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RP-HPLC method development and validation for simultaneous estimation of bromhexine and ciprofloxacin in tablet dosage form

Sanjay S. Pekamwar*, Tukaram M. Kalyankar and Seema. S. Kokate

School of Pharmacy, S R T M University, Nanded(MS), India

ABSTRACT

Bromhexineis a mucolytic agent, secretolytic while Ciprofloxacin is abroad spectrum antibacterial agent, belonging to the group of fluoroquinolones, both the drugs available in tablet dosage form as BRO 8 mg and CIP 500 mg. The chromatographic separation was achieved on reversed-phase C_{18} column (5µ, 250 x 4.6 mm, i.d.) in the isocratic mode using methanol: 0.05 M phosphate buffer +0.5 mL tri-ethylamine + 0.5 mL tetra hydrofuran (80:20, v/v), at pH 3.8 adjusted with orthophosphoric acid as the mobile phase at a flow rate 0.8 ml/min. Retention times of BRO and SIP were 4.78 and 3.01 min respectively. Quantitation was achieved with PDA detection at 217 nm. The linearity of BRO and CIP was in the range of 5-30 µg/mL and 50-500 µg/mL respectively. A simple high performance liquid chromatography method is developed to the simultaneous determination of Bromhexine and Ciprofloxacin. Developed method is economical in terms of the time taken and amount of solvent consumed for each analysis. The method is validated and successfully applied to the simultaneous determination of Bromhexine and Ciprofloxacin in bulk and pharmaceutical formulations.

Keywords: Bromhexine, Ciprofloxacin, RP-HPLC, Validation.

INTRODUCTION

Bromhexine is chemically 2, 4-Dibromo-6-{[cyclohexyl (methyl) amino] methyl} aniline. It is a mucolyticagent, secretolytic, increasing the production of serous mucus in the respiratory tract and makes the phlegm thinner and less viscous. Bromhexine acts on the mucus-secreting cells. Bromhexine disrupts the structure of acid muco-polysaccharide fibres in mucoid sputum and produces less viscous mucus, which is easier to expectorate. This contributes to a secretomotoric effect by helping the cilia transport the phlegm out of the lungs [1-3].

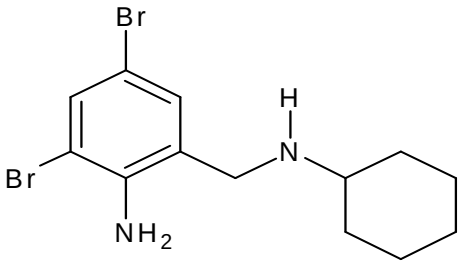

Figure 1: Structure of Bromhexine

Chemically Ciprofloxacin is (1cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7[1-piperazinyl]-3quinoline carboxylic acid) a broad spectrum antibacterial agent, belonging to the group of fluoroquinolones. Ciprofloxacin is active against a wide variety of gram-positive and gram-negative organism, use in the treatment of urinary tract infection, conjunctivitis, gonorrhea and respiratory tract infection [1-3].

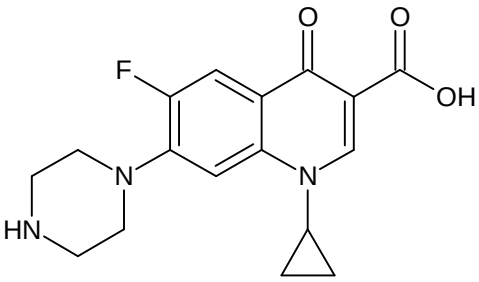

Figure 2: Structure of Ciprofloxacin

This paper is in continuation with our work [20-26], where we studied chromatographic method for single or multicomponent drugs. There are methods to estimate the drugs individually for Bromhexine and Ciprofloxacin or in combination with other drugs [4-17], but not a single method is reported for its simultaneous estimation. So here an attempt has been made to develop simple, accurate, sensitive, rapid and economic method for simultaneous determination of Bromhexine and Ciprofloxacin in combined dosage form using RP-HPLC. The proposed method was validated according to ICH guidelines [18, 19].

