Chemistry Research Journal, 2024, 9(2):77-88

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Validation of spectrometric method for the determination of assay of terbinafine hydrochloride drug substance

Desh Raj Singh[1*], Sachin K Jain[1], Sudha Vengurlekar[1]

[1]Faculty of Pharmacy, Oriental University, Near Aurobindo Hospital Sanwer Road Indore MP India 453555
Email: desh_rajdi@yahoo.in

Abstract

The proposed RP- HPLC method has the advantages of sensitivity, simplicity, precision, accuracy and convenience for the separation and quantization of terbinafine hydrochloride in tablet dosage form. The method was carried out using Zodiac C18 column (100 X 4.6 mm, 5µ) with mobile phase comprised of methanol: acetonitrilein the ratio of 60:40 (v/v/v). The flow rate was set at 1.5ml/min and effluent was detected at 220nm. Under these conditions the retention time of terbinafine hydrochloride was found to be 3.57minute. The method was validated for specificity, accuracy, precision, linearity, and limit of detection, limit of quantification, robustness and solubility stability. The sensitivity test results of terbinafine hydrochloride indicated that the method was sensitive enough to detect a concentration of 1.0µg/ml and able to quantify at a concentration of above 3.3µg/ml. The RSD values of intra-day and inter day precision were very low which reveals that the proposed method was very precise. Linearity of the method was achieved at the range of 20-80µg/ml which cans analysis in wide range of concentrations. Recovery and other validation results are satisfactory and the proposed method was successfully applied for quantitative determination of terbinafine hydrochloride in tablet dosage form.

Keywords: Terbinafine hydrochloride, Specificity, Accuracy, Precision, Linearity

Introduction

Analytical method validation is important to ensure that an analytical methodology is accurate, specific, reproducible and robust over the specified range that an analyte will be analysed. Validation provides an assurance of reliability during normal use and is sometime referred to as the process of providing documented evidence that the method does what it is intended to do [1-4].

Assay

An assay is done to determine the selected specific groups in the' whole sample. Assay is different from purity because purity is nothing but finding the percentage of the sample free from foreign substance. Assay is the characteristic feature of every drug. Assay finds immense importance for every drug before formulation. Various methods are available for the determination of assay, namely

- Titrimetric method
- Spectrometric method

• Chromatographic method etc.

Among these methods the easiest method and most simplest is the titrimetric method. Spectrometric method is less selective and sensitive. GC methods are more selective but they require derivatisation of the drug prior to analysis. Thin layer chromatography (Chromatographic techniques) and HPLC methods require sample cleanup procedures to remove proteins before injection, as liquid-liquid extraction [5-8] or solid-phase extraction.

Titrimetric method overcomes all the methods by its higher sensitivity and its selectivity and doesn't need any tedious procedures during analysis.

Titrimetric Analysis

It is a quantitative chemical analysis carried out by determining the volume of a solution of accurately known concentration which is required to react quantitatively with a measured volume of a solution of a substance to be determined. The solution of accurately known strength is called the standard solution. The weight of the substance to be determined is calculated from the volume of the standard solution used and relative molecular masses of the reacting compounds.

The term 'volumetric analysis' was formerly used for this form of quantitative determination but it has now been replaced by titrimetric analysis. It is considered that the latter expresses the process of titration rather better and the former is likely to be confused with measurements of volumes, such as those involving gases. In titrimetric analysis reagent of known concentration is called the titrant and the substance being titrated is termed the titrant. The alternative name has not been extended to apparatus used in the various operations, so the term volumetric glassware and volumetric flasks are still common, but it is better to employ the expressions graduated flasks. The standard solution is usually added from a long graduated tube called a burette. The process of adding the standard solution until the reaction is just complete is called titration, and the substance to be determined is titrated. The point at which this occurs is called the equivalence point or the theoretical (stoichiometric) end point. The completion of the titration is detected by some physical change, produced by the standard solution itself (e.g. the faint pink colour formed by potassium permanganate) or more usually by the addition of an auxiliary reagent known as an indicator. Alternatively, some other measurement may be used. After the reaction between the substances is practically complete the indicator should give a clear visual change (either a color change or the formation of turbidity) in the liquid being titrated. The point at which this occurs is called the end point of the titration. In the ideal titration the visible end point will coincide with the stoichiometric or theoretical end point. In practice, however a very small difference usually occurs and this represents the titration error. The indicator and the experimental conditions should be so selected that the difference between the visible end point and the equivalence point is as small as possible [9-13].

