Research Article

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

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ABSTRACT

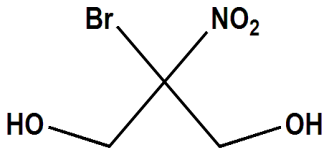
A simple and accurate colorimetric method has been developed for the estimation of Betahistin dihydrochloride in bulk and pharmaceutical dosage forms. This method was involving oxidation-reduction reactions where chloramine T has been used for oxidation of Bronopol in excess amount in presence of sulphuric acid. Remaining amount of oxidizing agents oxidizes standard amount of crystal violet which produces violet color. Absorbance maxima were found to be 585 nm. Linearity range was found 2-18 µg/ml of drug concentration. The method has been validated according to ICH Guidelines.

Key Words: Bronopol, chloramine T , 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]

Bronopol is a highly active antimicrobial, Bronopol was invented by The Boots Company PLC, Nottingham, England in the early 1960s and first applications were as a preservative for pharmaceuticals. Bronopol's low mammaliantoxicity (at in-use levels) and exceptional activity against bacteria (especially the troublesome Gram-negative species) ensured that it became popular as a preservative in many consumer products such as shampoos and cosmetics.[18] Bronopol was subsequently taken up as an effective antimicrobial in many industrial environments such as paper mills, oil exploration and production facilities, as well as cooling water disinfection plants.[2]



It is 2-bromo-2-nitropropane-1,3-diol and formula is $C_3H_6BrNO_4$. It is soluble in Water 28%, Methanol 89%, Ethanol 56%, Isopropanol 41%. Patterns of growth inhibition of Escherichia coli in the presence of 2-bromo-2-nitropropane-1,3-diol (bronopol) indicate a period of biocide-induced bacteriostasis followed by growth at an inhibited rate. The length of the bacteriostatic period, but not the subsequent growth inhibition, was reduced by the addition of excess cysteine. Patterns of growth inhibition were unaffected by catalase or superoxide dismutase. Catalytic oxidation of thiols in the presence of excess thiol leads to the creation of an anoxic state. Under these conditions, the slower reaction with thiols, which consumes bronopol, predominates. Consumption of bronopol by its reaction with thiols, without the involvement of oxygen, leads to the eventual removal of bronopol from treated suspensions and the resumption of growth.[3] Up to now there is HPLC and spectrophotometric method developed on Betahistine dihydrochloride.[4-5] 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 [6-7]

EXPERIMENTAL

Bronopol was determined spectrophotometrically in bulk and marketed formulation by using crystal violet as dye and chloramine-T as a strong oxidizing agent in presence of H_2SO_4.

1.Preparation of stock solution

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

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

2. Preparation of reagent

Preparation of 0.005M chloramine-T solution: weighed accurately 140.86 mg of chloramine-T and transferred to 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 (0.01%): Weighed accurately 100 mg of 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 0.005M chloramine-T solution, 1ml of 4M H_2SO_4 and 1 ml of drug solution (100µg/ml) were transferred into 10 ml volumetric flask and kept aside for 20 minutes for the completion of reaction. 1 ml crystal violet solution was added and made up the volume with distilled alcohol. Absorbance was taken against reagent blank.

3.1 Determination of absorbance maxima

Model: JASCO V-630
Band width: 1.5 nm
Measurement: 800-400 nm
λmax: 589 nm
Absorbance: 0.448

Procedure

0.5 ml of chloramine-T solution, 1 ml of 4M H_2SO_4, 1 ml of the bronopol working standard stock solution were added to the 10 ml volumetric flask. It was kept aside for 20 minute until the completion of reaction. Added 1 ml of crystal violet and made up the volume with distilled alcohol. Absorbance against reagent blank was recorded. These solutions were scanned in UV spectrophotometer between 400-800 nm. Graph was recorded in figure no: 1

4. Investigation

Experiments was 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 (chloramine-T)

Different concentrations of chloramine-T solution, 1 ml 4M H_2SO_4 and 1 ml of working standard of Bronopol were taken in different five volumetric flasks of 10 ml and kept aside for 20 minutes. 1 ml 0.01% crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank and recorded to Table no 4.1 and Figure no 4.2

Conclusion: Best absorbance was found in 0.5 ml of 0.005M Chloramine-T solution.

