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Research Article

Enantiomeric separation of some common controlled stimulants by capillary electrophoresis with contactless conductivity detection

CE methods with capacitively coupled contactless conductivity detection (C^4D) were developed for the enantiomeric separation of the following stimulants: amphetamine (AP), methamphetamine (MA), ephedrine (EP), pseudoephedrine (PE), norephedrine (NE) and norpseudoephedrine (NPE). Acetic acid (pH 2.5 and 2.8) was found to be the optimal background electrolyte for the CE-C^4D system. The chiral selectors, carboxymethyl-β-cyclodextrin (CMBCD), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DMBCD) and chiral crown ether (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid ($18C6H_4$), were investigated for their enantioseparation properties in the BGE. The use of either a single or a combination of two chiral selectors was chosen to obtain optimal condition of enantiomeric selectivity. Enantiomeric separation of AP and MA was achieved using the single chiral selector CMBCD and (hydroxypropyl)methyl cellulose (HPMC) as the modifier. A combination of the two chiral selectors, CMBCD and DMBCD and HPMC as the modifier, was required for enantiomeric separation of EP and PE. In addition, a combination of DMBCD and $18C6H_4$ was successfully applied for the enantiomeric separation of NE and NPE. The detection limits of the enantiomers were found to be in the range of 2.3–5.7 μmol/L. Good precisions of migration time and peak area were obtained. The developed CE-C^4D method was successfully applied to urine samples of athletes for the identification of enantiomers of the detected stimulants.

Keywords:
Capacitively coupled contactless conductivity detection / CE / Enantiomeric separation / Stimulants
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1 Introduction

Methamphetamine (MA), amphetamine (AP), ephedrine (EP), pseudoephedrine (PE), norephedrine (NE), and norpseudoephedrine (NPE) are basic compounds that stimulate the central nervous system. They are chiral molecules with one (or two) asymmetric C-atoms. These compounds have potential for abuse, and are thus controlled in most jurisdictions. In sports such stimulants provide an unfair advantage, such as increased alertness and diminished fatigue, and cardiovascular activation [1]. In competition, MA and AP are thus prohibited by the World Anti-Doping Agency (WADA). EP, PE, and NPE (cathine) have threshold values, as detected in urine. Levels above these constitute an adverse finding and the athlete is subject to sanctions. However, some stimulants such as NE (phenylpropanolamine) and caffeine are not considered as prohibited substances, but are subject to monitoring and reporting to the WADA. The WADA also requires the identification of the enantiomeric forms (*d*- and *l*-) of MA (but not of AP or other chiral compounds in the list of prohibited substances), since the sanction imposed for the use of *l*-MA can be of lesser severity. Nevertheless, the enantiomeric determination of chiral compounds can provide useful information about the source of the drug.

Enantiomeric separation of these stimulants has been reported by various analytical techniques, such as gas chromatography [2–4], high-performance liquid chromatography [5], and CE [2, 6–22].

CE has become a viable technique for the separation of enantiomers because of its high separation efficiency and short analysis time. Furthermore, the enantiomeric

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Abbreviations: **$18C6H_4$,** (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid; **AP,** amphetamine; **C^4D,** capacitively coupled contactless conductivity detection; **CMBCD,** carboxymethyl-β-cyclodextrin; **DMBCD,** heptakis(2,6-di-*O*-methyl)-β-cyclodextrin; **EP,** ephedrine; **HPMC,** (hydroxypropyl)methyl cellulose; **MA,** methamphetamine; **MDMA,** 3,4-methylenedioxymethamphetamine; **NDCC,** National Doping Control Center; **NE,** norephedrine; **NPE,** norpseudoephedrine; **PE,** pseudoephedrine; **TBME,** *t*-butylmethylether; **WADA,** World Anti-Doping Agency

separation by CE methods is relatively simple and of low cost because derivatization steps or expensive chiral columns, which are needed in GC and HPLC methods, are not necessary. The separation of chiral compounds by CE techniques requires only small amounts of chiral selectors, frequently cyclodextrins (CDs), which are added directly to the background electrolyte (BGE). The chiral analytes interact with these selectors to form inclusion complexes. The separation of chiral compounds is due to the differences in the formation constants of the inclusion complexes between the CD and the chiral forms of the analyte.

