International Journal of Pharmacy and Pharmaceutical Sciences

Print ISSN: 2656-0097 | Online ISSN: 0975-1491 Vol 12, Issue 11, 2020

Original Article

NEW DEVELOPED AND VALIDATED SPECTROSCOPIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TERBINAFINE HYDROCHLORIDE AND FLUCONAZOLE

BHUMIKA THAKUR[1*], INDER KUMAR[2]

[1*]Shiva Institute of Pharmacy, Bilaspur, HP, India, [2]School of Pharmacy, Abhilashi University, Mandi, HP, India
Email: bhumi.thakur26@gmail.com

Received: 09 Aug 2020, Revised and Accepted: 10 Sep 2020

ABSTRACT

Objective: A new, simple, precise, accurate, and reproducible method or simultaneous equation method was developed and validated for the simultaneous estimation of terbinafine hydrochloride (TH) and fluconazole (FLZ) in pure form.

Methods: Simultaneous estimation of terbinafine hydrochloride and fluconazole was estimated by the ultraviolet (UV) spectrophotometry method. The method was based on the measurement of absorbance at two wavelengths 222 nm and 239 nm, of terbinafine hydrochloride and fluconazole in 0.1N HCl respectively.

Results: Calibration curves terbinafine hydrochloride and fluconazole were found to be linear in the concentration ranges of 0.5-3.0 µg/ml and 80-400 µg/ml, respectively, with their correlation coefficient values (R^2) 0.999 and 0.998. LOD and LOQ of TH were found to be 0.067, 0.203 at 222 nm and 0.175, 0.531 at 239 nm, similarly for FLZ; 31.089, 94.210 at 222 nm and 94.380, 286.00 at 239 nm respectively. In the precision study, the % RSD value was found within limits (%). The percentage recovery at various concentration levels varied from 98.50 % to 103.96 % for TH and 97.27 % to 103.83 % for FLZ confirming that the expected method is accurate.

Conclusion: It could be concluded from the results obtained in the present study that this method for simultaneous estimation of TH and FLZ in pure is simple, precise, and economical. The proposed method can be applied successfully for the simultaneous estimation of TH and FLZ in the pure and pharmaceutical dosage form.

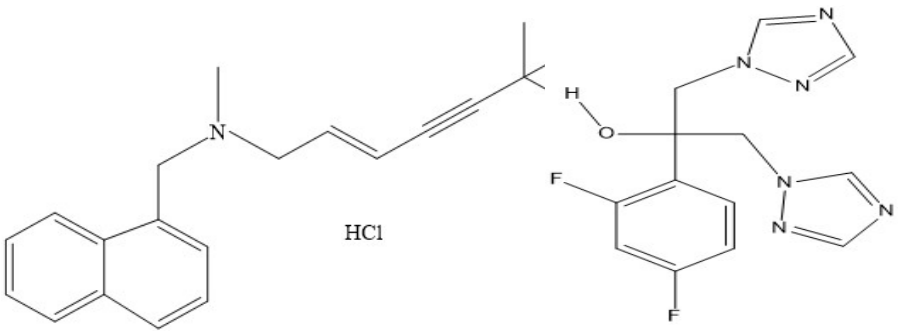
Keywords: Fluconazole, Terbinafine hydrochloride, UV-visible spectroscopy, Simultaneous estimation

DOI: http://dx.doi.org/10.22159/ijpps.2020v12i11.39344. Journal homepage: https://innovareacademics.in/journals/index.php/ijpps.

INTRODUCTION

Terbinafine hydrochloride (TH) is (E)-N,6,6-trimethyl-N-(naphthalen-1-ylmethyl) hept-2-en-4-yn-1-amine; hydrochloride. It is an allylamine, which has a wide spectrum of antifungal activity in fungal infections of the hair and skin such as Pityriasis Versicolor. Terbinafine, a synthetic allylamine antifungal agent, inhibits ergosterol biosynthesis via squalene epoxidase. When exposed to terbinafine, fungi accumulate squalene while becoming deficient in ergosterol, an essential component of fungal cell membranes [1, 2]. The inhibition of squalene epoxidase leads to fungistatic and fungicidal action. Terbinafine is an orally and topically active drug with primarily fungicidal activity against a broad spectrum of fungi, including dermatophytes, filamentous, dimorphic organisms, and some yeasts such as *Candida parapsilosis* [3, 4].

Fluconazole (FLZ) is 2-(2,4-difluorophenyl)-1,3-bis(lH-1,2,4-triazo1-yl)propan-2-ol. Fluconazole is a synthetic triazole with antifungal activity. It is a well-established first-line management option for both localized and systemic *C. albicans* infections. Fluconazole is used against superficial and systemic candidiasis and in the treatment of cryptococcal infections for patients with the acquired immunodeficiency syndrome [5, 6]. Fluconazole main targets the heme protein, which co-catalyzes cytochrome-P450-dependent 14 α-demethylation of lanosterol in the last stage of ergosterol biosynthesis in fungus. Fluconazole is effective against *C. albicans* infections at a wide range of body sites and tissues, irrespective of the patient's immune status [7, 8]. Therefore, the present study aims to develop and validate the simultaneous estimation method of terbinafine hydrochloride and fluconazole in pure form.



