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Simultaneous gas chromatographic determination of methamphetamine, amphetamine and their *p*-hydroxylated metabolites in plasma and urine

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Abstract

We report a method for the simultaneous determination of methamphetamine, amphetamine and their hydroxylated metabolites in plasma and urine samples using a GC–NPD system. The analytical procedures are: (1) adjust the sample to pH 11.5 with bicarbonate buffer, saturate with NaCl and extract with acetate; (2) back-extract the amines in the ethyl acetate fraction with 0.1 *M* HCl; (3) adjust the pH of the acid fraction to 11.5 and follow by extraction in ethyl acetate; (4) reduce the volume of ethyl acetate under nitrogen and derivatize the concentrate with trifluoroacetic anhydride or heptafluorobutyric anhydride before the GC analysis. The derivatives were separated on a GC–NPD system equipped with a HP-5 column of 25 m×0.32 m I.D. and a 0.52 μm film of 5% phenylmethylsilicone. The detection limit (taking a signal-to-noise ratio of 2) of heptafluorobutyl derivatives of methamphetamine and its metabolites in plasma and the trifluoroacetyl derivatives in urine was 1 ng/ml (22 pg on column). The limit of quantitation of the heptafluorobutyl derivatives in the plasma was 1 ng/ml (22 pg on column), and that of the trifluoroacetyl derivatives in urine was 20 ng/ml (73 pg on column). The between-day variation was from 0.9 to 17.4% and within-day variation from 0.9 to 8.3%. This method was used successfully in the quantitative determination of methamphetamine and its *p*-hydroxylated metabolites in the plasma and urine of human subjects.

Keywords: Methamphetamine; Amphetamine; *p*-Hydroxymethamphetamine; *p*-Hydroxyamphetamine

1. Introduction

Methamphetamine (MAMP) is a potent CNS stimulant and has been prescribed for the treatment of narcolepsy, obesity and hyperkinetic syndrome [1]. Like amphetamine (AMP), MAMP is often used and abused by drivers and athletes to improve their performance [2]. Recent reports [3,4] have shown a significant use of MAMP by smoking, and its abuse has become a serious drug problem in some regions of the world [3].

Methamphetamine is metabolized by two cyto-

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chrome P450-dependant oxidation processes: para-hydroxylation and deamination yielding *p*-hydroxy-methamphetamine (*p*-OH-MAMP) and *p*-hydroxy-amphetamine (*p*-OH-AMP) and AMP [5–9] which are less active pharmacologically. Wu [10] has shown that the cytochrome P450 2D6 (CYP2D6) mediates the hydroxylation of AMP and MAMP in human liver microsomes. Since the CYP2D6 gene is polymorphic (7% of Caucasians have a defective gene resulting in the lack of enzyme activity), this raised the possibility that the CYP2D6 phenotype may be a risk factor to AMP abuse liability. As a first step in understanding the role of CYP2D in MAMP abuse, the metabolism and pharmacokinetic properties of MAMP in humans were investigated [Ocampo et al., manuscript submitted].

Several analytical methods have been developed for the quantitative measurement of metabolites of MAMP and AMP over the past 20 years. These included the radioimmunoassay (RIA) [11], gas chromatography–mass spectrometry (GC–MS) [12–15], GC-nitrogen–phosphorus detection (GC–NPD) [2,4,16,17], GC-flame ionization detection (GC–FID) [18,19], GC-electron capture detection (GC–ECD) [20] and high-performance liquid chromatography chemiluminescence detection (HPLC–CD) [21] systems. The hydroxylated metabolites such as *p*-OH-MAMP and *p*-OH-AMP, have also been detected at the sub-nanogram levels by HPLC-electrochemical detection (HPLC–ED) [18,22] assay. Nakashima et al. [21] developed an HPLC–ECD method to determine MAMP metabolites in urine samples of MAMP addicts. Since their method involved a lengthy gradient elution (65 min) and a post-column derivatization, it is not practical for use in large clinical pharmacokinetic studies. Terada [17] also reported an analytical procedure using GC–NPD system to determine MAMP and its metabolites in rat tissues. However, the procedure involved the use of two different GC columns; one for the determination of MAMP and AMP and the other column for *p*-OH-MAMP and *p*-OH-AMP. We have developed a GC–NPD method which provides a simultaneous and quantitative analysis of MAMP and its metabolites, AMP, *p*-OH-MAMP and *p*-OH-AMP, in human urine and plasma samples in a single chromatographic run.

2. Experimental

2.1. Reagents and materials

MAMP hydrochloride was purchased from BDH (Toronto, Canada). AMP sulphate, 4-OH-MAMP hydrochloride and 4-OH-AMP hydrobromide were provided by the National Institute on Drug Abuse (Washington, DC, USA). *N*-Methylphenethylamine (I.S.) was obtained from Aldrich Chemical (Milwaukee, WI, USA). β-Glucuronidase (type H-1), trifluoroacetic anhydride (TFA) and heptafluorobutyric anhydride (HFBA) were purchased from Sigma (St. Louis, MO, USA). Glass distilled ethyl acetate was obtained from Caledon Laboratories (Georgetown, Canada). All other chemicals were of analytical grade.

