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Spectrophotometric Assay of Paracetamol in Pharmaceutical Preparations

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الملخص

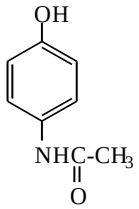
يتضمن البحث طريقة طيفية لتقديركميات متناهيه في الصغر من الباراسيتامول . تعتمد الطريقة على أزوتة بارا–امينو فينول الناتج من التحلل الحامضي للباراسيتامول وذلك بمفاعلته مع ايون النتريت بوجود حامض الهيدروكلوريك ثم اقتران ملح الدايازونيوم الناتج في وسط قاعدي مع كاشف الاقتران فلورواسيتوفينون لتكوين صبغة آزوية برتقالية مستقرة وذائبة في الماء وتم قياس شدة الامتصاص للصبغة الناتجة عند الطول الموجي 472 نانوميتر وكانت حدود قانون بير في مدى التركيز من 10 إلى 180 مايكروغرام من الـ باراسيتامول/25 مل وكانت قيمة الامتصاصية المولارية هي 2.16×10^4 لتر .م ول$^{-1}$.سم$^{-1}$، والخطأ النسبي تراوح بين -0.64و1.64+ % والانحراف القياسي النسبي بين 0.4 ± و1.24± % اعتمادا على مستوى تركيز الباراسيتامول. تم تطبيق الطريقة بنجاح في تقدير الباراسيتامول في المستحضرات الصيدلانية.

ABSTRACT

A spectrophotometric method for the assay of micro amounts of paracetamol has been developed. The method is based on the reaction of *p*–aminophenol which results from the acid hydrolysis of paracetamol, with nitrite ion to form the corresponding diazonium salt followed by coupling reaction in an alkaline medium with phloroacetophenone to form a stable and soluble orange azo dye. The intensity of absorbance for the resulting azo dye is measured at 472 nm and Beer's law is obeyed in the concentration range of 10–180 μg of paracetamol in a final volume of 25 ml, with a molar absorptivity of 2.16×10^4 l.mol^{-1}.cm^{-1}, a relative error of -0.64 to +1.64% and a relative standard deviation of ±0.4 to ±1.24 %, depending on the concentration level of paracetamol. The method has been successfully applied to the assay of paracetamol in various pharmaceutical preparations.

INTRODUCTION

Paracetamol [acetaminophen, N-acetyl-*p*-aminophenol, 4-acetamidophenol] [1] is used as analgesic and antipyretic agents (1)



[1]

Various spectrophotometric methods have been utilised for the determination of paracetamol, these include coupling with different diazotised reagents such as sulphanilic acid(2),2-nitroaniline(3),benzocaine(4)and sulphacetamide(5).

Paracetamol can be determined by diazotization of hydrolysed paracetamol and coupling with 1-naphthol (6),

Oxidative coupling reaction has been used in determination of paracetamol based in reaction with *o*-cresol (7), or *p*-xylenol after hydrolysis to *p*-aminophenol (8) with an oxidizing agent such as sodium periodate.

Paracetamol can be determined by nitration and subsequent reaction with acetone as nucleophilic reagent (9) or by complexation with Co(III) and Cu(II) ions (10). Another method based on reaction of hydrolysed paracetamol (*p*-aminophenol) at ambient temperature with sodium sulphide in the presence of Ce(IV) or Fe (III) to produce a methylene blue–like dye (11). Also, a charge transfer complex formation has been used for the determination using 2,3-dichloro- 5,6-dicyano-1,4-benzoquinone(12). Oxidation reduction reaction can be used in the determination of paracetamol using Fe (III) in the presence of 2,2`-bipyridyl(13).

Many chromatographic methods have been published. These included high performance liquid chromatography, gas chromatography and liquid chromatography (14-16).

The present method involves the diazotisation of hydrolysed paracetamol, followed by coupling with phloroacetophenone to form a highly coloured dye that has been applied successfully to the assay of paracetamol in pharmaceutical preparations.

Experimental

Instruments

All spectrophotometric measurements are performed on Shimadzu UV-Visible Recording Spectrophotometer UV-160 using 1- cm silica cells, pH meter type Philips PW 9420 is used for pH reading.

Reagents

All chemicals used in this investigation are of analytical – reagent grade, and paracetamol standard material is provided from general establishment for medical appliance and drugs / SDI – Samaraa / Iraq.

