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Note

Separation of nadolol diastereoisomers by reversed-phase high-performance liquid chromatography

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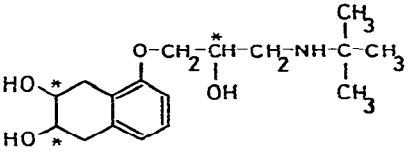
Nadolol, *cis*-5-{3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy}-1,2,3,4-tetrahydro-2,3-naphthalenediol, is a β-adrenergic receptor blocking agent that has been used clinically in the treatment of angina pectoris and hypertension. Nadolol has three asymmetric carbons in the molecule and the two hydroxyl groups in the cyclohexene ring have a *cis*-configuration, so it consists of four enantiomers. The mixture of side-chain (*d*)–cyclohexene ring (*l*) and side chain (*l*)–cyclohexene ring (*d*) is called racemate A and the mixture of side chain (*d*)–cyclohexene ring (*d*) and side chain (*l*)–cyclohexene ring (*l*) is called racemate B[1], and these are diastereoisomers of each other.



In recent years, much work has been published on the separation of diastereoisomers by thin-layer chromatography (TLC)[2–5], gas chromatography (GC)[6–9] and high-performance liquid chromatography (HPLC)[10–15]. The separation of nadolol diastereoisomers by TLC and GC has been examined but neither method succeeded.

This paper describes the development of a method for separating nadolol diastereoisomers by reversed-phase HPLC.

EXPERIMENTAL

Reagents

Nadolol was supplied by Squibb (Princeton, NJ, U.S.A.). Racemates A and B were prepared from nadolol according to published procedures[1], and had melting points of 139 and 154°C, respectively. Methanol, ethanol, isopropanol, dioxane, tetrahydrofuran, acetonitrile, acetic acid and sodium acetate (trihydrate) of analytical-reagent grade were supplied by Wako (Osaka, Japan).

Apparatus

A Waters Model ALC/GPC 204 liquid chromatograph, equipped with a

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Model 6000A pump, a Model 440 detector (254 nm) and a Model U6K injector (Waters Assoc., Milford, MA, U.S.A.), was used.

The column was a stainless-steel tube (300 × 4 mm I.D.) packed with Develosil ODS-5 (Nomura Chemical, Aichi, Japan).

Mobile phase

A mixture of sodium acetate buffer (pH adjusted with acetic acid) and different organic solvents was used.

Procedure

Nadolol was dissolved in methanol to a concentration of 5 mg/ml and racemates A and B in methanol to a concentration of 2.5 mg/ml; 3 μl of each solution were injected into the liquid chromatograph.

RESULTS

Effect of organic solvent

The effect of the different organic solvents in the mobile phase on the separation of nadolol diastereoisomers is shown in Table I. The retention times (t_R) and the resolution (R_s) were used as parameters for the separation. The separation was greatly influenced by the type of organic solvent in the mobile phase. Methanol was the most suitable for the separation of the diastereoisomers and dioxane was also effective. Ethanol, isopropanol and tetrahydrofuran were inferior to methanol and dioxane. Acetonitrile had no effect on the separation.

TABLE I

EFFECT OF DIFFERENT ORGANIC SOLVENTS IN THE MOBILE PHASE ON THE SEPARATION OF NADOLOL DIASTEREOISOMERS

Column, Develosil ODS-5 (300 × 4 mm I.D.); mobile phase, 0.1 *M* sodium acetate buffer (pH 6.0) plus different organic solvents; flow-rate, 0.8 ml/min.

Organic solvent	*Concentration of organic solvent (%)*	t_R *(min)*		R_s
		Racemate A	*Racemate B*	
Methanol	22	29.7	32.4	1.49
Dioxane	7	28.7	31.5	1.31
Ethanol	10	32.4	34.5	0.96
Isopropanol	5	29.9	31.5	0.84
Tetrahydrofuran	2.5	29.6	31.1	0.75
Acetonitrile	11	30.6	30.6	0

Effect of pH and methanol concentration

The effect of pH and the methanol concentration in the mobile phase on the separation of the diastereoisomers is shown in Figs. 1 and 2. As the pH increased, the resolutions and the retention times increased. Similarly, as the methanol concentration decreased, the resolutions and the retention times increased.

