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High Performance Liquid Chromatographic Method for Determination of BromhexineHydrochloride in Pharmaceutical Syrups Sample

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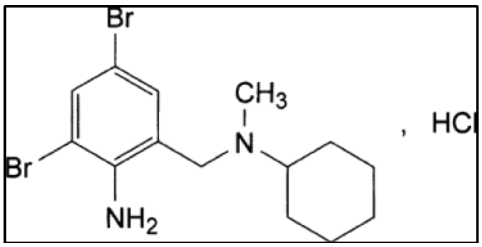
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Abstract: ***A simple, precise, rapid, and accurate high performance liquid chromatographic method has been developed for the determination of (BH.HCl) in pureform and in pharmaceutical formulations. The determination was carried out by M.N (C18) reversed-phase column (15cm × 4.6mm, 5 µm), using a mixture of buffer: acetonitrile: (20: 80 v/v) as a mobile phase at a flow rate of 1.5Ml.min^{1}. Detection was performed at 248 nm and at a ambient temperature. The retention time for (Rt) was found about 3.6 min. The calibration curve was over a concentration range linear from 10-70 ppm (r^2 = 0.9812). Limit of detection (LOD) and limit of quantification (LOQ) were found 0.13 ppm and 0.43 ppm respectively. The method was validated according to ICH guidelines for its, precision and accuracy. Recovery percentage (Rec%) was 103.2%andrelative standard deviation (RSD%) was precision to be 0.974% over the concentration range. The proposed method was successfully applied for the determination of (BH.HCl) in syrups.***

Keywords: High Performance Liquid Chromatography, Bromohexine Hydrochloride

1. Introduction

Bromhexine Hydrochloride is chemically known as1, N- (2-Amino-3, 5-dibromobenzyl) -N-methylcycloh exanamine hydrochloride figure [(1).]



Molecular formula: $C_{14}H_{20}Br_2N_2$, HCl = 412.6

Figure 1: Chemical structure of BromhexineHCl

It is a mucolytic used in the treatment of respiratory disorders associated with productive cough [(2).] It has been determined by different techniques including spectrophotometry [(3-5)] HPLC [(6-8),] colorimetry ([(9, 10),] TLC [(11),] Flow-injection spectrophotometry [(12),] Ion- Selective Electrode (ISE) [(13),] Hybrid Linear Analysis ([(14),] capillary isotachophoresis [(15),] Absorption Spectrophotometry and Electro-phoresis [(16, 17).]The present study aims to development a new high performance liquid chromatography (HPLC) method for determination of BH. HCl in pure and in different types of syrups.

2. Experimental

Reagents Chemicals

Methanol (GCC), Acetonitrile (GCC), Orthophosphoric acid (Flucka), Triethylamine (BDH), all the materials were HPLC grade.

Instrumentation

Chromatographic system consisted of a shimadzu HPLC model LC-20AD with UV detector model SPD-20A and C18 M.N column (15cm ×4.6mm, 5µm) was use to estimation of BH.HCL. HPLC condition are given in Table [1]

Preparation of standard stock solutions

One ml of 10 µg/ml of BH.HCl was Putled in a 10ml volumetric flask separately, dissolved in Methanol and the volume was made with Methanol to get final concentration of 1µg/ml.

Preparation of solutions for calibration curve

Aliquots volumes of Standard stock solution of BromhexineHCl were (0.1- 0.7ml) were diluted to 10 ml with Methanol. Further dilution to get solutions of final concentrations (10-70 ppm)

Preparation of solutions for standard addition method

Pipe 2.5 ml of sample was transferred to seven100 ml volumetric flasks add different volume of standard stock solution were add to each volumetric flask and completed the volumes to 100 ml with Methanol. to give 0- 60 ppm of standard solution.

Preparation of mobile phase

Buffer Solution:

0.05 ml of Orthophosphoric acid (85%) was added to 80 ml deionized water added and adjusted the pH to 7.0 (± 0.1) with Trimethylamine, the volume was completed to the mark with deionized water.

Mobile phase

Buffer – Acetonitrile (20: 80 v/v), were mixed and filtered through 0.45 µm membrane filter and sonicated on

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ultrasonic water bath.

Procedure

Procedure of pharmaceutical preparations (syrups) :
BH.HCl Syrup formulations (Solvodin 4mg/5ml produced by THE STATE COMPANY FOR DRUGS INDUSTRY AND MEDICAL COMPLIANCE SAMARRA) was selected the application of method 12.5 ml of sulvoden syrup was transferred to a volumetric flask and completed to the mark with methanol, and selected for analysis. The optimum conditions are given in Table (1)
For analysis of BH.HCL

Table 1: HPLC conditions

Column	M.N C18 (15cm×4.6mm), 5 µm
Wavelength	248 n.m
Mobile phase	Buffer – Acetonitrile (20 : 80 v/v)
Retention time	3.6 min.
Flow rate	1.5 ml/min
Temperature	Room temperature
Injection volume	20µL

3. Results and Discussion

RP- HPLC method for determition of Bromhexine hydrochloride was developed using C_{18} column. In order to determination, some chromatographic conditions were optimized as bellow

4. Optimum Conditions

1- Wavelength Selection
In order to obtain the better wavelength which gave the highest and narrower peak, many wave light were tested as in table 2, the wavelength 248 nm was selected because it gave optimal result as in fig. (1)

Table 2: λ_{max}

λ (nm)	Peak area	Rt min
220	1641.913	4.607
248	894.598	3.883
254	821.314	4.728
260	602.314	4.954

