— **Minireview** —

Chiral Capillary Electrophoresis of Amphetamine-Type Stimulants

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Amphetamine-type stimulants (ATS), having one or more asymmetric carbons, are important targets in forensic science field. For chiral analysis, capillary electrophoresis (CE) has advantages over other chromatographic methods, especially, in fastness, resolution, cost performance and simplicity. This review summarizes chiral separation techniques of ATS using CE with UV detection or CE/mass spectrometry mainly by the present authors' research group.

Key words —— capillary electrophoresis, chiral separation, amphetamine-type stimulant, seized drug, urine

INTRODUCTION

Amphetamine-type stimulants (ATS) are abused by about 34 million people in the world.[1)] In Japan, methamphetamine (MA), which is one of the ATS, accounts for the greater part of illicit drugs.[2)] ATS have one or more asymmetric carbons, and thus may occur in the form of different enantiomers. For some drugs, different enantiomers are controlled by different laws, so identification of which enantiomers are present is important in the forensic field. In the case of MA, most seized MA was the *d*-isomer,[3)] but mixtures of *d*- and *l*- isomers have also been observed.[4)] It is well known that each enantiomer of ATS has different pharmacological activities: *d*-MA is 5–10 times more potent than *l*-MA with respect to its effect on the central nervous system, so the chiral analysis of ATS is important in the toxicological as well as forensic science field. In addition, the seized MA sometimes includes dimethylamphetamine (DMA), ephedrine (EP) or its analogues, as impurities or additives. Furthermore, the use of 3,4-methylenedioxymethamphetamine (MDMA) and its analogues has been recently increasing. Therefore, a method for the simultaneous chiral analysis of ATS is needed.

This review describes chiral capillary electrophoresis (CE) separation methods for common real forensic samples (seized drugs and urine samples) for ATS analysis (Fig. 1).

CHIRAL SEPARATION BY CAPILLARY ELECTROPHORESIS

CE is a fast, effective, powerful, cost-effective, and high-resolution separation method. Chiral separation can be easily accomplished by adding a chiral selector, usually cyclodextrin (CD) or one of its derivatives, to the background electrolyte. The chiral separation mechanism in CE was described in our previous review.[5)]

The most widely used detection method of CE is UV absorption (UV detector or diode-array detector). Analytes are detected in the capillary, so it is possible to detect them without a decrease in the resolution. However, the sensitivity and selectivity of UV detection is not sufficient in some cases, especially in biological samples, because of small amounts of analytes and interference of matrix.

Recently, CE/mass spectrometry (CE/MS) has been used in various fields, because of the high-selectivity and sensitivity of the MS detector. The ana-

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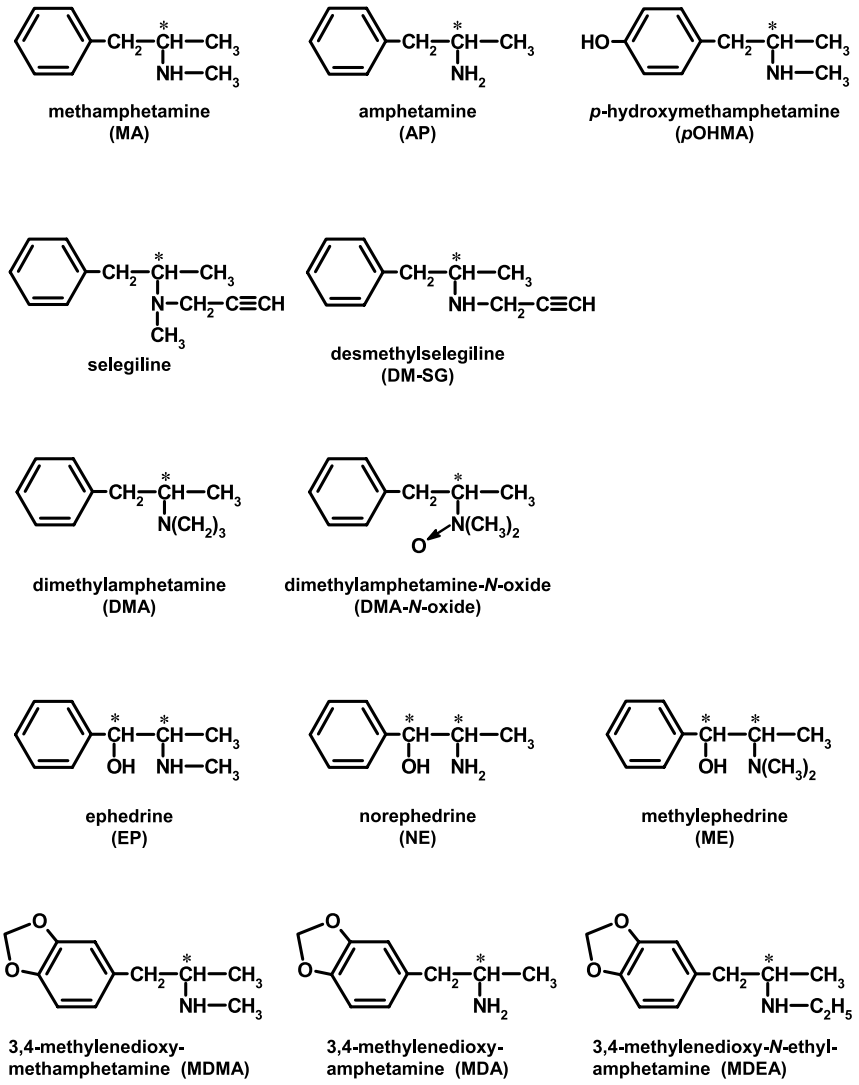

