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## Spectrophotometric and RP-HPLC methods for simultaneous estimation of ofloxacin and satranidazole from tablets

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### ABSTRACT

Two simple, accurate and sensitive validated UV spectrophotometric methods and one HPLC method for simultaneous estimation of ofloxacin and satranidazole in combined tablet dosage form has been developed. The first developed spectroscopic method employs "area under curve" using 292-296nm and 316-320nm for estimation of ofloxacin and satranidazole, respectively. The second spectrophotometric method employed was "Dual wavelength method" using 300.2, 338.8nm for ofloxacin and 276.2, 307.6nm for satranidazole. Beer's law is obeyed in the concentration range of 2.0 to 20.0 $\mu\text{g/mL}^{-1}$  and 3.0 to 30 $\mu\text{g/mL}^{-1}$  of ofloxacin and satranidazole, respectively. Developed HPLC method is reverse-phase chromatographic method using Jasco HPLC system with HiQ Sil C18W column (250 $\times$ 4.6mm i.d.), using acetonitrile: 0.005mol. L<sup>-1</sup> tetra butyl ammonium hydrogen sulphate 70:30 v/v as a mobile phase. The detection was carried out using UV detector set at 320nm. Clonazepam was used as internal standard. Beer's law is obeyed in the concentration range of 2.0 to 30.0 $\mu\text{g/mL}^{-1}$  and 3.0 to 45 $\mu\text{g/mL}^{-1}$  of ofloxacin and satranidazole, respectively. All the three methods have been successfully applied for the analysis of drugs in pharmaceutical formulations. Results of analysis were validated statistically and by recovery studies. © 2008 Trade Science Inc. - INDIA

### KEYWORDS

Ofloxacin;  
Satranidazole;  
Clonazepam;  
Area under curve;  
Dual wavelength method;  
HPLC.

### INTRODUCTION

Chemically ofloxacin is ( $\pm$ )-9- fluoro-2, 3-dihydro-3- methyl-10-(4- methyl-1-piperazinyl)-7-oxo-7H-pyrido(1,2,3-dec)-1,4-benzoxazine-6-carboxylic acid. It is a fluoroquinolone and used mainly as antibacterial for the treatment of urinary tract infection and sexually transmitted diseases.

It is official in U.S.P<sup>[1]</sup>. The method of analysis given in U.S.P is titrimetric analysis. Literature survey reveals that ofloxacin is estimated by spectrophotometry<sup>[2]</sup>, RP-HPLC<sup>[3-9]</sup>and spectrofluorometry<sup>[10,11]</sup>.

Chemically satranidazole is 1-methyl sulphonyl-3(1-methyl-5-nitro-2-imidazolyl)-2 imidazolidinone, which is a 5-nitroimidazole derivative. It is broad-spectrum antiprotozoal, antimicrobial, and antifungal used for the treatment of severe hepatic and intestinal amoebiasis. It is not official in I.P., U.S.P. and B.P. till date. A literature survey reveals that satranidazole is estimated by gas chromatography with electron capture detector<sup>[12]</sup>, spectrophotometry<sup>[13]</sup>.

This paper describes two simple, accurate and sensitive validated UV spectrophotometric methods and HPLC method for simultaneous estimation of ofloxacin

and satranidazole in combined tablet dosage form. The proposed methods are optimized and validated for linearity, accuracy, precision, limit of detection and limit of quantitation as per the 'International Conference on Harmonization' (ICH) guidelines.

## MATERIALS AND METHODS

### Equipment

UV-Vis double beam spectrophotometer of make Jasco, model V-530 with 1cm matched quartz cells was used for spectrophotometric method. For HPLC method, Jasco HPLC system, consisting of Jasco PU-2080 plus HPLC pump and Jasco UV-2075 plus UV/VIS detector was used for analysis. HiQ Sil C18W (250×4.6 mm i.d) column was used for analysis with a flow rate of 1ml/min. A Rheodyne injector with 20 $\mu$ l loop was used for injecting the sample. Shimadzu balance, AY-120 was used for weighing purpose in both the methods.

