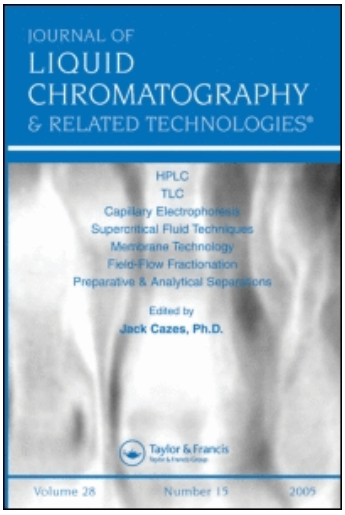


**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Quantitative Analysis of β-Blockers in Human Plasma by Reversed-Phase Ion-Pair High-Performance Liquid Chromatography Using a Microbore Column

J. Shen[a]; S. Wanwimolruk[a]; C. T. Hung[b]; A. R. Zoest[b]
[a] Department of Pharmacy, University of Otago Medical School, New Zealand [b] Zenith Technology Corporation Limited, New Zealand

**To cite this Article** Shen, J. , Wanwimolruk, S. , Hung, C. T. and Zoest, A. R.(1991) 'Quantitative Analysis of β-Blockers in Human Plasma by Reversed-Phase Ion-Pair High-Performance Liquid Chromatography Using a Microbore Column', Journal of Liquid Chromatography & Related Technologies, 14: 4, 777 — 793
**To link to this Article: DOI:** 10.1080/01483919108049287
**URL:** http://dx.doi.org/10.1080/01483919108049287

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(4), 777-793 (1991)

# QUANTITATIVE ANALYSIS OF β-BLOCKERS IN HUMAN PLASMA BY REVERSED-PHASE ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING A MICROBORE COLUMN

J. SHEN[1], S. WANWIMOLRUK[1]*,
C. T. HUNG[2], AND A. R. ZOEST[2]
[1]*Department of Pharmacy*
*University of Otago Medical School*
[2]*Zenith Technology Corporation Limited*
*Dunedin, New Zealand*



## ABSTRACT

HPLC assays for four β-blockers (namely atenolol, labetalol, metoprolol and propranolol) in human plasma have been developed using a reversed-phase microbore column. Sodium dodecyl sulphate (SDS) was chosen as the pairing ion. SDS concentration was varied to provide acceptable retention for the drugs of interest and good resolution from plasma interfering peaks. Based on these results, a general reversed-phase ion-pair HPLC elution scheme using microbore column (ODS-Hypersil) with fluorescence or UV detection was developed. The detection limits were 10, 5, 5 and 1 ng/ml for atenolol, labetalol, metoprolol and propranolol, respectively. The coefficients of variation for the drugs studied were less than 10%. These methods have been shown to be suitable for routine analysis in bioavailability studies and for studying the placental transfer of β-blockers in both human placental perfusion and the animal models.

* To whom correspondence should be addressed.

## INTRODUCTION

β-Blockers have proven efficacy in the treatment of hypertension, hyperthyroidism, atherosclerotic heart disease, and pregnancy-induced hypertension or pre-eclampsia (1-3). The estimation of concentrations of β-blockers in body fluids is required in studies aimed at understanding their pharmacokinetics and pharmacodynamics in humans and animals, as well as for therapeutic drug monitoring. Many gas chromatography (GC) and high-performance liquid chromatography (HPLC) methods for β-blockers have been published (4-12). However, some of them were found in this laboratory to be time-consuming and persistent endogenous plasma components that interfered with chromatography of β-blockers of interest. Chemically, β-blockers are all aryl(oxy)propranololamines and are bases. For basic compounds, the reversed-phase ion-pair HPLC has been successfully applied to a diverse selection of separation problems (13-15). This technique utilized a buffered aqueous-organic mobile phase containing a pairing ion which is available to form a lipophilic complex with the salt of a drug. This method involves the addition of an oppositely charged pairing ion to the chromatographic system, so that solute retention is affected by ion pairing via various mechanisms. Ion-pair reversed-phase HPLC, where the pairing ion is added the mobile phase, has been shown to have high flexibility in retention and selectivity control, particularly when the pairing ion is surface active (16, 17). Several different theories, for anionic pairing ions, have been reviewed elsewhere (18). Hung *et al* (19, 20, 21) have demonstrated that only the ion exchange desolvation mechanism provides a single explanation for both the maximum in the curve of capacity factor (k') against pairing ion for ionic solutes. The ion exchange desolvation approach considers that the decrease in k' observed at high pairing-ion concentrations is a direct result of coverage of alkylsilica surface with adsorbed pairing ion, causing a decrease in surface area to be available for desolvation of any solute. When dealing with reversed-phase ion-pair HPLC, conditions must be rationalized in order to obtain satisfactory resolution of the analytical separation. The paucity of information on the behaviour of the factors

