TITLE: DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF TERBINAFINE HYDROCHLORIDE AND MOMETASONE FUROATE IN COMBINED DOSAGE FORM

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ABSTRACT

A new simple, precise, accurate, specific and selective high performance thin layer chromatographic (HPTLC) method has been developed for the simultaneous estimation of Terbinafine hydrochloride (TH) and Mometasone furoate (MF) in cream dosage form. The chromatographic separation was achieved on Merck precoated silica gel aluminium plate 60 F254 using Toluene: Ethyl acetate: Glacial acetic acid (8: 4: 0.1 v/v) as mobile phase. The densitometric scanning was carried out at 248 nm. Response was found to be linear in the concentration range of 1000–3000 ng/band with correlation coefficient ($r^2 = 0.999$) for Terbinafine hydrochloride and 100-300 ng/band with correlation coefficient ($r^2 = 0.998$) for Mometasone furoate. All parameters of method, repeatability, precision, LOD, LOQ, % recovery were validated as per ICH guidelines. The proposed procedure was successfully applied for the simultaneous determination of both drugs in commercial cream preparation. The advantage of the method is simplicity, reasonable sensitivity, rapidity, excellent resolving power, low cost and is a more effective option than other chromatographic techniques in routine quality control.

Key Words: Terbinafine hydrochloride (TH), Mometasone furoate (MF), High performance thin layer chromatography (HPTLC).

INTRODUCTION

Terbinafine hydrochloride (TH) is an antifungal and enzyme inhibitor drug. Chemically, it is (2E)-N, 6, 6-Trimethyl-N-(naphthalen-1-ylmethyl) hept-2-en-4-yn-1-amine hydrochloride, clinically it is use in the treatment of dermatophyte infections of the toenail or fingernail caused by susceptible fungi. Also for the treatment of *tinea capitis* scalp ringworm) and *tinea corporis* (body ringworm) or *tinea cruris* jock itch). Mometasone furoate (MF) is a glucocorticoid having anti-inflammatory activity and chemically it is 9,21-Dichloro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate and it is also useful treatment of dermatological diseases. This combination of drugs is useful for relief of corticosteroid responsive dermatomes where fungal infections are present, suspected, or likely to occur. Both the drugs are marketed as combined dose cream formulation in the ratio of 10:1 w/w TH: MF. Literature survey reveals that Terbinafine hydrochloride (TH) can be estimated by spectrophotometrically, HPTLC and HPLC individually or with other drugs in bulk drugs and in human plasma[1-5] while Mometasone furoate (MF) can be estimated by spectrophotometrically, HPLC and HPTLC in combination with other drugs[6-10]. However, there is no analytical method has been reported for the estimation of TH and MF in a combined dosage formulation. Present work describes HPTLC method[4] for simultaneous estimation of TH and MF in cream formulation. Method validation was done according to ICH Q2 (R1) Guidelines.

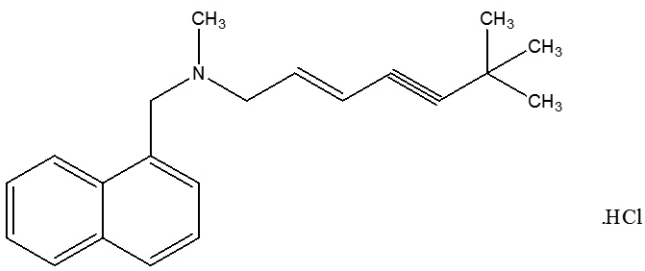

Figure 1: Structure of TH

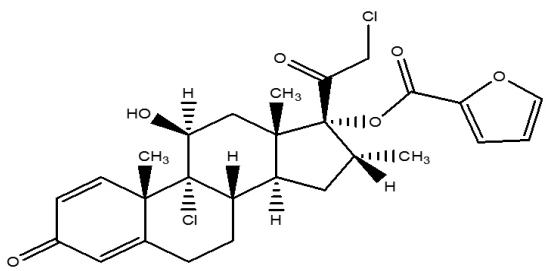

Figure 2: Structure of MF

MATERIALS AND METHODS:

Instrument

A CAMAG Linomat V sample applicator equipped with an applicator microsyringe (Hamilton, Bonaduz, Switzerland) and CAMAG UV cabinet with dual wavelength UV lamp (254 and 366 nm); densitometric scanning was performed at 248 nm with CAMAG TLC scanner IV operated in absorbance mode and controlled by Wincats 1.4.7 software.

