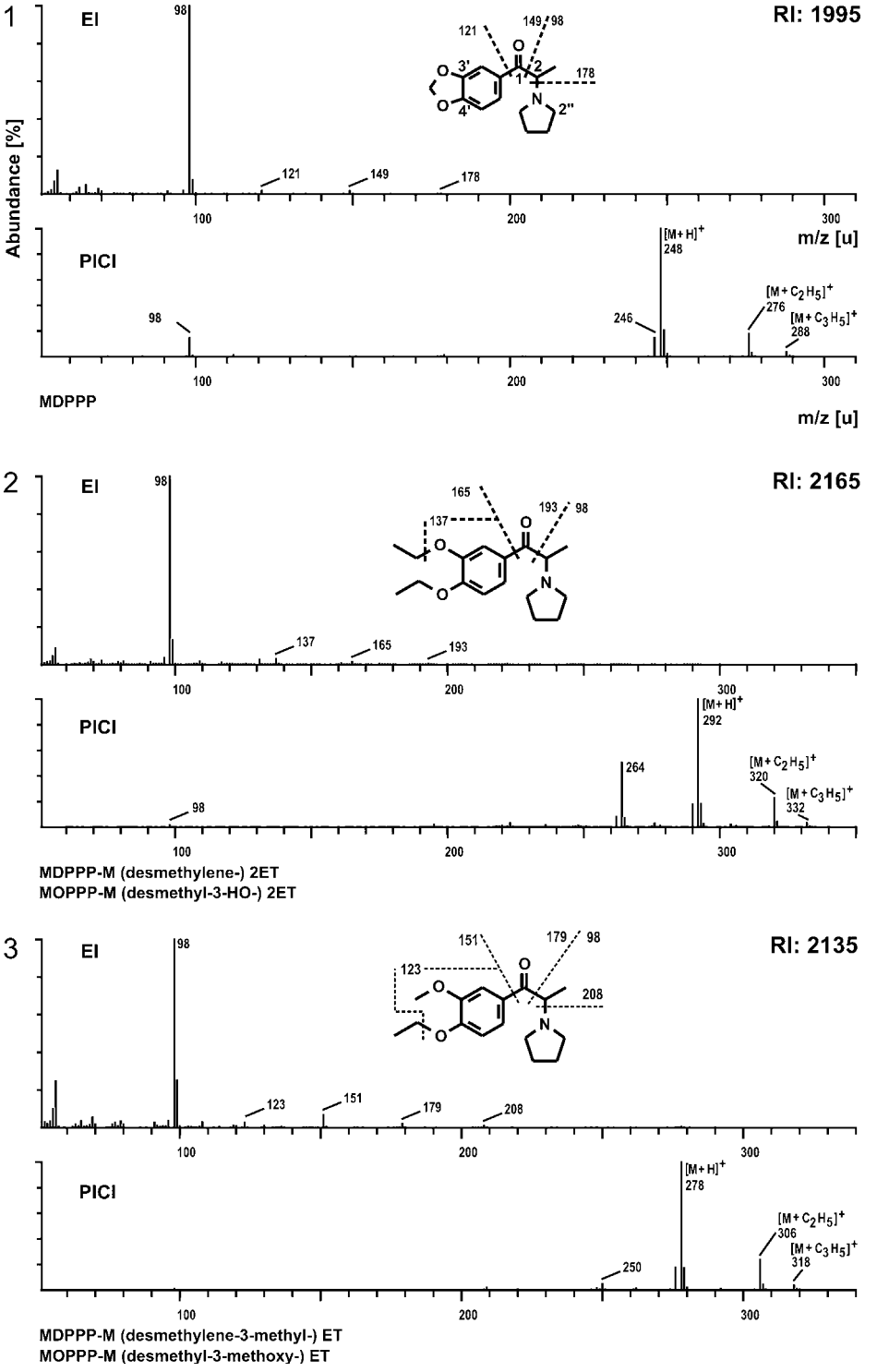
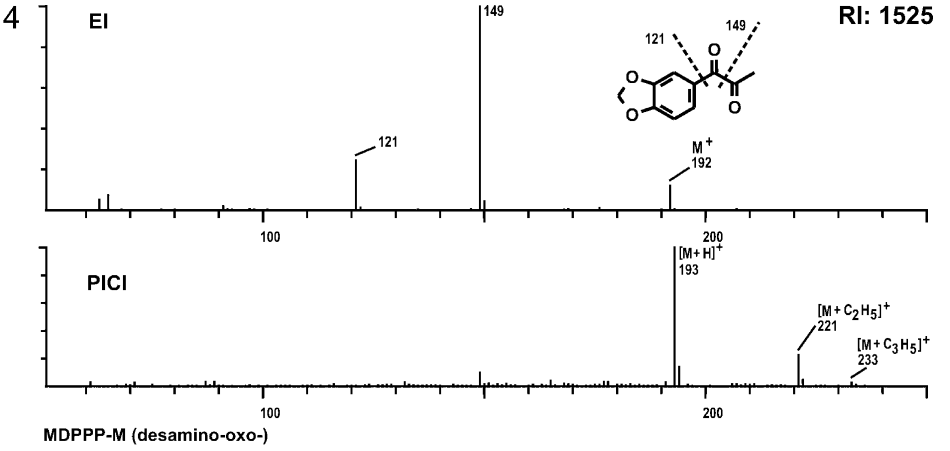
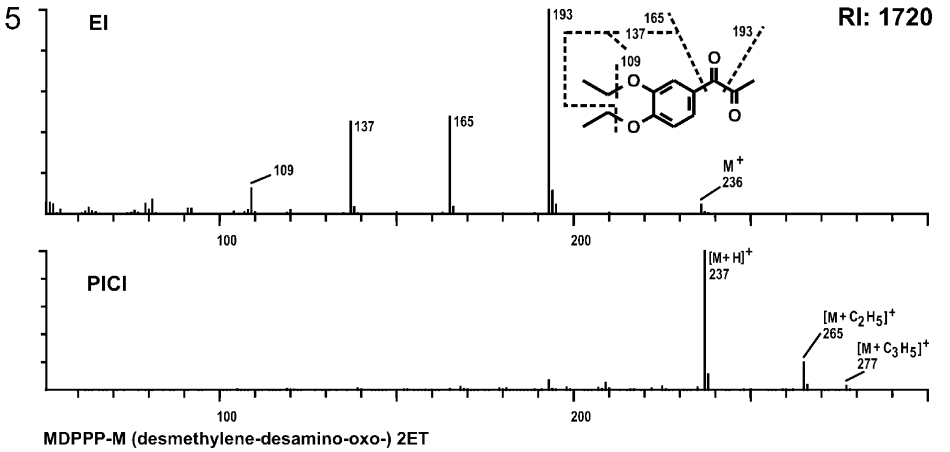


