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SEPARATION OF ISOMERIC COMPOUNDS AS CYCLODEXTRIN INCLUSION COMPLEXES ON A CYANOPROPYLSILICA STATIONARY PHASE

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SUMMARY

The retention behaviour of isomeric compounds (enantiomers, conformers, stereoisomers and structural isomers) on a cyanopropylsilica stationary phase was investigated using a methanol–water eluent containing α-, β- and γ-cyclodextrins as mobile phase additives. A stronger inclusion effect was observed than on an octadecylsilica. The selectivity of the separation depends on the size of the cavity of cyclodextrins and on the concentration of organic solvent and cyclodextrin in the eluent.

INTRODUCTION

Two different approaches have been widely used for the separation of isomeric compounds as cyclodextrin inclusion complexes by high-performance liquid chromatography (HPLC). The first method involves the use of cyclodextrins (CDs) as mobile-phase additives[1–6], and in the second the cyclodextrins are chemically bonded to the stationary phase surface, resulting in stable, derivatized high-performance packagings[7–12]. The technique using cyclodextrins as mobile phase additives was first introduced in thin-layer chromatography by Armstrong[13] and Hinze and co-workers[14,15]. The inclusion complex formation of enantiomers, geometrical isomers and other isomeric compounds using chemically bonded phases has also been reviewed[16].

In a recent paper[17] we reported the application on γ-cyclodextrin (γ-CD) as a mobile phase additive for the HPLC separation of two isomers (D- and L-) of norgestrel. Our experiments showed that γ-CD forms strong inclusion complexes with D- and L-norgestrel, and that baseline separation can be achieved. As a continuation of this work, the separation of optical isomers, stereoisomers and structural isomers by means of CDs differing in the size of the cavity (α-, β- and γ-CD) on a cyanopropylsilica stationary phase has been studied.

EXPERIMENTAL

A Liquochrom 2010 HPLC system (Labor-MIM, Esztergom-Budapest, Hungary) equipped with a variable-wavelength UV detector, a loop injector and a recorder was used. The separations were performed on pre-packed Nucleosil 10 CN

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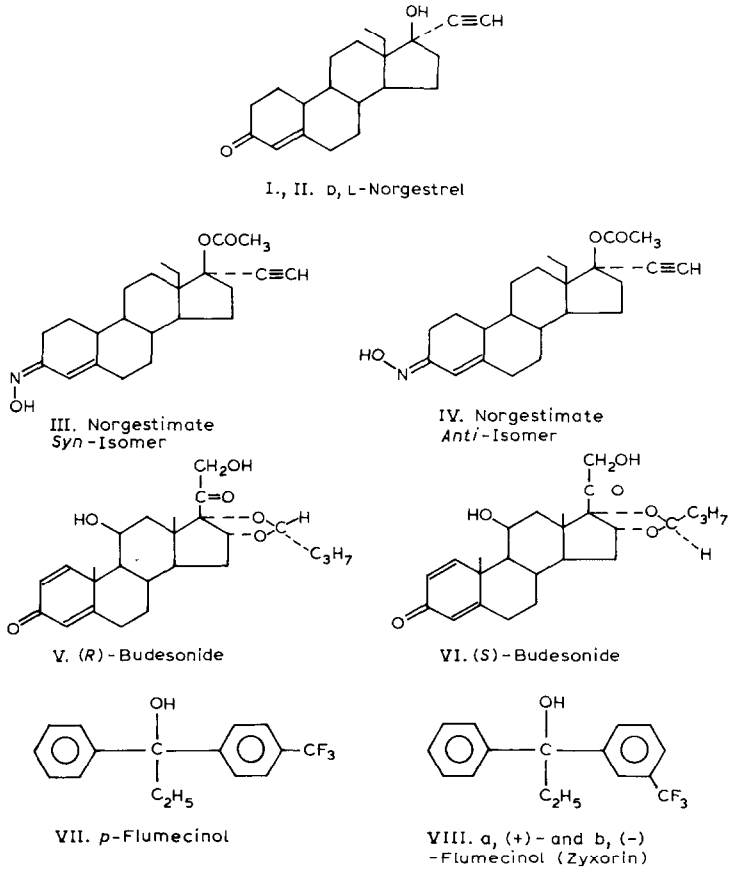

Fig. 1. Structures of the compounds investigated.