Solutions

Paracetamol solution , 1000 μg.ml^{-1}. This solution is prepared by dissolving 0.25 g of paracetamol in 10 ml of ethanol and diluted to 250 ml in a volumetric flask with distilled water.

Phloroacetophenone , 0.1% (w/v). This solution is prepared fresh daily by dissolving 0.1 g of phloroacetophenone (Fluka) in 100 ml distilled water.

Sodium nitrite solution, 1% (w/v). This solution is prepared by dissolving 1 g of sodium nitrite (BDH) in 100 ml distilled water.

Sulphamic acid solution, 3% (w/v). This solution is prepared by dissolving 3 g of sulphamic acid (Fluka) in 100 ml distilled water.

Hydrochloric acid solution, 1N. This solution is prepared by diluting 8.5 ml of concentrated acid (11.8 N) to 100 ml with distilled water.

Solution of hydrolyzed paracetamol, , 100μg.ml^{-1}.This solution is prepared by transferring 150 ml of 1000 ***μg.ml^{-1}***paracetamol into 250- ml round-bottomed flask provided with condenser,25ml of hydrochloric acid(11.8N) is added then refluxed for 1 hour, after that the cold solution is neutralised with 20% sodium carbonate and diluted to 250 - ml with distilled water in a volumetric flask(17). To prepare 100μg.ml^{-1} paracetamol, 16.6 ml of the above solution is diluted to 100 ml in a volumetric flask using distilled water.

Paracetamol tablets solution, 100μg.ml^{-1}. 10 tablets (each one contains 500 mg paracetamol) are weighed and finely powdered. An accurately weighed amount of powder equivalent to 0.25g paracetamol is dissolved in 10 ml ethanol, then 100-150 ml distilled water is added, shaking to increase the solubility, filtered into 250 ml calibrated flask, then the solution is completed to the volume with a distilled water, and proceed as mentioned above in preparation of hydrolysed paracetamol solution

Paracetamol syrup solution, 100μg.ml^{-1}. A 10.4 ml of syrup (each 5ml contains 120 mg paracetamol) is transferred into a 250 ml calibrated flask and the total volume is diluted with distilled water, and proceed as mentioned in preparation of hydrolysed paracetamol solution.

Paracetamol suppositories solution, 100μg.ml^{-1}. Weigh and mix well 4 suppositories (each suppository contain 250 mg paracetamol). An accurate weighed amount of mixture equivalent to 0.250 g paracetamol is dissolved in boiling distilled water, filtered, and the residues are washed with 10 ml ethanol and boiling distilled water and the volume is

completed to 250 ml in a calibrated flask with distilled water, and proceed as mentioned in preparation of hydrolysed paracetamol solution.

Procedure and calibration graph

To a series of 25 - ml calibrated flasks, transfer 0.1 – 2.2 ml of hydrolysed paracetamol solution (equivalent to 100 μg.ml-1 paracetamol) , then 0.5 ml of 1N hydrochloric acid and 0.5 ml of 1% (w/v) sodium nitrite solution are added and the mixture is allowed to stand for 5 minutes and then 0.2 ml of 3% (w/v) sulphamic acid solution is added with occasional shaking for 2 minutes. After that a 2 ml of 0.1% (w/v) phloroacetophenone solution and 2 ml of 1N sodium hydroxide are added . The volumes are completed to the mark with distilled water and the absorbance is read at 472 nm against the reagent blank after 10-minutes standing time. A linear calibration graph is obtained over the concentration range of 10 – 180 μg paracetamol / 25 ml and a concentration above 180 μg / 25 ml gives a negative deviation (Fig. 1).The molar absorptivity has been found to be 2.16×10^{4} $l.mol^{-1}.cm^{-1}$.

