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Research Article

DEVELOPMENT AND VALIDATION OF NEW COLORIMETRIC METHOD FOR THE ESTIMATION OF ISONIAZID IN BULK AND DOSAGE FORM

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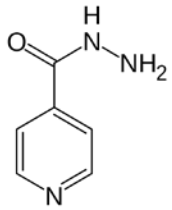
ABSTRACT

Four simple, sensitive, rapid and accurate analytical methods have been developed for the estimation of ISONIAZID in bulk and pharmaceutical dosage forms. Here the method was based on condensation reaction involving the formation of yellow colored hydrozone complex between isoniazid and ethyl vanillin in the presence of 0.5M NAOH, Which showed a linearity range 2 to 16 µg/ml at λmax of 410 nm. The colour was stable for more than 2 hour 30 min. The method was validated based on ICH guideline and Accuracy, Precision, Repeatability, Precision, Specificity, Detection Limit, Quantitation Limit, Linearity were recorded. They are simple, sensitive, and reliable and results are reproducible. Hence useful for the routine analysis of isoniazid.

Keywords: Isoniazid, Ethyl vanillin, NaoH, Distilled water.

INTRODUCTION

A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge of chemical substances comes from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample.[1] Isoniazid (Laniazid, Nydrazid), also known as isonicotinylhydrazine (INH), is an organic compound that is the first-line anti tuberculosis medication in prevention and treatment. It was first discovered in 1912, and later in 1951 it was found to be effective against tuberculosis by inhibiting its mycolic acid (wax coat). Isoniazid is never used on its own to treat active tuberculosis because resistance quickly develops. Isoniazid also has an antidepressant effect. The compound was first synthesized in the early 20th century, but its activity against tuberculosis was first reported in the early 1950s and three pharmaceutical companies attempted unsuccessfully to simultaneously patent the drug. [2-4] Up to now Isoniazid is estimate by colorimetry estimate by vanillin, colorimetric determination of micro quantities of isoniazid, spectrophotometric determination of isoniazid, liquid chromatography–tandem mass spectrometry, HPLC, Infra-red spectra of isoniazidhydrazones. [5-15]



Isoniazid is freely soluble in water, sparingly soluble in alcohol, very slightly soluble in ether. Its molecular weight is 137.[16] The aim of study is here to developed new colorimetric method. Estimation of Isoniazid was carried out by the reaction of functional group present in it with suitable agent like ethyl vanillin to formed colored products which are determined calorimetrically. Analysis of the drug is important for development of drugs in their formulation and their use in therapies, for which we require standard analytical procedures. The USP has published specific guidelines for method validation for compound evaluation. USP defines eight steps for validation: Accuracy, Precision, Specificity, Limit of detection, Limit of quantitation , Linearity and range, Ruggedness, Robustness[17-19] As quality control process is not static some form of validation/verification should continue till the validated procedure is in use. It should not be a concept that once the method is initially developed and validated it is forgotten.

MATERIALS AND METHODS

A colorimetric method was developed for Isoniazid by using ethyl vanillin as agent in presence of 0.5m NaoH.

Experimental

Instrumentation

All the experiments were carried out on Jasco V530 series UV-vis spectrophotometer using 1cm matched quartz cuvettes.

Preparation of standard stock solution of Isoniazid (1000µg/ml)

Stock solution of Isoniazid was prepared by weighing accurately 100mg of pure drug into a 100ml volumetric flask and dissolved in water and the volume was made up to mark with distilled water to get a concentration of 1000 µg/ml.

Preparation of working standard solution of Isoniazid (100µg/ml)

The working standard solution of Isoniazid was prepared by dilution of the stock solution suitably with distil water to get a concentration of 100µg / ml.

Preparation of reagents

2.1 Solution of Ethyl vanillin (3%)

The solution of Ethyl vanillin was prepared by dissolving 3 gms of ethyl vanillin in 100 ml of Ethanol.

2.2 2M Sodium hydroxide: 2gm + 100ml distilled water. [20]

Preliminary Investigation

To 1 ml of the drug solution containing 8 µg/ ml, 1 ml of 3% of ethyl vanillin was added, followed by 1 ml of 0.5M NaoH and volume was made up to 10ml with distilled water and reaction mixture was kept in water bath for 15 min for the completion of reaction. A yellow colored hydrazone was obtained. Corresponding reagent blank was prepared in the same manner omitting the drug...