(250 × 4.6 mm I.D.) columns (Chrompack, Middelburg, The Netherlands). The eluents were prepared with HPLC-grade solvents and were degassed prior to use.

α-, β- and γ-CD were obtained from Chinoin (Budapest, Hungary) and were used without further purification. The compounds to be tested were prepared at the Chemical Works of Gedeon Richter (Budapest, Hungary) and their quality was checked by HPLC before use.

The structures of the model compounds are shown in Fig. 1.

RESULTS AND DISCUSSION

The application of γ-CD to the separation of norgestrel isomers has been described previously[17]. The separation was performed on a chemically bonded octadecylsilica phase with reversed-phase eluents. The effects of the nature and concentration of organic solvents on the selectivity of the separation were also studied, and it was found that the resolution of the two isomers was considerably influenced by the nature of the organic solvent in the eluent. As the organic solvent tends to

compete, with the solutes for the preferred location in the hydrophobic cavity, the most efficient separation can be obtained with a methanol–water eluent system.

To improve the selectivity and efficiency of the separation of isomeric compounds, the effects of the following parameters were investigated in detail.

(a) The influence of the size of the cavity in the CD ring on the separation of guest molecules differing in molecular size was studied. Enantiomers (I and II), stereoisomers (III and IV), conformers (V and VI) and structural isomers (VII and VIII) were selected as model compounds and α-, β- and γ-CD as the host molecules.

(b) On the basis of our previous experiments[17], with a decrease in methanol concentration stronger inclusion complex formation was expected. For this reason, a cyanopropylsilica stationary phase was used instead of octadecylsilica to decrease the retention of the compounds investigated.

In Table I the retentions of the compounds investigated in the presence and absence of γ-CD on cyanopropylsilica and octadecylsilica are compared. It was found that significantly less methanol can be used on the cyanopropylsilica phase to achieve similar retentions with γ-CD at same concentrations.

Dependence of capacity ratios on the α-, β- and γ-CD concentrations

The influence of the α-CD concentration on the retention of model compounds is shown in Fig. 2. Only small changes in the retention of the model compounds occur when the α-CD concentration as varied. This change in retention, however, is insufficient for separation, except for III and IV The size of the cavity of α-CD is apparently too small for inclusion complex formation.

A greater change in the retentions with change in the β-CD concentration is shown in Fig. 3. All isomers with the exception of the enantiomers (I and II) can be well separated.

TABLE I

DEPENDENCE OF RETENTION ON THE POLARITY OF THE STATIONARY PHASE IN THE PRESENCE AND ABSENCE OF γ-CD

Conditions: columns, Nucleosil 10 CN (10 μm) (250 × 4.6 mm I.D.) and Hypersil ODS (5 μm) (250 × 4.6 mm I.D.); flow-rate, 1 ml/min; detection at 254 nm.

Compound	*Capacity ratio (k')*				
	Cyanopropylsilica, methanol–water (1:3)			*Octadecylsilica, methanol–water (3:2)*	
	Without γ-CD	*+ 10^{-3} mole/l γ-CD*	*+ 10^{-2} mole/l γ-CD*	*Without γ-CD*	*+ 10^{-2} mole/l γ-CD*
I	10.25	1.50	0.12	5.61	2.23
II	10.25	1.87	0.12	5.61	2.46
III	42.60	3.56	0.00	27.00	1.45
IV	48.00	3.87	0.00	28.20	1.60
V	11.75	7.01	1.62	6.15	4.84
VI	11.75	5.87	1.25	6.46	4.46
VII	21.50	19.10	7.75	14.70	14.70
VIII	21.50	19.10	8.56	12.20	12.20

