Spectrophotometric Determination of *p*-Aminobenzoic acid Results from Procaine Drug Using 2, 6-Dihydroxybenzoic acid as Coupling Agent

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Abstract

A spectrophotometric method for the trace determination of *p*-aminobenzoic acid (PABA) has been proposed. The method is based on the coupling of diazotised PABA with 2,6-dihydroxybenzoic acid in basic medium to form a yellow,stable and water soluble azo-dye which shows a maximum absorption at 438 nm with a molar absorptivity of 5.2745×10^4 $l.mol^{-1}.cm^{-1}$ and obeys Beer's law in the concentration range 10-100 μg of PABA in a final volume of 25ml i.e., 0.4-4 ppm with a relative error of +0.11 to -3.71 % and a relative standard deviation of ±2.11 to ±5.13 % depending on concentration level. The present method has been applied successfully to the determination of PABA which results from hydrolysis of procaine drug.

Introduction

4-Aminobenzoic acid (PABA) was used as a component of some medicines [e.g., analgesic or anethetic preparations), sunscreen agents and bentiromide (1,2). PABA is an essential factor for the growth of bacteria. It also is observed that it possessed an antisulfanilamide activity (3). Various spectrophotometric methods are used for the determination of PABA, most of these methods are based on diazotisation of PABA and coupling the correspoding diazonium salt with different coupling agent such as Braton Marshall reagent (4), 4-dimethylaminobenzaldehyde (5), N-(1-naphthyl)ethylendiamine dihydrochloride (6) and phloroglucinol (7)**.**

The oxidative coupling reaction with promethazine reagent is used for the determination of aniline and four of its substituents (8)**.**

An indirect spectrophotometric method for the determination of PABA is described,the method is based on the reaction of PABA with hypochlorite in acidic medium and the subsequent measurement of the residual chloride by using the well-known reaction with *o*-tolidine (9)**.**

The objective of the investigation reported in this paper is to evaluated a simple spectrophotometric method for the determination of PABA, the method involves the diazotisation of PABA and subsequent coupling with 2,6-dihydroxybenzoic acid to form a highly coloured dye that has proved successful for the assay of PABA results from degradation of procaine**.**

Experimental

Apparatus

Shimadzu UV-Visible Recording Spectrophotometric UV-160 with 1.0 cm matched cells is used in this investigation**.**

Reagent

All Chemicals used were of analytical-reagent grade**.**

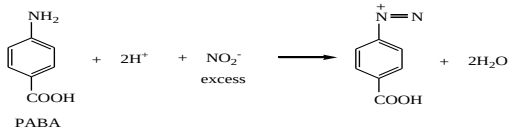
Standard p-aminobenzoic acid sollution, 50μgml-1 is prepared by dissolving 5mg of PABA in distilled water and then diluting to the mark in a 100-ml standard flask. A 1N hydrochloric acid solution is prepared by appropriative dilution of concentrated acid. 1% solution of sodium nitrite, 3% aqueous solution of sulphamic acid, 0.1% 2,6-dihydroxybenzoic acid and 1N sodium hydroxide solution (prepared by appropriate dilution of the concentrated volumetric (Fluka) solution with distilled water) are used for the subsequent experiments**.**

Procaine solution which is used in application part was prepared by dissolving 0.013g of procaine penicillin injection (PABA%=) in a mixture contain 5ml of 1N hydrochloric acid and 20ml of distilled water, then heating to boiling and the volume is completed to 50ml in a volumetric flask after cooling**.**

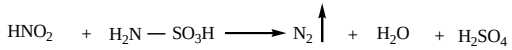
Results and Discussion

Principle of the method

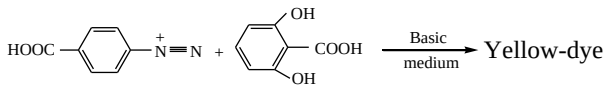
PABA reacts with excess amount of nitrite to form PABA diazonium salt in the presence of acid**:**



The residual nitrite (as nitrite acid) which is undesirable can be removed by adding sulphamic acid**:**



The coloured solution formed by coupling diazotised PABA with 2,6-dihydroxybenzoic acid in basic medium**:**



Study of the optimum reaction condition

The various parameters affecting and related to the formed dye have been studied and the optimum conditions have been selected**.**

Effect of diazotisation acid

It is well known that diazotisation reaction takes place in acidic medium. So hydrochloric, nitric, sulphuric and acetic acid (1N) with different amounts are studied as a possible acidic media. The results show that hydrochloric acid gives the best result, when added in a volume of 0.8ml (Table 1)**.**

