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Indirect spectrophotometric determination of paracetamol via oxidative coupling reaction using chlorocresol reagent

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الخلاصة

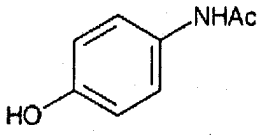
تم وصف طريقة طيفية لتقدير كميات مايكروغرامية من الباراسيتامول في المحلول المائي. تعتمد الطريقة على مفاعلة ناتج التحلل المائي الحامضي للباراسيتامول(بارا-امينوفينول) مع الكاشف كلوروكريسول بوجود الأوكسجين الجوي المذاب وكاربونات الصوديوم لتكوين ناتج ازرق (صبغة الاندوفينول) يمتلك أقصى امتصاص عند طول موجي 630 نانوميتر وبامتصاصية مولارية 8.63×10^3 لتر/مول. سم وأن قانون بير ينطبق ضمن مدى التراكيز 0.1-8.0 مايكروغرام/مللتر. وطبقت الطريقة بنجاح في تقدير الباراسيتامول في حالته النقية وفي بعض مستحضراته الصيدلانية وتم مقارنة النتائج مع الطرائق القياسية المعتمدة.

ABSTRACT

A spectrophotometric method is proposed for the determination of microgram amounts of paracetamol based on the reaction of hydrolytic product of paracetamol (p-aminophenol) with chlorochresol in the presence of dissolved atmospheric oxygen and sodium carbonate. The absorbance of the blue indophenol dye is measured at 630 nm and the molar absorptivity found to be 8.63×10^3 l.mol^{-1} .cm^{-1} and conforms to Beer's law obeyed over the range 0.1-8.0 µg ml^{-1}.The method is successfully employed for assay of paracetamol in various pharmaceutical formulations and results have been compared well with those obtained by the official methods indicating that the proposed method can be applied successfully in the assay of pharmaceutical preparations for paracetamol.

INTRODUCTION

Paracetamol (N-acetyl-p-aminophenol or acetaminophen) is an effective analgesic and tipyretic agent [1].



Numerous spectrophotometric methods have been reported for the determination of paracetamol (after its hydrolysis to p-aminophenol) in pure form, pharmaceutical preparations and biological fluids, using several coupling agents such as 8-hydroxyquinoline[2], o-cresol [3]and p-xylenol [4] in the presence of sodium periodate as oxidizing agent. Thymol has also been used as coupling reagent for the determination of paracetamol and its hydrolytic product in the presence of sodium metaperiodate[5], cerium(IV)sulphate[6] and potassium dichromate[7] as oxidizing agents. Also; charge transfer complex formation reaction has also been employed indirectly for the spectrophotometric determination of paracetamol using chloranil [8] and 2,3-dichloro-5,6-dicyano-p-benzoquinone(DDQ)[9] as π-acceptors. Spectrophotometric methods based on azo-dye formation reactions have also been used for indirect determination of paracetamol[10,11]. Recently, direct spectrophotometric method has been described for the determination of paracetamol by its reaction with Cu-NTA complex in aqueous solution leading to increase the complex absorbance at λmax=832 nm[12] Moreover, derivative[13] and differential [14] spectroscopic methods, gravimetric [15], titrimetric [16], fluorimetric [17], chromatographic [18] and electrochemical procedures [19] have also been reported. Some of these procedures suffer from interferences from other active ingredients or additives especially those carrying phenolic or amine functional groups, and chromatographic separation is usually required.

The present work describes an indirect spectrophotometric method for assay of paracetamol in pharmaceutical preparations based on oxidative coupling reaction with chlorocresol in the presence of dissolved atmospheric oxygen and sodium carbonate.

EXPERIMENTAL

Apparatus

All spectral absorbance measurements were carried out on double beam spectrophotometer Shimadzu (UV-160 A) using 1-cm silica cell.

Reagents

All chemicals used were of analytical reagent grade and paracetamol standard material was provided from the state company for drug industries and medical appliances-(SDI) Sammara-Iraq.

