Research Article

Development of New Method and Validation for Determination of Betahistin Dihydrochloride in Bulk and Marketed Formulation

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ABSTRACT

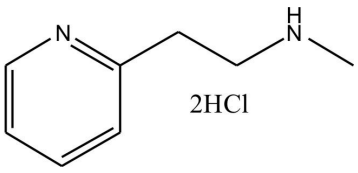
A simple and accurate colorimetric method has been developed for the estimation of Betahistin dihydrochloride in bulk and pharmaceutical dosage forms. Betahistine dihydrochloride was a colorimetric method based on oxidation-reduction reaction and indirect method, in this method Cerric Ammonium sulphate was use as oxidizing agent in present of H_2SO_4 after completion of reaction standard quantity of crystal violet was added. So some part of crystal violet was oxidized with reacting excess of cerric ammonium sulphate and remaining part gives violet color which showed a linearity range from 2-18 µg/ml at a λmax 588 nm. % rsd was found < 2%. Lod and loq were found 0.179 and 0.543 respectively. Correlation coefficient was found 0.999. Regression equation was found y = 0.0559x + 0.001.

Keywords: Betahistin dihydrochloride, Cerric ammonium sulphate, H_2SO_4.

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]

BetahistineDihydrochloride is an antivertigo drug. It was first registered in Europe in 1970 for the treatment of Ménière's disease. It is commonly prescribed to patients with balance disorders or to alleviate vertigo symptoms associated with Ménière's disease.[2] Betahistine Dihydrochloride is **-** 2-[2-(methylamino)ethyl]pyridine



It is soluble in Mithiline chloride and in water, freely soluble in chloroform and in alcohol; very slightly soluble in ether. Betahistine has a very strong affinity as an antagonist for histamine H_3 receptors and a weak affinity as an agonist for histamine H_1 receptors. Betahistine seems to dilate the blood vessels within the middle ear which can relieve pressure from excess fluid and act on the smooth muscle. Betahistine has two modes of action. Primarily, it has a direct stimulating (agonistic) effect on H_1 receptors located on blood vessels in the inner ear. This gives rise to local vasodilation and increased permeability, which helps to reverse the underlying problem of endolymphatichydrops. In addition, Betahistine has a powerful antagonistic effect at H_3 receptors, and increases the levels of neurotransmitters released from the nerve endings. This is thought to have two consequences; the increased amounts of histamine released from histaminergic nerve endings can stimulate H_1 receptors, thus augmenting the direct agonistic effects of Betahistine on these

receptors. This explains the potent vasodilatory effects of Betahistine in the inner ear, which are well documented. It is postulated that Betahistine increases the levels of neurotransmitters such as serotonin in the brainstem, which inhibits the activity of vestibular nuclei.[2] up to now there is HPLC and spectrophotometric method developed on Betahistine dihydrochloride.[3-4]

The USP has published specific guidelines for method validation for compound evaluation. USP defines eight steps for validation: Accuracy, Precision, Specificity, Limit of detection, and Limit of quantitation, Linearity and range, Ruggedness, Robustness[5-6].

EXPERIMENTAL

Betahistine Dihydrochloride was determined spectrophotometrically in bulk and marketed formulation by using crystal violet dye and ceric ammonium sulphate (CAS) as a strong oxidizing agent in presence of H_2SO_4.

1. Preparation of stock solution

Preparation of standard stock solution of Betahistine Dihydrochloride: standard stock solution was prepared by accurately weighing 100 mg of Betahistine Dihydrochloride in 100 ml calibrated volumetric flask and made up the volume with distilled alcohol up to 100 ml.

Preparation of working standard solution of Betahistine Dihydrochloride: working standard was prepared by transferring of 10 ml standard stock solution in to 100 ml calibrated volumetric flask and made up the volume with distilled alcohol to get concentration 100 µg/ml.

2. Preparation of reagent

Preparation of 0.5% CAS solution: Weighed accurately 0.5 gm CAS and transferring into 100 ml volumetric flask and made up the volume with distilled water.

Preparation of 4M H_2SO_4: Transferred 216 ml of concentrated H_2SO_4 into 1000 ml volumetric flask and made up the volume with distilled water.

