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Stability Indicating Development and Validation for Simultaneous Estimation of Bromohexine and Cephalexin RP-HPLC method

V. Mohan Goud* and N. Nidhi

Department of Pharmaceutical analysis, Joginpally B.R Pharmacy College, Moinabad, Telangana, India.

* Corresponding author: V. Mohan Goud, e-mail: mohanvanga@yahoo.com

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ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of the Bromhexine and Cephalexin in Tablet dosage form. Chromatogram was run through Std BDS 250 x 4.6 mm, 5µ. Mobile phase containing Buffer Perchloric acid: Acetonitrile taken in the ratio 48:52 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% OPA buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 260 nm. Retention time of Bromhexine and Cephalexin were found to be 2.637 min and 3.067 min. %RSD of the Bromhexine and Cephalexin were and found to be 0.5% and 1.2% respectively. %Recovery obtained was 99.98% and 99.57% for Bromhexine and Cephalexin respectively. LOD, LOQ values obtained from regression equations of Bromhexine and Cephalexin were 0.01, 0.27 and 0.02, 0.81 respectively. Regression equation of Bromhexine is y = 83150.x + 1901, and y = 20072x + 44518 of Cephalexin. Retention times were decreased and run time was decreased, hence the method developed was simple and economical that can be adopted in regular quality control test in industries.

Keywords: Bromhexine, Cephalexin, RP-HPLC.

1. INTRODUCTION

Bromhexine has a place with the class of natural mixes known as phenyl methylamines. These are mixes containing a phenylmethtylamine moiety, which comprises of a phenyl gather substituted by a methanamine. Bromhexine is an expectorant/mucolytic operator. Bromhexine is not accessible in the United States. It is advertised under the exchange name Bisolvon® in Germany, England, Belgium, France, Italy, Netherlands, Norway, Sweden, Australia, and South Africa.

2. MATERIALS AND METHODS

2.1 Apparatus and Chromatographic Parameters

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Bromhexine-Cephalexin solutions.

2.2 Drug Samples

The Bromhexine-Cephalexin drug used for estimation for this study was procured from tablets. The marketed under the exchange name Bisolvon® in Germany, England, Belgium, France, Italy, Netherlands, Norway, Sweden, Australia, and South Africa. The label claim was Bromhexine-Cephalexin250 mg in each tablet.

2.3 Reagent and solutions

HPLC grade Acetonitrile and Methanol, Phosphate buffer Potassium dihydrogen, Combination Bromhexine and Cephalexin drug was used in the study. A Mobile phase containing Buffer Perchloric acid: Acetonitrile taken in the ratio 48:52 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% OPA buffer, adjusted with Ortho phosphoric Acid and it is also used as a diluent for preparing the working solution of drug. The mobile phase was

degassed in ultrasonic water bath for 10 minutes and filtered through 0.45µm filter under vacuum filtration.

2.4 PREPARATION OF THE PREGABALIN & EPALRESTAT STANDARD & SAMPLE SOLUTION:

2.4.1 Standard Solution Preparation

Accurately weighed 4mg of Bromhexine, 25mg of Cephalexin and transferred to 100ml and 10ml individual volumetric flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (40µg/ml of Bromhexine and 2500µg/ml Cephalexin).

2.4.2 Sample Solution Preparation

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (40µg/ml of Bromhexine and 2500µg/ml of Cephalexin).

3. RESULTS AND DISCUSSION

3.1 METHOD DEVELOPMENT

Three trials were performed for the method development and the best peak with least fronting factor was found to be the third peak with RT=4.181 min (Fig 1).

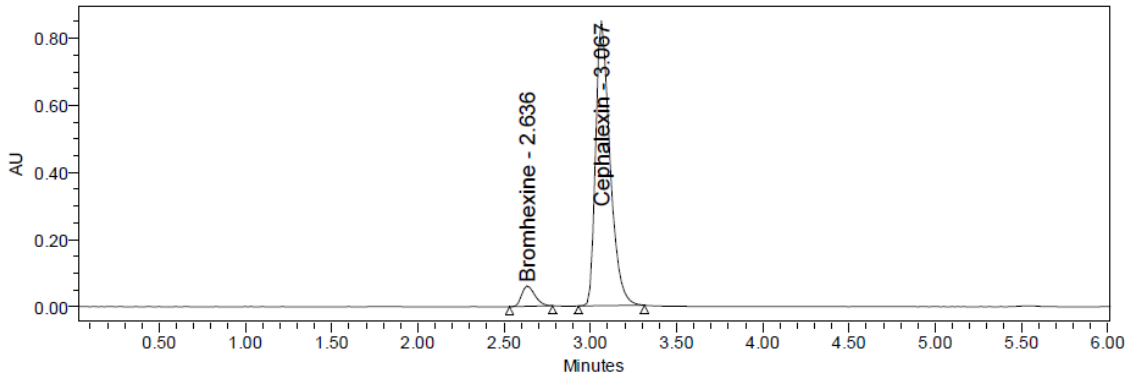


Figure 1. Chromatogram Peak of Bromhexine, Cephalexin.

3.2 METHOD VALIDATION

3.2.1 Precision

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

3.2.2 Acceptance Criteria

The % RSD for the area of five standard injections results (Table 1) should not be more than 2%.

Table 1: Precision results for Bromhexine and Cephalexin

S.NO	PEAK AREA OF BROMO HEXINE	% RSD	PEAK AREA OF CEPHALEXIN	% RSD
1	325560		4658205	
2	325153		4646564	0.5
3	331063	1.0	4603004	
4	327891		4601767	
5	324028		4635562	

3.2.3 Accuracy

Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner Amount added for forBromhexine and Cephalexin and the individual recovery and mean recovery values.

