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Toxicokinetics and analytical toxicology of amphetamine-derived designer drugs ('Ecstasy')

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Abstract

The phase I and II metabolites of the designer drugs methylenedioxyamphetamine (MDA), *R,S*-methylenedioxymethamphetamine (MDMA), *R,S*-methylenedioxyethylamphetamine (MDE), *R,S*-benzodioxazolylbutanamine (BDB) and *R,S*-*N*-methyl-benzodioxazolylbutanamine (MBDB) were identified by gas chromatography-mass spectrometry (GC-MS) or liquid chromotography-mass spectrometry (LC-MS) in urine and liver microsomes of humans and rats. Two overlapping pathways could be postulated: (1) demethylenation followed by catechol-*O*-methyl-transferase (COMT) catalyzed methylation and/or glucuronidation/sulfatation; (2) *N*-dealkylation, deamination and only for MDA, MDMA, MDE oxidation to the corresponding benzoic acid derivatives conjugated with glycine. Demethylenation was mainly catalyzed by CYP2D1/6 or CYP3A2/4, but also by CYP independent mechanisms. In humans, MDMA and MBDB could also be demethylenated by CYP1A2. *N*-demethylation was mainly catalyzed by CYP1A2, *N*-deethylation by CYP3A2/4. Based on these studies, GC-MS procedures were developed for the toxicological analysis in urine and plasma. Finally, toxicokinetic parameters are reviewed.

Keywords: Designer drugs; Methylenedioxy amphetamines; Toxicokinetics; Metabolism; GC-MS analysis

1. Introduction

The designer drugs methylenedioxyamphetamine (MDA), *R,S*-1-(3′,4′-methylenedioxyphenyl)-2-propanamine, 'Love Pills', *R,S*-methylenedioxymethamphetamine, 'Adam', 'Ecstasy' (MDMA) and *R,S*-methylenedioxyethylamphetamine; 'Eve' (MDE) as well as benzodioxazolylbutanamine (BDB); *R,S*-1-(1′,3′-benzodioxazol-5′-yl)-2-butanamine or *R,S*-1-(3′,4′-methylenedioxyphenyl)-2-butanamine and *R,S*-*N*-methylbenzodioxazolylbutanamine (MBDB) are so-called entactogens (Nichols, 1986), producing feelings of euphoria and energy and a desire to socialize. This may explain their current popularity as 'rave drugs' (Hegadoren et al., 1999). Although these drugs have the reputation of being safe, several experimental studies in rats and humans and epidemiological studies indi-

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cated risks to humans. Recent reviews summarize the current knowledge on hepatotoxicity (Jones and Simpson, 1999) and neurotoxicity, psychopathology and abuse potential of such designer drugs (Hegadoren et al., 1999). Since metabolites are claimed to be responsible for the hyperthermic, the neuro- and/or hepatotoxic effects (Hiramatsu et al., 1990; Carvalho et al., 1996), detailed knowledge on the metabolism is necessary.

In vivo studies in humans showed two main metabolic pathways: demethylenation and *N*-dealkylation (Ensslin et al., 1996a; Maurer, 1996). In vitro studies in rats and humans were described indicating that the demethylenation of MDMA to the toxic catechols and/or their oxidation products was catalyzed by polymorphic CYP2D1/6 (Tucker et al., 1994; Lin et al., 1997; Wu et al., 1997). However, since CYP2D1/6 deficient rats could also form such catechols (Colado et al., 1995), other CYP isoenzymes and/or other enzymatic and/or nonenzymatic mechanisms should be responsible for the demethylenation in vivo (Kumagai et al., 1991). Therefore, it was studied which metabolic pathway is catalyzed in humans or rats by the main CYP isoenzymes CYP1A2, CYP2C11/9, CYP2D1/6, CYP3A2/4 and/or by cytosolic enzymes. A comparison of human and rat metabolism was necessary to prove the applicability of toxicological data from rats (Chu et al., 1996) to risk assessment in humans.

Designer drugs may lead to more or less severe intoxications (Walubo and Seger, 1999) and impairment to drive a car (Moeller and Hartung, 1997). Therefore, in clinical and forensic toxicology, these illicit drugs are often to be analyzed. Commercial amphetamine immunoassays (e.g. radioimmunoassay, Cody, 1990), enzyme immunoassays (Kunsman et al., 1990) or

