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# Capillary electrophoretic enantioseparation of selegiline, methamphetamine and ephedrine using a neutral β-cyclodextrin epichlorhydrin polymer[1]

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**Abstract**

This paper describes the development of a capillary zone electrophoretic method for chiral separation of three basic compounds of the selegiline synthetic pathway: ephedrine, methamphethamine and selegiline. The method developed allows one to separate the studied compounds in one run using a neutral β-cyclodextrin epichlorhydrin polymer. The effect of various experimental parameters, such as chiral selector concentration, concentration and composition of background electrolyte, pH, temperature, and the addition of some organic solvents, on the resolution and migration time is discussed. For selegiline and methamphetamine, it is possible, under optimal conditions, to quantify less than 0.5% of the minor isomer in an excess of the major one.

*Keywords:* Capillary zone electrophoresis; Chiral separation; β-cyclodextrin polymer; Ephedrine; Epichlorhydrin cross-linking agent; Methamphetamine; Selegiline

## 1. Introduction

The separation of chiral compounds is currently an important subject of interest in the pharmaceutical and industrial fields. Individual enantiomers of chiral compounds often exhibit different pharmacological, toxicological or pesticidial properties in their interactions with live systems. This knowledge prompts the demand for their fast and reliable separation [1]. It is not easy to separate two optical isomers because they posses identical physicochemical properties in an achiral environment.

Currently the favourite techniques for an enantiomeric separation are mainly gas chromatography, high performance liquid chromatography, thin layer chromatography and the intensively developed technique of capillary electrophoresis (CE) that offers a lot of operational modes with

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Table 1
Effect of varying EP-β-CD concentration and amount of organic solvent (%, v/v) added to the BGE on resolution $R_s$ of the compounds studied[a]

| Compound | No solvent | | | | | MeOH[b] | | | | ACN[b] | | IPA[b] | | THF[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 10% | | 30% | | (10%) | | (10%) | | (10%) |
| | EP-β-CD (mg ml$^{-1}$) | | | | | | | | | | | | | |
| | 10 | 20 | 50 | 100 | 200 | 100 | 200 | 100 | 200 | 100 | 200 | 100 | 200 | 100 |
| Selegiline | <0.5 | 1.03 | 1.88 | 2.46 | 2.7 | 2.05 | 1.82 | 10.8 | 1.68 | 1.54 | 1.44 | 0.86 | 1 | 0 |
| Methamphetamine | <0.5 | 1.69 | 2.59 | 2.82 | 3.12 | 2.12 | 2.73 | 0.75 | 1.54 | 1.56 | 1.61 | 0.69 | 1 | 0 |
| Ephedrine | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.49 | 1.15 | 0 | 0 | 0 | 0 | 0 |

[a] Run conditions: sodium phosphate buffer (pH 2.5, 0.05 M) with EP-β-CD.
[b] Abbreviations: MeOH, methanol, ACN, acetonitrile, IPA, isopropyl alcohol, THF, tetrahydrofuran.

high efficiency and resolution power and a short analysis time [2]. All of these separation techniques are based on the interaction of enantiomers with a chiral selector before or during a separation process. Stable or unstable diastereomeric complexes are formed which permits chiral recognition [3]. Native or modified cyclodextrins are numbered among the most popular and most frequently used chiral selectors, especially in CE [4].

The compounds analysed—selegiline (deprenyl); methamphetamine and ephedrine—are widely used and are also known as drugs of abuse. These compounds represent particular steps of the pharmaceutical synthesis of (*R*)-(−) selegiline, where ephedrine is the starting compound and methamphetamine an intermediate. In medical practice selegiline is used as an antidepressant and an antiparkinsonian drug [5–7]. Methamphetamine (pervitin) is very well known among drug abuses in the Czech Republic.

The aim of the present work is to demonstrate a strategy for the development of a capillary zone electrophoretic (CZE) method for the chiral separation of the three basic compounds mentioned above, with the use of a new chiral selector: neutral epichlorhydrin β-cyclodextrin polymer (EP-β-CD) [8,9].

## 2. Experimental

### 2.1. Chemicals

Optically pure isomers of selegiline hydrochloride, methamphetamine hydrochloride and ephedrine hydrochloride of the purest obtainable quality were kindly provided by Farmakon (Olomouc, Czech Republic). The characteristics of the soluble β-cyclodextrin polymer were: MW, 3000–5000; cyclodextrin content, 50–60%; solubility in water, 40–50%; cross-linking agent, epichlorhydrin. EP-β-CD was obtained from Cyclolab (Budapest, Hungary). All other chemicals used were of analytical grade and were purchased from Merck (Darmstadt, Germany). The concentration of aqueous solutions of the studied compounds was 100 μmol l$^{-1}$.

