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Note

Determination of optical isomers in diltiazem hydrochloride by high-performance liquid chromatography

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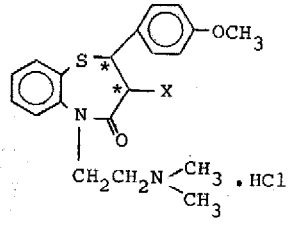
Optical isomers display different biochemical and pharmacological effects and it is therefore very important to be able to determine the optical isomer ratio in compounds with potential biochemical and pharmaceutical applications. Classically, optical purity is measured by comparing the rotation of a sample with the rotation of optically pure reference material[1]. This technique is not satisfactory for the accurate determination of optical purity, and for quantitative purposes chromatographic methods offer higher sensitivity.

The gas (GC) and liquid chromatographic resolution of racemates on columns prepared with chiral stationary phases has been reported[2–4]. More conventional is the resolution of diastereomeric derivatives of racemates by GC or high-performance liquid chromatography (HPLC)[5–8]. HPLC has been shown to be much more efficient because of its high resolution, high sensitivity and versatility. We therefore used the derivatization method, followed by HPLC, to determine the optical purity of diltiazem hydrochloride.



X = $-OCOCH_3$

I : *cis* form

II : *trans* form

d-I : Diltiazem hydrochloride

X = $-OH$

Deacetyl form

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Diltiazem hydrochloride has asymmetric carbon atoms at positions 2 and 3. Two geometric isomers, *cis*- (I) and *trans*- (II), exist, depending on the relative positions of the substituents at these positions. Each isomer also has optical isomers, the *d*- and *l*-forms. Diltiazem hydrochloride, the *d-cis*-isomer of acetoxy-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(*p*-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one hydrochloride, is a potent coronary vasodilator with additional hypotensive and negative chronotropic activities[9,10].

I and II can be separated by reversed-phase chromatography on a conventional column. To permit the chromatographic separation of enantiomers of diltiazem hydrochloride, another chiral species must be introduced. However, I lacks an active radical so it must first be converted into deacetyl-I by hydrolysis. A chiral species can then be introduced, establishing a diasteromeric relationship. The reaction of deacetyl-I and the chiral reagent was performed in the presence of pyridine, and the diastereomers were separated using reversed-phase chromatography.

For this method to be accurate, the reagent used must be optically pure and there must be no epimerization or racemization during the reactions that hydrolyse I and transform the enantiomeric mixture into a diastereomeric mixture.

EXPERIMENTAL

Materials

The syntheses of I and II have been reported previously[11–13]. 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 and UV-2 absorbance detector, monitoring at 240 and 254 nm, equipped with a Chromatopac EIA integrator. The test samples were applied to the chromatograph by a 20-μl loop injector. The mass spectrometer was a Hitachi M-80A equipped with an M-003 computer system and operated in the electron-impact mode at 20 eV.

HPLC conditions

Method A. The column was μBondapak C_{18} (30 cm × 4 mm I.D.) (Waters Assoc., Milford, MA, U.S.A.) and the mobile phase was 0.12 *M* sodium acetate solution containing 1.5 g of *d*-camphorsulphonic acid (pH 5.5)–acetonitrile–methanol (2:1:1). The flow-rate was 1.6 ml/min. A UV detector (240 nm) was used.

Method B. The column was Nucleosil $5C_{18}$ (20 cm × 4 mm I.D.) (Machery, Nagel & Co., Düren, G.F.R.) and the mobile phase was 0.01 *M* ammonium acetate solution (pH 6.6)–acetonitrile (1:9). The flow-rate was 1.5 ml/min. A UV detector (254 nm) was used.

Separation of I and II

I and II could be separated by reversed-phase chromatography (method A) and the chromatogram is shown in Fig. 1.

Diltiazem hydrochloride was analysed by these method and the following experiment was performed using material that had been ascertained not to include II.

Synthesis of derivatization reagent

d-2-(2-Naphthyl)propionic acid (III). A solution of 20.1 g of methyl 2-naphthylacetate and 10 ml of methyl iodide in 200 ml of dimethoxyethane was treated with 4.8 g of sodium hydride (50 % in oil) during 45 min. After stirring overnight, 5 ml of ethanol were added to destroy unreacted sodium hydride. The mixture was evaporated to about two thirds of its volume *in vacuo*. A solution of 10 g of sodium hydroxide in 20 ml of water was added and the solution heated at 50°C for 5 h. Water was added and neutral substances were removed by extraction with diethyl ether. After acidification with dilute hydrochloric acid, the product was extracted with diethyl ether–heptane (1:1). Evaporation of the solvent gave 21.0 g of white crystals.

