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Direct high-performance liquid chromatographic and high-performance liquid chromatographic–thermospray-mass spectrometric determination of enantiomers of methamphetamine and its main metabolites amphetamine and *p*-hydroxymethamphetamine in human urine

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Abstract

For the identification of drug abuse, a simple and rapid method which allows us to distinguish enantiomers of methamphetamine (MA) and its metabolites amphetamine (AP) and *p*-hydroxymethamphetamine (*p*-OHMA) in human urine was explored by coupling direct HPLC and HPLC–thermospray-mass spectrometry (HPLC–TSP-MS) both of which employ a β-cyclodextrin phenylcarbamate-bonded silica column. HPLC analysis was performed after the solid-phase extraction from the urine sample with Bond Elut SCX, and D- and L-enantiomers of MA, AP and *p*-OHMA could be separated well. The proposed conditions are as follows: eluent, acetonitrile–methanol–50 m*M* potassium phosphate buffer (pH 6.0) (10:30:60, v/v) flow-rate, 1.0 ml/min temperature, 25°C. The linear calibration curves were obtained for D- and L- MA and AP in the concentration range from 0.2 to 20 μg/ml; the relative standard deviation for D- and L-AP and D- and, L-MA ranged from 1.67 to 2.35% at 2 μg/ml and the detection limits were 50 ng/ml for D- and L-AP and D-MA and 100 ng/ml for L-MA. For the verification of the direct HPLC identification, HPLC–TSP-MS was also carried out under the same conditions except that acetonitrile–methanol–100 m*M* ammonium acetate (pH 6.0) (10:30:60, v/v) was used as an eluent. Upon applying the scan mode, 10 ng/ml for D- and L-AP and D-MA and 20 ng/ml for L-MA were the detection limits. Using the selected ion monitoring mode, 0.5 ng/ml, 0.8 ng/ml and 1 ng/ml could be detected for D- and L-AP, D-MA and L-MA, respectively.

Keywords: Enantiomer separation; Methamphetamine; Amphetamine; *p*-Hydroxymethamphetamine

1. Introduction

Drug abuse, which damages personality and also causes an increase in the number of criminals, has become one of the most serious problems all over the world and is still increasing rapidly. Therefore, the development of convenient and reliable analytical methods for detecting drugs and their metabolites in

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body fluids is urgently required and has become an important subject in forensic science.

At present, D-methamphetamine (D-MA) is extensively used in most cases of drug abuse in Japan (more than 99% among the cases) and its illicit use is evidenced by the detection of MA and its main metabolite amphetamine (AP). In this identification, optical activities of these two compounds are not considered, although it would be desirable. It is already known that some legitimate medicines which are not sold currently in Japan but are sold in Europe and the USA are metabolized to MA and AP and excreted in urine [1–8] and that some of them are in the L-form. Thus it is strongly recommended that the method we will develop should be able to distinguish between the D- and L- forms to differentiate the legitimate use of the medicines from illicit use of D-MA. Consequently, in the present study, we explored by high-performance liquid chromatography (HPLC) the identification of D- and L- forms of MA and its metabolites such as AP and *p*-hydroxymethamphetamine (*p*-OHMA) [9,10].

Previously reported analysis of enantiomeric MA and AP has utilized (1) HPLC coupled with chiral stationary phases after derivatization such as acetylation and benzoylation [11–17], (2) gas chromatography and HPLC coupled with chiral derivatization, for instance, with N-trifluoroacetyl-L-prolylchloride (L-TPC) [9,18–23], (*S*)-α-methoxy-α-trifluoromethylphenylacetyl chloride [24] and 2,3,4,6-tetra-O-acetyl-β-glucopyranosyl isothiocyanate (GITC) [10,25,26], and (3) capillary electrophoresis (CE) without derivatization processes [27]. Such derivatization is tedious and time-consuming. Particularly in chiral derivatization, the differences in the rate of the derivatization and enantiomeric purity of the reagents are difficult problems that hamper accurate analysis. Therefore, derivatization should be avoided in the improved method. It is also known that CE still lacks sensitivity and reproducibility. Another weak point of CE is that it is difficult to couple with mass spectrometry.

Recently, for direct HPLC analysis of drugs with chirality, various chiral-differentiating stationary phases have been developed, which allow us to avoid pre-column derivatization. Column materials recognizing the difference of the optical activities are cellulose derivatives [15–17,28], proteins such as bovine serum albumin [29], ovomucoid [30], and cyclodextrins (CD) [31,32]. Among them, CD-bonded columns are considered promising for drug abuse detection, because CD holds aromatic compounds such as MA in cavities through hydrophobic interactions and such a force, which strongly influences the retention, is highly dependent on the chirality of the compounds. Another advantage for CD-bonded columns is that it can be used both in aqueous and organic solvents and their mixtures and this allows us to use a wide variety of eluent modifiers. It is also attractive that derivatization of the CD stationary phase could improve the original recognition ability. Previously, a stationary phase bonded with unmodified CD has been used for the separation of enantiomeric MA and its analogs [33] and it has been reported that a separation factors (α) of less than 1.1 was obtained for enantiomeric MA with aqueous methanol as an eluent. This result implies the usefulness of a CD-bonded column in direct analysis of drug abuse although the separation must be much improved. Probably due to this rather poor resolution, no data on the urine samples of drug addicts have been reported so far.

