

KINETIC SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF MORPHINE, NALBUPHINE AND NALTREXONE DRUGS IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A novel study describes the development and validation of a selective and simple kinetic spectrophotometric method for the determination of some analgesic drugs namely, morphine (MOF), nalbuphine (NAB) and naltrexone (NAT) in bulk and in pharmaceutical formulations. This method was based on the reaction of the drugs under studying with alkaline KMnO_4 to form a water-soluble bluish green colored product with a maximum absorbance at 605 nm. Although, the determination of MOF, NAB and NAT drugs by rate constant, fixed-concentration and fixed time methods were feasible with the calibration equations obtained, the fixed time method has been found to be more applicable. The fixed-time method is adopted for constructing the calibration curves, which were found to be linear over the concentration ranges of $4 - 18 \mu\text{g mL}^{-1}$ MOF, $2 - 20 \mu\text{g mL}^{-1}$ NAB and $2 - 16 \mu\text{g mL}^{-1}$ NAT. Different experimental parameters which can affect the development and stability of the color were carefully studied and optimized. The fixed time method of 20 min was further applied to pharmaceutical formulations of each drug and the percentage recoveries were 99.85 ± 0.049 to 102.27 ± 0.024 . Statistical comparisons of the results with the reference methods show the excellent agreement and indicate no significant difference in accuracy and precision.

Keywords: Kinetic spectrophotometric, potassium permanganate, NaOH, morphine, nalbuphine, naltrexone, dosage forms.

INTRODUCTION

Morphine (MOF) ($5\alpha, 6\alpha$ -dihydro-4, 5-epoxy-17-methylmorphinan- 3, 6-diol) (Fig. 1a) is a therapeutic drug that is normally used for pain control and also abused as an illicit drug. It is recommended by the World Health Organization (WHO) for the relief of moderate cancer-related pains. However, it has lethal effect when abused. As, heroin is hydrolyzed into morphine by an organism; therefore, the determination of the morphine content of biological samples is helpful for clinical and forensic purposes.^{1,2} To avoid overdose-induced toxicity, it is necessary to accurately monitor the concentrations of morphine in a patient's blood or urine. Various analytical methods have been developed for determining morphine and its major metabolites. Typically, the common analytical techniques currently used for monitoring morphine's concentration include GC,^{3,4} HPLC,⁵⁻¹⁰ and their combination with other detection methods. Moreover, there are some other methods can be used as well like capillary electrophoresis,^{11,12} chemiluminescence,¹³ voltammetric,^{14,15} electrochemical,¹⁶ and spectrofluorometric.¹⁷ Nevertheless, little trials had been reported for using spectrophotometry for morphine detection.¹⁸

Nalbuphine (NAB) (-)-17-(cyclobutylmethyl)-4,5 α -epoxymorphinan-3,6 α ,14-triol (Fig. 1b) is a semisynthetic narcotic agonist-antagonist of the phenanthrene series. Structurally, it is closely related to naloxone, an antagonist of the opiate receptors and to oxymorphone, a narcotic agonist. Nalbuphine has been shown to be approximately equianalgesic to morphine,¹⁹ yet with a ceiling effect on ventilator depression,²⁰ and fewer adverse effects than pethidine or pentazocine.²¹ As an analgesic agent, it is almost as potent as morphine and has widely used in the treatment of acute and chronic pain.²²⁻²⁴ However, NAB shows some advantages over MOF including a ceiling effect of respiratory depression, a low tolerance liability and a lack of significant withdrawal symptoms.²⁵ It is accessible as an injection for intramuscular and intravenous administration. The usual recommended doses are 10-20 mg by intravenous or intramuscular injection every 3-4h. To the best of our knowledge, no official method for the assay of NAB in any pharmacopeia was reported.

A few methods have been described to detect nalbuphine in pharmaceutical formulations; they include GC coupled to electron-capture detection,²⁶ or mass spectrometry,²⁷ HPLC with electrochemical detection,²⁸⁻³⁴ and ion-selective electrode.³⁵ Although the GC methods,^{26,27} are sensitive, they involved expensive equipments and time consuming samples preparation and are not easily available for regular drug monitoring. On the other hand, the reported HPLC methods,³²⁻³⁴ do not provide adequate sensitivity.

