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Enantioselective determination of oxprenolol in human plasma using dialysis coupled on-line to reversed-phase chiral liquid chromatography[1]

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Abstract

A fully automated method for the determination of the enantiomers of oxprenolol in human plasma was developed, involving dialysis through a cellulose acetate membrane, clean-up and enrichment of the dialysate on a short precolumn and subsequent chiral liquid chromatographic (LC) analysis. All sample handling operations were executed automatically by a sample processor equipped with a robotic arm (ASTED system). The trace enrichment column (TEC) was packed with octadecylsilica. After conditioning of the TEC with the LC mobile phase and pH 3.0 acetate buffer, the sample was dialysed in the static-pulsed mode. The donor and acceptor solutions were made of pH 3.0 acetate buffer. After the enrichment step, the analyte was transferred by the LC mobile phase to the analytical column by means of a switching valve. The influence of different parameters of the dialysis process on the recovery of oxprenolol was first investigated using achiral LC conditions. The volume as well as the aspirating and dispensing flow rates of the acceptor solution were the main parameters studied. Oxprenolol was separated on a C18 stationary phase used for the enantioseparation of oxprenolol was a Chiralcel OD-R column which contained cellulose tris (3,5-dimethylphenylcarbamate) coated on silica as chiral selector. The corresponding mobile phase consisted of a mixture of pH 6.0 phosphate buffer containing $NaClO_4$ at 0.45 M concentration and acetonitrile (70:30 v/v). UV detection was performed at 273 nm. The method developed was validated. Recoveries for each enantiomer of oxprenolol were about 80%. The method was found to be linear in the 50–2500 ng ml^{-1} concentration range ($r^2 = 0.999$ for both enantiomers) and good results with respect to intra- and inter-day reproducibility as well as accuracy were obtained. © 1997 Elsevier Science B.V.

Keywords: Chiral analysis; Oxprenolol; Cellulose-based chiral stationary phase; Dialysis; Sample preparation; Plasma

1. Introduction

Oxprenolol is a non selective β-blocking drug marketed as a racemic mixture of its two enantiomers. As the β-adrenergic blocking activity is mainly dependent of the *S*-(−) enantiomer [1], it

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is important to measure plasma concentrations of the enantiomers separately.

Several methods for the determination of oxprenolol in biological fluids were described: they involve either gas chromatography [2–7], micellar electrokinetic capillary chromatography (MEKC) [8] or liquid chromatography (LC) [9–14].

For the enantioselective analysis of oxprenolol in biological samples, only two methods were reported: the first is a LC assay on a C-18 stationary phase for oxprenolol enantiomers in plasma using a chiral derivatization reagent (*S*-(−)-1-(1-naphtyl)-ethyl isocyanate) and fluorometric detection [15] and the second a capillary electrophoretic (CE) method for oxprenolol enantiomers in human urine using hydroxypropyl-β-cyclodextrin as chiral additive in the running buffer [16].

Generally the sample preparation was based on a liquid–liquid extraction (LLE) of the analyte, followed in most cases by a back-extraction into an acidic aqueous solution [9–13]. A method involving the isolation of oxprenolol by liquid–solid or solid-phase extraction (SPE) was also described [14].

An interesting alternative to these tedious and time-consuming techniques consists of using dialysis as sample preparation step prior to LC. This technique has been applied to plasma, serum, whole blood and tissue homogenate samples [17–22]. This on-line combination of dialysis to LC involves a clean-up and trace enrichment step, making it possible to load untreated plasma samples directly onto the LC autosampler [17]. The use of a cellulose acetate membrane (molecular weight cut-off: 15 000 Dalton) the elimination of proteins and other macromolecules from plasma samples. The dialysate is then flushed to a trace enrichment column (TEC) on which the analyte is concentrated while more polar compounds are eluted. By means of a switching valve, the analyte is desorbed from the TEC by the LC mobile phase and transferred to the analytical column. All these sample handling operations can be automatically performed by an ASTED system (automated sequential trace enrichment of dialysate).

For the determination of the enantiomers of β-adrenergic blocking agents in general, different chiral LC procedures were used [23]. Most of them were based on the use of a chiral stationary phase (CSP) in the normal or reversed-phase mode. Cellulose tris (3,5-dimethylphenylcarbamate) can be used in the reversed-phase mode (Chiralcel OD-R) and appears thus as a very useful CSP for enantioselective determinations in biological fluids. This chiral selector was previously used for the enantiomeric separation of various drugs by LC using mixtures of aqueous buffer and organic modifier as mobile phases [24–29].