(a) **(b)**

Fig. 1: Structure of (a) terbinafine hydrochloride (b) fluconazole

MATERIALS AND METHODS

Fluconazole and terbinafine hydrochloride were procured from Lark Pharmaceuticals, Bhiwandi, India as a gift sample. Hydrochloric acid, methanol, and other chemicals and solvents were procured from CDH Chemicals, Mumbai, India. All chemicals and solvents were used of analytical grade.

Preformulation studies

Various preformulation studies were carried out to identify the purity of the drug i.e. physical characterization, melting point, partition coefficients, solubility, UV, and FTIR studies [9, 10].

Determination of absorption maxima (λ_{max}) and preparation of calibration curve

Absorption maxima (λ_{max}) of TH in 0.1N HCl

Standard known concentration solution (3.0 µg/ml in HCl) was scanned in the range of 400-200 nm using a UV-visible spectrophotometer to get the absorption maxima.

Preparation of calibration curve of TH in 0.1N HCl

100 mg of TH was dissolved in 0.1N HCl in the 100 ml volumetric flask and a series of concentrations 0.5 µg/ml, 1.0 µg/ml, 1.5 µg/ml, 2.0 µg/ml, 2.5 µg/ml, 3.0 µg/ml of TH were prepared, and absorbance was measured at 222 nm in UV-visible spectrophotometer.

Determination of absorption maxima (λ_{max}) of FLZ in 0.1N HCl

Absorption maxima (λ_{max}) of FLZ was determined in 0.1N HCl by scanning the 400 µg/ml solution in a range of 200-400 nm using a UV-visible spectrophotometer.

Preparation of calibration curve of FLZ in 0.1N HCl

100 mg of FLZ was dissolved in 0.1N HCl in the 100 ml volumetric flask a series of concentration, 80 µg/ml, 120 µg/ml, 160 µg/ml, 200 µg/ml, 240 µg/ml, 280 µg/ml, 320 µg/ml, 360 µg/ml and 400 µg/ml of FLZ were prepared and absorbance was measured at 260 nm.

Compatibility studies

Both the drugs were uniformly mixed with dry powdered KBr in the ratio of 1:100 and the mixture was then compressed into the transparent disc under high pressure using special dies. The disc was placed in the FTIR spectrophotometer and the spectrum was recorded [11, 12].

Development and validation of simultaneous estimation method for TH and FLZ by UV-visible spectrophotometer

Q-Analysis method was developed and validated for the simultaneous estimation of TH and FLZ by UV-visible spectrophotometer

Standard stock solutions of TH and FLZ

Accurately weighed 100 mg of TH was dissolved in 0.1N HCl in 100 ml volumetric flask. The solution was sonicated for 10 min and the final volume was made up with the same solvent. From the standard stock solution, pipetting out 1 ml and dissolved it with 0.1N HCl to prepare the working standard solution of 100 µg/ml. Then, a series of concentrations were prepared using 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, and 3.0 ml working standard solution in 10 ml volumetric flasks.

The FLZ standard solution was prepared by weighed 100 mg of drug and dissolved in 0.1N HCl in 100 ml volumetric flask for 10 min sonication and make up the final volume. From the above solution 0.8 ml, 1.2 ml, 1.6 ml, 2.0 ml, 2.4 ml, 2.8 ml, 3.2 ml, 3.6 ml, and 4.0 ml were pipetted out in series of 10 ml volumetric flask and volume was made up with 0.1N HCl.

Selection of wavelength of maximum absorbance (λ_{max})

3.0 µg/ml solution was prepared from the working stock solution of TH in a 10 ml volumetric flask. For FLZ, 400 µg/ml solution was prepared by a standard stock solution of FLZ in a 10 ml volumetric flask. Both the solutions were scanned in 400-200 nm against 0.1N HCl as blank using a UV-visible spectrophotometer.

Determination of iso-absorptive point

1.5 µg/ml solution of TH and 200 µg/ml of FLZ and scanning in spectrum mode in the range of 400-200 nm. An overlain spectrum of TH and FLZ was found and an iso-absorptive point was determined [13, 14].

Linearity range

Linearity of TH and FLZ was determined by preparing different concentrations i.e. 0.5 µg/ml, 1.0 µg/ml, 1.5 µg/ml, 2.0 µg/ml, 2.5 µg/ml and 3.0 µg/ml for TH and 160 µg/ml, 200 µg/ml, 240 µg/ml, 300 µg/ml, 360 µg/ml and 400 µg/ml for FLZ from their working standard solution and absorbance was measured at 222 nm and 239 nm [15].

