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Chiral separations of β-blocking drug substances using the Pirkle-type α-Burke 1 chiral stationary phase

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Abstract

The Pirkle-type α-Burke 1 chiral stationary phase (CSP) column has been evaluated for the enantiomeric separation of β-blocking drug substances and suitability for the determination of enantiomeric purity of these substances. Eighteen β-blockers currently on sale in Denmark were investigated. By varying the amount of alcohol modifier in the mobile phase, a total of fifteen of the eighteen investigated β-blockers were successfully separated by straight-phase HPLC within acceptable analysis times (i.e. below 20 min). The separation factors ranged between 1.07 and 1.41, and the resolutions were in no case less than 0.9. The possible use of the column for a direct determination of enantiomeric purity of the drug substances was also investigated. Validation studies, in which the enantiomers of propranolol were used, were performed in accordance with the ICH guideline, Validation of Analytical Procedures. The limit of detection for *R*-propranolol in *S*-propranolol was estimated to be ca. 0.1% and the limit of quantification ca. 0.3%.

Keywords: Enantiomer separation; Chiral stationary phases, LC; β-Blockers

1. Introduction

In Denmark eighteen β-blockers (Table 1) are presently on sale, of which only three are marketed as the pure, active *S*-enantiomer (timolol, bunolol and penbutolol). The remaining fifteen β-blockers are marketed as the racemic mixtures.

Similarly, many synthetic drugs today are marketed as racemic mixtures, despite the fact that enantiomers may show different pharmacodynamic and pharmacokinetic characteristics. The growing awareness of the importance of chirality in this context is likely to cause an increasing number of pure enantiomeric substances being marketed as drugs in the future.

In applications for marketing authorizations, the regulatory authorities require thorough documentation for the drug substance purity including chiral purity and stability [1–3]. This creates a growing demand for rapid, precise and sensitive chiral methods for the determination of enantiomeric purity of chiral drug substances at levels down to 0.1%.

There are three possible approaches to enantiomeric resolution by use of HPLC:

1. Derivatization of the analyte with a chiral reagent and separation of the resulting diastereomers by conventional achiral chromatography.
2. Addition of chiral additives to the mobile phase and the use of an achiral stationary phase.

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Table 1
β-Blocking drug substances investigated and their sources

Compound	Company
Acebutolol	Rhône-Poulenc (Essex, UK)
Alprenolol	Hässle (Mölndal, Sweden)
Atenolol	ICI (Cheshire, UK)
Atenolol	Benzon Pharma (Hvidovre, Denmark)
Betaxolol	MEDA (Herlev, Denmark)
Betaxolol	Searle (Mölndal, Sweden)
Bevantolol	Parke-Davis (Ann Arbor, MI, USA)
Bevantolol	Benzon Pharma (Hvidovre, Denmark)
Bisoprolol	Merck (Darmstadt, Germany)
Bunolol	Allergan (Irvine, CA, USA)
Carazolol	Upjohn (West Sussex, UK)
Carteolol	Ercopharm (Vedbaek, Denmark)
Metipranolol	Ciba-Geigy (Basle, Switzerland)
Metoprolol	Hässle (Mölndal, Sweden)
Oxprenolol	Ciba-Geigy (Basle, Switzerland)
Penbutolol	Hoechst (Frankfurt, Germany)
Pindolol	Durascan (Odense, Denmark)
Pindolol	Benzon Pharma (Hvidovre, Denmark)
Pindolol	NM Pharma (Sundbyberg, Sweden)
Pindolol	Dumex (Copenhagen, Denmark)
Propranolol	Sigma (St. Louis, MO, USA)
Sotalol	Bristol-Myers (Evansville, IN, USA)
Tertatolol	Servier (Orleans, France)
Timolol	Merck, Sharp and Dohme (Rahway, NJ, USA)

3. The use of chiral stationary phases.

Derivatization reactions and the use of chiral additives in the mobile phase are described in a comprehensive review article [4] which includes 218 references.

