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Journal of Chromatography A, 818 (1998) 239–249

JOURNAL OF CHROMATOGRAPHY A

Effect of various cyclodextrin derivatives on the resolution of fencamfamine isomers with capillary electrophoresis and nuclear magnetic resonance

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Received 27 April 1998; received in revised form 30 June 1998; accepted 3 July 1998

Abstract

Even though it is known that the enantiomers of a drug can exhibit different pharmacokinetic and dynamic properties, fencamfamine has been used in clinical practice as a mixture of four isomers, arising from the two centres of chirality in the molecule. The purpose of this investigation was to establish a capillary electrophoretic method for the discrimination of the isomers of fencamfamine and to determine its isomeric composition. The chiral selectors studied were uncharged α-cyclodextrin (α-CD), β-CD, *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin, γ-CD and *octakis*(2,3-di-*O*-acetyl)γ-cyclodextrin, and the negatively charged sulfobutylether-β-CD. In order to optimize the separation conditions, the selector concentration, pH values and buffer strength were varied. With Diac-β-CD and γ-CD, a very high resolution of the four isomers was found. Interestingly, the migration order of the diastereomers was different for these two selectors. In each case NMR spectroscopic studies revealed multiple isomer–cyclodextrin complexes to be responsible for the good discrimination of the isomers. In contrast to the classical inclusion complexes mostly described for CDs, fencamfamine was found to interact mainly with the outside region of the CD cavity.

Keywords: Buffer composition; Enantiomer separation; Fencamfamine; Cyclodextrins

1. Introduction

Considerable differences in the physiological actions of enantiomers of a drug are often observed so that it is important to know the composition of isomeric drugs or the optical purity of chiral compounds. Numerous systems have been developed to discriminate between enantiomers, and cyclodextrin (CD) derivatives have been widely employed in high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) and nuclear magnetic resonance (NMR) spectroscopy [1]. As part of a greater project, in which the influence of substitution (e.g., acetylation) and size of CDs on the resolution of various racemic phenethylamines in clinical practice is being investigated [2–4], fencamfamine, whose phenethylamine moiety is a part of the rigid norbornan skeleton, was chosen as a test compound with a phenethylamine in a defined geometry. Fencamfamine, an ingredient of the psychoanaleptic preparation Reactivan [5] is characterized by two centres of chirality. Four isomers are usually ob-

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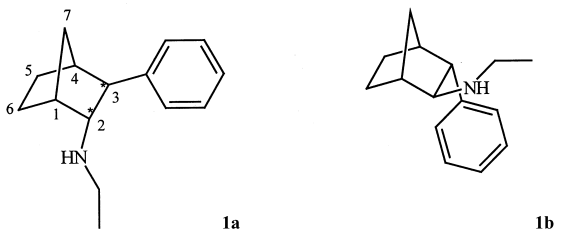
0021-9673/98/$19.00
PII: S0021-9673(98)00562-7



1a 2-endo-Ethylamino-3-exo-phenyl-bicyclo[2.2.1]heptane

1b 2-exo-Ethylamino-3-endo-phenyl-bicyclo[2.2.1]heptane

Fig. 1. Structural formula of the fencamfamine diastereomers.

tained from the non-stereoselective synthesis: 90% of the two enantiomers of 2-endo-ethylamino-3-exo-phenylbicyclo[2.2.1]heptane (**1a**=major isomer, Fig. 1) and 10% of the corresponding 2-exo-ethylamino-3-endo-phenylbicyclo[2.2.1]heptane enantiomers (**1b**=minor isomer, Fig. 1) [6]. The purpose of this study is to investigate the chiral recognition mechanism between the drug and the uncharged CDs, α-, β- and γ-CD as well as *heptakis*(2,3-di-*O*-acetyl)β-CD (Diac-β-CD) and *octakis*(2,3-di-*O*-acetyl)γ-CD (Diac-γ-CD) both as single isomers, and the charged sulfobutylether-β-CD (SBE-β-CD), to measure the exact ratio of the isomers by CE and to gain insight in the geometries of the various CD–fencamfamine complexes from NMR spectroscopic measurements.

2. Experimental

2.1. Chemicals

The fencamfamine isomers were synthesized and provided by Dr. Neugebauer [7], Pharmazeutisches Institut der Universität Bonn, and α-, β- and γ-CD were a gift from the Consortium für Elektrochemische Industrie (Munich, Germany). Diac-β-CD was synthesized according to Branch et al. [2] and Diac-γ-CD was obtained by an analogous synthesis pathway. SBE-β-CD (Advasep) was purchased from CyDex (Overland Park, KS, USA).

2.2. Capillary electrophoresis

All experiments were performed on a Beckman P/ACE 5500 system using a fused-silica capillary of 47 cm (detection length 40 cm)× 75 μm I.D. Samples were loaded by 5 s of pressure injection and separated at 25°C using a constant current of 50 μA. The fencamfamine solution had a concentration of 50 μg/ml and was detected using diode array detection (DAD) within 190 to 300 nm.

In order to optimize the separation conditions, different KH_2PO_4 buffers were tested between pH 3 and pH 7.5 with buffer concentrations of 0.025 *M* and 0.05 *M*. The β-CD and γ-CD- concentrations were varied in a range from 0.0015 to 0.018 *M*, the Diac-β-CD concentration in a range from 0.003 to 0.012 *M* and the Diac-γ-CD concentration from 0.0015 to 0.012 *M*.

