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Comparative study on camphor enantiomers behavior under the conditions of gas–liquid chromatography and reversed-phase high-performance liquid chromatography systems modified with α- and β-cyclodextrins

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Abstract

The dependence of retention and selectivity parameters of camphor enantiomers on the concentration of α- and β-cyclodextrins were studied under conditions of GLC (matrix solvent: Glycerol, 95°C) and RP-HPLC (matrix solvent: Aqueous methanolic, 20°C). It has been found that β-cyclodextrin forms complex of 1:1 stoichiometry and does not recognize enantiomers of camphor. In contrast α-CD forming complexes of 1:2 stoichiometry appeared to be very efficient chiral selector of (+) and (−)-camphor. Relatively considerable differences have been observed between stability constants determined by GLC and RP-HPLC, what may be explained by the various natures of the matrix solvents and the various temperatures of the measurements. On the contrary, the enantioseparation factor α observed at higher concentrations of α-cyclodextrin stabilizes on the very similar value $\alpha_{+/-}(\text{GLC}) \cong \alpha_{-/+}(\text{HPLC}) \cong 1.6$. Simple theoretical considerations focusing on the differences in the mechanisms of the studied processes have been performed. According to them the enantiomer forming the more stable complex with the cyclodextrin should be eluted from the RP-HPLC column first and GLC column last. This fact has been confirmed experimentally.

Keywords: Enantiomer separation; Stability constants; Camphor; Cyclodextrins

1. Introduction

The most characteristic property of cyclodextrins (CDs) is their remarkable ability to form stereoselective inclusion compounds with various organic and inorganic species of neutral as well as ionic nature. Moreover, as CDs are composed of D-glucose units—they are homochiral themselves and represent, therefore, a potential tool for the formation of diastereoisomers with other chiral compounds of diverse chemical characteristics. For that reason CD complexation processes can be considered as a procedure of choice for resolution of constitutional isomers and stereoisomers including enantiomers. Many chromatographic methods and related techniques have been modified by cyclodextrins. They were reported in numerous publications and summarized in monographic presentations [1–7].

In this study special attention is focused on chiral recognition achieved by applying α-CD or β-CD in GLC and RP-HPLC systems. Model tested com-

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pounds were the enantiomers of camphor. Such a choice of methodologies and tested compounds has been made for many reasons.

The argument concerning the methodologies is very simple, as the main purpose of our investigations was theoretical description and comparison of two methods. Both have been elaborated upon in our laboratory [8–11] and both of them deal with cyclodextrins in a dissolved state as stationary phase in GC and as mobile phase in HPLC, and thus they can be compared.

In favour of the selection of (+/−)-camphor as the model tested compound, is its volatility, solubility and spectroscopic properties, thus, enabling one to monitor it by UV detection at 280 nm. The fact that both enantiomers of relative high optical purity are commercially accessible was also the feature taken into account.

The comparative study of GC and HPLC methods modified with CDs has been undertaken keeping in mind the recognition mechanisms of the two processes, but not only these. An additional purpose of practical value was the answer to the question: How the chromatographic and complexation data could be foreseen and transferred from one method to the other?

2. Theoretical consideration

In the chromatographic systems modified with cyclodextrins the following equilibria should be considered:

$$K_R = \frac{[G]_s}{[G]_m}$$

where K_R is the equilibrium constant of transfer of solute G from the mobile to the stationary phase; $[G]_s$ and $[G]_m$ mean the concentrations of solute G in the stationary and mobile phase, respectively.

When a cyclodextrin complexation process occurs in the solution of an achiral solvent at the first step the following equilibrium should be taken into account:

$$G + CD \overset{K_1}{\Leftrightarrow} G \cdot CD \quad K_1 = \frac{[G \cdot CD]}{[G][CD]}$$

where G means guest–solute molecule and K_1 is the stability constant of dissolved CD complex.

When, apart from 1:1 stoichiometry, complexes of 1:2 stoichiometry are formed the following equilibria should be additionally considered:

$$G \cdot CD + CD \overset{K_2}{\Leftrightarrow} G \cdot CD_2 \quad K_2 = \frac{[G \cdot CD_2]}{[G \cdot CD][CD]}$$

which can be summarized as:

$$G + 2CD \overset{K_1 \cdot K_2}{\Leftrightarrow} G \cdot CD_2 \quad K_1 K_2 = \frac{[G \cdot CD_2]}{[G][CD]^2}$$

These steps in complexation should be respected examining both investigated systems: GC and HPLC.

