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Comparative investigation of disposition of 3,4-(methylenedioxy)methamphetamine (MDMA) in the rat and the mouse by a capillary gas chromatography–mass spectrometry assay based on perfluorotributylamine-enhanced ammonia positive ion chemical ionization*

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Abstract: A gas chromatography–mass spectrometry assay based on perfluorotributylamine-enhanced ammonia positive ion chemical ionization has been developed for MDMA and three of its primary metabolites in biological specimens; the assay is linear from 2 to 1000 ng ml^{-1}. Quantitatively, more of an administered dose of 10 mg kg^{-1} MDMA was excreted by the mouse (72%) than by the rat (35%); most in both species was excreted in urine and within 24 h. The difference in per cent excretion is entirely due to proportionally greater excretion of the parent drug by the mouse. 4-Hydroxy-3-methoxymethamphetamine (HMM) is the major urinary metabolite in both species. HMM and another primary metabolite, 4-hydroxy-3-methoxyamphetamine (HMA), were excreted mainly as glucuronide and sulphate conjugates (>85%).

Keywords: *MDMA; MDA; HMM; HMA; MDE; perfluorotributylamine-enhanced GC-NH_3–PCI–MS assay; comparative disposition studies; rats; mice.*

Introduction

3,4-(Methylenedioxy)methamphetamine (MDMA) has been banned from clinical use as an adjunct to psychotherapy because of numerous reports about its neurotoxic effects in several animal species, including rat [1], mouse [1], guinea pig [2] and primate species [3]. The drug selectively destroys serotonin-containing neurons and this serotonergic toxic effect of MDMA is species-dependent; among the animals investigated, the primate and the mouse are, respectively, the most and least sensitive to the neurotoxic effect of MDMA [1, 3].

Failure of intracerebroventricular injection of MDMA to elicit neurotoxicity in the rat [4] suggested that neurotoxicity depends upon bioactivation of MDMA. Studies to investigate the metabolism of MDMA in the rat [5, 6], the mouse [7] and the human [8] have identified a total of 16 metabolites of MDMA *in vivo* [5, 6, 9]; however, only two of these metabolites (2,4,5-trihydroxymethamphetamine and 2,4,5-trihydroxyamphetamine) are neurotoxic [10, 11]. Furthermore, the actual involvement of these two trihydroxyphenylisopropylamines in the neurotoxicity of MDMA remains to be established.

No qualitative differences between the mouse and the rat have been observed in the *N*-demethylation, *O*-dealkylation, deamination and *O*-methylation of MDMA [7]. The metabolites of MDMA that have been reported for mouse include HMM, HMA, 3,4-(methylenedioxy)amphetamine (MDA), 3,4-dihydroxymethamphetamine, (3,4-dihydroxyphenyl)acetone, (4-hydroxy-3-methoxyphenyl)acetone and [3,4-(methylenedioxy)-phenyl]acetone [7]. Whether the difference in susceptibilities of the rat and mouse to the neurotoxic effects of MDMA is related to quantitative differences in the disposition of MDMA is unknown.

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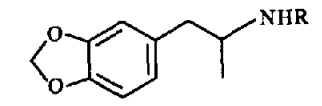
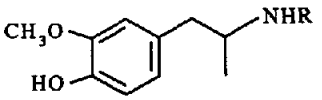
This paper describes development of an assay based on perfluorotributylamine-enhanced gas chromatography–ammonia positive ion chemical ionization mass spectrometry for quantitation of MDMA and three of its primary metabolites (Fig. 1) in biological specimens, and application of this assay to comparative investigation of disposition of MDMA in two animal species where differences in susceptibility to the neurotoxicity of MDMA have been documented.



MDA R = H
MDMA R = CH_3
MDE R = C_2H_5



HMA R = H
HMM R = CH_3

Figure 1
The structures of MDMA and three of its metabolites.

