Method Development and Validation for the estimation of VORICANOZOLE by RP-HPLC

मुंबई विद्यापीठ
University of Mumbai

Submitted in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy by

1. **16PH50 (Shaikh Mamuni Mujibar Marjina Begam)**
2. **16PH20 (Kazi Almas Hanif Rabia)**
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Under the Guidance of
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Certificate

Department of School of Pharmacy,
Anjuman-l-Islam's Kalsekar Technical Campus,
Khanda Gaon,
New Panvel, Navi Mumbai-410206

This is to certify that the project entitled **"Method Development And validation For The Estimation of voriconazole by RP-HPLC"** is a bonafide work of Shaikh Mamuni Mujibar Marjina Begam (16PH50), Kazi Almas Hanif Rabia (16PH20), Kazi Azmina Khairuddin Saira (16PH21), Ansari Rehnuma Parveen Irshad Ahmed Arab Jahan (16PH01) submitted for the appreciation of the degree of Bachelor of Pharmacy in Department of PHARMACY.

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Respected sir,

Subject :- Industrial project for B.Pharm Students

With reference to your request for providing the facilities at this institution I am glad to inform you that **1) Mamuni Shaikh 2) Rehnuma Parveeen Ansari 3) Afnan Kazi 4) Azmina kazi** Student of **AIKTC school Of Pharmacy ,New Panvel** under gone his project study of Voriconazole using RP HPLC and done analytical work regarding thesis.

Please note that facilities were provided purely keeping in mind the academic interest of the student. The date of the program starts from 26/12/2019 to 26 /12/2019

We hope that the student benefits from this industry interface in their curriculum

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1) Voriconzole HPLC Analysis Charges and Expriment	9000.00
Total	9000.00

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Approval for Bachelor of Pharmacy

This project entitled **"Method Development and Validation for The Estimation of VORICANOZOLE by RP- HPLC"** by Students

1. Shaikh Mamuni Mujibar Marjina Begam (16PH50)
2. Kazi Almas Hanif Rabia (16PH20)
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4. Ansari Rehnuma Parveen Irshad Ahmed Arab Jahan (16PH01)

Name is approved for the degree of Bachelor of Pharmacy in Department of PHARMACY.

Examiners	Supervisor
1.	1.
2.	2.

Declaration

I declare that this written submission represents my ideas in my own words and where others ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

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Abstract

A reverse phase high performance liquid chromatographic method is been used for the Validation of voriconazole. The chromatographical condition trial carried out on a Column Symmetry C18 (4.6 x 150mm) with the particle size of (5µm). The mobile phase use is of methanol,0.1%OPA with (pH 3.0) i.e. acidic and water (40+60% v/v). (0.7ml/min) is the flow rate and the following detection was carried out at 295nm. The retention time was 10min. The parameters like Linearity, robustness, accuracy, precision is within the specific limit as per ICH guideline. The Symmetry, plate number and RSD are as per limit and satisfactorily low which is required. This analytical experiment proved to be accurate, reliable, reproducible and consistency in its results. So, this proposed method can be successfully utilized in estimation of voriconazole in any quality control of Bulk dosage form and pharmaceutical formulations.

Contents

List of Figures

Keyword and Glossary

KEYWORDS: Voriconazole, RP-HPLC, validation

1. Analytical Procedure

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

2. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

3. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

3.1. Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

3.2. Intermediate precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

3.3. Reproducibility

- Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

4. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample

5. Robustness

The robustness of an analytical procedure 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.

6. System Suitability

System suitability should be determined by imitate analysis of the standard or before solution. System suitability is considered proper RSD, theoretical plates, tailing factor and resolution parameters.

Chapter-1

Introduction

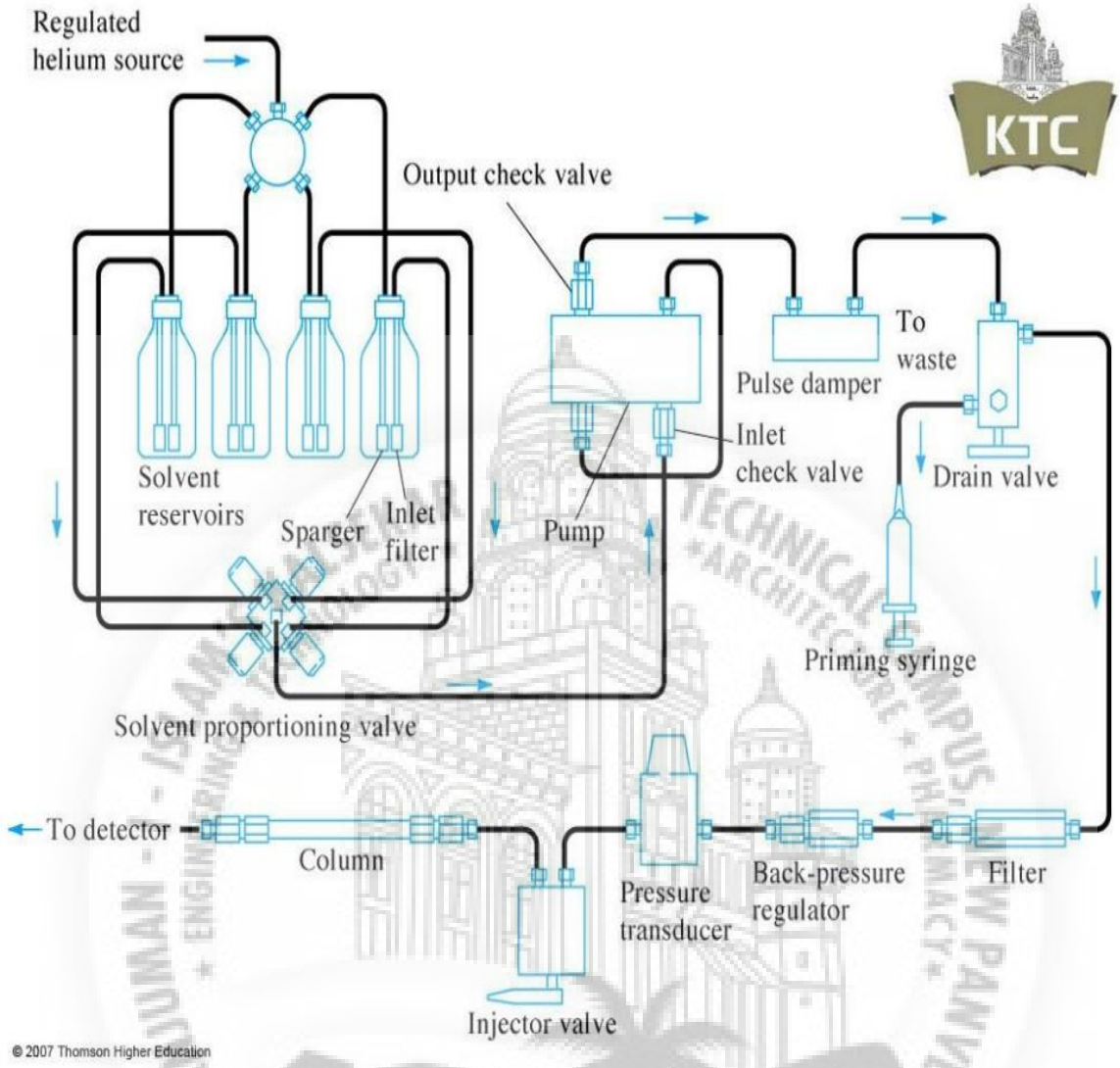
1.1) METHOD VALIDATION

- Method validation, according to ICH guideline is perform to ensure that an analytical methodology is accurate, specific, reproducible and robust over the specified range that an analyte will be analysed. Method validation provide an assurance of reliability.
- Mainly its means to prove something that is base on truth and which is acceptable with proper documentation report.
- Method validation is necessary to judge quality, reliability and consistency of any analytical results.

HPLC

- This method validation is done by RP-HPLC
- HPLC is chromatographic technique to separate individuals' components from any mixture solution for the purpose of Identification, purification and various studies
- HPLC separation can be done by two mean either by normal phase HPLC or RP-HPLC.
- Basically, we choose this RP-HPLC because: -

  i) wide range of compound can be tested.
  ii) Stabilize quickly
  iii)Inexpensive
  iv) Easy to operate.