2.3. ^1H NMR spectroscopy

All experiments were performed on a Varian XL 300 FT NMR spectrometer operating at 299.956 MHz with a sample temperature of 30°C. A varying number of scans (depending on the experiment) with a frequency range of 2200 Hz were collected into 65 000 data points, giving a digital resolution of 0.33 Hz/point. An appropriate Gaussian function was applied before Fourier transformation to enhance the spectral resolution. The CD derivatives were dried throughout in vacuo over P_2O_5 before use. Seven solutions having molar ratios of 9:1, 8:2, 6.67:3.33, 5:5, 3.33:6.67, 2:8 and 1:9 (cyclodextrin:ligand) were prepared in deuterated 0.05 *M* phosphate buffer (composed of 0.05 *M* KH_2PO_4 in deuterated water, equivalent to pH 4.5) in order to measure the induced chemical shifts of the CD and fencamfamine signals. All chemical shifts were referenced to the H^2HO signal at 4.650 ppm.

3. Results and discussion

3.1. CE studies

In order to optimize the separation conditions for all four isomers of fencamfamine, six CD derivatives, α-, β- and γ-CD, Diac-β-CD, Diac-γ-CD and SBE-β-CD as well as a mixture of Diac-β-CD and γ-CD were studied at varying pH values (cf. Refs. [8–10]), buffer concentrations (cf. Refs. [10–12]) and CD concentrations (cf. Refs. [10,12–14]). Organic modifiers (cf. Refs. [15,16]) were not used

Table 1
Migration times and resolution values using 0.012 *M* Diac-β-CD; buffer: 0.05 *M* KH_2PO_4

	pH			
	3	4.5	6	7.5
t_1	10.704	11.025	11.050	13.250
t_2	11.075	11.413	11.329	13.483
t_3	11.713	12.079		
t_4	11.800	12.167	11.904	14.008
R_{s1}	2.72	2.72	2.08	1.53
R_{s2}	4.80	4.70		
R_{s3}	0.62	0.59	<0.30	<0.30

Table 3
Migration times and resolution values using 0.003 *M* β-CD; buffer: 0.05 *M* KH_2PO_4

	pH			
	3	4.5	6	7.5
t_1	14.88	9.69	9.33	5.66
t_2	15.12	9.80		
R_{s1}	0.9	0.6		

because in previous studies, neither methanol, acetonitrile nor tetrahydrofuran (THF) were found to be favorable. Alternately one of the aforementioned parameters was varied whilst keeping the other parameters constant.

3.1.1. Variation of the buffer concentration

At 0.05 *M* KH_2PO_4 (pH 4.5) buffer concentration, the electroosmotic flow (EOF) was decreased compared to 0.025 *M* buffer and, thus, produced considerable increases in migration times [1,4]. However, the four isomers of fencamfamine could be partially separated with either γ- or Diac-β-CD using 0.05 *M* buffer within about 14 min.

3.1.2. pH variation

Going stepwise from pH 7.5 to 3.0, the EOF and migration rate of the protonated amines show opposite trends (Tables 1–3). Whereas the EOF governed the migration rate of the complexes at pH 7.5, the migration rate at pH 3.0 is governed mainly by the electrophoretic mobility caused by the charge of the fully protonated fencamfamine (pK_a=8.7 [17]). For this reason, the resolution of the fencamfamine isomers remained almost unaffected especially in the lower range of the pH value. At higher pH, when fencamfamine is not fully charged, migration rate of the complex is increased due to the reduction in positive charge, but decreased due to the increase in EOF. Interestingly this produces an increase in migration times with Diac-β-CD and γ-CD but a decrease in migration times with β-CD. This indicates that fencamfamine interacts more strongly with β-CD than with the other cyclodextrins (see Section 3.1.3). At 0.05 *M* KH_2PO_4 and 0.012 *M* CD concentration, the isomers were partially separated with γ- and Diac-β-CD, but not with α- and β-CD, Diac-γ-CD and SBE-β-CD.

Table 2
Migration times and resolution values using 0.012 *M* γ-CD; buffer: 0.05 *M* KH_2PO_4

	pH			
	3	4.5	6	7.5
t_1	9.629	7.908	9.633	11.542
t_2	9.771	8.004	9.746	11.663
t_3	10.233	8.358	10.142	12.100
t_4	10.396	8.467	10.263	12.208
R_{s1}	1.01	0.60	0.44	0.74
R_{s2}	3.34	2.37	2.17	1.95
R_{s3}	1.32	1.00	0.97	0.41

3.1.3. Variation of the selector concentration

Usually, the resolution of enantiomers and isomers can be enhanced by optimization of the selector concentration [1,4]. Often, an optimum in resolution can be found at a certain CD concentration [12].

In the case of Diac-β-CD, Diac-γ-CD and γ-CD, the migration time increases substantially with increased selector concentrations. Diac-β-CD (3–12 m*M*) easily separated the two major isomers of fencamfamine (the two enantiomers of **1a**, Fig. 1) and one of the other two minor isomers (see **1b**, Fig. 1). However, the second minor isomer is only partially resolved at 12 m*M* Diac-β-CD, which is the solubility limit of Diac-β-CD (Fig. 2a). The results for γ-CD show a different pattern because, although the two major isomers are a closely eluting pair and the two minor isomers are another closely eluting pair, the major and minor pairs are well resolved

