ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

ANALYTICAL CHEMISTRY

PROCESS VALIDATION OF FLUCONAZOLE

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ABSTRACT

Validation is the act of demonstrating and documenting that a procedure operates effectively. The U.S Food and Drug Administration (FDA) guidelines state that the process validation is the established documented evidence which provides a high degree of assurance that specific process.Fluconazole is a triazole antifungal drug used in the treatment and prevention of superficial and systemic fungal infections. In a bulk Powder form, it appears as a white crystalline powder, and it is very slightly soluble in water and soluble in alcohol. It is designated as 2,4-difluoro-bis(1H-1,2,4-triazol-1-ylmethyl) benzyl alcohol with an empirical formula of $C_{13}H_{12}F_2N_6O$ and molecular weight 306.3.

ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

KEYWORDS

Onychomycosis, Candida, Tinea corporis, Antifungal.

INTRODUCTION

CHEMICAL DATA :
Formula : $C_{13}H_{12}F_2N_6O$
Molecular Mass : 306.27 g/mol
PHARMACOKINETIC DATA:
Bioavailability : >90%
Protein binding : 11 to 12%

Assurance of product quality is derived from careful and systemic attention to a number of important factors like selection of quality parts and materials, adequate product and process design, process control and final product testing. Current Good Manufacturing Practices (CGMP) regulations demands written procedures for production and process controls to assure that the drag (Please check spelling is it 'drug'?) products have the identity, strength, quality, and purity.

U.S. FDA's CGMP guidelines state that control procedures should be established to monitor the product and to validate the performance of the manufacturing processes. Control procedures like weight variation, disintegration time, mixing time to assure uniformity and homogeneity, dissolution time and dissolution rate are related to the manufacturing and validation of solid dosage forms. Clarity, completeness and pH tests are related to the manufacturing and validation of liquid dosage forms [2].

Process Validation is to be done for the critical manufacturing steps laid down in the approved Master Formula as bulk stage and finished product stage.In the manufacturing processes, a minimum of three production runs or three repetitive steps to be selected

Like other imidazole- and triazole-class antifungal, fluconazole inhibits the fungal cytochrome P450 enzyme 14α-demethylase. Mammalian demethylase activity is much less sensitive to fluconazole than fungal demethylase. This inhibition prevents the conversion of lanosterol to ergo sterol, an essential component of the fungal cytoplasm membrane, and subsequent accumulation of 14α-methyl sterols. Fluconazole is primarily fungi static, however may be fungicidal against certain organisms in a dose-dependent manner. Interestingly, when fluconazole was in development at Pfizer, it was decided early in the process to avoid producing any chiral centers in the drug so that subsequent synthesis and purification did not encounter difficulties with enantiomer separation and associated variations in biological effect. A number of related compounds where found to be extremely potent teratogens and subsequently discarded.

The pharmacokinetic properties of fluconazole are similar following administration by the intravenous or oral routes. In normal volunteers, the bioavailability of orally administered fluconazole is over 90% compared with intravenous administration. Bioequivalence was established between the 100 mg tablet and both suspension strengths when administered as a single 200 mg dose.

Peak plasma concentrations (Cmax) in fasted normal volunteers occur between 1 and 2 hours with a terminal plasma elimination half-life of approximately 30 hours (range: 20-50 hours) after oral administration.

In fasted normal volunteers, administration of a single oral 400 mg dose of DIFLUCAN (fluconazole) leads to a mean Cmax

of 6.72 µg/mL (range: 4.12 to 8.08 µg/mL) and after single oral doses of 50-400 mg.

MATERIALS AND METHODS

FOR FLUCONAZOLE-III

STAGE	INGREDIENTS	STD. QTY
FI - III	FL –II	210KG
	Caustic potash flakes (CP Flakes)	150kg.
	Tri methyl sulfoxonium iodide (TMSI)	215kg
	1-H-1,2,4 Triazole	105kgs
	Citric Acid	5 Kg.
	Ethyl Acetate	60L
	Acetone	40L
	Toluene	750L
	Methanol	5 L
	Hydrochloric acid (HCL)	95-100kg
	D.M. Water	2200L

