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RP-HPLC method development and validation for estimation of voriconazole, azithromycin and metoprolol tartrate in pharmaceutical dosage form

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A b s t r a c t

Analytical methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation step. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. Voriconazole & Azithromycin was freely soluble in water and alcohol. Metoprolol Tartrate was freely soluble in alcohol and sparingly soluble in water. Methanol and potassium dihydrogen ortho phosphate (pH 3) was chosen as the mobile phase. The run time of the HPLC procedure was 10 minutes. The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration range of 10-100 µg / ml. The % recovery of Voriconazole, Azithromycin and Metoprolol Tartrate were found to be in the range of 99.22 % - 100.11 %. As there was no interference due to excipients and mobile phase, the method was found to be specific. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate and Mobile phase composition separately and analysis being performed by different analysts. Good agreement was seen in the assay results of Pharmaceutical formulation by developed method. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Voriconazole, Azithromycin and Metoprolol Tartrate in Bulk drug and Pharmaceutical formulation.

Keywords: Voriconazole, Azithromycin and Metoprolol Tartrate, HPLC.

A r t i c l e I n f o

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C O N T E N T S

1. Introduction

Drug Profile

Drug : Voriconazole

Synonyms : Voriconazolum, Voriconazol, Vfend, VCZ

Description : Voriconazole (Vfend®, Pfizer) is a triazole antifungal medication used to treat serious fungal infections. It is used to treat invasive fungal infections that are generally seen in patients who are immunocompromised. These include invasive candidiasis, invasive aspergillosis, and emerging fungal infections.

Solubility: aqueous solubility is very low at 0.7mg/mL at 25ºC.

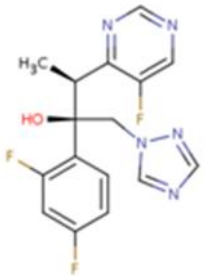
Physicalstate : crystalline solid

Meltingpoint: 127-130 °C

C**ASNO:** 137234-62-9

IUPAC name : (2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1H-1,2,4-triazol-1-yl)butan-2-ol

Structure :



Molecular formula: $C_{16}H_{14}F_3N_5O$

Molecular weight: Average: 349.3105, Monoisotopic: 349.1150447

Bioavailability: 96%

Half-life: dose dependant

Protein binding: 58%

Dose: Less than 40 kg: 100 mg orally every 12 hours, 40 kg or more: 200 mg orally every 12 hours

Category : Antifungal Agents,14-alpha Demethylase Inhibitors

Pharmacology: Voriconazole is well absorbed orally with a bioavailability of 96%, allowing patients to be switched between intravenous and oral administration. Being metabolized by hepatic cytochrome P450, voriconazole interacts with some drugs. Administration is contraindicated with some drugs (such as sirolimus, rifampin, rifabutin, and ergot alkaloids) and dose adjustments and/or monitoring when coadministered with others (including cyclosporine, tacrolimus, omeprazole, and phenytoin). Voriconazole may be safely administered with cimetidine, ranitidine, indinavir, macrolide antibiotics, mycophenolate, and prednisolone[1-3]. Because voriconazole is metabolized by the liver, the dose should be halved in patients with mild to moderate hepatic impairment (Child-Pugh score A or B). There is no data available for patients with severe hepatic impairment (Child-Pugh C). No dose adjustment is necessary for renal impairment or advanced age, but children seem to clear voriconazole faster than adults and drug levels may need monitoring.

Pharmacodynamics:

Mechanism of Action:

Voriconazole binds and inhibits ergosterol synthesis by inhibiting CYP450-dependent 14-alpha sterol demethylase. The inhibition of 14-alpha sterol demethylase results in a depletion of ergosterol in fungal cell membrane.

