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SIMPLE OF PARA AMINO BENZOIC ACID IN PURE FORM AND PHARMACEUTICAL PREPARATION BY DIAZOTIZATION AND CLOUD POINT EXTRACTION

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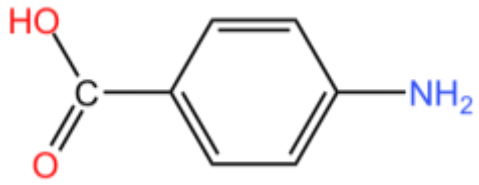
Abstract: Two simple, sensitive, and economical spectroscopic methods were developed for the quantification of pure para-aminobenzoic acid (PABA) and pharmaceutical preparation. The first method is based on the diazotation reaction of PABA, followed by conjugation with α-naphthol in an alkaline medium to form a stable, water-soluble, dark brown azo dye. The dye formed under optimized experimental conditions exhibited a maximum absorbance of 498 nm. The method showed a wide linear dynamic range of 1.0–12.0 µg/mL, with a correlation coefficient (R^2 = 0.997). Statistical evaluation of the results demonstrated excellent accuracy and clarity, with a mean recovery of 101.96% and a very low relative standard deviation (RSD) of 0.0083%. The calculated molar absorbance was 10450.0680 L/mol/cm². This was followed by the second photochemical method, which relies on cloud point extraction by using Trtion X-114 as surfactant .The product was measured at 498 nm. The dynamic range was 1.0 to 12.0 µg/mL, with a correlation coefficient (R^2 = 0.999). Statistical evaluation of the results showed excellent accuracy and clarity, with a mean recovery of 100.24% and a very low relative standard deviation (RSD) of 0.0047%. The calculated molar absorbance was 12219.1740 L/mol/cm². All variables that contribute to improving working conditions in order to obtain better results were studied, namely (type and size of acid, type and size of base, reagent concentration, reaction time and temperature) This also includes studying the (type and size of the active surface, as well as the effect of temperature and product stability time) within the cloud point extraction technique . Thanks to its simplicity and high accuracy, the developed method is very suitable for routine quality control and pharmaceutical analysis of para-aminobenzoic acid (PABA). It should be noted that both methods were applied successfully and no interference was observed from any additive used in the working method, and it did not affect the results at all.

Keywords: Cloud Point Extraction, PABA, α-Naphthol, Spectrophotometry, Azo dye, Diazotization, Quantitative analysis, Triton x-114.

1. INTRODUCTION

The drug para-aminobenzoic acid (PABA) (Table 1) [1][2]. is an aromatic compound Almost odorless or with a very faint, distinctive odor [3]. of structural importance, containing a primary amino group attached to a carboxyl group at the para position on a benzene ring [4].This dual functional nature gives it unique chemical reactivity, making it of great interest [5]. PABA is an essential precursor to folic acid in microorganisms [6]. It is also used pharmaceutically in skincare and UV protection products [7]. Para-aminobenzoic acid (PABA) is a pharmaceutical drug from the B-complex vitamin group. It is used in many sunscreens [8]. It can be synthesized indirectly within the human body due to the lack of necessary enzymes from the beneficial bacteria Escherichia coli (E. coli) present in the intestines. Therefore, it is considered an unnecessary component in food [9]. PABA is a potent antioxidant [10]. and acts as an essential coenzyme in the synthesis of folic acid (vitamin B9) by intestinal bacteria [11]. When applied topically, PABA absorbs harmful UVB radiation [12], preventing sunburn, protecting against premature skin aging, and reducing the risk of cancerous mutations [13]. PABA also acts as a cofactor. Vitamin B10 is essential for the body's enzymes, helping cells utilize proteins efficiently and supporting metabolism [14]. also synergistic antiviral effect when combined with chemical drugs and the properties of a direct anticoagulant [15]. It is also known for its therapeutic uses in treating eye conditions such as conjunctivitis and corneal ulcers. It helps reduce symptoms of conjunctivitis, including swelling, pain, redness, itching, and dryness of the eyes [16]. The dosage of vitamin B10 is an important factor for its safe and effective use [17]. The deficiency of PABA leads to several disorders like erratic white areas of skin, grey hair, fatigue, depression and irritability [18].

Table 1 : General properties of Para Amino Benzoic Acid (PABA).

Structure	Molecular formula Molecular Weight	Nomenclature
	Molecular formula is **($C_7H_7NO_2$)** molecular weight is **137.14 g/mol**	• para-Aminobenzoic acid • 4-aminobenzoic acid • 4-Carboxyaniline • Aminobenzoic acid • p-Aminobenzoic acid • p-Carboxyaniline • p-Carboxyphenylamine

2.MATERIALS AND METHODS

1.1. Apparatus

UV-Vis spectrophotometer: SHIMADZU, Double beam UV-Vis, model UV-1700 made in Japan. The range of wavelength (190-1100) nm, cell quartz with path 1cm., Water Bath : A thermostat water bath, VELP SCICTiFICa, Made in EUROPE, Electric Balance: Sartorius (0.0000), made in Germany, Centrifuge: Grammy Industrial corp, Modle:plc-03,Made in Taiwan.

1.2. Reagents and chemicals

All chemicals and reagents utilized in this study were of analytical reagent grade. Deionized water was employed throughout the work to prepare all solutions and serial dilutions.

1. PABA Stock Solution (1000 µg/mL): This standard solution was prepared by accurately weighing the pure p-Aminobenzoic acid (PABA), obtained from SD Fine Chem Ltd, and dissolving it in deionized water using a 100 mL volumetric flask.
2. α-Naphthol Solution: Prepared by dissolving the reagent, purchased from BDH, in deionized water to serve as the coupling agent.
3. Sodium nitrite (NaNO2): This is the pure salt provided by Scharlau, prepared in deionized water to serve as the diazotizing reagent.
4. Hydrochloric acid (HCl): Purchased from GCC (Gulf Chemical Co) and diluted to the required concentration value to provide the acidic medium.
5. Sulfamic Acid: Prepared from the pure reagent purchased from BDH in deionized water to function as a nitrite scavenger for the elimination of excess nitrous acid.
6. Potassium hydroxide (KOH) solution was prepared by dissolving the required amount of KOH pellets (Scharlau, purchased from BDH) in deionized water and diluting to volume in a 100 mL volumetric flask to provide an alkaline medium.
7. Triton x-114 (10 %) : Prepared by dissolving the surface surfactant, purchased from Amresco , by diluting 10 mL of Triton X-114 with water in a volumetric flask 100 mL.

