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Development and Validation of UV Spectrophotometric Methods for the Simultaneous Estimation of Itraconazole and Terbinafine Hydrochloride in Bulk Drug and Pharmaceutical Formulations

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Manzoor Ahmed, Sanjana N R*, Satishkumar Shetty A

Department of Pharmaceutical Analysis, National College of Pharmacy, Balraj Urs Road Shimoga-577201, Karnataka, India.

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ABSTRACT

In the present work two simple, precise, accurate, and economical spectrophotometric methods have been developed for the simultaneous estimation of Itraconazole and Terbinafine Hydrochloride in bulk drug and pharmaceutical dosage forms by using ethanol as a solvent. Method A is a Second-order derivative method, which involves the measurement of absorbances at two selected wavelengths 262 nm and 283 nm for the estimation of Itraconazole and Terbinafine Hydrochloride respectively. Method B is a Q Absorbance ratio method is based on the measurement of absorbances at two selected wavelengths 270nm (Isosbestic point) and 283 nm for the estimation of Itraconazole and Terbinafine Hydrochloride respectively. Beer's law was obeyed in the concentration range of 2-50 µg/mL for Itraconazole and 5-80 µg/mL for Terbinafine Hydrochloride. Working concentrations used in the range of 3-15µg/mL and 7.5-37.5µg/mL for of Itraconazole and Terbinafine Hydrochloride respectively. The% RSD for intra-day and inter-day precision was within 2% for both the methods.

INTRODUCTION

Itraconazole [2-4] is an antifungal drug, used to treat fungal infections of the toe and nails. Itraconazole oral solution is used to treat yeast infections of the mouth and throat or the oesophagus. Itraconazole is in a class of antifungals called triazoles. Chemically it is (±)-1-sec-Butyl-4-{4-[4-(4-{[(cis-2- (2,4-dichlorophenyl)-2-(1,2,4-triazol-1-ylmetyl)-1,3-dioxolan 4yl]methoxy} phenyl)piperazin-1-yl]phenyl}-4,5-dihydro-1,2,4-triazol-5-on[1] . Itraconazole interacts with 14- α demethylase, a cytochrome p-450 enzyme necessary to convert lanosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents. Itraconazole may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the transformation of yeasts to mycelial forms, inhibit purine uptake, and impair triglyceride and /phospholipid biosynthesis[2,3].

Terbinafine Hydrochloride [5-8] is an allylamine antifungal agent, it is mainly effective on the dermatophyte group fungi. **(**E)-N-(6,6-dimethyl -2-hepten -4ynyl) N-methyl-1-napthalene methanamine[4]. It is used topically for superficial skin infection such as jock itch, athlete'foot, and ringworm. Used to the treatment of onychomycosis, fungal nail infection. Terbinafine is hypothesized to act by inhibiting squalene monooxygenase [5], thus blocking the biosynthesis of ergosterol, an essential component of fungal cell membranes. This inhibition also results in an accumulation of squalene, which is a substrate catalyzed to 2,3-oxydo squalene by squalene monooxygenase. The resultant concentration of squalene and decreased amount of ergosterol are both thought to contribute to terbinafine antifungal activity [6].

The combination of Itraconazole and Terbinafine Hydrochloride is used for onychomycosis, oral antifungals, ringworm, skin infection [7].

On the Literature survey, it was found that no method has been reported for the simultaneous estimation of Itraconazole and Terbinafine Hydrochloride in combined dosage forms and no method is available in the pharmacopeias. Few analytical methods have been developed for the determination of Itraconazole and Terbinafine Hydrochloride individually and in combination with other drugs[8-12]. Hence in the view of the need for a suitable method for routine analysis in combined formulations, attempts were made to develop simple, precise, and accurate spectroscopic methods for simultaneous estimation of titled drugs and extend it

for their determination in pharmaceutical formulations. The present UV-Spectrophotometric methods were validated according to (ICH) guidelines[13,14].

MATERIALS AND METHODS:

INSTRUMENT

For UV-visible Spectroscopy methods, Shimadzu model 1800 double beam UV-visible Spectrophotometer with a spectral bandwidth of 1 ± 0.2 nm, wavelength accuracy of ± 0.3 nm and a pair of quartz cuvettes having 1cm path length was used.

