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Enantiomeric Separation of Methamphetamine and Related Analogs by Capillary Zone Electrophoresis: Intelligence Study in Routine Methamphetamine Seizures*

ABSTRACT: A method for simultaneous enantiomeric separation of ephedrine, pseudoephedrine, and methamphetamine (MA) in a single run by simple capillary zone electrophoresis (CZE) with β-cyclodextrin as a chiral selector is described. The effects of the buffer pH, phosphate concentration, β-cyclodextrin concentration, voltage and temperature on the peak resolution were examined. Good enantiomeric resolution was attained for each analyte under our optimized conditions: 15 mM β-cyclodextrin, 300 mM NaH_2PO_4 at pH 2.5 with an uncoated capillary (64.5 cm × 50 μm), applied potential at 20 kV and temperature at 30°C. Ultraviolet (UV) detection at a fixed wavelength (200 nm) was employed using a diode array detector. Using phentermine as an internal standard, migration times for all analytes are reproducible within 0.1% for intra-day and 0.6% for inter-day runs. Application of this method to the analysis of confiscated drugs is discussed.

KEYWORDS: forensic science, methamphetamine, ephedrine, pseudoephedrine, capillary zone electrophoresis

Methamphetamine (MA), a stimulant of the central nervous system (CNS), is one of the major drugs of abuse that has become a global problem (1). While D-(+)-MA is known to possess a strong CNS stimulatory effect, its L-(−) counterpart is used in a common nasal decongestant (e.g., Vick's inhaler) in the United States. In some countries, the enantiomeric purities of confiscated MA samples are an important consideration in prosecution. For instance, in the United States, possession of D-(+)-MA with high enantiomeric purity [>80% enantiomeric excess (*e.e.*)] would lead to severe penalties, according to the U.S. federal sentencing guidelines for "*ICE*" methamphetamine (2). The enantiomeric purities of seized drugs can also provide important clues about the possible preparative routes of the samples (3,4). Identification of racemic MA strongly indicates that the sample is derived from an achiral precursor phenylacetone using reductive amination. On the other hand, enantiomerically pure MA may suggest direct stereospecific reduction of enantiopure β-hydroxyphenethylamines (i.e., ephedrine or pseudoephedrine).

The statistics obtained from our laboratory showed that MA abuse in Hong Kong has increased rapidly in the past few years from 98 cases in 1994 to 822 cases in 2000. Over 85% of the samples confiscated by the law enforcement departments are in the form of crystals and as hydrochloride salt with a chemical purity of > 95%. Occasionally, ephedrine and pseudoephedrine are found in association with MA seizures; they are either present as a mixture with the MA or as pure compounds in separate exhibits.

In the United States (3) or Australia (5), the synthetic methods of MA can be revealed during an encounter of clandestine laboratories. However, no clandestine laboratories producing MA have ever been encountered in Hong Kong. In addition, the local legislation relating to illegal possession and trafficking of MA counts on the chemical purity of the sample, but not its optical purity. Therefore, the optical purities of the seized samples are rarely determined in routine analysis. The lack of knowledge on the enantiomeric purity of the MA results in the loss of valuable intelligence data relating to the possible route(s) of manufacture. If a simple, robust and inexpensive method could be established for the enantiomeric separation of (±)-ephedrine, (±)-pseudoephedrine and (±)-MA, more useful data for intelligence purposes could be obtained to indicate possible synthetic methodologies for MA seizures.

