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SIMULTANEOUS SEPARATION OF THIRD AND FOURTH GENERATION SIX FLUOROQUINOLONES BY ISOCRATIC HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: APPLICATION TO MOXIFLOXACIN DETERMINATION IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

This study was designed to develop and validate a simple, sensitive, precise, economical, reproducible and accurate isocratic reversed phase high performance liquid chromatographic method for separation and analysis of 6 fluoroquinolones in bulk and their pharmaceutical dosage forms. The effects of mobile phase composition, buffers, pH, and acetonitrile concentrations were investigated on the separation of third generation fluoroquinolones (levofloxacin, sparfloxacin, balofloxacin) and fourth generation fluoroquinolones (gatifloxacin, moxifloxacin, prulifloxacin) were assayed without any interference. RP-HPLC method was developed by using Welchrom C_{18} Column (4.6 X 250mm, 5µm), Shimadzu LC-20AT prominence liquid chromatograph. The mobile phase consisting of phosphate buffer pH-3.1 and acetonitrile in the portion of 70:30 v/v. Isocratic elution at a flow rate of 1ml/min was employed. The responses were measured at 293 nm using Shimadzu SPD-20A prominence UV-Vis detector. The method was successfully applied to Moxifloxacin pharmaceutical dosage form. During method validation parameters such as linearity, precision, specificity, robustness, ruggedness were evaluated from spiked tablet samples according to ICH guidelines, which remained within acceptable limits. The proposed method can also be extended for the determination of other five fluoroquinolones or their combinations. This method provides a fast simple method with good retention, excellent peak shape and high resolution.

KEYWORDS: Levofloxacin, sparfloxacin, balofloxacin, gatifloxacin, moxifloxacin, prulifloxacin.

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INTRODUCTION

The quinolones are a family of synthetic broad-spectrum antibacterial drugs[1,2]. The majority of quinolones in clinical have a fluorine atom attached to the central ring system, typically at the C_6 or C_7 position. Quinolones inhibit the topoisomerase II ligase domain, leaving the two nuclease domains intact. This modification, coupled with the constant action of the topoisomerase II in the bacterial cell, leads to DNA fragmentation. For many Gram-negative bacteria, DNA gyrase is the target, whereas topoisomerase IV is the target for many Gram-positive bacteria[3-5]. The six fluoroquinolones (Fig 1) are broad spectrum antimicrobials with potent activity against both Gram + ve and Gram – ve bacteria. Moxifloxacin, is 1-cyclopropyl-7-[(1S,6S)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy -4-oxo-quinoline-3-carboxylic acid, a fourth-generation synthetic fluoroquinolone antibacterial agent. It is effective against β-lactam and macrolide resistant bacteria and also for the treatment of acute bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis, community acquired pneumonia, complicated and uncomplicated skin structure infections, and complicated intra-abdominal infections[6]. The bactericidal action of moxifloxacin results from the interference with topoisomerase II and IV(DNA gyrase) Topoisomerases are essential enzymes that control DNA topology and assist in DNA replication,repair and transcription. Literature survey revealed that very few methods have been reported for the analysis of Moxifloxacin which include RP-HPLC[7-11], spectrophotometry[12], spectrofluorimetry[13], Lanthanide-sensitised Chemiluminescence[14], Voltammetry[15], Polarography[16], LC-MS/MS[17], Capillary Electrophoesis[18], Chiral liquid chromatography[19]. . Infact some times rate serious toxicity[20-22] associated with fluoroquinolones. Several HPLC methods had been developed for determination of these drugs individually or in combination with other drugs but no HPLC method for simultaneous estimation of these six drugs using C_{18} column with isocratic conditions has been reported till date. So the present research describes an isocratic reversed –phase high performance liquid chromatographic method for the rapid isolation of six fluoroquinolones namely levofloxacin, sparfloxacin, balofloxacin, gatifloxacin, moxifloxacin, prulifloxacin. The present paper also describes the quantification of fourth generation fluoroquinolones moxifloxacin. This method can also be extended for the determination of other five said fluoroquinolones. The method would help in assay of drugs in single run which reduces the time of analysis and does not require separate method for each drug. It can also be applied for routine analysis of either alone or of any combinations of the above mentioned drugs in dosage forms.

MATERIALS AND METHODS

Quantitative HPLC was performed on a high pressure gradient high performance liquid chromatograph (Shimadzu LC-20AT prominence liquid chromatograph) with two LC-20AT VP pumps, manual injector with loop volume of 20 µl (Rheodyne), programmable variable wavelength Shimadzu SPD-20A prominence UV-Vis detector and Welchrom C_{18} Column (4.6 X 250mm, 5µm). The HPLC system was equipped with “Spincotech” software. In addition an electronic balance (Essae-Teraoka Ltd.,) digital pH meter (Systronics model 802), Ultra sonic bath sonicator (spectra lab, model UCB 40), Double beam spectrophotometer (Systronics model-2203) were used in this study.

(i) Standards and chemicals used

Fluoroquinolone samples of balofloxacin and prulifloxacin were provided by Hetero Labs, levofloxacin and gatifloxacin by Aristo Pharma, sparfloxacin by Ananth Pharmaceuticals, and moxifloxacin by Torrent Pharmaceuticals. All chemicals were analytical grade. Potassium

dihydrogen orthophosphate and phosphoric acid from S.D Fine-Chem. Ltd., Mumbai, India. While acetonitrile (HPLC grade) and triethylamine (HPLC grade) from Merck Pharmaceuticals Private Limited, Mumbai, India. Commercial tablets of moxifloxacin were purchased from local market. Moxicip FC-400 (intra lab) manufactured by Cipla Ltd., Moxif-400mg tablets are manufactured by Torrent labs Ltd, India.

(ii) Preparation of mobile phase

A 10 mM phosphate buffer was prepared by dissolving 6.056 g of potassium dihydrogen orthophosphate in 445 ml of HPLC grade water. To this 55ml of 0.1M phosphoric acid was added and pH was adjusted to 3.1 with triethylamime. The above prepared buffer and acetonitrile were mixed in the proportion of 70: 30 v/v and was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

(iii) Preparation of calibration standards

About 100 mg of pure Moxifloxacin was accurately weighed and dissolved in 100 ml of mobile phase to get 1 µg/ml stock solution. Prepare five working standard solutions for calibration by adding defined volumes of the stock standard solution and diluting with mobile phase. The concentrations of Moxifloxacin are 2, 4, 6, 8, 10µg/ml, respectively. Similarly 10µg/ml of each standard fluoroquinolones were prepared from 1 mg/ml stock standard solutions of levofloxacin, prulifloxacin, gatifloxacin, sparfloxacin and balofloxacin respectively into each 10ml volumetric flask. Mix well.

(iv) Calibration curve of Moxifloxacin

Replicates of each calibration standard solutions 2, 4, 6, 8, 10 µg/ml were injected into the chromatogram, the retention times and average peak areas were recorded. The calibration data was presentede in table 4 . Calibration graph was plotted by taking concentration of moxifloxacin on X-axis and peak areas of standard moxifloxacin on Y-axis (Fig 9) and regression equations are computed Table 2. Retention times, peak areas and efficiency of six fluoroquinolones are presented in Table 3.

