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DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF AZITHROMYCIN, FLUCONAZOLE AND ORNIDAZOLE IN THE COMBINED DOSAGE FORM

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ABSTRACT

An isocratic, simple, precise, accurate, stability-indicating Reversed Phase - HPLC method for rapid separation with shorter runtime was developed and validated for the quantitative determination of Azithromycin, Fluconazole and Ornidazole in combined-dosage form. An Agilent Zorbax SB C_{18} (250mm x 4.6mm, 5µm) column with mobile phase containing Sodium dihydrogen orthophosphate (with pH - 5.2) : Acetonitrile in the ratio of 700:300(v/v) was used. The flow rate was 1.0 mL/min, column temperature was 30°C and eluted compounds were monitored at 236 nm. The retention times of Azithromycin, Fluconazole and Ornidazole were 4.402min, 8.399 min and 2.746min respectively. The correlation co-efficients for Azithromycin, Fluconazole and Ornidazole were found to be 0.99, 0.99 and 0.99 respectively. This newly developed method was validated as per ICH guidelines with respect to linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. Recovery of Azithromycin, Fluconazole and Ornidazole in formulations was found to be 100%, 100% and 100% respectively confirms the non-interferences of the excipients in the formulation. Good resolution between the peaks for degradation products and the analyte was achieved. Due to its simplicity, rapidness and high precision, this method was successfully applied to the estimation of Azithromycin, Fluconazole and Ornidazole in combined dosage form.

Keywords: RP-HPLC, Azithromycin, Fluconazole, Ornidazole estimation.

INTRODUCTION

Azithromycin: Azithromycin is a semi-synthetic macrolide antibiotic of the azalide class. It is derived from erythromycin, with methyl substituted nitrogen incorporated in to the lactone ring, thus making the lactone ring 15-membered. Azithromycin prevents bacteria from growing by interfering with their protein synthesis. It binds to the 50S subunit of the bacterial 70S ribosome and inhibits translation of mRNA. Its effects may be bacteriostatic or bacteriocidal depending on the drug concentration. Azithromycin is used to treat or prevent certain bacterial infections, most often those causing throat, pneumonia, typhoid, gastroenteritis, and sinusitis. In recent years, it has been used primarly to prevent bacterial infection in infants and those with weaker immune systems. It is also effective against certain sexually transmitted infections, such as non–gonococcal, urethritis, Chlamydia and cervicis.

Fluconazole: Fluconazole is designated chemically as 2, 4-difluoro-α, α^1-bis (1H-1, 2, 4-triazol-1-yl) methyl benzyl alcohol with an empirical formula of $C_{13}H_{12}F_2N_6O$. Fluconazole is an 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. Like other imidazole and triazole

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class antifungal, fluconazole inhibits the fungal cytochrome P450 enzyme 14α-demethylaseThis inhibition prevents the conversion of lanosterol to ergosterol, an essential component of the fungal cytoplasmic membrane, and subsequent accumulation of 14α-methyl sterols. Fluconazole is primarly fungistatic; however, it may dose dependent manner, specifically *Cryptococcus*.

Ornidazole (ORN): Ornidazole is chemically a-(chloromethyl)-2-methyl-5-nitroimidazole-1-ethanol, is a 5-nitro imidazole derivative, used as an anti infective agent. ORN is used in the treatment of susceptible protozoal infections and also in the treatment and prophylaxis of anaerobic bacterial infections.

EXPERIMENTAL

Materials and Reagents

The working standards (Authentic samples) of Azithromycin, Fluconazole and Ornidazole were provided as gift samples from Lara drugs Pvt Ltd., Hyderabad. Marketed formulation of combination was purchased from local pharmacy market. Ortho phosphoric acid of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Sodium dihydrogen orthophosphate and Acetonitrile of AR grade were obtained from S.D. Fine Chemicals Ltd., Mumbai and milli Q water.

HPLC Instrumentation

The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump and photodiode array detector and Empower 2 software. The chromatographic separation was performed using Agilent Zorbax SB C_{18} (250mmx4.6mm, 5µm). Separation was achieved using a mobile phase consisting of 0.01M sodium dihydrogen orthophosphate buffer adjusted to pH -5.2 with dil. Orthophosphoricacid : acetonitrile (700 : 300 v/v) solution at a flow rate of 1.0mL/min. The eluent was monitored using PDA detection at a wavelength of 236 nm. The column was maintained at ambient temperature and injection volume of 10 μL was used. The mobile phase was filtered through 0.45 μ membrane micron filter prior to use.

Preparation of stock and working standard solutions

Azithromycin: Accurately weighed quantity 1000 mg of Azithromycin was transferred into 100mL of volumetric flask dissolve and diluted to volume with mobile phase and sonicate for 15 min. From the above solution 5ml was transferred into 25ml volumetric flask and make up the volume with mobile phase.