2.2. Gas chromatography procedure

A Hewlett-Packard Model 5890 gas chromatograph (Palo Alto, CA, USA) fitted with a nitrogen–phosphorus detector (NPD) and a capillary column (25 m×0.32 m; 0.52 μm film of 5% phenylmethylsilicone as stationary phase; Hewlett-Packard) was used. The processed sample (2 μl) was injected, at room temperature, with an HP 7673A autosampler in the splitless mode (splitless injection with inlet insert purge time of 30 s, septum purge flow-rate of 2 ml/min and split vent flow-rate of 80 ml/min). Helium was used both as the carrier gas (flow-rate of 2.5 ml/min; column head pressure of 10 psi) and as the make-up gas (flow-rate of 27 ml/min). The oven temperature was initially set at 70°C and increased continuously at a rate of 30°C/min to 120°C, which was then increased to 185°C at a rate of 5°C/min to permit sufficient separation of various compounds of interest. Finally, the oven temperature was increased at a rate of 70°C/min to 300°C and was held at this temperature for 1.5 min to clean the column between the injections. The injector port and detector temperatures were set at 200°C and 300°C respectively. A mixture of H_2 (3.7 ml/min) and air (118 ml/min) was used as detector gas. The bead on the NP-detector was changed approximately every 2 months on a routine analysis, or when the signal-to-noise ratio of a standard HFBA-derivative of *p*-OH-

MAMP (22 pg on column) had decreased to less than 2, whichever occurred first. Data collection and handling were done by a HP 5895A GC Chemstation.

2.3. Sample preparation

Urine sample (0.2 ml) was hydrolyzed with 0.2 ml of β-glucuronidase (3500 U/ml in 0.2 *M* acetate buffer, pH 5.0) at 37°C for 24 h. After the addition of 100 μl of *N*-methylphenethylamine (I.S., 400 ng/ml) and 50 μl of 20% sodium carbonate in water, the aqueous layer (pH 11.5) was saturated with sodium chloride (approximately 0.3 g) and extracted with 4 ml of ethyl acetate. The sample was horizontally shaken for 30 min and centrifuged at 800 *g* for 5 min. The organic phase was back-extracted with 1 ml of 0.1 *M* HCl by vortexing for 5 min, followed by centrifugation at 800 *g* for 5 min. After centrifugation, the ethyl acetate layer was aspirated, and the aqueous layer was made alkaline (pH 11.5) with 0.35 ml of 20% sodium carbonate in water, saturated with sodium chloride (approximately 0.6 g) and extracted with 4 ml of ethyl acetate. The sample was vortexed for 10 min and centrifuged at 800 *g* for 5 min. The organic layer was transferred to a glass test tube and was concentrated to a volume of 0.2–0.4 ml (a precise volume was not critical for the subsequent process) under a stream of N_2 at 40°C. The concentrate was then transferred to a GC vial (1.5-ml capacity), the glass tube was rinsed once with ethyl acetate (0.1 ml), vortexed and added to the GC vial, capped with a teflon septum, and reacted with 50% TFA in ethyl acetate (0.1 ml) for 30 min at 60°C. When the reaction was completed, the mixture in the vial was evaporated to dryness under a gentle stream of N_2 at room temperature, and the resultant residue was immediately dissolved in ethyl acetate (110 μl). A 2-μl sample was injected into the GC instrument for the simultaneous and quantitative analysis of MAMP and its metabolites.

The plasma sample (1.0 ml) was hydrolyzed with 0.5 ml of β-glucuronidase (3500 U/ml) in a similar manner as the urine sample. After the addition of 100 μl of *N*-methylphenethylamine (I.S., 110 ng/ml), 100 μl of 20% sodium carbonate and 6 ml of ethyl acetate, the extraction procedure was the same as described above except that 2 ml of 0.1 *M* HCl, 0.5 ml of 20% sodium carbonate in water and 6 ml of ethyl acetate were used, respectively. The derivatization reagent was HFBA instead of TFA. After the reaction, the sample was dried under a stream of N_2 at room temperature, and the residue was immediately dissolved in 90 μl of ethyl acetate. A sample (2 μl) was used for injection into the GC instrument.

