Methyl acetoacetate as a Coupling Agent in Spectrophotometric Determination of p- Aminobenzoic Acid by Azo-Dye Formation Reaction

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Abstract:

A simple spectrophotometric method for the determination of p-aminobenzoic acid (PABA) in aqueous solution. The method is based on the reaction of PABA, with excess nitrite, in an acidic medium, to produce the corresponding diazonium salt. After the removal of residual nitrite with sulfamic acid, the diazonium salt is coupled with methyl acetoacetate reagent in basic medium to produce, an intense yellow coloured water-soluble and stable azo-dye which exhibits maximum absorption at 365nm. Beer's law is obeyed over the range 10-260μg of PABA in final volume of 25 ml i.e., 0.4-10.4 ppm with a molar absorptivity of 2.41×10^4 $l.mol^{-1}.cm^{-1}$ and Sandell sensitivity index of $0.0056\mu g.cm^{-2}$, a relative error of –0.70 to +0.43% and relative standard deviation of ±0.13 to ±1.01% depending on the concentration level. The method has been applied to the determination of PABA result from degradation of procaine-penicillin injection and from tablets of folic acid.

استخدام قواعد المثيل كعامل مساعد في التمثيل الضوئي لتقدير حامض امينوبنزيك باستخدام طريقة تفاعل الازوتة

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ملخص البحث :

يتضمن البحث طريقة طيفية بسيطة لتقدير بارا- امينو حامض البنزويك في الوسط المائي بطريقة الأزوتة والاقتران. تعتمد الطريقة على مفاعلة الـ PABA مع زيادة من النتريت في وسط حامضي لتكوين ملح الدايازونيوم المقابل وإزالة النتريت الزائد باستخدام حامض السلفاميك، ثم يتم اقتران ملح الدايازونيوم للـ PABA مع الكاشف مثيل اسيتواسيتات في وسط قاعدي ضعيف ليعطي صبغة آزوية ذات لون أصفر مستقرة وذائبة في الماء وتعطي أعلى امتصاص عند الطول الموجي ٣٦٥ نانوميتر. كانت حدود قانون بير في مدى التركيز ١٠-٢٦٠ مايكروغرام PABA في حجم نهائي ٢٥ مللتر أي ٠.٤-١٠.٤ جزء/مليون وكانت الامتصاصية المولارية للصبغة الناتجة ٢.٤١×١٠ ٤ لتر.مول$^{-١}$.سم$^{-١}$ ودلالة ساندل للحساسية ٠.٠٠٥٦ مايكروغرام.سم$^{-٢}$. الخطأ النسبي من -٠.٧٠ إلى +٠.٤٣% والانحراف القياسي النسبي من ±٠.١٣ إلى ±١.٠١% اعتماداً على مستوى التركيز وتم تطبيق الطريقة في تقدير الـ PABA في حقنة البروكائين- بنسلين وأقراص حامض الفوليك.

Introduction :

4 – Aminobenzoic acid or p- aminobenzoic acid (PABA), is a sunscreen agent commonly used in cosmetic products to filter out noxious radiations in sunlight [1]. PABA is used in preparing a number of local anesthetic such as benzocaine and it is used in topical application as a sunscreen agent [2]. PABA is formed as a result of slow degradations of procaine amide or fast degradation of procaine [3].

Different methods have been reported for the determination PABA. These include:

PABA is determined in serum by liquid chromatographic technique with fluorimetric detection [4].The high performance liquid

chromatography (HPLC) method is employed to measure PABA in human plasma and urine [5]. Another HPLC method is used in the determination of PABA in urine included alkaline hydrolysis for conversion of PABA metabolites to PABA[6].

A Spectrophotometric UV-method has been also used for the estimation of PABA in tablets based on the measurement of absorbance at 268 nm [7].

The Bratton Marshall reagent has been employed for the determination of water – soluble vitamins in pharmaceutical forms after separation and extraction by organic solvent then coupling the corresponding PABA diazotised with Bratton Marshall Reagent [8].

Another spectrophotometric method is applied for the determination of PABA, the method involves the diazotisation of PABA and subsequent coupling with phloroglucinol [9].

The oxidative – coupling reaction with promethazine reagent at pH 4.8 in the presence of hypochlorite has been used for the determination of PABA and other primary aromatic amine [10].

