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Quantification of 3,4-methylenedioxymetamphetamine and its metabolites in plasma and urine by gas chromatography with nitrogen–phosphorus detection[1]

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Abstract

A gas chromatographic method with nitrogen–phosphorus detection involving a solid–liquid extraction phase was developed and validated for the simultaneous quantification of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) in plasma. A modification of this method was validated for the analysis of MDMA, MDA, 4-hydroxy-3-methoxymethamphetamine (HMMA) and, 4-hydroxy-3-methoxyamphetamine (HMA) in urine. Under the analytical conditions described, the limits of detection in plasma and urine were less than 1.6 μg/l and 47 μg/l, respectively, for all the compounds studied. Good linearity was observed in the concentration range evaluated in plasma (5–400 μg/l) and urine (100–2000 μg/l) for all compounds tested. The recoveries obtained from plasma were 85.1% and 91.6% for MDMA and MDA, respectively. Urine recoveries were higher than 90% for MDMA and MDA, 74% for HMMA, and 64% for HMA. Methods have been successfully used in the assessment of plasma and urine concentrations of MDMA and its main metabolites in samples from clinical studies in healthy volunteers.

Keywords: 3,4-Methylenedioxymethamphetamine; 3,4-Methylenedioxyamphetamine

1. Introduction

MDMA (3,4-methylenedioxymethamphetamine) was synthesized by Merck in 1914 as an appetite suppressant. MDMA is structurally related with amphetamine and some hallucinogenic phenylalkylamines, such as MDA (3,4-methylenedioxyamphetamine) and mescaline, but the MDMA pharmacological profile appears to be different from pure stimulant or hallucinogenic substances [1,2] and can be considered as a representative of a new drug category, entactogens [3]. In the 1970s, it was shown that some subjective emotional effects

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induced by MDMA could be useful in psychotherapy [4].

Although in the last decade, the Drug Enforcement Administration (DEA) in the United States decided to restrict the use of MDMA [5], its recreational consumption continued and even increased among youth [6], and a number of cases of acute intoxication by entactogens has been reported in recent years [7,8]. In animal models, the administration of MDMA is followed by neurodegenerative effects on the central serotoninergic system [9,10]. The *O*-demethylenated metabolite of MDMA, 3,4-dihydroxymethamphetamine (HHMA), was found in the rat brain and postulated as being responsible for neurotoxicity [11,12]. *O*-Demethylenation in humans is catalyzed by cytochrome P450 isoenzyme, CYP2D6 also known as debrisoquine (a prototype substrate of this enzyme) 4-hydroxylase. This enzyme is expressed polymorphically in humans and about 5–9% of the Caucasian population is deficient for this enzyme activity [13]. Metabolism of MDMA (Fig. 1) involves *N*-demethylation to MDA, *O*-dealkylation to HHMA and to 3,4-dihydroxyamphetamine (HHA), *O*-methylation to 4-hydroxy-3-methoxymethamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA), and *O*-conjugation of *O*-dealkylated metabolites.