Accuracy

2.0 µg/ml, 2.5 µg/ml, 3.0 µg/ml and 240 µg/ml, 300 µg/ml, 360 µg/ml solutions were prepared for TH and FLZ, respectively. These were the three different levels 80 %, 100 %, and 120 % by considering 2.5 µg/ml and 300 µg/ml as 100 % for TH and FLZ, respectively. The recovery study was performed three times at each level [16].

Intra-day and inter-day precision

The intra-day and the inter-day precision study was performed by preparing the dilution of 2.0 µg/ml, 2.5 µg/ml, 3.0 µg/ml for TH and 240 µg/ml, 300 µg/ml and 360 µg/ml for FLZ and from the respective stock solutions for both the drugs, respectively and recording their absorbance at 222 nm and 239 nm against 0.1N HCl as a blank. The absorbance of these concentrations was measured three times a day at regular intervals of one hour for intra-day and day1, day 2, and day 3 for inter-day study [17, 18].

Repeatability

3.0 µg/ml and 240 µg/ml of concentrations were prepared from the stock solution of TH and FLZ, and absorbance was measured at 222 nm and 239 nm against 0.1N HCl as a blank. The standard deviation and relative standard deviation was calculated [19].

Reproducibility

2.0 µg/ml and 300 µg/ml solutions were prepared separately from stock solutions of TH and FLZ and analyzed by one analyst (analyst1) at 222 nm and 239 nm. The same solutions were prepared by another analyst (analyst2) and values obtained were evaluated using a t-test to verify their reproducibility [20, 21].

Limit of detection (LOD)

The limit of detection was calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined and calculated by the following formula.

$$\mathrm{LOD} = 3.3\,{}^{S}/_{b}$$

Where S is the standard deviation of the residuals around the regression line.

b is the slope of the regression line.

Limit of quantitation (LOQ)

The LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of the test [22-24]. The limit of quantitation can be calculated from the standard deviation of the response and the slope.

$$\mathrm{LOQ} = 10\,{}^{S}/_{b}$$

Where S is the standard deviation of the residuals around the regression line.

b is the slope of the regression line.

RESULTS AND DISCUSSION

Preformulation studies

Determination of λ_{max} and preparation of calibration curve

Determination of absorption maxima (λ_{max}) of TH and FLZ in 0.1N HCl

The absorption maxima of TH and FLZ were found at 222 nm and 260 nm respectively (fig. 2). The correlation coefficient, intercept, and slope for the calibration data for TH and FLZ also were calculated (table 1).

Compatibility study of TH and FLZ

For respective functional groups, the important peaks of TH and FLZ were present in the spectrum (fig. 4 and table 2). It concludes that the TH and FLZ are compatible with each other.

Development and validation of simultaneous estimation method of TH and FLZ by UV-visible spectrophotometer

Selection of wavelength of maximum absorbance (λ_{max}) wavelength

TH and FLZ showed wavelength maximums at 222 nm and 260 nm in 0.1N HCl respectively as shown in fig. 2.

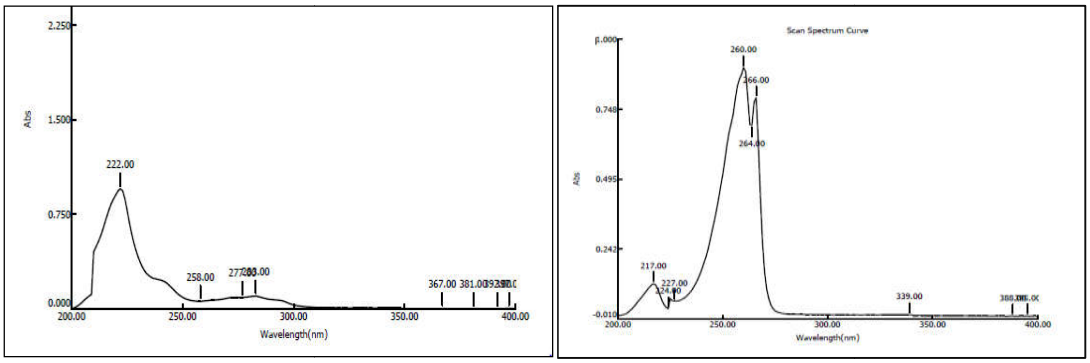
Determination of iso-absorptive point

1.5 µg/ml solution of TH and 200 µg/ml of FLZ were scanned in spectrum mode 200-400 nm and the overlain spectrum was found and it shows an iso-absorptive point at 239 nm as shown in fig. 5.

