Liquid Chromatographic Analysis of the Enantiomeric Impurities in Various (+)-Pseudoephedrine Samples

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An HPLC method has been developed for the separation of four stereoisomers of ephedrine using precolumn derivatization with S(+)-1-(1-naphthyl)-ethyl isocyanate. The formed derivatives are subsequently separated on a normal-phase column and are detected at a UV wavelength of 220 nm. This method was used to quantitate the differences in the enantiomeric impurity of various samples of (+)-pseudoephedrine. The reported method can differentiate between samples of (+)-pseudoephedrine which differ in their enantiomeric impurity by as little as 0.02%. Possible racemization of (+)-pseudoephedrine in aqueous solutions was also studied. Samples of (+)-pseudoephedrine from various suppliers and, indeed, different lots from the same supplier, differed significantly in their degree of enantiomeric impurity.

KEY WORDS: high-performance liquid chromatography; enantiomeric purity; optical purity; enantiomeric separation; ephedrine; pseudoephedrine; precolumn derivatization; S(+)-1-(1-naphthyl)-ethyl isocyanate.

INTRODUCTION

The liquid chromatographic determination of the enantiomeric purity of a drug may be carried out by direct or indirect methods. In the direct method, separation is carried out on a chiral column. The indirect method, on the other hand, is based on the formation of the derivative of each enantiomer with a chiral reagent, and subsequent separation of the derivatives on an achiral column. Both these methods suffer from certain shortcomings. For instance, separation on chiral columns, which are usually expensive, is sometimes very sensitive to several factors such as the composition of the mobile phase, temperature, flow rate, and pH (1). Furthermore, most of the compounds must be derivatized by a nonchiral reagent to enhance the separation on the chiral column. On the other hand, the absolute determination of the trace enantiomeric impurity by the indirect method is open to question until the enantiomeric purity of the reagent and its lack of racemization have been established.

Most of the commercially available reagents are not subject to spontaneous racemization and are relatively pure (>98%). However, even a small percentage of enantiomeric impurity can result in substantial errors when the reagent is used for the determination of small amounts of one enantiomer in the presence of its antipode. Therefore, in the absence of quantitative information on the purity of the reagent, the indirect method cannot be utilized for the determination of the absolute enantiomeric purity (2). However, this method may be used to determine quantitative differences between samples of varying enantiomeric purity; therefore, the method can be potentially useful for (a) comparing the samples obtained from different batches of the same drug prepared under identical conditions, (b) analyzing crystals obtained from media containing varying quantities of the opposite enantiomer (3), and (c) studying the rate of inversion of a drug in solution or a formulation (4).

Although recent literature describes many methods for the separation of ephedrine and pseudoephedrine enantiomers by indirect (5–8) and direct (9–13) methods, none of these studies reports the quantification of traces of the opposite enantiomer in the presence of a large quantity of its antipode. We report here an indirect method which can separate the four stereoisomers of ephedrine. Furthermore, the method can differentiate between samples of (+)-pseudoephedrine which differ by as little as 0.02% in their enantiomeric impurity. This method can also be extended to determination of the enantiomeric impurities in (−)-pseudoephedrine and (−)- and (+)-ephedrines.

MATERIALS AND METHODS

Materials

(+)-Pseudoephedrine was obtained from three sources [Sigma Chemical Company (St. Louis, MO), Aldrich Chemical Company Inc. (Milwaukee, WI), and Fluka Chemical Corp. (Ronkonkoma, NY]. The (−)- and (+)-ephedrine and (−)-pseudoephedrine were obtained from Sigma Chemical Company (St. Louis, MO), R(−)- and S(+)-1-(1-naphthyl)ethyl isocyanate and 2-methyl-1-propanol ACS, 99+%, were obtained from Aldrich Chemical Company Inc. (Milwaukee, WI). Hexane and isopropanol were of HPLC grade and were obtained from Fisher Scientific (Fair Lawn, NJ) and Mallinckrodt Inc. (Paris, KY), respectively.

