Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Rapid HPLC Determination of Norfloxacin, Levofloxacin, and Moxifloxacin Alone or in a Mixture

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Abstract

A simple, sensitive, and accurate chromatographic (RP-HPLC) method for simultaneous estimation of norfloxacin, levofloxacin, and moxifloxacin in their combined. Pharmaceutical dosage forms or individually. The HPLC separation was achieved on a Hypersil (C18, (150 mm x 4.6 mm,5 µm particle size) analytical column or equivalent. A Mixture of triethanolamine 1%: acetonitrile 80 % was used as the suitable mobile phase, at a flow rate of 1.2 mL/min and detector wavelength at 280 nm at ambient temperature (25 °C). In the HPLC method, the retention time of norfloxacin, levofloxacin, and moxifloxacin was found to be 1.854, 2.480, and 4.688 min, respectively, and the linearity of the method was obtained in the range from 25 to 80 µg/mL for norfloxacin, levofloxacin, and moxifloxacin. The Correlation coefficient for the method was greater than 0.999 RP-HPLC method validation parameter lay within its acceptance criteria as per the ICHq2 (R1) guideline Validation of analytical procedures. Hence it can be successfully used for routine analysis of norfloxacin, levofloxacin, and moxifloxacin in raw material or pharmaceutical dosage forms.

Keywords: Norfloxacin, Levofloxacin, Moxifloxacin, Validation, Simultaneous Estimation, HPLC with UV-Vis detection.

1. INTRODUCTION

The fluoroquinolones are a family of broad-spectrum, systemic antibacterial agents that have been used widely as therapy for respiratory and urinary tract infections. Fluoroquinolones are active against a wide range of aerobic gram-positive and gram-negative organisms. Gram-positive coverage includes penicillinase- and non-penicillinase-producing Staphylococci, Streptococcus pneumoniae, and viridans, Enterococcus faecalis, Listeria monocytogenes, and Nocardia species. Gram-negative coverage includes Neisseria meningitides and gonorrhoeae, Haemophilus influenzae, and most clinically important Enterobacteriaceae species, Pseudomonas aeruginosa and Vibrio species. The fluoroquinolones are believed to act by inhibition of type II DNA topoisomerases (gyrases) that are required for the synthesis of bacterial mRNAs (transcription) and DNA replication. They demonstrate little inhibition of human, host enzymes and have had an excellent safety record. The fluoroquinolones are indicated for the treatment of several bacterial infections, including bacterial bronchitis, pneumonia, sinusitis, urinary tract infections, septicemia and intra-abdominal infections, joint and bone infections, soft tissue and skin infections, typhoid fever, anthrax, bacterial gastroenteritis, urethral and gynecological infections, and pelvic inflammatory disease and several other infectious conditions.

The fluoroquinolones currently available include levofloxacin, moxifloxacin, and norfloxacin. These agents are well absorbed orally and well tolerated with a low rate of adverse effects. Several quinolones and fluoroquinolones were introduced but were subsequently withdrawn after spontaneous reports of severe adverse events including hepatotoxicity: temafloxacin (1992), gatifloxacin (2006), and trovafloxacin (1999). The currently available fluoroquinolones appear to cause idiosyncratic liver injury rarely, at an estimated rate of 1: 100,000 persons exposed. Idiosyncratic liver injury due to fluoroquinolones may be a “class” effect; the pattern of injury is similar, marked by an acute and often severe hepatocellular pattern of injury arising

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Receive Date: 26 February 2022, **Revise Date:** 29 May 2022, **Accept Date:** 11 June 2022, **First Publish Date:** 11 June 2022
DOI: 10.21608/EJCHEM.2022.124117.5536

within 1 to 4 weeks of starting therapy. The fluoroquinolones most frequently linked to liver injury are ciprofloxacin and levofloxacin, but these two agents also have been most widely used. Minor elevations in liver enzymes occur in 1% to 3% of patients receiving ciprofloxacin, norfloxacin, or ofloxacin. Rates with levofloxacin and moxifloxacin are less well defined but probably similar. The common side effects of fluoroquinolones are gastrointestinal disturbances, headaches, skin rash, and allergic reactions. Less common but more severe side effects include QT prolongation, seizures, hallucinations, tendon rupture, angioedema, and photosensitivity[2].

