Liquid Chromatographic Chiral Stationary Phases in Pharmaceutical Analysis: Determination of Trace Amounts of the (−)-Enantiomer in (+)-Amphetamine

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Abstract □ A rapid and accurate method was developed for the determination of the enantiomeric composition of amphetamine preparations. Amide derivatives of the amphetamine enantiomers are first formed by using achiral 2-naphthoyl chloride. The resulting enantiomeric amides are then chromatographed on a commercially available chiral stationary phase. The capacity factors (k') of (−)- and (+)-2-naphthoylamphetamine are 20 and 22, respectively, and the separation factor (α) for the two enantiomers is 1.08. The method allows detection of as little as 0.5% of the (−)-enantiomer in (+)-amphetamine and is applicable to both bulk drug and single-tablet analyses.

Keyphrases □ Enantiomers—(+)- and (−)-amphetamine, HPLC, trace amounts □ Chiral stationary phases—HPLC, determination of trace amounts of (+)- and (−)-amphetamine □ Amphetamine—(+)- and (−)-enantiomers, HPLC, trace amounts

Amphetamine (α-methylphenethylamine) is a pharmacologically active asymmetric molecule whose (S) and (R) enantiomeric forms, (+)- and (−)-amphetamine, respectively, have different biological activities (1). Accordingly, the resolution and quantification of the amphetamine enantiomers have received a great deal of attention.

The most common approach to this analytical problem involves the synthesis and separation by GC of diastereoisomeric amides of amphetamine (2–9). Although this technique has been successfully utilized, it suffers from a number of inherent problems which hinder the development of methods for the determination of trace amounts of one enantiomer in the presence of the other.

The most serious drawback is that the synthesis of diastereoisomers necessarily involves the use of a chiral derivatizing agent. Any isomeric impurity in this reagent will result in spurious results. Liu and Ku (9) have discussed this problem in detail for the case of L-prolyl-derivatives of amphetamine and were able to correct for it by resolving the diastereoisomers into their enantiomeric pairs on a GC chiral stationary phase. An additional complication is that enantiomers may have quite different rates and/or equilibrium constants when they react with another chiral molecule, resulting in the generation of two diastereoisomeric products in proportions that differ from the starting enantiomeric composition (10).

Both of these problems can be avoided by resolving the enantiomeric pair as enantiomers by utilizing chromatography on chiral stationary phases. In such a case, if a derivative is to be formed and if the derivatizing agent is not an optically active molecule, there is no question of trace isomeric contamination. Furthermore, all enantiomers react with an optically inactive reagent to the same extent, *i.e.*, they have the same rate constants and the same equilibrium constants (10). Therefore, complete reaction is not a requirement. Direct resolution of enantiomeric amphetamine derivatives has been accomplished by GC (9, 11) and most recently by Wainer and Doyle *via* high-performance liquid chromatography (HPLC) (12).

In this report the application of direct HPLC resolution (12) to the determination of the enantiomeric composition of bulk drug and commercial preparations of amphetamine is described. The method involves the synthesis of the 2-naphthoyl amide of amphetamine and its resolution by HPLC on a commercially available chiral stationary phase containing (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine. It is rapid and accurate and can detect the presence of as little as 0.5% of the (−)-enantiomer in (+)-amphetamine.

EXPERIMENTAL SECTION

Apparatus—The liquid chromatograph[1] was equipped with a data system[2], a fixed-wavelength detector[3] (280 nm), a temperature-controlled column compartment, and a 20-μL fixed-loop injector[4]. All analyses were performed with a 25-cm × 4.6-mm i.d. stainless steel column packed with a covalently bonded chiral stationary phase[5] and a 50-mm × 4.6-mm i.d. stainless steel guard column packed with 35–50-μm silica.

Materials—Hexane[6], isopropyl alcohol[6], and acetonitrile[6] were HPLC grade and were used as purchased. The 2-naphthoyl chloride[7], (+)-amphetamine sulfate (reference standard)[8], and (+)-, (−)-, and (±)-amphetamine sulfate (bulk drug)[9] were also used as purchased. Pharmaceutical preparations of (+)-amphetamine sulfate as capsule[10] and syrup[11], (+)-amphetamine-(±)-amphetamine sulfate capsule[12], and (±)-amphetamine sulfate capsule[13] were purchased commercially.