Experimental

Materials

MDMA, MDA and 3,4-(methylenedioxy)-ethylamphetamine (MDE) were purchased from Alltech Applied Science (Deerfield, IL, USA). HMM and HMA were synthesized according to methods reported previously [5]. Trifluoroacetic anhydride (>99%) was obtained from Pierce Chemical Co. (Rockford, IL, USA). All solvents obtained from Burdick and Jackson (Muskegon, MI, USA) were HPLC grade and glass-distilled. The following reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA): sodium hydroxide (98%), sodium acetate trihydrate (99%), anhydrous sodium sulphate (>99%), sodium chloride (>99%), sodium bicarbonate (99%), *N*-methyl-bis(trifluoroacetamide) (98%) and sodium carbonate (>99%). β-Glucuronidase (*Helix pomatia*, type H-1), β-glucuronidase (*E. coli* type VII, lyophilized) and sulphatase (limpets type V, lyophilized) were obtained from Sigma Chemical Co. (St Louis, MO, USA). Ammonium hydroxide and glacial acetic acid were purchased from VWR Scientific Co. (Salt Lake City, UT, USA).

Instrumentation

Quantitative determinations of MDMA and three of its primary metabolites in urine and faeces were performed on a Finnigan-MAT 4500 gas chromatograph–mass spectrometer system. The gas chromatograph was equipped with a DB-5 fused silica capillary column (15 m × 0.25 mm i.d., 0.25 μm film thickness; J & W Scientific, Rancho Cordova, CA, USA), routed through the separator oven (maintained at 250°C) and directly into the ion source of the mass spectrometer. The linear velocity of the carrier gas (hydrogen) was 60 cm s^{-1} at an oven temperature of 100°C. The Grob-type capillary injector was maintained at 260°C and operated in the splitless mode for 0.7 min following injection. After injection, the oven temperature was held at 100°C for 4 min, and then increased linearly to 300°C at 20°C min^{-1}.

The mass spectrometer was operated in the positive ion chemical ionization mode. Typical operating conditions were: electron energy, 80 eV; filament emission current, 15 mA; conversion dynode voltage, −3 kV; and indicated ion source temperature, 100°C. Before analysis, the mass spectrometer was tuned by opening the valve to the instrument's calibration compound inlet after methane was bled in to an indicated ion source pressure of 0.80 Torr; ion source voltages were adjusted so that the positive ion at *m/z* 414 from perfluorotributylamine (PFTBA) displayed a maximum ion current while retaining unit mass resolution and peak symmetry. During analyses, the CI gas consisted of a mixture of ammonia, PFTBA and methane. Ammonia was admitted to an indicated ion source pressure of 0.4 Torr; a trace amount of PFTBA was added by opening the calibrant inlet valve one-half turn, and finally, the indicated ion source pressure was raised to 0.8 Torr with methane.

The mass spectrometer was set to monitor ions at *m/z* 293 $(\{M + NH_4\}^+$ for the trifluoroacetyl {TFA} derivative of MDA), *m/z* 307 $(\{M + NH_4\}^+$ for the TFA derivative of MDMA), *m/z* 321 $(\{M + NH_4\}^+$ for the TFA derivative of MDE), *m/z* 391 $(\{M + NH_4\}^+$ for the TFA derivative of HMA), and *m/z* 405 $(\{M + NH_4\}^+$ for the TFA derivative of HMM). These ions were monitored using a sampling time of 0.05 s per mass and a total cycle time of 0.276 s. TFA derivatives of MDMA and related compounds (500 pg each) were injected to verify the sensitivity of the

GC–MS system before actual analysis of samples. All acquisitions and data reduction were performed with the Finnigan INCOS data system.

Treatment of animals

Male Sprague–Dawley rats (200–250 g, n = 6) and male CF-1 mice (25–30 g, n = 5) were deprived of food overnight prior to subcutaneous injection of 10 mg kg^{-1} MDMA (calculated as free base). The rats were then housed individually in metabolic cages in a room with controlled lighting (12-h light–dark cycle) and temperature (24°C); mice were placed together in one metabolic cage. All the animals had access to food and water for the duration of the study. Urine and faeces were collected at 24, 48, 72 and 96 h after drug administration. The samples were stored in polyethylene bottles at −20°C until analysed.