which affect the capacity factor (k') suggests that the variables governing retention for β-blockers on a reversed-phase ion-pair HPLC system are not well understood. The purpose of this study was to investigate these variables so that chromatographic conditions for β-blockers can be easily rationalized by a semi-empirical approach.

## MATERIALS AND METHODS

### Reagents and Chemicals

Propranolol hydrochloride, metoprolol succinate, oxprenolol, pindolol hydrochloride and atenolol were kindly supplied by Pacific Pharmaceutics Ltd (Auckland, New Zealand). Labetalol hydrochloride was purchased from Sigma Chemical Co (St. Louis, MO, USA). Sodium dodecyl sulphate (SDS), orthophosphoric acid, disodium hydrogenphosphate, HPLC-grade acetonitrile, n-butanol and ether were purchased from BDH Chemicals Ltd (Poole, England). Dichloromethane was obtained from M & B Australia Pty Ltd (Victoria, Australia). All chemicals used were analytical grade. Glassware was cleaned and silanized with 0.05% Aquasil$^{R}$ (Pierce Chemical Co, Rockford, IL, USA) before use.



### Chromatographic conditions

The analysis was was performed using a M6000A Waters pump (Waters Asso., Milford, MA, USA) equipped with either 1050 Hitachi fluorescence spectrophotometer (Hitachi, Tokyo, Japan) or variable wavelength UV detector (Spectroflow 757, Kratos Analytical Instruments, Ramsey, NJ, USA). The injections were made using a 7125 injector (Rheodyne, Cotati, CA, USA) with a 100-µl sample loop. A R-02 model chart recorder (Rikadenki Kogyo Co. Ltd, Japan) was used. Microbore columns (100 x 2 mm I.D.) were slurry-packed with a reversed-phase C18 material, 5 µm ODS-Hypersil (Shandon, London, UK) and typically provided in a plate efficiency in excess of 4,000 per 10 cm column length. The chromatographic system was operated at ambient temperature. The mobile phase was pumped at a flow rate of 0.5 ml/min.

For the determination of propranolol and metoprolol, a fluorescence detector was used at excitation wavelength of 225 nm, emission wavelength of 320 nm. Similarly, for the analysis of atenolol, a fluorescence detector was employed at an emission wavelength of 300 nm, excitation wavelength being 225 nm. A UV detector operated at a wavelength of 220 nm was used for the determination of labetalol.

The following systems were used to assess the optimal retention and obtain a satisfactory resolution of the β-blockers. System 1: For the development of a simple model to explain retention behaviour in ion-pair HPLC systems when SDS concentration was varied, acetonitrile-water (40 : 60, v/v) containing 10 mM disodium hydrogenphosphate, variable SDS concentrations (from 0 -100 mM) and adjusted to pH 2 with orthophosphoric acid. System 2: For the quantitative analysis of propranolol and metoprolol, a mobile phase consisting of acetonitrile-water (40 : 60, v/v) containing 10 mM disodium hydrogenphosphate and 100 mM SDS (pH 2.0). System 3: For the determination of labetalol, acetonitrile-water (50 : 50, v/v) containing 20 mM disodium hydrogenphosphate and 80 mM SDS (pH 2.0). System 4: For the determination of atenolol, a mobile phase composed of acetonitrile-water (15 : 85, v/v) containing 10 mM disodium hydrogenphosphate and 60 mM SDS (pH 2.0) was used.