Structural Determinations—Structural determinations were made with a 200-MHz NMR spectrometer[14], a gas chromatograph–mass spectrometer[15], and an IR spectrophotometer[16]. The ^{1}H-NMR spectra ($CDCl_3$) contained the following peaks: δ 1.24 (d, 3), 2.80–3.02 (m, 2), 4.44–4.58 (m, 1), 6.19 (d, 1), and 7.22–8.20 ppm (m, 12). The mass spectra contained a molecular ion peak at m/z 289 and a base peak at m/z 155. The IR spectra (CH_2Cl_2) contained a sharp peak at 1655 cm^{-1}. The following melting points for the derivatives were obtained: (+)-2-naphthoylamphetamine, 152–155°C; (−)-2-naphthoylamphetamine, 152–155°C; (±)-2-naphthoylamphetamine, 162–165°C.

General Procedure—The bulk drug (10 mg) or the pharmaceutical preparation (one 10-mg tablet or capsule or 10 mL of a 1-mg/mL syrup) was placed in 5 mL of 20% NaOH solution and ultrasonicated until dissolved. The solution was then transferred to a 30-mL separatory funnel. Ten milliliters of a 0.01 M solution of 2-naphthoyl chloride in dichloromethane was added, and the mixture was shaken for 1 min. The organic phase was transferred to another 30-mL separatory funnel. The aqueous phase was washed with a 5-mL

[1] Model SP3500 chromatograph; Spectra-Physics, Santa Clara, Calif.
[2] Model SP4000 data system; Spectra-Physics.
[3] Model SP8200 detector; Spectra-Physics.
[4] Valco injector; Spectra-Physics.
[5] Pirkle Covalent Phenylglycine column; Regis Chemical Co., Morton Grove, Ill.
[6] Distilled-in-glass; Burdick & Jackson Laboratories, Muskegon, Mich.
[7] Pfaltz & Bauer, Stamford, Conn.
[8] United States Pharmacopeial Convention, Rockville, Md.
[9] ICN K&K Laboratories, Plainview, N.Y.
[10] Dexedrine timed-release capsule, 10 mg; Smith, Kline, and French Laboratories, Philadelphia, Pa.
[11] Dexedrine syrup, 1 mg/mL; Smith, Kline, and French Laboratories.
[12] Biphetamine, capsule, 10 mg; Penwalt Pharmaceutical Division, Rochester, N.Y.
[13] Benzedrine capsule, 10 mg; Smith, Kline, and French Laboratories.
[14] Varian Model XL-200 NMR spectrometer; Varian Associates, Palo Alto, Calif.
[15] Finnigan Model 4023-T GC/MS/DS; Finnigan MAT, San Jose, Calif.
[16] Perkin-Elmer Model 599B IR spectrophotometer: Perkin-Elmer, Norwalk, Conn.

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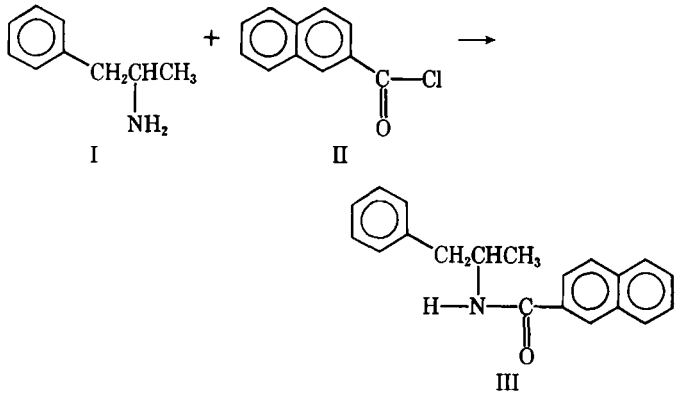

Scheme I—Reaction of amphetamine free base (I) and 2-naphthoyl chloride (II) to yield 2-naphthoylamphetamine (III).

portion of dichloromethane, and the organic layers were combined. The dichloromethane was extracted with two 5-mL portions of 0.01 M sulfuric acid, filtered through a cotton plug, and injected onto the HPLC column.

Chromatographic Conditions—The mobile phase was hexane–isopropyl alcohol–acetonitrile (97:3:0.5) at a flow rate of 2 mL/min. A column temperature of 20°C was maintained throughout the analyses.