(v) Assay of Marketed Formulations of Moxifloxacin

The content of twenty tablets was transferred into a mortar and ground to a fine powder. From this tablet powder a quantity equivalent to 100 mg of moxifloxacin was taken and the drug was extracted in 100 ml of mobile phase. The resulting solution was filtered through 0.25 µm nylon membrane filter and degassed by sonication. This solution was further suitably diluted for chromatography. The test solutions were injected into the system by filling a 20 µl fixed volume loop manual injector. The chromatographic run time of 10 min was maintained for the elution of the drug from the column. The elutes were monitored with UV detector at 293 nm. The amount of drug present in sample was computed from the calibration graph. The results are presented in Table 5 and the standard and sample chromatograms of moxifloxacin are shown in Fig. 10 and Fig. 11.

(vi) Method Validation

The present study illustrates development and validation of simple, sensitive, precise and accurate RP-HPLC method for the determination of new antibacterial fluoroquinolone, Moxifloxacin in bulk samples and pharmaceutical tablet dosage forms as per ICH guidelines[20]. The accuracy of the method is determined by calculating percentage recovery of Moxifloxacin. Known amount of moxifloxacin at 80%, 100%, and 120% was added to a pre quantified sample solution. The recovery studies are carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery of moxifloxacin at each level is not less than 99% and not more than 101%. Precision of the method is performed as intraday precision and interday precision. To study the intraday precision, six-replicate standard solutions of moxifloxacin were injected. The percent relative standard deviation (% RSD) was calculated, and it was found to be 0.127, which was within the acceptable criteria of not more than 2.0. The limit of detection

(LOD) and limit of quantitation (LOQ) were calculated using following formulae: LOD= 3.3(SD)/S and LOQ= 10(SD)/S, where SD= standard deviation of response (peak area) and S= average of the slope of the calibration curve. The HPLC system was stabilized for 40 minutes. One blank followed by six replicates of a single calibration standard solution of moxifloxacin was injected to check the system suitability. To ascertain the systems suitability for the proposed method, a number of parameters such as theoretical plates, peak asymmetry, retention time and parameters were taken.

The effect of wide range of excipients and other additives usually present in the formulations of moxifloxacin in the determinations under optimum conditions were investigated. The specificity of the RP-HPLC method was established by injecting the mobile phase and placebo solution in triplicate and recording the chromatograms. The common excipients such as lactose anhydrous, microcrystalline cellulose, and magnesium stearate have been added to the sample solution and injected. While the comparison of chromatograms there was no interference from placebo with sample peak. They did not disturb the elution or quantitation of moxifloxacin. Furthermore, the sharp peaks also indicate the specificity of the method. Therefore, it was concluded that the method was specific. Robustness of the proposed methods is evaluated by making small changes in flow rate (± 0.2 mL/min), detection wave length(± 5nm), Mobile phase composition (±5%), and pH of the buffer solution. The results were found to be not affected by these small alterations. The parameters were within the limit, which indicates that the method had robust and suitability for routine use. The method was shown to be very robust with consistent retention times.

RESULTS AND DISCUSSION

The mobile phase consisting of phosphate buffer pH-3.1 and acetonitrile 70:30 v/v. Isocratic elution was carried out with an optimized flow rate of 1 ml/min which gave sharp peak and a minimum tailing factor with short run time. The wave length of detection was selected at 293 nm from UV overlain spectra of these drugs. The retention times for levofloxacin, sparfloxacin, balofloxacin, prulifloxacin, gatifloxacin, moxifloxacin and were found to be 3.613 min, 4.250 min, 4.703 min, 5.497min, 5.880 min, 6.253 min respectively. The retention times and peak areas of six fluoroquinolones are shown in Table 3. The calibration curve for Moxifloxacin was found to be linear over the range of 2-10 µg/ml. Linear regression data of proposed method of moxifloxacin is shown in Table 2. The developed method was applied to the assay of moxifloxacin tablets. The results were very close to labeled value of commercial tablets. The representative separation chromatogram of third generation and fourth generation fluoroquinolones are shown in Fig.2. The comparative evaluation of retention times and peak areas of fluoroquinolone chromatograms of these six fluroquinolone standards detailed separately are shown in Fig 3 to 8 and the Chromatogram results of combination of fluoroquinolones are shown in Table 3. The regression equation was found to be Y= 42.10x - 0.9521 with correlation coefficient is r^2 = 0.999 which indicates this method had good linearity. The calibration of the plot of moxifloxacin is shown in Fig 9. The representative chromatograms indicating the standard and Sample of moxifloxacin are shown in Fig 10 and Fig 11 respectively. The specificity was studied for the examination of the presence of interfering components, while the comparison of chromatograms there was no interference from placebo with sample peak. They did not disturb the elution or quantitation of moxifloxacin, furthermore the symmetric peaks also indicate the specificity of the method. Specificity results are presented in Table 6. Precision was studied to find out intra and inter day variations in the test methods of moxifloxacin for the three times on the same day and different day. The intra-day and inter-day precision obtained was % RSD (< 2) indicates that the proposed method

was quite precise and reproducible. The relative standard deviation of the retention from the six consecutive runs was calculated to be less than 0.12 for all six compounds. The precision data of proposed method are presented in Table 7 and Table 8 for intraday and interday respectively.

Recovery studies of the drug were carried out for the accuracy parameter at three different concentrations levels i.e. multiple level recovery studies. A known amount of moxifloxacin standard was added into pre-analyzed sample and subjected them to the proposed HPLC method. The percentage recovery was found to be within the limits. Generally the mean percentage recovery of moxifloxacin at each level was not less than 99% and not more than 101%. The percent recovery of moxifloxacin was found to be between 99.024% and 100.054%. The recovery study data are presented in Table 9.Robustness of the proposed method was determined by small deliberate changes in change in composition of mobile phase, flow rate, detection wave length etc,. It is observed that there were no marked changes in the chromatograms. Infact the parameters were within the limit and results were found to be not affected by these small alterations, which indicate that the method was highly robust and suitable for routine use. The Robustness results indicate that the selected factors remain unaffected by small variations of the parameters. The method was shown to be very robust with consistent retention times. The results of robustness are presented in Table 10. The limit of detection (LOD) and limit of quantitation (LOQ) was calculated based on the standard deviation (SD) of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ. The limit of detection (LOD) was 0.256µg/ml and the limit of quantitation (LOQ) was 0.777µg/ml which showed that this method was very sensitive.

Table 1
Optimized chromatographic conditions and system suitability parameters for proposed HPLC method for Moxifloxacin

Parameter	Chromatographic conditions
Instrument	SHIMADZU LC-20AT prominence liquid chromatograph
Column	WELCHROM C_{18} Column (4.6 X 250mm, 5µm)
Detector	SHIMADZU SPD-20A prominence UV-Vis detector
Diluents	10mM Phosphate Buffer(pH-3.1): Acetonitrile (70:30 v/v)
Mobile phase	10mM Phosphate Buffer(pH-3.1): Acetonitrile (70 : 30 v/v)
Flow rate	1ml/min.
Detection wave length	By UV at 293nm.
Run time	10 minutes
Column back pressure	128-130(kg/cm^2)
Temperature	Ambient temperature(25^oC)
Volume of injection loop	20µl
Retention time (R_t)	5.880 min (moxifloxacin)
Theoretical plates[th.pl] (Efficiency)	14,912
Tailing factor (asymmetry factor)	1.03

Table 2
Linear Regression data of the proposed method of Moxifloxacin

Parameter	Method
Detection wavelength(λ max)	By UV at 293nm
Linearity range (µg/ml)	2-10 µg/ml
Regression equation (Y=a+bx)	Y= -0.9521+42.10x
Slope(b)	42.10
Intercept(a)	-0.9521
Standard deviation of slope (S_b)	3.274900787
Standard deviation of intercept (S_a)	0.540832065
Correlation coefficient (r^2)	0.999
% Relative standard deviation* i.e., Coefficient of variation (CV)	0.12736
Limit of detection (µg/ml)	0.25669634
Limit of quantitation(µg/ml)	0.777867696
(Confidence limits) 0.005significance level 0.001 significance level	 0.2197 0.0052