Quite successful investigations of separation and determination of enantiomeric purity of β-blockers have been performed previously in our laboratory, using both the derivatization technique [5,6] and different chiral stationary phases [7]. Separations performed by straight-phase HPLC [5] following derivatization with three different chiral derivatization agents showed that all eighteen β-blockers investigated (Table 1) were successfully separated by at least one of the three procedures. Reversed-phase HPLC [6] following derivatization with three different chiral derivatization agents proved almost as successful.

However, direct chromatographic enantioseparation by HPLC using chiral stationary phases has so far proven to be the most useful method for determination of enantiomeric purity. Using chiral stationary phases [7], three different commercially available columns were investigated: A modified cellulose phase (Chiralcel OD), an immobilised protein (AGP) and a β-cyclodextrin phase (Cyclobond I). The results for the Chiralcel OD column showed excellent separations of almost all eighteen β-blockers investigated, offering rugged methods and satisfactory low detection limits. The AGP column showed similar but less rugged results, while the Cyclobond I column showed poor separations.

The three above-mentioned references give thorough reviews and discussions of the existing literature on chiral separations of β-blockers.

The present investigation forms part of a series, in which various approaches towards the possibility of standardising HPLC-methods for testing the enantiomeric purity of β-blockers are evaluated. The purpose of the present work was to evaluate the Pirkle-type CSP, commercialised as α-Burke 1 column, for the enantiomeric separation power for β-blocking substances and suitability for the determination of the enantiomeric purity of these substances.

The α-Burke 1 CSP column material is a brush-type CSP derived from dimethyl-N-3,5-dinitrobenzoyl-α-amino-2,2-dimethyl-4-pentylphosphonate covalently bound to 5 μm mercaptopropyl silica (Fig. 1). The CSP was developed by rational design following synthesis and evaluation of numerous CSPs, as reported previously by C.J. Welch [8], who also gives a historical review of the design of CSPs in the Pirkle laboratories and an overview of important principles in CSP design. The α-Burke 1 CSP is especially designed for the enantioseparation of underivatized β-blockers; the structure–chromatographic activity relationship for some β-blockers, which have been successful enantioseparated, have also been discussed [9].

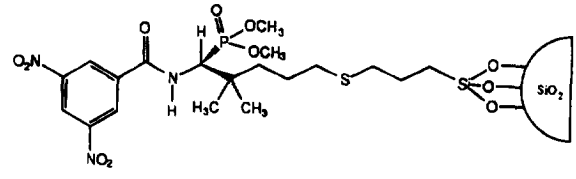

Fig. 1. Molecular structure of the Pirkle-type α-Burke 1 chiral stationary phase (CSP).

2. Experimental

2.1. Chemicals

Table 1 lists the compounds investigated and the companies from which they were obtained. Fig. 2 shows the molecular structures of the compounds.

The solvents dichloromethane and methanol and the reagent ammonium acetate were of analytical-reagent grade from Merck (Darmstadt, Germany). Ethanol was of pharmacopoeial grade.

2.2. Apparatus

The chromatographic system consisted of a Merck Hitachi L-6200A pump, a Merck Hitachi L-4250 detector, a Merck Hitachi AS-2000A autosampler and a Merck Hitachi L-5025 column thermostat.

Chromatograms were recorded on a Kipp and Zonen Model BD 8 recorder.

Data were collected on a Hewlett-Packard Model 3359A laboratory data system.

2.3. Chromatographic systems

Stationary phase: The Pirkle-type column was a (*R*)-α-Burke 1 column (5 μm, 25 cm×4.6 mm I.D.) from Regis (Illinois, USA).

Mobile phases: The eluent compositions were mixtures of dichloromethane and alcohol (ethanol or methanol) with the addition of ammonium acetate (0.5 g/l). The exact eluent compositions are given in Table 2.

Sample solutions: 1 mg/ml (as the free base) solutions were prepared by dissolving the compound in the alcohol component and diluting with dichloromethane (1:9).

