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Optimization of a simple method for the chiral separation of methamphetamine and related compounds in clandestine tablets and urine samples by β-cyclodextrine modified capillary electrophoresis: a complementary method to GC–MS

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Abstract

The chiral separation of (±)-methamphetamine, (±)-methcathinone, (±)-ephedrine and (±)-pseudoephedrine by means of β-cyclodextrine modified capillary electrophoresis is described. The distribution of enantiomers in clandestine tablets and urine samples were identified. Several electrophoretic parameters such as the concentration of β-cyclodextrin, temperature, the applied voltage and the amount of organic solvent required for successful separation were optimized. The method, as described herein, represents a good complementary method to GC–MS for use in forensic and clinical analysis.

Keywords: Capillary electrophoresis; Chiral separation; (±)-Methamphetamine; (±)-Methcathinone; (±)-Ephedrine; (±)-Pseudoephedrine

1. Introduction

Methamphetamine and its analogues are strong central nervous system stimulants and are classified as illicit drugs, and are currently a source of serious social problems in many countries. In general, (+)-methamphetamine can be synthesized from (−)-ephedrine or (+)-pseudoephedrine; (−)-methamphetamine can be produced from (+)-ephedrine or (−)-pseudoephedrine (Fig. 1) [1]. Thus, the separation and identification of these enantiomers are a great significance, not only with respect to providing valuable information concerning the clandestine conversion of norephedrine and norpseudoephedrine to amphetamine and ephedrine, and pseudoephedrine to methamphetamine [2–4] but also would be useful in clinical analysis. It is especially worthy noting that (−)-methamphetamine can also be extracted from a Vicks Inhaler [5] and that (−)-methamphetamine if used in certain prescription drugs [6]. To avoid errors in judgment, an enantiomeric analysis would be highly desirable. Currently, separation methods used for the separation of enantiomers either are chromatographic, such as gas chromatography (GC) [7], high performance liquid chromatography (HPLC) [8–10], or an elctrophoretic method such as capillary electrophoresis (CE) [11–14]. LeBelle et al. has also reported the chiral identification of (±)-ephedrine compounds by both GC after derivatization with (*R*)(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) and by nuclear magnetic resonance (NMR) using a chiral solvating agent ((*R*)(+)-1,1'-bi-2-naphthol) [7]. Derivatization of (±)-ephedrine, (±)-pseudoephedrine and related substances with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) permitted enantiomeric separations by micellar electrokinetic chromatography (MEKC), as reported in the literature [11]. The determination of (±)-ephedrine compounds in nutritional supplements has also been investigated by

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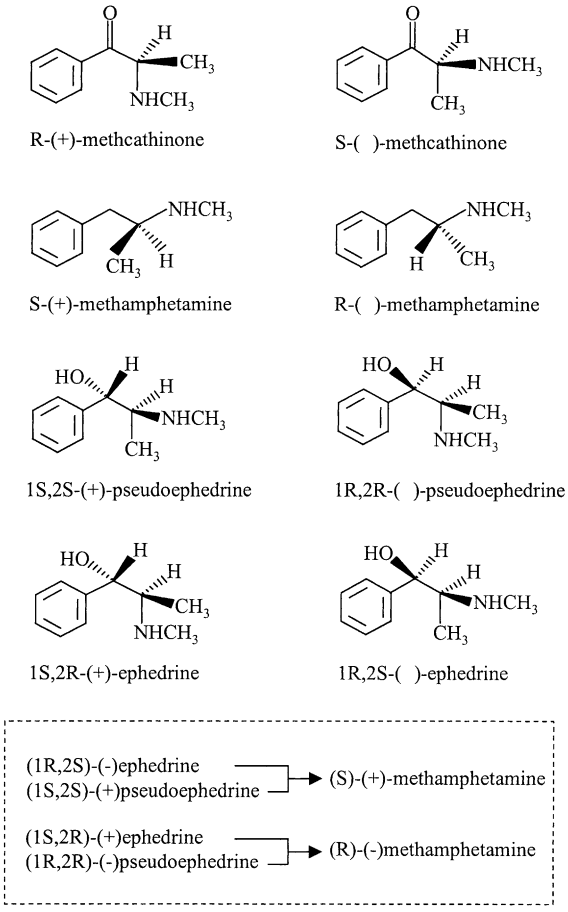