Fig. 1. EI and PICI mass spectra, the gas chromatographic retention indices (RIs), structures and predominant fragmentation patterns of MDPPP and its metabolites after ethylation or trimethylsilylation. The axes are only labelled for 1.





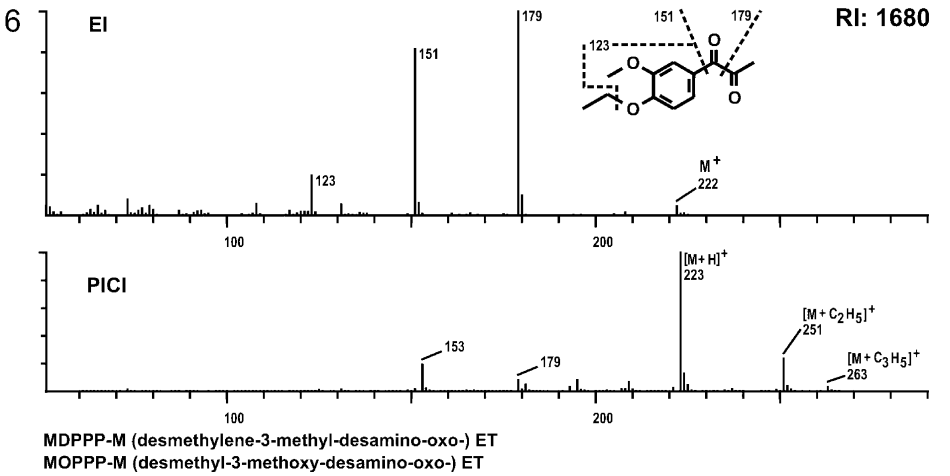


Fig. 1. (*continued*)

