Enantioresolution of Amphetamine, Methamphetamine, and Deprenyl (Selegiline) by LC, GC, and CE

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Tremendous advances have been made in the area of enantiomeric separations over the last decade. Separations and analyses that were unthinkable a few years ago have become straightforward. Today, scientists often have more than one technique available to solve problems involving the concentration and disposition of enantiomers. Amphetamine, methamphetamine, and deprenyl (selegiline) are chiral compounds that have important medicinal use. In some cases, they are widely abused drugs and their enantiomers are known to have different physiological properties. Several different approaches for their enantioresolution and analysis are demonstrated. Each approach has advantages and disadvantages in regard to speed, efficiency, selectivity, sensitivity, sample size, and sample preparation.

Amphetamine and methamphetamine are chiral sympathomimetic amines with central nervous system (CNS) stimulant activity (***F1***). Their peripheral actions include the elevation of systolic and diastolic blood pressure. They also have weak bronchodilator and respiratory stimulant action (l). The legitimate uses of amphetamine and methamphetamine include the treatment of attention deficit disorder with hyperactivity and the treatment of exogenous obesity. Both compounds have a high incidence of misuse and abuse as well.

It is well known that the enantiomers of amphetamine and methamphetamine can have different physiological effects (2-4). For example, (S)-(+)-amphetamine is several times more potent than the R-(-)-enantiomer in eliciting CNS effects. Conversely, R-(-)-amphetamine has somewhat more potent cardiovascular effects (1-4). (S)-(+)-methamphetamine is a widely abused, DEA schedule II, controlled substance, and R-(-)-methamphetamine has been used in an over-the-counter nasal decongestant (4,5).

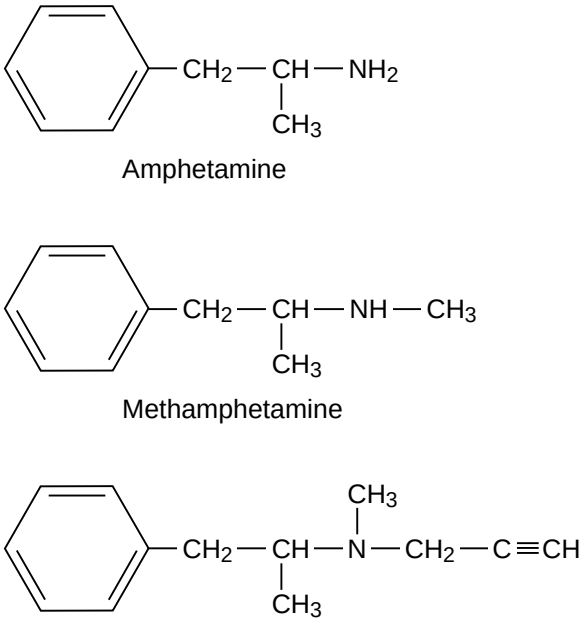
Depending on the method of synthesis and purification, amphetamine and methamphetamine can be produced as racemates or as enantiomerically enriched compounds (6-8). One method used to produce enantio-enriched methamphetamine for illicit street use involves the dehydroxylation of ephedrine and/or pseudoephedrine (***F2***), which are found in many readily available, over-the-counter pharmaceutical preparations (8).

Selegiline (a.k.a. deprenyl) is structurally related to amphetamine and methamphetamine (***F1***). The R-(-)-enantiomer is used in the treatment of Parkinson's disease (1). It is an irreversible inhibitor of monoamine oxidase (MAO), which is widely distributed throughout the body. Selegiline has a greater affinity for type B MAO (which is the predominant type in the brain) than for the type A MAO (the predominate type in the intestinal tract).

The rapid and effective enantioresolution of all the aforementioned compounds is important for pharmacological, toxicological, and forensic studies, as well as in production quality control. An analytical methodology that may be appropriate for one type of study, may be less appropriate for another. For example, capillary electrophoresis (CE) may be the best approach for the rapid analysis of small amounts of a relatively pure compound; however, the greater sensitivity and reproducibility of gas chromatography (GC) or liquid chromatography (LC) may be needed when analyzing low levels of a compound in physiological samples. Preparative and semiprep separations would be limited to LC. In this work, we report the enantioresolution of racemic amphetamine, methamphetamine, and deprenyl by three different and complimentary methods: LC, GC, and CE.

