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NEW DERIVATIZATION REAGENT FOR THE RESOLUTION OF OPTICAL ISOMERS IN DILTIAZEM HYDROCHLORIDE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

An highly optically pure derivatization reagent, *S*(−)N-1-(2-naphthylsulphonyl)-2-pyrrolidinecarbonyl chloride, has been developed for converting enantiomers into diastereomers for subsequent resolution by high-performance liquid chromatography. The diastereomers formed from four optical isomers of diltiazem hydrochloride and this chiral reagent by hydrolysis and Schotten–Baumann reaction were completely resolved by adsorption chromatography with ultraviolet detection. Three optical impurities in diltiazem hydrochloride were successfully determined with sufficient sensitivity by the method.

INTRODUCTION

High-performance liquid chromatography (HPLC) has been widely used for the separation of enantiomers. Recently much work on the direct resolution of enantiomers by HPLC has been reported, using either a chiral stationary phase[1–5] or a chiral mobile phase[6,7]. These methods are still in the development stage and it is very difficult to determine small quantities of optical antipodes in pharmaceutical compounds for quality control.

Therefore we have developed highly optically pure chiral reagents for converting enantiomers into diastereomers for resolution by HPLC. It is necessary for such a reagent to have the following features: high sensitivity to UV detection, high reactivity under mild conditions; resulting diastereomers should be readily resolved on a conventional column. Another favourable feature would be that the chiral reagent exists in the solid state at room temperature.

We synthesized several chiral reagents from L-amino acids. Of these, *S*(−)N-1-(2-naphthylsulphonyl)-2-pyrrolidinecarbonyl chloride was the most suitable. This paper describes its synthesis and its application to the determination of optical iomers in diltiazem hydrochloride by HPLC.

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EXPERIMENTAL

Materials

The synthesis of diltiazem hydrochloride (I) and its optical isomers has been reported previously[8–10]. Dichloromethane (special grade) was washed with water and dried over anhydrous calcium chloride prior to use.

All other solvents and reagents were of special grade.

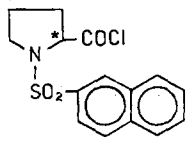
Apparatus

The instruments were a Shimadzu LC-3A liquid chromatograph and an UV-2 absorbance detector, monitoring at 254 nm, equipped with an IBM Series 1 computer. The samples were applied to the column by a 20-μl loop injector. An Hitachi M-80A mass spectrometer was equipped with a M-003 computer system and operated in the electron-impact mode at 20 eV. The column was a Zorbax Sil (15 cm × 4.6 mm I.D.) (DuPont, Wilmington, DE, U.S.A.) and the mobile phases were dichloromethane–ethyl acetate (100:9) (A) and chloroform–dichloromethane–methanol–diethylamine (200:50:30:0.1) (B).

Synthesis of derivatization reagent

S(−)N-1-(2-naphthylsulphonyl)-2-pyrrolidinecarboxylic acid (II). A solution of 24.5 g of 2-naphthalenesulphonyl chloride in 186 ml of diethyl ether was slowly added to a solution of 10.35 g of L-proline and 37.25 g of potassium carbonate in 216 ml of water cooled in ice, and the reaction mixture was stirred for 2 days. The aqueous layer was then separated from the ether layer, washed with diethyl ether, acidified to *ca.* pH 2 with 10% hydrochloric acid and the resulting crystalline product extracted with 500 ml of ethyl acetate. The organic layer was washed with water saturated with sodium chloride, dried over anhydrous sodium sulphate and evaporated. Recrystallization of the crude product from benzene gave 15.6 g product (m.p. 133–135°C).

