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Relevant Metabolic and Pharmacokinetic Aspects of Ecstasy Consumption

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Introduction

3,4-methylenedioxyamphetamine (MDMA, ecstasy) is a stimulant drug included in WADA proficiency testing scheme (in competition). MDMA has some relevant metabolic and pharmacokinetic properties (i.e. non-linear pharmacokinetics [1], enantioselective disposition [2-4]) to be considered when dealing with a MDMA positive sample.

A simplified scheme of MDMA metabolic disposition in humans is presented in Figure 1. Briefly, MDMA is mainly *O*-demethylated to 3,4-dihydroxymetamphetamine (HHMA) (a reaction partially regulated by CYP2D6) followed by *O*-methylation to 4-hydroxy-3-methoxymetamphetamine (HMMA) (a reaction regulated by catechol-methyltransferase). At a lower rate, MDMA is *N*-demethylated to 3,4-methylenedioxyamphetamine (MDA) (a reaction regulated by CYP2B6) that is further metabolized to the catechol intermediate (3,4-dihydroxyamphetamine, HHA) and finally *O*-methylated to 4-hydroxy-3-methoxyamphetamine (HMA) [5-7]. MDMA and MDA are detected by using standard analytical methods (i.e. GC/NPD procedure) for volatile nitrogen compounds [8]. If samples are submitted to an enzymatic hydrolysis, also HMMA and HMA are detected by GC/MS or by GC/NPD after their derivatization (i.e. acylation with MBTFA) [4].

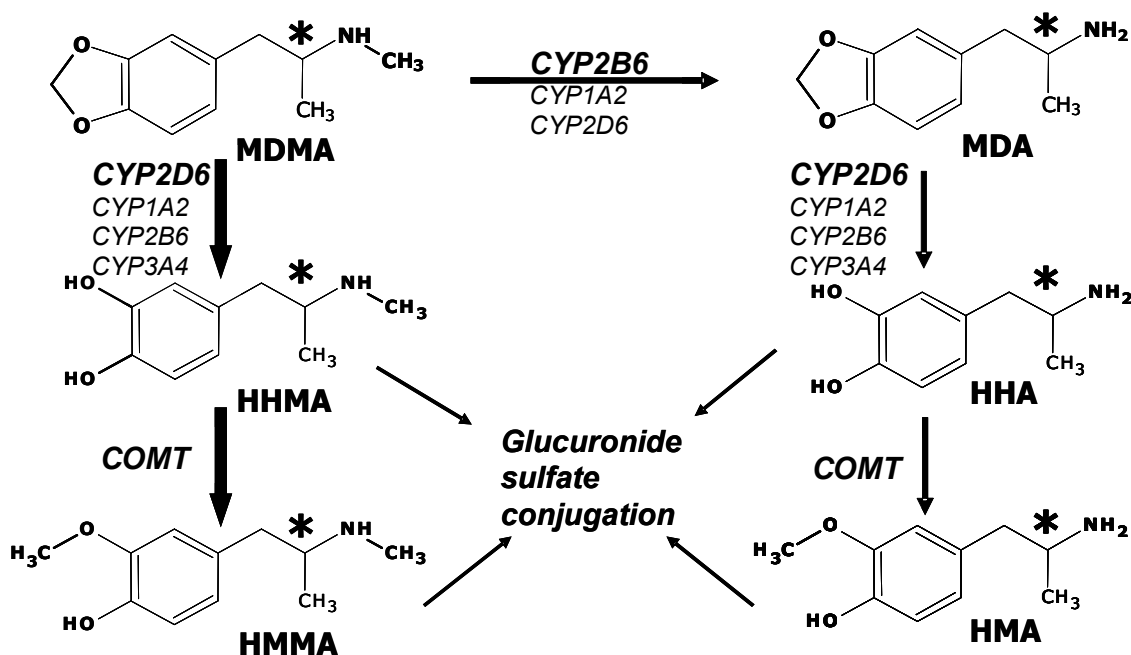


Fig. 1.- MDMA main metabolic pathways in humans. Enzymes regulating them are in italics and the most important emphasised in bold face.

MDMA, 3,4-methylenedioxy-methamphetamine; MDA, 3,4-methylenedioxyamphetamin;

HHMA, 3,4-dihydroxy-methamphetamine; HHA, 3,4-dihydroxyamphetamin,

HMMA, 4-hydroxy-3-methoxy-methamphetamine; HMA, 4-hydroxy-3-methoxyamphetamin.

