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Dissolution Studies and RP-HPLC Method for the Simultaneous Determination of Satranidazole and Ofloxacin in Pharmaceutical Dosage Form

S. Sharma[1], M. C. Sharma*

***School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (M.P) 452001, India**

[1]Department of Chemistry Chodhary Dilip Singh Kanya Mahavidyalya, Bhind (M.P) India

Corres.author: mukeshcsharma@yahoo.com

Abstract: A Stability indicating reverse phase high performance liquid chromatographic method has been developed for the simultaneous determination of Satranidazole and Ofloxacin in pharmaceutical formulation. The equipment was controlled by a PC workstation. Compounds were separated on a 25 cm × 4.6 mm i.d, 5-µm particle, Phenomenex Luna C18 column under reversed-phase partition chromatographic conditions. The mobile phase was a Mixture of acetonitrile: methanol: Glacial acetic acid: ammonia (60:15:10:15) was used as a mobile phase at a flow rate of 1.5 ml min^{-1}, was used to sharpen the peak. The run time was less than 15 min. Before analysis, both mobile phase and sample solutions were degassed by sonication and filtered through 0.2-µm filter paper. The analytes were monitored at 248 nm.The described method shows excellent linearity over a range of 60-300 µg ml^{-1} for Satranidazole, and 40-200 µg ml^{-1} for Ofloxacin. Norfloxcin was used as an internal standard. The proposed method was found to be suitable, accurate for quantitative determination and the stability study of Satranidazole and Ofloxacin in pharmaceutical preparations.
Keywords: Satranidazole, Ofloxacin RP-HPLC, Method Validation.

Introduction

Satranidazole (STZ), chemically 1-Methylsulfonyl-3-(1-methyl-5-nitro-2-imidazolyl)-2-imidazolidinone is an antiamoebic drug. It has been determined in pharmaceutical formulations by different methods like UV-Visible spectrophotometry [1] , HPTLC [2-3], HPLC [4] .Ofloxacin (OFLOX) chemically (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyridol[1,2,3-de]-1,4benzoxazine-6-carboxylic acid is a fluoroquinolone antibiotic and is used in the treatment for gonorrhea[5-6] . Several methods such as Spectrophotometry [7], HPLC [8], are reported in literature for determination of Ofloxacin in dosage form and in biological samples; combination of HPLC method.[9] Author of the article and his research team has developed a HPLC method development in different pharmaceutical dosage form [10-18]. In the present study attempts were made to develop a rapid, economical, precise and accurate method for the simultaneous determination of the ingredients of this combination in the presence of their degradates.

Materials and Methods

Chemicals and Reagents

The AR grade sodium lauryl sulphate, ortho phosphoric acid, sodium hydroxide, hydrochloric acid were purchased from Merck Fine Chemicals (Mumbai, India) and hydrogen peroxide was from Qualigens Fine Chemicals, Mumbai, India and high pure water prepared by using Millipore Milli Q plus purification system The 0.45µm-Pump nylon filter,Hypersil C_8,

column was procured from Thermo Electron Corporation.

Chromatography

HPLC was performed with a Shimadzu LC-10 AT VP solvent-delivery system, a Shimadzu SPD-10 AVP UV–visible photodiode-array detector, and a Rheodyne7725 I universal loop injector of injection capacity 20 µL. The equipment was controlled by a PC workstation. Compounds were separated on a 25 cm × 4.6 mm i.d, 5-µm particle, Phenomenex Luna C18 column under reversed-phase partition chromatographic conditions. The mobile phase was a Mixture of acetonitrile: methanol: Glacial acetic acid: ammonia (60:15:10:15) was used as a mobile phase at a flow rate of 1.5 ml min^{-1}, was used to sharpen the peak. The run time was less than 15 min. Before analysis, both mobile phase and sample solutions were degassed by sonication and filtered through 0.2-µm filter paper. The analytes were monitored at 248 nm.

Preparation of Standard stock solution

Solution (A)

Weighed accurately 100mg of Satranidazole working reference standard and transferred carefully in to a 50ml volumetric flask. Added 35ml of mobile phase and sonicated for 15min, cooled to room temperature and diluted 50ml with mobile phase. Mixed well.

