Determination of 3,4-methylenedioxymethamphetamine (MDMA) and metabolites in urine following administration to the greyhound

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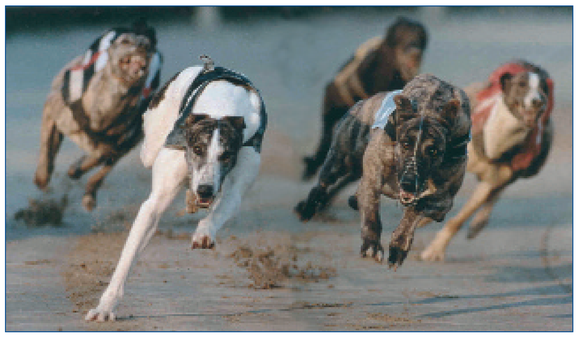
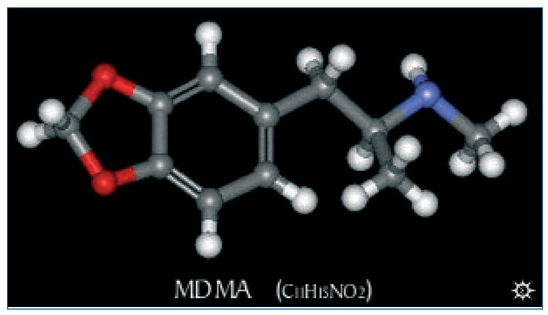
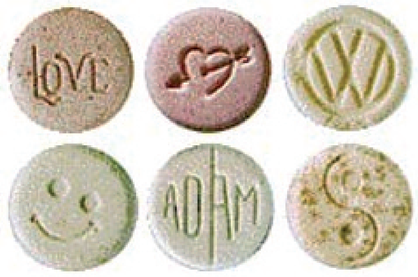
Introduction



With street drugs and drugs of abuse representing a potential source of doping agents in greyhound racing, it is necessary to have detection capability for many of these compounds. Stimulants are an important category of street drugs and include a number of compounds related to amphetamine, such as MDMA, a synthetic, psychoactive drug, commonly known as 'Ecstasy'. MDMA (3,4-methylenedioxymethamphetamine) is a ring-substituted derivative of phenethylamine and a structural analogue of amphetamine. Other drugs in this class of compounds include methamphetamine, 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxyethylamphetamine (MDEA). MDMA is a popular drug of abuse, which has both stimulant and hallucinogenic effects in humans but has associated with it a number of side effects including hyperthermia, memory loss, cognitive impairment and long-term neurochemical/brain cell damage. The ability to detect the administration of MDMA to the greyhound is therefore beneficial from both a doping control and welfare point of view.





Experimental

Animal administration

Six greyhounds in total were used and each dog received a single 10 mg oral dose of MDMA. Pre- and post administration urine samples were collected at regular intervals for the duration of the study (3 days). From these, blank, high, medium and low concentration pooled samples were prepared.

Sample preparation and extraction

Pooled post administration urines (4 ml) were hydrolysed overnight at 37°C using Helix pomatia (50 µl ≈ 7,000 units). The samples were extracted using Bond Elut Certify™ (500 mg, 6 ml) SPE cartridges (Varian, UK), preconditioned with methanol and water. Following addition of the sample, cartridges were washed with acetic acid and methanol before eluting with 2% ammonia in ethyl acetate. The eluates were reduced to dryness under nitrogen at 40°C. Trimethylsilyl (TMS) derivatives were formed prior to gas chromatographic-mass spectrometric (GCMS) analysis by addition of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) in toluene to the extracts (35% v/v, 50 µl) and heating for 30 min at 80°C.

Analysis

Derivatised sample extracts were analysed by GCMS using a Fisons MD800 bench-top mass spectrometer interfaced to a Thermoquest Trace 2000 series GC. The instrument was operated in full scan positive ion electron ionisation mode (EI+-GCMS); mass range 40-550 amu and scanning from 5–18 min. Analyte separation was achieved on an SGE BPX5 column (approximately 25 m, 0.22 mm id, 0.25 µm film thickness) with helium as the carrier gas. The derivatised extracts (1 ml) were injected in splitless mode (1 min) with injector temperature and interface temperatures at 260°C. The GC temperature programme was as follows: initial temperature 90°C, held for 1 min, ramping from 90°C to 340°C at 15°C min-1, with a final hold time of 4 min. Authentic, certified reference standards of MDMA, 4-hydroxy-3-methoxymethamphetamine (HMMA) and MDA were obtained from Cerilliant, USA and analysed alongside the extracts.

TABLE 1

Analyte	Fragment ions (m/z)	
MDMA *mono*-TMS (MW 265)	250	$[M]^{+\cdot}$-$CH_3^{\cdot}$
	130	$[TMS\text{-}NCH_3CHCH_3]^+$, α-cleavage ion
HMMA *bis*-TMS (MW 339)	324	$[M]^{+\cdot}$-$CH_3^{\cdot}$
	130	$[TMS\text{-}NCH_3CHCH_3]$+, α-cleavage ion
MDA *mono*-TMS (MW 251)	236	$[M]^{+\cdot}$-$CH_3^{\cdot}$
	116	$[TMS\text{-}NHCHCH_3]^+$, α-cleavage ion
HMA *bis*-TMS (MW 325)	310	$[M]^{+\cdot}$-$CH_3^{\cdot}$
	116	$[TMS\text{-}NHCHCH_3]^+$, α-cleavage ion

Results

Mass chromatograms of the ions m/z 116 and m/z 130 (representing α-cleavage ions from the isopropylamine and *N*-methylisopropylamine side chains of MDA and MDMA respectively) were constructed for MDMA and metabolites, namely HMMA, MDA and 4-hydroxy-3-methoxyamphetamine (HMA) in the pooled post administration samples. These are shown in Figure 1. The characteristic fragmentation ions for each analyte are detailed in Table 1. The mass spectra, shown in Figures 2 and 3 indicated the formation of *N*-TMS derivatives, which can be variable depending upon instrument conditions. In the absence of *N*-TMS derivative formation, the characteristic ions detected would correspond to the α-cleavage ions of m/z 58 for MDMA & HMMA and m/z 44 for MDA & HMA.

