OXIDATIVE DEGRADATION OF TAVABOROLE: DETERMINATION BY DERIVATIVE UV SPECTROPHOTOMETRY

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ABSTRACT

The aim of the present work was to investigate degradation behavior of tavaborole upon exposure to oxidative conditions using first order derivative UV spectrophotometric method. We have developed simple and fast analytical method that can be used as quality control tool for the determination of tavaborole. The method was based on oxidative studies over a period of time. The order of oxidative degradation reaction was calculated by using the log of recovered concentration obtained from the oxidative degradation study. The reaction was first order kinetics. The method was validated according to ICH guidelines. The linear response was found in the concentration range of 5-25 µg mL^{-1} at 271 nm. The % relative standard deviation (RSD) for precision studies of intraday and interday was <2 %.

Keywords: Tavaborole, oxidative degradation, first derivative, UV spectroscopy, validation, antifungal

INTRODUCTION

Tavaborole is one of the new antifungal drugs approved by the United States Food and Drugs Administration in 2014 for onychomycosis. Tavaborole 5 % V/V solution was marketed by Anacor Pharmaceutical Inc, Palo Alto, CA and it was approved in July 2014[1]. For patients where oral antifungal medications were undesired, the new topical antifungal presents an alternative treatment. Patients requiring multiple medications (e.g. the elderly), or with diabetes and/or autoimmune diseases, who are unable to use oral antifungal medications, will be benefitted from the topical antifungals[2]. Tavaborole is novel, chemically a small molecule used to treat the toenails onychomycosis disease[3] (Fig. 1). Onychomycosis is a serious fungal infection in nail and it may be caused by many infectious agents such as dermatophytes, yeast and molds. Onychomycosis is the term in which nails get disintegrated, discolored and thickened and may be detached from nail bed[4-5]. During the last few years, nail permeability has been fully investigated as a screening test for the development of new drugs or formulations[6]. Treatment options tavaborole and efinaconazole got approval for the treatment of dermatophytic onychomycosis and luliconazole, which is indicated for cutaneous dermatophytic infections[7-8]. The unpleasant appearance of onychomycosis is a cause of psychological distress for the patient and it may lead to depression, anxiety and social inaccessibility. Anacor Pharmaceuticals has patented its product in the USA, Australia, South Africa, New Zealand, and Russia which covers the methods of using tavaborole to treat onychomycosis[9].

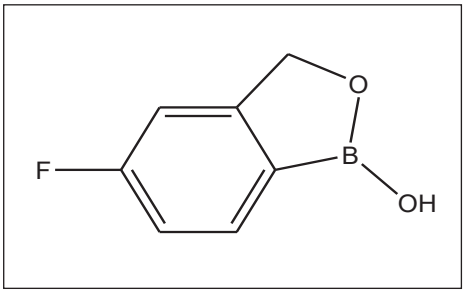

Fig. 1: Tavaborole structure

Tavaborole belongs to the family of oxaboroles, which are boron containing chemical compounds. The IUPAC name of tavaborole is 5-fluoro-1, 3-dihydro-1-hydroxy-2, 1-benzoxaborole with molecular formula and molecular weight of $C_7H_6BFO_2$ and 151.93Da, respectively. This low molecular weight of tavaborole allows high amount of penetration in thick human nails than any other antifungals such as terbinafine, fluconazole, itraconazole and others. 5-Fluoro group enhances the antifungal activity of the compound and 1-hydroxyl group attached

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to 1-phenyl group enhances water solubility than other benzoxaboroles[10].

Being a new antifungal drug, tavaborole receives importance in terms of clinical usage as well as research interests pertaining to analytical purposes. Literature review shows that the HPLC methods have been developed for quantization of tavaborole in *in vitro* studies samples and one method developed for the stability studies of tavaborole using HPLC-PDA system[11-13]. There were only two spectrophotometric methods reported for tavaborole, however we could not find any method using derivative spectroscopy[14,15]. During stress degradation studies of tavaborole using HPLC PDA, it was observed that the drug is very sensitive to oxidative degradation condition. Hence, we decided to explore simple spectrophotometric method for monitoring oxidative degradation of tavaborole.

MATERIALS AND METHODS

Reagents and chemicals

Pfizer Ltd. Mumbai, India provided the working standard tavaborole. Methanol (HPLC and AR grade) and 30 % w/V hydrogen peroxide (H_2O_2) were procured from Loba Chemie Pvt. Ltd., Mumbai, India. Distilled water was collected using water purifier system (EXTRAPURE LAB LINK).