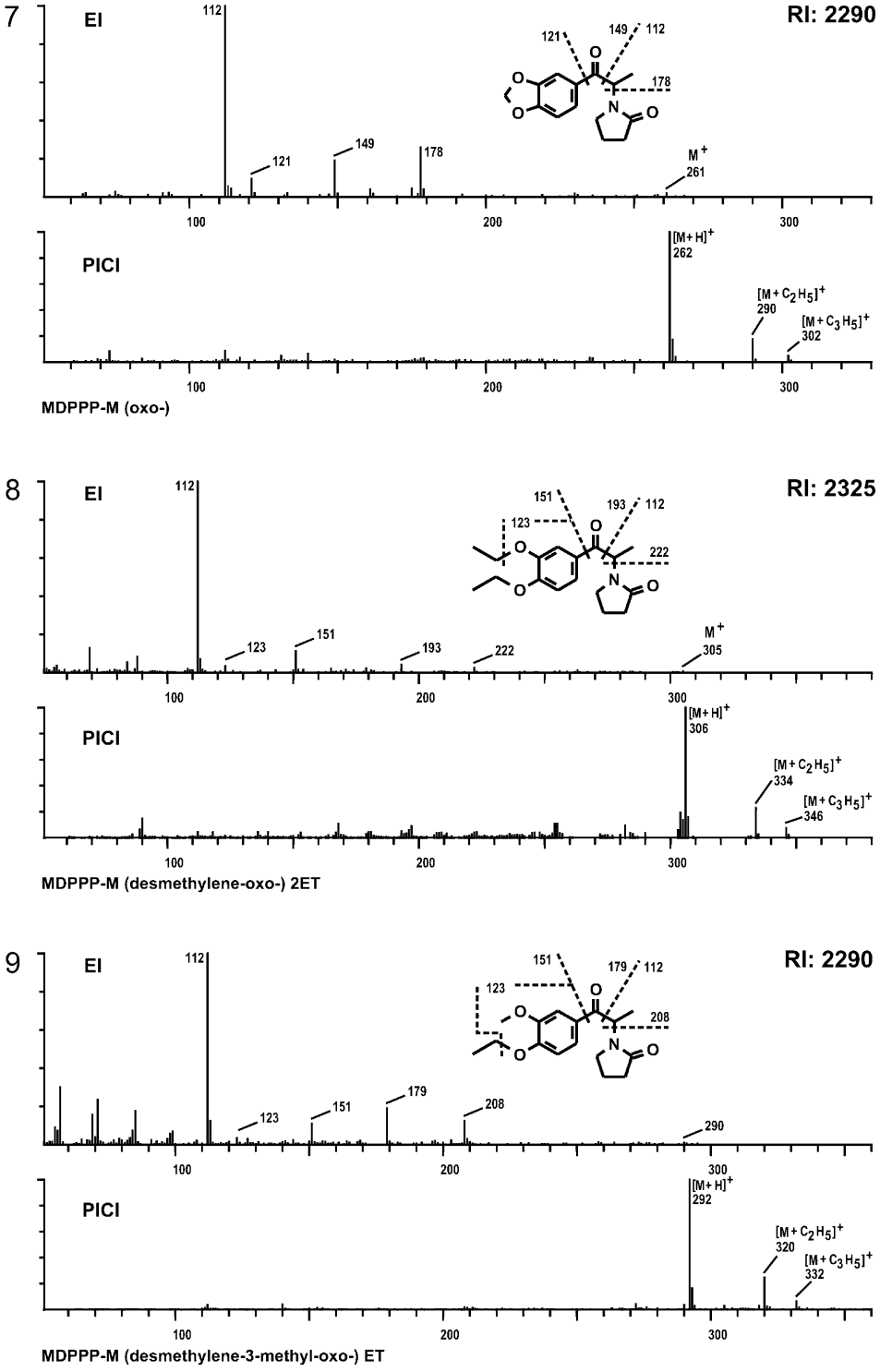


Fig. 1. (*continued*)

