Analysis of 3,4-Methylenedioxymethamphetamine (MDMA) and its Metabolites in Plasma and Urine by HPLC-DAD and GC-MS

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Abstract

In Europe, the compound 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy, Adam), in addition to cannabis, is the most abused illicit drug at all-night “techno” parties. Methods for the determination of MDMA and its metabolites, 4-hydroxy-3-methoxymethamphetamine (HMMA), 3,4-dihydroxy-methamphetamine (HHMA), 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxyamphetamine (HMA), and 3,4-dihydroxyamphetamine (HHA), in biological fluids were established. Plasma and urine samples were collected from two patients in a controlled clinical study over periods of 9 and 22 h, respectively. MDMA and MDA were determined in plasma and urine by reversed-phase high-performance liquid chromatography (HPLC-DAD) after solid-phase extraction on cation-exchange columns. Acidic or enzymatic hydrolysis was necessary to detect HMMA, HMA, HHMA, and HHA, which are mainly excreted as glucuronides. Gas chromatography-mass spectrometry (GC-MS) was used for confirmation. Sample extraction and on-disc derivatization with heptafluorobutyric anhydride (HFBA) were performed on Toxi-Lab SPEC solid-phase extraction concentrators. After administration of a single oral dose of 1.5 mg/kg body weight MDMA, peak plasma levels of 331 ng/mL MDMA and 15 ng/mL MDA were measured after 2 h and 6.3 h, respectively. Peak concentrations of 28.1 µg/mL MDMA in urine appeared after 21.5 h. Up to 2.3 µg/mL MDA, 35.1 µg/mL HMMA, and 2.1 µg/mL HMA were measured within 16–21.5 h. Conjugated HMMA and HHMA are the main urinary metabolites of MDMA.

Introduction

The recreational use of 3,4-methylenedioxymethylamphetamine (MDMA; Figure 1) dramatically increased in the United States and Europe by the mid to late 1980s. In Britain, more than 500,000 people are said to use the drug each week (1). Since 1986, MDMA has been an internationally controlled sub-

stance. It shows distinct psychotropic effects, which are different from those of the structurally related stimulant amphetamine or hallucinogenic phenylalkylamines (e.g., 3,4-methylenedioxymethamphetamine [MDA] and 3,4,5-trimethoxyphenethylamine [mescaline]) (2–6). MDMA is reported to possess antidepressant and anxiolytic properties and to evoke a controllable emotional experience with relaxation, peaceful feelings, increased empathy, and a drop in fear responses that are mostly without distortion of sensory perception and thought and without marked stimulation (5–10). A novel psychoactive substance class, which is named the “entactogens” (greco-latinic term: “produce (gen) an inner (en) touching (tact)”) was recently proposed for MDMA (3,5–7,11). The mechanism of action of MDMA is characterized by a high affinity at serotonin uptake sites, whereas at norepinephrine and dopamine uptake sites of central neurons the affinity is lower (12). Reviews of the pharmacology of MDMA have appeared in the literature (13,14).

The acute and chronic toxicity of MDMA is controversial. In animal experiments MDMA was shown to exert dose- and species-dependent neurotoxic effects on central serotonergic neurons in terms of degeneration of axon terminals (12,15–22). However, the relevance of these findings for humans is still unclear (23–25). There are no reports of individuals or drug-addicts who take frequent or large doses of MDMA for extended periods of time. The reason for this is that the positive effects of the drug seem to diminish and the negative effects seem to increase with time (5,8). Well-documented deaths related to the use of MDMA are exceptionally rare and are mostly the result of underlying cardiac diseases, cardiovascular complications, and hyperthermia (26–28). A recent postmortem study on seven men (age range 20–25) who died after the consumption of MDMA (“Ecstasy”) or 3,4-methylenedioxyethylamphetamine (MDEA, “Eve”) or both showed significant changes in the liver, heart, and brain (1). As the possible mechanism of damage, the authors suggested hyperthermia, direct toxic effects on organs, individual susceptibility to ring substituted amphetamines, abnormal metabolism, and water intoxication.

On the other hand, there is also an ongoing discussion about the therapeutic potency of MDMA. For example, MDMA is used...
in medicine as an adjunct in psychotherapy (5,7,9,29,30), where it can facilitate the therapeutic communication and increase patient insight and self-esteem (26), and as an analgesic for patients with terminal cancer (31).

