STEREOCHEMICAL ANALYSIS OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE AND ITS MAIN METABOLITES IN HUMAN SAMPLES INCLUDING THE CATECHOL-TYPE METABOLITE (3,4-DIHYDROXYMETHAMPHETAMINE)

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ABSTRACT:

3,4-Methylenedioxymethamphetamine (MDMA; “ecstasy”) is a designer drug commonly misused in large segments of young populations. MDMA is usually formulated in tablets of its racemate (1:1 mixture of its enantiomers) in doses ranging from 50 to 200 mg. MDMA has an enantioselective metabolism, the (S)-enantiomer being metabolized faster than the (R)-enantiomer. Different pharmacologic properties have been attributed to each enantiomer. The carbon responsible for MDMA chirality is preserved along its metabolic disposition. An analytical method has been developed to determine MDMA enantiomers and those from its major metabolites, 3,4-methylenedioxymphetamine (MDA), 3,4-dihydroxymethamphetamine (HHMA), and 4-hydroxy-3-methoxymethamphetamine (HMMA). It has been applied to the analysis of plasma and urine samples from healthy recreational users of MDMA who participated voluntarily in a clinical trial and received 100 mg (R,S)-MDMA · HCl orally. (R)/(S) ratios both in plasma (0–48 h) and urine (0–72 h) for MDA and MDMA were >1 and <1, respectively. Ratios corresponding to HHMA and HMMA, close to unity, deviate from theoretical expectations and are most likely explained by the ability of MDMA to autoinhibit its own metabolism. The short elimination half-life of (S)-MDMA (4.8 h) is consistent with the subjective effects and psychomotor performance reported in subjects exposed to MDMA, whereas the much longer half-life of the (R)-enantiomer (14.8 h) correlates with mood and cognitive effects experienced on the next days after MDMA use.

3,4-Methylenedioxymethamphetamine (MDMA; “ecstasy”) is a synthetic amphetamine derivative, commonly misused recreationally due to its entactogenic properties (Nichols, 1986). MDMA is an indirect serotonin agonist and a potent inducer of dopamine and norepinephrine release (White et al., 1996). The pharmacology of this compound has been extensively described in an excellent review (Green et al., 2003). MDMA has four different substituents in the α-carbon of its chemical structure that define a chiral center, preserved in all methylenedioxy amphetamine derivatives. MDMA and related compounds are consumed as racemates, a 1:1 mixture of its enantiomers. Experimental studies have shown that whereas the (S)-enantiomers of methylenedioxy amphetamines are more potent dopaminergic agents, (R)-enantiomers show higher affinity to serotonin receptors and, then, higher potency as psychostimulants (Johnson et al., 1986). In mice and rhesus monkeys (Fantegrossi et al., 2002, 2003; Meyer et al., 2002a,b), the MDMA enantiomers showed heterogeneous pharmacologic effects, including elevation of body temperature, induction of locomotor activity, and reinforcing properties. In humans, a study performed with 3,4-methyleneoxyethylamphetamine (MDEA) enantiomers, administered separately, showed that the (S)-enantiomer was associated with entactogen effects, whereas the (R)-enantiomer was responsible for dysphoria and somatic complaints (Spitzer et al., 2001).

The MDMA major metabolic pathway includes its O-demethylation to 3,4-dihydroxymethamphetamine (HHMA) (a reaction mainly regulated by CYP2D6) followed by O-methylation to 4-hydroxy-3-methoxymethamphetamine (HMMA) (a reaction regulated by cate-

