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# Short Communication

# Direct separation of nadolol enantiomers on a Pirkle-type chiral stationary phase

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ABSTRACT

The enantiomers of the β-adrenergic blocking drug nadolol may be separated on a chiral stationary phase following conversion to their 1-naphthylurea derivatives by reaction with the achiral reagent 1-naphthylisocyanate. A mixture of *n*-hexane, propan-2-ol and acetonitrile is used to elute the enantiomers from a column comprising (*R*)-N-(3,5-dinitrobenzoyl)-L-leucine covalently bound to 5-μm aminopropylsilica. Nadolol is a *cis*-diol structure which has three stereogenic centres, however the two diol carbons are held in a fixed configuration and it therefore has only four optical isomers. These are resolved in under 30 mn using the procedure described herein, which is therefore suitable for use in the quality control context.

## INTRODUCTION

The chromatographic separation of drug enantiomers has assumed greater importance with the realisation that pharmacologically significant differences exist between stereoisomers. The increased emphasis on research into enantiospecific drug action has been accompanied by increased activity in the field of chromatographic chiral separations. This has resulted in an upsurge in published methods for such separations and the development of an almost bewildering array of chromatographic chiral stationary phases (CSPs). The β-adrenergic blocking agents have received particular attention as they are a pharmacologically important class of drugs and consequently have been used as models in the development of a great many chiral separations systems. Nadolol is a distinctive β-blocker in that although it has three stereogenic centres it is a *cis*-diol and has only four optical isomers. Only one direct separation of all four has been reported by Lee *et al.* [1] who investigated the separation of nadolol using supercritical fluid chromatography under sub-critical conditions with carbon dioxide as an eluent and were unable to resolve more than three peaks when using Chiralcel-type or the Pirkle-type stationary phases. However, they obtained full resolution of all four optical isomers using an $\alpha_1$-acid glycoprotein column. As part of a wide-ranging programme examining the critical parameters that determine chiral separation on Pirkle-type phases a method has been successfully developed for the direct separation of all four optical isomers of nadolol on an (*R*)-N-(3,5-dinitrobenzoyl)-L-leu-

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cine CSP following achiral derivatisation of the analyte with 1-naphthylisocyanate.

## EXPERIMENTAL

### *Reagents and chemicals*

Bristol-Myers Squibb (Princeton, NJ, USA) standard reference samples of racemic nadolol, nadolol racemate A [(*RS*)–(*SR*)], nadolol racemate B [(*RR*)–(*SS*)], (*RS*)-nadolol, (*SR*)-nadolol and (*SS*)-nadolol were used. 1-Naphthylisocyanate (99%) and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ; 97%) were obtained from Aldrich (Poole, UK) whilst the chromatographic solvents (*n*-hexane, propan-2-ol, ethanol and acetonitrile) were obtained from BDH (Liverpool, UK) and were analytical-reagent grade.

### *Chromatographic conditions*

An eluent consisting of *n*-hexane–ethanol–acetonitrile (45:5:1, v/v/v) was mixed, degassed by helium sparging and pumped at 2 ml/min using a Constametric I pump (LDC-Milton Roy, Riviera Beach FL, USA), fitted with a Rheodyne 7037 pressure relief valve (Rheodyne, Cotati, CA, USA). A Rheodyne 7420 valve was used to apply 20-$\mu$l samples to the column, which contained (*R*)-N-(3,5-dinitrobenzoyl)-L-leucine covalently bound to aminopropylsilica. The absorbance of the eluent at 239 nm was monitored with a Shimadzu SPD-6A UV detector (Shimadzu, Duisburg, Germany) set at 0.16 a.u.f.s. All chromatograms were collected using both a Perkin-Elmer PE56 chart recorder (Perkin-Elmer, Norwalk, CT, USA) and the Beckman CALS PeakPro laboratory data system (Beckman Instruments, Fullerton, CA, USA).

