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# DETERMINATION OF *cis*- AND *trans*-ISOMERS OF DOSULEPINE AND DITHIADENE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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## SUMMARY

High-performance liquid chromatography (HPLC) can be applied successfully to the separation and the determination of stereoisomers of tricyclic antidepressants and antihistamines with propylidene side chains.

The contents of *cis*- and *trans*-isomers were determined in dosulepine and dithiadene by HPLC on a silica gel with chemically bonded propylamine groups, 10 $\mu$g graining (Silasorb). The results obtained were compared with those from gas chromatography. The contents of the *cis*-isomers ranged from 1 to 4%, the relative standard deviation of the determination being *ca.* 5% for manual evaluation of chromatograms. The analysis time was *ca.* 10 min.

## INTRODUCTION

Some of the compounds of the benzothiepine series, synthesized recently, have a significant biological activity and are used successfully in pharmacotherapeutical practice as psychopharmaceuticals or antihistamines. When an exocyclic double bond is present in their structure, two stereoisomeric forms are possible. These are 11-(3-dimethylaminopropylidene)-6,11-dihydrodibenzo[*b*,*e*]thiepine (I, see Table I; dosulepine[1], dothiepine[2]), 2-methyl-11-(3-dimethylaminopropylidene)-6,11-dihydrodibenzo[*b*,*e*]thiepine (II, medosulepine[1]) and 4-(3-dimethylaminopropylidene)-4,9-dihydrothieno[2,3-*b*]-benzo[*e*]thiepine (III, dithiadene[3]). A desirable configuration of compounds I–III is *trans*; however, preparations usually contain a significant amount of *cis*-isomers, which have somewhat different biological activities[4–6]. The *cis*-isomer in I has been determined by gas–liquid chromatography (GLC)[1,2], but a method suitable for other substances has not yet been proposed.

Within the last few years high-performance liquid chromatography has been proposed for the separation and the determination of tricyclic pharmaceuticals with a seven-membered central ring[7–11]. So far, only compounds containing sulphur as the heteroatom in the middle ring have been analyzed[12]. The HPLC of compounds I–III has not yet been described.

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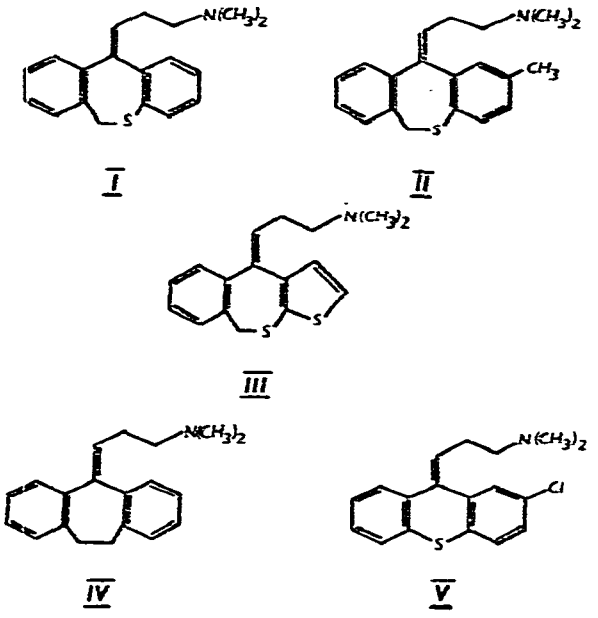


Fig. 1. Structure and notation of the drugs under analysis. The *cis*-configuration of the exocyclic double bond is shown.

## EXPERIMENTAL

### *Apparatus*

The high-performance liquid chromatograph used consisted of a HPP 40001 high-pressure pump (Laboratory Instruments, Prague, Czechoslovakia) and a single-beam UVM 4 UV detector with an 8-$\mu$l cell volume and adjustable wavelength over the range 240–400 nm (Development Workshops, Czechoslovak Academy of Sciences, Prague, Czechoslovakia). Some measurements were carried out with a monochromatic LCD 254 detector with an 8-$\mu$l cell volume (Laboratory Instruments). A TZ 21 S line recorder (Laboratory Instruments) was used to monitor chromatograms.