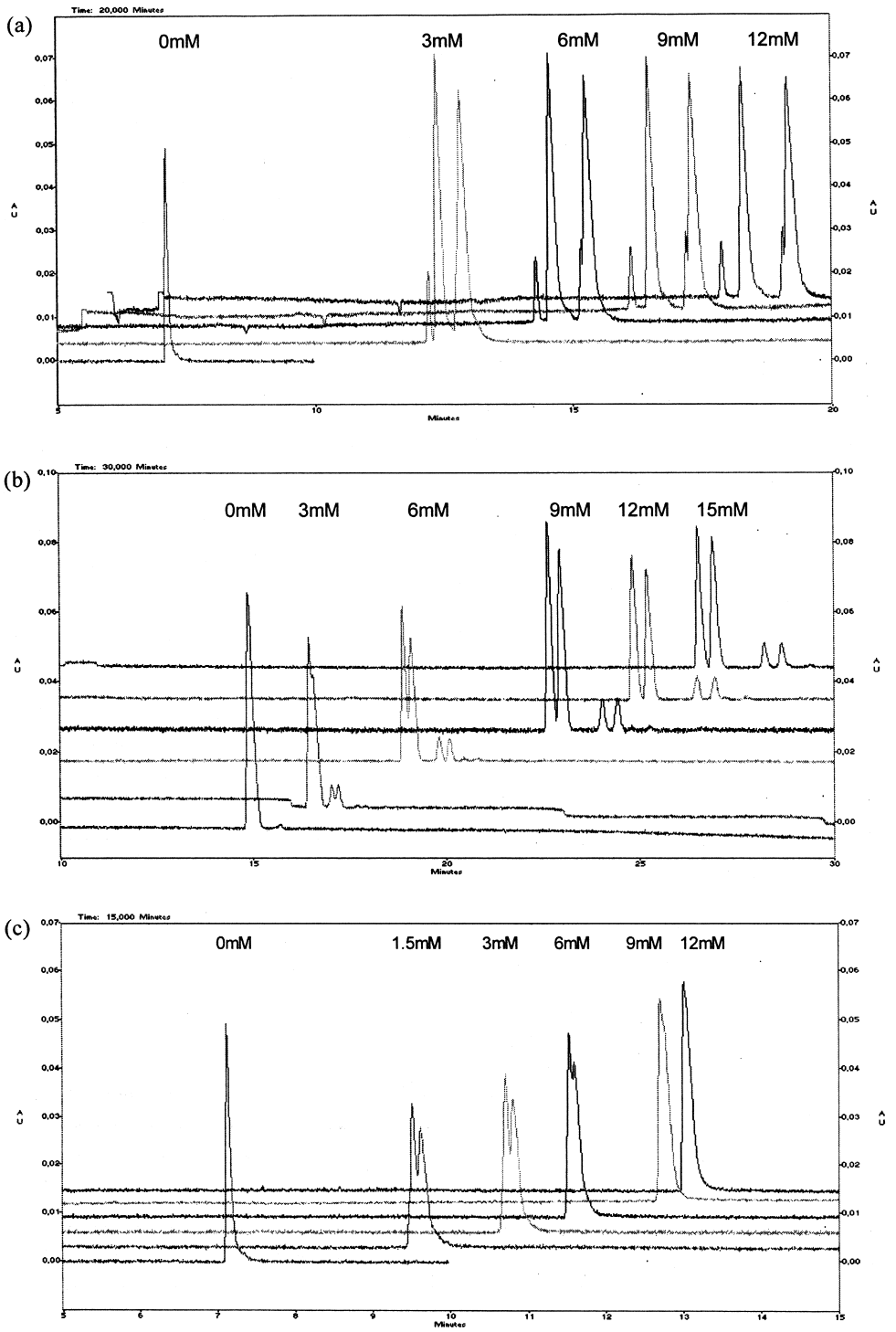


Fig. 2. Electropherograms of fencamfamine in presence of varying concentrations of (a) Diac-β-CD, (b) γ-CD and (c) β-CD added to the running buffer (0.05 *M* KH_2PO_4, pH 4.5).

from each other at γ-CD concentrations of 6 m*M* or higher. Excellent resolution of the four isomers was achieved at 12 and 18 m*M* (Fig. 2b). Since the resolution of the isomers was observed at relatively high CD concentrations and the migration time increased with increasing CD concentrations, it seems to be likely that more or less permanent complexes of different physicochemical properties, e.g., mobility, are formed. The difference in the migration order of the fencamfamine diastereomers upon complexation with Diac-β-CD and γ-CD is likely to be caused by a different complex geometry (see Section 3.2).

Since the enantiomers were clearly separated with Diac-β-CD, and the diastereomers with γ-CD, an attempt was made to enhance the separation of all isomers with a kind of hybrid CD containing the properties of the diacetylation of β-CD and γ-CD. The capability of the newly synthesized Diac-γ-CD to discriminate between all isomers was checked at pH 3.0 and 0.05 *M* buffer using selector concentrations in a range of 1.5 to 12 m*M*. At all concentrations, the enantiomers of the major diastereomer **1a** were clearly separated; at low selector concentrations, the enantiomers of the minor diastereomers **1b** were hidden under the peak of the enantiomer with the longer migration time. An increase in the Diac-γ-CD concentration resulted in a shift of the peak of one minor enantiomer with the second still being hidden under the peak of the enantiomer of the major diastereomer **1a** (Fig. 3). Thus, we obtained a mixture of the separation patterns of Diac-β-CD and γ-CD; the enantiomers of the major diastereomer were highly resolved ($R_s > 4$) whilst the enantiomers of the minor diastereomer were less separated ($R_s \approx 1.5$). Unfortunately, it was impossible to resolve the diastereomers completely.

In a similar approach, we prepared 1:3, 1:1 and 3:1 mixtures of Diac-β-CD:γ-CD with a total selector concentration of 12 m*M*. But none of the mixtures showed a better resolution than the single selectors (Fig. 4).

Interestingly, β-CD could discriminate between the isomers of fencamfamine only at a low con-

