Enantiomeric Determination of Methamphetamine, Amphetamine, Ephedrine, and Pseudoephedrine using Chiral Supercritical Fluid Chromatography with Mass Spectrometric Detection

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ABSTRACT: Supercritical fluid chromatography-single quadrupole mass spectrometry (SFC-SQD) was utilized for the enantiomeric determination of methamphetamine, amphetamine, ephedrine, and pseudo-ephedrine. The effects of varying stationary phases, alcohol modifiers, and amine additives were assessed. The optimal separation for methamphetamine was achieved by a Trefoil AMY1 (150 x 2.1 mm, 2.5 µm) column using a supercritical CO_2 mobile phase containing EtOH as the co-solvent and 1% cyclohexylamine as the amine additive. The method was successfully applied for determination of the chiral composition of illicit methamphetamine, even for samples with skewed ratios of enantiomers as low as 0.1% d- or 3% l-.

Introduction

The d- and l- enantiomers of methamphetamine have dramatically different pharmacological activity levels (1,2). Despite these differences, both enantiomers are Schedule II in the U.S., due to their widespread abuse[1]. In addition, samples containing 80% or more d-methamphetamine HCl are subject to higher sentences [3]. Therefore, the enantiomeric composition of methamphetamine is important from legal and medical perspectives. In addition, the chirality of methamphetamine can help indicate the possible synthetic route and precursor used in its synthesis. d-Methamphet-amine is most commonly produced either from l-ephedrine or d-pseudoephedrine, while racemic d,l-methamphetamine is most commonly produced from 1-phenyl-2-propanone (P2P; also known as phenylacetone and by several other, more obscure names and acronyms). Methamphetamine with a skewed ratio of d- and l- is usually synthesized from P2P with subsequent, incomplete resolution using d-tartaric acid or a similar agent [4]. Thus, the enantiomeric composition of methamphet-amine is also important for forensic and intelligence derivation purposes.

Since the enactment of the Combat Methamphet-amine Epidemic Act of 2005 [5], which implemented purchasing limits on l-ephedrine and d-pseudoephedrine, the chiral composition of methamphetamine seized in the U.S. has shifted

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[1] Pharmaceuticals containing low concentrations of l-methamphetamine, which are used as bronchodilators, are not controlled.

dramatically, from the d- enantiomer and the d,l-racemate to samples with skewed ratios of the d- and l- isomers. This shift confirms the current use of P2P and resolution efforts by illicit processors.

Chromatographic enantioseparations remain the most popular techniques for chiral analyses, and can be accomplished using HPLC, GC, CE, and supercritical fluid chromatography (SFC). Direct HPLC using a chiral stationary phase (CSP) is the most common technique for the separation of enantiomers [6-17]. However, this method consumes large quantities of HPLC-grade organic solvents (which are expensive), and can display significant peak broadening as well as longer equilibration and analysis times. In addition, multiple pilot columns with different CSPs are normally needed to determine the optimal column and conditions, another time consuming expense.

Indirect HPLC with chiral mobile phase additives has also been utilized for chiral analyses of phenethylamines; however, the results displayed low efficiency in enantioselectivity [18,19]. Similarly, CE with chiral mobile phase additives is effective, efficient, and rapid [20-24]; however, this method suffers from low reproducibility in migration times, and relative migration times versus internal standards are usually required.

Another common approach involves derivatization with a chiral reagent (to form diasteromers) with subsequent analyses with either HPLC or (more commonly) GC [25-34]. Though such analyses have a better enantioselectivity, efficiency, and reproducibility, the derivatization process is time consuming, more expensive, and technique sensitive, and the optical impurity in the chiral reagents often obscures or masks the detection of the low level isomer in a heavily skewed-ratio sample (chiral derivatizing reagents are typically about 98% enantiomerically pure).