Opiate addiction is a worldwide serious problem which is now spread to some societies.³⁶ One of the current treatments is to use narcotic antagonists which generally have chemical structures similar to those of opiates and can preferentially occupy the body's opiate receptors and accordingly block their euphoric effects. This makes opiate intake delight-less and removes the addicts' incentive in opiate using. Naltrexone (NAT) (17-cyclopropylmethylmethyl-6-

deoxy - 7, 8 - dihydro-14-hydroxy-6-oxo-17-normorphine), (Fig. 1c) is a long-acting synthetic opiate antagonist with few side effects that is efficacious when administered orally, either daily or three times a week for a sustained period of time.

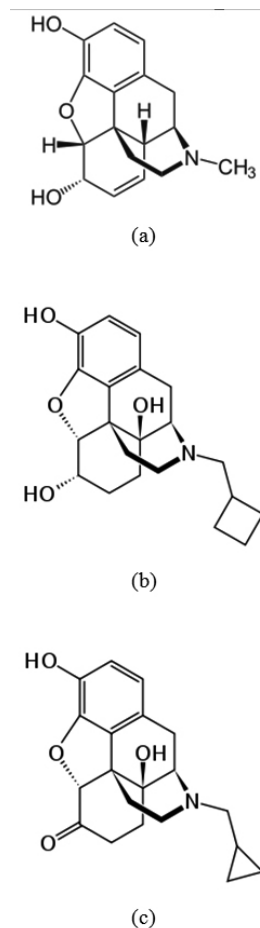


Fig. 1. Chemical structure of (a) morphine, (b) nalbuphine and (c) naltrexone.

Naltrexone has been determined by using a wide variety of analytical techniques, particularly chromatographic, such as TLC,³⁷ HPLC with electrochemical detection,³⁸⁻⁴² GC,^{43,44} and gas chromatography coupled with mass spectrometry (MS).⁴⁵ The most recent progress is to use LC with MS detection,⁴⁶ and LC-MS/MS,^{47,48} primarily for determination of naltrexone in animal plasma. However, these methods are time-consuming; they require off-line sample cleanup steps,^{39-42,47,48} including liquid-phase or solid-phase extraction steps, which is inconvenient for a routine therapeutic drug monitoring (TDM) survey aiming to report results within a single day after blood withdraw.⁴⁹

To our knowledge at the date of writing, there is no reference in the analytical literature reviews describing the kinetic spectrophotometric techniques for the assay of the studied drugs. For that reason, our goal is to develop a novel kinetic spectrophotometric method devise for the MOF, NAB and NAT drugs examination. In the present method we first oxidize each of the studied drugs with alkaline potassium permanganate and then run the subsequent measurement of absorbance at wavelength of 605 nm. The fixed time method was adopted after full investigation.

EXPERIMENTAL

Apparatus

All the absorbance spectral measurements were made using spectroscan 80 D double-beam UV/Vis spectrophotometer (Biotech Engineering Ltd., UK), with wavelength range 190–1100 nm, spectral bandwidth 2 nm, with 10 mm matched quartz cells. A water bath shaker was used to control the heating temperature for color development.

Reagents and Solutions

All chemicals and the reagents were analytical grade and used without further purification. Double distilled water with high purity was used throughout the experiments.

Unless otherwise specified, the stock and working standard solutions have to be freshly prepared and were prepared using distilled water. Standard stock solutions of MOF, NAB and NAT containing 200 µg mL⁻¹ were prepared separately in distilled water. MOF, NAB and NAT were kindly supplied from the Egyptian International Pharmaceutical Industries Company (EIPICO). Potassium manganate (Merck, Germany), 5×10⁻³ M aqueous solutions, should be freshly prepared and its molarity was checked titrimetrically. NaOH (BDH, UK), 1.0M aqueous solution was prepared by dissolving 4.0 g of the chemical in 100 mL of water.