A solution of 22.2 g of the above acid in 100 ml of acetone was treated with 32.6 g of cinchonidine suspended in 100 ml of methanol. The solution was boiled down to 120 ml and 130 ml of diethyl ether were added. The crystals formed were filtered and recrystallized a further twice, giving 13.4 g (m.p. 146–147°C).

A suspension of 13.4 g of the cinchonidine salt in diethyl ether–heptane (1:1) was shaken with dilute hydrochloric acid. The organic phase was separated, washed with water and evaporated. Crystallization from acetone–heptane gave 4.77 g of product (m.p. 140–142°C).

d-2-(2-Naphthyl) propionyl chloride (IV). Oxalyl chloride (6 ml) was added to a suspension of 4 g of III in absolute benzene and stirred at 35–40°C for 1 h. The benzene was evaporated *in vacuo* below 50°C and the residue obtained was dissolved in 30 ml of absolute benzene, evaporated *in vacuo* and the same procedure was

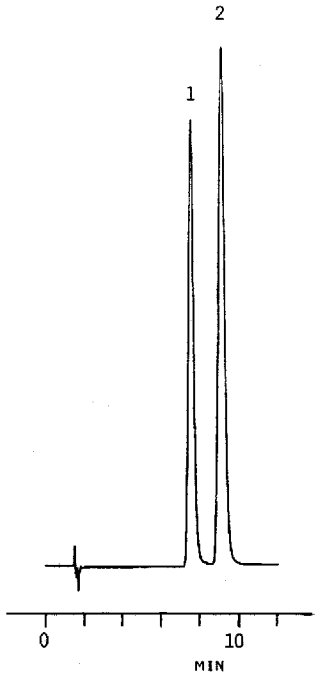
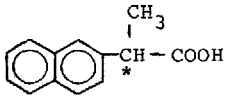


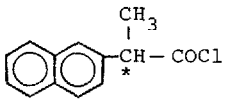
Fig. 1. Separation of (1) I and (2) II.

repeated to remove unreacted oxalyl chloride. Recrystallization of the crude product from hexane gave IV ($[\alpha]_D^{20}$ + 73.6° (c = 1, $CHCl_3$); m.p. 39–41 °C).

IV was converted to the diastereomeric amide with 1-proline methyl ester and chromatographed on a Zorbax-Sil column (15 cm × 4.6 mm I.D.). The optical purity proved to be greater than 99.8 %.



(III)



(IV)

Hydrolysis of dl-I

The optimum conditions for the quantitative hydrolysis of *dl*-I, without racemization or side-reactions, were examined. Hydrochloric acid (0.5 *N*) was added in the ratio of 2 ml to 60 mg of *dl*-I, the mixture was heated at 100°C and samples of the solution were taken at intervals of 10 min. In this solution, residual *dl*-I and the deacetyl *dl*-I produced were determined by method A. From Fig. 2 hydrolysis was complete at 100°C within 40 min.

Derivatization

Diltiazem hydrochloride (0.30 g) was weighed into a 20-ml volumetric flask, 10 ml of 0.5 *N* hydrochloric acid were added. The mixture was heated at 100°C for 50 min and, after cooling, water was added give a volume of 20 ml. Sodium chloride (1.6 g), 1 *N* sodium hydroxide solution (1.5 ml) and dichloromethane (20 ml) were added to a glass-stoppered centrifuge tube containing 4 ml of the above solution and equilibrated by shaking. The dichloromethane layer was filtered and 10 ml of the filtrate were measured into a glass-stoppered flask. The dichloromethane was evaporated *in*

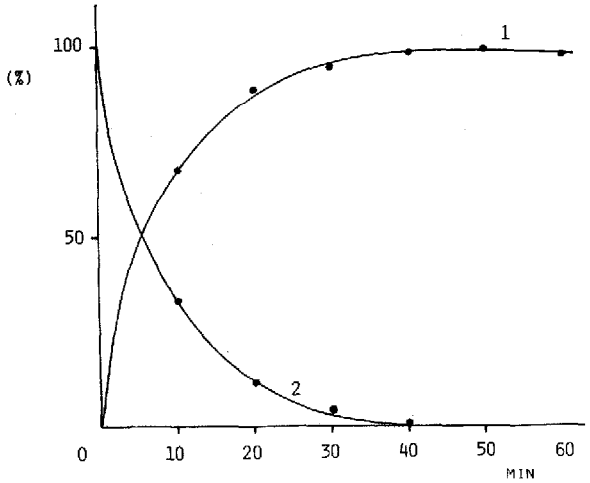


Fig. 2. Time course of *dl*-I hydrolysis. 1 = Deacetyl-*dl*-I; 2 = residual *dl*-I.