Preparation of crystal violet (0.02%): Weighed accurately 200 mg crystal violet and added in 1000 ml volumetric flask then diluted up to 1000 ml with distilled water.

3. Preliminary investigation

0.5 ml of 0.5% CAS solution, 1ml of 4M H_2SO_4 and 1 ml of betahistine dihydrochloride (100µg/ml) were taken in to 10 ml volumetric flask and kept aside for 20 minutes for the completion of reaction. 1 ml Crystal violet solution was added in volumetric flask and made up the volume with distilled alcohol. Absorbance against reagent blank was recorded.

3.1 Determination of absorbance maxima

Model: JASCO V-630
Band width: 1.5 nm
Measurement: 800-400 nm
λmax: 588 nm
Absorbance: 0.215

Procedure

0.5 ml of 0.5% CAS solution, 1 ml of 4M H_2SO_4, 0.5 ml of the Betahistine dihydrochloride working standard stock solution were taken in to 10 ml volumetric flask and kept aside for 20 minutes until the completion of reaction. 1 ml of crystal violet was added and made up the volume with distilled alcohol. Absorbance against reagent blank was taken. These solutions were scanned in UV spectrophotometer between 400-800 nm. λmax graph is recorded in figure no. 1.

4. Investigation

Experiments were carried out to ascertain the optimum concentrations of reagents needed for rapid and quantitative formation of greenish blue coloured species by measuring the absorbance of series of solutions in which one parameter was varied and others fixed.

4.1 Effect of concentration of oxidizing agent (CAS)

0.5 ml different concentrations of CAS solution were taken in 5 volumetric flasks of 10 ml. 1 ml 4M H_2SO_4 and 1 ml of working standard of Betahistine dihydrochloride were added in each volumetric flask and kept aside for 20 minutes. 1 ml 0.02% crystal violet was

added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 588 nm and records in table no. 1 and Figure no. 2.

4.2 Effect of volume of oxidizing agent (CAS)

Different volumes of 0.5% CAS solution were taken in 5 volumetric flask of 10 ml. 1 ml 4M H_2SO_4 and 1 ml of working standard of Betahistine dihydrochloride were added in each volumetric flask and kept aside for 20 minutes. 1ml 0.02% crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 588 nm and recorded in Table no: 2 and Figures no: 3.

4.3 Effect of concentration of H_2SO_4

1 ml of different concentrations of H_2SO_4 were taken in 5 volumetric flasks of 10 ml. 0.4 ml of 0.5% CAS solution and 1 ml of working standard of Betahistine dihydrochloride were added in each volumetric flask and kept aside for 20 minutes. 1 ml 0.02% crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank and recorded in table no: 3 and figures no: 4.

4.4 Effect of volume of 4M H_2SO_4

Different volumes of 4M H_2SO_4 were taken in 5 volumetric flasks of 10 ml. 0.4 ml of 0.5% CAS solution and 1 ml of working standard of Betahistine dihydrochloride were added in each volumetric flask and kept aside for 20 minutes. 1 ml 0.02% crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 588 nm and recorded in table no: 4 and figures no:5.

4.5 Effect of concentration of crystal violet

0.4 ml of 0.5% CAS solution, 1ml of 4M H_2SO_4 and 1 ml of working standard of Betahistine dihydrochloride were taken in 5 volumetric flasks of 10 ml and kept aside for 20 minutes. 1 ml of different concentrations of crystal violet were added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 588 nm and recorded in table no:5 and graphs no:6.

4.6 Effect of volume of crystal violet

0.4 ml of 0.5% CAS solution, 1ml of 4M H2SO4 and 1 ml of working standard of Betahistine dihydrochloride were taken in 5 volumetric flasks of 10 ml. and kept aside for 20 minutes. Different volumes of 0.02% crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 588 nm and recorded in table no 6 and graphs no:7.

1. Stability of colour

0.4 ml of 0.5% CAS solution, 1 ml of 4M H2SO4, 1 ml of the Betahistine dihydrochloride working standard stock solution were added to the 10 ml volumetric flask and kept aside for 20 minutes. 1.1 ml of 0.02% crystal violet was added and made up the volume with distilled alcohol. Take absorbance against reagent blank at 588 nm for every 10 minute intervals and recorded in table no: 7 and graph is given in Figure no 8.