In the present study, therefore, we employed a stationary phase immobilized with derivatized CD, such as a β-cyclodextrin phenylcarbamate-bonded silica chiral column. This paper describes a rapid, simple and accurate analysis of enantiomers of MA, AP and *p*-OHMA in urine samples by direct HPLC. Also the analytical conditions for HPLC–thermospray (TSP)-MS analysis, which is used for the confirmation of the results by direct HPLC, were explored.

2. Experimental

2.1. Materials

D-MA hydrochloride was obtained from Dainippon Pharmaceutical (Osaka, Japan). L-MA was extracted from Vicks inhaler and purified as the hydrochloride salt in our laboratory. DL-AP sulphate was obtained from Takeda Pharmaceutical Industries (Osaka, Japan). DL-*p*-OHMA hydrochloride was synthesized according to the procedure described in a previous paper [34]. Standard stock solutions of MA, AP and

p-OHMA were prepared in distilled water (1 mg/ml), and adjusted to the appropriate concentration with distilled water or human urine immediately prior to use. An internal standard (I.S.), N-ethyl aniline, was purchased from Wako Pure Chemical Industries (Osaka, Japan), and the I.S. solution was prepared in methanol (the concentration being 1 mg/ml). Methanol and acetonitrile were of HPLC-grade, and other chemicals used were of analytical grade. Bond Elut SCX and Bond Elut Certify cartridges were purchased from Varian Sample Preparation Products (Harbor City, CA, USA) and Sep-Pak C_{18} cartridges from Waters Associates (Milford, MA, USA).

2.2. Sample preparation

Urine samples were prepared as follows (Fig. 1): Bond Elut SCX cartridges were prewashed successively with 10 ml of 2% aqueous NH_3–methanol, 10 ml of methanol, and 30 ml of distilled water. Urine samples (10 ml) were mixed with 10 ml of 50 m*M* potassium phosphate buffer (pH 7.8) and loaded on the prewashed cartridge. Subsequently, the cartridge was washed with 2 ml of distilled water and then 10 ml of methanol. The retained compounds were eluted using 3 ml of 2% aqueous NH_3–methanol. Then, 30 μl of CH_3COOH and 30 μl of the I.S. solution were added to the eluates. The aliquots (5 μl) was injected into the HPLC system for direct HPLC. To the HPLC–TSP-MS system, 50 μl was applied since this system is used only for the confirmation of the data obtained.

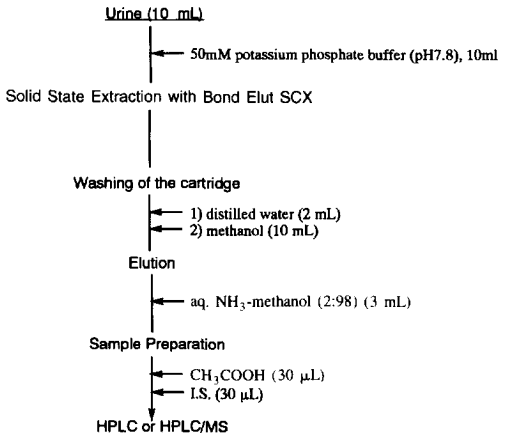

Fig. 1. Procedure for the preparation of urine samples.

2.3. High-performance liquid chromatography

Direct HPLC of enantiomers was carried out on an LC-10A liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with an SPD-10A UV detector (Shimadzu) set at 220 nm and a CS-300B temperature-controlled column compartment (Chromato Science, Osaka, Japan). An analytical column (a β-CD phenylcarbamate-bonded silica column) used was ULTRON ES-PhCD (150×6.0 mm I.D. and 5-μm particle size) (Shinwa Chemical Industries, Kyoto, Japan). Also, for comparison of the separation, a β-CD-bonded silica column (ULTRON ES-CD, Shinwa Chemical Industries) was used as a reference. The mobile phase consisted of acetonitrile–methanol–50 m*M* potassium phosphate buffer: Details appear in the text. The flow-rate of the mobile phase was 1 ml/min.

2.4. HPLC–TSP-MS analysis

HPLC–TSP-MS was performed with a Shimadzu LCMS-QP1100EX system equipped with a TSP ion source. The mass spectrometer was operated in the positive ion mode with the filament off. The TSP vaporizer temperature was 230°C and the ion source block temperature was 300°C. The chromatographic conditions were the same as those for direct HPLC except that the mobile phase was composed of acetonitrile–methanol–100 m*M* CH_3COONH_4 (pH 6.0) (10:30:60, v/v).