Standard Curves—Two standard curves were constructed by combining (+)-amphetamine reference standard and (±)-amphetamine in various proportions by weight. The enantiomeric composition of one standard curve ranged from 50 parts (−)-amphetamine sulfate–50 parts (+)-amphetamine sulfate to 10 parts (−)-amphetamine sulfate–90 parts (+)-amphetamine sulfate. The other curve measured from 10 parts (−)-amphetamine sulfate–90 parts (+)-amphetamine sulfate to 0.5 parts (−)-amphetamine sulfate–99.5 parts (+)-amphetamine sulfate. The ratio of the integrated peak area of the (−)-isomer to the integrated peak area of the (+)-isomer was plotted *versus* the known enantiomeric composition.

RESULTS AND DISCUSSION

The reaction of amphetamine (I) with 2-naphthoyl chloride (II) proceeded rapidly under Schotten–Baumann conditions. When USP reference standard (+)-amphetamine sulfate was reacted under these conditions, a single product was isolated. The product was recrystallized from methanol and identified as the 2-naphthoylamide [III-(+)] by NMR, MS, and IR analyses (Scheme I). The amide was chromatographed on the chiral stationary phase; the chromatogram contained a single sharp peak with a capacity factor (k') of 22.

A series of five reactions with the USP reference standard and 2-naphthoyl chloride was carried out, and the crude reaction mixture was analyzed for amide concentration by using the crystalline product prepared as described above as an external standard. In this manner, the yield of the reaction was determined to be 82%. No attempt was made to increase the yield of the reaction, since the use of an achiral acid chloride ensures that both enantiomers react equally.

Table I—Enantiomeric Composition of Commercial Amphetamine Preparations

Sample	Assay 1, %	Assay 2, %	Average, %	Label, %
(+)-Amphetamine (bulk drug)				
(−)-Isomer	8.9	9.6	9.3	0
(+)-Isomer	91.1	90.4	90.8	100
(−)-Amphetamine (bulk drug)				
(−)-Isomer	98.4	99.3	98.9	100
(+)-Isomer	1.6	0.7	1.2	0
(+)-Amphetamine (timed-release capsule)				
(−)-Isomer	1.2	1.5	1.4	0
(+)-Isomer	98.7	98.5	98.9	100
(+)-Amphetamine (syrup)				
(−)-Isomer	1.4	0.8	1.1	0
(+)-Isomer	98.6	99.1	98.9	100
(+)-,(±)-Amphetamine (capsule)				
(−)-Isomer	25.4	25.5	25.5	25
(+)-Isomer	74.7	74.5	74.6	75
(±)-Amphetamine (capsule)				
(−)-Isomer	49.1	49.5	49.3	50
(+)-Isomer	50.8	50.4	50.7	50

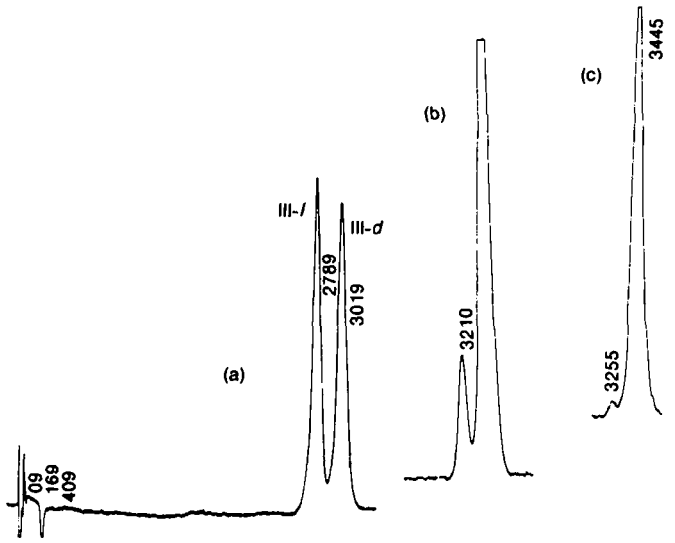

Figure 1—*Chromatograms of mixtures of (−)-2-naphthoylamphetamine [III-(−)] and (+)-2-naphthoylamphetamine [III-(+)]. Key: (a) III-(−)-III-(+), 50:50; (b) III-(−)-III-(+), 10:90; (c) III-(−)-III-(+), 1:99.*

