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Comparison of methamphetamine concentrations in oral fluid, urine and hair of twelve drug abusers using solid-phase extraction and GC-MS

Comparaison des concentrations en métamphétamine dans la salive, les urines et les cheveux de douze toxicomanes après extraction en phase solide et GC-MS

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Abstract – Introduction: Methamphetamine (MA) is the most abused drug in Korea. In order to investigate the correlation of MA disposition in oral fluid, urine and hair specimens, quantitative analysis for MA and its main metabolite amphetamine (AM) was performed. **Methods**: Twelve drug abuser's oral fluid, urine and hair samples, submitted by the Police for drug testing, were used. As the preliminary test, MA was screened in urine and oral fluid by fluorescence polarization immunoassay. Extraction for MA was performed using solid-phase extraction after dilution with phosphate buffer. Hair samples were finely cut and incubated for 20 h in 1 mL methanol. Samples were derivatized with pentafluoropropionic acid anhydride for oral fluids and urines and trifluoroacetic acid anhydride for hairs. Quantitation of MA and AM was performed by GC-MS using SIM mode for oral fluids and hairs and full scan mode for urines. **Results and conclusion**: Concentrations of MA and AM in twelve urine samples ranged 120.4–73 216.0 and 67.6–4238.4 ng/mL, respectively. For oral fluids, just one sample gave negative result, and concentrations of MA and AM in eleven samples ranged 104.2–4603.3 and 32.4–268.6 ng/mL, respectively. For hairs, the concentrations of MA and AM were 3.40–98.25 and 0.21–4.35 ng/mg, respectively in twelve samples. The average ratios of AM/MA in urine, oral fluid and hair for eleven samples were 0.12, 0.11 and 0.06, respectively. Two-way ANOVA showed a significant difference of AM/MA ratios between three specimens. Multiple comparison by Tukey method, AM/MA ratio of hair was significantly lower than urine and oral fluid.

Key words: Methamphetamine, oral fluid, urine, hair

Résumé – Introduction : La métamphétamine est la drogue la plus répandue en Corée. Afin de rechercher des traces de métamphétamine dans la salive, les urines et les cheveux des sujets, des analyses quantitatives portant sur la métamphétamine et sur son métabolite amphétaminique principal ont été réalisées. **Méthodes** : Les échantillons de salive, urine et cheveux de douze toxicomanes, fournis par la police pour dépistage, ont été examinés. Comme test préliminaire, la métamphétamine a été ciblée dans les urines et la salive à l'aide d'immunodosage par polarisation par fluorescence. L'extraction de la métamphétamine a été réalisée en phase solide après dilution à l'aide d'un tampon phosphate. Des échantillons de cheveux ont été finement coupés et placés pour incubation dans 1 mL de méthanol pendant 20 h. Les échantillons de salive et d'urine ont été traités avec de l'anhydride pentafluoropropionique, les cheveux avec de l'anhydride trifluoroacétique. Le dosage de la métamphétamine et de son métabolite a été réalisé par spectrométrie de masse en mode SIM pour la salive et les cheveux, et en mode full-scan pour les urines. **Résultats et conclusion** : Les concentrations en métamphétamine et son métabolite dans les douze échantillons d'urines ont varié respectivement de 120,4 à 73 216,0 ng/mg, et de 67,6 à 4238,4 ng/mg. Concernant la salive, un seul échantillon s'est révélé négatif, et les concentrations en métamphétamine et son métabolite dans les onze autres ont varié respectivement de 104,2 à 4603,3 ng/mL, et de 32,4 à 268,6 ng/mL. Concernant les cheveux, ces concentrations ont varié respectivement de 3,40 à 98,25 ng/mL, et de 0,21 à 4,35 ng/mL dans les douze échantillons. Les rapports moyens métabolite/métamphétamine dans les urines, la salive et les cheveux se sont élevés respectivement à 0,12, 0,11 et 0,06 (11 cas). Un test de variance

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a montré une différence significative des ratios métabolite/métamphétamine entre 3 matrices. Une seconde comparaison par le test de Tukey a montré que le ratio métabolite/métamphétamine pour les cheveux était significativement plus faible que pour la salive et les urines.