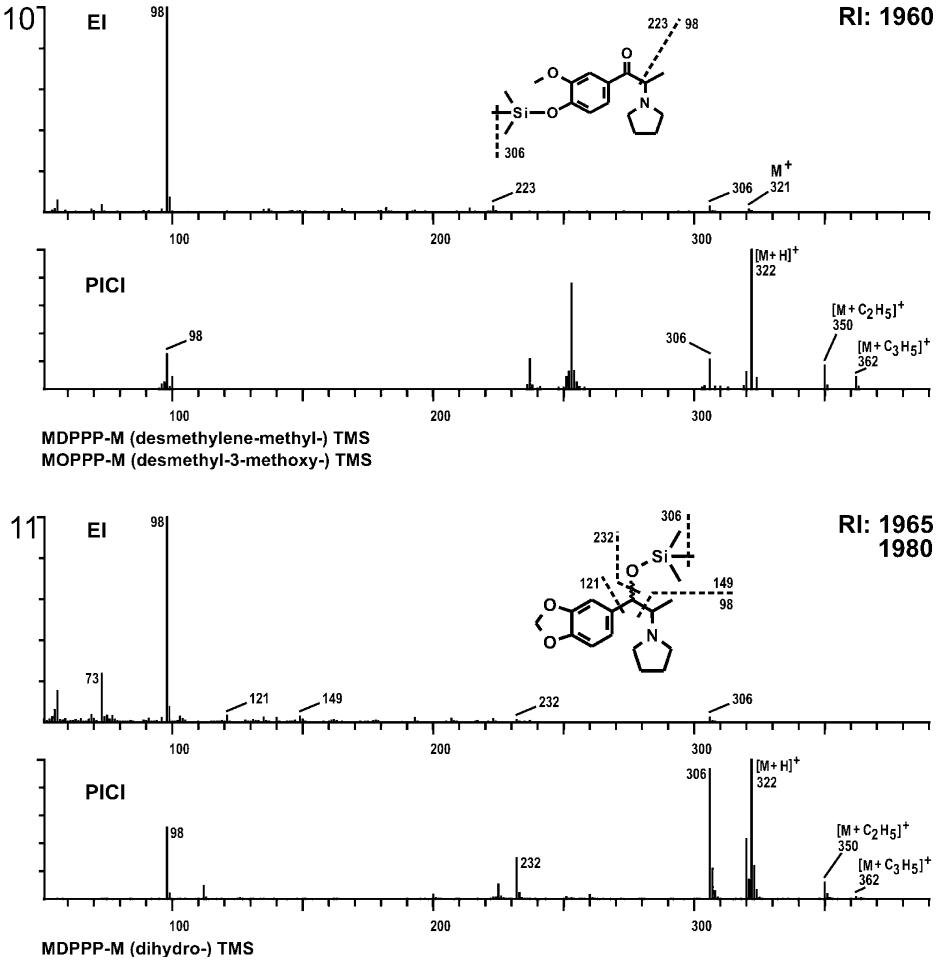


Fig. 1. (*continued*)

of other pyrrolidinophenone-type designer drugs [4,9].

However, for the toxicological detection procedure, common trimethylsilylation was preferred. In routine work, trimethylsilylation is safer and easier to handle and the reagent is commercially available. All expected target analytes in urine after intake of MDPPP showed good GC properties after trimethylsilylation.

3.2. Identification of metabolites

The urinary metabolites of MDPPP 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 the parent compound according to the rules described by, e.g., McLafferty and Turecek [24] and Smith and Busch [25]. 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 the EI spectra. In addition, adduct ions are produced typical for PICI using methane as reagent gas.

EI and PICI mass spectra, the gas chromatographic retention indices (RIs), structures and predominant fragmentation patterns of MDPPP and its ethylated or trimethylsilylated metabolites are shown in Fig. 1. No acetylated metabolites were detected,

indicating that no primary (i.e., double dealkylation of the pyrrolidine ring) or secondary amines (i.e., ring opening of the pyrrolidine ring) were created. Demethylenation of MDPPP leads to the formation of a vinylogous carboxylic acid. Consequently, these metabolites could not be acetylated either. Only one mass spectrum of the diasteriomeric compound (mass spectrum 11) is depicted. Their spectra are very similar, so that one can be used for identification of both peaks considering the given two different RIs.

The following metabolites of MDPPP (mass spectrum 1 in Fig. 1) could be identified after high dose application: 3′,4′-dihydroxy-PPP (mass spectrum 2), 4′-hydroxy-3′-methoxy-PPP (mass spectrum 3), 3′,4′-methylenedioxy-2-oxo-propiophenone (mass spectrum 4), 3′,4′-dihydroxy-2-oxo-propiophenone (mass spectrum 5), 4′-hydroxy-3′-methoxy-2-oxo-propiophenone (mass spectrum 6), 2″-oxo-MDPPP (mass spectrum 7), 3′,4′-dihydroxy-2″-oxo-PPP (mass spectrum 8) and 3′-hydroxy-4′-methoxy-2″-oxo-PPP. With an estimated share of 80% of the excreted MDPPP metabolites, 4′-hydroxy-3′-methoxy-PPP was the metabolite in greatest abundance. 3′,4′-Dihydroxy-PPP contributed to about 10%.

Many studies by the authors have demonstrated a high degree of qualitative correspondence of rat and human metabolism [17,18,22,23,26–28]. However, substances like cathinone [20], amfepramone [29] or metamfepramone [30] which are structurally related to MDPPP, were shown to be additionally excreted as dihydro metabolites (diasteromers) in humans to a considerable extent. Therefore, the data of dihydro-MDPPP (synthesized from MDPPP by sodium borohydride hydration according to Ref. [31]) were included in Fig. 1 (mass spectrum 11). The GC and MS data of those compounds and derivatives, which are not shown in Fig. 1, will also be included in the forthcoming update of the authors' handbook and library [16,32].