Figure 1: Ion chromatograms – post administration urine

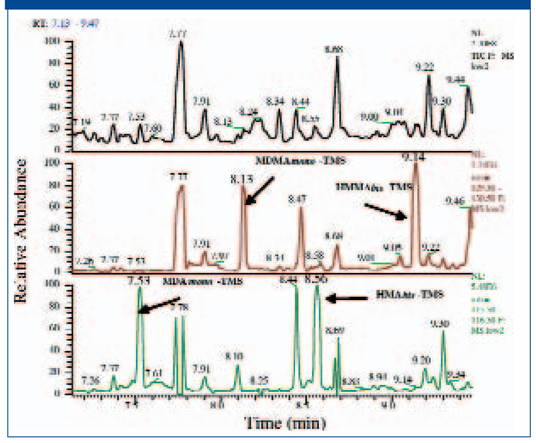
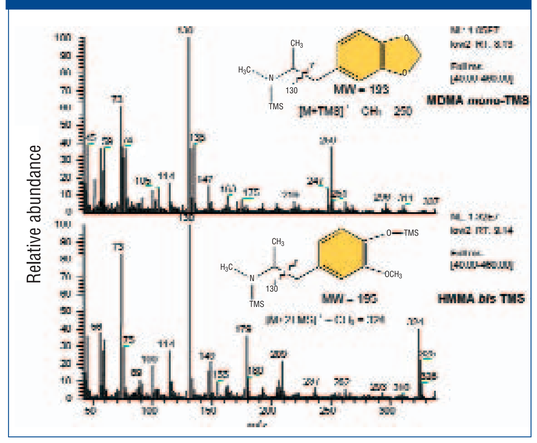


Figure 2: Mass spectra of MDMA and HMMA



MDMA – 3,4-methylenedioxymethamphetamine (MW 193.25)

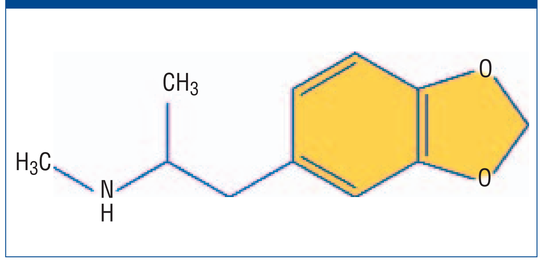
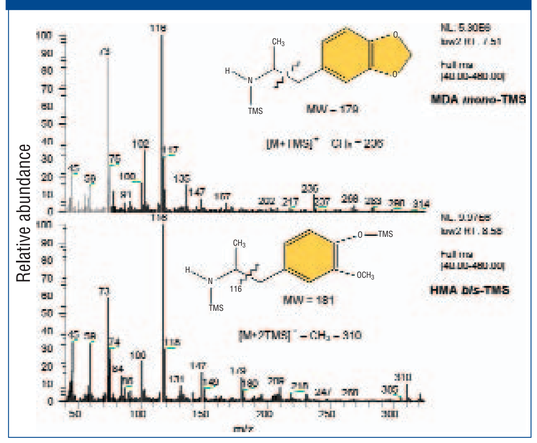


Figure 3: Mass spectra of MDA and HMA



For HMA, no authentic reference standards could be obtained for identification and comparison. A significant peak in the ion chromatogram of m/z 116 was present in the mass spectra of the post administration samples, corresponding to an α-cleavage ion from the isopropylamine side chain of MDA. This peak was not present in the pre-administration urine extract and did not correspond to any of the authentic reference standards. Chemical Ionisation (CI) analysis indicated that this compound had a molecular weight of 325. An ion at m/z 310 was also present in the EI+ mass spectrum, suggesting a loss of a methyl radical from the molecular ion. This analyte was therefore tentatively identified as the bis-TMS derivative of HMA.

Conclusions

Metabolism of MDMA in the greyhound was seen to occur by a number of Phase I biotransformation pathways, including *N*-demethylation to give 3,4-methylenedioxyamphetamine (MDA) along with postulated dealkylation and methylation reactions to give 4-hydroxy-3-methoxymethamphetamine (HMMA). MDA was also further metabolised via the same route to give 4-hydroxy-3-methoxyamphetamine (HMA). Parent MDMA was also readily detectable.

As well as being a metabolite of MDMA, MDA is a drug of abuse in its own right, producing HMA as a metabolite. Therefore, having the ability to independently detect HMA will also prove valuable in controlling any abuse of MDA. It is possible that HMA would also be formed as a metabolite following an administration of 3,4-methylenedioxyethylamphetamine (MDEA), thus providing greater detection coverage for this and potentially other drugs in this class of compounds.

Acknowledgements

- National Greyhound Racing Club (NGRC) for funding this work.
- Walter Hyde and Jackie Peterson (Iowa State University, USA) for the provision of post administration samples.

References

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