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Research paper

Kinetic spectrophotometric method for the determination of norfloxacin in pharmaceutical formulations

Nafisur Rahman*, Yasmin Ahmad, Syed Najmul Hejaz Azmi

Department of Chemistry, Aligarh Muslim University, Aligarh, India

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Abstract

A simple and sensitive kinetic spectrophotometric method is described, based on the oxidation of norfloxacin with alkaline potassium permanganate. The reaction is followed spectrophotometrically by measuring the rate of change of absorbance at 603 nm. The initial rate and fixed time (at 3 min) methods are utilized for constructing the calibration graphs to determine the concentration of the drug. The calibration graphs are linear in the concentration ranges 2.0–20 μg ml^{-1} and 1.0–20 μg ml^{-1} using the initial rate and fixed time methods, respectively. The results are validated statistically and through recovery studies. The method has been successfully applied to the determination of norfloxacin in commercial dosage forms. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision.

Keywords: Initial rate method; Fixed time method; Norfloxacin; Potassium permanganate; Pharmaceutical formulations; Spectrophotometry

1. Introduction

Norfloxacin,1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid is a synthetic broad spectrum antibacterial drug, which is very much active against many gram positive and gram negative bacteria. It is mainly used in the treatment of urinary tract infections.

The drug and its formulations are listed in the United States Pharmacopoeia [1] and European Pharmacopoeia [2]. The USP describes a high performance liquid chromatography (HPLC) method for its determination while the European Pharmacopoeia recommends a non-aqueous titration method. Other techniques used for its quantification include HPLC [3–13], high performance thin layer chromatography [14], fluorimetry [15–19], titrimetry [20] and differential scanning potentiometry [21].

Spectrophotometry still belongs to the most frequently used analytical techniques in pharmaceutical analysis, which provides practical and significant economic advantages over other methods. A review of the literature revealed that the charge transfer reactions of norfloxacin with π-acceptors such as 2,3-dichloro-5,6-dicyano-*p*-benzoquinone, 7,7,8,8,tetra cyano quinodimethane, *p*-chloranil and *p*-chloranilic acid have been used for its assay in bulk and dosage forms [22]. El Walily et al. [23]have developed two spectrophotometric methods based on the interaction of norfloxacin with 2,4-dinitrofluorobenzene in aqueous borate buffer and dimethylsufoxide medium to form colored chromophores, which absorb maximally at 365 and 410 nm, respectively. The drug content in pharmaceutical formulations has been determined by the formation of an oxidative coupling product of the drug with 3-methyl-2-benzothiazolinone hydrazone-HCl in the presence of cerric ammonium sulfate [24]. The quantification of norfloxacin [25] was done utilizing the reaction between drug and Fe(III) in sulfuric acid medium. Two extractive spectrophotometric methods [26] have been recommended based on chloroform extractable ion-association complex of the drug with each of the dyes such as supracene violet 3B and tropaeolin 000. The determination of norfloxacin was carried out on the basis of the formation of precipitate between drug and ammonium reineckate. The precipitated drug–reagent complex was dissolved in aqueous 50% acetone and absorbance measured at 524 nm [27]. However, many of these methods are limited in their applications or rather much tedious and time consuming. The literature is

* Corresponding author. Department of Chemistry, Aligarh Muslim University, Aligarh-202002 (U.P.), India. Tel.: +91-571-2703515.
E-mail address: cht17nr@yahoo.co.in (N. Rahman).

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still poor in analytical procedures based on kinetics, especially for the determination of drugs in commercial dosage forms. However, some specific advantages in the application of kinetic methods can be expected [28], such as selectivity due to the measurement of the evolution of the absorbance with the time of reaction. There is, therefore, a need for a simple and sensitive kinetic spectrophotometric method for the assay of norfloxacin in commercial dosage forms.

Our present communication describes a simple and sensitive kinetic spectrophotometric method for the determination of norfloxacin in drug formulations. The method involves the oxidation of drug with alkaline potassium permanganate at room temperature and subsequent measurement of absorbance at 603 nm. The initial rate and fixed rate methods are adopted for its determination in commercial dosage forms after full investigation.

2. Materials and methods

2.1. Apparatus

A Shimadzu UV-visible spectrophotometer (model-1601, Japan) with matched quartz cells was used to measure absorbance.

2.2. Reagents and standards

All chemicals used were of analytical or pharmaceutical grade. Norfloxacin was provided by Cipla, India and was used as received. A standard solution of norfloxacin (1.0 mg/ml) was prepared by dissolving 100 mg in 1.0 ml of 0.75 M NaOH, and further diluted to 100 ml with doubly distilled water. A working test solution of norfloxacin (0.2 mg ml^{-1}) was prepared by dilution of 10 ml of the standard solution to 50 ml with doubly distilled water. The tablets containing the norfloxacin such as Alflox (Alkem), Negaflox (Zydus Alidac), Norflox (Cipla), Uroflox (Torrent) were purchased locally. Aqueous solutions of sodium hydroxide (0.75 M) and potassium permanganate (0.006 M) were prepared in doubly distilled water. Potassium permanganate solution should be freshly prepared and its apparent purity was checked titrimetrically [29].

