CHROMSYMP. 2928

Influence of the structure of the alcoholic modifier on the enantioselective separation of nadolol

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ABSTRACT

The influence of the structure of the alcoholic modifier of the mobile phase on the liquid chromatographic separation of the optical isomers of nadolol on a Pirkle-type chiral stationary phase was investigated. The isomers were separated as their chiral 1-naphthylureides on a column consisting of 3,5-dinitrobenzoyl-L-leucine covalently bound to 3-μm aminopropylsilica, using an eluent consisting of *n*-hexane modified with an alcohol. A number of straight-chain, branched and unsaturated alcohols with carbon numbers ranging from 1 to 8 were evaluated. The choice of alcohol influenced both retention and selectivity, with optimum stereoselectivity being observed for C_3. The alcoholic modifiers selectively affected the retention and resolution of the two pairs of enantiomers that constitute nadolol. Thus chromatograms displaying two, three or four peaks could be obtained, depending on which modifier was employed. The best separation of all four isomers was achieved with ethanol as the modifier.

INTRODUCTION

The β-adrenoceptor-blocking drug nadolol is used as an antihypertensive agent in the treatment of high blood pressure. This molecule (Fig. 1) features three asymmetric carbon atoms in its structure although, as the two in the naphthalenediol ring are held in the *cis* configuration, there are only two chiral centres. Hence nadolol exists as four isomers, consisting of two pairs of enantiomers referred to as nadolol racemate A and nadolol racemate B, which are also diasteromeric pairs. Nadolol is produced by a non-stereoselective synthetic process and consequently the material contains all four isomers. A number of procedures have been reported for the analysis of nadolol [1], some of which are chirally discriminating.

During the investigation of stereoselective analytical methodology, we developed a procedure for the resolution of all four isomers on a Pirkle-type chiral stationary phase (CSP) [2]. The procedure required that the polar amino-function of nadolol was masked by derivatization prior to injection on to the column. The masking derivatization was based on reaction with isocyanates to form the corresponding ureide (Fig. 2). The isocyanate reagent used was achiral and the reaction proceeded rapidly under ambient conditions in unreactive solvents. The ureides produced were resolved on a Pirkle-type CSP comprising 3,5-dinitrobenzoyl-L-leucine (DNB-L-Leu) covalently bonded to 3-μm aminopropylsilica. The eluent employed was a mixture of *n*-hexane, ethanol and acetonitrile.

During the development of the procedure the effect of the hydrocarbons used in the eluent and of the substituent on the isocyanate reagent were investigated. These two "achiral" aspects had a

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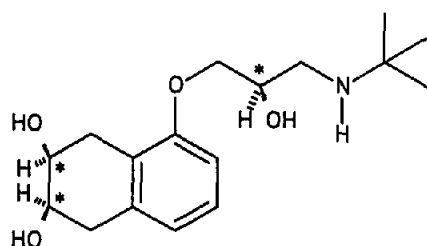
Compound Name		Sign of rotation		Compound Number
		Ring	Side-chain	
NADOLOL (SQ 11,725)	Racemate 'A' (SQ 12,181)	+	–	SQ 12,148
		–	+	SQ 12,150
	Racemate 'B' (SQ 12,182)	+	+	SQ 12,149
		–	–	SQ 12,151

Fig. 1. Structure of nadolol. Nadolol, (±)-2,3-cis-1,2,3,4-tetrahydro-5-(2-hydroxy-3-tert.-butylaminopropoxy)-2,3-naphthalenediol, features three asymmetric carbon atoms (marked with asterisks). However, the two within the tetrahydronaphthalene ring are held in a cis configuration, so there are only two chiral centres, one in the ring and one in the side-chain. This results in nadolol having four optical isomers, made up to two pairs of diastereomers.
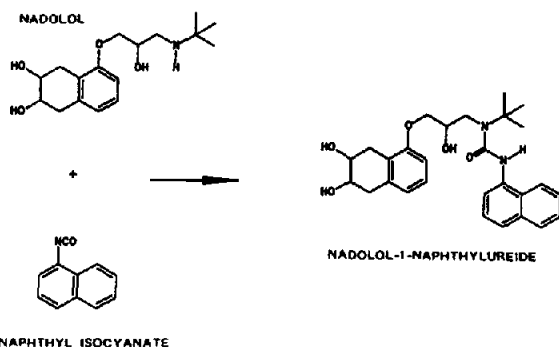

Fig. 2. Derivatization of nadolol with 1-naphthyl isocyanate to form the corresponding ureide. The amino group of nadolol reacts rapidly with 1-naphthyl isocyanate to yield the ureide derivative. The reaction goes almost to completion (>98% in 5 min) and does not require an excess of reagent. Although alcohols may also react with this reagent to form urethanes, under the reaction conditions employed (ambient temperature and no catalyst) reaction with the naphthalenediols does not occur.

profound influence on the chiral separation obtained [3,4]. This prompted an investigation of the alcoholic component of the eluent, itself also an achiral entity, and the results are presented herein.