General recommended procedure

Appropriate volumes of MOF, NAB or NAT stock solution (200 µg mL⁻¹) were transferred into a series of 10 mL standard flasks. To each flask 1.5 mL of 1.0M sodium hydroxide followed by 2.0 mL of 5×10⁻³ M potassium permanganate were added. The volume was made up to the mark using distilled water, mixed well for 20 min at room temperature for MOF and NAT, while with NAB, the reaction mixture was transferred to a thermostatically controlled water bath adjusted to 40±2°C, at fixed time of 20 min. Afterward, the absorbance of the solutions were measured at 605 nm against a "reagent blank" which was treated similarly. The calibration graph was then constructed by plotting the final concentration of each drug against the absorbance values which were measured at a fixed time. Alternatively, the corresponding regression equation was derived.

Procedures for pharmaceutical formulations

Procedure for ampoules

Five ampoules containing 20 mg of morphine (Manufactured by Misr Pharmaceuticals Co., Egypt) or five nalufin ampoules (20 mg mL⁻¹) of nalbuphine, (manufactured by Misr Pharmaceuticals Co., Egypt) were mixed. A volume equivalent to 20 mg of MOF or NAB was transferred to a 100 mL volumetric flask and made up to the mark with distilled water. Suitable dilution was made to fit the applicable concentration range and then the above described procedures were followed. The nominal content of the ampoules was calculated either from calibration graphs or using the regression equation.

Procedure for the tablets

Ten tablets of dextrexone (manufactured by Delta Pharmaceuticals Co., Egypt) each containing 50 mg of NAT were grinded to powder together. An accurately weighed portion, equivalent to 20 mg was dissolved well in about 10 mL of distilled water while any residue was removed by filtration. The resultant

solution was then transferred into a 100 mL calibrated flask and diluted to 100 mL with water. Appropriate dilutions were carried out with distilled water to achieve the applicable concentration within the range of calibration points range. The formerly described procedures were followed. The nominal content of the tablet was assayed from the calibration curve.

RESULTS AND DISCUSSION

Optimization of parameters

Oxidation-reduction reactions have been used as the basis for the development of simple and sensitive spectrophotometric methods for the determination of many pharmaceutical compounds. KMnO₄ is known as a strong oxidant, and its oxidation of the organic compounds is a pH dependent reaction. The reaction between the studied drugs and KMnO₄ in alkaline solution yields a bluish green color as a result of manganate species, which absorbs at wavelength of 605 nm (Fig. 2). The absorbance of the colored solution increases with time and hence, a kinetically-based spectrophotometric method was elaborated for their assay in 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 (KMnO₄ and NaOH), order of addition of reagents, temperature and time of heating.

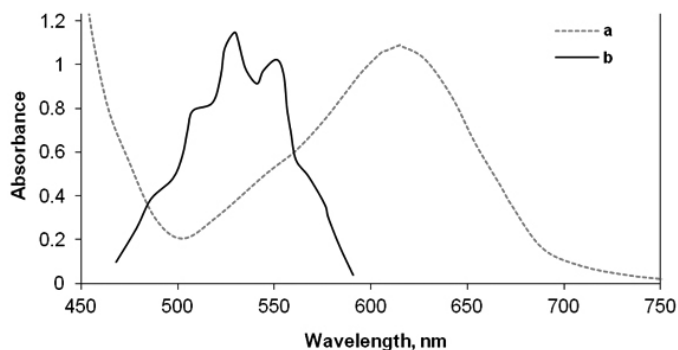


Fig. 2. Absorption spectrum of MOF (16 µg mL⁻¹) after reaction with KMnO₄: (a) manganate ions and (b) reagent blank.

Effect of KMnO₄ concentration

Potassium permanganate oxidizes MOF, NAB and NAT in the presence of sodium hydroxide to form the bluish green product resulting from the reduction of permanganate to manganate. The effect of KMnO₄ concentration on the initial rate of the reaction was studied in the range 1×10⁻⁴ to 1.25×10⁻³ M. The absorbance at 605 nm was measured at a fixed time of 20 min. Maximum absorbance was obtained when 2.0 mL of KMnO₄ solution was used. Further increase in the concentration had no effect of the reaction.

Effect of NaOH concentration

The influence of the NaOH concentration on the formation of MnO₄²⁻ was examined by taking varying volume (0.2–3.0 mL) of 1.0M NaOH. It was found that increasing the volume of 1.0M 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 1.0M NaOH was found to be the most suitable concentration for maximum absorbance. The effect of different Na salt buffers, particularly acetate, borate, carbonate, oxalate and phosphate, was investigated. No effect was observed when 0.01M of these buffers was added to a solution.