4.2 Effect of volume of oxidizing agent

Different volumes of 0.005M chloramine-T solution and 1 ml 4M H_2SO_4 and 1 ml of working standard of bronopol were taken in 5 volumetric flasks of 10 ml and Kept aside for 20 minutes. 1ml 0.01% crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 589 nm and recorded in Table no: 2 and Figure no: 3

4.3 Effect of concentration of H_2SO_4

Different concentrations of H_2SO_4 were taken in 5 volumetric flasks of10 ml. 0.5 ml of 0.005M chloramine-T solution and 1 ml of working standard of bronopol were added in each volumetric flask and kept aside for 20 minute. 1 ml 0.01% crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 589 nm and recorded in Table no:3 and Figure 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. 0.5 ml of 0.005M chloramine-T solution and 1 ml of working standard of Bronopol were added in each volumetric flask and kept aside for 20 minutes. 1 ml of 0.01% crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 589 nm and recorded in Table no:4 and Figure no:5.

4.5 Effect of concentration of crystal violet

0.5 ml chloramine-T solutions, 1ml of 4M H2SO4 and 1 ml of working standard of Bronopol were taken in 5 volumetric flasks of 10 ml and kept aside for 20 minutes. Different conc. of crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 589 nm and recorded in table no: 5 and Figure no: 6.

4.6 Effect of volume of crystal violet

0.5 ml of 0.005M chloramine-T solution, 1 ml of 4M H2SO4 and 1 ml of working standard of Bronopol were taken in 5 volumetric flasks of 10 ml and kept aside for 20 minutes. Add different volumes of 0.01% crystal violet in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 589 nm and recorded in Table no: 6 and Figure no: 7.

4.7 Stability of colour

0.5 ml of 0.005M chloramine-T solution, 1ml of 4M H_2SO_4 and 1 ml of working standard of Bronopol were taken in 5 volumetric flasks of 10 ml and kept aside for 20 minutes. 1 ml of 0.01% crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 589 nm and recorded in Table no: 7 and Figure no: 8.

5. Optical characters

51. 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.

5.2. Preparation of standard curve

Standard curve was prepared by using pure Bronopol in the concentration range of 4-20µg/ml by this method and selecting absorbance maximum at 589 nm.

Reagent and chemicals:

1. Working standard stock solution (100µg/ml)
2. 0.005M Chloramine-T solution
3. 4M H2SO4
4. 0.01% Crystal violet

Procedure

0, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6 and 1.8 ml of working standard of Bronopol were taken in 9 volumetric flasks of 10 ml and 1 ml of 4M H_2SO_4, 0.5 ml of 0.005M Chloramine-T were added in each volumetric flask and kept aside for 20 minutes. 1.2 ml 0.01% of crystal violet was added and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 589 nm. The result was recorded in table no.: 8 and graph was given in figure no:9.

6. Method validation

6.1. Linearity

Linearity was determined over the range of 4-20µg/ml. 0, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8 and 2 ml of working standard of Bronopol were taken in 1o volumetric flasks of 10 ml. 1 ml of 4M H_2SO_4 and 0.5 ml of 0.005M Chloramine-T were added in each volumetric flask and kept a side for 20 minutes. 1.2 ml 0.01% of crystal violet solution was added and made up the volume with ethanol. Absorbance was taken against reagent blank at 589 nm

and recorded in table no:8 and graph is given in figure no.:9.

6.2. %Recovery (Accuracy)

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

6.3. 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).

6.4. 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).

6.5. 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.

6.6. Analysis of marketed formulation

Bronopol is marketed as Hista-Q of 50mg/100ml syrup manufactured by Que pharma was taken for analysis.