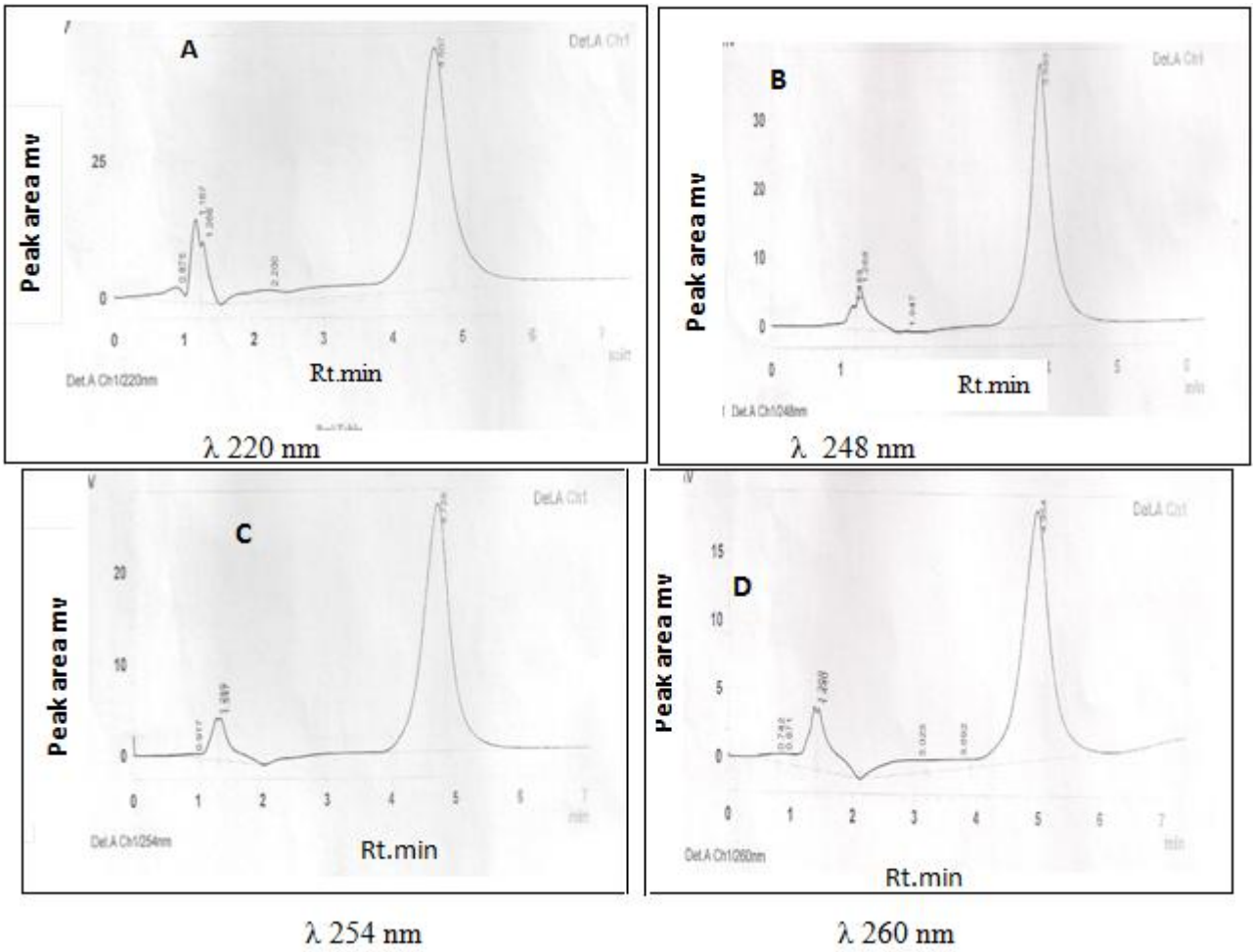


Figure 1: Chromatograms of selected Wavelength

2- Selection of Column
Through five columns were used as in table (3) and figure (2) The column $C_{18}$was selected the suitable Coloma, C_{18} gave lower retention time, while the column L_{10} didn't give any peak in the time program, so C_{18} was depended in the method.

Table 3: Selection of Column

Column	Peak area mv	Ret.Time (min.)
C 18	894.598	3.930
C 8	1075.662	9.226
L 3	4471.946	4.166 (Noise)
L 9	166.024	4.034 (Noise)
L 10	-	-