Studies with different doses of MDMA demonstrated that a linear increase of dose is translated into an exponential increase of maximum MDMA concentration. On the contrary, there are no changes in that of HMMA. In other words, MDMA has a non-linear pharmacokinetics, because the formation of an enzyme-metabolite complex in its first metabolic step. [1, 9]

MDMA has four different substituents in the α carbon of its chemical structure that define a chiral centre, preserved in its metabolites and in also all methylenedioxy amphetamine derivatives. MDMA and related compounds are consumed as racemates, a 1:1 mixture of its enantiomers. However, each MDMA enantiomer has specific properties and some studies point towards (*S*)-MDMA being more active in the central nervous system than (*R*)-MDMA.

[10] Also (*S*)-MDMA has been related to the neurodegeneration of the serotonergic system [11]. MDMA enantiomers are metabolized at different rates by CYP2D6, as this enzyme has higher affinity for (*S*)-MDMA than for its (*R*)-enantiomer. MDMA enantiomeric ratios in biological fluids may vary from 1 to higher than 1 and the magnitude of this ratio and those of MDMA metabolites may be of use to estimate time elapsed after MDMA administration.

The aim of the present study was to evaluate some parameters that may help to distinguish between an ergogenic use of MDMA versus its consumption for a recreational purpose. Analytical developments for the determination of MDMA and metabolites enantiomers presented in this manuscript may be easily adapted to the enantioselective analyses of other chiral amphetamine derivatives and substances which metabolism gives rise to amphetamine and/or methamphetamine.

Methodology

Urine samples from six healthy volunteers, recreational users of MDMA, who participated in a clinical trial (administered dose: 100 mg of (*R,S*)-MDMA·HCl) were analysed. Participants were phenotyped with dextromethorphan for CYP2D6 enzyme activity, and all were categorized as extensive metabolizers [12]. Urine was collected at the following periods: 0-24, 24-48 and 48-72 hours.

Diastereoselective analysis was performed by GC/MS in a 2%-phenylmethylsiloxane capillary column (12 m x 0.2 mm I.D. x 0.3 µm film thickness) after a solid-liquid extraction with Bond Elut Certify[®] columns (ethylacetate with 2% ammonium hydroxide as eluent mixture) and derivatization with *N*-methyl-bis(trifluoroacetamide), MBTFA. [4].

An enantioselective derivatization followed by a conventional GC/MS analysis was chosen for the enantiomeric approach (also a 2%-phenylmethylsiloxane capillary column with 12 m x 0.2 mm I.D. x 0.3 µm film thickness). Extraction procedure was the same than those of diastereoselective determination but for the analysis of MDMA and MDA enantiomers, enantioselective derivatization was performed with the pure *R* enantiomer of (*R*)-(-)- α -methoxy- α -trifluoromethylphenylacetyl chloride, (*R*)-MTPCl, also known as Mosher reagent, in 1:1 mixture of ethyl acetate and hexane with 15% of triethylamine (80°C, 20 min). For the derivatization of hydroxyl residues, a treatment with ammonium hydroxide after the reaction with Mosher reagent (80°C, 20 min) was required. After discarding ammonium salts by

centrifugation and decantation, solvents were evaporated to dryness. Dry residues were derivatized with 1,1,1,3,3,3-hexamethyldisilazane, HMDS (60°C, 60 min) [13].

Results and Discussion

There are different approaches for the study of chiral compounds, but in all cases, an enantioselective environment capable of distinguishing between a pair of enantiomers is needed. For the enantiomeric analysis of MDMA and its main metabolites' enantiomers, an enantioselective derivatization procedure and analysis by conventional GC/MS was chosen. The same extraction protocol used for diastereoselective determination was applied for the enantioselective approach. For the enantioselective derivatization of compounds with the methylenedioxy substituent, an incubation with (*R*)-MTPCl was sufficient. As this reaction generates hydrochloric acid that prevents the quantitative amine derivatization, it is very important the addition of small amounts of triethylamine in the reaction mixture, capable of neutralizing acid. This represented a substantial improvement of the reaction yield. For the inclusion of hydroxylated compounds in the analytical method, a treatment with ammonium hydroxide after the enantioselective derivatization of amines is required, as both amine and hydroxyl groups undergo derivatization with the Mosher reagent. Ammonium hydroxide breaks bonds formed with hydroxyl groups without affecting amine derivatives. Finally, hydroxyl groups are derivatized with a mild silylating reagent HMDS. Figure 2 shows the three-steps enantioselective derivatization procedure with MDMA and HMMA as an example of non-hydroxylated and hydroxylated compounds, respectively.