Parameter fixation

Determination of λmax:

An absorption maxima (or) λmax are the wavelengths at which maximum absorption takes place. It is important to know the absorption maxima of the substance under study, since it helps to avoid any interfering impurities.

λmax of coloured sample: as given in figure no. 1.

Model: V-530.

Band width: 2nm.

Response: Medium.

Measurement: 650-400nm.

No. of cycle: 1.

Sample: Isoniazid

λmax: 410 nm.

Stability of colour

The time taken for color development and stability was studied.

Procedure: To 0.4 ml of the solution containing Isoniazid taken in 10ml volumetric flasks, 1 ml of 3% ethyl vanillin and add 1ml 0.5M NaoH , the solution was made up to 10ml with distilled water, and kept in water bath for 15 min for color development. The absorbance was measured at different time intervals after dilution. The absorbance was measured against a reagent blank at 410 nm. The results are recorded in Table No: 1 and graph is given in figure No: 2.

Investigation

Effect of concentration of reagent (ethyl vanillin in 0.5M NaoH)

Experiment was carried out to ascertain the optimum concentration of reagents needed for rapid and quantitative formation of yellow colored hydrazones by measuring the absorbance of series of solutions in which one parameter was varied and others fixed.

Procedure: 1ml of the drug solution was placed in 5 different 10 ml volumetric flasks, to this different concentrations of ethyl vanillin in 0.5M NaoH was added followed by 1 ml of 0.5M NaoH, then was made up to 10ml with distilled water then reaction mixture was kept in water bath for 15 min for the completion of reaction for full color development and the absorbance was measured against reagent blank. The results were given is Table No.2 and the graph was given in figure No.3.

Effect of volume of ethyl vanillin in 0.5M NaoH

Procedure: To 1ml of the drug solution, taken in 5 different 10ml volumetric flasks, different volumes of 3 % of ethyl vanillin in 0.5M NaoH was added followed by 1 ml of 0.5M NaoH, then volume was made up to 10 ml with distilled Water then reaction mixture was kept in water bath for 15 min for the completion of reaction for color development and the absorbance was measured against a reagent blank. The results were given in table No .3 and graph was given in fig No.4.

Effect of volume of dilute 0.5M NaoH

Procedure: To 1ml of the drug solution placed in 5 different 10 ml volumetric flasks, to each volumetric flask 1 ml of 3% ethyl vanillin in 0.5M NaoH was added followed by different volumes i.e. 0.5, 1, 1.5, 2, 2.5 ,3 , 3.5 ,4 ml of 0.5M NaoH then volume was made up to 10ml with distilled Water then reaction mixture was kept in water bath for 15 min for the completion of reaction for full color development and the absorbance was measured against reagent blank. The result was given in table No.4 and graph was given in Figure No.5.

Optical characters

Determination of Concentration range

For spectrophotometric analysis, determination of the concentration range which obeys the Beer- Lambert's law is necessary for accuracy and reproducibility.

Preparation of standard curve

A standard curve was prepared by using pure Isoniazid in the concentration of 2-16 µg/ml by this method and selecting the absorption maxima at 404 nm.

Procedure: From the working standard drug solution of 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4 and 1.6 ml (which gives 2-16µg/ml) drug solution was placed in 8 different 10 ml volumetric flasks. Into this 1 ml of 1% of ethyl vanillin was added followed by 2 ml of 0.5M sodium hydroxide and then volume was made up to 10ml with distilled water and then reaction mixture was kept in water bath for 15 min for the completion of reaction for full color development and the absorbance was measured against a reagent blank. And the results were recorded in table No: 5 and the graph was given in the figure No: 6.

The six such linearities were taken for regression co-efficient and eight linearities were taken for standard deviation separately.

Analysis of Formulation

Isoniazid was procured from the local market as tablets of strength 300 mg and marketed with brand name of SOLONEX 300 and it was manufactured by Macleods pharmaceutical Ltd, Daman.

