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Ruggedness of enantiomeric separation by capillary electrophoresis and high-performance liquid chromatography with methylated cyclodextrins as chiral selectors

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Abstract

When applying cyclodextrin (CD) derivatives as chiral selectors for enantiomeric separation in different separation methods, the difference in degree of substitution (DS) and substitution pattern of the CD derivative may lead to complications. Seven different methylated β-cyclodextrin derivatives were tested as additives in the background electrolyte in capillary electrophoresis (CE) and as an additive to the mobile phase in high-performance liquid chromatography (HPLC). The substitution patterns of heptakis(2,6-di-O-methyl)-β-CD (DIMEB) and randomly methylated β-CD samples were compared using a simple thin-layer chromatography method. The CE method was sensitive to the quality of the chiral selector and it was solute dependent. While the enantiomeric separation of N-methylephedrine and terbutaline was excellent with all DIMEBs, in the case of hexobarbital, the separation failed with one DIMEB sample. The HPLC method was less sensitive to the quality of the chiral selector than the CE method.

Keywords: Enantiomer separation; Chiral selectors; Methylephedrine; Terbutaline; Hexobarbital; Cyclodextrins

1. Introduction

Cyclodextrins (CDs) and their derivatives, which are extremely versatile chiral selectors, are used for the separation of positional, structural and optical isomers in different analytical methods [1–4]. Although many separation problems can be solved with the natural CDs, the use of CD derivatives may increase the selectivity of the method. One of the most frequently used CD derivatives in analytical methods is heptakis(2,6-di-O-methyl)-β-CD (DIMEB) due to its good solubility and complex-forming ability [5–9].

Most of the CD derivatives, including DIMEB, however, are mixtures of compounds with different degrees and patterns of substitution. The average degree of substitution (DS) of DIMEB is fourteen, but the isomer compositions of products having DS= 14 are very different. The purity of the DIMEB CD isomer of commercially available DIMEB is in the range of 30–80%.

Pure DIMEB CD isomer has excellent complexation properties due to the presence of methoxy groups in C(2) and C(6) positions, which result in a deeper cavity than that of the parent β-CD [10]. Commercially available DIMEB samples contain at least two–three over- and undermethylated isomers in higher amounts, and many minor components besides the DIMEB isomer. The isomers with different DSs and the substitution patterns contain methoxy groups that are not only in the C(2) and C(6) positions, but also in the C(3) position, which is another reason for their heterogeneity [11–13]. The complex-forming ability of CD derivatives

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strongly depends on the number and position of the substituents. Therefore, when CD derivatives are used as the chiral selector in capillary electrophoresis (CE) or high-performance liquid chromatography (HPLC), the DS and the substitution pattern greatly influence the selectivity [14–18].

The quality of the chiral selector is one of the most important factors in the development of an enantiomeric separation method. The validation of enantiomeric separation was carried out successfully when the same batches of CD or its derivatives were used as chiral selectors [19,20]. However, when different batches of commercially available CD derivatives are used, a problem is that the purity, DS and substitution pattern are often not disclosed precisely by the manufacturer.

In this work, our aim is to examine the ruggedness of enantiomeric separation when commercially available methylated β-CD derivatives are used as chiral selectors in the background electrolyte in CE and as the mobile phase additive in HPLC, respectively.

2. Experimental

2.1. Chemicals

DIMEB samples were commercial products from different sources (DIMEB/1: Aldrich-Chemie, Steinheim, Germany; DIMEB/2: Sigma, St. Louis, MO, USA; DIMEB/3–5: Cyclolab, Budapest, Hungary). The average DS of these samples is fourteen (determined by NMR). The RAMEB/1 (randomly methylated β-CD) sample (DS=14.1) was also prepared by Cyclolab. RAMEB/2 (DS=12.8) is a product from Wacker Chemie (Munich, Germany). The racemic solutes (hexobarbital, terbutaline and N-methylephedrine) were of pharmaceutical grade. Their structures are shown in Fig. 1.

