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A validated RP-HPLC method for simultaneous estimation of ofloxacin and satranidazole from tablets

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ABSTRACT

A simple, selective, rapid, precise and accurate reverse phase high performance liquid chromatographic method has been developed for simultaneous estimation of ofloxacin and satranidazole in combined tablet dosage form. The method was carried out on Jasco HPLC system with HiQ Sil C18W column (250×4.6mm i.d.), using Acetonitrile: Ammonium acetate 65:35v/v as a mobile phase. The detection was carried out using UV detector set at 305nm. Clonazepam was used as internal standard. Beer's law is obeyed in the concentration range of 2.0 to 20.0 µg mL⁻¹ and 3.0 to 30 µg mL⁻¹ of Ofloxacin and Satranidazole, respectively. The method has been successfully applied for the analysis of drugs in pharmaceutical formulations. Results of analysis were validated statistically and by recovery studies.

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KEYWORDS

RP-HPLC;
Ofloxacin;
Satranidazole;
Clonazepam.

INTRODUCTION

Chemically Ofloxacin is (±)-9- fluoro-2, 3-dihydro-3- methyl-10-(4- methyl-1-piperazinyl)-7-oxo-7Hpyrido(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^[1]. The method of analysis given in U.S.P is titrimetric analysis. Literature survey reveals that Ofloxacin is estimated by spectrophotometry^[2], RP-HPLC^[3-9] and Spectrofluorometry^[10-11].

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 amoe-

biasis. 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^[12], spectrophotometry^[13]. 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.

EXPERIMENTAL

Equipment

Jasco HPLC system, consisting of Jasco PU-2080

Full Paper

plus HPLC pump and Jasco UV-2075 plus UV/VIS detector was used for analysis. HiQ Sil C18W (250 x 4.6mm i.d) column was used for analysis with a flow rate of 1 ml/min. A Rheodyne injector with 20 μ L loop was used for injecting the sample. Shimadzu balance, AY-120 was used for weighing purpose in this method.

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). Ammonium acetate was purchased from Sisco research Laboratories Pvt. Ltd. (Mumbai, India). 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 (300 mg); (Satracyl-O) were procured from the local market.

Procedure

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 (250x4.6 mm i.d) column was used in analysis. A Rheodyne injector with 20 μ L loop was used for injecting the sample. Detection of eluent was carried out using UV detector set at 305 nm. Mobile phase selected for this method consists of Acetonitrile: Ammonium acetate 65:35v/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 1. System suitability parameters for the developed method are given in TABLE 2.

Preparation of standard stock solution

Standard stock solutions of working standards were made separately in acetonitrile to obtain concentration of 200 μ g mL⁻¹ of Ofloxacin, 300 μ g mL⁻¹ of Satranidazole and 500 μ 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 10 ml volumetric flasks, appropriate dilutions were made from standard stock solutions to obtain final concentrations in the range of 2 to 20 μ g mL⁻¹ of ofloxacin and 3 to 30 μ 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 3000 rpm. 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 added in the same flask and the volume was made up to the mark with the mobile

TABLE 1: HPLC operating conditions

Mobile phase	Acetonitrile : Ammonium acetate (65: 35)
Flow rate	1.0mL/ min
Column temperature	Ambient
Injection volume	20 μ L
Detection wavelength	305 nm
	Ofloxacin 2.057
Mean retention time	Satranidazole 3.067
	Clonazepam 3.717

TABLE 2: System suitability parameters

Parameter	Ofloxacin	Satranidazole
Theoretical plates	2081.63	3886.08
Resolution	2.81	5.33
Asymmetry factor	1.42	1.33
Limit of detection	0.089(μ g mL ⁻¹)	0.031 (μ g mL ⁻¹)
Limit of ouantitation	0.27(μ g mL ⁻¹)	0.095 (μ g mL ⁻¹)
% RSD	0.79	0.74

phase. The solution was injected (20 μL) and a chromatogram was recorded. The injections were repeated five times and peak areas were recorded. A representative chromatogram is given in figure 1. 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.

