Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 1, 2014

Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TERBINAFINE HYDROCHLORIDE AND MOMETASONE FUROATE IN COMBINED DOSAGE FORM

MEHUL M. PATEL*, HETA D. PATEL

Ramanbhai Patel College of Pharmacy, Charotar University of Science and Technology, CHARUSAT Campus, Changa 388421, Ta. Petlad, Dist. Anand, Gujarat, India. Email: mehulpatel.ph@ecchanga.ac.in

Received: 15 July 2013, Revised and Accepted: 8 Oct 2013

ABSTRACT

Objective: To develop a validated analytical RP-HPLC method for simultaneous estimation of containing terbinafine hydrochloride and mometasone furoate for pharmaceutical formulation.

Method: The sample was estimated using methanol and water in the ratio 95:5 as mobile phase at a flow rate of 1.2 mL/min and detection at 248 nm. The retention time for terbinafine hydrochloride and mometasone furoate was found to be 6.9 min and 3.2 min respectively.

Result: The linearity of the developed method was tested in the range of 20-200µg/mL for terbinafine hydrochloride and 2-20µg/mL for mometasone furoate. The limit of detection was 5.57µg/mL for terbinafine hydrochloride and 0.07µg/mL for mometasone furoate, the % recoveries obtained were 101.18% and 99.67% respectively.

Conclusion: A simple, precise and accurate RP-HPLC method has been developed for the determination of terbinafine hydrochloride and mometasone furoate in pharmaceutical formulations.

Keywords: Mometasone furoate (MF), Reverse phase high pressure liquid chromatography (RP-HPLC), Terbinafine hydrochloride (TH)

INTRODUCTION

Terbinafine hydrochloride(TH) is a 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[1], clinically used in the treatment of dermatophyte infections of the toenail or fingernail caused by susceptible fungi. Also for the treatment of tinea capitis and tinea corporis[1]. 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[2]. Terbinafine hydrochloride (TH) is a antifungal drug and Mometasone furoate (MF) is a glucocorticoid. This combination of drug is useful for relief of corticosteroid responsive dermatoses 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 of TH : MF. Literature survey reveals that Terbinafine hydrochloride (Fig.1a) can be estimated by spectrophotometrically, by HPTLC and by HPLC individually or with other drugs in bulk drugs and in human plasma[6-9], while Mometasone furoate (Fig.1b) can be estimated by spectrophotometrically, HPLC and HPTLCin combination with other drugs[10-14]. But, there is no analytical method has been reported for the simultaneous estimation of TH and MF in a combined dosage formulation. Present work describes RP-HPLC method[5]for simultaneous estimation of TH and MF in cream formulation. Method validation was done according to ICH Guidelines[3-4].

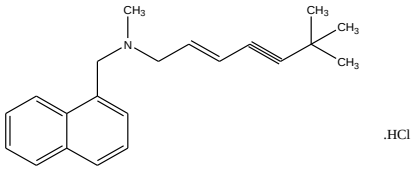

Fig. 1a: Structure of TH

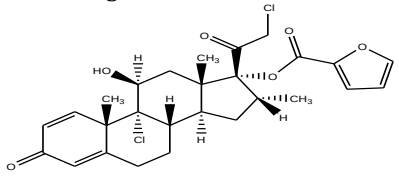

Fig. 1b: Structure of MF

MATERIALS AND METHODS

Instrumentation

HPLC: Shimadzu LC-20AT system equipped with LC solution software, PDA detector, injection volume: 20µL, column: C_{18} Enable (250 × 4.6 mm) 5 µm, flow rate: 1.2 mL/min, detection wavelength 248 nm, final optimized chromatographic conditions are Methanol : Water (95:5 v/v) as mobile phase.

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 (HPLC Grade) procured from Loba Chemie pvt ltd., Mumbai.

Preparation of standard solutions

Preparation of stock solution

Standard stock solution of 1000µg/mL of TH and 100µg/mL of MF were prepared by dissolving 100 mg of TH and 10 mg of MF in 100 mL of mixture of 95:5 v/v of Methanol: Water.

Preparation of working standard solutions

From Standard Stock Solution 25 mL of the solution is taken and further diluted to 50 mL with methanol to get mixed standard solution containing 500µg/mL of TH and 50µg/mL of MF.

