WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 8, 266-276. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF VORICONAZOLE AND ITS RELATED SUBSTANCE IN PARENTERAL DOSAGE FORM

Nitika V. Arrawatia*[1], Dr. Chirag J. Patel[2] and Dr. M. M. Patel[3]

[1]Department of Pharmaceutical Quality Assurance, Shree Swaminarayan Sanskar Pharmacy College, Ahmedabad, Gujarat, India.

[2]Professor, Department of Pharmaceutical Quality Assurance, Shree Swaminarayan Sanskar Pharmacy College, Gandhinagar, Gujarat, India.

[3]Principal, Shree Swaminarayan Sanskar Pharmacy College, Gandhinagar, Gujarat, India.

Article Received on
20 Feb. 2018,

Revised on 14 March 2018,
Accepted on 03 April 2018,

DOI: 10.20959/wjpr20188-11819

***Corresponding Author**
Nitika V. Arrawatia
Department of Pharmaceutical Quality Assurance, Shree Swaminarayan Sanskar Pharmacy College, Ahmedabad, Gujarat, India.

ABSTRACT

An Isocratic Reversed-Phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed and validated for the determination of Voriconazole and its related substance in parenteral dosage form. The method is simple, accurate, precise and capable of separating known impurities and degradant impurities from Voriconazole. Chromatographic separation has been achieved on Inertsil C_{18} (250*4.6mm, 5µm) column, mobile phase consisting of Ammonium phosphate dibasic buffer (6.6 gm of Ammonium phosphate dibasic into 1000ml water, adjust pH 6.0 with dilute ortho phosphoric acid): Acetonitrile (55:45%w/v), delivered at flow rate of 1.0ml/min with detection wavelength at 256nm. The drug was subjected to stress condition such as Acid, Base and Oxidative. The Degradation product was well resolved from the main peak and its Impurities and the mass balance was found close to 100.5%. The procedure was validated for Linearity (Correlation Co-efficient = 0.9999), % Recovery was found within the range of 98.60-105.9%. The percentage RSD for precision and accuracy of the method was found to be less than 5%. The method was found to be Robust. This method can be successfully employed for the quantitative analysis of Voriconazole in its parenteral dosage form.

KEYWORDS: Voriconazole, RP-HPLC, Forced Degradation Study, Validation.

INTRODUCTION

Voriconazole is chemically (2R, 3S)-2-(2, 4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1H-1, 2, 4-triazol-1-yl) butan-2-ol, chemical formula $C_{16}H_{14}F_3N_5O$, molecular weight 349.31 g/mol.[1,2] Voriconazole is triazole antifungal agent used to treat serious fungal infection such as aspergillosis, candidemia, esophageal candidiasis or other fungal infections.[3] The primary mode of action of Voriconazole is the inhibition of cytochrome P_{450} dependent enzyme 14-alpha-sterol demethylase, thereby disrupting the cell membrane and halting fungal growth.[4] Voriconazole in its active dosage form is official in United State Pharmacopoeia but unofficial in its parenteral dosage form.[5] There is several process or degraded impurities associated with the synthesis of Voriconazole.[6] Three of the known Voriconazole related substances have been mentioned here; chemical structures for Voriconazole and its related substances viz. Imp B, Imp C and Imp D.[7] The Forced degradation study has been carried out and validation parameters provide valuable information on linearity, accuracy, precision and robustness of related substances.[8-9]

The analytical method which has been reported are very few for the determination of Voriconazole in parenteral dosage form.[10-13] The present work deals with development and validation of simple, precise and accurate stability indicating RP-HPLC method for determination of Voriconazole and its related substance in Parenteral dosage form.

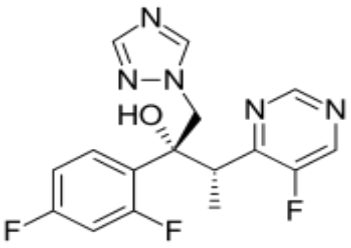

Fig. 1: Voriconazole

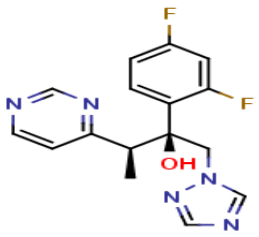

Fig. 2: Voriconazole related compound B

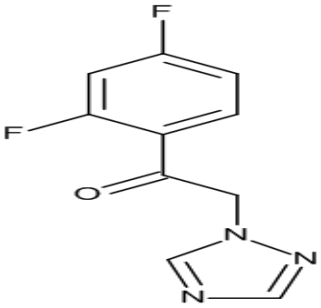

Fig. 3: Voriconazole related compound C.

