Colorimetric Determination of Paracetamol Using 9-Chloroacridine Reagent: Application to Pharmaceutical Formulations

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Abstract
Background: The majority of reported spectrophotometric methods for determination of paracetamol are depended on hydrolysis of the compound and applying oxidative coupling and diazotization coupling methods leading to the determination of produced p-aminophenol by different reagents. In addition to using of other methods such as Schiff bases formation, oxidation reaction and charge transfer complex formation reactions. However, some of these methods either not sensitive, carried on organic medium or suffered from interferences. Other analytical techniques have been also used for determination of paracetamol, such as HPLC, voltammetry, chemiluminescence in addition to the titrimetric method. These methods needed of highly sophisticated instruments.
Aim: To develop a simple and accurate method for determination of acetaminophen in pharmaceutical formulations.
Materials and methods: The method is based on the reaction between the hydrolyzed paracetamol and 9-chloroacridine reagent (9-CA).
Results: The spectra of the product show maximum absorption at 436 nm. Beer's law is obeyed in the concentration range of 0.25-11 μg/ml with molar absorptivity value 5.3×10^3 L/ mol. cm. The average recovery percentage (Rec%) is 99.27% and relative standard deviation (RSD) is ≤2.82 %. In addition, the stability constant has been determined and the reaction mechanism is proposed.
Conclusion: A simple, sensitive and accurate spectrophotometric method is developed for the quantitative determination of paracetamol. The method has been applied successfully for the assay of paracetamol in pharmaceutical formulations.

Keywords: Acetaminophen, Paracetamol; 9-Chloroacridine; Spectrophotometry

Introduction

Paracetamol (acetaminophen, N-acetyl-p-aminophenol, 4-acetamido phenol) is official in the United States [1] and British [2] European [3] German [4] and Japanese [5] pharmacopeias. The drug is widely used as a minor analgesic and antipyretic agent [6]. Its action is similar to aspirin and it is the most commonly used in pediatrics [7], although it has some cyclo-oxygenase inhibiting properties. Paracetamol is a synthetic non opiate derivative of p-aminophenol. Paracetamol is hydrolyzed in appropriate storage conditions such as high temperatures and acidic or basic media to p-aminophenol [8]. It is a harmful compound for a human

organism because it increases body temperature and remains active for a long time [9].

The majority of reported spectrophotometric methods for determination of paracetamol are depended on hydrolysis of the compound and applying oxidative coupling [10-13] and diazotization coupling [14-16] methods leading to the determination of produced p-aminophenol by different reagents. In addition to using of other methods such as Schiff bases formation [17], oxidation reaction [18,19] and charge transfer complex formation reactions [20,21]. However, some of these methods either not sensitive [18], carried on organic medium [20] or suffered from interferences [22]. Other analytical techniques have been also used for determination of paracetamol, such as HPLC [23,24], voltammetry [25,26], chemiluminescence [27,28] in addition to the titrimetric method [29]. These methods needed of highly sophisticated instruments. Thus this study was conducted to develop a simple and accurate method for determination of acetaminophen in pharmaceutical formulations.

Materials and Methods

Apparatus

Spectra and absorbance measurements were made with UV-Visible double beam spectrophotometers (Perkin-Elmer, lambda 25). with 1-cm matched silica cells. The pH measurements were made by using Cyber Scan 510 pc. pH meter with a combined glass electrode. Heating of solutions is carried out on a water bath of frost instruments, LTD). Weighing is carried out on a sensitive balance type of Mettler H 54AR. All calculations in the computing process were done in Microsoft Excel for Windows.

Reagents

All chemicals used are of the highest purity available which are provided by BDH, Fluka and Molekula companies. 9-Chloroacridine (Eastman chemical Company.) was used as the chromogenic reagent. Absolute ethanol is used (ROTH Co.). Sodium hydroxide (1×10^{-2} M) and hydrochloric acid (1×10^{-2} M) solution are prepared by appropriate dilution of the concentrated NaOH (1 M) or HCl (1 M) solutions with distilled water. 9-chloroacridine reagent *(1×10^{-3} M) (*9-CA*)*. The 25 ml solution is prepared by dissolving 0.0053 g of 9-chloroacridine in ethanol absolute and then the volume is completed to 25 ml in a volumetric flask. The solution is prepared daily and used immediately [28].