Wavelength selection

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. For determination of wavelength, standard solution of TH and MF were scanned over the range of 200-400 nm wavelengths. Wavelength of MF, 248nm was selected for analysis because of its low dose in formulation.

Calibration curve for TH and MF

Aliquot equivalent to 0.4, 1, 2, 3, 4 mL of Working Standard solution were transferred into 10 mL volumetric flasks and finally diluted up

to mark with mixture of 95:5 of Methanol: Water. 20 µL of each solution were injected in to HPLC system and analyzed. Calibration curve was obtained by plotting respective peak area against concentration in µg/mL and the regression equation was computed.

Validation of proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guideline[3].

System suitability

They are used to verify that resolution and reproducibility of chromatographic system are adequate for the analysis to be done. The tests include Resolution (R), Column efficiency (N), Tailing factor (T) and Precision of replicate injection. For system suitability test, standard mixture of 20µg/mL of TH and 2µg/mL of MF solution was injected 6 times and the results were recorded.

Linearity

Linearity was studied by preparing standard solutions at 5 different concentrations. Each concentration was repeated 6 times. The linearity for TH and MF were determined in the range of 20-200µg/mL and 2-20µg/mL respectively. Linearity was assessed in terms of slope, intercept and correlation coefficient.

Precision

Intraday and interday precision was determined by assay of sample solution three times in a day for three different concentrations for intraday and six different days for three different concentrations for interday.(Combined standard samples of concentrations 20, 100 and 200µg/mL for TH and 2, 10 and 20µg/mL for MF).

Accuracy

Accuracy of the method is determined by performing the recovery studies. Recovery study was performed by addition of known amount of standard drugs to pre analyzed commercial pharmaceutical product sample. Accuracy was performed at three levels 50, 100 and 150%. The experiment was repeated three times.

Limit of detection and limit of quantitation

According to the ICH recommendation, the approach based on the standard deviation (SD) of the response and slope was use for the determining the LOD and LOQ values.

$$LOD = 3.3\ \sigma/s \qquad LOQ = 10\ \sigma/s$$

Where,

σ = Standard deviation of response

S = Slope of calibration curve

Robustness

Change following parameters, one by one and observe their effect on system suitability test and assay.

- Change flow rate by 10%
- Change the organic phase ratio of mobile phase by ± 2 %
- Change in λ (± 2 nm)

Analysis of sample

The quantity of cream equivalent to 10 mg TH and 1 mg of MF was accurately weighed and transferred into 10 mL centrifugal tube, dissolved in methanol and centrifuged for 5 minute at 4000rpm. The solution was filtered through whattman filter paper and 5mL volume was taken in 50mL volumetric flask and volume adjusted up to the mark with methanol. This solution contains 100µg/mL of TH and 10 µg/mL of MF. From this solution, 5 mL was taken into 10 mL volumetric flask and volume was adjusted up to the mark with Methanol: Water. 20 µL of solution was injected into HPLC system and analyzed. The % assay was calculated using straight line equation.

RESULTS AND DISCUSSION

Optimization of mobile phase strength

Retention time for TH and MF was found to be 6.9 and 3.2 respectively (Fig.2).

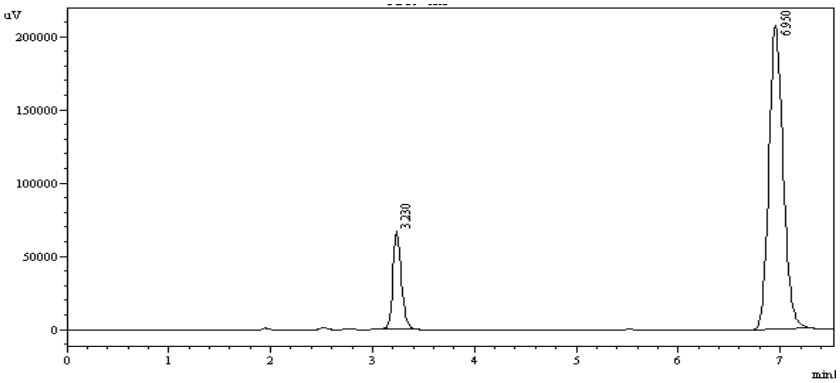

Fig. 2: Chromatogram of standard TH (100µg/mL) (6.9min RT) and MF (10µg/mL) (3.2min RT)

System suitability test

Result of no. of theoretical plate, less tailing and short run time and high resolution of peaks of TH and MF is shown below (Table 1).

