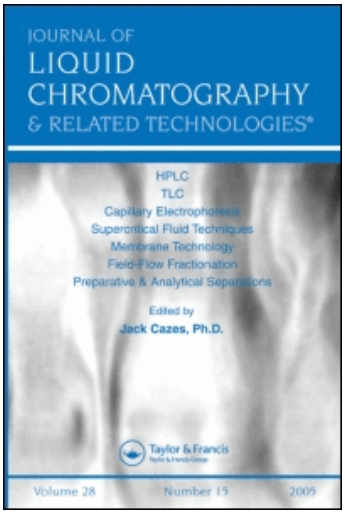


Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Resolution of Mephenytoin and Some Chiral Barbiturates into Enantiomers by Reversed Phase High Performance Liquid Chromatography via β-Cyclodextrin Inclusion Complexes

Danuta Sybilska[a]; Janusz Żukowski[a]; Jacek Bojarski[b]
[a] Institute of Physical Chemistry Polish Academy of Sciences, Warsaw, Poland [b] Department of Organic Chemistry, N. Copernicus Academy of Medicine, Kraków, Poland

To cite this Article Sybilska, Danuta , Żukowski, Janusz and Bojarski, Jacek(1986) 'Resolution of Mephenytoin and Some Chiral Barbiturates into Enantiomers by Reversed Phase High Performance Liquid Chromatography via β-Cyclodextrin Inclusion Complexes', Journal of Liquid Chromatography & Related Technologies, 9: 2, 591 — 606
To link to this Article: DOI: 10.1080/01483918608076655
URL: http://dx.doi.org/10.1080/01483918608076655

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(2&3), 591-606 (1986)

RESOLUTION OF MEPHENYTOIN AND SOME CHIRAL BARBITURATES INTO ENANTIOMERS BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY VIA B-CYCLO-DEXTRIN INCLUSION COMPLEXES

Danuta Sybilska[1], Janusz Żukowski[1], and Jacek Bojarski[2]

[1]*Institute of Physical Chemistry*
Polish Academy of Sciences
Kasprzaka 44/52
01-224 Warsaw, Poland
[2]*Department of Organic Chemistry*
N. Copernicus Academy of Medicine
Dzierżyńskiego 14B
30-048 Kraków, Poland

ABSTRACT

β-Cyclodextrin as the chiral mobile phase component was used for resolution of mephenytoin and some barbiturates (antidepressant drugs) into enantiomers on LiChrosorb RP 18 column. The effects of β-cyclodextrin concentration on the capacity factors were investigated and the stability constants as well as capacity factors of β-cyclodextrin complexes were calculated. It has been found that β-cyclodextrin complexation results in a distinct enantioselectivity in the case of mephenytoin and barbiturates containing a chiral center in the pyrimidine ring. Results are discussed in the light of two phenomena influencing resolution: adsorption of inclusion complexes on the stationary phase and complexation process in the mobile phase solution.

 0148-3919/86/0902-0591$3.50/0

INTRODUCTION

β-Cyclodextrin (β-CD) is torus shaped cyclic oligosaccharide made up of seven α-1,4 linked D-glucopyranose units; it is produced on a large scale from starch. The most characteristic property of β-CD is its remarkable ability to form inclusion compounds with various neutral and ionic species (1). These processes of inclusion (in hydrophobic cavity of β-CD of diameter 8 Å and volume 346 $Å^3$) are influenced mainly by the hydrophobicity and the shape of a guest compound i.e. by the fitness of a complexed molecule for the β-CD cavity. For that reason complexation with β-CD may be considered as a process of choice for separation of isomers.

β-CD is a chiral compound itself, like D-glucose of which it is constructed. β-CD is dextrorotatory of specific rotation α_D^{25} = +162.0 ± 0.5 (1). Thus β-CD complexation is also a potential tool for separation of other chiral compounds into enantiomers.

These inclusion properties of β-CD have been used in various separation techniques including classical liquid chromatography methods (2), but the classical columns containing usually polymers with β-CD molecules incorporated in their structure are of comparatively large dimensions and low efficiency due to the complex mechanism of sorption: gel permeation+molecular inclusion.