### 2.2. Apparatus

CZE experiments were carried out using a P/ACE 2200 instrument (Beckman Instruments, Fullerton, CA). All separations were performed in a thermostatted cartridge in an uncoated fused-silica capillary (37 cm × 50 μm i.d.) with the detector located 7 cm from the outlet end of the capillary. A constant field strength of 400 V cm$^{-1}$ was applied. The analytes were detected at 214 nm. The chiral selector EP-β-CD was only

Table 2
Effect of concentration of BGE, pH and temperature on resolution $R_s$ and migration time $t_m$ of methamphetamine and selegiline

| Parameter | | Methamphetamine | | Selegiline | |
|---|---|---|---|---|---|
| | | $R_s$ | $t_m$ (min) | $R_s$ | $t_m$ (min) |
| Concentration of BGE (mM)[a] | 10 | 1.61 | 28.46 | 1.48 | 36.17 |
| | 25 | 2.18 | 24.62 | 1.66 | 33.67 |
| | 50 | 2.63 | 22.92 | 1.93 | 26.65 |
| | 75 | 2.93 | 22.65 | 2.41 | 27.03 |
| | 100 | 3.31 | 24.13 | 2.81 | 28.26 |
| | 200 | 3.54 | 24.78 | 3.24 | 29.67 |
| pH[b] | (i) 2.5 | 2.61 | 22.89 | 1.93 | 26.61 |
| | (ii) 3.5 | 0.74 | 9.16 | 0.51 | 9.52 |
| | (iii) 4.5 | 0.84 | 9.47 | 0.58 | 9.38 |
| | (iv) 6 | 0 | 4.39 | 0 | 4.82 |
| Temperature (°C) | 20 | 2.61 | 22.87 | 1.94 | 26.59 |
| | 25 | 2.58 | 17.71 | 1.74 | 19.84 |
| | 30 | 2.55 | 16.45 | 1.44 | 18.76 |
| | 40 | 2.45 | 14.67 | 0.96 | 15.53 |
| | 50 | 2.07 | 9.75 | 1.76 | 10.86 |

[a–c] Run conditions:
[a] Sodium phosphate buffer (pH 2.5) with EP-β-CD (200 mg ml$^{-1}$), $T = 20$°C; ephedrine was not separated under these conditions.
[b] (i) Sodium phosphate buffer (pH 2.5, 0.05 M) with EP-β-CD (200 mg ml$^{-1}$); (ii) sodium phosphate buffer (pH 3.5, 0.05 M) with EP-β-CD (200 mg ml$^{-1}$); (iii) sodium acetate buffer (pH 4.5, 0.05 M) with EP-β-CD (200 mg ml$^{-1}$); (iv) sodium phosphate buffer (pH 6.0, 0.05 M) with EP-β-CD (200 mg ml$^{-1}$); $T = 20$°C.
[c] Sodium phosphate buffer (pH 2.5, 0.05 M) with EP-β-CD (200 mg ml$^{-1}$).

present inside the capillary. Samples were injected by pressure (0.5 psi) for 8 s.

### 2.3. Description of enantioseparation

For a quantitative description of mutual separation of two analytes the resolution $R_s$ was calculated using the classical equation [3].

## 3. Results and discussion

The enantiorecognition of the compounds studied is based on the inclusion of the phenyl group in the hydrophobic cavity of the β-cyclodextrin (β-CD) selector and on hydrogen bonding of polar groups in the molecule with the secondary hydroxyl groups at the entrance of the β-CD cavity. The stabilities of the resulting inclusion complexes, together with the effective mobilities of individual enantiomers and thus also final resolution, are significantly influenced by a number of experimental parameters. It is necessary to make an appropriate choice of applied voltage, temperature during separation, concentration of chiral selector, suitable composition (the nature of cationic and anionic components), concentration and pH of background electrolyte (BGE), and whether or not modifiers should be added to the electrolyte system. Tables 1–3 demonstrate the necessity of optimising these parameters to obtain successful separation of the optical isomers.

Owing to its very good solubility in water solutions (40–50%, w/w) in comparison to native β-CD (2%, w/w), EP-β-CD allows high concentrations to be used and consequently the excellent separation of methamphetamine and selegiline (Table 1). However, in contrast with native β-CD (unpublished data) it is not possible to separate the enantiomers of ephedrine without the addition of organic modifier. The discrepancy in the enantioseparation of ephedrine using native β-CD or EP-β-CD is probably connected with changes in the size of the entrance to the cavity surrounded

