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A Simple Kinetic Spectrophotometric Method For The Determination of Certain 4-Quinolones in Drug Formulations.

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Abstract :

An accurate simple and selective kinetic procedure for the determination of certain 4-quinolones namely, norfloxacin (I), ofloxacin (II), enrofloxacin (III), fleroxacin (IV), ciprofloxacin (V) and pefloxacin (VI) is described. The procedure is based on reacting the studied compounds in acidic media (0.1M HCL) with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in presence of cerium (IV) ammonium sulphate as an oxidant at room temperature for a fixed time of 20 min. for (I), (III), 12 min for (II) and 30 min for IV , V and VI, then the absorbance of the reaction product is measured at 630 nm. The concentration of the studied compounds is computed using the corresponding calibration curve equation for the fixed-time method. The absorbance-concentrations plot is rectilinear over the range 20-100 $ug.ml^{-1}$ for (I), 2 - 20 $ug.ml^{-1}$ for (II), 10 - 74 $ug.ml^{-1}$ for (III), 10 - 60 $ug.ml^{-1}$ for IV, 10 - 50 $ug.ml^{-1}$ for IV and 8 - 40 $ug.ml^{-1}$ for VI.

The determination of the studied compounds by the fixed-concentration and rate constant methods is feasible with the calibration equation obtained, but the fixed-time method proved to be more applicable.

The procedure was applied successfully to commercial tablets and ampoules and the results obtained were compared statistically with the reference methods.

Keywords:

Kinetic determination, 4-quinolone antibiotics, 3-methyl-2-benzothiazolinone hydrazone HCL (MBTH), Ce. (IV), pharmaceutical analysis.

Introduction :

The studied 4-quinolones are antibacterial agents, structurally related to nalidixic acid but with a wider antibacterial spectrum and greater activity[1]. Various techniques have been utilized for the determination of the studied 4- quinolone compounds either per se or in dosage forms. The most commonly used methods for the determination of the studied compounds were reported in the recently published review[2]. Also, spectrophotometric[3-7], spectrofluorimetric[8-11], conductimetric[12] and HPLC[13] methods were reported for their determinations .

Kinetic-based methods of pharmaceutical analysis are not widely applied, although they offer certain advantage of elimintating the additive interferences which are likely affect other methods such as titrimetry or direct spectrophotometric methods, beside, it offers certain selectivity .

The literatures are still poor in analytical procedures based on kinetics especially for pharmaceutical or biological fluids.

Furthermore some specific advantages in the application of kinetic methods can be expected[14];

1- Possibility of no interference of the coloured and or turbidity background of the samples, and ...

2- Possibility of no interference of other active compounds present in the commerical product if they are resisting the chemical reaction conditions established for the proposed kinetic method.

A kinetic method is proposed for the determination of studied compounds based on the formation of a coloured reaction product with 3-methyl 2-benzothiazolinone hydrazone HCL (MBTH) in the presence of Ce (IV) as an oxidant and measuring the absorbance at 630 nm in an acidic medium. MBTH has different advantages [15,16] including : a) good solubility in water , b) oxidative coupling reactions over a wide pH range, c) highly sensitive colour reaction .The proposed method is more sensitive than the titrimetric methods adopted by USP[17] for the drug in bulk and pharmaceutical preparations. The method is simple, rapid and readily adoptable to both the bulk drug and unit dose determinations.

EXPERIMENTAL

Apparatus:

The spectrophotometric measurements were made on the UV. Visible/ Spectrophotometer, model UV-1601 Shimadz recording Spectrophotometer (P/N 206 - 67001) equipped with kinetic accessory provide with temperature controlled cell(Tcc - 240 A) Thermoelectrically temperature. Recording range 0 to 1, wavelength (nm) 630, factor = 1, number of cell 1 , reaction time 40 minutes, cycle time 0.1.

Materials:

- Norfloxacin and ciprofloxacin (Memphis Chemical Co. Cairo, Egypt), ofloxacin (Hoechst Orient Pharmacautical Co. Cairo, Egypt), fleroxacin (Hoffman- La Roche, (Nutley, NJ) U.S.A) , pefloxacin (Amriya for Pharmaceutical Industries; Alex. Egypt) and enrofloxacin (Bayer-Drugofa, Abt.Drugofa Gmbh, Germany).

The purity of these compounds was established by applying the recommended official[17] or reference methods[22-26]. Pharmaceutical preparations containing the studied compounds were purchased from commercial sources in the local market.

