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Enantioseparation of antiarrhythmic drugs propafenone and diprafenone, their metabolites and analogs by capillary electrophoresis

The enantioseparation of antiarrhythmic drugs propafenone (PF), diprafenone (DF), their metabolites and analogs was studied by capillary electrophoresis (CE). Several cyclodextrin-type chiral selectors, such as native α, β, and γ-cyclodextrin (CD), selectively and randomly substituted neutral and charged derivatives of β-CD were not suitable for the enantioseparation of PF. The acceptable simultaneous enantioseparation of PF and its major metabolites, 5-hydroxypropafenone (5-OH-PF) and *N*-despropyl-propafenone (*N*-Des-PF), was achieved with a designed mixture of randomly substituted carboxymethyl-β-CD (CM-β-CD) and sulfated β-CD (SU-β-CD). The method was also developed for the simultaneous enantioseparation of DF and its major metabolites, 5-hydroxydiprafenone (5-OH-DF) and *N*-desalkyldiprafenone (*N*-Des-DF) as well as other chiral compounds with a similar structure, such as *N*-ethylpropafenone and alprafenone.

Dedicated to Prof. H. Oelschläger on the occasion of his 80th birthday

1 Introduction

Propafenone (PF) with the chemical name 2′-(2-hydroxy-3-propylamino-propoxy)-3-phenylpropiophenone hydrochloride is a class Ic antiarrhythmic drug with some negative inotropic and β-adrenoceptor blocking activity. It is widely used for the management of supraventricular and ventricular arrhythmias [1]. PF is a chiral drug which has been in clinical use as the racemate since 1978. The enantiomers of PF do not significantly differ in their inotropic activity on the sodium channels [2−4] but the *S*-enantiomer exhibits a 50−100 times higher β-adrenoceptor blocking activity [2, 3, 5]. In addition, recent studies indicate stereoselective renal clearance [6−8], oxidative metabolism [6, 9, 10], and glucuronidation [11] of the PF enantiomers as well as significant interactions between the

enantiomers leading to non-linear pharmacokinetic and pharmacodynamic effects [12].

In order to follow the concentration of the parent drug and its two major metabolites, 5-hydroxy-propafenone (5-OH-PF) and *N*-despropyl-propafenone (*N*-Des-PF), in biological fluids an enantioselective method is required. Hollenhorst and Blaschke studied the simultaneous enantioseparation of PF, 5-OH-PF, and *N*-Des-PF by HPLC using various chiral stationary phases (CSP) [13]. The best simultaneous separation was achieved using a combination of two polysaccharide-type chiral columns, Chiralcel OD and Chiralpak AD. This method allowed the separation of all three compounds and baseline resolution of the enantiomers of PF and *N*-Des-PF. However, no enantioseparation was observed for 5-OH-PF. Recently, several indirect [6] and direct HPLC methods have been published with chiral mobile phase additives [14] or chiral stationary phases [15−20] for the simultaneous enantioseparation of PF and its metabolites. Only one of these studies involved the enantiomers of PF and both of its major phase I metabolites. However, no simultaneous enantioseparation of the enantiomers of PF, 5-OH-PF, and *N*-Des-PF was achieved [19]. Capillary electrophoresis (CE) represents an effective alternative to chromatographic methods for enantioseparation of chiral analytes [21−23]. Several unsuccessful attempts were made in CE in order to resolve the enantiomers of PF [24−28]. To the best of our