1.3. Pharmaceutical sample preparation

The analytical applicability of the developed method was assessed using PABA capsules (500 mg each capsule, NOW Foods, USA). 0.1 g dissolved in deionized water. The resulting mixture was then filtered through Whatman filter paper to remove insoluble excipients and was transferred into a 100 mL volumetric flask. The volume was finally brought to the mark with deionized water to prepare the sample stock solution for analysis.

1.4. General procedure

1.4.1 First Method (diazotization–coupling reaction)

The PABA was determined by a diazotization–coupling reaction performed in 10 mL volumetric flasks as follows: A precise volume of the PABA solution was placed into the volumetric flask containing stirring bar, followed by the sequential additions of 1 mL of HCl and then 1 mL of $NaNO_2$. Quantitative diazotization was achieved by immediately placing the flask in an ice bath (0–5 °C) for 5 min. After incubation, Sulfamic acid (1 mL) was immediately added while shaking to ensure all unused nitrous acid was neutralized. Azo-coupling was then started by adding 1 mL of α-Naphthol,

and KOH was introduced (1 mL) to form the final chromophore. Deionized water was used to dilute this mixture to the 10 mL mark. The absorbance of the formed maroon azo dye was determined at its λmax498 nm as compared to a reagent blank prepared under identical conditions. Maximum molar absorptivity and signal stability over time were ensured by strict adherence to the chronological order of reagent addition.

1.4.2 Second Method (Cloud Point Extraction)

cloud point extraction needs the subsequent step:Taking volumetric flask (10mL) having the optimal conditions for diazotization and coupling reaction of [PABA] gotten from first batch with add [10%(v/v) surfactant] then ending it to the blot by ethanol and the comfortable of volumetric flask transmission to centrifuge test tube . The mix is shuddered for 1min and left in thermos bath at 60 Co for 20 min, then detached by centrifuge at 4000rpm for 20 min. Test tube was set in ice bath to rise thickness micelles coat , at that point the informal detached. The outstanding micellar was softened by 1mL ethanol later that the absorbance is unhurried spectrophotometrically UV-VIS at maximum wavelength.

2. RESULTS AND DISCUSSION

2.1. First Method : Spectrophotometric determination of PABA by azo-coupling reaction

PABA was spectrophotometrically ascertained on the basis of diazotization and azo-coupling reaction with α-Naphthol to create a maroon colored dye. The emergence of this color is a chemical indication of a successful reaction and the creation of the colored complex, which forms the foundation of the spectrophotometric measurement. Based on the absorption spectrums in Figure (1) the resulting complex registered its maximum absorption λ max at 498 nm. The development of this peak at the visible region is attributed to the development of an extended conjugated system in the azo dye. This resulted in the production of a new chromophore that absorbs light in the visible spectrum rather than being limited to the ultraviolet spectrum, physically describing why the color of the solution changed to maroon following the reaction. Moreover, the absorbance of the sample (blue line) is much higher than that of the reagent blank (green line) within the measurement region. This proves that the spectral signal is solely the presence of PABA and is not affected by the reagents or the medium in which the reaction occurs. The large spectral separation between the sample and the empty base provides the approach with high selectivity and sensitivity. The azo-coupling reaction with α-Naphthol is an accurate and dependable method of qualitative and quantitative determination of the PABA, due to the stability of the colored product and the characteristic absorption peak.

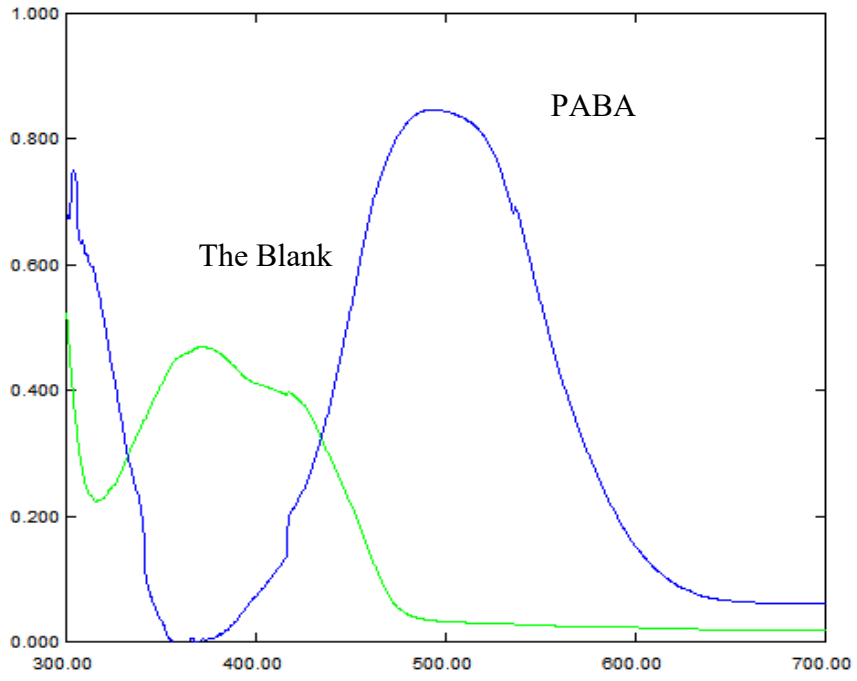


Figure 1: Ultraviolet-visible absorption spectra of the colored product resulting from the reaction of PABA with α-Naphthol

2.1.1. Study the optimal conditions for the reaction

The effects of various parameters on the absorbance of the formed azo dye were studied to establish the optimal conditions for the determination of PABA.

