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Research Paper

Novel analytical method development and validation of several third and fourth generation fluoroquinolone antibacterials in pharmaceutical dosage forms by RP-HPLC

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***ABSTRACT*:-** *For the first time simple, selective, sensitive RP-HPLC method was developed for the separation and quantitative development of Levofloxacin (LEVO), Prulifloxacin (PRFX), Gatifloxacin (GATI), Sparfloxacin (SPAR), Moxifloxacin (MOXI) and Balofloxacin (BALO) relating to fluoroquinolone anti bacterials in pharmaceutical dosage forms. The most important advantage of developed method was that the 6 separate drugs can be determined on a single chromatographic system without modifications in detection wavelength and mobile phase by RP-HPLC. The chromatographic separation of the selected drugs was carried out on Welchrom C_{18} column consisting of 250 mm X4.6 mm, 5 µm particle size utilizing mixture of 10 mM phosphate buffer (pH 3.1): Acetonitrile in the ratio of 70:30,v/v as mobile phase at the flow rate of 1mL/min with detection wave length at 293 nm by using UV spectrophotometric detector with total run time of 10 minutes and 3.613, 4.230, 4.707, 5.497, 5.880 and 6.253 minutes of retention time, 12,261, 12,554, 13,157, 14,761, 14,912 and 15,916 of plate number, 1.106, 1.067, 1.040, and 1.073, 1.030 and 1.086 tailing factors were obtained for LEVO, PRFX, GATI, SPAR, MOXI and BALO respectively. All calibration curves for six drugs showed indicated linearity over a concentration range of 2-10µg/mL. The results regarding to limit of detection (LOD) and limit of quantitation (LOQ) for LEVO, PRFX, GATI, SPAR, MOXI and BALO were found to be 0.116 µg/mL and 0.348 µg/mL; 0.152 µg/mL and 0.460 µg/mL; 0.084 µg/mL and 0.255 µg/mL; 0.186 µg/mL and 0.558 µg/mL, 0.162 and 0.493, 0.112 and 0.390 respectively. These results clearly show low values of LOD and LOQ. The said developed method was ultimately utilized for quantification of marketed formulation. The mean assay values for LEVO, PRFX, GATI, SPAR, MOXI and BALO were arrived at 99.317±0.990%, 99.9±0.04%, 99.9±0.02%, 99.45±0.01%, 99.945±0.056% and 99.68±0.09% respectively.*

Keywords:- *Levofloxacin, Prulifloxacin, Gatifloxacin, Sparfloxacin, Moxifloxacin, Balofloxacin.*

I. INTRODUCTION

Fluoroquinolones (FQs) are fluorinated analogues of nalidixic acid and vital class of synthetic anti-bacterial extensively used in anti-infective chemotherapy owing to their highly remarkable broad spectrum activity. The third and fourth generation FQs such as Levofloxacin (LEVO), Prulifloxacin (PRFX), Gatifloxacin (GATI), Sparfloxacin (SPAR), Moxifloxacin (MOXI) and Balofloxacin (BALO) has numerous advantages over the earlier ones. They not only exhibit stronger and expanded activity against both gram negative and gram positive bacteria and anaerobes but also have improved pharmacokinetic properties. Particularly, these novel fluoroquinolones consisting of maximum oral bioavailability with plasma drug concentrations, long elimination half-lives and good tissue penetration when compared to earlier drugs first and second generation. The third and fourth generation fluoroquinolones have very good safety profile and fewer side effects compare too many other antibiotics.

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In this connection relevant nemerous literature on these drugs were compiled and thoroughly examined before development of this method to have adequate knowledge in this regard. The author deeply felt that a novel single method for quantification of all the above said drugs to be resolved and estimated on single chromatographic system without any minor changes in detection wavelength and mobile phase composition is direly needed. Majority of HPLC methods were applied in determination of FQs in human plasma[1-5], edible animal products, feeds and to a lesser extent in pharmaceutical formulations [6-9]. Most of the reported methods involve troublesome mobile phase and difficult detection methods (fluorescence or mass detectors).

Particularly the chief aim of the author is to develop a method that allowed determining each agent in tablet form without seeking the necessity for development of separate and different methods for each agent However, few RP - HPLC methods so far have been reported in this regard but the methods available hitherto are poorly validated, uneconomical and consume longer runtimes. Keeping in view the complete evaluation of the above reported methods, the author developed a novel RP - HPLC method which is considered to be accurate, simple, precise, rapid with shorter runtime as well as economical for the separation and estimation of the above said fluoroquinolones in tablet dosage forms. The proposed method out and out permitted for determination and quantification of the six fluoroquinolones in a single chromatographic run without effecting any modifications relating to optimized conditions of relevant parameter for each proposed compound separately in chromatographic system which make the proposed method more precise, economic, specific and faster. The chemical structures of the drugs used in the present study are given in **Fig. 1.** The **Table 1** shows the details of brand names of six tablet formulations selected for the present study.

II. EXPERIMENTAL

2.1. Materials and methods

All the chemicals and reagents used in the present study were of Anal R grade and solvents were of HPLC grade. The details of procured materials are shown in **Table 2** and the details of instruments used are shown in **Table 3.**

2.2. Preparation of reagents and standards

2.2.1. Preparation of phosphate buffer pH 3.1

Phosphate buffer with 10 mM was prepared duly dissolving 6.056 g KH_2PO_4 in 445 mL of HPLC grade water. To this said solution 55 mL of 0.1M H_3PO_4 was added to adjust the pH 3.1 with triethyl amine as column modifier.

2.2.2. Preparation of mobile phase

The above stated prepared phosphate buffer (pH 3.1) 500 mL (70 %) and acetonitrile 200 mL (30 %) were mixed completely in the proportion of 70: 30 v/v and it was filtered through 0.45 µm nylon membrane filter and then degassed by sonication. The prepared solution was used as mobile phase.

2.2.3. Preparation of standard stock solution

Standard stock solution was prepared by dissolving 100 mg for each drug of LEVO, PRFX, GATI, SPAR, MOXI and BALO in 100 mL mobile phase in six separate individual 100 mL volumetric flasks to get 1 mg/mL stock solution for each drug.

2.2.4. Preparation of sample solution of method

The content of twenty tablets of proposed different brands namely LEVO, PRFX, GATI, SPAR, MOXI and BALO were separately transferred into a mortar and ground to a fine powder. From this tablet powder a quantity equivalent to 100 mg of LEVO, PRFX, GATI, SPAR, MOXI and BALO was taken individually in different calibration flasks 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.

III. RESULTS AND DISCUSSION

3.1. Selection of common wavelength for detection (Determination of λ_{max})

The UV spectra of fluoroquinolones in a mixture of Phosphate buffer (pH 3.1), acetonitrile (70:30 v/v) was scanned in the region between 200 - 400 nm. UV overlain spectra **(Fig. 2)** of these drugs showed that they absorbed appreciably at 293 nm, so that it was selected as the detection wave length.

3.2. Optimization and Method Development

Numerous trials were examined duly varying the commonly used solvents for obtaining optimizing separation conditions for six drugs under isocratic mode. Mixtures of commonly used solvents like HPLC grade water, methanol and acetonitrile with or without buffers in different combinations were tested as mobile phases. Based on the nature of the drugs, a C_{18} column was preferred as stationary phase for reversed phase HPLC system. Trials of optimization were taken up by altering one parameter at a time and all other parameters kept constant.

Eventually from all trials, better reproducibility of the results and good resolution good peak shape, short runtime, minimal peak tailing were well identified when analytical column of Welchrom RP - C_{18} Column (4.6 mm X 250 mm, 5µ), Shimadzu LC - 20 AT Prominence Liquid chromatograph with mobile phase constituted of 10 mM Phosphate buffer of pH 3.1: acetonitrile (70:30, v/v) were found to be the most suitable for the method adopted by the author. A mobile phase flow rate of 1.0mL/min was found to be most suitable and gives the maximum retention time for each drug with base line stability. Ambient temperature and detection wave length at 293 nm were also found to good for effective analysis of the LEVO, PRFX, GATI, SPAR, MOXI and BALO samples.

Based on the above optimized chromatographic condition for the proposed method, the retention times of the LEVO, PRFX, GATI, SPAR, MOXI and BALO were found to be 3.613, 4.230, 4.707, 5.497, 5.880 and 6.253 minutes respectively. A model chromatogram that shows how the peaks of these six drugs are well separated and is presented in **Fig.3.** Ultimately satisfactory good results were obtained with the following optimized chromatographic parameters and the values of each parameter are shown below of their corresponding column of the **Table.4.**

3.3. Method validation

In pursuance of the guide lines of International Conference on Harmonization guidelines[10] this method was validated for system suitability, specificity, linearity, precision, accuracy, robustness and LOD and LOQ.

3.3.1. System suitability

This test was conducted to verify different parameters namely column efficiency, resolution, retention time and peak tailing of the chromatographic system are sufficiently enough for analyzing them. The results so obtained are mentioned in **Table 4.**

3.3.2. Specificity

To examine the interferences of the excipients available in the formulation while a chromatographic analysis Specificity study was held. In the current work, specificity for analyzing the proposed six individual drugs were assessed by comparing the chromatograms which were obtained during the analysis of standard sample together with most commonly used excipients in the tablet formulations such as lactose anhydrous, magnesium stearare, microcrystalline cellulose, pregelatinized starch, purified talc, hydroxyl propyl methyl cellulose (HPMC), polyvinyl alcohol, polyethylene glycol, titanium dioxide. The blank solution was prepared by adopting same procedure as in the case of test solution by mixing the excipients in the diluents. Drug to excipients ratio was taken on par with commercial formulations. Before injection, the mixtures were filtered through 0.45 µ membrane filters. The results of specificity study table are shown in **Table 5.**

3.3.3. Linearity

Standard drug solutions aliquots of LEVO, PRFX, GATI, SPAR, MOXI and BALO (100 µg/mL; 0.2 to 1 mL) were poured in to a series of 10 mL of 6 calibrated flasks and the volume was filled with diluent to get the six different concentrations of the said drugs ranging from 2 - 10 µg/mL. By obtaining three replicate measurements at five different points of concentration, the response of peak area was decided for each drug. By plotting average peak areas (n=3) against concentration of standard drug, linearity plot was constructed for each of the drug. Linearity data of LEVO, PRFX, GATI, SPAR, MOXI and BALO is shown in **Table 6.** The least square analysis method was adopted for achieving the slope, intercept and correlation coefficient, regression data values. The statistical parameters of data relevant linear regression analysis for the said six drugs are presented in **Table 7.**

The representative chromatograms for separate calibration standards for each analyzed drug pertaining to LEVO, PRFX, GATI, SPAR, MOXI and BALO are shown in **Fig. 4 to 9.** Linearity plots for above mentioned six drugs are shown in **Fig. 10 to 15** respectively.