6. Optical characters

6.1 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.

6.2. Preparation of standard curve

Standard curve was prepared by using pure Aceclofenac in the conc. range of 2-18 µg/ml by this method and selecting absorbance maximum at 585 nm.

Reagent and chemicals:

1. Working standard stock solution (100µg/ml)
2. 0.5% Cerric ammonium sulphate solution (CAS)
3. 4M H_2SO_4
4. 0.02% Crystal violet

Procedure
0, 0.2, 0.6, 1, 1.4, and 1.8 ml of working standard of Betahistine dihydrochloride were taken in 6 volumetric flasks of 10 ml. 1 ml of 4M H_2SO_4, 0.4 ml of 0.5% CAS were added in each volumetric flask and kept aside for 20 minutes. 1.1 ml of 0.02% of crystal violet solution was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 585 nm and recorded in table no:8 and graph was given in figure no: 9.

7. Method validation
7. 1 Linearity
Linearity was determined over the range of 2-18 µg/ml. 0, 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, and 1.8 ml of working standard of Betahistine dihydrochloride were added in 10 different volumetric flasks of 10 ml. 1 ml of 4M H_2SO_4 and 0.4 ml of 0.5% CAS were added in each volumetric flasks and Kept a side for 20 minutes. 1.1 ml 0.02% of crystal violet solution was added and made up the volume with ethanol. Absorbance was taken against blank at 588 nm and recorded in table no: 8 and graph is given in figure no: 9.

7.2. %Recovery (Accuracy)
The accuracy of the methods was determined by calculating % recovery of Betahistine dihydrochloride by standard addition method. Known volumes of standard solutions of Betahistine dihydrochloride were taken for recovery studies in 3 different levels 50%, 100%, 150% and recovery study was carried out.

7.3. Method precision (% Repeatability)
The precision of the methods was checked by repeated measurement of the absorbance of standard solutions (n = 6) of 10 µg/ml without changing the parameters for the method. The repeatability was expressed in terms of relative standard deviation (RSD).

7.4. Intermediate precision (Reproducibility)
The intraday and interday precision of the proposed methods were performed by analysing the corresponding responses three times on the same day and on three different days over a period of one week for three different concentrations of standard solutions of Bronopol (8, 10, 12 µg/ml). The results were reported in terms of relative standard deviation (RSD).

7.5 Limit of detection and Limit of quantification
The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated by International Conference on Harmonization (ICH) guideline:

LOD = 3.3 σ/S and LOQ = 10 σ/S

Where, σ = the standard deviation of the response,
S = slope of the calibration curve.

7.6. Analysis of marketed formulation
Betahistine dihydrochloride is marketed as serc of 16mg capsules manufactured by solvay were taken for analysis.
Reagent and chemicals

- Working standard stock solution (100µg/ml)
- 0.5% CAS solution
- 4M H_2SO_4
- 0.02% Crystal violet

Preparation of sample solution
Capsule powder equivalent to 100 mg was weighed accurately and transferred into 100ml volumetric flask and made up the volume with distilled alcohol to get 1000 µg/ml concentration. This solution was further diluted to get concentration 100 µg/ml.

7.7 Recovery experiments
To keep an additional check on accuracy of developed assay method, analytical recovery experiments were performed. The different solutions of different concentrations like 5, 10 and 15 µg/ml were prepared in case of both pure drug solution and formulation extract solution and these solutions were subjected to analysis by the above developed method as mentioned above. The six such samples were prepared and average of that readings taken for calculation of %

recovery. This is reported in following table no: 9.

RESULT AND DISCUSSION

Carric ammonium sulphate is a strong oxidizing agent. It reacts with Betahistine Dihydrochloride in presence of acidic medium. When CAS was added in excessive amount it oxidises Bronopol and Remaining CAS reacts with crystal violet & crystal violet was oxidised by CAS. Crystal violet which was left after oxidization it produces different colours accordingly which indirectly indicates the amount of drug present.