Preparation of sample solution

20 tablets were weighed and crushed properly using a mortar and pestle. Then Powder weight equivalent to 100mg was weighed and transferred to 100ml of volumetric flask and dissolved in distil water and filtered through whatmann filter paper in to another 100ml volumetric flask and made up to mark with same diluent which give the solution of 1000µg/ml conc., Further dilution was performed to get a concentration of 100µg/ml.

Validation parameter:[21]

Linearity

A linear relationship should be evaluated across the range of the analytical procedure. It was demonstrated directly on the drug substance (by dilution of a standard stock solution) and using the proposed procedure.

This method obeys the Beer- Lambert's law in the concentration range of 2-16 µg/ml. as given in table no.5.

Accuracy

Accuracy was established across the specified range of the analytical procedure.

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 20 tablets were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels taking into consideration percentage purity of added bulk drug samples. As given in table no. 7.

Repeatability

Standard solutions of Isoniazid (2, 4, 6, 8, 10, 12, 14 and 16 µg/ml) were prepared and a spectrum was recorded. Absorbance was measured at 410 nm taking the mixture of ethyl vanillin and water as the blank. The absorbance of the same concentration solution was measured six times and RSD was calculated and recorded in table no. 8 and 9.

Limit of Detection (LOD) & Limit of Quantitation (LOQ)

The limit of detection and quantification of the drugs were calculated with the standard deviation and slop. And given in table no. 10.

$$LOD = \frac{3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

σ= standard deviation

s= slope of the calibration curve

Specificity and Selectivity

As given in table no.11.

Reproducibility

Reproducibility is assessed by means of an inter-laboratory trial.

The absorbance readings were measured at 410nm at different laboratory using another spectrophotometer...and it's recorded in table no. 12.

Intra and Inter day Precision

Variation of results within the day (intra-day), variation of results between days (inter day) were analyzed.

Intraday precision was determined by analyzing Isoniazid for three times in the same day at 410 nm.

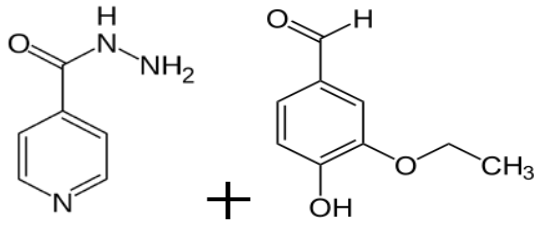
Inter day precision was determined by analyzing the drug different day for three days at 410 nm. Data recorded in table no. 13.

✓ Summary of validation parameters of spectrophotometry is given in table no. 14.

RESULT AND DISCUSSION

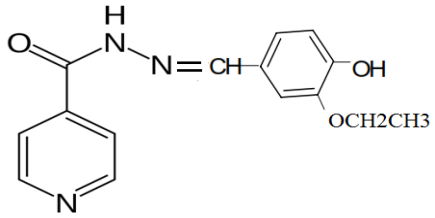
In This method Isoniazid was estimated based on the reaction of ethyl vanillin in the presence of 0.5M sodium hydroxide. The probable reaction takes place was condensation reaction, resulting in the formation of yellow coloured hydrozone which showed λmax at 410 nm. The colour was stable for more than 2 hour 30 min. The method obeyed Beer-Lambert's law in the concentration range of 2-16 µg/ml.

The probable reaction for the development of hydrazone is as per follow



Isoniazid ethyl vanillin

0.5M NAOH



YELLOW HYDRAZONE

A colorimetric method was developed for Isoniazid by using ethyl vanillin as reagent in presence of 0.5M Naoh. All Tables with all data are given as below

Table 1: Stability of colored species

S. No.	Volume of drug solution (100µg / ml)	Time (Minutes)	Absorbance At 410 nm
1.	0.4 ml	0 min	0.204
2.	0.4 ml	15 min	0.204
3.	0.4 ml	30 min	0.203
4.	0.4 ml	45 min	0.200
5.	0.4 ml	60 min	0.199
6.	0.4 ml	75 min	0.193
7.	0.4 ml	90 min	0.193
8.	0.4 ml	105 min	0.192
9.	0.4 ml	120 min	0.190
10.	0.4 ml	135 min	0.189
11	0.4 ml	150 ml	0.189