ABBREVIATIONS: MDMA, 3,4-methylenedioxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; MDEA, 3,4-methylenedioxymethylamphetamine; HHMA, 3,4-dihydroxymethamphetamine; HMMA, 4-hydroxy-3-methoxymethamphetamine; MDA-D₃, 1-[3,4-(methyleneoxy)phenyl]-2-(1,2-dideutero-3,3,3-trideuteromethylaminopropane); MDA-D₅, 1-[3,4-(methyleneoxy)phenyl]-2-(1,2,3,3,3-pentadeuteroaminopropane); MTP, (R)-(→)-α-methoxy-α-trifluoromethylphenylacetyl derivative; TMS, trimethylsilyl derivative; DHBA, 3,4-dihydroxybenzylamine; GC/MS, gas chromatography/mass spectrometry; CE, capillary electrophoresis; SCX, strong cation exchange; BEC, bond elut certify; IS, internal standard; COMT, catechol-O-methyltransferase; Cₘₜ₉₈, maximum concentration; tₘₜ₉₈, maximum time; AUC, area under the curve; AUC₀–₄₈ h, area under the curve from 0 to 48 h; AUC₀–₉₈ h, area under the curve from 0 to infinity; kₑₕ, elimination rate constant; tₑₕ, elimination half-time.
chol-methyltransferase). At a lower rate, MDMA is N-demethylated to 3,4-methylenedioxyamphetamine (MDA) (a reaction regulated by CYP2B6), which is further metabolized to the catechol intermediate (3,4-dihydroxyamphetamine) and finally O-methylated to 4-hydroxy-3-methoxyamphetamine (HMA) (Maurer et al., 2000; Segura et al., 2001; de la Torre et al., 2004). Similar metabolic pathways have been reported for other methylenedioxy derivatives of amphetamine, such as MDAE (Ensslin et al., 1996). In both cases, and along these metabolic pathways, the α-carbon responsible for stereochemical properties is not affected and all the metabolites are chiral compounds that may be presented as a mixture of their enantiomers. In addition to these major compounds, some other minor metabolites derived from the activity of MAO on the amine residue are also formed.

Studies in rats have shown an enantioselective metabolism in MDMA N-demethylation to MDA, the elimination half-life of the (R)-MDMA being shorter than that of the (S)-enantiomer, as opposed to MDA, in which the elimination half-life of the (S)-enantiomer is shorter than that of the (R)-enantiomer (Fitzgerald et al., 1990). These data are not consistent with enantioselectivity studies of MDMA in humans carried out in cases of MDMA intoxication (Moore et al., 1996; de Boer et al., 1997; Hensley and Cody, 1999) and in controlled settings (Lanz et al., 1997; Fallon et al., 1999; Pizarro et al., 2002b).

This is mainly due to the fact that enantioselectivity in rats is associated with N-demethylation, a metabolic pathway that accounts for only 8 to 9% of the MDMA concentration in human beings (de la Torre et al., 2000a). In humans, the enantioselective step is the O-demethylation, mainly regulated by CYP2D6.

The study of the enantioselective disposition of MDMA has been limited to the determination of MDMA and MDA. However, MDA is not involved in the O-demethylation pathway. Chiral analysis of the more polar metabolites HHMA and HMM would be more relevant because these metabolites are involved in the O-demethylation pathway. The unavailability of reference substances and the physico-chemical properties of HHMA and HMM render difficult the application of the same analytical strategy followed for MDMA and MDA.

Additionally, the fact that in animal models, MDMA-related neurotoxic effects are not produced after intracerebral injection of the drug and only develop after systemic administration further increases the interest in the assessment of the enantioselective disposition of MDMA metabolites (Green et al., 2003), as well as its relationship with mid-long-term neurotoxic effects. The present study was therefore designed to determine the concentrations of enantiomers of MDMA and its metabolites in plasma and urine samples from healthy volunteers. To this purpose a modification of a previously described GC/MS chiral method (Pizarro et al., 2003) was developed to be able to quantify simultaneously the enantiomers of MDMA, HHMA, HMM, HMA, and HMA. Pharmacokinetic data of all determined enantiomers are reported.

Materials and Methods

Plasma and Urine Samples. Biological samples were obtained from seven male healthy volunteers who were recreational users of MDMA who were given a single 100-mg oral dose of (R,S)-MDMA - HCl. All participants gave written informed consent to participate in the study, which was approved by the institutional review board and authorized by the Spanish Ministry of Health. MDMA was obtained from the Spanish Ministry of Health and MDMA soft gelatin capsules were prepared and supplied by the Department of Pharmacy of Hospital del Mar (Barcelona, Spain). Participants were phenotyped with dexamethasone for CYP2D6 enzyme activity and all were categorized as extensive metabolizers (Schmid et al., 1985).