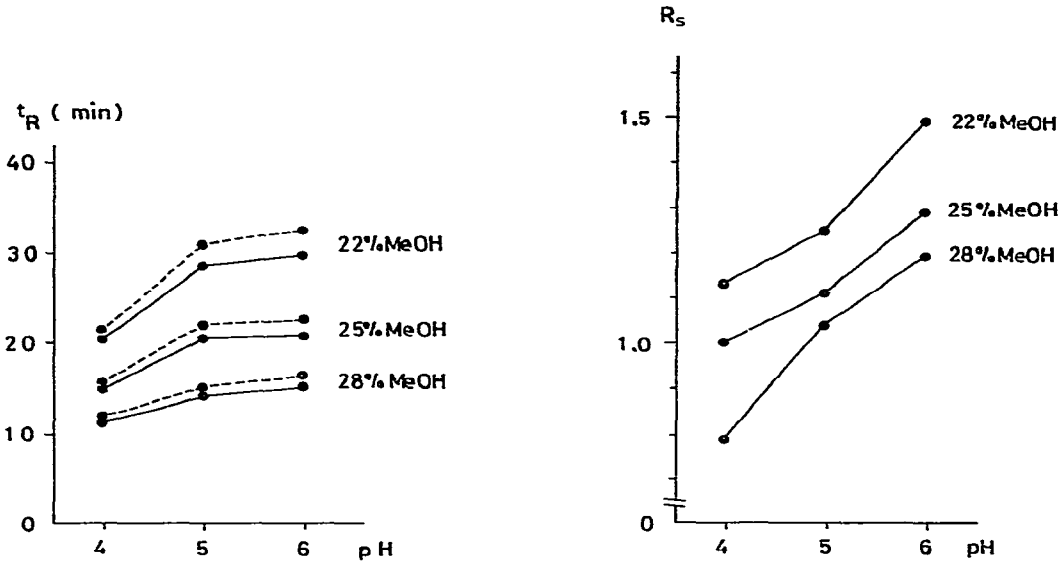

Fig. 1. Effect of pH and the methanol (MeOH) concentration in the mobile phase on the retention times of nadolol diastereoisomers: solid lines, racemate A; broken lines, racemate B. Column, Develosil ODS-5 (300 × 4 mm I.D.); mobile phase, 0.1 *M* sodium acetate buffer–methanol; flow-rate, 0.8 ml/min.

Fig. 2. Effect of pH and the methanol concentration in the mobile phase on the resolution (R_s) of nadolol diastereoisomers. Column, Develosil ODS-5 (300 × 4 mm I.D.); mobile phase, 0.1 *M* sodium acetate buffer–methanol; flow-rate, 0.8 ml/min.

Effect of buffer concentration

The effect of the concentration of sodium acetate buffer (pH 6.0) in the mobile phase on the separation of the diastereoisomers is shown in Table II. As the concentration of sodium acetate buffer increased, the retention times were increased slightly but the resolutions did not change.

Consequently, it was found that nadolol diastereoisomers were completely separated by using 0.1 *M* sodium acetate buffer (pH 6.0)–methanol (78:22) as the mobile phase and racemate A was eluted faster than racemate B (Fig. 3).

TABLE II

EFFECT OF CONCENTRATION OF SODIUM ACETATE BUFFER IN THE MOBILE PHASE ON THE SEPARATION OF NADOLOL DIASTEREOISOMERS

Column, Develosil ODS-5 (300 × 4 mm I.D.); mobile phase, sodium acetate buffer (pH 6.0)–methanol (75:25); flow-rate, 0.8 ml/min.

Concentration of sodium acetate buffer (M)	t_R *(min)*		R_s
	Racemate A	*Racemate B*	
0.05	21.0	22.6	1.00
0.1	22.0	23.7	1.04
0.2	23.3	25.0	1.00

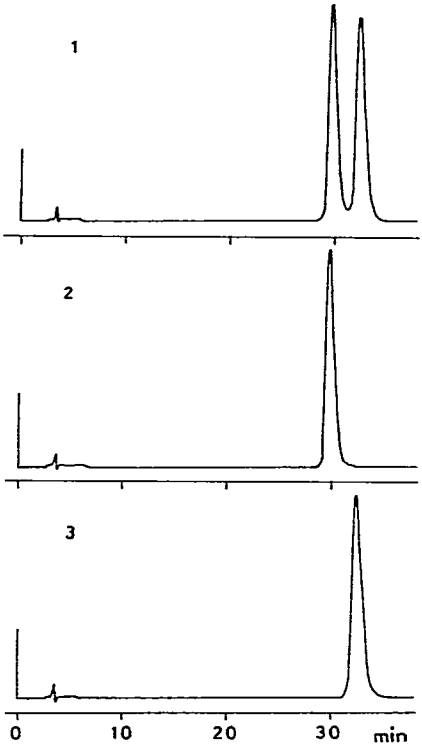

Fig. 3. Chromatograms of nadolol diastereoisomers on Develosil ODS-5 (300 × 4 mm I.D.). Mobile phase, 0.1 *M* sodium acetate buffer (pH 6.0)–methanol (78:22); flow-rate, 0.8 ml/min; pressure, 2200 p.s.i. 1 = Nadolol; 2 = racemate A; 3 = racemate B.

