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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING METHOD OF RP-HPLC FOR QUANTIFICATION OF TAVABOROLE RELATED SUBSTANCES: APPLICATION TO 5% TOPICAL SOLUTION

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ABSTRACT
Novel stability indicating analytical method development for estimation of Tavaborole related substances such as Known and Unknown impurities in antifungal Drug product of 5% Topical solution. The optimum separation was achieved on reverse phase HPLC reverse phase column (250 mm X 4.6 mm, 3 µm) with Perchloric acid (TFA), methanol and acetonitrile as mobile phase using simple gradient elution technique, and with UV detection analysis which can helps in quantification of impurities during shelf life. All impurities were separated with good resolution and preparation of Tavaborole was exposed to various environments like acid treatment, alkali treatment, peroxide treatment, photo, thermal irradiation and injected in the developed analytical method to assess the stability indicating nature. Detection was monitored using PDA detector, this method was found to be sensitive, precise, and accurate with detection limit is below 0.02µg/mL, limit of quantification 0.05µg/mL for all its impurities.

KEYWORDS: Tavaborole, Related substances, Method development, HPLC, stability, Topical solution.

INTRODUCTION
Tavaborole topical 5%v/v solution was invented by Anacor Pharmaceutical, inc, Palo Alto, CA and it has been approved by Food and drug administration in July 2014.[1] Tovaborole is novel, very small molecule chemical structured used to treat the toenails onychomycosis disease.[2] Onychomycosis is a chronic fungal infection in nail and it may be caused by many infection agents such as dermatophytes, yeast and molds. Onychomycosis is characteristically categorized by discolored, thickened and disintegrating nails that may be detached from nail bed.[3,4] The unpleasant appearance of onychomycosis is base of Psychological distress for the patient and Patient may cause to loss of self-possession, depression, anxiety and social inaccessibility.[5,7] Anacor pharmaceuticals has been taken in the USA (US patent Nos. 7 582 621 and 7 767 567), Australia, New Zealand, South Africa and Russia which covers the methods of using tavaborole treat onychomycosis and formulations contains the Tavaborole's.[8]

Chemistry of Tavaborole is belongs to family of oxaboroles which are boron contained chemical compounds. The IUPAC name of Tavaborole is 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole with molecular formula and molecular weight are $C_7H_6BFO_2$ and 151.93Da respectively. This low molecular weight of tavaborole allows high amount of penetration to high thickness of human nails than any other antifungals such as Terbinafine, Fluconazole, Iitraconazole and etc. 5-fluoro group enhance the antifungal activity of the compound and 1-hydroxyl group attached to 1-phenyl group enhance water solubility than other benzoxaboroles.[9]

Being a successful novel antifungal drug, Tavaborole receiving importance in terms of clinical usage as well as research interests pertaining to analytical purposes, literature review in this domain yields few articles which highlight these studies. Tracey Vlahovicet al stated that they used HPLC method for quantification of Tavaborole in in-vitro release test, but no discussions about method in details.[10] Tampucci, Silvia, et al developed for quantification of Tavaborole in in vitro studies samples.[11] Quantification of Tavaborole impurities by HPLC is not published.

However, no method are reported in public domain as well as in USP, EP and any other pharmacopeia for estimation of impurities.

The current study covers the novel development of valid analytical method for related substances with boron functional groups such as Tavaborole related impurities in API and 5% topical solutions is economic. The stability indicating method of this analytical method provides extra advantage for determination of any possible new impurities which may arise during the shelf-life and metabolites during clinical studies.

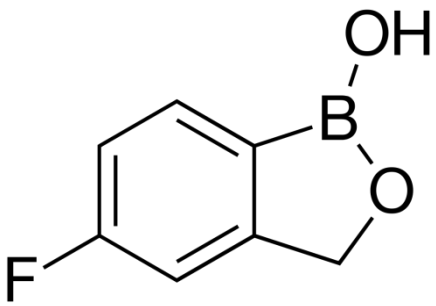

Fig. 1: Tavaborole structure.