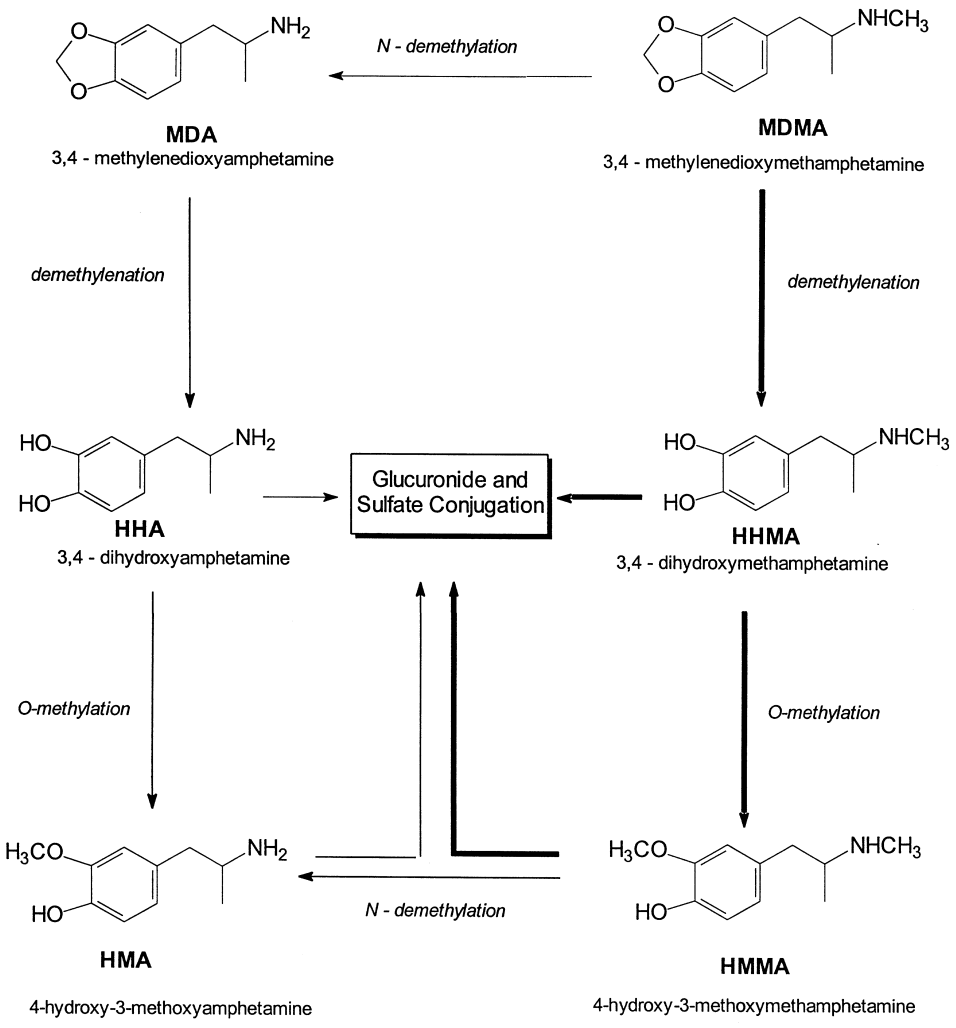
There are a few reports on in vivo MDMA metabolism in humans. Free and/or conjugated MDA, HMMA, and HMA appear to be major MDMA metabolites in urine [14–17]. In plasma MDA has been identified [18] and represents 10% of MDMA concentrations [16]. Gas chromatography coupled to mass spectrometry has been the instrumental technique more often used for the identification of MDMA urinary metabolites [16,19–24]. High-performance liquid chromatography either with diode array (HPLC–DAD) [16,25,26] or electrochemical detection (HPLC–ED) [27,28] has been used for MDMA and MDA determination in plasma and blood. Extraction procedures described use multiple liquid–liquid extraction [19,24,27] or solid–liquid extraction steps [16,21]. Gas chromatographic techniques include the formation of different chromatographic derivatives in order to improve the chromatographic behavior of primary and secondary amines [16,19–24]. These techniques, however, have been never used in large-scale pharmacokinetic studies requiring a high number of plasma and urine determinations. In addition there are no validated methods designed for the simultaneous quantification of MDMA and its main metabolites (MDA, HMMA, HMA) in plasma and urine.

We describe a validated method for measuring MDMA and MDA in human plasma by capillary gas–liquid chromatography and selective nitrogen–phosphorus detection (GC–NPD) after solid-phase extraction. Under these conditions, low detection limits and reliable reproducibilities are achieved. A modification of this method was validated for the analysis of MDMA, MDA, HMMA, and HMA in urine. This method was used for the determination of MDMA and its main metabolites in plasma and urine samples from clinical studies in healthy volunteers.

2. Materials and methods

2.1. Chemicals and reagents

MDMA and MDA were purchased from Radian (Austin, TX, USA). 3,4-Methylenedioxypropylamphetamine (MDPA), internal standard) was supplied from Alltech-Applied Sciences (State College, PA, USA). HMMA and HMA were kindly provided by Dr. Rudolf Brenneisen (Institute of Pharmacy, University of Berne, Switzerland) [29,30]. Pholedrine (4-hydroxymethamphetamine) was generously given by Prof. Manfred Dönike (Deutsche Sporthochschule, Biochemistry Department, Cologne, Germany). Methanol and chloroform, HPLC grade, and acetic acid, glacial reagent grade, were obtained from Scharlau (Barcelona, Spain). *N*-Methyl-bis(trifluoroacetamide) (MBTFA), gas chromatography grade, was purchased from Macherey–Nagel (Düren, Germany). Sodium acetate trihydrate, disodium hydrogen phosphate anhydrous, sodium hydrogen phosphate monohydrate, and ammonia solution were reagent grade and purchased from Merck (Darmstadt, Germany). β-Glucuronidase from *Helix pomatia* (HP-2) was obtained from Sigma (St. Louis, MO, USA). Phosphate buffer 0.1 *M*, pH 6, was prepared by mixing adequate volumes of two solutions of 0.1 *M* disodium hydrogen phosphate and 0.1 *M* sodium hydrogen phosphate. Ultra pure water was obtained using a Milli-Q purification system (Milli-



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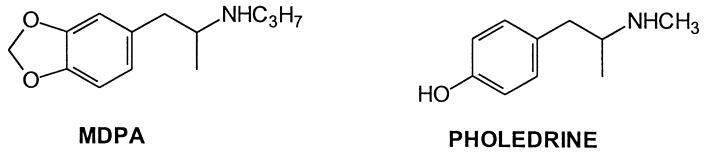

Fig. 1. Metabolism of 3,4-methylenedioxymethamphetamine and I.S. structures.

pore, Molsheim, France). Bond Elut Certify® solid-phase extraction columns (10 ml/130 mg) were obtained from Varian Sample Preparation Products (Harbor City, CA, USA) and mounted on a Vac-Elut vacuum manifold (Supelco, Bellefonte, PA, USA). MDMA for human administration was obtained from the Spanish Ministry of Health (Plan Nacional sobre Drogas, Madrid, Spain). Drugs were prepared and

supplied by the Pharmacy Department of Hospital del Mar (Barcelona, Spain).