The method described here for the enantioselective determination of oxprenolol in human plasma involves on-line sample preparation using dialysis followed by precolumn clean-up and enrichment of the dialysate and subsequent chiral LC analysis on a cellulose tris (3,5-dimethylphenylcarbamate) (Chiralcel OD-R) based stationary phase.

The influence of different parameters of the dialysis procedure on the recovery of oxprenolol was studied using achiral LC conditions. The volume, the aspirating and dispensing flow rates of the dialysis liquid were the main parameters investigated. The breakthrough volumes of oxprenolol from trace enrichment columns were determined for three types of sorbents. These different parameters were optimized with respect to oxprenolol recovery.

In chiral LC separation of oxprenolol, the effect of sodium perchlorate concentration in the mobile phase on the enantioseparation of oxprenolol was more particularly investigated. Finally, dialysis and chiral LC conditions were combined and this fully automated method developed for the determination of oxprenolol enantiomers in plasma was validated.

2. Experimental

2.1. Chemical and reagents

Racemic oxprenolol was purchased from Sigma Chemical (Saint-Louis, MO, USA) and used without further purification. *R*-(+)-oxprenolol and *S*-(−)-oxprenolol were prepared using the simulated moving bed (SMB) technology [30–32] and were then purified by recrystalization. Each enantiomer was identified by polarimetric measurement.

Sodium acetate, glacial acetic acid, sodium dihydrogen phosphate dihydrate, disodium hydrogen phosphate dihydrate, sodium hydroxide and sodium perchlorate monohydrate were all of analytical grade from Merck (Darmstadt, Germany).

Methanol and acetonitrile were of HPLC grade from Janssen Chimica (Geel, Belgium). Water used in all experiments was of Milli-Q quality (Millipore, Bedford, MA, USA). The LiChroCart analytical column used during the development of the dialysis process was prepacked with Superspher 100 RP-18 (particle size: 4 μm) and the LiChroCart guard column with LiChrospher 100 RP-18 (5 μm) from Merck.

The chiral stationary phase used for the enantioseparation of oxprenolol was a Chiralcel OD-R column filled with cellulose tris (3,5-dimethylphenylcarbamate) coated on silica, from Daicel Chemical (Tokyo, Japan). The latter was preceded by a LiChroCart guard column, prepacked with LiChrosper 100 DIOL (5 μm) from Merck.

The sorbents used in the trace enrichment column were Hypersil RP-18 (10 μm) prepacked TECs from Gilson Medical Electronics), Nucleosil CN (30 μm) from Macherey-Nagel (Düren, Germany) and LiChrospher 100-CN (5 μm) from Merck.

2.2. Apparatus

The chromatographic instrumentation comprised a model 305 pump from Gilson (Villiers-le-Bel, France) and a model Dynamax UV-1 Variable Wavelength UV/Visible Absorbance detector from Rainin Instrument (Woburn, MA, USA), which was set at a wavelength of 273 nm.

The sample preparation system was a Gilson ASTED XL unit consisting of an autosampler, two 1 ml model 401 dilutors and a dialyser with a donor channel volume of 360 μl and an acceptor channel volume of 650 μl, fitted with a Cuprophan membrane (cellulose acetate) with a molecular cut-off of 15 000 Daltons.

Three kinds of trace enrichment columns (TECs) prepacked with cyanopropyl silica (11 × 4 mm i.d.—Nucleosil CN), with cyanopropyl silica (4 × 4 mm i.d.—Lichrospher 100-CN) or with octadecylsilica (10 × 4 mm i.d.—Hypersil RP-18) were tested. An automated six-port valve (model 7010, Rheodyne, Berkeley, CA, USA) was used to connect the TEC either to the acceptor channel of the dialysis cell or the mobile phase used in the analytical column.

In the achiral determination of oxprenolol, a Manu-Cart system which consisted of a LiChroCart analytical column (125 × 4 mm i.d.) and a short LiChroCart guard column (4 × 4 mm i.d.) from Merck was thermostatted at 35 ± 0.1°C in a model 20B/VC Julabo waterbath (Seelbach, Germany). For the analysis of oxprenolol enantiomers, the LC system consisted of a Chiralcel OD-R column (250 × 4.6 mm i.d.) from Daicel and a short LiChroCart guard column (4 × 4 mm i.d.) from Merck. This system was maintained at 25 ± 0.1°C using the same waterbath as in the achiral LC system.