Titrimetric methods are normally capable of high precision (1 part in 1000) and wherever applicable possess obvious advantages over gravimetric, methods. They need simpler apparatus and are generally quickly performed Tedious and difficult separation can often be avoided. The following apparatus is required for titrimetric analysis: (i) calibrated measuring vessels, including burettes, pipettes and measuring flasks (ii) substances of known purity for the preparation of standard solutions (iii) a visual indicator or an instrumental method for detecting the completion of the reaction [14-15].

Experimental

Preparation of standard stock solution

Standard stock solution of terbinafine hydrochloride pure drug (1mg/ml) was prepared by accurately weighing about 100 mg of each drug in 100 ml volumetric flasks separately. Then the drugs were dissolved with 25ml ofmethanol, and sonicated to dissolve it completely and made up to the mark with the same solvent. The contents were mixed well and filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper. Appropriate volumes of these solutions were further diluted with mobile phase to get at concentrations of 50-100µg/ml separately. Equal quantities of the two drug solutions were mixed and the resultant solution was used for simultaneous analysis.

Preparation of sample solution

terbinafine hydrochloride tablets were purchased from local pharmacy. Ten tablets were weighed and average weight was calculated. Then they were ground to a fine,uniform size powder. An amount of drug equivalent to 10 mg of terbinafine hydrochloride was accurately weighed and quantitatively transferred into 100 ml volumetric flask. Approximately 30 ml methanol was added and the solution was sonicated for 15 min. The flask was made up to volume with mobile phase, and mixed well. Then the solution is filtered through 0.45µm nylon 6 membrane filter paper. The solution results 100 µg/ml of terbinafine hydrochloride drugs. Then an amount of the solution was diluted to a concentration of 70 µg/ml of terbinafine hydrochloride.

Method development

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose.

Detection wavelength

The spectrum of diluted solutions of the terbinafine hydrochloride in methanol was recorded. The absorption spectrum of terbinafine hydrochloride obtained by scanning the sample separately on UV spectrophotometer in UV region (200-400nm) in spectrum mode showed that the drug has maximum absorbance at 272nm. Analysis was carried out by adjusting the UV detector of the HPLC system at 243nm.

Choice of stationary phase

Preliminary development trials have performed with octadecyl columns with different types, configurations and from different manufacturers. Finally, the expected separation and shapes of peak was succeeded Analytical column Inertsil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size.

Selection of the mobile phase

Several systematic trials were performed to optimize the mobile phase. Different solvents like methanol, water and acetonitrile in different ratios and different P^H values of the mobile phase ratios by using different buffer solutions in order to get sharp peak and base line separation of the components and without interference of the excipients. Satisfactory peak symmetry, resolved and free from tailing was obtained in mobile phase methanol: acetonitrile: 0.1%orthophosphoric Aid in the ratio of 75:20:05 (V/V/V) in isocratic condition.

Selection of the mobile phase flow rate

Flow rates of the mobile phase were changed from 0.5 – 1.2 ml/min foroptimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1ml/min flow rate was ideal for the successful elution of the analyte.

Optimized chromatographic conditions

After completion of several systematic trials to optimize the chromatographic conditions, a sensitive, precise and accurate RP-HPLC method was developed for the analysis of terbinafine hydrochloride in pharmaceutical dosage forms. The optimized chromatographic conditions were shown in table. The chromatograms of blank, standard and formulation were shown in figure.

