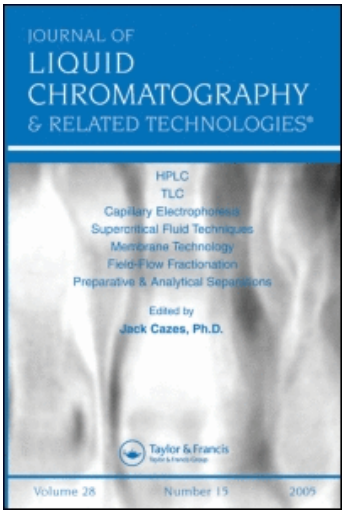

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ONE-RUN CHIRAL SEPARATION OF METHAMPHETAMINE AND ITS RELATED METABOLITES BY CAPILLARY ELECTROPHORESIS

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ABSTRACT

Capillary zone electrophoretic method for the one run enantioseparation of methamphetamine and its related metabolites (amphetamine, 4-hydroxymethamphetamine, 4-hydroxyamphet-amine, norephedrine and 4-hydroxy-norephedrine) was developed. Six different cyclodextrins (α-cyclodextrin, β-cyclodextrin, hydroxypropyl-β-cyclodextrin, dimethyl-β-cyclodextrin, epi-chlorhydrin-β-cyclodextrin polymer and γ-cyclodextrin) were tested.

The best chiral separation of the set of studied compounds was reached under the following condition: Tris-phosphate buffer (pH 2.5, 100mmol.L^{-1}) with 20 mmol.mL^{-1} hydroxypropyl-β-cyclodextrin and V=200 V.cm^{-1}.

The applicability of the developed method was tested by analyses of treated urine samples of healthy volunteers.

INTRODUCTION

Methamphetamine together with marijuana belongs to the most abused drugs in the world. On the illegal market, there is according to preparations available, racemic mixtures as well as D-(+)-methamphetamine. It is well known that D-(+)-isomers of methamphetamine and its metabolites exhibit greater central nervous system activity than the L-(–)-isomers.[1] Differences in the pharmacodynamic profiles of drug enantiomers may be, in part, due to differences in their disposition kinetics.[2]

For better understanding of the effect of methamphetamine and its metabolites, the metabolism of single enantiomers should be studied. Such a study is possible only by using an appropriate analytical method.

The enantiomeric separation of amphetamine analogues can be performed by numerous techniques (HPLC,[3-6] GC,[7-8] as well as CZE[9-14]). Up to now, we have found only HPLC procedures[15-18] that have been applied for the investigation of methamphetamine enantiomer metabolism.

The aim of this paper is to work up rapid capillary zone electrophoretic method that can be used for a study of metabolism of methamphetamine enantiomers.

EXPERIMENTAL

Chemicals

Optically pure isomers of studied compounds were a gift of FARMAKON (Olomouc, Czech Republic) (methamphetamine hydrochloride) and VÚFB (Prague, Czech Republic) (amphethamine hydrochloride, 4-hydroxy-methamphetamine hydrochloride, 4-hydroxyamphetamine hydrobromide, norephedrine hydrochloride, and 4-hydroxynorephedrine hydrochloride). The chiral selectors α-cyclodextrin, β-cyclodextrin, hydroxypropyl-β-cyclodextrin, dimethyl-β-cyclodextrin and γ-cyclodextrin were part of the eCAP™ Chiral Methods Development Kit (Beckman Instruments, Fullerton, CA) and soluble β-cyclodextrin polymer (M_W: 3000-5000, cyclodextrin content: 50-60%, cross-linking agent: epichlorhydrin) 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 studied compounds was 100 μmol.L^{-1}. 100 mmol.L^{-1} phosphate buffer was prepared by titration of 100 mmol.L^{-1} phosphoric acid using Tris(hydroxymethyl)aminomethane.

Apparatus

CZE experiments were performed on a modular system of Spectra-PHORESIS 100 (Thermo Separation Products) equipped with a fast scanning SpectraFocus detector (Thermo Separation Products). All separations were performed in an uncoated fused-silica capillary (75 μm i.d. x 375 μm o.d., Polymicro Technologies Inc.) with UV detection over the range 190-340 nm at ambient temperature (25°C). The capillary had an effective length of 45 cm (total length 75cm). A constant field strength of 200 V.cm^{-1} was applied. The chiral selector was only present inside the capillary.