Reagents:

- HCL, H_2SO_4 were spectroscopically pure A R grade, 3-methyl-2-benzothiazolinone hydrochloride 0.5% aqueous solution (Aldrich, Germany) and cerium (IV) ammonium sulphate 1% prepared by dissolving 1.0 gm of the reagent in 0.72ml conc H_2SO_4 and completing to 100 ml with distilled water.

Stock Solutions:

- Standard solutions 10.0 mg of the studied compounds were prepared freshly every day in 0.1M HCL and were successfully diluted to contain the reported concentration range in ug. ml^{-1} (Tab.5) .

General Procedure:

Transfer aliquots 1.0 ml of stock solution of each drug into a series of 10ml calibrated flasks. Add to each flask 1ml of 0.3% MBTH and 2.3 ± 0.3 ml of 1% Ce (IV), shake well and let to stand

for 20 min. for (I), (III), 12 min for (II) and 30 min. for IV,V and VI complete to the mark with distilled water. Measure the absorbance of the resulted solution at 630 nm with time during 40 minutes at ambient temperature (25°C). A blank experiment is prepared simultaneously. Determine the drug concentration during the first 40 minutes of reaction by using the appropriate graphs. Plot log reaction rate versus log concentration of the drugs to get order of the reaction. For calibration curves , carry out the same procedure. Measure the absorbance of the solution at 630 nm. Plot the values of absorbance against the concetration of each drug in $ug.ml^{-1}$ at the specified fixed time. Alternatively, the regression equation is derived (Tab.4). Alternatively the regression equation is derived.

Procedure for Spectrophotometric Determination of the Studied Compounds in Dosage Forms.

For tablets :

Weigh and pulverize 10 tablets, weigh accurately an amount of the pulverized tablets equivalent to 20.0 mg of the studied compounds and transfer into 100 ml conical flask. Extract the drug three times each with 30 ml of 0.1M HCL. Filter the extract into a 100 ml measuring flask. Wash the conical flask with few mls of 0.1M HCL and pass the washings into the same volumetric flask. Complete to volume with 0.1M HCL. Transfer aliquots of these solutions and continue as previously stated above (Tab.6) .

For ampoules :

Transfer aliquot of peflacin mesylate ampoule equivalent to contain 20.0 mg of VI into 100 ml measuring flask dilute and complete to volume with 0.1 M HCl. Transfer aliquots of this solution within the concentration range cited in table 6 into a series of 10 ml calibrated flask. Proceed as mentioned under general procedure . Calculate the nominal content of the ampoules either from a previously plotted calibration graph or using the regression equation.

Results and Discussion:

Kinetic and Optimization of the Reaction Conditions:

The reaction between the studied 4 - quinolones and MBTH in acidic medium in the presence of Ce (IV) as an oxidant yields a green colour. The oxidation product shows maximum absorbance at 630 nm (Fig.1).

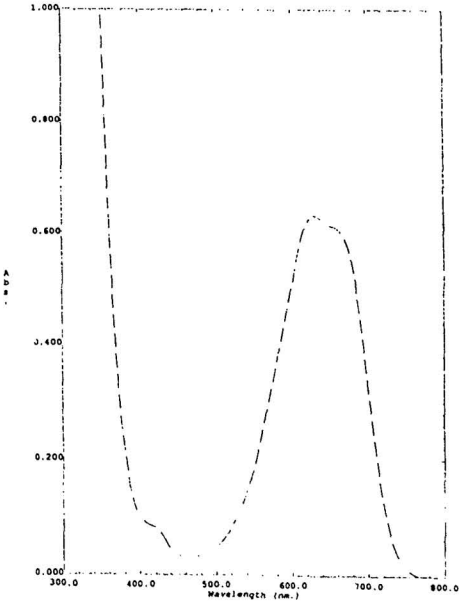


Fig.1 : Absorption spectrum of norfloxalin 80 $ug.ml^{-1}$ after reaction with MBTH and Ce (IV).

The reaction was studied under various concentrations of both the reagent and oxidant for varying time at room temperature or under the effect of heat.

It was found that the colour intensity reaches maximum after leaving at room temperature. Room temperature was preferred as upon heating the reaction mixture, precipitatition takes place .

The influence of cerium (IV) ammonium sulphate on the reaction rate was studied in the range 1-4%. It was found to be 2.3 $\pm$ 0.3 ml. of 1% solution of Ce (IV) was optimum for development of the colour reaction and maximum formation of the product .