High-performance liquid chromatography (HPLC) with electrochemical (32,33), UV (34), or diode array detection (DAD) (35) and gas chromatography with nitrogen-phosphorus detection (GC-NPD) (36) or mass spectrometry (GC-MS, GC-MS-MS, GC-MS-MS-MS) (32,37-44) have been used for the detection of MDMA in biological specimens.

Several animal studies (rat and mouse) have shown that MDMA is metabolized by N-demethylation, O-dealkylation, deamination, O-methylation, and O-conjugation to glucuronides and/or sulfates. Metabolites detected in urine are 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxyamphetamine (HMA), 3-hydroxy-4-methoxyamphetamine, 4-hydroxy-3-methoxyamphetamine (HMA), 3-hydroxy-4-methoxyamphetamine, 4-hydroxy-3-methoxyphenylacetone, 3,4-methylenedioxyphenylacetone, and 3,4-dihydroxyphenylacetone (32,36,37,41,42). Most of these metabolites are also present in the blood. In addition, 6-hydroxy-3,4-methylenedioxymethamphetamine and 6-hydroxy-3,4-methylenedioxymethamphetamine have been found in rat plasma and brain (39), whereas 2,4,5-trihydroxyamphetamine and 2,4,5-trihydroxyamphetamine have been identified in rat liver (40).

Very few MDMA studies have been performed in man. In a postmortem urine specimen (with unknown dosage), HMMA was detected as the main urinary metabolite of MDMA. Other metabolites found were MDA, HMA, 3,4-dihydroxymethamphetamine (HHMA), 3-hydroxy-4-methoxymethamphetamine, and 3,4-methylenedioxyphenylacetone (38). In a single patient, controlled study, unchanged MDMA was the major urinary excretion product, and MDA was the only metabolite identified after the ingestion of 50 mg MDMA. Of the dosage given, 72% was recovered from the urine within 72 h. The MDMA plasma level peaked at about 106 ng/mL 2 h after administration and declined to about 5 ng/mL by 24 h (43).

It was the aim of the present study to establish the analytical methodology for monitoring MDMA and its metabolites in body fluids and to investigate the pharmacokinetic behavior of MDMA in man under controlled conditions.
Experimental

Standards and chemicals
(±)-3,4-Methylenedioxyamphetamine hydrochloride (MDMA) was purchased from Eprova (Schaffhausen, Switzerland); (±)-3,4-Methylenedioxyamphetamine hydrochloride (MDA) and (+)-methamphetamine hydrochloride (MA) were obtained from Sigma (Buchs, Switzerland). (±)-4-Hydroxy-3-methoxymethylamphetamine (HMMA) was synthesized by reaction of methyamine hydrochloride, sodium cyanoborohydride, and 4-hydroxy-3-methoxyphenylacetone, which was prepared by the oxidation of 4-hydroxy-3-methoxyphenyl-2-nitropropane (45). (±)-4-Hydroxy-3-methoxymethylamphetamine (HMA) was synthesized by the reduction of 4-hydroxy-3-methoxyphenyl-2-nitropropane, which was prepared by reaction of 4-hydroxy-3-methoxybenzaldehyde with nitroethane (45). (±)-3,4-Dihydroxymethylamphetamine (HHMA) was synthesized by demethylation of 3,4-dimethoxyamphetamine with borotribromide (46). 3,4-Dimethoxymethamphetamine was prepared by reacting 3,4-dimethoxyphenylacetone, methylamine hydrochloride, and sodium cyanoborohydride (45). (±)-3,4-Dihydroxymethylamphetamine (HMA) was synthesized by reaction of 3,4-dibenzoylbenzaldehyde with nitroethane to yield 3,4-dibenzoylphenyl-2-nitropropane, followed by reduction to 3,4-dibenzylamphetamine and debenzylation to HHA (47). All synthesized standards were prepared as hydrochlorides, and synthesized by demethylation of 3,4-dimethoxyamphetamine with borotribromide (46). 3,4-Dimethoxymethylamphetamine was prepared by reacting 3,4-dimethoxyphenylacetone, methylamine hydrochloride, and sodium cyanoborohydride (45). All synthesized standards were prepared as hydrochlorides, and the structures were confirmed by MS, infrared spectroscopy (IR), proton (1H-), and carbon nuclear magnetic resonance spectroscopy (13C-NMR). β-Glucuronidase/sulfatase type II was purchased from Sigma. All other chemicals and reagents were of HPLC or analytical grade and were purchased from Merck (Zurich, Switzerland) or Fluka (Buchs, Switzerland).