The CE technique has been reported in previous publications for the enantiomeric separation of AP [7, 8, 10–14, 18–21], MA [6–8, 11–14, 17–21], EP, and related compounds [11, 14–18, 20, 21]. Various types of CDs have been used as the chiral selectors, such as β-CD [7, 11, 15, 18], heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DMBCD) [6, 10, 14, 16–18], 2-hydroxypropyl-β-CD [2, 8, 15], and carboxymethyl-β-CD [12, 14]. Single wavelength or diode array UV-absorbance (DAD) detectors have been commonly used [2, 6–8, 10–12, 14–18]. In addition, the highly selective and sensitive mass spectrometer (MS) has been employed in some applications, especially for biological samples, because of the small amounts of analyte and complex matrices [19–21]. However, the MS detector is expensive and is not commonly available for CE.

Capacitively coupled contactless conductivity detection (C^4D) is a universal and low-cost detection system. Its application in CE has been reviewed in 2009 [23]. In 2010, the application of CE-C^4D was reported for the analysis of seven AP analogues, AP, MA, dextroamphetamine (DexAP), and 3,4-methylenedioxymethamphetamine (MDMA) using a BGE consisting of 75 mmol/L acetic acid and 25 mmol/L sodium acetate at pH 4.55 and 30 mmol/L 2-hydroxypropyl-β-CD as the chiral selector [24]. Not all enantiomers of the AP analogues, as well as AP and MA, could be separated with this condition, but the enantiomeric separation of MDMA was obtained. The developed CE-C^4D method was applied to illicit street-grade samples of *Ecstasy* (MDMA) and *Dexie* (DexAP). However, to our knowledge, the enantiomeric separation of AP, MA, EP, PE, Ne, and NPE has not been reported for CE-C^4D.

In this work, CE-C^4D methods have been developed for (i) the enantiomeric separation of AP and MA, (ii) the enantiomeric separation of EP and PE, and (iii) the enantiomeric separation of NE and NPE. The three CE-C^4D methods developed were applied to the trace analysis of some of the enantiomers in urine sample of athletes.

2 Materials and methods

2.1 Instrumentation

Separations using the conventional capillaries were carried out with an instrument constructed in-house. The instrument employs a high-voltage power supply with interchangeable polarity (CZE 2000) from Spellman (Hauppauge, NY, USA). The contactless conductivity detector consists of two electrodes of 4 mm in length, surrounding a PEEK tubing with id of about 400 μm, with a detection gap of 1 mm and Faradaic shielding. The input voltage was a sine wave at 300 kHz and amplitude of 280 V_{pp} (peak-to-peak). The resulting AC current was converted into voltage, then amplified, rectified, and low-pass filtered. Further details can be found in [25–29]. The signal was digitized with a data acquisition system from eDAQ (Denistone East, NSW, Australia). Fused-silica capillaries of 50 μm id and 365 μm od (Polymicro Technologies, Phoenix, AZ, USA) and total and effective lengths of 50 and 44 cm respectively, were employed for the electrophoretic separations.

2.2 Reagents and chemicals

The standard compounds, *d*-methamphetamine hydrochloride and *l*-methamphetamine and *l*-amphetamine methanol solution were obtained from Sigma (St. Louis, MO, USA). *d*-Amphetamine hydrochloride was obtained from Lipomed (MA, USA). *d,l*-Amphetamine hydrochloride was obtained from the National Measurement Institute, Sydney, Australia. (+)-Pseudoephedrine hydrochloride, (−)-pseudoephedrine, (+)-ephedrine hydrochloride, and (−)-ephedrine hydrochloride were obtained from Fluka (Buchs, Switzerland). L(−)-NE was obtained from Fluka (St. Louis, MO, USA). D-(+)-Norephedrine hydrochloride was obtained from Aldrich (Steinheim, Germany). (1R, 2R)-(−)-NPE and (1S, 2S)-(+)-norpseudoehedrine was obtained from Aldrich (Buchs). 2-Phenyl-ethylamine was obtained from Fluka (Steinheim, Germany). The chiral selector, DMBCD, was obtained from Sigma (Tokyo, Japan). Carboxymethyl-β-cyclodextrin (CMBCD) was supplied by Cyclolab (Budapest, Hungary) and (+)-(18-Crown-6)-2,3,11,12-tetra carboxylic acid ($18C6H_4$) was obtained from Fluka. (Hydroxypropyl)methyl cellulose (HPMC, viscosity of a 2% solution, 35–65 mPa s at 25°C) and acetic acid were obtained from Fluka). The BGE was filtered through a 0.2 μm nylon filter before use. The pH values of the buffers were measured with a pH meter (Model 744, Metrohm, Herisau, Switzerland), and a combination electrode calibrated with standard buffers, of pH 4 and 7.