Table 3
Influence of cationic or anionic component of the background electrolyte on selegiline separation parameters[a]

| Component | Migration time $t_m$ (min) | Resolution $R_S = \Delta t/4\sigma$ | Efficiency $N = t_m^2/\sigma^2$ | Separation factor $\alpha = t_2/t_1$ | Peak symmetry $A_S = b/a$ |
|---|---|---|---|---|---|
| Sodium | 28.26–29.79 | 2.81 | 68 823–44 219 | 1.05 | 2.21–2.76 |
| Ammonium | 27.83–29.31 | 2.28 | 44 856–30 639 | 1.05 | 1.95–2.27 |
| Triethylamine | 32.69–34.41 | 3.17 | 98 357–64 496 | 1.05 | 1.46–1.93 |
| Tris | 29.22–30.68 | 3.65 | 128 097–90 015 | 1.05 | 20.4–2.58 |
| Phosphate | 28.19–29.74 | 2.83 | 69 228–46 456 | 1.05 | 2.19–2.69 |
| Sulphate | 28.12–29.63 | 2.76 | 64 746–42 313 | 1.05 | 1.82–2.43 |
| Nitrate | 28.59–30.16 | 2.96 | 79 541–56 582 | 1.06 | 1.09–1.15 |
| Perchlorate | 29.65–30.82 | 1.49 | 28 644–25 090 | 1.04 | 1.19–1.35 |

[a] Run conditions: buffer (pH 2.5; 0.1 M) with EP-β-CD (200 mg ml$^{-1}$), $T = 20$°C.

by secondary hydroxyl groups and with steric hindrance due to the polymerisation of β-CD units.

For practical CZE analysis EP-β-CD can be used up to a concentration of 250 mg ml$^{-1}$, because in more concentrated solutions, owing to the increase in Joule heating, it precipitates. The addition of organic solvent to the background electrolyte can influence the separation of enantiomers owing to the change in solubility and solvation of analytes as well as the chiral selector. For higher EP-β-CD concentrations the addition of methanol to the BGE (20%, v/v) allows the attainment of almost complete separation of ephedrine (Table 1). It is possible that the solvataion of secondary hydroxyl groups and spacer arms with methanol molecules leads to a decrease in hydrogen bonding interactions with hydroxyl groups and consequently to an extension of the

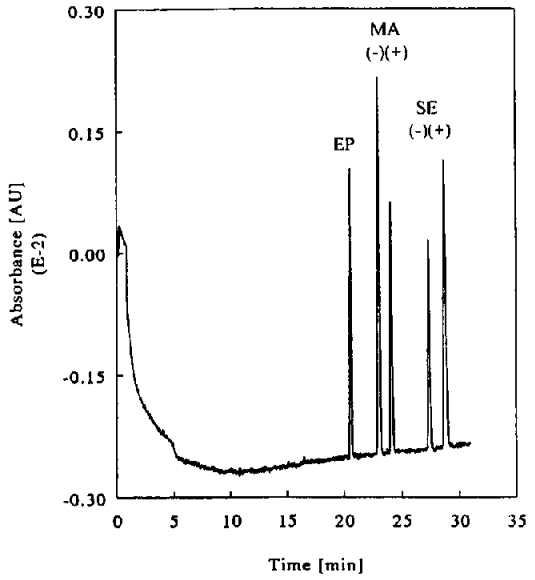


Fig. 1. Electropherogram representing the chiral separation of a mixture of compounds under the best conditions found for methamphetamine (MA) and selegiline (SE). Ephedrine (EP) was not resolved in this run. Run conditions: Tris–phosphate buffer (pH 2.5; 0.1 M) with EP-β-CD (200 mg ml$^{-1}$); $T$ = 20°C.

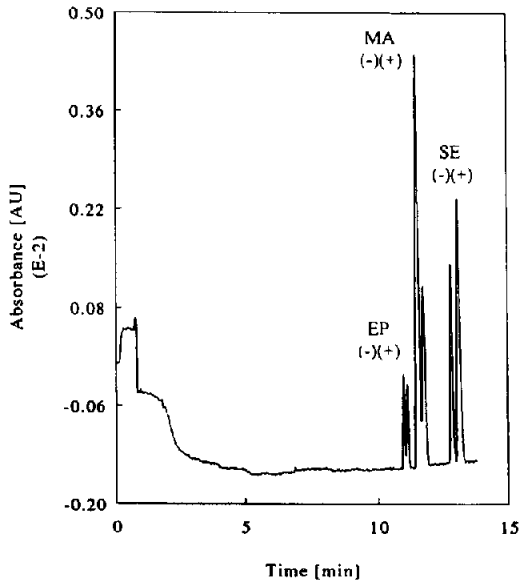


Fig. 2. Electropherogram representing the effects of column temperature and the addition of methanol to the BGE on the enantiomeric resolution of EP, MA and SE. Run conditions: methanol–Tris–phosphate buffer (pH 2.5; 0.1 M) (20:80, v/v) with EP-β-CD (300 mg ml$^{-1}$); $T$ = 50°C.