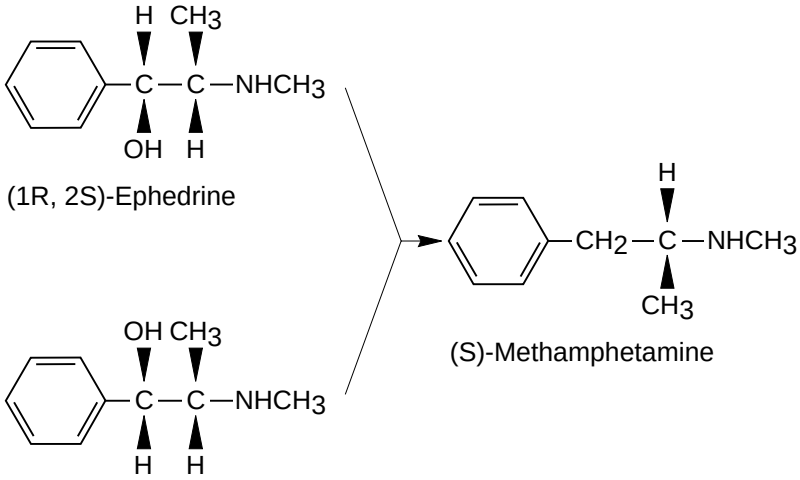
F1

Structures of amphetamine, methamphetamine, and deprenyl.



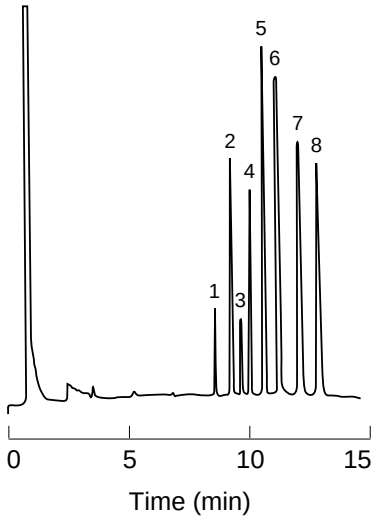
F2

Enantiomerically enriched methamphetamine can be produced by dehydroxylation of appropriate isomers of ephedrine and/or pseudoephedrine. (S)-methamphetamine is a widely abused, controlled substance. The chiral starting materials that can be used to produce it have been available in over-the-counter products but they may soon be restricted.



F3

GC enantioseparation of: 1. (-)-ephedrine, 2. (R)-amphetamine, 3. (+)-ephedrine, 4. (S)-amphetamine, 5. (S)-methamphetamine, 6. (R)-methamphetamine, 7. (+)-pseudoephedrine, and 8. (-)-pseudoephedrine. The separation was done on a 30 m x 0.25 mm Chiraldex G-PN column with He carrier gas @ 35 psi and FID. The oven temperature was 130°C (isothermal) and the split ratio was 120:1. All compounds were separated as their trifluoroacetyl derivatives.



Experimental

Materials

All LC and CE solvents were obtained from Fisher Scientific (St. Louis, MO). The AccQ·Fluor (AQC) reagent kit was obtained from Waters (Bedford, MA). The hydroxypropyl-ß-cyclodextrin, Cyclobond I 2000 LC column, Cyclobond I 2000 RSP LC column, Cyclobond I 2000 DMP LC column, Cyclobond I 2000 RN LC column, Cyclobond I 2000 SN LC column, Chiraldex ß-DM capillary GC column and Chiraldex G-PN GC column were obtained from Advanced Separation Technologies, Inc. (Whippany, NJ). All other buffers and reagents were obtained from Aldrich (Milwaukee, WI).

