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QUANTITY DETERMINATION OF DERIVATIVE DRUGS PHENOLIC COMPOUNDS BY COUPLING REACTION WITH P- AMINO BENZOIC ACID

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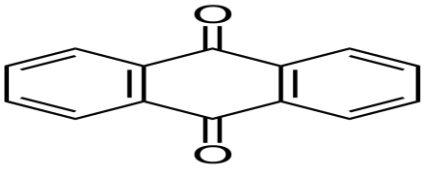
Abstract*: Development of an analytical method to determine Anthraquinones (derivative drugs phenolic compounds) by coupling reaction with diazotized solution of p- amino benzoic acid in the presence of buffer solution Na_2HPO_4 (pH= 10.8) to gave a compound with a single azo dye salt having orange color soluble in water with high absortivity at a wave length 449 nm , an early study to have a perfect optimum condition was made for the determination. A calibration curve for a range of concentration ($6.21x10^{-4}$ – $2.21x10^{-2}$ $\mu g.mL^{-1}$) was taken and the value of molar absortivity was $2.1x10^{4}$ $L.mol^{-1}.cm^{-1}$, with a relative standard deviation more than 1.35% and a recovery 97.66%. The described procedure is very simple, low-time-consuming, provides high throughput of examinated samples, and could be used for routine screening and confirmatory analyses as well. The method was successfully validated to the analysis of the (derivative drugs phenolic compounds) in biological samples.

Keywords **–** Drugs phenolic compounds (Anthraquinones), Coupling reaction, Biological samples, Azo dyes, Spectrophotometric.

1. INTRODUCTION

Anthraquinones are commonly found as glycosides in the living plant, and several groups are distinguished based on the degree of oxidation[1] of the nucleus and whether one or two unites make up the core of the molecule. The anthrones and less oxygenated than the anthraquinones and the dianthrones are formed from two anthrone units. Studies using dianthrone glycosides such as sennosides A and B suggest most of these compounds [2] pass through the upper GI tract without any change, however they are subsequently metabolized in the colon by the natural flora (mainly bacteria) of the GI tract.

Anthraquinones Fig.[1] act directly on the intestinal mucosa, influencing several pharmacological targets, and their laxative effect is largely due to increased peristalsis of the colon, reducing transit time and consequently the reabsorption of water from the colon making the stool more liquid and easing bowel movements. Additionally the **stimulation of active chloride secretion into the gut** increase osmosis, and results in a subsequent increased excretion of water. Overall the result is an increase in fecal volume and GI pressure.



Fig[1]: Chemical Structure of Anthraquinones.

The diazotizing and coupling reactions is considered one of the good, easy, fast and useful method for analytical applications for samples [4] especially the samples which is difficult to measure by original methods due to the interference that effect the determination operation. This method depends on the formation of a coloured azo dye [5] which absorbed light at a specific wave length which is considered on of the sensitive method for the determination with a wide applications as a diazotizing 4- aminoantipyrine compound used in the determination of many compound like using it in determine of little amount of pyrrole in dilute solution this method depends on the reaction of pyrrole with diazotized compound for 4- aminoantipyrine compound in the present of sodium acetate to produce a single stable azo dye soluble in water with a dark yellow color that can determine the dye absorbance at a wave length about 420 nm [6].

There is additional method for the determination of sulfonamide medicine , and its coupling reaction with phenol in basic media. To form a yellow azo dye which have a maximum absorbance at 425 nm. As there is a colorful method to determine phenols in drink water [7] which depend on the reaction of nitric acid with phenol to give nitro phenol , the last compound couples with phenol to give colored product. This method have a specific properties as its easy and fast also can be applicable in a substituted phenol in p-position there for we can determine some of the pharmaceutical cosmetics which have a subtitled phenols.

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Several analytical methods have been developed for the analysis and determination of Pyridoxine hydrochloride including Liquid Chromatography [8], Liquid Chromatography–Mass Spectrometry (LC-MS) [9,10], Enzyme-Linked Immunosorbent Assay (ELISA) [11], High Performance Liquid Chromatography (HPLC) [12], and Spectrophotometry [13].

In this paper we proposed a suitable procedure for the micro determination of Anthraquinones, based on the coupling reaction of Anthraquinones with diazotized compound of p- amino benzoic acid in basic media.

