US Department of Health and Human Services, Food and Drug Administration

NMR Regulatory analysis: enantiomeric purity determination for (*R*)-(–)-desoxyephedrine and antipode methamphetamine

G. M. HANNA

Received March 22, 2005, accepted March 30, 2005

George M. Hanna, Food and Drug Administration, Northeast Regional Laboratory, 158-15 Liberty Avenue, Jamaica, New York, 11435-1034, U.S.A.

Pharmazie 61: 188–193 (2006)

Regulatory enantiomeric purity direct determination for (*S*)-(+)-methamphetamine, the widely abused DEA schedule II controlled substance, and (*R*)-(−)-desoxyephedrine, over-the-counter nasal inhaler decongestant were developed using 400 MHz ^{1}H NMR spectroscopy. The efficient enantiomeric differentiation was obtained using a diamagnetic chiral solvating agent to dissimilarly perturb the NMR spectra of the enantiomeric solutes. Nonequivalence behavior was studied in terms of all variables that affect population and intrinsic spectra of the fast diastereomeric solvates. Assignment of enantiomer configuration was based on the relative field position of the resolved enantiomeric signals. Optimization of experimental conditions provided significant resolved enantiomeric signals suitable for quantification. Utilizing the relative intensities of the corresponding enantiomeric signals of the $N-CH_3$ assigned to (*S*)-(+)-methamphetamine and (*R*)-(−)-desoxyephedrine, the analysis of synthetic enantiomeric mixtures by the proposed methods demonstrated excellent agreements with the known values of the enantiomers present. The mean ± SD recovery values for the (*R*)-(−)-enantiomer was 99.9 ± 0.4% of added antipode ($n = 7$). The optically pure enantiomer was used to establish the minimum amount detected by the proposed NMR spectroscopic method.

1. Introduction

Consistent with the current focus on issues of stereo selectivity, regulatory agencies have placed emphasis on safety and efficacy of stereo isomers in drug research and development (Rauus and Groen 1994). Therefore, there is a growing need for methods to determine drug enantiomeric configuration and purity. This has been one of the most difficult and challenging issues in analytical science, because the two enantiomers have identical chemical and physical properties, except for the opposing directions in which they rotate plane-polarized light. Development of new and improved methods suitable for chiral drugs is more than an academic exercise. Several important chiral drugs demonstrate stereo selective disposition in humans and large differences in therapeutic relevance and toxicity.

Desoxyephedrine synthesis from ephedrine can lead to the formation of two enantiomers. (*S*)-(+)-Desoxyephedrine (methamphetamine) has a strong central nervous system activity. It is a schedule II drug of abuse. The antipode (*R*)-(−)-desoxyephedrine is used as an over-the-counter nasal inhalant decongestant exempted from control in many countries. (1*R*, 2*S*)-(−)-Ephedrine or (1*S*, 2*S*)-(+)-pseudoephedrine and their derivatives have the same absolute α-carbon configuration so that they can both be used as starting substances for methamphetamine manufacturing (Cho 1990). Other methods of synthesis are based on the condensation of phenylacetone with methylamine followed by reduction. Desoxyephedrine obtained by this approach used to be rather less enantiomerically pure or racemic. The mechanism of stimulant effect persists for hours. The inhalation exposure to (*S*)-(+)-desoxyephedrine (methamphetamine) has similar pharmacological characteristics as the intravenous route of administration (Meng et al. 1999). It represents a dangerous agent for abuse by those seeking psycho stimulation (Cho 1990). Death due to overdose has been reported after inhalation intake of methamphetamine and its related compounds. Therefore, the development of convenient and reliable methods for detecting methamphetamine and its antipode (*R*)-(−)-desoxyephedrine for their enantiomeric purity is urgently required and has become an important safety subject in regulatory sciences.

Several methods have been reported for the enantiomeric separation of racemic desoxyephedrine using GC or GC-MS coupled with chiral derivatization, for instance, with N-trifuoroacetyl-L-prolylchloride (Nakahara et al. 1991; Gunne 1967; Liu and Ku 1981; Eiceman et al. 1984; Fitzgerald 1988; Cody and Schwarzhoff 1993) and (*S*)-α-methoxy-α-trifluoromethylphenylacetyl chloride (Mori et al. 1991). Many methods previously reported for the enantiomeric separation of racemic desoxyephedrine by HPLC were carried out by derivatizing the analytes with a chiral reagent, and then separating the diastereomers formed on an achiral stationary phase (Sukbuntherng et al. 1995; Hutchaleelaha et al. 1994; Barksdale and Clark 1985; Miller et al. 1984; Zhou and Krull 1993; Desai and Gal 1993; Sukbuntherng et al. 1995; Gao and Krull 1989; Miller et al. 1984; Noggle Jr and Clark 1986; Matin et al. 1973; Rohl and Trager 1973; Nichols et al. 1973; Gal