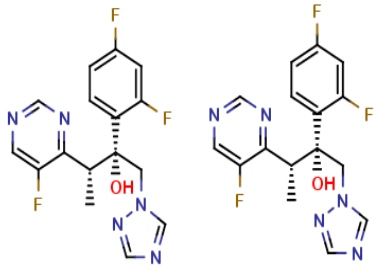

Fig. 4: Voriconazole related compound D.

MATERIALS AND METHODS

Chemicals and Reagents

Acetonitrile and ortho Phosphoric acid of HPLC grade produced by Merck Life science, Mumbai were used and Ammonium Phosphate Dibasic and other reagents used in the study were of analytical reagent grade. Working standard and sample (Voriconazole for Injection 200mg/vial) was provided by Zydus Cadila, Moraiya, Gujarat, India.

Instrumentation

The separation was carried out on Agilent-1200 Series HPLC using Chromeleon software. The column used in development is Inertsil C_{18} (250*4.6mm, 5µm). The mobile phase consists of Ammonium phosphate dibasic buffer (6.6 gm of Ammonium phosphate dibasic into 1000ml water, adjust pH 6.0 with dilute ortho phosphoric acid): Acetonitrile (55:45%w/v), with flow rate of 1.0ml/min, column oven temperature of 25°C, injection volume of 20µl, with isocratic elution at 256nm detection and the run time was of 30 min.

Table 1: Chromatographic parameters and condition.

Mobile phase:	Buffer (6.6 gm of Ammonium phosphate dibasic into 1000ml water, adjust the pH 6.0 with dilute Orthophosphoric acid): ACN (55:45%v/v)
Column:	Inertsil C_{18} (250mm*4.6mm) 5µm
Wavelength:	256nm
Flow rate:	1.0 ml/min
Injection volume:	20µl
Column temperature:	25ºC

Preparation of Solution

Preparation of diluent

Mobile Phase is used as diluent.

Preparation of standard stock solution (100ppm)

Transfer an accurately weighed quantity of 25mg of Voriconazole API to a 50ml volumetric flask, add about 20ml of diluent and sonicate to dissolve. Make up the volume upto the mark with diluent and mix dilute 5.0ml of above solution to 25ml with diluent and mix.

Preparation of impurities stock solution

Voriconazole Related Compound C (25ppm): Weigh 1.25mg of Voriconazole Related Compound C impurity into 50ml volumetric flask, add 30ml diluent and sonicate to dissolve.

Make up volume with diluent (25ppm). Further dilute 2ml of above solution into 25ml volumetric flask and make up the volume with diluent (2.0ppm).

Voriconazole Related Compound D (25ppm): Weigh 1.25mg of Voriconazole Related Compound D impurity into 50ml volumetric flask, add 30ml diluent and sonicate to dissolve. Make up volume with diluents (25ppm). Further dilute 2ml of above solution into 25ml volumetric flask and make up the volume with diluents (2.0ppm).

Spiked impurities mixture

Weigh 25mg of Voriconazole API to a 50ml volumetric flask, spike 2ml of impurity stock solution of Voriconazole Related Compound C (25ppm) and 2ml of Voriconazole Related Compound D (25ppm) into it and make up the volume with diluent.

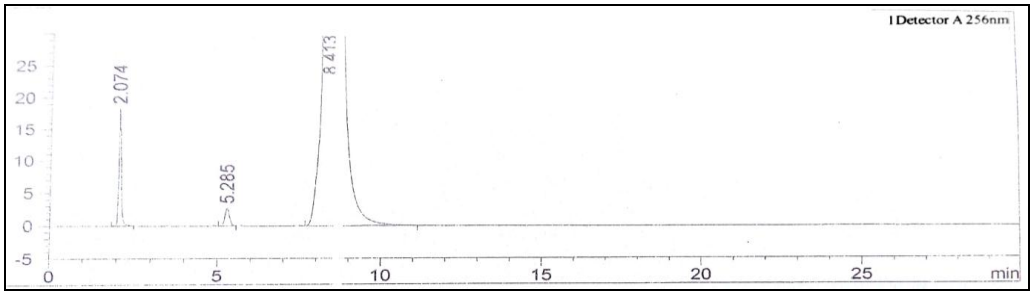

Fig. 5: Impurity Mixture + API.

