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Spectrophotometric Kinetic Methods for the Determination of Paracetamol in Pure Form and Pharmaceutical Preparations

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Abstract

Simple and sensitive kinetic methods are developed for the determination of Paracetamol in pure form and in pharmaceutical preparations. The methods are based on direct reaction (oxidative-coupling reaction) of Paracetamol with o-cresol in the presence of sodium periodate in alkaline medium, to form an intense blue-water-soluble dye that is stable at room temperature, and was followed spectrophotometriclly at λmax= 612 nm. The reaction was studied kinetically by Initial rate and fixed time (at 25 minutes) methods, and the optimization of conditions were fixed. The calibration graphs for drug determination were linear in the concentration ranges (1-7 $\mu g.ml^{-1}$) for the initial rate and (1-10 $\mu g.ml^{-1}$) for the fixed time methods at 25 min. The methods were applied successfully for the determination of Paracetamol in pharmaceutical.

Keywords: Paracetamol, ortho-Cresol, oxidative-coupling reaction, kinetic spectrophotometry.

طرائق طيفية حركية لتقدير الباراسيتامول بصورته النقية و في المستحضرات الصيدلانية

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الخلاصة

طورت طرائق طيفية حركية بسيطة وحساسة لتقدير دواء الباراسيتامول في صورته النقية وفي المستحضرات الصيدلانية. اعتمدت الطرائق على التفاعل المباشر (تفاعل الأزدواج التاكسدي) للدواء مع كاشف الأورثو–كريسول بوجود بيريودات الصوديوم كعامل مؤكسد في محيط قاعدي لتكوين صبغة مائية ذائبة زرقاء ومستقرة عند درجة حرارة الغرفة، قيست طيفيا عند الطول الموجي الأعظم = 612 نانومتر. وتمت دراسة حركية التفاعل بوساطة طريقتي معدل السرعة الأبتدائية والزمن الثابت (عند 25 دقيقة) وتم تثبيت الظروف المثلى للتفاعل وكان مدى الخطية لتقدير الدواء باستخدام طريقة معدل السرعة الأبتدائية بين (1 – 7 مايكروغرام/ مل) وباستخدام طريقة الزمن الثابت عند 25 دقيقة (1 – 10 مايكروغرام/مل). طبقت الطريقتين بنجاح في تقدير دواء الباراسيتامول في المستحضرات الصيدلانية.

Introduction

Paracetamol (Acetaminophen) is the name given to N-acetyl-p-aminophenol a formula molecule $C_8H_9NO_2$ where its chemical structure is shown in figure-1 [1] :

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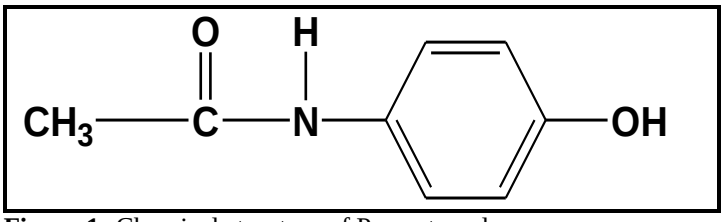


Figure 1- Chemical structure of Paracetamol

Its molecular weight is 151.17 g mol^{-1}, it is a white, fine crystalline powder slightly soluble in ether, ethylene chloride and water, more soluble in alcohol, it melts at 168-172^{o}C [2].

Paracetamol is an analgesic and antipyretic derived from phenacetin. It is widely used (alone or associated with other active substances such as caffeine) due to the lack of gastric upsets often associated with other analgesics such as acetyl salicylic acid [3].

Various methods have been reported for the determination of Paracetamol in pharmaceutical preparations, including HPLC [4-6], electrochemical methods [7-10], chemiluminescence [11, 12], spectrofluorimetric[13,14], flow injection [15-18] , spectrophotometry [19-25] and many other methods. The British Pharmacopoeia (BP) method describes a titrimetric procedure for paracetamol determination in pharmaceutical formulations using Ce (IV) in acidic media and 1, 10-phenanthroline-iron (II) complex (ferroin) to determine the end point. The literature is still poor in analytical procedures based on kinetics, especially for drugs in pharmaceuticals or biological fluids. However, some specific advantages in the application of kinetic methods can be expected such as, selectivity due to the measurement of the evolution of the absorbance with the time of the reaction instead of the measurement of absorbance value. Potassium permanganate has been frequently utilized for kinetic measurements in the field of pharmaceutical analysis. Many pharmaceutical compounds have been determined kinetically through this approach such as fluvoxamine [26], tetracycline hydrochloride [27], cephalosporins [28].

A norfloxacine [29] was determined by its reaction with acetaldehyde and 2,3,5,6 – tetrachloro – 1, 4 -benzoquinone to give a colored product. Ketoprofen [30] was determined kinetically by oxidative coupling reaction of the drug with MBTH reagent in the presence of Ce (IV) in acidic medium. Ramipril has also determined kinetically based on the reaction of the carboxylic group of the drug with a mixture of potassium iodate and potassium iodide and the reaction was followed spectrophotometrically [31]. The present work involves development of simple, accurate and sensitive spectrophotometric methods based on reaction between Paracetamol and ortho-cresol in the presence of sodium periodate; kinetically in an attempt to evaluate the drug in pharmaceutical preparations. Initial-rate and fixed-time methods were adopted after a full investigation.

Experimental

Apparatus : All spectral and absorbance measurements were carried out on a Shimadzu UV-Visble-260 digital double-beam recording spectrophotometer (Tokyo-Japan), using 1-cm quartz cells; Digital pH-meter (CD 330) WPA; Melttler balance H80.

