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Kinetic spectrophotometric determination of Para-amino benzoic acid in pharmaceutical preparation by diazotization-coupling reaction

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***Abstract*— Modest and delicate kinetic approaches are industrialized used for the resolve of Para-amino benzoic acid in unadulterated form and in pharmaceutical measures. The process is based on diazotization of benzoic acid and coupling with 1,2,3-Trihydroxybenzene in natural medium to form a brown-reddish water-soluble azo dye, that has a maximum absorption at 446nm. We used the constant time method (15 minutes) with an initial rate of speed as a reference. We also scrutinized the reaction kinetics and determined the most favorable conditions for studying the reaction kinetics in 15 minutes. Drug kinetics were calculated from the linear drug kinetics using a calibration curve, with the initial velocity rate method and constant time method both having the concentration range (1*10-4-1*10-5 M). Pharmaceutical preparations utilized effortless methods to determine (PABA) using workable techniques.**

***Keywords*— PAPA; Spectrophotometric; Kinetic; 1,2,3-Trihydroxybenzen; Diazotization-Coupling Reaction.**

I. INTRODUCTION

Amino benzoic acids are mixtures that consume carboxyl and amino sets in a straight line, with the aromatic ring being added to the mixture and ingredients that are consumed in a straight line to the aromatic ring, and the carbohydrates that are consuming amino acids in a straight line are mixing amino and amino acids [1].The molecular formula of Para-amino benzoic acid (PABA) is C7H7NO2 and it has an aromatic moiety, also known as Para-amino benzoic acid [2-3]. The 4-aminobenzoic acid also yields a different type of dietary fiber, such as 6-aminobenzoic acid in 4-aminobenzoic acid-based DNA sequences. [4-5]. PABA has x been extensively used for long time in the chemical manufacturing process by means of a precursor substantial for the base material of folic acid, a vital vitamin for DNA production and repetition [6 -7].Its target is also a product of the UV defense and sun-protection due to its potential in harnessing UV energy, which it utilizes in the production of hair colorants and sunblock[8 -9]. PABA is a harmless substance ingested only in the digestive system, and its byproducts perform various biological functions [10-11]. The medications encompassing PABA supports are supposed to be fine accepted [12]. PABA, a vitamin A, is an important and easily accessible component in the production of folic acid [13]. It is derived from a range of foods, such as meat, milk, grins, eggs and more, and is made into different variety of foods. Excluded folic acid discontinuation, it is formed in the humanoid body and is named after the folic acid which is produced in the coelomate, lysoemic nervous system [14]. PABA is not generated in humans or mammals, but due to the supply of food and interrelated bacterial species (Escherichia coli), which continuously produce PABA in their diets, and it is a constant metabolic rate component in humans and the mammals [15]. PABA's chemistry incorporates the practical sets of reactions, including carboxylic acid and amino acids [14]. Acetic anhydride is used to procedure an amide through acylation in the amino set, which is also a process commonly involved in many amino acids. Utilizing alcohol is used to an esterification reaction, the esterification reaction is then used as a rebound in the carboxyl set [16]. PABA also suffers oxidation by potassium permanganate or hydrogen peroxide and results from 4-benzoquinone or 4-nitrobenzoic acid oxidation, in which it is fatal without benefit of PABA in particular. Many different moieties have been synthesized as of PABA through diverse methods, among other thing From PABA, the chief mixtures that have been arranged are Quinolone the Schiff base, parole, thiourea, azide, pyridine, indole, etc. PABA dismiss procedural organized multiplexes through metal ions such as copper and zinc [18]. In addition, these multiplexes have displayed antibacterial acts and act as an antibacterial agent [19]. PABA has a wide range of chemical characteristics and is an essential component in range of industrial and biological processes, as its production is critical. [20]. Benzoic acid isomers such as m-amino benzoic acid, anthracitic acid, and p-amino benzoic acid are the most significant mixtures in this set [21]. Specifically, their products from aliphatic, halogen, or aromatic exchanges, which are general existence rummage-sale activities in chemistry and industry. PABA has been recognized as a chemical compound since 1863 and it has been used as a vitamin drug with vitamins since 1939 [22].The presence of significant differences between the cytotoxic properties of

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PABA can be deduced from substitutions in its chemical mother nature, such as the chemical Mother Nature or changes in individual peptides [23]. In chemistry with PABA, the inductive conclusion, fascinating outcome, and responsibility dissemination are the main problems that highlight the inductive result, fascinating outcome, and responsibility dissemination [24].The statement remains ambiguous. Typical examples of mixtures that provide binary protonation spots against amino and carboxyl sets are PABA and esterified products, which are well-planned and contain binomial and carboxyl sets. PABA is too a biologically energetic multifaceted that is regularly originate as a construction chunk for medications through customs reaching after antimicrobial to UV protectants.