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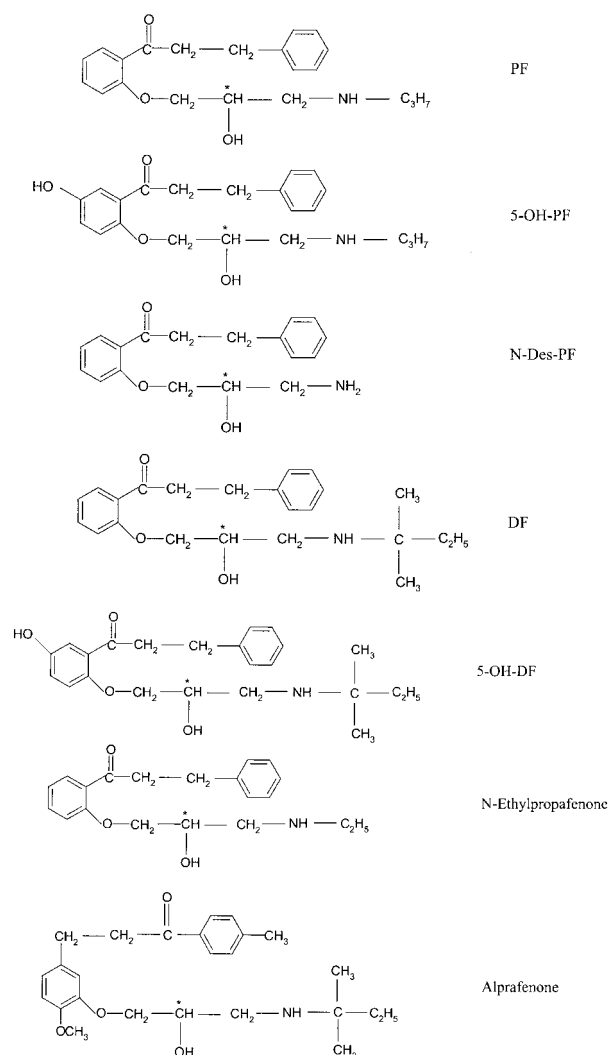
Figure 1. Structure of PF, 5-OH-PF, *N*-Des-PF, DF, 5-OH-DF, *N*-ethylpropafenone, and alprafenone.

knowledge no attempt was made in CE to resolve the enantiomers of 5-OH-PF and *N*-Des-PF as well as the antyarrhythmic drug DF [28–31], its major metabolites, 5-hydroxydiprafenone (5-OH-DF), *N*-desalkyl diprafenone (*N*-Des-DF) and compounds with a similar structure such as alprafenone and *N*-ethylpropafenone.

The goal of this study was to develop a method for the simultaneous enantioseparation of PF and its major phase 1 metabolites 5-OH-PF and Des-PF as well as for DF, 5-OH-DF, and *N*-Des-DF by CE (**Figure 1**).

2 Materials and methods

2.1 Chemicals and reagents

PF, 5-OH-PF, *N*-Des-PF, alprafenone, and *N*-ethylpropafenone were obtained from Knoll (Ludwigshafen, Ger-

many). The enantiomers of PF were obtained by diastereomeric crystallization with (−)-di-*O-O′-p*-tolyl tartaric acid as previously described [28]. DF, 5-OH-DF, and *N*-Des-DF were obtained from Helopharm KG (Berlin, Germany). α-, β-, γ-CD, carboxymethyl β-CD (CM-β-CD) with the averaged degree of substitution (DS) 3.5 was a gift from Wacker Chemie (Munich, Germany). Heptakis (2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) was from Sigma (Deisenhofen, Germany). Single isomer heptakis(6-sulfo)-β-CD (HS-β-CD) [32], heptakis(2,3-diacetyl-6-sulfo)-β-CD (HDAS-β-CD) [33], and heptakis(2,3-dimethyl-6-sulfo)-β-CD (HDMS-β-CD) [34], were kindly donated by Prof. Gy. Vigh from Texas A & M University (College Station, TX, USA). Randomly substituted sulfated β-CD (SU-β-CD, DS = 14) was from Aldrich Chemie (Steinheim, Germany) and randomly substituted sulfobutyl β-CDs with the DS 4 and 7 were from Cydex (Overland Park, KS, USA). Analytical grade triethanolamine and concentrated phosphoric acid were purchased from Merck (Darmstadt, Germany).

2.2 CE separations

CE separations were performed using Beckman P/ACE 5010 capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) equipped with a UV detector. The samples (0.1 mg/mL solutions) were injected by pressure, 0.5 p.s.i. A fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 31.2 cm total length, 21.0 cm effective length and 50 μm ID was used. The enantioseparations were performed in 100 mM triethanolamine phosphate buffer at pH 2.5. The detection was performed at 214 nm. Other experimental conditions are indicated in the legends to the figures.

3 Results and discussion

3.1 Enantioseparation of PF, its metabolites and analogs, DF and its metabolites with various CDs in CE

The enantioseparation of PF was studied with native α-, β-, and γ-CD, TM-β-CD and charged randomly substituted (SU-β-CD, CM-β-CD, SBE-β-CD) and single-isomer (HS-β-CD, HDAS-β-CD, HDMS-β-CD) β-CD derivatives. Triethanolamine phosphate buffer was used as it appeared to be advantageous for the separation of cationic analytes compared to alkali metal phosphates [35].