Mots clés : Métamphétamine, salive, urine, cheveux

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1 Introduction

Methamphetamine (MA), known as "speed", "crystal" or especially "philopons" in Asia, has been the most abused in Korea [1]. For drug testing, urine is still the best specimen, recently oral fluid has been used as an alternative to urine. Compare to urine, oral fluid can be collected easily, provide non-invasive specimen for drug testing and reduce or eliminate adulteration and substitution [2]. Hair, another alternative specimen, can also provide a reliable tool for proving chronic drug use [3]. In Korea, we usually have performed MA analysis in urine and hair samples. Hair analysis of MA was performed since 1993, and we, National Institute of Scientific Invetigation (NISI), performed about 3200 hairs analysis annually for the past seven years (2001–2007). In 2007, we analyzed 3847 hair samples and 3484 urine samples for drug testing [4]. And recently we published research papers about MA analysis from Korean abusers' hair and urine samples [5–7]. Even though oral fluid analysis has been performed worldwide in the field of driving under the influence of drug (DUID) and work place drug testing [8, 9], drug analysis including MA in oral fluid is not performed yet in Korea. So in this study we established the analytical method for MA and its main metabolite, amphetamine (AM) in oral fluid. And we also performed quantitative analysis of MA and AM in oral fluid, urine and hair samples of twelve Korean drug abusers and investigated the correlation of MA disposition between these specimens.

2 Experimental

2.1 Standards and reagents

MA·HCl (as free base; 1 mg/mL in methanol), AM·HCl (as free base; 1 mg/mL in methanol), MA-D_5 (100 μg/mL in methanol) and AM-D_5 (100 μg/mL in methanol) were purchased from Cerilliant Co., USA. Trifluoroacetic anhydride (TFAA) and pentafluoropropionic anhydride, 99% (PFPA) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). 0.1 M Phosphate buffer, pH 6.0 (Dissolve 13.6 g KH_2PO_4 in 900 mL of distilled water. Adjust the pH to 6.0 with 1 N KOH and make up to 1000 mL with distilled water.) and 5N HCl (Add 206 mL of concentrated HCl to 200 mL of distilled water. Make up to 500 mL with distilled water) were used for extraction. All other chemicals and solvents were of analytical grade.

2.2 Preparation of working standards and internal solution

Each ampule of MA and AM was diluted to 100 μg/mL with methanol (standard stock solution), and working standard solutions were diluted to appropriate concentrations as needed. For internal standards, three concentration solutions of MA-D_5 and one concentration solution of AM-D_5 were prepared with methanol; *i.e.*, 1 μg/mL (for hair analysis), 5 μg/mL (for oral fluid analysis) and 10 μg/mL (for urinalysis) of MA-D_5, and 1 μg/mL of AM-D_5 (for three specimens) were used as internal standards for quantitative analysis.

2.3 Sampling

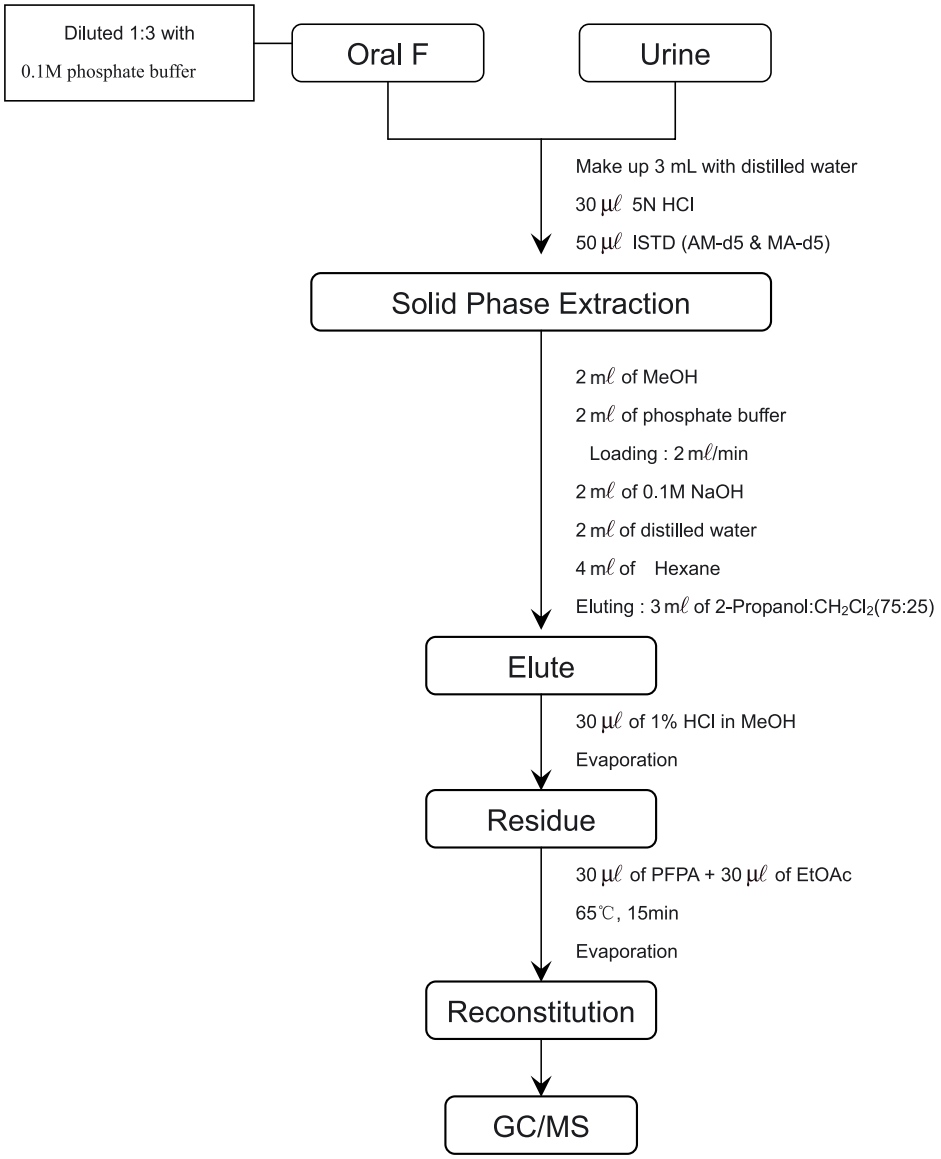
Twelve drug abusers' oral fluid, urine and hair samples were used, and they were submitted to NISI by the Police for drug testing. All of them were men and their age ranged from 33 to 49 (ave. 39.8). Three specimens of twelve drug abusers were obtained simultaneously. Oral fluid was collected by Salivette® (Sarstedt, USA), and its production was stimulated by a cotton swab treated with 20 mg of citric acid. Cotton swab was filtered and centrifuged for further experiment. Urine was collected by plastic container and hair was taken from the posterior vertex region of the scalp by cut closely to skin or pull out.