S(−)N-1-(2-naphthylsulphonyl)-2-pyrrolidinecarbonyl chloride (III). Oxalyl chloride (17.5 ml) was added to a solution of 15.6 g of compound II in absolute benzene and stirred overnight at 40–50°C. The benzene was evaporated *in vacuo* and recrystallization of the crude product from benzene–hexane gave compound III [m.p. 107–109°C; $[\alpha]_D^{20}$ − 81.6° (*c* = 1, chloroform); calculated for $C_{15}H_{14}ClNO_3S$: C, 55.77%; H, 4.37%; N, 4.34%; found: C, 55.60%; H, 4.34%; N, 4.30%; IR spectrum (Nujol): 1780, 1340, 1205 and 1150 cm^{-1}; mass spectrum: *m/z* 325 and 323 (M^+)].



(III)

Derivatization

Method A. L-Proline methyl ester (20 mg) was weighed into a 100-ml volumetric flask, dissolved in dry dichloromethane and made up to 100 ml in the same

solvent. A 3-ml volume of this solution was measured into a glass-stoppered flask, and 20 μl of triethylamine and 10 mg of compound III were added. After mixing, the mixture was allowed to stand at room temperature for 10 min. Chloroform was added to give a volume of 10 ml. Then an aliquot was injected into the liquid chromatograph.

Method B. Diltiazem hydrochloride (0.1 g) was weighed into a 50-ml volumetric flask, 5 ml of 1 *M* sodium hydroxide solution were added and the volume adjusted to 50 ml with methanol. After shaking, the mixture was allowed to stand at room temperature for 1 h. This solution (10 ml) was placed in a centrifuge-tube and methanol was evaporated *in vacuo* at room temperature. Chloroform (10 ml) and water (20 ml) were added and equilibrated by shaking. The chloroform layer (1.5 ml) was measured into a glass-stoppered flask. The chloroform was evaporated and the residue obtained was dried *in vacuo* for 1 h. The residue was dissolved in 0.5 ml of dry dichloromethane, 20 μl of pyridine and 20 mg of compound III were added and the resulting solution was allowed to stand at room temperature for 15 min. Chloroform was then added to give a volume of 20 ml. An aliquot was injected into the liquid chromatograph.

RESULTS AND DISCUSSION

The applicability of several chiral reagents prepared from L-amino acids for the resolution of the optical isomers of diltiazem hydrochloride by HPLC was investigated. It was shown that the reaction of deacetyl-I with chiral reagent III proceeded under weekly basic conditions without any formation of by-products. There-

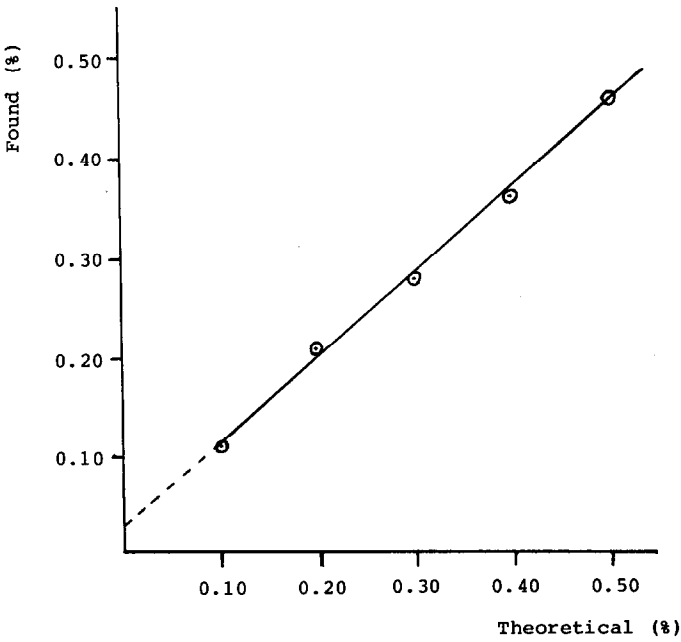


Fig. 1. Relationship between the known content (%) of L-proline methyl ester and that found by HPLC.

