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Chiral separation of deprenyl and its major metabolites using cyclodextrin-modified capillary zone electrophoresis

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Abstract

A capillary electrophoretic method for the enantiomer resolution of deprenyl and its main alkaline metabolites amphetamine, methamphetamine and propargylamphetamine is described. An acidic separation buffer with a suitable chiral complexing agent, heptakis-(2,6-di-O-methyl)-β-cyclodextrin, was used and the optimum separation conditions were determined by changing the concentration of the chiral selector, the applied electric field and the concentration of methanol.

1. Introduction

It is well known, that many drugs display enantioselectivity in their pharmacological activity and metabolism. Using the optical isomer having the therapeutic effect is highly required to avoid unnecessary burdening of the body with the xenobiotic. Since a great number of drugs consist of enantiomers, chiral separation of these appears to be important.

Selegiline [*R*-(−)-deprenyl] has been used in the treatment of Parkinson's disease. This drug is the antipode of a chiral compound having the higher pharmacological efficacy [1]. Two of its main metabolites are amphetamine and methamphetamine [2,3]. These compounds have strong psychostimulant effects, the *S*-(+)-enantiomers being more active [4]. It is an issue of debate if the metabolites of deprenyl significantly contribute to its pharmacological effects [5,6]. Also, it has still to be proved that the more efficacious enantiomers of the metabolites are not formed in the body. This necessitates a chiral separation method of these compounds.

As indicated by the many papers published in this field, there is a growing interest in the application of capillary electrophoresis (CE) for enantiomer separations. The chiral selectors are usually applied as buffer additives, the most often used being cyclodextrin (CD) derivatives (uncharged, charged, polymers) [7–10], crown ethers [11], bile salts [12], other optically active detergents [13] or biopolymers [14]. Advantages offered by capillary electrophoresis are direct chiral resolution, high efficiency of the separation, speed, and low cost.

A micellar electrokinetic capillary chromatographic method for the separation of enantiomers of amphetamine, methamphetamine and other phenylethylamine compounds has been developed by Lurie [15]. Successful use of cyclodextrin derivatives for the chiral resolution of phenylethylamines has also been demonstrated [7]. In the present study we elaborated a cyclodextrin-modified CE method for the separation of deprenyl and its main metabolites.

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2. Experimental

2.1. Apparatus

A Crystal 300 (ATI Unicam, Cambridge, UK) capillary electrophoresis system equipped with a variable-wavelength UV absorbance detector set at 190 nm was used. CE separations were performed in a 70 cm × 75 μm I.D. uncoated fused-silica capillary; the length to the detection window was 55 cm. Samples were introduced by electrokinetic injection. Axxiom 727 software was used for data collection.

2.2. Chemicals

Tris–phosphate pH 2.8 (20 m*M*) containing 0.1% or 0.5% hydroxypropylmethylcellulose (HPMC) was used as running buffer. Tris and phosphoric acid were purchased from Reanal (Budapest, Hungary), and hydroxypropylmethylcellulose from Sigma (St. Louis, MO, USA). The chiral selectors β-cyclodextrin and heptakis-(2,6-di-O-methyl)-β-cyclodextrin (DIMEB) were obtained from Cyclolab (Budapest, Hungary).

The sample mixture contained *S*-(+)- and *R*-(−)-deprenyl, *S*-(+)- and *R*-(−)-methamphetamine, *R*-(−)-amphetamine, and *R*-(−)-propargylamphetamine. Only one of the antipodes of the two latter compounds were available. These test compounds were kindly provided by Chinoin Pharmaceutical and Chemical Works (Budapest, Hungary). The chemical structure of deprenyl and the metabolites are shown in Fig. 1.

3. Results and discussion

Achiral separation of deprenyl and its three metabolites was achieved using low pH background electrolyte (Fig. 2.). All of the components are ionized at this pH and their migration is in the direction of the electroosmotic flow. Application of HPMC in the separation buffer improved the resolution of the sample components and the reproducibility of migration times as well.

3.1. Use of chiral additives

For the separation of the enantiomer pairs, the dimethyl substituted derivative of β-cyclodextrin is suitable. The unsubstituted β-cyclodextrin does not fit the chiral resolution of these compounds even in its saturating concentration. Both the deeper hydrophobic cavity of DIMEB and its different capability of hydrogen bond formation [16] may contribute to the different complex formation constants of the enantiomer pairs necessary to achieve their chiral resolution.