1977; Kikura et al. 1992; Noggle Jr and Clark 1986; Noggle Jr et al. 1990; Noggle Jr et al. 1986; Foster and Gilbert 1998). These methods may cause serious error if any optical impurity in derivative reagents is present, since both the diastereomers of RS′ and SR′ have the same chromatographic character. Other chromatographic methods for the enantiomeric separation of racemic desoxephedrine by HPLC were to derivatize desoxyephedrine with an achiral reagent and then separate the derivatives formed on a chiral stationary phase (Wainer et al. 1984; Lee et al. 1986; Hayes et al. 1987; Nagai et al. 1989; Nagai and Kamiyama 1990; Nagai et al. 1992; Lee and Henion 1986; Rizzi et al. 1994). Enantiomeric separation of desoxyephedrine by HPLC using β-cyclodextrin immobilized stationary phase with the addition of organic solvents such as methanol or acetonitrile during the procedure has been reported (Lemur et al. 1996; Katagi et al. 1996). Yukiko et al. (1998) separated desoxyephedrine enantiomers by HPLC on a chiral crown ether-coated stationary phase and mobile phase of pH 1.8 containing $HClO_4$. All the methods mentioned above however are time-consuming and tedious, with low sensitivity detection owing to the complicated procedures involved. Particularly in chiral derivatization, the difference in the rate of the derivatization and enantiomeric purity of the reagents are difficult problems that hamper accurate analysis. Therefore, in an improved stereo specific methodology derivatization and other problems should be avoided.

Nuclear magnetic resonance spectroscopy (NMR) could be used as a routine method for drug analyses. NMR has several advantages over other techniques, including stereo chemical differentiation and its ability to analyze volatile material. Furthermore, the need to use high temperature injectors with techniques may lead to problems such as the thermal decomposition of the components being analyzed. NMR spectroscopic approach enables the discrimination on the differential interactions of each enantiomer with a chiral probe. Chiral lanthanide shift reagents have been employed to resolve the ^1H NMR spectra of desoxyephedrine enantiomers for the determination of the enantiomeric composition (optical purity) (Liu et al. 1981). Salient favorable features of this method were economy of the reagents and procedural steps, non-reliance on reference standards, and freedom from potential racemization. However, it requires the use of absolutely anhydrous reagents, solvents of high purity, and completely anaerobic working environment.

The purpose of this report is to describe an alternative ^1H NMR spectroscopic method free from the stringent requirements imposed by the earlier method. The required resolution of the enantiomeric resonance lines is accomplished through interaction of the enantiomeric mixture with a chiral solvating agent. The NMR signals observed in these binary selector-solute solutions are the time-averaged signals of both the complexed and uncomplexed substances. Optimization of experimental conditions can give rise to the shift displacements coupled with shift nonequivalence. In addition to permitting further simplification of the analytical procedure, this approach is also suitable for establishing the absolute configuration of the enantiomers.

2. Investigations, results and discussion

The chemical shift difference in the NMR spectra between the diastereomeric solvates of corresponding enantiomers may be induced by a chiral solvating agent (CSA) through combination of several factors. First, the diastereomeric solvates may have intrinsically non-identical spectra. Second, if a chemical shift perturbation occurs upon solvation, a difference in equilibrium constants such that one solute enantiomer is solvated to a greater extent than the other can also result in chemical shift nonequivalence. More convoluted situation might be encountered. The solute enantiomers might themselves associate, thus forming a different type of diastereomeric complex, various complexes possibly having no identical spectra or formation constants. Since solute-solute association is a second (or higher) order in solute concentration, whereas solute-CSA association is a first order in solute concentration, the extent to which these two processes compete depends on the concentration of solute enantiomer and the chiral solvating agent. In this study, the effect of solute-solute interaction was kept to a minimum and can be ignored by the combined use of an excess of strong solvating agent with a concentration of solutes that was just enough to produce adequate signal strength. In this situation, when there is enough excess of CSA present to drive the solvation essentially to completion, as contributions originating from a partially resolved solute or differential association constants become minimal. By evaluating nonequivalence under these conditions, one confines its origin to the intrinsic spectral differences of the diastereomeric solvates.

The acidic carbinol function of the chiral solvating agent is expected to interact strongly with a hydrogen bond receptor such as the unshared electron pairs of the nitrogen of the amino group in the enantiomeric solutes. The carbinyl hydrogen, also somewhat acidic because of the electronegative character of the perfluoroalkyl substituent, is predicted to seek interaction with a secondary basic site such as the π electrons clouds of the aromatic rings in the enantiomeric solutes (Alfred 1962). Evidence for conformational control by the interamolecular variation of this type of interactions has been reported (Pirkle et al. 1974). The spectral nonequivalence may be explained in the following way: rapid reversible simultaneous interactions with the hydroxyl and carbonyl hydrogens of the chiral solvating agent with the substrate enantiomers afford conformational mobile diastereomeric solvates. Accordingly, the diastereomer formed from (*S*)-TFAE and (*R*)-desoxyephedrine has the *N*-methyl group cis to the anthryl causing it to experience shielding relative to the trans position. In the related conformation the diastereomer formed from (*S*)-TFAE and (*S*)-desoxyephedrine (methamphetamine) solvate, the N-methyl position is reversed. Consequently, the $N-CH_3$ resonance of the (*S*)-(+)-desoxyephedrine (methamphetamine) solvated with (*S*)-(+)-TFAE appeared at lower field than in the (*R*)-(−)-desoxyephedrine solvated with (*S*)-(+)-TFAE. The opposite was observed for (*R*)-(−)-TFAE as shown in Table 1. The construction and