Effect of time

To study the effect of time, a fixed concentration of the studied drugs was made to react with 2.0 mL of 5×10⁻³ M KMnO₄ solution and the absorbance readings were recorded at different time in range of 2.0 - 60 min at room temperature. The oxidation reaction was completed in 20 min with MOF and NAT, as with NAB, the interaction need more time, even up to the end of the oxidation reaction. The color was stable up to 60 min in the presence of the reaction product(s) (Fig. 3).

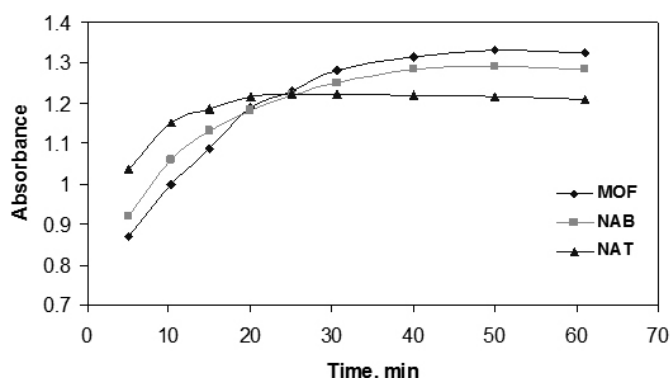


Fig. 3. Effect of mixing time on the reaction between the investigated drugs and alkaline potassium permanganate at room temperature.

Effect of temperature

The effect of temperature was studied in the range of 25–60°C. We found the rate of reaction of NAB with potassium permanganate increased with increasing temperature up to 40 °C. According to Fig 4, lower absorbance was obtained at higher temperatures than 50°C, for NAB. Therefore, 40°C was selected as the optimum temperature (Fig. 4). However, heating the reaction mixture of MOF and NAT lead to the precipitation of MnO_2 . Therefore, room temperature (25±2°C) was selected as the optimum temperature for MOF and NAT.

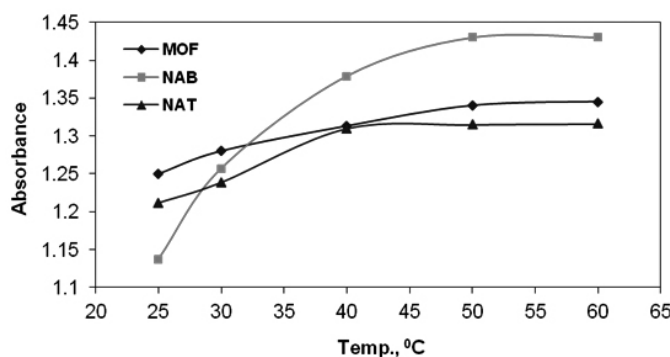


Fig. 4. Effect of temperature on the reaction between the investigated drugs and alkaline potassium permanganate.

Order of addition

The order of addition of reagents is an essential part of the experiment. 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 (drug, NaOH and KMnO_4), results in maximum absorbance. On the other hand, any other addition order, will lead to a lower absorption values.

Stoichiometric ratio

The stoichiometric ratio between studied drugs and potassium permanganate was determined by using the limiting logarithmic method.⁵⁰ Typically, in this method two sets of experiments are taking place. In the first set, the concentration of drug was varied while the concentration of KMnO_4 was set to a fixed value. Afterward, in the second set of experiment, the concentration of drug was set to a fixed value, 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 . Subsequently, the stoichiometric ratio is obtained from the slope of these two plots. The slopes of the two straight lines were calculated and found to be unity in each case. Accordingly, we set the stoichiometric ratio between each analyzed drug and potassium permanganate to 1:1.

Evaluation of the kinetic methods

The quantitative determination of MOF, NAB and NAT under the optimized experimental conditions outlined above, results in a pseudo-first order reaction with respect to their concentration. Accordingly, the drug concentration can be carried out from the pseudo-first order rate equation, shown in equation (1).

