

Application of H-Point Standard Addition Method in Simultaneous Kinetic Spectrophotometric Determination of Paracetamol and Salicylamide

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Abstract

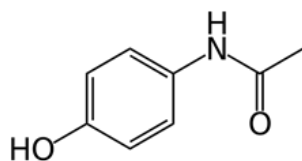
A simple spectrophotometric method for the simultaneous kinetic determination of Paracetamol (PAR) and Salicylamide (SAL) by applying the H-point standard addition method (HPSAM) has been developed. The method depends on the difference in the oxidation time of the two medicinal compounds by the ferric ion Fe(III) and the reaction of the resulting ferrous ion Fe(II) with 2,2'-bipyridyl reagent (BPY) reagent to form an orange complex that has a maximum absorption at 522 nm. PAR as analyte and SAL as interferent were determined in the mixture, Beer's law applies within concentrations 1-5 $\mu\text{g.mL}^{-1}$ for PAR and 10-90 $\mu\text{g.mL}^{-1}$ for SAL in their mixture, with weight ratios of 1:90 and 5:20 PAR: SAL, relative standard deviation range between 1.04 and 2.4 and a recovery % ranging from 98.2%-102.1%, at the pair time t_6-t_{16} , indicating that the method is precise and accurate. It should be corrected as: The method is free from interferences due to the excipients present in pharmaceutical formulation. The method is free from excipients present in pharmaceutical formulation. The proposed method was also successfully applied for the determination of PAR and SAL in pure forms and synthetic pharmaceutical preparation as Rinomicine.

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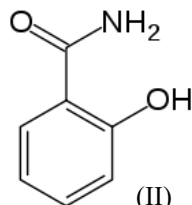
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Introduction

Paracetamol (PAR, N-acetyl-p-aminophenol or acetaminophen, I), is also known as acetaminophen. It was known in 1878 by Mors [1] and first used clinically by von Mering in 1887[2]. Since 1970, PAR became one of the most popular medications in the world and the most used to treat fever, and perhaps more common to treat pediatrics[3,4]. It is used to treat diseases and is also used during pregnancy and lactation. It is usually used to relieve mild to moderate pain and use for more severe diseases such as cancer and pain after surgery. It is used either in the form of orally or in the form of anal suppositories by rectum or in an intravenous injection[5], PAR replaces aspirin in patients who suffer from infectious diseases such as ulcers[6]. Salicylamide (SAL), also known as (o-hydroxybenzamide, II) is an over-the-counter medication with antipyretic and analgesic effects. Salicylamide usage is similar to aspirin [7] and is used in conjunction with aspirin, acetaminophen, and caffeine. It served as a phenacetin alternative in later Australian formulations of Vincent's powders.



(I)

C₈H₉NO₂, Mwt = 151.2 g/mol

(II)

C₇H₇NO₂, Mwt= 137.1 g/mol

For determining PAR and SAL in their binary combination, various methods have been described. These include spectrofluorimetric[8] electrochemical [9] and high performance liquid chromatography HPLC [10] and flow injection[11] methods.

For the determinations of PAR and SAL in their mixture, two procedures were described. The methods relied on using the partial least squares (PLS) and the H-point standard addition method (HPSAM)[12]. To determine the amounts of PAR and SAL in their mixture, a kinetic spectrophotometric approach utilizing HPSAM was also described. The method was based on the oxidation of PAR and SAL by Fe³⁺ ion followed by the complexation of the produced Fe(II) ion with 1,10-phenanthroline and measuring the absorbance of the formed complexes at 510 nm [13]

Several methods have been reported for simultaneous determination of PAR or SAL in combined mixtures with other drugs, such as PAR, Phenylephrine hydrochloride and Chlorpheniramine maleate[14], PAR and Chlorzoxazone[15] using HPSAM, paracetamol, and Caffeine using HPSAM[16] and derivative spectrometry[17], PAR, Tramadol Hydrochloride and Domperidone using RP-HPLC[18], PAR and Meloxicam using simultaneous equation[19] and PAR and Metoclopramide using a simultaneous equation, absorbance ratio and correction methods[20]. SAL, Acetylsalicylic acid, and Salicylic Acid were determined in a combined mixture using a multi-wavelength spectrophotometric method[21]. SAL, Aspirin, and Caffeine using Target factor analysis by HPLC[22].

Principle of HPSAM

In 1991 the HPSAM was developed by Falco and his group for application in kinetic reactions in the estimation of drug compounds without errors in their binary or ternary mixtures. For this purpose, two variables were used to process the kinetic data[23]. One of them is applied to the rate constant of the two components that depends on time, and the other variable depends on the difference in the reaction speed of the two components so that one of the two components reacts faster than the other or the other component does not react at the same time at all. In the first case, the interaction of the components X and Y with the reagent in the mixture evolves with time, and C_x (the concentration of the analyte) and A_H (the absorption of the interfering substance) can be calculated by plotting the relationship between the difference in absorption at t₁ and t₂ against the concentration of X at wavelength, which both have the same Y absorption (A_y).

As for the second case of the method, it assumes that analyte X is the only one whose interaction evolves with time, while the interfering substance Y is not affected with time. In this case, two times t₁ and t₂ are chosen during which the interfering Y is not affected and its absorption must be constant during the two times chosen[24]. The absorbances are measured at t₁ and t₂ and expressed by the following equations:

$$A_{t1} = b_0 + b + M_{t1}C_i \dots\dots\dots(1)$$

$$A_{t2} = A_0 + A' + M_{t2}C_i \dots\dots\dots(2)$$

Where A_{t1} and A_{t2} represent the mixture's absorbance at times t₁ and t₂, respectively. The absorbance of X at A_{t1} and A_{t2}, respectively, is represented by b₀ and A₀ (b₀ ≠ A₀). The absorbance of Y at A_{t1} and A_{t2}, respectively, is represented by b and A'. C_i is the added X concentration, and M_{t1} and M_{t2} are the slopes of the standard addition calibration curves at t₁ and t₂, respectively. The so-called H-point is the intersection of the two obtained straight lines (-C_H, A_H). The reasoning above indicates that C_H is independent of interferent concentration at the H-point, and thus A_H is independent of analyte concentration[25].

Therefore, the goal of this work is to develop a spectrophotometric method that is rapid, accurate, precise, and simple for the determination of PAR and SAL in their binary mixture. The method depends on the different oxidation periods of the drug compounds with Fe(III) ion, followed by complexation of resulting Fe(II) with 2,2'-bipyridyl (BPY). Then absorbance is measured at 522 nm using HPSAM.