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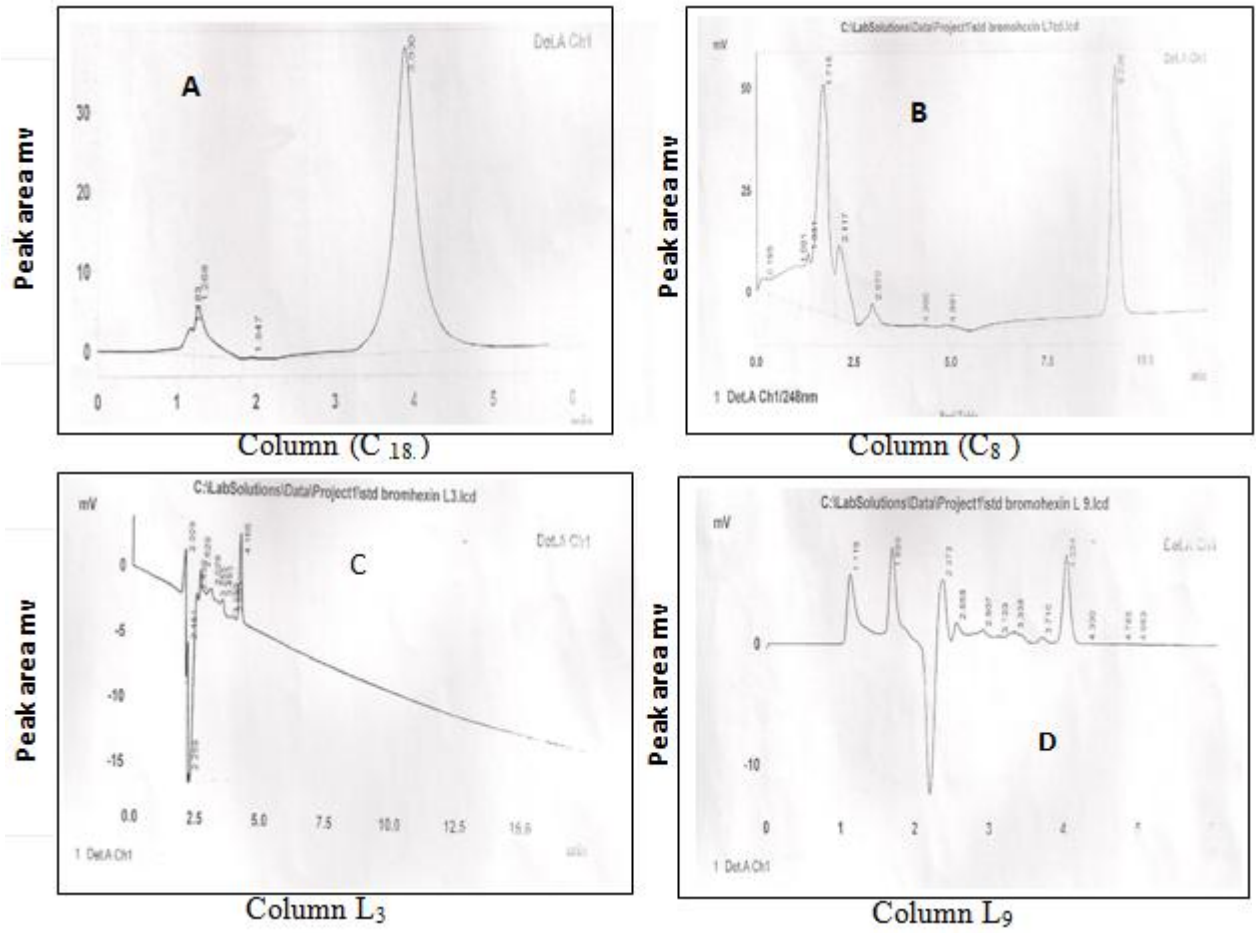


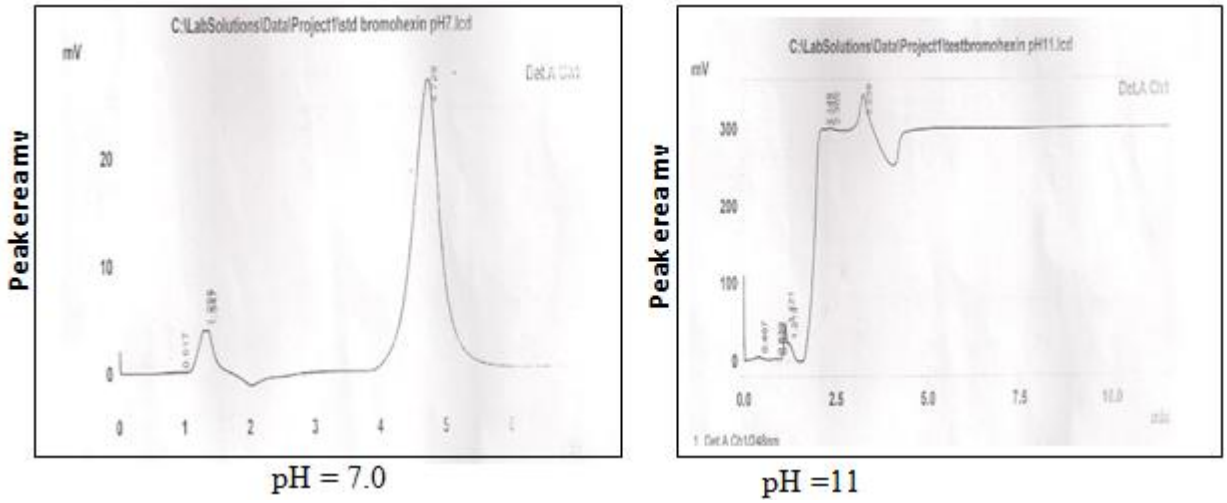
Figure 2: Chromatograms of selected the Column

3- Selection of the Buffer Solution pH

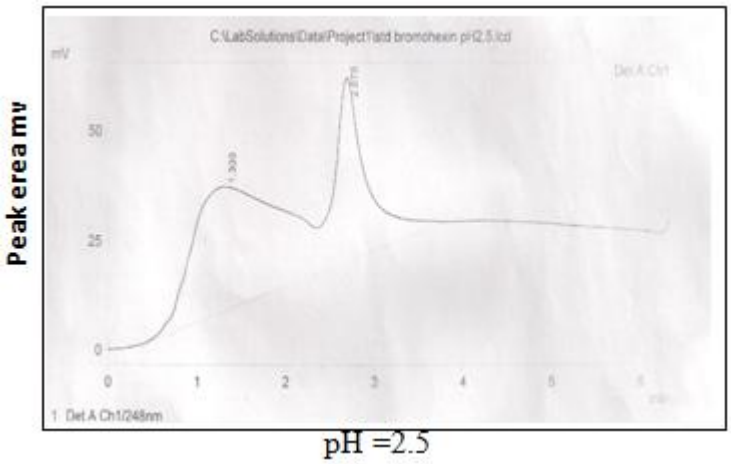
The pH of the Buffer Solution was investigate and pH =7 was found the beast media as in table 4 and Figure 3

