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Kinetic spectrophotometric method for the determination of some fourth generation fluoroquinolones in bulk and in pharmaceutical formulations

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Abstract   A kinetic spectrophotometric method for accurate and sensitive determination of gemifloxacin (GMFX) and gatifloxacin (GTFX) has been described. The method is based on the reaction of the studied drugs with potassium permanganate in the presence of sodium hydroxide to form a water-soluble green product which shows maximum absorbance at 604 nm. The determination of GMFX and GTFX drugs by rate constant, fixed-concentration, and fixed time methods was feasible with the calibration equations obtained but the fixed time method had been found to be more applicable. The concentration of the selected drugs is calculated using the calibration equation for the fixed time method. The absorbance–concentration plot is linear over the range of 4–36 µg mL^{-1} and 4–40 µg mL^{-1} with correlation coefficient of 0.9998 and 0.9991, for GMFX and GTFX, respectively. The molar absorptivity, Sandell sensitivity, detection and quantification limits were also calculated. The different experimental parameters affecting the development and stability of the color were carefully studied and optimized. The intra- and inter-day RSD values indicated the ruggedness of the method. The proposed method has been successfully applied to pharmaceutical formulations of each drug. Statistical comparison of the results with a well established reported method showed excellent agreement and proved that there is no significant difference in the accuracy and precision.
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1. Introduction

Fluoroquinolones, as a group, have shown excellent activity against the most frequently occurring gram-positive and -negative ocular pathogens [1,2,3,4,5]. Earlier generation fluoroquinolones, such as ciprofloxacin and ofloxacin, have been used widely to treat various pathogenic conditions.
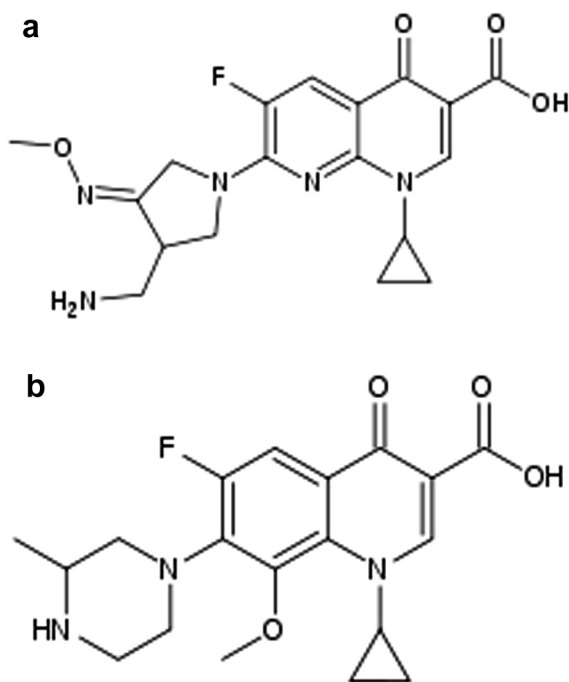
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Figure 1 Chemical structure of (a) gemifloxacin and (b) gatifloxacin.

However, the development of a resistant strain against these fluoroquinolones has been reported [6,7]. Gemifloxacin and gatifloxacin are fourth-generation fluoroquinolones, possess an improved antibacterial spectrum, particularly against resistant staphylococcus and streptococcus pathogens, compared with older fluoroquinolones [8,9].

Gemifloxacin (GMFX) (R,S)-7(3-aminomethyl-4-syn-methoxyimino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1, 4 dihydro-4-oxo-1, 2 naphthyridine-3-carboxylic acid (Fig. 1a) [10]. Gemifloxacin is an antibacterial compound with enhanced affinity for bacterial topoisomerase IV and is being used for the treatment of respiratory and urinary tract infections. The compound has a broad spectrum of activity against gram-positive and gram-negative bacteria. Gemifloxacin mesylate is not official in any pharmacopoeia.

Literature survey revealed that few analytical methods have been reported for the estimation of GMFX in pharmaceutical preparations or human plasma by visible spectrophotometry [11,12], capillary electrophoresis [13], high performance liquid chromatography–tandem mass spectrometry [14,15], and microchip electrophoresis [16]. These methods were related with some major drawbacks such as having inadequate sensitivity, being time-consuming, tedious, and dedicated to sophisticated and requiring expensive instruments.

Gatifloxacin (GTFX) (1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid), (Fig. 1b) [17]. It is widely used in the treatment of urinary tract infection, acute bacterial sinusitis, community acquired pneumonia, and acute bacterial exacerbation of chronic bronchitis [18]. Gatifloxacin is an antibacterial drug having selective antimicrobial activity against streptococcus pneumoniae and penicillin-resistant pneumococci. It is also active against anaerobic pathogen, bacteroides fragilis, and mouth anaerobes [19]. It is available in the tablet form and not official in any pharmacopoeia.

