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Indirect Spectrophotometric Assay of Paracetamol in Pharmaceutical Preparations

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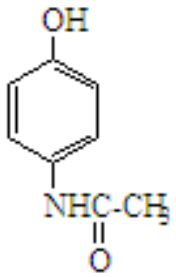
ABSTRACT

A simple ,accurate and sensitive indirect spectrophotometric method for the determination of paracetamol (PAR) in various formulations has been suggested. The method was based on acid hydrolysis of PAR to produced p-aminophenol(PAP), after that diazotization of PAP with nitrite ion to form the corresponding diazonium salt followed by coupling reaction with 2,7-dihydroxy naphthalene (2,7-DHN) in alkaline medium to produced a colored azo dye that showed maximum absorbance at 481 nm. . Beer's law was obeyed in the concentration range of 1-14 µg.ml^{-1} . The molar absorpitivity and Sandell's sensitivity of the colored dye were 1.058x10^{4} l.mol^{-1}.cm^{-1} and 0.0142 µg.cm^{-2} respectively. Limit of detection(LOD) 0.006 µg.ml^{-1} ,limit of quantitation (LOQ) 0.023 µg.ml^{-1}.A relative error ranged from1.5 to 1.8%, and the relative standard deviation was not more than ±3.1% depending on the concentration of the drug. The method was successfully applied to the determination of PAR in pure form, and its pharmaceutical preparations.

Keyword: paracetamol, p-aminophenol, 2,7-dihydroxy naphthalene, diazotisation, spectrophotometric determination

INTRODUCTION

Paracetamol [acetaminophen, N-acetyl-*p*-aminophenol, 4-acetamidophenol],it has analgesic and antipyretic properties with week anti-inflammatory activity, it is used in the symptomatic management of moderate pain and fever. PAR is available in different pharmaceutical preparations such as: tablet, drops, capsules, injection and syrup [1].PAR has the following chemical structure[2].



Paracetamol, *N*-(4-Hydroxyphenyl) acetamide , M.wt = 151.2 g/mol).

Literature survey reveals various(direct and indirect) methods for quantitative determination of PAR, these methods included: HPLC and RP-HPLC[3-5], HPTLC and RP- HPLC[6] ,Voltammetric[7-10] and electrochemical methods [11]. According to the active groups in the chemical structure of paracetamol and the hydrolysed product(PAP), so that various methods have been used in spectrophotometric determination[12-21]. Another method included carbon nano-tubes supported Pd nano particles has been used[22].

The present indirect method involves the diazotisation of PAP which results from acid hydrolysis of PAR, followed by coupling with 2,7-DHN(this reagent was first used in the spectral estimation of paracetamol) in alkaline medium to form a highly colored azo dye ,that has been applied successfully for the assay of paracetamol in pharmaceutical preparations.

EXPERIMENTAL

Instruments

All spectrophotometric measurements were performed on Jasco V-630 Spectrophotometer using 1 cm quartz cells, pH meter type HANNA PH 211 was used for pH reading.

Reagents

All chemicals used in this investigation are of analytical – reagent grade, and paracetamol standard material was provided from General Establishment for Medical Appliance and Drugs / SDI – Samaraa / Iraq.

Solutions

Paracetamol solution,1000 µg.ml^{-1}. This solution was prepared by dissolving 0.25 g of paracetamol in 10 ml ethanol , then the solution was completed to 100 ml in a volumetric flask with distilled water[23].

Solution of hydrolyzed paracetamol(HPAR), PAP , 100 µg.ml^{-1}. This solution was prepared by transferring 150 ml of 1000 µg.ml^{-1} PAR into 250- ml round-bottomed flask provided with condenser,25ml of hydrochloric acid(11.8N) was added, then refluxed for 1 hour, after that the cold solution was neutralized with 20% of sodium carbonate solution and diluted to 250 ml with distilled water in a volumetric flask. To prepared 100 µg.ml^{-1}paracetamol, 16.6 ml of the above solution was diluted to 100 ml in a volumetric flask using distilled water[23].