An IBM compatible computer (CPU type 80486) equipped with '715 HPLC System Controller' and the '772 Keypad' softwares from Gilson was used to control the LC and the ASTED systems, respectively.

2.3. Chromatographic conditions

The mobile phase used for the achiral LC determination of oxprenolol consisted of a mixture of acetonitrile, 2-aminoheptane and 0.01 M acetate buffer adjusted to pH 3.0 with acetic acid (8:0.5:92 v/v/v).

The mobile phase used for the enantioseparation of oxprenolol consisted of a mixture of a 50 mM phosphate buffer containing sodium perchlorate (0.45 M) adjusted to pH 6.0 with a 10% NaOH solution if necessary and acetonitrile (70:30 v/v).

Before use, the mobile phases were degassed for 15 min in an ultrasonic bath. The flow rate was 1.0 ml min^{-1} in achiral LC and 0.9 ml min^{-1} in chiral LC.

2.4. Dialysis conditions

The priming solutions for the donor and acceptor sides of the dialysis cell consisted of 0.01 M acetate buffer of pH 3.0.

2.5. Standard solutions

2.5.1. Solutions used for the determination of breakthrough volumes

The stock solution was prepared by dissolving 50 mg of racemic oxprenolol in 50 ml of methanol. This solution was stored in a refrigerator at 4°C.

The solution used for the determination of breakthrough volumes was prepared by diluting 200 μl of the stock solution into a final volume of 300 ml of pH 3.0 acetate buffer or of pH 8.0 phosphate buffer (0.67 μg ml^{-1}).

2.5.2. Solutions used for the development of the dialysis process

The stock solution of racemic oxprenolol was diluted with water to obtain a final concentration of 2 μg ml^{-1}.

2.5.3. Solutions used for the development of the chiral chromatography

The stock solution of racemic oxprenolol was diluted with water to obtain a final concentration of 10 μg ml^{-1}.

2.5.4. Solutions used for method validation

The stock solution of *R*-(+)-oxprenolol was prepared by dissolving 10 mg of this enantiomer in 10 ml of methanol. This solution was then diluted with water to obtain a final concentration of 5 μg ml^{-1}. The solution of *S*-(−)-oxprenolol was prepared in the same way.

Three solutions were prepared by diluting the stock solution of racemic oxprenolol with water to achieve concentrations of 200, 20 and 2 μg ml^{-1}, respectively. These three solutions were used to spike plasma samples (2.0 ml) for calibration curves (from 50 to 2500 ng ml^{-1} of each enantiomer).

2.6. Sample preparation

After centrifugation of the plasma sample at 4000 rpm for 10 min, a 500 μl volume of plasma was transferred into a vial placed on the appropriate rack of the ASTED XL system. All sample handling operations were then executed automatically by the sample processor.

2.6.1. Washing

2.0 ml of pH 3.0 acetate buffer were used for washing the donor channel of the dialyser (flow rate: 3.0 ml min^{-1}) while the acceptor channel was washed with 2.0 ml of the same buffer.

2.6.2. TEC conditioning

The TEC was first conditioned twice with 0.995 ml of HPLC mobile phase (flow rate: 3.0 ml min^{-1}) and then with 0.995 ml of pH 3.0 acetate buffer.

2.6.3. Dialysis

Dilutor 1 was used to introduce the plasma samples into the donor channel of the dialysis cell. A 370 μl volume of plasma was aspirated (1.0 ml min^{-1} by the autosampler needle and dispensed in the donor channel (3.0 ml min^{-1}). The sample was then kept static while dilutor 2 pumped ten portions of 1 ml of the acceptor solution into the dialyser (1.0 ml min^{-1} and dispensed simultaneously the dialysate onto the TEC in the pulse mode (1.0 ml min^{-1}).

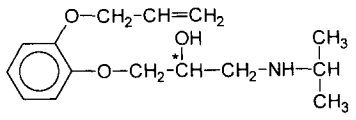
2.6.4. Injection and washing

When dialysis was discontinued, the analyte retained on the TEC was eluted in the back-flush mode with the LC mobile phase by rotation of the switching valve for 2 min. While the analyte was chromatographed on the analytical column, the TEC was regenerated and the preparation of a new sample was started.