Assay procedure

All pieces of glassware were vapour-phase silanized [12], rinsed with methanol, and oven-dried before use. To a 10-ml test tube with a Teflon-lined screw cap was added 0.5 ml of urine or 10,000 g-faeces supernatant (30% w/v, prepared in 0.9% saline solution) and 200 ng MDE. The tube was capped and allowed to equilibrate at room temperature by gentle rocking for 15 min. The pH of the sample was adjusted to 5 by addition of 20 μl of 6 M hydrochloric acid and 0.5 ml of 1 M sodium acetate buffer (pH 4.8) containing 2000 units of β-glucuronidase. Enzymatic hydrolysis of the sample was allowed to proceed for 16 h at 37°C. After hydrolysis, the sample was adjusted to pH 2–3 with 105 μl of 6 M hydrochloric acid and extracted with 4 ml of ethyl acetate by gentle rocking for 15 min. After the mixture was centrifuged at 1280 ***g*** for 5 min, the organic phase was removed by aspiration. The remaining aqueous phase was adjusted to pH 9 by addition of 40 μl of 10 M sodium hydroxide and 0.5 ml 1 M sodium bicarbonate–sodium carbonate buffer (pH 9). The sample was saturated with sodium chloride and then extracted with 4 ml of chloroform–*n*-butanol (3:1) by gentle rocking for 15 min. Phase separation was achieved by centrifugation as above and the organic layer was transferred to a 15-ml glass test tube. The remaining aqueous layer was adjusted to pH 12 with 53 μl of 10 M sodium hydroxide, and extracted with 4 ml of chloroform by gentle rocking for 15 min. After another phase separation, the organic phases were pooled and washed with 4 ml of 0.1 M ammonium hydroxide for 10 min with gentle rocking. Following centrifugation, the aqueous layer was removed by aspiration and the organic layer was dried over anhydrous sodium sulphate and transferred to a disposable 10-ml test tube after another centrifugation. The extract was evaporated to dryness under a gentle stream of air at 50°C after addition of 200 μl of 1% hydrochloric acid in methanol.

To the residue in the test tube were added 100 μl of ethyl acetate and 200 μl of trifluoroacetic anhydride. The tube was tightly capped, hand vortexed for 5 s, and heated at 80°C for 20 min. Following evaporation to dryness under a gentle stream of nitrogen at 50°C, 50–200 μl of 5% *N*-methyl-bis(trifluoroacetamide) in toluene was added and the mixture was reheated at 80°C for 5 min. After cooling to room temperature, an aliquot of 1 μl was injected into the GC–MS for analysis.

Working standards

Separate 100-μg ml^{-1} stock solutions of MDMA, MDA, HMA, HMM and MDE were prepared by dissolving an amount equivalent to 1 mg of the free base in 10 ml of methanol. Working solutions containing all four analytes (MDMA, MDA, HMA and HMM), each at concentrations of 20, 50, 100, 250, 500, 1000, 2500, 5000 and 10,000 ng ml^{-1}, were prepared by serial dilution of the stock solutions with water. The internal standard, MDE, was only diluted 10-fold with water to give a working solution with a concentration of 10,000 ng ml^{-1}. All the working solutions were freshly prepared for each analysis.

Calibration curves

Calibration standards containing 1.0, 2.5, 5.0, 12.5, 25, 50, 125, 250 and 500 ng of each analyte (MDMA, MDA, HMM and HMA) were prepared by addition of 50 μl of each of the analyte working solutions to 0.5 ml urine or faeces supernatant. The samples were then processed as described in the assay procedure. Calibration curves were constructed by plotting the ratios of the peak areas of the analyte to the internal standard (MDE) versus concentrations of the analyte. Quality control samples containing 2.5, 5 and 250 ng of each compound in 0.5 ml biological fluid were prepared by addition of 50, 100 and 5000 ng, respectively,

of each compound to 10-ml of drug-free, pooled control rat urine or faeces supernatant; the spiked controls were aliquoted (0.5 ml) into test tubes and frozen at −20°C until analysis.

