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Research Article

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR FLUCONAZOLE AND IVERMECTIN IN TABLET FORMULATION BY USING RP-HPLC

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ABSTRACT

A simple, efficient and reproducible RP-HPLC method for the simultaneous determination of Fluconazole and Ivermectin in bulk and tablet formulation has been developed and validated. The separation was carried out using Insertil C_{18} column (250 mm x 4.6 mm, 5 µ) using Acetonitrile:Methanol:Water (75:15:10 v/v/v) as mobile phase. The flow rate was 1.5 ml/min and detection was carried out 254 nm. The retention time of Fluconazole and Ivermectin were 2.313 and 9.617 respectively. The linear was established in the range of 50-150 µg/ml and 52-156 µg/ml for Fluconazole and Ivermectin respectively. Percentage recoveries for Fluconazole and Ivermectin were found to be 99.830±1.079 and 100.814±1.99 respectively. All the analytical validation parameters were determined and found in the limit as per ICH Guidelines which indicate the validity of the method. The developed method is also found to be precise and robust for the simultaneous determination of Fluconazole and Ivermectin in tablet formulation.

KEYWORDS: Fluconazole, Ivermectin, RP-HPLC, Tablet Dosage Form, Analytical Method Development and Validation.

INTRODUCTION

Azole antifungal agents are the largest class of antimycotic available today with more than 20 drugs on the market. Some are primary used topically to treat superficial dermatophytic and yeast infection, whereas others are administered orally for the treatment of systematic fungal infection.[1] Fluconazole is chemically 2-(2,4-diflurophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)-2-propranolol, a synthetic triazole derivatives antifungal agent that has been shown to be effective against a wide range of systemic and superficial fungal infection.[2] It has desirable pharmacological properties including a relatively long half-life, the ability to be administered either orally or parenterally.[3] Like other imidazoles and triazoles-class antifungals, Fluconazole inhibits cytochrome P450 enzyme 14α-demethylase. Major advantage of Fluconazole over other antifungal agents is that it can cross the blood-brain barrier.[1]

Antiparasitic are a class of medications which are indicated for the treatment of parasitic diseases such as nematodes, cestodes, trematodes, infectious protozoa, and amoebas. Ivermectin is a broad-spectrum antiparasitic avermectin medicine. Chemically, Ivermectin is 22,23-dihydroavermectin B_{1b}. Avermectins are basically a series of 16-membered marcocyclic lactone derivatives with potent anthelmintic and insecticidal properties. Ivermectin is traditionally used against worm infections. It also finds it application in veterinary medicine.[4]

Literature survey reveals a few spectrophotometric and chromatographic methods for the estimation of both drugs as a single component and in combination with other drugs.[5-8] However no method has been reported for analysis of these drugs in combined dosage form. The objective of present communication is to develop simple, rapid and precise RP-HPLC method for the estimation of Fluconazole and Ivermectin in combined tablet formulation.

MATERIALS AND METHODS

Chemicals

Standard Fluconazole and Ivermectin were collected from Molecule Laboratory, Ahmedabad. Analytical grade solvents and reagents were purchased from SD Fine Chemicals Ltd. Mumbai, India. Nuforce – Plus, Mankind were purchased from local market; each tablet was labeled to contain 150 mg of FLUC and 6 mg of Ivermectin.

Instrumentation

The HPLC method was performed on a Shimadzu HPLC System equipped with LC-20 ATVP Solvent Delivery System pump, SPD M-10 AVP photodiode array detector and Rheodyne Injector system fitted with 20 µl loop.

Chromatographic Condition

The HPLC analysis was performed on reversed-phase high performance liquid chromatographic system with isocratic elution mode using a mobile phase Acetonitrile:Methanol:Water (75:15:10 v/v/v) on Insertil C18 Column (250 mm x 4.6 mm, 5 µ) with 1.5 ml/min flow rate at 254 nm using PDA detector. Spinchrome software was used for data interpretation.

Preparation of Standard Stock Solution

An accurately weighed 100 mg of Fluconazole and 100 mg of Ivermectin were transferred to 10 ml separate volumetric flask and volume was adjust to mark with distilled water to obtain concentration of 1000 µg/ml for Fluconazole and Ivermectin.