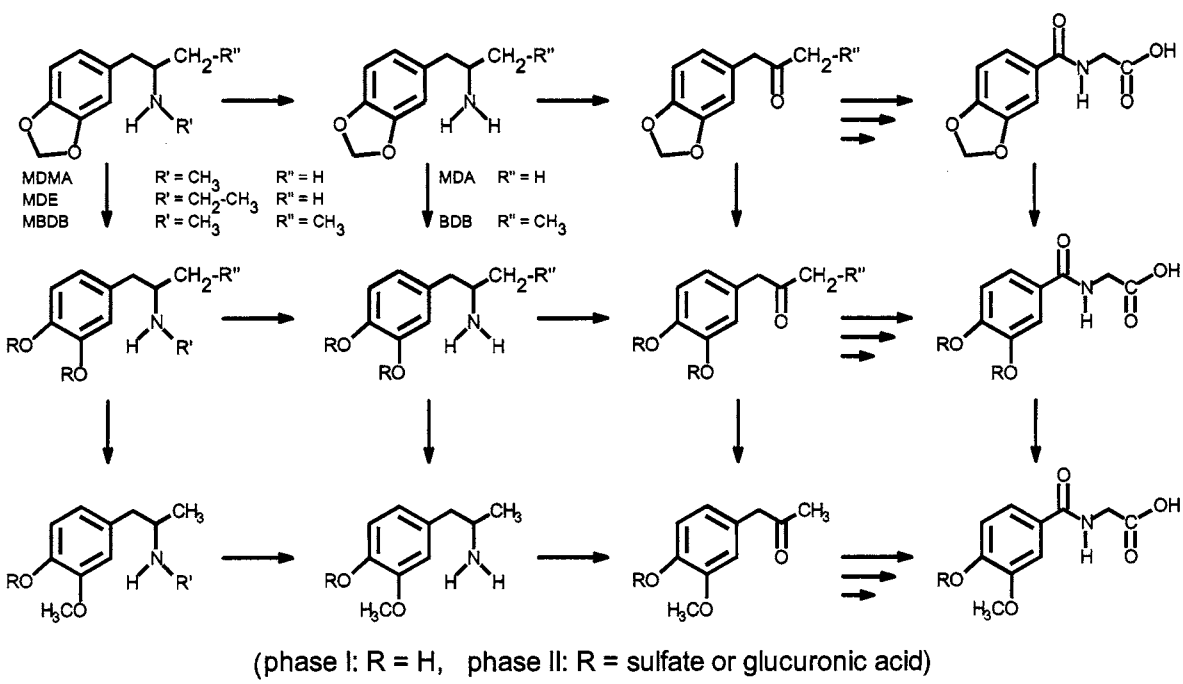

Fig. 1. Proposed scheme for the metabolism of the *R*,*S*-methylenedioxyamphetamine (MDA), *R*,*S*-methylenedioxymethamphetamine (MDMA), *R*,*S*-methylenedioxyethylamphetamine (MDE), *R*,*S*-benzodioxazolylbutanamine (BDB) and *R*,*S*-*N*-methylbenzodioxazolylbutanamine (MBDB) in humans and rats. The glycine conjugates were only formed by the propylamines. The hydroxy metabolites were also present as glucuronic acid and/or sulfate conjugates in urine.

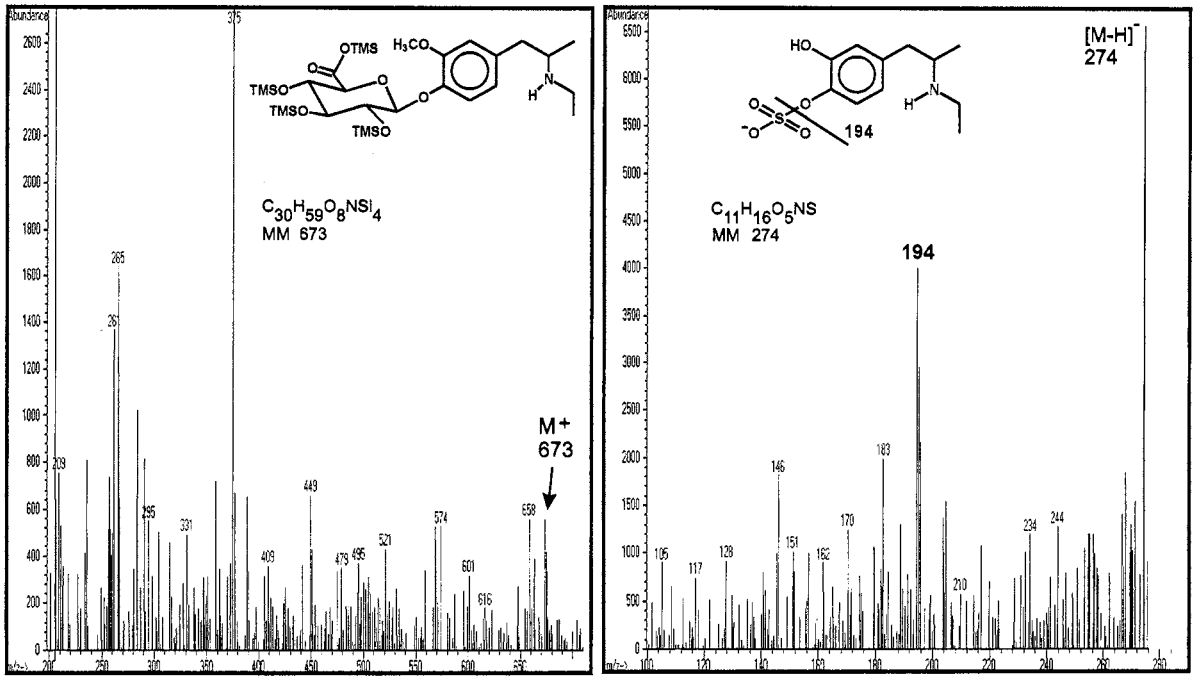

Fig. 2. Electron-impact (EI)-gas chromatography-mass spectrometry (GC-MS) spectrum of the silylated hydroxymethoxy ethylamphetamine (HME) glucuronide (left) and the ESI-liquid chromatography-mass spectrometry (LC-MS) spectrum of the dihydroxy ethylamphetamine (DHE) sulfate (right) recorded in urine extracts.

fluorescence polarization immunoassays (FPIA) (Kunsman et al., 1990; Ensslin et al., 1996b) were successfully used for screening of methylenedioxyamphetamine derivatives. For example, intake of 140 mg of MDE led to FPIA positive results up to 60 h after ingestion (Ensslin et al., 1996a). However, more specific procedures are necessary for confirmation and for differentiation, since non-scheduled therapeutics may also lead to positive results (Kraemer and Maurer, 1998). Confirmation and quantification methods using gas chromatography-mass spectrometry (GC-MS), HPLC, CE or TLC were recently reviewed (Kraemer and Maurer, 1998).

