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Effect of alcohol concentration in Diffusion medium on diffusion of Terbinafine gel through artificial membranes

Chamarthi R P Kishore*, Dr. G V Krishna Mohan

Department of Chemistry, K L University, Guntur-522502, Andhra Pradesh, India.

A B S T R A C T

The aim of the present experiment was to compare the diffusion property of Terbinafinein diffusion medium containing ethanol in different concentrations. Diffusion study was conducted on artificial membranes with different pore size of 0.22µ. A simple, accurate and sensitive HPLC method was developed and validated for the estimation of Terbinafine in diffusion study. This methodology can be useful in the determination of bioequivalence between topical formulations. 0.2% Triethyl amine buffer with pH adjusted to 7.5 and Acetonitrile in the ratio of 15:85 was used as a mobile phase. Flow rate was set to 1.5mL/min and detection was carried at 280nm. 100µl sample and standard solutions were injected into150x 4.6mm 2.7µ, Ascentis express C18 HPLC column. Linearity was established in the concentration range of 0.02 ppm– 3ppm.Recovery studies were done and satisfactory results were observed.

Keywords: Diffusion study, artificial membranes, gel, bio-equivalence and development.

A R T I C L E I N F O

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***Corresponding Author**
Chamarthi R P Kishore
Department of Chemistry,
K L University, Guntur-522502,
Andhra Pradesh, India
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1. Introduction

Terbinafine is an ally amine antifungal drug (1) administrated orally (250 or 500mg per day) or topically (1% cream applied twice daily), and is marketed under the trademark Lamisil.It is effective against several cutaneous fungal infections. In the treatment of cutaneous fungal infections, topical application is more effective compared to oral administration. Topical application has very less side

effects, but also, for the same reason, making the measures of systematic availability very difficult. Survey of literature shows several HPTLC [13-15], HPLC [1-5&17-18], non-aqueous volumetric [6-7], spectrometric [8-12] and ion-pair RP chromatography [7] methods have been used for assay of Terbinafine in raw material and dosage forms. These methods are simple and rapid but due low sensitivity of them, their use is limited. Reported spectrophotometric and chromatographic [16,19] methods estimates Terbinafine in presence of its photodegradant or metabolites.In-vitro studies are the best alternatives to in-vivo experiments. Franz diffusion cell apparatus is suitable for the diffusion studies of gel formulations. This apparatus consists of two compartments (donor and receptor). Donor compartment contains active agent or gel formulation and receptor contains receiving media. A membrane separates these two compartments. Receiver compartment jacketed in water bath to maintain temperature. Receptor contains stirred solution and donor contains unstirred one. Active agent passes through the membrane and enters into the acceptor cell. By measuring the concentration of drug in acceptor, we can estimate the amount of drug diffused through the membrane. It has been reported that using a liquid receptor in transdermal experiments with human cadaver skin may result in skin breakdown, especially if these experiments run for more than 24hrs. Hence, it is potentially beneficial to use more physiologically relevant in-vitro experiments, particularly with respect to excessive hydration effects of the membrane resulting from water exposure. It would be our interest to study the effects of various media on the diffusion of drug.

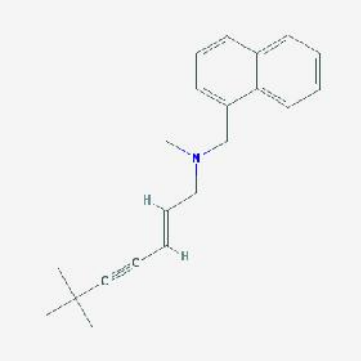

Figure 1: Terbinafine structure

2. Experimental

Diffusion study for the Terbinafine gel was performed by Franz diffusion apparatus. Experiments were performed with different media in receptor cell.Gel was applied on 0.22micron membrane and placed in donor cell. Acceptor cell jacketed to maintain the temperature of the media. Experment was performed for 24hrs and amount of drug penetrated through the membrane and entered into the media was estimated by HPLC analysis. Residue of the drug retained on the membrane was also estimated.

Chemicals, reagents and solutions:

Mobile phase consists of 0.2% Triethyl amine with pH adjusted to 7.5and acetonitrile mixed in the ratio of 15:85 was used. Ascentis express C18, 150 x 4.6mm 2.7µ column was used.100µL sample and standard solutions were injected and response was measured at 280nm.Column compartment temperature was maintained at 30°C throughout the analysis. Diffusion mediums were prepared with water and ethanol in different ratio (20/80, 40/60 and 70/30)

Standard and sample solutions:

Standard solution preparation: Weighed and dissolved about 20.0mg of Terbinafine working standard into 100mL volumetric flask. Dissolved and diluted to volume with the respective diffusion medium.

Sample preparation:

Weighed and applied approximately about 250mg of Terbinafine 1% gel on 0.22µ membrane and kept on donor cell. Acceptor cell was filled with 11mL of diffusion medium containing ethanol and pH 7.5 buffer in different ratio (80/20, 70/30 and 30/70). Experiment was performed for 24hrs on automated franz-diffusion apparatus and sampling was collected at pre-determined time points.

