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SIMULTANEOUS ANALYSIS OF RPHPLC METHOD DEVELOPMENT AND VALIDATION OF TERBINAFINE AND BEZAFIBRATE DRUGS IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, sensitive and accurate reversed phase high performance liquid chromatographic method for terbinafine and bezafibrate drugs. This method is developed for terbinafine and bezafibrate drugs. Reversed phase chromatographic separation of the two drugs was performed a C_{18} column is used with different mobile phases of methanol, water, ammonium dihydrogen phosphate and methanol, acetonitrile, orthophosphoric acid respectively. The detection of wave length is 225 nm for terbinafine and 232 nm for bezafibrate. The percentage of recovery 99.51% for terbinafine and 99.94% for bezafibrate. The proposed method is validated for linearity, accuracy and precision, limit of detection (LOD) and limit of quantification (LOQ) as per the guide lines of International Conference on Harmonization (ICH).

Keywords: Terbinafine, Bezafibrate, Ammonium dihydrogen phosphate, Fungal infection, Propionic acid.

INTRODUCTION

Terbinafine[1-7] chemically known as [(2*E*)-6, 6-dimethylhept-2-en-4-1ylmethyl)] amine and molecular formula is $C_{21}H_{25}N$. It is used to treat many fungal infections i.e., fingernail or toenail. Bezafibrate is chemically known as 2-(4-{2-[(4-chlorobenzoyl) amino] ethyl} phenoxy)-2-methylpropanoic acid. Molecular formula is $C_{19}H_{20}ClNO_4$. It is used for medication is along with a diet and an exercise program to treat high cholesterol levels. This proposed method is simple and accurate for the analysis of above drugs in pharmaceutical dosage form. The structures of above drugs are shown in figure1a and figure1b.

MATERIALS AND METHODS

Instrument

The high pressure liquid chromatographic system consisted of Shimadzu HPLC model (VP series) contains LC-10 AT pump variable wave length programmable UV/Visible detector and rheodyne injector (7725 i) with 20 µl fixed loop. Chromatographic analysis was performed using Intersil C-18 analytical column with 250×4.6 mm.

Reagents and Materials

Methanol of HPLC grade, acetonitrile, orthophospharicacid and ammonium dihydrogen phosphate is commercially available in the

market was purchased from Dr.Reddys laboratory, Hyderabad, India.

Chromatographic Conditions

Chromatographic analysis was carried out at ambient temperature. Separation of two drugs terbinafine and bezafibrate were achieved by gradient elution of C_{18} column and mobile phases are methanol, water, ammonium dihydrogen phosphate (60:15:25 v/v), methanol, acetonitrile, orthophospharic acid (35:55:10 v/v) are respectively. These mobile phases are sonicated about five minutes, filtered through 0.45 µm nylon membrane. The injection volume was 20 µl. The mobile phase flow rate is 1.0 ml/min for terbinofine and 1.0 ml/min for bezafibrate. The analysis was carried out at 225 nm and 232 nm wave length of respective drugs.

Preparation of Sample Solution of Terbinafine

The sample solution was prepared by accurately weighed 1mg of this drug and it is transferred in 25 ml volumetric flask and 10 ml of mobile phase .Then the solution was ultrasonicated for five minutes and filtered through 0.45 µm nylon membrane**.**

Preparation of Bezafibrate Sample Solution

The sample solution of bezafibrate drug was prepared about 0.1 mg of drug was dissolved in 100 ml mobile phase.

RESULTS AND DISCUSSION

We have done an analytical RP HPLC analysis for terbinafine in a pharmaceutical formulation was developed and validated as per the guidelines of ICH. The UV spectra showed that the terbinafine drug absorbs at 235 nm and bezafibrate[8-12] drug absorbs at 232 nm was selected as the detection wave length in high performance liquid chromatography. The above two drugs mobile phases performed based on asymmetric factor and peak area obtained. Different mobile phases were tried for satisfactory separation, well resolved and good symmetrical peaks and a sharp typical chromatogram are shown in figure (2a & 2b) obtained with the mobile phase of terbinafine is methanol, water, ammonium dihydrogen phosphate (60:15:25 v/v) and methanol, acetonitrile, orthophospharic acid, (35:55:10 v/v) is the mobile phase of bezafibrate respectively. The retention time of terbinafine is 5.1 minutes and bezafibrate for 6.0 minutes and the number of theoretical plates is terbinafine 7901 and for bezafibrate is 18782.The tailing factor of terbinafine is 1.16 and for bezafibrate is 1.17.

Method Validation

This developed method was validating linearity, LOD, LOQ, precision, and accuracy stipulated by the ICH guidelines.

