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Development and validation of simple colorimetric methods for assessing norfloxacin in pure form, in pharmaceutical products and in biological material

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Abstract

Two straightforward, affordable, accurate, and spectrophotometric techniques gross developed to assess norfloxacin, formulations, and biological samples. The oxidation of norfloxacin will be done in technique (A) in acid solution with help of Fe(III). A wavelength of 511 nm with a correlation coefficient of 0.9879 was produced by the resulting Fe(II) coupled with 1,10-phenanthroline and the red colour complex. A uniform absorbance ranging from 1 to 30 µg/mL was discovered. Similarly, in procedure (B), in an acidic medium, Ce(IV) was added to norfloxacin. After reacting with a specific amount of methyl orange, the residual Ce(IV) is then determined. An absorbance measurement at 508 nm and a correlation coefficient of 0.9966 indicate a straight-line relationship between the two variables for concentration range is 1–15 µg/mL. The procedures were developed after a careful analysis of the several elements that influence the reaction process. After calculations, the LOD (limits of detection) and LOQ (limit of quantification) were determined to be 1.098 and 1.111 µg/mL for method A and 2.875 and 3.368 µg/mL for method B respectively. The method B has also been applied for the determination of norfloxacin in spiked human plasma and urine samples. The percentage recoveries ranged from 98.74 to 103.43% and from 98.17 to 100.85% for plasma and urine samples, respectively. Proposed techniques have been successfully used for the examination of biological fluids, formulations for medicines as well as pure norfloxacin following statistical validation through recovery studies.

Keywords Norfloxacin, 1,10-phenonthroline, Methyl orange, Spectrophotometry, Pharmaceutical formulations, Ce(IV), Redox reaction

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Introduction

Antimicrobial medicines called quinolones are commonly used to treat infections in both people and animals. These drugs are essential for treating diseases of the respiratory system, skin, and urinary tract. Nalidixic acid is the precursor to the broad-spectrum synthetic antibacterial class drugs called fluoroquinolones. Their primary use since their accidental discovery in 1962 has been to treat a variety of infectious diseases [1–4]. Fluoroquinolones are efficiently absorbed by the digestive system. They are capable of good extended elimination half-lives, tissue penetration, and low protein binding. Adults can tolerate these antibiotics well and they can be used to treat a wide range of infections [5, 6]. Norfloxacin (NFX), made from fluoroquinolone carboxylic acid, is prescribed to treat urinary tract and respiratory infections [7]. Rapid and precise analytical methods are needed to identify NFX because of its extensive use as well as the necessity of clinical and medical research.

There are several methods for NFX analysis in various pharmacological preparations and biological materials. High pressure liquid chromatography [8–10], thin layer chromatography [11], liquid chromatography [12], voltammetric technique [13], DPP [14], fluorometry [15] as well as PT [16] are few of given approaches. For identifying (NFX), most of published studies on spectrophotometric [17–21] approaches include a lot of steps, expensive reagents, poor selectivity, and low linear ranges. Some of the approaches are completely described in the literature. Spectrophotometry is taken into consideration as the preeminent analytical methods for studying allopathic products due to its accessibility, affordability, and availability. Goal of the manuscript is to introduce two simple, inexpensive, rapid, accurate, and environmentally unaffected techniques that enable excellent sensitivity and high reproducibility of (NFX) detection.

Experimental

Reagents and material

Every reagent used was of the higher quality and utilized straight away, without further purification. The given supplies have been used in the scheme: Methyl orange (MO) (Fisher Chemical UK Limited), 1,10-phenanthroline (Scharlau Chemi S. A., Barcelona, Spain), methanol (Merck, Darmstadt, Germany), H_2SO_4 (Riedel-deHaen, Germany), 95 – 77% pure $FeCl_3 \times 6H_2O$, and 65% nitric acid (Sigma-Aldrich). The standard reference norfloxacin was providing by the pharmaceutical manufacturer. Noroxin tablets 400 mg (manufactured by OBS Pakistan (Pvt) Ltd, C-4 S.I.T.E, Karachi), (Utinor tablets 400 mg, manufactured by Standpharm Pakistan (Pvt) LTD), Alenbit tablets 400 mg, manufactured by Meezab International, Karachi, Pakistan and Norocin tablets 400 mg manufactured by Bosch Pharmaceutical Pvt) Ltd. Korangi Industrial Area Karachi Pakistan) were purchased locally.