3. Results and discussion

3.1. Chromatographic conditions for direct HPLC analysis

To optimize the separation of D- and L-enantiomers of MA, AP and *p*-OHMA on a β-CD phenylcarbamate-bonded silica column (ULTRON ES-PhCD, referred to as ES-PhCD), suitable mobile phase composition was explored using the mixture of acetonitrile and/or methanol and potassium phos-

phate buffer. As samples, artificial mixtures of the three sets of the enantiomers were used.

Before using ES-PhCD, we tested a reference column of ULTRON ES-CD (referred to as ES-CD) to reexamine if sufficient resolution can not be produced on this column: Note that for a similar purpose, a stationary phase bonded with unmodified CD has been used previously for the separation of enantiomeric MA with aqueous methanol as an eluent and a poor α value was obtained [33]. In the present study, using ES-CD, the isomers eluted in the order of L-AP, D-AP, L-MA and D-MA, and fairly good separation of enantiomers of both MA and AP was obtained (data not shown). For urine samples from suspected drug abusers, however, D-*p*-OHMA eluted between D-AP and L-MA with insufficient resolution and this problem could not be overcome with the various solvent systems used here. This result implies the necessity for column improvement.

On the other hand, probably due to the large hydrophobicity of the stationary phase introduced by the phenyl groups bonded with CD, ES-PhCD gave a sufficient separation of the enantiomers of MA, AP and *p*-OHMA, as shown later. The elution order of the isomers was always in the order of the polarity: *p*-OHMA, AP and MA.

In the separation on ES-PhCD, it was found that the separation of the enantiomers is largely dependent on the solvent composition. Therefore, we explored the optimization of the eluent starting with methanol–potassium phosphate buffer (50 m*M*, pH 6.0). The methanol concentrations were varied between 50 and 70% and k values of the enantiomers were measured. As shown in Fig. 2, the higher methanol concentration led to smaller k values and worse separations for all the D- and L-isomers. At the methanol concentration of 60% (referred to as eluent A), all the components eluted within 20 min, and the best separation was achieved in this eluent system. With this composition, however, the separations between L-*p*-OHMA and D-AP and between L-AP and D-MA were not sufficient for practical drug abuse detection, and required further improvement.

In the acetonitrile–potassium phosphate buffer (pH 6.0) system, the concentration of acetonitrile was varied from 10 to 30%. As depicted in Fig. 2, smaller k values and worse separations were produced as the acetonitrile concentration increased. At the acetonitrile concentration of 20% (referred to as eluent B), when all the components eluted within 20 min and the best separation in this eluent system was achieved, sufficient separations were obtained only for L-*p*-OHMA, D-AP and D-MA.

Since the sole use of methanol or acetonitrile as

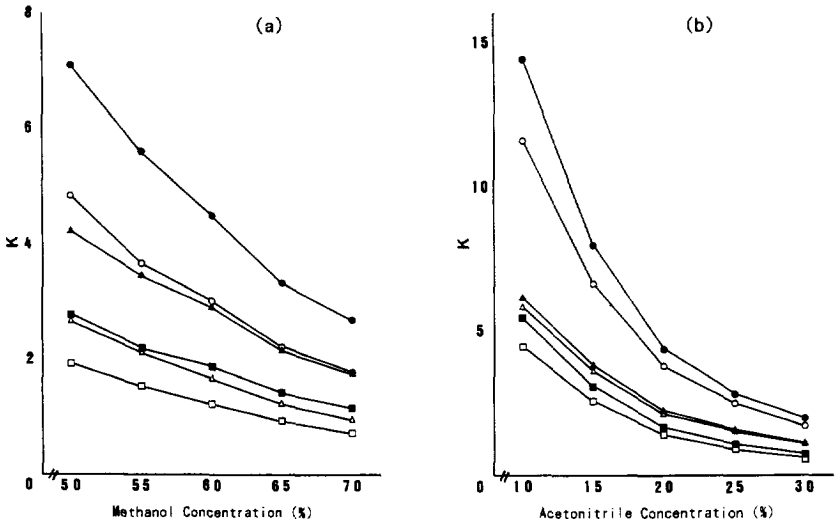

Fig. 2. Effect of (a) methanol and (b) acetonitrile concentrations in eluents on the k values of enantiomers of MA, AP and *p*-OHMA. These organic modifiers were mixed with 50 m*M* potassium phosphate buffer (pH 6.0). (●) L-MA, (○) D-MA, (▲) L-AP, (△) D-AP, (■) L-*p*-OHMA, (□) D-*p*-OHMA.

modifiers did not lead to the desired separation of the enantiomers, the optimum composition of the mobile phase was explored by mixing eluents A and B, since the results shown in Fig. 2 suggest that these two modifiers can work complementarily. The composition of eluent A and eluent B was varied between 40 and 60% eluent A, and k and α values and the resolution (R_s) were measured. As shown in Table 1 and Fig. 3, the larger α and R_s values were obtained for every enantiomer and the separation was worse for L-*p*-OHMA and D-AP as the ratio of eluent A increased. Finally the best separation was achieved when the ratio was 50%. The final composition was acetonitrile–methanol–50 m*M* potassium phosphate buffer (10:30:60, v/v). The effect of pH was also examined but no significant improvement was achieved.