Reagents and Solutions

All chemicals used were of analytical reagent grade. Standard Paracetamol was provided from the state company for drug industries and medical appliances (SDI) Sammara-Iraq.

1. Paracetamol solution (1000 $\mu g.ml^{-1}$)= 6.615 x 10^{-3}M
Prepared by dissolving 0.25 g of pure Paracetamol in 10 ml ethanol then transferred into 250 ml volumetric flask, diluted to the mark with distilled water, to prepare the concentratio100 $\mu g.ml^{-1}$ by transferred 10 ml stander solution into 100 ml volumetric flask then diluted to the mark volume with distilled water.

2. Ortho cresol (Hopkin & Williams) solution (5 x 10^{-3} M).
The amount of reagent purification by distillation at it's boiling point (191 ^{O}C) and preserved quantity distilled in a dark bottle away from light. Prepared by dissolving 0.1351 gm of pure reagent distilled in 5 ml ethanol then transferred into 250 ml volumetric flask, diluted to the mark with distilled water and kept in dark place.

3. Sodium periodate (B.D.H) solution (3 ×10^{-2}M).
Prepared by dissolving 3.208 gm with distilled water then completed the volume to 500 ml with the same solvent and kept in dark place.

4. Buffer solution:

Prepared by dissolving 6.4 gm of ammonium chloride (Fluka) in distilled water followed by addition of 57 ml of concentrated ammonium hydroxide (Fluka, 20%), transferred into 100 ml volumetric flask, then completed the volume with the same solvent. The pH of the solution is measured by a pH- meter device and then edit using concentrated ammonium hydroxide where the pH of the resulting solution up to pH = 10 ± 0.2.

Solutions of Pharmaceutical Preparations.

1. Tablets samples (500 mg Paracetamol- (SDI) Sammara-Iraq):

Ten tablets were weighed, grinded and the powder was mixed. An accurately weighed of the powder equivalent to 0.25 g of Paracetamol was dissolved in to 10 ml of ethanol, and then transferred into 250 ml volumetric flask, diluted to the mark with distilled water. The solution was filtered twice to obtain 1000 μg ml^{-1} of standard solution, and then prepared 100 μg ml^{-1} solution of Paracetamol by simple dilution with distilled water.

2.Suppositories Samples (Revanine / 125 mg Paracetamol, 10 mg Phenobarbitone – The Arab Pharamaceutical manufacturing Co. Sult-Jordan):

Five suppositories were weighed, mixed well. An accurately weighed of the mixture equivalent to 0.25 g of pure Paracetamol was dissolved in a little amount of hot water well then filtered, the residue was washed by a mixture of ethanol (10 ml) and hot distilled water, diluted to the mark volume of volumetric flask 250 ml with distilled water to obtain 1000 μg ml^{-1} of standard Paracetamol solution, then prepared 100 μg ml^{-1} solution of Paracetamol by simple dilution with distilled water.

3. Paracetamol injections (300mg paracetamol/ Shanghai pharm. Co. LTD. China):

The contents of five injections were mixed. An aliquot corresponding to 250 mg of paracetamol (1.7 ml) was shaken with 20 mL of ethanol and diluted to 250 ml with distilled water to obtained 1000 μg ml^{-1} of paracetamol, then prepared 100 μg ml^{-1} solution of Paracetamol by simple dilution with distilled water.

Analytical procedure for calibration

In to a series of 25 ml volumetric flask, transfer increasing volumes (0.25-1.75 ml) of standard stock solution (100 $\mu g.ml^{-1} = 6.615\times10^{-4}$ M) of Paracetamol to cover the range of the calibration graph (1.0-7.0 $\mu g.ml^{-1}$) for the initial-rate method and (0.25 -2.5 ml) of Paracetamol to cover the range of the calibration graph (1.0-10.0 $\mu g.ml^{-1}$) for the fixed-time method, to this solutions added 5 ml of Sodium periodate (3×10^{-2} M) shake thoroughly, then 2 ml of (5×10^{-3} M) of ortho cresol and 2 ml of pH= 10 were added then the contents were dilute to the mark with distilled water and shake well and transferred to a spectrophotometer cell. The absorbance of the colored product was measured as a function of time (after 10 minutes and after 25 minutes for the two methods respectively), at 612 nm against a reagent blank (prepared by added 5 ml of Sodium periodate (3×10^{-2} M) then 2 ml of (5×10^{-3} M) of ortho cresol and 2 ml of pH= 10then the contents were dilute to the mark with distilled water and shake).

Preliminary Investigations

Throughout the preliminary investigations of oxidative coupling reaction between Paracetamol with ortho-cresol in the presence of sodium periodate to give a soluble blue color dye that have a maximum absorbance at 612 nm. The absorbance of the colored product was measured versus reagent blank increases with time and then remains stable for at least 180 min. This was used as a basis for a useful kinetic method for the determination of Paracetamol in pharmaceutical preparations. Initial studies were directed towards the optimization of the experimental conditions in order to establish the optimum conditions necessary for quantitative formation of the product with maximum sensitivity.

Optimization of the Experimental Conditions

The effect of various variables on the color development was tested to establish the optimum conditions for determination of Paracetamol. In subsequent experiments, 1000 μg ml^{-1} of Paracetamol was taken into a 25 ml final volume and the absorbance was measured at room temperature (25°C) for series of solutions by varying one and fixing the other parameters at 612 nm versus reagent blank (prepared by added 5 ml of Sodium periodate (3×10^{-2} M) then 2 ml of (5×10^{-3} M) of ortho cresol and 2 ml of pH= 10±2 then the contents were dilute to the mark with distilled water and shake).

