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Spectrophotometric Determination of Salicylamide and Paracetamol in Biological Samples and Pharmaceutical Formulations by a Differential Kinetic Method

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Abstract

A rapid, simple and sensitive spectrophotometric method is presented for the determinations of salicylamide and paracetamol in mixture. The method is based on their complexation and oxidation reactions by Fe^{3+} ion. Two sets of conditions were established such that in one set of conditions only salicylamide reacts with Fe^{3+} ion, but in the other set of conditions both of the salicylamide and paracetamol are oxidized by Fe^{3+} ion in the presence of 1,10-phenantroline. In both sets the reactions can be monitored spectrophotometrically by measuring the absorbance of the produced copmplexes at 510 nm. The data were evaluated by proportional equations. The method allowed the determination of salicylamide and paracetamol at concentrations between 2.0 – 300 μg mL^{-1} and 0.50 - 10 μg mL^{-1} with relative standard deviations of 2.58% and 3.47%, respectively. The method was applied to the determination of salicylamide and paracetamol in human serum and pharmaceutical formulations.

Key words: salicylamide, paracetamol

1. Introduction

Differences in kinetic behavior have been used extensively for the determination of two or more components in mixtures. Many differential kinetic methods have been proposed for the analysis of mixtures of closely related species without prior separation.[1-5] Proportional-equation method is a mathematical method of wide use in differential kinetic methods for the resolution of closely related species. This method depends on changing the ratio of two rate constants by varying the reaction medium or the conditions.[5]

Salicylamide (*o*-hydroxy benzamide, SAL) and paracetamol (*N*-acetyl-*p*-aminophenol, PRC, also known as acetaminophen) are important and extensively used antipyretic-analgesic drugs. They are frequently prescribed in mixture with each other or with other related drugs. Therefore their determinations in mixtures are required.

Several methods have been reported for determination of salicylamide and paracetamol in mixtures. These include HPLC,[6] spectrofluorimetric[7] electrochemical[8] and spectrophotometric[9] methods.

Recently we reported a spectrophotometric method for the determination of salicylamide and paracetamol in mixtures using partial least squares regression (PLS) and H-Point standard addition method (HPSAM).[10]

In this paper we describe a simple, precise and accurate method for rapid spectrophotometric determination of salicylamide and paracetamol in mixture. The method is based on their complexation and oxidation reactions with Fe^{3+} ion at two different sets of conditions. Under both sets of conditions the reactions were monitored spectrophotometrically by measuring the increase in absorbance at 510 nm.

2. Results and discussion

2.1. Preliminary Investigations

In pH 3.5 acetate buffer solution and at 25 °C, salicylamide reacts with Fe^{3+} ion and forms a colored complex (Eq. 1). This reaction was performed through the possession of a free phenolic group adjacent to the COR group (R=NH_2). The spectrum of complex is shown in Fig. 1. Under this set of conditions paracetamol does not react with Fe^{3+} ion even up to 500 μg mL^{-1}.

$$\text{Salicylamide} + Fe^{3+} \longrightarrow \text{colored complex} \quad (1)$$

But in pH 4.5 acetate buffer solution and at 60 °C salicylamide and paracetamol are oxidized by Fe^{3+} ion and Fe^{2+} ion is produced (Eq. 2). The produced Fe^{2+} ion reacts with 1,10-phenantroline and forms a colored complex which is called ferroin (Eq. 3).[11] The spectrum of produced ferroin is shown in Fig. 1.

Paracetamol / Salicylamide + Fe^{3+} ⟶ Fe^{2+} + Oxidized products (2)

Fe^{2+} + 3 Phen ⟶ $Fe(Phen)_3^{2+}$ (Ferroin) (3)