Table 1: Optimized chromatographic conditions of terbinafine hydrochloride

Standard Concentration	70µg/ml	
Pump mode	Isocratic	
Mobile phase	Methanol: Acetonitrile: 0.1%Orthophosphoric Aid in the ratio of 75:20:05 (V/V/V)	
Mobile Phase P^H	4.8	
Wavelength	243nm	
Column	C18 column (250 X 4.6 mm, 5µ)	
Column Temp	Ambient	
Diluent	Methanol	
Injector	Rheodyne	
Injection Volume	20µl	
Flow rate	1 ml/min	
Retention Time	Terbinafine hydrochloride	3.30 min
Run time	10min	
Peak Area	terbinafine hydrochloride	271253
Theoretical plates	terbinafine hydrochloride	7684
Tailing Factor	terbinafine hydrochloride	1.90
Pump Pressure	9.5±5MPa	

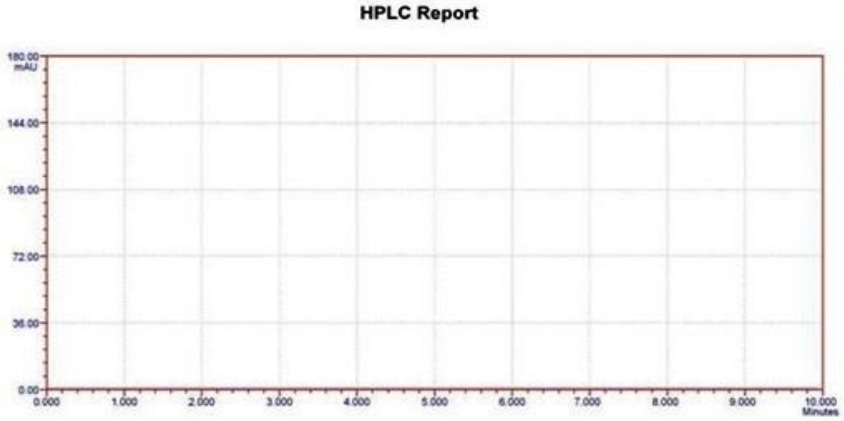

Figure 1: Chromatogram of Blank

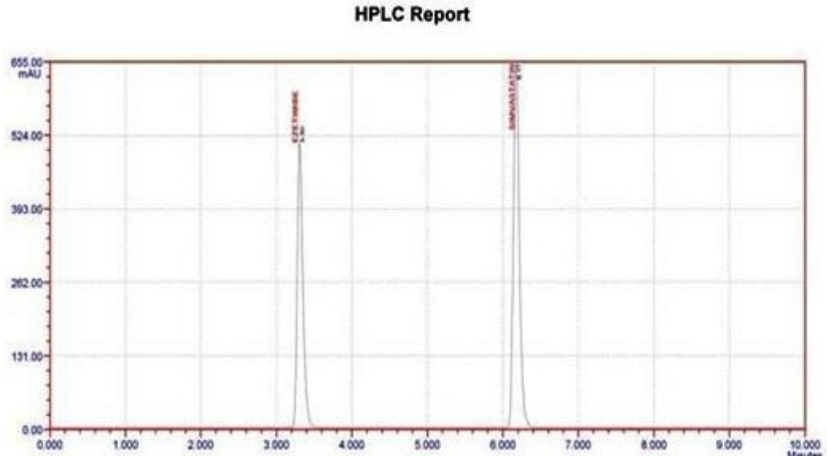

Figure 2: Chromatogram of Standard

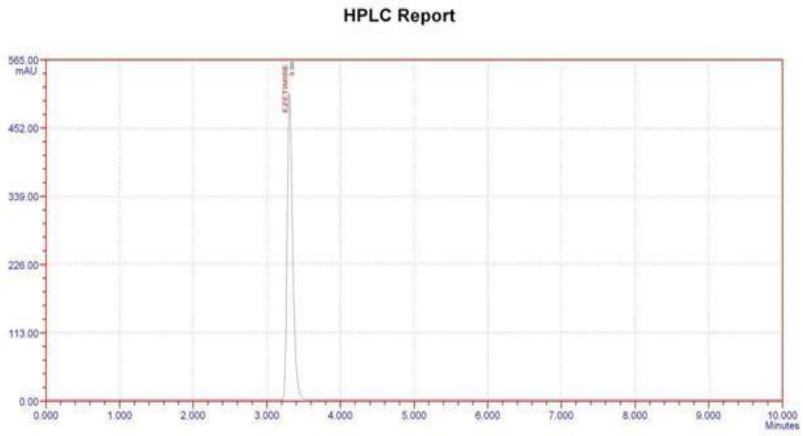

Figure 3: Chromatogram of terbinafine hydrochloride single

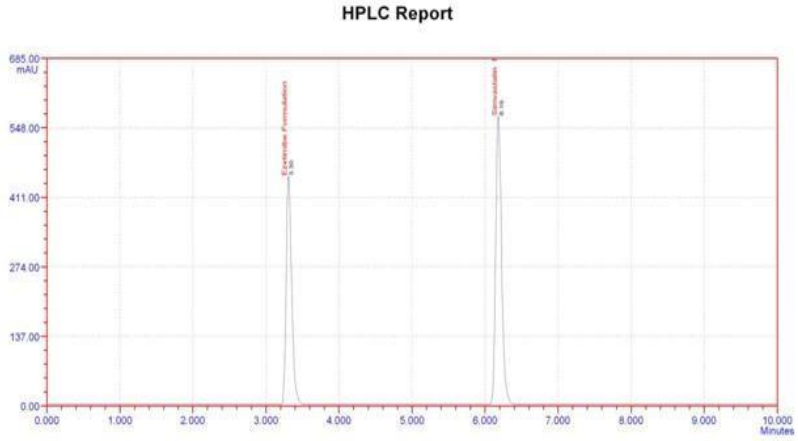

Figure 4: Chromatogram of Formulation

Validation of the Proposed Method

The proposed method was validated as per ICH guidelines. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, and system suitability, limit of detection and limit of quantification.

Specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure was able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. It has been observed that there were no peaks of diluents and placebo at main peaks. This confirms that the chromatographic system used for the simultaneous estimation of terbinafine hydrochloride is selective and specific. Specificity studies indicating that the excipients did not interfere with the analysis. The standard solution showed symmetric peak with retention times of 3.30 minutes for terbinafine hydrochloride. There is no interference of excipients in the chromatogram. This indicates that the proposed method is specific.

Table 2: Result of Specificity analysis

Name of the solution	Retention Time in Min
Blank	No peaks
terbinafine hydrochloride	1.65minutes

System suitability

