American Journal of Advanced Drug Delivery

www.ajadd.co.uk

Original Article

Stability Indicating RP-HPLC Method Development and Validation of Norfloxacin

Raghabaendra Narayan Singh*, Shisir Sahoo, Umashakar Mishra, Bamakanta Garnaik, Sudhir Kumar Sahoo, Deepak Hati

Laboratories of Royal College of Pharmacy and Health Sciences, Berhampur-760 002, Orissa, India.

Date of Receipt- 15/12/2013
Date of Revision- 27/12/2013
Date of Acceptance- 29/12/2013

Address for Correspondence
Laboratories of Royal College of Pharmacy and Health Sciences, Berhampur-760 002, Orissa, India.
Tel. +91-9218085658
E-mail: raghabendrasingh@gmail.com

ABSTRACT

A stability indicating high performance liquid chromatographic method has been developed for the determination of norfloxacin. Optimum separation was achieved in less than 10 minutes using Phenomenex ODS C_{18} (250x 4.6mm packed with 5µ) column. The analyte was resolved by using a mobile phase 20 mmol L^{-1} ammonium formate and acetonitrile (70:30) pH adjusted to 4.0 with formic acid at flow rate 1 ml/min on a isocratic high performance liquid chromatographic system at a wavelength of 280 nm. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, ruggedness and robustness and can be successfully applied for determination of this drug in commercial tablets. For stress studies the drug was subjected to acid, alkali and neutral hydrolysis, oxidation, dry heat and photolytic degradation. The degradation studies indicated the drug to be susceptible to acid, alkali hydrolysis and oxidative degradation. The analytical conditions and solvent developed provided good resolution within a short analysis time and economic advantages. The proposed method not required highly sophisticated and expensive instrumentation.

Keywords: Norfloxacin, RP-HPLC, Validation, Stability, Degradation.

INTRODUCTION

Norfloxacin is the first generation fluoroquinolone antibacterial drug. It was available in market for the treatment of urinary tract infection for many years. Chemically, it is 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid[1-4]. The bactericidal action of norfloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are

required for bacterial DNA replication, transcription, repair and recombination[5]. The mechanism of action of fluoroquinolones, including norfloxacin, is different from that of penicillins, cephalosporins, aminogly-cosides, macro-lides, and tetracyclines; therefore, microorganisms resistant to these classes of drugs may be susceptible to and other fluoroquinolones[6]. The focus of present study was to develop and validate a rapid, stable and economic RP-HPLC method for the estimation of norfloxacin in pure.

Several analytical methods for norfloxacin have been described in scientific literature such as UV spectrophotometry, liquid chromatography, high performance thin layer chromatography etc, amongst others[7-12]. The high performance Liquid Chromatography (HPLC) has become an important tool for the routine determination of anti microbial drugs, with specific emphasis on fluoroquinolones, in various animal products, antimicrobial activity, biological fluida, pharmaceutical products, with emphasis on fluoroquinolones[13-16].

In the literature there are some references about the determination of norfloxacin using HPLC methodology. Mascher *et al*. described a method for the determination of norfloxacin in human plasma and urine is described. Plasma samples were deproteinized using acetonitrile. The supernatant was analysed by C18 HPLC[17]. Espinosa-Mansilla *et al*. described the fluorescence emission of the fluoroquinolone norfloxacin. An HPLC method has been developed, for the determination of these fluoroquinolones, based in the separation of the formed irradiation photoproducts[18]. Christodoulou *et al*. proposed methods for the determination of norfloxacin in chicken muscle and egg yolk. Two different HPLC systems were used comparatively and the respective methods were fully validated[19].

Our investigation involved the optimization of the method described above using a reliable stability indicating and one new development, as wel as validating a simple, sensitive accurate and reproducible HPLC method for the determination of norfloxacin pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals & Reagents

Analytically pure Norfloxacin was obtained as a gift sample from Aurobindo Pharma, Hyderabad, India. Commercial tablet formulations were purchased from the local market. All chemicals and reagents used were of AR/HPLC grade, obtained from Merck, Qualigens and Loba Chemie.

Instrument

A High Performance Liquid Chromatographic system, with Spinchrom data handling system (Shimadzu-LC 2010) with Analytical Column- Phenomenex ODS C18 (250 X 4.6 mm, 5 micron particle size), equipped with quaternary isocratic pump, 2010C UV-VIS detector in isocratic mode was used for the analysis. Calibrated electronic single pan balance (Sigma 200/A Super), pH Meter (Thermo Fisher scientific), RK 102 CH Iiter 3,0 Ultrasonicator were also used during the analysis.

Chromatographic conditions

A reverse phase C-18 column was equilibrated with the mobile phase Ammonium formate: Acetonitrile (70:30) and pH adjusted 4.0 with formic acid. Mobile phase flow rate was maintained at 1ml/min and eluents were monitored at 280nm for norfloxacin. The sample was injected using a 20 µl fixed loop. The determination was performed at 30^0C for a run time of 10min.

Preparation of mobile phase and Standard Stock Solution

Mobile phase was prepared by mixing 700 ml of 20mM ammonium formate solution with 300 ml of HPLC grade acetonitrile to get the proportion of 70:30 v/v and finally the pH was adjusted to 4.0 with formic acid. The mobile phase was sonicated for 10 minutes and filtered through 0.45µ membrane filter. The standard stock solution of Norfloxacin was prepared by dissolving 50mg norfloxacin in 50ml of mobile phase to get a concentration of 1000µg/ml volume was made up to the mark.

Calibration curve for Norfloxacin

Appropriate aliquots of standard stock solution of the drug was taken in 10 ml volumetric flask and diluted up to the mark with mobile phase in such a way that final concentration of drug was the range of 10-150 µg/ml Norfloxacin respectively. The solution was injected using a 20 µl fixed loop system and chromatogram was recorded. Calibration curve was plotted by taking peak area on y-axis and respective concentration of drug on x-axis.