3.3.4. Precision

The experiments pertaining to precision were conducted by determining the intra-day and inter-day precisions of the method. The intra-day precision was examined by estimating corresponding responses thrice on the same day as well as in three separate days for the concentration of 10 µg/mL. The percent relative standard deviation was computed which is within the range of agreeable criteria and it was found not more than 2.0.The results of precision study were affirmed in terms of % RSD and it is presented in **Table 8.**

3.3.5. Accuracy (Recovery studies)

Standard addition method was adopted to determine accuracy of proposed drugs. Recovery tests were held by analyzing mixtures of LEVO, PRFX, GATI, SPAR, MOXI and BALO with varying compositions. At three variant levels of 80 %, 100 % and 120 %, Known amount of standard drugs were added to a pre-analyzed sample and the mixed standard solutions were analyzed in triplicate at every level as per the proposed method. At each level for the all drugs the percent of individual recovery and % RSD are given in **Table 9.**

3.3.6. Robustness

To decide the robustness of the proposed method the experimental conditions such as flow rate (± 0.1 mL/min), detection wavelength (± 5 nm) and Mobile phase composition (± 2 %) were deliberately altered to know their effect on the peak area, peak tailing as well as number of theoretical plates. It was observed that all the conditions were within the agreeable limits. The results obtained are summarized in **Table 10.**

3.3.7**.** LOD and LOQ:

The LOD was determined by multiplying the ratio between the standard deviation to the slope with 3.3 and the LOQ was decided by multiplying the same ratio with 10. Keeping in view the ICH guidelines then the results of LOD and LOQ were got for the proposed drugs LEVO, PRFX, GATI, SPAR, MOXI and BALO as mentioned against each are shown in the **Table 11.**

3.3.8. Assay

20 tablets of LEVO, PRFX, GATI, SPAR, MOXI and BALO were taken separately for analysis and transferred into a mortar and grounded in smooth powder. The said powder of each drug equivalent to one tablet weight was taken, out of the said powder of 20 tablets of each drug and then transferred to volumetric flask consisting of the mobile phase. The volume was then made up to the mark with the diluents and filtered through 0.45 µm nylon filters. There after the dilution was prepared based on the required concentrations of the each drug and kept for quantifications of all drugs from their calibration plots. The assay was carried out six times and the amount of the drug exist in each tablet of relevant drug was estimated from calibration graph. The results are exhibited in the following **Table 12.** Representative sample chromatograms for assay of Glevo, Pruflox, Segat, Sparcip, Moxicip, Balo-100 tablets are shown in **Fig. 16 to 21** respectively.

3.4. DISCUSSION

The core object of the present study is to develop a simple, new, rapid, precise and accurate RP-HPLC method for simultaneous separation and quantification of six fluoroquinolones LEVO, PRFX, GATI, SPAR, MOXI and BALO. The main aim and goal of the developing a new method is to obtain a consistent reproducible separation by selecting a highly reproducible RP-HPLC method which is necessary to attain the desired result subject to ICH Q2 (R1) guide lines. Prior to identifying the best operational and environmental conditions for optimizing suitable methods for separations, trial and error methods were undertaken. Before development of this method extensive studies were taken up relating to properties of sample of each drug, drug profiles and the appropriate analytical methods for estimation of each individual drug considering its primary factors of physical and chemical properties. In support of development of this method the nature of the samples, molecular weight, pKa values, stability and other physical constant, information of the drugs were kept in view which are very helpful to decide the initial optimum separation conditions.

RP-HPLC method was selected for the proposed drugs considering their relative polarity for the initial separation process preferring C_{18} column with 4.6 mm internal diameter, 250 mm length and 5 micron particle size and a number of trials were held duly utilizing different buffer solutions of different pH ranges with different compositions of mobile phases, variable flow rate and column temperature. Eventually an optimum separation condition was obtained with a mixture of phosphate buffer (pH 3.1) 500 mL (70 %) and acetonitrile 200 mL (30 %) were mixed thoroughly in the proportion of 70: 30 v/v. A mobile phase flow rate adjusted at 1mL/min, a common detection wavelength was established at 293 nm for all the six drugs. Subsequent to the adjustment of such operational parameters optimum values, precise chromatographic peak were achieved with characterizes of proper resolution, good symmetry and minimal peak tailing.

The proposed method was validated according to the ICH Q2 (R1) guidelines with regard to specificity, linearity, precision, accuracy, robustness and LOD and LOQ. Pursuant to the prescribed guidelines, system suitability was performed to ensure the suitability of entire testing system for this intended method. Consequently, system suitability parameters such as resolution, number of theoretical plates, tailing factor of the peaks were counted for the optimized chromatographic condition for the said method. From this experiment, 3.613, 4.230, 4.707, 5.497, 5.880 and 6.253 minutes of retention time, 12,261, 12,554, 13,157, 14,761, 14,912 and 15,916 of plate number, 1.106, 1.067, 1.040, and 1.073, 1.030 and 1.086 tailing factors were obtained for LEVO, PRFX, GATI, SPAR, MOXI and BALO respectively. The resolution values for PRFX, GATI, SPAR, MOXI and BALO were found to be 4.508, 2.866, 4.604, 2.056 and 2.017 respectively. Since the above results clearly shows that they are within the acceptable limit, the method is aptly suitable for the proposed purpose of analysis.

Linearity of the proposed method was examined by choosing five points concentrations. All calibration curves for six drugs indicated linearity over a concentration range of 2 - 10 µg/mL. The concerned correlation coefficient were also calculated from the linear regression analysis and found at 0.999 in all cases of six drugs and the calculated results clearly proved that they were within the agreeable limits. These linearity results illustrate that there was strong linear relationship between concentrations of each drug and their peak areas.

Following the guidelines of ICH a mixture of pure drug samples mixed with suitable excipients were injected to the system to test specificity of the proposed method to quantify the six drugs namely LEVO, PRFX, GATI, SPAR, MOXI and BALO without the interference of used excipients. In the same way synthetic mixture solutions and blank solution along with the excipients existed in the said formulations were also injected individually. The peak response so obtained for the drug and the blank were compared with the corresponding drug. The results showed that there is no interference due to the commonly used excipients. Hence the method becomes perfectly specific to estimate the above said drugs.

Precision of the method was decided by using intra-day and inter-day precision studies. Three different concentration levels of triplicate samples were obtained for the experiment and assessed for the repeatability and intermediate precision of the method. The utilized samples belong to standard quality control. After words the values of % RSD for each drug were calculated and intra and inter day precision and the % RSD for all six drugs showed less than 2 % which clearly indicates that the present method is said to be highly precise.

Regarding the accuracy of the proposed method the known amount of pure standard drugs to pre-analyzed samples at 80 %, 100 % and 120 % (three levels) were combined and the recovery levels were carefully observed. All solutions of the above said drugs were prepared and analyzed in triplicate. The same procedure was applied for all the LEVO, PRFX, GATI, SPAR, MOXI and BALO and got the high recovery values. It was also noticed that the % RSD were also found to be less than 2 % for each drug which shows that the method is totally accurate.

Robustness was decided by evaluating the effect of slight modifications in the operation parameters such as flow rate, mobile phase composition and lambda max. It was noticed that in the chromatograms the author found that there were no any changes and the results were also within the agreeable limits. Thus the method said to be robust.

The results pertaining to limit of detection (LOD) and limit of quantitation (LOQ) for LEVO, PRFX, GATI, SPAR, MOXI and BALO were found to be 0.116 µg/mL and 0.348 µg/mL; 0.152 µg/mL and 0.460 µg/mL; 0.084 µg/mL and 0.255 µg/mL; 0.186 µg/mL and 0.558 µg/mL, 0.162 and 0.493, 0.112 and 0.339 respectively. These results lucidly states that the method possesses relatively low values of LOD and LOQ.

The said developed method was finally used for quantification of marketed formulation. The mean assay values for LEVO, PRFX, GATI, SPAR, MOXI and BALO were arrived at 99.317 ± 0.990 %, 99.9 ± 0.04 %, 99.9 ± 0.02 %, 99.45 ± 0.01 %, 99.945 ± 0.056 % and 99.68 ± 0.09 % respectively. Hence this method evolved by the author was found to be most suitable for estimating the commercial formulations as well as bulk drugs.