1- Effect of the coupling reagent (5×10^{-3} M).

The effect of the reagent solution was investigated by carrying out the reaction using different reagents such as ortho, meta, para-cresol, 8-hydroxy quinoline and ortho, meta, para-amino phenol by

using different oxidizing agent, and the reagent o-cresol seams to be the best coupling reagent so The effect of different volumes of (5×10^{-3} M) o-cresol (0.25 – 6 ml) was examed and the maximum absorbance obtained upon using 2 ml of (5×10^{-3} M) ortho-cresol solution as shown in figure-2.

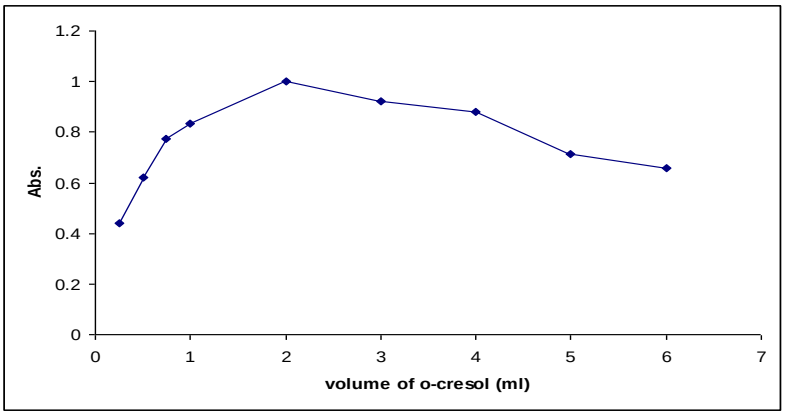


Figure 2- Effect of volume of (5×10^{-3} M) o-cresol

2- Effect of volume of Sodium periodate solution (3×10^{-2} M).

The effect of volume of the oxidant solution was studied by carrying out the reaction using different volumes of sodium periodate solution ranging from (0.5-10 ml). An increase in absorbance was obtained upon using 5 ml of oxidizing solution (3×10^{-2} M) sodium periodate as shown in figure-3.

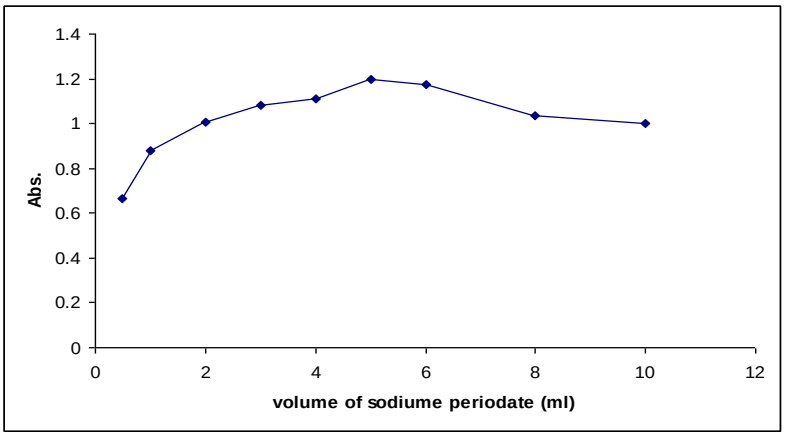


Figure 3- Effect of volume of (3×10^{-2} M) sodium periodate

3- Effect of PH

The effect of PH on the oxidative coupling reaction was studied by carrying out the reaction using different PH, the volumes of buffer solution ranging from (0.5-8 ml). The study show that the absorbance of the dye remains constant at PH = 10 ± 0.2 by using 2 ml of buffer solution as shown in figure-4.

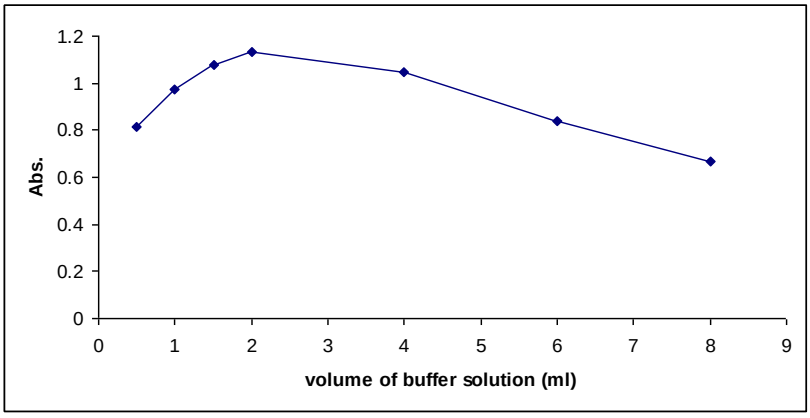


Figure 4- Effect of volume of buffer pH= 10±2

4- Effect of temperature

The effect of temperature on the oxidative coupling reaction study show that the absorbance of the dye remains constant at room temperature (25 C^o) for more than 90 min, and decrease at 0 C^o and 45 C^o, as shown in figure-5.

