VALIDATED STABILITY-INDICATING HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF OFLOXACIN AND SATRANIDAZOLE FROM PHARMACEUTICAL DOSAGE FORM

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ABSTRACT: The present paper describes development of stability- indicating RP- HPLC method for the simultaneous determination of Ofloxacin and Satranidazole in presence of its degradation products, generated from forced degradation studies. Ofloxacin and Satranidazole and their combination drug product were exposed to acid, base, neutral hydrolysis; oxidation, dry heat, photolytic stress conditions and the stressed samples were analyzed by proposed method. The proposed HPLC method utilizes HiQ sil C18W column (250mm × 4.6mm i.d., 5µm) of KYA TECH, Corporation and a mobile phase comprising of acetonitrile: phosphate buffer (pH3) in ratio of 35:65v/v with flow rate of 1ml/min. The retention time of OFLX and STZ was found to be 2.85min and 6.25min respectively. Quantitation was achieved with UV detection at 296nm for OFLX and 320nm for STZ. The method has been validated for ofloxacin and satranidazole in terms of accuracy, precision, linearity, LOD, LOQ and robustness. The developed validated stability-indicating HPLC method was found to be simple, specific, accurate and reproducible for the determination of instability of these drugs in bulk and commercial products.

Keywords: RP-HPLC, stability indicating method, forced degradation studies, degradation product, Satranidazole and Ofloxacin

INTRODUCTION

The "Stability" of a drug dosage form refers to the ability of a particular formulation, in a specific container, to maintain its physical, chemical, therapeutic and toxicological specification presented in the monograph on identity, strength, quality, and purity. The stability of a drug product should ordinarily be demonstrated by its manufacturer by methods appropriate for the purpose. Obviously, a stability testing problem is never simple [1-3].

Stability testing is an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors, such as temperature, humidity, and sun light and enables recommendation of storage conditions, retest periods, and shelf lives to be established. Two main aspects of a drug product that play an important role in shelf life determination are assay of the active drug and degradation products generated during the stability study. The drug product in a stability test sample needs to be determined using a stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines and U.S. pharmacopoeia (USP) [4].

Literature survey reveals that many analytical methods are reported for determination of ofloxacin and satranidazole; individually, however no method has been reported for simultaneous estimation of these two drugs by reverse phase HPLC [5-20].

The aim of present work is to develop simple, specific, accurate, repeatable stability-indicating HPLC method for the simultaneous determination of ofloxacin and satranidazole from API and marketed formulation in presence of possible degradation products. The proposed method was validated as per ICH guidelines.

Ofloxacin [9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1, 2,3-de]-1,4-benzoxacine-6-carboxylic acid] is a pale yellow or bright yellow, crystalline powder, freely soluble in water, slightly soluble in glacial acetic acid, methyl chloride and in methanol.

Ofloxacin is broad spectrum antibacterial of quinolone derivative.

Satranidazole [1-Methanesulphonyl-3-(1-methyl-5-nitro-2-imidazolyl)-2-imidazolidinone] is a light lemmon yellow crystals. Soluble in methanol and insoluble in water. It is not official in any of the pharmacopoeia. It is more active towards anaerobes than many 5-nitroimidazoles because its relatively high redox potential may make it more resistant to inactivation by oxygen. It shows activity against *E. hystolytica, T. vaginalis* & *Giardia* [21, 22].

EXPERIMENTAL:

Chemicals and reagent:

Pure drug sample of ofloxacin and satranidazole of pharmaceutical grade were kindly supplied as a gift sample by Alkem Laboratories Pvt. Ltd. (Mumbai, Maharashtra, India). Satrogyl-O, tablet formulation was purchased from local shop, (1 tablet equivalent to 200mg of OFLX and 300mg of STZ. HPLC grade acetonitrile was procured from Sisco Laboratories Pvt. Ltd, (Mumbai). Potassium Dihydrogen Phosphate (anhydrous) and all other chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

HPLC instrumentation:

