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Research Article

Method development and validation of a new stability indicating HPLC and LC-ESI-MS/MS methods for the determination of Tavaborole

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Abstract

Tavaborole, a topical antifungal agent containing Boron is used for the treatment of onychomycosis, an infection of the nail and nail bed caused by *Trichophyton rubrum* or *Trichophyton mentagrophytes* infection. Tavaborole is chemically known as 5 - Fluoro -1,3 - dihydro -2,1-benzoxaborol -1-ol. It acts by inhibiting Leucyl-tRNA synthetase an essential fungal enzyme required for protein synthesis. AB SCIEX Instruments LC-ESI-MS/MS (Model no. 5068379-Y) QTRAP Enabled Triple Quad 5500+ with Agilent Zorbox C18 (150 mm x 4.6 mm x 3 µm) column and PDA detector was employed for the present study. The total run time was 10 mins and the detection wavelength was 254 nm. A mixture of 5 mM Ammonium formate: Methanol (30: 70) was used as mobile phase on isocratic mode with 1 ml/min as flow rate. Tavaborole has shown linearity over the concentration range 0.5-100 µg/ml and the proposed method was validated as per ICH guidelines. The proposed method is found to be simple, precise, accurate and suitable for the quantification of the marketed formulations of Tavaborole. Stress degradation studies were performed and the method is found to be selective and specific.

Keywords: Tavaborole, Stability indicating, LC-ESI-MS/MS, Validation.

INTRODUCTION

Tavaborole (Figure 1) is an antifungal agent used for the fungal infection of nail and nail bed. FDA has given approval for the treatment of onychomycosis[1-3] in July 2014. Tavaborole ($C_7H_6BFO_2$; Mo. Wt. 151.93 g/mol) is chemically 5 - Fluoro -1,3 - dihydro -2,1-benzoxaborol -1-ol. It acts by inhibiting an essential fungal enzyme required for protein synthesis. This inhibition of protein enzyme (aminoacyl transfer ribonucleic acid) synthesis leads to cell growth termination and causes death of the cell which finally eliminates the fungal infection.

Tavaborole was earlier studied by spectrophotometry[4-5] and HPLC[6-7] and in the present study a new stability indicating LC-APCI-MS/MS method has been proposed for the estimation of Tavaborole and the method was validated as per ICH guidelines[8].

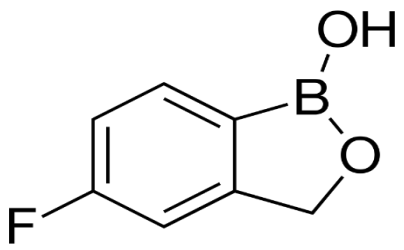


Figure 1: Chemical structure of Tavaborole

MATERIALS AND METHODS

Tavaborole API was obtained as gift sample from Zydus Lifesciences Ltd (India) and it is available as 5% topical solution from Alembic Pharma, Viona Pharmaceuticals etc.

Instrumentation

AB SCIEX Instruments Linear Ion Trap Quadrupole LC-MS/MS Mass Spectrometer (Model no. 5068379-Y) QTRAP Enabled Triple Quad 5500+ with ExionLC Binary Gradient AD Pump, Autosampler AD Autosampler, Column Oven AC Column Oven and Agilent column PDA detector was employed for the present study. Agilent ZORBOX C18 (150 mm x 4.6 mm x 3 µm) column was employed and the injection volume was 20 µL and the total run time was 10 mins (Detection wavelength 254 nm). A mixture of 5 mM Ammonium formate: Methanol (30: 70) was used as mobile phase on isocratic mode with 1 ml/min as flow rate. 0.1% Formic acid: Acetonitrile was used as mobile phase on gradient mode with flow rate was 1 ml/min.

MS Conditions

AB SCIEX Instruments

Triple Quad 5500+ QTRAP

Model	:5068379-Y
Ionization mode	:ESI positive

Source temp.	:650ºC
Source type	:Turbo spray
Heater gas (GS1)	: 60 ºC)
Nebulizer gas (GS2)	:65 ºC)
Collision energy	:35 V

Preparation of stock solution

25 mg of Tavaborole API was accurately weighed and carefully transferred into a 25 ml volumetric flask and was dissolved in HPLC grade Methanol (1000 µg/ml) and this stock solution was sonicated for 30 mins and then diluted with the diluent as per the requirement.

Method validation[8]

0.5-100 µg/ml Tavaborole solutions were prepared from the stock solution (1000 µg/ml) on dilution with the mobile phase consisting of a mixture of 5 mM Ammonium formate: Methanol (30: 70) and each solution was injected (n=3) into the system and the average peak area from the respective chromatograms was calculated. A calibration graph was drawn by plotting the concentration of the drug solutions on the x-axis and the corresponding peak area of the chromatograms on the y-axis. The intraday precision studies were conducted on the same day at different equal time intervals and the inter-day precision studies were conducted on three successive days (Day 1, Day 2 and Day 3) and the % RSD was calculated. Accuracy studies were performed by spiking the formulation solution with 50%, 100% and 150% API solution and thereby the percentage recovery was calculated with the help of regression equation. The percentage relative standard deviation was calculated in all the validation parameters.

Assay of Tavaborole

Tavaborole is available as topical solution (Label claim: 5%) Alembic Pharmaceuticals Ltd, Viona Pharmaceuticals, Zydus Lifesciences Ltd, Encube Ethicals Pvt. Ltd. in India. Two different brands of Tavaborole were collected and extracted with methanol and after sonication diluted with the mobile phase as per the requirement. The resulting solution was filtered through 0.24 µm membrane filter and 10 µL of these formulation solutions were injected in to the HPLC system. The peak area of the chromatogram (n =3) was noted and the percentage purity was determined.

Stress degradation studies[9]

During the acidic degradation study Tavaborole solution was heated with 0.1N HCl at 70ºC for about 30 mins and neutralized with 0.1N NaOH solution. The contents were diluted with mobile phase and the resultant solution was injected into LC-MS/MS system and the peak area of Tavaborole was noted from the respective chromatogram and the mass spectrum was recorded.

