LIQUID CHROMATOGRAPHIC SIMULTANEOUS SEPARATION AND ESTIMATION OF OFLOXACIN AND SATRANIDAZOLE IN PHARMACEUTICAL FORMULATION

Vijaya Kumar Meher [1], Girendra Kumar Gautam [2], Saroja Kumar Patro[3]*

Abstract:
A simple, novel, economical and precise Liquid chromatography method has been developed and subsequently validated for the simultaneous determination of Ofloxacin and Satranidazole in pure drug and formulations. The best separation was achieved by employing the C_{18} column and mobile phase consisting of the Ortho-phosphoric acid buffer (pH 3±0.1) and Acetonitrile in the ratio of 70:30.The flow rate was 1.5ml/minute and the effluent was monitored at 278nm. The retention times of Ofloxacin and Satranidazole were found 1.730 and 5.810minute respectively. The linearity plots of Satranidazole and Ofloxacin shows linearity in the concentration ranges of 10.5 to 24 and 7 to 16 µg/ml respectively. The validation of the proposed method has been done statistically and on the basis of the ICH guidelines. The LOD and LOQ of the method were calculated to be 0.59 µg/ml and 1.44 µg/ml of Ofloxacin and Satranidazole respectively. The Precision was estimated by employing repeatability; intra-day and inter-day studies and the results were calculated as %RSD values and were found to be within the acceptable limits. Recovery of both drugs was found to be in the range of 98 and 102 percent, which establishes the accuracy of the method. As the chromatogram for Satranidazole and Ofloxacin in formulation does not show any other peaks except that corresponding to the drugs, it was revealed that common pharmaceuticals excipients used in the formulation were not interfering with the proposed method. So the proposed method is highly suitable for routine analysis of formulations containing of Ofloxacin and Satranidazole.

Keywords: RP-HPLC, Ofloxacin, Satranidazole, Validation, ICH guidelines.

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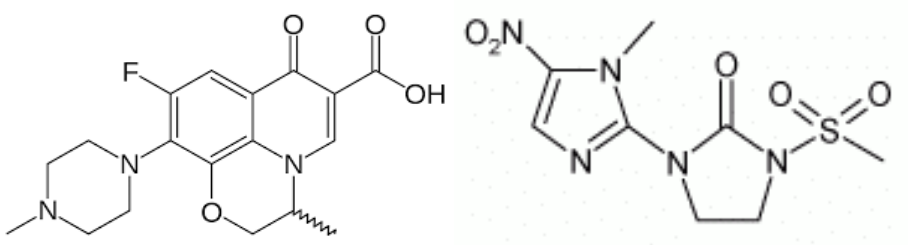
INTRODUCTION:

Ofloxacin is a fluoroquinolone derivative and Chemically it is (RS)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)- 7-oxo2,3-dihydro-7H-pyrido[1,2,3-de]-1,4- benzoxazine- 6-carboxylic acid. It is used in the treatment of urinary tract infection and sexually transmitted diseases. Satranidazole is a 5-nitro imidazole derivative. Chemically it is 1-(1-Methyl-5-nitro-1H-imidazol-2-yl)-3-(methylsulfonyl)-2-imidazolidinone. It is used in amoebic liver abscess, Trichomoniasis, Giardiasis. The chemical structure of Ofloxacin and Satranidazole are shows in the Fig 1 (a) Ofloxacin (b) Satranidazole[1]. The literature survey reveals the reported methods are available for estimation of Ofloxacin and Satranidazole by UV-visible spectrophotometric [2-3,5], HPTLC [4], & HPLC methods [7-16]. In comparison to the reported methods of RP-HPLC, the proposed method is having less retention time for Ofloxacin and best resolution between the two analytes. Both the analytes are eluted within the 7minutes and which is less time in comparison to all the reported methods.