Table 4: Selection of the pH Buffer

PH	Peak area	Rt. (min.)
2.5	821.314	3.407
7.0	933.600	4.728
11.0	Noise	Noise



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pH =2.5

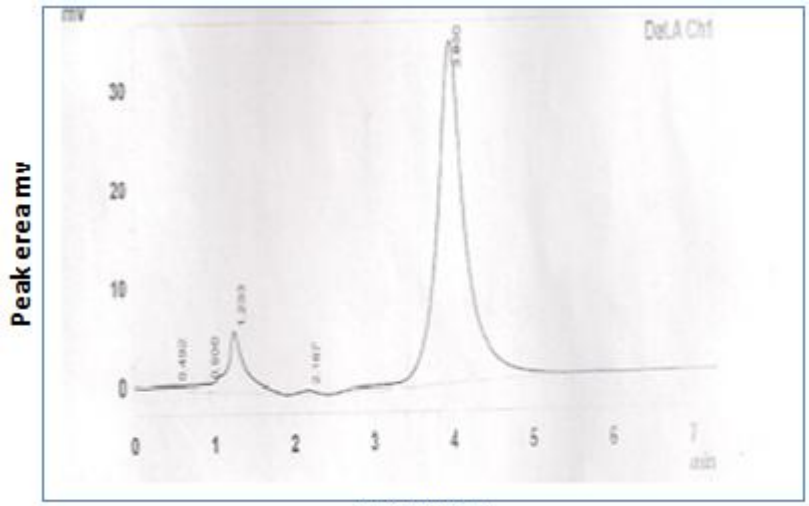
Figure 3: Chromatograms of different pH values

4- Selection of Mobile Phase Ratio

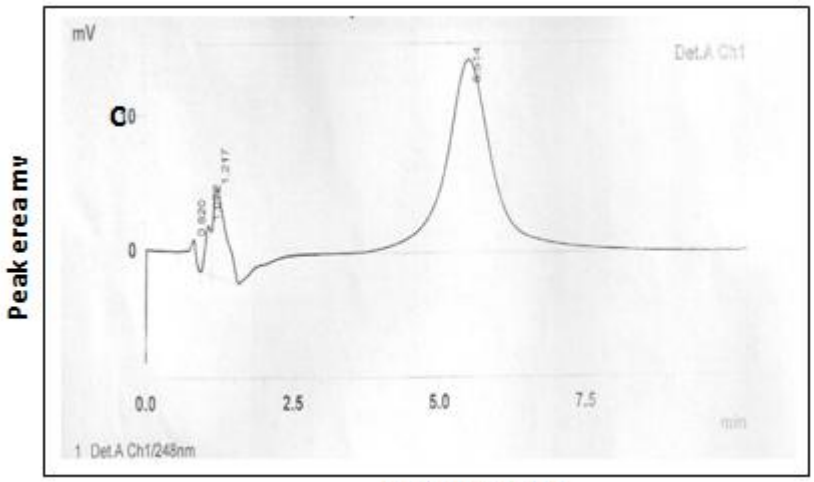
The ratio 20 of Buffer and 80 of Acetonitrile prefect peak occurred, the value given in Tablet (5) and fig. (4)

Table 5: Selection of Mobile Phase Ratio

Ratio Buffer : Acetonitrile	Peak area	Ret.Time (min.)
30 : 70	891.323	3.850
50 : 50	826.804	5.514
80 : 20	Noise	Noise

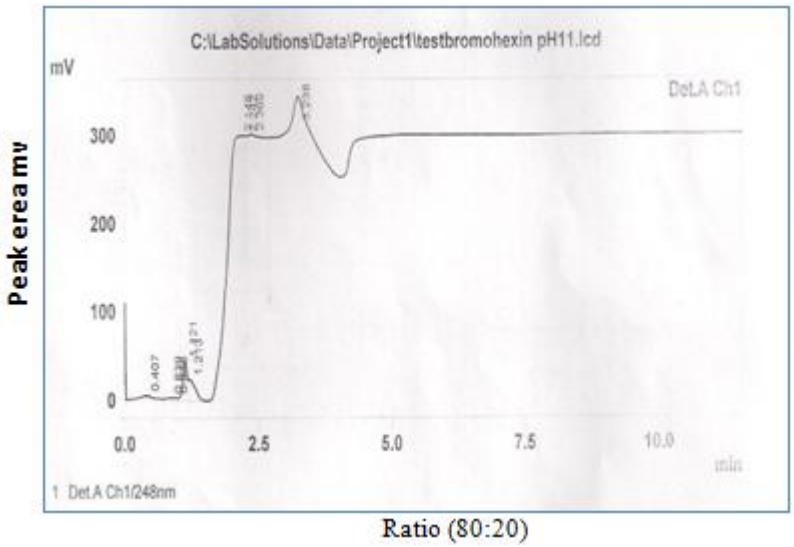


(30:70)Ratio



Ratio (50:50)

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Ratio (80:20)

Figure 4: Chromatograms of selected Mobile Phase Ratio

5- Selection of flow rate

The flow rate 1.5 ml/min occurred the peak a good retention time and good pressure, the value given in Tablet (6) and fig. (5)