Blood samples were obtained before drug administration (baseline) and at 20, 40, 60, and 90 min and at 2, 3, 4, 6, 8, 10, 24, 28, and 48 h after drug administration. The heparinized blood was centrifuged at 1100g for 10 min, and plasma was transferred to polypropylene tubes containing 30 μl of sodium bisulfite (250 mM) and stored at −20°C until analysis. Urine samples were collected before and after drug administration at 0 to 2, 2 to 6, 6 to 12, 12 to 24, 24 to 48, and 48 to 72 h time periods, acidified with HCl, and stored at −20°C until analysis.

Materials and Reagents. All chemicals (analysis or reagent grade) were obtained from Lipomed (Arlesheim, Switzerland), Radian Research (Lafayette, IN), Aldrich Chemical (Steinheim, Germany), Merck (Darmstadt, Germany), Scharlab Chemie (Barcelona, Spain), Sigma-Aldrich (St. Louis, MO), and Varian, Inc. (Palo Alto, CA). Pholedrine (4-hydroxyamphetamine) was generously given by the Deutsche Sporthochschule, Biochemistry Department (Cologne, Germany). Enantiomerically enriched standards for (S)-MDMA, (S)-HMM, and (S)-HHMA were synthesized in our laboratory (Pizarro et al., 2002a). Drug-free plasma was supplied by the blood bank of our hospital and drug-free urine was obtained from healthy volunteers.

Working Standards. Solutions of racemic MDMA, MDA, HHMA, and HMM (1 mg/ml) were prepared by dissolving 10 mg of each substance in 10 ml of methanol. Working solutions of 0.1, 1, and 10 μg/ml of each compound were prepared further diluting the corresponding starting solutions. MDMA and MDA pentadeuterated (D5) analogs were used as internal standards (ISs) for their analysis. Pholedrine (4-hydroxyamphetamine) was used as IS in HMM analyses and 3,4-dihydroxybenzylamine (DBHA) was the IS for HMA. A total of 20 μl of a mixture containing 5 μg/ml of both MDMA-D5 and pholedrine, and 0.5 μg/ml of MDA-D5, and 40 μl of a 1 μg/ml solution of DBHA were added to each sample. Enantiomerically enriched standard solutions of 100 μg/ml (S)-MDMA, (S)-HMM, and (S)-HHMA were used for enantiomeric identification. MDA and MDA-D5 enantiomers were identified as 1 and 2 according their elution order.

Calibration and Quality Control Samples. Calibration curves and quality control samples were prepared by adding appropriate volumes of working racemic solutions to test tubes, each containing 1 ml of drug-free plasma or urine. Quality control samples were prepared with solutions different from those used for the preparation of calibration curves. Final racemate concentrations in the calibration curves were 0.01, 0.05, 0.1, 0.15, and 0.2 μg/ml (plasma) and 0.2, 0.5, 1, 1.5, and 2 μg/ml (urine) for MDMA and MDA; 0.001, 0.005, 0.01, 0.015, and 0.02 μg/ml (plasma) and 0.02, 0.05, 0.1, 0.15, and 0.2 μg/ml (urine) for MDA; and 0.01, 0.025, 0.05, 0.1, and 0.15 μg/ml (plasma) and 0.1, 0.25, 0.5, 1, and 1.5 μg/ml (urine) for HHMA. Control plasma and urine samples containing appropriate racemic analytes at different concentrations were prepared in drug-free samples and kept frozen at −20°C in 1-ml aliquots. The concentrations of quality control samples were as follows: 0.012, 0.08, and 0.16 μg/ml (plasma) and 0.3, 0.8, and 1.6 μg/ml (urine) for MDMA and MDA; 0.032, 0.08, and 0.16 μg/ml (plasma) and 0.08, 0.16 μg/ml (urine) for MDMA-D5; 0.012, 0.04, and 0.12 μg/ml (plasma) and 0.12, 0.4, and 1.2 (urine) for HHMA.

