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 (I). 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 predominant 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.
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-
ity for this particular class of com-
ounds. The ability to rapidly deter-
mine enantiomeric ratios of methamphetamine and its possible pre-
cursors, ephedrine and pseudoephedrine, in a single run can be useful for forensic identi-
fication of illicit street drugs and the laboratories that produce them. For most routine pharmaceutical analy-

Reversing the elution order of enantiomers also can be done in GC. Previously, it was shown that CSPs made of different cyclodex-
trin derivatives or different size cy-
cloextrins could reverse the enan-
tiometric retention order of many compounds (10). F5 shows an addi-
tional approach where trifluoroace-
tylated amphetamine has the oppo-
site enantiomeric elution order as
acetylated amphetamine on the same CSP.

Hydroxypropyl derivatized β-
cyclodextrin is an effective solu-
tion-based chiral selector for these stimulants. F6 shows the LC enan-
tioresolution of amphetamine and methamphetamine using a hydroxypropyl-β-cyclodextrin CSP (CBI 2000 RSP) versus the analogous separation by capillary elec-
trophoresis (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-naphthylethylcar-
bamate functionalized β-cyclodextrin (CBI 2000 SN) (F7B).

When doing physiological or biological studies on extremely small amounts of highly water sol-

cule chiral compounds, such as the amphetamine and metham-
phetamine hydrochlorides, there are usually two additional problems. One is sensitivity and the other in-
volves isolating and concentrating the analyte from a complex biological matrix. Both problems are eas-
ily solved by reacting the analyte(s) with an easy-to-use, achiral fluores-
cent tagging agent. The use of fluo-
rescence detection in HPLC can en-
hance sensitivity by three orders of magnitude. Also, the derivatized analytes are much more hydropho-
bic allowing them to be more easily and effectively concentrated and re-
covered by solid phase extraction (11-20). A variety of fluorescent and/or electroactive “tagging-
agents” have appeared in the litera-
ture, including o-phthalaldehyde (OPA), naphthalene-dialdehyde (NDA), aromatic anhydrides (AA), 9-fluorenylethylchloroformate (FMOC), and 6-aminoquinolyl-N-
hydroxysuccinimidyl carbamate (AQC) (10-19). For chiral amines and amino acids where enan-
tioseparations are also involved, AQC, AA, and FMOC are the preferred fluorescent “tagging-agents”. F8 shows the LC enantiomeric
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.
**References**

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