Research Note

Journal of Food and Drug Analysis, Vol. 16, No. 5, 2008, Pages 97-101

藥物食品分析　第十六卷　第五期

Assessment of Methamphetamine Abuse Patterns in Southern Taiwan by Immunoassay and Quantification by Gas Chromatographic-Mass Spectrometric Analysis of Urine Samples

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(Received: April 26, 2007; Accepted: March 27, 2008)

ABSTRACT

Methamphetamine (MA) is one of the most commonly abused drugs in Taiwan. In order to investigate the abuse patterns of MA in the Southern Taiwan, urine samples were collected from specified residents of Chiayi City (CCI), Chiayi county (CCO), Tainan City (TCI), Tainan County (TCO), Kaoshiung City (KCI), Kaoshiung County (KCO) and Pingtung County (PCO), representing seven southern cities and counties. A total of 5452 urine samples was collected from July 2006 to December 2006 and subjected to screening for MA with enzyme immunoassay (EIA) and confirmed with GC-MS. As a result, MA positive percent and concentration were 10.9 ± 2.3% and 16535 ± 5682ng/mL, respectively. The positive percent for each location was TCI 14.4 %, CCI 13.2%, PCO 11.3%, KCO 11.2%, CCO 9.0%, TCO 8.7% and KCI 8.7%. The mean concentrations of MA were: TCI 23892, TCO 20566, CCO 17877, KCO 17750, PCO 16260, KCI 13343 and CCI 6058 ng/mL.

Key words: methamphetamine, drug abuse, immunoassay, GC-MS

INTRODUCTION

Methamphetamine is a sympathomimetic drug used to treat narcolepsy, attention deficit disorder and obesity[(1)]. Unfortunately, MA is widely abused, causing social problems in many countries including Taiwan[(2-5)]. MA is classified as a Schedule 2 drug under the Controlled Drugs Act and listed as a restricted ingredient that cannot be produced and traded in Taiwan[(4)]. Under the street names such as speed, ice, crystal, or glass, MA is abused by injection, snorting, smoking, or oral intake. MA is highly addictive and its withdrawal symptoms include depression, anxiety, fatigue, paranoia, aggression, and intense cravings for the drug. Chronic MA use can cause violent behavior, anxiety, confusion, and insomnia. Users may also exhibit psychotic behavior including auditory hallucinations, mood disturbances, delusions, and paranoia, possibly resulting in homicidal or suicidal attempts[(2)]. In fact, the damage to the brain caused by MA use is similar to the damage caused by Alzheimer's disease, stroke, and epilepsy[(6-10)].

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Most MA distributed in the black market is produced in clandestine laboratories in Taiwan. The ease of clandestine synthesis, combined with tremendous profits, has resulted in wide availability of illicit MA. In addition to local clandestine manufacturing, significant amounts of MA have been smuggled into Taiwan[(4)].

Urinary drug testing is commonly used as an objective tool to identify the recent drug use[(11-12)] and drug abuse surveillance. For example, Richardson and Morein[(13)] conducted a urine screening program on prisoners and reported that their results reflected local drug use patterns. Understanding the drug abuse patterns is an important issue for central and local government. This study investigated the abuse pattern of MA in southern Taiwan. In this study, urine samples were collected from the specified residents of Chiayi City (n=204), Chiayi County (n=305), Tainan City (n=243), Tainan County (n=369), Kaoshiung City (n=1601), Kaoshiung County (n=2203) and Pingtung County (n=477), representing seven southern cities and counties in southern Taiwan. Samples were first screened using enzyme immunoassay (EIA) and confirmed of presumptive positive using gas chromatography and mass spectrometer (GC-MS).

MATERIALS AND METHODS

I. *Sampling*

Urine samples were collected from the specified residents of CCI, CCO, TCI, TCO, KCI, KCO and PCO. In each city or county, urine samples were collected from high-risk individuals who have been released from rehabilitation centers. A total of 5452 urine samples were collected from July 2006 to December 2006. Samples were kept refrigerated at 4-6°C prior to analysis.