Apparatus and conditions

The instrument used for analysis was a double beam UV-spectrophotometer (JASCO V- 730) with 1 cm matched quartz cells. The spectra in the presented study were recorded at spectral band width of 1.0 nm with the scanning speed 400 nm min^{-1} and data pitch 1 nm. Range for scanning wavelength was 200-400 nm.

Stock solution and working standard preparation

Accurately weighed 10 mg of tavaborole was transferred to 10 mL volumetric flask, and the volume was made with methanol up to 10 mL, to get standard stock solution of tavaborole (1000 µg mL^{-1}). From the standard stock solution, working standard solution was prepared using water as final diluent.

Solution for oxidative degradation product

Sample solution was prepared by addition of 1 mL of stock solution (200 µg mL^{-1}) to 1 mL of 1 %w/V H_2O_2. Volume was made with water up to 10 mL. At room temperature, solution was kept for 60 min. and UV spectrum was recorded to check the spectral difference from the standard.

Selection of analytical wavelength

A solution of 20 µg mL^{-1} was prepared from standard stock solution of tavaborole (1000 µg mL^{-1}) and scanned over 200- 400 nm in the UV– spectrophotometer. The

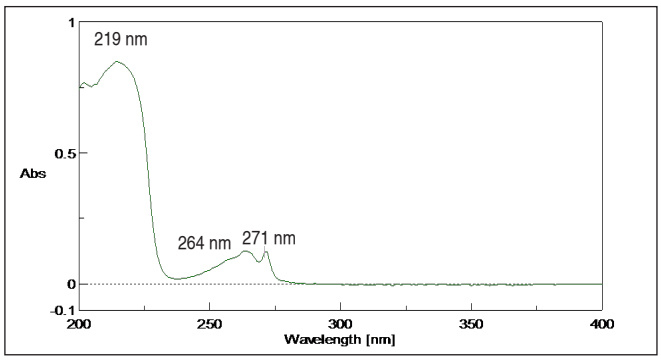

Fig. 2: UV-Spectrum of tavaborole (20 µg mL^{-1})

maximum absorbance was shown at 219, 264 and 271 nm. To maintain method specificity, the wavelength of 219 was not chosen. 271 nm was selected for studies. The UV spectrum is given in Fig. 2.

First derivative study

A sample solution was prepared by addition of 1 mL of stock solution (200 µg mL^{-1}) to 1 mL of 1 %w/V H_2O_2. Volume was made with water up to 10 mL. At room temperature, solution was kept for 60 min. and zero order

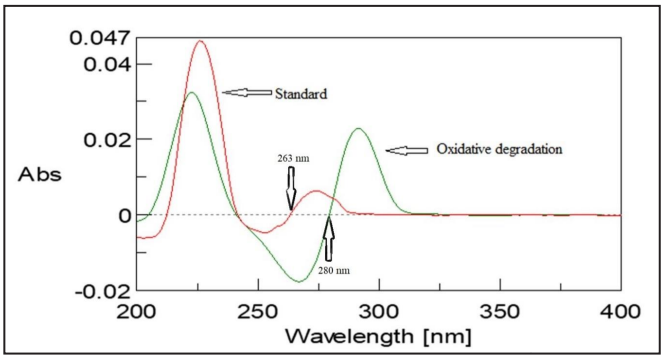

Fig. 3: Overlay of first order UV- spectrum of standard (20 µg mL^{-1}) and oxidized solution after 1 h (20 µg mL^{-1})

UV spectrum was recorded. The obtained zero order spectrum of oxidative solution as well as working standard were converted to first derivative and overlapped to get spectral differences between standard and degradation solution. The UV spectrum as shown in Fig. 3.

Oxidative degradation studies

To detect degradation of tavaborole in oxidative condition, dilutions were prepared from standard stock

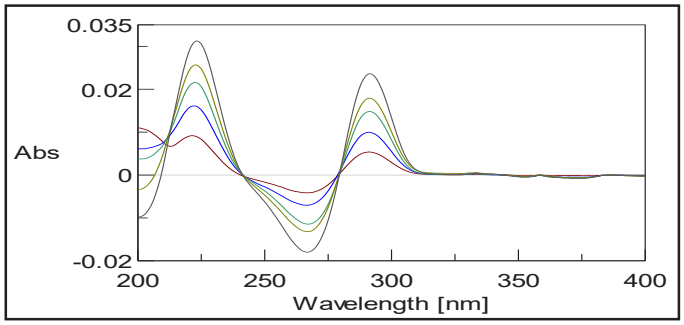

Fig. 4: First order overlay spectra of 1 % w/V H_2O_2 for 10 min-50 min.