2.4 Immunoassay

As the preliminary test, oral fluid and urine samples were screened for amphetamines by TDxFLx® (Abbott Co., USA), which was fluorescence polarization immunoassay (FPIA) and calibration range was 0–8000 ng/mL as *d*-amphetamine. For screening, 100 μL of oral fluid and urine samples were applied to TDxFLx. Cut-off level of oral fluid in this study was set to 50 ng/mL as *d*-amphetamine, which was the recommendation level by DHHS (Department of Health and Human Service, USA) [10]. In urine, the cut-off level was 250 ng/mL, following guideline of NISI [11]. Hair samples were directly analyzed for MA by GC-MS without preliminary test.

2.5 Sample preparation

Oral fluid and urine extraction for MA and AM was performed on automatic SPE equipment, RapidTrace™ (Zymark Co., USA) and CLEAN SCREEN® column (130 mg/3 mL



Scheme 1. Extraction procedure for MA and AM in oral fluid and urine.

for oral fluid and 200 mg/3 mL for urine) was purchased from UCT Co. (Bristol, PA, USA). As the sample preparation, 250 μL of oral fluids were diluted with 750 μL of 0.1 M phosphate buffer, pH 6.0 (1:3 volume). Diluted oral fluid (1 mL) and urine samples (~1 mL) were made up to 3 mL with distilled water, and 30 μL 5N-HCl and 50 μL internal standards were added. Column was preconditioned by adding 2 mL methanol and 2 mL 0.1 M phosphate buffer, and sample was loaded on to column at a rate of 2 mL/min. After loading column was washed with 2 mL 0.1N–NaOH, 2 mL distilled water and 4 mL hexane in sequence. MA, AM and internal standards were eluted with 3 mL methylene chloride/isopropanol (25:75). Elute was evaporated under N_2 gas followed by addition of 30 μL 1% HCl in methanol. Residue was derivatized with 30 μL PFPA and 30 μL ethylacetate at 65 °C for 15 min. The excess derivatizing reagent was removed under N_2 gas, and was reconstituted with 50 μL of ethylacetate (Scheme 1).

Sample preparation for hair was the same as in the former study [5]. Briefly, 10–20 mg hair samples were washed twice with 3 mL of distilled water and twice with 3 mL of methanol. Then, they were dried, cut into very small pieces of less than 1 mm and incubated for 20 hr in 1 mL methanol containing 1% HCl in the presence of MA-D_5 and AM-D_5 as internal standards. The solvent was evaporated to dryness under N_2 gas and the residue was derivatized with 30 μL TFAA at 65 °C for 15 min. After evaporation of excess derivatization reagent, and methanol was added for GC-MS analysis. In order to avoid contamination from extraction procedure, different derivatization reagent, TFAA, was used in hair analysis instead of PFPA.