Other chromatographic conditions were always as follows: The injected volume was 20 μl, the flow-rate was 2 ml/min, the detection wavelength was 230 nm and the experiments were performed at 25°C.

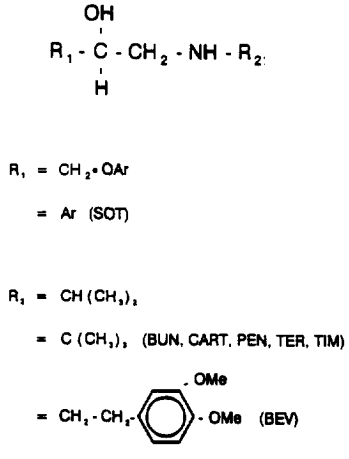

Fig. 2. Molecular structures of the β-blockers investigated.

3. Results and discussion

The manufacturer of the column suggests mixtures of dichloromethane–ethanol (19:1) with the addition of ammonium acetate 0.5 g/l or acetonitrile–ethanol (38:6) with ammonium acetate 0.02 g/l as the mobile phases for the separation of β-blockers. In the present work we used dichloromethane as the main eluent component. In order to decrease retention times to practical levels without damaging the enantiomeric separation of the β-blockers, we investigated the influence of the alcohol modifier in the mobile phase. Investigations of both the nature and the concentration of the alcohol modifier were performed in order to obtain acceptable times of analysis (i.e. below 20 min).

Previous work has shown that the temperature influences the chiral separations using the α-Burke 1 CSP, but that varying concentrations of ammonium acetate in the mobile phase does not drastically alter the enantioselectivity [9]. Thus, we performed all separations at 25°C with a concentration of ammonium acetate fixed at 0.5 g/l.

Table 2 summarises the chromatographic results giving capacity factors (k'), separation factors (α) and resolutions (R_S) as obtained from the separations of the eighteen β-blockers.

In the first instance a mobile phase consisting of dichloromethane–ethanol (19:1) with ammonium acetate 0.5 g/l was selected and, with this mobile phase, nine of the β-blockers (ALP, BET, BIS, BUN, METI, METO, OXP, PRO and TER) were separated within acceptable retention times (below 20 min) exhibiting α-values of 1.10 to 1.41.

Table 2
Retention (capacity factors, k'), separation factors (α) and resolution (R_S) of eighteen β-blockers separated on the Pirkle-type α-Burke 1 CSP

Compound		Eluent	$k'(1)$	$k'(2)$	α	R_S
Acebutolol	ACE	B	12.38	13.23	1.07	0.9
Alprenolol	ALP	A	7.61	9.09	1.20	1.6
Atenolol	ATE	C	12.08	13.00	1.08	0.9
Betaxolol	BET	A	8.58	10.12	1.18	1.5
Bevantolol	BEV	*				
Bisoprolol	BIS	A	8.19	9.44	1.15	1.4
Bunolol	BUN	A	9.15	10.46	1.14	1.5
Carazolol	CARA	D	13.31	15.15	1.14	1.8
Carteolol	CART	C	9.15	10.23	1.12	1.4
Metipranolol	METI	A	8.78	10.45	1.19	1.8
Metoprolol	METO	A	9.68	11.18	1.15	1.8
Oxprenolol	OXP	A	8.46	9.31	1.10	1.2
Penbutolol	PEN	A**		11.24		
Pindolol	PIN	D	12.54	14.85	1.18	2.5
Propranolol	PRO	A	10.33	14.59	1.41	4.1
Sotalol	SOT	***				
Tertatolol	TER	A	13.49	16.98	1.26	2.7
Timolol	TIM	B	3.38	3.69	1.09	1.0

Eluent compositions:
A: dichloromethane–ethanol (95:5) with ammoniumacetate 0.5 g/l.
B: dichloromethane–methanol (95:5) with ammoniumacetate 0.5 g/l.
C: dichloromethane–methanol (90:10) with ammoniumacetate 0.5 g/l.
D: dichloromethane–methanol (85:15) with ammoniumacetate 0.5 g/l.
*Peak-splitting problems.
**The *S*-enantiomer elutes as a single peak; the *R*-enantiomer was not available.
***No or very poor separation with all mobile phases tested.