II. Experimental

Device: Using a shimadzu UV-Visible-1800 digital double-beam recording spectrophotometer (Tokyo-Japan), a spectrophotometer with 1cm quartz cells was used to measure spectra and absorbance using a shimadzu Digital pH-meter; Metter balance. This page discusses the spectrum of microwave energy, including the Mass and Hnmr spectrum., Folic acid pure, Para-amino benzoic acid, Ethanol 99% purity, $NaNO_2$, NaOH, HCl, $NaCl_2.6H_2O$.

A. Reagent and solutions

The analytical-reagent mark, reagent and solutions, was used to identify the primary ingredient used in Wholly chemicals recycled through the laboratory reaction was. By dissolving (1.37) g of PABA in distilled water and diluting it to the point of formation in a 100 ml standard flask, Standard P-amino benzoic acid solutions, (0.01mole), were produced. A 1M HCl solution was produced by appropriating dilution of concentrated acid.0.02-mole solution of $NaNO_2$, 0.02 mole of 1,2,3-Trihydroxybenzene and 1M NaOH (appropriately dilution of the concentrated volumetric (Fluka) solution with distilled water), $NiCl_2$ (0.237) g in 50 ml distilled water, and combining it with 0.239 moles of concentrated acid dissolved in 100-mole solution [25].

B. Pharmaceutical preparations of PABA

To prepare the folic acid solution used in study, a mixture consisting of 5ml of 1M HCl and 20 of distilled water were mixed with 1.237 g of the folic acid tablet, and then boiled until it reached 100 ml in a volumetric flask after cooling.

C. Analytical procedure for calibration

An aliquot of a standard solution (1×10^{-4}M) containing (0.25-3.5) ml of PABA was transferred into series of 25 ml calibrated flask for the initial-rate method and (0.25-1.75) ml for the fixed-time technique. Then, additional equimolar of $NaNO_2$ (1×10^{-4}M) and the acidity was attuned with 3 ml of 1M HCl solution were added to the final solution. The solution was stunned methodically. Formerly ,2 ml of (1×10^{-4}M) of 1,2,3-Trihydroxybenzen and 3ml of NaOH solutions were added and the innards were diluted to the spot with distilled water and varied well. After 5 min, the absorbance of the colored azo dye was measured at (446) nm in contradiction of the conforming reagent blank.

III. D. Recommended procedure for the determination of Para amino benzoic acid for the assay of pharmaceutical preparations

This solution was prepared by devastating 10 tablets (every tablet having 5 mg folic acid). The powder was then dissolved in 50 ml of diluted NaOH solution (0.1) M and then 1ml of this solution was added to 100 ml volume flask with 75 ml distilled water. After that, 18 ml of HCl (0.1 M)diluted with distilled water to the mark. A 75 ml of final prepared solution was transported to conical flask and (0.237) g of nickel metal was added and then left shaking for 15 min. Then, the solution was filtered and isolated the first 10 ml of filtered solution was taken. The remaining of the filtered solution was composed and kept in a dark vessel (Fig 1).