As it can be seen from the results summarized in **Table 1**, the enantiomers of PF can be resolved only with CD derivatives containing the sulfate and carboxymethyl groups such as randomly substituted SU-β-CD, SBE-β-CD, CM-β-CD, and single-isomer HS-β-CD. The most acceptable

Table 1. Enantioseparation of PF and DF and their metabolites and analogs in capillary electrophoresis with CDs as chiral selectors.

Analyte	CD	Conc. mg/ml	t_1, min	t_2, min	t_2/t_1
PF	achiral	–	8.0		
	β-CD	18	25.8	25.8	1.00
	α-CD	150	16.4	16.4	1.00
	γ-CD	150	31.8	31.8	1.00
	TM-β-CD	150	27.4	27.4	1.00
	HDAS-β-CD	0.2	9.0	9.0	1.00
		0.5	9.7	9.7	1.00
		1.0	11.1	11.1	1.00
	HDMS-β-CD	10	13.7	13.7	1.00
	HS-β-CD	0.5	14.8	15.0	1.02
		0.8	30.3	34.5	1.14
	CM-β-CD	2.5	51.0	52.1	1.02
	SU-β-CD	1.0	14.8	15.2	1.03
		1.5	16.8	17.7	1.05
	SBE-β-CD	1.5	17.0	17.7	1.03
5-OH-PF	SU-β-CD	1.5	17.9	22.8	1.27
N-Des-PF	SU-β-CD	1.5	19.5	19.7	1.01
	CM-β-CD	1.0	25.9	26.2	1.01
		2.0	41.7	42.8	1.02
		2.5	50.8	52.3	1.03
DF	SU-β-CD	1.0	22.4	22.7	1.02
		3.0	31.5	32.8	1.04
5-OH-DF	SU-β-CD	1.7	24.4	22.7	1.12
		3.0	29.3	37.5	1.28
N-Des-DF	SU-β-CD	3.5			
Alprafenone	SU-β-CD	1.5	17.1	17.8	1.04
N-Ethylpropafenone	SU-β-CD	1.5	13.1	13.3	1.02
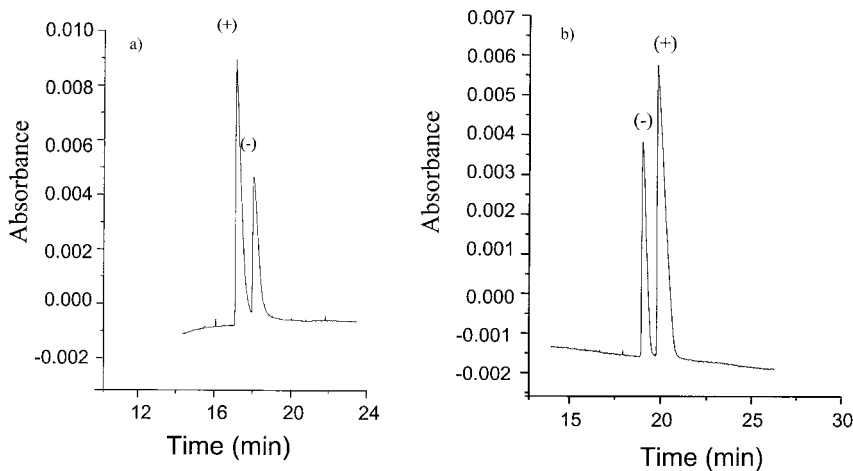
Figure 2. Enantioseparation of (±)-PF with 1.5 mg/mL SU-β-CD. The sample was PF as a free base in the case a) and PF as hydrochloride salt in the case b) spiked with a double excess of the dextrorotatory enantiomer in both cases. Separation capillary was as described in Materials and methods. Buffer: 100 mM triethanolamine phosphate adjusted to pH 2.5. UV detection was performed at 214 nm.

enantioseparation was observed with SU-β-CD in relatively low concentrations of 1.5–2.0 mg/mL.

