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Development and validation of a stability indicating uplc method for determination of Voriconazole in pharmaceutical formulation

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ABSTRACT

Simple, rapid, sensitive, accurate, robust & rugged stability indicating analytical method for determination of voriconazole in pharmaceutical formulations is developed and validated by using UPLC & applied the developed and validated method for determining the assay of voriconazole in tablets (Vfend®), as there is no official monograph & no analytical method by UPLC. Chromatography was performed with mobile phase containing sodium dihydrogen ortho phosphate & acetonitrile in the ratio of 50:50,adjusted to pH 5.50±0.05 with dilute NaOH, with a flow rate of 0.5mL/min, C-18 column & UV detection at 254nm.The method was validated for linearity, accuracy, ruggedness, robustness, precision & bench top stability of sample & standard solution. Voriconazole tablets were subjected to different stress conditions like acid, alkali, peroxide, thermal, water & UV studies and checked for its specificity, degradation &stability. The developed method was very rapid with a run time of 1 min, accurate, robust, rugged and stable.

Keywords: Voriconazole, Assay method, UPLC, Stability indicating method.

INTRODUCTION

Voriconazole is designated chemically as (2*R*,3*S*)-2-(2,4-difluorophenyl)-3-(5-fluoro-4-pyrimidinyl)-1-(1H-1,2,4-triazol-1-yl)-2-butanol with an empirical formula of $C_{16}H_{14}F_3N_5O$ and a molecular weight of 349.3.(Fig.1)[1]. Voriconazole (Vfend) (Pfizer) was approved in 2002 for the treatment of invasive aspergillosis, fusarium and scedosporium infections as well as the treatment of resistant candidiasis. It is referred to as a second generation triazole[2].Like other

azole antifungal agents, such as fluconazole and itraconazole, its primary mode of action is by inhibiting of the fungal cytochrome P450-dependent 14α-sterol demethylase, an essential enzyme in ergosterol biosynthesis [3].Voriconazole is moderately lipophilic (log D7.4=18) and a single diastereomer with R- and S-stereochemistry by virtue of two chiral centers (2R, 3S) as shown in Fig.1. Voriconazole is solid, white to off-white powder with a pKa of 2.72 & 11.54, melting point at 128.5^{o}C and boiling Point at 508.6°C at 760 mm Hg[4,5]. It is freely soluble in acetone and in methylene chloride, soluble in methanol and in chloroform, very slightly soluble in water [6].A few methods for the determination of voriconazole in pharmaceutical formulations by HPLC and UV appear in literature. So far no systematic UPLC method has been reported for determination of voriconazole in pharmaceutical formulations. This paper reports a rapid and sensitive UPLC method with UV detection, useful for routine quality control of voriconazole in pharmaceutical formulations. The method was validated by parameters such as linearity, accuracy, precision, robustness, ruggedness, sample and standard solution stability and forced degradation studies[7].

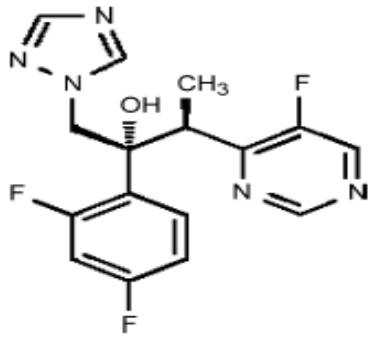


Fig.1.

MATERIALS AND METHODS

Reagents

HPLC grade Acetonitrile (HPLC Grade, Merck), Sodium dihydrogen orthophosphate (AR, Fisher), Hydrochloric Acid (AR, Rankem) Sodium hydroxide (AR, Rankem),hydrogen peroxide (AR, Rankem),Water (Milli Q water). Voriconazole pure drug substance was kindly supplied by MSN Laboratories Limited, India. Ingredients used for placebo were Lactose monohydrate, pregelatinized starch, cross carmellose sodium, povidone, magnesium stearate.

Instrumentation

A liquid chromatograph (Waters Acquity) system equipped with an injection valve (Rheodyne), & PDA detector. The UPLC system was well equipped with Empower 2 software for data processing. Other instruments like Sartorius Analytical Balance, Metrohm pH Meter and Biotechnics sonicator were used in sample and standard preparations and for forced degradation studies.