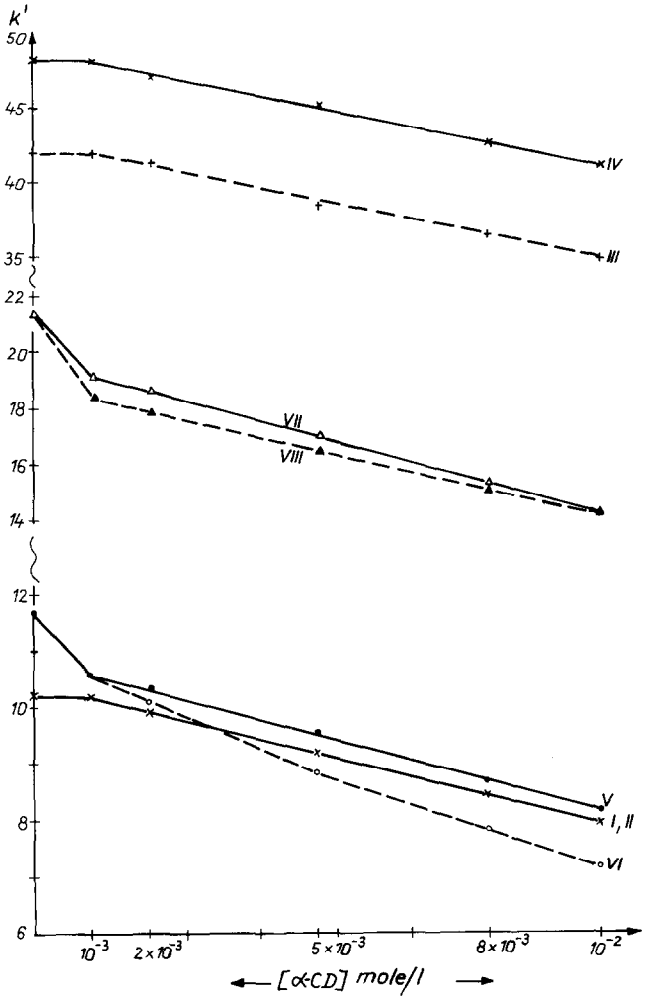

Fig. 2. Dependence of retention on α-CD concentration. Conditions: column, Nucleosil 10 CN (10 μm) (250 × 4.6 mm I.D.); eluent, methanol–water (1:3) containing different amounts of α-CD; flow-rate, 1 ml/min; detection at 254 nm.

The effect of γ-CD concentration on the separation is shown in Fig. 4.

Comparing the data in Figs. 2–4, it can be concluded that the enantiomeric test compounds of norgstrel can be separated only by using γ-CD for complexation. The best separation of flumecinol isomers (VII and VIII) is achieved in the presence of β-CD in the eluent. The *R*- and *S*-isomers of of budesonide can be separated with both β- and γ-CD, although in the form of γ-CD complexes these compounds have smaller k' values. For the separation of norgestimate isomers (III and IV), the use of β-CD in the eluent seems to be optimal.

Effect of concentration of methanol in the eluent on the selectivity of the separation

The dependences of the capacity ratios and selectivity factors on the methanol

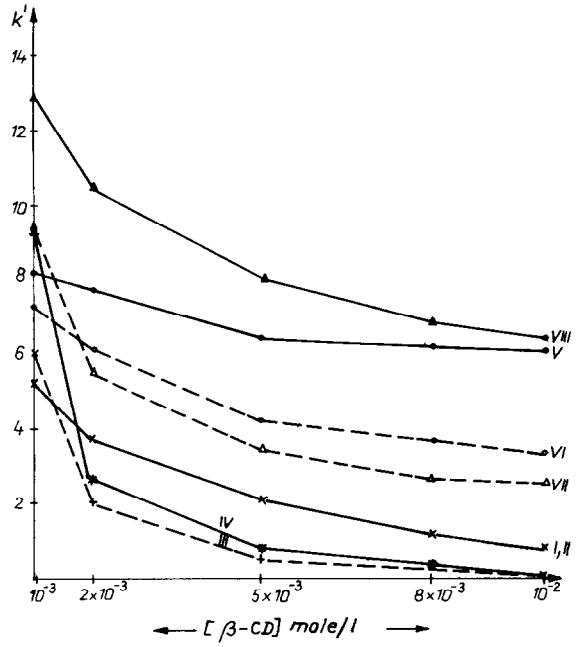

Fig. 3. Dependence of retention on β-CD concentration. Eluent, methanol–water (1:3) containing different amounts of β-CD. Other conditions as in Fig. 2.

concentration of the eluent with the use of β-CD in the eluent are illustrated in Fig. 5. The following conclusions can be drawn:

(1) The retentions of flumecinol isomers (VII and VIII) are almost independent of the methanol concentration, indicating stronger inclusion complex formation in a methanol-lean eluent. The stronger inclusion complex formation (decreased retention) is compensated for by the lower methanol content (increased retention), resulting in only a slight change in retention. The concept of stronger inclusion complex formation is supported by the separation of the enantiomers of VIII (VIIIa and b).