(a) **(b)**

Fig1. Chemical Structure of Ofloxacin(a) and Satranidazole (b)

Sl. No.	Column	Mobile Phase Composition	Retention time (min) Ofloxacin& Satranidazole	Reference No.
1.	Phenomenex Luna C18	Phosphate buffer (**pH** 6): Acetonitrile (70: 30 % v/v)	3.720, & 6.130min	7
2	Kromasil-100 C18	10mM phosphate buffer (pH3) and methanol (50:50).	2.59 & 4.0 min	8
3	Kromasil C18 Column	20mM potassium dihydrogen phosphate pH4: acetonitrile 60 : 40 (v/v) containing 0.1% glacial acetic acid	2.29 & 4.80 min	9
4	HiQSil C18W	acetonitrile: phosphate pH 3 (35 : 65 v/v)	2.85 & 6.28min	10
5	Inertsil ODS 3V	Tri-ethylamine (pH3.5): Acetonitrile (50:50 V/V)	2.837 & 4.953 min	11
6.	Inertsil ODS 3V	Methanol: Acetonitrile: Water (50:20:30 V/V))	4.41& 2.552 min	12
7	C18	Acetonitrile and ammonium di-hydrogen orthophosphate buffer (pH 7) (50:50V/V)	---	13
8	Kromasil C18	0.05 M phosphate buffer: acetonitrile (65:35 v/v)	2.63, & 5.94 min.	14
9	Phenomenex Luna C18	acetonitrile: methanol: Glacial acetic acid (pH3): ammonia (60:15:10:15)	8.03 & 6.22 min	15
10	HiQ Sil C18Wcolumn	acetonitrile: 0.005mol. /L tetra butyl ammonium hydrogen sulphate (70:30 v/v)	2.057 & 3.067 min	16
11	Kromasil C-18	Phosphoric acid buffer with Acetonitrile (70:30)	1.730 & 5.810minute	Proposed

MATERIALS AND METHOD:

All analytical works were performed on HPLC Agilent 1260 affinity 2, equipped with Quaternary constant flow pump, auto injector, UV-visible detector version 1260 and open Lab software 4.02. Kromasil C-18 Octadecyl silane (250mm×4.6mm×5µ) column forms stationary phase. pH Meter- Orion Star A211, Sonicator-PCI Analyst, Analytical balance: Mettler Toledo, Ortho-phosphoric acid (AR), Water (HPLC

grade), tri–ethylamine (AR), Acetonitrile (HPLC grade)

Preparation of Buffer pH 3.0: 1.6M of Ortho phosphoric acid was used as buffer for the preparation of mobile phase. The pH of the buffer was adjusted to 3.0±0.1 using tri-ethylamine solution.

Preparation of Mobile Phase: The mobile phase was prepared by mixing the Acetonitrile and buffer pH (3±0.1) in the ratio of 300:700 and then it was filtered with 0.45µm filter paper. It was then degassed by ultra-sonication for 10minutes.

Preparation of Standard Stock Solution: An accurately weighed quantity of 100mg of Ofloxacin (working standard) and about 150mg of Satranidazole (working standard) were transferred into a 100ml volumetric flask, add 50ml of Acetonitrile sonicate for two minutes and add 20 ml of buffer pH 3.0 and again sonicate for five minutes, dissolve and dilute to volume with buffer pH 3.0. Then again transfer 5ml of this solution into another 50ml volumetric flask and then the volume was further diluted up to the volume with mobile phase to produce 100µg/ml and 150µg/ml of Ofloxacin and Satranidazole respectively.

Optimized chromatography conditions:
RP-HPLC analysis was performed by isocratic elution with flow rate 1.5ml/min. The mobile phase consisting of buffer pH 3.0 and acetonitrile in the ratio of 700:300 to obtain well resolved peaks of Ofloxacin and Satranidazole as shown in Fig: 1. Injection volumes of 20µl each of standard solution was injected into the column. The detection wavelength and chromatographic run time were selected at 278nm and 10 minutes respectively.