2.4. Stability of the compounds in β-glucuronidase hydrolysis

The stability of MAMP, AMP, *p*-OH-MAMP and *p*-OH-AMP in β-glucuronidase hydrolysis was determined in the plasma. To plasma samples (1.0 ml each) were added MAMP (15 ng), AMP (15 ng), *p*-OH-MAMP (7 ng), *p*-OH-AMP (7 ng) and 0.5 ml of β-glucuronidase (3500 U/ml in 0.2 *M* acetate buffer, pH 5.0). The mixture was incubated at 37°C for 24 h and was processed according to the method described (see above) after the addition of 100 μl of I.S. (110 ng/ml). In a parallel experiment, 0.5 ml of β-glucuronidase (3500 U/ml in 0.2 *M* acetate buffer, pH 5.0) was added to the plasma samples (1.0 ml) and incubated at 37°C for 24 h. Thereafter, the mixture was spiked with MAMP (15 ng), AMP (15 ng), *p*-OH-MAMP (7 ng), *p*-OH-AMP (7 ng) and 100 μl of I.S. (110 ng/ml) and derivatized with HFBA (as described) for GC analysis.

2.5. Determination of recovery

Both relative and absolute recoveries of MAMP, AMP, *p*-OH-MAMP and *p*-OH-AMP in the plasma and urine were studied. For the determination of relative recovery, MAMP, AMP, *p*-OH-MAMP, *p*-OH-AMP and I.S. were put in the distilled H_2O, plasma or urine, and the samples were processed according to the method described (see above). MAMP (600 ng), AMP (600 ng), *p*-OH-MAMP (60 ng), *p*-OH-AMP (60 ng) and I.S. (40 ng) were added to the urine samples. For the plasma samples, MAMP (30 ng), AMP (30 ng), *p*-OH-MAMP (15 ng), *p*-OH-AMP (15 ng) and I.S. (11 ng) were used. The relative recovery was obtained by comparing the peak area of H_2O samples to that of the urine or plasma samples and was expressed as percentage of

that of the water. For the determination of absolute recovery, two paradigms were used: (1) A known amount of MAMP, AMP, *p*-OH-MAMP, *p*-OH-AMP and I.S. was dissolved in methanol and was transferred into a glass tube. After the addition of 10 μl of 0.1 *M* HCl, the content of the tube was evaporated to dryness under a stream of N_2 at room temperature. The residue was dissolved in 100 μl of ethyl acetate and transferred to a GC vial. The glass tube was rinsed with 100 μl of ethyl acetate twice and added to the GC vial. This was then reacted with 100 μl of HFBA (the plasma samples) or 100 μl of 50% TFA in ethyl acetate (the urine samples). After evaporation, the residue was immediately dissolved in 110 μl of ethyl acetate, 2 μl of which were used for GC analysis. (2) The I.S. and 10 μl of 0.1 *M* HCl were pipetted into a small glass test tube where it was evaporated to dryness under a stream of N_2 at room temperature. The residue was dissolved in 100 μl of ethyl acetate and transferred to a GC vial. To this vial was added the ethyl acetate extract of the urine or plasma that had been spiked with MAMP, AMP, *p*-OH-MAMP and *p*-OH-AMP, and the volume of ethyl acetate in the GC vial was reduced to approximately 200 μl by evaporation under a stream of N_2. This was then reacted with HFBA (plasma samples) or TFA (urine samples) and used in the GC analysis as described above. The peak area of each compound and the I.S. of the first and the second paradigms were used to calculate the absolute recovery. MAMP (600 ng), AMP (600 ng), *p*-OH-MAMP (60 ng), *p*-OH-AMP (60 ng) and I.S. (40 ng) were used to spike the urine samples for the analysis while MAMP (30 ng), AMP (30 ng), *p*-OH-MAMP (15 ng), *p*-OH-AMP (15 ng) and I.S. (11 ng) were used in the plasma samples. The reason for differences in the amount of compounds used in the determination of the recovery in the urine and plasma was to simulate the expected drug concentrations in the respective body fluids.

3. Results and discussion

N-methylphenethylamine was used as an internal standard by Cook et al. [4] in a GC analysis of amphetamine, and we also used this compound as an I.S. in our study. The I.S. behaves similarly to MAMP and its metabolites throughout the processing, and it shows similar relative and absolute recoveries to MAMP and its metabolites from the plasma and urine samples. The absolute recovery of the I.S. is not affected by the range of pH 9.6–11.8 with an average recovery of 83.8±5.5%. Therefore, the I.S. was added to all samples in the subsequent studies.

Ethyl acetate was used to extract MAMP and its metabolites based on an earlier study by Terada [17] who demonstrated that MAMP and its hydroxylated metabolites could be efficiently extracted (93.5–115.4%) by ethyl acetate at pH 9.5. However, in the present experiments, the extraction efficiency of MAMP and AMP remained constant over the pH range of 9.5–11.8 but *p*-OH-AMP and *p*-OH-MAMP were extracted 45% and 36% more efficiently over the pH range 11.0–11.5 when compared with that of pH 9.6 (Fig. 1). Since the absolute recovery of the I.S. is unaffected in this range of pH, the increase in the relative recovery of *p*-OH-MAMP and *p*-OH-AMP must be due to increase in the efficiency of extraction. Therefore, MAMP and its metabolites can be effectively recovered by ethyl acetate after adjusting the sample to pH 11.5 with 20% sodium carbonate.