Chromatographic conditions:

The analytical column used was Waters, BEH C-18, 50*2.1, 1.7µm .The mobile phase was sodium dihydrogen ortho phosphate & acetonitrile in the ratio of 50:50, adjusted to pH

5.50±0.05 with dilute NaOH, with a flow rate of 0.5mL/min, injection volume of 1µL, column oven temperature of 40°C, run time of 1 min, with isocratic elution, sample tray temperature was ambient & UV detection at 254nm.

Standard, sample, mobile phase and diluent preparation:
Diluent: 10ml of Milli Q water was taken in to a 100mL volumetric flask and made up to the mark with Acetonitrile and used as diluent.

Mobile Phase: Dissolved 7.80g of sodium dihydrogen orthophosphate in 1 litre Milli Q water and adjusted the pH to 5.50±0.05 with diluted sodium hydroxide solution. Filtered through 0.22µm filter. Mixed the buffer and acetonitrile in the ratio of 50:50 and sonicated for 5 minutes to degas.

Standard Preparation: 50.0mg of voriconazole was accurately weighed in to a 100mL volumetric flask dissolved with diluents and made up to the mark with the same. Further 10mL of the above solution was diluted to 25mL with diluent and filtered through 0.45µm filter.

Sample Preparation: Weighed 20 tablets and determined the average weight. Crushed the tablets in to a fine powder in mortar and pestle. Accurately weighed the sample equivalent to 50.0mg of Voriconazole in to a 100mL volumetric flask and added 70mL of diluent and sonicated for 20min with intermittent shaking. Allowed it to cool to room temperature and made up to the mark with diluent and filtered through 0.45µm filter. Further 10mL of the above solution was diluted to 25mL with diluent.

RESULTS AND DISCUSSION

Specificity:
Specificity was demonstrated by injecting a blank, placebo and standard solution. No interference was seen at the retention time of analyte. The specificity was also demonstrated by induced degradation of voriconazole formulation and placebo samples to acid degradation, alkali degradation, peroxide degradation, thermal degradation, water degradation, U.V. degradation. Purity angle is less than purity threshold for all the stress conditions. The results are tabulated in Table No.:1.Figures 2-13 represents different stress conditions.

System suitability Testing:
System suitability testing is used to verify that the reproducibility of the system is adequate for the analysis to be performed. System suitability is done by preparing and injecting the standard solution 5 times and calculating its RSD. Other parameters like tailing and theoretical plates should also be taken in to consideration. Results are tabulated in Table No.:2

Linearity:
The linearity of the test method was performed by plotting a graph between concentration of the test solution on X-axis and response of the corresponding solutions on Y-axis from 50% to 150% of test concentration and calculated the correlation coefficient, it was found to be 0.999.The results are tabulated in Table No.:3 and the graphs are represented as Fig No.:14,15,16.

Limit of detection (LOD) and limit of quantification (LOQ):
Calculated the LOD & LOQ, with the calculations obtained from evaluation of the calibration curve of the linearity.

LOD and LOQ values are less than the minimum linearity concentration. The calculations and results are tabulated in Table. No.:4

Bench top stability of standard & test preparation:
Performed the assay of voriconazole as per the test method in duplicate and kept the standard and test solutions on the bench top for 48 Hrs. Injected at initial, 24 Hrs and 48 Hrs. Calculated the difference between initial and bench top stability samples for % assay of voriconazole for test solutions and similarity factor for standard solutions were found to be with in limits. The results are tabulated in Table No.:5

Accuracy:
Performed the accuracy of test method using voriconazole placebo at 50%, 75%, 100%, 125%, 150% spike levels. The % assay at each spike level was found to be between 98.0-102.0% of the labeled amount. The results are tabulated in Table No.:6

Table No.:1

VORICONAZOLE FORCED DEGRADATION		
Stress Condition	**Purity Angle**	**Purity Threshhold**
Acid Stress	23.379	46.69
Alkali Stress	20.269	49.83
Peroxide Stress	21.762	48.245
Water Stress	19.475	49.318
Heat Stress	20.407	49.452
U.V. Stress	0.353	0.76
Acceptance Criteria	Peak Purity shall pass	