CHEMICALS AND REAGENTS

Ethanol.

DRUG SAMPLE

Standard Itraconazole and Terbinafine Hydrochloride were obtained as gift samples from Anvik Biotech, Sonipat, Haryana.

METHODS:

Preparation of Standard solutions.

100 mg of Itraconazole and 100 mg Terbinafine Hydrochloride was weighed and transferred into two different 100 ml volumetric flask. Both the drugs were dissolved in 70 ml of ethanol by ultra-sonication and then the volume was made up-to-the-mark with ethanol to obtain the final concentration of 1000µg/ml (Stock A and A' solution).

From the above Stock A and A' solution 10ml of aliquot was pipetted out into two different 100 ml volumetric flask and volume were made up-to-the-mark with the ethanol to obtain a concentration of 100µg/ml (Stock B and B' solution).

From the above Stock B and B' solution further dilution was made to get concentration from 3-15µg/ml of Itraconazole and 7.5-37.5µg/ml of Terbinafine Hydrochloride.

Preparation of Sample solutions

Commercially available formulation TERBOL-IT was purchased which contains 250mg of Terbinafine Hydrochloride and 100mg of Itraconazole. From this formulation, 100mg of drug equivalent to Terbinafine Hydrochloride was taken which also contains 40mg of Itraconazole and transferred to 100 mL volumetric flask, dissolved in 70 mL of ethanol and the contents were kept in a sonicator for 15min. The solution was filtered through Whatman filter paper No.41, finally, the volume was made up-to-the-mark with ethanol to get a concentration of 1000 µg/mL of ITRA and 400 µg/mL of TERB and this solution was used as stock "A" solution of the sample.

From the above stock "A" solution, 10 mL of the aliquot was pipetted out and transferred to a 100 mL volumetric flask. The volume was made up to 100 mL with distilled ethanol to obtain a concentration of 100 µg/mL of TERB and 40µg/mL of ITRA (stock "B" solution of the sample).

Appropriate aliquots were pipetted out from the sample stock "B" solution into a series of 10 mL volumetric flasks. The volume was made up to the mark with the ethanol to get a set of solutions having the concentration range of 3, 6, 9, 12, and 15 µg/mL of ITRA and 7.5, 15, 22.5, 30 and 37.5 µg/mL of TERB.

METHODOLOGY

Method A (Second order derivative method)

The second-order derivative method involves the measurement of absorbances at selected wavelengths. i.e. 262nm and 283nm for the estimation of Itraconazole and Terbinafine Hydrochloride respectively.

The standard solutions of both the drugs were scanned in the spectrum mode from 400-200nm using UV-Spectrophotometer. These spectrums were converted to Second order derivative spectra by using derivative mode in UV probe software. The absorbance spectra, thus obtained were derivatized to remove the interference of absorbing species. The two wavelengths selected should be such that at each wavelength the absorbance difference between the components should be as large as possible. From the examination of the Second-

order derivative spectra of Itraconazole and Terbinafine Hydrochloride 262 nm and 283 nm were selected as working wavelengths for the second-order derivative spectroscopy.

Method B (Q-Analysis or Absorbance ratio method)

The absorbance ratio method is based on the simultaneous equation procedure. It depends on the property of the substance, which obeys Beer's law at all wavelengths, the ratio of absorbance at any wavelength is a constant value independent of concentration or wavelength. This ratio is also referred to as a **Q-value.** In the quantitative assay of two components in admixture by the absorbance ratio method, absorbances were measured at two wavelengths. One being the λ_{max} of one of the components (λ_2) and other being a wavelength of equal absorptivity of the two components (λ_1), i.e. an iso-absorptivity point (Pernarowski1961).

From the above Stock solutions, both the drugs are scanned in the wavelength range of 400-200nm using UV-Spectrophotometer. With the help of an overlay spectrum absorbance of the solutions was measured at 283.0 nm (λmax of TERB) and 270.0nm (Isoabsorptive point).