2.6 GC-MS analysis

The GC-MS system consisted of a Hewlett Packard 7683 series injector, HP 6890 series GC system, and HP

5973 mass selective detector. HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used for separation. The injector was operated in the splitless mode and the injection volume was 1 μL. The injector temperature was 250 °C. The ionization energy was 70 eV; the transfer line temperature was 300 °C. For oral fluid and urine, the initial GC oven temperature was 100 °C (1 min hold) and increased at a rate of 15 °C/min to 160 °C (3 min hold) and increased at a rate of 30 °C/min until the final temperature of 280 °C. The final temperature was held for 10 min. For hair analysis, temperature programmed from 100 °C (1 min hold) to 270 °C (10 min hold) at 20 °C/min. Identification and quantitation of MA and AM for oral fluid and hair were performed by SIM mode following ions; MA-PFP, *m/z* 204, AM-PFP, *m/z* 190, MA-D_5-PFP, *m/z* 208, and AM-D_5-PFP, *m/z* 194 [oral fluid], MA-TFA, *m/z* 118, and 154, AM-TFA, *m/z* 118, and 140, MA-D_5-TFA, *m/z* 158 and AM-D_5-TFA, *m/z* 144 [hair], respectively. In urine, quantitation of MA and AM were monitored from full-SCAN mass spectrum; *m/z* 91, 160, and 204 for MA-PFP, *m/z* 91, 118, and 190 for AM-PFP, *m/z* 208 for MA-D_5-PFP and *m/z* 194 for AM-D_5-PFP (the underlined ions were used for quantitation).

2.7 Validation of the method in oral fluid

Method validation was carried out by establishing linearity, intra- and inter-assay accuracy and precision, limit of detection (LOD), limit of quantitation (LOQ) and recovery. For calibration curves of MA and AM, the standard stock solution (100 μg/mL) of MA and AM were diluted to 0.1–10 μg/mL of working standard solutions with methanol. The standard curves of MA and AM were made by spiking 1 mL of dilutes blank oral fluid (1:3 diluted with 0.1M phosphate buffer) with corresponding working standard solutions to obtain two calibration concentration ranges. One is low concentration range from 1–100 ng/mL and the other is high concentration range from 50–1000 ng/mL. Internal standards of MA and AM were used of 5 μg/mL and 1 μg/mL in methanol, respectively. Inter- and intra-assay accuracy and precision data for MA and AM were determined at two different concentrations 5 and 100 ng/mL at low calibration range, 50 and 1000 ng/mL at high calibration range. Three diluted oral fluids which were spiked with MA and AM were extracted by SPE and analyzed by GC-MS (Scheme 1). The extraction procedure was repeated independently on three successive days. Samples were accepted for quantification the value were within ± 15%. The absolute and relative recoveries were also determined at two calibration ranges (5 and 100, 50 and 1000 ng/mL). For absolute recovery, oral fluid samples, which were spiked two different concentrations were extracted and calculated as concentration corresponding to aliquot of standard solution in methanol. Relative recovery of spiked oral fluid was calculated as concentration corresponding to matrix free sample, like as spiked water. In both recoveries, internal standards were added in the last step of extraction procedure and derivatized with PFPA and injected to GC/MS. Identification and quantitation of AM and MA for method validation were the same as the above mentioned section of 2.6 GC-MS analysis.

3 Results and discussion

3.1 Identification of MA and AM in oral fluid, urine and hair samples of drug abuser

Figure 1 showed extraction ion chromatograms (EIC) of AM and MA of oral fluid (Fig. 1A and B), urine (Fig. 1C and D) and hair sample (Fig. 1E and F) from one drug abuser (sample ID 28324). In our condition, AM and MA were well detected with retention time of 5.4 and 6.6 min in oral fluid and urine samples. In hair, retention times of AM and MA were 4.5 and 5.2, respectively. Since MA and AM existed in different concentrations among these specimens, we used different concentrations of internal standards for quantitative analysis. 50 ng of AM-D_5 (Fig. 1A, peak 1) and 250 ng of MA-D_5 (Fig. 1B, peak 3) used for oral fluid, and 50 ng of AM-D_5 (Fig. 1C, peak 1) and 500 ng of MA-D_5 (Fig. 1D, peak 3) for urinalysis, and 50 ng of AM-D_5 (Fig. 1E, peak 1) and MA-D_5 (Fig. 1F, peak 3) for hair analysis were used, respectively.

