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Plasma, oral fluid and sweat wipe ecstasy concentrations in controlled and real life conditions

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Abstract

In a double-blind placebo controlled study on psychomotor skills important for car driving (Study 1), a 75 mg dose of $\pm$ 3,4-methylenedioxymethamphetamine (MDMA) was administered orally to 12 healthy volunteers who were known to be recreational MDMA-users. Toxicokinetic data were gathered by analysis of blood, urine, oral fluid and sweat wipes collected during the first 5 h after administration. Resultant plasma concentrations varied from 21 to 295 ng/ml, with an average peak concentration of 178 ng/ml observed between 2 and 4 h after administration. MDA concentrations never exceeded 20 ng/ml. Corresponding MDMA concentrations in oral fluid, as measured with a specific LC-MS/MS method (which required only 50 μ l of oral fluid), generally exceeded those in plasma and peaked at an average concentration of 1215 ng/ml. A substantial intra- and inter-subject variability was observed with this matrix, and values ranged from 50 to 6982 ng/ml MDMA. Somewhat surprisingly, even 4–5 h after ingestion, the MDMA levels in sweat only averaged 25 ng/wipe.

In addition to this controlled study, data were collected from 19 MDMA-users who participated in a driving simulator study (Study 2), comparing sober non-drug conditions with MDMA-only and multiple drug use conditions. In this particular study, urine samples were used for general drug screening and oral fluid was collected as an alternative to blood sampling. Analysis of oral fluid samples by LC-MS/MS revealed an average MDMA/MDEA concentration of 1121 ng/ml in the MDMA-only condition, with large inter-subject variability. This was also the case in the multiple drug condition, where generally, significantly higher concentrations of MDMA, MDEA and/or amphetamine were detected in the oral fluid samples. Urine screening revealed the presence of combinations such as MDMA, MDEA, amph, cannabis, cocaine, LSD and psilocine in the multiple-drug condition.

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Keywords: MDMA; Controlled study; Oral fluid; Sweat; Driving under the influence; LC-MS/MS

1. Introduction

The widespread use of ecstasy ($\pm$ 3,4-methylenedioxymethamphetamine; MDMA) by young people in most western countries has caused concern due to its associated accident risks [1]. In a recent roadside study in Belgium, blood

analysis of drivers suspected of impairment by the police revealed the presence of MDMA in 35% of the cases. Multiple drug use was also quite common, with MDMA often associated with amphetamine, cocaine or cannabis [2].

The present paper reports on the toxicological data obtained from two separate studies [3–5] investigating the effects of MDMA alone and the effects of multiple drug use, i.e. MDMA and a variety of other compounds, on driving behaviour. Since pharmacokinetic data on MDMA are scarce, especially for alternative specimens, the purpose of this paper is to compare biological indices of MDMA-use from different

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body fluids, i.e. plasma, oral fluid, sweat and urine and to assess their applicability for future use in actual traffic surveillance (e.g. application of suitable cut-off values) and in forensic accident analysis. In Study 1, 12 experienced MDMA-users were administered MDMA 75 mg. Oral fluid and sweat concentrations of MDMA were monitored during the first 5 h after administration and compared to the corresponding blood and urine concentrations. In Study 2, 19 subjects were instructed to use only one tablet of MDMA before going to a party; they were unrestricted during the party and used anything they liked. Urine and oral fluid samples were collected when subjects visited the institute of the department of psychology in Groningen where they performed a driving simulator test before and after they went out and on a control night when no drugs were allowed.

2. Experimental design

2.1. Study 1

Twelve experienced MDMA-using but otherwise healthy volunteers (eight male/four female, aged 21–30) were administered MDMA 75 mg (Lipomed, Arlesheim, Switzerland), alcohol 0.5 g/kg or placebo according to double-blind, cross-over design on three separate occasions, spaced 2 weeks apart. MDMA was dissolved in 25 ml of bitter orange syrup, which was ingested at once. The study was approved by the Hospital Ethics Committee of the University of Maastricht. Before participation written informed consent was obtained. Drug use was restricted 2 weeks prior to and alcohol 1 day prior to the experimental sessions. A Lion SD-3 Breath-Alcohol Analyser test and an on-site screening test for the detection of drugs of abuse in urine (Syva Rapidtest, Dade Behring) were performed before the start of the experiment.