Sample Preparation

The method of sample preparation is a modification of a method reported for analysis of propafenone enantiomers (14). To 200 μl of the drug solution of known concentration (e.g., 0.5 mg/ml for purity determinations) in a glass test tube 200 μl of a saturated solution of sodium carbonate was added. Then 4 ml of hexane:isopropanol (95:5, v/v) mixture was added to the aqueous solution, which was then vortexed for 1 min. The tubes were centrifuged at 2000 rpm for 5 min. The organic phase was transferred to another glass tube and evaporated under nitrogen. To the residue in the tube 100 μl of the reagent [0.1% of S(+)-1-(1-naphthyl)-ethyl isocyanate in hexane] and 20 μl of isopropanol were added and the mixture was vortexed for 5 sec. The tubes were then allowed to stand at room temperature for 2 min. Twenty microliters

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of the above solution was then injected onto the HPLC column.

Chromatographic Method

The HPLC system (Waters, Milford, MA) included a 510 HPLC pump, a U6K manual injector, and a 994 photodiode array detector equipped with a printer and plotter. The detection wavelength was set at 220 nm. The mobile phase consisted of hexane:isopropanol:2-methyl-1-propanol (96:2:2, v/v) and the flow rate was 1.5 ml/min. The HPLC column was a silica column (Partisil 5 RAC II; dimensions, 4.6 × 10 cm) obtained from Whatman Inc. (Clifton, NJ).

Statistical Analysis

All data were analyzed by ANOVA ($\alpha = 0.05$). Individual means were compared by the Bonferroni procedure ($\alpha = 0.05$) (15) for multiple comparisons in a completely randomized design. Data are reported as mean ± SD.

RESULTS AND DISCUSSION

Separation of the Four Ephedrine Stereoisomers

The four stereoisomers, *viz.*, (+)- and (−)-pseudoephedrine and (+)- and (−)-ephedrine, can be separated on the silica column after derivatization with *S*(+)-1-(1-naphthyl)-ethyl isocyanate (Fig. 1). The capacity factors (k') and the separation factors (α) are given in Table I. If the retention times of the peaks of the two enantiomers of a drug are close, it is preferable that the impurity elutes before the major component. This order allows more accurate integration of the impurity peak (16) and a lower limit of detection and may be accommodated by the proper selection of the appropriate enantiomer of the reagent. In our experiments, the use of the *R*(−) enantiomer instead of the *S*(+) enantiomer of the reagent reversed the elution order of the enantiomers. However, in both cases, the isomers of pseudoephedrine eluted before those of ephedrine.

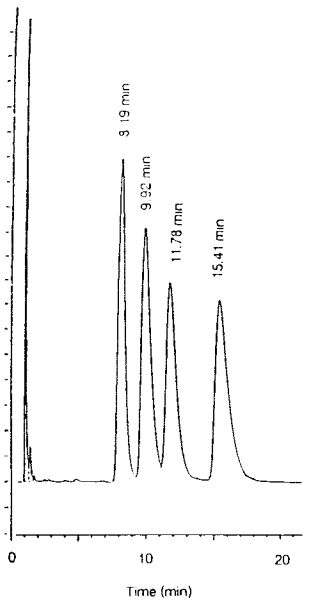

Fig. 1. Separation of the four isomers of ephedrine and their retention times, in the eluting order, (−)-pseudoephedrine (8.19 min), (+)-pseudoephedrine (9.92 min), (−)-ephedrine (11.78 min), and (+)-ephedrine (15.41 min).