Results from urine samples are summarized in table 1 and 2. Mean urinary recoveries (μmol , % dose) of the six volunteers who participated in the clinical trial and concentration ranges (minimum value-maximum value) ($\mu\text{g/mL}$) of MDMA, MDA and HMMA measured at the different time frames after 100 mg of MDMA·HCl racemate administration (n=6) are presented. A high interindividual variability in MDMA and MDA urinary concentrations is observed. As stated before, the six participants were phenotyped as extensive metabolizers for CYP2D6. Nevertheless, although the performed phenotyping test is capable of detecting poor metabolizers, it is unable to distinguish between ultra-rapid, extensive and intermediate metabolizers and all of them are categorized as "extensive metabolizers". An accurate genotyping of the volunteers for CYP2D6 activities may help to explain the variability in the metabolites recoveries.

Because variability is so high, table 1 values should be taken carefully. Table 2 contains maximum and minimum measured concentration values of MDMA, MDA and HMMA obtained for the 6 volunteers. This table may be used as a guide to the know what may be the expected concentration values for the MDMA, MDA and HMMA in a MDMA positive urine.

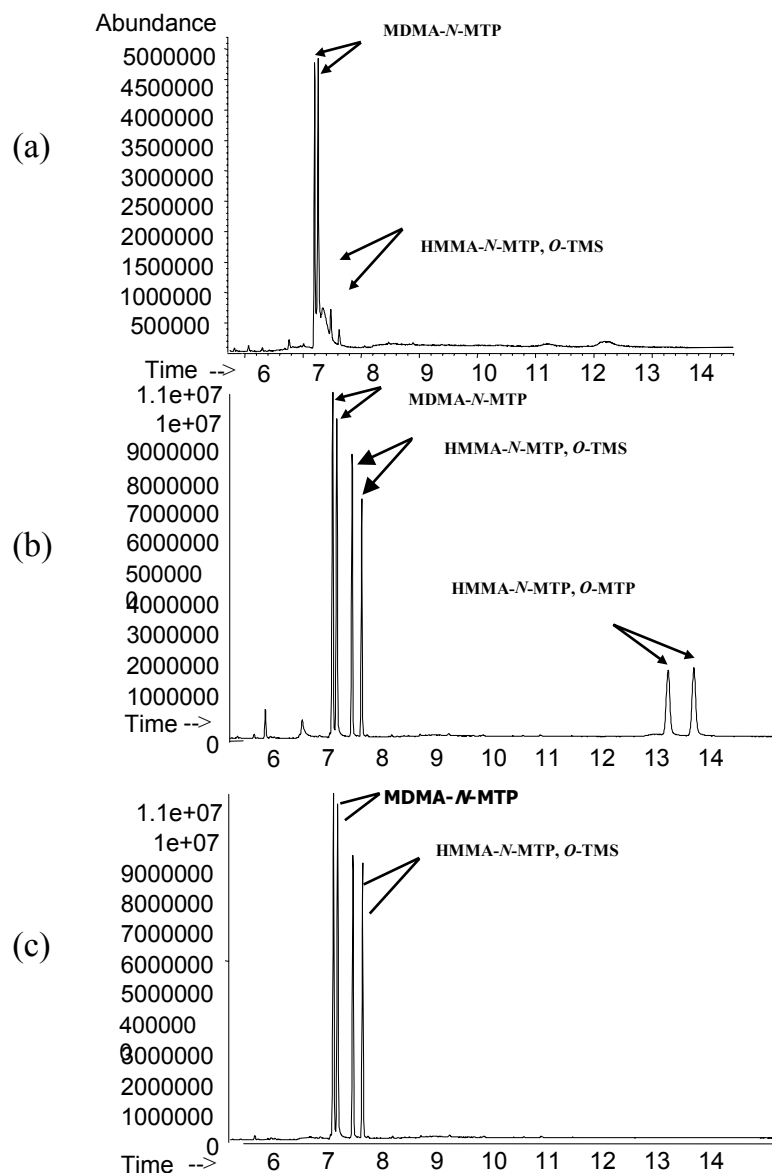


Fig. 2.- chromatograms showing derivatives obtained during the enantioselective derivatization procedure of MDMA and HMMA after incubation with 1)(*R*)-MTPCl in 1:1 mixture of ethylacetate/hexane, 2) HMDS (a); 1) (*R*)-MTPCl in 1:1 mixture of ethylacetate/hexane with triethylamine 2) HMDS (b); 1) (*R*)-MTPCl in 1:1 mixture of ethylacetate/hexane with triethylamine, 2) NH₃ and 3) HMDS (c)