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

Equation (1) was the basis for several experiments, which were carried out to obtain drug concentration. By taking logarithms of rates and concentrations (Table 1), the above equation becomes:

$$\log K = \log \Delta A / \Delta t = \log k' + n \log C$$

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:

$$\begin{aligned} \log K &= \log \Delta A / \Delta t = 0.4251 + 0.7724 \log C, & r &= 0.9979 \text{ for MOF} \\ \log K &= \log \Delta A / \Delta t = 1.9161 + 1.0641 \log C, & r &= 0.9976 \text{ for NAB} \\ \log K &= \log \Delta A / \Delta t = 0.3413 + 0.7045 \log C, & r &= 0.9918 \text{ for NAT} \end{aligned}$$

Table 1. Values of logarithms of rates and concentrations of the studied drugs with alkaline KMnO_4 .

Drug	$\log \Delta A / \Delta t$	$\log [\text{Drug}]$	Regression equation, $\log \Delta A / \Delta t = \log k' + n \log C$	Correlation coefficient (r)
MOF	-3.2534	-4.8538	$\log \Delta A / \Delta t = 0.4251 + 0.7724 \log C$	0.9979
	-2.9929	-4.5528		
	-2.8747	-4.3757		
	-2.7878	-4.2510		
	-2.7222	-4.1542		
NAB	-3.4578	-4.9913	$\log \Delta A / \Delta t = 1.9161 + 1.0641 \log C$	0.9976
	-3.0118	-4.6925		
	-2.8009	-4.5157		
	-2.7796	-4.3914		
	-2.7224	-4.2941		
NAT	-3.1799	-4.9746	$\log \Delta A / \Delta t = 0.3413 + 0.7045 \log C$	0.9918
	-2.9498	-4.6736		
	-2.7871	-4.4989		
	-2.7769	-4.2765		
	-2.6411	-4.1972		

A straight line with slope values of ($n \approx 1$) was obtained confirming that the reaction is following first order. In this study, we used the rate constant, fixed-concentration, and fixed time methods,^{51,52} aiming to select the most suitable analytical method taking into account the applicability, the sensitivity, the correlation coefficient (r), and the intercept.

Fixed-time method

In order to achieve the most suitable time interval for measurement, we measured the calibration curve of absorbance versus initial concentration of the investigated drugs at fixed time of (2, 5, 8, 11, 14, 17, 20, 25, 30 and 35 min), as shown in (Figs. 5-7). The regression equation associated with them assembled in Table 2. We found that the slope increases with time. However, the most acceptable values of the correlation coefficient (r) and the intercept were obtained for a fixed time of 20 min. We chose this time as the most suitable time interval for the subsequent measurement.

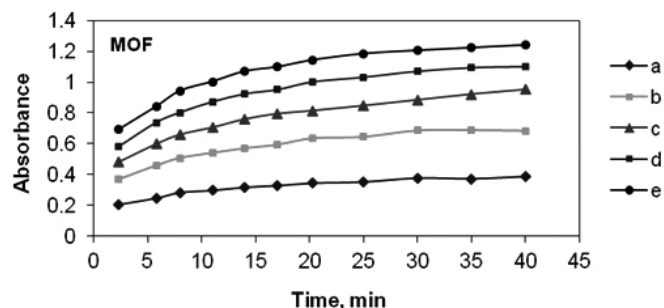


Fig. 5. Absorbance versus time graph for the reaction of MOF and alkaline potassium permanganate at 25 °C showing the dependence of the reaction on MOF concentration a) 1.40×10^{-5} (b) 2.80×10^{-5} (c) 4.21×10^{-5} (d) 5.61×10^{-5} and (e) 7.01×10^{-5} M, $\lambda_{\max} = 605$ nm.