3. Results and discussion

3.1. Achiral and chiral LC conditions

Oxprenolol is a basic compound ($pK_a = 9.5$) containing a secondary amino group (Fig. 1) and therefore having a tendency to interact with the free silanol groups at the surface of the alkyl-bonded stationary phase. The addition of 2-aminoheptane distilled twice [33] was found to decrease the retention of oxprenolol and improve the symmetry of the corresponding peak. The acetonitrile content of the mobile phase was adjusted to obtain sufficient retention for oxprenolol



Oxprenolol

Fig. 1. Structure of oxprenolol.

and avoid interferences from endogenous components in the achiral LC analysis of this compound in plasma [34].

In chiral reversed-phase LC systems, with Chiralcel OD-R as CSP, the pH, the nature of buffer ions and the organic modifier concentration of the mobile phase were found to influence the retention and enantioseparation of basic and acidic chiral compounds [24]. The enantiomeric separation of acidic compounds was generally obtained at low pH (pH < 3.0) where these compounds were mainly present in uncharged form and the presence of the anion perchlorate resulted in good enantioselectivity for most basic compounds in the pH range where they were fully ionized.

Table 1 illustrates the effect of the concentration of sodium perchlorate added to the mobile phase (pH 6.0 phosphate buffer—acetonitrile, 70:30 v/v) on the retention and separation of oxprenolol enantiomers. An increase of the sodium perchlorate concentration gives rise to an increase in the capacity ratios of both enantiomers but also to an improvement of enantioselectivity and enantioresolution. By using sodium perchlorate in the higher concentration range (0.4–0.5 M), resolution values higher than two were obtained for oxprenolol enantiomers. Since only a very limited increase in chiral resolution was observed at perchlorate concentrations higher than 0.45 M, the latter concentration was selected. The influence of sodium perchlorate concentration on retention and enantioselectivity, which is particularly important in these chiral LC systems, could be explained either by an ion-pairing process, related to the concept of chaotropicity [24] or by an salting-in effect which could modify hydrophilic interactions between solute and mobile phase [29].

3.2. Breakthrough volumes and type of TEC sorbent

The breakthrough volume of compound can be defined as the minimum volume of liquid necessary to elute this compound from a column. It depends on the composition of the eluting liquid and its flow rate as well as on the nature of the solid phase and the dimensions of the column.

The measurement of breakthrough volumes is useful to determine the maximum volume of liquid that can be allowed for the dialysis of biological samples without any significant loss of analyte by elution from the trace enrichment column.

The breakthrough volumes of oxprenolol were determined on three kinds of trace enrichment columns with different sorbents and lengths, using pH 3.0 acetate buffer or pH 8.0 phosphate buffer as dialysis liquid (Table 2).

As it can be seen in Table 2, an increase of the lengths of the column results, as expected, in larger breakthrough volumes. This is particularly

Table 1
Influence of sodium perchlorate concentration in the mobile phase on retention and enantioseparation of oxprenolol on Chiralcel OD-R

	0.01 M	0.02 M	0.05 M	0.1 M	0.4 M	0.45 M	0.5 M
k'_1	0.44	0.75	1.03	1.23	1.54	1.62	1.66
k'_2	0.50	0.88	1.23	1.49	1.92	2.05	2.22
α	1.14	1.17	1.20	1.21	1.25	1.27	1.34
Rs	—	1.02	1.23	1.40	2.01	2.25	2.28

k'_1 and k'_2: capacity ratios of the first and second eluting enantiomer—α: selectivity factor—Rs: resolution.
Chromatographic conditions: stationary phase: Chiralcel OD-R 250 × 4.6 mm i.d. (10 μm)—mobile phase: pH 6.0 phosphate buffer containing $NaClO_4$/acetonitrile (70:30 v/v)—flow rate: 0.9 ml min^{-1}—UV detection: 273 nm—sample: aqueous solution of racemic oxprenolol (10 μg ml^{-1}).