Solution of hydrolyzed paracetamol (25 µg/ml): 2000 µg/ml solution was prepared by dissolving 0.20 g of pure paracetamol in 10 ml ethanol and shaking to increase the solubility, then filtered into 100 ml calibrated flask and the solution was completed to the volume with a distilled water (the solution was equivalent to 2000 µg/ml paracetamol). Seventy five ml of this solution was transferred into 250 ml round bottom flask provided with condenser and 25ml of Conc. hydrochloric acid was added then reflux for 1 hour. After that the cold solution was neutralized by 4.5 ml of 20% sodium carbonate and diluted to 250 ml with distilled water in a volumetric flask. To prepare 25 µg/ml paracetamol, a 4.16 ml of above solution was diluted to 100 ml in a volumetric flask using distilled water.

Recommended procedure

To a series of 5 ml calibrated flasks, increasing volumes of the working hydrolyzed paracetamol solution (25µg/ml) were transferred to cover the

concentration range 0.25-11 µg/ml, add 2 ml of 1×10^{-3} M 9-CA . The solutions were diluted to the mark with absolute ethanol. The solutions were kept at 40°C for 30 min in a water bath and the absorbance was measured at 436 nm against reagent blank after cooling to room temperature.

Procedure for paracetamol assay in tablet and suspension

Tablet

Weighted and finely powdered 10 tablets (each one contain 500 mg paracetamol), an accurately weighed amount of powder equivalent to 0.2 g paracetamol was dissolved in 10 ml ethanol, shaking to increase the solubility and filtered into 100 ml calibrated flask, then the solution was completed to the volume with the distilled water (the solution was equivalent to 2000 µg/ml paracetamol), then followed the above procedure of acid hydrolysis of paracetamol**.** A 16.7 ml of resulting solution was diluted to 100 ml in a volumetric flask using distilled water to obtain 100 µg/ml.

Suspension solution

A 10 ml of syrup (each 5ml contain 125 mg paracetamol) was transferred into a 250 ml calibrated flask and the total volume was diluted with distilled water and proceed the procedure as mentioned in hydrolyzed paracetamol solution from tablets.

Results and discussion

In the preliminary investigation work, it was found that 9-CA reagent reacted selectively with paracetamol after hydrolyzed paracetamol, in alcoholic medium of ethanol and produced a yellowish-green colored solution immediately with maximum absorption at 436 nm. The intensity of this color increased when the reaction mixture was heated and in contrast to the reagent blank which shows a maximum absorption at 390 nm (Fig. 1). However, the wavelength of maximum absorption 436 nm was used in all subsequent experiments.

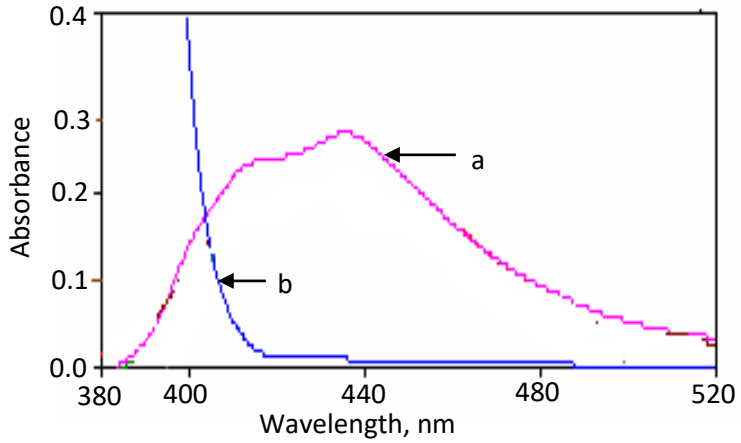


Figure 1. Absorption spectra of (a) hydrolyzed paracetamol (6.0 µg/ml) product with 9-CA (1×10^{-3} M) against reagent blank and (b) reagent blank against ethanol at optimum conditions.

The optimum conditions

The effect of various parameters on the absorption intensity of the colored 9-CA–hydrolyzed paracetamol product has been investigated and the reaction conditions have been optimized.

Effect of pH

The effect of pH on the color intensity at range between 2.51 and 11.35 pH value in the final volume (5) ml, by addition of 0.01 M of HCl and NaOH, was examined. It was found that the sensitivity of product was not affected by HCl addition but decreased in the presence of NaOH. However, the pH of the final dilution was measured in the absence of HCl and NaOH and found 9.42. Different buffer solutions (bicarbonate, borate and phosphate of pH 9.42) were also examined. These showed a negative effect on the absorbance of the product 9 (Fig. 2).