2.3 Separation procedures

A new capillary was first rinsed with 0.1 M HCl solution (5 min), followed by deionized water (5 min), 0.1 M NaOH solution (5 min) and then deionized water (5 min) before conditioning with the BGE (10 min). Before commencement of each experiment, the capillary was rinsed with NaOH solution (5 min), deionized water (5 min) and BGE (10 min). After every electrophoretic run, the capillary was rinsed for 2 min with the BGE to maintain reproducibility of

the analysis. Standards and samples were injected hydrodynamically into the capillary by raising the sample container 10 cm for 15 or 20 s. After sample introduction, for separation a positive separation voltage of 15 kV was applied at the inlet end of the capillary.

2.4 Urine samples

Urine samples containing AP and MA, or the ephedrines (EP, PE, NE, NPE), were provided by the National Doping Control Center (NDCC), Mahidol University, Bangkok, Thailand. The urine samples had been previously analysed using a routine GC-NPD/MS procedure [30] for volatile nitrogen-containing compounds. Briefly, the urine sample (5 mL) was spiked with 10 μL of diphenylamine (1 mg/mL) as the internal standard, and extracted with 2 mL *t*-butylmethylether (TBME). The TBME organic layer was separated and 200 μL was used for the GC-NPD analysis. The remainder of the organic layer was dried under nitrogen gas at room temperature and redissolved in 100 μL TBME. Then, 2 μL was injected for GC-MS analysis. The remainder of the sample in the GC-vial was dried under N_2 gas and sent to the University of Basel for the CE-C^4D analysis. This dried part of the sample was redissolved in 50 μL or 100 μL of water and analysed using the CE-C^4D system.

3 Results and discussion

3.1 BGE

MA, AP, EP, PE, NE, and NPE are basic chiral compounds containing a benzene ring and amine group side chain. The pK_a values of these basic compounds are between 9.05 and 9.94. The separation of basic compounds in CE commonly requires a pH of the BGE below the pK_a of the compounds so that the amine group remains protonated. The compounds are then positively charged and separated as cations. In this work, acetic acid at pH 2.5 or 2.8 was employed as BGE in order to give a stable background signal for the C^4D.

3.2 Chiral selectors

CD and its derivatives are commonly used as the chiral selectors for the separation in CE [31, 32]. When using conductivity detection it is necessary to keep the background conductivity of the buffer low, and thus neutral CDs, or species that are weak electrolytes and neutral at the pH used, have previously been employed for the separation of amines in their protonated form, and these have also been combined with the chiral crown ether (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid in order to achieve the required separation (18C6H$_4$) [22, 33–36]. In this work, a neutral modified CD (DMBCD), and the anionic CD (carboxymethyl-β-CD or CMBCD), as well as 18C6H$_4$ were investigated for their separation ability for the chiral compounds of interest.