Preparation of Sample Solution

Twenty tablets were taken; average weight was determined and mixed well fine powder. Amount equivalent to 100 mg FLUC and 100 mg IVR was taken in 100 ml volumetric flask. This was dissolved in water and sonicate for 3 minutes. The volume was made up to mark with water and filtered through Whatman Filter Paper No. 41. Filtrate was further diluted with solvent to get the final concentration of both drugs on the working range.

Method Validation

The developed analytical method was validated according to the International Conference on Harmonization (ICH) Guidelines for validation of analytical procedures.[9-10]

Linearity

A series of dilution were prepared in the concentration range of 50-150 µg/ml for FLUC and 52-156 µg/ml for IVR, as per ICH Guidelines. All the solutions were filtered through 0.2 µm membrane filter and injected. A calibration graph was plotted between the mean peak area v/s respective concentration and regression equation was generated using graph. According to ICH Guidelines, Correlation Coefficient should be Not Less Than 0.99% [9-10]

Accuracy

To a pre-analyzed sample solution (100µg/ml for FLUC and 104 µg/ml for IVR), a definite concentration of mixed standard drugs were added. Spiking of concentration of the pre-analyzed sample was done by 80 µg/ml, 100 µg/ml and 120 µg/ml for FLUC and for IVR was done by 83 µg/ml, 104 µg/ml and 124 µg/ml. Triplicate injections for each spiked concentration were injected.[9-10]

Precision

The repeatability study of the drug was performed for three times in the concentration range of 50-150 µg/ml for FLUC and 52-156 µg/ml for IVR. The intermediate precision was performed by doing day to day variation and analyst to analyst variation. Intermediate precision study of the drug was performed for three times in the concentration range of 50-150 µg/ml for FLUC and 52-156 µg/ml for IVR. As per ICH norms, %RSD for area should be Not More Than 2%.[9-10]

Robustness

As per ICH norms, small but deliberate variations by altering the operating conditions such as flow rate and/or ratio of the mobile phase were made to check the method's capacity to remain unaffected. Three injections of 100 µg/ml for FLUC and 104 µg/ml for IVR were injected and flow rate was varied by ±0.2 ml/min. Change in the ratio of mobile phase was done by varying the concentration in range of ±5%. Triplicate injections of formulation were injected and results were analyzed. As per ICH norms, %RSD for area should be Not More Than 2%.[9-10]

RESULT AND DISCUSSION

The goal of this study was to develop a rapid HPLC method for analysis of Fluconazole and Ivrmectin in its bulk and Tablet formulation using a commonly used reverse phase C_{18} column. To develop an effective method for the analysis of drug, preliminary tests were performed in order to select adequate and optimum condition parameter such as detection wavelength, ideal mobile phase and its combination. Separation of drugs was achieved by using HPLC system with UV absorbance detector set at 254 nm. Mobile phase used for separation was Acetonitrile:Methanol:Water (75:15:10 v/v/v). Inertsil C_{18} column (250 mm x 4.6mm, 5 µ) was used a stationary phase containing silica gel. Linearity was observed over a concentration range of 50-150 µg/ml for Fluconazole and 52-156 µg/ml for Ivermectin. Correlation coefficient was observed 0.999 for both the drugs. In order to validate the accuracy of method, recovery studies were performed. 99.830% and 100.814% amount were recovered for Fluconazole and Ivermectin respectively. The %RSD for area was less than 2, which indicate the accuracy of the method. Precision studies were carried out by studying repeatability studies and intraday precision. %RSD values for areas were observed to be less than 2% and hence it indicates that method is precise. Small and deliberate variations were applied to method parameters in order to assess robustness of the method. %RSD values for area was observed to be less than 2%. This value indicates that method is robust.

CONCLUSION

The following research was done in order to develop a simple, precise and accurate method for analysis of Fluconazole and Ivermectin in bulk and tablet formulation using RP-HPLC. The validation parameters for the analytical method suggest that method is economical and simple and can be used for routine analysis of the drugs.