Fig. 1. Molecular structures of (±)-methamphetamine, (±)-methcathinone, (±)-ephedrine and (±)-pseudoephedrine and their relationship to the major synthetic pathway.

cyclodextrin-modified capillary electrophoresis, using hydroxypropyl-β-cyclodextrin (HP-β-CD) [11].

Thus far, GC–MS still remains the officially prescribed method. However, for the chiral separation of methamphetamine related compounds it is necessary to derivatize the analytes prior to their injection into the GC system. All of these procedures are time consuming. Furthermore, hundreds of samples are frequently involved in routine testing and, as a result, a simple and rapid method which is also reliable and complementary to GC–MS for use in forensic analysis would be highly desirable.

In this study, the optimum conditions for the separation and determination of (±)-methamphetamine, (±)-methcathinone, (±)-ephedrine and (±)-pseudoephedrine using native β-CD in conjunction with CE is described. Several electrophoretic parameters, such as concentration of β-CD and the amount of organic solvent needed for the separation were optimized. The distribution of each single enantiomer in clandestine tablets and suspect urine samples were identified.

2. Experimental

2.1. Reagents

(±)-Methamphetamine, (±)-methcathinone, (±)-ephedrine and (±)-pseudoephedrine obtained from Radian International (Austin, TX, USA). β-Cyclodextrin and acetonitrile were purchased from Sigma (St. Louis, MO, USA) and Acros (Belgium), respectively. All of the tablets and urine samples were generously donated by Command of the Army Force of Military Police, Forensic Science Center, Taiwan.

2.2. CE apparatus

A capillary electrophoresis system (Hewlett-Packard ^{3D}CE system, Germany) equipped with a photodiode array detector was used for the enantiomeric separations. The wavelength used for the detection was 210 nm. UV spectra (200–300 nm) were collected for each peak for purpose of identification. A 50-μm i.d. fused silica capillary column (J&W Scientific, CA, USA) was used for the separation (total length: 58.5 cm; effective length: 50 cm). The sample injection was performed hydro-dynamically with a pressure (50 mbar) for 3 s. Prior to use, the capillary was conditioned with 0.1 M NaOH for 10 min, purified water for 10 min, and then with the electrolyte solution for 10 min.

2.3. GC–MS apparatus and methods

A gas chromatograph (Hewlett-Packard 6890 GC: Palo Alto, CA) equipped with a mass spectrometer (Hewlett-Packard 5973 mass selective detector) and an auto-injector (Model 7683) was used. A capillary column (30 μm × 0.32 μm i.d.) with an HP-5 MS (cross-linked 5% phenylmethylsilicone) bonded stationary phase film 0.25 μm thickness (Agilent Technologies, USA) was used. The temperatures of the quadrupole, injector and interface were maintained at 150, 250 and 280 °C, respectively. The temperature program for the column oven was as follows: 70 °C for 1 min, a linear ramp to 200 °C at 15 °C/min and a 2 min held. Finally, the temperature was ramped linearly to 260 °C at 20 °C/min with a 12.3 min hold. The total analysis time was 27 min. Helium carrier gas was used at a constant flow-rate of 1.0 ml/min (at splitless mode). Data were collected using the Hewlett-Packard Chem-Station software. The mass conditions were as follows: ionization energy, 70 eV; ion source temperature, 230 °C; full-scan, 40–450 amu at 1.84 scans/s.

