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Analytical method development and method validation of simultaneous determination of tinidazole and fluconazole by RP-HPLC

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ABSTRACT

A simple and sensitive RP-HPLC method for the simultaneous quantification of tinidazole and fluconazole in pharmaceutical formulation has been developed and validated. Chromatographic separation achieved on an octylsilane column (250 x 4.6 mm, 5µm) and 0.2% Triethylamine Buffer (pH 3.5): Acetonitrile (80:20 v/v) as the mobile phase, at a flow rate of 1.0 ml/min. The detection Isobestic point was fixed at 260 nm, the total runtime was 17 min. The calibration curve was linear over the concentration range of 0.1-0.3 µg/ml for tinidazole, 0.15-0.45 µg/ml fluconazole. The analysis was found to be linear, specific, precise, sensitive and accurate. Twenty commercially available tinidazole and fluconazole tablets were analyzed showing good % recovery and % RSD.

Keywords: RP-HPLC, Method development, Tinidazole, Fluconazole.

1. INTRODUCTION

Fluconazole (Figure 1) is azoles inhibit the fungal cytochrome P450 3A enzyme, lanosine 14α-demethylase, which is responsible for converting lanosterol to ergosterol, the main sterol in the fungal cell membrane. The resulting depletion of ergosterol alters the fluidity of the membrane, and this interferes with the action of membrane-associated enzymes. It is almost completely absorbed from the GI tract irrespective of food or gastric acidity. Only 10% of drugs in circulation is protein bound. Renal excretion accounts for >90% of elimination, with a t1/2 of ~25 hours. Tinidazole (Figure 2) is imidazole derivative, It requires reductive activation of the Nitro group by susceptible organisms. Its selective toxicity toward anaerobic and microaerophilic pathogens such as the amitochondriate protozoa T. vaginalis, E. istolytica, and G. lamblia and various anaerobic bacteria derives from their energy metabolism, which differs from that of aerobic cells. These organisms, unlike their aerobic counterparts, contain electron transport components such as ferredoxins, small Fe-S proteins that have a sufficiently negative redox potential to donate electrons to etronidazole. The single electron transfer forms a highly reactive Nitro radical anion that kills susceptible organisms by radical-mediated mechanisms that target DNA and possibly other vital biomolecules. From the literature survey, it was found that there are few analytical methods are reported for the Tinidazole, Fluconazole in reverse phase HPLC method. There is no simple method reported for the determination of Tinidazole and Fluconazole in combination of pharmaceutical dosage form. So it was felt that there is a need to develop analytical method for determination of Tinidazole and Fluconazole in simultaneously single step process. So, the present work is aimed at development of reverse phase HPLC method for the simultaneous determination of Tinidazole and Fluconazole in tablet dosage form and the validation of the developed method.

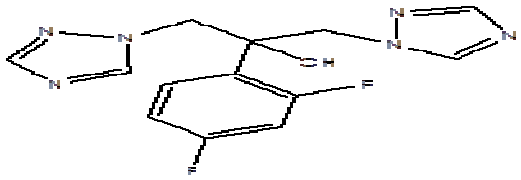

Figure - 1: Structure of fluconazole

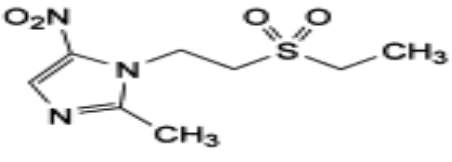

Figure - 2: Structure of tinidazole

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Reference standards of Tinidazle (99.0 %), Fluconazole (99.48%) were obtained from Madras pharmaceuticals Pvt Ltd. (Chennai, India). Their chemical structures are presented in Fig. 1. Water was prepared using a Milli Q water purification system from Millipore (Bangalore, India). Acetonitrile (HPLC grade) were purchased from J. T. Baker (Phillipsburg, USA). Ortho phosphoric acid was purchased from M/S SD Fine Chemicals (Mumbai, India).