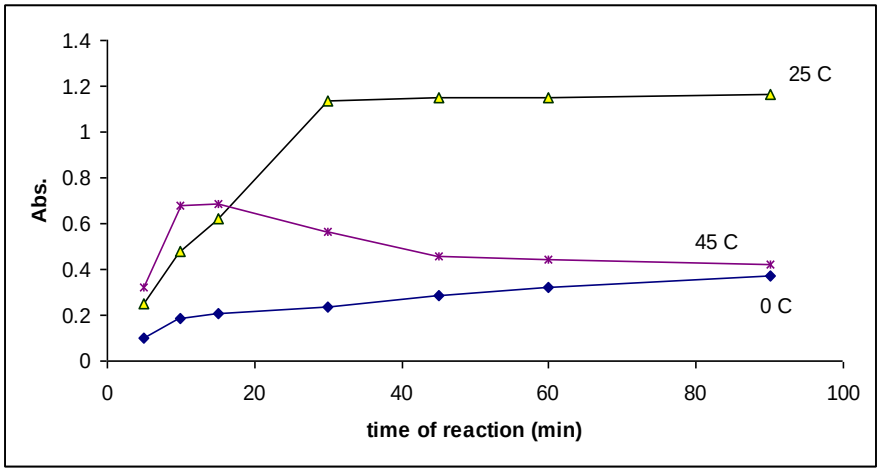


Figure 5- Effect of temperatures on reaction stability.

Absorbance Spectra

After obtaining the optimum conditions for the formation of the product, the absorbance spectra of the product solution versus reagent blank and reagent blank versus distilled water were recorded within 300 to 700 nm figure-6. The maximum absorbance of the product was found at 612 nm, which was the same as found in the preliminary investigations, and it was used in all subsequent experiments.

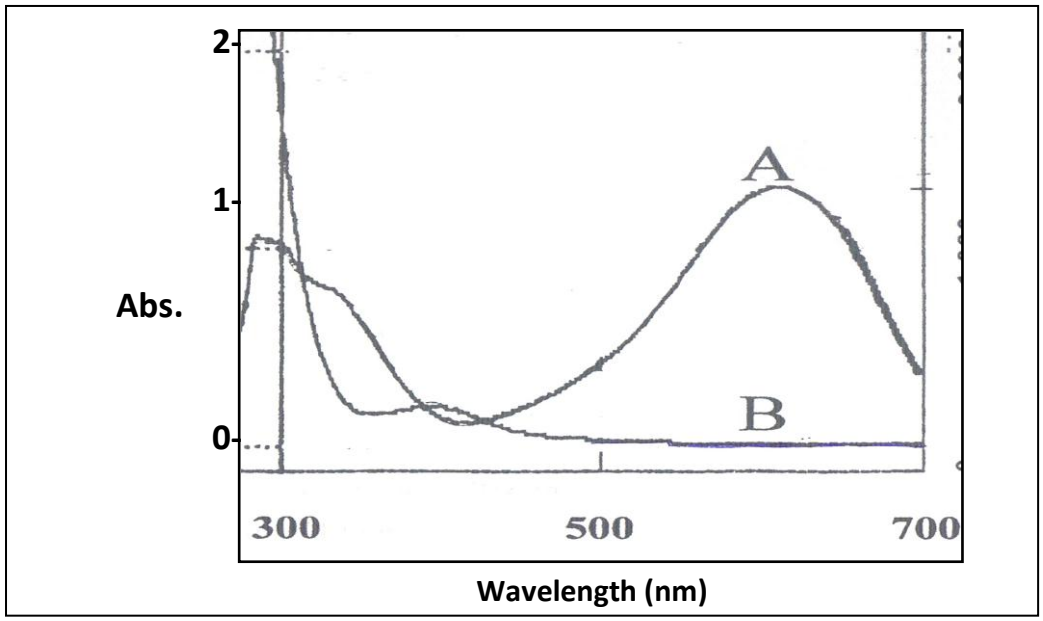


Figure 6- Absorbance spectra of color species against reagent blank (A) and reagent blank (prepared by added 5 ml of Sodium periodate (3×10^{-2} M) then 2 ml of (5×10^{-3} M) of ortho-cresol and 2 ml of pH= 10 then the contents were dilute to the mark with distilled water and shake) against distilled water (B).

Stoichiometry of the Reaction

Stoichiometry of the reaction, combining ratio between paracetamol and ortho cresol, was established by limiting the logarithmic method [32], using two sets of experiments. In the first set, the paracetamol concentration was varied while keeping a constant ortho cresol concentration (5×10^{-3} M); in the second set, the ortho cresol concentration was varied while keeping a constant concentration of paracetamol (6.6×10^{-4} M).

A plot of log absorbance versus log [paracetamole] (a) and log [ortho cresol] (b) gave straight lines; the values of the slopes were 0.9823 and 0.9471, respectively figure-7. Hence, it is concluded that, the molar reactivity of the reaction is 0.9823 / 0.9471, i.e. the reaction proceeds in the ratio of 1:1 (paracetamol: ortho-cresol) as shown in scheme-1.