3.2.1 CMBCD as the single chiral selector

CMBCD is a commercial anionic CD. Below pH 4, the carboxyl groups are protonated resulting in an electrically neutral CD. CMBCD had been successfully employed for the enantiomeric separation of AP and MA, the metabolites of selegiline, in urine samples [12] and was thus investigated for use with CE-C^4D. Low concentrations (2, 3 and 4 mmol/L) of CMBCD were added to 150 mmol/L acetic acid at pH 2.8. The C^4D-signal (mV) and peak resolution of the enantiomers of AP and MA increased with increasing concentration of CMBCD (Fig. 1). The best peak shape and good peak height of all enantiomers were obtained using 3 mmol/L of CMBCD. However, the peak resolution of enantiomers needed to be improved. Application of HPMC as the modifier in BGE has been reported for improving the resolution and the reproducibility of migration times [6]. Various concentrations (0, 0.1, 0.2, 0.3% w/v) of HPMC were thus added to the BGE and the results are shown in Fig. 2. The peak resolutions and migration times of AP and MA enantiomers increased with increasing concentration of HPMC, but the peak heights of all enantiomers decreased. Therefore, the optimum concentration of 0.2% w/v HPMC was chosen and added to 150 mmol/L acetic acid containing 3 mmol/L of CMBCD for the separation of the AP and MA enantiomers. In addition, the separation of the enantiomers of EP, PE, NE, and NPE was investigated using this separation conditions. The enantiomers of EP, PE, NE, and NPE could be resolved for individual pairs; however, the selectivity of the system was not sufficient if all were present concurrently. Comigration of (−)-NPE and (+)-NE, as well as (−)-NE and (−)-PE, was observed (Fig. 3A). Further optimization was thus necessary.

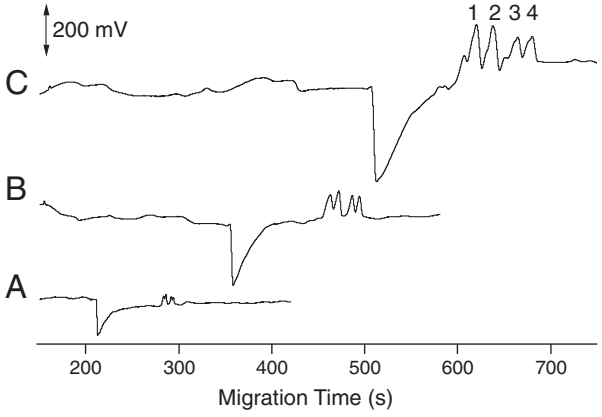

Figure 1. Electropherograms of a standard mixture of 50 μmol/L of (+)-AP, (−)-AP, (+)-MA and (−)-MA separated in 150 mmol/L of acetic acid containing CMBCD at various concentrations: (A) 2 mmol/L, (B) 3 mmol/L, and (C) 4 mmol/L. Peaks: 1, (−)-AP; 2, (+)-AP; 3, (−)-MA; 4, (+)-MA.

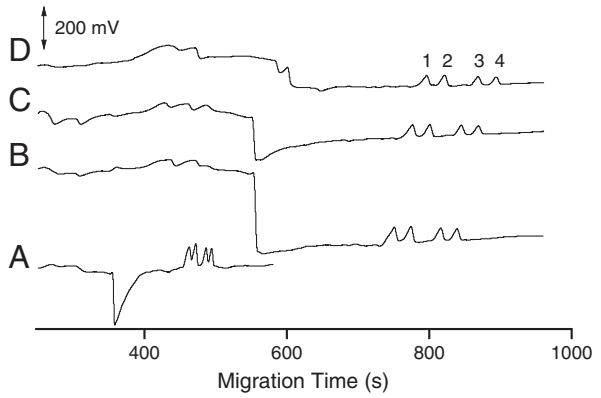

Figure 2. Electropherograms of a standard mixture of 50 μmol/L of (+)-AP, (−)-AP, (+)-MA and (−)-MA separated in 150 mmol/L of acetic acid containing 3 mmol/L of CMBCD and HPMC at various concentrations: (A) 0 (% w/v), (B) 0.1 (% w/v), (C) 0.2 (% w/v), and (D) 0.3 (% w/v). Peaks: 1, (−)-AP; 2, (+)-AP; 3, (−)-MA; 4, (+)-MA.