It is known that optimum temperature frequently exists for the separation of enantiomeric compounds. Particularly, upon usage of chiral stationary phases, good resolution is often attained at relatively low temperature. In the present study, column oven temperature was varied. However, no significant improvement in the separation and resolution was obtained and, therefore, temperature was set at 25°C in the following measurements.

3.2. Sample preparation by solid-phase extraction

To avoid disturbance in the detection of small peaks, sample preparation is one of the most essential processes in HPLC analysis. Particularly when samples are urine which contains a number of unknown compounds, suitable sample preparation would give rise to good results. Recently, to extract compounds such as MA and AP from urine, solid-phase cartridges such as Sep-Pak C_{18} [35,36], Bond Elut SCX [37] and Bond Elut Certify [10,38] have been used successfully. In the present study, these three extraction cartridges were tested using urine samples of a healthy volunteer and the resultant HPLC chromatograms compared (Fig. 4). Extraction with Sep-Pak C_{18} [35] and with Bond Elut Certify [10] was performed according to the methods de-

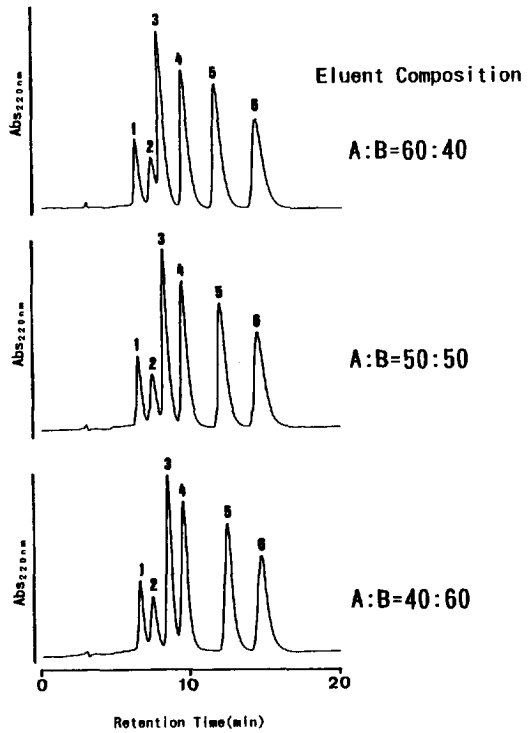

Fig. 3. Chromatograms obtained for a fortified urine with a mixture of eluents A and B as an eluent. Eluent A: methanol–50 m*M* potassium phosphate buffer (pH 6.0) (60:40, v/v). Eluent B: acetonitrile–50 m*M* potassium phosphate buffer (pH 6.0) (20:80, v/v). The concentrations of the samples added to the urine: D-*p*-OHMA and L-*p*-OHMA, 0.2 μg/ml; D-AP, L-AP, D-MA and L-MA, 2 μg/ml. Other chromatographic conditions appear in the Experimental section. Peaks: 1=D-*p*-OHMA; 2=L-*p*-OHMA; 3=D-AP; 4=L-AP; 5=D-MA; 6=L-MA.

Table 1
Effect of eluent composition on optical resolution of *p*-OHMA, AP and MA

	A:B = 60:40				A:B = 50:50				A:B = 40:60			
	k_D	k_L	α	R_s	k_D	k_L	α	R_s	k_D	k_L	α	R_s
p-OHMA	1.15	1.49	1.30	1.32	1.19	1.50	1.26	1.24	1.22	1.50	1.23	1.06
AP	1.72	2.23	1.30	1.70	1.80	2.21	1.23	1.48	1.86	2.19	1.18	1.28
MA	2.94	3.83	1.30	2.24	3.06	3.88	1.27	2.00	3.16	3.92	1.24	1.86

Eluents: (A) CH_3OH–50 m*M* KH_2PO_4 (pH 6.0) (60:40, v/v); (B) CH_3CN–50 m*M* KH_2PO_4 (pH 6.0) (20:80, v/v).

