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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF BROMHEXINE BY RP-HPLC METHOD

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ABSTRACT

The objective of the present work is to develop an efficient, precise, accurate, linear, simple, rapid, reproducible and sensitive RP- HPLC method for the estimation of Bromhexine in tablet dosage forms. The developed method was validated as per ICH guidelines. The HPLC method was developed using waters C_{18} column (250mm×4.6mm; 5_{μ} id) and flow rate 1.0ml /min. Detection was carried out at by absorption at 226nm and injection volume is 20µl. The mobile phase used was methanol, acetonitrile and 0.1% orthophospharic acid 75:24:01. The calibration curve was linear over the range of 10-60µg/ml. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Bromhexine in tablet dosage forms.

KEYWORDS: Bromhexine, tablet dosage form, RP-HPLC method, validation.

I. INTRODUCTION

Bromhexine is chemically 2,4-Dibromo-6-{[cyclohexyl(methyl)amino]methyl}aniline. Bromhexin is a mucolytic drug used in the treatment of respiratory disorders associated with viscid or excessive mucus. Bromhexine is intended to support the body's mechanisms for clearing mucus from the respiratory tract. It is secretolytic, increasing the production of serous mucus in the respiratory tract, which makes the phlegm thinner and less viscous. Bromhexine is a synthetic derivative of the herbal active ingredient. It has been shown to

increase the proportion of serous bronchial secretion, making it more easily expectorated. It is indicated as "secretolytic therapy in bronchopulmonary diseases associated with abnormal mucus secretion and impaired mucus transport".

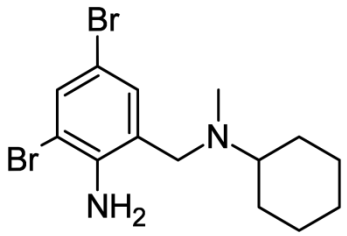

Figure 1: Structure of Bromhexine.

A literature survey revealed that a number of analytical methods have been developed for the determination of Bromhexine alone and in combination in various dosage forms and biological samples using HPLC, liquid chromatography and spectrophotometry techniques.

We have developed a new accurate and precise RP-HPLC method for the determination of Bromhexine in tablet dosage form. The developed method is validated as per ICH guidelines.

2. MATERIALS AND METHODS

2.1 Drugs, chemicals and solvents: HPLC grade water was purchased from Merck chemicals, Mumbai. HPLC grade methanol and all other laboratory grade chemicals were purchased from Merck chemicals, Mumbai.

2.2 Equipment and chromatographic conditions: Agilent 1100 series HPLC with Quaternary G1311 A pump, COLCOM G1316A thermostat column temperature control, Thermostatic auto sampler G 1329A with sample volume of 0. 1 – 1500 µL and variable programmable UV detector G 1314 A. The instrument was operated and integrated with Agilent chem. station LC software. The LC was coupled with Water mass detector model LAA 1369. Mobile phase used was methanol: acetonitrile: 0.1% orthophosphoric acid in the ratio of 75:24:01. All the chromatographic runs were carried out in isocratic elution mode with a flow rate of 1ml/min and the sample injection volume was 20 µL. The detector wavelength was set at 226nm.

2.3 Preparation of the mobile phase and diluent: Methanol: acetonitrile: 0.1% orthophosphoric acid are mixed in the ratio of 75:24:01 after that, the mobile phase was degassed in sonicator for 10min it is filtered by vaccum filtration using 0.4 micron filter

paper and employed as the mobile Phase. The same solution was also used as the diluents for preparing drug dilutions.

2.4 Preparation of working standard solution of bromhexin: About 10mg of drug was weighed accurately and transferred into a 10ml volumetric flask. Methanol was added to it to dissolve the drug. The volume was made up to the quantity with the diluents and mixed well. This was used as a standard stock solution. 1.0 ml of the stock solution was transferred to 10ml volumetric flask and made up to the volume using diluents to get a 100µg/ml of bromhexin. This was used as working standard solution.

2.5 Estimation of the drug from the tablet dosage form: Ten tablets of drug Bromhexin (Bisolvon – 8mg) were grounded to finely powdered material. Powder equivalent to 10mg of drug was taken into a 10 ml of volumetric flask containing 10ml of mobile phase and was shaken to dissolve the drug and then filtered through Nylon membrane filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 1000µg/ml.

3. RESULTS AND DISCUSSION

During the method optimization studies trails were carried out for an ideal separation of the drug using different mobile phases and different chromatographic conditions. Finally the following conditions were found to be optimum after evaluating the column efficiency by parameters like theoretical plates and tailing factor.

Table 1: Optimized conditions for the proposed HPLC method.

Stationary phase	**Waters, C18 column, (250mm×4.6mm; 5µ)**
Mobile phase	Methanol: acetonitrile: 0.1% orthophosphoric acid in the ratio of 75:24:01
Flow rate	1.0 ml/min
Column temperature	Ambient
Injection volume	20 µl
Detection wavelength	226nm
Run time	10 min
Retention time of the drug	4.6 min

Optimum wavelength was selected by injecting standard solution of drug into HPLC with UV detector G 1314 A and the wave length which gives higher response for the compound is selected. The wavelength was found to be 226nm. Under the optimized conditions the retention time of bromhexin was found to be 4.6min.