Table 2
Breakthrough volumes of oxprenolol

TEC sorbent	Acetate buffer pH 3.0	Phosphate buffer pH 8.0	LC mobile phase
LiChrospher 100-CN (4 mm)	0.45 ml	2.15 ml	0.10 ml
Nucleosil CN (11 mm)	0.8 ml	10.0 ml	0.27 ml
Hypersil RP-18 (10 mm)	17.3 ml	>70 ml	0.50 ml

LC Mobile phase: pH 3.0 acetate buffer—acetonitrile—2-aminoheptane (92-8-0.5 v/v/v).

pronounced when the pH 8.0 buffer is used. On the other hand, the type of sorbent used in the column is also of prime importance. A proper selection of this sorbent give rise to a gain in selectivity and analyte recovery [22].

Table 2 shows that the breakthrough volumes obtained with the TEC containing octadecyl silica were much larger than those obtained with the TECs filled with cyanopropyl silica. Finally, the C-18 was selected because it gave a sufficiently high breakthrough volume with pH 3.0 acetate buffer which was chosen as dialysis liquid (acceptor solution).

It should also be noted that the volumes of LC mobile phase (achiral system) necessary to elute oxprenolol from any TEC tested were generally very low (not higher than 0.50 ml).

The content of acetonitrile in the chiral LC mobile phase (30%) was even more favourable for rapid elution and transfer of oxprenolol from the TEC to the chiral column. Therefore the dialysis step could easily be coupled to chiral LC under the conditions selected for the LC assay of oxprenolol enantiomers.

3.3. Dialysis

Three parameters likely to influence the dialysis process were mainly investigated: the volume and the aspirating flow rate of the acceptor solution and the dispensing flow rate of the dialysate on the TEC. These investigations were made using an achiral HPLC system and aqueous solutions of oxprenolol, since previous experiments have shown that analyte recoveries obtained with plasma samples were not significantly different from those found with aqueous solutions [22].

The influence of the volume of acceptor solution on dialysis recovery for oxprenolol is shown in Fig. 2. The aspirating and dispensing flow rates were set at 1.0 ml min^{-1} and 3.0 ml min^{-1}, respectively. Dialysis recoveries were expressed here in terms of relative recoveries (%), calculated by comparing analyte peak areas found with dialysed samples to those obtained when aqueous solutions of oxprenolol at the same concentration were injected directly onto the TEC.

An increase of the volume of acceptor solution from 1 to 14 ml gives rise to better relative recoveries for oxprenolol. However, when using volumes of acceptor solution, the increase in relative recovery becomes very limited. A 10 ml volume of pH 3.0 acetate buffer was therefore selected giving a recovery after dialysis of about 80%.

The influence of the aspirating flow rate of the dialysis liquid on the recovery of oxprenolol was also investigated (cf. Table 3). Aspirating flow rates higher than 1.0 ml min^{-1} were found to decrease the relative recoveries of the analyte. On the other hand, it is not advisable to select too low aspirating flow rates because the time needed for sample preparation would become exceedingly long. An aspirating flow rate of 1.0 ml min^{-1} was finally found to be a good compromise.

Table 3 also shows the influence of the dispensing flow rate of the dialysate onto the trace enrichment column on the recovery of oxprenolol. By contrast with the aspirating flow rate, the increase of the dispensing flow rate does not seem to have a detrimental influence on the recovery (about 80% in all cases). Higher dispensing flow rates would in principle be preferable since they can reduce the analysis time. However, flow rates higher than 3.0 ml min^{-1} are not recommended

