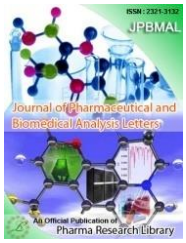


Journal of Pharmaceutical and Biomedical Analysis Letters
CODEN (USA): JPBAC9 | ISSN: 2347-4742
Home Page: https://pharmaresearchlibrary.org/journals/index.php/jpbmal
DOI: https://doi.org/10.30904/j.jpbmal.2025.4795
J. Pharm, Biomed. A. Lett., 2025, 13(1): 07-13

Development of a Robust RP-HPLC Method for the Analysis of Ofloxacin and Nitazoxanide in Fixed-Dose Combination Products

J. Chiranjeevi[1], Mahesh.M*[2], O. Sreenivasakrishna[2]
[1]Department of Pharmaceutical Analysis, JNTUA-Oil Technological and Pharmaceutical Research Institute, Jawaharlal Nehru Technological University Anantapur (JNTUA), Ananthapuramu-515001, Andhra Pradesh, India.
[2]Assistant Professor, Department of Pharmacy, JNTUA-Oil Technological and Pharmaceutical Research Institute, Jawaharlal Nehru Technological University Anantapur (JNTUA), Ananthapuramu-515001, Andhra Pradesh, India

A b s t r a c t
A new method was established for simultaneous estimation of Ofloxacin and Nitazoxanide by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Ofloxacin and Nitazoxanide by using Xterra C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wavelength was 255nm. The instrument used was Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.399mins and 3.907mins. The % purity of Ofloxacin and Nitazoxanide was found to be 100.7% and 101.4% respectively. The system suitability parameters for Ofloxacin and Nitazoxanide such as theoretical plates and tailing factors were found to be 1.3, 5117.5and 1.4, 3877.3 the resolution was found to be 5.31. In compliance with ICH criteria, the analytical procedure was verified (ICH, Q2 (R1)) 4. According to the linearity investigation, the concentration ranges of ofloxacin and nitazoxanide were 20µg–100µg and 10µg–50µg. The correlation coefficient (r2) was 0.999 and 0.999, and the percentage mean recovery was 100% and 100.5%. The repeatability RSD was between 0.2 and 0.4 percent. For intermediate precision, the corresponding RSDs were 0.5 and 0.1
Keywords: Ofloxacin and Nitazoxanide, RP-HPLC method

A r t i c l e I n f o

Corresponding Author:
Mahesh. M
Assistant Professor, Department of Pharmaceutical Analysis, JNTUA-OTPRI, Ananthapuramu, A.P, India
Mail ID: meghavath9@gmail.com

Article History:
Received : 14 Mar 2025
Revised : 31 Mar 2025
Accepted : 22 April 2025
Published : 05 May 2025

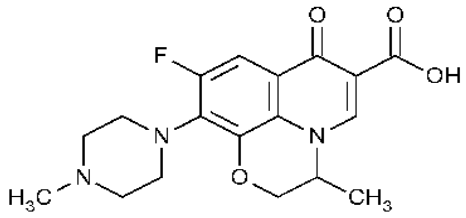
Citation: *J. Chiranjeevi, et al. (2025) Development of a Robust RP-HPLC Method for the Analysis of Ofloxacin and Nitazoxanide in Fixed-Dose Combination Products. J. Pharm, Biomed. A. Lett., 13(1): 07-13.*