Reagent and chemicals

1) Working standard stock solution (100µg/ml)
2) 0.01M Chloramine-T solution
3) 2M H_2SO_4
4) 0.02% Crystal violet

Preparation of sample solution

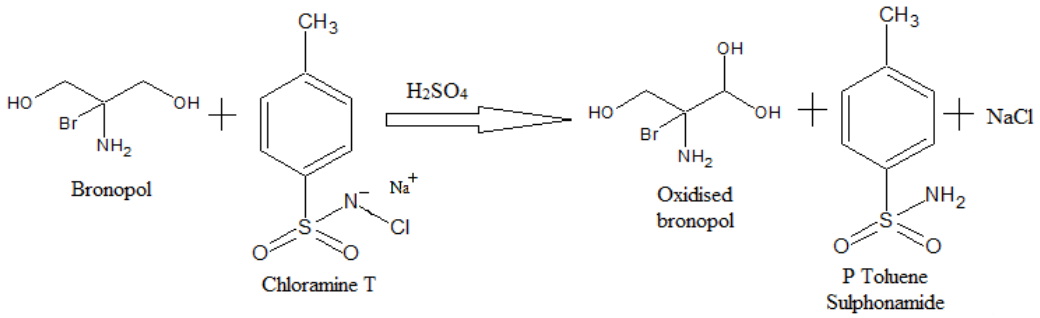
Syrup equivalent to 10 mg or 20 ml was taken accurately and transferred in to 100 ml volumetric flask and made up the volume with distilled alcohol to get 100 µg/ml conc.

Recovery experiments

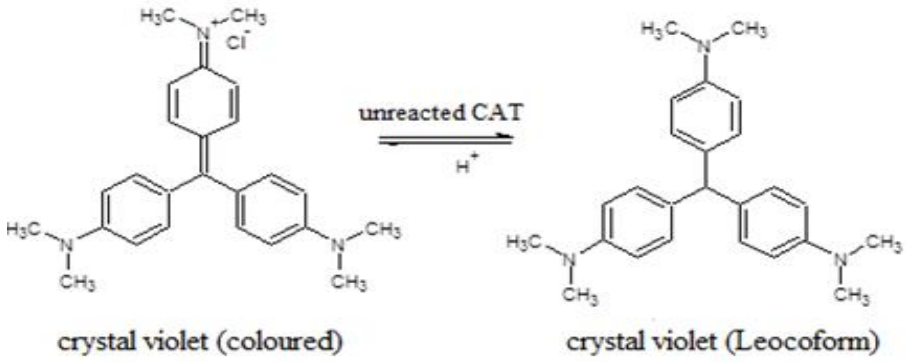
To keep an additional check on accuracy of developed assay method, analytical recovery experiments were performed. The different solutions of different conc. like 5, 10 and 15 µg/ml were prepared in case of both pure drug solution and formulation extract solution and these solutions were subjected to 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. Result was recorded in table no:9

RESULT AND DISCUSSION

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



Reaction of Chloramine T with Bronopol



Reaction of Chloramine T with Cristal violet

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. Bronopol is official in Indian Pharmacopoeia. A very few analytical method appeared in the literature for the determination of Bronopol. In view of the above fact, some simple analytical method was planned to develop with sensitivity, accuracy, precision and economical. In the present investigation, colorimetric method for the quantitative estimation of Bronopol in bulk drug and pharmaceutical formulations has been developed.

ACKNOWLEDGEMENT

The author wishes to thanks mates who helped me lot for my work. And how can I forget staff of Srinivas College of pharmacy, Mangalore who suggest me in all way.

