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UV-Visible Spectroscopic Assay Method Development And Validation For Determination Of Itraconazole In Itraconazole Table

Bhaskar O. Aher, Jyoti R. Gadhe*, Parag Pathade, Vinod A.Bairagi

Department Of Pharmaceutical Quality Assurance,K.B.H.S.S.Trust's Institute Of Pharmacy &Research Center,Affiliated To Savitribaiphule university, Bhayegaon,Malegaon,Nashik,Maharashtra, India.

Department Of Pharmaceutical Quality Assurance,K.B.H.S.S.Trust's Institute Of Pharmacy &Research Center, Affiliated To Savitribaiphule university, Bhayegaon, Malegaon, Nashik, Maharashtra, India.

Department Of Pharmaceutical Quality Assurance,K.B.H.S.S.Trust's Institute Of Pharmacy &Research Center, Affiliated To Savitribaiphule university, Bhayegaon, Malegaon, Nashik, Maharashtra, India.

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ABSTRACT:
A simple, precise, quick, and affordable UV spectrophotometric technique is created and proven to be effective for figuring out the dosages of itraconazole in tablets and bulk. The drug is soluble in organic solvents like methanol, ethanol, and dimethyl forma-amide. Itraconazole in tablet form can be measured simply, rapidly, and economically with the UV method. UV spectroscopic analysis are conduct at an absorption maximum of 262 nm using methanol as a solvent. In accordance with ICH criteria, the suggested approach was verified for linearity, precision, accuracy, sensitivity, and robustness. This review is really comprehensive, and the procedure for measuring itraconazole is simple and accurate method.

KEYWORDS: Itraconazole, UV Spectrophotometer, HPLC, Assay method, Method development, Validation,

INTRODUCTION

In pharmaceutical industries, the validation of analytical method is used to demonstrate that the method is fitted for its purpose; it must follow a plan which includes scopes, Performance characteristics, and acceptance limits. Analyticalmethods need to be validated or revalidated prior totheirintroductionintoroutineanalyses.Chromatographyisananalyticaltechniquesbasedonthe separation of molecules due to differences in their structure and/or composition. In general, Chromatography involves moving a sample through the system over a stationary phase. The molecules in the samples will have different affinities and interaction with the stationary support, leading to separation of Molecules [1]. Samples components that display stronger interaction with the stationary phase will move more slowly through the column than components with weaker interaction. Different compounds can be separated from each other as they move through the column. Chromatographic separation can be carried types of liquid Chromatography used to separate

and quantify dissolved in Solution.

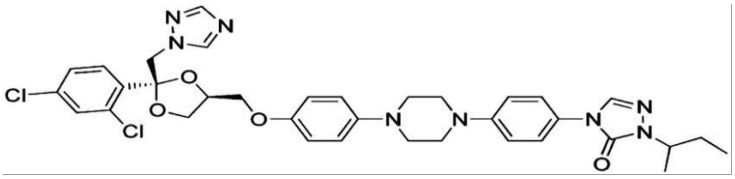


Figure 1: Chemical structure of Itraconazole

Table 1: Drug profile of itraconazole

Generic Name	**Itraconazole**
Brand Name	Sporanox, Onmel.
IUPAC Name	2-butan-2-yl-4-[4-[4-[4-[[(2R,4S)-2-(2,4-dichlorophenyl)-2-(1,2,4-triazol-1-ylmethyl)-1,3-dioxolan4-yl] methoxy] phenyl] piperazin1-yl] phenyl]-1,2,4-triazol-3-one
Chemical formula	$C_{35}H_{38}Cl_2N_8O_4$
Molar Mass	705.6 g.mol
Synonyms	Sporanox, Onmel, Tosura
Melting point	168-170 ^oC
Solubility	Insoluble in Water and soluble in dilute acidic solutions
Protein binding	99.8%
Elimination half life	34 o 40 hrs

Material and method

Vidisha analytical presented the Puredesidustat medication as a gift. Itraconazole analytical reagent was purchased from Merck and Siddhi Lab. Acetonitrile, methanol, and water from (qualigens) were of HPLC grade

Instrumentation: An HPLC-1260 infinity II (Agilent) was used for the method development and validation HPLC binary gradient system, Detector (DEAX02386), Double beam UV visible spectroscopy (Jasco), Weighing Balance (CY224C) from Aczet for sample weighing, Bio-technic Ultra Sonicator (13.5L) and pH meter from Lab Man used for sample preparation.

Selectionofsolven:

DMSOwasselectedasthesolventfordissolvingItraconazole.

PreparationofstandardstocksolutionsforUVscan

In order to prepare stock solution, weighed accurately 20mg Itraconazole and transferred into 20mlvolumetricflask,added2mlofDMSOandsonicatedtodissolvethestandardcompletely and diluted up to the mark with methanol(1000 PPM Furtherdiluted0.4 mLto20(20 PPM)

PreparationofblanksolutionofUVscan:(Solution1)

Added 2 ml of DMSO in 20 mL of volumetric flask and volume made up to the mark with methanol. Further diluted 0.4 mL to 20 mL with methanol.

Selectionofanalyticalwavelength

Solution 1 as a blank and Itraconazole standard solution (20 PPM) was scanned from 400nm to 200nm. Absorption maxima was determined for drug. Itraconazole showed maximum absorbance at 262 nm shown in results.

Selectionoftest/WorkingConcentration:

When we analyzed 20 ppm of Itraconazole between 400 nm to 200 nm. 20 ppm solutionshowed0.5876absorbanceatitsabsorptionmaxima(262nm).Hencewehaveselected25ppmasates t concentration. 25 ppm of Itraconazole will shows absorbance about 0.7345 and when we will perform

thelinearityfrom80%to120%onUV,theabsorbanceof120%levelwillnotgoabove1,becauseabsorbancesh

ouldnotbemorethan1inUV-spectroscopyasitisoneofthelimitationofUV-spectroscopy. 25ppmselectedastestconcentrationfor UVandHPLCanalysis.

RESULTAND DISCUSSION

Solubility study

TableNo. 2:Solubility study Of Itraconazole

Sr. No.	Nameof Solvent	Observation	Conclusion	Summary
1	Water	DrugParticlesseenafter sonication	Drugwasnotfoundsoluble in water.	DMSOusedas a diluent for preparing stock solution.
2	Methanol	DrugParticlesseenafter sonication	Drugwasnotfoundsoluble inmethanol.	
3	Ethanol	DrugParticlesseenafter sonication	Drugwasnotfoundsoluble inEthanol.	
4	Acetonitrile	DrugParticlesseenafter sonication	Drugwasnotfoundsoluble in Acetonitrile	
5	0.1 N HCl	DrugParticlesseenafter sonication	Drugwasnotfoundsoluble in 0.1 N HCl.	
6	0.1N NaOH	DrugParticleseen after sonication	Drugwasnotfoundsoluble in 0.1 N NaOH.	
7	DMSO	NoDrugParticlesseenafter sonication	Drugwasfoundsolubleiin DMSO.	

VALIDATIONOFUVMETHODFORITRACONAZOLE

1) FILTRATIONSTUDY:

Filtration study of an analytical procedure checks the interference extraneous components from filter, deposition on filter bed and compatibility of filter with sample. Performed on capsule sample

TableNo.3:Results of filter study

Sample description	Area	% Absolute difference
Unfiltered	0.7246	NA
0.45µ PVDFfilter	0.7215	0.43
0.45 µ Nylon filter	0.7189	0.79

Acceptance criteria:% Absolute difference of filtered samples NMT 2.0 w.r.t. Unfiltered sample.
SOLUTION STABILITY:.Thesolution was stored at normal illuminated laboratory conditions and analyzed at initial, after 12 hours and 24 hours.

TableNo. 4 :Results of Solution stability

Sample solution			Standard solution		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**%Absolute difference**
Initial	0.7249	NA	Initial	0.7362	NA
12 Hours	0.7195	0.74	12 Hours	0.7315	0.64
24Hours	0.7146	1.42	24 Hours	0.7279	1.13

Acceptancecriteria:%AbsolutedifferenceofStabilitysolution:NMT2.0w.r.t.Initial solution.

2) SPECIFICITY: Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present.Blankandplacebosolution prepared and scanned from 400nmto200 nm.

Results of Specificity.

TableNo.5: Results ofSpecificity

Description	Observation
Blank	NointerferenceatAbsorptionmaximaofItraconazoledue to blank
Placebo	NointerferenceatAbsorptionmaximaofItraconazoledue toplacebosolution

UV-spectrum:

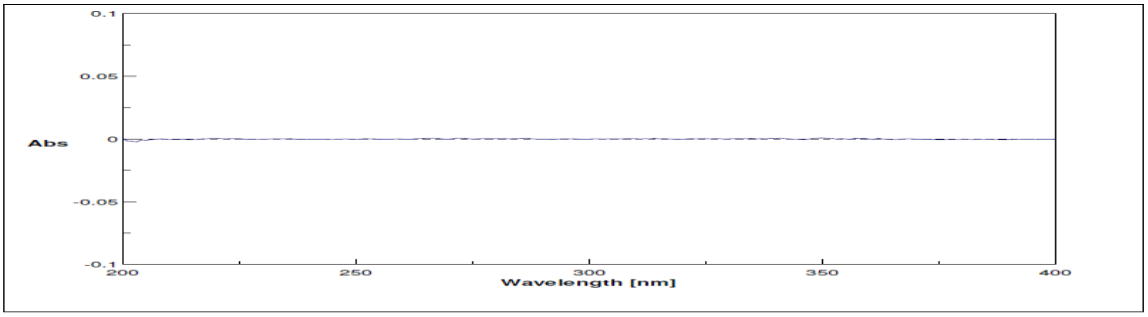


Fig.No.8.6.3.1TypicalUV-spectrum ofBlank solution.

