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Note

α-, β- and γ-cyclodextrins as mobile phase additives in the high-performance liquid chromatographic separation of enantiomeric compounds

I. Separation of optical isomers of D,L-norgestrel

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The separation or resolution of enantiomeric compounds is of great importance in pharmaceutical analysis. The demand for an efficient, accurate technique for the determination of the optical purity of biologically active compounds has resulted in a great amount of chromatographic research in this area. One of the methods in this field is the use of cyclodextrins to form inclusion complexes between the solute to be tested and cyclodextrin molecule. Based on the selective interaction of α-, β- and γ-cyclodextrins with molecules, the methods can be divided into two groups.

In the first group the cyclodextrins are chemically bonded to the stationary phase surface, resulting in stable, fully derivatized, high-performance packings[1–8]. In most of these experiments β-cyclodextrin and its derivatives have been bonded to silica[3,5–7] because of the poorer resolution for the same components obtained on α-cyclodextrin-coated stationary phases[8].

The second group involves the use of cyclodextrins as mobile phase additives, a technique first introduced in thin-layer chromatography[9–11]. In high-performance liquid chromatography (HPLC) the extensive work of Debowski and Sybilska[12–16] can be mentioned: they used β-cyclodextrin as a chiral agent in the eluent.

The first successful application of this method was reported by Japanese workers[17] for the separation of prostaglandin isomers.

Generally it has to be noted that no data in the literature can be found for the use of γ-cyclodextrin as mobile phase additive in HPLC.

The main aim of our work was to clarify the role of different cyclodextrins in the HPLC separation of steroid optical isomers, which has not been studied before. The two isomers (D and L) of norgestrel seemed to be an excellent model, because the optical purity of D-norgestrel can be checked merely by measuring its optical rotation. D,L-Norgestrel is official in the *United States Pharmacopoeia*, D-norgestrel in the *United States Pharmacopoeia* and *British Pharmacopoeia* as Levonorgestrel[18–20].

The two isomers differ only in the stereochemistry on the C-13 ethyl group. To improve the selectivity and efficiency of the separation, the dependence of the resolution on the concentration of γ-cyclodextrin in the mobile phase, as well as on the nature and concentration of organic solvents in the eluent, has also been studied.

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EXPERIMENTAL

A Liquochrom 2010 high-pressure liquid chromatograph (Labor MIM, Esztergom-Budapest, Hungary) equipped with a variable-wavelength UV detector, a loop injector and a recorder (Labor MIM) was used. The separations were performed on prepacked LiChrosorb RP-18 (10 μm and 5 μm) (Chrompack, Middelburg, The Netherlands), Nucleosil 5 C_{18} (Chrompack), Hypersil ODS (5 μm) (Chrompack), and Ultrasphere ODS (5 μm) (Beckmann, Wien, Austria) columns. The size of the columns was 250 × 4.6 mm I.D. The eluents were prepared with HPLC-grade solvents and were degassed prior to use.

Cyclodextrins (α, β and γ) were obtained from Chinoin (Budapest, Hungary) and were used without further purification. The steroids to be tested were prepared at Chemical Works of Gedeon Richter (Budapest, Hungary): they were of pharmacopoeial quality.

RESULTS AND DISCUSSION

Dependence of capacity ratios on the γ-cyclodextrin concentration

The separation system was optimized by investigating the effect of the concentration of γ-cyclodextrin (γ-CYD) in the eluent on the capacity ratios of various steroids (Fig. 1). As it can be seen, the retentions of the compounds are considerably diminished with increasing γ-CYD concentration, indicating strong formation of more polar inclusion complexes. It is also apparent that the separation of D- and L-isomers of norgestrel can be carried out in the concentration range from $5 \cdot 10^{-3}$ to 10^{-2} mol/l γ-CYD.

These experiments were performed on the 10-μm LiChrosorb RP-18 column; however, for further optimization of chiral separation of norgestrel isomers, the 5-μm Hypersil ODS column and a methanol–water eluent were used.