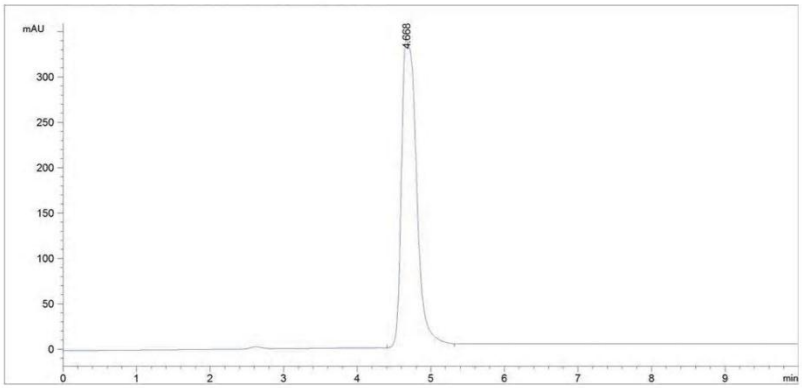

Figure 2: Chromatogram of standard Bromhexine.

3.1 Linearity: The regression of the plot was computed by least squares method and is shown in Figure 3. The calibration curve of the drug was linear over the concentration range of 10-60µg/ml with the correlation coefficient 0.999 and the % RSD for each component was less than 2.

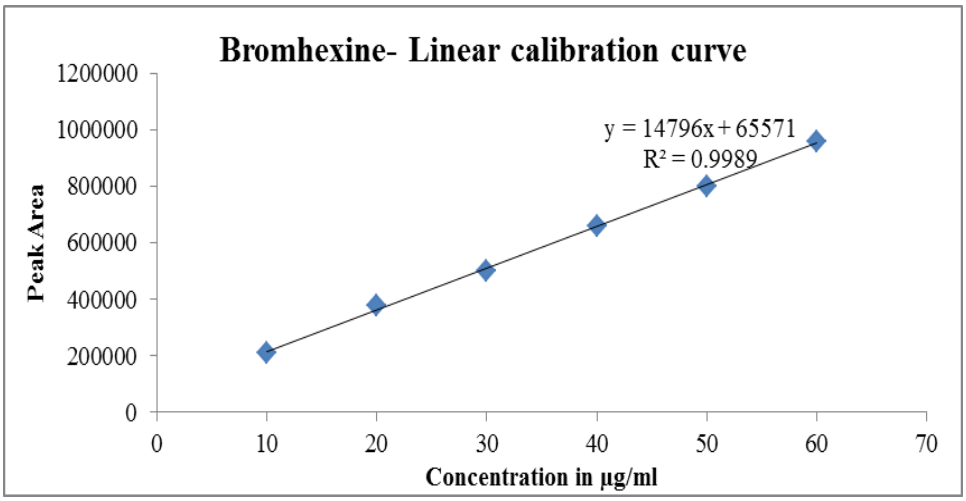

Figure 3: Linearity of detector response graphs for bromhexin.

3.2 Accuracy and precision: The accuracy of the method was determined by recovery experiments. Individual percentage recovery, mean percentage recovery, percentage RSD and squares correlation coefficient for linearity of the test method were calculated and the results were presented in table 2. The high percentage recovery indicates that the developed method is highly accurate. The precision of the method was demonstrated by intraday variation studies. Six replicate injections of sample solutions were made and the percentage RSD was calculated and presented in Table 3. From the data obtained the developed RP-HPLC method was found to be precise.

Table 2: Accuracy data of developed method.

Level	Concentration in µg/ml			Peak Area observed	Amount of Recovered (µg/ml)	% Recovery
	Target	spiked	Total			
50 %	20	10	30	490576	29.48	98.26
	20	10	30	496582	29.84	99.496
	20	10	30	497286	29.88	99.60
100%	20	20	40	652749	39.71	99.27
	20	20	40	651791	39.65	99.12
	20	20	40	654069	39.79	99.47
150%	20	30	50	799586	49.95	99.90
	20	30	50	798274	49.87	99.73
	20	30	50	796301	49.74	99.49

Table 3: Precision data of developed method.

S.No	Retention time	Area
1	4.674	657573
2	4.673	656928
3	4.677	656604
4	4.669	659124
5	4.670	658967
6	4.672	657493
Avg area		657781
%RSD		0.16

3.3 System suitability: System suitability parameters were studied with six replicates of standard sample solution and the corresponding values are presented in Table 4.

Table 4: System suitability parameters of developed method.

Parameter	Value
Retention time (min)	4.6
Tailing factor	1.17
Theoretical plates	4491

3.4 Limit of detection (LOD) and Limit of quantification (LOQ): LOD and LOQ in the sample were determined with acceptable precision and accuracy. The results were presented in Table 5.

Table 5: Limit of detection and limit of quantification data.

S.No	Sample name	LOD		LOQ	
		Conc (µg/ml)	Retention time	Conc (µg/ml)	Retention time
1.	Bromhexine	0.03	4.675	0.10	4.668

3.5 Robustness: Robustness of the proposed analytical method was determined by varying flow rate and mobile phase composition. Percentage RSD was given in Table 6.

Table 6: Robustness of proposed method.

Variability		%RSD
pH	5.4	1.6
	5.6	1.27
Organic phase	70:29:01	0.97
	80:19:01	1.75
Wave length	221nm	0.42
	231nm	0.36

3.6 Method Suitability: The commercial tablet formulation, bisolvon-8, was analyzed by the proposed method and the average percent recovery was found to be 99.92. The value is in good agreement with the labeled amount, which confirms the suitability of the method for the analysis of bromhexin in pharmaceutical dosage forms.

4. CONCLUSION

The developed RP-HPLC method is simple, sensitive, precise and accurate and can be used for the estimation of bromhexin in the tablet dosage form for quality control analysis and the method is validated by ICH guidelines.

5. ABBREVIATIONS

HPLC- High performance liquid chromatography, µg- microgram, ml- milli litre, %- Percent.

6. ACKNOWLEDGEMENT

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