Instrumentation. A gas chromatograph (6890 N; Agilent Technologies, Palo Alto, CA), equipped with a mass selective detector (5973 Network; Agilent Technologies), and an autosampler injector (7683 series; Agilent Technologies) were used. Gas chromatography conditions for chiral analysis included splitless injection mode (1 min); column, Phenomenex (Torrance, CA) cross-linked 5% phenyl/95% dimethylpolysiloxane (15 m × 0.25 mm × 0.25 μm film thickness); injection port temperature, 280°C; carrier gas, helium; flow rate, 1 ml/min; temperature program, from 150°C to 290°C at 20°C/min; initial time, 1 min; final time, 7 min; and injection volume, 3 μl. Mass spectrometry conditions were: selected ion monitoring mode; electron impact, 70 eV; ion source temperature, 280°C. Mass/charge (m/z) values selected for identification of analytes were as follows: 136, 264, 167 (quantification) for MDMA; 135, 260, 162 (quantification) for MDMA-N-MTP; 136, 264, 162 (quantification) for MDA-N-MTP; 274, 483, 236 (quantification) for HHMA-N-MTP-O-TMS; 226, 295 (quantification) for HHMA-N-MTP-O-bis-TMS; 136, 164, 278 (quantification) for MDMA-D5-N-MTP; 136, 264, 167 (quantification) for MDA-D5-N-MTP; 179, 274, 206 (quantification) for pholedrine-N-MTP-O-TMS; and 179, 499, 268 (quantification) for DHBA-N-MTP-O-bis-TMS.

Plasma and Urine Sample Preparation. Achiral sample preparation and quantification were performed as described previously (Pizarro et al., 2002b). Chiral quantification was performed by GC/MS after a chiral derivatization. Sample volumes were 500 μl for plasma and 100 μl for urine; final volumes of 1 ml were obtained by dilution with the corresponding blank. Extraction was
done as described by Segura et al. (2001). To reconstituted extracts (in 200 μl of methanol), 4 ml of ethyl acetate/NH₃ (2%) and an excess of Na₂SO₄ anhydrous were added. Tubes were stacked in an end-to-end mixer at 40 movements per min for 20 min and then centrifuged at 3500 rpm for 10 min. The solvent phase was transferred to a clean tube. This procedure was repeated twice and the final volume of solvent was taken to dryness under nitrogen steam at 40°C, 15 psi. Dried extracts were kept in a vacuum oven (40°C) for 30 min. Finally, derivatization was performed with minor modifications following a previously described procedure (Pizarro et al., 2003): the amine was derivatized using (R)-(−)-α-methoxy-α-trifluoromethylen acetyl chloride in ethyl acetate/hexane (50:50) that contained 0.015% triethylamine, and phenols were derivatized with 1,1,1,3,3,3-hexamethyldisilazane at 60°C for 1 h.

Validation Procedure. Validation of the GC/MS chiral method was performed according to a 4-day protocol. Linearity of the method was evaluated in the working ranges described previously. Calculations were performed with peak area ratios between compounds and internal standard. A weighted (1/C- concentration) least-squares regression analysis was used (SPSS computer software package, version 11.5 for Windows; SPSS Inc., Chicago, IL). By quantifying a quadruplicate of the lower concentration of the calibration curves, we estimated the limits of detection and quantification as 3 and 10 S.D. of the calculated concentrations, respectively. Intermediate precision was calculated with the relative S.D. of concentrations calculated for quality control samples, and the interassay accuracy was the relative error of the calculated concentrations.

Pharmacokinetic Data. The noncompartamental analysis (estimation of $C_{\text{max}}$, $t_{\text{max}}$, AUC₀→₄₈ h, and AUC₀→∞, $k_e$, and $t_{1/2}$) was performed using Microsoft Excel (PK Functions for Microsoft Excel, Joel I. Usansky, Ph.D., Atul Desai, M.S., and Diane Tang-Liu, Ph.D., Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA; http://www.boomer.org/pkin/soft.html). Pharmacokinetic parameters were estimated separately for each subject and the mean of these estimations (and the standard deviation) are presented in Table 1. Pharmacokinetic data were calculated for mixture of enantiomers obtained with the achiral procedure and also for separated enantiomers. Although the method allowed quantification of MDMA and all its metabolite enantiomers, pharmacokinetic data were only calculated for MDMA, MDA, HHMA, and HMMA.