System suitability tests were carried out on freshly prepared standard stock solution of terbinafine hydrochloride. Equal volume of standard concentration was mixed well. From the prepared solution 20 µl of the sample was injected into HPLC system and the results obtained were used to express the system suitability of the developed method. System suitability resultswere shown in Table.

Table 3: System suitability results

Retention Time	terbinafine hydrochloride	1.65 min
Peak Area	terbinafine hydrochloride	432056
Theoretical plates	terbinafine hydrochloride	5926
Tailing Factor	terbinafine hydrochloride	1.53
Resolution Factor	terbinafine hydrochloride	…….

Linearity

In to a series of seven standard test tubes, varying amount of standard stock solution of terbinafine hydrochloride was taken and made up to variousconcentrations of 50-100 µg/ml. 20µl was injected from each flask. The peak area responses of the solutions were recorded at 243 nm. The plot of peak area versus the respective concentrations of terbinafine hydrochloride were found to be linear in the range of 50-100µg/ml with coefficient of correlation (r^2) 0.999 for terbinafine hydrochloride, regression equation of Y=3829X+3285 for terbinafine hydrochloride.

Table 4: Linearity results of terbinafine hydrochloride

S. No.	Conc µg/ml	Area of terbinafine hydrochloride
1	50	199726
2	60	238655
3	70	271253
4	80	305211
5	90	342830
6	100	388644

Accuracy

Accuracy of the method was determined by direct addition method. The standard addition method was performed at 50 %, 100 % and 150 % levels. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and was calculated and results are presented in table. Satisfactory recoveries ranging from 98.9 to 100.38 % for terbinafine hydrochloride for ezetimibe were obtained by the proposed method. The values of recovery justify the accuracy of the method. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients. This indicates that the proposed method was accurate.

