3,4-Dihydroxymethamphetamine (HHMA). A Major in Vivo 3,4-methylenedioxymethamphetamine (MDMA) Metabolite in Humans

ARTICLE *in* CHEMICAL RESEARCH IN TOXICOLOGY · OCTOBER 2001

Impact Factor: 3.53 · DOI: 10.1021/tx010051p · Source: PubMed

3,4-Dihydroxymethamphetamine (HHMA). A Major in Vivo 3,4-methylenedioxymethamphetamine (MDMA) Metabolite in Humans

Mireia Segura,[†] Jordi Ortuño,[†] Magí Farré,[†,‡] James Alexander McLure,[†,§] Mitona Pujadas,[†] Nieves Pizarro,[†] Amadeu Llebaria,[∥] Jesús Joglar,[∥] Pere Nolasc Roset,[†,‡] Jordi Segura,[†,⊥] and Rafael de la Torre*[,†,‡]

Pharmacology Research Unit, Institut Municipal d'Investigació Mèdica (IMIM), Universitat Autònoma de Barcelona, Department of Biological Organic Chemistry, Institut d'Investigacions Químiques i Ambientals (CID-CSIC), Universitat Pompeu Fabra, Barcelona, Spain

Received March 2, 2001

There is evidence that some heavy users of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) show signs of neurotoxicity (a cognitive dysfunction, a larger incidence of psychopathology). It has been postulated that the catechol intermediates of methylenedioxyamphetamines such as 3,4-dihydroxymethamphetamine (HHMA), a metabolite of MDMA, may play a role in their neurotoxicity by formation of thioether adducts. This study describes the first validated method for HHMA determination in plasma and urine by strong cation-exchange solid-phase extraction high-performance liquid chromatography/electrochemical detection (HPLC/ED) analysis. The method has been applied for the determination of HHMA in plasma and urine samples from a clinical study in healthy volunteers of MDMA and provides preliminary kinetic data on this metabolite. HHMA appeared to be a major MDMA metabolite with plasma concentrations as high as the parent compound. Thus, HHMA C_{max} (154.5 μg/L) and AUC_{0-24h}(1990.9 μg/L h) were similar to those obtained in previously published reports for MDMA (181.6 μg/L and 1465.9 μg/L h, respectively). The 24-h urinary recovery of HHMA accounted for 17.7% of the MDMA dose administered and increases the total 24 h recovery of MDMA and metabolites to 58% of the 100 mg dose administered. The determination of HHMA in plasma and urine samples is of interest in order to establish its relevance in MDMA metabolism and its possible contribution to MDMA neurotoxicity in humans. Its validation showed appropriate accuracy and precision for its use in pharmacokinetic studies.

Introduction

Studies in rats and primates have shown selective neurotoxic effects on the central serotonergic system after the administration of single and repeated doses of MDMA. This toxicity is secondary to long-lasting depletion of serotonine (5-HT)[1] and damage of 5-HT axon terminals with loss of 5-HT uptake sites (*1*−*3*). Clinical studies on MDMA long-term toxicity in humans are relatively scarce. There is mounting evidence that some MDMA users show a cognitive dysfunction (memory deficits) and an increased incidence of psychopathology (*4*). It is unknown whether some or all of these clinical signs of neurotoxicity disappear with abstinence.

In animal models, the direct injection of MDMA into the brain does not reproduce neurotoxic effects observed after peripheral administration (*5*−*7*). This fact suggests a possible implication of MDMA metabolites in the development of neurotoxicity. It has been postulated that the catechol intermediates of methylenedioxyamphetamines, such as 3,4-dihydroxymethamphetamine (HHMA), the major metabolite of MDMA in rat and human liver microsomal preparations may play a role in MDMA neurotoxicity by formation of adducts with glutathione and other thiol-containing compounds (*8*). The injection of these putative toxic agents into the brain in animal models has been able to reproduce some of the pharmacologic and toxicologic effects attributed to MDMA (*9*−*11*).