2. Material and experimental part

2.1 Standard solutions

- Anthraquinones solution (50 µg.mL^{-1}).

It prepared by dissolving a 0.50 µg of Anthraquinones (from BDH ANALAR company) in 50 mL absolute ethanol then complete it with distal water to 250 mL in volumetric flask.

-Diazotizing solution for p- amino benzoic acid (100 µg.Ml^{-1}).

We prepared it by weighting the compound from (BDH ANALAR) then dissolve it in 40 mL of distilled water, then heat the solution to increase the solubility then added 4 mL of 0.7M standard hydrochloric acid with cooling to zero degree by using an ice bath then added 0.0077 gm sodium nitrate with mixing. After 5 minute pour the diazonium final solution to 250 mL volumetric flask, and complete the volume by using cooling distil water to (4˚C) and keep the solution in the freezer. The final solution will be stable after 1 hr in room temperature which is (22˚C).

-Hydrochloric acid (0.7M) from (FLUKA A.G company).

We prepared it with an approximate concentration by dilution of concentrated hydrochloric acid then titrate it with standard sodium carbonate to fix the concentration to (0.7M).

-Sodium carbonate Na_2CO_3 (0.7M) from (BDH company).

We prepared it by dried sodium carbonate for an hour in (115˚C) in watch glass then cool it and weight 3.32449 gm, then we dissolve it in distilled water and complete to the final volume which is 250 mL in volumetric flask.

-Solution (8M) of sodium hydroxide (from Fluka A.G company).

We prepared it from standard ammonia solution, to determine the exact molarity we prepare (8M) of NaOH according to dilution equation.

2.2 Buffer solution

We prepared a buffer solution with pH=3.2 and 6.4 respectively. Prepare a (0.1M) solution of Na_2HPO_4 and (0.2M) solution of citric acid. The Table (1) below shows the preparation of buffer solution (buffer citrate):

Table (1) prepare buffer solution (buffer citrate)

PH	0.1 M Na_2HPO_4(mL)	0.2 M Citric acid(mL)
3.4	3.3	18.72
7.7	17.65	2.24

-We prepared a buffer solution with pH=9.8 and 11.10 respectively by prepared a (0.2M) solution of sodium carbonate and a (0.2M) solution of sodium bicarbonate, the Table (2) represent the preparation of the buffer solution (carbonate and bicarbonate buffer).

Table (2) prepare the buffer solution (carbonate buffer).

pH	0.2 M Na_2CO_3(mL)	0.2 M $NaHCO_3$(mL)
8.9	3	5.5
10.9	9.1	3.4

-We prepared a phosphate buffer solution with pH=11.6 by taking 70 mL of (0.3M)of Na_2HPO_4 solution and added to it 5.5 mL of (0.2M) sodium hydroxide solution then dilute to 250mL by using distilled water.

2.3. Experimental method.

Determination of Anthraquinones by coupling reaction:

The coupling operation done by using (50 µg.mL^{-1}) of Anthraquinones with (100 µg.mL^{-1}) of 4- amino benzoic acid in present of phosphate buffer solution (pH=10.80) as we added 3mL of diazotized compound to 6mL of buffer solution then added different volume of Anthraquinones then added to it 500mL distilled water after that measure the absortivity (A) for this solution against the blank solution at a wave length 455 nm and illustrated in Fig. 2, as we draw calibration curve between the absorbance and the concentration we have a straight line.

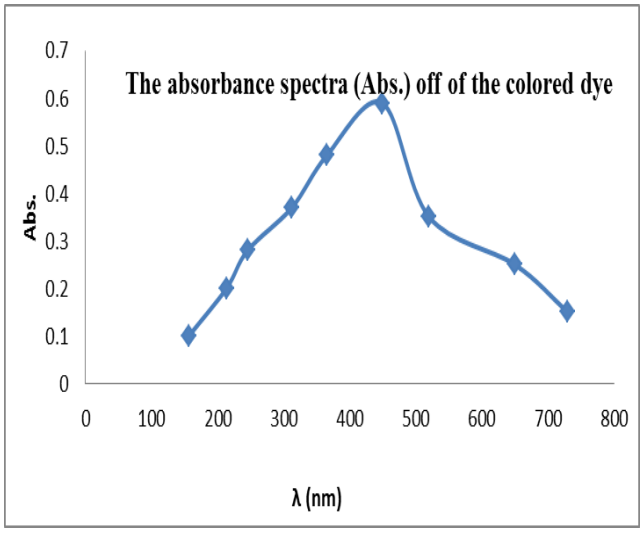

Fig. 2. The absorbance spectra of the colored product.