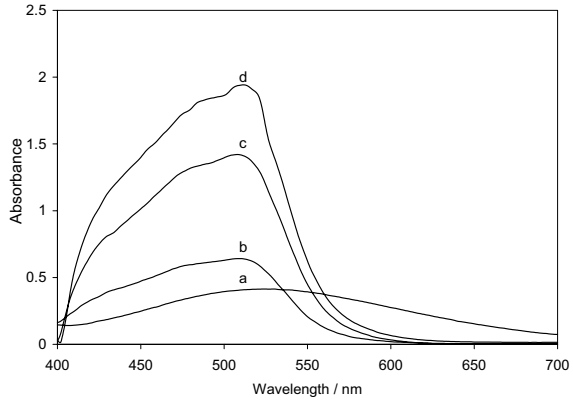

Figure 1. Absorption spectra of (a) complex of salicylamide with Fe^{3+} ion and the ferroin produced from oxidation reaction of (b) salicylamide (c) paracetamol and (d) their mixture by Fe^{3+} ion. Conditions for (a) : salicylamide, 40 μg mL^{-1};pH, 3.5; Fe^{3+}, 2.58×10^{-3} M; T= 25 °C; for (b): salicylamide, 20 μg mL^{-1}; paracetamol, 5.0 μg mL^{-1}; pH, 4.5; 1,10-phenanthroline, 1.8×10^{-3} M; Fe^{3+}, 6.45×10^{-4} M; T=60 °C.

The reactions could be monitored spectrophotometrically by measuring the absorbance of the solution at 510 nm with time.

Based on the above results, salicylamide and paracetamol could be determined in the presence of each other by choosing suitable conditions. Two simultaneous equation were solved to give the salicylamide and paracetamol concentration.

2.2. Effect of Variables

The effect of reaction variables was studied separately for salicylamide and paracetamol and their optimum values for procedures were selected. Two sets of conditions must be fulfilled. Under the first set of conditions (Procedure 1) only salicylamide reacted and under the second set of conditions (Procedure 2) both the salicylamide and paracetamol reacted.

The effect of pH on the reaction of salicylamide and paracetamol with Fe^{3+} was studied in the range of 2-7. The results are shown in Fig. 2. As Fig. 2 shows under the first set of conditions only salicylamide forms complex with Fe^{3+} ion and paracetamol did not react.

Even when its concentration was in 10-fold excess over salicylamide. The absorbance for salicylamide reaction increased with increasing pH up to 3.5 and decreased at higher pH values**.** Therefore a pH of 3.5 was chosen for the first set. Fig. 2 shows the effect of pH on both the oxidation reactions of salicylamide and paracetamol with Fe^{3+} ion under the second set of conditions. As Fig. 2 shows the absorbance for both

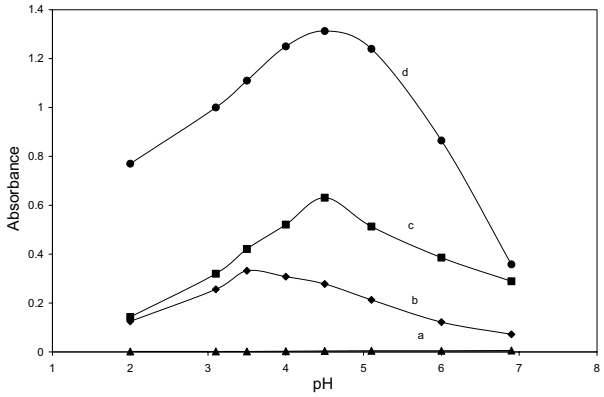

Figure 2. Effect of pH on the complex formation reaction of (a) paracetamol and (b) salicylamide with Fe^{3+}; and oxidation of (c) salicylamide and (d) paracetamol. Conditions for (a) and (b): salicylamide, 30 μg mL^{-1}; paracetamol, 300 μg mL^{-1}; Fe^{3+}, 2.58×10^{-3} M; T= 25 °C; for (c) and (d): salicylamide, 20 μg mL^{-1}, paracetamol, 5.0 μg mL^{-1}; 1,10-phenanthroline, 1.8×10^{-3} M; Fe^{3+}, 6.45×10^{-4} M; T=60 °C.

the reactions increased by increasing pH up to 4.5 and decreased at higher pH values**.** Therefore a pH 4.5 was chosen for second set.

The effect of Fe^{3+} ion concentration on the reaction of salicylamide and paracetamol was studied separately. The results are shown in Fig. 3. As the results showed under the first set of conditions even at high concentrations of Fe^{3+} only salicylamide reacted with Fe^{3+} ion and paracetamol did not react even when its concentration was 10-fold excess over salicylamide. The effect of Fe^{3+} ion concentration on the complex formation reactions of salicylamide in the range of 6.45×10^{-5}– 5.81×10^{-3} M was studied.