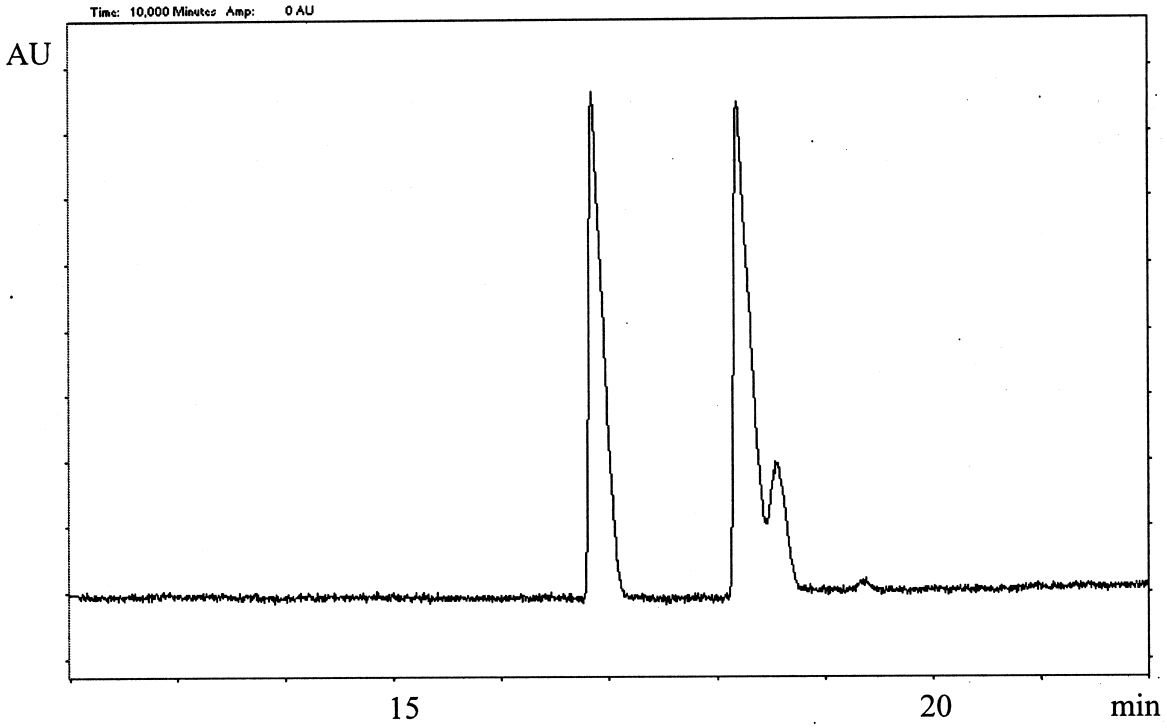


Fig. 3. Electropherogram of fencamfamine in presence of 0.003 m*M* Diac-γ-CD added to the running buffer (0.05 *M* KH_2PO_4, pH 4.5).

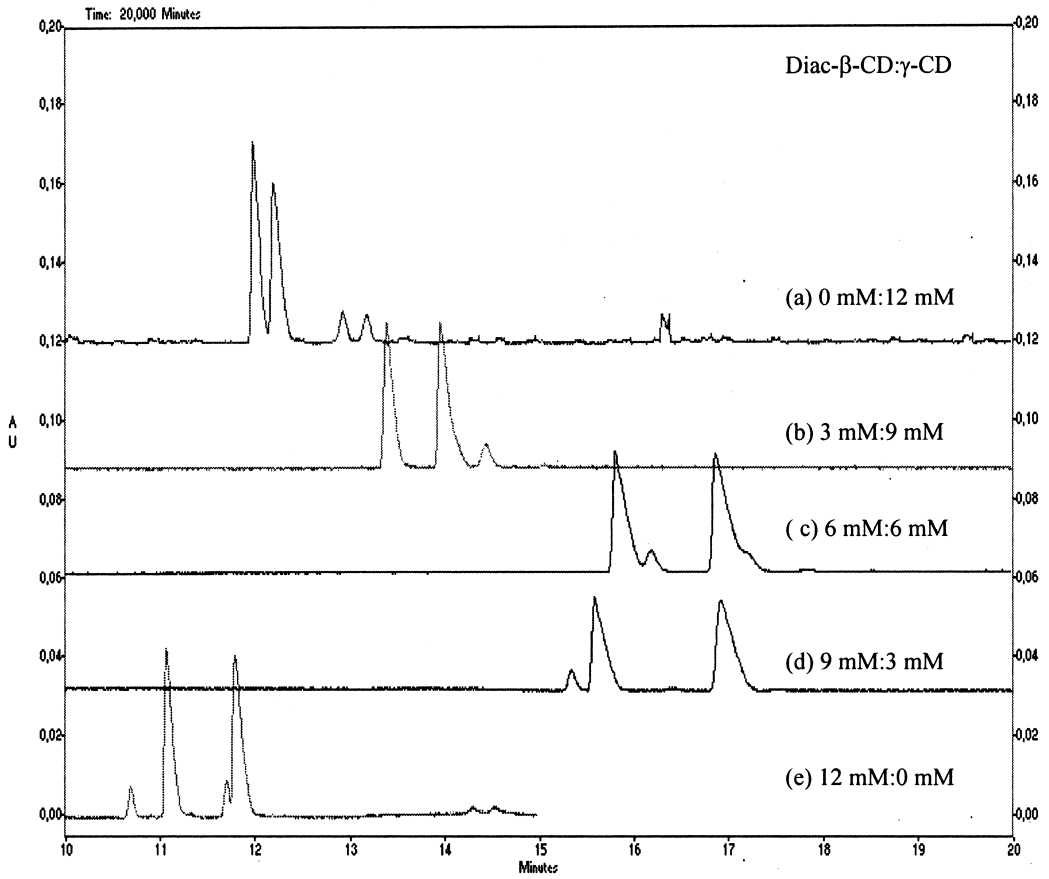


Fig. 4. Electropherograms of fencamfamine in presence of mixtures Diac-β-CD and γ-CD added to the running buffer (0.05 *M* KH_2PO_4, pH 4.5): (a) 12 m*M* Diac-β-CD, (b) 9 m*M* Diac-β-CD and 3 m*M* γ-CD, (c) 6 m*M* Diac-β-CD and 6 m*M* γ-CD, (d) 3 m*M* Diac-β-CD and 9 m*M* γ-CD and (e) 12 m*M* γ-CD.