### Chemicals and reagents

Acetonitrile (HPLC grade) was purchased from Merck specialties pvt. Ltd. (Worli, Mumbai, India) and Water (HPLC grade) was purchased from Loba Chemie (Mumbai, India). Tetra butyl ammonium hydrogen sulphate (TBAHS) was purchased from Sisco research Laboratories Pvt. Ltd. (Mumbai, India). All other reagents used were of analytical grade for spectrophotometric method and of HPLC grade for HPLC method. Working standard of ofloxacin (percent purity=99.5%) and satranidazole (percent purity=99.225%) were provided by Alkem Ltd., Mumbai, India and clonazepam was obtained from Torrent pharmaceuticals Ltd., Indrad, Gujarat, India.

### Pharmaceutical formulation

Commercial tablets, each containing ofloxacin (200mg) and satranidazole(300mg); (Satragyl-O) were procured from the local market.

### Procedure

#### Method 1: Area under curve method<sup>[14]</sup>

Ofloxacin (OFLOX) and satranidazole (SATRA) were dissolved separately in 0.1N HCL so as to get 1000 $\mu$ g mL<sup>-1</sup> concentration of each drug. These solu-

tions were further diluted in distilled water and both solutions were scanned in the wavelength range of 400-200nm.

The  $\lambda_{\max}$  values for ofloxacin and satranidazole were 294nm & 318nm respectively. The spectrum is given in figure 1.

Wavelengths selected for calculation of 'Area Under Curve' were 292-296nm and 316-320nm, for OFLOX and SATRA since they show significant absorption at these wavelengths respectively. The Beer's law is obeyed by ofloxacin over the concentration range 2 to 20 $\mu$ g mL<sup>-1</sup> at 294nm and by satranidazole over the concentration range 3 to 30 $\mu$ g mL<sup>-1</sup> at 318nm.

The estimation of OFLOX and SATRA has been done at 292-296nm and 316-320nm respectively using its area under the curve values, which show linearity over the concentration range 2 to 20 $\mu$ g mL<sup>-1</sup> and 3 to 30  $\mu$ g mL<sup>-1</sup> for OFLOX and SATRA respectively. Regression analysis results for this method are mentioned in TABLE 1.

### Procedure for analysis of tablet formulation

Twenty tablets were weighed accurately; the average weight was determined and then ground to a fine powder. A quantity equivalent to 50mg of OFLOX was transferred to 50ml volumetric flask. 20ml of 0.1N HCL was added to the same flask and shaken vigorously for 5 minutes and sonicated for 5 min. The volume was made up to the mark with 0.1N HCL. The solution was centrifuged for 5 minutes at 3000rpm. Supernatant solution was pipetted out and diluted with distilled water to obtain the dilution within the Beer's law range.

The area values at 292-296nm and 316-320nm for both the drugs were determined over a concentration range 2-20 $\mu$ g mL<sup>-1</sup> for working standard of ofloxacin

**TABLE 1: Regression analysis of calibration curves for method 1**

Parameter	Ofloxacin	Satranidazole
Wavelength range (nm)	292-296	316-320
Beer's law limit( $\mu$ g mL <sup>-1</sup> )	2-20	3-30
R*	0.9993	0.9997
Linear regression equation <sup>a</sup>	y = 0.357x - 0.082	y = 0.106x + 0.0329
S.D. <sup>b</sup>	0.04	0.04041
Limit of detection( $\mu$ g mL <sup>-1</sup> )	0.3697	1.2580
Limit of quantitation( $\mu$ g mL <sup>-1</sup> )	1.120	3.8122

\*Correlation coefficient; <sup>a</sup>With respect to y=mx+c, where y is the absorbance and x is the concentration ( $\mu$ g mL<sup>-1</sup>); <sup>b</sup>Standard deviation.