2.1.1.1. Acidity optimization

Figure (2a) results show that the absorbance intensity is greatly affected by the type of acidic medium with HCl showing the highest absorbance at 0.407. This may be explained by the fact that a strong acid is able to provide the necessary level of protons to generate the nitrosonium ion (NO^+), which is the electrophile in the diazotization reaction. Figure (2b) indicated that the peak absorbance was 0.8 mL. This optimum volume can be scientifically accounted by the subsequent decrease in absorbance following this optimum volume through the protonation of the functional groups in the reactants under the highly acidic conditions. This protonation decreases their nucleophilic potentiall , thus inhibiting the azo-coupling mechanism and making the resultant maroon dye unstable. Therefore
0.8 mL of HCl was chosen as the best amount to achieve a high reaction rate and stability of the product.

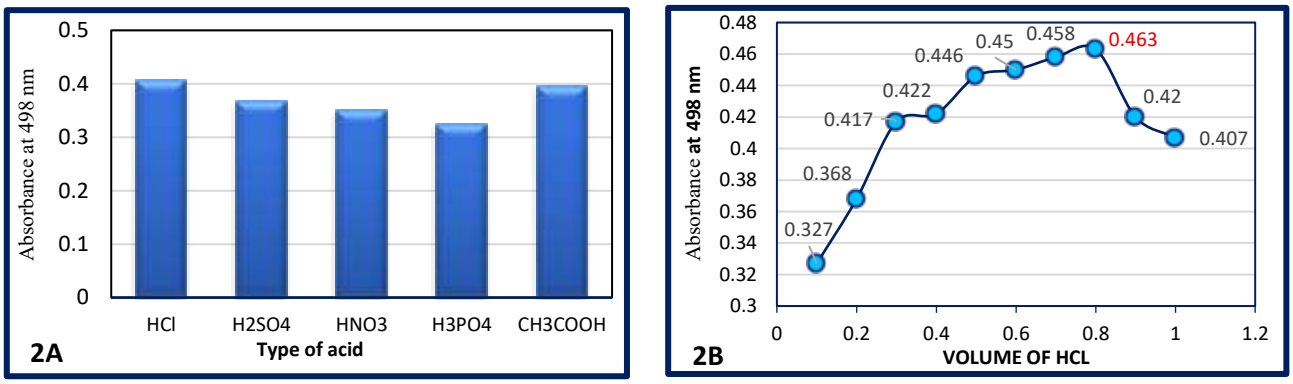


Figure 2: Optimization of acidic conditions for the diazotization reaction: (a) effect of acid type; (b) effect of HCl volume

2.1.1.2. Diazotizing agent study

Figure (3a) indicates that, the absorbance rises slowly with the addition of sodium nitrite up to the highest point of 0.651 at a volume of 0.6 mL. This is chemically explained by the fact that sufficient concentration of nitrosonium ions to completely diazotize all PABA molecules in the solution is formed. The small decrease that is seen after this volume is probably because of excess nitrite causing side reactions or interfering with the stability of the resultant azo dye. The result in Figure (3b) shows that the highest concentration of sulfamic acid is 0.3 mL, which has the highest absorbance of 0.728. Sulfamic acid can additively eliminate any leftover nitrite (NO_2) that was not used; any remaining nitrite may react with the coupling reagent (alpha-naphthol) or cause any second-order reactions that reduce the intensity of the dye color. Thus, by establishing the optimal volume, the highest reaction efficiency and stability can be achieved, as a clean medium is available for the next coupling step.

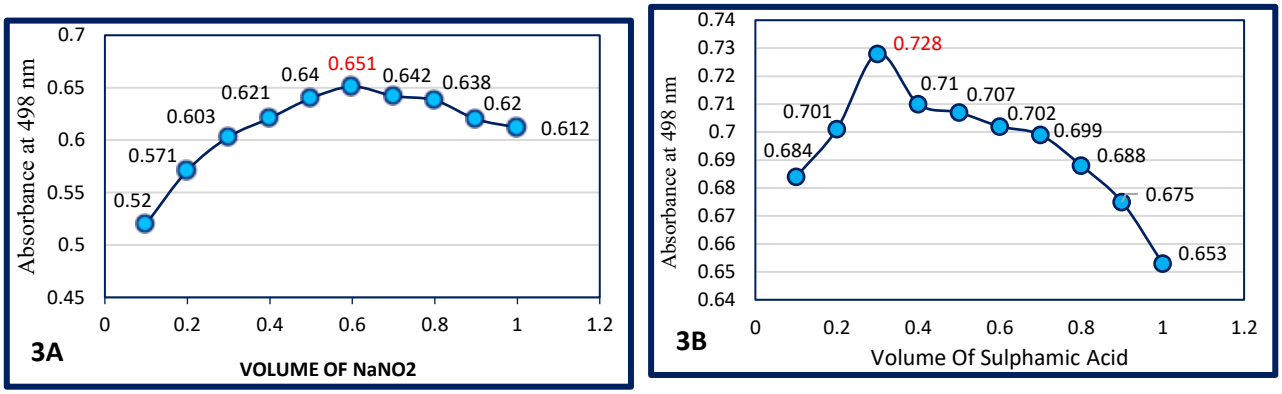
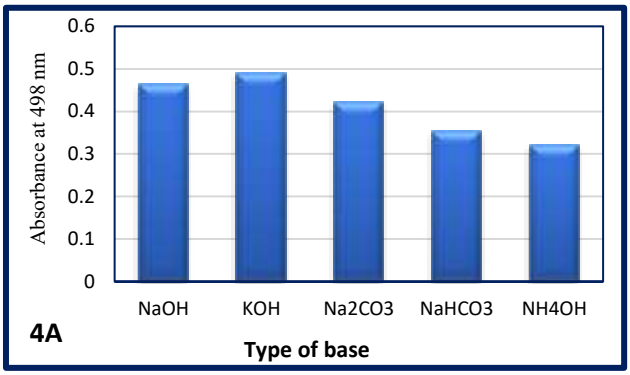
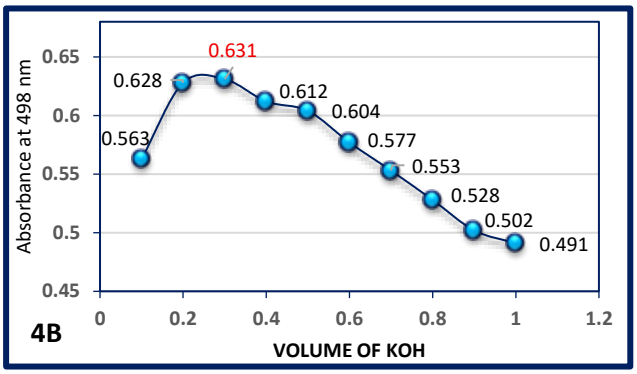