Fluconazole: Accurately weighed quantity 150 mg of Fluconazole was transferred into 100ml of volumetric flask dissolve and diluted to volume with mobile phase and sonicate for 15 min. From the above solution 5ml was taken into 25ml volumetric flask and make up the volume with mobile phase.

Ornidazole: Accurately weighed quantity 750 mg of Ornidazole was transferred into 100ml of volumetric flask dissolve and diluted to volume with mobile phase and sonicate for 15 min. From the above solution 5ml was taken into 25ml volumetric flask and make up the volume with mobile phase.

A standard stock solution of 1 mg/mL of ornidazole, azithromycin and fluconazole were prepared separately used mobile phase as solvent. In order to get the required ratio (15:20:3) of the drugs ornidazole, azithromycin and fluconazole, appropriate quantities of respective solutions of each drug were mixed and diluted with the mobile phase. The flask containing standard solution was sonicated for 10 minutes to degas it. The standard solution was then filtered with 0.45 µm membrane filter paper. A series of different dilutions (100-3000 µg/mL) were prepared using above stock solution with selected mobile phase and analyzed using the same chromatographic conditions as those of the target compounds and a calibration curve was generated.

Sample preparation

Accurately weighed Quantity of sample powder equivalent to 750mg of ornidazole, 1000mg of azithromycin and 150 mg of fluconazole was transferred into 100ml of volumetric flask added 50ml of water and sonicated for 30mins and make up the volume with mobile phase and filtered through the 0.45µm membrane filter paper. From the above solution, take 5ml into 25ml volumetric flask make up the volume with mobile phase. An aliquot of this solution was injected into HPLC system.

Method Validation

Linearity

Linearity was established by the least squares linear regression analysis of the calibration curve. The linearity of response for the drugs Azithromycin, Fluconazole and Ornidazole assay method was determined by preparing and injecting the solutions of various concentrations of the drugs. The responses were measured as peak areas and plotted against concentrations.

Precision

Precision of the method was measured by repeatability and intermediate precision studies .The precision of the method was carried out by using six replicate injections of standard concentration and six replicate injections of sample concentration. The intermediate precision of the method was measured checked by repeating the same process on three different days.The %RSD values were calculated.

Robustness

Robustness of the method was studied by varying single condition in the optimized chromatographic conditions such as mobile phase composition, pH, column

temperature, flow rate and wavelength at a time keeping all other parameter constant. The effect of the above changes on system suitability parameters like tailing factor, number of theoretical plates and on peak area were studied. Robustness of the method was carried out with the variation of flow rate ±0.1 mL/min and temperature ±5∘C.

Limit of Detection and Limit of Quantification

LOD is defined as the lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method.LOQ is determined by the analysis of samples with the known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. LOQ is the concentration that can be quantitated reliably with the specified level of accuracy and precision.

Consider the following:
Limit of Detection = ($\sigma \times 3.3$) / S,
Limit of Quantitation = ($\sigma \times 10$) / S,

Where
σ = the standard deviation of the response.
S = the slope of the calibration curve (of the analyte).

Accuracy

. Accuracy of the developed method was determined by using standard addition method. A known amount of the standard drug was added to fixed amount of pre-analyzed sample solution. The recovery studies were carried out at three concentration levels. The standard addition method was performed at 50%, 100%, and 150% levels of sample solution. The percentage recovery and standard deviation of the percentage recovery were calculated.

The resulting solutions were analyzed measured in triplicate at each level as per the ICH guidelines

Specificity

Specificity is the ability of the analytical method to measure the analyte which is free from the interference due to other components like impurities, degradants, matrix, etc. The specificity was established by preparing a Azithromycin, Fluconazole and Ornidazole standard at 0.5% level of test concentration and injected 6 times into HPLC system as per the test procedure. Specificity was measured by the comparison of the test results obtained from the analysis of sample solution containing ingredients with the test results obtained from the standard drug. Purity of the sample was measured by the comparison of the spectra at peak start, peak apex and peak end positions of the band.

Forced Degradation Studies

For determining whether the analytical method and assay were stability-indicating, the standard drugs were stressed under various conditions to conduct forced degradation studies. Intentional degradation was attempted to stress conditions of photolytic degradation, acid hydrolysis (using 1N HCl), base hydrolysis (1N NaOH), oxidative degradation (20% H_2O_2), and thermal treatment to evaluate the ability of the proposed method to separate the drugs from its degradation products.

Acid and Alkaline Degradation

Forced degradation in acidic media was performed by taking the pure drugs in the volumetric flasks followed by the addition of 1N HCl and diluent and the mixture was heated under reflux for 3 hrs at 55^0C, and the volume was made up to the mark with diluent and filtered. The resultant solution was diluted to obtain 100μg/Ml solutions and 10μL was injected into the system and the chromatograms were recorded to assess the stability of sample. Similarly, forced degradation in basic (alkaline) medium was performed by using 1N NaOH.