Preparation of Standard Calibration Curve for Ofloxacin and Satranidazole:
The calibration curve of an analytical method was performed to know the range, to provide results that are directly, or through a mathematical transformation, proportional to the concentration of the analyte.3.5, 4.5, 5.0, 5.5, 6.0, 6.5, & 8mlof standard stock solution were taken in seven different 50ml volumetric flask and diluted up to mark with mobile phase to obtain final concentration of the drug shown in the below Table 1. The solutions were injected automatically and chromatograms were obtained. Calibration curve was drawn by plotting average peak area versus concentration. The linearity data of Ofloxacin and Satranidazole is shown in Table-1. The linearity curve of Ofloxacin and Satranidazole are shown in the below Fig 2 and 3 respectively. The linear regression equation of Ofloxacin is Y = 5,818,530.5172X + 1,127,661.7069 with r^2 = 0.999. The linear regression equation of Satranidazole is Y = 1,346,582.9216X + 91941.2712 with r^2 = 0.9998

Table 1: Linearity data of Satranidazole and Ofloxacin

Sl. No.	Satranidazole		Ofloxacin	
	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
1	0	0	0	0
2	10.5	14237597	7	42594296
3	13.5	18278372	9	54291117
4	15	20293168	10	60054798
5	16.5	22355486	11	65841799
6	18	24456488	12	70995260
7	19.5	26555498	13	75985263
8	24	32109123	16	93104141