2.3. Recommended procedure for norfloxacin determination

2.3.1. Initial rate method

Aliquots of 0.1–1.0 ml of 0.02% norfloxacin test solution were pipetted into a series of 10-ml standard flasks. Two milliliters of 0.75 M NaOH followed by 2.0 ml of 0.006 M potassium permanganate were added to each flask and then diluted with doubly distilled water at 30 ± 1 °C. The contents of the mixture of each flask were mixed well and the increase in absorbance at 603 nm was recorded as a function of time. The initial rate of the reaction (ν) at different concentrations was obtained from the slope of the tangent to the absorbance time curve. The calibration graph was constructed by plotting the logarithm of the initial rate of the reaction (log ν) versus the logarithm of the molar concentration of norfloxacin (log C). The amount of the drug in each sample was calculated either from the calibration graph or the regression equation.

2.3.2. Fixed time method

In this method, the absorbance of each sample solution at a preselected fixed time was accurately measured and plotted against the final concentration of the drug. The content of the drug was estimated either from the calibration graph or regression equation.

2.4. Determination of norfloxacin in dosage forms

An accurately weighed quantity of the mixed contents of the tablets, equivalent to 50 mg of the drug was extracted into 50 ml of 1,2-dichloromethane with shaking and the residue was filtered using Whatmann No. 42 filter paper. The filtrate was evaporated to dryness and the residue was dissolved in 1.0 ml of 0.75 M NaOH and transferred to a 50-ml standard volumetric flask, diluting to volume with doubly distilled water. The stock solution of drug was diluted according to need and analyzed by the recommended procedures.

3. Results and discussion

The absorption spectrum of aqueous potassium permanganate solution in the alkaline medium exhibited an absorption band peaking at 530 nm. The addition of norfloxacin to this solution produced a new characteristic band at 603 nm (Fig. 1). This band is due to the formation of manganate ion, which resulted in the oxidation of

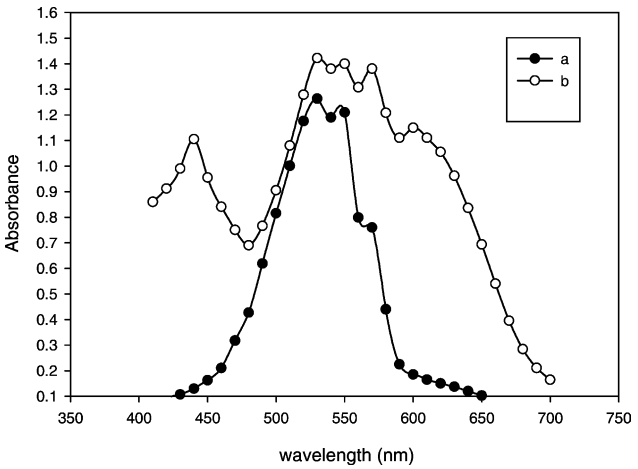


Fig. 1. Absorption spectra of (a) 2.0 ml of 0.75 M NaOH + 1 ml of 0.006 M $KMnO_4$; (b) 20 μg ml^{-1} norfloxacin + 2.0 ml NaOH (0.75 M) + 2 ml $KMnO_4$ (0.006 M) in double distilled water.

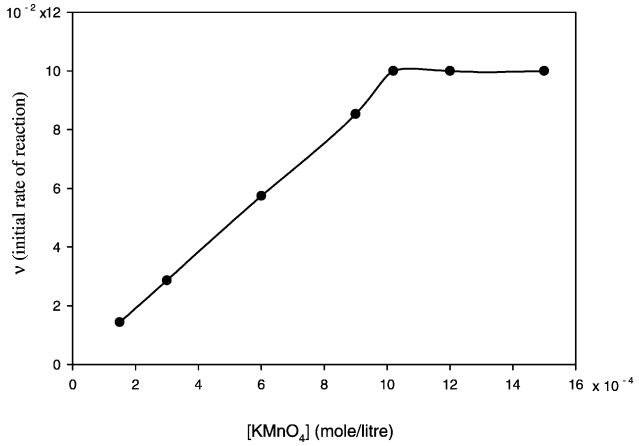


Fig. 2. Effect of the concentration of $KMnO_4$ (3×10^{-4} to 15×10^{-4} M) on the initial rate of reaction (ν) with 20 μg ml^{-1} norfloxacin and 2.0 ml NaOH (0.75 M) in double distilled water.

norfloxacin by potassium permanganate in alkaline medium. The intensity of the color increases with time, and therefore, a kinetically based method was developed for the determination of norfloxacin in drug formulations. The optimum conditions affecting the formation of manganate ion were studied and maintained throughout the experiment.