IV. FIGURES AND TABLES

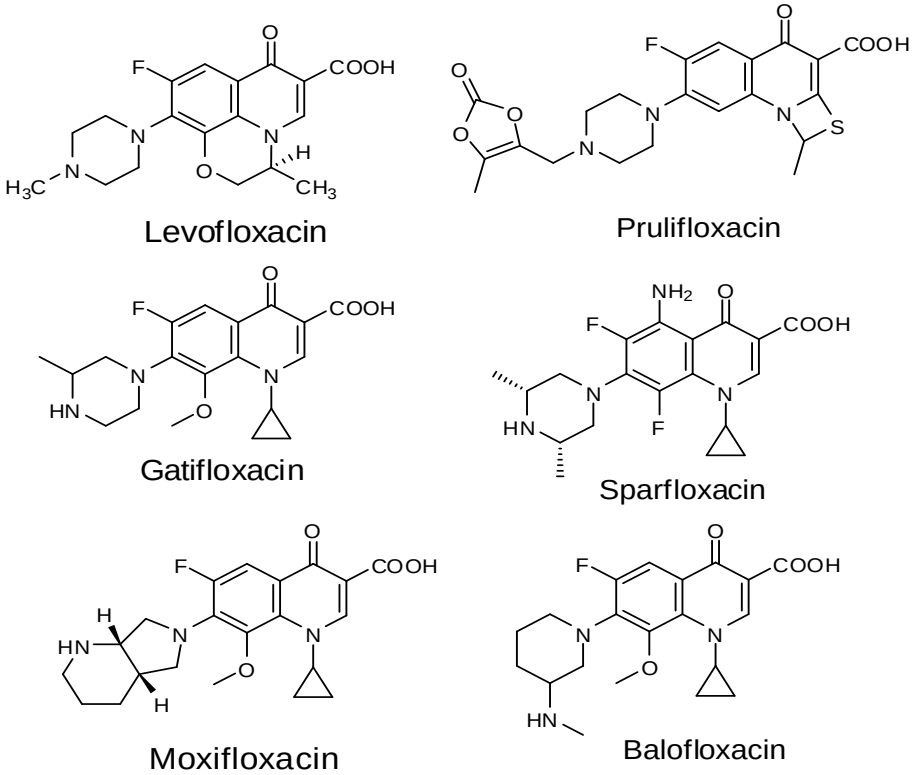

Figure 1. Chemical structures of the drugs used in the present study.

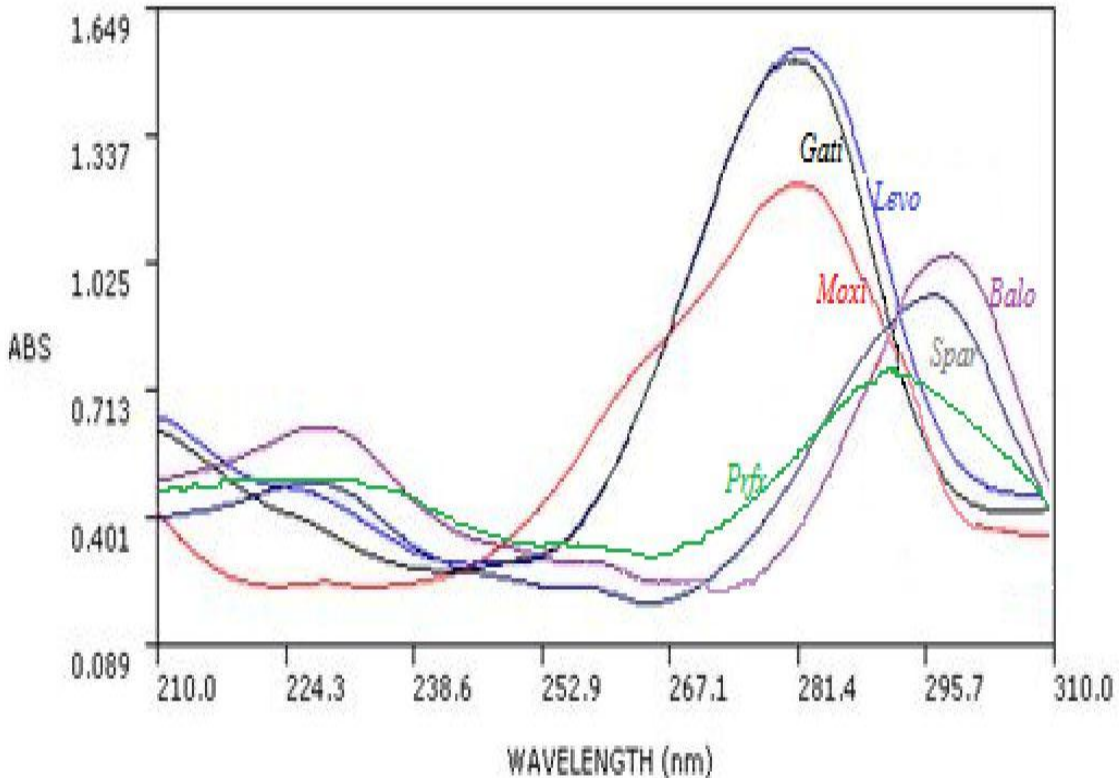

Figure 2. UV overlain spectra of the six fluoroquinolones compounds.

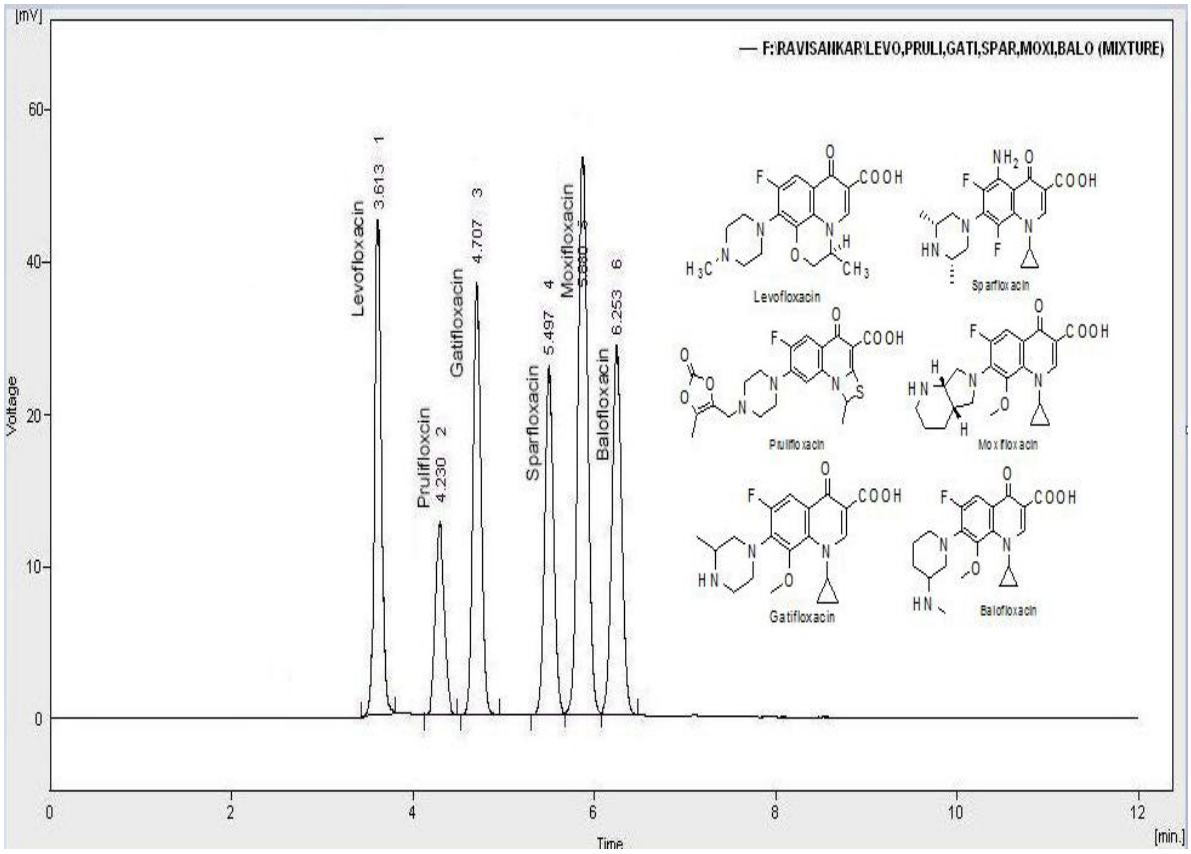

Figure 3. A typical chromatogram showing the separation of LEVO, PRFX, GATI, SPAR, MOXI and BALO in synthetic mixture.

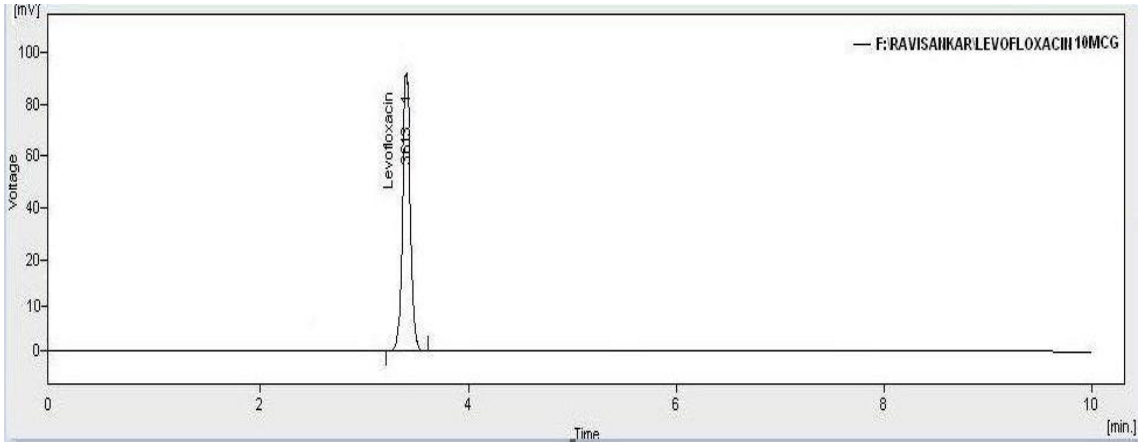

Figure 4. Standard chromatogram relating to LEVO (10 µg/mL).