The chromatography was performed with Jasco HPLC system consisted of pump (model JASCO; PU – 2080 plus) with universal loop injector (Rheodyne 7725 i) of injection capacity 20µl. It was equipped with Photodiode array detector (MD-2010 plus, JASCO) LC-Net (II/ADC, JASCO) system. The column HiQ Sil C18W (250mm × 4.6mm i.d., 5µm) of KYA TECH Corporation, (Japan) was used at ambient temperature. The Data obtained was integrated using Jasco Chrompass version 1.7.403.1 and. The photo stability chamber (THERMO LAB) was equipped with an illumination bank on inside top consisting of a combination of two black light UV lamps (OSRAM L18W/73) and four white fluorescent lamps (OSRAM L18W/20) in accordance with option two of International Conference on Harmonization (ICH) guideline. The samples were placed at a distance of 9 in. from the light bank. Both fluorescent and UV lamps were put on simultaneously. Thermal stability study was carried out in dry air oven (Innovative DTC 96, New Delhi, India). Other equipments used were sonicator Spectra lab (UCB-30) and An Electronic balance BL 220H of Shimadzu Corporation, (Japan) was used for weighing.

Preparation of mobile phase:

Mobile phase comprising of acetonitrile: phosphate buffer (pH3) in ratio of 35:65v/v with flow rate of 1ml/min. Mobile phase was prepared by mixing 350ml of acetonitrile and 650ml of dihydrogen phosphate buffer and pH of mix. was adjusted at pH3 by using o- phosphoric acid. Phosphate buffer was prepared by weighing accurately 1.36gm of anhydrous potassium dihydrogen phosphate was dissolved in 1000ml of distilled water. It was filtered through 0.45µm membrane filter and degassed by sonication with ultrasonic bath. All determination was carried out at room temperature.

Preparation of standard stock solution:

50mg each of OFLX and STZ were taken in 50ml volumetric flask and dissolved in minimum amount of mobile phase and diluted up to the mark to obtain 1000µg /ml concentration separately. Working standard solution were prepared from the above stock solution 10ml of solution was pipetted out and transferred to 100ml volumetric flask and diluted this to the mark with mobile phase to get concentration of 100µg/ml of each drug. Further dilutions were made by using this stock solution to obtain final concentrations 5, 10, 20, 30, 40, 50µg/ml of both the drugs.

Preparation of sample solution of Marketed Formulation:

Twenty tablets (Satrogyl-O, Alkem Laboratories Ltd., Mumbai) each containing 200mg of OFX and 300mg of STZ were weighed and powder equivalent to 200mg of OFX and 300mg of STZ were (Eq.wt 0.322gm of powder) weighed accurately and transferred to 100ml volumetric flask and dissolve in distilled water by shaking the flask for 10min. the solution was filtered through 0.45µ filter. From this solution, further dilutions were made using distilled water to get the final concentration of 20µg/ml of OFX and 30µg/ml of STZ. Areas were recorded at wavelength 296nm and 320nm. The concentration of drug was then calculated by using calibration curve method.

Forced Degradation Studies of API and formulation:

Stress studies were carried by using 50µg/ml of each solution in different conditions. Acidic and alkaline hydrolysis was carried out in 0.1N HCl and 0.1N NaOH, respectively, whereas neutral hydrolysis was performed in water. All hydrolytic studies were conducted at 80^0C. The oxidative study was carried out in 10% H_2O_2 for 24hrs. and recently prepared 3% H_2O_2 for OFLX and STZ respectively. Photolytic studies on the drug in the solid state were carried out by exposure of UV and fluorescent lamps in a photostabilty chamber set at accelerated condition of temperature and humidity (40^0C, 75% RH). For thermal stress testing, both the drugs were spread in petridish and placed in the oven at 50^0C for 21 days. Samples were withdrawn periodically and subjected to analysis after suitable dilution.

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HPLC studies of OFLX and STZ under different stress conditions indicated the following degradation behavior.

Acidic condition

Both OFLX and STZ showed sufficient degradation at 1hr, in 0.1N HCl. The major degradation product formed for OFLX was at retention time (Rt) 2.47min and at 2.3min for STZ. It has shown in Fig.2.