2.4. Liquid–liquid extraction procedures

2.4.1. Tablet

Tablets were grounded into a fine powder and approximately 30 mg was dissolved in 3.0 ml 0.2 N KOH solutions by shaking for 5 min. The solution was extracted with 3.0 ml ethyl acetate (containing diphenylamine at 0.5 mg/ml as the

internal standard) by shaking for 5 min. The mixture was centrifuged for 5 min at 3,000 rpm and a 2.0 ml aliquot of the organic layer was transferred to an autosampler vial. The sample was analyzed (see GC–MS procedure above) on the day of extraction. For the CE experiments, the tablet power (1 mg) was extracted with methanol (1 ml). After 2 min of sonication and a 2 min centrifugation at 5,000 rpm at room temperature, the upper layer was collected and was then used directly.

2.4.2. Urine

Two milliliter of an urine sample was made alkaline by the addition of excess K_2CO_3. The free bases were then extracted into 4 ml of a hexane:CH_2Cl_2 (3:1 (v/v)) solution by stirring the suspension for 1 min. After centrifugation, the upper layer was collected and this organic phase was then evaporated to dryness. The residue was dissolved in 20 μl of methanol for the subsequent CE separation.

3. Results and discussion

3.1. Identification of standards

Fig. 1 shows the molecular structures of (±)-methamphetamine, (±)-methcathinone, (±)-ephedrine and (±)-pseudoephedrine. These compounds possess a chiral center: the (+) and (−) forms have different pharmacological activities [15–17].

In the case of CE separation, β-CD is most commonly used chiral additive. However, less soluble (1.8 g per 100 ml at 250 °C) in water [18], highly sulfated cyclodextrins have been developed by Beckman (http://www.beckmancoulter.-com/) to improve solubility. Various chiral selectors of derivatized β-CDs have also been reported by Sabine et al. [19], Schmitt and Engelhardt [20], and Nagai et al. [21]. Although these modified β-CDs provide unique advantages, for routine analyses, the use of the native β-CD is convenient, if an optimized CE buffer and separation conditions can be found. For this purpose, we investigated the optimum conditions for the separation by investigating several parameters, such as the concentration of β-CDs, temperatures, applied voltages, and organic solvents. In Figs. 2 and 3, the electropherogram a shows a typical UV ($\lambda_{ab} = 210$ nm) electropherogram of methamphetamine related compounds in capillary zone electrophoresis (CZE) mode. The sample concentration was 25 ppm for each. The applied voltage was 20 kV; temperature was 15 °C. The CE buffer was water–acetonitrile (95:5 (v/v)) solution that included 150 mM phosphate (pH = 2.5).Under these conditions, the migration order was: methcathinone < methamphetamine < pseudo ephedrine < ephedrine. Electropherogram b shows the UV-electropherogram, where 17.5 mM β-CD was added, showing that the enantiomers were separated completely. This buffer is very simple and useful for the separation of methamphetamine related analytes. However, the peak corresponding to (+)-amphetamine overlaps slightly with (+)-pseudoephedrine. Thus, care needs to be exercised when both (+)-amphetamine and (+)-pseudoephedrine are present in a sample. This can be readily solved by the use of a longer (total: 80 cm) capillary. The complete separation of these 10 analytes is shown in the inset.

In order to investigate the effect of β-CD, under exactly the same experimental conditions, concentrations of 5, 10 and 15 mM β-CD were used and the findings show that the separation is improved when higher concentrations of β-CD are used, as shown in frame A (electropherograms a–c). In order to investigate the effects of organic solvents, under exactly the same experimental conditions, an aqueous solution, 10 and 15% acetonitrile mixed solutions were used.

Table 1
Distributions of methamphetamine and related compounds in 524 seized tablets from the Taiwan illicit market during 2001 by GC–MS

Methcathinone	Methamphetamine	Pseudoephedrine	Ephedrine	Ketamine	Caffeine	Numbers
	√					4
	√			√		12
	√				√	6
	√			√	√	20
	√	√		√		1
	√	√		√	√	5
	√		√			3
	√		√	√		1
	√		√	√	√	4
	√	√	√	√		1
		√			√	1
		√		√	√	19
			√		√	1
					√	255
			√	√		20

√: Detected.