FLUCONAZOLE-FINAL

STAGE	INGREDIENTS	STD. QTY
FI – IIIH	FL –III	210KG
	Citric Acid	13Kg.
	Ethyl Acetate	100L
	Acetone	200L
	Toluene	10L
	Hydrocholirc Acid	150kg
	Methyl dichloride	400L
	Activated Carbon	8kg
	Hyflowe	3kg
	EDTA	4kg
	Ammonia Solution	120kg
	D.M. Water	1325L

FLUCONAZOLE-I

		STD Qty	Actual Qty
1,3-Difulora Benzene (1,3-DFB)	-	125 kgs	125 kgs
Aluminum Chloride	-	160 kgs	160 kgs
4-Amino, 1,2,4-Triazole	-	140 kgs	145 kgs
HCL	-	60 kgs	60 kgs
Acetonitrile	-	1100 lts	100+800 lts
M.D.C	-	1000 lts	100+800 lts
D.M Water	-	1000 lts	100+950 lts
ICE blocks	-	12 no's	11 no's

METHODOLOGY

- Charge 100Lts of MDC in GLR -206.Cool to 5-7^{0}C
- Charge 125kgs of 1,3 – Diflugenzene in GLR -206 at 5-7^{0}C
- Charge 160kgs of Aluminum Chloride in GLR-206 at 5-7^{0}C
- Add slowly 140kgs of CAC over a period of 4-5 hrs at 5-45^{0}C
- Rise the Mass temperature to 20-25^{0}C
- Maintain the R.M. for 4hrs at 20-25^{0}C
- Rise the R.M. temperature to 30-35^{0}C by hot water circulation.
- Maintain the RM for 7 hrs at 30-35^{0}C
- Send a sample of R.M. to Qc for G.C analysis.
- (Limit : -1,3- Difluro benzene NMTO. 5%)
- If reaction is not complete , Maintain for 60 min max and recheck the
- 1,3 – Difulro benzene content by G.C
- If Reaction is complete , cool the RM to 10-5^{0}C
- Charge 12No's of Ice blocks & 60kgs of Hcl In PPFRP reactor (PPR-212).
- Transfer the RM slowly over period of 60-90min below 25^{0}C from GLR-206 to PPR -212
- Rise (GLR – 206) with 100 lts of MDC and transfer the wasting
- Stir for 30min and settle for 30min
- Separate the organic layer in the PPRC-212 A.
- Charge 200lts of MDC to aqueous layer in PPR-212A.
  Stir for 30min and stir for 30min
- Separate the organic layer in to PPRc -212A.
- Charge 200 lts of MDC to aqueous layer in PPRC -212A.
- Stir for 30min and settle for 30min
- Separate the organic layer in to PPRC-212A.
- Charge 200lts of MDC to aq layer in PPR-212.
- Stir for 30 min and settle for 30min
- Separate the organic layer in to PPRC-212 a and drain the aqueous layer in ETP
- Charge the total organic Layer in PPR-212.
- Charge 300ltr D.M. water.
- Stir for 30min and settle for 30 min
- Separate the organic layer in SR -207/SSR-205.
- Charge 200lts MDC to aqueous layer in PPR – 212
- Stir for 30min and settle for 30min
- Separate the organic layer into SSR – 207/SSR-205.
- Distilled out MDC at 50-55^{0}C (Use hot water circulation)
- Remove the MDC traces at 50-55^{0}C under vacuum over a period of 2 hrs
- Cool R.M. temperature to 25-30^{0}C

- Charge 1000lts of 4-Amino 1,2,4-Trizole at 258-30^{0}C in SSR-207/SSR-205
- Charge 1000 lts of Acetourtirle in to SSR -207/SSR-205
- Maintain the R.M. for 2 hrs at 25-30^{0}C
- Heat the RM temperature to 76-80^{0}C (Reflux)
- Maintain the RM under reflux at 76-80^{0}C for 26 hours
- Send a sample RM to QC for TLC Cheek.
- (Limit =- Acetyl Compound NMT 0.5%)
- If reaction is not complete, Maintain the RM under Reflux at 76-80^{0}C for 2
- hers more and recheck TLC.
- if reaction is complete, cool the RM to 5-10^{0}C
- Maintain the RM for 30 min at 5-10^{0}C

Centrifugation :

- Centrifuge the material in centrifuge CF -202.
- Wash the material with 100lts of chilled acetorutile.
- Spin dry the wet cake for 1 hour
- Stop the centrifuge and Unload the wet cake in double poly bag need
- fiber drums
- Label the container with detailed of product Batch no. etc., Wet weight = 341 kgs.