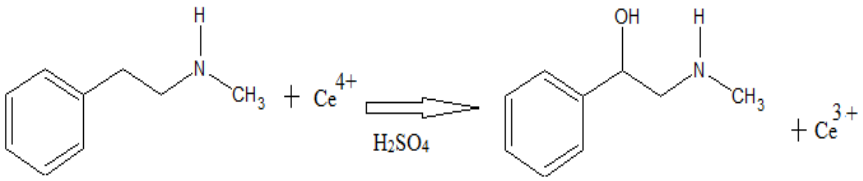
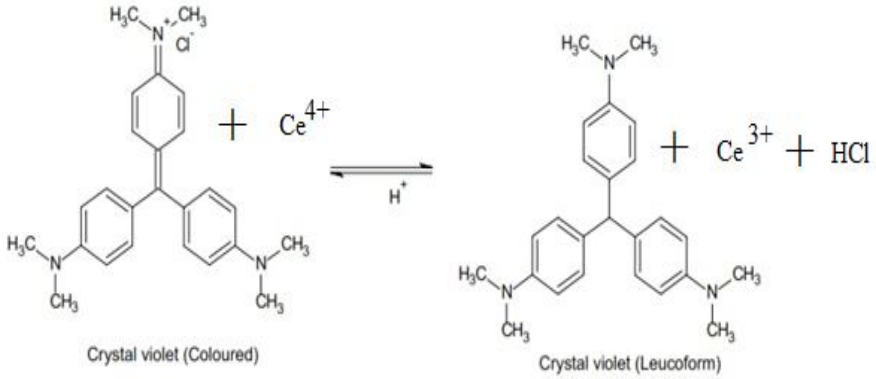


Figure no 6.5 Reaction of Betahistine dihydrochloride with CAS in presene of Sulphuric acid



Reaction of crystal violet with CAS in presence of sulphuric acid

CONCLUSION

For routine analytical purpose, it is always necessary to establish methods capable of analysing huge number of samples in a short time period with due accuracy and precision. A very few analytical methods appeared in the literature for the determination of Betahistine dihydrochloride includes HPLC, HPTLC, and UV-Visible spectrophotometric methods. In the present investigation, colorimetric method for the quantitative estimation of Betahistine dihydrochloride in bulk drug and pharmaceutical formulations has been developed validated.

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Table 1: Effect of Conc. of CAS for estimation of Betahistine dihydrochloride

S. No.	conc. CAS in %	Absorbance
1	0.01	0.003
2	0.05	0.022
3	0.5	0.392
4	1	0.122
5	2	0.039

Table 2: Effect of Volume of 0.5% CAS for estimation Betahistine dihydrochloride

S.No.	Volume of 0.5% CAS in ml	Absorbance
1	0.2	0.276
2	0.4	0.482
3	0.6	0.255
4	0.8	0.119
5	1	0.025

Table 3: Effect of conc. of H_2SO_4 for Estimation of Betahistine dihydrochloride

S.No.	Conc. of H_2SO_4 in molarity(M)	Absorption
1	0.1	0.001
2	1	0.015
3	2	0.112
4	4	0.483
5	6	0.488

Table 4: Effect of Volume of 4M H_2SO_4 for estimation of Betahistine dihydrochloride

S. No.	Volume of 4M H_2SO_4 in ml	Absorbance
1	0.5	0.164
2	1	0.484
3	1.5	0.486
4	2	0.488
5	2.5	0.488

Table 5: Effect of Conc. of crystal violet for estimation of Betahistine dihydrochloride

S. No.	Conc. of crystal violet in %	Absorbance
1	0.0001	0.001
2	0.001	0.012
3	0.01	0.253
4	0.02	0.486
5	0.04	0.210

Table 6: Effect of Volume of crystal violet for Betahistine dihydrochloride

S. No.	Volume of crystal violet in ml	Absorbance
1	0.8	0.321
2	0.9	0.443
3	1	0.507
4	1.1	0.571
5	1.2	0.578

Table 7: Stability of colour for Betahistine dihydrochloride

S. No.	Drug Conc. in µg/ml	Time in minute	Absorbance
1	10	10	0.574
2	10	20	0.574
3	10	30	0.574
4	10	40	0.573
5	10	50	0.573
6	10	60	0.573
7	10	70	0.572
8	10	80	0.572
9	10	90	0.571
10	10	100	0.571
11	10	110	0.570
12	10	120	0.570