Oxidative Degradation

Oxidative degradation was performed by taking each of 100 mg of pure drugs in 100mL volumetric flask then 1mL of 30%H_2O_2 and 70mL of diluents were added and the mixture was heated under reflux for 3 hrs at 55^0C, and the volume was made up to the mark with diluent. Appropriate aliquot was taken from the above solution and diluted with the diluent to get the final concentration of 100μg/mL. The chromatogram was recorded with the help of HPLC for each drug.

Photostability

The photo-stability degradation studies was performed by exposing the pure drug to sunlight for 6 days and it was transferred into 100mL volumetric flask and the volume was made up to the mark with diluent. Appropriate aliquot was taken from the above solution and diluted to obtain a final concentration of 100μg/mL. The chromatogram was recorded to assess the stability of sample.

Thermal Degradation

The standard drugs were placed in an oven at 105^0C for 72 hrs to study dry heat degradation. For the HPLC study, the resultant solution was diluted to 100μg/mL solution and 10μL was injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

Method Development

The ultimate target of this chromatographic method was to achieve the separation of all drugs along with the degradation products. The maximum absorption wavelength of the reference drugs and the forcibly degraded drug solution is 236 nm; hence this wavelength

was selected as the detection wavelength for the analysis. Pure drug along with its degraded products was injected and run in different solvent systems. For ideal separation of the drug isocratic conditions, mixtures of commonly used solvents with or without different buffers in different combinations were tested as mobile phases. Finally the mobile phase consisting of sodium dihydrogen orthophosphate buffer and acetonitrile (700 : 300 v/v) with pH – 5.2 adjusted with ortho phosphoric acid was selected for validation purpose and stability studies. Several preliminary chromatographic runs were performed to investigate the suitability for drug.

By using this proposed developed method, system suitability parameters, USP tailing factor, resolution of drugs and the degradation products were calculated and were found to be in the specified range. The method was also validated with respect to parameters including linearity, limit of detection (LOD), and limit of quantitation (LOQ), recovery, precision, accuracy, robustness, and specificity.

Linearity and Range

The plot of peak area versus the respective concentrations of Azithromycin, Fluconazole and Ornidazole were found to be linear in the concentration range of 1000-3000µg/mL, 150-450 µg/mL and 750-2250 µg/mL respectively. The regression equation for Azithromycin is *y = 40366x* with a coefficient of correlation (R^2) of 0.99. The regression equation for Fluconazole is *y = 47895x* with a coefficient of correlation (R^2) of 0.99 The regression equation for Ornidazole is *y = 13485x* with a coefficient of correlation (R^2)of 0.99. Linear regression least square fit for the data obtained from the above calibration curves. The linear regression data values are shown in Table. 14. The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. The results for calibration curves are given in Fig.4,5&6.

Robustness

Under all the deliberately altered chromatographic conditions (flow rate and temperature), all peaks were adequately resolved and elution orders remained unchanged which indicates that the method is robust. The results are summarized in Tables 4, 5 & 6.

Precision

Interday analysis was carried out by repeating the experiments on three different days, whereas intraday analysis was done for 6 times on the same day. The system precision was carried out by injecting the standard drug solutions six times and the method precision was carried out by injecting the sample drug solutions for six times. The %RSD for repeatability of both standard and sample solutions were found to be <2.0% . The %RSD for intermediate precision of both standard and sample solutions were also found to be <2.0%. This shows that precision of the method is satisfactory as % relative standard deviation is not more than 2.0% and the developed RP-HPLC method was found to be precise. The results are depicted in Table 7(a) and 7(b), respectively.

Accuracy

The accuracy of the method was established by recovery studies. The recovery of the drugs by the proposed method was very satisfactory. The mean recoveries of Azithromycin, Fluconazole and Ornidazole were found to be 100%, 100% and 100%. The results indicated good accuracy of the method for the determination of analysed drugs as revealed by mean recovery data (Tables.8,9&10). The chromatograms of three different levels shown in Fig. 7, Fig. 8 & Fig. 9.

Limit of Detection and Limit of Quantification

LOD and LOQ values were evaluated by the standard deviation method and the following values were found to be the LOD and LOQ values for mentioned drugs. These results are depicted in Table.11, Table.12& and Table.13 respectively. Chromatograms of LOD and LOQ study were shownin Fig. 10 & Fig.11.

Specificity

This developed method was declared as specific, as there no interfering peaks were observed at the retention times of the drugs. The drugs peaks were well resolved from the peaks of all the possible degradation products. The typical chromatogram of standard and sample for the specificity study is shown in Fig.12 and Fig.13 respectively. These results were indicating the specificity of the developed method.