Chlorocresol solution, 1×10^{-2} *M*

Prepared by dissolving 0.1425 g of 4-chloro-3-methylphenol in distilled water and then the solution was made up to 100 ml in volumetric flask with distilled water.

Sodium carbonate solution, 0.1 M

Prepared by dissolving 1.06 g of sodium carbonate in distilled water and completed the volume to 100 ml in volumetric flask with distilled water.

Paracetamol solution, 2000 μg ml^{-1}

Prepared by dissolving 0.2 g of pure paracetamol powder in 10 ml of ethanol and the solution was made up to 100 ml in a volumetric flask with distilled water.

Stock solution of hydrolyzed paracetamol (100μg ml^{-1})

Accurately 75 ml of 2000 μg ml^{-1}paracetamol was transferred into 250 ml round buttom flask provided with condenser, 25 ml of 11.8 M HCl was added and refluxed for 1houre. Then cooled and the solution was neutralized by 20% Na_2CO_3 and diluted to 250 ml with distilled water in a volumetric flask, then 16.7 ml from this solution was transferred to 100 ml volumetric flask and completed to the mark with distilled water to obtain a concentration of 100μg ml^{-1} [8].

Preparation of samples

Tablets

Weighed and finely powdered 10 tablets. An accurately weighed amount of powder equivalent to 0.2 g of paracetamol was transferred into 100 ml calibrated flask, dissolved in 5 ml of ethanol and completed the volume with distilled water. Filtered through a Whatmann 41 filter paper, and proceed the procedure as mentioned under the stock solution of hydrolyzed paracetamol.

Syrup

Accurately a volume of syrup sample was measured equivalent to 0.2 g of paracetamol and transferred it to a 100 ml calibrated flask, and proceed the procedure for the stock solution of hydrolyzed paracetamol.

Suppositories

Weighed and mixed well 5 suppositories. An accurately weighed amount of mixture equivalent to 0.2 g of paracetamol was dissolved in boiling water, filtered and the residues were washed by 10 ml of ethanol and distilled water, cooled and diluted to 100 ml into calibrated flask with distilled water, and proceed the procedure for the stock solution of hydrolyzed paracetamol.

General assay procedure

Transfer different amounts of hydrolyzed paracetamol standard sample solution into a series of 25-ml volumetric flasks covering the range 0.1-8.0μg ml^{-1}in final volume. Add 1ml of 0.1M sodium carbonate solution followed by 4ml of chlorocresol reagent solution. Stopper the flasks and shake well, then allow them to stand for 25 min in a water bath adjusted at 60°C. Cool

and dilute to the mark with distilled water, then the absorbance is measured at 630 nm against the reagent blank.

RESULTS AND DISCUSSION

Absorption spectra

p-Aminophenol (the hydrolyzed product of paracetamol) was allowed to react with chlorocresol in the presence of dissolved atmospheric oxygen and a base to produce a blue colour (λ_{max}=630 nm). The absorption spectra of the reaction product is shown in Fig.1. Colourless reagent blank was obtained.