Precision studies

Intra-assay validation was conducted by analysing on the same day three sets of quality control samples ($n = 5$) at concentrations of 5, 10 and 500 ng ml^{-1}. The concentrations were calculated from the linear regression calibration curves. A five-day inter-assay validation was carried out by analysing three independently prepared quality control samples at concentrations of 5, 10 and 500 ng ml^{-1} on each day; 1 ml of each quality control sample was prepared daily for this study. Routinely, nine calibration standards and three previously aliquoted quality control standards (5, 10 and 500 ng ml^{-1}) were analysed along with samples from rats and mice dosed with MDMA.

Enzymatic hydrolysis

The amount of enzyme required to completely hydrolyse the conjugate of each phenolic amine metabolite of MDMA was determined by analysis of pooled 24-h dosed rat urines treated with β-glucuronidase (*H. pomatia* type H-1) at three different concentrations (500, 2000 and 8000 units ml^{-1}). The amount of each phenolic amine metabolite excreted in dosed-rat urine was established as total, sulphate and glucuronide conjugates according to a published procedure [13] which involved hydrolysis of the dosed-rat and mouse urine with each of the following enzymes: β-glucuronidase (*H.pomatia* type H-1, 2000 units ml^{-1}), sulphatase (limpets type V, lyophilized, 250 units ml^{-1}) and β-glucuronidase (*E. coli* type VII, lyophilized, 2000 units ml^{-1}), respectively. Phenolic amine metabolites in the free form were measured by analysis of unhydrolysed urine samples.

Recovery studies

Recoveries of MDMA and three of its metabolites from urine were determined at concentrations of 10 and 500 ng ml^{-1}. The spiked urine samples were processed as described in the assay procedure except that an accurately measured fraction of the organic phase was removed in each extraction step and the internal standard (MDE, 200 ng) was added to the organic extract rather than to the urine sample. The peak area ratios obtained from these samples were compared to those obtained when equal amounts of MDMA and three of its metabolites were derivatized without prior extraction. Recovery of MDE at concentration of 400 ng ml^{-1} was carried out as above except the internal standard was MDMA (200 ng).

Results and Discussion

GC–MS assay development

In the development of a sensitive and specific GC–MS assay for MDMA and its major metabolites, chemical ionization of the TFA derivatives with various Cl reagent gases was investigated. Methane was found to be unsuitable because it caused considerable fragmentation of the derivatized amines but not the derivatized phenolic amines, even though all the derivatized compounds were ionized with high efficiencies. A mixture of methane and ammonia is often used in this laboratory for quantitative analysis of drugs. With this combination of reagent gases, basic compounds are generally ionized with high efficiency and give Cl mass spectra consisting of a single major peak corresponding to the protonated molecule; nonbasic compounds are ionized with much lower efficiency and often give prominent ammonia adduct ions ($M{\cdot}NH_4^+$) [14]. Addition of a trace amount of an efficient electron-capturing compound, such as perfluorotributylamine or carbon tetrachloride, to the reagent gas mixture is known to provide more efficient formation of positive ions under ammonia chemical ionization conditions [15–17] although the actual mechanism of this phenomenon is uncertain [16]. For the analysis of TFA derivatives of MDMA and its related compounds, the addition of perfluorotributylamine to the methane–ammonia reagent gas mixture resulted in a 10- to 20-fold increase in the intensity of the $M{\cdot}NH_4^+$ ion as shown in Fig. 2. In addition to the $M{\cdot}NH_4^+$ ion, the Cl mass spectra also showed trace amounts of ammonia dimer adduct ions ($M{\cdot}NH_4NH_3^+$) and protonated molecule ions (MH^+).