System Suitability Studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ±3% standard deviation range during routine performance of the method.

Forced Degradation Studies

The results obtained for stress testing studies indicated a high degree of selectivity of the method. The chromatograms obtained from stressed samples are shown in Fig. 14.The drugs was unstable under acid and alkaline stress conditions when kept for 3 hrs at 55^0C. The drugs were degraded approximately to 11.6% and 10% in acidic and alkaline conditions respectively.

The drugs Azithromycin, Fluconazole and Ornidazolewere found to be degraded around 7%, 18%, 6% respectively when kept under oxidative stress conditions with 30% H_2O_2 for 3 hrs at 55^0C .When the

solid drugs was exposed to light for 7days, the drug underwent 5%,6% and 3% of degradation Azithromycin, Fluconazole and Ornidazole respectively. Azithromycin, Fluconazole and Ornidazole were found to be degraded 4%, 5% and 3% respectively nearly when they kept for 72 hrs at 105^0C. The results of the assay indicating that the developed method was selective for the assay of Azithromycin, Fluconazole and Ornidazole without interference of the excipients used in the capsules Tables 15,16 & 17.

Table 1. Linearity data of Azithromycin

S.No	Concentration (µg/mL)	Peakarea	
1	1000	2015241	Slope = 40366 C.C = 0.99
2	1500	3022089	
3	2000	4033745	
4	2500	5048069	
5	3000.00	6058513	

Table 2. Linearity data of Fluconazole

S.No	Concentration (µg/mL)	Peakarea	
1	150	2390265	Slope = 47895 C.C = 0.99
2	225.00	3592909	
3	300.00	4788788	
4	375	5983305	
5	450	7188885	

Table 3. Linearity data of Ornidazole

S.No	Concentration (µg/mL)	Peakarea	
1	750	673342	Slope = 13485 C.C = 0.99
2	1125.00	1014472	
3	1500.00	1346288	
4	1875	1684425	
5	2250	2024030	

Table 4. Robustnessfor Azithromycin

S No	Sample name	Change	Name	RT	Area	Tailing	Plate count
1	Flow1	-10%	Azithromycin	5.046	5055322	1.189	6518
2	Flow2	+10%	Azithromycin	3.426	3305492	1.139	4617
3	Temp1	-5^oC	Azithromycin	3.949	3944676	1.139	6621
4	Temp2	$+5^oC$	Azithromycin	4.065	3988229	1.126	5149

Table 5. Robustness for Fluconazole

S No	Sample name	Change	Name	RT	Area	Tailing	Plate count
1	Temp2	$+5^0C$	Fluconazole	7.975	4845246	1.143	10808
2	Flow1	-10%	Fluconazole	10.578	5966630	1.173	10455
3	Flow2	+10%	Fluconazole	7.157	4004573	1.138	7539
4	Temp1	-5^0C	Fluconazole	8.560	4807084	1.183	9063

Table 6. Robustness for Ornidazole

S No	Sample name	Change	Name	RT	Area	Tailing	Plate count
1	Flow1	-10%	Ornidazole	3.463	1743594	1.432	10057
2	Temp2	$+5^0C$	Ornidazole	2.772	1335302	1.345	10108
3	Flow2	+10%	Ornidazole	2.325	1131296	1.479	8030
4	Temp1	-5^0C	Ornidazole	2.789	1353857	1.386	9385

Table 7(a). Precision Studies (intra – day) for Azithromycin, Fluconazole and Ornidazole

S.No	Sample Weight	Azithromycin	Fluconazole	Ornidazole	% Assay Azithromycin	% Assay Fluconazole	% Assay Ornidazole
1	2240.10	4030733	4783930	1344145	100	99	100
2	2240.10	4034770	4780227	1344674	100	99	100
3	2240.10	4035909	4781515	1340083	100	99	100
4	2240.10	4031685	4785021	1349972	100	99	100
5	2240.10	4039770	4784077	1344044	100	99	100
6	2240.10	4035119	4787217	1343567	100	99	100
AvgAssay:					100	99	100
STD					0.08	0.05	0.23
%RSD					0.08	0.05	0.24

Table 7(b). Precision Studies (inter- day) for Azithromycin, Fluconazole and Ornidazole

S.No	Sample Weight	Azithromycin	Fluconazole	Ornidazole	% Assay Azithromycin	% Assay Fluconazole	% Assay Ornidazole
1	2240.10	4032451	4784521	1346548	100	100	100
2	2240.10	4032154	4782365	1342647	100	100	100
3	2240.10	4036432	4784578	1341254	100	100	100
4	2240.10	4031275	4782456	1342634	100	100	100
5	2240.10	4036248	4786235	1343641	100	100	100
6	2240.10	4033791	4787596	1346687	100	100	100
AvgAssay:					100	100	100
STD					0.05	0.04	0.17
%RSD					0.05	0.04	0.17