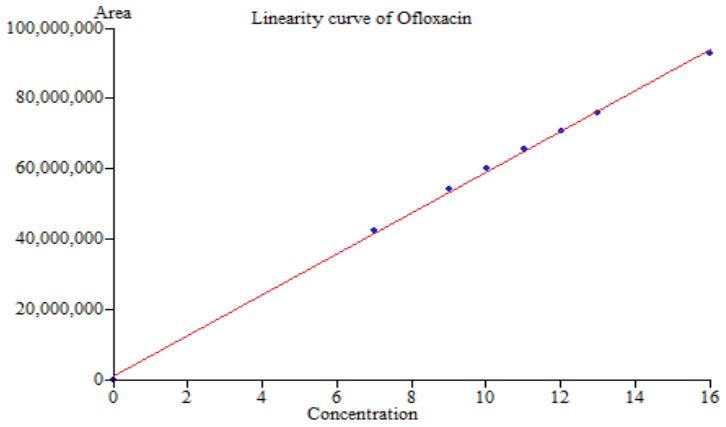


Fig 2: The linearity curve of Ofloxacin

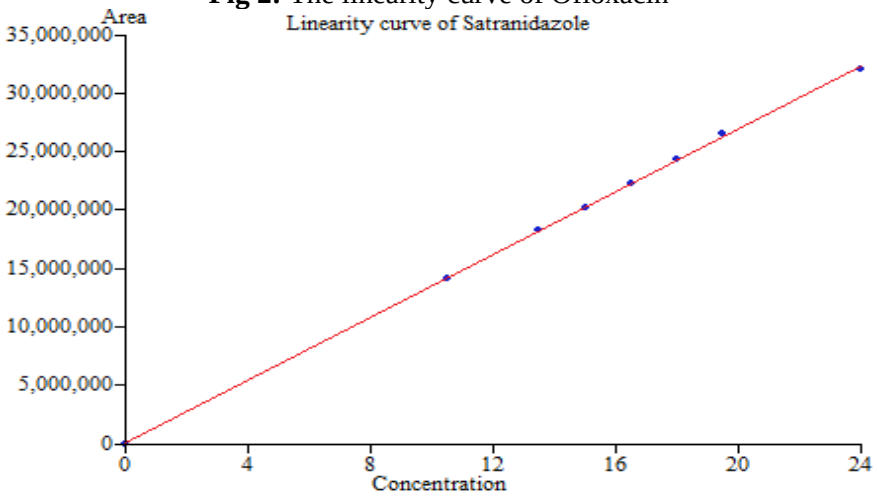


Fig 3: The linearity curve of Satranidazole

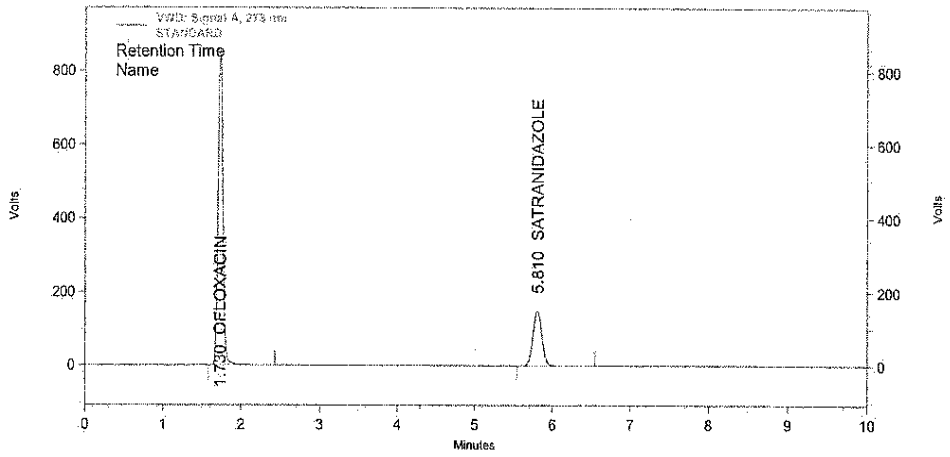


Fig: 4. Representative Chromatogram of Ofloxacin and Satranidazole (9 and 13.5µg/ml)

Assay of Tablets:

Sample Solution Preparation:

An accurately powder sample of tablet equivalent to 100 mg of Ofloxacin and 150mg of Satranidazole was transferred into a 100ml volumetric flask, add 50ml of Acetonitrile and sonicate for two minutes and add 20 ml of buffer pH 3 and again sonicate for five minutes, dissolve and dilute to volume with buffer pH 3.0. Transfer 5ml of this solution into another 50ml volumetric flask and then the volume was made up to the mark with the mobile phase. Then 3.5ml of this stock solution was transferred into another 50ml volumetric flask and then the volume was made up to the mark. This solution claimed to contain 7 and 10.5µg/ml of Ofloxacin and Satranidazole was injected and analyzed using proposed method to measure the area. Repeat the same procedure for 5 times and recorded the area. The concentration of the tablet sample solution was computed by utilizing the linear regression equations. The results of tablet analysis are given in the Table 2.

Table 2: Analysis of commercial Tablet (Satrogyl-O®) (*n=5)

Analyte	Label claim (mg/Tablet)	Amount Found (mg/Tablet)	C.I.	% RSD
Ofloxacin	200	200.230	100.153± 0.316	0.254
Satranidazole	300	300.272	100.091 ±0.1963	0.157

C.I.: Confidence Interval within which true value may be found at 95% confidence level = R ± ts/√n, R: Mean percent result of analysis (n = 5). Theoretical 't' values at 95% confidence level for n - 1 degrees of freedom t (0.05, 4) = 2.776

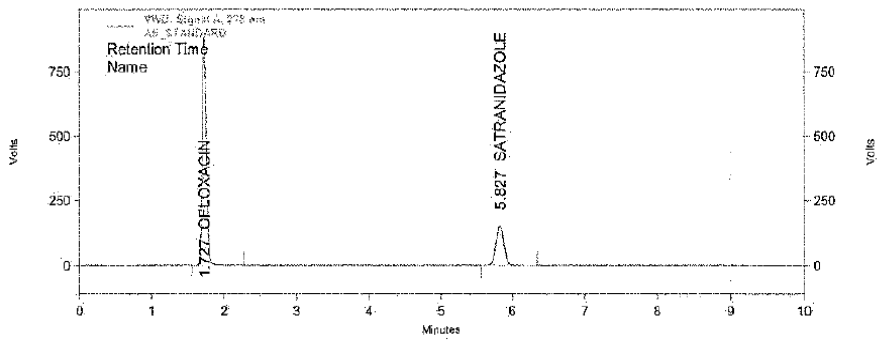


Fig 5: Representative Chromatogram of tablet sample of Ofloxacin and Satranidazole(7 and 10.5 µg/ml)

Method Validation:[6]

The proposed method was validated in terms of accuracy, linearity, precision, sensitivity and robustness in accordance with ICH Q2 (R1) guideline and system suitability test as per USP.

