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DEVELOPMENT OF VALIDATED NEW ANALYTICAL RP-HPLCMETHOD FOR THE ESTIMATION OF FLUCONAZOLE IN BULK AND PHARMACEUTICAL DOSAGEFORMS

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Research Article

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Abstract: various methods have developed and validated regarding to the fluconazole using different spectrophotometric and chromatographic techniques using different organic solvents. My aim is to develop a cost effective method for analyzing the fluconazole in the bulk and pharmaceutical dosage forms by using solvents of low cost and easily available in the market. The objective of the proposed method is to develop a simple and accurate method for the Estimation of the Fluconazole in Bulk and pharmaceutical dosage forms by using liquid chromatographic techniques like High performance liquid chromatography (HPLC) for the estimation of fluconazole in bulk and pharmaceutical dosage forms the above proposed method is precise and accurate and the method is validated with suitable validation parameters like linearity, accuracy, precision, LOD, LOQ, and the results obtained are within the limits as per ICH guidelines . A simple, sensitive, rapid and accurate chromatographic method was developed for the estimation of the Fluconazole in bulk and pharmaceutical formulation. The analysis was carried out on a C18 (Octadecyl Saline) (4.6mm x 250mm) reversed-phase column, with a uv – visible detector using a mixture of Acetonitrile: Water (90:10 %v/v) as the mobile phase using an isocratic mode with flow rate at 2ml/min. The injection volume was 20µl.The retention time of the drug was 2min for Fluconazole. The method produced linear responses in the concentration range of 50 to 300µg/ml for Fluconazole correlation coefficient was found to be 0.9998 which is within the limits and . The Tailing factors of Fluconazole were found to be 1 - 1.3 respectively. The method was found to be applicable for determination of the drug in bulk and pharmaceutical dosage forms the accuracy of the method was found to be98.0%. The precision of this method reflected by relative standard deviation of replicates was found to be 1.382 with limit of detection of 0.013 µg/mL and limit of quantification of 0.039 µg/mL

Keywords: fluconazole, High performance liquid chromatography, Retention time,

Introduction:

Fluconazole[1] is Triazole antifungal agent that is used to treat oropharyngeal candidiasis and cryptococcal meningitis in AIDS. It appears as a white crystalline powder, and it is very slightly soluble in water and soluble in alcohol .It[1] interacts with 14- alpha demethyllase, a cytochrome P-450 enzyme necessary to convert lanosterol to ergosterol. It inhibits endogenous respiration, interact with membrane phospholipids, and inhibits the transformation of yeasts to mycelial forms. The objective of the proposed method is to develop simple and accurate method for the Estimation of Fluconazole in Bulk and pharmaceutical dosage forms by using liquid chromatographic techniques like High performance liquid chromatography (HPLC)

A few methods are reported in literature survey by spectrophotometry[3-9] for determination of fluconazole in bulk and pharmaceutical dosage forms. A few methods are reported in literature survey by RP-HPLC[5-6] technique for determination of fluconazole in bulk and pharmaceutical dosage forms. In the present study a rapid, sensitive, accurate and precise HPLC method for the estimation of fluconazole in bulk and pharmaceutical dosage form samples form is proposed.

MATERIALS AND METHODS:

Chromatographic conditions:

The analysis of the drug was carried out on a Shimadzu SPD 20 A HPLC system equipped with a reverse phase Octadecyl Silane C18 column (250x4.6 mm. 5 µm), a 20 µl injection loop, PDA detector 2996 and running on LC solutions software.

Mobile phase preparation:

Prepare the mobile phase by weighing 90ml of acetonitrile and 10ml of water (HPLC grade) with a measuring cylinder and mix them in a 100ml beaker and sonicate for about 180 seconds

Sample preparation:

Standard stock solution preparation:

Weigh 10µgm of sample place it in a 10ml of volumetric flask and make it up to mark with by using 9ml of acetonitrile and 1ml of water

Procedure:

Take 10µgm/ml of standard solution in 10ml volumetric flask and sonicated before use. It was pumped through the column at a flow rate of 2.0 ml/min. The detection of the drug was monitored at 261.6 nm. The run time was set at 10 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 2.9 min. A typical chromatogram showing the separation of the drug is given in Fig 2.

Validation[10] of the proposed method:

1. Linearity:

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. Standard plots were constructed with six concentrations in the range of 50-300µg/ml to test linearity. The peak area of fluconazole was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis Results are furnished in Table.1

2. Precision:

The precision of the assay was studied with respect to both repeatability and intermediate precision

Repeatability:

It was calculated from five replicate injections of freshly prepared valsartan test solution in the same equipment at a same concentration. Peak area of valsartan was determined and precision was reported as % RSD and the results are furnished in Table-2

3. Accuracy:

The accuracy of the HPLC method was assessed by taking accurate sample stock solution at various concentrations ranging from 50% to 250%.sample solutions were placed in triplicate form for each concentration. The % RSD and the results are furnished in table-3

4. Limit of detection (LOD):

Limit detection of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value. Limit of detection corresponds to the concentration

LOD = 3.3σ /S

The **LOD** value was found to be **0.013µg/ml.**

5. Limit of quantification (LOQ):

Limit quantification of an individual analytical procedure is the lowest amount of analyte in a sample that can be determined quantitatively. Limit of quantification corresponds to the concentration

LOQ = 10 σ/S

The **LOQ** value was found to be **0.039µg/ml.**

RESULTS AND DISCUSSION:

A new isocratic reverse-phase high performance liquid chromatographic with UV-detection at 261.6 nm was developed for quantitative determination of fluconazole in pure form. The mobile phase used was of acetonitrile and water (HPLC grade) in the proportion of 90:10v/v. The chromatographic method was performed on C_{18} column at a flow rate of 2 ml/min.The validated parameters were Precision, Accuracy, linearity, LOD, LOQ. The resulting chromatograms exhibited retention time at 2.600

The numbers of theoretical plates were more than 2000, for it was 2279 .The tailing factor was less than 2 that is 1.37 for fluconazole. Hence all the parameters were with the specified limits.