As the intensity of the colour increases with time, (Fig.2) this was used as a usefull kinetic method for the determination of studied 4-Quinolones either in pure form or in pharmaceutical preparations.

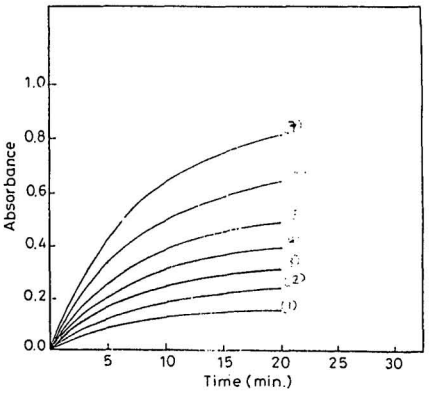


Fig. 2 : Effect of time on the absorbance of norfloxacin :

(1)6.265x10^{-5}M, (2) 9.397x10^{-5}M, (3) 1.253x10^{-4} M, (4) 1.566x10^{-4} M (5) 1.879x10^{-4}M, (6) 2.506x10^{-4}M, (7) 3.132x10^{-4}M.

Other oxidants are tried e.g. iron (III) ammonium sulphate, this proves to be unappropriate , since it forms a coloured complex with norfloxacin[3] .

The rate of the reaction was also found to be concentration dependent. The reaction rate was followed at room temperature with various concentrations of the studied compounds over the range -100 ug.ml^{-1} for I , 2-30 ug.ml^{-1} for II and 10-74 ug.ml^{-1} for III , 10 - 60 ug.ml^{-1} for IV, 10 - 5- ug.ml^{-1} for V and 8 - 40 ug.ml^{-1} for VI, keeping MBTH and Ce (IV) concentrations constant. (Fig.3).

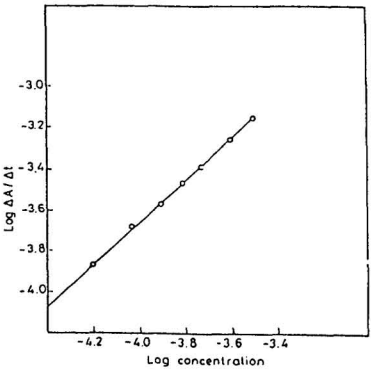


Fig. 3 : Plot of log absorbance versus log concentration of norfloxacin at 630mm.

The graph shown in figure 3 clearly indicates that, the reaction rate of the studied compounds obeys the following equation:-

$$Rate = K^{-} [Drug]^{n} \quad (1)$$

Where K^{-} is the pseudo order rate constant and n is the order of the reaction.

The rate of the reaction may be estimated by the variable-time-method measurement[18] as $\Delta A/\Delta t$, where A is the absorbance and t is the time in seconds. Taking logarithms of rates and concentrations (tables 1A ,1B, 1C and 1D) and figure 5, equation (1) is transformed into.

$$Log (rate) = Log \Delta A/\Delta t = Log K^{-} + n \log [drug] \quad (2)$$

Regression of log (rate) versus log [norfloxacin] gave the following regression equation:

$$Log\ rate = 0.339 + (1.001) \log C \quad (r = 0.9995)$$

Hence K^{-} = 2.18 Sec. and the reaction is first order (n=1.001), with respect to norfloxacin concentration.

Table :1. A.

Logarithms of rates for different concentrations of norfloxacin at room temperature at 630 nm.

Log ΔA/Δt	Log [norfloxacin]
-3.875	-4.203 M
-3.681	-4.027 M
-3.569	-3.902 M
-3.464	-3.805 M
-3.398	-3.726 M
-3.273	-3.601 M
-3.163	-3.504 M

Table: 1.B.

Logarithms of rates for different concentration of ofloxacin at room temperature at 630 nm.

Log ΔA/Δt	Log [ofloxacin]
-3.830	-5.257 M
-3. 554	-4.859 M
-3.380	-4.558 M
-3.279	-4.479 M
-3.208	-4.382 M
-3.149	-4.257 M
-3.109	-4.160 M
-3.009	-4.081 M

$$Log.\ rate = -0.2368 + 0.6834 \log [\ ofloxacin] \quad (r = 0.9971)$$

Hence k^{-} = 0.5796 Sec. and the reaction is first order (n= 0.683), with respect to ofloxacin concentration.