A spectrofluorometric method to determine levofloxacin is proposed and applied to determine the substance in tablets and spiked human urine and serum. The fluorimetric method allows the determination of 20–3000 ng mL^{-1} of levofloxacin in an aqueous solution containing acetic acid–sodium acetate buffer (pH 4) with λexc=292 and λem=494 nm [3]. A rapid, specific, and economic UV spectrophotometric method has been developed using a solvent composed of water: methanol: acetonitrile (9:0.5:0.5) to determine the levofloxacin content in bulk and pharmaceutical dosage formulations. At a pre-determined λ max of 292 nm, it was proved linear in the range of 1.0–12.0 µg/mL and exhibited a good correlation coefficient (R2=0.9998) and excellent mean recovery (99.00–100.07%) [4]. For the first time, a simple, selective, and sensitive liquid chromatography method was developed and validated for the simultaneous determination of levofloxacin (LEV), pazufloxacin (PAZ), gatifloxacin (GAT), moxifloxacin (MOX) and trovafloxacin (TRO) in human plasma. Samples were pre-treated with acetonitrile for precipitation of plasma proteins followed by evaporation and reconstitution steps. Chromatographic separation of the analytes and norfloxacin, used as internal standard (IS), was performed under gradient elution on a LiChroCART® Purospher Star C18 column (55 mm × 4 mm, 3 µm). The mobile phase comprised a mixture of 0.1% aqueous formic acid adjusted to pH 3.0 with triethylamine, acetonitrile and methanol pumped at a flow rate of 1.0 mL/min. The detector was set at excitation/emission wavelengths of 260/455 nm[5]. Treatment of multidrug-resistant tuberculosis (MDR-TB) is challenging due to the high treatment failure rate and adverse drug events. This study aimed to develop and validate a simple LC-MS/MS method for simultaneous measurement of five TB drugs in human plasma and to facilitate therapeutic drug monitoring (TDM) in MDR-TB treatment to increase efficacy and reduce toxicity. Moxifloxacin, levofloxacin, prothionamide, pyrazinamide, and ethambutol were prepared in blank plasma from healthy volunteers and extracted using a protein precipitation reagent containing trichloroacetic acid. Separation was achieved on an Atlantis T3 column with a gradient of 0.1% formic acid in water and acetonitrile [6]. Two simple, rapid, and sensitive spectrophotometric methods for the determination of levofloxacin, norfloxacin, and ciprofloxacin have been performed in pure form, pharmaceutical tablets, and spiked human urine. Both methods are based on the formation of a binary complex between the drugs and one of the two xanthene dyes, eosin Y or merbromin in the aqueous buffered medium. Under the optimum conditions, the binary complexes showed absorption maxima at 547 nm for eosin Y and 545 nm for merbromin. Using eosin Y, the calibration graph was linear over the range 2–8 µg mL^{-1} for the three drugs [7]. There are different analytical methods available to determine norfloxacin applied in the quality control of this medicine to ensure its effectiveness and safety. The authors present an overview of the fourth generation of quinolones, followed by the properties, applications, and analytical methods of norfloxacin. These results show several existing analytical techniques that are flexible and broad-based methods of analysis in different matrices[8]. A new high-performance liquid chromatography assay method was developed and validated for the determination of the above-mentioned drugs in small samples of human plasma. After protein precipitation with acetonitrile: methanol (1:1, vol/vol), satisfactory separation was achieved on a Hypersil BDS C18 column (250 × 4.6 mm, 5 µm) using a mobile phase comprising 20 mM sodium dihydrogen phosphate-2 hydrate (pH = 3.2) and acetonitrile at a ratio of 75:25, vol/vol; the elution was isocratic at ambient temperature with a flow rate of 1.5 mL/min. The ultraviolet detector was set at 260 nm[9]. selective and ultrasensitive analytical method for simultaneous determination of 11 fluoroquinolones (FQ) antibiotics in environmental and wastewater samples. The method employs offline solid-phase extraction (SPE) and reversed-phase high-performance liquid chromatography with fluorescence detection (HPLC-FLD). A weak cation exchange SPE protocol was developed with a novel loading volume optimization algorithm and a methanol cleanup step to remove background organic matter. Various parameters were optimized to recover FQs from water/wastewater and analyte recovery was generally greater than 80%. Chromatographic separation of the 11 FQs was achieved on a 150 mm pentafluorophenyl column using a gradient elution scheme with methanol, acetonitrile, and 20 mM phosphate buffer (pH = 2.4). Excitation and emission wavelengths were individually optimized for each FQ using fluorescence spectroscopy; the excitation and emission wavelengths were 276–296 nm and 444–506 nm, respectively. Instrumental quantitation limits were 20–100 pg of mass injected[10].

High performance liquid chromatography, or HPLC, is an analytical testing instrument used to separate components of a sample to identify **and** quantify the ingredients within a formulation. It has many uses in the medical, polymer, coatings, pharmaceutical, and other commercial or industrial industries. For those regulated by the FDA or governed by industrial standards, HPLC analysis is an industry tool for product validation and can be used for both qualitative and quantitative analysis. An HPLC analysis can be used to test both the raw materials and the finished goods. When first finding a raw material supplier or for routine quality control checks it is important to test the raw materials to ensure you have received the correct purity and grade of the product. Many raw materials look and feel alike, but to truly understand if you are receiving exactly what you are paying for they should be tested and verified. Manufacturers use laboratories with HPLC capabilities to reverse engineer formulations, solve product failure problems, perform competitor product analyses, and look for contaminants or other impurities. These types of testing can be performed on both the raw materials and the finished product. Often once the manufacturing process is finished, the final product is tested again to verify it contains the proper amount of active ingredients as claimed on the label. And since this type of testing will identify any contaminates or other product defects that may have occurred with the batch during the manufacturing process, performing these tests will reduce the chance of having the product recalled. HPLC analysis can also be used in conjunction with additional analytical instrumentation to determine the stability and shelf life of products. The advantages of using HPLC for analysis are that it requires a small sample size, and testing can be modified depending on the level of quantification needed[11,12].

