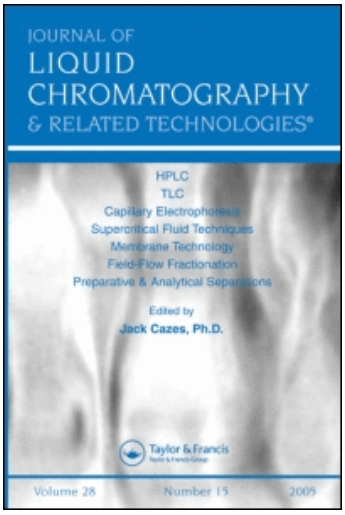

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STABILITY INDICATING HPLC ASSAY OF PROPRANOLOL FOR TRANSDERMAL PERMEATION STUDY

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ABSTRACT

A stability indicating reversed phase high-performance liquid chromatographic assay of propranolol (PP) was developed. The assay enabled the quantitation of PP in both diffusate and human skin extract samples of permeation study, at as low as 0.1 µg/ml with a signal noise ratio of 7. Baseline resolution of PP from internal standard, Verapamil (VRP) and degraded products was achieved with a cyano column and a mobile phase of 47% acetonitrile in water with 5 mM heptane sulfonic acid as the ion pairing agent. The linear range was established at 0.5-10 µg/ml, and 1-15 µg/ml, for diffusate and skin extract samples, respectively. The assay was reproducible with low within-day (2.2 and 8.8 % for diffusate and skin extract, respectively) and between-day (2.2 and 7.7%, respectively) variations. The applicability of the assay for transdermal permeation studies of PPHCl and PP was demonstrated.

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INTRODUCTION

Propranolol hydrochloride (PPHCl) is a nonselective adrenergic beta receptor blocking agent (1). Its chemical structure is shown in Figure 1. It is indicated for the treatment of hypertension, angina pectoris, cardiac arrhythmias, hyperthyroidism, anxiety, hypertropic obstructive cardiomyopathy and pheochromocytoma (2). However, the drug undergoes extensive first pass metabolism in the liver and leads to upto 20-fold variability in plasma concentration after oral administration of comparable doses. Transdermal route of drug administration, in general, bypasses the first pass hepatic metabolism, and may alleviate the problem of variability. In order to evaluate the feasibility of transdermal delivery of propranolol as related to its permeation characteristics, it is a prerequisite to develop a sensitive and specific assay to monitor the drug in permeation diffusate and skin extract samples.

In literature, there are several assays of PP reported for biological fluids. These include radioreceptor assay (3), analysis with ion-pair extraction (4,5), radioimmunoassay (6), gas-chromatography (GC,7-10), GC-mass spectrometry (11-12), thin layer chromatography (TLC,13), Spectrofluoremetry (14) and high performance liquid chromatography (HPLC, 15-20). These assays applied to plasma (4,9,10,12-20), urine (5,15,17) or brain (8), and none is for skin tissue

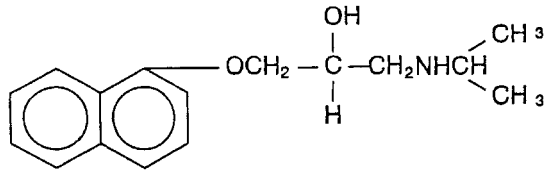

Figure 1: The chemical structure of propranolol hydrochloride.

which requires extensive homogenization and extraction procedures.

In transdermal screening of PP, it is necessary to quantitate the PP retention in the skin, in addition to the PP permeation. First, this information is indicative of the extent of PP binding in the skin. The drug molecules retained in the skin act as a depot reservoir after the topical dosage form or transdermal patch is removed, and have clinical relevance. Secondly, any modification of PP skin retention in the presence of a potential permeation enhancer may provide clue in elucidating the mechanism of the enhancer. The modes of enhancer action are known to increase one or combination of the following parameters: skin retention, partitioning coefficient or permeability coefficient of the drug. However, the skin is a complex membrane composed of lipids and proteins; therefore, it is required to develop a specific assay free of interference from the skin components. To date, no such an

assay for monitoring PP in diffusate and human skin samples has been published. In this report, a specific and stability-indicating assay was developed, and its application for transdermal permeation study was demonstrated.

