Spectrophotometric Determination of Paracetamol Using Diazotization Coupling Reaction

Enas S. Thanoon
Department of Chemistry/ College of Science/ University of Mosul
E-mail: enashamoo1981@gmail.com

ABSTRACT

An accurate, simple, and sensitive indirect spectrophotometric method which proposed and developed for the determination of paracetamol in different pharmaceutical preparations. The proposed method was based on acid hydrolysis of PAR to produced p-aminophenol (PAP), PAP was diazotization with nitrite ion to form the corresponding diazonium salt, followed by coupling with histidine reagent in alkaline medium to produced azo dye that showed maximum absorbance at 430 nm. Beer's law was obeyed in the concentration rage of 10-500 µg/20 ml (i.e. 0.5-25 ppm). The molar absorpitivity and Sandell's sensitivity of the dye were. 1.118×10^4 l.mol^{-1}.cm^{-1} and 0.0135 µg.cm^{-2} respectively. The method successfully has been applied for the determination of PAR in pure form,and its pharmaceutical preparations (tablets, syrup and injection).

Keywords: Paracetamol, Indirect Spectrophotometric, Histidine, P-aminophenol.

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20/   500-10   .   430

  $^{1-}$.$^{1-}$. ^{4}10× 1.118   /   (25-0.5)

                                                   $^{2-}$.   0.0135

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INTRODUCTION

Paracetamol [N-(4-hydroxy-phenyl) acetamide] is a chemical compound and drug that is commonly used for headaches, other minor aches and pains of the management more sever pains where it gives room for additional non-steroidal anti-inflammatory drugs which have to be used at lower doses, for minimizing side effect (Chandka *et al.*, 2013). Paracetamol (PCM) is used as analgesic and antipyretic and anti-inflammatory which is effect of paracetamol due to the inhibiting prostaglandin synthesis cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (Kamble and Singh, 2012; Indian Pharmacopeia, 2007). Also paracetamol known as acetaminophen (USA) which is called by different trade names, including panadol, tylenol (derived from N-acetyl-p-aminophenol), and panadol extra among many other names (Marta and Jerzy, 2014).

Several analytical method were reported for the determination of paracetamol in pharmaceuticals such as, spectrophotometric (Hana and Nagham, 2018; Ahmed, 2017; Raymond *et al*., 2017; Sharma *et al*., 2015, Swetha *et al*., 2015; Ahmed and Abedlatif, 2015; Eglal *et al*., 2016; Omar, 2014; Vinay *et al*., 2013), HPLC and RP-HPLC (Chandra and Sharma, 2013; Ruchita et al., 2014; Fayek and Eglal, 2016), voltammetric (Ying *et al*., 2010; Chitravathi and Munichandraiah, 2016), chromatography (Dungl and Hail, 2016).

The present work is devoted to indirect spectrophotometric method for the determination of paracetamol included the diazotisation of p-amiophenol which results from acid hydrolysis of PAR, followed by coupling reaction with histidine reagent in the presence of alkaline medium to yield an azo dye that shows maximum absorbance at 340 nm, that has been applied successfully for the determination of paracetamol in pharmaceutical preparations.

EXPERIMENTAL

Spectral and absorbance measurements were carried out using Jasco V-630 computerized double beam spectrophotometer and 1-cm plastic cells, while the pH measurements were carried out with HANNA pH 24.

Reagents

All chemicals used are of the highest purity available.

Paracetamol solution (1000µg.ml^{-1}): 0.25 g of paracetamol was dissolved in 10 ml ethanol then the solution was completed with distilled water using a 250 ml volumetric flask (Raymond *et al*., 2017).