Table 1: Effect of Conc. of Chloramine T for Bronopol

S. No.	Conc. Chloramine T	Absorbance
1	0.0001	0.004
2	0.0005	0.012
3	0.001	0.050
4	0.005	0.280
5	0.01	0.044

Table 2: Effect of volume of 0.005M Chloramine T for Bronopol

S. No.	Volume of 0.005M chloramine T in ml	Absorbance
1	0.1	0.728
2	0.3	0.432
3	0.5	0.284
4	0.7	0.102
5	0.9	0.048

Table 3: Effect of Conc. Of $H_2SO_4$for estimation of Bronopol

S. No.	Conc. Of H_2SO_4 in molarity (M)	Absorption
1	0.1	0.001
2	1	0.005
3	2	0.094
4	4	0.285
5	6	0.287

Table 4: Effect of volume of 4M H_2SO_4 for estimation of Bronopol

S. No.	Volume of 4M H_2SO_4 in ml	Absorbance
1	0.5	0.092
2	1	0.280
3	1.5	0.281
4	2	0.284
5	2.5	0.281

Table no: 5 Effect of Conc. of crystal violet for estimation of Bronopol

S. No.	Conc. Of crystal violet in %	Absorbance
1	0.0001	0.001
2	0.001	0.012
3	0.01	0.282
4	0.02	0.180
5	0.04	0.120

Table 6: Effect of volume of crystal violet for estimation of Bronopol

S. No.	Volume of crystal violet in ml	Absorbance
1	0.6	0.090
2	0.8	0.140
3	1	0.284
4	1.2	0.448
5	1.4	0.721

Table 7: Stability of colour for Bronopol

S.No.	Drug Conc.in µg/ml	Time in minute	Absorbance
1	10	10	0.448
2	10	20	0.448
3	10	30	0.448
4	10	40	0.448
5	10	50	0.447
6	10	60	0.447
7	10	70	0.447
8	10	80	0.447
9	10	90	0.446
10	10	100	0.446
11	10	110	0.445
12	10	120	0.445

Table 8: standard curves for estimation Bronopol

S. No.	Vol. of working standard drug	Conc. Of drug (µg/ml)	Absorbance
1	0.4 ml	4	0.171
2	0.8 ml	8	0.362
3	1.2 ml	12	0.549
4	1.6 ml	16	0.717
5	2 ml	20	0.885

Table 9: Recovery study for estimation of Bronopol

S.No.	Sample	Labelled amount	Amount found	% Recovery
1	Bronopol	50 mg/100 ml	48.9 mg/100 ml	97.8

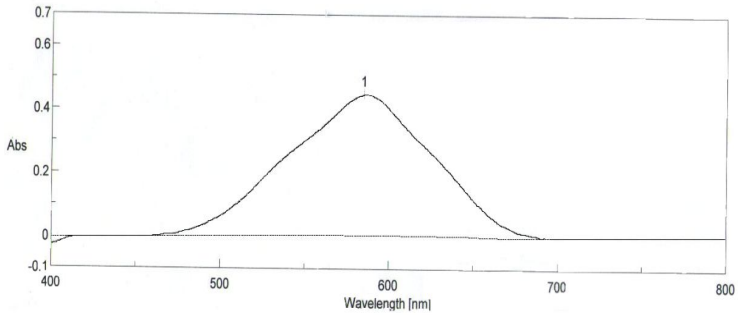
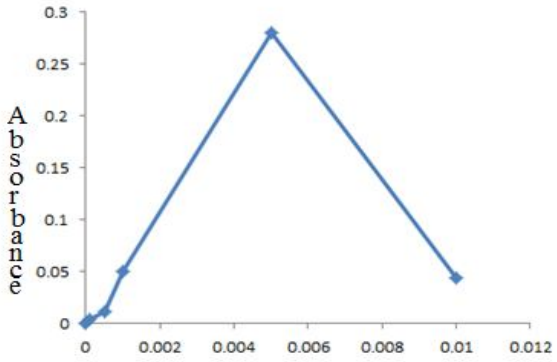
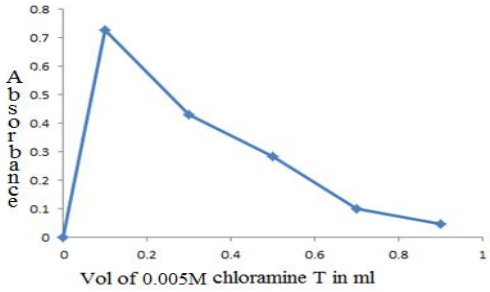


Fig. 1: absorption maximum



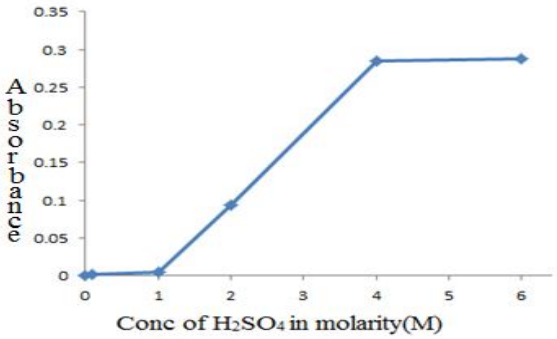
Conclusion: Best absorbance was found in 0.5 ml of 0.005M Chloramine-T solution.