Solution (B)

Weighed accurately 100mg of Ofloxacin working reference standard and transferred carefully in to a 50ml volumetric flask. Added 35ml of mobile phase and sonicated for 15min, cooled to room temperature and diluted 50ml with mobile phase. Mixed well. Diluted 5ml of Solution (A) and 5ml Solution (B) to 50ml with mobile phase.

Buffer preparation

Dissolve 2.5 g of sodium dihydrogen orthophosphate in to 1000 mL of Milli Q water and adjust pH 3.0 with orthophosphoric acid. Filtered it through 0.45 µ HVLP nylon filter.

Applied method to compare dissolution profiles

The description of the in vitro dissolution profiles was calculated by using model-independent method [19-21]. In this study, as model-independent approaches, two fit factors were applied to the dissolution data that compare the dissolution profiles of a pair of drug product. These fit factors directly compare the difference between the percent drug dissolved per unit time for a test and reference product. The fit factors are f_1 (difference factor) and f_2 (similarity factor).

Preparation of Sample solution

Weighed and finely powdered not less than 20 tablets. Transferred an accurately weighed portion of the powder equivalent to about 20 mg Norfloxcin, 100mg to 100ml volumetric flask, and added 70ml of mobile phase. Sonicated for 15min and cooled to room temperature. Diluted to 100ml with mobile phase. Mixed well and filtered through Whatman No.1 filter paper. Discarded first few ml of the filtrate. Injected separately 20µl of the standard preparation in to the equilibrated HPLC system in 5 replicate and measured the response of the major peak due to Satranidazole and Ofloxacin. Then injected separately 20µl of the sample preparation in to duplicate and measured the response of the major peak due to Satranidazole and Ofloxacin.

Limit of Detection and Limit of Quantification

Calibration curves for both Satranidazole and Ofloxacin were plotted individually by taking the peak areas of drug/Internal standard versus concentration. The slopes of the plots for Satranidazole and Ofloxacin were determined by the method of least square regression analysis. The LOD and LOQ for Satranidazole and Ofloxacin by the proposed method were determined using calibration curves. LOD and LOQ were calculated as 3.3 s/S and 10 s/S, respectively, where S is the slope of the calibration curve and s is the standard deviation of y-intercept of regression equation (n=6).

Accuracy and Precision

Intraday reproducibility precision and accuracy were assessed for three different concentrations of 200, 350 and 500 ng/ml of Satranidazole and Ofloxacin with replicates of 6 were analyzed on the same day and the concentrations were calculated by extrapolating peak area ratios to standard curves obtained on that day. The procedure was repeated on three separate days to allow the determination of interday precision and accuracy. Accuracy was expressed as the mean % error and precision was expressed as the coefficient of variance (%).

Table-1. Summary of validation parameters Satranidazole and Ofloxacin

Parameters	Satranidazole	Ofloxacin
LOD (µg mL^{-1})	1.8 µg mL^{-1}	0.9 µg mL^{-1}
LOQ (µg mL^{-1})	5.1 µg mL^{-1}	2.8 µg mL^{-1}
Accuracy (%) ± % RSD	101.00± 0.015	99,98± 0.076
Precision (% RSD)		
Inter-day (n = 2)	0.43	0.26
Repeatability (% RSD)	0.1	0.21

Table 2. System suitability test parameters and Regression analysis

Parameters	Satranidazole	Ofloxacin
Retention time	6.22	8.03
Tailing factor	1.24	1.19
Theoretical plates	7492	9251
Slope	13154	17651
Intercept	5482	7652
Correlation coefficient (r)	0.9989	0.9995

Table 3: Results of analysis of degradation

Stress Condition	Satranidazole	Ofloxacin
Acid degradation	98.89	99.99
Base degradation	99.96	100.07
Peroxide degradation	100.05	99.99
Heat degradation	100.12	100.26