### *Columns and eluents*

Stainless-steel columns (see Table I) were packed with Silasorb (Lachema, Brno, Czechoslovakia), a spherical silica gel with bonded propylamine groups (particle diameter 10 $\mu$m, specific surface area 600 m$^2$/g) by the viscosity technique using cyclohexane with 5% of methanol as a suspension medium. The flow-rates varied between 0.4 and 1.1 ml/min. Columns were operated at ambient temperature (20–25°). Samples to be analyzed were prepared by dissolving the hydrochloride of the respective base in methanol to give solutions containing 1–10 mg/ml. A 1–3-$\mu$l volume of the solution was injected into the column through a septum using a Hamilton 7005 N microsyringe.

## RESULTS

### *Separation of stereoisomers*

It follows from the retention data in Table I that the ratios of the capacity factors of corresponding pairs of isomers do not change substantially with change in composition of the mobile phase. *n*-Propanol, *n*-butanol or methylene chloride used for homogenization of other components may also be replaced with amyl alcohol or cyclohexanol without greatly affecting the separation of the pairs of isomers. A suitable amount of acid is crucial since a low concentration caused fronting, a high concentration tailing of all the peaks. Systems 1–3 proved to be less effective for the separation of isomers III owing to peak broadening, while system 4 was suitable for the separation of all the pairs of isomers discussed above. Examples of separations are shown in Fig. 2.

TABLE I

RETENTION OF DRUGS FOR DIFFERENT MOBILE PHASES

Suppliers: I, Chemopharma, N.E. (Ústí n/L, Czechoslovakia); II, III and IV, Pharmaceuticals, N.E. (Prague, Czechoslovakia); V, Farmakon, N.E. (Olomouc, Czechoslovakia). Chromatographic systems: 1, column 100 × 4 mm, mobile phase *n*-hexane–methanol–*n*-propanol–70% $HClO_4$ (70:32:10:0.010); 2, column 100 × 4 mm, mobile phase *n*-hexane–methanol–methylene chloride–70% $HClO_4$ (70:32:10:0.010); 3, column 200 × 6 mm, mobile phase *n*-hexane–methanol–*n*-propanol–70% $HClO_4$ (65:35:10:0.020); 4, column 200 × 4 mm, mobile phase *n*-hexane–methanol–*n*-butanol–water–70% $HClO_4$ (56:30:13:1:0.025).

| *Notation* | *Drug* | *Configuration* | *Capacity factor with system* | | | |
|---|---|---|---|---|---|---|
| | | | *1* | *2* | *3* | *4* |
| I | Dosulepine | *cis* | 3.9 | 2.4 | 6.8 | 4.0 |
| | | *trans* | 4.8 | 3.2 | 8.2 | 4.6 |
| II | Medosulepine | *cis* | — | — | — | 3.2 |
| | | *trans* | — | — | — | 3.7 |
| III | Dithiadene | *cis* | 5.4 | 3.1 | 8.3 | 4.5 |
| | | *trans* | 6.3 | 3.6 | 9.3 | 5.2 |
| IV | Amitriptyline | — | 2.7 | 1.7 | 4.9 | 3.0 |
| V | Chlorprothixene | *cis* | — | — | 4.3 | 3.8 |