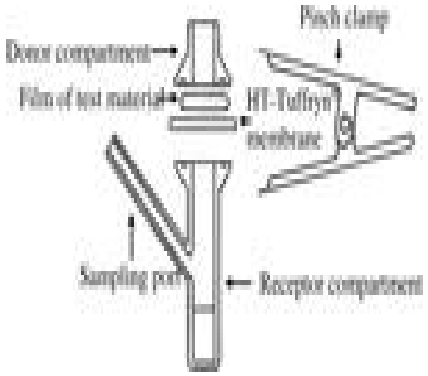

Figure 2: Typicalfranz diffusion cell

Apparatus and chromatographic conditions:

Analysis was performed on Agilent 1100 series equipped with UV detector. Empower software was used to record the data.

Instrument calibration:

Linearity solutions are prepared in the range from 0.01-3ppm and injected into HPLC. The linearity of method was calibrated by calculating the slope, intercept and correlation coefficient. Results are tabulated in Table-1.

Table 1: Calibration curve of standard

Linearity levels	Concentration of Terbinafine in µg/ml	Terbinafine Peak Area (µV*Sec)
LOQ	0.020	1086
50%	0.503	25263
100%	1.007	49327
200%	2.014	97809
300%	3.021	150266
600%	6.042	295028
900%	9.063	443584
Slope		48891.77
Intercept		411.20
Correlation coefficient		0.9999

Specificity:
Placebo and blank samples are injected into HPLC with the final chromatographic conditions. Results are tabulated in Table-2.

Table 2: Blank interference

Sample No.	Peak found at the retention time of
1	No
2	No

Recovery:
Recovery studies were done on sample after diffusion study. Samples retained on filter were collected into 100mL volumetric flask and dissolved in diluent. Results are tabulated in Table-3.

Table 3: Recover

S.No.	Spike level	'mg' added	'mg' found	Individual recovery
1.	100%	1.0070	(recovered)	96.0
2.	100%	1.0070	0.9665	96.1
3.	100%	1.0070	0.9681	92.3

Determination of LOD and LOQ:
LOD and LOQ were determined by injecting a series of solutions with different concentrations. LOD concentration was derived which gave signal to noise ratio about 2.8 (2.0-3.4) and LOQ Concentration which gave signal to noise ratio about 9.5 (9.0-11.4).Six spiked sample solutions of Terbinafine were prepared at LOQ concentration and injected into HPLC and calculated the % RSD of µg/mL of Terbinafine found. Results are tabulated in Table-4 and 5.

Table 4: LOD and LOQ

Name	Test Name		Signal to Noise ratio	
	LOD	LOQ	LOD	LOQ
Terbinafine	0.007	0.02	2.8	9.5

Table 5: Repeatability at LOQ level

Sample No	Concentration in ppm
01	0.019
02	0.019
03	0.021
04	0.019
05	0.020
06	0.022
Average	0.020
%RSD	7.0

3. Results and Discussion
Linearity of the detector was established from LOQ level (0.02ppm) to 300% of the test concentration (9ppm).Correlation coefficient between concentration and response was found to be 0.9999.Hence we can conclude that the method is linear within the above-specified range.
Recovery studies were performed on the gel samples remained in membranes. Sample was collected and transferred carefully without losing any gel into 100mL volumetric flask.20mL of Tetrahydrofuran added. Dissolved the gel completely by shaking manually for 5mins.Diluted to volume with Acetonitrile/water in the ratio of 9:1.LOQ and LOD values are established based on S/N ratio. LOQ values are very less compared to the amount of gel diffused into the receptor medium. Hence we can estimate very less quantity of gel penetrated into receptor cell very accurately. Diffusion study was done on the sample in diffusion mediums containing different alcoholic concentrations. It was observed that the amount of gel diffused into acceptor medium was increased with the increase of alcohol content in medium. Results are reported in Table-6.

Table 6: % Drug release of Terbinafine in diffusion mediums with different ethanol concentration

Time (hr)	% Drug release		
	Buffer:EtOH (7:3)	Buffer:EtOH (2:8)	Buffer:EtOH (6:4)
0.5	0.06	9.1	1.27
1	0.08	19.9	3.03
2	0.11	54.3	5.83
4	0.13	72.5	8.27
6	0.14	80.3	10.12
8	2.03	84.0	11.57

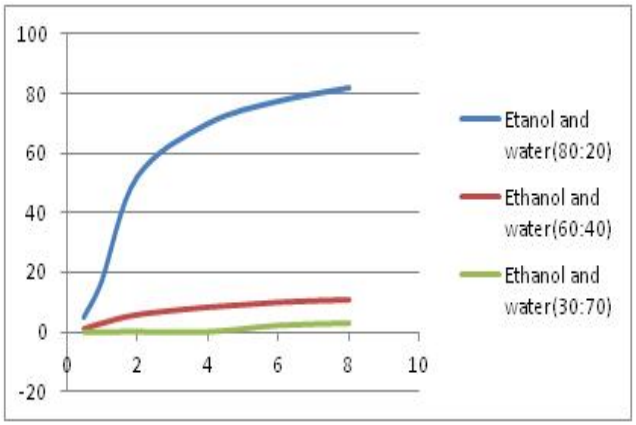

Figure 3: Comparison between %releases in different mediums:

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