Sample collection and preparation

Plasma and urine samples. The plasma and urine specimens were obtained from a female (40-year-old subject A) and a male patient (23-year-old subject B) treated with MDMA by a psychiatrist (J.S., one of the authors) from The Swiss Association for Psycholytic Therapy (SAPT) under the authorization of the Swiss Federal Office for Public Health. Blood samples (10–20 mL each) were obtained through an indwelling catheter at 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 170, 190, 220, 250, 310, 380, 450, and 530 min after the oral administration of 1.5 mg MDMA (calculated as base) per kg body-weight. The heparinized blood samples were centrifuged for 10 min at 2000 rpm; the plasma was transferred to polypropylene tubes and stored at −20°C.

Urine samples from subject A were collected 0, 1.25, 1.5, 3.75, 4.25, 5, 5.5, 6.4, 8.4, 10.5, 12.5, 16, and 21.5 h after the administration of MDMA. The urine samples were stored at −20°C.

Preparation of plasma samples for HPLC. The solid-phase extraction (SPE) and cleanup of plasma samples were performed on an automated SPE system (ASPEC, Gilson Medical Electronics France S.A., Synmedic, Zurich, Switzerland). Frozen plasma samples were warmed up to room temperature in an ultrasonic bath and centrifuged, if necessary. To an aliquot of 1.2 mL plasma 100 μL of the internal standard solution (800 ng/mL MA in water) and 0.6 mL 75 mM H3PO4 (85%) were added, sonicated for 1 min in a stoppered 2.5-mL vial, and centrifuged for 10 min at 2000 rpm. The supernatant (1.5 mL) was then applied to the Adsorbex SCX (100 mg) cation-exchange SPE column (Merck, Zurich, Switzerland), which was preconditioned with 2 mL methanol, 1 mL water, and 2 mL 25 mM KH2PO4. After drying the column with 6 mL of air, the plasma interferences were removed by washing with 1.5 mL of 25 mM KH2PO4 and 1 mL of methanol, followed by 1.6 mL of air. The elution was done twice with 1.25 mL of methanol–HCl 7.3% (97.5:2.5), followed by 1 mL of air. After addition of 90 μL of 1M K2HPO4 to a 2-mL aliquot of the eluate, the solution was concentrated to about 100 μL under a stream of nitrogen and reconstituted to exactly 200 μL with water. After filtration through the tip of a Pasteur pipette filled with cotton wool, 25 μL of the filtered solution was used for HPLC.

Preparation of urine samples for HPLC. SPE and cleanup of urine samples were carried out according to the procedure described earlier (35), but modified for the use on the ASPEC system. Frozen urine samples were warmed up to room temperature in an ultrasonic bath and centrifuged if necessary. To an aliquot of 1.4 mL urine 14 μL of the internal standard solution (1.24 mg/mL MA in water) and 0.6 mL 75 mM KH2PO4 were added. The pH was adjusted to 5 with 200 mM H3PO4 when necessary and sonicated for 1 min in a stoppered 2.5-mL vial. An aliquot (1.5 mL) of this sample was then applied to the Adsorbex SCX (100 mg) cation-exchange SPE column, which was preconditioned with 2 mL methanol, 1 mL water, and 1 mL 25 mM KH3PO4. After drying the column with 6 mL of air, the urine interferences were removed by washing with 1.5 mL of 25 mM KH2PO4 and 1 mL of methanol, followed by 1.6 mL of

![Figure 2. HPLC profile of a plasma extract; subject B, 120 min after an oral dose of 1.5 mg/kg 3,4-methylenedioxyamphetamine (MDMA). Peaks: 1, 3,4-Methylenedioxyamphetamine (MDA, 3.9 ng/mL); 2, methamphetamine (MA, internal standard); 3, 3,4-Methylenedioxyamphetamine (MDMA, 331.3 ng/mL).](image)
air. The elution was done twice with 1 mL methanol–HCl 7.3% (97.5:2.5), followed by 1 mL of air. After the addition of 68 µL 1M K2HPO4 to a 1.5-mL aliquot of the eluate, the solution was concentrated to about 100 µL under a stream of nitrogen and reconstituted to exactly 150 µL with water. After filtration through the tip of a Pasteur pipette filled with cotton wool, 3 µL of the filtrated solution was used for HPLC.