MATERIALS

PPHCl and verapamil hydrochloride (VRP) were purchased from Sigma Chemical Co. (St. Louis, MO). PP base was prepared by neutralization of PPHCl with equimolar sodium bicarbonate, and verified with melting point measurement. HPLC grade acetonitrile and heptane sulfonic acid were from Fisher Scientific Co. (Fairlawn, NJ). Human cadaver skin (65-year old Caucasian male) was obtained from the Texas Medical Center, Houston, TX.

METHODS

High Performance Liquid Chromatography

A liquid chromatograph (Consta-Metric I, Laboratories Data Control, Riviera Beach, FL.), equipped with a 50 ul sample loop (Rheodyne, Berkeley, CA), a variable wavelength U.V. detector (Spectro Moniter III, Laboratories Data Control), a reversed phase cyano (CN, 4.6 mm X 150 mm, particle size, 5 μm) column was used. The isocratic mobile phase was 47% acetonitrile in water with 5 mM sodium heptane sulfonic acid as the ion pairing agent (pH 6.96). The U.V. detection was at 230

nm, with a flow rate of 1.9 ml/min. The internal standard was VRP (15 µg/ml).

Calibration Curves

Calibration curves were constructed in the range of 0.5 to 10 µg/ml and 1 to 15 µg/ml for diffusate and skin extract samples, respectively. PP and VRP stock solutions of 100 µg/ml were prepared. When required, appropriate dilutions were made to obtain the standard solutions.

Validation of the Assay

The assay was validated by establishing the within-day and between-day variations. Five sets of calibration curves were made on the same day for within-day variation. To establish the between-day variation, the assay was repeated with freshly prepared samples over a period of 2 years.

Forced Degraded Samples

PP solution was boiled for 3 minutes in 0.1 N HCl or 0.1 N NaOH for the extremely acidic and alkaline conditions, respectively. The solutions were analyzed by the HPLC assay.

In vitro Permeation Study (21)

The Franz diffusion apparatus was used for the

permeation study. The defatted full-thickness skin sample was mounted between the donor and receptor chambers of Franz diffusion cell (FDC 108 Series with FDC 128 manifold and FDC 127 magnetic bar, Crown Glass Co., Somerville, N.J.).

The stratum corneum side was exposed to the ambient condition and the dermal side was oriented towards the saline-phosphate buffer (pH 7.4) in the receptor chamber. The buffer was stirred with a magnetic stirrer. Two hundred microliters of test solution of PPHCl (60 mg/ml) or PP base (44.5 mg/ml) in propylene glycol (PG) was applied to the exposed skin. The solution of the receptor chamber was equilibrated by circulating water at 37°C. Samples were taken at predetermined time intervals upto 72 hours and analyzed by the HPLC assay.

Extraction Procedure

Using a polytron homogenizer (Polytron, Kinematic GmbH), defatted skin of about 300 mg was homogenized three times with five milliliters of chloroform each time. The homogenates were combined, filtered through Whattman-1 filter paper and evaporated to dryness. The residue was reconstituted with 1 ml of the mobile phase and analyzed by HPLC.

Data Analysis

The permeation parameters of lag time (T), perme-

ability coefficient (Kp), the diffusion constant (D/d^2), and the partition coefficient (Km*d) were calculated from the permeation data. A graph of amount permeated versus time was plotted. The X intercept of the extrapolated linear region of the curve gave T. D was calculated from T using equation 1:

$$D/d^2 = 1/6T \qquad \text{(Equation 1)}$$

The slope of the linear portion of the profile, determined by linear regression analysis, gave the flux (Jx). Because Jx = (Km * D * Cs)/d = Kp * Cs, where Cs is the drug concentration and Kp = (Km * D)/d. Kp and Km*d were calculated by equations 2 and 3, respectively:

$$Kp = Jx/Cs = Slope/Cs \qquad \text{(Equation 2)}$$

$$Km * d = (Kp)/ (D/d^2) \qquad \text{(Equation 3)}$$

RESULTS AND DISCUSSION

The typical chromatograms of PP and VRP with baseline separation, were shown in Figure 2. The retention times of PP and VRP were 7.2 and 9.6 minutes, respectively. The sensitivity of the developed assay was 0.1 μg/ml at a signal noise ratio of 7. The chromatogram of blank skin sample had no interfering peak with PP or VRP. The chromatograms of PP after forced degradation at extreme pH's were shown in Figure 3. Baseline resolution of PP from all degradation products was achieved.

