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The implementation of the detection of the abuse of 3,4-methylenedioxyamphetamine and analogues in doping control screening procedures

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Introduction

The abuse of amphetamine designer drugs is in Europe an increasing problem in the field of Drug-Of-Abuse (DOA). It is a problem which affects not only the society in general, but also sports in particular. Although not in order to improve their sport performance, athletes may use amphetamine designer drugs for recreational reasons. Therefore it would be of interest to implement the detection of the abuse of these designer drugs in doping control screening procedures.

Classification

The different amphetamine designer drugs can be classified into 3 groups (Figure 1).

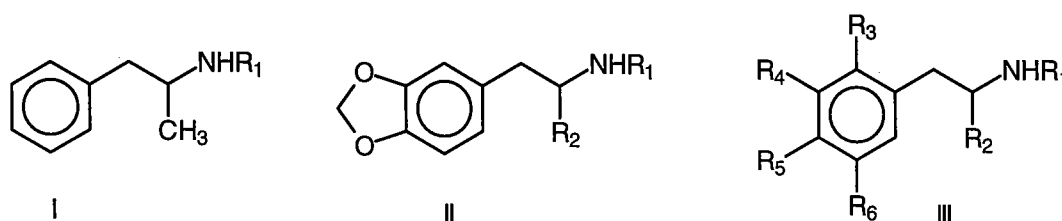


Figure 1 The molecular structures of (I) amphetamines, (II) 3,4-methylenedioxy-phenylalkylamines and (III) polymethoxyphenylalkylamines; R₁ = H, CH₃ or CH₂CH₃; R₂ = H or CH₃; R₃₋₆ = H and/or OCH₃.

The first group are the classical amphetamines (*phenylisopropylamines*). These compounds have a stimulating action and are in sport traditional doping agents. The second group are the 3,4-methylenedioxyphenyl*alkylamines*, which have a so-called entactogenic effect. The last group are the polymethoxyphenyl*alkylamines*, which have at least 2 methoxy groups combined with either an *ethylamine* or an *isopropylamine* group, and possess primarily a hallucinogenic action.

For the 3,4-methylenedioxyphenyl*alkylamines* the *alkylamine* group is either an *isopropylamine* group as with amphetamines or a *2-butanamine*. A selection of the different members of both the 3,4-methylenedioxyphenyl*isopropylamines* and 3,4-methylenedioxyphenyl-*2-butanamines* is shown in Figure 2. This selection is purely based on the occurrence of these compounds in Ecstasy tablets. In principal numerous structural analogues can be synthesized [1]. It is however believed that the compounds shown are the ones with the highest desired pharmacological activity. This article will only focus on the 3,4-methylenedioxyphenyl*isopropylamines*.

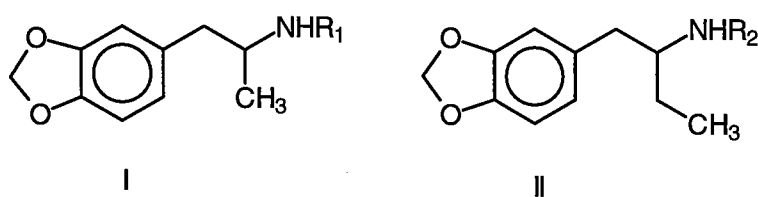


Figure 2 The molecular structures of (I) 3,4-methylenedioxyphenyl*isopropylamines*; $R_1 = \text{H}$: MDA = 3,4-methylenedioxyamphetamine; $R_1 = \text{CH}_3$: MDMA = 3,4-methylenedioxyamphetamine; $R_1 = \text{CH}_2\text{CH}_3$: MDEA = 3,4-methylenedioxyethylamphetamine; $R_1 = \text{OH}$: MDAOH = 3,4-methylenedioxy-*N*-hydroxyamphetamine. (II) 3,4-methylenedioxyphenyl-*2-butanamines*; $R_2 = \text{H}$: BDB = 3,4-methylenedioxyphenyl-*2-butanamine*; $R_2 = \text{CH}_3$: MBDB = 3,4-methylenedioxyphenyl-*N*-methyl-*2-butanamine*.

Metabolism

In order to detect the abuse of the 3,4-methylenedioxyphenylisopropylamines the metabolism must be considered first. The metabolism of for example MDMA (= 3,4-methylenedioxy-*N*-methylamphetamine or 3,4-methylenedioxymethamphetamine) has been well described [2]. MDEA (= 3,4-methylenedioxy-*N*-ethylamphetamine) and MBDB (= 3,4-methylenedioxyphenyl-*N*-methyl-2-butan-amine) are metabolized through similar routes [2], sometimes leading to identical metabolites as for MDMA. MDA (= 3,4-methylenedioxyamphetamine), MDAOH = 3,4-methylenedioxy-*N*-hydroxyamphetamine and BDB (= 3,4-methylenedioxyphenyl-2-butanamine) are for example besides metabolites of MDMA, MDEA or MBDB, also pharmacologically active and possible parent compounds in Ecstasy tablets.