Currently HPLC (6) and GC (7) techniques mostly have been employed for determining the enantiopurity of MA. They require either prior derivatization with chiral reagents to form diastereomers or the use of an expensive chiral stationary or mobile phase. Capillary electrophoresis, in contrast, has shown itself to be a superior tool for chiral drugs analysis because of its low cost and very high separation efficiency (8). Lurie et al. first reported the separation of (±)-amphetamine, (±)-MA and their related hydroxyphenethylamine precursors after derivatization to their diastereomers by the MEKC method (9). Recently, with the use of substituted cyclodextrins, separation of six chiral phenethylamines and three achiral neutral impurities, which are commonly found in illicit methamphetamine, has been achieved in a single run without derivatization

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(10). Tagliaro et al. have developed a simple CZE method for simultaneous chiral resolution of amphetamine, MA, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDE), 3,4-methylenedioxyamphetamine (MDA) and ephedrine using β-cyclodextrin as a chiral selector (11,12). However, in Tagilaro's work, the resolution of (±)-pseudoephedrine, which can also be a precursor for the synthesis of MA, was not reported because of the unavailability of authentic standards (11).

Herein we describe the optical separations of racemic ephedrine, pseudoephedrine, and MA using the CZE method with β-cyclodextrin as a chiral selector. The conditions for optical resolution have been optimized and subsequently applied to routine analysis of confiscated MA and related samples.

Materials and Methods

Reagents and Standards

Anhydrous sodium dihydrogen orthophosphate (BDH), β-cyclodextrin (Sigma Chemicals) and phosphoric acid (Eastman Kodak Company) were obtained commercially and used as received. (+)/(−)-Ephedrine, (+)/(−)-pseudoephedrine, (+)/(−)-MA, and phentermine were purchased from Sigma Chemicals. Standard solutions at a concentration of 1 mg/mL were prepared in water and stored at 4°C. Deionized water from a Millipore Milli-Q System was used for buffer preparations. All the buffers and samples were filtered through a 0.2 μm pore size nylon membrane prior to sample injection into the CE.

Instrumentation

An HP^{3D} capillary electrophoresis system equipped with a photodiode array UV detector was employed. An untreated fused-silica capillary of 50 μm inside diameter (ID) and 65 cm in length from HP was used for all the experiments. The capillary was conditioned before use by successively washing for 30 min with 1 M NaOH, 10 min 0.1 M NaOH and 10 min water, followed by a 30 min flushing with the run buffer. The capillary was also flushed with the run buffer for 5 min between injections.

Sample Preparation

(+)/(−)-Ephedrine, (+)/(−)-pseudoephedrine, (+)/(−)-MA, either the standards or the samples, were dissolved directly in water (*c.a.* 0.1 mg/mL) in the presence of 0.1 mg/mL of phentermine as an internal standard.

Electrophoretic Conditions

Experimental conditions such as background phosphate buffer concentration, buffer pH, cyclodextrin concentration, applied voltage and temperature had been varied for method optimization; and the optimized conditions were found to be: 15 mM β-cyclodextrin, 300 mM NaH_2PO_4 at pH 2.5 with an uncoated capillary (64.5 cm × 50 μm), applied potential at 20 kV, and with the temperature at 30°C. Detection was performed at a fixed wavelength of 200 nm for all measurements. All injections were accomplished by applying pressure at 50 mbar for 4 seconds.

Results and Discussion

As reported by Tagilaro recently (12), with the use of 15 mM β-cyclodextrin as a chiral selector and a 100 mM phosphate buffer at pH 2.5, baseline enantiomeric separations of ephedrine, MA and some amphetamine-related substances have been achieved. Applying similar conditions (except a column length of 64.5 cm was employed in this work versus 45 cm as quoted in Tagilaro's work), we found that all constituents were well resolved apart from (−)-pseudoephedrine and (+)-ephedrine for which there was peak overlap. In order to attain complete resolution for all the compounds, we have examined the effects of buffer concentration, buffer pH, and other experimental parameters (e.g., applied potential, operating temperature).

It is known that the migration time reproducibility for each analyte can be improved by adding a suitable internal standard in each run. In Tagilaro's work, (−)-MA was employed as the internal standard since this compound was rarely found in the clandestine market in Italy (12). The resultant effect was a significant improvement in both the precision of the migration time and quantitation of all the analytes. Since this work involved the separation of (+)- and (−)-MA, phentermine (an achiral phenethylamine with a chemical structure similar to MA) was used as an internal standard.