SFC employs mobile phases comprised of super-critical CO_2 mixed with organic modifiers (e.g., alcohols) and additives (e.g., acids or bases); this results in higher diffusivity and lower viscosity versus an HPLC mobile phase. As a result, SFC offers high throughput capacity, much lower solvent consumption, and a "greener," less expensive technology. Recently, with improved instrumentation and wider availability of chiral columns with small particle sizes, SFC has become a viable alternative chromatographic technique for enantiomeric analyses [35-44].

The use of SFC on chiral amylose (AMY) or cellulose (CEL) columns for enantomeric determination of methamphetamine, amphet-amine, ephedrine, and pseudoephedrine is presented herein. Chiral analyses of seized methamphetamine samples with skewed enantiomer ratios are also presented. The effects of varying the stationary phases, co-solvents, and additives are discussed.

Experimental

Chemicals
All phenethylamine standards were obtained from this laboratory's reference materials collection. Ammonium hydroxide solution, ammonium acetate, trifluoroacetic acid (TFA), and cyclohexylamine (CHA) were analytical grade and obtained from Sigma (St. Louis, MO). HPLC grade solvents, including isopropyl alcohol (IPA), ethanol (EtOH), methanol (MeOH), and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO). Carbon dioxide (CO_2, beverage grade) was from Air Gas (Chantilly, VA).

Columns
Trefoil AMY1 (tris-(3,5-dimethylphenyl-carbamate) 2.1 x 50 mm, 2.5 µm), Trefoil CEL1 (tris-(3,5-dimethylphenylcarbamate)-cellulose, 2.1 x 50 mm, 2.5 µm), Trefoil CEL2 (tris-(3-chloro-4-

methylphenylcarbamate)-cellulose, 2.1 x 50 mm, 2.5 μm), and Trefoil AMY1 (2.1 x 150 mm, 2.5 μm) columns were provided by the Waters Corporation (Milford, MA).

Instrumentation
All experiments were performed on a Waters Acquity Ultra Performance Convergence Chromatography (UPC^2) System. The system was equipped with a binary solvent manager, an autosampler with a partial loop volume injection system, a 2-position column oven compatible with 150 mm length columns, and was interfaced with a PDA detector and an SQD with an ESI source. A 515 pump system (an isocratic solvent manager) was used as a make-up pump, and was positioned before the mass detector. The main flow stream was split by a flow-splitter assembly before the SQD. Empower 3® software was used for system control and data acquisition.

Screening experiments were performed at 40⁰C with a flow rate of 1.2 mL/min. The system back pressure was set at ABPR = 2000 psi, except as noted. The gradient elution was varied to compensate for different modifiers, as specified for each experiment. The injection volume was 2 μL.

The compounds were detected in positive ESI mode with the following parameters: The ion source temperature was 150⁰C; nitrogen was used as the desolvation gas at a flow rate of 600 L/hr and at 400⁰C; the capillary voltage was 3 kV. The makeup flow was 0.6 mL/min with MeOH. In this study, Single Ion Recording (SIR) was utilized for better sensitivity and selectivity (mass spectral parameters are listed in Table 1).

Standard and Sample Preparation
Stock solutions of each standard were prepared at 1 mg/mL in MeOH. Working solutions of individual standards or their mixtures were prepared by diluting their stock solutions with

IPA. For samples, a 1 mg/mL stock solution in MeOH was prepared. A working sample solution at 200 μg/mL was prepared by diluting its stock solution with IPA.

Table 1. Mass Spectral Parameters.

Compound	[M+H]$^+$	Cone Voltage (kV)
Amphetamine	135.8	20
Methamphetamine	150.2	20
Ephedrine	166.2	15
Pseudoephedrine	166.2	15

Results and Discussion

Screening of Chiral Columns with Different Co-Solvents and Additives
Initial screening of stationary phases was attempted using methamphetamine on 50 mm Trefoil "pilot" columns (CEL1, CEL2, and AMY1). Based on Waters' strategy for Trefoil chiral method development, the following four step process was implemented: 1. AMY1-EtOH/IPA/ACH (33/33/33)-20 mM AmAc (B1); 2. CEL1-MeOH/IPA (50/50)-0.2% TFA (B2); 3. CEL2-EtOH/ACN (50/50)-0.2% TFA (B3); 4. AMY1-EtOH/IPA (50/50)-0.2% TFA (B4). The instrument was equilibrated at 3% B for 0.5 min, then linear gradient to 60% B over 1.5 min, then hold at 60% B for 5 min.