A fluorimetric method is developed for the determination of PABA by reaction with 4-methyl-*m*-phenylene diamine. The fluorescence was measured at 462nm. The method was suitable for the determination of seven other aromatic amines [11].

The objective of the investigation reported in this paper is to evaluate a simple spectrophotometric method for the determination of PABA. The method involves the diazotisation of PABA and subsequent coupling with methyl acetoacetate reagent as a coupling agent to form a highly coloured dye. This dye has been proved to be successful for the estimation of the PABA resulted from degradation of procaine and folic acid.

Experimental

Apparatus

All measurements are performed using Shimadzu UV-Visible Recording Spectrophotometer UV-160 with 1 − cm matched silica cells.

Reagents

All chemicals used are of the highest purity available.

Working PABA solution, 100 μg / ml. A 0.01 g of PABA supplied by (BDH) is dissolved in 2 ml ethanol and 30 ml distilled water (heating is necessary to increase solubility), and the volume is completed to 100 ml in a volumetric flask, and this solution is stoppered and kept in a brown bottle in a refrigerator. Under these conditions the solution should be stable for at least one week.

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

Sodium nitrite solution, 1 %. This solution is prepared by dissolving 1 g of sodium nitrite in 100 ml distilled water.

Sulphamic acid solution, 3 %. A 3 g of sulphamic acid is dissolved in 100 ml distilled water.

Methyl acetoacetate solution, 1 %. This solution is prepared by mixing 1.0 g of methyl acetoacetate with methanol in a 100 ml calibrated volumetric flask.

Sodium hydroxide solution, 1N. This solution is prepared by appropriate dilution of the concentrated volumetric (Fluka) solution with distilled water and then transferred to a plastic bottle

Procaine solution. A 0.0286g of procaine penicillin forte injection (France) was dissolved in mixture containing 5ml of 1N HCl and 20 ml

of distilled water. The resulting solution was heated to boiling then cooled and the volume was completed to 50 ml in a volumetric flask.

Folic acid tablets solution

Weight and finely powder 10 tablets (each one contains 5 mg folic acid). The powder is dissolved in 50 ml sodium hydroxide solution (0.1N) then 1ml of solution is transferred to 100 ml volumetric flask followed by adding 75 ml distilled water,18 ml hydrochloric acid (0.1N) and 1ml of gelatin solution and the volume is completed to 100 ml with distilled water(to prepare 10μg folic acid/ml), then 75 ml of the above solution mixed with 500 mg zinc in a conical flask with shaking for 15 minutes , filtered and keep the filtered in black flask (each ml contain 3.1μg PABA).

Recommended Procedure and Calibration Graph

To a series of 25 ml volumetric flasks aliquots covering the range of 10 – 260 μg PABA are transferred, 0.25 ml of 1 N HCl is then added and the mixtures are shaken. Then 0.3 ml of 1 % sodium nitrite solution is added and the mixtures are allowed to stand for 2 minutes. Then 0.2 ml of 3 % sulphamic acid solution is added and the mixtures are occasionally stirred for 2 minutes. Then 0.25 ml of 1 % methyl acetoacetate solution is added and after 5ml of sodium bicarbonate solution (1N) added the volumes are completed to the mark with distilled water. The absorbances are measured at 365 nm against blank solution or distilled water, using 1 – cm matched cells. (Fig. 1) shows the calibration curve which indicates that Beer's law is obeyed over the concentration range 10 – 260 μg / 25 ml final volume, i.e., 0.4- 10.4 ppm and above 260 μg / 25 ml gives negative deviation . The molar absorptivity is 2.41×10^4 $l.mol^{-1}.cm^{-1}$.