The system suitability test was carried out on 9 and 13.5µg/ml of Ofloxacin and Satranidazole respectively by five replicate injections. The system suitability results are given in the Table 3.

Table 3: The results of System suitability test

Parameter	Ofloxacin	Satranidazole	Acceptance range
Retention time (R_t)	1.730	5.810	---------
Asymmetric factor (As)	1.29	1.41	Not more than 1.5
Theoretical plate	5289	4270.169	Not less than 3000
Resolution (Rs)	____	2.67	more than 2

The sample and standard sample solution stability studies were done for a period of 48 hours at room temperature. The retention time and peak area of both the drugs i.e. Ofloxacin and Satranidazole have no much variation (%RSD less than 1.5). So it indicates that there was no significant degradation of both the standard and sample solution drug within 48 hours at room temperature. The representative chromatogram of tablet sample solution stability is shown in the Fig 6.

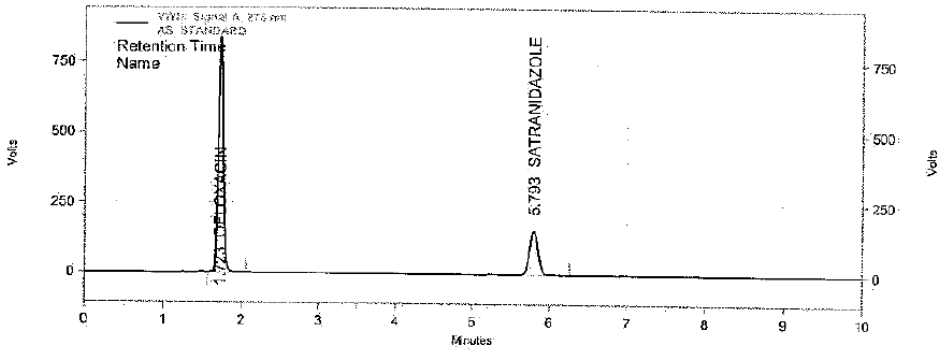


Fig 6: The representative chromatogram of tablet sample solution stability (within 24-48hour)

Precision was evaluated at system, method and intermediate precision levels. For repeatability analysis, six independent portions of a sample solution for Ofloxacin and Satranidazole (9µg/ml and 13.5µg/ml) were processed through the full analytical method and results were evaluated obtaining a %RSD value less than 0.1. Intermediate precision was evaluated with a new series of six portions of the same sample used in the repeatability assay, processed on a different day within three days. The corresponding % RSD was less than 0.3.

Specificity was performed by injecting of the placebo to demonstrate the absence of interferences with the signals of Ofloxacin and Satranidazole. On the other hand, the chromatogram of the solution of sample with the two compounds showed clear, compact and well separated peaks of Ofloxacin and Satranidazole,

as shown in Fig 4. No other peaks are eluted besides the two active compounds. Therefore, the method was considered specific.

The sensitivity of method was estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ). LOD and LOQ of the newly proposed method were calculated from the standard deviation of the response and slope of the calibration curve of drugs using the formula of ICH guideline, Limit of detection = $3.3 \times \sigma/S$ and Limit of quantitation = $10 \times \sigma/S$. Where, "σ" is standard deviation of y intercepts of regression lines, "S" is Slope of calibration curve.The LOD and LOQ of the method were calculated and found to be 0.59µg/ml and 1.44µg/ml of Ofloxacin and Satranidazole respectively.