Sample introduction was accomplished by vacuum injection (0.5 sec). All samples were filtered through a 0.45 μm membrane filter (Millipore, Milford, MA) before assay.

Description of Enantioseparation

For a quantitative description of mutual separation of two analytes the resolution R_S was calculated using the classical equation.[19]

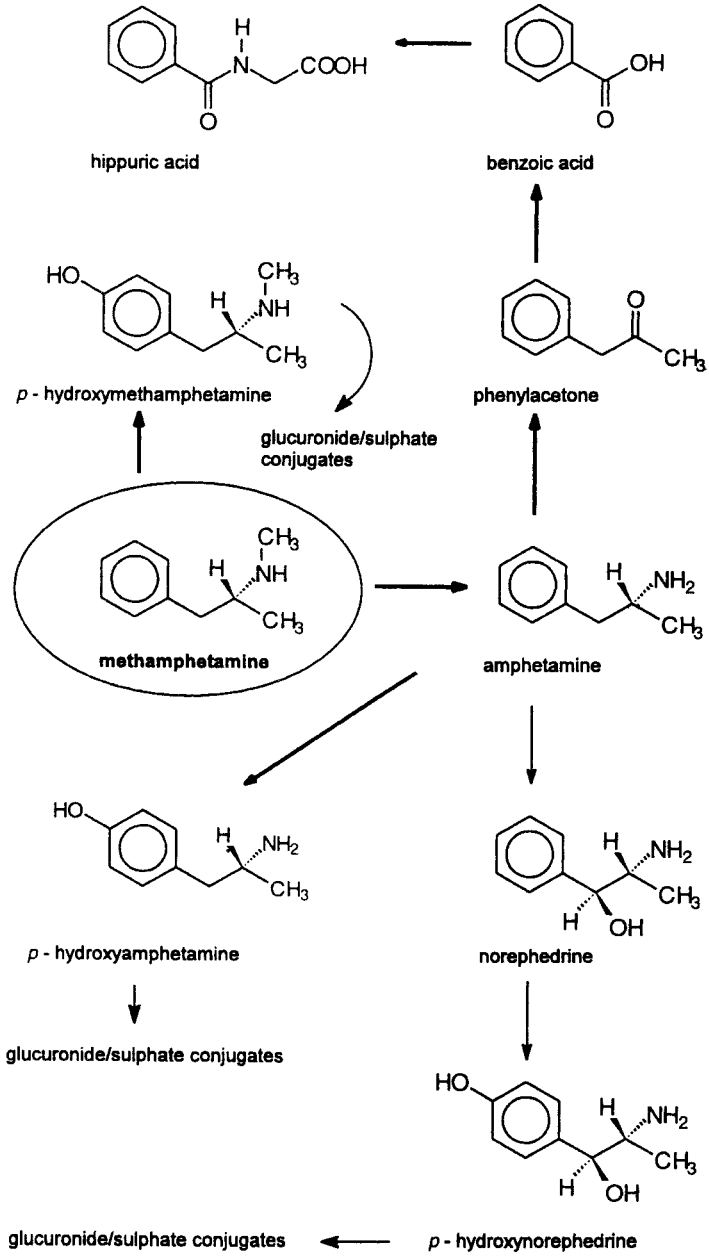

Figure 1. Metabolic pathway of methamphetamine.

Table 1

Effect of Different Kinds of β-Cyclodextrins on Resolution R_S of the Compounds Studied

Chiral Selector	MAP	AP	PMAP	PAP	NEP	PNEP
200 mg.mL^{-1} EP-β-CD	2.86	3.1	3.6	3.8	1.15	1.10
20 mmol.L^{-1} HP-β-CD	1.62	1.48	1.74	1.55	0	1.45
15 mmol.L^{-1} β-CD	1.18	1.2	1.28	1.30	<0.5	0.7
20 mmol.L^{-1} DM-β-CD	0.77	0.68	<0.5	0.71	<0.5	<0.5

Run conditions: Tris-phosphate buffer (pH 2.5, 100 mmol.L^{-1}) with appropriate chiral selector, V = 200 V.cm^{-1}, ambient temperature.

