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Direct determination of methamphetamine enantiomers in urine by liquid chromatography with a strong cation-exchange precolumn and phenyl-β-cyclodextrin-bonded semi-microcolumn

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Abstract

Methamphetamine enantiomers in drug abusers' urine were clearly separated by semi-microcolumn liquid chromatography using a column-switching system. The system consists of two separation processes: firstly, a strong cation-exchange precolumn removes neutral and anionic substances in urine, and then methamphetamine enantiomers trapped in the column are transferred to and separated in a phenyl-β-cyclodextrin-bonded semi-microcolumn (Chiral Drug™, 150 mm×1.5 mm I.D.). (*S*)-(+)-Methamphetamine was baseline separated from (*R*)-(−)-methamphetamine within 25 min, directly from urine samples. The detection limit for both enantiomers was 0.1 μg/ml.

Keywords: Enantiomer separation; Methamphetamine

1. Introduction

The continuous world-wide increase in the illicit manufacture, trafficking and abuse of amphetamine-type substances, such as methamphetamine (MA) and 3,4-methylenedioxymethamphetamine (MDMA), has been a major development in the global drug situation in the past few years. These designer drugs have an asymmetric center in their structure. Since enantiomer analysis may provide valuable information on the original substrate in the metabolic pathway or on the geographical sources of different samples, development of chiral determination of abused drugs is of interest to forensic chemists.

As reviewed by Kraemer and Maurer [1], a number of attempts have been reported for MA analysis to date. A relatively new complexity in forensic analysis of MA is the existence of deprenyl, a drug for Parkinson's disease. Deprenyl is known to be metabolized to (*R*)-(−)-MA, which is excreted in urine [2,3]. For forensic purposes, it is necessary to distinguish the illicit use of (*S*)-(+)-MA from medical use of deprenyl in some cases.

Several different approaches have been reported for the enantiomeric resolution of MA by HPLC. Derivatization of the two enantiomers with an optically pure reagent afforded the corresponding dia-

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stereomers, which could be separated with a common reversed-phase column [4,5]. A β-cyclodextrin (CD)-type column could baseline separate the two enantiomers successfully [6,7]. All of these methods, however, require tedious sample pretreatment, either by clean-up or chemical derivatization.

We have already reported methods for the direct analysis of MA from urine by means of semi-micro-column HPLC, where columns were arranged in tandem to minimize sample pretreatment, and MA was sensitively detected via the concentrating effect of narrow-bore columns [8–10]. The use of a commercially available phenyl-β-CD-bonded semi-microcolumn and a selective spectrometric detector, such as a photodiode array or mass spectrometer, has proven to be useful for sensitive and enantio-selective analysis of MA.

As a continuing study, the present paper describes an attempt to improve the enantiomeric separation of MA by using a different phenyl-β-CD-bonded semi-microcolumn and a strong cation exchange pre-column to attain highly selective separation with ultraviolet adsorption detection.

2. Experimental

2.1. Apparatus

A Shiseido Nanospace liquid chromatograph, equipped with a photodiode array detector linked to a data system (S-MC, Shiseido, Tokyo, Japan), was used for data acquisition and storage. A photodiode array detector was connected in series with a UV detector. The eluent was monitored by measuring UV absorbance at 210 nm. The system consisted of two Inert pumps, a column oven, an autosampler, an H-P valve, and a degassing unit (Fig. 1).

2.2. HPLC column

The column used for the initial separation was a cartridge column (10 mm×2.0 mm I.D., Shiseido, Tokyo, Japan) packed with a strong cation-exchange resin (SCX, Shiseido). The column used for the primary analytical separation was a phenyl-β-CD-bonded semi-microcolumn (Chiral Drug™, 150 mm×2.0 mm I.D., Shiseido).