Pharmacokinetic Properties

Absorption and Bioavailability

Voriconazole is rapidly and almost completely absorbed following oral administration, with maximum plasma concentrations (Cmax) achieved 1 to 2 hours after dosing. The oral bioavailability of voriconazole is estimated to be 96%. Bioequivalence has been established between the 200 mg tablet and the 40 mg/mL oral suspension when administered as a 200 mg dose. When multiple doses of voriconazole are administered with high fat meals, Cmax and AUCτ of the tablets are reduced by 34% and 24%, respectively, and Cmax and AUCτ of the suspension are reduced by 58% and 37%, respectively. The absorption of voriconazole is not affected by changes in gastric pH.

Distribution

The volume of distribution at steady state for voriconazole is estimated to be 4.6 L/kg, suggesting extensive distribution into tissues. Plasma protein binding is estimated to be 58%. Cerebrospinal fluid samples from eight patients in a compassionate programme showed detectable voriconazole concentrations in all patients

Metabolism and Elimination

Hepatic. The major metabolite of voriconazole is the N-oxide, which accounts for 72% of the circulating radiolabelled metabolites in plasma. Since this metabolite has minimal antifungal activity, it does not contribute to the overall efficacy of voriconazole.

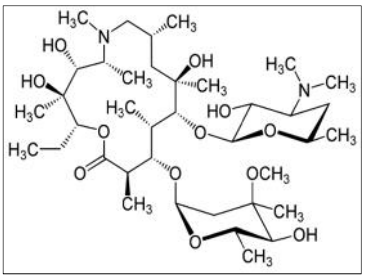
Adverse Effects:

Transient visual disturbances, fever, rash, vomiting, nausea, diarrhea, headache, sepsis, peripheral edema, abdominal pain.

Storage: Store voriconazole tablets at room temperature, between 59 and 86 degrees F (15 and 30 degrees C). Do not refrigerate or freeze.

Brand Name: VORAZE

Azithromycin



Chemical Data

IUPAC Name : (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-11-{[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy}-2-ethyl-3,4,10-trihydroxy-13-{[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-

yl]oxy}-3,5,6,8,10,12,14-heptamethyl-1-oxa-6-azacyclopentadecan-15-one

Chemical formula : $C_{38}H_{72}N_2O_{12}$
Molecular weight : 748.9845
CAS No : 83905-01-5
pKa : 9.6

Physical Data

Description: Azithromycin is a semi-synthetic macrolide antibiotic of the azalide class. Like other macrolide antibiotics, azithromycin inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit of the bacterial 70S ribosome. Binding inhibits peptidyl transferase activity and interferes with amino acid translocation during the process of translation. Its effects may be bacteriostatic or bactericidal depending of the organism and the drug concentration. Its long half life, which enables once daily dosing and shorter administration durations, is a property distinct from other macrolides.

Category: Anti-Bacterial Agents, Anti-Infective Agents.

Mechanism of action: Azithromycin binds to the 50S subunit of the 70S bacterial ribosomes, and therefore inhibits RNA-dependent protein synthesis in bacterial cells.

Pharmacodynamics : Azithromycin, a semisynthetic antibiotic belonging to the macrolide subgroup of azalides, is used to treat STDs due to chlamydia and gonorrhea, community-acquired pneumonia, pelvic inflammatory disease, pediatric otitis media and pharyngitis, and Mycobacterium avium complex (MAC) in patients with advanced HIV disease. Similar in structure to erythromycin. azithromycin reaches higher intracellular concentrations than erythromycin, increasing its efficacy and duration of action.

Absorption: Bioavailability is 37% following oral administration. Absorption is not affected by food. Azithromycin is extensively distributed in tissues with tissue concentrations reaching up to 50 times greater than plasma concentrations. Drug becomes concentrated within macrophages and polymorphonucleocytes giving it good activity against Chlamydia trachomatis[4-8].

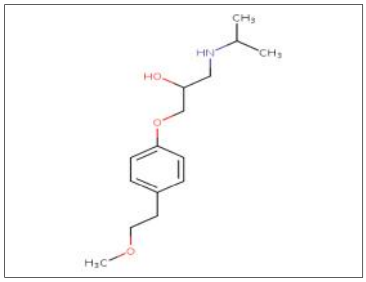
Route of elimination: Biliary excretion of azithromycin, predominantly as unchanged drug, is a major route of elimination.