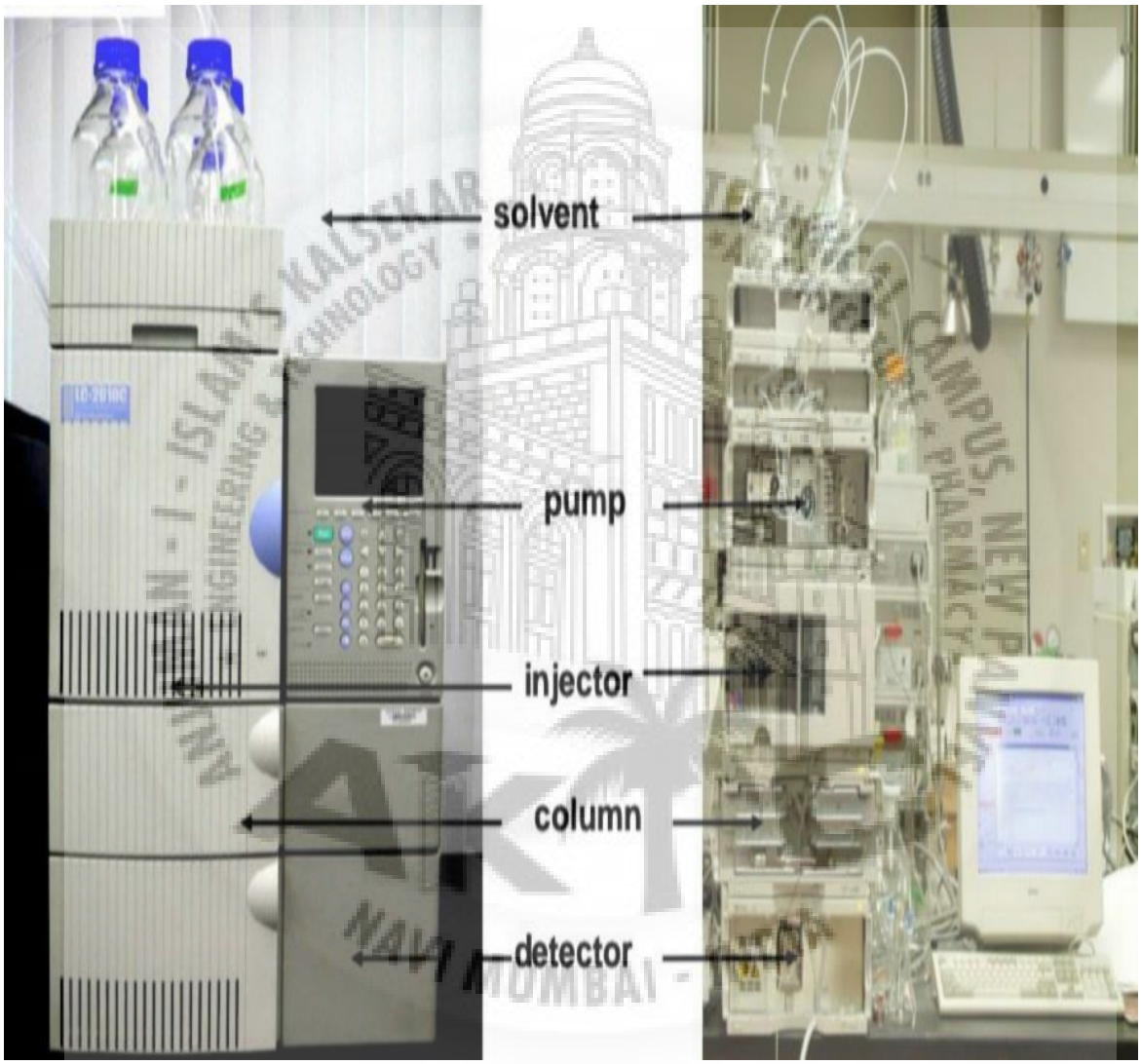


~~Figure-1: Working of HPLC~~

1.2) Instrumentation of HPLC

Components of HPLC

i) mobile phase reservoir
ii) pump
iii) sample
iv) column
v) detector



~~Figure-2: Components of HPLC~~

i) Mobile phase

Mobile phase reservoir will carry the mobile phase solution and through the siphon it will siphoned into the column. This development is as per the gravitational force. The Mobile phase reservoir contain dissolvable of various polarities, for example, water, methanol and repository channel at (2-10mm) supply end of the conveyance lines.

ii) Pump

The work of siphon is to put the fluid (called the mobile phase) through the section at a predefined flow rate mL/min.A siphon can convey a mobile phase (isocratic) or expanding versatile stage arrangement (gradient).On the Agilent 1100 ,we have a quaternary pump. Which means up to four dissolvable can be siphoned or blended in with one another. Before conveying mobile phase to the segment, the siphon blends the dissolvable in both of the steady extent (isocratic) or in fluctuating extent (gradient).

iii) Sample Injector

a) **Autosampler and injector**

The injector injects the sample into the mobile phase. The auto sampler measures the proper sample volume, infuses the sample, and flushes the injector to be prepared for the following sample. This allows unattended automatic operation. The autosampler holds 1.5ml sample vials. It contains a 100µl sample loop. It can inject volumes from 0.1 to 100 µl. To prevent carry over, it is recommended to use the needle wash vial in vial 91 to clean outside of needle. If you still see carry over, you may need to run a blank injection in between injections.

b) **Elution method: -**

- **Isocratic elution**: A partition that employes a solitary dissolvable or dissolvable blend of constant composition.
- **Gradient elution**: Here at least two dissolvable System that contrasts in polarities are utilized. After elutions is started the apportion in the dissolvable is fluctuated in the customized way,sometime persistently and at some point in a progression of division productivity is incredibly upgraded by slope elution.

iv) Columns

- **Liquid-Chromatographic Columns: -**
- Column segments are developed from the hardened steel tubing, although glassor tygon(lower pressure application(<600psi).
- Length from 10 to 30 cm and have within diameter measurement from 2 to 5mm.
- Particle size of 3 to 10 micrometre.
- Micro-columns have inside width(diameter) of 1 to 4.6 mm and length of 7.5 cm and compact with 3 to 5 um molecule contains upwards of 100000 plates/m have favorable position of speed and insignificant dissolvable utilization.

v) Detectors

Diode Array Dectors (DAD)

DAD vary from UV-VIS finders in that light from the lights is shone legitimately onto the stream cell, light that goes through the stream cell is scattered by the diffraction grating, and the measure of the scattered light is evaluated for every frequency in the photodiode clusters.

The cluster may contain a large number of diodes and the yield from every diode is outinely inspected by a PC and put away on a hard plate. Toward the finish of the run, the yield from any diode can be chosen and a chromatogram delivered utilizing the UV frequency that was falling on that specific diode.

During chromatographic turn of events, the yield of one diode is recorded progressively delivering a continuous chromatogram.

It is seen that by taking note of the hour of a specific pinnacle, a range can be gotten by reviewing from memory the yield of the considerable number of diodes at that specific time. Contrasted and a UV-VIS indicator, the DAD has the accompanying burdens:

- Commotion is huge on the grounds that the measure of light is little; the DAD is likewise powerless.
- To different changes, for example, light variances, on the grounds that the reference light can't be gotten.

DAD (Diode Array Detectors)

Affectability: -

Relies upon the molar assimilation coefficient of the compound.

Points of interest:

a) An exceptionally particular indicator which will identify just such solutes that explicitly assimilate UV/obvious radiation e.g., alkenes, aromatics and mixes having various bonds between C, O, N and S.