**Sample preparation**

To 0.5 ml of plasma sample in a silanized tube, 100 µl of the internal standard solution was added (oxprenolol 50 µg/ml in water for metoprolol and propranolol assay; propranolol 0.5 µg/ml for labetalol assay and 5 µg/ml pindolol for atenolol assay). The mixture was basicified by adding 100 µl of 0.5 N NaOH for propranolol and metoprolol, 0.1 N NaOH for atenolol and 3 M $NH_4OAc$ for labetalol assay. The contents were shaken with an appropriate organic solvent for 10 min. Five ml of ether was used for the extraction of propranolol, metoprolol and labetalol. 5 ml of a mixture of dichloromethane/n-butanol (4 : 1, v/v) was used as an extraction solvent for atenolol. The samples were centrifuged at $4^0C$ for 10 min at 1,500 g. The organic layer was transferred to a tapered centrifuge tube containing 100 µl of an acidic aqueous solution (0.05 N

$H_2SO_4$ for propranolol, metoprolol and atenolol; and 0.1 N HCl for labetalol assay). The mixture was then shaken for 10 min. After centrifugation at $4^0C$ for 10 min at 1,500 g, the organic solvent was aspirated and discarded. A 50-µl aliquot of the aqueous solution was injected onto the HPLC column.

## RESULTS AND DISCUSSION

The chromatographic behaviour of metoprolol, propranolol, labetalol and atenolol have been studied in a reversed-phase ion-pair HPLC system with a microbore C18 column. Before the development of these HPLC assays, the UV and fluorescence spectra were determined for each β-blocker. The wavelength of 216 nm (12) and 233 nm (22) have been employed to measure labetalol concentration in plasma. In our study, the wavelength was chosen at 220 nm as this provided a maximum sensitivity for labetalol and no interference with endogenous components from plasma was found. For other β-blockers, fluorometric detection was used successfully. Excitation and emission wavelengths were selected to obtain the optimal conditions (see MATERIALS AND METHODS). The maximun response for the drugs of interest was achieved at these wavelengths and there was no endogenous interference from plasma.

Assay development in liquid chromatography has usually been conducted by trial and error. This is probably due to its theoretical complexity and also the lack of a commonly accepted mathematical model (23, 24). In the case of ion-pair chromatography, the optimization process is less straight forward. The chromatographic selectivities are governed by a number of variables, such as pH, organic modifier content, ion-pair reagents and concentration, as well as the buffers and organic counterion content (19-21, 25-27). Zoest (28) developed a complicated mathematical model to predict experimental conditions for retention optimization of ionic solutes with different variables. In the present study, a semi-empirical approach for rationalizing retention of β-blockers was developed in a reversed-phase ion-pair HPLC system when SDS concentration was varied, with fixed organic modifier

concentration and pH. The advantage of using reversed-phase ion-pair HPLC in the analysis of β-blockers relies on the fact that β-blockers are basic compounds. Ionic suppression techniques which are often applied to increase the retention of acidic compounds, are not practical under these circumstances. This is because the high pH value (pH 9-10) which required to suppress the ionization of β-blockers will dissolve the silica support of the stationary phase. In this study, pH of the mobile phase was adjusted to pH 2, β-blockers were fully ionized at this pH. The column displays significantly longer lifetime when used in the pH range of 2-4 (16). Reversed-phase ion-pair HPLC was therefore adopted in the quantitation of β-blockers in plasma. The retention time of β-blockers was achieved by the addition of the anionic hydrophobic pairing ion, SDS, to the mobile phase at low pH. SDS was chosen as the pairing ion because of its good solubility in the mobile phase (20). The variation of capacity factors (k') of β-blockers and internal standards as a function of the mobile phase SDS concentrations in the System 1 is presented in Fig. 1A. All four β-blockers go through the predicted maxima. Such chromatographic responses can be adequately modelled by Horvath's equation (29)

$$k'=(ko'+B[S])/(1+K1[S])(1+K2[S]) \qquad \text{Eq. 1}$$

where ko' is the capacity factor of β-blockers in the absence of pairing ion, K1 is the association constant for β-blockers and pairing ion, K2 is the binding constant of the pairing ion to the stationary phase, [S] is the concentration of pairing ion (SDS) in the mobile phase and the meaning of B depends on the underlying physicochemical equilibria controlling retention, such that for ion-pair formation in the mobile phase followed by distribution to the stationary phase $B=K1K3$, where K3 is the ion-pair distribution constant, and for dynamic ion-exchange mechanisms $B=K2K4$, where K4 is the formation constant for the solute-adsorbed pairing ion complex. Eq. 1 is in the form of a parabolic dependency for k' on [S] provided $K1^{-1}>(K2[S])^{-1}$. A plot of k' vs. [S] yields a parabola (Fig. 1A). Fig. 1A shows that atenolol is independent of pairing ion concentration in this system over 40 mM of SDS. This means atenolol has