The mass spectra 3, 6, 9 and 10 of Fig. 1 do not identify the position of the methoxy group. However, as catechol-*O*-methyl transferase (COMT) can only methylate the hydroxy groups in position 3 of catechols, the hydroxylation must have taken place in position 3′ of the 3′,4′-dihydroxy-PPP. Ethylated 3′-hydroxy-4′-methoxy-PPP would probably yield almost the same mass spectrum as mass spectrum 3, but it is likely, that this compound would have a different RI. A second metabolite was not detected, so that 4′-hydroxy-3′-methoxy-PPP was the only positional isomer to be identified. Mass spectra 7–9 did not identify the position of the carbonyl group in the pyrrolidine ring. However, as other compounds carrying a pyrrolidine ring are also excreted as their lactam metabolite (e.g., MPPP or nicotine), we postulate the same metabolic pathway for MDPPP. The parent compound MDPPP was not detected, although the limit of detection (S/N 3) was 100 ng/ml. In addition, the extraction efficiency for MDPPP was 94±5% (n=5) measured at 1000 ng/ml.

Based on the identified metabolites of MDPPP, the following partly overlapping metabolic pathways could be postulated (Fig. 2): demethylenation of the methylenedioxy group (2, 3, 5, 6, 8 and 9), 3′-methylation of these demethylenated compounds by COMT to the corresponding demethylene-methyl compounds (3, 6 and 9), oxidative desamination to the corresponding 2-oxo compounds (4–6) and/or hydroxylation of the 2″-position of the pyrrolidine ring followed by dehydrogenation to the corresponding lactams (7–9). In contrast to the metabolism of the structurally related compound MPHP, reduction of the keto group to the corresponding secondary alcohol or hydroxylation of the side chain did not result in excretion of detectable amounts of metabolite. As the peaks of the metabolites 2, 3, 5, 6, 8 and 9 were more abundant after glucuronidase and sulfatase hydrolysis, it can be concluded that they are partly excreted as glucuronides and/or sulfates.

3.3. Toxicological detection by GC–MS

MDPPP metabolites were separated by GC and identified by full-scan EI MS after fast enzymatic hydrolysis, SPE and trimethylsilylation. Only fraction 2, where—among others—the MDPPP main metabolite 4′-hydroxy-3′-methoxy-PPP was eluted, was needed for the toxicological detection. MS with the following ions was used to detect the presence of MDPPP and/or its metabolites: m/z 98, 112, 121 and 306.

The selected ion m/z 98 was used for monitoring the presence of compounds with unchanged

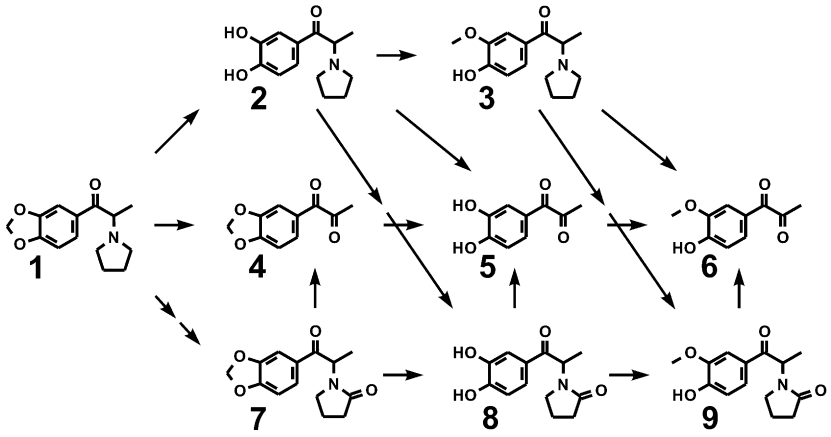


Fig. 2. Proposed scheme for the metabolism of MDPPP in rats. The numbering of the compounds corresponds to that of the mass spectra of the corresponding derivative in Fig. 1.

pyrrolidine ring (mass spectra 1, 10 and 11 in Fig. 1), *m*/*z* 112 for compounds with oxidized pyrrolidine ring (mass spectrum 7 in Fig. 1), *m*/*z* 121 for compounds with an unchanged 3′,4′-methylene-dioxy-phenyl moiety (mass spectra 1, 4, 7 and 11) and *m*/*z* 306 for trimethylsilylated demethyene-methyl-MDPPP and dihydro-MDPPP (mass spectra 10 and 11 in Fig. 1).