Linearity

Linearity was evaluated by analysis of standard solution contains terbinafine drug. Six standard concentration of ranging from 2 ppm to 12 ppm a standard calibration curve of terbinafine was constructed by plotting area versus concentration is shown figure 3a. The calibration curve of bezafibrate drug was showed in figure 3b. Slope, Intercept and correlation coefficient, data are listed in table1.

Limit of Detection and Limit of Quantification

Limit of detection (LOD) of terbinafine drug is 0.5 ppm and limit of quantification (LOQ) of is 1.15 ppm and for bezafibrate is LOD is 0.01 ppm and LOQ is 0.04 ppm.

Precision

The precision of the chromatographic analysis of the above two drugs by measuring the repeatability (Intra- day precision) and the intermediate precision (Inter-day precision). The repeatability was evaluated by assay six samples at same concentration on the same day and the intermediate precision was calculated on consecutive three days. The relative standard

deviations (RSD) value was obtained less than 2 of each concentration.

Accuracy

The accuracy of the method evaluated by calculating recovery of terbinafine and bezafibrate drugs. The recovery amount of was estimated by measuring the peak, with known concentration. These values fitting from calibration curve. The recovery studies were carried out three times of the same concentration range and amount of was estimated. From the above estimation, percentage of drug recovery was calculated. The results of system suitability and validation parameters are given in table2.

CONCLUSION

In conclusion the developed method is simple, accurate, precise and specific assay for the analysis the said drugs in pharmaceutical dosage forms. This method was validation good results and presented good linearity, accuracy and precision of these drugs. The RSD values for all parameters were found to be less than 2, which indicates the validity of method and results obtained by this method are in fair agreement. Finally this method can be used for better analysis and pharmaceutical formulations of the above two drugs.

Table 1: Data of the calibration curve

Parameters	Terbinafine Values	Bezafibrate Values
Calibration range	2 to 12 ppm	0.2 to 1.4 ppm
Slope	25161.18	88313.17
Intercept	4382.4	12817.9
Correlation coefficient	0.999	0.996

Table 2: System suitability and validation parameters

Parameters	Terbinafine Values	Bezafibrate Values
Theoretical plates (N)	7901	18782
Retention (min)	5.1	6.0
LOD	0.5 µg/ml	0.01 ppm
LOQ	0.15 µg/m	0.04 ppm
Accuracy (%)	99.54 %	99.80 %
RSD (%)	0.751	0.410

