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Indirect Spectrophotometric Assay of Paracetamol in Pharmaceutical Preparations via Oxidative Coupling Reaction with 1-Naphthol and Metaperiodate

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الخلاصـــة

تم تطوير طريقة طيفية غير مباشرة لتقديـــر الباراســيتامول بهيئتـــه النقيـــة وفـــي مستحضراته الصيدلانية . اعتمدت الطريقة على اجراء تحلل مائي حامضي للباراسيتامول إلى بارا-أمينوفينول يتبعها تفاعل الاقتران التأكسدي مع الكاشف 1-نفثول بوجود ميتـــابيريودات الصوديوم في وسط قاعدي لتكوين صبغة الاندوفينول الزرقاء يقاس امتصاصـــها عنـــد 600 نانوميتر . لقد كان قانون بير يسري ضمن مدى التراكـــيز 1.2-24 مـــايكروغرام/ مللـــتر بامتصاصية مولارية 9.65×10^3 لتر. مول$^{-1}$. سم$^{-1}$ وبلغت دقة الطريقـــة (معـــدل نســـبة الاسترجاع) 100.91% والتوافق (الانحراف القياسي النسبي) اقل مـــن 2.5% . أظـــهرت النتائج عدم حدوث تداخل في الطريقة المطورة من قبل مواد السّواغ بوصفها مضافـــات فـــي المستحضرات الصيدلانية . طبقت الطريقة بنجاح في تقدير الباراسيتامول في المســـتحضرات الصيدلانية ، إذ كانت النتائج متفقة مع طريقتي دستور الادوية البريطاني و S.D.I القياســيتين وكذلك مع المحتوى الاصيل للمستحضرات الصيدلانية.

ABSTRACT

Development of indirect spectrophotometric method for the determination of paracetamol in pure form as well as dosage form is described. The method is based on the acid hydrolysis of paracetamol to p-aminophenol followed by oxidative coupling reaction with 1-naphthol in the presence of sodium metaperiodate in alkaline medium to form a blue indophenol dye which absorbs at 600nm. Beer's law is obeyed in the concentrations range of 1.2-24 μg/ml with a molar absorptivity of 9.65×10^3 l. mol^{-1}. cm^{-1}., accuracy (average recovery) is 100.91% and precision (RSD) is less than 2.5%. Common excipients used as additives in pharmaceuticals do not interfere in the proposed method. The method is successfully employed for the determination of paracetamol in

pharmaceutical preparations and the results agree favourably with British pharmacopoeia and S.D.I. methods and also with the certified values.

INTRODUCTION

Paracetamol (4-hydroxyacetanilide), also known as acetaminophen is widely used as an analgesic and antipyretic agent (1). By reviewing the developed available colorimetric procedures for the analysis of paracetamol, one can easily recognize that most of these methods mainly based on the acidic or basic hydrolysis of paracetamol to p-aminophenol (PAP). These methods include spectrophotometric methods such as flow injection analysis after microwave assisted alkaline hydrolysis and reacting PAP with 8-hydroxyquinoline (8-quinolinol) in the presence of periodate (2), o-cresol in 3.5 M NaOH (3) or iron (III) and sulphide (4), a reaction of acidic hydrolysis product of paracetamol with thymol and periodate (5), bromanil (6), sodium 1,2-naphthoquinone -4-sulphonate and cetyltrimethyl ammonium bromide (7) or p-xylenol catalyzed by periodate (8). Ratio spectra derivative spectrophotometry and chemometric technique (9) or liquid chromatography (10), a flow-through solid phase UV spectrophotometric detection (an optosensor) (11) and near infrared transimittance spectroscopy (12) are described for the direct analyzing paracetamol in pharmaceutical preparations.

Oxidative coupling organic reactions seem to be one of the most suitable spectrophotometric determination of drugs such as catecholamines (13), propranolol (14), minocycline (15), folic acid (16) and salbutamol (17). The aim of the present work is to develop a simple and sensitive method for the determination of paracetamol in pharmaceutical preparations using oxidative coupling reaction. This analytical procedure was based on the reaction of acidic hydrolysis product (PAP) of paracetamol with 1-naphthol in the presence of sodium metaperiodate and in alkaline medium to form an intense blue colour product.