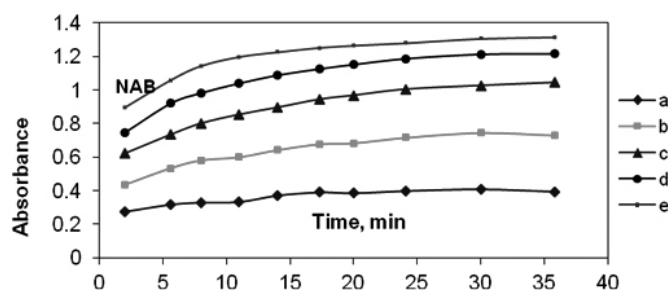


Fig. 6. Absorbance versus time graph for the reaction of NAB and alkaline potassium permanganate at 40 °C showing the dependence of the reaction on NAB concentration a) 1.02×10^{-5} (b) 2.03×10^{-5} (c) 3.05×10^{-5} (d) 4.06×10^{-5} and (e) 5.08×10^{-5} M, $\lambda_{\max} = 605$ nm.

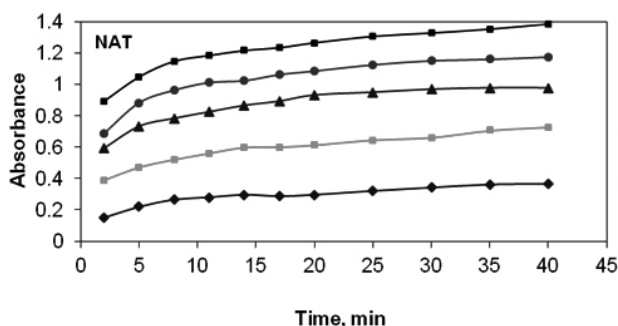


Fig. 7. Absorbance versus time graph for the reaction of NAT and alkaline potassium permanganate at 25 °C showing the dependence of the reaction on NAT concentration a) 1.06×10^{-5} (b) 2.12×10^{-5} (c) 3.17×10^{-5} (d) 5.29×10^{-5} and (e) 6.35×10^{-5} M, $\lambda_{\max} = 605$ nm.

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

Drug	Time, min	Regression equation* $A=a+bC$	Correlation coefficient (r)
MOF	2	$A=-0.1305+0.0594C$	0.9951
	5	$A=-0.1612+0.0737C$	0.9917
	8	$A=-0.1718+0.081C$	0.9947
	11	$A=-0.1907+0.0873C$	0.9935
	14	$A=-0.2041+0.0931C$	0.9937
	17	$A=-0.1985+0.0951C$	0.9988
	20	$A=-0.197+0.0984C$	0.9996
	25	$A=-0.2165+0.1027C$	0.9960
	30	$A=-0.1782+0.1022C$	0.9982
	35	$A=-0.1955+0.10546C$	0.9951
NAB	2	$A=-0.1824+0.0777C$	0.9980
	5	$A=-0.2222+0.0935C$	0.9969
	8	$A=-0.2481+0.1016C$	0.9958
	11	$A=-0.2800+0.1085C$	0.9937
	14	$A=-0.2647+0.1117C$	0.9955
	17	$A=-0.2080+0.1086C$	0.9974
	20	$A=-0.2232+0.1131C$	0.9991
	25	$A=-0.1998+0.1117C$	0.9982
	30	$A=-0.1917+0.1131C$	0.9990
	35	$A=-0.2235+0.1164C$	0.9752
NAT	2	$A=-0.3457+0.0886C$	0.9915
	5	$A=-0.3619+0.1031C$	0.9924
	8	$A=-0.367+0.1102C$	0.9959
	11	$A=-0.3571+0.1129C$	0.9939
	14	$A=-0.3323+0.1132C$	0.9916
	17	$A=-0.3624+0.1177C$	0.9990
	20	$A=-0.3664+0.1204C$	0.9997
	25	$A=-0.3564+0.1225C$	0.9992
	30	$A=-0.3416+0.1151C$	0.9991
	35	$A=-0.3082+0.1219C$	0.9901

*A is the absorbance at 605 nm and C is the concentration in $\mu\text{g mL}^{-1}$

Rate constant method

Graphs of $\log(\text{absorbance})$ versus time over the concentrations range of 1.4×10^{-5} to 7.01×10^{-5} M, 1.02×10^{-5} to 5.09×10^{-5} M and 1.06×10^{-5} to 6.35×10^{-5} M for MOF, NAB and NAT, respectively 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:

$$\begin{aligned} K &= -0.00077 + 5.005C, & r &= 0.9760 \text{ for MOF} \\ K &= -0.00054 + 2.008C, & r &= 0.9724 \text{ for NAB} \\ K &= -0.00069 + 3.931C, & r &= 0.9354 \text{ for NAT} \end{aligned}$$

where A is the absorbance at 605 nm and C is the molar concentration. The method suffered from poor linearity as indicated from r value, therefore this method was excluded.