MATERIALS AND METHODS

Apparatus

The liquid chromatographic system of Perkin Elmer series 200 (Mumbai, India) containing quaternary gradient pump, variable wavelength programmable PDA detector, and auto sampler with 10 μL fixed loop wasused. For analysis a hypersil C18 column with 250 ×4.6 mm i.d. and 5 μm particle size was used as stationary phase.

Reagents and Materials

Pharmaceutical grade Bromhexine and Ciprofloxacin were pursued as a gift sample from Aarati Drug Limited (Mumbai), India. All chemicals and solvents of HPLC grade and were purchased from Rankem Pvt. Ltd., Mumbai, India. Marketed formulation Cinor BR Forte tablet containing Bromhexine 8 mg and Ciprofloxacin 500 mg was used as sample; purchased from local market (Orison Pharmaceutical, Himachal Pradesh).

Preparation of Mobile Phase and Stock Solution

Mobile phase was prepared by accurately weighing 1.360 g of potassium dihydrogen phosphate +0.5 mL triethylamine + 0.5 mL tetra hydrofuran and dissolving in 200 mL of water. It was mixed with 800 mL of methanol, and pH was adjusted to pH 3.8 using orthophosphoric acid (OPA). The solution was filtered through a 0.45 µ membrane filter. The solution was sonicated for 15 min for degassing prior to use.

Stock solutions were prepared by accurately weighing 10 mg of BRO and 100 mg of CIP and transferring to two separate 100 mL volumetric flasks containing 30 mL of mobile phase. The flasks were sonicated for 10 minutes to dissolve the solids. Volumes were made up to the mark with mobile phase, which gave 100 µg/mL and 1000 µg/mL of the BRO and CIP respectively. A figure 3 and 4 represent the typical chromatogram of standard Bromhexine and Ciprofloxacin respectively.

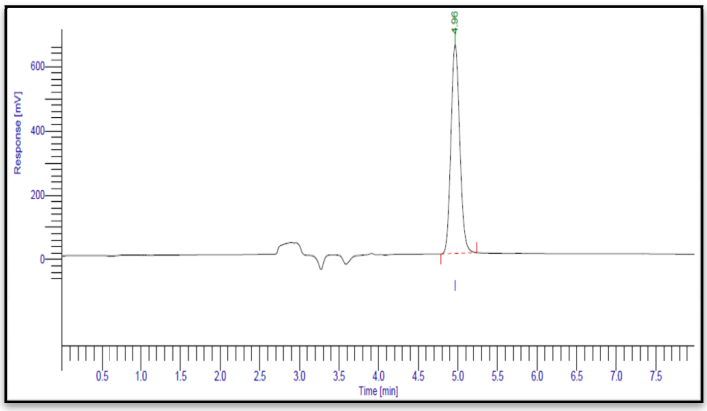

Figure 3: HPLC chromatogram of standard Bromhexine

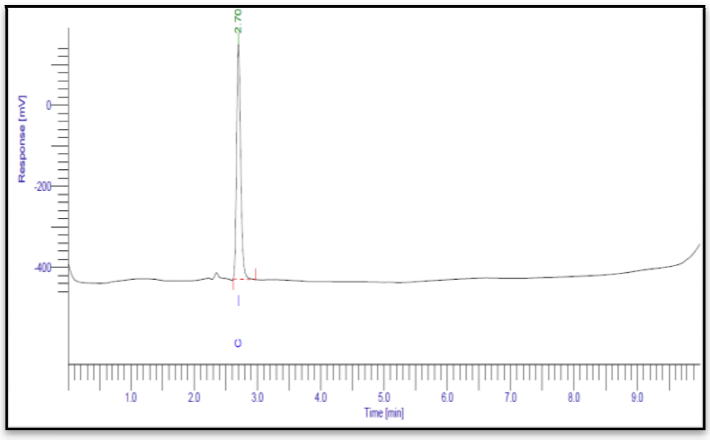

Figure 4: HPLC chromatogram of standard Ciprofloxacin

Chromatographic Conditions

The hypersil C_{18} column (5 μ × 250 mm × 4.6 mm) equilibrated with mobile phase Methanol: 0.05 M phosphate buffer: (0.5 mL triethylamine + 0.5 mL tetrahydrofuran) (80:20 v/v), pH 3.8 adjusted with orthophosphoric acid was used. The flow rate was maintained at 0.8 mL/min, eluents were monitored with PDA detector at 217 nm, and the injection volume was 10 μL. Total run time was kept for 10 min.