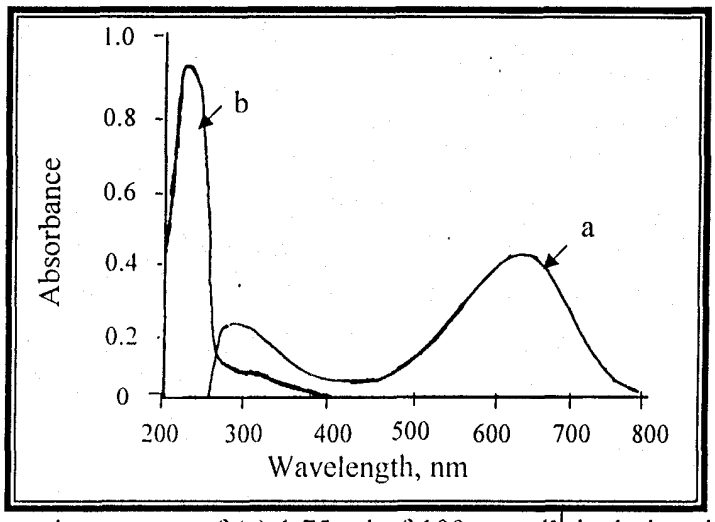


Fig.1 Absorption spectra of (a) 1.75 ml of 100 μg ml^{-1} hydrolyzed paracetamol with 4ml of 1×10^{-2} M chlorocresol and 1ml of 0.1M Na_2CO_3 in final volume of 25-ml versus blank, and (b) 4ml of 1×10^{-2} M chlorocresol and 1ml of 0.1M Na_2CO_3 in final volume of 25-ml versus distilled water.

Effect of base

Various bases were examined in order to obtain high sensitivity, and it was found that 1ml of 0.1M of sodium carbonate gave maximum colour intensity (Table1) which is used in all subsequent experiments. In addition, it was found that the final pH of reaction mixture is 10.1. Therefore various buffers having the same pH including $NaHCO_3$-Na_2CO_3 (B1), $Na_2B_4O_7$-NaOH (B2), NaCl-NaOH (B3) and NaH_2PO_4-NaOH (B4) were tried and found that there is negative effect on the colour intensity, in addition, it was found that B1buffer solution leading to increase the absorption of the blank solution at λ_{max} of the product(Table 2).

Table (1) Effect of bases on the intensity of the product colour

Base (0.1M)	NaOH	KOH	Na_2CO_3	NH_4OH
Absorbance	0.198	0.215	0.237	0.098

Table (2) Effect of buffer solutions on the intensity of the product colour

Buffer solution (1.0ml)	B1	B2	B3	B4
Absorbance	0.231	0.222	0.184	0.186

Effect of chlorocresol concentration

The effect of changing the chlorocresol concentration on the absorbance of solution containing 4μg ml^{-1} hydrolyzed paracetamol was studied. From Fig.2, it is evident that the absorbance increases with increasing in chlorocresol concentration but reached maximum when using 4ml of 1×10^{-2}M chlorocresol. Therefore, this amount was used in all subsequent experiments.

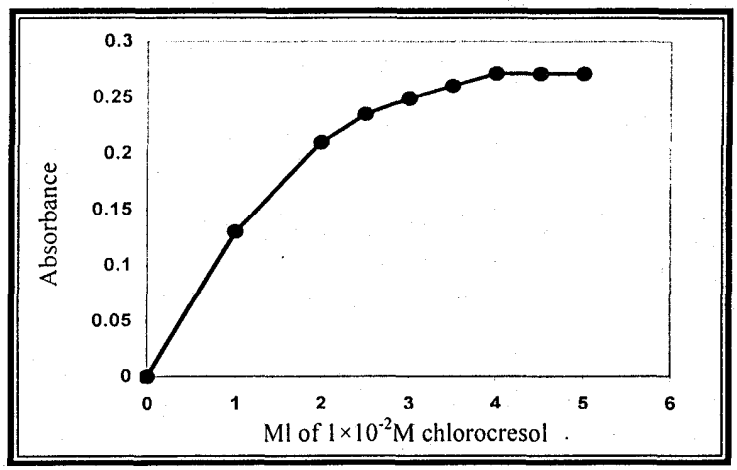


Fig.2 Effect of chlorocresol amount on the absorption intensity of the reaction product of 4μg ml^{-1} hydrolyzed paracetamol

Effect of temperature and reaction time

The reaction time was determined by following the colour development at room temperature and in thermostatically controlled water-bath at different temperatures. The absorbance was measured at 5min intervals against reagent blank treated similarly. It was observed that the absorbance reached maximum after 25min at 60°C and remain constant more than 3hours. Thus this temperature and reaction time were chosen for colour development.