For separation of chiral compounds into enantiomers by high performance liquid chromatography (HPLC) CD - inclusion phenomena have been recently applied in two different ways:

- by using chemically bonded β-CD - silica stationary phases; the resolutions of various chiral compounds including some barbiturates have been reported recently (3,4,5),

- by applying β-CD as a mobile phase component in the reversed phase (RP) system (6,7,8).

The latter way has been exemplified by resolution of mandelic acid and some of its derivatives substituted in the side chain and/or in the aromatic ring. It has been found that the enantioselectivity arising from inclusion in β-CD molecules is substantial only for compounds containing an intact carboxylic group and another polar group (e.g. OH, NH_2) at the chiral carbon atom able to form a hydrogen bond with the secondary hydroxyl groups of β-CD. It was also assumed that the insertion of a phenyl group in the cavity of β-CD provides third point of contact indispensable for achieving a chiral recognition according to the "three points of attachment" concept of Dalgliesh (9).

These results have encouraged us to undertake further studies which are reported in the present work on application of the same procedure for resolutions of mephenytoin and some barbiturates into enantiomers. All the compounds studied are well-known and widely used therapeutic agents.

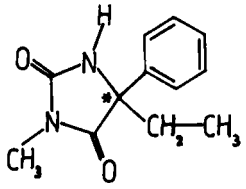
EXPERIMENTAL

Reagents

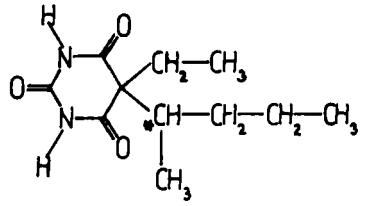
β-CD was supplied by Chinoin (Budapest, Hungary) and was purified by recristallization from water. All solvents and reagents were of analytical-reagent grade and were used without purification. Mephenytoin and barbituric acid derivatives which formulae are collected in Table 1 were commercial drug products. Pure enantiomers of methylphenobarbital were kindly provided by Professor J. Knabe (Saarbrücken). Enantiomers of mephenytoin and hexobarbital were prepared by means of micro-preparative

TABLE 1

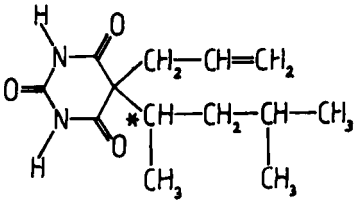
Structural formulae of mephenytoin and barbiturates studied



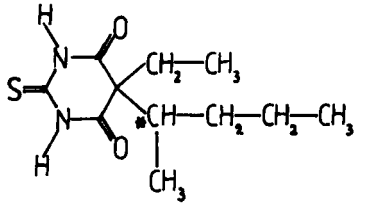
Mephenytoin



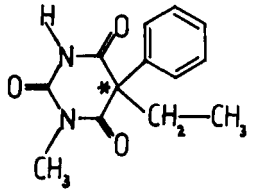
Pentobarbital



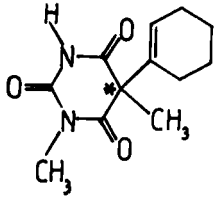
Secobarbital



Thiopental



Methylphenobarbital



Hexobarbital

chromatographic method using β-CD solution as described below.

Apparatus and procedures

A Type 302 HPLC apparatus (Institute of Physical Chemistry, Warsaw, Poland) equipped with UV detector (254 nm) and injector (5 µl) was used.

Chromatographic measurements of k' values versus β-CD concentration were carried out with a column (100×4.5 mm i.d.) slurry-packed with LiChrosorb RP 18 10 μm (E.Merck, Darmstadt, F.R.G.). Dead volume determined by D_2O was 1.00 ml. Mobile phases were ethanol-buffer solutions of β-CD (concs. 0,1,2,4,8 and 12 mM) of pH 2.0. Ethanol-buffer solution was prepared by mixing 200 ml of ethanol, 6 ml of acetic acid and 7.1 g of Na_2SO_4 with 750 ml of water; then the pH was adjusted to 2.0 with H_2SO_4 and the volume diluted to 1000 ml with water. The flow-rate was 0.9 ml/min.

