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Indirect spectrophotometric determination of paracetamol via oxidative coupling reaction with 4-(2-pyridylazo)-resorcinol

Thabit S. Al-Ghabsha Theia'a N. Al-Sabha Amal M. Saeed

Chemistry department, college of Education
Mosul University
Mosul, Iraq

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الخلاصـــة

تم وصف طريقة طيفية سهلة وسـريعة وحـساسة لتقـدير كميـات ضـئيلة مـن الباراسيتامول في المحلول المائي. تعتمد الطريقة على أكسدة ناتج التحلل المـائي الحامـضي للباراسيتامول بواسطة بيريودات البوتاسيوم واقترانه مع الكاشـف 4-(2-بريـديل أزو)-ريسورسينول بوجود هيدروكسيد الصوديوم لتكوين ناتج قهـوائي مـصفر يمتلـك أقـصى امتصاص عند طول موجي 450 نانوميتر وبامتصاصية مولارية 5252 لتر/مول. سم وأن قانون بير ينطبق ضمن مدى التراكيز 0.4-22 مايكروغرام/مللتر. وطبقت الطريقة بنجاح في تقدير الباراسيتامول في حالته النقية وفي الحبوب وتم مقارنة النتائج مـع الطريقـة القياسـية المعتمدة.

Abstract

A rapid, sensitive and simple spectrophotometric method is proposed for the determination of paracetamol. The method is based on the oxidation of the hydrolytic product of paracetamol by potassium periodate and coupled with 4-(2-pyridylazo)-resorcinol in the presence of sodium hydroxide. The absorbance of the yellowish-brown product is measured at 450 nm and the molar absorptivity found to be 5252 $l.mol^{-1}.cm^{-1}$. The product conforms to Beer's law over the range 0.4-22.0μg ml^{-1}. The method is successfully employed for assay of paracetamol in tablets and results have been compared with those obtained by the official method.

Keywords: Paracetamol; oxidative coupling; spectrophotometry.

Introduction

Paracetamol (N-acetyl-p-aminophenol or acetaminophen) is an effective analgesic and antipyretic agent. p-Aminophenol is the hydrolytic product of paracetamol and is reported to have significant nephrotoxicity and teratgenic effects [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 reagents such as 8-hydroxyquioline[2], o-cresol [3] and p-xylenol [4] in the presence of sodium periodate as oxidizing agent. Thymol has 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 reagents. Also; charge transfer complexes using chloranil [8], 2,3-dichloro-5,6-dicyano-p-benzoquinone [9] and bromanil [10] as π-acceptors have been employed indirectly for the spectrophotometric determination of paracetamol. Azo-dye formation reactions have also been used for the indirect spectrophotometric determination of paracetamol [11,12], Moreover, derivative[13] and differential [14] spectroscopic methods have been described for the determination of paracetamol. Copper-NTA complex is used for determination of paracetamol by its enhancement the sensitivity of the this complex[15]. Near-infrared (NIR) reflectance spectroscopy was used to determine the content of paracetamol in bulk batches of intact Sterwin 500 mg tablets by collecting NIR spectra in the range 1100–2500 nm and using a multiple linear regression calibration method[16].A flow injection method is described for determination of paracetamol in pharmaceutical dosage forms based on the nitration of paracetamol with sodium nitrite, and the absorption of the product was measured at 430 nm in alkaline medium[17]. Some of these procedures suffer from interferences from other active ingredients or additives especially those carrying phenolic or amine functional groups.

The present work describes an indirect spectrophotometric method for assay of paracetamol in pharmaceutical preparations based on oxidative coupling reaction with 4-(2-pyridylazo)-resorcinol reagent in the presence of potassium periodate and sodium hydroxide.

Experimental

Apparatus

All spectral absorbance measurements were carried out on Shimadzu (UV-210) Double-beam Spectrophotometer using 1-cm silica cells.

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, Sammara-Iraq.

4-(2-Pyridylazo)-resorcinol solution: 1×10^{-3} M prepared by dissolving 0.1366 g of 4-(2-pyridylazo)-resorcinol monosodium dehydrate (Hopkins & Williams) in distilled water and then the solution was made up to 500 ml in volumetric flask with the same solvent and kept in amber bottle. This solution is stable at least for two weeks.

Potassium periodate solution: 0.01 M prepared by dissolving 0.230g of potassium periodate in distilled water and complete the volume to 100 ml in volumetric flask with distilled water.

Sodium hydroxide solution: 0.1M prepared by dissolving 0.4g of sodium hydroxide in 100-ml volumetric flask and complete the volume to the mark with distilled water.