RESULTS AND DISCUSSION

The major and minor metabolic pathways of methamphetamine[20] (Figure 1) determine which main chiral compound should be analyzed in one capillary zone electrophoretic run. It is, specifically, methamphetamine that is mainly excreted in unchanged form, with 4-hydroxymethamphetamine, amphetamine and 4-hydroxyamphetamine. In low concentration, norephedrine and 4-hydroxynorephedrine can be extracted and should be determined too.

For chiral separation of above mentioned set of six enantiomeric pairs, with regard to their molecular structures, some cyclodextrin derivatives could be suitable. These chiral selectors are also easily commercially available. In this study the enantioseparation using cyclodextrins is based on the formation of inclusion complexes of different stability where the difference in the stability constants of enantiomers determines the final resolution. The various cyclodextrins have different affinity to single enantiomers of analyzed compounds. In the presented work, we tested six different kinds of cyclodexrins: α-cyclodextrin, β-cyclodextrin, hydroxypropyl-β-cyclodextrin, dimethyl-β-cyclodextrin, epichlorhydrine-β-cyclodextrin polymer, and γ-cyclodextrin. The use of α-cyclodextrin and γ-cyclodextrin as chiral selectors did not allow enantiodiscrimination of any enantiomeric pair from tested amphetamines.

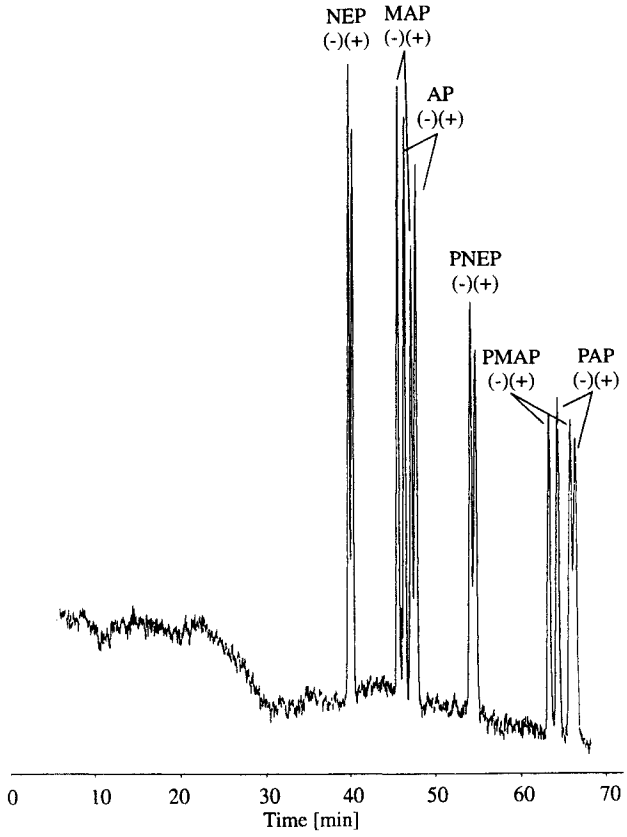

Figure 2. Electropherogram representing the chiral separation of a tested mixture. Run conditions: Tris-phosphate buffer (pH 2.5, 100 mmol.L^{-1}) with EP-β-CD (200 mg.mL^{-1}), V=200 V.cm^{-1}, ambient temperature, λ=190 nm.

The failure of separation is probably connected with cyclodextrin cavity inner diameter and geometry that, in both cases, do not allow the formation of enough stable inclusion complexes. Much better results were reached using β-cyclodextrins. Found values of resolution of single enantiomeric pairs of studied amphetamines are shown in Table I. The observed different R_S values for enantiomers using native β-cyclodextrin and its three derivatives are the result of the change of the cyclodextrin cavity and its surroundings due to various derivatizations.