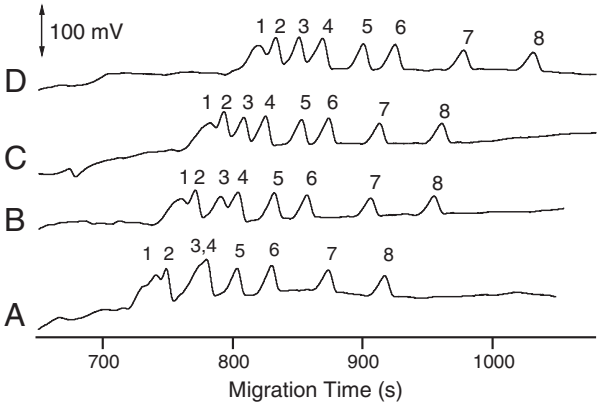

Figure 3. Electropherograms of a standard mixture of 50 μmol/L of (+)-EP, (−)-EP, (+)-PE, (−)-PE, (+)-NE, (−)-NE, (+)-NPE, and (−)-NPE separated in 150 mmol/L of acetic acid containing 0.2 (%w/v) of HPMC, 3 mmol/L of CMBCD and DMBCD at various concentrations: (A) 0 mmol/L, (B) 2 mmol/L, (C) 3 mmol/L, and (D) 4 mmol/L. Peaks: 1, (−)-NPE; 2, (+)-NE; 3, (−)-NE; 4, (+)-PE; 5, (+)-EP; 6, (−)-EP; 7, (+)-NPE; 8, (+)-PE.

3.2.2 Dual chiral selectors using CMBCD and DMBCD

As adequate separation with a single additive could not be achieved, the combination of selectors was investigated. The simultaneous enantiomeric separation of the three EP derivatives (NE, EP, and PE) has been reported using a mixture of two CDs (CMBCD and DMBCD) [14]. Therefore, this combination was also tested for the simultaneous separation of the four ephedrines (EP, PE, NE, and NPE) and the use of conductivity detection. The electrolyte based on CMBCD that was found to give best results above was used as the basis and increasing amounts of DMBCD were added to this solution. The peak resolution of comigrating (−)-NPE and (+)-NE, as well as (−)-NE and (−)-PE was found to improve when increasing the DMBCD concentration. However, the comigration of (−)-NPE and (+)-NE could not be resolved even when the concentration of DMBCD was increased to 4 mmol/L. The BGE of 150 mmol/L of acetic acid containing 0.2% w/v of HPMC and 3 mmol/L of CMBCD and 3 mmol/L of DMBCD was adopted for the enantiomeric separation of EP and PE only because the comigration of (−)-NE and (−)-PE could be resolved within a short separation time (Fig. 3C). Therefore, the enantiomeric separation of NE and NPE was investigated using a different separation electrolyte.

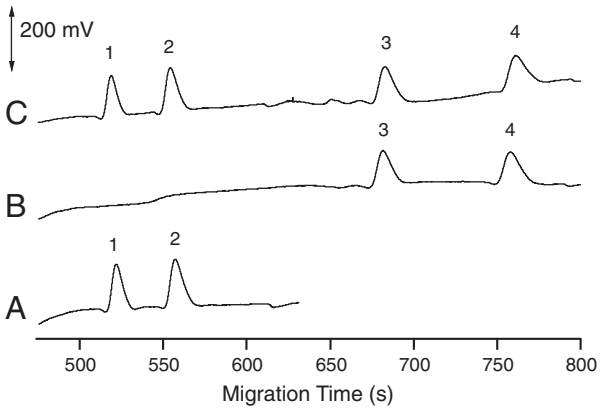

Figure 4. Electropherograms (inverted signal) of (A) a standard mixture of 100 μmol/L of (+)-NPE, and (−)-NPE, (B) a standard mixture of 100 μmol/L of (+)-NE, and (−)-NE, and (C) a standard mixture of 100 μmol/L of (+)-NE, (−)-NE, (+)-NPE, and (−)-NPE separated in 500 mmol/L of acetic acid containing 5 mmol/L of DMBCD and 5 mmol/L of 18C6H_4. Peaks: 1, (+)-NPE; 2, (−)-NPE; 3, (−)-NE; 4, (+)-NE.

3.2.3 Dual chiral selectors using DMBCD and the chiral crown ether 18C6H_4

The combination of the chiral crown ether 18C6H_4 and DMBCD had been used successfully for the analysis of enantiomers of small underivatized amines by CE with contactless conductivity detection [36] and also for the separation and detection of stereoisomers of di-, tri-, and tetrapeptides [34]. The chiral crown ether interacts with the amine group through hydrogen bonding, while the CD interacts with the lipophilic part of the peptides. Therefore, the use of the combination of these chiral selectors was investigated for the enantiomeric separation of NE and NPE in our work. The separation electrolyte of 500 mmol/L of acetic acid containing 5 mmol/L of DMBCD and 5 mmol/L of 18C6H_4 was found to be successful for the enantiomeric separation of NE and NPE (Fig. 4).