The enantiomer migration order in the case of PF deserves especial care. This drug belongs to the rather few examples of chemical compounds for which the sign of optical rotation reverts when free base is transformed into the hydrochloride salt with preservation of the absolute configuration [36]. It is known that (+)-PF as a free base possesses the *S*-configuration and (−)-PF as a free base
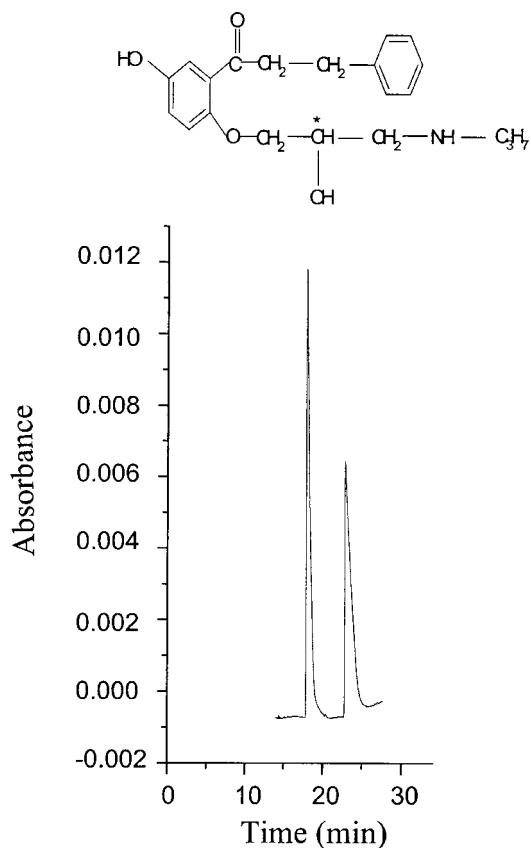
Figure 3. Enantioseparation of 5-OH-PF with 1.5 mg/mL SU-β-CD. Other conditions were the same as in the experiment shown in Figure 2.

Figure 4. Enantioseparation of *N*-Des-PF with 2.5 mg/mL CM-β-CD. Other conditions were the same as in the experiment shown in Figure 2.

the *R*-configuration. In the experiment shown in **Figure 2.a** the sample was the free base and it was spiked with the dextrorotatory enantiomer. The migration order is (+)- before (−)-enantiomer. In the experiment shown in **Figure 2.b**, PF as hydrochloride salt was used as the sample and was also spiked with the dextrorotatory enantiomer. The migration order was (−)- before (+)-enantiomer. Thus, in this particular case, the use of the incorrect descriptor (the sign of optical rotation) for the absolute configuration may lead to the erroneous "reversal" of the enantiomer migration order. The more tightly bound enantiomer of PF to SU-β-CD is R-PF in both cases shown in Figure 2.

The enantiomers of 5-OH-PF were successfully resolved with SU-β-CD (**Figure 3**). It was most difficult to achieve the enantioseparation of Des-PF. Many of the native and derivatized CDs did not allow an acceptable enantioseparation. However, a baseline resolution was achieved with CM-β-CD in relatively low concentrations (**Figure 4**). Unfortunately, CM-β-CD in the concentration range suitable for Des-PF did not result in the enantioseparation of the parent drug PF and its other metabolite, 5-OH-PF.
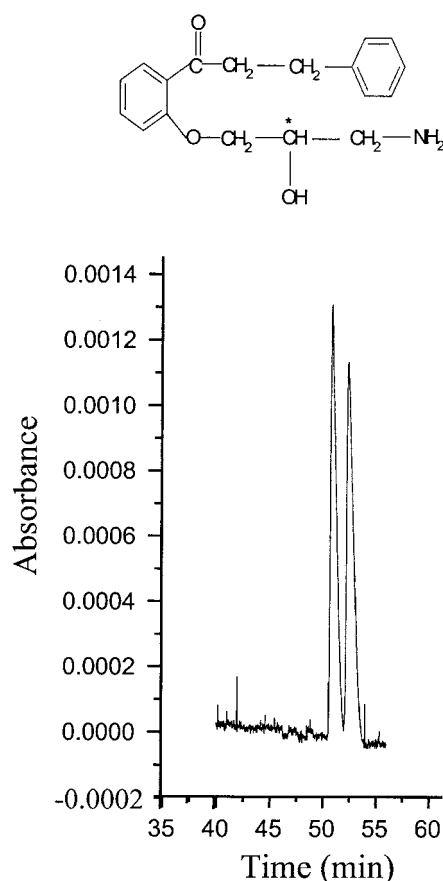
The enantiomers of alprafenone and *N*-ethylpropafenone were resolved with SU-β-CD at a concentration of 2 mg/mL (**Figure 5**).