Table 1: Shift data of the $N-CH_3$ protons signals of the diastereomeric solvates of methamphetamine and (*R*)-(−)-desoxyephedrine solvated with 7 molar equivalents of CSA at 28 °C

	(*S*)- (+)-Desoxyephedrine (Methamphetamine)		(*R*)-(−)-Desoxyephedrine		
CSA	δ	Δδ	δ	Δδ	ΔΔδ
(*R*)- (−)-TFAE	1.727	−0.656	1.749	−0.634	0.022
(*S*)- (+)-TFAE	1.749	−0.634	1.727	−0.656	0.022

[a] Enantiomeric mixture concentration in $CDCl_3$ was 0.02 M

examination of suitable ball-and-stick molecular models of the diastereomeric solvates formed can facilitate the assignments of the absolute configuration. In this fashion, the induced spectral nonequivalence arises from the formation of short-lived diastereomeric solvates that have nonidentical spectra as a consequence of population of rather specific conformations. Knowledge of the structure of these conformations and the absolute configuration of the solvating agent allows assignment of absolute configuration to each of the solute enantiomers on the basis of the sense of nonequivalence induced by the chiral solvating agent. It is on this basis the configuration can be assigned. The NMR approach of absolute configuration determination is demonstrated to be reliable and positive.

The upfield region of the ^{1}H NMR spectra of a mixture of (−)-desoxyephedrine and (+)-methamphetamine with combined concentration of 0.02 M in $CDCl_3$ at 28 °C is shown without CSA and with 0.15 M (*S*)-(+)-TFAE in Fig. 1a and 1b, respectively. Selected expansion of the resolved N−CH_3 enantiomeric signal is shown in Fig. 2. The nonequivalence magnitude was found to depend upon the experimental conditions utilized. It is always desired to obtain nonequivalence magnitudes large enough for accurate quantitative measurements. The effect of varying CSA molar equivalents on the chemical shift, induced shift Δδ and the differential induced shift ΔΔδ of the N−CH_3 signals of (*S*)-(+)-desoxyephedrine and its (*R*)-(−) antipode diastereomeric solvates is shown in Table 2. The induced shift Δδ and the differential induced shift, ΔΔδ, continued to increase with increasing CSA molar equivalents and then tended to level off at higher values. The plots of the induced shift Δδ and differential induced shift ΔΔδ, for the N−CH_3 signals of (*S*)-(+)-desoxyephedrine and its (R)-(-)antipode versus molar equivalents of (*S*)-(+)-TFAE are shown in Fig. 3a and 3b, respectively. The ΔΔδ between the diastereomeric solvates might arise from at least two, (probably mutually dependent) interactions: (a) the differences in equilibrium constants for formation of the various possible diastereomeric solvates between enantiomeric solutes and the CSA and (b) the distinct geometry of resulted solvates. The data in Table 2 provide a qualitative support to conclude that there was only one main contribution of the two types of interactions to the observed ΔΔδ values. The signal for the N−CH_3 of the (*R*)-(−)-enantiomer was shifted upfield to a greater extent than that of the (*S*)-(+)-enantiomer after solvation with (*S*)-(+)-TFAE. The nonequivalence was definitely a reflection of difference in the geometries of the solvates formed and not simply the result of difference in equilibrium constants. The sense of nonequivalence for this particular CSA-solute combination was mainly dependent on the configuration of each component.

The enantiomeric purity of the CSA was found to affect only the magnitude of spectral nonequivalence but not the relative size of the signals stemming from the diastereomeric solvates. No nonequivalence was observed when a racemic CSA was used. Since the CSA need not to be enantiomeric pure for nonequivalence to arise, enantiomeric purity determination by this method was found to be absolute in the sense that no reference to standard of known optical purity was required. Using more enatiomeric pure CSA than the one used, >98%, will contribute only a negligible effect to Δδ or ΔΔδ. Because the amount of intermolecular hydrogen bonding to the CSA-solute interaction is an important factor, dilution induced a dramatic effect on Δδ and ΔΔδ. Slopes of all curves were different at low CSA concentrations for different solute concentrations despite equal CSA/solute ratios. Addition of a small quantity of a polar material such as dimethyl sulfoxide or methanol severely reduced and eliminated nonequivalence. Obviously, the polar material competed with the solute for CSA and probably altered conformations of the solvates that give rise to nonequivalence.