An in-dwelling venous catheter was placed in a forearm vein from which blood was drawn at 60 min intervals. Blood samples were taken using Vacutainer[®] tubes with sodium fluoride and potassium oxalate as anticoagulant (Becton–Dickinson Benelux, Belgium) from time 0 to 5 h after administration. Blood samples were cooled to +4 °C and centrifuged after each experiment. Following centrifugation, samples were stored at –20 °C until analysis. Additionally, at the same time points, an oral fluid sample (obtained by spitting in a polypropylene tube), a urine sample, and a sweat sample (by wiping a 5 cm × 5 cm cotton-based fleece—Securetec, Germany—moistened with 0.5 ml of 70% of isopropanol over the forehead) were collected. Oral fluid, urine and sweat samples were frozen immediately.

2.2. Study 2

A total of 19 subjects, 14 male and 5 female, participated in a study on the effects of ecstasy during an evening on which they already intended to use MDMA. Subjects bought ecstasy for their own purpose (and mailed an extra tablet to

the laboratory for substance analysis), using MDMA in a self-determined dosage. During the night of a party two rides in a driving simulator were made, one approximately 1 h after ingestion of MDMA, and one after the party when subjects normally would go home or to an after-party. Subjects were asked to take no more than one tablet before the first test ride. Between the first and second ride, subjects were allowed to take any psychoactive substance in any combination and dosage they would normally use. On a separate evening a non-drugs control test ride was completed at the same hour as the first MDMA ride, and on that day no drugs were allowed. The experimental design was approved by the Ethical Committee of the Department of Psychology of the University of Groningen. Questionnaires on used drugs were administered and subjects were asked to supply a urine sample to perform a general toxicological screening. The study protocol did not allow blood sampling because it was considered too invasive, but oral fluid samples were collected by spitting into polypropylene tubes.

3. Analysis of biological samples

3.1. Plasma and urine

All urine samples were screened with FPIA (Abbott Diagnostics Division, Belgium) for amphetamines, cocaine, cannabis, opiates, and benzodiazepines. Systematic toxicological analysis was performed with GC-MS-EI after solid phase extraction and separate elution of the acidic/neutral fraction and the basic fraction [6]. Specific screening and/or confirmation methods were applied to detect LSD and GHB in urine samples of subjects that reported the use of these drugs [7,8]. The amph and the designer amphetamines were extracted from urine and plasma using Bond Elut Certify columns (Varian, St. Katelijne-Waver, Belgium) according to the manufacturer's instructions [2]. They were derivatised with hepta-fluorobutyric anhydride (HFBA). Quantitative analyses were performed using the deuterated analogues of the analytes of interest and an Agilent 6890 gas chromatograph equipped with an autosampler (HP7673A) and interfaced with an Agilent 5973 mass selective detector (Agilent Technology, Woluwe, Belgium). Analytical conditions were optimised for the detection of amphetamine, methamphetamine, 3,4-methylenedioxy-*N*-amphetamine (MDA), MDMA, 3,4-methylenedioxy-*N*-ethyl-amphetamine (MDEA), *N*-methyl-1-(3,4-methylene-dioxy-phenyl)-2-butanamine (MBDB) and ephedrine. The MS was operated in SIM mode. At least three ions were monitored for the analytes and two ions for the internal standards. Limits of quantitation (LOQ) were 20 ng/ml for MDA and 10 ng/ml for the other amphetamines.

3.2. Oral fluid and sweat

After thorough centrifugation of the oral fluid samples, which were often viscous, 50 µl of clear supernatant was

transferred to an Eppendorf vial using a positive displacement pipette. The internal standards were added to obtain a final concentration of 40 ng/ml of amphetamine- d_{11} , methamphetamine- d_5 , MDA- d_5 , MDMA- d_5 , MDEA- d_6 and ephedrine- d_5 (Promochem, Hertfordshire, UK).

The amphetamines were isolated from oral fluid using a simple methanol clean-up procedure and subsequently analysed using reversed phase HPLC-MS/MS. A Quattro Ultima (Micromass Ltd., UK) tandem mass spectrometer fitted with a z-spray ion source was used for the analyses. The instrument was operated in electrospray positive ionisation mode and was coupled to a Waters 2690 Alliance HPLC system. The mobile phase (A: 10 mM ammonium acetate/B: 95% acetonitrile, 5% 10 mM ammonium acetate) (85/15) was delivered at a flow rate of 0.3 ml/min. A Hypersil BDS C_{18} column (100 mm $\times$ 2.1 mm, 3.5 μ m) was used. Quantification of the drugs and their deuterated analogues was performed using multiple reaction monitoring (MRM). The developed method has a chromatographic run time of <5 min. LOQ were 1–5 ng/ml, estimated by analysis of 50 μ l of blank oral fluid samples spiked with a mixture of amphetamines, where the accuracy was >80% and coefficient of variation <20%. Intra-day and inter-day coefficients of variation for a number of quality control samples varying from 10 to 1000 ng/ml were <10%.