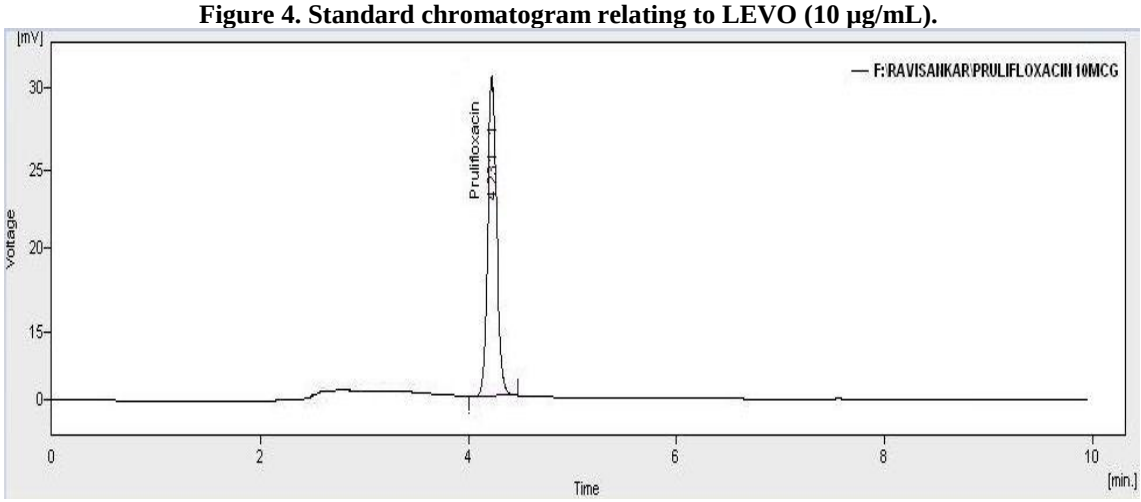

Figure 5. Standard chromatogram relating to PRFX (10 µg/mL).

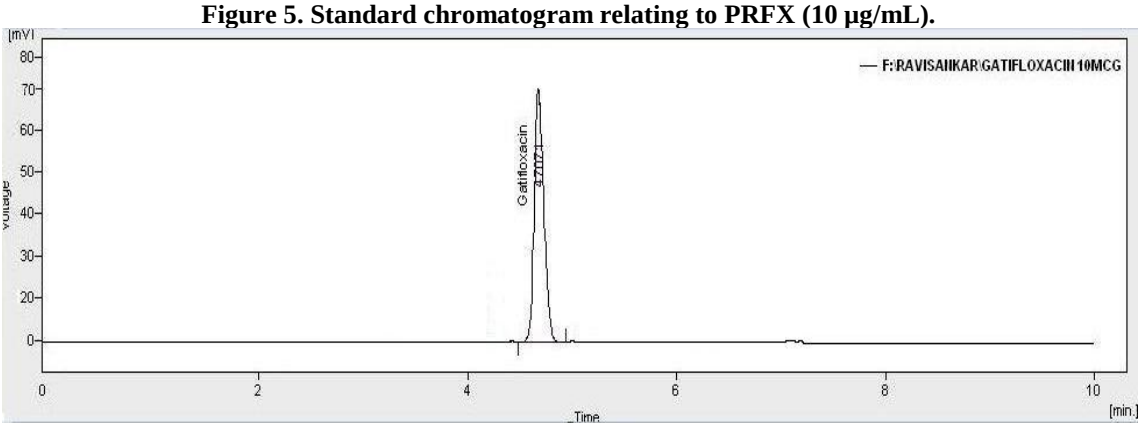

Figure 6. Standard chromatogram relating to GATI (10 µg/mL).

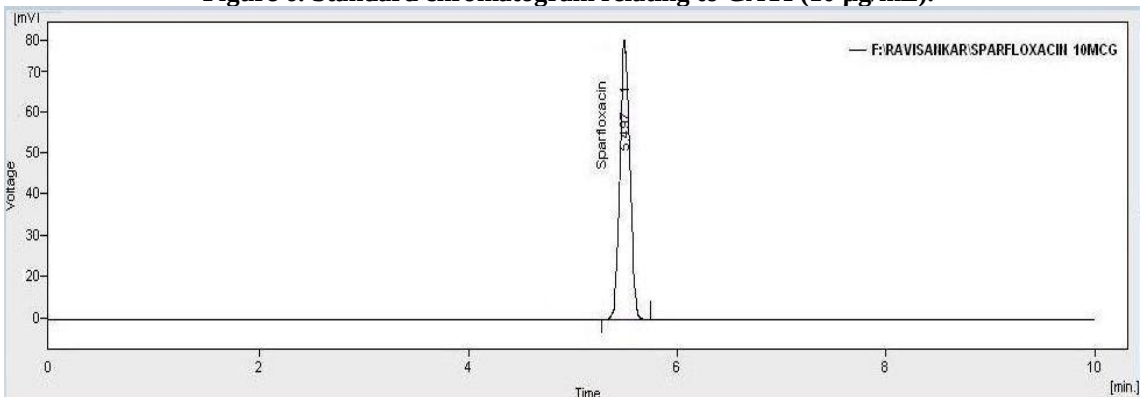

Figure 7. Standard chromatogram relating to SPAR (10 µg/mL).

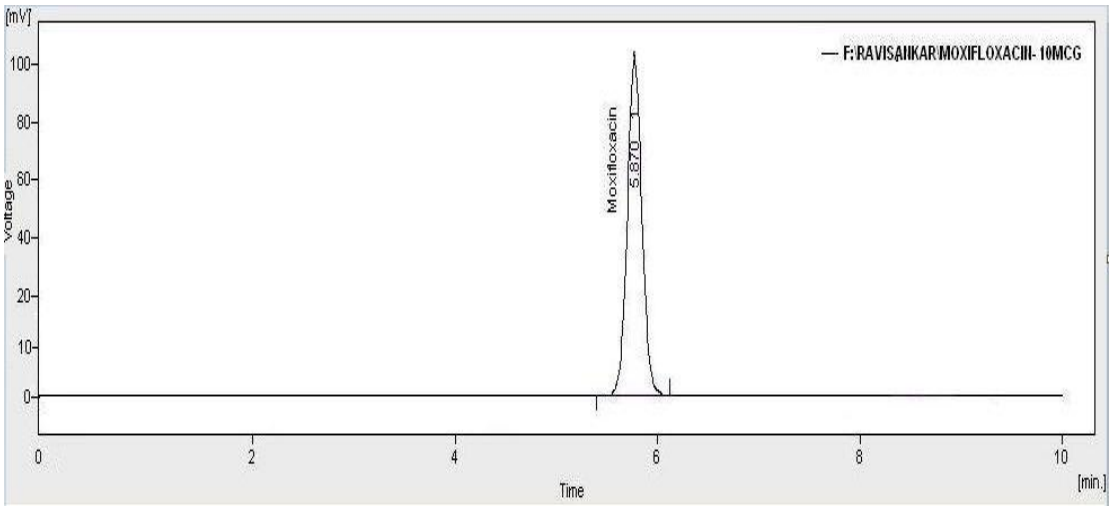

Figure 8. Standard chromatogram relating to MOXI (10 µg/mL).

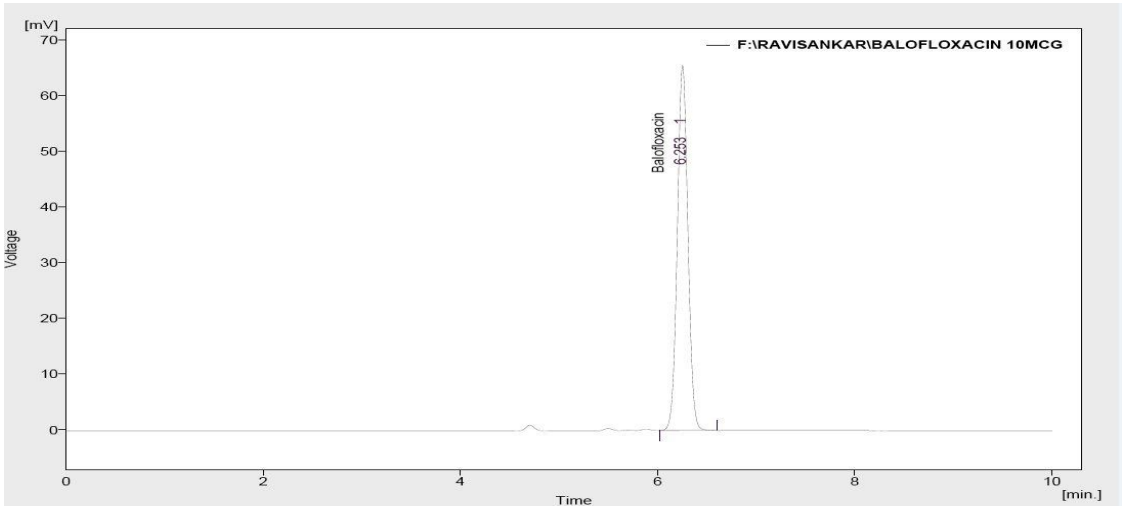

Figure 9. Standard chromatogram relating to BALO (10 µg/mL).

