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Short Communication

Improved chiral recognition of some compounds via the simultaneous use of β-cyclodextrin and its permethylated derivative in a reversed-phase high-performance liquid chromatographic system

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ABSTRACT

Two chiral additives, β-cyclodextrin and (2,3,6-tri-O-methyl)-β-cyclodextrin, were applied to improve the enantioselectivity of reversed-phase high-performance liquid chromatography for methylphenobarbital, glutethimide, mephenytoin and morsuximide. It was found that the joint use of these two additives leads to an improved enantioselectivity, except for mephenytoin.

INTRODUCTION

For a long time chiral discrimination has received close attention in organic chemistry, biology, pharmacology and other natural sciences and the problem of how biological chiral receptors operate is still under discussion. In consequence, the development and extension of methods for enantioselective synthesis and chiral separations have become an important issue.

During the last decade, chromatography has proved to be a major technique for the resolution of stereoisomers, including enantiomers [1–7]. The basic rule, which is generally accepted, states that two enantiomeric species can be recognized exclusively by their interactions with a chiral resolving agent, and a variety of optically active separating agents have been applied in this respect. For chromatographic enantioselectivity the "three-point attachment" concept was proposed by Dalgliesh [8] in the early 1950s. Depending on the localization of the discriminating agent in the chromatographic system, chiral stationary, coated and mobile phases could be distinguished.

Unfortunately, the basic thermodynamic differentiation of enantiomers in many chiral systems is

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usually poor, *i.e*, the enantioselectivity (α) reaches values very close to 1.00. Hence, the requirements for column efficiency are severe, frequently making the separation unrealizable because of technical limitations. To enhance enantioselectivity, various approaches have been reported, mainly via derivatization of chiral stationary phases, of chiral additives or of the solute molecules themselves [1–7]. Recently, a new idea for a combined method was proposed, using simultaneously a chiral stationary phase and a chiral additive in the mobile phase solution [9]. This method permitted the enhanced resolution of acidic compounds.

In this paper we report an analogous idea for the simultaneous use of the two chiral selectors, but in a different manner. We propose the use of two chiral additives able to form molecular inclusion compounds in a reversed-phase high-performance liquid chromatographic (RP-HPLC) system. These two selectors are β-cyclodextrin (β-CD) and (2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD).

EXPERIMENTAL

Reagents

β-CD and TM-β-CD were supplied by Chinoin (Budapest, Hungary). All other reagents and solvents were of analytical-reagent grade and were used as received.

The model compounds tested, which included well known therapeutic drugs, were methylphenobarbital, glutethimide, mephenytoin and morsuximide (Fig. 1).

Apparatus and procedures

Chromatographic experiments were performed on a Type 310 HPLC unit (Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland) equipped with a 0.5-μl injector and a UV detector (254 nm) containing a 1-μl flow cell. The columns (250 × 1 mm I.D.) were packed with 5-μm LiChrosorb RP-18 by the viscosity method. The mobile phases were aqueous ethanolic solutions containing various amounts of the appropriate CD; a pH of 2 was maintained with phosphoric acid solution. Experiments were carried out on the columns equilibrated with the mobile phase solutions at 20 ± 1°C. Experimental data were collected and processed using the Chromblues software package (Insitute of Physical Chemistry, Polish Academy of Sciences).

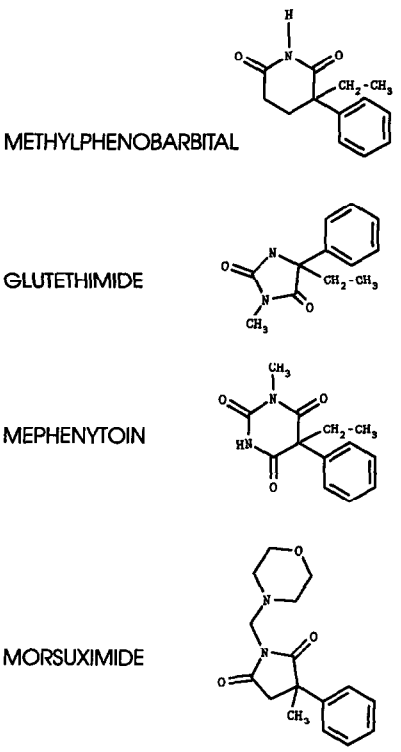

Fig. 1. Structural formulae of the compounds investigated.

RESULTS AND DISCUSSION

Experimental examinations

The values of the capacity factors (k') and enantioselectivity factors (α) for the four compounds investigated are given in Table I; they were determined with β-CD and TM-β-CD used separately and as a mixture.

For all the compounds except mephenytoin an improvement in enantioselectivity followed the joint use of the two chiral additives. These overall effects may be more or less distinct, depending on the solute under investigation and the starting values of the selectivity factor [α] found for the two additives separately. The enantioselectivity of β-CD complexation, undetectable under the conditions of the experiment with glutethimide, really exists and is revealed when β-CD is used together with TM-β-CD.

