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VALIDATED HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF OFLOXACIN AND SATRANIDAZOLE IN A TABLET DOSAGE FORM

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ABSTRACT

A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of ofloxacin and satranidazole in combined dosage forms. The stationary phase used was Pre coated silica gel $60F_{254}$. The mobile phase used was a mixture of n-butanol: Methanol: Ammonia 7:3:3%v/v). The detection of spots was carried out at 315 nm. The method was validated in terms of linearity, accuracy, precision and specificity. The linear regression data showed a good linear relationship over a concentration range of 40 to 120 µg/spot for ofloxacin and 60 to 180 µg/spot for satranidazole. The limit of detection was found to be 4 ng and 18 ng for ofloxacin and satranidazole respectively and Limit of Quantification was found to be 8 ng and 60 ng for ofloxacin and satranidazole respectively. The proposed method can be successfully used to determine the drug content of marketed formulation.

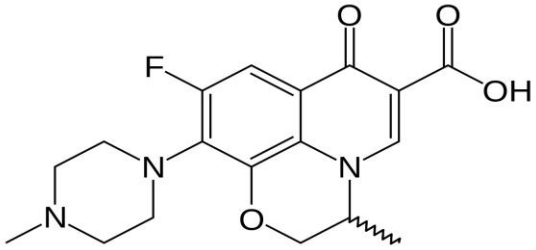
KEY WORDS: Ofloxacin, Satranidazole, HPTLC, Validation.

INTRODUCTION

Ofloxacin is a quinolone/fluoroquinolone antibiotic. Ofloxacin is bactericidal and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase, which allows the untwisting required to eplicate one DNA double helix into two. Soluble in aqueous solutions with pH between 2 and 5. It is sparingly to slightly soluble in aqueous solutions with pH 7 (solubility falls to 4 mg/mL) and freely soluble in aqueous solutions with pH above 9. Ofloxacin acts on DNA gyrase, an enzyme which, like human topoisomerase, prevents the excessive supercoiling of DNA during replication or transcription [1,2].

Satranidazole belongs to nitroimidazole group of drugs. It is highly potent, well tolerated and clinically useful against common protozoa, it is twice as active as other nitroimidazole against giardiasis and amoebiasis. It is also significantly more active against anaerobes than other nitroimidazoles. Soluble in 1:4 Dioxan, in Dimethyl formamide, Insoluble in water. Satranidazole, is a novel nitroimidazole possessing a C-N linkage at C_2 of the imidazole ring, during reduction, which damages DNA of the organism. Used to treat Intestinal and hepatic amoebiasis, giardiasis, trichomoniasis, anaerobic infections

Structure of Ofloxacin



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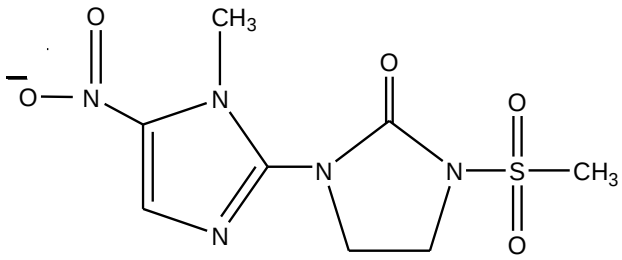
Structure of Satranidazole



MATERIALS AND METHODS

CAMAG Linomat V sample applicator, CAMAG TLC Scanner 3 with win CATS software, Pre coated silica gel 60F254 on aluminium sheets, n-butanol, Methanol, Ammonia, UV Spectrophotometer and ultrasonicator were used during study.

Validation [3-5]

The validation of the developed method was carried out in terms of linearity, accuracy, limit of detection (LOD), limit of Quantification(LOQ), inter and intra day precision, repeatability of sample application and stability studies.

Preparation of Standard solution

An accurately weighed quantity of 10 mg of Ofloxacin and 15 mg of Satranidazole RS was transferred into 50 ml volumetric flask, dissolved completely using chloroform and further made it to volume with same solvent to get the stock solution. From this suitable dilutions are made to obtain a final concentration of 20µg/ml of Ofloxacin and 30µg/ml of Satranidazole.

Preparation of Sample solution

Twenty tablets, each containing 200mg of Ofloxacin and 300 mg of Satranidazole average weight was calculated. Quantity equivalent to 20 mg of Ofloxacin was transferred to a 100 ml volumetric flask. The drugs were extracted with chloroform and filtered through Watman filter paper. Finally the volume was made up to 100 ml with chloroform. From the stock solution 1ml has taken in to 10ml volumetric flask volume was made up with the same solvent.