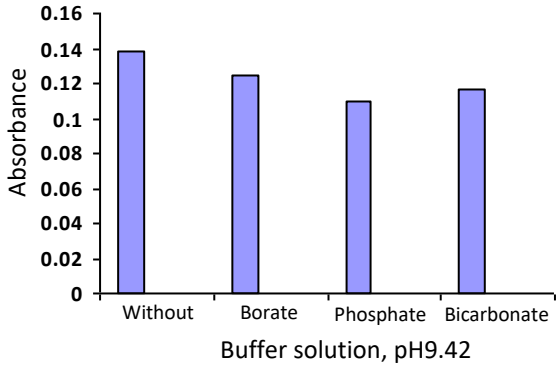


Figure 2: **Effect of buffer solutions on the absorbance of 10 µg/ml hydrolyzed paracetamol with 9-CA reagent**

Effect of reagent concentration

Different volumes of (1×10^{-3} M) 9-CA were added to a solution containing 2.5 µg/ml of paracetamol in final volume of 5 ml. The absorbance was measured at 436 nm after 10 min at room temperature against reagent blank. It was evident that the absorbance increases with increasing reagent concentration and reached maximum on using a volume of 2.0-3.0 ml of 9-CA (Fig.3) and 2 ml was selected in the subsequent experiments.

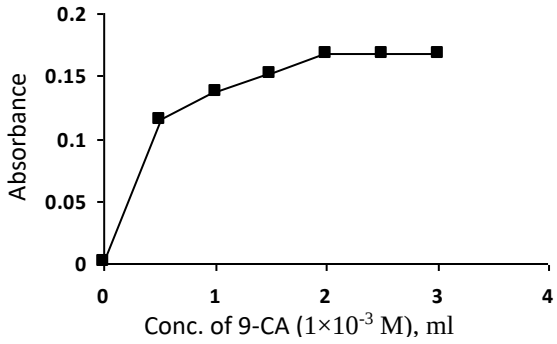


Figure 3: Effect of 9-CA reagent concentration

Effect of surfactants

Effect of various surfactants including SDS, CTAB, Tween-80 and Triton x-100, of 0.2 % concentration, on the absorption intensity of the paracetamol – 9-CA product has been investigated as shown in Fig. 4 there is a negative effect of these surfactants on the absorbance of paracetamol–9-CA product.

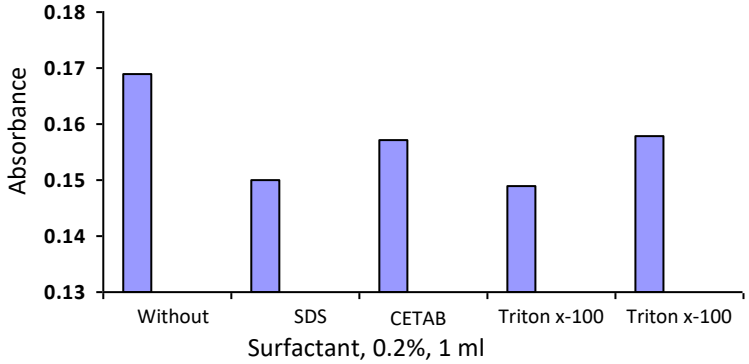


Figure 4: Effect of surfactant on the absorption of 2.5 µg/ml of paracetamol

Effect of temperature and time

The effect of temperature on the rate of reaction for paracetamol–9-CA product was studied at room temperature (22°C), 40°C and 50°C at the previous optimum reaction conditions. The results indicated that the product was formed after the addition of reagent immediately and reached its maximum absorbance at 40°C after 30 min and remain constant for 50 min after which the absorbance was decreased indicating dissociation (Fig. 5). Whereas, a decrease in absorbance with increased temperature was noticed indicating dissociation.