In humans, the presence of HHMA has only been demonstrated in in vitro studies and HHMA appears as a major metabolite of MDMA in microsomal preparations. This metabolic pathway is catalysed partially by P4502D6 (debrisoquine 4-hydroxylase) (*12*−*15*). This enzyme is expressed polymorphically in humans and about 5−9% of the Caucasian population is deficient for. In this way, poor and extensive metabolizers could have different susceptibilities to the acute and neurotoxic effects of MDMA. In humans, conjugated 4-hydroxy-3-methoxymethamphetamine (HMMA), a compound resulting from the O-methylation of HHMA, appears as a major in vivo metabolite both in plasma and urine samples. 3,4-Methylenedioxyamphetamine (MDA) and 4-hydroxy-3-

* To whom correspondence should be addressed. Phone: +34-93-2211009. Fax: +34-93-2213237. E-mail: rtorre@imim.es.
[†] Pharmacology Research Unit.
[‡] Universitat Autònoma de Barcelona.
[§] Present address: Flinders University of South Australia, Department of Clinical Pharmacology, Flinders Medical Centre, Bedford Park, SA 5042, Australia.
[∥] Department of Biological Organic Chemistry.
[⊥] Universitat Pompeu Fabra.

[1] Abbreviations: HHMA, 3,4-dihydroxymethamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; HMMA, 4-hydroxy-3-methoxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; HMA, 4-hydroxy-3-methoxyamphetamine; DHBA, 3,4-dihydroxybenzylamine; 5-HT, serotonine; AUC, area under the curve.

10.1021/tx010051p CCC: $20.00
Published on Web 08/02/2001

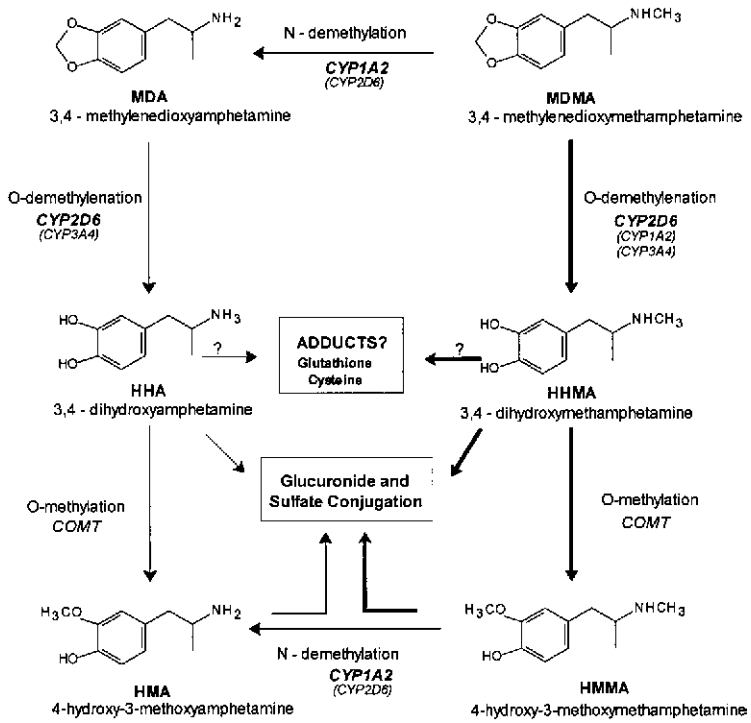

Figure 1. Pathways involved in the metabolic disposition of MDMA in humans (information obtained in part from ref *20*). Major pathways are outlined with thicker lines.

methoxyamphetamine (HMA) have been identified as minor metabolites (*16*−*19*). Metabolic pathways described for MDMA in humans as well as enzymes regulating each one are shown in Figure 1. HHMA, HMMA, and HMA are found both in plasma and in urine almost exclusively as conjugates. MDMA has also been found in urine as benzoic acid derivative conjugated with glycine after oxidation of its deaminated byproduct (*20*).

The determination of HHMA in plasma and urine samples is of interest in order to establish its relevance on MDMA metabolism and its possible contribution to MDMA neurotoxicity in humans. The detection of HHMA in biological fluids has been limited by its catecholamine-like physicochemical properties making extraction from biological samples difficult. Its qualitative detection in human urine has only been documented in some forensic studies and spot samples from MDMA users (*16*, *18*). Defining pharmacokinetic parameters of HHMA trough quantitative analysis is important for a more complete understanding of MDMA metabolism in vivo. Therefore, this study describes a validated method for HHMA determination in plasma and urine by strong cation-exchange solid-phase extraction and HPLC analysis with electrochemical detection (ED). This method has been applied to determine HHMA concentrations in plasma and urine samples from a clinical study in healthy volunteers. Preliminary kinetic data on HHMA are reported.

Material and Methods

Clinical Studies. Plasma and urine samples were obtained from four healthy male subjects (age 25.5 ± 5.0 years, weight 69.5 ± 4.4 kg, height 182.0 ± 9.7 cm, all were recreational users of MDMA and other stimulants and cannabis) who were given a single 100 mg oral dose of MDMA. The study protocol was approved by the Institutional Review Board and by the Spanish Ministry of Health. Subjects were phenotyped for P4502D6 activity using dextromethorphan. The dextromethorphan/dextrorphan metabolic ratio (*21*) was used to classify subjects as extensive or poor metabolizers. All participants were extensive metabolizers.

