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DETERMINATION OF (*R*)- AND (*S*)-PROPRANOLOL IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING N-BENZOXYCARBONYLGLYCYL-L-PROLINE AS CHIRAL SELECTOR IN THE MOBILE PHASE

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SUMMARY

A normal-phase chromatographic method for the determination of (*R*)- and (*S*)-propranolol in plasma is described The chiral separation is performed by adding an optically active complexing agent, N-benzoxycarbonylglycyl-L-proline, to the mobile phase (dichloromethane) The solid phase is LiChrosorb DIOL After adjustment of the pH of the plasma, the propranolol enantiomers are extracted into hexane–dichloromethane–*n*-butanol (72 18 10) The organic phase is evaporated and the residue dissolved in the mobile phase before injection on to the column Quantifications are performed by using internal standardization, giving a precision of better than 2% (coefficient of variation) The method employs 1-ml plasma samples and has linear calibration graphs ($r=0\,999$) over the concentration range studied, 9 2–288 nmol/l Injections of sample solutions with a composition different from that of the mobile phase gave system peaks that might affect the shape of the solute peaks Several possibilities for avoiding these disturbing system peaks in the chromatogram by changing the mobile phase composition are discussed

INTRODUCTION

Propranolol [1-isopropylamino-3-(1-naphthoxy)-2-propanol] (Fig 1) is a non-selective β-adrenergic blocking agent used in the treatment of, e g , hy-

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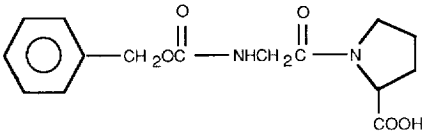
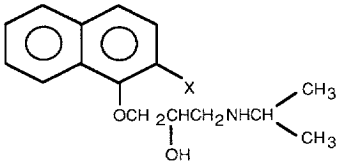


N-benzoxycarbonyl-glycyl-L-proline



X = H Propranolol

X = CH_3 2 -Methylpropranolol

Fig 1 Structures of compounds

pertension, angina pectoris and cardiac arrhythmias [1]. The *S*-enantiomer has a β-adrenergic blocking activity that is 60–100 times higher than that of the *R*-enantiomer [2]

Several methods for the determination of propranolol enantiomers in plasma and urine have been reported which involve the formation of diastereomeric derivatives N-Trifluoroacetyl-L-propyl chloride and N-heptafluorobutyryl-L-prolyl chloride are reagents applied in gas chromatography [3] and N-trifluoroacetyl-L-prolyl chloride [4,5], L-1-phenylethyl isocyanate [6] and Boc-L-alanine anhydride [7] have been used in liquid chromatography for derivatization of (*R*, *S*)-propranolol Indirect separations of enantiomers as diastereomeric derivatives have some serious limitations, e.g , risk of racemization during derivatization or unequal reaction rates of enantiomers in the formation of diastereomers [8] An enantiomeric impurity in the derivatization reagent may also give rise to undetectable errors The direct separation of enantiomers using a chiral stationary phase or a chiral mobile phase additive avoids these potential errors [9,10]. The propranolol enantiomers have been resolved on several chiral stationary phases [11–14]. The Pirkle phase based on (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine has also been applied to the determination of (*R*)- and (*S*)-propranolol in human serum [11] Derivatization of propranolol to form 2-oxazolidone derivatives was required before chromatography

Direct separation of enantiomers can also be achieved by use of a chiral additive, e g , a counter ion, in the mobile phase [15,16] The resolution of the enantiomers is based on the fact that interaction with the counter ion gives

two diastereomeric complexes (ion pairs) with different stabilities The separation can also be due to differences in the distribution of the diastereomeric ion pairs between the organic mobile phase and the adsorbing stationary phase

Recently, N-benzoxycarbonylglycyl-L-proline (L-ZGP) (Fig. 1) was introduced as a chiral additive for the separation of enantiomeric 1,2-amino alcohols [17,18]. L-ZGP gave high stereoselectivity with separation factors (α) between 1 2 and 1 5 for several β-adrenergic blocking agents (e.g , propranolol, metoprolol, alprenolol, atenolol, oxprenolol and practolol).