Results

The pharmacokinetics of MDMA and its main metabolites (MDA, HHMA, and HMMA) after both achiral and chiral quantification in plasma and urine samples from seven healthy volunteers are reported. With regard to the achiral analysis of HHMA, plasma concentrations are not reported since there was not enough of a sample volume to perform GC/MS analysis of MDMA and metabolites except HHMA (Pizarro et al., 2002b). Chiral quantification was performed combining the extraction procedure described for HHMA (Segura et al., 2001) with the modified two-step chiral derivatization procedures developed for noncatechol MDMA main metabolites (Pizarro et al., 2002b). After extraction, samples were taken to dryness, and an intermediate treatment of the precipitated salts with a mixture of ethyl acetate that contained NH₃ (2%) was needed. A baseline enantiomer separation was obtained for all the studied compounds in a single run (see Fig. 1).

Validation results were as follows: limits of detection and quantification of MDMA, HMMA, and HHMA enantiomers in plasma were lower than 0.014 and 0.041 μg/ml, respectively, and lower than 0.0016 and 0.0047 μg/ml for MDA. For urine analysis, limits of detection and quantification were lower than 0.03 and 0.09 μg/ml, respectively, for MDMA, HMMA, and HHMA enantiomers, and lower than 0.002 and 0.007 μg/ml for the enantiomers of MDA. Intermediate precision and interassay accuracy for all quantified enantiomers in plasma were lower than 15.5% and 19.5%, respectively. For urine analysis, intermediate precision was lower than 19.4% and interassay accuracy lower than 15.6% in all cases.

To check the appropriate fitting between chiral and achiral approaches, results obtained using the achiral method were compared with data of chiral analysis [sum of the corresponding (R)- and (S)-enantiomers] by correlation analysis. For all compounds for which this analysis was performed (MDMA, MDA, and HMMA), the correlation coefficient was higher than $r = 0.92$ both in plasma and urine samples.

Calculated pharmacokinetic parameters for MDMA and its main metabolites as a mixture of its enantiomers and also as each enantiomer taken separately are presented in Table 1. The time course of plasma concentrations of MDMA, MDA, HMMA, and HHMA was monitored for 48 h (see Fig. 2). Urine collection was performed until 72 h post-ingestion, and recoveries of MDMA and metabolites are reported in Table 2. The variability observed is quite acceptable, taking into account enzymes involved in MDMA disposition in humans and not attributable to chemical assays that meet international requirements for bioanalytical method validation. Two enzymes crucial in the metabolic disposition of MDMA, CYP2D6 and COMT, are highly polymorphic in humans. Subjects participating in the study were all phenotypically extensive metabolizers for CYP2D6, whereas COMT phenotype was not examined. Because within the phenotypic label of extensive metabolizers we are including subjects 1) homozygous for the wild-type allele, 2) heterozygous for a mutant and a wild-type allele, and finally, 3) heterozygous/homozygous for less functional alleles *10, *17, and *41, a relatively high variability in