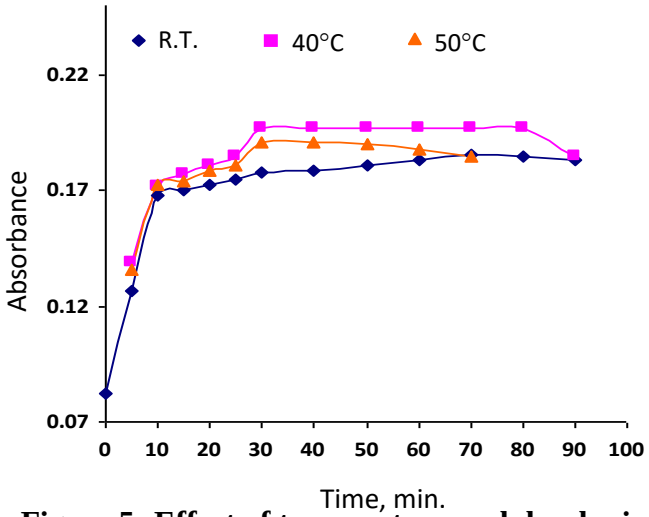


Figure 5: Effect of temperature and developing time on the absorption of 2.5 µg/ml paracetamol

Quantitation

The results for the determination of paracetamol by 9-CA reagent are summarized in Table 1. The Beer's law limits and molar absorptivity value were evaluated and indicated that the method is sensitive. The linearity was represented by the regression equation and the corresponding correlation coefficient for drug determined by the proposed method represents excellent linearity. The relative standard deviation (RSD) and accuracy (average recovery percentage (Rec%)) for the analysis of four replicates of each three different concentrations for paracetamol indicated that the method is precise and accurate. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the following equations:

$$\mathbf{LOD = 3.3\sigma/b \quad and \quad LOQ = 10\sigma/b}$$

where σ is the standard deviation of five reagent blank determinations and b is the slope of the calibration curve. The obtained results are in the accepted range below the lower limit of Beer's law range.

Table 1: Optical characteristics and statistical data for the proposed method

Parameter		
λ_{max} (nm)		**436**
Linearity range (µg/ml)		**0.25 – 11**
Molar absorptivity (l.mol^{-1}. cm^{-1})		**5.3×10^3**
LOD (µg/ml)		**0.668**
LOQ (µg/ml)		**2.026**
Average recovery (%)*		**99.27**
Correlation coefficient		**0.998**
Regression equation (Y)**	**Slope, a**	**0.018**
	Intercept, b	**0.069**
	RSD**	**≤ 2.82**

***Average of four determinations**

****Y = a X + b,* where *X* is the concentration of paracetamol in µg ml^{-1}.**

Study of Interferences

The extent of interference by some excipients which often accompany pharmaceutical preparations were studied by measuring the absorbance of solutions containing fixed amount of drug (5.5 µg/ml) and various amounts of excipients in a final volume of 5 ml. It was found that the studied excipients did not interfere seriously (Table 2). Slight positive interference was observed in the presence of large excess of excipients. However; an error of 5.0 % in the absorbance readings was considered tolerable. Typical results are given in Table (2). This was indicated that the method was free from interferences.

Table (2). Effect of excipients on the determination of 5.5 µg/ml paracetamol

Excipient	Recovery percent 5.5 µg/ml in the presence of excipient in µg/ml						
	40	100	140	180	220	260	300
NaCl	97.97	98.99	100.00	102.03	103.55	104.06	106.09
Lactose	98.48	99.49	100.00	101.52	104.55	105.56	-
Glucose	101.52	102.53	103.03	103.54	104.04	105.05	-
Acacia	98.98	101.02	102.54	104.57	106.59	-	-
Sucrose	97.98	98.99	100.51	102.02	103.55	104.04	106.06

Nature of the colored product and stability constant

Continuous variations introduced by Job's and molar ratio methods [30] have been employed to establish the stoichiometry of the colored product.

Job's method

A $1x10^{-3}$ M standard solutions of hydrolyzed paracetamol and 9-CA reagent were used. A series of solutions were prepared in which the total volume of paracetamol and reagent was kept at 2 ml. The reagents were mixed in various proportions diluted to volume in a 5-ml calibrated flask with absolute ethanol and the general procedure followed.

As shown in Figure (6,a), the result indicated that the stoichiometric composition of the product was 1:1 paracetamol : 9-CA. This indicated that aromatic amino group presented in the hydrolyzed paracetamol (p-aminophenol) was responsible for the formation of the product.

Mole ratio method

A $1x10^{-3}$ M 9-CA was added to the fixed volume (1ml) of $1x10^{-3}$ M hydrolyzed paracetamol, then the solution was diluted to the mark in 5-ml volumetric flask with absolute ethanol and the general procedure followed. The intersections of the obtained straight lines indicated the molar ratio of the product. As shown in Figure (6,b), the result also proved the formation of the 1:1stochiometry.