C o n t e n t s

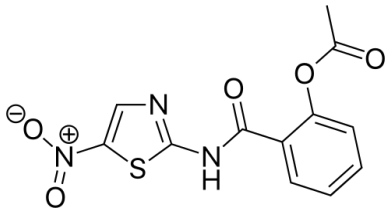
1. Introduction

Ofloxacin is a quinolone/fluoroquinolone antibiotic. Ofloxacin is bactericidal and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase. Ofloxacin with IUPAC name as 7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.0^{5} ,^{13}] trideca-5(13),6,8,11-tetraene-11-carboxylic acid having Chemical formula as $C_{12}H_9N_3O_5S$ and Molecular weight of 361.3675 g/mol. It is freely soluble in methanol and in lower alcohol Solvents but is practically insoluble in water. Its approximate elimination half-life is 9 h. It is marketed under the brand name Floxin. Nitazoxanide, a synthetic nitrothiazolyl-salicylamide derivative and anti-protozoal medication, is also marketed under the names Alania and Nizonide. It is

authorised 16 to treat infectious diarrhoea in individuals aged 1 year and up that is brought on by Giardia lamblia and Cryptosporidium parvum. 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethan-1-ol is its IUPAC designation; its molecular weight is 307.282g/mol, and its chemical formula is C18H20FN3O4. 11. It is quickly hydrolysed to its active metabolite, tizoxanide, which is 99% protein bound, after oral treatment. After delivery, peak concentrations are seen 1-4 hours later. It is insoluble in chloroform, only weakly soluble in methanol, and somewhat soluble in water. Its half-life elimination is roughly 7.3 hours.



a)



b)

Fig1: Chemical structure a) Ofloxacin and b) Nitazoxanide

For the simultaneous estimation, stability analyses, and impurity profiling of ofloxacin and nitazoxanide in pharmaceutical formulations, a number of RP-HPLC techniques have been created and approved. A high-performance liquid chromatographic analytical method was created and verified by Vania Maslarska et al. to detect ofloxacin and metronidazole in a synthetic mixture at the same time. 2. Using a LiChrosorb RP-18 (250 × 4.6 mm) column, an isocratic mode, and a mobile phase comprising 0.3% o-phosphoric acid solution (0.02:20:80 v/v/v), triethylamine, and acetonitrile, the separation was carried out at 30 °C. The eluent flow rate was 1.0 ml/min, and it was measured at 290 nm. For ofloxacin and metronidazole, the calibration curves were linear in the concentration range of 12.5 to 100.0 µg/ml, and the regression coefficients were greater than 0.999.

Ofloxacin and metronidazole had respective recovery rates of 100.01% and 100.04%. Selective fast accurate methods were developed by Soumya Jyoti Ghosh et al. for the simultaneous estimate and validation of Ornidazole and Ofloxacin. It was performed on an RP 18 column using a mobile phase that had a pH of 3.0 and a 40:60 ratio of acetonitrile to mixed phosphate buffer. Using a mobile phase of MeOH: 0.025M KH2PO4 adjusted to pH 3 using ortho-phosphoric acid (20:80, v/v) at room temperature, Mahmoud M. Sebaiy et al. created 10 an isocratic RPHPLC method for the rapid simultaneous separation and determination of sparfloxacin, gatifloxacin, metronidazole, and tinidazole in pure form or in the presence of some impurities in less than five minutes using Chromolith Performance RP-18e (100 2 x 4.6 mm). The maximum absorption was observed at 290 nm, and the flow rate was 4 ml/min. The retention times for sparfloxacin, gatifloxacin, metronidazole, and tinidazole were found to be 4.3, 3, 1.8, and 1.2 minutes, respectively, indicating shorter analysis times, and the standard curve was linear in the concentration range of 1-80 µg/mL for all medications.

Inference:

Based upon the literature review, a new method development was envisaged to improve the analytical parameters for Ofloxacin and Nitazoxanide and validation in formulations by RP HPLC method.

2. Methodology

2.1Materials: The materials used in the study comprised both Ofloxacin and Nitazoxanide sourced from KP labs pvt Ltd. Potassium dihydrogen phosphate ($KH_2 PO_4$) was obtained from MERCK. High-performance liquid chromatography (HPLC) grade water and methanol were supplied by MERCK, while acetonitrile for HPLC was procured from MERCK. Ortho-phosphoric acid was also sourced from MERCK.

