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The enantiomeric separation of metipranolol and desacetylmetipranolol on a cellulose tris-3,5-dimethylphenyl-carbamate chiral stationary phase

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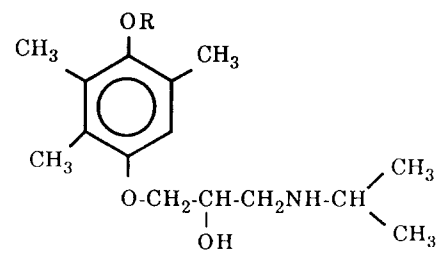
Abstract: A high-performance liquid chromatographic (HPLC) method is described for direct separation of the enantiomers of metipranolol and its principal degradation product and main metabolite, desacetylmetipranolol. Separations are performed on a chiral stationary phase of cellulose tris-3,5-dimethylphenyl carbamate (Chiralcel OD), with hexane–propan-2-ol–diethylamine elution and UV detection at 278 nm. The method affords identification and determination of the optical purity of the bulk drug and its formulated ophthalmic products.

Keywords: *Metipranolol; desacetylmetipranolol; enantioseparation; Chiralcel OD; ophthalmic formulation; radiation sterilization.*

Introduction

Since Philips and associates [1] first reported in 1967 that the β-receptor blocking agent propranolol reduced intraocular pressure, more and more compounds of this group have been investigated and developed to enhance the choice of drugs for the treatment of glaucoma. One of these substances is metipranolol [1-(4-acetoxy-2,3,5-trimethylphenoxy)-3-isopropylaminopropan-2-ol]. Metipranolol is efficacious in the topical treatment of intraocular pressure in patients with chronic open angle glaucoma or ocular hypertension [2]. Desacetylmetipranolol which is the principal degradation product and main metabolite of metipranolol [3] does not significantly differ from metipranolol in its pharmacological activity [4]. Both metipranolol and desacetylmetipranolol exist as enantiomers owing to the presence of an asymmetric carbon atom in the alkanolamine chain.

Due to its known therapeutic effect, metipranolol was selected as a candidate drug for the development of a novel ophthalmic delivery system (NODS) recently launched by Chauvin Pharmaceuticals. The NODS is a solid device for delivering a unit dose of a drug in a non-traumatic way. In formulating this product the drug substance is sterilized by exposure to γ-radiation to ensure minimal microbial growth in the drug suspension during manufacture. Thus, a need was identified for an enantioselective assay for quality control purposes and to investigate the possible effect of γ-radiation sterilization on the enantiomeric composition of metipranolol.



R = CO CH3 (I)
R = H (II)

Structure 1
(I) metipranolol and (II) desacetylmetipranolol.

Over the past decade, a large number of chromatographic methods for the resolution of racemic β-adrenergic blocking agents have been reported and these have been reviewed by a number of authors [5–8]. The separation of β-blockers appears to have been dominated

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by the use of protein-based chiral stationary phases (CSP). Early versions of α_1-acid glycoprotein columns suffered from severe stability and efficiency problems; however, later versions have given significantly improved performance [9]. The direct resolution of racemic β-blocking agents using an unusual polar organic phase in conjunction with cyclodextrin-bonded phases has also been reported [10]. In 1986, Okamoto *et al.* [11, 12] developed and extensively evaluated CSPs based upon cellulose triphenylcarbamate derivatives. It emerged that dimethylphenyl- and dichlorophenyl-carbamates substituted at 3,4- or 3,5-positions displayed significant chiral discrimination towards several β-adrenergic blockers. Surprisingly, however, only two publications have been found that refer to the separation of the isomers of metipranolol, both of which involve the derivatization of metipranolol with a chiral derivatizing reagent and the subsequent separation of the resulting diastereomers on achiral stationary phases [13, 14].

In the present study, the use of cellulose tris-3,5-dimethylphenylcarbamate as a CSP has been investigated for the direct resolution of the enantiomers of metipranolol and desacetylmetipranolol.

Experimental

Materials

(±)-Metipranolol and reference samples of R-(+)- and S-(−)-metipranolol, and (±)-desacetylmetipranolol were obtained from Dr Gerhard Mann (Chem.-pharm. Fabrik GmbH, D-1000 Berlin (Spandau), Germany). Samples of racemic metipranolol, placed in glass containers, were exposed to radiation doses of 25, 50, 100 and 200 kGy at Isotron Ltd (Swindon, UK). Similarly, small amounts of R-(+)- and S-(−)-metipranolol were treated with a radiation dose of 50 kGy.