2.2. Instrumentation

A gas chromatograph (HP5890 series II, Hewlett Packard, Palo Alto, CA, USA) equipped with a nitrogen–phosphorus detector and autosampler (HP7673A, Hewlett Packard) was used. Separation of MDMA and metabolites was carried using a cross-linked 5% phenyl-methylsilicone capillary column, 12 m×0.2 mm I.D. and 0.33 μm film thickness (Ultra-2, Hewlett Packard). Helium was used as carrier gas at a flow-rate of 0.80 ml/min (measured at 180°C) and as make up gas at a flow-rate of 24 ml/min. Air and hydrogen detector flows were set at 100 and 4 ml/min, respectively. A temperature program for plasma MDMA and MDA separation starting at 70°C, was maintained during 2 min and programmed to 100°C at 30°C/min, then to 200°C at 20°C/min, and finally to 280°C at 25°C/min. Urinary metabolites were separated starting at 100°C and programmed to 280°C at 15°C/min. Total run time including equilibration time was 12 min in both cases. Plasma samples were injected in the splitless mode (30 s of purge off time) and urine samples were injected in the split mode with a split ratio 1:10. Insert liners packed with silanized glasswool were used. Injector and detector temperatures were set at 280°C.

2.3. Clinical studies

Plasma and urine specimens were obtained from healthy male recreational users of MDMA who were given a single oral dose of MDMA under authorization of the Spanish Ministry of Health (Ref. DGFPS Nr. 95/297) and the local Ethical Committee. All subjects were CYP2D6 extensive metabolizers after dextrometorphan phenotyping [31]. MDMA was prepared by the Pharmacy department of Hospital del Mar as soft-gelatine capsules, and administered in the fasting state with 200 ml of tap water. Blood samples were obtained through a catheter inserted in a peripheral vein before drug administration and at 0, 15, 30, 45, 60 and 90 min, and 2, 3, 4, 6, 8, 10 and 24 h after drug administration. The heparinized blood was centrifuged 10 min at 1100 *g* and plasma was transferred to polypropylene tubes and stored at −20°C until analysis. Urine samples were collected in four different time periods (basal, 0–4, 4–8, and 8–24 h) after drug administration and stored at −20°C until assay.

2.4. Working standards

Working solutions of 1 and 10 mg/l of MDMA, MDA, HMMA, and HMA were prepared from stock solutions of 100 mg/l by dilution with methanol. MDPA working solution of 5 mg/l was prepared from a stock solution of 1 g/l by dilution with methanol for plasma analysis. A mixture of MDPA (62.5 mg/l) and pholedrine (25 mg/l) was prepared as I.S. working solution from stock solutions of 1 g/l for urine specimen analysis.

2.5. Preparation of plasma calibration and quality control samples

Calibration curves were prepared for each analytical batch in blank plasma. Appropriate volumes of working solutions were added to test tubes containing 1 ml of drug-free plasma and vortexed vigorously. Final concentrations were 20, 50, 100, 200, and 400 μg/l for MDMA and 5, 10, 25,50, and 100 μg/l for MDA. Control plasma samples containing MDMA and MDA were prepared at two different concentrations (low control: 45 μg/l for MDMA and 24 μg/l for MDA, and high control: 300 μg/l for MDMA and 75 μg/l for MDA) and kept frozen at −20°C in 1-ml aliquots. Control samples were included in each batch.

2.6. Preparation of urine calibration and quality control samples

Calibration curves were prepared for each analytical batch in blank urine. Appropriate volumes of working solutions were added to test tubes containing 2 ml of drug-free urine and mixed vigorously. Final concentrations were 0.25, 0.50, 0.75, 1, and 2 mg/l for MDMA and MDA; 0.1, 0.25, 0.50, 1, and 1.5 mg/l for HMMA, and 0.1, 0.25, 0.50, 0.75, and 1 mg/l for HMA. Control urine samples containing

MDMA, MDA, HMMA, and HMA were prepared at three different concentrations (low control: 0.3 mg/l for MDMA and MDA and 0.2 mg/l for HMMA and HMA; medium control: 0.8 mg/l for MDMA and MDA and 0.6 mg/l for HMMA and HMA; and high control: 1.5 mg/l for MDMA and MDA, 1.2 mg/l for HMMA, and 0.9 mg/l for MDA) and kept frozen at −20°C in 1-ml aliquots until assay. Control samples were included in each batch.

2.7. Hydrolysis procedure

Undiluted and diluted 1:10 (with blank urine) urine specimens were processed before and after hydrolysis. Enzymatic hydrolysis was performed in urine (1 ml). The pH was adjusted to 5 with 1 ml of 1.1 *M* acetate buffer (pH 5.2), and 20 000 Fishman units of β-glucuronidase were added. Samples were incubated overnight at 37°C.

