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High-performance liquid chromatographic method for direct resolution of the indobufen enantiomeric components

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ABSTRACT

A high-performance liquid chromatographic method for the determination of (*S*)-(+)- and (*R*)-(−)-indobufen as diastereoisomeric derivatives has been applied and validated for stability control in tablet formulations. In order to obtain a less troublesome and more rapid method the direct resolution of the enantiomeric components by chiral stationary phases was investigated. The direct separation of the enantiomers was achieved using the cellulose tris(3,5-dimethylphenylcarbamate) derivative Chiralcel OD as a chiral stationary phase. The method was validated for the determination of the optical purity of (*S*)-(+)-indobufen as a quality control method for finished dosage forms.

INTRODUCTION

Indobufen, 2-[*p*-(1-oxo-2-isoindolinyl)phenyl]butyric acid (Fig. 1), is a racemic compound, active in the cardiovascular field as a platelet anti-aggregant and synthetized by Carlo Erba. Since the pharmaceutical activity is due only to the (*S*)-(+)-enantiomer, a new finished dosage form containing only the dextrorotatory isomer has been prepared in our laboratories. Consequently, studies were necessary for both the separation and identification of the two enantiomeric compounds, thus ensuring the optical purity of the new formulation.

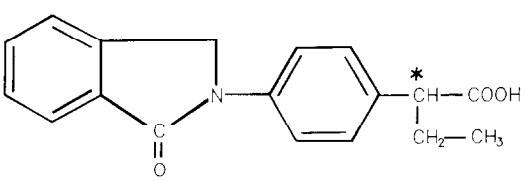

Fig. 1. Structural formula of indobufen.

A high-performance liquid chromatographic (HPLC) method for the determination and separation of (*S*)-(+)- and (*R*)-(−)-indobufen as diasteroisomers after derivatization by means of ethyl chloroformate and leucinamide [1] was validated and previously applied for stability control purposes. In order to obtain a more rapid method, the direct resolution of the enantiomeric components using chiral stationary phases was studied.

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In a first attempt, the separation mechanism of protein columns based on bioaffinity principles was investigated. An EnantioPac column was tested, varying the chromatographic conditions such as pH and organic modifier concentration. Subsequently, the capability of cyclodextrins of determining stereoselective inclusion complexes was tested by using β-cyclodextrins as chiral additives in the mobile phase. Finally, a new cellulose phase developed by Daicel (Tokyo, Japan) was tested for the separation of indobufen enantiomers, leading to a great improvement in peak resolution. The (*S*)-(+) and (*R*)-(−)-enantiomers were completely separated on cellulose tris(3,5-dimethylphenylcarbamate) stationary phase.

EXPERIMENTAL

Derivatization procedure

The method, reagents and chemicals were as reported elsewhere [1].

The derivatized samples were analysed using the following chromatographic conditions: column, Waters Assoc. μBondapak C_{18} (300 × 3.9 mm I.D.) (average particle size 10 μm); mobile phase, phosphate buffer (pH 6.4)–acetonitrile (60:40, v/v); mobile phase flow-rate, 1.5 ml/min; pump, Spectra-Physics Model SP 8770; detector, Spectra-Physics Model SP 8440; analytical wavelength, 275 ± 1 nm; and integrating recorder, Shimadzu Model C-R3A.

A typical chromatogram of (*S*)-(+)-indobufen leucinamide derivative containing (*R*)-(−)-indobufen as an impurity, obtained under the above analytical condi-

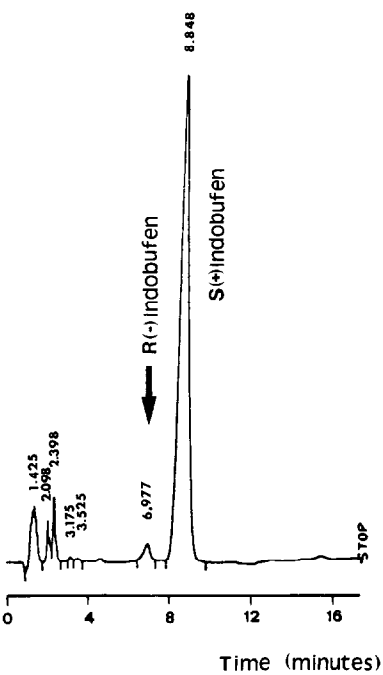

Fig. 2. Typical chromatogram of (*S*)-(+)- and (*R*)-(−)-indobufen after derivatization with ethyl chloroformate and leucinamide.

tions, is shown in Fig. 2. The capacity factors, k', determined for the (*R*)-(−)- and (*S*)-(+)-indobufen derivatives gave a separation factors $\alpha = k'_{(S)\text{-}(+)}/k'_{(R)\text{-}(-)} = 1.23$.