Figure 3 : Effect of diazotizing agent parameters: (a) influence of $NaNO_2$ volume; (b) effect of sulfamic acid volume





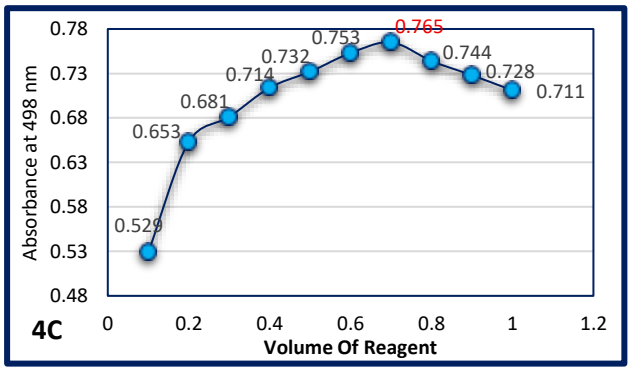


Figure 4 : Optimization of the coupling reaction: (a) effect of base type; (b) effect of base volume; (c) effect of α-Naphthol volume

2.1.1.3. Coupling reaction parameters

As shown in Figure (4a), the highest absorbance (0.49) was found with KOH. The great level of efficiency is attributed to the strong alkalinity of KOH that ensures complete conversion of α-Naphthol to the naphtholate ion, which is the most active in electrophilic coupling with aromatic compounds. Figure (4b) shows that the maximum formation of the colour was at 0.3 mL. This sharp decrease in absorbance when extra volume was added can be explained by the fact that too high level of alkalinity may lead to the breakdown of diazonium salt or impairment of the structure of maroon dye. The results in Figure (4c) show that the absorbance of the solution rises with the volume of the reagent up to 0.7 mL where the absorbance is 0.765. This ensures that there is enough stoichiometric amount to the coupling process. This is likely to reduce further and is likely caused by either dilution or unwanted reagent molecules, which may cause molecular aggregation or spectral overlap to decrease the net absorbance.

2.1.1.4. Temperature and time stability

Figure (5a) indicates that the absorbance rises with rising temperature and reaches its highest value at 40 ℃. Chemically, this temperature is moderate enough to give energy required to bring the coupling process effectively. The loss of absorbance at elevated temperatures, though, is possibly because of the partial thermal degradation of the diazonium salt or the instability of the ensuing azo dye to heat stress. As shown in Figure (5b), the highest colour intensity is obtained after 40 minutes. This implies that a certain time period is needed to ensure that the PABA molecules and the coupling reagent have been in contact. This minimal reduction in absorbance after this point can be rationally explained by the gradual fading of the chromophore or changes in stability of the dye in the solution, and set 40 minutes as the time at which consistent and stable readings could be achieved .

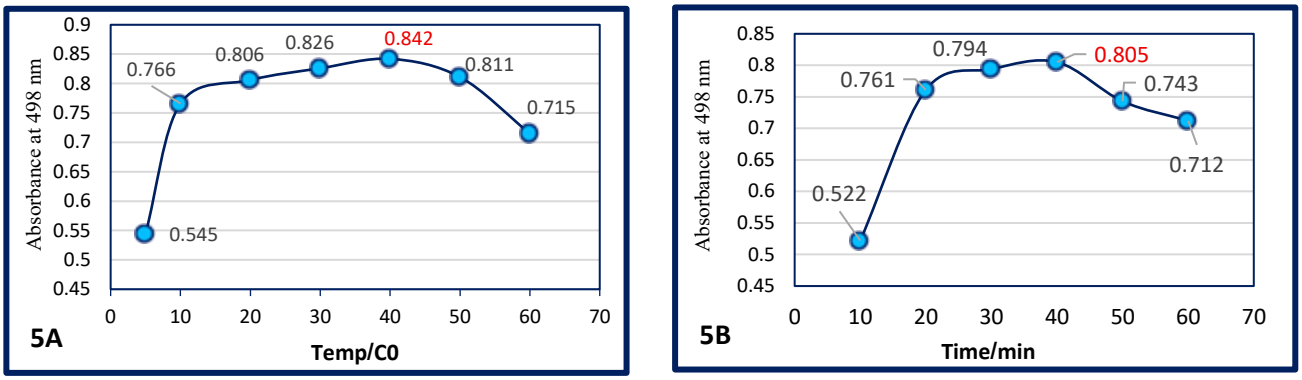
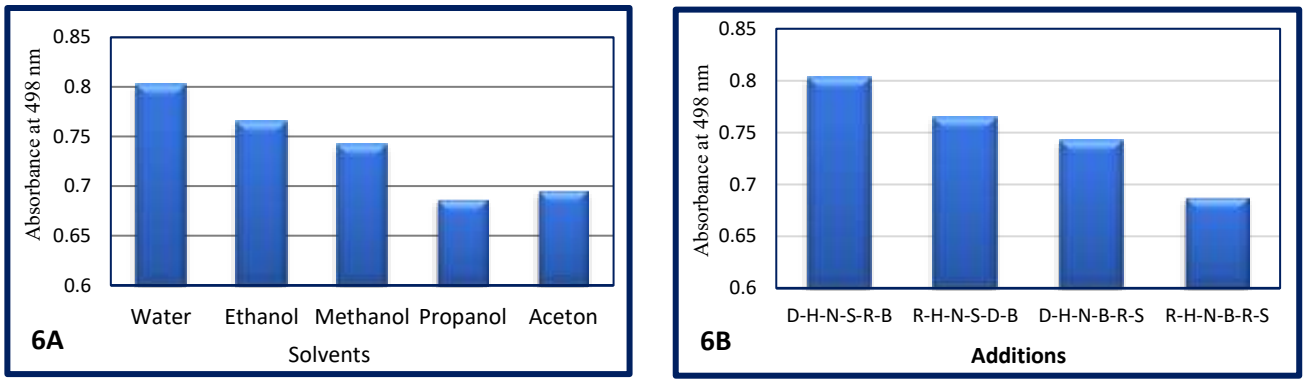


Figure 5: Optimization of reaction conditions: (a) effect of temperature on azo dye stability; (b) effect of reaction time

2.1.1.5. Solvent and addition sequence studies

Figure (6a) shows that water had the highest absorbance (0.803) when compared to organic solvents such as ethanol and methanol. The reason is that, the water is very polar and it stabilizes the ionic species of the dye, and facilitates the effective interaction of the molecules. Additionally, the water is used in line with the concept of green chemistry since the method is more cost-effective and sustainable. Findings in Figure (6b) show that the best order to use to get the highest color intensity is (D–H–N–S–R–B). This particular sequence is to make sure that diazotization is done initially, excess nitrite is removed and then the coupling reaction is done in an alkaline medium. Violation of this sequence will lead to a decrease in absorbance since there might be side reactions or the diazonium salt might decompose itself before time.