TABLE 2: Linearity for dual wavelength method

Concentration of ofloxacin $\mu\text{g mL}^{-1}$	Absorbance at		Absorbance difference	Concentration of satranidazole $\mu\text{g mL}^{-1}$	Absorbance at		Absorbance difference
	300.2nm	338.8nm			276.2nm	307.6nm	
2	0.14850	0.05726	0.09124	3	0.02765	0.07824	0.05059
4	0.28290	0.11215	0.17076	6	0.04906	0.14838	0.09932
6	0.41068	0.16328	0.24740	9	0.07310	0.21411	0.14101
8	0.57393	0.22933	0.34460	12	0.10364	0.29563	0.19199
10	0.69877	0.27589	0.42288	15	0.12657	0.36716	0.24059
12	0.87156	0.34315	0.52841	18	0.15840	0.44897	0.29019
14	0.94822	0.37364	0.64250	21	0.18114	0.52457	0.34343
16	1.16774	0.45782	0.71900	24	0.20790	0.59555	0.38765
18	1.33556	0.52401	0.81155	27	0.23539	0.66964	0.43425
20	1.43608	0.56276	0.87332	30	0.25362	0.73296	0.47934

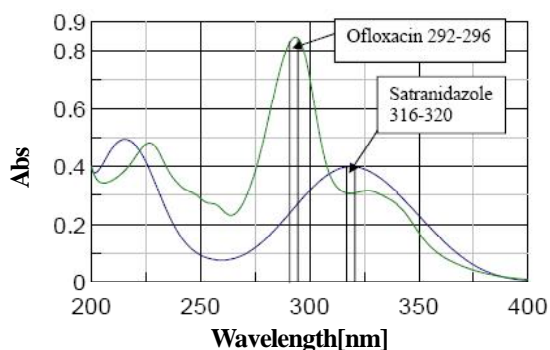


Figure 1: Overlain spectra of OFLOX and SATRA indicating area under curve

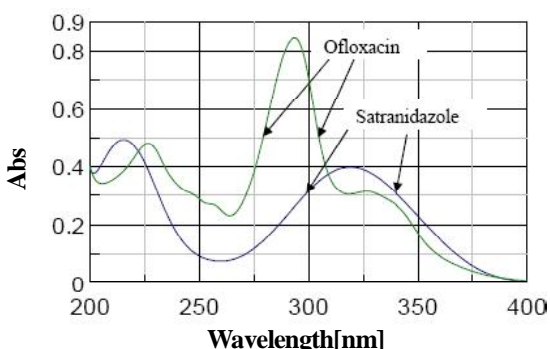


Figure 2: Dual wavelength indicated on spectrum of OFLOX and SATRA

and over a concentration range 3-30  $\mu\text{g mL}^{-1}$  for working standard of aatranidazole.

The areas were measured at 292-296nm and 316-320nm for the sample solution. The concentration was calculated using the following formula

$$C_{\text{OFLOX}} = \frac{X^S_{292-296} * \text{AUC}_{316-320} - X^S_{316-320} * \text{AUC}_{292-296}}{X^O_{316-320} * X^S_{292-296} - X^O_{292-296} * X^S_{316-320}}$$

$$C_{\text{SATRA}} = \frac{X^O_{316-320} * \text{AUC}_{292-296} - X^O_{292-296} * \text{AUC}_{316-320}}{X^O_{316-320} * X^S_{292-296} - X^O_{292-296} * X^S_{316-320}}$$

Where,  $X^O$  &  $X^S$  are constants which calculated using following formula

$$X = \frac{\text{AUC of component between selected wavelength range}}{\text{Concentration of that component in gm/lit}}$$

### Method 2: Dual wavelength method<sup>[15]</sup>

Ofloxacin and satranidazole were dissolved separately in 0.1N HCL so as to get 1000  $\mu\text{g mL}^{-1}$  concentration of each drug. These solutions were further diluted in distilled water and were scanned in the wavelength range of 400-200nm.