Paracetamol solution :2000μgml^{-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 conical flask provided with condenser, add 25 ml of 11.8 M HCl and refluxed for 1hour. 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 of this solution was transferred to 100 ml volumetric flask and complete the volume with distilled water to obtain a concentration of 100μg ml^{-1} .

Preparation of tablet samples

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 complete the volume with distilled water. Filtered through a Whatmann 41 filter paper, and proceed the procedure as 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 0.4-22.0 μg ml^{-1} in final volume. Add 3ml of 4-(2-pyridylazo)-resorcinol reagent solution followed by 1ml of potassium periodate solution and 1ml of sodium hydroxide, then dilute to the mark with distilled water. Stopper the flasks and shake well, then allow them to

stand for 20 min at room temperature, and the absorbance is measured at 450 nm against the reagent blank.

Results and discussion

Absorption spectra

p-Aminophenol (the hydrolyzed product of paracetamol) was allowed to react with 4-(2-pyridylazo)-resorcinol in the presence of potassium periodate as an oxidizing reagent and sodium hydroxide to produce a yellowish-brown colour with maximum absorption at 450 nm while the reagent blank shows low absorption at this wavelength (Fig.1).

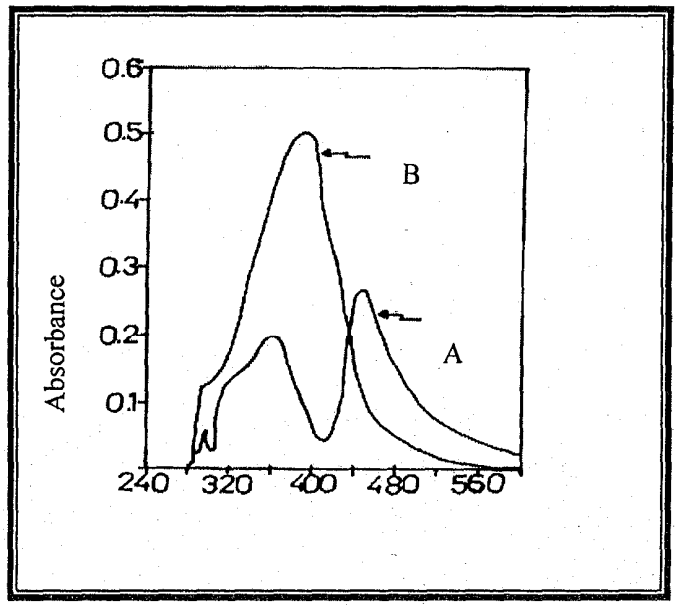

Fig.1 Absorption spectra of (a) 2ml of 100μg ml^{-1} hydrolyzed paracetamol with 3ml of 1×10^{-3} M 4-(2-pyridylazo)-resorcinol and 1ml of 0.01M potassium periodate solution and 1ml of sodium hydroxide in final volume of 25-ml versus blank, and(b) 3ml of 1×10^{-3} M 4-(2-pyridylazo)- resorcinol in final volume of 25-ml versus distilled water.

Effect of amounts of potassium periodate

Increasing volumes of 0.01M potassium periodate are added to the fixed amounts of hydrolytic product of paracetamol (8μg/ml) and NaOH solution(1ml of 0.1M). It was found that 1ml of potassium periodate gave maximum colour intensity which is used in all subsequent experiments.

Effect of pH and buffers

The effect of pH ranged from 4 to 12 on the absorption of the product was studied using different concentrations of HCl and NaOH. It was found that the product formed in the final pH of 9 using 0.1 M of NaOH (Table1). Therefore different buffers of pH_9 are prepared such as phosphate, borate and carbonate to examine the sensitivity. It was found that the these buffers decrease the absorbance (Table2).

Table 1. Effect of pH on the absorption of the product

Final pH	4	6	7	8	9	10	11	12
Absorbance	0.173	0.192	0.209	0.267	0.280	0.251	0.249	0.223

Table 2: Effect of buffers on the absorption of the product

Buffer type(pH=9)	Absorbance
Without*	0.201
Phosphate	0.234
Borate	0.224
Carbonate	0.216
Sodium hydroxide	0.280

*Without addition of buffer or sodium hydroxide

Effect of NaOH concentration

Increasing volumes of 0.1M NaOH are added to the fixed amounts of hydrolytic product of paracetamol (8μg/ml) and KIO_4(1ml of 0.01M). As shown in table 3 it was found that 1ml of NaOH gave high absorbance and used in all subsequent experiments.