Chiral affinity stationary phase

An HPLC chiral stationary phase composed of α_1-acid glycoprotein bonded to diethylaminoethylsilica [2] (EnantioPac) was tested. The separation mechanism is based on principles of bioaffinity: it includes hydrophobic interactions, interactions of polar groups and steric effects. In order to reduce the retention time and to improve the stereoselectivity of the compounds under analysis, uncharged mobile additives were used. The retention can be considerably reduced by using monovalent alcohols such as 2-propanol.

Better results with regard to stereoselectivity were obtained using diols. Compared with alcohols, diols give low-energy interactions with proteins and can be used at relatively high concentrations without a risk of protein denaturation. The chromatographic conditions were as follows: column, LKB EnantioPac (100 × 4.0 mm I.D.) (average particle size 10 μm); mobile phase, 0.02 *M* phosphate buffer (pH 7.0) con-

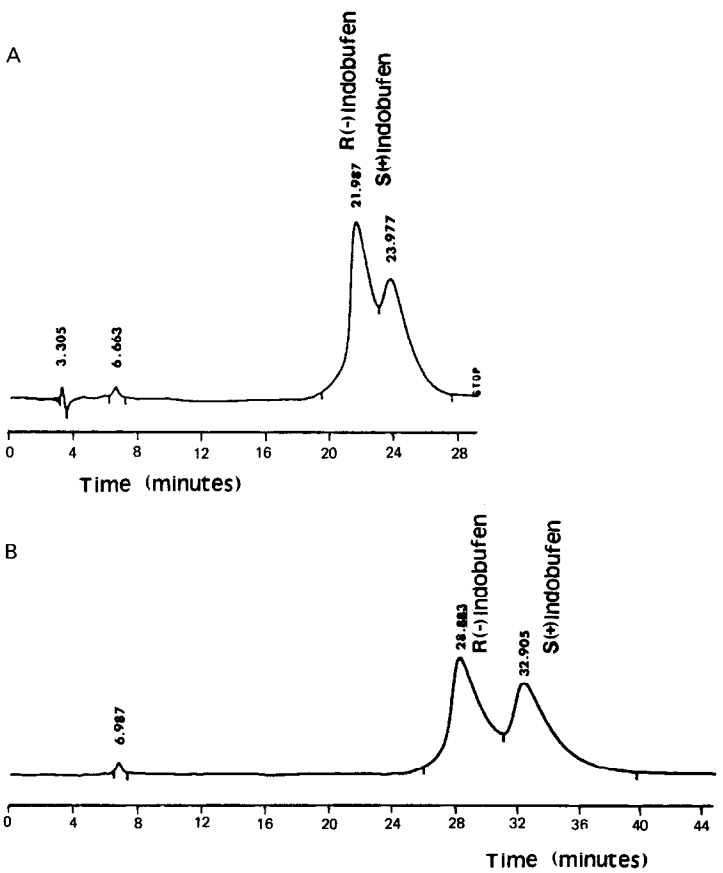

Fig. 3. Chromatograms obtained with EnantioPac column and mobile phase containing propylene glycol at concentrations of (A) 0.50 and (B) 0.25 *M*.

taining 0.50 *M* propylene glycol and 0.1 *M* sodium chloride; mobile phase flow-rate, 0.3 ml/min; pump, Spectra-Physics Model SP 8770; detector, Spectra-Physics Model SP 8440; analytical wavelength, 282 ± 1 nm; and integrating recorder, Shimadzu Model C-R3A.

The chromatograms in Fig. 3A and B were obtained using 0.50 and 0.25 *M* propylene glycol, respectively.

HPLC chiral separation by adding cyclodextrins to the mobile phase

Owing to the very strong adsorption of methylated β-cyclodextrins on hydrophobic stationary phases, a chiral stationary phase is generated dynamically by adding dimethyl-β-cyclodextyrin to the mobile phase [3,4]. Fig. 4 shows a chromatogram of (*R,S*)-indobufen obtained by using 0.025 *M* heptakis(2,6-di-O-methyl)-β-cyclodextrin. The chromatographic conditions were as follows: column, Waters Assoc. μBondapak C_{18} (300 × 3.9 mm I.D.) (Average particle size 10 μm); mobile phase, acetate buffer (pH 6.4)–acetonitrile (85:15, v/v) containing 0.025 *M* heptakis(2,6-di-O-methyl)-β-cyclodextrin; mobile phase flow-rate, 1.0 ml/min; pump, Spectra-Physics Model SP 8770; detector, Spectra-Physics Model SP 8440; analytical wavelength, 282 ± 1 nm; and integrating recorder, Shimadzu Model C-R3A.