TABLE I

CAPACITY FACTORS (k') AND SELECTIVITIES (α) AS A FUNCTION OF THE ELUENT COMPOSITION

Compound	$k'_{1,2}$[a]	β-CD[b]			TM-β-CD[c]			TM-β-CD + β-CD[d]		
		k'_1	k'_2	α	k'_1	k'_2	α	k'_1	k'_2	α
Methylphenobarbital	18.0	6.5	7.1	1.09	13.3	16.0	1.20	4.2	5.4	1.30
Glutethimide	23.0	3.4	3.4	1.00	20.7	22.4	1.08	1.8	2.1	1.12
Mephenytoin	16.0	9.1	10.2	1.13	7.4	7.4	1.00	4.5	5.0	1.13
Morsuximide	6.1	2.2	2.2	1.00	4.2	4.6	1.08	1.2	1.4	1.16

[a] 20% ethanol; pH 2.
[b] 20% ethanol; $1.5 \cdot 10^{-2}$ M β-CD; pH 2.
[c] 20% ethanol; $5 \cdot 10^{-4}$ *M* TM-β-CD; pH 2.
[d] 20% ethanol; $1.5 \cdot 10^{-2}$ *M* β-CD, $5 \cdot 10^{-4}$ *M* TM-β-CD; pH 2.

Typical data concerning changes in the resolution (R_s) and the analysis time for methylphenobarbital presented in Table II, and the chromatograms shown in Fig. 2, demonstrate the beneficial role of the use of two chiral additives under appropriate conditions.

It has been found previously [7,10] that additions of β-CD to a solution are always accompanied by a decrease in the efficiency of the column. This phenomenon may explain why the R_s value remains approximately constant for methylphenobarbital (Table II) in spite of the improved enantioselectivity. Generation of enantioselectivity by β-CD always proceeds at the expense of the number of theoretical plates. This problem, which is difficult to follow experimentally when inclusion compounds are involved, has not yet been explored sufficiently. Nevertheless, the present data indicate that by using two additives together one may obtain a suitable resolution in a much shorter time. The latter effect seems to be unachievable using simple organic solvent additions.

The exceptional behaviour of mephenytoin can

TABLE II

RESOLUTION (R_s) AND TIME OF RESOLUTION (t) ACHIEVED FOR METHYLPHENOBARBITAL DEPENDING ON THE ELUENT COMPOSITION

Chiral additive	R_s	t (min)
β-CD[b]	0.9	36.3
TM-β-CD[c]	2.1	76.6
β-CD + TM-β-CD[d]	2.0	28.5

[b,c,d] Conditions as in Table I.

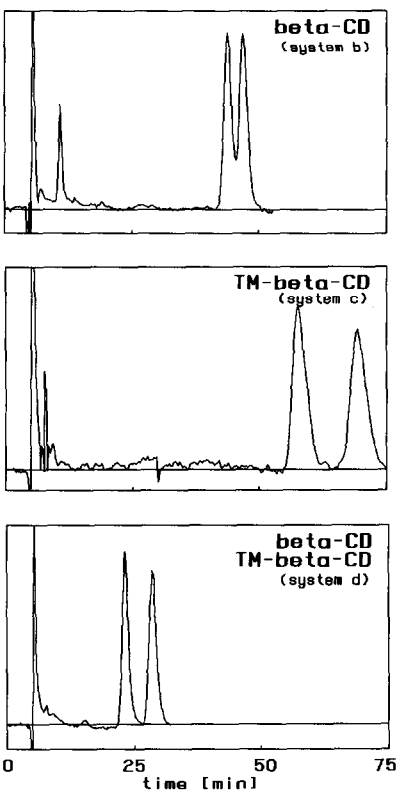

Fig. 2. Chromatograms obtained on a column (250 × 1 mm I.D.) filled with 5-μm LiChrosorb RP-18. Systems b, c and d: for conditions, see footnotes *b*, *c* and *d* in Table I.

be explained by its unusual mechanism of separation that occurs on an ODS column in the presence of β-CD [10]. In such a system two different phenomena may initiate enantioselectivity: a differentiation of stability constants of complexes formed in the bulk mobile phase solution and a differentiation of capacity factors of these complexes on the ODS phase. Most frequently the first phenomenon predominates, but mephenytoin represents a rare example of where the second factor plays the major role and the separation of enantiomeric mephenytoins is due to the difference in adsorption of β-CD diastereoisomeric complexes on the ODS phase.

In conclusion, it has been shown that the simultaneous use of two chiral additives, β-CD and TM-β-CD, may offer under appropriate conditions better enantioselectivity and better resolution in a shorter time of analysis. Moreover, in contrast to the previously described method [9], which is useful for acidic compounds, the present method using CDs should be more universal, *i.e.*, applicable to compounds of various nature, acidic, basic and neutral. The questions of when and why these synergistic effects occur are under study and a theoretical treatment will be presented in the near future [11].

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