The reaction of (−)-amphetamine bulk drug and 2-naphthoyl chloride afforded, on recrystallization, a product with the same NMR, MS, and IR spectral features as (+)-2-naphthoylamphetamine; this product was identified as the (−)-enantiomer [III-(−)]. The HPLC chromatogram of the recrystallized product contained two sharp peaks with capacity factors of 20 and 22, respectively. The first peak in the chromatogram, that of the (−)-enantiomer, contained 99% of the integrated area of the chromatogram, and the second peak, that of the (+)-enantiomer, contained 1% (Table I).

Two series of mixtures of (+)- and (−)-amphetamine were prepared by combining USP reference standard (+)-amphetamine sulfate and (±)-amphetamine sulfate in various proportions by weight. Enantiomeric proportions in the first series ranged from (±)-amphetamine sulfate (a 50:50 mixture of the two isomers) to a (+)-amphetamine sulfate–(−)-amphetamine sulfate mixture of 90:10. Proportions in the second series ranged from a (+)-amphetamine sulfate–(−)-amphetamine sulfate mixture of 90:10 to a 99.5:0.5 mixture. All solutions were derivatized and analyzed, and standard curves were constructed (Fig. 1). The curves were linear over both concentration ranges. The first standard curve (50:50–90:10) had a standard error of 0.0170 and a correlation coefficient of 0.998. The second curve had a standard error of 0.0077 and a correlation coefficient of 0.982. The separation factor (α) averaged 1.08.

Four pharmaceutical preparations of (+)-amphetamine and of (+)-amphetamine–(−)-amphetamine mixtures were purchased commercially and analyzed (Table I). A single 10-mg capsule or 10 mL of a 1-mg/mL syrup was used in the assays. By this method 1% of the (−)-isomer was detected in the (+)-amphetamine preparations; the expected enantiomeric ratios were detected in the other preparations.

The assay is a rapid (requiring no more than 2 h) and accurate method for the determination of the isomeric content of amphetamine bulk drug and commercial pharmaceutical preparations. It utilizes an achiral derivatizing reagent, 2-naphthoyl chloride, and a chiral stationary phase HPLC column which are both commercially available.

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Dipole Moments and Conformational Equilibrium in Some Substituted 1-Phenyl-1-fluoro-2-halogenoethanes

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Abstract □ Experimental dipole moments of substituted 1-phenyl-1-fluoro-2-halogenoethanes are compared with the vectorially and the theoretically calculated values using the CNDO/2 method. Results support the existence of a conformational mixture of these compounds as solutes; *gauche* structures are the prevailing conformations as in the related catecholamines.

Keyphrases □ Phenylfluorohalogenoethanes—substituted, dipole moments, conformations as solutes □ Dipole moments—substituted phenylfluorohalogenoethanes as solutes □ Conformations—phenylfluorohalogenoethanes as solutes

Potentially antiarrhythmic drugs can be prepared from substituted 1-phenyl-1-fluoro-2-halogenoethanes, because the latter are structurally related to catecholamines. However, conformations of these intermediates have to be portrayed. For this purpose, their experimental dipole moments and the vectorially as well as the theoretically calculated moments were compared in this report.

EXPERIMENTAL SECTION

Materials—The following compounds were studied: 1-phenyl-1-fluoro-2-chloroethane (I), 1-phenyl-(4′-bromo)-1-fluoro-2-chloroethane (II), 1-phenyl-(4′-chloro)-1-fluoro-2-chloroethane (III), 1-phenyl-(2′-bromo)-1-fluoro-2-bromoethane (IV), 1-phenyl-(2′-chloro)-1-fluoro-2-bromoethane (V), 1-phenyl-(2′,4′-dichloro)-1-fluoro-2-chloroethane (VI), 1-phenyl-(2′,-5′-dichloro)-1-fluoro-2-chloroethane (VII), and 1-phenyl-(2′,6′-dichloro)-1-fluoro-2-bromoethane (VIII). These compounds were prepared from the corresponding alcohols, in accordance with the process previously described (1).

