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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF OFLAXACIN AND SATRINIDAZOLE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Ofloxacin and Satrinidazole in combination. The separation was carried out using a mobile phase consisting of phosphate buffer (0.05M) and Acetonitrile with pH 4.6 adjusted with orthophosphoric acid in the ratio of 70: 30%v/v. The column used was Symmetry C_{18} (4.6 x 150mm, 5µm, Make: Waters) or equivalent with flow rate of 0.8 ml / min using PDA detection at 301 nm. The retention times of Ofloxacin and Satrinidazole were found to be 2.0 and 4.7 min respectively. Results of analysis were validated statistically and by recovery studies. The developed method was validated as per ICH guidelines using the parameters such as accuracy, precision, linearity, LOD, LOQ, ruggedness and robustness. The developed and validated method was successfully used for the quantitative analysis of ofloxacin and Satranidazole in bulk and combined tablet dosage forms.

KEY WORDS

Ofloxacin, Satrinidazole, Satrogyl-O tablet dosage form and HPLC.

INTRODUCTION

Ofloxacin is a synthetic broad spectrum antibacterial agent. Chemically ofloxacin a fluorinated carboxy quinolone, Chemically it is found 7-fluoro- 2- methyl- 6- (4- methylpiperazin- 1- yl)- 10- oxo- 4- oxa- 1azatricyclo[7.3.1.0{5,13}]trideca5(13),6,8,11-tetraene-11-carboxylic acid. It is a broad spectrum antibacterial agent, belonging to the group of fluoroquinolones. Ofloxacin is active against awide variety of gram-positive and gram-negative organism, use in the treatment of urinary tract infection, conjunctivitis, gonorrhoea and respiratory tract infection.

Satranidazole is an anti-amoebic, antiprotozoal, and anti-bacterial medicine which belongs to the group of ritroimidazole drug Chemically it is found 1 - (1-Methyl- 5- nitroimidazol- 2- yl) - 3 - (methylsulfonyl)- 2-imidazolidinone. Satranidazole is a novel nitroimidazole possessing a C-N linkage at C2 of the imidazole ring has been examined, during reduction, for its ability to damage DNA. Physical damage to DNA was measured by viscometry, thermal denaturation and renaturation, and hydroxyapatite chromatography.

Literature survey shows that various methods have been reported for estimation of OFL and SAT individually and in combination with other drugs. Available techniques are high performance thin-layer chromatography HPTLC, HP liquid chromatography (LC), and colorimetric methods. OFL and SAT in combination with each other have been estimated by spectroscopy alone. The aim of this study was to develop a HPLC method for the combined dosage form OFL and SAT. Present work describes method development and validation of both drugs (i.e., OFL and SAT in combination according to ICH guidelines.

MATERIALS & METHODS

Materials: Ofloxacinand Satrinidazole were received gift samples from Alkem Laboratories, Ltd., were procured from local market (Mumbai, India). The pharmaceutical preparation of combination of ofloxacin and satrinidazole that is SATROGYL-O tablets contains 200mg of OFA and 300 mg of Satrinidazole. The solvents used was Methanol AR Grade, HPLC grade Acetonitrile (manufacturer Linchrosolv, Batch No: DBBDF63024) and water for HPLC. The analytical reagent grade potassium di hydrogen phosphate (Qualikems fine chemicals pvt.ltd, vadodara) and orthophosphoric acid was used to prepare the mobile phase which is filtered through a nylon 0.45µm membrane filter paper.

CHROMATOGRAPHIC CONDITIONS:

Method was developed using a Symmetry C_{18} (4.6 x 150mm, 5µm, Make: Waters) Mobile phase used was potassium dihydrogen phosphate buffer (0.05 M, pH 4.6adjusted with 0.5% orthophosphoric acid): acetonitrile (70:30 v/v) at flow rate is 0.8 ml/min. Samples were injected using Auto injector with 20µl loop.

PREPARATON OF STANDARD STOCK SOLUTION:

Accurately weighed and transferred 10 mg of Ofloxacin and 15mg of Satrinidazole working standard into a 10ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent finally the concentration found 30µg/ml of OFA and 45µg/ml of SAT.

PREPARATION OF SAMPLE SOLUTION:

Accurately weighed and transferred equivalent to 640 mg of Ofloxacin and Satrinidazole sample into a 100ml clean dry volumetric flask add about 70ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of Ofloxacin and Satrinidazole of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents finally the concentration found 30µg/ml of OFA and 45µg/ml of SAT.

METHOD VALIDATION: The developed RP-HPLC method was validated as per ICH guidelines.