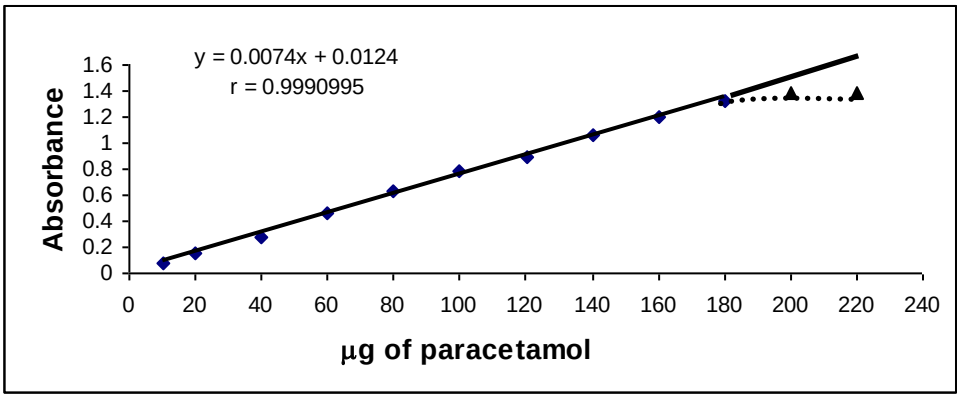


Fig. 1. Calibration graph of paracetamol determination

Results and Discussion

During the investigation, hydrolysed paracetamol solution equivalent to 100 $\mu g.ml^{-1}$ paracetamol, is taken and the final volumes are brought to 25 ml with distilled water.

Effect of diazotisation acid

Different amounts and types of acids have been used in diazotisation of paracetamol, the results show that the solution of 1N hydrochloric acid gives the best results when added in a volume of 0.5 ml (Table 1).

Table 1. Effect of diazotisation acids

1N Acid solution used	Absorbance and colour contrast / ml of acid used							
	0.25		0.5		1.0		1.5	
	A*	Δλ**,nm	A	Δλ, nm	A	Δλ, nm	A	Δλ, nm
HCl	0.774	147	0.786	148	0.754	113	0.685	112
HNO_3	0.638	129	0.667	146	0.630	120	0.623	110
H_2SO_4	0.645	154	0.753	148	0.741	147	0.749	113
CH_3COOH	0.424	110	0.493	106	0.515	115	0.491	108

* No diazotisation acid A =0.668

**$\Delta\lambda = \lambda_{max}S - \lambda_{max}B$ Where S = The dye , B = Blank

Effect of sodium nitrite amount and time

The maximum absorbance reading is obtained by adding 0.2 ml of 1% sodium nitrite with 5 minutes reaction time (Table2).

Table 2. Effect of sodium nitrite amount and time

ml of 1% $NaNO_2$ solution	Absorbance / minute standing time						
	0	1	2	3	4	5	7
0.1	0.525	0.434	0.452	0.457	0.584	0.680	0.683
0.2	0.706	0.662	0.696	0.726	0.730	0.736	0.740
0.3	0.773	0.691	0.712	0.714	0.719	0.734	0.741
0.4	0.748	0.695	0.664	0.699	0.694	0.718	0.72
0.5	0.793	0.760	0.765	0.768	0.775	0.789	0.781
0.7	0.607	0.536	0.545	0.577	0.541	0.548	0.545

Effect of sulphamic acid amount and time

The excess of nitrite can be removed by the addition of sulphamic acid solution (18).The effect of sulphamic acid amount and time has been studied.
(Table 3)

Table 3 Effect of sulphamic acid amount and time

ml of 3% Sulphamic acid solution	Sample type	Absorbance/minute standing time						
		0	1	2	3	4	5	7
0.00	Standard = S	0.434	0.505	0.497	0.536	0,508	0.458	0.396
	Blank = B	0.108	0.111	0.104	0.098	0.090	0.096	0.074
0.10	S	0.746	0.604	0.629	0.658	0.699	0.677	0.672
	B	0.072	0.091	0.080	0.089	0.072	0.090	0.094
0.20	S	0.778	0.774	0.788	0.786	0.766	0.768	0.758
	B	0.011	0.023	0.023	0.023	0.004	0.011	0.008
0.30	S	0.694	0.719	0.723	0.730	0.737	0.730	0.716
	B	0.011	0.008	0.007	0.006	0.009	0.005	0.019

The results in Table 3 indicate that 0.2 ml of sulphamic acid solution (3%, w/v) with 2 minute standing time for the reaction, give the most suitable effect on the intensity of the dye.

Effect of phloroacetophenone amount

The effect of different amounts of phloroacetophenone solution (0.1%) on the intensity of absorbance at different amounts (5-200 μg) of paracetamol/25ml has been studied. A 2 ml of phloroacetophenone solution (0.1%) in a total volume of 25 ml give the higher sensitivity and higher value of correlation coefficient (r), therefore it has been selected for subsequent experiments (Table 4).