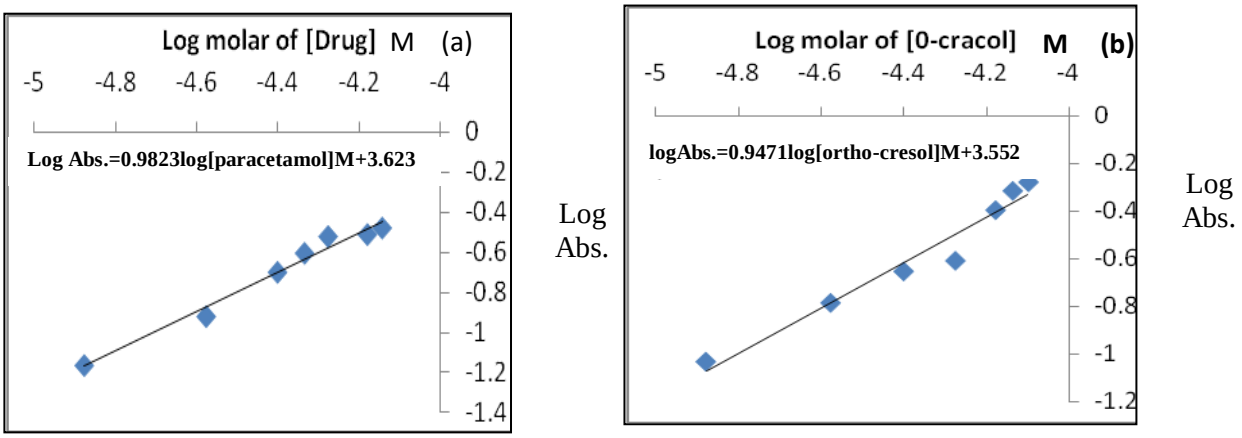
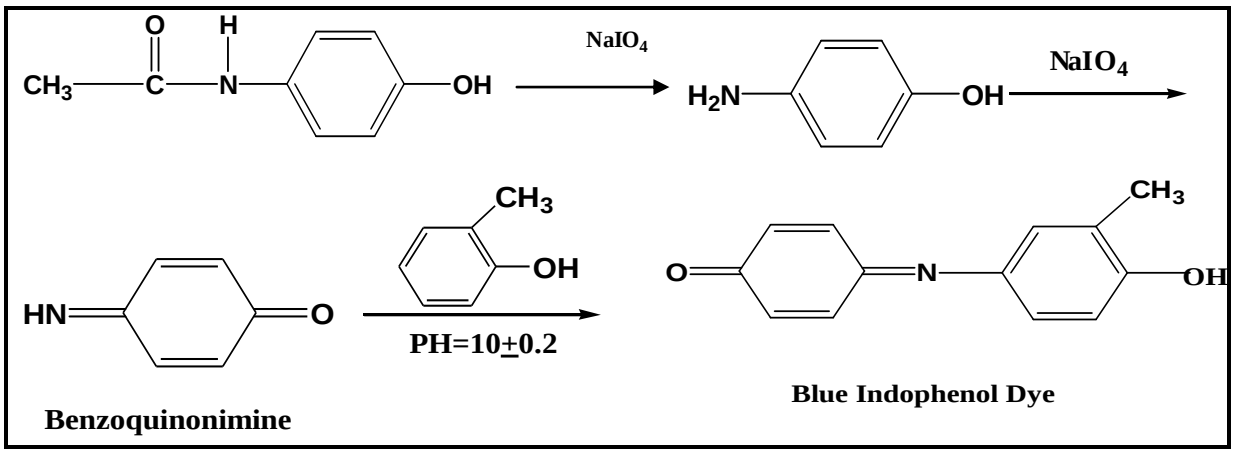


Figure 7- Limiting logarithmic plots for the molar ratio.

Based on the obtained molar reactivity, a reaction subsequent based on the above results is shown in scheme-1.



Scheme 1- Proposed mechanism for reaction sequence between paracetamol and o-cresol to form a blue indophenol dye

Evaluation of the Kinetic Methods

The quantization of paracetamol under the optimized experimental conditions outlined above would result in a pseudo- first order with respect to its concentrations where ortho cresol and periodate, were at least 15 time of the concentration of paracetamol. However, the rate was directly proportional to paracetamol concentration in a pseudo-first order equation as follows:

$$\text{Rate} = k' [\text{paracetamol}] \quad (1)$$

Where k' is the pseudo-first order rate constant.

Several experiments were then carried out to obtain paracetamol concentration from the rate data according to equation (1). Initial rate, fixed time methods [33, 34] were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the intercept and the correlation coefficient (r^2).

1- Initial rate method

The initial rates of the reaction were determined by measuring the slopes of the initial tangents of the absorbance time curves for the first 10 minutes Figure-8. Further more, logarithmic analysis of the reaction rate R (R= log ΔA /Δt) was plotted against log concentration of the drug Figure-9.