Quantification

Under the proposed experimental conditions, a linear relation between the absorbance and concentration of hydrolyzed paracetamol was observed over the concentration range 0.1- 8.0 μg ml^{-1} (Fig.3) and negative deviation from Beer's law was observed at higher concentration of paracetamol , The average molar absorptivity is 8.63×10^{3} $l.mol^{-1}$ $.cm^{-1}$ and the correlation coefficient of 0.9997 and intercept of 0.00021.

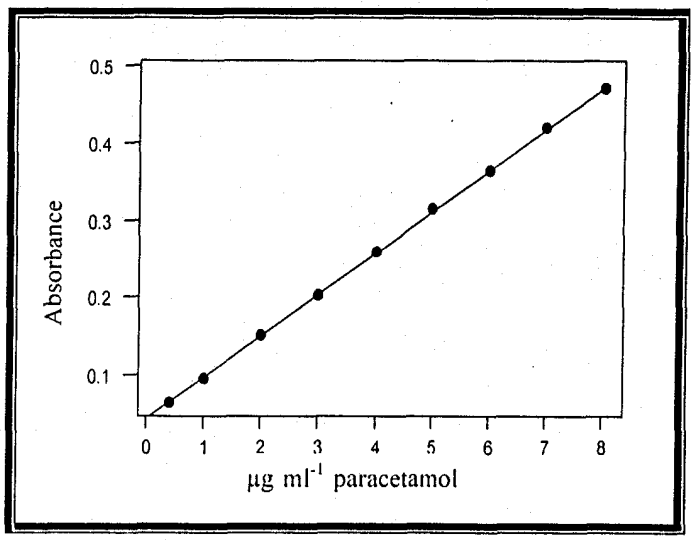


Fig.(3) Calibration graph of hydrolyzed paracetamol

Accuracy and precision

To determine the accuracy and precision of the method, paracetamol was determined at three different concentrations. The results shown in table(3) indicating that satisfactory precision and accuracy could be obtained with the proposed method.

Table(3) Accuracy and precision of the proposed method

Amount of hydrolyzed paracetamol taken/μg ml^{-1}	Recovery* (%)	Relative standard deviation* (R.S.D %)
2	100.2	0.219
4	100.1	0.152
6	99.8	0.100

*Average for five determinations

Nature of product and reaction mechanism

The stoicheiometry of the reaction between the hydrolyzed paracetamol and chlorocresol in the presence of atmospheric oxygen and sodium carbonate was investigated using Job's method [20]; the results obtained (Fig.4) show that 1:1 is the drug to reagent ratio.