HPLC grade hexane and propan-2-ol and AR grade ethanol and diethylamine were obtained from Merck Ltd (Poole, UK). Other chemicals used were of AR grade. A 300 × 4.6 mm column packed with 10-μm Chiralcel OD [cellulose tris-3,5-dimethylphenylcarbamate coated on silica gel] was purchased from J.T. Baker (UK) Ltd. All mobile phases were pumped through using an in-line 2-μm filter without prior degassing or filtration. Solutions of (±)-metipranolol (0.6 mg ml^{-1}) were prepared in propan-2-ol. Similarly, quantities of about 3 mg of R-(+)- and S-(−)- were separately dissolved in 10 ml of propan-2-ol.

Hydrolysis of metipranolol

About 6 mg of each of the enantiomers of metipranolol and 12 mg of racemic metipranolol were separately dissolved in 25 ml of methanol–aqueous 0.2% w/v trisodium phosphate (hydrate) (1:1, v/v) with the aid of an ultrasonic bath. The pH value of each solution was 11.3. These solutions were kept in the dark at ambient temperature for about 48 h to effect complete hydrolysis. A 2-ml aliquot of each of the hydrolysates was individually transferred to a small separating funnel. After the addition of one drop of 5 M sodium hydroxide, each solution was shaken successively with 10 and 5-ml volumes of dichloromethane. The lower dichloromethane layer was collected over anhydrous sodium sulphate. The combined extract, in each case, was evaporated to dryness under a gentle stream of nitrogen and reconstituted with 1 ml of propan-2-ol.

Apparatus

The HPLC system comprised a Perkin–Elmer 410 pump (quaternary solvent delivery system) with an Applied Biosystem 757 detector or a Philips 4025 pump with a Philips 4015 UV detector or a Merck-Hitachi L6200 pump with a Merck-Hitachi L6000 UV detector, and a Rheodyne 7125 injector operated manually or via a Gilson 231/401 autoinjector. A Trilab 2000 was used for data handling.

Results and Discussion

Resolution of metipranolol enantiomers

Effect of mobile phase composition on retention and enantioselectivity of metipranolol. The capacity factor (k'), separation factor (α) and resolution (R) of solutes can be regulated by the additon of polar modifiers to the hydrocarbon mobile phases normally used with cellulose-based chiral phases.

Effect of propan-2-ol content of the mobile phase upon enantioselectivity. The effect of propan-2-ol on the peak characteristics of metipranolol enantiomers was investigated by increasing the propan-2-ol concentration of the mixed hexane–propan-2-ol phase from 1 to 10% (v/v). The results obtained are presented in Table 1.

Table 1
Effect of concentration of propan-2-ol in the mobile phase on the separation of metipranolol enantiomers*

Chromatographic parameters	Propan-2-ol concentration (% v/v)			
	1	2	5	10
k'_1	No elution	7.5	1.7	0.7
k'_2	No elution	8.6	2.0	0.8
α	No elution	1.15	1.18	1.14
R	No elution	1.37	0.67	0.38

*Column; Chiralcel OD (30 cm × 4.6 mm). Mobile phase; hexane–propan-2-ol with 0.1% (v/v) diethylamine. Flow-rate; 0.5 ml min^{-1}. Temperature; ambient. Detection; 278 nm.
†k'_1 = capacity factor of first-eluted peak; k'_2 = capacity factor of second-eluted peak; α = separation factor; R = resolution.

Table 2
Effect of ethanol content, with or without propanol-2-ol, in the mobile phase on the retention and separation of metipranolol enantiomers*

Chromatographic parameters†	Concentration (% v/v) Ethanol	propanol-2-ol	Ethanol	propanol-2-ol	Ethanol	propanol-2-ol	Ethanol	propanol-2-ol
	1	2	1	1	2	0	3	0
k'_1	3.6		5.5		5.8		2.8	
k'_2	4.0		6.0		6.3		2.9	
α	1.11		1.09		1.09		1.03	
R	0.69		0.56		0.72		0.29	

*Column; Chiralcel OD (30 cm × 4.6 mm). Mobile phase; hexane–ethanol/propan-2-ol with 0.1% (v/v) diethylamine. Flow-rate; 0.5 ml min^{-1}. Temperature; ambient. Detection; 278 nm.