II. *Samples Analysis*

MA metabolites were screened using EIA on a Hitachi 717 automatic analyzer (Hitachi Co. Ltd., Tokyo, Japan) using DRI^R Amphetamines EIA reagents (Diagnostic Reagents, Inc., Sunnyvale, CA.), with a cut-off concentration of 500 ng/mL. Positive specimens were then derivatized with 4-CB (4-carboethoxyhexafluorobutyryl chloride) and analyzed by GC-MS for confirmation.

III. *Pretreatment of Urine Samples and GC-MS Quantification*

All screened positive specimens were subjected to confirmation by GC-MS. The procedure of Wang *et al.*[14] for pretreatment of urine samples was followed with modification. One milliliter of urine was transferred to screw-capped glass test tube and thoroughly mixed with 1 mL of carbonate buffer solution (1.5 M, pH 10.0), 4 mL of 1-chlorobutane and 50 μL of 10,000 ng/mL AM-d8 and MA-d8 (Cerilliant Co., Austin, TX) were used as deuterated internal standards. The solution was continuously shaken for 10 min and centrifuged (2500 rpm/min) for a further 10 min. After centrifugation, the 1-chlorobutane layer was withdrawn and mixed with 100 μL of 4-CB (Sigma-Aldrich Company Ltd., Dorset, UK) followed by incubation at 70°C for 15 min for derivative formation. Then, the solution was withdrawn, flushed with nitrogen at 45°C to dryness, and re-dissolved in 150 μL of ethyl acetate prior to GC-MS analysis.

The GC-MS analysis followed the method of Xie *et al.*[15] with minor modification. A GC 6890 series gas chromatograph (Agilent Inc., San Jose, CA) interfaced with a 5973 quadrupole mass spectrometer (Agilent) was used for GC-MS analysis. A HP-5MS, 95% dimethylpolysiloxane column (30 m × 0.25 mm i.d., 0.25 μm film thickness), was used for all analyses. Chromatographic conditions were as follows: injector port temperature of 190°C with a injection volume of 1 μL in a splitless mode; Helium at a constant flow rate of 1 mL/min. Initial temperature 120°C was held for 2 min and increased at 20°C /min to 280°C and held for an additional 2 min. Analysis was performed in the selected ion monitoring (SIM) mode. Quantification was based on the first ion listed and the other ions were used for confirmation purpposes. The ions monitored were as follows: *m/z* 294, 266 and 248 for AM, *m/z* 297 and 269 for AM-d8, *m/z* 308, 280 and 262 for MA, *m/z* 315 and 287 for MA-d8.

IV. *Statistics*

For quantification of amphetamines, triplicate determinations were conducted and the mean of determinations with standard deviation reported.

RESULTS AND DISCUSSION

Mandatory urine testing for opiates and amphetamines have been implemented on many groups such as bus drivers and public construction workers in Taiwan. These testing programs follow the same analytical approaches currently adopted in the U.S., i.e., a two-step testing protocol utilizing immunoassays (IAs) for preliminary screens and GC-MS methodologies to confirm those tested positive in the IA test step[3].

As reported by Lua *et al.*[16], 3 different IA reagents (DRI^R; TDx^R and Syva^R) were evaluated for MA screening, all of the IAs were acceptable for the initial screening of MA. As a result, the false positive rates were Syva^R 27.7%, TDx^R 13.9% and DRI^R 11.9%. Accordingly, DRI^R AM EIA reagents were used in this study to screen MA and metabolites. Since IAs can not differentiate structurally similar drugs, most forensic urine drug testing protocols require screening of the sample