Table 5: Precision results for terbinafine hydrochloride

Recovery	Conc. of sample	Terbinafine hydrochloride estimated	Terbinafine hydrochloride % of recovery
50%		50.33	100.66
		50.11	100.22
	50ppm	49.88	99.76
75%		74.92	99.89
		74.88	99.5
	75ppm	75.01	100.1
100 %		99.1	99.1
	100ppm	99.6	99.6
		99.5	99.5
Mean			99.81

Precision

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over a period of time. The repeatability study was conducted on the solution having the concentration equal to standard concentration. Precision of the method was performed as intraday precision, Inter day precision.

Intra-day precision

To study the intra-day precision, six replicate standard solutions (70µg/ml) of terbinafine hydrochloride was injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.25 for terbinafine hydrochloride, which are well within the acceptable criteria of not more than 2.0. It was confirmed that the analytical technique showed good repeatability. Results of system precision studies are shown in Table.

Table 6: Intra-day precision results of ezetimibe and simvastatin

Conc.	Injection No.	terbinafine hydrochloride peakarea response
70µg/ml	1	271253
	2	272219
	3	272481
	4	271685
	5	272051
	6	273272
	RSD	**0.25**

Inter day precision

To study the inter day precision, six replicate standard solutions (70µg/ml) of terbinafine hydrochloride was injected on three consecutive days. The percent relative standard deviation (% RSD) was calculated and it was found to be 1.23 for terbinafine hydrochloride, which are well within the acceptable criteria of not more than 2.0. It was conformed that the analytical technique showed good repeatability. Results of system precision studies are shown in Table.

Table 7: Inter day precision results of terbinafine hydrochloride

Conc.	Injection No.	Terbinafine hydrochloride peak area response
70µg/ml	1	274666
	2	266547
	3	268688
	4	271303
	5	272481
	6	268039
	RSD	**1.23**

Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It showed the reliability of the analysis with respect to deliberate variations in method parameters. Robustness test was carried out by small variation in the chromatographic conditions at a concentration equal to standard concentration i.e 70µg/ml and percentage change in the results wascalculated. Here robustness was performed by change in mobile phase ratio, mobile phase flow rate and wavelength of the detector. terbinafine hydrochloride at 70µg/ml concentration were analyzed under these changed experimental conditions. Percentage change in the results was calculated and was found to be within the acceptance criteria of below 2. This indicates that the proposed method is valid. Results were shown in Table.

Table 8: Robustness results of ezetimibe and simvastatin

S. No.	Parameter	Condition	Terbinafine hydrochloride	
			Area	% of change
1	Standard	Standard conditions	271253	……..
2	Mobile phase	MeOH: ACN:0.1 %O.P.A70:25:05	432954	0.73
		80:15:05	273252	0.74
3	Mobile phase pH	5.0	270644	0.225
		4.6	273625	0.87
4	Wavelength	274 nm	269963	0.48
		249nm	270583	0.25

Ruggedness

Inter day variations were performed by using six replicate injections of standard solution of 70µg/ml concentration which were prepared and analyzed by different analyst on three different days over a period of one week. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.21 for terbinafine hydrochloride, which are well within the acceptable criteria of not more than 2.0. It was concluded that the analytical technique showed good repeatability. Results of system precision studies are shown in table.

Table 9: Ruggedness results of terbinafine hydrochloride

Conc.	Injection No.	Ezetimibe peak area response
70µg/ml	1	271443
	2	270794
	3	270954
	4	271511
	5	270833
	6	272268
	RSD	**0.21**

Limit of Detection

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio 2:1 is generally considered acceptable for estimating the detection limit. LOD is found to be 1.2µg/ml for terbinafine hydrochloride.

Quantization Limit

The quantization limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. LOQ is found to be 4µg/ml for terbinafine hydrochloride.

Formulation

From the prepared formulation solution, 20µl of sample solution was injected into HPLC system and peak area of the detector response was used to calculate % assay in the prepared solution. The % assay was found to be 99.85% for terbinafine hydrochloride. Results were shown intable.

