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UV-Spectrometric Method Development and Validation of Tavaborole

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ABSTRACT

There is a need to develop a reliable, cost effective, and methodologies for a routine analytical method of active pharmaceutical ingredients being an essential part of pre-formulation, formulation research and development. A new, economical, sensitive, simple, rapid UV spectrophotometric method has been developed for the estimation of Tavaborole in pure form. Tavaboroleis an antifungal agent formulated for the treatment of toenail onychomycosis. This UV method was developed using methanol as a solvent. Tavaborole has shown absorption maxima at 272 nm and 265 nm in all the methods. UV-Visible double beam spectrophotometer (Jasco V-730) was used to carry out the spectral analysis.The ICH guidelines were used to validate the method. The method was validated for linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ. Beer's law was obeyed over the concentration range of 20-100 µg/ml, using regression analysis. The linear equation y=0.0049x+0.0024, y=0.0054x-0.0026 with a correlation coefficient of R^2=0.9981, R^2=0.9979 at wavelength 272 nm and 265 nm respectively was obtained. The limit of detection was found to be 103.749 µg/mL, 105.289 µg/mL and the limit of quantification was found to be 314.392 µg/mL, 319.058 µg/mL at 272 nm and 265 nm respectively. Precision was calculated with intra and inter-day variation. Recovery study was performed on formulations and % RSD value was found to be less than 2. The proposed UV spectroscopic method was found to be linear, accurate, precise, stable, specific and simple for quantitative estimation. Hence the present UV spectroscopic method is suitable for the routine assay of tavaborole in bulk and pharmaceutical formulations.

Keywords: Tavaborole; UV-spectroscopy; Analysis; Method validation; Onychomycosis

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INTRODUCTION

Tavaborole is a small, boron-based molecule capable of effectively penetrating the nail unit as described below. It is the first molecule in this class of drugs to achieve FDA approval in July 2014 as tavaborole (formerly AN2690). It is formulated for the treatment of toenail onychomycosis as 5% topical solution. Tavaborole is a novel broad-spectrum antifungal pharmaceutical agent of the oxaborole class of boron-containing compounds. These antifungal compounds were designed from a previous class of borinic acid quinolone ester compounds having antibacterial properties and they were specifically selected based on their antifungal properties. The broad-spectrum antifungal activity of these compounds was optimized by the addition of a 5-fluoro group and their hydrophilicity which was optimized by the substitution of a 1-phenyl group for a 1-hydroxy group. 1-hydroxy-5-fluoro-1,3-dihydro-2,1 benzoxaborole is an optimal compound that has also been shown to maintain its activity in presence of keratin [1] and also claim to have better *in-vitro* nail penetration than other oxaboroles at a 10% concentration in ethanol and

ciclopirox 8% nail lacquer [2]. This compound formerly AN2690, was selected for future drug development for the topical treatment of onychomycosis and was subsequently named tavaborole (Figures 1 and 2).