Instruments

Shimadzu UV-1800, ENG 240 V dual beam spectrophotometer with 1 cm quartz cells were employed to determine the absorbance. Additionally, a water bath is used and digital analytical balance (OHAUS Corporation USA).

Preparation a solution of reagents

$FeCl_3.6H_2O$ (0.054 g) dissolved in water that had been deionized to create 0.002 mol/L Fe(III) solution, and then further diluted to 100 mL. 0.0594 g of the reagent was dissolved in 5.0 mL of ethanol to create 0.006 mol/L 1,10-phenanthroline solution, and then diluted it further using distilled water, up to about 50 mL.

Nitric acid 8.02 mL solution in 50 mL of distilled water was used to create 2.5 mol/L. Similarly, 0.0606 g of $Ce(SO_4)_2.4H_2O$ was added to 50 mL of 2.5 mol/L nitric acid, and the mixture was diluted with deionized water until it reached 50 mL. This resulted in a 3.0×10^{-3} mol/L Ce(IV) solution. 0.1304 g MO was dissolved in distilled water (50 mL) to create an 8×10^{-3} mol/L solution.

Making standards

0.005 g of standard norfloxacin was dissolved in 50 mL distilled water by heating at 60 °C on a water bath to make a stock solution of 100 µg/mL. To make new standard solutions with the necessary concentrations, the stock solution was diluted with deionized water every day before use.

Suggested method for calibration curve construction

Method-A: NFX stock solution was added in the proper amounts to separate test tubes till an ultimate concentration of 1–30 µg/mL is achieved. 10 mL of deionized water was used to dilute the mixtures after 1.5 mL of 0.014 mol/L 1,10-phenanthroline and after adding 0.002 mol/L Fe^{3+} solution about 0.5 mL, the mixture was heated using water bath for 15 min at 100 °C. Spectrophotometer was used to detect the ratio of absorbance at 511 nm to a reagent blank that had been precisely replicated from drug sample.

Method-B: 10 milliliter volumetric flasks were filled with diluted norfloxacin stock solutions of the appropriate volumes, making the ultimate concentration ranging from1 and 20 µg/mL. Then they were filled with 2.5mL of 2.5 mol/L nitric acid and 1.5mL of 3.0×10^{-3}mol/L Ce(IV). Flasks remained left alone for 15 min while being continuously shaken, followed by the addition of 1.5 mL of 8.0×10^{-3} mol/L MO solution. Each solution's absorbance at 508 nm is measured in contrast to a reagent blank that was the same but did not contain the drug.

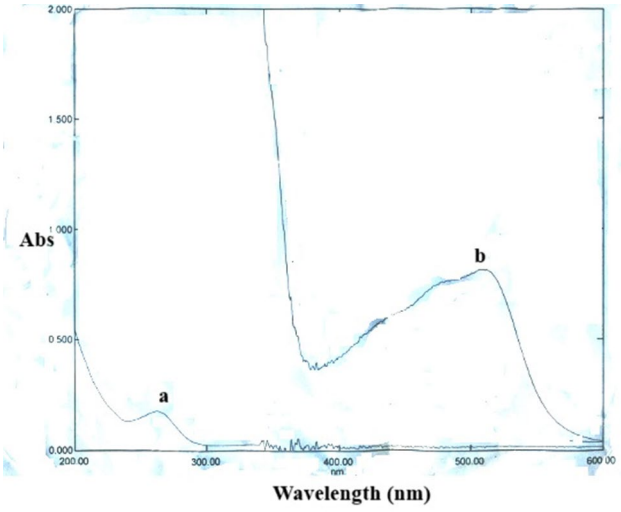

Fig. 1A Absorption spectrum of norfloxacin (a), Absorption spectrum of color complex (b). Condition: 1.0 mL of Fe^{3+} (0.002 mol/L), 30 µg/mL of norfloxacin, 1.0 mL of 1,10-phenanthroline (0.006 M), heated at 100 ° C for 15 min, diluted to 10 mL