Thin-layer chromatography (TLC) chromatograms were developed on a Kieselgel 60 plate (Merck, Darmstadt, Germany, 20×20 cm) with chloroform–acetone–formic acid–ethanol (68:16:8:8, v/v). Visualization: charring by heating with 50% conc. sulphuric acid in ethanol [13]. The solvents used for TLC were of analytical grade.

The buffer used for the CE separation was 20 m*M* NaH_2PO_4 solution containing 15 m*M* DIMEB or RAMEB. The pH of the buffer was adjusted to pH 9 with a 3 *M* NaOH solution. In the case of terbutaline, 0.033 *M* phosphoric acid, pH 3 (adjusted with 3 *M* NaOH), containing the CD derivative was used. The buffers were filtered prior to analysis. All buffer reagents were of analytical reagent grade and purchased from Merck.

The HPLC mobile phases consisted of 0.01 *M* aqueous phosphoric acid containing 1 m*M* DIMEB or RAMEB and 10% ethanol (LiChrosolv, Merck).

2.2. Instruments

UV spectra of DIMEB and RAMEB solutions were measured using a Hewlett-Packard HP8452 A diode array spectrophotometer using the HP 89532Q Quant software Rev. A.00.00. The samples were dissolved in double distilled water to a concentration of 1% (w/v) and the absorbance was recorded over the range of 200 to 510 nm (1 cm cell).

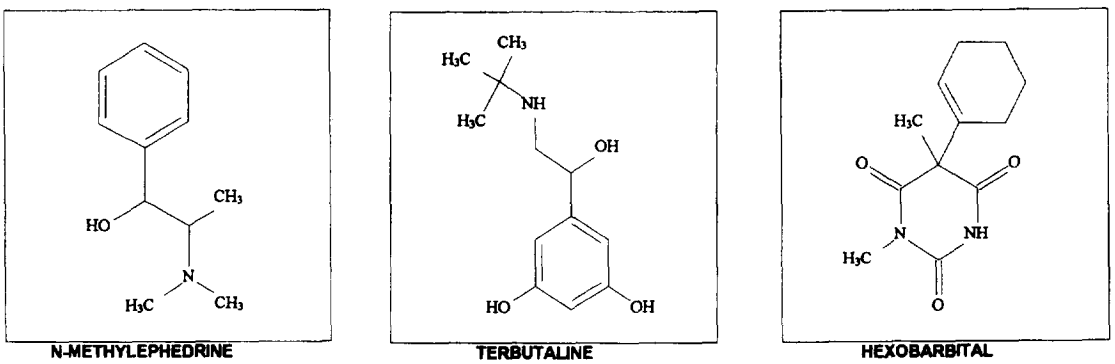

Fig. 1. Structures of the model molecules.

The CE experiments were performed on a Hewlett-Packard ^{3D}CE System (Hewlett-Packard, Waldbronn, Germany). A fused-silica capillary (Composite Metal Services, Hallow, UK) [58.5 cm (effective length 50 cm)×50 μm I.D.], thermostated to 25°C, was used. The applied voltage was 30 kV. Samples were introduced by applying 50 mbar for 3 s. The detection wavelength was 202 nm. Test samples were dissolved in methanol (0.1 mg/ml).

The HPLC experiments were performed on a Hewlett-Packard 1050 HPLC system. A Nucleosil 300-5 C_4 column (100×4 mm, Macherey-Nagel, Düren, Germany) was used at 26°C. The detection wavelength was 220 nm. The flow-rate was 0.8 ml/min. The test substance, hexobarbital was dissolved in 10% aqueous ethanol (0.1 mg/ml, with an injection volume of 20 μl).

3. Results and discussion

3.1. TLC test

TLC is a very simple and fast method for comparing both the DS and the substitution pattern of CD derivatives [13]. Purified DIMEB/5 sample contains only 3 minor fractions besides the DIMEB spot.