3.2 Analytical method validation results

The parameters of the validated method for salivary analysis of MA and AM are shown in Table I. Extracted calibration curve of MA and AM were linear over the two concentration range of 1–100 and 50–1000 ng/mL with correlation coefficient of above 0.999. LOQ of MA and AM was 1 and 3 ng/mL, respectively. The limits of detection of MA and AM, which were defined as the detection limits of the target and qualifier ion peaks on each mass chromatogram ($S/N = 3$), was estimated to be 0.1 and 0.7 ng/mL in the SIM mode, respectively. Precision was calculated by performing a one-way ANOVA test [12]. The intra- and inter-day run precisions (CV) for MA and AM were less than 10%, and the accuracies (bias) for MA and AM were also less than 10% at the two different concentrations 5 and 100 ng/mL at low calibration range, 50 and 1000 ng/mL at high calibration range. The absolute recoveries of MA and AM at low and high calibration ranges were more than 82% and 75%, respectively (Table II).

3.3 MA and AM concentrations in oral fluid and urine

Immunoassay and GC-MS results of twelve drug abusers' oral fluid were shown in Table III. Total volume of oral fluids was less than 2 mL in twelve drug abusers. By TDxFLx screening, just one sample gave negative result for amphetamines, and concentrations of MA and AM by GC-MS in eleven oral fluid samples ranged 104.2–4603.3 and 32.4–268.6 ng/mL, respectively (Table III). Average metabolite ratio to parent drug (AM/MA ratio) was 0.11 (range, 0.03–0.31). Amphetamines concentrations by TDxFLx were consistent with those by GC-MS in eleven oral fluid samples. The average amphetamines concentration ratio of TDxFLx/GC-MS was 1.08 (range, 0.92–1.48). By regression analysis, it showed good linearity with a correlation coefficient, 0.9767 (Fig. 2).

While all urine samples were positive to amphetamines by TDxFLx. MA and AM concentrations in twelve urines by GC-MS showed large variety in amount, ranged 120.4–73216.0 ng/mL and 67.6–4238.4 ng/mL, respectively (Table IV). AM/MA ratios were 0.03 to 0.56 (ave. 0.12).