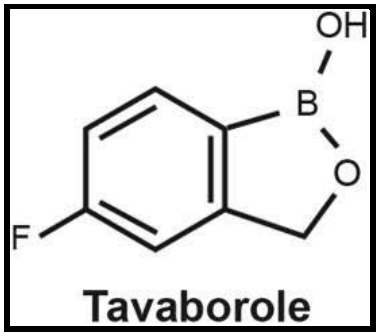

Figure 1: Structure of Tavaborole

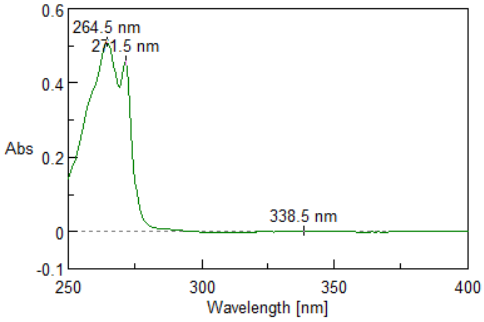

Figure 2: UV-spectrometric graph of tavaborole

Tavaborole has low molecular weight (approximately half of the most antifungal medications such as terbinafine and efinaconazole) which permits optimal nail plate penetration, higher to that of an existing topical antifungal medication [3]. The amount of 5% solution tavaborole penetrating an ex vivo cadaver fingernail plate was 250-fold higher than ciclopirox 8% solution in a head-to-head study with ciclopirox, for daily application after 14 days (524.7 µg/cm^2 versus 13.0 µg/cm^2, respectively) [4,5]. *In vitro* nail penetration studies, it also shows that tavaborole is able to penetrate human nails better compared with ciclopirox 8% and amorolfine 5% nail lacquers. Tavaborole acts by inhibiting synthesis of protein in the fungus. It inhibits an enzyme known as cytosolic aminoacyl transfer RNA synthetase (AARS) with 1000 times' greater selectivity than for mammalian AARS (Also known as leucyl-transfer RNA synthetase, or LeuRS) which is the enzyme that plays a key role in fungal essential protein synthesis. Termination of protein synthesis leads to inhibition of fungal cell growth which ultimately leads to death of fungus [6,7]. Tavaborole exhibits broad-spectrum activity against a variety of fungi, including non-dermatophytes, dermatophytes and yeasts. It was also tested for an *in-vitro* activity against a panel of different fungal strains including *dermatophytes T. rubrum, T. mentagrophytes, Cryptococcus neoformans, C. albicans* and *Aspergillusfumigatus*. Tavaborole exhibited an inhibitory activity to all of the fungal strains tested (1 mg/mL) [8].Tavaborole safety have been studied, results found no evidence of teratogenicity in pregnant rats and rabbits [9]. 5% tavaborole solutions have demonstrated a good safety profile across Phase II, Phase III studies, with most adverse events considered mild and unrelated to study drug. None of the serious adverse events reported were considered treatment-related [10].The FDA in 2014 approved a 5% nail solution of tavaborole which is to be applied daily for the period of 48 weeks and should completely cover the entire surface of the toenail and underneath the

toenail tip. A new, economical, sensitive, simple, rapid UV spectrophotometric method has been developed in this article for the estimation of Tavaborole in pure form and the ICH guidelines were used to validate the method.

MATERIALS AND METHODS

Instrument and Chemical Reagents

The following chemicals and instruments were used for the analytical process (Table 1).

Table 1: List of materials and instruments used

Materials		**Source**
Drug	Tavaborole	As a gift from Enaltec Pharma Research Pvt. Ltd.
Solvent	Methanol	Research-Lab fine chem. Industry, Mumbai.
List of Instruments used		
Instruments		**Source**
UV-Spectrophotometer		Double beam UV-Spectrophotometer Jasco V-730
Electronic analytical balance		BL-220 H Electronic Balance Shimadzu Corporation
Sonicator		Ultrasonic Cleaner bath (Coslab ISO 9001)

Method Development

Preparation of stock solutions: 100 mg of standard tavaborole was accurately weighed and transferred into 1000 ml volumetric flask and dissolved with 10 mL of methanol. The flask was shaken and volume was made up to the mark with distilled water to prepare a solution containing 1000 µg/ml (stock solution A 100 ppm) and sonicated for 1 min.

Selection of wavelength: To determine the wavelength for measurement, Tavaborole (100 µg/ml) solution was scanned at the range of 200-400 nm against methanol as blank. Wavelength of maximum absorption was determined for the drug. Tavaborole showed a maximum absorption at 272 nm and 265 nm.

Selection of analytical concentration ranges: From the standard stock solution A of Tavaborole, appropriate aliquots were pipetted out into 10 ml volumetric flasks and dilutions were made with distilled water to obtain working standard solutions of concentrations from 20 to 100 ppm. Absorbance for these solutions was measured at absorption maxima of 272 nm and 265 nm. For the standard solution analytical concentration range were found to be 20-100 µg/ml and those values were reported in Table 2.