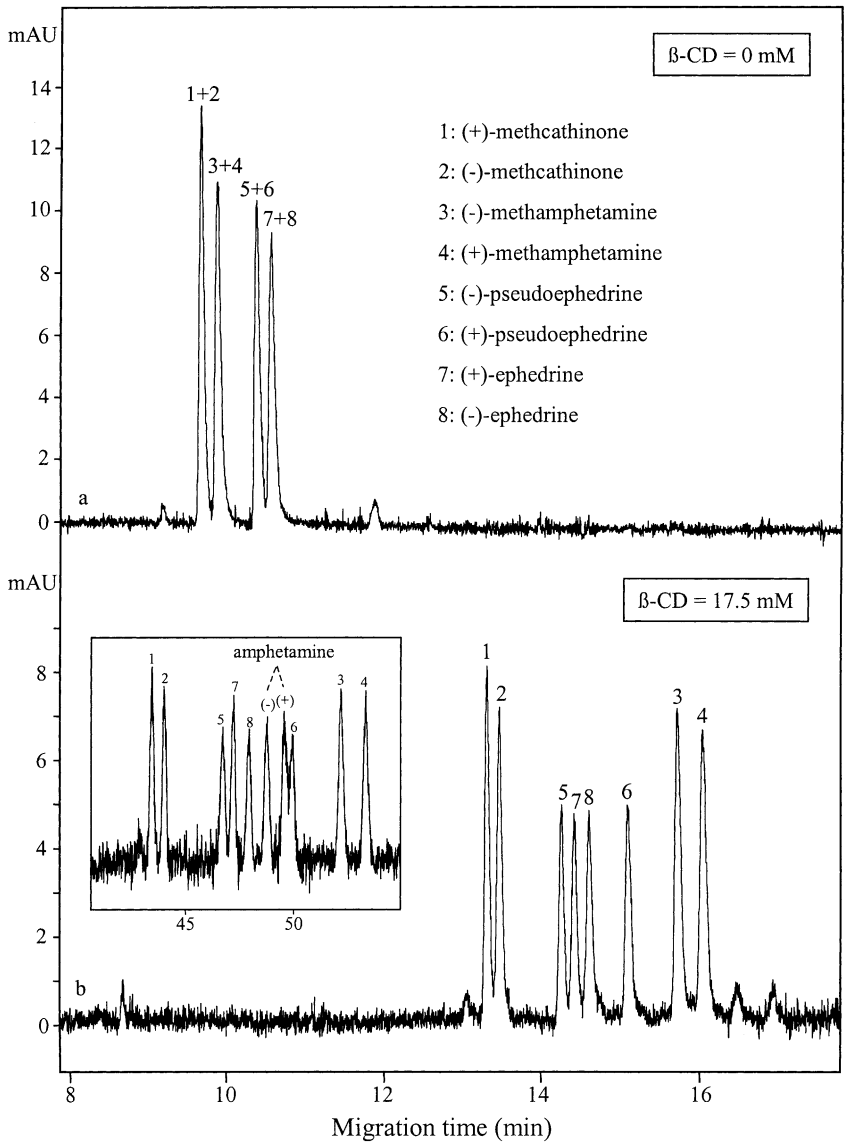

Fig. 2. UV electropherograms of (±)-methamphetamine, (±)-methcathinone, (±)-ephedrine and (±)-pseudoephedrine standards. The sample concentrations were 25 ppm. CE conditions: capillary, 58.5 cm (50 cm to detector); detection wavelength, λ_{ab} = 210 nm. Running buffers: (a) 150 mM H_3PO_4; water:acetonitrile = 95:5 (v/v); (b) the same buffer as described earlier and the addition of 17.5 mM β-CD. The inset shows the separation of (±)-methamphetamine, (±)-methcathinone, (±)-ephedrine, (±)-pseudoephedrine and (±)-amphetamine standards when an 80 cm capillary was used.