Parameters Optimization

The effect of phosphate buffer concentration on the chiral separation is illustrated in Fig. 1. It is evident that the electroosmotic flow (EOF) decreases as the phosphate concentration increases (50 mM to 300 mM). Hence the analyte would have a longer time to interact with the β-cyclodextrin present in the buffer, thereby improving the resolution (R) of (−)-pseudoephedrine and (+)-ephedrine significantly from 0.6 at 50 mM buffer to 1.1 at 300 mM buffer.

As depicted in Fig. 2, poor peak resolutions of all the analytes resulted upon lowering the β-CD concentration from 15 mM to 5 mM. This is not surprising since the number of chiral selector molecules interacting with analytes had decreased. Not only was no improvement to resolution observed with the increase of the β-cyclodextrin concentration, but an actual decrease in resolution between peaks a and b (Fig. 2) is obtained due to the saturation of the run buffer by the β-cyclodextrin with respect to the analyte.

The effect of buffer in the range of pH 2.5–5.5 was studied. We found that the migration time at pH 2.5 was nearly doubled compared with that at pH 5.5, and higher buffer pH had no detrimental effect on the peak resolutions. However, we found that shortening of the migration time was counterbalanced by poor migration time reproducibility (for instance, RSD of (+)MA at pH 2.5 was 1.0% while at pH 5.5 the RSD was 4.6%). This can be reasoned that increasing the buffer pH would raise the operating current (from 125 μA at pH 2.5 to 170 μA at pH 5.5), leading to significant joule heating. Optimal results were obtained with buffer pH at 2.5. A study of the peak resolutions by varying the applied potential was conducted within the 10–25 kV range (Fig. 3). The peak resolutions increased as applied potentials were increased from 10 to 20 kV. Further increases in the applied voltage beyond 20 kV resulted in the drop in peak resolution. Joule heating generated by increases in the operating current at high potential may result in a decreased resolution. Thus, 20 kV was selected as the optimal applied voltage. The peak resolutions are largely invariant to the operating temperature within the range of 20°C to 30°C. Although generally an increase in temperature can shorten analysis time (due to a decrease in viscosity which would result in an increase of EOF), an operating temperature of 30°C was chosen as a compromise to balance peak resolution and analysis time.

Under the optimized conditions (see Electrophoretic Conditions), baseline separation of (+)/(−)-ephedrine, (+)/(−)-