Following the addition of 20 ng of the relevant deuterated standards to the sweat wipes, the amphetamines were recovered from the cotton fleece with 5 ml of methanol, which was concentrated to approximately 100 μ l using vacuum centrifugation (Jouan RC 10.22, Merck Eurolab, Leuven, Belgium). After addition of 100 μ l of LC mobile phase and after filtration through an HPLC-filter, 10 μ l of filtrate was injected into the LC-MS/MS, using identical chromatographic conditions as for oral fluid. LOQs were estimated by using moistened cotton fleeces wiped on the forehead of

non-drug users and spiked with decreasing concentrations of amphetamines; for MDMA the LOQ was 5 ng/wipe.

4. Results and discussion

4.1. Study 1

Ethanol Breath Analyser tests were all negative. All urine samples screened negative for cannabinoids, cocaine, opiates and benzodiazepines. MDMA levels in urine varied from 0.32 mg MDMA/g creatinine to over 50 mg MDMA/g creatinine, with an average value of 13 ± 8 mg MDMA/g creatinine at time 5 h. At time 0 h, all samples screened negative for the amphetamine group except one sample which contained 0.16 mg MDMA/g creatinine.

The plasma concentrations within 5 h after administration of 75 mg of MDMA varied from 21 to 295 ng/ml and reached a maximum at 2–4 h after administration with an average value of 178 ± 52 ng/ml (Fig. 1). This concentration is in agreement with the results of a previously published controlled study [9] and is within the lower range of MDMA concentrations reported after blood analysis of impaired drivers (49–1510 ng/ml) [2]. MDA concentrations never exceeded 20 ng/ml. The legal limit for driving under the influence of ecstasy in Belgium is 50 ng MDMA/ml plasma.

Oral fluid concentrations of MDMA were generally higher than plasma concentrations, but the inter-subject variability was approximately three times higher for oral fluid, ranging from 50 to 6982 ng/ml. This could partly be due to the nature of the samples which differed widely in viscosity and volume from one subject to another, and even within a particular subject. All of the samples would have been classified as “positive” taking the new cut-off values proposed by SAMHSA into account [10]. Peak oral fluid

Fig. 1. Average plasma concentrations of MDMA as a function of time, after controlled administration of 75 mg of MDMA to 12 healthy volunteers. Error bars = S.D.

Fig. 2. Average oral fluid concentrations of MDMA as a function of time, after controlled administration of 75 mg of MDMA to 12 healthy volunteers. Error bars = S.D.

concentrations of MDMA averaged 1215 ± 944 ng/ml (Fig. 2). This value is lower than the one reported in a similar study [11] where average oral fluid concentrations peaked at 3375 ng/ml. The dose of MDMA administered in that study was 100 mg, administered as two soft-gelatin capsules. On the other hand, the maximum concentration of MBDB in oral fluid after a controlled administration of 100 mg to one subject was 1083 ng/ml [12]. Fig. 3 shows that the concentration-time course of the average levels of MDMA in plasma and in oral fluid was quite similar. However, in individual subjects, the correlation between concentrations of MDMA in plasma and in oral fluid was poor, resulting in variable saliva-to-plasma ratios (S/P) within a particular subject as a function of time. Moreover, inter-subject variability was high since average S/P ratios per subject ranged from 0.8 to 22.4. Average S/P ratios as a

function of time are shown in Fig. 4. The maximum value is 12 ± 6 , reached after 1 h, then declining to 4 ± 3 at 4–5 h after intake. Since the dose of MDMA was dissolved in 25 ml of orange syrup, contamination of the buccal cavity 60–120 min after administration seems highly unlikely. Saliva-to-plasma ratios >1 have been reported for MDMA in non-controlled studies of impaired drivers [13], and a median S/P ratio of 9 ± 8 was determined from 180 impaired drivers tested during the course of the European Commission project Rosita (Roadside Testing Assessment) in Belgium [14].

With the exception of two individual data points, MDMA levels obtained by extracting the sweat wipes with methanol remained <50 ng/wipe. A single administration of 75 mg of MDMA resulted in average sweat concentrations not exceeding 25 ng MDMA/wipe within the first 5 h (Fig. 5).

Fig. 3. Average plasma and oral fluid concentrations of MDMA as a function of time, after controlled administration of 75 mg of MDMA to 12 healthy volunteers: (■) plasma; (◇, dotted line) oral fluid.

Fig. 4. Average saliva (oral fluid) to plasma concentration ratio (S/P) as a function of time, after controlled administration of 75 mg of MDMA to 12 healthy volunteers. Error bars = S.D.