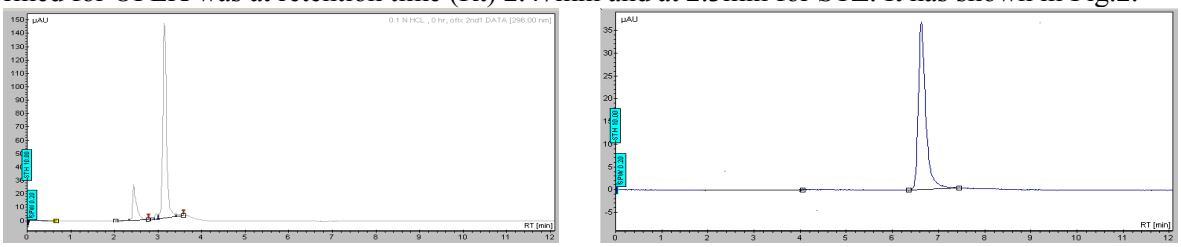

Figure. 2. *Degradation behavior in Acidic condition of (a) OFLX and (b) STZ*

Degradation in alkali

Both the drugs were shown sufficient degradation in alkaline hydrolysis in 0.1N NaOH at 80^{0}C. OFLX decomposed within 15min. while, STZ showed degradation within 30min. The degraded product appeared at Rt 2.41min for OFLX and at 2.3min for STZ. It has shown in fig. 3.

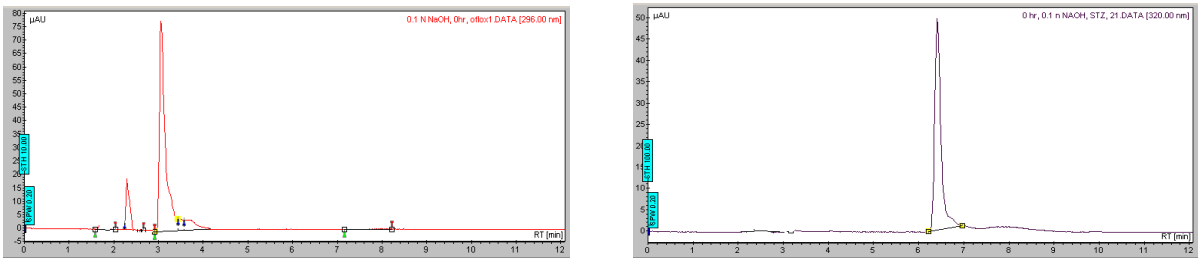

Figure. 3. *Degradation behavior in alkaline condition of (a) OFLX and (b) STZ*

Neutral (water) degradation

Degradation product of OFLX was found nonchromophoric upon refluxing for 12hrs. at 80^{0}C and STZ showed no degradation when refluxing for 12hrs. at 80^{0}C. It has shown in fig. 4.

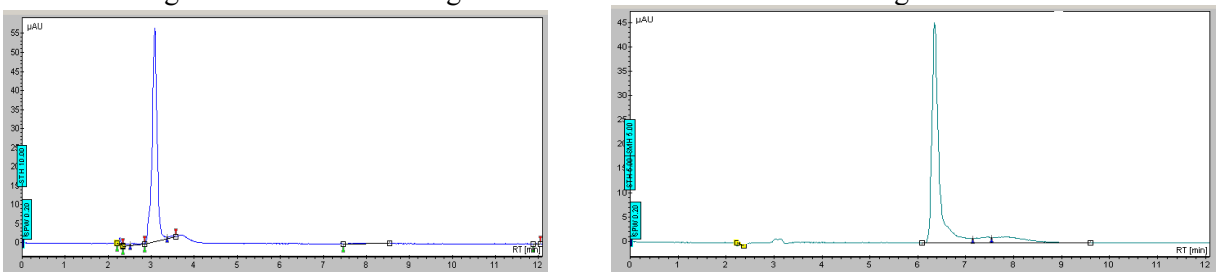

Figure. 4. *Degradation behavior in Neutral condition of both drugs (a) OFLX and (b) STZ*

Oxidative degradation

The drug OFLX showed sufficient decrease in height of the peak, but no degradation product was found to be (nonchromophoric), when it was degraded in 3% H2O2 for 6 h. and drug peak was observed to be stable for STZ in 30% H2O2 for 24hrs at room temperature. It has shown in fig. 5.