Table 4. Effect of coupling agent amount on absorbance

ml of phloroacetophenone solution (0.1%)	Absorbance/μg Paracetamol present in 25 ml										r
	10	20	40	60	80	100	120	140	160	180	
0.5	0.073	0.153	0.320	0.472	0.652	0.793	0.935	0.990	1.012	1.349	0.9865829
1	0.069	0.138	0.297	0.477	0.636	0.751	0.927	1.060	1.206	1.379	0.9996299
2	0.081	0.173	0.314	0.466	0.627	0.790	0.910	1.049	1.218	1.368	0.9996669
3	0.074	0.162	0.296	0.450	0.613	0.753	0.899	1.032	1.168	1.359	0.9995789

Effect of base

Previous experiments have shown that the coloured azo dye formed in alkaline medium, therefore different amounts and types of strong and weak bases have been studied (Table 5). The results indicate that the strong bases give high intensity and high colour contrast and the formed orange azo dye is stable while sodium bicarbonate gives turbid solution

after 30 minutes. A volume of 2 ml of 1N sodium hydroxide (final reaction mixture pH=11.7) has been selected for the subsequent experiments.

Table 5. Effect of bases on absorbance and colour contrast

Base used (1N)	Variable	A / ml of base used			
		1	2	3	4
NaOH	A	0.749	0.795	0.780	0.756
	$\Delta\lambda^*$	155.5	154.0	154.5	154
KOH	A	0.735	0.725	0.745	0.779
	$\Delta\lambda$	154	151.5	151	153
Na_2CO_3	A	0.106	0.404	0.468	0.568
	$\Delta\lambda$	146	150	154	152
$NaHCO_3$ **	A	1.363	1.042	0.803	0.793
	$\Delta\lambda$	30	30	31	44

* $\Delta\lambda = \lambda_{max}S - \lambda_{max}B$ Where S = The dye, B = Blank

** Turbid solution after 30 minutes

Effect of time

The coloured azo dye developed rapidly after addition of base and the stability period (at least one hour) is sufficient to allow several measurements to be performed and the results are given in Table 6.

Table 6. The effect of time and paracetamol amount on absorbance

µg of Paracetamol present	Absorbance / minute standing									
	0	5	10	15	25	30	35	45	55	60
50	0.423	0.413	0.406	0.403	0.401	0.401	0.400	0.400	0.397	0.397
100	0.935	0.819	0.808	0.807	0.804	0.804	0.804	0.804	0.803	0.804

Final absorption spectrum

The absorption spectrum of the orange azo dye formed from coupling of diazotised *p*-aminophenol with phloroacetophenone in alkaline medium shows

a maximum absorption at 472 nm. The reagent blank has very nill absorption(0.023) at this wavelength (Fig. 2).

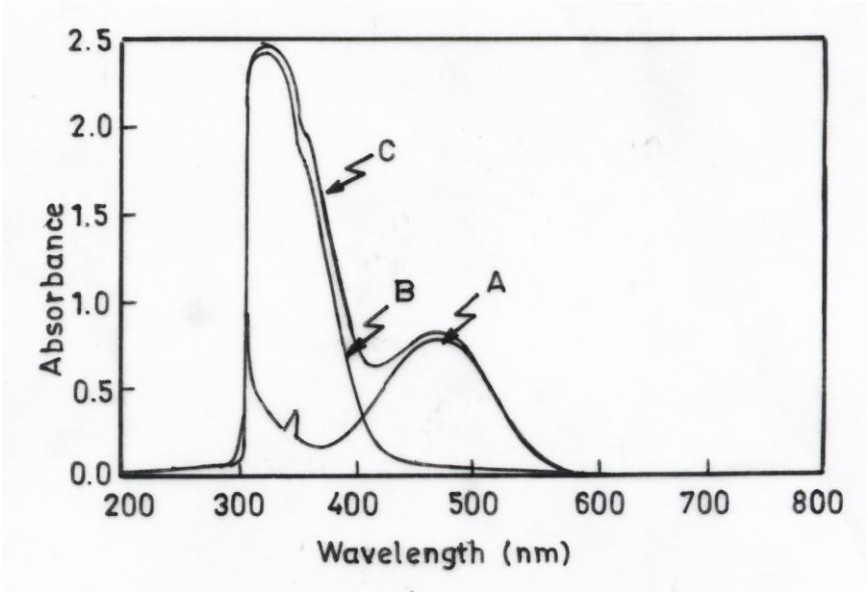
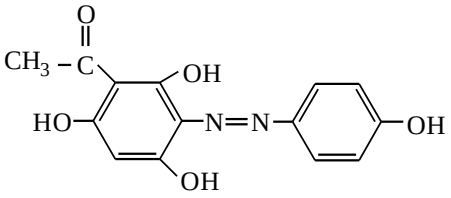