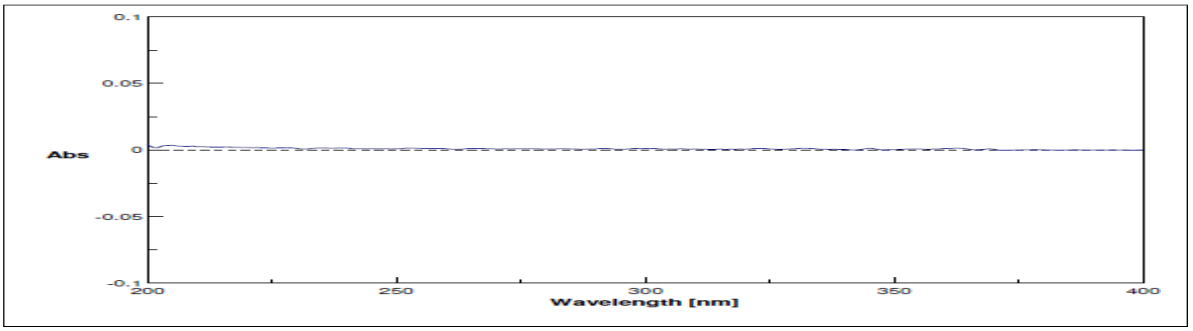


Fig.No.8.6.3.2TypicalUV-spectrum ofPlacebosolution.

Acceptance criteria:

Blank:%InterferenceatAbsorptionmaximaofItraconazoleisNMT1.0%

Placebo:%Interference atAbsorptionmaximaofItraconazoleisNMT2.0%

3) LINEARITY ON UVSPECTROPHOTOMETER:

Linearity of an analytical method is itsability toelicit test results that areproportional to the concentration of analyte in samples within a given range.

ResultsofUVLinearity forItraconazole:

TableNo. 5:Linearity Data

Level	Conc(µg/mL)	Absorbance	Mean	% RSD
80%	20.00	0.5785	0.5784	0.148
		0.5792		
		0.5775		
90%	22.50	0.6642	0.6647	0.084
		0.6653		
		0.6646		
100%	25.00	0.7356	0.7364	0.132
		0.7362		
		0.7375		
110%	27.50	0.8141	0.815	0.106
		0.8152		
		0.8158		
120%	30.00	0.8839	0.884	0.020
		0.8842		
		0.8839		

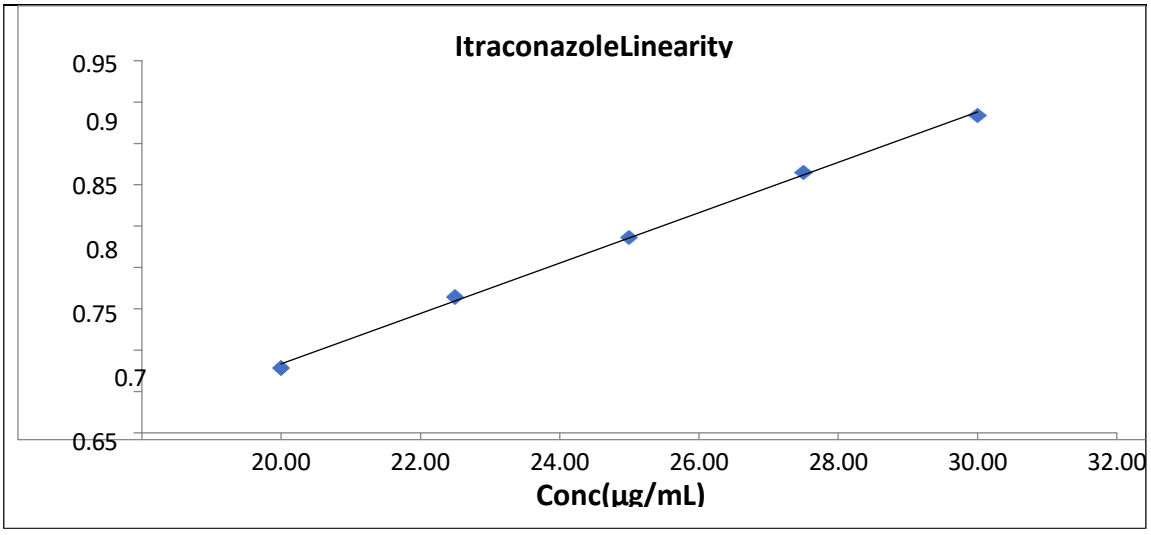


Fig.No.6 CalibrationcurveofItraconazoleonUV

SummaryofUV-linearityof Itraconazole:

TableNo .6.:Linearity Summary

Srno.	Parameter	Result value	Acceptancecriteria
1	Beer'slinearityrange	20.00-30.00µg/mL	NA
2	Correlationcoefficient(R^2)	0.99933	NLT0.98
3	Intercept	-0.0258	To be report
4	Slope	0.03046	To be report

5	%RSDforareaateachlevel	NA	NMT 2.0

TherespectivelinearequationforItraconazolewas

Y = M X+C

Y=0.03046 x +-0.0258

where,x=concentrationofAnalyteinµg/Ml

y=is Absorbance.

M = Slope

C=Intercept

4) LIMITOFDETECTION(LOD)ANDLIMITOFQUANTITATION(LOQ):

σ=0.00441(Residualstandarddeviationofaregressionline) s = 0.03046 (Slope)

Detectionlimit(LOD):

LOD=3.3σ/ S

LOD=3.3 x 0.00441 / 0.03046

LOD= 0.478 µg/mL

Quantitationlimit(LOQ):

LOQ=10σ/ S

LOQ=10 x 0.00441 / 0.03046

LOQ= 1.448 µg/mL

5) ACCURACY (RECOVERY):

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method is determined by applying the method to analyzed samples to which known amounts of analyte have been added.

TableNo.7:ResultandstatisticaldataofAccuracyof Itraconazole

Level (%)	Absorbance	Recovered conc (µg/mL)	Addedconc (µg/mL)	% Recovery	Mean Recovery	% RSD
80	0.5862	19.91	20.13	98.91	99.25	0.565
	0.5843	19.84	20.05	98.95		
	0.5913	20.08	20.10	99.90		
100	0.7382	25.07	25.05	100.08	99.91	0.677
	0.7428	25.22	25.10	100.48		
	0.7284	24.74	24.95	99.16		
120	0.8792	29.86	30.03	99.43	100.14	0.625
	0.8864	30.10	29.98	100.40		
	0.8896	30.21	30.03	100.60		

OverallRecovery:99.77%

%RSDforOverallRecovery: 0.673

Acceptancecriteria:

%Recovery foreach levelandoverall recovery:98.0to 102.0%

%RSDforeachlevelandoverallrecovery:NMT2.0

TableNo .8:Linearity Summary

Srno.	Parameter	Result value	Acceptancecriteria
1	Beer'slinearityrange	20.00-30.00µg/mL	NA
2	Correlationcoefficient(R^2)	0.99933	NLT0.98
3	Intercept	-0.0258	To be report
4	Slope	0.03046	To be report

5	%RSDforareaateachlevel	NA	NMT 2.0

6) PRECISION

Precisionofananalyticalmethodisthedegreeofagreementamongindividualtestresultswhen the procedure is applied repeatedly to multiple samplings of a homogenous sample

Table No.9 : Result of Intra- day and Inter- Day Precision for Itraconazole test sample assay

	Sample	**TestSample (mg)**	**Absorbance**	**% Assay**
Repeatability	Sample1	175.2	0.7282	98.97
	Sample2	175.5	0.7176	97.36
	Sample3	175.4	0.7251	98.44
	Sample4	175.8	0.7239	98.05
	Sample5	174.6	0.7194	98.11
	Sample6	174.9	0.7312	99.55
	Mean			**98.41**
	STD DEV			**0.7663**
	% RSD			**0.779**
Intermediate precision (Inter-Day)	Sample1	175.4	0.7265	98.63
	Sample2	175.4	0.7219	98.00
	Sample3	175.6	0.7309	99.11
	Sample4	175.8	0.7188	97.36
	Sample5	174.9	0.7145	97.27
	Sample6	175.3	0.7235	98.27
	Mean			**98.11**
	STD DEV			**0.7178**
	% RSD			**0.732**
Repeatability PlusInter-day	**Mean**			**98.260**
	STD DEV			**0.7258**
	% RSD			**0.739**

Acceptancecriteria:% Assay: % Assay value for each sample (Individual sample) and mean assay value for precision (6 samples), mean assay value intermediate precision (6 samples), and mean assay value for precision plus intermediate precision sample (12 samples): 90-110%

%RSD:%RSDforprecisionstudysamples(6samples),Intermediateprecisionstudysamples (6 samples) and precision plus intermediate precision sample (12 samples): NMT 2.0

7) ROBUSTNESS:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Followingchangesmade underRobustness:

- ChangeinSonication time
- Changein Wavelength

A. Changesin Sonicationtimefortestsamplepreparation by+5 min (20minutes)

Twosamplespreparedbychangeinthisparameter.Summaryas follows

TableNo.10:ChangesinSonication timefortestsamplepreparationby+5 min

Sample	Powderwt. (mg)	Dilutedto (mL)	Volume taken	Dilutedto (mL)
Sample1	175.2	100	0.5	20
Sample2	175.6	100	0.5	20

TableNo. 11:Results ofChangeinsonication timeby +5minutes(20 minutes)

Sample	Absorbance	% Assay	Abs difference w.r.t.Precision assay value
Sample1	0.7242	98.43	0.57
Sample2	0.7172	97.25	
Mean		**97.84**	
STD DEV		**0.8344**	
% RSD		**0.853**	