The accuracy of the method was found out by recovery study using standard addition method. Known amounts of standard Ofloxacin and Satranidazole were added separately to pre-analyze samples at a level from 50 and 100 percent (4 replicate each concentration). The result of recovery for 16 such independent determinations for both Ofloxacin and Satranidazole by the proposed method are shown in Table 4. The Percent Recovery of labeled amount of Satranidazole and Ofloxacin was not less than 98.0% and not more than 102.0%. The recovery study results are shown in the Table 4.

Table 4: Results of recovery study Ofloxacin and Satranidazole

Analyte	Conc in Preanalyzed Sample (µg/ml)	Amount of drug added (µg/ml)	Amount of drug found (R) (µg/ml)	CI	%RSD	t
OFL	7	3.5	10.548	100.457±0.686	0.550	0.138
	7	7	13.956	99.68±2.49	1.866	0.724
SAT	10.5	5.25	15.842	100.584±2.26	1.812	0.513
	10.5	10.5	21.122	100.581±1.324	1.060	0.29

C.I.: Confidence Interval within which true value may be found at 95% confidence level = $R \pm ts/\sqrt{n}$, R: Mean percent result of analysis of Recovery study (n = 3). Theoretical't' values at 95% confidence level for n - 1 degrees of freedom t (0.05,3) = 3.182

Robustness study was conducted by deliberate changes in the chromatographic conditions and found to be unaffected by small changes like flow rate ±10%, pH of buffer ±0.2. The difference in average result between flow rate 1.35 and 1.65 and buffer pH 2.8 & 3.2 was not more than 2 % assay values.

RESULTS & DISCUSSION:

The method was tried by utilizing different types of mobile phase compositions and different ratios of buffer and acetonitrile i.e. 40+60, 50+50, 60+40. A mobile phase consisting of buffer pH 3.0 & acetonitrile in the ratio 70:30 gave a well resolved and sharp peak for Ofloxacin and Satranidazole with retention time 1.730 and 5.810 min respectively. The flow rate was 1.5ml/min and effluent was monitored at 278nm. A Kromasil Octadecylsilane C-18 column with run time was 10minutes temperature for the HPLC system was found to be best for analysis.

The protocols adopted in experimentation were successfully validated as per analytical procedures laid down in routine. The proposed method was validated by preliminary analysis of standard sample and by recovery studies. The percentage of average recoveries was obtained in the range of 99.1 to 100.1. The results of average recoveries obtained in each instance were compared with the theoretical value of 100 percent by means of Student's t-test. As the calculated 't' values are less than the theoretical t values (table-4), it is concluded that the proposed method were in 100 percent in agreement for each analyte. The lower limit of detection and limit of quantitation were found to be 0.59µg/ml and 1.44µg/ml respectively. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. Less than 1% RSD for all the parameters in the robustness study, this proves that method is robust.

All the system suitability parameter for both the drug passes the general acceptance criteria. The results of specificity studies indicated no interference from excipients, impurities and assured that the two peak response were due to the combination of two analytes or drug. The %RSD was less than 1 in intraday, inter-day precision, recovery and in each parameter of robustness.

CONCLUSION:

The combination formulation of Ofloxacin and Satranidazole is not official in Pharmacopoeia. So the proposed liquid chromatographic method can

be used for the routine concurrent analysis in combination formulations without going for individual drug determination separately as per established procedures. The proposed method is new, simple, accurate, precise, and robust and a have a great scope of adoptability in routine analysis in pharmaceutical industries and drug testing laboratories.