2.1. Gas chromatography model

Assuming that

1. between the gaseous and liquid stationary phases above all the distribution of G is significant and, thus, it plays the main role in equilibration,
2. while an inclusion of the matrix solvent in the CD cavity and adsorption of CD on the surface of the solid inert support are negligible and can be omitted,
3. moreover the range of G concentrations comprises of the segment of the linear distribution isotherm,

then the model of the GC process could be simplified to the following phenomenological description presented in Fig. 1.

When the complexes formed by G with CD are of 1:1 stoichiometry then the relation between retention time and CD concentration is described by the linear equation

$$k' = k'_R(1 + K_1[CD]) \tag{1}$$

where k'_R and k' mean, respectively, the retention factor of G on the two columns — the one containing pure matrix solvent and the second with CD dissolved in the same matrix.

If the solute G forms complexes with CD of 1:2 stoichiometry the relation k' versus CD concentration changes to parabolic.

$$k' = k'_R(1 + K_1[CD] + K_1K_2[CD]^2) \tag{2}$$

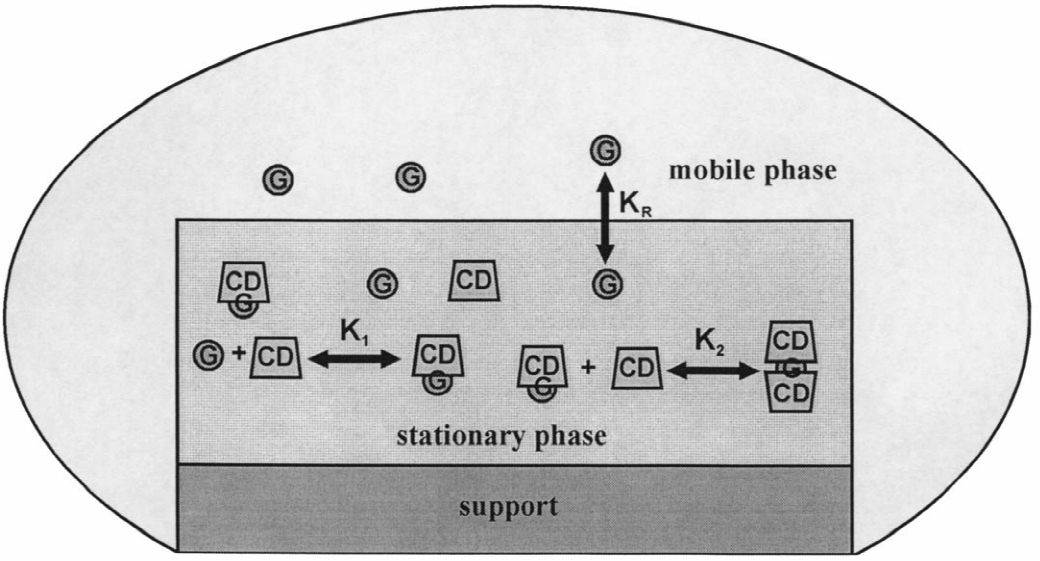

Fig. 1. Scheme of equilibrium in gas chromatography system modified with cyclodextrins; K_R-distribution constant, K_1,K_2-stability constants of complexes formed between the guest molecule and the cyclodextrins of 1:1 and 1:2 stoichiometry respectively.

2.2. HPLC model

With assumptions that

1. adsorption of CD at the surface of the stationary phase is very small and, therefore, the CD does not influence its properties [12,13],
2. the solute molecule (G) is adsorbed at the surface of the stationary phase and complexed by the CD in the bulk mobile phase solution,
3. depending on the conditions the complexes of 1:1 and 1:2 stoichiometry between solute G and CD may be formed,
4. adsorption of these complexes (G–CD and G–CD_2) on the stationary phase is negligible, then the chromatographic process may be simplified to the following phenomenological equilibrium presented in Fig. 2.

By joining the formulae for distribution and complexation constants with the definition of the retention factor, k', the final equation for complexes of 1:1 stoichiometry is as follows [3,14,15]:

$$k' = \frac{k'_R}{1 + K_1[\mathrm{CD}]} \tag{3}$$

where k'_R is the retention factor observed in the system without the CD and K_1 means the stability constant of 1:1 complexes.