Table I. Capacity Factors (k') and Selectivity Values (α) for the Four Isomers of Ephedrine

	Capacity factor	Selectivity value
(−)-Pseudoephedrine	7.02	
		1.24
(+)-Pseudoephedrine	8.72	
		1.21
(−)-Ephedrine	10.54	
		1.33
(+)-Ephedrine	14.10	

Reproducibility of the Method

(+)-Pseudoephedrine base (Sigma Chemical Company, lot 86F-0441) recrystallized from water was chosen as the reference material since it was the purest available commercially. The enantiomeric impurity level in the reference material was determined after 1 and 7 days. Table II gives the percentage area fraction calculated by Eq. (1) on different days.

$$\% \text{ area fraction} = \frac{\text{area under (}-\text{)-pseudoephedrine peak}}{\text{area under (}-\text{)-pseudoephedrine peak} + \text{area under (}+\text{)-pseudoephedrine peak}} \times 100 \quad (1)$$

No statistically significant difference was observed in the area fraction measured. This also suggests that the enantiomeric inversion of the reagent in hexane over a period of 7 days is very slow, if it occurs, and cannot be detected by this method.

Sensitivity of the Method

To determine the sensitivity of the method for differentiating between samples of (+)-pseudoephedrine containing different quantities of the opposite enantiomer [(−)-pseudoephedrine], a known quantity of the latter was added to the solution of a pure reference drug substance. The area fractions were then calculated from the peak areas according to Eq. (1). Initially, about 0.1% of the enantiomeric impurity was added. The amount added to the subsequent samples was decreased until no statistically significant difference was seen between the area ratios of the impurity added samples

Table II. Day-to-Day Reproducibility of the Amount of (−)-Pseudoephedrine Present as an Impurity in the Reference Sample of (+)-Pseudoephedrine[a] Measured by the Reported Method

Time of measurement (day)	% area fraction measured (mean ± SD)[b]
0	0.234 ± 0.008
1	0.242 ± 0.013
7	0.229 ± 0.008

[a] See text for details of the reference material.
[b] $n = 6$.

and the reference material. The minimum impurity difference that the method can detect is defined as the sensitivity of this method. The results of the experiments with differing values of added impurity are presented in Fig. 2, which shows the plot of percentage impurity added and percentage area fraction measured. The difference in the area fractions is statistically significant when the percentage impurity added is >0.023%. However, the method was not able to detect any statistically significant differences when the samples differed by 0.011%. So the method can differentiate between samples which differ in their enantiomeric impurity by as little as 0.023% at or above 0.2% level. The relationship between the percentage impurity added (x) and the area fractions (y) could be described by Eq. (2):

$$y = 0.228 + 1.20\,x \quad (r^2 = 0.990) \qquad (2)$$

Using this relationship, the back-calculated values for the percentage impurity added are 0.108 ± 0.015, 0.054 ± 0.009, and 0.025 ± 0.009 for the samples which actually contained 0.107, 0.052, and 0.023% of the added impurities, respectively. The measured impurity values are in excellent agreement with the added values which attests to the accuracy of the method. The intercept value (0.228) is a combination of the enantiomeric impurity in the reagent and/or in the sample. The inversion of the reagent and/or the sample during derivatization may also contribute to the intercept value.

Figure 3 depicts a chromatogram of the reference material to which no impurity had been added. The small peak eluting at a retention time identical to the derivatized (−)-pseudoephedrine may be ascribed to the presence of the enantiomeric impurity in the reference material and/or in the reagent.

Possible Racemization in Aqueous Solutions

To investigate the possibility of racemization, an aqueous solution containing 0.5 mg/ml (+)-pseudoephedrine was prepared and maintained at 22°C. Samples were taken on days 1, 2, and 7 and analyzed for the enantiomeric impurity. Table III gives the percentage area fraction measured at 22°C over a period of 7 days. The inversion was very slow and about 0.065% inversion had taken place in 7 days. The measured value after 1 day was slightly greater than the initial value, but this difference was not statistically significant. No diastereomeric impurities were detected after 7 days. Since our crystal engineering experiments involve heating the solution to about 60°C, an experiment was also carried out to determine the extent of enantiomeric inversion when the samples were maintained at 60°C for 2 hr. The percentage areas measured before and after 2 hr of heat treatment at 60°C were 0.229 ± 0.008 (n = 6) and 0.234 ± 0.009 (n = 6), respectively; the difference was not statistically significant. Therefore, it appears that heating in our crystal engineering experiments, to be reported elsewhere, does not cause any detectable inversion.