The results were not satisfactory and attempts to improve the separating power of this kind of chiral system are in progress.

Direct resolution on cellulose derivative stationary phase

A cellulose stationary phase developed by Daicel was tested for the separation of indobufen enantiomers. The (*S*)-(+)- and (*R*)-(−)-enantiomers were effectively resolved on cellulose tris(3,5-dimethylphenylcarbamate) stationary phase available form Daicel as Chiralcel OD. Its chemical constitution is that of a linear poly-β-D-1,4-glucoside coated on silica gel. The optimization of the mobile phase on this column provided a very satisfactory HPLC method. A typical chromatogram determined

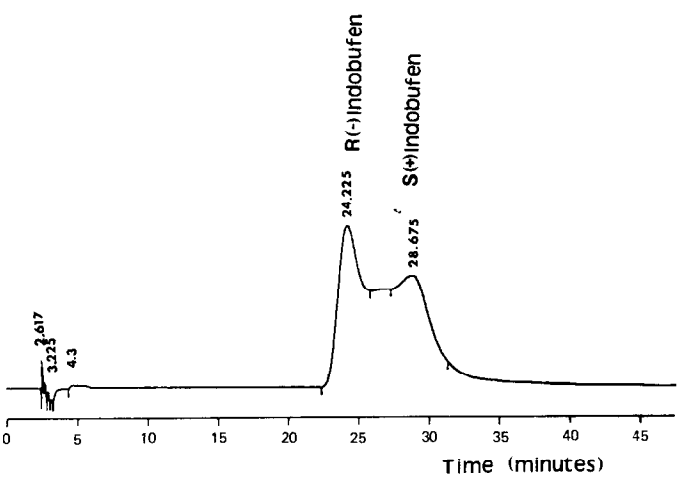

Fig. 4. Typical chromatogram for (*R,S*)-indobufen separation obtained by adding 0.025 *M* heptakis(2,6-di-O-methyl-β-cyclodextrin) to the mobile phase.

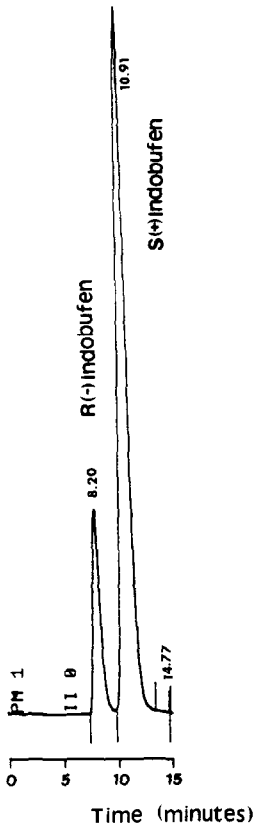

Fig. 5. Typical chromatogram of (*S*)-(+)- and (*R*)-(−)-indobufen separation obtained with the Chiralcel OD column.

under the following chromatographic conditions is shown in Fig. 5: column, Daicel Chiralcel OD (250 × 4.6 mm I.D.) (average particle size 10 μm); column temperature, ambient (22 ± 2°C); mobile phase, hexane–2-propanol–formic acid (160:40:1, v/v/v); mobile phase flow-rate, 1.5 ml/min; pump, Gilson Model 302; detector, Knauer Model 87 (Wissenschaftliche Geräte, Bad Homburg, F.R.G.); analytical wavelength, 270 ± 1 nm; and integrating recorder, Spectra-Physics Model SP 4270.

DISCUSSION

The HPLC method based on the Daicel column achieved the aim of developing a less time consuming and troublesome method than the derivatization procedure. The enhanced sensitivity and reproducibility are shown by the following method performances: linearity, $r = 0.9987$; precision, repeated determination on six different samples showed a relative standard deviation of 0.48%; sensitivity, the method proved to be sensitive to the injection on-column of about 2 ng of (*R*)-(−)-indobufen, corresponding to about 0.1% of the amount of (*S*)-(+)-indobufen usually analysed; the calculated k' values gave a separation factor $\alpha = 1.33$.

The HPLC method validated for the determination of the optical purity of (*S*)-(+)-indobufen has been shown to be suitable for application during stability trials for the shelflife determination and for quality control on the final dosage forms.

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