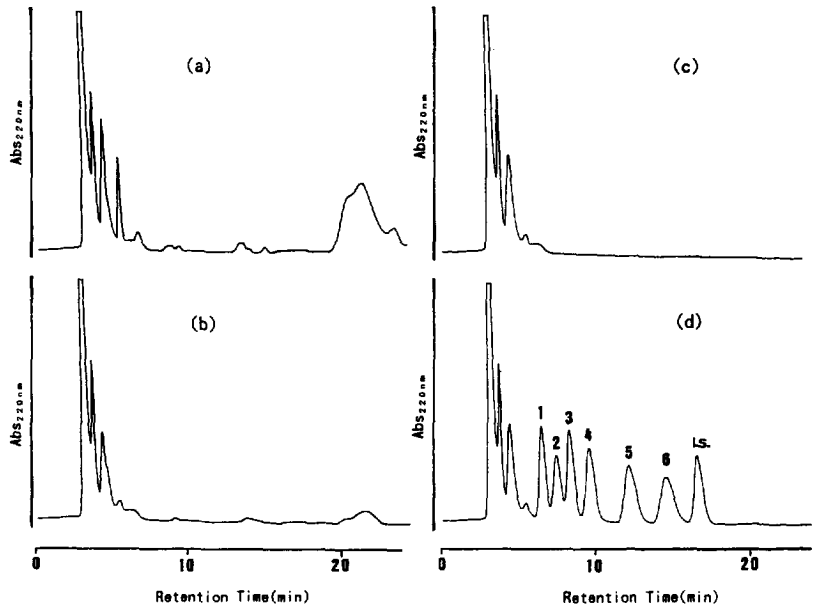

Fig. 4. Chromatograms obtained for a urine sample of a healthy volunteer after the sample preparation with (a) Sep-Pak C_{18}, (b) Bond Elut Certify and (c) Bond Elut SCX. The chromatogram in (d) was obtained for a fortified urine sample after preparation with Bond Elut SCX. Eluent: acetonitrile–methanol–50 m*M* potassium phosphate buffer (pH 6.0) (10:30:60, v/v). The concentrations of the samples added to the urine: D-*p*-OHMA and L-*p*-OHMA, 1 μg/ml; D-AP, L-AP, D-MA and L-MA, 5 μg/ml. Other chromatographic conditions appear in the Experimental section. Peaks: 1=D-*p*-OHMA; 2=L-*p*-OHMA; 3=D-AP; 4=L-AP; 5=D-MA; 6=L-MA.

scribed in previous papers. Extraction with Bond Elut SCX was conducted according to the method described in Experimental in this paper.

The chromatograms shown in Fig. 4a, b and c were obtained for urine samples which were prepared by extracting with Sep-Pak C_{18}, Bond Elut Certify and Bond Elut SCX cartridges, respectively. One can see large impurity peaks in the chromatogram in Fig. 4a and, therefore, Sep-Pak C_{18} was ruled out. Also small impurity peaks appeared in the chromatogram in Fig. 4b, suggesting that Bond Elut SCX is preferred to Bond Elut Certify. To compare the other two cartridges in more detail, we measured the recovery of D-MA from them by direct HPLC of the urine samples containing only D-MA. The values obtained were 88.5% and 98.2% for Bond Elut Certify and Bond Elut SCX, respectively. Based on the above comparison, we finally chose Bond Elut SCX in the present study. The chromatogram obtained for a fortified urine sample after the sample preparation with this cartridge is also depicted in Fig. 4d, where all the enantiomers are eluted in well-separated peaks without any disturbance from impurities in the urine. The recoveries calculated from the peak areas were 98.2% for D-MA, 98.5% for L-MA, 96.5% for D-AP, and 96.9% for L-AP.

3.3. HPLC–TSP-MS of the enantiomers

We have established overall direct HPLC identification of the urine of D-MA addicts. In the present study, in addition to this, we explored the method by which we can confirm the results from the direct analysis. For this purpose, HPLC–TSP-MS was taken. Instead of non-volatile potassium phosphate buffer which cannot be used for HPLC–TSP-MS, 100 m*M* CH_3COOH buffer was used, and excellent separation was obtained for all the enantiomers in fortified urine samples, as depicted in Fig. 5: Acetonitrile–methanol–100 m*M* CH_3COOH (pH 6.0) (10:30:60, v/v) was finally chosen. Three sets of enantiomers in a fortified urine sample were separated well, as shown in Fig. 5, and each peak was identified by analyzing the TSP mass spectrum by taking the $[M+H]^+$ ion as the base peak.