In this study, a method utilizing L-ZGP as a mobile phase additive has been developed for the determination of picomole amounts of (*R*)- and (*S*)-propranolol in plasma. No significant changes in the chromatographic performance over time were observed despite several hundred injections of bioanalytical samples The work-up procedure consisted of liquid–liquid extraction, evaporation and dissolution of the residue in the mobile phase. The assay utilized one enantiomer of 2′-methylpropranolol as an internal standard

In bioanalysis it is often necessary to inject the solutes in a solution with a composition different from that of the mobile phase This may give rise to the formation of system peaks that can affect the elution of the solutes [19] The influence of co-eluting system peaks on the separation and quantification of propranolol enantiomers and possibilities for regulating their retention times are discussed

EXPERIMENTAL

Apparatus

The liquid chromatographic system consisted of a ConstaMetric III pump (LDC, Riviera Beach, FL, U S A) and Model 7125 injector (Rheodyne, Cotati, CA, U.S A) with a 20- or 120-μl loop. The detectors were a Model LS-4 fluorescence detector (Perkin-Elmer, Beaconsfield, U K.) and a Spectromonitor III UV detector (LDC).

The analytical columns (150 mm × 3.0 mm I.D.) were made of stainless steel with a polished inner surface, equipped with Swagelock connectors and Altex 250/21 stainless-steel frits The column and solvent reservoir were thermostated at 25 ± 0 1 °C with a Heto type 02 pt 923 C water-bath (Birkerød, Denmark). The organic solvents in the sample work-up were evaporated using a Thermolyne dry bath (Sybron, U.S.A)

The pH measurements were made with an AG 9100 Metrohm 632 pH meter (Herisau, Switzerland) with a Type 1014 glass pH electrode A Beckman Model 25 UV spectrophotometer was utilized for the spectrophotometric measurements

A Surfasil solution (2% in hexane) was used for silanization of all glass equipment.

Chemicals

All chemicals were of analytical-reagent grade, except where indicated otherwise, and were used without further purification

LiChrosorb DIOL (5 μm) (batches vv-415 399, vv-349 799 and vv-1022), dichloromethane (LiChrosolv), sodium hydroxide, phosphoric acid, *n*-butanol and 4-Å molecular sieves were obtained from E Merck (Darmstadt, F R G) (*R*)-, (*S*)- and (*R,S*)-propranolol were supplied by ICI (Macclesfield, U K) (*R,S*)-Methylpropranolol (H86/81) was a gift from Hassle (Mölndal, Sweden) Hexane (HPLC grade) was obtained from Ratherburn Chemicals (Walkerburn, U K.) Surfasil was purchased from Pierce (Rockford, IL, U S A), triethylamine from Janssen Chimica (Beerse, Belgium), L-ZGP from Fluka (Buchs, Switzerland) and dimethyloctylamine (DMOA) from ICN Biomedicals (Plainview, NY, U S A)

Column preparation and chromatographic technique

The columns were packed by a slurry technique using chloroform as the suspending medium. The test solutes, toluene and 2-phenylethanol, were eluted with hexane–1-butanol (199 1) as the mobile phase Only columns giving a reduced plate height, $h = H/d_p$ (H = plate height and d_p = particle diameter), of less than 10 and asymmetry factors of less than 1 5 were accepted.

Dichloromethane was freed from water before use by 4 Å molecular sieves The water content was less than 35 ppm [17] Mobile phases with a higher water content were prepared by mixing dry dichloromethane and water-saturated dichloromethane The columns were washed with 200 ml of dichloromethane (containing 500 ppm of water) before introducing the mobile phase The determinations of propranolol enantiomers were accomplished at 25 0 °C using 2 5 m*M* N-benzoxycarbonylglycyl-L-proline and 0 4 m*M* triethylamine in dichloromethane (containing 500 ppm of water) as the mobile phase unless stated otherwise The flow-rate was 1 0 ml/min The mobile phase was not recirculated