The effect of varying the temperature on the enantiomeric separation was evaluated with a mixture of 0.02 M (*S*)-(+) and (*R*)-(−) enantiomers, solvated with 0.15 M (*S*)-(+)-TFAE in $CDCl_3$. As shown in Fig. 3c and 3d, the degree of Δδ and ΔΔδ of the N−CH_3 signals of the diastereomeric solvates respectively increased in a proportional manner by decreasing the temperature. However, at lower temperatures than −15 °C these signals started to broaden. As it can be seen in Table 3, sufficiently large ΔΔδ values

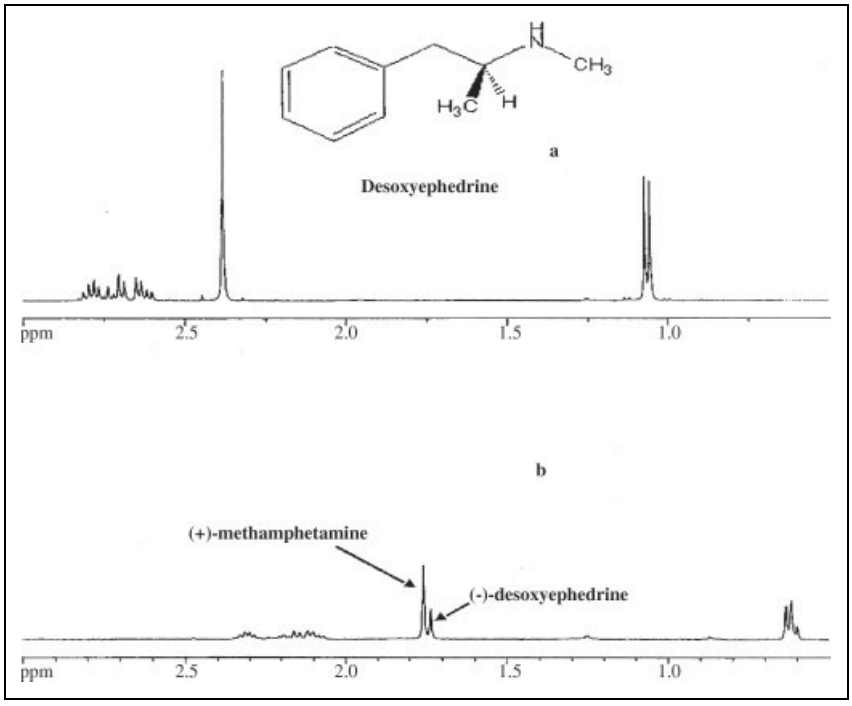

Fig. 1:
Upfield region of the ^{1}H NMR spectra of a mixture of (−)-desoxyephedrine and (+)-methamphetamine (combined concentration 0.02 M) in $CDCl_3$; (a) without CSA and (b) with (*S*)-(+)-TFAE (0.15 M) at 30 °C

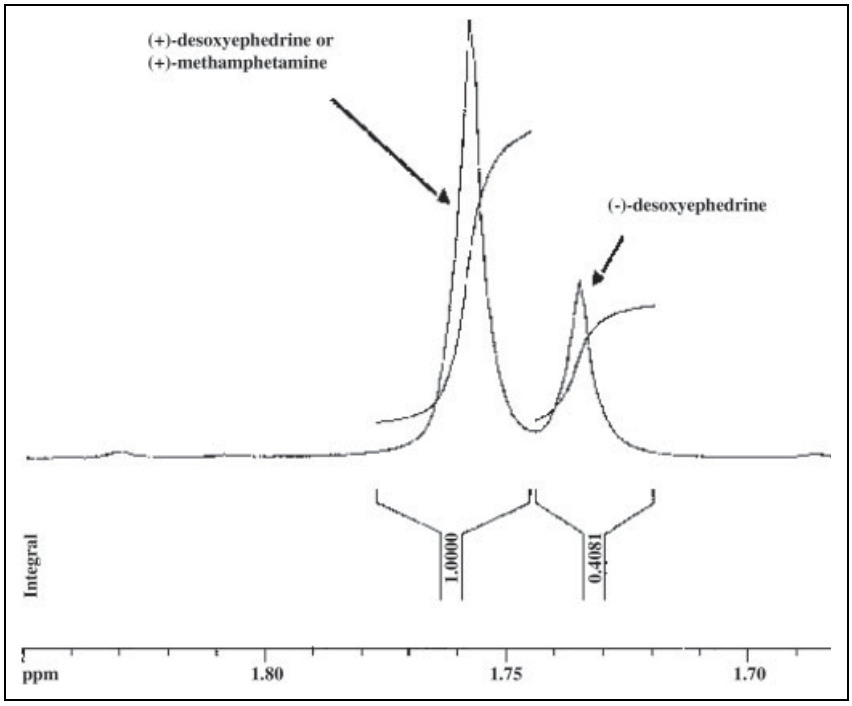

Fig. 2:
Selected expansion of the ^{1}H NMR spectra of a mixture of (−)-desoxyephedrine and (+)-desoxyephedrine (combined concentration 0.02 M) in $CDCl_3$ solvated with (*S*)-(+)-TFAE (0.15 M) at 30 °C