Table 8: Standard curves for estimation of Betahistine dihydrochloride

S. No.	Vol. of working standard drug	Conc. of drug (µg/ml)	Absorbance
1	0.2ml	2	0.124
2	0.6 ml	6	0.333
3	1 ml	10	0.566
4	1.4 ml	14	0.795
5	1.8 ml	18	0.995

Table 9: Recovery study for estimation of Betahistine dihydrochloride

S. No	Sample	Labelled amount	Amount found	% Recovery
1	Betahistine dihydrochloride	16 mg	15.78 mg	98.62%

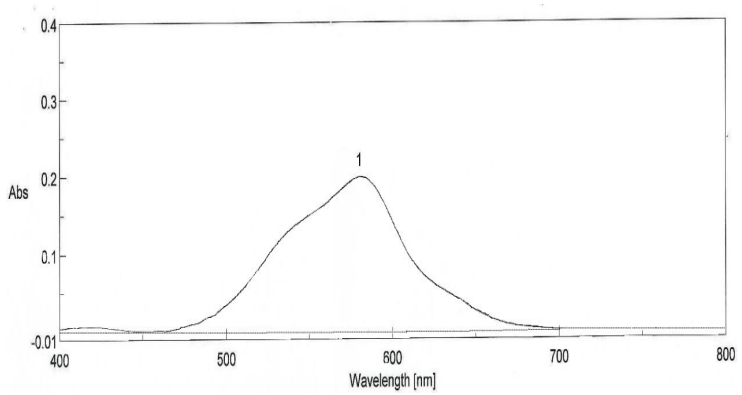
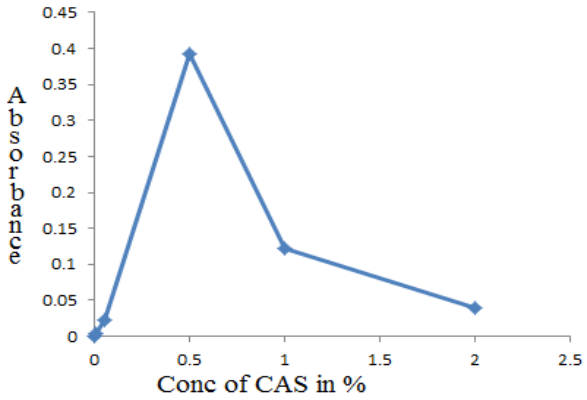
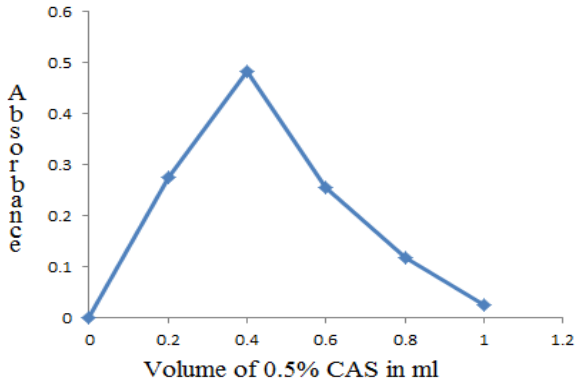


Fig. 1: λmax graph for Betahistine dihydrochloride



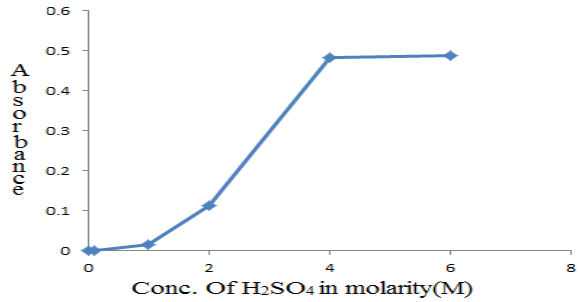
Conclusion: Best absorbance found in 0.5% CAS solution.

