Chiral Separation of Methamphetamine and Related Compounds using Capillary Electrophoresis with Dynamically Coated Capillaries

Ira S. Lurie*, Joseph S. Bozenko Jr., Li Li, Erin E. Miller, and Stephanie J. Greenfield
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[email: ira.s.lurie -at- usdoj.gov]

ABSTRACT: The chiral differentiation of the dextro- and levo- isomers of methamphetamine and certain precursor and/or byproducts in methamphetamine exhibits is obtained at levels down to 0.2% relative to total methamphetamine. Dynamic coating of the capillary surface is accomplished by rapid flushes of 0.1N sodium hydroxide, water, a buffer containing a polycation coating reagent, and a reagent containing a polyanionic coating reagent plus hydroxypropyl-β-cyclodextrin. The methodology has been successfully applied to samples which contain skewed ratios of d- and l-methamphetamine even at trace levels.

KEYWORDS: methamphetamine, chiral analysis, capillary electrophoresis, dynamically coated capillaries, forensic chemistry

The determination of the enantiomers of methamphetamine, its precursors, and/or by-products is important for legal and intelligence purposes [1]. Under federal sentencing guidelines, sentencing enhancement depends on whether the sample contains dextro-methamphetamine hydrochloride over 80% (ice) [2]. Isomer determination can help identify synthetic methodologies. For example, the presence of dextro-pseudoephedrine and dextro-methamphetamine could indicate the methamphetamine was produced from the reduction of pseudoephedrine.

Gas chromatography (GC) [3-5], High Performance Liquid Chromatography (HPLC) [6,7], and Capillary Electrophoresis (CE) [8-10] have all been used to determine enantiomers of phenethylamines in methamphetamine exhibits. There are some limitations for GC and HPLC for the simultaneous analysis of the above solutes. Derivatizations with chiral reagents are often required. Enantiomerically impure reagents often mask detection of low level isomers in a skewed-ratio sample. Over the past few years, the occurrence of non-racemic mixed enantiomer methamphetamine samples has been identified by the DEA laboratory system. Although chiral GC and HPLC columns are available, derivatization has been required for capillary GC. In addition, HPLC columns (chiral and achiral) typically have relatively low plate counts, which can result in poor resolution and/or long analysis times. CE can be performed without prior derivatization by employing chiral additives in the run buffer. Neutral [8] and charged cyclodextrins [9] and mixtures of these reagents [9] have been used. Dynamically coating the capillary, which gives rise to a relatively high and robust electroosmotic flow at lower pH values compared to uncoated capillaries [10-13], is well suited for chiral analysis of basic solutes. Although a dual dynamic coating procedure allowed baseline resolution of the dextro- and levo- isomers of amphetamine, methamphetamine, and pseudoephedrine, the enantiomeric separation of ephedrine and the resolution of the individual enantiomers from each other was lacking [10].

An improved dual dynamic coating procedure in terms of overall separation as well as sensitivity is presented for the analysis of methamphetamine exhibits.

Experimental

Chemicals, Material, and Reagents

Standards were obtained from the reference collection of this laboratory. Sodium hydroxide 0.1N, CElixir A (pH 2.5), CElixir B (pH 2.5), CElixir B (pH 2.5) with hydroxypropyl-β-cyclodextrin (HPBCD)[1] (Custom Chiral2 Buffer), and injection solvent concentrate (75 mM sodium phosphate, pH 2.5) were all acquired from MicroSolv Technology (Long Branch, NJ). Deionized and high purity water (HPLC-grade water) were obtained from a Millipore Synergy 185 water system (Bedford, MA).