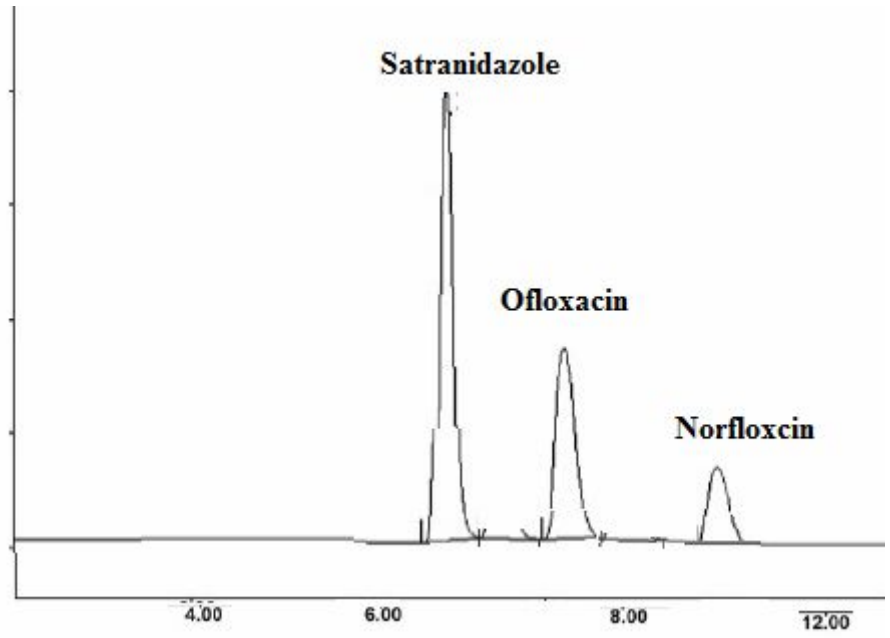

Fig. A typical chromatogram of Satranidazole and Ofloxacin

Results and discussion

The method was validated in terms of linearity, accuracy, precision and specificity of the sample applications. The linearity of the method was investigated by serially diluting the stock solutions of Satranidazole and Ofloxacin and measured the absorbance at 248nm. Calibration curves where constructed by plotting the area against the concentration. Satranidazole shows the linearity in the concentration range from 60-100 µg/ml with correlation coefficient of 0.9998 and Ofloxacin shows the linearity in the concentration range from 60-100 µg/ml with correlation coefficient of 0.9996. Both the calibration curves pass through the origin, which justifies the use of single point calibration. The regression equation for Satranidazole and Ofloxacin were Y = 0.8742 X + 0.1265 and Y= 0.6531 X + 0.3264 (Y=peak area drug/IS peak area ratio and X = concentration) respectively. Recovery studies were carried out to study the accuracy of the proposed method and ascertained by standard addition method. A known amount of drug was added to preanalysed tablet powder, at three level and the percentage recoveries were calculated. Precision was found to be lower than 1%. Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by different analysts using similar operational and environmental conditions. Hence methanol is used as a diluent and the chromatographic elution was carried out in acetonitrile than in methanol due to poor selectivity. To develop the stability-indicating method different stationary phases like C_{18}, C_8, CN different mobile phases containing buffers like phosphate, ammonium acetate and ortho phosphoric acid with different pH (3–5) and organic modifier (acetonitrile) were used. Our objective of the chromatographic method development was to achieve a peak tailing factor <2.0, retention time in between 3 min to 15 min, along with a resolution among Satranidazole and Ofloxacin. From the development studies, it was determined that 2.5 nM sodium Lauryl sulphate and 5.0 ml of ortho phosphoric acid in water and acetonitrile in the ration of 70:30(v/v), had a mobile phase flow rate of 1.0 mL min^{-1} and a column temperature of 40°C. The analytes of this combination had adequate retentions, peak shape, less tailing, more resolution and the chromatographic analysis time was less than 18 min. But retention time consistency is not observed due to Sodium lauryl sulphate buffer, so we changed the mobile phase as 20nM with an adjustment of pH 2.8 with ortho phosphoric acid and little bit changed the chromatography parameters. In optimized conditions Satranidazole and Ofloxacin their degradants were well separated. The precision of the method was studied by determining the concentrations of each drug in the tablets six times. The assay % RSD for Satranidazole and Ofloxacin were 0.43 and 0.26. The results of the precision study indicate that the method is reliable (% RSD < 2.0).Intermediate precision of the method was determined by analyzing the samples six times on different days. The percentage assay was calculated using calibration curves. The assay results of Satranidazole and Ofloxacin were 100.23 and 101.18. The linearity of an analytical procedure is its ability (with in a given range i.e. 20% - 120% levels) to obtain test results, which are directly proportional to the concentration of the analyte in the sample. The calibration curve solutions contained 60–300 µg mL^{-1} of Satranidazole, 40–200 µg mL^{-1} Ofloxacin. The robustness