Validation of the methods

Both methods were validated according to ICH guidelines by carrying out an analysis of six replicate samples. Recovery studies were carried out at three different levels i.e., 80%, 100%, and 120% by adding the pure drug to the previously analyzed sample. From the amount of the drug found, percentage recovery was calculated.

RESULTS AND DISCUSSION

The estimation of ITRA and TERB in bulk and the pharmaceutical formulation was found to be accurate and reproducible with a linearity range of 3-15 µg/ml and 7.5-37.5 µg/ml respectively for both the methods and the correlation coefficient was found to be 0.9999 and 0.9999 for the method A and 0.9998 and 0.9999 for the method B respectively. The optical characteristics such as linearity range, molar absorptivity, percentage relative standard deviation of recovery studies, and precision in each method were calculated and the results were reported in Table 1 for both the methods. Also, the regression characteristics like slope (m), intercept (c), and correlation coefficient (r^2) were calculated and are presented in Table 1 for both the methods. The accuracy was found by recovery studies at three different levels i.e.

80%, 100%, and 120%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The % RSD value was less than 2, indicative of the accuracy of the methods. The results for formulation were reported in Table 2. The absorption spectra of Itraconazole, Terbinafine Hydrochloride and formulation by Second order derivative method were shown in (Fig. 3) and the calibration curve was shown in (Fig. 4, 5).