Instrumentation and Procedures

An Agilent Model HP^{3D} CE Capillary Electrophoresis System fitted with a diode array detector (Waldbronn, Germany) was used for CE separations. New, bare silica capillaries were conditioned following the same procedure used for regular analysis. Capillaries were first flushed with 0.1 N sodium hydroxide for 1 minute, followed by water for 1 minute, then CElixir Reagent A for 1 minute, and finally the run buffer for 2 minutes. 1.0 mL polypropylene vials were used as reservoirs for 0.1N sodium hydroxide solution and for the run buffer; while 2.0 mL glass vials were used as reservoirs for the remaining flush solutions, waste vials, and samples. 0.1N sodium hydroxide and run buffer vials were fill with 500 µL of liquid, while samples and flush vials containing CElixir Reagent A and water, respectively, were filled with 1000 µL of liquid. Waste vials were filled with 500 µL of water.

Standard and Sample Preparation

An injection solvent concentrate was diluted with 1:20 HPLC-grade water. For standard solutions, an appropriate amount of standard dextro and levo isomers of methamphetamine HCl, amphetamine sulfate, ephedrine HCl,

[1]Since different lots of a cyclodextrin can vary in both the degree of substitution and the position of substituents, each time a new batch of HPBCD is received, a test mixture is analyzed; and, if necessary, a small change is made in the concentration of the HPBCD (original concentration 78 mM).

and pseudoephedrine HCl were weighed into an appropriate volumetric flask and diluted with injection solvent (after the addition by pipetting of internal standard) in order to obtain a final concentration of approximately 0.10 mg/mL of each isomer of methamphetamine, 0.008 mg/mL of the other target isomers, and 0.10 mg/mL of a racemic mixture of the internal standard (n-butylamphetamine). For sample solutions, an appropriate amount of weighed material was added into an appropriate volumetric flask and diluted with injection solvent (after the addition by pipetting of the internal standard) in order to obtain a final achiral methamphetamine concentration of approximately 0.20 mg/mL and an internal standard concentration of 0.10 mg/mL.

Capillary Electrophoresis Conditions

Either a 50mm ID 64.5 cm (56.0 cm to the detector) fused silica capillary obtained from Polymicro Technologies (Phoenix, AZ) or a pre-made capillary (Agilent) with the same dimensions was used at 15°C. The run buffer consisted of CElixer B (pH 2.5) with or with out HPBCD. For all CE runs, a 50 mbar pressure injection of 16 second duration was used, followed by a 35 mbar pressure injection of water for 1 second. For electrophoresis, an initial 0.5 minute linear voltage ramp from 0 V to the final voltage of either 20 kV (run buffer B reagent) or 30 kV (run buffer B reagent + HPBCD) was used.

Results and Discussion

An improved separation over previously reported methodology [10] for the dextro- and levo- isomers of methamphetamine, amphetamine, ephedrine, and pseudoephedrine was obtained by a combination of approximately doubling the length of capillary and increasing the concentration of HPBCD. As shown in Figure 1, the individual enantiomers of these solutes, as well as the enantiomers of a structurally related internal standard (n-butylamphetamine), are well resolved in less than 17 minutes.

Highly precise run-to-run separations were obtainable as demonstrated by migration time, relative migration time (relative to the 2nd internal standard peak), corrected area (area/migration time), and relative corrected area precision (relative to the 2nd internal standard peak) (%RSD ≤ 0.13, ≤ 0.05, ≤ 2.0, and ≤ 0.92, respectively, n = 5). Because of the narrow peaks and the possibility of larger shifts in migration time, identification can be difficult based on migration time alone. Therefore, the use of relative migration times or co-injection is suggested for compound identification. Day-to-day and capillary-to-capillary reproducibility is also greater using relative migration times versus absolute migration times. Relative migration time data (relative to the 2nd internal standard peak) of solutes found in methamphetamine exhibits is given in Table 1. In addition, the combination of a relatively large sample concentration and injection preceded by the stacking effect of the water plug on the large methamphetamine peak(s), allows for the determination of individual enantiomers at levels down to 0.2% relative to total methamphetamine. In comparison to previously reported dynamically coated methodology [10], this represents an approximately 4 fold improvement in detection limits.

Since implemented for routine use for intelligence analysis, thousands of samples have been successfully analyzed. An electropherogram of a sample containing *d*-methamphetamine and *d*-pseudoephedrine is shown in Figure 2.

