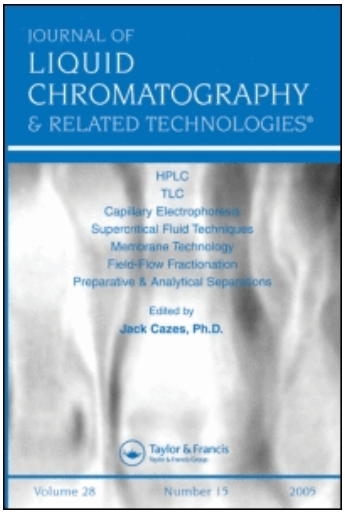

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DIRECT HPLC SEPARATION OF INDENOLOL ENANTIOMERS USING A CELLULOSE CHIRAL STATIONARY PHASE

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ABSTRACT

Racemic indenolol was resolved into its individual enantiomers by high performance liquid chromatography (HPLC) using a commercially available cellulose tris (3,5-dimethylphenyl carbamate) chiral stationary phase, known as Chiralcel OD. Indenolol contains two positional isomers, giving it a total of four enantiomers. The enantiomeric ratio was validated and peak identification for each enantiomer was established according to their optical rotation sign.

INTRODUCTION

Indenolol and other β-blockers are one drug class of many, whose enantiomers have been analyzed via chiral high performance liquid chromatography (HPLC).[1,2,3] The asymmetric pharmacological actions present in the corresponding enantiomers of a racemic drug have been well documented.[4] Research in chirality has been going on for quite some time but the area is still evolving as pharmaceutical companies and other research establishments channel their efforts into marketing therapeutically important

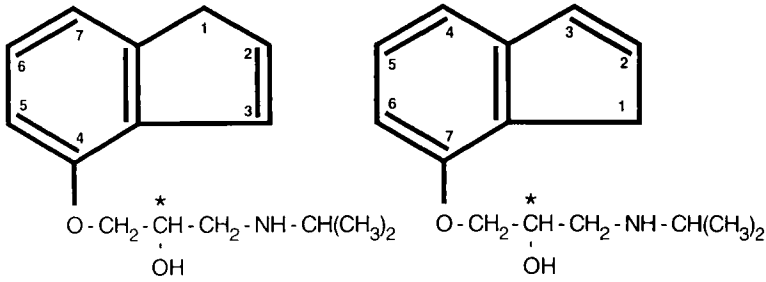

Figure 1. The tautomeric chemical structures of indenolol. (a) 4-indenyloxy and (b) 7-indenyloxy isomers. Asterisk denotes the position of the chiral carbon.

compounds as single enantiomers. Chiral chromatography can achieve two results with the successful separation of a racemate's enantiomers: (1) the determination of optical purity and (2) the obtention of the enantiomeric ratio present in the racemic mixture. The analysis also isolates the respective enantiomers which can then be further studied depending on the interests of the researcher.

This paper describes a direct, simple, HPLC gradient method for the enantiomeric separation of racemic indenolol, 1-(inden-4(or 7)-yloxy)-3-isopropylamino-2-propanol. Indenolol is a non-selective β-adrenoceptor antagonist that consists of two positional isomers, whose chemical structures are shown in Figure 1. The racemic drug exists as a tautomeric mixture of the 7- and 4- indenyloxy isomers in the ratio of 2:1. There are a total of four enantiomers and the method outlined later achieves baseline separation throughout most of the chromatographic run. The enantiomeric ratio was verified by determining the elution order (peak identification) and comparing the areas under each peak to establish the composition of the racemic mixture.

EXPERIMENTAL

Chemicals

Racemic indenolol hydrochloride (lot # PUP-A504) was a generous gift from Yamanouchi Pharmaceutical Corporation (Tokyo, Japan). HPLC grade organic solvents and reagent grade diethylamine were purchased from Fisher Scientific (Springfield, NJ, USA).

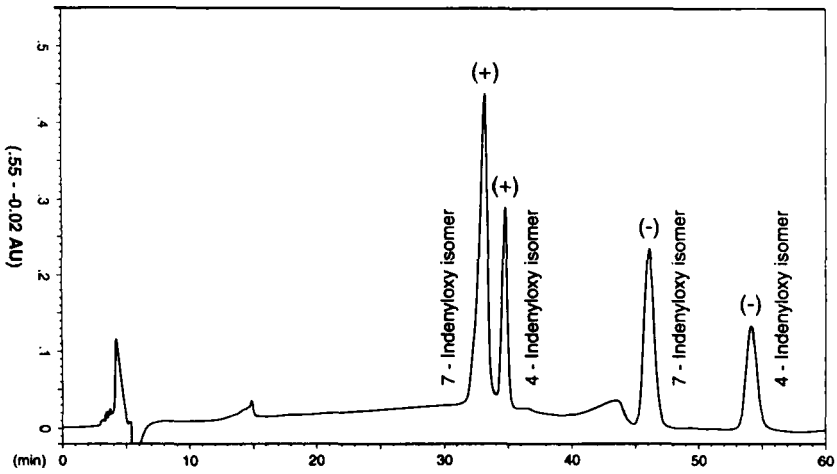

Figure 2. HPLC enantiomeric separation of racemic indenolol hydrochloride. Column: Chiralcel OD (25 cm x 4.6 mm id); Mobile Phase: Solvent A- hexane : ethanol : diethylamine, 99:1:0.2 (v/v/v); Solvent B- ethanol : diethylamine, 100:0.2 (v/v); Linear Gradient Profile: 0 min, 100% A; 20 min, 100% A; 60 min, 80% A, 20% B; Flow Rate: 1 mL/min; Initial Pressure: 198 PSI; Temperature: 23°C; Chart Speed: 0.5 cm/min; Detection: UV at 250 nm; Sample Quantity: 10 nmoles.