Table 6: Selection the Flow Rate

ml/min	Peak area	Ret.Time (min.)
0.5	2510.488	11.243
0.8	2402.784	8.533
1.0	1663.630	7.037
1.5	834.884	3.698
2.0	689.734	3.413

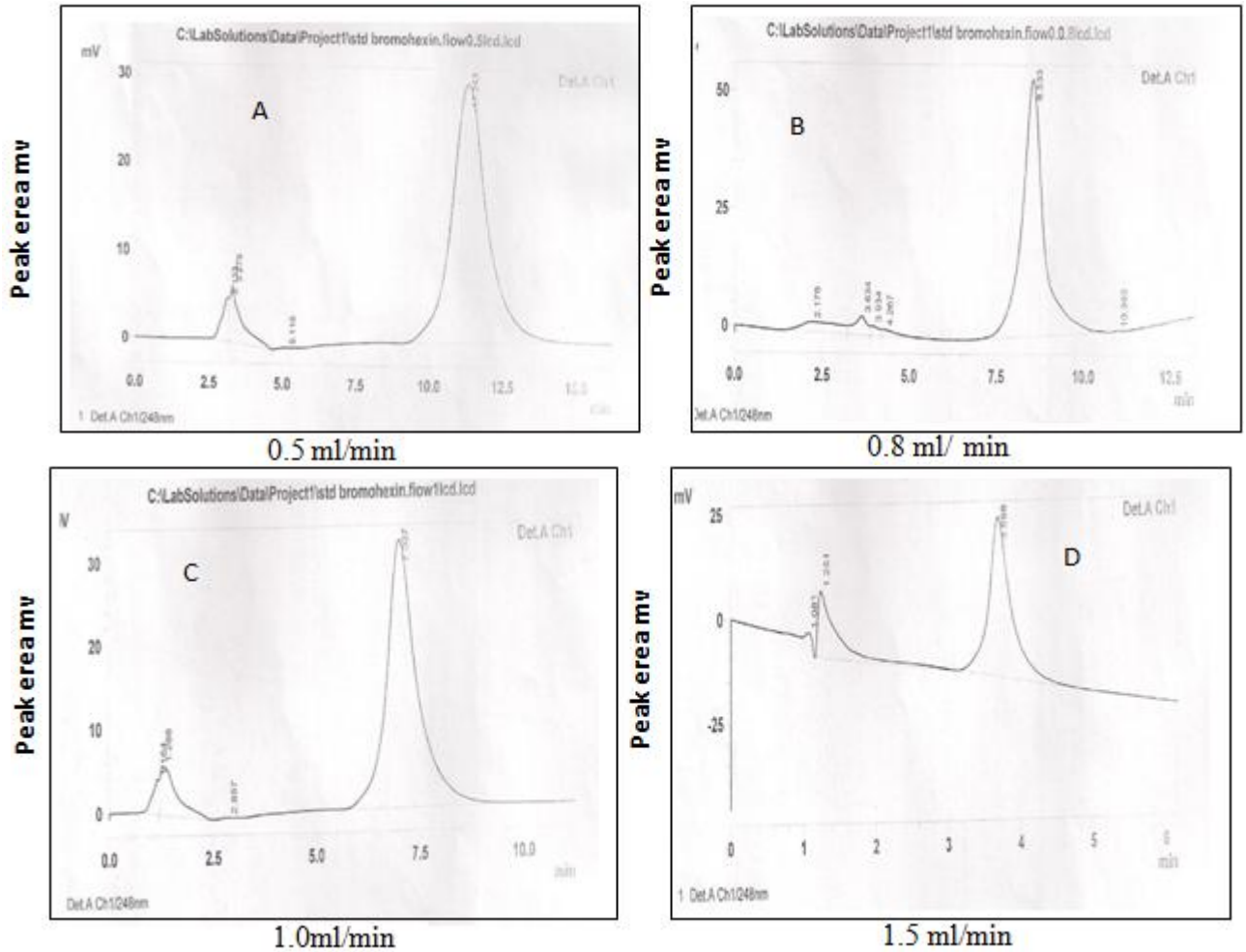


Figure 5: Chromatograms of selected Flow Rate

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Calibration Curve of HPLC method

Under the optimum experimental conditions, the analyte peaks were well defined and free from tailing. BH. HCl was determined by measuring the peak area. A plot of peak area against concentration gave a linear relationship (r^2=0.9812) over the concentration range 10-70 µg/ml Using regression analysis, the linear equation Y=21.939x+15.54 was obtained where Y is the mean peak area and X is the concentration in µg/ml fig (6) and the values given in Tablet (7)