Effect of using ethanol as a mobile phase modifier. The effect of the concentration of ethanol on the separation was studied by varying its concentration from 1 to 3% (v/v) with and without propan-2-ol, in the mobile phase. The results are given in Table 2.

The effects upon chromatographic performance of changes of propan-2-ol and ethanol content of the mobile phase summarized in Tables 1 and 2 indicate that no elution (up to about 90 min) from the column was observed when a mobile phase containing 1% (v/v) propan-2-ol was used. Increasing the concentration of propan-2-ol to 2% (v/v) resulted in the elution of a pair of almost resolved peaks. Further increases in the proportion of propan-2-ol resulted in increasingly sharp peaks with a progressive decrease in the values for capacity factor (k'). The resolution between the peaks due to the metipranolol enantiomers decreased from 1.37, with 2% (v/v) propan-2-ol, to 0.38 with 10% (v/v) propan-2-ol as mobile phase modifier. The addition of 1% ethanol to the hexane-propan-2-ol (98:2, v/v) eluent also led to a reduction of the capacity factors and the resolution of the peaks. With 1% each of propan-2-ol and ethanol, the separation factor (α) was almost identical to that with a mobile phase comprising hexane–propan-2-ol–ethanol (97:2.1, v/v/v). However, the broad nature of the peaks caused a further loss in resolution. The mobile phase compositions containing only ethanol as the polar modifier did not yield encouraging results, although the resolution factor increased markedly from 0.29 to 0.72 by reducing the proportion of ethanol from 3 to 2% (v/v).

Effect of using diethylamine as a residual silanol deactivator. The influence of diethylamine on the enantioseparation of metipranolol was assessed by varying its proportion (0.0–0.2%) in the mobile phase consisting of hexane–propan-2-ol (98:2, v/v). The results are shown in Table 3.

A resolution factor (R) of only 0.26 and tailing peaks were observed without diethylamine present in the mobile phase. A concentration of 0.1% (v/v) diethylamine in the mobile phase effected the separation of the optical antipodes of metipranolol with a resolution factor (R) of about 1.4. There was no apparent further improvement in resolution between the peaks on doubling the concentration of diethylamine. The peaks were almost

Table 3
Effect of diethylamine on the chromatography of metipranolol enantiomers*

Chromatographic parameters†	Diethylamine (% v/v)		
	0.0	0.1	0.2
k'_1	7.9	7.5	7.8
k'_2	9.0	8.6	8.8
α	1.14	1.15	1.13
R	0.26	1.37	1.40
T	‡Tailing peaks	1.1	1.1

*Column; Chiralcel OD (30 cm × 4.6 mm). Mobile phase; hexane–propan-2-ol (98:2, v/v). Flow-rate; 0.5 ml min^{-1}. Temperature; ambient. Detection; 278 nm.

†k'_1, k'_2, α and R as in Table 1. T = tailing factor at 5% of the peak height.

‡The tailing factor could not be measured because of the overlapping peaks.

Table 4
Effect of flow-rate on the retention behaviour of metipranolol enantiomers*

Chromatographic parameters†	Flow-rate (ml min^{-1})		
	0.5	0.7	0.9
k'_1	7.5	5.3	6.9
k'_2	8.6	6.6	8.1
α	1.15	1.25	1.18
R	1.37	2.00	1.90

*Column; Chiralcel OD (30 cm × 4.6 mm). Mobile phase; hexane–propanol-2-ol–diethylamine (98:2:0.1, v/v/v). Temperature; ambient. Detection; 278 nm.

†k'_1, k'_2, α and R as in Table 1.

symmetrical with a tailing factor (T) of only 1.1 with 0.1 or 0.2% (v/v) diethylamine in the mobile phase. These results clearly demonstrate the effectiveness of the masking of the residual silanols in the stationary phase by the secondary amine.

A concentration of up to 1% diethylamine can be incorporated in the mobile phase, according to information provided by the supplier of Chiralcel columns. However, the maximum concentration was restricted to 0.2% (v/v) to minimize any destructive effects of the basic mobile phase on the ester groups contained in the CSP.

Effect of mobile phase flow-rate. A mobile phase comprising hexane–propan-2-ol-diethylamine (98:2:0.1, v/v/v) was pumped at flow-rates of 0.5, 0.7 and 0.9 ml min^{-1}. The retention behaviour of the enantiomers of metipranolol under these conditions is shown in Table 4.