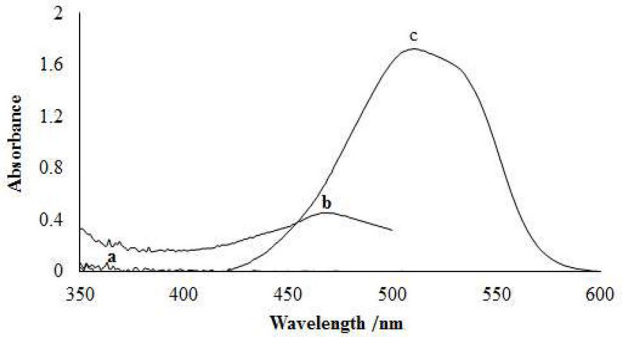

Fig. 1B Absorption spectra of (a) Norfloxacin, (b) Ce(IV)-Methyl orange, (c) Norfloxacin-Ce(IV)-Methyl orange. Condition: 20 µg/mL of norfloxacin, 3.0×10^{-3}molL $^{-1}$ of cerium(IV), and 8×10^{-3} mol/L of MO

Application to pharmaceutical formulations

The contents of four 400 mg tablets—Norocin, Utinor, Noroxin, and Alenbit—were weighed individually to determine the mean weight of one tablet. Next, 100 µg/mL solution was prepared by dissolving 0.005 g of norfloxacin powder in distilled water while being shaken. Following filtering, 50 mL volumetric flasks were filled with the resultant solutions, and deionized water was used to adjust the contents. Next, the accurate amount of this stock solution was created by diluting 100 µg/mL norfloxacin solution. To ascertain the precise norfloxacin content of the provided sample, following that, using the methods described to create the calibration curve, the volumes of these solutions were examined.

Application to biological fluids

The method B can also be applied for the biological fluids. 1 mL of blood plasma or urine was mixed with 6 mL of acetonitrile and 2.5 mL of standard norfloxacin in a test tube and placed in centrifuge tube. The mixture was centrifuge for 10 min at 3500 rpm. After deproteinization clear liquid was diluted to 25 mL with distilled water in volumetric flask. Then 1, 2 and 3 µg/mL of drug were taken in three separate 10 mL volumetric flasks and added all the optimized reagents and allowed to stand for 15 min and then diluted to 10 mL with distilled water and absorbance was measured at 508 nm.

Outcome and discussion

Method-A: Ferric salt is an essential part of the analysis of pharmaceutical drugs; the concentration of the drug is indicated by the amount of ferric salt that oxidizes to ferrous salt. The drug's decrease of Fe(II) is considered using 1,10-phenonthroline, and a spectrophotometric technique can be developed using this property that measures NFX in pharmaceutical formulations. Norfloxacin reacts with 1,10-phenonthroline and Fe(III) to form tris(1,10-phenonthroline) iron(II) chelate, an orange-red color complex with a maximum absorbance of 511 nm (Fig. 1A).

For Method B: Using spectrofluorimetric [22–24], spectrophotometric [25–27] and chemiluminescence [28] methods, a variety of medicinal compounds have been identified, Ce(IV) is utilized as an oxidizing agent in each of them. The proposed method is based on the addition of Ce(IV) to norfloxacin in acidic medium, followed by measuring the residual Ce(IV) by reacting with methyl orange and the absorbance was measured at 508 nm (Fig. 1B(c)). As shown in Fig. 1B(a), the norfloxacin show very low absorbance in the ultraviolet region. The Fig. 1B(b) show the absorption spectra of Ce(IV) and methyl orange. The Fig. 1B(c) show the absorbance spectra of Norfloxacin-Ce(IV) and methyl orange.

Optimization of reaction conditions

Several distinct experimental conditions that impact the results of both techniques development and stability of color products formation were thoroughly analyzed and optimized. One by one, these characteristics were altered while the others stayed the same.

Method-A

Impact of heating time and temperature

Effects of duration and temperature on complexation reaction were examined. At temperatures between 60 and 100ºC, the effects of heating times ranging from 5 to 30 min were investigated. Based on the findings, it was determined that heating the reaction to 100 ºC for around 15 min increased the amount of color creation (Figs. 2 and 3).