MATERIALS AND METHODS

Materials

Perchloric acid (70-72%) EMSURE® was purchased from Merck, Billerica, USA. Acetonitrile HPLC grade, Methanol HPLC grade and Tetrahydrofuran (THF) were purchased from Merck specialties Pvt limited, Mumbai, and India. ~35% Hydrochloric acid, emplura was purchased from Finar chemicals limited, Ahmedabad, India. HPLC grade water was used from in-house water purification systems Thermo scientific TKA, Germany and Mill-QR, Millipore (India) private limited, Bangalore, India. Other standards, excipients and samples were provided by Dr. Reddy's Laboratories Ltd., Hyderabad, India.

Methods

Instrumentations and chromatographic conditions

Waters HPLC system (Waters Corporation, USA) equipped with photo diode-array (PDA) detector was used. The data were acquired via Empower 2 software Built 2154 from Waters Corporation. 0.05% v/v Perchloric acid in water was used as mobile phase A, and 0.05% v/v Perchloric acid in acetonitrile, Methanol and THF in the ratio of 50: 47:03 respectively were used as mobile phase B. Total a 60 minutes run time gradient elution, the ratio of time (min) /mobile phase B (% v/v) changes as 0/25, 5/25, 40/50, 50/50, 52/25 and 60/25 with constant flow rate of 1.0 mL/min. The injection volume was 10 µL and the detection wavelength was set at 210 nm for Tavaborole, Debromo impurity, Desfluro impurity, Methyl impurity, Methanol impurity, Dimer impurity and Benzyl acetate impurity. Samples were maintained at 22°C within the system and analysis was performed at 40°C using Phenomenex Luna phenyl-hexyl column, 250 mm length, 4.6 mm inner diameter and with 3 µm particle size (part no: 00A-4040-B0) (Phenomenex Inc., USA).

Standard and sample preparations

Standard solutions preparations

Standard stock solution (200 ppm)

20 mg of Tavaborole standard was weighed and transferred in to 100 mL volumetric flask. 75 mL of 8:2 ratios of water and mixture of acetonitrile and methanol (50:50) as diluent added to it and sonicated for 5 minutes to dissolve and made up the volume with diluent.

Diluted standard solution for RS (2 ppm)

2 mL of standard stock solution was transferred into 200 mL volumetric flask and made up the volume with diluents.

Sample solutions preparations

2 g of Tavaborole topical solution 5%w/w was weighed into 100 mL volumetric flask and 50 mL of diluent was added to it. This was sonicated for 15 minutes and finally made up the volume with diluent. Some portion of sample solution was centrifuged at 2000 rpm for 15 minutes and filtered through 0.22µ PTFE filter with minimum of 3 mL discarded volume.

RESULTS AND DISCUSSIONS

Method Development

Selection of mobile phase

Tavaborole Log P value is 1.28 and pKa value is 8.36 gets ionization in basic solution. Acidic mobile phase can control ionization of Tavaborole and it was retained in reverse phase chromatography. The combination of 0.05% of perchloric acid in water as solution A and 0.05% perchloric acid in Acetonitrile and methanol in the ratio of 50:50 solutions achieved the better separation among the Tavaborole and its related impurities.

Column selection

Column choice is the most critical factor for separation of similar nature peaks such as related impurities in LC method development.[12] Since their high polar nature in protonated form in basic pH, it is not easy to maintain control retention and separate the acidic and small molecules in reverse phase hydrophobic stationary phases such as C18 and C8.[13] Methyl impurity was not well separated from Tavaborole peak in inertsil ODS 3V 150 mm x 4.6 mm, 3 µm column as well as in Agilent eclipse plus C18 100 mm x 4.6 mm, 3.5 µm column. All impurities separated from each other along with Tavaborole peak achieved in Phenomenex Luna phenyl hexyl 150 mm x 4.6 mm, 3 µm columns which offers not only bulky dispersion interactions but also π-π exchanges with phenyl group in Tavaborole and its impurities.

Diluent selection

Tavaborole is small polar molecule which can soluble in combination of water and polar organic solvents such as Methanol and Acetonitrile. Tavaborole 5% propylene glycol medium solution and it is also soluble in water. Hence mixture of water and 50:50 ratios of acetonitrile and methanol used as diluent and it is well compatible

with initial mobile phase gradient composition in order to gives symmetrical peaks.