The addition of 5% acetonitrile was absolutely necessary for this separation, as shown in frame B (electropherograms d–f). Frame C (electropherograms g–i) and D (electropherograms d–f) show the effects of temperature (11, 25 and 30 °C) and applied voltage (15, 20 and 25 kV), respectively. As a result, a higher voltage and temperature provided resulted in shorter separation times and better resolution. Thus, the complete, optimal separation of methamphetamine related compounds can be achieved with phosphate buffer (150 mM) containing β-CD (15–17.5 mM) in a water–acetonitrile solution (95:5 (v/v)) at 15–30 °C; applied voltage, 20–25 kV.

3.2. Identification of clandestine tablet: a comparison of CE with GC–MS

Table 1 shows results of the analysis of 524 clandestine tablets, which were seized from the Taiwan illicit market during 2001, by GC–MS. The experimental conditions and methods are described in Section 2.3. Most of

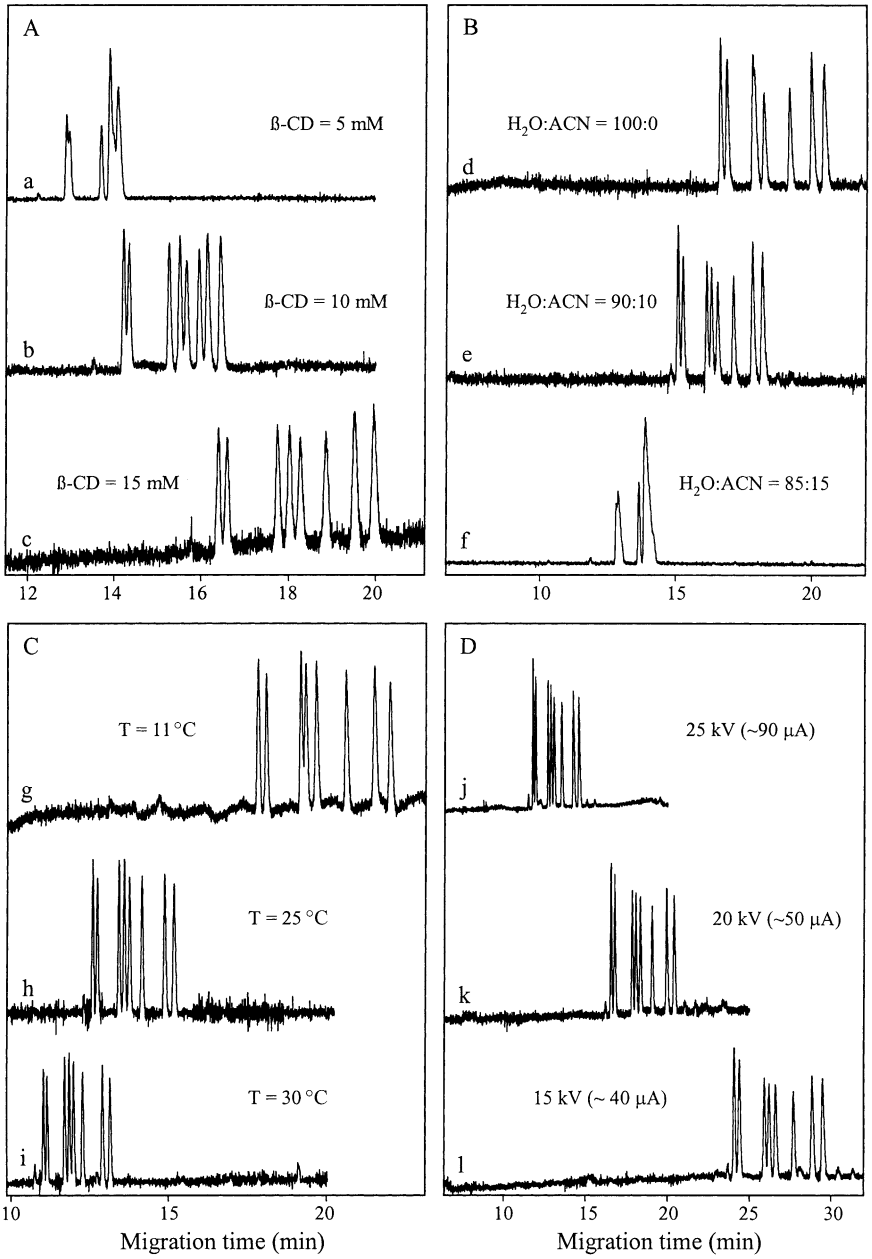

Fig. 3. Effects of different parameters on CE separation: (A) 5, 10 and 15 mM of β-CD (electropherograms a–c); (B) different solutions (water–acetonitrile): 100:0, 90:10 and 85:15 (v/v) (electropherograms d–f); (C) 11,25 and 30 °C (electropherograms g–i); (D) 15, 20 and 25 kV (electropherograms j-1).

the clandestine tablets contained multi-components, including methamphetamine, caffeine and/or ketamine. Some contained ephedrine and/or pseudoephedrine; no methcathinone was found in any of the samples. However, 255 tablets contained caffeine only and these can be considered to be fake amphetamine tablets. In Table 1, not all of these data show the distribution of enantiomers. Herein, we selected one of the clandestine tablets as an example and examined it by GC–MS and β-CD modified CE. Fig. 4A shows the result obtained by GC–MS. The peaks having migration times of 6.18, 8.0, 8.1 and 12.7 min were assigned as methamphetamine, ephedrine, pseudoephedrine and ketamine, respectively, based on their mass spectra (data not shown). The result obtained by CE is shown in Fig. 4B. Each peak was identified by spiking with standards and by comparisons with on-line