Method Validation[20-22]

1. **Linearity:** Various working standard solution was prepared and the linearity range was calculated from the observation.
2. **Accuracy:** The accuracy of the method was determined by calculating recoveries of drug by method of standard addition. Known amount of standard drug corresponding to 80%, 100%, and 120% of the label claim was added to pre quantified sample solution, and the amounts of drug were estimated by measuring the peak areas and by fitting these values to the straight line equation of calibration curve.
3. **Precision:** The intraday and interday precision studies of the drug was carried out by estimating the corresponding responses on the same day and consecutive three days respectively. The results were reported in terms of standard deviation and %RSD.
4. **Specificity:** The specificity of the proposed RP-HPLC method was determined by complete separation of two peaks with parameters like retention time (R_t), resolution (R_s) and tailing factor (T).
5. **Robustness:** Robustness of the method was studied by deliberate variations of the analytical parameters such as flow rate (1.0$\pm$0.1 ml/min), concentration of acetonitrile (30$\pm$2%).
6. **Ruggedness:** Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions, expressed as %RSD. The conditions include different laboratory conditions and different analysts.
7. **Sensitivity:** The sensitivity of the method was determined with respect to LOD and LOQ. Calibration curves were plotted by using concentration in the expected detection limit range (0.1-5 µg/ml) for each drug. The standard deviation of y-intercept of regression line was determined and substituted in the following equation for the determination of detection limit and quantification limit. Detection limit = 3.3 σ/s; quantification limit = 10 σ/s; where σ is the standard deviation of y-intercept of regression line and s is the slope of the calibration curve.

Forced Degradation Studies[20-22]

The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, thermal, photolytic, and UV degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the

degradation products from the pure active ingredient.

1. Degradation in Neutral Condition

About 10mg of pure drug was accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of acetonitrile. Then the volume was made up to the mark with water and kept at 70^0C. At different time interval solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

2. Degradation in Acidic Condition

About 10mg of pure drug was accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of acetonitrile. Then the volume was made up to the mark with 1N HCl and kept at 70^0C. At different time interval solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

3. Degradation in Basic Condition

About 10mg of pure drug was accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of acetonitrile. Then the volume was made up to the mark with 1N NaOH and kept at 70^0C. At different time interval solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

4. Oxidative Degradation

About 10mg of pure drug was accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of acetonitrile. Then the volume was made up to the mark with 6% w/v H2O2 and kept at 70^0C. At different time interval solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

5. Photolytic Degradation

About 100 mg of pure drug was taken in a clean petridish and exposed to day light. Sampling was done at an interval of 10h, 1week and 2weeks. From these samples, different solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

6. UV- Degradation

About 100 mg of pure drug was taken in a clean petridish and subjected to UV illumination of $1.2x10^6$ lux hours. Sampling was done at an interval of 12h, 24h, and 48h and from the sample, different solutions were prepared and 20µl of the sample solution was injected into the HPLC system.

7. Thermal Degradation

About 100 mg of pure drug was taken in three separate clean petridishes and subjected to dry heat at 70^0C. Sampling was done at intervals of 10 days, 20 days and 30 days. Solutions of the drug were prepared and 20 µl of the sample solution was injected into the HPLC system.

RESULTS AND DISCUSSION

Calibration Curve

The peak areas for the different concentrations (10-150 µg/ml) were recorded at 280 nm. The calibration curve (Figure 2) and the HPLC chromatogram (Figure 3) is shown in Table 1.

Accuracy

The percentage recovery was found to be in the range of 99.97% to 100.11% as shown in Table 2.

Precision

From Table 3, the %RSD for precision was found to be 0.59% and 0.73%.

Sensitivity

The LOD was found to be 0.35µg/ml and the LOQ was found to be 1.16µg/ml at 280 nm respectively.

Intraday and Interday Assay

The %RSD for Intraday and Interday Assay was found to be 0.72% to 1.35% and 0.64% to 1.48% respectively. Low values of %RSD indicate that the proposed method is accurate. The data is shown in Table 4 and 5.

Ruggedness

To evaluate ruggedness of the developed method, deliberate variations were made in the method parameters such as analysts and temperature of the system. The results are found to be %RSD of 1.01% to 2.57% as presented in Table 6.

Robustness

To evaluate robustness of the developed method, deliberate variations were made in the method parameters such as the flow rate of the mobile phase and ratio of mobile phase. The %RSD for different pH was 0.57% to 0.95% and flow rate was 0.03% to 1.49% are presented in Table 7 and 8.

Stability Results

The results obtained in acidic degradation, alkaline degradation, neutral degradation, thermal degradation, oxidative degradation, photolytic degradation and UV degradation are depicted as chromatograms and given in figure 4, 5, 6, 7, 8 and 9 respectively and represented in Table 9.

CONCLUSION

From the results of method development it is found that the developed method is simple, reliable, sensitive and accurate. The developed RP-HPLC method was found suitable for the analysis of selected drug in its pure and dosage form in presence of their respective degradants since the resolution between the drug with their corresponding degradants is better. The optimized chromatographic condition for the selected drug was a reverse phase C-18 column, mobile phase Ammonium Formate solution: Acetonitrile (70:30) pH adjusted to 4.0 with formic acid, flow rate was maintained at 1ml/min and eluents was monitored at 280nm for norfloxacin. The sample was injected using a 20 µl fixed loop. The determination was performed at 30^0C for a run time of 10 min.

The method was found to be fast, simple, reliable, sensitive, economical, accurate and precise. The method was found to be linear within the range of 10mcg/ml to 150mcg/ml with regression coefficient of 0.999. The method was found to be accurate with % recovery within for norfloxacin with the standard deviation and percentage standard deviation was less than 1. The method was found to be precise according to the repeatability data, intraday precision data and interday precision data with the standard deviation and % relative standard deviation less than 2. The method was rugged and robust with the standard deviation and % relative standard deviation less than 2.

Degradation studies for norfloxacin were carried out in different stress conditions. The drug was found to undergo 8.62%, 14.75 % and 18.9% degradation in neutral, acidic and basic stress conditions respectively. On oxidative degradation norfloxacin in presence of hydrogen peroxide showed 31.5% degradation after 11days. Norfloxacin was found to degrade up to 11.6% after 11days of exposure to day light. Degradation carried out in presence of UV light showed 12.5 % degradation after 11days. The thermal degradation study showed a degradation of 13.68% after 11days.