2. Experimental part

2.1 Devices

Jenway 6800 UV-visible double beam spectrophotometer equipped with 1 cm silica cells was used to measure absorbance. Solutions are heated in a water bath using frost instrumentation. The samples were weighed using an electronic balance made by KERN & SOHN GmbH company. firm KERN & Sohn GmbH Using an electronic balance, the samples were weighed.

Materials and Reagents

The purity of all the chemicals and reagents utilized was extremely high.

Preparation of standard solutions

Paracetamol and Salicylamide ($100\mu\text{g mL}^{-1}$)

The standard solutions of $100\mu\text{g mL}^{-1}$ of each PAR and SAL were prepared by dissolving 0.01g of pure substance, PAR in double distilled water, and SAL in 5 mL of ethanol then the volume was completed to 100 mL, separately with the distilled water in a volumetric flask.

Bipyridyl solution (0.01 M)

This solution was prepared by dissolving 0.1562 g of 2, 2'-bipyridyl in 5 mL of ethanol, and then adding distilled water to the solution to dilute it to 100 mL in a volumetric flask. The solution was freshly made.

Ferric chloride hexahydrate solution (0.008 M)

This solution was prepared by dissolving 0.2162 g of ferric chloride hexahydrate in 100 mL of distilled water. Just prior to the experiment, the solution was freshly prepared and stored in an amber reagent container.

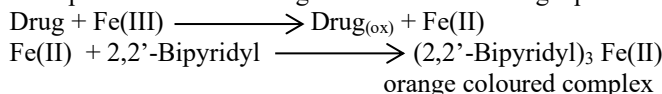
3. The recommended procedure for the determination of PAR and SAL in a synthetic mixture

Amounts ranging between 20 and $90\mu\text{g mL}^{-1}$ of SAL (Interferent) were placed into 10 mL volumetric flasks and then amounts varying between 1 and $5\mu\text{g mL}^{-1}$ PAR was added (Analyte). Then, increasing amounts of the analyte ($0-5\mu\text{g mL}^{-1}$), 1 mL of $8\times 10^{-3}\text{ M FeCl}_3$, and 1.5 mL of $1\times 10^{-2}\text{ M BPY}$ were added. The volume was diluted to the mark with distilled water and the absorbance at a wavelength of 522 nm was measured after 6 and 16 minutes. By calculating the value of C_H by resolving the two linear equations, the concentration of PAR was determined, while the concentration of SAL was found through the values of A_H and by solving the linear equation of SAL.

Results And Discussion

Preliminary study

Ferric chloride is known as an oxidizing agent that is used in the oxidation of some medicinal compounds, which in turn is reduced to ferrous ion Fe(II), and reacts with the 2,2'-bipyridyl (BPY) to form an orange-colored complex, which has an absorption in the visible region as in the following equations [26].



The analysis of drugs by utilizing BPY is in two steps. At first, the solution of the drug is allowed to react with ferric chloride. During this process, the drug gets oxidized and results in the generation of ferrous iron. In the second step, the ferrous complexes with BPY form an orange-colored complex having maximum absorbance at 522 nm.

Among the results shown by this study is that the oxidation of PAR with the FeCl_3 reagent is very fast and with high absorbance, while the oxidation of SAL is slow and has a low absorbance. However, depending on the difference in the oxidation time for the above drugs, the H-point standard addition method was used to simultaneously determine PAR and SAL in their mixture, and the optimum conditions for this were investigated.

Optimization of conditions

The impact of several factors on the intensity of the absorption of Fe (II) – BPY complex, in the presence of $5\mu\text{g mL}^{-1}$ PAR and $30\mu\text{g mL}^{-1}$ SAL in a mixture, were studied to reach the best conditions for the determination of drug compounds in their mixture.

Effect of the FeCl_3 concentration and volume

Solutions of FeCl_3 at different concentrations (5×10^{-4} - 5×10^{-2} M) were prepared and their effect on the intensity of absorbance of the colored complex formed was studied by adding a fixed volume (0.5 mL) of each prepared concentration to volumetric flasks containing fixed amounts of PAR ($5.0 \mu\text{g.mL}^{-1}$) and SAL ($30 \mu\text{g.mL}^{-1}$) in the mixture, followed by addition 1.5 mL of BPY (0.01 M). The volume was diluted to 10ml with distilled water in volumetric flasks, and the absorbance was measured at 522 nm after 10 min against the reagent blank. The results of this study, as shown in Figure (1) indicate that the concentration of 8×10^{-3} M of the oxidizing agent is the most appropriate in the estimation of PAR and SAL and was used in subsequent experiments. However; The results which are cited in Figure (2) indicated that 1.0 mL of FeCl_3 is the best volume and considered in the following experiments.

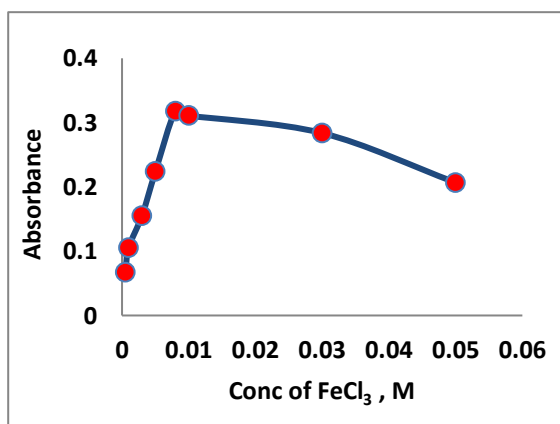


Figure 1: Effect of FeCl_3 conc. on the absorbance of Fe(II)-BPY complex in the presence of PAR and SAL mixture

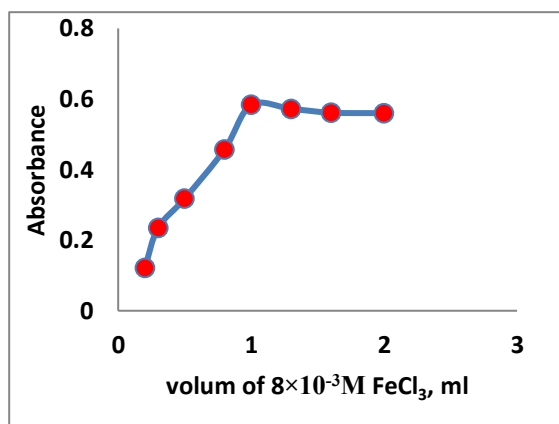


Figure 2: Effect of 8×10^{-3} M FeCl_3 volume

Effect of the BPY Concentration and Volume

Different concentrations of 1.5 mL BPY reagent in the range (5×10^{-5} - 5×10^{-2} M) were examined to obtain high sensitivity for the estimating of PAR and SAL in their admixture, in the presence of optimum amount of FeCl_3 . Figure (3) indicated that 1×10^{-2} M of BPY gave high absorbance, and the results in Figure (4) indicated that 1.5 mL of 1×10^{-2} M BPY is optimal which gave high absorbance and depended on subsequent experiments.