Ideal Experimental Conditions

Stationary Phase: Pre coated silica gel $60F_{254}$ on aluminium sheets

Mobile phase: n-butanol: Methanol: Ammonia (7:3:3%v/v)

Chamber saturation	: 30 minutes
Migration distance	: 85 mm
Band width	: 6 mm
Slit dimension	: 5 X 0.45mm
Source of radiation	: Deuterium lamp
Wave length scanning	: 315 nm

R_f values

Ofloxacin	: 0.59 ± 0.02
Satranidazole	: 0.78 ± 0.02

Linearity Range

Aliquots of 2, 3, 4, 5 and 6.0 µl of standard solution containing ofloxacin (20 µg/ml) and satranidazole (30 µg/ml) were applied on the plate. The linear regression data showed a good linear relationship over a concentration range of 40 to 120 µg/spot for ofloxacin and 60 to 180 µg/spot for satranidazole (Table 1). The slope, intercept and correlation co-efficient values(r) for ofloxacin were found to be 39.474, 4.085 and 0.9998 respectively (Fig. 1). The slope, intercept and correlation coefficient values for satranidazole were found to be 12.337, 21.014 and 0.9977 respectively (Fig. 2).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of the drugs were determined by applying decreasing amounts of the drugs on the plate. The lowest concentration at which the peak is detected is called the 'limit of detection' which was found to be 4 ng and 18 ng for ofloxacin and satranidazole respectively (Fig. 3 and Fig. 4). The lowest concentration at which the peak is quantified is called 'Limit of Quantification' which was found to be 8 ng and 60 ng for ofloxacin and satranidazole respectively (Fig. 5 and Fig. 6).

Accuracy

Recovery studies of the drugs were carried out for determining accuracy parameter. It was done by mixing known quantity of standard drugs with the preanalysed sample formulation and the contents were reanalyzed by the proposed method. This was carried out at 80, 100 and 120% levels. The percentage recovery and its % RSD were calculated (Table 2).

Precision

Precision of the method was demonstrated by

i) Intra day precision
ii) Inter day precision
iii) Repeatability
a. *Repeatability of sample application*
b. *Repeatability of measurement*

i) Intra day precision

Intra day precision was found out by carrying out the analysis of the standard drugs at any one concentration in the linearity range of drugs for six times on the same day. Concentration was applied in duplicate and % RSD was calculated (Table 3).

ii) Inter day precision

Inter day precision was found out by carrying out the analysis of the standard drugs at three different concentrations in the linearity range of drugs for three days and % RSD was calculated (Table 4).

iii) Repeatability [6-9]

a. Repeatability of sample application

Repeatability of sample application was assessed by spotting 3.2 µl of drug solution six times on pre coated TLC plate followed by development of plate and %RSD was calculated (Table 5).

b. Repeatability of measurement

Repeatability of measurement of peak area was determined by spotting 1 µl of standard drug solutions on pre coated TLC plate. After development of the plate the separated spots were scanned six times without changing position of the plate and %RSD was calculated (Table 6).

Estimation

The sample solution was spotted on the TLC plate with the help of Linomat V spotting system.The chromatographic plate was developed in a twin trough chamber containing the Mobile Phase. The Chromatograms were recorded and Rf values were determined for ofloxacin and satranidazole. The amount of drug present was calculated by comparing the peak area values of standard with that of sample as follows. The results were as shown in Table 8 and fig. 7.

$$\text{Amount of drugs in a Tablet} = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \frac{\text{standard dilution factor}}{\text{sample dilution factor}} \times \text{Avg wt}$$

RESULTS AND DISCUSSION

The proposed method shows that the chromatographic layer gives best separation of the two components in the mobile phase consisting of n-butanol: Methanol: Ammonia (7: 3: 3) and Rf values was found to be 0.59 ± 0.02 and 0.78 ± 0.02 for Ofloxacin and Satranidazole respectively.

The linearity was evaluated by plotting peak area as a function of analyte concentration for Ofloxacin and Satranidazole. The graphical representation is given in Figure 1 & 2. From the linearity studies the specified range determined was 40-120ng/spot, 60-180ng/spot for Ofloxacin and Satranidazole respectively. The linearity data are presented in Table 1.

The precision for the Ofloxacin and Satranidazole were evaluated by using homogenous sample in six times determination (100% of target). The data are presented in Table 3 to Table 6. Overall assay percentage for Ofloxacin is 99.65% and 95% confidence interval of ± 0.44 and for Satranidazole are 99.27 and 95% confidence interval of ± 0.87.