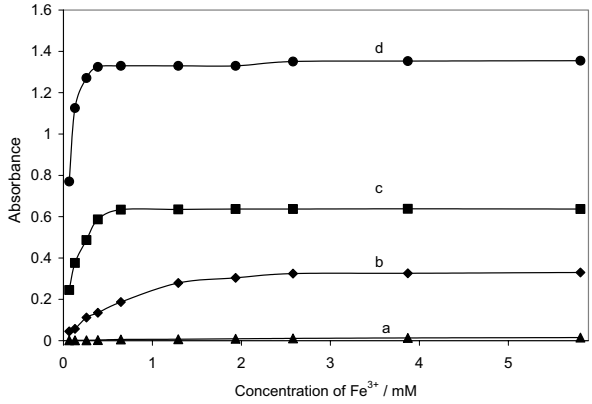

Figure 3. Effect of Fe^{3+} ion concentration on the complex formation reaction of (a) paracetamol and (b) salicylamide with Fe^{3+}, and oxidation reaction of (c) salicylamide and (d) paracetamol. Conditions for (a) and (b): salicylamide, 30 μg mL^{-1}; paracetamol, 300 μg mL^{-1}; pH, 3.5; T= 25 °C; for (c) and (d): salicylamide, 20 μg mL^{-1}; paracetamol, 5.0 μg mL^{-1}; 1,10-phenanthroline, 1.8×10^{-3} M; pH, 4.5; T=60 °C.

The absorbance for salicylamide reaction increased with increasing Fe^{3+} ion concentration up to 1.90×10^{-3} M and remained constant at higher concentrations**.** Therefore a concentration of 2.58×10^{-3} M Fe^{3+} ion was chosen for the first set of conditions. Fig. 3 also shows the effect of Fe^{3+} ion concentration on the oxidation reactions of salicylamide and paracetamol with Fe^{3+} ion under the second set of conditions in the range of 6.45×10^{-5}– 5.81×10^{-3} M. As Fig. 3 shows the absorbance for paracetamol and salicylamide reactions increased by increasing Fe^{3+} ion concentration up to 3.87×10^{-4} and 6.45×10^{-4} M, respectively, and remained constant at higher concentrations. Therefore, a concentration of 6.45×10^{-4} M Fe^{3+} ion was used for second set of conditions.

The effect of 1, 10- phenanthroline concentration on the absorbance of the solution under the second set of conditions was studied in the range of 6.06×10^{-5} – 2.424×10^{-3} M. The results are shown in Fig. 4. As Fig. 4 shows the absorbance for both the reactions increased by increasing 1,10-phenanthroline concentration up to 1.30×10^{-3} M and remained nearly constant at higher concentrations. Therefore, a concentration of 1.8×10^{-3} M 1,10- phenanthroline was selected as optimum.

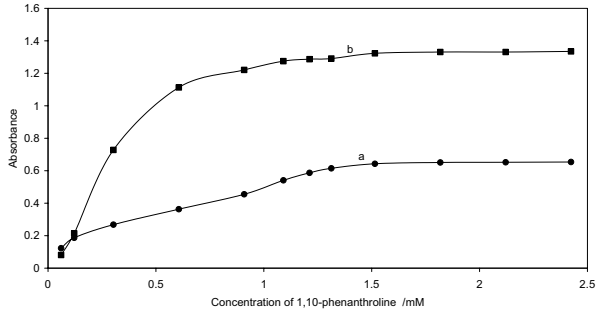

Figure 4. Effect of 1,10-phenanthroline concentration on the absorbance of the ferroin produced from the oxidation of (a) paracetamol and (b) salicylamide with Fe^{3+}. Conditions: salicylamide, 20 μg mL^{-1}; paracetamol, 5.0 μg mL^{-1}; pH, 4.5; Fe^{3+}, 6.45×10^{-4} M; T= 60 °C.

The effect of temperature on the reaction of salicylamide and paracetamol were studied in the range of 15-80 °C. As Fig. 5 shows that the absorbance remained constant with changing temperature. This meant that temperature had no effect on the complexation reaction of salicylamide with Fe^{3+} and paracetamol did not react with Fe^{3+} in the investigated temperature range. Therefore, a temperature of 25 °C was chosen for the first set of conditions. Fig. 5 also shows the effect of temperature on the oxidation reactions of salicylamide and paracetamol with Fe^{3+} ion under the second set of conditions. As Fig. 5 shows by increasing temperature the absorbance for both the reactions increased rapidly up to 60 °C and slowly at higher temperatures. Therefore, a temperature of 60 °C was chosen for second set of conditions.