Two separate columns were employed in the study. The first was a 250 mm × 4.6 mm I.D. commercially available column containing 5-$\mu$m packing (CHI-1-LEU, Hichrom, Reading, UK). The second column was prepared in the laboratory by passing a solution of 5 g of the chiral selector (*R*)-N-(3,5)-dinitrobenzoyl)-L-leucine and 5 g of the peptide-coupling agent EEDQ in tetrahydrofuran through a 150 mm × 4.6 mm I.D. column packed with 3-$\mu$m aminopropylsilica (HPLC Technology, Macclesfield, UK). The column was then washed, in turn, with tetrahydrofuran and with dichloromethane, before 100 ml of dichloromethane containing 10 g of trifluoroacetic anhydride were pumped through to derivatise any unreacted propylamine groups. The column was washed with dichloromethane before being equilibrated with the eluent.

### *Derivatisation procedure*

The test solute was dissolved in eluent at a concentration of 0.5–2.0 mg/ml and a five-fold molar excess of 1-naphthylisocyanate was added. The solution was mixed and allowed to stand for 15 min before being injected onto the column.

## RESULTS AND DISCUSSION

The conversion of $\beta$-blockers to their urea derivatives by means of isocyanates (see Fig. 1) has been previously reported by this group [2–4] and others [5–11]. The 1-naphthylurea function was identified as one likely to enhance chiral separation on Pirkle phases, so this was selected for use with nadolol for that reason. Alcohols also react with isocyanates, forming urethanes although under the conditions employed (ambient temperature, short reaction time) the naphthalenediol alcohol groups are unlikely to react.

Using an optimised mobile phase composition (as described below) the separation of the four enantiomers on the 5-$\mu$m column is almost complete (Fig. 2), with baseline resolution between

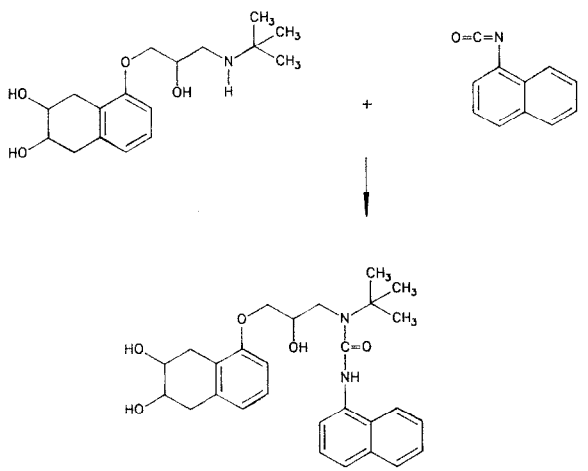


Fig. 1. Reaction between nadolol and 1-naphthylisocyanate leading to the formation of the naphthylurea derivative of the $\beta$-blocker.