B. Changesin Sonicationtimefortestsample preparationby-5 min(10 minutes)

Twosamplespreparedby changeinthisparameter.Summaryas follows

TableNo.12:Changesin Sonicationtimefortest samplepreparationby-5 min

Sample	Powderwt. (mg)	Dilutedto (mL)	Volume taken	Dilutedto (mL)
Sample1	175.4	100	0.5	20
Sample2	173.6	100	0.5	20

TableNo.13 :Results ofChangein sonicationtimeby-5minutes(10 minutes)

Sample	Absorbance	% Assay	Absdifferencew.r.t. Precisionassay value
Sample1	0.724	98.29	0.13
Sample2	0.7164	98.26	
Mean		**98.28**	
STD DEV		**0.0212**	
% RSD		**0.022**	

C. **Changesinwavelengthby-3 NM**

Note: First twosamples ofP recisionstudy analyzedatthis wavelengthand calculated its assay value. Abs difference calculated for assay value w.r.t. Precision assay value (Mean value)

Resultsofchangeinwavelengthby-3 NM

TableNo 14:Systemsuitabilityat259 nm

SrNo.	Standardsolution	Absorbanceat259nm
1	Standard_1	0.7291
2	Standard_2	0.7285
3	Standard_3	0.7304
4	Standard_4	0.7285
5	Standard_5	0.7294
Mean		**0.7292**
STD Dev		**0.0008**
% RSD		**0.11**

TableNo.15:Results of Test samplesby changein–3 nmwavelength

Sample	Absorbance	% Assay	Abs difference w.r.t.Precision assay value
Sample1	0.7156	98.19	0.49
Sample2	0.7128	97.64	
Mean		**97.92**	
STD DEV		**0.3899**	
% RSD		**0.398**	

D. Resultsofchange in wavelength by+3 NM

Note: First twosamples ofPrecisionstudy analyzedatthis wavelengthand calculated its assay value. Abs difference calculated for assay value w.r.t. Precision assay value (Mean value)

TableNo. 16:Systemsuitabilityat259 nm

SrNo.	Standardsolution	Absorbanceat265nm
1	Standard_1	0.7215
2	Standard_2	0.7225
3	Standard_3	0.7231
4	Standard_4	0.7216
5	Standard_5	0.7229
Mean		**0.7223**
STD Dev		**0.00074**
% RSD		**0.10**

TableNo. 17:Results of Test samplesby changein+3nmwavelength

Sample	Absorbance	% Assay	Absdifferencew.r.t. Precisionassay value
Sample1	0.7082	98.10	0.64
Sample2	0.7046	97.44	
Mean		**97.77**	
STD DEV		**0.4706**	
% RSD		**0.481**	

MethodDevelopmentbyRP – HPLC
Optimizationofmethod Trial 1:
Trial 1:
Chromatogram:

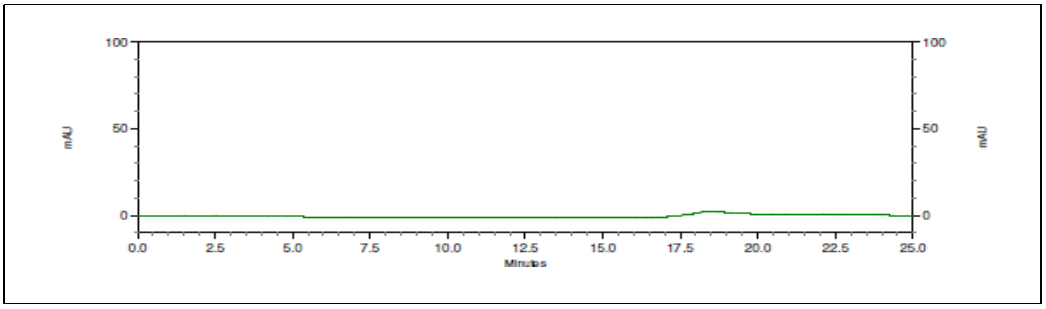


Fig.No.07TypicalchromatogramofTrial1

Observation: Itraconazole not eluted till 25 minutes.
Conclusion: Method rejected.
Trial 2:
Chromatogram

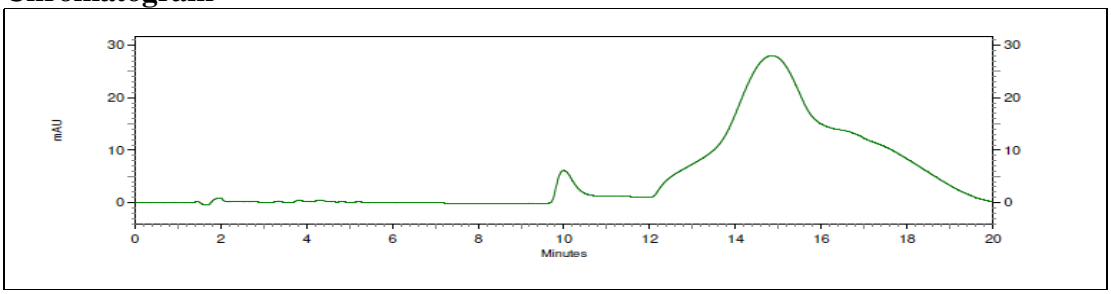


Fig.No.08 TypicalchromatogramofofTrial 2

Observation: Itraconazoleeluted atabout15 minuteswith verybroad peak(chromatography is not acceptable)
Conclusion: Method rejected
Trial 3:
Chromatogram

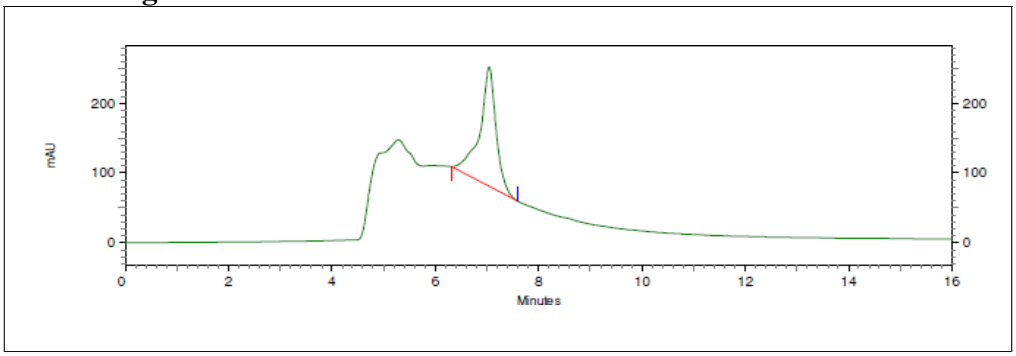


Fig.No.09 TypicalchromatogramofTrial 3

Observation: Itraconazoleelutedatabout7minuteswithunacceptablechromatography(peak eluted on hump)
Conclusion: Method rejected.
Trial 4:
Chromatogram:

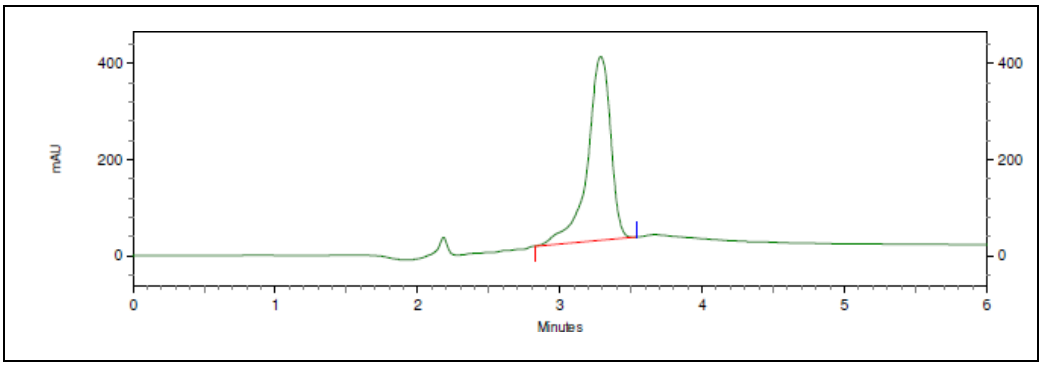


Fig.No.10 TypicalchromatogramofTrial 4

Observation: Itraconazole eluted at about 3.3 minutes with unacceptable chromatography (peakfrontingobserved,asymmetry:0.71,peakshapeisalsonotsharptheoreticalplates1942)
Conclusion: Method rejected.
Trial 5:
Chromatogram:

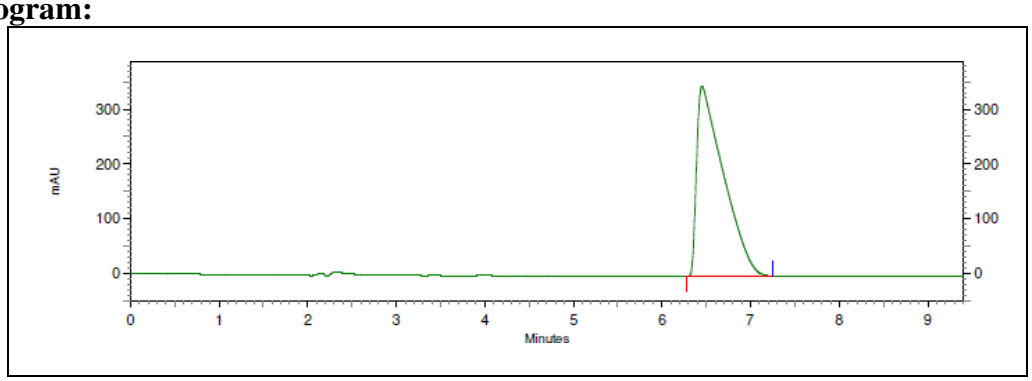


Fig.No.11 TypicalchromatogramofTrial 5

Observation: Itraconazole eluted at about 6.4 minutes with unacceptable chromatography (peak tailing observed, asymmetry: 3.08, peak shape is also not sharp theoretical plates 1761)
Conclusion: Method rejected.
Trial6:
Chromatogram:

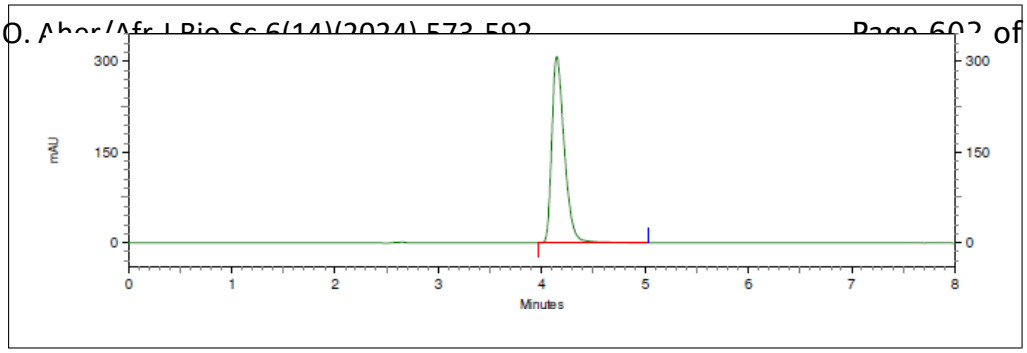


Fig.No.12 TypicalchromatogramofTrial 6

Observation: Itraconazole eluted at about 4.1 minutes with acceptable chromatography

Conclusion: Fromtheobservationsoftrialsfirsttosix,itwasconcludedthatchromatographic conditionsintrialsixgivesbetterpeak.

TableNo.18:OptimizedChromatographic Conditions

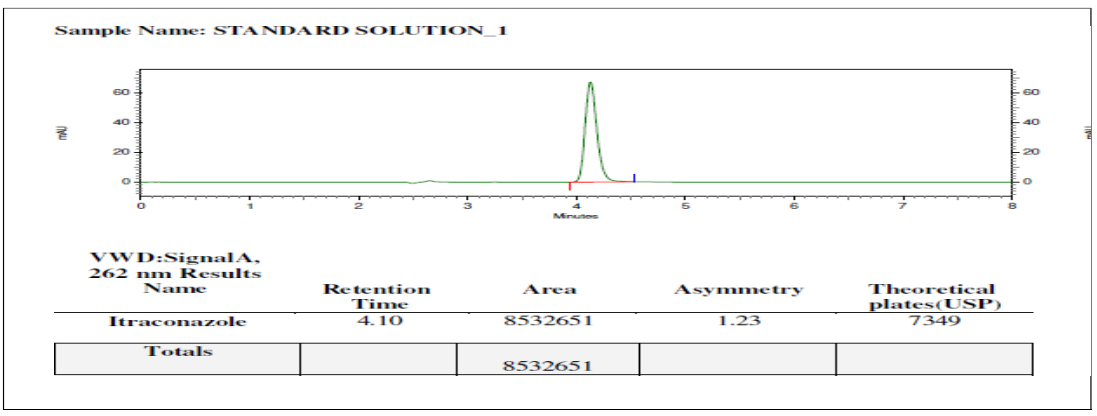
Parameter	**Description**
Mode	Isocratic
ColumnName	PhenomenexC18,250mm X4.6mmID,5 µm
Detector	UVDetector
InjectionVolume	20 µl
Wavelength	262 nm
ColumnOventemp	40ºC
Mobile Phase	Acetonitrile:0.1% TFAAin water(80:20%v/v)
FlowRate	1.0 ml/min
Run time	08 Minutes

Systemsuitability test

TableNo.19:ResultsforSystem SuitabilityTestof Itraconazolefor HPLC

Sr.No.	**Standardsolution**	**Area**	**Asymmetry**	**Theoreticalplates**
1	Standard_1	8532651	1.23	7349
2	Standard_2	8533419	1.23	7352
3	Standard_3	8536529	1.22	7362
4	Standard_4	8531024	1.23	7359
5	Standard_5	8533416	1.22	7346
Mean		**8533408**	**1.23**	**7354**
STD Dev		**1999.833**		
% RSD		**0.02**		

1. **Fig.No.13:TypicalchromatogramofStandardsolution1ofsystemsuitabili**



solution

VALIDATIONOFRP-HPLCMETHOD

1) **FILTRATIONSTUDY:**

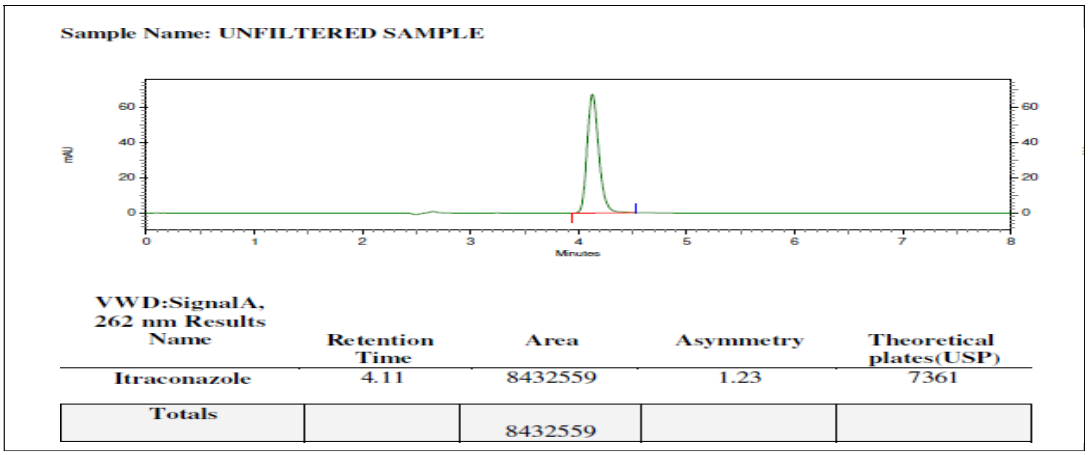
Filtration study of an analytical procedure checks the interference of extraneous components from filter, deposition on filter bed and compatibility of filter with sample. Performed on tablet test sample.

TableNo.20:Resultsof Filterstudy

Sampledescription	Area	% Absolutedifference
Unfiltered	8432559	NA
0.45µ PVDFfilter	8416871	0.19
0.45 µ Nylon filter	8373981	0.69

Chromatograms:

Fig.No.14Typicalchromatogram ofunfilteredsample.



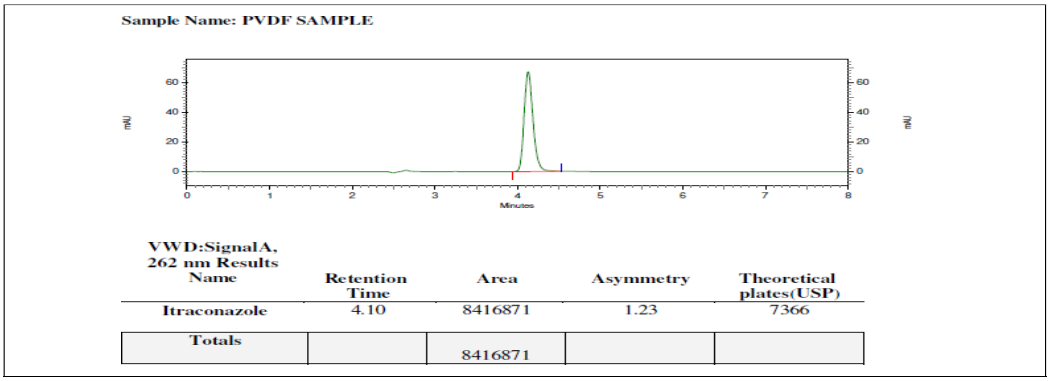


Fig.No.15Typicalchromatogram ofsamplefilteredthrough0.45µPVDF filter.

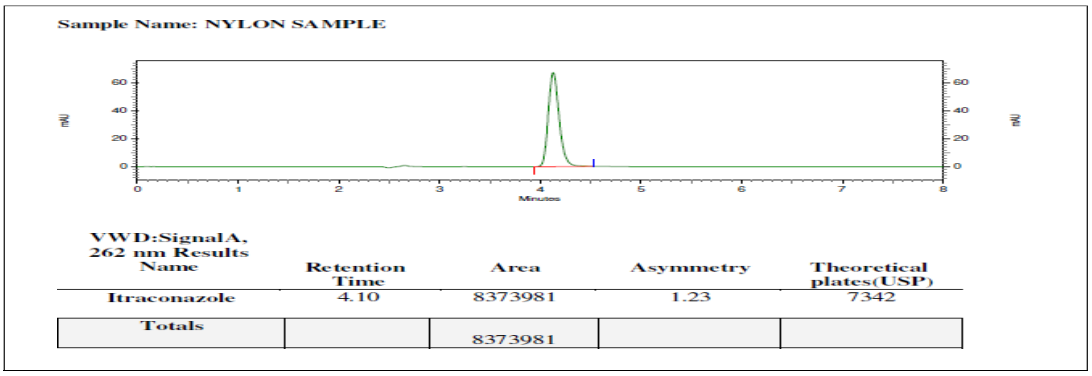


Fig.No.16.Typicalchromatogramofsamplefilteredthrough0.45µfilter

Acceptance criteria: % Absolute difference of filtered samples NMT 2.0 w.r.t. Unfiltered sample.

2) SOLUTION STABILITY:

Stability study was conducted for Standard as well as Test Sample.Stabilitystudywasperformedatnormallaboratoryconditions.

