Journal of Chromatography, 553 (1991) 391–397
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2170

Liquid chromatographic separation of the enantiomers of metoprolol and its α-hydroxy metabolite on Chiralcel OD for determination in plasma and urine

KARIN BALMÉR*, ANNIKA PERSSON, PER-OLOF LAGERSTRÖM and BENGT-ARNE PERSSON

Bioanalytical Chemistry, AB Hässle, S-431 83 Mölndal (Sweden)

and

GÖRAN SCHILL

Department of Analytical Pharmaceutical Chemistry, University of Uppsala, S-751 23 Uppsala (Sweden)

ABSTRACT

The two enantiomers of metoprolol and the four enantiomeric forms of α-hydroxymetoprolol were separated by liquid chromatography on a Chiralcel OD column containing a cellulose tris(3,5-dimethylphenylcarbamate) chiral stationary phase. The column efficiency was strongly dependent on the flow-rate and the enantioselectivity was influenced by temperature. Of utmost importance for the chiral separation was the water content of the mobile organic phase. The separation system was used for the separation and determination of the enantiomers in plasma and urine samples. The metoprolol enantiomers could be determined by fluorescence down to 10 nmol/l of each in plasma with a relative standard deviation of less than 15%.

INTRODUCTION

In recent years there has been an increased interest in chiral drugs with regard to pharmacokinetics and pharmacodynamics of the separate enantiomers. The development of new tools for chiral separation, mainly by liquid chromatography, has contributed greatly to the possibility of such studies [1].

Metoprolol, a β-adrenoceptor blocking drug, is one of numerous examples of drugs administered as a racemate, where the two antipodes do not have identical pharmacological properties. We have studied the separation of the enantiomers by liquid chromatography on an α_1-acid glycoprotein chiral stationary phase, Chiral AGP [2], which has found wide applications in drug separations. We have also presented an enantioselective assay for metoprolol in plasma samples at levels down to 2 nmol/l [3].

A family of chiral stationary phases of cellulose triphenylcarbamate derivatives on silica gel has attracted considerable interest during the last few years [4–6]. Recently the separation and determination of metoprolol enantiomers were demonstrated

0021-9673/91/$03.50

using Chiralcel OD [7–9], a stationary phase of cellulose tris(3,5-dimethylphenylcarbamate) polymer coated on macroporous silica, from Daicel. We also examined this phase, not only for separating metoprolol isomers but also for the separation of the isomers of the α-hydroxy metabolite in human urine and in dog plasma. There are two asymmetric carbons in this metabolite, making four isomers to be separated. The aim of this study was to optimize the separation conditions. We examined the influence of flow-rate, temperature and mobile phase water content on column efficiency and selectivity. It was found essential to control the water content of the organic mobile phase as it had a significant influence on the enantioselectivity of the Chiralcel OD phase.

EXPERIMENTAL

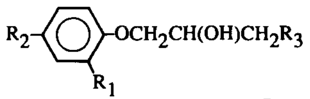
Chemicals

Metoprolol as tartrate, α-hydroxymetoprolol as *p*-hydroxybensoate, (*S*)-alprenolol as chloride, (*S*)-metoprolol as sorbate and H 93/82 (a diol) (Fig. 1) were obtained from the Department of Organic Chemistry, Hässle (Mölndal, Sweden). Hexane and dichloromethane of high-performance liquid chromatographic (HPLC) grade were from Rathburn (Walkerburn, U.K.), diethyl ether of pro analysi grade from May & Baker (Dagenham, U.K.), diethylamine from Fluka (Buchs, Switzerland), 1-propanol and buffer substances from E. Merck (Darmstadt, Germany) and water from a Milli-Q system (Millipore, Molsheim, France).

Instrumentation and conditions

The liquid chromatograph consisted of an LKB (Bromma, Sweden) Model 2150 pump, a WISP 710 B autosampler (Waters Assoc., Milford, MA, U.S.A.) and a Model 821 FP fluorescence detector (Jasco, Tokyo, Japan) operated at 272 nm (excitation) and 306 nm (emission). A laboratory-built thermostat controlled the temperature of the column. The chromatograms were recorded on an SP4400 integrator (Spectra-Physics, San José, CA, U.S.A.). A Metrohm (Herisau, Switzerland) 684 KF coulometer was used to measure the water content of the mobile phase.