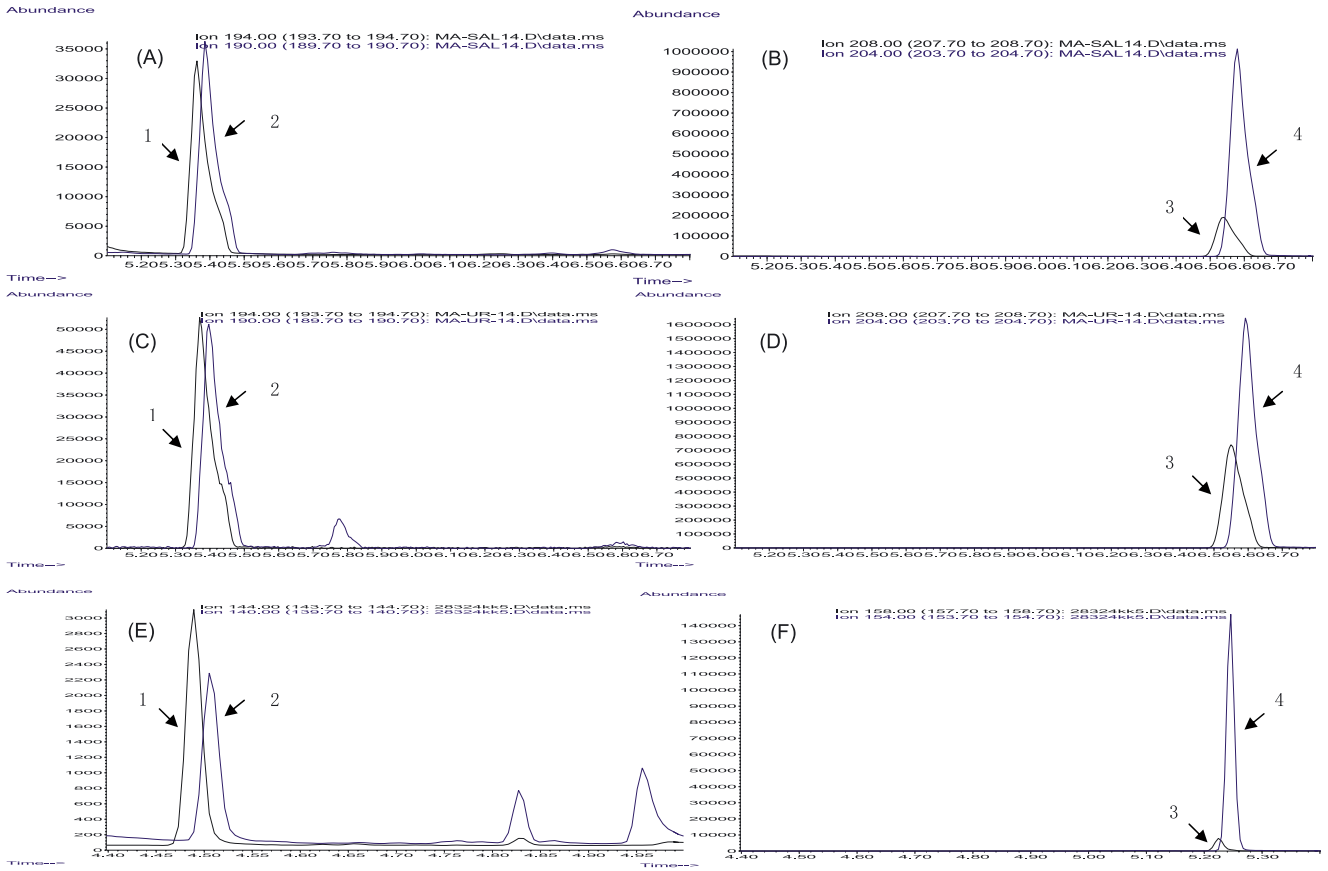


Fig. 1. Extract ion chromatograms of amphetamine and methamphetamine of oral fluid (A and B), urine (C and D) and hair (E and F) from one drug abuser (sample ID: 28324). In three specimens, AM and MA were detected with peak 2 and 4, and peak 1 and 3 were internal standards of AM-D_5 and MA-D_5, respectively.

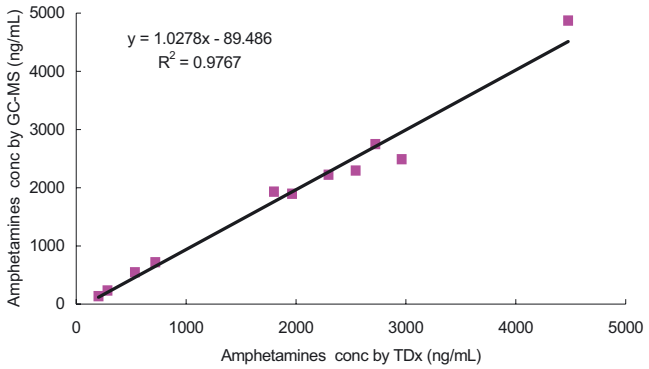


Fig. 2. Correlation of amphetamines concentration between immunoassay and GC-MS analysis.

3.4 Comparison of MA and AM concentrations between oral fluid and urine

Urinary MA and AM concentrations were higher than those in oral fluid in eleven drug abusers, with the average MA and AM ratios of urine/oral fluid was 15.6. (range, 2.5–30.5) and 16.7 (range, 2.7–30.1), respectively (data was not shown). Salivary MA and AM concentrations showed a tendency to increase as urinary MA and AM concentrations increased. However R^2 values of MA and AM were low, 0.176 and 0.227, respectively by linear regression analysis.

3.5 MA and AM concentrations in hair samples

Hair samples were directly analyzed for MA by GC-MS without immunoassay. All twelve hair samples was positive to MA and AM, concentrations of MA and AM ranged 3.40–98.25 and 0.21–4.35 ng/mg, respectively (Table V). Average AM/MA ratio was 0.06 (range, 0.01–0.12).

In former study [7], we determined the AM/MA ratios in the hair of 2444 MA abusers, reported the AM/MA ratios ranged 0.004–1.16 (ave. 0.09). We divided six groups based on MA concentrations in hair (n = 2389), and investigated the relationship between MA concentrations and AM/MA ratios. There was a statistically significant difference among six range groups, namely, in groups of higher MA concentrations, lower ratios of AM/MA were found [7]. In this study, we also investigated the relationship between MA concentrations and AM/MA ratios in hair. We divided three groups based on MA concentrations in twelve samples, with less than 10 ng/mg (group I, n = 3), 11–40 ng/mg (group II, n = 6), and more than 40 ng/mg (group III, n = 3). The average ratios of AM/MA in three groups were 0.085, 0.067 and 0.050, respectively (data was not shown). It showed a similar pattern with the former

Table I. Validation data of MA and AM for established method in oral fluid.