Initial investigation of the TFA derivative of the hydrolysed basic extract of MDMA-spiked rat urine by the perfluorotributylamine-enhanced GC–CI–MS assay was unsuccessful because compounds from the biological matrix

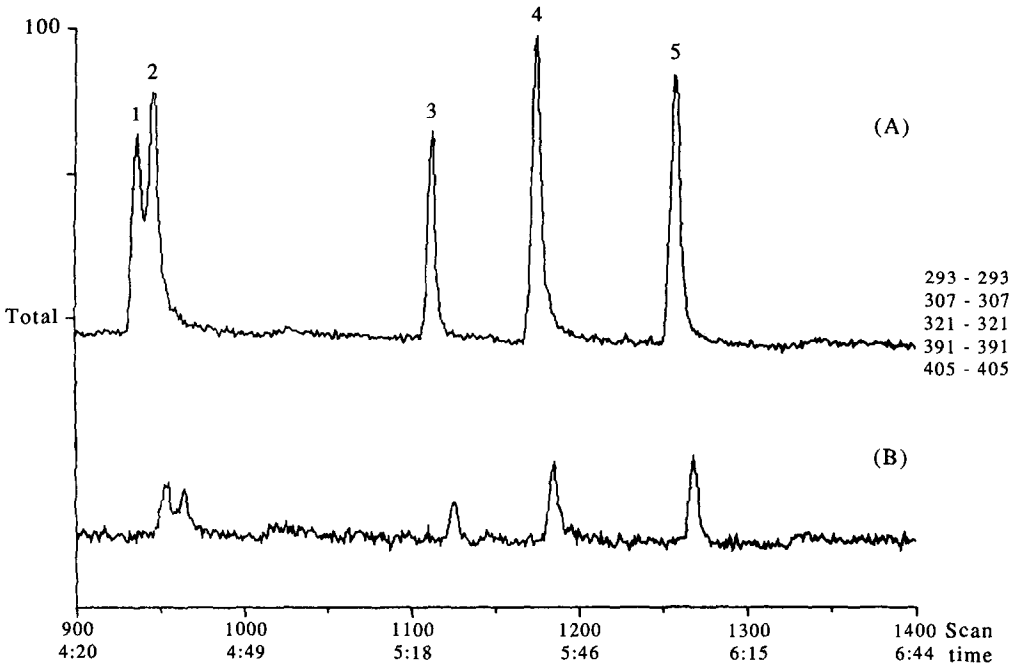


Figure 2
Total ion current profiles from gas chromatographic–positive ion chemical ionization mass spectrometric analysis of the trifluoroacetyl derivatives of MDA (1), HMA (2), HMM (3), MDMA (4) and MDE (5) under selected ion monitoring mode: (top) CH_4-NH_3-perfluorotributylamine mixture as reagent gas; (bottom) same analysis but without the perfluorotributylamine. Amount of each compound injected on column was 500 pg.

co-eluted and interfered with the measurement of these analytes. Fortunately most of these interfering compounds could be removed from the hydrolysed urine by simple extraction into ethyl acetate after acidification. Differences in polarities and pK_as among amines and amphoteric phenolic amine compounds also complicate the development of an extraction scheme that will give both a clean extract and a high extraction efficiency for all the analytes. With these problems in mind, we developed a two-step extraction scheme consisting of initial extraction of the amphoteric phenolic amine compounds at pH 9 with a polar solvent mixture of chloroform–*n*-butanol (4:1), followed by adjustment of the pH to 12 and extraction with chloroform to extract any amine compounds remaining in the aqueous phase after the first extraction at pH 9.