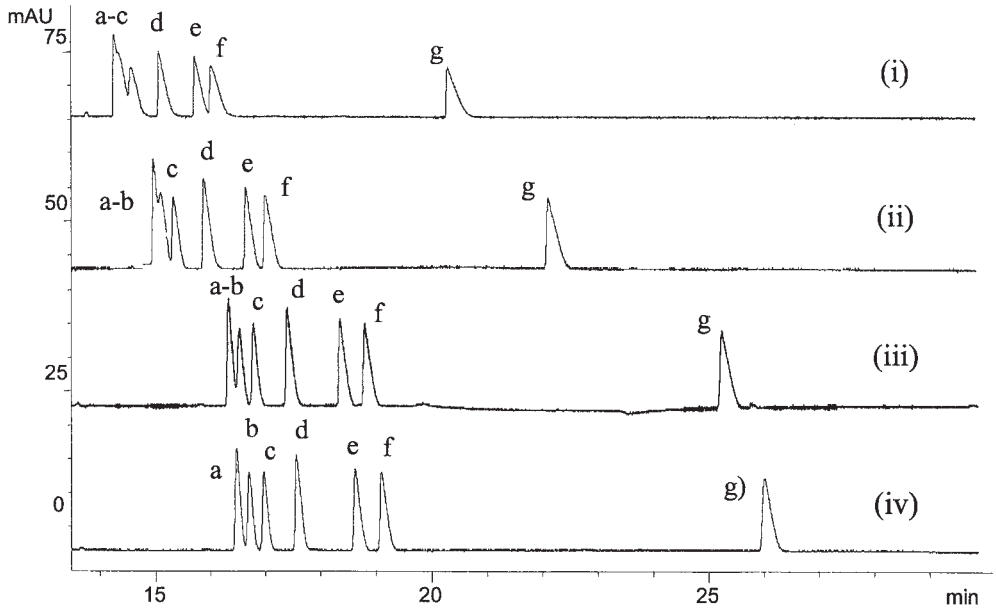

FIG. 1—*Effect of phosphate concentration on CZE separation of a) (−)-pseudoephedrine, b) (+)-ephedrine, c) (−)-ephedrine, d) (+)-pseudoephedrine, e) (−)-MA, f) (+)-MA, g) phentermine (internal standard). Conditions: 15 mM* β*-cyclodextrin, phosphate concentration: (i) 50 mM, (ii) 100 mM, (iii) 200 mM, (iv) 300 mM at pH 2.5 with an uncoated capillary (64.5 cm* × *50* μ*m), applied potential at 20 kV, and temperature at 30°C.*