2.8. Plasma and urine extraction

Test and control samples were allowed to thaw at room temperature. Aliquots of 1 ml of plasma or urine (unaltered or previously hydrolyzed) were pipetted into 15-ml screw-capped tubes and processed together with a calibration curve after addition of 100 ng of MDPA as internal standard in plasma samples, or 500 ng of pholedrine and 1250 ng of MDPA in urine. Extraction was carried out using Bond Elut Certify® columns. The pH of the samples was adjusted to 6 by adding 1 ml of 0.1 *M* phosphate buffer and passed through the columns that had been previously conditioned by passing sequentially 2 ml of methanol and 2 ml of 0.1 *M* phosphate buffer. Columns were washed consecutively with 1 ml of 1 *M* acetic acid and 6 ml of methanol. MDMA, MDA, and MDPA were eluted from plasma with 2 ml of chloroform with 2% of ammonium hydroxide. Eluates were evaporated to dryness at 30°C under a nitrogen stream and the residue was redissolved in 100 μl of methanol. Finally, 1 μl was injected onto the chromatographic system. Columns for the determination MDMA and its main metabolites in urine were eluted with 2 ml of ethyl acetate with 2% of ammonium hydroxide.

2.9. Derivatization of urine samples

Urine eluates were evaporated to dryness under a nitrogen stream at 50°C with the previous addition of 20 μl of MBTFA to prevent metabolite losses, and kept in a vacuum oven with di-phosphorus pentoxide during at least 2 h at 50°C. Trifluoroacyl derivatives were formed by redissolving the dry extracts with 50 μl of MBTFA and heating test tubes at 70°C during 45 min. Finally, 1 μl was injected into the chromatographic system. The structure of the TFA derivatives of the analytes was verified by gas chromatography coupled to electron impact mass spectrometry. The following derivatives were obtained: MDMA, *N*-TFA; MDA, *N*-TFA; pholedrine, *O*-TFA, *N*-TFA; MDPA, *N*-TFA; HMMA, *O*-TFA, *N*-TFA; and HMA, *O*-TFA, *N*-TFA.

3. Results

3.1. GC–NPD analysis

With the aforementioned chromatographic conditions, a good separation was obtained between MDA, MDMA, and the internal standard in plasma samples and between MDMA and its metabolites in urine samples. Chromatographic behavior was excellent and good peak shapes were obtained for secondary or primary amines of MDA, HMA, MDMA, and HMMA, respectively. The selectivity of the extraction procedure together with the nitrogen–phosphorus detector produced very clean chromatograms free of background interferences at the retention time of MDMA and its metabolites. Figs. 2 and 3 show typical chromatograms from plasma and urine obtained from samples of one volunteer who was given 75 mg MDMA orally. Prior to the application of the method to real samples, the following validation protocol was employed.

3.2. Recovery

The recoveries of MDMA and MDA were calculated by comparing the peak areas that were obtained when calibration samples were analyzed by adding the reference substances and the internal standard in blank plasma prior to and after extraction. The