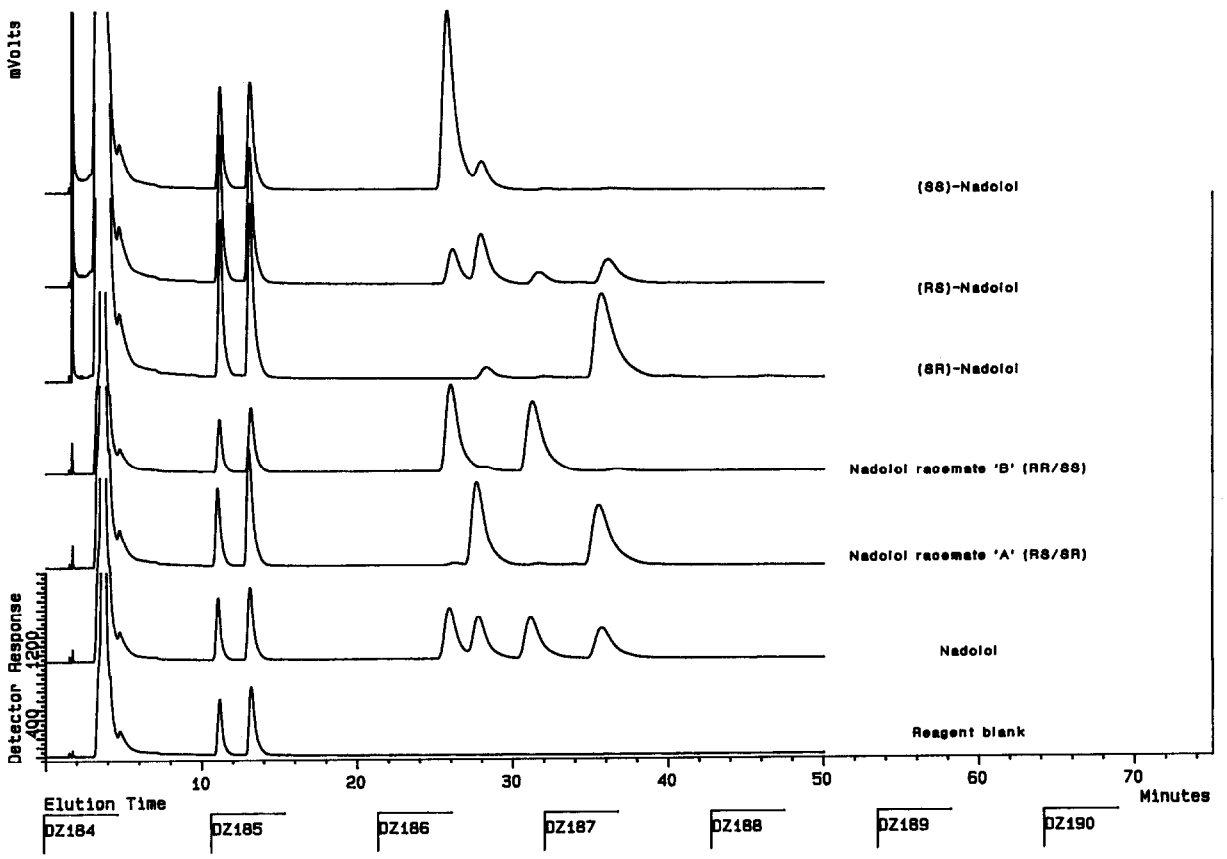


Fig. 2. Chromatograms for injections of nadolol standard materials (for details of see *Chromatographic conditions*).

peaks 2, 3 and 4 and approximately 80% separation of peaks 1 and 2. The elution order was determined using reference standard materials and is summarised in Table I. Although only three of the four isomers were available, assignment was based on a comparison with known racemic mixtures. Two system peaks are seen to elute at 11 and 13 min, neither being unreacted reagent which elutes with the solvent front. They are products of reaction between excess derivatising reagent and the eluent as they increase in size if solutions (including the blank) are left to stand for a time.

The separation achieved for the racemic sample of nadolol is sufficient for quantitation of the enantiomers, although the run time is rather long, necessitating some 40 min between injections. Preliminary attemps to optimise the separation have been undertaken based on modifications to the mo-

TABLE I

CAPACITY FACTORS FOR THE OPTICAL ISOMERS OF NADOLOL

For details see *Chromatographic conditions*.

| Component | Capacity factor ($k'$) | | | |
|---|---|---|---|---|
| | Peak 1 | Peak 2 | Peak 3 | Peak 4 |
| (*SS*)-Nadolol | 16.2 | | | |
| (*RS*)-Nadolol | | 17.7 | | |
| (*SR*)-Nadolol | | | | 22.8 |
| (*RR*)-Nadolol[a] | – | – | – | – |
| Nadolol racemate A[b] | | 17.4 | | 22.7 |
| Nadolol racemate B[c] | 16.3 | | 19.9 | |
| Nadolol | 16.3 | 17.5 | 19.7 | 22.8 |

[a] Not available.
[b] Nadolol racemate A = (*RS*)-(*SR*)-nadolol.
[c] Nadolol racemate B = (*RR*)-(*SS*)-nadolol.