In the following, in vivo and in vitro studies on the metabolism of designer drugs necessary for the toxicological risk assessment will be presented as well as GC-MS screening, identification and quantification procedures necessary for clinical and forensic toxicology. For toxicological interpretation of the analytical results, toxicokinetic parameters were selected from the literature.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were obtained from E. Merck, Darmstadt, Germany, Sigma–Aldrich, Deisenhofen, Germany, or Biomol, Hamburg, Germany, and deuterated standards from Promochem, Wesel, Germany.

2.2. GC-MS and liquid chromatography-mass spectrometry (LC-MS) apparatus

Hewlett Packard (HP, Waldbronn, Germany) 5890 Series II GC combined with an HP 5989B MS Engine (for details see Ref. Kraemer et al., 1997). MS conditions: full scan mode or single ion monitoring (SIM) mode; electron-impact (EI) mode or positive chemical ionization (PCI) mode using methane. Quantification in microsomes or plasma was performed in the SIM mode. The concentrations were calculated as the relation of

the peak areas to those of the internal standards (IS) methaqualone or, if available, the deuterated drugs.

An HP 5989B MS Engine electrospray LC-MS (for details see Ref. Maurer et al., 1997) was used as follows. Column: HP Hypersil RP-18 (100 × 2.1 mm; 3 μm). Mobile phase: 23:77 mixture (v/v) acetonitrile–50 mM aqueous triethylammonium acetate, flow-rate: 350 μl/min.

2.3. Work-up procedures

Urine samples, 5 ml, (from rats, from clinical cases or for MDE from a clinical study, Gouzoulis et al., 1992) were worked up by enzymatic or acid cleavage of conjugates, extraction, acetylation and/or methylation (for details see Ref. Maurer, 1996).