3.3 Analytical characteristics of the CE-C^4D method

Three different CE-C^4D methods for the enantiomeric separations of (i) AP and MA, (ii) EP and PE, and (iii) NE and NPE have thus been developed. The separation of the MA and AP enantiomers, as well as of the EP and PE

Table 1. Analytical characteristics of the CE-C^{4}D method for enantiomeric separation of (1) AP and MA, (2) EP and PE, (3) NE and NPE

Separation electrolyte	Enantiomer	Concentration range tested (μmol/L)	Coefficient of determination (r^2)	LOD, μmol/L (μg/mL)	Reproducibility (% RSD)	
					Migration time	Peak area
1	(−)-AP	5–75	0.996	4.5 (0.61)	1	2.1
	(+)-AP	5–75	0.998	3.6 (0.49)	1	3.2
	(−)-MA	5–75	0.997	3.9 (0.58)	1	1.9
	(+)-MA	5–75	0.996	4.4 (0.66)	1	3
2	(−)-EP	5–100	0.999	2.6 (0.43)	0.4	2.9
	(+)-EP	5–100	0.996	5.7 (0.94)	0.4	4.1
	(−)-PE	5–100	0.998	4.1 (0.68)	0.4	4
	(+)-PE	5–100	0.999	2.4 (0.40)	0.4	2.8
3	(−)-NE	5–100	0.999	2.7 (0.41)	1.6	4.1
	(+)-NE	5–100	0.999	3.3 (0.50)	1.7	5.2
	(−)-NPE	5–100	0.999	3.3 (0.50)	1.4	4
	(+)-NPE	5–100	0.999	3.1 (0.47)	1.3	4.7

Separation electrolytes: (1) 150 mmol/L of acetic acid (pH 2.8) containing 0.2% w/v of HPMC and 3 mmol/L of CMBCD. (2) 150 mmol/L of acetic acid (pH 2.8) containing 0.2% w/v of HPMC, 3 mmol/L of CMBCD, and 3 mmol/L of DMBCD. (3) 500 mmol/L of acetic acid (pH 2.5) containing 5 mmol/L of DMBCD and 5 mmol/L of $18C6H_4$.

enantiomers, was achieved by using the BGE containing the single CMBCD chiral selector and HPMC modifier. However, the complete separation of NE and NPE enantiomers could not be achieved with this electrolyte. In urine samples from athletes where all four EP compounds (EP, PE, NE, and NPE) may be found, the combination of two chiral selectors (CMBCD and DMBCD) and HPMC modifier is used to separate all the enantiomers of EP, PE, NE, and NPE. However, comigration of (−)-NPE and (+)-NE was observed (see Section 3.2.2). For complete enantiomeric separation of NE and NPE, the dual chiral selector system using DMBCD and chiral crown ether was successfully applied (see Section 3.2.3). Moreover, the enantiomers of EP and PE did not comigrate with NE and NPE. The analytical characteristics for all enantiomers are shown in Table 1. The reproducibility of the migration time of each enantiomer was in the range from 0.4 to 1.7% RSD. The reproducibility of the peak area was in the range from 2.1 to 5.2% RSD. The calibration curves in the tested range were linear with coefficients of determination (r^2) between 0.996 and 0.999. The limits of detection ($3 \times S/N$) were in the range from 2.4 to 5.7 μmol/L (0.40–0.94 μg/mL). Note that these values apply to the solution analysed, not considering any prior extraction procedure for samples.