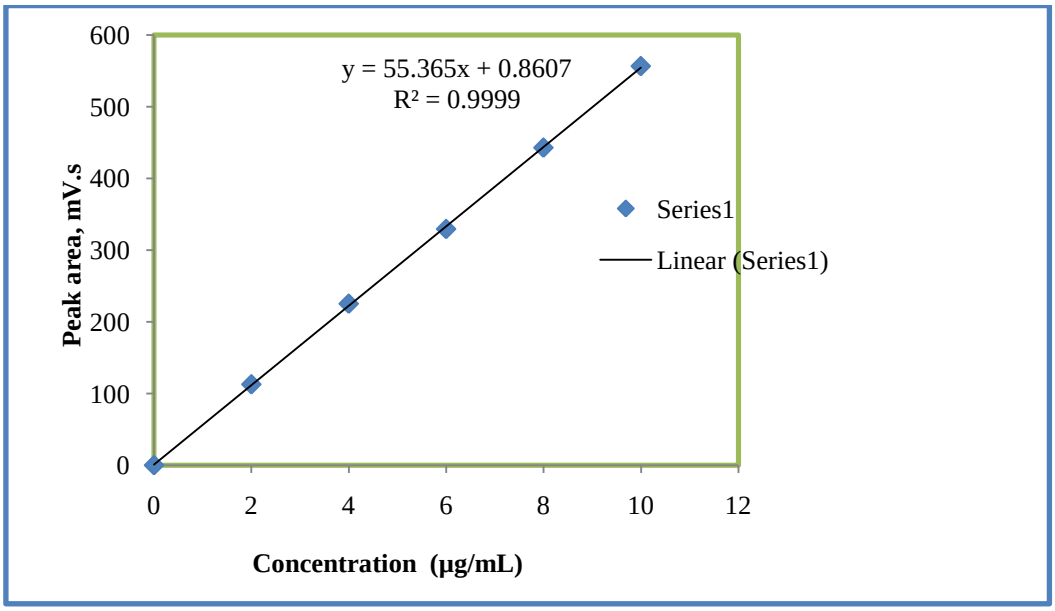

Figure 10. Linearity plot pertaining to LEVO.

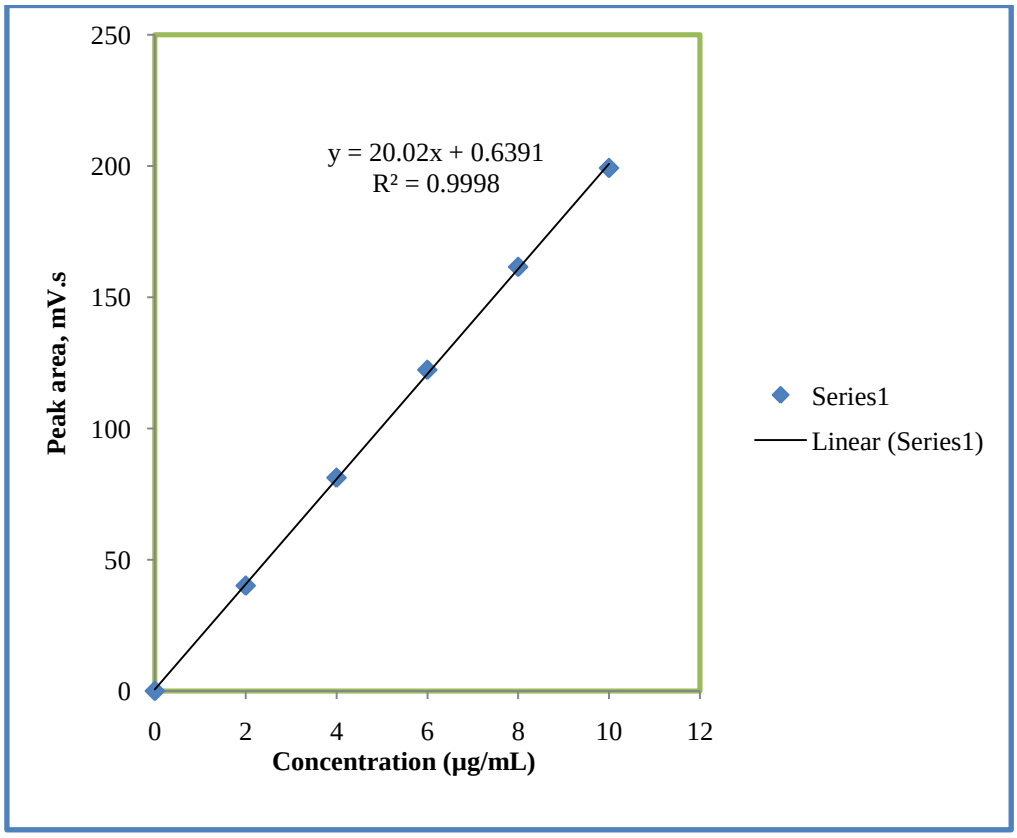

Figure 11. Linearity plot pertaining to PRFX.

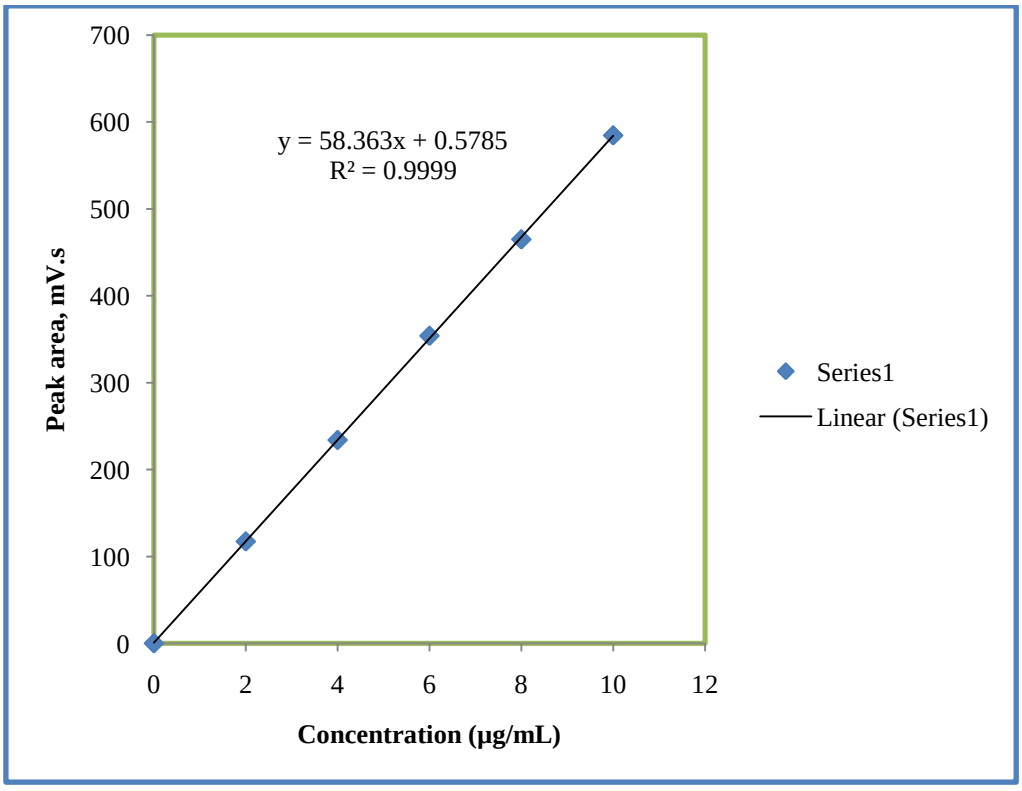

Figure 12. Linearity plot pertaining to GATI.

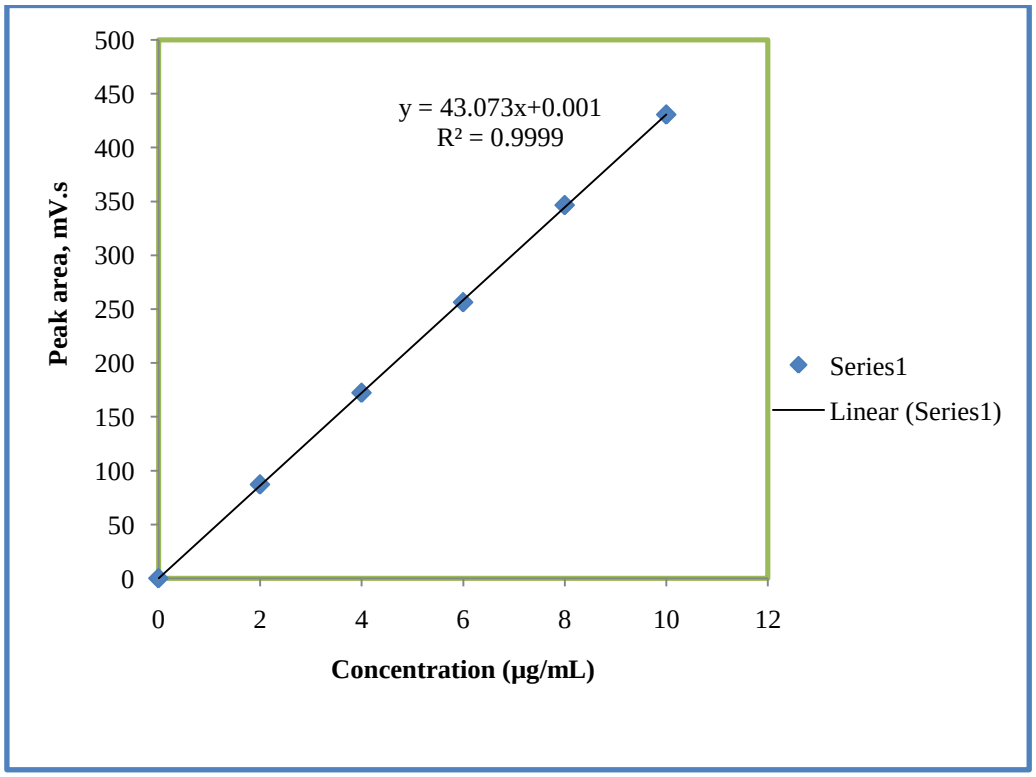

Figure 13. Linearity plot pertaining to SPAR.