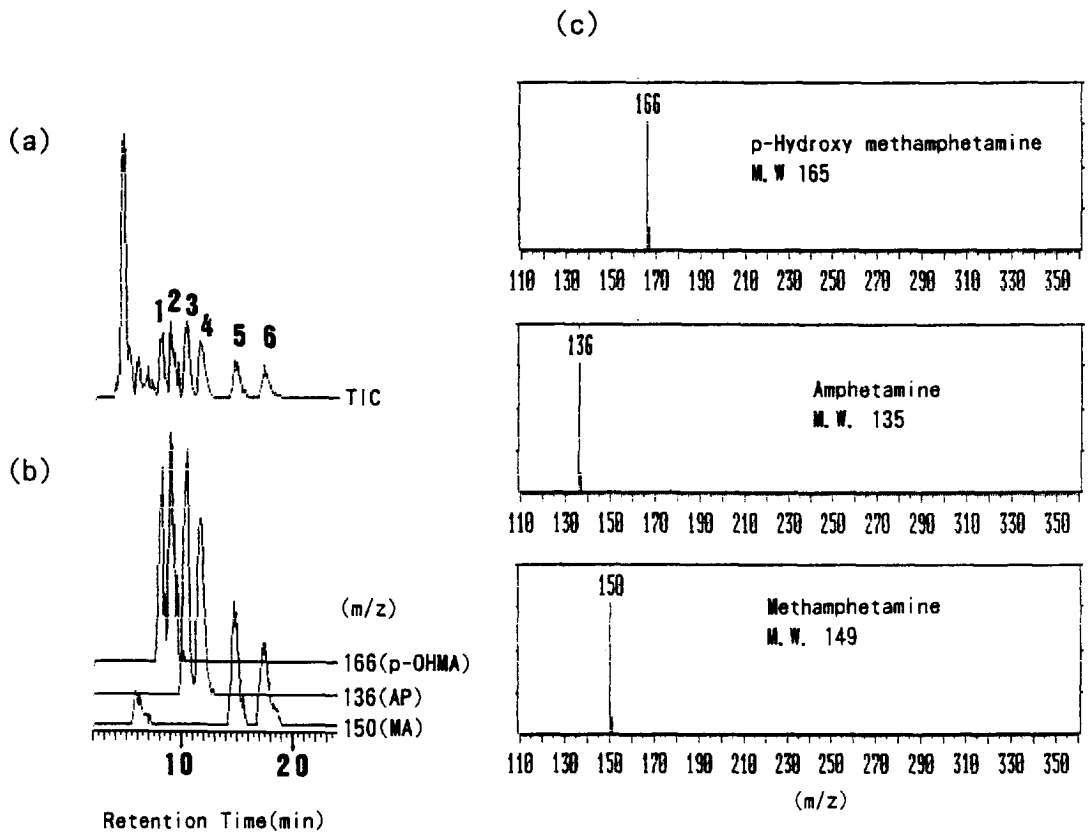

Fig. 5. (a) Total ion chromatogram (TIC); (b) mass chromatograms obtained for a fortified urine sample; (c) mass spectra produced from peaks of MA, AP and *p*-OHMA. Eluent: acetonitrile–methanol–100 m*M* CH_3COONH_4 (pH 6.0) (10:30:60, v/v). The concentrations of all the samples added to the urine were 2 μg/ml. Peaks in (a): 1=D-*p*-OHMA; 2=L-*p*-OHMA; 3=D-AP; 4=L-AP; 5=D-MA; 6=L-MA .

3.4. Quantitative analysis and resultant detection limits

To see how the methods established here work reliably, quantitative measurements were carried out in the presence of the I.S. for fortified urine samples in which the concentrations of the added enantiomers were varied. Direct HPLC analysis showed good linearity throughout the concentration range from 0.2 to 20 μg/ml for both the enantiomers of AP and MA (D-AP; $y=0.079x+0.003$, $r^2=0.999$, L-AP; $y=0.078x+0.002$, $r^2=0.998$, D-MA; $y=0.080x+0.001$, $r^2=0.999$, L-MA; $y=0.078x$-0.001, $r^2=0.999$). The relative standard deviation obtained at the sample concentrations of 2 μg/ml was 1.75% for D-AP, 2.20% for L-AP, 1.67% for D-MA, and 2.36% for L-MA. Below the concentration of 0.2 μg, the calibration curve deviated slightly from the linear relationship. However, it was sufficient for the detection of the compounds. The detection limits were calculated to be 50 ng/ml for D- and, L-AP and D-MA, and 100 ng/ml for L-MA. These values attained for 10 ml of urine may be insufficient in some cases. In such cases, HPLC–MS detection should be used for samples from suspected addicts.

For the scan mode of HPLC–TSP-MS, the detection limits were estimated to be 10 ng/ml for D-AP, L-AP and D-MA and 20 ng/ml for L-MA. *p*-OHMA had nearly the same limits as for MA. When the selected ion monitoring (SIM) technique, in which the $[M+H]^+$ ion (*m/z* 136 for AP and 150 for MA) was chosen, was applied, the limits were 0.5 ng/ml for D- and L-AP, 0.8 ng/ml for D-MA and 1.0 ng/ml for L-MA.