Stress study and selectivity

Samples were treated to stress environments of (a) heating at 90°C for 4 Hrs, (b) exposure to UV light at 200 W.h/m^2 for 1.2 million lux hours, (c) treatment with 2 N Hydrochloric acid (HCl) at 80°C for 2 Hrs, (d) 2 N Sodium hydroxide (NaOH) at 80°C for 2 Hrs, and (e) 6% Hydrogen peroxide (H2O2) at 80°C for 2 Hrs. Treated samples were then analysed by HPLC-PDA. The Selectivity was established by spiking the concentrations of impurities in the sample. No interference due to blank and placebo were observed at the peaks of interest. The homogeneity of the peaks was ensured. All interested peaks have passed the criteria of peak purity in wavelength range of 200 nm to 400 nm. Results of stress study indicate that no significant degradation was observed. However, an unknown RRT at 0.40 was found to be 100% and main peak completely degraded when the sample was subjected to peroxide treatment. Stress condition of peroxide was optimised to 1% Hydrogen peroxide (H2O2) at room temperature for 1 Hrs and the unknown impurity found to be 9.8% at 0.40 RRT. Peak purity of Tavaborole peak was ensured to pass the criteria of purity angle less than the purity threshold.

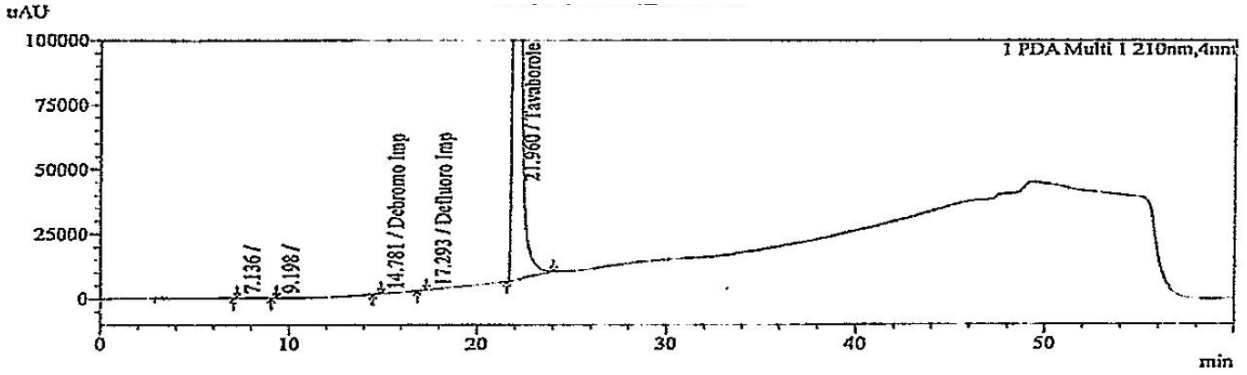

Fig. 2: Control sample chromatogram.