Several techniques have been proposed for the quantification of GTFX in pure, pharmaceutical dosage forms and in biological fluids by titrimetry (Marona et al., 2003), voltammetry [20,21], chromatography [22,23,24,25,26,27], capillary electrophoresis [28], atomic absorption spectrometry [29], chemiluminescence [30], fluorimetry, [31]; and [32] and spectrophotometry [29,33,34,35,36]. The titrimetry is insensitive and time consuming. The voltammetric, chromatographic, electrophoretic, atomic absorption spectrometric and chemiluminometric methods utilized dedicated and/or expensive instruments that are not available in most quality control laboratories' analytical technique. Spectrophotometry is considered the most convenient analytical technique, because of its inherent simplicity, low cost, and wide availability in most quality control laboratories [37]. However, few spectrophotometric methods were reported for the determination of GTFX in its pharmaceutical dosage forms [29,33,34,35,36]. These methods were associated with some major drawbacks such as decreased selectivity due to measurement in ultraviolet region, [33] and/or decreased simplicity of the assay procedure e.g. tedious precipitation, [29] or liquid–liquid extraction steps are based on the formation of ion-pair complex [36].

The kinetic spectrophotometric method offers an easy, less time consuming, sensitive analysis, by using simple and available reagents, which are able to be used for routine determinations of drug substances. Therefore kinetic spectrophotometric analysis is one of the major interests of analytical pharmacy. This work represents the first attempt at assaying gemifloxacin (GMFX) and gatifloxacin (GTFX) in pharmaceutical preparations by the use of the kinetic spectrophotometric method. The method is based on oxidizing the drugs with alkaline potassium permanganate. The reaction is followed up spectrophotometrically and the rate of change of absorbance at 604 nm is measured. The fixed time method is adopted after full investigation and understanding of the kinetics of the reaction. The proposed method is simple, accurate and sensitive.

2. Experimental

2.1. Apparatus

All the absorbance spectral measurements were made using spectroscan 80 D double-beam UV/Vis spectrophotometer (Biotech Engineering Ltd., UK), with a wavelength range of 190–1100 nm, spectral bandwidth 2.0 nm, with 10 mm matched quartz cells. A water bath shaker was used to control the heating temperature for color development.

2.2. Reagents and solutions

All chemicals and reagents used were of analytical grade. High purity double distilled water was used throughout.

i. Standard stock solutions of GMFX and GTFX containing 200 µg mL^{-1} were prepared separately in distilled water. GMFX and GTFX were kindly supplied from the Egyptian International Pharmaceutical Industries Company (EIPICO), Egypt. Samples of adrenergic blocker drugs were generously supplied by their respective manufacturers and were used without further purification. The stock and working standard solutions must be freshly prepared.

ii. Commercial dosage forms of GMFX (320 mg/tablet Floxgurad, product of Al-Debeiky Pharma, Al-Obour City, Egypt) and GTFX (Tymer, sterile ophthalmic solution 0.3% produced by JamJoom Pharmaceuticals, Jeddah, Saudi Arabia).

iii. Potassium manganate (Merck, Germany), 5×10^{-3} M aqueous solutions, should be freshly prepared and its molarity was checked titrimetrically.

iv. NaOH (BDH, UK), 1.0 M aqueous solution was prepared by dissolving 4.0 g of the chemical in 100 mL of water.

2.3. General recommended procedure

Accurate volumes of GMFX or GTFX working solution over the concentration range of 4–36 μg mL^{-1} and 4–40 μg mL^{-1}, respectively were transferred into a series of 10 mL standard flasks. To each flask 1.5 mL of 1.0 M sodium hydroxide followed by 2.0 mL of 5×10^{-3} M potassium permanganate was added. Complete to volume with water and mix well. After mixing, the reaction mixture was transferred to a thermostatically controlled water bath adjusted to 70 ± 2 °C or 50 ± 2 °C, at fixed time of 15 min for GMFX and GTFX, respectively. Cool and then, measure the absorbance of solutions at 604 nm against reagent blank treated similarly. Construct the calibration graph by plotting the final concentration of the drug against the absorbance values, measured at a fixed time of 15 min. Alternatively, derive the corresponding regression equation.

2.4. Procedures for pharmaceutical formulations

2.4.1. Procedure for the tablets

Ten tablets of floxguard each containing 320 mg of GMFX were crushed, powdered, weighed out and the average weight of one tablet was determined. An accurately weighed portion, equivalent to 20 mg was dissolved in about 10 mL of distilled water and any remaining residue was removed by filtration. The filtered solution was then transferred into a 100 mL calibrated flask and diluted to 100 mL with water. The nominal content of the tablet was assayed from the calibration curve.