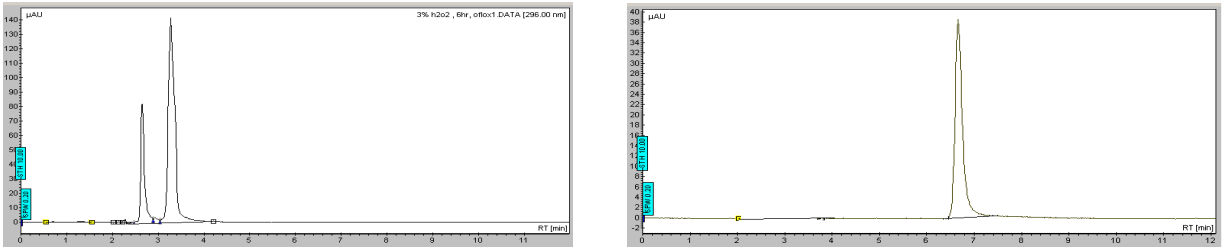

Figure. 5*. Degradation behavior in Oxidative condition of (a) OFLX and (b) STZ*

Photolytic and thermal degradation

Solid state studies showed that OFLX and STZ both were unstable in light and sufficient degradation was observed in dark and light after 1 day in photo stability chamber at 0.12million lux hrs. at RT 2.8min for OFLX and 3.4min for STZ. Also both the drugs were found to be thermally stable. Shown in fig.6.

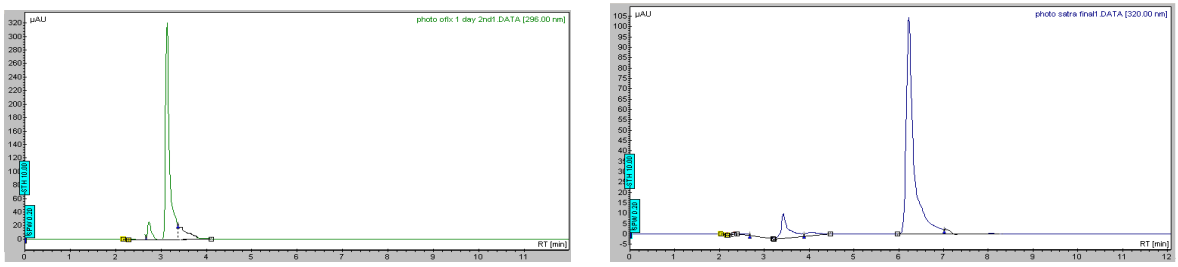

Figure. 6. *Degradation behavior in Photolytic condition of (a) OFLX and (b) STZ*

Development and optimization of the stability-indicating HPLC method:

To develop accurate, precise and specific stability HPLC method for simultaneous estimation of OFLX and STZ in stressed sample as well as in standard drug solution. Various mobile phases with different composition and flow rate were tried. After number of trial experiments, it was established that methanol: buffer (pH3) has high eluting power.

Initially different ratio of methanol: buffer (pH3) in isocratic mode in ratio of (45:55) was tried but no good resolution of OFLX and STZ from their degradation peaks were found. Finally, ratio of (35:65) was found necessary to optimize the separation of drugs from major degradation products formed under various stress conditions, because it was found to ideally resolve the peaks of OFLX (retention time, t_R =2.853min) and STZ (t_R = 6.257min), can give complete separation of both drugs from their degradation products and impurities at a flow rate of 1ml/min. UV detection at 296nm and 320nm for OFLX and STZ respectively, with injection volume of 20µL and ambient temperature (25^0C) for column were found to be best for analysis.

Validation of the developed stability-indicating method

The developed stability indicating method was validated according to ICH[1] guidelines. The validation parameters addressed were linearity, precision (inter-day, intra-day and intermediate precision), accuracy and LOD, LOQ, robustness [24].

Linearity:

Linearity was established over the concentration ranges 5-50µg/ml at 296nm for OFLX and 320nm for STZ. Peak area (y) of ofloxacin and satranidazole was plotted versus their respective concentrations (x) and linear regression analysis performed on the resultant calibration curves. Correlation coefficient (R^2) was found to be more than 0.99 for both the analytes. Typically, the mean (± SD) of the regression equation were: y = 2.4334x + 8.6879 for ofloxacin and y = 0.556x + 0.1338 for satranidazole, respectively.