The metabolites of MDMA can be divided into chiral and non-chiral metabolites. In our studies regarding these metabolites the Netherlands Institute Drugs and Doping Research is synthesizing these metabolites itself [4]. In the first part of the metabolic steps of MDMA the chiral center remains intact (Figure 3). The steps involve demethylation at the amine function and the 3,4-methylenedioxy ring, followed by methylation at the 3-hydroxy group of the phenyl-ring and/or hydroxylation at the amine function. All these metabolites have a basic nature and those which have a hydroxy group will be conjugated with glucuronic acid or sulfate. The dihydroxy and *N*-hydroxy metabolites are relatively unstable and are difficult to detect.

The biotransformation of MDEA is very similar to that of MDMA. The only difference is the formation of HHEA (= 3,4-dihydroxy-ethylamphetamine) and HMEA (3-methoxy-4-hydroxy-ethylamphetamine). It has been reported that the demethylation of the 3,4-methylenedioxy ring of MDEA is more prominent than that of MDMA, making the step which leads to MDA a more minor metabolic route [3].

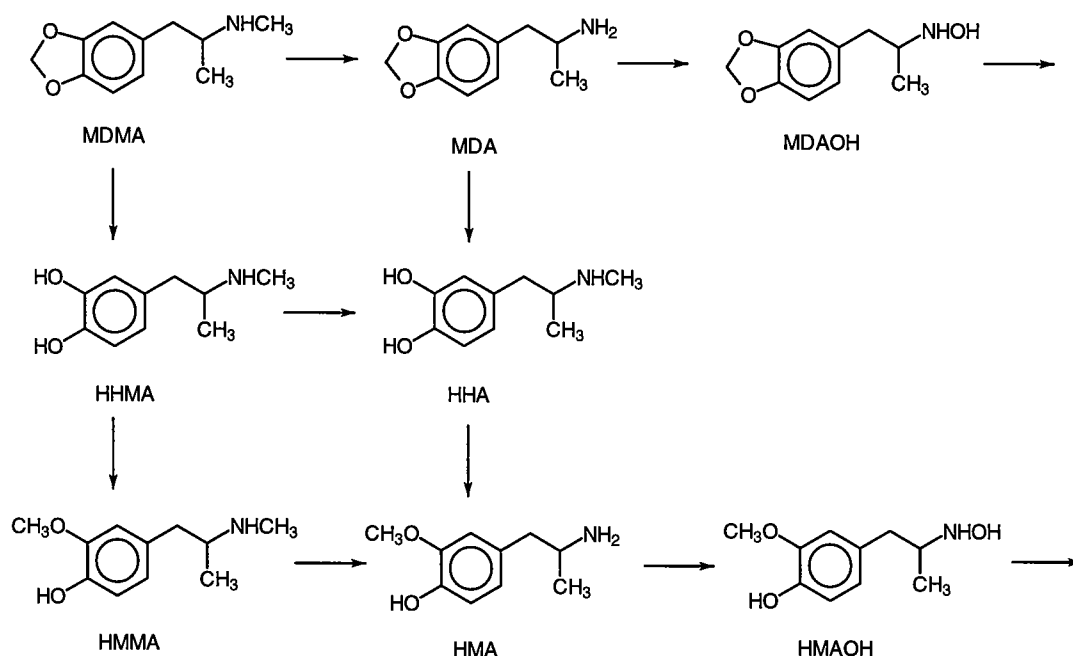


Figure 3 First part of the metabolism of MDMA. MDMA = 3,4-methylenedioxy-methamphetamine; MDA = 3,4-methylenedioxyamphetamine; MDAOH = 3,4-methylenedioxy-*N*-hydroxyamphetamine; HHMA = 3,4-dihydroxy-methamphetamine; HHA = 3,4-dihydroxyamphetamine; HMMA = 3-methoxy-4-hydroxymethamphetamine; HMA = 3-methoxy-4-hydroxyamphetamine; HMAOH = 3-methoxy-4-hydroxy-*N*-hydroxyamphetamine.

The second part of the metabolism of 3,4-methylenedioxyphenylisopropylamines is identical for the different analogues. The amine function is oxidized and the chiral center is lost (Figure 4). Further oxidation followed by conjugation with glycine finally results in hippuric acid metabolites. Besides the intermediary neutral metabolites, the non-chiral metabolites are of acidic nature. The neutral metabolites can be conjugated with glucuronic acid or sulfate at the hydroxy position if present or with sulfate at the keto position in order to enolic conjugated metabolites.