The analytical column (250 × 4.6 mm I.D.) was a Chiralcel OD from Daicel (Tokyo, Japan) which was used between 20 and 40°C with flow-rates of 0.25–1.75 ml/min. The mobile phase contained 0.1% of diethylamine, 200–1500 mg/l of water, 4–10% of 1-propanol and hexane.



		R_1	R_2	R_3
I	Metoprolol	H	$CH_2CH_2OCH_3$	$NHCH(CH_3)_2$
II	S–Alprenolol	$CH_2CH{=}CH_2$	H	$NHCH(CH_3)_2$
III	α–OH–metoprolol	H	$CH(OH)CH_2OCH_3$	$NHCH(CH_3)_2$
IV	H 93/82 (diol)	H	$CH_2CH_2OCH_3$	OH

Fig. 1. Structures of metoprolol, metabolites and the internal standard.

Calibration and test solutions

Standard solutions of metoprolol, (*S*)-metoprolol, α-hydroxymetoprolol and (*S*)-alprenolol were made up in 0.01 *M* hydrochloric acid at concentrations of 10, 10, 75 and 20 μmol/l, respectively. Test solutions in the organic mobile phase were prepared by extraction with diethyl ether–dichloromethane (4:1) from alkalinized reference solutions. After phase separation (freezing of aqueous phase) and evaporation under nitrogen the amines in base form were dissolved in the mobile phase.

Analytical procedure

A 1-ml volume of human or dog plasma or urine is mixed with 0.050 ml of internal standard solution [20 μmol/l (*S*)-alprenolol in 0.01 *M* hydrochloric acid] and 0.10 ml of 1 *M* sodium hydroxide solution and the mixture is extracted with 5 ml of diethyl ether by shaking for 10 min. After centrifugation for 5 min the organic phase is evaporated under nitrogen and the residue is dissolved in 0.250 ml of mobile phase, 0.050 ml of which is injected onto the chromatographic column. When α-hydroxymetoprolol is determined, 0.5 g of sodium chloride is added prior to extraction with diethyl ether–dichloromethane (4:1) [10].

Reference samples for calibration are included in the daily series of analyses. A 0.100-ml volume of a standard solution, 2 μmol/l in 0.01 *M* hydrochloric acid, is added to 1 ml of blank plasma in five replicates which, together with one blank plasma sample, are processed in parallel with authentic samples and with daily quality control samples.

Calculations

Peak-height ratios between the analytes and the internal standard, (*S*)-alprenolol, are calculated for each chromatogram by an electronic integrator, which uses the median value of the calibration samples to estimate plasma concentrations of the authentic samples. Full calibration graphs in the range 10–1000 nmol/l are determined at intervals of 1 month during analysis periods.

The parameters used in the evaluation of the column and separation efficiency are the plate number, *N*, the capacity factor, *k'*, and the separation factor, α:

$$N = 5.54(t_R/W_{1/2})^2$$
$$k' = (t_R - t_o)/t_o$$
$$\alpha = k'_S/k'_R$$

where $W_{1/2}$ = width at peak half-height in minutes, t_R = retention time in minutes and t_o = retention time of injected toluene, which was assumed to be non-retained.

RESULTS AND DISCUSSION

The work-up procedure for metoprolol in plasma was similar to that used in two previous studies [3,10]. The extract from plasma in diethyl ether or diethyl ether–dichloromethane was concentrated by evaporation prior to dissolution in the mobile phase and injection. The two metoprolol enantiomers were separated from each other and from the internal standard, (*S*)-alprenolol, which was eluted in between. Representative chromatograms from blank plasma, and from an authentic human plasma sample are shown in Fig. 2. The metoprolol antipodes are well isolated from plasma components and, despite the relatively simple work-up [7–9], the measurement of small amounts is possible.

The presence of diethylamine in the mobile phase was needed to permit an acceptable chromatographic performance for metoprolol and other amines. The column efficiency, *i.e.*, the plate number, was strongly dependent on the flow-rate (0.25–1.75 ml/min), but temperature (20–40°C) had little influence. The capacity factors decreased with increase in temperature, more rapidly for the later eluting *S* form than for the *R* form, and the separation factor α decreased.