Table: 1.C.

Logarithms of rates for different concentrations of enrofloxacin at room temperature at 630 nm.

Log ΔA/Δt	Log [enrofloxacin]
-4.019	-4.556
3.717	-4.255
-3.548	-4.078
3.439	3.954
-3.262	-3.807
-3.135	3.686

Log. rate = 0.5757 + 1.009 log [enrofloxacin] (r = 0.9993)

Hence k^{-} = 3.8 Sec. and the reaction is first order (n= 1.009), with respect to enrofloxacin concentration.

Table: 1.D.

Logarithms of rates for different concentrations of pefloxacin mesylate at room temperature at 630nm.

Log ΔA/Δt	Log [pefloxacin mesylate]
- 4.336	- 4.618
- 4.131	- 4.442
- 3.819	- 4.220
- 3.594	- 4.016
-3.492	-3.919

Log. rate = 1.32 + 1.22 log [pefloxacin mesylate]

Hence K^{-} = 20.8 sec. and the reaction is first order (n = 1.22), with respect to pefloxacin concentration

Evaluation of the Kinetic Methods:

The quantitation of the norfloxacin, ofloxacin, enrofloxacin, fleroxacin, ciprofloxacin and pefloxacin, under the optimized experimental conditions outlined above, where MBTH concentration was 40 times the initial concentration of norfloxacin and 200 time the concentration of ofloxacin would result in a pseudo first order reaction with respect to their concentrations.

However, the rate will be directly proportional to the drug concentration in a pseudo first order rate equation as follows:

Rate = K^{-} (any of the studied4- quinolones) (3)

Where K^{-} is the pseudo-first order constant. Several experiments were then carried out to obtain the drug concentration from the rate data according to equation (3). Intial rate, rate constant, fixed-concentration and fixed time[19,20] methods were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity (i.e. the slope of the calibration graph), the intercept and the correlation coefficient (r).

Rate-Constant Method:

Graphs of logarithms the absorbance versus time over the concentration range $6.265x10^{-5}$ to $3.133x10^{-3}$M for I, $5.534x10^{-6}$ to $8.300x10^{-5}$M for II and 2.783×10^{-5} to 2.059×10^{-4} M for III, 2.717×10^{-5} to 1.223×10^{-4} M for IV, 4.218×10^{-5} to 1.928×10^{-4}M for V and 2.41×10^{-5} to 1.205×10^{-4} M for VI, were plotted (Fig. 4) and all appeared to be rectilinear, pseudo-first order rate constants (K^{-}) corresponding to different drug concentration (C) were calculated from the slopes multiphied by -2.303 (Fig. 4) and are presented in tables 2A , 2B , 2C and 2D.

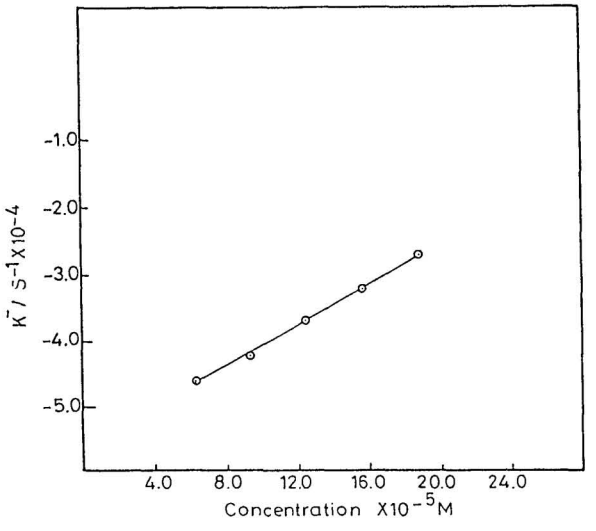


Fig. 4 : Plot of $\bar{k}/S^{-1} \times 10^{-4}$ versus concentration of norfloxacin(M) at 630mm.

Table : 2.A.

Values of K^{-} calculated from slopes of log A versus t for norfloxacin

K^{-}/S^{-1}	[norfloxacin] /M
$-4.606x10^{-4}$	$6.260 x10^{-4}$
$-4.283 x10^{-4}$	$9.390 x10^{-4}$
$-3.170 x10^{-4}$	$1.253 x10^{-4}$
$-3.212 x10^{-4}$	$1.566 x10^{-4}$
$-2.700 x10^{-4}$	$1.879 x10^{-4}$

$K^{-} = -4.64x10^{-4} + 1.487C$ (r = 0.9964)

For norfloxacin over the concentration range. $6.26x10^{-5}$ to $1.879x10^{-4}$M.