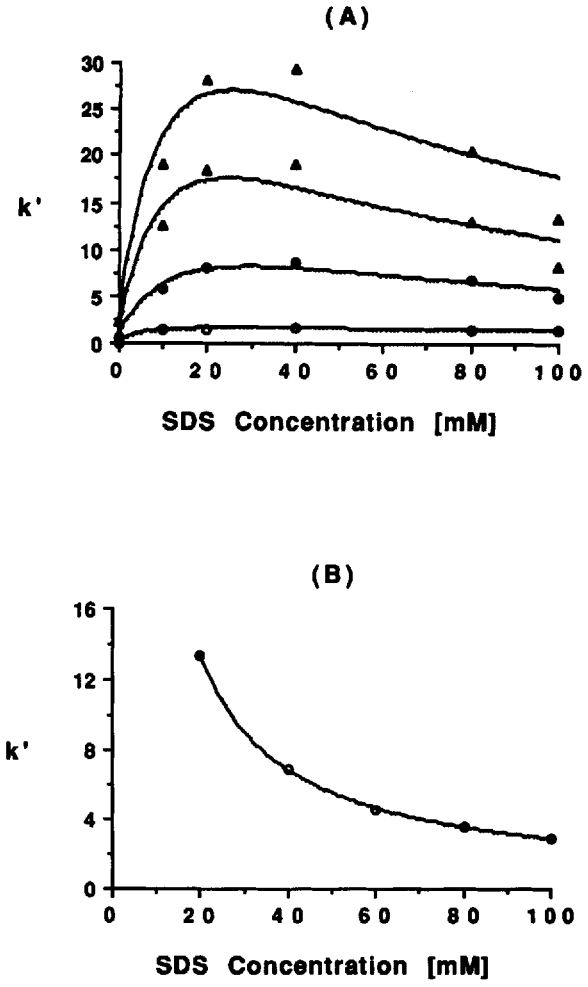


FIGURE 1.

Effect of paring ion (SDS) concentration on capacity factors (k') of β-blockers, under the conditions of System 1 (A) and System 4 (B). Conditions and compositions of mobile phase in System 1 and System 4 are described in Materials and Methods. (▲) propranolol, (Δ) labetalol, (●) metoprolol and (o) atenolol.

**TABLE 1**

Parameters Estimated for β-Blockers in System 1* with Eq. 1 and Kp Values

| Drug | K1 | K2 | K3 | K4 | B | Kp** |
|---|---|---|---|---|---|---|
| Atenolol | 0.004 | 0.255 | 127.5 | 2.0 | 0.51 | 0.015 |
| Metoprolol | 0.015 | 0.072 | 80.0 | 16.7 | 1.20 | 0.980 |
| Labetalol | 0.019 | 0.091 | 173.2 | 36.2 | 3.29 | 11.50 |
| Propranolol | 0.012 | 0.141 | 504.2 | 42.9 | 6.05 | 20.20 |

* System 1 is referred to the mobile phase used - see Materials and Methods.
** Kp (partition coefficient) values are from reference 31.



different mechanism from other β-blockers in this system. When changing acetonitrile from 40% to 15% in the mobile phase, a hyperbolic relationship between k' and [SDS] can be established for atenolol (Fig. 1B). The chromatographic condition selected often contains a mobile phase pairing ion concentration that produces a maximum solute retention (k'max) (21). This approach offers the advantage that retention of the ionic solutes is relatively insensitive to small changes in the mobile phase pairing ion concentration.

In order to validate Eq. 1, the experimental data were analysed by a Macintosh curve-fitting computer program ("Minim", by Dr. R. D. Purves, Department of Pharmacology, University of Otago, New Zealand). Marquardt's method was used for unweighted nonlinear least squares regression. A numerical central difference approximation was used for the derivatives. The appropriate parameters were estimated from fitting curves and are given in TABLE 1.