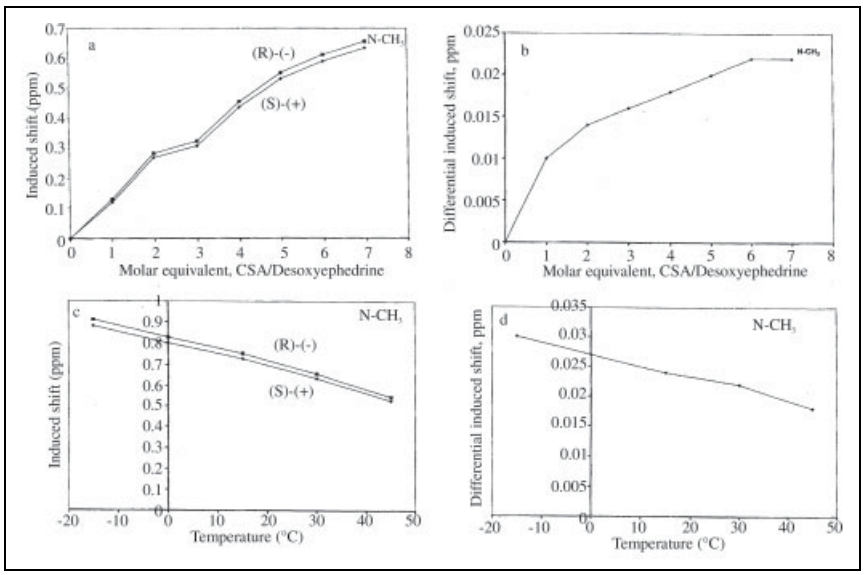

Fig. 3:
Plots of molar equivalent of CSA/desoxyephedrine and temperature versus induced and differential induced shift for (*S*)-(+)-methamphetamine and (*R*)-(−)-desoxyephedrine (combined concentration 0.02 M) in $CDCl_3$

Table 2: Influence of CSA[a] molar equivalents on chemical shift, δ, induced shift, Δδ, and differential induced shift, ΔΔδ, of the N−CH_3 protons signals of the diastereomeric solvates of methamphetamine and (*R*)-(−)-desoxyephedrine at 28 °C

	(*S*)- (+)-Desoxyephedrine Methamphetamine		(*R*)-(−)-Desoxyephedrine		
CSA molar equivalents	δ	Δδ	δ	Δδ	ΔΔδ
0	2.383	0	2.383	0	0
1	2.262	−0.121	2.253	−0.130	0.011
2	2.113	−0.270	2.100	−0.283	0.013
3	2.072	−0.311	2.056	−0.327	0.016
4	1.943	−0.440	1.925	−0.458	0.018
5	1.852	−0.531	1.831	−0.552	0.021
6	1.791	−0.592	1.769	−0.614	0.022
7	1.749	−0.634	1.727	−0.656	0.022

[a] Chiral solvating agent (*S*)-(+)-TFAE
[b] Total enantiomeric mixture concentration in $CDCl_{3\ was}$ 0.04 M

were obtained in the temperature range between 30 °C and −15 °C. Although the increase in nonequivalence magnitude with reduction of temperature can be attributed to an increase in the equilibrium constants for CSA-solute association but not in this case where the CSA was present in such excess to cause essentially complete solvation. Accordingly, temperature reduction increased spectral difference of the diastereomeric solvates by increasing the populations of specific conformations that gave rise to nonequivalence.

^{1}H NMR spectra of 0.02 M solutes and 0.15 M CSA in $CDCl_3$ provided sufficient strength and well separated signals at probe temperature 28 °C convenient for chiral recognition and optical purity determinations. Using (*S*)-(+)-TFAE under the stated conditions, the enantiomeric N−CH_3 signals of the two enantiomers were clearly resolved into two singlets: the downfield signal was assigned to the N−CH_3 methamphetamine or (*S*)-(+)-desoxyephedrine and the upfield signal was assigned to the corresponding N−CH_3 protons of (*R*)-(−)-enantiomer. The

Table 3: Influence of probe temperature on the chemical shift, δ, the induced shift, Δδ, and the differential induced shift, ΔΔδ, of the N−CH_3 protons of methamphetamine and its (*R*)-(−)-antipode[a] solvated with 7.0 molar equivalents of (*S*)-(+)-TFAE

	(*S*)-(+)-Desoxyephedrine, (Methamphetamine)		(*R*)-(−)-Desoxyephedrine		
Temperature, (°C)	δ	Δδ	δ	Δδ	ΔΔδ
45	1.852	−0.531	1.841	−0.542	0.011
28	1.749	−0.634	1.727	−0.656	0.022
15	1.649	−0.734	1.626	−0.757	0.023
0	1.581	−0.802	1.551	−0.832	0.030
−15	1.502	−0.881	1.471	−0.912	0.031