centration of 3 m*M* β-CD (Fig. 2c). Lower and higher concentrations led to a lower resolution. A complete separation of the four isomers could not be achieved. The phenomenon of resolution at low CD concentration was recently explained by a different incorporation time of the isomers into the cyclodextrin. Vespalec et al. [18] and Wren et al. [12,19] additionally found the difference in complex mobility formed between the CD and the enantiomers to be dependent on the equilibrium constants K_1 and K_2 of either diastereomeric complex: $\Delta\mu = 1/(K_1K_2)^{1/2}$. According to this simple and rough model, it may be concluded that β-CD forms stronger complexes with fencamfamine than Diac-β-CD and γ-CD. This is additionally in good agreement with the observation of decreased migration time at high pH values (see above), and with previous investigations [3], in which the dissociation constants of phenethylamine–Diac-β-CD complexes were found to be too weak to be determinable whereas the constants for phenethylamine–β-CD complexes were in a range of 13 to 15 M^{-1}. This effect observed with β-CD suggests a rather different complex geometry occurring between fencamfamine with β-CD compared to either Diac-β-CD or γ-CD.

In conclusion, the optimized CE conditions for the discrimination of fencamfamine isomers can be summarized as follows: with Diac-β-CD and γ-CD, the best resolution can be achieved at pH 3, a buffer strength of 0.05 *M* and a CD concentration of 12 m*M*; with Diac-γ-CD, a mixture of the resolution pattern of Diac-β-CD and γ-CD was obtained; with

β-CD, the best result is only a slight resolution at similar pH and buffer strength, with β-CD concentration of 3 m*M*. The α-CD and SBE-β-CD showed no resolution, only a broadening of the peak, at either CD concentration. The ratio of diastereomers of the fencamfamine batch studied here amounted to 84:16 (%).

3.2. NMR studies

The NMR spectra were measured to find out the reason for the different pattern of separation with Diac-β-CD and γ-CD. Even though high enantioselectivity was observed in the NMR spectra, Diac-β-CD was found to form weak 1:1 complexes with the previously studied phenethylamines. The analysis of the variation of the chemical shifts of both the phenethylamine and the CD derivatives upon complexation clearly showed that the aromatic part of the phenethylamines was included in the CD cavity whereas the side chain was located at the wider rim of all cyclodextrins [2,3].

The NMR spectra were measured with each diastereomer of fencamfamine separately in different CD:ligand ratios: 9:1, 8:2, 6.67:3.33, 5:5, 3.33:6.67, 2:8 and 1:9. The analysis of the chemical shift variations ($\Delta\delta$ values) of both the CD selector and fencamfamine led to an indistinct picture in either case. In the case of the Diac-β-CD, considerable $\Delta\delta$ values can be found for the hydrogens of both partners of the complex (Table 4). Since it is known that weak complexes, typical of Diac-β-CD-ligand pairs [4], result in curved Job plots [1], the $\Delta\delta$ values of all CD hydrogens were evaluated. In Fig. 5a a maximum of complexation-induced chemical shifts (CICSs) at a molar refraction of 0.8 was found. Since the Job plot is not symmetrical, it is difficult to derive the stoichiometry of the Diac-β-CD–endo/exo-fencamfamine (**1a**) complex. Interestingly, the hydrogens H6 and H4 of the CD, which are located outside the cavity, are shifted upfield the most, whereas the $\Delta\delta$ values of the hydrogen inside the cavity, H3 and, with restrictions, H5 are shifted to a lesser extent (Table 4). Inspection of the $\Delta\delta$ values

Table 4
^{1}H NMR chemical shifts and $\Delta\delta$ values of Diac-β-CD–endo/exo-fencamfamine **1a** complex at varying ratios

		CD/ligand ratio													
	Buffer	(1:9)		(1:4)		(1:2)		(1:1)		(2:1)		(4:1)		(9:1)	
	δ	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
CD															
$CO\text{-}CH_3$	2.065	2.056	0.009	2.057	0.008	2.064	0.001	2.064	0.001	2.064	0.001	2.064	0.001	2.065	0.000
$CO\text{-}CH_3$	2.077	2.064	0.013	2.064	0.013	2.065	0.012	2.064	0.013	2.069	0.008	2.072	0.005	2.076	0.001
H6	3.862	3.769	0.093	3.721	0.141	3.769	0.093	3.783	0.079	3.799	0.063	3.807	0.055	3.861	0.001
H4	3.913	3.801	0.112	3.807	0.106	3.817	0.096	3.827	0.086	3.843	0.070	3.854	0.059	3.901	0.012
H5	*	3.936													
H2	4.843	4.836	0.007	4.836	0.007	4.837	0.006	4.839	0.004	4.84	0.003	4.836	0.007	4.842	0.001
H1	5.175	5.149	0.026	5.151	0.024	5.157	0.018	5.16	0.015	5.166	0.009	5.151	0.024	5.173	0.002
H3	5.264	5.235	0.029	5.237	0.027	5.245	0.019	5.249	0.015	5.260	0.004	5.252	0.012	5.267	−0.003
1a															
CH_3	1.126	1.134	−0.01	1.146	−0.020	1.178	−0.052	1.181	−0.055	1.189	−0.063	1.146	−0.020	1.194	−0.068
H7	1.392	1.406	−0.01	1.412	−0.020	*		1.417	−0.025	1.46	−0.068	1.412	−0.020	1.480	−0.088
H5	1.501	*		1.527	−0.026	1.552	−0.051	1.553	−0.052	1.588	−0.087	1.527	−0.026	1.578	−0.077
H6	1.664	*		1.68	−0.016	1.684	−0.020	1.695	−0.031	1.702	−0.038	1.680	−0.016	*	
H7	1.823	1.824	−0.001	1.829	−0.006	1.844	−0.021	1.844	−0.021	1.845	−0.022	1.829	−0.006	1.851	−0.028
H3	2.189	2.190	−0.001	2.197	−0.008	2.197	−0.008	*		*		2.197	−0.008	*	
H4	2.606	*		2.668	−0.062	2.673	−0.067	2.675	−0.069	*		*	*		
H1	2.667	*		2.708	−0.041	2.732	−0.065	2.772	−0.105	*		*	*		
$CH_2\text{-}N$	2.892	2.910	−0.02	2.925	−0.033	2.929	−0.037	2.939	−0.047	2.946	−0.054	2.940	−0.048	2.956	−0.064
H2	3.753	*		*		*		*		*		*	*		
Aromat	7.316	7.327	−0.01	7.332	−0.016	7.334	−0.018	7.347	−0.031	7.338	−0.022	7.332	−0.016	7.343	−0.027