**TABLE 1**
Pharmacokinetic parameters

<table>
<thead>
<tr>
<th></th>
<th>$C_{\text{max}}$ ng/ml</th>
<th>$t_{\text{max}}$ h</th>
<th>AUC₀→₄₈ h</th>
<th>AUC₀→∞</th>
<th>$k_e$ h⁻¹</th>
<th>$t_{1/2}$ h</th>
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<tbody>
<tr>
<td><strong>Achiral Results</strong></td>
<td></td>
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<tr>
<td>MDMA</td>
<td>208.7 ± 17.1</td>
<td>1.6 ± 0.4</td>
<td>3108.5 ± 329.8</td>
<td>3223.4 ± 425.5</td>
<td>0.07 ± 0.03</td>
<td>11.8 ± 4.4</td>
</tr>
<tr>
<td>MDA</td>
<td>13.0 ± 2.3</td>
<td>6.6 ± 1.9</td>
<td>308.4 ± 73.1</td>
<td>361.8 ± 90.9</td>
<td>0.04 ± 0.01</td>
<td>17.7 ± 6.2</td>
</tr>
<tr>
<td>HHMA</td>
<td>163.8 ± 71.4</td>
<td>2.8 ± 0.8</td>
<td>2293.2 ± 881.5</td>
<td>2293.2 ± 881.5</td>
<td>0.07 ± 0.01</td>
<td>10.4 ± 2.4</td>
</tr>
<tr>
<td><strong>Chiral Results</strong></td>
<td></td>
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<tr>
<td>(R)-MDMA</td>
<td>116.7 ± 14.3</td>
<td>3.5 ± 2.1</td>
<td>2158.8 ± 297.5</td>
<td>2292.8 ± 490.8</td>
<td>0.06 ± 0.04</td>
<td>14.8 ± 9.2</td>
</tr>
<tr>
<td>(S)-MDMA</td>
<td>88.8 ± 17.0</td>
<td>1.9 ± 0.5</td>
<td>773.0 ± 83.3</td>
<td>773.0 ± 83.3</td>
<td>0.16 ± 0.07</td>
<td>4.8 ± 1.7</td>
</tr>
<tr>
<td>(R)-MDMA</td>
<td>1.7 ± 0.6</td>
<td>15.9 ± 10.5</td>
<td>52.6 ± 22.0</td>
<td>52.6 ± 22.0</td>
<td>0.05 ± 0.03</td>
<td>18.3 ± 10.6</td>
</tr>
<tr>
<td>(S)-MDMA</td>
<td>7.7 ± 3.9</td>
<td>6.7 ± 2.5</td>
<td>130.6 ± 43.4</td>
<td>145.2 ± 37.2</td>
<td>0.07 ± 0.04</td>
<td>11.9 ± 5.4</td>
</tr>
<tr>
<td>(R)-HHMA</td>
<td>38.9 ± 12.4</td>
<td>2.4 ± 1.9</td>
<td>653.5 ± 22.2</td>
<td>1104.3 ± 362.2</td>
<td>0.06 ± 0.05</td>
<td>42.6 ± 56.3</td>
</tr>
<tr>
<td>(S)-HHMA</td>
<td>90.9 ± 38.8</td>
<td>2.3 ± 1.8</td>
<td>999.2 ± 459.0</td>
<td>1099.7 ± 538.7</td>
<td>0.10 ± 0.04</td>
<td>7.9 ± 2.7</td>
</tr>
<tr>
<td>(R)-HHMA</td>
<td>65.0 ± 26.1</td>
<td>2.9 ± 0.7</td>
<td>868.9 ± 453.3</td>
<td>946.2 ± 501.3</td>
<td>0.06 ± 0.02</td>
<td>13.5 ± 4.1</td>
</tr>
<tr>
<td>(S)-HHMA</td>
<td>62.1 ± 21.6</td>
<td>2.6 ± 0.6</td>
<td>585.3 ± 216.6</td>
<td>588.2 ± 216.0</td>
<td>0.12 ± 0.02</td>
<td>5.9 ± 1.0</td>
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(1)-MDA, first eluted MDMA enantiomer; (2)-MDA, second eluted MDMA enantiomer.
drugs in which metabolism is regulated by CYP2D6 is expected. The picture is further compounded by the contribution of COMT. Enantiomeric ratios for MDA have been calculated assuming that peaks identified as 1 and 2 correspond to the (\(R\))- and (\(S\))-enantiomer (as for MDMA and other metabolites measured).

**Discussion**

Chiral analysis of plasma and urine samples was carried out by combining the extraction procedure developed for the high performance liquid chromatography analysis method for HHMA quantification (Segura et al., 2002) and derivatization steps developed for GC/MS determination of enantiomers of MDMA, MDA, HMMA, and HMA (Pizarro et al., 2003). Extraction and derivatization coupling was not achieved easily because chemical properties of extracted samples make it impossible for the target compounds to be derivatized. The presence of considerable amounts of HCl in the elution mixture was responsible for the formation of the corresponding amine chlorhydrate salts making amine reaction unfeasible. Various attempts to neutralize acid excess with different types of bases (organic and inorganic, and also a basic functionalized resin) were unsuccessful (data not shown). The evaporation of extracts to eliminate HCl before the first derivatization step gave rise to the precipitation of antioxidant (metabisulphite) and antichelant (EDTA) reagents required for the extraction. These reagents were insoluble in the mixture of solvents required for derivatization and compounds were still in their chlorhydrate form, preventing the correct reaction. All these findings resulted in the development of a procedure that could eliminate the salts before derivatization and was able to return all substances to their corresponding alkaline structures. Treatment of the precipitated salts with a mixture of ethyl acetate that contained \(\text{NH}_3\) (2%) allowed the recovery of the target compounds and made them ready for the derivatization procedure.