Blood samples were obtained through a catheter inserted into a peripheral vein before drug administration and at 20, 40, 60, and 90 min, and 2, 3, 4, 6, 8, 10, and 24 h after drug administration. Collection tubes were stored at 4 °C before its use. The heparinized blood was centrifuged 10 min at 1100*g* and 4 °C. Plasma was transferred to polypropylene tubes in the presence of 30 μL of 250 mM sodium bisulfite to prevent HHMA oxidation and stored at −20 °C until analysis. Urine samples were collected at four different time periods (predose or basal, 0−4, 4−8, 8−12, 12−24 h) after drug administration, acidified, and stored at −20 °C until assay.

Chemical and Reagents. HHMA (3,4-dihydroxymethamphetamine) racemate was synthesized in the laboratories of Centro de Investigación y Desarrollo of Centro Superior de Investigaciones Científicas (CID-CSIC, Barcelona, Spain). 3,4-Dihydroxybenzylamine (DHBA) was used as internal standard. DHBA and methylcatechol were purchased from Aldrich Chem Co. (Milwaukee, WI) and Sigma (St. Louis, MO), respectively.

Ultrapure water was obtained using a Milli-Q purification system (Millipore, Molsheim, France). HPLC-grade acetonitrile, methanol, hydrochloric acid, perchloric acid, ortho-phosphoric acid (85%), sodium acetate, potassium hydrogen phosphate, potassium dihydrogen phosphate, and sodium hydroxide were obtained from Merck (Darmstadt, Germany). EDTA and 1-octanesulfonic acid were supplied by Fluka (Buchs, Switzerland). Sodium bisulfite was from Sigma (St. Louis, MO). Bond Elut SCX (strong cation exchange) columns were purchased from Varian (Harbor City, CA) and mounted on a Vac Elut vacuum manifold (Supelco, Bellefonte, PA). Drug-free (blank) urine was purchased from Bio-Rad (Hercules, CA). Blank plasma was supplied by the blood bank of Hospital del Mar, Barcelona, Spain.

Synthesis of 3,4-Dihydroxymethamphetamine (HHMA). Details for the preparation of standards are described elsewhere (*22*). Briefly, HHMA was synthesized from commercially available 3,4-dibenzyloxybenzaldehyde upon condensation with nitroethane. Resultant amine reacted with ethyl chloroformate yielding a carbamate that was then reduced with lithium

