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A New Analytical Method Development and Validation for the Determination of Fluconazole and Tinidazole in Pure Form and Marketed Pharmaceutical Dosage form by using RP HPLC

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ABSTRACT

A simple, specific, precise, and efficient method for the Simultaneous estimation of Fluconazole and Tinidazolein pure and pharmaceutical dosage forms by a Reverse Phase-High Performance Liquid Chromatography method is developed and validated. Selected mobile phase were in a combination of Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v). Optimized column is a Develosil C18 (4.6mm×250mm) 5µm particle size and at a flow rate of 1.0mL/min with detection wavelength at 238nm for Fluconazole and Tinidazole. In our study, the validation of analytical method for determination of Fluconazole and Tinidazolein pure and pharmaceutical dosage forms was performed in accordance the parameters including-system suitability, specificity, linearity of response, accuracy, precision (reproducibility & repeatability), robustness (change of wave length±2 nm). The method is validated according to ICH guidelines.In RP-HPLC method, the calibration graphs were linear in the concentration range of 10-30µg/ml for Fluconazole and 30-90µg/ml for Tinidazole with percentage recoveries are within the limits. The results obtained by RP-HPLC methods are rapid, accurate and precise. Therefore, proposed method can be used for routine analysis of Fluconazole and Tinidazolein the pure form as well as in combined pharmaceutical dosage form.

Keywords: Fluconazole and Tinidazole, RP-HPLC, Validation, ICH Guidelines

INTRODUCTION

Analytical chemistry[1] is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and the quantitative measurement of the substances present in bulk and pharmaceutical preparation.

Measurements of physical properties of analytes such as conductivity, electrode potential,light absorption or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of variety of inorganic and biochemical analytes. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior

to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as instrumental methods of analysis. Most of the instrumental methods fit into one of the three following categories viz spectroscopy, electrochemistry and chromatography

Introduction to hplc

HPLC[3] is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch. The rate of distribution of drugs between Stationary and mobile

phase is controlled by diffusion process. If diffusion is minimized faster and effective separation can be achieved .The techniques of high performance liquid chromatography are so called because of its improved performance when compared to classical column chromatography advances in column chromatography into high speed,efficient ,accurate and highly resolved method of separation. For the recent study Clonazepam and Propranolol was selected for estimation of amount of analyte present in formulation and bulk drug. The HPLC method is selected in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages
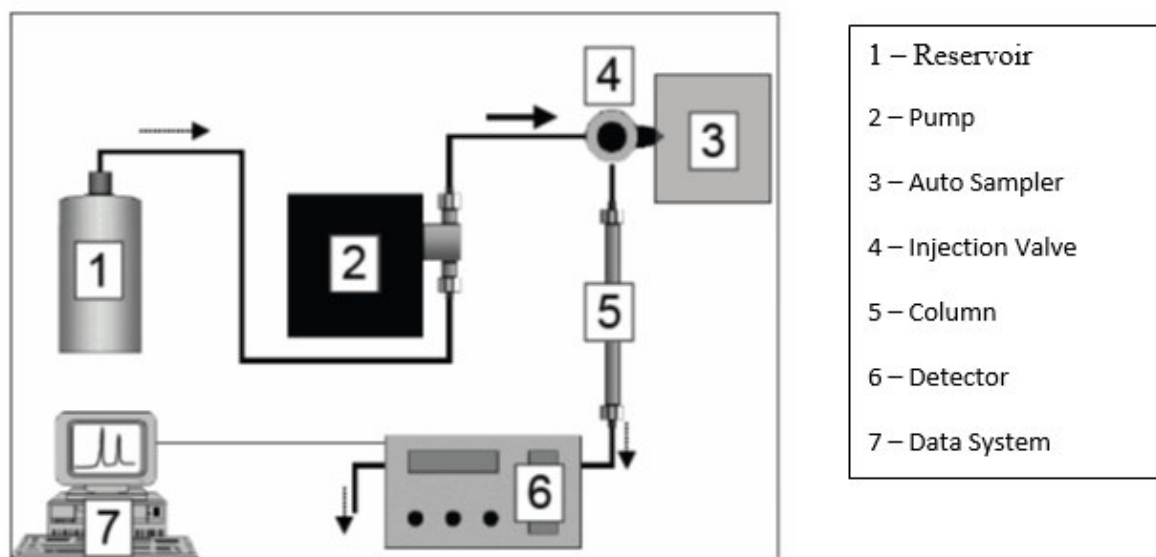
Hplc Basic Instrumentation