Fig. 3 shows reconstructed mass chromatograms indicating the presence of MDPPP metabolites in a trimethylsilylated extract of rat urine after administration of 1 mg/kg body mass of MDPPP. This dose

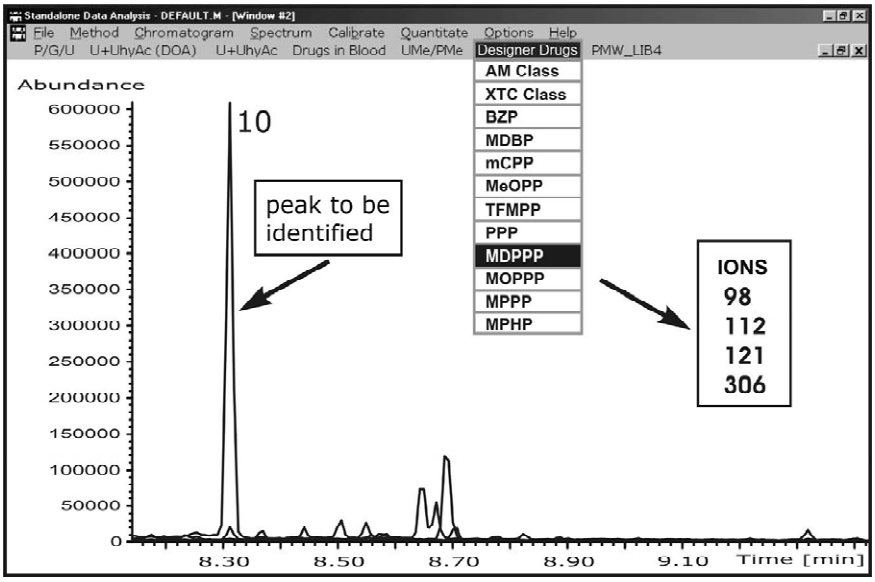


Fig. 3. Typical mass chromatograms with the ions *m*/*z* 98, 112, 121 and 306. They indicate the presence of MDPPP metabolites in a trimethylsilylated extract of a rat urine sample collected over 24 h after ingestion of 1 mg/kg body mass of MDPPP. The numbering of the peaks corresponds to that of the mass spectra of the corresponding derivative in Fig. 1. The merged chromatograms can be differentiated by their colors on a color screen.

was chosen as it should approximately correspond to a dose ingested by abusers. Seized tablets of the related designer drug PPP always have been shown to contain approximately 40 mg. In addition, the chosen dose is in the same range as that of the structurally related medicament amfepramone. The identity of the peak in the mass chromatograms was confirmed by computerized comparison of the underlying mass spectrum with reference spectra recorded during this study [16]. Fig. 4 illustrates the mass spectrum underlying the marked peak in Fig. 3, reference spectrum (10 in Fig. 1), structure, and the drug list found by computer library search. The gas chromatographic RIs provide preliminary indications, allow to distinguish between the above mentioned positional isomers and/or diastereomers and may be useful to gas chromatographers without an MS facility. Therefore, RIs are also provided in Fig. 1. They were recorded during the GC–MS procedure (Section 2.5) and calculated in correlation with the Kovats' indices [33] of the components of a standard solution of typical drugs which is measured daily for testing the GC–MS performance [34,35]. The reproducibility of RIs 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 MDPPP were available. However, the same metabolites found in rat urine are also likely to be in human urine samples. As already discussed above, the diasteromeric dihydro metabolites (mass spectrum 11) might additionally be detectable in human urine. However, as mass m/z 98 would also indicate the presence of dihydro-MDPPP, its mass