Table 1. Effect of diazotization acids on absorbance

Acid used 1N	Absorbance*/ml acid added					
	0.1	**0.2**	**0.4**	**0.6**	**0.8**	**1**
HCl	0.601	0.632	0.656	0.658	0.680	0.636
HNO_3	0.579	0.630	0.645	0.672	0.678	0.676
H_2SO_4	0.603	0.639	0.654	0.662	0.670	0.668
CH_3COOH	0.591	0.636	0.640	0.666	0.661	0.665

* Absorbance without addition of acid is = 0.492

Effect of nitrite amount and time

The amount of sodium nitrite required to obtain maximum absorbance was studied. The yellow azo-dye reached the maximum intensity on using 0.3 ml of 1% sodium nitrite solution and 1minute reaction time (Table 2)**.**

Table 2. Effect of nitrite amount and time on absorbance

ml of 1% $NaNO_2$ solution	Absorbance/minute standing time				
	0	**1**	**2**	**3**	**5**
0.1	0.280	0.257	0.265	0.272	0.270
0.2	0.267	0.265	0.268	0.264	0.265
0.3	0.649	0.656	0.643	0.654	0.645
0.4	0.627	0.627	0.653	0.652	0.648
0.5	0.648	0.648	0.638	0.645	0.637
1.0	0.091	0.041	0.053	0.085	0.053

Effect of sulphamic acid amount and time

The residual amount of nitrite is undesirable according to its side reaction (10). Therefore, it should be removed by adding sulphamic acid. The results of adding different amounts of sulphamic acid indicated that 0.2ml of 3% sulphamic acid with 2 minutes standing time gave the most suitable result (Table 3)**.**

Table 3. Effect of sulphamic acid and time on absorbance

Ml of 3% sulphamic acid	Absorbance/minute standing time				
	0	**1**	**2**	**3**	**5**
0.1	0.073	0.057	0.044	0.052	0.083
0.2	0.645	0.638	0.660	0.620	0.616
0.3	0.589	0.610	0.618	0.614	0.614
0.4	0.622	0.596	0.617	0.594	0.610
0.5	0.615	0.609	0.600	0.609	0.619

Effect of coupling agent amount

The volume of the coupling agent (2, 6-dihydroxybenzoic acid) for maximum colour intensity and for high correlation coefficient is studied and it is found that 2 ml produce the highest intensity of azo-dye with high value of correlation coefficient (Table 4)**.**

Table 4. Effect of 2,6-dihydroxybenzoic acid amount on absorbance

Ml of 1% 2,6-dihydroxybenzoic acid	Absorbance/µg of PABA									r
	10	**20**	**30**	**50**	**70**	**90**	**100**	**130**	**160**	
1	0.132	0.219	0.346	0.609	0.710	1.087	1.202	1.435	1.732	0.99536
2	0.115	0.241	0.476	0.675	0.910	1.140	1.281	1.543	1.852	0.99582
3	0.112	0.213	0.329	0.540	0.812	1.061	1.279	1.607	1.711	0.99165
4	0.117	0.138	0.171	0.348	0.505	0.963	1.093	1.411	1.660	0.98690

Effect of bases

The effect of different types of base solution (which is necessary for developing the chromophore) on the colour intensity and the colour contrast was investigated. The colour of azo dye became most intense and stable with high colour contrast when 4 ml of 1N sodium hydroxide solution(pH=11.98) is used(Table 5)**.**

Table 5. Effect of bases on absorbance and on colour contrast

Base solution used (1N)	Absorbance/ml of base used					Δλ*
	1	2	3	4	5	
NaOH	0.091	0.708	0.757	0.794	0.703	139±4
KOH	0.066	0.687	0.606	0.574	0.560	129±3
Na_2CO_3	0.044	0.365	0.526	0.567	0.543	130±2
$NaHCO_3$	0.033	0.159	0.206	0.191	0.172	90±7
CH_3COONa	0.028	0.026	0.041	0.050	0.051	78±6

*Colour contrast = $\Delta\lambda=\lambda^{S}_{max}-\lambda^{B}_{max}$ where S = the dye and B = the blank

Effect of time on colour development

The colour of the formed azo-dye reached the full intensity within not more than 5 minutes and it is stable for at least 1 hour (Table 6).