Fig. 2 shows the dependence of the most important chromatographic parameters (k', α, H and R_s) on the concentration of γ-CYD. It can be concluded that the best separation can be achieved by using γ-CYD in a concentration of 10^{-2} mol/l: the resolution somewhat poorer, but the separation can be carried out within a reasonable time.

To elucidate the retention mechanism, the possible adsorption of γ-CYD on the stationary phase was studied by means of breakthrough curves, as well as by the effect of the presence and absence of γ-CYD in the eluent on the capacity ratios. Table I shows the retention behaviour of norgestrel isomers in the presence and absence of γ-CYD in the eluent when the stationary phase is loaded and unloaded with γ-CYD. From these results we deduce that liquid–liquid partition of the inclusion complexes formed in the mobile phase should be an acceptable model for the retention mechanism, because a significant increase of the measured capacity ratios is observed when a γ-CYD-loaded column and an eluent without γ-CYD were used.

Effect of the nature and concentration of organic solvents on the selectivity and efficiency of the separation

Fig. 3 shows the dependence of the capacity ratios on concentration of the organic solvent in the eluent, with methanol, ethanol, 2-propanol, acetonitrile or tetrahydrofuran as the organic solvent. Fig. 4 shows the changes in selectivity factor, resolution and column efficiency using the same solvent systems.

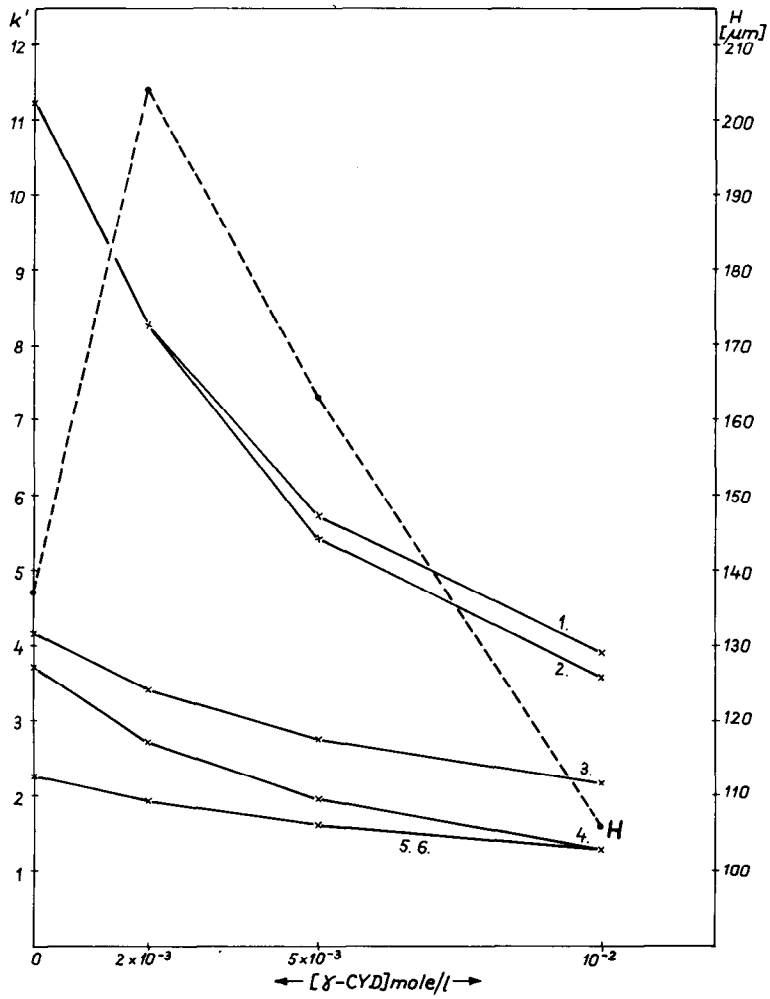

Fig. 1. Dependence of chromatographic parameters on γ-CYD concentration. Column, LiChrosorb RP-18, 10 μm (250 × 4.6 mm I.D.); eluent, water–methanol (3:4); flow-rates, 1 cm³/min; detection at 240 nm. Compounds: 1, L-norgestrel; 2, D-norgestrel; 3, hydrocortisone acetate; 4, triamcinolone acetonide; 5, prednisolone; 6, hydrocortisone.