Application of DIMEB in the buffer increased the migration time of each sample component.

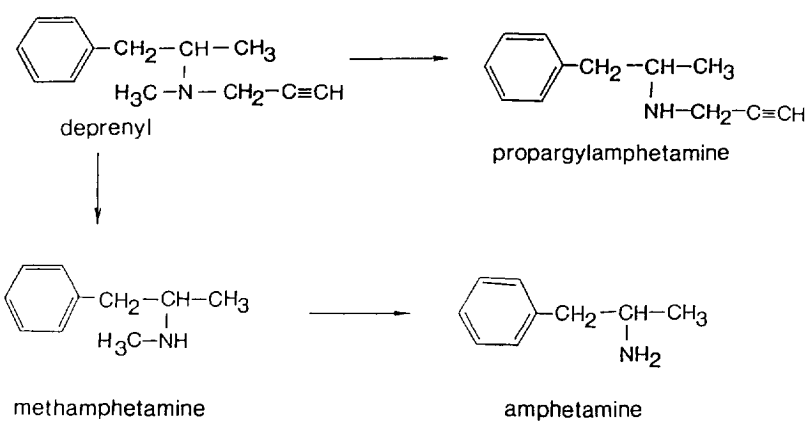

Fig. 1. Chemical structures of deprenyl and its metabolites.

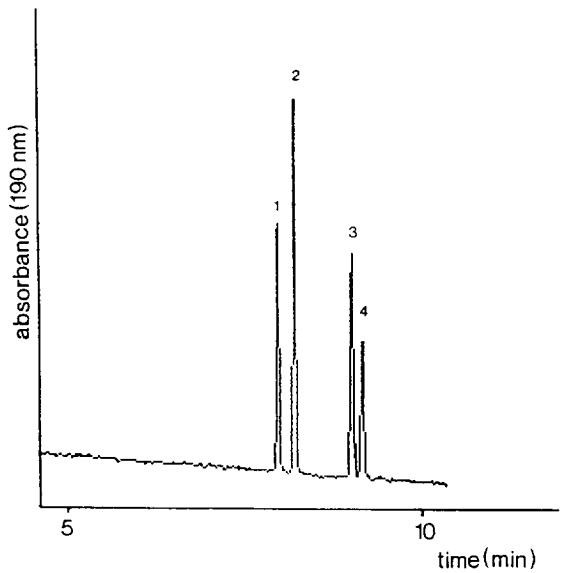

Fig. 2. Achiral separation of mixture of racemic deprenyl and its metabolites. Capillary: uncoated fused-silica 70 cm × 75 μm I.D. (55 cm to detector). Buffer: 20 m*M* Tris–phosphate–0.1% HPMC pH 2.8. Sample: 1 = (−)-amphetamine; 2 = (±)-methamphetamine; 3 = (±)-deprenyl; 4 = (−)-propargylamphetamine; 10^{-5} *M* each. Injection 3 kV. 12 s. Constant voltage 21 kV; current 32 μA.

This indicated the inclusion complex formation of the analytes with the chiral additive. The complexes formed have lower electrophoretic mobilities than the uncomplexed analytes. The increased migration time results primarily from the complex formation, whilst the slightly increased viscosity of the buffer and the slightly decreased electroosmotic flow do not significantly contribute. Fig. 3. shows the migration times of the sample components in the presence of 3–24 m*M* DIMEB. The non-linear change observed is characteristic of this kind of separations, just as the existence of a concentration optimum of the chiral selector for each analyte [17,18]. The decline in resolution when using the chiral selector above its optimum concentration has not been really explained so far. Inversion of the migration order of the enantiomers was found and different separation mechanisms at low and high concentrations of the chiral selector have been suggested by Schmitt and Engelhardt [19]. Non-specific hydrophobic interactions [20] and dimerization of CDs [21] have also been discussed as a reason for the loss in resolution at very high concentrations of the CD derivatives.