Chemicals Formula

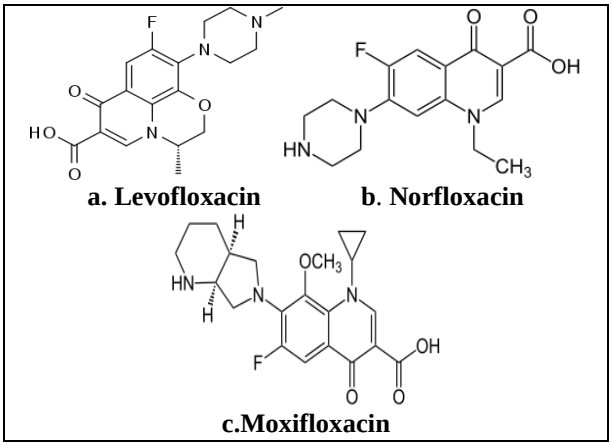


Fig (1) Chemical structures of (a) Levofloxacin, (b) Norfloxacin, (c) Moxifloxacin

2. Experimental

Instrument

All the peak area measurements were made using Shimadzu high performance liquid - chromatography consisting of a pump model LC-20AD, UV detector model SPD 20A, auto sampler model, 20AC, Oven model CTO -20A9 Japan, HPLC with UV-Vis detection spectrophotometer Shimadzu model 1650 (Japan) Waters column heater and a Rheodyne injector with a 20 µL loop. Electronic balance model kern ABJ (UK) 4 - digit number, Buffer solution were measured using instrument pH meter (model 3510 Jenway).

Reagent and Chemicals

All chemicals and materials used were of pharmaceutical grade and were standardized (BP 2018), distilled water was used throughout the investigation. norfloxacin, levofloxacin, and moxifloxacin are supplied by Artri (Mumbai, India). Acetonitrile (HPLC grade) and Nylon Filter membranes (diameter = 47 mm, pore size = 0.45 µm) were obtained from Merck (Germany), and Triethanolamine was purchased from Fisher (U.K).

Table (1) Chemicals reagent

Reagent Name	M.Wt	Formula
Levofloxacin	361.368	$C_{18}H_{20}FN_3O_4$
Norfloxacin	319.331	$C_{16}H_{18}FN_3O_3$
Moxifloxacin	401.431	$C_{21}H_{24}FN_3O_4$
Acetonitrile	41.05	C_2H_3N
Triethanolamine	149.188	$C_6H_{15}NO_3$

Chromatographic Condition

The Mobile phase consists of

Table (2) Mobile phase consisting

No	Time(min)	Acetonitrile (80%)	Triethanolamine (1%)
1	0.00	3 %	97 %
2	3.00	3 %	97 %
3	8.00	15 %	85 %

An Inertsil C18 column ((25cm x 4.6 mm, 5µm) GL Science, Japan) was used as the stationary phase and ambient temperature(25°C). Isocratic elution was performed with a mobile phase. Was filtered through a nylon disc filter of 0.45 µm (Millipore) and sonicated for 20 min before use. the flow rate was 1.2 mL/min and the injection volume was 20 µl. The UV detector was performed at 280 nm and the separation was achieved at ambient temperature (25°C).

Preparation of Standard Solution

Accurately 5 mg of each drug (levofloxacin, norfloxacin, moxifloxacin) was weighed and then transferred to a 10 mL volumetric flask, each amount of drug was dissolved and diluted up to mark with

mobile phase to obtain the final concentration of 500 µg /mL (levofloxacin, norfloxacin, moxifloxacin), then the solution was filtered through 0.20 µm filter paper and sonicated for 5 min. then take 1 mL into a 10 mL volumetric flask. Add diluted up to mark with mobile phase to obtain a final concentration (levofloxacin, norfloxacin, moxifloxacin). 50 µg /mL and repeat this step for the other drugs.

preparation of standard mixture solution of norfloxacin, levofloxacin& moxifloxacin (1:1:1)

Accurately 5 mg of norfloxacin, levofloxacin, and moxifloxacin was weighed then transferred to a 10 mL volumetric flask, dissolved, and diluted up to mark with mobile phase to obtain a final concentration of 500 µg /mL Norfloxacin. then the solution was filtered through 0.20 µm membrane filter paper and sonicated for 5 min. then take 1 mL into a 10 mL volumetric flask. Add diluted up to mark with mobile phase to obtain final concentrations of norfloxacin, levofloxacin, and moxifloxacin (concentration 50:50:50 µg /mL).

Validation of Developed Method

Validation of Developed Method was carried out according to ICH [(1)] Guideline for validation of analytical procedure Q2(R1).

Specificity

Defining its ability to measure accurately and specifically the analyte of interest without interferences from blank: solution contains 50 µg/mL norfloxacin,50 µg/mL levofloxacin, and 50 µg/mL moxifloxacin. prepared solutions were analyzed as per the proposed method. The mobile phase was taken as blank and was analyzed as per the proposed method. Interferences from blank to measure accurately and specifically the analyte of interest were checked.