Drying :

- Load the wet material into TD_101.
- Dry the wet material at 85-90^{0}C
- Record the temperature for every 60 min
- Send a sample to QC for determination of LOD initially after 4 hours .
- Stop the dryer and unload the material in to clean double poly bag lined contains
- Load the wet material into TD_101.
- Dry the wet material at 85-90^{0}C
- Record the temperature for every 60 min
- Send a sample to QC for determination of LOD initially after 4 hours.
- Stop the dryer and unload the material in to clean double polybag containers.

METHODOLOGY FOR FLUCONAZOLE –II

		STD.QTY	ACTUAL.QTY
Sodium nitrite	-	280 kgs	327 kgs wet
Ammonia Solution	-	75 kgs	71.5 kgs
Hcl	-	160 kgs	140 kgs
D.M water	-	1110 lts	970+140 lts

- Charge 200kgs of Hcl at 25-30^{0}C in GLR -208
- Stir for dissolution (Solution should be clear)
- Cool the solution to 10-15^{0}C
- Simultaneously Prepare a solution of sodium nitrate by dissolving 75 kg
- of Sodium nitrite in 230 ltrs of DM water in two HDPE drums.
- Add slowly the sodium nitrite solution over a period of 4-5 hrs at 10-15^{0}C
- Maintain the RM for 30min at 10-15^{0}C
- Send a sample to RM to Qc for TLL Check (Limit : FL -1 NMT =0.5%)
- If Reaction is not complete, Maintain the RM for 30min more at 10-15^{0}C
- Send a sample of RM to Qc for TLC Restrict (Limit FL-1NMT 0.5%)
- If TLC Complies , Rise the RM Temp to 20-25^{0}C.
- Maintain the RM for 4 hrs at 20-25^{0}C

- Adjust the P^H of RM to 8.0 – 8.5 with Ammonia solution at 20-25^{0}C
- Maintain the RM for 30 minutes at 20-25^{0}C
- Cool the RM To 10-15^{0}C
- Maintain the RM to 10-15^{0}C

Centrifugation :

1. Centrifugation the material in centrifuge ef – 203.
2. Wash the material with 100the of DM Water
3. Spin dry the wet cake 1 hour
4. Stop the centrifuge and unload there wet cake in double poly bag lived fiber drums
5. Label the contain with details of product Batch No.
6. Record the wet weight of the FL-II.

Wet Weight = 253 kgs.

Drying

1. Load the Wet material in to TD -101
2. Dry the wet material 55-60^{0}C
3. Record the temperature every 60minutes.
4. Send a sample to QC for determination of LOD initially, after 12 hours and then after every 2 hours till % of LOD comes below the limit (NMT 0.5%, W/W).
5. Stop the dryer and unload the material in the clean double poly bag lined contained. Dry weight = 216 kgs.

METHODOLOGY FOR FLUCONAZOLE – III

		Std.Qty	Actual.Qty
Caustic Potash Flakes	-	150 kgs	160 kgs
Atric acid	-	5 kgs	7 kgs
Ethyl acetate	-	60 lts	70 lts
Acetone	-	40 lts	45 lts
Toluene	-	750 lts	1000 lts
Methanol	-	750 lts	1000 lts
Hcl -		5 lts	5 lts
DM water	-	2200 lts	1825 lts

Processing :

1. Charge 1260 lts of DM Water and 150kg of C.P. Flakes at 25-30^{0}C in SSR . Stir for dissolution Charge 215 kgs of Trimethyl sulfoxominum Iodide at at 25-30^{0}C in SSR – 210.
2. Charge 105kgs of 1-H, 1, 2,4 trizole at 25-30^{0}C in SSR – 209/SSR-210.
3. Charge 210kgs of Fl-II at 25-30^{0}C in SSR -209/SSR-210.
4. Rise the RM Temperature to 60^0 – 65^{0}C
5. Maintain the RM for 24hrs at 60-65^{0}C
6. Send a sample of RM to QC for TLC Check. (Limit : Epoxy compound NMT0.5%) If Reaction is not complete, Maintain for 2hrs more and recheck TLC.
7. If Reaction is complete cool the RM to 25-30^{0}C
8. Charge 750 lts of toluene into the RM in SSR-209/SSR-210.
9. Adjust the P^H of RM to 10.0 – 10.5 with Dil Hcl (1:1) at 25-30^{0}C
10. Stir for 30min at 25-30^{0}C
11. Cool RM to 0-5^{0}C
12. Maintain the RM for 4hrs at 0-5^{0}C

Centrifugation :

1. Centrifuge the material in centrifuge CF-203.
2. Wash the material with 200lts of DM Water
3. Spin Dry the wet cake for 1 hour
4. Stop the centrifuge and unload the wet cake in double poly bag lined fiber drums.
5. Label the contain with details of product Batch No. etc.
6. Record the net weight of the material net weight = 256 kgs.