Hydrochloric acid solution, 1N. This solution was prepared by diluting 8.5 ml of con.HCl (11.8 N) to 100 ml with distilled water in a volumetric flask.

Aqueous solutions of (0.1%,0.031 M) 2,7- DHN , (1% ,w/v) sodium nitrite and (3% ,w/v) sulphamic acid s were prepared by dissolving an appropriate weight in 100 ml distilled water.

Paracetamol tablets solution, 100 µg.ml^{-1}. Weight and finely powder 10 tablets (each one contains 500 mg PAR). An accurately weighed amount of powder equivalent to 0.25g PAR was dissolved in 10 ml ethanol, then100-150 ml distilled water was added, shaking to increased the solubility, filtered into 250 ml calibrated flask, then the solution was completed to the mark with distilled water, and proceed as mentioned above in preparation of HPAR solution.

Paracetamol syrup solution, 100 µg.ml^{-1}

A 10.41 ml of antipyrol syrup(each 5ml contain 120 mg PAR) was diluted to 250 ml with distilled water in a volumetric flask, then 150 ml was taken and proceed as mentioned above in preparation of HPAR solution.

Paracetamol injection solution, 100 µg.ml^{-1}

The contents of 3 injections were mixed, a 2.5 ml equivalent to 250 mg paracetamol was diluted to 250 ml with distilled water in a volumetric flask, then 150 ml was taken and proceed as mentioned above in preparation of HPAR solution.

Procedure and calibration graph

To a series of 10 - ml calibrated flasks, transfer 0.1 – 1.2 ml(100 µg.ml^{-1}) of PAP solution , then 0.5 ml of 1N hydrochloric acid and 0.7 ml of 1% sodium nitrite solution were added ,the mixture was allowed to stand for 5 minutes, and then 0.3 ml of 3% sulphamic acid solution was added with occasional shaking for 2 minutes. after that a 1 ml of 2,7- DHN solution(0.1%) and 2 ml of 1N sodium hydroxide solution were added . The volumes were completed to the mark with distilled water and the absorbance was read at 481 nm against the reagent blank . A linear calibration graph was obtained over the concentration range of 1 – 14 µg PAR . ml^{-1} (Fig. 1).

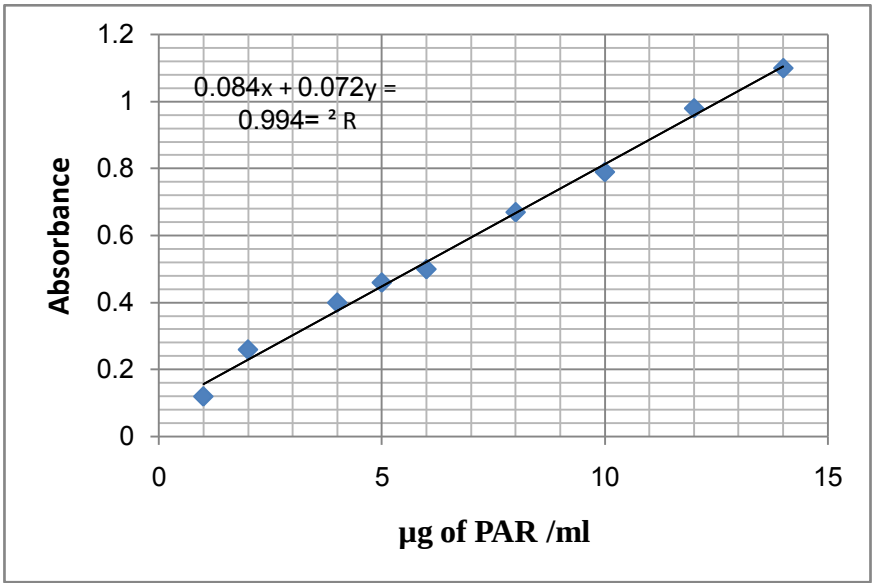


Fig. 1: Calibration graph of PAR determination.

The characteristic and the results of statistical analysis of the experimental data were summarized in (Table 1).

