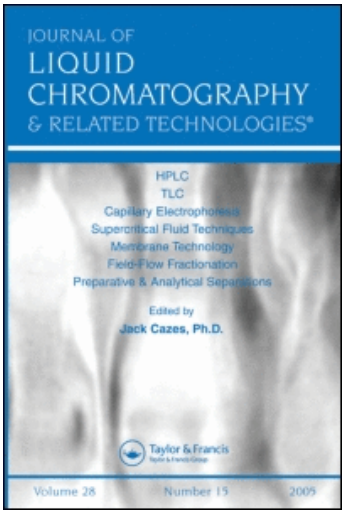


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High Performance Liquid Chromatographic Assay of Terbutaline for Preformulation Studies

S. N. Tenjarla[a]; R. Allen[a]; B. Mitchell[a]
[a] Department of Pharmaceutical Sciences, Mercer University Atlanta, Georgia

To cite this Article Tenjarla, S. N. , Allen, R. and Mitchell, B.(1995) 'High Performance Liquid Chromatographic Assay of Terbutaline for Preformulation Studies', Journal of Liquid Chromatography & Related Technologies, 18: 8, 1603 — 1615
To link to this Article: DOI: 10.1080/10826079508009298
URL: http://dx.doi.org/10.1080/10826079508009298

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(8), 1603-1615 (1995)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY OF TERBUTALINE FOR PREFORMULATION STUDIES

S. N. TENJARLA*, R. ALLEN, AND B. MITCHELL
Department of Pharmaceutical Sciences
Mercer University
Atlanta, Georgia 30341-4415

ABSTRACT

A reversed phase, stability indicating high performance liquid chromatographic (HPLC) assay was developed for the evaluation of terbutaline (TL) as a potential candidate for transdermal drug delivery. TL was quantitated in the permeation diffusate and human skin extract samples. The isocratic mobile phase was 30 % acetonitrile in pH 5.6 phosphate buffer. The effluent was monitored at 225 nm. Propranolol (PP, 2.5 μg/ml) was used as the internal standard. Baseline separation of TL and PP was attained with a cyano column. The linear range for the calibration curve was established at 5-15 μg/ml. The assay was reproducible with low inter-day and intra-day variation of the slopes of the calibration curves (3.5 and 4.2 % for diffusate samples and 5 and 5.5 % for the human skin extract samples respectively). The lowest detectable quantity was 0.1 μg/ml with a signal noise ratio of 4. The application of the assay in various preformulation studies was demonstrated.

* To whom correspondence should be addressed

INTRODUCTION

Terbutaline (1-(3,5-dihydroxyphenyl)-2-tertiarybutyl amino ethanol is is a beta-2 selective bronchodilator. Its structure is shown in Figure 1. It is indicated for the long term treatment of obstructive airway diseases and in the treatment of bronchospasm. It is the only selective beta-2 bronchodilator used parenterally in the emergency treatment of asthmaticus. It is also indicated as a tocolytic agent. It is currently delivered by the oral, inhalation and subcutaneous route. It has a bioavailability of 14 ± 2 %, a clearance of 3.4 ± 0.6 ml.min^{-1} and a volume of distribution of 1.8 ± 0.3 liters/kg. The low bioavailability can be overcome by the noninvasive transdermal route of drug administration. The advantages of transdermal route of drug administration are well documented (1). This route increases bioavailability by bypassing the hepatic first pass metabolism. It provides prolonged duration of action. It improves patient compliance by eliminating multiple dosing. In addition the dosage can easily be discontinued if toxic side effects occur. Thus a transdermal dosage form of terbutaline would be of great advantage. A number of preformulation studies are required to evaluate the potential of transdermal delivery of TL. These include partition coefficient, solubility studies, pH stability and extent of drug permeation through the skin. In addition determination of the amount of drug retained in