During the acidic degradation study Tavaborole solution was heated with 0.1N NaOH at 70ºC for about 30 mins and neutralized with 0.1N HCl solution. The contents were diluted with mobile phase and the resultant solution was injected into LC-MS/MS system and the peak area of Tavaborole was noted from the respective chromatogram and the mass spectrum was recorded.

During the thermal degradation study Tavaborole solution was heated at 70ºC for about 30 mins and the contents were diluted with mobile phase and the resultant solution was injected into LC-MS/MS system and the peak area of Tavaborole was noted from the respective chromatogram and the mass spectrum was recorded.

During the oxidative degradation study Tavaborole solution was heated with hydrogen peroxide at 70ºC for about 30 mins and then diluted with mobile phase and the resultant solution was injected into LC-MS/MS system and the peak area of Tavaborole was noted from the respective chromatogram and the mass spectrum was recorded.

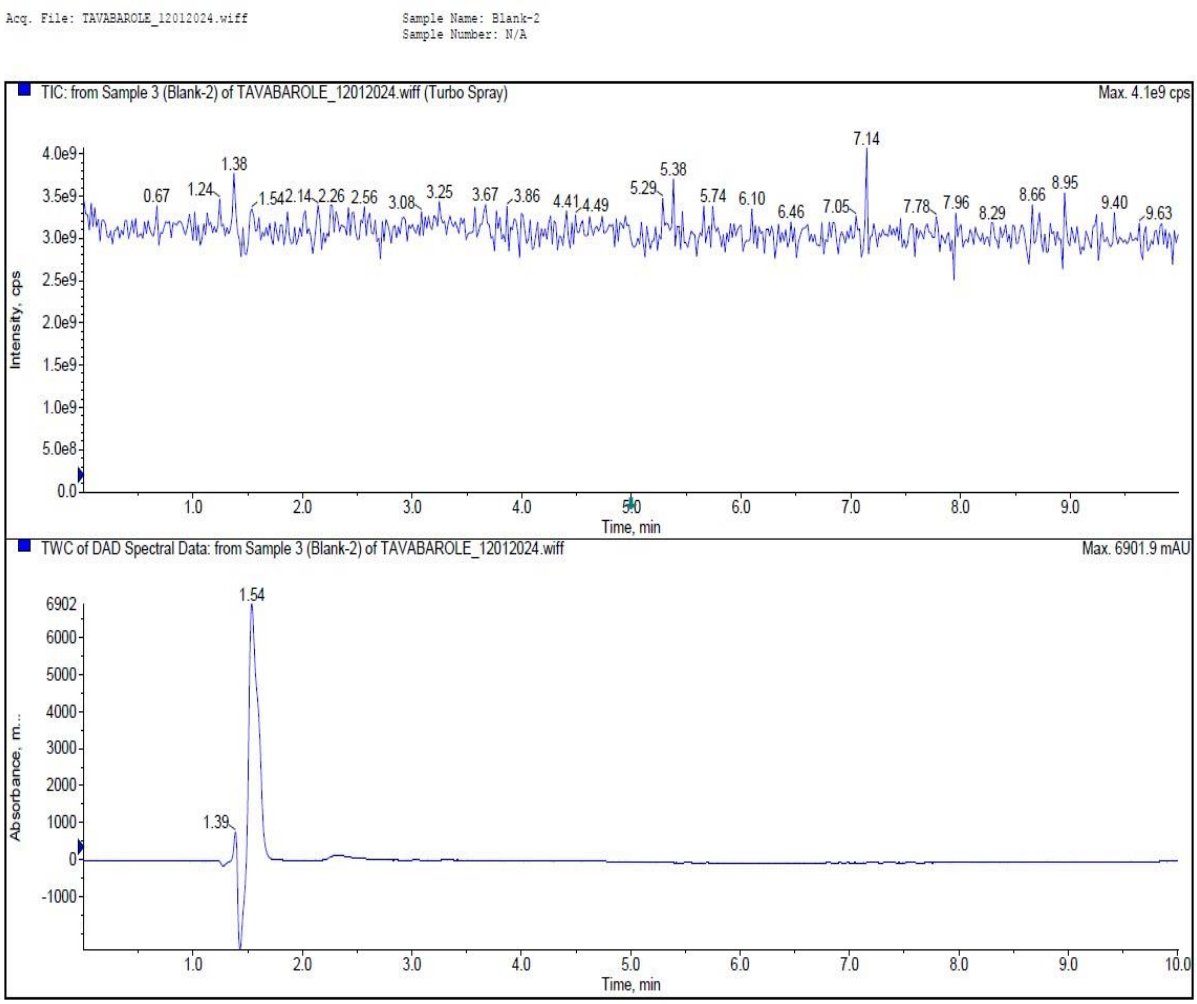
RESULTS AND DISCUSSION

A new stability-indicating LC-ESI-MS/MS method has been proposed for the estimation of Tavaborole in pharmaceutical formulations using AB SCIEX Instruments LC-ESI-MS/MS (Model no. 5068379-Y) QTRAP Enabled Triple Quad 5500+ with Agilent Zorbox C18 (150 mm x 4.6 mm x 3 µm) column and PDA detector. The total run time was 10 mins and the detection wavelength was 254 nm. A mixture of 5 mM Ammonium formate: Methanol (30: 70) was used as mobile phase on isocratic mode with 1 ml/min as flow rate. A brief review of the reported methods was summarized and some of the parameters were compared with the present proposed method and the details were given in Table 1.