Methods—The dipole moments of the compounds as solutes in anhydrous benzene were measured at 25.00 ± 0.05°C. The permittivity[1] and refractive index[2] of the solutions were extrapolated to infinite dilution according to Guggenheim (2) and Smith (3). The quantity $(\epsilon_{12} - n_{12}^2) - (\epsilon_1 - n_1^2)$ was plotted *versus* the molar concentration, C, of the solute. The slope of the curve at $C = 0$ was then used to calculate the dipole moment, μ, by:

$$\mu^2 = \frac{9kT}{4\pi N} \cdot \frac{3}{(\epsilon_1 + 2)(n_1^2 + 2)} \cdot \frac{(\epsilon_{12} - n_{12}^2) - (\epsilon_1 - n_1^2)}{C} \qquad \text{(Eq. 1)}$$

where k is the Boltzmann constant, N is Avogadro's number, T is the absolute temperature, and ϵ_i and n_i are the permittivities and refractive indexes, respectively, of the solutes (Index 1) and of the solutions (Index 12).

RESULTS AND DISCUSSION

The molecular geometry of the compounds studied is schematized in Fig. 1. Hence, six limit-conformers have to be considered, because of the presumable restricted rotation around the C_1—C_2 bond. These conformers, presented in Fig. 2, are noted (A) when bonds are staggered and (B) when bonds are opposed. The following bond increments were then used for vectorial calculations: H—$C_{sp}3$, 0.25 *D* (4); $C_{sp}3$—Br, 1.38 *D*; $C_{sp}3$—Cl, 1.46 *D*; $C_{sp}3$—F, 1.41 *D* (5); $C_{sp}3$—$C_{sp}2$, 0.4 *D*; $C_{sp}2$—Br, 1.54 *D*; and $C_{sp}2$—Cl, 1.58 *D* (6).

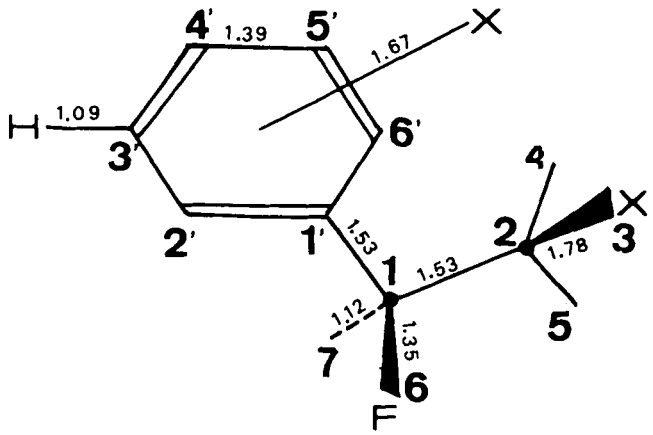

Figure 1—*Molecular geometry of the compounds studied.*

As regards the vectorial pattern of the molecules, it must be emphasized that spatial orientation of the phenyl ring *versus* the C_1—F bond is of no importance, except with the *ortho*-substituted derivatives. In the latter case, four possible orientations must be taken into account in accordance either with

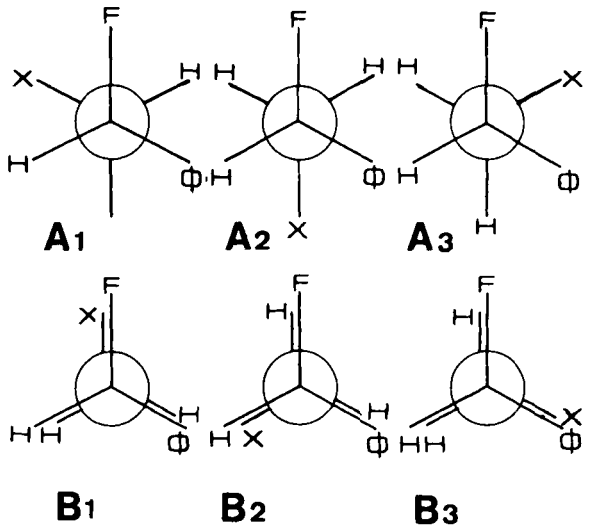

Figure 2—*Schemes of the staggered and the opposed limit-conformers used in the calculations.*

[1] W.T.W. DM 01 dipolmeter with a DFL 1 cell.
[2] O.P.L. Abbe type refractometer.

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