Linearity

Linearity was performed by preparing 5 different concentrations of (25,40,50,60, and 75 µg/mL) of norfloxacin, levofloxacin, and moxifloxacin standard. The sample was prepared by weighing about 100 mg of norfloxacin, levofloxacin, and moxifloxacin standard and dissolved in 1000 mL of the mobile phase in a volumetric flask (stock solution). Subsequently, serial dilutions were prepared from the stock solution to obtain the final concentrations. the mean area with its standard deviation and % relative standard deviation of peak area was calculated mean AUC against concentration were plotted to obtain the calibration curve. regression equations and correlation coefficients were computed from calibration curves.

Accuracy and recovery studies

Accuracy was calculated by the addition of standard drugs to the pre-analyzed sample at 3 different concentration levels and computing percentage recoveries. accuracy was assessed using 9 determinations over 3 concentration levels covering the specified range (eg.,3 concentrations and 3 replicates each of the total analytical procedure),

Prepared solutions were analyzed as per the proposed method % recoveries were calculated from the absorbance ratio. The mean percentage recovery with its standard deviation and % relative standard deviation was computed at each level.

Precision

The Precision of the method was computed by two means: repeatability and intermediate precision:

Repeatability: System Precision and Method Precision

System precision

A solution containing a mixture of 50 µg/mL norfloxacin,50 µg/mL levofloxacin, and 50 µg/mL moxifloxacin (100% test concentration) was then prepared from their respective working mixture solution prepared solution was analyzed. five times as per the proposed method. The mean peak area with its standard deviation was computed for the drugs.

Method precision

Six replicate solutions containing a mixture of 50 µg/mL norfloxacin,50 µg/mL levofloxacin, and 50 µg/mL moxifloxacin (100% test concentration) was prepared from their respective working mixture solution prepared. The Prepared solution was analyzed as per the proposed method the mean peak area with its standard deviation and relative standard deviation were computed for the drugs.

Ruggedness: Replication in Different Days

Six replicate solutions containing a mixture of 50 µg /mL norfloxacin,50 µg/mL levofloxacin, and 50 µg /mL moxifloxacin (100% test concentration) are implemented on the first day, and then on a second day, five replicates of freshly prepared norfloxacin, levofloxacin, moxifloxacin are analyzed. the same analyst performs both tests.

Robustness

A solution containing a mixture of 50 µg/mL norfloxacin,50 µg/mL levofloxacin, and 50 µg/mL moxifloxacin (100% test concentration) was prepared from their respective working mixture solution prepared as described above, and a prepared solution was analyzed as per proposed method with small but deliberated change in chromatographic condition as liked below:

The Change included column temperature ± 2°C, and flow rate ±0.005 mL/min.

The mean peak area with standard deviation and % relative standard deviation was computed at each level.

3. Results and Discussion

Method Development

The HPLC method was accurate and precise for the simultaneous determination of norfloxacin, levofloxacin, and moxifloxacin. good resolution of both components was obtained with tri ethanol amine: acetonitrile at a ratio of 97:3 v/v for 3 minutes and 85:15 v/v from 3 to 8 minutes the flow rate of 1.2 mL/minutes was optimum. UV detection was made at 280 nm. At this wavelength, norfloxacin, levofloxacin, and moxifloxacin can is quantified. hence,280 nm determined empirically is to be optimum the average retentions times for norfloxacin, levofloxacin, and moxifloxacin were found to be 1.854,2.480, and 4.688 minutes, respectively, and the mixture (fig. 2,3,4). The system suitability parameter for the chromatogram area follows.

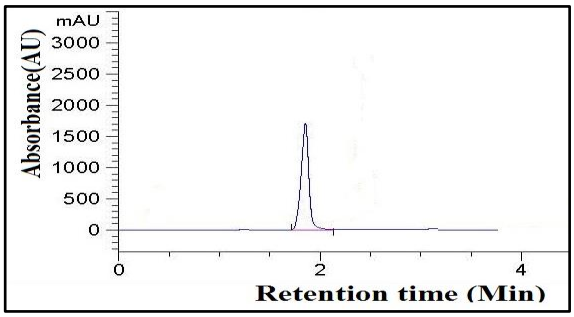


Fig (2) Chromatogram of norfloxacin

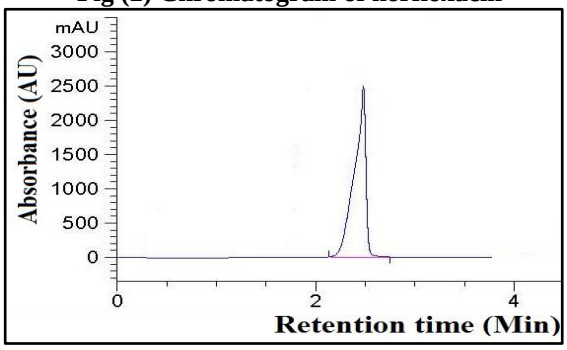


Fig (3) Chromatogram of levofloxacin

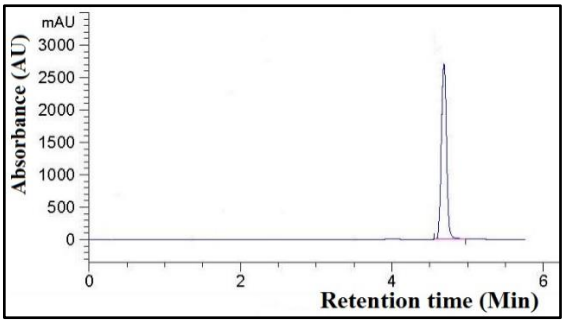


Fig (4) Chromatogram of moxifloxacin

Method Validation

Analytical parameters

Table (3) Analytical parameter for levofloxacin, norfloxacin, moxifloxacin

Parameter	Norfloxacin	Levofloxacin	moxifloxacin
Wavelength (nm)	280	280	280
Linearity range, µg /mL	25-80	25-80	25-80
Slope(b)	1066.233	1628.330	3021.652
Intercept (a)	0.8228	0.8981	0.826
Correlation coefficient (R^2)	0.9999	0.9998	0.9995
LOD	0.0008	0.000110	0.00066
LOQ	0.0024	0.00334	0.002

