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Short communication

Simultaneous determination of methamphetamine and its metabolite, amphetamine, in urine using a high performance liquid chromatography column-switching method

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Abstract

We describe here a simple, precise, and highly sensitive method for the simultaneous determination of methamphetamine (MA) and amphetamine (AM) in urine using a high performance liquid chromatography (HPLC) column-switching method. A PK-2A (Shodex) column was used for extraction and deproteinization, and a CAPCELL PAK SCX semi-micro, polymer-coated cation-exchange column was employed for separation. The urine sample was mixed with an equal volume of borate buffer (0.1 M, pH 9.4), and then 100 μl of the mixture was injected into the HPLC column. The column was switched for 6 min, and then 10 min later detection was performed at 210 nm. Recovery yields of the MA and AM spiked in the urine were 93.0–100.4% with a coefficient of variation of less than 1%. The calibration curves of MA and AM were in the range of 0.1–10 μg/ml with good linearity ($r^2 = 0.999$), with the limit of qualification being 0.005 μg/ml. This method of using HPLC with column-switching can be used for both qualification and quantification of MA and its metabolite, AM, in urine, especially in forensic cases.

Keywords: HPLC; Column-switching; Methamphetamine; Amphetamine; Urine

1. Introduction

Methamphetamine (MA) is one of the most commonly abused stimulant drugs in the world. About 20,000 people were reportedly arrested in the last 10 years in Japan due to the abuse of illegal drugs; more than 85% of all cases involved MA abuse [1]. Amphetamine (AM), a metabolic product of MA, is easily formed in the body of the MA abuser. Since both of these compounds are excreted in the urine, it is necessary to be able to detect them there in order to confirm an individual as an abuser.

Various analytical methods such as thin-layer chromatography, gas chromatography, gas chromatography–mass spectrometry (GC–MS), high performance liquid chromatography–ultraviolet detection (HPLC–UV), HPLC–chemiluminescence detection, and HPLC–MS have been used for the assay of MA, AM and their related compounds in biological samples [2–8]. Recently an HPLC–tandem mass spectrometric method has been developed for the identification as well as the quantification of MA and its metabolites such as AM, *p*-hydroxy-MA-glucuronide and *p*-hydroxy-MA-sulfate in an MA abuser's urine [9].

Automated forensic analysis methods using HPLC to determine drugs in a biological matrix have also been recently reported [10–13]. These methods involve the capacity to extract, separate, and detect drugs in urine or serum via an on-line connection with or without mixing with a buffered solution. The process of liquid–liquid extraction or solid phase extraction followed by concentration, which involves a loss of target drugs as the total analysis standard deviation increases, is not required. In addition, these methods are time saving, can be performed using a small sample volume, and are free from tedium. We, however, established a simple, accurate, and sensitive system of analysis that used HPLC–UV with column-switching. In our

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work we used this technique for the simultaneous qualification and quantification of MA and its major metabolite, AM, in urine for the confirmation of MA abuse.

2. Material and methods

2.1. Reagents

MA·HCl (>98.5%) was purchased from Dainippon Pharmaceutical (Osaka, Japan). AM·H_2SO_4 (>ca. 98.5%) was obtained from the Ministry of Health, Labour, and Welfare (Japan). Acetonitrile (HPLC grade) and all other reagents used here were purchased from Wako Pure Chemical Co. (Tokyo, Japan). 0.1 M phosphate buffers (pH 4.0–9.0), 0.1 M borate (pH 9.4), and 0.1 M borate-NaOH buffers (pH 10.0, 11.0) were prepared. Drug-free urine pH 5.4–7.1 (pH 6.4 ± 0.56, $n = 6$) was collected from healthy male volunteers without any history of stimulant use after we had obtained their informed consent. A stock solution (500 μg/ml) of MA and AM was prepared in distilled water. Working solutions were prepared from stock solutions by dilution with distilled water or drug-free urine. All prepared solutions were stored at 4 °C until analysis. Urine samples were obtained from subjects suspected of having used MA. The MA and AM were qualified by the GC–MS method according to a previous report after liquid–liquid extraction and trifluoroacetyl derivatization [2]. The main operation conditions for GC–MS were as follows: apparatus GCMS-QP5050A (Shimadzu Corp., Kyoto, Japan), column SPB-17 (0.25 mm i.d. × 30 m, film thickness 0.25 μm, Sigma–Aldrich Japan, Tokyo, Japan), temperature: 120 °C for column, 230 °C for injection and interface ionisation, electron ionization (70 eV).