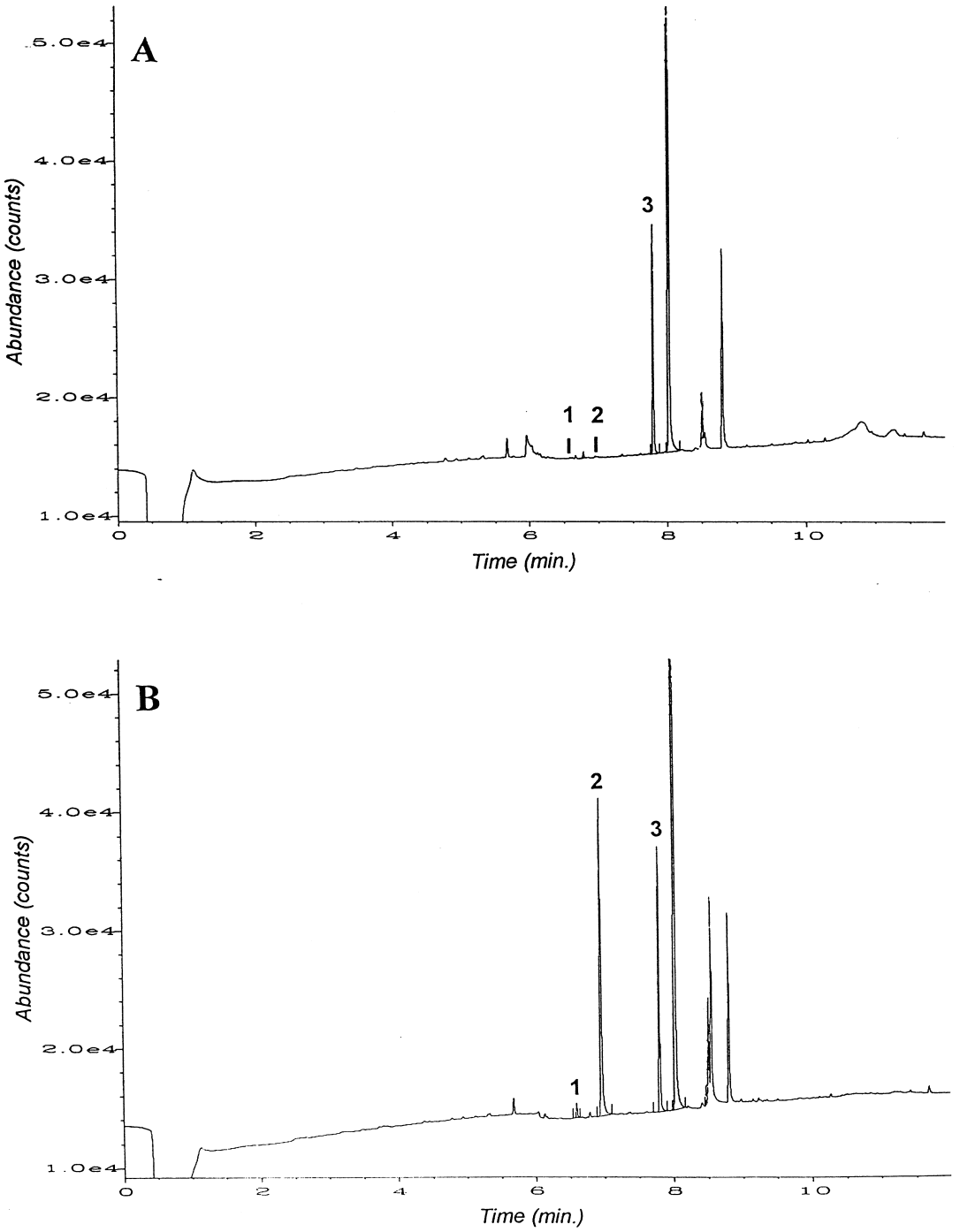

Fig. 2. GC–NPD chromatogram of plasma samples from a volunteer administered with 75 mg of MDMA. (A) Basal plasma obtained just before drug administration. Expected retention times for MDA (1) and MDMA (2) are indicated ; peak numbered (3) corresponds to the internal standard (MDPA). (B) Plasma obtained 1.5 h after drug administration, concentrations calculated for MDMA and MDA were 137.5 and 5.75 μg/l.