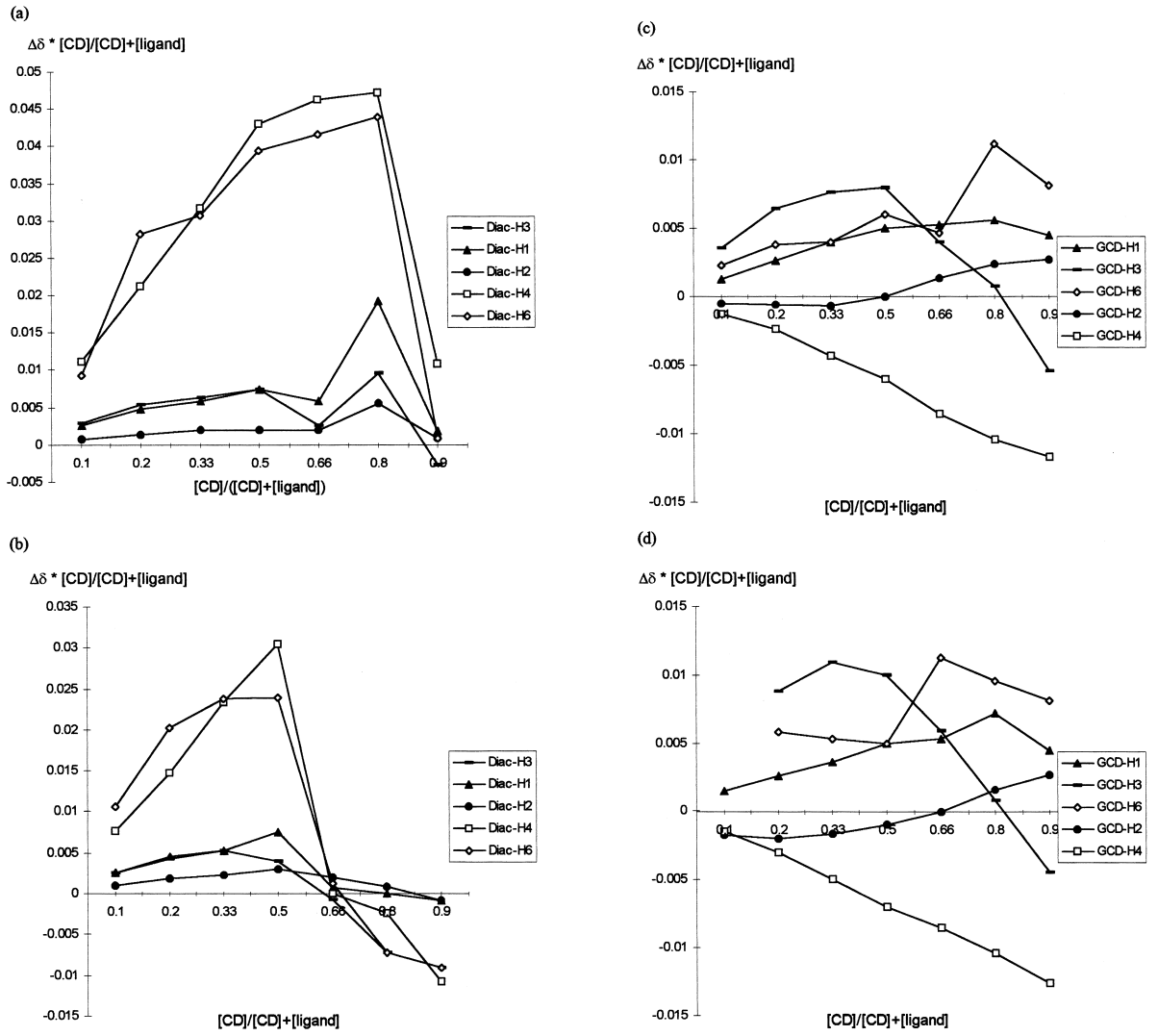


Fig. 5. Job plot of the interaction between Diac-β-CD and endo/exo-fencamfamine (a), or exo/endo-fencamfamine (b), γ-CD and endo/exo-fencamfamine (c) or exo/endo-fencamfamine (d); *y*-axis=$\Delta\delta$ values of the various CD hydrogens, *x*-axis=mol. refraction.

of the fencamfamine hydrogens shows that the less substituted cyclopentane ring of the norbornane moiety and the N-ethyl group are especially involved in the complexation although the aromatic signals are also shifted somewhat. In addition, only one of the two protons at H7 displays a great upfield shift on complexation. It would thus appear from these results that a mixture of complexes, with the ligand either inside or outside the cavity, are formed, the latter predominating.