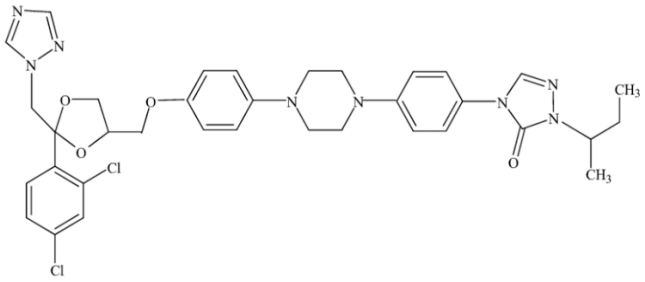

Figure No. 1: Chemical structure of Itraconazole

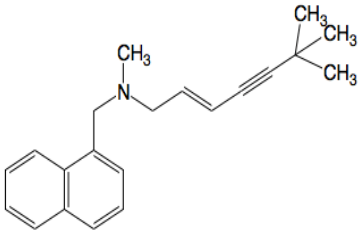

Figure No. 2: Chemical structure of Terbinafine

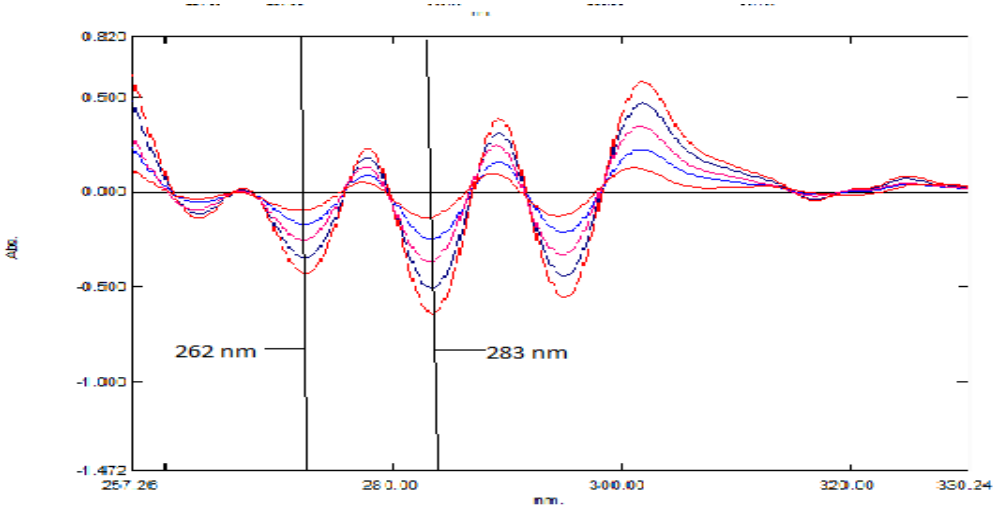

Figure No. 3: Overlay Spectrum of Mixture in ethanol

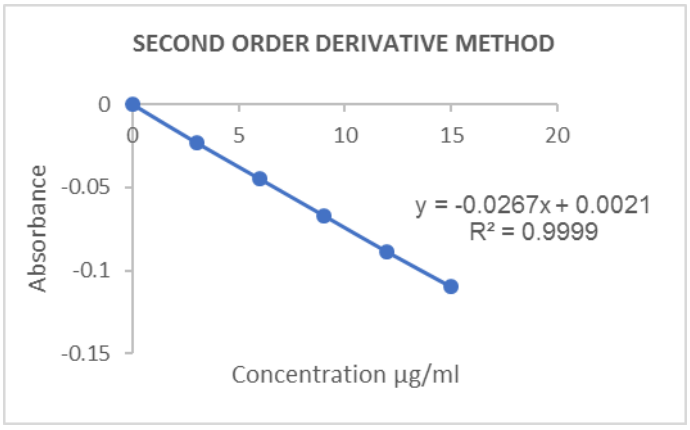

Figure No. 4: Calibration curve for ITRA at 262.0 nm

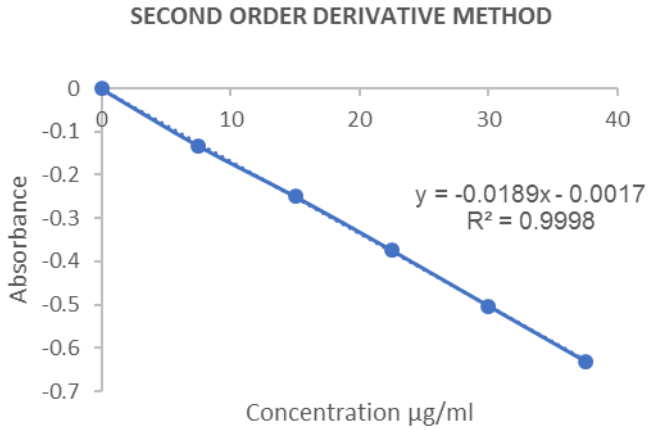

Figure No. 5: Calibration curve for TERB at 283.0 nm

The spectra of Itraconazole and Terbinafine Hydrochloride were reported by Q-Absorbance ratio method (Fig. 6) and the calibration curve was plotted (Fig. 7, 8).

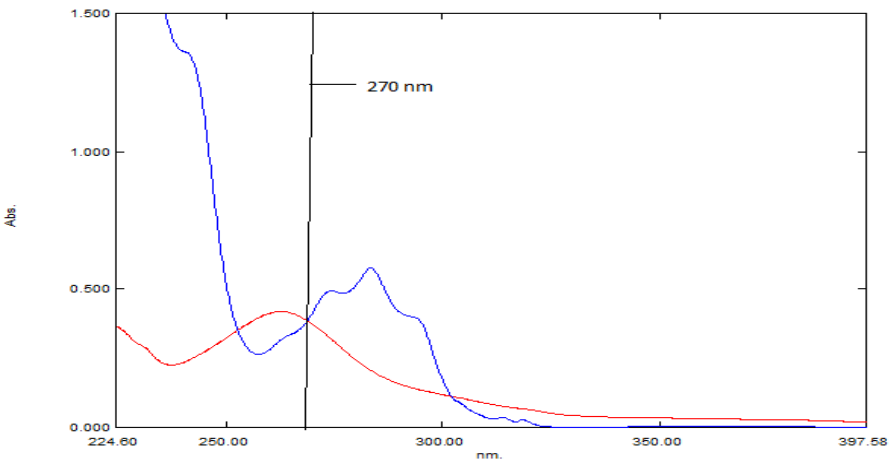

Figure No. 6: Isoabsorptive point of ITRA and TERB.