2.2. Operation conditions of HPLC and sample preparation

The details of the column-switching HPLC–UV system used here were as follows: PU-2085 pump (for separation), PU-2080 pump (for extraction), DG-2080-53 degasser, AS-2059 auto-sampler, CO-2065 column oven, HV-2080-01 six-ports valve, HSS-2000/Borwin LC workstation (Jasco Corp., Tokyo, Japan), L-7400 UV-detector (Hitachi High-Technologies Corp., Tokyo, Japan), column CAPCELL PAK SCX UG80 (1.5 mm i.d. × 150 mm, polymer coat type strong cation-exchange phase, Shiseido, Tokyo, Japan) for analysis, Mspak PK-2A (2.0 mm i.d. × 10 mm, hydrophilic copolymers containing *N*-vinyl acetamide resin, Shodex, Tokyo, Japan) for extraction.

The two mobile phases used were as follows: 5 mM of phosphate buffer (pH 6.8, flow rate at 0.7 ml/min) for extraction, and 20 mM of phosphate buffer (pH 5.0) combined with acetonitrile (25/75, v/v, flow rate at 0.2 ml/min) for analysis. The column temperature was room temperature for extraction and 40 °C for analysis. After 500 μl of urine sample was mixed with an equal volume of 0.1 M borate buffer (pH 9.4), the mixture was filtered through a membrane filter (0.45 μm pore size), and then 100 μl of the filtrate was injected into the HPLC column. Column-switching was performed for 6 and 10 min, respectively, and then determined at 210 nm. The extraction process was 0–6 min, the elution process in the back-flushed mode was 6–10 min, and the stabilization process was 10–26 min for the extraction column. The elution and separation process performed using the analysis column was 6–26 min. A high concentration of MA (>10 μg/ml) in urine samples was required to be diluted by distilled water.

3. Results and discussion

Several analytical methods such as GC–MS [2–4], HPLC–UV [5], and HPLC–MS [8,9,13] have been reported for the assay of MA and AM and related compounds in biological samples. Most of the methods involve sample pretreatment by extraction as well as through a concentration process. A few studies reported a column-switching HPLC–UV method to screen out many drugs including AM [12], and a column-switching HPLC–UV method for the determination of MA enantiomers in urine [10]. The column-switching HPLC–UV method used in the present study is the first approach that simultaneously determines both MA and AM in urine. The semi-micro column was used here to reduce the volume of the mobile phase. We believe that the present method is particularly appropriate for the routine analysis of these compounds in forensic cases.

3.1. Optimization of the HPLC determination

Fig. 1(a) shows the UV-spectra of MA and AM dissolved in a mobile phase that is composed of a 20 mM phosphate buffer of pH 5.0; acetonitrile (25/75, v/v) was used for elution and separation. The wavelength of 210 nm was used here because high absorbance was obtained at this wavelength.

Fig. 1(b–d) shows the typical chromatograms obtained from blank urine, urine spiked with MA and AM (1.0 μg/ml), and forensic urine, respectively. The MA and AM peaks were clearly separated at retention times of 17.4 and 19.2 min, respectively, and there were no interference peaks observed in the chromatogram of blank urine. In general, MA and AM analysis using HPLC is performed by reverse phase columns for separation [2,7]. Here we used a polymer-coated cation-exchange column. This column has the capability of performing both cation-exchange and reverse phase action. Therefore, the separation of MA and AM was found to be quite good using this column, at levels acceptable for forensic cases. The HPLC system and operating conditions examined here provides sufficient separation of MA and AM with no interference of the urinary matrix.

Fig. 2 shows the effects of pH on the% recovery of MA- and AM-spiked urine (5.0 μg/ml). The% recovery is the area value of MA and AM obtained by the column-switching system divided by the area value of these substances obtained from the HPLC system with and without using an extraction column. The recovery yields of MA and AM in the spiked urine were over 95% when the mixture with the buffer was at a pH of 9.4–11.0, but the recoveries of MA and AM were decreased gradually by lowering the pH in the buffer. We therefore used borate buffer (0.1 M, pH 9.4) due to its high recovery yields and stability and because of its the simple preparation. The pH value of the urine sample mixed with borate buffer (0.1 M, pH 9.4) was adjusted to the range of 8.9–9.2 (pH 9.0 ± 0.12, $n = 6$).