Average of six determinations

Table 3
Chromatogram results of proposed combination of fluoroquinolones

Compound name	Peak ID	Retention time (min)	Asymmetry	Efficiency(theoretical plates)	Resolution
Levofloxacin	1	3.613	1.23	12306	—
Prulifloxacin	2	4.250	1.03	12354	4.508
Gatifloxacin	3	4.703	1.04	13115	2.866
Sparfloxacin	4	5.497	1.02	14711	4.604
Moxifloxacin	5	5.880	1.03	14912	2.056
Balofloxacin	6	6.253	1.06	15916	2.017

Table 4
Calibration data of the proposed HPLC method of moxifloxacin

S.No	Concentration, µg/ml.	Retention time, (R_t) min.	Peak area, mV.s.
1.	0	-	0
2.	2	5.882	84.488
3.	4	5.880	168.974
4.	6	5.882	243.462
5.	8	5.881	337.949
6.	10	5.880	422.436

Table 5
Assay results of moxifloxacin formulations

S.No	Formulations	Standard Peak area	Sample Peak area	Labeled amount	Amount found	% Assay ±RSD*
1	Moxicip FC (intralab)	423.346	423.112	400mg	399.77mg	99.945±0.056

Average of six determinations

Table 6
Specificity study

Name of the solution	Retention time (R_t) min.
Mobile phase	No peaks
Placebo	No peaks
moxifloxacin 10 µg/ml	5.880 min.

Table 7
Results of Precision study (Intraday)

Sample	Concentration (µg/ml)	Injection no.	Peak area	%RSD(acceptance criteria < 2.0)
Moxifloxacin	10	1	423.346	0.1263632
		2	422.478	
		3	422.789	
		4	421.956	
		5	422.587	
		6	421.932	

Table 8
Results of Precision study (Interday)

Sample	Concentration (µg/ml)	Injection no.	Peak area	%RSD(acceptance criteria < 2.0)
Moxifloxacin	10	1	423.346	0.131881
		2	422.487	
		3	422.798	
		4	423.956	
		5	422.537	
		6	422.932	

Table 9
Recovery data of the proposed Moxifloxacin by RP-HPLC method

Recovery level	Amount added (mg)	Total amount (mg)	Amount found (mg)	Amount recovered (mg)	% recovery	Mean % Recovery ± SD	%RSD[#]
80%	79.85	179.85	179.34	79.34	99.36	99.519± 0.495	0.4980
	79.89	179.89	179.95	79.95	100.07		
	79.78	179.78	179.08	79.08	99.12		
100%	99.92	199.92	199.94	99.94	100.02	99.853 ± 0.454	0.4549
	99.87	199.87	199.21	99.21	99.33		
	99.76	199.76	199.96	99.96	100.20		
120%	119.82	219.82	219.86	119.86	100.03	100.044± 0.009	0.0096
	119.88	219.88	219.94	119.94	100.05		
	119.9	219.9	219.96	119.96	100.05		

[#]acceptance criteria < 2.0.
**SD is standard deviation*
[#] % RSD is percentage of relative standard deviation.

Table 10
Robustness results of moxifloxacin HCl

S. no	Parameter [a]	Optimized	Used	Retention time (t_R), min	Plate count[$]	Peak asymmetry[#]	Remark
1.	Flow rate (±0.2 ml/min)	1.0 mL/min	0.8 mL/min	6.020	15276	1.028	*Robust
			1.2 mL/min	5.642	14724	1.036	*Robust
2.	Detection wavelength (±5 nm)	235 nm	230 nm	5.854	14927	1.032	Robust
			240 nm	5.877	14908	1.029	Robust
3.	Mobile phase composition (±5 %)	50:50, v/v	55:45, v/v	5.996	15142	1.028	*Robust
			45:55, v/v	5.624	14736	1.034	*Robust

Acceptance criteria (Limits):
[#]Peak Asymmetry < 1.5, [$] Plate count> 3000
****significant change in Retention time.***

Table 11
Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection(LOD)	0.25669634 µg/ml
Limit of Quantitation(LOQ)	0.77786769 µg/ml

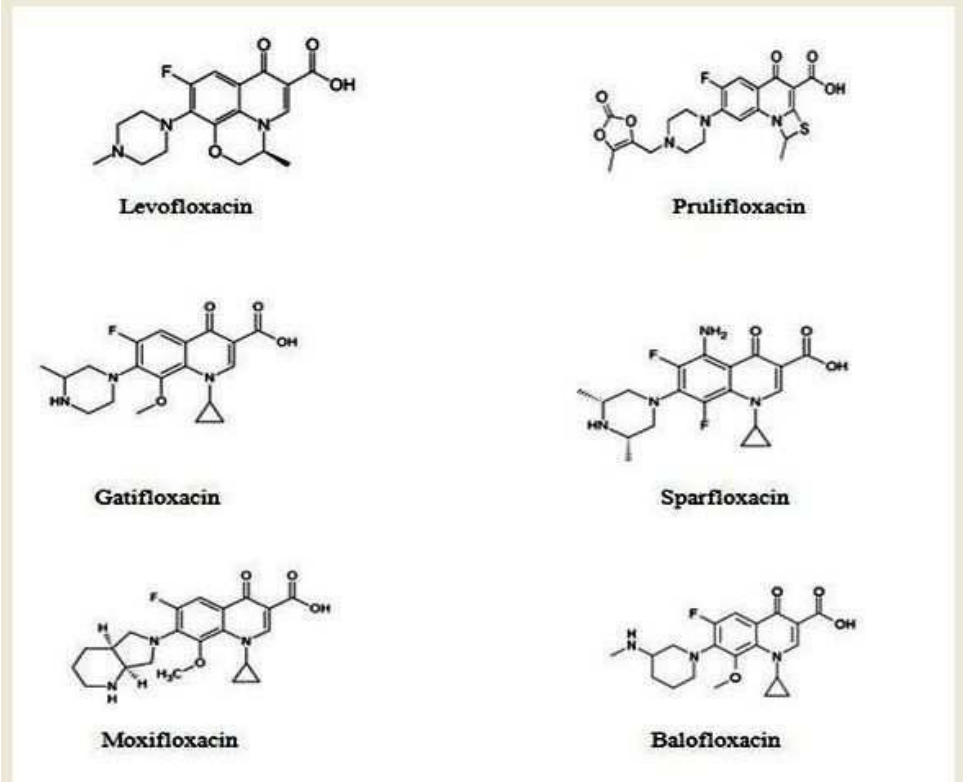

Figure 1
Chemical structures third and fourth generation fluoroquinolones investigated in this study