2.2 Instruments:

The instruments used in the study included a WATERS HPLC system. Software: Empower, 2695 separation module.2996 PDA detector. A LABINDIA UV 3000+ UV/VIS spectrophotometer was employed for spectroscopic analysis, while pH measurements were conducted using Lab India pH meter. Sonicator (Model N0. Ultrasonic cleaner power sonic 420). Semi micro balance was Sartorius ME235P Additionally, Thermo lab GMP was used as Constant temperature water bath.

2.3 Preparation of Solutions:

Preparation of Phosphate buffer :(PH: 4.6):

Weighed 6.8 grams of KH_2PO_4 was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 4.6 with ortho phosphoric acid.

Preparation of mobile phase:

A mixture of pH 4.6 Phosphate buffer 300 mL (30%), 700 mL of ACN (70%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

Mobile phase is used as Diluent.

Preparation of the individual Ofloxacin standard preparation: 10mg of Ofloxacin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the individual Nitazoxanide standard preparation: 10mg of Nitazoxanide working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is

added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluent. (Stock solution).Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted up to the mark with diluent.

Procedure:

20µL of the standard, sample are injected into the chromatographic system and the areas for Ofloxacin and Nitazoxanide peaks are measured and the %Assay are calculated by using the formulae.

Preparation of standard solution (Ofloxacin and Nitazoxanide): Accurately weighed 10mg of Ofloxacin and 10mg of Nitazoxanide working standard were transferred into a 10mL and 100ml of clean dry volumetric flasks. About 7mL and 70ml of Diluents are added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further 3ml and 0.3ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluents.

Preparation of Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration): Accurately 5mg of Ofloxacin and 5mg of Nitazoxanide working standard were weighed and transferred into 10mL and 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock Solution). Further 3ml and 0.3ml of the above Ofloxacin and Nitazoxanide stock solution were pipetted into a 10ml volumetric flask and diluted up to the mark with diluent.

2.4 Methodology

The study involved developing an RP-HPLC method for the concurrent analysis of Rosuvastatin and Fenofibrate. A Waters HPLC system with a symmetry C18 Column (4.6x150mm x 5 µm) was used. The mobile phase comprised 30% phosphate buffer and 70% methanol, delivered at a flow rate of 1 mL/min. Detection was performed at 254nm using a UV detector. Sample preparation involved dissolving standard drugs in the mobile phase, followed by sonication and filtration. The method was validated for system suitability, linearity, Intermediate precession, precision, accuracy, robustness, LOQ and LOD per ICH guidelines. The method demonstrated good resolution, linearity, and reproducibility, making it suitable for pharmaceutical analysis.

Method Validation

The analytical method was validated as per ICH Q2 (R1) guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, LOD, and LOQ.

System suitability

In order to measure system suitability appropriateness characteristics, such as theoretical plates, retention time, tailing factor, and percentage RSD, the prepared standard solution is injected six times.

Linearity:

It is the method's ability to produce a linear response when the analyte concentration is within the predetermined range. For concentrations of 10% to 50% of Ofloxacin and 5% to 25% of Nitazoxanide, the linearity was tested. For both analytes, the coefficient of determination was 0.999.

Accuracy

The percentage recovery was found to be within the limit (97-103%). The results obtained for recovery at 80%, 100%, 120% are within the limits. Hence the method is accurate.

Precision

Five injections of nitazoxanide and ofloxacin were the subject of the precision investigation. 7.

A chromatographic system received each standard injection. The percentage RSD was calculated using the area of each Standard injection. The results are listed in Table 4 along with the chromatograms.

Intermediate precision/ruggedness:

Intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by using different make columns of same dimensions. %RSD of five different sample solutions should not more than 2. The %RSD obtained is within the limit, hence the method is rugged.

LOD and LOQ

Limit of Detection and Limit of Quantitation for the calculations that determined the limits of detection (LOD) and quantification (LOQ), the signal-to-noise ratio was set at 3:1 for detection and 10:1 for quantification.