Specificity

Method specificity was determined for levofloxacin, norfloxacin, moxifloxacin samples, and placebos of a chemical mixture. levofloxacin, norfloxacin, moxifloxacin, and placebo spectra show no interference for norfloxacin, levofloxacin, and moxifloxacin peaks. Therefore, data obtained for norfloxacin, levofloxacin, and moxifloxacin will is considered acceptable for method specificity.

As it can be seen from the respective chromatogram (figure 5-9)

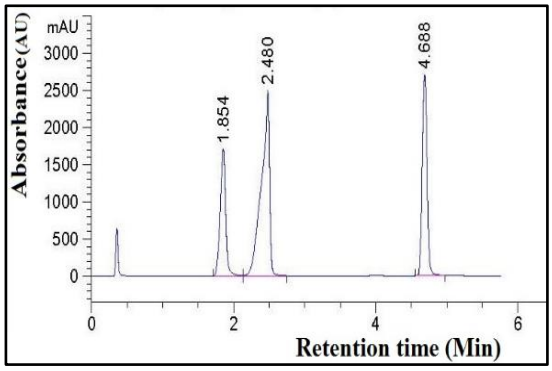


Fig (5) Chromatogram of mixture STD

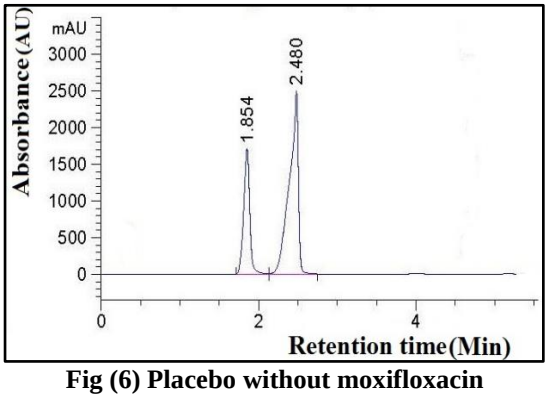


Fig (6) Placebo without moxifloxacin

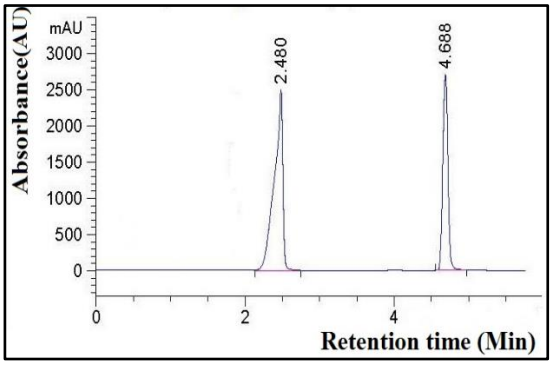


Fig (7) Placebo without norfloxacin

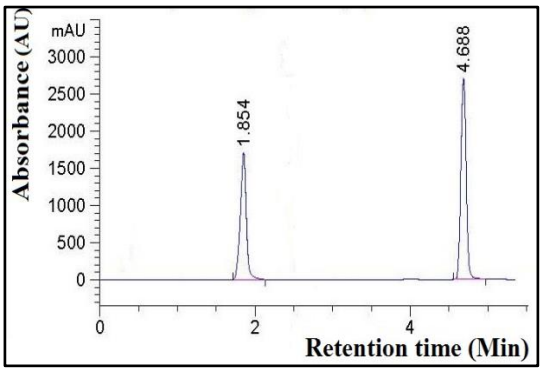


Fig (8) Placebo without levofloxacin

Absorbance (AU)