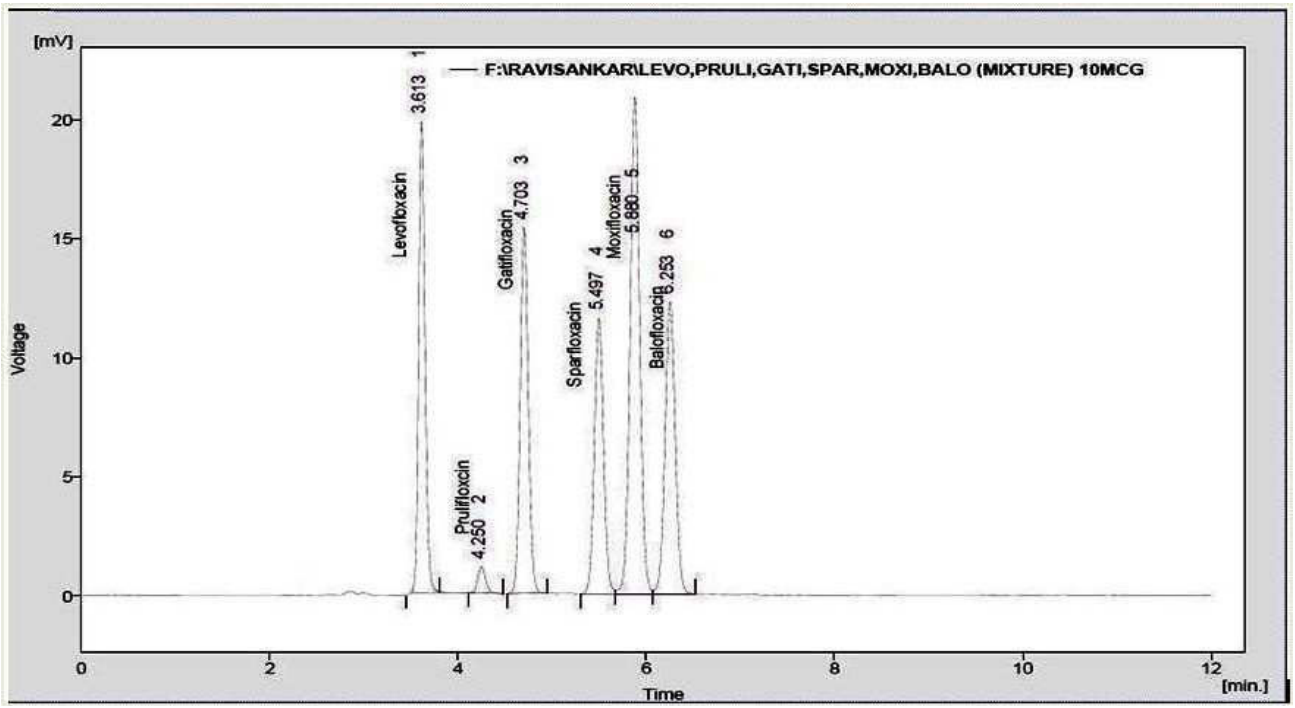

Figure 2

The separation of the six III[rd] and IV[th] generation fluoroquinolones anti-bacterials. The peaks at 3.61 min, 4.25 min, 4.70 min, 5.49 min, 5.88 min, 6.25 min corresponds to levofloxaxin, prulifloxacin, gatifloxacin, sparfloxacin, moxifloxacin, balofloxacin respectively.

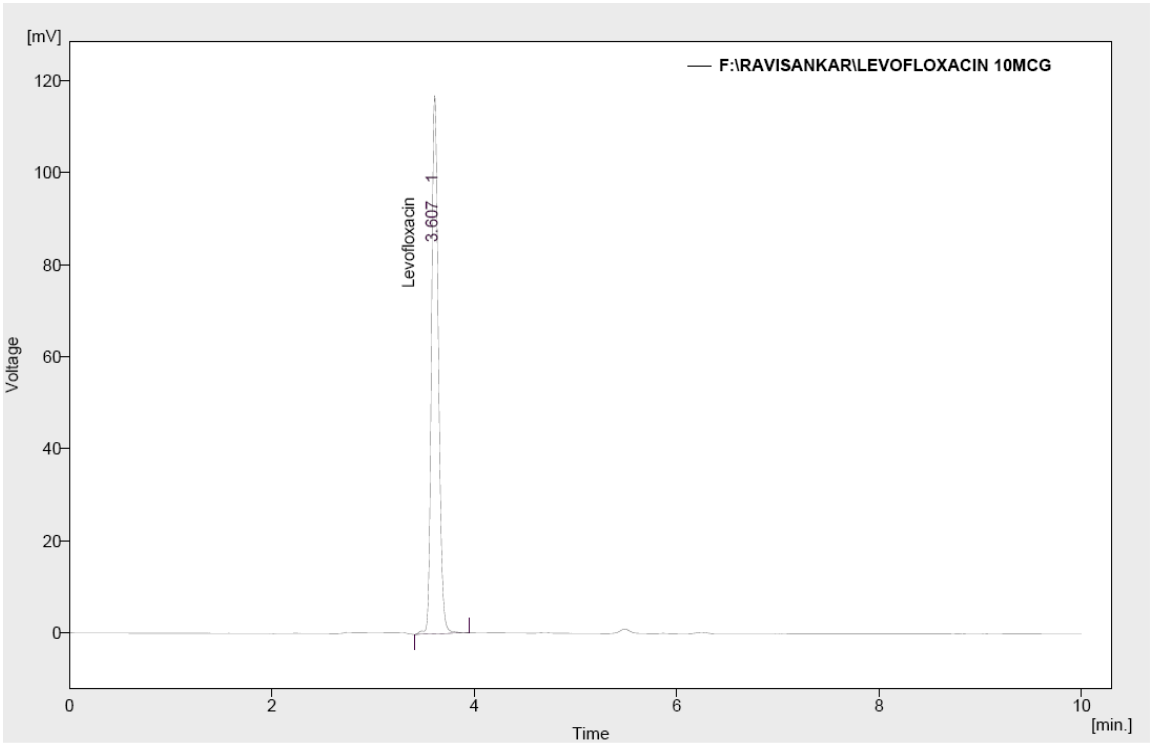

Figure 3

Standard chromatogram of levofloxacin 10 µg/ml.