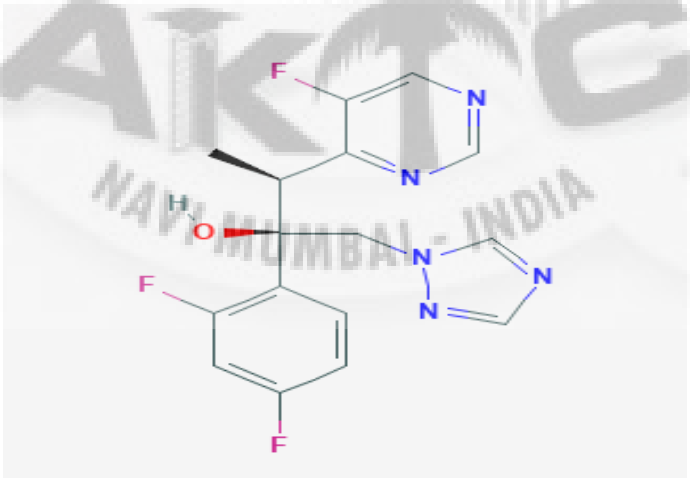
b) The portable stage utilized in a perfect world must not retain any radiation. Light source Flow cell Diffraction grinding W light the light from the two lights is blended, and shone onto the stream cell. D light Photo diode clusters (1,024 pieces). The light force is changed over into the electrical sign for every frequency.

Disadvantages:

- Compound without UV action can't be distinguished (eg. sugars).
- Solvents and eluents must not ingest at the recognition frequency.

1.3) Drug Profile

- Categories: acids, carbocyclic.
- Mol. Wt. 439.57
- IUPAC Name: (2S)-2-ethoxy-3-[4-(2-{-2-methyl-5[4-(methyl sulfinyl) phenyl]-1H-pyrrol-1-yl}ethoxyl)phenyl]propanoic acid.
- pka (acidic): 3.73
- pka (basic): -4.1
- class: pyrroles
- super class: organ heterocyclic compound
- direct parent: phenylpyrrole
- Solvability: drug was seen as sparingly dissolvable in methanol, chloroform, DMSO.
- Chemical Function: Bronsted corrosive a sub-atomic element equipped for giving a hydron to an acceptor (Bronsted base) by means of oxoacid.
- Biological Function: Ppargamma agonist, a PPAR (Peroxisome Proliferator-Actuated Receptor) modulator which enacts the peroxisome proliferator-initiated receptor-gamma.
- Application: Hypoglycemic agent a drug which lowers the blood glucose level.



~~Figure No 3: voriconazole~~

- **About Voriconazole**

A monocarboxylic acid that is (2S)-2-ethoxy-3-(p-ethoxyphenyl) propanoic acid in which one of the methyl hydrogens of the p-ethoxy substituent has been replaced by the nitrogen of 2-methyl-5-[4-(methylthio)phenyl]-1H-pyrrole. An agonist at the subtypes α and γ of the PPAR (Peroxisome proliferator-activated receptor with predominant pparα activity, it is used in the treatment of type 2 diabetes.

- **Mechanism of Action:**

Glitazars are another class of oral antidiabetic operators that initiate atomic receptors known as peroxisome proliferator–actuated receptors (PPAR). Three PPAR subtypes have been described: PPAR-α, - γ, and - β/δ. Upon ligand bonding, every receptor subtype intercedes unmistakable physiological consequences for glucose homeostasis and lipid digestion . Actuation of PPAR-γ lessens insulin opposition and improves glycemic control, though enactment of PPAR-α decreases fatty substance levels and expands groupings of HDLC. voriconazole is a double (α/γ) PPAR activator in t he glitazar class, prevalently PPAR-α agonist with moderate PPAR-γ agonistic movement, in this manner improving hyperglycemia and lipid variations from the norm (i.e., lessening fatty substances and expanding HDL-C) at the same time. This medication focuses on the PPAR-α and - γ receptors, which are right now anguished independently in the thiazolidinediones for type 2 diabetes (pioglitazone and rosiglitazone), and the fibrates for treatment of dyslipidemia (fenofibrate and gemfibrozil)respectively. This methodology of anguishing both the sub sorts of an atomic receptor PPAR was likewise foreseen to help decrease the pill trouble and improve medicine adherence in type 2 diabetic patients.

Chapter-2

Review of Literature

Title	Author	Year	Journal	Column	Mobile Phase	Λ	Rt
development of spectrophotometric method of voriconazole in bulk and pharmaceutical formulations using 1, 10 - phenanthroline	Man usha d.karad and v.d.barhate	vol. 4(3), jul - sep, 2015	international journalof advances in pharmacy, biologyand chemistry			510 nm	
validated stability indicating rp – hplc method development for the determination of voriconazole in bulk and pharmaceutical dosage form	sureshbabu kapavarapu, ramu golkonda, rambabu chintala	vol 5, issue 03, 2015	indo american journal of pharmaceutical research	waters hplc system equipped with uv visible detector, altima ods c18 (150 mm x 3.9 mm; 5µ) column	mixture of disodium hydrogen phosphate buffer and acetonitrile in a ratio of 40:60 v/v at a flow rate of 1.2ml/min	294 nm	2.827 min
method development and validation for the estimation of voriconazole in bulk and pharmaceutical dosage form by rp-hplc	hanumantha rao k1, lakshmana rao a, chandra sekhar kb.	july - september 2015; 2(3);150-154	indian journal of pharmacy and pharmacology,	kromasil c18 column (150 mm x 4.6 mm i.d., 5 µm particle size).	0.1% orthophosphoric acid buffer: 45:55 v/v 1 ml/min	295 nm	3.430 min.
stability indicating rp-hplc method development and validation for estimation of voriconazole in bulk and tablet dosage form	p. sripriya, g. naga sowjanya, a. ajitha, and v. uma maheswara rao	vol 4, issue 08, 2015. 2361-2372.	world journal of pharmaceutical research	on a ods c18 (ods) column (250 mm x 4.6 mm i.d. particle size 5)	acetonitrile: triethylamine buffer ph4.6:methanol (70:20: 10 v/v) as a mobile phase at a flow rate of 1 ml/ min	292 nm	2.443 min
method development and validation for the estimation of voriconazole in bulk and pharmaceutical dosage form by	rucha v. pancham, dr. milind j. umekar and r. t. lohiya	vol 7, issue 8, 2018 965-987	world journal of pharmacy and pharmaceutical sciences	shimadzu hplc system equipped with uv visible detector, ace 3c18-ar- hplc column (80×4.6mm) column	mobile phase of mixture of disodium hydrogen phosphate buffer and acetonitrile in a ratio of 58:42 v/v at a	295 nm	3.141 min

rp-hplc					flow rate of 1.ml/min		
development and validation of hptlc method for estimation of voriconazole in bulk and pharmaceutical dosage form	patel nm, mehta fa, shah da and chhalotiya uk	volume 2 issue 2 – 2015 ,1035	austin journal of analytical and pharmaceutical chemistry	aluminum plates precoated with silica gel 60 f254 were used as the stationary phase,	while the solvent system was n-butanol: ammonia (7:3 v/v).	… …	the rf value was observed to be 0.55 ± 0.02.
method development and validation for the estimation of voriconazole in bulk and pharmaceutical dosage form by rp-hplc	hanumantha rao k, lakshmana rao a, chandra sekhar kb			kromasil c18 column (150 mm x 4.6 mm i.d., 5 µm particle size).	using 0.1% orthophosphoric acid buffer:acetonitrile as the mobile phase in the ratio of 45:55 v/v	295 nm	3.430 min
identification of degradant products of voriconazole by uplc tandem mass spectroscopy and attenuated total reflection ftir techniques	t n v ganesh kumar1, , s vidyadhara 1, niteen ashok narkhede2, n yamini sai silpa1, m rajya lakshmi1	vol 52 | issue 4 | oct-dec, 2018	indian journal of pharmaceutical education and research	kromasil 100-5c18 (250×4.6 mm, 5µm) column.	acetonitrile and phosphate buffer (ph 7.4, 50:50 v/v) as a mobile phase 1.0 ml/min	294 nm	
development and validation of uv spectrophotometric method for voriconazole tablets	ekta h. amin , dr.dilip g. maheshwari	volume 4, issue 5: 2014 (312-315)	journal of pharmaceutical science and biosientafic research	shimadzu uv – visible double beam spectrophotometer (model-1800)			

Chapter-3
Aim and Objective

Aim: -

- **Method Development and Validation for The Estimation of Voriconazole By Rp-HPLC**

Objective: -

- To develop method for drug voriconazole in the form of bulk and solid dosage form.
- To validate, accuracy, precision, linearity, robustness as per ICH guidelines.
- The objective of the present study is to establish and generate inheriting Validation data for voriconazole used the wavelength of UV spectrophotometer.
- To obtained consistent, reliable and accurate data.
- To obtained result, that result from method validation is judge the quality of product.