EXPERIMENTAL

The chromatographic procedure for the separation of the four nadolol isomers has been reported previously [2]. In this system, acetonitrile is used as a modifier in the mobile phase, where it has some effect on the retention and separation of the isomers. However, it relies on the ethanol to remain in solution with the major component, n-hexane. Varying the nature of the alcohol used would vary the content of acetonitrile in the eluent, which would consequently influence the chromatographic result. To reduce the number of variables in the experiments and thus facilitate the evaluation of the resulting data, the eluents employed consisted only of a mixture of n-hexane and the alcohol under investigation.

The samples used were nadolol, a racemic mixture of all four isomers, racemate A and racemate B. Solutions of each were prepared at ca. 1 mg/ml in the test eluent. 1-Naphthyl isocyanate was added at 1 μl/ml and the samples were left to stand under ambient conditions for 15 min before being injected on to the column.

The column was prepared using a procedure that has been reported previously [2]. Briefly, 3,5-dinitrobenzoyl-L-leucine was covalently bound to 3-μm aminopropylsilica already packed into a 150 mm × 3.9 mm I.D. column, which was conditioned with the test eluent before use.

The test eluent was pumped at a rate of 2 ml/min and a detection wavelength of 239 nm was employed. Chromatograms were recorded using the PeakPro software feature of the Beckman CALS LIMS system and stored on an HP1000 computer. The test eluents consisted of mixtures of n-hexane and an alcohol at a ratio of 4:1; when the alcohol was a liquid under ambient conditions, it was added on a volume basis, but when it was a solid, it was added on a mass basis.

The eluents were degassed by sparging with

helium before use and 100-ml volumes were passed through the column to equilibrate it before any injections were made.

RESULTS

The chromatograms were each evaluated in terms of retention, selectivity and separation as follows.

For retention, the capacity factor quoted, k'_{med}, is the median capacity factor observed.

Three selectivity factors (α) were calculated. The factor $\alpha_{2/1}$ is the selectivity of separation of the first two peaks eluting. These are the least retained enantiomers of racemates B and A, compounds SQ 12151 and SQ 12150, respectively. This is therefore a diastereoselectivity factor. The factor $\alpha_{3/1}$ is the selectivity factor for the separation of the first- and third-eluting peaks. These are the enantiomers of racemate B, compounds SQ 12151 and SQ 12149, respectively. This is therefore an enantioselectivity factor.

Likewise, $\alpha_{4/2}$ is the enantioselectivity factor for the separation of the second- and fourth-eluting peaks, which are the enantiomers of racemate A, compounds SQ 12150 and SQ 12148, respectively.

The quality of the separation attained is indicated by coding the peaks observed. Each peak observed is given a letter, e.g., ABCD = four peaks, ABC = three peaks. The symbols between the peaks indicate the degree of separation: no symbol, e.g., AB, = very poor separation (shoulder); solidus, e.g., A/B, = poor separation (10–50%); comma, e.g., A,B, = good separation (50–95%); point, e.g., A.B, = baseline separation. The separation makes use of the Kaiser peak separation factor. This quantifies the separation as the depth of the valley as a percentage of the median peak height. Thus baseline separation yields a factor of 100%.

The resulting data for the test eluents are presented in Table I. An example chromatogram depicting the calculation of the chromatographic criteria is presented in Fig. 3.

TABLE I

RETENTION, SELECTIVITY AND SEPARATION DATA OBTAINED USING THE TEST ELUENTS

See text for experimental details.

Type of alcohol	Alcohol	Capacity factor, k'_{med}	Selectivity factor			Separation type
			$\alpha_{2/1}$	$\alpha_{3/1}$	$\alpha_{4/2}$	
Primary	Methanol[a]	45.52	1.064	1.118	1.154	A.B,C.D
	Ethanol	5.95	1.069	1.312	1.393	A,B.C.D
	1-Propanol	10.04	1.072	1.397	1.518	A/B.C,D
	2-Propen-1-ol	5.95	1.044	1.279	1.363	A/B.C,D
	1-Butanol	14.83	1.060	1.389	1.485	AB,C/D
	1-Pentanol	23.37	1.000	1.282	1.374	A,BC
	2,2-Dimethyl-1-propanol	101.39	1.000	1.463	1.463	A,B
	3-Methyl-1-butanol	30.98	1.000	1.262	1.362	A/BC
	1-Heptanol	41.70	1.000	1.242	1.242	A/B
	1-Octanol	43.99	1.000	1.013	1.209	AB/C
Secondary	2-Propan-2-ol	25.82	1.130	1.567	1.697	A,B.C,D
	(±)-2-Butanol	43.83	1.108	1.594	1.706	A/B.C,D
	(±)-2-Pentanol	68.74	1.056	1.448	1.573	AB,C,D
	(±)-3-Methyl-2-butanol	160.30	1.000	1.692	2.382	A.B.C
Tertiary	2-Methyl-2-propanol	199.25	1.164	1.715	1.910	A/B,C,D
	2-Methyl-2-butanol	239.63	1.193	1.655	1.789	A/B,C,D