2.4.2. Procedure for eye drops

The contents of five tymer samples (each 1.0 mL contains 3 mg of GTFX) were mixed. A volume equivalent to 20 mg of GTFX was transferred to a 100 mL volumetric flask and made up to the mark with water. Suitable dilution was made to fit the applicable concentration range and the above described procedures were followed. The nominal content of the bottles was calculated either from calibration graph or using the regression equation.

3. Results and discussion

3.1. Optimization of parameters

The absorption spectrum of aqueous potassium permanganate solution in alkaline medium exhibited an absorption band at 530 nm. The additions of any of the studied drugs to this solu-
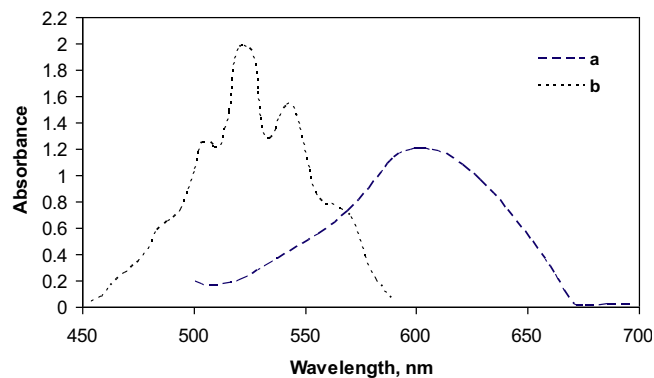

Figure 2 Absorption spectrum of GMFX (36 μg mL^{-1}) after reaction with KMnO$_4$ (1×10^{-3} M): (a) manganate ions and (b) reagent blank.

tion produce a new characteristic band at 604 nm (Fig. 2). This band is due to the formation of manganate ion, which resulted from the oxidation of GMFX by potassium permanganate in alkaline medium. The intensity of the color increases with time; therefore a kinetically based method was developed for the determination of GMFX and GTFX in their pharmaceutical formulations. The various experimental factors affecting the development and stability of the reaction product were studied and optimized. Such factors which were changed individually, include concentration of the reagents (KMnO$_4$ and NaOH), order of addition of reagents, temperature, time of heating and buffer solutions.

3.1.1. Effect of KMnO$_4$ concentration

Potassium permanganate oxidizes GMFX and GTFX in the presence of sodium hydroxide to form the green product resulting from the reduction of permanganate to manganate. Different concentrations of potassium permanganate from 1×10^{-4} to 1.25×10^{-3} M were studied. The absorbance at 604 nm was measured at a fixed time of 15 min. The reaction increased substantially with increasing the concentration of KMnO$_4$ (Fig. 3). Maximum absorbance was obtained when 2.0 mL of KMnO$_4$ solution was used (the concentration in

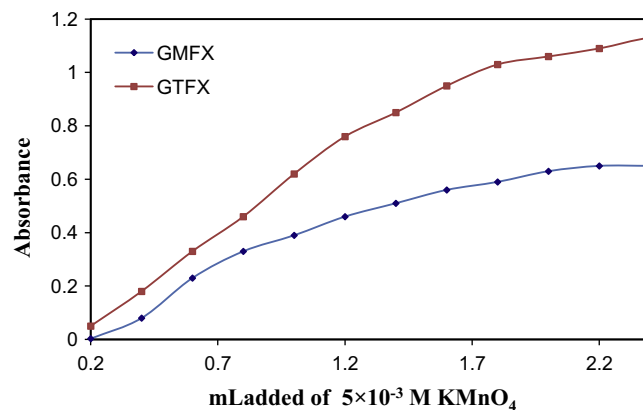

Figure 3 Effect of the volume of 5×10^{-3} M KMnO$_4$ on the reaction of GMFX (36 μg mL^{-1}) and GTFX (40 μg mL^{-1}) with alkaline potassium permanganate. The reactions were carried out at room temperature (25 ± 2 °C).

the final assay solution was 1.0×10^{-3} M). Further increase in the concentration had no effect on the reaction.

3.1.2. Effect of NaOH concentration

NaOH concentration on the reaction rate was studied using 0.2–3.0 mL of 1.0 M NaOH. It was found that increasing the volume of 1.0 M NaOH, would increase the absorbance of the reaction product up to 1.0 mL. It was also observed that there was no significant difference in the absorbance of reactant solutions at NaOH concentrations above 1.0 mL, while decreasing NaOH concentration resulted in lower absorbance values. Therefore, 1.5 mL of 1.0 M NaOH was found to be the most suitable concentration for maximum absorbance (Fig. 4). The effect of different Na salt buffers, particularly acetate, borate, carbonate, oxalate and phosphate, was investigated. No effect was observed when 0.01 M of these buffers was added to a solution.

