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SPECTROPHOTOMETRIC DETERMINATION OF ISONAIZID BY COUPLING-OXIDATIVE IN PHARMACEUTICAL DRUG

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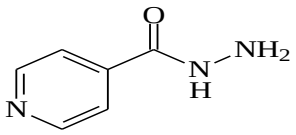
Abstract: *Isoniazide is considered to be one of the most important antibiotics for TB bacteria because of its high efficacy, relatively low toxicity and its low price. 1 The drug was discovered by chance when the researcher Chorine proved in 1945 that nicotine amyotene (nicotinamide) has the ability to stop the effectiveness of tuberculosis bacilli. The discovery was not given importance until 1948 when Mckenzie and his group observed the same effect. A study was conducted on the effect of isonicotinic acid similar to nicotine ammide. It was noted that most pyridine derivatives have almost the same ability to stop the effectiveness of tuberculosis bacilli. In 1952, Fox discovered the isoniazid while trying to collect the active substances used to treat tuberculosis.23 Isoniazide is an organic compound with several scientific names:*

Keywords: *isonicotinic acid hydrazide; isonicotinylhydrazide; tubazid; 4-pyridinecarboxylic acid hydrazide*

INTRODUCTION

The isoniazid has the following structure:



$C_6H_7N_3O$ M.wt 137.1 g/mol, m.p. 170-174 °C

It is a white crystalline or white powder that is fast soluble in water and has a solubility in alcohol, chloroform and ether. Its sweet taste is sweet and then turns into a bitter taste (6). The isoniazide is kept in sealed, non-light-sealed cannons (7). The isoniazid has a selective effect against mycobacterium tuberculosis, preventing the manufacture of the basic components of the bacterial cell wall. It also inhibits the synthesis of the necessary DNA for the division of the bacillus (10,9,8). The isoniazide is rapidly absorbed by the gastrointestinal tract and reaches its highest plasma concentration after about It is spread through the tissues, organs and fluids of the body, reaches the spinal cord fluid, passes through the placenta and is excreted with breast milk. [11] It is undermined in the liver by acetylation and dehydrazination, 70-50 of the isoniazid through Al-Adrar 24 hours as material metabolic Mottagodh. (12) The isoniazade is given individually to prevent tuberculosis 13 and isoniazade was estimated in several ways, including corrective methods (15,14), spectral methods (18,17,16), fluorine methods (20.19), chromatographic methods (22,21)), Electric methods (24,23) and chemical luster methods (26.25).

CONCLUSION

The method is based on the oxidation of the drug using the triangular ferric ion in a acidic medium and the addition of the 10.1-phenanthrolene reagent solution. After completion of the additives, the final product of the direct red-color reaction, which has stabilized more than 30 minutes long enough to perform many measurements. The highest absorption was given at a wavelength of 510 nm and followed the BIR code in the range of concentrations 5-26 µg / ml. The molar absorption value was 11107.53 lm. -1.2 cm -1 and the sandal significance was 0.01234 µg, cm -2. The method of accuracy and compatibility was 99.9170% and the relative standard deviation 0.1817-0.3628%. The method was successfully applied to isoniazade in some pharmaceutical preparations.

PRACTICAL PART

2.1 Devices used

The following devices were used for measurements:

1. Single-beam spectrometer type CECIL single beam CE1021.

2-type double-beam spectrometer device

Shimadzu UV-Visible Spectrophotometer UV-160

3 - quartz cells and silica width of 1 cm

4 - water bath type Galleahomb.

5 - acid function measuring device type pH meter 3310 (Jenway)

6. Sensitive Balance Type Sartorius BL 210S

2.2 Reagents and chemicals used:

The chemicals and analytical reagents used all have a high degree of purity.

2.2.1. The standard isoniazide solution is 1000 µg / mL

Prepare this solution by dissolving 0.1000 g of isoniazide powder and soluble in distilled water and then complete the mark in a 100 ml bottle

2.2.2 Standard isoniazide solution 250 µg / mL

The solution was prepared 250 µg / mL by diluting 25 ml of the solution of 1000 µg / ml to 100 ml using distilled water.