With the current trend of enantiomeric enrichment of methamphetamine isomers [14], chiral capillary electrophoresis enables the chemist to identify even the most subtle enrichment. Trace and non-trace determination of minor isomers, both dextro and levo, is essential in determining the route of synthesis and/or post-processing techniques employed by clandestine laboratory chemists. Electropherograms of three methamphetamine exhibits seized in the same case are shown in Figure 3. The exhibits, based on their skewed ratios of *l*- and *d*-methamphetamine (see Figures 1 and 3B-D), appear to be processed using a trace enrichment procedure. However, the non-racemic ratios do not eliminate the possible use of mixed precursor material or the post-production mixing of different batches.

A question can arise whether the minor peaks in Figure 3 are *d*- or *l*-methamphetamine. The identity of the peaks is supported by the achiral profile of one of the exhibits, (similar profile for other two exhibits) which indicates that the sample only contains one peak other than the internal standard (see Figure 3A).

A significant number of submitted samples to this

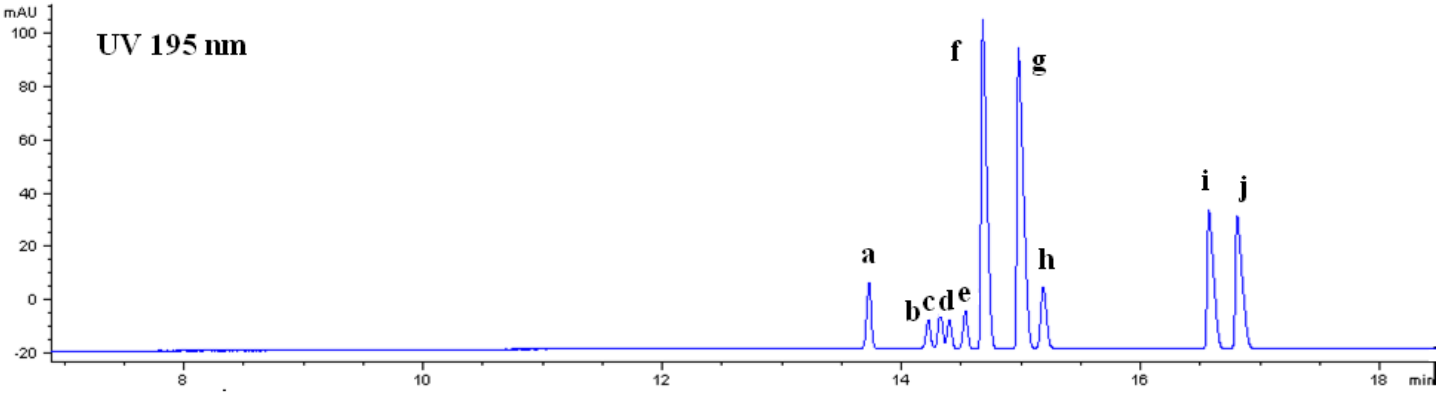

Figure 1 - Dynamically coated CE separation of standard mixture of (a) *l*-pseudoephedrine, (b) *d*-ephedrine, (c) *l*-amphetamine, (d) *l*-ephedrine, (e) *d*-amphetamine, (f) *l*-methamphetamine, (g) *d*-methamphetamine, (h) *d*-pseudoephedrine, (i) n-butylamphetamine (1), and (j) n-butylamphetamine (2). CE conditions are described in the experimental section.