Table : 2.B.

Values of K^- calculated from slopes of log A versus t for ofloxacin

K^-/S^{-1}	[ofloxacin] /M
$-5.182x10^{-4}$	$1.380\ x10^{-5}$
$-4.606\ x10^{-4}$	$2.767\ x10^{-5}$
$-4.380x10^{-4}$	$3.320x10^{-5}$
$-4.005\ x10^{-4}$	$4.150\ x10^{-5}$
$-3.450\ x10^{-4}$	$5.534\ x10^{-5}$
$-2.303\ x10^{-4}$	$8.300\ x10^{-5}$

$K^- = -5.75x10^{-4} + 4.163C$ (r = 0.9999)

For ofloxacin over the range $1.38x10^{-5}$ to $8.3x10^{-5}$M

Table : 2.C.

Values of K calculated from slopes of log A versus t for enrofloxacin.

K^- / S^{-1}	[enrofloxacin] / M.
-4.564×10^{-4}	2.783×10^{-5}
-3.685×10^{-4}	5.565×10^{-5}
-3.358×10^{-4}	8.347×10^{-5}
-2.519×10^{-4}	1.558×10^{-4}
-2.003×10^{-4}	2.059×10^{-4}

$K^- = -4.345 \times 10^{-4} + 1.153\ C$ (r = 0.9992)

For Enrofloxacin over the range 2.783×10^{-5} to 2.059×10^{-4} M.

Table : 2.D.

Values of K^- calculated from slopes of log A versus t for pefloxacin mesylate

K^- / S^{-1}	[pefloxacin mesylate] / M.
-5.313×10^{-4}	2.410×10^{-5}
-4.797×10^{-4}	6.025×10^{-5}
-4.428×10^{-4}	9.640×10^{-5}
-3.904×10^{-4}	1.205×10^{-5}

$K^- = -5.663 \times 10^{-4} + 1.397\ C$ (r=0.9910)

For pefloxacin over the range 1.205×10^{-5} to 2.410×10^{-5} M.

Fixed Absorbance Method :

Reaction rates were recorded for different drug concentrations over the range $9.397x10^{-5}$ to $2.506x10^{-4}$ M for I, over the range $3.3204x10^{-5}$ to $5.534x10^{-5}$ M for II, $8.348x10^{-5}$ to $1.558x10^{-4}$ M for III, $7.337\ x10^{-5}$ to $1.087\ x10^{-4}$ M for IV, $9.038\ x10^{-5}$ to $1.506\ x10^{-4}$ M for V and over the range

6.025 $x10^{-5}$ to 1.205 $x10^{-4}$ M for VI. A preselected value of the absorbance was fixed, and the time was measured in seconds. The reciprocal (1/t) versus the initial concentration of the studied compounds (Tables 3A, 3B,3C and 3D), was plotted and the following equation of the calibration graph was obtained.

Table: 3.A.

Values of reciprocal of time taken at fixed absorbance (0.3) for different rates of variable concentrations of norfloxacin at constant concentration of MBTH and Ce (IV) at room temperature.

1/t /Sec^{-1}	[norfloxacin] /M
$1.515x10^{-3}$	$9.397x10^{-5}$
$2.777x10^{-3}$	$1.253x10^{-4}$
$4.166x10^{-3}$	$1.879x10^{-4}$
$5.550x10^{-3}$	$2.506x10^{-4}$

$1/t = -5.93x10^{-3} + 24.905\ C$ (r = 0.9927).

Table : 3.B.

Values of reciprocal of time taken at fixed absorbance (0.4) for different rates of variable concentrations of ofloxacin at constant concentration of MBTH and Ce (IV).

1/t /Sec^{-1}	[ofloxacin]/M
$3.400x10^{-3}$	$3.3204x10^{-5}$
$4.629x10^{-3}$	$4.1505x10^{-5}$
$7.575x10^{-3}$	$5.5340x10^{-5}$

$1/t = -3.082x10^{-3} + 191.089\ C$ (r = 0.9959)

Table : 3.C.

Values of reciprocal of time taken at fixed absorbance (0.3) for different rates of variable concentrations of enrofloxacin at constant concentration of MBTH and Ce (IV).