2.2.3-Hydrolyzed Chloride Solution 1 × 2-10 molar

Prepare this solution by dissolving 0.2703 g FeCl3.6H2O in a quantity of distilled water and full-size to the mark with distilled water in a 100 mL volume vial.

2.2.4-reagent solution, 110-phenanthrolene 1 × 10-2 molar

Prepare this solution by dissolving 0.18021 g of reagent in 5 ml of ethanol and then complete the volume to 100 ml with distilled water.

2.2.5-Solution of isoniazide 300 mg / ml

The pharmaceutical product Isoniziad (production of the general company for medicine and medical supplies - SDI - Samarra - Iraq) in the form of grain and each grain contains 100 mg of isoniazid has been prepared solutions as follows:

Ten grains were finely grinded after the weight of each individual pill, and the weight of one grain was obtained. It was equivalent to 0.1000 g of isoniazade, dissolved in a quantity of distilled water, then filtered, washed several times, 100 ml, full size to the mark with distilled water, take 25 ml and put in a 100 mL volume vial and complete the volume to the mark with distilled water to obtain a 250 µg / ml solution.

2.2.6. Solution of approximate 0.1 mL hydrochloric acid

Prepare a 0.1 mL molar hydrochloric solution by diluting 0.8 ml of concentrated acid (11.8 molar) in distilled water and then complete to a mark in a 100 mL distilled water vial.

2-2.7-Ammonium hydroxide solution approx. 0.1 molar

Prepare an ammonium hydroxide solution by diluting 1.5 mL of the concentrated base (7.71 molar) in distilled water and complete the size to the mark in a 100 ml volume vial.

Results and discussion

2.3.1. Preliminary study

Add 1 ml of oxidized ionic ferric acid at a concentration of 1 × 10-2 molar to 1 ml of isoniazide at 250 µg / ml and leave solution 5 minutes to complete the oxidation, then add 2 ml of reagent 1, 10-phenanthrolen at 1 × 10-2 molar concentration, To 25 mL with distilled water, its absorbance spectra was measured against its photolysis, and its highest uptake was at a wavelength of 510 nm while the photoreceptor gave very little absorption at the same wavelength.

2.3.2. Optimal Conditions Study:

Subsequent experiments were performed using 1 mL of isoniazide with a concentration of 250 µg / ml for a final volume of 25 mL and a measure of the absorption of solutions at a wavelength of 510 nm versus their solution.

2.3.2-a-Effect of acid

For the purpose of determining the optimal acidic function to obtain the highest absorption, different volumes (2.5-0.5 mL) of hydrochloric acid were added at a concentration of 0.1 molar to 1 mL of isoniazade solution at 250 µg / mL and 1 mL of 1 × 10-1 mmolar iron solution and 5 minutes for oxidation 2 ml of the 1 × 10-2molar reagent solution was applied. The solutions were diluted to the point of the mark with distilled water, and their absorption was measured against their photolysis at 510 nm and the acid function was measured. The results were as shown in Table (1).

Table 1: Effect of acid in absorption

ml of 0.1M HCl	Absorbance	PH
0.0	0.738	3.57
0.5	0.775	2.78
1.0	0.809	2.50
1.5	0.781	2.39
2	0.743	2.30
2.5	0.721	2.22

The results shown in Table 1 show that the addition of different volumes of acid resulted in the highest absorption at 1 mL and then began to decrease gradually. Therefore, 1 mL will be taken in subsequent experiments.

2.3.2-B - Effect of the quantity of the base

The effect of the amount of alkaline on absorption was studied by adding increasing volumes (0.5-2.5) mL of ammonium hydroxide solution at 0.1 molar to 1 ml of 250 mg / ml solution and 1 ml of ferric solution at 1 × 10-2 mmolar concentration. The solutions left 5 minutes for oxidation, Of the reagent solution at a concentration of 1 × 10-2molar. The solutions were diluted to the extent of the mark with distilled water, and the absorption was measured at 510 nm wavelength compared to their photolysis. The acidic function was measured and the results are shown in Table (2).

