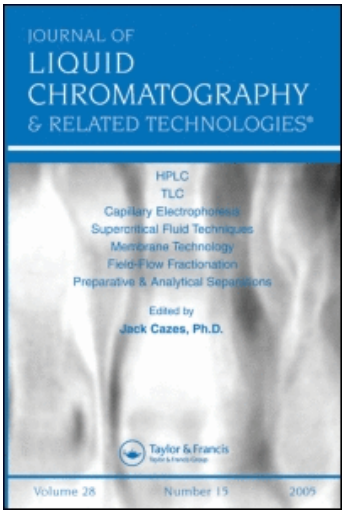

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Aboul-Enein, Hassan Y. and Abou-Basha, Laila I.(1996) 'HPLC Separation of Nadolol and Enantiomers on Chiralcel OD Column', Journal of Liquid Chromatography & Related Technologies, 19: 3, 383 — 392
To link to this Article: DOI: 10.1080/10826079608001222
URL: http://dx.doi.org/10.1080/10826079608001222

J. LIQ. CHROM. & REL. TECHNOL., 19(3), 383–392 (1996)

HPLC SEPARATION OF NADOLOL AND ENANTIOMERS ON CHIRALCEL OD COLUMN

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ABSTRACT

Nadolol (SQ 11725) is a β-adrenergic blocker which has two chiral centers, this allows for the presence of two racemates known as racemate A (SQ 12181) and racemate B (12182) and a total of four enantiomers. A simple isocratic HPLC method is developed for the separation of nadolol racemate A (SQ 12181) to its corresponding enantiomers RSR-nadolol (SQ 12148) and SRS-nadolol (SQ 12150) and racemate B (SQ 12182) to its corresponding enantiomers RRS-nadolol (SQ 12149) and SSR-nadolol (SQ 12151). Hexane with different percentage of ethanol containing 0.4% diethylamine is used as a mobile phase.

The effluents are monitored by ultraviolet detector set at 254 nm. The method showed good efficiency in identification, separation and optical purity determination of nadolol racemates and individual enantiomers in bulk materials and pharmaceutical formulations.

INTRODUCTION

The increased emphasis on research into enantiospecific drug action has been accompanied by increased activity in the field of chromatographic chiral separations. The β-adrenergic blocking agents have received particular attention as they are a pharmacologically important class of drug (cardiovascular drugs). They are used in treatment of angina pectoris, cardiac arrthymias and other heart conditions.

Nadolol 2, 3 cis-1, 2, 3, 4-tetrahydro-5 [2-hydroxy-3-(tert-butylamino) propoxy-2, 3 napthalenediol], is a long acting β-blocker used for the treatment of hypertension. Although nadolol possesses three chiral centres, the ring hydroxy groups at position 2 and 3 of tetrahydronaphthalene ring are in the cis configuration. In turn, there are only four enantiomers available since there are only two stereogenic centers. Nadolol (SQ 11725) is marketed and administered as a mixture of two racemic mixtures, racemate A (SQ 12181) and racemate B (SQ 12182). Racemate A (SQ 12181) consists of two enantiomers RSR (SQ 12148) and SRS (SQ 12150), while racemate B (SQ 12182) is composed of two enantiomers RRS (SQ 12149) and SSR (SQ 12151). The chemical structure of nadolol, its racemate and four available enantiomers are shown in Figure 1. Nadolol is stable to long exposure of normal levels of heat and light.[1] Several methods for the assay of nadolol in bulk material and pharmaceutical formulation based on colorimetry,[2] titration,[3] HPLC[1] and NMR[4] have been reported. Mutsutera et al.[5] described a method for the separation of nadolol diastereomers by reverse phase HPLC.

Recently, the enantiomers of several β-blockers are successfully separated using cellulose tris (3, 5-dimethylphenyl carbamate) known as Chiralcel OD as the chiral stationary phase (CSP).[6,7,8,9,10]

The aim of this study is to present a direct and simple separation of the four nadolol enantiomers on Chiralcel OD CSP under isocratic conditon for the purpose of identification and quality control of nadolol in bulk material.