**Enzymatic hydrolysis.** For the enzymatic hydrolysis of the conjugates, 0.6 mL 75 mM KH2PO4, containing 10000 units/mL urine of β-glucuronidase/sulfatase (from *Helix pomatia*, type H-I) was added to 1.4 mL of urine with 14 µL internal standard solution. The mixture was then incubated for 16 h at 37°C. After centrifugation, 1.5 mL of the supernatant solution was extracted as described previously, and a 3-µL aliquot of the filtrated extract was used for HPLC.

**Acidic hydrolysis.** HCl (0.4 mL; 37%) was added to 2 mL of urine with 20 µL internal standard solution, and the mixture was incubated for 15 min at 120°C and 10⁶ Pa pressure. After cooling to room temperature, the mixture was centrifuged (2000 rpm for 5 min), and 1.5 mL of the supernatant solution was applied to the Bakerbond C-18 (300 mg) SPE column (PH. Stehelin & Co., Basel, Switzerland). The column was preconditioned with 3 mL methanol and 6 mL water. Urine interferences and the excessive hydrochloric acid were removed by washing with 6 mL water. After drying the column with 6 mL of air, the elution was done with 1.5 mL of methanol–75mM KH2PO4 (95:5) followed by 5 mL of air. The methanol was evaporated to 50 µL under a stream of nitrogen and reconstituted to 1.75 mL with 50 mM KH2PO4. An aliquot of 1.5 mL was extracted on the Adsorbex SCX (100 mg) cation-exchange SPE column as described previously. After the addition of 68 µL of 1M K2HPO4, the eluate was concentrated to 200 µL, and a 3-µL aliquot of the filtrated extract was used for HPLC.

**Preparation of urine extracts for GC–MS.** Extraction and cleanup of urine specimens and the preparation of HFBA derivatives by On-Disc Derivatization™ (ODD) with heptafluorobutyric anhydride were performed by using TOXI-LAB® SPE VC MP1 (15 mg) microcolumns (Toxi-Lab, Irvine, CA; Spectronex AG, Basel, Switzerland) and following the instructions of the manufacturer for the extraction of amphetamine and methamphetamine. After derivatization, 200 µL water was added to the hexane–HFBA mixture and strongly shaken. The water phase was discarded, and 200 µL of 4% NH4OH was added. The mixture was shaken again. A 1-µL aliquot of the hexane layer was used for GC–MS analysis. The enzymatic and acidic hydrolysis were done as described for HPLC analysis.

**HPLC analysis**

The HPLC system consisted of a Hewlett-Packard (HP) (Waldbonn, Germany) 1090M liquid chromatograph with an HP 1040M DAD and an HP HPLC® Chemstation (software version A.02.00). The separation of plasma and urine samples was performed isocratically at 40°C on a 150 × 4.6-mm internal diameter column with a 20 × 4.0-mm internal diameter precolumn packed with 3-µm Spherisorb ODS-1. The mobile phase was acetonitrile–water (96:904, v/v) containing 5.0 mL (8.5 g) orthophosphoric acid (85%) and 0.28 mL (0.22 g) hexylamine per 1000 mL. The flow rate was 1 mL/min. The quantitation of MDMA, MDA, HMMA, and HMA before and after enzymatic or acidic

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**Figure 3.** HPLC profiles of an urine extract before (A) and after enzymatic (B) or acidic (C) hydrolysis; subject B, 21.5 h after an oral dose of 1.5 mg/kg 3,4-methylenedioxymethamphetamine. Peak identification: 1, 3,4-dihydroxymethamphetamine (HHMA); 2, 4-hydroxy-3-methoxymethamphetamine (HMA, 2.1 µg/mL, after acidic hydrolysis); 3, 4-hydroxy-3-methoxymethamphetamine (HMMA, 3.1 µg/mL); 4, 3,4-methylenedioxyamphetamine (MDA) (1.6 µg/mL); 5, methamphetamine (MA, internal standard); 6, 3,4-methylenedioxyamphetamine (MDMA, 18.1 µg/mL).
hydrolysis was performed at 200 nm by measuring the peak areas and using the internal standard method.