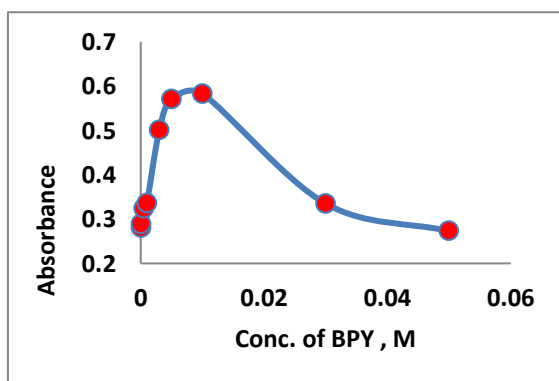


Figure (3) effect of BPY reagent conc.

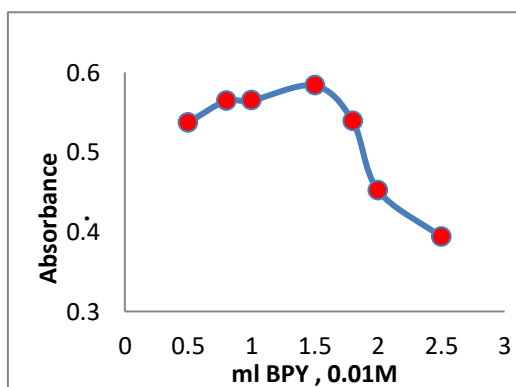


Figure (4) Effect of 1×10^{-2} M BPY volume

Effect of Time on the Absorbance of the Complex in the Presence of PAR and Individually

The effect of time was studied on the formation of the complex. The selection of the optimal time for oxidation of drugs separately in the presence of optimum amounts of FeCl_3 and BPY reagents. Then, against a reagent blank, absorbance was measured at 522 nm after each period of time at room temperature. It was observed that the absorbance of the complex was increased with increasing time. However, 30 min for PAR and 100 min for SAL were selected for absorption spectra and calibration curves.

The final absorption spectra

Final absorption spectra of the Fe(II)-BPY complex in the presence of PAR and SAL, separately, were plotted under the optimum conditions (Table 1) in the range 400-600 nm against the blank solution after 30 min for PAR and 100 min for SAL. Figure (5) shows that the maximum absorption is 522 nm for PAR and SAL respectively and considered in this procedure.

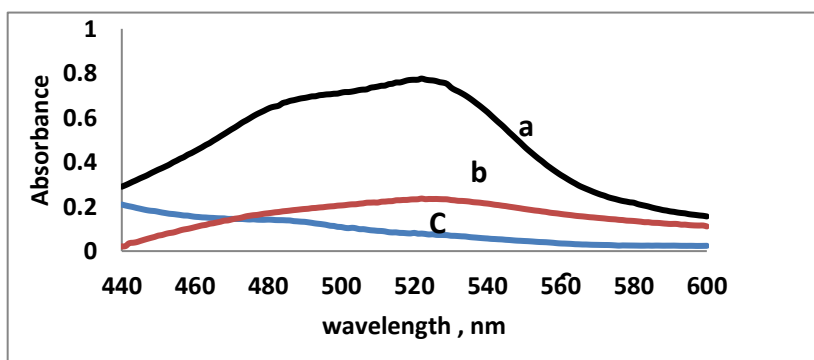


Figure (5) Absorbance spectra of Fe(II)-BPY complex in the presence of (a) $5 \mu\text{g mL}^{-1}$ PAR, (b) $30 \mu\text{g mL}^{-1}$ SAL, and (c) blank against water

Calibration plots of PAR and SAL

Under the optimum conditions described in Table (1), increasing volumes (ml) of $100 \mu\text{g mL}^{-1}$ of PAR and SAL were added separately into two sets of 10 mL volumetric flasks, followed by the addition of the optimum amounts of FeCl_3 and BPY reagent solutions, and the volume diluted to the mark with distilled water. After 30 and 100 minutes, respectively, for PAR and SAL, the absorbance of the complex, at 522 nm, was measured against a reagent blank. Standard curves were obtained by plotting the absorbance versus the concentration. As shown in Figure 6 (a & b), Beer's law was followed in the range ($1-8 \mu\text{g mL}^{-1}$) for PAR and ($5-100 \mu\text{g mL}^{-1}$) for SAL, and there is a negative deviation after the upper limits of determination. The correlation coefficient was more than 0.99 for both drugs, Table (1) indicates that they have excellent linear characteristics.

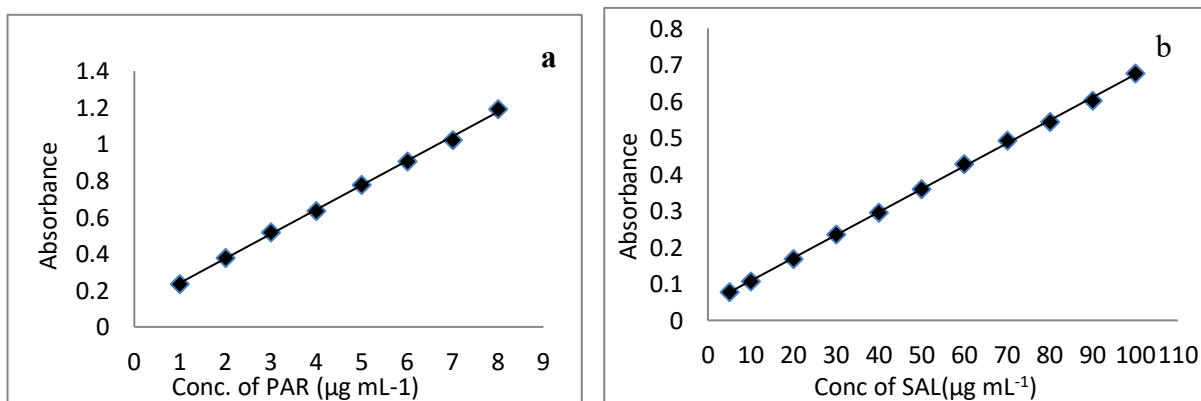


Figure (6): Calibration plots of PAR (a) and SAL (b)