Preparation of internal standard, 2′-methylpropranolol-I

The high-performance liquid chromatographic (HPLC) system developed for the determination of (*R*)- and (*S*)-propranolol in plasma could also be used to resolve racemic 2′-methylpropranolol (Fig. 1). The second-eluted enantiomer of 2′-methylpropranolol (2′-methylpropranolol-II) is not useful as an internal standard as it has almost the same retention time as (*R*)-propranolol Therefore, mobile phase fractions containing the first-eluted enantiomer of 2′-methylpropranolol (2′-methylpropranolol-I) were collected and used for internal standardization The contamination by 2′-methylpropranolol-II in the collected fractions of 2′-methylpropranolol-I was determined by HPLC to be less than 1% Appropriate concentrations of the internal standard, 2′-methylpropranolol-I, were obtained by diluting the collected fractions of 2′-methylpropranolol-I with hexane–dichloromethane–*n*-butanol (72 18 10)

Determination of distribution ratios

The determinations of the distribution ratios of propranolol were performed by shaking equal phase volumes of carefully pre-equilibrated phases in centrifuge tubes The experimental temperature was 25 0°C and the agitation time was 30 min After centrifugation the organic phase was removed with a glass capillary siphon The concentrations of propranolol were determined in both phases by photometric measurements

Liquid–liquid extraction efficiency and reproducibility

Six different plasma samples were spiked with 0 62 μM (*R*)- and (*S*)-propranolol and analyses of each sample were made in duplicate The enantiomers were extracted into the organic phase (hexane–dichloromethane–*n*-butanol) as described below The organic phase was injected directly on to the column The extraction recoveries were determined by comparison of peak heights with those obtained on injecting standards of (*R*)- and (*S*)-propranolol dissolved in the organic phase.

Calibration graphs

Standard solutions for the calibration graphs were prepared by adding (*R*)- and (*S*)-propranolol dissolved in 100 μl of 0 01 *M* hydrochloric acid to pooled blank plasma (1 00 ml) These standards were treated as described below Peak-height ratios were determined by comparing the appropriate propranolol peak with that of the internal standard Calibration graphs were constructed by plotting peak-height ratio versus concentration of the standards. The concentration of the internal standard was adjusted to give a peak-height ratio between 0 2 and 7

Assay procedure

Plasma samples of 1 00 ml were rendered alkaline by addition of 0 50 ml of 1 *M* sodium hydroxide solution. The internal standard solution (100 μl) and 4 00 ml of hexane–dichloromethane–*n*-butanol (72 18 10) were added to the aqueous phase This mixture was equilibrated on a vortex mixer for 2 min The glass tubes were centrifuged (10 min at 1500 *g*) and placed in a refrigerator in order to facilitate phase separation The organic phase was transferred into a new glass tube and evaporated (37°C) under a stream of air. The residue was dissolved in the mobile phase (100–250 μl) A 20- or 120-μl aliquot was injected for analysis by HPLC

RESULTS AND DISCUSSION

Liquid–liquid extraction

The degree of extraction of a solute from an aqueous phase (e g , plasma) into an organic phase is controlled by the phase volume ratio (V_{org}/V_{aq}) and

TABLE I

DETERMINATION OF DISTRIBUTION CONSTANT, K_D

Aqueous phase, phosphate buffer ($\mu = 0.1$), organic phase, hexane–dichloromethane–*n*-butanol

pH range	Hexane–dichloromethane–*n*-butanol (%, v/v)	C_{Baq} (10^{-4} *M*)	C_{Borg} (10^{-3} *M*)	Log K_D
7.08–7.81	4 1 0	1.17–2.30	0.62–1.84	1.68
6.73–7.44	72 18 10	9.20–20.2	15.8–32.9	2.58

the distribution ratio, D (the ratio of the total concentrations of the solute in the two phases). The distribution ratio for propranolol (B) when extracted in uncharged form is given by the equation [20]

$$1/D_B = 1/K_{D(B)} + a_{H^+}/(K_{D(B)} \cdot K'_{HB^+}) \quad (1)$$

where D_B = distribution ratio, $K_{D(B)}$ = distribution constant $\{K_{D(B)} = [B]_{org}/[B]\}$, K'_{HB^+} = acid dissociation constant of HB^+, a_{H^+} = hydrogen ion activity. The distribution ratio of the base B is governed by pH and K_D, i.e., the organic phase [20]. The distribution constant, $K_{D(B)}$, can be determined experimentally from the intercept or slope by plotting $1/D_B$ versus a_{H^+} (eqn. 1).