3.1. Effect of $KMnO_4$ concentration

The effect of $KMnO_4$ concentration on the initial rate of the reaction was studied in the range 3.0×10^{-4} to 15.0×10^{-4} M. The initial rate of reaction (Fig. 2) increased with increasing the concentration of $KMnO_4$ and became

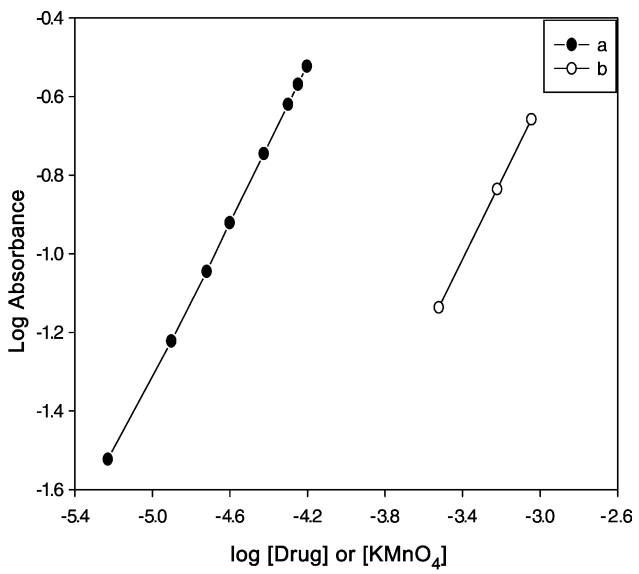
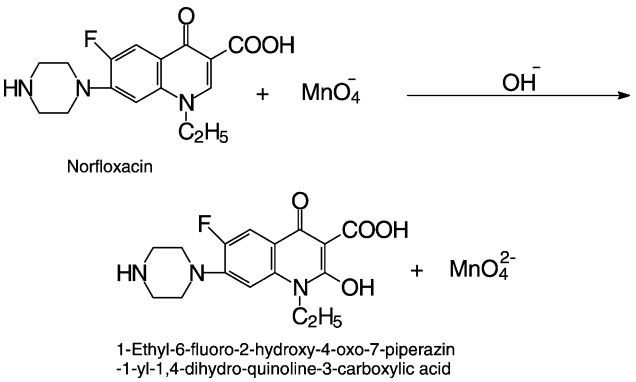


Fig. 3. Limiting logarithmic plot for molar combining ratio between norfloxacin and $KMnO_4$: (a) log A vs. log [Drug]; (b) log A vs. log [$KMnO_4$].



Scheme 1.

constant at 10.2×10^{-4} M. Thus, the adoption of 12.0×10^{-4} M $KMnO_4$ in the final solution proved to be adequate for the maximum concentration of norfloxacin used in the determination process.

3.2. Effect of NaOH concentration

The influence of the NaOH concentration on the formation of MnO_4^{2-} was also examined by taking 20 μg ml^{-1} norfloxacin, 2.0 ml of 0.006 M $KMnO_4$ solution and varying volume (0.25–2.0ml) of 0.75 M NaOH. The maximum absorbance was obtained with 1.5 ml of 0.75 M NaOH, after which further increase in volume of NaOH caused no change in absorbance. Hence, 2.0 ml of 0.75 M NaOH was used as an optimum value.

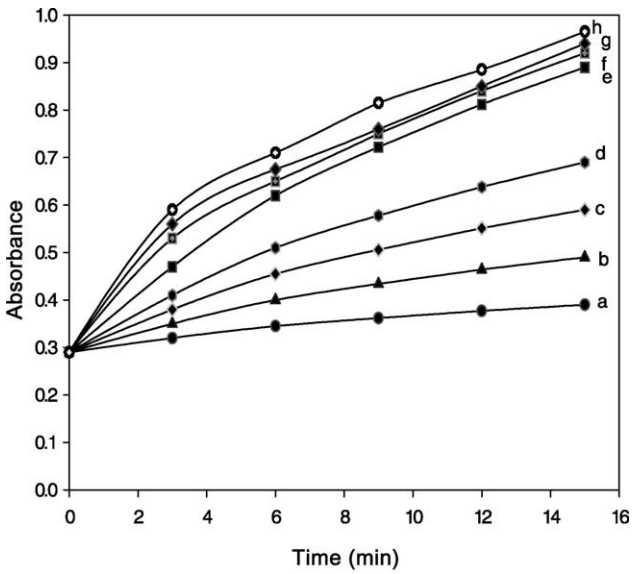


Fig. 4. Absorbance vs. time curves for the reaction between norfloxacin and $KMnO_4$: $KMnO_4$, 1.2×10^{-3} mol l^{-1} and norfloxacin (a) 6.263×10^{-6} mol l^{-1}; (b) 1.253×10^{-5} mol l^{-1}; (c) 1.879×10^{-5} mol l^{-1}; (d) 2.505×10^{-5} mol l^{-1}; (e) 3.758×10^{-5} mol l^{-1}; (f) 5.010×10^{-5} mol l^{-1}; (g) 5.637×10^{-5} mol l^{-1}; (h) 6.263×10^{-5} mol l^{-1}.