ACKNOWLEDGEMENT

The authors are grateful to Prof. (Dr.) P. N. Murthy, Royal College of Pharmacy

and Health Sciences, Berhampur, Odisha, India-760002 for their encouragement, support and providing all the necessary instrumental facility to carry out the research work.

We express sincere thanks to Prof. Sunakar Panda, Head, P.G. Dept. of Chemistry, Berhampur University, Bhanja Bihar, Berhampur, Odisha, given us constant support throughout the research work.

REFERENCES

1. "The Merck Index: An encyclopedia of chemicals, drugs, biologicals", 14th edition, USA: Merck & co, *Inc* 2006, pp1087.
2. "Indian pharmacopoeia 2010 vol.3", Ghaziabad: *The Indian Pharmacopoeia commission*, C 2007, pp-1793-1795.
3. "2007 The United States Pharmacopoeia 30/The National Formulary 25 vol-3", United States: *The united state pharmacopoeia convention*, C2006 pp2778-2779.
4. Florey, Klaus, "Analytical Profiles of Drug Substances, Vol. 20", Delhi: Academic Press/ an imprint of Elsevier, C2004, pp557-558.
5. "Goodman and Gillman's The Pharmacological Basis of Therapeutics", edited by Laurence, L. Brunton, 11th ed., Mc Graw Hill, New York, C2006, pp.1119-1122.
6. Rang, H.P.; Dale, M.M.; Ritter, J.M. and Flower, R.J., "Rang and Dale's Pharmacology", 6th ed., Churchill Livingstone, *Philadelphia*, 2007, pp.672-674.
7. Mascher H.J., Kikurta C., Journal of Chromatography A, 812, 1-2, 1998, 381-385.
8. Lee H.B., Peart T.E., Journal of Chromatography A, 1139, 1, 2007, 45-52.
9. Barbosa B., Berges R., Nebot V. S., *Journal of Chromatography A*, 719, 1, 1996, 27-36.
10. Xiao Y., Chang H., Hu J., Jia A., *Journal of Chromatography A*, 1214, 1-2, 2008, 100-108.
11. Rahman N., Ahmed Y. *European Journal of Pharmaceutics and Biopharmaceutics*, 57, 2, 2004, 359-367.
12. Simonovska, B.,Andrensek, S., Vovk, I. and Prosek, M., High-performance thin-layer chromatography method for monitoring norfloxacin residues on pharmaceutical equipment surfaces, *Journal of Chromatography A*, 862, 2, 1999, 209-215.
13. Gigosos P.G., Revesado P. R., *Journal of Chromatography A*, 871, 1-2, 2000, 31-36.
14. Galarini R., Fioroni L., Angelucci F., *Journal of Chromatography* A, 1216, 46, 2009, 8158-8164.
15. Simonovska B., Andren S., Vovk I., *Journal of Chromatography A*, 862, 2, 1999, 209-215.
16. Alkaysi H. N., Abdel-Hay, M.H, Sheikh Salem, M, Gharaibeh, A.M, Na'was, T.E, *International Journal of Pharmaceutics*, 87, 1-3, 1992, 73-77.
17. Mascher, H. J. and Kikuta, C., Determination of norfloxacin in human plasma and urine by high-performance liquid chromatography and fluorescence detection, *Journal of Chromatography A*, 812, 1-2, 1998, 381-385.
18. Espinosa-Mansilla, A., Peña, A. M., Gómez, D. G. and Salinas, F., HPLC determination of enoxacin, cipro-floxacin, norfloxacin and ofloxacin with photoinduced fluorimetric (PIF) detection and multiemission scanning: Application to urine and serum, *Journal of Chromatography B*, 822, 1-2, 2005, 185-193.
19. Christodoulou, E. A., Samanidou, V. F. and Papadoyannis, I. N., Validation of

an HPLC-UV method according to the European Union Decision 2002/657/EC for the simultaneous determination of 10 quinolones in chicken muscle and egg yolk, *Journal of Chromatography B*, 859,2, 2007, 246-255.

20. ICH, Q2A (R1) Validation of analytical procedure, *International Conference on Harmonization*, 1995.
21. ICH, Q1A (R2) Stability testing of new drug substances and products, *International Conference on Harmonization*, 2003.
22. ICH, Q2B Validation of analytical procedure: methodology, *International Conference on Harmonization*, 1997.

Table 1. Calibration Table of norfloxacin for RP-HPLC Method

Conc. (µg/ml)	PA1	PA2	PA3	PA4	PA5	PA6	Mean	Stdev	%Rsd
10	371.368	368.989	369.893	367.009	369.478	367.729	369.078	1.56	0.42
20	749.391	748.112	749.732	748.364	749.495	749.722	749.136	0.71	0.10
30	1048.190	1049.091	1049.205	1048.3	1046.56	1049.47	1048.470	1.06	0.10
40	1405.105	1406.890	1405.38	1405.21	1409.28	1404.39	1406.040	1.79	0.13
50	1811.76	1818.882	1812.79	1811.45	1816.32	1815.23	1814.403	2.92	0.16
60	2176.370	2174.441	2177.92	2175.33	2175.87	2174.21	2175.690	1.37	0.06
70	2600.881	2601.391	2601.39	2598.11	2598.09	2601.12	2600.161	1.61	0.06
80	2954.650	2952.361	2958.03	2957.45	2955.82	2958.77	2956.183	1.40	0.08
90	3351.631	3352.890	3354.55	3354.44	3353.03	3354.15	3353.451	1.14	0.03
100	3716.062	3717.090	3717.71	3718.01	3711.78	3718.34	3716.503	1.45	0.07
150	5649.931	5647.221	5644.11	5646.07	5647.31	5641.38	5646.012	1.95	0.05
								1.71	0.12

Abbreviation as follows:
Conc.= Concentration
PA= Peak Area (1, 2, to 6)
Stdev= Standard Deviation
Rsd= Relative Standard Deviation