Fig. 1. Chemical Structures and Abbreviations of ATS Described in this Review

lytical conditions of CE/MS were different from those of CE with UV detection (CE/UV): the electrolyte of CE/MS consisted of volatile acid and/or volatile salt, and a sheath liquid was required for electrospray ionization. In chiral analysis, CD, which is nonvolatile, was prevented from flowing into the ion source of the MS. The techniques are also described in the following chapter.

ANALYSIS OF AMPHETAMINE-TYPE STIMULANTS

Drug Samples

A number of chiral analysis methods for ATS using CE/UV have been reported.[6,7] However, few reports have focused on DMA, even though the analysis of DMA is required in Japanese drug abuse situations.

The authors' group developed a CE method for the simultaneous chiral analysis of 9 ATS (18 enantiomers), MA, amphetamine (AP), DMA, EP, norephedrine (NE), methylephedrine (ME), MDMA, 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA).[8] The electrolyte was Tris buffer (pH 2.5) containing β-CD and heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD). This method successfully separated all 18 enantiomers simultaneously (Fig. 2). The detection limits of all enantiomers were 0.1 μg/ml (referring to concentration of the injected sample).

A CE/MS method for 7 ATS (MA, AP, DMA, EP, NE, ME and pseudoEP) was developed by Iio *et al.*[9] The electrolyte was 1 M formic acid containing

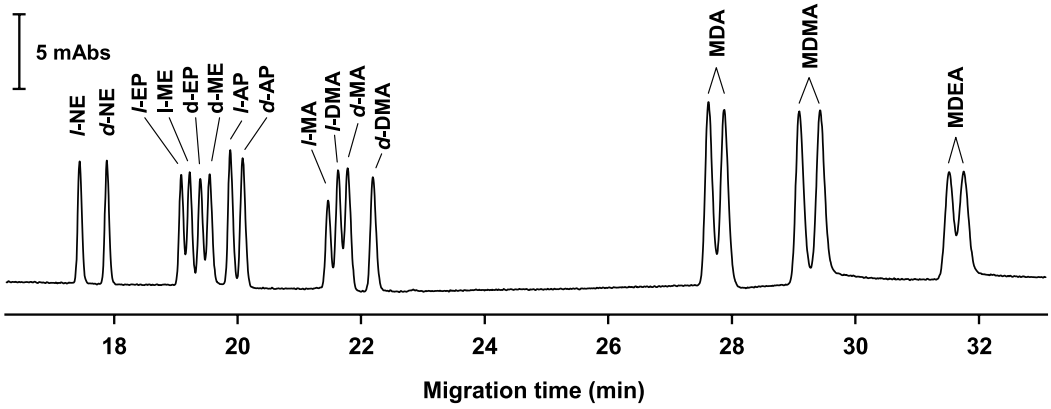

Fig. 2. Electropherograms of 18 Enantiomers by CE/UV
Enantiomers are 5 μg/ml each except for *l*-MA (4 μg/ml). Redrawn from [8].