Chapter-4
Experiment Methodology/Procedure

4.1) Procurement of Drug/Material

- Voriconazole pure form of drug obtain from RSITC laboratory Maharashtra, India.
- A marketed formula (dosage form) is taken from local pharmacy, Brand name - LIPAGLY (Zydus candilla healthcare Ltd).

4.2) Mobile Phase Composition

- Preparation of mobile phase: -
- The mobile phase was set up by blending methanol and water in proportion of (40:60 v/v) at pH3.
- The filtration of mobile phase was done by utilizing (0.4 micrometer) pore size and degasification done by ultrasonic vibrations before use.
- Flow rate = 1mL/min.
- All assurance performed at room temperature.

4.3) Procedures of validation parameters

1.1 Chromatographic Trial Conditions: -

Standard stock solution

- Standard stock solution of voriconazole (1000microgrm/ml) was prepared by accurately weighing the powder of voriconazole on weighing machine and transfer the weighed 10 mg voriconazole working standard, into 10ml volumetric flask as about dilute water prepared and make up the volume up to the mark with the same solvent to get (1000microgrm/ml)
- placed on the sonicator and Sonicate it for 15 min to dissolve it filter through the filter paper of pore size (0.45 micrometre).
- From the resulting solution pipette out 0.1 ml solution and transfer to 10 ml volumetric flask and make up the volume with water till mark.
- Carry out the trial indifferent PH conditions such as 3,4,5 and different mobile phase composition of methanol +0.1% OPA in ratio (80+20%v/v), (70+30%v/v), (50+50% v/v), (40+60%v/v).

Standard Stock Solution (Voriconazole)

- Accurately weigh the powder on weighing machine and transfer the the weighed 10 mg voriconazole working standard, into 10 ml volumetric flask as about diluent methanol completely and make up the volume up to the mark in the same solvent to get (1000 microgram/ml) standard stock solution.
- Place it on the sonicator and Sonicate it for 15 min to dissolve it, filter through the filter paper of pore size (0.45micrometr).
- From the resulting solution pipette out 0.1ml solution and transfer to 10 ml volumetric flask and make up the volume til mark with mobile phase methanol: water (0.1%OPA PH 3) prepared in (40 ml MEOH :60 ml water v/v) solvent.

- Inject to the Agilent C18 column (100 mm × 4.6mm) ,2.5 micrometre particle size with each of the injection volume 20 microliter with maximum wavelength of 295 nm.

Linearity

- Preparation standard stock arrangement: -
- The standard arrangement was set up by appropriate weakening of the essential stock arrangement with mobile phase to obtain working standard. All the estimations were performed at room temperature.
- The absorbance of the arrangement containing voriconazole was resolved at greatest wavelength of 295 nm using an appropriate blank.
- For linearity study, dilutions were made for voriconazole in the scope of 10 to 50 microgram/ml fixation were set up by dilution the stock arrangement with methanol: water.
- The calibration curve set up at a similar length by plotting chart between the area of peak and following concentration.

Preparation of sample solution: -

- It is carried out with preparation of five different concentration of (10,20,30,40,50 mcg).
- For concentration of 10 mcg/ml pipette out 0.1 ml from stock solution and dilute it with Mobile phase and make up the volume into 10 ml volumetric flask.
- For concentration of 20 mcg/ml pipette out (0.2 ml) of stock solution, for 30 mcg/ml pipette out (0.3ml), for 40 mcg/ml pipette out (0.4ml) ,for 50 mcg/ml pipette out (0.5 ml) stock solution and make up the volume with Mobile phase till mark in 10 ml volumetric flask.
- The % RSD value for slope any Y- intercept of calibration curve was calculated.

Precision

- For intermediate precision study, three sample solutions containing 10 mg of voriconazole with three different concentration (10 ,30 ,60 mcg/ml) voriconazole were analyzed twice time on the same day and %RSD was calculated.
- For concentration of 10 mcg/ml ,pipette out (0.1ml) of stock solution, for 30 mcg/ml pipette out (0.3ml) stock solution, for 60 mcg/ml pipette out (0.6 ml) stock solution and dilute it with mobile phase make up the volume till mark in 10 ml volumetric flask.

Repeatability

- Repeatability of system was determined with the sample.
- Concentration of 30 mcg/ml was made by pipette out (0.3ml) stock solution thrice and make up the volume with Methanol: water up till the mark in 10 ml volumetric flask.
- Replicas of sample solution containing 30 mcg of voriconazole were injected and peak area were measured and %RSD was calculated and repeated for thrice.

Degradation Studies

- Preparation of sample for degradation study
- Degradation studies is the parameter of validation in which sample solution is heated in different analytical condition.

- From the stock solution, sample is pipette out diluted and further heated in different conditions: -
    1) 0.1N HCl heating for 1 hour.
    2) 0.1N NaOH heating for 1 hour.
    3) At 3% H_2O_2 heating for 1 hour.
    4) Neutral heating for 2 hours.

Accuracy

- The prepared three standard solutions (8,10,12 microgram/ml) were injected at three times at different level as a test sample.
- Pipette out 0.08ml from stock solution for making 8mcg/ml concentration, for 10 mcg/ml pipette out (0.1 ml) stock solution, for 12 mcg/ml pipette out (0.12ml) and spiking is done.
- Appropriate samples were tested for percentage purity and recovery test at three different level (80% ,100% ,120%).
- The percentage of recoveries for impurities were calculated from slope and y-intercept of calibration curve obtained from linearity.

Robustness

- To determine the robustness of developed method, experimental condition was deliberately altered.
- The mobile phase composition was changed in (±1ml) proportion in mobile phase composition and the Flow rate was (±1ml/min) and change in the detection wavelength (±1nm) and effect of result were examined, and it was performed using 20 microgram/ml solution of voriconazole.
- To study the effect the flow rate in range between (0.6-0.8ml), wavelength in range between (294 - 296nm), mobile phase in range between (MEOH 39% +61% 0.1OPA pH 3 water - MEOH 41%+59%0.1 OPA pH 3 water) used.