Since the majority of the phenolic amine metabolites of MDMA are excreted in urine as conjugates [5], it was necessary to subject the urine to enzymatic hydrolysis before extraction. Replicate aliquots of pooled 24-h urine from rat injected with 10 mg kg^{-1} of MDMA were hydrolysed with 500, 2000 and 8000 units of β-glucuronidase. No significant differences were found in the measured concentrations of MDMA, MDA, HMM or HMA when the urine samples were hydrolysed with different amounts of β-glucuronidase. Consequently, 500 units of enzyme was considered sufficient for complete hydrolysis of conjugated metabolites.

With the extraction procedure described here, we obtained acceptable extraction efficiencies for MDMA and three of its metabolites from rat urine. Within the range of concentrations examined, the mean recoveries of MDMA, MDA, HMM and HMA were 96, 81, 66 and 73%, respectively. For each analyte, the relative standard deviation (RSD) was less than 15% at each concentration, and extraction efficiency appeared to be independent of concentration for all compounds. Furthermore, the internal standard (MDE) was extracted with comparable efficiency as those of MDMA and its metabolites; a recovery of 71% (RSD = 3.25%, n = 4) was obtained.

For each analyte, the computer-generated regression line of the calibration curve was linear from 2 to 1000 ng ml^{-1} and gave an r^2 of at least 0.99. The limit of quantitation (LOQ) was defined as the lowest concentration of analyte that could be quantitated within 20% of the target value and with an RSD of

≤15%. On the basis of this definition, all of the analytes could be measured at concentrations as low as 2 ng ml^{-1}.

Because a deuterium-labelled analogue of MDMA was not available, we used a homologue of MDMA (MDE) as the internal standard for quantitation of each of the analytes. Before the GC–MS analysis of either rat or mouse urine extracts, calibrator and quality control samples were injected. Only if the quality control samples measured within 20% of their target values was analysis of the rat or mouse urine extracts begun. The quality control sample of lowest concentration was reanalysed after analysis of 10 rat urine extracts. By following this protocol we were able to achieve the precision and accuracy reflected in the results listed in Table 1.

Comparative disposition

The mouse resembles the rat in the metabolism of MDMA, except for a notable difference in the aromatic hydroxylation pathway; that is, this pathway is dose-dependent in the mouse [6, 7]. In view of the similarity in metabolism of MDMA between the two species, we investigated whether differences in the susceptibilities of mice and rats to the neurotoxic effects of MDMA can be attributed to quantitative differences in the disposition of MDMA. The GC–CI–MS assay was applied to quantitation of MDMA and three of its metabolites in hydrolysed and derivatized extracts of urines and faeces from both rats and mice. Reconstructed ion current profiles corresponding to the four respective $M{\cdot}NH_4^+$ ions derived from analysis of 72-h urine from a dosed rat are shown in Fig. 3. The comparative disposition data for rats and mice are compared in Table 2.

The combined amount of MDMA and the three metabolites eliminated in urine and faeces over 96 h in mice (72%) was double that eliminated by the rat (36%) following administration of 10 mg kg^{-1} MDMA (as free base). In faeces only, over 96 h both species excreted comparable amounts of MDMA and the three metabolites corresponded to less than 0.5% of the administered dose. The presence of only trace amounts of phenolic amine metabolites (HMM and HMA) in faeces suggested that there is no appreciable biliary excretion of these compounds even though their glucuronide conjugates exceed the molecular weight of 325 daltons typically required for biliary excretion [18].

Of the quantities of MDMA and the three metabolites recovered in excreta, >99% of the material was present in the urinary fraction. Furthermore, most elimination occurs within 24-h; 24 h urinary excretion accounted for 98% of the 96-h total for rats, and 90% for mice. The difference in 24-h excretion between the two species is due entirely to the excretion of parent drug because comparable amounts of each of the three metabolites were excreted. This discrepancy in urinary excretion cannot be explained by a difference in urinary pH (Table 2). The greater excretion of the parent drug in mice may explain the relative resistance of mice to development of MDMA-induced neurotoxicity; this conclusion is consistent with observations that MDMA-induced neurotoxicity [1] and formation of precursors of the potent neurotoxic trihydroxyphenylisopropylamine metabolites of MDMA [unpublished data] are dose-dependent in mice.