(2) A similar effect can be observed with the *R*-isomer of budesonide. However, owing to the significant dependence of the capacity ratio of the *S*-isomer of budesonide on the methanol concentration, the selectivity of the separation is highly dependent on the water content of the eluent.

The separations of model compounds in the optimal eluent system are shown in Figs. 6–8.

CONCLUSIONS

These experiments show that isomeric compounds can be advantageously separated on a cyanopropylsilica stationary phase in the presence of CDs. When the separations are performed on a cyanopropylsilica stationary phase, the inclusion complex formation is more effective than on octadecylsilica. As a consequence, lower concentrations of methanol and CD can be used in the eluent, clearly demonstrating

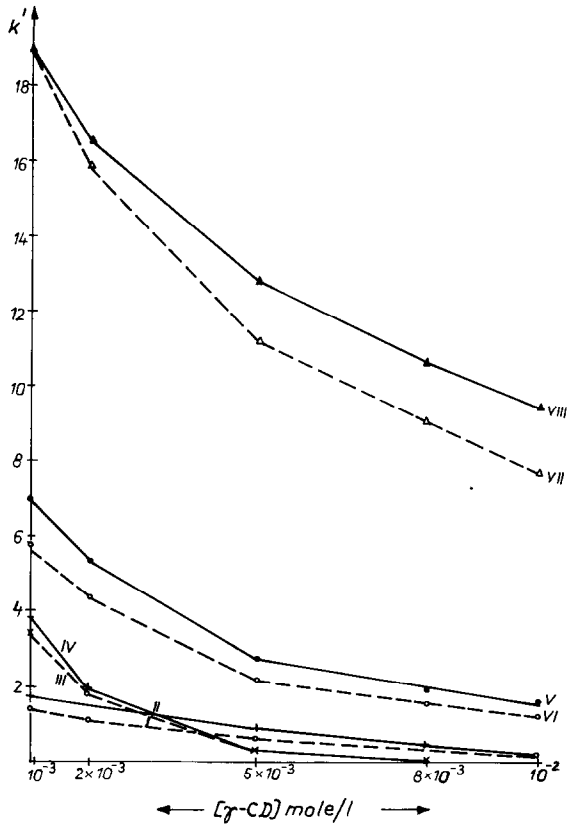

Fig. 4. Dependence of retention on γ-CD concentration. Eluent, methanol–water (1:3) containing different amounts of γ-CD; other conditions as in Fig. 2.

the important effect of methanol concentration on inclusion complex formation. The selectivity of the separation is highly influenced by the size of the cavity in the CDs. Norgestrel isomers can be separated only in the presence of γ-CD, whereas the isomers of norgestimate and flumecinol can be separated in the presence of β-CD. The budesonide isomers can be resolved by using either β- or γ-CD.

From the results, it can be concluded that the size of the cavity of α-CD is too small for the model compounds. The size of the cavity of β-CD is appropriate for inclusion complex formation in every case, but enantiomeric separation of norgestrel isomers can be achieved only with γ-CD. This can be explained by the more intimate contact of the isomers with the chiral γ-CD cavity.

The polarity of the complexes formed depends on the number of glucose units in the CD rings, the most polar complexes being formed by γ-CD.

With decreasing methanol concentration (resulting in increasing chromatographic retention), the capacity ratios of the compounds should remain constant when stronger inclusion complexes are formed (resulting in diminished retention), as illustrated by the example of flumecinol isomers. With methanol-lean eluents only a slight change in retention (VIIIa and b) can be achieved.