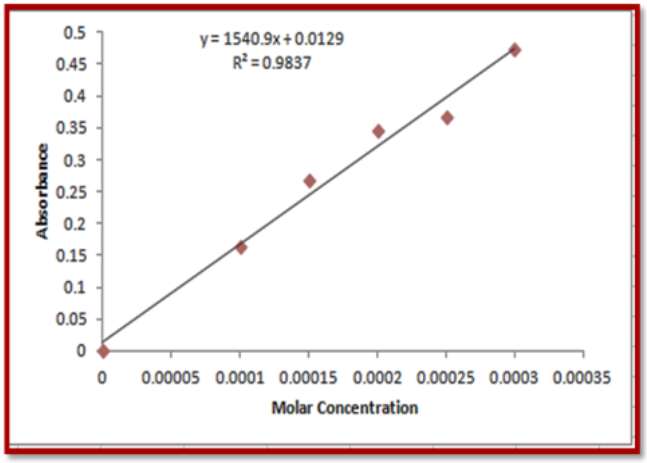

Fig 1: Calibration graph of PABA determination

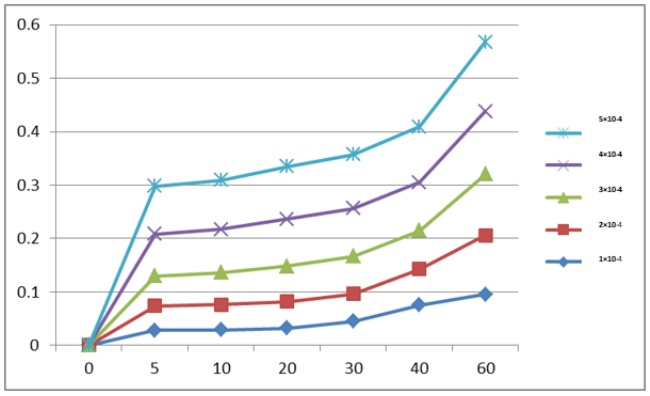

Fig .2: The absorption curve illustrates how reactants react based on the concentration of folic acid

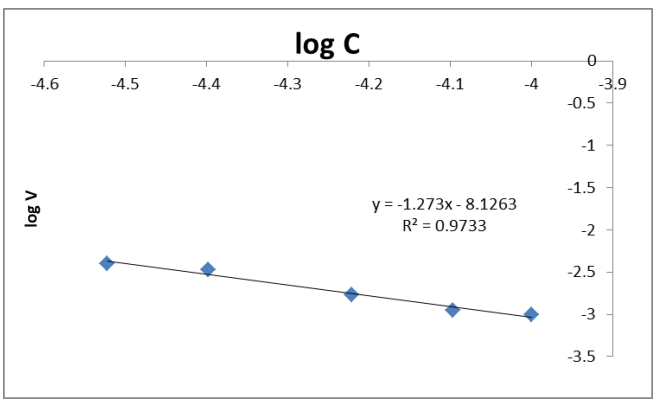

Fig. 3: The log (rate) is influenced by the difference between log Folic acid and log (rate).

Different concentrations of PABA in the range of (1×10^{-4} - 3×10^{-5} M) at room temperature (25C) were used to shadow the reaction rate, while maintaining constant control with respect to reagent and metal concentration. The next equation was applied to the reaction rate, with the resultant equation indicating that the following equations should be used:

$$\text{Rate} = K^{\backslash}[\text{folic acid}]^{n} \quad \ldots\ldots\ldots (2)$$

Where n is the order of the reaction and k is the pseudo-order rate constant with a zero-interval still necessary. $\Delta A/\Delta t$ can be used to assess the rate of the reaction using Variable time methods for different interval methods such as the energy method with low return rate and the adaptive stochastic approached [28]. The absorption of energy is represented by A and the time in minutes by t. Logarithms of rates and concentration represented by logarithms of concentration and rate That were used later to transform equation (3) into:

$\text{Log(rate)} = \log\Delta A/\Delta t = \log K^{\backslash} + n \log \text{PABA}]$

Login rate curves for log (rate) and log [PABA] were applied to solve for the regression equation:

Log (rate) =8.1263 +1.273 log C

The correlation coefficient's value (r=0.9733) indicates poor linearity, which leads to the abandonment of this method. Table 1 highlights the main points.

Table (1): Analytical data analysis for the offered kinetic initial rate method is provided, along with analytical statistics.

Linear range(M)	**Leastsquare equation**	**Correlation coefficient(r)**	**LOD(M)**	**LOQ(M)**
	$(\boldsymbol{log\, V = log\, K' + n\, log\, C})$	0.9733	2.997591946	9.083611958
$\mathbf{3\times10^{-5}\_1\times10^{-4}}$	Intercept (log k) 8.1263 , slope(n) =1.273			

IV. Fixed Time Method

This technique utilized variables such as the absorption of the reaction solution containing long-term PABA and fixed time-fixed assortment. During the reaction time intervals between PABA stimulation and binding to a powder, absorbance plots were created using the results of a chemical absorption experiment. (Table2) reveals the coefficients of correlation and limits of detection (LOD) in the regression equation, as calculated by the regression equation. The time-to-time addition exhibited slopes that were noticeable and the correlation coefficient at 15 minutes was the highest. Therefore, the fixed time of 15 minutes was deemed the most appropriate time for the analytical procedure.