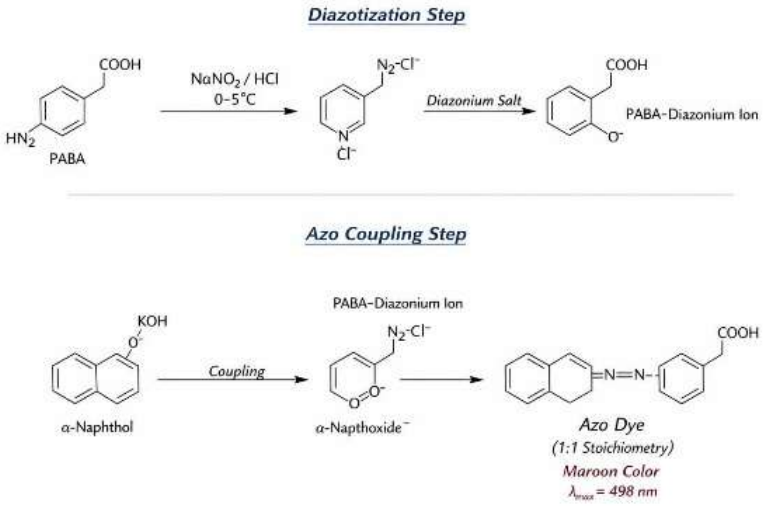


Note: D : PABA; H: Hydrochloride acid; N: Sodium nitrite; S: Sulfamic acid; R: a-Naphthol; B: KOH

Figure 6: Solvent and addition sequence studies: (a) effect of different solvents; (b) influence of the addition sequence on the absorbance

2.1.2. Mechanism of the diazotization and coupling reaction

As shown shown in Scheme 1, the formation of the colored species proceeds through a concerted two-step chemical pathway. The reaction starts with the diazotization of the primary aromatic amine group in PABA with sodium nitrite in an acidic medium (HCl) at 0 to 5°C to give the unstable diazonium salt intermediate. This process is followed by the electrophilic coupling of the formed cation with α-naphthol in alkaline conditions (KOH) to produce a stable azo derivative have marone colour . The origin of this colour has been explained due to the extended π-conjugation system due to azo linkage (-N=N-) which shifts the electronic transitions into the visible region at λmax=498 nm. Due to the conversion of α-naphthol to the more reactive phenoxide form at optimal alkaline conditions, a nearly instant and quantitative coupling occurs under the above-mentioned conditions.



Scheme 1: Proposed reaction mechanism

2.1.3. Stoichiometry of the reaction

The stoichiometry of the maroon azo dye formed was determined using Job's method of continuous variations [19]. Such a methodology is essential for determination of the cation coupling agent binding fraction. Corresponding Job's plot, of the absorbance against the PABA mole fraction, is presented in Figure 7. Maximum absorbance has reached at mole fraction of 0.5, which is strong evidence for the establishment of 1:1 stoichiometric ratio of drug and α-naphthol, Figure 8. This equimolar interaction suggests
that the coupling process is a localized electrophilic attack from a single site. These findings not only confirm the quantitative nature of the reaction but also are in total agreement with the reaction pathway proposed.

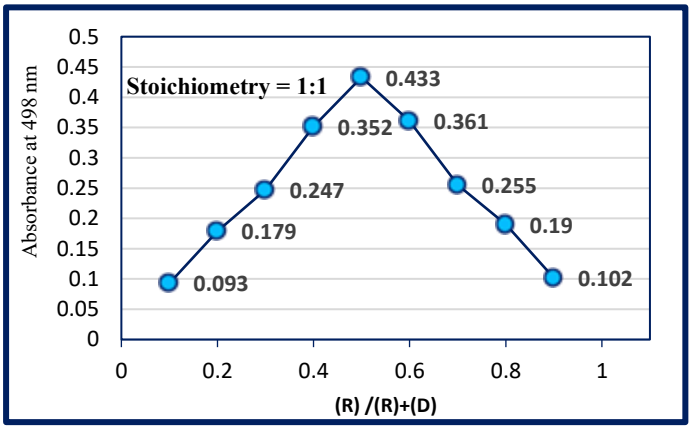


Figure 7: Job's Plot for PABA with α-Naphthol

2.1.4. Mole ratio method

For this experiment, the concentration of PABA was maintained while the concentration of the α-naphthol reagent was changed Figure 8. shows the plot of the corresponding absorbance against the molar ratio ([Reagent]/[Drug]). The straight inflection point of the curve at 1:1 molar ratio reveals the results of Job's method. The agreement between two independent methods for determining the stoichiometry of the reaction constitutes strong evidence that when the reactants are present in equimolar amounts,
the reaction is at its highest stability and at its greatest intensity [20].