3.1.3. Effect of time

To study the effect of time, a fixed concentration of the studied drugs $(36\,\mu g\,mL^{-1})$ was made to react with 2.0 mL of 5×10^{-3} M KMnO$_4$ solution; absorbance readings were recorded at different times in the range of 2.0–40 min. The oxidation reaction was completed in 35 min and the color was stable up to 60 min in the presence of the reaction product(s) (Fig. 5).

3.1.4. Effect of temperature

Preliminary test proved that a complete color formation was achieved by heating the resulting solution in a thermostatically-controlled water bath. Different temperature settings were used with constant heating time. Increasing temperature of the water bath was found to produce a proportional increase in absorbance (Fig. 6). So, trials have been done to carry out the reaction at higher temperatures but unwanted chemical changes e.g. precipitation of manganese (II) dioxide might occur. To avoid this and for the sake of good results, the optimum temperature of $70 \pm 2\,°C$ and $50 \pm 2\,°C$ was selected for the determination of GMFX and GTFX, respectively. The time of heating is an essential part of the experiment. Different time intervals were tested to ascertain the time after which the solution attains its highest absorbance. It was found that heating for 15 min gave the highest absorbance readings
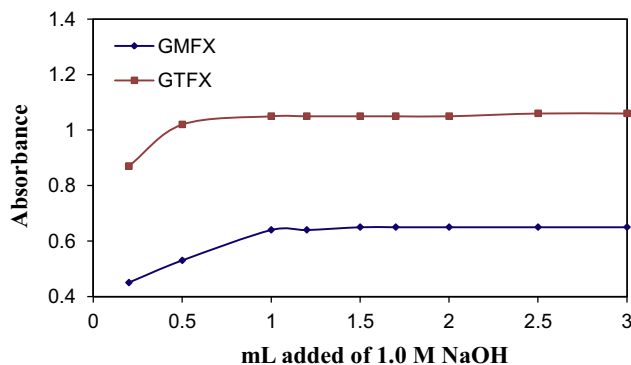

Figure 4 Effect of the volume of 1.0 M NaOH on the reaction of GMFX $(36\,\mu g\,mL^{-1})$ and GTFX $(40\,\mu g\,mL^{-1})$ with alkaline potassium permanganate. The reactions were carried out at room temperature $(25 \pm 2\,°C)$.

Figure 5 Effect of time on the reaction of GMFX $(36\,\mu g\,mL^{-1})$ and GTFX $(40\,\mu g\,mL^{-1})$ with alkaline potassium permanganate. The reactions were carried out at room temperature $(25 \pm 2\,°C)$.
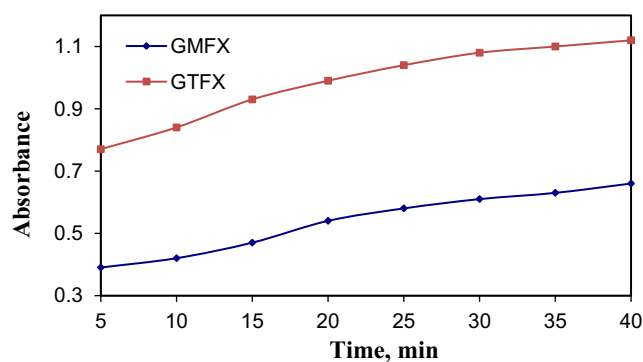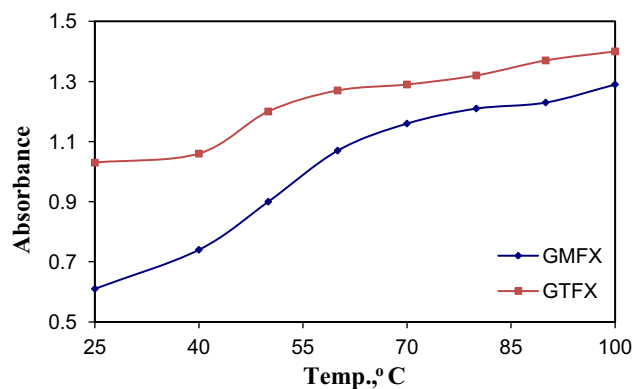