There was no noticeable enantiomeric separation of any of the phenethylamines with any of the screening columns and conditions. Distorted peaks were observed, possibly due to the acidic character of the large percentage of CO_2 in the mobile phase. According to a study by Ye *et al.*, addition of an amine raises the pH of the mobile

phase, thereby deprotonating the analytes and improving their peak shapes and resolution [36]. Thus, three organic co-solvents (MeOH, EtOH, or IPA) doped with NH_4OH or CHA were investigated. Only the AMY1 column using an alcohol and CHA demonstrated any chiral separations (and those were slight). Multiple experiments were conducted (not presented herein); based on the results, a longer AMY1 column (150 x 3.5 mm, 2.1 µm) with various alcohols as a co-solvent and CHA as an additive was selected for method optimization.

Screening of Co-Solvents with an AMY1 Chiral Column and using CHA as an Additive
As amphetamine, ephedrine, and pseudoephedrine can also be present in seized methamphetamine samples, it is useful to resolve all four compounds as well as their enantiomers in a single run, if possible. A standard mixture containing 100 µg/mL of all four phenethylamines was prepared in IPA. The effects of MeOH, EtOH, and IPA were separately assessed, each using CHA as an additive. The results are illustrated in Figures 1, 2, and 3. All four phenethylamines were separated using any of the alcohols; however, none of the alcohols resolved all four pairs of enantiomers in a single run. IPA and EtOH are both adequate co-solvents for enantiomeric separation of amphetamine; indeed, d- and l-amphetamine were well resolved using IPA, with resolution greater than 2. The four ephedrine and pseudoephedrine diastereomers were partially resolved by each of the alcohols, with MeOH giving the highest discrimination. Based on these results, MeOH/EtOH (50/50) and 0.3% CHA was utilized, and baseline resolved all four ephedrine/ pseudoephedrine diastereomers (Figure 4). The amphetamine enantiomers were also baseline resolved; however, the methamphetamine enantiomers were not quite fully resolved, with the best separation being achieved using EtOH as a co-solvent, with a resolution of 0.9.

Effects of CHA Concentration and Optimization of the Chiral Separation for Methamphetamine
In order to fully resolve the separation of d- and l-methamphetamine, the CHA concentration was raised from 0.3% to 0.5% to 1%, using EtOH as the co-solvent. The flow rate was also increased to 2.5 mL/min for higher efficiency. As illustrated in Figure 5, EtOH with 1.0% CHA separated d- and l- methamphetamine with a resolution of 1.2. Under these conditions, the amphetamine enantiomers were also baseline resolved; however, the separations of ephedrine and pseudoephedrine deteriorated at 1% CHA. Higher percentages of CHA were not investigated due to ion suppression effects.

Detection Limits and Linearity of Methamphetamine Analyses
Using the optimized conditions (SIR mode), the detection limit was 0.2 µg/mL and the linearity range was 0.5 - 200 µg/mL. The detection limits for the minor isomer in a methamphetamine sample with a skewed ratio of enantiomers was also studied. Trace d-methamphetamine could be detected at 0.1%; however, trace l-methamphetamine could only be detected at 3%, due to peak tailing and limited resolution (Figure 6). In contrast to these results, the detection levels for both d- and l- methamphetamine are 0.1% using this laboratory's current CE method [37].

Chiral Determination of Methamphetamine in Seized Samples
Six illicit methamphetamine samples were analyzed using EtOH doped with 1% CHA. The chromatograms are illustrated in Figure 7. The results are compared against the analyses performed using our current CE method in Table 2. To summarize, SFC offers faster and more consistent analyses, but is not well suited for detecting trace levels of l-methamphetamine due to its lower efficiency and resolution.

