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Nitroso-R-salt as a sensitive spectrophotometric reagent for the determination of paracetamol in pharmaceutical preparations

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Abstract

Nitroso-R-salt is proposed as a sensitive spectrophotometric reagent for the determination of paracetamol in aqueous solution. The method is based on the reaction of paracetamol with iron(III) and subsequent reaction with nitroso-R-salt to yield a green colored complex with maximum absorption at 720 nm. Optimization of the experimental conditions was described. The calibration graph was linear in the concentration range of 0.1 – 2.0 μg mL^{-1} paracetamol with a molar absorptivity of 6.9×10^4 L mol^{-1} cm^{-1}. The method was successfully applied to the determination of paracetamol in pharmaceutical preparations without any interference from common excipients. The method has been statistically evaluated with British Pharmacopoeia method and no statistical difference between methods was found at the 95% confidence level.

Key words: Paracetamol; Spectrophotometry; Nitroso-R-salt; Pharmaceutical preparations.

Introduction

Nitroso-R-salt is the disodium salt of α-nitroso-β-naphthol disolfonic acid. This reagent was used for qualitative identification of ferrous ion and for copper and nickel ions at low pH [1].

Paracetamol (acetaminophen) has the chemical name N-(4-hydroxyphenyl) acetamide. It is important and extensively used as antipyretic-analgesic drug [2].

Various methods have been reported for the determination of paracetamol in pharmaceutical preparations. These include spectrophotometry [3-7], polarography [8], micellar electrokinetic chromatography [9], flow injection-spectrofluorimetry [10], flow injection fourier-transform infrared spectrometry [11], flow injection-spectrophotometry [12, 13], first derivative spectrofluorimetry [14], square-wave voltammetry [15], flow injection-biamperometry [16], high-performance thin-layer chromatography [17], and reversed-phase capillary electrochromatography [18].

Most of spectrophotometric methods need either pre-hydrolysis step [19] or temperature and pH controls [20]. Therefore, development of a simple and sensitive spectrophotometric method seems to be desirable. The present paper describes a simple and sensitive spectrophotometric method for the evaluation of paracetamol in pharmaceutical preparations. The method is based on the reaction of paracetamol with iron(III) and subsequent reaction with nitroso-R-salt to yield a green colored complex. A comparative study of this method with

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the official method reported in pharmacopoeia was made. The present method offers the advantages of simplicity, no need for extraction or heating, in addition to higher sensitivity in comparison with some of the existing spectrophotometric methods.

Materials and methods:

Apparatus

A Shimadzu UV-visible 260 digital double-beam recording spectrophotometer (Shimadzu, Kyoto, Japan) was used for all spectral and absorbance measurements with matched 1-cm quartz cells.

Reagents

All chemicals used were of analytical reagent grade. Paracetamol standard material was provided from the State Company for Drug Industries and Medical Appliances (SDI), Samarra – Iraq.

1- Paracetamol stock standard solution 1000 µg mL^{-1} was prepared by dissolving 0.1000 g of pure paracetamol in 5 ml of ethanol and diluting to the marked with distilled water in 100 mL volumetric flask. Working standard solutions were prepared by suitable dilution of the stock standard solution.
2- Ferric sulfate solution 1 mM was prepared by dissolving 0.0562 g of ferric sulfate in distilled water and diluting to the marked in 100 mL volumetric flask.
3- Nitroso-R-salt solution 10 mM was prepared by dissolving 0.3773 g of nitroso-R-salt in distilled water and diluting to the marked in 100 mL volumetric flask.

Pharmaceutical preparations of paracetamol

Pharmaceutical preparations were obtained from commercial sources.

1- Paracetol tablets (SDI, Iraq): 500 mg paracetamol for each tablet.
2- Panadol Extra tablets (Smithkline Beecham, Ireland): 500 mg paracetamol, 65 mg caffeine for each tablet.
3- Panatol tablets (Global Pharma, UAE): 500 mg paracetamol for each tablet.
4- Emidol tablets (Global Pharma, UAE): 500 mg paracetamol for each tablet.
5- Kanagesic tablets (Kanawati Medical Products, Syria): 450 mg paracetamol and 35 mg orphenadrine citrate for each tablet.
6- Hayamol injections (Ibn Hayyan Pharmaceutical HOMS, Syria): 375 mg paracetamol for each injection (5 mL).

Calibration graph

Aliquot of standard paracetamol solution (2.5 – 50.0 µg) was transferred into 25 mL calibrated flasks. To each flask, 1.5 mL of ferric sulfate solution (1 mM), shake well and followed by 3 mL of nitroso-R-salt solution (10 mM). The contents were diluted to the mark with distilled water and mixed; after 45 min, the absorbance value at λ_{max} 720 nm was measured against a reagent blank and a calibration graph was constructed.