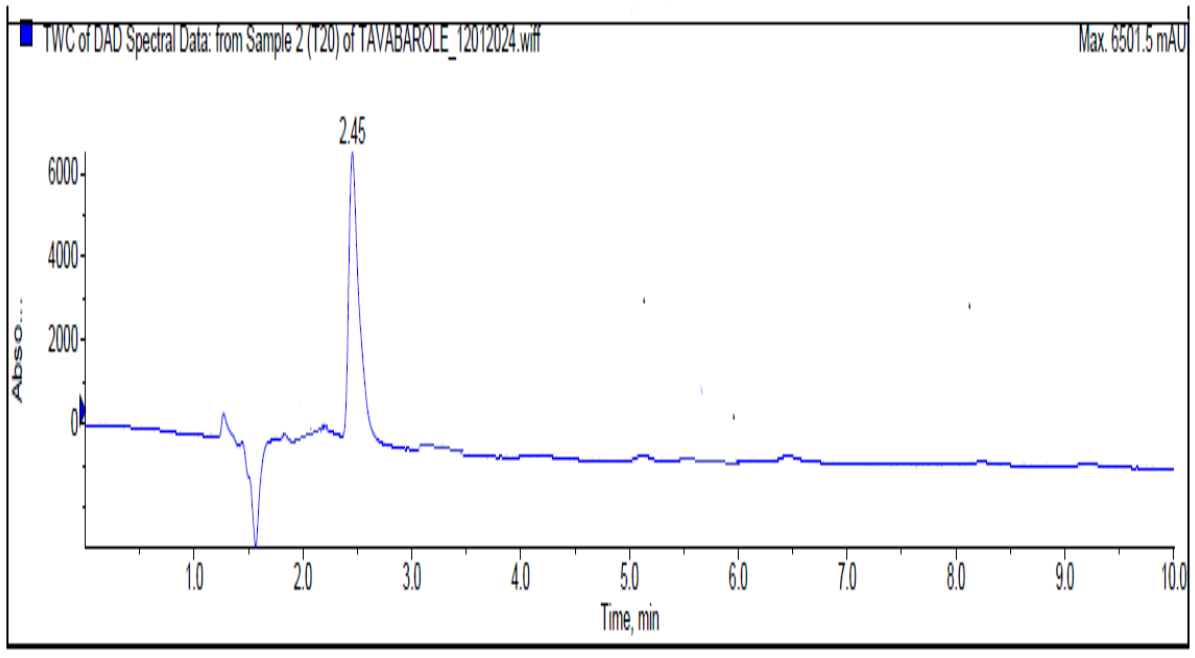
Table 1: Literature survey

Method	Mobile phase (v/v) / Reagent	λ_{max} (nm)	Linearity (µg/mL)	Ref
Spectrophotometry	Distilled water	272 265	20-100	4
Spectrophotometry	Methanol, Water, HCl, NaOH, Phosphate buffers (pH 2.0, 4.0 & 7.0) Borate buffer (pH 9.0)	271	1-100 1-80	5
RP-HPLC	10 mM Phosphoric acid (pH 2.0): Acetonitrile (70:30)	220	-	6
RP-HPLC (Gradient mode)	Methanol: Acetonitrile (50:50) 0.05% of aq. Perchloric acid: 0.05% Perchloric acid in Acetonitrile & Methanol (50:50)	-	0.05- 4	7
LC-ESI-MS/MS	10 mM Ammonium formate: Methanol (30: 70) (Isocratic mode)	254	0.5-100	Present method

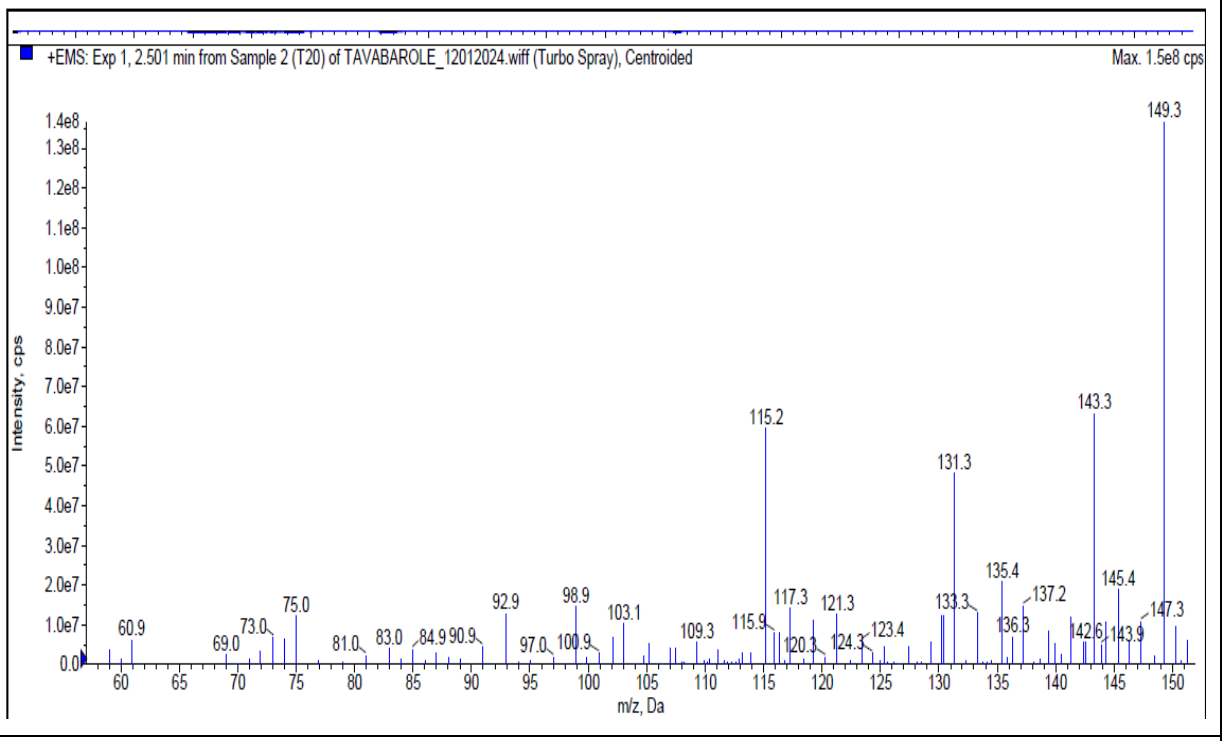
Tavaborole was eluted at Rt 2.45 min with theoretical plates more than 2000 and tailing factor less than 1.5 and the corresponding chromatograms of blank, Tavaborole API and the mass spectrum of Tavaborole with the optimized chromatographic conditions were shown in Figure 2.