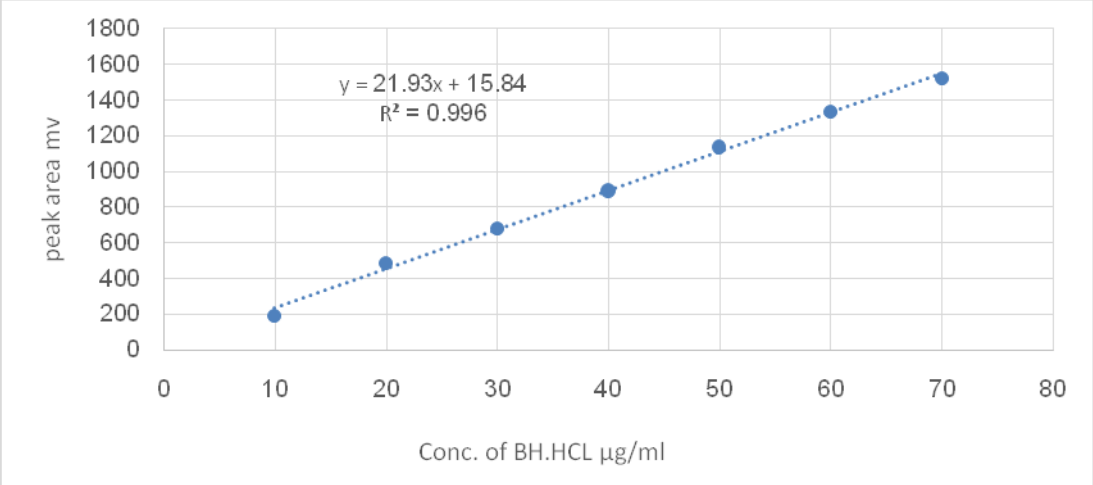
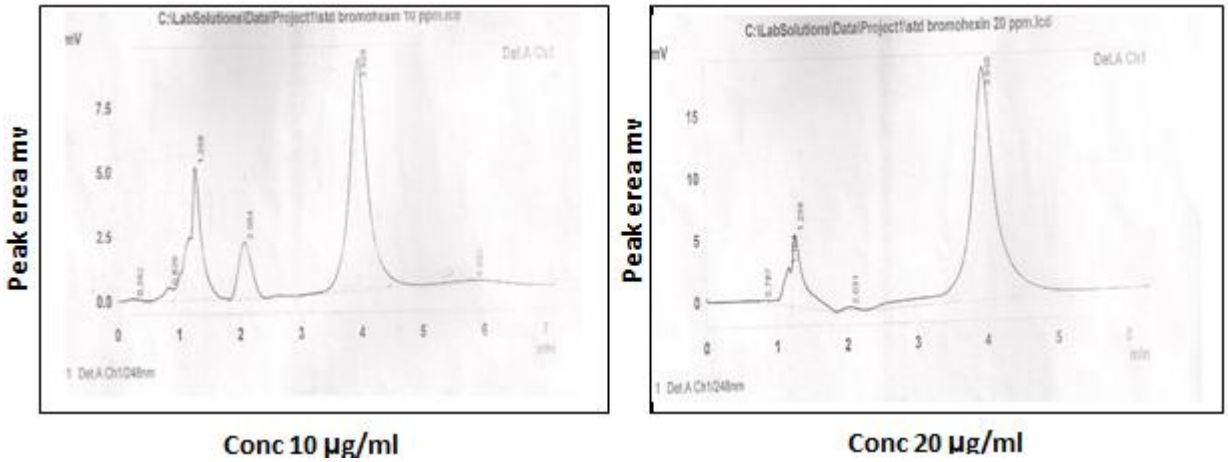


Figure 6: Calibration Curve of BH.HCL Over Concentration 10-70 µg/ml

Tablet 7: The value of Calibration graph

Conc. ppm	Peak area	Rt. (min.)
10	193.568	3.928
20	486.721	3.940
30	681.782	3.929
40	894.598	3.883
50	1137.54	3.793
60	1337.267	3.670
70	1522.224	3.796



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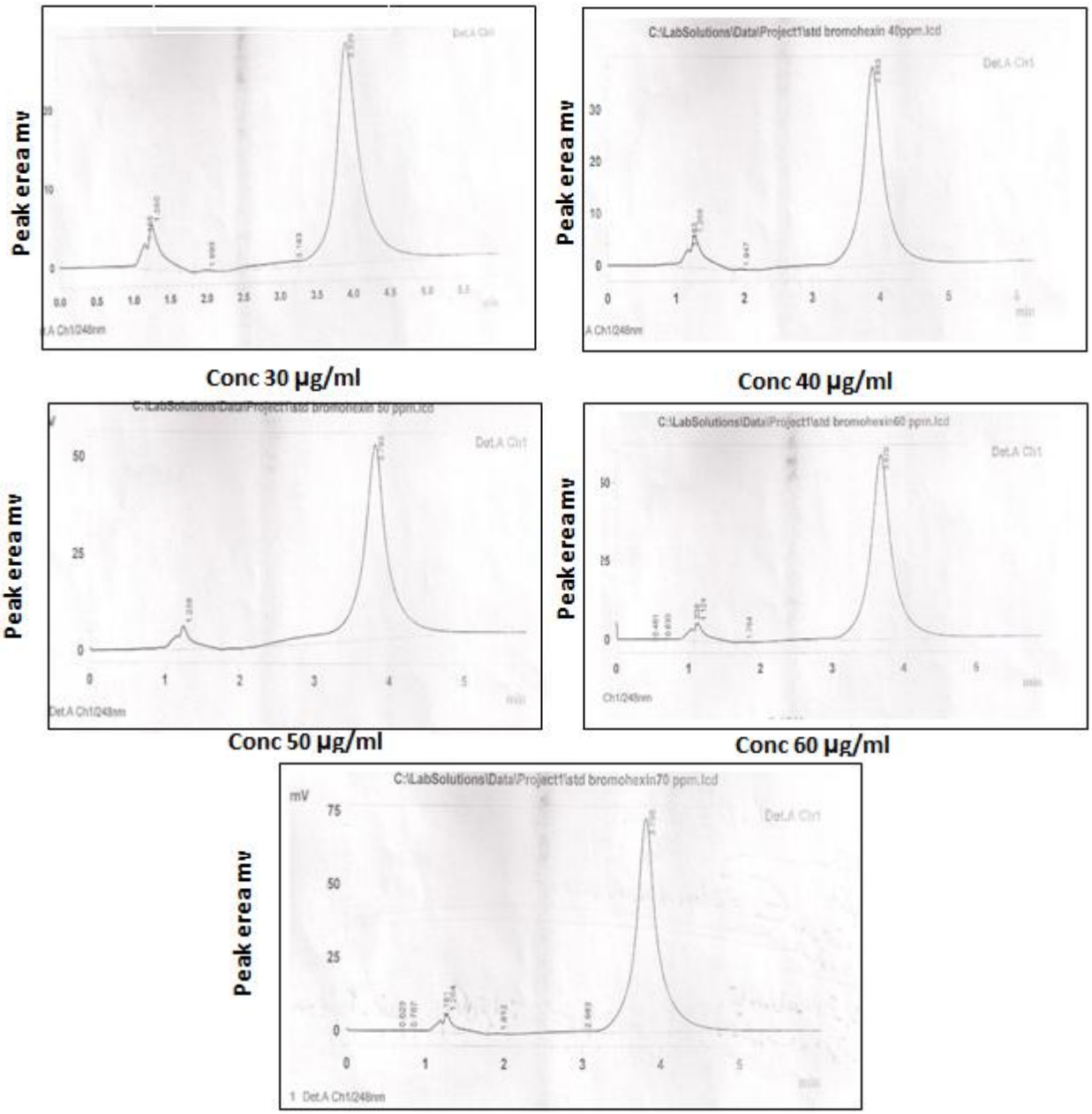