Figure 6 Effect of temperature on the reaction of GMFX $(36\,\mu g\,mL^{-1})$ and GTFX $(40\,\mu g\,mL^{-1})$ with alkaline potassium permanganate.
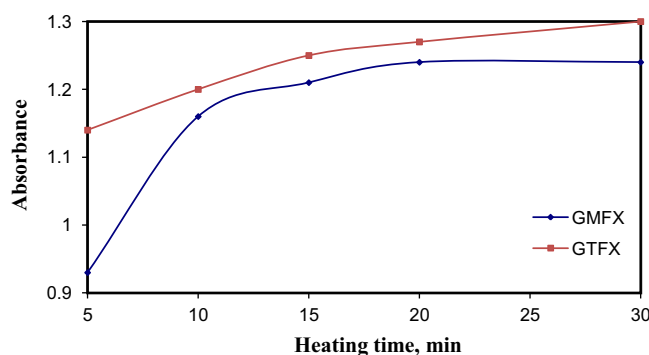
Figure 7 Effect of heating time on the reaction of GMFX $(36\,\mu g\,mL^{-1})$ and GTFX $(40\,\mu g\,mL^{-1})$ with alkaline potassium permanganate.

for each drug. Excessive heating time did not produce a significant increase in absorbance readings (Fig. 7).

3.1.5. Order of addition

The experimental parameters were fixed, and further experiments were performed to test the influence of the order of the addition of reactants. It was found that the order (KMnO$_4$, NaOH and drug), results in maximum absorbance. Addition

orders, other than those described in the procedure, gave lower results.

3.1.6. Stoichiometric ratio

The stoichiometric ratio between studied drugs and potassium permanganate was determined by the limiting logarithmic method, [38] by performing two sets of experiments. In the first set, the concentration of drug was varied keeping a constant concentration of $KMnO_4$. In the second set of experiment, concentration of drug was kept constant while varying the concentration of $KMnO_4$. The logarithm of the absorbance was plotted against the logarithm of the respective varied concentration of drug or $KMnO_4$ (Figs. 8 and 9). The slopes of the
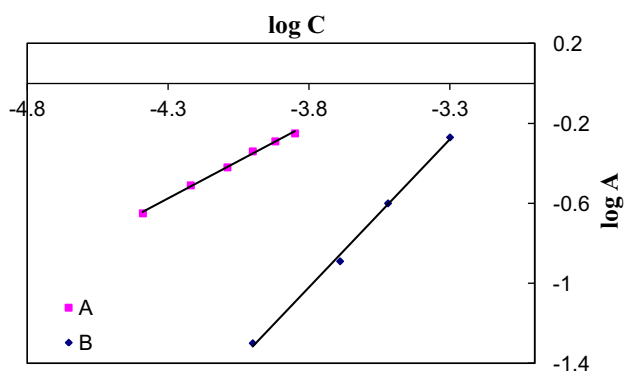

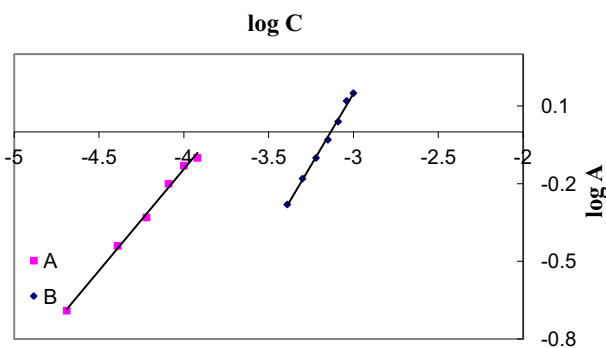**Figure 8** Limiting logarithmic plots for the molar ratio: (A) log A vs. log [$KMnO_4$], (B) log A vs. log [GMFX].

Figure 9 Limiting logarithmic plots for the molar ratio: (A) log A vs. log [$KMnO_4$]; (B) log A vs. log [GTFX].

two straight lines were calculated and found to be unity in each case. Thus, the stoichiometric ratio between each drug and potassium permanganate was found to be 1:1.

3.2. Evaluation of the kinetic methods

The quantitative determination of GMFX and GTFX under the optimized experimental conditions outlined above would result in a pseudo-first order reaction with respect to their concentration where, $KMnO_4$ concentration was at least 30 times the concentration of each drug, and NaOH concentration was at least 600 times the initial concentration of each drug. However, the rates will be directly proportional to drug concentration in a pseudo-first order rate equation as follows:

$$\text{Rate} = K' + [C]^n \quad (1)$$

Eq. (1) was the basis for several experiments, which were carried out to obtain drug concentration. The rate constant, fixed-concentration, and fixed time methods [39,40] were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the correlation coefficient (r), and the intercept. Taking logarithms of rates and concentrations (Table 1), the above equation becomes:

$$\log K = \log \Delta A / \Delta t = \log k' + n \log C \quad (2)$$

where A is the absorbance, t is the time in seconds and K is the pseudo-first order rate constant. Regression of log (K) versus log [C] gave the regression equations:

$$\log K = \log \Delta A / \Delta t = -0.2524 + 0.6389 \log C,$$
$$r = 0.9971 \text{ for GMFX}$$

$$\log K = \log \Delta A / \Delta t = 0.7526 + 0.8835 \log C,$$
$$r = 0.9997 \text{ for GTFX}$$

A straight line with slope values of ($n \approx 1$) was obtained confirming that the reaction was first order.