Blank



Chromatogram of Tavaborole (API) (20 µg/ml); Rt 2.45 min



Mass spectrum of Tavaborole (M+: m/z 151.95)

Figure 2: Typical chromatograms and mass spectra of Blank & Tavaborole (API)

Linearity, Precision, Accuracy and Robustness

Tavaborole obeys Beer-Lambert's law over the concentration range 0.5-100 µg/ml (Table 2) and the linear regression equation was found to be y = = 3316.9x + 79.061 (R^2 = 0.9999) Figure 3). The LOD and LOQ values were found to be 0.4129 µg/ml and 1.3721 µg/ml respectively.

The % RSD in intraday precision (0.52), interday precision (0.62-0.82) (Table 3) was found to be less than 2.0% stating that the method is precise. In the accuracy study the % RSD was found to be 0.27-0.72 (<2) (Table 4) with a recovery of 99.58.99-99.92 indicating that the method is accurate.

Table 2: Linearity

Conc. (µg/ml)	*Mean peak area
0	0
0.5	1773
1	3339
5	16601
10	33238
20	66483
40	132791
50	167021
80	263158
100	333015

*Mean of three replicates

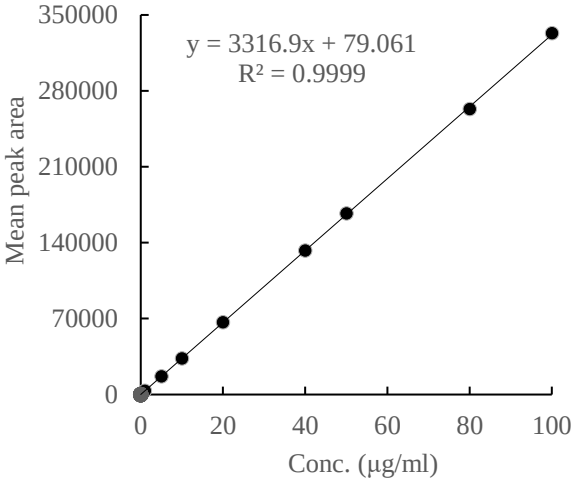


Figure 3: Calibration curve

Table 3: Precision study

Intraday precision study		
Conc. (µg/ml)	**Mean peak area**	***Mean peak area ± SD (% RSD)**
10	33238	33233.5 ± 172.81 (0.52)
10	33291	
10	33206	
10	33224	
10	33219	
10	33223	

Interday precision study				
Conc. (µg/ml)	**Day 1**	**Day 2**	**Day 3**	***Mean peak area ± SD (% RSD)**
10	33238	33238	33238	33225.33 ± 262.48 (0.79)
50	167021	166984	167172	167059 ± 1369.88 (0.82)
100	333015	332984	333133	333044 ± 2064.87 (0.62)

*Mean of three replicates

Table 4: Accuracy study

Spiked conc. (µg/ml)	Formulation (µg/ml)	% Recovery	% RSD
5 (50 %)	10	99.58	0.27
10 (100 %)	10	99.92	0.61
15 (150 %)	10	99.69	0.72

*Mean of three replicates

Assay of Tavaborole

The assay of Tavaborole topical solution was performed using the proposed method with the optimized conditions and the percentage of purity of Tavaborole was found to be 98.20-99.20 (Table 5).

Table 5: Assay of Tavaborole

S. No.	Brand name	Label claim (5%)	*Observed amount (mg/ml)	% Recovery*
1	Brand I	4.96	0.992	99.20
2	Brand II	4.91	0.982	98.20

*Mean of three replicates

Stress degradation studies

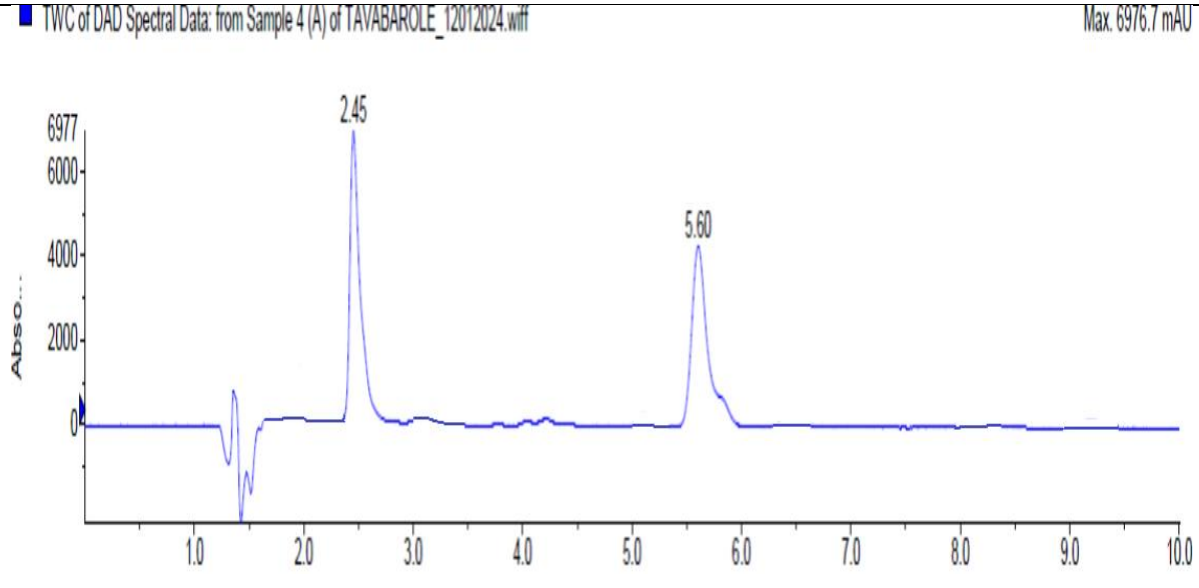
Tavaborole (20 µg/ml) was exposed to different stress conditions under the optimized chromatographic conditions and then injected in to the system.

During the acidic degradation, Tavaborole was eluted at Rt 2.45 min and a degradant was eluted at 5.60 mins and about 31.78 % has undergone decomposition. The mass spectra of Tavaborole and its degradant were shown in Figure 4.