1/t /Sec^{-1}	[enrofloxacin]/M
$2.220x10^{-3}$	$8.348x10^{-5}$
$3.333x10^{-3}$	$1.113x10^{-4}$
$4.386x10^{-3}$	$1.391x10^{-4}$
$4.760x10^{-3}$	$1.558x10^{-4}$

$1/t = -7.069x10^{-4} + 35.792\ C$ (r = 0.9959)

Table 3.D.

Values of reciprocal of time taken at fixed absorbance (0.3) for different rates of variable concentrations of pefloxacin at constant of MBTH and ce (IV).

1/t /Sec^{-1}	[pefloxacin]/M
$5.952\ x10^{-4}$	$6.025\ x10^{-5}$
$1.111\ x10^{-3}$	$9.640\ x10^{-5}$
$1.389\ x10^{-3}$	$1.205\ x10^{-5}$

$1/t = 5.4x10^{-4} + 6.598\ C$ (r = 0.9886)

Fixed Time Method:

Reaction rates were determined for different concentrations of the studied 4-quinolones. At a preselected fixed-time, which was accurately determined, the absorbance was measured. Calibration graphs of absorbance versus initial concentrations of the studied compounds at fixed times, with the regression equations assembled in tables 4A, 4B, 4C and 4D.

Table : 4.A.

Regression equations at different fixed times for norfloxacin in the range $6.265x10^{-5}$ to $0.1325x10^{-4}$M at constant concentration of MBTH and Ce (IV) at room temperature.

Time/min	Regression Equation	Correlation Coefficient (r)
5	A = -0.001 + 0.0040 C	r = 0.9997
10	A = -0.0008 + 0.0060 C	r = 0.9989
15	A = -0.0022 + 0.0070 C	r = 0.9998
20	A = -0.0016 + 0.0080 C	r = 0.9994

Table :4.B.

Regression equations at different fixed times for ofloxacin in the range $5.534x10^{-6}$ to $5.534x10^{-5}$M at constant concentration of MBTH and Ce (IV) at room temperature.

Time/min	Regression Equation	Correlation Coefficient (r)
2	A= 0.0610 + 0.0178 C	r = 0.9899
4	A= 0.0830 + 0.0231 C	r = 0.9928
6	A= 0.098 0+ 0.0259 C	r = 0.9944
8	A= 0.1108 + 0.0274C	r = 0.9965
10	A= 0.1189 + 0.0284C	r = 0.9948
12	A= 0.1195 + 0.0285 C	r = 0.9999

Table :4.C.

Regression equations at different fixed times for enrofloxacin in the range $2.783x10^{-5}$ to $2.059x10^{-4}$M at constant concentration of MBTH and Ce (IV) at room temperature.

Time/min	Regression Equation	Correlation Coefficient (r)
5	A= 0.0002 + 0.0078 C	r = 0.9992
10	A= -0.0197 + 0.0116 C	r = 0.9991
15	A= -0.0205 + 0.0133 C	r = 0.9991
20	A= -0.0017 + 0.0135 C	r = 0.9999

Table :4.D.

Regression equations at different fixed times for pefloxacin in the range $2.41x10^{-5}$ to $1.205x10^{-4}$M at constant concentration of MBTH and Ce (IV) at room temperature.

Time/min	Regression Equation	Correlation Coefficient (r)
15	A= 0.0172 + 0.0092 C	r = 0.9985
20	A= - 0.0038 + 0.0123 C	r = 0.9998
25	A= 0.0142 + 0.0058 C	r = 0.9994
30	A= 0.0023 + 0.0155 C	r = 0.9999
35	A= 0.0007 + 0.0168 C	r = 0.9982

Application of the Fixed Time Method for the Determination of the Studied Compounds in pure form.

After optimizing the reaction condition, the fixed time method was applied to the determination of the studied compounds in pure form.