The water content of the organic mobile phase was not discussed in previous papers [7–9] but is of great importance for the chiral separation, as shown in Fig. 3. On going from 200 to 1400 mg/l of water the retention of the *R* enantiomer was only slightly affected whereas the k' value of the *S* form was almost halved and consequently the separation factor, α, decreased from 2.4 to 1.4. The water content of the organic solvents constituting the mobile phase, in particular the alcohol, may vary considerably and in certain instances differences in air humidity are probably of significance.

Overall we found it important to control the water content of the mobile phase in order to ensure a reproducible chiral separation. This was obvious from the sep-

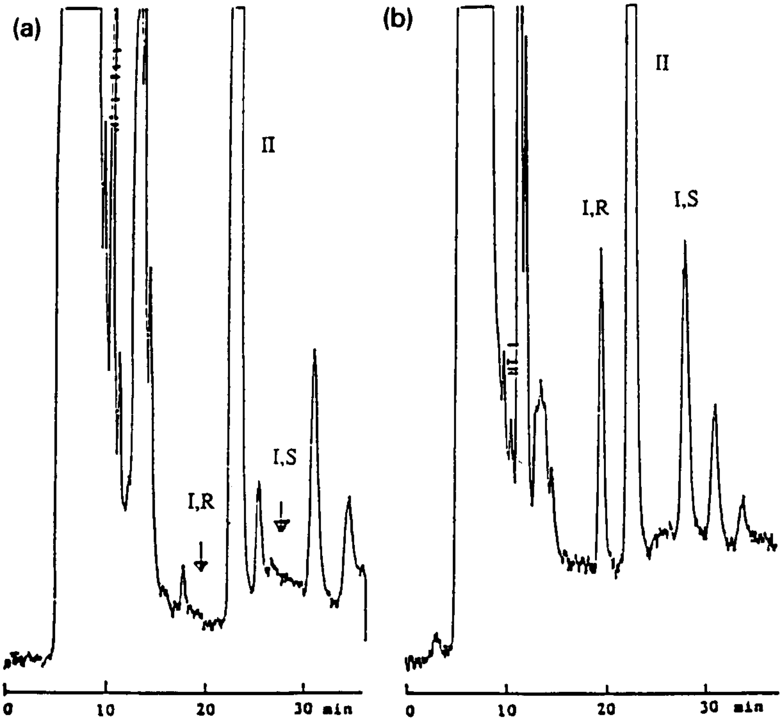


Fig. 2. Separation of metoprolol (I) enantiomers in human plasma. Mobile phase: 4% 1-propanol, 0.1% diethylamine and 1500 mg/l water in hexane; flow-rate, 0.5 ml/min; temperature, 35°C. Integrator attenuation: 8. (a) Blank plasma; (b) authentic plasma, *R* enantiomer 37 nmol/l, *S* enantiomer 47 nmol/l.

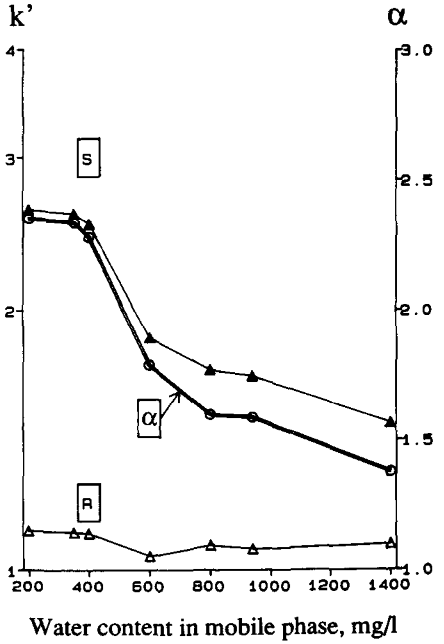


Fig. 3. Effect of water content in the mobile phase on retention (k') and separation factor (α) of (*R*)- and (*S*)-metoprolol. Mobile phase: 10% 1-propanol and 0.1% diethylamine in hexane; temperature, 30°C; flow-rate, 0.75 ml/min.

aration of the four isomers of α-hydroxymetoprolol, the capacity factors of which are plotted in Fig. 4. As can be seen, the water content has to be in the range 600–800 mg/l to enable a complete separation. Even here the *R* forms were influenced to a lesser extent than the *S* isomers. This study was performed with an authentic urine