When apart from 1:1 stoichiometry, 1:2 stoichiometry is obtained then the equation for k' changes as follows [16,17]:

$$k' = \frac{k'_R}{1 + K_1[\mathrm{CD}] + K_1K_2[\mathrm{CD}]^2} \tag{4}$$

After rearranging the equations we have as follows:

$$\frac{1}{k'} = \frac{1}{k'_R} + \frac{K_1[\mathrm{CD}]}{k'_R} \tag{5}$$

or

$$\frac{1}{k'} = \frac{1}{k'_R} + \frac{K_1[\mathrm{CD}]}{k'_R} + \frac{K_1K_2[\mathrm{CD}]^2}{k'_R} \tag{6}$$

It is seen that contrary to the GC model for 1:1 stoichiometry complexes, that is described by the linear relation between k' and [CD] — in HPLC the reciprocal of k' is a linear function of [CD]. In the case of 1:2 stoichiometry complexes, the relation of $1/k'$ versus [CD] becomes parabolic.

In summing up, this reasoning should be verifiable experimentally by the following behavior: Of the two species, the one forming more stable complexes with

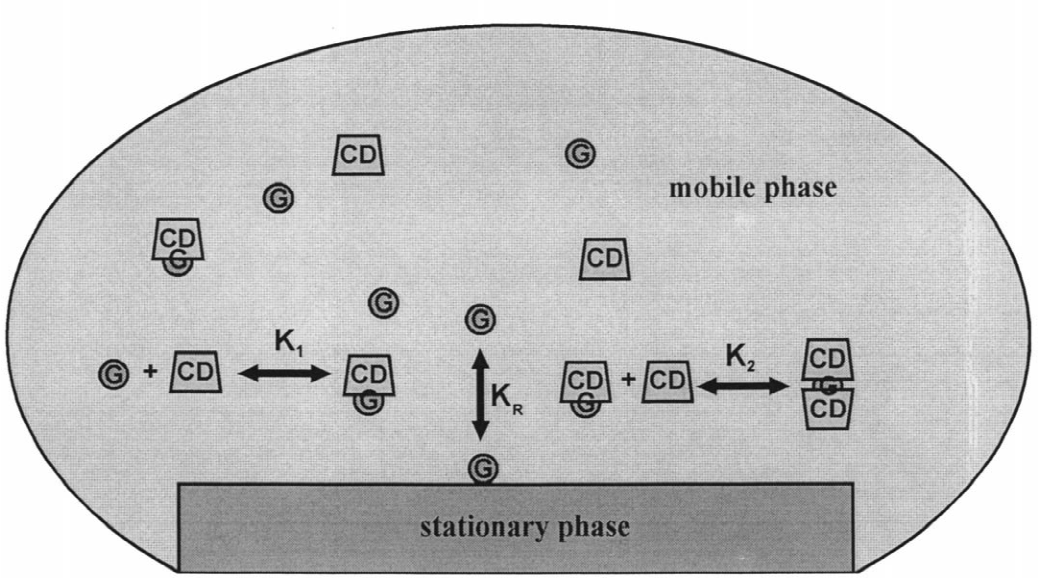

Fig. 2. Scheme of the equilibrium in RP-HPLC system with the mobile phase modified with cyclodextrins; K_R-distribution constant, K_1,K_2-stability constants of complexes formed between the guest molecule and the cyclodextrins of 1:1 and 1:2 stoichiometry respectively.

the CD should be eluted from the HPLC column first, while from GC last.

3. Experimental

3.1. Reagents

α-Cyclodextrin (α-CD) and β-cyclodextrin (β-CD) were supplied by Chinoin (Budapest, Hungary); (+) and (−)-camphor were from Fluka (Buchs, Switzerland).

All other reagents and solvents were of analytical reagent grade and were used as received.

3.2. Apparatus and procedures

Gas chromatographic studies were performed using a Hewlett-Packard Model 5890 gas chromatograph equipped with a dual flame ionization detector. The peak areas and retention times were measured by means of a Hewlett-Packard 3390 A integrator.