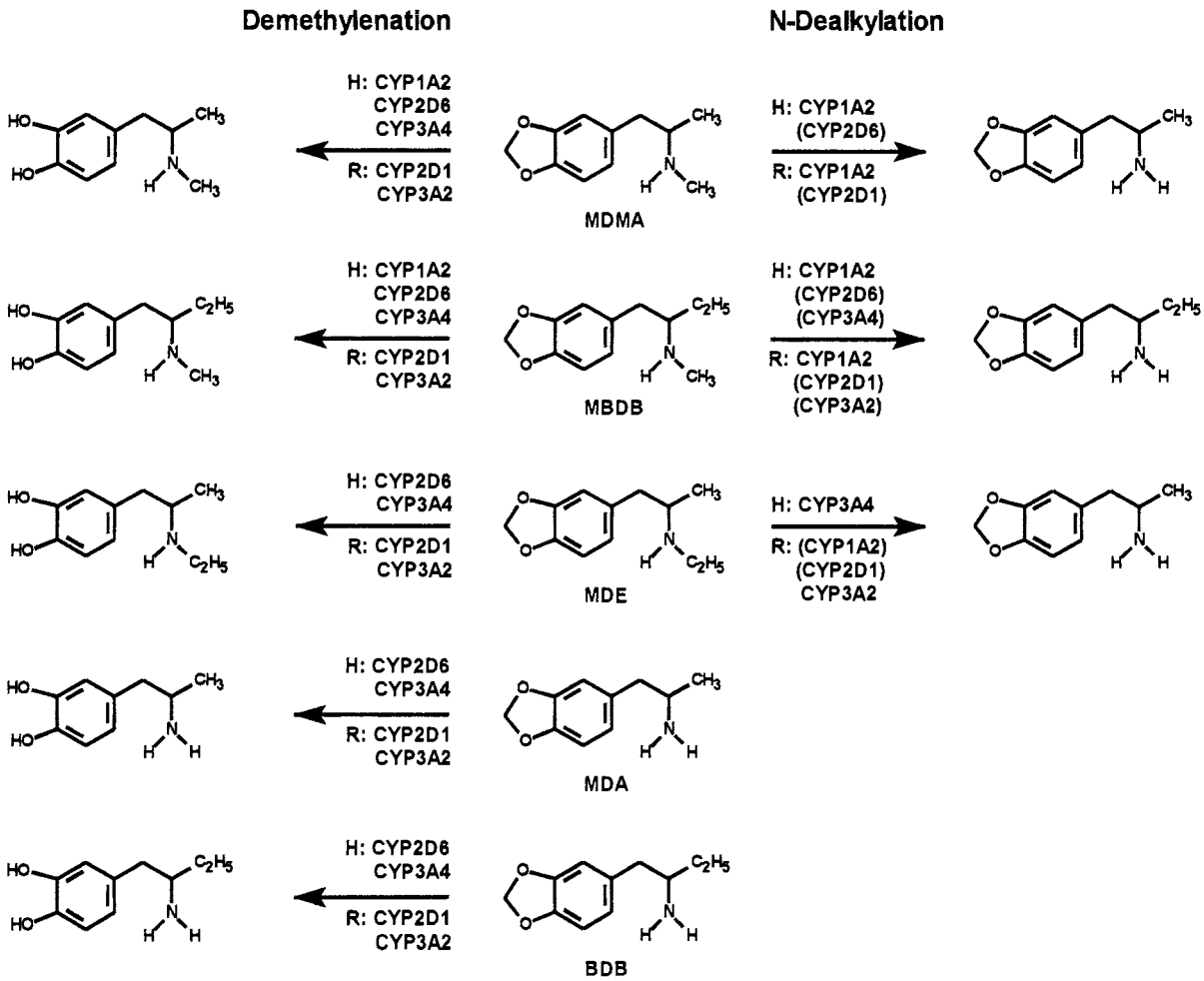

Fig. 3. Isoenzyme dependent major metabolic pathways of *R,S*-methylenedioxyamphetamine (MDA), *R,S*-methylenedioxymethamphetamine (MDMA), *R,S*-methylenedioxyethylamphetamine (MDE), *R,S*-benzodioxazolylbutanamine (BDB) and *R,S-N*-methylbenzodioxazolylbutanamine (MBDB) in humans (H) and rats (R). Metabolism by CYPs given in brackets is of minor extent.

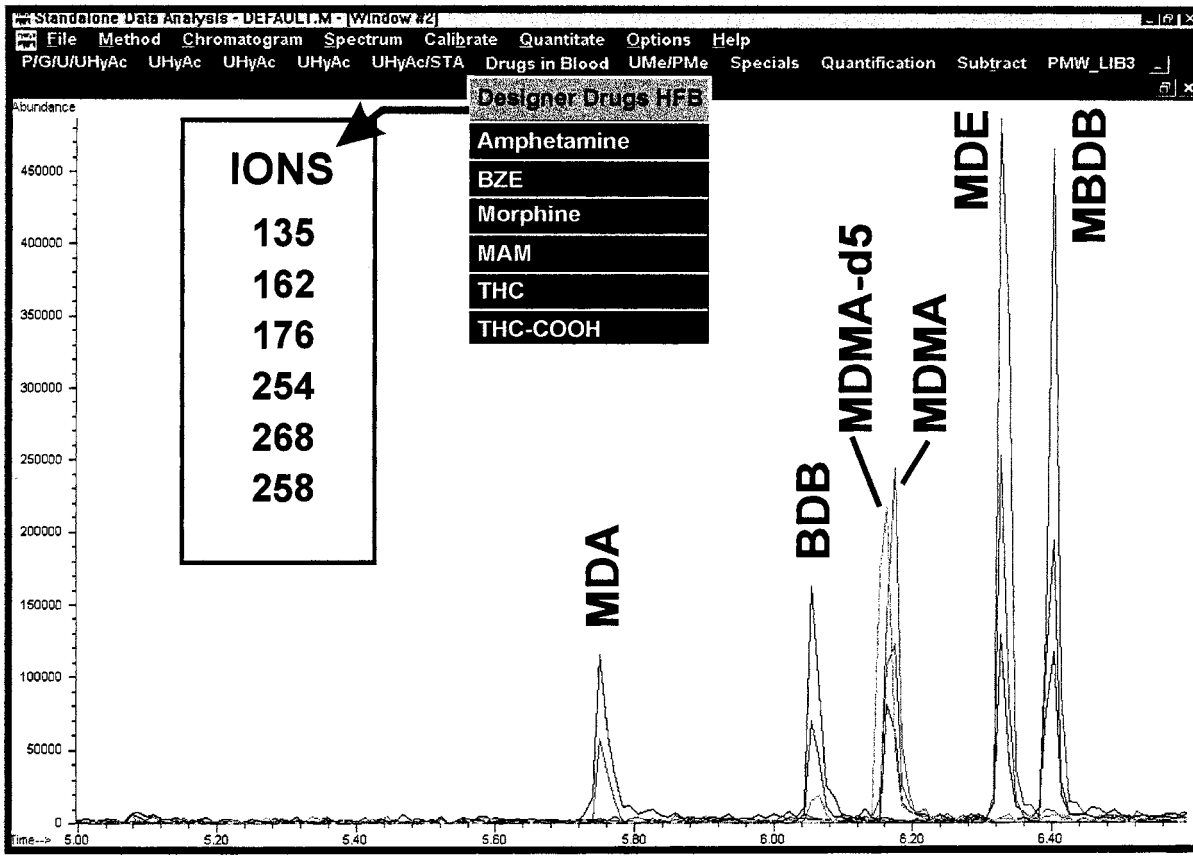

Fig. 4. Typical mass chromatograms with the ions m/z 135, 162, 176, 254, and 258 indicating the presence of methylenedioxyamphetamine (MDA), benzodioxazolylbutanamine (BDB), d5-MDMA (IS), *R,S*-methylenedioxymethamphetamine (MDMA), *R,S*-methylenedioxyethylamphetamine (MDE) and *R,S*-*N*-methyl-benzodioxazolylbutanamine (MBDB), spiked into plasma (100 ng/ml each) after solid phase extraction (SPE) and heptafluorobutyric anhydride (HFB) derivatization.

2.4. Glucuronide and sulfate conjugates were isolated from urine by SPE on Varian Bond Elut C18 with methanol elution. For GC-MS of glucuronides, the extract was trimethylsilylated (Maurer, 2000)

Plasma samples, 1 ml, were worked up after addition of deuterated standards by solid phase extraction (SPE; pH 6, ICT (Bad Homburg, Germany) Isolute Confirm HCX, ethyl acetate-ammonia, 98:2) followed by derivatization using heptafluorobutyric anhydride (HFB; 30 min, 56°C).

