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THE PHARMA RESEARCH
An International Peer Reviewed Cited Journal

METHOD DEVELOPMENT AND VALIDATION OF BRONOPOL IN DRY SYRUP OF AMOXYCILLIN TRIHYDRATE AND CLAVULANIC ACID FORMULATION BY RP-HPLC

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ARTICLE INFO

Published on: 15-12-2017

ISSN: 0975-8216

Keywords:

RP-HPLC method, PDA detector, Bronopol, ICH, Validation

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ABSTRACT

A RP- HPLC method was developed for estimation of Bronopol in dry syrup of Amoxycillin Trihydrate and Clavulanic Acid formulation using C18 column (250 X 4.6mm, 5µm) and a mobile phase of 0.1% Orthophosphoric acid : Methanol with gradient programming, at flow rate 1.0 ml/min with PDA detector at 210 nm . The retention time of Bronopol, Amoxycillin Trihydrate and Clavulanic Acid were found to be 12.3 minutes, 15.7 minutes and 22.6 minutes respectively. The proposed method was validated for System Suitability, specificity, Pricision, Linearity, Accuracy, LOD and LOQ. All the parameters were found to be within the acceptable limits. Linearity of Bronopol was in the range of 0.5-10µg/ml . RP-HPLC method was simple, accurate, precise and suitable for the analysis of Bronopol in the dry syrup formulations.

INTRODUCTION

Bronopol (2-brmo-2-nitropropane-1, 3-diol) is one of the most advanced bactericides available in the market. Bronopol was recommended as suitable for the preservation of oral medicament and also in many consumer products such as shampoo and Cosmetics. The molecular structure of Bronopol is shown in figure 1.

Chemical Structure of Bronopol:

Formula: $C_3H_6BrNO_4$ (2-Bromo-2-nitro-1,3-propanediol)

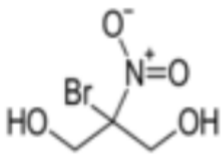


Fig-1

The objective of this work was to develop a simple and rapid RP-HPLC method which would be accurate and precise. The method was validated according to ICH guidelines.

METHOD DEVELOPMENT

CHEMICALS AND REAGENTS

Orthophosphoric acid (Fischer scientific), Methanol (Fischer scientific, HPLC grade), water (HPLC grade), Bronopol.

INSTRUMENTATION

A Shimadzu LC solution 2010 C HPLC System with PDA detector equipped with an auto sampler with LC solution software. Column was employed in the method was Zodiac, C18 Stainless Steel Column (250mm x 4.6 mm, 5µm).The flow rate selected was 1.0 ml/min. The detection was done at 210 nm. The temperature and run time were 25°C and 42.0 minutes respectively.

All the weighing in the experiments was done with Sartorious electronic balance capable of measuring with an accuracy of 0.01 mg. The solubility was enhanced by sonication on an Ultrasonic bath.

GLASSWARE

All the volumetric glassware used in the study was grade A quality Borosil.

Table-1 Chromatographic Conditions:

Parameters	Description
Detector	UV-210nm
Injection Volume	20µl
Flow rate	1ml/minutes
Temperature	25°C
Mobile Phase (Gradient)	Mobile Phase A : 0.1% v/v Orthophosphoric Acid Mobile Phase B : Methanol
Diluent	0.1% v/v Orthophosphoric Acid : Methanol (95:5)
Run time	42.0 minutes

PREPARATION OF ORTHOPHOSPHORIC ACID SOLUTION (0.1% V/V ORTHOPHOSPHORIC ACID)

The 0.1% v/v Orthophosphoric acid solution was prepared by adding 1ml of orthophosphoric acid in 1000 ml HPLC grade water.

GRADIENT PROGRAMMING

Time (Minutes)	Mobile Phase A (%)	Mobile Phase B (%)
0	95	5
20	95	5
25	40	60
35	40	60
37	95	5
42	95	5

PREPARATION OF DILUENT

The diluent was prepared by mixing Orthophosphoric acid solution and methanol (HPLC grade) in the ratio of 95: 5 V/V. This solution was used for diluting the drug solution in the study.

PREPARATION OF BRONOPOL STANDARD SOLUTION (2µG/ML):

Approx. 20.0 mg of Bronopol weighed & transferred to a 100 ml volumetric flask. To this 60 ml of diluent was added and sonicated for five minutes to ensure the complete dissolution of Bronopol. Made up the volume with diluent to obtain standard stock solution (200 µg/ml) of Bronopol, and dilute 1.0 ml of this solution to 100 ml with diluent. 20l of this solution was injected into HPLC system.