Fig1: Schematic diagram of a basic HPLC system.

Validation Parameters
1. Specificity
2. Precision
- System precision
- Method precision
3. Accuracy
4. Linearity
5. Robustness
- Flow rate variation
- Temperature variation
- Mobile phase variation
- pH variation
6. Ruggedness

- System to system variation
- Analyst to analyst variation
- Column to column variation
1. Filter validation

Aim and Objective

❖ Review of literature for Fluconazole and Tinidazole gave information regarding its physical and chemical properties, various analytical methods that were conducted alone and in combination with other Fluconazole and Tinidazole.

- ❖ Literature survey reveals that certain chromatographic methods were reported for simultaneous estimation of Fluconazole and Tinidazole and single method is available for such estimation by RP-HPLC.
- ❖ In view of the need for a suitable RP-HPLC method for routine analysis of Fluconazole and Tinidazole in formulations, attempts were made to develop simple, precise and accurate analytical method for simultaneous estimation of Fluconazole and Tinidazole and extend it for their determination in formulation.
- ❖ Validation is a necessary and important step in both framing and documenting the capabilities of the developed method.
- ❖ The utility of the developed method to determine the content of Fluconazole and Tinidazole in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient.

- ❖ The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness, limit of detection and limit of quantification. This method provides means to quantify the component. This proposed method was suitable for the analysis of Pharmaceutical dosage forms.

The primary objective of proposed work is

- To develop new simple, sensitive, accurate and economical analytical method for the simultaneous estimation of Fluconazole and Tinidazole in pure form and its pharmaceutical dosage form.
- To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Fluconazole and Tinidazole in dosage form.
- Experimental Methods

Instruments Used

Table1 : Instruments used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Chemicals Used

Table 2: Chemicals used

S.No.	Chemical	Brand names
1	Fluconazole (Pure)	Sura labs
2	Tinidazole (Pure)	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

Optimized Chromatographic Conditions

Instrument used : Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature : Ambient
Column : Develosil C18 (4.6mm×250mm) 5µm particle size Column

Mobile phase	:	Acetonitrile and Acetate buffer(pH-4.3) (35:65% v/v)
Flow rate	:	1ml/min
Wavelength	:	238nm
Injection volume	:	20μl
Run time	:	6minutes

RESULTS AND DISCUSSION

Trails

Table 3: Peak Results for trail 1

S.No	Peak Name	R_t	Area	Height	USP Tailing	USP Plate count
1	Fluconazole	2.610	1167502	10236	1.7	710
2	Tinidazole	3.248	35682	46055	2.6	415

Observation

In this trial it shows less plate count, improper separation of two peaks and shows improper baseline, resolution in the chromatogram. So it's required more trials to obtain good peaks.
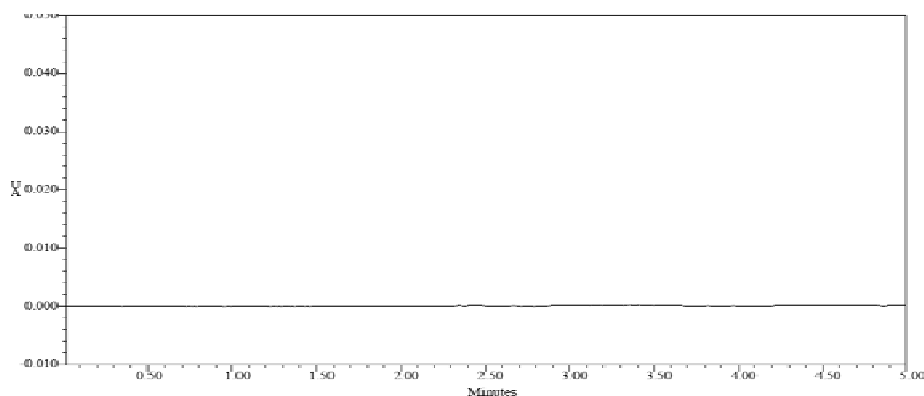
Method Validation