By changing the nature of the alcohol component from ethanol to methanol the retention was decreased, and another two of the β-blockers (ACE and TIM) were separated within the defined acceptable time of analysis, exhibiting α-values of 1.07 and 1.09.

By increasing the concentration of the methanol component from 5% to 10% or 15% methanol, the retention was further decreased, and another four of the β-blockers (ATE, CARA, CART and PIN) were separated within the defined acceptable time of analysis, exhibiting α-values of 1.08 to 1.18.

Fig. 3 shows typical chromatograms of the separation of racemic propranolol and pindolol using ethanol and methanol as the alcohol modifier in different concentrations.

From Table 2 it appears that the chromatography is influenced by both the nature and the concentration of the alcohol modifier. With increasing solvent strength and concentration of the alcohol modifier the retention in general is significantly decreased, whereas the resolution is less influenced, as shown in Fig. 4 using carteolol as the model substance. This provides possibilities for the optimization of the methods.

Table 2 also shows that the column is capable of separating fifteen of the eighteen investigated β-blockers with acceptable retention times (below 20 min), separation factors between 1.07 and 1.41 and resolutions of not less than 0.9.

Two of the investigated β-blockers (BEV and SOT) could not be enantioseparated. This may be due to differences in the chemical structure of these compounds compared with the other β-blockers. Sotalol does not have an oxygen atom connected to the aromatic group close to the chiral centre, and bevantolol has two aromatic groups which may cause steric hindrance, which is considered to be an important potential interaction mechanism between β-blockers and the CSP [9].