Table (1): Linearity, slope, intercept, and correlation coefficient of the standard curves for each of PAR and SAL

Drug compound	Linearity ($\mu\text{g mL}^{-1}$)	Slope	Intercept	Correlation coefficient
PAR	1-8	0.1337	0.1064	0.9988
SAL	5-100	0.0063	0.0454	0.9995

Accuracy and reliability

The accuracy (recovery %) and relative standard deviation (RSD) of the proposed method were calculated for three different concentrations of PAR (2,4,6 $\mu\text{g.mL}^{-1}$) and SAL (20, 50, 80 $\mu\text{g.mL}^{-1}$) for four replicates. The results included in Table (2) indicated the method is accurate and precise, as the average recovery % values were 99.33% and 97.63%, while the average RSD values were found to be 2.53 and 2.59 for each of PAR and SAL, respectively.

Table (2): Accuracy and precision of the method

Drug	Amount added ($\mu\text{g.mL}^{-1}$)	Amount found ($\mu\text{g.mL}^{-1}$)	Recovery * (%)	Average recovery (%)	RSD * (%)	Average RSD
PAR	2	1.96	98.13	99.33	3.2	2.53
	4	4.05	101.36		2.22	
	6	5.91	98.5		2.16	
SAL	20	19.71	98.57	97.63	2.25	2.59
	50	48.52	97.05		2.82	
	80	77.82	97.28		2.71	

*Average of four determinations.

H-point standard addition method (HPSAM) in simultaneous kinetic spectrophotometric determination of PAR and SAL in admixture

Previous experiments in the studying of the optimum conditions of PAR and SAL in their mixture were studied and applied to estimate the above two drugs individually. However, it was found that both compounds oxidized by the FeCl_3 reagent and the produced Fe(II) interacted with BPY to produce an orange complex that had a maximum absorption at 522 nm. It was observed that there is a difference in the oxidation time of the two compounds, where it was found that PAR is oxidized directly, while SAL needs time to start oxidation, so through this difference in kinetic behavior, it was resorted to using the method for simultaneous kinetic assay of PAR and SAL by HPSAM. This is based on the optimum conditions that have been reached for the two compounds in this study, Table (1).

Selecting the appropriate time in the application of the HPSAM

To choose the appropriate time for the application of the HPSAM, the following conditions must be followed:

- 1- When choosing the times, the analyte should be absorbed linearly with concentration, while the absorption of the interferent substance should be constant even in increasing the concentration of the analyte.
- 2- The mixture's absorption must equal the sum of the absorptions of each analyte and the interfering material individually.
- 3- To achieve high accuracy, the slope difference measured at times t_1 and t_2 for both straight lines should be as large as possible.

In order to prove the above conditions, the following work steps have been taken:

30 and 5 $\mu\text{g.mL}^{-1}$ of SAL and PAR were added separately in a 10 ml volumetric flask, followed by the addition of optimal amounts of FeCl_3 as an oxidizing agent, and BPY as a complexing agent, Table (1), then the solution was diluted to the mark with distilled water. Then absorbance was measured at 522 nm per 1 min for a period of 20 min.

As for the mixture, 30 and 5 $\mu\text{g.mL}^{-1}$ of SAL and PAR were added in a 10 ml volumetric flask, followed by the addition of optimum amounts of oxidizing agent and BPY reagent, Table (1), then complete the volume with distilled water to the mark. The absorbance was measured at 522 nm per 1 min for a period of 20 min. Figure (7) shows the obtained results.

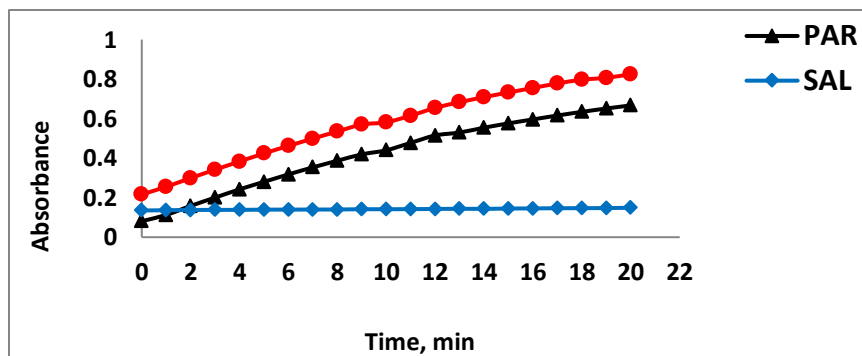


Figure (7) Absorbance vs. time of 5nm PAR, 30 nm SAL, and their

It was found through Figure (7) that the absorption of the interfering substance SAL is constant during the period from 0-20 min, while the absorption of the PAR analyte substance is continuously increasing during that period. In addition, it was found that the absorption of the mixture was equal to the total absorptions of each PAR and SAL individually at the same time, which is one of the conditions that must be provided in the application of the HPSAM.

Appropriate times (t_1 , t_2) selection in applying HPSAM

In the suggested system PAR was regarded as an analyte and SAL as an interferent. Therefore, Due to the disparity in the rates at which the two medications under study reacted with BPY, HPSAM was used to conduct a simultaneous kinetic analysis of both pharmaceuticals. By choosing the conditions where the slope values of the two straight lines differ as much as possible, the proper pair of times (t_1 and t_2) can be determined. Therefore the following procedure was used:

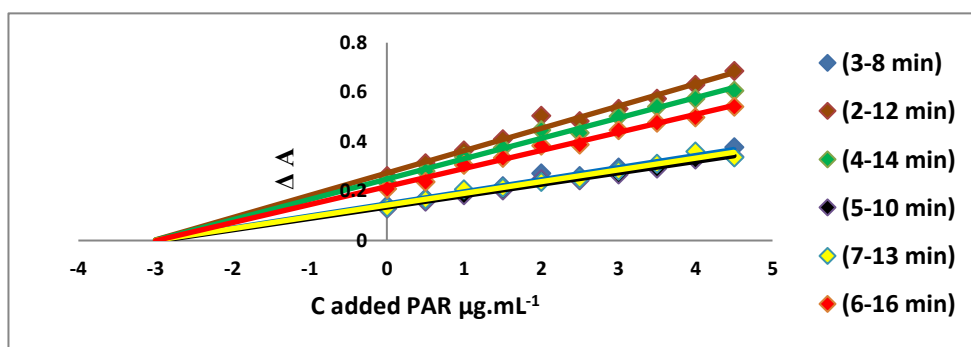
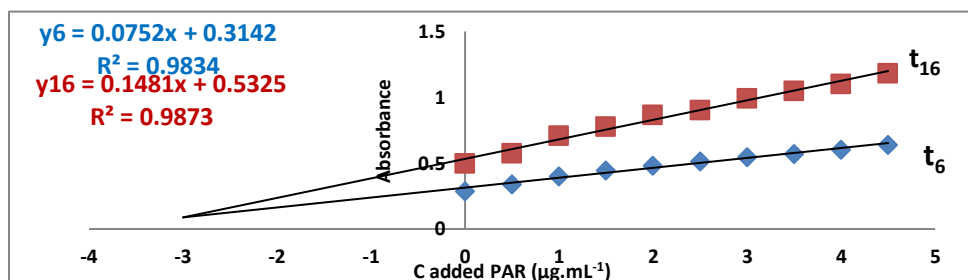
30 $\mu\text{g.mL}^{-1}$ of SAL and 3 $\mu\text{g.mL}^{-1}$ of PAR were added in a 10 ml volumetric flask followed by the addition of increasing concentrations of PAR as the analyte (0–4.5 $\mu\text{g.mL}^{-1}$). Then optimum quantities of the oxidizing agent FeCl_3 and BPY reagent were added, Table (1), and the volume was diluted with distilled water to the mark. The absorbance of solutions was measured against the reagent blank at different time pairs (t_1 – t_2) of 7–13, 6–16, 3–8, 4–14, 5–10, and 2–12 min. Then the corresponding H-point plots were plotted. As shown in Table (3), all the selected times gave good recovery % for PAR, and to choose the exact time, the change in absorbance was studied by drawing standard curves between the change in absorption (ΔA) versus the concentration (C) ($\Delta A_{t_1-t_2}$ versus C) and for each pair of times mentioned in Table (4). It was found that the best standard curve for the pair (t_1 – t_2) is 6-16 min (Table 4 and Figure 8), which gave the biggest slope increment, best recovery, and the shortest time for analysis had been recommended in this study. However, Figure 9 shows the plot of HPSAM at the time (6-16 min).

Table (3): selection of appropriate time

Time min.	A - C Equation	R^2	Amount taken ($\mu\text{g.mL}^{-1}$)		Amount found ($\mu\text{g.mL}^{-1}$)	Recovery (%)	Absorbance (A_H)
			PAR	SAL	PAR		SAL
7-13	$t_{7\text{min}} = 0.0867x + 0.3366$ $t_{13\text{min}} = 0.1341x + 0.4797$	0.9752 0.9867	3.0	30	3.018	100.63	0.0749
6-16	$t_{6\text{min}} = 0.0752x + 0.3142$ $t_{16\text{min}} = 0.1481x + 0.5325$	0.9834 0.9873	3.0	30	2.99	99.82	0.0890
3-8	$t_{3\text{min}} = 0.0464x + 0.2211$ $t_{8\text{min}} = 0.0953x + 0.3692$	0.9437 0.982	3.0	30	3.02	100.95	0.0806
4-14	$t_{4\text{min}} = 0.0544x + 0.2545$ $t_{14\text{min}} = 0.1367x + 0.5029$	0.9529 0.9816	3.0	30	3.01	100.61	0.0903
5-10	$t_{5\text{min}} = 0.0649x + 0.2862$ $t_{10\text{min}} = 0.1109x + 0.4217$	0.9589 0.9813	3.0	30	2.94	98.19	0.0950
2-12	$t_{2\text{min}} = 0.0357x + 0.188$ $t_{12\text{min}} = 0.1258x + 0.4611$	0.8319 0.9776	3.0	30	3.03	101.04	0.0798

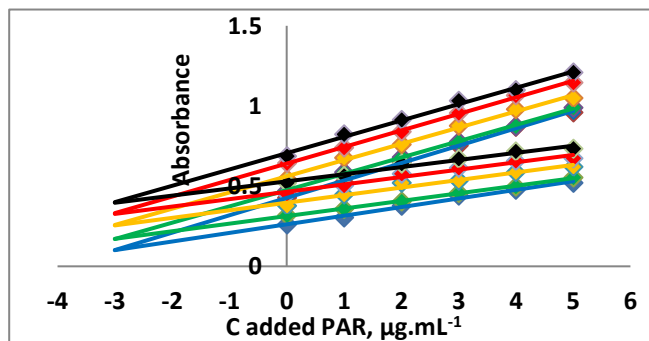
Table (4) Linear equations for standard curves

Time (min)	A-C Equation ΔA	R ²
3-8	$Y=0.0488x+0.1481$	0.9782
5-10	$Y=0.0461x+0.1355$	0.9856
4-14	$Y=0.0823x+0.2484$	0.9858
7-13	$Y=0.0475x+0.1431$	0.9656
6-16	$Y=0.0729x+0.2183$	0.9866
2-12	$Y=0.0901x+0.2731$	0.9805

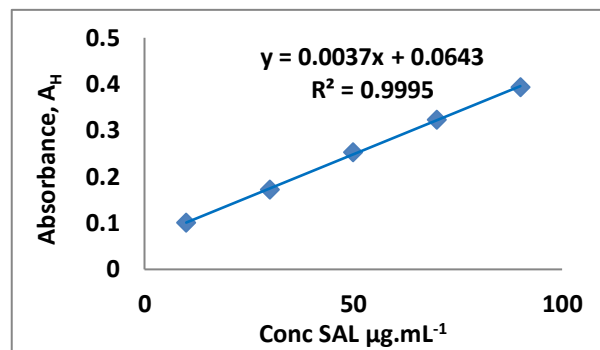
Figure(8): ΔA vs. the conc. of PAR added at different pairs of time at 522 nm for a mixture containing $3 \mu\text{g.mL}^{-1}$ each of PAR and $30 \mu\text{g/ml}$ SAL.Figure (9). Plot of HPSAM for the determination of $3.0 \mu\text{g mL}^{-1}$ PAR and $30.0 \mu\text{g mL}^{-1}$ SAL simultaneously.