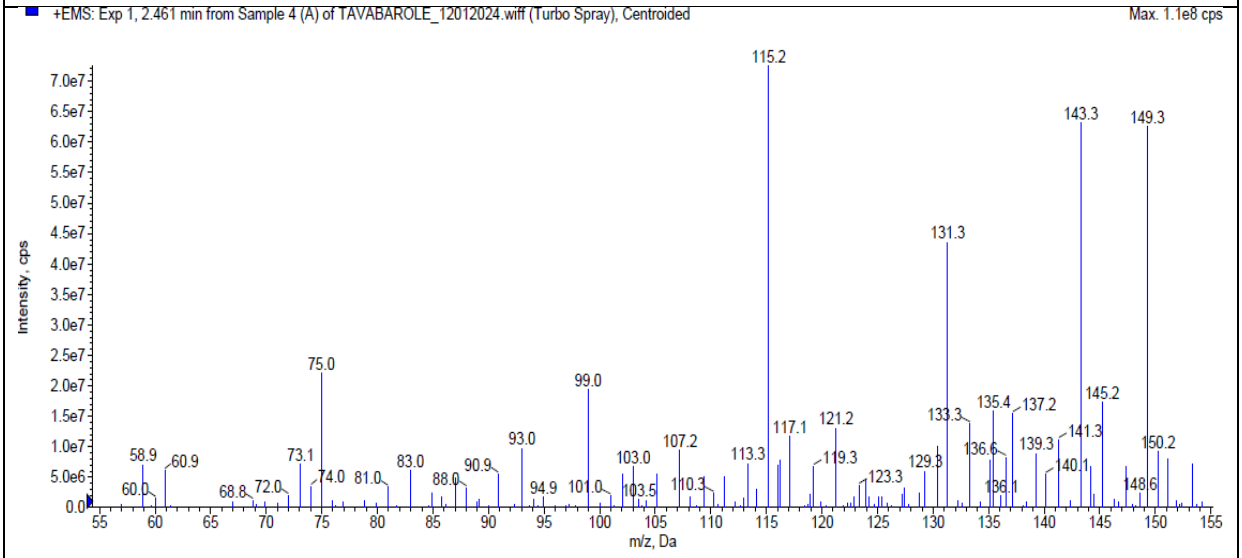
Table 6: Stress degradation studies

Condition	R_t (min)	*Mean peak area	% Recovery*	% Drug degradation
Standard drug	2.45	66483	100	-
Acidic hydrolysis	2.45	45353	68.22	31.78
Thermal degradation	2.45	63469	95.47	4.53
Alkaline hydrolysis	2.45	49360	74.25	25.75
Oxidative degradation	2.39	40189	60.45	39.55

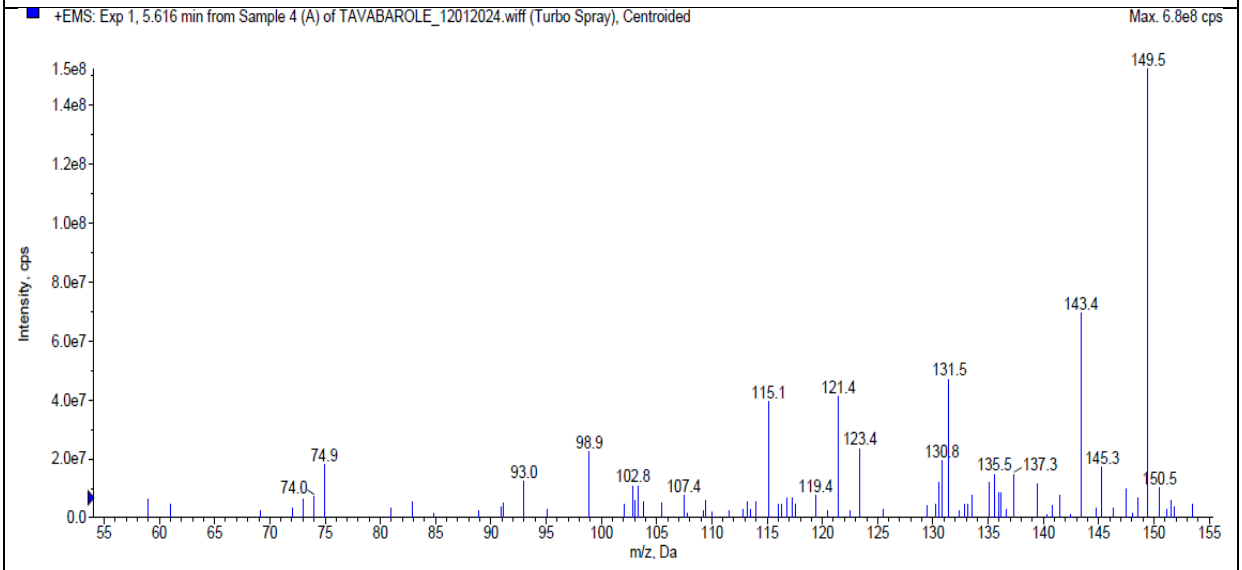
*Mean of three replicates



Typical chromatogram of Tavaborole (Rt 2.45 min) (Degradant at Rt 5.60 min)