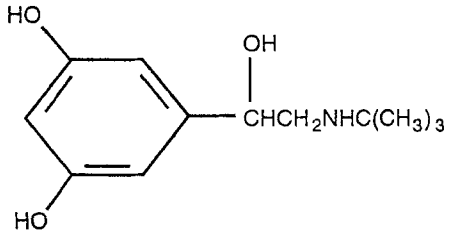


Figure 1: Chemical Structure of Terbutaline

the skin also provides useful information as to the depot effect of the drug or in elucidating the mechanism of skin penetration enhancers. The prerequisite for all these studies is a sensitive, stability indicating assay method. There a number of assay methods reported in literature for the quantitation of TL in plasma samples and solutions (2-9). These include coupled column, GCMS, electrochemical detection. None of these methods is specific for the quantitation of the drug in the skin. The goal of this study is to develop a simple, sensitive, stability indicating HPLC assay which can be used in all the preformulation studies.

MATERIALS

All chemicals and solvents were purchased from Sigma Chemical CO. (St. Louis, MO). The human cadaver skin (abdominal area, 65 white male) was obtained from the local hospital.

METHODS

High Performance Liquid Chromatography

The liquid chromatograph (Consta-Metric I) was from Laboratories Data Control, Riviera Beach, FL. It has a 100 μl injection sample loop (Rheodyne, Berkeley, CA) and a variable wavelength ultra violet detector (Spectro Monitor III, Laboratories Data Control). The cyano column used (Sphersorb, 4.6 X 250 mm, 5 μm particle size) was from AllTech. The isocratic mobile phase was 30 % acetonitrile in pH 5.6 buffer. The effluent was monitored at 225 nm. The flow rate was 1.4 ml/min. Propranolol HCl was used as the internal standard at a concentration of 2.5 μg/ml.

Degraded Samples from Extreme pH and Heat Conditions

A 15 μg/ml TL solution was boiled for 3 minutes in 0.1 Normal HCl or 0.1 Normal NaOH to obtain the extreme acidic and alkaline conditions respectively. The solutions were analyzed by the developed HPLC assay.

Calibration Curves

Calibration curves were constructed in the range of 5-15 μg/ml for diffusate and skin extract samples. TL and PP stock solutions in deionized water (100 μg/ml) were prepared. Appropriate dilutions were

made either with the diffusate or the skin extract solution to obtain standard solutions of cocentration 5, 7, 10, 12 and 15 μg/ml and an internal standard concentration of 2.5 μg/ml.

Extraction Efficiency of TL From the Skin (10)

The slope of the calibration curve (obtained by adding known amount of TL and PP to 400 mg of skin and homogenizing the samples) was determined (X). The same amounts of TL and PP were then added to the blank skin extract and its slope of the calibration curve obtained was calculated. (Y). The extraction efficiency of the TL was calculated from the equation:

Extraction efficiency (%) = (X/Y) X 100

Inter and Intra Day Variations of the Slopes of Calibration Curves

The slopes of six calibration curves (in saline phosphate buffer) constructed on the same day (inter-day) were determined and the coefficient of variation among them was calculated. Similarly the intra-day variation was determined by evaluating the slopes of six calibration curves constructed on different days over a period of 8 months. The inter and intra day variation was also determined for the calibration curves constructed with human skin extract homogenate. Approximately 300 mg of skin was weighed and the appropriate

amount of internal standard and drug solution was added to it. The membrane was then homogenized with methanol. (with 5 ml of methanol four times). The homogenate was combined filtered, evaporated to dryness, diluted with the mobile phase and quantitated by the developed assay.

In vitro Skin Permeation Study (11)

To evaluate the extent of TL permeation through the human skin, Franz diffusion cells (FDC 108 Series with FDC 128 manifold and FDC 127 magnetic bar, Crown Glass CO., Somerville, N.J.). The receptor chamber was filled with saline phosphate buffer (pH 7.4) to simulate the physiological pH. A circulating water bath maintained the cell at 37°C. The defatted human cadaver skin was placed between the donor and the receptor chambers of the difusion cell with the stratum corneum facing outward and the dermis facing the buffer solution. 200 μl of the test solution (TL in propylene glycol with or without penetration enhancer) was applied to the stratum corneum. Aliquot samples were taken at predetermined time intervals and analyzed for drug content by the developed HPLC assay. An equal volume of buffer solution was always added back to the receptor chamber to ensure contact between the buffer and the skin.

