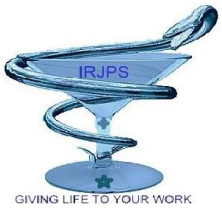
Original Research



VALIDATED UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF TOLNAFTATE

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ABSTRACT

A new UV Spectrophotometric method was developed for determination of tolnaftate in bulk drug and pharmaceutical formulaton. Tolnaftate, an antifungal drug of thiocarbamate class, is used topically in 1% topical formulations as fungicide. Though its exact mechanism is unknown, it is believed to prevent ergosterol biosynthesis by inhibiting squalene epoxidase. It has also been reported to distort the hyphae and stunt mycelial growth in susceptible organisms. The spectra of Tolnaftate in Acetonitrile showed maximum wave length at 258nm and obeyed Beer's law in the concentration range of 2-12 µg/ml. Standard curve depict line equation y = 0.1633x + 0.0218 with correlation coefficient of 0.9992 (shown in Table 1 and Figure 1). The developed method was validated with respect to linearity, precision (method precision, intermediate or inter-day precision), accuracy, stability in analytical solution, robustness or system suitability. The developed method exhibited the best results in terms of the aforesaid validation parameters. The other components and additives did not interfere in their determinations. The objective of present work is to develop simple, precise and accurate UV Spectrophotometric method for estimation of Tolnaftate in organic solvent. The organic solvent acetonitrile is used was AR grade. This method can also be used for routine analysis of Tolnaftate in bulk and its marketed formulations.

KEY-WORDS: Tolnaftate, UV Spectrophotometry, bulk drug, marketed formulation.

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INTRODUCTION

Tolnaftate is chemically O-naphthalen-2-yl methyl (3-methyl phenyl) thiocarbamate and it belongs to the class of antifungal and used topically in the treatment or prophylaxis of superficial dermatophyte infections.[7,8] There is no official method for determination of tolnaftate neither in bulk nor in pharmaceutical formulations. However, different UV Spectrophotometry methods are available for the pharmaceutical formulation of same drug in combination but not alone. But no method has been developed so far for tolnaftate which is widely employed in various topical skin care formulations. Therefore, it was found worthwhile to develop an appropriate validated UV Spectrophotometry method for estimation of tolnaftate in bulk and marketed formulations. A successful attempt was made in this context and the present paper aims to report the same.

MATERIALS AND METHODS

Reagents and Chemicals: The solvents (acetonitrile) was of AR grade standard. Magnesium stearate, Talc, Lactose was of AR Grade, Lobachemie Pvt. Ltd. The standard samples of tolnaftate was obtained from Aarti drugs Pvt. Ltd., marketed formulation of TINADERM® (Fulford India Ltd., a subsidiary of Merck & Co.) was procured from local market.

Mobile phase: Acetonitrile of AR grade was employed in the study.

Preparation of the standard stock solutions: An accurately weighed amount of Tolnaftate (100 mg) was transferred to 100 ml volumetric flask. Volume was made up with Acetonitrile (concentration-100µg/ml).The stock solution was stored at room temperature.[9,10]

Preperation of the standard dilution: 0.2ml of the stock solution was diluted upto 10ml with acetonitrile resulting in a solution of concentrations 2µg/ml. In the same way, solution of concentrations 2,4,6,8,10,12 µg were prepared.[9,10]

Preparation of the sample solution (Using marketed formulation,Tinaderm): The lotion procured from market contained tolnaftate. The lotion equivalent to 10 mg of tolnaftate was weighed and transferred to a 100 ml volumetric flask, 60 ml acetonitrile was added into it and, sonicated (PCI 6.5 lit) for 15minutes, and made the volume up to the mark with acetonitrile. The resultant solution was filtered with Whatmann filter paper (No.1), and the filtrate was collected for use in the study discarding the first portions of the filtrate. A sample solution of concentration 10 µg/ml is formed.[11]

Linearity: Linearity for tolnaftate was determined in the concentration range 10 to 100 % µg of working concentration of standard. The peak area responses were plotted against the corresponding concentrations and the r2 values were calculated.[3,4]

Validation of UV Spectrophotometric method : In order to confirm method suitability during routine quality control use, the proposed method was checked critically for the following validation characteristics as per ICH guidelines.