Similar but even more confusing are the $\Delta\delta$ values found for the exo/endo-fencamfamine (**1b**) complex with Diac-β-CD (Fig. 5b, Table 5): again, the cyclodextrin hydrogens located outside the cavity are the most shifted upfield, with the H6 signal being shifted more than H2. The stoichiometry cannot be derived. The $\Delta\delta$ values of the hydrogens of exo/endo-fencamfamine molecule **1b** are rather small but clearly indicate that, in this complex, the N-ethyl group and the aromatic ring and, to a certain extent,

Table 5
^{1}H NMR chemical shifts and $\Delta\delta$ values of Diac-β-CD–exo/endo-fencamfamine **1b** complex at varying ratios

		CD/ligand ratio													
	Buffer	(1:9)		(1:4)		(1:2)		(1:1)		(2:1)		(4:1)		(9:1)	
	δ	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
CD															
CO-CH_3	2.065	2.058	0.007	2.06	0.005	2.061	0.004	2.063	0.002	2.064	0.001	2.065	0.000	2.065	0.000
CO-CH_3	2.077	2.06	0.017	2.06	0.017	2.061	0.016	2.069	0.008	2.076	0.001	2.078	−0.001	2.079	−0.002
H6	3.862	3.756	0.106	3.761	0.101	3.790	0.072	3.814	0.048	3.860	0.002	3.871	−0.009	3.822	0.040
H4	3.913	3.837	0.076	3.839	0.074	3.842	0.071	3.852	0.061	3.913	0.000	3.916	−0.003	3.925	−0.012
H5	*	3.919	−3.919	3.919	−3.919	3.918	−3.918	3.903	−3.903	*		*	*		
H2	4.843	4.833	0.010	4.834	0.009	4.836	0.007	4.837	0.006	4.840	0.003	4.842	0.001	4.844	−0.001
H1	5.175	5.150	0.025	5.152	0.023	5.159	0.016	5.160	0.015	5.174	0.001	5.175	0.000	5.176	−0.001
H3	5.264	5.239	0.025	5.243	0.021	5.248	0.016	5.256	0.008	5.265	−0.001	5.273	−0.009	5.274	−0.010
1b															
CH_3	1.115	1.123	−0.008	1.121	−0.006	1.143	−0.028	1.137	−0.022	1.141	−0.026	1.140	−0.025	1.154	−0.039
b	1.392	*		*		*		*		*		*	*		
c	1.501	*		*		*		*		*		*	*		
H7	1.531	1.514	0.017	1.516	0.015	1.538	−0.007	1.531	0.000	*		*	*		
H6	1.694	1.697	−0.003	1.700	−0.006	*		*		*		*	*		
H7	1.811	1.817	−0.006	1.817	−0.006	1.818	−0.007	1.824	−0.013	*		1.817	−0.006	*	
H4	2.443	2.449	−0.006	2.451	−0.008	2.448	−0.005	2.428	0.015	*		*	*		
H1	2.510	2.514	−0.004	2.519	−0.009	2.523	−0.013	2.535	−0.025	2.542	−0.032	*	*		
N-CH_2	2.844	2.892	−0.048	2.895	−0.051	2.913	−0.069	2.940	−0.096	*		*	*		
H3	3.168	3.194	−0.026	3.211	−0.043	*		*		*		*	*		
H2	3.408	3.437	−0.029	3.436	−0.028	*		*		*		*	*		
Aromat	7.328	7.330	−0.002	7.334	−0.006	7.329	−0.001	7.338	−0.010	7.346	−0.018	7.339	−0.011	7.254	0.074

the higher substituted cyclopentane of the norbornane are close to the CD, presumably near the outside of the cavity.

The Job plot of the endo/exo-fencamfamine (**1a**)–γ-CD complex is so indistinct that no stoichiometry can be derived (Fig. 5c). H4 located at the outside of the cavity shows consistent downfield shifts (Table 6) whereas H3 is shifted more strongly at low CD:ligand ratios. H6 moves further than H2, which is virtually unaffected, indicating interaction of the ligand with the narrow rim of the CD. The signals of the fencamfamine molecule do not change much upon complexation with γ-CD, indicating a very weak complex in which the hydrogens of the less substituted cyclopentane ring are involved (H1, H5 and H7 are downfield shifting the most). In contrast to the endo/exo-fencamfamine (**1a**)–Diac-β-CD complex, neither the N-ethyl group nor the phenyl ring of the corresponding γ-CD complex is influenced by complexation.

The Job plot of the exo/endo-fencamfamine (**1b**) complex with γ-CD utilizing the CD hydrogens does not give any information about the complex stoichiometry (see Fig. 5d); it is similar to the **1a**–γ-CD plot. Interestingly, the $\Delta\delta$ values of the fencamfamine hydrogens in this complex (Table 7) clearly indicate that the highly substituted cyclopentane ring and both substituents, the N-ethyl group and the phenyl ring are involved in the complexation.

No splitting of any signal was observed in either diastereoisomer, for either bornane or aromatic hydrogens, upon addition of either cyclodextrin derivative, thus isomers could not be discriminated by NMR in contrast with the CE method.

Taking the NMR investigations of each complex together, it can be stated firstly that only weak complexes were formed and secondly that fencamfamine forms a mixture of classical inclusion complexes [3,4], and complexes in which the ligand is bound externally. With Diac-β-CD and γ-CD, the endo/exo-fencamfamine **1a** seems to be located at the narrow rim. External attachment of a ligand to

Table 6
^{1}H NMR chemical shifts and $\Delta\delta$ values of γ-CD–endo/exo-fencamfamine **1a** complex at varying ratios