Method Validation

The method was validated for accuracy, precision, sensitivity, recovery, linearity and robustness. The method validation was performed as per ICH guidelines.

Linearity

Appropriate aliquots of the standard stock solutions of BRO and CIP were pipette out and transferred to a series of 10 mL volumetric flasks respectively. The volume was made up to the mark with mobile phase to obtain working standard solutions of BRO of concentrations 5 µg/mL to 30 µg/mL and for CIP of concentrations 50 µg/mL to 500 µg/mL. The calibration curves were found to be linear and in adherence to Beer's law over the concentration range of 5-30 µg/mL for BRO and 50-500 µg/mL for CIP. The solutions were injected using a 10 μL fixed loop system, and chromatograms were recorded. Calibration curves were constructed by plotting peak area Vs concentrations of the drug and regression equations were computed for BRO and CIP.

The standard calibration tables and graphs for BRO and CIP are shown in Table No. 1 and 2, Figure No. 6 and 7 respectively.

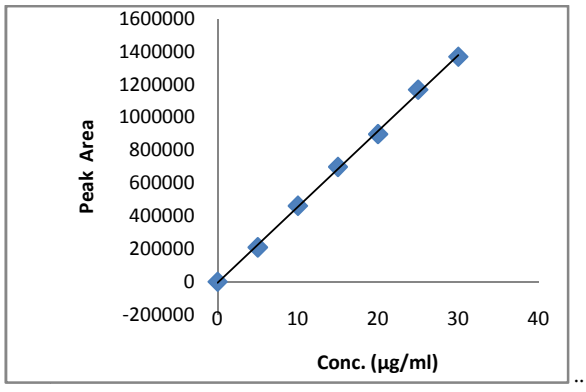

Figure 6: Calibration curve for BRO

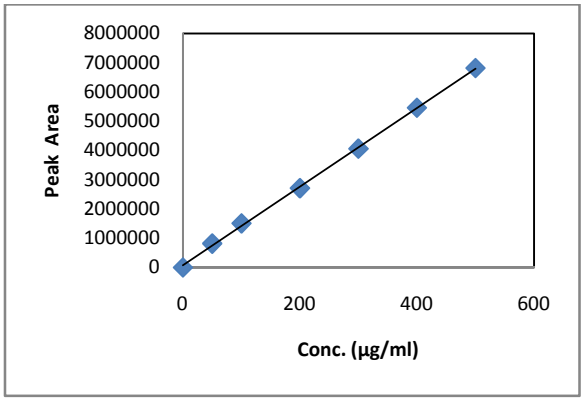

Figure 7: Calibration curve for CIP

Table 1: Calibration table for BRO

Sr. No.	Concentration of BRO (µg/ml)	Area*
1	5	209060
2	10	462337
3	15	698889
4	20	898798
5	25	1169122
6	30	1369705
Slope		46184
Y-intercept		5909
Correlation coefficient (r)		0.999

Table 2: Calibration table for CIP

Sr. No.	Concentration of CIP (µg/ml)	Area*
1	50	814121
2	100	1504122
3	200	2706521
4	300	4058040
5	400	5452783
6	500	6808989
Slope		13423
Y-intercept		77094
Correlation coefficient (r)		0.999

Precision

The repeatability studies were carried out by estimating response of BRO (20 μg/mL) and CIP (500 μg/mL) six times and results were reported in terms of relative standard deviation. The intraday and interday precision studies (intermediate precision) were carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for three different concentrations of BRO (10, 15, and 20 μg/mL) CIP (250, 375, and 500 μg/mL), and the results were reported in terms of relative standard deviation.

Accuracy

Accuracy was performed by recovery studies. The recovery studies were carried out at three concentration level 80%, 100%, 120% by standard addition method. The percentage recovery and standard deviation were calculated and reportedin table No. 3.

Sensitivity

The sensitivity of measurement for BRO and CIP was estimated in terms of the limit of quantitation (LOQ). The smallest amounts detected under the chromatographic conditions used were estimated in terms of the limit of detection (LOD). LOQ and LOD were calculated by use of the equations

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where σ is the standard deviation of the peak areas of the drugs, taken as a measure of noise, and S is the slope of the corresponding calibration curve.