Table No.:2

VORICONAZOLE SYSTEM SUITABILITY									
Injection No.:	1	2	3	4	5	Mean	STDEV	RSD	*Limits*
Standard Area:	522174	522671	520511	522265	521998	521924	827	0.2	RSD NMT 2.0%
USP tailing	1.54	1.54	1.54	1.54	1.54	1.54	0	0.0	*NMT 2.0*
RT	0.454	0.454	0.454	0.454	0.454	0.454	0	0.0	

Method precision:
Determined the precision of the test method by preparing & injecting 6 test solutions of voriconazole formulations in to the chromatograph and recorded the results. The average % assay was found to be 101.3 with % RSD of 0.13. The results are tabulated in Table No.:7

Intermediate precision:
Performed the assay of voriconazole by following the same procedure as that of Method precision but on a different day and by a different analyst. The average % assay was found to be

100.6% with % RSD of 0.32.Overall RSD when compared with Method precision is 0.43. The results are tabulated in Table No.:8&9

Table No.:3

VORICONAZOLE-LINEARITY						
Run	% Conc.	Conc. Of voriconazole	Area of voriconazole	Slope	Y-intercept	R^2
1	50%	100.78	263727	2587.3	3700.6	1.000
	75%	151.17	396140			
	100%	201.56	524857			
	125%	251.95	655241			
	150%	302.34	786056			
2	50%	100.78	263527	2596.2	2421.2	1.000
	75%	151.17	396682			
	100%	201.56	525082			
	125%	251.95	654533			
	150%	302.34	788708			
3	50%	100.78	263599	2636.8	-10032	0.998
	75%	151.17	374414			
	100%	201.56	525829			
	125%	251.95	656403			
	150%	302.34	786941			
Average				2606.763908	-1303.4	0.9994361
Standard Deviation				26.37	7586.21	0.00
Acceptance criteria: Coefficient of correlation shall be NLT 0.999						

Table No.:4

VORICONAZOLE- Limit of detection (LOD) & Limit of Quantification (LOQ)				
S.No.	Injection No.	Slope	Y-Intercept	R^2
1	Inj-1	2587.3368	3700.6	0.999
2	Inj-2	2578.3151	4221.2	0.999
3	Inj-3	2636.7791	-10032	0.998
Average		2600.8103	-703.4000	0.9987
STDEV		31.475	8082.997	0.001
LOD=3.3 x σ/S				
σ = Standard deviation of y-intercepts of regression line				
S= slope of the linearity curve				
LOD	10.3	ppm		
LOQ=10 x σ/S				
σ = Standard deviation of y-intercepts of regression line				
S= slope of the linearity curve				
LOQ	31.1	ppm		
Acceptance Criteria: LOD & LOQ values shall be less than the minimum linearity concentration				

Table No.:5

VORICONAZOLE STANDARD AND TEST SOLUTION STABILITY					
Time (Hrs)	Std. Wt.	Response	Fresh Std. Wt.	Response Fresh Std.	Similarity Factor
Initial	55.8	573505			
24 Hrs	55.75	576172	55.75	571681	0.99
48 Hrs	55.46	581407	55.46	566411	0.98

Table No.:6

VORICONAZOLE-ACCURACY						
Spike level	Wt.of sample taken in mg	Sample area	mg/mL added	mg/mL found	% Recovery	Average
50%_01	26.5	276975	0.10518	0.10619	101.0	101.1
50%_02	26.44	278182	0.10495	0.10665	101.6	
50%_03	26.49	276359	0.10514	0.10596	100.8	
70%_01	38.37	398364	0.1523	0.15273	100.3	100.3
70%_02	38.41	398944	0.15246	0.15295	100.3	
70%_03	38.4	398406	0.15242	0.15275	100.2	
100%_01	51.04	532640	0.20259	0.20421	100.8	100.8
100%_02	51.02	532789	0.20251	0.20427	100.9	
100%_03	51.06	532193	0.20267	0.20404	100.7	
125%_01	62.51	649035	0.24811	0.24884	100.3	100.3
125%_02	62.49	649559	0.24804	0.24904	100.4	
125%_03	62.52	647703	0.24815	0.24833	100.1	
150%_01	75.58	785193	0.29999	0.30104	100.4	100.4
150%_02	75.55	784430	0.29987	0.30075	100.3	
150%_03	75.55	786894	0.29987	0.30169	100.6	
Acceptance criteria:% Average recovery shall be between 98.0% -102.0%						