For estimation of a drug eg. A, two such wavelengths were selected where the absorbance of other drug eg. B (interfering compound) is similar, so that the absorbance difference at these wavelengths were directly proportional to the concentration of drug A. Wavelengths selected for estimation of OFLOX were 300.2nm, 338.8nm and for SATRA 276.2nm and 307.6nm. The spectrum is given in figure 2. The calibration curves for OFLOX and SATRA were plotted by considering the difference in absorbance values at the selected wavelengths against concentration. The Beer's law is obeyed by OFLOX for the difference in absorbance at 300.2 nm and 338.8 nm over the concentration range 2 to 20  $\mu\text{g mL}^{-1}$  and by SATRA over the concentration range 3 to 30  $\mu\text{g mL}^{-1}$  for the difference in absorbance at 276.2 nm, 307.6 nm. Linearity data for this method is mentioned in TABLE 2.

### Procedure for analysis of tablet formulation

Twenty tablets were weighed accurately; the average weight was determined and then ground to a fine powder. A quantity equivalent to 50mg of OFLOX was transferred to 50ml volumetric flask, 20ml of 0.1N HCL was added to the same flask and shaken vigorously for

5 minutes. The solution was sonicated for five minutes. The volume was made up to the mark with 0.1N HCl. The solution was centrifuged for 5 minutes at 3000rpm. Supernatant solution was pipetted out and diluted with distilled water to obtain the dilution within the Beer's law range.

The absorbance values were determined for sample solution at 276.2nm, 300.2nm, 307.6nm and 338.8nm. Absorbance values were measured at 300.2, 338.8nm over a concentration range 2-20 $\mu\text{g mL}^{-1}$  for working standards of ofloxacin. Absorbance values were measured at 276.2, 307.6nm over a concentration range 3-30 $\mu\text{g mL}^{-1}$  for working standards of satranidazole. The concentration of drug in sample solution was calculated using regression equation for the difference in absorbance at two selected wavelengths vs. concentration.

The concentration was calculated using the following regression equations

For OFLOX:  $y=0.0451x - 0.0109$

For SATRA:  $y=0.016x + 0.0012$

### Method 3: High-performance liquid chromatographic method

For HPLC method, Jasco HPLC system, consisting of Jasco PU-2080 plus HPLC pump and Jasco UV-2075 plus UV/VIS detector was used for analysis. HiQ Sil C18 W(250 $\times$ 4.6mm i.d) column was used in analysis. A Rheodyne injector with 20 $\mu\text{L}$  loop was

used for injecting the sample. Detection of eluent was carried out using UV detector set at 320nm. Mobile phase selected for this method consists of acetonitrile: 0.005mol. L<sup>-1</sup> tetra butyl ammonium hydrogen sulphate 70:30v/v(filtered through 0.45 micron membrane filter) at a flow rate of 1 ml/min. Method was developed using Clonazepam as internal standard. HPLC details are presented in TABLE 3. System suitability parameters for the developed method are given in TABLE 4.

### Standard stock solution

Standard stock solutions of working standards were made separately in acetonitrile to obtain concentration of 200 $\mu\text{g mL}^{-1}$  of ofloxacin, 300 $\mu\text{g mL}^{-1}$  of satranidazole and 500 $\mu\text{g mL}^{-1}$  of clonazepam and filtered through a 0.2 micron membrane filter.

### Preparation of calibration curve

To prepare the drug solutions for the calibration curve, in a series of 10ml volumetric flasks, appropriate dilutions were made from standard stock solutions to obtain final concentrations in the range of 2 to 30 $\mu\text{g mL}^{-1}$  of ofloxacin and 3 to 45 $\mu\text{g mL}^{-1}$  satranidazole. In each flask, 0.1ml of stock solution of standard clonazepam was added and the volume was made up to the mark with the mobile phase. Each solution was injected and a chromatogram was recorded. Mean retention time of ofloxacin, satranidazole and clonazepam were found to be 2.057min, 3.067min and 3.758min respectively.

