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HPLC enantiomeric resolution of nebivolol on normal and reversed amylose based chiral phases

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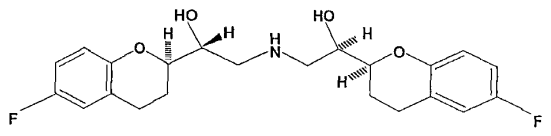
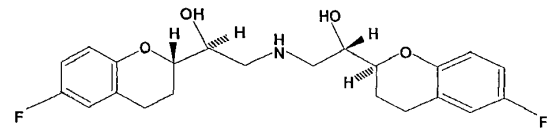
Racemic nebivolol, a β-adrenergic blocker showing very promising β-adrenergic antagonist properties in comparison to other β-adrenergic blockers has been resolved by HPLC under normal and reversed phase modes. The columns used were Chiralpak AD and Chiralpak AD-RH containing amylose tris (3,5-dimethyl phenyl carbamate) as the chiral selector. The mobile phases used were pure ethanol and 1-propanol. The flow rates used were 0.5, 1.0 and 1.5 ml/min. The best resolution was achieved at 0.5 ml/min. flow rate with ethanol and 1-propanol on both Chiralpak AD and Chiralpak AD-RH stationary phases. The values of α for both alcohols on Chiralpak AD were 1.38 while on Chiralpak AD-RH these values were 1.41 and 1.38 respectively. The values of Rs for ethanol and 1-propanol were 2.63 and 1.71 on Chiralpak AD and 1.73 and 1.76 on Chiralpak AD-RH respectively.

1. Introduction

The therapeutic efficacy and safety of β-adrenergic blockers are well known in hypertension, angina pectoris and heart failure [1]. The most commonly known β-adrenergic receptors antagonists such as atenolol, bisoprolol, carvedilol and metoprolol are used in the treatment of hypertension [2], congestive heart failure [3, 4] and to reduce morbidity and mortality after myocardial infarction [5]. The use of these β-adrenergic blockers is associated with a variety of side effects [6] and metabolic adverse side effects [7]. To overcome such problems, new β-adrenergic antagonists such as labetalol and nebivolol have been developed [8]. Among these, nebivolol (Fig. 1) is a very promising β-adrenergic antagonist agent with selective β-adrenergic receptor antagonizing properties and lack of intrinsic sympathomimetic activity [9]. In addition, nebivolol relaxes coronary arteries [10] causes an immediate fall in arterial blood pressure, improves both left ventricular systolic and diastolic functions and lowers peripheral resistance and hence it is considered to be the best antagonist with vasodilating properties [11–13]. Nebivolol has four chiral centers and it is interesting to note that it exists in five pairs of enantiomers only. This is due to the fact that the other three pairs of enantiomers contain a center of symmetry and hence are optically inactive (meso form). Out of ten available enantiomers only (+)-RRRS and (−)-SSSR are the biologically active. It has been reported that the (+)-RRRS enantiomer of nebivolol is 100 times more active than the (−)-SSSR enantiomer [14]. However, Janssen, et al. [15] reported that the (−)-SSSR enantiomer potentiates the hypotensive effect of the (+)-RRRS nebivolol enantiomer.



(+)-RRRS



(-)-SSSR

Fig. 1: Stereochemical Formulae of (+)-RRRS and (−)-SSSR Enantiomers of Nebivolol

Polysaccharide based chiral stationary phases i.e. cellulose and amylose CSPs have been widely used for the enantiomeric resolution for a large variety of racemates by liquid chromatography [16–21]. The enantiomers of several β-blockers were resolved using cellulose tris (3,5-dimethyl phenyl carbamate), known as Chiracel OD, as the CSP [22–25]. It has also been reported that the amylose CSP is a better chiral selector than cellulose due to its more helical structure [26]. This paper describes the enantiomeric resolution of racemic nebivolol on amylose tris (3,5-dimethyl phenyl carbamate), known as Chiralpak AD and Chiralpak AD-RH, with normal and reversed modes respectively.