Half life: 68 hours

BRAND NAME : Actimycin

Metoprolol

Structure :



IUPAC name: 1-[4-(2-methoxyethyl)phenoxy]-3-[(propan-2-yl)amino]propan-2-ol

Cas No: 37350-58-6

Molecular formulae : $C_{15}H_{25}NO_3$

Molecular Weight: 267.3639 g/mol

Category: Antihypertensive Agents

Description: This compound belongs to the class of organic compounds known as tyrosols and derivatives. These are compounds containing a hydroxyethyl group attached to the C4 carbon of a phenol group.

Solubility : soluble in water.

Melting point: 120^0c

Mechanism of action: Metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at beta(1)-adrenergic receptors in the heart. Beta(1)-receptor blockade results in a decrease in heart rate, cardiac output, and blood pressure[9].

2. Methodology

Selection of mobile phase

- pH 3 phosphate buffer : Methanol (55 : 45% v/v)
- Buffer pH should be between 2 to 8.
- Below 2: siloxane linkages are cleaved.
  Above 8: dissolution of silica.
- pH selected: 3 ±0.05
- pH controls the elution properties by controlling the ionization characteristics.
- Reasons: To decrease the retention and improve separation. Good Response, Area, Tailing factor, Resolution.

Selection of wavelength

10 mg of Voriconazole, Azithromycin and Metoprolol Tartrate was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Voriconazole, Azithromycin and Metoprolol Tartrate. The isobestic point was taken as detection wavelength.

Selection of column

Heart of HPLC made of 316 grade stainless steel packed with stationary phase.

Silica based columns with different cross linking's in the increasing order of polarity are as follows:

Non-polar----------moderately polar--------Polar

C_{18}< C_8< C_6< Phenyl < Amino <Cyano< Silica

- In reverse phase chromatography, hydrophobic interaction between drug molecule and the alkyl chains on the column packing material.
- Column is selected based on solubility, polarity and chemical differences among analysts and Column selected: i.e Agilent (4.6×150mm)5µ.
- Reasons : Better separation,
  Good tailing factor.

4. Selection of solvent delivery system

- Always preferable solvent delivery system.
- More chance of getting reproducible result on retention time of analytes.

- More economic than gradient technique.

5. Selection of flow rate

Acceptable limit: - Not more than 2.5 ml/min

- Flow rate selected was 1ml/min
- Flow rate is selected based on
  1. Retention time
  2. Column back pressure
  3. Peak symmetry.
  4. Separation of impurities.

Reasons:

- For earlier elution of analyte and elution of all impurities within 6.0 min.
- Information from the reference method in literature.

6. Selection of diluent

- Selection of diluent is based on the solubility of the analyte
- Diluent selected: Methanol : phosphate buffer pH 3 (55 : 45v/v)

Reason: good peak area, retention time, peak symmetry

7 Selection of column temperature:

- Preferable temperature is ambient or room temperature.

Reasons:

- To elute all impurities along with analyte with in 10.0 min of run time.
- Less retention time
- Good peak shape
- Higher theoretical plates.
- Good resolution.

8. Selection of test concentration and injection volume

Test concentration is finalized after it is proved that API is completely extractable at the selected test concentration[10]

Test concentration is fixed based upon the response of API peak at selected detector wavelength.

- And the test concentration selected is 10 ppm.
- Injection volume selected was 10µL.