Robustness

Robustness was assessed by slightly modifying the parameters of the approach and tracking the impact on the method through the results of system compatibility tests. The %RSD obtained for change of flow rate, variation in mobile phase was found to be below 1, which is within the acceptance criteria. Hence the method is robust.

3. Results and Discussion

The chromatographic method development for the simultaneous estimation of Ofloxacin and Nitazoxanide were optimized by several trials for various parameters such as different columns, flow rate and mobile phase, finally the optimized chromatographic method was selected for the separation and quantification of Ofloxacin and Nitazoxanide in API and pharmaceutical dosage form by RP-HPLC method optimized chromatogram shown in fig 2.

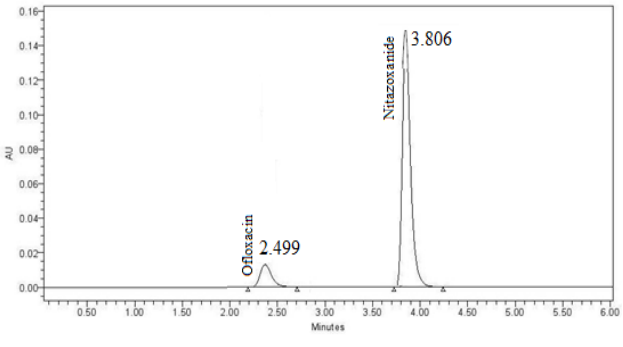


Figure 2: Optimized chromatogram of Ofloxacin and Nitazoxanide

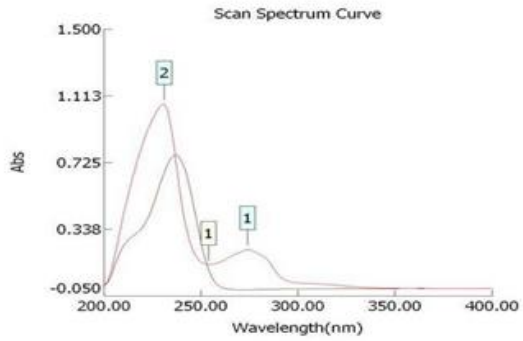


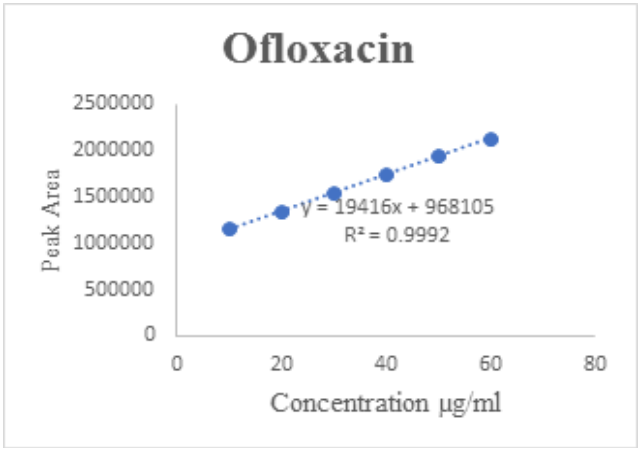
Fig. 2.1: Overlay spectrum of Ofloxacin and Nitazoxanide

System suitability:

System suitability is evaluated by injecting the prepared standard solution six times and analyzing parameters such as theoretical plates, retention time, tailing factor, resolution 7.1 and %RSD. All the results shown in table 1

Linearity:

The linearity study was performed for the concentration of 10µg/ml to 60µg/ml and 20 µg/ml to 120 µg/ml level. Each level was injected into a chromatographic system. The area of each level was used for calculation of correlation coefficient. The results are tabulated in Table. 2 Calibration graph for Ofloxacin and Nitazoxanide are shown in Fig. 3.