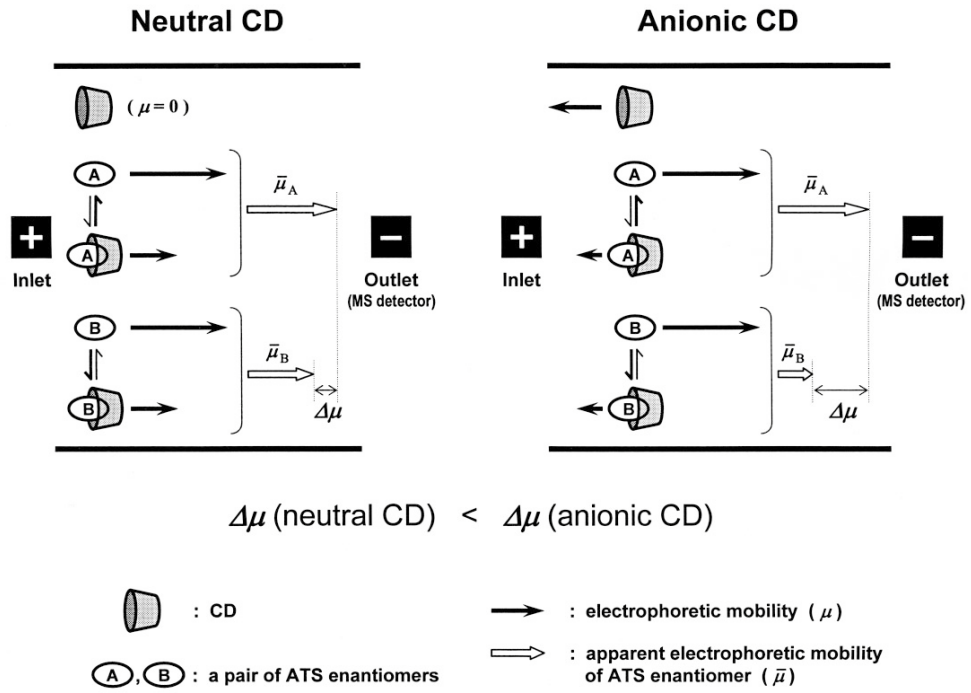

Fig. 3. Chiral Separation Model in CE/MS using Neutral CD (Left) and Anionic CD (Right)

heptakis(2,6-diacethyl-6-sulfato)-β-CD (DAS-β-CD). DAS-β-CD is anionic so it has negative mobility and does not flow into the MS detector. In addition, the fact that the direction of mobility of DAS-β-CD is opposite to that of ATS means that DAS-β-CD can give a higher chiral resolution than do neutral CDs (Fig. 3).[10] The detection limits of the enantiomers were 0.01–0.02 μg/ml using selected-ion monitoring (SIM).

Iwata *et al*.[11] reported a CE/MS/MS method for 9 ATS (MA, AP, EP, pseudoEP, NE, pseudoNE, MDMA, MDA and MDEA) using anionic CD, sulfated (XIII)-γ-CD. This method was used to analyze seized *d*-MA samples, and successfully identified the impurities (*l*-EP and *d*-pseudoEP).

Urine Samples (with Extraction)

In CE analysis, when an ion-rich sample, such as urine extract, is used, the migration times of the peaks frequently shift. For example, when urine containing only *d*-enantiomers is analyzed and a shift arises, all enantiomers might be misidentified as *l*-enantiomers, because the migration times of *d*- and *l*-enantiomers of each compound are close. Therefore, to carry out a reliable chiral analysis of actual urine samples, it is essential to correct for shifts in

the migration time.

The authors reported a CE/UV method that corrects the peak shift and that is applicable to urine analysis.[12] The CE/UV conditions were similar to those described in the previous report.[8] The migration times of shifted peaks were corrected by using two internal standards, 2-phenylethylamine (PEA) and 1-amino-4-phenylbutane (APB). PEA appeared just before the first target peak, and APB appeared just after the last target peak. The amount of analyte was also quantified by the internal standard method. Urine was pretreated by liquid-liquid extraction. In this report we also describe the simultaneous chiral separation of the metabolites of ATS, *p*-hydroxymethamphetamine (*p*OHMA), *p*-hydroxyamphetamine and *p*-hydroxynorephedrine. This method was applied to the analysis of urine samples of MA addicts and patients under selegiline pharmacotherapy.