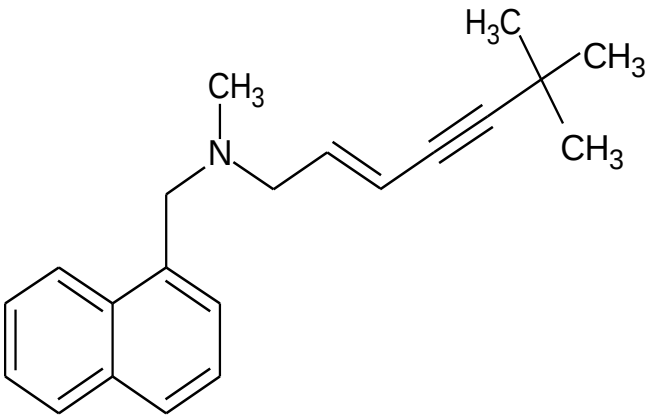


Figure1a: Structure of Terbinafine

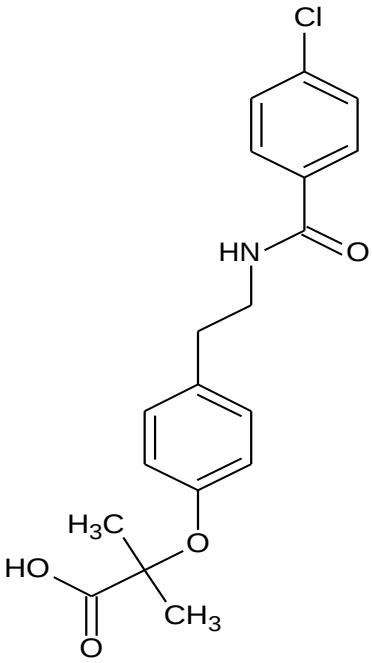


Figure1b: structure of Bezafibrate

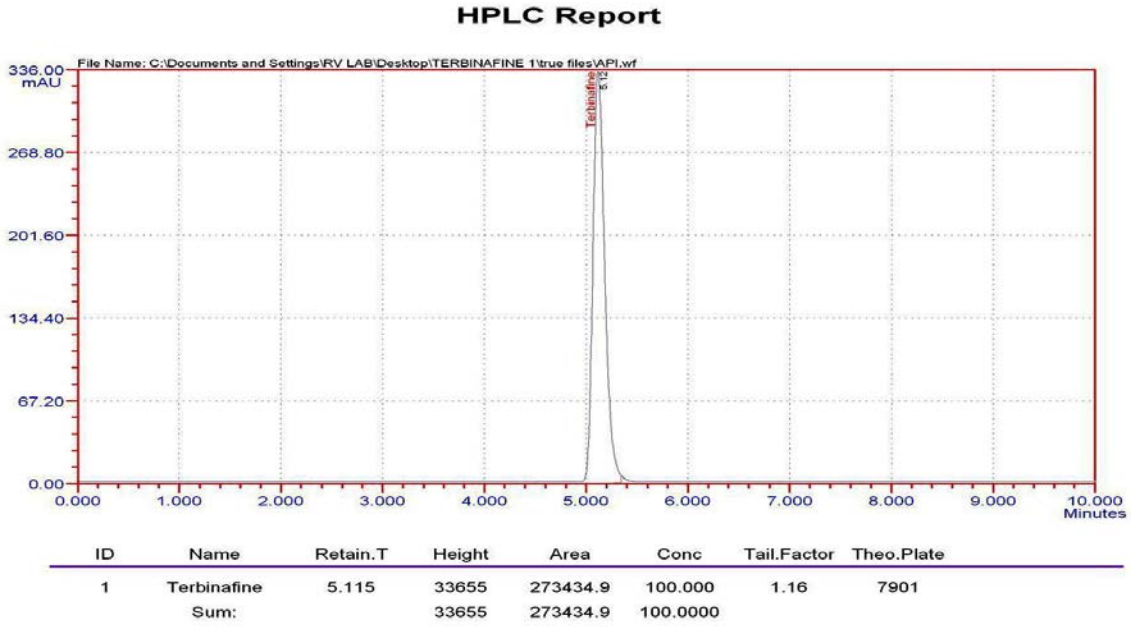
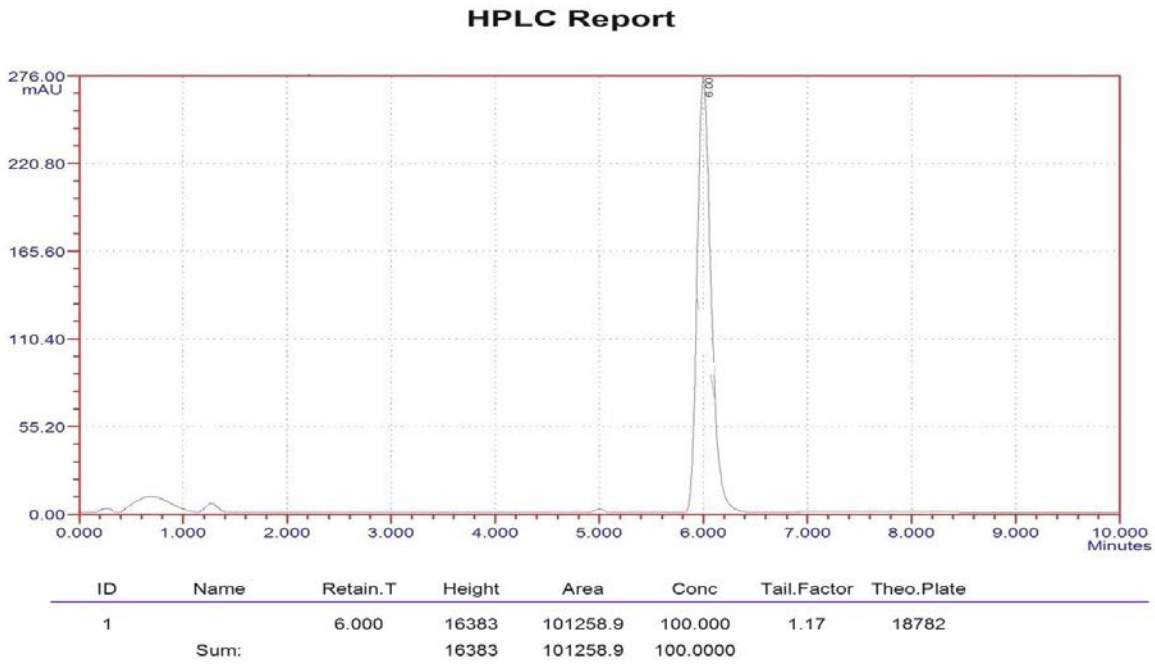
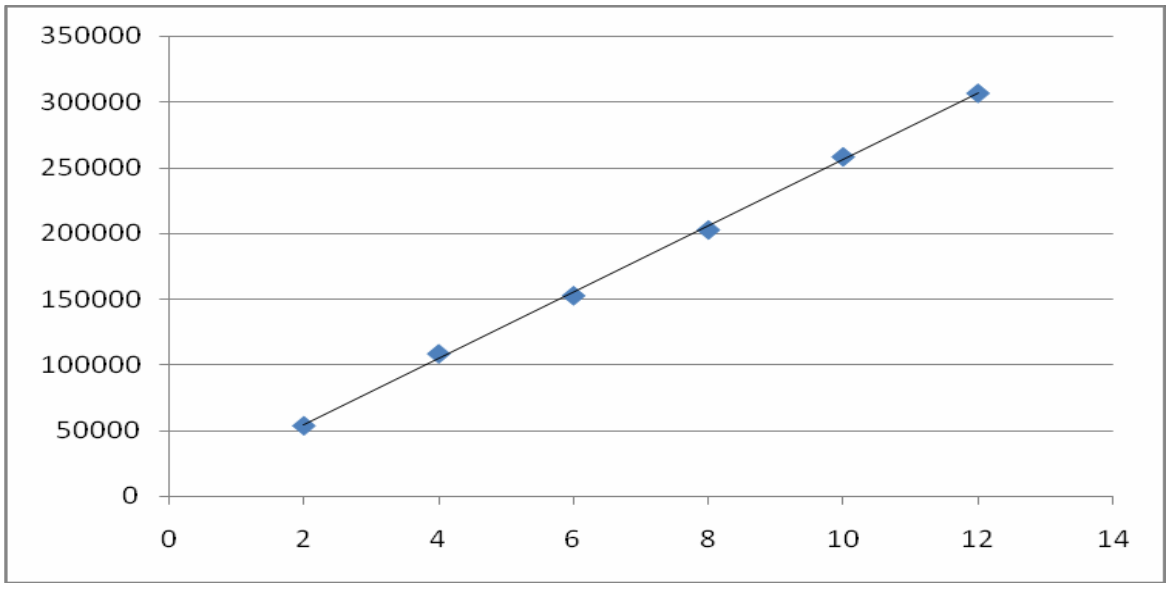