HMM instead of the previously proposed major metabolite MDA [19] appears according

Table 1
Precision and accuracy for determination of MDMA, MDA, HMM and HMA in spiked rat urine

Target conc. (ng ml^{-1})	Mean measured conc. [ng ml^{-1} (RSD*)]			
	MDMA	MDA	HMM	HMA
Intra-assay				
5.0	4.7 (8.2)	5.1 (5.5)	5.9 (11.6)	5.7 (16.0)
10.0	8.3 (11.7)	10.0 (8.3)	11.2 (6.5)	11.5 (6.9)
500.0	440.3 (9.5)	488.1 (6.1)	507.4 (10.0)	516.5 (10.3)
Inter-assay				
5.0	5.2 (14.0)	5.2 (10.3)	4.3 (10.9)	4.7 (11.3)
10.0	10.0 (3.9)	10.0 (3.3)	9.8 (7.7)	10.4 (8.8)
500.0	490.8 (6.4)	489.6 (1.4)	519.3 (5.5)	535.1 (8.9)

*RSD = relative standard deviation (n = 6).

Table 2
Excretion profiles of MDMA and three of its metabolites in urines and faeces from rats and mice injected subcutaneously with 10 mg kg^{-1} MDMA

Specimen	Time (h)	Urine pH	% of dose recovered (±SD*)				% total recovery
			MDMA	MDA	HMM	HMA	
Rat urine	24	7.3 ± 0.6	16.3 ± 5.6	6.3 ± 3.3	10.3 ± 2.9	2.1 ± 0.9	35.0
	48	6.7 ± 0.4	0.02 ± 0.01	0.01 ± 0.001	0.4 ± 0.1	0.1 ± 0.02	0.4
	72	7.0 ± 0.9	0.01 ± 0.01	0.003 ± 0.001	0.1 ± 0.04	0.01 ± 0.004	0.1
	96	6.6 ± 0.2	0.01 ± 0.003	0.001 ± 0.001	0.04 ± 0.02	0.01 ± 0.004	0.1
Rat faeces	24		0.1 ± 0.08	0.04 ± 0.03	0.02 ± 0.03	0.003 ± 0.003	0.2
	48		0.004 ± 0.003	ND	0.002 ± 0.001	ND	0.01
	72		0.003 ± 0.001	ND	ND	ND	0.003
	96		0.003 ± 0.002	ND	ND	ND	0.003
Mice urine†	24	6.0	43.0	7.8	11.9	2.5	65.2
	48	6.0	0.8	0.4	1.3	0.4	2.9
	72	6.1	0.2	0.1	0.5	0.1	0.9
	96	7.8	1.0	0.3	1.3	0.3	2.9
Mice faeces†	24		0.1	0.1	0.1	0.02	0.3
	48		0.004	0.001	0.002	0.001	0.008
	72		0.003	0.001	0.001	ND	0.01
	96		0.01	0.001	0.01	0.002	0.02

*SD = standard deviation (n = 6).
†Mice urine and faeces, pooled from five mice.
ND = not detected.