The mobile phase of hexane–propan-2-ol–diethylamine (98:2:0.1, v/v/v) delivered at a flow rate of 0.7 ml min^{-1} produced a resolution of 2.0 between the peaks of the metipranolol enantiomers, with capacity

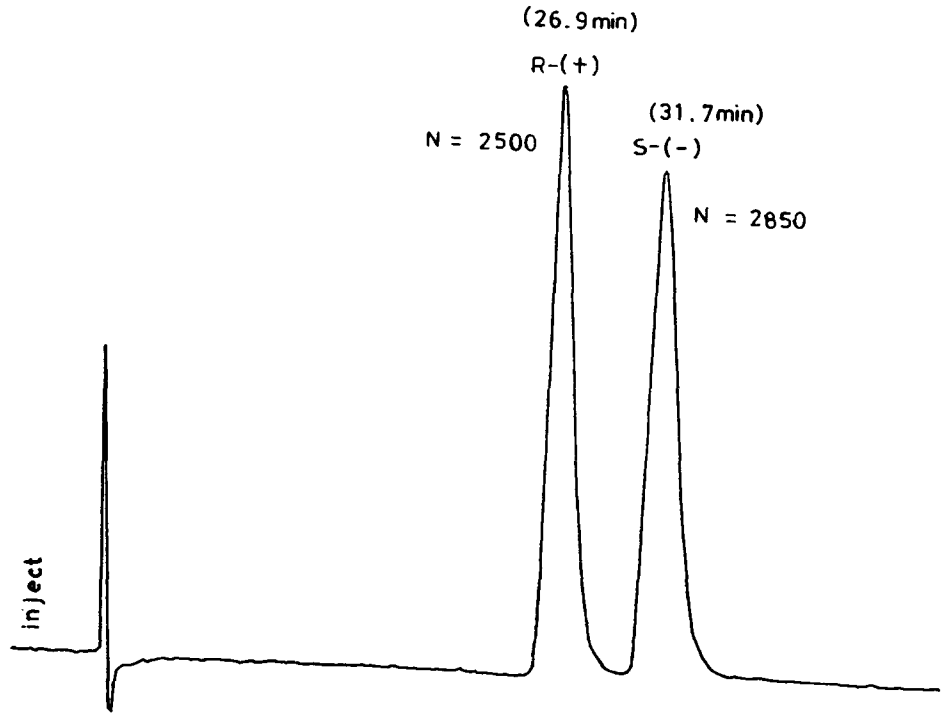

Figure 1
Enantiomeric separation of metipranolol. Column; Chiralcel OD (30 cm × 4.6 mm). Mobile phase; hexane–propan-2-ol–diethylamine (98:2:0.1, v/v/v). Flow-rate; 0.7 ml min^{-1}. Detection; 278 nm.

factors 5.3 and 6.6, respectively, compared to a resolution factor of 1.37 at a flow-rate of 0.5 ml min^{-1}. The separation achieved by operating the Chiralcel OD column under these conditions, shown in Fig. 1, was considered to be adequate for the study of the effects of radiolysis. By comparing the retention characteristics of the peaks in the above chromatogram with those of the authentic stereoisomers of metipranolol, the first eluting peak ($k' = 5.3$) was assigned to R-(+)-metipranolol and the second peak ($k' = 6.6$) was assigned to S-(−)-metipranolol.

Stereoselective separation of desacetylmetipranolol

The separation of enantiomers of desacetylmetipranolol was investigated using mobile phases containing 10 and 5% (v/v) propan-2-ol delivered at 0.5 ml min^{-1} and containing 6% (v/v) propan-2-ol, but delivered at 0.8 ml min^{-1}. The resolving power of the Chiralcel OD column for the two optical isomers of desacetylmetipranolol under the three arbitrarily chosen operating conditions is illustrated by the data given in Table 5.

