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Journal of Chromatography B, 749 (2000) 111–118

JOURNAL OF CHROMATOGRAPHY B

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Simultaneous chiral analysis of methamphetamine and related compounds by capillary electrophoresis

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Received 1 March 2000; received in revised form 7 July 2000; accepted 7 July 2000

Abstract

A capillary electrophoretic method for the simultaneous chiral analysis of nine cationic drugs (18 enantiomers) has been developed. These drugs are methamphetamine (MA), amphetamine, dimethylamphetamine, ephedrine (EP), norephedrine, methylephedrine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyamphetamine and 3,4-methylenedioxy-*N*-ethylamphetamine. The chiral selector, which was added to the electrolyte, was a mixture of β-cyclodextrin and heptakis(2,6-di-*O*-methyl)-β-cyclodextrin. The detection limits of all enantiomers were 0.1 μg/ml, and the intermediate precisions of migration time and peak area of within-run assays ($n=6$) were under 0.3% and 1.4%, respectively. The calibration curves of the peak area of (1*R*,2*S*)-(−)-EP and *S*-(+)-MA were linear in the range 0.2–500 μg/ml. This method was applicable to urine analysis.

Keywords: Enantiomer separation; Methamphetamine; Ephedrine; 3,4-Methylenedioxymethamphetamine

1. Introduction

Chiral analysis of methamphetamine (MA) and related compounds is important for forensic analysis to discriminate between MA use and dosage of legal medicines, which are metabolized to MA. In Japan, most (ca. 72%) of drug-related arrests involve MA, and in more than 99% of the cases in which MA is detected, the MA is *S*-(+)-enantiomer [1]. Consequently, detection of *S*-(+)-MA and its main metabolite *S*-(+)-amphetamine [*S*-(+)-AP] is evidence of illegal use of MA. On the other hand, the usage of legal medicines which are metabolized to *R*-(−)-MA (e.g. selegiline), are distinguished by detection of *R*-(−)-MA [2]. However, there has been a recent increase in the illegal use of dimethylamphetamine (DMA) in Japan, and in almost all cases, the enantiomer is *S*-(+)-DMA. *S*-(+)-DMA is metabolized to *S*-(+)-MA, while unchanged DMA is also excreted in urine [3]. Therefore, a simple method for

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0378-4347/00/$ – see front matter
PII: S0378-4347(00)00392-3

the simultaneous separation of enantiomers of MA, AP and DMA is needed.

Capillary electrophoresis (CE) is a powerful tool for chiral analysis, because of its high resolution and efficiency. A number of CE methods using cyclodextrins (CDs) as the chiral selector to analyze MA and related compounds have been reported [4–9], but none of these methods involves DMA as its target. Other related drugs whose illegal use is increasing in Japan are ring-substituted congeners of MA, such as 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA). In addition, for routine analysis of urine samples, it is necessary to discriminate ephedrine (EP), norephedrine (NE) and methylephedrine (ME), which are raw materials of MA and AP and also legal antitussives.

The goal of this work was to develop a simple chiral CE method for the simultaneous determination of MA, AP, DMA, MDMA, MDA, MDEA, EP, NE and ME, and to apply it to the analysis of urine samples.

2. Experimental

2.1. Reagents and chemicals

All standard drugs were from the reference collection of the Forensic Science Laboratory, Ishikawa Prefectural Police Headquarters. The compounds used were hydrosulfates of *S*-(+)-AP and racemic AP, and hydrochlorides of *S*-(+)-MA, *R*-(−)-MA, *S*-(+)-DMA, racemic DMA, (l*R*,2*S*)-(−)-EP, racemic EP, (l*R*,2*S*)-(−)-NE, racemic NE, (1*R*,2*S*)-(−)-ME, racemic ME, racemic MDMA, racemic MDA and racemic MDEA. Tris(hydroxymethyl)aminomethane (Tris), phosphoric acid, β-CD and heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD) were obtained from Nacalai Tesque (Kyoto, Japan). Heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) and (2-hydroxy)propyl-β-CD (HP-β-CD) were obtained from Hewlett-Packard (Waldbronn, Germany). All other reagents used were of analytical reagent grade. Ultrapure water provided by a Milli-RX12α and Milli-Q SP system (Millipore, Bedford, MA, USA) was used for all procedures.