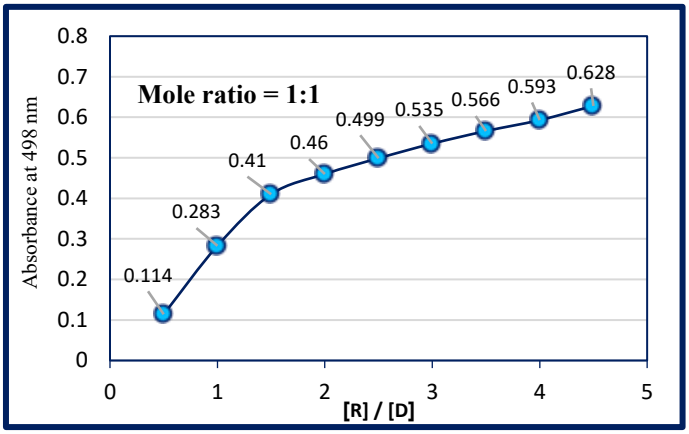


Figure 8: Plot of mole ratio PABA with α-Naphthol

2.1.5. Calibration curve for PABA with α-Naphthol

The calibration curve of Figure 9 indicates that there is a strong linear relationship, which means that strict adherence to the Law of Beer is followed at the range of 112 µg/mL. The chemical basis of this linearity is the proportional growth of the azo dye up to the extent of concentration of PABA; the greater the number of PABA molecules the greater the amount of chromophores formed and the greater the light absorption at λ max. These results are statistically validated by a great correlation coefficient (R^2 = 0.997) and regression equation (y = 0.0762x + 0.1007), which shows that the regression fits the data and precisely quantifies unknown samples.

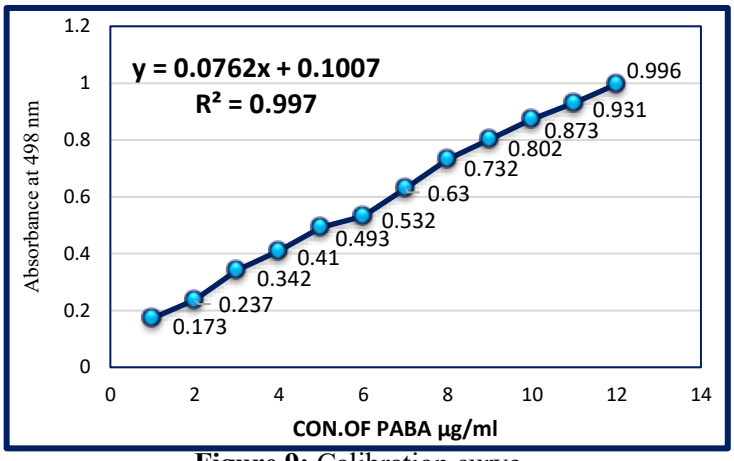


Figure 9: Calibration curve

2.1.6. Accuracy and precision

As indicated in Table (2) , the results yielded an extraordinary average recovery of 101.96% illustrating a strong correlation between measured and theoretical concentrations. Furthermore, the RSD% was lower than 0.0083%, which also indicates the excellent repeatability and reproducibility of the procedure developed.

Table 2: Precision and accuracy of the proposed method for the determination of PABA

Concentration (µg mL⁻¹)	Measured Value (µg mL⁻¹) ± C.L (99%)	Mean Recovery (%)	RSD % (n=5)
3	3.1475 ± 0.10760	**101.96579**	**0.008325**
6	6.1158 ± 0.02877		
9	9.0525 ± 0.18409		
12	12,159.11162		

[*]= Average of five determinations

2.1.7. Application of the proposed method for the determination of PABA in pharmaceutical preparation

These results presented in Table (3) show the successful application of the proposed method in order to quantify the content of para-aminobenzoic acid (PABA) in the pharmaceutical products of NOW Company. This good agreement between the added and recovered concentrations, indicates the efficiency of the analytical process as well as stability of the coloured product obtained. The absorbance of this chromophore is very high and it shows a good spectrophotometric response that is linear in the range of concentration studied and is in agreement with the Beer-Lambert's law. The same method has been found to be accurate and reliable in pharmaceutical products with good recovery values from 93.81 to 99.56% (average 96.05%). The relative standard deviation (RSD) of the method have very low values (0.0011-0.0166%), which means that the method has very good repeatability and precision.

Table 3: Determination of PABA in pharmaceutical preparation by the proposed method

Amount of PABA µg mL-1	*Found	Recovery %	Average Recovery %	Erel%	Average Erel%	RSD%
12	11.9476	99.5633	96.0505	- 0.4367	- 3.9494	0.0011
9	8.5718	95.2422		-4.7578		0.0088
6	5.7350	95.5833		-4.4167		0.0023
3	2.8144	93.8133		-6.1867		0.0166

[*]= Average of five determinations

2.1.8. Comparison with the reported methods

To evaluation the analytical efficiency of the proposed method, a comparative performance of different techniques reported for PABA determination was in Table (4). The comparative data presented in Table 4 show the advantage of the proposed method for its analytical Characteristics, wider dynamic linear range (1.0–12.0 µg/mL), high precision with a very low RSD% 0.0083. Moreover, α-Naphthol is used as a stable and economical coupling reagent in the method, which ensures both sensitivity and applicability of PABA determination in routine. The choice of α-Naphthol is also

advantageous, since it displaces the absorption maximum to the visible region (498 nm), therefore considerably reducing possible spectral interferences.

Table 4: Comparison of the proposed method with other reported methods for PABA determination

Method	Reported Method 1	Reported Method 2	Reported Method 3
Reagent	1-NASA	2,4,6-THBA	alpha-Naphthol
λmax (nm)	525nm	416nm	498nm
Linear Range (μg mL^{-1})	0.25-7.0	0.125-5.0	1.0-12.0
Molar Absorptivity ($L.mol^{-1}.cm^{-1}$)	3.60×10^4	3.79×10^4	1.04×10^4
Comparative Analytical Performance	Moderate stability and sensitivity.	High sensitivity but lower wavelength.	Remarkable precision (RSD 0.0083%) and excellent chromophore stability.
Reference	[21]	[22]	Current study

2.2.Second Method : Spectrophotometric determination of PABA by using Cloud Point Extraction Technique

2.2.1. Study the effect of materials used in Cloud Point Extraction Technique

The effects of different materials used in cloud point extraction technology on absorption accuracy were studied to determine the optimal conditions for PABA determination.