Processing

1. charge 400lts of DM water at 25-30c in SSR-211
2. Charge net material at 25-30^{0}c in SSR-211
3. Charge 60 lts of Ethyl acetate, 40 lts of Acetone, 5 lts of methanol not at 25-30^{0}c in SSR-211.
4. Charge 5kg of citric avid at 25-30^0 in SSR-211
5. Stir for dissolution
6. Heat the RM to 65-70^{0}c(Reflux)
7. Maintain the RM at 65-70^{0}c under reflux for 60 min
8. Cool the RM to 0-5^{0}c
9. Maintain the RM for 30 min at 0-5^{0}c

Drying:-

1. Load the wet material in to TD-202
2. Dry the wet material at 85-90^{0}c
3. Record the temperature for every 60 minutes
4. Send a sample to Qc for determination of LOD initially, after 8 hours and then after every 2 hrs fill % of LOD comes below the limit (NMT 0.5% w/w)
5. Stop the dryer and unload the material in to clean double polybag lined containers
   Net of dry material =166 kgs.
   Expected yield = 160-170 kgs.

Centrifugation:-

1. Centrifuge the material in centrifuge eF-203
2. Wash the material with 100 lts of DM water
3. Spin dry the cake for 1 hours
4. Stop the centrifuge and unload the net cake in double poly bag lined fiber
   Label the container with details of product Batch No. etc
5. Record the net weight of the material FL-III
   Net weight = 208 kgs.

METHODOLOGY FOR FLUCONZOL FINAL

		Std.Qty	Actual.Qty
Citric acid	-	180kg	176 kg
Ethyl acetate	-	8 kg	7 kg
Acetone	-	60 lts	70 lts
Toluene	-	200 lts	300 lts
Methanol	-	5 lts	5 lts
Hcl -		400 lts	300 lts
EDTA -		3 kg	3 kg
Ammonia solution	-	120 kgs	4 kgs
D.M water	-	800 lts	350lts +

Processing :

1. Charge 425 lts of DM water and wet material at 25-30^{0}C in GLR -202
2. Charge 150 kg of HCL at 25-30^{0}C in GLR -202
3. Stir for 15mm at 25-30^{0}C
4. Charge 100lts of Toluene at 25-30^{0}C in GLR -202.
5. Stirr for 30minutes and settle for 30 min.
6. Separate the aqueous layer in GLR
7. Charge 100lts of toluene at 25-30^{0}C in GLR -208
8. Still for 30minutes and settle for 30minutes
9. Separate the aqueous layer in GLR-2002, Unload the Organic layer into drums
10. Charging 100lts of MDC in GLR -202
11. Stir for the 30minutes and settle for 30minutes
12. Separate the Organic layer and unloading into drums
13. Charge of 100lts of MDC in GLR-202
14. Stir for 30minutes separate the Organic layer
15. Charge 100 lts of MDC in GLR-202
16. Charge 100lts of MDC in GLR -202
17. Stir for 30 minutes and settle for 30 minutes
18. Separate the Organic layer and unloading into drums
19. Heat the agues layer to 45-50^{0}C In GLR -202
20. Maintain the RM for 15 minutes at 45 – 50^{0}C
21. Transfer the RM into SSR-103
22. Charge the 8kg of activated Carbon into RM at 45^{0}C
23. Stir the RM for 30minutes at 45^{0}C
24. 50lts of DM water to Carbon bed in LF – 202 and transfer clear filtrate to the reactor GLR -301
25. Cool the aqueous layer to 25^{0}C
26. Charge 60lts of acetone into SSR -204
27. Charge 60lts of ethyl estate into SSR – 204
28. Charge the 5 lts for methanol into SSR -204
29. Charges Citric acid + EDTA Solution into SSR -202
30. Charge the above solution in GLR -301 through MF -201 at 25^{0}C
31. Stir the RM for 50 minutes at 25^{0}C
32. Adjust the P^H is 7.02,7.5 with 20kg of Ammonia Solution at 25-35^{0}C
33. Send the Sample to QC