Methods

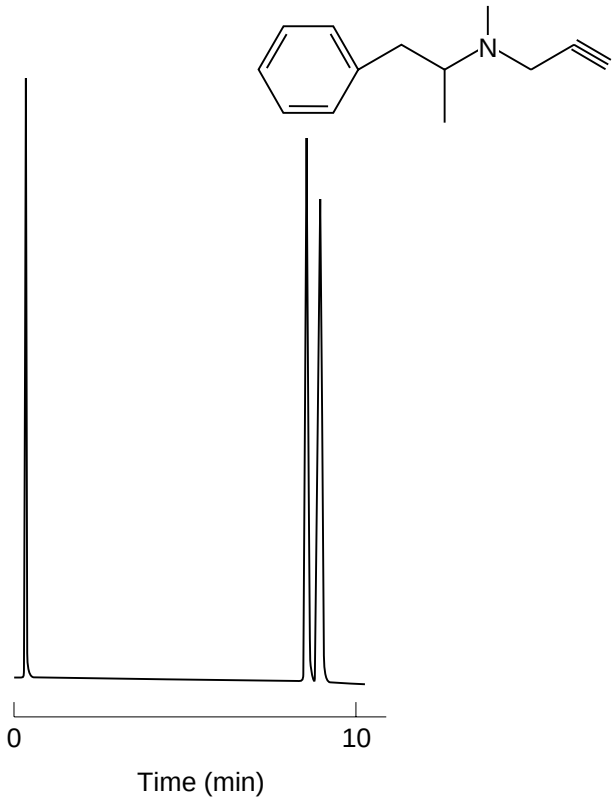
All LC separations were done on a BAS (West Lafayette, IN) modular liquid chromatograph with a PM-80 solvent delivery system and a UV-116A UV-Vis detector. All chromatograms were run at room temperature (~22°C). The GC separations were done either on a Varian Model 3700 gas chromatograph or a Shimadzu Spl-G9 gas chromatograph with a CRIB chromatopac recorder. Split ratios were 1:100 unless noted otherwise. CE separations were done at 25°C on a Beckman P/ACE System 2100. UV detection was used at either 214 or 254 nm. Voltages were 10 kV, and a 27 cm x 50 µ (i.d) capillary was used (20 cm to the detector).

Results and Discussion

Gas chromatography (GC) on derivatized cyclodextrin chiral stationary phases (CSPs) is a highly effective approach for resolving most compounds with a phenylisopropylamine-base-structure (9). This is shown in ***F3*** (for amphetamine, methamphetamine, ephedrine, and pseudoephedrine) and in ***F4*** (for deprenyl). Capillary GC on appropriate CSPs still offers the best combination of efficiency, sensitivity, selectivity, and peak capac-

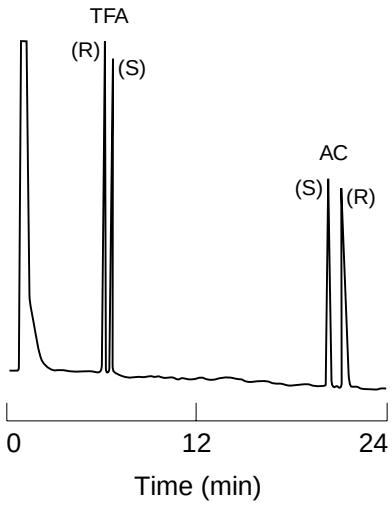
F4

GC enantioseparation of deprenyl (selegiline) on a 20 m x 0.25 mm Chiraldex ß-DM column. The first eluted peak is the (R)-(-)-enantiomer and the second peak is the (S)-(+)-enantiomer. H_2 carrier gas was used at 20 psi with FID. The oven temperature was 120°C (isothermal) and the split ratio was 100:1.



F5

Gas chromatogram showing that trifluoroacetylated amphetamine (TFA) has the opposite enantiomeric elution order as acetylated amphetamine (AC). This separation was done on a 30 m x 0.25 mm Chiraldex G-PN column using FID. Helium was the carrier gas @ 35 psi. The oven temperature was 140°C (isothermal).



ity for this particular class of compounds. The ability to rapidly determine enantiomeric ratios of methamphetamine and its possible precursors, ephedrine and pseudoephedrine, in a single run can be useful for forensic identification of illicit street drugs and the laboratories that produce them. For most routine pharmaceutical analyses or legitimate pharmacological studies, it is not necessary to simultaneously resolve and identify all of these compounds.