CHAPTER-5
RESULTS & DISCUSSIONS

Chromatographic trial

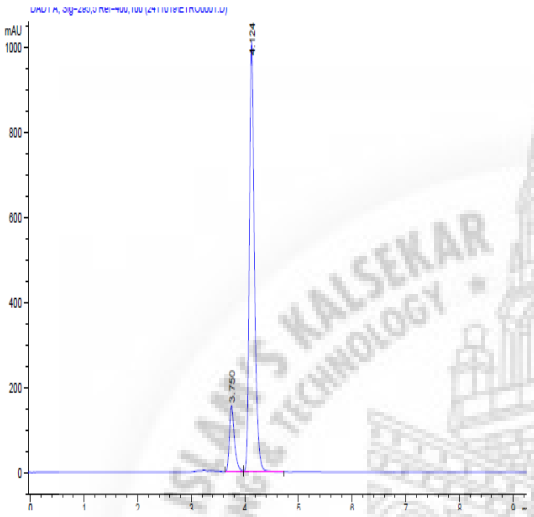


Fig. no.3 Chromatogram of Trial 1

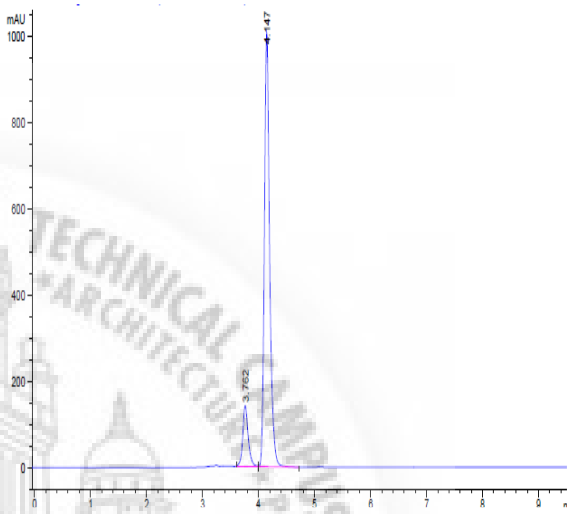


Fig. no. 4 Chromatogram of Trial 2

Ret. Time [min]	Symm.	Plates.
3.762	0.80	8588
4.147	0.71	10555

Table. No. 6 Result for Chromatogram of Trial 1

Ret. Time [min]	Symm.	Plates.
3.762	0.80	8588
4.147	0.71	10555

Table. No. 7. Result for Chromatogram of Trial 2

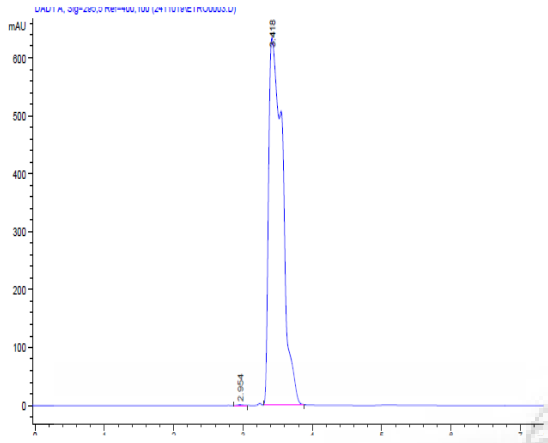


Fig. no. 5. Chromatogram of Trial 3

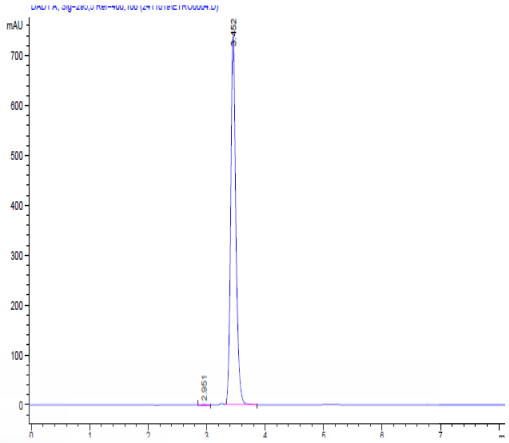


Fig. no. 6. Chromatogram of Trial 4

Ret. Time [min]	Symm.	Plates.
2.954	0.65	6579
3.418	0.32	1212

Table. No.8. Result for Chromatogram of Trial 3

Ret. Time [min]	Symm.	Plates.
2.951	0.72	7360
3.452	0.81	8270

Table. No. 9. Result for Chromatogram of Trial 4

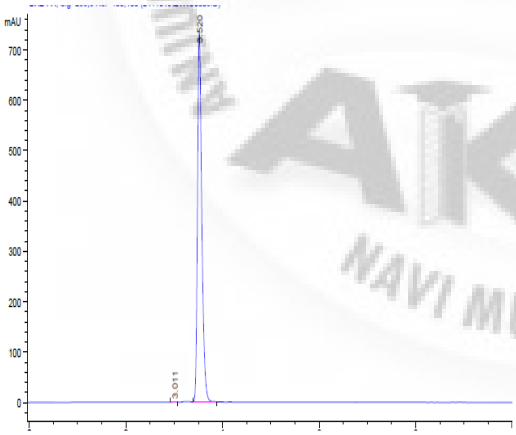


Fig. no. 7. Chromatogram of Trial 5

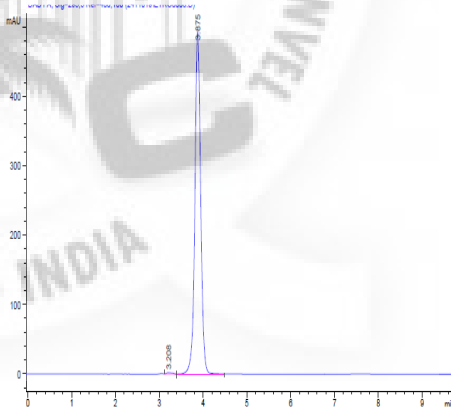


Fig. no. 8. Chromatogram of Trial 6

Ret. Time [min]	Symm.	Plates.
3.011	0.99	7533
3.520	0.70	8111

Table. No. 10. Result for Chromatogram of Trial 5

Ret. Time [min]	Symm.	Plates.
3.208	0.42	2228
3.875	0.91	4527

Table. No.11. Result for Chromatogram of Trial 6

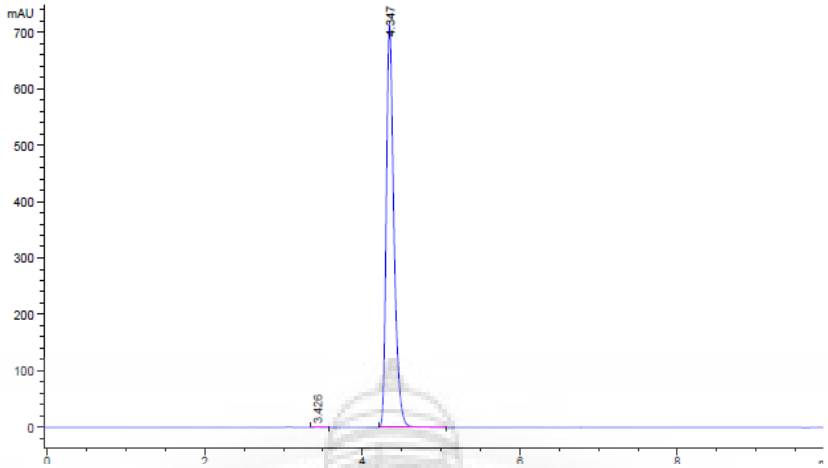


Fig. no. 9. Chromatogram of Trial 7

Ret. Time [min]	Symm.	Plates.
3.426	0.58	11786
4.347	0.68	10949

Table. No. 12. Result for Chromatogram of Trial 7

5.1) Linearity

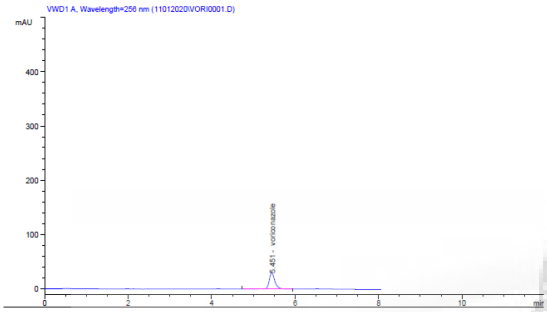


Fig.No.10. Chromatogram of linearity 10 mcg

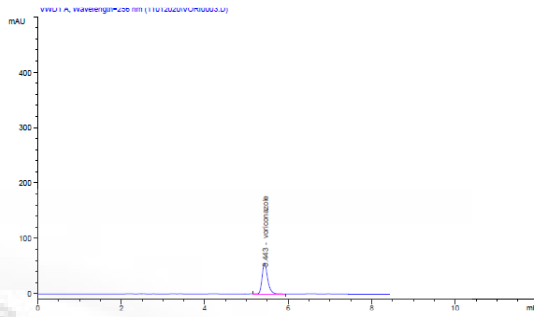


Fig.No.11. Chromatogram of linearity 20 mcg

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.381	1819.39	0.79	7902

Table no 13: Chromatogram of linearity 10 mcg

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.364	3683.16	0.75	5648

Table no 14: Chromatogram of linearity 20 mcg

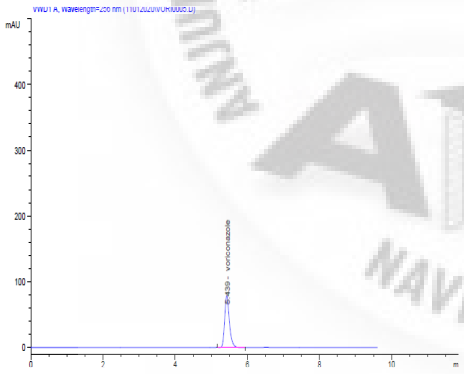


Fig.No.12. Chromatogram of linearity 30 mcg

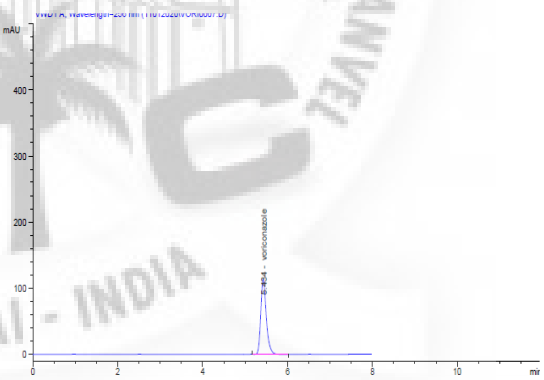


Fig.No.13. Chromatogram of linearity 40 mcg

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.381	5411.00	0.69	9345

Table no 15: Chromatogram of linearity 30 mcg

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.384	7395.08	0.71	11138

Table no 16: Chromatogram of linearity 40 mcg

Result for Linearity

By plotting the graph of concentration vs absorbance, we get straight line that which shows linearity is been obtain the value of r^2 (linear regression) is obtain to be 0.999. Symmetry and theoretical plate no. are as per limits.

Acceptance criteria

According to ICH guideline the r^2 (linear regression) value should be greater than 0.95 or equal to 1. The symmetry and plate no. Should be less than 2 and more than 2000 respectively.

Inference

The linearity test is been pass as it specified the test limits as per ICH guideline.

5.2) Accuracy

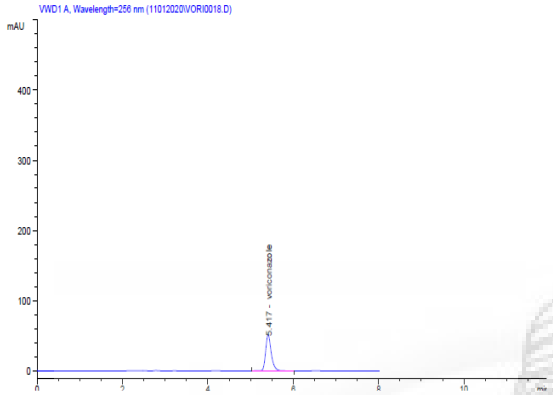