3.4 Analysis of extracted urine samples of athletes

3.4.1 Analysis of the enantiomers of AP and MA in extracted urine samples

Two extracted urine samples (samples A and B) and an aqueous standard mixture of AP and MA enantiomers were analysed by the CE-C^{4}D method as described in Section 3.2.1, see the electropherograms in Fig. 5. The peak for

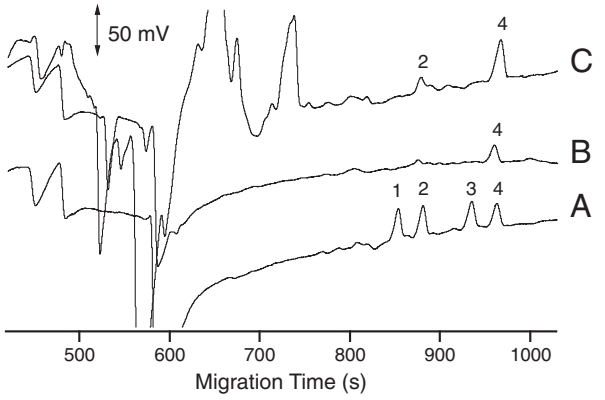

Figure 5. Electropherograms of (A) a standard mixture of (+)-AP, (−)-AP, (+)-MA, (−)-MA, (B) the extracted urine sample **A**, (C) the extracted urine sample **B** separated in 150 mmol/L of acetic acid containing 0.2 (% w/v) of HPMC and 3 mmol/L of CMBCD. Peaks: 1, (−)-AP; 2, (+)-AP; 3, (−)-MA; 4, (+)-MA.

(+)-MA was found in both samples (A, Fig. 5B and B, Fig. 5C). The peak of (+)-AP was found in only sample B, (+)-AP being the metabolite of (+)-MA.

3.4.2 Analysis of the enantiomers of NE and NPE in extracted urine samples

An extracted urine sample (sample C) and an extracted blank urine sample spiked with a standard mixture of NE, NPE enantiomers, together with the EP, PE enantiomers were analysed by the CE-C^{4}D method as described in Section 3.2.3 (see the electropherograms in Fig. 6B and C). An aqueous standard mixture of NE and NPE was also analysed by this CE-C^{4}D method as shown in Fig. 6A. From the NDCC data, sample C contains PE and NPE (metabolite of PE). The peak for (+)-NPE was seen in sample C

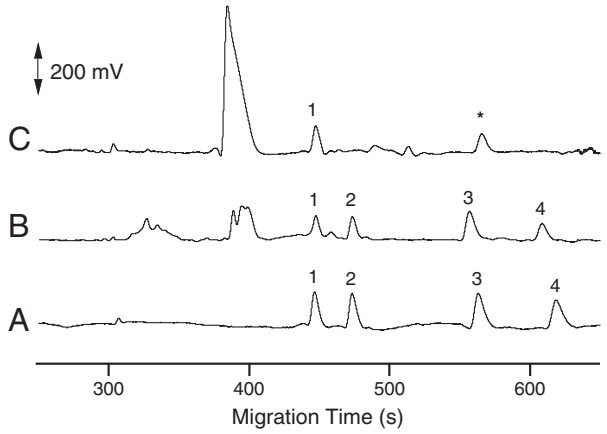

Figure 6. Electropherograms (inverted signal) of (A) a standard mixture of (+)-NE, (−)-NE, (+)-NPE, (−)-NPE, (B) the extracted blank urine sample spiked with a standard mixture of (+)/(−)-NE, (+)/(−)-NPE, (+)/(−)-EP, (+)/(−)-PE, (C) the extracted urine sample **C** separated in 500 mM acetic acid containing 5 mmol/L of DMBCD and 5 mmol/L of $18C6H_4$. Peaks: 1, (+)-NPE; 2, (−)-NPE; 3, (−)-NE; 4, (+)-NE.

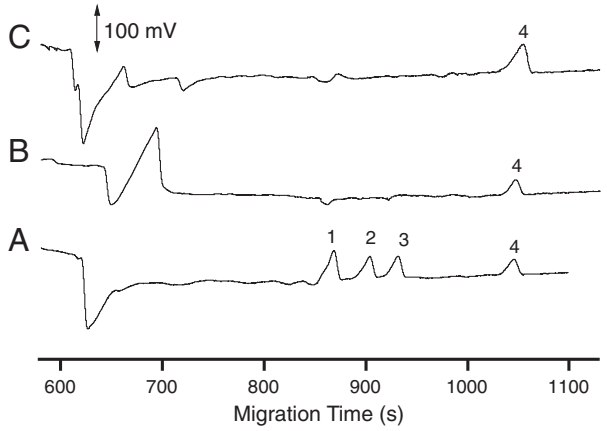

Figure 7. Electropherograms of (A) a standard mixture of (+)-EP, (−)-EP, (+)-PE, (−)-PE, (B) the extracted urine sample **D**, (C) the extracted urine sample **C** (diluted 10 times) separated in 150 mmol/L of acetic acid containing 0.2 (% w/v) of HPMC, 3 mmol/L of CMBCD, and 3 mmol/L of DMBCD. Peaks: 1, (−)-PE; 2, (+)-EP; 3, (−)-EP; 4, (+)-PE.