Table 2. Comparison of the chiral analysis of 6 illicit methamphetamine samples.

| Sample	By SFC	By CE
1	d-	0.5% l-
2	d-	0.7% l-
3	d-	0.4% l-
4	l-	l-
5	d-	d-
6	35.4% l-	35.6% l-

Conclusions

Using MeOH/EtOH (50/50) and 0.3% CHA, all four phenethylamines were resolved, and all but the methamphetamine enantiomers were baseline resolved (methamphetamine 0.9). Using EtOH and 1% CHA, the methamphetamine enantiomers were fully resolved (1.2). Both experiments were carried out in less than 7 minutes.

Using SQD in positive ESI and SIR mode, the detection limits were as low as 0.2 µg/mL for methamphetamine. The method also gave a good linearity range for methamphetamine (0.5 to 200 µg/mL). However, the method can only detect l-methamphetamine at 3% or greater. Despite this limitation, the method is an excellent alternative to other GC, HPLC, and CE methods for rapid chiral analyses, with good sensitivity, selectivity, and reliability.
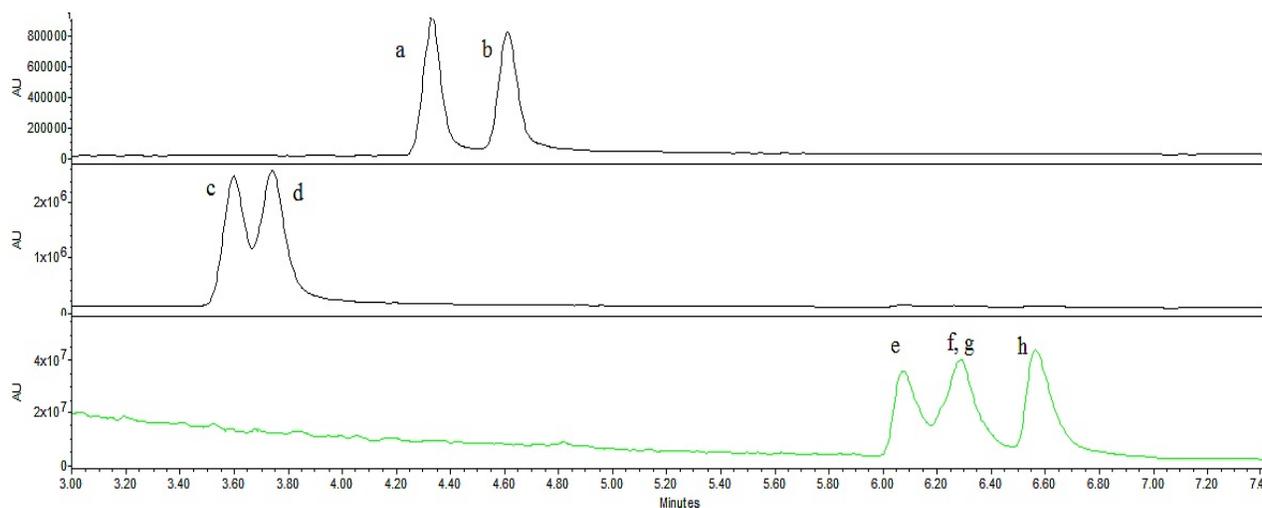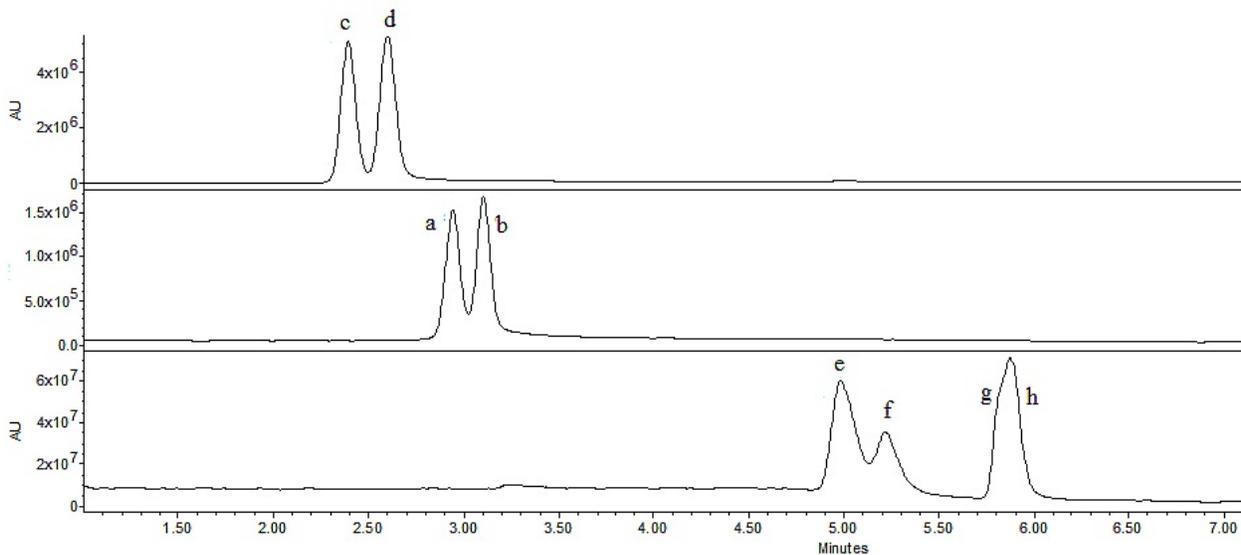
Figure 1. SFC chromatograms of co-solvent screening. Co-solvent: IPA with 0.6% CHA; and gradient: 