Fixed absorbance method

Reaction rates were determined for different concentrations of the investigated drugs. A pre-selected absorbance value was fixed (0.5 for each drugs MOR, NAB and NAT) for different concentrations of the studied drugs, in the range of 2.80×10^{-5} to 8.41×10^{-5} M for MOR, 2.03×10^{-5} to 5.07×10^{-5} M for NAB and 3.17×10^{-5} to 5.29×10^{-5} M for NAT 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:

$$\begin{aligned} 1/t &= -0.00319 + 122.71C, & r &= 0.9967 \text{ for MOF} \\ 1/t &= -0.0154 + 787.71C, & r &= 0.9951 \text{ for NAB} \\ 1/t &= -0.0082 + 317.94C, & r &= 0.9972 \text{ for NAT} \end{aligned}$$

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

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)
MOF	1.40×10^{-5}	-7.001×10^{-4}	$K = -7.7 \times 10^{-4} + 5.005C$	0.9760
	2.80×10^{-5}	-6.287×10^{-4}		
	4.21×10^{-5}	-5.987×10^{-4}		
	5.61×10^{-5}	-4.606×10^{-4}		
	7.01×10^{-5}	-4.329×10^{-4}		
NAB	1.02×10^{-5}	-6.540×10^{-4}	$K = -5.4 \times 10^{-4} + 2.008C$	0.9724
	2.03×10^{-5}	-5.021×10^{-4}		
	3.05×10^{-5}	-4.675×10^{-4}		
	4.06×10^{-5}	-4.582×10^{-4}		
	5.08×10^{-5}	-4.375×10^{-4}		
NAT	1.06×10^{-5}	-8.751×10^{-4}	$K = -6.9 \times 10^{-4} + 3.391C$	0.9354
	2.12×10^{-5}	-6.079×10^{-4}		
	3.17×10^{-5}	-5.527×10^{-4}		
	5.29×10^{-5}	-5.227×10^{-4}		
	6.35×10^{-5}	-4.145×10^{-4}		

Linearity

The kinetic curves obtained at different concentrations of MOF, NAB and NAT under the optimized conditions was processed by the fixed-time method. Calibration graphs of absorbance versus initial concentrations of each drug 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 20 min for each drug which was therefore, chosen as the most suitable time intervals for measurement. The calibration graphs were linear over the concentration range of $4 - 18 \mu\text{g mL}^{-1}$ for MOF, $2 - 20 \mu\text{g mL}^{-1}$ for NAB and $2 - 16 \mu\text{g mL}^{-1}$ for NAT. 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.

The limit of detection (LOD) and quantitation (LOQ) were calculated using statistical treatment of calibration data at ten concentration levels by the following equation.⁵³

$$\text{LOD} = 3.3\sigma/s \text{ and } \text{LOQ} = 10\sigma/s$$

where σ is the standard deviation of five reagent blank determinations and s is the slope of the calibration curve. The LOD and LOQ were found to be 0.0678 and $0.2257 \mu\text{g mL}^{-1}$, respectively. This low value confirmed the good sensitivity of the method and consequently its capability to determine low amounts of each drug.

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) by determining the content of MOF, NAB, and NAT in quality control samples. Three different concentration levels (low, medium, and high)

of the studied drugs were analyzed in five replicates during the same day (intra-day precision) and for seven consecutive 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 2.95% (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.

Table 4. Analytical data for the kinetic spectrophotometric determination of MOF, NAB and NAT by fixed time method.