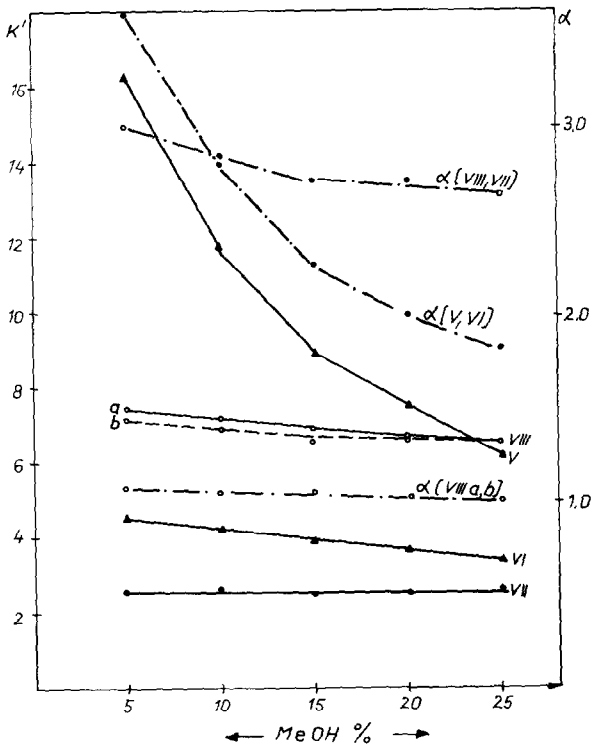

Fig. 5. Dependence of retention and selectivity on the methanol (MeOH) concentration in the eluent. Eluent: various mixtures of methanol and water containing 10^{-2} mole/l of β-CD. Other conditions as in Fig. 3.

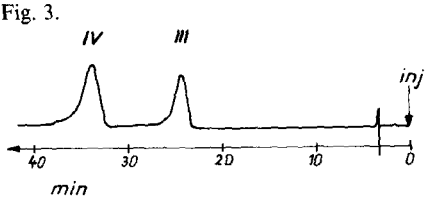

Fig. 6. Separation of *syn*- and *anti*-isomers of norgestimate. Eluent, methanol–water (1:3) containing 10^{-3} mole/l of β-CD; other conditions as in Fig. 3.

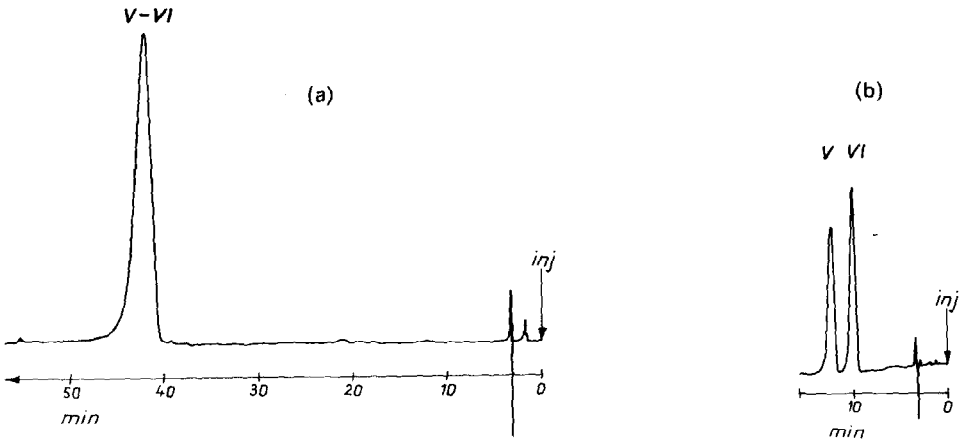

Fig. 7. Separation of *S*- and *R*-isomers of budesonide. Eluent, (a) methanol–water (1:3) without γ-CD and (b) methanol–water (1:3) containing $5 \cdot 10^{-3}$ mole/l of γ-CD; other conditions as in Fig. 3.

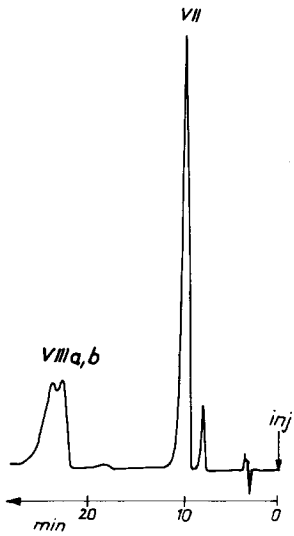

Fig. 8. Separation of flumecinol isomers. Eluent, methanol–water (15:85) containing 10^{-2} mole/l of β-CD; other conditions as in Fig. 3. Compounds VIIIa and b: (+)- and (−)-isomers.

Further studies of the inclusion complex formation between CDs and the model compounds are in progress.

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