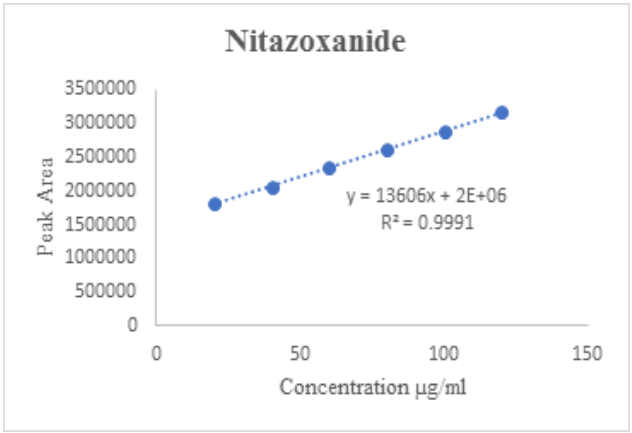


Figure 3: Calibration graph for a) Ofloxacin
b) Nitazoxanide

Accuracy:

To determine accuracy, three different concentrations of the analyte i.e., 50%, 100%, 150% were prepared separately while maintaining a constant amount of the marketed formulation. Chromatograms were recorded accordingly. The accuracy of the analytical method was evaluated based on the percentage of recovery. Each solution was prepared in triplicate, and the results are presented in Tables 3 and 4, and chromatogram shown in fig 4-6.

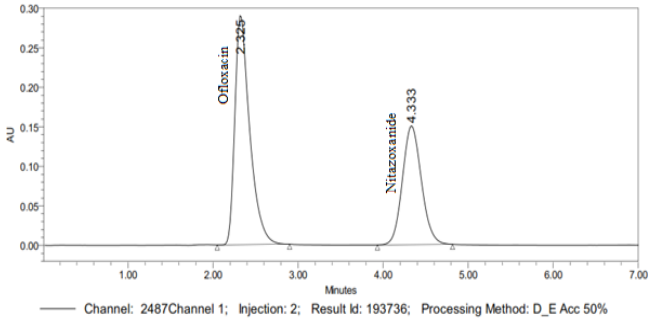


Fig. 4 Chromatogram showing accuracy 50%

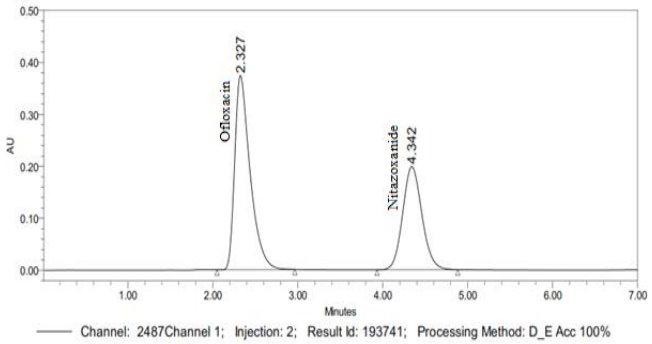


Fig 5 Chromatogram showing accuracy 100%

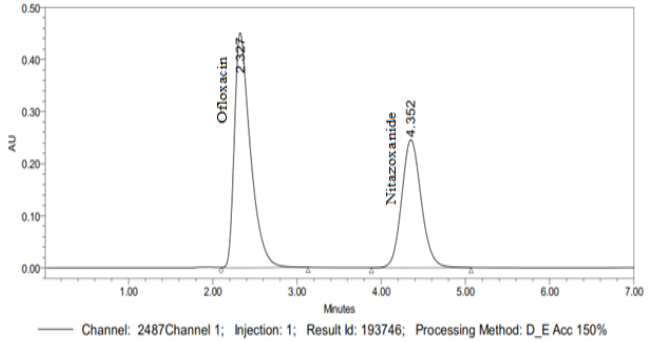


Fig 6 Chromatogram showing accuracy 150%

Precision:

The precision study was performed for five injections of Ofloxacin and Nitazoxanide. Each standard injection was injected into a chromatographic system. The area of each Standard injection was used for calculation of % RSD. The chromatograms are shown in results are tabulated in Table 4.