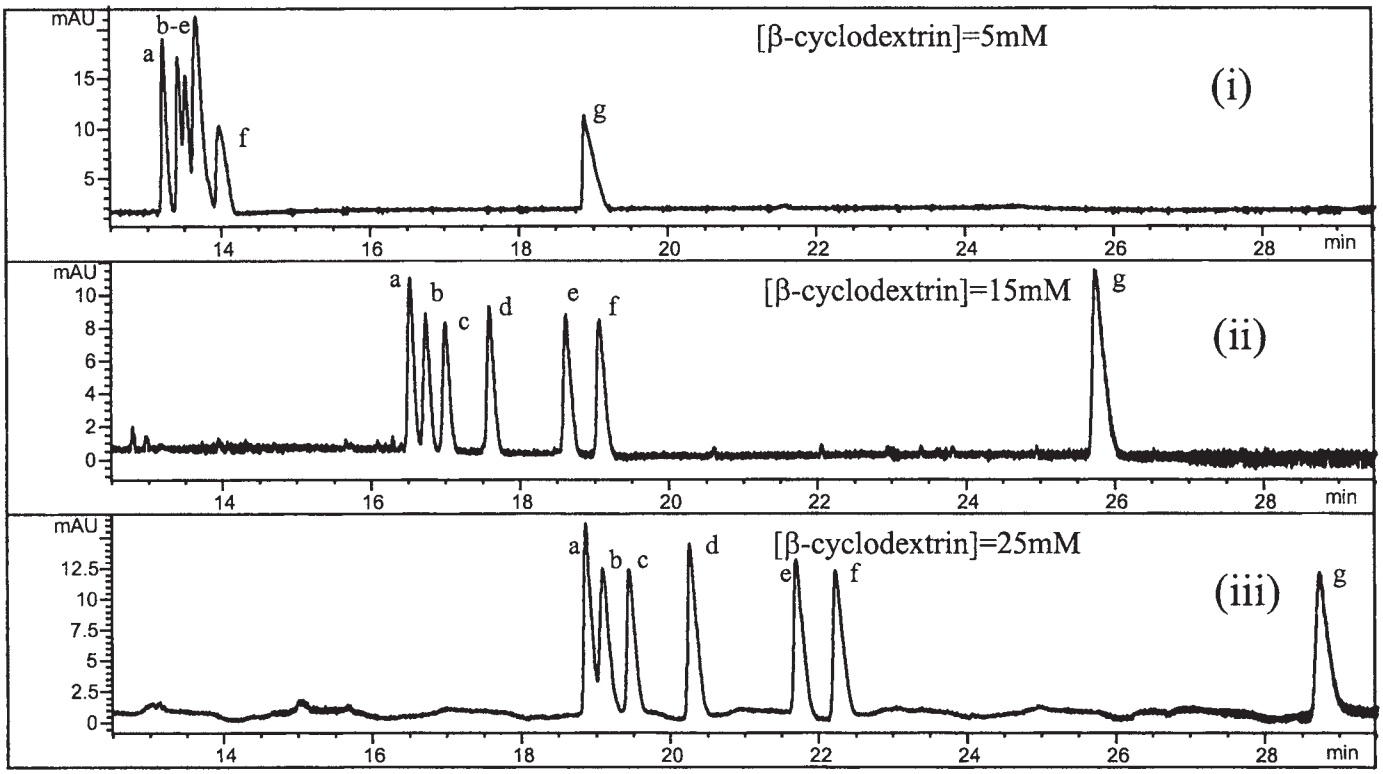

FIG. 2—*Effect of* β*-cyclodextrin concentration on CZE separation of a) (−)-pseudoephedrine, b) (+)-ephedrine, c) (−)-ephedrine, d) (+)-pseudoephedrine, e) (−)-MA, f) (+)-MA, g) phentermine (internal standard). Conditions:* β*-cyclodextrin concentration: (i) 5 mM, (ii) 15 mM, (iii) 25 mM, 300 mM phosphate at pH 2.5 with an uncoated capillary (64.5 cm* × *50* μ*m), applied potential at 20 kV, and temperature at 30°C.*

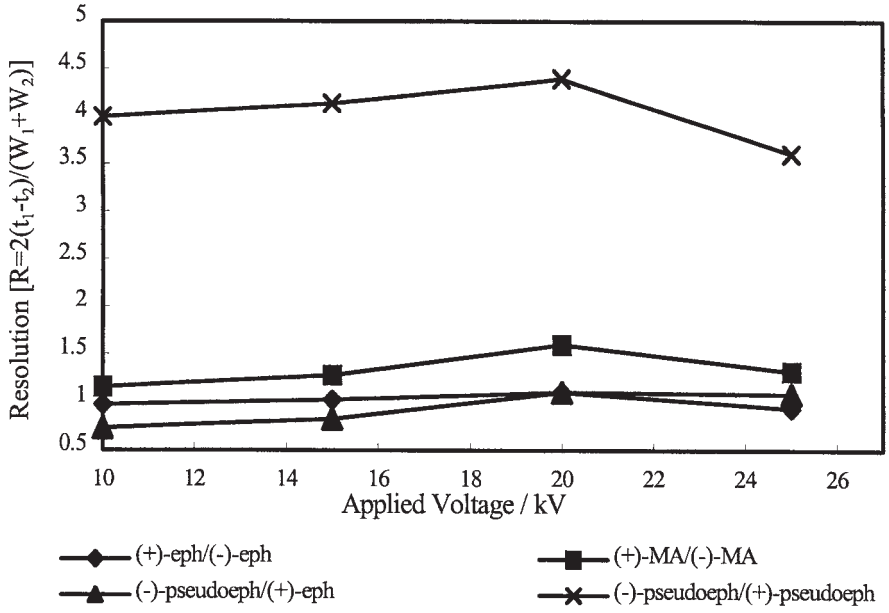

FIG. 3—*Plots of resolution of (±)-pseudoephedrine, (±)-ephedrine, (±)-MA versus applied potential. Conditions as in Fig. 2ii except for the applied potential.*

pseudoephedrine and (+)/(−)-MA and good resolution of their respective enantiomers using phentermine as an internal standard was accomplished in less than 30 min (Fig. 2ii). The chiral resolutions of ephedrine, pseudoephedrine and MA were found to be 1.1, 4.4 and 1.6, respectively. Without sample preconcentration, the sensitivity was 3 μg/mL with the signal-to-noise ratio equal to 3. A good linear calibration curve was obtained in the concentration range of 5–250 μg/mL. For all the enantiomers, the correlation coefficients (r^2) are equal to or greater than 0.9993, and the practical quantitation limit in our application is 5 μg/mL. By running a standard solution containing (+)-MA and (−)-MA in a ratio of 99:1, optical purity down to 2% *e.e.* could be detected. For all analytes, using phentermine as an internal standard, the intra-day average RSD of the relative migration times ($n = 9$) is below 0.1% and the inter-day average RSD of relative migration times is less than 0.6% (10 days; $n = 20$).