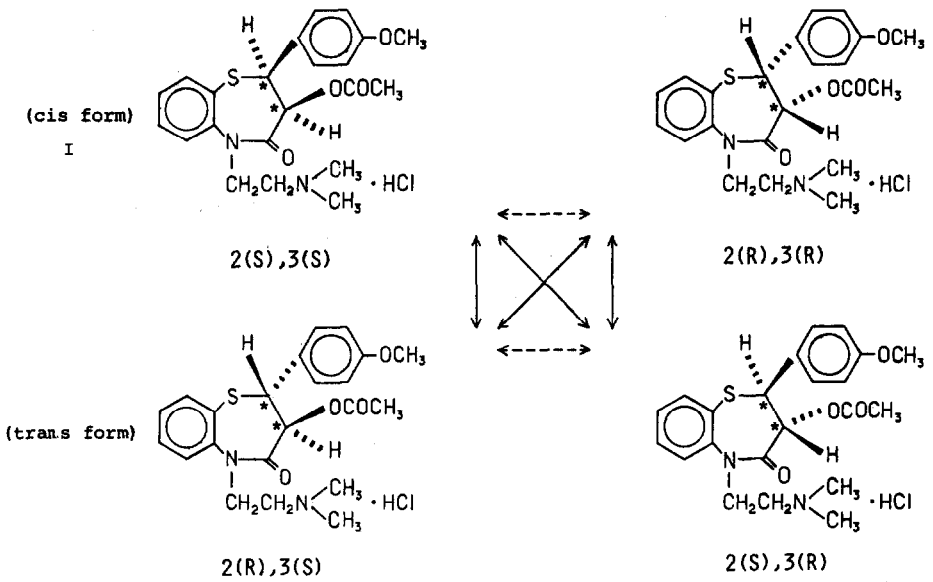


Fig. 2. Diastereomeric (↔) and enantiomeric (<------>) relationships among the optical isomers of compound I.

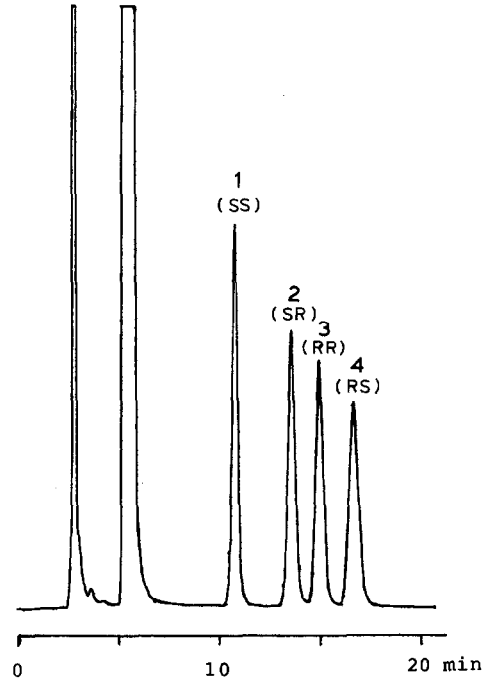


Fig. 3. Separation of the diastereomeric esters prepared from *dl-cis*-I, *dl-trans*-I and chiral reagent III. Peaks: 1 = *d-cis*-I-III; 2 = *l-cis*-I-III; 3 = *d-trans*-I-III; 4 = *l-trans*-I-III.

TABLE I

RECOVERY (%) OF EACH ISOMER ADDED TO *d-cis*-I

Isomer	*Added**	*Recovery***	*Difference*
l-cis-I	0.50	0.53	+0.03
	1.01	0.99	−0.02
	2.01	1.91	−0.10
	3.03	3.00	−0.03
	4.00	4.07	+0.07
	5.06	4.72	−0.34
d-trans-I	0.50	0.43	−0.07
	1.00	0.94	−0.06
	1.99	1.89	−0.10
	3.00	2.88	−0.12
	3.97	3.85	−0.12
	5.02	4.44	−0.58
l-trans-I	0.50	0.35	−0.15
	1.00	0.85	−0.15
	1.99	1.71	−0.28
	3.00	2.67	−0.33
	3.97	3.57	−0.40
	5.02	4.36	−0.66

* Calculated by weight.
** Calculated from the peak area ratio.

fore we used this reagent for the determination of the optical isomers of compound I.