Although DIMEB/1 and DIMEB/2 samples were obtained from different suppliers, the component distribution of these samples was found to be very similar (Fig. 2). Some spots in the higher R_F region are from some overmethylated isomers (DS:15). The components that appear close to the DIMEB spot are the isomers with DS=14, but they also contain some methyl groups in the C(3) position.

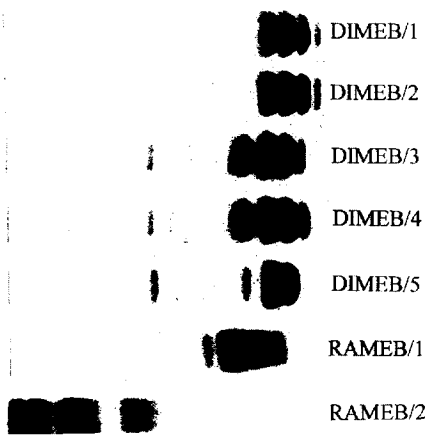

Fig. 2. TLC chromatogram of methylated β-CD derivatives.

DIMEB/3 and DIMEB/4 samples contain components both in the higher and lower R_F regions. The distribution of spots on the TLC plate is similar for these samples, which confirms the similarity of different batches prepared by the same synthetic route in Cyclolab.

Although the DS of RAMEB/1 is 14.1, as determined from NMR spectra, the lower R_F values on the TLC plate suggest a lower DS. This fact was confirmed in Ref. [13], where DS 13.3 was calculated for this sample after fragmentation analysis. The wide distribution of RAMEB spots on the TLC plate indicates that there are notable differences in the substitution patterns of these samples.

3.2. UV spectra

UV transparency is also an important factor for additives used in CE or HPLC methods, because UV detection is used normally. Therefore, the absorbance level has to be controlled.

Fig. 3 shows the UV spectra of a 1% aqueous solution of the tested samples. The absorption of all DIMEB samples decreases below 0.1 absorbance unit (AU) with increasing wavelength. The absorbance of DIMEB/3 and DIMEB/4 solutions is less than 0.2 AU at 200 nm, whereas when the wavelength is 220 nm, the absorbance is lower than 0.02 AU. These low values give excellent sensitivity for the low wavelength analysis often used in CE. DIMEB/1, DIMEB/2 and DIMEB/5 samples show much higher absorption at 200 nm (0.5, 0.3 and 0.5 AU, respectively) than DIMEB/3 and DIMEB/4. These low levels of impurities met the requirements of both CE and HPLC experiments.

RAMEB/1 and RAMEB/2 appeared to show higher levels of absorbance in the whole wavelength range than did DIMEBs. Consequently, RAMEB samples can only be used for analytical purposes after appropriate purification.

3.3. Capillary electrophoresis

N-Methylephedrine, terbutaline and hexobarbital

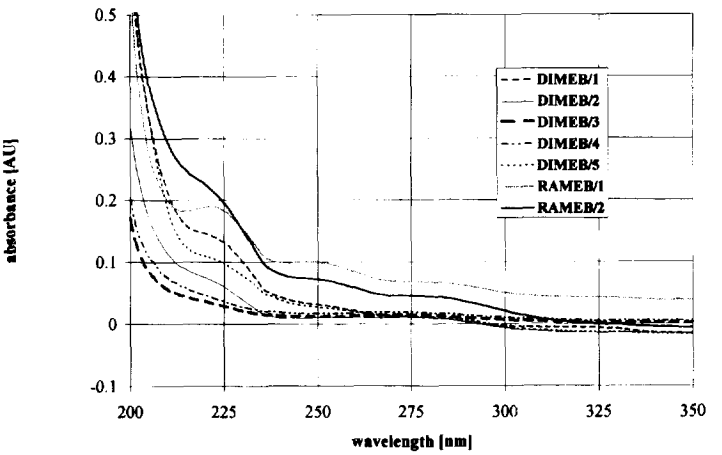

Fig. 3. UV spectra of methylated β-CD derivatives.

are appropriate, often used, model molecules for enantiomeric separation using CDs as chiral selectors [2].