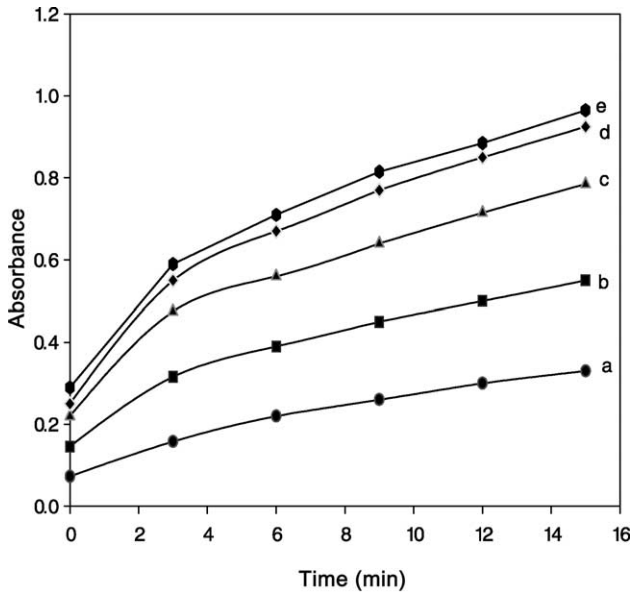


Fig. 5. Absorbance vs. time curves for the reaction between norfloxacin and $KMnO_4$: norfloxacin, 6.263×10^{-5} mol l^{-1} and $KMnO_4$ (a) 3×10^{-4} mol l^{-1} (b) 6×10^{-4} mol l^{-1} (c) 9×10^{-4} mol l^{-1} (d) 10.2×10^{-4} mol l^{-1} (e) 12×10^{-4} mol l^{-1}.

3.3. Stoichiometry and reaction mechanism

The stoichiometric ratio between potassium permanganate and norfloxacin was determined by the limiting logarithmic method [30]. This was established by performing two sets of experiments. In the first set, $KMnO_4$ concentration was varied while keeping a constant concentration of norfloxacin. In the second set, the $KMnO_4$ concentration was kept constant while varying the concentration of norfloxacin. The logarithms of the absorbance were plotted against the logarithm of the respective varied concentration (Fig. 3). The slopes of the two straight lines were calculated and found to be unity in each case. Thus, it is concluded that the combining molar ratio between norfloxacin and $KMnO_4$ is 1:1.

A review of the literature indicated that norfloxacin undergoes oxidation when treated with oxidants such as chloramine-B and *N*-chlorobenzotriazole [31]. In this study, the potassium permanganate oxidizes norfloxacin in alkaline medium. Therefore, the following reaction mechanism (Scheme 1) is proposed on the basis of literature background and our experimental findings.

3.4. Initial rate method

The initial rates of the reaction were determined from absorbance time curves (Figs. 4 and 5) by measuring the slopes of the initial tangent to the absorbance time curves. The order with respect to permanganate was determined by studying the reaction at different concentrations of permanganate with fixed norfloxacin concentration. The plot of initial rate, dA/dt, against the initial absorbance was linear passing through the origin indicating that the initial order of the reaction with respect to permanganate was 1. The order with respect to norfloxacin was evaluated by plotting the logarithm of the initial rate of the reaction versus the logarithm of the molar concentration of norfloxacin and was found to be 1.

Under the optimized experimental conditions, the concentration of norfloxacin was determined using an excess of $KMnO_4$ and NaOH solution with respect to the initial concentration of norfloxacin. As a result, a pseudo zero order condition was obtained with respect to their concentrations. However, the initial rate of the reaction would follow a pseudo first order and was found to obey the following equation

$$\text{Rate} = \Delta A/\Delta t = K' C^n$$

where K' is the pseudo first order rate constant, C is the concentration of norfloxacin, n is the order of the reaction.

Table 1
Optical and regression characteristics of fixed time method

Parameters	Fixed-time method				
	3 min	6 min	9 min	12 min	15 min
Beer's law limit (μg ml^{-1})	1.0–20	1.0–12	1.0–12	1.0–12	1.0-12
Regression equation	$A = 2.905 \times 10^{-1} + 1.500 \times 10^{-2}C$	$A = 2.899 \times 10^{-1} + 2.756 \times 10^{-2}C$	$A = 2.898 \times 10^{-1} + 3.604 \times 10^{-2}C$	$A = 2.907 \times 10^{-1} + 4.346 \times 10^{-2}C$	$A = 2.907 \times 10^{-1} + 4.996 \times 10^{-2}C$
S_a	3.318×10^{-4}	3.925×10^{-4}	5.212×10^{-4}	4.571×10^{-4}	4.234×10^{-4}
$\pm tS_a$	7.846×10^{-4}	1.090×10^{-3}	1.447×10^{-3}	1.269×10^{-3}	1.175×10^{-3}
S_b	2.821×10^{-5}	5.906×10^{-5}	7.843×10^{-5}	6.878×10^{-5}	6.372×10^{-5}
$\pm tS_b$	6.671×10^{-5}	1.639×10^{-4}	2.177×10^{-4}	1.909×10^{-4}	1.769×10^{-4}
Coefficient of correlation (r)	0.9999	0.9999	0.9999	0.9999	0.9999
Detection limit (μg ml^{-1})	0.08	0.05	0.05	0.04	0.03
Variance (S_0^2)	3.214×10^{-7}	2.913×10^{-7}	5.136×10^{-7}	6.285×10^{-7}	3.390×10^{-7}

$\pm tS_a$ = confidence limit for intercept; $\pm tS_b$ = confidence limit for slope.

