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Enantiomeric separation of amphetamine related drugs by capillary zone electrophoresis using native and derivatized β-cyclodextrin as chiral additives

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Abstract

Amphetamine, methamphetamine and several ring-substituted analogs which are under governmental regulations have been separated by capillary zone electrophoresis employing native and various substituted β-cyclodextrins as additives to the background electrolyte. The following chiral selectors were used: native β-cyclodextrin, heptakis-(2,6-di-O-methyl)-β-cyclodextrin, heptakis-(2,3,6-tri-O-methyl)-β-cyclodextrin, (2-hydroxy)propyl-β-cyclodextrin and carboxymethyl-β-cyclodextrin. The amphetamines were separated without derivatization. Separations are reported with respect to the kind of chiral selector. Native β-cyclodextrin and carboxymethyl-β-cyclodextrin turned out to give optimal resolutions within only a few minutes. This direct method is compared with the indirect method separating the diastereomeric Marfey's derivatized amphetamines by means of non-chiral sodium dodecylsulfate micelles.

1. Introduction

Because of the increasing popularity of amphetamine, methamphetamine and various ring-substituted amphetamines as drugs which act as central nervous stimulants, these compounds are receiving more and more attention in clinical, pharmacological and toxicological science [1]. As the enantiomers of amphetamine have different pharmacological and toxicological potential [2,3]–and a similar situation can be expected for their derivatives–separation and quantitation of the single enantiomers are required. The enantiomeric composition is also of interest in forensic analysis, as in some countries the controlling regulations for the optical isomers are different. The enantiomer pattern is further supposed to lead to information about the origin and the way of preparation of the administered drug samples [4].

Recently enantioseparation has been reported for nine amphetamine related drugs using HPLC [5]. Direct separation by means of a chiral stationary phase consisting of immobilized β-cyclodextrin (β-CD) was obtained within analysis times of ca. 20 to 30 min. Some of the compounds could be separated without derivatization, for some others derivatization prior to enantiomeric separation was required. Alterna-

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tively, most of the analytes could be separated also by simple non-chiral reversed-phase chromatography after derivatization of the analytes with optical pure Marfey's reagent to form diastereomeric compounds.

Beside the HPLC methods, capillary zone electrophoresis (CZE) has become a powerful tool for enantiomer separation, particularly because of the shorter analysis times which often can be achieved [6]. Direct methods of enantioseparation can be performed by simply adding a chiral selector to the background electrolyte (BGE). Cyclic oligosaccharides, especially cyclodextrins, are widely used as chiral selectors [7–11]. The present paper deals with the direct enantioseparation of nine different amphetamines (see Fig. 1), covering amphetamine, methamphetamine and ring-substituted derivatives of both, by using native β-CD or several derivatized β-cyclodextrins as additives to the BGE. The use of modified chiral selectors allows to achieve different enantioselectivity coefficients due to differences in the host–guest complexation constants caused by differences in the cavity size and shape, differences in the hydrophobicity and introduction of additional interaction sites. The amphetamines are analyzed without any derivatization using their native UV absorbance. The method thus becomes even more simple, cheap and fast, especially concerning sample preparation. The aim of the present work is to specify conditions where all of the amphetamines can be enantioseparated and to study the influence of different selector substituents and analyte structure on the enantioseparation.

The results obtained with the proposed method are finally compared with the results of an alternative electrophoretic method proposed previously [12], i.e. the indirect enantioseparation of the amphetamines after derivatizing the enantiomers to diastereomers by optically pure Marfey's reagent. These uncharged derivatives are separated by use of micellar electrokinetic chromatography (MEKC) employing non-chiral SDS micelles in alkaline solutions.