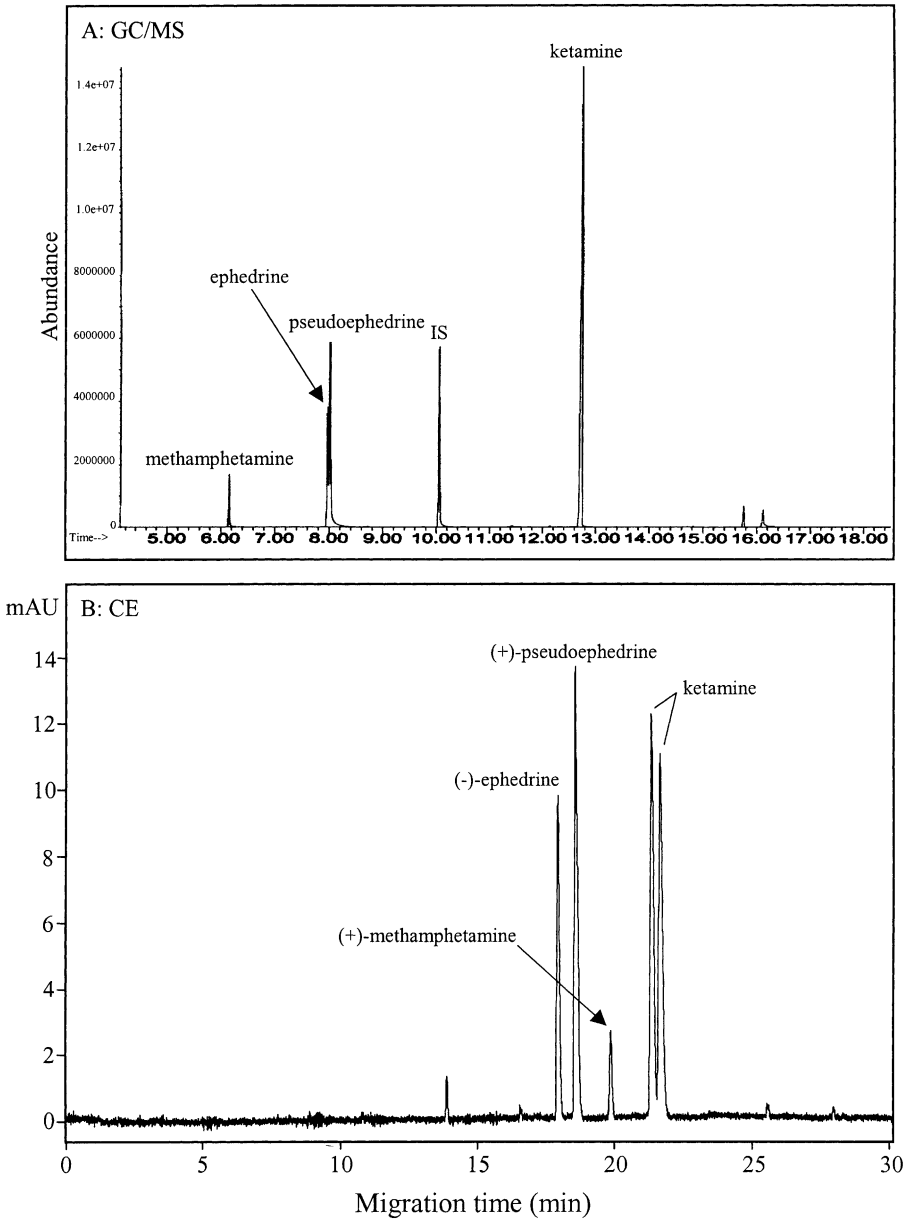

Fig. 4. (A) GC–MS chromatograph of a tablet extract; (B) CE electropherogram of the same tablet extract.

Table 2
Distributions of enantiomers of (±)-methamphetamine and related compounds in 22 clandestine tablets by β-CD modified CE

Methcathinone		Methamphetarnine		Pseudoephedrine		Ephedrine		Numbers
(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	
		√						11
			√					6
							√	1
		√	√					4
		√					√	2
		√		√			√	1
								1

√: Detected.