Table 8. Accuracy for Azithromycin

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	1120.10	2013623	997.045	995.43	100	100
50%	1120.10	2019468	997.045	998.32	100	
50%	1120.10	2011693	997.045	994.48	100	
0%	1120.10	2017849	997.045	997.52	100	
50%	1120.10	2018132	997.045	997.66	100	
50%	1120.10	2019045	997.045	998.12	100	
100%	2240.10	4031797.00	1994.00	1993.12	100	100
100%	2240.10	4035920	1994.00	1995.17	100	
100%	2240.10	4032543	1994.00	1993.49	100	
150%	3360.20	6055738	2991.045	2993.65	100	100
150%	3360.20	6052912	2991.045	2992.26	100	
150%	3360.20	6059701	2991.045	2995.61	100	
150%	3360.20	6051534	2991.045	2991.58	100	
150%	3360.20	6058073	2991.045	2994.81	100	
150%	3360.20	606690	2991.045	2994.13	100	

Table 9. Accuracy of Fluconazole

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	1120.10	2392005	148.657	148.54	100	100
50%	1120.10	2398373	148.657	148.93	100	
50%	1120.10	2396378	148.657	148.81	100	
0%	1120.10	2399311	148.657	148.99	100	
50%	1120.10	2397590	148.657	148.88	100	
50%	1120.10	2393257	148.657	148.61	100	
100%	2240.10	4787858	297.300	297.31	100	100

100%	2240.10	4785399	297.300	297.16	100	
100%	2240.10	4786421	297.300	297.22	100	
150%	3360.20	7188984	445.957	446.41	100	100
150%	3360.20	7187498	445.957	446.32	100	
150%	3360.20	7189155	445.957	446.42	100	
150%	3360.20	7187128	445.957	446.30	100	
150%	3360.20	7186666	445.957	446.27	100	
150%	3360.20	7186030	445.957	446.23	100	

Table 10. Accuracy for Ornidazole

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	271.50	3985264	792.800	792.55	100	100
50%	271.50	3984333	792.800	792.37	100	
50%	271.50	3983900	792.800	792.28	100	
50%	271.50	3988285	792.800	793.15	100	
50%	271.50	3986263	792.800	792.75	100	
50%	271.50	3987635	792.800	793.02	100	
100%	543.10	7979453	1585.892	1586.88	100	100
100%	543.10	7972398	1585.892	1585.48	100	
100%	543.10	7973162	1585.892	1585.63	100	
150%	814.60	11918737	2378.692	2370.29	100	100
150%	814.60	11919264	2378.692	2370.39	100	
150%	814.60	11959344	2378.692	2378.36	100	
150%	814.60	11947991	2378.692	2376.10	100	
150%	814.60	11969725	2378.692	2380.43	100	
150%	814.60	11909255	2378.692	2368.40	100	

Table 11. LOD and LOQ of Azithromycin

LOD	2.7100
LOQ	9.0334

Table 12. LOD and LOQ of Fluconazole

LOD	2.990
LOQ	9.967

Table 13. LOD and LOQ of Ornidazole

LOD	2.724
LOQ	9.080

Table 14. System Suitability Parameters (Regression characteristics of the Linearity plot of Azithromycin, Fluconazole and Ornidazole)

Parameters	**Azithromycin**	**Fluconazole**	**Ornidazole**
Correlation coefficient	0.99	0.99	0.99
Regression equation	y = 40366x	y = 47895x	y = 13485x
LOD	2.7100	2.990	2.724
LOQ	9.0334	9.967	9.080
Theoritical plates	5713	9474	9651
Tailing	1.115	1.132	1.387

Table 15. Degradation studies for Azithromycin

Stress condition	**Sample weight**	**Area**	**% Assay**	**% Deg.**
Acid	2240mg	3606318	89	-11
Base	2240mg	3723043	92	-8

Peroxide	2240mg	3768791	93	-7
Light	2240mg	3834322	95	-5
Heat	2240mg	3853856	95	-4

Table 16. Degradation studies for Fluconazole

Stress condition	Sample weight	Area	% Assay	% Deg.
Acid	2240mg	3753623	78	-21
Base	2240mg	4008691	83	-16
Peroxide	2240mg	3916398	81	-18
Light	2240mg	4491644	93	-6
Heat	2240mg	4543924	94	-5

Table 17. Degradation studies for Ornidazole

Stress condition	Sample weight	Area	% Assay	% Deg.
Acid	2240mg	1201044	88	-11
Base	2240mg	1228550	90	-9
Peroxide	2240mg	1267555	93	-6
Light	2240mg	1299328	96	-3
Heat	2240mg	1308496	96	-3