The linearity for fluconazole from concentration range of 0.5-3 µ g/ml was established by constructing the calibration curve with concentration on x-axis and peak area on y-axis with the correlation coefficient of 0.998.Accuracy of the method was 98-100%, The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were found to be 0.013 µg/ml and 0.039 µg/ml respectively.

System Precision was determined by preparing the standard solution at working concentration and analysis was carried for six replicate injections. The percentage relative standard deviation (% RSD) was calculated for the peak areas of fluconazole was found to be 1.382 respectively which was within the acceptance criteria of not more than 2.0%.

CONCLUSION:

An accurate, simple, fast and reproducible RP-HPLC method was developed and validated as per ICH guidelines for the estimation of Fluconazole. After development of the method it was validated for

accuracy, linearity, precision, LOD, LOQ and .The value of theoretical plates, tailing factor, retention time and peak area was found to be within limits.

Hence the developed chromatographic method for Fluconazole is said to be rapid, simple, precise and accurate. Therefore the proposed method can be effectively applied for routine analysis of Fluconazole in bulk and pharmaceutical dosage forms.

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Fig- 1: Structure of fluconazole

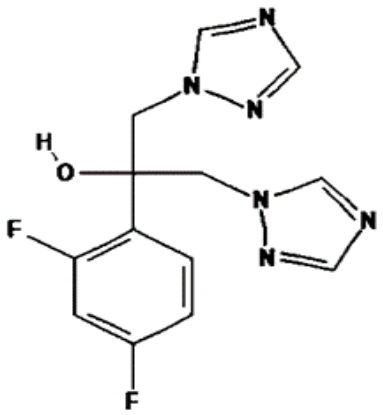

Fig 2: Chromatogram of Fluconazole

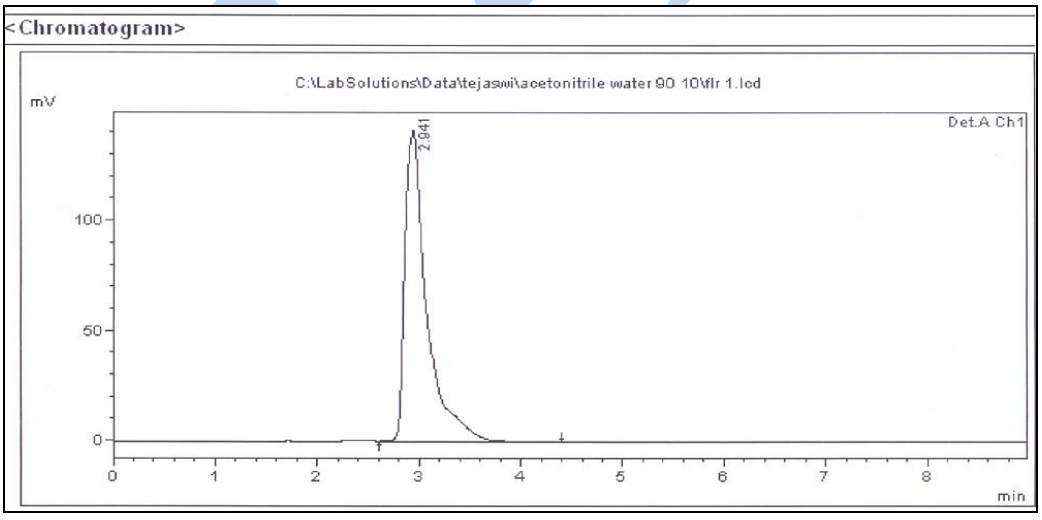

Fig 3: Calibration plot of Fluconazole

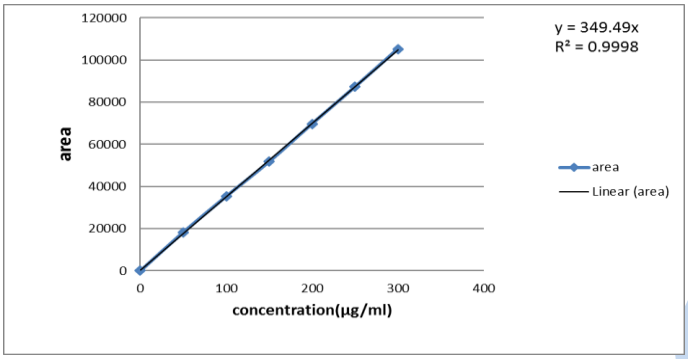

Table 1: Linearity data

S.no	Concentration (µg/mL)	Peak Area
1	50	18295
2	100	35295
3	150	51743
4	200	69712
5	250	87205
6	300	105201
Slope		349.49
R^2		0.9998

Table 3: Precision

Sample no	Concentration (µg/ml)	Area
1	2.5	840289
2	2.5	840674
3	2.5	857388
4	2.5	857399
5	2.5	866509
6	2.5	866510
Mean		5128769
SD		11813.46
%RSD		1.382

Table 3: Accuracy

S.N0	Concentration (%)	Amount added	Amount found	Percentage recovery	Mean recovery
1	50%	150	150	100	98.6
2	50%	150	147.7	98.4	
3	50%	150	146.2	97.4	
4	100%	200	193.1	96.5	98.3
5	100%	200	198.7	99.3	
6	100%	200	198.6	99.3	
7	150%	250	242.1	96.8	97.1
8	150%	250	240.8	96.3	
9	150%	250	245.7	98.2	98.0