[a] Enantiomeric mixture concentration in $CDCl_3$ was 0.02 M

Table 4: Assay results of synthetic mixtures of (*S*)-(+)-desoxyephedrine (methamphetamine) and (*R*)-(−)-desoxyephedrine[a] by ^{1}H NMR spectroscopy[b] using solvating agent[c]

			(*R*)-(−), %		
Mixture	(*S*)-(+), mg	(*R*)-(−), mg	Added	Found	Recovered[d]
1	0	5.321	100.0	99.3	99.3
2	0.213	4.876	95.8	95.4	99.6
3	0.312	4.765	93.9	94.1	100.2
4	0.376	4.432	91.1	91.0	99.9
5	0.432	4.756	91.7	91.1	99.4
6	0.653	4.549	87.4	87.5	100.1
7	0.752	4.283	85.1	85.6	100.6
Av					99.9
SD					0.4

[a] Total concentration of methamphetamine and its (*R*)-(−)-antipode was 0.02 M in $CDCl_3$
[b] Probe temperature 28 °C
[c] (*S*)-(+)-TFAE concentration was 0.14 M
[d] Recoveries were calculated from (amount found X 100)/amount added

advantages of using ^{1}H NMR spectroscopy for measuring enantiomeric composition lie in the high sensitivity of the ^{1}H nucleus and in the fact that relative signal intensities directly reflect the relative number of resonating nuclei and hence relative enantiomeric populations. Eight mixtures of methamphetamine and (*R*)-(−)-desoxyephedrine antipode, made up in proportions shown in Table 4 were mixed with the specific amounts of chiral solvating agent (*S*)-(+)-TFAE, and dissolved in $CDCl_3$, to yield solutions with ca 0.02 M and 0.15 M solute and CSA concentrations, respectively. The results of enantiomeric composition calculated based on the integrals of the N−CH_3 protons were found in close agreement with the known values. Average recovery ±SD for the (*R*)-(−)-enantiomer was 99.9 ± 0.4%. The optically pure was found to contain 0.7% of (*S*)-(+)-enantiomer.

3. Experimental

3.1. Apparatus

^{1}H NMR spectra were obtained on a Bruker AMX-400 spectrometer equipped with a carbon/proton 5 mm probe (Bruker Instruments, Inc., Billerica, MA, USA). The ^{1}H NMR spectra were obtained under the following conditions: acquisition time, 2.03 s; data point resolution, 0.492 Hz/point; pulse width, 7.0 μs and tip angle 30°; relaxation delay, 2.0 s; number of scans, 32. Chemical shifts were referred to $CHCl_3$ (δ 7.26).

3.2. Materials

Deuterochloroform ($CDCl_3$, 99.8 atom% D, stabilized with Ag foil), and (*R*)-(−)- and (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl) ethanol (TFAE; >99% chemical purity and >98% optical purity), (−)-desoxyephedrine and (+)-methamphetamine HCl (>99%) were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). The samples were analyzed by polarimetry and the proposed NMR method.

3.3. Sample preparation

Salts were converted to the free base form as follows: a quantity of the drug, accurately weighed was dissolved to the extent of 1.5 ml of D_2O. Drop of 1.5 M NaOD was added to $CDCl_3$ (1.0 ml). Both solutions were bubbled with argon and then combined. The $CDCl_3$ layer was removed and evaporated to dryness. The sample was dried in vacuum at 50 °C for approximately 30 min and weighed.

3.4. Enantiomeric resolution studies

Conditions for the determination of the enantiomeric composition were explored by observing the N−CH_3 proton signal and study : (a) the effect of varying the chiral solvating agent, CSA, molar equivalents and (b) the effect of probe temperature on chemical shift δ, induced chemical shift Δδ, and differential induced chemical shift ΔΔδ.

The required changes in CSA molar equivalents were obtained by first preparing stock solutions of enantiomeric mixture of desoxyephedrine sample (ca. 30.0 mg/ml) and (*S*)-(+)-TFAE (ca.110.6 mg/ml) in $CDCl_3$. A 0.1 ml of sample (3.0 mg) solution and the appropriate amount of (*S*)-(+)-TFAE solution (50, 100, 150, 200, 250, 300, and 350 μL) were added to a 5-mm NMR tube. The final volume was adjusted with $CDCl_3$ to 1.0 ml. The NMR tube was capped with a Teflon cap; its contents were mixed by inversion, allowed to stand for 10 min, and then placed in the spectrometer to obtain the ^{1}H NMR spectrum. The additions and spectral recording were repeated until an appropriate number of spectra were available for properly defining the effects of CSA molar equivalents on the enantiomeric spectral lines.