Solutions of hydrolyzed paracetamol (HPAR) (100µg.ml^{-1}): a 150 ml of 1000 µg.ml^{-1} PAR was transferred to 250 ml round bottomed flask provided with 20 ml of 4 M of HCl, then refluxed for 1 hour, after that the cold solution was neutralized with 20% of sodium carbonate solution then diluted with distilled water using a 250 ml volumetric flask. 16.6 ml of the above solution was diluted with distilled water in a 100 ml volumetric flask to prepare 100 µg.ml^{-1} PAR (Raymond *et al*., 2017).

Hydrochloric acid solution (4 M): This solution was prepared by diluting 21.8 ml of concentrated HCl with distilled water in a 200 ml volumetric flask (Raymond *et al*., 2017).

Histidine reagent solution (1×10^{-3} M): This solution was prepared by dissolving 0.015 g of hstidine (Fluka) in distilled water and completed to 100 ml with distilled water using a volumetric flask. Then transferred it to a brown bottle where it remains stable for at least one week.

Aqueous solution of (1% w/v) sodium nitrite and (3% w/v) sulphamic acid were prepared by dissolving an appropriate weight in 100 ml distilled water.

Paracetamol tablets solution (100 µg.ml^{-1}):
10 tablets were weight and powdered (each one contains 500 mg PAR). An equivalent weight to 0.25 g PAR was weighed and dissolved in 10 ml ethanol, then 100-150 ml distilled water was added, shaking well and filtered into 250 ml calibrated flask, then the solution was completed to mark using distilled water, and proceed as mentioned above in preparation of HPAR solution (Nabeel and Safaa, 2007).

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 with distilled water a 250 ml volumetric flask, then the 150 ml was taken and proceed as mentioned above in preparation of HPAR solution (Nabeel and Safaa, 2007).

Paracetamol solution syrup (100 µg.ml^{-1}): 10.41 ml of antipyrol syrup (each 5 ml contain 120 mg PAR) was diluted with distilled water in a 250 ml volumetric flask, then 150 ml was taken and proceed as mentioned above in preparation of HPAR solution (Nabeel and Safaa, 2007).

RESULTS AND DISCUSSION

The maximum absorbance value of the colored azo dye formed in an alkaline medium of 100 µg.ml^{-1} paracetamol (PAR) solution with histidine reagent was observed at 430 nm, in the final volume was brought to 20 ml with distilled water.

Effect of diazotisation acid:

In diazotisation of PAP, different types of acids have been selected (HCl, H2SO4, HNO3 and CH3COOH). The experiment showed that the solution of 1 N hydrochloric acid was the best, and another experiment showed that 1.5 ml of 1 N HCl as optimum volume as it showed high intensity of the azo dye, therefore 1.5 ml was fixed in the sequent experiment.

Effect of sodium nitrite amount and time:

The effect of NaNO2 (1%) has been studied with different amounts and standing times. The results in (Table 1) showed a maximum absorbance reading by adding 0.75 of (1%) sodium nitrate immediately.

Table 1: Effect of NaNO2 amount with time on absorbance

ml of 1% NaNO2	Absorbance / minute					
	0	**1**	**2**	**3**	**4**	**5**
0.25	0.353	0.342	0.362	0.382	0.331	0.310
0.50	0.391	0.353	0.342	0.301	0.272	0.261
0.75	0.410	0.387	0.393	0.332	0.330	0.324
1.0	0.350	0.326	0.282	0.273	0.188	0.105

Effect of sulphamic acid amount and time:

The effect of sulphamic acid amount and time has been studied. The optimum amount was 0.2 ml of sulphamic acid solution (3%) with 2 minute standing time for complete reaction, (Table 2).

