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# DETERMINATION OF PROPRANOLOL IN PERITONEAL DIALYSIS FLUID BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITHOUT EXTRACTION

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## SUMMARY

A rapid, sensitive method for the high-performance liquid chromatographic determination of propranolol in peritoneal dialysis fluid is described. An extraction step is replaced by the use of a $C_{18}$ Sep-Pak® cartridge for sample preparation. The procedure offers an acceptable alternative to sample extraction and will allow for pharmacokinetic studies of propranolol in patients undergoing peritoneal dialysis for chronic renal failure.

## INTRODUCTION

Propranolol·HCl is a well known beta antagonist having a wide variety of indications in medical practice [1]. Its use in the therapy of hypertension in normal individuals and in patients with end-stage renal disease is well documented [2—5]. Propranolol pharmacokinetics have been studied in patients undergoing hemodialysis [6—9], but no information is available concerning kinetics of the drug in patients undergoing peritoneal dialysis.

Analytical procedures utilizing high-performance liquid chromatography (HPLC) have been described for the determination of propranolol·HCl in plasma and urine [10—16]. These procedures call for extraction of the drug from the biological fluid, at a basic pH, into an organic solvent. While some published procedures then use an additional acid extraction from the organic solvent [10—13], most procedures call for evaporation of the organic solvent followed by reconstitution of the residue with another solvent followed by injection into an HPLC system. One published report utilizes protein precipitation rather than extraction [17]. Almost all published HPLC procedures use a fluorescence detector with a variety of wavelengths for excitation

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and emission [10—17]. No analytical procedures have been described for the determination for propranolol in peritoneal dialysis fluid.

This paper describes a new procedure for the HPLC determination of propranolol in peritoneal dialysis fluid obtained from patients undergoing continuous ambulatory peritoneal dialysis [18] for end-stage renal disease. Previously described extraction procedures were found to give unreliable results when used with dialysis fluid. Consequently the extraction step was replaced by the use of a $C_{18}$ Sep-Pak cartridge (supplied by Waters Assoc., Milford, MA, U.S.A.). The stability of propranolol in dialysis fluid was also studied.

## EXPERIMENTAL

### *Instrumentation*

A high-performance liquid chromatograph equipped with an M-45 pump, U6K injector and reversed-phase $C_{18}$ μBondapak column (all from Waters Assoc.) was used. The detector was a Spectra/Glo fluorometer (Gilson Electronics, Middleton, WI, U.S.A.) equipped with a 45-μl quartz flow-through cell and a 280-nm light source and filter for excitation. An emission filter of 330—380 nm was used. Detector output was recorded with a single-pen 25-cm strip-chart recorder (Linear Instrument, Irvine, CA, U.S.A.).

### *Chemicals and reagents*

Glacial acetic acid and propyl-paraben were reagent grade. Acetonitrile and methanol were HPLC grade (J.T. Baker, Phillipsburg, NJ, U.S.A.). Propranolol·hydrochloride was supplied courtesy of Ayerst Laboratories (New York, NY, U.S.A.). Peritoneal dialysis fluid (Dianeal-137® with 4.25% dextrose, Travenol Labs., Deerfield, IL, U.S.A.) was obtained from renal failure patients undergoing continuous ambulatory peritoneal dialysis as part of their normal medical therapy.

### *Mobile phase*

The mobile phase was acetonitrile—methanol—glacial acetic acid—deionized water (30:5:1:64), which is a modification of that reported by Pritchard et al. [12]. After filtration and sonification for 15 min the degassed mobile phase was pumped through the column at a flow-rate of 2 ml/min.

### *Assay standards*

A stock solution of propranolol·HCl in methanol (1.00 mg/ml) was prepared. For assay work the stock solution was diluted with methanol so as to contain 4.00 ng/ml. Both stock solution and dilution were prepared fresh monthly and stored at 4°C. The internal standard, propyl-paraben, was prepared in methanol (0.499 mg/ml). It was also prepared monthly and stored at 4°C. No changes in the chromatograms, or extra peaks, were noted during use of the standard solutions over a period of 30 days.

### *Analytical procedure*

Dry $C_{18}$ Sep-Pak cartridges were prepared for use by passing through approx-

imately 2 ml of methanol followed by approximately 4 ml of deionized water. Sep-Pak cartridges were used only once and then discarded because Sep-Pak cartridges which had been cleaned and re-used gave unreliable results.