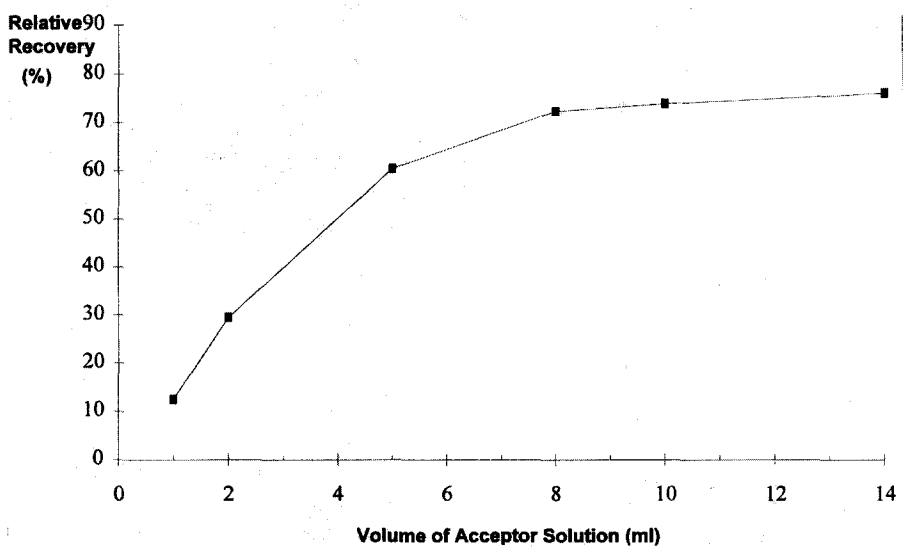


Fig. 2. Influence of the volume of acceptor solution on recovery for oxprenolol. Dialysis: static—pulsed mode—acceptor solution: pH 3.0 acetate buffer—aspirating flow rate: 1.0 ml min^{-1}—dispensing flow rate: 3.0 ml min^{-1}—-dialyser: donor channel: 370 μl—acceptor channel: 650 μl—sample loading volume: 370 μl of aqueous solution of oxprenolol (2 μg ml^{-1})—trace enrichment column: hypersil RP-18 (10 μm), 10 × 4 mm i.d.

since they may lead to a deterioration of the dialysis membrane [35].

The aspirating and dispensing flow rates have in any case less influence on the recovery of oxprenolol than the total volume of acceptor solution which is obviously the most important parameter to be optimized in the dialysis step.

Table 3
Influence of the aspirating and dispensing flow rates of the acceptor solution on dialysis recovery

Aspirating flow rate (ml min^{-1})	Dispensing flow rate (ml min^{-1})	Relative recovery ($n = 3$, %)
0.25	3.0	80.8
0.5	3.0	78.6
1.0	3.0	76.2
2.0	3.0	69.9
4.0	3.0	63.7
1.0	1.0	79.8
1.0	2.0	79.2
1.0	3.0	79.3
1.0	4.0	81.3
1.0	5.0	79.9

3.4. Method validation

3.4.1. Selectivity

A typical chromatographic trace of a plasma extract containing racemic oxprenolol is shown in Fig. 3. Under the conditions selected for the LC enantioseparation of oxprenolol, the mean retention times for the first and the second enantiomer were 9.6 and 11.0 min, respectively ($n = 20$).

As can be seen from the blank plasma in the Fig. 3, no endogenous sources of interference were observed at the retention times of the enantiomers of oxprenolol. The order of elution of enantiomers of oxprenolol was determined by injecting separately solutions of each enantiomer.

3.4.2. Absolute recovery

The absolute recovery is defined as the ratio between the peak area obtained from freshly prepared sample extracts and the peak areas found by direct injection of an aqueous standard solution at the same concentration, using the same autosampler but equipped with a loop of 100 μl instead of the trace enrichment column [36]. The