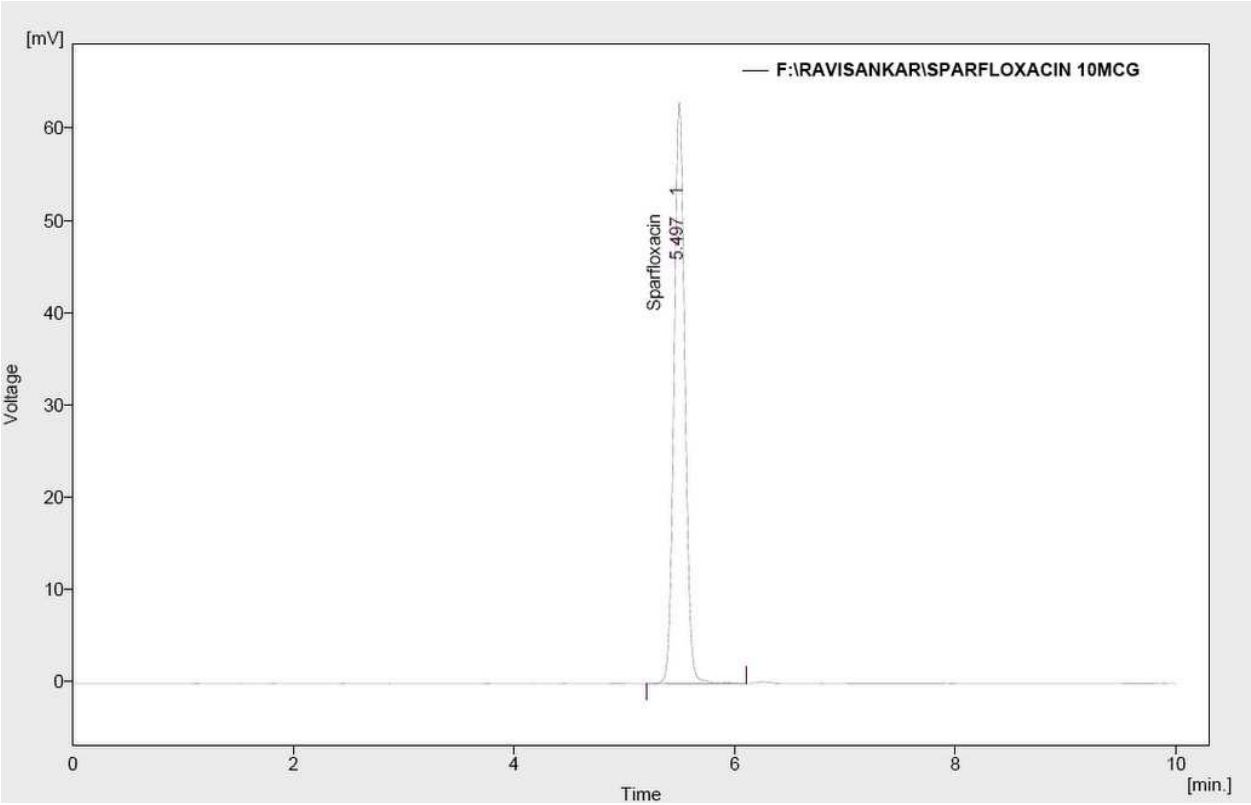

Figure 4
Standard chromatogram of sparfloxacin 10 µg/ml.

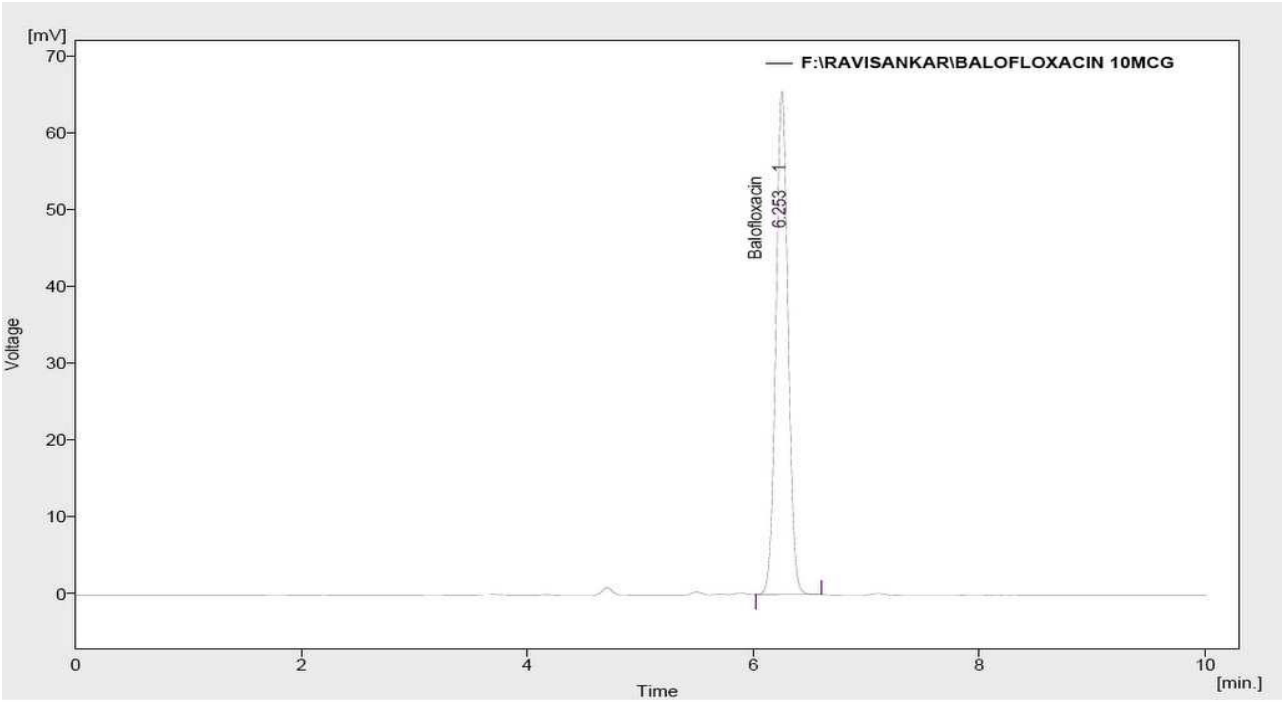

Figure 5
Standard chromatogram of balofloxacin 10 µg/ml.

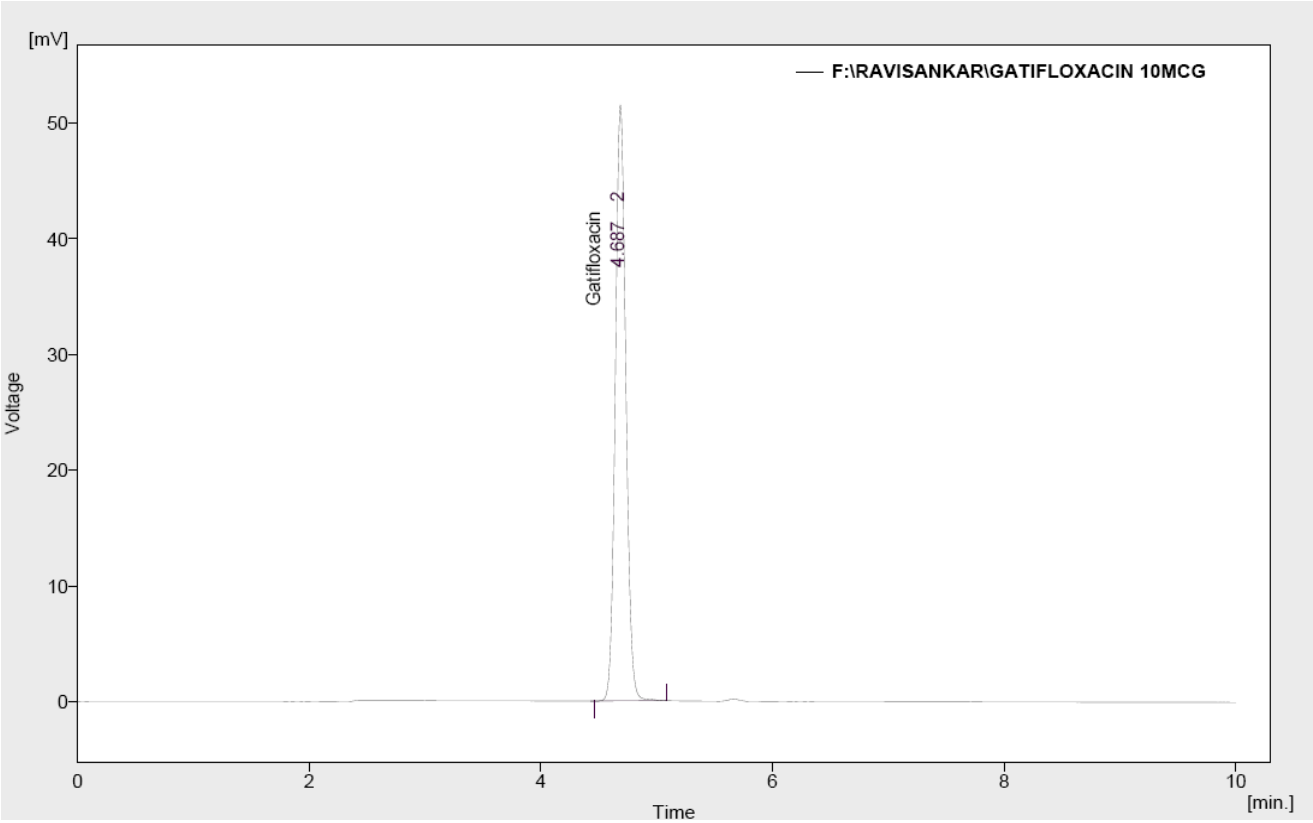

Figure 6
Standard chromatogram of gatifloxacin 10 µg/ml.