Materials

Standard gift sample of Terbinafine hydrochloride was provided by Aarti Drugs sales, Mumbai and Mometasone furoate was povided by Glenmark Generics pharmaceutical, Nasik. Combined dose Terbinafine hydrochloride and Mometasone furoate cream sebifin™ plus was purchased from local market. Methanol (AR Grade), Toluene (AR Grade), Ethyl acetate (AR Grade) and Glacial acetic acid (AR Grade) were used as solvents, procured from Loba Chemie pvt ltd., Mumbai.

Optimized Parameters for Chromatographic Condition

As stationary Phase, Silica gel 60F254 TLC Precoated Plates was selected and after trials of various mobile phase, final mobile phase selected was Toluene: Ethyl aceate: Glacial acetic acid (8: 4: 0.1 v/v/v). Plate development technique was simple ascending at ambient room temperature and chamber saturation time was 20min. Run distance was 80mm and detection wavelength was 248 nm. Spotting Parameters were band width of 6 mm and syringe used was of 100µl capacity. Scanning Parameters were set of slit dimension of 6 × 0.45 mm, detection was done at wavelength 248 nm and scanning speed was 100 mm/sec. In integration Parameters, baseline correction was done, peak threshold, height was 10AU, peak threshold, area was 50 and peak threshold, slope was kept 5.

Stock solutions

Stock solution: Standard stock solutions of TH (1000 µg/ml) and MF (1000 µg/ml) were prepared in methanol.

Working solutions

Working solution: Standard working solutions of TH (500 µg/ml) and MF (50 µg/ml) were prepared in methanol and used for the analysis.

Method validation

The developed method was validated for linearity, range, specificity, precision, accuracy and robustness as per ICH guidelines.

Linearity and range

Each concentration mixture in the range of 1000-3000 ng /spot for TH and 100-300 ng/spot for MF was spotted five times on individual plates and response was measured after scanning. For evaluation of linearity, peak area and concentrations were subjected to least square regression analysis to calculate calibration equation and correlation coefficient.

Specificity

The specificity of the method was ascertained by analyzing TH and MF in presence of excipients of TH and MF in cream formulations. The bands of TH and MF in the sample were confirmed by comparing R_f values and respective spectra of the sample with those of the standards. The peak purity of TH and MF was assured by comparing the spectra at three different levels, that is, peak-

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start (s), peak-apex (m) and peak-end (e) positions.

Precision

Precision was measured by using standard solutions containing TH and MF at concentrations covering the entire calibration range. The precision of the method was evaluated by calculating %RSD of mean peak areas obtained from each spot of TH and MF. Same procedure was performed at different time intervals on the same day and on different days.

Accuracy

The accuracy of the method was determined by recovery studies using standard additions at three different levels (approximately 80, 100 and 120% of label claim), i.e. multiple level recovery studies. This was done to check for the recovery of the drug at different levels in the formulations.

Robustness

Robustness was assessed by deliberately changing the chromatographic conditions and studying the effects on the results obtained.

Limit of detection and Limit of Quantification

Limits of detection and limit of quantification were determined on the basis of the mathematical terms mentioned in ICH guidelines for method validation from triplicate results of linearity.

LOD= 3.3 σ/s

LOQ= 10 σ/s

Limit of detection was determined using equation 3.3 σ/s and limit of quantification was determined using equation 10 σ/s, where σ is the slope of calibration curve and s is standard deviation of responses.

Application of the proposed method for the determination of TH and MF in cream

1gm cream was weighed which is equivalent to 10 mg TH and transferred into 10 ml centrifugal tube, dissolved in methanol and centrifuged for 5 minute at 4000 rpm. The solution was filtered through whatman filter paper. Take 5ml from this and adjust the volume up 1 ml with methanol. This solution contains 500µg/ml of TH and 50 µg/ml of MF.