Skin Permeation Data Analysis(11)

A plot of the amount of drug permeated vs time was constructed. The slope of the linear phase of the profile yielded the flux (J) of the drug. The X-intercept of the linear phase was the lag time (T). "C" was the concentration of the drug solution added to the skin. The thickness of the memebrane (d) was assumed to be 50 μm. The diffusion coefficient (D), permeatbility coefficient (Kp), partition coefficient between the solution and the skin (Km) was calculated from the follwing equations:

$D/d^2 = 1/6T$

$Kp = J/C$

$Km*d = Kp/(D/d^2)$

Reservoir Effect Study

The amount of drug retained in the skin at the end of the skin permeation study provide insight into the mechanism of skin enhancer. The drug exposed skin was washed thrice with 3 ml of water to remove any residual drug. The skin was then homogenized with 5 ml of methanol using a polytron homogenizer. The homogenate was filtered and the process was repeated thrice with the residue. The filtrates (15 ml) was combined, evaporated to dryness. The residue was reconstituted with the mobile phase, internal standard solution added and suitably diluted and analyzed for drug content.

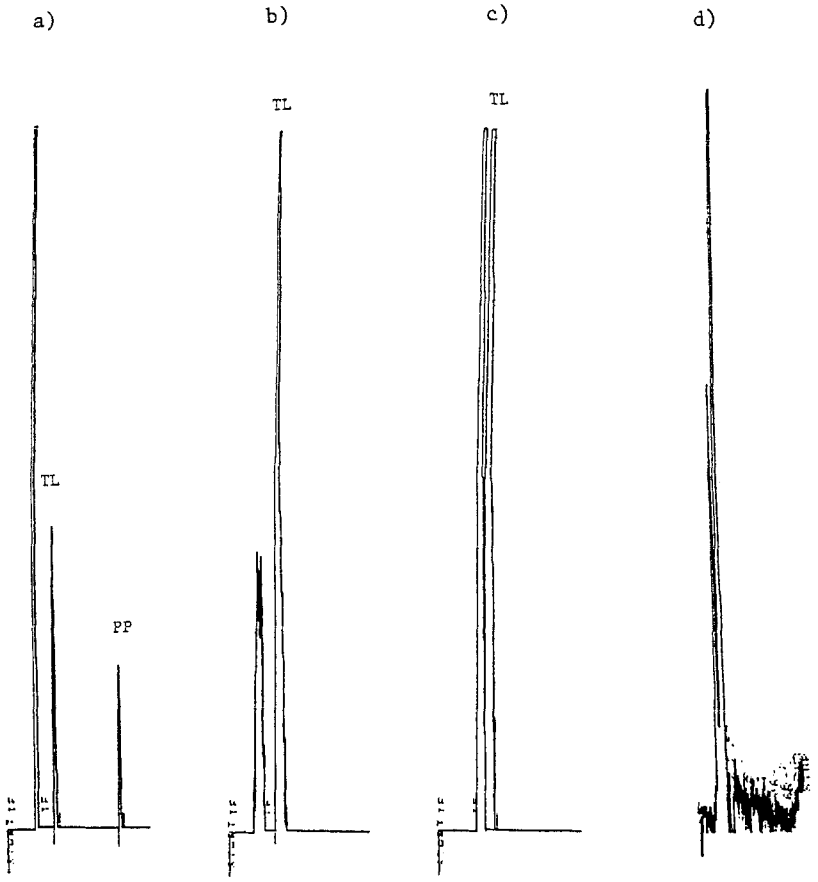
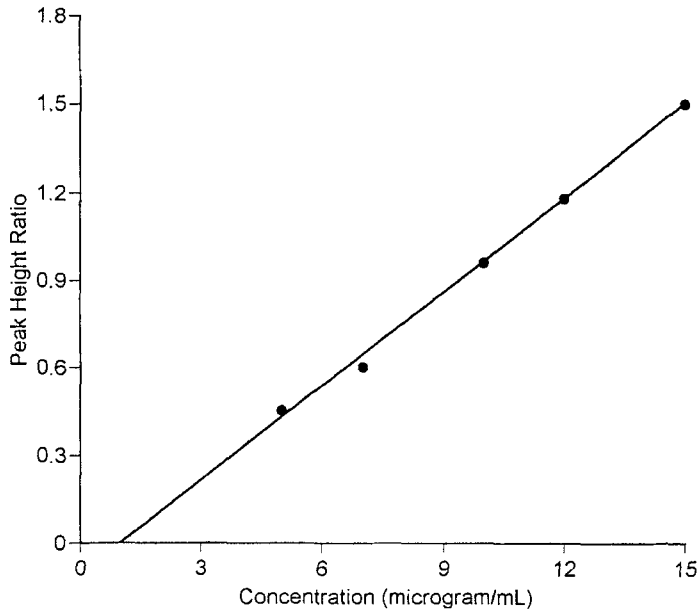