3.2.1. Fixed-time method

Reaction rates were determined for different concentrations of the investigated drugs. At a preselected fixed time, which was accurately determined, the absorbance was measured. Calibration graph of absorbance versus initial concentration of drugs was established at fixed time of 2, 5, 7, 10, 13, 15, 20, 25 and 30 min (Figs. 10 and 11) with the regression equation assembled in Table 2. It is clear that the slope increases with time and the most acceptable values of the correlation coefficient (r) and the intercept were obtained for a fixed time of

Table 1 Relation between reaction rates and concentrations.

Drug	log $\Delta A/\Delta t$	log [drug]	Regression equation, log $\Delta A/\Delta t$ = log k^- + n log C	Correlation coefficient (r)
GMFX	−3.041	−4.387	log $\Delta A/\Delta t$ = −0.2524 + 0.6389 log C	0.9971
	−2.886	−4.289		
	−2.762	−4.211		
	−2.674	−4.143		
	−2.638	−4.086		
	−3.380	−4.371		
	−3.100	−4.274		
GTFX	−2.951	−4.195	log $\Delta A/\Delta t$ = −0.7526 + 0.8835 log C	0.9997
	−2.848	−4.128		
	−2.758	−4.070		

Figure 10 Absorbance–time curves for the reaction between GMFX and $KMnO_4$ in aqueous medium: 2.0 mL of 5×10^{-3} M $KMnO_4$ and GMFX (a) 4.10×10^{-5} (b) 5.13×10^{-5} (c) 6.15×10^{-5}, (d) 7.18×10^{-5} and (e) 8.20×10^{-5} M.
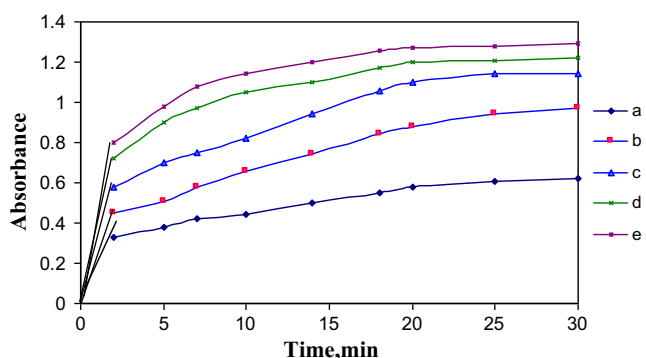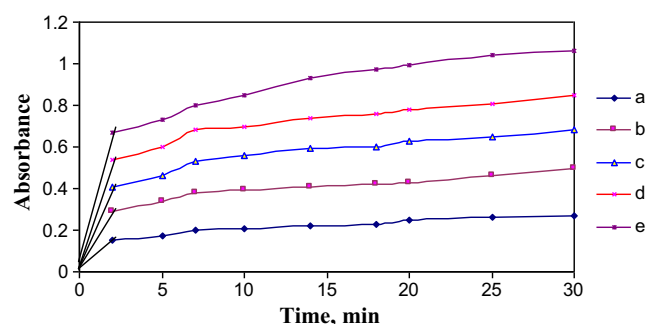

Figure 11 Absorbance–time curves for the reaction between GTFX and $KMnO_4$ in aqueous medium: 2.0 mL of 5×10^{-3} M $KMnO_4$ and GTFX: (a) 4.25×10^{-5}, (b) 5.32×10^{-5} (c) 6.38×10^{-5}, (d) 7.44×10^{-5} and (e) 8.51×10^{-5} M.

15 min, which was therefore chosen as the most suitable time interval for measurement. The analytical parameters for the determination of drugs in pure form by the fixed time method are shown in Table 2. After optimizing the reaction conditions, the fixed time method was applied to the determination of the studied drugs in pure form over the concentration range of 4–36 and 4–40 µg mL^{-1} for GMFX and GTFX, respectively.

3.2.2. Rate constant method

Graphs of log (absorbance) versus time for GMFX concentrations in the range of 2.05×10^{-5}–1.02×10^{-4} M and GTFX concentrations in the range of 2.12×10^{-5}–1.06×10^{-4} M were plotted. Pseudo-first-order rate constants (K) corresponding to different concentrations of the investigated drugs [C] were calculated from the slopes multiplied by -2.303 (Table 3). Regression of K values versus [C] gave the equations:

$$K = -0.0011 + 5.533C, \quad r = 0.9839 \text{ for GMFX}$$

$$K = -0.00085 + 4.739C, \quad r = 0.9821 \text{ for GTFX}$$

where A is the absorbance at 604 nm and C is the molar concentration. The method suffered from poor linearity as indicated from r value, therefore this method was excluded.