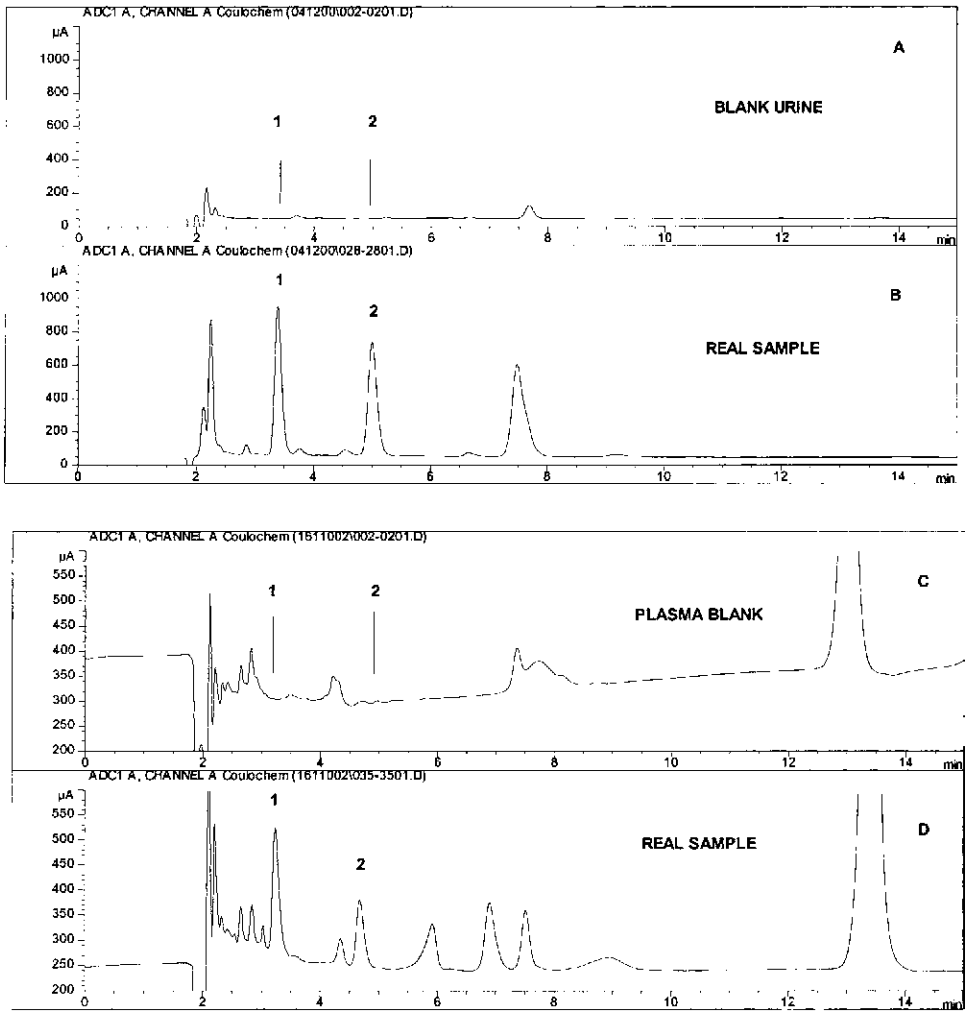

Figure 2. Chromatograms of blank samples (traces A and C for urine and plasma) and real samples (traces B and D for urine and plasma). Peak 1 corresponds to DHBA and peak 2 to HHMA.

aluminum hydride. Finally, the hydrochloride of the methylamine obtained was hydrogenated catalytically providing the desired HHMA.

Instrumentation. Chromatographic analysis was carried out using a 1050 liquid chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a model 5100A Coulochem electrochemical detector (ESA, Bedford, MA). Compounds were monitored through a model 5011 dual porous graphite electrode cell with potential electrodes 1 and 2 set at +0.05 and +0.35 V, respectively. A guard cell set at +0.40 V was positioned between the solvent delivery pump and the injector to condition the mobile phase (model 5021). The gain of the detector was set at 60 μA and the response-time was 4 s. The signal of detector 2 was connected to a HP Chemstation for LC (Rev. A.06.03[509]).

HPLC Methodology. Separation was carried out using a 4.6 mm × 250 mm Kromasil 100 C_4 column (Teknokroma, Barcelona, Spain). Mobile phase was 0.1 M sodium acetate (containing 0.1 mM 1-octanesulfonic acid and 4 mM EDTA) pH 3.1/acetonitrile (82:18, v/v). The mobile phase was filtered through a 0.45 μm Hewlett-Packard nylon filter before use. The flow-rate of the mobile phase was maintained at 1 mL/min. Peak areas of HHMA and DHBA were measured and the resultant ratios (HHMA versus DHBA) were used for all calculations.

Urine Sample Preparation. Urine was diluted 1:15 (v/v) with water. One milliliter of the diluted urine was pipetted into 15-mL screw-capped glass tubes and 25 μL of the ISTD solution (DHBA 10 μg/mL in methanol), 100 μL of 250 mM sodium bisulfite, and 50 μL of 250 mM EDTA were added. Acidic hydrolysis was performed by adding 200 μL of 6 M hydrochloric acid. Samples were incubated at 100 °C for 30 min and then cooled to room temperature. The pH was adjusted to 5.5−6 with 1 mL of 1 M potassium phosphate buffer (pH 6) and approximately 200 μL of NaOH 5 M. After hydrolysis, samples were processed applying a solid-phase extraction procedure using SCX columns (benzenesulfonic acid). The columns were activated and conditioned with 2 mL of methanol and 2 mL of 1 M potassium phosphate buffer (pH 6) avoiding columns to dry. Samples were forced to pass through columns at no more than 15 mm Hg of vacuum pressure. After application of the samples, columns were washed with 1 mL of water and 4 mL of methanol. Columns were dried applying vacuum (maximum 15 mm Hg) for 2 min. Analytes were then eluted with 2 mL of methanol/HCl (99:1) containing 3% v/v 250 mM EDTA and 3% v/v 250 mM sodium bisulfite. Eluates were evaporated to dryness under a stream of nitrogen in a water-bath at 40 °C. The dried extracts were reconstituted in 200 μL of mobile phase by vigorous vortex mixing and transferred into 200 μL injection vials. Volumes of 30 μL were injected into the chromatographic system.