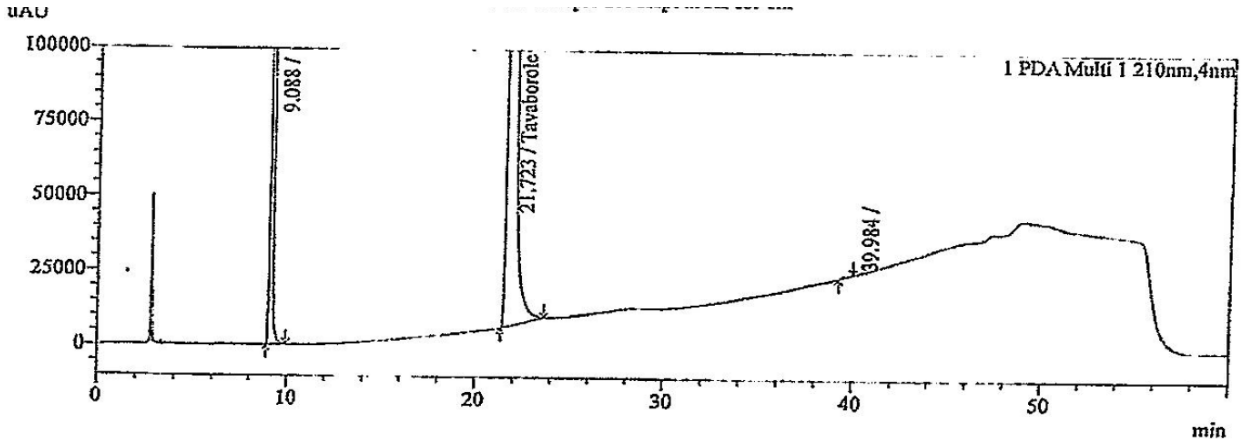

Fig. 3: Peroxide stress sample chromatogram.

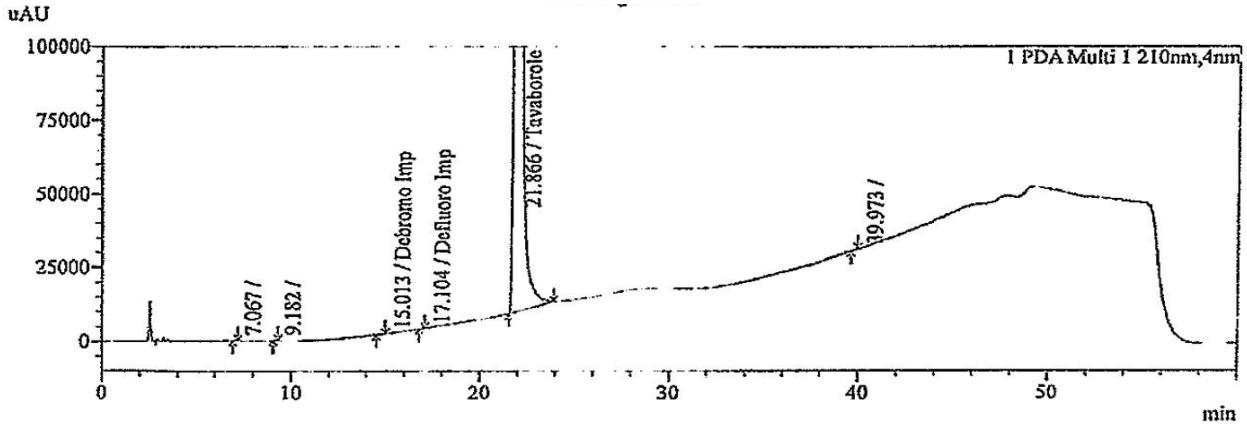

Fig. 4: Acid stress sample chromatogram.

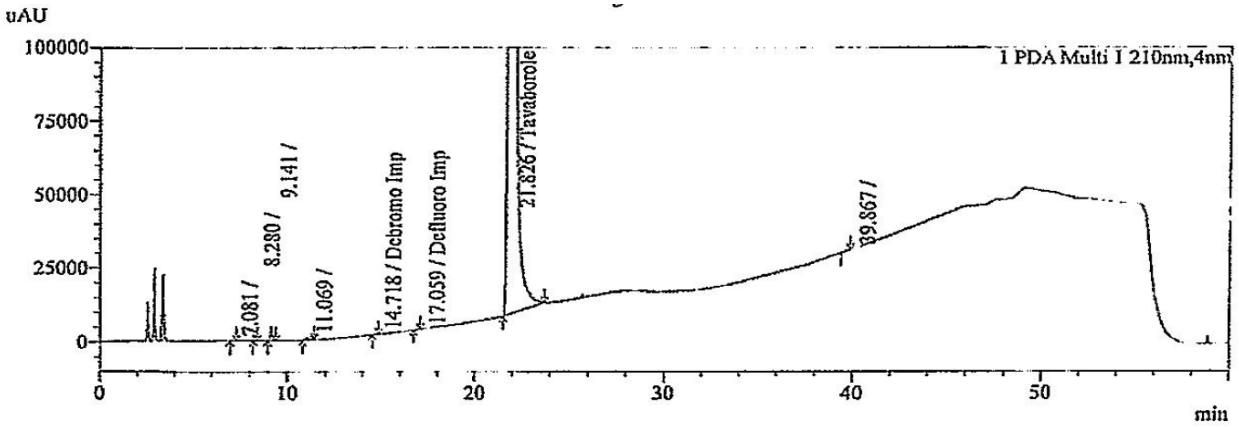

Fig. 5: Base stress sample chromatogram.