The accuracy of Ofloxacin and Satranidazole was determined by fortifying sample and standard drug substances at concentration from 80 to 120% of target level. The data are presented in Table 2. Overall recovery for Ofloxacin is 99.64 and 95% confidence interval of ± 0.22 and for Satranidazole is 99.72 and 95% confidence interval of ± 0.42.

The amount of drug present was calculated by comparing the peak area values of standard with that of sample was found to be for ofloxacin 199.31 mg and for Satranidazole 298.08 mg respectively.

All the above parameters are precise and ensure the use of proposed method in the assay of pharmaceutical dosage form containing this combination.

Table 1. Linearity

S.No.	Ofloxacin		Satranidazole	
	Conc. (ng/spot)	Peak Area	Conc. (ng/spot)	Peak Area
1	40	1586.5	60	731.8
2	60	2387.3	90	1087.0
3	80	3181.8	120	1406.2
4	100	3978.7	150	1766.6
5	120	4770.1	180	2214.6

Table 2. Accuracy

Level	% of Recovery		% RSD*	
	Ofloxacin	Satranidazole	Ofloxacin	Satranidazole
80%	99.93	100.26	0.252	0.474
100%	99.58	99.59	0.126	0.281
120%	99.41	99.31	0.196	0.389

CI for Ofloxacin = ±0.22 and for Satronidazole = ±0.42

*average of three observations, CI= Confidence interval.

Table 3. Intra day precision (first day)

Volume applied (µl)	Concentration (µg/ml)		Area		% RSD	
	Ofloxacin	Satranidazole	Ofloxacin	Satranidazole	Ofloxacin	Satranidazole
4	20	30	3178.4	1398.4	0.418	0.597
			3150.8	1385.6		
			3167.1	1400.1		
			3172.4	1396.0		
			3144.5	1410.9		
			3169.9	1403.2		

Table 4. Inter day precision (second day)

Volume applied (µl)	Concentration (µg/ml)		Area		% RSD	
	Ofloxacin	Satranidazole	Ofloxacin	Satranidazole	Ofloxacin	Satranidazole
4	20	30	3171.6	1382.0	0.256	0.414
			3184.5	1390.3		
			3166.2	1379.5		
			3175.4	1386.5		
			3162.5	1375.1		
			3179.0	1388.0		

Table 5. Repeatability of Sample application

Volume applied (µl)	Concentration (µg/ml)		Area		% RSD	
	Ofloxacin	Satranidazole	Ofloxacin	Satranidazole	Ofloxacin	Satranidazole
4 µl	20	30	3164.0	1397.4	0.419	0.832
			3157.8	1381.0		
			3168.3	1405.1		
			3151.3	1386.9		
			3144.4	1372.6		
			3132.5	1390.1		

Table 6. Repeatability of Measurement

Volume applied (µl)	Concentration (µg/ml)		Area		% RSD	
	Ofloxacin	Satranidazole	Ofloxacin	Satranidazole	Ofloxacin	Satranidazole
2µl	20	30	1987.5	702.5	0.403	0.834
			1975.0	697.8		
			1980.1	694.1		
			1969.3	705.4		
			1965.6	689.1		
			1971.1	698.3		

Table 7. Validation of the Method

Parameters	Observation	
	Ofloxacin	Satranidazole
LOD	4 ng	18 ng
LOQ	8 ng	60 ng
Linearity **r value**	40–120 ng 0.9998	60-180 ng 0.997
Precision **Intra day (% RSD)** **Inter day (% RSD)**	 0.418 0.256	 0.597 0.414

Repeatability		
Sample application	0.419	0.832
Measurement	0.403	0.834
Recovery		
80 % level	0.252	0.474
100 % level	0.126	0.281
120 % level	0.196	0.389

Table 8. Analysis of formulation

Drug*	Amount(mg / Tablet)		% Label claim	% RSD**	CI
	Labeled amount	Estimated			
Ofloxacin	200	199.31	99.65	0.421	0.44
Satranidazole	300	298.08	99.27	0.831	0.87