8% to 15% in 4 min; 15% to 40% at 6 min. Flow rate: 1.5 mL/min. a, d-methamphetamine; b, l-methamphetamine; c, d-amphetamine; d, l-amphetamine; e, d-pseudoephedrine; f, l-pseudoephedrine; g, d-ephedrine; h, l-ephedrine. [Note: Peaks were identified via analyses of individual enantiomers.]
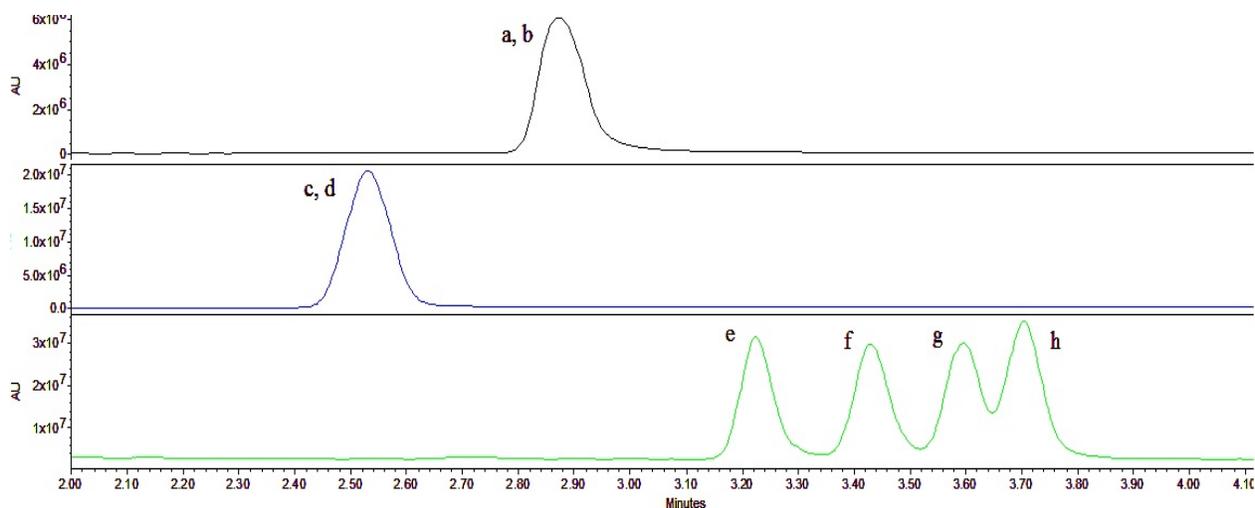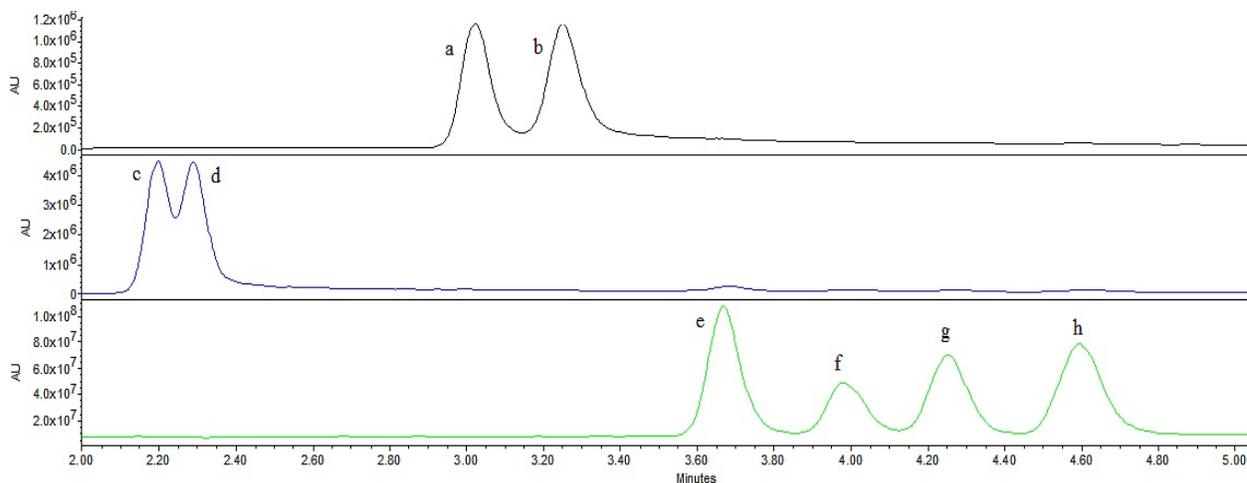
Figure 2. SFC chromatograms of co-solvent screening. Co-solvent: MeOH with 0.3% CHA; and gradient: 8% to 15% in 6 min. Flow rate: 1.2 mL/min.
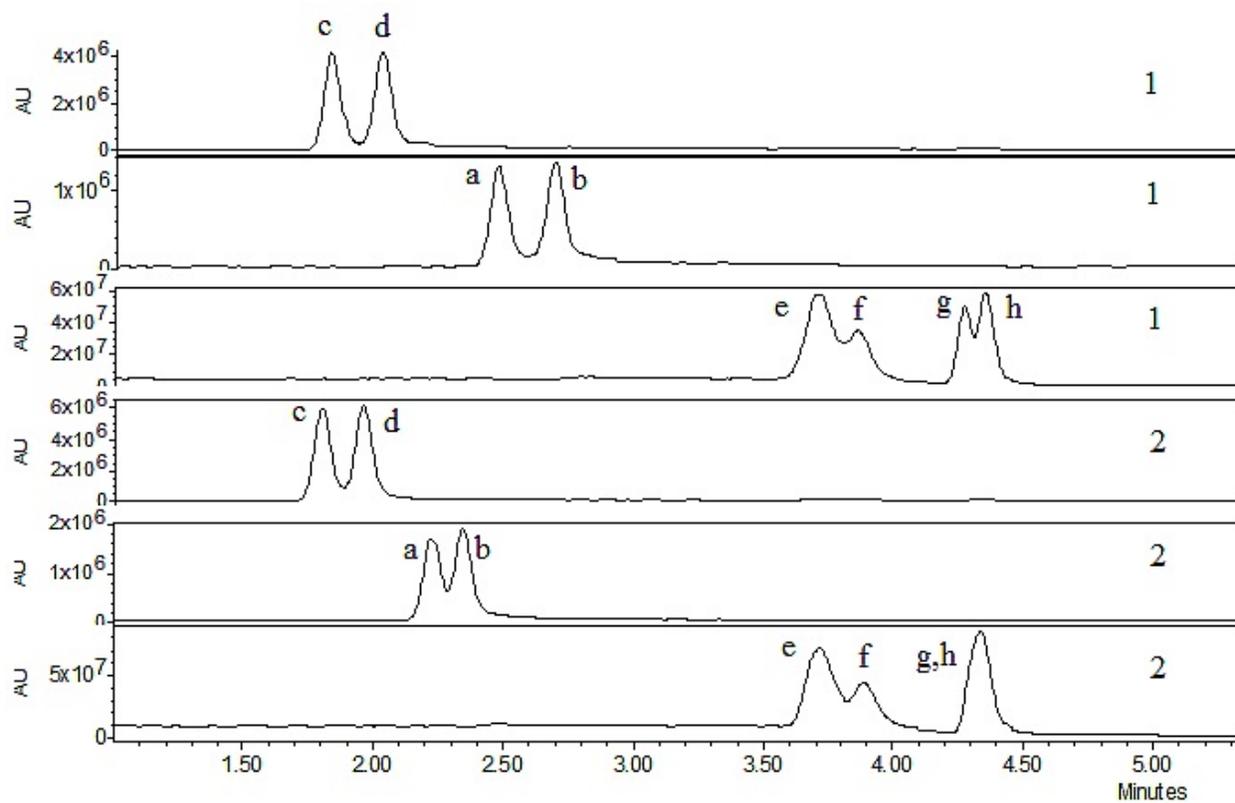