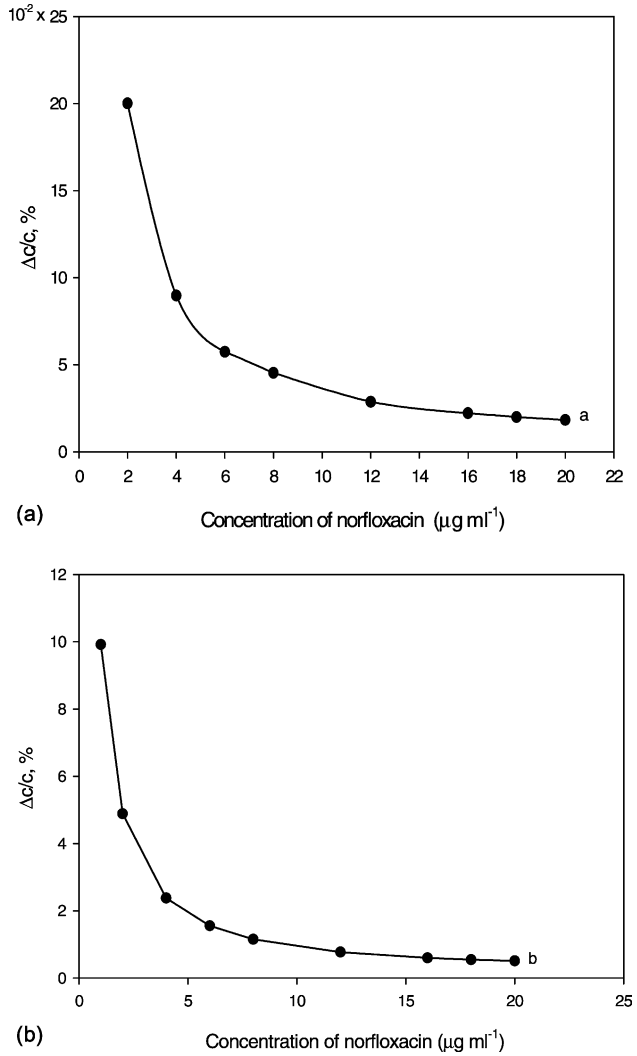


Fig. 6. Variation of the confidence limit at 95% confidence level in the form of uncertainty percentage on the concentration of norfloxacin with (a) initial rate method (b) fixed time method (3 min).

The logarithmic form of the above equation is written as,

$$\text{Log (rate)} = \log k' + n \log C$$

The linear regression analysis using the method of least square was made to evaluate slope, intercept and correlation coefficient. Under the optimized experimental conditions a calibration curve was constructed by plotting log initial rate versus log molar concentration of norfloxacin (log C), which showed a linear relationship over norfloxacin concentration range of 2.0–100μg ml^{-1}. The regression of log rate versus log C gave a linear regression equation

$$\text{Log(rate)} = 3.213 + 0.9975 \log C$$

With a correlation coefficient (r) of 0.9999. The value of n in the regression equation also confirmed that the reaction was first order with respect to the norfloxacin concentration. The confidence limits for the slope of the line of regression and intercept were computed using the relation $b \pm tS_b$ and $a \pm tS_a$ [32] at 95% confidence level and found to be $0.9975 \pm 3.557 \times 10^{-3}$ and $3.213 \pm 1.632 \times 10^{-2}$, respectively. This indicated the high reproducibility of the proposed method. The limit of detection was established using the equation [33].

$$\text{Limit of detection} = \left(S_o^2 \times \frac{n-2}{n-1}\right)^{1/2} \frac{t}{b}$$

where t is the value of student's t for $n-2$ degree of freedom at 95% confidence level and S_0^2 = variance. The variance and detection limit were calculated and found to be 1.833×10^{-6} and 3.100×10^{-3} μg ml^{-1}, which confirmed negligible scattering of experimental data points around the line of best fit and good sensitivity of the method, respectively.