Procedure for the assay of pharmaceutical preparations

1- Tablets solution (1000 µg mL^{-1})

The average tablet weight was calculated from the contents of 20 tablets that had been finely powdered and weighed. A portion of this powder, equivalent to 100 mg of paracetamol, was accurately weighed, shaken with 5 mL of ethanol, diluted with distilled water in to a 100 mL volumetric flask and was filtered.

2- Injections solution (1000 µg mL^{-1})

The contents of five injections were mixed. An aliquot corresponding to

100 mg of paracetamol (1.3 mL) was shaken with 5 mL of ethanol and diluted to 100 mL with distilled water in a volumetric flask.

Further appropriate solutions of pharamaceutical preparations were made by using simple dilution with distilled water. Two different concentrations of each solution of pharmaceutical preparations were analyzed in five replicate using the above procedure.

Results and discussion

The proposed spectrophotometric method for the determination of paracetamol is based on the oxidation reaction of paracetamol with iron(III) and subsequent chelation of iron(II) with nitroso-R-salt to form a green colored product.

Spectral characteristics

A green product is formed when paracetamol was allowed to react with iron(III) salts and subsequent reaction of iron(II) produced with nitroso-R-salt. The soluble colored complex has a maximum absorption at 720 nm as shown in Fig. (1).

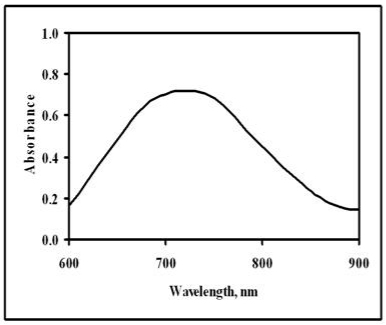


Fig. (1): Absorption spectrum of complex of nitroso-R-salt with iron(II) which produced from oxidation reaction of paracetamol (1.5 µg mL^{-1}) with iron(III)

Optimum conditions for complex formation

In order to establish the optimum conditions necessary for a rapid and quantitative formation of the colored product with maximum stability and sensitivity, the investigators measured the absorbance of a series of solutions by varying one and fixing the other parameters at 720 nm. It was found that a 1 mM solution of ferric sulfate in the range 0.5 – 4.0 mL and a 10 mM solution of nitroso-R-salt in the range 0.5 – 5.0 mL were necessary to achieve a maximum color intensity of the product. Fig. (2) shows that 1.5 mL of ferric sulfate solution (1 mM) and 3 mL of nitroso-R-salt solution (10 mM) were enough to obtain the maximum absorbance. Therefore, 1.5 mL of ferric sulfate and 3 mL of nitroso-R-salt were recommended for all measurements.

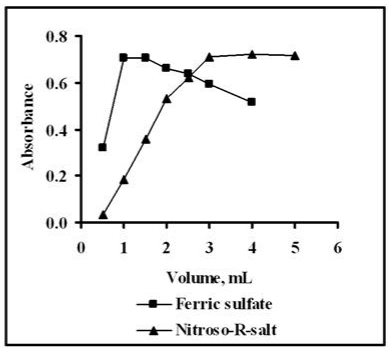


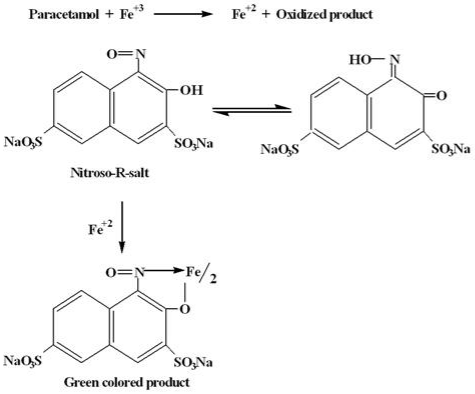
Fig. (2): Optimum conditions for determination of paracetamol

The reaction between paracetamol and iron(III) in the presence of nitroso-R-salt was found to be instantaneous. However, the reaction is complete within 45 min at room temperature (25 ^{o}C).

The effect of temperature on the colored product was studied at 5, 25 and 45 ^{o}C and the results obtained indicated that the color was stable for at least 120 min at 25 ^{o}C and was used in the recommended procedure.

Reaction mechanism

Paracetamol reduces iron(III) salts in aqueous medium to form iron(II) salts, which subsequently chelate with nitroso-R-salt to form a green colored product as given in the following equations [1, 21]:



Analytical parameters

Employing the conditions described under analytical procedure, a linear calibration graph [Fig. (3)] for paracetamol was obtained. It shows that Beer's law was obeyed in the range given in Table (1). The analytical and regression parameters [22] of proposed spectrophotometric method are compiled in Table (1), and demonstrate the highly sensitivity of the method.