(Fig. 6C). The peak designated with an asterisk in Fig. 6C with migration time 565 s, near the migration time of (−)-NE, is an artifact from the matrix in the urine sample, since analysis by NDCC has shown that NE was not present in this sample. The large peak with migration time of 370 s observed in sample C is the unresolved PE enantiomers (see Section 3.4.3).

3.4.3 Analysis of the enantiomers of EP and PE in urine samples

Two extracted urine samples (sample C diluted tenfold and sample D) and an aqueous standard mixture of EP and PE enantiomers were analysed by the CE-C^4D method for EP and PE enantiomers, as described in Section 3.2.2. The electropherograms are shown in Fig. 7. From the NDCC data, sample D contains only PE, with no cathine (NPE) present. For this CE-C^4D method, the peak for (+)-PE was found in both samples (C, Fig. 7C and D, Fig. 7B). Thus, the large peak found in electropherogram Fig. 6C of sample C was confirmed to be (+)-PE.

3.4.4 Quantification of stimulants in extracted urine samples of athletes

At NDCC, Bangkok, the concentrations of prohibited stimulants in urine samples are determined by a WADA-accredited GC-NPD method, with diphenylamine as the internal standard to correct for the variation in extraction recovery. The identification of the compound is subsequently confirmed by GC-MS using the same type of GC-column and the same temperature program following a further sample preparation step (compare Section 2.4). In this work, the quantification of enantiomers of MA, AP, EP, PE, NE, and NPE by CE-C^4D was then carried out after drying the remainder of the extract used for the GC-MS analysis for shipping from Bangkok to Basel and reconstitution prior to analysis. Note that the extraction procedure leads to a preconcentration for the CE-measurement (considering the consumption of aliquots in the prior analysis steps, the maximum factor is 88.2). Thus, the effective LODs as applied to the urine samples are approximately 0.05 μmol/mL or 0.01 μg/mL. The results

Table 2. Quantification of stimulants in the urine samples of athletes by CE-C^4D method compared with GC-NPD method

Sample	CE-C^4D			GC-NPD	
	Analyte	Concentration in the reconstituted solution (μg/mL)	Concentration in urine (μg/mL)	Analyte	Concentration in urine (μg/mL)
A	(+)-MA	5.7 ± 0.3	0.13 ± 0.01	MA	0.22
B	(+)-AP	1.1 ± 0.2	0.024 ± 0.004	AP	0.12
	(+)-MA	7.5 ± 0.4	0.17 ± 0.01	MA	1.0
C	(+)-PE	16.0 ± 0.5	1.8 ± 0.1	PE	3.2
	(+)-NPE	12.3 ± 1.5	0.14 ± 0.02	NPE	0.20
D	(+)-PE	7.8 ± 0.4	0.87 ± 0.05	PE	0.86

are shown in Table 2. Note also, that the GC-NPD method did not allow the separation of the enantiomers. The agreement for samples A, C, and D (four enantiomers found) is reasonable considering the accumulated uncertainties of the further sample treatment steps following the quantification and the fact that no internal standard was employed to account for any losses. The reason for the larger discrepancy for sample B is not clear, but must be due to a non-recognized systematic error.

4 Concluding remarks

The CE-C^4D method developed provides a simple method for the enantiomeric separation of chiral stimulants. Only low concentrations of chiral selectors added to the low-pH BGE of acetic acid are required to achieve enantioselectivity. While it probably is not possible to find an appropriate BGE with selectors that allow the separation of all 16 enantiomers in a single run, these could be successfully separated with three different reagent solutions containing dual combinations of selectors. The CE-C^4D method may be successfully applied as a low-cost screening method for urine samples of athletes for the rapid identification of the enantiomeric forms of MA and other chiral compounds in doping control.

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