Precision:

Repeatability or Intraday Precision: The repeatability or intraday precision of the method was determined by three triplicate analysis of tolnaftate from standard dilutions, as per the proposed method, by same analyst on a single day. [5,6]

Intermediate Precision or Inter-day Precision: The intermediate or inter-day precision of the method was determined by three triplicate analysis of tolnaftate

from standard dilutions, as per the proposed method, by same analyst on three different days. The average drug content and the % RSD were calculated in each case. [5,6] The formula employed to calculate % RSD is :

$$\%RSD = (SD / Mean) \times 100$$

Accuracy (Recovery Studies): The accuracy (recovery studies) of the method was determined by calculating the recoveries of Tolnaftate by the method of standard addition. Known amount of the standards (80, 100and 120 %) were spiked in to the pre-analyzed sample solutions (30µg/ml), and the amounts of these standards were estimated by measuring the peak areas and by fitting these values to the straight-line equation of the calibration curves. [5,6]

Stability of analytical solution: The sample solution was prepared and injected into the system and analyzed as per the proposed method, initially and at 4 h time intervals up to 24 h, at room temperature and at 8 °C. [5,6]

Robustness (system suitability): The robustness study was done by making small change in the maximum wavelength at which observations were made as indicated in Table 5. [5,6]

LOD and LOQ: Detection limit and Quantitation limit of the drug is calculated by using the calibration curve standards. DL and QLwas calculated from the equation 3.3σ/S and 10σ/S respectively, where σ is the standard deviation of Y-intercept and S is the slope of the calibration curve.

Application of the Method for the Assay in Marketed Formulations (TINADERM®): The lotion procured from market contained tolnaftate. The lotion equivalent to 10 mg of tolnaftate was weighed and transferred to a 100 ml volumetric flask, 60 ml acetonitrile was added into it and, sonicated (PCI 6.5 lit) for 15minutes, and made the volume up to the mark with acetonitrile. The resultant solution was filtered with Whatmann filter paper (No.1), and the filtrate was collected for use in the study discarding the first portions of the filtrate. A sample solution of concentration 10 µg/ml is formed. [5,6,9]

RESULTS & DISCUSSION

Linearity: The calibration curve drawn by using the proposed method was found to be linear in the range (2-12µg/ml). Table 1 shows the calibration data with regression coefficient 0.9992.

Regression equation	y = 0.1633x + 0.0218
Regression coefficient (R^2)	0.9992
Range	2-12 mcg/ml
Slope	0.1633
Intercept	0.0218
LOD	0.0178 mcg/ml
LOQ	0.0595 mcg/ml

Table 1 Result of Linearity Studies

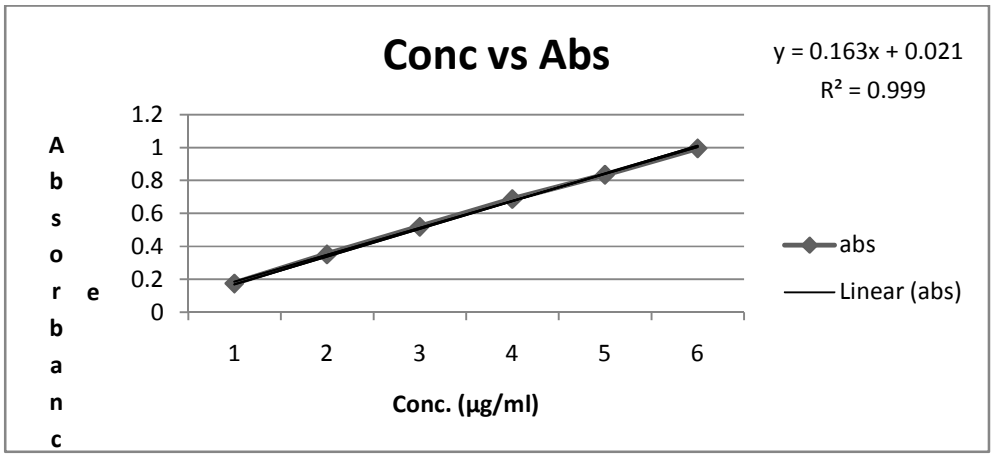

Figure 1 UV Linearity curve of Tolnaftate

Precision: Repeatability was assessed by analyzing three different concentrations. The method passed the test as the %RSD was found to be less than 2. Intermediate precision was assessed by analyzing three different concentrations for three different days. The method passed the test as the %RSD was found to be less than 2. Results are shown in table 2 and table 3.

Conc	Abs1	Abs 2	Abs 3	Mean	SD	%RSD
2	0.182	0.181	0.181	0.181333	0.000577	0.318392
6	0.522	0.521	0.521	0.521333	0.000577	0.110745
10	0.834	0.835	0.833	0.834	0.001	0.119904

Table 2 Repeatability or Intraday Precision

Conc.	Abs1	Abs2	Abs3	Mean	SD	RSD%
DAY 1						
2	0.183	0.181	0.182	0.181333	0.000577	0.256221
6	0.538	0.537	0.537	0.537333	0.000577	0.107447
10	0.837	0.836	0.836	0.836333	0.000577	0.069034
DAY 2						
2	0.181	0.180	0.180	0.180333	0.000577	0.320157

6	0.529	0.528	0.528	0.528333	0.000577	0.109278
10	0.838	0.837	0.838	0.837667	0.000577	0.068924
DAY 3						
2	0.177	0.176	0.176	0.176333	0.000577	0.32742
6	0.531	0.532	0.531	0.531333	0.000577	0.108661
10	0.836	0.835	0.836	0.835667	0.000577	0.069089

Table 3 Intermediate Precision or Inter-day Precision

Accuracy (recovery): Accuracy of the method was determined by using standard addition method and % recovery was found in the range 101-102% and %RSD was within the range. Table 4 shows the results of accuracy studies.