Analytical resolutions of racemates were performed using a column (250×4.0 mm i.d.) slurry-packed with LiChrosorb RP 18 10 μm (dead volume 1.98 ml). Mobile phase was 30 mM β-CD ethanol-acetate buffer solution of pH 5.0. Ethanol-buffer solution was prepared by mixing 180 ml of ethanol and 8.2 g sodium acetate with 800 ml of water; then the pH was adjusted to 5.0 with acetic acid and diluted to 1000 ml with water. The flow-rate was 0.6 ml/min. Temperature of measurements and separations was maintained at 25 ± 0.1 °C using water jacket.

For preparative separations three connected together columns (250×8.0 mm i.d.) packed with LiChroprep RP 18 15-25 μm (E. Merck, Darmstadt, F.R.G.) were used. The mobile phase containing 30 mM β-CD in ethanol-buffer solution was prepared as for analytical resolutions. 10 mg samples dissolved in acetone were injected using loop injector (100 μl). The flow-rate was 1.2 ml/min. The optical purity of collected fractions was tested using analytical column.

EQUILIBRIA AND EQUATIONS

Stoichiometry in β-CD complexes is usually 1:1 but inclusion compounds with other host to guest ratios also exist (1).

In chromatographic RP system containing CD two phenomena are involved in the equilibria: adsorption of free and bound to CD solutes and complexation in mobile phase solution.
The pH of the mobile phase used in this work was much smaller than the pKa values of the investigated compounds (10), therefore only one specie of solute i.e. neutral molecule (G) should be taken into account. It is also supposed that only 1:1 stoichiometry complexes are formed (11).
The third assumption is that β-CD does not influence the properties of RP stationary phase.
The following simplified scheme for description of the equilibria can be derived from these assumptions:

$$G_m + \beta\text{-CD}_m \overset{K_G}{\rightleftharpoons} (G\cdot\beta\text{-CD})_m$$

$$G_s \overset{k'_G}{\rightleftharpoons} G_m$$

$$(G\cdot\beta\text{-CD})_s \overset{k'_{(G\cdot\beta\text{-CD})}}{\rightleftharpoons} (G\cdot\beta\text{-CD})_m$$

where the subscripts s and m denote the stationary and mobile phases respectively, K_G is the stability constant of (G·β-CD) complex, and k'_G, $k'_{(G\cdot\beta\text{-CD})}$ are the capacity factors of the free guest molecule and its β-CD complex. For such system the apparent capacity factor k' can be expressed as:

$$k' = \frac{k'_G + k'_{(G\cdot\beta\text{-CD})} \cdot K_G \cdot [\beta\text{-CD}]}{1 + K_G \cdot [\beta\text{-CD}]} \qquad 1$$

This equation 1, describing a simple RP system, is analogous to that one derived by Uekama et al. (12) for determination of the stability constants of some CD complexes with various ionic species by ion-exchange chromatography. The equation 2 arises from eq. 1 by simple transformation:

$$k' = \frac{k'_G - k'}{[\beta\text{-CD}] \cdot K_G} + k'_{(G \cdot \beta\text{-CD})} \qquad 2$$

The stability constants K_G and the capacity factors $k'_{(G \cdot \beta\text{-CD})}$ of the investigated compounds were evaluated in this work by the least squares method using eq. 2.

RESULTS AND DISCUSSION

The observed changes of capacity factor values k' with β-CD concentration in the mobile phase solution are exemplified in Fig. 1 by the behavior of methylphenobarbital enantiomers. The similar influence of β-CD was observed for all investigated compounds; β-CD additions were always followed by a decrease in the apparent capacity factor values k'. This result suggests that the adsorption of the complex is smaller than that of the corresponding free molecule:

$$k'_G > k'_{(G \cdot \beta\text{-CD})}$$

if the above assumption mentioned, that β-CD does not change RP stationary phase properties, is valid.