Figure2a: Sharp Typical Chromatogram of Terbinafine



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		6.000	16383	101258.9	100.000	1.17	18782
	Sum:		16383	101258.9	100.0000		

Figure2b: Sharp Typical Chromatogram of Bezafibrate



On x – axis: concentration and on y – axis: area.

Figure3a: Calibration curve of Terbinafine

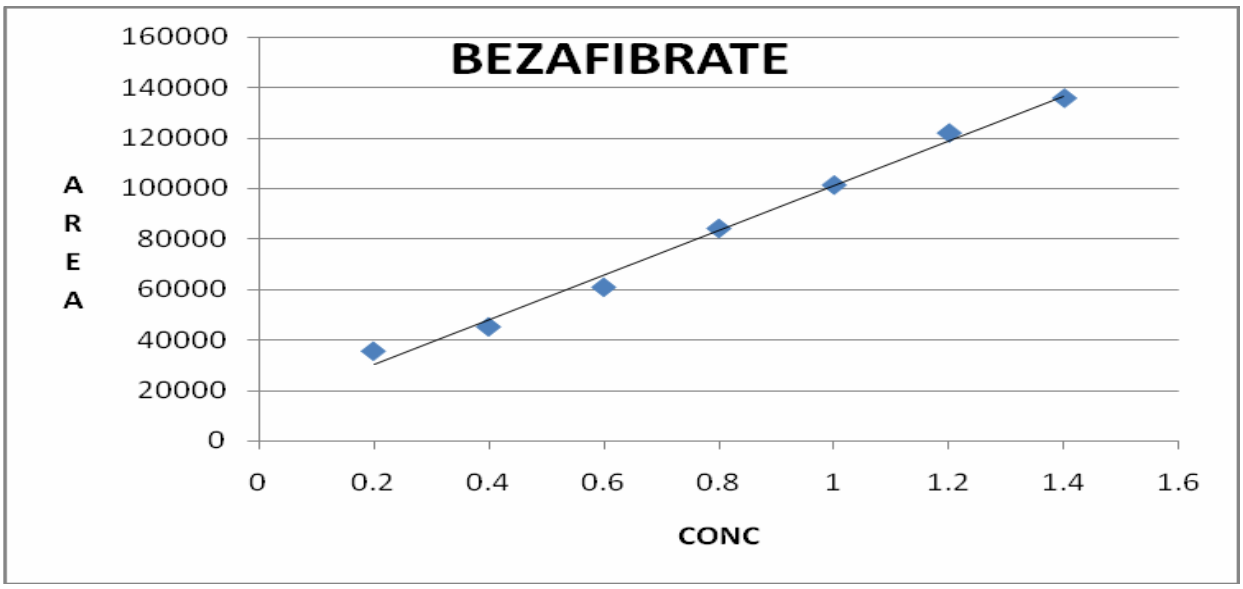


Figure3b: Calibration curve of Bezafibrate

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