Figure 7: Calibration chart of HPLC

5. Standard Addition Method

The value of some pharmaceuticals is estimated by the method of standard additions and the table (8) shows the results obtained which showed the efficiency and success of the method used

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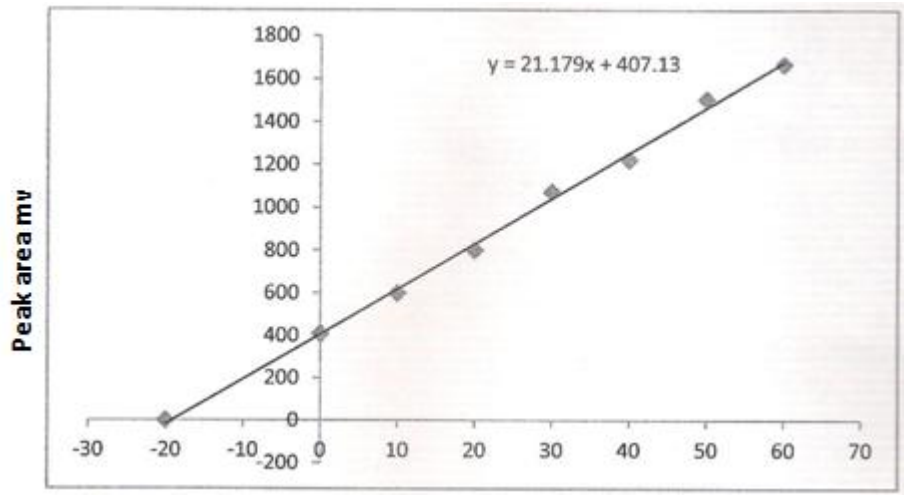
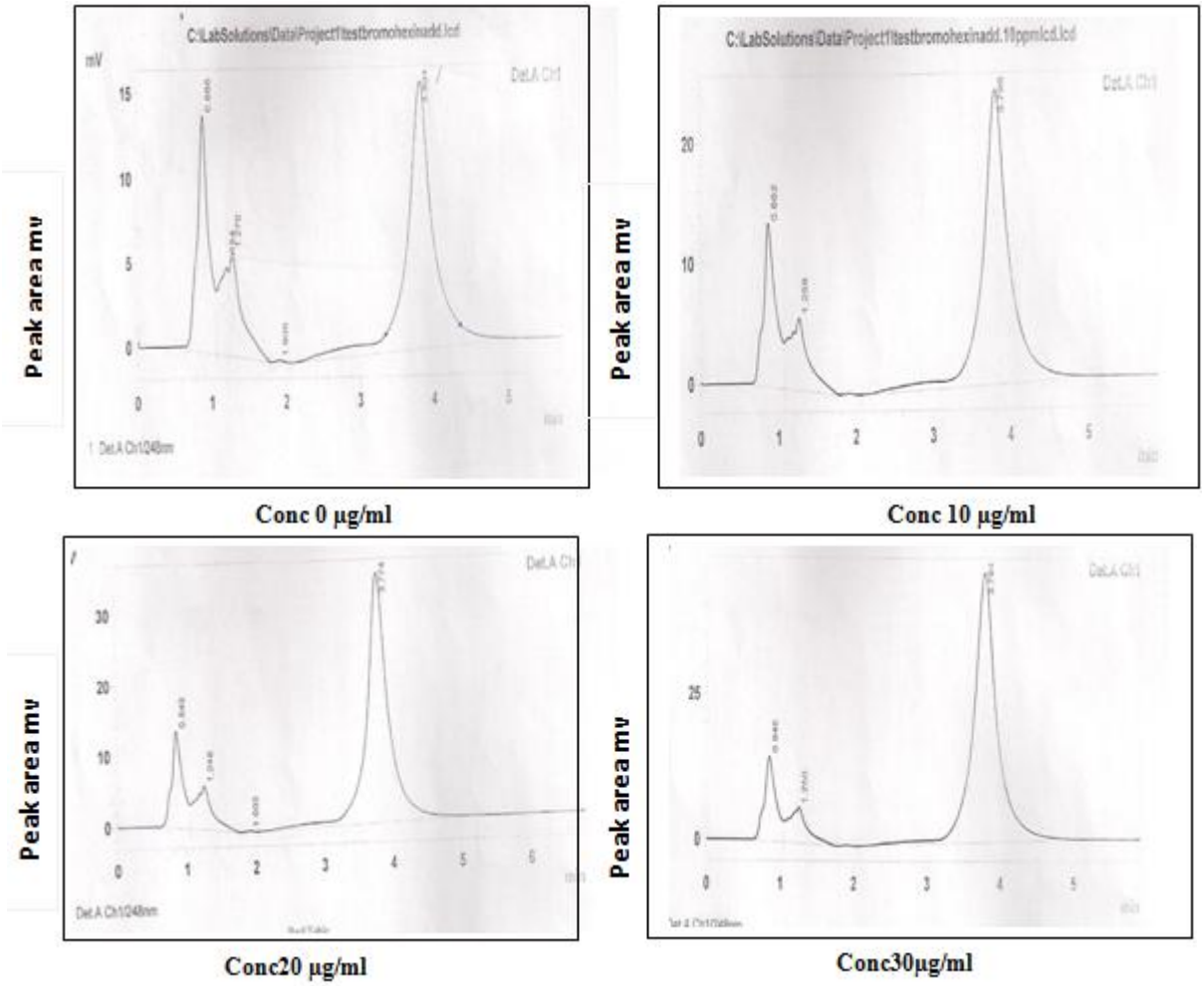