Table 2: Effect of the amount of base in absorption

ml of 0.1M NH_4OH	Absorbance	pH
0.0	0.738	**3.57**
0.5	0.553	**3.01**
1	0.521	**3.03**
1.5	0.503	**3.06**
2	0.488	**3.08**
0.5	0.451	**3.10**

The results shown in Table 2 show that the addition of the base resulted in a decrease in absorption. This is in addition to the addition of the volume of acid, which led to an increase in absorption of 1 mL of acid, so the addition of the base was excluded from subsequent experiments.

2.3.2-C - Effect of the amount of the oxidizing agent

The effect of the oxidant factor was studied by adding increasing volumes (0.2-3 mL) of ferric ion at a concentration of 1 × 10-2 mmolar to the solution of the drug and adding 1 ml of hydrochloric acid at a concentration of 0.1 molar. The solutions then left 5 minutes for oxidation. 10 - phenanthrolin at a concentration of 1 × 10-2 mmolar, and was diluted with distilled water to the extent of the mark and absorption measure and the results are shown in Table (3).

Table 3: Effect of Oxidation Factor Quantity

ml of 1×10^{-2} M $FeCl_3.6H_2O$	Absorbance
0.2	0.885
0.5	0.765
1	0.809
1.5	0.853
2	0.616
2.5	0.520
3	0.446

As shown by the table, when adding an increase of the oxidizing factor, it is the highest absorption at the size of 1.5 ml and then begin to decrease gradually, so will adopt 1.5 ml in subsequent experiments.

2-3-2-D-effect of reagent quantity, 110- finanthrolin 1 × 10-2 molar

The effect of the reagent quantity at a concentration of 1 × 10-2 mmolar in the absorption was studied by adding increasing volumes of reagent (3.0 - 0.5 ml) to different sizes of the 100 mg / mL solution and the results are shown in Table (4).

Table 4: Effect of Reagent Quantity, 110- Finnethrolin

ml of 1×10^{-2}M 1,10- phenanthrolin	**Absorbance, µg/ml Isonaizid acid**				**^{2}R**	**Slope**
	5	10	20	30		

0.5	0.074	0.151	0.272	0.381	0.996	**0.012**
1	0.237	0.408	0.544	0.790	0.979	**0.020**
1.5	0.445	0.615	0.820	1.166	0.988	**0.027**
2	0.625	0.853	1.291	1.721	0.999	**0.043**
2.5	0.776	1.273	1.734	2.402	0.985	**0.061**
3	0.828	1.387	2.258	2.853	0.985	**0.080**

Table (4) shows that the use of 3 ml of 1 × 10-2 mmolar of the reagent solution is sufficient to give the highest absorption and the highest tendency indicates that this volume gives the highest sensitivity so this volume was used in subsequent experiments.

2.3.2-e. Effect of the quantity of the regulated solution

The effect of the regulated solution (27) on acidic function (2.50), consisting of (potassium phthalate, hydrochloric acid) was studied by adding different sizes of (0.5 - 2 ml) The solution is regulated to 1 ml of concentrated isoniazide solution 250 µg / mL and 1.5 ml of 1 × 10-2 molar concentrated ion solution and 3 ml of reagent solution 1, 10 - finnaturel at 1 × 10-2molar concentration The first on 1 ml of 0.1 molar hydrochloric acid and not containing the structured solution) and then completed the pilgrimage To mark an end with distilled water, then measured the absorption of solutions at the wavelength of 450 nm versus mock solution for each of them and the results are shown in Table 5.

Table 5: Effect of the solution type in the absorption

Buffer solution	Absorbance	pH
0.0	1.387	2.5
0.5	1.312	2.8
1	1.288	3.5
1.5	1.256	4.1
2	1.220	4.7

*0.0 With out Buffer solution

Note from the results of Table (5) that the addition of the regulated solution resulted in a decrease in absorption and an increase in the value of the acidic function. Therefore, it was excluded in subsequent experiments.