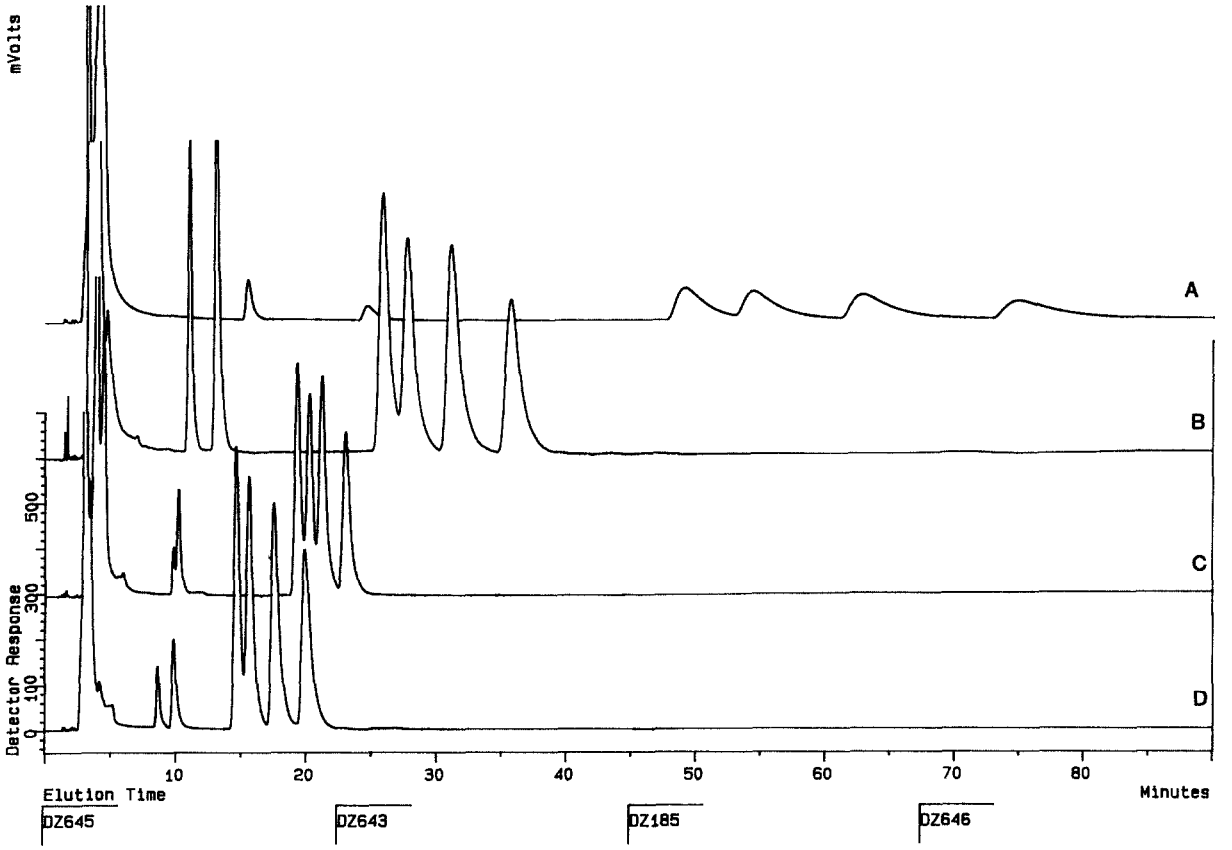


Fig. 3. Chromatograms showing effect of eluent on separation of nadolol enantiomers using (A) *n*-hexane–propan-2-ol–acetonitrile (45:5:1, v/v/v), (B) *n*-hexane–ethanol–acetonitrile (45:5:1, v/v/v), (C) as for (B) in the proportions 45:5:2.5, v/v/v and (D) as for (B) in the proportions 45:7.5:1, v/v/v.

bile phase composition. Substituting the ethanol with propan-2-ol leads to slight improvements in resolution, but with an unacceptable increase in the retention time to approximately 80 min for the slowest eluting enantiomer (Fig. 3). The use of methanol instead of ethanol leads to a two-phase eluent and although the upper (*n*-hexane) phase can be removed and used, this shows rapid elution of all components with almost total loss of resolution. Increasing the ethanol content or the acetonitrile content both speed up elution, although in the latter case there is significant loss of resolution between peaks 2 and 3 and a worsening of the overlap between peaks 1 and 2.

The separation can be improved further by reducing the particle size of the silica, as can be seen from the superior resolution observed for a given eluent on the 3-$\mu$m column (Fig. 4) compared with that for the 5-$\mu$m column with the same eluent.