TableNo. 21:Results ofSolution stability

Samplesolution			Standardsolution		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**% Absolute difference**
Initial	8430526	NA	**Initial**	8539462	NA
12 Hours	8416957	0.16	**12 Hours**	8524859	0.17
24 Hours	8332637	1.16	**24 Hours**	8462419	0.90

Chromatograms:

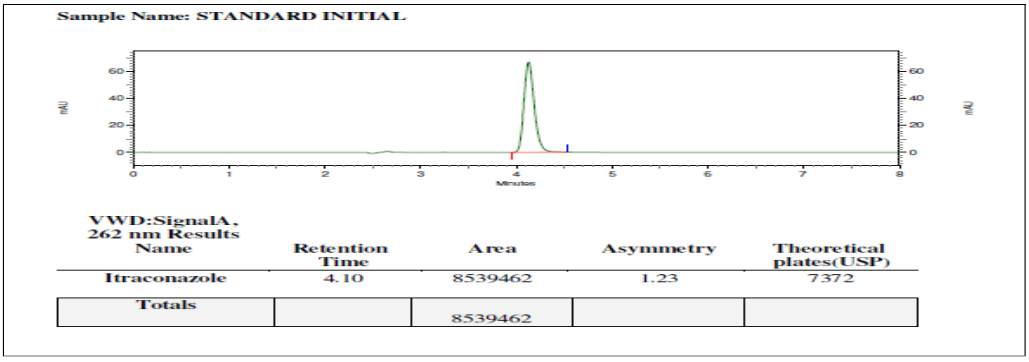
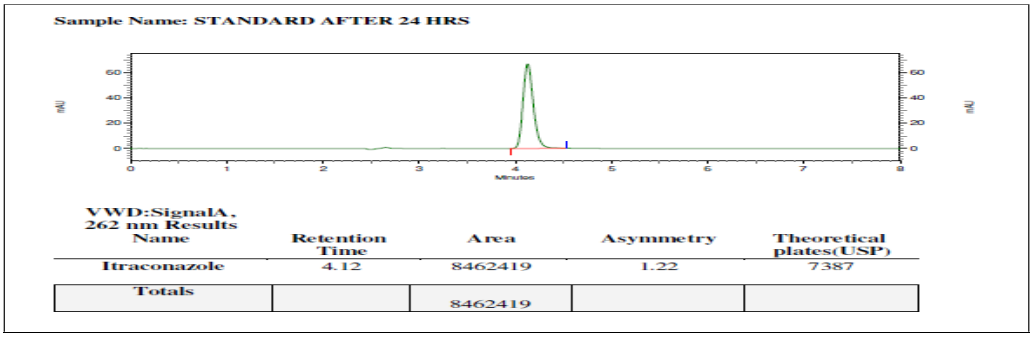
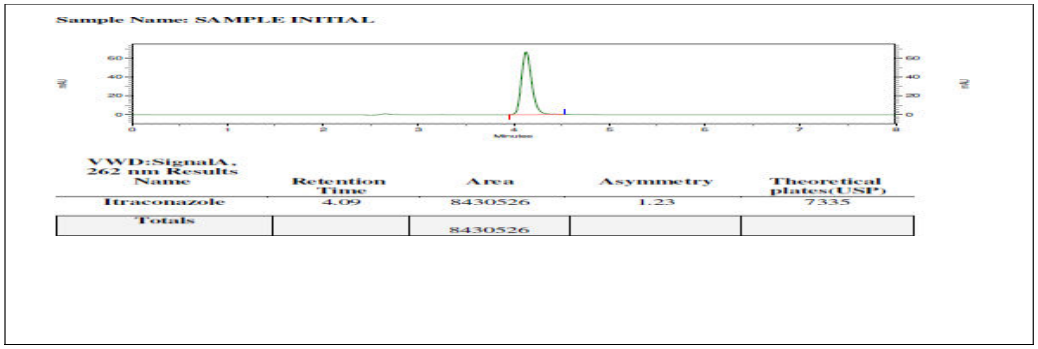


Fig.No.17 TypicalchromatogramofStandardsolution Initial.



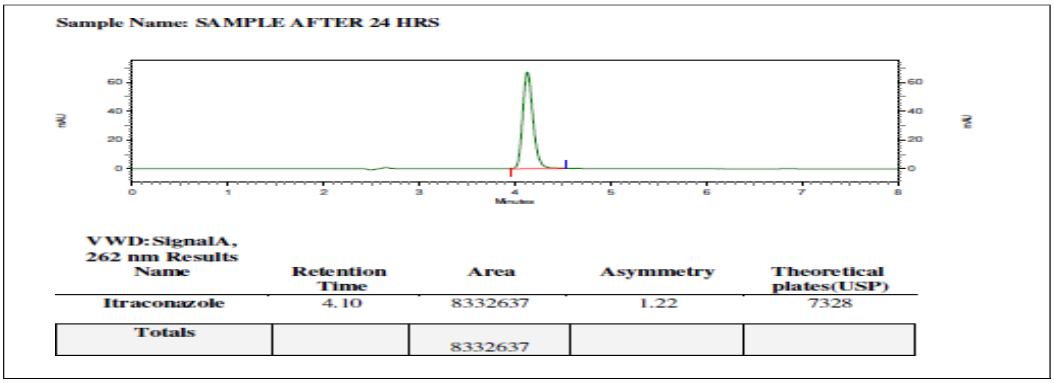
VWD:SignalA, 262 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates(USP)
Itraconazole	4.12	8462419	1.22	7387
Totals		8462419		

Fig.No.18Typicalchromatogram of StandardsolutionAfter24 Hrs.



VWD:SignalA, 262 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates(USP)
Itraconazole	4.09	8430526	1.23	7335
Totals		8430526		

Fig.No.29Typicalchromatogram ofTestsolutionInitial.
Fig.No.20TypicalchromatogramofTestsolutionAfter24Hrs.



VWD:SignalA, 262 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates(USP)
Itraconazole	4.10	8332637	1.22	7328
Totals		8332637		

Acceptancecriteria

%AbsolutedifferenceofStabilitysolution:NMT2.0w.r.t.Initial solution.

3) **SPECIFICITY:**Specificityistheabilitytoaccessunequivocallytheanalyteinthe presence of components which may be expected to be present.Blank,standardsolutionpreparedandinjectedtocheckpeakpurity.

TableNo. 22:Results ofSpecificity

Description	**Observation**
Blank	NointerferenceatR.T.ofItraconazoleduetoblank
Placebo	NointerferenceatR.T.of Itraconazoleduetoplacebo
Standard solution	Peakpuritywas0.999
Test Solution	Peakpuritywas0.998

Chromatograms:

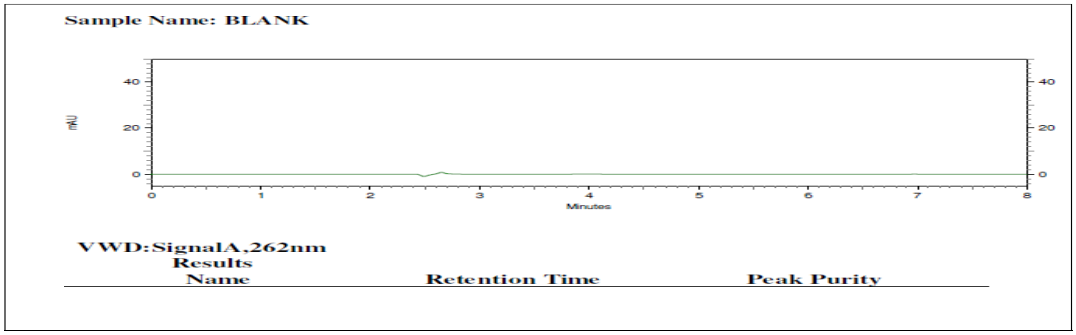


Fig.No.21Typical chromatogram ofBlank solution.

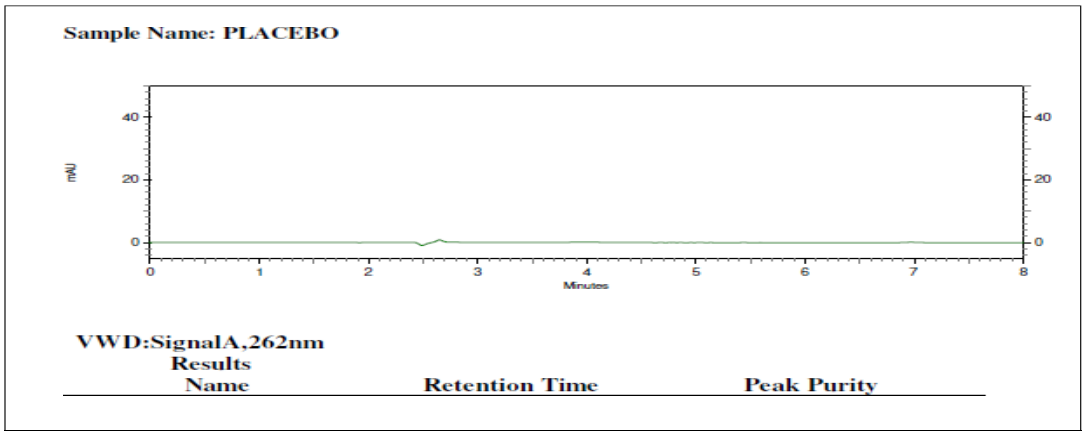


Fig.No.22Typicalchromatogram ofPlacebosolution.