Limit of Detection and Quantitation:

The limit of detection (LOD) and quantitation (LOQ) for both OFLX and STZ were determined according to ICH guideline Q2B[1]. LOD was defined as 33σ/S and LOQ was 10 σ/S based on standard deviation of the response and slope of the calibration curve specially constructed in a low region of 0.05 to 1.0% of the target analyte concentration. The standard deviation of y- intercepts of regression lines was used as σ (standard deviation of the response) and S is the slope of calibration curve. The signal: noise ratio of 3:1 and 10:1 were considered as LOD and LOQ respectively. The LOD and LOQ were found to be 0.10 µg/ml and 0.50µg/ml respectively for OFLX and 0.22µg/ml and 0.78µg/ml for STZ respectively.

Accuracy /recovery:

Accuracy of the method was determined by standard addition method at 50%, 100%, 150%. Known amounts of preanalysed solution containing mixture of 20µg/ml of OFLX and 30µg/ml of STZ were added to standard. The % recovery and % RSD were calculated for each concentration. The method was found to be accurate with percent recoveries between 98.5 and 101 and % RSD was < 2.

Precision:

Six injections of three different concentrations were given on the same day and the percent relative standard deviations (%RSD) were calculated to determine intra-day precision. These studies were also repeated on three consecutive days to determine inter-day precision.

Robustness:

To evaluate the robustness of the developed HPLC method, small deliberate variation in the optimized method parameters were done. The effect of change in flow rate, mobile phase ratio and column temperature on the retention time and tailing factor were studied. The method was found to be unaffected by small changes like ± 0.1 change in pH, ±0.1 change in flow rate and ± 0.1 change in mobile phase.

Table 1: System Suitability Parameters

PARAMETER	OFLX	STZ
Calibration range (µg/ml)	5-50	5-50
Linearity	0.9982	0.9975
Precision (Intra-day)%RSD	0.9975	1.13
Precision (Inter-day)%RSD	1.46	1.22
Precision(Intermediate)%RSD	0.993	0.994
Mean Recovery 50%	99.22	99.82
100%	99.75	99.43
150%	99.74	99.87
Theoretical plates	7381.40	3132.45
Retention time	2.853	6.283
Area (µ AU)	195	26.08
Resolution	----	2.02
Tailing Factor	1.42	2.06
% R.S.D.	0.627885	0.112016
LOD(µg/ml)	0.10	0.22
LOQ(µg/ml)	0.50	0.78