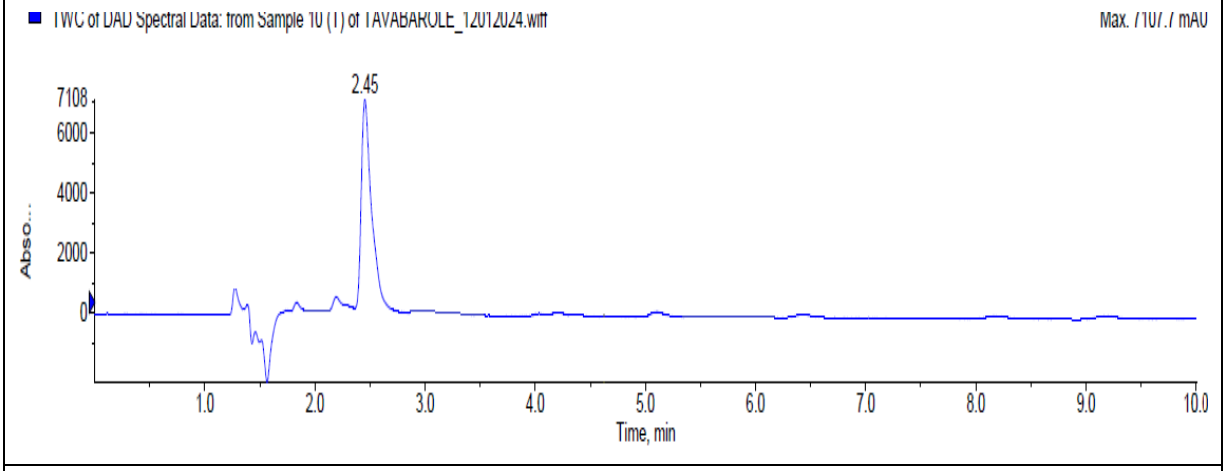
Mass spectrum of Tavaborole (Rt 2.461 min)



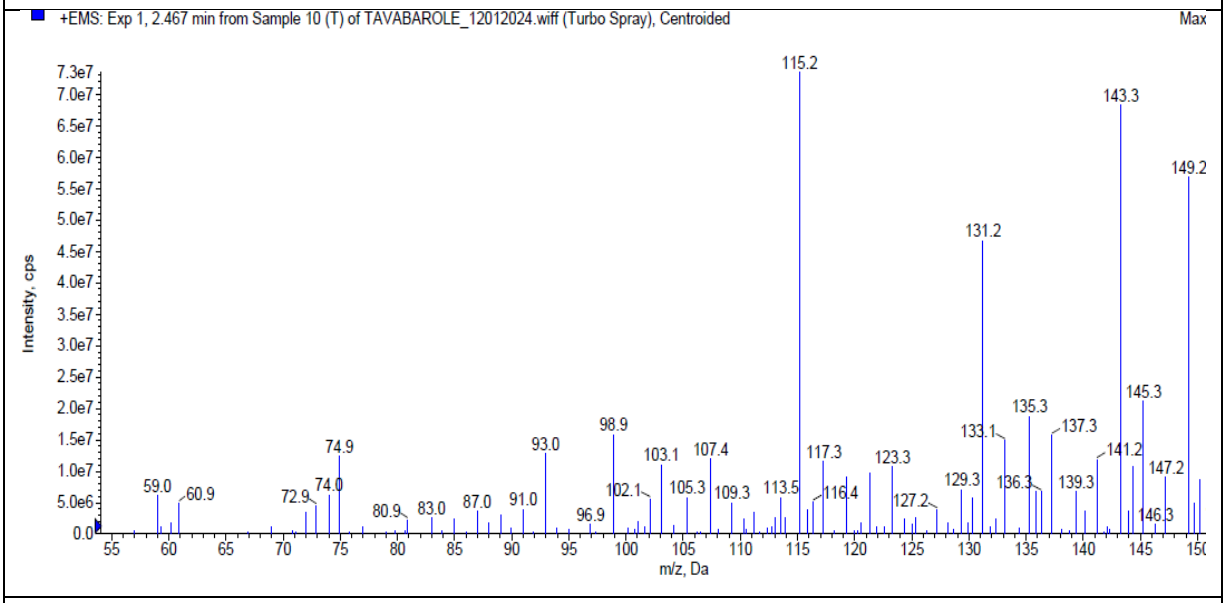
Mass spectrum of Tavaborole degradant (Rt 5.616 min)

Figure 4: Typical chromatogram and mass spectra of Tavaborole during acidic degradation

During the thermal degradation, Tavaborole was eluted at Rt 2.45 min and no degradants were observed. about 4.53 % has undergone decomposition. The mass spectra of Tavaborole was shown in Figure 5.



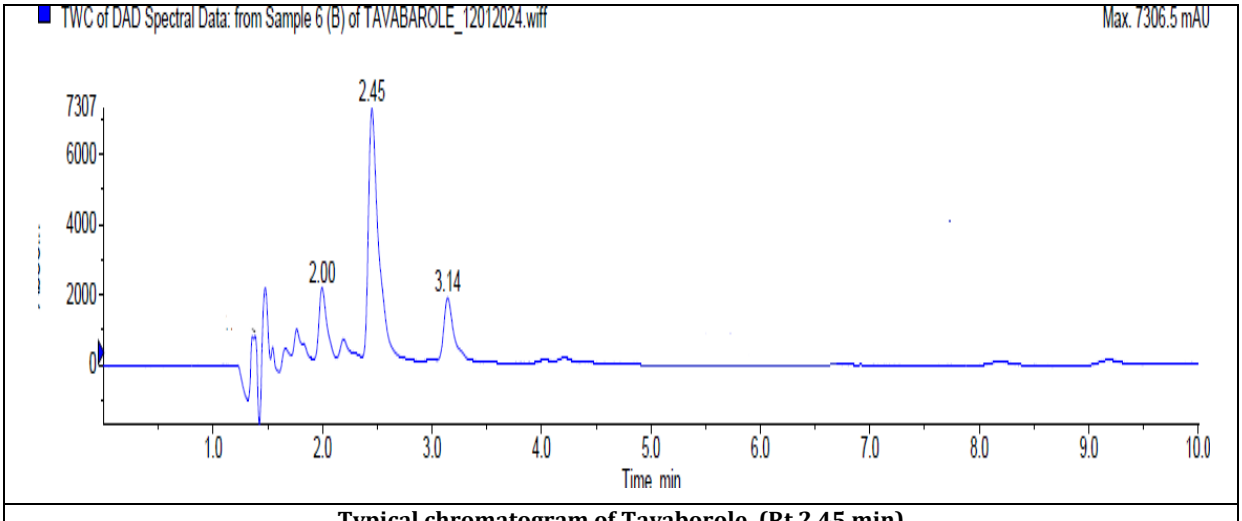
Typical chromatogram of Tavaborole (Rt 2.45 min)