**GC–MS analysis**

The GC–MS system consisted of a HP 5990 GC with a HP 5970 mass selective detector (MSD). A 1-µL aliquot of the derivatized urine extracts was separated on a J&W Scientific (HSP Friedlid Co., König, Switzerland) DB-5 bonded-phase capillary column (20 m × 0.18-mm i.d.; 0.40-µm film thickness) directly inserted into the ion source. The injector and transfer line temperatures were 260 and 280°C, respectively. The oven temperature was programmed from 70°C (held 1 min) to 200°C at 10°C/min and from 200°C (held 1 min) to 280°C (held 14 min) at 15°C/min. Helium was used as the carrier gas at a flow rate of 0.7 mL/min. The MS was operated in the scan and the SIM mode. The scan range was m/z 33-780, and the scan-rate was set at 0.57 scans/sec. The characteristic ions were m/z 389, 254, 210, 162, 135 (MDMA-HFBA); 375, 240, 162, 135 (MDA-HFBA); 587, 360, 254, 210 (HMMA-di-HFBA); 573, 360, 240, 163 (HMA-di-HFBA); 769, 542, 515, 254, 210 (HHMA-tri-HFBA); and 755, 542, 515, 240, 210 (HHA-tri-HFBA).

**Results and Discussion**

**HPLC analysis**

The same SPE procedure, which can be automated for serial analysis, has been used for the preparation of plasma and urine samples for HPLC analysis. As shown by Figures 2 and 3, most of the endogenous matrix is eliminated by the highly selective cation-exchange column, whereas about 98 and 99% of MDMA is recovered from plasma and urine, respectively. The recovery of MDMA was determined using two plasma samples spiked with 49 and 493 ng/mL MDMA and two urine samples spiked with 1800 and 8800 ng/mL MDMA. The extraction efficiency for the MDMA metabolites (MDA, HMMA, HMA) was determined to be good to excellent. Urine samples were spiked with 400 ng and 800 ng/mL MDA, 725 and 7250 ng/mL HMMA, and 800 and 4000 ng/mL HMA. The recoveries were 100, 90, and 68% for MDA, HMMA, and HMA, respectively. Methamphetamine (MA) was chosen as the internal standard for plasma and urine. Amphetamine was too polar and interfered with the biological matrix.

For the determination of the conjugated mono- and dihydroxylated MDMA metabolites (HMMA, HMA, HHMA, etc.) enzymatic or acidic hydrolysis was necessary prior to SPE. The hydrolysis with hydrochloric acid was faster (completed within 1 h), more efficient (see Figure 3) and less expensive than with β-glucuronidase/sulfatase.

The chromatographic system described here was originally developed for the analysis of methadone in pharmaceutical preparations (48). Subsequently, it was routinely used in our laboratory for the analysis of psychotropic substances in biological matrix (e.g., determination of cathinone and its metabolites in plasma [49] and urine [50] as well as for MDMA and MDA in urine [35]). The selectivity of this universal HPLC method can easily be optimized for basic
analytes of a wide range of polarity (even for zwitter ions) by modifying the ratio of acetonitrile-water and the concentration of hexylamine. The latter serves as a modifier and masking agent for residual silanol groups on the C\textsubscript{18} reversed-phase material, resulting in improved peak shape and smaller $k'$ values. Figure 3 shows the chromatogram of an urine collected 21.5 h after the oral administration of 1.5 mg/kg-body weight MDMA, which indicates that even the more polar metabolites, HHMA, HMA, and HMMA, are separated and do not interfere with the endogenous matrix.

Peak identification was achieved by comparing retention times and DAD-UV spectra of standards. The UV spectrum of the methylenedioxy-type amphetamines (MDMA, MDA, etc.) is characterized by maxima at 200, 236, and 284 nm, whereas the hydroxy/methoxy ring-substituted derivatives (HMMA, HMA, etc.) show maxima at 200, 228, and 278 nm. The correct peak assignment was confirmed by GC-MS. The low UV cutoff of the mobile phase allows the sensitive detection at 200 nm, where MDMA and metabolites exhibit their main UV maximum. The resulting limit of quantitation (LOQ) of MDMA was 7 ng/mL in plasma and urine with a signal-to-noise ratio of greater than 3. The LOQ of MDA was 5 ng/mL in plasma and urine.