of a method is the ability to remain unaffected by small changes in parameters. To determine robustness (tailing factor, % RSD) of the method, experimental conditions were purposely altered. To study the effect of flow rate on the tailing factor of Satranidazole and Ofloxacin. The effect of column temperature was studied at 20 and 30°C instead of 25°C, while other mobile phase components were held constant. The LOD and LOQ for Satranidazole and Ofloxacin were determined at a signal to- noise ratio of 5:1 and 15:1, respectively, by injecting a series of dilute solutions with known concentrations. The LOD values for Satranidazole and Ofloxacin were 1.8 µg mL^{-1} ,0.9 µg mL^{-1} , and the LOQ values were 5.1 µg mL^{-1} , 2.8 µg mL^{-1} respectively for 10 µL injection volume. The stability of the standard and test solution was tested at intervals of 24 and 48 h. The % assay values were within 3.0 up to 48 h. The results indicate that the solutions were stable for 48 h at ambient temperature as there was no formation of any unknown peak and solution remained stable. Study of Forced degradation of each drug product was carried out under thermolytic, Humidity, photolytic, acid, base, hydrolytic and oxidative stress conditions. This change was observed in 24 h of irradiation. A second photolytic stress test experiment with greater irradiation time was in 48 h. Sample preparation was prepared by open and transferred ten capsules content into a 250 ml volumetric flask, 10ml of 0.1N HCl added and the mixture kept reflux for 30 min at 60°c .The solution was allowed to attend ambient temperature, then it was neutralized with 0.1N NaOH to pH 7, 250 mL of diluent was added, and the solution was sonicated for 60min with intermittent shaking, maintained sonicator temperature at 25°C, followed by shaking of 15 mints. Then the volume was made up with diluent and centrifuged at 1500 rpm for 15 min. The centrifuged solution filtered through a 0.45-µm filter. From the filtered solution, 15 mL were transferred into a 100 mL volumetric flask and diluted to volume with diluent. Sample preparation was prepared by open and transferred ten capsules content into a 250 ml volumetric flask, 10ml of 0.1N NaOH

added and the mixture kept reflux for 30 min at 60°c .The solution was allowed to attend ambient temperature, then it was neutralized with 0.1N HCl to pH 7 150 mL of diluent was added, and the solution was sonicated for 30min with intermittent shaking, maintained sonicator temperature at 25°C, followed by shaking of 15 mints. Then the volume was made up with diluent and centrifuged at 1500 rpm for 15 min.Sample preparation was prepared by open and transferred ten capsules content into a 250 ml volumetric flask, 10ml of 5.0% H_2O_2 added and the mixture kept reflux for 30 min at 60°c .The solution was allowed to attend ambient temperature, then it was solution was sonicated for 30min with intermittent shaking, maintained sonicator temperature at 25°C, followed by shaking of 15 mints. Then the volume was made up with diluent and centrifuged at 2000 rpm for 5 min. The centrifuged solution filtered through a 0.45-µm filter. From the filtered solution, 5 mL were transferred into a 25 mL volumetric flask and diluted to volume with diluent. In conclusion forced degradation method developed for the analysis of Satranidazole and Ofloxacin in their pharmaceutical preparations is precise, accurate and with a short run time. The method was fully validated showing satisfactory data for all the method validation parameters tested.

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