Pharmacokinetic data of the achiral analysis of MDMA and its main metabolites are similar to those in previous reports (de la Torre et al., 2000a). Nevertheless, to our knowledge this is the longest period of time (48 h) ever studied for these compounds and, also, the first time that kinetics of HHMA and HMMA enantiomers have been reported.

The enantiomeric ratios \((R)/ (S)\) for MDMA is around 2.9, and this result is very close to the ratio of 2.4 reported in a previous study (Fallon et al., 1999). The elimination half-life of the (\(R\))-enantiomer is 3 times higher than that of the (\(S\))-enantiomer (14.8 h versus 4.8 h) and quite similar to the elimination half-life calculated under achiral conditions (11.8 h). This result confirms that (\(R\))-MDMA is the major component of the calculated racemic MDMA elimination half-life. Moreover, the half-life of the (\(S\))-enantiomer fits very well with the kinetics of subjective effects, psychomotor performance, neuroendocrine-induced changes, and cardiovascular effects observed in humans after the use of MDMA in controlled studies (Mas et al., 1999; Cami et al., 2000). In contrast, the longer half-life calculated for the (\(R\))-enantiomer may explain mood and cognitive effects experienced by MDMA consumers on the next days after ingestion (Curran and Travill, 1997). Our current knowledge on the pharmacological activity of MDMA major metabolites HHMA and HMMA is very limited and
Fig. 2. Time course of MDMA analyzed by the GC/MS achiral method (A1) and its enantiomers analyzed by the GC/MS chiral method (A2), MDA analyzed by the GC/MS achiral method (B1) and its enantiomers analyzed by the GC/MS chiral method (B2), HHMA-separated enantiomers analyzed by the GC/MS chiral method (C), and HMMA analyzed by the GC/MS achiral method (D1) and its enantiomers analyzed by GC/MS chiral method (D2).
their impact on its pharmacology is unknown. Therefore, it would be very speculative to correlate their enantiomers with any pharmacological effect. Thioeucts derived from HHMA may play a role not only in the development of neurotoxicity but also in some behavioral effects. Their formation in vivo has been reported very recently (Monks et al., 2004), and further studies are guaranteed to investigate their involvement in the pharmacology of MDMA in humans, including chiral aspects of their biological activity and disposition.

MDA enantiomeric ratios <1 are opposed to those observed for MDMA. These results most probably reflect changes in the availability of MDMA enantiomers rather than to an enantioselectivity of this metabolic pathway. Urinary recoveries at 24 h and up to 72 h are close to unity, further confirming the lack of enantioselectivity of this pathway.

Enantiomeric ratios for HHMA are, as expected, just the reverse of those observed for MDMA. However, they are lower (around 0.65) than expected. This observation is most likely related to the nonlinearity of MDMA pharmacokinetics (de la Torre et al., 2000b) due to inhibition of the CYP2D6 enzyme (responsible for enantioselectivity) as a result of the formation of an enzyme-metabolite complex (Delaforgue et al., 1999). When CYP2D6 becomes inactivated, the enantioselectivity of the pathway is lost because other cytochrome P450 isoenzymes (CYP1A2, CYP3A4, and CYP2B6) that begin to be involved in the reaction (Kreth et al., 2000) probably lack this chiral selectivity. In fact, enantiomeric ratios observed for MDMA should be greater than those observed in the absence of this process of autoinhibition of the enantioselective pathway.