2.5. Methods for isoenzyme differentiation

Liver samples (adult male Wistar rats or human samples from liver carcinoma resections) were homogenized and centrifuged at $10\,000 \times g$ and $100\,000 \times g$. Microsome pellets and cytosol were stored at -80°C (for details see Ref. Kraemer et al., 2000).

Microsome and/or cytosol preparations (1.5 mg protein/ml each) were incubated for 90 min at 37°C with the designer drugs (120–140 μM each), 1.2 mM NADP, 2 U isocitrate dehydrogenase, 5 mM isocitrate, 5 mM $MgCl_2$ and/or 5 mM NAD and 1.15 mM *S*-adenosyl-methionine in 0.1 M phosphate buffer (pH 7.4) (final volume 1 ml). Incubation was stopped by adding the extraction mixture. For inhibition studies, the following inhibitors dissolved in methanol were added: naphthoflavone (18 μM) for CYP1A2, sulfaphenazole (10 μM) for

CYP2C9 (human), cimetidine (50 μM) for CYP2C11 (rat), quinine (25 μM) for CYP2D6 (human) and CYP2D1 (rat), and/or ketoconazole (9.4 μM) for CYP3A (human and rat).

After addition of 100 μl of methanolic methaqualone and 100 μl of methanolic d5-MDA (0.01 mg/ml each), the incubation mixtures were extracted and acetylated as described for urine (Maurer, 1996).

3. Results and discussion

3.1. Metabolism of designer drugs studied in human and rat urine

The metabolites detected in urine were identified by interpretation of the mass spectra and, if available, by comparison with synthesized reference substances (Ensslin et al., 1996b). PCI was used for confirmation of the molecular mass. As shown in Fig. 1, the methylenedioxyphenylalkylamine designer drugs undergo two overlapping metabolic pathways: *O*-demethylenation of the methylenedioxy group to dihydroxy derivatives followed by methylation of one of the hydroxy groups and successive degradation of the side chain to *N*-dealkyl and deaminooxo metabolites (Ensslin et al., 1996a; Maurer, 1996). The propylamines MDA, MDMA and MDE are additionally metabolized to glycine conjugates of the corresponding 3,4-disubstituted benzoic acids (hippuric acids). However, 3,4-dihydroxy hippuric acid and 3-methoxy-4-hydroxy hippuric acid can

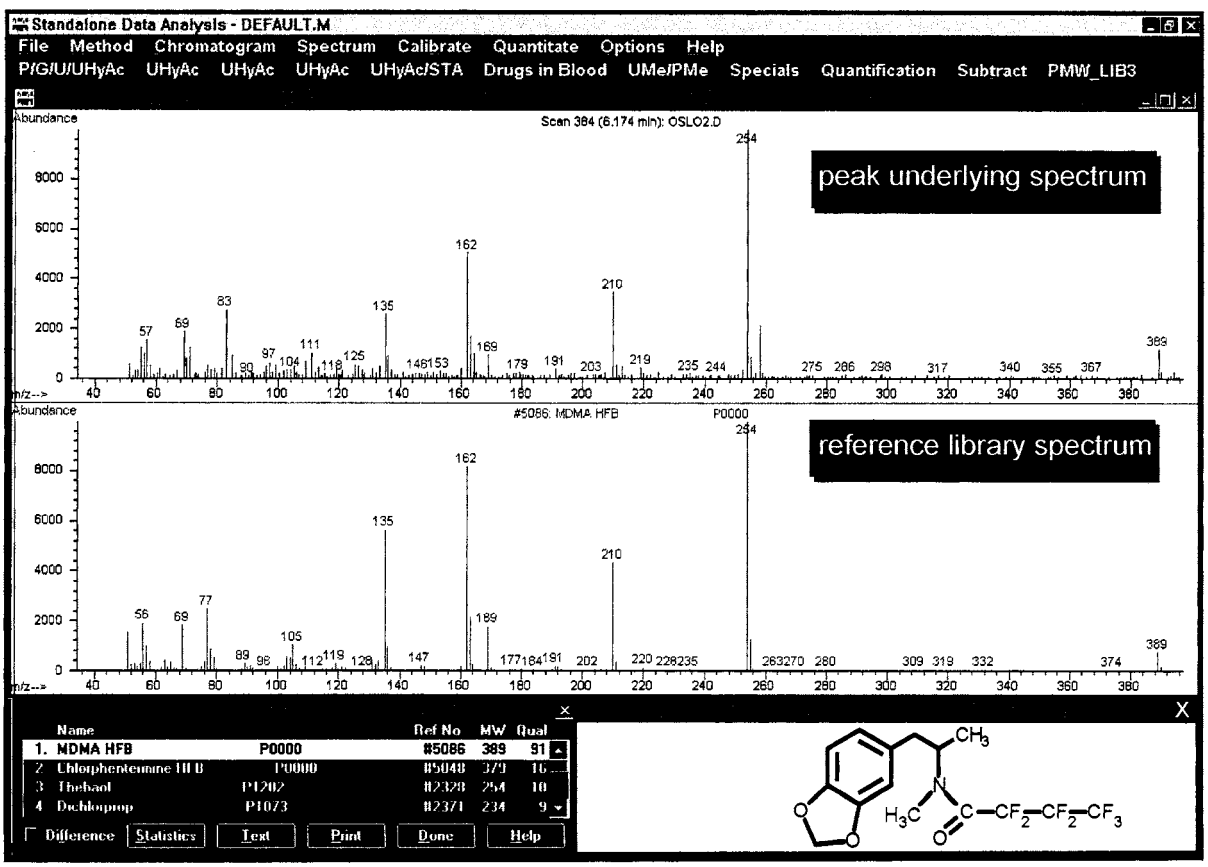

Fig. 5. Mass spectrum underlying the *R,S*-methylenedioxymethamphetamine (MDMA) peak in Fig. 4, the reference spectrum, the structure and the hit list found by library search in (Pfleger et al., 2000).