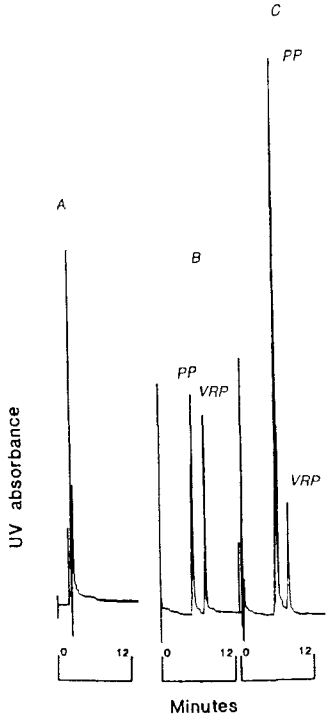

Figure 2: Authentic HPLC chromatograms of (A) blank skin extract, (B) PP and VRP in diffusate and (C) skin extract. PP, propranolol, 7.2 min and VRP, verapamil, 9.6 min.

Linearity was established ($r^2 > 0.99$) for the calibration curves at 0.5-10 and 1-15 μg/ml, for PP in diffusate of saline phosphate buffer and in the homogenized skin extract, respectively (Figure 4). The assay was reproducible with low within-day and between-day variations, 2.2% for diffusate samples and 7.7-8.8% for skin samples (Table 1). The extraction efficiency of PP

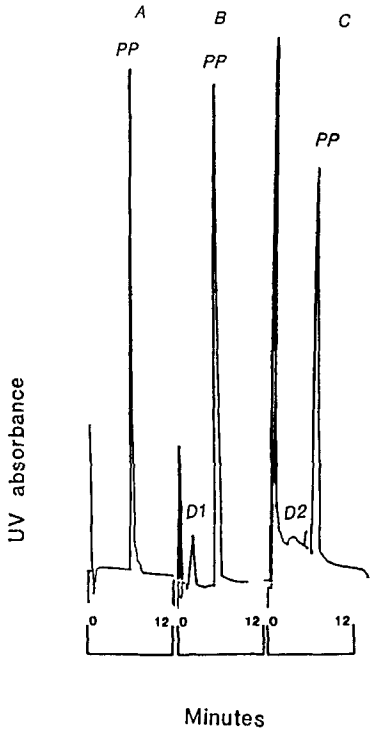

Figure 3: Authentic HPLC chromatograms of (A) undegraded PP sample and samples degraded at (B) pH < 2 and (C) pH > 8. PP 7.2 min, unidentified degradation products: D1, 3.0 min and D2, 4.2 min.

from human skin was 82.1 ± 5.0% (n=5). No metabolite or degradation product was detected in skin samples.

The application of the developed HPLC assay in transdermal permeation study was demonstrated. The developed HPLC assay was suitable for the comparative permeation evaluation of PPHCl and PP from PG solutions

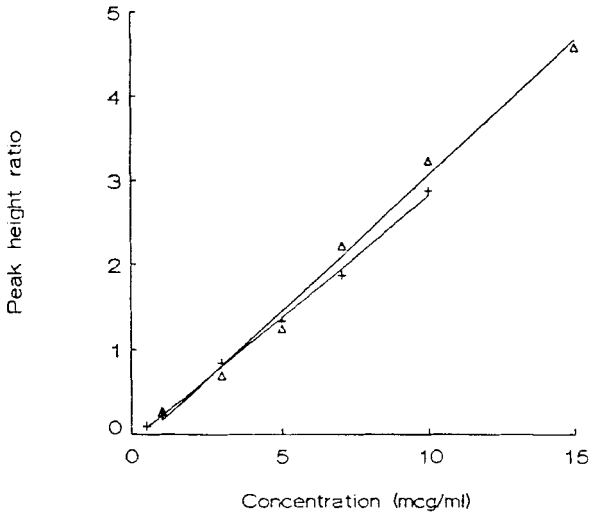

Figure 4: Propranolol calibration curves for diffusate and skin extract samples, n=6 each.(+) in diffusate, (Δ) in skin extract samples.