Fig.no. 14. Chromatogram of Accuracy 80%

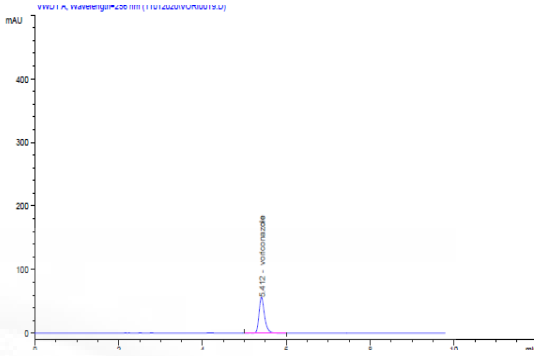


Fig.no. 15 Chromatogram of Accuracy 100%

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.377	3345.92188	0.69	10613

Table no 20: Chromatogram of Accuracy 80%

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.340	3698.87549	0.68	9406

Table no 21: Chromatogram of Accuracy 100%

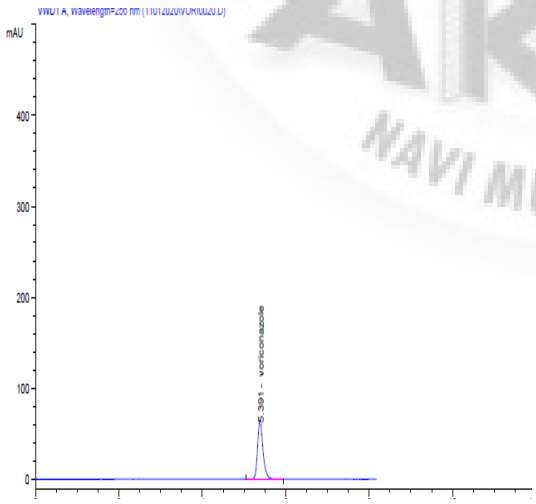


Fig.16. Chromatogram of Accuracy 120%

Drug	Sr No	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	Amt. found ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
SAR	1	80%	10	08	18.06 ±0.01	8.06 ±0.01	100.75 ±0.18
	2	100%	10	10	20.01± 0.04	10.01 ±0.04	100.89±0.38
	3	120%	10	12	21.94 ±0.06	11.94± 0.06	99.51± 0.55

Table no 23. Result of Recovery data for voriconazole

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.388	4030.18945	0.69	9616

Table no 22: Chromatogram of Accuracy 120%

Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation*	% RSD
80%	voriconazole	100.75	0.18	0.18
100%	voriconazole	100.09	0.38	0.38
120%	voriconazole	99.51	0.55	0.55

Table.24: Statistical Validation of Recovery Studies of voriconazole

Result For Accuracy

Here we done spiking that for three different concentration 80%,100% and 120% of concentration

Theoretical plate no. Is 9616, symmetry 0.69. Percent mean recovery of (100.76,100.09,99.51) respectively. %RSD is of (0.18%,0.38%,0.55%) respectively as per show in above result table.

Acceptance criteria

The mean recuperation will be in between 90 to 110% or of 100 ± 2% is common for a test of a functioning fixing in a drug according to the guidelines. The %RSD in precision ought to be ≤2 or ≤1

Inference -

The particularly accuracy test is been pass as per specified test limit give by ICH guideline

5.3) System Suitability

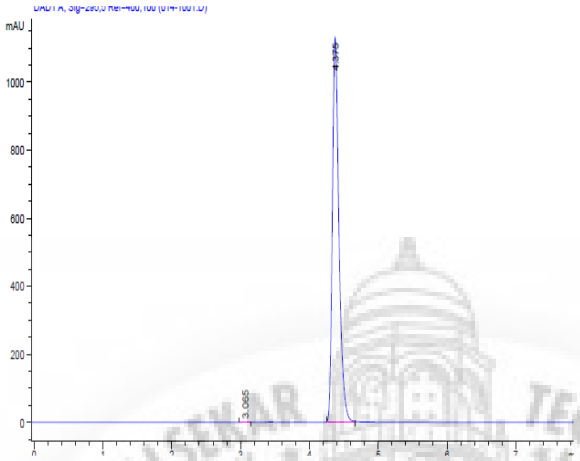


Fig No.17: Chromatogram of System suitability No- 1

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.352	5425.10254	0.70	9703

Table No.25: Chromatogram of System suitability No- 1

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.365	5434.29004	0.68	9514

Table No.26: Chromatogram of System suitability No- 2

Sr. No.	Concentration of voriconazole (mg/ml)	Peak area	Amount found (mg)	% Amount found
1	30	5425.10		
2	30	5434.29	29.74	99.14
	Mean	5429.70		
	SD	4.59		
	%RSD	0.12		

Table No.27: Repeatability studies on voriconazole

Result For System Suitability

We get is well resolve and sharp peak with the Symmetry value of 0.70and 0.68 respectively and the theoretical plate no. is 9703 and 9514 respectively.

Acceptance criteria

According to ICH guideline the symmetry and plate no. Should be less than 2 and more than 2000 respectively.

Inference

The system suitability test is been pass as it specified the test limits as per ICH guideline and also show repeatability in its study.