Blank

Fig 2: Chromatogram showing blank (mobile phase preparation)
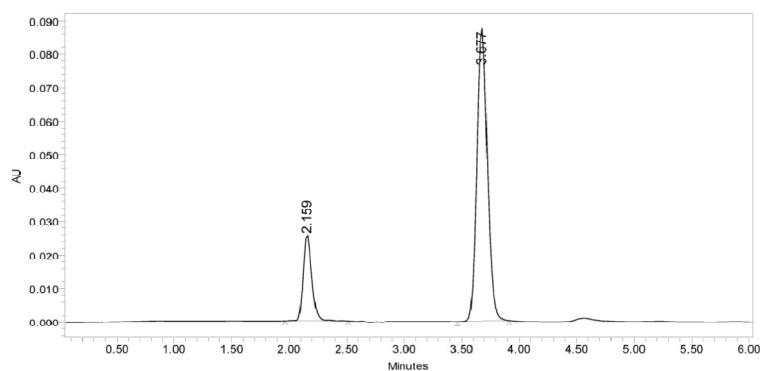
Linearity

Fig 3: Chromatogram showing linearity level-1

Chromatographic Data For Linearity Study Of Fluconazole

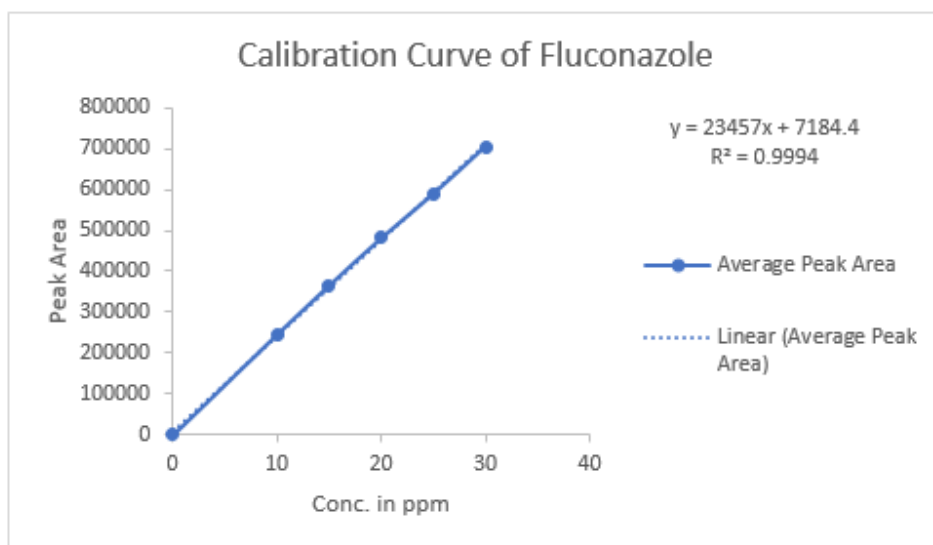| Concentration µg/ml	Average Peak Area
10	245899
15	365687
20	481526
25	589854
30	705882

Fig4: Calibration Graph of Fluconazole

Linearity Plot

The plot of Concentration (x) versus the Average Peak Area (y) data of Fluconazole is a straight line.
Y = mx + c
Slope (m) = 23457
Intercept (c) = 7184
Correlation Coefficient (r)   = 0.999

Validation Criteria

The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion

Correlation Coefficient (r) is 0.99, and the intercept is 7184. These   values meet the validation criteria.