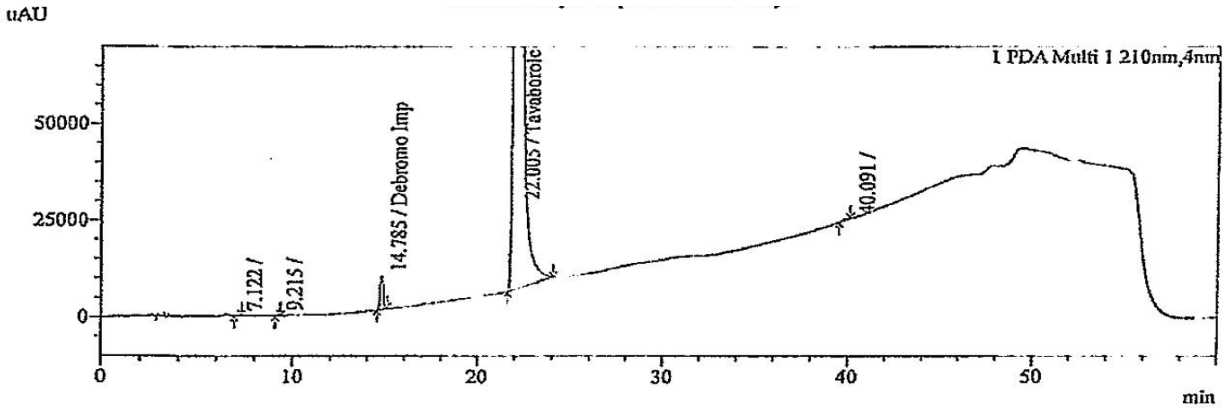

Fig. 6: Heat stress sample chromatogram.

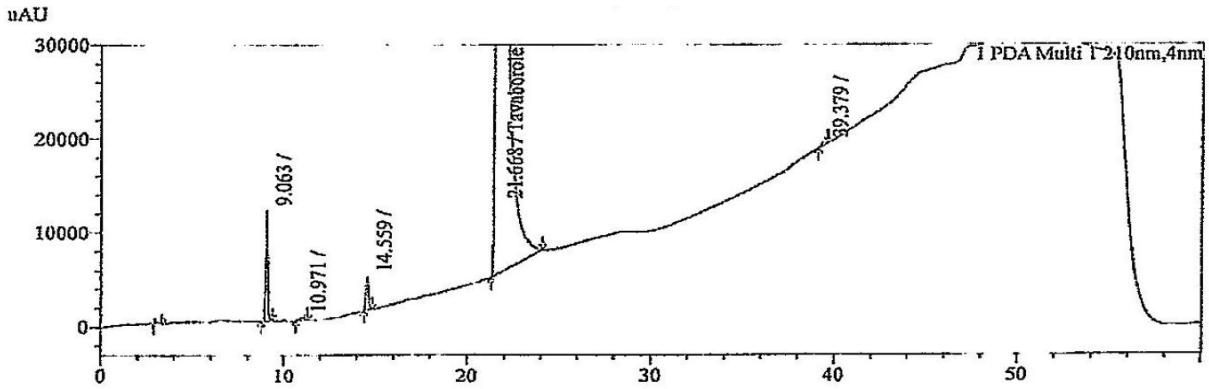

Fig. 7: Light stress sample chromatogram.

Method Validation

Validation was performed as per ICH guideline Q2 (R1) [14] and USP <1225>.

Precision (Repeatability)

Precision was accomplished by spiking the known impurities to the sample solution at 100% level with six intervals and measured the Mean % Recovery and % RSD. Results met with criteria, depicted in table 2.