Organic phases with a high content of dichloromethane, the main component in the mobile phase, were chosen to make possible direct injections of organic extracts. Addition of hexane gave an organic phase with a lower density than plasma and this facilitates the transfer of the organic phase in the work-up procedure. Hexane will also decrease the distribution constant of the analytes and *n*-butanol had to be added to compensate for this loss of extraction ability of the organic phase (Table I). Extraction of propranolol ($pK_a = 9.32$ [21]) with hexane–dichloromethane–*n*-butanol (72 18 10) should give a recovery of more than 99% at pH > 11 and a phase volume ratio of 2.7. Quantitative extraction (> 99 ± 5%) of propranolol enantiomers from spiked plasma was confirmed. The distribution constant for the internal standard, 2′-methylpropranolol-I, has not been determined. However, quantitative extraction of the methyl analogue of propranolol is assumed as addition of a methylene group, e.g., in an alkyl chain, generally increases the distribution constant three- or four-fold [20].

Chromatography

The direct separation of (*R*)- and (*S*)-propranolol was accomplished by using L-ZGP as a chiral additive in the mobile phase. The structures of the compounds are given in Fig. 1. The resolution is based on the formation of diastereomeric complexes (ion pairs) in an organic mobile phase of low polarity [15,16]. The presence of an amine, e.g., triethylamine, in the mobile phase was

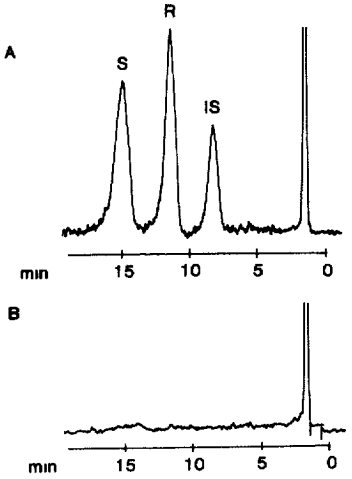


Fig 2 Determination of (*R*)- and (*S*)-propranolol in plasma Solid phase LiChrosorb DIOL, 5 μm (batch vv-415 399) Mobile phase 2 50 m*M* L-ZGP–0 20 m*M* triethylamine in dichloromethane (containing 500 ppm of water) Solute (A) (*R,S*)-propranolol (9 6 nmol/l of each enantiomer in plasma), (B) blank plasma Fluorimetric detection exitation 292 nm, emission 340 nm

found to be necessary in order to elute the propranolol enantiomers within a reasonable time The retention of the enantiomeric solutes depends on the concentration of L-ZGP and of triethylamine [17].

(*R,S*)-Propranolol was transferred into an organic phase by liquid–liquid extraction as described above. Determinations of high propranolol concentrations (>0.1 μmol/l) were possible by direct injection of the organic extract However, for measuring small amounts of (*R*)- and (*S*)-propranolol, evaporation of the organic phase and redissolution in a small volume of the mobile phase were necessary.

Despite a high water content (500 ppm) in the mobile phase [17], it was still possible to resolve (*R*)- and (*S*)-propranolol completely (Fig. 2). The high water content was used to make the system less prone to changes in the chromatographic performance when bioanalytical extracts with high water concentrations were injected The peaks had capacity factors (k') of 8 1 and 11 0, respectively, a separation factor (α) of 1 35 and a resolution factor (R_s) of 2.1. The propranolol peaks were well separated from the internal standard, 2′-methylpropranolol-I, and no interfering peaks were observed in the chromatogram from pooled blank plasma (Fig 2).

Stability and reproducibility of chromatographic system

More than 300 samples from plasma extractions and standard solutions have been injected without any significant changes in the retention or stereoselectivity (Fig. 3). A further illustration of the high stability and reproducibility

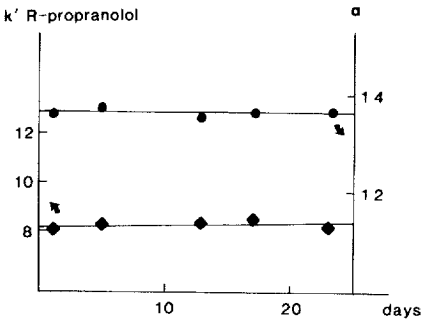


Fig 3 Stability of chromatographic system Solid phase LiChrosorb DIOL, 5 μm (batch vv-415 399) Mobile phase 2 50 m*M* L-ZGP–0 40 m*M* triethylamine in dichloromethane (containing 500 ppm of water)