EXPERIMENTAL

Chemicals

Nadolol (SQ 11725), the two racemates, racemate A (SQ 12181), racemate B (SQ 12182) and the four enantiomers (SQ 12148, 12150, 12149, 12151) are kindly supplied by Bristol-Myers, Squibb Pharmaceutical Research

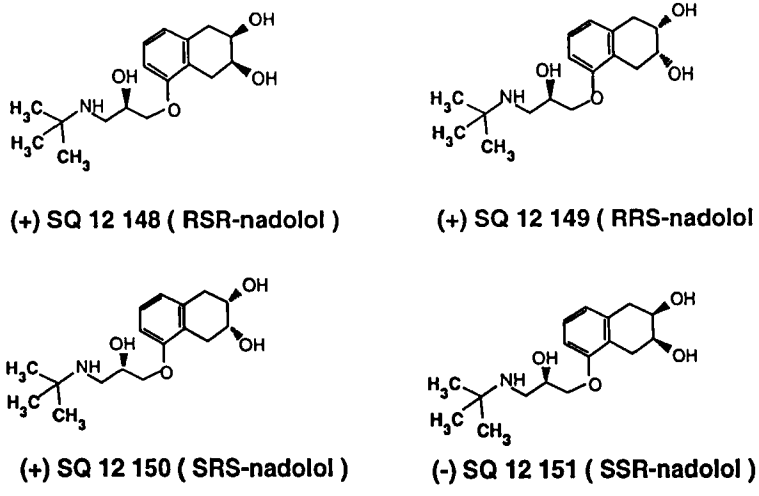

Figure 1. Chemical structure of nadolol enantiomers.

Institute (Princeton, NJ, USA). HPLC grade hexane was obtained from Fisher Scientific (Fairlawn, New Jersey, USA). Ethanol and diethylamine were purchased from BDH Chemicals (Poole, England).

Apparatus

The HPLC analysis is performed at room temperature with a Bio-Rad 1350 Solvent Delivery Pump, a Rheodyne Model 7125 Injector, a Waters Lambda Max 481 Variable Wavelength Detector set at 245 nm and a Hewlett-Packard 3394A Integrator. The cellulose tris (3,5-dimethylphenyl carbamate) Chiralcel OD column (25 cm x 4.6 i.d. coated on silica gel of 10 μm particle size) was obtained from Daicel Chemical Industries, Tokyo, Japan.

Chromatographic characteristics:

The separation factor (α), which represents a measure of relative peak separation is expressed as follows:

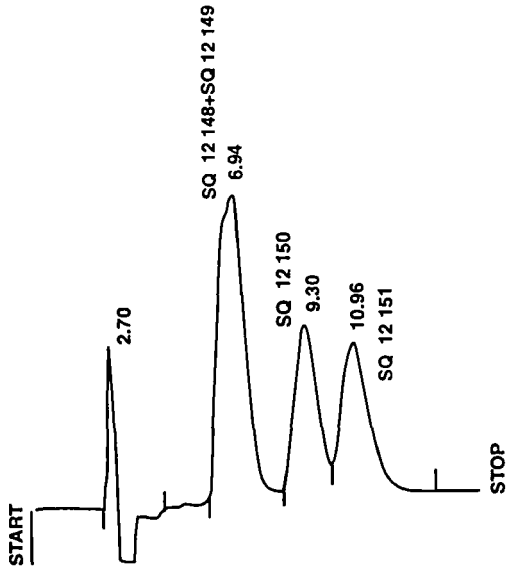

Figure 2. Chromatogram of nadolol SQ 11725. Column: Chiralcel OD (250 x 4.6 mm I.D.); mobile phase: hexane:ethanol:diethylamine (85:15:0.4); flow rate: 1 ml/min; chart speed: 0.5 m/min; temperature: 23°C; detector UV 254 nm; sensitivity; 0.01 aufs; sample quantity 10 nmol.