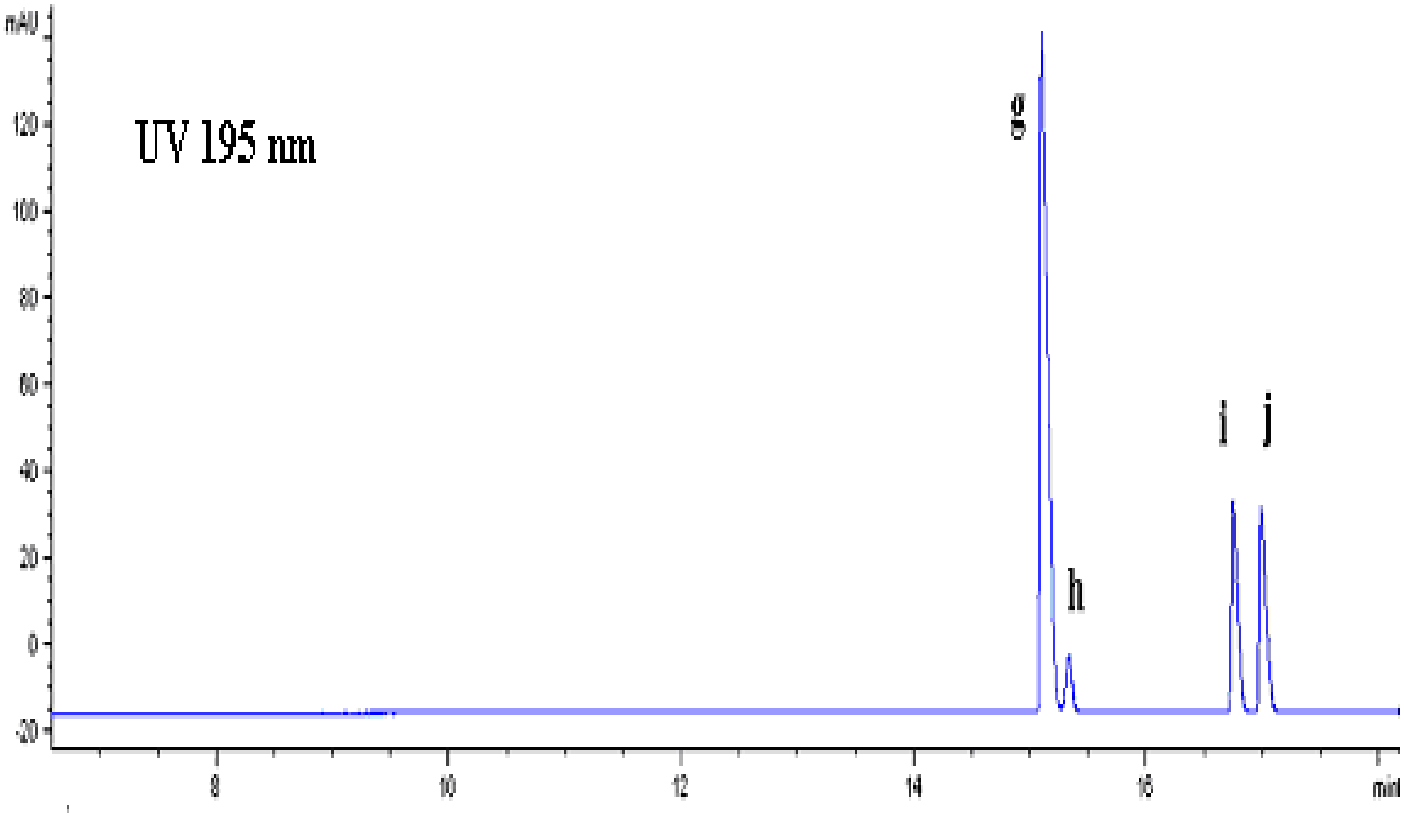

Figure 2 - Dynamically coated CE separation of a methamphetamine exhibit containing (g) d-meth-amphetamine, (h) d-pseudoephedrine, (i) n-butyl-amphetamine (1), and (j) n-butylamphetamine (2). CE conditions are described in the experimental section.