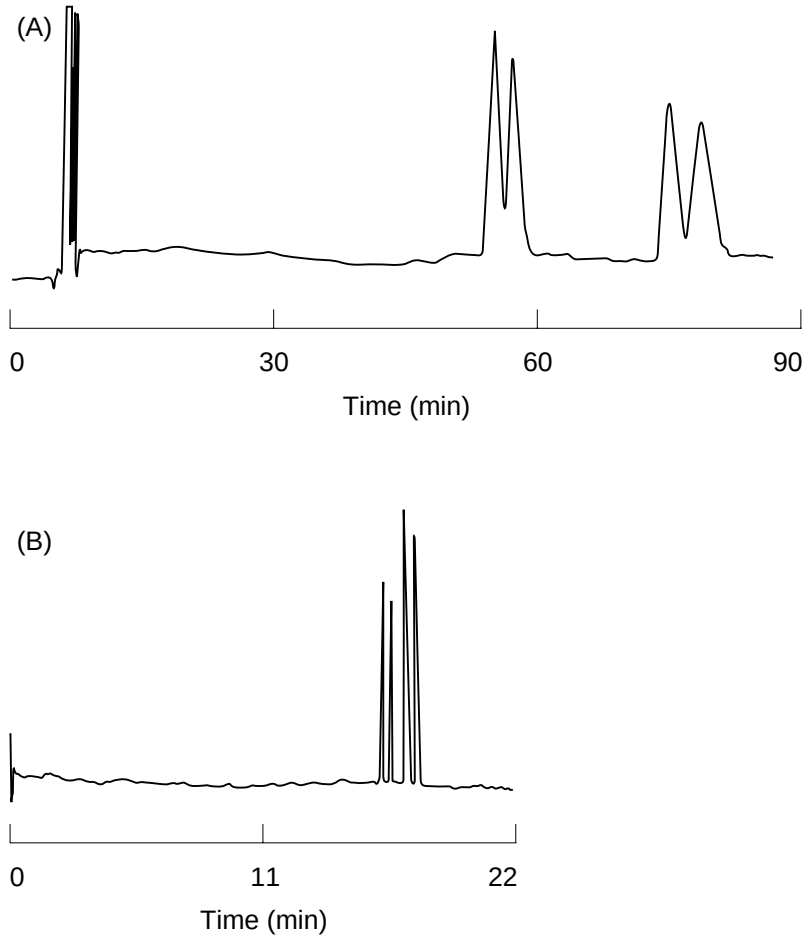
Reversing the elution order of enantiomers also can be done in GC. Previously, it was shown that CSPs made of different cyclodextrin derivatives or different size cyclodextrins could reverse the enantiometric retention order of many compounds (10). ***F5*** shows an additional approach where trifluoroacetylated amphetamine has the opposite enantiomeric elution order as acetylated amphetamine on the same CSP.

Hydroxypropyl derivatized ß-cyclodextrin is an effective solution-based chiral selector for these stimulants. ***F6*** shows the LC enantioresolution of amphetamine and methamphetamine using a hydroxypropyl-ß-cyclodextrin CSP (CBI 2000 RSP) versus the analogous separation by capillary electrophoresis (CE). In this case, the greater efficiency of CE provides baseline resolution and in a shorter time. However, deprenyl is not as easily resolved by CE (***F7A***). A far better separation is achieved in a shorter time with LC using a CSP consisting of S-naphthylethylcarbamate functionalized ß-cyclodextrin (CBI 2000 SN) (***F7B***).

When doing physiological or biological studies on extremely small amounts of highly water soluble chiral compounds, such as the amphetamine and methamphetamine hydrochlorides, there are usually two additional problems. One is sensitivity and the other involves isolating and concentrating the analyte from a complex biological matrix. Both problems are easily solved by reacting the analyte(s) with an easy-to-use, achiral fluorescent tagging agent. The use of fluorescence detection in HPLC can enhance sensitivity by three orders of magnitude. Also, the derivatized analytes are much more hydrophobic allowing them to be more easily and effectively concentrated and recovered by solid phase extraction (11-20). A variety of fluorescent and/or electroactive "tagging-agents" have appeared in the literature, including o-phthaldehyde (OPA), naphthalene-dialdehyde (NDA), aromatic anhydrides (AA), 9-fluorenylmethylchloroformate (FMOC), and 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) (10-19). For chiral amines and amino acids where enantioseparations are also involved, AQC, AA, and FMOC are the preferred fluorescent "tagging-agents". ***F8*** shows the LC enantiomeric