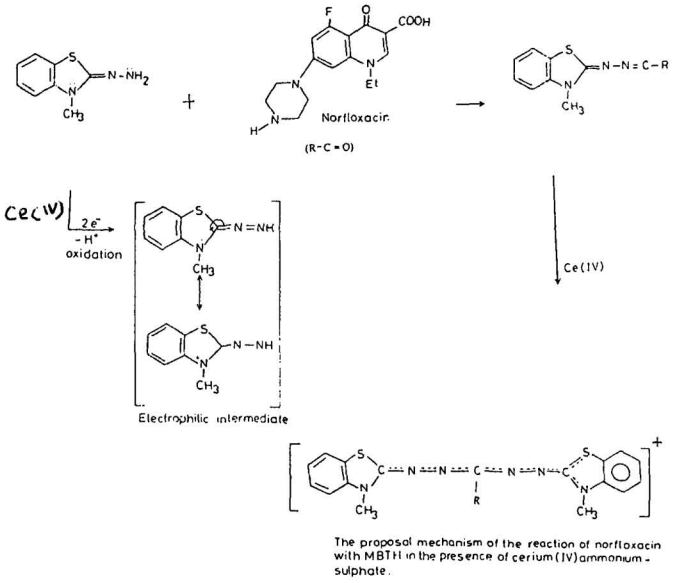
A = -0.0016 + 0.0080 C (r = 0.9994)
For norfloxacin over the range 20-100ug.ml^{-1}
A = 0.1195 + 0.0285 C (r = 0.9999)
For ofloxacin over the range 2-20 ug.ml^{-1}
A= -0.0017 + 0.0135 C (r = 0.9999)
For enrofloxacin over the range 10-74 ug.ml^{-1}.
A = - 0.0007 + 0.0102 C (r=0.9997)
For fleroxacin over the range 10 - 60 ug.ml^{-1}.
A = - 0.0196 + 0.0164 C (r=0.9995)
For ciprofloxacin over the range 10 - 50 ug.ml^{-1}.
A = 0.0023 + 0.0155 C (r=0.9999)
For pefloxacin over the range 8 - 40 ug.ml^{-1}.

Statistical analysis[21] of the result obtained by the proposed and official or reference methods[2226], revealed no significant difference between the performance of the two methods regarding the accuracy and precision. The validity of the method was evaluated by statistical analysis of the regression data[21]. The results are shown in table 5.

Application of the Fixed Time Method to the Determination of the Studied Compounds in Dosage forms.

The proposed method was further applied to the determination of the studied 4-quinolones in pharmaceutical preparations. Common tablet excepients such as talc, magnesium stearate, lactose and starch did not interfere with the assay. The results are shown in table 6. The reaction between the studied 4-quinolones and MBTH in acidic medium in the presence of Ce (IV) as an oxidant yields a green colour. The oxidation product shows maximum absorbance at 630 nm (Fig.1). due to oxidation of MBTH by Ce (IV) accompanied by a simultaneous loss of a proton and two electrons forming an electrophilic intermediate which has been postulated to be the active coupling species. In the second step, an electrophilic reaction between the keto group of the drug and the electrophilic intermediate with the formation of coloured product and elimination of one molecule of water.The mechanism of interaction of the studied 4-quinolones with MBTH was determined via molar ratio

method[27] and It was found that using equimolar concentration of the studied drugs and MBTH at constant concentration of Ce (IV), show that the drugs interact via the carbonyl group with the hydrazone group of MBTH by forming formazan (show max. absorbance at 630 nm) in a molar ratio of 2:1 (MBTH : drug) (Fig. 5).



The proposal mechanism of the reaction of norfloxacin with MBTH in the presence of cerium(IV)ammonium-sulphate.

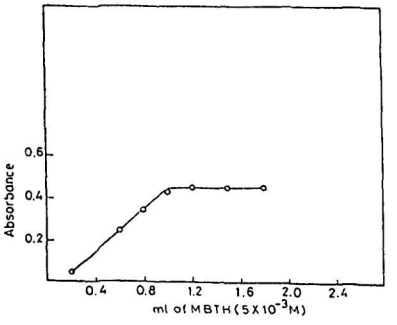


Fig. 5 : Stoichiometry of the reaction between norfloxacin-MBTH (5×10^{-3} M) via molar ratio method.

Conclusion :

The kinetically-based method proposed in this work for the quantitation of the studied compounds is a direct method and more sensitive compared to USP method. Furthermore, the proposed method doesn't require elaboration of treatment and procedures which are usually associated with the chromatographic methods.

Table 5 :

Analytical parameters for the determination of the studied compounds in pure form using fixed time method.