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

Table1: Results of system suitability parameters

Ofloxacin					Nitazoxanide				
Injection	**RT (min)**	**Peak area**	**TP**	**TF**	**Injection**	**RT (min)**	**Peak area**	**TP**	**TF**
1	2.5263	124652	1554.31	1.28	1	3.901	434308	4315.31	1.17
2	2.767	127376	1634.55	1.31	2	4.016	436736	4232.73	1.17
3	2.764	122803	1623.37	1.31	3	4.012	436821	4372.54	1.17
4	2.808	125382	1622.73	1.23	4	4.140	435350	4354.17	1.17
5	2.789	122153	1460.39	1.32	5	4.077	425462	4322.22	1.17
6	2.782	126345	1634.88	1.27	6	4.056	438085	4328.19	1.18
Mean		123634	-	-	Mean		44531.3	-	-
SD		631.0	-	-	SD		1257.3	-	-
%RSD		0.6	-	-	%RSD		0.3	-	-

Table 2: Results of Linearity by RP-HPLC method

Sr. No.	**Ofloxacin**		**Nitazoxanide**	
	Conc.range	**Peak Area response (mV)**	**Conc.range**	**Peak Area response (mV)**
1	10	1164173	20	1810101
2	20	1342535	40	2054287
3	30	1555931	60	2357133
4	40	1757973	80	2612279
5	50	1942319	100	2869778
6	60	2122991	120	3174561
Regression Equation		Y= 19416x + 968105	Y=13606x + 2E+06	
R^2		0.9992	0.9991	

Table 3: Accuracy results of Ofloxacin

%Concentration (at specification Level)	**Area**	**Amount added(mg)**	**Amount found(mg)**	**% Recovey**	**Mean Recovery**
50%	1332744	20	20.10	101.8%	100.5%
100%	1747697	40	39.99	99.9%	
150%	2118997	60	59.9	99.1%	

Table 4: Accuracy results of Nitazoxanide

%Concentration (at specification level)	**Area**	**Amount Added(mg)**	**Amount Found(mg)**	**% Recovery**	**Mean Recovery**
50%	2153867	40	40.0	101.3%	100.0%
100%	2535088	80	79.94	99.4%	
150%	3101798	120	119.8	99.2%	

Table 5: Precision results of Ofloxacin & Nitazoxanide

S. No.	**Ofloxacin**		**Nitazoxanide**	
	Intraday precision Area	**Interday precision Area**	**Intraday precision Area**	**Interday precision Area**
1	1501417	1456296	2235319	2194758
2	1486940	1457422	2240678	2195700
3	1490656	1456513	2249490	2196191
4	1487329	1454579	2245822	2195326
5	1490384	1451483	2251694	2200951
Mean	1491345	1455259	2244601	2196585
StdDev	5881.4	2347.6	6656.8	2496.0
%RSD	0.39	0.16	0.32	0.11

Table 6: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Ofloxacin	41	125	3.04
Nitazoxanide	41	121	2.95

Table 7: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Ofloxacin	41	412	10.0
Nitazoxanide	41	405	9.87

Table 8.a System suitability results For Ofloxacin (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	1748.5	1.22
2	1.0	1548.2	1.2
3	1.2	1948.0	1.2

Table 8.b System suitability results for *Nitazoxanide* (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	883.3	1.56
2	1.0	1234.0	1.1
3	1.2	969.2	1.6

Table 9.a System suitability results For Ofloxacin (Mobile Phase)

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	1748.5	1.22
2	Actual	1548.2	1.2
3	10% More	1948.0	1.2

Table 9.b System suitability results for Nitazoxanide (Mobile phase)

	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	883.3	1.56
2	Actual	1234.0	1.1
3	10% More	969.2	1.6