Robustness

Robustness of the method was studied by deliberately changing the experimental conditions like flow rate, pH, temperature and percentage of mobile phase ratio. The study was carried out by changing 5% of the mobile phase ratio and 0.1 mL/min of flow rate.

Solution Stability

The solutions were prepared and solution stability was checked for 3, 9, 12, and 24 hrs by checking the area over the period of time, using the different analysts and the same instrument.

System Suitability

A system suitability test was an integral part of the method development to verify that the system is adequate for the analysis of BRO and CIP tobe performed. System suitability test of the chromatography system was performed before each validation run. Five replicate injections of a system suitability standard and one injection of a check standard were made. Area, retention time (R_t), tailing factor, asymmetry factor, and theoretical plates for the five suitability injections were determined.

Analysis of Marketed Formulation

Twenty tablets were accurately weighed and finely powdered. Tablet powder equivalent to 8 mg BRO, and 500 mg of CIP was taken in 100 mL volumetric flask and to this 12 mg of standard Bromhexine was added by standard addition method. Mobile phase (50 mL) was added to the above flask and the flask was sonicated for 15 minutes. The solution was filtered through 0.45 µm membrane filter paper and volume was made up to the mark with the mobile phase. Appropriate volume of the aliquot was transferred to a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase to obtain a solution containing 20 μg/mL of BRO and 500 μg/mL of CIP. The solution was sonicated for 10 min. It was injected as per the above chromatographic conditions and peak areas were recorded. The quantifications were carried out by keeping these values to the straight line equation of calibration curve.

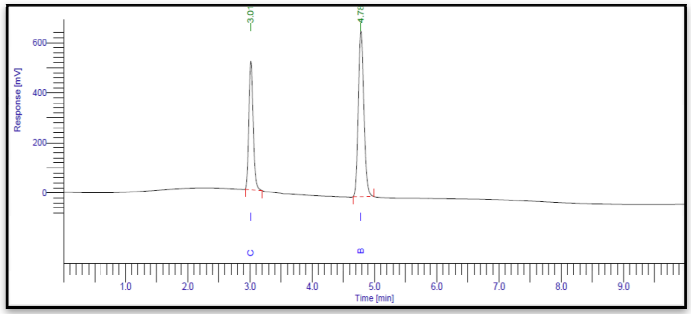

Figure 5: HPLC chromatogram for mixture of Bromhexine and Ciprofloxacin in tablet

RESULTS AND DISCUSSION

Optimization of Mobile Phase

Optimization of mobile phase was performed based on resolution of the drugs and degradation products, asymmetric factor, and theoretical plates obtained for BRO and CIP. The mobile phase consisting methanol: acetic acid, acetonitrile: potassium dihydrogen phosphate buffer, methanol: potassium dihydrogen phosphate buffer, methanol: ammonium acetate were tried in order to find the optimum conditions for the separation of BRO and CIP. After several trials, mobile phase of methanol:0.05M phosphate buffer + 0.5 ml triethylamine + 0.5 ml tetrahydrofuran in ratio (80:20 v/v), pH 3.8 adjusted with orthophosphoric acid was selected which gave sharp, well-resolved peaks for BRO and CIP (Figure 3,4).

The retention times for BRO and CIP were 4.78 and 3.01min, respectively. The asymmetric factors for BRO and CIP were 1.326 and 1.231, respectively.

Method Validation

The calibration curve for BRO was found to be linear in the range of 5-30 μg/mL with a correlation coefficient of 0.999. The calibration curve for CIP was found to be linear in the range of 50-500 μg/mL with a correlation coefficient of 0.999. Instrument precision was determined by performing injection repeatability test and the RSD values for BRO and CIP were found to be 0.079 % and 0.971%, respectively. The intraday and interday precision studies were carried out and the results are reported in Table 3. The low RSD value indicates that the method is precise.

The accuracy of the method was determined by calculating recoveries of BRO and CIP by method of standard addition. The recoveries were found to be 99.29-100.06 % and 99.14-99.95 % for BRO and CIP, respectively. The results are reported in Table 3.