Table 2: Effect of concentration of ethyl vanillin in 0.5M NaoH

S. No.	Concentration Of ethyl vanillin in 0.5M NaoH	Absorbance At 410 nm
1.	1 %	0.427
2.	2 %	0.516
3.	3 %	0.606
4.	4 %	0.550
5.	5 %	0.500

Table 3: Effect of Volume of ethyl vanillin in 0.5M NaoH

S. No.	Volume of ethyl vanillin in 0.5M NaoH.	Absorbance At410 nm
1.	0.5 ml	0.674
2.	1 ml	0.601
3.	1.5 ml	0.573
4.	2 ml	0.517
5.	2.5 ml	0.217

Table 4: Effect of volume of 0.5M NaoH

S. No.	Volume of 0.5M NaoH	Absorbance At 410 nm
1.	0.5 ml	0.200
2.	1 ml	0.394
3.	1.5 ml	0.414
4.	2 ml	0.450
5.	2.5 ml	0.418

Table 5: Absorbance of Different concentration of Isoniazid Obeying beer's law

S. No.	Volume of drug 100µg/ml	Concentration of drug taken in µg/ml	Absorbance At 410 nm
1.	0.2 ml	2 µg	0.101
2.	0.4 ml	4 µg	0.200
3.	0.6 ml	6 µg	0.307
4.	0.8 ml	8 µg	0.412
5.	1 ml	10 µg	0.530
6.	1.2 ml	12 µg	0.636
7.	1.4 ml	14 µg	0.740
8.	1.6 ml	16 µg	0.844

Table 6: Assay Results of Marketed Formulation

Formulation	Actual concentration of Isoniazid (µg/ml)	Amount obtained of Isoniazid (µg/ml)	% Isoniazid
Tablet	5	4.9	98

Table 7: Determination of Accuracy

Amt of sample Isoniazid µg/ml	Amt. of drug added Isoniazid µg/ml	Amt. of drug recovered Isoniazid µg/ml	% Recovery Isoniazid
10	0	9.90	-
10	5	4.90	98
10	10	9.95	99.5
10	15	14.90	99.33

Table 8: Repeatability data for Isoniazid at 410 nm

Concentration	2 µg/ml	4 µg/ml	6 µg/ml	8 µg/ml	10 µg/ml	12 µg/ml	14 µg/ml	16 µg/ml
Absorption	0.101	0.200	0.307	0.412	0.530	0.636	0.740	0.844
	0.100	0.200	0.307	0.413	0.530	0.635	0.740	0.841
	0.101	0.201	0.306	0.412	0.531	0.634	0.741	0.843
	0.101	0.201	0.306	0.411	0.532	0.635	0.740	0.844
	0.100	0.202	0.307	0.412	0.530	0.636	0.739	0.845
	0.100	0.200	0.305	0.412	0.531	0.636	0.741	0.844
Mean.	0.100	0.200	0.306	0.411	0.530	0.635	0.740	0.843
Std. Dev.	0.000548	0.000816	0.000816	0.000632	0.000816	0.000816	0.000753	0.001378
Coefficient variation	0.0054	0.0040	0.0026	0.0015	0.0015	0.0012	0.0010	0.0016
% RSD	0.54	0.40	0.26	0.15	0.15	0.12	0.10	0.16

n = 6 determination

Table 9: Repeatability of sample application data for Isoniazid

Concentration	Isoniazid 5 µg/ml
Absorption	0.251
	0.250
	0.251
	0.251
	0.249
	0.250
Mean.	0.250
Std. Dev.	0.0008164
Coefficient variation	0.0032
% RSD	0.32

n = 6 determination

Table 10: Lod and Loq

LOD	LOQ
0.052	0.158

Table 11: Specificity and Selectivity study

Study	Isoniazid
Specificity	Specific
Selectivity	Selective

Table 12: Reproducibility data for Isoniazid at 410 nm

Instrument 1	Instrument 2	Inference
0.102 ± 0.002708	0.101 ± 0.001751	Not significant difference