The enantiomeric separation of N-methylephedrine and terbutaline has not been found to be dependent on a change in the composition of the chiral selector (Figs. 4 and 5). Only insignificant differences can be observed in the mobility and resolution (R_s) using DIMEB/1–4 in the background electrolyte (BGE). These DIMEB samples have fairly similar substitution patterns. The highest migration time value is found with DIMEB/5, which contains the highest amount of pure DIMEB isomer. While the separation of enantiomers appears to be the best with DIMEB/5 in the case of N-methylephedrine (R_s=2.52), the resolution of terbutaline is worse (R_s=4.79), compared to the other DIMEB samples.

Shorter migration times and rather poor resolution of N-methylephedrine enantiomers are obtained using RAMEB samples in the BGE, compared to those when DIMEB samples were used. The conductivity values of buffers prepared with RAMEB/1 and RAMEB/2 were found to be different. This phenomenon may be explained by the presence of some ionic impurities in RAMEB samples, which affected the separation and the peak shapes. The mobility of the solutes was shorter using RAMEB/1, but the resolution was higher with RAMEB/1 than with RAMEB/2.

The migration times are also shorter with RAMEBs than with DIMEB samples in the case of terbutaline, however, the R_s values are significantly higher than those with DIMEBs. A possible explanation of these findings is that terbutaline forms a more stable complex with some other isomers than it does with DIMEB.

The influence of the quality of the chiral selector was evaluated by calculating the relative standard deviation (R.S.D.) of parallel measurements. The precision of the separation of terbutaline enantiomers was calculated using five parallel prepared buffers with DIMEB/5 sample. R.S.D.s of 1.4–1.8% were achieved for the corrected peak areas, while the R.S.D. of the resolution was only 0.60% (Table 1). The differences in the R_s values were higher when the five different batches of DIMEB were used; for terbutaline, R.S.D.=9.01% and for N-methylephedrine, R.S.D.=3.64% (Table 2).

The separation of hexobarbital enantiomers is more sensitive to the chiral selector. Almost the same R_s values (1.33–1.36) are found when using DIMEB/3, DIMEB/4 and DIMEB/5 as the additive in the BGE (Fig. 6). Although the substitution pattern of DIMEB/2 is very similar to that of DIMEB/1, surprisingly, no resolution of hexobarbital enantiomers was obtained when DIMEB/2 was used as the chiral selector. Repeated series of experiments were carried out to clarify this phenomenon. However, no resolution was achieved with DIMEB/2 samples (Lot 84H0723 Sigma) in three repeated test runs. The different peak shapes of hexobarbital enantiomers with RAMEBs verify that