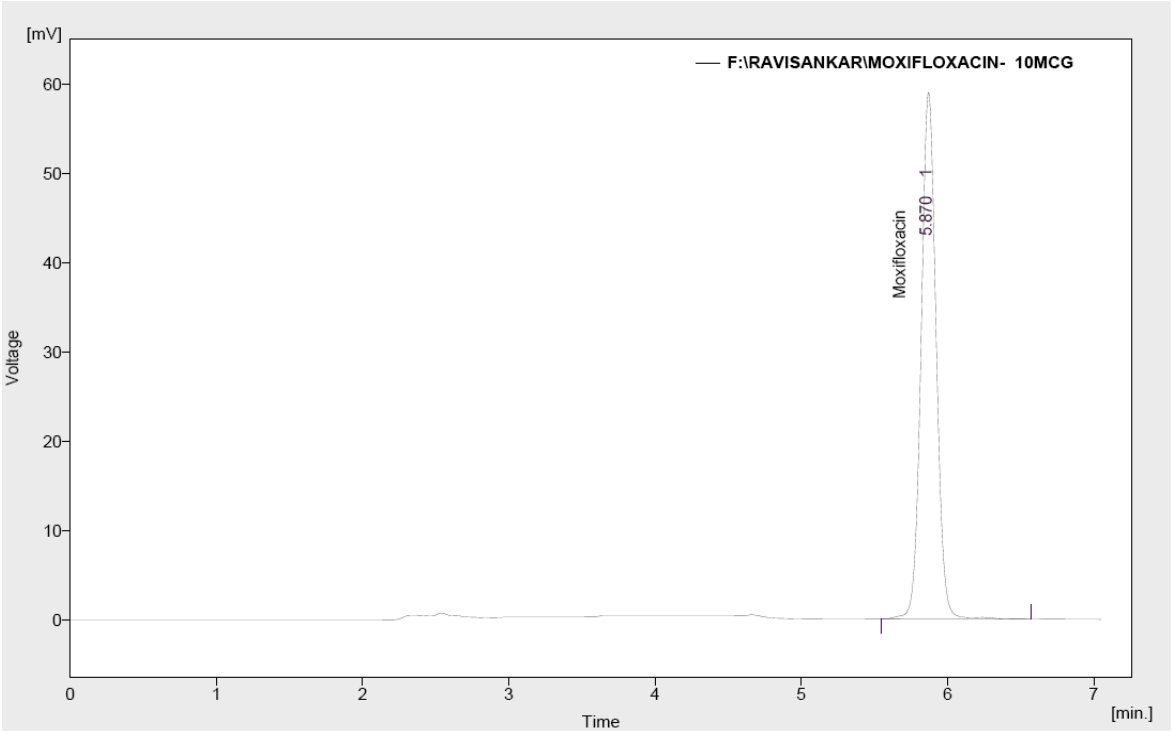

Figure 7
Standard chromatogram of Moxifloxacin 10 µg/ml.

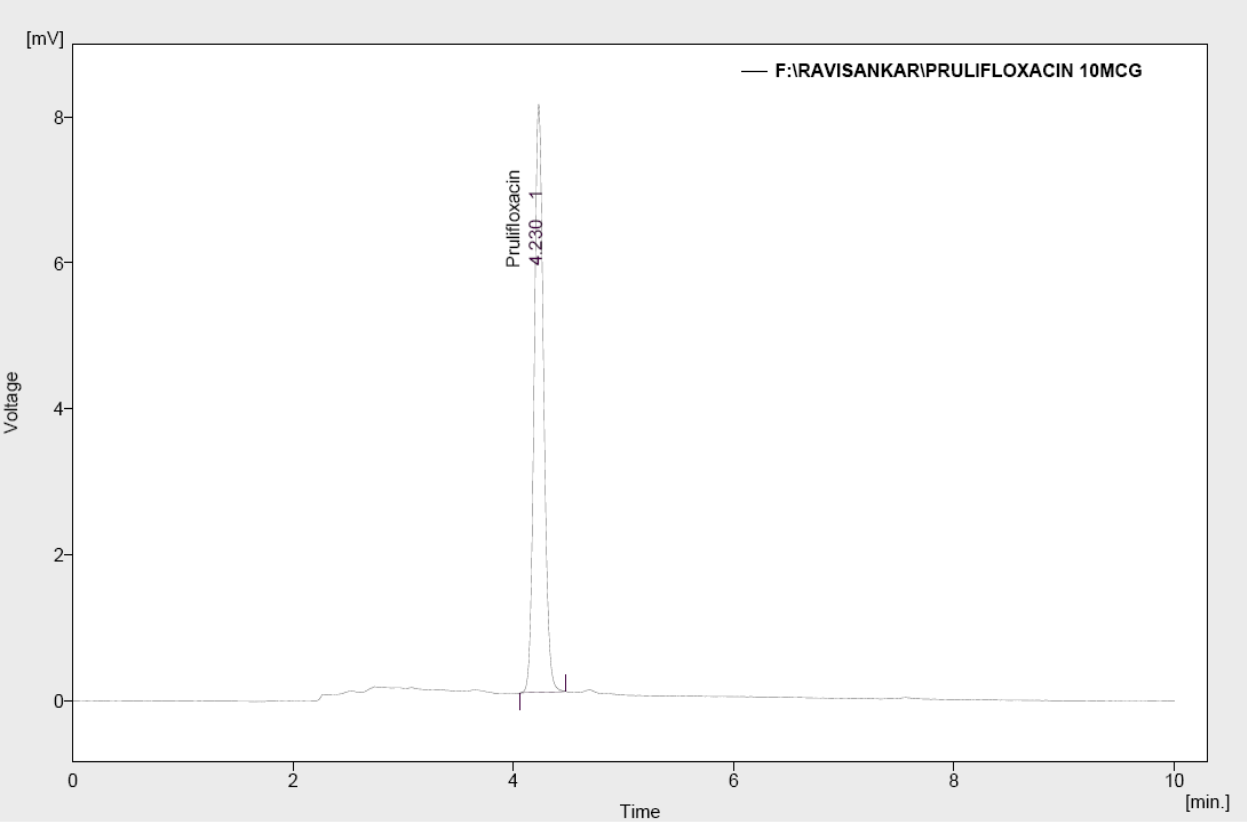

Figure 8
Standard chromatogram of prulifloxacin 10 µg/ml.