* At 95% confidence interval

Table 13: Precision data for Isoniazid at 410 nm

Conc. µg/ml	Intra-day (n=3)	CV	%RSD
10	0.100 ± 0.000547	0.0054	0.54
20	0.203 ± 0.001303	0.0064	0.64
30	0.306 ± 0.000836	0.0027	0.27

Conc. µg/ml	Inter day (n=3)	CV	%RSD
10	0.101 ± 0.001940	0.0190	1.9
20	0.199 ± 0.001211	0.0060	0.60
30	0.304 ± 0.001788	0.0058	0.58

Table 14: Summary of Validation Parameters of Spectrophotometry

Parameter	Result
λmax (nm)	410.0
Beer's law limits (µg/ml)	2-16
Molar absorptivity (1/mol.cm)	7.102 x 10^3
Sandell's sensitivity (µg.cm^2/0.001 Au)	0.0192
Regression equation (y=a+bc)	
Slope (b)	0.052
Intercept (a)	-0.007
Correlation coefficient (r^2)	0.999
% Relative Standard deviation	0.23
Limit of Detection (µg/ml)	0.052
Limit of Quantitation (µg/ml)	0.158

Figures

A colorimetric method was developed for Isoniazid by using ethyl vanillin as reagent in presence of 0.5M NaoH. All figures are given as below

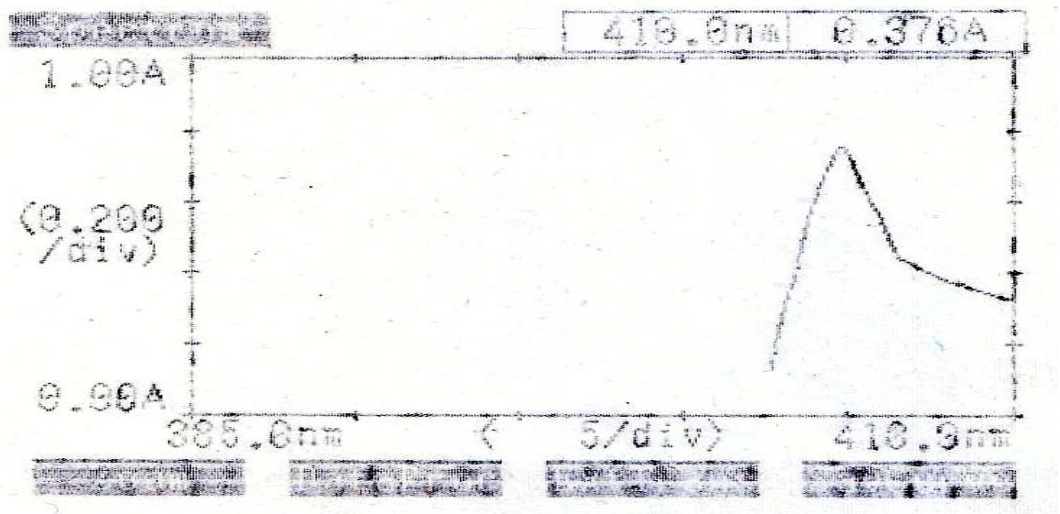


Fig. 1: Ethyl vanillin with drug with 0.5M NaoH

Conclusion: The λmax of the colour sample was found to be 410nm.