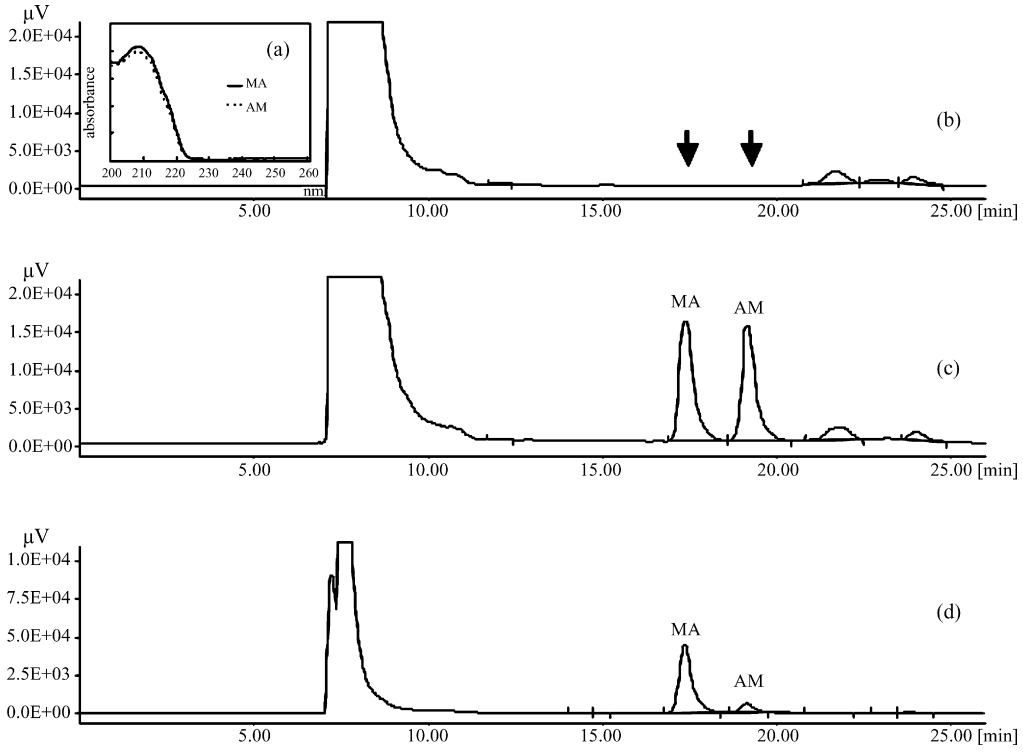

Fig. 1. UV spectra of methamphetamine (MA) and amphetamine (AM) in the mobile phase (a) and chromatograms obtained from drug-free urine (b), urine spiked with MA and AM (1.0 μg/ml) (c), forensic urine (MA 32.1 μg/ml and AM 4.6 μg/ml) diluted by 10-fold (d).

3.2. Limit of detection and calibration curves

Urine controls to which MA and AM had been added were serially diluted (0.02–10 μg/ml) and analyzed in five replicates to determine the limits of quantification and to produce calibration curves. The limits of detection (a signal-to-noise ratio of 5:1) for MA and AM in urine were both estimated 0.005 μg/ml. The both calibration curves were obtained from the concentration range of 0.1–10 μg/ml with a high correlation coefficient ($r^2 = 0.999$) that was based on the absolute calibration curve method. The equations were $y = 311300x + 9100$ for MA and $y = 311357x + 4412$ for AM (x = the analyte concentration (μg/ml), y = peak area). These results showed an acceptable level of sensitivity and a quantification that was higher than the cut-off values of 0.25 and 0.1 μg/ml for MA and AM, respectively [14].

3.3. Accuracy and precision

Table 1 shows the accuracy and precision (within-day and day-to-day) of the method, which were examined using five replicate analyses of spiked urine at 0.2, 1.0 and 10 μg/ml. In each concentrate examined, the recovery yields of MA and AM were more than 96%, although the AM yield at 0.2 μg/ml was about 94%. The recovery yields of MA and AM in urine mixed with borate buffer were high and stable in the wide ranges of those concentrations. The values of coefficient variation of the method both within-day and day-to-day were less than 1%.

3.4. Application of forensic toxicological cases

Fig. 3 shows the concentration of MA and AM in each urine sample obtained from 158 forensic cases of individuals sus-

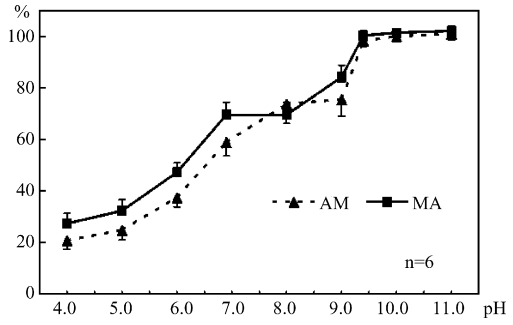

Fig. 2. Effect of pH on recovery of methamphetamine (MA) and amphetamine (AM) in spiked urine (5.0 μg/ml).

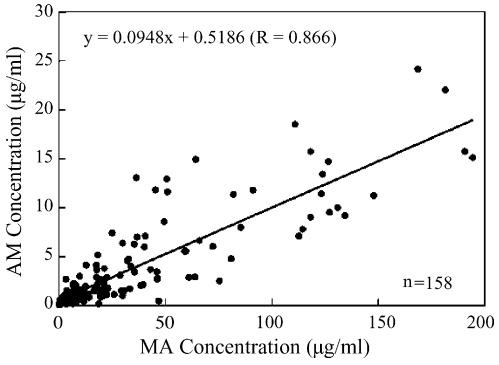

Fig. 3. Correlation coefficient between methamphetamine (MA) and amphetamine (AM) in forensic urine of MA abusers.