Table 2. Accuracy Data of the RP-HPLC Method for Norfloxacin

No. of Preparations	Concentration (µg/ml)		% Recovery	Statistical Analysis
	Formulation	Pure Drug		
S1 : 80 %	30	24	100.405	Mean=100.11 SD=0.27 %Rsd=0.27
S2 : 80 %	30	24	99.894	
S3 : 80 %	30	24	100.017	
S4 : 100 %	30	30	99.884	Mean=99.97 SD=0.08 %Rsd=0.078
S5 : 100 %	30	30	99.997	
S6 : 100 %	30	30	100.034	
S7 : 120 %	30	36	99.992	Mean=100.08 SD=0.15 %Rsd=0.15
S8 : 120 %	30	36	100.251	
S9 : 120 %	30	36	100.002	

Abbreviation as follows:
S= Sample (1, 2 to 9)
SD= Standard Deviation
Rsd= Relative Standard Deviation

Table 3. Precision Data Showing Repeatability of the RP-HPLC Method for Norfloxacin

Sl. No	Concentration (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis
1	30	1066	29.7352	Mean=29.90 SD.=0.22 %Rsd=0.73
2	30	1068.44	29.7999	
3	30	1081.44	30.1435	
4	40	1459.26	40.1334	Mean=40.08 SD.=0.24 %Rsd=0.59
5	40	1447.47	39.8217	
6	40	1464.97	40.2844	

Abbreviation as follows:
Calc. Amt. = Calculated Amount
SD= Standard Deviation
Rsd= Relative Standard Deviation

Table 4. Intraday Precision Data of the RP-HPLC Method for Norfloxacin

Sl. No	Conc.(µg/ml)	Peak Area1	Peak Area2	Peak Area3	Statistical Analysis
1	30	1061.55	1079.53	1088.11	Mean=29.95 SD=0.22 %Rsd=0.72
2	30	1065.13	1081.34	1073.2	
3	30	1066.83	1074.12	1075.55	
	Mean	1064.5	1078.33	1078.95	
	Calc. Amt.	29.6957	30.0613	30.0778	
1	40	1451.55	1479.53	1438.11	Mean=40.29 SD=0.54 %Rsd=1.35
2	40	1445.13	1451.34	1451.2	
3	40	1466.83	1464.12	1445.55	
	Mean	1486.08	1464.99	1444.95	
	Calc. Amt.	40.8427	40.2852	39.7553	

Abbreviation as follows:
Conc.= Concentration
SD= Standard Deviation
Rsd= Relative Standard Deviation

Table 5. Interday Precision Data of the RP-HPLC Method for Norfloxacin

Sl. No	Conc.(µg/ml)	Day1	Day2	Day3	Statistical Analysis
1	30	1081.55	1089.53	1088.11	Mean=30.02 SD=0.19 %Rsd=0.64
2	30	1075.13	1061.34	1091.2	
3	30	1066.83	1062.12	1075.55	
	Mean	1074.5	1070.99	1084.95	
	Calc. Amt.	29.9601	29.8674	30.2365	
1	40	1477.49	1482.28	1438.02	Mean=40.18 SD=0.60 %Rsd=1.48
2	40	1455.72	1443.93	1451.62	
3	40	1433.81	1431.68	1442.12	
	Mean	1486.54	1452.63	1443.92	
	Calc. Amt.	40.8549	39.9583	39.7279	

Abbreviation as follows:
Conc.= Concentration
SD= Standard Deviation
Rsd= Relative Standard Deviation

Table 6. Ruggedness Data of the RP-HPLC Method by Different Analysts for Norfloxacin

Analyst-1				Analyst-2			
Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis	Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis
30	1071.3	29.88	Mean=30.21 SD.=0.29 %Rsd=0.97	30	1098.97	30.61	Mean=29.93 SD.=0.7 %Rsd=2.57
30	1088.6	30.33		30	1079.77	30.10	
30	1091.99	30.42		30	1041.78	29.09	
40	1459.26	40.13	Mean=39.90 SD.=0.20 %Rsd=0.51	40	1469.13	40.39	Mean=40.19 SD.=0.41 %Rsd=1.01
40	1447.47	39.82		40	1443.55	39.72	
40	1444.97	39.76		40	1471.04	40.45	

Abbreviation as follows:
Conc.= Concentration
Calc. Amt. = Calculated Amount
SD= Standard Deviation
Rsd= Relative Standard Deviation

Table 7. Robustness Data of the RP-HPLC Method at Different pH for Norfloxacin

pH-3.9				pH-4.1			
Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis	Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis
30	1098.01	30.58	Mean=30.27 SD.=0.41 %Rsd=1.35	30	1065.42	29.72	Mean=29.85 SD.=0.17 %Rsd=0.57
30	1068.75	29.81		30	1068.12	29.79	
30	1091.94	30.42		30	1077.66	30.04	
40	1459.19	40.13	Mean=40.19 SD.=0.13 %Rsd=0.33	40	1448.03	39.84	Mean=40.28 SD.=0.38 %Rsd=0.95
40	1467.23	40.34		40	1473.1	40.50	
40	1458.12	40.10		40	1472.99	40.50	

Abbreviation as follows:
Conc.= Concentration
Calc. Amt. = Calculated Amount
SD= Standard Deviation
Rsd= Relative Standard Deviation

Table 8. Robustness Data of the RP-HPLC Method at Different Flow Rate for Norfloxacin

Flow Rate 0.9ml/min				Flow Rate 1.1ml/min			
Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis	Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis
30	1078.06	30.05	Mean=29.71 SD.=0.30 %Rsd=1.02	30	1094.98	30.50	Mean=30.00 SD.=0.45 %Rsd=1.49
30	1059.91	29.57		30	1069.64	29.83	
30	1056.99	29.50		30	1063.07	29.66	
40	1449.32	39.87	Mean=40.08 SD.=0.20 %Rsd=0.51	40	1461.12	40.18	Mean=40.19 SD.=0.01 %Rsd=0.03
40	1457.41	40.08		40	1460.88	40.18	
40	1464.64	40.28		40	1461.73	40.20	

Abbreviation as follows:
Conc.= Concentration
Calc. Amt. = Calculated Amount
SD= Standard Deviation
Rsd= Relative Standard Deviation