Parameters		Methamphetamine	Amphetamine
Linearity (n = 4)			
Low calibration range		$Y = 0.0197x + 0.0135$	$Y = 0.0183x + 0.008$
(1–100 ng/mL)		(r^2 = 0.9999)	(r^2 = 0.9999)
High calibration range		$Y = 0.0022x - 0.0197$	$Y = 0.0020x - 0.0197$
(50–1000 ng/mL)		(r^2 = 0.9993)	(r^2 = 0.9995)
LOD (ng/mL)		0.1	0.7
LOQ (ng/mL)		1	3
Accuracy (%)			
Low calibration range			
	5 ng/mL	6.01	6.99
	1000 ng/mL	–1.56	–1.48
High calibration range			
	50 ng/mL	–2.36	0.90
	1000 ng/mL	–0.48	9.80
Precision (%)			
Low calibration range	5 ng/mL		
Intra-day		2.23	5.90
Inter-day		1.09	1.47
Low calibration range	100 ng/mL		
Intra-day		2.11	2.16
Inter-day		1.35	1.46
High calibration range	50 ng/mL		
Intra-day		5.64	9.29
Inter-day		4.17	4.50
High calibration range	1000 ng/mL		
Intra-day		8.23	4.71
Inter-day		2.41	1.93

n: number of replicate

Table II. Recoveries of MA and AM for the established method in oral fluid.

Recoveries		Methamphetamine	Amphetamine
Absolute recovery (%) (n = 5)			
Low calibration range			
	5 ng/mL	91.9	80.8
	100 ng/mL	91.6	83.4
High calibration range			
	50 ng/mL	93.1	90.3
	1000 ng/mL	82.1	75.1
Relative recovery (%) (n = 5)			
Low calibration range			
	5 ng/mL	95.9	98.9
	100 ng/mL	102.6	101.3
High calibration range			
	50 ng/mL	100.0	101.0
	1000 ng/mL	103.5	100.5

study, MA concentrations were reciprocally proportional to the ratios of AM/MA. However there was no statistically significant difference among three groups by one-way ANOVA (p = 0.387).

3.6 Comparison of AM/MA ratios in oral fluid, urine and hair samples

The average ratios of AM/MA in urine, oral fluid and hair for eleven samples were 0.12, 0.11 and 0.06, respectively. Two-way ANOVA by RCBD (randomized complete block design) showed a significant difference of AM/MA ratios between three specimens (p = 0.008). Using SAS and multiple comparison by Tukey method, they were divided two groups, one was hair the other was urine and oral fluid, which means AM/MA ratio of hair was significantly lower than those of urine or oral fluid.

Typically, oral fluid is more acidic than is plasma, thus producing higher concentrations of MA in saliva compared to plasma. Regarding MA concentrations between oral fluid and plasma or blood, Schepers *et al.* [13] compared MA and AM

Table III. MA and AM concentrations in twelve drug abusers' oral fluid.

No	Sample ID	Amphetamines conc. by TDxFLx (ng/mL)		Amphetamines conc. by GC-MS (ng/mL)		AM/MA ratio	Total vol. (mL)
				AM	MA		
1	25946	3.13	(–)	n.d.	n.d.	-	1.6
2	14641	202.19	(+)	32.4	104.2	0.31	0.6
3	11466	2296.31	(+)	156.7	2067.0	0.08	0.4
4	19969	1799.53	(+)	84.6	1846.9	0.05	1.4
5	21654–1	534.37	(+)	49.3	496.9	0.10	1.0
6	21654–2	1964.68	(+)	157.5	1738.7	0.09	0.8
7	22816	284.93	(+)	47.7	186.6	0.26	0.8
8	23280	718.69	(+)	57.8	660.9	0.09	0.4
9	25956–1	2722.27	(+)	204.1	2542.5	0.08	0.6
10	25956–2	2542.29	(+)	207.3	2088.4	0.10	0.8
11	25956–3	2961.71	(+)	75.8	2411.8	0.03	1.0
12	28324	4475.68	(+)	268.6	4603.3	0.06	0.4

n.d.: not detected

Table IV. MA and AM concentrations in twelve drug abusers' urine.