Table No.:7

VORICONAZOLE METHOD PRECISION								
Std. wt. & Dilution	50.31 100	10 25	Tablet Wt. 612	Spl. wt. & Dilution	Wt.of sample taken 100	10 25	Label claim (mg) Potency (%)	200 99.5
Std. No.	Standards	USP Tailing	Weight of sample taken	Area of sample	Assay %	Average(%)	STDEV	% RSD
1	522174	1.54	154.90	534066	101.19	101.3	0.12790	0.13
2	522671	1.54	156.63	540529	101.28			
3	520511	1.54	156.60	541290	101.44			
4	522265	1.54	154.90	533425	101.07			
5	521998	1.54	156.67	540506	101.25			
Average	521924	1.54	156.72	541104	101.33			
STDEV	827.47	0.00	Limits	% RSD of 6 replicate injections is notmore than 6				
%RSD	0.2	0.0						

Table No.:8

VORICONAZOLE INTERMEDIATE PRECISION								
Std. wt. & Dilution	50.39	10	Tablet Wt.	Spl. wt. & Dilution	Wt.of sample taken	10	Label claim (mg)	200
	100	25	612		100	25	Potency (%)	99.5
Std. No.	**Standards**	**USP Tailing**	**Weight of sample taken**	**Area of sample**	**Assay %**	**Average(%)**	**STDEV**	**% RSD**
1	516147	1.55	154.60	522491	100.26	100.6	0.322	0.32
2	518410	1.55	155.35	528521	100.93			
3	516354	1.55	155.63	526038	100.28			
4	516531	1.55	154.63	523421	100.42			
5	518292	1.55	155.45	529189	100.99			
Average	517147	1.55	155.45	526495	100.48			
STDEV	1108.43	0.00	Limits	% RSD of six replicate assay results is NMT 2.0.&				
%RSD	0.2	0.0						

Table No.:9

VORICONAZOLE METHOD AND INTERMEDIATE PRECISION COMBINEDLY							
Method Precision			**Intermediate Precision**				
S.No.	**% Drug content**	**S.No.**	**% Drug content**	**Difference**	**Average of both Method & Intermediate precision**	**STDEV of both Method & Intermediate precision**	**%RSD of both Method & Intermediate precision**
1	**101.2**	**1**	**100.3**	**0.9**	**100.9**	**0.434**	**0.43**
2	**101.3**	**2**	**100.9**	**0.4**			
3	**101.4**	**3**	**100.3**	**1.2**			
4	**101.1**	**4**	**100.4**	**0.6**			
5	**101.3**	**5**	**101.0**	**0.3**			
6	**101.3**	**6**	**100.5**	**0.9**			
Limits:Acceptance criteria:Overall RSD when compard with Method Precision should be not more than 2.0%							

Robustness:

Robustness was performed by injecting the voriconazole standard solution in to the UPLC by altering the Flow rate, Column oven temperature and also by changing the pH of the buffer & composition of the organic solvent from the normal chromatographic conditions. The results are tabulated in Table No.:10