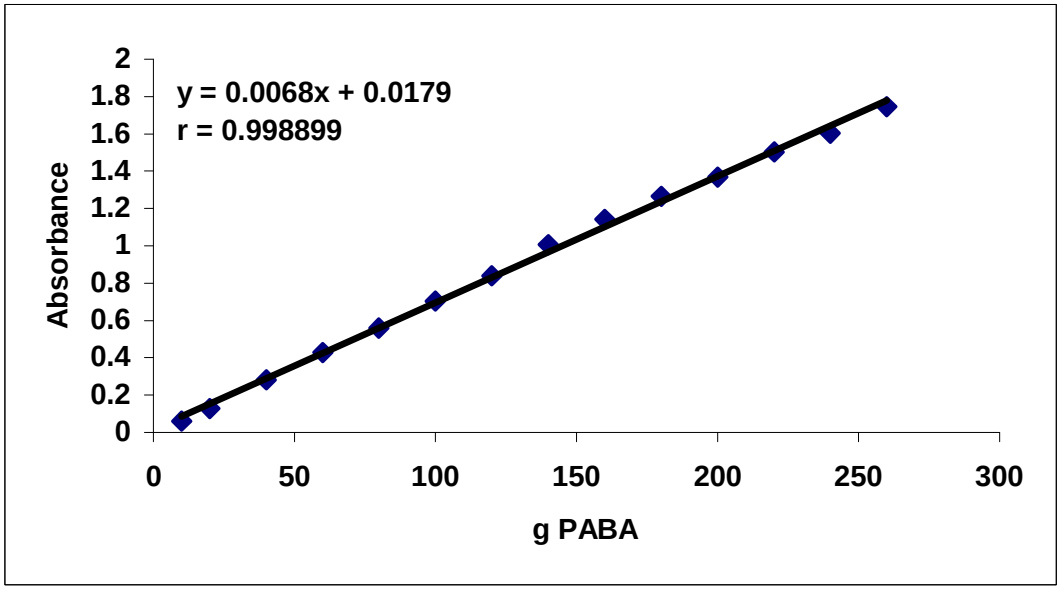


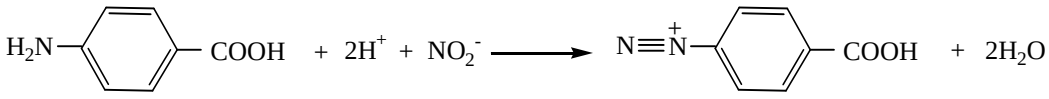
Fig1. Calibration graph for PABA determination using as a coupling reagent

Results and Discussion

For the subsequent experiments, 100 μg of PABA is taken in 25 ml final volumes and absorbance measurements are performed at 365 nm.

Principle of the method

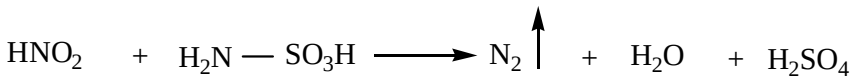
PABA is reacted with excess nitrite in acidic medium to form the corresponding diazonium salt:



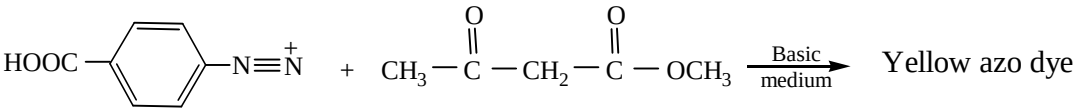
p-Aminobenzoic acid

Diazotised *p*-aminobenzoic acid

The residual nitrite (as nitrite acid) which was undesirable can be removed by adding sulphamic acid



The coloured solution formed by coupling diazotised PABA with methyl acetoacetate in a lkaline medium:



Diazotised *p*-aminobenzoic acid Methyl acetoacetate

Study of the Optimum Reaction Conditions

The various parameters affecting and related to the color intensity of the dye have been studied and optimum conditions are selected.

Effect of diazotisation acid

The effect of the amount of different acids (weak and strong) for the diazotisation of PABA, have been investigated. The results are indicated that 0.25 ml of 1N HCl produces the highest intensity for the dye, so it has been selected in the subsequent experiments.

Effect of nitrite amount and time

The effect of nitrite amount and its reaction time with PABA have been investigated to verify its optimum amount which gives the higest intensity of the resulting azo-dye. A 0.3 ml of 1 % nitrite solution with 2 minutes reaction time has been incorporated for the subsequent steps.

Effect of sulphamic acid amount and time

The presence of unreacted nitrite is undesirable in diazotisation reaction. Therefore, it should be removed by sulphamic acid which fastly reacts with nitrite. The results indicated that 0.2 ml of 3 % sulphamic acid solution with 2 minutes standing time are considered to be the most suitable, and therefore are selected subsequently.