Fig. 2: Absorbance Vs Conc. Of CAS for Betahistine dihydrochloride



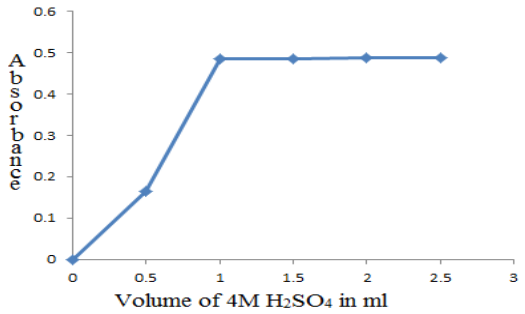
Conclusion: Best absorbance was found in 0.4 ml of 0.5% CAS solution.

Fig. 3: Absorbance Vs Volume of 0.5% CAS for Betahistine dihydrochloride



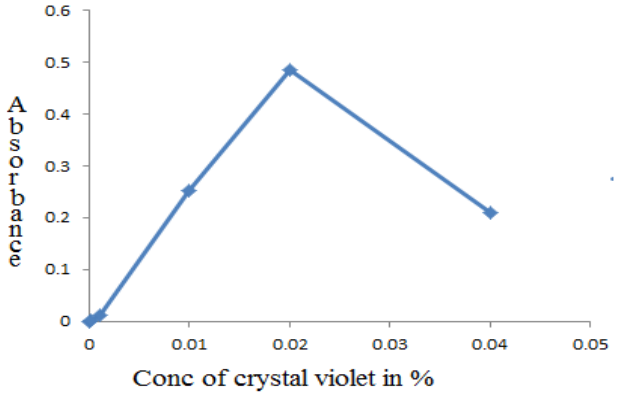
Conclusion: Best absorbance was found in 4M H_2SO_4

Fig. 4: Absorbance Vs Conc. Of H_2SO_4 for Betahistine dihydrochloride



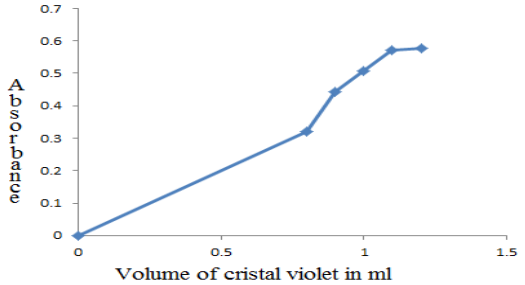
Conclusion: Best absorbance was found in 1ml of 4M H_2SO_4

Fig. 5: Absorbance Vs Volume of 4M H_2SO_4 for Betahistine dihydrochloride



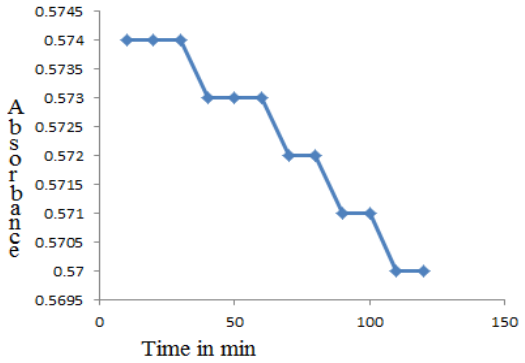
Conclusion: Best absorbance was found in 0.02% conc. of crystal violet solution.

Fig. 6: Absorbance Vs Conc. of crystal violet for Betahistine dihydrochloride



Conclusion: Best absorbance was found in 1.1 ml of (0.02%) crystal violet solution

Fig. 7: Absorbance Vs Volume of crystal violet for Betahistine dihydrochloride



Conclusion: Stability study of colour was performed and from graph it proved that colour is stable for at least 2 hours.

Fig. 8: Absorbance Vs Time in minute for Betahistine dihydrochloride

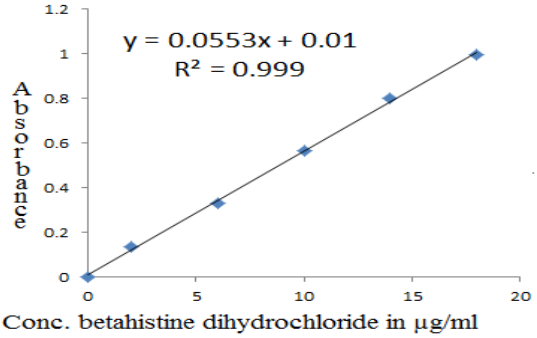


Fig. 9: Standard curves for Betahistine dihydrochloride

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