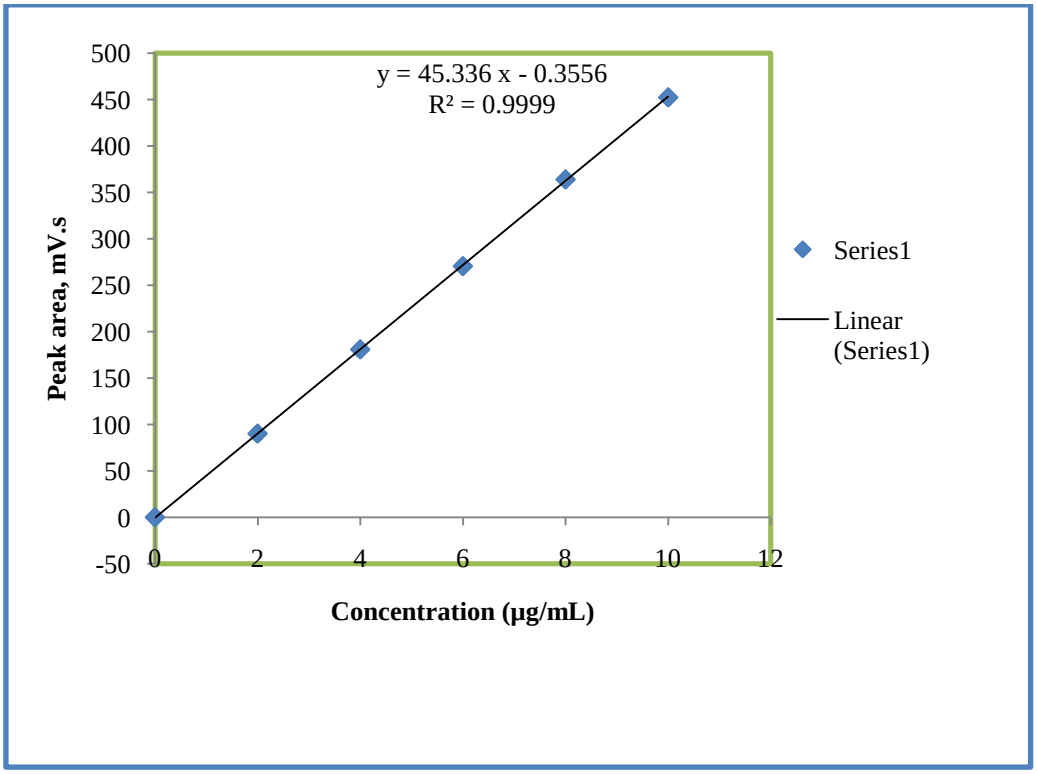

Figure 14. Linearity plot pertaining to MOXI.

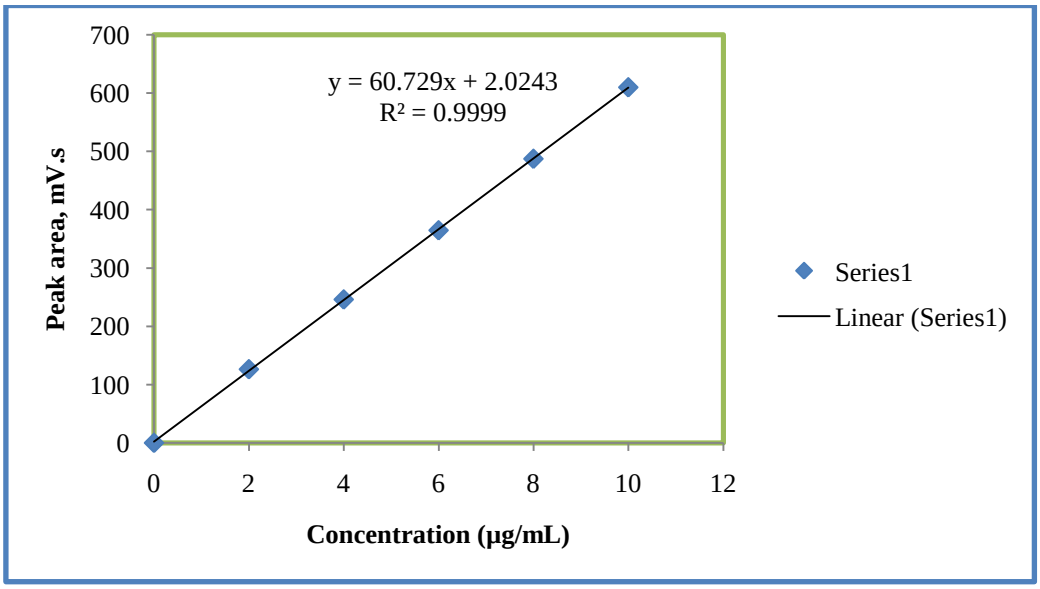

Figure 15. Linearity plot pertaining to BALO.

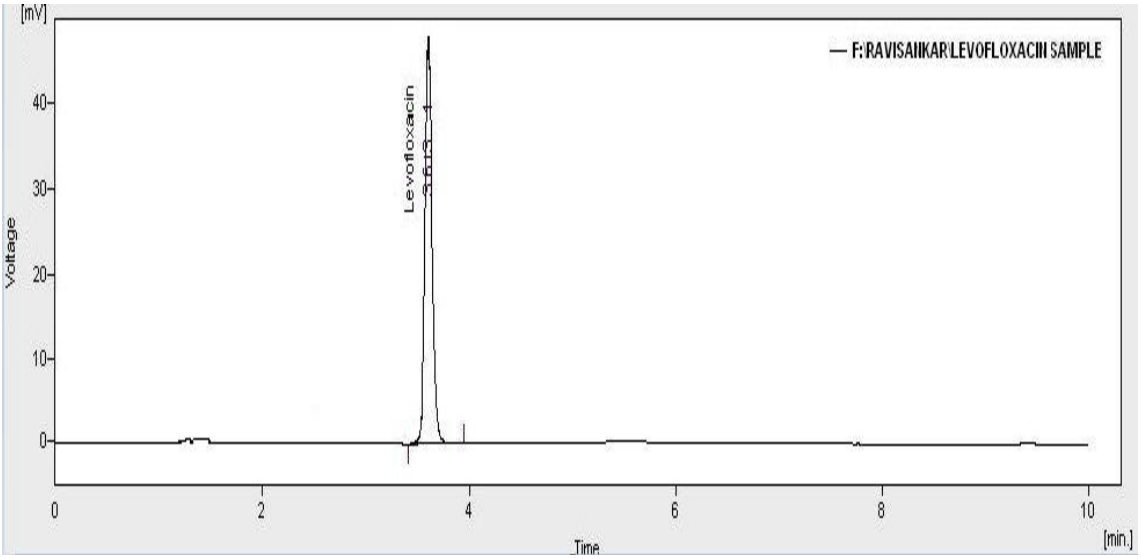

Figure 16. Sample chromatogram relating to LEVO.

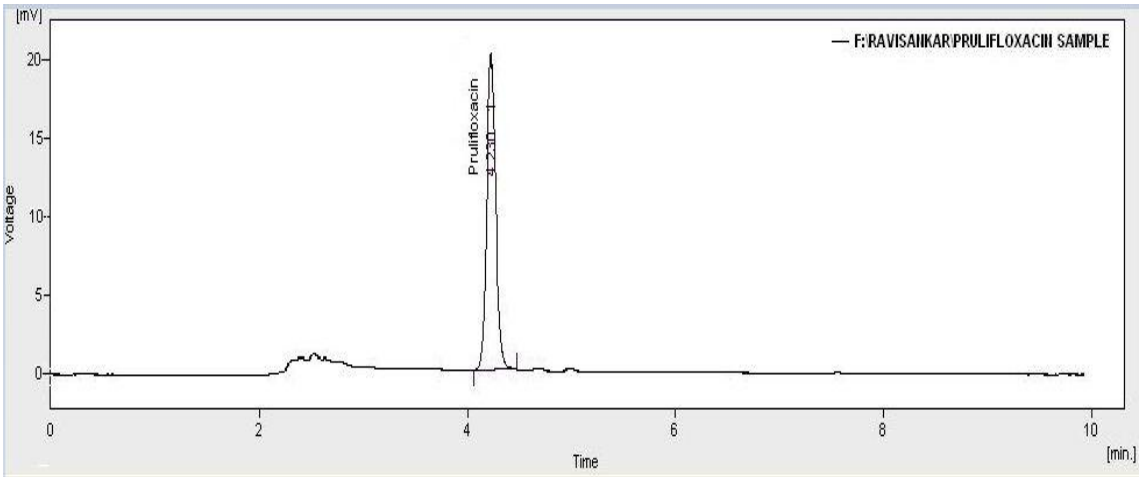

Figure 17. Sample chromatogram relating to PRFX.

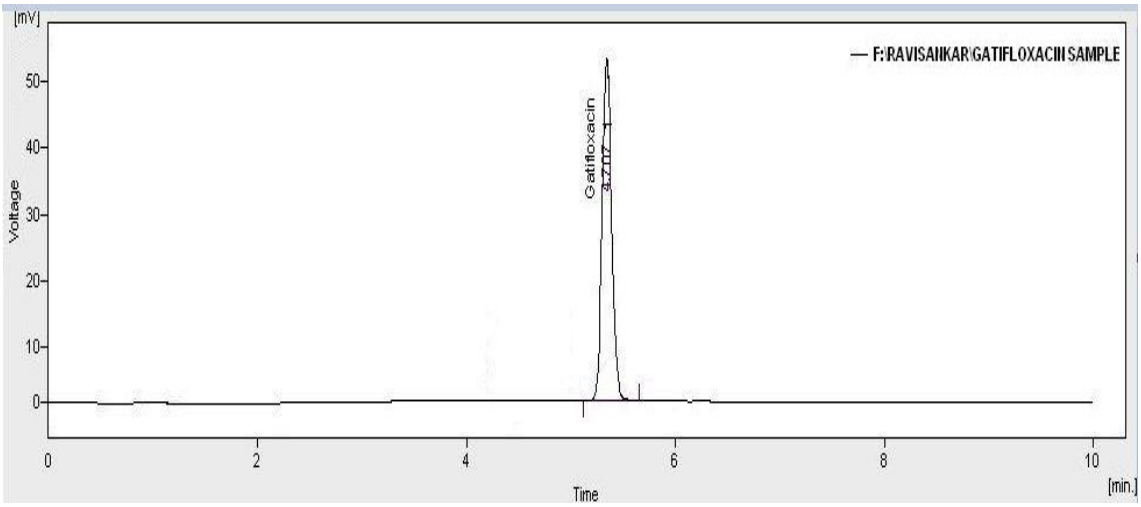

Figure 18. Sample chromatogram relating to GATI.