Table 2: Linearity result of Tavaborole at 272 nm and 265 nm

S/No.	**Concentration (µg/mL)**	**λ=271 nm**	**λ=265 nm**
1	Blank	0.0116	0.0146
2	Stock Solution	0.4991	0.5418
3	20	0.1021	0.1039
4	40	0.2107	0.2203
5	60	0.2857	0.3039
6	80	0.3925	0.4223
7	100	0.4996	0.5431

Calibration curve for Tavaborole: Appropriate volume of aliquots from standard Tavaborole stock solution A was transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with distilled water to obtain concentrations of 20,40,60,80 and 100 µg/ml. Absorbance spectra of each solution against methanol as blank were measured at 272 nm and 265 nm and the graphs of absorbance against concentration were plotted and shown in Figures 3 and 4 respectively. The regression equation and coefficient of determination was determined.

Method Validation

The method was validated for several parameters like Linearity, Accuracy, Precision, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ). The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [11].

Linearity (calibration curve): The developed method validates as per ICH guidelines. The plot of absorbance verses concentration is shown in Figures 3 and 4. It can be seen that plot is linear in the concentration range of 20-100 µg/ml with correlation coefficient (r2) of 0.9981 and 0.9979 for 272 nm and 265 nm respectively.

Accuracy (recovery study): Recovery studies were carried out by adding a known amount of pure drug to the pre-analysed formulations and the proposed method was followed. From the quantity of drug found, percentage recovery was calculated as per ICH guidelines. Accurately weighed formulation standard drug solution sample equivalent at 3 different concentration levels of 80%, 100% and 120% of Tavaborole sample were dissolved in 10 ml of methanol and further volume make up with distilled water. The solutions were analysed and the data were presented in Tables 3 and 4. The percentage recovery was determined by using the formula [8].

Table 3: Optical parameters for determination of Tavaborole

S/N	Parameter	Data	Data
1.	λ max	272 nm	265 nm
2.	Beer's law limit	20-100 µg/mL	20-100 µg/mL
3.	Correlation coefficient (r2)	R^2=0.9981	R^2=0.9979
4.	Regression equation (y=mx+c)	y=0.0049x+0.0024	y=0.0054x-0.0026
5.	Slope (m)	0.00492	0.00536
6.	Intercept (c)	0.00242	-0.0026
7.	Standard Deviation	0.15469	0.17112
8.	Limit of detection (µg/mL)	103.749	105.289
9.	Limit of quantification (µg/mL)	314.392	319.058

Table 4: Accuracy result of Tavaborole at 272 nm and 265 nm

Conc. Added (µg/mL)	Absorbance λ=272 nm	Amount recovered (µg/mL)	%Recovery	Absorbance λ=265 nm	Amount recovered (µg/mL)	%Recovery
80	0.3960	79.99	99.99	0.4290	80.47	100.60
80	0.3960	79.99	99.99	0.4292	79.93	99.91
80	0.3932	79.42	99.28	0.4261	79.93	99.91
100	0.5026	101.66	101.66	0.5374	100.68	100.68
100	0.5054	102.23	102.23	0.5407	101.29	101.29
100	0.5067	102.49	102.49	0.5425	101.63	101.63
120	0.5953	120.49	100.41	0.6537	122.37	101.97
120	0.6051	122.49	102.08	0.6542	122.46	102.05

120	0.5955	120.54	100.45	0.6536	122.35	101.96

Percentage Recovery=Amount of drug recovered/Amount of drug added × 100

Precision (repeatability): The parameter was validated by assaying number of aliquots samples of Tavaborole and its validity was estimated using parameters such as Standard deviation and Relative Standard deviation. Intra-day and inter-day precision was determined by measuring the absorbance of the sample for three times on same day and on two or three different days. The obtained results are presented in Tables 5 and 6 respectively (Figures 3 and 4).