TableNo.:10

VORICONAZOLE ROBUSTNESS								
Change in Flow Rate(0.45mL/min)			**Change in Flow Rate(0.55mL/min)**			**Change in Column Oven Temp.(35°C)**		
Std. No.	**Standards**	**USP Tailing**	**Std. No.**	**Standards**	**USP Tailing**	**Std. No.**	**Standards**	**USP Tailing**
1	583610	1.54	1	476503	1.57	1	519129	1.53
2	587293	1.54	2	479864	1.57	2	518659	1.52
3	585444	1.54	3	478923	1.57	3	519390	1.52
4	582025	1.54	4	478136	1.57	4	520220	1.52
5	585179	1.54	5	480015	1.57	5	520230	1.52
Average	584710	1.54	Average	478688	1.57	Average	519526	1.52
STDEV	1990.59	0.00	STDEV	1438.58	0.00	STDEV	690.12	0.00
%RSD	0.34	0.0	%RSD	0.30	0.0	%RSD	0.13	0.3
Change in Column Oven Temp.(45°C)			**Change in pH of Moblie Phase(5.3)**			**Change in pH of Moblie Phase(5.7)**		
Std. No.	**Standards**	**USP Tailing**	**Std. No.**	**Standards**	**USP Tailing**	**Std. No.**	**Standards**	**USP Tailing**
1	520368	1.54	1	515377	1.54	1	516431	1.54
2	519836	1.54	2	515950	1.54	2	517250	1.54
3	521408	1.54	3	516117	1.54	3	515286	1.54
4	522618	1.54	4	515951	1.54	4	516404	1.54
5	521457	1.54	5	512120	1.54	5	517655	1.54
Average	521137	1.54	Average	515103	1.54	Average	516605	1.54
STDEV	1078.56	0.00	STDEV	1691.01	0.00	STDEV	912.27	0.00
%RSD	0.21	0.0	%RSD	0.33	0.0	%RSD	0.18	0.0
Change in Org Phase comp(110%)			**Change in Org Phase comp(90%)**					
Std. No.	**Standards**	**RT**	**Std. No.**	**Standards**	**USP Tailing**			
1	530722	0.437	1	535711	0.51			
2	527971	0.436	2	531482	0.511			
3	534078	0.435	3	531147	0.511			
4	528416	0.435	4	528871	0.511			
5	531186	0.434	5	528107	0.512			
Average	530475	0.44	Average	531064	0.51			
STDEV	2452.43	0.00	STDEV	2971.92	0.00			
%RSD	0.46	0.3	%RSD	0.56	0.14			

Acceptance Criteria:
System suitability should pass

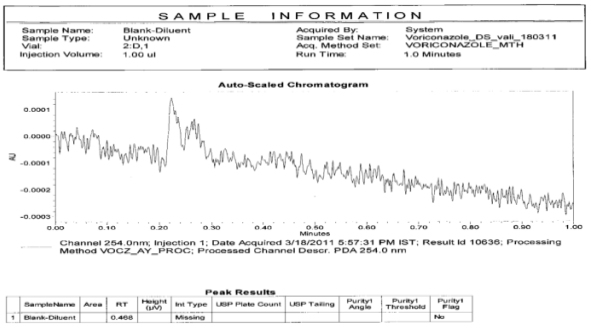


Fig. No.:02 Blank Solution

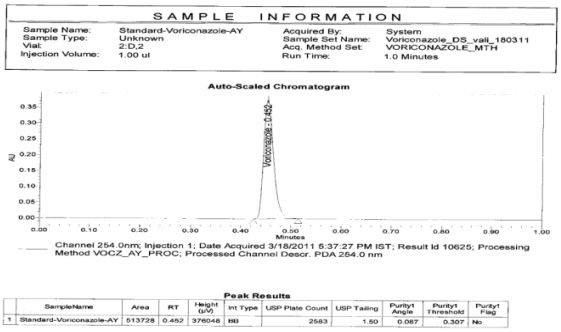


Fig. No.:03 Standard Solution

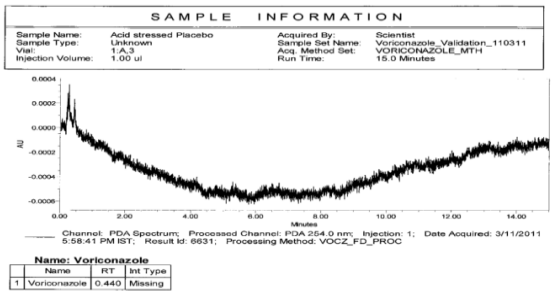
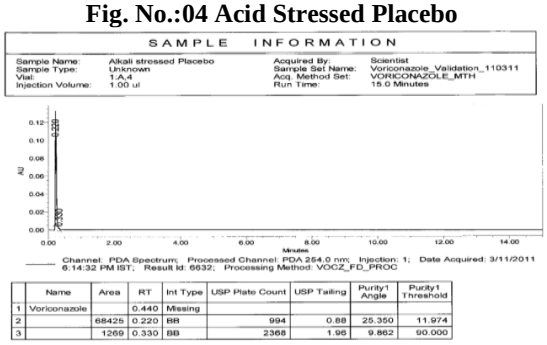
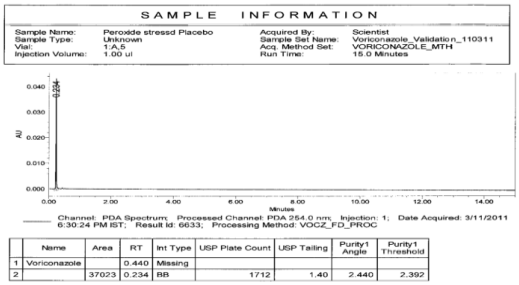