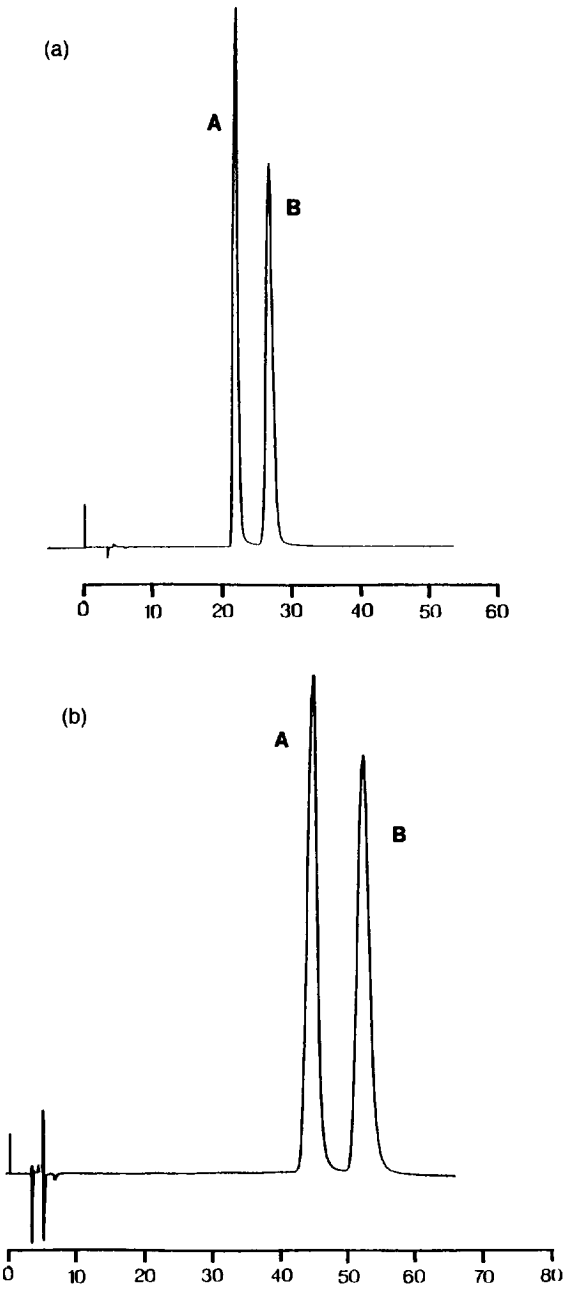

Fig. 3. (a) Chromatogram of racemic propranolol; injected volume: 20 μl; sample concentration: 1 mg/ml; detection wavelength: 230 nm; Peak A: *S*-propranolol; Peak B: *R*-propranolol. (b) Chromatogram of racemic pindolol; injected volume: 20 μl; sample concentration: 1 mg/ml; detection wavelength: 230 nm; Peak A: *S*-pindolol; Peak B: *R*-pindolol.

Penbutolol was available as the *S*-form only and eluted as a single peak, so no information can be given on the possible enantioseparation of racemic penbutolol.

The enantiomeric elution order was determined by chromatographing the individual enantiomers of all racemic compounds under similar conditions. Thus, the elution order was established as *S*–*R* for all the separated compounds, and no inversion of the elution order was observed when varying the eluent composition.

The possible use of the Pirkle-type α-Burke 1 CSP column for the determination of enantiomeric purity of drug substances was validated regarding specificity, reproducibility, accuracy, linearity, limit of detection, limit of quantification and ruggedness according to the ICH guideline, Validation of Analytical Procedures [10]. Propranolol was chosen as the model compound. Fig. 5 shows a chromatogram of (*S*)-propranolol with the addition of 1% of (*R*)-propranolol. The reproducibility at this level was calculated to be ca. 3.7% ($R.S.D._{n=6}$). The linearity of the detector response of (*R*)-propranolol added to the (*S*)-form was observed up to a content of 8%. The regression equation (area counts versus percentage added) was $y=41974x-3288$ ($r=0.999$), which indicates a small content of the (*R*)-enantiomer in the (*S*)-enantiomer. The limit of detection was estimated to be ca. 0.1% and the limit of quantification ca. 0.3%. Concerning stability, a sample solution of (*S*)-propranolol (1 mg/ml) proved stable for at least 1 week when stored at room temperature. No extra peaks appeared in the chromatogram and no detectable racemisation occurred.

The investigations of ruggedness showed that the systems should be equilibrated by eluting with the actual mobile phase for about 1 h before starting the analysis and about 15 min following a change in the mobile-phase composition. Minor changes in retention time may occur and can be explained by evaporation of primarily dichloromethane, thereby causing a slight increase in the polarity of the eluent.

Compared with the Chiralcel OD column investigated previously [7], the α-Burke 1 column turned out to be less effective regarding both resolution and reproducibility.

Work is in progress to investigate the impact of temperature changes on the chiral separations of

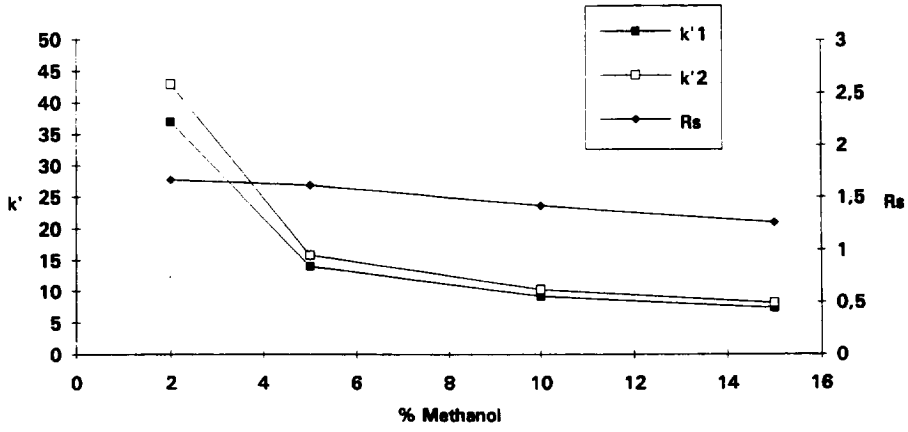

Fig. 4. Effect of increasing concentrations of methanol in the mobile phase on the retention (k') and resolution (R_S) of carteolol.