2. Investigations, results and discussion

The chromatographic parameters, capacity factor (k′), separation factor (α) and resolution factor (Rs) for the resolved (+)-RRRS and (−)-SSSR enantiomers of nebivolol on amylose CSPs under normal and reversed phases are presented in the Table. The resolved enantiomers were identified by running the chromatograms for the individual (+)-RRRS and (−)-SSSR enantiomers under the same chromatographic conditions. It was found that (−)-RRRS enantiomer eluted first and then the (−)-SSSR enantiomer. The Table shows that racemic nebivolol was resolved to the corresponding enantiomers on both Chiralpak AD and Chiralpak AD-RH columns by simply using ethanol and 1-propanol at different flow rates. Although the baseline resolution of nebivolol enantiomers was achieved using pure ethanol and 1-propanol at all the three reported flow rates (Table), the best resolution was achieved at 0.5 ml/min flow rate on both Chiralpak AD and Chiralpak AD-RH columns. The chromatograms are shown in Fig. 2. The chromatographic parameters were varied in order to obtain the best resolution and to optimize the chromatographic conditions. Other alcohols such as methanol and 1-butanol

Table: Chromatographic parameters, capacity factor (k′), separation factor (α) and resolution factor (Rs) for enantiomeric resolution of (±)-nebivolol on chiralpak AD and Chiralpak AD-RH chiral stationary phases

	Flow rate (ml/min)	k'_1	k'_2	α	Rs
Chiralpak AD					
	EtOH				
1.	0.5	(+)-4.57	(−)-6.32	1.38	2.63
2.	1.0	(+)-4.33	(−)-6.06	1.40	2.28
3.	1.5	(+)-4.18	(−)-5.77	1.38	1.40
	1-PrOH				
1.	0.5	(+)-4.22	(−)-5.84	1.38	1.71
2.	1.0	(+)-2.10	(−)-3.03	1.44	1.45
3.	1.5	(+)-1.09	(−)-1.86	1.71	1.21
Chiralpak AD-RH					
	EtOH				
1.	0.5	(+)-5.65	(−)-7.94	1.41	1.73
2.	1.0	(+)-3.00	(−)-4.27	1.42	1.10
3.	1.5	(+)-2.60	(−)-3.75	1.44	1.15
	1-PrOH				
1.	0.5	(+)-8.36	(−)-11.5	1.38	1.76
2.	1.0	(+)-2.61	(−)-3.83	1.47	1.20
3.	1.5	(+)-1.10	(−)-1.75	1.59	1.10

For details see experimental section.

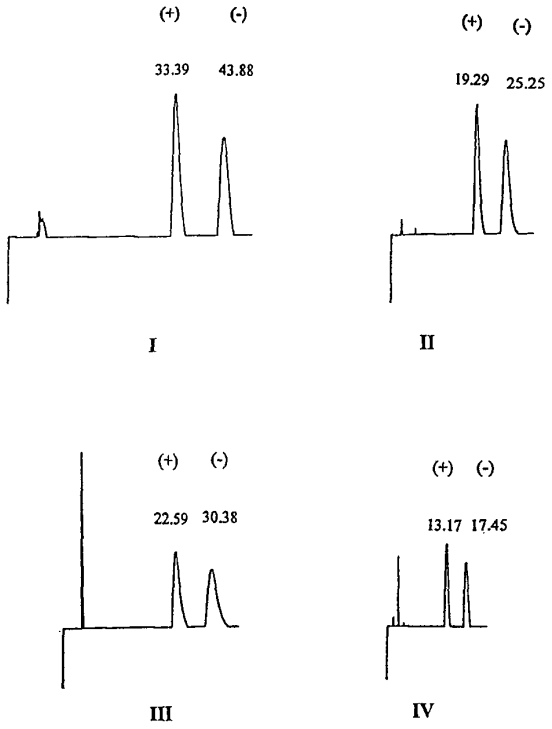


Fig. 2: Chromatograms of resolved enantiomers of (±)-nebivolol I & II: Chiralpak AD column with ethanol (I) and 1-propanol (II) and III & IV: Chiralpak AD-RH column with ethanol (III) and 1-propanol (IV) Flow rates: 0.5 ml/min of each mobile phase See details in the experimental section

were also tried but good resolution could not be achieved. Various ratios of ethanol and 1-propanol with other solvents such as hexane (in normal phase mode) and acetonitrile (in reversed phase mode) were tried but none of these trials improved the resolution. Since there was only partial resolution when using methanol and 1-butanol it may be concluded that the enantiomeric resolution of nebivolol is controlled by both the polarities and the viscosities of the alcohols. Therefore, one can assume that the polarities and viscosities of ethanol and 1-propanol are suitable for the enantiomeric resolution of nebivolol enantiomers.

In both normal and reversed phase conditions, the values of α were in the order of 1-PrOH > EtOH which indicates the same behavior of enantiomers with both stationary phases. Generally, the values of α for the resolved enantiomers increased with an increase in flow rate, because the retention of enantiomers decreases with increasing flow rates. The values of α for enantiomeric resolution in all cases (using ethanol and 1-propanol at all flow rates) is greater with the reversed stationary phase than the normal stationary phase. This shows that the reversed stationary phase is better than the normal stationary phase. It is also important to note that the differences of the values of α for ethanol and 1-propanol at different flow rates is greater in normal phase mode than in the reversed phase one. This may be due to the fact that Chiralpak AD in normal mode is affected by the flow rate more comparised with Chiralpak AD-RH in reversed mode.