For a given pairing ion, Eq. 1 predicts that k' should increase with increasing concentration of the pairing ion up to the point and then decrease as the concentration of the pairing ion is increased. Theoretically it has been argued that these large hydrophobic ions exert their action either via a dynamic ion-exchange event, or via ion pairing in the mobile phase followed by distribution to the stationary support, or

via a combination of both effects (30). Hung and Taylor (19) proposed that the ion-exchange desolvation mechanism accounts for the experimentally observed variation of k' with pairing ion concentration because of the different mobile phase pairing ion concentrations which produces different retention maxima (Pmax). In this study, the formation constant for β-blocker-adsorbed pairing ion complex, K4, was correlated ($R^2$=0.876) with partition coefficient (Kp) of the β-blockers as shown in Fig. 2A. The Kp values for the β-blockers were taken from the report of Woods and Robinson (31). Fig. 2B shows good linear relationship between B and Kp ($R^2$=0.986). This indicates that the equilibrium of the chromatographic process in this system may be directly related to the hydrophobicity of β-blockers. The solute hydrophobicity appears to be an important factor for controlling the retention, suggesting that the Kp values of the β-blockers could be used to predict solute retention in ion pair HPLC. However, K1, K2 and K3 were not correlated with Kp. This would suggest that ion-pair formation are largely independent of solute hydrophobicity. The values of Pmax obtained from our study for atenolol, labetalol, metoprolol and propranolol are 30.3, 24.2, 30, and 26.3 mM, respectively (FIGURE 1). These results are in agreement with the previous observations by Hung and Taylor (19) and Zoest (28) that the Pmax values are dependent on the solutes. All these findings are suggested as being useful for prediction of retention behaviour of β-blockers.

In the present study, mobile phase containing different concentrations of SDS were used for different β-blockers as they provide acceptable retention for the drugs of interest and internal standard. The easy regulation of the retention of β-blockers by SDS concentration in the mobile phase has made these chromatographic system highly suitable for the pharmacokinetic studies. A suitable SDS concentration can be easily obtained from the plot of k' versus SDS concentration curves.

The best chromatographic conditions showing the compositions of acetonitrile, phosphate buffer and SDS are outlined in Materials and Methods for each β-blocker studied. Under these chromatographic conditions, no interference from plasma endogenous substances was

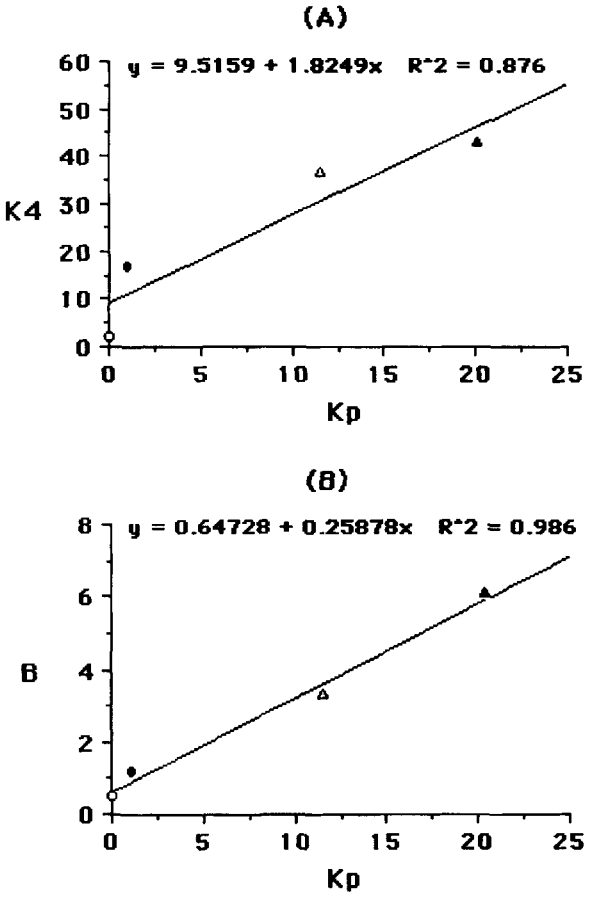




FIGURE 2.

(A) Relationship between the formation constant for solute-adsorbed pairing ion complex (K4) and the partition coefficient (Kp) of β-blockers.

(B) Relationship between the constant B and partition coefficient (Kp) of β-blockers.

(▲) propranolol, (Δ) labetalol, (●) metoprolol and (o) atenolol.

The values of K4, B and Kp are derived and given in TABLE 1.

R^2 is the coefficient of determination.