2.3.2-Stability of colored output

The concentration of the colored product was taken by taking 1 ml of the drug at 250 µg / ml and then adding the optimal quantities of the solution. After that, the distilled water was diluted to the mark limit in 25 mL volume tanks. The absorption of the colored solutions was measured after a specific time in minutes at 510 nm As compared to its visual analysis and the results are shown in Table (6).

Table 6: Stability of the reaction product

ml of **Isonaizid** 250µg/ml	Absorbance/ min. standing time								
	5	10	15	20	25	30	40	50	**60**
1	1.387	1.429	1.463	1.480	1.515	1.501	1.480	1.451	**1.424**

It was observed from the results shown in the above table that the absorption of the product gives the highest absorption after 25 minutes and that the solution remains stable for 30 minutes and then begins to decrease gradually and 30 minutes on this is enough time to conduct many experiments.

2.3.2-g-Effect of temperature

The effect of temperature on the absorption and stability of the formed product was studied using temperatures between 5 ° C and 50 ° C and the results are recorded in Table (7).

Table 7: Effect of temperature on absorption

Temperature	15	20	25	30	35	40	45	50
Absorbance	1.463	1.465	1.467	1.450	1.409	1.371	1.350	**1.321**

The results shown in Table (6) show that the temperature of 15-25 ° C gives the highest absorption of the product and room temperature is adopted in subsequent experiments.

2.3.2-H. Effect of solvent

The solvent effect was studied on the resulting complex spectra by diluting solutions with these solvents instead of distilled water and then waiting for 25 minutes to complete the final reaction. The absorbance spectra of these solutions were measured against their photoreceptors and the results are shown in Table 8 and Figure 1.

Table 8: Effect of solvent

Solvent	λ_{max} (nm)	Absorbance	
DMSO	510	2.284	A
Ethanol	510	1.740	B
2-Propanol	510	1.573	C
Water	510	1.515	D
Methanol	510	1.187	E
Aceton	510	1.183	F
DMF	510	1.125	G

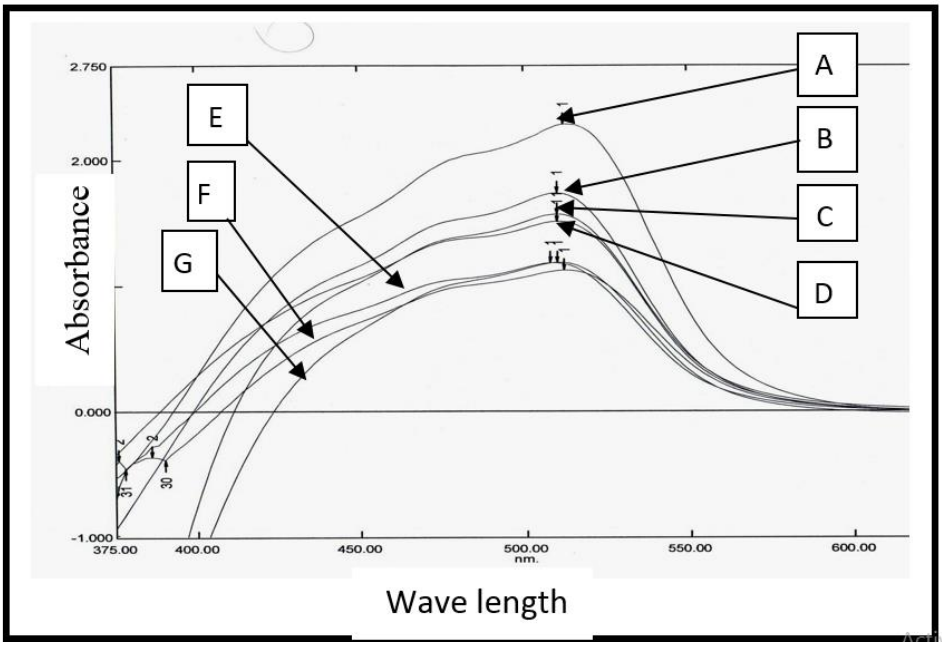


Fig 1: Effect of solvent

The results shown in Figure (1) and Table (8) indicate that water is a good medium of reaction and gives a high absorption value at 510 nm wavelength as well as its availability and cheap price, so it was used as the best solvent.