REFERENCES:

1. Mochizuki, Y., Oishi, M., Nishiyama, C., and Iida, T., Active leprosy treated effectively with ofloxacin., Internal medicine., **1997,** 35(9), 749-751.
2. Wankhede, S. B., Prakash, A., and Chitlange, S. S., Simultaneous spectrophotometric estimation of ofloxacin and satranidazole in tablet dosage form., Asian Journal of Research in Chemistry, **2008,** 1(1), 9-11.
3. Wankhede, S. B., Prakash, A., and Chitlange, S. S., Simultaneous spectrophotometric estimation of Satranidazole in tablet dosage form., Res. J. Pharm. Technol., **2008,** 1(4), 441-443.
4. Balammal, G., Saravana, K.A., Validated HPTLC Method for Simultaneous Estimation Of Ofloxacin And Satranidazole in a Tablet Dosage Form., International Journal of Innovative Drug Discovery., **2014,** 4(3), 170-176.
5. Patel, B. D., and Patel, S. A., Development and Validation of Dual Wavelength Spectrophotometric Method for Simultaneous Estimation of Ofloxacin and Prednisolone Acetate in Pharmaceutical Formulation., World Journal of Pharmaceutical Research., **2017,** 6(4), 1345-1354.
6. ICH guidance, Validation of Analytical method: definition and terminology. International Conference on Harmonization, Q2A: Geneva.
7. Boopathy, D., Prakash, M., Mathew, B., Kumar, S. S., and Perumal, P., Method development and validation of simultaneous determination of Ofloxacin and Satranidazole in pharmaceutical dosage form by RP-HPLC., Der Pharmacia Letter., **2010**, 2(2), 22.
8. Bhoir, S. I., Gaikwad, P. V., Parab, L. S., Shringarpure, R. N., Savant, S. S., and Verma, P. J., RP-HPLC method development and validation for the simultaneous estimation of satranidazole and ofloxacin in pharmaceutical dosage form., Journal of chromatographic science., **2011**, 49(1), 84-87.
9. Sachin, R. S., Suvarna, I. B., Namdev, S. P., Suman, B. Y., and Ashok, M. B., Simultaneous estimation of satranidazole and ofloxacin in tablet dosage form by high performance liquid chromatography., E-Journal of Chemistry., **2010**, 7(1), 198-202.
10. Godse, V. P., Bafana, Y. S., Deshapande, S. Y., Vyas, M. R., and Bhosale, A. V., Validated stability-indicating HPLC method for simultaneous estimation of ofloxacin and satranidazole from pharmaceutical dosage form., International Journal of Applied Biology and Pharmaceutical Technology., **2010**, 1(3), 1220-1229.
11. Rose, M. F., Peruboina, R., Hamid, H. E., and Parthipan, P., Development of new RP-HPLC method for the simultaneous estimation of Satranidazole and Oflaxacin pharmaceutical dosage form., Indian Journal of Research in Pharmacy and Biotechnology., **2017**, 5(5), 325-328.
12. Talha, M. A., and Noorulla, K. M., A New Method Development And Validation For The Simultaneous Estimation Of Satranidazole Altrexone And Ofloxacin By Rp-Hplc In Liquid Dosage Form., International Journal of innovative pharmaceutical sciences and research., **2017,** 5(8), 65-74. Doi: 10.21276/ IJIPSR. 2017.05.08.569.
13. Venkatachalam, T., Narendiran, S., Selvi, P. K., and Kumar, P. D., RP-HPLC method for simultaneous determination of ofloxacin and satranidazole in tablet dosage form., Asian Journal of Research in Chemistry., **2009,** 2(4), 469-471.
14. Sb, W., Prakash, A., and Chitlange, S. S., Simultaneous RP-HPLC estimation of Ofloxacin and satranidazole in tablet dosage form., Int. J. PharmTech Res., **2009**, 1(4), 1136-1138.
15. Sharma, M. C., and Sharma, S., Dissolution Studies and RP-HPLC Method for the Simultaneous Determination of Satranidazole and Ofloxacin in Pharmaceutical Dosage Form., Int. J. Chem. Tech. Resear, **2011,** 3(2), 1003-1007.
16. Godge R.K., Bhat L. R., Vora A. T., Damle M. C., Spectrophotometric and RP-HPLC methods for Simultaneous estimation of Ofloxacin and Satranidazole from tablets., ACAIJ., **2008,** 7(1), 48-53