TABLE II

PROPERTIES OF THREE DIFFERENT BATCHES OF LICHROSORB DIOL

Mobile phase 2 50 m*M* L-ZGP and –0 40 m*M* triethylamine in dichloromethane (containing 500 ppm of water)

Solute	vv-415 399				vv-349 799				vv-1022			
	k'	h	asf[a]	α^b	k'	h	asf[a]	α^b	k'	h	asf[a]	α^b
Internal standard	5 57	26 8	1 05		5 71	22 3	1 05		3 79	15 8	1 05	
(*R*)-Propranolol	8 00	23 6	1 09		8 14	27 5	1 21		5 27	14 6	1 07	
(*S*)-Propranolol	10 0	22 8	1 12	1 35	11 2	22 0	1 06	1 37	7 07	15 4	1 05	1 34

[a]asf = asymmetry factor (measured at 10% of the peak height)
[b]$\alpha = k'_{(S)\ \text{propranolol}} / k'_{(R)\ \text{propranolol}}$

is the fact that introducing a freshly prepared mobile phase every fourth day had no effect on the capacity or separation factors (Fig 3)

Of interest is the possibility of reproducing the separation of propranolol enantiomers when using different batches of the support material, LiChrosorb DIOL The stereoselectivity was unchanged for all batches, but the retention times for the propranolol enatiomers and the internal standard varied (Table II) As discussed previously, it is possible to adjust the capacity factors by changing the concentrations of L-ZGP and /or triethylamine

Influence of system peaks

For one batch only (LiChrosorb DIOL vv-1022) we observed split peaks (Fig 4) The plasma samples were treated according to the general work-up procedure described above The peak splitting could not be attributed to any impurities or endogenous compounds from plasma (cf., blank plasma in Fig 4) However, injections of solutes or a solution with a composition only slightly

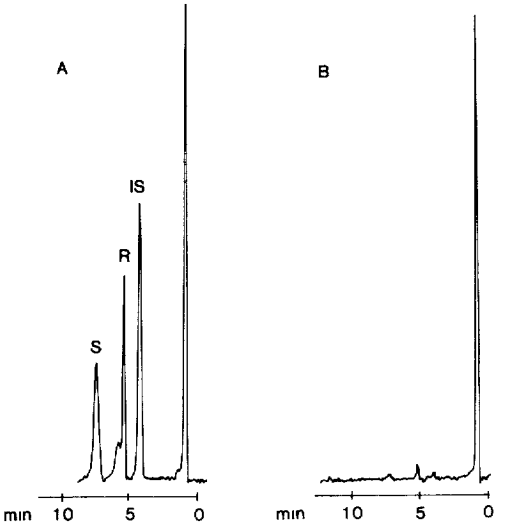


Fig 4 Influence of system peak on the analysis of (*R*)- and (*S*)-propranolol Solid phase Li-Chrosorb DIOL, 5 μm (batch vv-1022) Mobile phase as in Fig 3 Solute (A) (*R,S*)-propranolol (9 6 nmol/l of each enantiomer), (B) blank plasma Fluorimetric detection excitation 292 nm, emission 340 nm

different from that of the mobile phase will disturb the established distribution equilibria in the column These disturbances will give rise to migrating zones with a composition that deviates from the mobile phase These peaks originating from mobile phase components are called system peaks [19] Solute peaks co-eluting with a system peak may be compressed or deformed, depending on their position within the system peak The peak compression induced by system peaks was utilized by Nilsson and co-workers [22,23] to enhance the detection sensitivity in reversed-phase chromatography

Injection of propranolol dissolved in a mixture of the mobile phase and *n*-butanol (9 1) gave a compressed peak for (*R*)-propranolol, (Fig 5) The change in the L-ZGP concentration was simultaneously recorded using a UV detector The UV trace showed that the (*R*)-propranolol was eluting in a zone with increasing L-ZGP concentration The injected *n*-butanol desorbed the complexing agent, L-ZGP, from the solid phase, giving a peak with high UV absorbance in the front of the chromatogram (peak A in Fig 5) The origin of peak B has not been elucidated but it might be a system peak due to changes in the triethylamine concentration A migrating zone with a deficiency of the UV-absorbing L-ZGP (peak C in Fig 5) appeared when L-ZGP was readsorbed from the mobile phase to the solid phase A breakthrough front with increasing concentration of L-ZGP was formed after re-equilibration of the column with L-ZGP, as shown by the increased UV response of the mobile phase. The L-