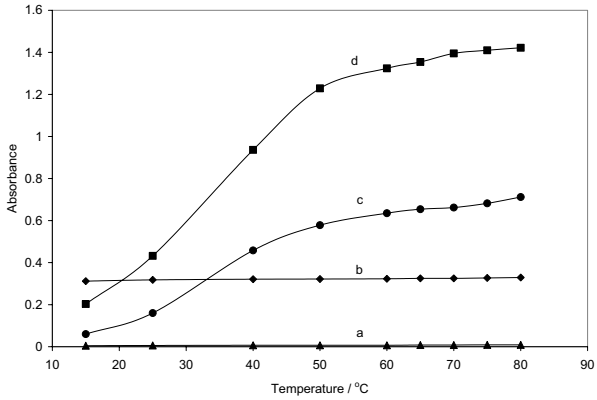

Figure 5. Effect of temperature on the complex formation reaction of (a) paracetamol and (b) salicylamide with Fe^{3+}, and oxidation reaction of (c) paracetamol and (d) salicylamide with Fe^{3+}. Conditions for (a) and (b):salicylamide, 30 μg mL^{-1}; paracetamol, 300 μg mL^{-1}; pH, 3.5; Fe^{3+}, 2.58×10^{-3} M; for (c) and (d): salicylamide, 20 μg mL^{-1}; paracetamol, 5.0 μg mL^{-1}; pH, 4.5; Fe^{3+}; 6.45×10^{-4} M.

The effect of paracetamol concentration on the reaction of 30 μg mL^{-1} salicylamide under the first set of conditions was studied in the range of 0.0 – 1000 μg mL^{-1}. The results showed that paracetamol had no effect on the reaction of 30 μg mL^{-1} salicylamide with Fe^{3+} up to 700 μg mL^{-1} and interfered slightly at higher concentrations.

2.3. Analytical Parameters

Under the optimum conditions described above, calibration graphs for salicylamide and paracetamol were constructed by plotting absorbance values as a function of the analyte concentration. The calibration graphs for the individual determinations were linear in the range of 2.0-300 μg mL^{-1} for salicylamide under the first set of conditions and 1.0-40 μg mL^{-1} for salicylamide and 0.50- 10 μg mL^{-1} for paracetamol under the second set of conditions. Paracetamol had no contribution to the absorbance under the first set of conditions. The results are summarized in Table 1.

Table 1. Characteristics of the calibration graphs for the determination of salicylamide and paracetamol

Analyte	Procedure	Range (μg mL^{-1})	Slope (mL μg^{-1})	Intercept	R^2
Salicyl-amide	1	2.00 - 300	0.0091	0.0455	0.9988
	2	1.0 - 40	0.019	0.2443	0.9984
Paracet-amol	2	0.50 - 10	0.25	0.0864	09974

The absence of synergistic effects ensured that the absorbance values obtained at 20 min after initiation of the reaction for a mixture of the two analytes were the sum of the absorbance values obtained for each individual analyte. Therefore the following equations can be used for the determination of salicylamide and paracetamol in mixture.

$$A_1 = 0.0455 + 0.0091C_{\text{paracetamol}} \quad (4)$$

$$A_2 = 0.33 + 0.25C_{\text{paracetamol}} + 0.019\,C_{\text{salicylamide}} \quad (5)$$

where A_1 and A_2 are the absorbance for the first and second set of conditions, respectively, and the concentrations are in μg mL^{-1}.

To evaluate the precision and the detection limit, a series of independent standard samples was used. The relative standard deviation for five replicate measurement of salicylamide and paracetamol was 3.47% and 2.58% in a 30 : 1 mixture salicylamide = 30 μg mL^{-1} and paracetamol = 1.0 μg mL^{-1}).

The limit of detection which can be calculated on the basis of $Y_{LOD} = Y_B + 3S_B$, in which Y_{LOD}, Y_B and S_B are signal of limit of detection, signal of blank and standard deviation of blank,[12] respectively was 0.134 μg mL^{-1} for salicylamide under the first set of conditions and 0.27 and 0.19 μg mL^{-1} for salicylamide and paracetamol respectively, under the second set of conditions.