Fig. No.:04 Acid Stressed Placebo



	Name	Area	RT	Int Type	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	Voriconazole		0.440	Missing				
2		68425	0.220	BB	994	0.88	25.350	11.974
3		1269	0.330	BB	2368	1.96	9.862	90.000

Fig. No.:05 Alkali Stressed Placebo



	Name	Area	RT	Int Type	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	Voriconazole		0.440	Missing				
2		37023	0.234	BB	1712	1.40	2.440	2.392

Fig. No.:06 Peroxide Stressed Placebo

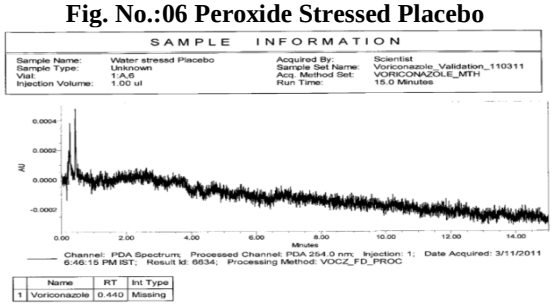
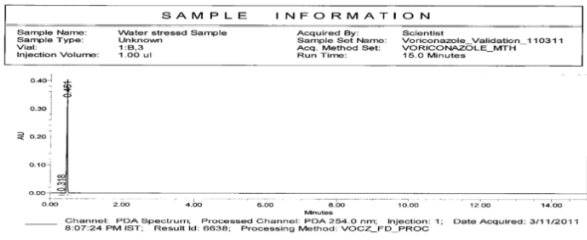


Fig. No.:07 Water Stressed Placebo



	Name	Area	RT	Int Type	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	Voriconazole	552309	0.461	BB	2621	1.52	19.475	49.318
2		1168	0.318	BB	1605	1.41	70.402	90.000

Fig. No.:12 Water Stressed Sample

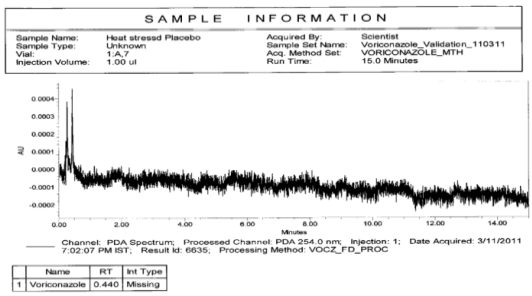
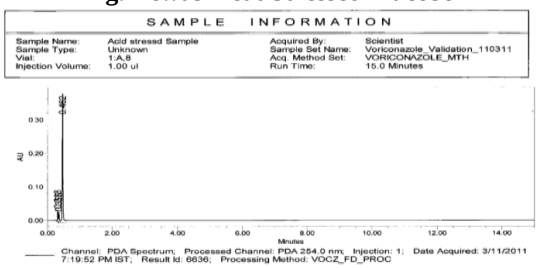
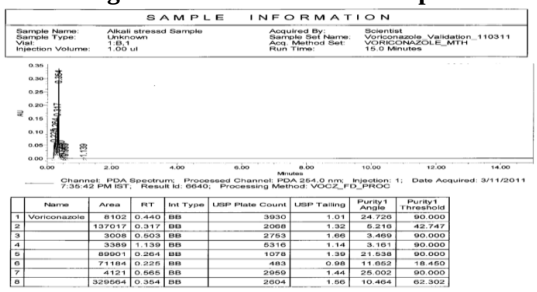


Fig. No.:08 Heat Stressed Placebo



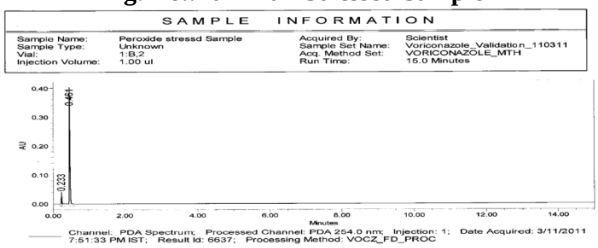
	Name	Area	RT	Int Type	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	Voriconazole	507153	0.462	BB	2741	1.51	23.379	49.690
2		31005	0.318	BB	1970	1.32	45.851	90.000
3		18894	0.355	BB	2826	1.58	71.755	90.000