The HPLC operating conditions for the enantio-discrimination of desacetylmetipranolol were not optimized. However, the limited experimental data obtained, as summarized in Table 5, indicate that the elution rate and resolution of the peaks of the desacetylmetipranolol pair are strongly influenced by the presence of propan-2-ol in the mobile phase. The resolution of the isomers of desacetylmetipranolol were as high as 3.14 and 4.12 with 10% (v/v) and 5% (v/v) propan-2-ol in the mobile phase, respectively, compared to 0.38 and 0.67 in the case of metipranolol. It appears that a concentration higher than 10% (v/v) propan-2-ol in the mobile phase delivered

Table 5
Chromatographic functions illustrating the separation of enantiomers of desacetylmetipranolol*

Chromatographic parameters†	Operating conditions A	B	C
k'_1	0.8	3.9	5.1
k'_2	1.4	6.7	9.0
α	1.75	1.72	1.77
R	3.14	4.12	4.48

*Column; Chiralcel OD (30 cm × 4.6 mm). Mobile phase; hexane–propan-2-ol–diethylamine, A — 90:10:0.1 (v/v/v) 0.5 ml min^{-1}, B — 95:5:0.1 (v/v/v) 0.5 ml min^{-1} and C — 94:6:0.1 (v/v/v) 0.8 ml min^{-1}. Temperature; ambient. Detection; 278 nm.

†k'_1, k'_2, α and R as in Table 1.

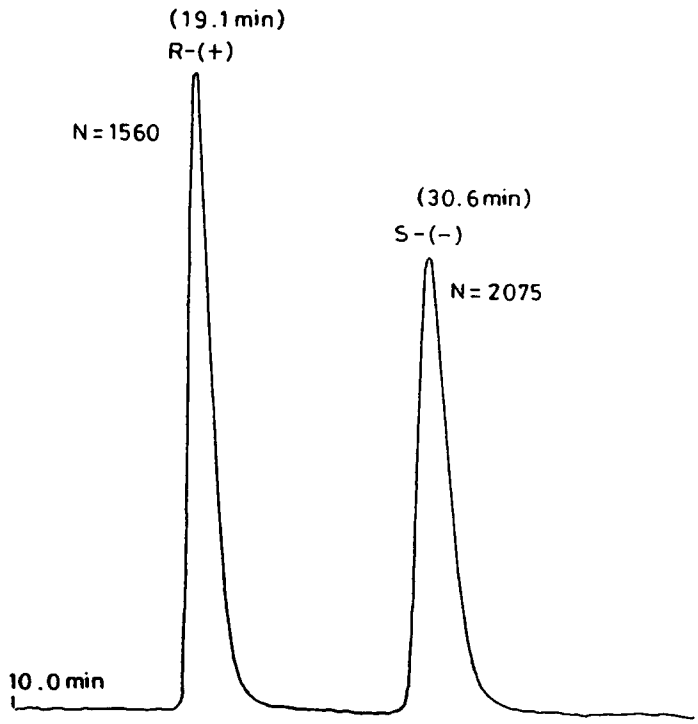

Figure 2
Enantiomeric separation of desacetylmetipranolol. Column; Chiralcel OD (30 cm × 4.6 mm). Mobile phase; hexane–propan-2-ol–diethylamine (94:6:0.1, v/v/v). Flow-rate; 0.8 ml min^{-1}. Detection; 278 nm.

at a flow-rate of 0.5 ml min^{-1} can be tolerated for a baseline separation between the two peaks due to desacetylmetipranolol. The enantio-resolution of desacetylmetipranolol with a mobile phase composition of hexane–propan-2-ol–diethylamine (94:6:0.1, v/v/v) pumped at a flow-rate of 0.8 ml min^{-1} is illustrated in Fig. 2.

Identification of the peaks for R- and S-desacetylmetipranolol. It is well known that an ester group can be subjected to hydrolysis by a base in an aqueous-methanolic medium. Hydrolysis proceeds at ambient temperature with the rate increasing with temperature. Thus, samples of the individual isomers and racemic metipranolol were base hydrolysed to produce the corresponding forms of desacetylmetipranolol, which were then extracted from the aqueous phase into dichloromethane. It was assumed that hydrolysis did not affect the chirality. The residue remaining after evaporation of the dichloromethane was reconstituted with propan-2-ol. Chromatography of the extracts together with that of a solution of a sample of desacetylmetipranolol was performed using a Chiralcel OD column with a mobile phase of hexane–propan-2-ol–diethylamine (95:5:0.1, v/v/v) at a flow-rate of 0.8 ml min^{-1}. The capacity factors of the major peaks observed in chromatograms of the above solutions are given in Table 6.