Effect of methyl acetoacetate amount

The effect of methyl acetoacetate amount on the intensity of the dye has been studied. From the results, it can be observed that 0.25 ml of 0.1% methylaceto acetate is the more suitable amount which gives the highest value of intensity for the azo dye formed and the highest value of correlation coefficient(Table 1)**.**

Table1.The effect of methyl acetoacetate amount

Ml of reagent (0.1%)	Absorbance / μg of PABA/25 ml					r
	10	**40**	**80**	**140**	**200**	
0.1	0.042	0.229	0.444	0.896	1.193	0..9977
0.25	0.049	0.231	0.471	0.898	1.200	0.9984
0.5	0.050	0.225	0.462	0.846	1.010	0.9902
0.75	0.052	0.223	0.460	0.847	1.000	0.9890

Effect of time on color development

The effect of time on the development and stability period of the coloured dye is investigated under optimum conditions of determination of PABA .From the experimental data, it can be shown that the formation of the coloured dye formed is complete immediately and the absorbance remained constant for, at least 60 minutes(Table2).

Table 2.The effect of time on absorbance

μg of PABA/25 ml	Absorbance / minute standing time							
	5	**10**	**20**	**30**	**40**	**50**	**55**	**60**
50	0.356	0.356	0.357	0.355	0.355	0.354	0.354	0.353
100	0.705	0.704	0.707	0.705	0.707	0.705	0.704	0.703
200	1.420	1.422	1.422	1.420	1.419	1.417	1.416	1.416

Effect of base

The preliminary experiments have shown that dizotised PABA can give coloured dye with methyl acetoacetate only in alkaline medium. Different bases (strong and weak) have been used , the results indicate that the coloured dye need a weak basic medium which give high intensity and accepted value of colour contrast($\Delta\lambda$), therefore 1 ml of 1N sodium bicarbonate solution has been recommended for the subsequent experiments(Table3).

Table 3.The effect of base on the absorbance and colour contrast

Solution 1N base used	Variable	Absorbance / ml of base use						
		0.5	**1**	**1.5**	**2**	**3**	**5**	**7**
NaOH	A	0.574	0.595	0.609	0.617	0.620	0.631	0.590
	$\Delta\lambda^*$, nm	150	158	157	157	159	159	159
KOH	A	0.689	0.661	0.673	0.669	0.658	0.645	0.660
	$\Delta\lambda$	99	99	98	98	98	100	102
Na_2CO_3	A	0.715	0.716	0718	0.715	0.710	0.711	0.710
	$\Delta\lambda$	108	95	95	100	100	99	101
$NaHCO_3$	A	0.705	0.708	0.710	0.713	0.711	0.715	0.709
	$\Delta\lambda$	149	143	143	143	149	150	150
NH_4OH	A	0.627	0.638	0.661	0.668	0.683	0.688	0.680
	$\Delta\lambda$	141	143	145	144	150	149	151

$\Delta\lambda^* = \lambda_{max}S - \lambda_{max}B$ S = the dye B = blank

Final Absorption Spectra

Under the above optimized conditions, absorption spectra of the dye formed from the reaction of PABA with methyl acetoacetate in alkaline medium against its corresponding reagent blank which shows no absorption in the visible region (Fig. 2). The wavelength of maximum

absorption of the colored dye at 365 nm has been used in all subsequent experiments.