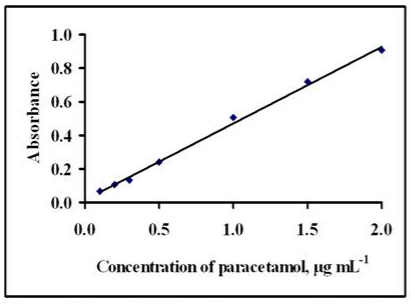


Fig. (3): Calibration graph for paracetamol

Table (1): Analytical and regression parameters of proposed method Accuracy and precision

Parameter	Value
λ_{max}, nm	720
Beer's law limits (µg mL^{-1})	0.1 – 2
Regression equation y = a + b x; y = absorbance, x = concentration (µg mL^{-1})	y = 0.0165 + 0.4566 x
Correlation coefficient, r	0.9980
Correlation of determination, r^2	0.9960
Intercept, a	0.0165
Slope, b (mL µg^{-1})	0.4566
Standard deviation of the residuals, $S_{y/x}$	0.0230
Standard deviation of the intercept, S_a	0.0135
Standard deviation of the slope, S_b	0.0130
Molar absorptivity, ε (L mol^{-1} cm^{-1})	6.902 × 10^4
Sandell's sensitivity, S (ng cm^{-2}) per 0.001 absorbance unit	2.1902
Limit of detection, LOD (µg mL^{-1}) LOD = 3 $S_{y/x}$ / b	0.1513
Limit of quantification, LOQ (µg mL^{-1}) LOQ = 10 $S_{y/x}$ / b	0.5044

To determine the accuracy and precision of the method, pure paracetamol solution was determined at two different concentrations. The results shown in Table (2), indicate that a satisfactory precision and accuracy could be obtained with the proposed method.

Interference

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 lactose, talc, starch, magnesium stearate and polyvinylpirrolidone (PVP) that are usually present in dosage forms. The criterion of interference was an error of not more than ± 1% in the absorbance. In this study, a wide range of concentrations was used in which the determination of the 2 µg mL^{-1} level of a drug was performed. Experimental results showed that there was no interference from additives or

excipients for the examined method up to 10-fold excess as shown in Table (3).

Table (2): Accuracy and precision of the proposed method

Concentration of paracetamol (μg mL^{-1})		Erel., %	Rec., %	RSD, %
Taken	Found*			
0.3000	0.2992	– 0.267	99.733	1.273
1.0000	1.0153	+ 1.530	101.530	0.807
Erel. is relative error. Rec. is recovery. RSD is relative standard deviation. * Mean value of five determinations.				

Table (3): Determination of 2 μg mL^{-1} of paracetamol in the presence of excipients

Excipient, 20 μg mL^{-1}	Conc. of paracet-amol, μg mL^{-1}	Erel., %	Rec., %	RSD, %
	Found*			
Lactose	2.0037	+ 0.185	100.185	0.612
Starch	1.9920	– 0.400	99.600	0.251
Talc	2.0191	+ 0.955	100.955	0.337
Mg stearate	1.9963	– 0.185	99.815	0.427
PVP	2.0005	+ 0.025	100.025	0.221
* Mean value of five determinations.				

Analytical applications

The proposed method was applied successfully to the determination of paracetamol in pharmaceutical preparations [Table (4)]. The results were compared statistically using Student's t-test for accuracy, and a variance ratio F-test for precision [22] with the official method [23] at the 95% confidence level [Table (5)]. The results showed that the t- and F-values were less than the theoretical value, indicating there was no significant difference between both methods in ***accuracy***, but the proposed method was ***more precise*** than the official method. Consequently, we advise to adopt the proposed method in routine analysis and for quality control purposes for paracetamol and related pharmaceutical preparations.

Conclusion

The proposed method is found to be economical, simple, fairly and highly sensitive than other spectrophotometric methods [Table (6)]. It has the advantage of being accurate, does not require the removal of excipients, temperature control, pH control, solvent extraction step and pre-hydrolysis step. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the proposed method. It can be applied successfully to different pharmaceutical preparations.