3.2.3. Fixed absorbance method

Reaction rates were determined for different concentrations of the investigated drugs. A pre-selected absorbance value was fixed (0.5 for both GMFX and GTFX) for different concentrations of the studied drugs, in the range of 2.05×10^{-5}–1.02×10^{-4} M for GMFX and 2.12×10^{-5} to 1.06×10^{-4} M for GTFX and the time required for each concentration to reach the preselected absorbance value was measured in seconds. The reciprocal of time ($1/t$) versus drug concentrations was plotted and the following equations were obtained by linear regression:

$$1/t = -0.00057 + 162.28C, \quad r = 0.9960 \text{ for GMFX}$$

$$1/t = -0.0044 + 131.57C, \quad r = 0.9965 \text{ for GTFX}$$

The concentration ranges giving the most satisfactory calibration graphs were limited, therefore this method was abandoned.

Table 2 Regression equations for the studied drugs of different concentrations at different time intervals using the fixed time method.

Drug	Time, min	Regression equation* $A = a + bC$	Correlation coefficient (r)
GMFX	2	$0.1296 + 0.0322C$	0.9984
	5	$0.2135 + 0.03668C$	0.9959
	7	$0.2583 + 0.03803C$	0.9954
	10	$0.3197 + 0.04148C$	0.9916
	13	$0.4259 + 0.04173C$	0.9993
	15	$0.4764 + 0.04285C$	0.9998
	20	$0.4992 + 0.0429C$	0.9939
	25	$0.5400 + 0.0415C$	0.9894
	30	$0.5612 + 0.04083C$	0.9865
	2	$-0.0210 + 0.03195C$	0.9995
	5	$0.0058 + 0.03605C$	0.9983
	7	$0.0244 + 0.0391C$	0.9985
GTFX	10	$0.0366 + 0.0417C$	0.9974
	13	$0.0590 + 0.04325C$	0.9987
	15	$0.0616 + 0.0456C$	0.9991
	20	$0.0697 + 0.04678C$	0.9991
	25	$0.0777 + .04828C$	0.9990
	30	$0.0928 + 0.0483C$	0.9987

* A is the absorbance at 610 nm and C is the concentration in µg mL^{-1}.

Table 3 Values of K calculated from slopes of log A versus t graphs multiplied by -2.303 for different concentrations of the studied drugs.

Drug	[Drug]	K	Regression equation	Correlation coefficient (r)
GMFX	2.05×10^{-5}	-9.442×10^{-4}	$K = -0.0011 + 5.533C$	0.9839
	4.10×10^{-5}	-8.728×10^{-4}		
	6.15×10^{-5}	-6.701×10^{-4}		
	8.20×10^{-5}	-6.125×10^{-4}		
	1.02×10^{-4}	-5.066×10^{-4}		
	2.12×10^{-5}	-7.622×10^{-4}		
	4.25×10^{-5}	-6.310×10^{-4}		
GTFX	6.38×10^{-5}	-5.135×10^{-4}	$K = -0.00085 + 4.739C$	0.9821
	8.51×10^{-5}	-4.652×10^{-4}		
	1.06×10^{-4}	-4.490×10^{-4}		

Table 4 Analytical parameters for the determination of GMFX and GTFX in pure form using the fixed time method.

Parameters	GMFX	GTFX
λ_{max}, nm	610	610
Temperature, °C	70 ± 2	50 ± 2
Heating time, min	15	15
Beer's law limit, µg mL^{-1}	4–36	4–40
Molar absorptivity, L mol^{-1} cm^{-1}	1.21×10^4	1.18×10^4
Sandell's sensitivity, ng cm^{-2}	29.72	31.97
Correlation coefficient (r)	0.9998	0.9991
Regression equation*		
Slope (b)	0.0299	0.0312
Intercept (a)	0.2930	0.1482
$S_{y/x}$	8.36×10^{-3}	1.85×10^{-3}
SD of slope (S_b)	1.22×10^{-3}	2.72×10^{-4}
SD of intercept (S_a)	7.79×10^{-3}	1.73×10^{-3}
LOD, µg mL^{-1}	0.0778	0.0778
LOQ, µg mL^{-1}	0.2951	0.2591

* Regression equation: $A = a + bC$, where C is the concentration of drug (µg mL^{-1}).