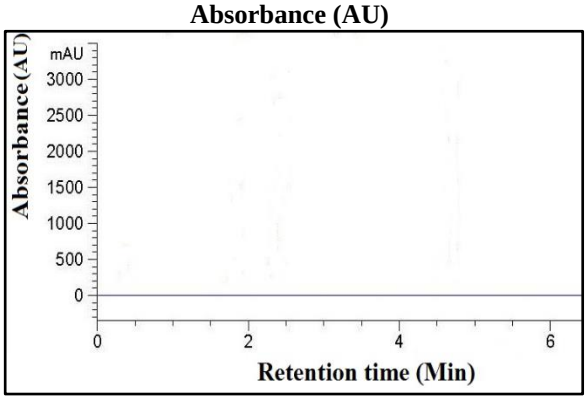


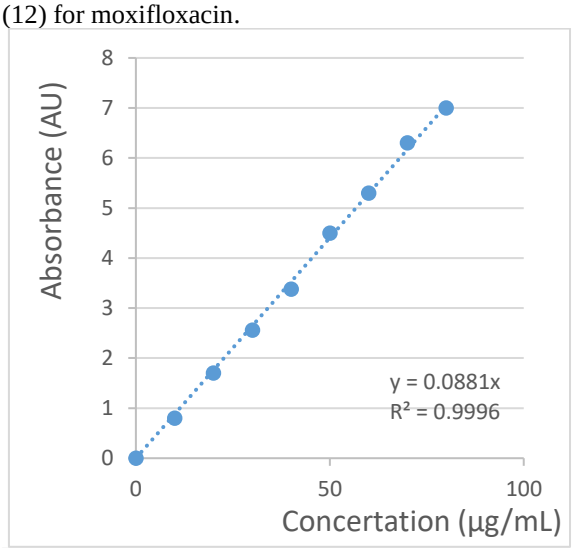
Fig (9) Placebo without three drugs

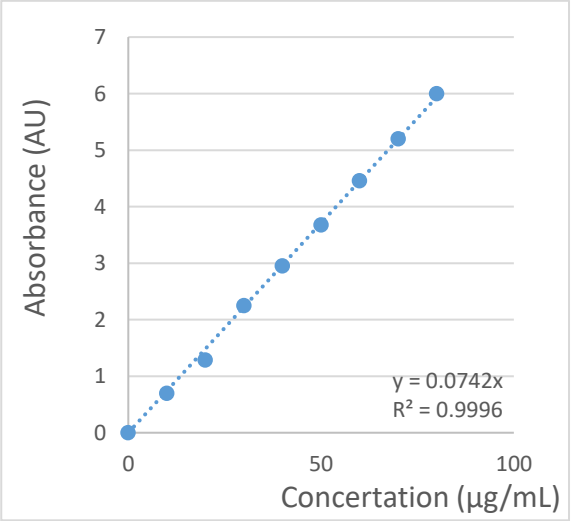
Effect of Interference

The use of interference materials such as saccharin sodium, lactose, microcrystalline cellulose, magnesium stearate, titanium dioxide, talc, and sodium starch glycolate are colloidal anhydrous silica, by adding 10 times excess with 5 mg/mL concentration of each drug show that there is no interference with each one.

Linearity and Range

Standard solutions are prepared using different concentrations of norfloxacin, levofloxacin, and moxifloxacin, (25-80 µg /mL) one the range of 50% to150% of the theoretical quantity of norfloxacin, levofloxacin, and moxifloxacin. the criteria of good linearity are determined by obtaining a correlation coefficient not less than 0.999 of concentration versus peak area graphs. calibration plots are given in fig (10), for norfloxacin, fig (11) for levofloxacin, and fig (12) for moxifloxacin.





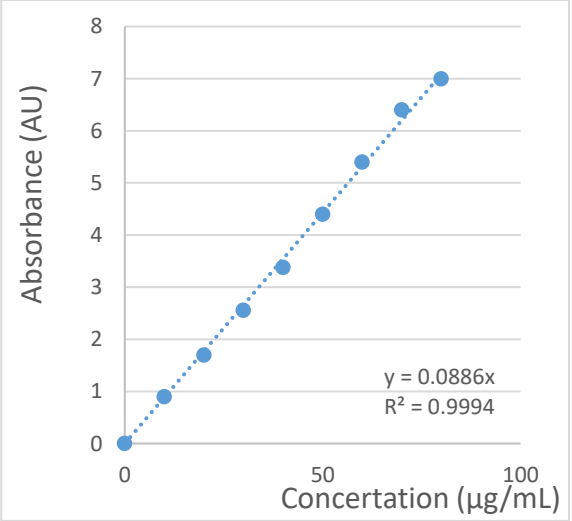


Fig (10) Calibration curve for norfloxacin Fig (11) Calibration curve for levofloxacin Fig (12) Calibration curve for moxifloxacin

Accuracy and Recovery Studies

Samples are spiked by adding know quantities of norfloxacin, levofloxacin, and moxifloxacin standard to the placebo matrix containing all excipients of the product. the measurements are made at a concentration that is to be 100% of the target concentration and at 50% and 150%, the accuracy of the method is determined by the percentage recovery of each concentration concerning the real values.

Precision

System Precision

Six replicates of same solution from 50 µg/mL levofloxacin ,50 µg/mL norfloxacin, and 50 µg/mL moxifloxacin. (RSD not more than 1%) (table 5).

Method precision

Six replicates of same solution from 50 µg/mL levofloxacin ,50 µg/mL norfloxacin, and 50 µg/mL moxifloxacin. (RSD not more than 1%) (table 6).

Ruggedness:

Six replicates of same solution from 50 µg/mL levofloxacin ,50 µg/mL norfloxacin, and 50 µg/mL moxifloxacin. are implemented in the first-day table (7) and then on a second-day table (8) the method is rugged as the % (RSD not more than 3 %).

Robustness

Six replicates of single sample norfloxacin, levofloxacin, and moxifloxacin. are implemented with a change in temperature ± 2°C and flow rate ±0.005 mL/min, and the same analyst performs both tests. The method is rugged as the %RSD is not more than 2%) table 9, 10 11&12. So, the obtained results indicate that the method is robust.