From this solution, 5µl was spotted which gives 2500 ng/spot of TH and 250 ng/spot of MF concentration.

RESULTS AND DISCUSSION

Selection of best solvent system is the critical step in HPTLC method development. From the different solvent systems tried, the mobile phase consisting of toluene, ethyl acetate and glacial acetic acid in ratio of 8:4:0.1 v/v/v gave good separation between TH and MF selected as optimized mobile phase. R_f value of symmetric peaks of TH and MF were obtained at 0.62 and 0.35 respectively (Fig.3). Well-defined bands were obtained when the chamber was saturated with mobile phase for 20 min at ambient temperature. Reproducible responses were obtained. For quantitative purpose, the densitometric scanning was carried out at wavelength 248 nmThe proposed method could be employed for routine quality control of TH and MF in combined cream dose formulation.

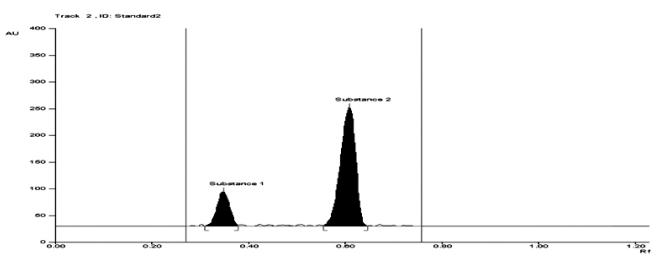

Figure 3: Typical HPTLC chromatogram of 2500ng/spot of TH and 250ng/spot MF

Linearity and Range

Linearity was observed over the concentration range 1000-3000 ng/spot for TH and 100-300 ng/spot for MF confirming adherence of the system to Beer's law. Calibration curve was taken (Fig.4).The regression analysis equation was y=3.688x+1559 for TH and y=13.09x+88.35 for MF and, correlation coefficient (r^2) was 0.999 for TH and 0.998 for MF respectively. Linearity data were taken (Table 1, 2 and 3).

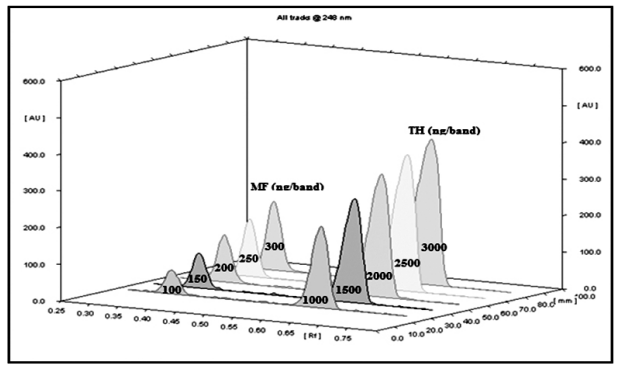

Figure 4: 3-D view of calibration curve of TH and MF

Table 1: Linearity data of TH (n=6).

Sr No.	Conc. (ng/spot)	Mean (area) ± SD	% RSD
1	1000	5143.37 ± 34.24	0.67
2	1500	7186.11 ± 40.30	0.56
3	2000	9029.54 ± 33.75	0.37
4	2500	10733.39 ± 43.63	0.41
5	3000	12591.21 ± 54.38	0.43

Table 2: Linearity data of MF (n=6).

Sr No.	Conc. (ng/spot)	Mean (area) ± SD	% RSD
1	100	1398.76 ± 5.56	0.40
2	150	2032.54 ± 13.10	0.64
3	200	2765.15 ± 18.34	0.66
4	250	3297.78 ± 26.30	0.80
5	300	4039.02 ± 29.61	0.73

Table 3: Linear regression data of TH and MF for HPTLC method.