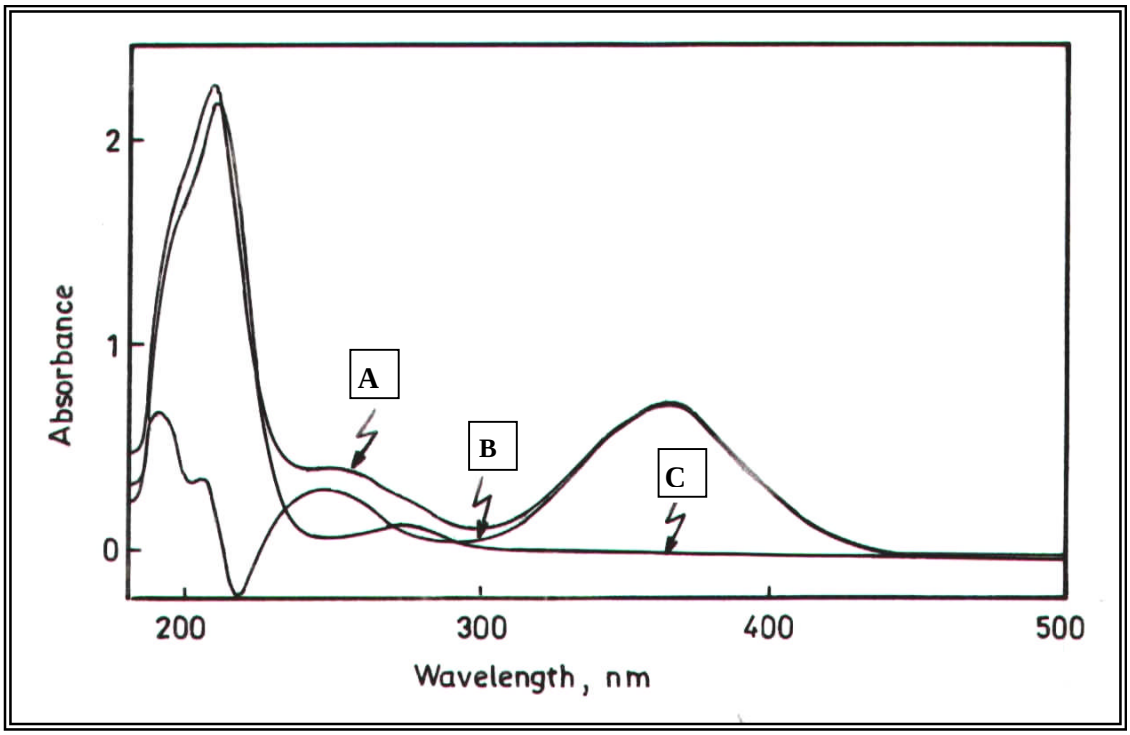


Fig. 2: Absorption spectra of 100 μg PABA / 25 ml treated according to the recommended procedure and measured against (A) blank, (B) distilled water and (C) blank measured against distilled water.

Accuracy And Precision

Three different concentrations of PABA are used in the determination of the accuracy and precision of the method, the results shown in Table 4 indicate that the method has good accuracy and precision.

Table 4. Accuracy and precision of the method.

Amount of *p*-aminobenzoic acid taken, μg	Relative error, %*	Relative standard deviation, %*
40	-0.67	±1.01
120	-0.70	±0.44
200	+0.43	±0.13

* Average of five determinations

Nature of the Dye

The composition of the intense yellow dye that results from the reaction of PABA and methyl acetoacetate has been established using the continuous variations and the mole – ratio methods, the results indicate that the dye has a combination 1:1 ratio of diasotized PABA to methyl acetoacetate(Fig. 3 and 4).