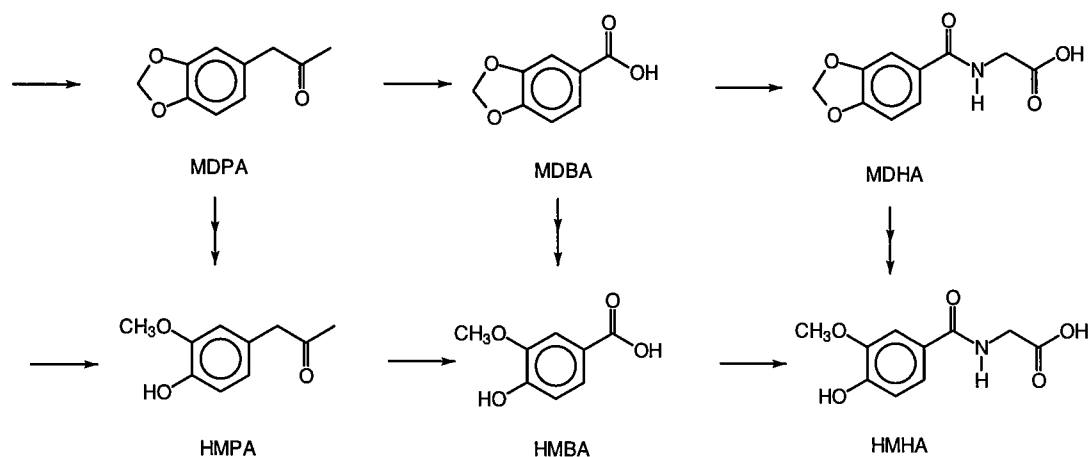


Figure 4 Second part of the metabolism of 3,4-methylenedioxyisopropylamines. MDPA = 3,4-methylenedioxyphenylacetone; MDBA= 3,4-methylenedioxybenzoic acid; MDHA = 3,4-methylenedioxyhippuric acid; HMPA = 3-methoxy-4-hydroxyphenylacetone; HMBA = 3-methoxy-4-hydroxybenzoic acid; HMHA = 3-methoxy-4-hydroxyhippuric acid.

The presence of acidic 3-methoxy-4-hydroxyphenyl metabolites is not specific for the abuse of 3,4-methylenedioxyphenylisopropylamines as vanillin (3-methoxy-4-hydroxybenzaldehyde) also is converted into these metabolites (Figure 5). Identification of the abuse of 3,4-methylenedioxyphenylisopropylamines can therefore not be based on these acidic 3-methoxy-4-hydroxyphenyl metabolites.

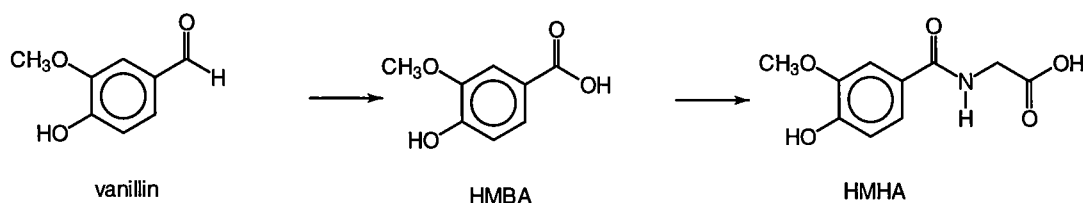


Figure 5 Metabolism of vanillin. HMBA = 3-methoxy-4-hydroxybenzoic acid; HMHA = 3-methoxy-4-hydroxyhippuric acid.

It is believed that the potential toxicity of MDMA is mainly caused by metabolites and not by the parent compound. Regarding the neurotoxicity of MDMA several

metabolites with 2 or even 3 hydroxy groups at the phenyl ring are of special interest. Hepatotoxicity on the other hand may be correlated to MDAOH [4].

Implementation in doping analysis

Depending on the compound analyzed, the implementation of the detection of the abuse of the 3,4-methylenedioxyphenylisopropylamines designer drugs in doping control can be achieved in several screening procedures. Table 1 presents an overview of common screening procedures and the respective compounds which can be detected by that procedures.