Chromatographic Data For Linearity Study Of Tinidazole

Concentration µg/ml	Average Peak Area
30	863094
45	1249397

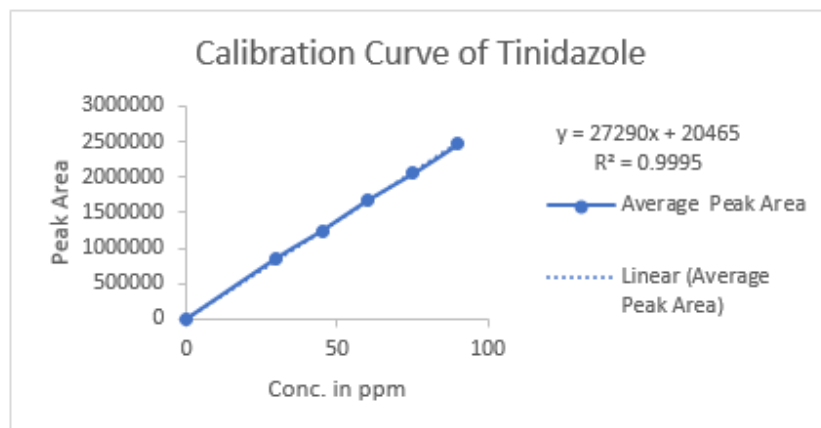|	
60	1678592
75	2050412
90	2468444

Fig 5: Calibration Curve of Tinidazole

Linearity Plot:

The plot of Concentration (x) versus the Average Peak Area (y) data of Tinidazole is a straight line.
Y = mx + c
Slope (m) = 27290
Intercept (c) = 20465
Correlation Coefficient (r)   =   0.99

Validation Criteria

The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion

Correlation Coefficient (r) is 0.99, and the intercept is 20465. These   values meet the validation criteria.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table 4: Results of repeatability for Fluconazole

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Fluconazole	2.157	513568	78546	1.2	4528
2	Fluconazole	2.159	513685	78541	1.2	4572
3	Fluconazole	2.186	513659	79852	1.2	4598
4	Fluconazole	2.160	513254	78498	1.3	4529
5	Fluconazole	2.170	513647	77898	1.2	4572
Mean			513562.6			
Std.dev			177.9475			
%RSD			0.03465			

Acceptance criteria

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 5: Results of repeatability for Tinidazole

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Tinidazole	3.603	1635625	265325	1.1	7985
2	Tinidazole	3.608	1658744	264588	1.1	7859
3	Tinidazole	3.600	1652985	265985	1.2	7845
4	Tinidazole	3.696	1645898	264898	1.1	7969
5	Tinidazole	3.629	1652364	268489	1.1	7846
Mean			1649123			
Std.dev			8811.631			
%RSD			0.534322			

Accuracy

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table 6: The accuracy results for Fluconazole

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	245954	10	10.179	101.79%	
100%	483747	20	20.316	101.58%	101.36%
150%	715961	30	30.	100.72%	

Acceptance Criteria

- The percentage recovery was found to be within the limit (98-102%).

Table 7: The accuracy results for Tinidazole

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	842287	30	30.114	100.38%	
100%	1659744	60	60.068	100.113%	100.26%
150%	2483885	90	90.268	100.297%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

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CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Fluconazole and Tinidazole in bulk drug and pharmaceutical dosage forms. Fluconazole was found to be soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide, Slightly soluble in water 1mg/mL. Soluble in ethanol (61mg/mL), ethyl acetate and methanol, soluble in alcohol and acetoneand very slightly soluble in toluene.Tinidazole was found to be soluble in organic solvents such as ethanol, DMSO, and

dimethyl formamide (DMF), practically insoluble in water, soluble in acetone and in methylene chloride, sparingly soluble in methanol.Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v)was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed inTablesfor RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Fluconazole and Tinidazole in bulk drug and in Pharmaceutical dosage forms.

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