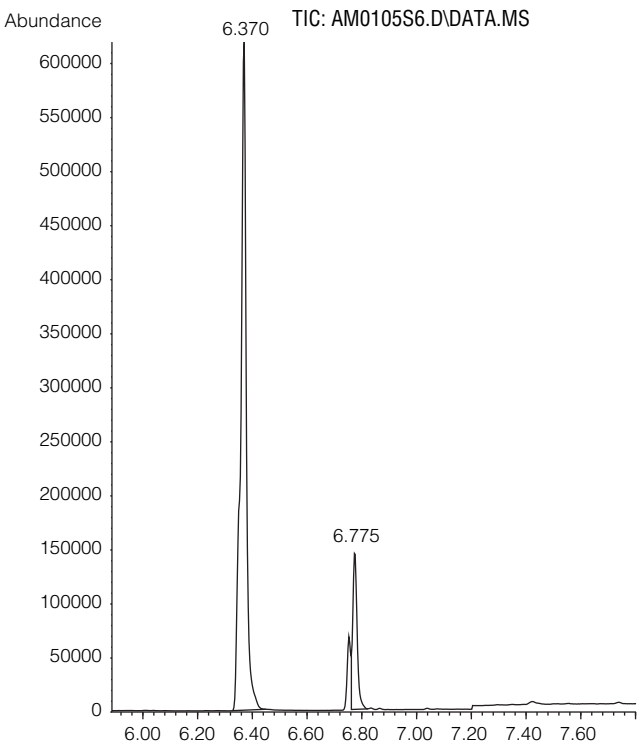

Figure 1. The GC chromatogram of the CB-AM-d8 (retention time = 6.352min), CB-AM (retention time = 6.370min), CB-MA-d8 (retention time = 6.754min), and CB-MA (retention time = 6.775min)

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by IA and confirmation and quantification of the suitable processed sample by GC-MS.

For GC/MS analysis, chemical derivatization of the two analytes (AM and MA), one primary amine and one

Table 1. Intra-day and inter-day precision data for the GC/MS analysis of amphetamine and methamphetamine

Concentration	Intra-day			Inter-day		
(ng/mL)	Mean*	S.D.	R.S.D**	Mean*	S.D.	R.S.D**
Amphetamine						
500	475	11.8	2.5	457	19.1	4.2
800	779	11.3	1.5	763	23.7	3.1
1000	1069	26.9	2.5	985	46.3	4.7
Methamphetamine						
500	521	5.7	1.1	512	7.5	1.5
800	820	1.0	0.1	821	12.1	1.5
1000	1011	2.5	0.3	1012	6.7	0.7