An enantioseparation of DF was observed with SU-β-CD at a concentration of 3.0 mg/mL (**Figure 6.a**). The same CD derivative at a lower concentration of 1.5 mg/mL allowed the baseline enantioseparation of 5-OH-DF (**Figure 6.b**) and at higher concentration almost baseline enantioseparation of *N*-Des-DF. It must be noted that the *N*-desalkyl metabolites of both PF and DF possess the same structure.

3.2 Simultaneous enantioseparation of PF and its major metabolites

As shown in Table 1 no single CD could resolve PF and both of its major metabolites, 5-OH-PF and Des-PF with acceptable selectivity. A variation of the concentration of the most promising SU-β-CD as well as using the carrier-mode at relatively high concentrations of this CD did not result in satisfactory results. As the next optimization step,
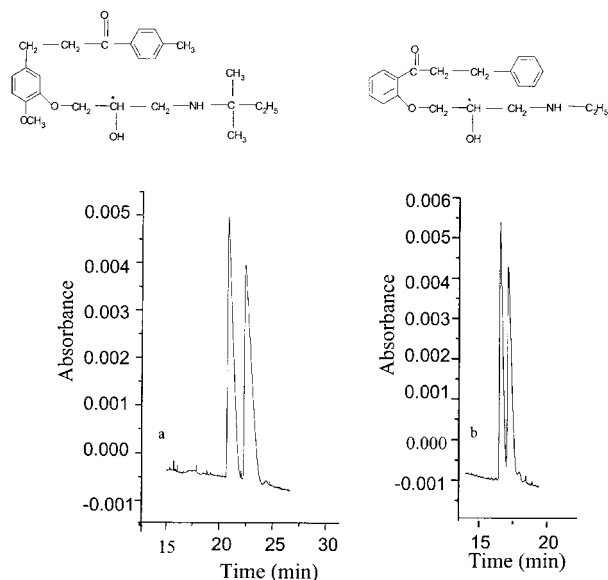
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Figure 5. Enantioseparation of alprafenone (a) and *N*-ethyl-propafenone (b) with 2 mg/mL SU-β-CD. Other conditions were the same as in the experiment shown in Figure 2.
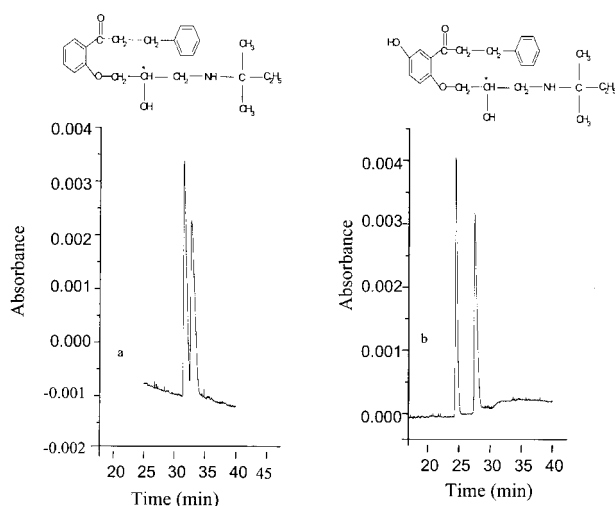

Figure 6. Enantioseparation of DF (a) with 3 mg/mL SU-β-CD and 5-OH-DF (b) with 1.7 mg/mL SU-β-CD, respectively. Other conditions were the same as in the experiment shown in Figure 2.

a mixture of SU-β-CD and CM-β-CD was applied as chiral selector. The ratio of the two chiral selectors was calculated based on the separation results when both of them were used as single chiral selector. The total concentration of both CDs of 7.9 mg/mL with the ratio SU-β-CD : CM-β-CD = 1:4 resulted in almost baseline separations of all components of the sample (**Figure 7**). The peaks are rather narrow and symmetrical although the analysis time is long. Despite the last disadvantage the method seems to be acceptable because currently no alternative method exists allowing the simultaneous enantiosepara-
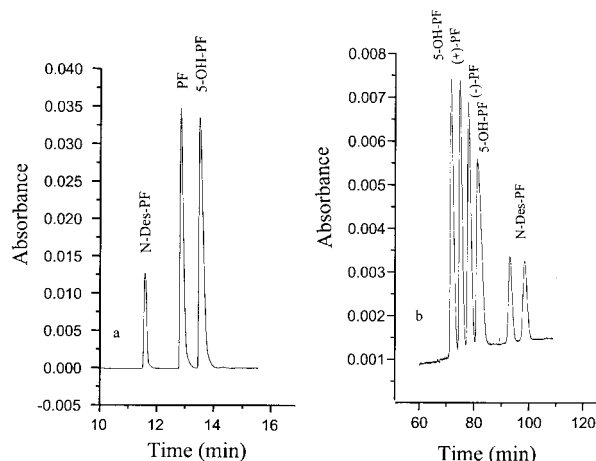