In this derivatization method it is very important to check the optical purity of the chiral reagent. We tested the optical purity of reagent III using L-proline methyl ester. DL-Proline methyl ester was added to L-proline methyl ester to give a concentration of D-proline methyl ester in the range 0.1–0.5%. Following derivatization method A and HPLC with mobile phase A, the optical purity was shown to be greater than 99.95% (Fig. 1).

We have already reported the determination of the optical isomers of diltiazem hydrochloride by two different HPLC methods[11]. In the present study we tried to determine these optical isomers by use of a single HPLC method. When the chiral reagent III reacts with the four optical isomers of compound I, (*SS*)-deacetyl-I-(*S*)-III, (*RR*)-deacetyl-I-(*S*)-III, (*RS*)-deacetyl-I-(*S*)-III and (*SR*)-deacetyl-I-(*S*)-III are produced. *dl-cis*-I[(*SS*)-I and (*RR*)-I] and *dl-trans*-I[(*SR*)-I and (*RS*)-I] (Fig. 2) were obtained by the derivatization method B. It was found that the diastereomers could be separated more easily by adsorption chromatography (mobile phase B) than by reversed-phase chromatography (Fig. 3). The diastereomeric esters produced from the four optical isomers of I each showed a single, symmetrical peak, indicating excellent chromatographic properties. The excess of the derivatization reagent and pyridine were eluted near the solvent front without causing any interference.

The peaks eluted from the HPLC column were collected to confirm the identity of the diltiazem hydrochloride derivatives. The mobile phase was evaporated and the residue dissolved in chloroform. An aliquot was injected into the ion source of the

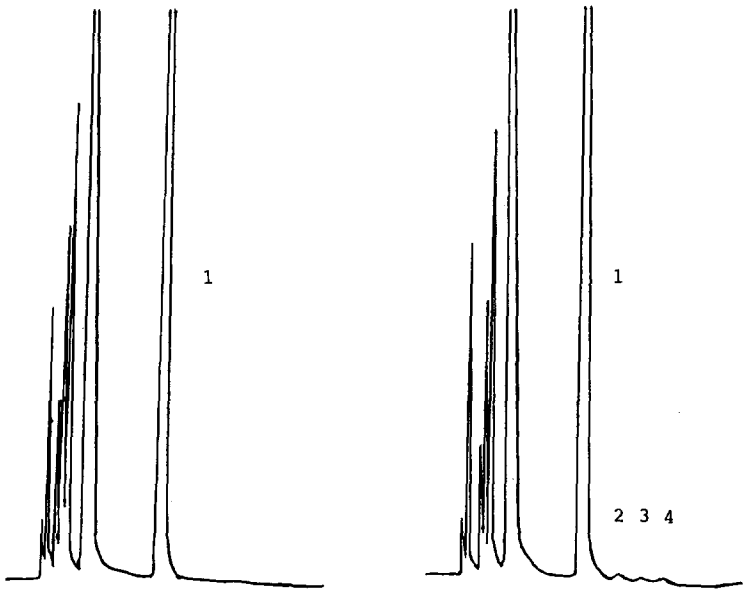


Fig. 4. Chromatograms of recovery tests with various isomer ratios in *d-cis*-I.