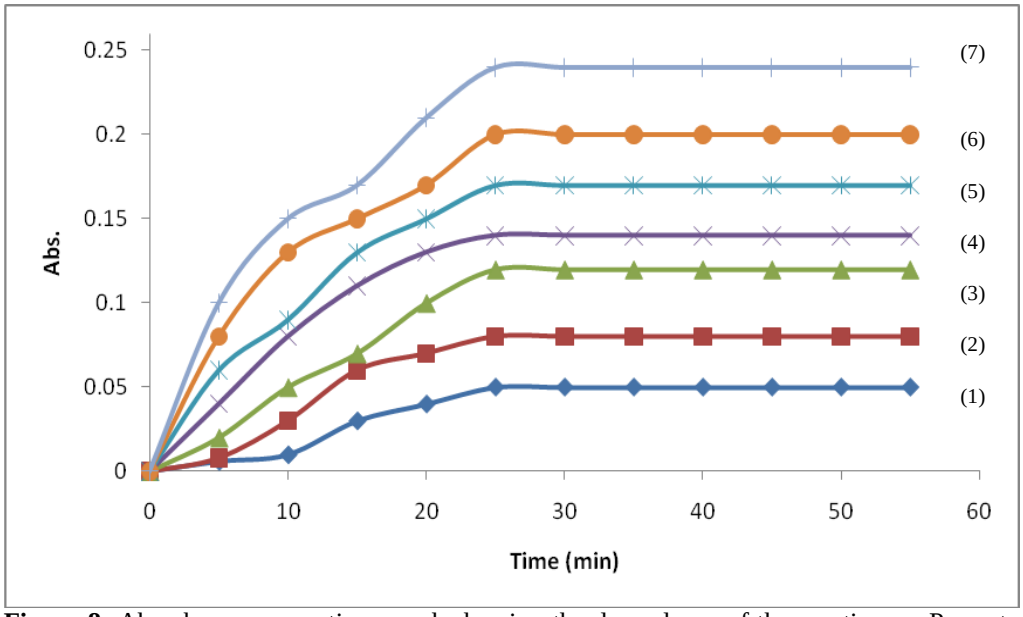


Figure 8- Absorbance verses time graph showing the dependence of the reaction on Paracetamol concentration (1-7 µg.ml^{-1}**)** (1)6.6×10^{-6}M, (2)1.32×10^{-5}M, (3)1.98×10^{-5}M, (4)2.64×10^{-5} M, (5)3.3×10^{-5}M, (6) 3.96×10^{-5}M and (7) 4.62×10^{-5}M, these absorbance measured against reagent blank.

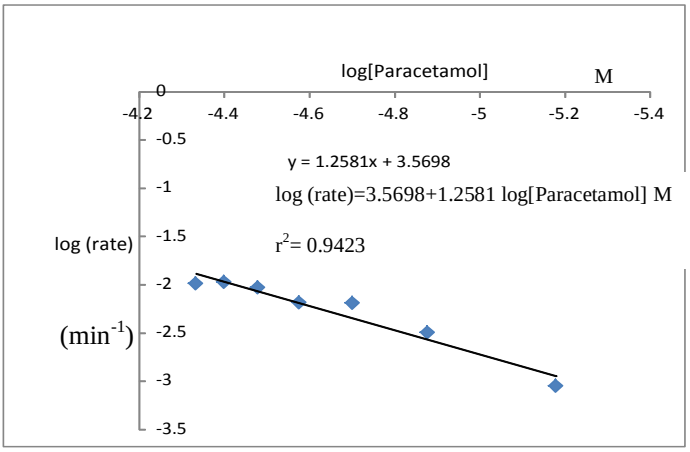


Figure 9- Effect of variation of log [Paracetamol] on log (rate)

The rate of reaction was also found to be dependent on paracetamol concentrations; the rates were followed at room temperature (25 ^{o}C) with various concentration of paracetamol in the range of 1-7 µg ml^{-1} keeping the reagent and the oxidant concentration constant. The reaction rate was found to obey the following equation:

$$\text{Rate} = k^{\backslash}[\text{paracetamol}]^{n} \quad (2)$$

Where $k^{\backslash}$ 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 [35] (differential initial rate method) [36] as $\Delta A/\Delta t$, where A is the absorbance and t is the time in minutes. Taking logarithms of rates and concentration, equation (3) is transformed into:

$$\text{Log (rate)} = \log \Delta A/\Delta t = \log k^{\backslash} + n \log [\text{paracetamol}] \quad (3)$$

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

Log (rate) = 3.5698 + 1.2581 logC

Where (r=0.9707).

Hence k = 3713.6 min^{-1} = 62 sec^{-1} and the reaction is first order (n = 1.2581) with respect to Paracetamol concentration.

2- Rate Constant Method

The best way to obtain an average K` value for the reaction, is to plot the log (A) versus time for Paracetamol in the concentration range 1-7 µg.$ml^{-1}$ ($6.6x10^{-6}$–$4.62x10^{-5}$ M) as shown in Figure-8, obtained pseudo first rate constant K`corresponding to different Paracetamol concentrations. These K` values were calculated from the slops of curves multiplied by (-2.303), as shown in Table-1.

Table 1- values of K\` calculated from slops of log A versus t graphs at 612nm.

[Drug] µg.ml^{-1}	Equation	K` / min^{-1}
1	Log A = 0.0486 t – 2.894	-0.1119
2	Log A = 0.0332 t – 1.942	-0.7645
3	Log A = 0.0273 t – 1.718	-0.0628
4	Log A = 0.0203 t – 1.395	-0.0467
5	Log A = 0.0182 t – 1.313	-0.0419
6	Log A = 0.0124 t – 1.086	-0.0285
7	Log A = 0.0108 t – 0.9691	-0.0248

Regression of [Paracetamol] versus K` gave the following equation:

$K^{\backslash}$=0.0135[paracetamol]-0.1102

Where r^2 = 0.9112 as shown in Figure-10.

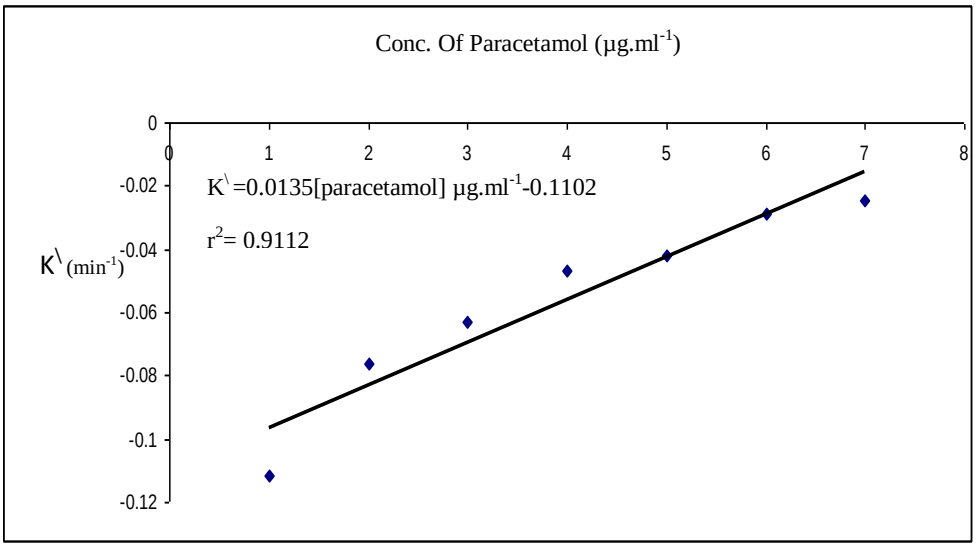


Figure 10- a plot of rate constant K` (min^{-1}) versus conc. of Paracetamol (µg.ml^{-1})