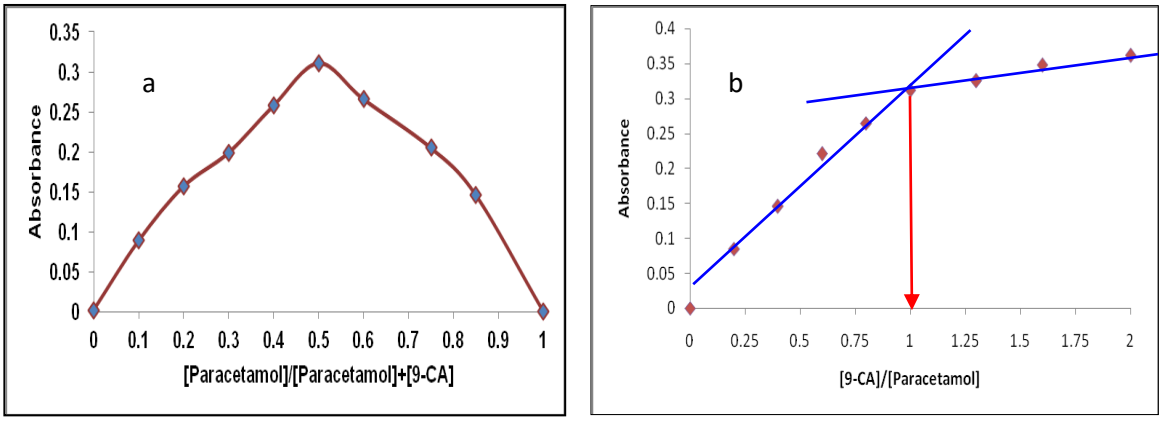


Figure (6) : Continuous variation (a) and mole ratio (b) plots for the hydrolyzed paracetamol– 9-CA

Stability Constant

The apparent stability constant was estimated by comparing the absorbance of a solution containing stoichiometric amounts of the paracetamol and 9-CA reagent (As) to one containing an excessive amount of 9-CA reagent (Am). The average conditional stability constant of the product was calculated, according to the 1:1 ratio, by the following equation :