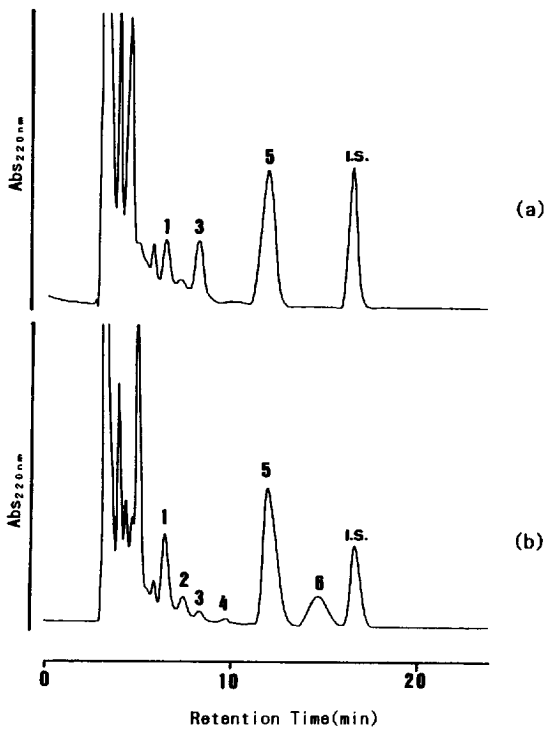

Fig. 6. Chromatograms obtained for urine samples taken from MA addicts which contained only (a) D-isomers and (b) both D- and L-isomers. Eluent: acetonitrile–methanol–50 m*M* potassium phosphate buffer (pH 6.0) (10:30:60, v/v). Other chromatographic conditions appear in the Experimental section. Peaks: 1=D-*p*-OHMA; 2=L-*p*-OHMA; 3=D-AP; 4=L-AP; 5=D-MA; 6=L-MA. The concentrations estimated are (a) 3.1 μg/ml for D-AP and 19 μg/ml for D-MA and (b) 1.2 μg/ml for D-AP, 0.55 μg/ml for L-AP, 27 μg/ml for D-MA and 14 μg/ml for L-MA.

3.5. Analysis of urine samples from MA addicts

The combination of direct HPLC and HPLC–TSP-MS analyses was applied to the identification of 100 MA addict urine samples, which were already identified as positive by GC–MS in our laboratory. HPLC separation of the enantiomers of MA, AP and *p*-OHMA was carried out in the optimized eluent system after careful sample preparation, as described earlier. As shown in Fig. 6, direct HPLC detected all of the D- and L-isomers of MA, AP and *p*-OHMA within 20 min. The L/D ratios for MA and AP estimated using the calibration curve are summarized in Table 2. For 99 samples, only D-isomers of MA, AP and *p*-OHMA were detected, and for the remaining one, both D- and L- isomers found. This result indicates that D-MA is used in most cases of MA abuse in Japan, as we mentioned in the Introduction.

4. Conclusion

A rapid, simple and accurate detection method of enantiomers of MA, AP and *p*-OHMA in urine was developed by coupling direct HPLC and HPLC–TSP-MS analyses in the present study. Both in direct HPLC and HPLC–TSP-MS analyses, a β-cyclodextrin phenylcarbamate-bonded silica column offers good separation of these enantiomers in less than 20 min and provides good linearity between the peak areas and a wide range of solute concentrations, high sensitivity, and reliable reproducibility of the results. Using direct HPLC supported by HPLC–TSP-MS, optical isomers of MA and its metabolites in urine samples can be identified conveniently and accurately leading to the reliable identification of MA addicts.

An antiparkinsonism drug, deprenyl (DPN), which is to be sold in the near future in Japan, is known to be metabolized in the human body to MA and AP and excreted in urine [4,5]. The excreted MA and AP are L-isomers. It will then be very necessary to be able to distinguish legitimate use of DPN from illicit use of D-MA. The method described in this paper will become a useful tool for solving such problems.

Table 2
Optical activity of *p*-OHMA, AP and MA detected in 100 urine samples of MA addicts

Number of Samples	*p*-OHMA		AP		MA	
	Isomer	L/D Ratio	Isomer	L/D Ratio	Isomer	L/D Ratio
99	D	0	D	0	D	0
1	D, L	–	D, L	0.46	D, L	0.52