* n = 3.
** R.S.D (relative standard deviation) = S.D./Mean × 100%

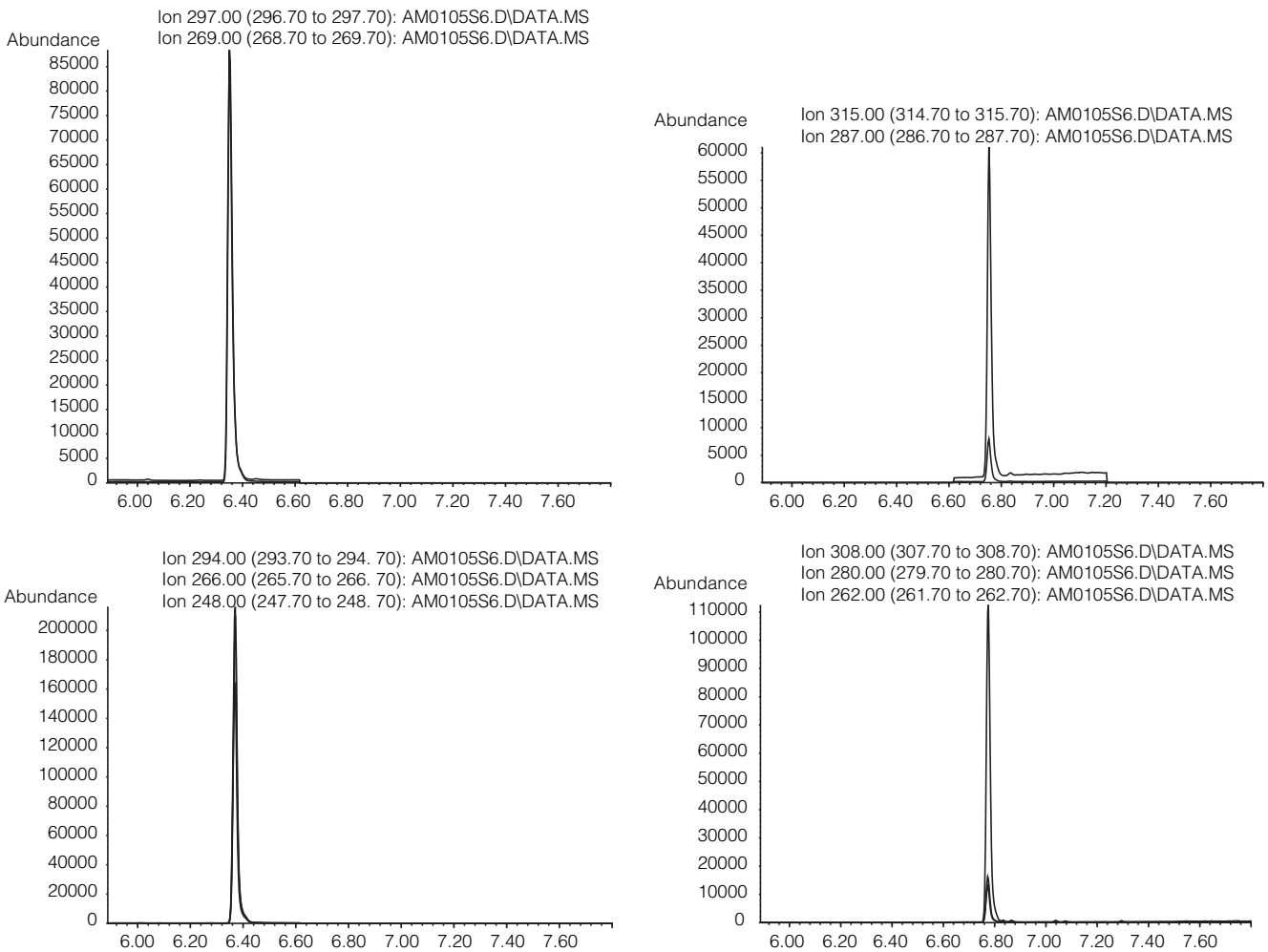

Figure 2. The selected ion chromatograms of CB-AM-d8(ions 297, 269), CB-AM(ions 294, 266, 248), CB-MA-d8 (ions 315, 287), and CB-MA(ions 308, 280, 262).

secondary amine, with 4-CB has not only enhanced their instrumental responses and mass-spectral uniqueness but also enabled easier selection of qualifier and quantifier ions[17]. Hence, GC/MS analysis achieved more evidential qualification and quantification in this study. The results showed that the recoveries calculated for AM and MA were 86.7 % and 89.9 %, respectively. The LOQs were 40 ng/ mL for AM and MA. LODs were 20 ng/mL for AM and 40 ng/mL for MA. The intra-day and inter-day precisions were typically below 4.7% (Table 1).

Shown in Figure 1 are the ion chromatogram of the CB-derivatized analytes and their internal standards (CB-AM and CB-AM-d8 and between CB-MA and CB-MA-d8). The reconstructed GC-MS SIM chromatograms resulting from the analysis of the standards are shown in Figure 2. For quantitative analysis, isotope-dilution method was employed in this study. A specimen was reported positive for MA if its concentration was higher than the MA cutoff (500 ng/mL) and also with 200 ng/mL amphetamine (AM).