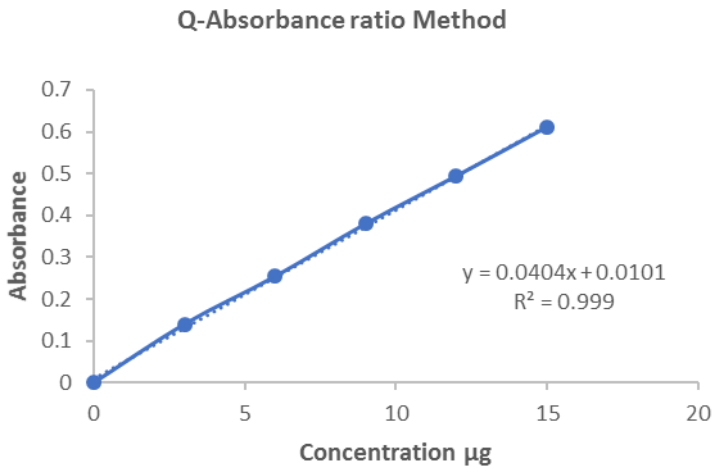

Figure No. 7: Calibration curve for ITR at 270.0 nm

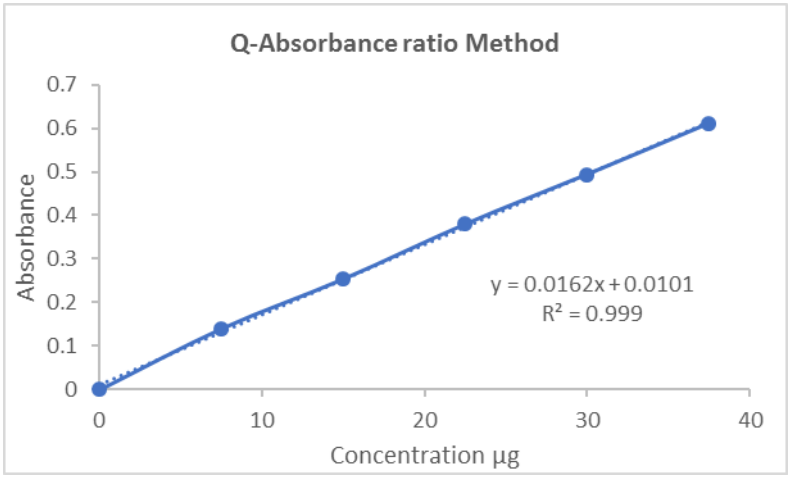

Figure No. 8: Calibration curve for TERB at 283.0 nm

Table No. 1: Optical and validation parameters of UV Spectrophotometric methods.

UV Spectroscopy		Third Order Derivative Method		Q Absorbance Ratio Method	
Parameters		**ITRA**	**TERB**	**ITRA**	**TERB**
Linearity range (µg/ml)		3-15	7.5-37.5	3-15	7.5-37.5
λmax / wavelength range (nm)		262	283	270	283
Coefficient of correlation		0.9998	0.9999	0.9999	0.9999
Slope*(m)		0.0267x	0.0018x	0.0404x	0.0162x
Intercept*(c)		0.002	0.0017	0.0101	0.0101
Accuracy (% RSD)	**80%**	0.4943	0.2425	0.2253	0.2558
	100%	0.1358	0.2669	0.6699	0.2832
	120%	0.0346	0.2699	0.1020	0.1224
Precision (% RSD)	**Intra-day**	1.2083	0.3063	1.2083	0.3063
	Inter-day	0.9297	0.2832	0.9297	0.2832
Limit of Detection (µg/ml)		0.1562	0.2818	0.1771	0.2165
Limit of Quantification (µg/ml)		0.4732	0.8541	0.5366	0.6562

Table No. 2: Results of formulation

Method	Brand name	Label claim (mg)		%Recovery*		%RSD*		Standard Error*	
		ITRA	**TERB**	**ITRA**	**TERB**	**ITRA**	**TERB**	**ITRA**	**TERB**
A	**TERBOL-IT**	100	250	99.95	99.91	1.2083	0.3063	0.0031	0.0030
B		100	250	99.80	99.93	1.2083	0.3063	0.0029	0.0041

CONCLUSION

The developed Second order derivative and Q-Absorbance ratio methods were found to be simple, precise, specific, and accurate and can be used for routine analysis of Itraconazole and Terbinafine Hydrochloride. Both the methods were validated as per ICH guideline

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