3.5. Determination of enantiomeric purity

A quantity of desoxyephedrine sample of approximately 5 mg was converted to the free base as described above. The dry residue was dissolved in 0.5 ml $CDCl_3$, and the solution was transferred to a dry NMR tube containing approximately 52 mg of (*S*)-(+)-TFAE. The final volume was adjusted to 1.0 ml and then the tube was capped, inverted several times to effect solution, allowed to stand for 10 min, and then used to obtain the ^{1}H NMR spectrum. The intensities of enantiomeric N−CH_3 protons enantiomeric signals at approximately δ1.749 and δ1.727 corresponding to methamphetamine and (*R*)-(−)-antipode, respectively, were measured and the percentage of each enantiomer was calculated based on the contribution of each resonance to the sum of both resonances as follows:

$$\%(R)\text{-}(-)\text{-desoxyephedrine} = \frac{100 \times A_{(-)}}{A_{(-)} + A_{(+)}} \quad (1)$$

$$\%\ \frac{(S)\text{+}(-)\text{-desoxyephedrine}}{(\text{methamphetamine})} = \frac{100 \times A_{(+)}}{A_{(+)} + A_{(-)}} \quad (2)$$

where $A_{(+)}$ = area of the resonance signal for the N−CH_3 of the (*S*)-(+)-enantiomer (methamphetamine), and $A_{(-)}$ = area of the resonance signal for the N−CH_3 protons of the (*R*)-(−)-enantiomer.

References

Barksdale JM, Clark CR (1985) Liquid chromatographic determination of the enantiomeric composition of amphetamine and related drugs by diastereomeric derivatization. J Chromatogr Sci 23: 176–180.

Cho AK (1990) Ice: A new dosage form of an old drug. Science 249: 631–634.

Cody JT, Schwarzhoff R (1993) Interpretation of methamphetamine and amphetamine enantiomer data. J Anal Toxicol 17: 321–326.

Desai DM, Gal J (1993) Enantiospecific drug analysis via the ortho-phthalaldehyde/homochiral thiol derivatization method. J Chromatogr 629: 215–228.

Eiceman GA, Leasure CS, Selim SL (1984) Quantitative investigation of rapid injector port derivatization of amphetamine using trifluoroacetic anhydride with packed and capillary column GC and GC/MS methods. J Chromatogr Sci 22: 509–513.

Fitzgerald RL, Ramos JM Jr, Bogema SC, Poklis A (1988) Resolution of methamphetamine stereoisomers in urine drug testing: urinary excretion of *R*(−)-methamphetamine following use of nasal inhalers. J Anal Toxicol 12: 255–259.

Foster BS, Gilbert DD (1998) Enantiomeric determination of amphetamine and methamphetamine in urine by precolumn derivatization with Marfey's reagent and HPLC. J Anal Toxicol 22: 265–269.

Gal J (1977) Stereochemistry of metabolism of amphetamines: use of (−)-alpha-methoxy-alpha-(trifluoromethyl)phenylacetyl chloride for GLC resolution of chiral amines. J Pharm Sci 66: 169–1732.

Gao CX, Krull IS (1989) Determination of enantiomeric drugs in physiological fluids using on-line solid phase derivatization and reversed-phase liquid chromatography. J Pharm Biomed Anal 7: 1183–1198.

Gunne LM (1967) The urinary output of d- and l-amphetamine in man. Biochem Pharm 16: 863- 869.

Hayes SM, Liu RH, Tsang WS, Legendre MG, Berni RJ, Pillion DJ, Barnes S, Ho MH (1987) Enantiomeric composition analysis of amphetamine and methamphetamine by chiral phase high-performance liquid chromatography-mass spectrometry. J Chromatogr 398: 239–246.

Hutchaleelaha A, Walters A, Chow H, Mayersohn M (1994) Sensitive enantiomer-specific high-performance liquid chromatographic analysis of methamphetamine and amphetamine from serum using precolumn fluorescent derivatization. J Chromatogr B 658: 103–112.

Katagi M, Nishika H, Nakajima K, Tsuchihashi H, Fujima H,Wada H, Nakamura K, Makino K (1996) Direct high-performance liquid chromatographic and high-performance liquid chromatographic-thermo spray-mass spectrometric determination of enantiomers of methamphetamine and its main metabolites amphetamine and *p*-hydroxymethamphetamine in human urine. J Chromatogr B 676: 35–43.

Kikura R, Ishigami A, Nakahara Y (1992) Studies on comparison of metabolites in urine between deprenyl and methamphetamine. III. Enantiomeric composition analysis of metabolites in mouse urine by HPLC with GITC chiral reagent. Jpn J Toxicol Environ Health 38 : 136–141.

Lee ED, Henion JD, Brunner CA, Wainer IW, Doyle TD, Gal J (1986) High-performance liquid chromatographic chiral stationary phase separation with filament-on thermo spray mass spectrometric identification of the enantiomer contaminant (*S*)-(+)-methamphetamine. Anal Chem 58: 1349–1352.

Lemur K, Jirovsky D, Seveik J (1996) Effect of some parameters on enantiomer separation of ephedrine, methamphetamine and selegiline using HPLC with β-cyclodextrin stationary phase. J Liq Chromatogr Relat Technol 19: 3173–3191.