Plasma Sample Preparation. One milliliter of plasma was pipetted into 15-mL screw-capped glass tubes and 75 μL of the ISTD solution (DHBA 1 μg/mL in methanol), 50 μL of methylcathecol 1 mg/mL, 200 μL of 250 mM sodium bisulfite, and 50 μL of 250 mM EDTA were added. Acidic hydrolysis was performed by adding 1 mL of 0.5 M hydrochloric acid. Samples were incubated 30 min at 100 °C and then cooled at room temperature. After hydrolysis, proteins in plasma samples were precipitated with 100 μL of perchloric acid and centrifuged 10 min at 2200*g*. The supernatant was adjusted to pH 5.5−6 with 1 mL of 1 M potassium phosphate buffer (pH 6) and approximately 130 μL of NaOH 10 M. Solid-phase extraction procedure

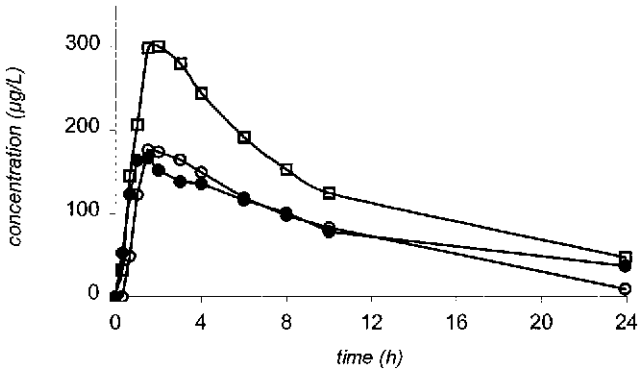

Figure 3. Plasma concentrations versus time curves of MDMA (○), HHMA (●), and HMMA (□).

Table 1. Pharmacokinetic Parameters of MDMA and Its Main Metabolites (n = 4, mean ± SD)

parameter	MDMA	HMMA	HHMA
C_{max} (ng/mL)	181.6 ± 24.5	307.1 ± 85.5	154.5 ± 76.6
T_{max} (h)	1.9 ± 0.2	2.0 ± 0.0	1.2 ± 0.3
K_a (h^{-1})[a]	2.5 ± 0.1	2.1± 0.2	5.3 ± 2.9
K_e (h^{-1})	0.099 ± 0.015	0.084 ± 0.013	0.063 ± 0.026
$T_{1/2}$ (h)	7.1± 1.3	8.3 ± 1.3	13.4 ± 8.1
AUC_{0-24} (ng/mL h)	1465.9 ± 705.1	3190.9 ± 714.5	1990.94 ± 647.1

[a] For HMMA and HHMA K_a stands for formation rate constant.

using SCX columns was carried out according to the procedure described above for urine samples. The dried extracts were reconstituted in 200 μL of mobile phase by vigorous vortex mixing and transferred into 200 μL injection vials. Volumes of 30 μL were injected into the chromatographic system.

The method was tested following a validation protocol before measuring HHMA in the human volunteers samples.

The presence of MDMA, MDA, HMMA, and HMA in urine and plasma was determined by using a standardized previously published method (*17*) involving solid-phase extraction and GC/MS detection.

Data Analysis. Areas under plasma concentrations of MDMA and metabolites vs time curves (AUC_{0-24h}) were determined by the linear trapezoidal rule. Other pharmacokinetic calculations were performed using PKCALC software (*23*).

Results

The extraction of HHMA from urine and plasma by using a SCX column followed by a HPLC/ED analysis provided clean chromatograms free of background interferences at the retention time of HHMA and DHBA (see Figure 2). Calibration curves were linear over the 50−1000 μg/L and 10−300 μg/L concentration ranges for urine and plasma samples, respectively. HHMA estimated limits of quantification (μg/L) and of detection were 31.8 and 10.5 μg/L for urine and 8.9 and 5.7 μg/L for plasma. Recoveries of HHMA and DHBA were around 50%. Interday precision (expressed as coefficient of variation for specific added target concentrations) and accuracy (expressed as percentage error of concentration found as compared with target added concentration) were lower than 6.8 and 13.2% for urine and below 8.9 and 13.8% for plasma, respectively.

All urine and plasma samples were processed following the method here presented for HHMA analysis. Plasma concentrations over time curves and calculated pharmacokinetic parameters for MDMA, HMMA, and HHMA in the four volunteers studied are shown in Figure 3 and Table 1. Table 2 summarizes MDMA, HMMA, MDA, HMA, and HHMA urinary recoveries obtained at different time collection periods between 0 and 24 h after administration of 100 mg of MDMA for the four volunteers studied. The urinary recovery of HHMA in 24 h accounted for a 17.7% of the 100 mg MDMA dose and increased the total 24 h recovery of MDMA and metabolites to 58% of the dose administered.