The absolute recovery (determined from four separate experiments) of the urine was MAMP (68±1.6%), AMP (70±1.4%), *p*-OH-MAMP (90±1.1%), *p*-OH-AMP (90±1.2%) and I.S. (86±2.6%). The relative recovery (determined from four separate experiments) of the MAMP and AMP was 84±2.7% and 88±2.4% respectively, and that of *p*-OH-MAMP and *p*-OH-AMP was 93±6.9% and 92±5.5% in the urine samples. In the plasma samples, the absolute recovery was MAMP (54±7.1%), AMP (67±3.8%), *p*-OH-MAMP (67±4.4%), *p*-OH-AMP (62±2.8%) and I.S. (105±9.7%). The relative recovery was MAMP (79±4.6%), AMP (80±7.9%), *p*-OH-MAMP (60±.2.2%) and *p*-OH-AMP (56±6.1%). The results (Table 1) indicate that these compounds can be effectively extracted by ethyl acetate at pH 11.5 from both urine and plasma samples. Since smaller amounts of the compounds were added to the plasma samples, the recovery was usually poorer than that of the urine samples which contained a higher concentration of the compounds. Table 1 also shows that

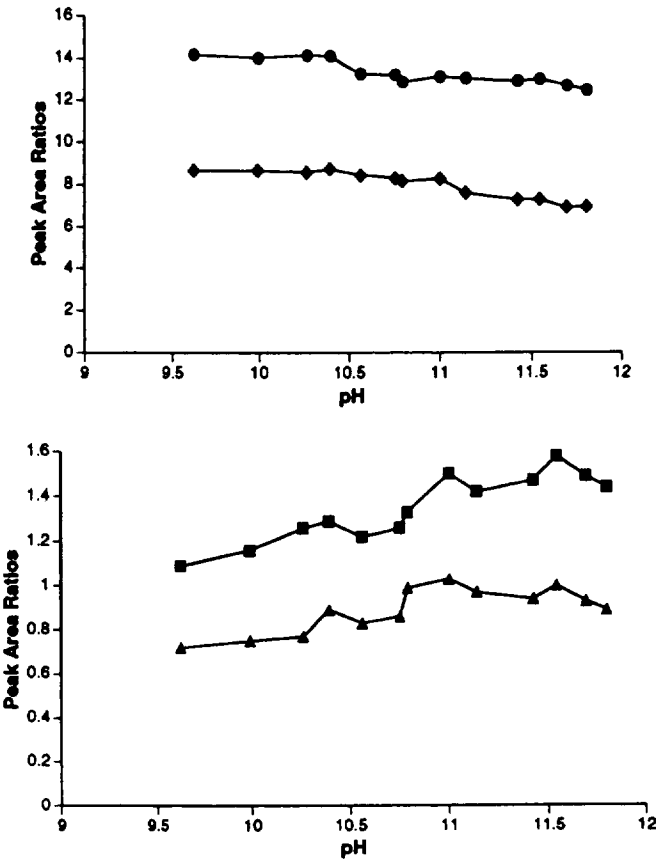

Fig. 1. Effect of pH on the recovery of MAMP (●), Amp (♦), *p*-OH-MAMP (■) and *p*-OH-AMP (▲) during the extraction. The recovery of MAMP and AMP was not affected by change within the range pH 9.5–11.8. However, a higher recovery of *p*-OH-AMP and *p*-OH-MAMP was found from pH 11.0–11.8. Therefore, a pH value of 11.5 was used in the initial extraction to recover MAMP, AMP, *p*-OH-MAMP and *p*-OH-AMP from the biological fluids.

Table 1
Absolute and relative recoveries of MAMP and its metabolites

Compound	Absolute recovery (%)	C.V. (%)	Relative recovery (%)	C.V. (%)
Plasma				
MAMP	54	7.1	79	4.6
AMP	67	3.8	80	7.9
p-OH-MAMP	67	4.4	60	2.2
p-OH-AMP	62	2.8	56	6.1
Urine				
MAMP	68	1.6	84	2.7
AMP	70	1.4	88	2.4
p-OH-MAMP	90	1.2	93	6.9
p-OH-AMP	90	1.1	92	5.5

The methods used in the determination of the absolute and relative recoveries were described in the text. These results are the mean±S.D. (C.V.) of four different experiments.

the recovery for the hydroxylated metabolites (>90%) is greater than that of the parent compounds (<70%) in the urine samples whereas the recovery does not differ markedly in the plasma samples. The explanation for the difference in the recovery from the urine and the plasma is not apparent, besides the higher concentration of compounds in the urine samples.

These compounds are stable in the β-glucuronidase solution for 24 h. The stability study showed that MAMP (98.5±9.0%), AMP (95.1±10.8%), *p*-OH-MAMP (98.3±8%), *p*-OH-AMP (90.5±20%) and I.S. (103.0±6%) could be recovered after hydrolysis. Therefore, the hydrolysis of the conjugated metabolites by β-glucuronidase in our study does not affect the stability of the compounds in the plasma or urine samples.