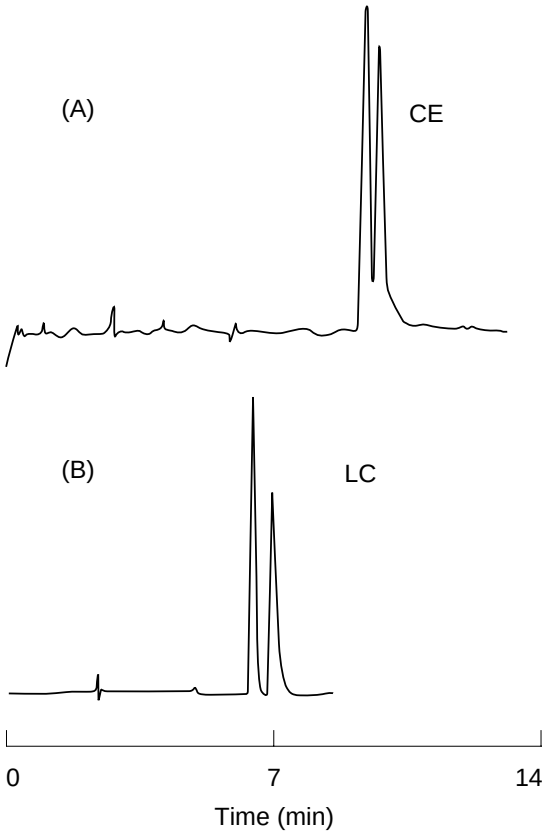
F6

A comparison of the (A) reversed phase LC resolution of amphetamine (first eluted pair of peaks) and methamphetamine on a 25 x 0.46 cm hydroxypropyl-ß-cyclodextrin column (Cyclobond I 2000 RSP), flow rate 1.0 mL/min, mobile phase = 95:2.5:2.5 (v:v:v) 0.5% pH 6.8 triethylammonium acetate buffer: acetonitrile: methanol, and (B) CE resolution of amphetamine (first eluted pair of peaks) and methamphetamine using 50 mM hydroxypropyl-ß-cyclodextrin in pH 2.5, 0.1 M phosphate buffer. UV detection at 254 nm and 214 nm, respectively, was used.



F7

Comparison of the (A) CE and (B) LC enantioseparations of deprenyl. The CE separation utilized a 27 cm (50 µm, i.d.) capillary (20 cm to detector) containing a run buffer of pH 3.5, 0.05 M phosphate buffer + 50 mM hydroxypropyl-ß-cyclodextrin (10 kV). UV detection at 214 nm was used. The LC separation was done on a Cyclobond I 2000 SN column at a flow rate of 0.5 mL/min. The mobile phase was 25:75 (v:v) acetonitrile: 1%, pH 4.1 triethylammonium acetate buffer. UV detection at 254 nm was used.