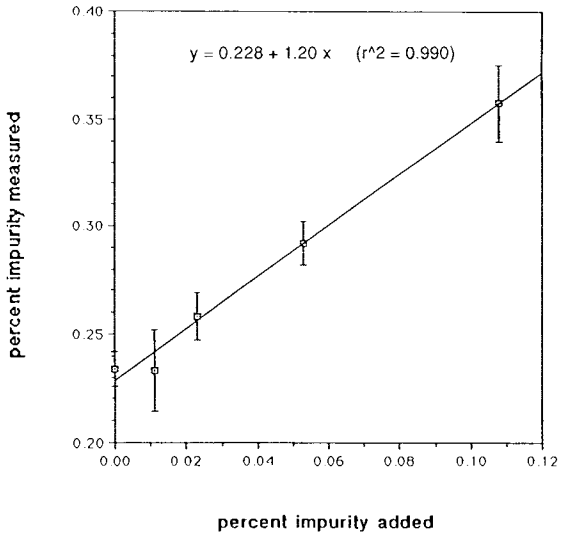

Fig. 2. Effect of the percentage of (−)-pseudoephedrine present as an added impurity in (+)-pseudoephedrine on the resulting percentage area fraction measured by the reported method.

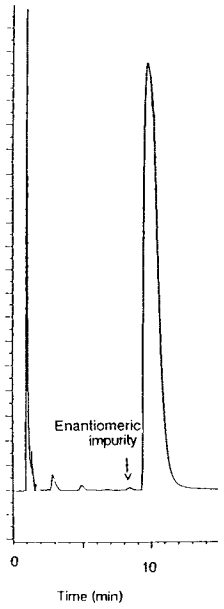

Fig. 3. Chromatogram of the (+)-pseudoephedrine reference material showing the presence of a trace quantity of the opposite enantiomer, (−)-pseudoephedrine.

Enantiomeric Impurities in (+)-Pseudoephedrine Samples from Various Sources

(+)-Pseudoephedrine from the various sources mentioned in the materials section were analyzed for the enan-

Table III. Percentage Area Fraction Due to (−)-Pseudoephedrine Measured over a Period of 7 Days in Aqueous Solutions of the Reference Sample of (+)-Pseudoephedrine[a]

Time maintained at 22°C (day)	% area fraction measured (mean ± SD)[b]
0	0.229 ± 0.008
1	0.240 ± 0.010
7	0.294 ± 0.007

[a] See text for the details of the reference material.
[b] n = 6.

Table IV. Comparison of the Level of (−)-Pseudoephedrine, Present as the Enantiometric Impurity, in (+)-Pseudoephedrine Samples from Various Suppliers

Supplier and lot No.	Percentage area fraction measured (mean ± SD)[a]
Sigma, lot 86F-0441	0.229 ± 0.008
Sigma, lot 107F-0269	0.252 ± 0.007
Aldrich, lot EX 10423 KW	0.290 ± 0.020
Fluka, lot 470220	0.273 ± 0.013

[a] $n = 6$.

tiomeric impurity. The results are summarized in Table IV. Of particular interest is the difference in the enantiomeric purity between two lots, *viz.*, lot 86F-0441 and lot 107F-0269, from the same supplier, Sigma Chemicals. The two lots differ statistically from each other. The sample from Sigma Chemical Company (lot 86F-0441) contained a significantly smaller percentage of the opposite enantiomer than the samples from Aldrich and Fluka Chemicals. This shows the potential utility of this type of method for monitoring enantiomeric impurities in different batches of pharmaceutical raw materials.

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