From the data illustrated in Figs. 3 and 4 the following general conclusions can be drawn:

(1) When different organic solvents were used the elution order was unchanged, and the selectivity was not been significantly affected (the D-isomer is eluted first).

(2) The resolution of the two isomers is considerably influenced by the column efficiency, which is highly dependent on the nature of organic modifier in the eluent. The organic solvent tends to compete with the solutes for the preferred location in the hydrophobic cavity, resulting in various degrees of interaction of the compounds to be tested with γ-CYD.

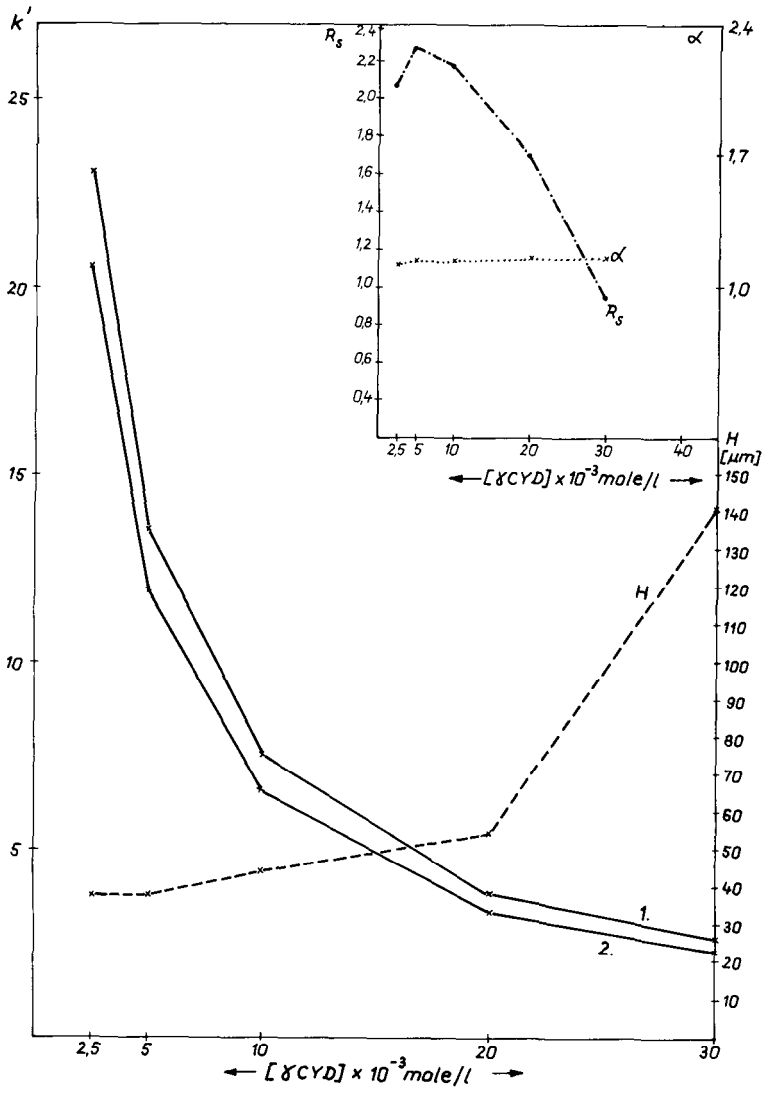

Fig. 2. Effect of γ-CYD concentration on the capacity ratios of D,L-norgestrel. Column, Hypersil ODS, 5 μm; eluent, water–methanol (6:4); other conditions as in Fig. 1.