The determined k' values satisfied the linear relation:

k' vs. $(k'_G - k')/[\beta\text{-CD}]$ from eq. 2

as it is shown in Fig. 2, what indicates that β-CD complexes formed by all investigated compounds under the experimental conditions are in fact of 1:1 stoichiometry.

The linear relationships enabled evaluation of the stability constants K_G and the capacity factors $k'_{(G \cdot \beta\text{-CD})}$ for β-CD complexes: the determined values are collected in Table 2.

It can be seen that $k'_{(G \cdot \beta\text{-CD})}$ values of barbiturates are slightly negative. This systematic error i.e. the reduction of $k'_{(G \cdot \beta\text{-CD})}$ calculated values may arise from two phenomena:

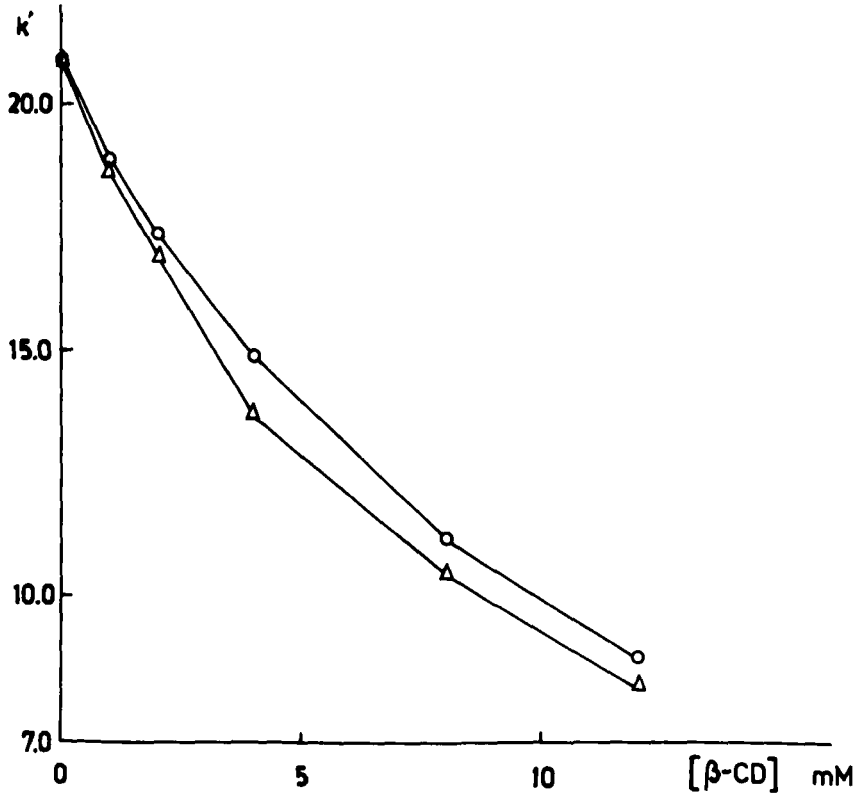


FIGURE 1. Plots of capacity factors k' vs. β-CD conc. of methylphenobarbital enantiomers: Δ-R(-), O-S(+). column: 100×4.5 mm i.d. LiChrosorb RP 18 10 μm; mobile phase: 20% ethanol-buffer solution of pH=2.0 containing various β-CD concentrations.

- either β-CD influences hydrophobic properties of RP stationary phase,
- or some pores of this serbent are not accessible for such large molecules as β-CD complexes (molecular weight ca. 1300) in comparison to small penetrating them D_2O molecules (used for the dead volume determination).

The second suggestion seems to be more plausible.