PREPARATION OF BRONOPOL SAMPLE SOLUTION:

Sample solution was prepared in duplicate by dissolving weight eq. to 0.2 mg of Bronopol freshly prepared constituted suspension in each of 100 ml volumetric flask, To this add 60 ml of diluent & sonicate for 30 minutes then made up to the mark with diluent. 20l of this solution was injected into HPLC system.

VALIDATION OF HPLC METHOD

SELECTIVITY/SPECIFICITY:

This parameter was performed to assess and ensure that the impurities, degraded products and diluents do not affect the samples analyzed. 20µl of diluent, placebo and standard solution were injected into the system and chromatograms recorded. Specificity of the method was demonstrated by calculating the resolution between different peaks eluted during the run and the tailing factor. It was found that the resolution between the peaks of

Bronopol, Clavulanic acid and amoxycillin is greater than 2, indicating a good resolution between the peaks. The tailing factor for component was also calculated and found to be less than 2, indicating symmetric peaks. The following components were injected to demonstrate selectivity which is a measure of retention between various components on the chromatograph.

Table -2 System suitability study:

S.No.	Components	Retention Time (Min.)	Resolution (NLT 2.0%)	Tailing Factor (NMT 2.0%)
1	Bronopol	12.33	--	1.2
2	Clavulanic Acid	15.74	7.15	1.2
3	Amoxycillin Trihydrate	22.64	10.388	1.3