Calibration curve of interfering substance SAL (A_H)

To determine the limit of the interfering substance SAL (A_H), increasing amounts of SAL ($10\text{--}90 \mu\text{g mL}^{-1}$) were added in 10 mL volumetric flasks, after that a fixed amount of PAR ($3.0 \mu\text{g mL}^{-1}$) was added, then increasing amounts of PAR ($0\text{--}5 \mu\text{g mL}^{-1}$) was added followed by the addition of optimum amounts of reagents FeCl_3 and BPY, and the volume was diluted to the mark with distilled water. Then absorbance was measured at the time pair ($t_6\text{--}t_{16}$) at the wavelength of 522 nm at the laboratory temperature. Figure (10) shows the relationship between A_H and the concentration of SAL, it was found that Beer's law followed the quantities from $10\text{--}90 \mu\text{g mL}^{-1}$ and that there was a negative deviation after the upper limit, Figure (11). At the same time, the accuracy (Recovery %) of the method was found and cited in Table (5).



Figure(10): HPSAM graph showing the value of A_H for interferent in the presence of PAR



Figure(11): Calibration plot of SAL

Table (5): Linear equations at t_{6min} , t_{16min} and recovery% of PAR(C_H) and SAL(A_H) in the mixture

A - C Equation	R^2	Amount taken ($\mu\text{g.mL}^{-1}$)		Amount found ($\mu\text{g.mL}^{-1}$)		Recovery %	
		PAR	SAL	PAR	SAL	SAL	PAR
$t_6 = 0.0537x + 0.262$ $t_{16} = 0.1085x + 0.4266$	0.9897 0.9987	3	10	3.00	9.84	100.12	98.4
$t_6 = 0.0473x + 0.3133$ $t_{16} = 0.1016x + 0.4751$	0.9978 0.9986	3	30	2.98	29.21	99.32	97.4
$t_6 = 0.0472x + 0.3966$ $t_{16} = 0.1011x + 0.5614$	0.9680 0.9933	3	50	3.06	50.81	101.92	101.6
$t_6 = 0.046x + 0.4647$ $t_{16} = 0.1031x + 0.641$	0.9658 0.9979	3	70	3.09	69.83	102.92	99.77
$t_6 = 0.0444x + 0.5298$ $t_{16} = 0.1018x + 0.7057$	0.9703 0.9929	3	90	3.06	89.02	102.15	98.9

Calibration curve of the PAR (C_H)

To know the linearity range of the analyte (PAR, C_H), the same previous procedure was applied. A fixed amount of the interfering substance SAL ($30 \mu\text{g.mL}^{-1}$) was added in 10 ml volumetric flask. Followed by the addition of increasing amounts of the PAR ($1-5 \mu\text{g.mL}^{-1}$), and increasing amounts ($0-5 \mu\text{g.mL}^{-1}$) of analyte was added, then the addition of the optimum quantities of reagents FeCl_3 and BPY, and the volume was diluted to the mark with distilled water. The absorption was measured at the two times (t_6 - t_{16}) at 522 nm at the laboratory temperature. It is observed from Figure (12) the HPSAM graph that includes the C_H values of the PAR analyte and the A_H value of the interfering substance SAL, and from Figure (13) shows the calibration curve of PAR indicate that Beer's law applies within concentrations ($1-5 \mu\text{g mL}^{-1}$) and that there was a negative deviation after the upper limit. From the results obtained in Table (6), It is evident that the method has high accuracy. and is suitable for choosing the time (t_6 - t_{16}) in determining the concentrations of above medicinal compounds in their mixture.

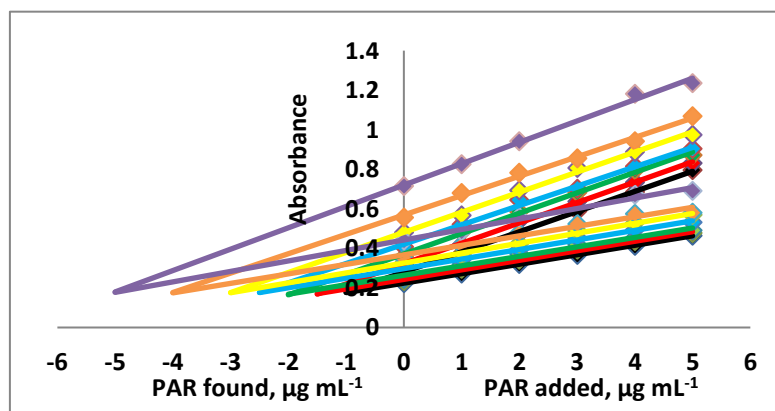


Figure (12): HPSAM graph showing the value of C_H for analyte in the presence of SAL

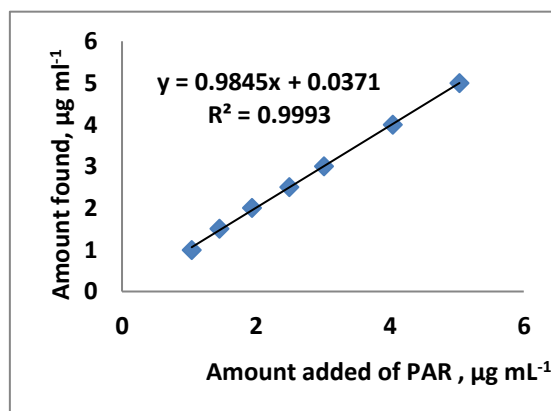


Figure (13): Calibration plot for PAR

Table (6): Linear equations at $t_{6 \text{ min}}$, $t_{16 \text{ min}}$ and recovery% of PAR($-C_H$) and SAL(A_H) in the mixture

A - C Equation	R^2	Amount taken ($\mu\text{g.mL}^{-1}$)		Amount found ($\mu\text{g.mL}^{-1}$)		Recovery %	
		PAR	SAL	PAR	SAL	PAR	SAL
$t_6=0.0479x+0.2242$ $t_{16}=0.1019x+0.2804$	0.9998 0.9978	1	30	1.04	29.74	104.1	99.1
$t_6=0.0482x+0.2432$ $t_{16}=0.103x+0.3229$	0.9951 0.9985	1.5	30	1.45	29.40	96.95	98
$t_6=0.0478x+0.264$ $t_{16}=0.1032x+0.3714$	0.9777 0.9962	2	30	1.93	28.92	96.9	96.4
$t_6=0.0484x+0.2976$ $t_{16}=0.0978x+0.4212$	0.9952 0.9931	2.5	30	2.50	30.32	100.1	101.1
$t_6=0.0501x+0.326$ $t_{16}=0.1015x+0.4811$	0.9937 0.9944	3	30	3.01	29.87	100.6	99.6
$t_6=0.0481x+0.3673$ $t_{16}=0.098x+0.569$	0.9595 0.9934	4	30	4.04	29.34	101.1	97.8
$t_6=0.053x+0.4425$ $t_{16}=0.1081x+0.7202$	0.9826 0.9918	5	30	5.03	30.02	100.8	100.1