Parameter	TH	MF
Straight line equation	y=3.688x+1559	y=13.09x+88.35
Correlation co-efficient	0.999	0.998
Slope	3.688	13.09
Intercept	1559	88.35
SD of intercept	47.18	10.14

Specificity

Specificity of the method for TH and MF were proved from the spectral scan (Fig 5 and 6) for TH and MF in bulk and in cream formulations indicate that there is no merging or co-elution of interfering peaks with TH and MF, so there is no interference from any excipients present in cream formulation.

Precision

For determination of precision of TH and MF by the proposed method, same homogeneous samples of TH and MF (real samples) were prepared repeatedly and analyzed. Intermediate precision was evaluated at different times on same day, on different days. Low values of RSD (less than 2%) obtained in the precision studies (Table 4, 5 and 6) indicate that the method is precise and reproducible.

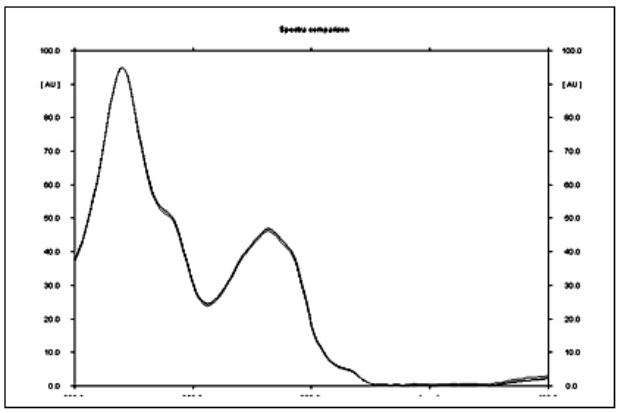

Figure 5: Spectra of specificity of 2500ng/spot TH

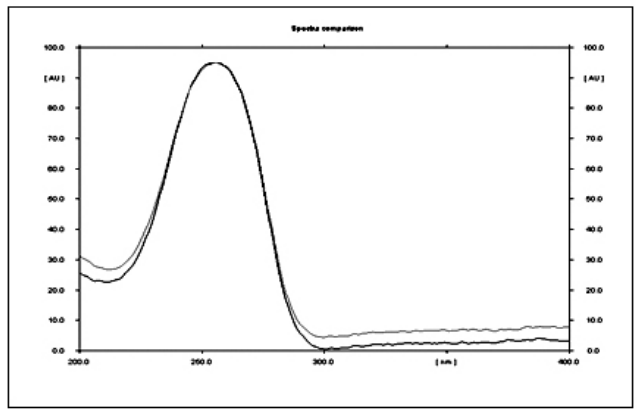

Figure 6: Spectra of specificity of 250ng/spot MF

Table 4: Intraday precision data for TH and MF (n=6).

Conc. (ng/spot)		Area (Mean ± SD)		%RSD	
TH	MF	TH	MF	TH	MF
1000	100	5145.59 ± 33.06	1402.31 ± 5.57	0.64	0.40
2000	200	9045.13 ± 39.54	2775.75 ± 18.65	0.44	0.67
3000	300	12625.89 ± 41.72	4055.81 ± 23.72	0.33	0.58

Table 5: Interday precision data for TH and MF (n=6).

Conc. (ng/spot)		Area (Mean ± SD)		%RSD	
TH	MF	TH	MF	TH	MF
1000	100	5176.99 ± 41.46	1384.79 ± 21.68	0.80	1.57
2000	200	9022.55 ± 47.77	2739.53 ± 26.28	0.53	0.96
3000	300	12517.92 ± 104.93	3976.25 ± 36.79	0.84	0.93

Table 6: Repeatability data of TH and MF(n=6).

Drug	TH	MF
Conc. (ng/spot)	1000	100
MEAN (Area)	5130.76	1402.34
SD	7.17	5.51
%RSD	0.14	0.39

Accuracy

Accuracy of the proposed method was studied by standard addition to sample solution at three levels 80, 100 and 120% of the label claim. % Mean recovery for TH (Table 7) and MF (Table 8) were between ±2% indicating that the developed method was accurate for the determination of TH and MF in pharmaceutical formulations.

Table 7: Accuracy data of TH (n=3).