2.4. Complexation Reaction of Salicylamide

The stochiometry and formation constant of complex of salicylamide with Fe^{3+} ion were determined spectrophotometrically. The stochiometry was determined using the Job's method and the formation constant was determined using the variation of the absorbance of the complex with the metal to ligand mole ratio and non-linear least-squares curve fitting program KINFIT.[13] The results show that the stochiometry of complex of salicylamide with Fe^{3+} ion was 1:1 and complex formation constant was log $K_f = 5.23 \pm 0.09$.

2.5. Determination of Salicylamide and Paracetamol in Synthetic Mixtures

Various mixtures of standards of salicylamide and paracetamol solutions were prepared and tested according to the recommended procedure. The test was carried out covering concentrations within the dynamic ranges of the species, using different concentration ratio of these ions. The results are given in Table 2. As Table 2 shows the relative error of the measurements were ≤ 6.0%, which confirms the good accuracy of the proposed method.

Table 2. Analyses of mixtures of salicylamide and paracetamol by the proposed method

Saicylamide: paracetamol	Salicylamide (μg mL^{-1})		Relative error (%)	Paracetamol (μg mL^{-1})		Relative error (%)
	Taken	Found		Taken	Found	
30:1	30	30.2	+0.70	1.0	0.948	-5.2
80:1	40	41.2	+3.0	0.50	0.48	-4..0
4:1	20	19.3	-3.5	5.0	4.9	-2.0
1:1	5.0	4.89	-2.1	5.0	5.3	+6.0
2:5	2.0	2.1	+5.0	5.0	4.7	-6.0
5:2	10	10.4	+4.0	4.0	4.2	+4.2

2.6. Application

To evaluate the analytical applicability of the proposed method, it was applied to the determination of salicylamide and paracetamol in pharmaceutical preparations and in human serum. The results are given in Table 3 and Table 4. The recoveries are close to 100% which indicates that there is no serious interference in the determination of salicylamide and paracetamol in such samples.

Table 3. Determination of paracetamol and salicylamide in pharmaceutical preparations.

Sample[a]	Nominal value (mg)		Found[b] (mg)	
	Salicylamide	Paracetamol	Salicylamide	Paracetamol
Yendol granular packet (Faes)	500	200	495.2	208.6
Rinomicine pellets (Fardi)	50	50	48.5	52.2
Coricidin Capsules (Schering plough)	190	-	192.9	-

[a] Composition: Yendol: Salicylamide, 500 mg; paracetamol, 200 mg; Chlorpheniramine maleate, 3 mg; Caffeine, 30mg; Saccharin, 10mg; Saccharose, 6.5 g; Rinomicine: Paracetamol, 50 mg; Chlorpheniramine maleate, 4.0 mg; Salicylamide, 50 mg; Lactose, 10 mg; Saccharose, 225 mg. ; coricidin : Salicylamide . 190 mg; Chlorphenir amine maleate , 4 mg; caffeine , 30mg; as corbic acid, 50mg.
[b] mean+ S.D. (n=3)

Table 4. Determination of paracetamol and salicylamide in human serum.

Spiked / μg mL^{-1}		Found / μg mL^{-1}		Recovery (%)	
Salicylamide	Paracetamol	Salicylamide	Paracetamol	Salicylamide	Paracetamol
30.0	1.00	31.0	0.970	103	97.0
20.0	5.00	19.4	5.23	97.0	104.2
5.00	10.0	4.81	10.16	96.2	101.6
10.0	4.00	10.3	3.88	103	97.0
25.0	3.00	24.8	3.11	99.2	104

3. Conclusion

Determination of salicylamide and paracetamol in mixture by proposed method is based on their complexation and oxidation reactions by Fe^{3+} ion. Two sets of conditions were established that in one set of conditions only salicylamide forms complex with Fe^{3+} ion, but in the other set of conditions both the analytes are oxidized by Fe^{3+} ion in the presence of 1,10-phenanthroline as indicator. In both sets the reactions can be monitored spectrophotometrically by measuring the increase in absorbance at 510 nm. The proposed method offers good selectivity, accuracy and precision that can be applied for a wide range of paracetamol and salicylamide in synthetic and real samples.

4. Experimental

4.1. Apparatus

A Pharmacia model LKB3 UV-visible Ultraspect (III) single beam Spectrophotometer that connected to a Pentium II computer with 1- cm quartz cells was used for absorbance measurements. All spectral measurements were performed using the blank solution as a reference. Measurements of pH were made with a Jenway C15 pH- meter using a combined glass electrode.