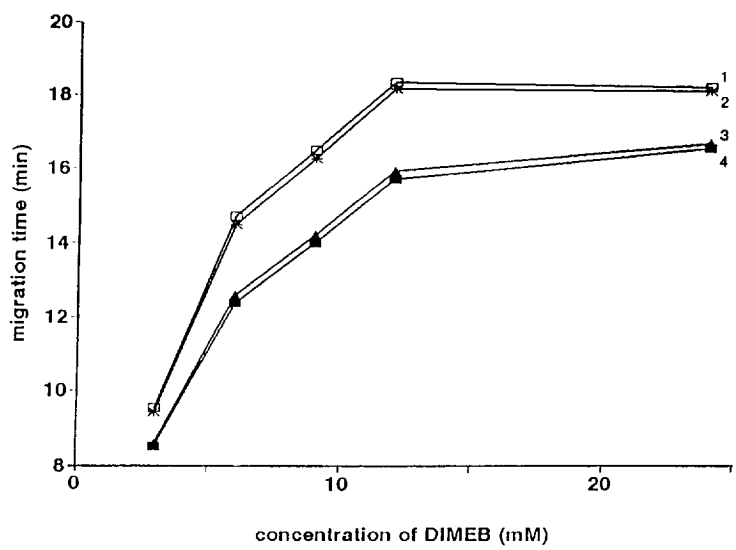

Fig. 3. Plot of migration time vs. concentration of DIMEB in the separation buffer: 1 = (+)-deprenyl; 2 = (−)-deprenyl; 3 = (+)-methamphetamine; 4 = (−)-methamphetamine.

Table 1
Effect of heptakis-(2,6-di-O-methyl)-β-cyclodextrin (DIMEB) concentration on separation selectivity and resolution of enantiomer pairs

Concentration of DIMEB (m*M*)	Selectivity[a]		Resolution[b]	
	MA[c]	D[d]	MA	D
3	1.007	1.010	0.8	0.9
6	1.014	1.015	2.4	2.9
9	1.014	1.014	2.0	2.2
12	1.014	1.010	2.2	2.4
15	1.010	1.008	1.2	0.7
24	1.008	1.007	0.4	0.3

[a] Selectivity = μ_1/μ_2, where $\mu_{1,2}$ are the electrophoretic mobilities.
[b] Resolution = $2 \cdot (t_2 - t_1)/(w_1 + w_2)$, $t_{1,2}$ are the migration time, and $w_{1,2}$ are the peak widths of the enantiomers, respectively.
[c] Methamphetamine.
[d] Deprenyl.

The dependence of the selectivity and resolution of enantiomer pairs on the concentration of the chiral selector is listed in Table 1. For both compounds the (+)-isomers have lower electrophoretic mobility, indicating that their inclusion complex formation constants are higher compared to those of the (−)-isomers. The optimum concentration of the chiral selector for the separation of both enantiomer pairs of deprenyl and methamphetamine was found to be between 6 m*M* and 12 m*M* in 20 m*M* Tris–phosphate, pH 2.8 buffer containing 0.1% HPMC.

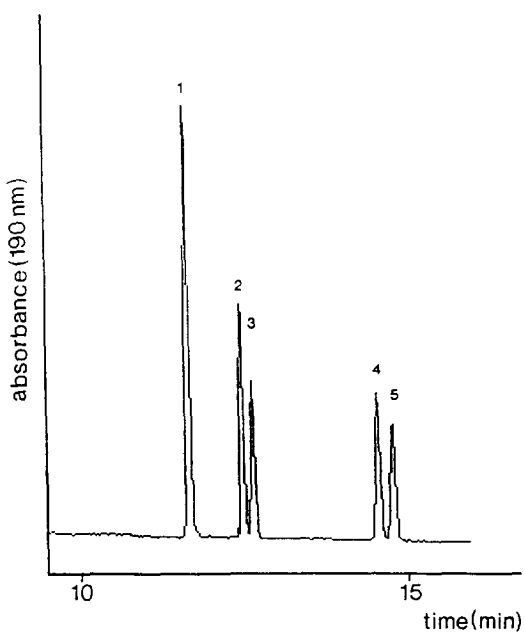

Fig. 4. Chiral separation of the mixture of racemic methamphetamine and deprenyl. Separation buffer as in Fig. 2, containing 6 m*M* DIMEB. Sample: 1 = (−)-amphetamine; 2 = (−)-methamphetamine; 3 = (+)methamphetamine; 4 = (−)-deprenyl; 5 = (+)-deprenyl; 10^{-5} *M* each. Injection 3 kV, 12 s. Constant voltage 21 kV; current 32 μA.