(3) As the concentration of the organic solvent in the eluent increases, the capacity factors are significantly diminished because of the decreased interaction between the inclusion complexes and the stationary phase.

The most efficient separation can be obtained with an alcohol as the organic solvents compared to acetonitrile and tetrahydrofuran. Maximum resolution is achieved in a water–methanol mixture.

Effect of the size of the cavity in the CYD-ring on the selectivity of the separation

As the formation of inclusion complexes of steroids with cyclodextrins should

TABLE I

EFFECT OF THE PRESENCE OF γ-CYD IN THE ELUENT ON THE CAPACITY RATIO

Column, Hypersil ODS, 5 μm (250 × 4.6 mm I.D.); flow-rates, 1 cm^3/min; detection at 240 nm. A = unloaded column; eluent, water–methanol (1:1); B = loaded column; eluent, water–methanol (1:1) containing 10^{-2} mol/l γ-CYD; C = loaded column; eluent, water–methanol (1:1).

Compound	*Capacity ratio**		
	A	*B*	*C*
D-Norgestrel	19.6	4.04	19.6
L-Norgestrel	19.6	4.54	19.6

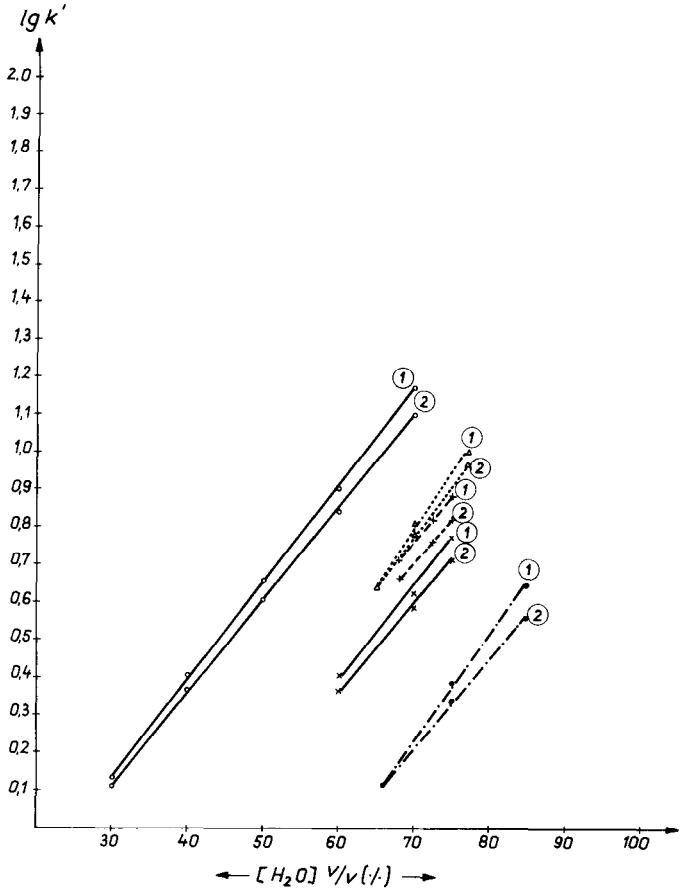

Fig. 3. Dependence of capacity ratios on the nature and concentration of organic modifier used in the eluent. γ-CYD concentration, 10^{-2} mol/l; other conditions as in Fig. 2. 1 = (−)-L; 2 = (+)-D. ○, Methanol; ×, ethanol; ●, isopropanol; +, acetonitrile; △, tetrahydrofuran.