Table 1.- Mean urinary recoveries (μmols , % dose) (n=6) of MDMA, MDA and HMMA after the administration of 100 mg of MDMA·HCl racemate

μmols (%dose)	0-24h	24-48h	48-72h
MDMA	91.3 \pm 48.0 (17.6%)	20.0 \pm 13.5 (3.9%)	3.4 \pm 5.2 (0.6%)
MDA	6.0 \pm 62.3 (1.2%)	2.8 \pm 1.6 (0.5%)	0.7 \pm 1.0 (0.1%)
HMMA	52.2 \pm 54.7 (10.1%)	13.8 \pm 7.2 (2.7%)	8.4 \pm 4.8 (1.4%)

Table 2.- Concentration ranges (minimum measured value-maximum measured value) ($\mu\text{g/mL}$) of MDMA, MDA and HMMA measured for the 6 volunteers after the administration of 100 mg of MDMA·HCl racemate of MDMA.

$\mu\text{g/mL}$ (minimum-maximum)	0-24h	24-48h	48-72h
MDMA	4.6-48.2	4.1-37.1	0.3-13.8
MDA	0.1-4.2	0.6-4.2	0-2.3
HMMA	1.5-37.0	6.3-27.8	4.1-17.4

Figure 3 shows (*R*)/(*S*) enantiomeric ratios of MDMA (a), HMMA (b) and MDA (c), respectively, obtained by the enantioselective analysis of urine samples. Although MDMA is taken as a racemate, this 1 to 1 initial enantiomeric ratio increases after drug administration because of the enantioselective metabolism of CYP2D6. MDMA enantiomeric concentrations gives rise to ratios close to 2 but after that, enantiomeric ratios increases because (*S*)-MDMA is practically undetected and almost all MDMA present in urine corresponds to (*R*)-MDMA. Figure 4 shows pharmacokinetics of MDMA enantiomers, and confirms that 24h post-administration almost all MDMA present in urine corresponds to (*R*)-MDMA, detectable until 72h post-administration [14].

MDA ratio is reverse to those of MDMA at first 24h and then, ratios experience a change from lower than 1 to higher than 1. In any case, MDA concentrations are very low compared to those of MDMA and HMMA, as biotransformation of MDMA to MDA is a minor metabolic pathway.

Finally, HMMA ratio for the first 24h is close to 1 and then ratio increases, because (*S*)-HMMA concentrations are very low compared to those of its *R* enantiomer. It is important to notice that HMMA enantiomeric ratio is also higher than 1, although according to enantioselectivity in this metabolic pathway, an inversion of enantiomeric ratio would be expected and the metabolic inhibition of CYP2D6 may be responsible for these ratios [14]