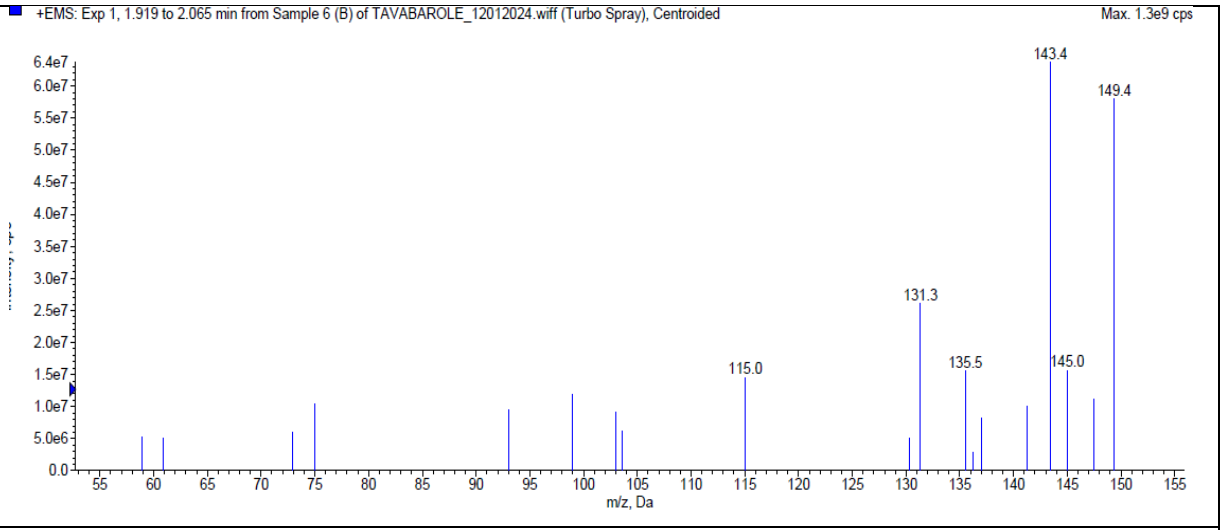
Mass spectrum of Tavaborole (Rt 2.467 min)

Figure 5: Typical chromatogram and mass spectrum of Tavaborole during thermal degradation

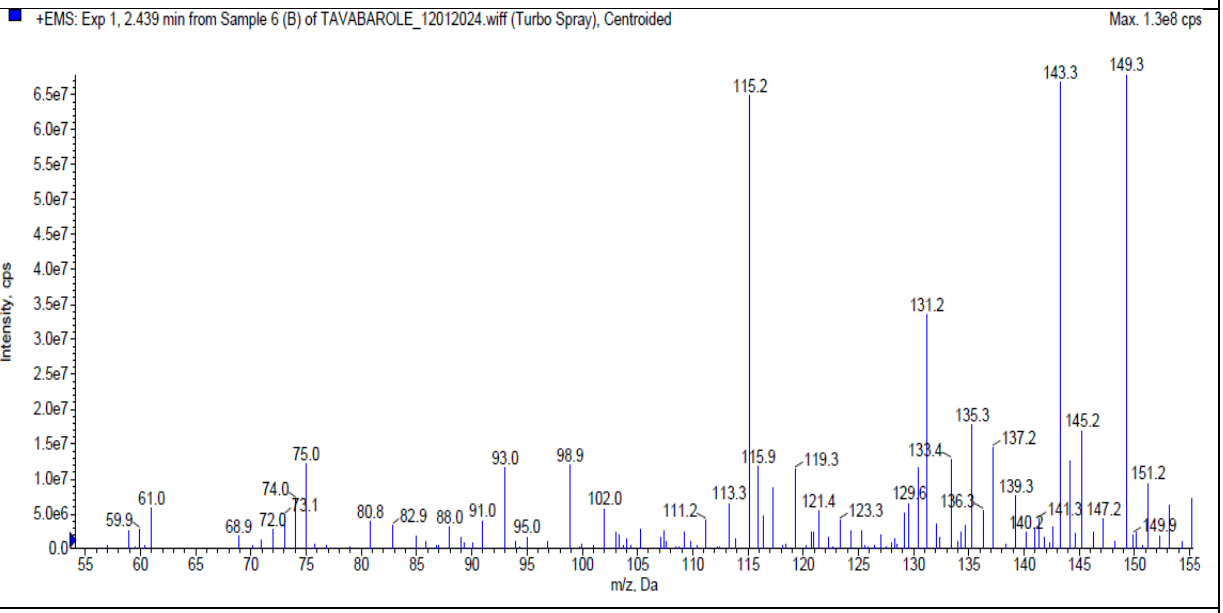
During the basic degradation, Tavaborole was eluted at Rt 2.45 min and two degradants were eluted at 2.00 mins and 3.14 mins. about 39.55 % has undergone decomposition. The mass spectra of Tavaborole and its degradants were shown in Figure 6.



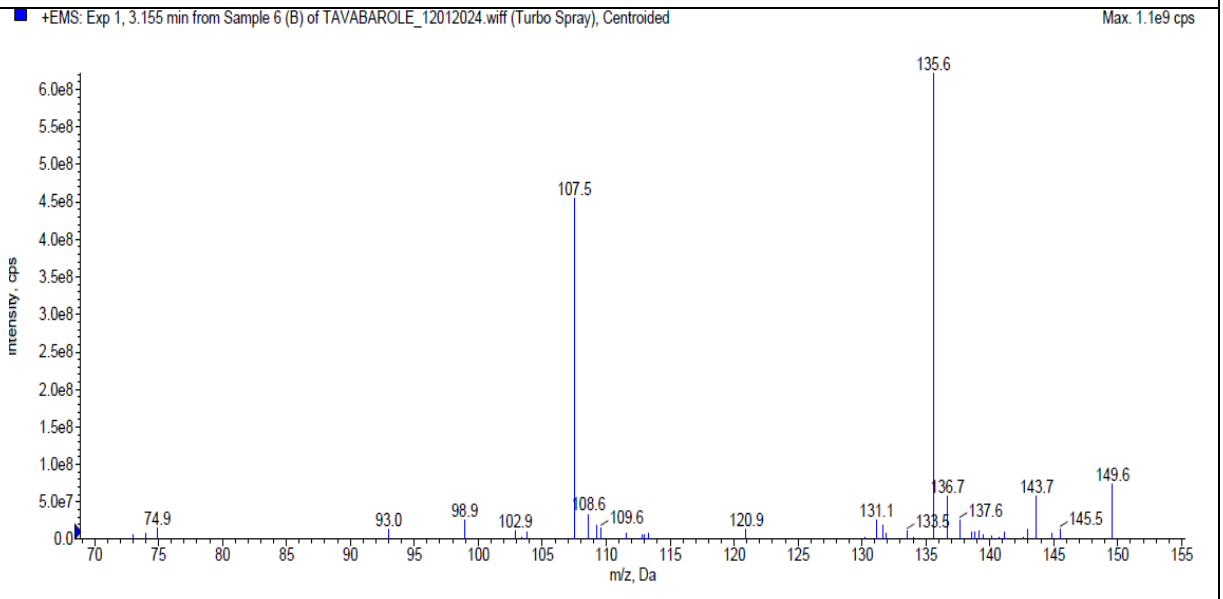
Typical chromatogram of Tavaborole (Rt 2.45 min)