Glass columns (2 m×4 mm I.D.) packings were: Chromosorb (60–80 mesh) coated with glycerol alone and glycerol solutions of α- or β-cyclodextrin of appropriate concentration. In the case of α-cyclodextrin, to improve its solubility, small amount of lithium nitrate ($LiNO_3$) was added to the solution. The GC measurements were performed at 95°C.

HPLC experiments were carried out using a Waters (Vienna, Austria) Model 590 pump, a Rheodyne type injector and a Waters UV–Vis detector Model 490 (280 nm).

One column 250×1 mm I.D. packed with 5 μm LiChrosorb RP-18 was used.

The mobile phases were aqueous methanolic solutions (35% (v/v) MeOH–water) with an appropriate concentration of native cyclodextrin. The HPLC measurements were performed at 20°C.

To simplify GC calculations, the cyclodextrins concentrations have been expressed as molal solutions.

4. Results and discussion

Retention and selectivity parameters for enantiomers of camphor were studied depending on the kind (α- or β-) and the concentration of cyclodextrins.

4.1. Retention data

Fig. 3 presents k' and $1/k'$ values as the functions of α- or β-CD concentrations determined respective-

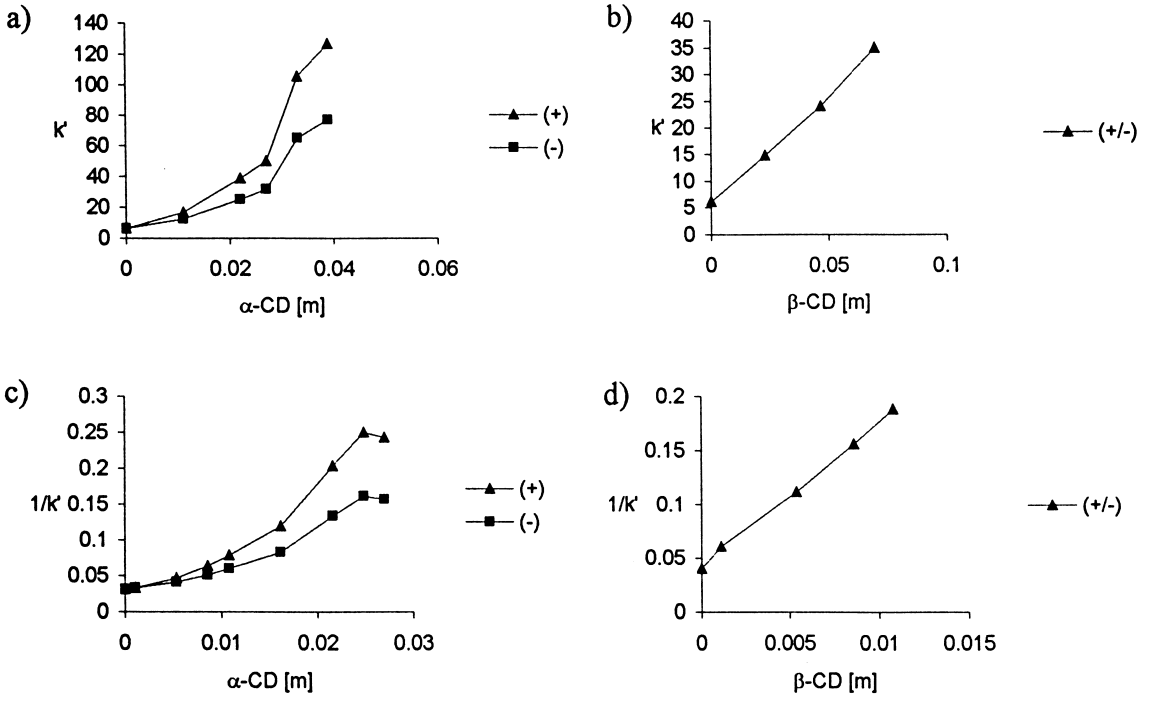

Fig. 3. Relationship between k' for the GC system and $1/k'$ for the RP-HPLC system of (+) and (−)-camphor versus α-CD concentration (a and c), and β-CD concentration (b and d).

ly in GC (a and b) and HPLC (c and d) systems. It is seen that with a growing degree of complexation, the retention factors of camphor get down or up depending on the system (RP-HPLC and GC).