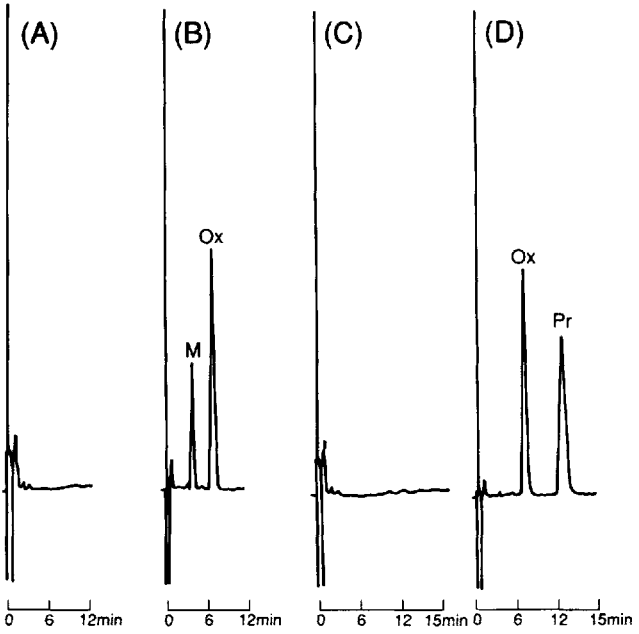


FIGURE 3.

Chromatograms of human blank plasma (A and C); plasma spiked with 25 ng/ml metoprolol and 50 μg/ml of internal standard, oxprenolol (B); and plasma spiked with 10 ng/ml of propranolol and 50 μg/ml of internal standard, oxprenolol (D). The chromatographic conditions are given in Materials and Methods.

Peaks: M = metoprolol; Ox = oxprenolol; Pr = propranolol.

observed. β-Blockers of interest and their internal standards were well resolved as shown in Fig. 3 and 4.

For the analysis of metoprolol and propranolol, oxprenolol was used as an internal standard. The mobile phase consisted of acetonitrile-water (40 : 60, v/v) containing 10 mM $Na_2HPO_4$ and 100 mM SDS (pH 2.0). Under these chromatographic conditions, retention times for metoprolol, oxprenolol and propranolol were 5.5, 8.0 and 13 min, respectively.

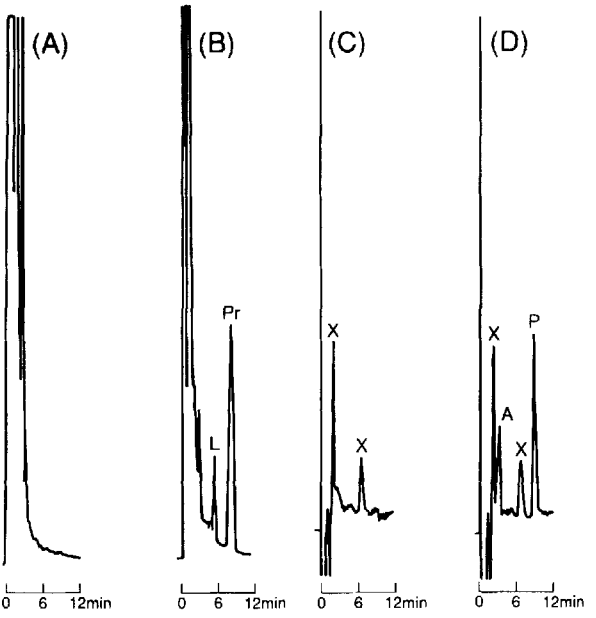


FIGURE 4.

Chromatograms of human blank plasma (A and C); plasma spiked with 25 ng/ml labetalol and 0.5 μg/ml of internal standard, propranolol (B); and plasma spiked with 25 ng/ml of atenolol and 5 μg/ml of internal standard, pindolol (D). The chromatographic conditions are given in Materials and Methods.

Peaks: L=labetalol; A=atenolol; Pr=propranolol; P=pindolol; X=plasma endogenous peak.

Typical chromatograms of human plasma spiked with metoprolol and propranolol are illustrated in Fig. 3. The detection limits of the assays were 5 and 1 ng/ml for metoprolol and propranolol, respectively. The calibration curves constructed from the peak height ratios versus metoprolol and propranolol concentrations were linear in the concentration range tested (5 to 500 ng/ml for metoprolol and 1 to 500 ng/ml for propranolol).