In contrast to oral fluid, the results for sweat do not agree with reported MDMA levels obtained during the Rosita-project in Belgium [14]. Much higher concentrations of MDMA were reported (88 to >20,000 ng/wipe) in sweat samples obtained in a similar way (by wiping the forehead with the same type of cotton fleece) with a median value of 1813 ng/wipe. Obviously, in that study, external contamination of the skin (e.g. by accidental touching of the forehead with contaminated hands), and especially, repeated dosing of MDMA during a certain interval before sampling, could not be excluded. In the current study, MDMA was administered under controlled conditions, with no possibility of external contamination. Fig. 5 shows that average concentrations are slowly increasing as a function of time, which could result in higher MDMA levels in sweat at a later time after administration. Therefore, more studies are needed on the toxicokinetics of MDMA in sweat wipes, extending the

detection window and evaluating repeated dosing of MDMA. Kintz [12] reported sweat patch concentrations of MBDB in the low nanogram-range the first 4 h after administration, peaking at 44 ng/patch at 36 h.

4.2. Study 2

4.2.1. Analyses of amphetamine and designer amphetamines

Subjects suffered from dry mouth and found it difficult to provide an oral fluid sample, especially in the multiple drug condition. Thus, the sample volume was extremely low in this study, requiring the use of a technique with a suitably low LOQ for the detection of the amphetamine group. Table 1 shows the concentrations of MDMA, MDEA and amphetamine in oral fluid specimens obtained in the MDMA-only test condition and in the multiple-drug condition. Before the

Fig. 5. Average sweat wipe concentrations of MDMA as a function of time, after controlled administration of 75 mg of MDMA to 12 healthy volunteers. Sweat samples were taken by wiping the forehead with a cotton fleece, moistened with 70% of isopropanol. Error bars = S.D.

Table 1

Oral fluid concentrations of amphetamines (ng/ml) in Study 2 with an estimation of the amount of active substance taken before the test ride in the MDMA-only condition, and the number of extra tablets taken during the time interval between the two drug conditions

ID	“XTC” (mg)	MDMA-only oral fluid			Extra tablets	Time interval	Multiple drug oral fluid		
		MDMA	MDEA	amph			MDMA	MDEA	amph
1	37	2639			0	4.0	1354		
2	25	2003			0	4.0	1081		382
3	–	33			4.5	5.0	2027		753
4	–	248			1.5	5.0	1815		257
5	50	704			1.5	5.0	5519		
6	80	2466			1	4.0	7077		
7	58	1252			0	4.5	2190		
8	80	787			0.5	5.1	564	322	
9	63			15	0.5	3.4	70	2479	407
10	50	1181			3.5	5.2	1147		
11	32		25		0.5	5.1	38	1318	
12	70	190		60	0	3.4	986		129
13	25	352			2	6.2	1664		
14	54	2031			0.5	5.4	1454		395
15	58	3533			0	6.1	65		
16	44	228			0	4.0	1020		
17	63	55	2468		0.5	22.1	19	1132	
18	95	252			0.5	21.5	423	331	
19	83	900			1.5	5.1	3778	3320	

MDMA-only ride, most subjects reported taking one tablet according to the instructions. Two subjects did not send in the extra tablet for analysis. The average consumed dosage of stimulant of the other 17 subjects was 57 ± 20 mg. In one case (subject 9), only amphetamine was taken; in two cases (subjects 11 and 17), the ingested dose consisted mainly of MDEA. Oral fluid samples in the MDMA-only condition were taken at an average time of 62 ± 23 min after intake of the ecstasy tablet. Concentrations of stimulant ranged from 15 ng/ml of amphetamine to 3533 ng/ml of MDMA (average 1121 ng/ml). Table 1 also shows the time interval between oral fluid sampling in the MDMA-only test condition and in the multiple drug test condition and the self-reported number of “ecstasy” tablets that were taken between the two test rides. In six cases, no extra tablets were reported. In subjects 1, 2 and 15, a decrease in oral fluid MDMA concentration was observed in the multiple-drug condition after, respectively, 4, 4 and 6.1 h. In subjects 7, 12 and 16, however, an increase in the oral fluid MDMA concentration was observed although no additional MDMA intake was reported. This is either due to discrepancies in the self-reported drug use, intra-subject variability in the oral fluid concentrations of MDMA, as reported in Study 1, or incomplete absorption of MDMA in plasma and oral fluid at the time of the first test ride, which was 47, 47 and 40 min after intake of the tablet in subjects 7, 12 and 16, respectively. In the other 13 subjects, extra consumption of amphetamines in the time interval between the two drug conditions was reported, either additional MDMA, MDEA, or amphetamine, resulting in oral fluid concentrations exceeding the 50 ng/ml cut-off for at

least one analyte. Concentrations ranged from 19 to 7077 ng/ml MDMA, 322 to 3320 ng/ml MDEA and 129 to 753 ng/ml amphetamine.