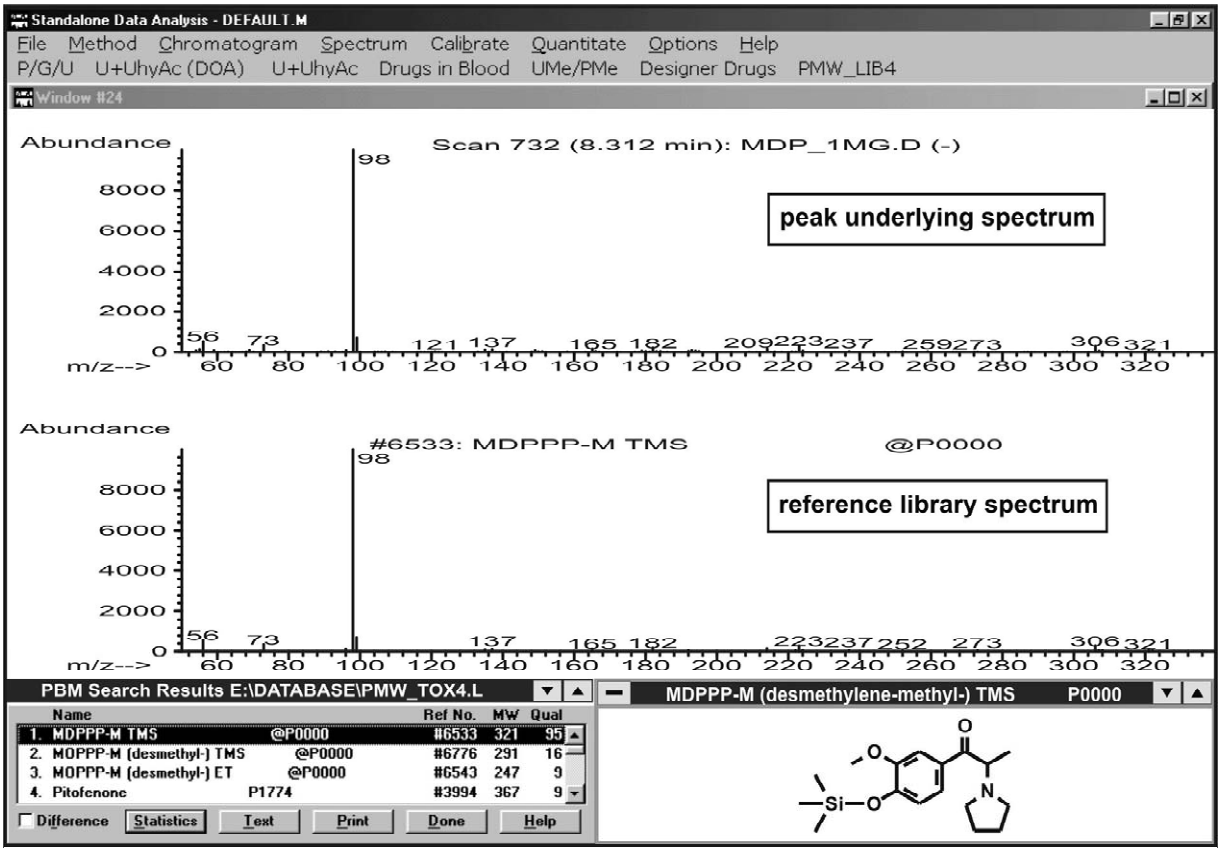


Fig. 4. Mass spectrum underlying the marked peak in Fig. 3, the reference spectrum, the structure, and the hit list found by computer library search.

spectrum was included in Fig. 1. This metabolite is also likely to be detectable in human urine. The extraction efficiency for trimethylsilylated dihydro-MDPPP was 69±6% measured at 1000 ng/ml and the limit of detection (S/N 3) was 50 ng/ml (n=4). Extrapolating the rat data, the described screening procedure should be sensitive enough to detect an intake of an illicit dose of MDPPP.

It should be mentioned that 3′,4′-dihydroxy-PPP, 3′-methoxy-4′-hydroxy-PPP and 3′-methoxy-4′-hydroxy-2-oxo-propiophenone are common metabolites of MDPPP and MOPPP [36]. Intake of small doses of MOPPP in combination with MDPPP can easily be differentiated via the unique main metabolite of MOPPP, 4′-hydroxy-PPP. Differentiation of the ingestion of small doses of MDPPP with MOPPP might be hard, if only 3′-methoxy-4′-hydroxy-PPP as the MDPPP main metabolite was present, as 3′-methoxy-4′-hydroxy-PPP is also detectable after the intake of MOPPP alone. Special attention must be paid to the detection of unique MDPPP metabolites in this instance. If all members of the pyrrolidinophenone designer drug class were excreted as their respective dihydro metabolites to a considerable extent in humans, as discussed above, incontestable statements on the ingested parent compound(s) can be made. The presented screening procedure has already been proven to be suitable for other designer drugs of the α-pyrrolidinophenone type.

4. Conclusions

The presented studies revealed that the new designer drug MDPPP was extensively metabolized by the rat. Screening must, therefore, be focused on metabolites. The described screening procedure should be suitable for detection of MDPPP and/or its metabolites in human urine in clinical or forensic cases.

Acknowledgements

The authors thank Thomas Kraemer, Frank T. Peters, Roland F. Staack, Simone Schaefer, Gabriele Ulrich and Armin A. Weber for their support.