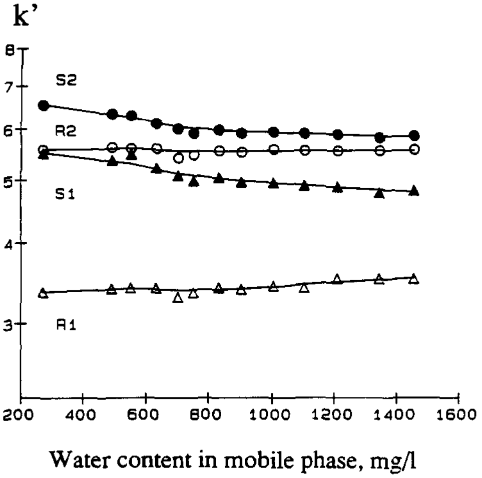


Fig. 4. Effect of water content on retention (k') of the isomers of (*R*)- and (*S*)-α-hydroxymetoprolol. Mobile phase: 10% 1-propanol and 0.1% diethylamine in hexane; temperature, 30°C; flow-rate, 0.75 ml/min.

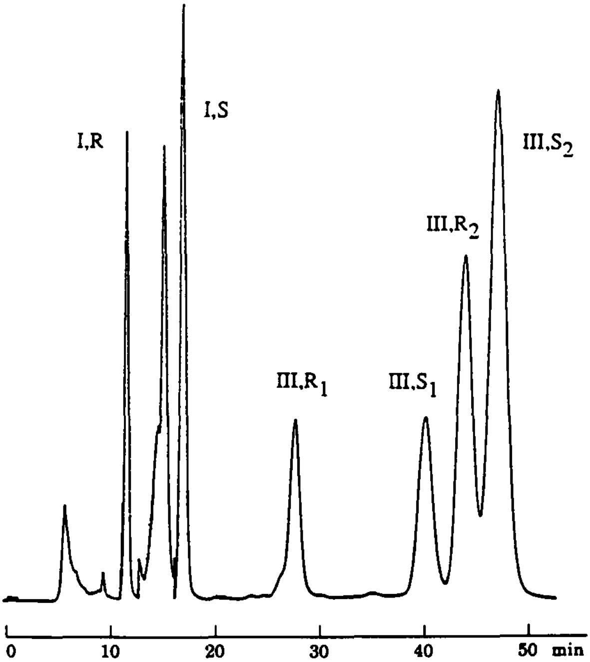


Fig. 5. Chromatogram from a human urine sample containing 1.2 μmol/l metoprolol (I) and 13 μmol/l α-hydroxymetoprolol (III). Integrator attenuation: 512 at start and 128 after 22 min. Mobile phase: 10% 1-propanol, 0.1% diethylamine and 600 mg/l water in hexane; temperature, 25°C; flowrate, 0.5 ml/min.

sample containing the four isomers in different concentrations. *R* and *S* refer to the corresponding metoprolol antipodes.

The concentration of α-hydroxymetoprolol is often low and lower than that of metoprolol in human plasma. The compounds are more easily assayed in human urine. A chromatogram from an authentic sample is shown in Fig. 5, where one pair of metoprolol enantiomers and two pairs of enantiomers of α-hydroxymetoprolol are separated.

In order to track the respective *R* and *S* isomers of α-hydroxymetoprolol, metoprolol racemate and (*S*)-metoprolol were given to two dogs on separate occasions. Chromatograms from the respective administration are shown in Fig. 6a and b and the *R* and *S* peaks can be localized. As can be seen, there is a huge peak in Fig. 6b which appears as a doublet in Fig. 6a. These peaks have the same retention as a diol metabolite, which is postulated as an intermittent degradation product in the dog [11,12] but has not been determined. The chromatogram in Fig. 6a provides evidence of the resolving capability of this Chiracel OD column. The Chiralcel OD columns were found to possess excellent long-term stability and could be used for more than 12 months.

Analytical validation

The absolute recovery was more than 95% for metoprolol and also for the α-hydroxymetabolite after addition of sodium chloride. The metoprolol enantiomers