Table 2: System suitability parameters in optimized condition.

Retention Time (min)	RRT*	Peak names	Plate	Tailing	Resolution
2.074	0.25	Voriconazole Related Compound C	22812	1.230	-
5.285	0.63	Voriconazole Related Compound D	40707	1.098	4.165
8.413	1.0	Voriconazole	60832	1.111	9.443

Note: *RRT- Relative Retention Time*

Forced degradation study

Acid degradation

Preparation of Sample solution

20ml reconstituted solution transfer into 100ml volumetric flask. Add approx 50ml of diluent and sonicate for 10 minute add 5ml of 5N HCl and heat for 30 minute in water bath at 80°C for acid hydrolysis. Then the solution was neutralized with 5N NaOH and made volume upto

mark with diluent. Then pipette out 5ml of solution in 50ml volumetric flask and make volume upto mark with diluent.

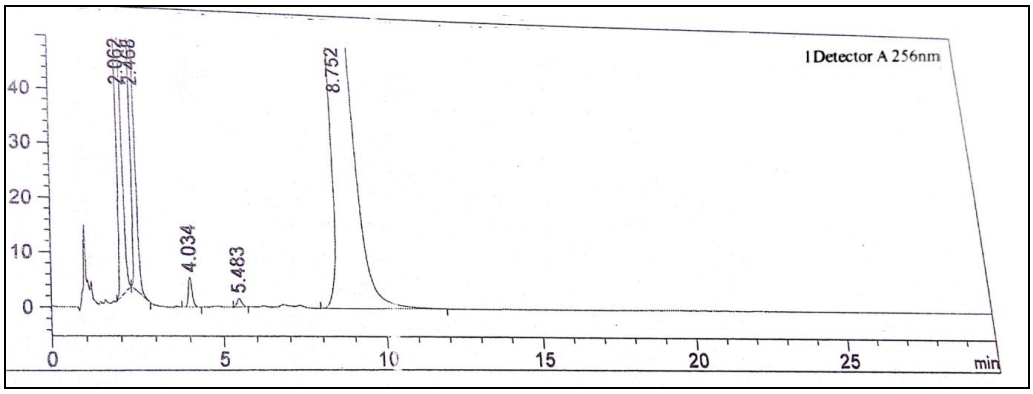

Fig. 6: Sample Acid degradation (5 N HCl_5 ml_80°C_30min).

Base Degradation

Preparation of Sample solution

20ml reconstituted solution transfer into 100ml volumetric flask. Add approx 50ml of diluent and sonicate for 10 minute add 5ml of 5N NaOH and heat for 10 minute at Critical Room Temperature (CRT) for base hydrolysis. Then the solution was neutralized with 5N HCl and make volume upto mark with diluent. Then pipette out 5ml of solution in 50ml volumetric flask and make volume upto mark with diluent.

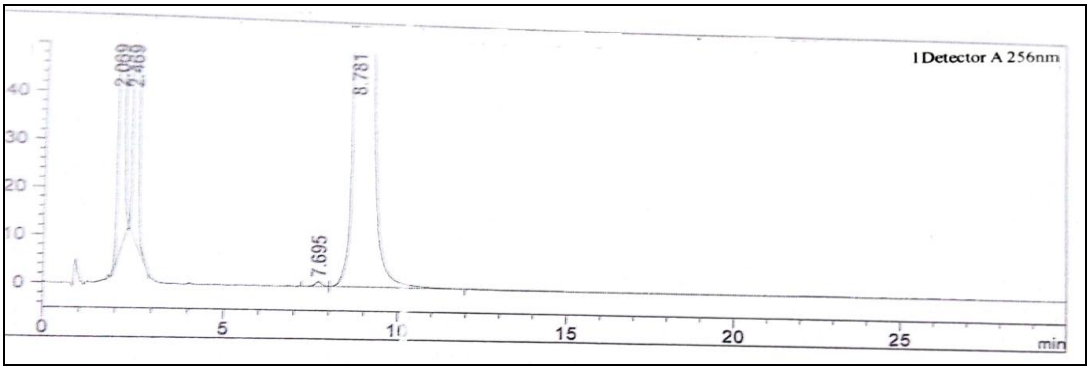

Fig. 7: Base degradation (5N NaOH_5 ml_ CRT_10min).