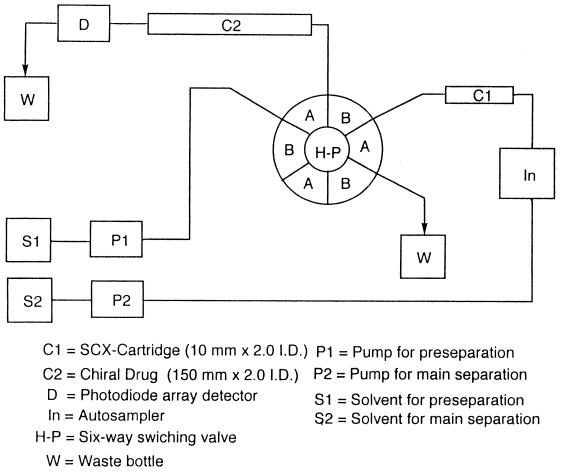

Fig. 1. Schematic diagram of the HPLC system.

2.3. Reagents

MA was purchased from Dainippon Pharmaceutical (Osaka, Japan). Other amphetamine-type substances were supplied by the Ministry of Health and Welfare of Japan. All other solvents for the mobile phases were of HPLC grade. Water was purified with a Milli-Q system (Nihon Millipore Kogyo, Tokyo, Japan).

2.4. Mobile phase

The mobile phases used in the pre- and main separation were 50 mmol/l KH_2PO_4–CH_3CN (85:15), and 50 mmol/l KH_2PO_4–CH_3CN (70:30), respectively. The pH of the 50 mmol/l was 4.6. The flow-rate for the initial separation column was maintained at 0.2 ml/min, and that for the main separation column was set at 0.1 ml/min. The column switching program was arranged so that the 4.0–6.0 min fraction in the pre-separation was transferred to the analytical column. Both separations were carried out at 20°C. Other chromatographic details are described in the corresponding figure captions.

2.5. Urine sample

Urine samples were collected from drug abusers at Kanto-Shin'etu Regional Narcotic Control Office. Urine samples of patients treated with deprenyl were collected from patients suffering from Parkinson's disease at the School of Medicine, Juntendo University. Urine samples were filtered with PURADISC (0.2 μm, Arbor Technologies, Ann Arbor, MI, USA).

2.6. Validation

Linearity was examined by using standard aqueous solutions of both MA enantiomers with concentrations of 5, 10, 20, and 40 μg/ml. Precision and accuracy were examined with urine spiked with (*S*)-(+)-MA at 10.0 μg/ml and (*R*)-(−)-MA at 17.0 μg/ml.

3. Results and discussion

HPLC using narrow-bore columns has been known to provide a tremendous concentrating effect, which leads to an increase in concentration-sensitive detection for HPLC [11,12]. It has been demonstrated to be a powerful technique for analyzing biological samples with limited availability [13]. In its application to biological samples available in large volume (e.g., of the order of dozens of microliters or more), however, mere miniaturization of columns may not lead to any benefit in terms of analytical chemistry. The limited loadability of narrow-bore columns cannot exploit sample availability, and the concentrating effect may enhance the sensitivity for both substances of interest and interfering substances.

As a technique to make narrow-bore liquid chromatography applicable to dilute sample solutions, a column-switching system was reported [14]. In this method, a precolumn of unique geometry fractionated an analyte-containing region in a crude separation, and pre-focused it for the subsequent sensitive analysis with a semi-microcolumn. This method seemed suitable for forensic analysis of MA, since residual MA usually exists in the order of μg/ml in urine collected from abusers, which is available in volumes of the order of milliliters or more.

In this work, a strong cation-exchange column is used for the pre-separation and a Chiral Drug column for the main separation. Silica-based cation exchange was found to be useful for selective analysis of cationic substances [15]. The precolumn packed with the cation exchanger trapped the MA molecules and allowed other neutral and acidic substances to pass through it to be discarded under the initial valve setting. The elution profile of MA with the precolumn is shown in the upper chromatogram of Fig. 2.