3. Result and discussion:

3.1 Primary test:

We noticed that in mixing of Anthraquinones compound with 4- amino benzoic acid in basic media, a single azo dye with orange color soluble in water formed, the reaction involve two step which is :

1- 4-amino benzoic acid compound reacted in acidic media in 0˚C with equal amount of sodium nitrate to give diazonium salt.

2- When we added Anthraquinones to the diazotizing compound in basic media a single azo dye with orange color formed which give a high absortivity at a wave length 455nm.

3.2 Types of parameters influenced on the reaction:

3.2.1 Effect of PH:

We reached the optimum condition (high sensitivity, fast reaction and low absorbance) for the blank solution, a study of Anthraquinones with diazotizing compound 4-amino benzoic acid in neutral, acidic and basic media was mad as we notice that no azo dye is formed in neutral and acidic media and the best media for the coupling reaction is the basic media which pH=10.91 as Table (3) represent the effect of pH on coupling reaction.

Table (3) represents the effect of pH on coupling reaction.

Kind of Buffer	pH	λ Max.(nm)	Absorbance
Na_2HPO_4	1.9	217	0.143
Na_2HPO_4	6.45	398	0.245
Na_2CO_3-$NaHCO_3$	8.8	394	0.143
Na_2CO_3-$NaHCO_3$	9.34	398	0.148
Phosphate Buffer	10.91	455	0.596
Hydroxide-Chloride Buffer	12.4	412	0.146

A 5mL of buffer solution was used for every 25 mL of the final solution, the Table (3) shows that the best buffer solution for coupling reaction is phosphate buffer.

3.2.2 Type of Buffer solution:

The effect of buffer solution kind after the perfect value for the PH was know, which is equal to (10.80) to know the effect of the buffer solution on Absorptivity which is phosphate buffer NaOH – Na_2 HPO_4 and KCL- NaOH buffer as the Table (4) represent the kind of the buffer solution.

Table (4) represents the kind of the buffer solution

Kind of Buffer	pH	λ Max.(nm)	Absorbance
Na_2 HPO_4-NaOH	10.91	455	0.596
KCL- NaOH	10.91	455	0.246

3.2.3 The effect of the buffer solution on the intensity of absorbance:

We fixing the optimum condition for the reaction. An experiment was made to gave the perfect volume for the buffer solution which gave a high sensitivity as Table (5) shows that 4mL is the perfect volume for the buffer solution which gave a highest sensitivity as Table (5) shows that 4 mL is the perfect volume for the buffer solution.

Table (5) represents the volume of the buffer solution

Volume of the Buffer solution(mL)	Absorbance
2	0.196
2	0.259
3	0.354
4	0.596
5	0.345
6	0.216

3.2.4 Measurements the amounts of diazotized compound:

We measured the amount of diazoting compound for 4-amino benzoic acid compound, an experiment was made to show the effect of diazoting solution size on the absortivity. The final result shown in Table (6) as the perfect size for the diazoting agent is 3 mL.

Table (6) shows the effect of diazoting solution size on the absortivity

Size of diazotizing solution(mL)	Absorbance
1	0.198
3	0.596
5	0.243
6	0.178

3.2.5 Priority of Addition

We measured the order addition after fixing the diazotizing agent for p-amino benzoic acid compound and using a phosphate buffer solution , as for the important of order addition measurement for the solution and its effect on the intensity color of the formed azo day compound so we study order addition .The table (7) represent the result.

Table (7) shows the effect of order addition on the absortivity

Order number	Reaction component	Abs.
1	A+D+W	0.596
2	W+D+A	0.342
3	W+A+D	0.257

(D=buffer solution), (W= Anthraquinones), (A=diazotizing agent) As we consider 1 as number one because it a high sensitivity.