3. Results and Discussion

Table 1: Chromatogram values for System suitability a) Voriconazole

Injection	R_t	Peak Area	USP Plate count	USP Tailing
1	2.402	1250765	2487	1.63
2	2.403	1248867	2489	1.56
3	2.404	1256849	2485	1.63
Mean		1251460		
SD		3860.678		
% RSD		0.30823		

Acceptance Criteria:

1). Tailing factor Obtained from the standard injection is 1.7

2). Theoretical Plates Obtained from the standard injection is 2496

Table no 2: Chromatogram values for System b) Azithromycin

Injection	R_t	Peak Area	USP Plate count	USP Tailing	USP Resolution
1	2.421	940528	2273	1.54	3.03
2	2.422	931462	2245	1.43	3.02
3	2.423	940305	2367	1.42	3.05
Mean		937464.6			
17SD		5384.94			
% RSD		0.475408			

Acceptance Criteria:

1) Tailing factor Obtained from the standard injection is 1.51

2) Theoretical Plates Obtained from the standard injection is 2281

Table 3: Linearity results for Voriconazole

S.No	Linearity Level	Concentration(µg/ml)	Area
1	I	10 ppm	339286
2	II	20 ppm	667774
3	III	30 ppm	986474
4	IV	40 ppm	1339994
5	V	50 ppm	1639065
Correlation Coefficient			0.999

Table 4: Linearity results for Metoprolol Tartrate

S.No	Linearity Level	Concentration (µg/ml)	Area
1	I	12.5	231737
2	II	25	453615
3	III	37.5	659796
4	IV	50	895191
5	V	62.5	1094356
Correlation Coefficient			0.999

Table no 5: Chromatogram values for intermediate Precision: Voriconazole

Injection No	Peak Area	R_t
1	912412	4.412
2	913062	4.413
3	909642	4.414
4	916881	4.416
5	914005	4.415
Mean	913200.4	
SD	2621.886	
% RSD	0.388	

Table no- 6: Chromatogram values for intermediate Precision: Azithromycin

Injection No	Peak Area	R_t
1	914922	7.084
2	909335	7.085
3	913266	7.086
4	909418	7.087
5	911496	7.088
Mean	911687.4	
SD	2432.859	
% RSD	0.4657	

Table 7: Chromatogram Values for Accuracy of Voriconazole

Sample.No.	Accuracy	Amount added(mg)	Amount found(mg)	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.13%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.69%

Table 8: Robustness results for Voriconazole (flow rate)

S.No	Drug	Flow Rate ml/min		
		0.8ml/min R_t	1ml/min R_t	.1.2m l/min R_t
1	**/Azithromycin Robustness Results**	2.475	2.482	2.488
USP Plate count		2279	2232	2185
USP Tailing		1.47	1.49	1.51

Table 9: Robustness results for Azithromycin (flow rate)

S.No	Drug	Flow Rate ml/min		
		0.8ml/min R_t	1ml/min R_t	1.2 m l/min R_t

1	**Metoprolol Tartrate Robustness Results**	3.488	3.190	2.877
USP Plate count		2346	2556	2096
USP Tailing		1.28	1.24	1.27

Table 10: Limit of detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Voriconazole	371877.10	563365963	2.03
Azithromycin	431401.80	476884400	0.0365
Metoprolol Tartrate	287058.10	376884400	1.84

Table no 11: Results of LOQ

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Voriconazole	371877.10	563365963	4.53
Azithromycin	431401.80	476884400	5.75
Metoprolol Tartrate	287058.10	376884400	3.84

4. Conclusion

For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation step. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool[10]. Voriconazole & Azithromycin was freely soluble in water and alcohol. Metoprolol Tartrate was freely soluble in alcohol and sparingly soluble in water. Methanol and potassium dihydrogen ortho phosphate (pH 3) was chosen as the mobile phase. The run time of the HPLC procedure was 10 minutes. The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay[11-15]. The method shows linearity between the concentration range of 10-100 µg / ml. The % recovery of Voriconazole, Azithromycin and Metoprolol Tartrate were found to be in the range of 99.22 % - 100.11 %. As there was no interference due to excipients and mobile phase, the method was found to be specific. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate and Mobile phase composition separately and analysis being performed by different analysts. Good agreement was seen in the assay results of Pharmaceutical formulation by developed method. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Voriconazole, Azithromycin and Metoprolol Tartrate in Bulk drug and Pharmaceutical formulation.

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