Table 3: Summary of validation parameters

Parameters	BRO	CIP
Detection limit (μg/mL)	0.071	0.245
Quantitation limit (μg/mL)	0.236	0.810
Accuracy(%)	99.29-100.06%	99.14-99.95%
Precision (RSDa,%)		
Intraday precision (n = 3)	0.012-0.559%	0.156-0.776%
Interday precision (n = 3)	0.255-0.822%	0.292-0.513%
Instrument precision (RSD a)	0.079%	0.971%

[a]RSD is relative standard deviation and "n" is number of determinations.

The high values indicate that the method is accurate. The detection limits for BRO and CIP were found to be 0.071 µg/mL and 0.245 µg/mL respectively while quantitation limits were found to be 0.236 μg/mL and 0.810 μg/mL respectively. Robustness study was performed by deliberately changing the experimental conditions like flow rate from 0.6 mL/min to 0.8 mL/min and 1.0 mL/min. The composition of mobile phase was changed by varying the proportion of methanol by 2%, pH and temperature was changed. In both conditions the recoveries ofboth drugs were determined and the RSD was found to beless than 2%.The results are reported in Table 6.System suitability parameters such as the number of theoreticalplates, resolution, and tailing factor were determined.System suitability test was carried out and the results aresummarized in Table 4.

Table 4: System suitability test parameters for the proposed method

System suitability parameters	BRO	CIP
Retention time (min)	4.78	3.01
Theoretical plates/ meter	8987	6808
Asymmetric factor	1.326	1.231
Resolution	9.673	

Asymmetric factors for BRO and CIP are 1.326 and 1.231, respectively.

Stability of standard and sample solution of BRO and CIP were evaluated at room temperature. The solutionsof the two drugs were found to be stable for 0, 3, 6, 12 and24 hrs. The results are reported in Table 5.

Table 5: Solvent stability study

Time (Hrs.)	Area (n = 3)		Result %	
	BRO 20 (μg/mL)	CIP 500 (μg/mL)	BRO	CIP
0	898491	6808089	99.96	99.98
3	897042	6806891	99.80	99.96
6	893120	6780778	99.36	99.58
12	889921	6750472	99.01	99.14
24	879243	6724389	97.82	98.75

Two drugs were found to be stable with a recovery of more than 97%.

Analysis of Marketed Formulations

The proposed method was successfully applied to the determination of BRO and CIPin their combined dosage form. The % recovery± S.D. was found to be 99.77 ± 0.294 and 99.95±0.827, respectively, for BRO and CIP (Table 7) which were comparable with the corresponding labeled amounts.

Table 6: Data derived from robustness for proposed method

Parameters	Normal condition	Change in condition	Change in % RSD	
			BRO	CIP
Flow Rate	0.8 mL/min	0.6 mL/min	0.586	0.719
		1.0 mL/min	0.435	0.297
pH	3.8	3.6	0.769	0.594
		4.0	0.790	0.089
Mobile phase ratio	80:20	18:82	0.149	0.391
		22:78	0.776	0.596
Temperature	28 ^{O}C	26 ^{O}C	0.024	0.827
		30 ^{O}C	0.294	0.198

Table 7: Assay results of tablet dosage form using proposed method.

Formulations	Labelled amount (mg)		% Recovery [c]	
	BRO	CIP	BRO	CIP
Cinor BR Forte	8	500	99.77±0.294	99.95±0.827

[c] mean value ±standard deviation of determinations; Tablet formulation Cinor BR Forte (Orison Pharmaceutical, Himachal Pradesh) containing labeled amount of Bromhexine 8 mg and Ciprofloxacin 500 mg.

CONCLUSION

The objective of the work was to develop the simple, accurate, precise and sensitive HPLC method for the estimation of Bromhexine and Ciprofloxacin in bulk and multicomponent formulations. From the results obtained by all parameters, it is concluded that developed RP-HPLC method is suitable for the simultaneous estimation of Bromhexine and Ciprofloxacin in bulk and multicomponent formulation.

The concentration of BRO and CIP in pharmaceutical dosage form could be satisfactorily determined using isocratic RP-HPLC system with PDA detector.

This method has been found suitable for the routine analysis of pharmaceutical dosage forms in QC and R & D Laboratories for product of similar type and composition.

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