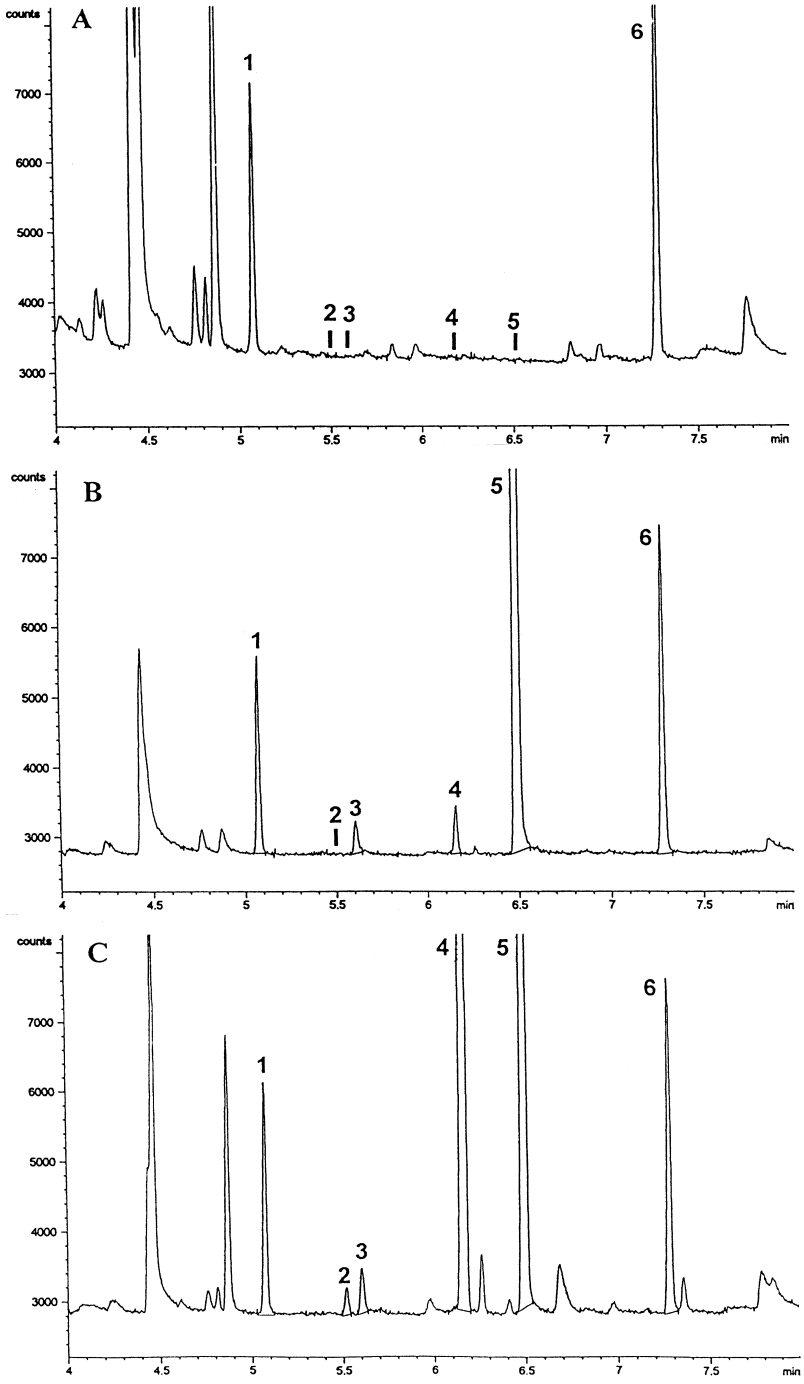

Fig. 3. GC–NPD chromatogram of urine samples from a volunteer given 75 mg of MDMA. (A) Basal urine obtained just before drug administration. Expected retention times for HMA, *O*-TFA, *N*-TFA (2), MDA, *N*-TFA (3), HMMA, *O*-TFA, *N*-TFA (4) and MDMA, *N*-TFA (5) are indicated; peaks numbered (1) and (6), correspond to I.S., Pholedrine, *O*-TFA, *N*-TFA and MDPA, *N*-TFA, respectively. (B) Non-hydrolyzed urine sample from a time period 0–4 h after drug administration, concentrations calculated were: MDMA, 6.83 mg/l; MDA, 0.19 mg/l; HMMA, 0.32 mg/l; and HMA was not detected. (C) Hydrolyzed urine sample from a time period of 0–4 h after drug administration, concentrations calculated were MDMA, 6.60 mg/l; MDA, 0.16 mg/l; HMMA, 11.1 mg/l; and HMA 0.23 mg/l.

recoveries obtained from plasma were 98.3% for MDPA, and 85.1% and 91.6% for MDMA and MDA, respectively. Urine recoveries were higher than 90% for MDMA and MDA, 74% for HMMA, and 64% for HMA. The recoveries obtained for pholedrine and MDPA were 71% and 93%, respectively.

3.3. Linearity

Linearity in plasma samples was determined by checking different calibration curves in duplicate at five different concentrations between 20 and 400 μg/l for MDMA and between 5 and 100 μg/l for MDA. Peak area ratios between compounds and internal standards (MDPA) were used for calculations. A weighted least square regression analysis was used (GraFit 3.01, R.J. Leatherbarow). The calibration curves obtained showed good determination coefficients of 0.9959±0.0011 for MDMA and 0.9924±0.0011 for MDA and reproducible slopes (0.0099±0.0011 for MDMA; 0.0103±0.0013 for MDA). Linearity in urine specimens was determined in the same way between 0.25 and 2 mg/l for MDMA and MDA, 0.1 and 1 mg/l for HMA, and 0.1 and 1.5 mg/l for HMMA. Peak area ratios between each compound and the internal standard (MDPA for MDMA and MDA, and pholedrine for HMMA and HMA) were used for calculations. Mean determination coefficients were 0.9968±0.008 for MDMA, 0.9964±0.0011 for MDA, 0.9980±0.0025 for HMMA, and 0.9972±0.0030 for HMA.

3.4. Limits of detection and quantification

Six replicate analyses were performed with spiked plasma samples containing 20 μg/l of MDMA and 10 μg/l of MDA. The standard deviation (SD) of quantitative values was used as an estimation of the ''noise'' of the analytical system for the calculation of the limits of detection (three standard deviations, 3 SD) and quantification (ten standard deviations, 10 SD). In plasma, the detection and quantification limits for MDMA were 1.6 and 5.3 μg/l and for MDA 0.8 and 2.7 μg/l, respectively. The limits of detection and quantification in urine were calculated from the variability (SD) of quantitative values of spiked samples containing 0.25 mg/l for MDMA and MDA, and 0.1 mg/l for HMMA and HMA. Detection and quantification limits were MDMA, 0.038 and 0.126 mg/l; MDA, 0.047 and 0.157 mg/l; HMMA, 0.029 and 0.097 mg/l; and HMA, 0.028 and 0.093 mg/l, respectively.

3.5. Precision and accuracy (Tables 1 and 2)

Six replicates of blank plasma spiked with 20, 100, and 400 μg/l of MDMA and 5, 25, and 100 μg/l of MDA were used to determine the intra-assay precision and accuracy in plasma. The inter-assay precision and accuracy were determined using all calibration points analyzed during the study. Six replicates of blank urine spiked with 0.25, 0.75, and 2 mg/l of MDMA and MDA; 0.1, 0.5, and 1.5 mg/l of HMMA; and 0.1, 0.5 and 1 mg/l of HMA were used to determine intra-assay accuracy and precision in urine. Precision is expressed as the relative SD of the concentrations calculated by the calibration graphs. Accuracy is expressed as the relative error of the estimated concentrations.

3.6. Quality control samples

One quality control sample of each level was included in each analytical batch. The mean calculated concentrations together with their relative SD and relative errors obtained during the study period are shown in Tables 3 and 4. Results show the stability of the samples under the storage conditions and the reproducibility and accuracy of the determinations.