EXPERIMENTAL

Apparatus

A Shimadzu UV-vis 210 digital double beam spectrophotometer with 1-cm matched quartz cells was used for all spectra and absorbance measurements.

Reagents

Chemicals were all analytical grade and used without further purification. Paracetamol was supplied by S.D.I-Iraq, 1-naphthol and sodium hydroxide by BDH, sodium metaperiodate, sodium carbonate and

hydrochloric acid (36%) by Fluka. Distilled water was used to prepare all solutions except 1-naphthol was prepared in ethanol.

Solutions

A stock solution of paracetamol (1000 μg/ml) was prepared by dissolving 250 mg of pure paracetamol powder in 250 ml distilled water using water bath to ensure a complete dissolution. This solution was kept in a dark bottle, and was highly stable for long time. Solutions of 0.02 M ethanolic 1-naphthol solution, 0.015M sodium metaperiodate and 0.1 M sodium hydroxide were used.

Acid hydrolysis of Paracetamol

A 150 ml of a stock solution of paracetamol and 25 ml of concentrated hydrochloric acid (11.8M) was refluxed 1 hour, cooled and made up to 250 ml with distilled water to produce stock solution with concentration 600 μg/ml PAP. From this solution all appropriate solutions were prepared by neutralization suitable aliquots to pH7 with 20% sodium carbonate solution and then diluting with distilled water.

Recommended Procedure for Calibration

Appropriate aliquots of acid hydrolysis yield (PAP) of pure paracetamol (containing 30-600 μg) were transferred into a series of 25ml calibrated flasks, to which 2ml of 0.02M 1-naphthol, 0.5ml of 0.015M sodium metaperiodate and 1.5ml of 0.1M sodium hydroxide were added and the contents were diluted to the mark and mixed well. After 5min, the absorbances of the resulting solutions were measured at the wavelength of maximum absorption (600nm) against reagent blank treated similarly. Finally the calibration plot was constructed and the regression equation was derived.

Procedure for the Assay of Paracetamol In Pharmaceutical Preparations

Six pharmaceutical preparations containing paracetamol, excipients and other active ingredients were analyzed by the developed procedure. Table (1) indicates the composition and company of these preparations.

Tablets

Ten tablets were accurately weighed and ground to a fine powder using a mortar. A weighed amount of the powder equivalent to 250 mg of the pure paracetamol was dissolved in hot water, cooled and made up to 250ml with distilled water. The resulting solution was filtered off and was treated as described above under sections of acid hydrolysis of paracetamol and recommended procedure.

Suppositories

Paracetamol suppositories were assayed by developed method. For this purpose, five suppositories were weighed and mixed. The required amount (250mg) of sample corresponding to a stock solution of a concentration of 1000 μg/ml was dissolved in hot water, filtered through a fine-pore filter paper which then washed with hot ethanol-water mixture (10ml). The final filtrate was diluted to 250ml with distilled water and manipulated as described above under the acid hydrolysis of paracetamol and recommended procedure.

Table 1. Composition and company of the pharmaceutical preparations analyzed.

Pharmaceutical preparation	**Composition (mg per unit)**	**Company**
Paracetamol tablets	Paracetamol 500	Meheco-China
Paracetol tablets	Paracetamol 500	S.D.I. Iraq
Colden tables	Paracetamol 450 Promethazine HCl 5 Phenylephrine HCl 5	S.D.I. Iraq
Algesic tablets	Paracetamol 350 Codien phosphate 10	S.D.I.-Iraq
Myogesic tablets	Paracetamol 450 Orphenadrine citrate 35	Dar-Al-Dawa, Jordan
Antipyrol suppositories	Paracetamol 120	S.D.I.-Iraq

RESULTS AND DISCUSSION

Reaction of Paracetamol with 1-naphthol

The hydrolysis of paracetamol to PAP can be carried out in aqueous solutions, both in acidic and alkaline media (18). In an alkaline medium, in the presence of a suitable oxidizing agent (sodium metaperiodate), produces a reactive benzoquinocimine which can be coupled with 1-naphthol to produce a blue indophenol dye with maximum absorbance at 600nm (19) (Fig. 1) which can be used for he determing paracetamol.