2.4 The final absorption spectra

After setting the ideal conditions for the reaction of 1 ml of 250 mg / ml solution and 1.5 ml of ferric solution at 1 × 10-2molar concentration, the solutions left 5 minutes for oxidation, then add 1 ml of 0.1 mM hydrochloric acid, add 3 ml of 1 × 10-2molar reagent solution and wait 25 minutes To complete the final interaction. The absorption spectra of the red-color solution versus the photoluminescence was measured at a range of 200-600 nm wavelengths. The color output showed the highest absorption value at 510 nm wavelength while the photoreceptor gave weak absorption at the same wavelength, 2) eBay N spectra of final absorption.

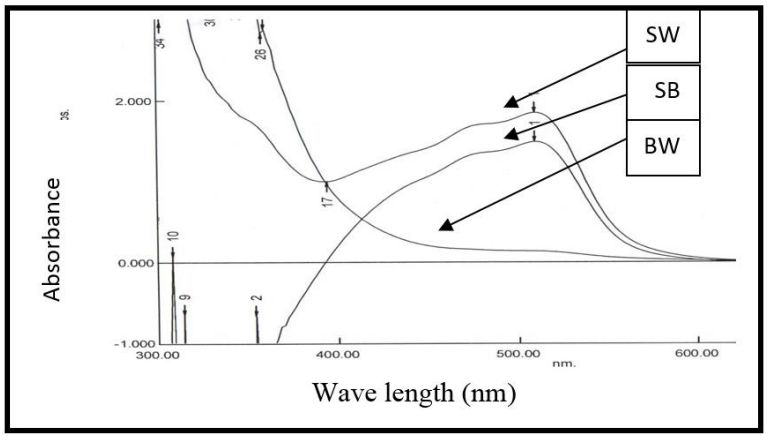


Fig 2: The final absorption spectra

SW represents the absorption spectrum of isoniazide versus distilled water.

SB represents the absorption spectrum of isoniazide versus the photolysis.

BW represents the absorption spectrum of the solution compared to the distilled water.

2.5. The approved method of work and the calibrating factor

I attended a series of 25 mL volumetric bottles by taking volumes (0.5-1.9-1.1-1.7 -2.3-2.6) in the solution of the isoniazid solution at a concentration of 250 µg / ml corresponding to 5-9-11-17-23-26 µg / mL. 5 mL of oxidized agent solution at 1 × 10-2molar concentration. The solutions were left for 5 minutes for oxidation, then 1 ml of hydrochloric acid at 0.1 molar concentration. All bottles were added 3 ml of the reagent solution at 1 × 10-2molar concentration and then supplemented with distilled water and then left for 25 minutes to complete Reaction, and then the absorption was measured at 510 nm for all solutions versus the solution. Figure (3) and Figure (4) represent the standard curve.