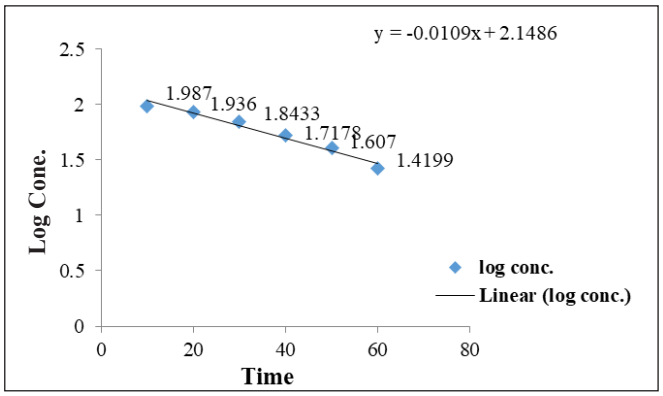

Fig. 5: First order chemical kinetics

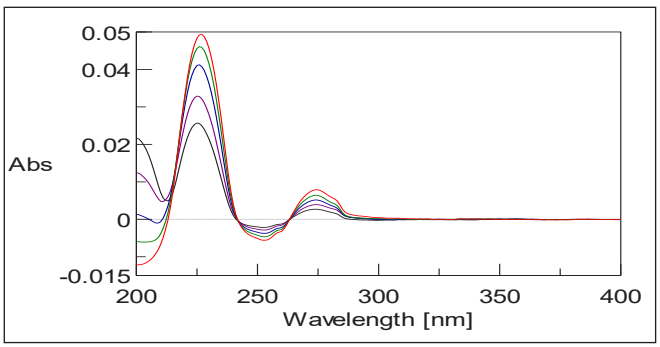

Fig. 6: First order linearity overlay of tavaborole (5-25 µg mL^{-1})

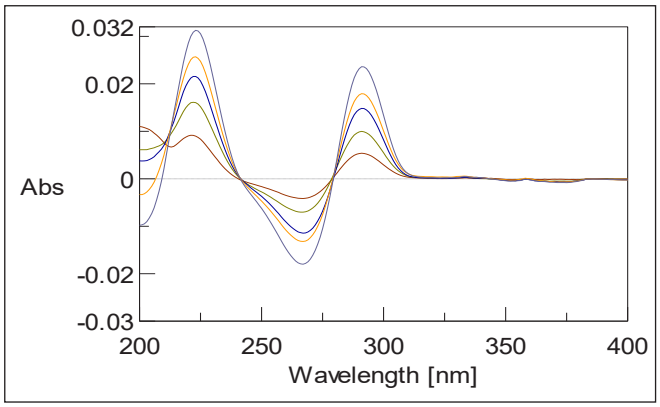

Fig. 7: First order linearity overlay of oxidative solution (5-25 µg mL^{-1})

solution (1000 µg mL^{-1}) in the range 5-25 µg mL^{-1} and spectrum were recorded at first derivative. Similarly prepared were oxidative samples in range 5-25 µg mL^{-1} and the spectra were recorded at first derivative. The first derivative spectrum of standard shows 263 nm as zero crossing point (ZCP) as shown in Fig. 5, and 280 nm as ZCP of oxidative degradation solution as shown in Fig. 6. Hence, the degradation of tavaborole in oxidative condition was detected as it shows absorbance at the ZCP of standard in first derivative. To study the oxidative degradation of tavaborole, the sample solution was prepared by addition of 1 mL of 1 %w/V H_2O_2 to 1 mL of stock solution (200 µg mL^{-1}) and volume was made with water. For the determination of degradation of tavaborole, initially the study was planned for 30 min, 60 min, 120 min, 240 min, and 24 h. But it was found that the drug was completely degraded after 120 min. So, the study time was reduced to 1 h. The spectra were recorded at 10 min, 20 min, 30 min, 40 min, 50 min and 60 min and the results are shown in Fig. 4. The blank was prepared using 1 mL solution of 1 % w/V H_2O_2 and diluted with water up to 10 mL.