ASSAY:

Twenty tablets were weighed and crushed to fine powder. The tablet powder equivalent to 10mg of OFA and 15 mg of SAT was transferred to a 100 ml volumetric flask and dissolved in mobile phase and the content was kept in ultra sonicator for 15 min. The flask was allowed to stand for 5 min at room temperature and the volume was adjusted up to the mark with mobile phase. The solution was filtered through a nylon 0.45 µm membrane filter paper. The solution was suitably diluted with mobile phase to get a final concentration of 30µg/ml and 45µg/ml of OFA and SAT respectively. The % assay of the drugs was calculated and the results are given in**Table-1**

ACCURACY:

The accuracy of the method was determined by calculating the recovery studies at three levels (50%, 100% and 150%) by standard addition method. Known amounts of standard OFA and SAT were added to the pre quantified samples and they were subjected to proposed HPLC method. The results of the recovery studies are given in **Table-3**

PRECISION:

Precision study was performed to find out intra-day and inter-day variations. In this process the combined solution (30µg/ml and 45µg/ml of OFA and SAT respectively) analyzed by same day (Intra-day precision) and different days (Inter-day precision). The %relative standard deviation (RSD) for intra-day precision was 0.6% of OFA and 0.2% of SAT and for inter-day precision was 0.2% of OFA and 0.1% of SAT respectively, which is less than 2% indicating high degree of precision.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ):

The LOD and LOQ for OFA and SAT were separately determined by based on calculating the signal-to-noise ratio (S/N is 3.3 for LOD and 10 for LOQ) and from the calibration curves the standard deviation of the y-intercepts and slope of the regression lines

were used. Results of LOD and LOQ are given in **Table-2**. The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response,
S = slope of the calibration curve.

LINEARITY:

An accurately weighed quantity of OFA 10mg and SAT 15mg (Working standard drugs) were transferred to into a separate 10ml clean and dry volumetric flasks and dissolved in mobile phase and finally each volumetric flask volume was adjusted up to the mark with mobile phase respectively. From this stock solution prepare 10, 20, 30, 40 and 50µg/ml of OFA and 15,30,45,60 and 75µg/ml of SAT concentrations respectively. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results were shown in **Table-4.**

ROBUSTNESS:

The robustness study was done by making small changes in the optimized method parameters like changing in flow rate by ± 1 ml/min and the organic composition in the mobile phase was varied from 40% to 60%. No significance change was observed.

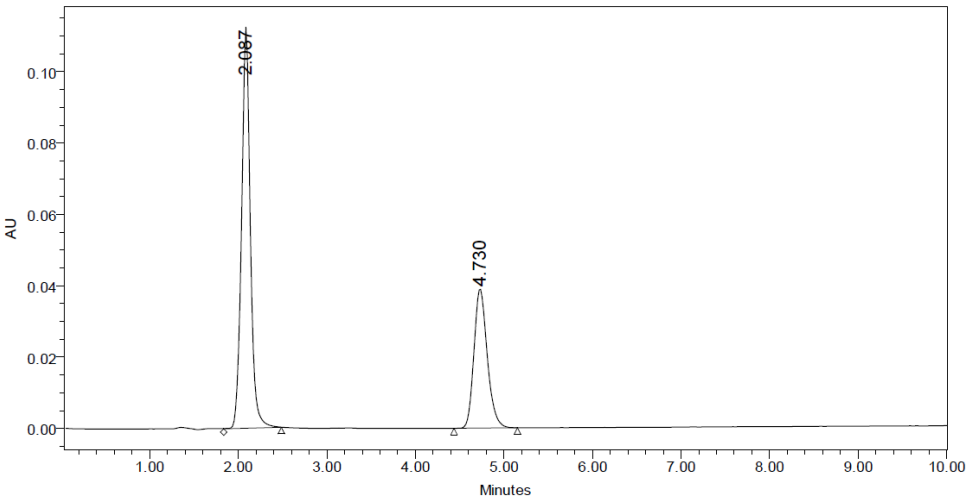


Fig 1: Chromatogram of OFA and SAT in Tablet Analysis

Marketed formulation	Drugs	Label claim (mg)	% Assay
Satrogyl-O Tab	OFA	200	99.6
	SAT	300	99.4

Table-1: Assay Parameters

Validation parameters	OFA	SAT
Theoretical plates	2801.1	5455.7
Resolution	-	11.3
Tailing factor	1.5	0.9
Intra-day precision (%RSD)	0.6	0.2
Inter-day precision (%RSD)	0.2	0.1
LOD (µg/ml)	2.98	2.94
LOQ (µg/ml)	9.96	10.0

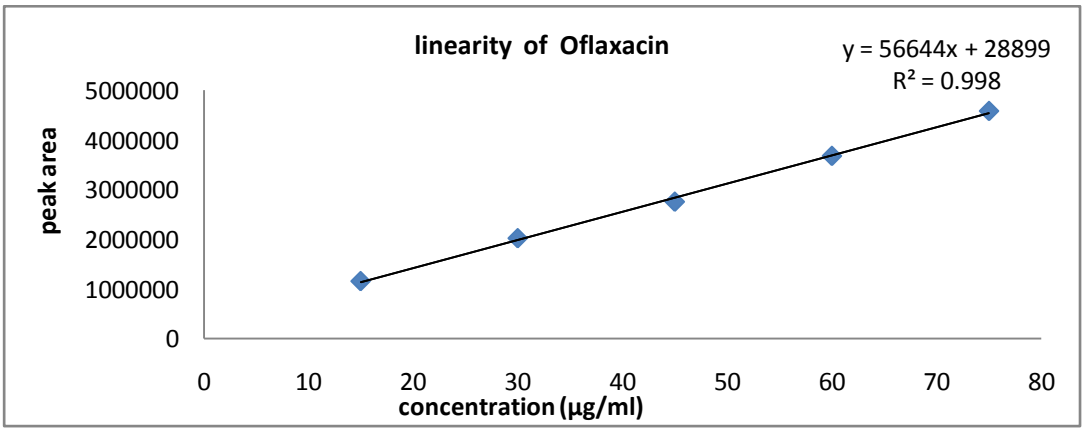
Table -2: Results from validation and system suitability studies