Unfortunately, the ranges of concentration of the CDs investigated by both techniques were not strictly the same. This fact may be a source of some doubts but it could not be avoided because of limitations in solubility of the CDs in glycerol and water–methanol mixtures.

In the case of the β-CD the linear relation k' and $1/k'$ values against the CD concentration has been observed respectively under conditions of GC and HPLC. This behavior indicates a 1:1 stoichiometry [camphor–β-CD] complex according to Eqs. (1) and (5).

Within the limit of the β-CD concentrations in the experiments — this statement seems to be well proven and, thus, the determined relations have been used as the basis for stability constants evaluation according to Eqs. (1) and (5). Thus evaluated from retention data, the stability constants have been collected in the Table 1.

The complex β-CD–camphor is about five times

Table 1
The stability constants of complexes formed by the camphor enantiomers with the α- and β-cyclodextrin

	K_1 (m^{-1}), (+/−)-Camphor–β-CD	K_1K_2 (m^{-2})	
		(+)-Camphor–α-CD	(−)-Camphor–α-CD
GC (temp. 95°C, glycerol)	60	5500	3250
RP-HPLC (temp. 20°C, water–methanol)	350	8770	5260

more stable under the conditions of HPLC than in GC. These results are in accordance with expectation as a consequence of there being about a 75°C difference in the temperature of measurements between HPLC (20°C) and GC (95°C) and different matrix solvent.

α-CD behaves otherwise. In contrast to β-CD, parabolic relations have been found between k' (GC) and $1/k'$ (HPLC) and α-CD concentration.

Such dependence suggests the formation of 1:2 stoichiometry complexes. In Fig. 3, showing α-CD behavior, the segment of linear relation is not visible. Maybe it does not exist at all or it only appears at very dilute solutions. But in the area of very low concentrations the changes of retention factor values will be very small, not detectable by the chromatographic techniques being applied.

On the basis of Eqs. (2) and (6) and retention data the stability constants for complexes camphor–α-CD have been also evaluated. The stability constants for complexes of a 1:2 stoichiometry formed between enantiomers of camphor and α-CD have been presented together with β-CD data in Table 1. In this situation comparing the stability constants K_1K_2 for both techniques we do not observe such considerable differences as in the case of β-CD for K_1 values. The stability constants for HPLC are only about 1.5 higher than for GC even if the difference in the temperatures of measurements is the same (75°C). The reason for such a phenomenon can be the dissimilar influence of the matrix-solvent of the cyclodextrins (glycerol in GC and water–methanol in HPLC) on the dissociation of the G–CD_2 complex.

4.2. Selectivity

Fig. 4 presents relations of the enantioseparation factor against the α-CD concentration. β-CD does not recognize enantiomers of camphor, at least to such an extent so as to be distinguishable by our chromatographic techniques.

Contrary to β-CD behavior, α-CD resolves (+) and (−) camphor and observed enantioseparation factors are remarkable. This property seems to be due to the formation of 1:2 stoichiometry complexes. As our results linking the enantioseparation with the stoichiometry concern not only camphor but also

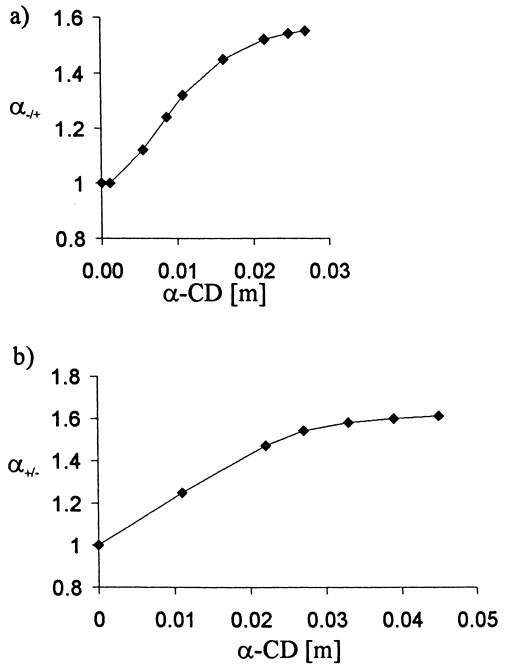

Fig. 4. Relationship between the separation factor, α, of enantiomers of camphor and the concentration of the α-cyclodextrin for the GC system (a) and the RP-HPLC system (b).

many other terpenoic compounds this theme will be described separately [18].