Table I: Summary of oxidative degradation (first derivative)

Condition	Time (min)	% Recovery
Oxidative degradation	10 min	95%
	20 min	86.30%
	30 min	73%
	40 min	61.35%
	50 min	41.40%
	60 min	26.37%

Table II: Details of recovery studies

Drug	% Level	Initial amount (µg mL^{-1})	Amount added (µg mL^{-1})	% Recovery
Tavaborole	50	10	5	99.41
	100	10	10	100.62
	150	10	15	101.01

Table III: LOD and LOQ details of tavaborole

Sr. No.	Parameter		Concentration (µg mL^{-1})
1	LOD	Using y-intercept	0.19
		Using response at lowest conc.	0.06
2	LOQ	Using y-intercept	1.04
		Using response at lowest conc.	0.36

Table IV: Summary of validation parameters

Sr. No.	Validation parameter		Tavaborole
1	Linearity range		5-25 µg mL^{-1}
2	Intraday precision (% RSD)		1.15
	Interday precision (% RSD)		1.39
3	Assay (%)		99.96
4	Accuracy		% Recovery
	50 % Level		99.41
	100 % Level		100.62
	150 % Level		101.01
5	LOD (µg mL^{-1})	Using y-intercept	1.04
		Using response at lowest Conc.	0.36
6	LOQ (µg mL^{-1})	Using y-intercept	0.19
		Using response at lowest Conc.	0.06
7	Robustness		Robust

As 263 nm and 280 nm are the zero crossing points of degradation and standard, respectively. So, for the calculation of percent recovery the mean wavelength (271 nm) was used. The zero order spectra were converted to first order derivative spectra and absorbances were recorded at 271 nm. The results are summarized in Table I.

Chemical kinetics

The order of the oxidative reaction is calculated using the graphical method. The time for degradation was plotted against the log percent concentration. The straight line was obtained with negative slope which shows that the reaction is first order kinetics. In first order, reaction rate is directly proportional to the concentration of the drug undergoing reaction. Percent degradation increases as the time increases[16]. The chemical kinetics plot is given in Fig. 5.

RESULTS

Method validation

The validation of the developed method was performed for quantitative study of tavaborole. It was validated using ICH Q2 (R1) guidelines.

Linearity and range

The dilutions were prepared from standard stock solution (1000 µg mL^{-1}). The absorbances were recorded for first derivative at 280 nm for standard as it is ZCP of oxidative degradation solution. Linearity was observed over the range of 5-25 µg mL^{-1}. The procedure was repeated for 5 times to get the linear regression equation. The correlation coefficient R^2 was 0.998 with the regression equation y = 0.0002x + 0.0012. The first order linearity overlay is given in Fig. 6.

Similarly, the dilutions were prepared for oxidative degradation solution in the range 5- 25 µg mL^{-1} by addition 1 mL of 1 % w/V H_2O_2 to stock solution and volume was made with water up to 10mL. Absorbances were recorded for first derivative at 263 nm as it is zero crossing point of standard. The correlation coefficient R^2 was found to be 0.997 with regression equation y = -0.0006x - 0.0009. The first order linearity overlay of degradation is given in Fig. 7.

Assay

From the prepared formulation of tavaborole (5% solution-10 mL), 0.2 mL solution (10 mg drug) was pipetted out and volume was made with water up to 10 mL. For assay results, 6 replicates of same concentration were evaluated. The % recovery obtained was 99.1 %.

Accuracy

Accuracy of the method was determined by method of standard addition. Known amount of API to be analyzed was added to the prepared formulation (topical solution) of tavaborole. In the assay solution, standard drug solution was spiked at 50 %, 100 % and 150 % level. The 3 replicates of 3 concentrations were evaluated to calculate % recovery. The results obtained are depicted in Table II.

Precision

The precision was checked, both for intraday and inter day study. The precision was assessed using 6 replicates of 10 µg mL^{-1} within the same day and consecutive days (% RSD was less than 2 %). The % RSD for intraday and inter day precision were found to be 1.15 and 1.39, respectively.

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

The limits were calculated from values of the regression equation. Both LOD and LOQ were determined using formula 3.3 σ/S and 10 σ/S, respectively. Here, S is slope of calibration curve and σ is standard deviation of absorbance at lowest concentration or y-intercept. The results obtained are depicted in Table III.

Robustness

Robustness was performed by doing small and deliberate changes to developed system. Absorbances were checked after doing the changes to wavelength and changing meniscus while making dilutions. The optimized method is robust as % RSD is below 2 %.

The results of validation proved that the established method complies with validation parameters and data is summarized in Table IV.