Drug	%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
	50%	1397368	5	5.0	100.9%	
OFA	100%	2759947	10	9.96	99.6%	100.3%
	150%	4170885	15	15.0	100.4%	
	50%	890834.7	7.0	7.0	100.7%	
SAT	100%	1886146	15	14.9	99.5%	100.4%
	150%	2874526	22.5	22.7	101.1%	

Table-3: Accuracy

S.NO	Concentration(µg/ml)		Peak area	
	OFA	SAT	OFA	SAT
1	10	15	1158130	681435
2	20	30	2021532	1289755
3	30	45	2755148	1889611
4	40	60	3675783	2523209
5	50	75	4579316	3073639
Correlation Coefficient			0.998	0.999

Table-4: Linearity



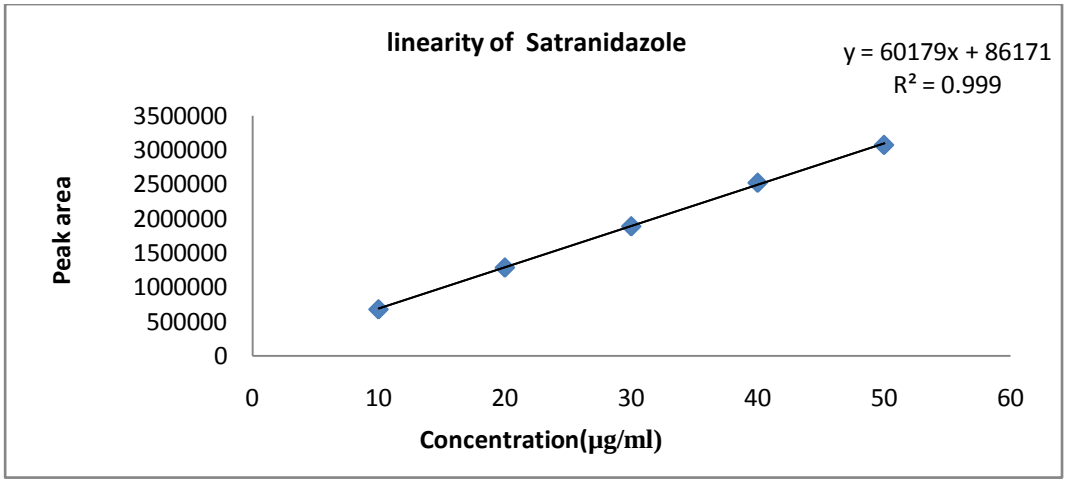


Fig-2: Linearity of OFA and SAT

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for OFA and SAT was obtained using Symmetry C18 (4.6 x 150mm, 5µm, Make: Waters) or equivalent with a mobile phase consisting of potassium dihydrogen phosphate buffer (0.05 M, pH 4.6 adjusted with 0.5% orthophosphoric acid): acetonitrile (70:30 v/v) at flow rate is 0.8 ml/min, PDA detection was performed at 301 nm. The retention times of ofloxacin and satrinidazole were found to be 2.07 min and 4.73 min respectively shown in (**Fig1**). The amount of OFA and SAT present in the sample solutions were determined respectively and the results obtained were comparable with the corresponding labelled claim (**Table 1**). The results of system suitability testing are given in Table 2. The %RSD of OFA and SAT for intra-day precision and inter-day precision was less than 2% it reveals that the proposed method is precise (**Table 2**). The sensitivity of method LOD and LOQ is shown in (**Table 2)**. The % recovery was found to be 98-102% within the limits for OFA and SAT (**Table 3**) which indicates high degree of accuracy of developed method. Linear correlation was obtained between concentration versus peak area of OFA and SAT in the concentration ranges of 10-50(µg/ml) and 15-75(µg/ml) respectively (**Table 4**). The correlation co-efficient (' *r2* ' value) for OFA and SAT was 0.998 and 0.999 respectively (**Fig 2**). The results of the robustness study also indicated that the method is robust and is unaffected by small variations in the chromatographic conditions.

CONCLUSION

The proposed study describes RP-HPLC method for the estimation of OFA and SAT in bulk drugs as well as in tablet formulation. The method was validated according to the ICH guidelines. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise, and selective and it can be employed successfully for the estimation of OFA and SAT in their bulk drugs and tablet formulation in routine analysis.

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