2.2. Apparatus and conditions

Experiments were carried out using an HP^{3D} CE system (Hewlett-Packard). Samples were injected by applying a pressure of 50 mbar for 3 s. The separations were performed in an uncoated fused-silica capillary of 75 μm I.D.×80.5 cm (72 cm effective length) equipped with a built-in, 200 μm I.D., extended light path detection window (bubble cell) (Hewlett-Packard). The applied voltage was +30 kV, the capillary temperature was maintained at 25°C, and the detection wavelength was 195 nm. The electrolyte was 75 m*M* Tris buffer (pH 2.5 adjusted by phosphoric acid) containing 10 m*M* DM-β-CD and 3 m*M* β-CD. The run electrolyte was filtered with a 0.45 μm filter before use.

2.3. Urine samples

The urine samples of MA addicts were taken from suspects arrested by the Ishikawa Prefectural Police under ''Voluntary Presentation''. The urine samples of patients under selegiline pharmacotherapy were collected from the patients with their informed consent by letter. Control urine samples were collected from healthy individuals who had not taken any legal or illegal drugs. Urine samples were stored at +4°C for several days until analysis.

2.4. Pretreatment of urine samples

To 10 ml of urine in a tube, 1 ml of 10% sodium carbonate solution and 10 ml of chloroform–isopropanol (3:1, v/v) were added and the mixture (pH 9–10) was shaken vigorously for 2 min. After centrifugation (1000 *g*, 10 min), the organic phase was transferred. This procedure was repeated two more times. All the organic phases were combined and dehydrated with ca. 1 g of sodium sulfate and decanted. After addition of several drops of 10% hydrochloric acid, the solution was dried in a stream of nitrogen, and the residue was dissolved in 0.5 ml

of water [10]. The solution was applied to the CE system.

3. Results and discussion

3.1. Choice of chiral selector

In order to simultaneously determine all 18 enantiomers, a capillary zone electrophoretic method using an electrolyte containing a chiral selector was adopted. β-CD, DM-β-CD, TM-β-CD and HP-β-CD were examined as chiral selectors. For this experiment, each CD was added to 50 m*M* Tris buffer (pH 2.5 adjusted by phosphoric acid), and the solution was used as an electrolyte. The capillary was 50 μm I.D.×48.5 cm (effective length 40 cm) uncoated fused-silica equipped with a bubble cell of 150 μm I.D. (Hewlett-Packard). The sample was injected with a pressure of 50 mbar for 6 s. Other CE conditions are described in the Experimental section. The results are shown in Fig. 1. None of the CDs could separate all enantiomers simultaneously, and none of them could completely separate the MA and DMA peaks. β-CD could achieve good chiral separation except for NE, EP and ME (Fig. 1 a), and DM-β-CD could achieve good separation except for MDA and MDEA (Fig. 1 b). Therefore, various mixtures of β-CD and DM-β-CD were examined (Fig. 2). A mixture of 3 m*M* β-CD and 12 m*M* DM-β-CD succeeded in the separation of all enantiomers (Fig. 2a). Hence, a mixture of β-CD and DM-β-CD was used in the following experiments.

3.2. Optimization of CE conditions

CE conditions were optimized using Tris buffer including 3 m*M* β-CD and 12 m*M* DM-β-CD as electrolyte. First, the effect of the pH was examined by varying the pH of the electrolyte from 2.5 to 7. Electro-osmotic flow increased with increasing pH, resulting in a degradation of the peak resolution. Therefore pH 2.5 was selected. Then, the inside diameter and the length of the capillary were examined. The inside diameter of the capillary was normally 50 or 75 μm, and capillaries of 50 μm I.D.×48.5 cm (40 cm effective length) and 75 μm I.D.×80.5 cm (72 cm effective length) gave similar peak resolutions and migration times, but the resolution of the latter capillary was slightly better than that of the former, and thus was used for further experiments. Different combinations of applied voltage (+10–30 kV) and Tris concentration (50–100 m*M*) were examined. A combination of (+30 kV) and 100 m*M* Tris gave the best resolution and reproducibility, but required a longer analysis time, and sometimes resulted in the formation of bubbles in the capillary, which disturbed the analysis. Therefore, a combination of (+30 kV) and 75 m*M* Tris concentration was chosen. Capillary temperatures ranging from 15 to 30°C were then examined. At lower temperatures, the peak resolution was slightly better, but the run time was longer (e.g., the migration time of MDEA, which was the longest, was 41 min at 15°C and 28 min at 30°C). For use as a routine analytical method, the method should have a short analytical time. However, the resolution of the MA and DMA peaks declined significantly at 30°C, and so a temperature of 25°C was selected. Lurie et al. reported that the addition of methanol to the electrolyte improved resolution [11]. We found that methanol concentrations of 5–10% improved the separation of MDA, MDMA and MDEA, but resulted in poor separation of the other enantiomers.