Figure 8: Standard Additions calibration of BH.H

Conc.	Std	10ppm	20ppm	30ppm	40ppm	50ppm	60ppm
Peak area	409.057	600.026	801.229	1072.475	1223.826	1506.332	1668.127



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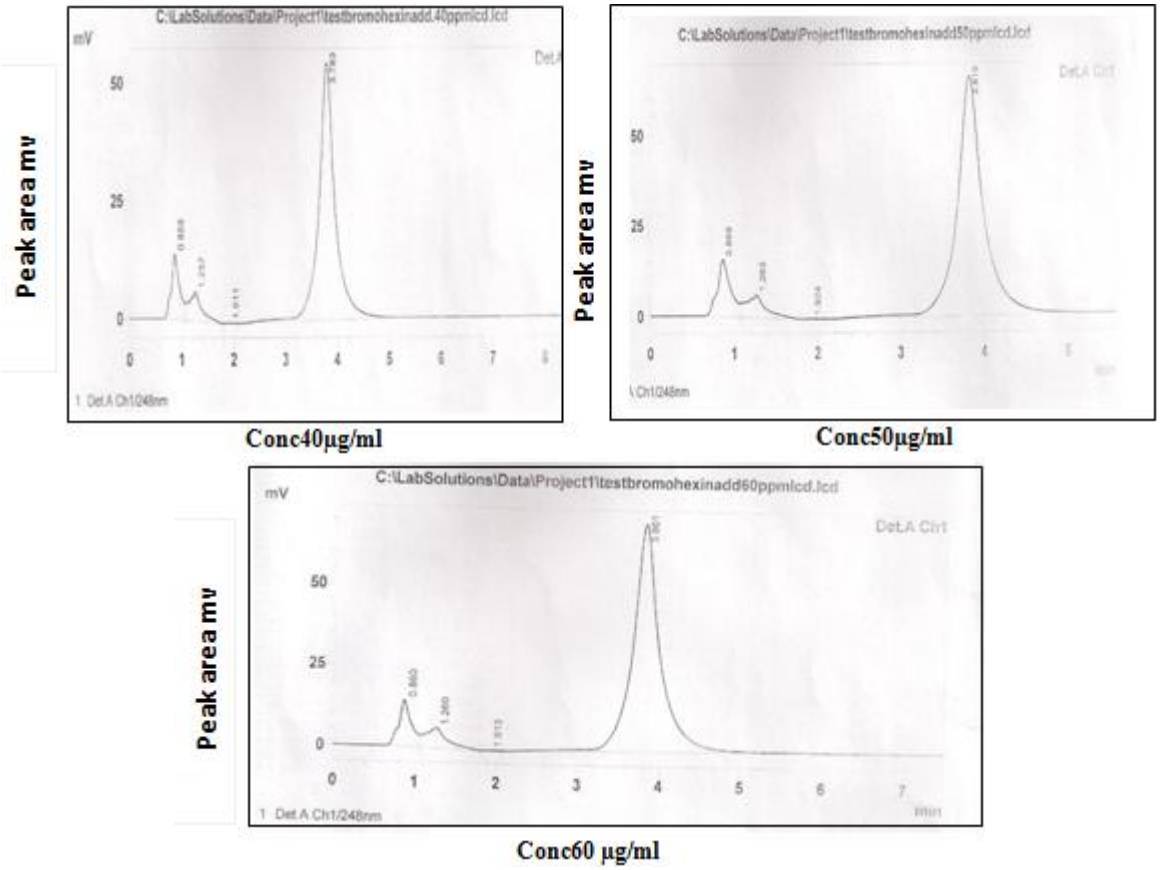


Figure 9: Standard Addition chart of HPLC

6. Conclusion

The method developed enables the quantification of Bromhexine HCl. The validation data demonstrate good precision and accuracy, which prove the reliability of the proposed method. Hence, this HPLC method can be used routinely for quantitative estimation of component in oral dosage form. High performance liquid chromatography (HPLC) can be used for determination of drugs and for purposes of control throughout the entire manufacturing process of drugs, as well as quality control of the finished product. It has the advantages of being sensitive, selective, rapid, accurate and reproducible.

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