*Dialysis fluid*

To 6 ml of peritoneal dialysis fluid were added 100 μl of internal standard solution and an aliquot of the dilute propranolol standard containing 40—400 ng of drug as the hydrochloride salt (10—100 μl of standard dilute solution). Each sample was then vortexed for 10 sec and a 5-ml aliquot was passed through a prepared Sep-Pak at a rate of approximately 100 drops per min. Drug and internal standard were then eluted from the Sep-Pak by passing 2 ml of a wash solution through the Sep-Pak. The wash solution was acetonitrile—methanol—deionized water—glacial acetic acid (40:20:39:1). Injections of 200 μl were made from this wash solution using a 500-μl syringe (Hamilton, Reno, NV, U.S.A.). The chromatograms were recorded at a chart speed of 15 cm/h and separation was adequate for measurement of peak heights (see Fig. 1). Peak height ratios (propranolol to internal standard) were calculated and plotted versus propranolol concentration expressed as ng/ml.

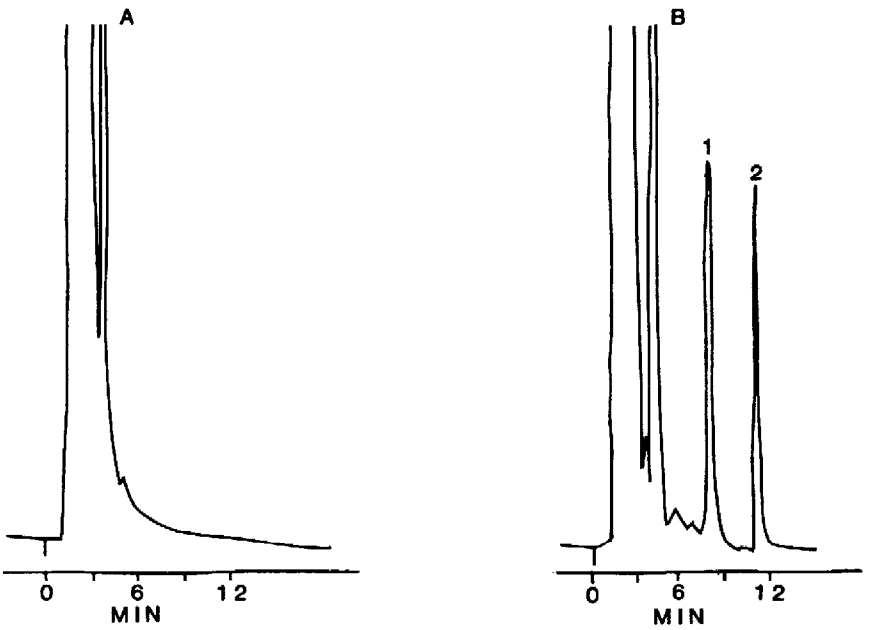


Fig. 1. Assay of propranolol in peritoneal dialysis fluid obtained from renal failure patients undergoing continuous ambulatory peritoneal dialysis. (A) Propranolol-free dialysis fluid; (B) dialysis fluid with added propranolol. Peaks: 1 = propranolol, 32 ng/ml; 2 = propylparaben (internal standard).

*Stability study*

Volumes of 500 ml of dialysis fluid containing 16 and 64 ng/ml of propranolol were prepared and divided into thirty, 15-ml samples for each concentration. The samples were placed in 50-ml, glass, screw-top bottles and

fifteen samples of each concentration were stored at refrigerator temperature (5°C) and −60°C. Three samples at each concentration and storage condition were assayed at days 0, 3, 7, 10 and 14.

*Recovery study*

Recovery of both propranolol and internal standard was studied in dialysate. The procedure used for the study is shown in Table I. Sample 2 represents 100% recovery of drug since the propranolol was added to the wash solution after the wash was passed through the Sep-Pak. Likewise sample 3 represents 100% recovery of internal standard. The procedure followed for sample 1 follows that used for the standard curves as run in dialysis fluid. Mean peak height ratios were determined for each sample and percent recovery of propranolol and internal standard determined by comparing samples 1 and 2 and samples 1 and 3, respectively.