### *Calculation of the weight fraction of the cis-isomer of dithiadene*

In order to calculate the weight fraction of the *cis*-isomer, $g_x$, from the chromatogram, the relationship applied to the internal normalization technique[13] can be utilized

$$g_x = \frac{w_x}{w_x + w_y} = \frac{A_x \cdot \mathrm{RMR}_{y/x}}{A_x \cdot \mathrm{RMR}_{y/x} + A_y} \qquad (1)$$

where $w_x$ and $w_y$ are the weights of the *cis*- and the *trans*-isomers, respectively, with $w = w_x + w_y$ being the total weight of the sample of the pure preparation. $A_x$ and $A_y$ are the areas of the peaks of the individual isomers and $\mathrm{RMR}_{y/x}$ is the ratio of the molar response of the *trans*-isomer to that of the *cis*-isomer, measured under the same conditions. The latter ratio is given by

$$\mathrm{RMR}_{y/x} = \frac{A_y \cdot w_x}{A_x \cdot w_y} = \frac{b_y \cdot h_y \cdot w_x}{b_x \cdot h_x \cdot w_y} = K_{y/x} \cdot \frac{b_y}{b_x} \qquad (2)$$

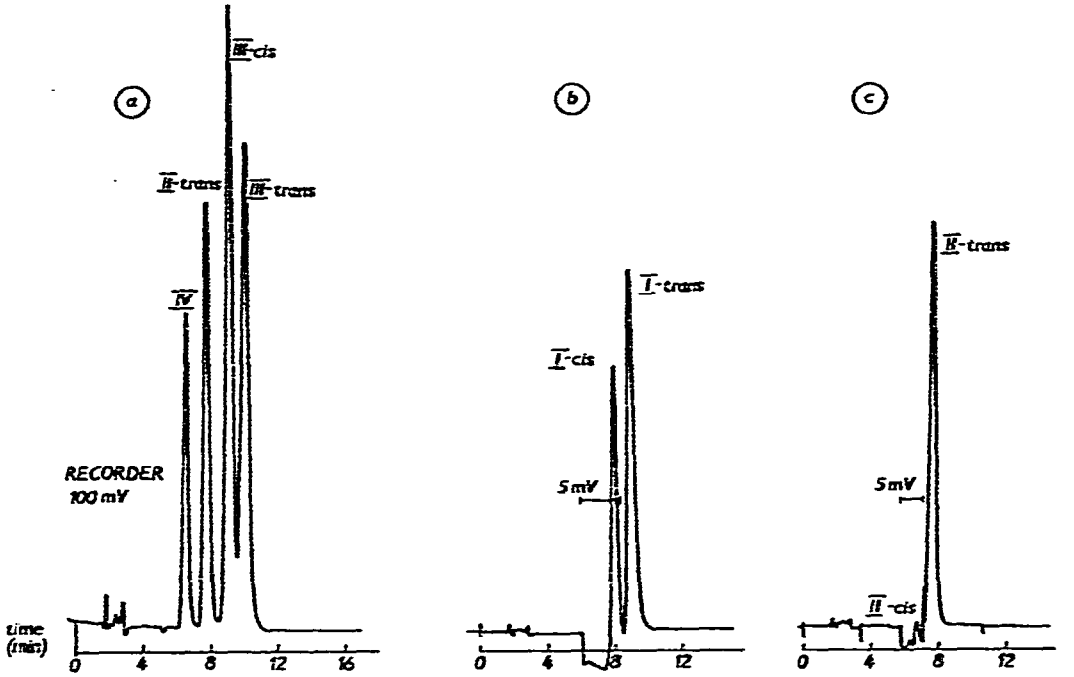


Fig. 2. Separation of the pairs of isomers of III (a), I (b) and II (c). For notation of peaks see Fig. 1. For the mobile phase see Table I, system 4. Sorbent: Silasorb, particle diameter 10 μm, surface area 600 m²/g. Flow-rate, 0.8 ml/min; pressure, 0.8 MPa. Detector: LCD 254; $8 \cdot 10^{-2}$ a.u.f.s./10 mV.

where $h_x$ and $h_y$ denote the heights of the peaks of the individual isomers, $b_x$ and $b_y$ the peak widths and $K_{y/x}$ the searched correction factor defined by:

$$K_{y/x} = \frac{h_y \cdot w_x}{h_x \cdot w_y} \tag{3}$$

Since it is more rapid, and in the case of symmetric peaks often more accurate[13], to evaluate the chromatograms by measuring peak heights, relationship[4]

$$g_x = \frac{h_x \cdot K_{y/x}}{h_x \cdot K_{y/x} + h_y} \tag{4}$$

derived from eqns. 1–3 can be used to advantage in order to calculate the weight fraction of the isomer.