In spite of very different conditions (solvent, temperature) at higher concentrations of the α-CD enantioseparation factors reach a very similar value: $\alpha_{+/-}(\mathrm{GLC}) \cong \alpha_{-/+}(\mathrm{HPLC}) \cong 1.6$.

This interesting phenomenon requires further investigations to be undertaken.

According to the simple theoretical considerations described above, (+)-camphor eluted from the RP-HPLC column first, last in GC (see Figs. 5 and 6) and forms more stable complexes with the α-cyclodextrin.

5. Conclusions

1. β-CD forms inclusion complexes with camphor of a 1:1 stoichiometry that are relatively stable even at 95°C. Nevertheless, throughout this complexation process it does not recognize the enantiomers of camphor,
2. α-CD forms complexes, stable at 95°C, of a 1:2

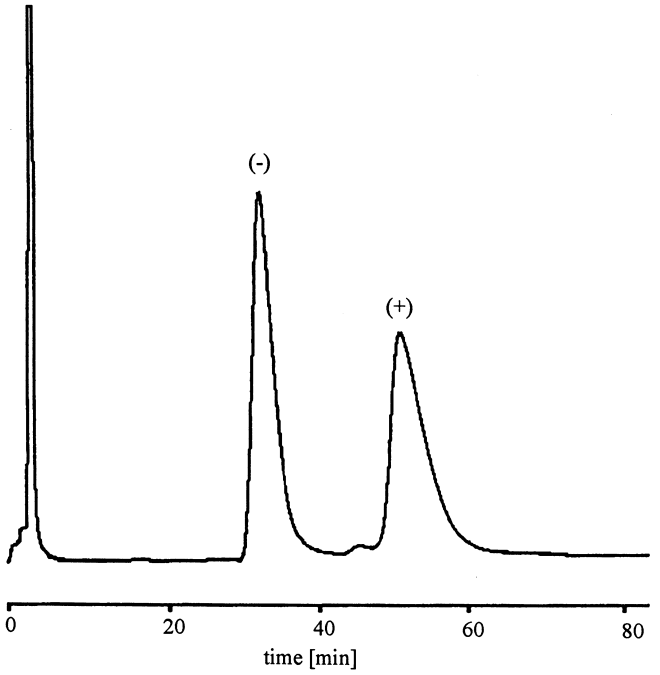

Fig. 5. Chromatogram of the enantiomers of camphor obtained on a GC column 2 m×4 mm I.D. filled with Chromosorb W 60–80 mesh coated with 0.027 *m* solution of α-cyclodextrin in glycerol; temperature 95°C, flow-rate 40 ml/min.

stoichiometry and enables efficient baseline separations of camphor optical antipodes,

3. According to the previous theoretical considerations — the order of elution of the camphor enantiomers in RP-HPLC and GLC appeared opposite,
4. The described relations may be of some use in designing further studies on the optimization of

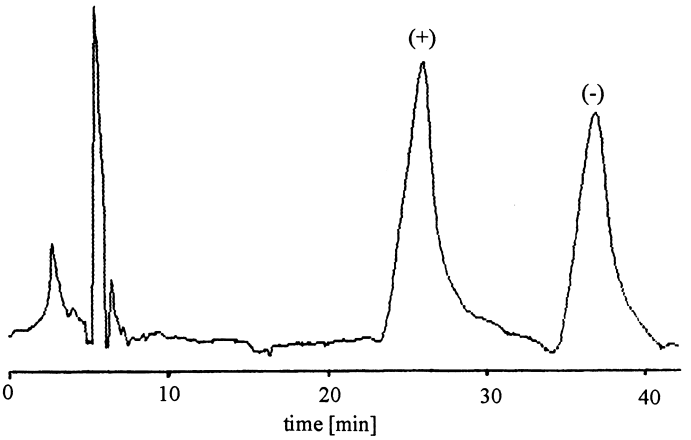

Fig. 6. Chromatogram of enantiomers of camphor obtained on a RP-HPLC column 250×1 mm I.D. packed with 5 μm LiChrosorb RP-18, mobile phase: Water–methanol (65:35, v/v) containing 0.027 *m* α-CD; temperature 20°C, flow-rate 40 μl/min.

chromatographic resolution and the determination of stability constant values.

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