2.2. Instrumentation and chromatographic conditions

Chromatographic separation was carried out on an Agilent 1100-UV Detector with a HYPERSIL BDS C8 (250 x 4.6mm, 5µ) and a mobile phase 0.2 % of Triethylamine pH 3.5 using orthophosphoric acid. Buffer: Acetonitrile (80:20 v/v) delivered at a flow rate of 1.0 ml/min. The Injection volume was 20*µl.*

2.3. Sample preparation

Crush 20 tablets. From the powdered tablets, weigh accurately about 135.0 mg of powdered tablets into a 25 ml volumetric standard flask, and add 15 ml diluent, and sonicate for 30 minutes and make up to 25 ml with diluents. Shake well and filter the solution. Use this filtrate for fluconazole sample solution. From the above filtrate pipette out 5.0 ml into a 100 ml standard volumetric flask and make up to 100 ml with diluents for a Tinidazole sample solution.

3. RESULTS AND DISCUSSION

The combination of Tinidazole and fluconazole is increasingly finding use in the treatment of antifungal. Hence it was felt necessary to develop a sensitive method for simultaneous determination of Tinidazole and fluconazole, literature reviews does not show the simple method for simultaneous determination in this combined dosage formulation. HPLC Agilent 1100 auto sampler separation module with UV detector and column used is Hypersil BDS C8 (250 x 4.6mm, particle size. Injection volume of 20µl is injected and eluted with the mobile phase of 0.2% triethylamine and acetonitrile (80:20) ratio, which is pumped at the flow rate of 1ml/min and detected by UV detector at 260 nm. The peaks of Tinidazole and Fluconazole are well separated at 10.2 and 15.0 min respectively. The net retention time for the two compounds in the reported method is about 17 min. Thus, this method provides shorter analysis time and conserves mobile phase system. The method was validated based on United States pharmacopoeia and ICH parameters. The parameters are Accuracy, Precision, Specificity, Linearity, Ruggedness and Robustness. The validation of the proposed reverse phase HPLC method was further verified by recovery studies. The percentage recovery was found to be within 99.62-101.98 w/v for Tinidazole, 99.54-101.54 w/v for fluconazole. This serves a good index of accuracy and reproducibility of the proposed method and data's is reported in table1&2. The precision of the method was determined by replicating injections of standard solution. The percentage of RSD of the assay was to be 0.118 for fortinidazole, 0.621 for fluconazole, which was within the acceptance criteria of NMT 2%. Thus the proposed method was found to be providing high degree of precision and reproducibility and the data are reported in table 3&4. The specificity of the method was confirmed by injecting the placebo and placebo with mixed standard and observed that there was no interference due to placebo. The data regarding linearity of the two drugs are shown in table no 5&6 the linearity studies the specified range was determined for two drugs 0.20 mcg/ml for tinidazole, 0.30 mcg/ml for fluconazole and linearity coefficient was found to be 0.997 for tinidazole, 0.998 for fluconazole. The method is further confirmed by analyzing the product day to day and analyst to analyst. The percentage RSD values of the above parameters are found within the limit. The data for Ruggedness are shown in table 7&8. The analysis is also determined by varying the flow rate, P^H, etc., the data are predicted in the respective tables 9and 10.

Table - 1: Accuracy-Tinidazole

Conc (µg/ml)	Sam. Wt (mg)	Area	Recovery (%)
50	12.2	1438581	100.09
50	12.4	1455406	99.62
50	12.3	1455488	100.94
100	22.7	2727373	101.98
100	22.9	2695219	99.90
100	22.8	2694483	100.31
150	31.6	3872757	101.48
150	31.5	3743940	100.88
150	31.7	3767044	100.87
		Mean	100.67
		SD	0.765
		% RSD	0.760