Results of validation parameters of simultaneous estimation method of TH and FLZ by UV-visible spectrophotometer

Linearity curve

The linearity curve for TH was constructed by plotting concentration versus absorbance (fig. 6). The linearity of TH was found in a concentration range of 0.5-3.0 µg/ml at 222 nm and 239 nm. The linearity of FLZ was found in the concentration range 160-400 µg/ml at 222 nm and 239 nm. A linear graph was obtained between absorbance and concentration in the range given in table 3.



(a) **(b)**

Fig. 2: UV spectra of (a) TH (b) FLZ in 0.1N HCl

Table 1: Calibration curve data of TH and FLZ in 0.1N HCl

S. No.	Concentration (µg/ml) TH	Absorbance	Concentration (µg/ml) FLZ	Absorbance
1	0	0	0	0
2	0.5	0.178±0.0005	80	0.165±0.0024
3	1.0	0.345±0.0005	120	0.243±0.0010
4	1.5	0.498±0.0008	160	0.318±0.0014
5	2.0	0.661±0.0005	200	0.396±0.0013
6	2.5	0.802±0.0006	240	0.475±0.0018
7	3.0	0.972±0.0008	280	0.569±0.0007
8			320	0.650±0.0010
9			360	0.720±0.0008
10			400	0.778±0.0017

*mean±SD; n=3, SD=standard deviation, TH=terbinafine hydrochloride, FLZ=fluconazole

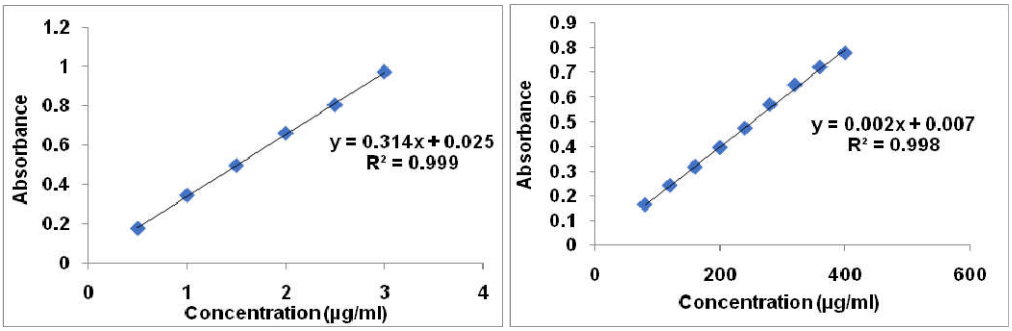


Fig. 3: Calibration curve of TH (left) and FLZ (right) in 0.1N HCl

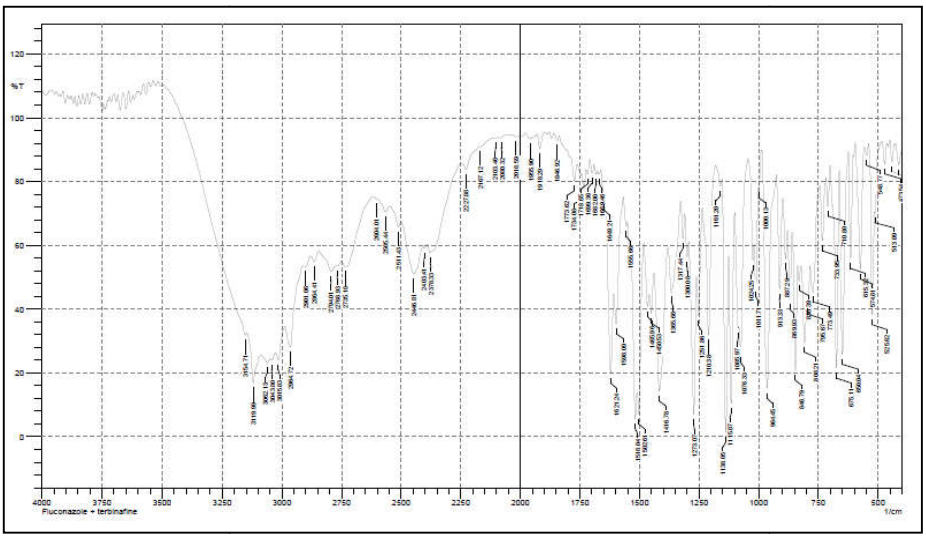


Fig. 4: FTIR spectra of a mixture of TH and FLZ

Table 2: Interpretation of FTIR spectra of a mixture of TH and FLZ

Functional groups	Standard values (cm^{-1})	Observed vales (cm^{-1})
-OH stretching	3200-2800	3119.99
-SH stretching	2800-2400	2446.81
-CN stretching	2400-2000	2227.88
-C=O stretching	1800-1600	1621.24
-CH bending	1600-1400	1465.96
-COOH stretching	1400-1200	1365.66
-S=O stretching	1200-1000	1076.33
-CH bending	1000-800	808.21
-C-Cl stretching	800-600	773.21
-OH stretching	3320.96	2964.72
-C-F stretching	1209.42	1210.38
-C=N stretching	1620.00	1621.24