Figure 3. SFC chromatograms of co-solvent screening. Co-solvent: EtOH with 0.5% CHA; and gradient: 8% for 4 min; 8% to 30% at 9 min. Flow rate: 2.0 mL/min.
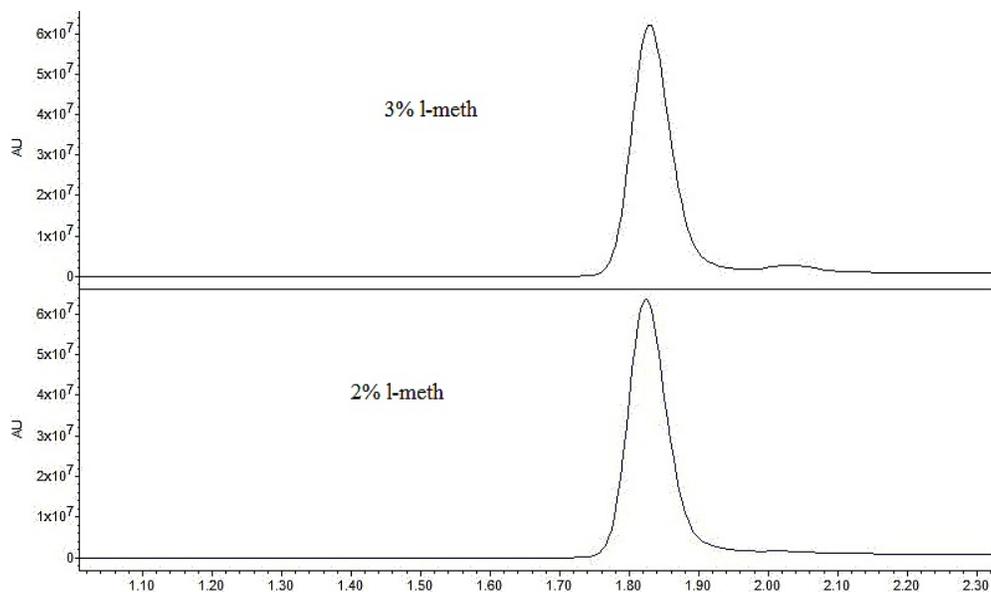
[Figures Continued, Next Two Pages]