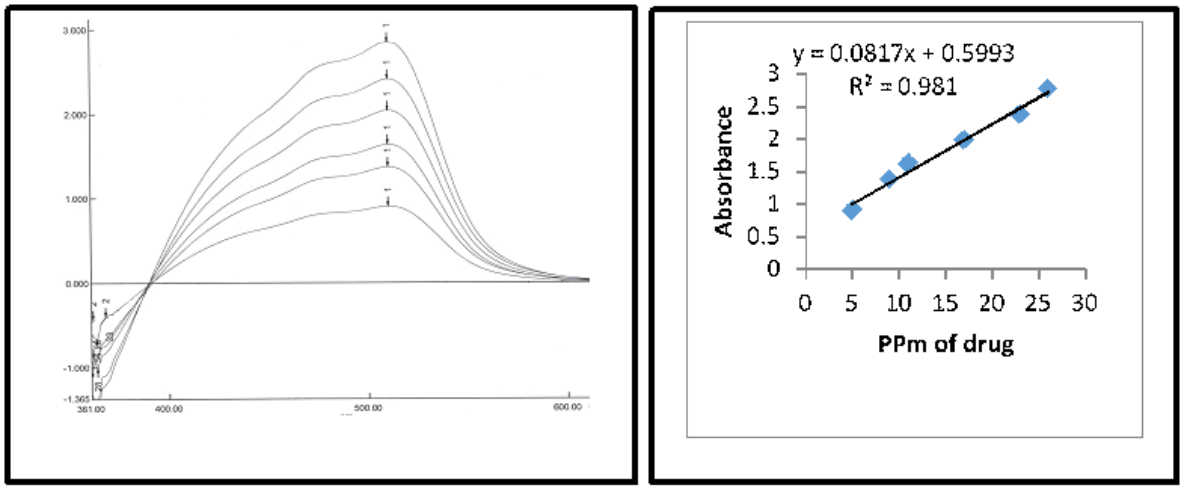


Fig 3: Calibration curve for concentrations between 5 - Figure (4) Absorption spectrometer for calibration titers 26 µg / ml. For concentrations of 5-26 µg / mL.

2.6 Accuracy and compatibility

The optimum conditions were used in the method of work to test the accuracy of the calibration curve and its compatibility. Six readings of three different amounts of isoniazade solution were taken within the limits of the BER law in the calibration curve. The regression rate and the relative standard deviation were calculated. The method was found to be of high accuracy. 9).

Table 9: Accuracy and compatibility

Conc.of Isonaizid ml/ µg	RE*%	Recovery*%	Average recovery%	RSD*%
5	-0.4479	99.5520	99.9179	+0.3628
17	+0.2016	100.2016		+0.1817
26	+o.ooo1	100.0001		+0.1908

*Average of six determinations

2.7-Limit detection

The detection limit was calculated by measuring the absorption of 11 prepared solution for the lowest concentration (5 µg / ml) in the calibration curve and within the Bier law. Under the same conditions (optimum conditions) the detection limit was 0.0535 micrograms / ml (5 µg / ml) (10).

Table 10: Detection Limit

Concentration µg/ml	$\overline{X}$	*S*	*D.L µg/ml*
5	0.894	0.003190	0.0535

2-8 - The nature of the resulting product

In order to determine the nature of the red product, the color formed and the ratio of the property bond with the detector applied the method of Job and the percentage method. In both methods, the concentration of the isoniazide solution and the detector solution is equal to 1.8 × 10-3 molar, in the JUP method 28 in a 25 ml volume bottle. Different sizes of the solution were placed between 1 - 9 ml and 1.5 ml of solution Oxidation factor at a concentration of 1.8 x 10-3 mmolar and 1 ml of acid at 0.1 molar concentration. The solutions were left for 5 minutes to complete the oxidation. The supplementation was added to 10 ml of the reagent solution and the mark was diluted to distilled water and then waited for 25 minutes to complete the reaction. These solutions are at 510 nm wavelength versus solution My pictures are each and the figure for figure (5) shows that the ratio is 1: 1.