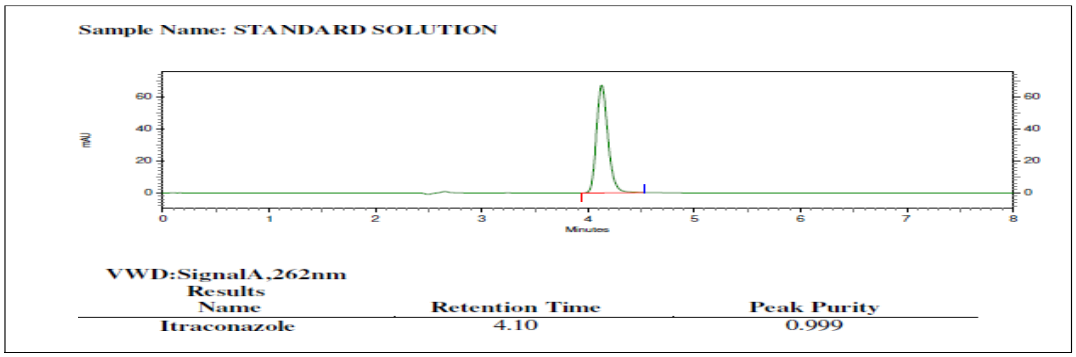


Fig.No.23:Typicalchromatogram ofPeakpurityofStandardsolution

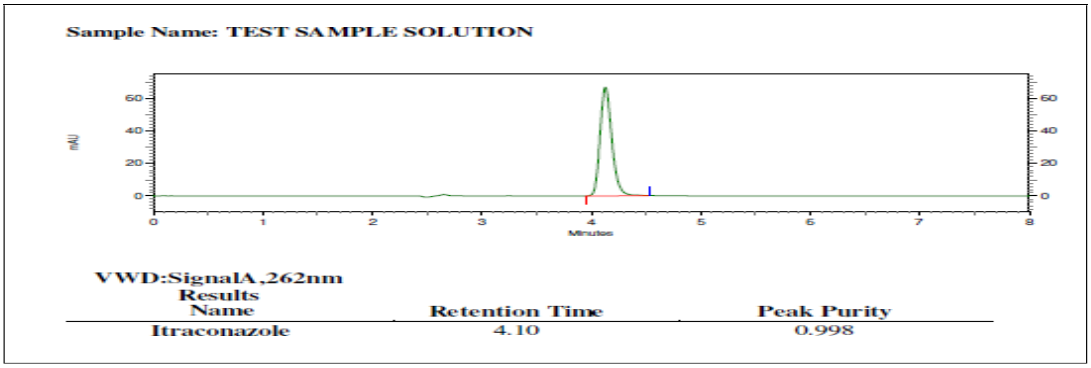


Fig.No.24TypicalchromatogramofPeakpurityofTestsamplesolution.

Acceptance criteria:

Blank: There should be no Interference at R.T. Itraconazole Standard and Test purityNLT 0.95

4) LINEARITYAND RANGE

Linearity of an analytical method is itsability toelicit test results that areproportional to the concentration of analyte in samples within a given range.

TableNo.23:LinearityDatafor Itraconazole

Level	Conc(µg/mL)	Area	Mean	% RSD
10%	2.50	869413	872925	0.354
		875234		
		874129		
50%	12.50	4285329	4282595	0.064
		4279864		
		4282593		
100%	25.00	8434129	8433368	0.041
		8429563		
		8436413		
125%	31.25	10563529	10567270	0.033
		10567862		
		10570420		
150%	37.50	12672410	12678838	0.047
		12679854		
		12684251		

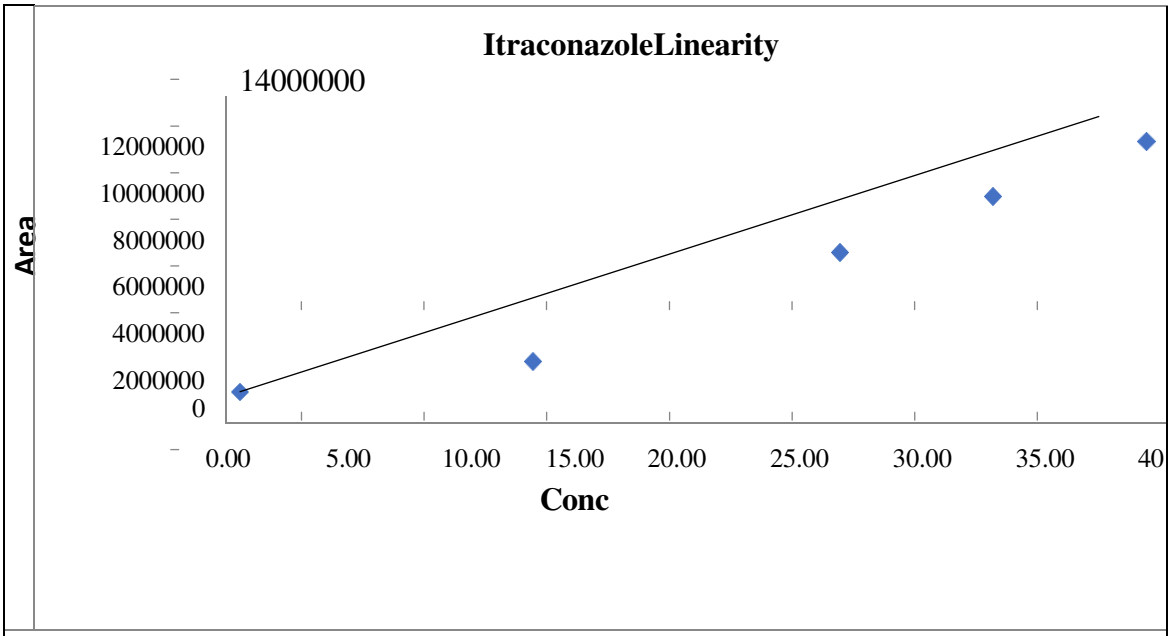


Fig.No.25: Calibration curveofItraconazole on HPLC

TableNo.24 :Dataoflinearityof Itraconazole

Srno.	**Parameter**	**Result value**	**Acceptancecriteria**
1	Beer'slinearityrange	2.50-37.50µg/mL	NA
2	Correlationcoefficient(R^2)	0.999989	NLT0.98
3	Intercept	43020.39	To be report
4	Slope	336734.66	To be report
5	%RSDforareaateachlevel	NA	NMT 2.0

TherespectivelinearequationforItraconazolewas

Y = M X+C

Y=336734.66x +43020.39

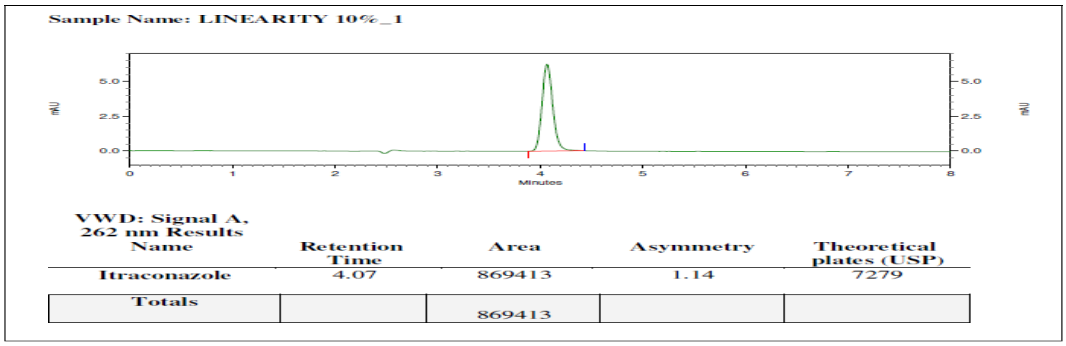
where, x=concentrationofAnalyteinµg/mL

y=is area of peak.

M = Slope

C=Intercept

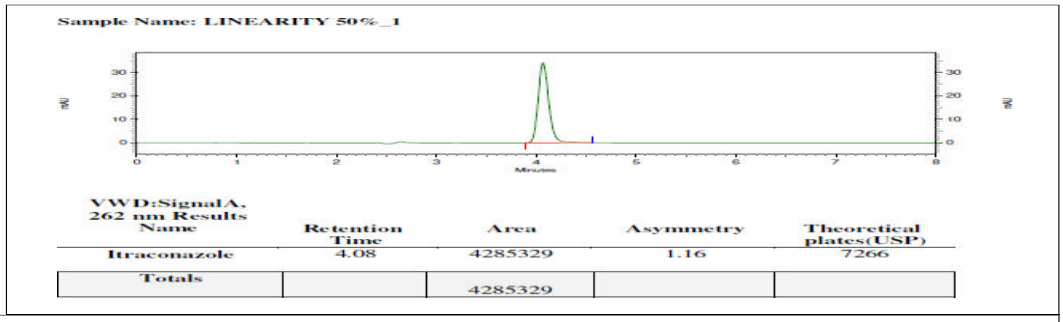
Chromatograms:



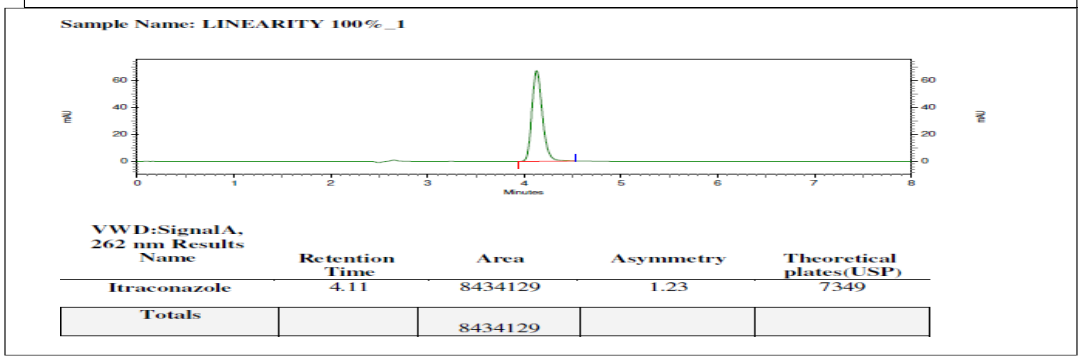
VWD: Signal A, 262 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Itraconazole	4.07	869413	1.14	7279
Totals		869413		

Fig.No.26Typical chromatogram ofLinearity 10%

Fig.No.27Typical chromatogram ofLinearity 50%.