Table 2: Effect of sulphamic acid amount with time

Ml of 3% sulphamic acid solution	Absorbance / minute				
	0	**1**	**2**	**3**	**4**
0	0.237	0.290	0.294	0.286	0.272
0.1	0.244	0.253	0.331	0.293	0.242
0.2	0.282	0.315	0.418	0.410	0.395
0.3	0.372	0.385	0.399	0.356	0.337
0.5	0.368	0.303	0.332	0.321	0.315
0.7	0.349	0.338	0.330	0.325	0.321
1.0	0.345	0.317	0.311	0.309	0.305
1.5	0.326	0.310	0.309	0.305	0.307
2.0	0.322	0.298	0.287	0.281	0.275

Effect of histidine amount:

Different volumes of 1×10^{-3} M histidine solution were added to (10-250 μg) of PAR while other conditions being kept constant. A 3 ml of the reagent solution has been found to be optimum volume since the linearity (determination coefficient) is good and the sensitivity of the colour reaction is fair, (Table 3).

Table 3: Effect of histidine amount

ml of 1×10^{-3} M histidine solution	Absorbance / ml of PAR								R^2
	0.1	0.3	0.5	0.7	1.0	1.5	2.0	2.5	
1.0	0.011	0.015	0.105	0.136	0.188	0.194	0.273	0.291	0.942169
2.0	0.035	0.097	0.190	0.193	0.324	0.432	0.548	0.609	0.984428
2.5	0.065	0.112	0.177	0.262	0.389	0.509	0.599	0.801	0.994408
3.0	0.091	0.125	0.213	0.347	0.426	0.601	0.842	1.01	0.997323
4.0	0.011	0.055	0.091	0.191	0.157	0.254	0.664	0.747	0.99433

Effect of base:

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

Table 4: Effect of base on absorbance

Solution of 1N base used	Absorbance / ml of base added					
	0.5	1.0	1.5	2.0	3.0	4.0
NaOH	0.125	0.153	0.382	0.423	0.392	0.382
KOH	0.123	0.150	0.235	0.385	0.296	0.299
Na_2CO_3	0.113	0.115	0.125	0.243	0.233	0.237
$NaHCO_3$	0.152	0.159	0.163	0.169	0.495	0.199

The results in (Table 4) indicate that it is very clearly that the reaction needs a strong alkaline medium and NaOH gives the best result ,so the amount of NaOH has been studied and 2 ml gives the high intensity of the azo dye, therefore it selected and fixed on the subsequent experiments.

Effect of time:

A study to time on the absorbance of the final reaction show that the maximum absorbance is obtained after 20 minutes and remains stable for at least 24 hrs. (Table 5).

Table 5: Effect of time on the stability of colored azo dye

Time, minutes	Absorbance
0	0.288
5	0.350
10	0.387
15	0.416
20	0.428
25	0.429
30	0.428
35	0.429
40	0.427
45	0.428
50	0.429
55	0.428
60	0.429

Final absorption spectra:

Under the above established conditions, absorption spectra of a yellow azo dye formed from coupling of diazotized PAP with histidine reagent in alkaline medium showed a maximum absorption at 430 nm.
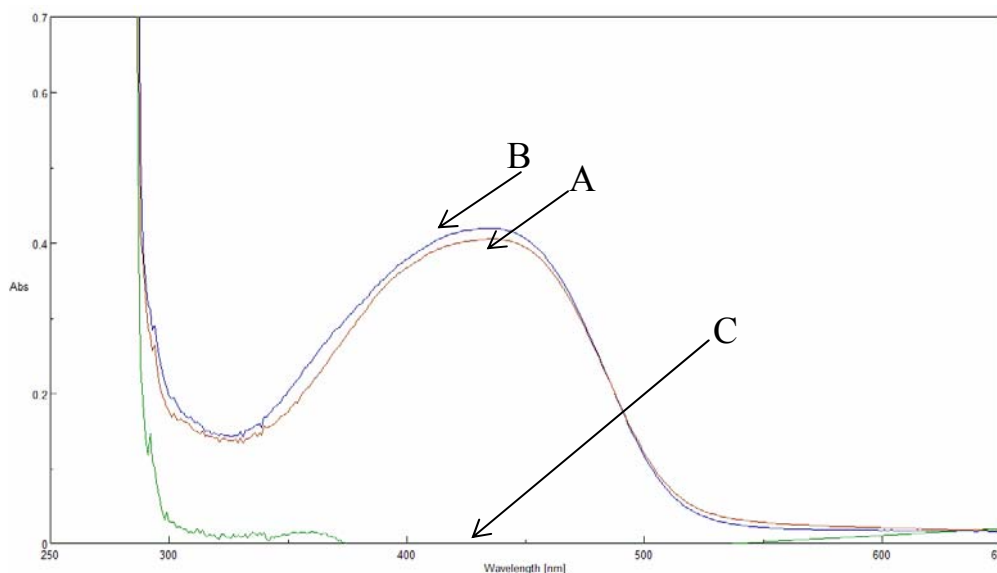