Table 1
Toxicokinetic data of designer drugs[a]

Drugs	Dose (mg)	$C_{P\,max}$ (ng/ml)	$C_{U\,max}$ (ng/ml)	t_{max} (h)	$t_{1/2}$ (h)	AUC (ng/ml/h)	Ref.
MDMA	100–120	331	28 100	2	5–10		(Helmlin et al., 1996)
MDA		15	2300				
MDMA	75	126		2	9.5	995	(de-la-Torre et al., 1997)
MDMA	125	226		2	9.1	2235	(de-la-Torre et al., 1997)
MDE	140	203–465	24 900	1.5–3		675–1935	(Ensslin et al., 1996a; Brunnenberg et al., 1998)
MDA		7–33	6700	3.3		18–120	
HME		67–670	89 690	1.5–4		218–3028	
MBDB BDB	100		18 577 751				(Kintz, 1997)

[a] $C_{P\,max}$, maximal plasma concentration; $C_{U\,max}$, maximal urine concentration; t_{max}, time after ingestion, at which the plasma concentration is maximal; $t_{1/2}$, plasma elimination half life; AUC, area under the curve (plasma concentration vs. time after ingestion).

also be metabolites, e.g. of food preservatives (Liebich and Forst, 1990). The hydroxy metabolites were found to be excreted in conjugated form. For example, Fig. 2 shows the EI GC-MS spectrum of the silylated hydroxymethoxy ethylamphetamine (HME) glucuronide (upper part) and the electrospray LC-MS spectrum of the dihydroxy ethylamphetamine (DHE) sulfate, both recorded in human urine.

3.2. Influence of cytochrome P450 isoenzymes on the metabolism of designer drugs studied in human and rat liver microsomes

The isoenzyme dependent major metabolic pathways of racemic MDA, MDMA, MDE, BDB and MBDB in humans (H) and rats (R) are summarized in Fig. 3. *N*-Demethylation of MDMA and MBDB was predominantly catalyzed in rats and humans by CYP1A2 and only in minor extent by the polymorphic CYP2D1/6. MBDB was additionally *N*-demethylated by CYP3A2/4. *N*-Deethylation of MDE was predominantly catalyzed in rats and humans by CYP3A2/4, and in rats additionally to a minor extent by CYP1A2 and CYP2D1. This is in accordance with other studies on *N*-dealkylation of other drugs (Coutts et al., 1994), which showed that *N*-demethylation was often catalyzed by CYP1A2 and *N*-deethylation by CYP3A2/4. Both, rat and human microsomes showed a high variability in *N*-dealkylation, which is due to the different amount of each isoenzyme.

Demethylenation of all tested drugs to the catechols was catalyzed in humans and rats predominantly by CYP2D1/6 and CYP3A2/4 isoenzymes. Demethylenation of MDMA and MBDB was additionally catalyzed in humans by CYP1A2. First, we were unable to demonstrate in microsomes the influence of CYPs on the demethylenation to the corresponding catechols, since no catechols were detectable. To check whether this effect was due to the instability of the catechols, we incubated the drugs with both, microsomes and cytosol plus *S*-adenosyl-methionine. Thus, the catechols could be stabilized in *statu nascendi* by catechol-*O*-methyl-transferase (COMT) catalyzed methylation. Using this trick, we were able to indirectly determine the catechols as their 3-methyl derivatives.

Interestingly, it has been found that at least MDMA, MDE and MBDB could also be demethylenated during cytosolic incubation in the absence of microsomes. In the presence of NAD the extent of this demethylenation was higher than without NAD. This is in accordance to studies which described that the demethylenation of (methylenedioxy)phenyl derivatives was also

mediated by hydroxy radicals (Kumagai et al., 1991). Further studies will show, whether such demethylenation could also be possible in the brain, thereby contributing to the neurotoxic effects as claimed by Hiramatsu et al. (1990). Massive release of catecholic neurotransmitters by designer drugs together with catecholic designer drug metabolites may lead to an overabundance of catechols, which might not be quantitatively detoxified by low active COMT (Lavigne et al., 1997). Therefore, it was suspected that low activity of COMT may also influence the hepatotoxic risk of designer drugs.

3.3. GC-MS procedure for screening and identification of designer drugs and their metabolites in urine

Based on the metabolism studies, a full scan GC-MS procedure was developed for simultaneous screening and identification of these designer drugs and/or their metabolites in urine. A work-up procedure was used that has proven to be successful for several classes of drugs (Maurer, 1992; Maurer et al., 1997). Cleavage of conjugates and derivatization was performed to extend the duration of detectability. The procedure for screening and confirmation in urine was explained elsewhere (Maurer, 1996). This procedure allowed simultaneous detection of most of the toxicologically relevant drugs in the same GC-MS run (Maurer, 1992, 2000).

3.4. GC-MS procedures for screening, identification and quantification of designer drugs in plasma

For screening, identification and quantification in plasma, another work-up was necessary to improve the signal to noise ratio. Since only a limited number of quite similar compounds had to be isolated, SPE was preferred resulting in rather clean extracts. However, to compensate for the known batch-to-batch differences of SPE columns, deuterated analytes were used as internal standard. HFB derivatization was preferred to reach the sensitivity necessary for determination of low blood levels.