Fig. 2. Absorption spectra of 100μg paracetamol / 25ml treated according to the recommended procedure and measured against (A) reagent blank, (B) distilled water and (C) reagent blank measured against distilled water

Nature of the dye

The stoichiometry of the formed azo dye between diazotised *p*-amino- phenol and phloroacetophenone is investigated by applying the continuous- variations method and mole- ratio method. The results indicate that the azo-dye has formed in the ratio of 1:1 diazotised *p*-aminophenol to phloroacetophenone, and the azo dye may have the following suggested structure:



Orange azo-dye

Accuracy and precision

To check the accuracy and precision of the calibration graph, three different concentrations of paracetamol are determined. The results shown in Table 7 indicate that the method is satisfactory.

Table 7. Accuracy and precision

Amount of paracetamol taken, μg	Relative error, %*	Relative standard deviation, %*
40	+1.64	± 1.24
100	-0.64	± 0.50
160	+0.51	± 0.40

* Average for five determinations.

Analytical application

The proposed method is applied to determine paracetamol in different pharmaceutical preparations. On applying proposed procedure, good recovery is obtained as shown in table 8.

Table8. Analytical applications

Pharmaceutical preparation	μg paracetamol present/25ml	μg paracetamol found/25ml	Recovery* (%)
Paracetamol tablets 500 mg (S.D.I-Iraq)	40	38.6	96.72
	100	98.20	98.21
	160	160.00	100.00
Antipyrol-syrup 120mg/5ml (S.D.I-Iraq)	40	40.90	102.40
	100	101.80	101.80
	160	164.80	103.02
Antipyrol suppositories250mg (Medico laboratory Syria)	40	39.30	98.36
	100	97.50	97.50
	160	157.9	98.72

*Average for five determinations

Comparison of the methods

A comparison between the present method and British pharmacopeia standard method (1) for the determination of paracetamol in tablets drug, is based on the t-test to show the ability of using the present method in the determination of investigated drug (Table 9).

Table 9. Comparison of the methods and experimental t-test values

Drug	Pharmaceutical preparation	Recovery*,%		t.exp
		Present method	British pharmacopeia method	
Paracetamol	Tablet	100.035	98.932	1.9964

*Average for five determinations

The results in Table 9 indicate that the calculated experimental t-value is less than its value in the statistic table at confidence level (95%) and for four degrees of freedom (2.776).These result indicated that there is no significant difference between the present method and the standard method.

According to the difficulties of availability of some reagents used in standard method for determination of paracetamol in syrup and suppositories ,so we used standard addition method in order to prove that the proposed method can be applied to determination of paracetamol in syrup and suppositories without interferences (Table 10)

Table 10. The results of standard addition method

Pharmaceutical preparation	µg paracetamol present/25ml	µg paracetamol found/25ml	Recovery* (%)
Antipyrol-syrup 120mg/5ml (S.D.I-Iraq)	40	40.90	102.25
	60	61.01	101.68
Antipyrol suppositories250mg (Medico laboratory Syria)	40	38.80	97.00
	60	59.10	98.50

*Average for three determinations

The results in Table 10 indicated that the proposed method can be used in determination of paracetamol in syrup and suppositories with satisfactory results.

Table 11 shows the comparison between some of analytical variables obtained from the present method with that of another Literature spectrophotometric method.

Table11.The comparison of the methods.

Analytical parameters	**Present method**	**Literature method[5]**
pH	11.77	…
Temperature (C°)	At room temperature	At room temperature
λ_{max} (nm)	472	432
Medium of reaction	Aqueous	Aqueous
Type of reaction	Diazotisation and coupling	Diazotisation and coupling
Reagent	Acetophloroglu-cinol	Diazotised sulphacetamide
Beer's law range (ppm)	0.4-7.2	0.3-2
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	2.16×10^4	7.62×10^4
Colour of the dye	Orange	Yellow – orange
Nature of the dye	1:1	1:1
Application of the method	Pharmaceutical preparations	Pharmaceutical preparations

The results indicate that the proposed method has a good sensitivity compared with the above literature method which is more sensitive.

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