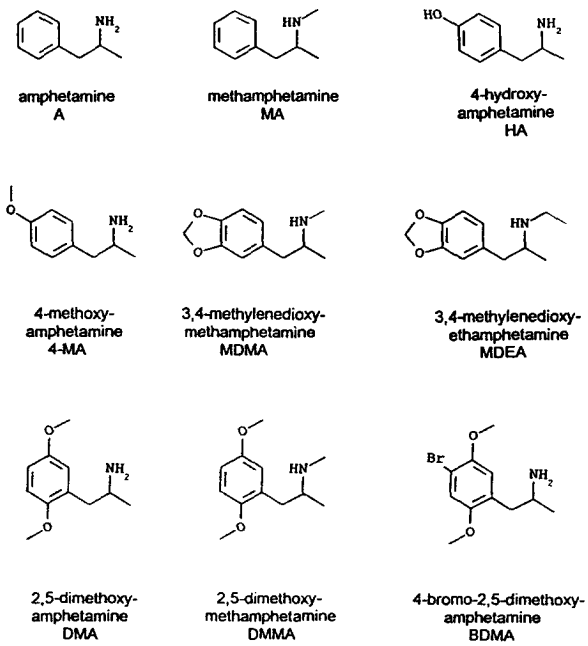

Fig. 1. Structure of amphetamines and abbreviations used.

2. Experimental

2.1. Chemicals

Sodium hydroxide, sodium dihydrogenphosphate and phosphoric acid were purchased from E. Merck (Darmstadt, Germany). Amphetamine standards were gifts from the UNIDO laboratories (Vienna, Austria). Native β-cyclodextrin (β-CD) was a gift from the Department of Chemistry of the Polish Academy of Sciences (Warsaw, Poland). Heptakis-(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) containing about 14 methoxy-groups per β-CD molecule), heptakis-(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD) containing 21 methoxy-groups per β-CD molecule, (2-hydroxy)propyl-β-cyclodextrin (HP-β-CD) containing about 6.3 hydroxypropyl-groups per β-CD ring, and carboxymethyl-β-cyclodextrin (CM-β-CD) containing 2.5–3 carboxymethyl groups per β-CD ring) were purchased from

Cyclolab R&D Laboratory (Budapest, Hungary).

Sodium dodecylsulfate (SDS) was purchased from Fluka (Buchs, Switzerland), and Marfey's reagent from Sigma (Deisenhofen, Germany).

2.2. Apparatus and electrophoretic conditions

All experiments were carried out using an HP-3D capillary electrophoretic instrument (Hewlett-Packard, Waldbronn, Germany), equipped with a diode-array detector monitoring a wavelength of 214 nm. A non-coated fused-silica capillary (Hewlett-Packard; 48.5 cm total length, 40 cm effective length, 50 μm I.D.) was kept at a constant temperature of 20 ± 0.1°C and the applied voltage was 20 kV, unless stated otherwise.

The background electrolyte (BGE) consisted of an aqueous solution of 50 m*M* sodium dihydrogenphosphate at pH 2.5 (adjusted with phosphoric acid) and 10 m*M* selector (β-CD or derivatives).

3. Results and discussion

3.1. Enantioseparation with different substituted β-cyclodextrins

Enantioselectivity coefficients, r, for the amphetamines listed in Fig. 1 obtained by using different β-CD based selectors are given in Table 1. Enantioselectivity coefficients (separation factors) are calculated as the ratios of the effective mobilities of the enantiomers. The migration order of the amphetamines has not been determined in this paper. The pH of 2.5 chosen for all analyses reported allowed to keep the electroosmotic flow completely suppressed and all of the analytes protonated.

Native β-CD

With native, nonderivatized β-CD all of the amphetamines investigated could, at least partially, be resolved. Baseline resolution was not achieved for all compounds, although in all instances the separation was sufficient to provide

Table 1
Migration times and enantioselectivity coefficients (r) measured for amphetamines employing different chiral selectors

Amphetamine	Chiral selector									
	β-CD		DM-β-CD		TM-β-CD		HP-β-CD		CM-β-CD[a]	
	t (min)	r	t (min)	r	t (min)	r	t (min)	r	t (min)	r
A	8.50	1.015	9.39	1.007	6.07	<1.003	6.48	1.011	13.07	1.028
MA	8.29	1.015	8.56	1.010	6.42	1.003	6.82	1.011	13.00	1.027
HA	10.09	1.017	6.36	1.008	6.61	1.011	6.06	1.011	19.03	1.043
4-MA	9.85	1.011	11.22	1.000	7.02	<1.003	7.60	1.011	17.55	1.027
MDMA	10.90	1.021	12.42	1.010	6.84	1.016	9.15	1.026	16.96	1.028
MDEA	12.01	1.017	13.32	1.007	7.38	1.020	10.31	1.025	30.76	1.026
DMA	8.30	1.013	10.24	1.020	6.96	1.015	8.61	1.004	12.50	1.016
DMMA	8.92	1.013	10.75	1.021	7.60	1.027	8.19	1.007	14.72	1.017
BDMA	8.32	1.005	9.19	1.004	7.83	1.000	7.54	1.000	11.94	1.000