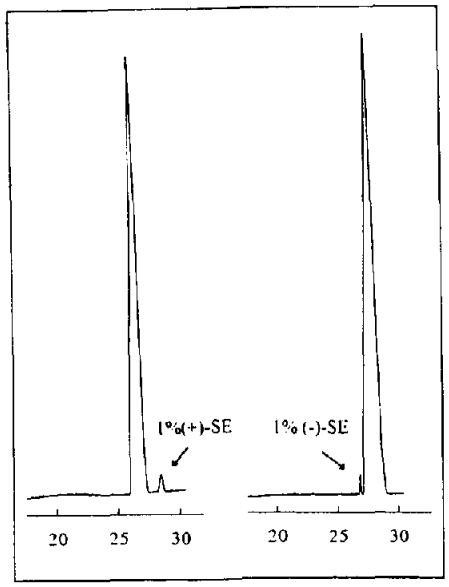


Fig. 3. Electropherograms representing chiral separation of the enantiomeric impurity in selegiline. Run conditions: Tris–phosphate buffer (pH 2.5; 0.1 M) with EP-$\beta$-CD (200 mg $ml^{-1}$); $T = 20°C$.

entrance to the EP-$\beta$-CD cavity. It certainly allows deeper penetration of ephedrine into the cavity, sufficient for the beginning of enantiodiscrimination.

The addition of solvents, with the exception of acetonitrile, led to poorer separations than those obtained with no solvent present. Other changes in system selectivity can be achieved through modification of the electrolyte system used.

The changes in ionic strength, composition and pH of BGE (Table 2) modify the background environment polarity, the charge of the analytes, their ionic interactions, electrodispersion and adsorption as well as the electroosmotic flow. They all influence the final resolution.

The nature of the cationic and anionic components has a significant influence on chiral separation (Table 3). Of the electrolyte cations examined, tris(hydroxymethyl)aminomethane (Tris) was selected as it gave the best efficiency and resolution. As an anionic component phosphate was chosen for its protective effect against interactions of cationic analytes with the capillary wall at low pH values [10].

Thus the best electrolyte system, Tris–phosphate buffer (pH 2.5, 0.1 M) with EP-$\beta$-CD (200 mg $ml^{-1}$), was chosen for enantioseparation of methamphetamine and selegiline (Fig. 1). It is interesting to more that at 50°C the resolution of selegiline enantiomers is greatly improved compared with the earlier decrease, in resolution (Table 2). Also, some degree of separation takes place at this temperature in the case of ephedrine (unpublished results). The increase in capillary temperature means a lower complex formation constant and, in most cases, a decrease in resolution. The positive effect referred to could perhaps be ascribed to conformational changes of EP-$\beta$-CD. Optical isomers of all three compounds can be successfully separated on the basis of data in this study (Table 1; see results for addition of methanol (30%, v/v) and EP-$\beta$-CD (200 mg $ml^{-1}$) to BGE). The positive effects of methanol addition and higher temperatures on the enantioseparation of ephedrine as well as selegiline (Fig. 2) favour a shortening of the analysis time without a substantial decrease in resolution. However, the negative effects of adding the organic solvent and of higher temperature led to a decrease in the resolution of methamphetamine enantiomers. The high recognition power of EP-$\beta$-CD allows the optical purity of selegiline to be determined (Fig. 3) as well as that of methamphetamine at levels of the minor isomer content of $< 1\%$.

The following values were found for (*S*)-(+)-selegiline (chiral impurity of the final product (*R*)-(−)-selegiline): limit of detection, $20\,\mu mol\,l^{-1}$; limit of quantitation (the lowest concentration of (*S*)-(+)-selegiline that could be determined in a purity determination), 0.25%, w/w; recovery, $y = (0.97 \pm 0.04)x - (0.02 \pm 0.01)$, $r^2 = 0.997$ $(n = 8)$; reproducibility for (*S*)-(+)-selegiline (0.5% w/w in (*R*)-(−)-selegiline (0.01 M) RSD = 2% $(n = 6)$; linearity, $y = (0.03 \pm 0.001)x + (0.017 \pm 0.019)$, $r^2 = 0.9986$.

## 4. Conclusion

Evaluation of the influence of experimental parameters permits optimal conditions to be found for successful enantiomer separation of selegiline, methamphetamine and ephedrine. The

CZE method developed using the soluble neutral epichlorhydrin $\beta$-cyclodextrin polymer as chiral selector allows very sensitive determination of the chiral purity of methamphetamine and selegiline. The addition of methanol to the background electrolyte enables the optical isomers of all these compounds to be resolved in one run.

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