For the analysis of labetalol, propranolol was used as an internal standard. The separation of labetalol and propranolol from the plasma endogenous peaks was achieved by using a mobile phase consisted of acetonitrile-water (50 : 50, v/v) containing 20 mM $Na_2HPO_4$ and 80 mM SDS (pH 2.0). Retention times were 5.6 and 8.3 min for labetalol and propranolol, respectively. Representative chromatograms of human plasma spiked with metoprolol and propranolol are presented in Fig. 4 (A). The detection limit of the assay were 5 ng/ml. The calibration curves constructed from the peak height ratios versus metoprolol concentration were linear in the concentration range tested (5 to 500 ng/ml).

For the determination of atenolol, pindolol was used as an internal standard. The elution of the analytes of interest was achieved by using a mobile phase comprised of acetonitrile-water (15 : 85, v/v) containing 10 mM $Na_2HPO_4$ and 60 mM SDS (pH 2.0). Under these chromatographic conditions, the retention times for atenolol and pindolol were 4.6 and 8. 3 min, respectively. Typical chromatogram of human plasma spiked with atenolol and pindolol is shown in Fig. 4 (B). The detection limit of the assay were 10 ng/ml. The calibration curves were found to be linear in the concentration range tested (10 to 500 ng/ml).

Correlation coefficients of the calibration curves for these four β-blockers were greater than 0.999. The detection limit was defined on a basis of a signal-noise ratio (4:1). The extraction recoveries of β-blockers and their internal standards were assessed by comparing the peak heights of the drugs and their internal standards with those obtained by direct injection of the compounds of equivalent quantities (TABLE 2). The mean recoveries of atenolol, metoprolol, labetalol and propranolol at two concentrations studied were all greater than 82%. Data showing the intra-assay reproducibility and precision of the assays for atenolol, metoprolol, labetalol and propranolol in human plasma are summarized in TABLE 3. The inter-assay precision (between days) of the methods were reasonable with a C.V. less than 10% (n=5) at the same concentrations as those used for the intra-assay reproducibility studies.

The plasma samples containing either atenolol, metoprolol, labetalol or propranolol stored at $-20^0C$ for up to 3 months showed no signs of

**TABLE 2**

Absolute Recovery (Mean±S.D.) for Atenolol, Metoprolol, Labetalol and Propranolol

| Drug | Concentration (ng/ml) | Recovery (%) | Coefficient of Variation (%) |
|---|---|---|---|
| Atenolol | 25 | 91±3.8 | 4.2 (n=6) |
| | 100 | 93±3.7 | 3.8 (n=6) |
| Metoprolol | 5 | 96±4.3 | 4.5 (n=5) |
| | 100 | 101±0.3 | 0.3 (n=5) |
| Labetalol | 10 | 82±2.5 | 3.0 (n=6) |
| | 100 | 87±2.9 | 3.3 (n=6) |
| Propranolol | 5 | 100±2.7 | 2.7 (n=4) |
| | 100 | 101±2.1 | 2.1 (n=4) |



**TABLE 3**

Reproducibility and Precision of the Assay of β-Blockers in Human Plasma

| Spiked Concentration (ng/ml) | Observed Concentration (ng/ml)[1] | Coefficient of Variation (%) | Accuracy (%)[2] |
|---|---|---|---|
| **Atenolol** (n=6) | | | |
| 25 | 24.4±1.1 | 4.5 | 97.6 |
| 100 | 98.7±0.5 | 0.5 | 98.7 |
| **Metoprolol** (n=5) | | | |
| 5 | 4.9±0.2 | 4.5 | 98.0 |
| 100 | 99.3±0.3 | 0.3 | 99.3 |
| **Labetalol** (n=6) | | | |
| 10 | 9.8±0.1 | 1.2 | 98.0 |
| 100 | 99±1.1 | 1.1 | 99.0 |
| **Propranolol** (n=5) | | | |
| 5 | 4.9±0.1 | 2.2 | 98.0 |
| 100 | 98.5±1.2 | 1.2 | 98.5 |

1 Results given are mean ± S.D.

2 $\text{Accuracy}(\%) = \frac{\text{(Observed concentration) X 100}}{\text{Spiked concentration}}$

decomposition and practically the similar concentration values were obtained (n=5). This indicates that atenolol, metoprolol, labetalol and propranolol are stable in human plasma samples under these storage conditions for at least 3 months.

Basic compounds often exhibit tailing and column-to-column variations in retention. This problem was overcome by using microbore column. In addition, the use of microbore column enhances mass sensitivity and is more economical because less mobile phase is consumed (32, 33).