Centrifugation :

1. Centrifuge the material in centrifuge CF – 301
2. Wash the material with 60lts of DM water
3. Slurry the wet cake with 20lts of DM Water
4. Wash wet Cake with 60lts of DM water
5. Send the sample to QC for solubility of Cake in chloroform.
6. If complies spin dry the wet cake for 60minutes
7. Stop the centrifuge and unload the wet cake in double poly bag lined fiber drums and then record the weight of fluconazole

Drying :

1. Load the wet material into V.T.D -301
2. Dry the wet material at 85 to 90^{0}C
3. Record the Temperature at 60^{0}C
4. Sand the Sample to QC for the determination of LOD (NMT-0.5% W/W)
5. Stop the dryer unload the material into clean double ploy bag lined containers

RESULTS AND DISCUSSION

S.No.	Tests	Specifications	Results of Analysis		
			FL –III04001	**FL –III04002**	**FL –III04003**
1.	**Description**	**A pale of yellow to off white crystalline powder**	**Almost white Crystalline powder**	**Almost white Crystalline powder**	**Almost white Crystalline powder**
2.	Solubility	Freely Soluble in Methanol	Complies	Complies	Complies
3.	Loss on Drying	NMT : 0.50% w/w	0.30% w/w	0.23% w/w	0.25% w/w
4.	Melting Range	130^0C to 136^0C	132.3^0C – 134^0C	131.8^0C – 133.7^0C	133.1^0C - 133.2^0C

S. No	Tests	Specification	Results of Analysis		
			FL04001	**FL04002**	**FL04003**
1.	Description	White crystalline powder	White fine crystalline powder	White fine crystalline powder	White fine crystalline powder
2.	Solubility	Slightly soluble in water, freely soluble in Methanol	Complies	Complies	Complies
3.	Identification				
	a. Test I(By IR)	The IR spectrum should be Concordant with reference standard Sample	Complies	Complies	Complies

	b. Test II (Melting range)	136^0C to 140^0C	137.2^0C 139.3^0C	137.5^0C 139.4^0C	137.6^0C 139.5^0C
	c. Test III (TLC)	The principle spot in the chromatogram obtained with the test solution is similar in position and size to the principle spot in the chromatogram obtained with reference solution A.	Complies	Complies	Complies
4.	Appearance of the solution	Clear and colourless	Complies	Complies	Complies
		Imp B (in house) : NMT0.20%	---	---	---
		Any other IMP : NMT 0.10%	0.04%	0.04%	0.08%
		Total Impurity : NMT 0.50%	0.20%	0.28%	0.26%
		NMT 0.50%	0.20%	0.28%	0.26%
5.	Sluphated Ash	NMT 0.10%	0.05%	0.04%	0.05%
6	Assay by chemical (On dryings)	99.00% to 101.00%	99.49%	99.45%	99.51%
7	Residual Solvents				
	Methanyl	NMT 200 ppm	BDL	BDL	BDL
	Aceton	NMT 200ppm	BDL	BDL	BDL
	Methyl dichloride	NMT 600 ppm	BDL	BDL	BDL
	Ethyl Acetone	NMT 500 ppm	100 ppm	90 ppm	80 ppm
	Tolune	NMT 500 ppm	200 ppm	158 ppm	169 ppm

CONCLUSION

Three batches of Fluconazole Crude are FI-II04002 and FL-11104003 and Fluconazole Final are FI04001, FI04002 and FL 04003 are taken for performing prospective validation of the process.

All the Critical process Parameters and In-process results were well within the approved standard parameters and limits.

All the Quality Attributes of Intermediate and Finished API are very well within the already established approved specified limit.

Manufacturing facility (Critical Instruments, utilities) and other support system were checked for their functionality and found ok.

Fluconazole, when manufactured by using the approved Master production and control records, Produces a consistent quality of the product . All the three batches were found within the specifications by following predetermined Critical Process Parameters, Inprocess Tests and by Raw materials and Intermediates of Specified quantity.

Therefore, the process for manufacturing Fluconazole considered to be validated prospectively.

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