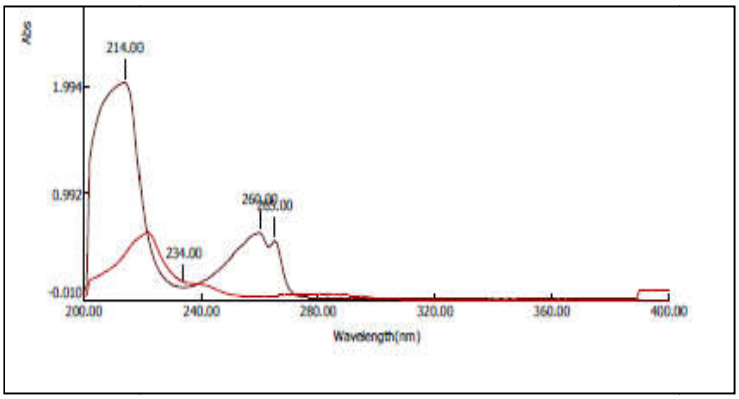


Fig. 5: Overlain spectra of TH and FLZ in 0.1N HCl

Table 3: Linearity data of TH and FLZ at 222 nm, 239 nm, and in 0.1N HCl

Conc. (µg/ml) TH	Conc. (µg/ml) FLZ	Absorbance at 222 nm TH	Absorbance at 239 nm	Absorbance at 222 nm FLZ	Absorbance at 239 nm
0.5	160	0.178±0.0020	0.035±0.0010	0.265±0.0005	0.065±0.0012
1.0	200	0.345±0.0010	0.077±0.0015	0.334±0.0012	0.082±0.0012
1.5	240	0.498±0.0010	0.102±0.0006	0.431±.0010	0.105±0.0010
2.0	300	0.661±0.0020	0.140±0.0012	0.524±0.0005	0.126±0.0005
2.5	360	0.802±0.0010	0.178±0.0010	0.628±0.0015	0.154±0.0011
3.0	400	0.972±0.0010	0.217±0.0012	0.717±0.0012	0.176±0.0015

*mean±SD; n=3, SD=standard deviation, TH=terbinafine hydrochloride, FLZ=fluconazole

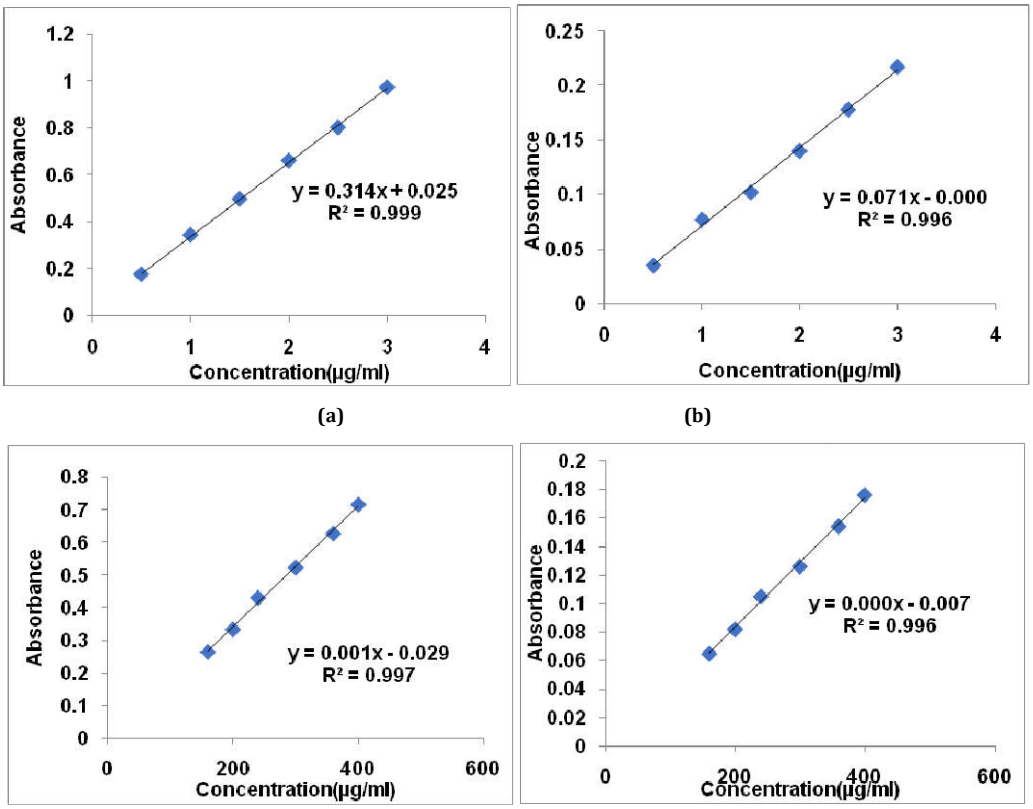


Fig. 6: Linearity curve of TH and FLZ at 222 nm and 239 nm in 0.1N HCl by Q-Analysis method, (a) TH at 222 nm (b) TH at 239 nm (c) FLZ at 222 nm (d) FLZ at 239 nm