2.2.1.1. Effect of Surfactant

The surfactant plays a crucial role in the turbidity point extraction method. Since all surfaces remain opaque, depending on the micelle centers, several 10 mL volumetric bottles were taken, and in each bottle (1 mL of para-aminobenzoic acid, 0.8 mL of hydrochloric acid , 0.6 mL of sodium nitrite, 0.3 mL of sulfamic acid, 0.7 mL of α-Naphthol, and 0.3 mL of potassium hydroxide) were placed. Then, 1 mL of each surfactant was added to each volumetric bottle containing the two drugs [Tween 20, Tween 80, Triton X-114, Triton X-100, CTAP, SDS].The mixture was then heated to 60°C for 20 minutes and centrifuged at 4000 rpm for 20 minutes. The active surface-rich phase and the solute (the substance to be extracted) were then separated by dissolution in 1 ml of ethanol. The absorbance was measured at the established wavelength for para-aminobenzoic acid (PABA) at 498 nm. The effect of surfactant

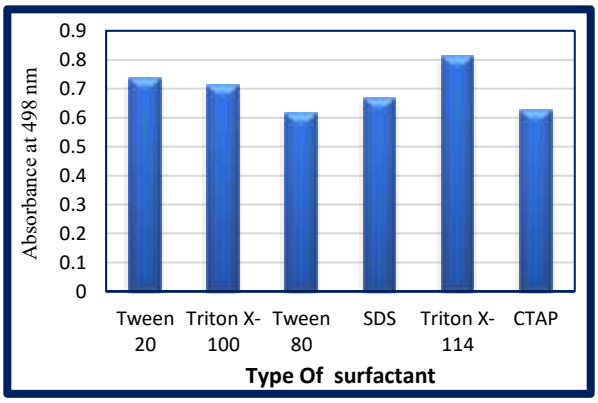


Figure 10 : effect of Surfactant type

2.2.1.2. Effect of Triton X-114 Volume

The same solution was prepared in a 10 ml glass flask as in the paragraph (2.2.2) , then varying volumes of 10% Triton X-114 were added to it, the volume was completed with distilled water, and it was heated at 60 °C for 20 minutes. Then the components of the solution were transferred into a test tube and placed in a centrifuge at 4000 rpm for 20 minutes. The active surface-rich phase and the drug to be extracted were separated by 1 ml of ethanol. The absorption Was then measured at the fixed wavelength of para-aminobenzoic acid at 498 nm. It was found that the optimal volume of the active surface was 1 ml, which achieved the highest absorption, as shown in Figure 11.

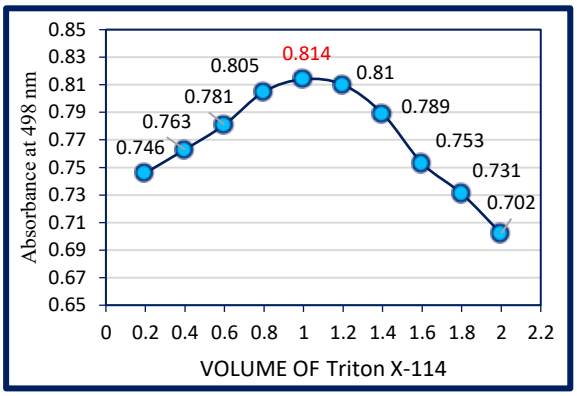


Figure 11 : effect of Triton X-114 Volume

2.2.1.3. Effect of Equilibrium Temperature

In a series of 10 mL volumetric flasks, 1 mL of para-aminobenzoic acid, 0.8 mL of hydrochloric acid, 0.6 mL of sodium nitrite, 0.3 mL of sulfamic acid, 0.7 mL of α-naphthol, 0.3 mL of potassium hydroxide, and 1 mL of 10% (v/v) Triton X-114 were added, and the volume was completed with distilled water. The temperature was varied within a range of 35–65°C, and the incubation time was 5–35 minutes for each of the
two drugs. The absorbance was then measured and
recorded at the maximum wavelength , The results are shown in the figure 12.

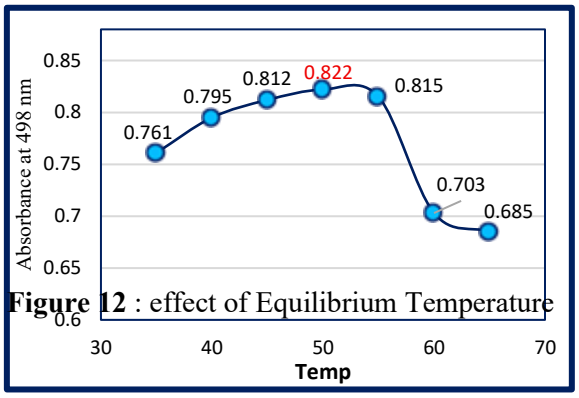


Figure 12 : effect of Equilibrium Temperature

2.2.1.4. Effect of the Incubation Time

In a series of 10 mL volumetric flasks, 1 mL of para-aminobenzoic acid, 0.8 mL of acetic acid, 0.6 mL of sodium nitrite, 0.3 mL of sulfamic acid, 0.7 mL of α-naphthol, 0.3 mL of potassium hydroxide, and 1 mL of 10% (v/v) Triton-X114 were added, and the temperature was maintained at 50°C. The volume was then topped up with distilled water. The temperature was maintained at 50°C for varying times of 5–35 minutes for each substance. Para-aminobenzoic acid was analyzed at a maximum wavelength of 498 nm. The results are shown in the figure 13.

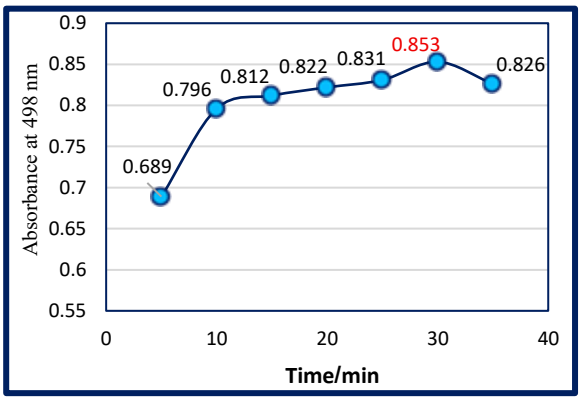


Figure 13 : effect of the Incubation Time

2.2.2. Preparation of Calibration Curve in CPE

After establishing the optimal conditions in previous experiments for the determination of para-aminobenzoic acid, a standard titration curve was prepared by adding increasing volumes of para-aminobenzoic acid (0.1-1.2 mL) at a concentration of (1-12 µg mL^{-1}), 0.8 mL of hydrochloric acid, 0.6 mL of sodium nitrite, 0.3 mL of sulfamic acid, 0.7 mL of α-naphthol, 0.3 mL of potassium hydroxide
, and 1 mL of 10% (v/v) Triton X-114 to a 10 mL volumetric flask. The volume was then completed with distilled water, and the solution
was heated in a water bath to the cloud point at the optimal temperature and stability time. Separation was then performed by centrifugation at 4000 rpm for 20 minutes. The rich liquid layer is dissolved by 1 ml of ethanol, then at a maximum wavelength of 498 nanometers as shown in the figure 14 .