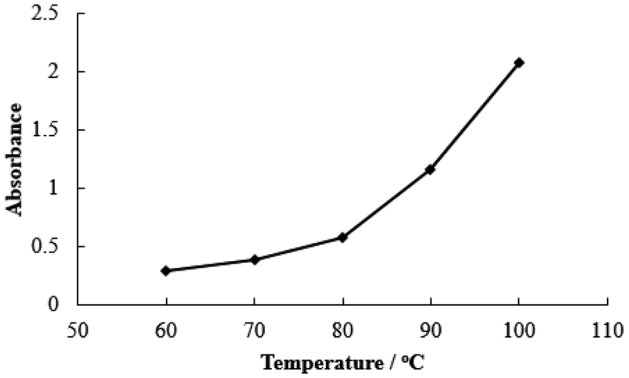

Fig. 2 Temperature's impact on norfloxacin's spectrophotometric characteristics

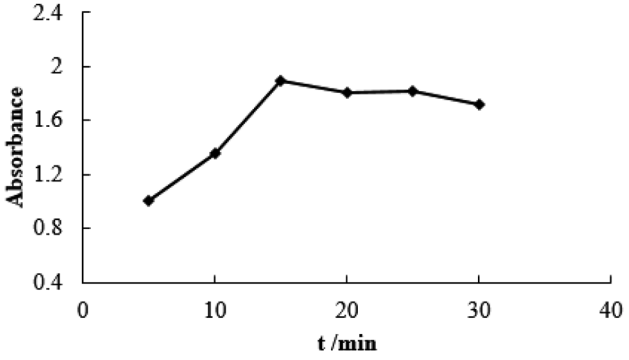

Fig. 3 Effect of heating time on the determination of norfloxacin

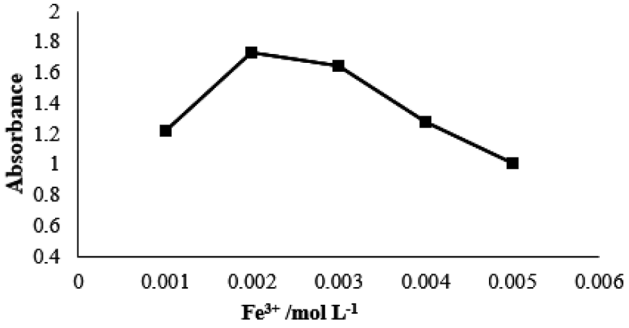

Fig. 4 Effect of Fe(III) concentration on the norfloxacin determination

Effect of concentration and volume of Fe^{3+} solution

The effect of Fe^{3+} concentration was investigated in the range of 0.001–0.005 mol/L (Fig. 4). A 0.002 mol/L Fe^{3+} solution was found to form the most colorful formation.

The influence of the total amount at 0.002 mol/L of the Fe^{3+} solution has been investigated by the addition of various concentrations of Fe^{3+}. The solution with the highest absorbance, 0.5 mL, was used.

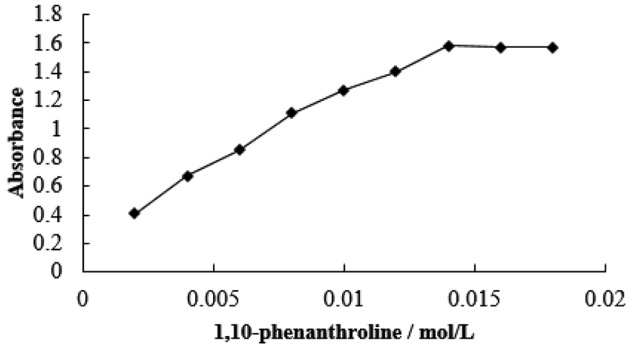

Fig. 5 Effect of concentration of 1,10 phenanthroline

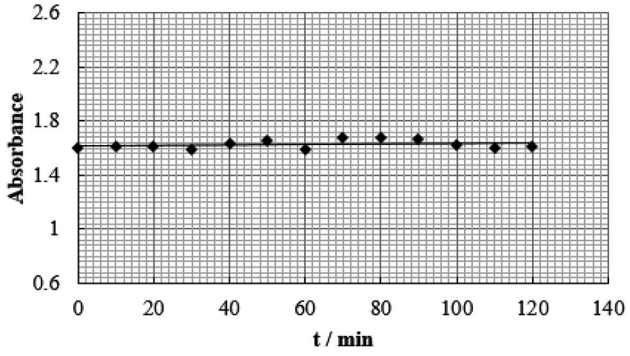

Fig. 6 Time's impact on the colored product's stability

Impact of 1,10-phenanthroline solution's concentration and volume

Effect of 1,10-phenanthroline has been studied in the 0.002–0.018 mol/L range, as well as the influence of the solution's volume and to produce colored complexes.