Enantioselectivity coefficients are obtained by dividing the effective mobility of the faster migrating enantiomer, μ_1, by that of the slower one, μ_2. Experimental conditions as specified in the Experimental section. BGE: aqueous solution of 50 m*M* sodium dihydrogenphosphate, pH 2.5; selector concentration, 10 m*M*; temperature, 20°C; applied voltage, 20 kV.
Abbreviations: β-CD = nonderivatized (native) β-cyclodextrin; DM-β-CD = heptakis-(2,6-di-O-methyl)-β-CD; TM-β-CD = heptakis-(2,3,6-tri-O-methyl)-β-CD; HP-β-CD = (2-hydroxy)propyl-β-CD; CM-β-CD = carboxymethylated β-CD.
[a] Applied voltage: 15 kV.

information whether racemic or non-racemic samples are investigated. Fairly well resolution is achieved for all compounds exhibiting enantioselectivity coefficients larger than 1.014 (cf. Fig. 2).

Methylated β-CDs

Both methylated cyclodextrins, i.e. DM-β-CD and TM-β-CD, gave only low enantioselectivity coefficients for most of the amphetamines. The large and bulky di-substituted analytes DMA and DMMA are the two exceptions. Both of them are baseline separated with DM-β-CD and best selectivity coefficients could be achieved for DMA in this system, whereas for DMMA TM-β-CD showed the highest selectivity. It is a matter of efficiency that DMMA was separated to the baseline only with DM-β-CD.

From the mechanistic point of view it is obvious that methylation of two (DM-β-CD) or three (TM-β-CD) hydroxyl groups per glucose unit in the CD ring leads to increased hydrophobicity in the CD ring which simply enlarges the size and width of the host molecule. It is likely that the good resolution achievable with methylated β-CDs, particularly for the bulky dimethyl-substituted amphetamines which are supposed not to be able to completely penetrate the hydrophobic interior of the cavity of native β-CD [5], can mainly be attributed to the reduced polarity of the rims of the cavity and the enlargement of the host molecule.

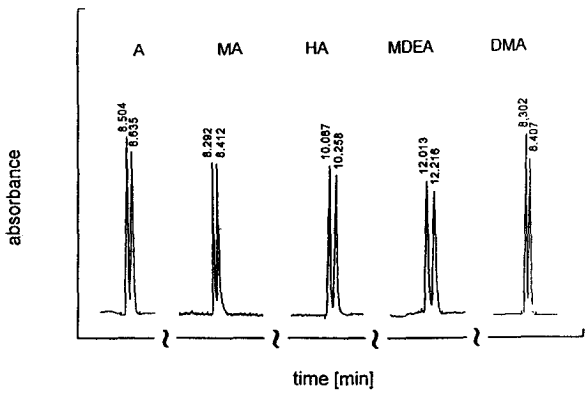

Fig. 2. Electropherograms of five well separated amphetamines using native β-CD as selector. Abbreviations as in Fig. 1. Experimental conditions: Non-coated fused-silica capillary with 50 μm I.D., total length of 48.5 cm and effective length of 40 cm. Detection wavelength, 214 nm. BGE: aqueous solution of 50 m*M* sodium dihydrogenphosphate at pH 2.5, 10 m*M* β-CD; temperature, 20°C, applied voltage, 20 kV.

Hydroxypropyl-β-CD

This selector molecule is used with a substitution degree of about 6.3. This means that on the average each glucose unit in the β-CD molecule carries one substituent. The use of the hydroxypropylated selector gave good results for the bicyclic amphetamines. For all other analytes no improvement in resolution was observed compared to the nonderivatized selector. Especially for the bulky amphetamines DMA, DMMA and BDMA–which are not able to penetrate the cavity–the resolving power was low.