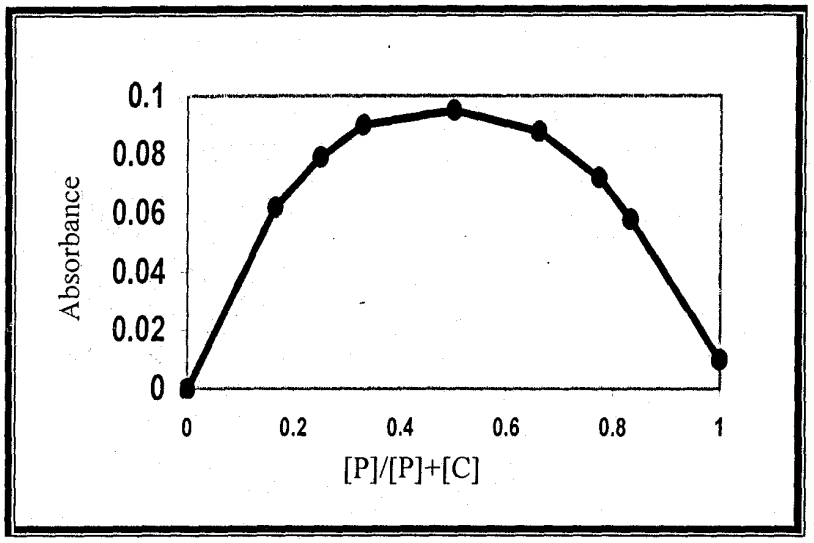
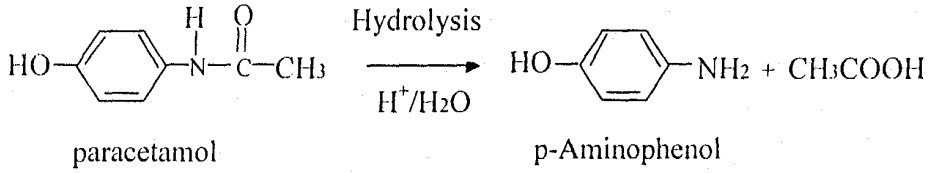
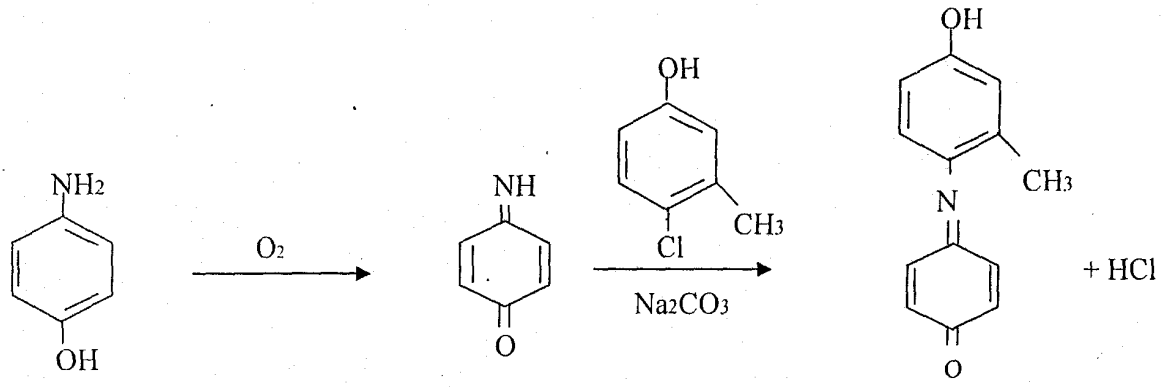


Fig.(4) Job's plot of hydrolyzed paracetamol–chlorocresol product ratio (*[P],[C]* are the concentration of hydrolyzed paracetamol and chlorocresol respectively).

Therefore the following scheme of reactions may be suggested



paracetamol p-Aminophenol



p-Benzoquinone imine Indophenol blue dye

The product formed was water soluble. The liberation of chloride ion was proved by addition drops of silver nitrate to the solution mixture and a turbidity was observed. The apparent stability constant was calculated by comparing the absorbance of a solution containing stoicheiometric amount of hydrolyzed paracetamol and chlorocresol with that of solution containing excess amount of chlorocresol reagent(4ml of 1×10^{-2}M). The average conditional stability constant of the product in water under the described experimental conditions was 3.544×10^{3} L.mol^{-1} .

Interferences

Compounds which are usually dispensed with paracetamol such as poly vinyl pyrrolidine, starch maize, talc, magnesium state, glycine, propylhydroxy benzoate and methylhydroxyl benzoate, were examined on the absorption of 100µg paracetamol in final volume of 25 ml. It was found that addition of 1000µg of these excipients did not interfere (Table 4).

Table(4) Interference effect of excipients(1000 µg) on the recovery of 100µg of paracetamol

Excipients	Recovery %
Talc	96.26
Starch maize	102.50
Magnesium stearate	97.50
Glycerin	98.75
Poly vinyl pyrrolidine	103.75
Propylhydroxy benzoate	98.75
Methylhydroxy benzoate	95.00

Applications

The proposed method was applied to determine paracetamol in pure form and in pharmaceutical formulations after its hydrolysis to p-aminophenol. Several commercially available paracetamol formulations including tablets, syrup and suppositories were analyzed for their contents of paracetamol without further treatment. On applying the proposed procedure, good recoveries were obtained with these formulations (Table 5), and the results obtained were compared favorably with the official standard methods (Table 6).