A 1,10-phenanthroline solution was found to achieve the highest absorbance at 0.014 mol/L (Fig. 5). To investigate the effect of the volume of the solution, several mL of 1,10-phenanthroline (0.014 mol/L) were added. Solution's maximum capacity for absorption was measured using 1.5mL.

Reaction product's stability

For up to two hours, absorbance measurements were taken every 10 min to assess reaction product's stability.

The product's absorbance remained unchanged. Absorbance stays constant and has no effect on the results of the analysis, even if it is tested two hours after the dilution (Fig. 6).

Method-B

Effects of acid concentration and kind

To prevent the precipitation of hydrated ceric oxide ($CeO_2.H_2O$), an acidic media was used for the oxidation reaction. A range of acids, such as H_2SO_4, HNO_3, and HCl, were examined to ascertain which ones would

be most effective for a reaction. The greatest amount of colour was produced by HNO_3 (2.5 mL of 2.5 mol/L).

Impact of Ce (IV) concentration

Outcomes of Ce(IV) concentrations that vary from 1×10^{-3} to 5×10^{-3} mol/L were examined in this study. The higher colour product formation was observed in the 3×10^{-3} mol/L Ce(IV) solution (Fig. 7). The volume of the Ce(IV) solution is optimized, the highest creation of products takes place with 1.5mL of Ce(IV) solution (3×10^{-3} mol/L).

Effect of MO concentration

Effect of various MO concentrations were investigated between 2×10^{-3} and 1.2×10^{-2} mol/L. The highest color production was seen in Fig. 8 when 8×10^{-3}mol/L MO has been employed. Additionally, result of the methyl orange solution's volume has been studied, as well as and 1.5 mL of 8×10^{-3} mol/L MO solution was determined to have the highest absorbance.

Analytical figures of merit

In ideal experimental circumstances for suggested techniques, the study demonstrates a linear connection between norfloxacin absorbance and concentration. Technique A demonstrated an excellent correlation coefficient of 0.9879 within range of concentrations of 1–30 µg/mL, method B, on the other hand, showed a significant correlation coefficient of 0.9966. The findings show that Beer's law is followed.

Techniques A and B's calibration curves for the measurement of NFX are displayed in Figs. 9 and 10, respectively. The LOD, which was calculated using the least amount (3.3 × (Sy/S)) at which NFX can be consistently recognized, remained found to be 1.098 µg/mL for approach A and 1.111 µg/mL for approach B.

Using lowest norfloxacin concentration (10 × (Sy/S)) that could be measured with high accuracy and precision to estimate the LOQ; for technique A, this was 2.875 µg/mL, and for method B, it was 3.368 µg/mL. Intercept, relative standard deviation, λ max, slope, linear regression equation, standard deviation, correlation coefficient, and molar absorptivity are among the optical characteristics of for method A and method B are listed in Table 1.

Specificity of the methods

Using recommended methods for measuring norfloxacin in pharmaceutical formulations containing antibiotic, the degree of selectivity was demonstrated. The interference brought on by widely utilized excipients such as glucose, sorbitol, lactose, fructose, starch, and Mg stearate has been also investigated.

Fixed amount of NFX solution was mixed with one excipient in every following ratio: 1:1, 1:2, 1:4, 1:6, 1:8, 1:10, and 1:20. The recommended procedure was then used to inspect the mixture. It was discovered that these excipients did not interfere with anything (Fig. 11).