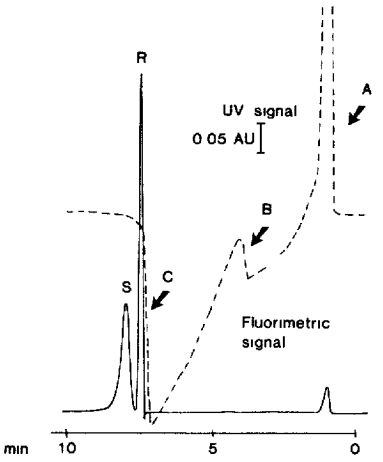


Fig 5 Peak compression effect of system peak Solid phase LiChrosorb DIOL, 5 μm (batch vv-1022) Mobile phase as in Fig 3 Solute (*R,S*)-propranolol, 3 34·10^{-7}*M* in 2 50 m*M* L-ZGP-0 40 m*M* triethylamine in dichloromethane (containing 500 ppm of water)-*n*-butanol (90 10, v/v) Fluorimetric detection excitation 292 nm, emission 340 nm

ZGP gradient compressed the eluting (*R*)-propranolol peak giving an observed reduced plate height of $h=1$

A change in the relative retention of the propranolol enantiomers and the system peak will limit the effect of the system peak Previously, it has been shown that L-ZGP and triethylamine concentrations are variables that control the retention [17]. Fig 6 shows the influence of ZGP on the retention of the propranolol enantiomers, the internal standard and the system peak The capacity ratios of the system peak were calculated from the peak minimum of the negative zone The retention of the system peak was found to be less sensitive to changes in concentration of L-ZGP than the retention of the solute peaks. Unfortunately, a relatively low concentration of L-ZGP and thus a high retention of solutes is necessary in order to separate the solute peaks from the system peak effect. The decrease in L-ZGP concentration is accompanied by a minor decrease in stereoselectivity However, the long retention times impaired the detection limits for the propranolol enantiomers

The hydrophobicity and concentration of the amine in the mobile phase will also affect the retention of solute and system peaks (Table III). Replacement of triethylamine with DMOA decreased the system peak retention whereas the retention times of the solutes increased, i e , the selectivity was improved A possible explanation is that the amine in the mobile phase is competing for the limited adsorption capacity of the solid phase in addition to the ion-pair formation with L-ZGP A more hydrophobic amine, e g , DMOA, would thus be a less efficient competitor and increase the retention times for the propranolol enantiomers However, determinations of (*R*)- and (*S*)-propranolol could be

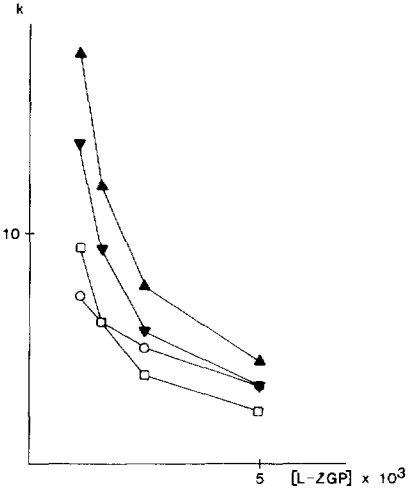


Fig 6 Influence of L-ZGP concentration on the capacity factors of solute and system peaks Solid phase LiChrosorb DIOL, 5 μm (batch vv-1022) Mobile phase L-ZGP-0 40 mM triethylamine in dichloromethane (containing 500 ppm of water) ▲, $k'_{(S)\ \text{propranolol}}$, ▼, $k'_{(R)\ \text{propranolol}}$, □, k'_{IS}, ○, $k'_{\text{system peak}}$