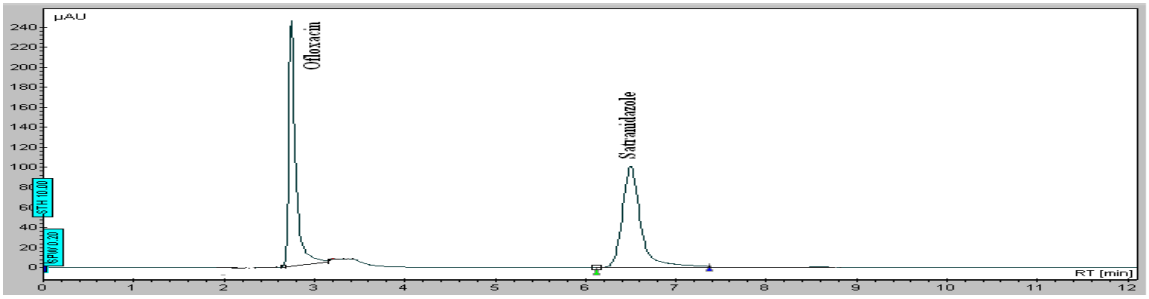

Fig. 7. *Chromatogram of mixture of untreated drug combination.*

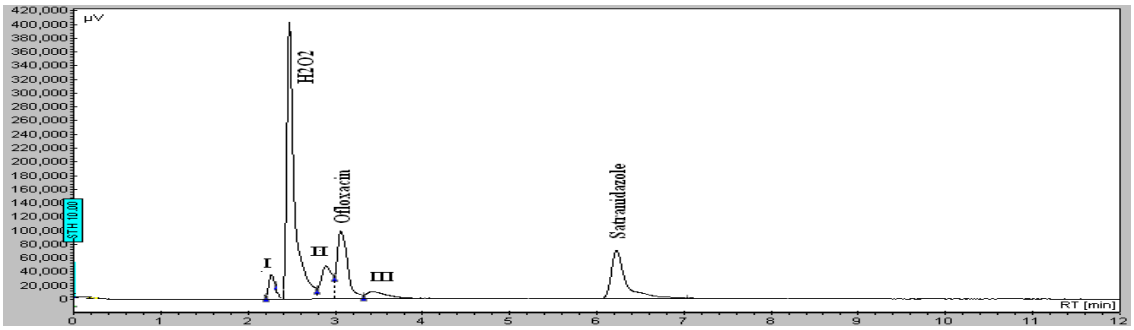

Fig. 8. *Chromatogram of mixture of stressed samples*

Table 2. Chromatogram of all degradation studies as observed in fig. 8

RTs of degradation product	Degradation Behavior
2.39min (I)	Acidic, Alkaline (OFLX)
2.81min (II)	Photolytic(OFLX)
3.42min (III)	Photolytic(STZ)

Table 3: Results of forced degradation study using the proposed method

Name of drug	Stress condition	% degradation	Retention time
OFLX	Acidic condition/ 0.1N / 1 hr.	50%	2.47min.
STZ	Acidic condition/ 0.1N/ 1 hr.	58.3%	2.3min
OFLX	Basic condition/ 0.1N/ 3hr.	25%	2.41min.
STZ	Basic condition/ 0.1N/ 5hr.	83.3%	2.3min
OFLX	Neural condition/ 800C/ 3hr.	---	---
STZ	Neural condition/ 800C/ 3hr.	---	---
OFLX	Oxidative condition/ 30% / 24hr.	No	----
STZ	Oxidative condition/ 3% / 0hr.	No	---
OFLX	Photo condition / 0.12 million lux h	53.3%	2.8min
STZ	Photo condition / 0.12 million lux h	83.3%	3.4min
OFLX	Thermal condition/ 500C/ 21 days	No	---
STZ	Thermal condition/ 500C/ 21 days	No	---

Application of the developed method to marketed formulations containing OFLX and STZ:
The developed method was used to analyze marketed formulations containing the two drugs. A clear resolution of the drugs was achieved even for all formulations tested, with no interference from excipient. In almost all the cases, chromatographic pattern was similar to the one shown in Fig. 9. This indicated that the method could be extended for the study of available drug content in commercial products. The data is given in Table 4, and chromatogram has shown in fig. 9.

Table 4: Analysis of formulation containing OFLX and STZ combination

Component	Label Claim (mg)	Mean*	Standard Deviation	% R. S. D.	Standard Error
OFLX	200	99.50	0.82462	0.73657	0.3121
STZ	300	99.74	0.18168	0.16481	0.0654

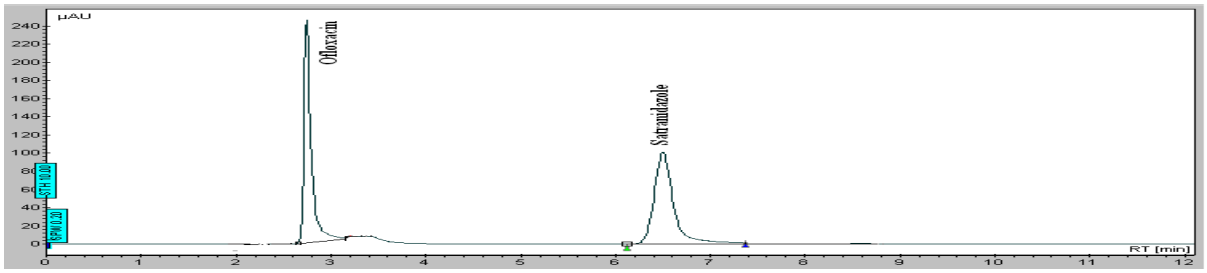

Figure. 9. *Chromatogram of Marketed formulation.*

Conclusion:

The developed HPLC technique is simple, accurate, specific and reproducible stability indicating for quantitative analysis of simultaneous determination of ofloxacin and satranidazole in pharmaceutical formulations, without any interference from the excipient and in the presence of its acidic, alkaline, neutral, oxidative, thermal and photolytic degradation products. The method was validated as per ICH guidelines. Statistical tests indicate that the proposed HPLC and SIAM methods reduce the duration of analysis and appear to be equally suitable for routine determination of ofloxacin and satranidazole in pharmaceutical formulation in quality control laboratories, where economy and time saving are essential. As the method separates the drug from its degradation products, it can be employed as a stability indicating method.

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