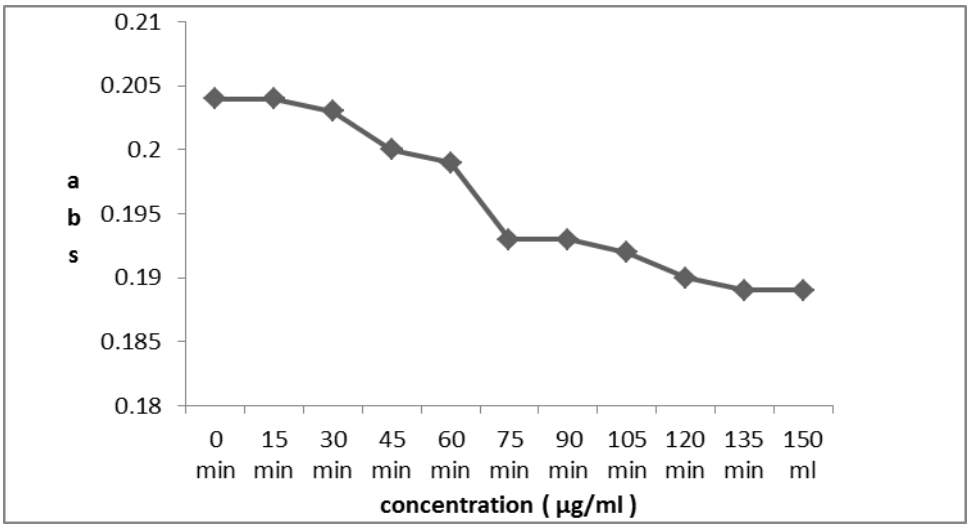


Fig. 2: Stability of color

Conclusion: The stability of color was found to be stable for 2 hr 30 min.

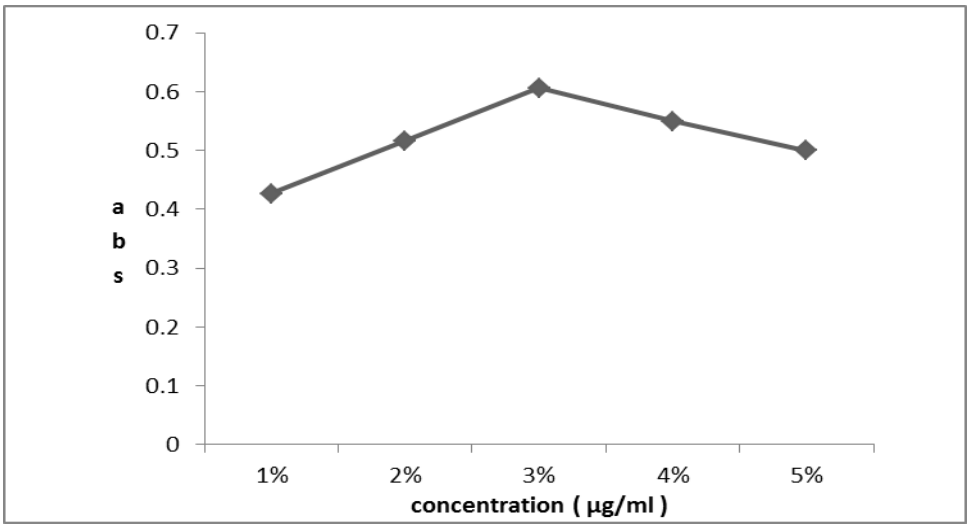


Fig. 3: Effect of concentration of ethyl vanillin in 0.5M NaoH

Conclusion: The maximum absorbance was obtained at the conc. of 3% of ethyl vanillin in 0.5M NaoH

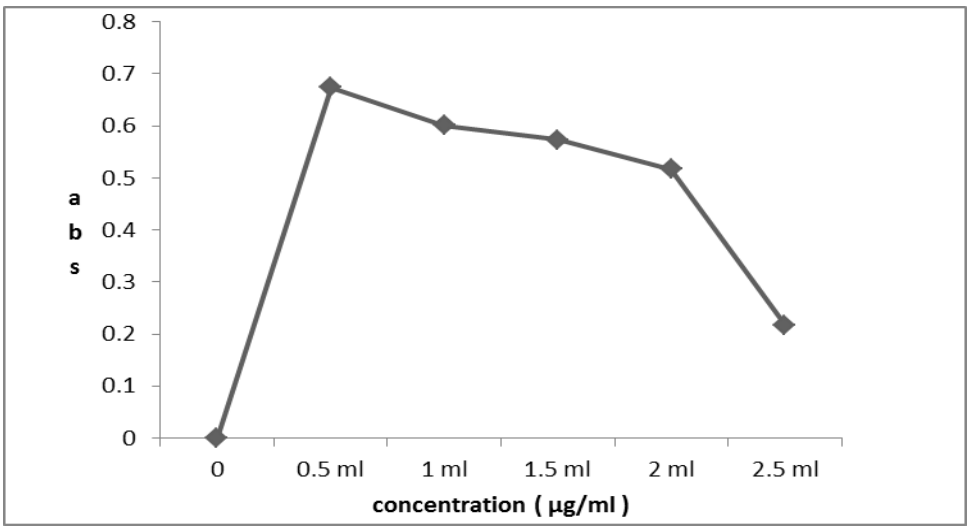


Fig. 4: Effect of Volume of ethyl vanillin in 0.5M NaoH

Conclusion: The maximum absorbance was obtained at the 1.0 ml of 3% of ethyl vanillin in 0.5M NaoH.