References

[1] A.H. Beckett, G.T. Tucker and A.C. Moffat, J. Pharm. Pharmacol, 19 (1967), 273.
[2] J. Marsel, G. Doring, G. Remberg and G. Spiteller, Z. Rechtsmed., 70 (1972) 245.
[3] R.D. Budd and N.C. Jain, J. Anal. Toxicol., 2 (1978) 241.
[4] G.P. Reynolds, J.D. Elsworth, K. Blau, M. Sandler, A.J. Lees and G.M. Stern, Br. J. Clin. Pharmacol., 6 (1978) 542.
[5] T. Inoue and S. Suzuki, Xenobiotica, 16 (1986) 691 and 17 (1987) 965.
[6] F. Karoum, L.W. Chuang, T. Eisler, D.B. Calne, M.R. Liebowiz, F.M. Quitkin, D.F. Klein and R.J. Wyatt, Neurology, 32 (1982) 503.
[7] K. Takahashi, A. Ishigami, M. Shimamine, M. Uchiyama, T. Ochiai, K. Sekita, Y. Kawasaki, T. Furuya and M. Tobe, Bull. Natl. Inst. Hyg. Sci., 105 (1987) 1.
[8] J. Mallol, L. Pitarch, R. Coronas and A. Pons, Jr., Arzneim., Forsch., 24 (1974) 1301.
[9] Y. Nakahara, K. Takahashi, A. Ishigami, R. Kikura and M. Shimamine, Jpn. J. Toxicol. Environ. Health, 37 (1991) 473.
[10] R. Kikura, A. Ishigami and Y. Nakahara, Jpn. J. Toxicol. Environ. Health, 38 (1992) 136.
[11] I.W. Wainer, T.D. Doyle and W.M. Adams, J. Pharm. Sci., 73 (1984) 1162.
[12] E.D. Lee, J.D. Henion, C.A. Brunner, I.W. Wainer, T.D. Doyle and J. Gal, Anal. Chem., 58 (1986) 1349.
[13] S.M. Hayes, R.H. Liu, W.S. Tsang, M.G. Legendre, R.J. Berni, D.J. Pillion, S. Barnes and M.H. Ho, J. Chromatogr., 398 (1987) 239.
[14] M.C. Alembic and I.W. Wainer, J. Assoc. Off. Anal. Chem., 71 (1988) 530.
[15] T. Nagai, M. Sato, T. Nagai, S. Kamiyama and Y. Miura, Clin. Biochem., 22 (1989) 439.
[16] T. Nagai and S. Kamiyama, J. Chromatogr., 525 (1990) 203.
[17] T. Nagai, S. Kamiyama, A. Kurosu and F. Iwamoto, J. Legal Med., 46 (1992) 244.
[18] L.M. Gunne, Biochem Pharm., 16 (1967) 863.
[19] C.E. Wells, J. Assoc. Off. Anal. Chem., 53 (1970) 113.
[20] J.H. Liu and W.W. Ku, Anal. Chem., 53 (1981) 2180.
[21] G.A. Eiceman, C.S. Leasure and S.L. Selim, J. Chromatogr. Sci., 22 (1984) 509.
[22] R.L. Fitzgerald, J.M. Ramos, Jr., S.C. Bogema and A. Poklis, J. Anal. Toxicol., 12 (1988) 255.
[23] J.T. Cody and R. Schwarzhoff, J. Anal. Toxicol., 17, (1993) 321.
[24] A. Mori, I. Ishiyama, H. Akita and T. Oishi, Jpn. J. Legal Med., 45 (1991) 1.
[25] F.T. Noggle, Jr. and C.R. Clark, J. Forensic Sci., 31 (1986) 732.
[26] F.T. Noggle, Jr., J. Deruiter and C.R. Clark, J. Chromatogr. Sci., 25 (1987) 38.
[27] M. Onodera, T. Yokokawa, H. Ishii, Y. Arakida and Y. Ushio, Abs. Annual Meeting of Forensic Chemistry at National Research Institute of Police Science, Tokyo, 1992, p.7.
[28] R. Isaksson, P. Erlandsson, L. Hansson, A. Holmberg and S. Berner, J. Chromatogr., 498 (1990) 257.
[29] R.A. Thompson, S. Andersson and S.G. Allenmark, J. Chromatogr., 465 (1989) 263.
[30] J. Haginaka, C. Seyama, H. Yasuda, H. Fujima and H. Wada, J. Chromatogr., 592 (1992) 301.
[31] J. Haginaka and H. Wakai, Anal. Chem., 62 (1990) 997.
[32] H. Kitagawa, H. Fujima, H. Kitamura, C. Nakamura, M. Yamamura and H. Wada, Chromatography (Japan), 15 (1994) 17.
[33] A.M. Rizzi, R. Hirz, S. Cladrowa-Runge and H. Jonsson, Chromatographia, 39 (1994) 131.
[34] A. Buzas and C. Dufour, Bull. Soc. Chim. Fr., (1950) 139.
[35] S. Suzuki, T. Inoue and T. Niwaguchi, Rep. Natl. Res. Inst. Police Sci., 35 (1982) 125.
[36] N. Takayama, H. Kobayashi and A. Tsuji, Jpn. J. Toxicol. Environ. Health, 30 (1984) 14.
[37] T. Kumazawa, K. Sato, H. Seno and O. Suzuki, Jpn. J. Legal Med., 47 (1993) 129.
[38] B.K. Gan, D. Baugh, R.H. Lira and S. Walia, J. Forensic Sci., 36 (1991) 1331.