Table - 2: Accuracy-Fluconazole

Conc (µg/ml)	Sam.Wt (mg)	Area	Recovery (%)
50	17.7	443084	100.06
50	17.6	440893	100.07
50	17.5	444554	101.54
100	32	800954	100.05
100	32.1	808952	100.74
100	32.3	804320	99.54
150	47.9	1205196	100.57
150	47.8	1201550	100.48
150	47.9	1216742	101.54
		Mean	100.51
		SD	0.682
		% RSD	0.679

Table - 3: Method precision for tinidazole

Weight (mg)	Area	Assay (mg)	Assay (%)
144.3	2571006	1007.8	100.78
140.3	2504410	1009.76	100.98
137.5	2449386	1007.69	100.77
137.9	2455678	1007.35	100.73
139.4	2488153	1009.69	100.97
		Mean	100.84
		SD	0.119
		% RSD	0.118

Table - 4: Method precision for fluconazole

Weight (mg)	Area	Assay (mg)	Assay (%)
140.3	799089	75.8	101.0
139.8	793283	75.58	100.78
137.5	775981	75.17	100.23
137.9	773844	74.75	99.66
139.4	782133	74.73	99.65
		Mean	100.26
		SD	0.622
		RSD	0.621

Table - 5: Linearity for Tinidazole

Conc (%)	Conc (µg/ml)	Area
50	0.10	971606
80	0.16	1571315
100	0.20	2003745
120	0.24	2484218
150	0.30	3213842

Table - 6: Linearity for fluconazole

Conc (%)	Conc (µg/ml)	Area
50	0.15	352565
80	0.24	575604
100	0.30	731973
120	0.36	899211
150	0.45	1162304

Table -7: Ruggedness test results for tinidazole

Parameters	Assay (%)	S.D	% RSD
	100.33		
Day-1	100.93		
	101.17		
DAY-2	101.99	0.688	0.68
	100.33		
Analyst-1	100.93		
	100.17		
Analyst-2	101.99	0.688	0.68

Table -8: Ruggedness test results for fluconazole

Parameters	Assay (%)	S.D	% RSD
	100.78		
Day-1	100.90		
	101.17		
DAY-2	100.73	100.90	0.197
	100.73		
Analyst-1	100.78		
	100.90		
Analyst-2	101.17	100.90	0.197

Table - 9: Robustness test results for tinidazole

Parameters		Assay (%)	Average (%)	SD	% RSD
		100.33			
	Actual	100.98			
		99.61			
	Low	99.70			
		99.29			
pH	High	101.82	100.29	0.962	0.96
		100.33			
	Actual	100.98			
		99.36			
	Low	101.29			
		101.48			
Flow	High	100.07	100.59	0.81	0.81

Table - 10: Robustness test results for fluconazole

Parameters		Assay (%)	Average (%)	SD	% RSD
		100.78			
	Actual	100.90			
		99.68			
	Low	99.18			
		99.70			
pH	High	100.94	100.20	0.766	1.36
		100.78			
	Actual	100.90			
		99.17			
	Low	99.00			
		100.26			
Flow	High	101.80	100.32	1.08	1.11

4. CONCLUSION

An HPLC method is developed for simultaneous estimation of Tinidazole and Fluconazole combined dosage form using high performance chromatography. HPLC Agilent 1100 auto sampler separation module with UV detector and column used is HYPERSIL BDS C8 (250 x 4.6mm) ID column (Agilent) with 5-micron particle size. Injection volume of 20 µl is injected and eluted with the mobile phase of 0.2 % triethylamine and Acetonitrile (80: 20) ratio, which is pumped at the flow rate of 1ml/min and detected by UV detector at 260 nm. The peaks of Tinidazole and fluconazole are well separated at 10.2 and 15.0 min respectively. The developed method is validated for various parameters as per ICH guidelines like accuracy, precision, linearity, specificity, system suitability, ruggedness and robustness. The results obtained are within the acceptable criteria. The proposed method is applied for determination of Tinidazole, Fluconazole respectively. Hence the proposed method was found to be satisfactory and could be used for the routine analysis of Tinidazole and Fluconazole in tablet dosage form.

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