HHMA enantiomeric ratios should follow the same trend as those of HHMA: (R)/(S) ratio <1. In practice, however, they follow a trend close to that observed for MDMA [(R)/(S) ratio >1]. In a recent report in which enantiomeric ratios of MDMA and HHMA in urine were determined (Pizarro et al., 2002b), results confirmed MDMA enantioselective disposition, but HHMA (R)/(S) ratios were also close to 1 (first 24 h). Both results may be explained by the autoinhibition of CYP2D6 that, over time and after several metabolic steps (O-demethylation and O-methylation), make differences between enantiomers minimal. Studies in humans, in which racemic MDEA was administered and MDEA, MDA, and 4-hydroxy-3-methoxyamphetamine (the equivalent compound to HHMA in MDMA metabolism) and enantiomers were measured, found that 4-hydroxy-3-methoxyamphetamine ratios were lower than 1, although only the first 0 to 4 h in blood samples were assessed (Brunenberg and Kovar, 2001). This finding is similar to ratios <1 found in our study. The shift in the R/S ratios in the last phase of the kinetics and in urinary recoveries may indicate a certain degree of enantioselectivity of the catechol methyl transferase, an assumption that has to be further substantiated experimentally.

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References


TABLE 2

Urinary recovery

Values are µmol with percentage of dose recovered calculated in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>0–2 h</th>
<th>2–6 h</th>
<th>6–12 h</th>
<th>12–24 h</th>
<th>24–48 h</th>
<th>48–72 h</th>
<th>Overall (0–72 h)</th>
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<tr>
<td>Achiral Results</td>
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<tr>
<td>MDMA</td>
<td>9.0 ± 4.1</td>
<td>28.0 ± 11.0</td>
<td>22.9 ± 12.4</td>
<td>31.4 ± 16.6</td>
<td>20.0 ± 13.5</td>
<td>3.4 ± 5.2</td>
<td>114.2 ± 38.1</td>
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<td>MDA</td>
<td>0.2 ± 0.2</td>
<td>1.3 ± 0.5</td>
<td>1.6 ± 1.0</td>
<td>2.9 ± 1.4</td>
<td>2.8 ± 1.6</td>
<td>0.7 ± 1.0</td>
<td>9.5 ± 4.2</td>
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<td>HHMA</td>
<td>7.7 ± 4.0</td>
<td>16.2 ± 5.3</td>
<td>14.3 ± 11.2</td>
<td>14.0 ± 7.8</td>
<td>13.8 ± 7.2</td>
<td>8.4 ± 4.8</td>
<td>74.4 ± 32.7</td>
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<tr>
<td>(R)-MDMA</td>
<td>5.6 ± 2.6</td>
<td>18.9 ± 7.0</td>
<td>13.9 ± 6.1</td>
<td>24.8 ± 14.5</td>
<td>17.1 ± 11.8</td>
<td>3.5 ± 5.8</td>
<td>83.1 ± 28.9</td>
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<tr>
<td>(S)-MDMA</td>
<td>4.6 ± 2.3</td>
<td>12.8 ± 4.7</td>
<td>7.3 ± 2.7</td>
<td>7.2 ± 4.1</td>
<td>2.3 ± 1.3</td>
<td>2.3 ± 4.2</td>
<td>36.1 ± 10.5</td>
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<tr>
<td>(R)-HHMA</td>
<td>3.3 ± 2.1</td>
<td>6.3 ± 4.9</td>
<td>8.8 ± 9.0</td>
<td>10.2 ± 10.2</td>
<td>13.3 ± 7.4</td>
<td>3.8 ± 3.8</td>
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<td>(S)-HHMA</td>
<td>8.9 ± 3.2</td>
<td>14.6 ± 9.5</td>
<td>15.0 ± 9.1</td>
<td>12.3 ± 12.0</td>
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<td>(R)-HMMA</td>
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<td>8.8 ± 3.8</td>
<td>6.3 ± 4.3</td>
<td>9.9 ± 5.4</td>
<td>10.5 ± 7.7</td>
<td>3.5 ± 3.8</td>
<td>44.2 ± 23.8</td>
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<td>8.8 ± 3.8</td>
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<td>0.9 ± 0.9</td>
<td>0.2 ± 0.1</td>
<td>25.2 ± 9.9</td>
</tr>
</tbody>
</table>

(1)-MDA, first eluted MDA enantiomer; (2)-MDA, second eluted MDA enantiomer.


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