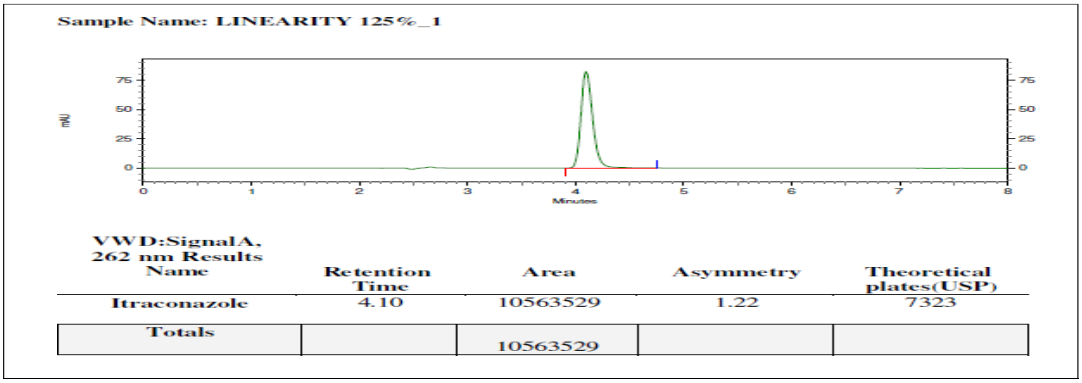
VWD:SignalA, 262 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates(USP)
Itraconazole	4.08	4285329	1.16	7266
Totals		4285329		



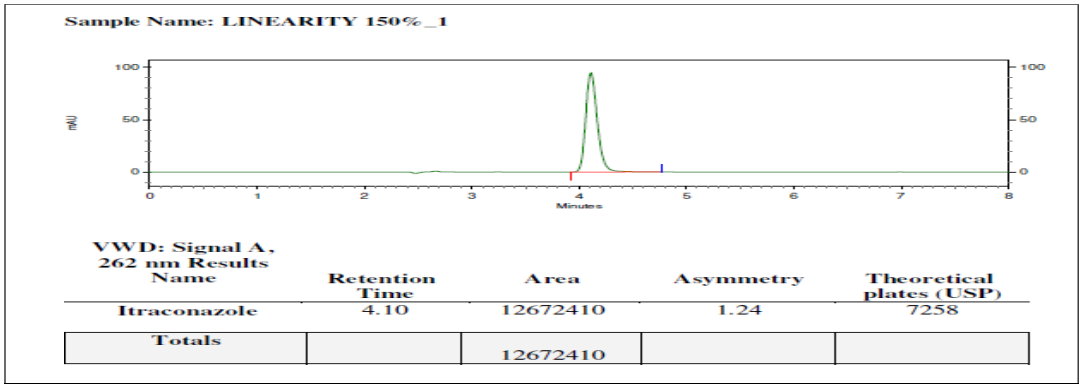
VWD:SignalA, 262 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates(USP)
Itraconazole	4.11	8434129	1.23	7349
Totals		8434129		

Fig.No.28Typical chromatogram ofLinearity 100%.

Fig.No.29Typical chromatogram ofLinearity 125%.



VWD:SignalA, 262 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates(USP)
Itraconazole	4.10	10563529	1.22	7323
Totals		10563529		



VWD: Signal A, 262 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Itraconazole	4.10	12672410	1.24	7258
Totals		12672410		

Fig.No.30Typical chromatogram ofLinearity 150%.

5)LimitofDetection(LOD)andLimitofQuantitation (LOQ):

σ=21915.04(Residualstandarddeviationofaregressi
online) s = 336734.66(Slope)

Detectionlimit(LOD):

LOD=3.3σ/ S

LOD=3.3 x 21915.04/ 336734.66

LOD= 0.215 µg/mL

Quantitationlimit(LOQ):

LOQ=10σ/ S

LOQ=10 x 21915.04/ 336734.66

6) ACCURACY (RECOVERY):

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value.

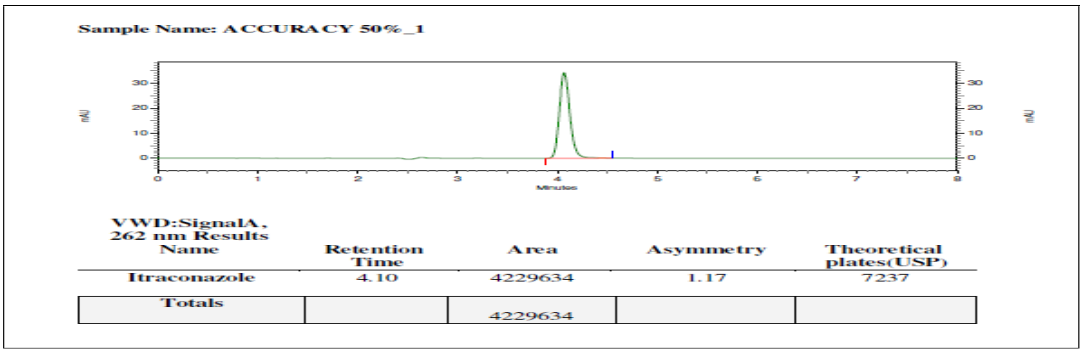
TableNo.25:ResultandstatisticaldataofAccuracyof Itraconazole

Level(%)	Area	Recovered conc(µg/mL)	Added conc(µg/mL)	% Recovery	Mean Recovery	% RSD
50	4229634	12.39	12.55	98.73	99.84	1.041
	4316529	12.65	12.65	100.00		
	4336531	12.70	12.60	100.79		
100	8473534	24.82	25.05	99.08	99.19	0.528
	8476523	24.83	25.15	98.73		
	8529461	24.99	25.05	99.76		
150	12650341	37.06	37.60	98.56	99.41	0.822
	12759634	37.38	37.58	99.47		
	12840659	37.62	37.55	100.19		

OverallRecovery:99.48%

%RSDforOverallRecovery: 0.771

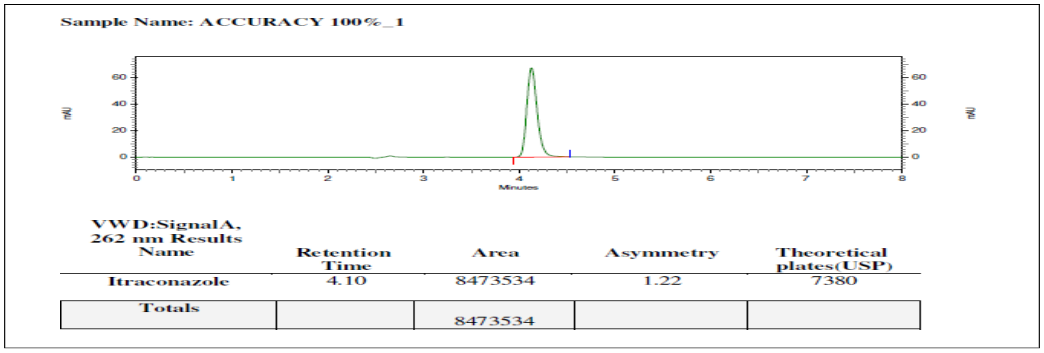
Chromatograms:



VWD:SignalA, 262 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates(USP)
Itraconazole	4.10	4229634	1.17	7237
Totals		4229634		

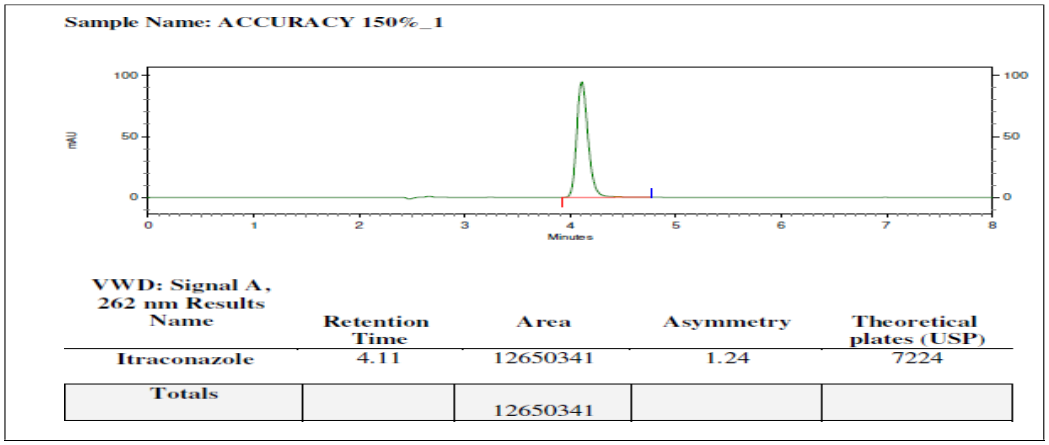
Fig.No.311:Typicalchromatogram ofAccuracy50%.



VWD:SignalA, 262 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates(USP)
Itraconazole	4.10	8473534	1.22	7380
Totals		8473534		

Fig.No.32:Typicalchromatogram ofAccuracy100%.



VWD: Signal A, 262 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Itraconazole	4.11	12650341	1.24	7224
Totals		12650341		

Fig.No.33:Typicalchromatogram ofAccuracy150%.