Table 1 Implementation of MDMA and some metabolites in doping control screening procedures

procedure no.	specification of procedure	type of compound and/or metabolites detected
procedure I	volatile stimulants and narcotics - no hydrolysis and basic extraction - GC/NPD	MDMA, MDA, MDEA
procedure II	stimulants, β -blocking agents, narcotics - hydrolysis and basic extraction - <i>N</i> -TFA- <i>O</i> -TMS derivatization - GC/MS	all chiral metabolites and neutral non-chiral metabolites
procedure V	diuretics, pemoline, dextropropoxyphene - extractive methylation or basic and acidic extraction - <i>N,O</i> -methyl derivatives - GC/MS	all metabolites
procedure VI	miscellaneous - immunoassays - no extraction/ sometimes a wash step - several commercial assays available	depending on assay specificity

The non-conjugated basic compounds MDMA, MDA and MDEA can easily be detected with screening procedure I. The other basic compounds are in principal conjugated and will only be observed using screening procedure II. Some mass spectrometric characteristics of MDMA, MDA, MDAOH, HMMA and HMA are shown in Table 2. Depending on the isolation procedure neutral metabolites may also be found with screening procedure II [3], but because up to now these compounds are considered to be less important no mass spectrometric characteristics are shown in Table 2.

The same compounds as well as the acidic metabolites may appear in the screening procedure V as their *N,O*-methyl derivatives. Because screening procedure II is more important for the respective compounds no mass spectrometric characteristics of the *N,O*-methyl derivatives of the different compounds are given.

Immunoassays (screening procedure VI) are sometimes used in order to obtain screening results rapidly. Commonly used commercial immunoassays for amphetamine-like drugs are for example FPIA (fluorescence polarization immunoassay) or EMIT® (enzyme multiplied immunoassay technique). The antibodies applied in different immunoassays for detecting abuse of amphetamine-like drugs vary in specificity and have varying degrees of cross-reactivity to other compounds. The antibodies, which have produced, are primarily directed against (*S*)-(+)-amphetamine or (*S*)-(+)-methamphetamine. Information on the immunogen structures used and the specificities of the antibodies obtained, have allowed insight in structure-specificity [5,6]. The assays intended for example to detect either (*S*)-(+)-amphetamine or (*S*)-(+)-methamphetamine with minimal cross-reaction, employ immunogens with amphetamine or methamphetamine derivatized via the *para*-position of the phenyl ring. Such assays theoretically show minimal cross-reactivity with other secondary or tertiary amines and respective (*R*)-(-)-enantiomers, but may strongly cross-react with phenyl ring substituted analogs, including the 3,4-methylenedioxyamphetamines. On the other hand, assays intended for detection of both (*S*)-(+)-amphetamine and (*S*)-(+)-methamphetamine employ amphetamine, rather than methamphetamine, derivatized via its amino group as an immunogen. Such assays theoretically show minimal cross-reaction with other tertiary amines and with phenyl-substituted amphetamines. The FPIA and EMIT® based assays proved to possess significant cross-reactivity with the amine-like parent compounds and metabolites, except for the 3-methoxy-4-hydroxyphenylisopropylamine-like metabolites, for which the assay

antibodies had low or no cross-reactivity.

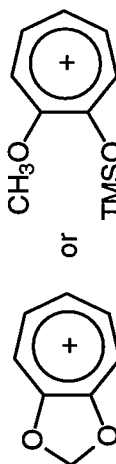
Conclusions

1. Besides the parent compounds several kinds of metabolites can be found in urine after the abuse of 3,4-methylenedioxymethamphetamine and analogues.
2. Depending of the procedure the detection should be focussed on certain metabolites.
3. Current commercial immunoassays are only specific for those metabolites, which still have their amine function, except those which also have both the 3-methoxy and 4-hydroxy substituent.

Table 2 Mass spectrometric data of 3,4-methylenedioxyphenylisopropylamines designer drugs in doping control screening procedures

derivatized compound	MW	M ⁺	[M - CH ₃] ⁺	[M - CF ₃ CONHR] ⁺	I ⁺ §	[I - CH ₂ O] ⁺	[M - I] ⁺
<i>N</i> -TFA- <i>O</i> -TMS-HMA	349	349(15)	335(5)	236(17)	209(100)	179(25)	140(9)
<i>N</i> -TFA- <i>O</i> -TMS-HMMA	363	363(10)	348(5)	236(47)	209(100)	179(21)	154(27)
<i>N</i> -TFA-MDA	275	275(15)	n.f.	162(38)	135(100)	n.f.	140(7)
<i>N</i> -TFA-MDMA	289	289(12)	n.f.	162(69)	135(56)	n.f.	154(100)
<i>N</i> -TFA- <i>O</i> -TMS-MDAOH	363	363(3)	348(1)	162(100)	135(85)	n.f.	228(24)

§ structure of I⁺ (tropylium ion)



explanation of abbreviations used:

HMA = 3-methoxy-4-hydroxy-amphetamine
 HMMA = 3-methoxy-4-hydroxymethamphetamine
 MDA = 3,4-methylenedioxyamphetamine
 MDAOH = 3,4-methylenedioxy-*N*-hydroxyamphetamine
 MDMA = 3,4-methylenedioxy-methamphetamine
 TFA = trifluoroacetyl
 TMS = trimethylsilyl
 n.f. = not formed

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