TABLE 1

Validation of HPLC Assay of Propranolol

	Diffusate	Skin Extract
Conc. Range (μg/ml)	0.5-10	1-15
Slope	0.28	0.32
Intercept	0.164	-0.038
Correlation coefficient	0.999	0.985
Within-day variation (%)	2.168	8.757
Between-day variation (%)	2.157	7.725

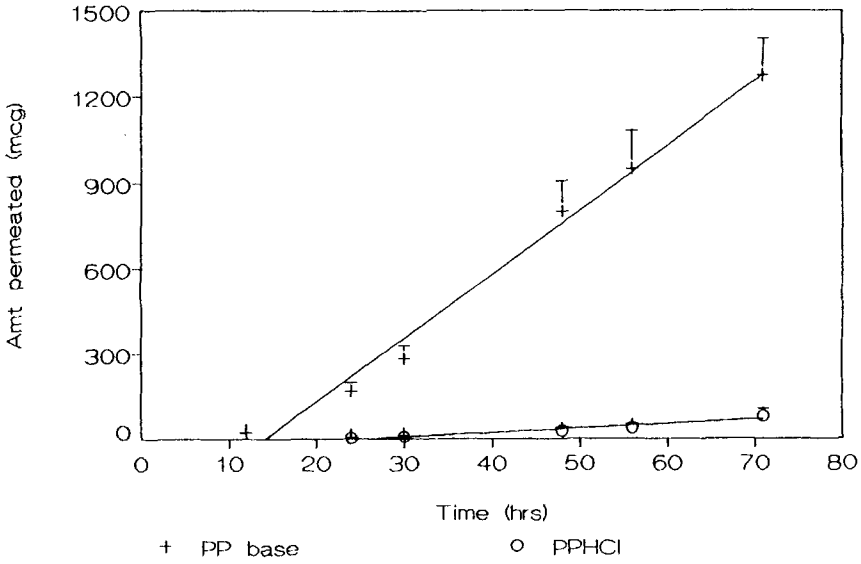

Figure 5: Comparison of PPHCl and PP permeations through human cadaver skin, n=4 each.

through the human cadaver skin (Figure 5). The permeation parameters were evaluated (Table 2). The flux of PP was significantly higher than that of PPHCl (37.8 vs 2.6 $\mu g/cm^2/hr$). The 14.5-fold difference was due to the combined effects in diffusion constant, D/d^2 (1.7-fold) and partition coefficient, Km*d (9.1-fold). The permeability coefficient of PPHCl, 4.4×10^{-5} cm/hr, was comparable to that reported through rabbit skin from a gel formulation consisting of 21.2% PG, 32.3% ethanol and 3% Carbopol in water, 5.2×10^{-5} cm/hr (22).

The amount of PP permeated was 15-fold of that of PPHCl, 10.6% vs 0.7% of the applied dose. In addition,

TABLE 2

Propranolol Permeation Parameters through Human Cadaver Skin from PPHCl/PG and PP/PG

Parameters	PPHCl/PG*	PP/PG*
Jx ($\mu g/cm^2/hr$)	2.6	37.8
Cs (mg/ml)	60.0	44.5
T (hr)	28.3	16.9
Kp (10^{-5} cm/hr)	4.4	63.0
D/d^2 (10^{-3} hr^{-1})	5.9	9.9
Km *d (10^{-3} cm)	7.0	64.0
% dose in diffusate	0.7	10.6
% dose in skin	0.8	9.5

* n = 4

the skin retention of PP was also significantly higher than that of PPHCl, 9.5% vs 0.8%. Convincingly, PP will be a more promising candidate than PPHCl for transdermal delivery. Dueball (22) reported the permeation enhancement of PPHCl by fatty acids of various chain lengths. The maximal enhancement is achieved with 2.3% of lauric acid (C12), with the Jx increased from 1.56 to 23.65 $\mu g/cm^2/hr$, which is still significantly lower than that of PP base, 37.8 $\mu g/cm^2/hr$ (Table 2).

Other potential applications of the HPLC assay include the characterization of the enhancer effect on PPHCl or PP permeation, evaluation of the drug permeation from various transdermal dosage forms including controlled release patches, and the determination of PP

partition coefficient between skin and the topical dosage forms.

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