The retention characteristics of the two major peaks in the chromatogram of hydrolysed (±)-metipranolol appeared to be identical to the corresponding peaks in the chromatogram of a solution of racemic desacetylmetipranolol. It should also be mentioned that additional peaks were detected near the system peak in the chromatograms of hydrolysed metipranolol, probably because of further degradation of desacetylmetipranolol in the alkaline condition of the reaction. However, the peak counts of the two major peaks in the hydrolysed racemic metipranolol were similar indicating that the hydrolysis reaction proceeded at the same rate for both isomers of metipranolol. The capacity factors of peaks due to hydrolysed R-(+)- and S-(−)-metipranolol corresponded to those of the first and second peak, respectively, of racemic desacetylmetipranolol and the hydrolysed racemic metipranolol. There was no evidence of the presence of S-(−)-enantiomer in the chromatogram of hydrolysed R(+)-metipranolol and vice versa.

The Chiralcel OD column exhibited effective chiral recognition towards both metipranolol and desacetylmetipranolol. Although the binding energies due to the pi-pi- interaction of phenyl groups on the CSP with the aromatic group of the solutes and partial inclusion may have been important, the mechanism of enantio-separation appears to be due mainly to interaction of the solute with the polar carbamate functions of the CSP via hydrogen bonding with —NH and C=O groups and the dipole–dipole interaction on C=O. The presence of a phenolic group in the desacetylmetipranolol provides an additional point of interaction for hydrogen bonding which may explain the longer retention and greater enantio-discriminating power of Chiralcel OD towards desacetylmetipranolol compared to metipranolol, as indicated by resolution data shown in Fig. 3.

Simultaneous separation of the enantiomers of metipranolol and desacetylmetipranolol

Isocratic elution. A solvent mixture of hexane–propan-2-ol (98:2, v/v) containing 0.1% diethylamine produced a baseline separation of the enantiomers of metipranolol after 30 min, whereas the desacetylmetipranolol enantiomers required a further 100 min for elution. Thus, the total run time for isocratic separation of the pairs of metipranolol and desacetylmetipranolol was about 130 min. In

Table 6
Capacity factors of the principal peaks observed for hydrolysed R-(+)-, S-(−)- and (±)-metipranolol, and for (±)-desacetylmetipranolol*

	Hydrolysed metipranolol			
	R-(+)	S-(−)	Racemate (±)	Desacetylmetipranolol racemate (±)
k'_1	4.8	—	4.8	4.8
k'_2	—	8.7	8.7	8.7

*Column; Chiralcel OD (30 cm × 4.6 mm). Mobile phase; hexane–propan-2-ol–diethylamine (95:5:1.0, v/v/v). Flow-rate; 0.8 ml min^{-1}. Detection; 278 nm.