Figure 2: Typical Chromatograms of terbutaline a) in diffusate samples b) base degraded sample c) acid degraded sample d) blank skin extract

RESULTS AND DISCUSSION

Baseline separation of TL from the internal standard PP was obtained with the cyano column and the mobile phase used. Typical chromatogram for TL and PP in the diffusate are shown in Figure 2a.



Figrue 3: Calibration curve of terbutaline in the diffusate medium

The retention times of TL and PP were 3.3 and 7.9 minutes respectively. The calibration curve was linear in the range of 5-15 μg/ml with a $r^2 > 0.98$ (Figure 3).

Forced Degraded Samples

Baseline separation of TL was obtained for the base-degraded sample (Figure 2b) and acid-degraded sample (Figure 2c), demonstrating the stability of the assay under those conditions of degradation. There was no interference from the blank skin extract at

the retention times of either the drug or the internal standard (Figure 2d).

Assay Sensitivity

The sensitivity of the assay was determined to be 0.1 μg/ml when 5 μl of the sample was injected. A higher sensitivity can be attained by increasing the volume of the injection.

Linearity

The calibration curves were linear in the range of 5-15 μg/ml for the diffusate and skin extract samples. The regression coefficient was 0.98 and 0.96 for the diffusate and skin extract samples respectively.

Inter and Intra Day Variation

The inter and intra-day variation was 3.5 and 4.2 % respectively for the diffusate standard curves. The corresponding values for standard curves constructed with human skin extract samples were 5 and 5.5 % respectively.

Extraction Efficiency

The extraction efficiency of TL from the human skin was calculated to be 92 $\pm$ 11.5 %. This suggests that both the drug and the internal standard were well extracted from the skin.