5.4) Precision

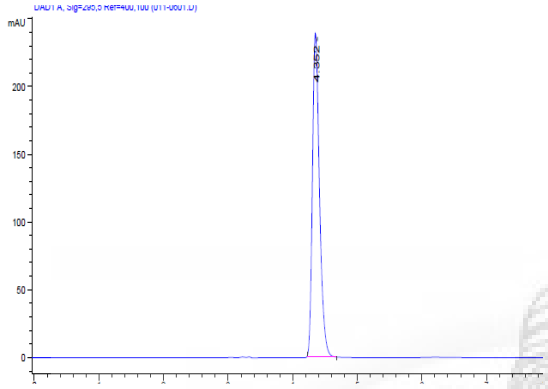


Fig No .18: Chromatogram of Precision 10 mcg-01

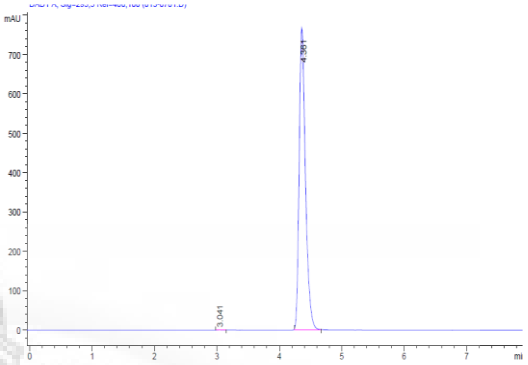


Fig No .19: Chromatogram of Precision 30 mcg-01

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.352	1872.49	0.71	8169

Table no 28: Chromatogram of Precision 10 mcg-01

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.361	5418.17	0.70	9740

Table no 29: Chromatogram of Precision 30 mcg-01

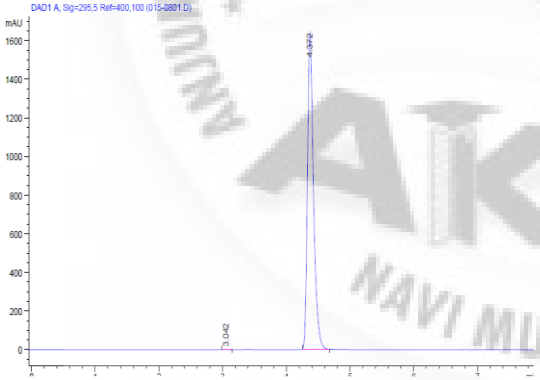


Fig No .20: Chromatogram of Precision 60 mcg-01

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.372	10757.4	0.70	11488

Table no 30 : Chromatogram of Precision 60 mcg-01

Drug	Concⁿ(µg/ml	Intraday Precision		
		Mean± SD	%Amt Found	%RSD
voriconazole	10	1872.79± 0.30	97.94	0.89
	30	5421.08 ±1.68	98.68	0.03
	60	10759.65 ±2.25	99.38	0.02

Table No 31: Result of Intraday and Inter day Precision for voriconazole

Result For Precision

According to result the theoretical plate no. Is 9740 and symmetry is 0.70 respectively. The %RSD is 0.89%,0.03% and 0.02% as per different concentration.

Acceptance criteria

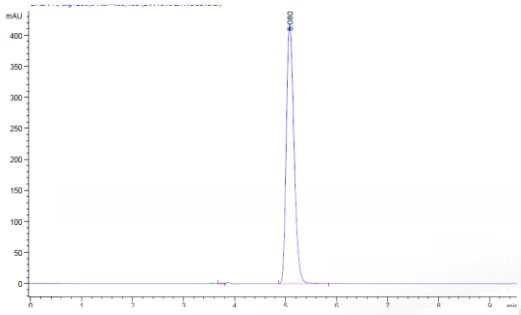
The FDA expresses that the run of the mill rsd ought to be 1% for sedate substances and medication items 2% for mass medications and completed items.

Inference

The precision test is been pass as per specified limits.

5.5) Robustness

Flowrate



FigNo.21. Chromatogram of Flow rate change 0.6 ml

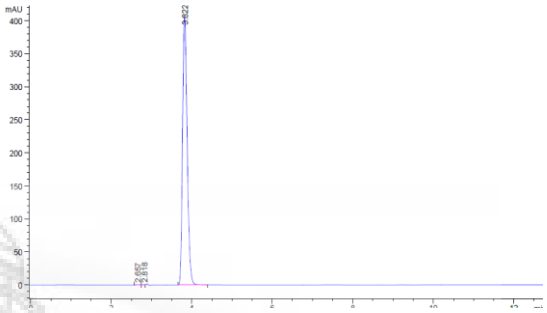


Fig No 22. Chromatogram of Flow rate change 0.8 ml

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
5.080	4382.56	0.74	5584

Table no 32: Chromatogram of Flow rate change 0.6 ml

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
3.822	3257.72	0.75	5467

Table no 33: Chromatogram of Flow rate change 0.8 ml

Wavelength

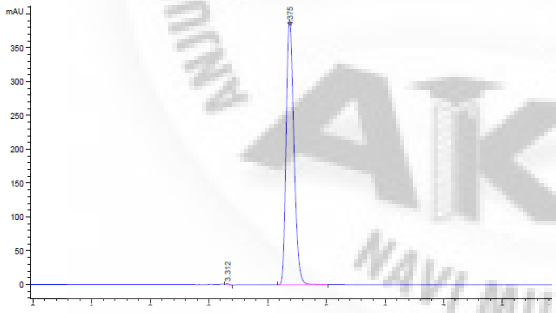


Fig. No 23: Chromatogram of comp change wavelength change 294 nm

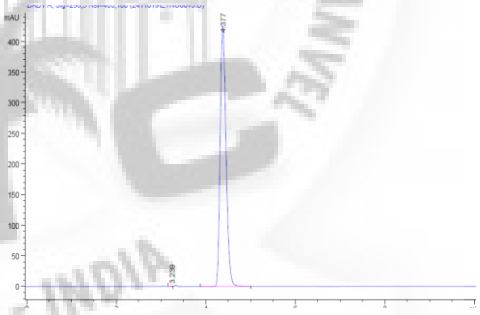


Fig. no 24: Chromatogram of comp change wavelength change 296 nm

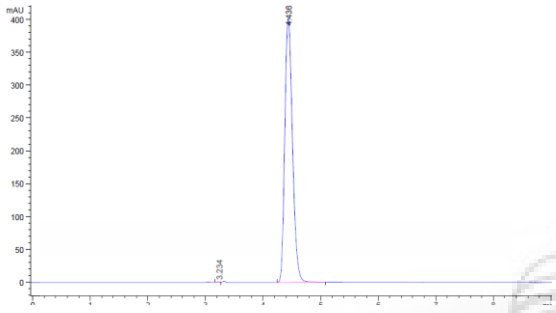
Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.375	3618.31	0.75	5241

Table No 34: Chromatogram of comp change wavelength change 294 nm

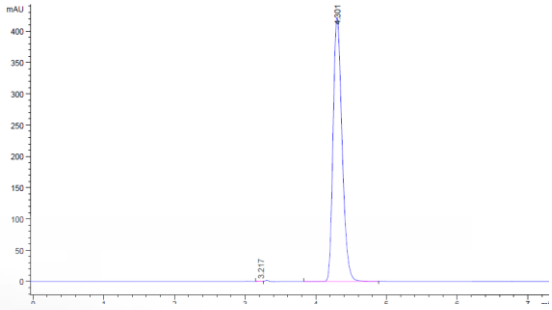
Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.377	3881.50	0.75	5590

Table No 35: Chromatogram of comp change wavelength change 296 nm

Mobile phase



FigNo.25. Chromatogram of mobile phase rate change MeOH 39 % + 61 % (0.1 % OPA water pH 3)



FigNo.26. Chromatogram of mobile phase rate change MeOH 41 % + 59 % (0.1 % OPA water pH 3)

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.436	3745.39	0.75	5389

Table no 36: Chromatogram of mobile phase change MeOH 39 % + 61 % (0.1 % OPA water pH 3)

Ret. Time [min]	Area [mAU*a]	Symm.	Plates.
4.301	3733.65	0.74	5764

Table no 37: Chromatogram of mobile phase change MeOH 41 % + 59 % (0.1 % OPA water pH 3)

Result For Robustness

During vigor check, the robustness were found to shift in the range ±1% though the maintenance time was additionally found to change in the range ± 1min. The parameters like wavelength,flowrate and mobile phase they were not altogether influenced when checked by fluctuating the boundaries.