A chiral CE/MS method using two neutral CDs, β-CD and DM-β-CD, was reported by Iio *et al.*[13] Because neutral CDs have no electro-mobility, suppression of electro-osmotic flow (EOF) by adjusting the pH value of the electrolyte to less than 3 could prevent the CDs from flowing into the ion source of the MS. Therefore, the authors examined volatile electrolyte compositions with pH values under 3 and found that the optimum CE/MS electrolyte was 1 M formic acid (pH 1.7) containing 3 mM β-CD and 10 mM DM-β-CD. This electrolyte gave the best separation of all enantiomers of MA, AP, *p*OHMA and DMA. Even after overnight operation of CE/MS using this electrolyte, little of the electrospray efficiency was lost. The detection limits were 0.03 μg/ml for the enantiomers of MA and AP and 0.05 μg/ml for the enantiomers of *p*OHMA using selected ion monitoring. In the analysis of healthy adult urine samples spiked with MA, AP and *p*OHMA, the detection yields utilizing solid phase extraction were 95–105%. This method was applicable to the analysis of urine samples of MA addicts and DMA addicts.

Urine Samples (with Direct Injection)

In CE, analytes are usually separated in an open tubular capillary without a stationary phase. Therefore, a sample easily pretreated can be analyzed. The above CE/UV method[12] is simple as a system, but requires that the analytes be extracted from the urine sample. Therefore the procedure is not necessarily rapid compared with other methods such as GC or HPLC. To establish a simple and rapid analysis method for urine samples, the authors developed a CE/UV method with direct injection of urine.[14] In this method, to separate urine matrix peaks and to make it possible to run continuous analyses, two different electrolytes were used, one for filling the capillary and the other for filling the inlet and outlet

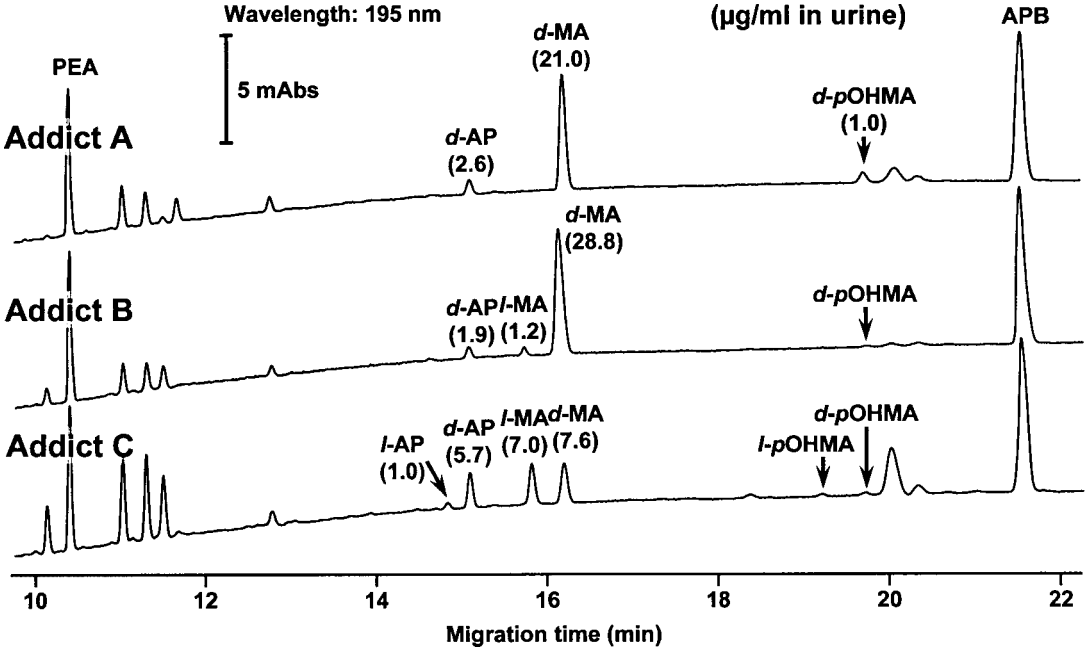

Fig. 4. Electropherograms of MA Addicts' Urine Samples by CE/UV with Direct Injection
Values in parentheses indicate concentrations (μg/ml) of ATS in urine. Redrawn from [14].