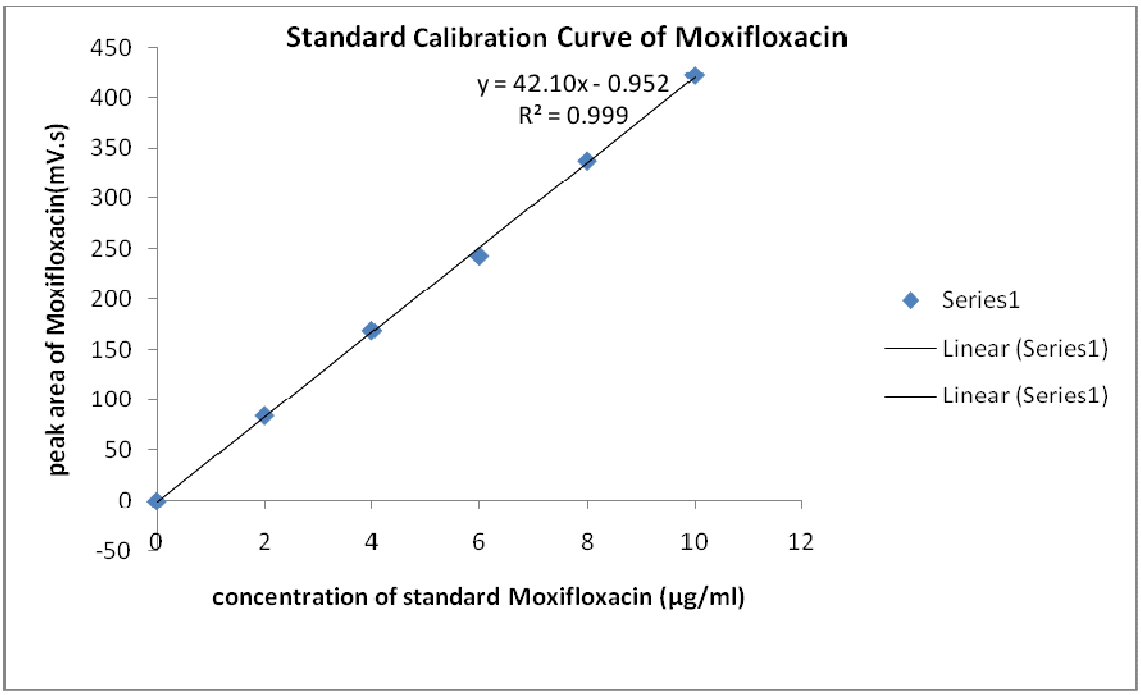

Figure 9
Calibration Plot of Moxifloxacin

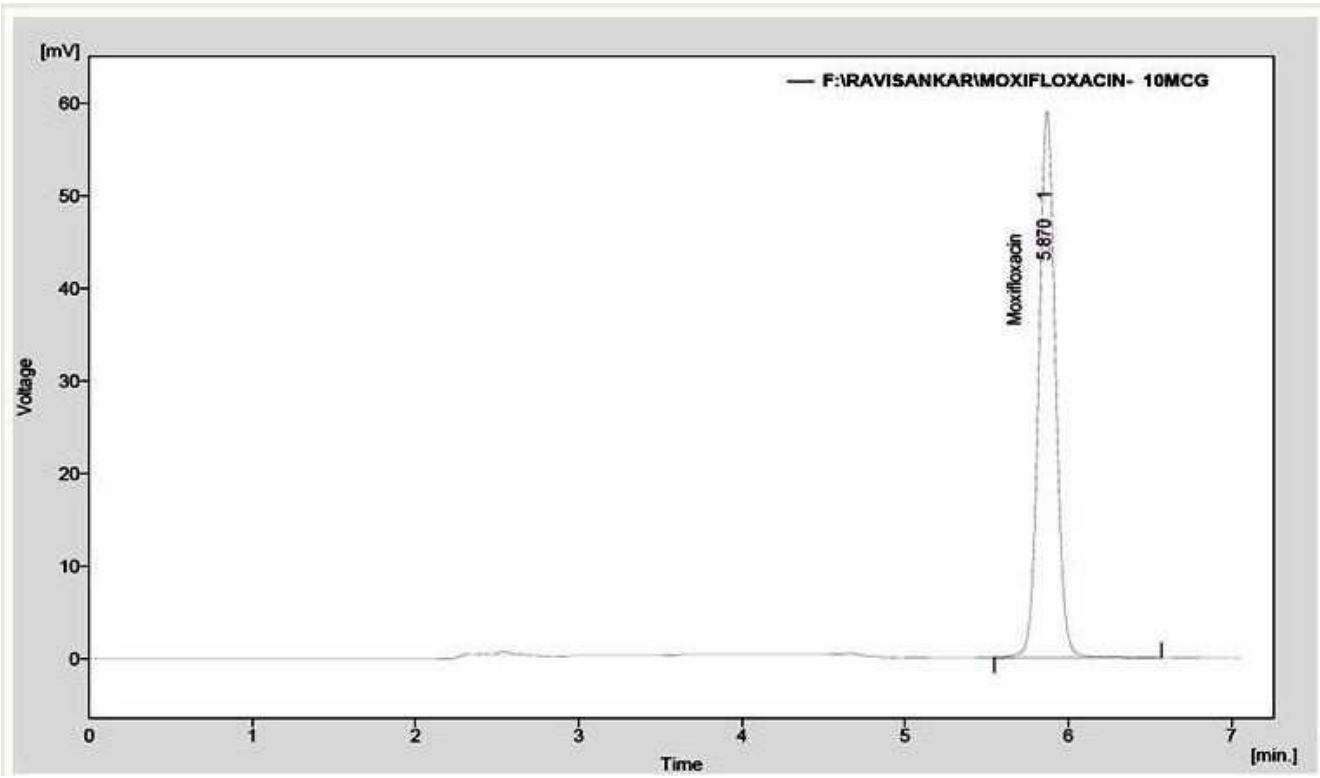

Figure 10
Standard Chromatogram of Moxifloxacin (10 µg/ml.)