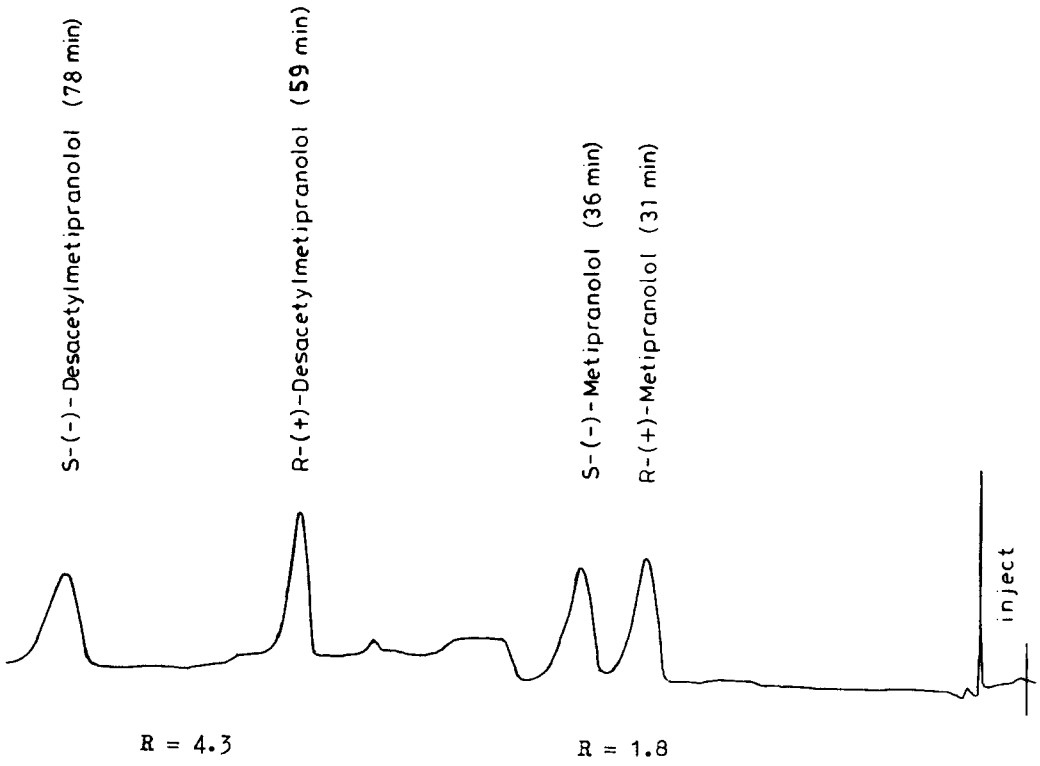

Figure 3
Solvent programming: simultaneous separation of the pairs of metipranolol and desacetylmetipranolol. Column: Chiralcel OD 30 cm × 4.6 mm. Detection: 278 nm.

	(min)	Hexane containing 0.1% diethylamine	Propan-2-ol	Flow-rate (ml min^{-1})	Solvent composition curve
Step 1	30	98	2	1	
Step 2	50	95	5	1	0 (immediate change)

Table 7
The effect of γ-radiation on the enantiomeric composition of racemic metipranolol*

Radiation dose (kGy)	% Peak area	
	R-(+)-metipranolol	S-(−)-metipranolol
0	49.5	50.5
	50.2	49.8
Mean	49.9	50.1
25	50.1	49.9
	50.5	49.5
Mean	50.3	49.7
50	49.7	50.3
	50.2	49.8
Mean	50.0	50.0
100	49.9	50.1
	49.7	50.3
Mean	49.8	50.2
200	50.6	49.4
	50.3	49.7
Mean	50.5	49.6

*Column; Chiralcel OD (30 cm × 4.6 mm). Mobile phase; hexane–propan-2-ol–diethylamine (98:2:0.1, v/v/v). Flow-rate; 0.7 ml min^{-1}. Temperature; ambient. Detection; 278 nm.