Under the above CE conditions (capillary size, applied voltage, Tris concentration, pH, and temperature), we again examined the effects of different mixtures of β-CD and DM-β-CD, and found that a mixture of 3 m*M* β-CD and 10 m*M* DM-β-CD gave the best separation of all enantiomers. Two detection window types, the normal straight cell and the bubble cell, were compared. The former showed a slightly better resolution, but the latter gave a better sensitivity (ca. three times better than the former). Therefore, the capillary with the bubble cell was chosen. Finally, the sample injection volume was examined. The standard sample was injected by applying a pressure of 50 mbar for 1–6 s. The peak area increased in proportion to the injection time, but when the injection time was more than 3 s, the peak resolution declined. Hence, an injection time of 3 s was selected. An electropherogram using the final CE conditions is shown in Fig. 3a.

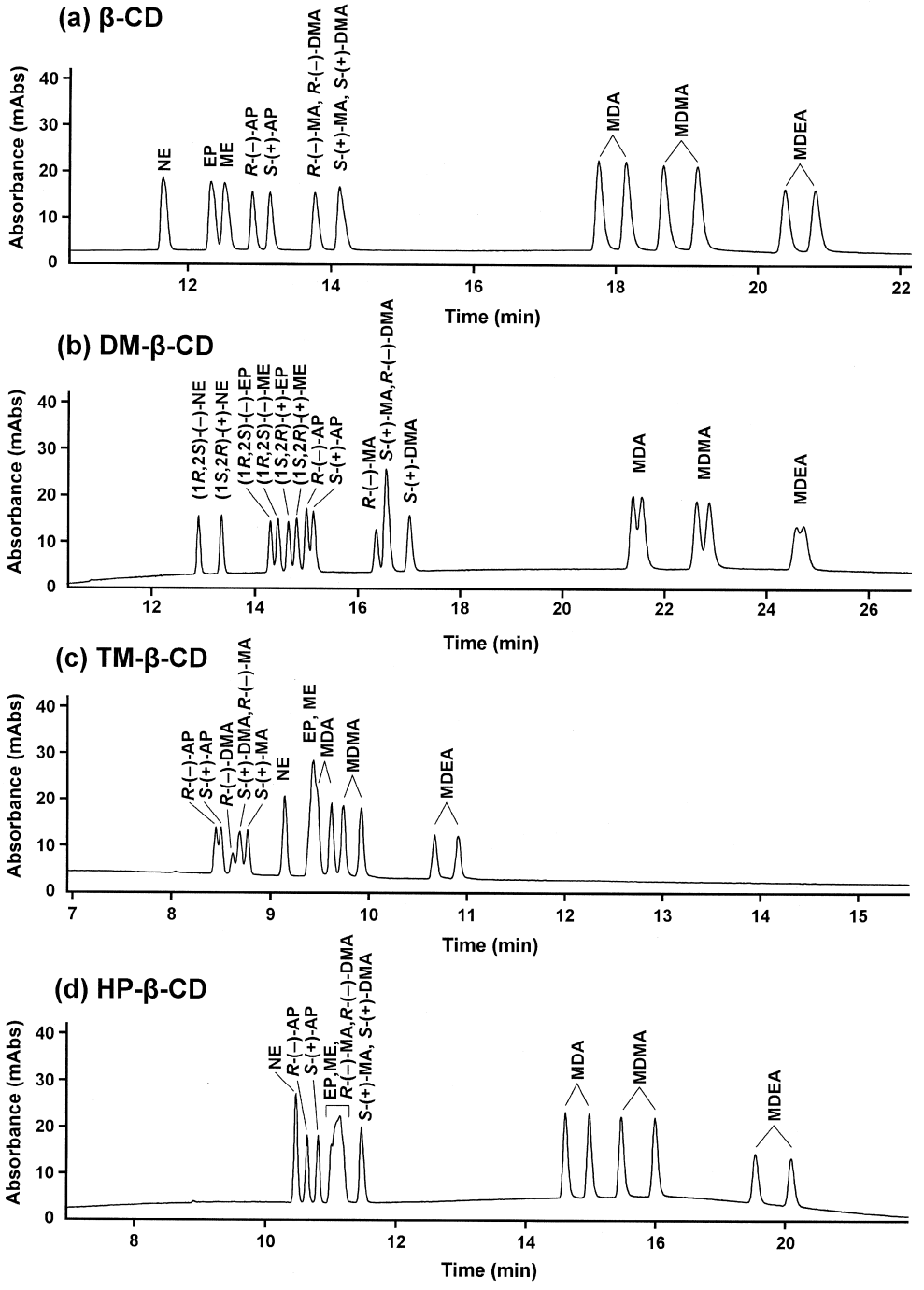

Fig. 1. Electropherograms of 18 enantiomers using chiral selectors (15 m*M* each) of (a) β-CD, (b) DM-β-CD, (c) TM-β-CD and (d) HP-β-CD. Concentrations: 10 μg/ml each except for (a,c.d) *R*-(−)-MA (8 μg/ml), *S*-(+)-DMA (5 μg/ml) and *R*-(−)-DMA (5 μg/ml) or (b) *R*-(−)-MA (8 μg/ml). Conditions: capillary, 50 μm I.D.×48.5 cm (effective length 40 cm) uncoated fused-silica equipped with a bubble cell of 150 μm I.D.; applied voltage, +30 kV; temperature, 25°C; detection wavelength, 195 nm; sample injection, 50 mbar for 6 s; electrolyte, 50 m*M* Tris buffer (pH 2.5) containing each chiral selector.