Testing to Forensic Exhibits

(a) Applicability—In order to examine the applicability and robustness of this method for the analysis of illicit MA samples, 138 samples that contained MA were randomly taken from 126 cases for analysis. They were in the form of liquids tablets and crystalline solids. The presence of MA and/or related analogs has been confirmed for all the samples using GC-MS or FTIR.

In these analyses, unequivocal separation of (+)- and (−)-MA was achieved. Of these samples, 80% were found to contain optically pure (+)-MA (>98% *e.e.*) and 10% contained optically pure (−)-MA (>98% *e.e.*). The remaining 10% contained a variable combination of the two enantiomers (varying from −55% to +92% *e.e.* based on (+)-MA) (Table 1). In the case where (−)-MA was detected, a repeated run with the test sample being spiked with an aliquot of standard (+)-MA was performed for confirmation.

Similarly, the method has been used to resolve the (±)-ephedrine and (±)-pseudoephedrine in some cases that contained MA. In one of the cases, two samples were found to contain (+)-pseudoephedrine as a minor constituent in the presence of optically pure (+)-MA. In another case, a sample of chemically and optically pure (−)-ephedrine was identified where the (+)-MA sample was found in a separate exhibit.

TABLE 1—*Distribution of % e.e. of MA racemic mixtures (14 casework samples).*

% *e.e.* (Enantiomeric Excess Based on (1)-MA)	Number of Cases
−98 to −75	0
−75 to −40	2
−40 to −5	2
−5 to +5	0
+5 to +40	5
+40 to +75	3
+75 to +98	2

(b) Potential intelligence on MA manufacture—The value for developing the separation method lies in its ability to generate information to provide clues on the manufacturing route of MA. This could be useful in linking the origin of an MA sample to a clandestine laboratory, and hence perhaps the trafficking route. In the two cases mentioned above, the (−)-ephedrine and (+)-pseudoephedrine found could have been the precursors used in their respective syntheses. It is known that optically pure (+)-MA could be obtained through their respective reduction in a reaction using red phosphorus/hydrogen/iodine or lithium/ammonia. This throws light on the possible synthetic routes employed by certain clandestine laboratories and facilitates the investigation by law enforcement officers. The analysis of the 10% of samples containing varying amounts of the two enantiomers (Table 1) revealed that their relative amounts were not in a 1:1 ratio. This indicates that they were unlikely to have been produced by the chemical syntheses via phenylacetone purely on a single run, as a ratio of 1:1 would be expected (3). Such findings could be accounted for probably by

a physical mixing of the two isomers or of either one with a racemate (±)-MA in different proportions.

There have been, as yet, no clandestine laboratories for MA manufacture identified in Hong Kong. MA is smuggled into Hong Kong from neighboring areas. In this study, the finding of relatively pure (−)-MA in seizures is perplexing. This compound is not registered for medicinal use in Hong Kong, (as opposed to being used in certain nasal decongestant preparations in the United States). It is known to be a precursor for the manufacture of (+)-selegiline (deprenyl), an effective antiparkinsonian and antidepressant. The Hong Kong laws, which levy heavy penalties on MA trafficking and manufacture, do not differentiate the penalties between the two enantiomers of MA. It is puzzling that traffickers would risk heavy penalties and antagonize their clientele by smuggling the less potent form of ineffective CNS stimulant, i.e., (−)-MA. It would be interesting to see if its occurrence has been experienced in clandestine laboratories elsewhere.

Conclusion

A simple CZE method using unsubstituted β-CD as a chiral selector has been developed and optimized for the enantiomeric separations of (±)-ephedrine, (±)-pseudoephedrine and (±)-MA. Its robustness has been tested with casework samples of MA (of a range of matrices including liquids tablets and crystalline solids) and found to be satisfactory. The method has the merit of achieving very respectable migration time reproducibility and without the need of sample pretreatment. The enantiomeric compositions of the seized materials provide information on possible precursors used in their manufacture and the chemical process involved.

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