Fig.1. Structure of Azithromycin

Fig.2. Structure of Fluconazole

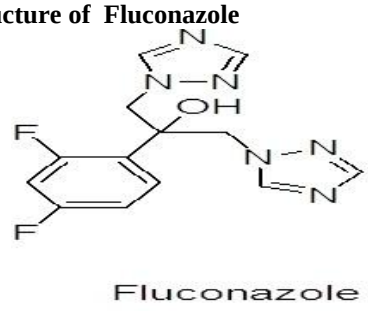


Fig. 3. Structure of Ornidazole

Fig. 4. Linearity Curve for Azithromycin

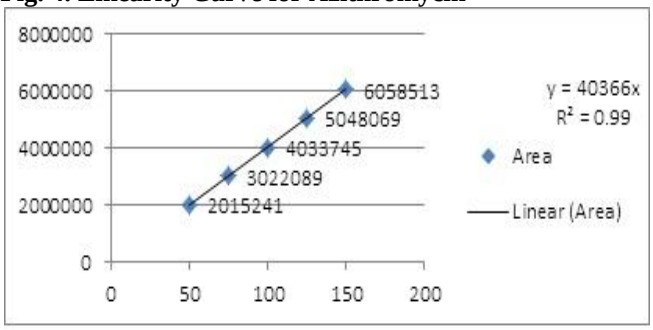


Fig.5.Linearity curve for Fluconazole

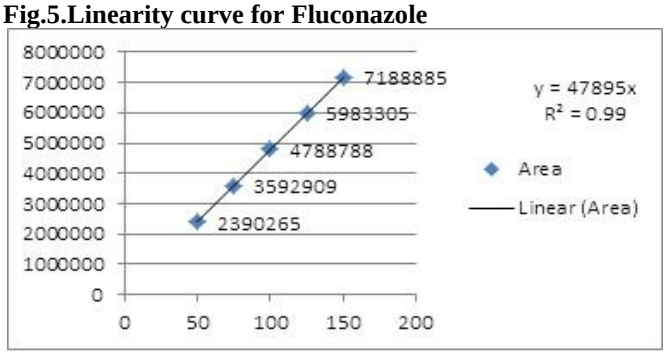


Fig. 6. Linearity Curve for Ornidazole

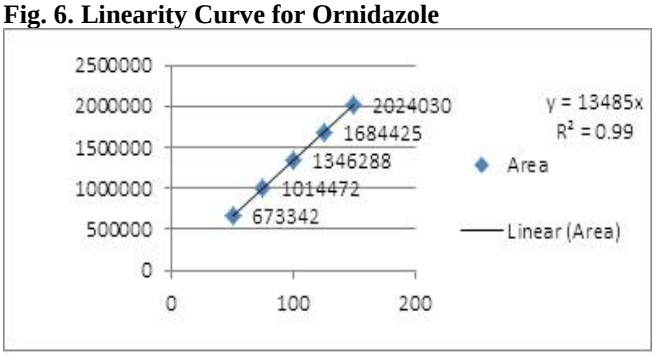


Fig. 7. Accuracy Chromatograms-50% of Azithromycin, Fluconazole and Ornidazole

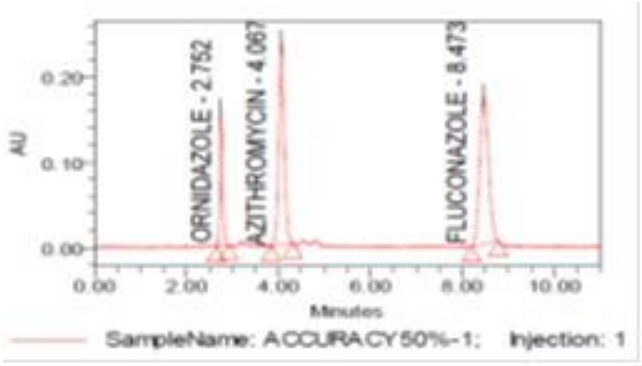


Fig. 8. Accuracy Chromatograms-100% of Azithromycin, Fluconazole and Ornidazole

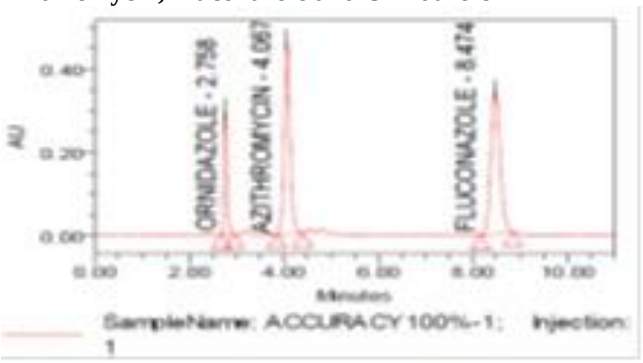


Fig. 9. Accuracy Chromatograms-150% of Azithromycin, Fluconazole and Ornidazole