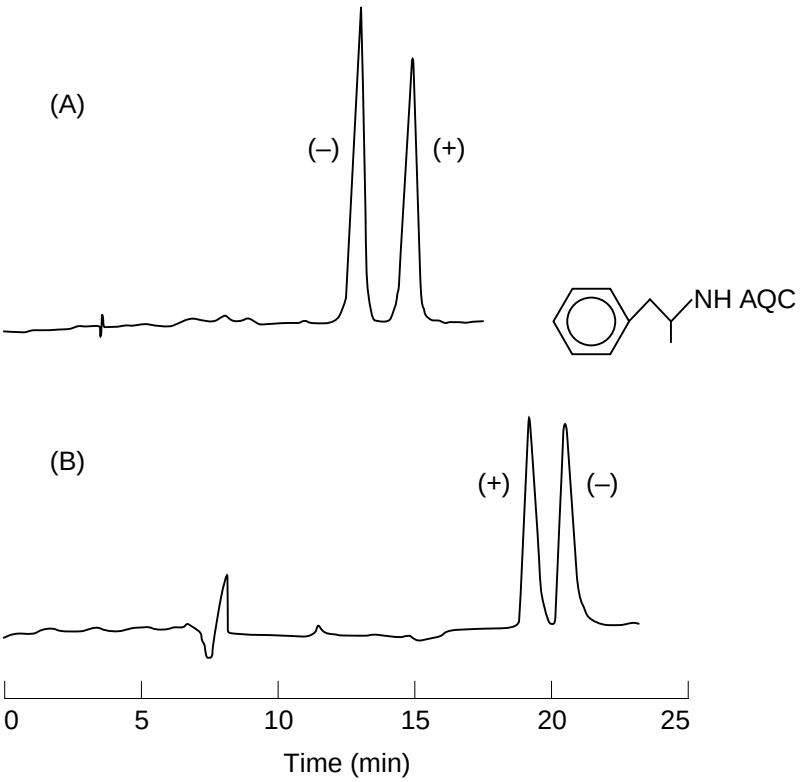
separation of AQC-amphetamine in both (A) the reversed phase mode and (B) the polar-organic mode. Although both are excellent separations, note that the enantiomeric elution order has been reversed in the two modes. This indicates that the chiral recognition mechanism is not the same. Both of these modes can be used in coupled column assays where there is an initial achiral, reversed phase separation (12-20). ***F9*** shows the analogous LC separation of AQC-methamphetamine. Note that although amphetamine and methamphetamine differ only by a methyl group, their enantioselectivity and retention can differ significantly. It is not uncommon for some closely related chiral compounds to require different CSPs and/or approaches for optimum separations.

Conclusions

A little over a decade ago, virtually any relatively rapid, somewhat efficient enantiomeric separation was considered highly noteworthy. Rapid advances in the development and understanding of enantioselective interactions and separations have made many analyses, which were once thought to be impossible, now routine. Today, this science and technology has evolved to the point where there are often several different separation approaches to choose from. This was demonstrated in the present work with amphetamine, methamphetamine, deprenyl, and other related compounds. Clearly, different approaches are useful for biological assays *vs*. forensic assays *vs*. quality control of a pharmaceutical product. Depending on the goal of the investigator and the nature of the problem to be solved, one chiral separation method may be superior to another. Thus, it is beneficial to have several different, effective approaches available when faced with problems involving the analysis of enantiomers.

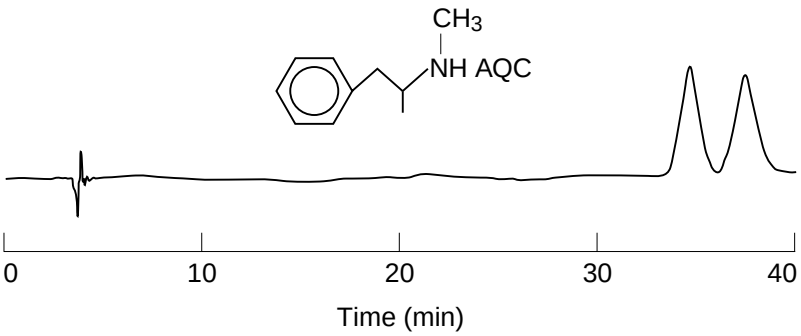
F8

LC resolution of AQC-tagged amphetamine in (A) the reversed phase mode using a 25 x 0.46 cm Cyclobond I 2000 DMP column. The mobile phase was 45:55 (v:v) acetonitrile: 0.1%, pH 4.1 triethylammonium acetate buffer. The flow rate was 1.0 mL/min and (B) in the polar-organic mode using a 25 x 0.46 cm Cyclobond I 2000 column. The mobile phase was 98:2:0.5:0.4, acetonitrile: methanol: glacial acetic acid: triethylamine. The flow rate was 0.5 mL/min. In both cases, UV detection at 254 nm was used. Note that the elution order of the enantiomers is different.



F9

LC resolution of AQC-methamphetamine. A 25 x 0.46 cm Cyclobond I 2000 DMP column was used at a flow rate of 1.0 mL/min. The mobile phase was 35:65 (v:v) acetonitrile: 0.1%, pH 4.1 triethylammonium acetate buffer. UV at 254 nm detection was used.



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