$\alpha = k_2'/k_1'$ where k_1' and k_2' are capacity factor for the first and second eluting enantiomers, respectively. The capacity factor (k′) is calculated as follows:

$k_1' = (t_{R1} - t_{R0})$ and $k_2' = (t_{R2} - t_{R0})$ where t_{R0}, t_{R1} and t_{R2} refer to the retention time in seconds for the solvent peak, the first and second eluting enantiomers, respectively.

The stereochemical resolution factor (Rs) is calculated by following formula:

$Rs = 2 (t_{R2} - t_{R1}) / (w_1 + w_2)$ where w_1 and w_2 are the peak width for the first and second eluting enantiomer peaks, respectively.

Determination of elution order

Peak identification for nadolol and its four enantiomers were established using Shodex OR-1 optical detector (JM Sciences, NY, USA) under the same chromatographic conditions described in the figures. It is of interest to mention that the peaks eluted with lower capacity factors were the dextroratatory (+) enantiomers followed by levorotatory (-) enantiomers (see Results and Discussions).

RESULTS AND DISCUSSION

Nadolol is a peculiar β-blocker in that it has three chiral centers, the 2-3 hydroxy groups are fixed in the cis configuration thus it possesses two chiral centers which allow a total of four enantiomers. Lee et al.[11] reported the direct separation of these four enantiomers using supercritical liquid chromatography under subcritical conditions with carbon dioxide as an eluent and were unable to resolve more than three enantiomers when using Chiralcel type or Pirkle type stationary phases. However, they obtained full resolution of all four enantiomers using an α_1 - acid glycoprotein.[11] Following a chiral derivatization of nadolol with 1-napthylisocyanate, a method has been developed for the separation of all four nadolol enantiomers on (R)-N-(3-5-dinitrobenzoyl)-L-leucine CSP.[12]

The method described by Lacroix et al.[13] can be used to determine total nadolol and related compounds and racemate composition of drug raw material. Numerous HPLC assays had been reported for determination of nadolol in biological fluid.[14,15,16,17] The Chiralcel OD-CSPs has been used for the resolution of several racemic β-adrenergic blockers that belong to the aryloxyaminopropane-2-ol class of compounds including propranolol[18] and others[6,20] into their corresponding enantiomers in normal phase mode. Nadolol is partially resolved into three peaks on Pirkle type[19] phases known as Chirex 3018 and 3022.[19]

In the method described Chiralcel OD column in the normal phase mode was used for separation of nadolol SQ 11725 which shows three separate peaks (Figure 2). These peaks were identified by the injection of individual enantiomers onto the HPLC system under the same chromatographic conditions. The two enantiomers SQ 12148 and SQ 12149 were overlapping in the first peak. We tried to optimize the separation of these overlapping peaks under isocratic condition but it was unsuccessful. However, the method can separate the enantiomers of the racemate A (SQ 12181), and the

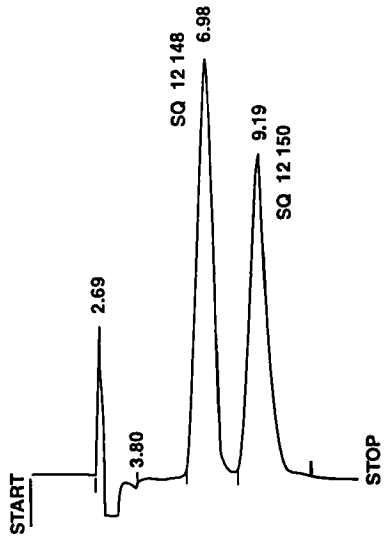

Figure 3. Chromatogram of nadolol SQ 12181. Column: Chiralcel OD (250 x 4.6 mm I.D.); mobile phase: hexane:ethanol:diethylamine (80:20:0.4);other chromatographic conditions are the same as in Figure 2.