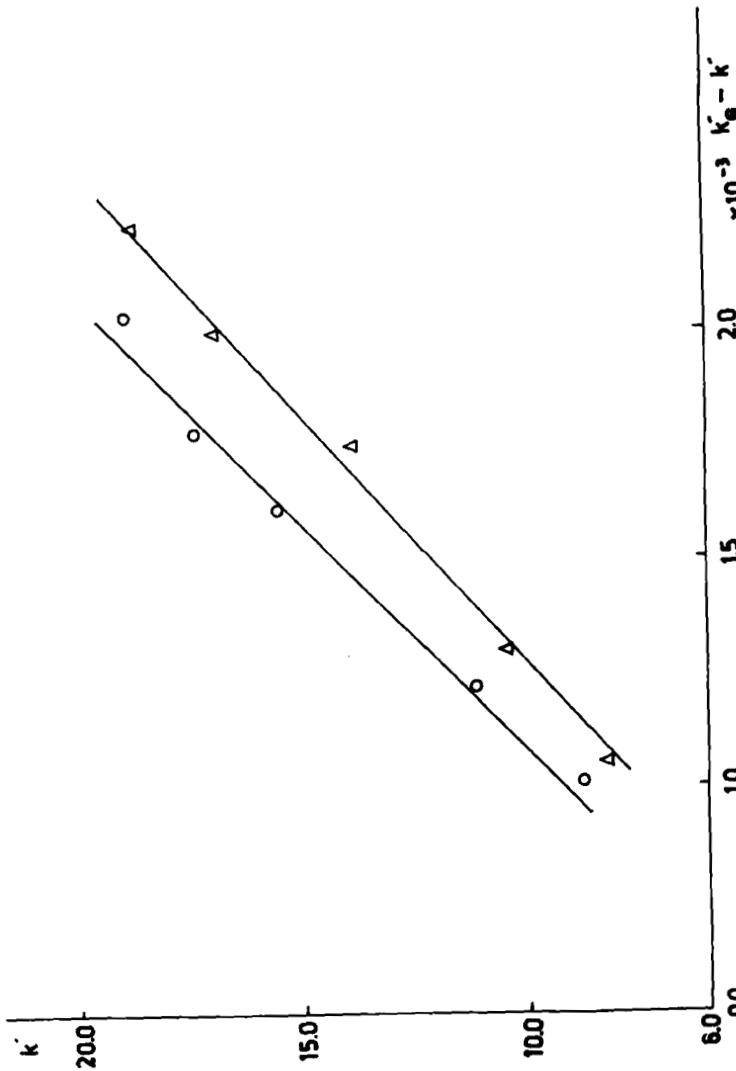


FIGURE 2. Capacity factors k' vs. $(k'_G - k')/[\beta\text{-CD}]$ for methylphenobarbital enantiomers. Chromatographic conditions as in Fig.1.

TABLE 2

Capacity factors k'_G, calculated capacity factors of complexes $k'_{(G\cdot\beta\text{-CD})}$ and their stability constants K_G and regression coefficients r for mephenytoin and barbiturates

Compounds	k'_G	$k'_{(G\cdot\beta\text{-CD})}$	K_G	r
Mephenytoin	15.0	2.0±1.0 5.9±0.3	43±4 49±2	0.9643 0.9926
Pentobarbital	36.9	-0.7±2.2	138±12	0.9497
Secobarbital	58.1	-1.9±0.4	180±2	0.9994
Thiopental	68.0	-1.7±1.8	195±9	0.9872
Methylphenobarbital	20.9	-1.3±0.5 -1.0±0.8	112±3 101±5	0.9942 0.9848
Hexobarbital	18.1	-0.4±0.3 -0.4±0.2	151±4 131±2	0.9957 0.9984

As it has already been mentioned in RP systems with aqueous mobile phase solutions containing β-CD the resolution of enantiomers on the column can be achieved due to two various effects, it can arise:

- from differences of stability constants of β-CD complexes ($K_{(-)G}$ and $K_{(+)G}$)
- and/or from differences in adsorption of β-CD complexes ($k'_{(-)G\cdot\beta\text{-CD}}$ and $k'_{(+)G\cdot\beta\text{-CD}}$) on the solid phase.

These factors may influence elution of enantiomers in an additive or substractive manner.

From the data quoted in Table 2 it follows that only stability constants differentiation is responsible for resolutions of barbituric enantiomers.

The adsorption of their complexes, practically equal to zero, has no influence on their separations.

Contrary to this behavior the resolution of mephenytoin enantiomers arises from the differentia-

tion in the adsorption of diastereoisomeric β-CD complexes. Calculated separation factor α for these complexes is ca. 3. The small difference in their stability constants acts just in the opposit direction.