Carboxymethylated β-CD

The degree of substitution of the carboxymethyl-β-CDs was ca. 2.5–3 carboxymethyl groups per CD ring. The application of carboxymethyl-β-CD gave very good selectivities. Particularly compared to native β-CD, the separation factors were higher with CM-β-CD in all cases except for BDMA. This compound and the other two bulky dimethoxy amphetamines were the only exceptions; they are better separated by a different selector, e.g. DM-β-CD and to some extent TM-β-CD. However, even for two of these analytes baseline resolution (DMMA) or near-baseline resolution (DMA) was achieved with the carboxymethylated selector. However, some improvement in resolution can be expected when improving the efficiency by increasing the voltage. Best separation of all amphetamines was found for the hydroxyl-substituted amphetamine HA.

The particularly high selectivity coefficients achieved with this selector can be considered as follows: First, the carboxy group serves as an additional type of site allowing for strong interactions with basic and acidic groups in the analytes, depending on the degree of dissociation. Ion-pairing might be included. Strong interactions like these, particularly when occurring near the chiral centers, very often enhance the

selectivity of the system. Furthermore, at the used pH of 2.5 the carboxymethyl groups of the host are at least partially dissociated as can be concluded from the increased current compared to the other selectors. In contrast to the positively charged analytes the partially charged selector molecule thus migrates to the anode. This causes an additional increase of the separation factor independent of the selectivity of the host–guest complexation itself.

When regarding not only the selectivity but also the efficiency it becomes obvious that it is often the latter which determines the achievable resolution. This is illustrated in Fig. 3 which shows the electropherograms of the two bicyclic amphetamines MDMA and MDEA. They are very similar in structure and only differ in the substituent at the amino group (methyl- vs. ethyl-). However, in all systems better efficiency due to better peak symmetry is found for MDEA. As a consequence, MDEA is better resolved in all cases, although the systems with β-CD, HP-β-CD and CM-β-CD selectors are more selective for MDMA. The higher noise in the electropherograms with modified CDs corresponds to the lower analyte concentrations chosen to reduce peak tailing and formation of triangular peaks.

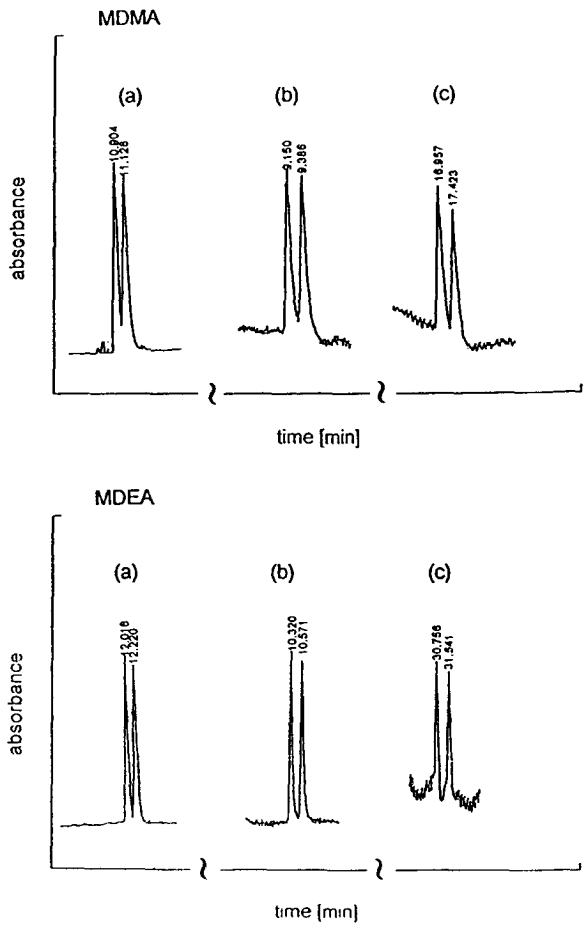

Fig. 3. Electropherograms of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyethamphetamine (MDEA) employing different selectors. (a) 10 m*M* β-CD, (b) 10 m*M* HP-β-CD, (c) 10 m*M* CM-β-CD. For other experimental conditions see Fig. 2.