Table 10: Formulation results of terbinafine hydrochloride

S. No.	Drug	Tablet	Dosage	Sampleconc	Amount found	% of Drug Estimated in Tablet
1	Ezetimibe	Lemicil	10 mg	70µg/ml	69.87 µg/ml	99.85

Discussion on the Results

A simple, specific, accurate, precise and sensitive reverse phase high performance liquid Chromatographic method has been developed for the quantification of terbinafine hydrochloride. Wavelength of the two drugs that absorb maximum light was confirmed by using spectrophotometer. Selection of stationary and mobile phases were carried out by random change in the different ratios of mobile phases and different stationary phases that separate the drugs with high resolution and high theoretical plates and less tailing factor. Finally it was succeeded at ODS C18 column with a mobile phase ratio of methanol: acetonitrile: 0.1%orthophosphoric Aid 75:20:05 (v/v/v), detection wavelength 243nm was found to be most suitable conditions for the simultaneous analysis of terbinafine hydrochloride. The optimized chromatographic conditions were shown in table.

The linear regression response was obtained for a series of concentration in the range of 50-100µg/ml and it was found to be linear. The calibration curve equation was found to be Y=3829X+3285 for terbinafine hydrochloride with correlation coefficient (r^2) value 0.999 for terbinafine hydrochloride. Terbinafine hydrochloride showed best response on the regression equation with less intercept and high correlation coefficient. The data of regression analysis of the calibration curves are shown in table. Selectivity and specificity were studied for the examination of various excipients generally present in the tablet dosage form of terbinafine hydrochloride. The results indicated that they did not interfere in the assay.

A suitability test was applied to representative chromatograms for various parameters. It was found that high number of theoretical plates for terbinafine hydrochloride was observed with less tailing factor and high resolution. Both the compound elute with in 3min with high resolution and short run time. The results obtained were within acceptable limits of tailing factor ≤2.0 and theoretical plates >2000, resolution factor >2 (Table 5.3). Results indicated that the developed method having shortest run time with high resolution. This confirms the easy usage of the method, less time for analysis. The proposed methods were validated as per the ICH guidelines.

The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample with in the day (intraday) and next consequent three days for inter day precision. For each cases % RSD was calculated within the acceptable range of 2 in intra-day and inter day precision for terbinafine hydrochloride. This showed that the precision of the methods are satisfactory.

The accuracy is the closeness of the measured value to the true value for the sample. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. The recovery technique was performed to study the accuracy and reproducibility of the proposed methods. For this, known quantities of the terbinafine hydrochloride solution were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed. The total amount of terbinafine hydrochloride was determined by using the proposed methods and the amount of added drug was calculated by the difference. Recovery was carried out by standard addition method of 50%, 100% and 150% addition to standard, pre analyzed sample of 20µg/ml in triplicates. Percentage recovery for each case was calculated and was found to be 98.05 to 101.76% for terbinafine hydrochloride. This was found to be well within the acceptance criteria of 98-102%. This showed that the recoveries of terbinafine hydrochloride by the proposed methods were satisfactory. Recovery results were shown in table.

Robustness test was carried out by small variation in the chromatographic conditions and percentage change in the results was calculated. Here robustness was performed by change in mobile phase ratio, mobile phase P^H and wavelength of the detector. terbinafine hydrochloride at 70µg/ml concentration were analyzed under these changed experimental conditions. Percentage change in the results was calculated and was found to be within the acceptance

criteria of below 2. Robustness results indicate that there is no considerable change in the results with change in the developed conditions. Hence the developed method is robust in nature. Robustness results were shown in table.
Ruggedness performed by using six replicate injections of standardsolution of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.21 for terbinafine hydrochloride, which are well within the acceptable criteria of not more than 2.0. It was concluded that the analytical technique showed good repeatability. Ruggedness results were shown in table.
A signal-to-noise ratio 2:1 is generally considered acceptable for estimating the detection limit. LOD is found to be 1.2µg/ml terbinafine hydrochloride and LOQ is found to be 4µg/ml for terbinafine hydrochloride.
The validated method was applied for the assay of commercial tablets of terbinafine hydrochloride. Peak area of the detector response was used to calculate % assay. The % assay was found to be 99.85 % for terbinafine hydrochloride. Results were shown in table. The results presented good agreement with the labeled content.
Thus the method developed in the present investigation was simple, sensitive, accurate, rugged, robust, rapid and precise. The absence of additional peaks in the chromatogram indicated that there is no interference of the common excipients used in the tablets. Hence, the above said method can be successfully applied for the estimation of terbinafine hydrochloride in tablet dosage forms.