Figures 3, 4 and 5 show the resolutions of racemic mephenytoin, methylphenobarbital and hexobarbital, respectively.

Under the experimental conditions the resolutions of racemic pentobarbital, secobarbital and thiopental were not observed. It seems that β-CD complexation results in a distinot enantioselectivity in case of mephenytoin and barbiturates which have a chiral center in the pyrimidine ring.

It has already been suggested (13) that the adsorption on the RP phase of CD complexes of molecules which are entirely immersed in the CD cavity is practically none; the definite adsorption arises only from this part of the molecule which is outside the cavity. Taking into account this fact and the remarkable difference in the adsorption of β-CD mephenytoin diastereoisomers one may conclude that the significant difference must exist between the immersion of the mephenytoin enantiomers in the β-CD cavity.

The results obtained recently using stable commercial CD stationary phase prove the remarkable power of this new sorbent for resolution of chiral compounds into enantiomers (3, 4, 5).

However our procedure using CD complexation in the mobile phase solution of RP HPLC systems seems to have also some advantages as a simple, cheap and enabling evaluation of stability constant values of the inclusion complexes. Moreover, sometimes it involves another factor, besides differentiation through

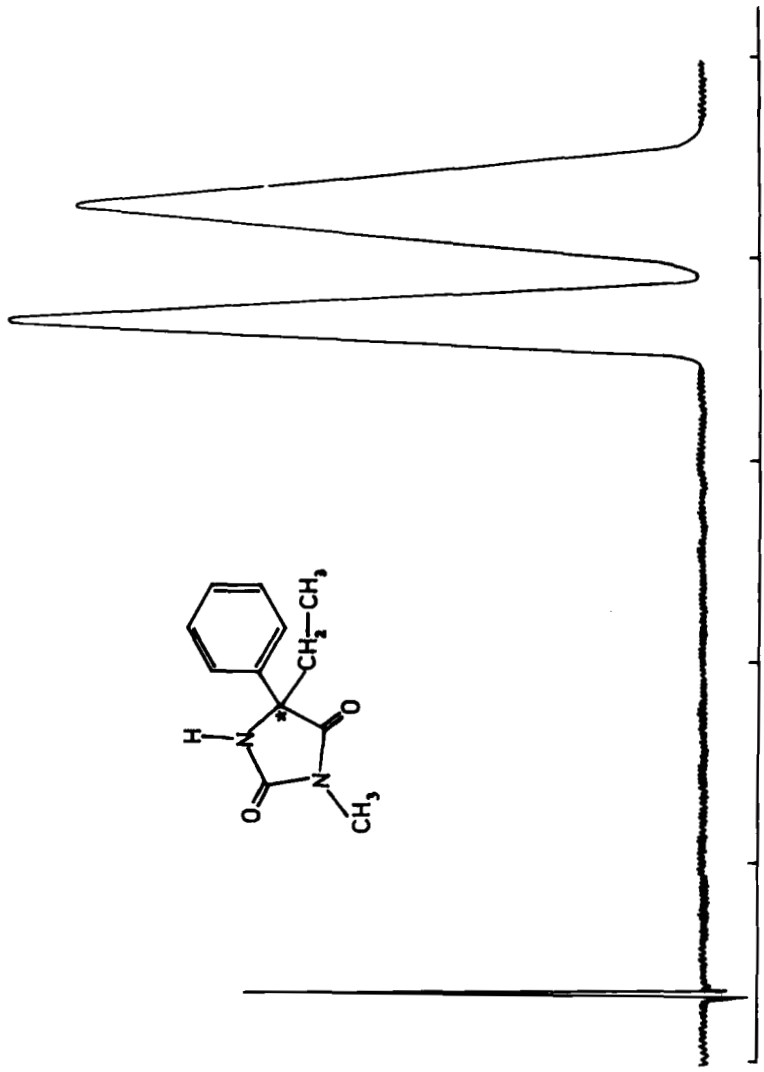


FIGURE 3. Resolution of mephenytoin enantiomers on 250x4.0 mm i.d. column packed with LiChrosorb RP 18 10 μm; mobile phase: 30 mM β-CD in 18% ethanol-acetate buffer solution of pH=5.0, flow-rate: 0.6 ml/min.