Conc. (µg/ml)	Excess Drug added to the analyte (%)	Theoretical Content(µg/ml)	Mean Conc.	SD	RSD %	Recovery %
4	50	6	0.531333	0.000577	0.108661	101.64
4	100	8	0.681667	0.000577	0.084697	101.45
4	150	10	0.836667	0.000577	0.069006	101.96

Table 4 Accuracy (Recovery Studies)

Robustness: Robustness studies were performed by varying the detection wavelength (±1). The method was found to be robust. Results are shown in table 5.

λ_{max} (±1 nm)	Conc.	Abs1	Abs2	Abs3	Mean	SD	RSD%
257	10	0.820	0.821	0.821	0.820333	0.000577	0.07038
259	10	0.827	0.826	0.826	0.826333	0.000577	0.069869

Table 5 Robustness Studies

Specificity : Specificity studies were performed by spiking the drug with the exipients like magnesium stearate, talc, lactose using two tailed unpaired t-test. Table 6 shows the results of specificity studies. No interference from other components or excipients was found during determination.

Conc. (µg/ml)	Abs 1	Abs 2	Abs 3	Mean	% RSD	p-value	Significantly different (P < 0.05)
2	0.180	0.181	0.181	0.180667	0.128	0.006618	NO
2*	0.179	0.179	0.178	0.178667	0.099		
6	0.527	0.525	0.524	0.525333	0.081	0.0310654	NO

6*	0.526	0.525	0.523	0.524667	0.147		
10	0.832	0.831	0.831	0.831667	0.045	0.02371	NO
10*	0.831	0.830	0.830	0.830333	0.041		

Table 6 Specificity

Note : 2, 4, 6 are unspiked / standard drug, while 2, 4*, 6* are spiked with talc, magnesium stearate & lactose.*

Application of the Method for the Assay in Marketed Formulations (TINADERM®) : The overall % of recovery and % RSD for tolnaftate in marketed formulation indicated that there is no significant difference in percentage of recovery.

Conc	Abs1	Abs2	Abs3	Mean	SD	RSD%	Recovery %
10	0.832	0.832	0.831	0.831667	0.000577	0.069421	101.29

Table 7 Application of the Method for the Assay in Marketed Formulations (TINADERM®)

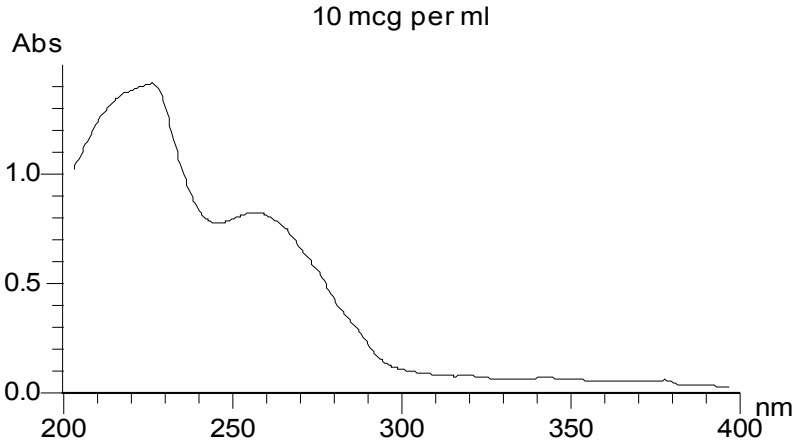

Figure 2 UV Scan of Tolnaftate

CONCLUSION

In the present research paper, UV Spectrophotometric method have been developed in-house for Tolnaftate in bulk drug and marketed formulation,Tinaderm. The analytical method developed on UV Spectrophotometry for Tolnaftate is simple, reliable, accurate, precise and reproducible in accordance with ICH Q2R1 Guideline.

The special features of developed method are:-

- **Linearity** (Inter and Intra day) on days of analysis, as demonstrated by the calibration curve, thus obeying Beer's Law.
- **Accuracy** (recovery) in the range of test concentration i.e. 98% -102%.
- Under the specificity studies, the method was evaluated for its ability to separate the drug peak from the excipients and no interference of the two was observed during analysis.
- The method is **reliable** and gives **reproducible** results.
- The method has further been successfully used to analyse the drug in topical dosage forms, without any complicated pre-treatment steps, yielding % recovery was 101.29%, precision with % RSD less than 2%.

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AUTHOR'S CONTRIBUTIONS

Mr. Ashish Kumar Sarawal carried out entire work starting from the procurement of drug, its identification, UV method development and validation and application on a marketed formulation.

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