Table 5: Intra-day precision results of Tavaborole at 272 nm and 265 nm

S/N	Conc. (µg/ml)	Absorbance λ=272 nm	Absorbance λ=265 nm
1.	100	0.504	0.5399
2.	100	0.5029	0.5392
3.	100	0.5064	0.5422
4.	100	0.5067	0.5428
5.	100	0.5095	0.5442
6.	100	0.5063	0.5423
7.	Blank	0.0142	0.0173
8.	Average	0.506	0.5418
9.	SD	0.002306	0.001873
10.	%RSD	0.4557	0.3456

Table 6: Inter-day Precision results of Tavaborole at 272 nm and 265 nm

S/N	Conc. (µg/ml)	Absorbance (λ=272 nm) Day 1	Absorbance (λ=272 nm) Day 2	Absorbance (λ=265 nm) Day 1	Absorbance (λ=265 nm) Day 2
1.	100	0.5040	0.5067	0.5399	0.5425
2.	100	0.5029	0.5026	0.5392	0.5374
3.	100	0.5064	0.5063	0.5422	0.5425
4.	100	0.5067	0.5058	0.5428	0.5433
5.	100	0.5095	0.5087	0.5442	0.5449
6.	100	0.5063	0.5043	0.5423	0.5399
7.	Blank	0.0142	0.0190	0.0173	0.0166
8.	Average	0.5060	0.5057	0.5418	0.5418
9.	SD	0.00231	0.00209	0.00187	0.00268
10.	%RSD	0.4557	0.4142	0.3456	0.4938

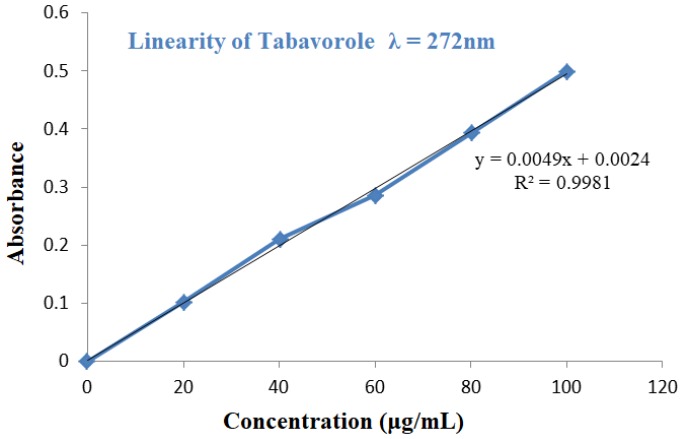

Figure 3: Linearity of Tavaborole at λ=272 nm

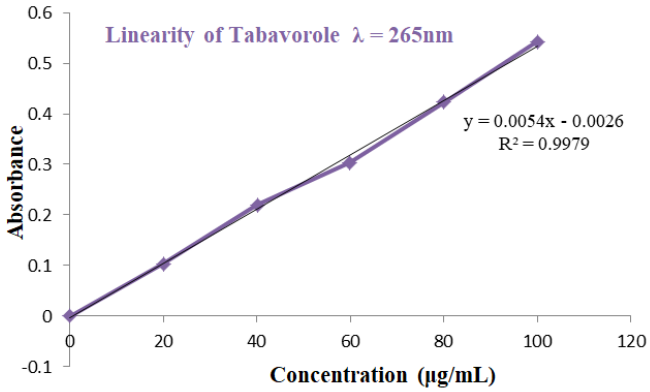

Figure 4: Linearity of Tavaborole at λ=265 nm

Robustness: Robustness is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameter and provides an indication of its reliability during normal usage. Robustness was carried out on two different analysts. The obtained results are presented in Table 7.

Table 7: Robustness results of Tavaborole at 272 nm and 265 nm

S/N	Conc. (µg/ml)	Absorbance λ=272 nm	Absorbance λ=265 nm
1.	80	0.346	0.369
2.	80	0.346	0.3692
3.	80	0.3432	0.3661
4.	80	0.3497	0.3729
5.	80	0.3486	0.372
6.	80	0.3452	0.3668
7.	Blank	0.0216	0.0244
8.	Average	0.34645	0.36933
9.	SD	0.00235	0.00271

10.	%RSD	0.67957	0.73488

Ruggedness: The degree of reproducibility of test results of the same sample within different laboratory and different analyst under the same condition with the same concentration.

LOD and LOQ: The limit of detection (LOD) and limit of quantification (LOQ) of the drug were separately determined based on method of the intercept and the average value of slope. (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by International Conference on Harmonization (ICH) guideline.