3-Fixed-time method

At a pre-selected fixed time, Calibration graphs of absorbance versus initial concentration of Paracetamol were established at fixed times of 5, 10, 15, 20, 25, 30, 35, 40, 50 and 60 min with regression equations assembled in Table-2.

Table 2- Regression equations for paracetamol at different fixed time over range ($6.6x10^{-6}$ – $4.62x10^{-5}$ M) at room temperature.

Time (min)	Regression equation	r^2
5	Y = 0.014 x – 0.011	0.9519
10	Y=0.0218x-0.0083	0.9827
15	Y=0.0239x+0.005	0.9901
20	Y=0.0285x+0.0092	0.9916
25	Y = 0.0324x + 0.0117	0.9918
30	Y = 0.0324x + 0.0117	0.9918
35	Y = 0.0324x + 0.0117	0.9918
40	Y = 0.0324x + 0.0117	0.9918
50	Y = 0.0324x + 0.0117	0.9918
60	Y = 0.0324x + 0.0117	0.9918

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 25 min, which was therefore, chosen as the most suitable time interval for measurement. After optimizing the reaction conditions, the fixed time method was applied to the determination of Paracetamol in pure form over the range (1-10 µg.ml^{-1}), as shown in Figure-11.

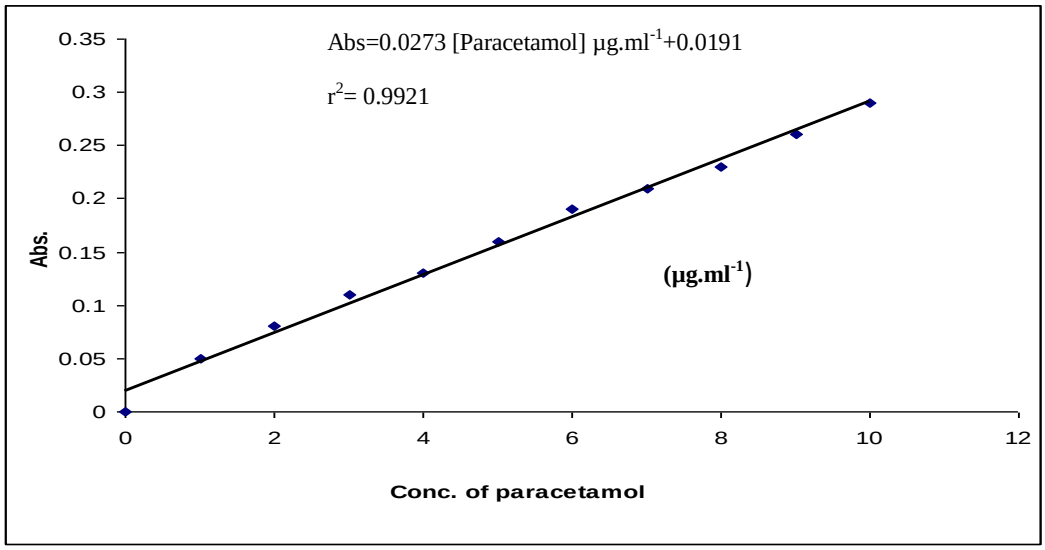


Figure 11- Calibration graphs of paracetamol at fixed time 25 min

Analytical values of statistical treatments for the calibration graphs are summarized in Table-3.

Table 3- analytical values of statistical treatments for the calibration graph of the fixed time method

Parameters	value
Correlation coefficient, r	0.9960
Correlation of determination , r^2	0.9921
Beer's low limits (µg.ml^{-1})	1-10
Regression equation	y=0.0273x+0.0191
Slop, b (ml.µg^{-1})	0.0273
Intercept, a	0.0191
Standard deviation of the residuals, $S_{y/x}$	0.0069
Standard deviation of the slop, S_b	0.00076
Standard deviation of the intercept, S_a	0.00469
Molar absorptivity Ɛ (l.mol^{-1}.cm^{-1})	$4.535x10^4$
Sandell's sensitivity S (µg.cm^{-2})	$3.333x10^3$
Limit of detection, LOD (µg.ml^{-1})	0.7549
Limit of quantification, LOQ (µg.ml^{-1})	2.5164

Accuracy and Precision

The accuracy and precision of Paracetamol was determined in five replacements of three different concentrations. The results shown in Tables-4, -5, indicate that a satisfactory precision and accuracy could be obtained with the proposed method.

Table 4- Accuracy and precision of the initial–rate method

Concentration of paracetamol µg.ml^{-1}		Error %	Recovery %	Relative standard deviation (R.S.D)%
Present	Found*			
2.000	2.031	+1.550	101.550	1.309
4.000	4.015	+0.375	100.375	0.815
6.000	5.969	-0.516	99.484	0.675

* For five repeated measurements.

Table 5- Accuracy and precision of the fixed-time method

Concentration of Paracetamol µg.ml^{-1}		Error %	Recovery %	Relative standard deviation (R.S.D)%
Present	Found*			
3.000	2.980	-0.660	99.340	0.998
6.000	5.980	-0.333	99.667	0.451
9.000	9.030	+0.330	100.330	0.324

* For five repeated measurements.