Summary and Conclusions

The development of HPLC methods for the determination of drugs has received great attention in analytical research because of their importance in the quality control. HPLC is the unique, versatile, universal, basic instrument and well utilized by the researchers because of its ease in the operation, availability and in terms of cost. In the present work, an attempt was made to develop a simple and rapid HPLC method for the routine analysis of eleven drugs in bulk and tablet formulations. For this purpose, the analytical column, solvent selection, mobile phase composition, flow rate, and detector wavelength were studied. The developed method conditions are subjected forvalidation under ICH guidelines.

Mainly deals with the drug analysis. In this the chemical analysis includes classical and instrumental analysis used in the pharmaceutical drug analysis is discussed. It concerns about methods involved for the estimation of pharmaceutical formulations using chromatic graphic techniques and also consists of brief discussion on high performance liquid chromatography and its instrumentation, method development followed by general method validation procedures and validation procedure for assay methods as per ICH guidelines.

Deals with the development and validation of terbinafine hydrochloride for the estimation in bulk samples and pharmaceutical formulations. The method development was conducted with Zodiac C18 column (250 X 4.6 mm, 5µ) with the flow rate of 1.0mL/min. the optimized mobile phase conditions were methanol, water and acetonitrile in the ratio of 50:30:20 (v/v). Column temperature was ambient and the wavelength was 218nm. The method found to be linear, accurate, rugged and robust for validated parameters. The linearity range was determined by external standard calibration method in the concentration range of 2µg/ml to 10µg/ml (r2= 0.999). The LOD and LOQ were found to be 0.05µg/ml and 0.165µg/ml respectively. Thus the method condition is very sensitive that can analysis in the nano gram level of concentration also. The system suitability parameters like capacity factor, asymmetric factor, tailing factor, and number of theoretical plates were tested. The amount of recovery was calculated as 99.5 to 101.2% and it was observed that all the values are within the limits. Further the precision of the method was confirmed by the repeatable analysisof formulation. The % RSD was found to be 0.588 for intraday and 0.918 for inter day. It indicated that the method has good precision. The low % RSD value indicated that there is no interference due to excipients used in formulation. Hence, the accuracy of the method was confirmed. This method offers better turnaround of analytical values. Using the same method, assay was performed for individual samples and found that values are between good agreements. Hence this will be an excellent method for the assay determination and content uniformity of terbinafine hydrochloride in oral solid dosage form. The method has many advantages, e.g., simplicity, isocratic conditions, shorter run time, low injection volume, smaller particle size, and

less flow rate, inexpensive mobile phases. Under these conditions, the retention timeof terbinafine hydrochloride was about 7.05min, with a good peak shape (peak tailing factor < 2), and the run time was 10min.