5.6) Degradation Studies

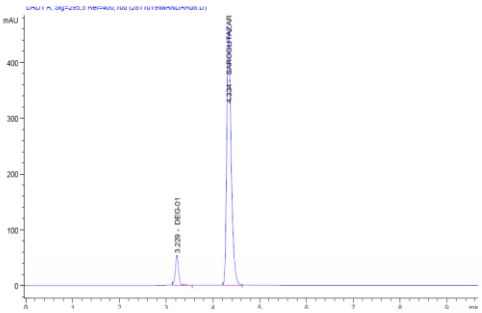


Fig. no. 27: Chromatogram of comp change at 0.1N HCL heating after 1 hours

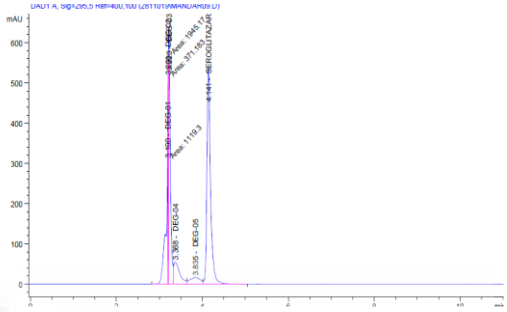


Fig. no 28: Chromatogram of comp change at 0.1N naoh heating after 1 hr.

Ret. Time [min]	Area [mAU*a]	Area % [mAU*a]	Name
3.229	263.05923	7.9540	DEG-01
4.334	3044.18140	92.0460	voriconazole
TOTAL	3307.24063	.....	.....

Table. No. 40 Result of Degradation Study of voriconazole at 0.1N HCL heating after 1 hours.

Ret. Time [min]	Area [mAU*a]	Area % [mAU*a]	Name
3.190	1119.30396	15.0509	DEG-01
3.203	371.18298	4.9912	DEG-02
3.220	1945.16565	26.1560	DEG-03
3.368	538.76117	7.2446	DEG-04
3.835	271.59583	3.6521	DEG-05
4.141	3190.76416	42.9052	voriconazole
TATAL	7436.77374	.....	.....

Table no. 41 Result of Degradation Study of voriconazole at 0.1N NaOH heating after 1 hours

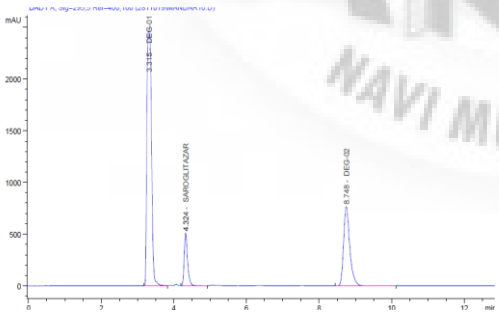


Fig. no. 29. Chromatogram of comp change at 3% H2O2 heating after 1 hours

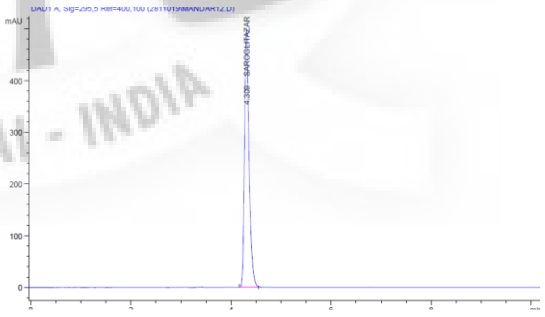


Fig no. 30. Chromatogram of comp change at Neutral heating after 2 hours

Ret. Time [min]	Area [mAU*a]	Area % [mAU*a]	Name	
3.315	2198.55	64.0544	DEG-01	
4.324	3310.64307	9.6455	voriconazole	
8.748	9026.99414	26.3001	DEG-02	
TOTAL	34323.1	.....	.....	

Table no. 42. Result of Degradation Study of voriconazole at 3% H2O2 heating after 1 hours

Ret. Time [min]	Area [mAU*a]	Area % [mAU*a]	Name
4.309	3165.68579	100.00	voriconazole
TOTAL	3165.68579	.....	.....

Table no. 43. Result of Degradation Study of voriconazole at Neutral heating after 2 hours

Result for Degradation Studies

i) Degradations study of Voriconazole with 0.1N HCl

Voriconazole was found to degrade with 0.1N HCl in acidic condition in an one hour of heating, as an extra impurities peak was detected.

ii) Degradations study of Voriconazole with 0.1N NaOH

Voriconazole was found to be quickly degrade with 0.1N NaOH in Alkaline conditioning and one hour of heating, as an extra sharp peak was found to be detected that means degradation occur rapidly

.

iii) Degradations study of Voriconazole with 3% H_2O_2

Voriconazole was found to degrade with 3% H_2O_2 under oxidative condition heating for an one hour, as an extra impurities peak was detected.

iv) Degradations study of Voriconazole with neutral conditions.

Voriconazole with neutral condition and heating for a two hour, no extra peak is detect mean degradation doesn't occurs.

CHAPTER-6
CONCLUSION

- Method validation and development for the estimation of voriconazole by RP-HPLC is established.
- Linearity, Precision, Accuracy, Robustness these parameters are come under acceptance criteria within the specified limits of ICH guideline.
- The system suitability studies that contains the symmetry and theoretical plate no. are as per limits provides by ICH guideline.
- Also, the standard deviation is satisfactorily low which is required.
- This analytical experiment is proved to be accurate, reliable, reproducible and consistent in its results.
- This method is also an economically effective to perform.
- So, this proposed method can be successful utilized in estimation of voriconazole in any formulation and bulk dosage form.

CHAPTER-7
FUTURE SCOPE

- RP-HPLC was known by its high sensitivity and readily adaptability to it quantitative determination.
- This can be widely use for separation of volatile and non-volatile substance.
- The selected estimation method has great advantage in terms of speed, resolution of drug.
- RP-HPLC large impact on pharmaceutical work, mainly for the separation of highly volatile compound.
- It is also a less time-consuming technique.
- The agonistic activity of voriconazole towards both the subtype of nuclear receptor PPAR will be helpful in further treatment of diabetes.

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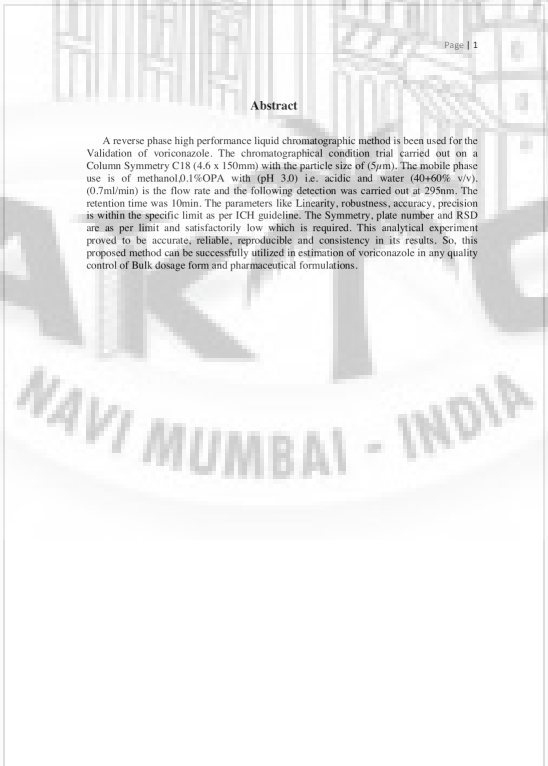

Abstract

A reverse phase high performance liquid chromatographic method is been used for the Validation of voriconazole. The chromatographical condition trial carried out on a Column Symmetry C18 (4.6 x 150mm) with the particle size of (5µm). The mobile phase use is of methanol,0.1%OPA with (pH 3.0) i.e. acidic and water (40+60% v/v), (0.7ml/min) is the flow rate and the following detection was carried out at 295nm. The retention time was 10min. The parameters like Linearity, robustness, accuracy, precision is within the specific limit as per ICH guideline. The Symmetry, plate number and RSD are as per limit and satisfactorily low which is required. This analytical experiment proved to be accurate, reliable, reproducible and consistency in its results. So, this proposed method can be successfully utilized in estimation of voriconazole in any quality control of Bulk dosage form and pharmaceutical formulations.