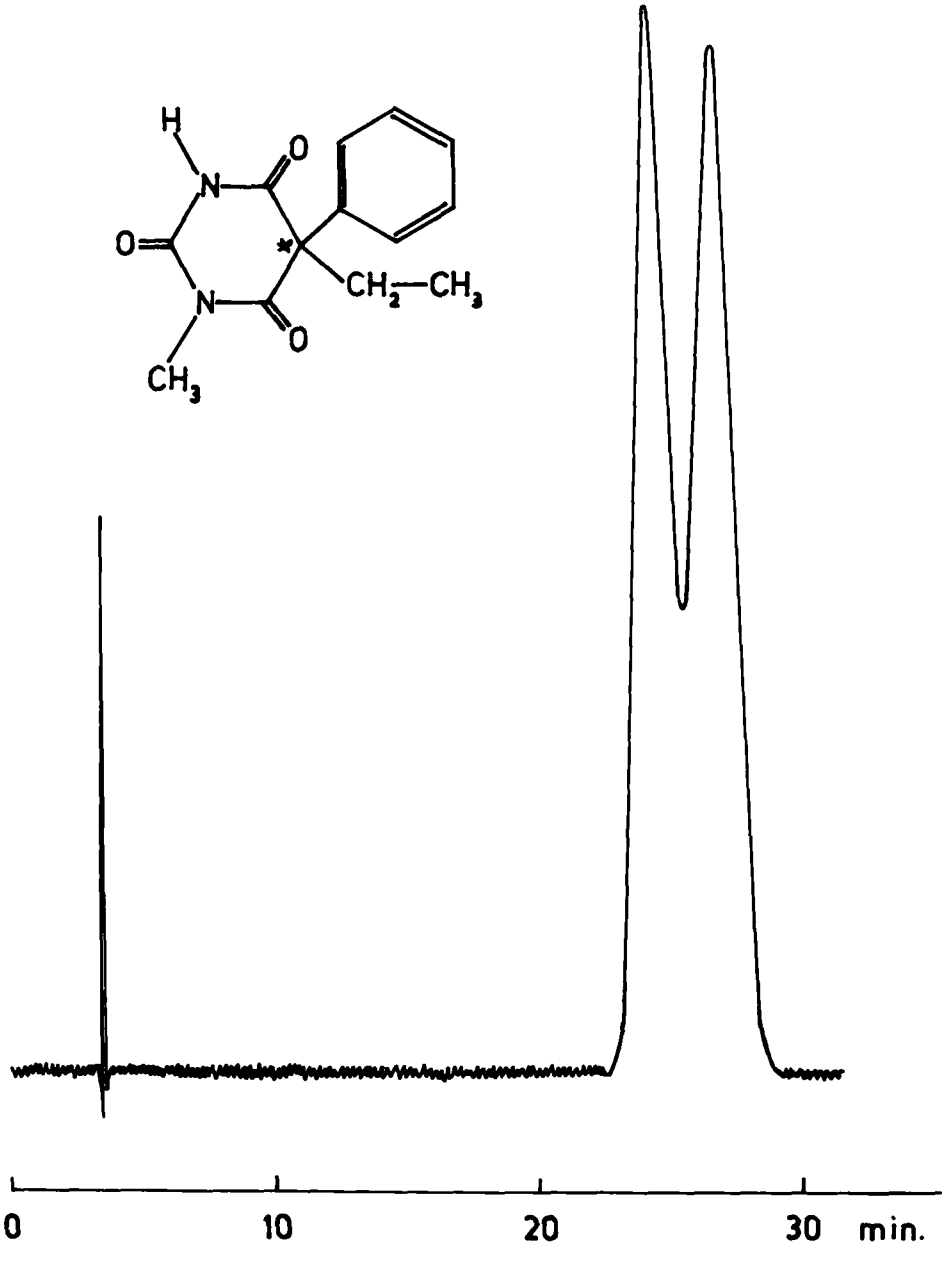


FIGURE 4. Resolution of methylphenobarbital enantiomers. Chromatographic conditions as in Fig.3.