% Level	Sample conc. (ng/spot)	Std. added (ng/spot)	Total conc. of TH (ng/spot)	Amt. recovered (ng/spot)	Mean % Recovery ±SD	% RSD
80	1000	800	1800	1811.84	100.83 ± 16.48	0.91
				1800.12		
				1832.66		
100	1000	1000	2000	2006.08	100.56 ± 23.76	1.18
				2036.97		
				1990.26		
120	1000	1200	2200	2228.13	101.10 ± 13.97	0.63
				2208.82		
				2268.50		

Table 8: Accuracy data of MF (n=3)

% Level	Sample conc. (ng/spot)	Std. added (ng/spot)	Total conc. of MF (ng/spot)	Amt. recovered (ng/spot)	Mean % Recovery ±SD	% RSD
80	100	80	180	178.35	99.72 ± 1.1074	0.62
				180.56		
				179.58		
100	100	100	200	196.96	99.27 ± 2.2735	1.15
				197.50		
				201.14		
120	100	120	220	219.09	99.10 ± 2.0356	0.93
				216.97		
				221.04		

Limit of detection and Limit of Quantification

LOD and LOQ were found by using equations (Table 9).

Table 9: LOD and LOQ

Drug	TH	MF
LOD (ng/spot)	42.22	2.56
LOQ (ng/spot)	127.93	7.75

Robustness

Acceptable %RSD values obtained after making small deliberate changes in the developed HPTLC method indicate that the method is robust for the intended purpose (Table 10 and 11).

Table 10: Robustness study of TH

Parameter	Mean Peak area (n=3)			%RSD	
	Optimized	Changed condition		Changed condition	
Run distance (cm)	**8**	**(-10%)**	**(+10%)**	**(-10%)**	**(+10%)**
	7186.11	7287.79	7197.18	0.54	1.29
Saturation time (min)	**20**	**(-10%)**	**(+10%)**	**(-10%)**	**(+10%)**
	7186.11	7118.81	7124.16	0.70	1.03
M.P. ratio (Toluene)	**8**	**(-10%)**	**(+10%)**	**(-10%)**	**(+10%)**
	7186.11	7011.64	7103.63	1.88	1.74
M.P.ratio (Ethyl acetate)	**4**	**(-10%)**	**(+10%)**	**(-10%)**	**(+10%)**
	7186.11	7042.743	7192.66	1.79	1.78

Table 11: Robustness study of MF

Parameter	Mean Peak area (n=3)			%RSD	
	Optimized	Changed condition		Changed condition	
Run distance (cm)	**8**	**(-10%)**	**(+10%)**	**(-10%)**	**(+10%)**
	2032.54	2059.40	2023.07	0.94	1.44
Saturation time (min)	**20**	**(-10%)**	**(+10%)**	**(-10%)**	**(+10%)**
	2032.54	1979.72	2068.22	1.52	1.31
M.P. ratio (Toluene)	**8**	**(-10%)**	**(+10%)**	**(-10%)**	**(+10%)**
	2032.54	2107.10	2028.63	1.57	1.62
M.P. ratio (Ethyl acetate)	**4**	**(-10%)**	**(+10%)**	**(-10%)**	**(+10%)**
	2032.54	2082.35	2133.10	1.25	1.53

Method application

The proposed, developed and validated HPTLC method was successfully applied for determination of TH and MF in marketed formulation. The assay results obtained were satisfactory, accurate and precise as indicated by %RSD values (Table 12). The good performance of the method indicates that it can be used for the determination of TH and MF in drug substances and pharmaceutical preparations.

Table 12: Assay of marketed formulation by HPTLC method (n=5).

Labeled claim (ppm)		Conc. found (ppm)		%Assay	
TH	**MF**	**TH**	**MF**	**TH**	**MF**
2500	250				
MEAN		2518.22	248.18	100.73	99.27
SD		11.81	1.63	0.47	0.65
% RSD		0.47	0.66	0.47	0.66

CONCLUSION

The developed methods were validated as per ICH guidelines and were found to be within the prescribed limit. It concludes that the developed method is simple, accurate, sensitive and precise and suitable for routine quality contol analysis for both authentic and cream dosage form.

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