Table (4): Determination of paracetamol in pharmaceutical preparations using the proposed method

Pharmaceutical Preparation	Concentration of paracetamol (μg mL^{-1})		Erel., %	Rec., %	RSD, %
	Taken	*Found**			
Paracetol tablets	0.5000	0.4925	– 1.500	98.500	0.563
	1.5000	1.4930	– 0.467	99.533	0.269
Panadol Extra tablets	0.5000	0.4957	– 0.860	99.140	0.204
	1.5000	1.5021	+ 0.140	100.140	0.126
Panatol tablets	0.5000	0.4882	– 2.360	97.640	1.570
	1.5000	1.5193	+ 1.287	101.287	0.226
Emidol tablets	0.5000	0.4877	– 2.460	97.540	1.380
	1.5000	1.5321	+ 2.140	102.140	0.350
Kanagesic tablets	0.5000	0.5005	+ 0.100	100.100	0.505
	1.5000	1.5160	+ 1.067	101.067	0.351
Hayamol injections	0.5000	0.4979	– 0.420	99.580	0.426
	1.5000	1.5021	+ 0.140	100.140	0.370
* Mean value of five determinations.					

Table (5): The comparison of the proposed method with standard official method using t- and F-statistical tests

Pharmaceutical preparation	Proposed method		Official method		s	Value	
	Rec.% * $(x_i)_1$	$(x_i-\overline{x})^2_1$	Rec.% * $(x_i)_2$	$(x_i-\overline{x})^2_2$		t (theor.)	F (theor.)
Paracetamol pure	99.757	0.0004	100.000	0.3446	1.482	0.409 (2.18)	2.266 (4.284)
Paracetol tablets	99.017	0.5184	98.881	0.2830			
Panadol Extra tablets	99.640	0.0094	100.726	1.7240			
Panatol tablets	99.464	0.0745	99.025	0.1505			
Emidol tablets	99.840	0.0106	99.242	0.0292			
Kanagesic tablets	100.584	0.7174	98.700	0.5084			
Hayamol injections	99.860	0.0151	99.314	0.0098			
	$\overline{X}_1$ = 99.737	Σ = 1.3458	$\overline{X}_2$ = 99.413	Σ = 3.0495	$(n_1 + n_2 - 2) = 12$	$(n_1 - 1) = 6$ $(n_2 - 1) = 6$	
* Mean value of five determinations.							

Table (6): Comparison of the proposed method with other spectrophotometric methods

Reagents	λ_{max}, nm	Linear range, µg mL^{-1}	ε, L mol^{-1}cm^{-1}	Remarks	Ref.
$HNO_3 + H_2SO_4$	355	$(0.2 - 1) \times 10^3$	4.747×10^3	Treated with acetone and KOH solution	24
Cerium(IV) sulfate in 5M H_2SO_4	410	30 – 160	8.112×10^2	Required heating at 80ºC for 90 min	25
Ammonium molybdate	670	0.1 – 6	2.600×10^4	Required strongly acidic medium	26
Sodium 1,2-naphthoquinone-4-sulfonate and cetyltrimethyl ammonium bromide	570	1 – 20	1.118×10^4	Required pre-hydrolysis of paracetamol	27
o-Cresol + $NaIO_4$ + ammonium buffer (pH 10)	612	2 – 44	7.024×10^3	Time consuming (50 min)	28
$NaNO_2$ + HCl	430	180 – 300	1.038×10^4	Treated with NaOH solution in flow injection system	29
Fe(III) + 1, 10-phenanthroline + acetate buffer (pH 4.5)	510	0.5 – 10	3.779×10^4	Required heating at 60ºC	20
Fe(III) + nitroso-R-salt	720	0.1 – 2	6.902×10^4	Most sensitive, rapid and a facile work	Proposed method

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التقدير الطيفي للباراسيتامول في المستحضرات الصيدلانية باستعمال ملح النتروزو-R ككاشف طيفي حساس

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الكلمات المفتاحية: باراسيتامول، التقدير الطيفي، ملح النتروزو-R، المستحضرات الصيدلانية.

الخلاصة

يتضمن البحث تطوير طريقة طيفية حساسة لتقدير الباراسيتامول في المحلول المائي باستعمال كاشف طيفي حساس (nitroso-R-salt). تعتمد الطريقة على تفاعل الباراسيتامول مع ايونات الحديديك، ثم تفاعل ايونات الحديدوز الناتجة مع الكاشف (nitroso-R-salt) لتكوين معقد اخضر يمتلك أقصى امتصاص عند طول موجي 720 نانومتر. تم تثبيت الظروف التجريبية الفضلى للتفاعل. كان مدى الخطية من0.1 إلى 2.0 مايكروغرام مل$^{-1}$ باراسيتامول و قيمة الامتصاصية المولارية مساوية إلى 6.9×10^4 لتر مول$^{-1}$ سم$^{-1}$. طبقت الطريقة بنجاح لتقدير الباراسيتامول في المستحضرات الصيدلانية، و تم مقارنة النتائج مع الطريقة القياسية المعتمدة من قبل الدستور البريطاني لتقدير الأدوية، و وجد أن الطريقتين القياسية و المقترحة لا تختلفان معنوياً في الدقة و المصداقية.