**** Average of six observations**

Fig. 1. Linearity graph of Ofloxacin

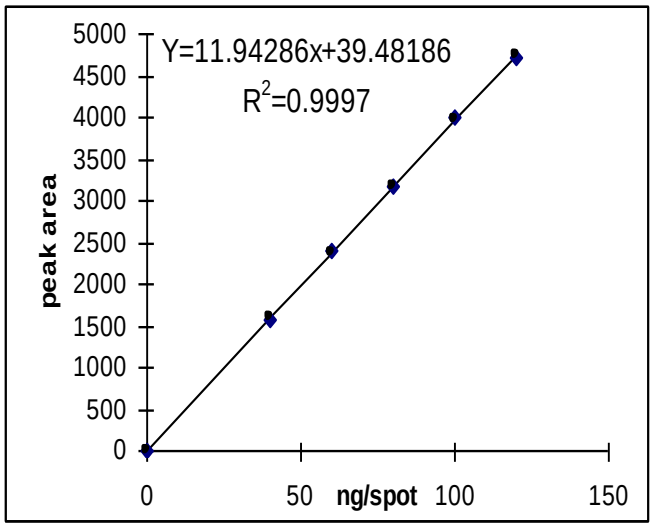


Fig. 2. Linearity graph of Satranidazole

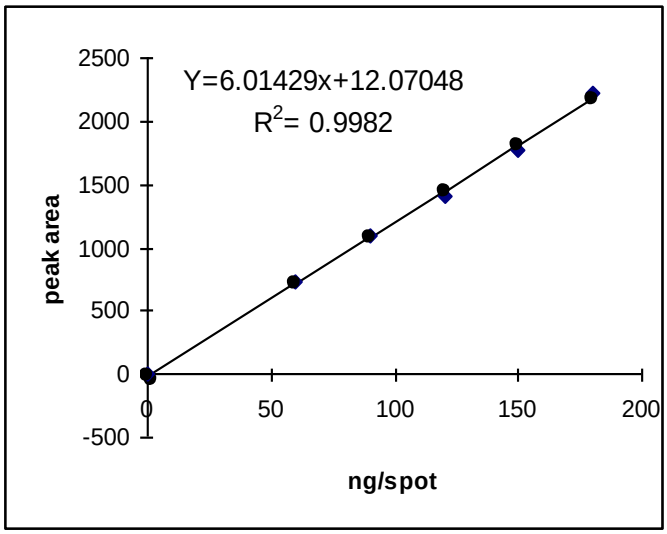


Fig. 3. LOD for Ofloxacin

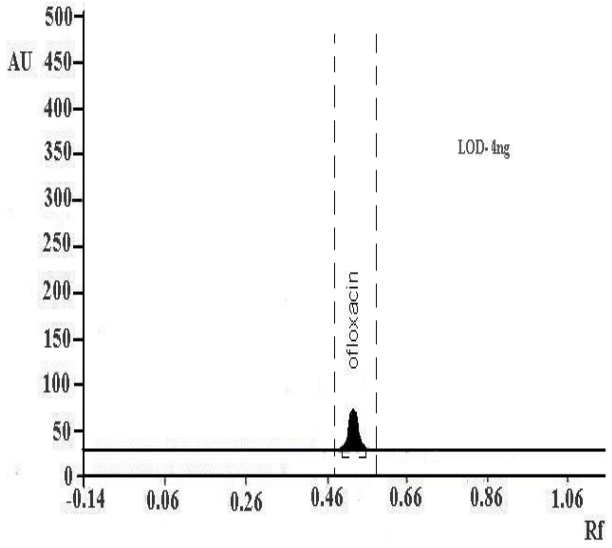


Fig. 4. LOD for Satranidazole

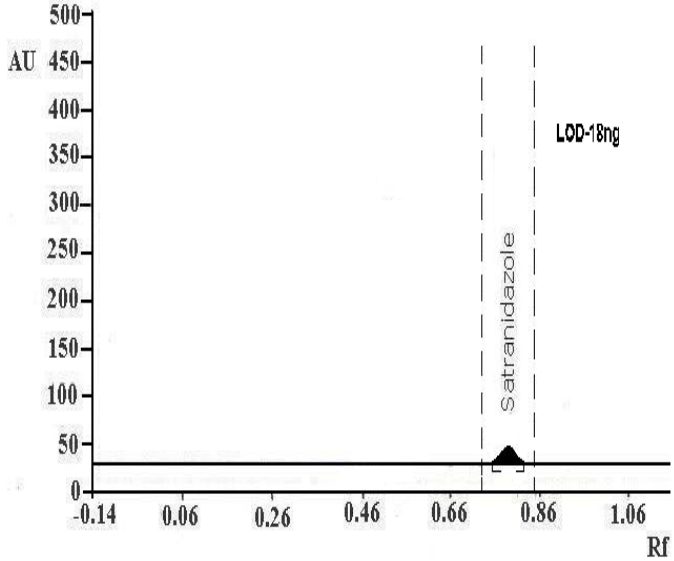


Fig. 5. LOQ for Ofloxacin

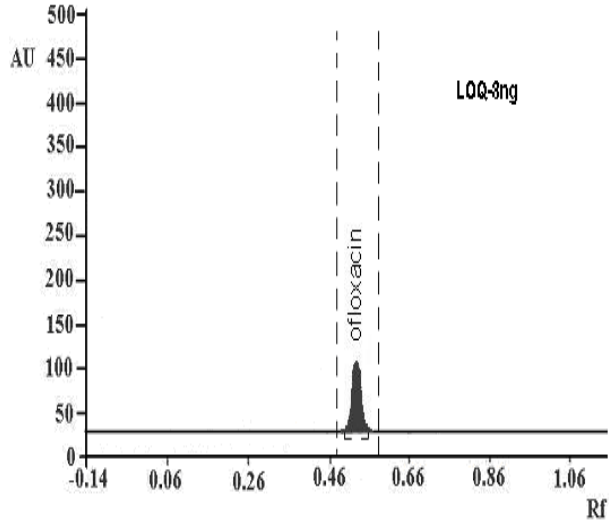


Fig. 6. LOQ for Satranidazole

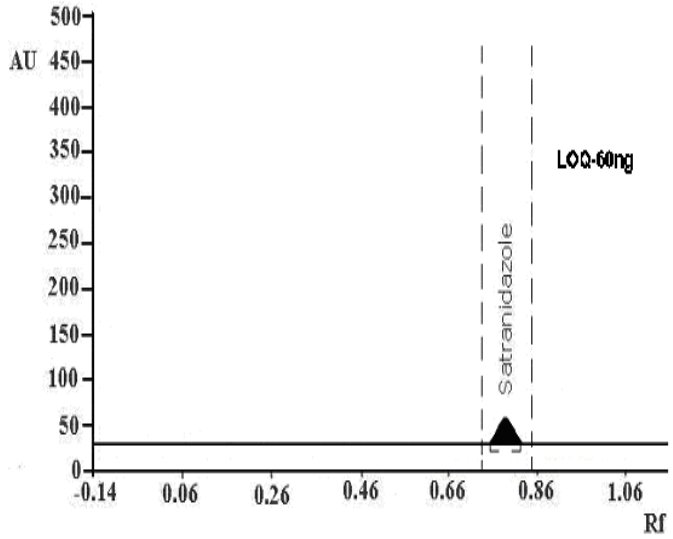
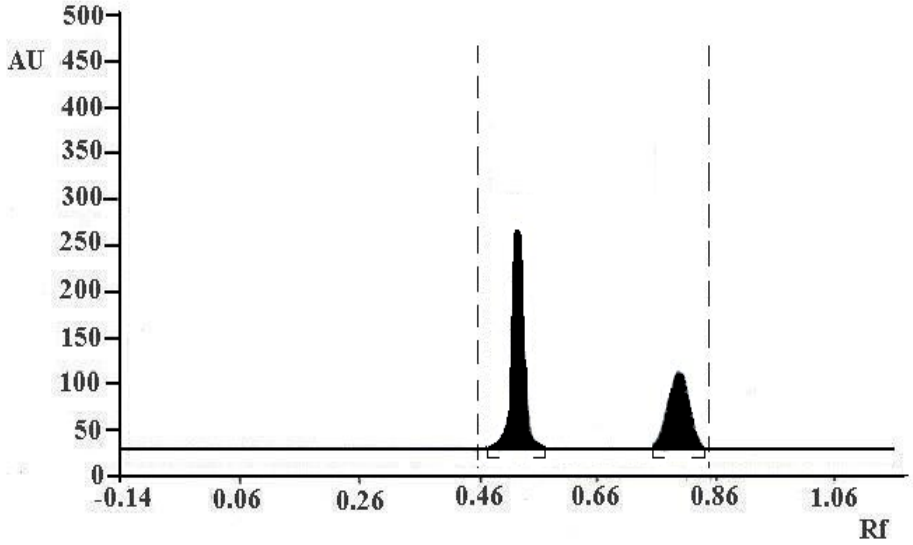


Fig. 7. Densitogram of sample formulation



CONCLUSION

The proposed HPTLC method has been evaluated over the linearity, precision, accuracy,specificity and proved to be convenient and effective for the quality control of Ofloxacin and Satranidazole in a tablet dosage form. The measured signal was shown to be precise, accurate, and linear over the concentration range tested with a correlation coefficient of 0.999 for both drugs and shows an acceptable limit of RSD. Thus the proposed method is rapid, selective, requires a simple sample preparation procedure, Moreover, the lower solvent consumption leads to a cost effective.

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