Fig. 1: Absorption spectra of 100 μg PAR /20 ml measured against (A) blank, (B) distilled water, (C) reagent blank against distilled water

Recommended procedure and calibration graph:

A liquots containing (10-500) μg of PAP solution were placed into 20 ml volumetric flask. To each PAR solution, 1.5 ml of 1N hydrochloric acid, 0.75 ml of 1% sodium nitrite solution and 0.2 ml of 3% sulphamic acid solution were added with occasional shaking for 2 minutes. After that a 3 ml of histamine solution (1×10^{-3}) M and 2 ml of 1M sodium hydroxide solution were added. The volume were diluted with distilled water to the mark and the absorbance value was read at 430 nm against the reagent blank. Beer's law was obeyed within the range of (10-500) μg / 20 ml (i.e. 0.5-25 ppm) (Fig. 2), the molar absorptivity and Sandell's sensitivity are 1.118×10^{4} l.mol^{-1}.cm^{-1} and 0.0135 μg.cm^{-2} respectively.
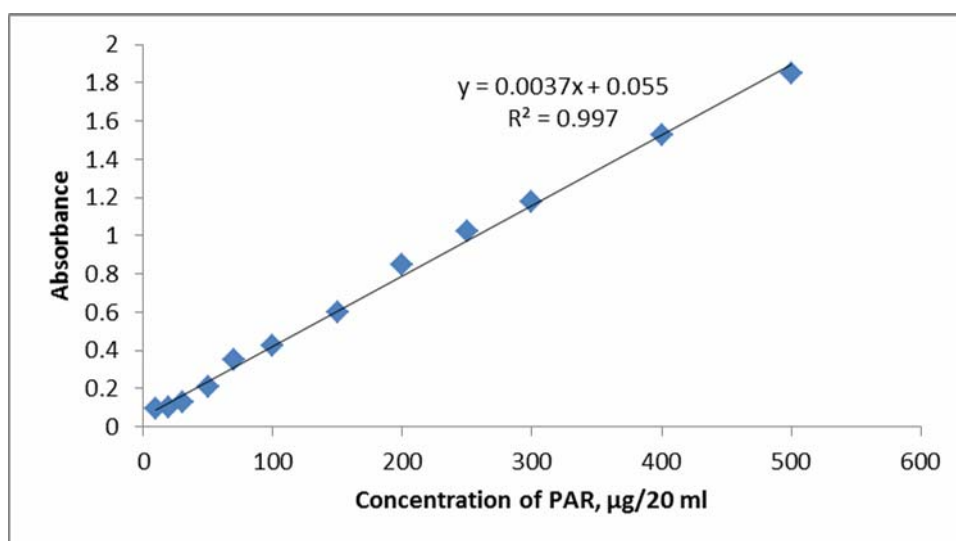

Fig. 2: Calibration graph of PAP determination

Nature of the dye:

   Job's method of continuous variations (Delvic, 1997), has been studied to find stoichiometry of the formed azo dye between diazotized PAP and histidine . The results indicate that the azo – dye has formed in the ratio of 1:1 diazotised PAP to histidine. Fig (3).
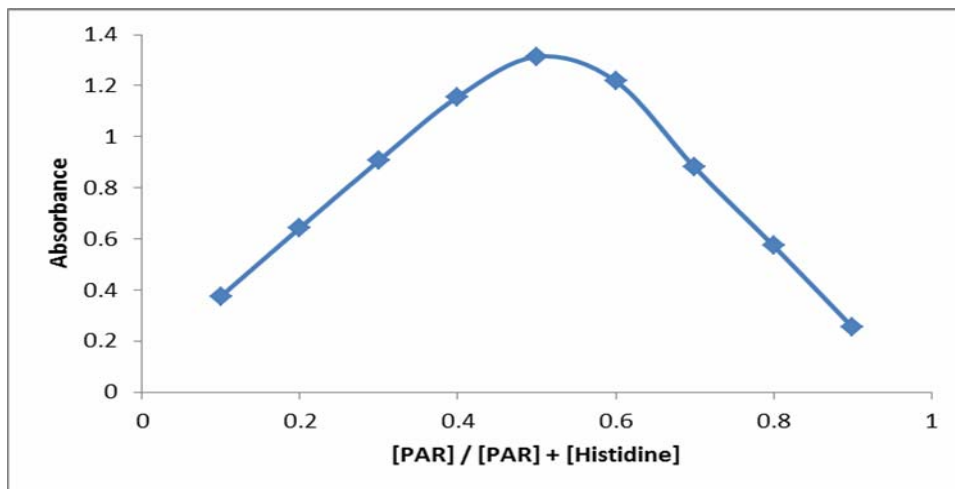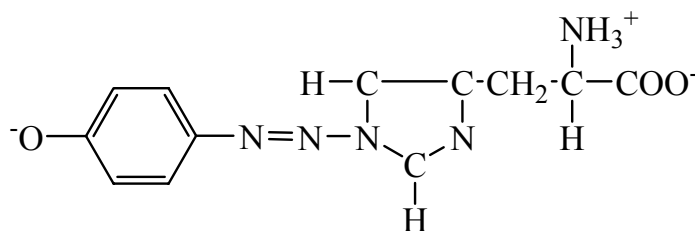

Fig. 3: Job's plot for PAP – Histidine

The suggested illustrated below

Analytical application:

   The suggested method applied for the determination of PAR in different pharmaceutical formulations. On applying proposed procedure, good recovery was obtained as shown in (Table 6).

Table 6: The analytical application of the proposed method

Pharmaceutical preparation	µg paracetamol / 20 ml	Recovery * (%)
Paracetamol tablets/ 500 µg S.D.I-Iraq	50	100.4
	100	99.8
	200	99.9
Panda tablets/ 1000 µg Paracetamol (Joswe)	50	100.7
	100	100.6
	200	99.9
Paracetamol syrup- S.D.I-Iraq	50	100.2
	100	99.5
	200	99.3
Paracetamol injection/ 500 mg/5 ml Pharmaceutical/India	50	100.5
	100	100.3
	200	100.1

* Average of five determination

Comparison of method:

Table (7) shows that the comparison between the analytical parameters of the proposed method with those of literature method for paracetamol determination.

Table 7 : Comparison of method

Analytical parameters	Proposed method	Literature method (Omar, 2014)	Literature method (Dixit and Patel, 2017)
Reagent	Histidine	Thymol	8-hydroxyquinoline
Medium	Alkaline	Alkaline	Alkaline
λ_{max} (nm)	430	600	470
Beer's law range ($\mu g .ml^{-1}$)	0.5-25	1-14	2-10
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	1.118×10^4	0.613×10^4	1.9×10^4
Color of the product	Yellow	Blue	Yellow-orange
Application of the method	Pharmaceutical preparations	Pharmaceutical preparations	clinical samples

CONCLUSION

The proposed spectrophotometric method is simple, sensitive and low cost, it does not involve solvent extraction step, and gives accurate and precise results. The method successfully has been applied to the determination of paracetamol in different types of dosage formulation without extraction of separation.

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