O'Brien et al. [23] showed that more than 50% of free MAMP and AMP was lost when the organic phase containing MAMP and its metabolites was evaporated to dryness under a stream of N_2 at room temperature. High volatility was observed even when the amines were converted to acetate salt by acetic acid before evaporation [12]. We also found that the reproducibility of the assay is poor when the organic phase containing the amines is evaporated to dryness and the reproducibility does not improve even after the conversion of amines to their corresponding acetate salts before drying: MAMP (30% C.V.), AMP (14% C.V.), I.S. (42% C.V.), *p*-OH-MAMP (31% C.V.) and *p*-OH-AMP (16% C.V.). Therefore, in our subsequent experiments, the volume of ethyl acetate containing MAMP and its hydroxylated metabolites was reduced to 0.2–0.4 ml under a stream of N_2 at 40°C before the derivatization. It is also important to make sure that over-drying must be avoided after derivatization with TFA or HFBA. Since the derivatives are very volatile, over drying of the samples results in poor recovery as shown by Terada [17]. As a result of these modifications, the reproducibility of the assay is much improved; the between-day variation of analysis is 1.6 to 12.0% (C.V.), and the within-day variation is 6.4 to 17.6% (C.V.) for the human plasma samples (Table 2). Similar results are obtained for the human urine samples; the between-day variation of the analysis is 0.9 to 16.8% (C.V.), and the within-day variation is 0.9 to 8.3% (C.V.) (Table 3). The larger variation in the analysis of the plasma samples is probably because lower concentrations of drugs were used.

HFBA-derivatization seems to give a higher sensitivity in the detector system: increased peak areas for HFBA-AMP (17%), HFBA-I.S. (21%), HFBA-MAMP (14%), HFBA-*p*-OH-AMP (54%), and HFBA-*p*-OH-MAMP (32%) over their corresponding TFA derivatives. The reason for these increases is not clear. Although HFBA- and TFA-derivatives offer excellent sensitivity in the electron capture detector system, the nitrogen–phosphorus detector may offer added selectivity because these compounds contain a nitrogen moiety. Fig. 2A shows the chromatogram of a plasma sample spiked with I.S. (11 ng), and the Fig. 2B shows the result of chromatographic separation of AMP, MAMP, *p*-OH-AMP and *p*-OH-MAMP in the form of HFBA derivatives. For plasma samples, the analysis was linear from 1 to 30 ng/ml (22–660 pg on column) for MAMP and AMP, and from 1 to 15 ng/ml (22–330 pg on column) for hydroxylated metabolites. These are also the concentration ranges used in our study. The linear regression analysis of the standard curves yields the following relationships: $Y=10.66X-0.02$ (MAMP, $\gamma=0.9993$); $Y=15.89X-0.2$ (AMP, $\gamma=0.9986$); $Y=15.30X-0.23$ (*p*-OH-MAMP, $\gamma=0.9996$); and $Y=24.14X-1.97$ (*p*-OH-AMP; $\gamma=0.9968$), where Y is the concentration of the drug (ng/ml), and X is the peak area ratio. Since there was a small interfering peak immediately adjacent to the HFBA-*p*-OH-AMP peak on the chromatogram, the Y intercept was approximately 2 ng/ml. The limit of quantitation is defined as twice the lowest concentration in the linear portion of the standard curve. Therefore, the limit of quantitation for *p*-OH-AMP is approximately 4 ng/ml in the plasma samples. For the other three compounds, the detection limit is 1 ng/ml (22 pg on column) which gives a signal-to-noise ratio of 2 for the MAMP and its metabolites, with the exception of *p*-OH-AMP, in the plasma. Fig. 2C shows the chromatogram of a plasma sample drawn from a human subject 2.5 h after an oral administration of 10 mg MAMP–HCl. In this subject, the AMP level in the plasma is 3.3 ng/ml, and the *p*-OH-MAMP is 26.3 ng/ml but *p*-OH-AMP is below the detection limit of 1 ng/ml.