Table(5) Application of the proposed method for determination of paracetamol in pharmaceutical formulations

Pharmaceutical formulation	Certified value (mg)	Drug content found (mg)	Recovery* (%)	R.S.D* (%)
Paracetamol tablet	500	496.5	99.3	0.841
Algesic tablet	350	353.7	101.15	1.061
Antipyrol suppositories	250	249.7	99.88	0.911
Antipyrol syrup	120	118.2	98.51	1.232

*Average for five determinations

Table(6) Comparison of the proposed method with standard methods

Drug formulations	Recovery %	
	Proposed method	Standard methods
Paracetamol tablet	99.3	101.0 [a]
Algesic tablet	101.15	99.0 [b]
Antipyrol syrup	98.51	99.7 [b]
Antipyrol suppositories	99.88	100.9 [c]

[a] B.P(British Pharmacopoeia)
[b] S.D.I (Sammara drug industries)
[c] U.S.P (United state pharmacopoeia)

CONCLUSION

A spectrophotometric method has been proposed for the determination of paracetamol in pure form. It has been shown that the proposed method is rapid, simple and sensitive for the determination of paracetamol in its pharmaceutical preparations without interference from commonly used excipients. It provides accurate and precise results and could be easily used in a quality control laboratory for assay of paracetamol.

REFERENCES

1. Katzung B.G.," Basic and clinical pharmacology", Appleton and Lange A Simon and Schuster Company, New Jersy, U.S.A, p.743 (1998).
2. Bouhsain Z., Garrigues S., Morales A. and Guardia M., Anal.Chim.Acta, 330,59 (1996).
3. Criado A., Cardenas S. and Callego M., Talanta,53,417 (2000).
4. Afshari J.T. and Liu T.Z., Anal.Chim.Acta, 443, 165 (2001).
5. Al-Ward H.S.J., "Analytical pharmaceutical applications on oxidative coupling reaction", M.Sc.Thesis, University of Baghdad (2002).
6. Sultan S.M., Al-Zamil I.Z. and Al-Rahman A.M.A,Analyst,11,919 (1986).
7. Sultan S.M., Talanta , 34 , 605 (1987).
8. Al-Ghabsha T.S., Al-Abachi M.Q. and Ahmad A.K., Microchem. J., 35, 66 (1987).
9. Al-Enizzi M.S., "Development of spectrophotometric method for the determination of paracetamol,histamine and some sulpha drugs", M.Sc. thesis, Mosul University (2002).
10. Belal S.F., El-Sayed A.H. and El-Waily A, Pharm.Sci., 68, 750 (1979).
11. Gesso S.G., "Development of spectrophotometric method for trace determination of some organic compounds in pharmaceutical preparations", M.Sc.Thesis, University of Mosul (1991).
12. Al-Ghabsha T.S., Al-Sabha T.N. and Amal M. S., J.Educ.and Sci., accepted for publishing (2005)

13. Sharaf El-Din M. K., Abuijeie M. A. and Abdel Hay M. H., Anal. Lett.,24, 2187 (1991).
14. El-Kheir A.A., Belal S. and El-Shanawani, Pharmazie, 40, 62 (1985).
15. Yesiada A., Erdogen H. and Ertan M., Anal. Lett.,24,129 (1991).
16. Parimco P., Sethuraman R.R., Amalraj A. and Seshadari N., Indian Drugs, 26, 1450 (1989).
17. Calatayud J.M. and Benito C.G., Anal. Chim. Acta, 231,259 (1990).
18. Krieger D.J., J. Assoc. Off. Anal. Chem., 67,339 (1984).
19. Lau O., Luk S. and Cheung Y., Analyst, 114,1047 (1989).
20. Christian G.D., Analytical Chemistry, 5th Edn., Wiley, New York, pp. 385 & 386(1994).