CHROMATOGRAMS OF STUDY

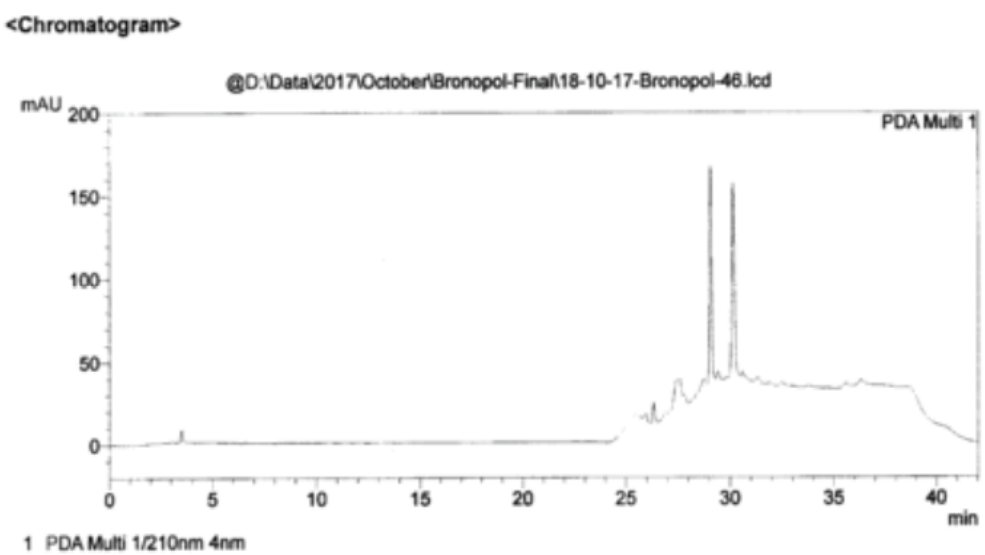


Fig No. 1: Blank Chromatogram

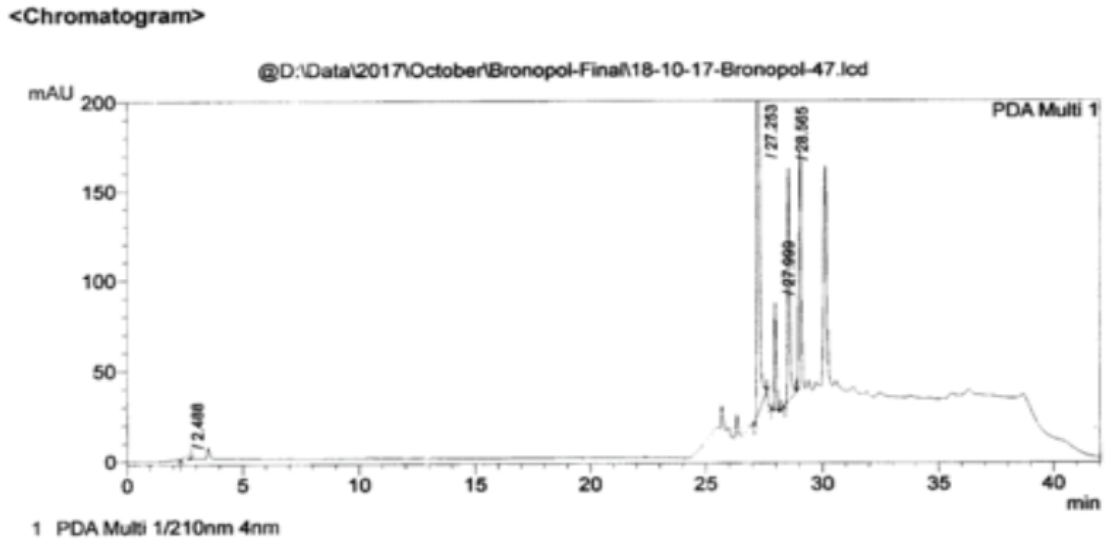


Fig No. 2: Placebo Chromatogram

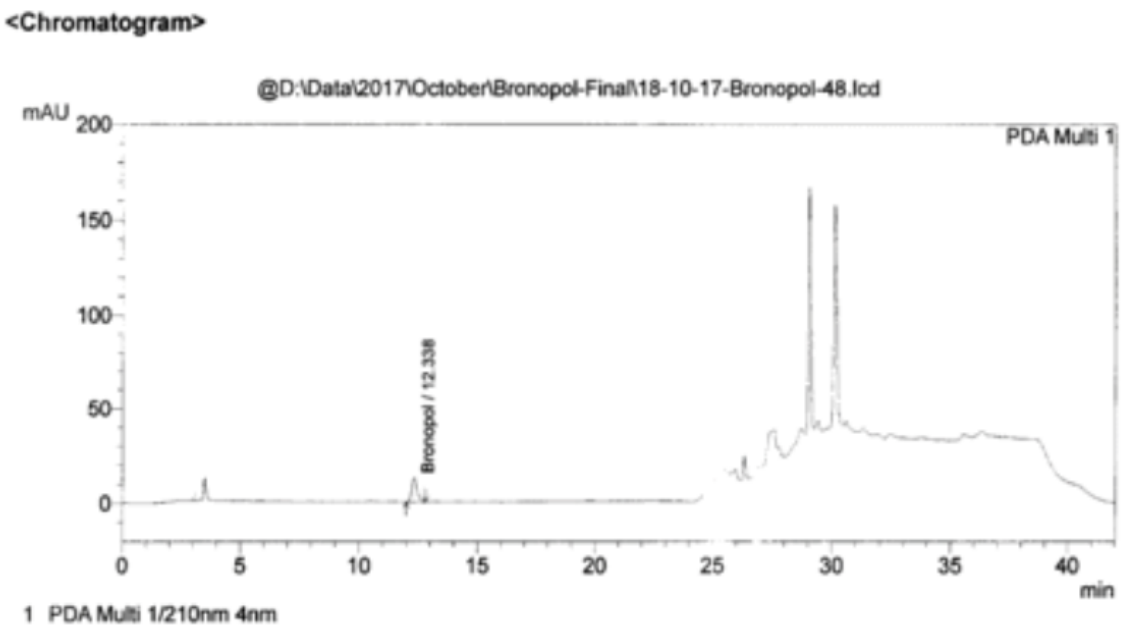


Fig No. 3: Standard (Bronopol) Chromatogram

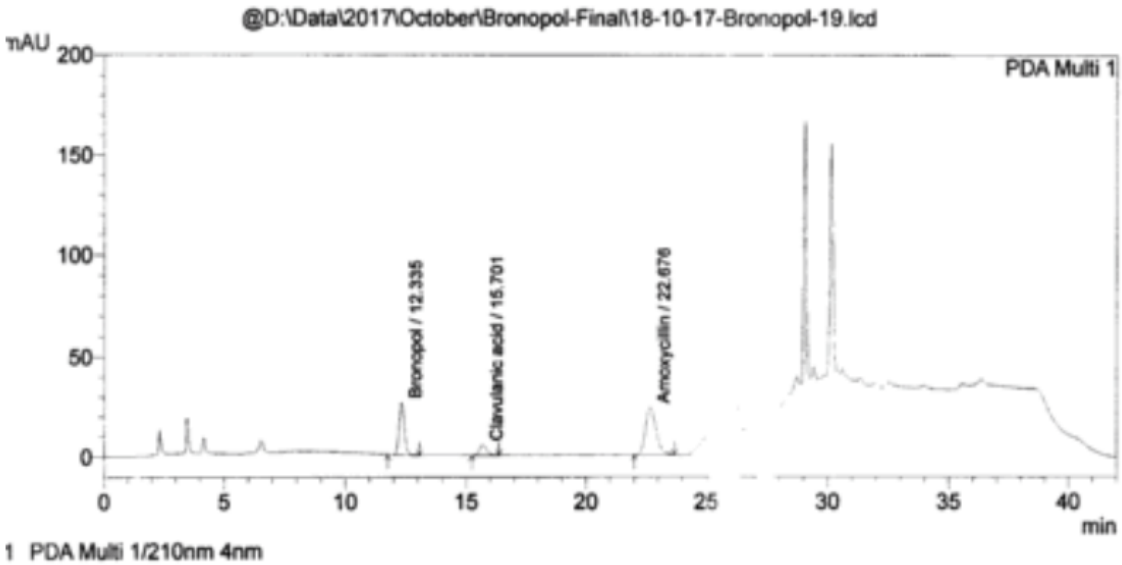


Fig No. 4: Chromatogram of Bronopol, Clavulanic Acid and Amoxycillin Trihydrate

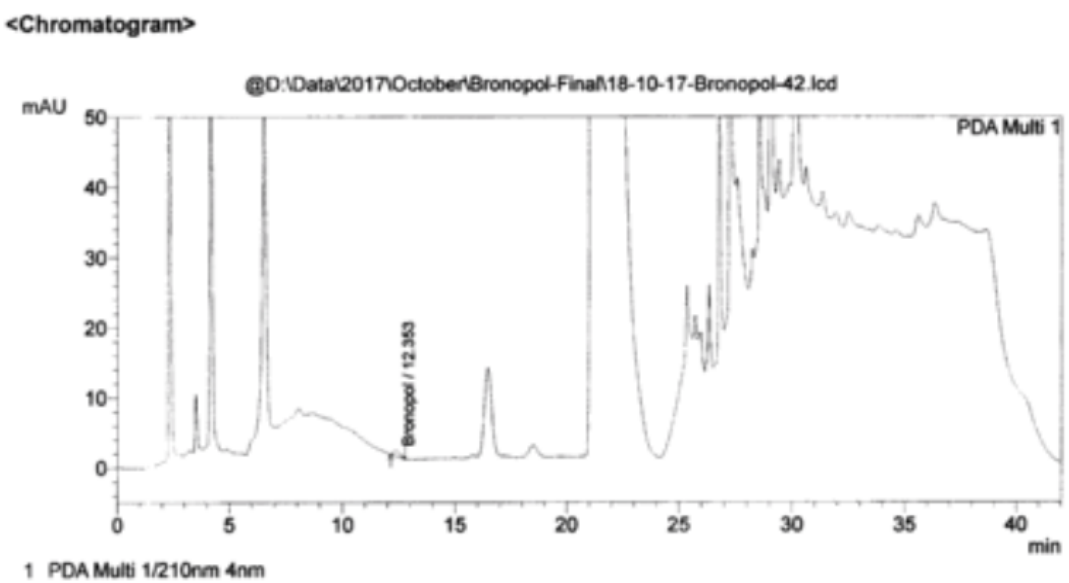


Fig No. 5: Chromatogram of Dry syrup Suspension (sample)

LINEARITY:

A linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to concentration of analyte. The linearity of the method was determined using different concentrations of Bronopol. Calibration graphs were found to be linear from 0.5 µg/ml to 10 µg/ml for Bronopol.

Table- 3 Bronopol Linearity

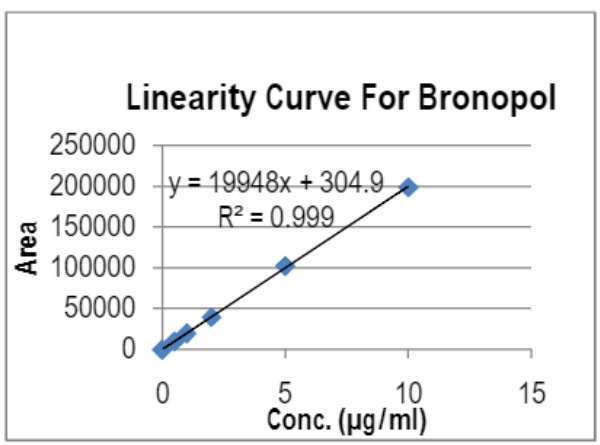
S. NO	Component	Values of X and Y variables						Correlation coefficient
		Variable	1	2	3	4	5	
1	Bronopol	X (Conc. In µg/ml)	0.5	1.0	2.0	5.0	10.0	0.999
		Y(Area)	10059	19845	39703	102604	198657	

Note: **X is the concentration of the respective component in µg/ml.**

Y is the peak response of the respective component in area counts.