Table (4) Accuracy and recovery result

Drugs	Taken (µg /mL)	Found (µg/mL)	Recovery (n=3) %	SD	RSD%
NORFLOXACIN	25	25.89	99.01	1.44	1.450
	40	41.14	101.1	0.543	0.533
	80	81.81	100.12	0.505	0.505
LEVOFLOXACIN	25	23.7	99.89	0.629	0.630
	40	37.89	100.56	0.614	0.611
	80	79.44	100.21	0.533	0.532
MOXIFLOXACIN	25	23.05	99.24	0.87	0.870
	40	38.06	99.75	0.40	0.401
	80	78.73	99.76	0.081	0.083

Table (5) System Precision Result

Item	Mean	SD	RSD
NORFLOXACIN	50.12	0.376	0.752
LEVOFLOXACIN	50.12	0.487	0.9720
MOXIFLOXACIN	50.05	0.414	0.827

Table (6) Method Precision Result

Item	Mean	SD	RSD
NORFLOXACIN	49.97	0.466786	0.934132
LEVOFLOXACIN	49.93	0.432178	0.865568
MOXIFLOXACIN	50.1	0.483046	0.96416

Table (7) Ruggedness Day 1

Item	Mean	SD	RSD
NORFLOXACIN	50.21	0.455705	0.907597
LEVOFLOXACIN	50.35	0.485913	0.96507
MOXIFLOXACIN	50.1	0.881917	1.760314

Table (8) Ruggedness Day 2

Item	Mean	SD	RSD
NORFLOXACIN	50.04	0.93832	1.87514
LEVOFLOXACIN	49.81	1.050344	2.108701
MOXIFLOXACIN	49.92	0.761285	1.525011

Table (9) Robustness temperature 27°C

Item	Mean	SD	RSD
NORFLOXACIN	50.13	0.566200	1.392015
LEVOFLOXACIN	50.07	0.921950	1.999779
MOXIFLOXACIN	50.03	0.872505	1.77001

Table (10) Robustness temperature 23°C

Item	Mean	SD	RSD
NORFLOXACIN	49.85	0.773590	1.352081
LEVOFLOXACIN	49.88	0.785580	0.985620
MOXIFLOXACIN	49.96	0.859620	0.985918

Table (11) Flow rate change effect 1.205mL/min

Item	Mean	SD	RSD
NORFLOXACIN	50.20	0.883289	1.378015
LEVOFLOXACIN	49.50	0.852500	1.889779
MOXIFLOXACIN	49.80	0.756240	1.559701

Table (12) Flow rate change effect 1.195mL/min

Item	Mean	SD	RSD
NORFLOXACIN	50.10	0.555203	1.55420
LEVOFLOXACIN	50.02	0.482500	0.98962
MOXIFLOXACIN	49.90	0.463520	0.92305

Table (13) Determination of drugs in pharmaceutical industrial samples

Drug Name	Taken (µg/mL)	Found (µg/mL)	Recommended Method	% Recovery
Norfloxacin (Epinor tablets 400 mg) Eipico, Egypt	5 mg	4.98 mg	4.99 mg	99.92
Levofloxacin (Tavanic tablets 500 mg) Sanofi Winthrop Industries, France	5 mg	5.06 mg	5.01 mg	99.38
Moxifloxacin (AVALOX 400MG TAB) BAYER SCHERING PHARMA AG	5mg	4.92 mg	4.97 mg	100.1

Recommended method referring to pharmacopeia (BP 2018)

4. Analysis of synthetic formulation product

Synthetic Formulation product contains 5 mg norfloxacin, levofloxacin, and moxifloxacin takes weight equivalent to 5 mg of three drugs then transferred to 10 mL volumetric flask added mobile phase to volume and flask was sonicated for 5 min. the solution was then filtered through 0.20 µm membrane filter paper. The filtrate solution was suitably diluted with the mobile phase to get a final concentration of 50 µg/mL of norfloxacin, levofloxacin, and moxifloxacin. The prepared solution was injected into the system with stated chromatographic conditions as described above. The chromatogram was stopped after separation was achieved completely. Peak areas were recorded. The concentration of synthetic formulation products was computed by putting the value of their peak areas in the respective standard regression equation obtained from the calibration curve. the analysis procedure was repeated three times with a synthetic formulation product.

5. Applications

The proposed procedures which are sensitive and accurate can be determined by the studied substances in their synthetic pharmaceutical formulations.

6. Conclusion

Stability indicating RP-HPLC method is a sample, provident, sensitive and accurate has been developed and validated for the simultaneous estimated of norfloxacin, levofloxacin, moxifloxacin individually or into a chemical mixture or separated pharmaceutical formulation and clustered we can use method for routine work of analysis into pharmaceutical factories for determined the three drugs so we can rename, studies showed that the method was precise, sensitive, selective, robust, and linear over the concentration range of 25-80 µg/mL for norfloxacin, levofloxacin, and moxifloxacin. This method was also free from interference from the excipients used in the formulations the method became the general method for determining three drugs without any interference.