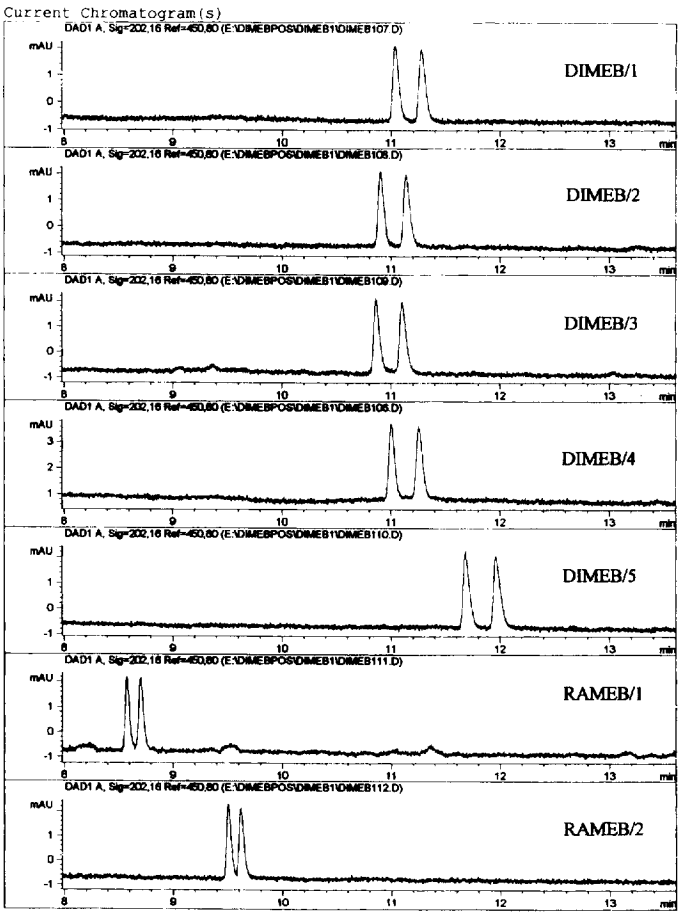

Fig. 4. Separation of N-methylephedrine by CE using different methylated β-CD derivatives as the chiral selector in the BGE. For conditions see Section 2.

this analyte is very sensitive to the quality of the chiral selector.

3.4. HPLC

For the HPLC experiments, hexobarbital was selected to demonstrate the differences in enantiomeric separation when methylated β-CD derivatives with similar DS but different substitution patterns were used in the mobile phase.

The retention times and resolution of hexobarbital enantiomers are very similar using different DIMEB samples as the mobile phase additive (Fig. 7). The resolution appears to be the best (R_s=2.0) with purified DIMEB/5, which has the highest DIMEB isomer content (Table 2). The retention times are also higher than those obtained with other DIMEB samples, suggesting that the separation takes place on a modified surface of stationary phase that is formed due to the strong absorption of DIMEB [8,9]. The HPLC method was found to be more rugged compared to the CE method; the R.S.D. of the resolution was only 1.08% when five different DIMEB samples were used as the mobile phase additive. The resolution with RAMEB/1 was decreased, but acceptable (1.86), whereas it was below 1.5 when RAMEB/2 was used as the chiral selector.