TABLE III

INFLUENCE OF COMPETING AMINE ON THE CAPACITY FACTORS OF SOLUTE AND SYSTEM PEAKS

Solid phase LiChrosorb DIOL, 5 μm (batch vv-1022) Mobile phase (I) 1 57 mM L-ZGP-0 40 mM triethylamine in dichloromethane (containing 500 ppm of water), (II) 1 50 mM L-ZGP-0 32 mM dimethyloctylamine in dichloromethane (containing 500 ppm of water)

Solute	k'	
	I	II
System peak	6 4	5 3
Internal standard	6 30	7 86
(R)-Propranolol	9 40	11 7
(S)-Propranolol	12 3	14 9

accomplished within less than 15 min (Fig 7) Hence batch vv-1022 of LiChrosorb DIOL could also be used as the adsorbing stationary phase, provided that DMOA is present in the mobile phase The system peak was well separated from the solute peaks, as shown by the UV trace in Fig 7 It should be stressed that no disturbances from system peaks were observed when using batch vv-415 399 or vv-349 799 of LiChrosorb DIOL

The influence of system peaks on the chromatographic performance was related to the injection volume (Table IV) An increase in the sample volume

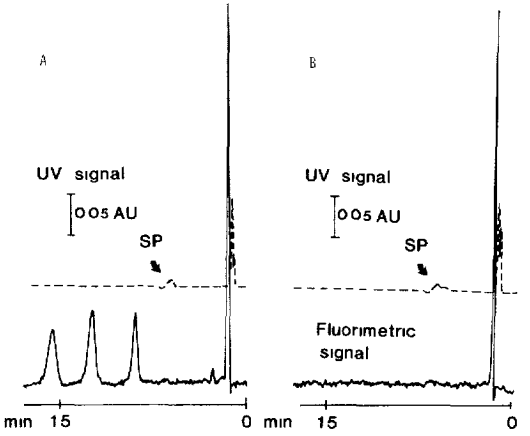


Fig 7 Determination of (*R*)- and (*S*)-propranolol in plasma using dimethyloctylamine as competing amine Solid phase LiChrosorb DIOL, 5 μm (batch vv-1022) Mobile phase 1 50 m*M* L-ZGP-0 32 m*M* dimethyloctylamine in dichloromethane (containing 500 ppm of water) Solute (A) (*R,S*)-propranolol (11 nmol/l of each enantiomer), (B) blank plasma SP = System peak Fluorimetric detection excitation 292 nm, emission 340 nm

TABLE IV

SYSTEM PEAK AND INJECTION VOLUME

Solid phase LiChrosorb DIOL, 5 μm (batch vv-1022) Mobile phase 1 50 m*M* L-ZGP-0 32 m*M* dimethyloctylamine in dichloromethane (containing 500 ppm of water)

Solute	20 μl				120 μl			
	k'	*h*	asf	α	*k'*	*h*	asf	α
System peak	5 3				5 3			
Internal standard	7 86	14 8	1 20		7 96	45 3	0 66	
(*R*)-Propranolol	11 7	13 4	1 10		11 7	17 7	1 03	
(*S*)-Propranolol	14 9	13 6	1 08	1 27	14 9	15 9	1 16	1 27

did not change the capacity factor of the system peak but gave a broader peak. The peak shape of the internal standard was severely distorted, with a reduced plate height of 45.3 and an asymmetry factor of 0.66 (Table IV) Therefore, the possibility of improving detection limits in the system with L-ZGP as chiral selector by injection of large volumes might be limited

However, the improved efficiency of the solute peak induced by the system peak, as discussed above, might be used to improve the detection limits Preliminary studies indicated possibilities of improving the detection sensitivity by using an L-ZGP gradient (Fig 8) The separation of (*R*)- and (*S*)-propranolol was highly reproducible Further, a relatively rapid reconditioning of