In the urine analysis, MAMP and metabolites were derivatized by TFA instead of HFBA due to interfer-

Table 2
Between-day and within-day variations in human plasma

Compound	Actual concentration (ng/ml)	Mean concentration (ng/ml)	C.V. (%)
Between-day (n=9)			
MAMP	2.5	2.6±0.25	9.6
	5.0	5.1±0.26	5.1
	10.0	10.0±0.39	3.9
	15.0	14. 9±0.55	3.7
	20.0	19.9±0.44	2.2
	30.0	30.0±0.49	1.6
p-OH-MAMP	2.5	2.5±0.27	10.8
	5.0	5.1±0.46	9.0
	7.5	7.4±0.50	6.8
	10.0	10.1±0.80	7.9
	15.0	14.9±0.59	3.9
AMP	2.5	2.7±0.21	7.8
	5.0	5.1±0.32	6.3
	10.0	10.0±0.57	5.7
	15.0	15.2±0.67	4.4
	20.0	19.6±0.89	4.4
	30.0	30.0±0.73	2.4
p-OH-AMP	2.5	2.7±0.21	7.8
	5.0	4.9±0.59	12.0
	7.5	7.5±0.49	6.5
	10.0	9.9±0.65	6.6
	15.0	15.0±0.43	2.9
Within-day (n=7)			
MAMP	5.0	4.9±0.42	8.7
	20.0	19.5±1.25	6.4
p-OH-MAMP	2.5	2.4±0.41	17.3
	10.0	9.9±0.95	9.6
AMP	5.0	5.1±0.62	12.1
	20.0	19.6±2.06	10.5
p-OH-AMP	2.5	2.4±0.42	17.6
	10.0	9.7±1.17	17.6

ing peaks in the urine samples when HFBA was used. Fig. 3A shows that there is a good separation of the I.S. from other compounds in the urine. The chromatogram (Fig. 3B) clearly indicates the separation of TFA-derivatives of AMP, MAMP, *p*-OH-AMP and *p*-OH-MAMP extracted from a urine sample. The analysis of MAMP and AMP in the urine is linear from 20 to 4000 ng/ml (73–14 600 pg on column) and that of the hydroxylated metabolites is 20–600 ng/ml (73–2190 pg on column). The linear regression analysis yielded the following relationships: $Y=230.74X+8.65$ (MAMP, $\gamma=0.9998$); $Y=347.27X+0.88$ (AMP, $\gamma=0.9999$); $Y=291.10X-2.08$ (*p*-OH-MAMP, $\gamma=0.9998$); and $Y=395.40X-4.17$ (*p*-OH-AMP, $\gamma=0.9997$), where Y is the concentration of the compound expressed in ng/ml, and X is the peak area ratio. The detection limit was 22 pg (on column) which gave a signal-to-noise ratio of 2 for all four compounds in the urine. The reproducibility of this assay is shown in Table 3. The within-day variation was between 0.9 and 8.3% (C.V.), and the between-day variation was from 0.9 to 17.4% (C.V.). Fig. 3C shows the chromatogram of a 24–48 h urine sample collected from a human subject who was given an oral dose of MAMP–HCl (10 mg). MAMP, AMP and *p*-OH-MAMP are readily detectable but the *p*-OH-AMP is not in this urine sample.

A comparison of the present method to those reported previously, clearly shows that our method represents an improvement over others with respect to the ability to analyze MAMP and some of its

Table 3
Between-day and within-day variations in human urine

Compound	Actual concentration (ng/ml)	Mean concentration (ng/ml)	C.V. (%)
Between-day (n=5)			
MAMP	200.0	200.0±19.0	9.6
	500.0	500.0±21.0	4.2
	1000.0	990.0±37.0	3.7
	2000.0	2010.0±120.0	5.9
	4000.0	4050.0±59.0	1.4
p-OH-MAMP	20.0	21.0±3.5	16.8
	50.0	51.0±5.4	10.6
	100.0	105.0±4.9	4.7
	200.0	202.0±8.1	4.0
	400.0	413.0±18.9	4.7
AMP	200.0	190.0±33.0	17.4
	500.0	500.0±35.0	7.1
	1000.0	1010.0±26.0	2.6
	2000.0	2050.0±127.0	6.2
	4000.0	4010.0±40.0	0.9
p-OH-AMP	20.0	20.0±3.3	16.6
	50.0	54.0±5.7	10.5
	100.0	105.0±8.3	7.9
	200.0	205.0±12.3	6.0
	400.0	385.0±13.7	3.6
Within-day (n=7)			
MAMP	1000.0	970.0±34.0	3.5
	4000.0	4210.0±147.0	3.5
p-OH-MAMP	100.0	100.0±7.2	7.2
	400.0	390.0±16.2	4.2
AMP	1000.0	980.0±34.0	3.5
	4000.0	3980.0±87.0	2.2
p-OH-AMP	100.0	105.0±8.7	8.3
	400.0	380.0±3.4	0.9

metabolites simultaneously. This analytical procedure has been used in a clinical study of MAMP metabolism [Ocampo et al., manuscript submitted]. Urine samples collected from a subject 0–10, 10–24 and 24–48 h after an oral administration of 10 mg of MAMP–HCl and 7.2 g of $NaHCO_3$. The analysis of the 24–48 h urine sample (Fig. 3C) shows the presence of MAMP (605 ng/ml), AMP (415 ng/ml) and *p*-OH-MAMP (400 ng/ml). However, the TFA derivative of *p*-OH-AMP was not detectable in this sample or in the 0–10 h and 10–24 h samples. Since the sensitivity of the detection of *p*-OH-AMP was 20 ng/ml, we conclude that there is very limited metabolism of MAMP to *p*-OH-AMP in this human subject.