Discussion

This study presents a novel description of the pharmacokinetics in plasma and urinary recovery of HHMA after the administration of MDMA in a controlled setting. The analytical method developed for the extraction of HHMA from plasma and urine has shown sufficient specificity to remove the interfering and coeluting compounds. The sensitivity achieved is adequate for HHMA concentrations encountered in biological fluids. The method has been validated and shows adequate accuracy and precision for its use in pharmacokinetic studies. The selection of strong cation exchange columns for sample extraction has proven to be suitable in front of other alternatives, such as alumina or the formation of boronates described for the analysis of catecholamines (*24*−*26*). The extraction procedure when coupled to an analytical method of HPLC with electrochemical detection provides a highly sensitive and selective assay procedure for HHMA. When samples are adjusted to pH 5.5−6, adequate adsorption of HHMA and DHBA onto SCX columns was obtained. In these conditions, columns can be washed with 1 mL of water and 4 mL of methanol, so that the polar and nonpolar components are washed off without affecting recoveries. Eluting columns with MeOH:HCl (99:1) containing 3% v/v EDTA 250 mM and 3% v/v sodium bisulfite 250 mM gives acceptable recoveries of HHMA. The addition of antioxidants during the whole analytical procedure is crucial for preventing HHMA from oxidation and degradation.

To our knowledge, this is the first method designed and validated for the quantification of HHMA in urine and plasma. HHMA appears to be a major MDMA metabolite. Plasma and urinary concentrations of HHMA are equivalent to those measured for MDMA. Pharmacokinetics and excretion parameters calculated in this study for MDMA and its metabolites are in agreement with previously reported data (*27*, *28*). For HHMA the C_{max} (154.5 μg/L)

Table 2. Urinary Excretion of MDMA and Its Main Metabolites (n = 4)

	urinary collection period[a]				
compd	0−4 h	4−8 h	8−12 h	12−24 h	0−24 h
MDMA	22.7 ± 9.9	15.5 ± 6.1	8.8 ± 2.9	30.8 ± 15.2	77.8 ± 25.4 (15.0%)[b]
HMMA	35.4 ± 9.2	30.3 ± 9.5	11.7 ± 5.6	40.0 ± 28.1	117.4 ± 40.0 (22.7%)
HHMA	30.9 ± 10.2	21.4 ± 9.7	12.8 ± 6.2	26.6 ± 9.39	91.8 ± 23.8 (17.7%)
MDA	1.4 ± 0.5	1.1 ± 0.2	0.8 ± 0.3	4.6 ± 2.8	7.8 ± 3.0 (1.5%)
HMA	1.5 ± 0.4	1.4 ± 0.3	0.9 ± 0.5	3.2 ± 1.0	7.0 ± 1.6 (1.35%)

[a] Mean ± STD, values given in micromoles. [b] Recoveries (0−24 h) as a percentage of the administered dose.

and AUC_{0-24h} (1990.9 μg/L h) were similar to those obtained for MDMA 181.6 μg/L and 1465.9 μg/L h, respectively. Even though if differences in T_{max} are not statistically significant, the fact that HHMA precedes MDMA T_{max} (see Table 1) suggests a relevant first-pass hepatic metabolism of MDMA. After processing the urine samples, HHMA appears as one of the major metabolites and contributes around 18% of the total MDMA dose recovery.