Table 9. Stability Study Results of Norfloxacin

Conditions	Conc. (µg/ml)	Time	% Degraded
Acidic Degradation	100	11days	14.75
Alkaline Degradation	100	11days	18.9
Neutral Degradation	100	1 week	8.62
Thermolytic Degradation	100	11days	13.68
Oxidative Degradation	100	11days	31.5
Photolytic Degradation	100	11days	11.6
UV Degradation	100	11days	12.5

Abbreviation as follows:
Conc.= Concentration

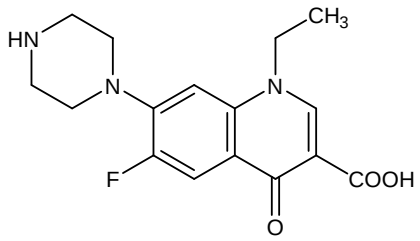

Figure 1. Chemical Structure of Norfloxacin

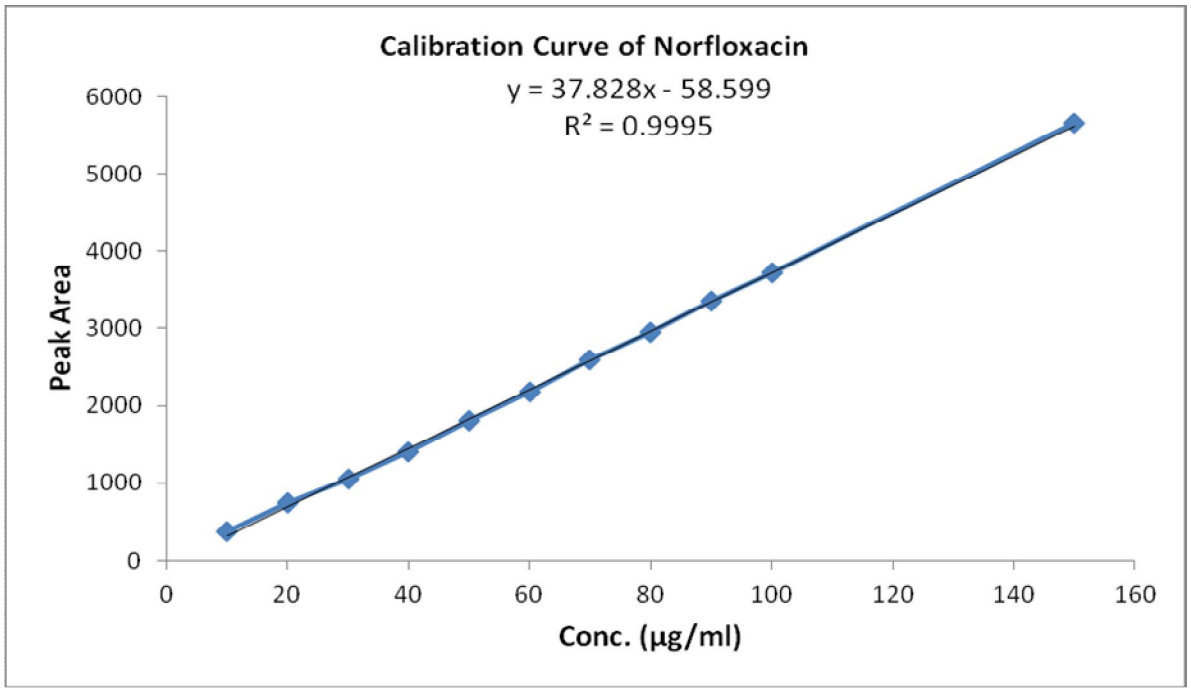

Figure 2. Calibration Curve of Norfloxacin

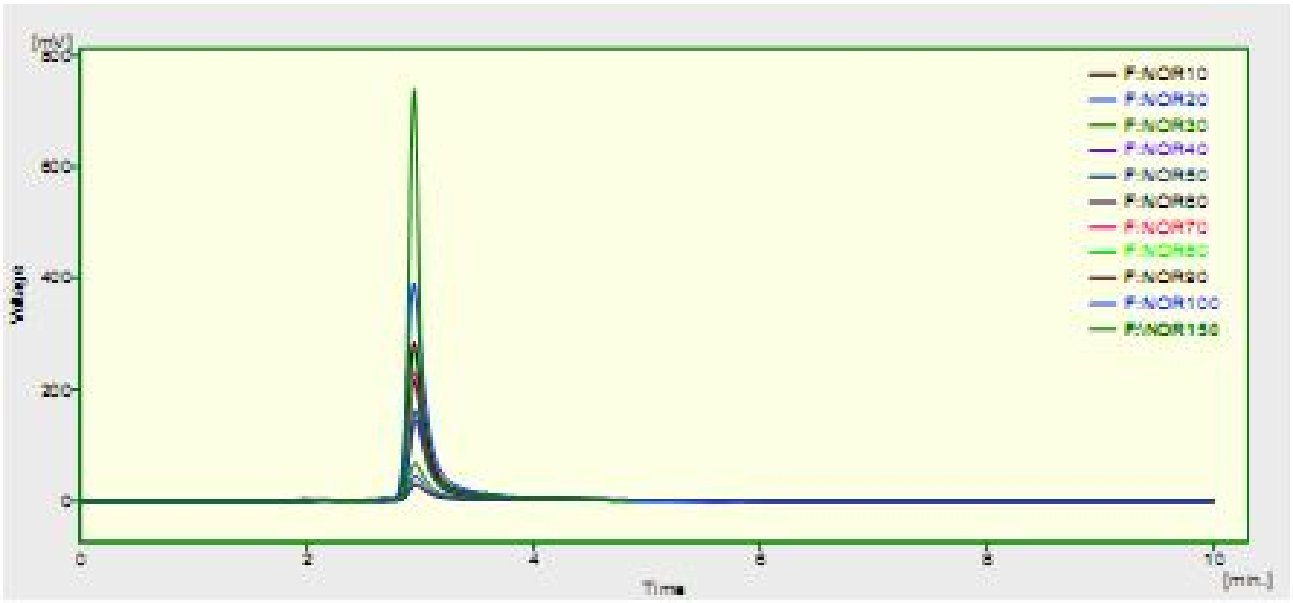

Figure 3. Typical HPLC Overlay Chromatogram of Norfloxacin

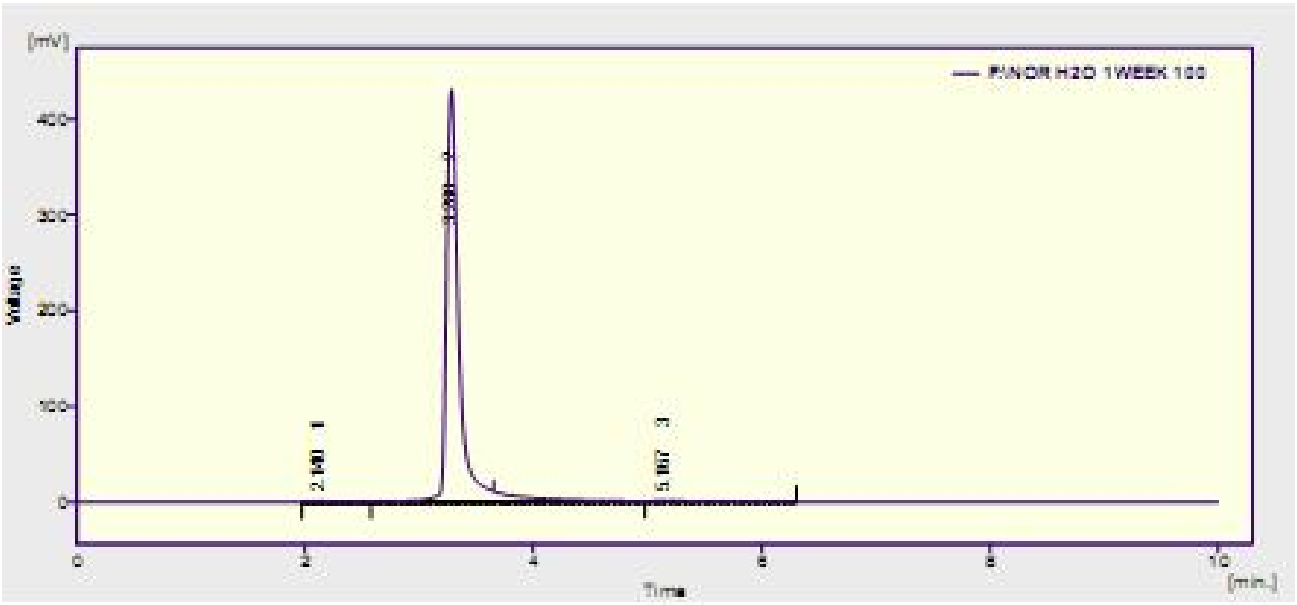