Accuracy

Accuracy results obtained in table 4 showed excellent mean recovery percent values, close to 100 %, and low standard deviation values (SD<1.0) which indicate high accuracy of the proposed analytical methods. Percent recovery of TH was found in between of 98.50 %-103.96 % and FLZ it was found between 97.27 %-103.83 % in all recovery level and both wavelengths.

Table 4: Result of accuracy of TH at 222 nm and 239 nm by Q-Analysis method

% recover level	Conc. of drug added	Abs	Mean	% RSD	% Recov	Abs	Mean	% RSD	% Recov
TH	(µg/ml)	TH at 222 nm				TH at 239 nm			
		0.605				0.138			98.50%
80%	2.0	0.607	0.608±0.0360	0.592	103.40%	0.139	0.139±0.0010	0.719	
		0.612				0.140			
		0.733				0.176	0.176±0.0006	0.341	100%
100%	2.5	0.739	0.735±0.0032	0.435	100%	0.177			
		0.734				0.176			
		0.915				0.212	0.211±0.0017	0.805	99.90%
120%	3.0	0.916	0.917±0.0026	0.283	103.96%	0.212			
		0.920				0.209			
FLZ	(µg/ml)	FLZ at 222 nm				FLZ at 239 nm			
	240	0.429	0.430±0.0010	0.233	103.36%	0.101	0.102±0.0006	0.588	101.19%
80%		0.430				0.102			
		0.431				0.102			
	300	0.519	0.520±0.0015	0.288	100%	0.125	0.126±0.0010	0.769	100%
100%		0.522				0.126			
		0.520				0.127			
	360	0.607	0.607±0.0025	0.412	97.27%	0.159	0.157±0.0015	0.955	103.83%
120%		0.605				0.157			
		0.610				0.156			

*mean±SD, n=3, SD=standard deviation, %Recov =percent recovery, Abs=absorbance, conc=concentration, %RSD=relative standard deviation, TH=terbinafine hydrochloride, FLZ=fluconazole

Intermediate precision (Intra-day and inter-day precision)

Intra-day and inter-day precision were assessed using triplicate analysis of three different concentrations. The calculated relative standard deviation values were found to be very small below 2 % indicating good repeatability and reliability of the proposed methods. The results and their statistical analysis are summarized in table 5.

Table 5: Intraday and interday precision of TH and FLZ at 222 nm and 239 nm

Conc. (µg/ml)	Intra-day	% RSD	Inter-day	% RSD	Intra-day	% RSD	Inter-day	% RSD
TH	Absorbance at 222 nm				Absorbance at 239 nm			
2.0	0.646±0.0017	0.216	0.641±0.0031	0.483	0.146±0.0012	0.822	0.144±0.0010	0.694
2.5	0.790±0.0045	0.569	0.790±0.0015	0.189	0.181±0.0012	0.662	0.182±0.0035	0.019
3.0	0.911±0.0064	0.702	0.917±0.0031	0.338	0.216±0.0006	0.277	0.217±0.0010	0.555
FLZ	Absorbance at 222 nm				Absorbance at 239 nm			
240	0.450±0.0023	0.511	0.454±0.0021	0.465	0.104±0.0006	0.576	0.108±0.0006	0.555
300	0.562±0.0015	0.267	0.556±0.0010	0.180	0.129±0.00058	0.387	0.127±0.0012	0.945
360	0.658±0.0023	0.349	0.656±0.0015	0.229	0.154±0.0012	0.779	0.152±0.0010	0.658

*mean±SD; n=3, SD=standard deviation, %RSD=relative standard deviation, conc=concentration, TH=terbinafine hydrochloride, FLZ=fluconazole

Repeatability

The repeatability result indicates the precision under the same operating conditions over a short interval of time and % RSD was found less than 2 % which indicates good repeatability. % RSD was in the range of 0.016-0.0193 %, which were less than the standard value and show significant results (table 6).

Reproducibility

For the proposed method, the t-value found from the t-test indicates good reproducibility, and results are shown in table 7. The T-value of both the drugs was 0.8857, 0.4000 at 222 nm, and 239 nm in TH, wherein FLZ was 0.8000, 0.5066 respectively. Both the drug shows no significant difference.