Parameters	MOF	NAB	NAT
λ_{max} , nm	605	605	605
Temperature, °C	25±2	40±2	25±2
Beer's law limit, $\mu\text{g mL}^{-1}$	4-18	2-20	2-16
Molar absorptivity, $\text{L mol}^{-1} \text{cm}^{-1}$	1.96×10^4	2.67×10^4	3.35×10^4
Sandell's sensitivity, ng cm^{-2}	14.579	14.755	11.262
Correlation coefficient (r)	0.9996	0.9991	0.9997
Regression equation*			
Slope (b)	0.0598	0.0605	0.0833
Intercept (a)	0.1667	0.1498	0.0759
$S_{y/x}$	8.89×10^{-3}	1.20×10^{-2}	1.23×10^{-2}
SD of slope (S_b)	6.86×10^{-3}	1.13×10^{-3}	1.18×10^{-3}
SD of intercept (S_a)	0.0213	0.0360	0.0202
LOD, $\mu\text{g mL}^{-1}$	0.0678	0.0678	0.0678
LOQ, $\mu\text{g mL}^{-1}$	0.2257	0.2257	0.2257

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

Table 5. Evaluation of intra- and inter-day precision and accuracy of the reaction of studied drugs by fixed time method.

Frequency of analysis	Drugs	Taken, $\mu\text{g mL}^{-1}$	Recovery, %	RSD ^a , %	Er ^b %	SE ^c
Intra	MOF	6	99.999	2.843	-0.001	7.870×10^{-3}
		12	99.999	0.646	-0.001	2.881×10^{-3}
		18	99.999	0.804	-0.001	4.756×10^{-3}
Enter		6	99.999	1.248	-0.001	3.595×10^{-3}
		12	99.999	0.577	-0.001	3.945×10^{-3}
		18	99.999	1.435	-0.001	9.517×10^{-3}
Intra	NAB	6	99.999	2.249	-0.001	5.557×10^{-3}
		12	99.999	2.351	-0.001	1.006×10^{-2}
		18	99.999	0.553	-0.001	3.489×10^{-3}
Enter		6	99.999	2.902	-0.001	6.700×10^{-3}
		12	99.999	2.946	-0.001	1.314×10^{-2}
		18	99.999	1.377	-0.001	8.249×10^{-3}
Intra	NAT	6	99.999	0.376	-0.001	1.036×10^{-3}
		12	99.999	0.608	-0.001	2.971×10^{-3}
		18	99.999	1.761	-0.001	1.103×10^{-2}
Enter		6	99.999	1.815	-0.001	4.814×10^{-3}
		12	99.999	2.957	-0.001	1.427×10^{-2}
		18	99.999	1.583	-0.001	9.607×10^{-3}

^aRelative standard deviation for five determinations.^bEr, Relative error.^cStandard error.**Table 6.** Application to the determination of MOF, NAB and NAT in dosage forms by fixed time method.

Drug	Formulations	Nominal value	Recovery ^a , \pm SD	t-test	F-test	Reported methods
MOF	Morphine ^b Injection	20 mg mL ⁻¹	99.85 \pm 0.049	0.814	1.169	99.55 \pm 0.053
NAB	Nalufin ^b ampoule	20 mg mL ⁻¹	99.93 \pm 0.057	0.289	1.678	100.2 \pm 0.044
NAT	Deltrexone ^c ampoule	50 mg/tablet	102.27 \pm 0.024	1.246	1.777	96.56 \pm 0.018

The theoretical values of *t* and *F* at *P* = 0.05 are 2.31 and 6.39, respectively.^aAverage of five determinations.^bManufactured by Misr Pharmaceuticals Co., Egypt.^cManufactured by Delta Pharmaceuticals Co., Egypt.

Analytical applications

The applicability of the fixed-time method for the assay of MOF, NAB, and NAT in drug formulations has been tested on commercially available dosage forms. The concentrations of MOF, NAB, and NAT were calculated using the corresponding calibration equation shown in Table 6 at a fixed time. No interference from the common excipients was observed. Statistical analysis of the results obtained by both the proposed method and reported methods.^{18,36,45} 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).

CONCLUSION

The proposed kinetic method is simple, sensitive, inexpensive, and suitable for routine quality control of MOF, NAB, and NAT in pharmaceutical dosage forms. The analytical procedure is very easy to perform within a short time, in comparison with the elaborate treatment and sophisticated instrumentation required for chromatographic methods. Moreover, the proposed method is a

direct one, applicable in aqueous medium and avoiding interference of colored and/or turbidity background of samples because it measures the increase in absorbance with time against blank treated similarly.

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