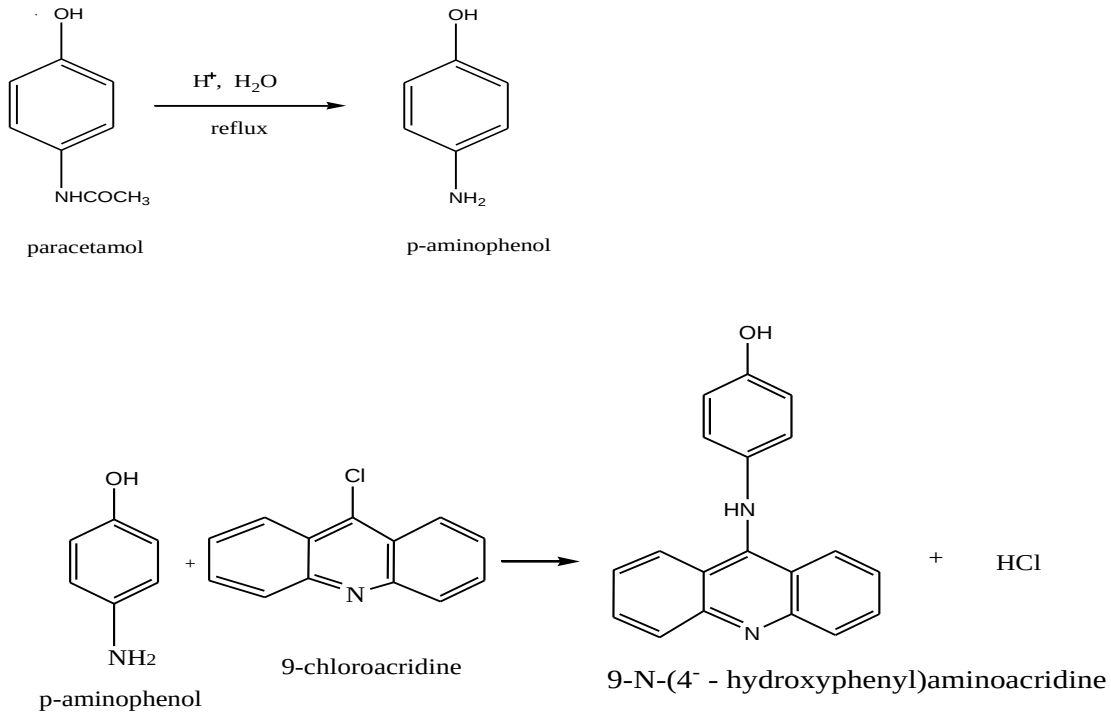
$$\mathbf{Kc=1-\alpha/\ \alpha^2\ C}$$

$$\mathbf{\alpha=Am-As/Am}$$

where Kc is the stability constant ($l.mol^{-1}$), α the dissociation degree and C the concentration of the product which is equal to the concentrations of paracetamol. However; the average of stability constant for three different concentration was found 6.34×10^5 $l.mol^{-1}$ for the paracetamol-9-CA product indicating high stability.

Reaction mechanism

The color produced from the reaction of hydrolyzed paracetamol with 9-CA suggested that a free amino function in the molecule is necessary for the reaction. This finding is in agreement with the reaction of primary aromatic amines and/or aromatic hydroxylamine's with the acridine [31-34] to form highly colored solutions. However, the reaction mechanism has been explained in Scheme (1).



Scheme 1: **Proposed reaction mechanism for assay of the paracetamol by 9-CA**

Analytical applications

The proposed method was successfully applied to determine paracetamol in pharmaceutical tablets and suspension preparations. The obtained results were compared statistically by a Student's *t*-test for accuracy and a variance ratio *F*-test for precision with the official method procedure[100] at the 95% confidence level with four degrees of freedom, As cited in Table (3), the results showed that the experimental *t*-test and *F*-test were less than the theoretical value (*t*= 3.182, *F*= 9.12), indicating that there was no significant difference between the proposed method and official method.

Table(3): Assay of paracetamol in pharmaceutical formulations

Procedure applied	Pharmaceutical preparation	Drug amount present (μg/ml)	Recovery [a] (%)	Average recovery (%)	Drug content detected (mg)	Certified value (mg)
Proposed 9-CA method	Paracetamol tablet [c]	2.5 5 8	99.37 103.00 96.13	99.5	497.50 (1.01, 2.13)[b]	500
	Paracetamol Oral suspension[d]	2.5 5 8	97.72 101.12 97.13	98.66	123.33 (2.47, 2.08)[b]	125
British Pharmacopoeia	Paracetamol tablet	7.5	101.00		505.00	500

[a] Average of four determinations.
[b] Figures in parenthesis are the calculated values for t and F respectively.
[c] Iraq- Samara[SDI] , [d] Turkey - Istanbul

Comparison of the Methods

Table (4) shows the comparison between some of analytical spectrophotometric methods using different reagents with the proposed method using 9-CA reagent. As seen in Table 3, the present method is more sensitive than the cited methods, accurate, have no interferences and carried on aqueous medium.

Conclusion

A simple, precise, selective and sensitive spectrophotometric method was developed for the determination of microgram amounts of paracetamol based on the reaction of hydrolyzed paracetamol with 9-CA reagent to form colored product having maximum absorption at 436 nm in aqueous medium. The proposed method was successfully applied for the assay of the pharmaceutical formulations as tablets and suspension of paracetamol.

Table (4): Comparison of the proposed method with other spectrophotometric methods

Analytical parameters	Reagent			
	Present method	Literature method		
	9-CA	sodium bismuthate, HCl[18]	3-chloro-7-hydroxy-4-methyl-2H-chromen-2-one[20]	Fe(III) - 2,4,6-tris(2-pyridyl)-S-triazine [22]
λ_{max}(nm)	436	550	545	593
pH	Alkaline	Acidic	Alkaline	Acidic
Temp.(°C)	40	RT	40	RT
Development time (min)	30	Immediately	10	15
Stability period (min)	50	-	-	15
Beer's law (µg/ml)	0.25-11	100-300	10.0–60.0	25-400
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	5.3×10^3	100.0	1.2×10^3	1.2×10^3
Recovery(%)	99.27	99.8	≤ 102.3	≤106.6
RSD(%)	≤ 2.82	1.70	≤ 1.50	-
Application	Tablet, Suspension	Tablet	Tablet	Serum, Plasma
Disadvantages	Need heating	Very poor sensitivity	Using of organic solvent	Suffered from interferences

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