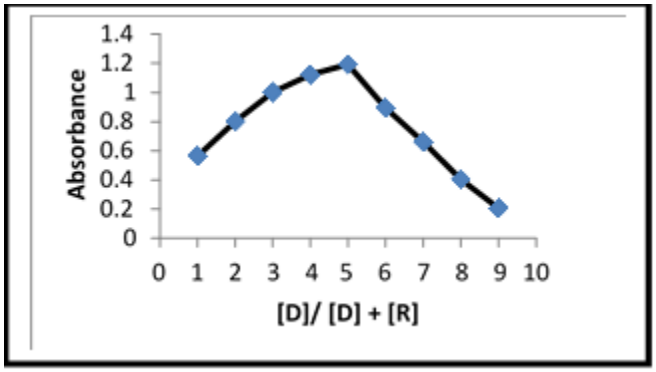


Fig 5: Job method

In the molar ratio method (29), 3 ml of the solution was placed in a 25 mL volume bottle with 1.5 ml of oxidized agent solution at a concentration of 1.8 x 10-3 mmolar and 1 ml of acid at 0.1 molar concentration. The solutions were then left for 5 minutes to complete the oxidation, (3.5-0.5) mL of the detector solution at a concentration of 2.4 × 10-2 mmolar. The volume was added to the mark with distilled water and then waited for 25 minutes to complete the reaction and measured the absorption of these solutions at the 510 nm wavelength compared to the solution of each solution, and found that the molar ratio corresponded to the JOB method and achieved the ratio 1: 1 as in Fig.

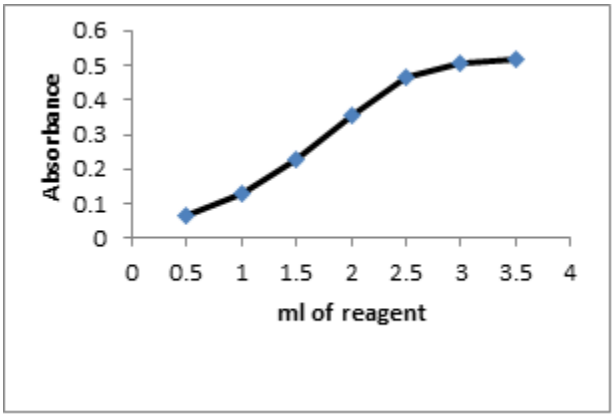


Fig 6: Method of percentage

APPLICATIONS

The method was applied to pharmaceutical preparations containing the isoniazid drug (Isonaizid 100 mg / ml).

Direct Method

Three different concentrations of Isonaizid solution were 5, 17 and 26 µg / ml. The solutions were treated with the same steps as the calibration curve. The absorption was measured at a wavelength of 510 nm compared to the solution. The rate of five measurements was calculated for each concentration. Blog in Table 11.

Table 11: Direct Method

Drug	Type of pharmaceutical	Conc., of Isonaizid µg/ml	RE*%	Recovery*%	Average recovery*%
Isonaizid	Tablet	5	-0.5152	99.4878	99.8597
		17	-0.3571	99.6428	
		26	+0.4485	100.4485	

*Average of 5 Determinations

The results of the above table show the success of the proposed method of estimating isoniazide in the pharmaceuticals containing it. The reaction rate was 99.8597% for the isoniazid tablet preparation.

Standard Addition Method

Apply the standard plugins method. The method was obtained by taking 0.7 mL of Isonaizid 250 mg / ml solution into six 25 ml volume bottles, then adding increasing volumes of the isoniazid standard solution (1 - 5 ml) with the remaining 6 bottles remaining without adding. The above solutions were then treated with the same method of work when the calibration curve was prepared and recorded at 510 nm. Figure (7) and Table (12) show the results obtained when applying the standard additions method.

Table 12: Standard Addition Method

Drug	Pharmaceutical Preparation	µg/ ml Isonaizid Present	µg/ml Isonaizid measured	Recovery*%
Isonaizid	Tablet	7	7.19	102.8218

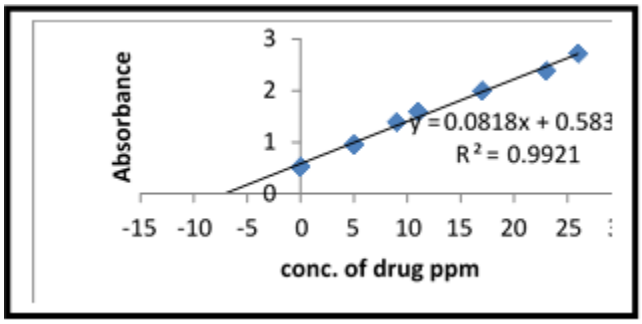


Fig 7: Method of standard additions

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