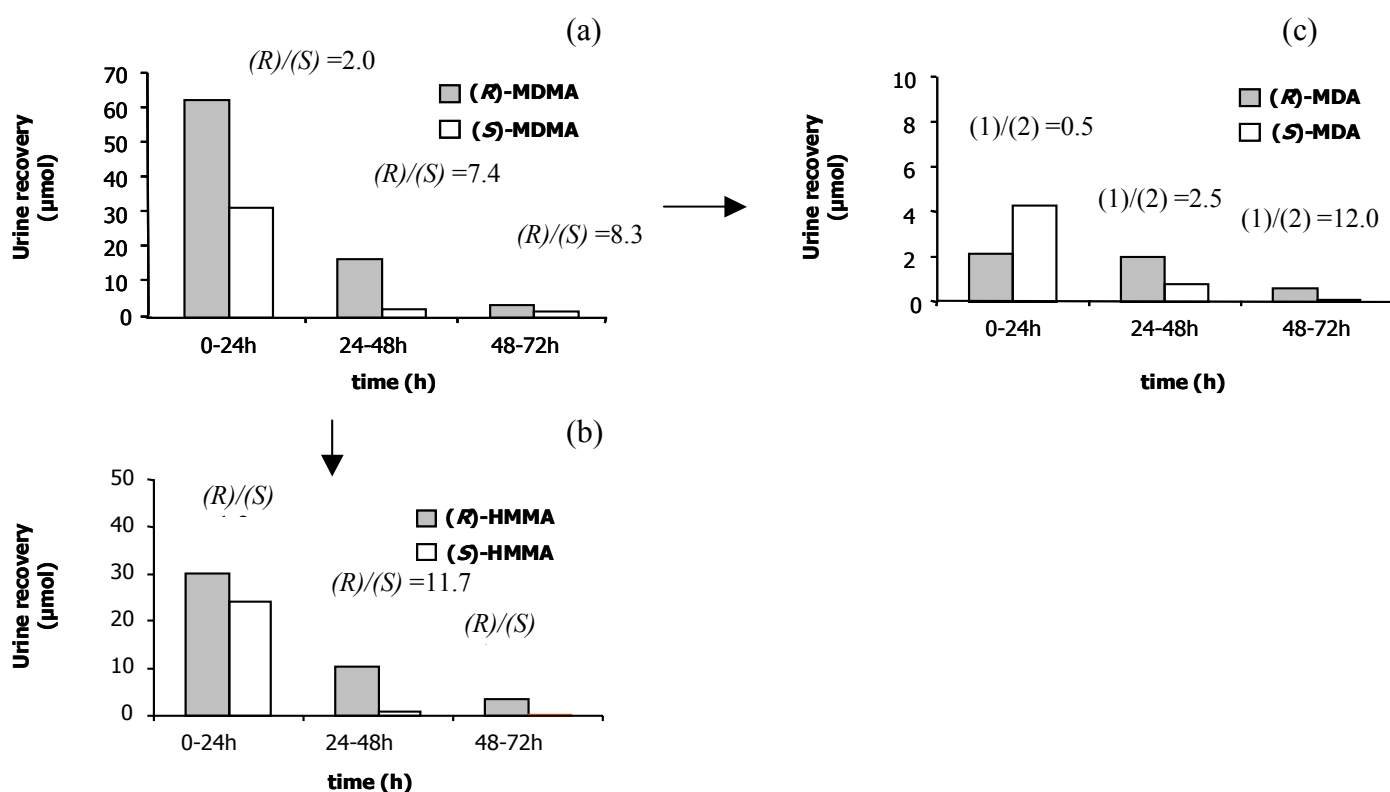


Figure 3.- (*R*)/(*S*) enantiomeric ratios of MDMA (a), HMMA (b) and MDA (c), (n=6, 100 mg MDMA·HCl racemate, p.o.)

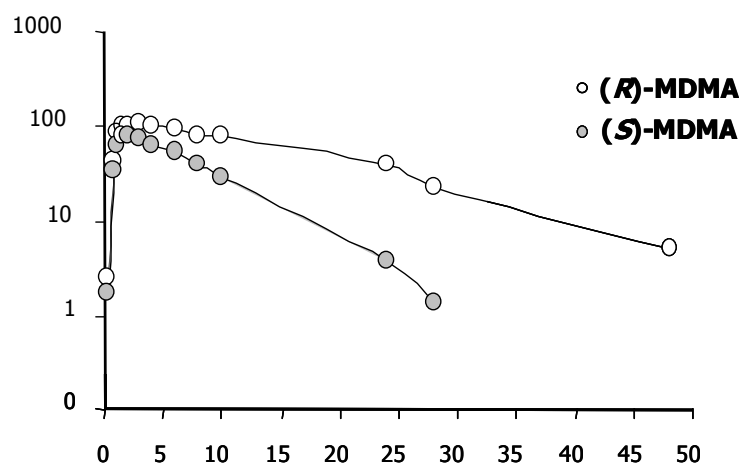


Figure 4.- pharmacokinetics of MDMA enantiomers (n=6, 100 mg MDMA·HCl racemate, p.o.)

Conclusions

MDMA and its main metabolites can be determined by enantioselective methods.

MDMA and its major metabolites (MDA and HMMA) enantiomeric ratios are influenced by some relevant aspects of human MDMA metabolism, like non-linear pharmacokinetics and enantioselectivity in MDMA major metabolic pathway.

The criteria for the interpretation of MDMA positive findings in terms of time elapsed after its ingestion (trying to differentiate a recreational use vs. an ergogenic effect) are:

- MDMA enantiomeric ratios in urine are close to 2 for the first 24h after drug administration. At 48 and 72h, *R/S* ratio is above 7.
- MDA enantiomeric ratios in urine change from lower than 1 (before first 24h) to higher than 2 (after 24h).
- HMMA enantiomeric ratios in urine are close to 1 at 24h after drug administration. The *R/S* ratios at 48 and 72h increase and they have the same order of magnitude than those observed for MDMA.

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