Table 6: Repeatability data for TH and FLZ at 222 nm, 239 nm

Drugs conc	TH		FLZ	
S. No.	3 µg/ml	3 µg/ml	300 µg/ml	300 µg/ml
	Abs at 222 nm	Abs at 239 nm	Abs at 222 nm	Abs at 239 nm
1	0.948	0.217	0.561	0.129
2	0.914	0.219	0.562	0.129
3	0.915	0.218	0.564	0.128
4	0.904	0.209	0.564	0.128
5	0.920	0.221	0.560	0.128
6	0.916	0.219	0.557	0.125
Mean	0.918±0.015	0.217±0.0042	0.561±0.0027	0.128±0.0015
% RSD	0.016	0.0193	0.481	0.0171

*mean±SD; n= 6, SD= standard deviation, %RSD= relative standard deviation, Conc= concentration, Abs= absorbance, TH= terbinafine hydrochloride, FLZ=fluconazole

Table 7: Reproducibility data for TH at 222 nm by Q-Analysis method

Analyst 1	Analyst 2	t-value	Inference	Analyst 1	Analyst 2	t-value	Inference
TH at 222 nm (2.0 µg/ml)				TH at 239 nm (2.0 µg/ml)			
0.643±0.0015	0.646±0.0030	0.8857	No significant difference	0.145±0.038	0.149±0.0035	0.4000	No significant difference
FLZ at 222 nm (300 µg/ml)				FLZ at 239 nm (300 µg/ml)			
0.566±0.0020	0.565±0.0040	0.8000	No significant difference	0.126±0.0025	0.128±0.0026	0.5066	No significant difference

*mean±SD; n=3, SD=standard deviation, TH=terbinafine hydrochloride, FLZ=fluconazole

Table 8: LOD and LOQ for TH and FLZ at 222 nm and 239 nm

Wavelength	222 nm	239 nm	222 nm	239 nm
Drugs	TH		FLZ	
LOD (µg/ml)	0.067	0.175	31.089	94.380
LOQ (µg/ml)	0.203	0.531	94.210	286.00
Mean of Residual values	0.00020	0.000074	0.001619	0.001034

*LOD=limit of detection, LOQ=limit of quantification, TH=terbinafine hydrochloride, FLZ=fluconazole

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection and limit of quantitation was calculated from the linearity curve of TH and FLZ at 222 nm and 239 nm. LOD and LOQ of TH were found to be 0.067, 0.203 at 222 nm and 0.175, 0.531 at 239 nm, similarly for FLZ; 31.089, 94.210 at 222 nm and 94.380, 286.00 at 239 nm respectively (table 8).

DISCUSSION

A new, simple, precise, accurate, and reproducible method or simultaneous equation method was developed and validated for the simultaneous estimation of terbinafine hydrochloride (TH) and fluconazole (FLZ) in pure form. The absorption maxima of TH and FLZ were found at 222 nm and 260 nm respectively. It shows an iso-

absorptive point at 239 nm. Compatibility studies show that both the drugs were compatible with each other. The linearity of TH was found in a concentration range of 0.5-3.0 µg/ml at 222 nm and 239 nm and the linearity of FLZ was found in the concentration range 160-400 µg/ml at 222 nm and 239 nm. Percent recovery of TH was found in between of 98.50 %-103.96 % and FLZ it was found between 97.27 %-103.83 % in all recovery level and both wavelengths. Percent recovery of TH and FLZ at all levels were found within the specific limit, which revealed that the method is accurate. The repeatability result indicates the precision under the same operating conditions over a short interval of time and % RSD was found less than 2 % which indicates good repeatability. Interday and intraday precision studies were also within the specific limits, which depicts that method is correct. The T-value of both the drugs was 0.8857, 0.4000 at 222 nm, and 239 nm in TH, wherein FLZ was 0.8000, 0.5066 respectively [23]. Both the drug shows no significant difference. LOD and LOQ of TH were found to be 0.067, 0.203 at 222 nm and 0.175, 0.531 at 239 nm, similarly for FLZ; 31.089, 94.210 at 222 nm and 94.380, 286.00 at 239 nm respectively [25]. All the parameters were done as per the ICH guidelines.