DISCUSSION

It was reported by Salto[13] that the separation of penicillin diastereoisomers was greatly dependent on the pH and the methanol concentration in the mobile phase. A similar effect has been found for the separation of nadolol diastereoisomers, and the pH and the methanol concentration had a great effect on R_s.

Usually resolution can be expressed by the equation[16]

$$R_s = \frac{1}{4} \cdot (\alpha - 1) \cdot \frac{\bar{k}}{1 + \bar{k}} \cdot \sqrt{N}$$

where α is the separation factor, $\bar{k}$ is the average capacity factor and N is the number of theoretical plates. The three parameters in this equation can be considered to be mutually independent and can be varied separately in order to improve R_s. The individual contributions of these parameters to the improvement in R_s were determined with varying the pH, methanol concentration or type of organic solvent in the mobile phase. If k'_A and k'_B are the capacity factors for racemates A and B, respectively. $\alpha = k'_B/k'_A$ and $\bar{k} = (k'_A + k'_B)/2$; N is determined separately from the peaks in each chromatogram.

TABLE III

EFFECT OF pH AND METHANOL CONCENTRATION IN THE MOBILE PHASE ON SELECTIVITY TERM, CAPACITY TERM AND RESOLUTION FOR NADOLOL DIASTEREOISOMERS

pH	$\alpha - 1$			$\frac{\bar{k}}{1+\bar{k}}$			R_s		
	22% CH_3OH	*25% CH_3OH*	*28% CH_3OH*	*22% CH_3OH*	*25% CH_3OH*	*28% CH_3OH*	*22% CH_3OH*	*25% CH_3OH*	*28% CH_3OH*
4.0	0.072	0.067	0.057	0.831	0.771	0.703	1.13	1.00	0.79
5.0	0.095	0.085	0.079	0.883	0.837	0.766	1.25	1.11	1.04
6.0	0.107	0.092	0.084	0.884	0.837	0.776	1.49	1.29	1.19

Table III gives values calculated for these parameters at various pH values and methanol concentrations in the mobile phase. The selectivity term ($\alpha - 1$) increased as the pH increased or the methanol concentration decreased. On the other hand, the capacity term [$\bar{k}/(1 + \bar{k})$] increased as the methanol concentration decreased and remained almost constant as the pH increased. Values of these parameters with various organic solvents in the mobile phase are shown in Table IV. It was found that there was a great difference in the selectivity term when the capacity term was kept constant by adjusting the concentration of orgaic solvent in the mobile phase. The selectivity term was greatest using methanol and was zero using acetonitrile.

From the above, it was concluded that the resolution of nadolol diastereoisomers was improved by the following: (1) increasing the pH of the mobile phase, thus increasing the selectivity term; (2) decreasing the methanol concentration, thus increasing both the selectivity term and the capacity term; and (3) using methanol as the organic solvent, thus increasing the selectivity term.

In conclusion, nadolol diastereoisomers were conveniently separated by reversed-phase HPLC and the separation was greatly influenced by the pH, the methanol concentration and the type of organic solvent in the mobile phase. It is interesting that it is necessary for the separation of nadolol diastereoisomers to use an organic solvent that has a hydroxyl group or ether linkage in the mobile phase.

TABLE IV

EFFECT OF DIFFERENT ORGANIC SOLVENTS IN THE MOBILE PHASE ON SELECTIVITY TERM, CAPACITY TERM AND RESOLUTION FOR NADOLOL DIASTEREOISOMERS

Organic solvent	*Concentration of organic solvent (%)*	$\alpha - 1$	$\frac{\bar{k}}{1+\bar{k}}$	R_s
Methanol	22	0.107	0.884	1.49
Dioxane	7	0.105	0.882	1.31
Ethanol	10	0.074	0.894	0.96
Isopropanol	5	0.065	0.884	0.84
Tetrahydrofuran	2.5	0.053	0.911	0.75
Acetonitrile	11	0	0.903	0

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