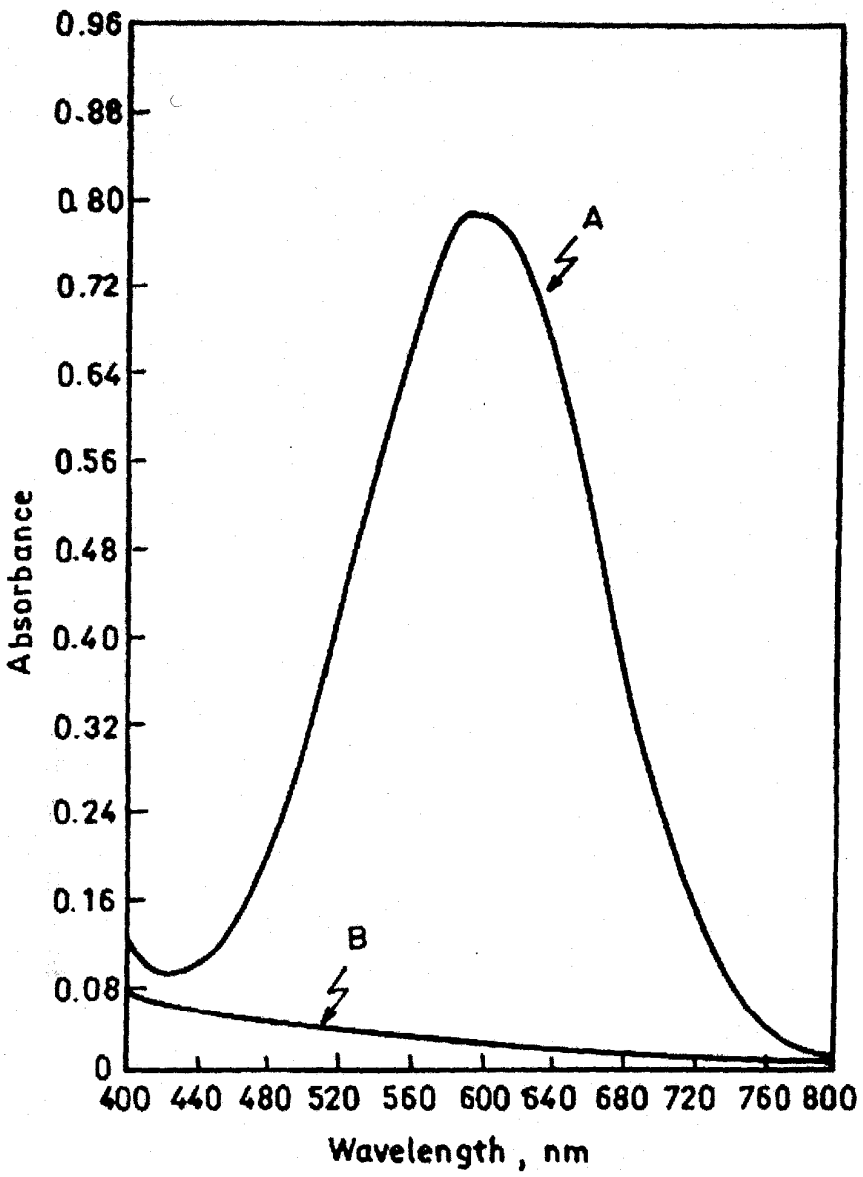


Fig. (1) Absorption spectra (A) of 12 μg/ml of paracetamol treated as described under procedure and measuring against reagent blank and (B) the reagent blank measured against distilled water. .

Optimization of Reaction Conditions

Spectrophotometric properties of the coloured product, as well as different experimental conditions affecting the colour development and its stability were carefully studied and optimized. The variables studied were 1-naphthol and sodium metaperiodate concentrations, pH temperature, development time and stability period.

Effect of pH

The effect of pH on the sensitivity of the coloured reaction product was investigated in the range of 4-13. The results obtained showed that the optimum pH value was more than 12.5 and this was performed by adding 0.1M sodium hydroxide solution and it was found that the optimum amount of 0.1M sodium hydroxide was 2ml.

Effect of Amounts of 1-naphthol and Sodium Metaperiodate Added

The effect of the amounts of 1-naphthol and sodium metaperiodate was studied and it was found that the optimum of 1-naphthol was 2ml of 0.2M and of sodium metaperiodate was 0.5ml of 0.015M.