CONCLUSION

Simultaneous estimation of fluconazole and terbinafine hydrochloride was developed and validated by UV-visible spectroscopy. All the statically analyses were within the standard limits. It proves that the method was repeatable and selective for the simultaneous estimation of TH and FLZ in pure and that it can be successfully used for simultaneous estimation of TH and FLZ in pharmaceutical dosage forms. The developed method was found to be simple, precise, specific, and accurate.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the author has contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Grant SM, Clissold SP. Fluconazole a review of its pharmacodynamics and pharmacokinetics properties and therapeutic potential in superficial and systemic mycoses. Drugs 1990;39:877-16.
2. Sander CS, Hipler UC, Wollina U, Elsner P. Inhibitory effect of terbinafine on reactive oxygen species (ROS) generation by candida albicans. Mycoses 2002;45:152-5.
3. Balfour JA, Faulds D Terbinafine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial mycoses. Drugs 1992;43:259-84.
4. Krishnan Natesan S. Terbinafine: a pharmacological and clinical review. Expert Opin Pharmacother 2009;10:2723-33.
5. Hollier LM, Cox SM. Fluconazole (Diflucan). Infect Dis Obstet Gynecol 1995;3:222-5.
6. Mikamo H, Matsumizu M, Nakazuru Y, Okayama A, Nagashima M, *et al.* Efficacy and safety of a single oral 150 mg dose of fluconazole for the treatment of vulvovaginal candidiasis in Japan. J Infect Chemother 2015;21:520-6.
7. Zervos M, Meunier F. Fluconazole (Diflucan): a review. Int J Antimicrob Agents 1993;3:147-70.
8. Indian Pharmacopoeia. Vol. II. Government of India, Ministry of health and family welfare, The Indian Pharmacopoeia Commission, Ghaziabad Publication; 2010.
9. Lathiya Tushar, D Akhilesh, P Prabhakara, Kamath JV. Preformulation studies of controlled/sustained release formulations: an overview. Int Res J Pharm 2012;3:95-9.
10. Dewangan A, Tomar B, Mehta P, Singhai AK. Development and evaluation of terbinafine hydrochloride for tablet formulation. Int J Pharm Bio Sci 2013;1:36-52.
11. Charoo N, Cristofoletti R, Graham A, Lartey P, Abrahamsson B, Groot DW, *et al.* Biowaiver monograph for immediate-release solid oral dosage forms: fluconazole. J Pharm Sci 2014;103:3843-58.
12. Das S, Samanta A, Bose A. Design, development and evaluation of fluconazole topical gel. Asian J Pharm Clin Res 2015;8:132-5.
13. Attia KAM, El-Abasawi NM, El-Olemy A, Abdelazim AH. Application of different spectrophotometric methods for simultaneous determination of elbasvir and grazoprevir in pharmaceutical preparation. Spectrochim Acta A Mol Biomol Spectrosc 2018;189:154-60.
14. Abdel Halim LM, Abd El Rahman MK, Ramadan NK, El Sanabary HF, Salem MY. Comparative study between recent methods manipulating ratio spectra and classical methods based on two-wavelength selection for the determination of binary mixture of antazoline hydrochloride and tetryzoline hydrochloride. Spectrochim Acta A Mol Biomol Spectrosc 2016;159:98-105.
15. Rote AR, Kumbhoje PA, Bhambar RS. UV-visible spectrophotometric simultaneous estimation of paracetamol and nabumetone by AUC method in combined tablet dosage form. Pharm Methods 2012;3:40-3.
16. Ilango K, Kumar PS. Validated spectrophotometric methods for the simultaneous determination of telmisartan and atorvastatin in bulk and tablets. Pharm Methods 2012;3:112-6.
17. ICH Q2 (R1). Validation of analytical procedures: text and methodology. International Conference on Harmonization, IFPMA, Geneva; 2005.
18. Sawant RL, Hadawale SD, Dhikale GK, Bansode CA, Tajane PS. Spectrophotometric methods for simultaneous estimation of rabeprazole sodium and aceclofenac from the combined capsule dosage form. Pharm Methods 2011;2:193-7.
19. Shawna Rekshmy D'dharan, Ganapathy D. Medical management of denture stomatitis. Asian J Pharm Clin Res 2016;9:14-6.
20. Attimarad M, Narayanswamy VK, Aldhubaib BE, SreeHarsha N, Nair AB. Development of UV spectrophotometry methods for concurrent quantification of amlodipine and celecoxib by manipulation of ratio spectra in pure and pharmaceutical formulation. PloS One Vol 2019;14:e0222526.
21. Wadher SJ, Pathankar PR, Puranik M, Ganjiwale RO, Yeole PG. Simultaneous spectrophotometric estimation of paracetamol and metoclopramide hydrochloride in solid dosage form. Indian J Pharm Sci 2008;70:393-5.
22. Validation of analytical procedures: Text and methodology Q2 (R1). ICH Harmonised Tripartite Guideline; 2005. p. 1-13.
23. Venkatachalam T, Lalitha KG. Spectrophotometric methods for simultaneous estimation of melatonin and zolpidem from the combined tablet dosage form. Pharmacophore 2014;5:252-7.
24. Pandey G, Mishra B. A new analytical Q-absorbance ratio method-development and validation for simultaneous estimation method for lamivudine and isoniazid. Int Scholarly Res Not 2013;1:1-5.
25. Pandey S, Pandey P, Dubey S, Chaturvedi U, Rai K Awani. Facile derivative UV spectroscopy method: simultaneous estimation of tinidazole and fluconazole in combined tablet dosage form. Thai J Pharm Sci 2012;36:55-62.