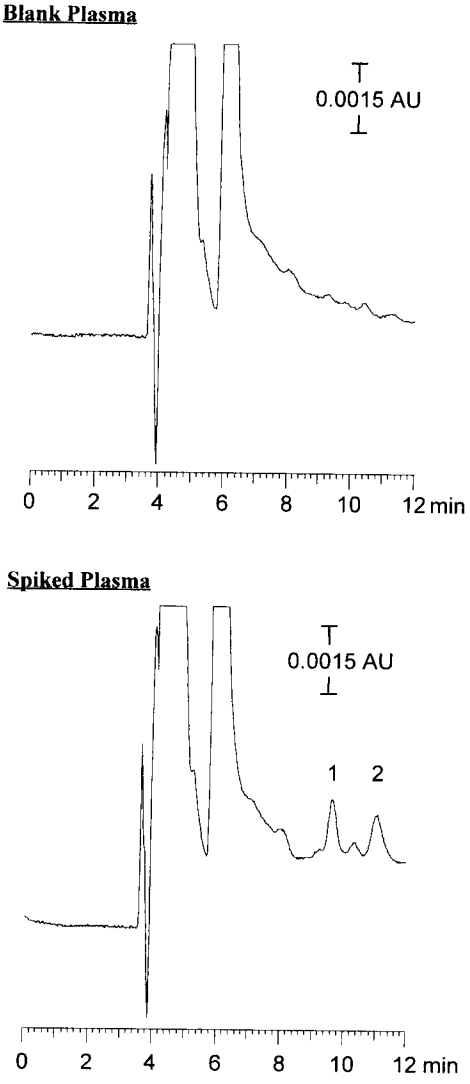


Fig. 3. Enantioseparation of oxprenolol in plasma, using dialysis and chiral LC. Chromatographic conditions: stationary phase: chiralcel OD-R 10 μm (250 × 4.6 mm i.d.)—mobile-phase: pH 6.0 phosphate buffer containing $NaClO_4$ (0.45 M)/acetonitrile (70:30 v/v)—flow rate: 0.9 ml min^{-1}—UV detection: 273 nm—sample: plasma spiked with racemic oxprenolol (100 ng ml^{-1} for each enantiomer). (1) *R*-(+)-Oxprenolol; (2) *S*-(−)-Oxprenolol.

absolute recovery for both enantiomers of oxprenolol was found to be 81% (cf. Table 4). A comparison with relative recoveries given in Table 3 indicates that no further losses of analyte occurred after passage of the dialysis membrane.

3.4.3. Linearity

The calibration curves were obtained in the concentration range 50–2500 ng ml^{-1} ($n = 6$; $k = 3$) and the following regression equations were found by plotting peak area (y) versus analyte concentration (x) in ng ml^{-1}:

$R-(+)$ – Oxprenolol: $y = 1939\,x - 18236$

$r^2 = 0.999$

$S-(-)$ – Oxprenolol: $y = 1866\,x - 27734$

$r^2 = 0.999$

The determination coefficients (r^2) obtained for the regression lines of both enantiomers of oxprenolol demonstrate the linearity of the relationship between peak area and concentration.

3.4.4. Limits of detection and quantitation

The limits of detection (LOD) and quantitation (LOQ) were determined as the concentrations of analyte giving rise to signal-to-noise ratios of 3 and 10, respectively. The LODs and LOQs for both enantiomers were found to be 17 ng ml^{-1} and 50 ng ml^{-1}, respectively.

3.4.5. Precision

The precision of the automated bioanalytical method was estimated by measuring the intra-day and inter-day reproducibilities of oxprenolol at three concentration levels, ranging from 50 to 2500 ng ml^{-1}. The mean values for the intra-day and inter-days reproducibilities were 3.3 and 4.6% for the first eluting enantiomer and 2.0 and 7.8% for the second eluting enantiomer, respectively.

3.4.6. Accuracy

The overall accuracy of the procedure was assessed by plotting the analyte amount found versus the amount introduced in the plasma sample at three concentration levels ($n = 6$) ranging from 50–2500 ng ml^{-1} ($r^2 = 0.998$ and 0.999 for *R*-(+) and *S*-(−) oxprenolol, respectively). *t*-Tests showed that the slopes of the regression lines were not significantly different from unity (calculated values of t were 0.72 and 0.57 for *R*-(+) and

Table 4
Validation of the automated method for the determination of oxprenolol enantiomers in plasma

Validation criterion	*R*-(+)-Oxprenolol	*S*-(−)-Oxprenolol	
Absolute recovery	(mean, $n=5$)	81%	81%
Linearity ($n=6$, $k=3$)	50–2500 ng ml^{-1}	$y=1939x-18236$ $r^2=0.999$	$y=1866x-27734$ $r^2=0.999$
LOD		17 ng ml^{-1}	17 ng ml^{-1}
LOQ		50 ng ml^{-1}	50 ng ml^{-1}
Reproducibility			
Intra-day	($n=6$; 1 day)		
	50 ng ml^{-1}	5.9%	3.5%
	500 ng ml^{-1}	1.7%	1.3%
	2500 ng ml^{-1}	2.3%	1.0%
	Mean	3.3%	2.0%
Inter-Day	($n=6$; 3 days)		
	50 ng ml^{-1}	3.3%	11.0%
	500 ng ml^{-1}	2.7%	6.0%
	2500 ng ml^{-1}	5.2%	6.6%
	Mean	4.6%	7.8%
Overall Accuracy			
	t-test for the slope	0.72	0.57
	t-test for the origin	0.56	1.76

S-(−) oxprenolol, respectively) and that intercepts were not significantly different from zero (calculated values of t were 0.56 and 1.76 for *R*-(+) and *S*-(−) enantiomers, respectively). The critical value of t was 2.12 ($p=0.05$). The automated HPLC procedure of the determination of the enantiomers of oxprenolol in human plasma using dialysis as sample preparation can thus be considered as accurate within the concentration range investigated.

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