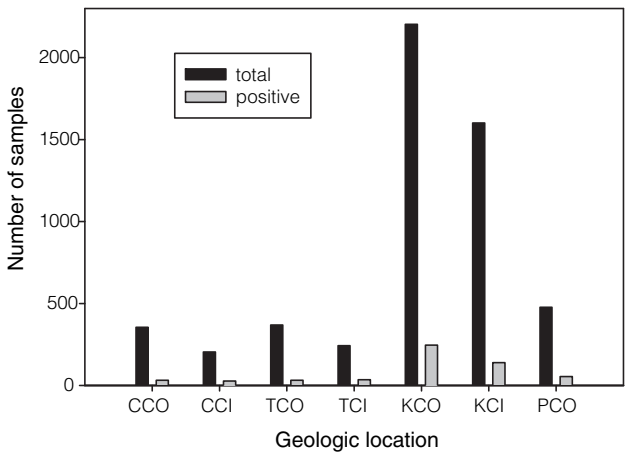

Figure 3. The positive samples for MA from Chiayi City (CCI), Chiayi County (CCO), Tainan City (TCI), Tainan County (TCO), Kaoshiung City (KCI), Kaoshiung County (KCO) and Pingtung County (PCO).
*Total number of samples analyzed is 5452.
GC/MS positive samples for methamphetamine is defined as follow: Methamphetamine: Methamphetamine ≧ 500 ng/mL and Amphetamine ≧ 200 ng/mL simultaneously.

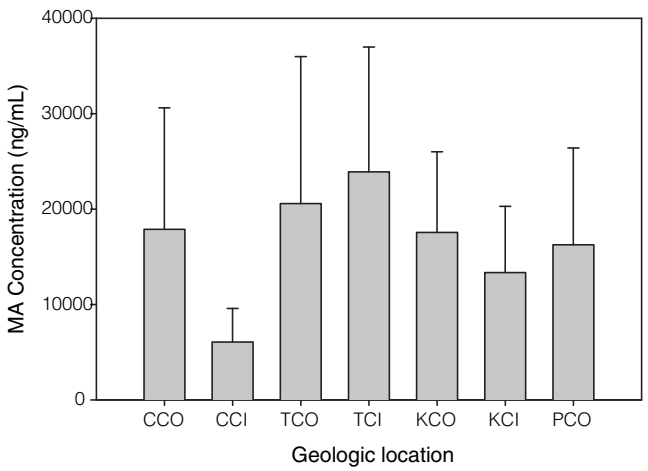

Figure 4. The concentrations of MA in urine samples from Chiayi City (CCI), Chiayi County (CCO), Tainan City (TCI), Tainan County (TCO), Kaoshiung City (KCI), Kaoshiung County (KCO) and Pingtung County (PCO).

As shown in Figure 3 and 4, the positive percents are as follows: TCI 14.4%, CCI 13.2%, PCO 11.3%, KCO 11.2%, CCO 9.0%, TCO 8.7% and KCI 8.7%. The mean concentrations are as follows: TCI 23,892, TCO 20,566, CCO 17,877, KCO 17,750, PCO 16,260, KCI 13,343 and CCI 6,058 ng/mL.

Liu *et al.*[18] reported that opiates and amphetamines were the major drugs of abuse in the detainee's samples, with detection rate of 40% and 38%, respectively. By contrast, lower rates of club drugs (MDMA/ketamine) were found in the sample groups (10% and 5%, respectively). Club drug users tended to be younger (mostly under 27 years old), better educated, and with a smaller gender gap (M/F ratio < 3.5). Club drug users were identified in only four relatively urbanized cities/counties and many arrests were of first offense. As reported by Lua *et al.*[19], the patterns of drug abuse were very different between the participants in disco-dancing club and the general public in Taiwan. In the club samples, MDMA was the most common drug detected (75.7%), followed by ketamine (47.0%) and MA (41.6%). In the detainee samples, MA (76.0%) was the most common drug detected, followed by opiates (37.0%), MDMA (6.0%) and ketamine (2.0%). In this study, urine samples were collected from the individuals released from rehabilitation centers for drug addicts. Although the result was lower than that of Liu and Lua, it showed that the recidivism was still high.

In conclusion, data hereby reported provide valuable information to policy development.

ACKNOWLEDGEMENTS

Assistance in the laboratory by C.Y. Chang, T.-L. Yeh, M.-H. Juan and M.-H. Chiang is acknowledged.

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