Oxidation Degradation

Preparation of Sample solution

20ml reconstituted solution transfer in 100ml volumetric flask. Add approx 50ml of diluent and sonicate for 10 minute and 5ml of 30% H_2O_2 was added and kept for 60 minute at critical room temperature. Then pipette out 5ml of solution in 50ml volumetric flask and make volume upto mark with diluent.

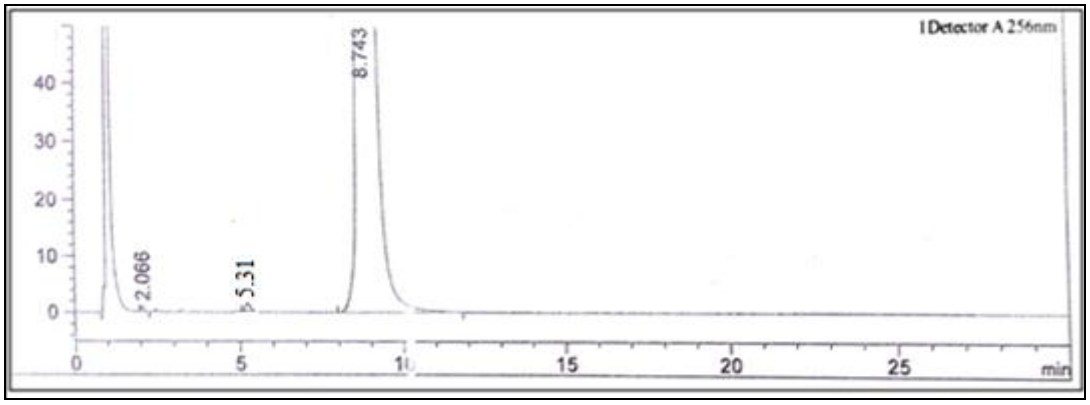

Fig. 8: Oxidation Degradation (30% H_2O_2_CRT_5 ml_60 min).

Table 3: Degradation summary.

Stress Condition	Duration	Area after Degradation	% Degradation	% Mass Balance
Acid Hydrolysis (5 N HCl_5 ml)	80°C_30min	233020	7.21	100.57
Base Hydrolysis (5 N NaOH_5 ml)	CRT*_10 min	201583	19.7	111.0
Oxidation Degradation (30% H_2O_2_5 ml)	CRT*_60 min	250130	0.39	99.19

Note: *CRT-Critical Room Temperature*

Method Validation

Related Compound C Impurity - Specification Limit (Not More Than 0.20%)

Linearity

According to specification limit, impurity should not be present more than 0.20%, so solutions of linearity of impurity prepared as following in which 0.20% of sample concentration is considered as 100% that is 2ppm (1000 ppm is sample concentration). From that consideration LOQ, 50%, 80%, 100%, 120% and 150% level solution are prepared.

Table 4: Linearity result of voriconazole related compound C.

Level	Concentration (ppm)*	Area
LOQ	0.5029	16924
50%	1.0058	34618
80%	1.6093	55242
100%	2.0117	69092
120%	2.4140	83615
150%	3.0175	105306

Note: *ppm-parts per million.*

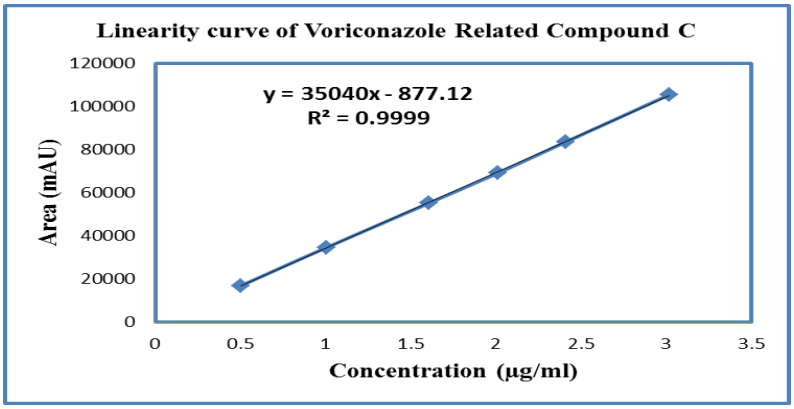

Fig. 9: Linearity curve of Voriconazole Related Compound C.