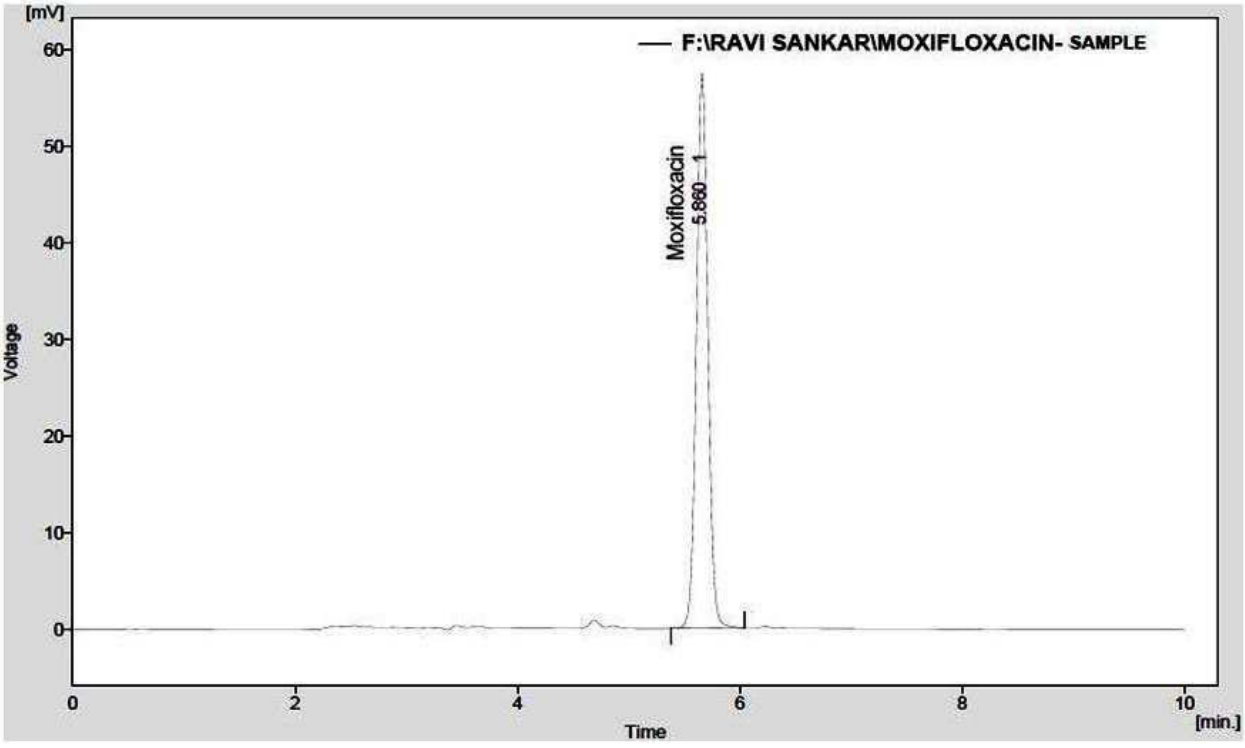

Figure 11
Sample Chromatogram of Moxifloxacin

CONCLUSION

The ultimate goal of any separation is to achieve acceptable resolution in a reasonable time. The proposed method showed excellent resolving and provided a very simple method for the separation of all six compounds. The mobile phase is economical and simple to prepare. The run time of 10 min indicates short analysis time. The analysis was performed at room temperature. All the fluoroquinolones were resolve within 6.5 min. The developed method provides good peak shapes and high resolving power. Hence by using this method six fluoroquinolones drugs can be separated with short retention time and this simple method gave excellent peak shape and provides excellent resolution. This highly reproducible method can be used for determination of small concentrations in a short period of time. The method overall provided to be simple, rapid, precise, sensitive, robust, highly reproducible, cost effective and can be conveniently applied for the determination of levofloxacin, sparfloxacin, balofloxacin, prulifloxacin, gatifloxacin, moxifloxacin and in bulk and pharmaceutical dosage forms. We hope that this method would also be applied for the combinations of said fluoroquinolone anti-bacterials, irrespective of their dose in pharmaceutical dosage forms.

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REFERENCES

1. Drug today medical journal, DT78,Vol 1, Lorina publication (India) inc., Delhi-91: 512-554, (2012).
2. Andersson MI, MacGowanAP, Development of the quinolones, J Antimicrob Chemother, 51(S1): 1–11, (2003).
3. Ivanov DV, Budanov SV, Ciprofloxacin and antibacterial therapy of respiratory tract infections, Antibiot Khimioter, 51(5): 29–37, (2006).
4. Hooper DC, Emerging mechanisms of fluoroquinolone resistance, Emerg Infect Dis, 7(2): 337–341, (2001).
5. Eliopoulos G.M, Activity of Newer Fluoroquinolones Invitro Against Gram-Positive Bacteria, Drugs, 58(2): 23-28, (1999).
6. Catherine M Oliphant, Gary M Green, Quinolones: A Comprehensive Review, Am Fam Physician. 65(3): 455-465, (2002).
7. Boubakar B. Baa, Renaud Etiennea, Dominique Ducinta, Claudine Quentinb, Marie-Claude Sauxa, Determination of moxifloxacin in growth media by high-performance liquid chromatography, Journal of Chromatography B: Biomedical Sciences and Applications, 754(1): 107–112, (2001).
8. Predrag Djurdjevic, Andrija Ciric, Aleksandra Djurdjevic, Milena Jelikic Stankov, Optimization of separation and determination of moxifloxacin and its related substances by RP-HPLC, Journal of Pharmaceutical and Biomedical Analysis, 50(2): 117–126, (2009).
9. Santoro MI, Kassab NM, Singh AK, Kedor-Hackmam ER. Quantitative determination of gatifloxacin, levofloxacin, lomefloxacin a

nd pefloxacin fluoroquinolonic antibiotics in pharmaceutical preparations by high-performance liquid chromatography, J Pharm Biomed Anal, 40(1): 179-84, (2006).
10. Yu H, Tao Y, Chen D, Pan Y, Liu Z, Wang Y, Huang L, Dai M, Peng D, Wang X, Yuan Z, Simultaneous determination of fluoroquinolones in foods of animal origin by a high performance liquid chromatography and a liquid chromatography tandem mass spectrometry with accelerated solvent extraction, J Chromatogr B Analyt Technol Biomed Life Sci, 885-886: 150-9, (2012).
11. Razzaq SN, Khan IU, Mariam I, Razzaq SS, Stability indicating HPLC method for the simultaneous determination of moxifloxacin and prednisolone in pharmaceutical formulations, Chem Cent J, 6(1): 94, (2012).
12. Sanjay K. Motwani, Shruti Chopra, Farhan J. Ahmad, Roop K. Khar, Validated spectrophotometric methods for the estimation of moxifloxacin in bulk and pharmaceutical formulations, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 68(2): 250–256, (2007).
13. Juan A. Ocaña, Francisco J. Barragán and Manuel Callejón, Spectrofluorimetric determination of moxifloxacin in tablets, human urine and serum, Analyst, 125(12): 2322-2325 (2000).
14. Juan A. Ocaña, Francisco J. Barragán, Manuel Callejón and Fernando De la Rosa, Application of Lanthanide-Sensitised Chemiluminescence to the Determination of Levofloxacin, Moxifloxacin and Trovafloxacin in Tablets, Microchimica Acta, 144(1-3): 207-213 (2004).
15. Erk N, Voltammetric behaviour and determination of moxifloxacin in pharmaceutical products and human plasma, Analytical and Bioanalytical Chemistry, 378(5): 1351-1356, (2004).
16. RecaiİnamaHülya Mercana, EmineYılmaza, Bengi Uslub, Differential Pulse Polarographic Determination of Moxifloxacin Hydrochloride in Pharmaceuticals and Biological Fluids, Analytical Letters, 40(3): 529-546, (2007).
17. Vu DH, Koster RA, Alffenaar JW, Brouwers JR, Uges DR, Determination of moxifloxacin in dried blood spots using LC-MS/MS and the impact of the hematocrit and blood volume, J Chromatogr B AnalytTechnol Biomed Life Sci, 879(15-16): 1063-1070, (2011).
18. Cruz LA, Hall R, Enantiomeric purity assay of moxifloxacin hydrochloride by capillary electrophoresis, J Pharm Biomed Anal, 38(1): 8-13 (2005).
19. Radhakrishna T, Rao DS, Vyas K, Reddy GO, Enantiomeric separation of a moxifloxacin intermediate by chiral liquid chromatography using cellulose based stationary phases, J Pharm Biomed Anal, 22(4): 691-7, (2000).
20. Rashmi R, Jayakumar k, Narayana swamy M, Avinash bhat, Acute oral yoxicity study of Norfloxacin in female rats, Int J Pharm Bio Sci , Vol 3(1): 98-101, (2012).
21. Rashmi R, Jayakumar K, Narayana Swamy H.D, Bayer M.D, Organ directed toxicity of norfloxacin in a repeated dose 28 day oral toxicity study in female rats, Int J Pharm Bio Sci ,Vol 3 (1): 81-95, (2012).
22. Gopal Muthu, Arumugam Suresh, Gnadesikan Sumathy,Ramesh Srivani, Studies on antimicrobial susceptibility pattern of salmonella isolates from Chennai, India. Int J Pharm Bio Sci, Vol2(2): 435-442, (2011).
23. ICH, Harmonized Tripartite Guideline. Validation of Analytical Procedure: Methodology (Q2B). International Conference on Harmonization, (1997).