3.5. Fixed time method

In this method, the absorbance of a green colored solution containing varying amounts of norfloxacin was

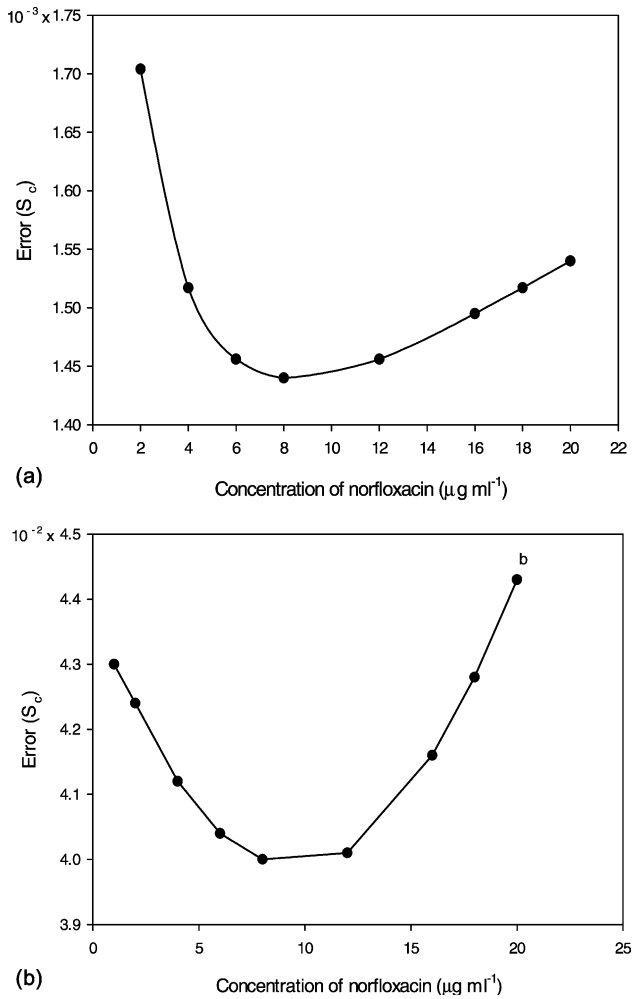


Fig. 7. Error in the determination of the concentration of norfloxacin using statistical analysis of standard calibration data (a) initial rate method (b) fixed time method (3 min).

Table 2
Intra day assay: evaluation of the accuracy and precision of the initial rate and fixed time methods

Proposed methods	Amount taken ($\mu g\ ml^{-1}$)	Amount found ± S.D.[a] ($\mu g\ ml^{-1}$)	R.S.D. (%)[a]	SAE[b]	C.L.[c]
Initial rate method	4.0	4.02 ± 0.043	1.09	0.019	0.054
	12.0	12.02 ± 0.043	0.36	0.019	0.054
	20.0	20.06 ± 0.080	0.40	0.036	0.100
Fixed time method	4.0	04.02 ± 0.056	1.39	0.025	0.069
	12.0	12.02 ± 0.056	0.47	0.025	0.069
	20.0	20.02 ± 0.056	0.28	0.025	0.069

[a] Mean ± S.D. for five determinations.
[b] SAE, standard analytical error.
[c] C.L., confidence limit at 95% confidence level and four degrees of freedom ($t = 2.776$).

Table 3
Intra day assay: test of precision of the proposed methods in pharmaceutical formulations

Formulations	Initial rate method					Fixed time method				
	Amount taken ($\mu g\ ml^{-1}$)	Amount found ± S.D.[a] ($\mu g\ ml^{-1}$)	R.S.D. (%)[a]	SAE[b]	C.L.[c]	Amount taken ($\mu g\ ml^{-1}$)	Amount found ± S.D.[a] ($\mu g\ ml^{-1}$)	R.S.D. (%)[a]	SAE[b]	C.L.[c]
Alflox (Alkem)	20.00	20.02 ± 0.080	0.40	0.035	0.099	20.00	20.01 ± 0.060	0.30	0.027	0.074
Negaflox (Zydus Alidac)	20.00	20.06 ± 0.080	0.40	0.035	0.099	20.00	20.02 ± 0.056	0.28	0.025	0.070
Norflox (Cipla)	20.00	20.00 ± 0.085	0.43	0.038	0.105	20.00	19.99 ± 0.060	0.28	0.027	0.074
Uroflox (Torrent)	20.00	20.02 ± 0.080	0.40	0.035	0.099	20.00	20.01 ± 0.060	0.28	0.027	0.074

[a] Mean ± S.D. for five determinations.
[b] SAE, standard analytical error.
[c] C.L., confidence limit at 95% confidence level and four degrees of freedom ($t = 2.776$).

Table 4
Inter day assay: evaluation of the accuracy and precision of the initial rate and fixed time methods

Proposed methods	Amount taken ($\mu g\ ml^{-1}$)	Amount found ± S.D.[a] ($\mu g\ ml^{-1}$)	R.S.D. (%)[a]	SAE[b]	C.L.[c]
Initial rate method	4.0	4.04 ± 0.052	1.28	0.023	0.064
	12.0	12.03 ± 0.049	0.41	0.022	0.061
	20.0	20.00 ± 0.160	0.80	0.072	0.199
Fixed time method	4.0	4.01 ± 0.076	1.89	0.034	0.094
	12.0	12.01 ± 0.076	0.63	0.034	0.094
	20.0	20.05 ± 0.087	0.43	0.039	0.108

[a] Mean ± S.D. for five determinations performed over a period of 5 days.
[b] SAE, standard analytical error.
[c] C.L., confidence limit at 95% confidence level and four degrees of freedom ($t = 2.776$).