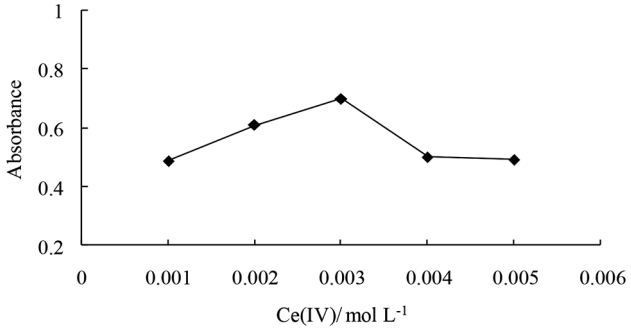

Fig. 7 Cerium (IV) concentration's effect

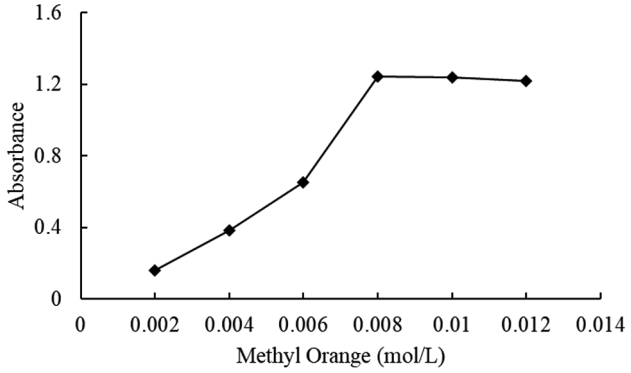

Fig. 8 Impact of concentration of MO

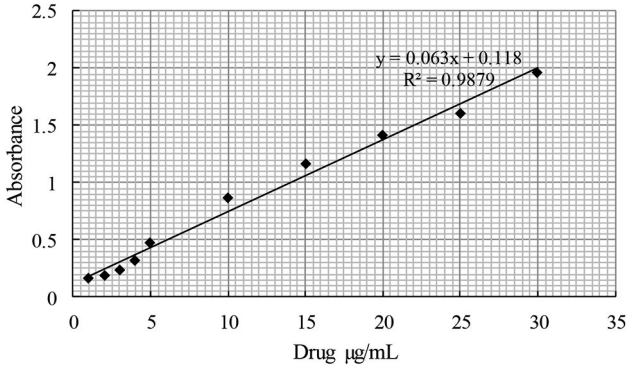

Fig. 9 Norfloxacin concentration's impact on absorbance. Conditions: 1–30 µg of norfloxacin per milliliter with a λ_{max} of 511 nm, Subsequently, 15 min of heating at 100 °C, 1.0 mL of Fe^{3+} (0.002 mol/L), 1.5 mL (0.014 mol/L), as well as 1,10 phenanthroline were diluted to 10 mL

Reliability

Through triplicate measurements of NFX in both pure form and at three distinct concentration levels and pharmaceutical formulations that adhere to Beer's law, the precision of the recommended techniques was shown. The results for pure form are listed in Table 2 and for pharmaceutical preparations are listed in Table 3. The

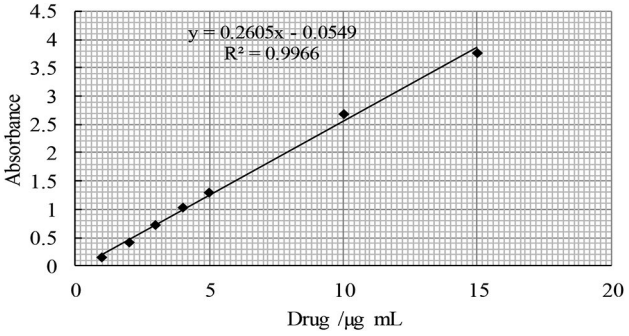

Fig. 10 Norfloxacin concentration's impact on absorbance. Conditions; The mixture contained Ce(IV) (1.5 mL; 3.0×10^{-3} mol/L), MO (1.5 mL; 8.0×10^{-4} mol/L), Norfloxacin (1–15 µg/mL), and HNO_3 (2.5 mL; 2.5 mol/L). It had a λ_{max} of 508 nm after being diluted to 10 milliliters

Table 1 Spectrophotometric methods' analytical parameters for measuring norfloxacin

Parameter	Method A	Method B
λ_{max} (nm)	511	508
Molar absorptivity (L mol^{-1} cm^{-1})	5.4×10^4	4.53×10^4
Linear range (µg/mL)	1–30	1–15
Limit of detection 3.3 × (Sy/S) (µg/mL)	1.098	1.111
Limit of quantification 10 × (Sy/S) (µg/mL)	2.875	3.368
Regression equation (y)	Y = 0.063X + 0.118	Y = 0.2605X − 0.0549
Slope (b)	0.063	0.2605
Intercept (a)	0.118	-0.0549
Correlatin coefficient (r^2)	0.987	0.996
Standard deviation (µg/mL)	0.087	0.056
Relative standard deviation (%)	5.08	6.21