Linearity and Range

Data of linearity of TH and MF were performed (Table 2). The chromatogram of standard mixture of TH and MF was taken (Fig.3). The linearity of developed method was achieved in the range of 20-200 µg/mL for TH with correlation coefficient of 0.999 and 2-20 µg/mL for MF with correlation coefficient of 0.999. The result shows that linearity lie within its specific acceptance criteria.

Precision

The % RSD for replicate sample solutions for intraday and interday study were less than 2.0 % for TH and MF, which met the acceptance criteria established for HPLC method (Table 3). This confirms that the method is precise.

Accuracy

Accuracy study was performed at three levels for TH and MF (Table 4). The values of % recovery were found in the acceptance limit of 98-102 % with low % RSD, which justifies that, the method is accurate and free from the interference of excipients used in formulation and is applicable for analysis of marketed formulation.

Robustness

The average values of % RSD of response for determination of TH and MF at changed conditions were less than 2 % which revels the robustness of the method (Table 5).

Analysis of marketed formulation

Acceptability of the proposed method was tested by analyzing the commercially available formulation Sebifin™plus cream (Table 6). The % assay of TH and MF were calculated by taking 5 replicates of

test sample. The % mean assay of TH and MF were found to be 100.12 % and 99.61 % respectively, which lies within the acceptable limit of 98-102 %, which shows that the method is applicable for analysis of marketed formulation.

Table 1: Result of System Suitability Test (n=6)

Parameters	Compounds	
	TH	MF
Retention time (min)	6.98	3.23
Resolution (Rs)	14.34	
Tailing factor (T)	1.24	1.09
%RSD (Retention time)	0.23	0.12
%RSD (Injection repeatability)	0.71	0.99
Theoretical plate	6832.29	4761.73

Table 2: Linear regression data of TH and MF

Parameter	TH	MF
Straight line equation	y=19320 x+90320	y=38226 x+20131
Correlation co-efficient	0.999	0.999
SD of intercept	32588.95	850.07
LOD (µg/mL)	5.57	0.07
LOQ (µg/mL)	16.87	0.22

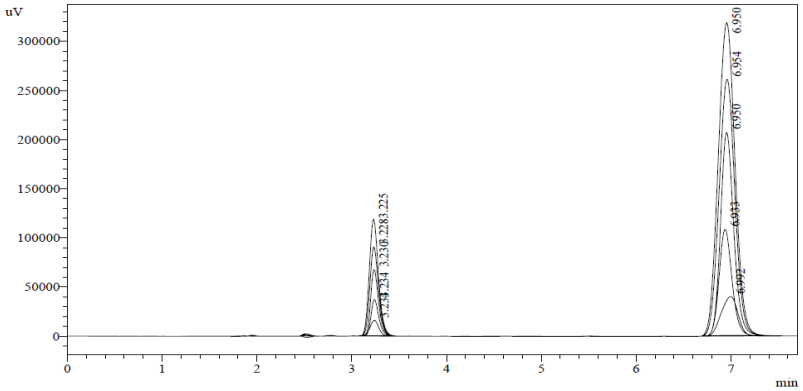

Fig. 3: Overlay of chromatogram of TH and MF

Table 3: Intraday and interday precision data of TH and MF

Concentration (µg/mL)		%RSD (Intraday) (n=3)		%RSD (Interday) (n=6)	
TH	MF	TH	MF	TH	MF
20	2	0.72	0.90	1.48	1.24
100	10	0.60	0.91	1.17	1.15
200	20	0.35	0.60	1.28	1.59

Table 4: Accuracy data of TH and MF (n=3)

% Level	TH				MF			
	Sample conc. (µg/mL)	Std. added (µg/mL)	Mean % Recovery ±SD	% RSD	Sample conc. (µg/mL)	Std. added (µg/mL)	Mean % Recovery ±SD	% RSD
50	60	30	101.51± 0.5116	0.56	6	3	100.46±0.551	0.61
100	60	60	101.33±1.0085	0.83	6	6	99.32±0.0755	0.63
150	60	90	100.70±1.4484	0.96	6	9	99.25±0.0306	0.21