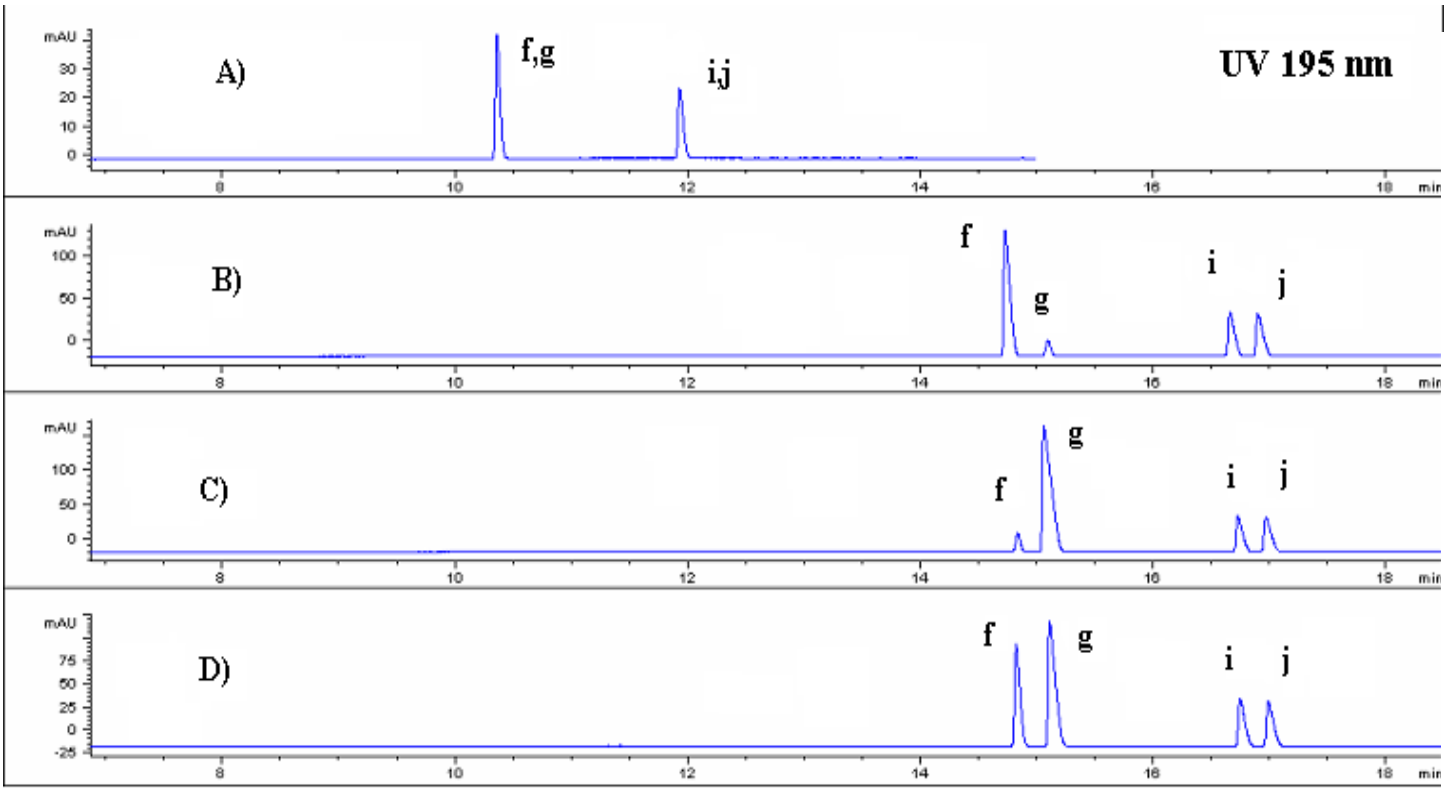

Figure 3 - Dynamically coated CE separations of methamphetamine exhibits containing (f) *l*-methamphetamine, (g) *d*-methamphetamine, (i) n-butylamphetamine (1), and (j) n-butylamphetamine (2).