4. Conclusion

The simple and fast TLC method is suitable for

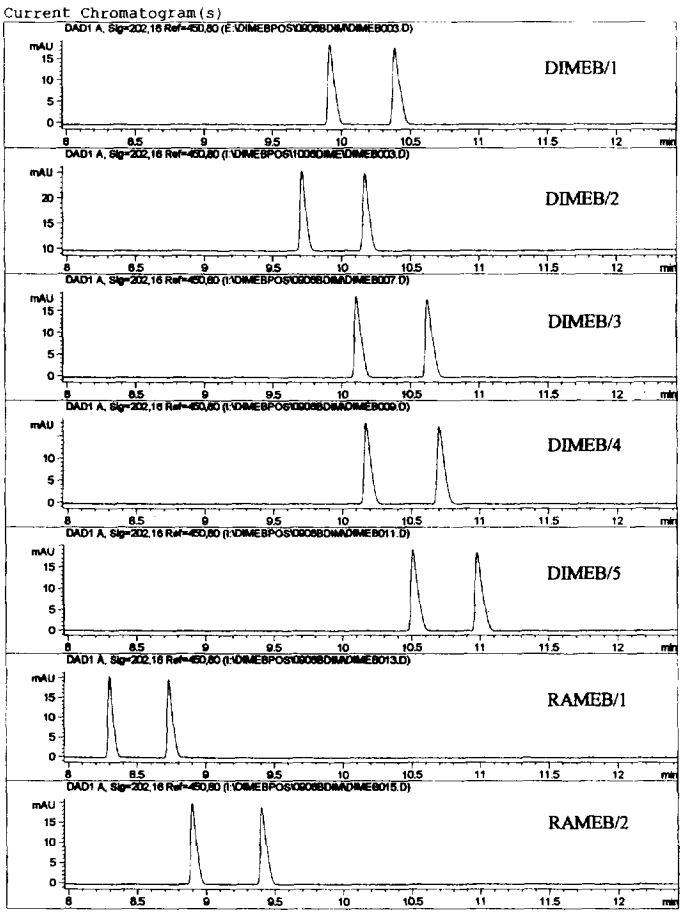

Fig. 5. Separation of terbutaline by CE using different methylated β-CD derivatives as the chiral selector in the BGE. For conditions see Section 2.

comparing the degree of substitution and isomer distribution of the different batches of methylated β-CD derivatives. Samples prepared by the same synthesis route can be clearly identified.

Table 1
Precision of the separation of terbutaline enantiomers using parallel prepared buffers with DIMEB/5 as the chiral selector

	Corrected peak area (1/min)		Resolution
Calculated values	0.198	0.196	4.95
	0.192	0.188	4.91
	0.193	0.192	4.96
	0.196	0.185	4.89
	0.192	0.189	4.92
Average ± S.D.	0.194±0.003	0.192±0.004	4.93±0.03
R.S.D.	1.38%	1.84%	0.60%

The enantiomeric separation potency of methylated β-CD derivatives strongly depends on the amount of pure DIMEB isomer in the sample. In some cases, however, the solute forms a stronger complex with another isomer, resulting in better resolution with a heterogeneous mixture of CD derivatives. Slight differences in the substitution pattern usually cause only slight differences in the enantiomeric separation potency of DIMEB samples, both in CE and HPLC.

When the same batch of DIMEB is used in the background electrolyte or in the mobile phase, both CE and HPLC separation methods are reproducible. In this case, the R.S.D. values of resolution are excellent. CE separation is more sensitive to the quality of the chiral selector than is HPLC. Batch-to-

Table 2
Resolution of enantiomers using different DIMEB and RAMEB samples as the chiral selector

Chiral selector	Resolution			
	CE			HPLC:
	N-Methylephedrine	Terbutaline	Hexobarbital	Hexobarbital
DIMEB/1	2.29	5.20	1.14	1.98
DIMEB/2	2.36	6.06	0	1.97
DIMEB/3	2.35	5.67	1.21	1.95
DIMEB/4	2.42	5.71	1.74	1.95
DIMEB/5	2.52	4.79	1.13	2.00
RAMEB/1	1.67	6.25	1.58	1.86
RAMEB/2	1.43	6.48	1.42	1.49
For DIMEBs				
Average±S.D.	2.39±0.09	5.49±0.49	1.04±0.64	1.97±0.02
R.S.D.	3.64%	9.01%	61.2%	1.08%