Figure 7. Separation of PF, 5-OH-PF and Des-PF (a) and their simultaneous enantioseparation (b) with a mixture of SU-β-CD and CM-β-CD (b). Total CD concentration was 7.9 mg/mL in the ratio SU-β-CD/CM-β-CD 1/4 (*w/w*). Other conditions were the same as in the experiment shown in Figure 2.

tion of all sample components shown in Figure 7. The potential advantage of the proposed method is that it can be extended to polar phase II metabolites (glucuronides) of PF and its phase I metabolites. Apparently, glucuronides play an important role in the overall pharmacological effect and the toxicology of PF [11].

The separation shown in Figure 7 was achieved with a complex array of chiral selectors. SU-β-CD and CM-β-CD both are mixtures of components with various degrees of substitution and positions of the substituents. Obviously, the mixture of these two CDs is even more complex. This aspect needs to be considered when separations like the present one are validated. Together with the aforementioned critical point this study clearly illustrates the high flexibility of CE. Thus, the coupling of two chiral columns was used in order to achieve a simultaneous enantioseparation of PF and its metabolites by Hollenhorst and Blaschke [13]. However, even this rather complex method does not allow the enantioseparation of 5-OH-PF. In addition, column coupling in HPLC is associated with certain technical problems, such as high back-pressure, increased dead volumes, etc.). Moreover, the ratio of the chiral selectors in HPLC is defined by the column composition and can not be changed. A combination of chiral selectors in CE is much easier, cost-effective, extremely flexible (any ratio of chiral selectors is allowed limited just by their solubility in buffer) and more effective. Thus, the method for the simultaneous enantioseparation of PF and its major metabolites was developed in a rather short time.

In the frame of this study a method was also developed for the simultaneous enantioseparation of the antiarrhythmic drug DF [28–31] and its major metabolites 5-OH-DF and
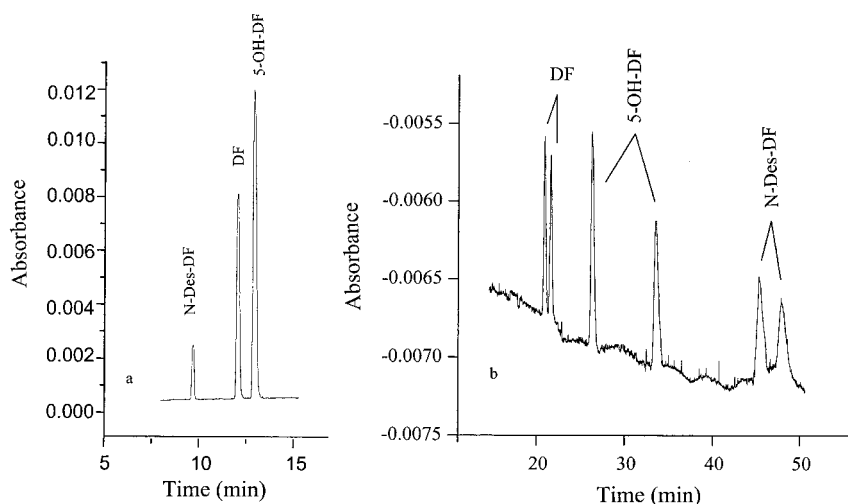
Figure 8. Separation of DF, 5-OH-DF and *N*-Des-DF (a) and their simultaneous enantioseparation (b) with 3.5 mg/mL SU-β-CD. Other conditions were the same as in the experiment shown in Figure 2.

N-Des-DF (**Figure 8**). This separation was possible with a single CD derivative, SU-β-CD. After the validation both methods described in this study may be applied for biomedical studies on the enantioselective biotransformation of the antyarrhythmic drugs PF and DF.

Acknowledgements

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