As described in the Introduction, the final separation provided optical resolution in addition to a high sensitivity. Chiral Drug, a phenyl-β-CD-based phase, has shown appropriate performance for chiral separation of MA in its single-column arrangement [16]. The column was employed as a secondary (main-separation) column in this experiment. The switching timing is shown in Fig. 2, which presents a

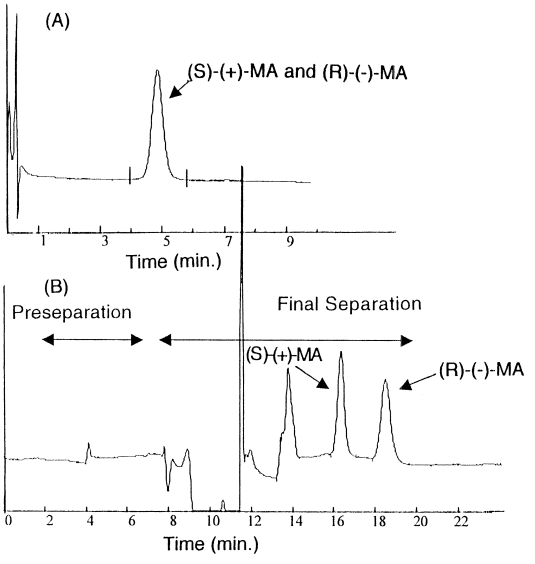

Fig. 2. Profiles of pre- and final separation of an authentic mixture of (*S*)-(+)-MA and (*R*)-(−)-MA. (A) Initial separation: SCX cartridge (10 mm×2.0 mm I.D.); flow-rate, 0.2 ml/min; 50 mmol/l KH_2PO_4–CH_3CN (85:15); monitoring wavelength, 210 nm. (B) Primary analytical separation: Chiral Drug (150 mm×1.5 mm I.D.); flow-rate, 0.1 ml/min; 50 mmol/l H_2PO_4–CH_3CN (70:30); monitoring wavelength, 210 nm.

representative chromatogram obtained with a standard solution of MA.

Urine samples collected from patients with Parkinson's disease and a drug abuser were run on the system. Chromatograms obtained from these samples have the same capability in identification of MA enantiomers as that obtained from the standard solution (Fig. 3). Other components in urine hardly interfered with the analyte peaks.

A photodiode array detector was connected to confirm the results obtained with a variable UV detector. A high level of spectral purity for all of these enantiomer peaks was confirmed in measurements with spiked urine samples (Fig. 4). The spectra of (*S*)-(+)- and (*R*)-(−)-MA peaks (10 and 17 μg/ml, respectively) matched those of standard 100 μg/ml solutions with matching values of 951 and 960 in the Hill and coworkers method [17], where a value of 1000 corresponds to a perfect match.

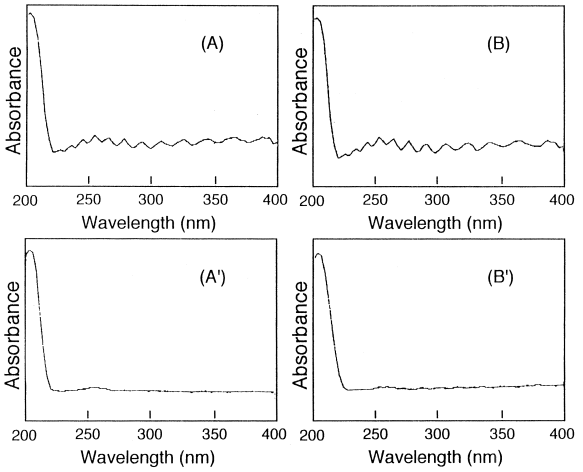

Fig. 4. Spectra of MA enantiomers of spiked urine and standard solutions. (A) Urine spiked with (*S*)-(+)-MA at 10 μg/ml. (A′) Standard solution of (*S*)-(+)-MA at 100 μg/ml. (B) Urine spiked with (*R*)-(−)-MA at 17 μg/ml. (B′) Standard solution of (*R*)-(−)-MA at 100 μg/ml.