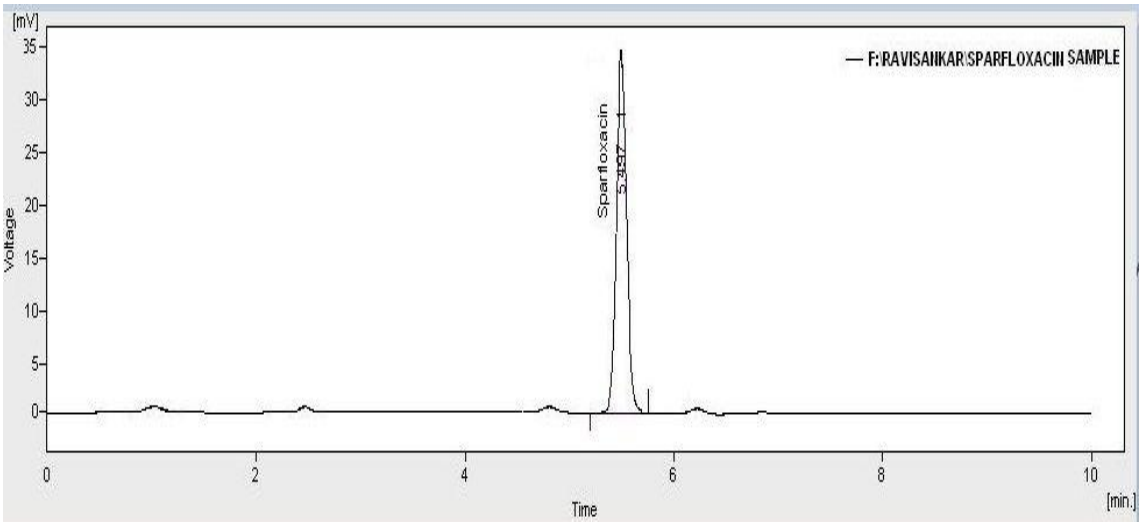

Figure 19. Sample chromatogram relating to SPAR.

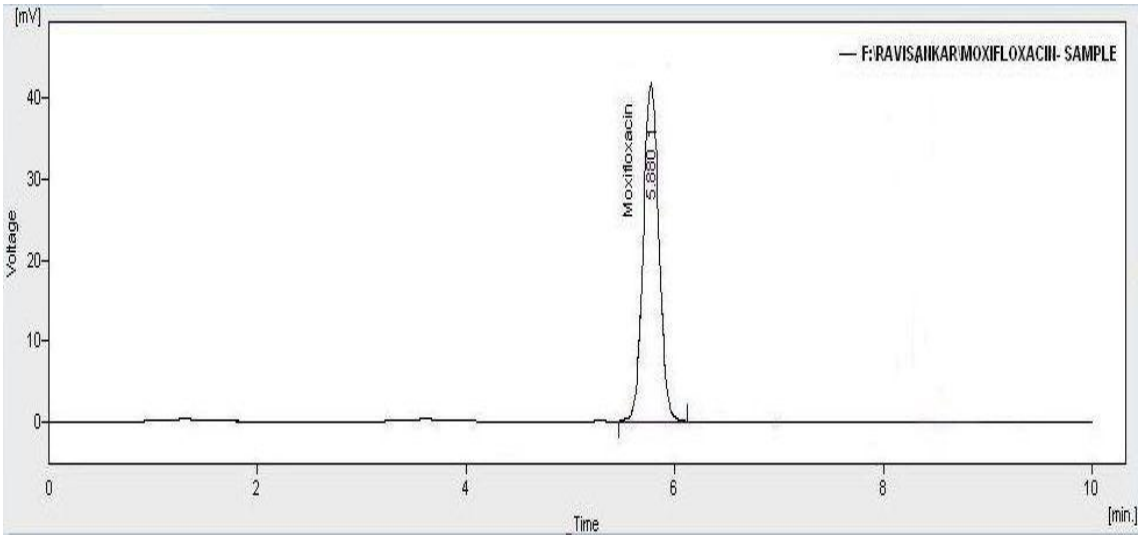

Figure 20. Sample chromatogram relating to MOXI.

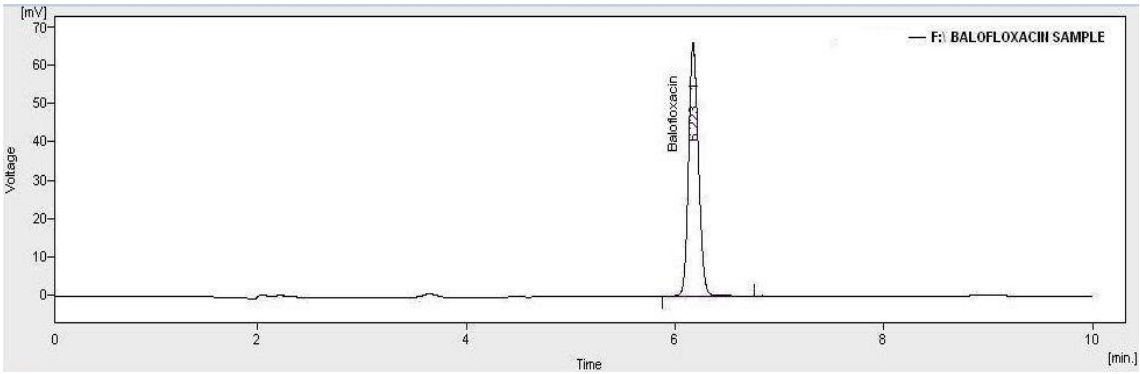

Figure 21. Sample chromatogram relating to BALO.

Table 1. Commercial brand names of LEVO, PRFX, GATI, SPAR, MOXI and BALO used for the present study.

Brand name	Formulation	Labeled amount (mg)	Manufacturer
Glevo	**Tablets**	**Levofloxacin - 500 mg**	**Glenmark Pharmaceuticals Ltd., Mumbai, India.**
Pruflox	**Tablets**	**Prulifloxacin - 600 mg**	**Cipla Ltd., Mumbai, India.**
Segat	**Tablets**	**Gatifloxacin - 400 mg**	**Secure health care Inc. India.**
Sparcip	**Tablets**	**Sparfloxacin - 100 mg**	**Cipla Ltd., Mumbai, India.**
Moxicip	**Tablets**	**Moxifloxacin - 400 mg**	**Intralabs, Bangalore, India.**
Balox-100	**Tablets**	**Balofloxacin - 100 mg**	**Lupin Ltd., Mumbai, India.**

Table 2. Materials used in the present study.

S.No.	Materials	Procured from
1.	Levofloxacin, Gatifloxacin	Aristo Pharmaceuticals Pvt.Ltd. Bombay.
2.	Prulifloxacin, Balofloxacin	Hetero Labs Ltd., Hyderabad
3.	Sparfloxacin	Anant Pharmaceuticals, Kamal, Haryana.
4.	Moxifloxacin	Torrent Pharmaceuticals, Ahmadabad.
5.	HPLC grade acetonitrile	Thermo Fisher Scientific India Pvt. Ltd., Mumbai.
6.	HPLC grade water	Merck Specialties Pvt. Ltd., Mumbai.
7.	HPLC grade methanol	Merck Specialties Pvt. Ltd., Mumbai.
8.	Dipotassium hydrogen phosphate	Thermo Fisher Scientific India Pvt. Ltd., Mumbai.
9.	Potassium dihydrogen phosphate	Glaxo Smith Kline Pharmaceuticals Ltd., Mumbai.
10.	O-Phosphoric acid	RFCL Ltd., New Delhi.
11.	Triethylamine	Merck Pharmaceuticals Private Limited, Mumbai.
12.	Concentrated Hydro chloric acid	Qualigens fine chemicals, Mumbai.
13.	Sodium Hydroxide	S.D Fine-Chem. Ltd., Mumbai.

Table 3. Instruments used in the present study.

S.No.	Instrument	Name of the company and model
1.	HPLC	Shimadzu LC-20 AT Prominence Liquid Chromatograph with Shimadzu SPD-20 A Prominence UV-Vis detector, Welchrom C_{18} Column (4.6 X 250 mm, 5 µm), with Rheodyne manual loop injector (20 µL) and Spinchrom data acquisition software.
2.	UV-Vis spectrophotometer	UV-Visible Spectrophotometer (Systronics model 2203). The UV-Vis spectrophotometer achieves a resolution of 1 nm with matched quartz cells of 1 cm path length.
3.	Weighing balance	Essae vibra AJ (0.001g), Essae-Teraoka Ltd.
4.	pH meter	Elico LI120 pH meter, Elico India Ltd.
5.	Ultrasonicator	Ultrasonic bath sonicator, PCI ltd., Mumbai.
6.	Vacuum pump	Single Stage Vacuum Pump.

Table 4. Optimized chromatographic conditions and system suitability parameters.