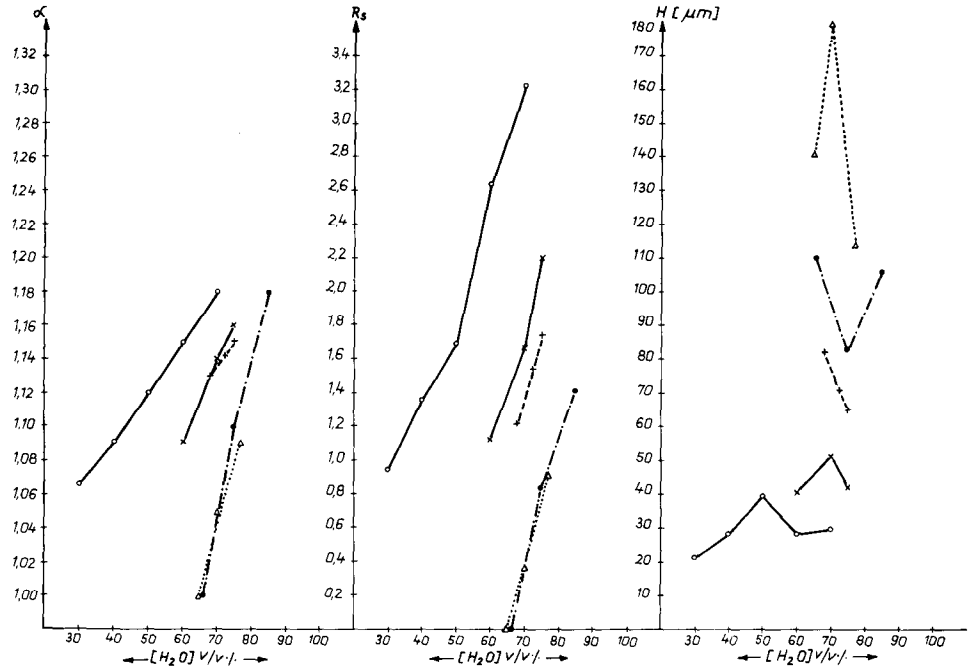

Fig. 4. Dependence of selectivity and efficiency on the nature and concentration of organic modifier used in the eluent. Conditions and key as in Fig. 3.

be influenced by the size of cavity of the CYD-ring, the use of α-, β- and γ-CYD in the eluent for the separation of D,L-norgestrel has been investigated. The results (Table II) show that no inclusion complexes are formed in the presence of α-CYD, and the retention time is the same as when no CYD is used in the eluent. In the presence of β-CYD inclusion complexes can be formed but no chiral recognition for D- and L-norgestrel occurs. When γ-CYD is applied a perfect resolution of enantiomeric compounds can be obtained. This can be explained by the intimate contact of a solute with the chiral CYD-cavity depending on the size and structure of the mol-

TABLE II

DEPENDENCE OF THE CAPACITY RATIOS ON THE SIZE OF CYD MOLECULES

Column, Ultrasphere ODS, 5 μm; flow-rate, 1 cm^3/min; detection at 240 nm.

Eluent	*Capacity ratio*		*L:D*
	D-Norgestrel	*L-Norgestrel*	
Water–methanol (1:1)	26.3	26.3	–
Water–methanol (1:1) + 10^{-2} α-CYD	26.3	26.3	–
Water–methanol (1:1) + $5 \cdot 10^{-3}$ mol β-CYD*	24.2	24.2	–
Water–methanol (1:1) + 10^{-2} mol γ-CYD	4.10	4.60	1.12

* No more β-CYD can be dissolved in the eluent.

ecule investigated. In the case of steroids the size of γ-CYD cavity is appropriate for the separation, whereas the α-CYD cavity is too small.