The peak area ratios of ofloxacin to clonazepam and satranidazole to clonazepam were calculated. calibration curves were obtained by plotting concentration of drug vs. peak area ratio of drug to internal standard.

### Procedure for analysis of tablet formulation

Twenty tablets were weighed accurately; the average weight was determined and then ground to a fine powder. Powder equivalent to 50mg of ofloxacin was weighed and transferred to a 50ml volumetric flask containing about 35ml of acetonitrile. The solution was sonicated. The solution was centrifuged for 5 minutes at 3000rpm. Supernatant solution was pipetted out and diluted with mobile phase to obtain the dilution within the Beer's law range.

From this solution 0.15ml was transferred into 10ml volumetric flask, 0.1ml of clonazepam solution was

TABLE 3: HPLC operating conditions

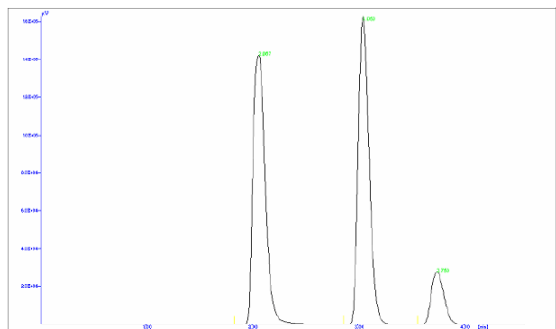
Mobile phase*	Acetonitrile : 0.005mol.L <sup>-1</sup> tetrabutyl ammonium hydrogen sulphate (70 :30v/v)
Flow rate	1.0mL/min.
Column temperature	Ambient
Injection volume	20 $\mu\text{L}$
Detection wavelength	320 nm
Mean retention time(min)	Ofloxacin 2.057
	Satranidazole 3.067
	Clonazepam 3.717

\*Filtered through  $\alpha$  0.45 $\mu$  membrane filter, degassed and sonicated

TABLE 4: System suitability parameters for method 3

Parameter	Ofloxacin	Satranidazole
Theoretical plates	2081.63	3886.08
Resolution	2.81	5.33
Asymmetry factor	1.42	1.33
Limit of detection ( $\mu\text{g mL}^{-1}$ )	0.3008	0.6045
Limit of quantitation ( $\mu\text{g mL}^{-1}$ )	0.9116	1.8318
% RSD	0.7994	0.74005

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**Figure 3:** Chromatogram of ofloxacin (2.057min), satranidazole (3.067min), clonazepam (3.758min)

added in the same flask and the volume was made up to the mark with the mobile phase. The solution was injected (20 $\mu$ L) and a chromatogram was recorded. The injections were repeated five times and peak areas were recorded. A representative chromatogram is given in figure 3. The peak area ratios of each of the drugs to the internal standard were calculated and the amount of each drug present per tablet was estimated from the respective calibration curves.

### Regression equations are as follows:

For OFLOX:  $y = 0.1754x + 0.2134$

For SATRA:  $y = 0.1212x + 0.1147$

### Recovery studies

To study the accuracy of the above methods, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample at 80%, 100% and 120% levels. The results are shown in TABLES 5, 6, and 7.

## RESULTS AND DISCUSSION

The proposed methods for simultaneous estimation of OFLOX and SATRA in combined tablet dosage form were found to be simple, accurate, rapid and economical. The values of percent RSD were not more than 1.5 and recovery was between 98.5 to 100.7%, indicating reproducibility and accuracy of methods.