Screening and identification is performed in the full scan mode using mass chromatography for screening and computer library search for identification as explained in the following. Fig. 4 shows typical mass chromatograms with the ions m/z 135, 162, 176, 240, 254 and 268 selected from the corresponding mass spectra indicating the presence of MDA, BDB, d5-MDMA (IS), MDMA, MDE and MBDB, spiked into plasma (100 ng/ml each), after SPE and HFB derivatization. As shown, all the drugs were sufficiently separated, of course with the exception of MDMA and its deuterated analogue. In the upper part of Fig. 5 the mass spectrum underlying the MDMA peak in the mass chromatograms in Fig. 4 is shown. In the lower part, the reference spectrum is shown with the structure and the hit list found by library search (Pfleger et al., 2000). Since the mass spectra are quite different, the given compounds can specifically be identified.

Quantification was performed in the SIM mode. The concentrations were calculated from the relation of the peak areas to those of the deuterated internal standards. The method was linear from 10 to 500 ng/ml with a recovery of better than 90%, a precision of better than 10% and a detection limit of 3 ng/ml (S/N 3).

3.5. Toxicokinetic parameters for interpretation of the analytical results

The few published toxicokinetic data are summarized in Table 1 for the interpretation of analytical results in clinical or forensic toxicology. However, when interpreting analytical results in correlation to such data, broad individual variations must be considered due to variations in metabolic or excretion rates or due to drug or food interactions.

4. Conclusions

Comparison of rat and human metabolism (in vitro and in vivo) of designer drugs resulted in only minor differences concerning N-dealkylation and demethylenation. The toxicologically relevant demethylenation is catalyzed not only by the poly-

morphic CYP2D1/6, but also by the non-polymorphic main isoenzyme CYP3A as well as by CYP independent mechanisms. Therefore, possible hepatotoxic and neurotoxic effects due to demethylenation seem to be independent from genetic polymorphism.

The GC-MS procedures described here allow the precise and sensitive detection of the designer drugs and their metabolites in plasma and urine and of other amphetamine derivatives as well as of most of the other toxicologically relevant drugs (Maurer, 1992). In principle, these methods should be suitable for toxicological analysis also of future designer drugs, if their metabolism will be studied in a similar way.

References

Brunnenberg, M., Lindenblatt, H., Gouzoulis, M.E., Kovar, K.A., 1998. Quantitation of *N*-ethyl-3,4-methylenedioxyamphetamine and its major metabolites in human plasma by high-performance liquid chromatography and fluorescence detection. J. Chromatogr. B 719, 79–85.

Carvalho, F., Remiao, F., Amado, F., Domingues, P., Correia, A.J., Bastos, M.L., 1996. d-Amphetamine interaction with glutathione in freshly isolated rat hepatocytes. Chem. Res. Toxicol. 9, 1031–1036.

Chu, T., Kumagai, Y., Distefano, E.W., Cho, A.K., 1996. Disposition of methylenedioxymethamphetamine and three metabolites in the brains of different rat strains and their possible roles in acute serotonin depletion. Biochem. Pharmacol. 51, 789–796.

Cody, J.T., 1990. Detection of D,L-amphetamine, D,L-methamphetamine, and illicit amphetamine analogs using diagnostic products corporation's amphetamine and methamphetamine radioimmunoassay. J. Anal. Toxicol. 14, 321–324.

Colado, M.I., Williams, J.L., Green, A.R., 1995. The hyperthermic and neurotoxic effects of 'Ecstasy' (MDMA) and 3,4 methylenedioxyamphetamine (MDA) in the Dark Agouti (DA) rat, a model of the CYP2D6 poor metabolizer phenotype. Br. J. Pharmacol. 115, 1281–1289.

Coutts, R.T., Su, P., Baker, G.B., 1994. Involvement of CYP2D6, CYP3A4, and other cytochrome P-450 isozymes in *N*-dealkylation reactions. J. Pharmacol. Toxicol. Methods 31, 177–186.

de-la-Torre, R., Ortuno, J., Farre, M., Mas, M., Rosert, P.N., Menoyo, E., Segura, J., 1997. Pharmacokinetics of ecstasy (MDMA) in healthy subjects. Ther. Drug Monit. 19, 585.

Ensslin, H.K., Kovar, K.A., Maurer, H.H., 1996a. Toxicological detection of the designer drug 3,4-methylenedioxyethylamphetamine (MDE, 'Eve') and its metabolites in urine by gas chromatography-mass spectrometry and fluorescence polarization immunoassay. J. Chromatogr. B 683, 189–197.

Ensslin, H.K., Maurer, H.H., Gouzoulis, E., Hermle, L., Kovar, K.A., 1996b. Metabolism of racemic 3,4-methylenedioxyethylamphetamine in humans. Isolation, identification, quantification, and synthesis of urinary metabolites. Drug Metab. Dispos. 24, 813–820.

Gouzoulis, E., Steiger, A., Ensslin, M., Kovar, A., Hermle, L., 1992. Sleep EEG effects of 3,4-methylenedioxyethamphetamine (MDE; 'Eve') in healthy volunteers. Biol. Psychiatry 32, 1108–1117.