ACCURACY

According to specification limit of impurity which is not more than 0.20%, amount of individual impurities will be 2.0ppm which is 0.20% of 1000ppm (sample concentration). Accuracy is performed in sample at 4 levels are LOQ, 50%, 100% and 150% in three sets.

Table 5: Recovery result of voriconazole related compound C

Set	ml Ad-ded	mg Add-ed	Area	mg Found	mg Recov-ered	% Recov-ery	% Mean Recov-ery	SD	% RSD
LOQ Level									
1	0.5	0.0127	20589	0.0160	0.0131	103.1%	103.9	160.0	0.76
2	0.5	0.0127	20987	0.0161	0.0132	103.9%			
3	0.5	0.0127	21177	0.0162	0.0133	104.7%			
50% Level									
1	1.0	0.0254	36193	0.0277	0.0248	97.6%	98.6	379.7	1.04
2	1.0	0.0254	36419	0.0279	0.0250	98.4%			
3	1.0	0.0254	36934	0.0283	0.0254	100.0%			
100% Level									
1	2.0	0.0508	70386	0.0539	0.0510	100.3%	100.7	243.9	0.34
2	2.0	0.0508	70872	0.0543	0.0514	101.1%			
3	2.0	0.0508	70591	0.0541	0.0512	100.7%			
150% Level									
1	3.0	0.0760	108579	0.0833	0.0804	105.8%	105.9	132.6	0.12
2	3.0	0.0760	108751	0.0834	0.0805	105.9%			
3	3.0	0.0760	108840	0.0835	0.0806	106.1%			

Note: *SD-Standard Deviation, RSD- Relative Standard Deviation*

Precision

Repeatability

According to specification limit of impurity which is not more than 0.20%, amount of individual impurity will be 2.0ppm which is 0.20% of 1000ppm (sample concentration). For

Repeatability sample containing impurity at 100% level injected for six times. Calculate RSD for response of impurity.

Table 6: Repeatability result of voriconazole related compound C.

Area	Mean	SD*	% RSD*
68549	68894.33	251.04	0.364
69091			
69003			
68793			
68872			
69148			

Note: *SD-Standard Deviation, RSD- Relative Standard Deviation.*

Intermediate Precision

Intraday Precision

According to specification limit of impurity which is not more than 0.20%, amount of individual impurity will be 2.0ppm which is 0.20% of 1000ppm (sample concentration). Intermediate Precision sample containing impurity at 50%, 100% and 150% level injected for Intraday in three sets at Morning and Evening.

Table 7: Intraday precision result of voriconazole related compound C.

Set	Level	Morning	Evening	Mean	SD*	%RSD*
50% Level						
1	50%	34765	34689	34727	53.74	0.154
2	50%	35093	35269	35131	124.45	0.353
3	50%	34917	35126	35021.5	147.78	0.421
100% Level						
1	100%	69084	69729	69406.5	456.08	0.657
2	100%	68893	69145	69019	178.19	0.258
3	100%	68994	69117	69055.5	86.97	0.125
150% Level						
1	150%	105913	105728	105820.5	130.81	0.123
2	150%	106193	106553	106373	254.55	0.239
3	150%	106199	106871	106535	475.17	0.446

Note: *SD-Standard Deviation, RSD- Relative Standard Deviation*

Interday Precision

According to specification limit of impurity which is not more than 0.20%, amount of individual impurity will be 2.0ppm which is 0.20% of 1000ppm (sample concentration). Intermediate Precision sample containing impurity at 50%, 100% and 150% level injected for Interday in three sets at Day-1 and Day-2.

Table 8: Interday precision result of voriconazole related compound C.