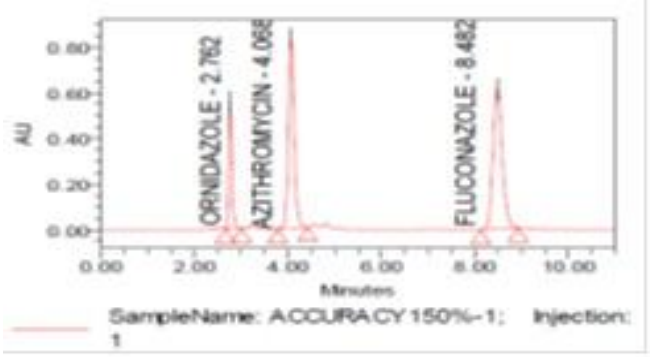


Fig. 10. LOD Chromatograms for Azithromycin, Fluconazole and Ornidazole

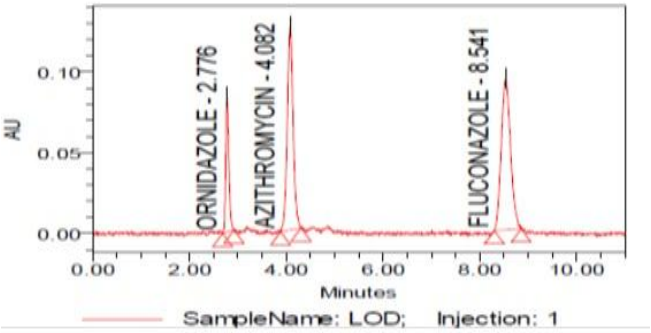


Fig. 11. LOQ Chromatograms for Azithromycin, Fluconazole and Ornidazole

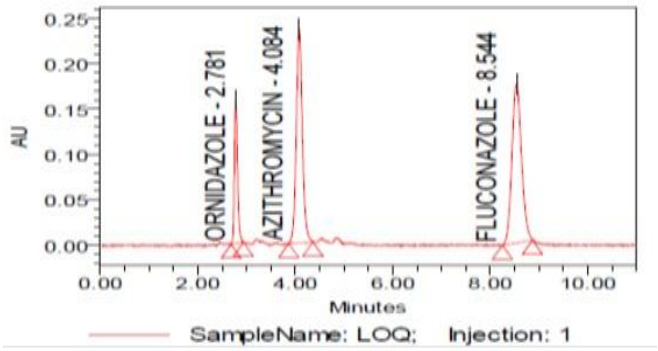


Fig. 12. Standard chromatogram for Azithromycin, Fluconazole and Ornidazole

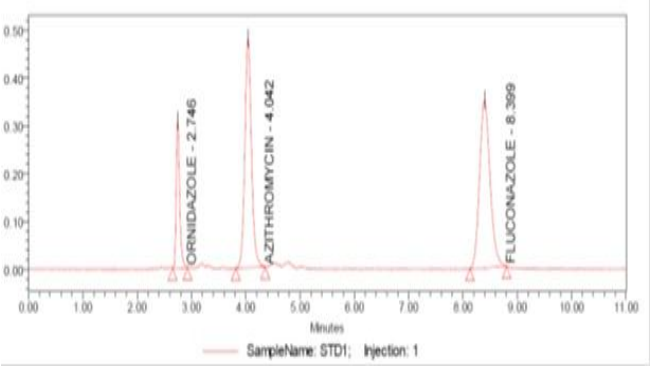


Fig. 13. Formulation chromatogram for Azithromycin, Fluconazole and Ornidazole

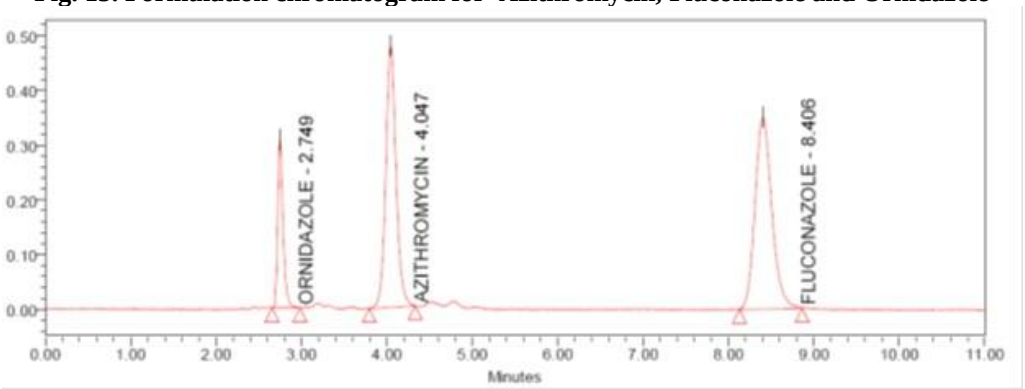
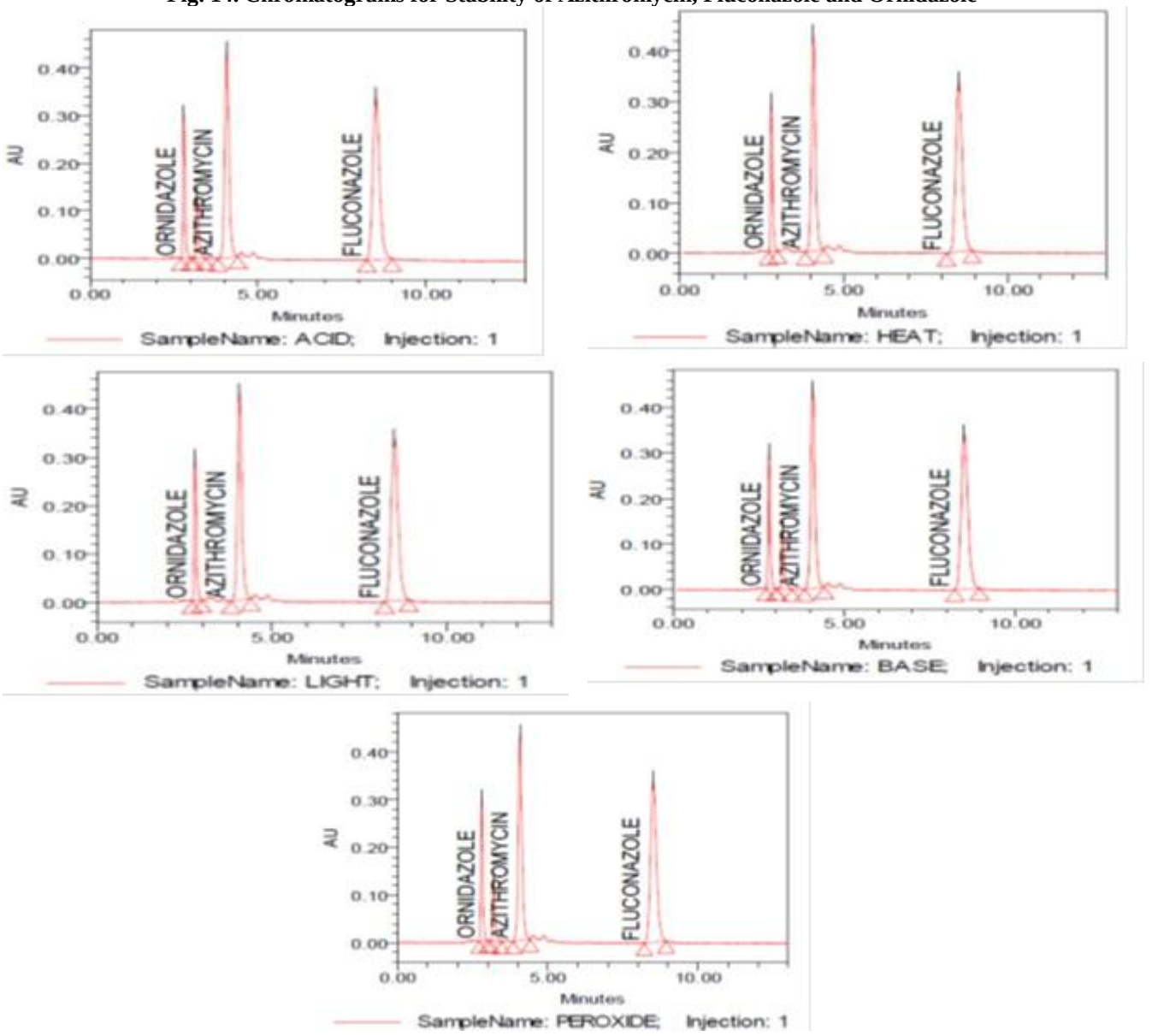


Fig. 14. Chromatograms for Stability of Azithromycin, Fluconazole and Ornidazole



CONCLUSION

The developed stability indicating RP- HPLC method was precise, specific, accurate, linear, sensitive and robust. The statistical analysis proves that this method was reproducible and selective for the simultaneous estimation and analysis of Azithromycin, Fluconazole and Ornidazole in pharmaceutical combined dosage form. Hence, this method can easily and conveniently adopt for routine quality control analysis of Azithromycin, Fluconazole and Ornidazole in pure and its pharmaceutical dosage forms. The method can be used to determine the purity of the drug available from various sources. As the method separates the drugs from its degradation products, it can be employed as stability-indicating.

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