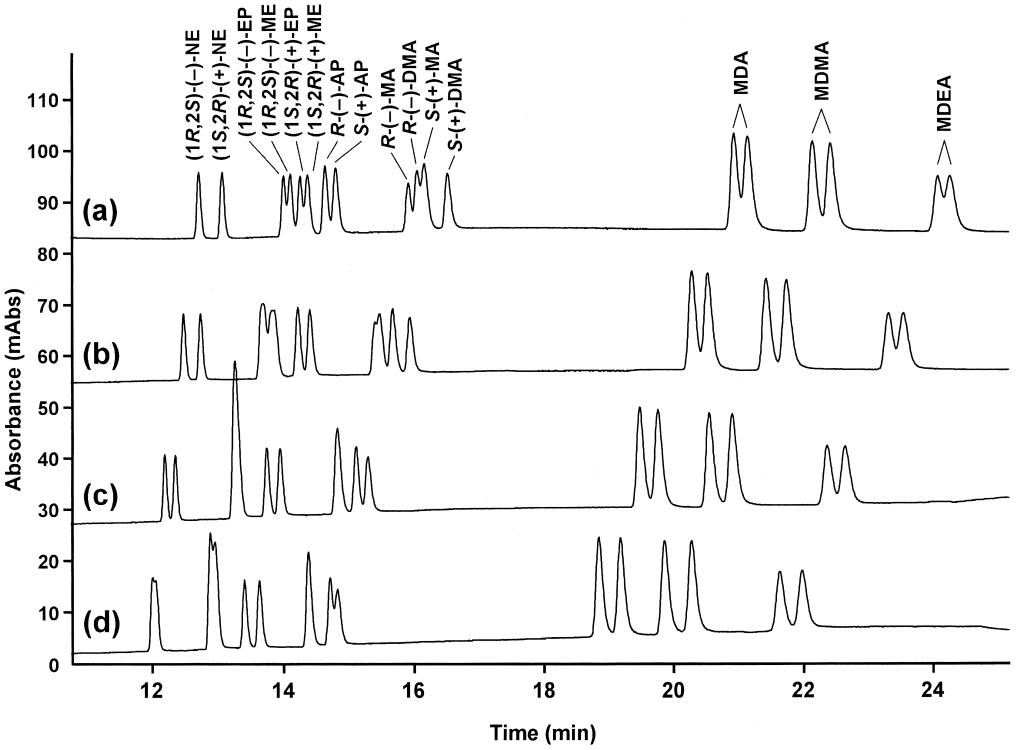

Fig. 2. Electropherograms of 18 enantiomers using chiral selectors of (a) 3 m*M* β-CD–12 m*M* DM-β-CD, (b) 6 m*M* β-CD–9 m*M* DM-β-CD, (c) 9 m*M* β-CD–6 m*M* DM-β-CD and (d)12 m*M* β-CD–3 m*M* DM-β-CD. Concentrations: 10 μg/ml except for *R*-(−)-MA (8 μg/ml). Conditions as in Fig. 1.

3.3. Detection limits, calibration curves and reproducibility for standard mixture

The detection limits of all enantiomers dissolved in water, defined as the concentration that produced a signal equal to 3–4 times the background noise level, were 0.1 μg/ml. An electropherogram of a mixture of (1*R*,2*S*)-(−)-EP and *S*-(+)-MA (0.1 μg/ml each) is shown in Fig. 4a. The calibration curves of (1*R*,2*S*-(−)-EP and *S*-(+)-MA are shown in Fig. 4b. The calibration curves based on peak areas were linear in the range of 0.2–500 μg/ml ($r^2=0.999$). On the other hand, the calibration curves based on peak heights were linear only in the range 0.2–10 μg/ml ($r^2=0.999$). This difference was due to the fact that the higher concentrations (>10 μg/ml) resulted in wider, shorter peaks. The intermediate precisions of within-run assays for the migration time and peak area of standard mixture are summarized in Table 1. The precision values for migration time and peak area were under 0.3% and 1.4%, respectively. This method gave good reproducibility without use of an internal standard.

3.4. Detection limits and reproducibility for urine

An electropherogram of the control urine extract is shown in Fig. 3b. No peaks were found at the migration times of the 18 enantiomers. AP and MA were detected clearly from the urine sample spiked with AP and MA (Fig. 3c). The detection limits of AP and MA spiked in urine were 10 ng/ml. The intermediate precisions of within-run assays for the migration time and peak area of extracts prepared from urine samples spiked with AP and MA are summarized in Table 2. The precision values of migration time for urine extracts were almost the same as those for standard mixture, but the values of peak area for urine extracts were worse than those for standard mixture. Therefore, an internal standard