The cellulose and amylose chiral stationary phases have been widely used for the enantiomeric resolution of a variety of racemates [19–21]. It has been reported that the amylose – CSP is more helical in nature and has well defined cavities, making it different from the corresponding cellulose analogue, which appears to be more linear and rigid in nature [26, 27]. The cellulose CSPs have been used for the enantiomeric resolution of other β-adrenergic agents such as tertatolol, oxprenolol, alprenolol, acebutolol, bisoprolol, tolamolol, bufuralol, metoprolol, and betaxolol among others [28–30]. The chiral recognition mechanism at a molecular level on the polysaccharide based CSPs is still unclear although it has been reported that chiral resolution by cellulose/amylose – CSP is achieved through differences in hydrogen bonding between the chiral stationary phase and the enantiomers among other interactions [19]. Amylose tris (3,5-dimethylphenyl carbamate) is a semi synthetic polymer which contain a polymeric chain of derivatized D–(+) glucose residues in α-1,4 linkage. These chains lie side by side in a helical fashion. The structure of nebivolol (Fig. 1) contains two hydroxyl groups, one secondary amine group and two aromatic rings. Therefore, the resolution of the nebivolol enantiomers occurred as a result of differences in hydrogen bondings between the carbonyl groups of carbamate moieties of the amylose – CSP and the hydroxyl and amine groups of the enantiomers of nebivolol. Furthermore, Francotte et al. [31] postulated that the chiral cavities of these CSPs have high affinity for aromatic compounds. Therefore, the two aromatic rings of nebivolol fit stereogenically in different fashions into the chiral cavities of the stationary phases and π-π interactions of different magnitude for the (+)-RRRS and (−)-SSSR enantiomers take place which result in the resolution of enantiomers.

The Table shows that the (+)-RRRS enantiomer eluted first followed by the (−)-SSSR enantiomer. This indicates that the (-)-SSSR enantiomer interacts with the amylose CSP through stronger hydrogen and π-π bonding than the (+)-RRRS enantiomer. Also the stereochemical configuration of the (−)-SSSR enantiomer fits better in the chiral cavities of the stationary phase than the (+)-RRRS enantiomer and hence (-)-SSSR ia able to form stronger hydrogen and π-π bond with amylose CSP than the (+)-RRRS enantiomer.

The reported HPLC system is simple, fast and reproducible. Accordingly, the method developed can be used for the resolution and enantiomeric excess determination of nebivolol. This method can also be applied to the analysis of nebivolol enantiomers in both pharmaceutical formulations and biological fluids.

3. Experimental

Nebivolol enantiomers i.e. (+)-RRRS (Product No: R85547) and (−)-SSSR (Product No.: R85548) and racemic nebivolol (Product No.: R67555) were kindly donated by Janssen Research Foundation, Beerse, Belgium. The absolute ethanol was obtained from E. Merck (Darmstadt, Germany). 1-Propanol was supplied by BDH, London, UK.

The stock solutions (0.1 mg/ml) of racemic nebivolol and its enantiomers were prepared in absolute ethanol. 20 µl of each of the solutions were loaded on to a HPLC system consisting of a Waters solvent delivery pump (model 510), Waters injector (model WISP 710B), Waters tunable absorbance detector (model 484) and Waters integrator (model 740). The columns used were Chiralpak AD (25 cm × 0.46 cm I.D., particle size 10 µm) and Chiralpak AD-RH (15 cm × 0.46 cm. I.D., particle size 5 µm) and were obtained from Daicel Chemical Industries, Tokyo, Japan. The mobile phases used in this study were pure ethanol and 1-propanol. The mobile phases were filtered and degassed before use. The flow rates of the mobile phases were 0.5, 1.0 and 1.5 ml/min respectively. The chart speed was kept at 0.1 cm/min. Detection of nebivolol was performed at 220 nm. All the experiments were carried out at 23 ± 1 °C. The chromatographic parameters such as capacity factor (k′), separation factor (α) and resolution factor (Rs) were calculated.