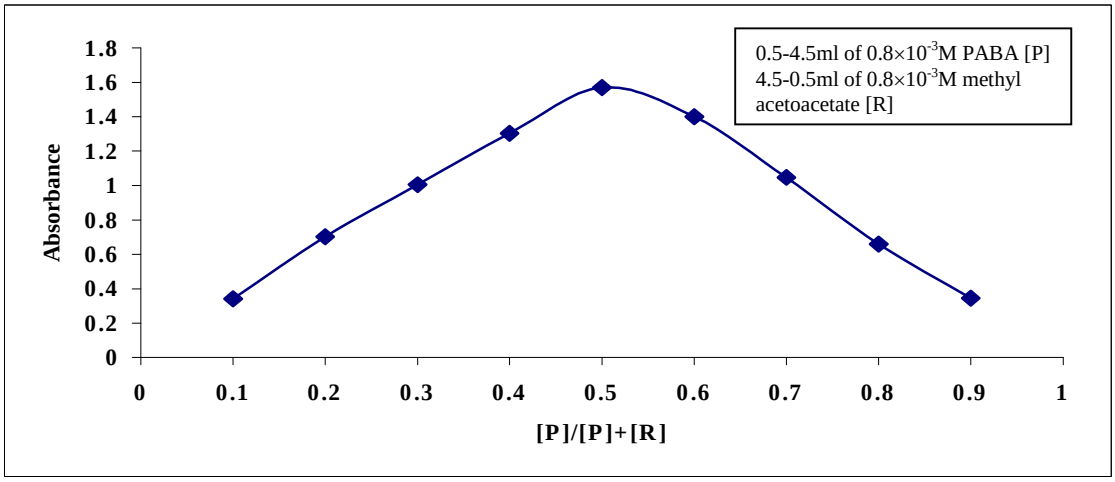


Fig.3: The continuous variations plot for diasotized PABA to methyl acetoacetate

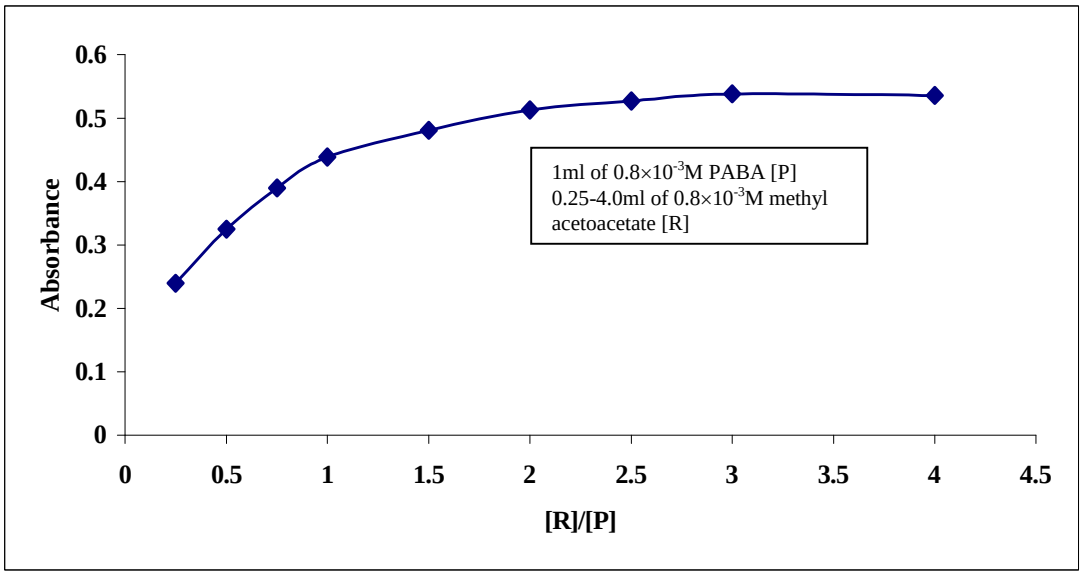
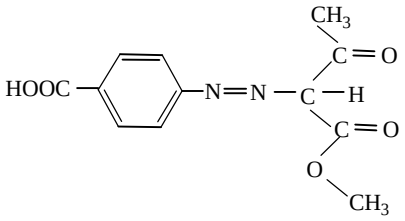


Fig.4: The mole - ratio plot for diasotized PABA to methyl acetoacetate The azo dye may be has the structure below[12]:



Yellow azo-dye

Application of the Method

To test the applicability of the present method, it has been applied to the determination of PABA in two pharmaceutical preparations. On applying proposed procedure, good recovery is obtained as shown in Table 5.

Table 5. Application of the method

Drug	µg PABA present / 25 ml	µg PABA measured / 25 ml	Recovery*, %
Procaine-penicillin	40	40.6	101.7
	100	100.6	100.6
	140	140.4	100.3
Folic acid	6.2	6.3	102.2
	12.4	13.0	105.0
	18.6	19.1	103.1

* Average of five determinations.

Comparison of Methods

Table 6 shows the comparison between the analytical variables obtained from the present method with those of a recent spectrophotometric method

Table 6: Comparison of the methods

Analytical parameters	Present method	Literature method[9]	Literature method[10]
pH	7.7	1.1	4.8
Temperature (^oC)	At room temperature	At room temperature	At room temperature
Development time (minutes)	0	5	5
λ_{max} (nm)	365	545	590
Medium of mthod	Aqueous	Aqueous	Aqueous
Reagent	Methyl acetoacetate	N-NED	Promethazine.HCl
Beer's law range (ppm)	0.4-10.4	0.4-4	0.4-6.4
Molar Absorptivity ($l.mol^{-1}.cm^{-1}$)	2.41×10^4	5.38×10^4	1.40×10^4
RSD (%)	$\leq\pm1.01$	$<\pm0.9$	<2
Stability of the color (minutes)	60	>60	>60
Colour of the dye	Yellow	Purplish-violet	Violet-blue
Stability constant(K_1), $Molar^{-1}$	4.43×10^5	0.92×10^6	1.44×10^2
Nature of the dye	1:1	1:1	1:1
Application of the method	Has been applied to the assay of PABA in procaine-pencilline and folic acid	Has been applied to the assay of PABA in procaine-pencilline	Has not been applied to any extent

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

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