Recommended procedure for determination of PAR and SAL in synthetic mixture

Amounts ranging between 20 and 90 $\mu\text{g.mL}^{-1}$ of SAL (Interferent) were added into 10 ml volumetric flasks, followed by the addition of amounts ranging from 1 and 5 $\mu\text{g.mL}^{-1}$ PAR (Analyte). Then, increasing amounts of the PAR analyte (0-5 $\mu\text{g.mL}^{-1}$) followed by the addition of the optimum amounts of the oxidizing agent and the BPY reagent. The mixture was diluted to the mark with distilled water, then absorbance was measured after t_6 and t_{16} at 522 nm. The amount of PAR was determined by finding the value of C_H by solving the two linear equations, while the concentration of SAL was found through the values of A_H and by solving the linear equation of SAL. The results included in Table(7) indicated the method is accurate.

Table (7): Results of analysis of PAR ($-C_H$) and SAL (A_H) in their mixtures containing different proportions by the HPSAM method

A-C Equation	R^2	The amount added ($\mu\text{g.mL}^{-1}$)		PAR		SAL	
		PAR	SAL	Amount found ($\mu\text{g.mL}^{-1}$)	Recovery %	Amount found ($\mu\text{g.mL}^{-1}$)	Recovery %
$t_6=0.0502x+0.3746$ $t_{16}=0.1074x+0.5191$	0.9963 0.9937	2.5	50	2.52	101	49.59	99.2
$t_6=0.0478x+0.4538$ $t_{16}=0.1043x+0.5691$	0.9833 0.9945	2	80	2.04	102	78.90	98.6
$t_6=0.0449x+0.3551$ $t_{16}=0.0947x+0.4308$	0.9909 0.9961	1.5	60	1.52	101.3	60.14	100.2
$t_6=0.0494x+0.235$ $t_{16}=0.1031x+0.341$	0.9895 0.9987	2	20	1.97	98.7	19.78	98.9
$t_6=0.042x+0.4553$ $t_{16}=0.0926x+0.6079$	0.9830 0.9866	3	70	3.01	100.5	71.44	102.1
$t_6=0.0418x+0.2186$ $t_{16}=0.0956x+0.2722$	0.9906 0.9955	1	30	0.99	99.6	30.44	101.5
$t_6=0.0501x+0.6479$ $t_{16}=0.0898x+0.8513$	0.9697 0.9862	5	90	5.12	102.5	88.35	98.2

Accuracy and precision

To study the accuracy and precision of this method, four replicates of three different mixtures of PAR and SAL were taken. The results included in Table (8), showed that the method has good accuracy and precision through the values of recovery % and the RSD of the binary mixtures.

interference effect of excipients

The impact of typical excipients present in PAR and SAL formulations as potential interferences on the absorbance of synthetic sample solutions containing mixtures of 3 and 30 $\mu\text{g.mL}^{-1}$ of PAR and SAL, respectively, was investigated in order to evaluate the potential analytical application of the proposed method. The recovery % results shown in Table (9) showed that none of the tested excipients caused any interference.

Table (8) : Accuracy and precision of the HPSAM method

A-C Equation	R^2	The amount taken $\mu\text{g mL}^{-1}$		The amount found $\mu\text{g mL}^{-1}$		Recovery%		RSD%	
		PAR	SAL	PAR	SAL	PAR	SAL	PAR	SAL
$t_6 = 0.0502x+0.3746$ $t_{16}= 0.1074x+0.5191$	0.9963 0.9937	2.5	50	2.52	49.59	101.05	99.18	1.38	1.68
$t_6= 0.0449x+0.3621$ $t_{16}= 0.0943x+0.485$	0.9652 0.9888	2.5	50	2.48	50.29	99.51	100.59		
$t_6= 0.0445x+0.3684$ $t_{16}= 0.0948x+0.4968$	0.9359 0.9940	2.5	50	2.55	51.48	102.11	102.98		
$t_6= 0.0511x+0.3801$ $t_{16}= 0.1005x+0.5025$	0.9957 0.9927	2.5	50	2.47	51.13	99.11	102.26		
$t_6=0.0494x+0.235$ $t_{16}=0.1031x+0.341$	0.9895 0.9987	2	20	1.97	19.78	98.7	98.9	2.05	2.40
$t_6=0.0522x+0.2442$ $t_{16}= 0.104x+0.3512$	0.9800 0.9982	2	20	2.06	19.47	103.28	97.4		
$t_6=0.0495x+0.2398$ $t_{16}= 0.0998x+0.343$	0.9654 0.9948	2	20	2.01	20.61	100.45	103.07		
$t_6=0.0472x+0.2354$ $t_{16}=0.1024x+0.3463$	0.9836 0.9963	2	20	2.05	19.98	102.58	99.92		

$t_6=0.0449x+0.3551$ $t_{16}=0.0947x+0.4308$	0.9909 0.9961	1.5	60	1.52	60.14	101.34	100.25	1.04	1.94
$t_6=0.0411x+0.3477$ $t_{16}=0.0964x+0.4323$	0.9919 0.9942	1.5	60	1.53	59.6	101.99	99.34		
$t_6=0.0447x+0.3473$ $t_{16}=0.1011x+0.4349$	0.9980 0.9967	1.5	60	1.55	57.72	103.55	96.2		
$t_6=0.0415x+0.3426$ $t_{16}=0.1013x+0.4334$	0.9963 0.9967	1.5	60	1.52	58.18	101.23	96.98		

Table (9): Recovery % of 3.0 $\mu\text{g.mL}^{-1}$ PAR and 30.0 $\mu\text{g.mL}^{-1}$ SAL in a solution mixture containing different excipients.