[a] This eluent consisted of methanol-saturated n-hexane. A true 1:4 (v/v) solution could not be achieved at ambient temperature.
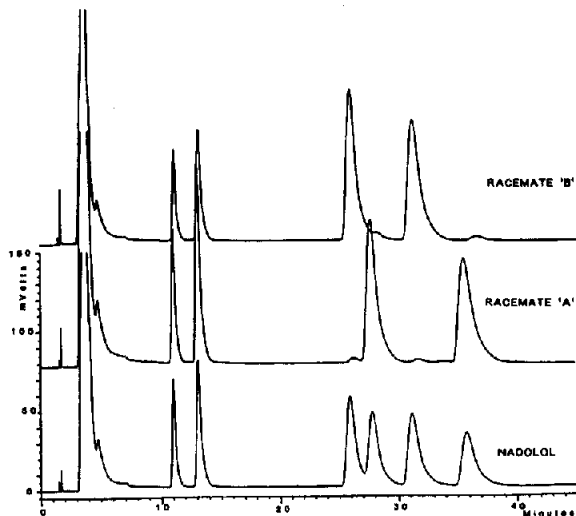
Fig. 3. Examples of chiral separations obtained for nadolol, racemate A and racemate B using the eluent containing 2-propanol as modifier. Conditions: eluent, n-hexane–2-propanol (4:1, v/v); flow-rate, 2 ml/min; column, DNB-L-Leu covalently bonded to 3-μm Hypersil APS (150 × 4.6 mm I.D.); injection volume, 20 μl; detection wavelength, 239 nm; sample, 1 mg of analyte dissolved in 1 ml of eluent and reacted with 1 μl of 1-naphthyl isocyanate under ambient conditions for 15 min before injection.

DISCUSSION

The data obtained indicate that the structure of the alcoholic modifier does influence the chromatographic performance. Increasing the length of the chain of primary alcohols consistently increases the retention, but the increased time on-column does not necessarily improve the resolution. For primary alcohols, the selectivity increases up to C_3 and thereafter it decreases. For secondary alcohols, where no C_1 or C_2 alcohol could be evaluated, the selectivity was a maximum at C_3 ($\alpha_{2/1}$) or C_4 ($\alpha_{3/1}$, $\alpha_{4/2}$). One case of unsaturation was studied, with 2-propen-1-ol (allyl alcohol). This alcohol exhibited decreased retention and separation in comparison with its saturated C_3 analogues. Few conclusions can be drawn from this isolated example, however.

Branching had a discernible effect, increasing both retention and selectivity (see 1-pentanol, 2-pentanol and 3-methyl-1-butanol). Hence it may be branching that is responsible for the observed differences in the performance of the primary and secondary alcohols. Use of the secondary alcohols consistently increased both retention and selectivity in comparison with their primary counterparts.

A peculiar complication arising out of the use of nadolol as a model system is that full separation requires both enantioselectivity and diastereoselectivity. The critical separation is of peaks 1 and 2, the first-eluting isomers of each racemate, i.e., a diastereomer separation. Where loss of selectivity occurred it invariably led to a loss of resolution of those peaks. Further loss of separation lead to two-peak chromatograms where peaks 3 and 4 co-eluted. This also represents loss of diastereoselectivity with enantioselectivity being retained throughout.

Hence demonstration of good enantioselectivity is not necessarily followed by good separation. For example, 2,2-dimethyl-1-propanol showed good enantioselectivity for each racemate ($\alpha = 1.463$) but totally failed to resolve the diastereomers and yielded only two peaks. In contrast, methanol yield lower selectivities ($\alpha = 1.2$) but all four peaks were separated.

In assessing the data it is important to bear in mind that the eluents were prepared on a volumetric or gravimetric basis rather than on a molar basis. The densities of the alcohols were all similar and consequently a 1:5 (v/v) mixture of a higher alcohol, such as 1-octanol, contains significantly less modifier than a 1:5 (v/v) mixture of 1-propanol when the two are considered in terms of their molar concentrations. It is possible that it is the concentration of hydroxyl functions alone which critically influences chromatographic performance, in which case the eluents should be prepared on a molar basis for a true comparison to be made.

Notwithstanding these considerations, the data are useful from an empirical point of view. They indicate that there is no straightforward correlation between time on-column (retention) and separation or selectivity. The fact that there are optimum chain lengths (C_3 or C_4) giving rise to optimum separations implies that the size and/or structure of the alcohols are factors in the enantioselective mechanism occurring at the molecular level.

Also on an empirical basis, the investigation revealed that methanol and ethanol gave the best separations. Although that with methanol was marginally the better, ethanol is the preferred modifier as it is not a saturated solution (*i.e.*, is more controllable) and gives a shorter analysis time.

CONCLUSIONS

The choice of modifier alcohol, an achiral entity, has a significant influence on the resulting chiral separation of nadolol isomers. Retention may be related to the polarity of the alcohol but the separation of isomers is affected by a more complex relationship. The separation is optimum for C_3 or C_4 alcohols. This indicates that the structure of the alcohol, an achiral entity, is involved in the chiral recognition process, although the nature of the mechanism is not clear.

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