The isomer ratio of the sample and also the ratio of the peak heights of these isomers, measured under the same conditions as in the analysis of the samples of unknown composition, must be known for the calculation of the factor $K_{y/x}$. Also, in order to secure sufficient accuracy of $K_{y/x}$ the ratio of the isomers in the selected sample should not differ significantly from unity. A sample of III, containing *ca.* 75% of the *cis*-isomer, was applied. The precise contents of the isomers were determined by liquid chromatography with the use of the standard addition technique based on

comparison with an added reference substance. The calculations were based on the peak heights[13]. Amitriptyline hydrochloride (IV) was used as reference substance, dithiadene hydrochloride (V), containing 99.0 ± 0.1% of the *trans*-isomer, was used as a standard for the addition of the *trans*-isomer, The amounts of the pure substances were of the order of tens of milligrams, with the precision of weighing being better than 0.05 mg. After dissolving the substances in methanol, the solutions were made up to 10 ml. One solution did not contain any of the *trans*-isomer, three more solutions contained different amounts of the *trans*-isomer. Four *ca.* 1-μl aliquots of each solution were injected into the chromatographic column with the use of system 4, Table I.

The calibration dependence can be described by

$$\frac{h'_y}{h'_r} \cdot \frac{w'_r}{w_r} \cdot \frac{w}{w'} = \frac{w_p \cdot 0.990 \cdot h_y}{w_y \cdot h_r} + \frac{h_y}{h_r} \qquad (5)$$

where primed symbols denote values for solutions enriched with the weight, $w_p$. Subscript r indicates the reference substance. The left-hand side of the equation is a dependent variable, $w_p$ is an independent variable, the other expressions in the first term at the right-hand side represent the slope and the second term at the right-hand side the intercept on the ordinate. Using the least-squares method, the following parameters of the regression line were calculated: intercept, $h_y/h_r = 0.5920 \pm 0.00020$; slope, $0.990 \cdot h_y/h_r \cdot w_y = 0.04711 \pm 0.00049$ mg$^{-1}$. The ratio $h_y/h_x$ was 0.288 ± 1.02% for $n = 4$. Hence according to eqn. 4, $K_{y/x} = 0.88 \pm 3.0\%$.

The ratio of the peak widths of the *cis*- and *trans*-isomers of dithiadene, $b_x/b_y$, was determined to be 0.893 ± 1.1% for $n = 4$.

By applying eqn. 2, the ratio of the relative molar extinction coefficients can be calculated as $RMR_{y/x} = 0.99 \pm 4\%$. The content of the *cis*-isomer in four dithiadene preparations was determined by use of relationship 4 and the calculated coefficient $K_{y/x}$. The results are summarized in Table II.

TABLE II

DETERMINATION OF THE CONTENT OF THE *cis*-ISOMER IN DITHIADENE

$\bar{x}$ = Weighted mean[14] of the determined contents of the *cis*-isomer; $\bar{s}_r$ = mean relative standard deviation; $N$ = total number of determinations of one batch; $k$ = number of series.

| *Lot (batch)* | $\bar{x}$ (%) | $\bar{s}_r$ (%) | $N$ | $k$ |
|---|---|---|---|---|
| 110676 | 3.2 | 3.9 | 12 | 3 |
| 020776 | 1.3 | 5.6 | 8 | 2 |
| 020177 | 1.8 | 5.4 | 9 | 2 |
| 600478 | 1.0 | 11 | 5 | 2 |