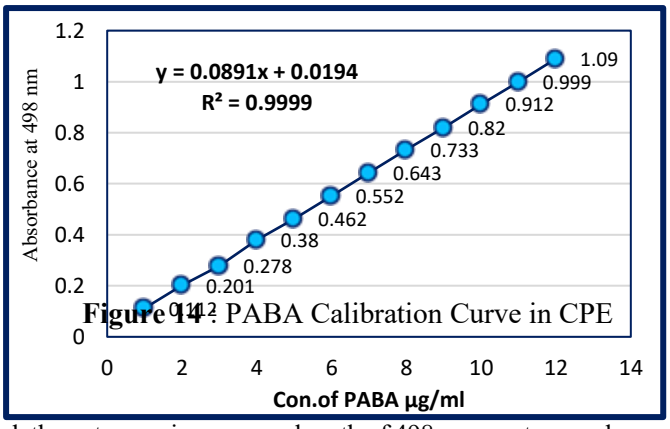


Figure 14 : PABA Calibration Curve in CPE

2.2.3. Accuracy and Precision:

The test results were excellent. Four different concentrations (3, 6, 9, and 12) of the drugs were used in five replicates, followed by the cloud point method under optimal conditions. These results are excellent because the technique is highly accurate and precise, with a retrieval rate of 100.24542, as shown in the table (5) .

Table 5: Precision and accuracy of the proposed method for the determination of PABA in CPE .

Amount Of PABA µg mL^{-1}	*Found	Recovery %	Average Recovery %	E_{rel}%	Average E_{rel}%	RSD%
12	12.0170	100.14167	100.24542	0.1417	0.2455	0.0007
9	9.0234	100.26000		0.2600		0.0012

6	**6.0068**	**100.11333**	**0.1133**	**0.0010**
3	**3.0140**	**100.46667**	**0.4667**	**2.0018**

[*]= Average of five determinations

2.2.3. Applications of the Cloud Point Extraction on Pharmaceticals

The drug PABA, in powder form, manufactured by NOW Foods, USA, containing 500 mg of para-aminobenzoic acid per capsule, was used. The results were good and highly reliable in the analysis of the pharmaceutical product samples. The results are shown in the table (6) .

Table 6. Determination of PABA in pharmaceutical preparation by the proposed method CPE

Amount of PABAL µg mL-1	*Found	Recovery %	Average Recovery %	Erel%	Average Erel%	RSD%
12	**11.9620**	**99.6833**	**97.8050**	**-0.3167**	**- 2.1950**	**0.0012**
9	**8.7324**	**97.0267**		**-2.9733**		**0.0056**
6	**5.8474**	**97.4567**		**-2.5433**		**0.0010**
3	**2.9116**	**97.0533**		**-2.9467**		**0.0018**

[*]= Average of five determinations

2.2.4. Comparison of the AZO-Coupling with Cloud Point Extraction Methods for PABA

The result of the AZO-Coupling with Cloud Point Extraction for PABA Methods shown in Table (7) .

Table 7: Comparison of the AZO-Coupling with Cloud Point Extraction Methods for PABA

Parameter	AZO-Coupling Method	CPE Method
Colour of Product	maroon	maroon
λ max	498 nm	498 nm
Regression equation	y = 0.0762x + 0.1007	y = 0.0891x + 0.0194
Standard deviation of regression	0.249515	0.321266
Correlation coefficient (r)	0.998	0.999
C.L for slop (b±tSb) at 99%	0.0762 ± 0.6588845	0.0891 ±0.1314916
C.L for Intercept (b±tSa) at 99%	0.1007 ± 0.0097845	0.019400±0.001952
Concentration range (µg mL-1)	(1-12) µg mL-1	(1-12) µg mL-1
Limit of Detection (µg mL-1)	0.0169	0.1489
Limit of Quantitative (µg mL-1)	0.0255	0.4146
Sandell's Sensitivity (µg mL-1)	0.01312	0.01122
Molar absorbance (L.mol-1.cm-1)	10450.0680	12219.1740
Composition of product	1:1	1:1
Recovery %	101.96579	100.24542
RSD%	0.008325	0.0047
C.L for con.12(µg mL-1)	12.0159 ± 0.11162	12.0170±0.0168539
C.L for con.9(µg mL-1)	9.0525 ± 0.18409	9.0234±0.0214772
C.L for con.6(µg mL-1)	6.1158 ± 0.02877	6.0068±0.0128091
C.L for con.3 (µg mL-1)	3.1475 ± 0.10760	3.0140±0.0112778

3. CONCLUSION

In this work, we present two novel spectrophotometric methods for the determination of para-aminobenzoic acid in its pure form and in pharmaceutical formulations. The results demonstrate that both methods are simple, highly sensitive, and inexpensive. The first technique involves the formation of a stable azo dye through a coupling reaction with α-naphthol in an alkaline medium. The improved method exhibited broad-range linearity ranging from 1 to 12 µg/ml (R^2 = 0.997). The accuracy and reliability of the method were further confirmed by high recovery rates and low relative standard deviations. The second technique involves the formation of micelles through the addition of a suitable active surface to form an organic layer. The improved method also exhibited broad-range linearity ranging from 1 to 12 µg/ml (R^2 = 0.999). The accuracy and reliability of the method were also confirmed by high recovery rates and low relative standard

deviations. In summary, the comparative study demonstrated that the proposed methods are a reliable alternative to more complex (and more expensive) techniques, with the added advantages of being environmentally safe and easy to apply in quality control laboratories.

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