β-blockers with the α-Burke 1 CSP in order to achieve further optimizations.

4. Conclusions

The preferable approach to enantiomeric resolution of chiral substances by HPLC is the use of chiral stationary phases. CSPs offer the opportunity of rapid, precise and sensitive methods for direct enantioseparations of underivatized substances and the determination of the enantiomeric purity of the substances.

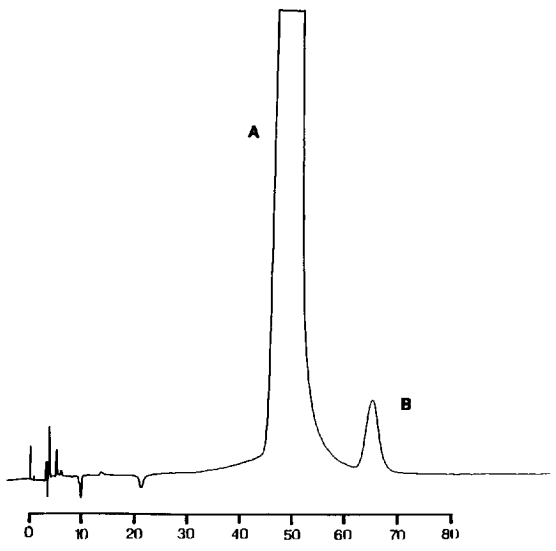

Fig. 5. Chromatogram of *S*-propranolol spiked with 1% *R*-propranolol; injected volume: 20 μl; sample concentration: 1 mg/ml; detection wavelength: 230 nm; Peak A: *S*-propranolol; Peak B: *R*-propranolol.

In the present work the Pirkle-type CSP, commercialised as the α-Burke 1 column, has been evaluated for the enantiomeric separation of β-blocking drug substances and suitability for the determination of the enantiomeric purity of these substances. By using a mobile phase containing dichloromethane as the main eluent component, ethanol or methanol as the modifier and ammonium acetate (0.5 g/l), the influence of the nature and the concentration of the alcohol modifier were investigated in order to obtain acceptable times of analysis (i.e. below 20 min). Ethanol or methanol were used in concentrations of 5%, 10% or 15%, and, thus, a total of fifteen of the eighteen investigated β-blockers were successfully separated within the defined acceptable time of analysis. The separation factors were found to range between 1.07 and 1.41, and the resolutions were not less than 0.9 (Table 2). The unsuccessful enantioseparations of bevantolol and sotalol may be due to differences in the chemical structure of these compounds compared to the other β-blockers. Penbutolol was available as the *S*-form only and eluted as a single peak.

It was shown that increasing solvent strength and concentration of the alcohol modifier in general decreases the retention significantly, while using carteolol as the model substance the resolution is less influenced (Fig. 4).

The elution order was identified as *S*–*R* for all the

compounds that were separated, and no inversion of the elution order was observed when changing the eluent composition.

The possible use of the column for the determination of the optical purity of drug substances was investigated and validated according to the ICH guideline, Validation of Analytical Procedures, using propranolol as the investigated compound (*S*-propranolol with the addition of 1% of *R*-propranolol). The limit of detection was estimated to be ca. 0.1%, and the limit of quantification ca. 0.3%.

The Pirkle-type α-Burke 1 column is concluded to be a suitable column for the enantiomeric separation of a wide range of β-blocking substances and the determination of the enantiomeric purity of these substances, but less effective compared to the Chiralcel OD column investigated previously [7].

Acknowledgments

We wish to thank the companies listed in Table 1 for donating the investigated β-blockers and their enantiomers.

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