DISCUSSION

A method to detect oxidation as well as determination of tavaborole in presence of oxidative degradation was developed. The results of this method indicate that the developed first order UV derivative spectrophotometric method is simple and economic. The aim of the study was to investigate the oxidative degradation behavior of tavaborole using simple first derivative spectroscopy instead of using sophisticated instruments like HPLC and HPTLC, which involve time consuming methods. Chemical kinetics proves that the order of reaction is first order. In first order reaction rate is directly proportional to the concentration of the drug undergoing reaction. Hence, it is concluded that use of this method can save much time and money and it can be used in laboratories for detection of oxidative degradation of tavaborole.

REFERENCES

1. Anacor Pharmaceuticals Inc. Kerydin (Tavaborole) topical solution, 5 % [Internet] 2014 [Accessed 2021 Aug 1.] Available at: <http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/204427s000lbl.pdf>.
2. Gupta A. K. and Paquet M.: Management of Onychomycosis in Canada in 2014, **J. Cutan. Med. Surg**, 2015, 19, 260–273.
3. Summary review of safety for regulatory action center for drug evolution and research for Kerydin 5 % Topical solution, Office of new drug, FDA, U.S, 2014 [Accessed on 01 August 2021.] Available at: <https://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/204427Orig1s000SumR.pdf>.
4. Gupta A. K. and Deanne D.: Tavaborole (AN-2690) for the treatment of onychomycosis of the toe nail in adults, **Anti-infect. Ther.**, 2014, 12(7), 735-742.
5. Gupta A. K. and Nakrieko K.A.: Molecular determination of mixed infections of dermatophytes and nondermatophyte molds in individuals with onychomycosis, **J. Am. Podiatr. Med. Assoc.,** 2014, 104(4), 330-336.
6. Matsuda Y., Sugiura K. and Hashimoto T.: Efficacy Coefficients Determined Using Nail Permeability and Antifungal Activity in Keratin-Containing Media Are Useful for Predicting Clinical Efficacies of Topical Drugs for Onychomycosis, **PLoS One** 2016, 11, 1–12.
7. Saunders J., Maki K. and Koski R.: Tavaborole, efinaconazole, and luliconazole; Three New Antimycotic agents for the treatment of dermatophytic fungi, **Int. J. Pharm. Pract.,** 2017, 30(6), 621-630.
8. Childs-Kean L. M. and Jourjy J.: Antifungal Penetration into the Nail and New Topicals for Onychomycosis, **Curr. Fung. Inf. Rep.**, 2016, 10, 24–29.
9. Markham A.: Tavaborole: first global approval, **Drugs**. 2014, 74(13), 1555-1558.
10. Baker S. J., Yang Z. and Tsutomu A.: Discovery of a new boron-containing antifungal agent, 5-fluoro-1, 3-dihydro-1-hydroxy-2, 1-benzoxaborole (AN2690), for the potential treatment of onychomycosis, **J. Med. Chem**., 2006, 49(15), 4447-4450.
11. Tracey V., Merchant T. and Chanda S.: *In vitro* nail penetration of tavaborole topical solution, 5%, through nail polish on *ex vivo* human fingernails, **J. Drugs Dermatol.,** 2015, 14(7), 675-678.
12. Tampucci S., Terreni E. and Burgalassi S.: Development and Validation of an HPLC–UV Method to Quantify Tavaborole during *in vitro* Transungual Permeation Studies, **J. AOAC. Int.,** 2018, 101(2), 437-443.
13. Tirukkovalluri S. R., Balireddi V. and Tatikonda K.: Analytical Method Development and Validation of stability indicating Method for RP-HPLC for Quantification of Tavaborole Related Substances: Application to 5% Topical Solution, **Eur. J. Pharm. Sci.,** 2018, 5(9), 545-550.
14. Avuthu S., Mukthinuthalapati M. and Rangisetty S. Y.: New validated analytical methods for the determination of Tavaborole (An anti-fungal agent), **Res. J. Pharm. Technol.,** 2020, 13(4), 1895-1900.
15. Indabawa A. H., Abubakar S. U. and Yahaya S. M.: UV-Spectrometric Method Development and Validation of Tavaborole, **J. Chem. Pharm.,** 2021, 13(5), 01-09.
16. Bramhankar D. M. and Jaiswal S. B.: Biopharmaceutics and Pharmacokinetics, 2nd Edition, Vallabh Prakashan, Delhi, 216-217.