Table 5: Robustness study of TH (20µg/mL) and MF (2µg/mL) (n=3)

Parameters	%RSD (TH)		%RSD (MF)	
	Changed condition		Changed condition	
Flow rate	(+10%)	(+10%)	(-10%)	(+10%)
(1.2 mL/min)	0.68	1.70	0.89	1.18
Mobile phase ratio	(-2%)	(+2%)	(-2%)	(+2%)
(95:5v/v)	1.41	1.70	1.31	1.38
λ (248 nm)	(-2nm)	(+2nm)	(-2%)	(+2%)
	1.62	1.57	1.44	1.89

Table 6: Assay of marketed formulation (n=5)

Labeled claim (%w/w)		%Assay	
TH	**MF**	**TH**	**MF**
1	**0.1**		
Mean		100.12	99.61
SD		1.0213	0.8360
% RSD		1.02	0.84

CONCLUSION

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

ACKNOWLEDGEMENT

The authors are very thankful to Dr. R.H PARIKH; Principal, Ramanbhai Patel College of Pharmacy, Changa, for providing the research facilities. We are also thankful to Aarti Drugs sales, Mumbai and Glenmark Generics pharmaceutical, Nasik for providing the gift samples of Terbinafine hydrochloride and Mometasone furoate respectively.

REFERENCE

1. Drug Profile of Terbinafine hydrochloride, October 2012, http://www.drugbank.ca/drugs/DB00764 (accessed on 20/10/2012)
2. Drug Profile of Mometasone furoate, October 2012, http://www.drugbank.ca/drugs/DB00857 (accessed on 20/10/2012)
3. "ICH Guidelines Q2 (R1)",October 2012, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf (accessed 10/10/12)
4. Ahuja S. Overview: Handbook of pharmaceutical analysis by HPLC. Separation Science and Technology. 6, 1-17, 2005.
5. Cardoso SG, Schapoval EES. High-performance liquid chromatographic assay of terbinafine hydrochloride in tablets and creams. Journal of Pharmaceutical and Biomedical Analysis. 19(5), 809-12, 1999.
6. Goswami PD, Stability-indicating RP-HPLC method for analysis of terbinafine hydrochloride in bulk and in tablet dosage form, International Journal of Pharmacy and Pharmaceutical Sciences, 5,(3), 536-540, 2013
7. Jain AJC, Patel SA, Patel ZN, Patel DT. Development and validation of the UV-spectrophotometric method for determination of terbinafine hydrochloride in bulk and in formulation Pharm Methods. 2(3), 198-202, 2011.
8. Suma.B.V., Kannan.K, Madhavan. V, Nayar CR. HPTLC Method for determination of Terbinafine in the Bulk drug and Tablet dosage form. International Journal of ChemTech Research. 3(2), 742-748, 2011.
9. Zehender H, Denouël J, Roy M, Le Saux L, Schaub P. Simultaneous determination of terbinafine (Lamisil) and five metabolites in human plasma and urine by high-performance liquid chromatography using on-line solid-phase extraction. Journal of Chromatography B: Biomedical Sciences and Applications. 664(2), 347-55, 1995.
10. Indian Pharmacopoeia.Ministry of health and family welfare, Government of India, New Delhi, (2), 1700-1702, 2010.
11. Rekha U, Manjula BP, Formulation and evaluation of microsponges for topical drug delivery of mometasone furoate, International Journal of Pharmacy and Pharmaceutical Sciences, 3 (4) , 133-137, 2011.
12. Vairale AVS, Hima V. B, Sivaswaroop P.Determination Of Mometasone Furoate By HPLC In Topical Preparations: Validation, 8(4), 2009.
13. Shaikh SM, Thusleem OA, Tahir M, Kondaguli AV. A simple RP-HPLC method for the simultaneous quantitation of chlorocresol, mometasone furoate, and fusidic acid in creams. International Journal of ChemTech Research. 47(2), 178-83, 2009.
14. Kulkarni AA, Ranjane MN, Ranjane PN. Simultaneous estimation of Nadifloxacin and Mometasone Furoatein topical cream by HPTLC method. Scholars Research Library. 2(3), 25-30, 2010.