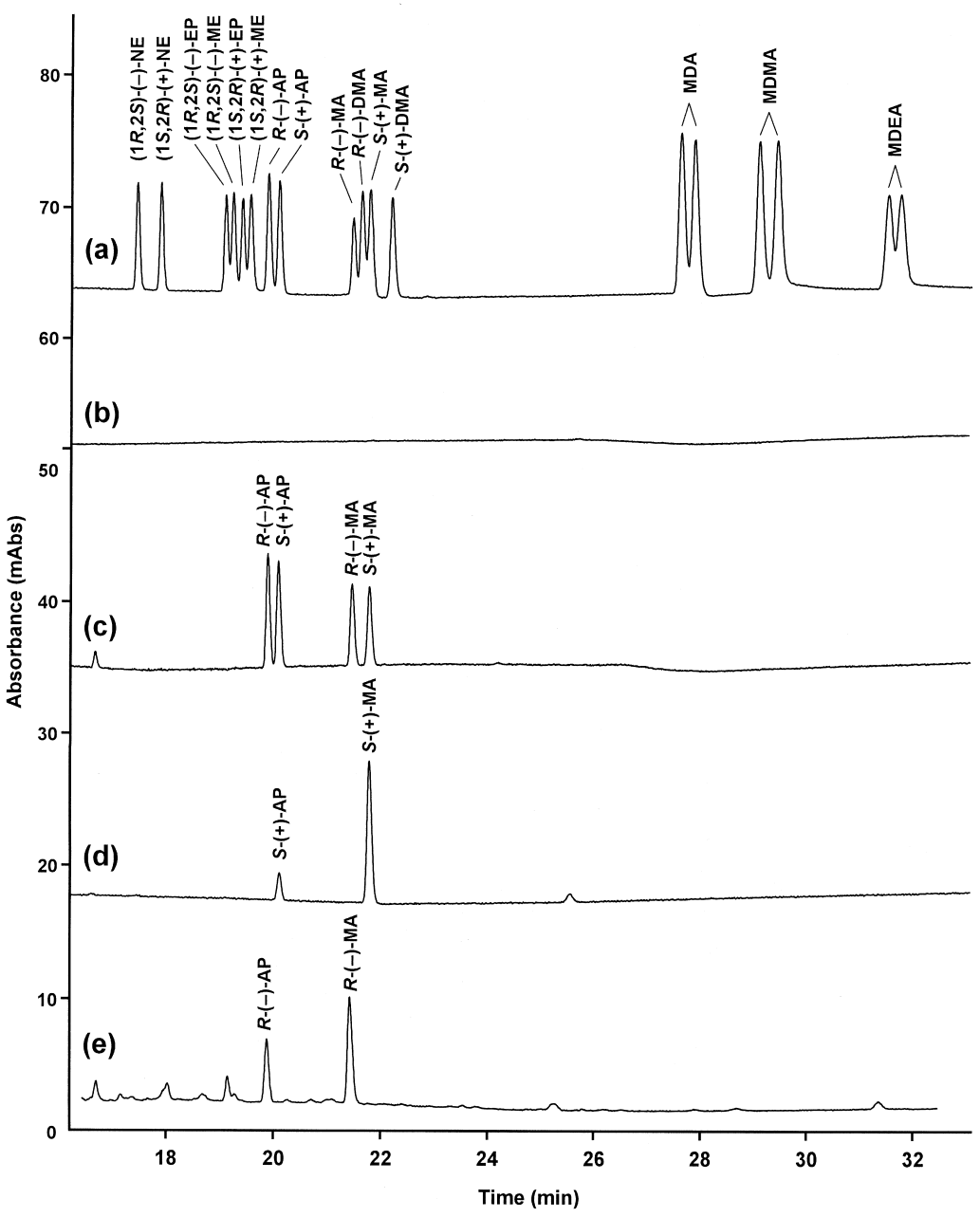

Fig. 3. Electropherograms of (a) 18 enantiomers (5 μg/ml each except for *R*-(−)-MA (4 μg/ml)) dissolved in water, (b) control urine extract, (c) extract of control urine spiked with 0.5 μg/ml racemic AP and 0.4 μg/ml racemic MA, (d) urine extract of an MA addict and (e) urine extract of a patient under selegiline pharmacotherapy. Conditions: capillary, 75 μm I.D.×80.5 cm (effective length 72 cm) uncoated fused-silica equipped with a bubble cell of 200 μm I.D.; applied voltage, +30 kV; temperature, 25°C; detection wavelength, 195 nm; sample injection, 50 mbar for 3 s; electrolyte, 75 m*M* Tris buffer (pH 2.5) containing 10 m*M* DM-β-CD and 3 m*M* β-CD.