Effect of Temperature

The reaction product concerned with proposed method was studied at different temperatures (0-50 °C). The results indicated that the colour of product intensified mostly at the room temperature, so that this temperature was chosen as the optimum temperature (Table 2).

Table 2. Effect of temperature.

Temp. °C	Absorbance/ min standing time				Absorbance/ hour standing time				
	5	15	30	45	1	2	3	6	12
0	0.790	0.791	0.791	0.792	0.788	0.780	0.773	-	-
Room temp.	0.810	0.810	0.812	0.811	0.811	0.812	0.813	0.814	0.814
40	0.805	0.805	0.800	0.804	0.804	0.793	0.789	-	-
50	0.764	0.776	0.755	0.754	0.730	0.711	0.693	-	-

Development Time and Stability Period

The optimum reaction time was determined by following the colour development at room temperature. Complete colour development was attained instantaneously and remained stable for at least 24h. Although the indophenol dye was formed instantaneously, constant absorbance readings were obtained after not less than 5min of standing.

Effect of Additions Order

The reactants involved in the reaction were mixed in various sequences, the best results (more sensitive) were obtained when they were mixed as follows:

Paracetamol, 1-naphthol, metaperiodate and sodium hydroxide.

Validation of Assay Procedure

At fixed optimum experimental conditions, the calibration graph for the investigated drug with 1-naphthol and metaperiodate was constructed. The least–squares method was used to derive the regression equation for the proposed procedure. Beer's plot at 600nm revealed very small intercept (0.0134) and good linear relationship (correlation coefficient, r=0.9995) between the absorbance and the drug concentrations over the range 1.2-24 μg/ml with molar absorptivity 9.65 x 10^3 $l.mol^{-1}$ cm^{-1} indicating the method is sensitive (Fig. 2). A relative standard deviation (RSD%) of less than 2.5% was obtained. The recovery experiments on samples containing three different concentrations (2.4, 12, 24 μg/ml) of the paracetamol were performed. A mean recovery of 100.91% was found.

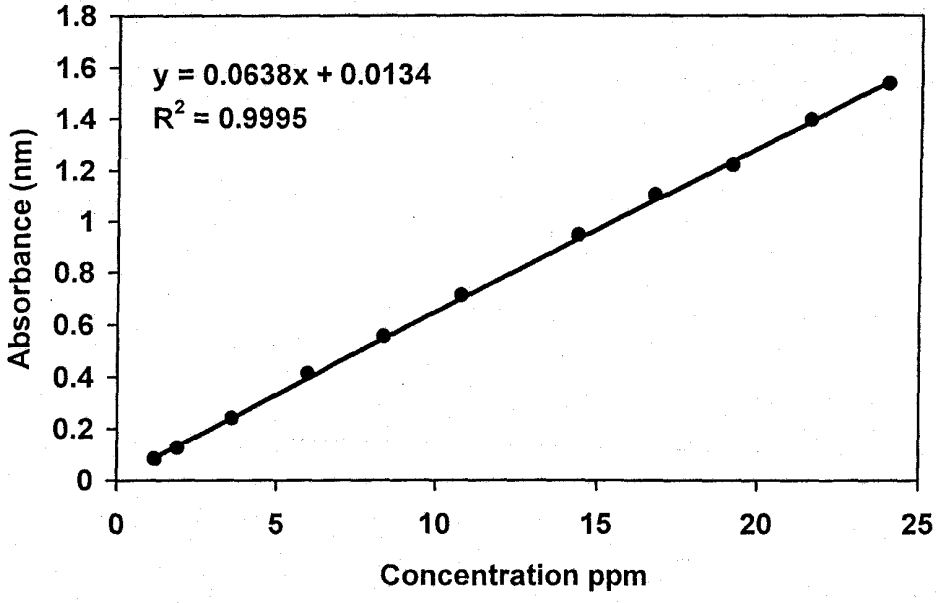
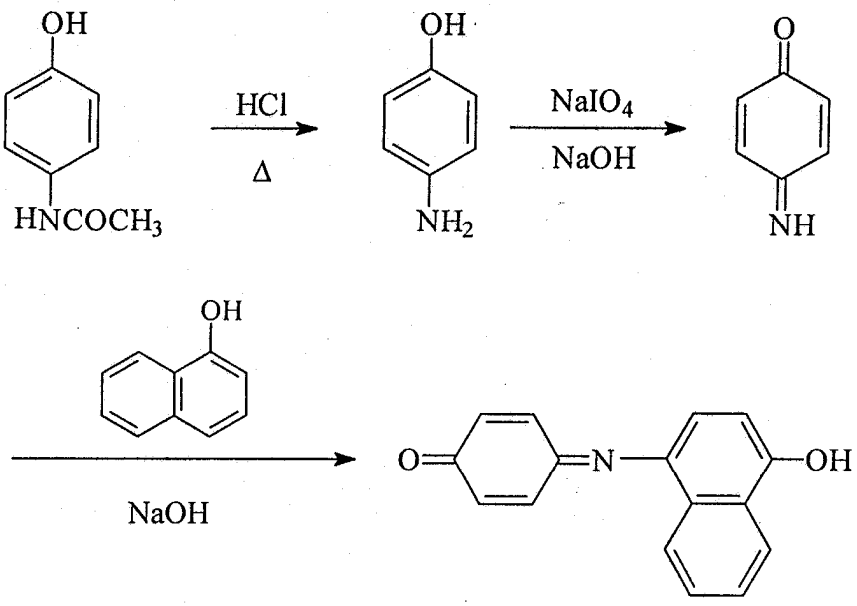