3.2.4 Acceptance Criteria

The % Recovery for each level (Table 2) should be between 98.0 to 102.0%.

Table 2: Recovery data

Concentration at specification level	Area of bromohexine	Area of cephalexin	%recovery of cephalexin	Mean recovery of bromohexine	Mean recovery of bromohexine	Mean recovery of cephalexin
50%	320148	4658205	101.27	99.77		
100%	325153	4646564	100.27	101.83	99.98%	99.57%
150%	331063	4603004	98.79	101.06		

3.3 Recovery Studies

To determine the accuracy and precision of the proposed method recovery studies were carried out. A fixed amount of sample was taken and standard drug was added at 50%, 100% and 150% levels. The results were analyzed and the results were within the limits. The % recovery, Mean recovery and %Relative standard deviation value for bromohexine and cepahlexin drug was found to be 101.27%,101.33%,99.10.and 99.77% 101.83%, 101.6%

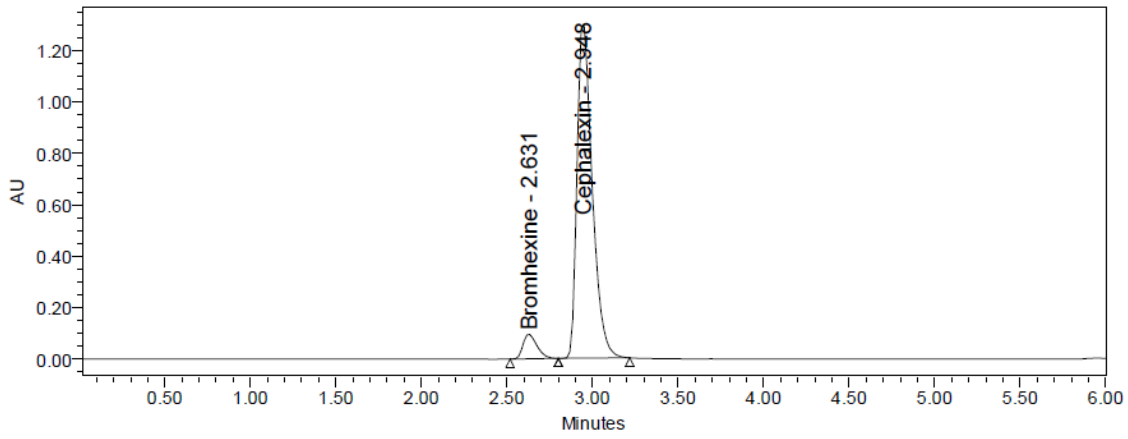
3.4 Linearity and Calibration Curve

To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 50ppm to 300ppm of Bromhexine and Cephalexin. Plot a graph to concentration versus peak area. Slope obtained was 3111 Y-Intercept was 5896 and Correlation Co-efficient was found to be 0.999

Table 3. Different concentration values of bromohexine and cepahlexin

bromohexine conc(µg/mL)	Area	Cephalexin conc(µg/mL)	Area
0	0	0	0
1	85089	62.5	1342082
2	168732	125	2504274
3	251923	187.5	3904409
4	335650	250	5011009
5	422747	312.5	6379679
6	495321	375	7514703

Linearity graph of Bromhexine and Cephalexin



3.5 Limit of Detection and Limit of Quantification

Limit of Detection (LOD) is the lowest concentration of an analyte in a sample that can be detected but not quantified. LOD is expressed as a concentration at a specified signal to noise ratio. The LOD will not only depend on the procedure of analysis but also on the type of instrument. In chromatography, detection limit is the injected amount that results in a peak with a height at least twice or thrice as high as baseline noise level. The LOD for bromohexine was found to be 0.01 and cephalexin 0.27

Limit of Quantification (LOQ) is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio. In chromatography, limit of quantification is the injected amount that results in a peak with a height, ten times as high as base line noise level. The LOQ for bromohexine was found to be 0.02 at 0.81

3.6 Robustness

Robustness is determined by making deliberate changes in the chromatographic conditions like change in flow rate, mobile phase composition and temperature and evaluated for the impact on the method. It was observed from the chromatograms that the results were within the limits. This indicates that the method developed is robust

A simple, rapid and precise method has been developed and validated for the drug bromohexine and cepahlexinThe estimation was carried out with A Combination Bromhexine and Cephalexin drug was

used in the study. A Mobile phase containing Buffer Perchloric acid: Acetonitrile taken in the ratio 48:52 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% OPA buffer, adjusted with Ortho phosphoric Acid Precision of the methods were studied by making repeated injections of the samples and system precision values were determined (Table 3, 4). The retention time was 4.181 min. The calibration curve was linear over the concentration range of 50- 90 µ g mL $^{-1}$. The LOD and LOQ values were found to be 0.20µg/ml and 0.60µg/ml. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method. Hence it was concluded that the RP-HPLC method developed was very much suitable for routine analysis.

Table 4. System precision values of bromohexine and cephalexin

S.No	parameters	Acceptance criteria	bromohexine	cepahlexin
1	Accuracy	95-105%	99.98%	99.57%
2	Precision	RSD with in 2%	0.5	1.2
3	Linearity	R^2 not less than 0.99	0.999	0.999
4	LOD	S/N=3	0.01	0.02
5	LOQ	S/N=10	0.27	0.27

4. CONCLUSION

The proposed study describes new and simple RP-HPLC method for the estimation of bromohexine and cephalexin The method validated was found to be simple, accurate and precise. Therefore the proposed study method can be used for quantification of bromohexine and cephalexin in bulk and pharmaceutical dosage form.

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