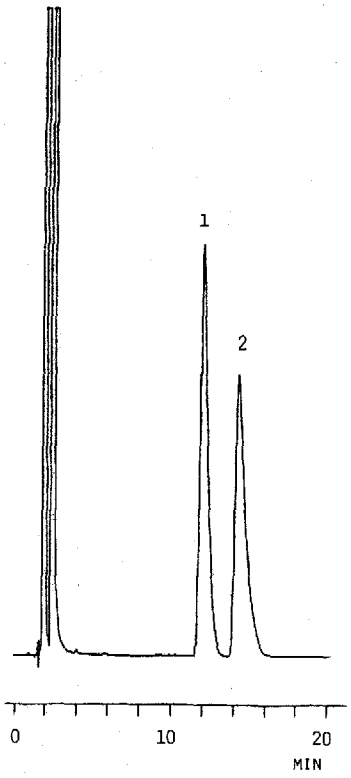


Fig. 3. Chromatogram of diastereomeric mixture. 1 = Deacetyl-*l*-I–IV ester; 2 = deacetyl-*d*-I–IV ester.

vacuo and the residue obtained was dried *in vacuo* for 1 h. The residue was dissolved in dry dichloromethane, 0.25 ml of dry pyridine and dry dichloromethane containing 75 mg of IV were added and the resulting solution was allowed to stand at room temperature for 15 min. After reaction, 5 ml of methanol were added and acetonitrile was added to give a volume of 50 ml. An aliquot was injected into the chromatograph.

Separation of deacetyl-d-I–IV ester and deacetyl-l-I–IV ester

dl-I was treated by the above procedure. The separation of diastereomers by HPLC was investigated with this test solution and the optimum separation (R_S = 2.2) was obtained by reversed-phase chromatography (method B). The chromatogram is shown in Fig. 3.

Repeated tests showed that the peak area ratio of deacetyl-*l*-I–IV ester relative to deacetyl-*d*-I–IV ester had a constant value close to 1.

Identification of the derivatives

The reacted mixture was spotted on a TLC plate and thin-layer chromatography was performed. The spots of the derivatives were scraped off from the TLC plate and extracted with methanol, which was subsequently evaporated. The residues were dissolved in chloroform and an aliquot was injected directly into the ion source of a

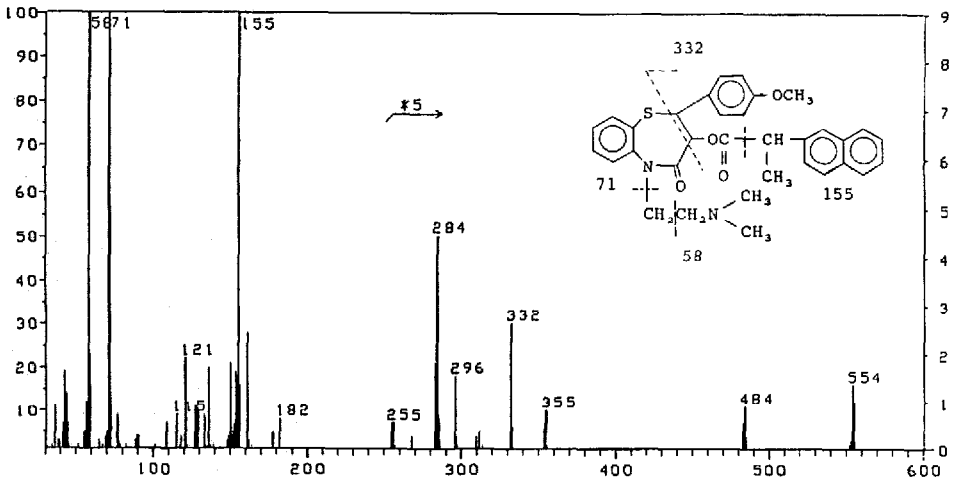


Fig. 4. Mass spectrum of diastereomer.

mass spectrometer. The mass spectrum is shown in Fig. 4. The M^+ peak at m/z 554 gave the molecular weight of the derivatives while the fragment ions identified the chemical structures.

Calibration graph

dl-I was added to *d*-I to give concentrations of *l*-I in the range 0.5–20%. The resulting mixtures were treated as described above and analysed by HPLC (method B). The relationship between the known content of *l*-I and the found value of deacetyl-*l*-I–IV ester was investigated. The theoretical content of *l*-I was calculated by eqn. 1 and the found value of deacetyl-*l*-I–IV ester was calculated by eqn. 2.

$$l\text{-I}\ (\%) = \frac{0.5\ W_2}{W_1 + W_2} \cdot 100 \tag{1}$$

$$\text{Deacetyl-}l\text{-I–IV}\ (\%) = \frac{A_2}{A_1 + A_2} \cdot 100 \tag{2}$$

where W_1 = weight of *d*-I, W_2 = weight of *dl*-I, A_1 = peak area of deacetyl-*d*-I–IV ester and A_2 = peak area of deacetyl-*l*-I–IV ester. Good linearity was obtained (Fig. 5).