4.2. Reagents

Triply distilled water and analytical-reagent grade chemicals were used. A 1000 μg mL^{-1} standard solution of salicylamide (Aldrich) was prepared by dissolving 0.1000 g salicylamide in 5% (v/v) ethanol and diluting to the mark in a 100-mL volumetric flask, working solutions were prepared by diluting the standard solution in water; this solution was stable at least for two weeks. A 1000 μg mL^{-1} standard solution of paracetamol (Merck) was prepared by dissolving 0.1000 g paracetamol in water and diluting to the mark in a 100-mL volumetric flask, working solutions were prepared by diluting the standard solution in water. A 0.02580 M Fe^{3+} ion solution was prepared by dissolving 0.69789 g $FeCl_3.6H_2O$ (Merck) in water and diluting to the mark in a 100-mL volumetric flask. A 1.212 $\times 10^{-2}$ M 1,10-phenanthroline solution was prepared by dissolving 0.2400 g 1,10-phenanthroline (Merck) in ethanol and diluting to the mark in a 100-mL volumetric flask. Acetate buffer solutions of pH 3.5 and 4.5 were prepared and pH checked by pH meter.

4.3. Determination of Salicylamide and Paracetamol in Mixture

Two runs are needed for each sample.

4.3.1. Procedure 1

An aliquot solution containing 2-300 μg of salicylamide and 0.5-20 μg of paracetamol was transferred into a 10 mL volumetric flask containing 1 mL of 2.58×10^{-2} M Fe^{3+} solution and 1 mL of pH 3.5 acetate buffer. The solution was diluted to the mark with triply distilled water. A portion of the solution was transferred into a glass cell to measure the increase in absorbance at 510 nm against a blank solution that was prepared in the same method except that distilled water was used instead of analytes. The dependence of the absorbance (A_1) on the concentration of salicylamide was found to conform the following equation:

$$A_1 = a_1 + b_1 C_{salicylamide} \quad (6)$$

4.3.2. Procedure 2

An aliquot solution containing 1-40 μg of salicylamide and 0.50-10 μg of paracetamol was transferred into a 10 mL volumetric flask containing 1.5 mL of 1.212×10^{-2} M 1,10-phenantroline and 1 mL of pH 4.5 acetate buffer solution. The solution was diluted to ca. 9 mL with triply distilled water and was placed in a water bath at 60 °C for 5 min. Then 1.0 mL of 6.45×10^{-3} M Fe^{3+} ion solution was added. The stop watch was started and a portion of the solution was transferred into a glass cell to measure the increase in the absorbance at 510 nm against a blank solution that was prepared in the same method except that distilled water was used instead of analytes at 20 min after initiation of the reactions. The dependence of the absorbance on the concentration of salicylamide and paracetamol was found to conform the following equations:

$$A_2 = a_2 + b_2 C_{salicylamide} + b_2' C_{paracetamol} \quad (7)$$

4.3.3. Preparation of Human Serum

Human serum was separated from blood by centrifugation at 3000 rpm for 10 min.[14] A 0.5 mL of the supernatant was transferred into a 10 mm × 75 mm glass tube containing 1.0 mL of ethyl acetate and a few crystals of NaCl and mixed vigorously for 30 s using a vortex mixer. Then exactly 0.5 mL of the upper organic layer was pipetted into a 10 mL volumetric flask. The determinations were carried out according to procedures 1 and 2.

5. References and Notes

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Povzetek

Predstavljena je hitra, enostavna in občutljiva spektrofotometrična metoda za določevanje salicilamida in paracetamola v zmesi. Metoda temelji na njuni oksidaciji z ionom Fe^{3+} in tvorbi kompleksov železa s salicilaminom in 1,10-fenantrolinom. Izbrani so bili taki reakcijski pogoji, da v prvem primeru reagira z Fe^{3+} samo salicilamin, v drugem pa Fe^{3+} oksidira obe spojini v prisotnosti 1,10-fenantrolina. V obeh primerih merimo koncentracijo nastalih kompleksov spektrofotometrično pri 510 nm. Metoda omogoča določevanje salicilamida in paracetamola v koncentracijskih območjih 2,0 do 300 μg mL^{-1} in 0,50 do 10 μg mL^{-1}, z relativnimi standardnimi odmiki meritev 2,58% oziroma 3,47%. Metoda je bila uporabljena za določevanje salicilamida in paracetamola v krvni plazmi in farmacevtskih pripravkih.