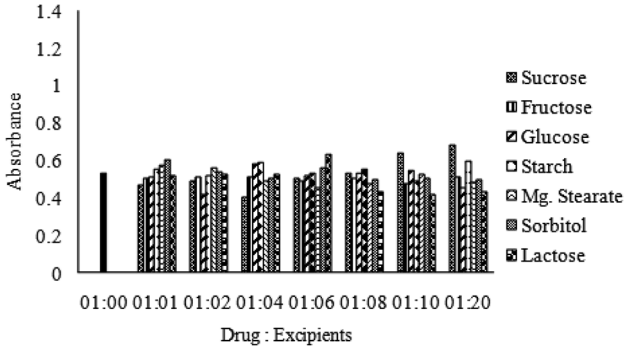

Fig. 11 Effects of common excipients on the recommended methods for determining norfloxacin

methods' low relative standard deviation values and good % recoveries show their great accuracy and exceptional precision.

The percentage recoveries for the standard for procedure A and B obtained were ranged from 96.80 to 102.30% and from 97.94 to 101.94% respectively. Similarly, the percent recoveries for pharmaceutical

Table 2 The suggested methods' accuracy and precision when utilizing a standard norfloxacin solution

Amount taken (µg/mL)	Method A	% Recovery ± RSD	Method B	% Recovery ± RSD
	Amount found (µg/mL)		Amount found (µg/mL)	
1.0	0.968	96.80 ± 3.96	0.979	97.94 ± 2.63
2.0	2.046	102.30 ± 1.74	2.038	101.94 ± 5.69
3.0	3.02	100.66 ± 0.76	2.983	99.43 ± 1.26
Mean		99.92		99.77
± SD		2.82		2.02
t-test		0.049 (4.303)		0.197 (4.303)

*Results are the averages of three separate analyses: RSD Relative standard deviation

Table 3 Evaluation of the current techniques' accuracy and precision in detecting norfloxacin in pharmaceutical formulations

Pharmaceutical preparations	Amount taken (µg/mL)	Method A	% Recovery ± RSD	Method B	% Recovery ± RSD
		Amount found (µg/mL)		Amount found (µg/mL)	
Noroxin	1.0	0.95	95.74 ± 3.01	0.98	98.17 ± 3.53
	2.0	2.05	102.53 ± 2.18	1.99	99.98 ± 3.93
	3.0	2.97	99.12 ± 0.93	2.98	99.51 ± 4.19
Utinor	1.0	0.97	97.16 ± 3.51	1.02	102.00 ± 3.57
	2.0	2.03	101.65 ± 1.30	1.96	98.06 ± 3.25
	3.0	2.96	98.88 ± 2.75	2.96	98.82 ± 1.75
Alenbit	1.0	0.98	98.00 ± 2.15	1.01	101.00 ± 1.32
	2.0	1.97	98.50 ± 1.70	2.01	100.50 ± 2.23
	3.0	2.98	99.30 ± 2.26	3.00	100.00 ± 2.54
Norocin	1.0	1.00	100.00 ± 1.78	1.04	104.44 ± 4.00
	2.0	1.98	99.00 ± 2.13	2.00	100.05 ± 3.82
	3.0	2.99	99.67 ± 1.65	2.96	98.94 ± 1.78

*Results are the averages of three separate analyses: RSD relative standard deviation

formulations for method A and B ranged from 95.74 to 102.53% and 98.06 to 104.44% respectively. Using four different brands of 400 mg NFX pills (Norocin, Utinor, Alenbit, and Noroxin), the standard addition method was also used to assess the procedure's accuracy. The recommended procedures were followed, and tablet solutions were combined with predetermined amounts of standard norfloxacin solution.