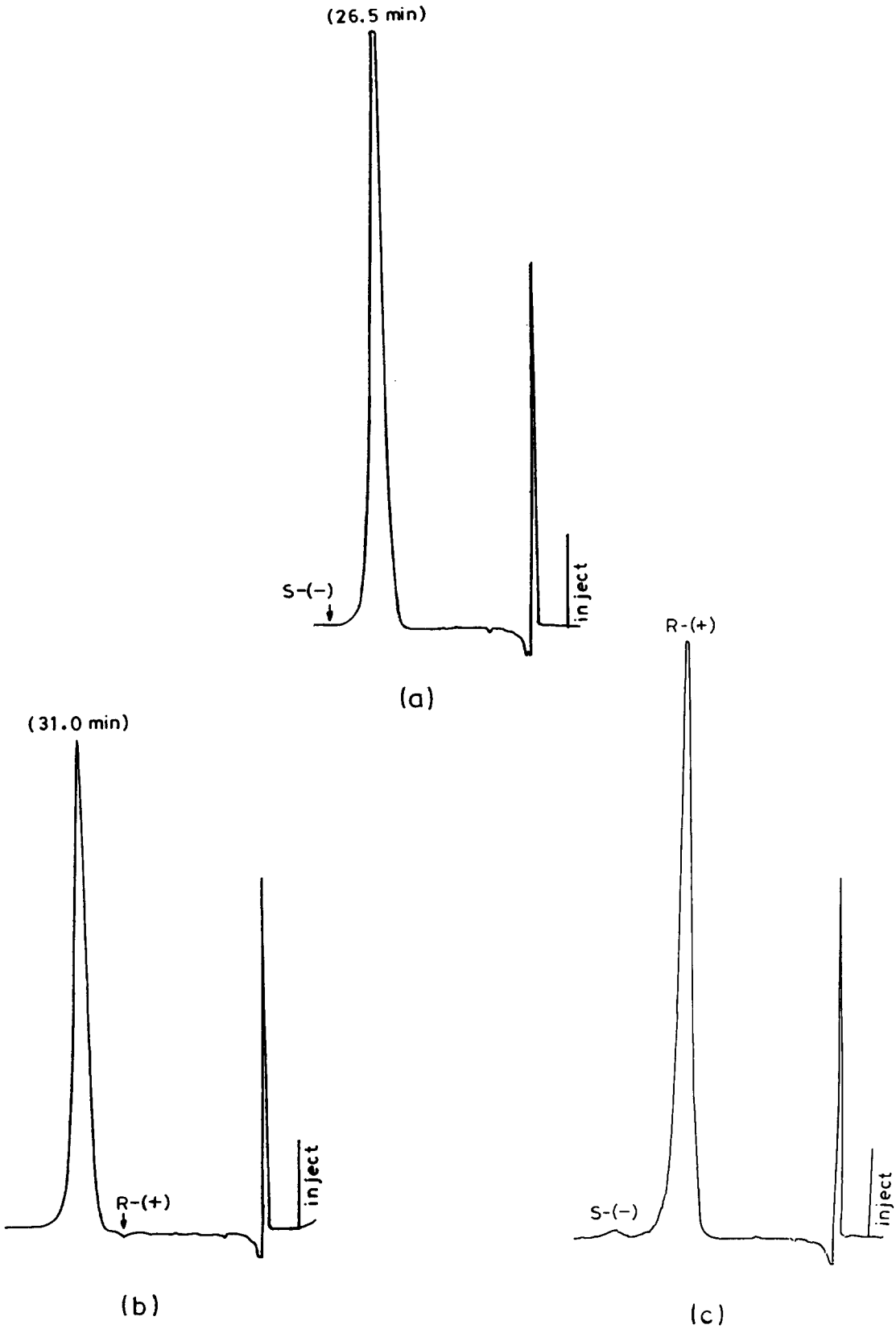

Figure 4
Chromatograms of (a) R-(+)- and (b) S-(−)- metipranolol treated with a radiation dose of 50 kGy, and (c) showing the detection of 2% w/w enantiomeric impurity. Column; Chiralcel OD (30 cm × 4.6 mm). Mobile phase; hexane–propan-2-ol–diethylamine (98:2:0.1, v/v/v). Flow-rate 0.7 ml min^{-1}. Detection; 278 nm.

order to speed up the separation a gradient elution routine was considered.

Solvent programming. The elution and separation of the pairs of metipranolol and desacetylmetipranolol with a step gradient programme is shown in Fig. 3.

Investigation of the effect of ionizing radiation on the enantiomeric ratio of metipranolol. Solutions of racemic metipranolol exposed to various doses of γ-radiation (0–200 kGy) were prepared in propan-2-ol (*ca* 0.6 mg ml^{-1}) and analysed by injecting 10 μl of solution under the selected HPLC conditions, namely a Chiralcel OD column with hexane–propan-2-ol–diethylamine (98:2:0.1, v/v/v) and elution at 0.7 ml min^{-1}. Only two peaks were observed in each of the chromatograms; the corresponding percent peak areas for the individual isomers are presented in Table 7. These results indicate that the enantiomeric composition of metipranolol is not affected by the levels of exposure to γ-radiation.

Study of the effect of ionizing radiation on stereo-inversion of a single enantiomer. Although the irradiation experiments involving the racemate suggest that no stereo-inversion has occurred the evidence is not conclusive. Accordingly the individual R- and S-enantiomers of metipranolol were subjected to a radiation dose of 50 kGy. In each case only one chromatographic peak was observed (Fig. 4) with no evidence of the other isomer. Under the conditions employed, independent experiments revealed that a minimum of 2% w/w of enantiomeric impurity would be readily detected. This evidence strengthens the conclusion that radiation sterilization does not affect the enantiomeric composition of the racemic mixtures of metipranolol normally used in ophthalmic dosage forms.

Conclusions

The direct HPLC separation of the enantiomers of metipranolol and desacetylmetipranolol was achieved using a Chiralcel OD CSP (cellulose tris-3,5-dimethylphenylcarbamate) column with hexane–propan-2-ol–diethylamine elution. In both cases, the R-(+)-enantiomer was eluted before the corresponding S-(−) isomer. The analysis of irradiated samples of racemic metipranolol (0–200 kGy) showed no evidence of a change in the enantiomeric composition. Furthermore, a radiation dose of 50 kGy was found to induce no chiral inversion. The chiral LC method offers a reliable means of identification of metipranolol and desacetylmetipranolol enantiomers and determination of isomeric purity in the bulk drug and in pharmaceutical dosage forms.

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