TABLE I

PROCEDURE FOR RECOVERY STUDY, PERITONEAL DIALYSIS FLUID

Samples 2 and 3 represent 100% recovery of drug and internal standard, respectively. Sample 1 represents the standard curve procedure.

| Sample No. | | |
|---|---|---|
| 1 | 2 | 3 |
| 1 ml I.S.* + 500 µl propranolol** + dialysate to a final volume of 50 ml | 1 ml I.S.* + 500 µl methanol + dialysate to a final volume of 50 ml | 1 ml methanol + 500 µl propranolol** + dialysate to a final volume of 50 ml |
| ↓ | ↓ | ↓ |
| 5 ml aliquot*** through Sep-Pak | 5 ml aliquot*** through Sep-Pak | 5 ml aliquot*** through Sep-Pak |
| ↓ | ↓ | ↓ |
| 2 ml wash§ through Sep-Pak | 2 ml wash§ through Sep-Pak | 2 ml wash§ through Sep-Pak |
| ↓ | ↓ | ↓ |
| add 100 µl methanol | add 50 µl propranolol** + 50 µl methanol | add 100 µl I.S.* |
| ↓ | ↓ | ↓ |
| 200 µl injected | 200 µl injected | 200 µl injected |

*Internal standard, 0.499 ng/ml.
**Dilute standard solution, 4 ng/ml.
***Repeated five times with new Sep-Paks.
§ Acetonitrile—methanol—deionized water—glacial acetic acid (40 : 20 : 39 : 1).

## RESULTS AND DISCUSSION

Recovery of both propranolol and internal standard from dialysate was 100%. Using Student's $t$ test there was no significant difference in peak height ratios when comparing samples 1 and 2 and samples 1 and 3 ($P > 0.05$). Reproducibility of recovery was good. The percent coefficient of variation in peak height ratios for sample 1 (five replications) was 3.1%. Recovery of propranolol from plasma using various extraction techniques has been reported as 80±5% [10,19] to 90% [11].

The pH of the propranolol solution does not seem to effect binding of the drug to the Sep-Pak. When 2 ml of a solution of propranolol in sodium carbonate (pH 11.4) was passed through the Sep-Pak no drug was detected in the effluent. When 2 ml of wash solution was then passed through the Sep-Pak recovery of propranolol was 100%. Likewise, when 2 ml of a solution of propranolol·HCl in dilute hydrochloric acid (pH 3.1) was passed through another new Sep-Pak no drug was detected in the effluent. However, when 2 ml of wash solution was passed through the Sep-Pak recovery of drug was approximately 80%. The reason for this change in binding is unknown. Studies of the effect of pH on binding of internal standard were not done.

Five standard curves (6.28–65.7 ng/ml) in dialysate were run over a period of fourteen days. Linear regression analysis was used to calculate the slope and intercept for each curve. Mean percent of theory for each curve was calculated using the technique of inverse estimation [20]. These results are shown in Table II. Curves 4 and 5 were run on a new $C_{18}$ μBondapak column which may account for the change in slope and intercept. Mean percent of theory for the five curves was 100.9% with a mean coefficient of variation of 5.1%. Day-to-day accuracy and precision of the assay are shown by the data in Table III. Mean retention times for propranolol and internal standard were 7.5 and 10.5 min, respectively. It was possible to decrease the lower limit of the assay to 1.79 ng/ml by doubling the volume of dialysate passed through the Sep-Pak from 5 to 10 ml. Three standard curves were run at approximately 30-day intervals using this procedure. Mean percent of theory was 98.9% with a mean coefficient of variation of 5.0% for these curves. Each curve included four

TABLE II

STANDARD CURVE DATA FOR ASSAY OF PROPRANOLOL·HCl IN PERITONEAL DIALYSIS FLUID

Five points (6.28–65.7 ng/ml) included for each curve. Data collected over fourteen days at approximately 2-day intervals.

| Curve | Correlation coefficient ($r$) | Slope | Intercept | Mean percent of theory | Coefficient of variation (%) |
|---|---|---|---|---|---|
| 1 | 0.999 | 0.0448 | −0.0655 | 100.3 | 3.0 |
| 2 | 0.999 | 0.0432 | −0.0645 | 101.5 | 4.5 |
| 3 | 0.999 | 0.0415 | −0.0502 | 101.2 | 6.3 |
| 4 | 0.997 | 0.0335 | −0.0918 | 103.3 | 9.8 |
| 5 | 0.999 | 0.0315 | 0.0203 | 99.3 | 2.0 |