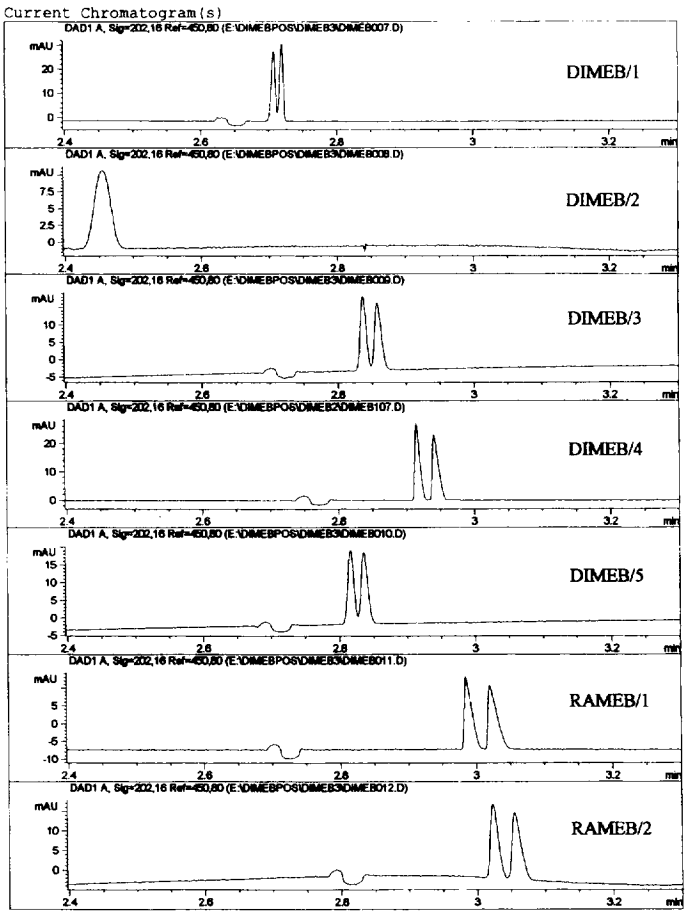

Fig. 6. Separation of hexobarbital by CE using different methylated β-CD derivatives as the chiral selector in the BGE. For conditions see Section 2.

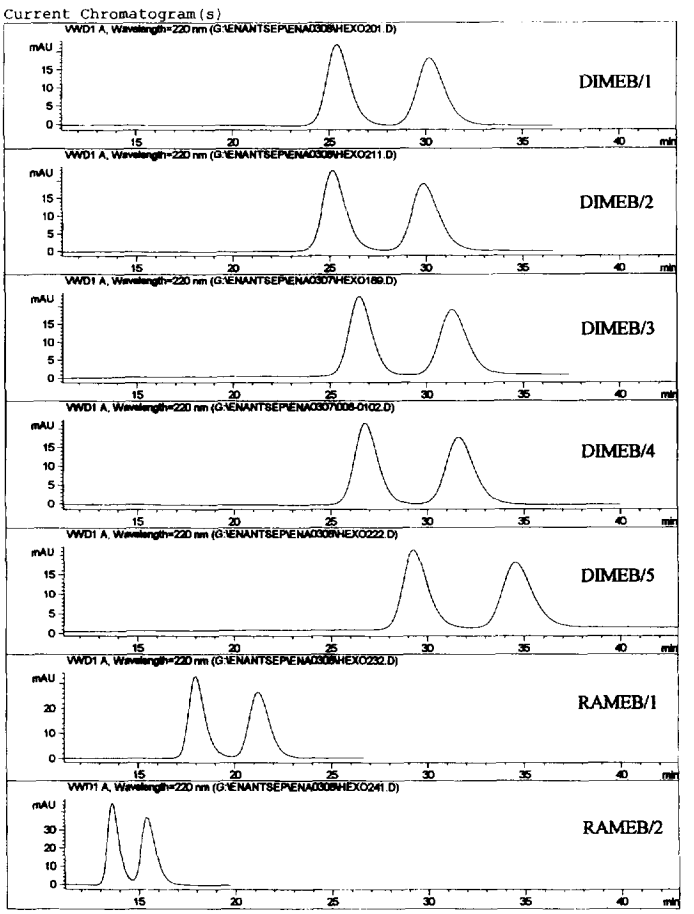

Fig. 7. Separation of hexobarbital by HPLC using different methylated β-CD derivatives as the chiral selector in the eluent. For conditions see Section 2.

batch reproducibility is acceptable in the HPLC method, however, it is impaired in CE.

The ruggedness of enantiomeric separation using DIMEB samples of similar isomeric composition strongly depends on the solute. For example, in the case of hexobarbital, different migration times and resolution were observed in spite of the similar substitution patterns of DIMEB batches, while the other tested solutes were not sensitive to the quality of the chiral selector. Therefore, for chiral separation, the use of CE-tested CD derivatives produced by identical and controlled production procedures is recommended.

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