Table 6. Effect of time on the intensity of azo-dye

μg of PABA/25ml	Absorbance/minute standing time							
	0	5	10	20	30	40	50	60
50	0.712	0.788	0.789	0.789	0.787	0.786	0.785	0.782
70	1.061	1.082	1.083	1.084	1.083	1.081	1.079	1.078

Procedure and calibration graph

An aliquots of the sample solution containing 10-120μg PABA are transferred into a series of 25-ml standard flasks. To these solutions 0.3 ml of 1% sodium nitrite added, and the acidity is adjusted with 0.8ml of 1N hydrochloric acid. After 1 minute, 0.2 ml of 3% sulphamic acid solution is added to each flask with occasional shaking for 2 minutes, then volumes of 2 ml of 2,6-dihydroxybenzoic acid and 4 ml of 1N sodium hydroxide are added, and the contents diluted to the mark with distilled water. After 5 minutes the absorbances of the coloured azo-dye are measured at 438 nm against the corresponding reagent balnk. A calibration graph is linear over the range 10-100μg PABA and above 100μg a negative deviation is shown (Fig. 1). The apparent molar absorptivity referred to PABA, has been found to be 5.2745×10^4 l. $mol^{-1}.cm^{-1}$.

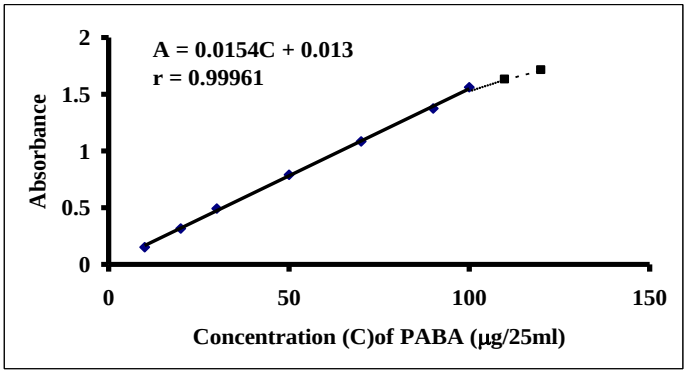


Fig. 1. Calibration graph for PABA determined using 2,6-dihydroxybenzoic acid as coupling reagent

Absorption spectra

Absorption spectrum of the coloured dye formed from the coupling of diazotised PABA with 2,6-dihydroxybenzoic acid reagent in basic medium shows a maximum absorption at 438nm, in contrast to the reagent blank which shows very nill absorption at the same wavelength (Fig. 2). The wavelength of maximum absorption 438nm is used in all subsequent experiments.

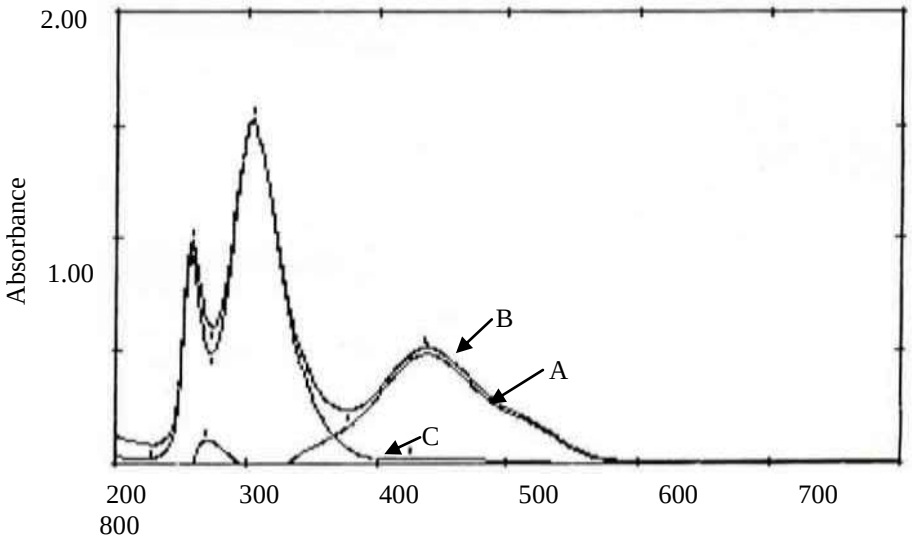


Fig. 2: Absorption spectra of 30 μg PABA/25ml treated according to the recommended procedure and measured against (A) blank, (B) distilled water and (C) blank against distilled water

Accuracy and precision

To determine the accuracy and precision of the method, PABA is determined at three different concentrations. The results are shown in Table 7 and indicate that satisfactory precision and accuracy can be attained using the proposed method.