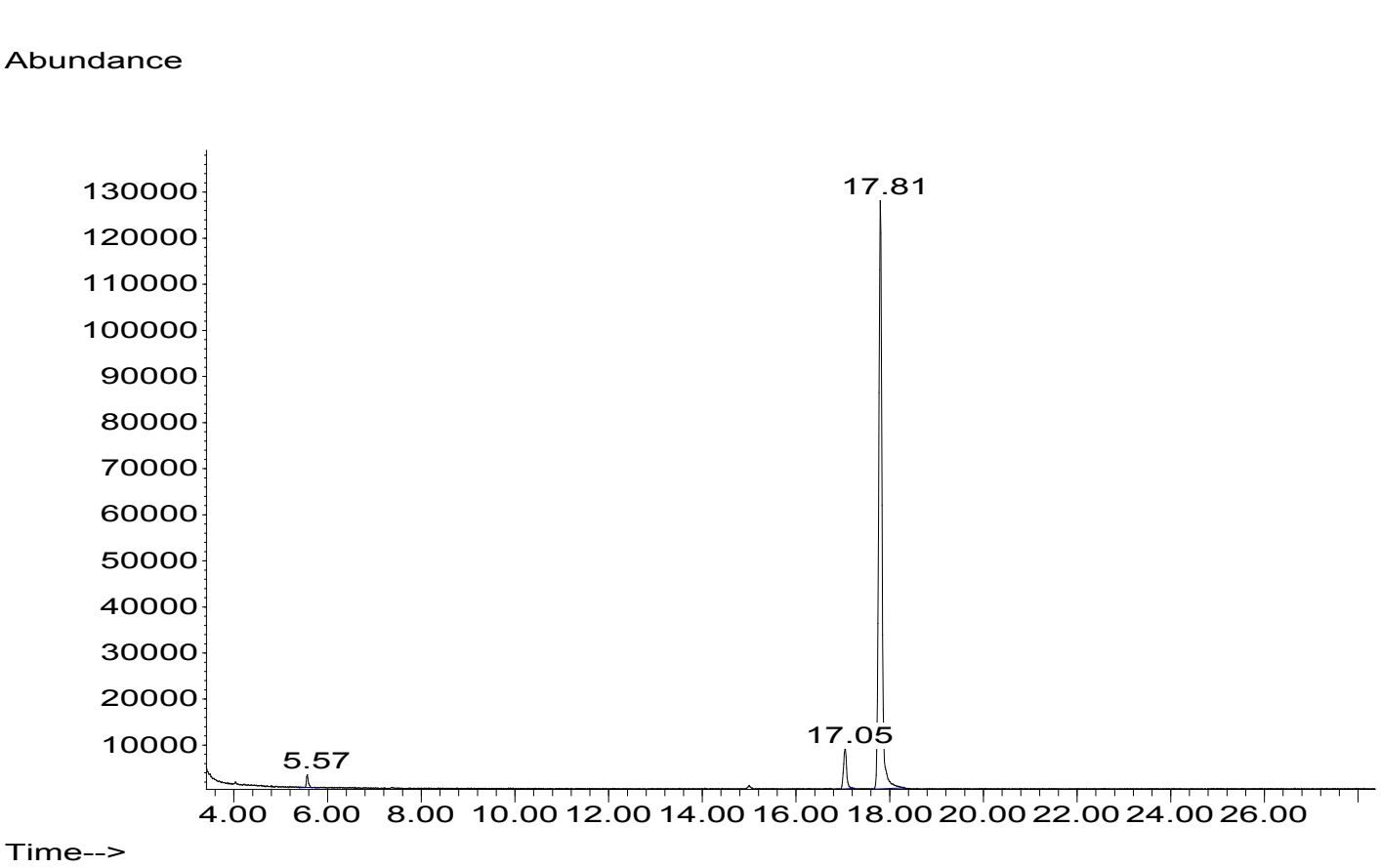

Figure 4 - GC TPC separation of d-methamphetamine. Capillary 30 m x 0.25um x 0.25mm DB-17, temperature programming