Table 2 shows the amphetamine results in urine, expressed as ng/ml to facilitate comparison with oral fluid quantitative data. There was a good qualitative correlation between the confirmatory results for amphetamines in urine and oral fluid. In only two cases, amphetamine was detected in urine but not in oral fluid. Table 2 also presents the GC-MS results of urine analyses in the non-drugs condition. In subjects 1, 5 and 18, the presence of MDMA in urine was confirmed. The corresponding oral fluid concentrations for MDMA were 516, 20 and 47 ng/ml, respectively. In subject 9, amphetamine was detected in urine, with a corresponding oral fluid concentration of 97 ng/ml. The 50 ng/ml cut-off level for oral fluid was thus exceeded in two subjects in the non-drug condition; one of them had reported the use of ecstasy the day before.

4.2.2. Analyses of other drugs

Urine analyses were performed to confirm the self-reported drug use of the subjects in the different study conditions and to detect non-reported additional psychoactive substances. Screening of urine samples in the multiple drug condition revealed the presence of amphetamine in 7 cases (6 reported), cannabis in 13 cases (16 reported), cocaine in 2 cases (2 reported), LSD in one case (1 reported), and psilocine in 2 cases (2 reported). GHB was never detected although reported twice, but the detection window of the analyte is <10 h, even in urine [15]. Cannabis was reported in 30% of the subjects in the non-drug condition.

Table 2

Urine concentrations of amphetamines (ng/ml) in the different test conditions in Study 2: the non-drug control condition, the MDMA-only condition and the multiple drug condition

ID	Non-drugs		MDMA-only condition			Multiple drug condition		
	MDMA	amph	MDMA	MDEA	amph	MDMA	MDEA	amph
1	6689		>20000			9510		
2			5540		1416	5480		1542
3			7530			7645		2490
4			1202			>20000		8780
5	2007		7900			3700		1965
6			1740			>20000		
7			1389			11650		
8			17800			7000	4721	
9		1274			1192	266	8414	1928
10			>20000			>20000		
11			517	3613		1621	>20000	
12			1184		284	>20000		4195
13			2196			>20000		
14			>20000			>20000		2276
15			13233			>20000		
16			9993			6255		
17			320	10390		591	>20000	
18	5405		>20000			>20000	8836	
19			>20000			>20000	16732	

Urine analysis revealed the presence of cannabinoids in 60% of the cases, but since carboxytetrahydrocannabinol can be detected in urine for several weeks in regular users, there was no proof of recent use in the extra 30%.

5. Conclusions

Since the number of experimental studies with MDMA is limited, the interpretation of MDMA concentrations in different matrices after a controlled administration of the drug is of considerable interest to the forensic toxicologist working in the field of drugs and driving and workplace drug testing. The results of Study 1 only represent a situation of recent abuse, showing toxicological data up to 5 h after administration. The average MDMA levels in plasma and oral fluid followed a similar time-course, but in individual subjects the intra- and inter-subject variability was much higher for oral fluid. Therefore, the interpretation of MDMA concentrations in oral fluid is extremely difficult without an additional quantitative confirmation of the corresponding blood sample. When a screening cut-off of 50 ng/ml of MDMA is applied, oral fluid offers a non-invasive alternative to urine testing with sufficiently high concentrations of MDMA detectable for at least 5 h after administration of a single dose of 75 mg of MDMA. This is in contrast to the results for sweat; very low concentrations of MDMA were detected in the collected forehead wipes in the first 5 h after intake. In this study, external contamination and repeated dosage were completely ruled out.

In Study 2, test conditions were far less controlled, but much more representative of real-life situations of ecstasy abuse. Unfortunately, the interpretation of the results was rather difficult because blood analysis could not be performed in this study. The mean MDMA concentration observed at approximately one hour after intake of a “street” tablet of ecstasy (average content 56 mg MDMA) in a non-controlled experimental study design, was in the same range as oral fluid concentrations obtained after a controlled administration of 75 mg of MDMA. However, as was the case in a controlled study design, the inter-subject variability was high. Oral fluid concentrations of amphetamines in a test situation of multiple drug use significantly exceeded the SAMHSA cut-off levels for amphetamines in oral fluid.

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