Parameter	Chromatographic conditions					
Instrument	Shimadzu LC-20AT Prominence liquid chromatograph					
Column	Welchrom C_{18} column (4.6 X 250 mm, 5 µm)					
Detector	Shimadzu SPD-20A prominence UV-VIS detector					
Mobile phase	10 mM phosphate buffer (pH 3.1) : Acetonitrile 70:30, v/v					
Flow rate	1mL/min					
wave length	UV at 293 nm					
Run time	10 minutes					
Temperature	Ambient temperature (25 ^{0}C)					
Injection volume	20 µL					
	LEVO	**PRFX**	**GATI**	**SPAR**	**MOXI**	**BALO**
Retention time (minutes)	3.613	4.230	4.707	5.497	5.880	6.253
Th.Pl (Efficiency)	12,261	12,554	13,155	14,761	14,912	15,916
Resolution	-	4.508	2.866	4.604	2.056	2.017
Tailing factor	1.106	1.067	1.040	1.073	1.030	1.086

Table 5. Results of specificity

Name of the solution	Method M_3					
	LEVO	**PRFX**	**GATI**	**SPAR**	**MOXI**	**BALO**
Mobile phase	No peaks	No peaks	No peaks	No peaks	No peaks	No peaks
Placebo	No peaks	No peaks	No peaks	No peaks	No peaks	No peaks
Separate injections of individual standard solutions	Peak for LEVO at 3.613 minutes	Peak for PRFX at 4.230 minutes	Peak for GATI at 4.707 minutes	Peak for SPAR at 5.497 minutes	Peak for MOXI at 5.880 minutes	Peak for BALO at 6.253 minutes

Table 6. Results relating to Linearity data

	LEVO		PRFX		GATI		SPAR		MOXI		BALO	
	Conc. µg/ML	Peak area, mV.s.	Conc. µg/ML	Peak area, mV.s.	Conc. µg/ML	Peak area, mV.s.	Conc. µg/ML	Peak area, mV.s.	Conc. µg/ML	Peak area, mV.s.	Conc. µg/ML	Peak area, mV.s.
1.	0	0	0	0	0	0	0	0	0	0	0	0
2.	2	112.736	2	40.124	2	117.211	2	105.342	2	90.102	2	126.302
3.	4	225.134	4	81.234	4	233.936	4	208.569	4	180.864	4	245.953
4.	6	329.242	6	122.324	6	353.85	6	299.802	6	270.56	6	364.604
5.	8	442.668	8	161.537	8	464.84	8	402.045	8	363.98	8	487.255
6.	10	556.325	10	199.213	10	584.52	10	499.25	10	452.436	10	609.906

Table 7. Regression analysis data relating to LEVO, PRFX, GATI, SPAR, MOXI and BALO

Parameter	LEVO	PRFX	GATI	SPAR	MOXI	BALO
Detection wavelength(λ_{max})	UV at 293 nm	UV at 293 nm	UV at 293 nm	UV at 293 nm	UV at 293 nm	UV at 293 nm
Linearity range (µg/mL)	2-10 µg/mL	2-10 µg/mL	2-10 µg/mL	2-10 µg/mL	2-10 µg/mL	2-10 µg/mL
Regression equation (Y = aX + b)	Y = 55.365X+0.860	Y = 20.02X+0.639	Y= 58.363X+0.578	Y= 43.07X+0.001	Y = 45.336X-0.3556	Y = 60.729X+2.024
Slope (a)	55.365	20.02	58.363	43.07	45.336	60.729
Intercept (b)	0.8607	0.6391	0.5785	0.001	-0.3556	2.0243
Standard error of slope (S_a)	0.32184	0.1537	0.24642	0.7290	0.0220	0.2464
Standard error of intercept (S_b)	1.9488	0.9310	1.49213	2.4277	0.00798	1.4922
Standard error of estimation (S_e)	2.6927	1.2864	2.06167	1.5001	0.02322	2.0618
Regression coefficient (R^2)	0.9999	0.9998	0.9999	0.9999	0.9999	0.9999
Percentage range of errors 0.05 significance level 0.01 significance level	 1.02904 1.6138	 1.15695 1.8144	 0.54992 0.86242	 0.12232 0.19183	 0.36373 0.57042	 1.150090 1.803643

Table 8. Results relating to intraday and interday precision.

Precision Study	Method M_3					
	LEVO	**PRFX**	**GATI**	**SPAR**	**MOXI**	**BALO**
	% RSD*	% RSD*	% RSD*	% RSD*	% RSD*	% RSD*
Intra-Day	0.3380	0.3554	0.1294	0.1145	0.3465	0.1964
Inter-Day	0.5087	0.4298	0.1347	0.1721	0.5033	0.3296

* = Number of determinations are six.

Table 9. Results relating to Accuracy (recovery)

Drug	**Parameter**	**Recovery level***		
		80 %	100 %	120 %
LEVO (M_3)	Mean % Recovery ± SD*	100.10 ± 0.364	100.05 ± 0.160	100.85 ± 0.153
	% RSD	0.225		
PRFX (M_3)	Mean % Recovery ± SD*	100.154 ± 0.245	99.0973 ± 0.710	100.569 ± 0.296
	%RSD	0.419		
GATI (M_3)	Mean % Recovery ± SD*	100.046 ± .243	100.02 ± 0.163	99.791 ± 0.063
	% RSD	1.156		
SPAR (M_3)	Mean % Recovery ± SD*	99.702 ± 0.461	100.456 ± 0.402	100.232 ± 0.423
	%RSD	0.290		
MOXI (M_3)	Mean % Recovery ± SD*	100.153 ± 0.238	100.456 ± 0.161	99.6308 ± 0.2089
	% RSD	0.202		
BALO (M_3)	Mean % Recovery ± SD*	100.216 ± 0.202	100.083 ± 0.110	100.839 ± 0.259
	% RSD	0.190		

*Mean of triplicate determination

Table 10. Robustness data of LEVO, PRFX, GATI, SPAR, MOXI and BALO

Parameter	**Flow rate (± 2 mL/min)**			**Detection wave length (± 5 nm)**			**Mobile phase composition (± 5%)**		
Used	**0.8 mL/min**	**1mL/min (optimized)**	**1.2 mL/min**	**288 nm**	**293 nm (optimized)**	**298 nm**	**65:35,v/v**	**70:30,v/v (optimized)**	**75:25,v/v**
Retention time (min)									
LEVO	3.848	3.613	3.325	3.613	3.613	3.613	3.596	3.613	3.712
PRFX	4.390	4.320	4.290	4.321	4.320	4.320	4.180	4.290	4.390
GATI	4.769	4.707	4.695	4.706	4.707	4.707	4.603	4.707	4.807
SPAR	5.499	5.497	5.427	5.496	5.497	5.497	5.477	5.497	5.523
MOXI	6.024	5.880	5.438	5.881	5.880	5.878	5.720	5.880	5.970
BALO	6.855	6.253	6.239	6.253	6.253	6.253	6.200	6.253	6.335
Plate count$									
LEVO	12,562	12,261	12,054	12,242	12,261	12,274	12,106	12,261	12,282
PRFX	12,598	12,554	12,454	12,555	12,554	12,559	12,588	12,554	12,564
GATI	13,168	13,155	13,135	13,153	13,155	13,155	13,165	13,155	13,195
SPAR	14,898	14,761	14,661	14,761	14761	14,762	14,757	14,761	14,797
MOXI	15,064	14,912	14,726	14,892	14,912	14,928	14,838	14,912	14,786
BALO	15,994	15,916	15,816	15,923	15,916	15,918	15,929	15,916	15,987
Peak asymmetry#									
LEVO	1.126	1.106	1.097	1.107	1.106	1.106	1.026	1.016	1.015
PRFX	1.069	1.067	1.068	1.067	1.067	1.067	1.066	1.067	1.069
GATI	1.052	1.042	1.048	1.043	1.042	1.042	1.142	1.042	1..69
SPAR	1.077	1.073	1.086	1.073	1.073	1.074	1.187	1.073	1.172
MOXI	1.134	1.030	1.021	1.038	1.030	1.036	1.168	1.030	1.184
BALO	1.090	1.063	1.068	1.063	1.063	1.063	1.065	1.063	1.063

Acceptance criteria (Limits):#Peak Asymmetry < 1.5, $ Plate count > 3000.

Table 11. Results relating to LOD and LOQ

PARAMETER	Method M_3					
	LEVO	PRFX	GATI	SPAR	MOXI	BALO
LOD µg/mL	0.116	0.152	0.084	0.186	0.162	0.112
LOQ µg/mL	0.348	0.460	0.255	0.558	0.493	0.339

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Table 12. Results relating to assay

Formulation (Tablets)	Labeled Amount (mg)	Amount Found (mg)	% Assay ± SD*
LEVO	500	496.58	99.317 ± 0.990
PRFX	600	599.40	99.9 ± 0.04
GATI	400	399.60	99.9 ± 0.02
SPAR	100	99.45	99.45 ± 0.010
MOXI	400	399.77	99.495 ± 0.056
BALO	100	99.67	99.68 ± 0.09

*Average of six determinations

V. CONCLUSION

The results of the said method were accurate and quite satisfactory. The optimum privilege of the proposed method was that all LEVO, PRFX, GATI, SPAR, MOXI and BALO fluoroquinolones can be estimated on single chromatographic system without minor modifications in detection wavelength. The mobile phase composition used in this analysis is similar for the above six drugs. This method is highly useful for evaluation of product quality in the form of tablets. This method enables to detect cross contamination of the said products. As selectivity found to be lacking in respect of microbiological, fluorimetric as well as paper chromatographic analysis the author opted to RP-HPLC method and gained optimum results. Statistical analysis lucidly proves that this method was very fast, precise, accurate sensitive, highly efficient and suitable than the existing methods so far in existence and utilized. Therefore this method invented by the author is aptly feasible for regular analysis of six fluoroquinolones individually in quality control laboratories.

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