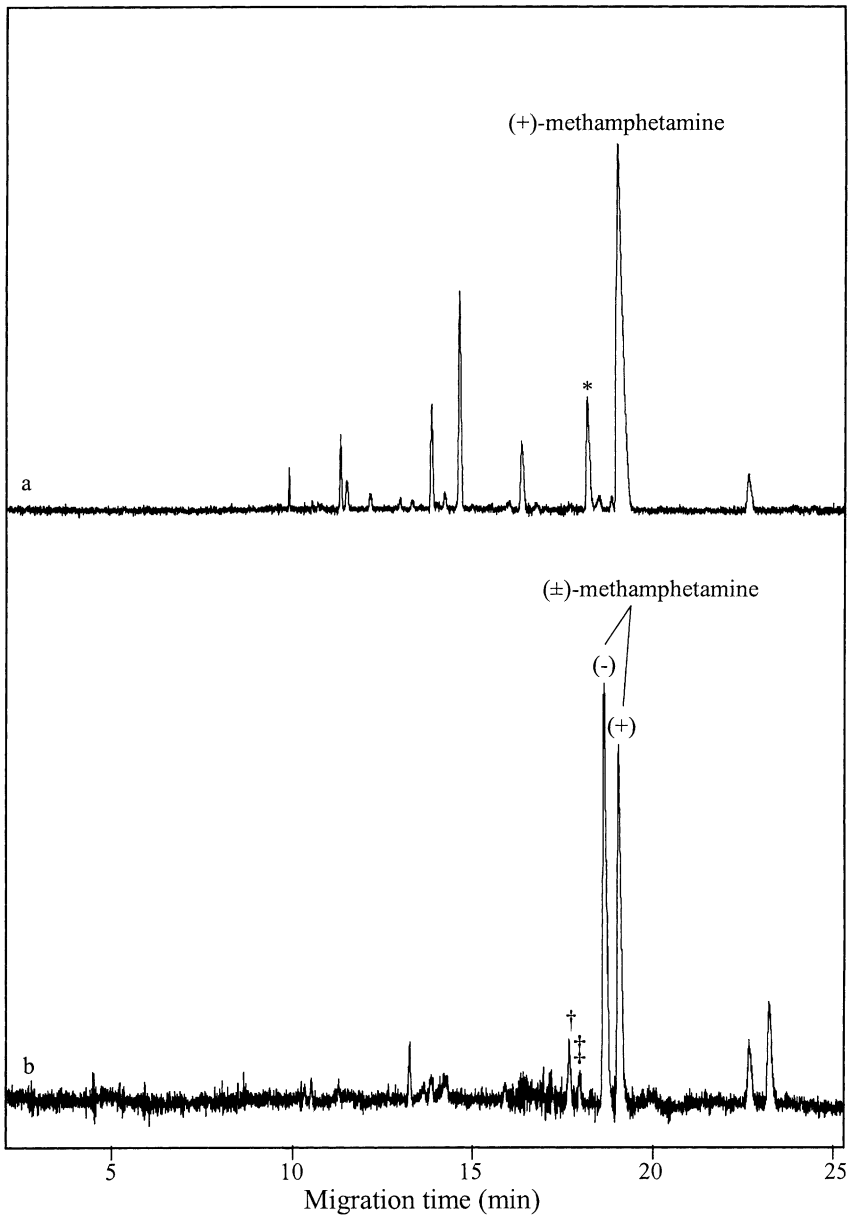

Fig. 5. Electropherograms of urine sample extracts: (a) contains only (+)-methamphetamine; (b) contains both (+)- and (−)-methamphetamine. CE conditions are as stated in Fig. 2 (chromatogram b).

UV spectra. Table 2 shows 22 results obtained by CE and these data show the enantiomeric distributions. In most of the cases, (+)-methamphetamine was present in the tablet because it is produced in the major synthetic pathway for the production of (+)-methamphetamine from (−)-ephedrine or (+)-pseudoephedrine. However, we also found that some (−)-methamphetamines were detected. Similar results have also reported by Nagai et al. [21]. Thus, care should be exercised in the identification of (+)- or (−)-methamphetamine.

3.3. Separation and identification of (±)-methamphetamine in urine samples

Using the same CE experimental conditions, urine extracts from two suspects were separated and the electropherograms are shown in Fig. 5 (electropherograms a and b). By spiking with standards, (+)-methamphetamine was detected in 10 of the samples which were donated for this research. The result of one of the samples is shown in electropherogram a. The peak indicated as "∗" was (+)-