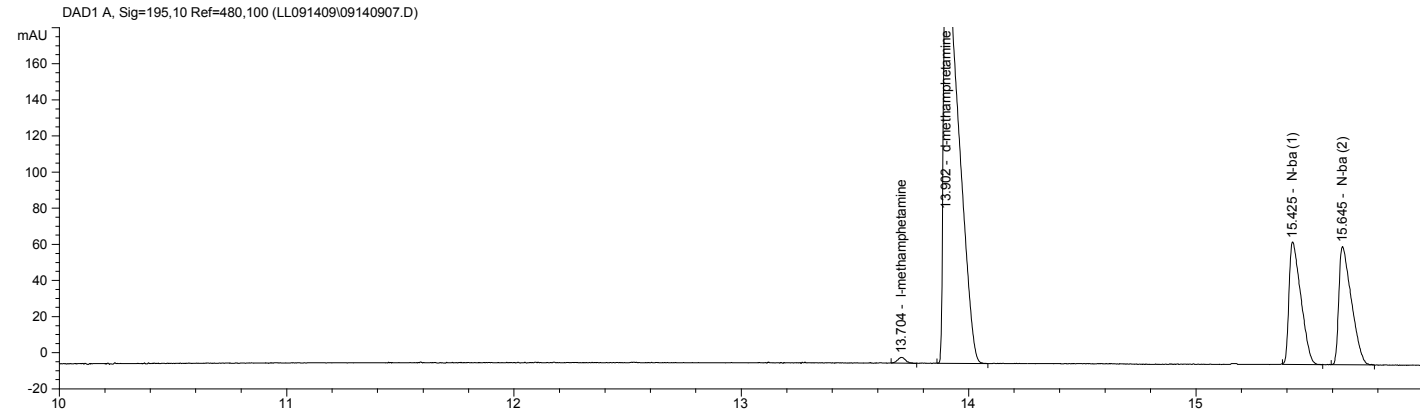

Figure 5 - Dynamically coated CE separation of methamphetamine sample containing *l*-methamphetamine, *d*-methamphetamine, n-butylamphetamine (1), and n-butylamphetamine (2). CE conditions are described in the Experimental section.

laboratory (both domestic and foreign) have contained skewed ratios of *d*- and *l*-methamphetamine contrary to the normal single enantiomer and racemate historically detected. A substantive number of these exhibits contain near-trace or trace amounts of an isomer. This instance poses analytical difficulty using traditional chemical derivatization agents such as (S)-(-)-N-(Trifluoroacetyl)prolylchloride (TPC or TFAP) which contain impurities, mainly the other enantiomer such as the (R)-prolyl, that effectively mask detection of the minor isomer in a skewed-ratio sample. In addition to the impurity's presence, the enantiomeric excess has been observed to degrade over time, thus diminishing the already hindered discrimination power. A number of re-analyses have shown that the TPC method failed to identify near-trace level isomers when compared to CE analysis. Capillary electrophoresis has no such "masking" problem and easily separates the enantiomers. A chromatogram depicting the analysis of standard *d*-methamphetamine using the TPC method (see Figure 4) indicates the presence of *l*-methamphetamine as an artifact at approximately the 8% level. In contrast using CE no *l*-methamphetamine is detected as an artifact (see Figure 2). An analysis not possible using the GC TPC method, i.e, the detection of the *l* isomer at the approximately 1% level relative to the *d* isomer of methamphetamine is shown in Figure 5.

Table 1 - Relative migration times (relative to the 2nd internal standard peak (n-butylamphetamine)) of solutes related to methamphetamine, CE conditions are described in the experimental section.