Table 1
Accuracy and precision of methamphetamine (MA) and amphetamine (AM) analysis from spiked urine and within-day and day-to-day variability

	MA and AM levels in spiked urine (μg/ml)		
	0.2	1.0	10
Recovery (%)*			
Within-day			
MA	98.3 ± 0.74	99.4 ± 0.59	99.9 ± 0.14
AM	94.8 ± 0.94	100.0 ± 0.73	99.8 ± 0.31
Day-to-day			
MA	96.7 ± 0.85	100.1 ± 0.66	97.3 ± 0.69
AM	93.0 ± 0.83	100.4 ± 0.84	96.3 ± 0.80
% Relative accuracy			
Within-day			
MA	−1.66	−0.57	−0.07
AM	−5.16	−0.05	−0.19
Day-to-day			
MA	−3.32	0.10	−2.73
AM	−7.03	0.40	−3.68
Precesion (% CV)			
Within-day			
MA	0.76	0.59	0.14
AM	0.99	0.73	0.31
Day-to-day			
MA	0.88	0.66	0.71
AM	0.89	0.83	0.84

$N = 5$; MA: methamphetamine; AM: amphetamine.
* Mean ± SD.

pected of abusing MA. MA and AM concentrations ranged from 0.14 to 426 and 0.13 to 46.7 μg/ml, respectively. These samples were qualitatively analyzed for the presence of MA and AM by GC–MS. Since MA and AM were simultaneously determined to be in the urine of all these cases by both HPLC–UV and by GC–MS analysis, we concluded that all cases illegally used MA. This conclusion also coincides with the results of police investigations. A relatively high correlation between MA and AM concentration in urine was observed in our cases ($r = 0.866$). The present results, furthermore, coincided with results from a previous report [15]. These high correlations indicate that the simultaneous qualification and quantification of MA and AM in urine refute the possibility of a legal claim that the results could come from either intentional or unintentional mixing.

4. Conclusions

In the present study, we described a column-switching HPLC–UV method for the analysis of MA and AM simultaneously in urine. This method is proven to be a highly precise and quite useful forensic method for analyzing the urine of suspected abusers.

References

[1] National Police Agency, White Paper on Police in Japan 2005, National Police Agency, Tokyo, 2005, p. 190.
[2] T. Nagata, K. Hara, in: A. Maehly, R.L. Williams (Eds.), Forensic Science Progress 4, Springer-Verlag, Berlin, Heidelberg, 1990, p. 25.
[3] A. Kankaanpää, T. Gunnar, K. Ariniemi, P. Lillsunde, S. Mykkänen, T. Seppälä, J. Chromatogr. B 810 (2004) 57.
[4] M. Nishida, A. Namera, M. Yashiki, T. Kojima, J. Chromatogr. B 789 (2003) 65.
[5] N.Y. Li, Y. Li, E.M. Sellers, Eur. J. Drug Metab. Pharm. 22 (1997) 427.
[6] K. Hayakawa, Y. Miyoshi, H. Kurimoto, Y. Matsushima, N. Takayama, S. Tanaka, M. Miyazaki, Biol. Pharm. Bull. 16 (1993) 817.
[7] K. Nakashima, Jpn. J. Forensic Toxicol. 21 (2003) 227.
[8] M. Katagi, H. Nishioka, K. Nakajima, H. Tsuchihashi, H. Fujima, H. Wada, K. Nakamura, K. Makino, J. Chromatogr. B 676 (1996) 35.
[9] N. Shima, H. Tsutsumi, T. Kamata, M. Nishikawa, M. Katagi, A. Miki, H. Tsuchihashi, J. Chromatogr. B 830 (2006) 64.
[10] Y. Makino, A. Suzuki, T. Ogawa, O. Shirota, J. Chromatogr. B 729 (1999) 97.
[11] H. Lee, J.S. Lee, H.S. Lee, J. Chromatogr. B 664 (1995) 335.
[12] M.T. Saarinen, H. Sirén, M.-L. Riekkola, J. Chromatogr. B 664 (1995) 341.
[13] A. Miki, M. Katagi, H. Tsuchihashi, J. Anal. Toxicol. 27 (2003) 95.
[14] Substance Abuse and Mental Health Services Administration (SAMHSA), Division of Workplace Programs, Draft #4 9/5/2001, http://workplace.samhsa.gov/ResourceCenter/DT/FA/GuidelinesDraft4.htm.
[15] C.E. Cook, A.R. Jeffcoat, J.M. Hill, D.E. Pugh, P.K. Patetta, B.M. Sadler, W.R. White, M. Petez-Reyes, Drug Metab. Dispos. 21 (1993) 717.