3.7. Clinical studies

All plasma samples were processed following the method described previously for MDMA and MDA quantification. Urine samples were processed to determine MDMA and its main metabolites. Fig. 2B and C correspond to a GC–NPD profile obtained from a 0–4 h urine after administration of 75 mg of MDMA processed before and after enzymatic hydrolysis. The four analytes were found in all hydrolyzed urine specimens. Conjugated HMMA was the major conjugated metabolite. Fig. 4 shows plasma profiles of MDMA and MDA, and the urinary

Table 1
Accuracy and precision of MDMA and MDA GC–NPD plasma determinations

Analyte	No.	Concentration targeted (μg/l)	Concentration estimated (μg/l)	SD (μg/l)	Precision (RSD, %)	Accuracy (error, %)
Intra-day						
MDMA	6	20	19.6	0.5	2.7	−2.0
	6	50	53.0	2.2	4.1	5.9
	6	200	197.8	13.7	6.9	−1.1
MDA	6	5	4.7	0.3	5.7	−5.9
	6	25	23.3	1.7	7.2	−6.6
	6	100	108.6	10.9	9.9	8.7
Inter-day						
MDMA	28	20	20.5	1.6	8.0	2.3
	25	50	49.9	3.9	7.7	−0.1
	30	100	100.8	5.9	5.8	0.8
	23	200	197.3	10.9	5.5	−1.3
	20	400	405.5	31.3	7.7	1.4
MDA	26	5	5.2	0.4	8.2	3.7
	20	10	9.9	0.7	7.5	−0.8
	26	25	24.3	2.0	8.2	−3.0
	24	50	47.8	3.8	8.1	−4.5
	23	100	101.8	4.3	4.5	1.8

metabolic excretion profile from a healthy volunteer after administration of 125 mg MDMA .

4. Discussion

Previous methods for the quantification of MDMA and MDA [17,19–24] used time-consuming liquid–liquid extraction procedures. In the present method, SPE with Bond Elut Certify® was used for MDMA and MDA extraction, introducing some minor modifications to a previous published method [21]. Elution with 2% ammonium hydroxide solution in chloroform gives very clean extracts and high specificity for MDA and MDMA in plasma samples, and no further back-extraction steps are needed. SPE combined with GC–NPD of underivatized MDMA and MDA provided a very fast and sensitive method. The possibility to form chromatographic derivatives, as reported by other authors [16,19–24], to improve the chromatographic behavior was considered. When analyzing plasma samples with the GC–NPD conditions described, a very good chromatography and adequate sensitivity of underivatized compounds was found. The same approach is not applicable in urine because of the presence of more polar metabolites of interest to be analyzed.

This is the first method designed and validated for the quantification of urinary MDMA together with its main metabolites. Urine hydrolysis for MDMA hydroxylated metabolites is necessary as more than 90% of these metabolites are excreted as conjugates, mainly as glucuronide conjugates. Elution with 2% of ammonium hydroxide in ethyl acetate instead of chloroform allows better recoveries for more polar metabolites (HMA, HMMA) and its trifluoroacyl derivatives can be very easily detected. MDMA and HMMA are present in urine at high concentrations. A 1:10 urine sample dilution is sufficient to adjust urine concentrations to the calibration curve dynamic range. MDA and HMA, which are present in lower concentrations, are better quantified in undiluted urine samples. This method has been validated and shows adequate accuracy, precision, and reproducibility for its use in pharmacokinetic studies. The use of MDPA (internal standard for MDMA and MDA) and pholedrine (internal standard for HMMA and HMA) for the analysis of MDMA and metabolites

Table 2
Accuracy and precision of MDMA and MDA GC–NPD urine determinations

Analyte	No.	Concentration targeted (mg/l)	Concentration estimated (mg/l)	SD (mg/l)	Precision (RSD, %)	Accuracy (error, %)
Intra-day						
MDMA	6	0.25	0.24	0.013	5.3	−4.8
	6	0.75	0.76	0.052	6.9	1.0
	6	2.00	1.99	0.118	5.9	−0.2
MDA	6	0.25	0.23	0.016	6.8	−8.8
	6	0.75	0.76	0.043	5.6	1.2
	6	2.00	2.04	0.171	8.3	2.3
HMMA	6	0.10	0.10	0.010	9.4	−4.0
	6	0.50	0.49	0.052	10.4	−0.4
	6	1.50	1.50	0.103	6.8	0.2
HMA	6	0.10	0.09	0.009	9.6	−2.7
	6	0.50	0.51	0.039	7.5	3.3
	6	1.00	0.99	0.050	5.1	−0.6
Inter-day						
MDMA	10	0.25	0.24	0.018	7.3	−4.1
	6	0.50	0.51	0.031	6.0	1.1
	10	0.75	0.76	0.060	7.8	1.9
	6	1.00	1.01	0.035	3.5	0.5
	10	2.00	1.99	0.100	5.0	−0.1
MDA	10	0.25	0.24	0.018	7.4	−4.6
	6	0.50	0.51	0.031	5.9	2.7
	10	0.75	0.81	0.060	7.3	9.1
	6	1.00	1.05	0.035	3.3	4.9
	10	2.00	2.02	0.100	4.9	1.2
HMMA	10	0.10	0.11	0.016	15.1	8.5
	6	0.25	0.26	0.013	5.1	3.1
	10	0.50	0.49	0.021	4.2	−1.1
	6	1.00	0.99	0.083	8.3	−1.1
	10	1.50	1.51	0.156	10.4	0.5
HMA	10	0.10	0.10	0.010	10.3	1.4
	6	0.25	0.23	0.014	6.0	−6.2
	10	0.50	0.52	0.033	6.3	3.7
	6	0.75	0.77	0.043	5.6	2.3
	10	1.00	0.99	0.040	4.1	−1.1