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References

1 Messerli, F. H.: Cardiovascular Drug Therapy. W. B. Saunders Co., Philadelphia, USA 1990
2 Dahlof, B.; Lindholm, L. H.; Hansson, L.; Schersten, B.; Ekbom, T.; Wester, O. P.: Lancet **338**, 1281 (1991)
3 CIBIS Investigators and Committees Circulation **90**, 1765 (1994)
4 Packer, M.; Bristow, M. R.; Cohn, J. N.; Colucci, W. S.; Fowler, M. B.; Gilbert, E. M.; Shusterman, M. H.: N. Engl. J. Med. **334**, 1349 (1996)
5 Yusuf, S.; Peto, R.; Collins, J.; Sleight, P.: Prog. Cardiovasc. Dis. **27**, 335 (1985)
6 Steiner, S. S.; Friedhoff, A. J.; Wilson, B. L.; Wecker, J. R.; Santo, J. P.: J. Hum. Hypertense **4**, 217 (1990)
7 Pollare, T.; Lithell, H.; Selenius, I. C.: Brit. Med. J. **298**, 1152 (1989)
8 Ruffolo, R. R. Jr.; Gellai, M.; Hieble, J. P.; Wilettee, R. N. Nichols, A. J.: Eur. J. Clin. Pharmacol. **38**, S82 (1990)
9 Janssen, W. J.; Van de Water, A.; Xhonneux, R.; Reneman, R. S.; Van Nueten, J. M.; Janssen, P. A. J.: Eur. J. Pharmacol. **159**, 89 (1989)
10 Gao, Y.; Nagao, T.; Bond, R. A.; Janssen, W. J.; Van Houte, P. M.: J. Cardiovasc. Pharmacol. **17**, 964 (1991)
11 Van de Water, V.; Xhonneux, R.; Reneman, R. S.; Janssen, P. A. J.: Eur. J. Pharmacol. **156**, 95 (1988)
12 Van Bortel, L. M. A. B.; de Hoon, J. N. J. M.: Kool, M. J. F.; Wijnen, J. A. G.; Vertommen, C. I. M., Van Nueten, L. G. M.: Eur. J. Clin. Pharmacol. **51**, 379 (1997)
13 Himmlmann, A.; Hender, T.; Snoeck, E.; Lundgren, B.; Hender, J.: Eur. J. Clin. Pharmacol. **51**, 259 (1996)
14 Cheymol, G.; Woestenborghs, R.; Snoeck, E.; Ianucci, R.; Le Moing, J. P.; Naditch, L.; Levron, J. C.; Poirier, J. M.: Eur. J. Clin. Pharmacol. **51**, 493 (1997)
15 Janssens, W. J.; Xhonneux, R.; Janssen, P. A. J.: Drug Investig. **3** (Suppl. 1), 13 (1991)
16 Yashima, E.; Yamamoto, C.; Okamoto, Y.: Polymer J. **27**, 856 (1995)
17 Tang, Y.; Reepmeyer, J. C.; Revelle, L. K.; Wilson, J. A.: J. Chromatogr. **752**, 93 (1996)
18 Yashima, E.; Sahavattanapog, P.; Okamoto, Y.: Chirality **8**, 446 (1996)
19 Yashima, E.; Yamada, M.; Kaida, Y.; Okamoto, Y.: J. Chromatogr. **694**, 347 (1995)
20 Chankvetadze, B.; Yashima, E.; Okamoto, Y.: J. Chromatogr. **694**, 101 (1995)
21 Yashima, E.; Kasashima, E.; Okamoto, Y.: Chirality **9**, 63 (1997)
22 Aboul-Enein, H. Y.; Islam, M. R.: Chirality **1**, 301 (1989)
23 Aboul-Enein, H. Y.; Islam, M. R.: J. Chromatogr. **511**, 109 (1990)
24 Aboul-Enein, H. Y.; Islam, M. R.: Anal. Lett. **23**, 83 (1990)
25 Aboul-Enein, H. Y.; Islam, M. R.: Anal. Lett. **23**, 973 (1990)
26 Ronden, N. G.; Nyquist, R. A.; Gillie, J. K.; Nicholson, L. W.; Goralski, C. T.: 4th Int. Symposium., Montreal, Canada, Abstract. **162**, 90 (1993)
27 Vogt, U.; Zugenmaier, P.: Ber. Bunsenges. Phys. Chem. **89**, 1217 (1985)
28 Aboul-Enein, H. Y.; Serignese, V.: J. Liq. Chromatogr. **16**, 197 (1993)
29 Aboul-Enein, H. Y.; Serignese, V.: Cellulose **2**, 215 (1995)
30 Cass, Q. B.; Tiritan, M. E.; Calafatti, S. A.; Matlin, S. A.: J. Liq. Chromatogr. & Rel. Technol. **22**, 3091 (1999)
31 Francotte, E.; Wolf, R. M.; Lohmann, D.; Mueller, R.: J. Chromatogr. **347**, 25 (1985)

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