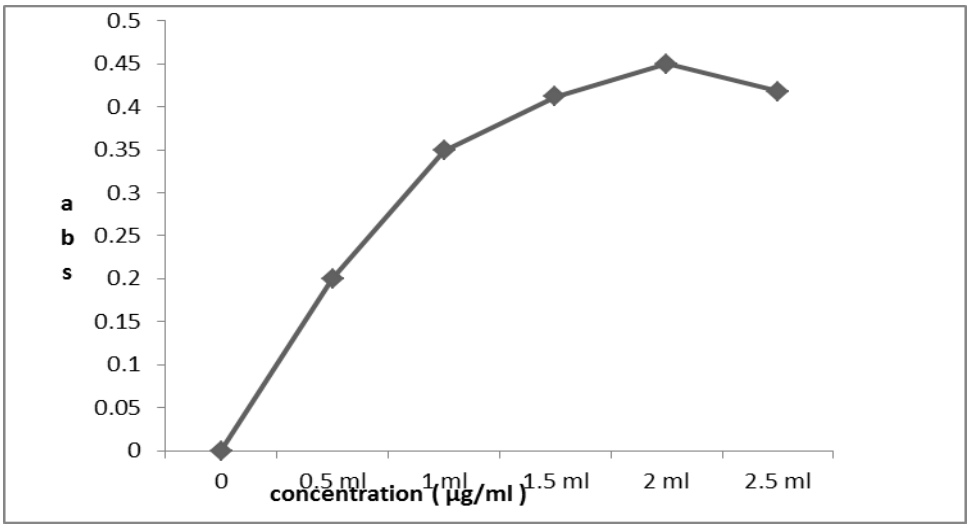


Fig. 5: Effect of volume of 0.5M NaoH

Conclusion: The maximum absorbance was obtained at the 2 ml of 0.5N NaoH.

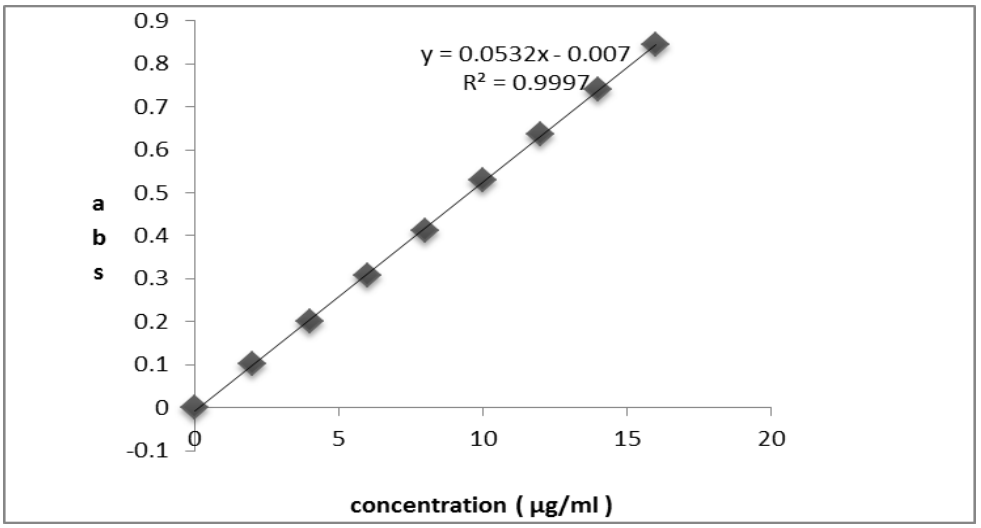


Fig. 6: Linearity of Isoniazid

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