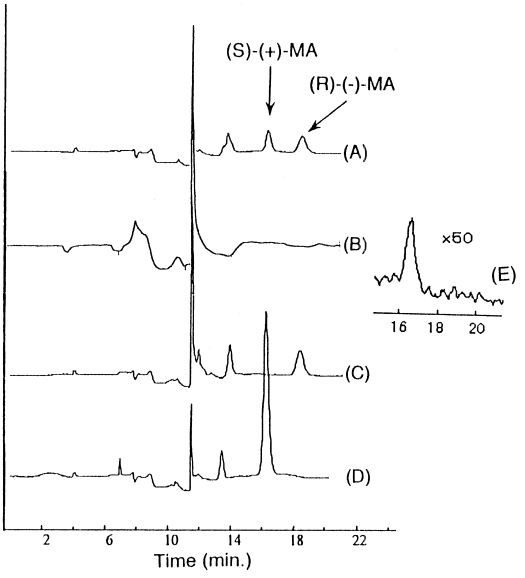

Fig. 3. Chromatograms of typical urine samples. (A) Authentic mixture of (*S*)-(+)-MA and (*R*)-(−)-MA (10 μg/ml). (B) Blank urine. (C) Parkinson's disease patient treated with (*R*)-(−)-deprenyl. (D) Methamphetamine abuser. (E) Authentic solution of (*S*)-(+)-MA (0.1 μg/ml). (i) Initial separation: SCX cartridge (10 mm×2.0 mm I.D.); flow-rate, 0.2 ml/min; 50 mmol/l KH_2PO_4–CH_3CN (85:15); monitoring wavelength, 210 nm. (ii) Primary analytical separation: Chiral Drug (150 mm×1.5 mm I.D.); flow-rate, 0.1 ml/min; 50 mmol/l KH_2PO_4–CH_3CN (70:30); monitoring wavelength, 210 nm.

Good linearity of the method was confirmed over the concentration range 5–100 μg/ml (R^2=0.999 for both enantiomers). The detection limit of (*S*)-(+)-MA was 0.1 μg/ml (S/N=3), as shown in Fig. 3. Also, the accuracy and precision observed in experiments with spiked urine samples seemed adequate (Table 1).

The separation of enantiomers of amphetamine with the phenyl-β-CD column (single-column arrangement) is shown in Fig. 5. The substance often exists in MA-related urine samples. It is favorable that these enantiomers are clearly baseline separated, and do not interfere with MA peaks in this method. The method has been applied to a number of actual forensic samples. Since no interfering peak has been observed so far, the method described here seems highly selective for MA enantiomers and adequately applicable to forensic analysis. Also, the Chiral Drug column used in this study will be useful for the enantiomeric separation of the several amines of interest in forensic chemistry.

Table 1
Accuracy and precision of the method[a]

	Spiked	Found					Average (accuracy)	RSD (%)
(*S*)-(+)-MA	10.0	10.15	10.20	10.17	10.14	10.13	10.16	0.24
(*R*)-(−)-MA	17.0	17.05	17.00	16.96	16.95	17.02	17.00	0.21

[a] Unit of concentration: μg/ml.

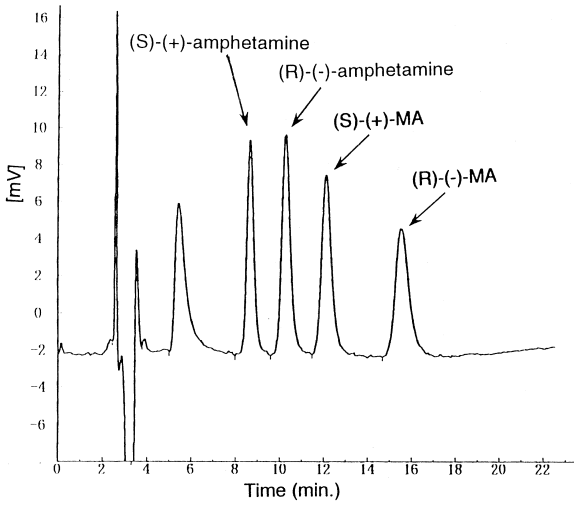

Fig. 5. Chiral separation of racemic amphetamine and methamphetamine. Conditions: column, Chiral Drug (150 mm×2.0 mm I.D.); mobile phase, 50 mmol/l KH_2PO_4–CH_3CN (60:40); detector, UV (210 nm); column temperature, 20°C; flow-rate, 0.1 ml/min.

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