Calculation of l-I content in d-I

The ratio of diastereomeric esters derivatized from *dl*-I was close to 1. Therefore, the deacetyl-I–IV ester content in the reacted mixture can be calculated by eqn. 2. The relationship between the added content of *l*-I (X) and the found value of deacetyl-*l*-I–IV ester (Y) has already been considered above. A regression line $Y = 0.99X$ was found with a correlation coefficient close to 1.0. A regression line with slope 1 shows that the ratio of the diastereomeric esters produced is equal to the ratio

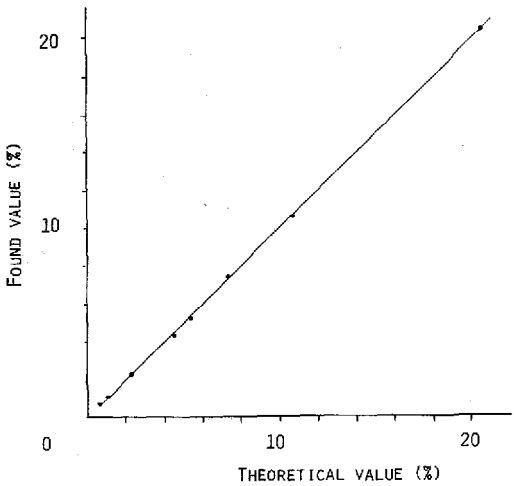


Fig. 5. Relationship between the known content (%) of *l*-I and the found content (%) of deacetyl-*l*-I–IV ester.

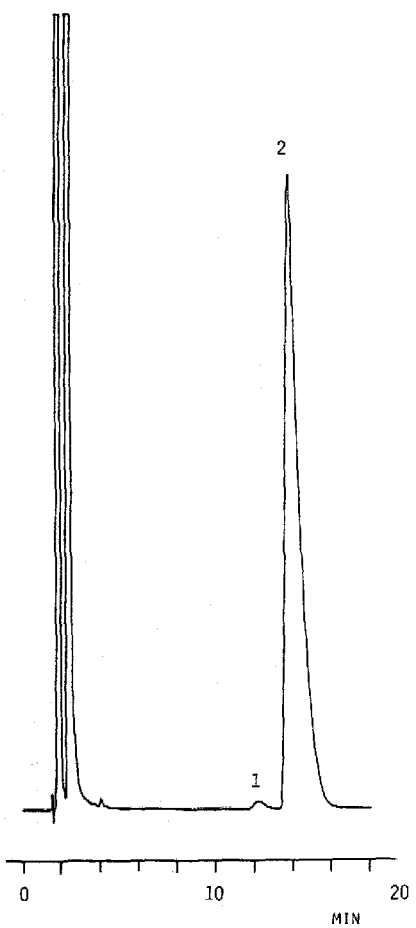


Fig. 6. Chromatogram of diastereomeric mixture prepared from *d*-I containing 1.0% of *l*-I. 1 = Deacetyl-*l*-I–IV ester; 2 = deacetyl-*d*-I–IV ester.

of enantiomers in diltiazem hydrochloride. Therefore, the *l*-I content in diltiazem hydrochloride can be calculated by eqn. 2.

Detection limit

dl-I was added to *d*-I to give concentrations of *l*-I in the range 0.05–1.0%. The quantitative detection limit was then investigated following the described procedure. The limit for detection of *l*-I in *d*-I was found to be 0.1%. Fig. 6 shows the chromatogram of a diastereomeric mixture prepared from *d*-I containing 1.0% of *l*-I.

RESULTS AND DISCUSSION

Hydrolysis of I and the esterification reaction of deacetyl-I with the chiral reagent (IV) were examined by HPLC. The hydrolysis was completed in about 50 min and the reaction proceeded within 5 min at room temperature under the conditions specified. No side-products were apparent and the lack of racemization and epimerization was confirmed by HPLC.

A plot of percentage of deacetyl-*l*-I–IV ester found experimentally against the added percent of *l*-I is shown in Fig. 4. This calibration graph with slope 1 clearly demonstrates that for each sample the ratio of the diastereomeric esters produced is identical with the ratio of enantiomers present in the initial sample. The amount of *l*-I contained in the sample of I could be determined at concentrations down to 0.1%, and consequently the optical purity of this pharmaceutical compound can be examined with sufficient sensitivity by this method.

Analyse were performed on a series of ten samples of diltiazem hydrochloride by this method and the results confirmed that the optical purity was excellent (more than 99.9%).

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