$LOD=3.3\ \sigma/S$
$LOQ=10\ \sigma/S$

Where,
σ=the standard deviation of the response.
S=slope of the calibration curve

RESULTS AND DISCUSSION

Determination of Wavelength and Calibration Graph

The λmax of Tavaborole was found to be at 272 nm and 265 nm. The absorbance was measured at both 272 nm and 265 nm against methanol. The calibration curve was prepared by plotting absorbance versus concentration of drug [11].

Linearity: The linear relation between absorbance and concentration of drug was evaluated over concentration range between 20-100 µg/mL. The wavelength for linearity was scanned at 272 nm and 265 nm. Linearity result was given at Table 2.

Accuracy: Accuracy of the method was confirmed by recovery studies at three different level 80%, 100% and 120%. The amount of tavaborole was calculated at each level and percentage recoveries were calculated shown at Table 4.

Precision: Precision of the developed method expressed in termsof the relative standard deviation of the absorbance. The solution was analyzed 6 replicates for intra-day and in two successive days for inter-day precision. The % RSD value was found to be less than 2. Results confirmed that the precision of the method was found to be acceptable. Precision results were given in Tables 5 and 6 for intra-day and inter-day respectively.

Robustness: By change in concentration and wavelengths i.e., 272 nm and 265 nm, % RSD is less than 2% which shows it's within the range. So the parameter was validated.

Ruggedness: By change in analyst and laboratory, there is no effect on absorbance with the same condition shown in Table 8. Hence the parameter was validated.

Table 8: Ruggedness results of Tavaborole at 272 nm and 265 nm

S/N	Conc. (µg/ml)	Analyst 1		Analyst 2	
		Absorbance (λ=272 nm)	Absorbance (λ=265 nm)	Absorbance (λ=272 nm)	Absorbance (λ=265 nm)
1.	10	0.0462	0.0451	0.0465	0.045
2.	10	0.0457	0.0445	0.0461	0.0448
3.	10	0.0455	0.0443	0.0454	0.0443

4.	10	0.0463	0.0449	0.0462	0.0449
5.	10	0.0459	0.0452	0.0457	0.0446
6.	10	0.047	0.0455	0.0463	0.0452
7.	Blank	0.0116	0.0146	0.0118	0.0144
8.	Average	0.0461	0.0449	0.046	0.0448
9.	SD	0.00053	0.00045	0.00041	0.00032
10.	%RSD	1.156	0.99979	0.88685	0.70587

CONCLUSION

Tavaborole is an antifungal agent formulated for the treatment of toenail onychomycosis. It is a small, boron-based molecule capable of effectively penetrating the nail unit. It is the first molecule in this class of drugs to achieve FDA approval in July 2014 as tavaborole (formerly AN2690). Tavaborole is chemically known as 5-Fluoro-1,3-dihydro-2,1-benzoxaborol-1-ol. Tavaborole has shown absorption maxima at 272 nm and 265 nm in all the methods. A calibration curve was drawn by taking the concentration on the X-axis and their respective absorbance on Y-axis for all the methods. Beers law is obeyed over the concentration range of 20-100 µg/ml, using regression analysis the linear equation y=0.0049x+0.0024, y=0.0054x-0.0026 with a correlation coefficient of R^2=0.9981, R^2=0.9979 at wavelength 272 nm and 265 nm respectively. The limit of detection was found to be 103.749 µg/mL,105.289 µg/mL and the limit of quantification was found to be 314.392 µg/mL, 319.058 µg/mL at 272 nm and 265 nm respectively. Precision was calculated with intra and inter-day variation. Recovery study was performed on formulations and % RSD was found. The optical parameters such as Beer's law limit, slope, and intercept values were calculated and given in Table 3. Method was validated for accuracy and precision. The accuracy of method was proved by performing recovery studies in formulations. The results were given in Table 4 and shows relative standard deviation of less than 2% was observed for analysis of three replicate samples, indicating precision and reproducibility.

DECLARATION OF INTEREST

The authors declare that there are no conflicts of interest.

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