Additive	Conc. of additive ($\mu\text{g mL}^{-1}$)	Recovery%	
		PAR	SAL
Lactose	25	103.4	98.9
	50	101	98.2
	100	100.7	102.9
Sucrose	25	102.9	100.3
	50	99.5	103.7
	100	101.8	102.6
Phenylephrine. HCl	25	100.4	102.3
	50	102.5	101.1
	100	101.8	101.0
Caffeine	25	103.4	97.5
	50	99.1	97.5
	100	103.2	97.3

Determination of PAR and SAL in the pharmaceutical formulation as Rinomicine tablet

Due to the unavailability of the pharmaceutical formulation (Rinomicine tablet) in the local markets, it was prepared based on the proportions of its components and the excipients included in its composition, which include PAR 50mg, Chlorpheniramine maleate 4.0mg, SAL 50mg; Lactose 10mg, Saccharose 225mg as follows: 0.05 gm of PAR, 0.05 gm of SAL, 0.01 gm of lactose, and 0.225 gm of saccharose were carefully weighed and mixed well, then dissolved in a small amount of ethanol and complete the volume with distilled water up to the mark in a 100 ml volumetric flask. However, Chlorpheniramine maleate was not available so it was not added. A stock solution was obtained of 500 $\mu\text{g mL}^{-1}$ of PAR and 500 $\mu\text{g mL}^{-1}$ SAL, then three different mixtures of PAR and SAL for four replicates were prepared by taking an appropriate amount from the solution in a 10 ml volumetric flask, and because the concentration of SAL is far from the standard curve for SAL. A known concentration of pure SAL was added and the HPSAM method was applied to the preparation mixtures[27,28]. Table (10) shows the quantities that were found and the recovery % for both PAR and SAL.

Table (10) Simultaneous determination of PAR and SAL in Rinomicine tablet

Rinomicine tablet													
Certified value mg		The amount present ($\mu\text{g.mL}^{-1}$)		Drug content found* ($\mu\text{g.mL}^{-1}$)		Recovery* (%)		Average Recovery%		Average Recovery % of mg		RSD	
PAR	SAL	PAR	SAL	PAR	SAL	PAR	SAL	PAR	SAL	PAR	SAL	PAR	SAL
50	50	5	15	4.98	15.10	99.70	100.67	100.87	99.26	50.44	49.63	1.038	1.08
		5	15	5.09	14.81	101.82	98.71						
		5	15	5.01	14.92	100.27	99.48						
		5	15	5.08	14.73	101.68	98.19						

50	50	2	50	2.07	48.39	103.67	96.78	100.82	98.27	50.41	49.14	1.99	2.54
		2	50	2.01	48.10	100.35	96.21						
		2	50	2.01	49.17	100.31	98.33						
		2	50	1.98	50.89	98.94	101.77						
50	50	3	70	3.12	70.10	103.85	101.42	102.86	98.74	51.43	49.37	1.48	2.08
		3	70	3.09	68.40	102.90	97.72						
		3	70	3.12	69.39	103.99	99.13						
		3	70	3.02	67.67	100.68	96.68						

Comparison between the present method and the literature method

Table (11) shows the comparison between the proposed method and another reported method. The literature method [13] depends on using Fe (III) and 1,10-phenanthroline reagents in the presence of buffer solution and heating at 60°C, and the absorbance was measured for two sets of solutions at 510 nm, then the amount of drugs was calculated by solving by simultaneous equations. The proposed method included the estimation of drug compounds based on the difference in the rate of their oxidation with Fe(III) in the presence of BPY reagent and measurement of absorbance at 522 nm at two different times, and the amount of drugs was found through the HPSAM. The proposed method did not require heating or the use of buffer solutions.

Table (11) Comparison between the present method and the literature method

Analytical parameters	Present method	Literature method
Reagents	Fe(III), BPY	Fe(III), 1,10-phenanthroline
λ (nm)	522	510
Temp.(°C)	RT	60
Development time (min)	6, 16	25
Beer's law ($\mu\text{g.mL}^{-1}$)		
PAR	1-5	2.0 – 300
SAL	10-90	0.50 – 10
Recovery (%)	≤ 103.55	≤ 103.30
RSD (%)	≤ 2.40	≤ 3.47
Application	Synthetic Rinomicine tablet	Synthetic Rinomicine tablet Human serum

Conclusion

A simple spectrophotometric method for the simultaneous kinetic determination of PAR and SAL by applying the HPSAM is described. The method does not need a heating process or the use of buffered solutions compared to the use of the same technique in the literature, as well as the method does not need a separation process in addition to being an economical method and does not need the use of complicated instruments. The method succeeded in the determination of PAR as analyte and SAL as interferent in their mixture, with weight ratios of 1: 90 and 5: 20 and a recovery % ranging from 98.2%-102.1%, at the pair time t_6 - t_{16} . The PAR and SAL in the synthetic pharmaceutical preparation known as Rinomicine were successfully determined using the suggested method as well.

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تطبيق طريقة الإضافة القياسية - النقطة المشتركة في التقدير الطيفي الحركي الآني للباراسيتامول والساليسيلاميد

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الخلاصة

تم وصف طريقة طيفية بسيطة للتقدير الطيفي الحركي الآني للباراسيتامول والساليسيلاميد من خلال تطبيق طريقة الإضافة القياسية - النقطة المشتركة. تعتمد الطريقة على الاختلاف في زمن أكسدة المركبين الدوائيين بواسطة أيون الحديدك (Fe(III) وتفاعل أيونات الحديدوز الناتجة (Fe(II) مع كاشف 2،2'-ثنائي البريدل لتكوين معقد برتقالي اللون يمتلك أقصى امتصاص عند الطول الموجي 522 نانومتر. تم تحديد الباراسيتامول كمادة محللة و الساليسيلاميد كمتداخل في الخليط ، حيث أمكن تطبيق قانون بير بحدود 1-5 مايكروغرام/مللتر للباراسيتامول و 10-90 مايكروغرام/مللتر للساليسيلاميد في مزيجهما، وبنسب وزنية بين 1 : 90 و 5 : 20 وبانحراف قياسي نسبي تراوح بين 1.04 و 2.4 وبنسبة استرداد تتراوح من 98.2%-102.1% ، عند زوج من الزمن t_6-t_{16} ، مما يدل على ان الطريقة ذات دقة وتوافق جيدتين. كما وجد أن الطريقة خالية من تأثير التداخلات من المواد المضافة في المستحضر الصيدلاني. تم تطبيق الطريقة المقترحة أيضاً بنجاح في تقدير الباراسيتامول والساليسيلاميد في صيغهم النقية وفي المستحضر الصيدلاني المحضر رينومايسين.