TABLE III

PRECISION AND ACCURACY FOR ASSAY OF PROPRANOLOL·HCl IN PERITONEAL DIALYSIS FLUID

Data obtained from standard curves run over a period of fourteen days at approximately 2-day intervals. Each experimental concentration is the mean of five determinations.

| Actual concentration (ng/ml) | Mean experimental concentration (ng/ml) | Mean percent of theory | Coefficient of variation (%) |
|---|---|---|---|
| 6.28 | 6.74 | 107.3 | 8.5 |
| 16.55 | 16.66 | 100.7 | 3.7 |
| 32.87 | 31.86 | 96.9 | 2.7 |
| 49.15 | 49.12 | 99.9 | 3.0 |
| 65.70 | 66.15 | 100.7 | 1.9 |
| Mean ± S.D. | | 101.1 ± 3.8 | 3.96 ± 2.6 |

TABLE IV

STABILITY DATA FOR PROPRANOLOL·HCl IN PERITONEAL DIALYSIS FLUID

Values represent the mean percent remaining ± S.D. of three determinations at the times indicated.

| Time (days) | Percent remaining | | | |
|---|---|---|---|---|
| | 5°C | | −60°C | |
| | 16 ng/ml | 64 ng/ml | 16 ng/ml | 64 ng/ml |
| 0 | 116.8 ± 1.0 | 105.9 ± 1.7 | 116.8 ± 1.0 | 105.9 ± 1.7 |
| 3 | 104.3 ± 6.07 | 105.4 ± 2.84 | 107.7 ± 3.03 | 107.3 ± 2.06 |
| 7 | 100.0 ± 3.92 | 105.0 ± 1.33 | 105.0 ± 0.50 | 105.6 ± 0.61 |
| 10 | 111.3 ± 2.55 | 98.9 ± 1.87 | 111.0 ± 4.45 | 100.5 ± 2.48 |
| 14 | 103.3 ± 2.51 | 104.3 ± 1.79 | 107.2 ± 5.31 | 115.2 ± 1.85 |

points and ranged in concentration from 1.79—33.12 ng/ml. The correlation coefficient was 0.999. These results in dialysate are comparable to previously published urine and plasma studies using extraction for sample preparation [12—14].

Propranolol, at a concentration of both 16 and 64 ng/ml, was stable in dialysis fluid for up to fourteen days when stored at either 5°C or −60°C (see Table IV). Previously published studies have shown propranolol to be stable in plasma for up to three days when frozen [7,21].

The use of $C_{18}$ Sep-Pak cartridges for sample preparation offers an acceptable alternative to extraction for the determination of propranolol in peritoneal dialysis fluid. The availability of a procedure to measure propranolol in peritoneal dialysis fluid will allow for studies of propranolol clearance in renal failure patients undergoing peritoneal dialysis as part of their medical therapy.

ADDENDUM

The use of a $C_{18}$ Sep-Pak cartridge for determination of propranolol in

plasma was investigated although this was not the primary purpose of this project. Protein precipitation was accomplished by adding acetonitrile containing internal standard to the plasma sample. After mixing and centrifugation an aliquot of supernatant was passed through a prepared Sep-Pak. Drug and internal standard were then eluted from the Sep-Pak as described for dialysate samples. Recoveries of drug and internal standard were 71.2% and 72.8%, respectively. The recovery study for plasma was similar to that described for dialysis fluid. Although incomplete, recovery was reproducible. The percent coefficient of variation in peak height ratios (five replications) was 4.4%. A five-point standard curve ranging from 18 to 190 ng/ml was run following the procedure described above. The correlation coefficient, mean percent of theory and coefficient of variation were 0.995, 104% and 7.3, respectively. It appears that this technique may be suitable for the determination of propranolol in plasma. Narasimhachari [22] recently evaluated $C_{18}$ Sep-Pak cartridges in the determination of tricyclic antidepressants in plasma and urine. He concluded that the cartridges were quite useful and provided a considerable saving in time.

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