3.2.6 Color Stability:

We studying the color stability for the formed complex from the reaction. An experiment was done to explain the stability the color of the complex formed due to the reaction between the Anthraquinones and 4-amino benzoic acid compound in present of phosphate buffer with optimum condition as shown in Table (8) the stability time for the color of the complex is 40 min

Table (8) shows the effect of the time of the stability of the complex

Absorbance	T (min.)
0.213	5
0.312	10
0.289	15
0.245	20
0.364	25
0.596	40
0.387	60
0.412	120
0.376	1440

3.2.7 Calibration curve assay:

We measured the calibration curve for a series of volumetric flask (250 mL) by adding 2mL of diazotizing agent solution , 4mL of phosphate buffer solution then pour to it (2-12 mL) of (50 $\mu g.mL^{-1}$) Anthraquinones solution after that complete with distilled water until reach the mark then we measure the absorbance against the blank solution at 455 nm after 1/2 hr. from reaching the final solution (250mL) and the fig. (3) show the calibration curve.

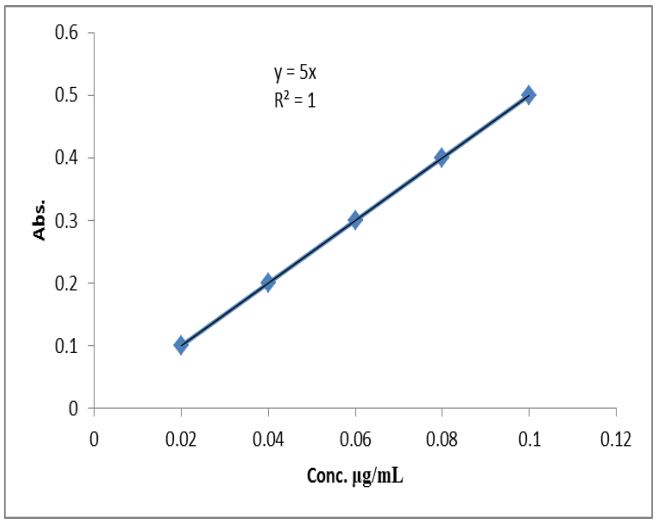

Fig. (3) the Calibration curve of Anthraquinones.

3.2.8 Study of accuracy and compatibility of method:

We studying the accuracy and compatibility for the method. We taking a different concentration of Anthraquinones (10,60,100) $\mu g.mL^{-1}$, by using an optimum condition, the Table (9) represent the result.

Table (9) represents the result of accuracy

Represent value	Found value	Error %	Recovery %	R.S.D. %
10	11.10	0.41	97.21	±1.78
60	59.65	1.45	99.23	±1.02
100	99.46	0.47	99.34	±1.37

4. EFFECT OF THE I INTERFERENCES

Talc powder, Acetyl salicylic acid, Chloramphenicol, Imidazol, Sulfamic acid, salicylic acid have been chosen as possible interfering species in the determination of Anthraquinones. Table (10) illustrate the effect of the interferences materials on the coupling reaction where some interferences effect whereas the other not effect.

Table (10) Effect of interferences on coupling reaction

Interferences Conc.	Talc powder, salicylic acid	Acetyl salicylic acid,	Chloramphenicol	Imidazol,	Sulfamic acid,
10	0.03	6.24	2.46	1.45	6.31
60	0.04	5.37	5.67	0.65	4.23
100	0.09	9.36	7.64	0.51	5.21

5. ANALYTICAL APPLICATION:

We investigated the characteristics of response recorded for the coupling reaction for micro determination of Anthraquinones involved (linear concentration range of determination, selectivity) revealed that this approached method can be used for the assessment of Anthraquinones in blood samples of persons taking this drug. The five samples of blood were supplied from the hospital, and used as collected, for the evaluation of (Anthraquinones). Therefore, the results obtained by using the coupling reaction between diazotized compound of 4- amino benzoic acid and our target material (Anthraquinones) show that it is a reliable tool for the evaluation of (Anthraquinones) in whole blood samples and Table (11) illustrate the result obtained.

Table (11) Recovery tests of pyridoxine hydrochloride in blood samples.

Recovery of Anthraquinones					
Samples Nr.	1	2	3	4	5
	98.9%	97.8%	99%	98.1%	99.12%

6. CONCLUSION:

The proposed method showed very good results for the recovery test which makes it a reliable tool for measuring Anthraquinones in whole blood samples, and this is very important for evaluation of trace amounts from Anthraquinones.

The assay of Anthraquinones in whole blood was reliable performed using the proposed coupling reaction based on coupling of diazotized 4- amino benzoic acid with Anthraquinones.

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