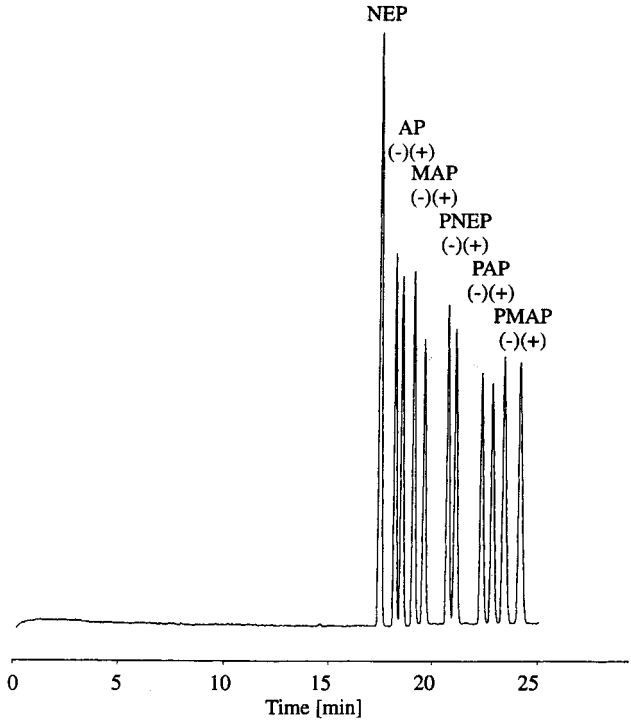

Figure 3. Electropherogram representing the chiral separation of a tested mixture. Run conditions: Tris-phosphate buffer (pH 2.5, 100mmol.L^{-1}) with HP-β-CD (20 mmol.mL^{-1}), V=200 V·cm^{-1}, ambient temperature, λ=190 nm.

For a one run analysis of a model mixture of six enantiomeric pairs the chiral selectors epichlorhydrine-β-cyclodextrin polymer and hydroxypropyl-β-cyclodextrin were chosen. On the basis of our proceeding studies on the effect of experimental parameters on the stability of formed cyclodextrin inclusion complexes,[12-14] we used the following optimized experimental conditions:

Background electrolyte: 100 mmol.L^{-1}, TRIS-phosphate buffer pH 2.5 containing 200 mg/mL, epichlorhydrine-β-cyclodextrin polymer or 20 mmol.L^{-1} hydroxypropyl-β-cyclodextrin, field strength: 200 V.cm^{-1}. Obtained electropherograms are shown in Figure 2 and Figure 3. The identity of all enantiomers was verified by spiking with individual isomers.

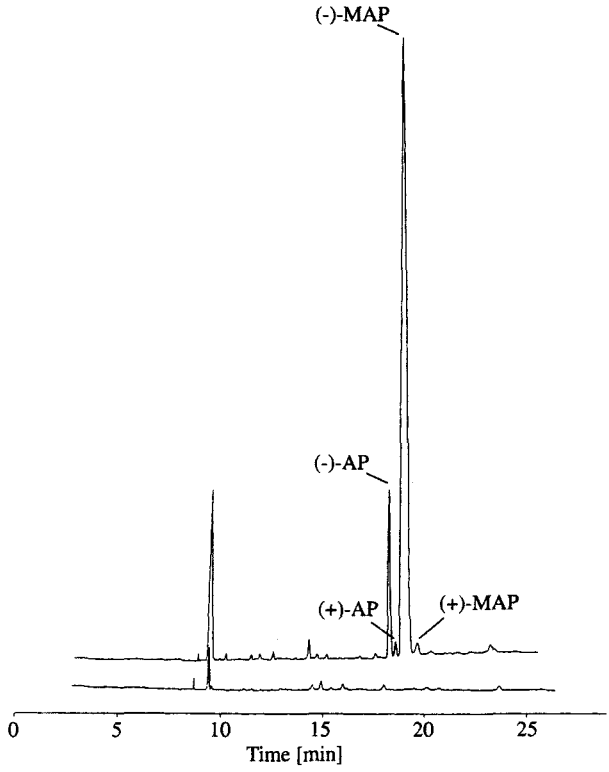

Figure 4. Electroferogram representing CZE separation of an urine sample of healthy volunteer 48 hours after application of (-) MAP. Note: The residual (+) MAP and (+) AP 120 hours after application of (+) MAP can be detected. For run condition see Figure 3.