This dramatic improvement should be treated with some caution, however, as the two columns may not be entirely comparable. Besides having different lengths and particle sizes, the two columns used different silica supports and were prepared in different ways. The 5-$\mu$m column was commercially packed with a chiral packing material based on Spherisorb silica whilst the 3-$\mu$m column was purchased as a commercially available achiral column (Hypersil aminopropylsilica) and then derivatised in the laboratory. However, for the 3-$\mu$m column the propan-2-ol again gave the better separation whilst the ethanol gave faster elution. Thus the choice of optimum eluent composition depends on the requirements for the speed and quality of the

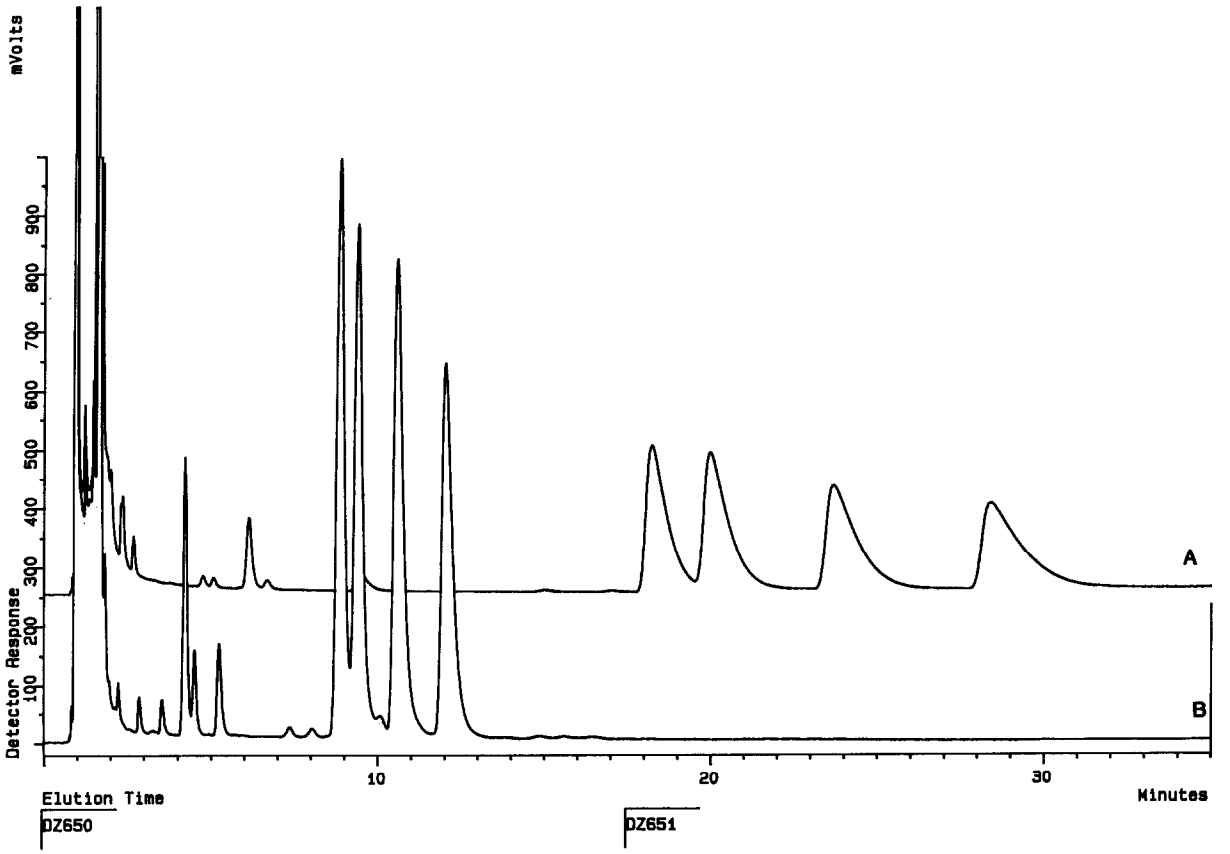


Fig. 4. Separation of nadolol enantiomers on a 3-$\mu$m column using an eluent containing (A) propan-2-ol or (B) ethanol (for details see *Chromatographic conditions*).

separation and on the particle size of the available column.

## CONCLUSIONS

The separation of the four optical isomers of nadolol may be achieved on a covalent Pirkle-type CSP following conversion to their 1-naphthylurea derivatives with the achiral reagent 1-naphthylisocyanate. The separation of the optical isomers may be optimised by adjusting the choice of alcohol modifier in the eluent, as well as the relative proportions of the eluent components. The use of a smaller (3-$\mu$m) particle size silica support affords significant improvements in both speed and extent of separation. This optimised method allows the enantiomers to be accurately quantitated in an analysis time of less then 30 min.

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