Percent recoveries for methods A and B ranged from 98.00 to 102.19% and 98.0–102.0%, respectively. The standard addition method compensates for the influence of the matrix by adding known quantities of the analyte to the sample. Used to determine the concentration of an analyte in samples with complex or unknown matrices, such as biological fluids, environmental samples, or food products. The recoveries were computed by comparing

Table 4 Standard addition method evaluation of norfloxacin recovery percentage in commercial formulations

Pharmaceutical preparations	Amount added (µg/mL)	Method A		Method B	
		Amount found (µg/mL)	% Recovery ± RSD	Amount found (µg/mL)	% Recovery ± RSD
Noroxin	1.0	0.98	98.00 ± 3.95	0.98	98.00 ± 3.45
	2.0	1.98	99.00 ± 2.79	1.97	98.50 ± 1.16
	3.0	3.02	100.67 ± 3.49	3.00	100.00 ± 2.54
Utinor	1.0	0.99	99.00 ± 2.52	1.00	100.40 ± 3.90
	2.0	2.01	100.50 ± 3.77	1.96	98.00 ± 1.05
	3.0	2.97	99.00 ± 2.83	3.01	100.34 ± 2.06
Alenbit	1.0	1.01	101.00 ± 3.23	1.01	101.00 ± 2.22
	2.0	1.99	99.50 ± 2.71	2.01	100.50 ± 1.79
	3.0	2.99	99.67 ± 2.27	2.97	99.00 ± 2.84
Norocin	1.0	1.02	102.00 ± 1.70	1.02	102.19 ± 0.16
	2.0	1.99	99.50 ± 3.15	1.93	96.43 ± 2.78
	3.0	3.01	100.34 ± 2.65	2.91	97.05 ± 3.06

*Results are the averages of three separate analyses: RSD Relative standard deviation

Table 5 Percentage of norfloxacin recovered using method B from spiked plasma and urine samples

Samples	(µg/mL) taken	(µg/mL) obtained	%Recovery ± RSD
Plasma	1	0.987	98.74 ± 3.77
	2	2.068	103.43 ± 3.84
	3	2.976	99.21 ± 3.34
Urine	1	0.990	98.17 ± 4.91
	2	2.017	100.85 ± 2.64
	3	3.019	100.65 ± 2.35

*Results are the averages of three separate analyses: RSD Relative standard deviation

Table 6 Determination of norfloxacin in pharmaceutical preparations

Brand name	Method A			Method B	
	Marked value	Obtained value	± t-test value (4.303)	Obtained value	± t-test value (4.303)
Noroxin	400	404.43 ± 2.52	0.37	396.67 ± 3.21	1.79
Utinor	400	395.45 ± 3.56	2.37	398.00 ± 4.07	0.85
Alenbit	400	390.87 ± 4.11	3.23	399.87 ± 2.37	1.86
Norocin	400	398.45 ± 3.77	1.87	390.67 ± 4.41	3.66

the outcomes prior to and following the addition of standard NFX solution (Table 4). To test technique B's accuracy, a recovery experiment was carried out using tampered-with plasma and urine samples. The percentage recoveries for plasma and urine samples varied from 98.74 to 103.43% and 98.17–100.85%, respectively (Table 5).

Applicability of the proposed methods

Four distinct pharmaceutical brands' levels of norfloxacin were successfully measured using the accepted methods. The suggested approach is a helpful tool for determining the amount of norfloxacin in pharmaceutical items, as showed by the results of the commended techniques that closely correspond to the label values (Table 6).

The methods' great selectivity and the lack of common excipient interference made them effective for detecting NFX in tampered-with human urine and plasma.

Conclusions

Using a variety of components, two sensitive, quick, economical, and selective approaches were generated to measure NFX in biotic samples as well as pharmaceutical preparations. It has been concluded that the proposed technique can precisely and accurately determine NFX concentrations down to 1 µg/mL and has a wider linear range. Time-consuming extraction processes and preparation are not necessary for the established methods, in contrast to other chromatographic and reported spectrophotometric approaches. The methods utilize affordable chemicals and easily accessible equipment. Furthermore, excipients commonly found in pharmaceutical formulations had no effect on the analysis. The techniques' high reproducibility and good recovery make them a valuable substitute for the numerous additional techniques currently employed to measure the amounts of NFX in commercial tablet formulations.

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Author contributions

Muhammad Adnan, Muhammad Naeem Khan, and Nusrat Bibi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Muhammad Adnan, Iftikhar Ali, Asif Kamal, Abd El-Zaher M.A. Mustafa, Muhammad Naeem Khan: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper. Muhammad Adnan, Muhammad Naeem Khan, Abd El-Zaher M.A. MustafaNusrat Bibi, Muhammad Adnan, Asif Kamal, Muhammad Naeem Khan: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agree to publish the article.

Competing interests

The authors declare no competing interests.

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