Mass spectrum of Tavaborole degradant (Rt 1.919-2.065 min)



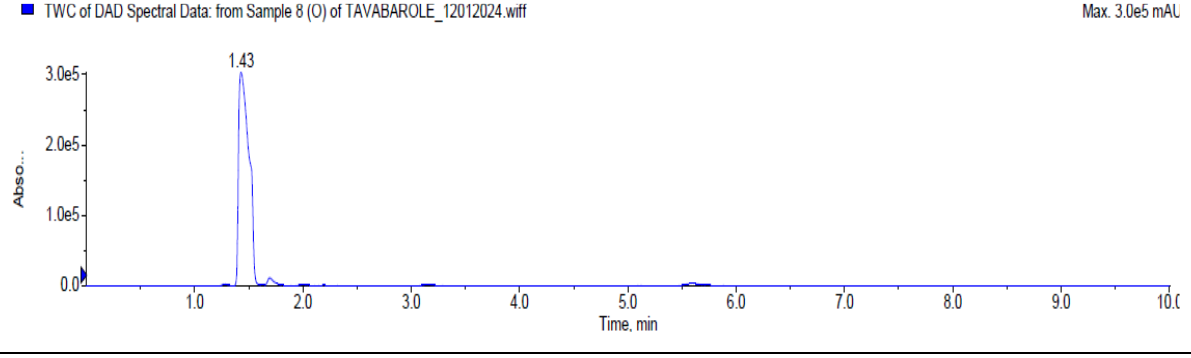
Mass spectrum of Tavaborole (Rt 2.439 min)



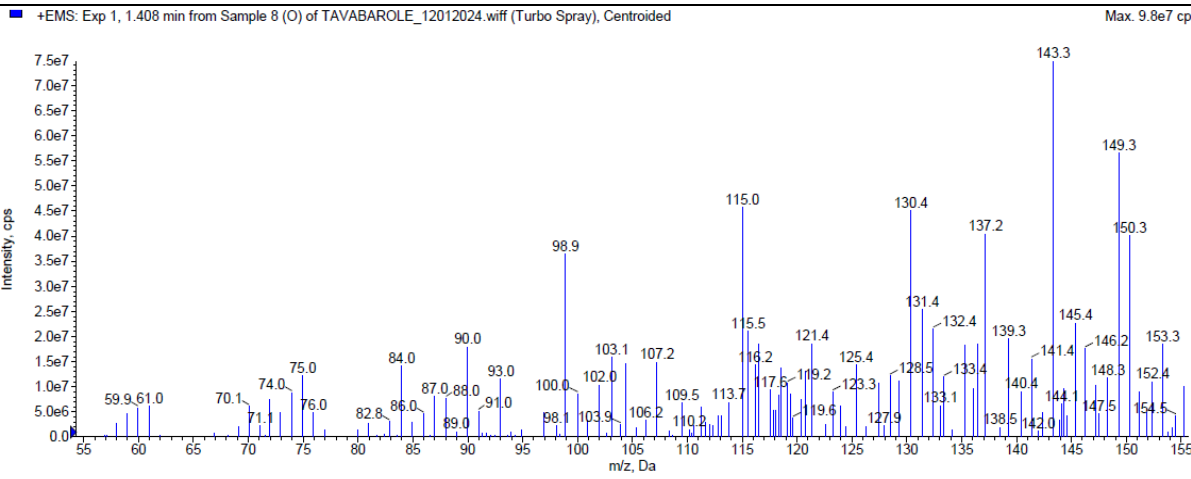
Mass spectrum of Tavaborole degradant (Rt 3.155 min)

Figure 6: Typical chromatogram and mass spectrum of Tavaborole during basic degradation

During the oxidative degradation, Tavaborole was eluted at Rt 2.45 min and a degradant was eluted at 1.43 mins. about 39.55 % has undergone decomposition. The mass spectra of Tavaborole and its degradant were shown in Figure 7.



Typical chromatogram of Tavaborole (Rt 2.45 min) (Degradant at Rt 5.60 min)



Mass spectrum of Tavaborole degradant (Rt 1.408 min)

Figure 7: Typical chromatogram and mass spectra of Tavaborole during Oxidative degradation

The details of the stress degradation studies of Tavaborole were shown in Table 6. It is observed that Tavaborole is highly sensitive towards the proposed acidic (31.78 %), alkaline (25.75 %) and oxidative conditions (39.55 %) and less than 5% degradation was observed during thermal degradation (4.53%).

CONCLUSION

The authors have developed a new stability indicating LC-ESI-MS/MS method for the estimation of Tavaborole and the method is simple, precise and accurate and used for the routine analysis of Tavaborole in pharmaceutical formulations and no interference of excipients was observed during the assay.

Conflicts of Interest: The authors declare no conflict of interest.

Acknowledgement: The authors are grateful to Zydus Lifesciences Ltd (India) for providing the gift samples of Tavaborole and the authors declare no conflict of interest.

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