Figure 4. Representative Chromatogram of Hydrolytic Degradation of Norfloxacin

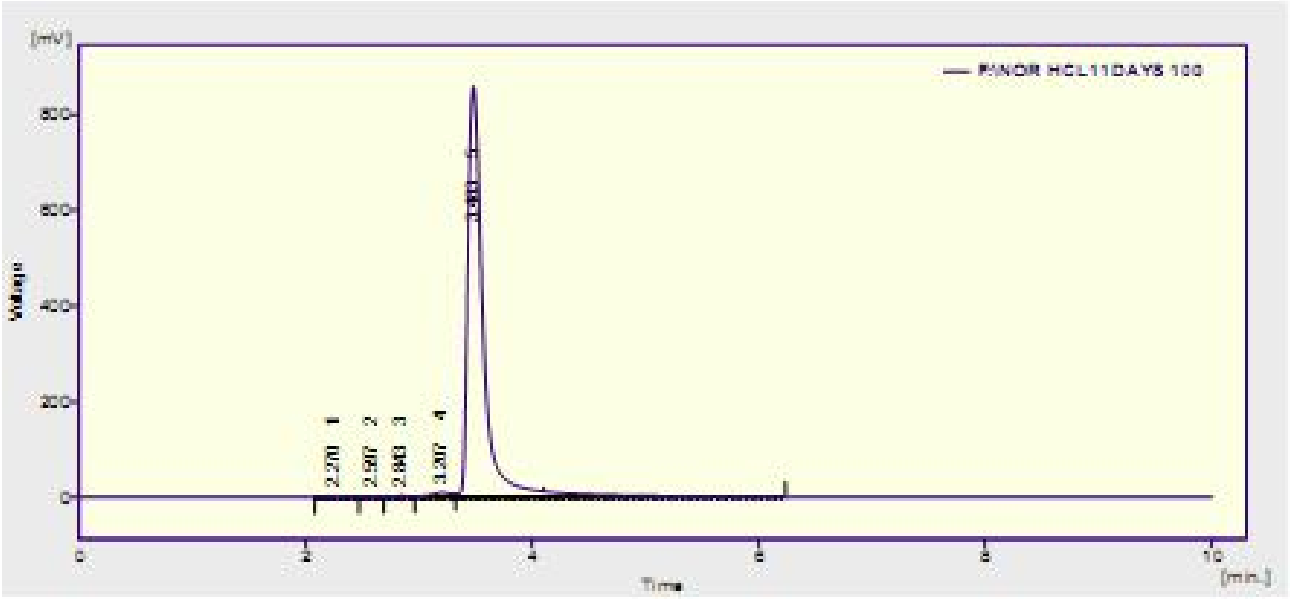

Figure 5. Representative Chromatogram of Acidic Degradation of Norfloxacin

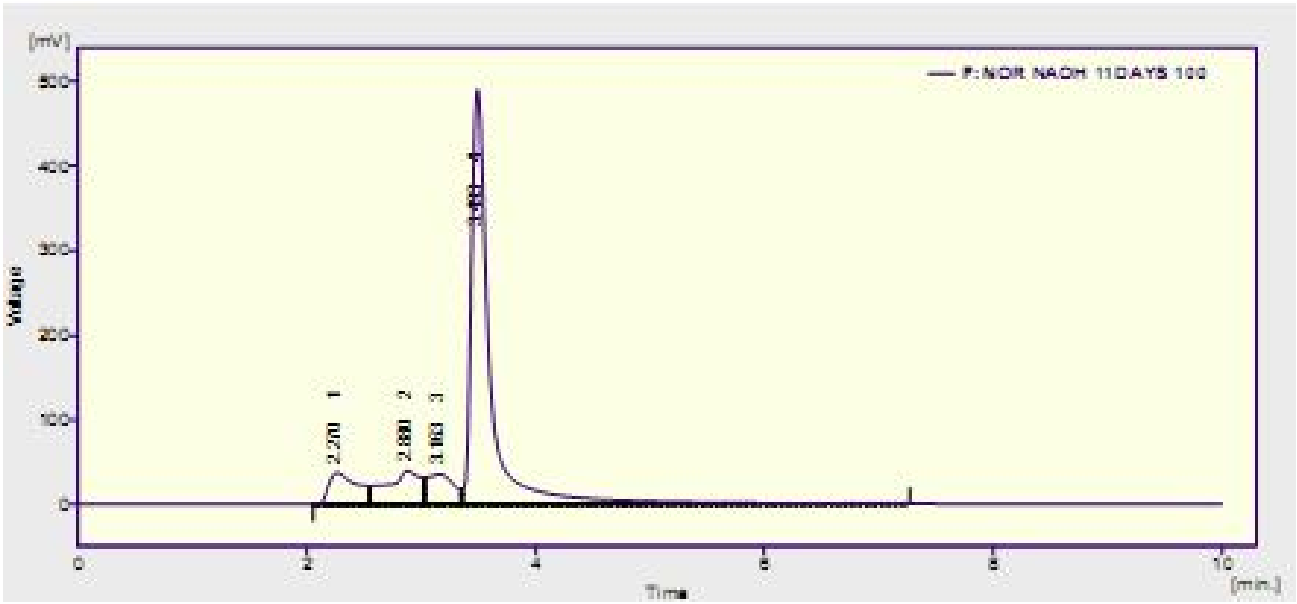

Figure 6. Representative Chromatogram of Basic Degradation of Norfloxacin

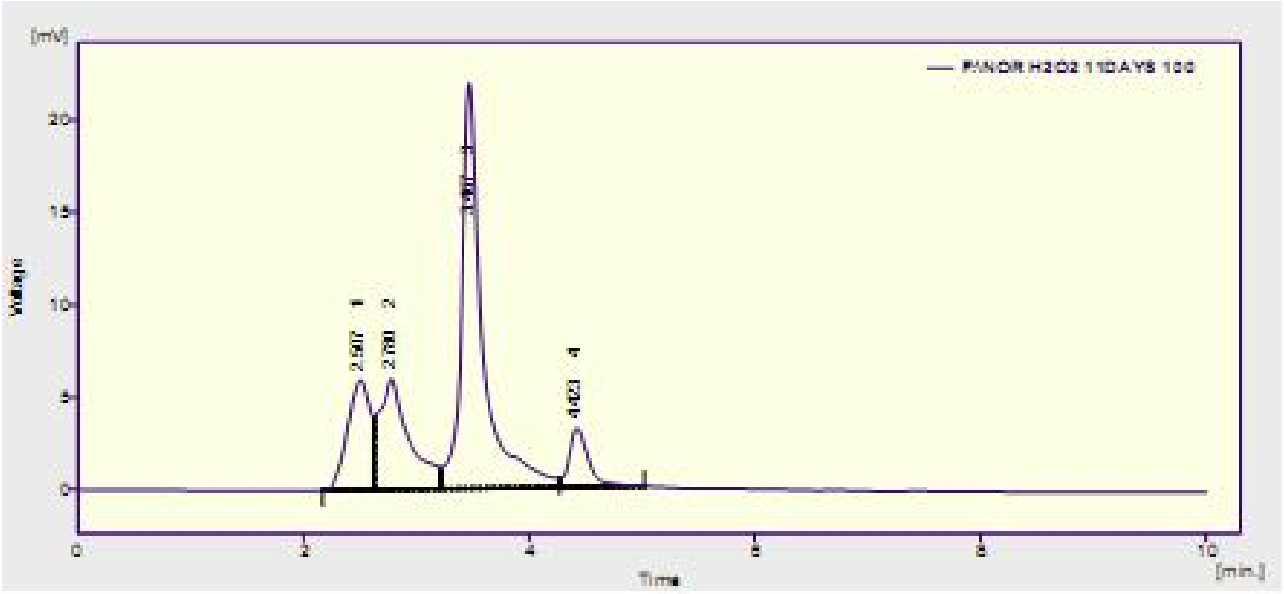

Figure 7. Representative Chromatogram of Oxidative Degradation of Norfloxacin