Fig. 1: Structure of Fluconazole[1]

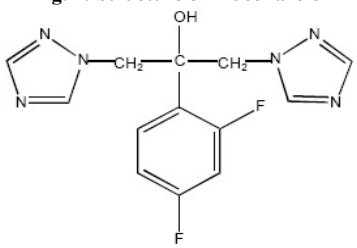

Fig. 2: Structure of Ivermectin[4]

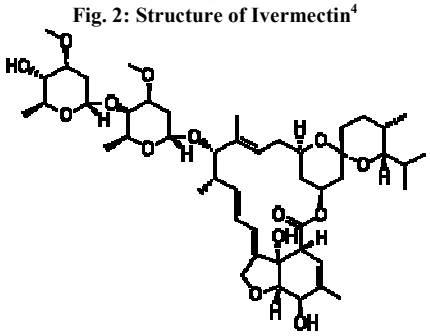

Fig.3: Chromatogram of Fluconazole and Ivermectin

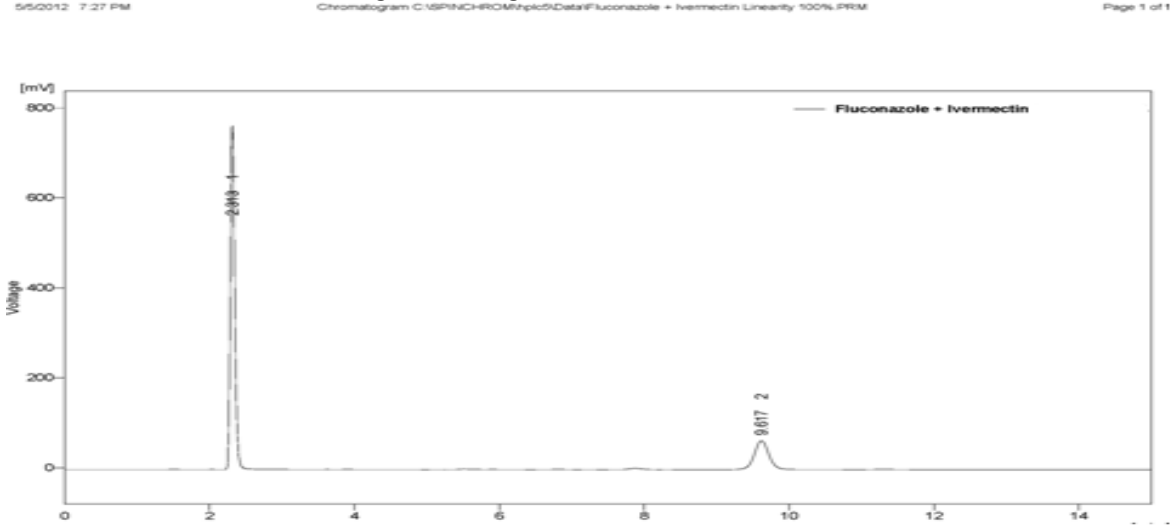

Fig.4: Calibration curve of Fluconazole

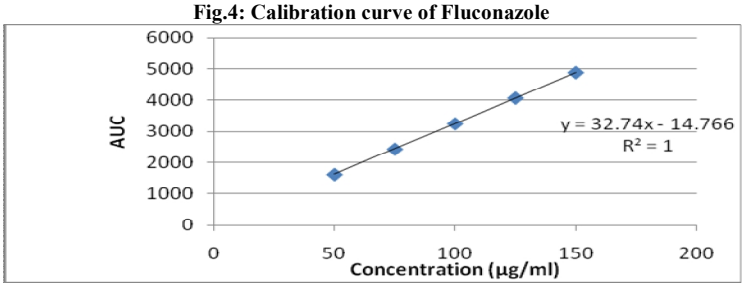

Fig.5: Calibration curve of Ivermectin

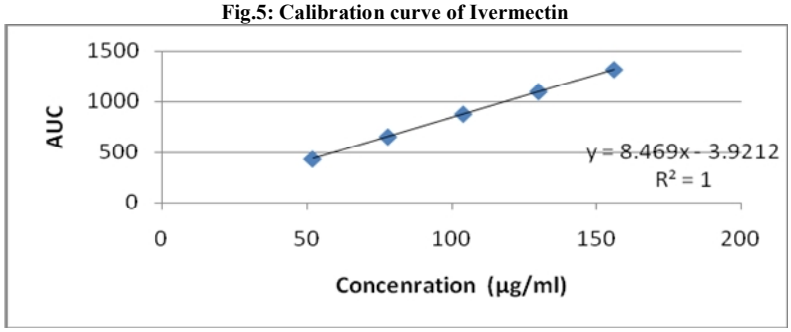

Table: 1 System suitability parameter of the proposed method

Parameter	Fluconazole	Ivermectin
Theoretical plates	6050	11257
Resolution	30.710	30.710
Asymmetric factor	1.4	1.1
Retention Time (min)	2.313	9.610
Linearity Range(µg/ml)	50-150	52-156
Regression Equation	Y=32.74x-14.76	Y=8.469x-3.921
Slope (m)	32.74	8.469
Intercept (c)	14.76	3.921
Correlation Coefficient	0.9999	0.9999