The molecular structure and the configuration around the chiral center is the main factor influencing the order of each isomer and also the overall enantiorecognition. In the case of norephedrine it can be stated that mostly the steric hindrance of substituents has not allowed its total chiral resolution. The presence of the 4-hydroxy group on the aromatic ring seemed to have a positive effect on forming of inclusion complexes by means of stabilization by hydrogen bonding which consequently caused the significant decrease of their effective mobilities. It is noticeable that a change of chiral agent (from EP-β-CD to HP-β-CD, Fig. 2, 3) switches the migration order of methamphetamine and amphetamine as well as 4-hydroxymethamphetamine and

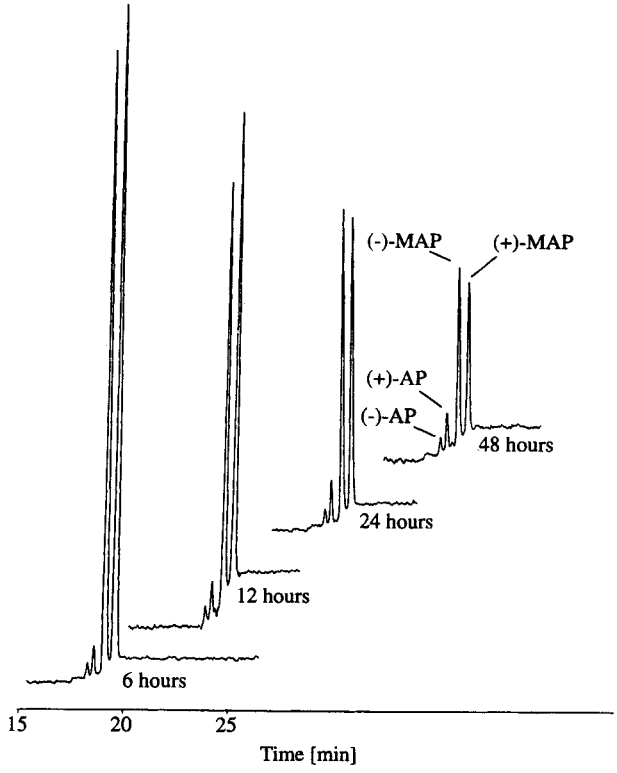

Figure 5. Electropherogram representing CZE separation of an urine samples of healthy volunter 6, 12, 24, and 48 hours after application of racemic MAP. Note: With time increase ratio of MAP enatiomers is changed. For run condition see Figure 3.

The migration order of discussed compounds for EP-β-CD can be probably explained due to the stabilization of inclusion complexes by hydrogen bonding between aliphatic 2-hydroxypropylene spacers of β-CD units in EP-β-CD polymer and primary amino group of amphetamine or its 4-hydroxyderivative, respectively. We can expect steric hindrance for the secondary amino group in methamphetamine and its hydroxyderivative molecules.

Although the use of epichlorhydrine-β-cyclodextrin polymer as a chiral selector gives very excellent resolution for individual enantiomer pairs of methamphetamine, amphetamine, and their 4-hydroxy derivatives, this selector

is not convenient for one run analysis due to overlapping of peaks of individual compounds (Figure 2). That is why for analyses of real samples we used hydroxypropyl-β-cyclodextrin that proved to lower enantio-discrimination but allows good chiral separation of studied compounds. The CZE method with 20 mmol.L^{-1} hydroxypropyl-β-cyclodextrin in the background electrolyte was used for the preliminary study of human methamphetamine metabolism. Electropherograms of urine samples of healthy volunteers (without enzymatic digestion and concentrated liquid-liquid extraction) are shown in Figure 4 and Figure 5. Further continuation on the investigation of mathamphetamine enantiomer metabolism is in progress and results will be published in the following papers.

CONCLUSION

The developed capillary zone electrophoretic method for enantioseparation of methamphetamine and its related main catabolites gives higher efficiency and resolution of compounds of interest in shorter analysis time over published HPLC procedures. Under described conditions no endogenous interferences were observed in real samples.

LIST OF SYMBOLS

AP	amphetamine
β-CD	β-cyclodextrin
DM-β-CD	dimethyl-β-cyclodextrin
EP-β-CD	epichlorhydrine-β-cyclodextrin polymer
HP-β-CD	hydroxypropyl-β-cyclodextrin
MAP	methamphetamine
NEP	norephedrine
PAP	4-hydroxyamphetamine
PMAP	4-hydroxymethamphetamine
PNEP	4-hydroxynorephedrine
TRIS	Tris(hydroxymethyl)aminomethane

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