Method validation

As per the ICH guidelines, the method validation parameters checked were linearity and range, accuracy, precision, limit of detection, limit of quantitation and robustness.

Linearity and range

Linearity of the method was checked using five different concentrations in the range of 2 to 30 $\mu\text{g mL}^{-1}$ (Ofloxacin) and 3 to 45 $\mu\text{g mL}^{-1}$ (Satranidazole). The linearity is indicated by regression equation.

For ofloxacin $y = 0.1754x + 0.2134$ ($r^2 = 0.997$)

For satranidazole $y = 0.1212x + 0.1147$ ($r^2 = 0.996$)

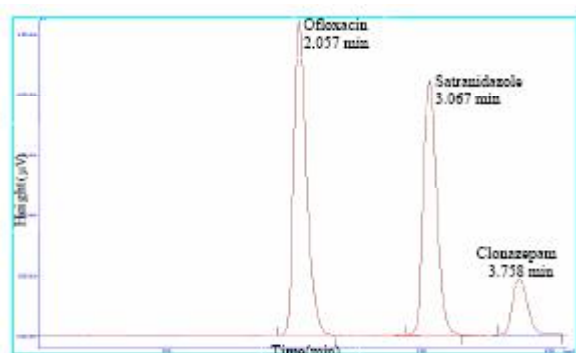


Figure1: Chromatogram of ofloxacin (2.057 min), Satranidazole (3.067 min), Clonazepam (3.758 min)

TABLE 3: Recovery studies of ofloxacin and satranidazole

Level of %	% mean recovery*		Standard deviation		%R.S.D.+	
recovery	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; +Is the relative standard deviation

TABLE 4 : Interday and studies for ofloxacin and satranidazole

Sr. no.	Concentration ($\mu\text{g mL}^{-1}$)	Interday studies (%RSD)		Intraday studies (%RSD)	
		Ofloxacin	Satranidazole	Ofloxacin	Satranidazole
1	2	0.99	0.11	0.68	0.12
2	3.75	0.69	0.006	0.72	0.65
3	7.5	0.6	0.064	0.98	0.24

Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 80, 100 and 120% and the percentage recovery was calculated and presented in TABLE 3. Recovery was within the range of $100 \pm 2\%$ which indicates accuracy of the method.

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, three different concentrations of the mixed standard were analyzed in a day and percentage RSD were calculated and was found to be less than 1.5%. In the inter day variation studies, three different concentrations of the mixed standard were analyzed on 3 consecutive days and percentage RSD were calculated and was found to be less than 1.5%. The results of interday and intraday studies are shown in TABLE 4. The data obtained indicates that the developed HPLC method is precise.

Limit of detection and limit of quantification

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula.

$$\text{LOD} = (3.3 \times \text{standard deviation}) / \text{Slope of calibration curve}$$

The LOD for ofloxacin and Satranidazole was found to be $0.089 \mu\text{g mL}^{-1}$ and $0.031 \mu\text{g mL}^{-1}$ respectively.

The limit of quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula.

$$\text{LOQ} = (10 \times \text{standard deviation}) / \text{Slope of calibration curve.}$$

The LOQ was $0.27 \mu\text{g mL}^{-1}$ and $0.095 \mu\text{g mL}^{-1}$ for ofloxacin and Satranidazole respectively.

Robustness

Robustness is checked by making slight deliberate change in the experimental procedures. It was determined by carrying out the analysis under conditions

Full Paper

during which mobile phase ratio and wavelength was altered and the changes on the R_t values and peak areas were noted. The method was found to be robust since the monitored parameters were not significantly affected.

RESULTS AND DISCUSSION

The proposed methods for simultaneous estimation of OFLOX and SATRA in combined tablet dosage form was found to be simple, accurate, rapid and economical. The values of percent RSD were not more than 0.7758 and recovery was between 98.91 to 100.47%, indicating reproducibility and accuracy of methods.

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.

CONCLUSION

The validated 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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