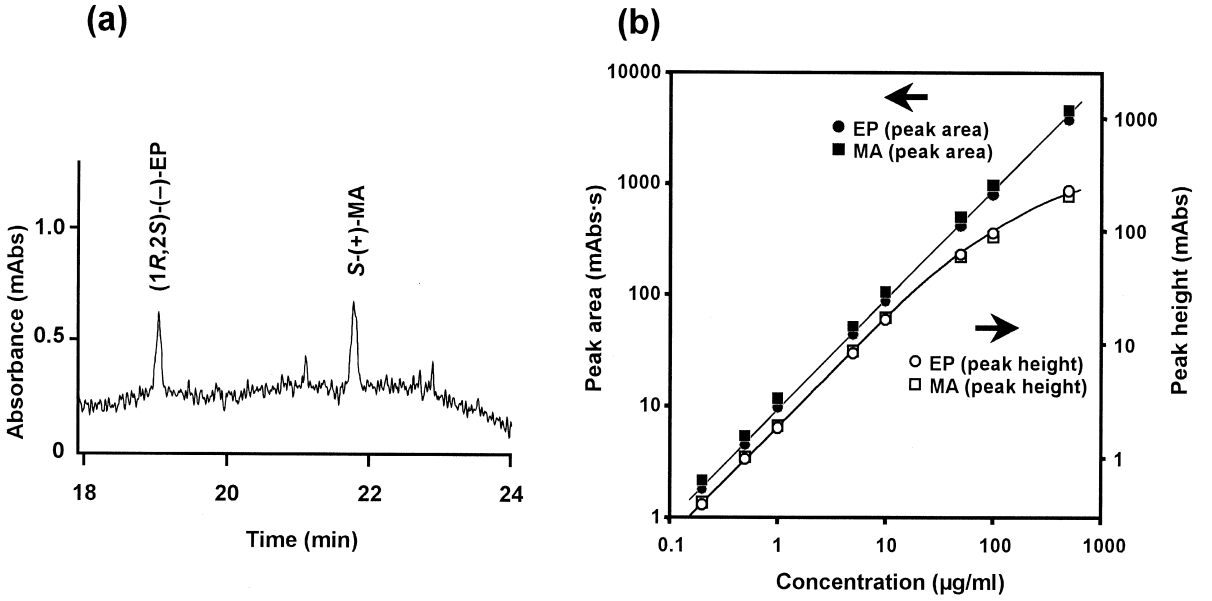

Fig. 4. (a) Electropherogram of a standard mixture of (1*R*,2*S*)-(−)-EP and *S*-(+)-MA (0.1 μg/ml each), and (b) calibration curves of (1*R*,2*S*)-(−)-EP and *S*-(+)-MA. Conditions as in Fig. 3.

Table 1
Intermediate precisions of within-run assays for migration time and peak area of standard mixture[a]

Compound	RSD (%)[b]	
	Migration time	Peak area
(1*R*,2*S*)-(−)-NE	0.14	0.94
(1*S*,2*R*)-(+)-NE	0.15	1.18
(1*R*,2*S*)-(−)-EP	0.17	1.19
(1*S*,2*R*)-(+)-EP	0.17	0.92
(1*R*,2*S*)-(−)-ME	0.17	1.16
(1*S*,2*R*-(+)-ME	0.16	0.76
R-(−)-AP	0.17	0.87
S-(+)-AP	0.17	1.29
R-(−)-AP	0.19	1.12
S-(+)-MA	0.19	1.12
R-(−)-DMA	0.19	0.82
S-(+)-DMA	0.20	1.27
MDA	0.26	1.10
	0.26	1.28
MDMA	0.27	1.13
	0.27	0.99
MDEA	0.26	1.36
	0.27	0.93

[a] 10 μg/ml each. Conditions as in Fig. 3.
[b] $n=6$.

Table 2
Intermediate precisions of within-run assays for migration time and peak area of urine extracts[a]

Compound	RSD (%)[b]	
	Migration time	Peak area
R-(−)-AP	0.23	2.48
S-(+)-AP	0.24	2.15
R-(−)-MA	0.26	3.90
S-(+)-MA	0.26	3.88

[a] Five healthy persons' urine samples spiked with *S* -(+)-AP, *R*-(−)-AP, *S*-(+)-MA and *R* -(−)-MA (0.5 μg/ml each). Conditions as in Fig. 3.
[b] $n=5$.

is needed for correct quantitative analysis of urine samples. The recoveries of *S*-(+)-AP, *R*-(−)-AP, *S*-(+)-MA and *R*-(−)-MA from urine (0.5 μg/ml each) were 93–97%, which were the same as reported using GC [10].

3.5. Analysis of AP and MA in urine

Urine samples from several dozen MA addicts and several patients under selegiline pharmacotherapy were analyzed. In all cases, the *S*-(+)-AP and the *S*-(+)-MA peaks were detected for the former, and

the *R*-(−)-AP and *R*-(−)-MA peaks were detected for the latter. All of the AP and MA peaks were free from interference. Typical electropherograms are shown in Fig. 3d and e.

Thus, the proposed method is a simple and selective method for the simultaneous chiral analysis of nine cationic drugs, and is applicable to analysis of urine samples of MA addicts. However, for forensic analysis, an internal standard is required to ensure the integrity of data, particularly for the quantitative analysis of biological samples. Experimental work is in progress for the determination of MA and related compounds in urine using internal standard.

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