Table 3: Effect of NaOH concentration on the absorption of the product

0.1M NaOH (ml)	0.3	0.5	0.7	1.0	1.3	1.5
Absorbance	0.235	0.243	0.262	0.281	0.260	0.257

Effect of 4-(2-pyridylazo)-resorcinol concentration

The effect of different 4-(2-pyridylazo)-resorcinol concentrations on the absorbance of solution containing 8μgml^{-1} hydrolyzed paracetamol was studied. It is evident that the absorbance increases with increasing 4-(2-pyridylazo)-resorcinol concentration and reached maximum on using

3ml of 1×10^{-3}M 4-(2-pyridylazo)-resorcinol. Therefore, this concentration was used in all subsequent work.

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 adjusted at 0, 40 and 50°C. The absorbance was measured at 5min intervals against reagent blank treated similarly. It was observed that the absorbance reached maximum after 20 min at room temperature and remains constant more than 6 hours and the absorbance decreased slowly thereafter. Hence room temperature and reaction time(20 min) were chosen for colour development.

Effect of order of addition

The effect of order of addition on the absorbance of the product was studied under the optimum experimental conditions, and the results are shown in the table(4) indicating that the product is formed with high sensitivity by the following order:

Hydrolyzed paracetamol + 4-(2-pyridylazo)-resorcinol + potassium periodate + sodium hydroxide

Table 4: Effect of order of addition

Order of addition	Absorbance
S+R+B+O	0.270
S+R+O+B	0.280
O+S+R+B	0.275
O+R+S+B	0.264
B+O+S+R	0.269
B+O+R+S	0.227
R+O+B+S	0.221
S+O+B+R	0.274

Assuming that:

S=sample , R= reagent , B= base , O= oxidizing agent

Quantitation

Under the proposed experimental conditions, a linear relation between the absorbance and concentration of hydrolyzed paracetamol was observed over the concentration range 0.4 - 22.0 $\mu g\ ml^{-1}$ (Fig.2), with correlation coefficient of 0.999 and intercept of 0.03406.A negative deviation from Beer's law was observed at higher concentrations of paracetamol. The molar absorptivity is 5252 $l.mol^{-1}.cm^{-1}$.

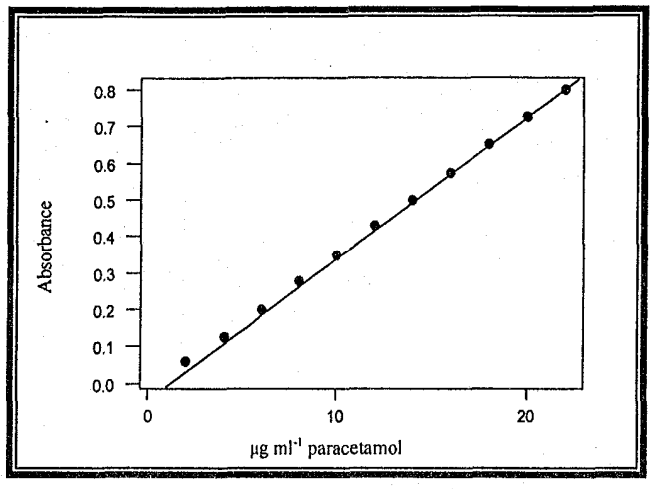

Fig.2 Calibration graph of hydrolyzed paracetamol

Accuracy and precision

To determine the accuracy and precision of the method, paracetamol was determined at three different concentrations. The results shown in table(5) referring a satisfactory precision and accuracy.

Table 5: Accuracy and precision of the proposed method

Amount of hydrolyzed paracetamol taken(µg ml^{-1})	Recovery* (%)	Relative standard deviation* (RSD %)
8	100.53	0.234
12	100.58	0.230
16	99.50	0.172

*Average for six determinations

Interferences

In order to assess the possible analytical applications of the proposed method, the effect of some common excipients usually found with paracetamol drug in pharmaceutical formulations, such as acacia, glucose, lactose, starch, sodium chloride and magnesium stearate was studied by analyzing synthetic sample solutions containing 10 µg/ml of paracetamol and excess amounts (10-fold excess) of each excipient, none of these substances interfered seriously (Table 6).

Drug formulations	Recovery %	
	Proposed method	Standard method*
Paracetamol tablet	99.23	101.0
Algesic tablet	101.13	99.0
Dolo tablet	100.32	99.7
Colden tablet	100.70	100.9

* British Pharmacopoeia (standard method) [19]

Conclusion

Indirect 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 assay of paracetamol in its pharmaceutical tablets without interference from commonly used excipients.

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