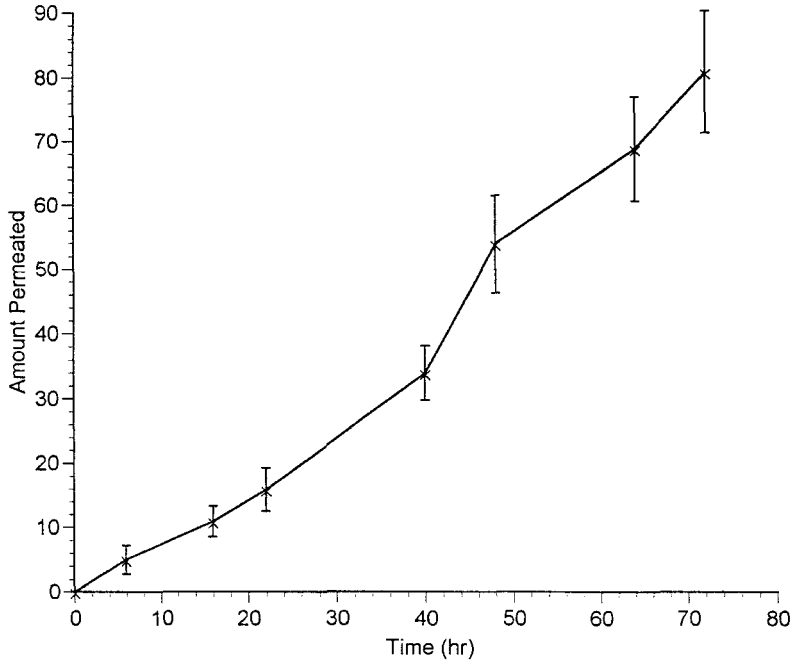


Figure 4: Permeation profile of terbutaline through the human cadaver skin

Table 1: Terbutaline Permeation Parameters through the Human Cadaver Skin

Flux(J) $\mu g/cm^2.hr$	Lag-time (T) hr	Permeability Coeff (Kp) cm/hr	Partiton Coeff (Km*d) cm	Diffusion Coeff. (D/d^2) cm^2/hr
1.3 ± 0.1	6.6 ± 2.2	0.34 ± .03	13.3 ± 4.5	.03 ± .02

Permeation Study

The permeation profile of terbutaline through the human cadaver skin was shown in Figure 4. The TL flux through the human skin was 1.2 $\mu g/cm^2$.hr. The permeation parameters were reported in Table I. Approximately 3.8 % of the dose was retained in the skin. Based on the preliminary studies it appears that terbutaline is a good candidate for transdermal delivery.

HPLC Assay Application

The developed HPLC assay was useful in the skin permeation study. The assay was also useful in quantitation of TL in the pH-stability study, solubility studies, partition coefficient study and in the evaluation of the rabbit skin as a suitable model for the human skin (12). The assay was also useful in screening of various skin penetration enhancers.

ACKNOWLEDGEMENTS

This work was supported by an Undergraduate Summer Research Grant (1991) from Mercer University, Atlanta.

REFERENCES

1) Y.W. Chien, Transdermal Controlled Systemic Medication, Marcel Dekker, Inc., New York, 1987

2) S. Bergquist and L.E.Edholm, J. Liq. Chromatogr. 6: 559-574 (1983)

3) K.A. Sagar, M.T. Kelly, M.R. Smyth. J. Chromatogr Biomed Appl: 115: 109- 116 (1992)

4) I.W. Wainer. J Pharm Biomed Anal 7: 1033-1038 (1989)

5) L.E. Edholm, C. Lindberg, J. Paulson, A. Walhagen, J Chromatogr Biomed Appl: 424: 61- 72 (1988)

6) J.G. Leferink, E. I. Wagemaker, R.A.A. Maes, H. Lamont, R. Pauwels. J. Chromatography Biomed Appl 143: 299-305 (1977)

7) D.A.Wiiliams, E.Y.Y. Fung, D.W. Newton, Journal of Pharmaceutical Sciences 71: 956-958 (1982)

8) J.S.Legge, J. Gaddie and K.N.V. Palmer, Brit. Med. J., 1, No. 201 (1971) 637.

9) L. Borgstrom, S.Newnan, a. Weigz, F.moren, Journal of Pharm. Sci, 81, 753-755 (1992)

10) S. N. Tenjarla and A. Tesggai. Journal of Clinical Pharmacy and Therapeutics 17: 37-42 (1992)

11) S.N.Tenjarla, R. Allen and A. Borazani. Drug Dev. and Indus. Pharmacy 20: 49-63 (1994)

12) S.N.Tenjarla, R. Allen. Submitted to Pharmaceutical Science Communications.

Received: October 17, 1994
Accepted: November 2, 1994