Table 1: Optical and regression characteristics of the present method

Parameter	Value
Beer's law(µg.ml^{-1})	1-14
λ_{max},(nm)	481
Molar absorptivity l.mol^{-1}.cm^{-1}	1.058x10^4
Relative standard deviation.	≤ ±3.1
Linear regression equation Slope = a Intercept = b	Y=ax* +b 0.072 +0.084
Determination coefficient(R^2).	0.9947
Limit of detection.(µg.ml^{-1})	0.006
Limit of quantitation.(µg.ml^{-1})	0.023

- Concentration in µg.ml^{-1}

RESULTS and DISCUSSION

During the investigation, PAP solution equivalent to 100 µg.ml^{-1} PAR, was taken and the final volumes were brought to 10 ml with distilled water.

Effect of diazotisation acid

Different types of acids(HCl,H_2SO_4,HNO_3 and CH_3COOH) have been selected in diazotisation of PAR, the results showed that the solution of 1N hydrochloric acid was the optimum acid, another experiment showed that 0.5 ml of 1N HCl was the optimum volume according to the high intensity of the azo dye, therefore 0.5 ml of 1NHCl was fixed in the subsequent experiments.

Effect of sodium nitrite amount and time

Different amount of $NaNO_2$(1%) with different standing time have been studded, the maximum absorbance reading was obtained by adding 0.7 ml of 1% sodium nitrite with 5 minutes reaction time (Table2).

Table 2: Effect of sodium nitrite amount and time on absorbance.

ml of (1%) $NaNO_2$	Absorbance/ minute				
	0	1	3	5	7
0.5	0.523	0.531	0.539	0.552	0.549
0.7	0.631	0.632	0.637	0.699	0.693
0.9	0.572	0.601	0.618	0.648	0.638

Effect of sulphamic acid amount and time

The presence of an excess of nitrite is undesirable due to its side reaction .Therefore, it should be removed by sulphamic acid which react more fast than urea[24,25].

$$HNO_2 + H_2N\text{-}SO_3H \xrightarrow{} N_2 + H_2O + H_2SO_4$$

The effect of sulphamic acid amount and time has been studied. The results showed that 0.3 ml of sulphamic acid solution (3%) with 2 minute standing time for complete reaction was the optimum amount and time.

Effect of 2,7- DHN amount

The effect of different amounts of 2,7- DHN solution (0.1%) on the intensity of the azo dye at different amounts (30-100 µg) of PAP has been studied. A 1 ml of 2,7- DHN solution in a total volume of 10 ml give the higher sensitivity and high value of determination coefficient (R^2), therefore it has been selected for the subsequent experiments (Table 3).

Table 3: Effect of coupling agent amount on absorbance.

2,7- DHN amount (ml)	Absorbance/µg PAP in 10 ml				
	30	**50**	**70**	**100**	**R^2**
0.3	0.210	0.332	0.391	0.612	0.888
0.5	0.299	0.401	0.620	0.791	0.9902
1.0	0.316	0.422	0.636	0.802	0.9980
1.25	0.272	0.365	0.455	0.615	0.9986

Effect of base

Previous experiments have been showed that the colored azo dye formed in alkaline medium, therefore different types of strong and weak bases have been studied (Table 4).

Table 4: Effect of bases on absorbance

Base	A	λ max,nm
NaOH	0.781	480
KOH	0.602	495

Na_2CO_3	0.409	484
$NaHCO_3$	0.311	474

The result in(Table 4) indicated that the reaction needs a strong alkaline medium and NaOH gives the best results ,also the amount of NaOH has been studied , the optimum volume was 2 ml, it gives the high intensity of the azo dye,so that it selected on the subsequent experiments.

Final absorption spectrum

The absorption spectrum of the orange azo dye formed from coupling of diazotized PAP with 2,7- DHN in alkaline medium showed a maximum absorption at 481 nm. (Fig. 2).