In summary, the present study has demonstrated the application of theoretical aspect of ion-pair liquid chromatography to predict the retention and to achieve optimal chromatographic conditions using pairing ion, SDS, for β-blocker assays. The procedures described here are simple, less time consuming and provides a sufficient sensitivity for the measurement of atenolol, metoprolol, labetalol and propranolol. The assay for each β-blocker has been shown to be suitable for the use in pharmacokinetic studies. We have employed these assays to measure concentrations of β-blockers in bioavailability studies, and to study the placental transfer of these drugs in human and guinea pig placental perfusions and in the pharmacokinetic studies in pregnant sheep.



## REFERENCES

1. Frishman, W. H. and Chesner, M. Am. Heart J., 115, 147, 1988

2. Frishman, W. H. and Silverman, R. Am. Heart J., 98, 119, 1979

3. Kaulhausen, H. and Wechsler, E. Clin. Exper. Hyper., B7, 213, 1988

4. Pflugman, G., Spahn, H. and Mutschler, E. J. Chromatogr., 416, 331, 1987

5. Sa'sa', S. I. J. Liq. Chromatogr., 11, 929, 1988

6. Smith, H. T. J. Chromatogr., 415, 93, 1987

7. Tsuei, S. E. and Thomas, J. J. Chromatogr., 181, 135, 1980

8. Devi, K. P., Rao, K. V. R. and Baverja, S. K. J. Chromatogr., 434, 265, 1988

9. Scales, B. and Copsey, P. B. J. Pharm. Pharmacol., 27, 430, 1975

10. Nation, R. L., Peng, G. W. and Chiou, W. L. J. Chromatogr., 145, 429, 1978

11. Ervik, M. Acta Pharmacol. Toxicol., 36, 136, 1975

12. Hidalgo, I. J. and Muir, K. T. J. Chromatogr., 305, 222, 1984

13. Pierson, S. L., Hanigan, J. J., Taylor, R. E. and McClurg, J. E. J. Pharm. Sci., 68, 1550, 1979

14. Ghanekar, A. and Das Gupta, V. J. Pharm. Sci., 67, 873, 1978

15. Lurie, I. S. and Demchuk, S. M. J. Liq. Chromatogr., 4, 337, 1981

16. Knox, J. H. and Jurand, J. J. Chromatogr., 125, 89, 1976

17. Bidlingmeyer, B. A. J. Chromatogr. Sci., 18, 525, 1980

18. Sorrel, R. H. A. and Hulshoff, A. Adv. Chromatogr., 21, 87, 1983

19. Hung, C. T. and Taylor, R. B. J. Chromatogr., 202, 333, 1980

20. Hung, C. T. and Taylor, R. B. J. Chromatogr., 209, 175, 1981

21. Hung, C. T., Taylor, R. B. and Paterson, N. J. Chromatogr., 240, 61, 1982

22 Dusci, L. J. and Hackett, L. P. J. Chromatogr., 175, 208, 1979

23 Taylor, R. B., Reid, R. and Hung, C. T. J. Chromatogr., 316, 279, 1984

24. Bartha, A. and Vigh, Gy. J. Chromatogr., 395, 503, 1987

25. Bartha, A. and Vigh, Gy. J. Chromatogr., 265, 171, 1983

26. Hung, C. T., Lim, J. K. C. Zoest, A. R. and Lam, F. C. J. Chromatogr., 425, 331, 1988

27. Bartha, A. Billet, H. A. H. Gallan, L. de. and Vigh, Gy. J. Chromatogr., 291, 91, 1984

28. Zoest, A. R. Master Thesis. Otago University, Dunedin, New Zealand. 1989

29. Horvath, C., Melander, W., Molnar, I. and Molnar, P. Anal. Chem., 49, 2295, 1977

30. Knox, J. H. and Laird, G. R. J. Chromatogr., 122, 17, 1976

31. Woods, P.B. and Robinson, M. L. J. Pharm. Pharmacol., 33, 172, 1980

32. Wong, S. H-Y., Cudny, B., Aziz, O., Marzouk, N. and Sheeham, S. R. J. Liq. Chromatogr., 11, 1143, 1988

33 Hyldburg, P. A., Sparacino, C. M., Hines, J. W. and Keller, C. D. J. Liq. Chromatogr., 10, 2639, 1987