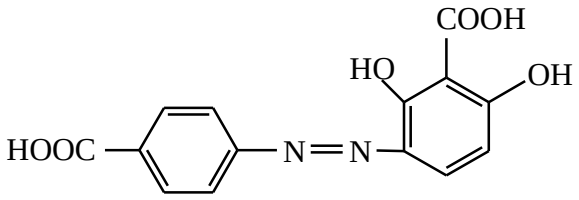
Table 7. Accuracy and precision

Amount of PABA taken, μg	Relative error*, %	Relative standard deviation*,%
30	-3.71	±5.133
50	-3.40	±2.110
70	+1.11	±3.812

* Average of five determinations

Nature of the dye

The stochiometry of the reaction between PABA and 2,6-dihydroxybenzoic acid is investigated by Job's method (11). The results obtained showed the existence of 1:1 PABA diazotized to 2,6- dihydroxybenzoic acid, therefore the structure of the formed dye may be written as follows:



Yellow azo-dye

Application of the method

The proposed method is applied to the quantitative determination of PABA which results from the degradation of procaine, the results are shown in (Table 8).

Table 8. The results of the application part

Drug	PABA present	
	Theoretical value	Practical value*
Procaine penicillin injection (Pakistan)	23.30 %	22.98 %

* Average of three determinations

From the results illustrated in Table 8, it can be shown that good agreement has occurred between theoretical and practical value.

Comparison of the method and t-test

A comparison between the proposed method and the literature method (7) for the determination of PABA which result from degradation of procaine (using procaine penicillin injection) is based on the t-test to show the ability of using the suggested method in the determination of investigated drug (Table 9).

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

Drug	PABA Present µg/25ml	PABA Recovery%		t-test
		Proposed method	Literature method	
Procaine penicillin injection	60.5	98.20	97.98	1.165

* Average of four determinations

The results illustrated above indicated that t-value which calculated experimentally (2.165) is less than it's value in the statistic table at confidence level (95%) and for three degrees of freedom (2.353), so that we can used the proposed method in determination of PABA with satisfactory results.

Comparison of Methods

Table (10) shows the comparison between the analytical variables obtained from the present method with that of a recent spectrophotometric method.

Table 10: Comparison of the methods

Analytical parameters	Literature method**(7)**	Present method
pH	12.57	11.98
Temperature (C^O)	At room temperature	At room temperature
Development time (minutes)	Immediately	5
Reagent used	Phloroglucinol	2, 6-Dihydroxybenzoic acid
l_{max} (nm)	419	438
Beer's law range (ppm)	0.4-5.6	0.4 – 4.0
Molar absorptivity ($l .mol^{-1}.cm^{-1}$)	4.7×10^4	5.27×10^4
RSD (%)	0.622	$< \pm 5.13$
Stability of the colour (minutes)	At least 60	60
Colour of the dye	Yellow	Yellow
Nature of dye	1:1	1:1
Application of the method	Has been applied to the assay of PABA in procaine	Has been applied to the assay of PABA in procaine

The results indicate that the present method is more sensitive, simple and has an application part

Conclusion

A simple and sensitive spectrophotometric method for the determination of micro amounts of PABA in aqueous solution, based on the coupling of PABA diazotized with 2,6-dihydroxybenzoic acid in basic medium, has been developed. The proposed method has been applied to the determination of PABA resulted from degradation of procaine.

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التقدير الطيفي لبارا أمينو حامض البنزويك الناتج من أدوية البروكائين باستعمال ٢ ، ٦ – ثنائي هيدروكسي حامض البنزويك كعامل اقتران

أنعام أحمد حمدون

قسم الكيمياء ، كلية العلوم ، جامعة الموصل ، الموص ، العراق

الخلاصة

يتضمن البحث طريقة طيفية لتقدير كميات متناهية في الصغر من بارا–أمينو حامض البنزويك (PABA). تعتمد الطريقة على اقتران بارا–أمينو حامض البنزويك المؤزوت مع عامل الاقتران 6,2– ثنائي هيدروكسي حامض البنزويك في الوسط القاعدي ليعطي صبغة آزوية صفراء ذائبة في الماء. أعطت هذه الصبغة أعلى امتصاص عند الطول الموجي ٤٣٨ نانوميتر وكانت قيمة معامل الامتصاص المولاري 5.2745×10^4 لتر.مول$^{-1}$.سم$^{-1}$.

وتم تطبيق قانون بير ضمن مدى التركيز ١٠–١٠٠ مايكروغرام PABA في حجم نهائي ٢٥ مل (٠,٤–٤ جزء/مليون) وتراوح الخطأ النسبي بين ٠,١١+ و ٣,٧١–% والانحراف القياسي النسبي بين ٢,١١± و ٥,١٣± اعتماداً على درجة التركيز. وتم تطبيق الطريقة المقترحة بنجاح في تقدير المركب الدوائي قيد الدراسة والناتج من تحلل البروكائين .