Table 5
Inter day assay: test of precision of the proposed methods in pharmaceutical formulations

Formulations	Initial rate method					Fixed time method				
	Amount taken ($\mu g\ ml^{-1}$)	Amount found ± S.D.[a] ($\mu g\ ml^{-1}$)	R.S.D. (%)[a]	SAE[b]	C.L.[c]	Amount taken ($\mu g\ ml^{-1}$)	Amount found ± S.D.[a] ($\mu g\ ml^{-1}$)	R.S.D. (%)[a]	SAE[b]	C.L.[c]
Alflox (Alkem)	20.00	20.00 ± 0.109	0.55	0.049	0.135	20.00	19.98 ± 0.056	0.28	0.025	0.069
Negaflox (Zydus Alidac)	20.00	20.02 ± 0.125	0.62	0.056	0.155	20.00	19.99 ± 0.076	0.38	0.034	0.095
Norflox (Cipla)	20.00	19.97 ± 0.105	0.53	0.047	0.130	20.00	19.99 ± 0.076	0.38	0.034	0.095
Uroflox (Torrent)	20.00	19.98 ± 0.109	0.55	0.049	0.135	20.00	20.02 ± 0.099	0.49	0.044	0.123

[a] Mean ± S.D. for five determinations performed over a period of 5 days.
[b] SAE, standard analytical error.
[c] C.L., confidence limit at 95% confidence level and four degrees of freedom ($t = 2.776$).

Table 6
Standard addition method for the determination of norfloxacin in dosage forms by initial and fixed time methods

Formulations	Initial rate method						Fixed time method					
	Amount ($\mu g\ ml^{-1}$) Taken	Added	Found ± S.D.[a]	Recovery (%) ±R.S.D. (%)[a]	SAE[b]	C.L.[c]	Amount ($\mu g\ ml^{-1}$) Taken	Added	Found ± S.D.[a]	Recovery (%) ±R.S.D. (%)[a]	SAE	C.L.[c]
Alflox	2.0	2.0	4.02 ± 0.046	100.56 ± 1.14	0.020	0.057	2.0	2.0	3.99 ± 0.060	99.82 ± 1.49	0.027	0.074
(Alkem)	6.0	6.0	12.02 ± 0.048	100.13 ± 0.36	0.020	0.054	6.0	6.0	11.99 ± 0.060	99.94 ± 0.50	0.027	0.074
Negaflox	2.0	2.0	4.02 ± 0.043	100.47 ± 1.09	0.020	0.054	2.0	2.0	4.02 ± 0.056	100.49 ± 1.39	0.025	0.070
(Zydus Alidac)	6.0	6.0	12.02 ± 0.043	100.19 ± 0.36	0.020	0.054	6.0	6.0	12.02 ± 0.056	100.16 ± 0.47	0.025	0.070
Norflox	2.0	2.0	4.02 ± 0.043	100.19 ± 1.09	0.020	0.054	2.0	2.0	4.02 ± 0.056	100.49 ± 0.06	0.025	0.070
(Cipla)	6.0	6.0	4.01 ± 0.047	100.18 ± 1.18	0.020	0.054	6.0	6.0	11.99 ± 0.060	99.94 ± 0.50	0.027	0.074
Uroflox	2.0	2.0	4.01 ± 0.047	100.18 ± 1.18	0.021	0.060	2.0	2.0	4.02 ± 0.056	100.49 ± 1.40	0.025	0.070
(Torrent)	6.0	6.0	12.02 ± 0.044	100.13 ± 0.36	0.020	0.054	6.0	6.0	12.02 ± 0.056	100.16 ± 0.47	0.025	0.070

[a] Mean ± S.D. for five determinations.
[b] SAE, standard analytical error.
[c] C.L., confidence limit at 95% confidence level and four degrees of freedom ($t = 2.776$).

Table 7
Point hypothesis: comparison of the proposed methods with the reference method at 95% confidence level

Formulations	Initial rate method				Fixed time method				Reference method	
	Recovery (%)	R.S.D.[a] (%)	t-value[b]	F-value[b]	Recovery (%)	R.S.D. (%)[a]	t-value[b]	F-value[b]	Recovery (%)[a]	R.S.D. (%)[a]
Alflox (Alkem)	100.11	0.40	0.39	1.91	100.03	0.30	0.89	1.08	100.20	0.29
Negaflox (Zydus Alidac)	100.30	0.40	1.40	3.22	100.10	0.28	0.52	1.58	100.02	0.22
Norflox (Cipla)	100.02	0.43	0.19	1.37	99.97	0.30	0.46	1.50	100.06	0.36
Uroflox (Torrent)	100.11	0.40	0.39	1.91	100.03	0.28	0.89	1.05	100.20	0.29

[a] Mean of five determinations.
[b] Theoretical t-value ($\nu = 8$) and F-value ($\nu = 4, 4$) at 95% confidence level are 2.306 and 6.39, respectively.