LINEARITY CURVE FOR BRONOPOL

Concentration (µg/ml)	Area
0.5	10059
1.0	19845
2.0	39703
5.0	102604
10.0	198657



PRECISION:

- The precision of an analytical method is the amount of scatter in the results obtained from multiple analysis of a homogenous sample (method precision).
- The precision was measured on six injections of standard solution containing Bronopol (system precision).

Table- 4 CALCULATION OF CORRELATION COEFFICIENT FOR BRONOPOL (INTRA-DAY PRECISION)

S. No.	Analyte (µg/ml)	Number of injections (Area Counts)						$\bar{x}$	S.D.	% COV
		1	2	3	4	5	6			
1.	Bronopol (2.0 µg/ml)	39483	39790	39590	39742	39241	39706	39592	204.8	0.52

$\bar{x}$ represents the average values of six replicates analysis.

SD is the standard deviation calculated on the six replicates.COV is the coefficient of variation.

CALCULATION OF CORRELATION COEFFICIENT FOR BRONOPOL (INTER-DAY PRECISION)

Table- 5

Days	Average Area
Day-1	39592
Day-2	39646
Day-3	39752
Average	39663
Standard Deviation	81.39
%RSD	0.20

The intra and inter day variation results, shows a low coefficient of variation. The % RSD for the area of standard injection for Bronopol was found to be 0.52 for Intra-day and 0.20 for inter day precision. This shows that the method is highly precise.

ACCURACY:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The Accuracy was performed by addition of known amounts of standard drug. The accuracy of method was determined by calculating percentage recovery of known added amount of analyte.

Table-6 Recovery studies of Bronopol at 100% level

S No.	Placebo sample taken (g)	Dilution (ml)	Std. Stock Conc. µg/ml	Std. volume spiked (ml)	Expected conc. (µg/ml)
1	1.887	100	209.8	1.0	2.098
2	1.978	100	209.8	1.0	2.098
3	1.956	100	209.8	1.0	2.098

Table-7

S. No.	Average std. Area	Average spiked Area	% Recovery
1.	39794	39660	99.66
2.	39794	39476	99.20
3.	39794	39431	99.09

Average Recovery : 99.32
Standard Deviation : 0.3
%RSD : 0.3

The percent recovery values were found to be in range between 99.09-99.66%, which is well within acceptance criteria.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION:

The detection limit was determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte could reliably be detected. The detection limits was calculated At S/N is equal to 3. ***The detection limits in µg/ml for the Bronopol was found to be 0.2 µg/ml.***

The quantitation limit was determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte could be quantified with acceptable accuracy and precision. The limit of Quantitation was calculated at S/N is equal

to 6.***The quantitation limits in µg/ml for Bronopol were found to be 0.5 µg/ml.***

DISCUSSION:

A chromatographic method involves demonstrating specificity, which is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. A mixture of three components was injected. Specificity of the method was demonstrated by calculating the resolution between different peaks eluted during the run and the tailing factor. The chromatographic parameters were fixed and HPLC system was studied for suitability of drug analysis. The developed method was performed for linearity, precision, Accuracy, specificity, LOD and LOQ.

CONCLUSION:

The present study was carried out to develop a Sensitive, Precise and Accurate RP-HPLC method for the analysis of Bronopol in dry syrup formulation. In order to develop a method under gradient conditions, mixture of 0.1% v/v Orthophosphoric acid and methanol in different combinations were tested as mobile phase on Zodiac ODS C18 (250 X 4.6mm) column. The retention time obtained for Bronopol is 12.33 minutes. The % RSD for standard was found to be 0.52. In order to test the linearity of the method, five dilutions of standard solutions of Bronopol in the range of 0.5 to 10 µg/ml were prepared. The method was validated by evaluation of the required parameters. The Bronopol content in the formulation was quantified by using the proposed analytical method. The low coefficient of variation in the recovery data indicates the reproducibility of the method in dosage forms. It is concluded that the method is specific for estimation of Bronopol in dry syrup formulations. The method is suitable for the analysis of Bronopol without any interference from the excipients. It may be extended to evaluate the Bronopol in bulk and in pharmaceutical formulation and also for the estimation of Bronopol in consumer products shampoos and Cosmetics.

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