Interferences

To test the efficiency and selectivity of the proposed analytical method to pharmaceutical preparations, a systematic study under the optimum experimental conditions was made for the effect of additives and excipients such as starch, talc, lactose, magnesium stearate and polyvinylpirrolidone (PVP) that are usually present in a dosage forms. Criterion of interference was an error of not more than ± 1 % in the absorbance. In this study, a wide range of concentration was used in which the determination of the 4 µg ml^{-1} level of a drug was performed. Experimental showed that there was no interference from additives or excipients for the examined method up to 10-fold excess as shown in Table-6.

Table 6- Determination of 4 µg ml^{-1} of paracetamol in the presence of excipients.

Excipient, 40 µg ml^{-1}	Concentration of paracetamol, µg ml^{-1}	Error %	Recovery %	RSD %
	Found*			
Starch	3.998	-0.05	99.95	0.314
Talc	3.978	-0.55	99.45	0.531
Lactose	4.007	+0.17	100.17	0.766
mg stearate	3.986	-0.35	99.65	0.213
PVP	4.017	+0.42	100.42	0.661

*Mean value of three repeated measurements**.**

Pharmaceutical Applications

The initial-rate and fixed-time methods were applied to the determination of paracetamol in pharmaceutical preparation by the analysis of two different concentrations of pharmaceutical preparations using the analytical procedures. The results are given in Table-7 and Table-8.

Table 7- Application of the proposed method of paracetamol in pharmaceutical preparations by the initial-rate method

Drug sample	Concentration of paracetamol (µg.ml^{-1})		Error%	Recovery%*	R.S.D%
	Present	Found*			
Paracetamol tables (500mg)	2.000 6.000	2.022 6.052	+1.100 +0.866	101.100 100.866	1.061 0.946
Suppositories Revanine (125mg Paracetamol)	2.000 6.000	2.024 6.089	+1.200 +1.483	101.200 101.483	1.773 1.316
Paracetamol injection (300mg)	2.000 6.000	2.009 6.018	+0.450 +0.300	100.450 100.300	1.310 1.795

*for five repeated measurements.

Table 8- Application of the proposed method of Paracetamol in pharmaceutical preparations by the fixed-time method

Drug sample	Conc. of paracetamol (μg.ml^{-1})		Error%	Recovery%*	R.S.D%
	Present	Found*			
Paracetamol tables (500mg Paracetamol)	4.000 8.000	3.930 7.940	-1.750 +1.630	98.250 101.630	0.856 0.624
Suppositories Revanine (125mg Paracetamol)	4.000 8.000	4.092 8.107	+2.300 +1.337	102.330 101.337	1.190 1.019
Paracetamol injection (300mg Paracetamol)	4.000 8.000	4.029 8.011	+0.725 +0.137	100.725 100.137	1.391 0.930

*for five repeated measurements.

The proposed method was compared successfully with the BP method [2] for both pure paracetamol and the pharmaceutical preparations using both initial-rate and fixed-time methods, good recoveries were obtained as shown in Table-9, Statistical F and t-test between Bp method and the proposed methods showed that there was no significant difference between Bp method and proposed methods, at 95% confidence level using the statistical low:

1- t-test

$$\mp t = \frac{\bar{X}_1 - \bar{X}_2}{S}\sqrt{\frac{n_1 n_2}{n_1 + n_2}}$$

Since n1,n2 =the number of values for both proposed and stander methods respectively, $\bar{X}_2$, $\bar{X}_1$ = the recoveries for both proposed and stander methods respectively and S= pooled standard deviation which calculated by:

$$S = \sqrt{\frac{\sum (X_{i1} - \bar{X}_1)^2 + \sum (X_{i2} - \bar{X}_2)^2}{n_1 + n_2 - 2}}$$

2- F-test

$$F = S_1^2 / S_2^2$$

Since S^1_1 and S^2_2 = the square of variance for both proposed and standard methods respectively

$S^2 = \frac{\sum_i (X_i - \bar{X})^2}{n-1}$, which X_i= analytical value, X\=average of values and n= number of values.

The calculated values for F and t-test for fixed time and initial-rate methods (1.238, 0.139 and 1.176, 0.07 respectively) , did not exceed the critical values of F=9.28 and t= 2.361 ($n_1 + n_2 - 2 = 6$). These confirming that there are no significant differences between the two proposed methods with BP method with respect to precision and accuracy in the determination of paracetamol in pharmaceutical preparations.

Table 9- Comparison of the proposed methods with standard method.

Drug sample	Recovery%*		
	Fixed-time method	**Initial-rate method**	**BP method**
Pure Paracetamol	99.666	99.483	100.000
Paracetamol tables (500mg Paracetamol)	100.866	100.985	99.530
Suppositories Revanine (125 mg Paracetamol)	101.483	99.861	100.608
Paracetamol injection (300mg Paracetamol)	100.300	100.925	101.128

*for five repeated measurements.

Conclusions

The proposed methods are showing good sensitivity. In addition, the proposed procedures show relevant selectivity allowing analysis without separation steps, and providing suitable alternative to the many chromatographic procedures proposed. The proposed methods are advantageous when they are compared with colorimetric methods in having higher sensitivity. The data given above reveal that the proposed methods are accurate and sensitive with good precision and accuracy. With this method, one can do the analysis with speed at low cost without losing accuracy. The proposed method can be used as alternative method to reported ones for the routine determination of Paracetamol in the pure form and in pharmaceutical preparations.

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