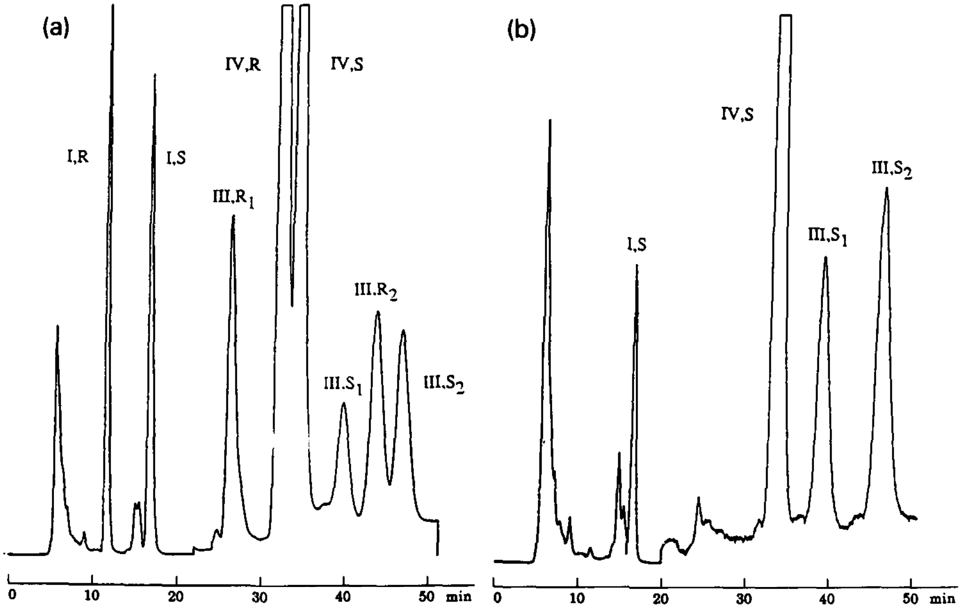


Fig. 6 (a) Chromatogram from dog plasma 0.5 h after dosing with 10 mg/kg of metoprolol racemate. Chromatographic conditions as in Fig. 5. Integrator attenuation: 1024 at start and 64 after 22 min. (b) Chromatogram from dog plasma 0.5 h after dosing with of 5 mg/kg of (*S*)-metoprolol. Chromatographic conditions as in Fig. 5. Integrator attenuation: 512 at start and 128 after 22 min. The numbers I–IV in the chromatograms refer to the compounds listed in Fig. 1.

could be measured in plasma at levels down to 10 nmol/l of each with a relative standard deviation of less than 15%. The calibration graph was linear between 10 and 1000 nmol/l, which covers the therapeutic range. The repeatability (within-day) for the assay of the *R* and *S* enantiomers of metoprolol was 1.3% and 2.2%, respectively, at a level of 100 nmol/l and 3.9% and 6.1%, respectively, at a level of 20 nmol/l (n = 7). The reproducibility (between-day) was 6.4% and 6.1% at 70 nmol/l (n = 10). The α-hydroxymetabolite could be measured at levels down to 50 nmol/l of each isomer. The repeatability for the assay averaged 9.8% at 200 nmol/l (n = 7).

REFERENCES

1 D. B. Campbell, *Acta Pharm. Nord.*, 2 (1990) 217.
2 K. Balmér, B.-A. Persson and G. Schill, *J. Chromatogr.*, 477 (1989) 107.
3 B. A. Persson, K. Balmér, P.-O. Lagerström and G. Schill, *J. Chromatogr.*, 500 (1990) 629.
4 Y. Okamoto, M. Kawashima and K. Hatada, *J. Chromatogr.*, 363 (1986) 173.
5 Y. Okamoto, M. Kawashima, R. Aburatani, K. Hatada, T. Nishiyama and M. Masuda, *Chem. Lett.*, (1986) 1237.
6 H. Y. Aboul-Enein and M. R. Islam, *J. Liq. Chromatogr.*, 13 (1990) 485.
7 D. R. Rutledge and C. Garrick, *J. Chromatogr.*, 497 (1989) 181.
8 M. S. Ching, M. S. Lennard, A. Gregory and G. T. Tucker, *J. Chromatogr.*, 497 (1989) 313.
9 R. J. Straka, K. A. Johnson, P. S. Marshall and R. P. Remmel, *J. Chromatogr.*, 530 (1990) 83.
10 K. Balmér, Y. Zhang, P.O. Lagerström and B.A. Persson, *J. Chromatogr.*, 417 (1987) 357.
11 K. O. Borg, E. Carlsson, K.-J. Hoffmann, T.E. Jönsson, H. Thorin and B. Wallin, *Acta Pharmacol Toxicol.*, 36, Suppl. V (1975) 125.
12 A. Arfwidsson, K. O. Borg, K.J. Hoffmann and I. Skånberg, *Xenobiotica*, 6 (1976) 691.