Table 8
Interval hypothesis: comparison of the proposed methods with the reference method at 95% confidence level

Formulations	Initial rate method		Fixed time method	
	Lower limit[a] (θ_L)	Upper limit[a] (θ_U)	Lower limit[a] (θ_L)	Upper limit[a] (θ_U)
Alflox (Alkem)	0.995	1.004	0.993	1.004
Negaflox (Zydus Alidac)	0.997	1.009	0.996	1.005
Norflox (Cipla)	0.993	1.007	0.993	1.005
Uroflox (Torrent)	0.995	1.004	0.993	1.004

[a] In pharmaceutical analysis, a bias, based on recovery experiments, of $\pm 2\%$ ($\theta_1 = 0.98$ and $\theta_2 = 1.02$) is acceptable[38].

measured at a preselected fixed time. Calibration plots of absorbance versus initial concentrations of norfloxacin were established at a fixed time of 3, 6, 9, 12 and 15 min. The regression equations, coefficient of correlation, detection limit, variance, relative standard deviation, standard analytical error are given in Table 1. The lowest detection limit was obtained with a fixed time of 15 min, whereas the fixed time of 3 min showed a wider concentration range for quantification. According to ICH guidelines [34], the detection limit is not required to be part of the validation procedure for assays. Therefore, on the basis of wider concentration range and less time of analysis, the fixed time of 3 min was recommended for determination.

Regression analyses of the calibration data also make it possible to evaluate the error, S_c, in the determination of a given concentration of norfloxacin using the equation [35].

$$S_c = \frac{S_o}{b}\left(1 + \frac{1}{n} + \frac{(y - \bar{y}^2)}{b^2(\sum C^2 - n\bar{C}^2)}\right)^{1/2}$$

where $\bar{y}$ and $\overline{C}$ are average initial rate of reaction or average absorbance at a fixed time of 3 min and concentration values, respectively, for n standard samples. It is clear from the graph in Fig. 6a,b that the error reaches a minimum, when the initial rate (v) (or actual absorbance) was equal to the average initial rate ($\overline{v}$) (or average absorbance) in the calibration graphs corresponding to 8.32 μg ml^{-1} and 9.65 μg ml^{-1} for the initial rate and fixed method, respectively. The value of S_c also allows one to establish the confidence limit at the selected level of significance for the determination of unknown concentrations by using the relation, $C_i \pm tS_c$. Fig. 7a,b shows the variation in the confidence limit in the form of percentage uncertainty [36] in the concentration at the 95% confidence level and used to calculate the relative uncertainty in the concentration over the full range of concentration tested. It is, therefore, a useful way to establish the confidence limit.

The accuracy and precision was determined by measuring the norfloxacin content of pure sample and dosage forms five times within 1 day by initial rate and fixed time methods (Tables 2 and 3). The daily precision was measured by assaying the pure samples and tablets on 5 consecutive days by both initial rate and fixed time methods (Tables 4 and 5). The standard deviations, relative standard deviations and standard analytical errors obtained in intra day and inter day assays can be considered to be very satisfactory.

The accuracy of the proposed methods was also checked by performing recovery experiments. For this, a known amount of the pure drug was added to preanalyzed dosage forms and then determined by the recommended procedures. The results (Table 6) obtained from the investigations showed that the mean recoveries and relative standard deviations were in the range 99.82–100.56% and 0.056–1.137%, respectively. No interference from the common excipients was observed.

The initial rate and fixed time methods for determining norfloxacin have been tested on commercial pharmaceutical formulations. The concentration of the drug was computed from its corresponding regression equations. The results of the proposed method (initial rate or fixed time) were compared with those of the reference method [23] using point hypothesis. Table 7 shows that the calculated t- and F-values [37] are less than theoretical ones, confirming no significant difference between the performance of the proposed method and the reference method at a 95% confidence level. The interval hypothesis tests [38] have also been performed to compare results of proposed method (initial rate or fixed time) with those of reference method at 95% confidence level (Table 8). It was decided that a bias of $\pm 2\%$ is acceptable. Therefore, the limit of acceptance interval is within $\theta_1 = 0.98$ and $\theta_2 = 1.02$. It is apparent from Table 8 that the true bias of all samples is smaller than $\pm 2\%$. The interval hypothesis leads to the same conclusion as the point hypothesis.

4. Conclusion

The initial rate and fixed time methods can be easily applied to the determination of norfloxacin in pure and dosage forms, which do not require elaborate treatment of the analyte and tedious extraction of the chromophore produced. The proposed method (initial-rate or fixed-time) is sensitive enough to enable determination of a lower amount of the drug. These advantages encourage the application of the proposed methods in routine quality control of norfloxacin in industrial laboratories.

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