HHMA has not been detected in its free form in plasma or in urine under the analytical conditions outlined. A first attempt of hydrolyzing samples with a standard enzymatic procedure (*17*, *18*) using β-glucuronidase/aryl sulfatase was unsuccessful for the recovery of HHMA from biological fluids. An acidic hydrolysis procedure from urine and plasma was required in order to release HHMA from its conjugated forms. Acidic hydrolysis conditions for plasma analysis of HHMA should be less aggressive than for urine because of the presence of proteins. Moreover, it was necessary after hydrolysis to precipitate plasma and submit the supernatant to the solid-phase extraction in order to avoid the presence of proteins. At this step, the addition of a high amount of 3-methylcathecol was necessary to prevent HHMA adsorption onto the protein precipitate. The lack of functional groups in methylcatechol chemical structure that could be ionized and interact with the resin (under the present analytical conditions) facilitates removal of methylcatechol during the solid phase extraction procedure. Findings made from sample enzymatic hydrolysis allow to discard a major presence of glucuronide conjugates of HHMA in biological fluids as in the same conditions HMMA is easily hydrolyzed. The presence of HHMA sulfate in urine has been described previously (*20*). Sulfate conjugation is a typical biotransformation pathway of catechol containing compounds. Surprisingly aryl-sulfatase activity already present in the enzyme preparation used in the hydrolysis procedure was unable to hydrolyze sulfate conjugates. Experiments have been repeated several times under different conditions and consistent results were obtained. However, under acidic conditions it was possible to hydrolyze HHMA conjugates. It may be assumed that sulfate conjugates were hydrolyzed more efficiently. Alternatively and complementarily to these observations, the formation of other type of conjugates, such as glutathione and thiol adducts that have been postulated as putative neurotoxic species of MDMA (*9*−*11*), cannot be excluded. HHMA has already shown a high reactivity with proteins. In vitro experiments (*14*) already suggest that O-demethylenation of MDMA (giving rise to HHMA) participates in the formation of a stable inhibitory P450 (P4502D6)−metabolite complex that may be involved in the nonlinear pharmacokinetics of MDMA observed in vivo in humans (*19*). Hence, in addition to COMT and isoforms of sulfotransferases, other enzymes or chemical reactions may be involved in HHMA disposition.

In summary, we have shown that HHMA is a relevant metabolic pathway in MDMA disposition in humans. HHMA has a high reactivity and is very unstable due to its chemical structure. As might be expected, HHMA is only present in conjugated form(s) in biological fluids. In addition to sulfate conjugation, the present findings do not allow to exclude the contribution of HHMA in MDMA neurotoxicity through the formation of thioether adducts.

Acknowledgment. This investigation was supported by FIS 97/1198 and 98/0081, CIRIT 99-SGR-242 and 99-SGR-00187, and Plan Nacional sobre Drogas (PNSD Madrid, Spain). We thank M. Pulido for editing the manuscript and editorial assistance.

References

(1) Schmidt, C. J., Levin, J. A., and Loumberg, W. (1987) In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. *Biochem. Pharmacol.* **36**, 747−755.

(2) Burdkin, J., Malyala, A., and Nash, J. F. (1993) Effect of acute monoamine depletion on 3,4-methylenedioxymethamphetamine-induced neurotoxicity. *Pharmacol. Biochem. Behav.* **45**, 647−653.

(3) Ricaurte, G. A., DeLanney, L. E., Wiener, S. G., Irwin, I., and Langston, J. W. (1988) 5-hydroxy-indoleaceticacid in cerebrospinal fluid reflects serotoninergic damage induced by 3,4-methylenedioxymethamphetamine in CNS of nonhuman primates. *Brain. Res.* **474**, 359−363.

(4) McCann, U. D., Eligulaschvili, V., and Ricaurte, G. A. (2000) (±) 3,4-Methylendioxymethamphetamine ('Ecstasy')-induced serotonin neurotoxicity: Clinical Studies. *Neuropsychobiology* **42**, 11−16.

(5) Gollamudi, R., Ali, S. F., Lipe, G., Newport, G., Webb, P., Lopez, M., Leakey, J. E., Kolta, M., and Slikker, W. (1989) Influence of inducers and inhibitors on the metabolism in vitro and neurochemical effects in vivo of MDMA. *Neurotoxicology* **10**, 455−466.

(6) McCann, U. D., and Ricaurte, G. A. (1991) Major metabolites of (±) 3,4-methylenedioxyamphetamine (MDA) do not mediate its toxic effects on brain serotonin neurons. *Brain Res.* **545**, 279−282.

(7) Chu, T., Kumagai, Y., DiStefano, E. W., and 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.* **22**, 789−796.

(8) Hiramatsu, M., Kumagai, Y., Unger, S. E., and 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.

(9) Bai, F., Lau, S. S., and Monks, R. J. (1999) Glutathione and N-acetylcysteine conjugates of α-methyldopamine produce serotonergic neurotoxicity: possible role in methylenedioxyamphetamine-mediated. *Chem. Res. Toxicol.* **12**, 1150−1157.

(10) Miller, R. T., Lau, S. S., and Monks, T. J. (1995) Metabolism of 5-(glutathion-S-yl)-α-methyldopamine following intracerebroventricular administration to male Sprague−Dawley rats. *Chem. Res. Toxicol.* **8**, 634−641.

(11) Miller, R. T., Lau, S. S., and Monks, T. J. (1996) Effects of intracerebroventricular administration of 5-(glutathion-S-yl)-α-methyldopamine on brain dopamine, serotonin, and norepinephrine concentrations in male Sprague−Dawley rats. *Chem. Res. Toxicol.* **9**, 457−465.