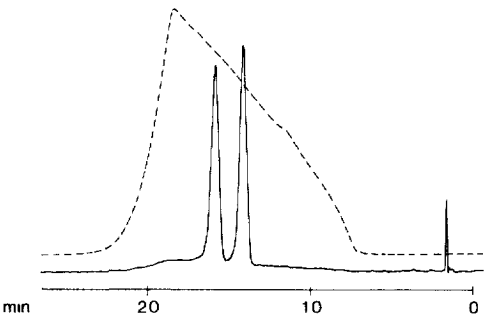


Fig 8 Gradient elution of (*R*)- and (*S*)-propranolol Solid phase LiChrosorb DIOL, 5 μm (batch vv-415 399) Mobile phase (A) 1 05 m*M* L-ZGP-0 020 m*M* triethylamine in dichloromethane (containing 80 ppm of water–*n*-pentanol (199 1), (B) 11 2 m*M* L-ZGP-0 020 m*M* triethylamine in dichloromethane (containing 80 ppm of water)–*n*-pentanol (199 1), gradient from A to B in 15 min Solute (*R,S*)-propranolol, 7 36 10^{-7} *M* Fluorimetric detection excitation = 292 nm, emission 340 nm

the column with L-ZGP was achieved, as shown by the recorded UV signal Analysis by gradient elution as described in Fig. 8 was five times more sensitive than the isocratic elution Further studies on gradient elution with L-ZGP are in progress

Quantitation and sensitivity

The accuracy and precision of the method were validated using spiked plasma samples (Table V) Two concentration levels of interest for pharmacokinetic studies after a single oral dose of (*R,S*)-propranolol were studied The method gave an intra-assay precision of 2% [coefficient of variation (C.V.)] for both the *R*- and *S*-enantiomers at high and low concentration levels

The calibration graphs were linear over the range studied, i e , 2.4–75 ng/ml of plasma; the correlation coefficients (*r*) were generally better than 0.999

TABLE V

QUANTIFICATION AND INTRA-ASSAY PRECISION

Solid phase LiChrosorb DIOL, 5 μm (batch vv-415 399) Mobile phase 2 50 m*M* L-ZGP-0 40 m*M* triethylamine in dichloromethane (containing 500 ppm of water)

Solute	Plasma concentration (ng/ml)	
	Added	Found (mean ± S D , $n = 12$)
(*R*)-Propranolol	2 36	2 21 ± 0 04
(*S*)-Propranolol	2 36	2 19 ± 0 04
(*R*)-Propranolol	23 6	22 8 ± 0 30
(*S*)-Propranolol	23 6	23 0 ± 0 30

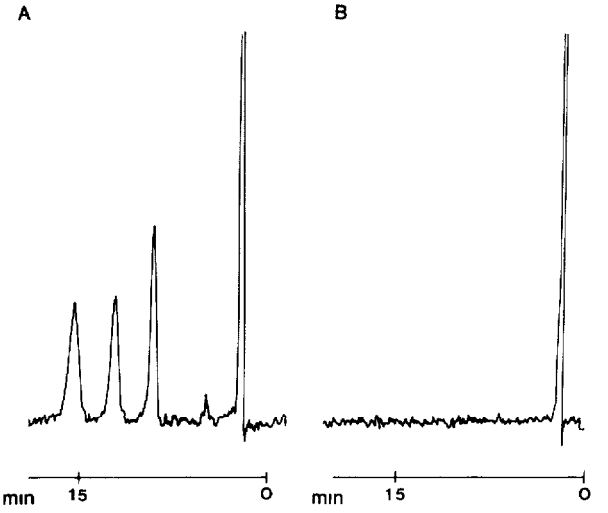


Fig 9 Determination of (*R*)- and (*S*)-propranolol in a healthy patient after a single oral dose of 80 mg Inderal Solid phase LiChrosorb DIOL, 5 μm (batch vv-1022) Mobile phase as in Fig 7 Solute (A) plasma sample 45 min after administration, (B) plasma sample before administration Fluorimetric detection excitation 292 nm, emission 340 nm

The equations of the lines were $y = -0\ 02 + 0\ 094x$ for the *R*-enantiomer and $y = -0\ 03 + 0\ 068x$ for the *S*-enantiomer

The minimum detectable quantities that have to be injected in order to give a response three times the noise were 0 23 ng of (*R*)-propranolol and 0 31 ng of (*S*)-propranolol DMOA was added to the mobile phase when using batch vv-1022 of LiChrosorb DIOL This amine eliminated the disturbances of the system peaks on the determination of the propranolol enantiomers as discussed above No peak deformation was observed when analysing plasma samples from a healthy volunteer (Fig 9). Plasma was analysed before and after an 80-mg single oral dose of Inderal

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