In conclusion, we demonstrate that MAMP and its metabolites in human body fluids can be identified and quantitated by converting them to HFBA- or TFA-derivatives followed by separation on a GC system equipped with a capillary column (5% phenylmethylsilicone) and a nitrogen–phosphorous detector. This procedure is reproducible and sensitive for the determination of MAMP and its metabolites in human subjects administered with methamphetamine.

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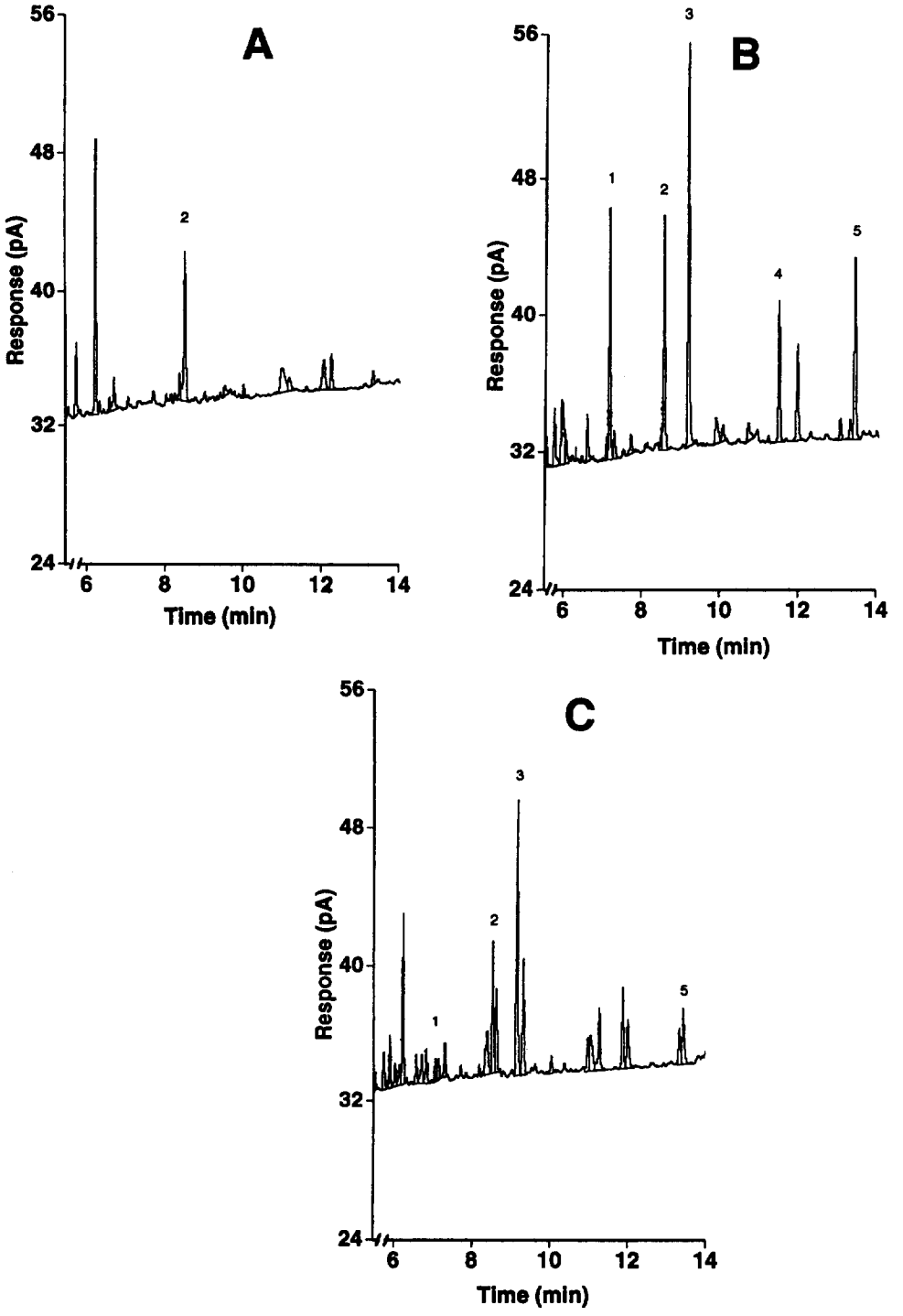

Fig. 2. Representative chromatograms from plasma (1.0 ml). (A) Plasma spiked with I.S. (11 ng; peak 2). (B) Plasma spiked with AMP (20 ng; peak 1), I.S. (11 ng; peak 2), MAMP (20 ng; peak 3), *p*-OH-AMP (10 ng; peak 4) and *p*-OH-MAMP (10 ng; peak 5). (C) Plasma drawn from a subject 2.5 h after oral administration of 10 mg MAMP hydrochloride. AMP (3.3 ng; peak 1), I.S. (11 ng; peak 2), MAMP (26.3 ng; peak 3) and *p*-OH-MAMP (6.0 ng; peak 5).