Fig. 2: Absorbance Vs Conc. of chloramine T for Bronopol



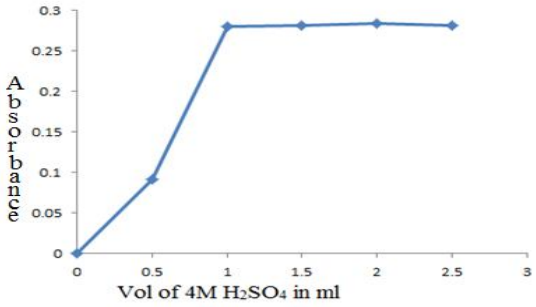
Conclusion: Best absorbance found in 0.5 ml of 0.005M of Chloramine-T solution.

Fig. 3: Absorbance Vs Volume of 0.005 M Chloramine T for Bronopol



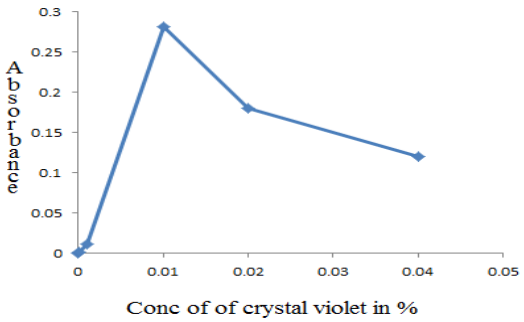
Conclusion: Best absorbance was found in 4M H_2SO_4

Fig. 4: Absorbance Vs Conc. Of H_2SO_4 for Bronopol



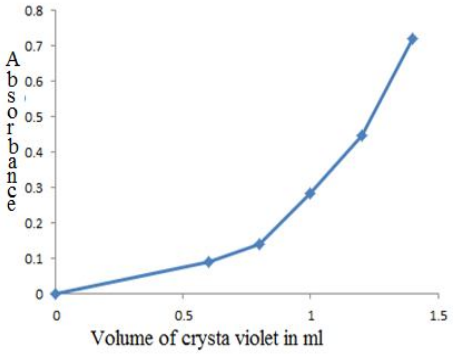
Conclusion: Best absorbance was found in 1mL of 4M H_2SO_4

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



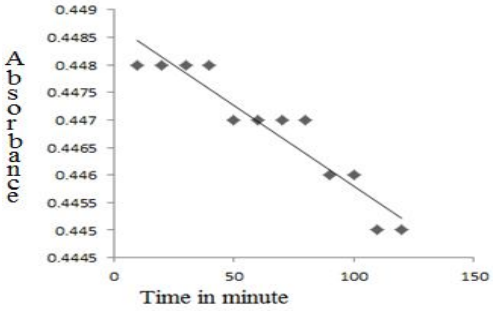
Conclusion: Best absorbance was found in 0.01% conc. of crystal violet solution

Fig. 6: Absorbance Vs Conc. Of crystal violet for Bronopol



Conclusion: Best absorbance was found in 1.2 ml of (0.01%) crystal violet solution

Fig. 7: Absorbance Vs Volume of crystal violet for Bronopol



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 bronopol

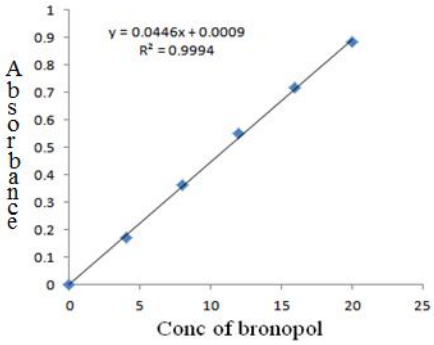


Fig. 9: Standard Curves for Bronopol

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