3.3. Linearity

The kinetic curves obtained at different concentrations of GMFX or GTFX, under the optimized conditions, were processed by the fixed-time method. Calibration graphs of absorbance versus initial concentrations of GMFX or GTFX were established at different fixed-time intervals. It was found that the slopes increase with time and the most acceptable values of the correlation coefficient (r) and the intercept were obtained at a fixed time of 15 min for both GMFX and GTFX which were, therefore, chosen as the most suitable time intervals for measurement. The calibration graphs were linear over the concentration range of 2.05×10^{-5}–1.02×10^{-4} M for GMFX and 2.12×10^{-5}–1.06×10^{-4} M for GTFX. Regression analysis indicates linear relationships with negligible intercepts. Table 4 presents the analytical parameters, molar absorptivity and the results of the statistical analysis of the experimental data: regression equations calculated from calibration graphs along with standard deviation of the slope (S_b) and intercept (S_a) on the ordinate and the standard deviation of residuals ($S_{y/x}$). The high values of the correlation coefficients of regression equations indicate good linearity and conformity to Beer's law.

3.4. Accuracy and precision

The accuracy and precision of the proposed kinetic spectrophotometric method were determined in terms of intermediate precision (intra-day and inter-day). Three different concentrations of the studied drugs were analyzed in five replicates during the same day (intra-day precision) and for seven con-

Table 5 Intra- and inter-day precision and accuracy of the reaction of GMFX and GTFX by the proposed kinetic spectrophotometric method.

Frequency of analysis	Drugs	Taken, µg mL^{-1}	Recovery, %	RSD[a],%	Er[b]%	SE[c]
Intra	GMFX	8	99.691	1.295	-6.0×10^{-3}	3.316×10^{-3}
		20	99.998	0.841	-2.0×10^{-3}	3.872×10^{-3}
		32	99.999	0.461	-2.0×10^{-3}	2.549×10^{-3}
Enter		8	99.999	1.391	-4.0×10^{-3}	3.674×10^{-3}
		20	99.999	0.491	-3.0×10^{-3}	2.236×10^{-3}
		32	99.999	0.519	0.012	2.915×10^{-3}
Intra	GTFX	8	99.999	0.634	-4.4×10^{-3}	1.224×10^{-3}
		20	99.998	0.976	-3.0×10^{-3}	3.741×10^{-3}
		32	99.999	0.118	-2.2×10^{-3}	6.244×10^{-3}
Enter		8	99.998	0.618	-0.025	1.224×10^{-3}
		20	99.999	0.405	-4.0×10^{-3}	1.581×10^{-3}
		32	99.998	0.340	-6.0×10^{-3}	1.870×10^{-3}

[a] Relative standard deviation for five determinations.
[b] Er, relative error.
[c] Standard error.

Table 6 Application of the proposed kinetic spectrophotometric method to the determination of GMFX and GTFX in dosage forms.

Drug	Formulations	Nominal value	Recovery[a], ±SD	t-Test	F-test	Reported methods
GMFX	Floxguard[b]	320 mg/tablet	99.996 ± 0.377	0.795	2.294	100.06 ± 0.571
GTFX	Tymer[c]	3 mg/ml	99.996 ± 0.493	1.117	2.211	99.60 ± 0.733

The theoretical values of t and F at P = 0.05 are 2.31 and 6.39, respectively.
[a] Average of five determinations.
[b] Product of Al-Debeiky Pharma, Al-Obour City, Egypt.
[c] Product of JamJoom pharmaceuticals, Jaddah, Saudi Arabia.

secutive days (inter-day precision). The analytical results obtained from the investigation are summarized in Table 5. The percentage relative standard deviation (RSD%) for the results did not exceed 1.4% (Table 5), proving the high reproducibility of the results and the precision of the method. This good level of precision was suitable for quality control analysis of the studied drugs.

3.5. Analytical applications

The fixed-time method has been successfully applied to determine GMFX in tablets and GTFX in eye drops. The concentrations of each drug were calculated using the corresponding regression equations at fixed time of 15 min for both GMFX and GTFX. The results obtained are presented in Table 6. Statistical analysis of the results obtained by both the proposed method and reported spectrophotometric methods, [11,12,36] revealed no significant difference in the performance of the two methods regarding accuracy and precision as revealed by t-test and F-test, respectively (Table 6).

4. Conclusion

The proposed method can be easily applied to the determination of GMFX and GTFX in pure and dosage forms, which do not require elaborate treatment of the analyte and tedious extraction of the chromospheres produced. The proposed method (fixed-time) is sensitive enough to enable determination of a lower amount of the drugs. These advantages encourage the application of the proposed method in routine quality control of the investigated drugs in industrial laboratories. No interference has been observed with excipients found in drug formulations.

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