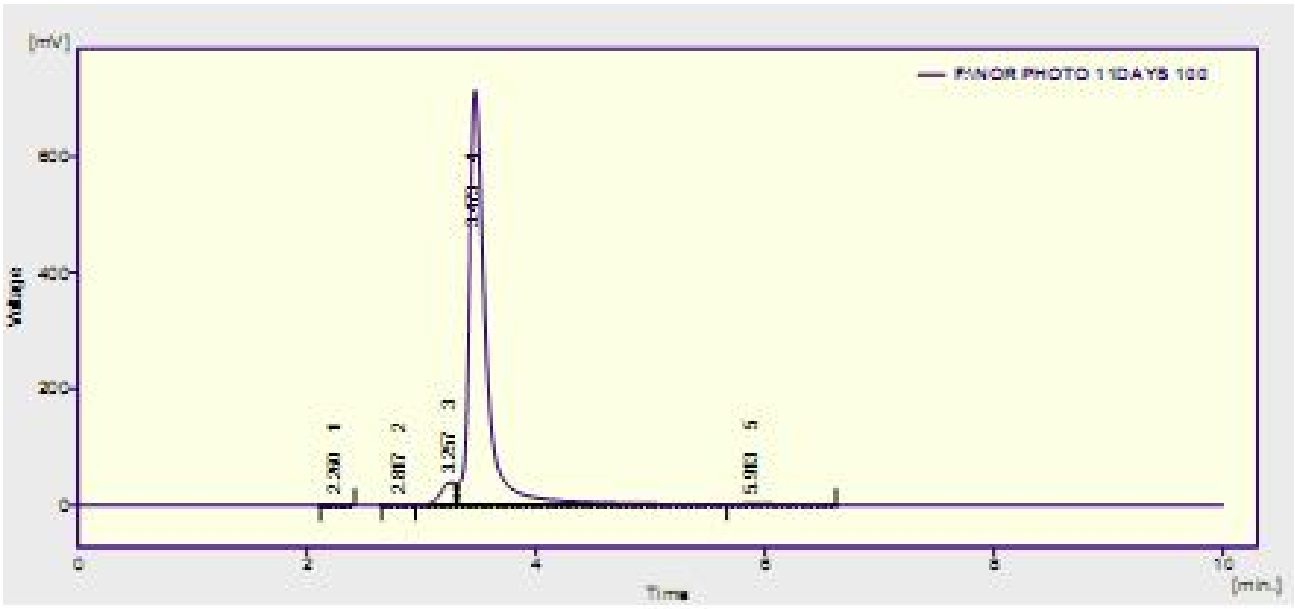

Figure 8. Representative Chromatogram of Photolytic Degradation of Norfloxacin

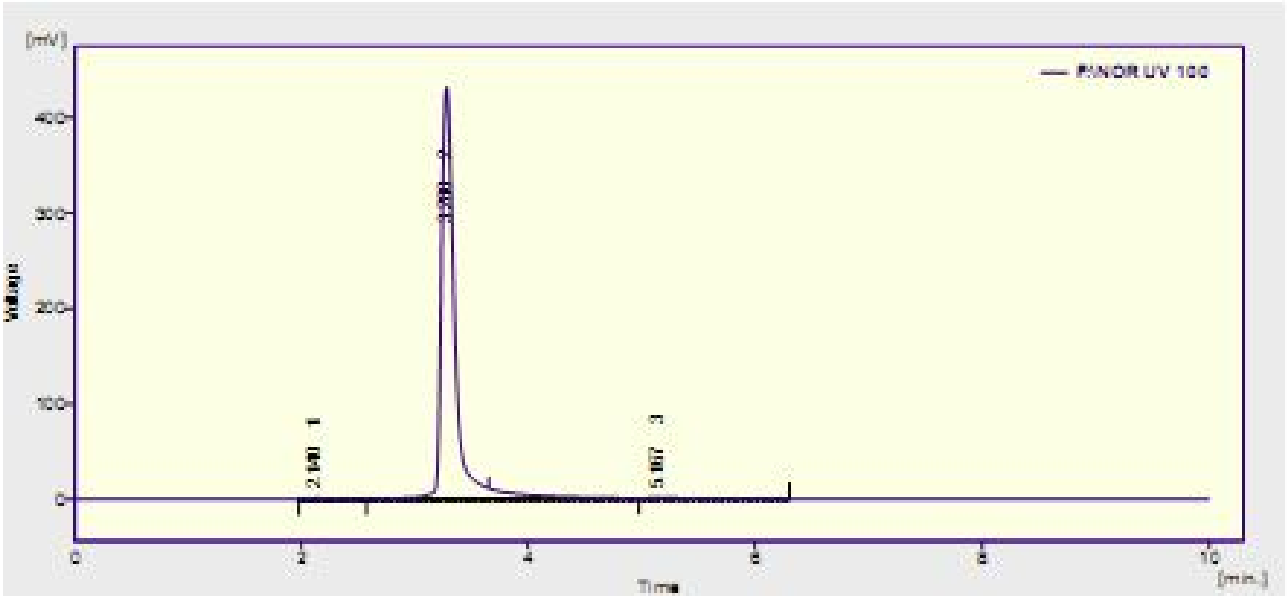

Figure 9. Representative Chromatogram of UV- Degradation of Norfloxacin

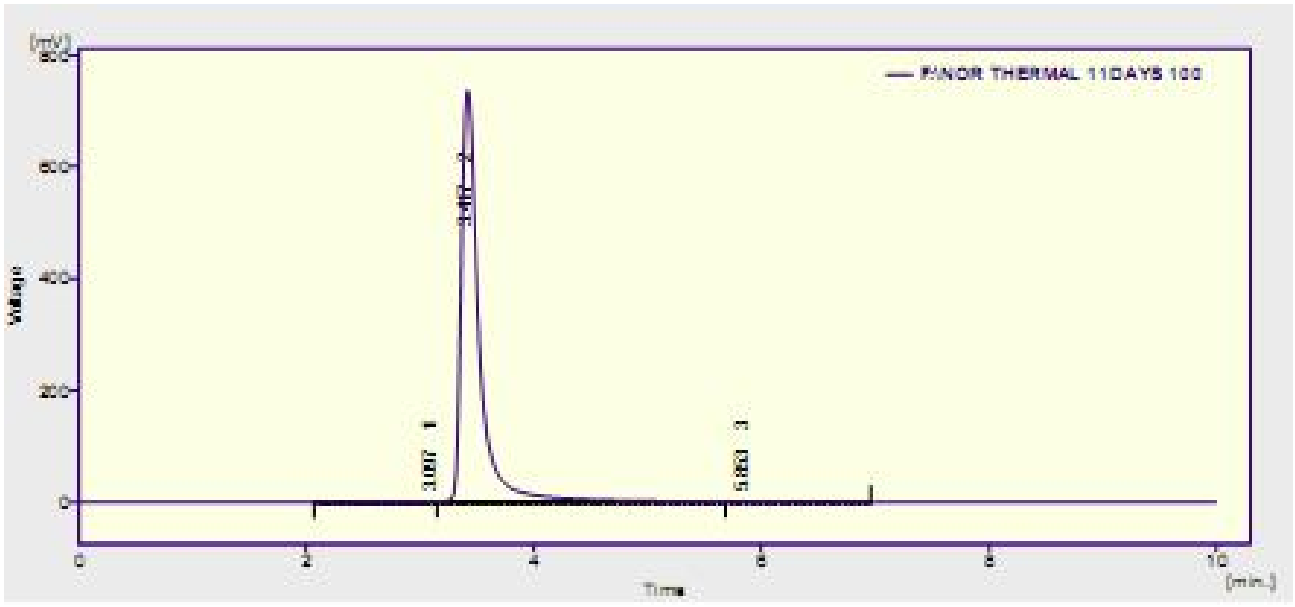

Figure 10. Representative Chromatogram of Thermal Degradation of Norfloxacin