(12) Tucker, G. T., Lennard, M. S., Ellis, S. W., Woods, H. F., Cho, A. K., Lin, L. Y, Hiratsuka, A., Schmitz, D. A., and Chu, T. Y. Y. (1994) The demethylenation of methylenedioxymethamphetamine ("ecstasy") by desbrisoquine hydroxylase (CYP2D6). *Biochem. Pharmacol.* **47**, 1151−1156.

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

(14) Delaforge, M., Jaouen, M., and Bouille, G. (1999) Inhibitory metabolite complex formation of methylenedioxymethamphetamine with rat and human cytochrome P450. Particular involvement of CYP 2D. *Environ. Toxicol. Pharmacol.* **7**, 153−158.

(15) Kreth, K. L., Kovar, K. A., Schwab, M., and Zangert, U. M. (2000) Identification of human cytochromes P450 involved in the oxidative metabolism of "ecstasy" related designer drugs. *Biochem. Pharmacol.* **59**, 1563−1571.

(16) Lim, H. K., and Foltz, R. L. (1989) Identification of metabolites of 3,4-(methylendioxy)methamphetamine in human urine. *Chem. Res. Toxicol.* **2**, 142−143.

(17) Ortuño, J., Pizarro, N., Mas, M., Brenneisen, R., Farré, M., Camí, J., Segura, J., and de la Torre, R. (1999) Quantification of MDMA and its main metabolites in plasma in urine by gas chromatography and nitrogen phosphorous detection. *J. Chromatogr.* **723**, 221−232.

(18) Helmin, H. J., Bracher, K., Bourquin, D., Vonlanthen, D., and Brenneisen, R. (1996) Analysis of 3,4-methylenedioxymethamphetamine (MDMA) and its metabolites in plasma and urine by HPLC-DAD and GC-MS. *J. Anal. Toxicol.* **20**, 432−440.

(19) De la Torre, R., Farré, M., Roset, P. N., Hernández López, C., Mas, M., Ortuño, J., Menoyo, E., Pizarro, N., Segura, J., and Camí, J. (2000) Pharmacology of MDMA in Humans. *Ann. N. Y. Acad. Sci.* **914**, 225−238.

(20) Maurer, H. H., Bickeboeller-Friedrich, J., Kraemer, T., and Peters, F. T. (2000) Toxicokinetics and analytical toxicology of amphetamine-derived designer drugs ('Ecstasy'). *Toxicol. Lett.* **112**−**113**, 133−142.

(21) Schmidt, B., Bircher, J., Preisig, R., and Küpfer, A. (1985) Polymorphic dextromethorphan metabolism: Co-segregation of oxidative o-demethylation with debrisoquin hydroxylation. *Clin. Pharmacol. Ther.* **38**, 618−624.

(22) Pizarro, N., de la Torre, R., Farré, M., Segura, J., Llebaria, A., and Joglar, J. (2001) Synthesis and Capillary Electrophoresis Analysis of enantiomerically enriched reference standards of MDMA and its main metabolites. *Bioorg. Med. Chem.* (accepted for publication).

(23) Shumaker, R. C. (1986) A basic interactive computer program for statistical and pharmacokinetic analysis of data. *Drug Metab. Rev.* **17**, 331−348.

(24) Macdonald, I. A., and Lake, D. M. (1995) An improved technique for extracting cathecolamines from body fluids. *J. Neurosci. Methods* **13**, 239−248.

(25) Huang, T., Wall, J., and Kabra, P. (1988) Improved solid-phase extraction and liquid chromatography with electrochemical detection of urinary catecholamines and 5-S-L-cysteinyl-L-dopa, *J. Chromatogr.* **452**, 409−418.

(26) Frayn, K. N., and Maycock, P. F. (1983) Sample preparation with ion-exchange resin before liquid-chromatographic determination of plasma catecholamines. *Clin. Chem.* **29**, 1426−1428.

(27) Mas, M., Farré, M., De la Torre, R., Roset, P. N., Ortuño, J., Segura, J., and Camí, J. (1999) Cardiovascular and neuroendocrine effects, and pharmacokinetics of MDMA in humans. *J. Pharmacol. Exp. Ther.* **290**, 136−145.

(28) De la Torre, R., Farré, M., Ortuño, J., Mas, M., Brenneisen, R., Segura, J., and Camí, J. (2000) Non-linear pharmacokinetics of MDMA ("ecstasy") in humans. *Br. J. Clin. Pharmacol.* **49**, 104−109.

TX010051P