Table (2): The fixed-time spectrophotometry method provided for PABA determination yields inconsistent results in terms of its analytical parameters.

Reaction Time(min)	**Linear Range (M)**	**Correlation Coefficient(r)**	**Slope(a)**	**Intercept(b)**	**LOD ($\mu g.ml^{-1}$)**	**LOQ ($\mu g.ml^{-1}$)**
5	3×10^{-5} - 1×10^{-4}	0.972	635.050	0.698	4.830	0.001
10	3×10^{-5} - 1×10^{-4}	0.921	679.880	0.695	8.364	0.003
15	3×10^{-5} - 1×10^{-4}	0.988	4308.500	0.529	3.025	9.169
20	3×10^{-5} - 1×10^{-4}	0.913	3187.200	0.578	8.790	0.003
25	3×10^{-5} - 1×10^{-4}	0.942	1417.700	0.526	7.072	0.002
30	3×10^{-5} - 1×10^{-4}	0.997	4400.000	0.470	1.398	4.237

A. Validation of the proposed methods

- *Accuracy and precision*

The accuracy and precision of PABA were determined through the substitution of five concentrations instead of three. Tables (3 and 4), however, indicate that an acceptable level of precision and accuracy could be achieved using the offered method, using an alternative method.

Table (3): Accuracy and precision on the initial-rate method with respect to precision.

Conc. of PABA M		**RE%**
Present	***found***	
1.000	1.031	103.100
3.000	3.015	100.500
4.000	4.969	124,225

Table (4): Accuracy and precision on the fixed-time method with respect to time.

Conc. of PABA M		**RE%**
Present	***found***	
2.000	1.980	99.000
5.000	4.980	99.600
8.000	8.030	100.375

B:Pharmaceutical applications

The practicality of using initial-rate and fixed-time approaches to analyze pharmaceutical preparation by analyzing two distinct concentrations of pharmaceutical preparations consuming the analytical processes was confirmed by the results presented in Table-5 and Table-6.

Table (5): Utilizing the initial-rate method in the preparation of drugs to apply the proposed PABA method in pharmaceutical preparations using.

Drug Sample	**Conc. of PABA(µg.ml^{-1})**		**RE%***
Folic acid tables	*Present*	*Found**	
	2.000	2.021	101.050
	6.000	6.050	100.933

*for five repeated measurements.

Table (6): Drug preparations using the fixed-time method of the proposed method of PABA.

Drug Sample	**Conc. of PABA(µg.ml-1)**		**Recovery%***
Folic acid tables	***Present***	***Found****	
	4.000	4.092	102.300
	8.000	8.011	100.137

* Five measurements made at a time for five repeated measurements.

Pure PABA and pharmaceutical preparations consumed both initial-rate and fixed-time methods, and the well-established BP method for these methods was easily demonstrated and reliable retrievals were established in Table (7).

Table (7): Utilizing the standard method and comparing the offered methods, the evaluated methods are analyzed using the standard method.

Drug Sample	**RE %***		
	Fixed-time method	**Initial-rate method**	**BP method**
Pure Folic acid(PABA)	99.555	99.473	99.917
Folic acid tables (PABA)	100.300	100.924	101.127

* Five measurements made at a time for five repeated measurements.

V. CONCLUSIONS

Based on the options offered, respectable sympathy is presented. Furthermore, the process offered ensures appropriate discrimination, enabling analysis despite absence of departure stages and offering alternative options to the suggested chromatographic processes. The methods in question, both quantitative and non-quantitative, lend themselves to practical application when used in conjunction with colorimetric methods due to their ability to offer greater sympathy. Stats afflicted above show that the proposed methods are both perfect and subtle, with acceptable precision and accuracy. This technique allows for swift and cost-effective analysis without the need for trailing accuracy. The method provided can be employed in conjunction with the method described for the monotonous determination of PABA in both the purified and semi-prescribed system and pharmaceutical preparation. Sodium nitrate was added to the reaction during the preparation of the reagent based on the traditional nitrogenation process.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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