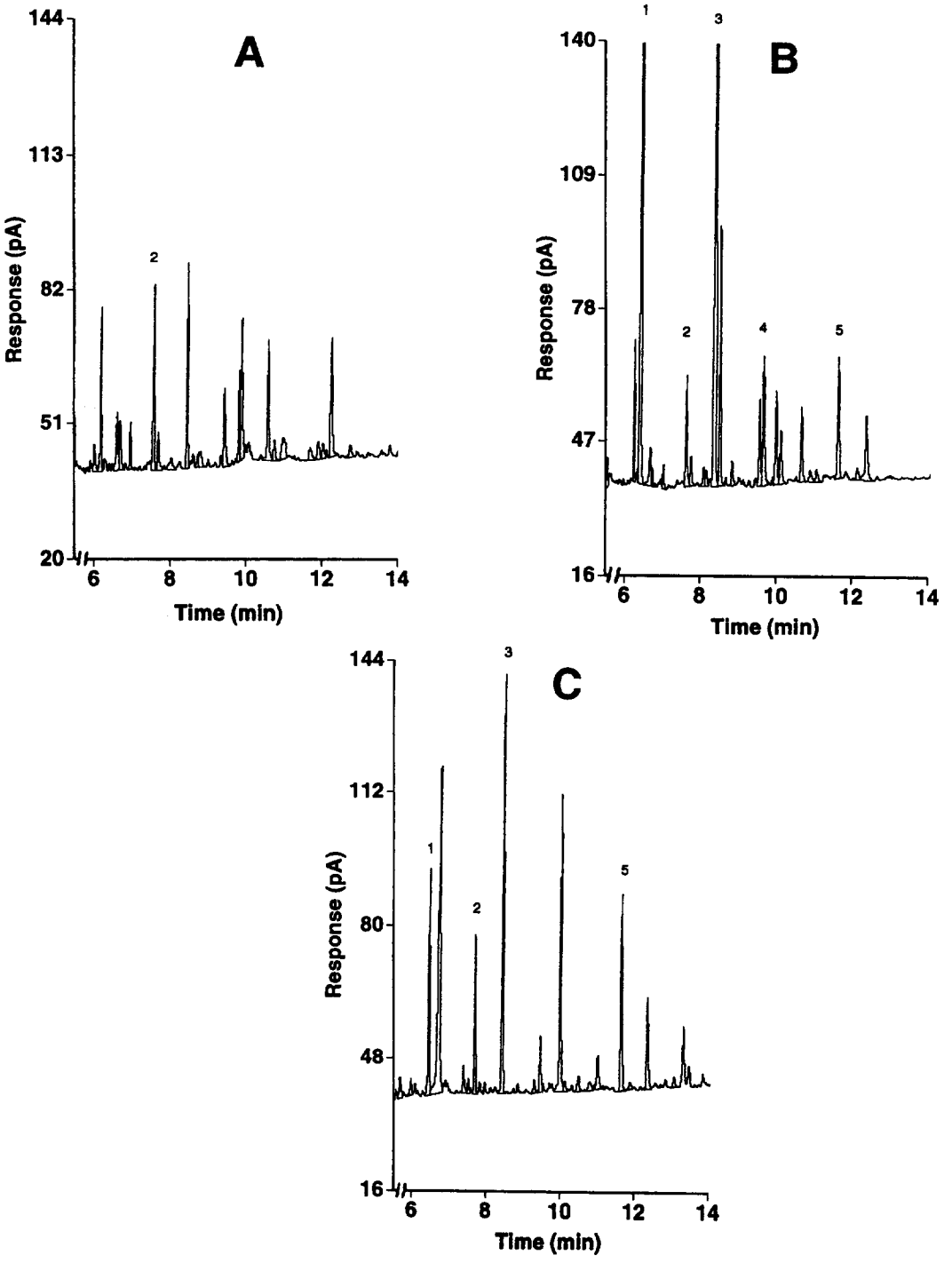

Fig. 3. Representative chromatograms of urine (0.2 ml). (A) Urine spiked with I.S. (40 ng; peak 2). (B) Urine spiked with AMP (600 ng; peak 1), I.S. (40 ng; peak 2), MAMP (600 ng; peak 3), *p*-OH-AMP (60 ng; peak 4) and *p*-OH-MAMP (60 ng; peak 5). (C) A 24–48 h urine sample from a subject after an oral dose of 10 mg MAMP hydrochloride and 7.2 g of sodium bicarbonate, AMP (415 ng; peak 1), I.S. (40 ng; peak 2), MAMP (3605 ng; peak 3) and *p*-OH-MAMP (400 ng; peak 5).

necessarily represent the policies and opinions of the Addiction Research Foundation.

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