Parameters	Norfloxacin	Ofloxacin	Enrofloxacin	Pefloxacin
λnm	630	630	630	630
Linearity range (ug. ml^{-1})	20 - 100	2 - 20	10-74	8 - 40
Linear regression				
Intercept (a)	-0.0016	0.1195	-0.0017	0.0023
Slope (b)	0.008	0.0285	0.0135	0.0155
Correlation coefficient	0.9994	0.9999	0.9999	0.9999
Sy/x	0.014	$2.125x10^{-3}$	$4.162x10^{-3}$	$2.445x10^{-3}$
S_a	$3.382x10^{-3}$	$1.147x10^{-3}$	$1.279x10^{-3}$	$1.119x10^{-3}$
S_b	$2.027x10^{-4}$	$1.456x10^{-4}$	$7.877x10^{-5}$	$1.008x10^{-4}$
% Er	0.51	0.27	0.31	0.36

N.B.

Sy/x = standard deviation of residuals

S_a = Standard deviation of intercept of regression line.

S_b = Standard deviation of slope of regression line.

Table :6

Spectrophotonetric determination of the studies compounds in pharmaceutical preparations by the proposed and refcrencc methods.

Pharmaceutical Preparations	ug taken	ug Found	% Recovery	Reference Methods[22 - 26]
1- Noroxin tablets (norfloxacin 400 mg/tablet)	20	20.0900	100.45	
	30	29.8406	99.47	
	40	39.8100	99.53	
	60	60.4100	100.68	
	80	79.8400	99.80	
$\bar{X}$ ± S.D.			99.99±0.49	99.84 ± 0.53
Student's t-value			0.41	(2.45)
Variance ratio F-test			1.17	(6.94)
2- Tarivid tablets (ofloxacin 200mg/ tablet).	5	5.049	10.98	
	8	7.854	98.18	
	10	10.100	101.00	
	12	11.933	99.44	
	15	15.008	100.05	
$\bar{X}$ ± S.D.			99.93 ± 1.05	98.60 ± 0.40
Student's t-value			2.03	(2.45)
Variance ratio F-test			6.87	(6.94)
3- Byatrile tablets (enrofloxacin 250 mg / tablet)	20.0	19.802	99.01	
	40.0	40.044	100.11	
	60.0	59.544	99.24	
	74.0	74.755	101.02	
$\bar{X}$ ± S.D.			99.85 ± 0.79	100.89 ±0.64
Student's t-value			1.86	(2.57)
Variance ratio F-test			1.52	(9.55)
4- Fleroxacin tablets (fleroxacin 400mg / tablet)	20.0	19.700	98.50	
	30.0	29.934	99.78	
	40.0	39.400	98.50	
	50.0	49.690	99.38	
	60.0	60.546	100.91	
$\bar{X}$ ± S.D.			99.41 ± 0.9	100.62 ± 1.07
Student's t-value			1.72	(2.45)
Variance ratio F-test			1.41	(6.94)
5- Cipro tablets (ciprofloxain 500 mg/tablet)	10.0	10.033	100.33	
	20.0	20.041	100.21	
	30.0	29.721	99.07	
	40.0	40.304	100.76	
	50.0	49.902	99.80	

$\bar{X}$ ± S.D.			100.03 ± 0.57	100.01 ± 0.26
Student's t-value			0.055	(2.45)
Variance ratio F-test			4.81	(6.94)
6-a Peflacin tablets	10.00	10.093	100.93	
(pefloxacin 400mg / tablet)	20.00	20.093	100.46	
	24.00	23.796	99.15	
	32.00	32.407	101.27	
	40.0	39.907	99.77	
$\bar{X}$ ± S.D.			100.32 ± 0.77	99.5 ± 0.46
Student's t-value			1.84	(2.45)
Variance ratio F-test			2.76	(6.94)
6-b Peflacin ampoules	10.0	10.116	101.16	
(pefloxacin 400mg / ampoule)	20.0	20.214	101.07	
	24.0	24.278	101.16	
	32.0	32.554	101.73	
	40.0	40.500	101.25	
$\bar{X}$ ± S.D.			101.27 ± 0.23	100.91 ± 0.36
Student's t-value			1.76	(2.45)
Variance ratio F-test			2.45	(6.94)

N.B:

Each result is the average of three separate determinations.

Figures in parentheses are the tabulated t and F values respectively at P= $(0.05)^{21}$.

1. Product of Eipico. Pharm. Co. Cairo, Egypt.
2. Product of Hoechest Orient Pharm. Co. Cairo, Egypt.

5.Product of Eipico-Pharm.Co-Cairo, Egypt.

6.Product of Amriya for Pharmaceutical Industries. Alex . Egypt.

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