**GC–MS analysis**

Extraction and cleanup for GC–MS analysis of urine samples were achieved by using the SPE disc technology. The SPEC MP1 (Solid Phase Extraction Concentrators, Mixed Phase) microcolumns consist of a rigid glass fiber disc coated with modified silica. Compared with the classic packed-bed cartridge SPE technique, less sample, solvents, and processing time are needed, and cleaner extracts with high analyte recovery result (51). The procedure is reproducible, inexpensive, and allows on-disc derivatization, but cannot be automated. To remove the relatively involatile excess of derivatizing reagent and reaction byproducts, which produce high background levels and shorten the column life, a wash step is recommended (52).

Figure 4 shows the GC–MS profile of a derivatized urine extract before and after enzymatic and acidic hydrolysis acquired in the full scan mode. By comparing the chromatogram with the HPLC profile of the same urine specimen (Figure 3) collected 21.5 h from subject B after administration of 1.5 mg/kg-body weight MDMA, a correlation can be observed, confirming the correct HPLC peak identification. HHA could not be identified by HPLC but was detectable in urine by GC-MS. Although the present GC–MS method has only been established for the qualitative profiling of urine, it may also be used with slight modifications in the SPE procedure for the quantitative analysis of urine and plasma samples.

**Plasma levels, urinary excretion, and metabolism of MDMA**

Figure 5 represents the plasma profiles of MDMA and MDA of subject A and B after administration of 1.5 mg/kg body weight MDMA. MDMA appeared in plasma within 15 (subject B) and 30 min (subject A) and reached the peak plasma levels of 330.3 (subject A) and 331.3 ng/mL (subject B) after 120 min. MDA, the only metabolite identified in plasma, was first detectable after 90 (subject B) and 135 min (subject A). Peak plasma concentrations of 15.3 (subject A) and 10.0 ng/mL (subject B) were
measured after 380 and 150 min, respectively. Significant amounts of MDMA were found 8 h after administration.

Figure 6 represents the urine excretion profiles of subjects A and B after administration of 1.5 mg/kg body weight MDMA. MDMA concentrations ranged from 13.09 to 28.14 μg/mL in the urine of subject A and from 0 to 18.12 μg/mL in the urine of subject B with peak values after 5 and 21.5 h, respectively. Up to 24.58 μg/mL HMMA (subject A) and 35.08 μg/mL (subject B) could be detected between 1.5 and 16 h. The MDA urine concentrations varied from 0.11 to 2.30 μg/mL (subject A) and from 0 to 1.58 μg/mL (subject B) with maximum levels occurring after 5 and 21.5 h. Concentrations of 0–1.20 μg/mL of HMA, the other minor MDMA metabolite, were measured for subject A and 0–2.05 μg/mL were measured for subject B. The highest concentrations appeared between 5.5 and 21.5 h. When comparing the urine profiles before and after hydrolysis (Figure 3), it is obvious that the metabolites with an open methylenedioxy ring (HMMA, HMA, HHMA) are excreted mainly as conjugates (glucuronides and/or sulfates). These urine data reflect the interindividual differences in the excretion pattern and that HMMA (as conjugate), and not MDA as found earlier (43), is the major urinary metabolite of MDMA.

The HMMA peak concentration may even exceed that of the parent compound MDMA. The metabolic scheme of MDMA is summarized in Figure 1. Our results confirm the findings of an uncontrolled study wherein one urine specimen from a fatally injured motorcyclist was analyzed by GC–MS (38). Although no quantitation was performed, it was suggested that HMMA is the major metabolite and HHMA, HMA, and MDA are formed as further metabolites.

The present study has been followed by a trial with six patients and slight modifications of the protocol (longer collection periods). The full pharmacokinetic and statistical data evaluation is currently in progress.

Conclusion

HPLC in combination with automated SPE is the method of choice for the pharmacokinetic profiling of MDMA and its conjugated and unconjugated metabolites in plasma and urine. GC–MS with SPEC-ODD sample preparation is recommended as the confirmation method. The controlled clinical trial with orally administered MDMA has shown that the main metabolic pathways of MDMA in man are cleavage of the methylenedioxy bridge, demethylation, and conjugation with HMMA and HHMA as major urinary metabolites.

Acknowledgment

This work was partly supported by grants from the Swiss Federal Office of Public Health (Foundation of Narcotics Research).

References


Manuscript received March 25, 1996; revision received May 14, 1996.