Effect of type of octadecyl silica packing on the separation

To investigate the general applicability of our method, the norgestrel isomers were separated on four different C_{18} columns with the same eluent composition. Fig. 5 shows that all four have the same resolving power, but the compounds are more

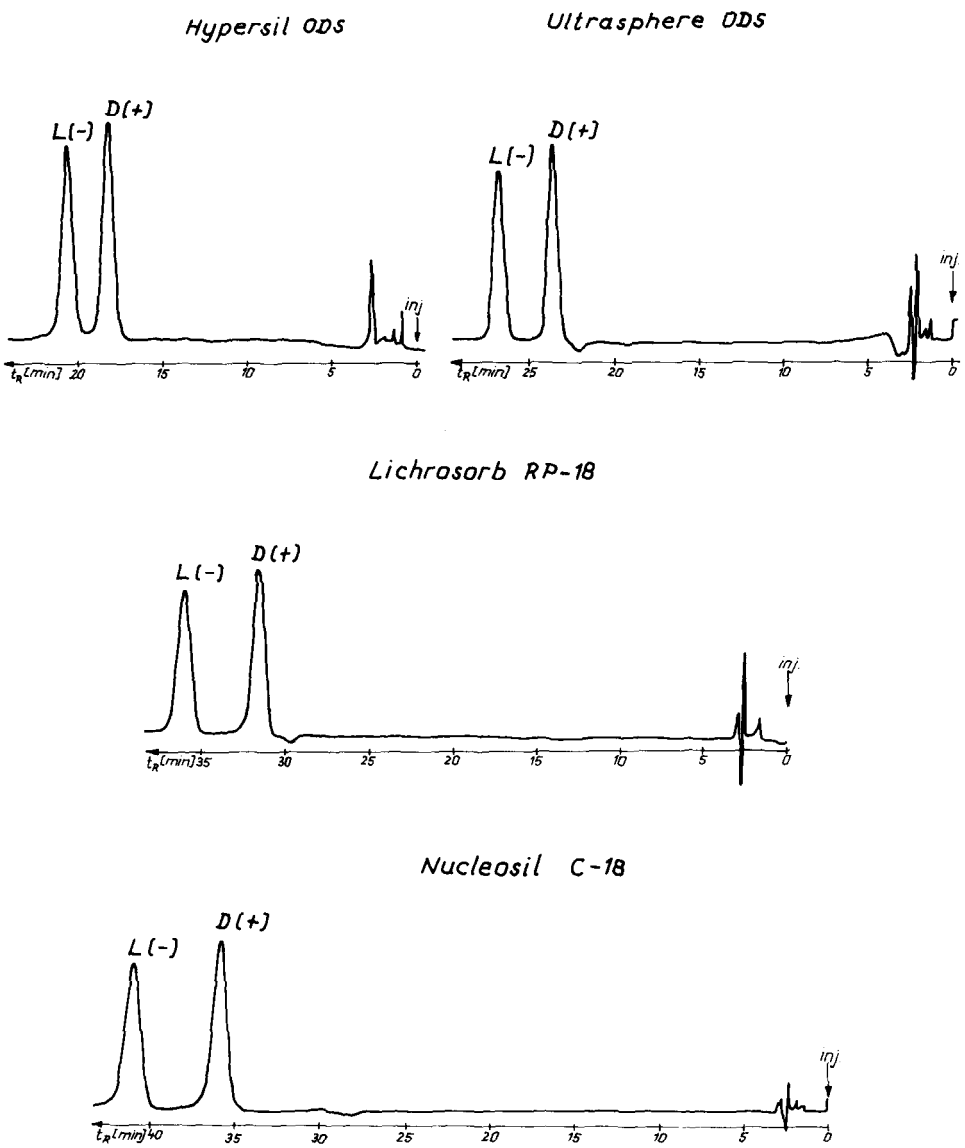

Fig. 5. Separation of D,L-norgestrel on different packing materials. Conditions as in Figs. 3 and 4.

retarded on LiChrosorb RP-18 and Nucleosil C_{18}, probably because of the higher carbon content in these two materials.

CONCLUSION

Our experiments show that γ-CYD forms strong inclusion complexes with D- and L-norgestrel, and baseline separation can be achieved. We think that the use of γ-CYD as a mobile phase additive shows promise presents for the effective separation chiral compounds of the size of steroids.

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