Solute	RM_t
nicotinimide	0.587
tripelennamine	0.607
quinidine	0.649
quinidine impurity	0.671
tripolidine	0.683
phenethylamine	0.729
chlorpheniramine (*l* or *d*)	0.733
chlorpheniramine (*l* or *d*)	0.743
carbinoxamine (*l* or *d*)	0.783
carbinoxamine (*l* or *d*)	0.791
l-pseudoephedrine	0.824
brompheniramine (*l* or *d*)	0.831
brompheniramine (*l* or *d*)	0.841
desloratadine (*l* or *d*)	0.848
d-ephedrine	0.852
l-amphetamine	0.857
xylazine (*l* and *d*)	0.857
d-dimethylephedrine	0.860
l-ephedrine	0.862
desloratadine (*l* or *d*)	0.866
ketamine (*l* or *d*)	0.867
d-amphetamine	0.868
l-phenylephrine	0.869
ketamine (*l* or *d*)	0.874
l-methamphetamine	0.879
papaverine	0.883
d-methamphetamine	0.897
d-dimethylamphetamine	0.902
d-pseudoephedrine	0.907
d-dimethylpseudoephedrine	0.909
Birch impurity	0.948
n-butylamphetamine (*l* or *d*)	0.987
n-butylamphetamine (*l* or *d*)	1.000
MDA (*l* or *d*)	1.019
MDA (*l* or *d*)	1.030
pyrilamine	1.031
MDMA (*l* or *d*)	1.037
MDMA (*l* or *d*)	1.051
MDEA (*l* or *d*)	1.074
MDEA (*l* or *d*)	1.085
reductive animation impurity	1.092
l-hycosamine	1.101
l-cocaine	1.104
hydroxyzine (*l* and *d*)	1.157
amitriptyline	1.160
doxepine (*l* or *d*)	1.162
doxepine (*l* or *d*)	1.169
loratadine	1.180

References

1. Perillo BA, Klein RFX, Franzosa ES. Recent advances by the US Drug Enforcement Administration in drug signature and comparative analysis. Forensic Sci Int 1994;69:1-6.
2. Federal Sentencing Guidelines 2002.
3. Liu JH, Ku WW, Tsay JT, Fitzgerald MP, Kim S. Approaches to drug sample differentiation. 111: A comparative study of the use of chiral and achiral capillary column gas chromatography/mass spectrometry for the determination of methamphetamine enantiomers and possible impurities. J Forensic Sci 1982;27:39-48.
4. McKibben TD. Separation and identification of drug enantiomers via N TFA (S) Prolyl Chloride Derivatization. J Clandest Lab Investig Chemists Assoc 1992;2:13-20.
5. Shin HS, Donike M. Stereospecific derivatization of amphetamines, phenol alkylamines, and hydroxyamines and quantification of the enantiomers by capillary GC/MS. Anal Chem 1996;68:3015-3020.
6. Noggle FT, DeRuiter J, Clark CR. Liquid chromatographic determination of the enantiomeric composition of methamphetamine prepared from ephedrine and pseudoephedrine. Anal Chem 1986;58:1643-1648.
7. Rizzi AM, Hirz R, Cladowa-Runge S, Jonsson H. Enantiomeric Separation of amphetamine, methamphetamine and ring substituted amphetamines by means of a B-cyclodextrin chiral stationary phase. Chromatographia 1994;39:131-137.
8. Varesio E, Gauvrit JY, Longeray R, Lanteri, P, Veuthey, JL. Central composite design in the chiral analysis of amphetamines by capillary electrophoresis. Electrophoresis 1997;18:931-937.
9. Iwata YT, Garcia A, Kanamori T, Inoue H, Kishi T, Lurie IS. The use of highly sulfated cyclodextrin for the simultaneous separation of amphetamine type stimulants by capillary electrophoresis. Electrophoresis 2002;23:1328-1334.
10. Lurie IS, Hays PA, Parker KP. Capillary electrophoresis analysis of a wide variety of seized drugs using the same capillary with dynamic coatings. Electrophoresis 2004;25:1580-1591.
11. Chevigne R, Janssens J. US Patent #5,611,903, 3/18/97.
12. Lurie IS, Bethea MJ, McKibben TD, Hays PA, Pellegrini P, Sahai R, Garcia AD, Weinberger R. Use of dynamically coated capillaries for the routine analysis of methamphetamine, amphetamine, MDA, MDMA, MDEA and cocaine using capillary electrophoresis. J Forensic Sci 2001;46:1025-1032.
13. Lurie IS, Cox KA. Rapid chiral separation of dextro- and levo-methorphan using capillary electrophoresis with dynamically coated capillaries. Microgram J 2005;3:138-141.
14. Bozenko JS Jr. Clandestine enantiomeric enrichment of *d*-methamphetamine via tartaric acid resolution. J Clandest Lab Investig Chemists Assoc 2008;18:2-6.