Remarks on the experimental conditions

Selectivity coefficients are known to be dependent on the selector concentration in the BGE [13]. The concentration at which optimum separation is achieved depends on the magnitude of the host–guest complexation constant. All experiments reported in Table 1 were carried out at a selector concentration of 10 m*M*. Without using buffer additives like urea to enhance the solubility this is about the highest concentration which can be used for β-CD. Reducing the β-CD concentration from 10 m*M* to 5 m*M* resulted in a decrease in selectivity in all instances, e.g. the selectivity coefficient of MA dropped from 1.015 to below 1.003. In spite of the increased water solubility of all the derivatized cyclodextrins all selector concentrations were kept at 10 m*M*.

For all measurements employing uncharged selectors a voltage of 20 kV was chosen. Typical currents observed at this voltage were ca. 30 μA. Increasing the voltage to 30 kV leads to improved efficiencies as expected from fundamental relationships but at the same time to a loss in selectivity which probably results from Joule heating generated in the capillary which cannot be controlled by the thermostating device. With the chosen electrolyte solution and the equipment described, a voltage of 20 kV should thus not be exceeded.

4. Conclusion

Various methods are presented that allow the enantioseparation of nine different amphetamine-related compounds of clinical and forensic interest employing different β-CD based chiral selectors as BGE additives. Native β-CD was the only selector which was able to resolve all the amphetamines investigated at least partially. The methyl-substituted cyclodextrins, DM-β-CD and TM-β-CD, gave improved selectivity only for the bulky disubstituted amphetamines DMA and DMMA. HP-β-CD gave no advantage compared to the native selector except for a slight improvement of the separation of the two bicyclic analytes MDMA and MDEA. However, the carboxymethylated β-CD was superior to β-CDs for all amphetamines, except for BDMA where no separation could be achieved. Native β-CD and CM-β-CD are thus the selectors of choice for routine screening of amphetamine samples. The analysis times are in most instances below 12 min and thus significantly shorter than those of the HPLC methods described previously [5], where up to 40 min were required in some instances. As for the CZE separation no derivatization is needed prior to the analysis, the speed of analysis is unsurpassed by the HPLC methods. The chiral selector additives used are significantly cheaper than chiral stationary HPLC phases.

The results obtained by the method based on β-CD selectors is unmatched also by the alternative CZE method based on indirect enantioseparation [12]. This alternative method yields separation of the diastereomeric derivatives of the amphetamines after derivatization with Marfey's reagent and employing SDS micelles. Electropherograms of successful separations are shown in Fig. 4. Especially the bicyclic amphetamines MDMA and MDEA could be separated with excellent selectivity coefficients (1.073 and 1.117) and baseline separation could also be achieved for MA and HA. However, all of the baseline resolutions could also be achieved by at least one of the direct methods described above. The time required for the micellar separations is about the same as that needed for the direct methods with β-CD. However, the derivatization step is rather time-consuming. Thus, no advantage can be seen for this alternative method, especially as the separations achieved for the two bulky dimethoxy amphetamines, DMA and DMMA, as well as for 4-MA were insufficient, and BDMA was not separated at all.

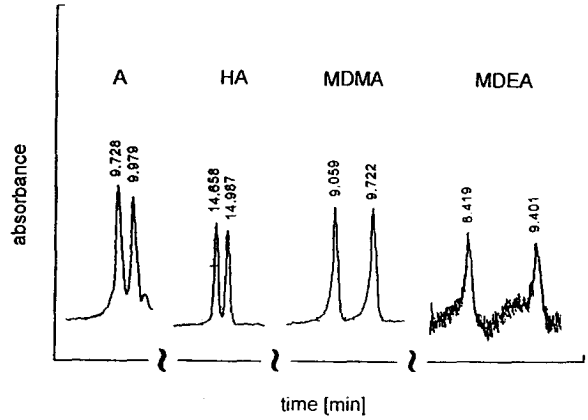

Fig. 4. Electropherograms of selected Marfey's reagent derivatized amphetamines applying nonchiral SDS micelles. Experimental conditions: Non-coated fused-silica capillary with 50 μm I.D., total length of 48.5 cm and effective length of 40 cm. Detection wavelength, 340 nm. BGE: 80% (v/v) aqueous solution of 5 m*M* sodium borate at pH 9.0, 100 m*M* SDS, 20% (v/v) methanol; temperature, 40°C; applied voltage, 30 kV.

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