*Determination of the content of the cis-isomer in dosulepine*

No suitable preparation of this substance was available which might be utilized in the same way as preparation V for the calculation of coefficient $K_{y/x}$. However, the coefficients were obtained by GLC analysis of three different batches (Research Institute for Pharmacy and Biochemistry, Prague, Czechoslovakia) which were used as reference substances. Since the contents of the *cis*-isomer were small and coefficient $K_{y/x}$ differs little from unity, this coefficient may be expressed by the relationship

given in Table III. It can be seen that this coefficient changes slightly with the composition of the mobile phase. With the use of the mobile phase from system 4, the ratio determined for $n = 4$ was $b_x/b_y = 0.91 \pm 1.2\%$. Hence $RMR_{y/x} = 1.01 \pm 6\%$.

TABLE III

RESULTS OF DOSULEPINE ANALYSIS

A = Batch No. 72500976; B = batch No. 72701076; C = batch No. 73201176. $\bar{x}'$ = Arithmetic mean of the contents of the *cis*-isomer assuming that $K_{y/z} = 1$, manual evaluation of the chromatogram and peak height calculation; $\bar{x}$ = arithmetic mean of the contents of the *cis*-isomer from GLC determination using FID and electronic integrator; $n$ = number of the determinations in the series; $s_r$ = relative standard deviation; $\bar{x}/\bar{x}'$ = average ratio of the results of GLC/HPLC for individual chromatographic systems detailed in Table I.

| *Chromatographic conditions* | *Batch* | $\bar{x}'$ (%) | $n$ | $s_r$ (%) | $\bar{x}/\bar{x}' = K_{y/x}$ |
|---|---|---|---|---|---|
| HPLC 1 | A | 3.3 | 5 | 1.6 | 1.05 |
| | B | 3.7 | 4 | 3.9 | |
| | C | 2.8 | 5 | 5.8 | |
| 2 | A | 3.3 | 4 | 5.0 | 1.06 |
| | B | 3.6 | 5 | 3.6 | |
| | C | 2.8 | 8 | 5.8 | |
| 3 | A | 3.6 | 4 | 4.2 | 0.96 |
| | B | 4.1 | 4 | 4.6 | |
| | C | 3.0 | 4 | 7.2 | |
| 4 | A | 3.6 | 3 | 8.1 | 0.92 |
| | B | 4.2 | 6 | 5.6 | |
| | C | 3.4 | 2 | 0.9 | |
| | | $\bar{x}$ (%) | | | |
| GLC | A | 3.2 | 13 | 1.9 | |
| | B | 3.8 | 3 | 0.6 | |
| | C | 3.2 | 3 | 1.3 | |

*Determination of the content of the cis-isomer in medosulepine*

No reference substance nor the results of another analysis were available for this substance. Using chromatographic system 4 and assuming $K_{y/x} = 1$, contents of 0.17, 0.29 and 0.77% of the *cis*-isomer were found for three different batches; the relative standard deviation reached *ca.* 10% for $n = 3$–4.

## DISCUSSION

The chromatographic system consisting of silica gel and a mixture of diisopropyl ether, dichloromethane, methanol, perchloric acid, and sodium perchlorate suggested[11] for the separation of the isomers of 10-hydroxy-nortriptyline proved to be unsatisfactory for the separation of the isomers under study. Mobile phases, described in this paper, were also not useful for the separation of isomers I–III on silica columns. The important influence of the sorbent having propylamine surface groups is caused by the fact that the derivative with a six-membered central ring, chlorprothixene (V, Table I), has a lower retention than any of the derivatives with a seven-membered

ring; this contradicts the results obtained previously by other authors[7,12] with similar mobile phases and compounds.

On the other hand, with all the isomeric pairs studied, the *cis*-isomer is eluted first, as in the separation of 10-hydroxy-nortriptyline isomers[11].

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