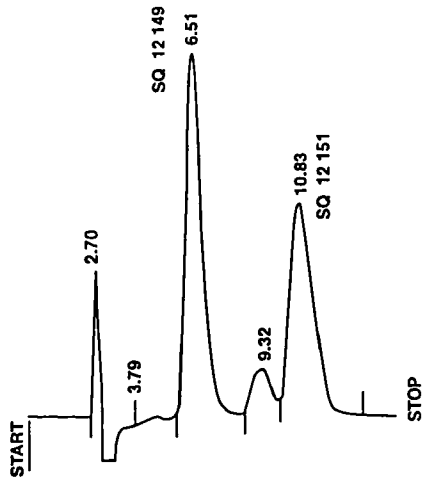

Figure 4. Chromatogram of nadolol SQ 12182. Column: Chiralcel OD (250 x 4.6 mm I.D.); mobile phase: hexane:ethanol:diethylamine (80:20:0.4); other chromatographic conditions are the same as in Figure 2.

enantiomers of racemate B (SQ 12182) (the chromatographic parameters were shown in Table 1) and verified by subsequent injection of the four individual enantiomers. It was found that SQ 12181 consists of 1:1 ratio of enantiomers SQ 12148 and SQ 12150 (Figure 3) while SQ 12182 consists of 1:1 ratio of enantiomers SQ 12149 and SQ 12151 (Figure 4). The mobile phase used contains 0.4 diethylamine to improve the peak sharpness, symmetry and to some extent the stereochemical resolution factor (Rs).[20]

Table 1

Chromatographic Parameters, Capacity (k′), Separation (α) and Resolution (Rs) Factors for the Nadolol Racemates.*

Nadolol Racemate	k'_1	k'_2	α	Rs
SQ12181 Racemate A	1.6 SQ 12148	2.42 SQ12150	1.5	2.6
SQ12182 Racemate B	1.4 SQ 12149	3.0 SQ 12151	2.14	5.4

* Mobile phase composition: Hexane:ethanol:diethylamine (80:20:0.4% v/v); see Figure 2 for other chromatographic conditions.

Shodex OR-1 optical detector was used to identify the optical rotation of nadolol enantiomers. It was found that the first two peaks are (+) dextrorotatory while the last peak is (-) levorotarory. The optical rotation was verified by injection of individual enantiomer under the same chromatographic condition (Table 2), enantiomer RSR nadolol (SQ 12148) and RRS nadolol (SQ 12149) which overlapped under the chromatographic condition used and represented the first peak both of these enantiomers are (+) dextrorotatory. The second (+) dextrorotatory peak belongs to SRS nadolol (SQ 12150) while the third peak is (-) levorotatory identified as SSR nadolol (SQ 12151). It is of interest to mention that there is no relationship between the sign of rotation and absolute configuration of the substance. Thus the method desribed can be used for the separation, identification and optical purity determination of nadolol racemates and enantiomers in the bulk material and pharmaceutical dosage forms.

Table 2

The Chromatographic Parameters; Capacity (k′) Separation (α) Factors and Optical Rotation of Nadolol enantiomers.

Nadolol Enantiomers	k′	α	Optical Rotation
SQ 12148	4.24	1.13	(+)
SQ 12149	3.73	0.87	(+)
SQ 12150	6.5	1.55	(+)
SQ 12151	8.25	1.25	(-)

Mobile phase composition: Hexane:ethanol:diethylamine (85:15: 0.4% v/v), see Figure 2 for other chromatographic conditions.

CONCLUSION

The two racemates and four enantiomers of nadolol were separated by a simple, isocratic and fast (required 15 minutes) method using Chiralcel OD column. The method can be used for the identification and optical purity determination of nadolol in bulk material and in pharmaceutical dosage form. It can also be used to separate the individual enantiomers of nadolol from their corresponding racemates on a preparative scale using a semi-preparative or preparative Chiralcel OD column.

ACKNOWLEDGMENTS

The authors would like to thank the Administration of King Faisal Specialist Hospital and Research Centre for their continuous support to the Bioanalytical and Drug Development research program. Thanks are also due to Bristol-Myers Squibb Pharmaceutical Research Institute for the kind supply of nadolol and their enantiomers used in this study.

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Received: June 27, 1995
Accepted: July 13, 1995
Manuscript: 3920