Development and validation of terbinafine hydrochloride for the estimation in bulk samples and pharmaceutical formulations. terbinafine hydrochloride is dopamine receptor agonist D2 receptors. terbinafine hydrochloride has a direct inhibitory effect on pituitary lactotroph (prolactin) cells. The method development was conducted with Symmetry C18; 250 mmx4.6 mm I.D; particle size 5µm with the flow rate of 1.0mL/min. The optimized mobile phase conditions were acetonitrile, 1% orthophosphoric acid and sodium dihydrogenphosphate in the ratio of 40:30:30 (v/v/v). Column temperature was ambient and the wavelength was 275nm. The method has significantly reduced runtime with better peak shape at 1.655 minutes and the runtime required for analysis is 5min. The method found to be linear, accurate, rugged and robust for validated parameters. The linearity range was determined by external standard calibration method in the concentration range of 5µg/ml to 25µg/ml (r^2= 0.999). It indicated that the concentrations of terbinafine hydrochloride had wide linearity range for analysis. The LOD and LOQ were found to be 0.4µg/mland 1.5µg/ml respectively. The system suitability parameters like capacityfactor, asymmetric factor, tailing factor, and number of theoretical plates were tested. The amount of recovery was calculated as 99.66 to 100.16% and it was observed that all the values are within the limits. Further the precision of the method was confirmed by the repeatable analysis of formulation. The % RSD was found to be 0.946 for intraday and 0.892 for inter day. It indicated that the method has good precision. The low % RSD value indicated that there is no interference due to excipients used in formulation. Hence, the accuracy of the method was confirmed. This method offers better turnaround of analytical values. Using the same method, assay was performed for individual samples and found that values are between good agreements. Hence this will be an excellent method for the assay determination and content uniformity ofterbinafine hydrochloride in oral solid dosage form. The method has many advantages, e.g., simplicity, isocratic conditions, shorter run time, low injection volume, smaller particle size, and less flow rate, inexpensive mobile phases. Under these conditions, the retention time of terbinafine hydrochloride was about 1.655min, with agood peak shape (peak tailing factor < 2), and the run time was 5min.

Development and validation of new reverse phase HPLC method for the estimation of terbinafine hydrochloride bulk samples and pharmaceutical formulations. The proposed RP- HPLC method has the advantages of sensitivity, simplicity, precision, accuracy and convenience for the separation and quantization of terbinafine hydrochloride in tablet dosage form. The method was carried out using Zodiac C18 column (100 X 4.6 mm, 5µ) with mobile phase comprised of methanol: acetonitrilein the ratio of 60:40 (v/v/v) .The flow rate was set at 1.5ml/min and effluent was detected at 220nm. Under these conditions the retention time of terbinafine hydrochloride was found to be 3.57minute. The method was validated for specificity, accuracy, precision, linearity, and limit of detection, limit of quantification, robustness and solubility stability. The sensitivity test results of terbinafine hydrochloride indicated that the method was sensitive enough to detect a concentration of 1.0µg/ml and able to quantify at a concentration of above 3.3µg/ml. The RSD values of intra-day and inter day precision were very low which reveals that the proposed method was very precise. Linearity of the method was achieved at the range of 20-80µg/ml which cans analysis in wide range of concentrations. Recovery and other validation results are satisfactory and the proposed method was successfully applied for quantitative determination of terbinafine hydrochloride in tablet dosage form.

HPLC method developed and subsequently validated for simultaneous estimation of terbinafine hydrochloride from their combination product. terbinafine hydrochloride is in a class of medications called cholesterol-lowering medications used to reduce the amount of cholesterol and other fatty substances in the blood. The proposed RP-HPLC method utilizes a Inertsil ODS C18 column (250 X 4.6 mm, 5µ) i.d. column, mobile phase consisting of methanol: acetonitrile: 0.1% orthophosphoric acid in the ratio of 75:20:05 (V/V/V) with apparent pH adjusted to 4.8, and UV detection at 243 nm using a UV detector. The described method has been validated, apart from specificity, for response function, accuracy, system suitability and precision. The nominal concentrations of standard and test solutions for terbinafine hydrochloride were 70µg/ml. The described method was linear over a range of 50-100µg/ml for terbinafine hydrochloride. %recovery for each case was calculated and was found to be 98.9 to 100.38 % for terbinafine hydrochloride. This was found to be well within the acceptance criteria of 98-102 %. A signal-to-noise

ratio 3:1 is generally considered acceptable for estimating the detection limit. LOD is found to be 1.2µg/ml for terbinafine hydrochloride and LOQ is found to be 4µg/ml for terbinafine hydrochloride. Chromatographic peak purity data of terbinafine hydrochloride indicated no co-eluting peaks with the main peaks of drugs which demonstrated the specificity of assay method for their estimation in presence of degradation products. The proposed method can be useful in the quality control ofcombination drug products.

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