Set	Level	Day 1	Day 2	Mean	SD*	%RSD*
50% Level						
1	50%	34765	35278	35021.5	362.74	1.035
2	50%	35093	35914	35503.5	580.53	1.635
3	50%	34917	35461	35189	384.66	1.093
100% Level						
1	100%	69084	69913	69498.5	586.19	0.843
2	100%	68893	69472	69182.5	409.41	0.591
3	100%	68994	69551	69272.5	393.85	0.568
150% Level						
1	150%	105913	106283	106098	261.62	0.246
2	150%	106193	106924	106558.5	516.89	0.485
3	150%	106199	107119	106659	650.53	0.609

Note: *SD-Standard Deviation, RSD- Relative Standard Deviation.*

Robustness

According to robustness, there is minor deliberate change made in chromatographic parameter with reference of Flow rate and Column temperature. To observe robustness, 100% level solution is used.

Change in flow rate

Inject the solution of 100% level of impurity at flow rate of 1.9 ml/min and 2.1 ml/min and calculate RSD for all responses of all impurities individually.

Change in Column Temperature

Inject the solution of 100% level of impurity at Column Temperature of 20°C and 30°C and calculate RSD for all responses of all impurities individually.

Table 9: Robustness result of voriconazole related compound C.

Parameter	Change	Area	Mean Area	SD*	%RSD*
	0.9	68762	68834.78	400.21	0.581
	0.9	67998			
	0.9	68493			
	1.0	69072			
Flow Rate (ml/min)	1.0	69118			
	1.0	68947			
	1.1	69384			
	1.1	68815			
	1.1	68924			
	20°C	68519			
	20°C	68583			
	20°C	68721			

	25°C	68896			
Column Temperature	25°C	69121	69158	551.92	0.798
	25°C	69223			
	30°C	69918			
	30°C	70054			
	30°C	69387			

Note: *SD-Standard Deviation, RSD- Relative Standard Deviation.*

CONCLUSION

The isocratic RP-HPLC method developed for quantitative analysis of Voriconazole and related impurities in parenteral dosage form is linear, accurate, precise and robust. Satisfactory results were obtained from validation of method. The method is stability-indicating and can be used for routine analysis of production samples, and to check the stability of Voriconazole in parenteral dosage form.

ACKNOWLEDGEMENT

Special thanks to Zydus Cadila, Moraiya for providing Voriconazole working standard and sample and also providing the facilities to complete the research work.

REFERENCES

1. Voriconazole chemical name. https://pubchem.ncbi.nlm.nih.gov/compound/Voriconazole#section=Top
2. Voriconazole Drug profile, https://www.drugbank.ca/drugs/DB00582.
3. Tripathi KD. Essential of Medical Pharmacology; 6th ed; Jaypeeb Rothersm Edical Publisher (Sp) Ltd, New Delhi, 2009; 757.
4. Sean C Sweetman. Martindale the Complete Drug Reference, 36th ed; Pharmaceutical Press, London, 1755.
5. Voriconazole official in pharmacopoeia. http://app.uspnf.com/uspnf/pub/index?usp=40&nf=35&s=1&officialOn=August%201,%202017.
6. ICH Q3A (R2) “International Conference on harmonization of Technical Requirements for registration of Pharmaceuticals for human use” Impurities in New Drug Substance, 2006.
7. Srinivas J et al. Novel Stability indicating RP-HPLC Method for the Determination of Assay of Voriconazole in Pharmaceutical Products. Med. Pharm. Sci., 2015; 1(3): 70-79.

8. ICH guidelines, Q1A (R2): Stability Testing of New Drug Substances and Products (revision2), International Conference on Harmonization, 2005.
9. ICH, Q2 (R1) "Validation of Analytical Procedure" Methodology, International Conference on Harmonization, IFPMA, Geneva, Switzerland, 2005.
10. Shaikh K A., Patil A T. A Validated Stability-Indicating Liquid Chromatographic Method for Determination of Degradation Impurities and Diastereomers in Voriconazole Tablets; Sci Pharm, 2012; 80: 879–888.
11. Rao E B et al. Method Development Validation and Degradation studies of Voriconazole Drug by RP-HPLC Method; IJCPS, 2014; 02(9): 1149-1154.
12. Gu Ping and Yuru Li. Development and Validation of A Stability-Indicating HPLC Method for Determination of Voriconazole and Its Related Substance; Journal of Chromatographic Science, 2009; 47: 594-598.
13. Verma MV, Patel CJ, Patel MM. Development and stability indicating HPLC method for Dapagliflozin in API and pharmaceutical dosage form. International Journal of Applied Pharmaceutics, 2017; 9(5): 33-41.