Liu JH, Ku WW (1981) Determination of enantiomeric N-trifluoroacetyl-L-prolyl chloride amphetamine derivatives by capillary gas chromatography/mass spectrometry with chiral and achiral stationary phases. Anal Chem 53: 2180–2184.

Liu JH, Ramesh S, Tsay JT, Ku WW, Fitzgerald MP, Angelos SA, Lins CLK (1981) Approaches to drug sample differentiation. II: Nuclear magnetic resonance spectroscopic determination of methamphetamine enantiomers. J Forensic Sci 26: 656–663.

Makino Y, Ohta S, Hirobe M (1996) Enantiomeric separation of amphetamine by high-performance liquid chromatography using chiral crown ether-coated reversed-phase packing: application to forensic analysis. Forensic Sci Int 78: 65–70.

Matin SB, Rowland M, Castagnoli Jr M (1973) Synthesis of N-pentafluorobenzoyl-*S*-(−)- prolyl 1-imidazolide, a new electron-capture sensitive reagent for determination of enantiomeric composition. J Pharm Sci 62: 821–823.

Meng Y, Dukat M, Bridgen DT, Martin BR, Lichtman AH (1999) Pharmacological effects of methamphetamine and other stimulants via inhalation exposure. Drug Alcohol Depend 53: 111–120.

Miller KJ, Gal J, Ames MM (1984) High-performance liquid chromatographic resolution of enantiomers of 1-phenyl-2-aminopropanes (amphetamines) with four chiral reagents. J Chromatogr 307: 335–342.

Nagai T, Kamiyama S (1990) Assay of the optical isomers of methamphetamine and amphetamine in rat urine using high-performance liquid chromatography with chiral cellulose-based columns. J Chromatogr 525: 203–209.

Nagai T, Kamiyama S, Kurosu A, Iwamoto F (1992) Identification of optical isomers of methamphetamine and its application to forensic medicine. J Legal Med 46: 244–253.

Nagai T, Sato M, Nagai T, Kamiyama S, Miura Y (1989) A new analytical method for stereoisomers of methamphetamine and amphetamine and its application to forensic toxicology. Clin Biochem 22: 439–442.

Nakahara Y, Takahashi K, Ishigami A, Kikurai R, Shimamine M (1991) Studies on comparison of metabolites in urine between deprenyl and methamphetamine administration. II. Enantiomeric composition analysis of metabolites in mouse urine administered with deprenyl and methamphetamines by GC-MS using chiral reagent. Jpn J Toxicol Environ Health 37: 473–479.

Nichols DE, Barfknecht CF, Rusterholz DB, Bwnington R, Morin RD (1973) Asymmetric synthesis of psychotomimetic phenylisopropylamines. J Med Chem 16: 480–483.

Noggle Jr. FT, Clark CR (1986) Resolution of some enantiomeric amines of forensic interest by high-performance liquid chromatography. J Forensic Sci 31: 732–742.

Noggle Jr FT, DeRuiter J, Clark CR (1990) Methods for the analysis and characterization of forensic samples containing amphetamines and related amines J Chromatogr Sci 28: 529–536.

Noggle Jr FT, DeRuiter J, Clark CR (1986) Liquid chromatographic determination of the enantiomeric composition of methamphetamine prepared from ephedrine and pseudoephedrine. Anal Chem 58: 1643–1648.

Pirkle WH, Beare SD, Muntz RL (1974) Assignment of absolute configuration of sulfoxides by NMR a salvation model. Tetrahedron Lett 26: 2295–2298.

Pohl LR, Trager WF (1973) A new chiral reagent for the determination of nantiomeric purity and absolute configuration of certain substituted arylethylamines. J Med Chem 16: 475–479.

Rauus AG, Groen K (1994) Current regulatory (draft) guidance on chiral medicinal products: Canada, EEC, Japan, United States. Chirality 6: 72–75.

Rizzi AM, Hirz R, Cladrowa-Runge S, Jonsson H (1994) Enantiomeric separation of amphetamine, methamphetamine and ring substituted amphetamines by means of a β-cyclodextrin-chiral stationary phase. Chromatographia 339: 31–37.

Sukbuntherng J, Hutchaleelaha A, Chow HH, Mayersohn M (1995) Separation and quantitation of the enantiomers of methamphetamine and its metabolites in urine by HPLC: precolumn derivatization and fluorescence detection. J Anal Toxicol 19: 139–147.

Wainer IW, Doyle TD, Adams WM (1984) Liquid chromatographic chiral stationary phases in pharmaceutical analysis: determination of trace amounts of the (−)-enantiomer in (+)-amphetamine. J Pharm Sci 73: 1162–1164.

Zhou FX, Krull IS (1993) Direct enantiomeric analysis of amphetamine in plasma by simultaneous solid phase extraction and chiral derivatization. Chromatographia 35: 153–159.