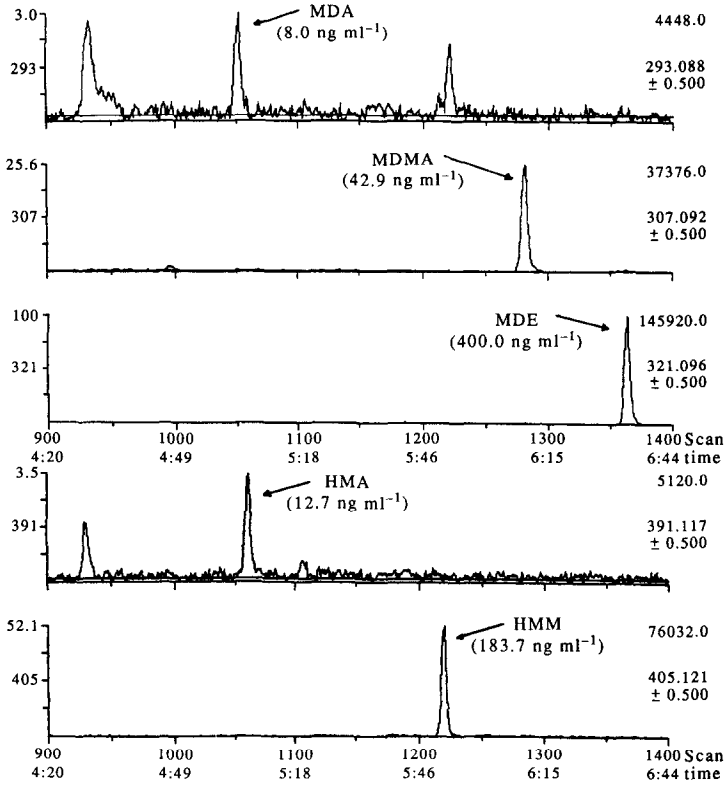


Figure 3
Selected ion current profiles from the gas chromatographic perfluorotributylamine-enhanced ammonia positive ion chemical ionization mass spectrometric analysis of a derivatized extract from a rat's hydrolysed urine collected 48–72 h after administration of 10 mg kg^{-1} MDMA.

Table 3
Recovery of various forms of HMM and HMA from 24 h urine from rats and mice injected subcutaneously with 10 mg kg^{-1} MDMA

	% mean recovery (±SD*)					
	Rats†			Mice‡		
MDMA metabolites	Free	Glucuronide	Sulphate	Free	Glucuronide	Sulphate
HMM	11.8 ± 6.8	42.2 ± 10.8	46.0 ± 4.8	5.7	52.4	41.9
HMA	14.4 ± 5.1	60.0 ± 6.7	25.6 ± 3.1	14.6	39.7	45.8

*SD = standard deviation (n = 6).
†Rats urine (n = 4).
‡Mice urine, pooled from five mice.

to the present data to be the major primary metabolite of MDMA in rat and mice. This discrepancy is attributed to the fact that the phenolic amine metabolites of MDMA had not been identified at the time of the earlier investigation [19]. The phenolic amine metabolites of MDMA (HMM and HMA) are excreted predominantly as conjugates in rat urine [5]; however, until now the relative amounts of the sulphate and glucuronide conjugates were unknown. Structural characterization and quantitation of phase II metabolites by GC–MS is often complicated by the susceptibility of derivatized conjugates (glucuronide and sulphate) to thermal degradation in the injection port of the GC. Consequently, traditional method of enzymatic hydrolysis with pure β-glucuronidase, sulphatase, or a mixture of the two prior to GC–MS analysis for quantitation of phenolic amine conjugates in 24-h urine was used (Table 3). In rats, as much as 88% of HMM and 86% of HMA were excreted in urine as conjugates; in mice, conjugates also represented the major frac-

tions of HMM (94%) and HMA (86%) excreted in the urine (Table 3). Furthermore, rats and mice excreted comparable amounts of HMM as glucuronide and sulphate conjugates. In contrast, rats excreted about twice the amount of glucuronide (60%) as sulphate (26%) conjugate of HMA in urine, although we observed no significant difference in the proportion of the two conjugates of HMA excreted in mice.

Conclusions

A capillary GC–MS assay based on the addition of an electron capturing agent (PFTBA) to increase the ionization efficiency of the ammonia reagent gas has been developed for MDMA and three of its primary metabolites in biological specimens; the assay has been successfully applied to an investigation of comparative disposition of MDMA in two mammalian species with different susceptibilities to MDMA-induced neurotoxicity. A greater fraction of the dose of MDMA is eliminated as parent drug by mouse than by rat. This difference may be related to the relative resistance of the mouse to neurotoxic sequelae of MDMA administration.

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