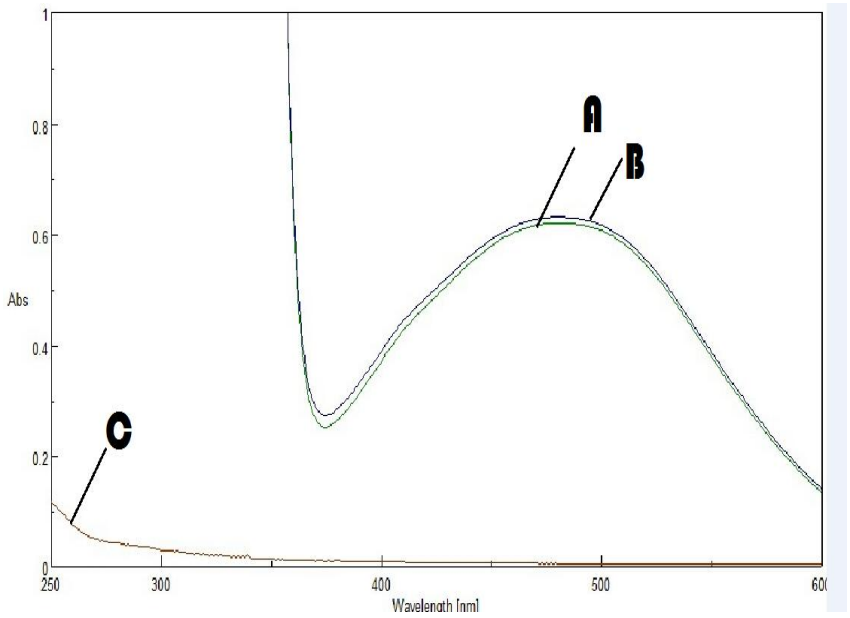
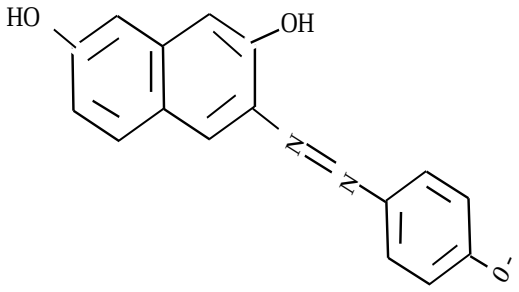


Fig. 2: Absorption spectra of 80µg PAR / 10ml treated according to the recommended procedure and measured against (A) reagent blank, (B) distilled water and (C) reagent blank measured against distilled water

Nature of the dye

The stoichiometry of the formed azo dye between diazotized PAP and 2,7-DHN was investigated by applying the continuous- variations method and mole- ratio method[26].The results indicate that the azo-dye has formed in the ratio of 1:1 diazotised PAP to 2,7- DHN, Since the ortho position of the hydroxyl group is not reserved, for that coupling of diazotized, it occurs at the ortho position[27] and the azo dye may have the following suggested structure:



Orange azo dye

Analytical application

The proposed method was applied to determine PAR in different pharmaceutical preparations. On applying proposed procedure, good recovery was obtained as shown in (Table 5).

Table 5. Analytical applications of the proposed method.

Pharmaceutical preparation	µg paracetamol present/10ml	µg paracetamol measured/10ml	Recovery*,%	RSD,%
Paracetamol tablets/ 500 mg S.D.I-Iraq	50	48.7	97.5	± 2.9
	100	102.5	101.5	± 2.1
Paracetamol tablets 1000 mg Barakat,Syria	50	48.7	97.5	± 1.5
	100	101.2	101.2	± 3.1
Sudefed Research Pharmaceuticals/India	50	51.2	102.5	± 1.7
	100	101.2	101.2	± 2.6
Antipyrol ,Syrup S.D.I-Iraq	50	50.2	100.4	±0.7
	100	100.5	100.5	±.1.2
Paracetamol injection 500mg/5ml harmaceuticals/India	50	50.3	100.6	±.0.9
	100	99.2	99.2	±.1.4

*****Average of five determinations.

CONCLUSION

The suggested procedure for PAR determination is sensitive , accurate and can be used in determination PAR in different types of dosage formulations without extraction or separation.

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