Hegadoren, K.M., Baker, G.B., Bourin, M., 1999. 3,4-Methylenedioxy analogues of amphetamine: defining the risks to humans. Neurosci. Biobehav. Rev. 23, 539–553.

Helmlin, H.J., Bracher, K., Bourquin, D., Vonlanthen, D., Brenneisen, R., 1996. Analysis of 3,4-methylenedioxymethamphetamine (MDMA) and its metabolites in plasma and urine by HPLC-DAD and GC-MS [see comments]. J. Anal. Toxicol. 20, 432–440.

Hiramatsu, M., Kumagai, Y., Unger, S.E., Cho, A.K., 1990. Metabolism of methylenedioxymethamphetamine: formation of dihydroxymethamphetamine and a quinone identified as its glutathione adduct. J. Pharmacol. Exp. Ther. 254, 521–527.

Jones, A.L., Simpson, K.J., 1999. Review article: mechanisms and management of hepatotoxicity in ecstasy (MDMA) and amphetamine intoxications. Aliment. Pharmacol. Ther. 13, 129–133.

Kintz, P., 1997. Excretion of MBDB and BDB in urine, saliva, and sweat following single oral administration. J. Anal. Toxicol. 21, 570–575.

Kraemer, T., Maurer, H.H., 1998. Determination of amphetamine, methamphetamine and amphetamine-derived designer drugs or medicaments in blood and urine [review]. J. Chromatogr. B 713, 163–187.

Kraemer, T., Vernaleken, I., Maurer, H.H., 1997. Studies on the metabolism and toxicological detection of the amphetamine-like anorectic mefenorex in human urine by gas chromatography-mass spectrometry and fluorescence polarization immunoassay. J. Chromatogr. B 702, 93–102.

Kraemer, T., Bickeboeller-Friedrich, J., Maurer, H.H., 2000. On the metabolism of the amphetamine-derived antispasmodic drug mebeverine-GC-MS studies on rat liver microsomes and on human urine. Drug Metab. Dispos. (in press).

Kumagai, Y., Lin, L.Y., Schmitz, D.A., Cho, A.K., 1991. Hydroxyl radical mediated demethylenation of (methylenedioxy)phenyl compounds. Chem. Res. Toxicol. 4, 330–334.

Kunsman, G.W., Manno, J.E., Cockerham, K.R., Manno, B.R., 1990. Application of the Syva EMIT and Abbott TDx amphetamine immunoassays to the detection of 3,4-methylene-dioxymethamphetamine (MDMA) and 3,4-methylene-dioxyethamphetamine (MDEA) in urine. J. Anal. Toxicol. 14, 149–153.

Lavigne, J.A., Helzlsouer, K.J., Huang, H.Y., Strickland, P.T., Bell, D.A., Selmin, O., Watson, M.A., Hoffman, S., Comstock, G.W., Yager, J.D., 1997. An association between the allele coding for a low activity variant of catechol-*O*-methyltransferase and the risk for breast cancer. Cancer Res. 57, 5493–5497.

Liebich, H.M., Forst, C., 1990. Basic profiles of organic acids in urine. J. Chromatogr. 525, 1–14.

Lin, L.Y., Di Stefano, E.W., Schmitz, D.A., Hsu, L., Ellis, S.W., Lennard, M.S., Tucker, G.T., Cho, A.K., 1997. Oxidation of methamphetamine and methylenedioxymethamphetamine by CYP2D6. Drug Metab. Dispos. 25, 1059–1064.

Maurer, H.H., 1992. Systematic toxicological analysis of drugs and their metabolites by gas chromatography-mass spectrometry [review]. J. Chromatogr. 580, 3–41.

Maurer, H.H., 1996. On the metabolism and the toxicological analysis of methylenedioxyphenylalkylamine designer drugs by gas chromatography-mass spectrometry. Ther. Drug Monit. 18, 465–470.

Maurer, H.H., 2000. Methods for GC-MS. In: Pfleger, K., Maurer, H.H., Weber, A. (Eds.), Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and their Metabolites, 3rd edition. Wiley-VCH, Weinheim.

Maurer, H.H., Arlt, J.W., Kraemer, T., Schmitt, C.J., Weber, A.A., 1997. Analytical development for low molecular weight xenobiotic compounds. Arch. Toxicol. 19 (Suppl.), 189–197.

Moeller, M.R., Hartung, M., 1997. Ecstasy and related substances — serum levels in impaired drivers [letter; comment]. J. Anal. Toxicol. 21, 591.

Nichols, D.E., 1986. Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class: entactogens. J. Psychoactive Drugs 18, 305–313.

Pfleger, K., Maurer, H.H., Weber, A., 2000. Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants and their Metabolites, 3rd edition. Hewlett Packard, Palo Alto (CA).

Tucker, G.T., Lennard, M.S., Ellis, S.W., Woods, H.F., Cho, A.K., Lin, L.Y., Hiratsuka, A., Schmitz, D.A., Chu, T.Y., 1994. The demethylenation of methylenedioxymethamphetamine ('ecstasy') by debrisoquine hydroxylase (CYP2D6). Biochem. Pharmacol. 47, 1151–1156.

Walubo, A., Seger, D., 1999. Fatal multi-organ failure after suicidal overdose with MDMA, 'ecstasy': case report and review of the literature. Hum. Exp. Toxicol. 18, 119–125.

Wu, D., Otton, S.V., Inaba, T., Kalow, W., Sellers, E.M., 1997. Interactions of amphetamine analogs with human liver CYP2D6. Biochem. Pharmacol. 53, 1605–1612.