Fig. (2) Calibration graph of paracetamol.

Nature and Stability Constant of the Dye Product

The stoichiometry of the reaction was investigated using Job's method of continuous variation (20). The results obtained show a 1:1 drug to the analytical reagent 1-naphthol was formed. The formation of the dye may probably occur according to the following chemical reactions:



The average stability constant of the dye product (obtained by following the equation cited in reference 20) was 3.85×10^4 $1.mol^{-1}$, which indicates a stable dye product is formed through the reaction of hydrolysis yield of paracetamol with 1-naphthol and in the presence of sodium metaperiodate.

Interference Studies

A systematic study of interferences has been carried out including the effects of six typical active principles which are currently present in pharmaceutical preparations (acetylsalicylic acid, citric acid, codeine, ascorbic acid, phenylephrine hydrochloride and promethazine hydrochloride) and 8 typical excipients (starch, glucose, lactose, fructose, sucrose, sodium chloride, talc and magnesium stearate). All those compounds are unable to form PAP under the experimental conditions and no peaks were obtained at 600 nm for concentrations of excipients ten-times higher than paracetamol.

Analytical Applications

The proposed method was applied successfully to the assay of commercial dosage forms containing paracetamol. The results in Table 3 are in accordance with those obtained by the official method (21, 22). In the t-and F-tests (23), no significant differences in precision and accuracy were found between the calculated and the theoretical values of the 95% confidence limit of both the proposed and official methods.

Table 3. Application of the proposed and official methods to the determination of paracetamol in pharmaceutical preparations.

Pharmaceutical preparations[a]	Certified value (mg)	Proposed method		Official method recovery (%)
		Amount found[b] (mg)	Recovery[b] (%)	
Paracetamol tablets	500	510.30	102.06	99.38[c]
Paracetol tablets	500	513.85	102.77	98.92[c]
Colden tablets	450	456.47	101.43	100.13[c]
Algesic tablets	350	355.24	101.50	102.32[c]
Myogesic tablets	450	464.37	103.19	102.07[c]
Antipyrol suppositories	120	118.23	98.53	99.24[d]

[a] see Table 1 for details

[b] for three determinations of 4.8, 12,16.8 μg/ml.

[c] British pharmacopia B.P.

[d] S.D.I. standard methods

[e] The calculated and tabulated values of t and F at the 95% confidence limit are 1.37, 1.22 and 2.23, 4.95 respectively.

CONCLUSION

The proposed method is found to be simple, rapid and economical and will compete with most of the spectrophotometric methods available in the literature. The proposed method is advantageous over many methods, as heating, pH control or extraction is avoided. The statistical parameters and recovery study data indicate the reproducibility and accuracy of the method. This method is recommended as a useful tool for the analysis of paracetamol in pharmaceutical preparations.

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