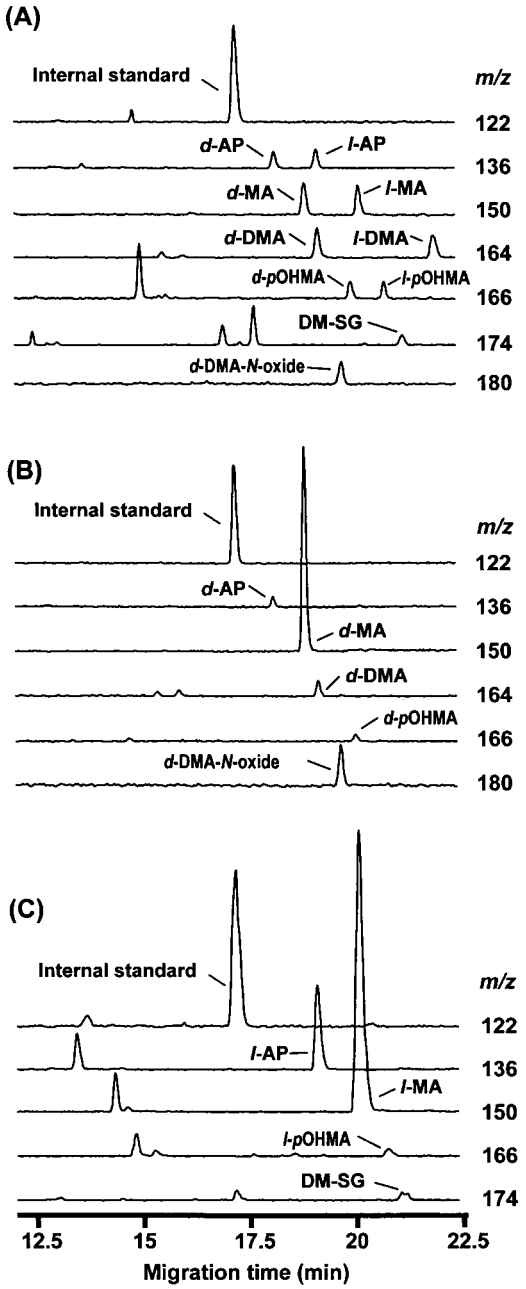

Fig. 5. Mass (Scan) Pherograms of (A) a Control Urine Sample Spiked with Racemic MA, AP, DMA, *p*OHMA, and DM-SG, *d*-DMA-*N*-Oxide (Each 0.5 μg/ml, which Corresponds to 1 μg/ml Urine), (B) a Typical Urine Sample from an Addict who Used a Mixture of *d*-MA and *d*-DMA, (C) a Typical Urine Sample from a Patient under Selegiline Pharmacotherapy
Redrawn from [15].

bottles, and a polyvinyl alcohol-coated capillary was used. The target analytes were MA, AP, DMA, *p*OHMA. Because selegiline and benzphetamine are metabolized to MA, we also analyzed desmethylselegiline, a major metabolite of selegiline and *p*-hydroxydesmethyl benzphetamine, a major metabolite of benzphetamine to distinguish MA use from selegiline or benzphetamine use. A urine sample was mixed with a four-fold volume of internal standards solution, filtered with 0.45 μm filter and then injected into the CE system. In the analyses of healthy persons' urine samples spiked with MA, AP and *p*OHMA, the detection limits in urine were 0.5, 0.5, and 0.3 μg/ml, respectively. The detection yields of each compound from urine were 95–105%. The proposed method was successfully applied to the chiral analysis of urine samples from MA addicts (Fig. 4).

A CE/MS method with direct injection of urine was reported by Iio *et al.*[15] The target compounds were MA, AP, DMA, EP, NE, ME, *p*OHMA, *d*-pseudoEP, desmethylselegiline and *d*-DMA-*N*-oxide (major metabolite of *d*-DMA), and the internal standard was *l*-1-phenylethylamine (*l*-1-PEA). The electrolyte was 1 M formic acid/1 M formic ammonium (10/0.2, v/v) (pH 2.0) containing 1.5 mM DAS-β-CD. The target compounds were completely separated within 30 min. A urine sample was mixed with the same volume of internal standard solution, filtered with 0.45 μm filter and then injected into the CE/MS system. The detection limits using SIM were 0.02 μg/ml urine. The detection yields of each enantiomer of MA, AP and *p*OHMA from urine were in the range of 97.7–108.8%. The proposed method was successfully used for the chiral analysis of urine samples from MA addicts (Fig. 5).

CONCLUDING REMARKS

Many ATS enantiomers can be chiral analyzed simultaneously with CE. The separation can be easily accomplished by adding a chiral selector to the background electrolyte. Various CDs, which have different chiral separation properties, are available for use as chiral selectors. This makes CE an effective tool for chiral analysis. Recently, the abuse of new drugs, including ATS, has been increasing. Therefore, continuous development is needed for simultaneous chiral analysis of ATS including the new compounds. We expect that CE will keep playing an important role in the future.

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