Fig. No.:09 Acid Stressed Sample



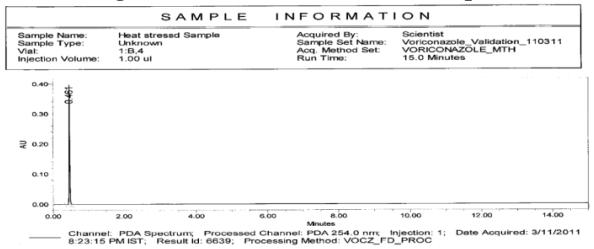
	Name	Area	RT	Int Type	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	Voriconazole	8102	0.440	BB	3930	1.01	24.726	90.000
2		137017	0.317	BB	2088	1.32	5.218	42.747
3		3006	0.503	BB	2753	1.66	3.469	90.000
4		3355	1.139	BB	5316	1.14	3.161	90.000
5		89901	0.264	BB	1078	1.39	21.538	90.000
6		71184	0.225	BB	483	0.98	11.662	18.450
7		4121	0.565	BB	2959	1.44	25.002	90.000
8		329564	0.354	BB	2604	1.56	10.464	62.302

Fig. No.:10 Alkali Stressed Sample



	Name	Area	RT	Int Type	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	Voriconazole	546565	0.461	BB	2684	1.52	21.762	48.245
2		34988	0.233	BB	1698	1.39	24.726	55.108

Fig.No.:11 Peroxide Stressed Sample



	Name	Area	RT	Int Type	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	Voriconazole	554073	0.461	BB	2521	1.51	20.407	49.452

Fig. No.:13 Heat Stressed Sample

Fig. No.:14

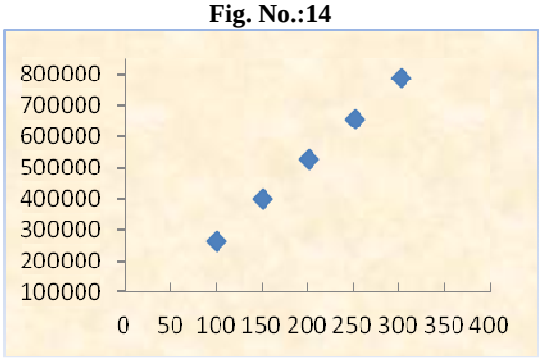


Fig. No.:15

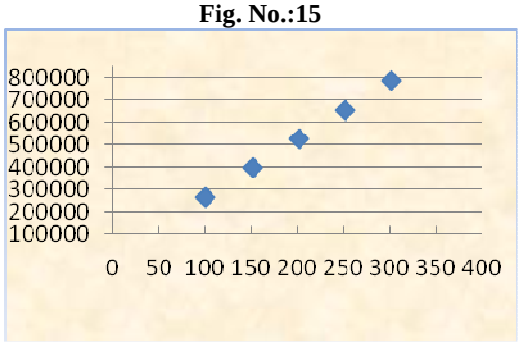
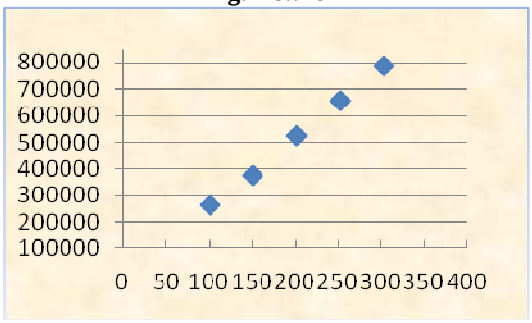


Fig. No.:16



CONCLUSION

The reported UPLC method was proved to be simple, rapid with a runtime of 1 min & reproducible. The validation data indicates good specificity, precision, accuracy & reliability of the method. The developed method has many advantages like isocratic mode of elution, easy sample preparation, short run time and can be used for routine quality control analysis of Voriconazole formulations.

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