amphetamine which is the main metabolite of (+)-methamphetamine. However, we also found that two of the urine samples contained both of (+)- and (−)-methamphetamine. One of the samples is shown in electropherogram b. The peaks indicated as "†" and "‡" were (−)-amphetamine and (+)-amphetamine, respectively. Thus, the method of β-CD modified CE separation provide a simple and rapid alternative to GC–MS.

4. Conclusions

We demonstrated here that a β-CD modified CE method can be successfully used for the separation and identification of: (±)-methamphetamine, (±)-methcathinone, (±)-ephedrine and (±)-pseudoephedrine, (±)-amphetamine in clandestine tablets and urine samples of suspects. The optimum CE conditions for the analysis of these analytes was achieved using a mixture of water–acetonitrile solution (95:5 (v/v)) containing phosphate (150 mM), β-CD (17.5 mM) at 15–30 °C; applied voltage, 20–25 kV. This method has successfully applied in the analysis of clandestine tablets and urine samples to realize the distributions of the enantiomers. Moreover, the method proposed here can provide results in <20 min without any complicated pretreatments and provided a ∼1 ppm detected limit for methamphetamine related compounds; whereas GC–MS requires a derivatization and additional sample handling for similar results. We conclude that the β-CD modified CE method provides a sensitive, accurate, rapid, simple, and economic complementary method to GC–MS for use in forensic and clinical analysis, as well as in related work.

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