The first method involving area under curve calculations requires both the drugs of combination to have reproducible area at both the  $\lambda_{max}$  values selected. This requirement was fulfilled by OFLOX and SATRA, thus concentration of both the components could be easily calculated. Good accuracy was indicated by recovery

**TABLE 5:** Recovery studies of oflox and satra by method 1

Level of recovery %	% Mean recovery*		Standard deviation		% R.S.D. <sup>a</sup>	
	OFLOX	SATRA	OFLOX	SATRA	OFLOX	SATRA
80	100.19	100.7	0.0047	0.001	0.02611	0.0037
100	100.03	99.58	0.0066	0.0117	0.033	0.039
120	100.6	100.56	0.006	0.0496	0.02727	0.1503

\*Avg. of three determinations; <sup>a</sup>Is the relative standard deviation

**TABLE 6:** Recovery studies of oflox and satra by method 2

Level of recovery %	% Mean recovery*		Standard deviation		% R.S.D. <sup>a</sup>	
	OFLOX	SATRA	OFLOX	SATRA	OFLOX	SATRA
80	99.10	99.7	0.0014	0.002	0.00778	0.00741
100	98.50	100.5	0.0006	0.0034	0.003	0.01133
120	99.5	98.19	0.0014	0.0032	0.00636	0.0097s

\*Avg. of three determinations, <sup>a</sup>Is the relative standard deviation

**TABLE 7:** Recovery studies of oflox and satra by method 3

Level of recovery %	% Mean recovery*		Standard deviation		% R.S.D. <sup>a</sup>	
	OFLOX	SATRA	OFLOX	SATRA	OFLOX	SATRA
80	99.13	99.65	0.2122	0.2915	1.1788	1.079
100	99.13	99.67	0.2706	0.7758	1.353	0.9193
120	98.91	100.47	0.2250	0.7561	1.022	0.77606

Avg. of three determinations, <sup>a</sup>Is the relative standard deviation values of 99.58 to 100.56%. Reproducibility is reflected in %RSD not more than 1.04.

The second method involved selection of two wavelengths for each drug such that the absorptivity value at these two wavelengths is the same. Then, the difference in the absorbance readings at these wavelengths is proportional to the concentration of the other drug. For OFLOX, the linearity of conc. versus difference in absorbance at 300.2nm and 338.8nm, was indicated by value of regression coefficient,  $r^2=0.997$ . For SATRA, the linearity of conc. versus difference in absorbance at 276.2nm and 307.6nm, was indicated by value of regression coefficient,  $r^2=0.999$ . Good accuracy was indicated by recovery values of 99.58 to 100.19%. Reproducibility is reflected in %RSD not more than 0.7188.

Third developed method for simultaneous estimation of two drugs from combined dosage form is RP-HPLC method utilizing C18 column and Acetonitrile: 0.005mol. L<sup>-1</sup> tetra butyl ammonium hydrogen sulphate as a mobile phase. The method was developed using Clonazepam as internal standard. The method was specific since excipients in the formulation did not interfere in the accurate estimation of OFLOX and SATRA. System suitability parameters are listed in TABLE 4.

Accuracy of the method was indicated by recovery values of 98.91 to 100.47%. Reproducibility is reflected in %RSD not more than 0.7758.

### Analytical data and calibration graphs

Straight-line calibration graphs were obtained over the calibration ranges 2 to 20  $\mu\text{g mL}^{-1}$  and 3 to 30  $\mu\text{g mL}^{-1}$  of OFLOX and SATRA respectively in method 1. TABLE 1 summarizes Beer's law limit, linear regression equation, correlation coefficient, standard deviations and limit of detection and limit of quantitation for method 1.

Similarly straight line calibration graphs were obtained over the calibration ranges 2 to 30  $\mu\text{g mL}^{-1}$  and 3 to 45  $\mu\text{g mL}^{-1}$  of OFLOX and SATRA respectively in method 2. TABLE 2 gives the details of linearity data.

### CONCLUSION

The validated spectrophotometric and HPLC methods employed here proved to be simple, fast, reliable, selective, and sensitive. Since none of the methods is reported for simultaneous estimation of OFLOX and SATRA from combined dosage form, these developed methods can be used for routine analysis of two components without prior separation.

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