Method

A stock solution of indenolol hydrochloride was prepared in hexane:ethanol, 1:1 (v/v), containing 1.5% diethylamine, of which 20 mL was injected for analysis using a Waters (Milford, Massachussetts, USA) HPLC system comprising a 600E multisolvent delivery pump, a multivolume U6K injector, a 5200 printer/plotter and a 990+ photodiode array detector with a NEC Powermate 2 computer module. The cellulose tris (3,5-dimethylphenyl carbamate), Chiralcel OD column (lot # 50-20-30318, 25 cm x 4.6 mm i.d., 10 μm particle size, coated on silica gel) was purchased from J.T. Baker, Inc. (Phillipsburg, NJ, USA). The analysis was done using a linear gradient mode with a run time of 60 minutes. The mobile phases were solvent A-hexane: ethanol:diethylamine, 99:1:0.2 (v/v/v) and solvent B-ethanol:diethylamine, 100:0.2 (v/v). The gradient composition was 100% A at 0 time, 100% A at 20 minutes, and 80% A, 20% B at 60 minutes. The flow rate was held constant at 1 mL/min. The wavelength of detection was 250 nm. All other chromatographic conditions are described in Figure 2.

Peak identification was established using the Shodex OR-1 optical rotation detector (JM Sciences, NY, USA), with the same chromatographic conditions as described above. Capacity factors (k`) were calculated using the equation $k` = (V_r - V_o)/V_o$, where V_r is the elution volume and V_o is the void volume. The separation factor (α) was calculated using the equation $\alpha = k`_2 / k`_1$, where $k`_2$ and $k`_1$ are the capacity factors for the second and first eluted peaks.

RESULTS AND DISCUSSION

Published reports[5,6] have described HPLC analyses of β-blocking agents with more than one chiral center, such as racemic nadolol, and achieved the separation of its four enantiomers. However, prior to injecting the sample onto the chromatographic system, a derivatization step was necessary. The present method, described above, does not require derivatization and, once the compound is dissolved in the mobile phase, the sample is directly injected onto the column.

The enantiomeric separation of racemic indenolol is shown in Figure 2. At the time of writing this manuscript, the authors could not determine the absolute configuration of the individual enantiomers. Thus, the chromatographic peaks were identified according to their optical rotation sign (results not shown). Referring to Figure 2, the first 2 eluting enantiomers carry the (+)-dextrorotatory sign while the latter 2 are both (-)-levorotatory. Furthermore, the enantiomers can be grouped under the isomer which they belong to. The manufacturer states that the composition of racemic indenolol is a tautomeric mixture of the 7- and 4-indenyloxy isomers in the ratio of 2:1. Quantitation of the peaks in Figure 2 by obtaining the area under each peak via integration gave the following results: The ratio of the total percent area of the first (+) and third (-) eluting enantiomers over that of the second (+) and fourth (-) eluting enantiomers was 1.97. This confirms the manufacturer's composition of racemic indenolol and concludes that the first and third eluting peaks are the corresponding enantiomers for the 7-indenyloxy isomer, and the second and fourth eluting peaks represent the enantiomers for the 4-indenyloxy isomer.

The chromatographic parameters calculated for the indenolol enantiomers are summarized in Table 1. Both the 4- and 7-indenyloxy isomers have the (+)-dextrorotatory enantiomer eluting first (lower capacity factor). Each isomeric pair of enantiomers is well separated, indicated by the high α values. When one looks at the chromatographic resolution in Figure 2, for those enantiomers of the same optical rotation sign, the (-)-levorotatory enantiomers are well

Table 1

Chromatographic Parameters, Capacity (k`) and Separation (α) Factors for the Indenolol Enantiomers.

Isomer	k_1`	k_2`	α
4-indenyloxy	8.36 (+)	13.73 (-)	1.64
7-indenyloxy	7.93 (+)	11.50 (-)	1.45

separated, whereas, the (+)- dextrorotatory enantiomers have similar k` values (Table 1). The isocratic mode (results not shown) did not separate the four individual enantiomers. A gradual introduction of the organic solvent (ethanol) was necessary to separate the first two enantiomers, but resulted in longer retention times for the last two eluting peaks.

Less polar organic modifiers such as 2-propanol or branched chain alcohols may improve the resolution of the first two eluting peaks but they may also cause a much prolonged run time which would require reoptimization of the gradient profile. This may prove difficult to do since the run time in the above analysis is 55 minutes and various attempts to improve the resolution of the first two eluting peaks could be limited by this parameter.

CONCLUSION

In this paper, the isomeric ratio of the two positional isomers present in racemic indenolol was verified by chromatographing the individual enantiomers via HPLC using cellulose tris (3,5-dimethylphenyl carbamate) chiral stationary phase, Chiralcel OD. Gradient elution was necessary to separate all four enantiomers of indenolol and peak identification was established according to the optical rotation sign. Although the above analysis may not be suitable for laboratories that require quick results, the assay can be used by quality control laboratories and for those who are interested in pursuing research with chiral drugs.

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