Acceptancecriteria:

%Recovery foreach levelandoverall recovery:98.0to102.0%

%RSDforeachlevelandoverallrecovery:NMT2.0

7) PRECISION

Precisionofananalyticalmethodisthedegreeofagreementamongindividualtestresultswhen the procedure is applied repeatedly to multiple samplings of a homogenous sample.

TableNo.26:ResultofIntra-dayandInter-DayPrecisionfor Itraconazoletestsampl assay

	Sample	**TestSample (mg)**	**Area**	**% Assay**
Repeatability	Sample1	175.2	8351034	97.92
	Sample2	175.8	8362519	97.72
	Sample3	175.4	8364859	97.97
	Sample4	175.6	8445965	98.81
	Sample5	174.8	8496451	99.85
	Sample6	174.3	8316529	98.02
	Mean			**98.38**
	STD DEV			**0.8113**
	% RSD			**0.825**
Intermedte precision (Inter-Day)	Sample1	175.0	8326504	97.74
	Sample2	175.3	8471393	99.27
	Sample3	175.4	8353622	97.84
	Sample4	175.6	8362519	97.83
	Sample5	174.9	8361558	98.21
	Sample6	175.2	8349630	97.90
	Mean			**98.13**
	STD DEV			**0.5805**
	% RSD			**0.592**
Repeatability PlusInter-day	**Mean**			**98.257**
	STD DEV			**0.6851**
	% RSD			**0.697**

Chromatograms:

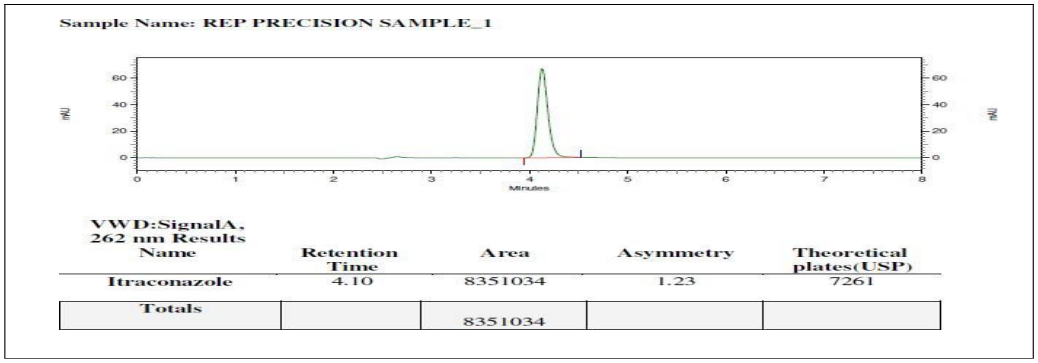
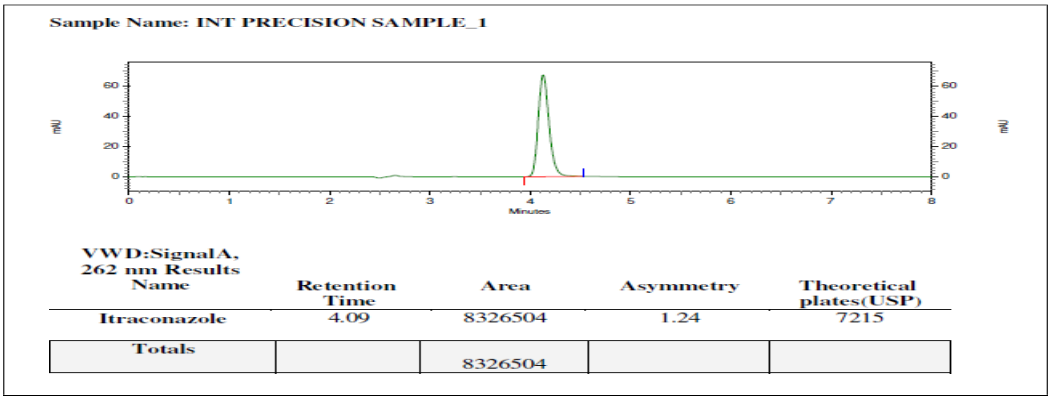


Fig.No.34:Typicalchromatogram of Repeatabilityprecision (Sample1)

Fig.No.35:Typicalchromatogram ofInter-dayprecision(Sample1).



Acceptancecriteria:

% Assay: % Assay value for each sample (Individual sample) and mean assay value for precision (6 sample), mean assay value intermediate precision (6 sample),and mean assay value for precision plus intermediate precision sample (12 sample): 90-110%

%RSD::%RSDforprecisionstudysamples(6sample),Intermediateprecisionstudysamples (6 sample) and precision plus intermediate precision sample (12 sample): NMT 2.0

8) ROBUSTNESS:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Followingchangesmade underRobustness:

- Changein Wavelength
- Changein flow rate
- Changein column oven temperature

TableNo.27:Result ofRobustness study

ChangeinParameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelengthby+3 NM(265 NM)	4.07	8042652	1.25	7634
Wavelengthby-3NM(259 NM)	4.07	7943521	1.21	7583

Flowrate by+10% (1.1mL/min)	3.67	7476226	1.20	7072
Flowrate by-10% (0.9mL/min)	4.49	9183648	1.23	7863
Columnoven temp by +2ºC (42ºC)	4.08	8546371	1.21	7459
Columnoven tempby-2ºC (38ºC)	4.11	8514567	1.24	7189

Chromatograms:

A. ChangeinWavelengthby +3 NM:

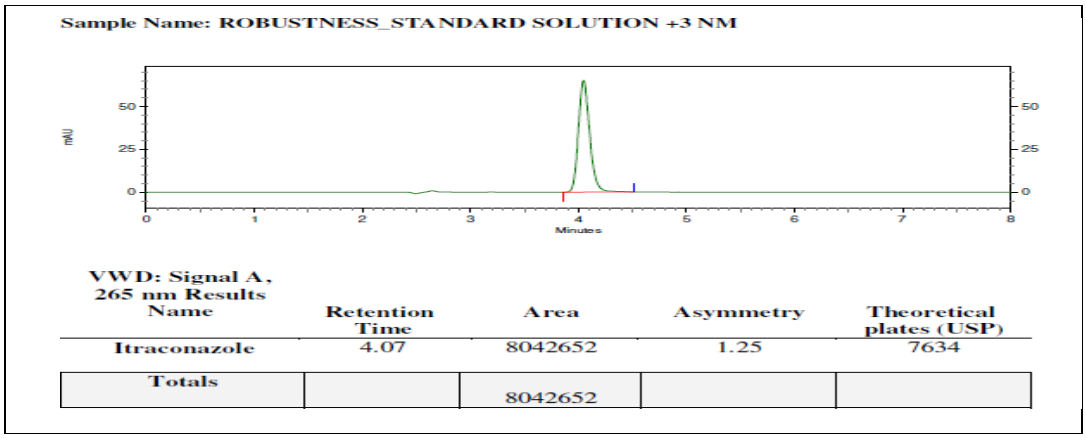


Fig.No.36: TypicalchromatogramofStandard+3 NM.

B. ChangeinWavelengthby-3 NM:

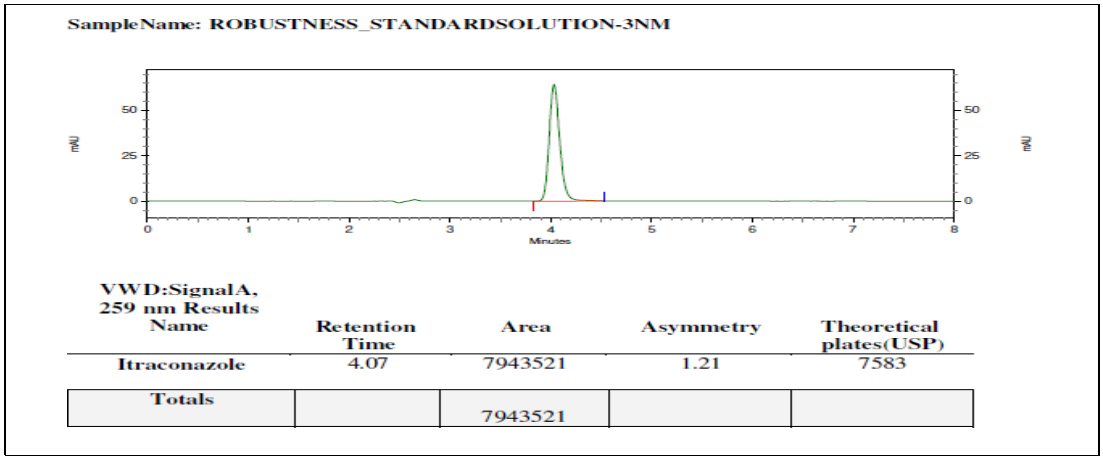


Fig.No.37:Typical chromatogram of Standard-3 NM.

CONCLUSIONS:

- The present work involved the development of simple, accurate, precise and suitable RP-HPLC method.
- Literature survey revealed that several methods have been reported for determination of Itraconazoleinbulkdrugorinpharmaceuticaldosageforms.Hence,inthepresentstudy, a new, sensitive and suitable reversed-phase high performance liquid chromatography method was developed and validated for the determination ofItraconazolein bulk drug and pharmaceutical dosage form.
- IndevelopedRP-

HPLCmethod,theanalytewereresolvedbyusingisocraticprogramand mobile phase was used Methanol : Water (75:25 % v/v) at a flow rate of 1.0 ml/min, on HPLC system containing UV- visible detector with Open lab EZ-Chrome Software and Kromasil C18, 250 mm X 4.6 mm, 5 µm. The detection was carried out at 258 nm.

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