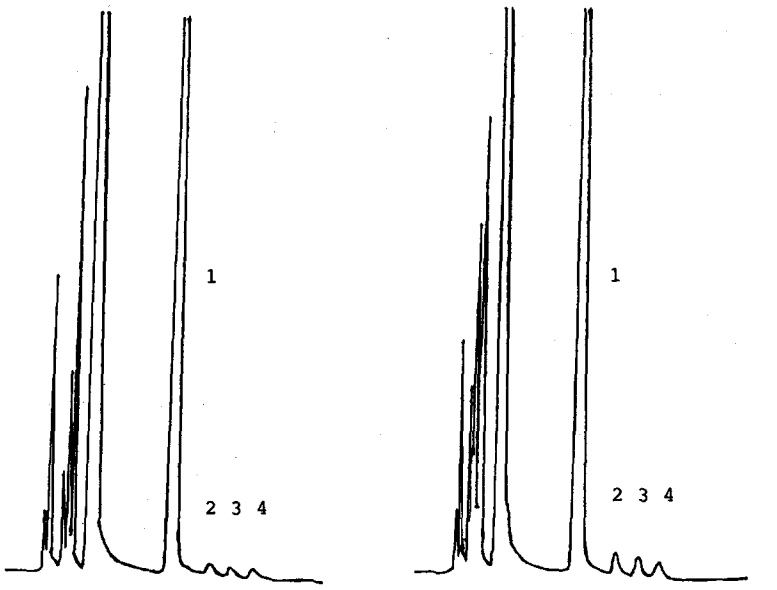


Fig. 5. Chromatograms of recovery tests with various isomer ratios in *d-cis*-I.

mass spectrometer. The mass spectra of the diastereomers were essentially identical. The M^+ peak at m/z 659 yields the molecular weight of the derivatives, while the fragment ions provide information on their chemical structures.

As the diastereomeric esters might possess different molar absorptivities, it was necessary to determine whether the enantiomeric purity could be measured directly from the peak area ratios or from the calibration curve. As a recovery test, *l-cis*-I [(*RR*)-I] and *dl-trans*-I [(*SR*)-I and (*RS*)-I] were added to *d-cis*-I [(*SS*)-I] to give concentrations in the range 0.5–10.0%. The resulting mixture was treated as described above and analysed by HPLC (mobile phase B). The ratio of the area of the peak for each diastereomeric ester to the sum of the areas of the peaks of the diastereomeric esters was compared with the percentage of each optical isomer calculated from the weights added. The results are presented in Table I. The data indicate that the estimation of enantiomeric purity from the area ratios is of sufficient accuracy, precision and linearity for drug analysis.

Typical chromatograms are shown in Figs. 4 and 5. The limit of detection of each optical isomer in *d-cis*-I [(*SS*)-I] was found to be 0.1%.

This method is particularly suitable for a semiquantitative test of quality control, where the desired result is a determination of whether or not an optical isomer is present in a sample in an amount exceeding a certain value.

REFERENCES

1 S. K. Yang and H. B. Weem, *Anal. Chem.*, 56 (1984) 2658.
2 J. B. Crowther, T. R. Covey, E. A. Dewey and J. D. Henion, *Anal. Chem.*, 56 (1984) 2921.
3 G. Gübitz and S. Mihellyes, *Chromatographia*, 19 (1984) 257.
4 L. R. Gelber and J. L. Neumeyer, *J. Chromatogr.*, 257 (1983) 317.
5 I. Benecke, *J. Chromatogr.*, 291 (1984) 155.
6 U. Forsmann, *J. Chromatogr.*, 303 (1984) 217.
7 S. Weinstein and S. Weiner, *J. Chromatogr.*, 303 (1984) 244.
8 H. Kugita, H. Inoue, M. Ikezaki and S. Takeo, *Chem. Pharm. Bull.*, 18 (1970) 2028.
9 H. Kugita, H. Inoue, M. Ikezaki, M. Konda and S. Takeo, *Chem. Pharm. Bull.*, 18 (1970) 2284.
10 H. Kugita, H. Inoue, M. Ikezaki, M. Konda and S. Takeo, *Chem. Pharm. Bull.*, 19 (1971) 595.
11 R. Shimizu, K. Ishii, N. Tsumagari, M. Matsumoto and I. T. Harrison, *J. Chromatogr.*, 253 (1982) 101.