Table 3
Results obtained from quality control plasma samples

Analyte	No.	Concentration targeted (μg/l)	Concentration estimated (μg/l)	SD (μg/l)	Precision (RSD, %)	Accuracy (error, %)
MDMA	12	45.0	44.4	3.9	8.8	−1.3
	12	300.0	293.4	13.7	4.7	−2.2
MDA	12	24.0	24.3	1.9	7.8	1.2
	12	75.0	75.2	4.5	6.0	0.3

Table 4
Results obtained from quality control urine samples

Analyte	No.	Concentration targeted (mg/l)	Concentration estimated (mg/l)	SD (mg/l)	Precision (RSD, %)	Accuracy (error, %)
MDMA	12	0.3	0.316	0.028	8.9	5.3
	12	0.8	0.778	0.061	7.8	−2.7
	12	1.5	1.550	0.088	5.6	3.3
MDA	12	0.3	0.299	0.035	11.7	−0.2
	12	0.8	0.769	0.076	9.9	−3.8
	12	1.5	1.435	0.113	7.9	−4.3
HMMA	12	0.2	0.219	0.017	7.9	9.4
	12	0.5	0.511	0.023	4.5	2.2
	12	1.2	1.180	0.116	9.8	−1.7
HMA	12	0.2	0.207	0.016	7.7	3.7
	12	0.6	0.563	0.036	6.4	−6.1
	12	0.9	0.882	0.074	8.4	−2.0

has proven to be a good choice. They are relatively inexpensive and share many physicochemical properties with analytes to be assayed because of their very similar chemical structure (Fig. 1), making them very suitable for this type of analysis.

After processing the plasma and urine samples,

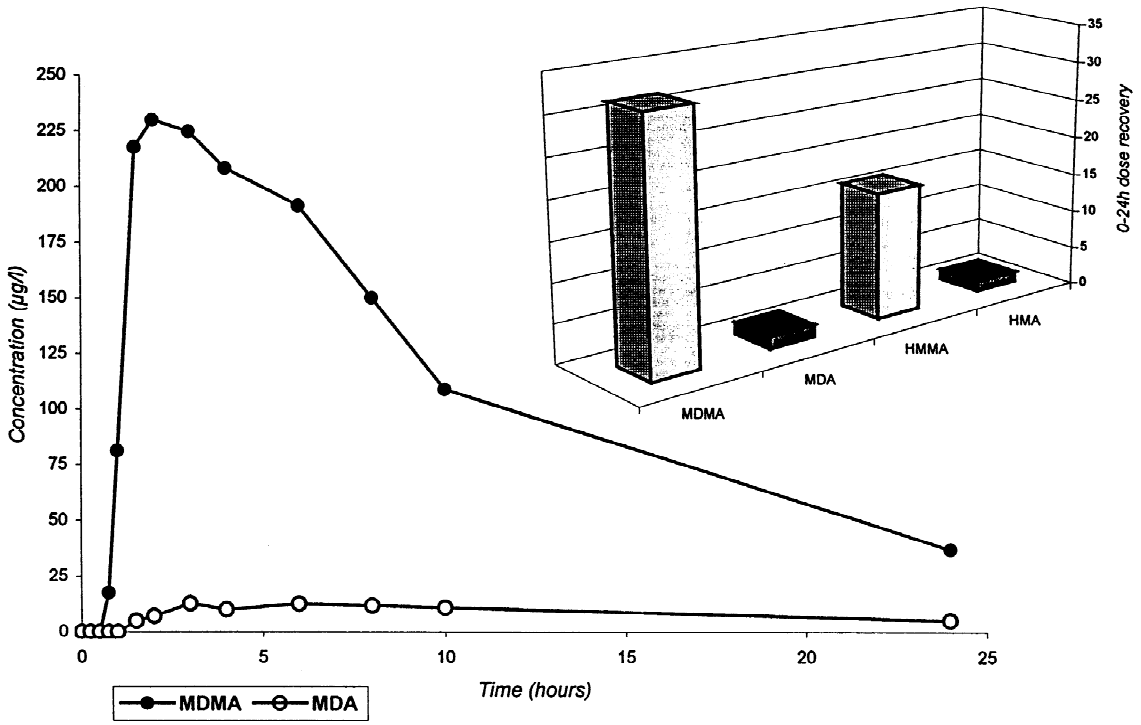

Fig. 4. MDMA and MDA plasma concentration versus time course and 0–24 h urinary recovery of the compounds studied from a volunteer administered with 125 mg of MDMA by the oral route.

MDA appears as the major unconjugated metabolite in plasma and urine [18], but only represents a low percentage of MDMA concentrations [17]. When hydrolyzing samples as was previously described [14–16], HMMA appears to be the major conjugated metabolite with HMA as a minor conjugated metabolite. The urinary recovery for the compounds studied in 24 h corresponds to about 50% of the MDMA dose administered. These results are in agreement with the recoveries reported for 3,4-methylenedioxyethylamphetamine (MDE) [32].

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