Figure 4. SFC chromatograms of co-solvent screening. Co-solvent: MeOH/EtOH (50/50) with 1.0% CHA; and gradient: 5-7.5% in 6 min. Flow rate: 2.5 mL/min.

Figure 5. Study of CHA concentration on methamphetamine chiral separation. Co-solvent: EtOH and CHA; and gradient: 8-30% in 6 min. Flow rate: 2.5 mL/min. 1: 1.0 % CHA; 2: 0.5% CHA.

Figure 6. Detection limit of l-methamphetamine (peak at ~2.03 minutes) with a skewed ratio of enantiomers. Co-solvent: EtOH and 1.0% CHA; and gradient: 8-30% in 6 min. Flow rate: 2.5 mL/min.
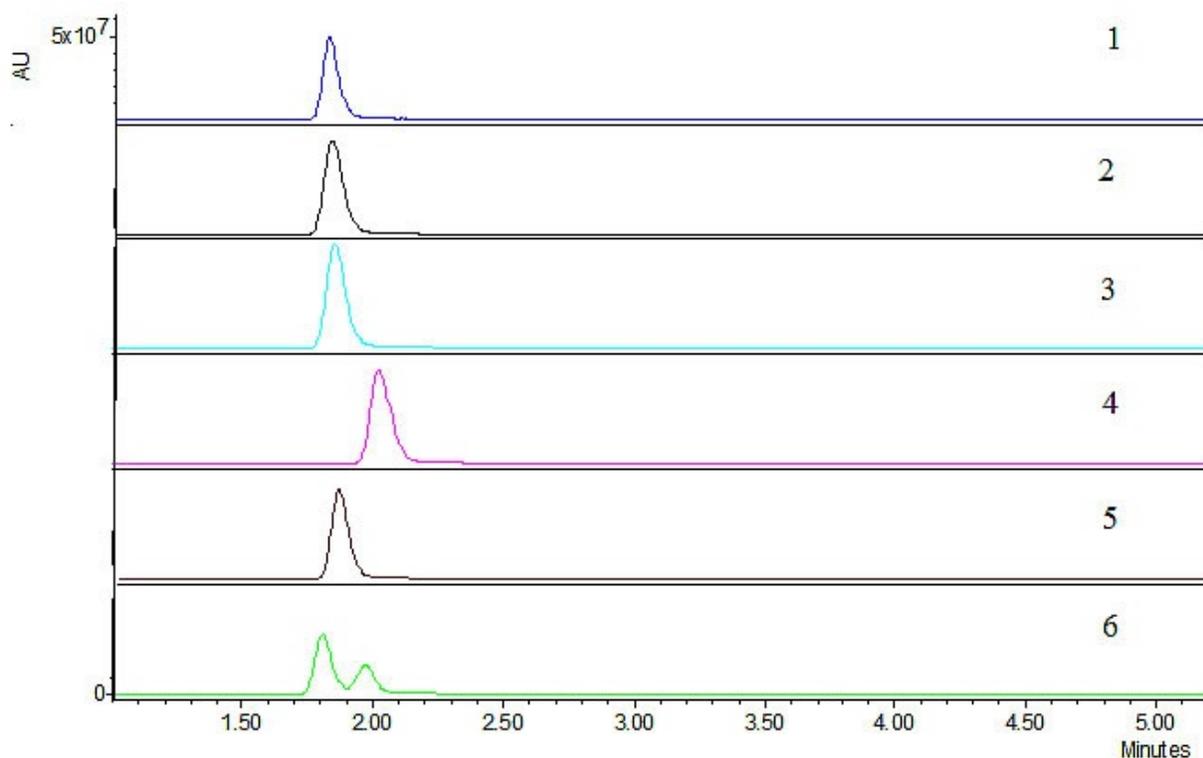
Figure 7. Chiral analysis of methamphetamine samples. Co-solvent: EtOH with 1.0% CHA; and gradient: 8-30% in 6 min. Flow rate: 2.5 mL/min.

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