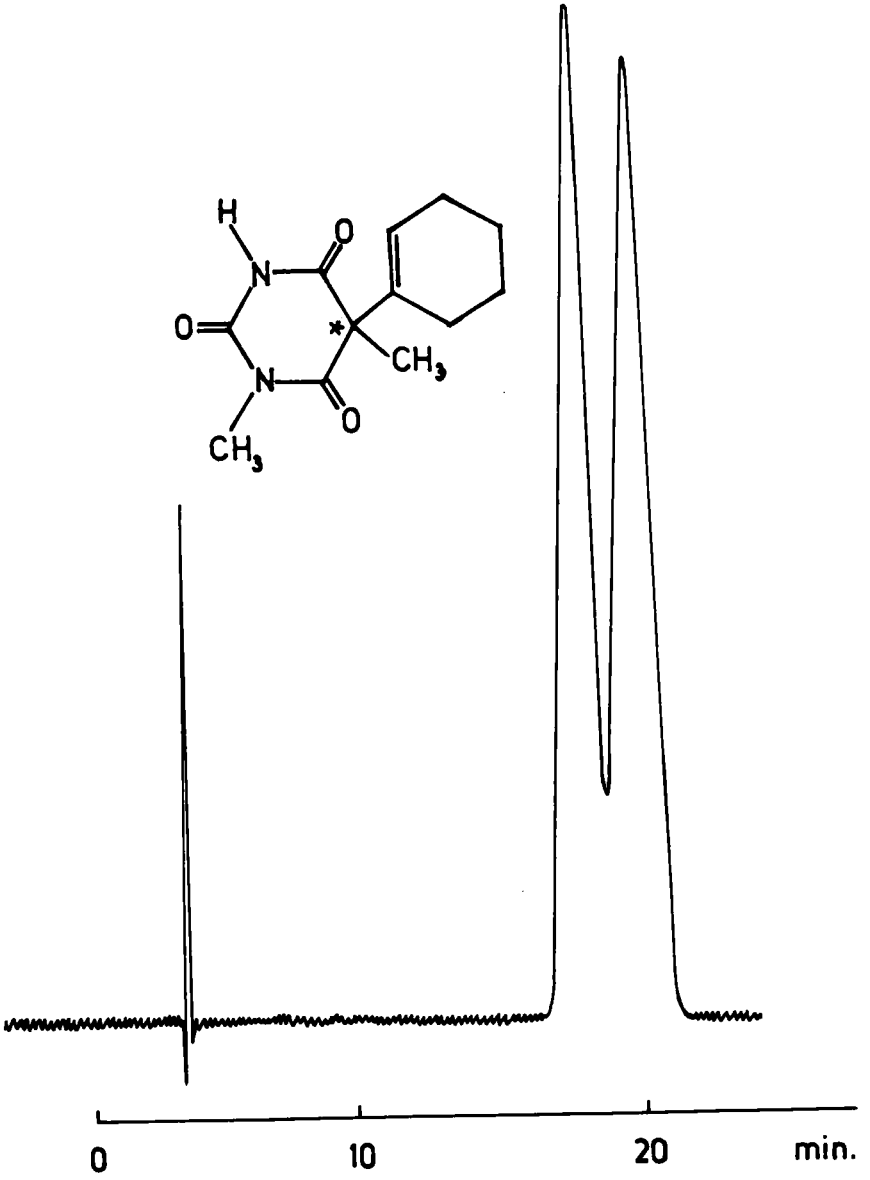


FIGURE 5. Resolution of hexobarbital enantiomers. Chromatographic conditions as in Fig. 3.

the stability constants of β-CD complexes, namely the differences of their adsorption on the RP phase, as it was exemplified in this work by mephenytoin enantiomers behavior.

ACKNOWLEDGMENTS

We are deeply indebted to Professor J. Szejtli (Chinoin, Budapest, Hungary) for kindly providing the β-CD. This study was supported within Polish Academy of Sciences project 03.10.

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