Table 2: Accuracy Test Results for Fluconazole

Sr. No.	Pre-analyzed sample (µg/ml)	Excess amount added (µg/ml) n=3	AUC	Conc. Found (µg/ml)	Amount recovered	% Recovery
1	100	80	5901.688	180.710	80.660	100.75
		80	5860.421	179.449	79.339	99.17
		80	5796.053	178.959	78.849	98.561
2	100	100	6557.438	202.408	102.298	102.298
		100	6511.572	199.338	99.228	99.228
		100	6466.023	197.946	97.836	97.36
3	100	120	7213.169	222.603	122.493	102.077
		120	7162.722	221.049	120.939	100.783
		120	7105.478	217.478	117.368	97.806
Mean						**99.830**
SD						**1.707**
RSD						**1.079**

Table 3: Accuracy Test Results for Ivermectin

Sr. No.	Pre-analyzed sample (µg/ml)	Excess amount added (µg/ml) n=3	AUC	Conc. Found (µg/ml)	Amount recovered	% Recovery
1	104	83	1588.003	187.974	83.7	100.95
		83	1576.927	186.662	82.48	99.37
		83	1559.409	184.594	80.412	96.88
2	104	104	1764.921	208.866	104.684	104.68
		104	1752.623	207.408	103.226	103.22
		104	1740.386	205.963	101.781	101.78
3	104	124	1944.709	230.089	125.907	101.537
		124	1930.611	228.425	124.243	100.195
		124	1915.268	226.606	122.424	98.72
Mean						**100.814**
SD						**1.99**
RSD						**1.99**

Table 4: Repeatability Studies

Sr. No.	Strength of Sample N=3		% Mean Amount Found	
	FLUC	IVR	FLUC	IVR
1	50	52	99.286	98.172
2	100	104	99.450	99.006
3	150	124	99.146	99.104
Mean			**99.294**	**98.761**
SD			**0.151**	**0.511**
RSD			**0.15**	**0.15**

Table 5: Intraday Precision Studies

Sr. No.	Strength of Sample N=3		% Mean Amount Found	
	FLUC	IVR	FLUC	IVR
1	50	52	98.926	98.593
2	100	104	98.923	98.564
3	150	124	98.780	97.630
Mean			**98.876**	**98.263**
SD			**0.08**	**0.548**
RSD			**0.8**	**0.5**

Table 6: Robustness Studies for Fluconazole (Change in Flow rate)

Sr. No.	Flow Rate n=3	Amount Found	% Amount Found	%Mean	SD	RSD
1	+0.2 ml/min	99.844	99.8	**99.225**	**0.623**	**0.627**
		99.234	99.234			
		98.598	98.598			
2	-0.2 ml/min	100.322	100.322	**100.00**	**0.673**	**0.673**
		100.458	100.458			
		99.23	99.23			

Table 7: Robustness Studies for Ivermectin (Change in Flow rate)

Sr. No.	Flow Rate n=3	Amount Found	% Amount Found	%Mean	SD	RSD
1	+0.2 ml/min	103.949	99.95	**98.504**	**1.58**	**0.16**
		102.699	98.749			
		100.688	96.815			
2	-0.2 ml/min	104.4	100.386	**99.813**	**0.611**	**0.6**
		103.139	99.172			
		103.896	99.9			

Table 8: Robustness Studies for Fluconazole (Change in Solvent Concentration)

Sr. No.	Solvent conc. n=3	Amount Found	% Amount Found	%Mean	SD	RSD
1	+5%	98.33	98.33	**97.810**	**0.537**	**0.5**
		97.844	97.84			
		97.257	97.257			
2	-5%	100.54	100.54	**100.064**	**0.737**	**0.736**
		100.437	100.437			
		99.215	99.215			

Table 9: Robustness Studies for Ivermectin (Change in Solvent Concentration)

Sr. No.	Solvent conc. n=3	Amount Found	% Amount Found	%Mean	SD	RSD
1	+5%	102.333	98.397	**97.277**	**1.539**	**1.582**
		101.829	97.912			
		99.343	95.522			
2	-5%	102.419	98.479	**98.364**	**0.979**	**0.9**
		101.226	97.332			
		103.253	99.281			

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