Linearity

Linearity of the individual known impurities were determined along with Tavaborole at 0.05, 0.6, 1, 2, 2.5, 3 and 4 ppm levels and plotted individual linearity curves against the respective concentrations. The solutions were injected and peak area responses were plotted against concentrations. The correlation coefficient(r) was found to be more than the acceptance criteria of 0.999. Relative response factors were calculated from the ratio of individual impurity slope to the Tavaborole slope and the y-intercept values tabulated in table 2.

LOQ and LOD

LOQ and LOD were predicted from linearity calibration curve, i.e. LOQ was 0.05 ppm whereas LOD was 0.02 ppm for all impurities and Tavaborole as well. Accuracy at LOQ was determined; all impurities along with Tavaborole were found in between 90% to 110% and results depicted in table 2.

Accuracy

The accuracy of the RS method was determined and anticipated by spiking the known impurities individually to sample at concentrations of 50%, 100%, and 200% with respect to 2 ppm. The recovery values were calculated (shown in Table 2) and it was found that the results were falling within the acceptance criteria from 90% to 110%.

Robustness

The robustness of the method was evaluated by deliberately changing the chromatographic parameters individually. The concentration of organic solvents were changed in mobile phase-B from 50:50 (Acetonitrile and methanol) to 50:45 and then to 50:55. Column temperature was changed in the range of 40°C ± 2°C and the flow rate was altered in the range of 1.0±0.1 mL/min. No significant change was observed on System suitability test (SST). Hence, the chromatography method was found to be robust within the working range.

Ruggedness

The ruggedness was demonstrated by testing the system suitability on an advanced Agilent HPLC (Agilent technologies, Germany) equipped Ultra violet (UV). The data was acquired via Empower 2 software Built 2154 from Waters Corporation**,** ruggedness results are tabulated in table 2.

Table 2: Summary chart of the validation.

Component	Precision (%RSD)			Relative response factor (RRF)	Accuracy				Linearity
					Recovery (%)				
	Repeatability	Ruggedness	LOQ		50%	100%	150%	LOQ	Correlation (r)
Debromo impurity	0.8	0.9	2.9	1.2	106.4	100.9	98.9	108.7	0.9998
Desfloro impurity	1.2	1.3	1.3	1.3	100.6	97.2	97.3	102.0	0.9997
Methyl impurity	1.3	0.8	3.0	1.0	98.0	101.0	102.8	95.6	0.9993
Methanol impurity	0.7	1.1	1.3	1.0	101.0	96.4	97.1	106.8	0.9997
Dimer impurity	1.6	1.2	0.9	2.4	101.3	96.8	96.1	104.9	0.9999
Benzyl impurity	2.3	1.6	6.8	0.8	98.1	101.6	94.4	100.4	0.9991
Tavaborole surrogate standard	1.1	0.6	2.7	Not applicable	101.5	102.0	101.6	102.8	0.9993

Solution stability

The sample and standard solutions were stable up to 3 days as confirmed with the results obtained from time defined injections. The percentage difference in the areas of impurities and Tavaborole at initial time point and final time point are below 2%.

Filter variability

Filter variability has been performed using Polyvinylidene fluoride (PVDF) 0.45µ, PVDF 0.2µ, Polytetrafluoroethylene (PTFE) 0.45µ, PTFE 0.22µ, Nylon 0.45µ and Nylon 0.2µ filters. It was observed that the variability was not more than 0.2% in comparison to the unfiltered sample with PTFE. However, less than 2% decrease in the area of Tavaborole and its impurities was observed with PVDF and Nylon**.**

CONCLUSIONS

The novel stability indicating HPLC method was precise, linear, accurate, specific and robust for quantification and identification of Tavaborole and its related impurities in Tavaborole 5% topical solution. The applicability of the method for identification, screening and estimation of impurities of Tavaborole obtained from various processes of Tavaborole synthesis and topical pharmaceutical products such as solution using the same method. Moreover, this method realizes the benefit of minimum solvent usage is economic, environment friendly and cost effectiveness.

This method can be useful for easy evaluation of any unknown impurities which may form in tavaborole drug substances and formulations which may arise during the product development, shelf-life and metabolites during clinical studies which can be useful for safe medication to healthy needs.

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