4. Conclusion

The simultaneous measurement of ofloxacin and nitazoxanide in fixed-dose combination products was accomplished with the development of a reliable and validated Reverse Phase 8 High-Performance Liquid Chromatography (RPHPLC) method. Using an Xterra C18 column and a mobile phase consisting of acetonitrile and phosphate buffer (pH 4.6), the procedure produced a clean separation with retention durations of 3.907 minutes for nitazoxanide and 2.399 minutes for ofloxacin. 20 The developed RP-HPLC technique satisfies all validation requirements as per ICH recommendations and is accurate, precise, linear, sensitive, robust, and appropriate for routine quality control analysis of ofloxacin and nitazoxanide in pharmaceutical formulations.

Acknowledgement

I am very thankful to Director, JNTUA-OTPRI, Ananthapuramu for providing the laboratory facilities, chemicals to carryout entire research work.

Conflict of Interest

We affirm that there are no conflicts of interest.

5. References

[1] G.R.Chatwal, S.K.Anand, Text book of Instrumental Methods of Chemicaln Analysis, Himalaya Publishing House, 5 Ed, 2002, p.2.566-2.570.

[2] G.W.Ewing, Text book of Instrumental Methods of Chemical Analysis, Mc Graw-Hill

Book Company, 5th Ed, p.375-385.

[3] B.K. Sharma, Textbook of Instrumental Methods of Chemical Analysis, GOEL publishing house, Meerut, 23 Ed, p.288-289.

[4] G.Vidyasagar, Textbook of Instrumental Methods of Drug Analysis, Pharmamed Press, 2009, p.106-120.

[5] H. H Willard, L. L Merritt, J. A Dean, and F. A Settle, Textbook of Instrumental Methods of Analysis, CBS publishers and distributors, New Delhi, 7 Ed,1986, p.592-596.

[6] H.H.Tackett, J.A.Cripe, G.Dyson, Positive displacement reciprocating pump fundamentals-power and direct acting types, Proceedings of the twenty-fourth international pump user's symposium,2008, p.45-58.

[7] D.A. Skoog, F.J.Holler, S.R.Crouch, Textbook of Instrumental Analysis,Brooks/Cole, Cengage Learning India Private Limited, 2007, p.900-906.

[8] R. E. Schirmer, Textbook of Modern Methods of Pharmaceuticals, CRC press,2 Ed, P.242-244.

[9] LR. Snyder, JJ Kirkland, LG.Joseph, Practical HPLC Method Development, Wiley Inter Science, New York, 2 Ed, 1997, p. 1-56.

[10] Ranjith singh, HPLC Method Development and Validation- an Overview, J Pharm. Educ. Res.4 (2013) 26-33.

[11] ICH: Q2B, Analytical Validation – Methodology (1996)

[12] Rapid RP-HPLC Method for Simultaneous Estimation of Sparfloxacin, Gatifloxacin, nitazoxanide and Tinidazole Asian J. Pharm. Res. 2011; Vol. 1: Issue 4, Pg 119-125

[13] Vania Maslarska1, rp-hplc method for simultaneous determination of metronidazole and ofloxacin in synthetic mixturemarch 23-25, 2016, prague, czech republic development and validation of spectroscopic method for the estimation of metronidazole and norfloxacin in syrup dosage form Revised on 29 March 2015,

[14] Soumya Jyoti Ghosh, Soumendra Darbar, Partha Pratim Chowdhury, Shyama Prasad Chattopadhyay and Matish Ranjan Chakraborty.Simultaneous determination and validation of Ofloxacin and Ornidazole in combined dosage pharmaceutical formulation. Int.J Pharm Tech Res. 2010; 2(1): 367-374.

[15] Pande V.V, Chandorkar J.G. A sensitive method HPLC method on determination of 2-methyl 5 nitro imidazole and reaction mass of intermediates of Ornidazole in Ornidazole bulk manufacturing. Int.J Pharm Tech Res. 2009;1(2): 310-312.