References

[1] P. Roesner, T. Junge, G. Fritschi, B. Klein, K. Thielert, M. Kozlowski, Toxichem. Krimtech. 66 (1999) 81.
[2] D. Springer, F.T. Peters, G. Fritschi, H.H. Maurer, in: F. Pragst, R. Aderjan (Eds.), Proceedings of the 12th GTFCh Symposium, Mosbach, Helm, Heppenheim, 2001, p. 156.
[3] D. Springer, F.T. Peters, G. Fritschi, H.H. Maurer, in: M. Balikova, E. Navakova (Eds.), Proceedings of the 39th International TIAFT Meeting, Prague, 2001, Charles University, Prague, 2002, p. 122.
[4] D. Springer, F.T. Peters, G. Fritschi, H.H. Maurer, J. Chromatogr. B 773 (2002) 25.
[5] M. Martinez, O. Mercado, A. Santamaria, S. Galvan, M. Vazquez, V. Bucio, C. Hall, R. Hernandez, A. Hurtazo, E. Pego, F. Rodriguez, R. Salvatierra, A. Sosa, C. Rios, Proc. West. Pharmacol. Soc. 41 (1998) 125.
[6] R.A. Glennon, M. Yousif, N. Naiman, P. Kalix, Pharmacol. Biochem. Behav. 26 (1987) 547.
[7] P. Kalix, R.A. Glennon, Biochem. Pharmacol. 35 (1986) 3015.
[8] S.G. Bryant, B.G. Guernsey, N.B. Ingrim, Clin. Pharm. 2 (1983) 525.
[9] D. Springer, F.T. Peters, G. Fritschi, H.H. Maurer, J. Chromatogr. B, in press.
[10] H.H. Maurer, J. Bickeboeller-Friedrich, J. Anal. Toxicol. 24 (2000) 340.
[11] A.F. McKay, W.L. Ott, G.W. Taylor, M.N. Buchanan, J.F. Crooker, Can. J. Res. 28 (Sec. B) (1950) 683.
[12] T. Kraemer, A.A. Weber, H.H. Maurer, in: F. Pragst (Ed.), Proceedings of the 10th GTFCh Symposium, Mosbach, Helm, Heppenheim, 1997, p. 200.
[13] H.H. Maurer, in: K. Pfleger, H.H. Maurer, A. Weber (Eds.), Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites, Part 4, Wiley–VCH, Weinheim, 2000, p. 3.
[14] H.H. Maurer, J. Bickeboeller-Friedrich, T. Kraemer, F.T. Peters, Toxicol. Lett. 112 (2000) 133.
[15] H.H. Maurer, Spectrosc. Eur. 6 (1994) 21.
[16] K. Pfleger, H.H. Maurer, A. Weber, Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites, Agilent Technologies, Palo Alto, CA, 2003, in preparation.
[17] R.F. Staack, G. Fritschi, H.H. Maurer, J. Chromatogr. B 773 (2002) 35.
[18] J. Bickeboeller-Friedrich, H.H. Maurer, Ther. Drug Monit. 23 (2001) 61.
[19] H.H. Maurer, Comb. Chem. High Throughput Screen. 3 (2000) 461.
[20] M. Pokrajac, B. Miljkovic, B. Misailovic, Rapid Commun. Mass Spectrom. 5 (1991) 59.
[21] R. Brenneisen, S. Geisshusler, Pharm. Acta Helv. 60 (1985) 290.
[22] T. Kraemer, J. Bickeboeller-Friedrich, H.H. Maurer, Drug Metab. Dispos. 28 (2000) 339.
[23] K. Pfleger, H.H. Maurer, A. Weber, Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites, Part 4, Wiley–VCH, Weinheim, 2000.

[24] F.W. McLafferty, F. Turecek, Interpretation of Mass Spectra, University Science Books, Mill Valley, CA, 1993.
[25] R.M. Smith, K.L. Busch, Understanding Mass Spectra—A Basic Approach, Wiley, New York, 1999.
[26] K. Pfleger, H.H. Maurer, A. Weber, Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites, VCH, Weinheim, 1992.
[27] H.H. Maurer, J. Bickeboeller-Friedrich, Ther. Drug Monit. 19 (1997) 583.
[28] T. Kraemer, H.H. Maurer, Ther. Drug Monit. 24 (2002) 277.
[29] A.H. Beckett, M. Stanojcic, J. Pharm. Pharmacol. 39 (1987) 409.
[30] S.L. Markantonis, A. Kyroudis, A.H. Beckett, Biochem. Pharmacol. 35 (1986) 529.
[31] K. Schwetlick, Organikum, Wiley–VCH, Weinheim, 2001.
[32] K. Pfleger, H.H. Maurer, A. Weber, Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites, Part 5, Wiley–VCH, Weinheim, 2003, in preparation.
[33] E. Kovats, Helv. Chim. Acta 41 (1958) 1915.
[34] R.A. de-Zeeuw, J.P. Franke, H.H. Maurer, K. Pfleger, 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, Weinheim, New York, Basle, 1992.
[35] DFG Senatskommission für Klinisch-Toxikologische Analytik, J. Clin. Chem. Clin. Biochem. 20 (1982) 699.
[36] D. Springer, G. Fritschi, H.H. Maurer, J. Chromatogr. B, submitted for publication.