3.2. Effect of the electric field

The effect of the electric field on the separation of the enantiomers was considerable. We obtained the best chiral resolution when the separation was performed at 300 V/cm. At a low field strength (100 V/cm) the chiral resolution was totally lost, due to the increased sample diffusion during the long migration time and to the decreased separation efficiency. At a high field strength (400 V/cm) the resolution also decreased, probably because of the effect of heat generated in the capillary. The formation constants of CD–analyte complexes are sensitive to temperature change [22]. With increasing temperature they become smaller and the separation selectivity decreases significantly [23–25].

Separation of the sample mixture with the optimum concentration of the chiral selector and field strength is shown in Fig 4. Although the

separation conditions are suitable for the enantiomer resolution of racemic deprenyl and methamphetamine, co-migration of *S*-(+)-deprenyl with *R*-(−)-propargylamphetamine was observed.

3.3. Effect of methanol

Addition of methanol to the separation buffer significantly improved the resolution of deprenyl and propargylamphetamine. This can be explained by the beneficial effect of reduced electroosmotic flow on the resolution of sample components migrating in the direction of the flow and by the differential change in the effective electrophoretic mobility of sample components of different hydrophobicity. However, the enantiomer resolution of methamphetamine and deprenyl slightly decreased as the methanol content of the separation buffer increased. Organic solvents usually reduce the hydrophobic interactions, and they may interact with the hydrophobic cavity of the cyclodextrins [26]. The presence of methanol can influence the complex formation of the analytes with DIMEB, which may also change the difference between the complex formation constants of the enantiomer pairs or the optimum concentration of the chiral additive. The effect of various methanol concentrations on the resolution of the sample components is shown in Fig. 5.

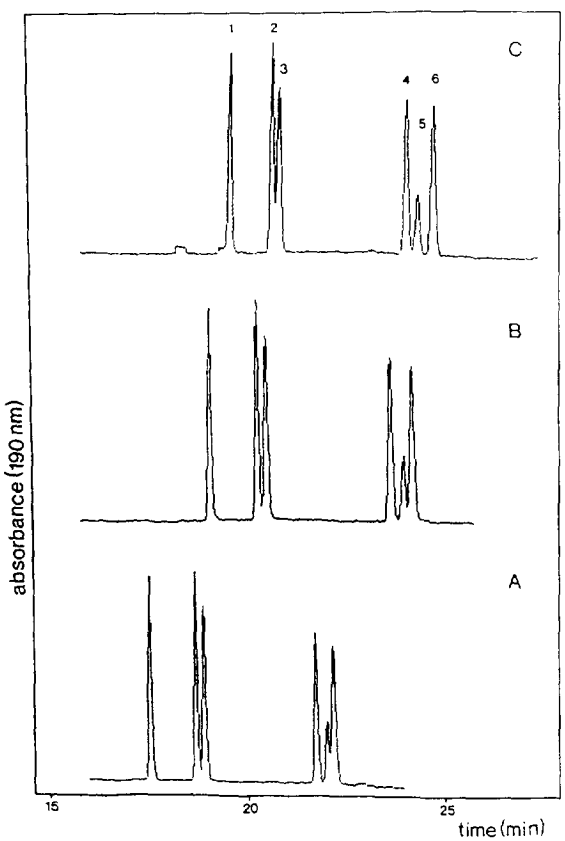

Fig. 5. Effect of methanol [(A) 10%, (B) 15%, (C) 20%] added to the separation buffer on the resolution of the sample components. Sample: 1 = (−)-amphetamine; 2 = (−)-methamphetamine; 3 = (+)methamphetamine; 4 = (−)-deprenyl; 5 = (+)-deprenyl; 6 = (−)-propargylamphetamine; 10^{-5} *M* each. Separation buffer as in Fig. 2, containing 12 m*M* DIMEB and various concentrations of methanol.

4. Conclusions

The chiral selector heptakis-(2,6-di-O-methyl)-β-cyclodextrin is thought to be an appropriate choice for the separation of optical isomers of deprenyl and its major metabolites by capillary electrophoresis. The use of methanol as an additive in the separation buffer was necessary to achieve the resolution of *S*-(+)-deprenyl and *R*-(−)-propargylamphetamine, although its presence slightly decreased the resolution of the enantiomer pairs of the two racemic compounds.

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