	Buffer	CD/ligand ratio (1:9)		(1:4)		(1:2)		(1:1)		(2:1)		(4:1)		(9:1)	
	δ	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
CD															
H4	3.502	3.515	−0.013	3.514	−0.012	3.515	−0.013	3.514	−0.012	3.515	−0.013	3.515	−0.013	3.515	−0.013
H2	3.588	3.593	−0.005	3.591	−0.003	3.590	−0.002	3.588	0.000	3.586	0.002	3.585	0.003	3.585	0.003
H5	3.776	*		*		*		*		*		*	*		
H6	3.805	3.782	0.023	3.786	0.019	3.793	0.012	3.793	0.012	3.798	0.007	3.791	0.014	3.796	0.009
H3	3.850	3.814	0.036	3.818	0.032	3.827	0.023	3.834	0.016	3.844	0.006	3.849	0.001	3.856	−0.006
H1	5.044	5.031	0.013	5.031	0.013	5.032	0.012	5.034	0.010	5.036	0.008	5.037	0.007	5.039	0.005
1a															
CH_3	1.126	1.128	−0.002	1.130	−0.004	1.132	−0.006	1.135	−0.009	1.139	−0.013	1.142	−0.016	1.146	−0.020
H7	1.392	1.394	−0.002	1.395	−0.003	1.399	−0.007	1.404	−0.012	1.412	−0.020	1.416	−0.024	1.416	−0.024
H5	1.501	1.506	−0.005	1.509	−0.008	1.512	−0.011	1.522	−0.021	*		*	*		
H6	1.664	1.668	−0.004	*		*		*		*		*	*		
e	1.823	1.821	0.002	1.820	0.003	1.816	0.007	1.819	0.004	1.822	0.001	1.822	0.001	1.824	−0.001
H3	2.189	2.186	0.003	2.181	0.008	2.179	0.010	2.177	0.012	2.183	0.006	2.181	0.008	2.188	0.001
H4	2.606	2.607	−0.001	2.607	−0.001	2.608	−0.002	2.613	−0.007	2.616	−0.010	2.617	−0.011	2.631	−0.025
H1	2.667	2.673	−0.006	2.674	−0.007	2.679	−0.012	2.686	−0.019	2.698	−0.031	2.697	−0.030	2.708	−0.041
CH_2-N	2.892	2.900	−0.008	2.898	−0.006	2.897	−0.005	2.896	−0.004	2.895	−0.003	2.895	−0.003	2.897	−0.005
H2	3.753	*		*		*		*		*		*	*		
Aromat	7.316	7.326	−0.010	7.324	−0.008	7.312	0.004	7.323	−0.007	7.312	0.004	7.315	0.001	*	

Table 7
^{1}H NMR chemical shifts and $\Delta\delta$ values of γ-CD–exo/endo-fencamfamine **1b** complex at varying ratios

	Buffer	CD/ligand ratio (9:1)		(1:4)		(1:2)		(1:1)		(2:1)		(4:1)		(9:1)	
	δ	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
CD															
H4	3.502	3.517	−0.015	3.517	−0.015	3.517	−0.015	3.516	−0.014	3.515	−0.013	3.515	−0.013	3.516	−0.014
H2	3.588	3.605	−0.017	3.598	−0.010	3.593	−0.005	3.590	−0.002	3.588	0.000	3.586	0.002	3.585	0.003
H5	3.776	*		*		*		*		*		*		*	
H6	3.805	*		3.776	0.029	3.789	0.016	3.795	0.010	3.788	0.017	3.793	0.012	3.796	0.009
H3	3.850	*		3.806	0.044	3.817	0.033	3.830	0.020	3.841	0.009	3.849	0.001	3.855	−0.005
H1	5.044	5.029	0.015	5.031	0.013	5.033	0.011	5.034	0.010	5.036	0.008	5.035	0.009	5.039	0.005
1b															
CH_3	1.115	1.116	−0.001	1.118	−0.003	1.125	−0.010	1.129	−0.014	1.135	−0.020	1.139	−0.024	1.142	−0.027
b	1.392	*		*		*		*		*		*		*	
c	1.501	*		*		*		*		*		*		*	
H7	1.531	1.512	0.019	1.513	0.018	1.519	0.012	1.524	0.007	1.534	−0.003	1.540	−0.009	1.546	−0.015
H6	1.694	1.696	−0.002	1.699	−0.005	*		*		*		*	*		
H7	1.811	1.816	−0.005	1.826	−0.015	1.841	−0.030	1.853	−0.042	1.862	−0.051	1.874	−0.063	*	
H4	2.443	2.438	0.005	2.435	0.008	2.420	0.023	2.417	0.026	2.414	0.029	2.417	0.026	2.422	0.021
H1	2.510	2.512	−0.002	2.517	−0.007	2.530	−0.020	2.537	−0.027	2.547	−0.037	2.556	−0.046	2.562	−0.052
N-CH_2	2.844	2.883	−0.039	2.879	−0.035	2.883	−0.039	2.868	−0.024	2.865	−0.021	2.883	−0.039	2.861	−0.017
H3	3.168	3.159	0.009	3.156	0.012	3.153	0.015	3.152	0.016	3.152	0.016	3.149	0.019	3.157	0.011
H2	3.408	3.404	0.004	3.401	0.007	3.398	0.010	3.396	0.012	3.396	0.012	3.397	0.011	3.398	0.010
Aromat	7.328	7.326	0.002	7.319	0.009	7.306	0.022	7.298	0.030	7.281	0.047	7.291	0.037	7.280	0.048

CDs has been recently reported by Owens et al. [20] for amlodipine with various CD derivatives. In contrast, Moyano et al. [21] observed an inclusion complex of gliclazide, which is also characterized by a corresponding rigid azabicyclic system, with β-CD.

Examination of the Job plots and the CICSs of the hydrogens of both the fencamfamine isomers and the CDs revealed the non-continuous increase with decreasing ligand/CD ratio. From this observation, it can be concluded that a multimodal complexation has taken place in either case. Similar observations were recently reported for the complexation of dimethindene with carboxymethyl-β-CD [22]. However, further investigations concerning the elucidation of the complex geometry are in progress.

4. Conclusions

In the present study, we have shown by means of CE that both Diac-β-CD and γ-CD are appropriate to discriminate between the four isomers of fencamfamine and, thus, can be used to determine the isomeric composition of the drug. Even though differences in the geometry of the diastereomeric complexes were found by NMR spectroscopic measurements, it was impossible to elucidate the exact mechanism of isomeric discrimination. It is likely that manifold weak complexes having different geometries cause the separation of the isomers in CE.

Acknowledgements

Thanks are due to Mrs. I. Knoblauch for measuring the NMR spectra and Dr. M. Neugebauer for providing fencamfamine. M.T. acknowledges a fellowship from the DAAD (ARC program).

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