Reference

1. The guideline, I. H. T. (2007). Validation of Analytical Procedures: Text and Methodology, Q2 (R1), Current Step 4 version, Parent Guideline dated 27 October 1994 (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005. In *International Conference on Harmonization, Geneva, Switzerland*.
2. British pharmacopeia, the stationary office, London (2018).
3. González, J. A. O., Mochón, M. C., & de la Rosa, F. J. B. (2000). Spectrofluorimetric determination of levofloxacin in tablets, human urine and serum. *Talanta*, *52*(6), 1149-1156.
4. Maleque, M., Hasan, M. R., Hossen, F., & Safi, S. (2012). Development and validation of a simple UV spectrophotometric method for the determination of levofloxacin both in bulk and marketed dosage formulations. *Journal of pharmaceutical analysis*, *2*(6), 454-457.
5. Sousa, J., Alves, G., Campos, G., Fortuna, A., & Falcão, A. (2013). First liquid chromatography method for the simultaneous determination of levofloxacin, pazufloxacin, gatifloxacin, moxifloxacin and trovafloxacin in human plasma. *Journal of Chromatography B*, *930*, 104-111.
6. Zheng, X., Jongedijk, E. M., Hu, Y., Kuhlin, J., Zheng, R., Niward, K., ... & Alffenaar, J. W. C. (2020). Development and validation of a simple LC-MS/MS method for simultaneous determination of moxifloxacin, levofloxacin, prothionamide, pyrazinamide and ethambutol in human plasma. *Journal of Chromatography B*, *1158*, 122397.
7. El-Brashy, A. M., Metwally, M. E. S., & El-Sepai, F. A. (2004). Spectrophotometric determination of some fluoroquinolone antibacterials by binary complex formation with xanthene dyes. *Il farmaco*, *59*(10), 809-817.
8. Chierentin, L., & Salgado, H. R. N. (2016). Review of properties and analytical methods

for the determination of norfloxacin. *Critical reviews in analytical chemistry*, *46*(1), 22-39.
9. Helmy, S. A. (2013). Simultaneous quantification of linezolid, tinidazole, norfloxacin, moxifloxacin, levofloxacin, and gatifloxacin in human plasma for therapeutic drug monitoring and pharmacokinetic studies in human volunteers. *Therapeutic drug monitoring*, *35*(6), 770-777.
10. He, K., & Blaney, L. (2015). Systematic optimization of an SPE with HPLC-FLD method for fluoroquinolone detection in wastewater. *Journal of hazardous materials*, *282*, 96-105.
11. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012–. Fluoroquinolones. 2020 Mar 10. PMID: 31643179.
12. Mando, H., & Hasan, A. (2015). Development and Validation of RP-HPLC Method for Determination of Levofloxacin in Tablet Using UV and Fluorescence Detectors Simultaneously. *International Journal of Pharmaceutical Sciences and Research*, *6*(4), 1401-1406.
13. Naveed, S., Sultana, N., Arayne, M. S., & Dilshad, H. (2014). A new HPLC method for the assay of levofloxacin and its application in drug-metal interaction studies. *Journal of Scientific and Innovative Research*, *3*(1), 91-96.
14. Mendes, C., Buttchevitz, A., Kruger, J. H., Bernardi, L. S., Oliveira, P. R., & Silva, M. A. S. (2015). Quantitative analysis of norfloxacin in β-cyclodextrin inclusion complexes—development and validation of a stability-indicating HPLC method. *Analytical Sciences*, *31*(10), 1083-1089.
15. Chierentin, L., & Salgado, H. R. N. (2016). Review of properties and analytical methods for the determination of norfloxacin. *Critical reviews in analytical chemistry*, *46*(1), 22-39.
16. Yıldırım, S., Karakoç, H. N., Yaşar, A., & Köksal, İ. (2020). Determination of levofloxacin, ciprofloxacin, moxifloxacin and gemifloxacin in urine and plasma by HPLC–FLD–DAD using pentafluorophenyl core–shell column: Application to drug monitoring. *Biomedical Chromatography*, *34*(10), e4925.
17. Hanh, L. T. M., Diep, N. T. M., Mai, P. T. N., & Truong, N. X. (2019). HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ASSAY FOR DETERMINATION OF MOXIFLOXACIN IN HUMAN PLASMA. *Vietnam Journal of Science and Technology*, *57*(3), 336.
18. Szerkus, O., Jacyna, J., Gibas, A., Sieczkowski, M., Siluk, D., Matuszewski, M., ... & Markuszewski, M. J. (2017). Robust HPLC–MS/MS method for levofloxacin and ciprofloxacin determination in human prostate tissue. *Journal of pharmaceutical and biomedical analysis*, *132*, 173-183.
19. Czyrski, A., Sokół, A., & Szałek, E. (2017). HPLC method for determination of moxifloxacin in plasma and its application in pharmacokinetic analysis. *Journal of Liquid Chromatography & Related Technologies*, *40*(1), 8-12.
20. Panchumarthy, R. (2013). Simultaneous Separation of Five Fluoroquinolones-Norfloxacin, Moxifloxacin, Enrofloxacin, Sparfloxacin, and Prulifloxacin Using an Isocratic HPLC: Application to Determination of Norfloxacin in Pharmaceutical Formulations. *International Journal*, *3*(2), 17-21.