No	Sample ID	Amphetamines conc. by TDxFLx (ng/mL)		Amphetamines conc. by GC-MS (ng/mL)		AM/MA ratio
				AM	MA	
1	25946	252.5	(+)	67.6	120.4	0.56
2	14641	1621.82	(+)	105.7	382.7	0.28
3	11466	Hi	(+)	427.5	5233.1	0.08
4	19969	Hi	(+)	1765.2	7865.3	0.22
5	21654–1	Hi	(+)	1483.0	15153.6	0.10
6	21654–2	Hi	(+)	4232.9	47684.6	0.09
7	22816	Hi	(+)	903.0	3756.8	0.24
8	23280	Hi	(+)	758.2	7397.8	0.10
9	25956–1	Hi	(+)	2879.4	33929.1	0.08
10	25956–2	Hi	(+)	4238.4	48414.0	0.09
11	25956–3	Hi	(+)	2192.8	73216.0	0.03
12	28324	Hi	(+)	1237.1	20640.4	0.06

Hi: >8000 ng/mL as *d*−amphetamine

Table V. MA and AM concentrations in twelve drug abusers' hair.

No	Sample ID	Amphetamines conc. by GC-MS (ng/mL)		AM/MA ratio	Hair length (cm)
		AM	MA		
1	25946	0.33	3.40	0.10	3–8
2	14641	0.77	6.44	0.12	9–11
3	11466	0.21	5.61	0.04	3–7
4	19969	1.03	16.36	0.06	4–6
5	21654–1	1.79	19.21	0.09	7–10
6	21654–2	1.77	22.48	0.08	2–4
7	22816	1.48	19.39	0.08	1–4
8	23280	3.11	48.74	0.06	7–9
9	25956–1	4.35	98.25	0.04	1–5
10	25956–2	1.28	16.78	0.08	3–10
11	25956–3	0.23	16.19	0.01	4–10
12	28324	3.74	88.89	0.04	4–6

concentrations between oral fluid and plasma after oral MA administration to human volunteers. They reported that oral fluid MA concentrations were two times higher than those in plasma, and AM concentrations in oral fluid were about one-tenth that of MA. Huestis and Cone [14] also investigated MA disposition in oral fluid, plasma and urine based on a comprehensive controlled dosing study in five healthy volunteers. In their study, MA and AM concentrations in oral fluid appeared to follow a similar time course in oral fluid as in plasma and were dose-proportional, and oral fluid concentrations exceed plasma concentrations. According to recent study by Raes *et al.* [15], the median and mean oral fluid/blood ratio of MA in six subjects were 5.19 and 8.05, respectively. In our study there was no data about MA concentrations in plasma, and the dose, time of last administration, and the duration of administration were unknown. Nevertheless our data for the AM/MA ratio in oral fluid (ave. 0.11) was consistent with Schepers report and MA and AM concentrations in oral fluid showed somewhat concentrations-proportional patterns as in urine.

Generally detection times or "windows of detection" for oral fluid, urine and hair are reported as 1–24 h, 6 h–3 d and >3 d–month/year, respectively [16]. The major advantage of hair testing are larger detection windows depending on the length of hair shaft and evaluation of long term history compared to short term history (*e.g.* oral fluid and urine). In our study, MA and AM were detected in hairs and urines of twelve drug abusers. Therefore all positive results for MA in twelve hair samples mean that they abused MA chronically as well as recent administration.

Finally, regarding lower AM/MA ratio in hair compare to oral fluid or urine, various possible factors could be considered. For examples, the differences of matrix type (solid and liquid), metabolism (adsorption and elimination) and human subject will be possible factors. Considering Nakahara and Hanajiri's research about the mechanism of incorporation into hair for MA and its analogue compounds [17], the difference of melanin affinity to hair between MA and AM also must be studied in further research. In addition to that, sample size can be an important factor to determine the AM/MA ratios. In this study we just analyzed twelve samples. Therefore in order to investigate the relationship of MA disposition in oral fluid, urine and hair specimens, more samples should be analyzed and it is necessary to get more information about the time of last administration, the dose and the route of administration and so on.

4 Conclusion

In this study, analytical method for the detection and quantitation of MA and AM in oral fluid by SPE and GC-MS was established. According to the method validation data, the analytical method is suitable for the detection and quantification of MA in oral fluid. When performed quantitative analysis MA in oral fluid, urine and hair samples of twelve drug abusers, all urines were positive to MA by immunoassay. For oral fluid, just one sample gave negative result. As MA concentrations compared in oral fluid with in urine, urinary MA concentration was about 15 times higher than those of oral fluid. Even though large variability in MA and AM concentrations were observed among three specimens, AM/MA ratios between urine and oral fluid were similar, whereas AM/MA ratio in hair was significantly lower than of other specimens ($p = 0.008$). While all positive results for MA in twelve hair samples mean that they abused MA chronically as well as recent administration. Comparison of drug concentrations in various specimens (*e.g.* oral fluid, urine, hair, etc.) can provide an useful tool for investigating drug disposition as well as evaluating short or long term history of drug abuses in forensic science.

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