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Journal of Chemical and Pharmaceutical Research, 2018, 10(7): 18-29

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

RP-HPLC Method Development and Validation for the Simultaneous Estimation of Diphenhydramine and Bromhexine in Tablet dosage forms

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Diphenhydramine and Bromhexine in Tablet dosage form. Chromatogram was run through Std Discovery 150 × 4.6 mm, 5m. Mobile phase containing Buffer 0.01N kh2po4: Acetonitrile taken in the ratio 50:50 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.01N kh2po4 buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 225 nm. Retention time of Diphenhydramine and Bromhexine were found to 2.458 min and 2.972. %RSD of the Diphenhydramine and Bromhexine were and found to be 0.5 and 0.3 respectively. %Recovery was obtained as 99.20% and 99.40% for Diphenhydramine and Bromhexine respectively. LOD, LOQ values obtained from regression equations of Diphenhydramine and Bromhexine were 0.07, 0.20 and 0.11, 0.33 respectively. Regression equation of Diphenhydramine is y = 9539x + 42940, and y = 9765x + 8034 of Bromhexine. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Diphenhydramine; Bromhexine; RP-HPLC; Validation.

INTRODUCTION

Diphenhydramine is a histamine H1 antagonist used as an antiemetic, antitussive, for dermatoses and pruritus, for hypersensitivity reactions, as a hypnotic, an antiparkinson, and as an ingredient in common cold preparations. It has some undesired antimuscarinic and sedative effects. Chemically diphenhydramine is [2-(diphenylmethoxy) ethyl] dimethylamine. Diphenhydramine competes with free histamine for binding at HA-receptor sites. This antagonizes the effects of histamine on HA-receptors, leading to a reduction of the negative symptoms brought on by histamine HA-receptor binding. [1-3].

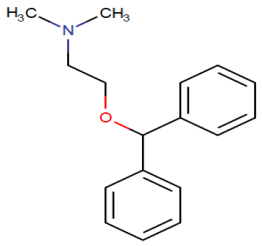

Figure 1: Structure of Diphenhydramine.

Bromhexine is an expectorant/mucolytic agent. Bromhexine is an oral mucolytic agent with a low level of associated toxicity. Bromhexine acts on the mucus at the formative stages in the glands, within the mucus-secreting cells.

Bromhexine disrupts the structure of acid mucopolysaccharide fibres in mucoid sputum and produces a less viscous mucus, which is easier to expectorate. Chemically Bromhexine is 2,4dibromo6 {[cyclohexyl(methyl)amino]methyl}aniline.[4-6].

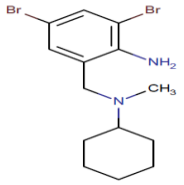

Figure 2: Structure of Bromhexine.

The literature review revealed that several analytical methods have been reported for Diphenhydramine and Bromhexine in UV-Spectrophotometry, RP-HPLC, individually and in combination. This research work implicates the simultaneous estimation of Diphenhydramine and Bromhexine by RP-HPLC in tablet dosage forms. This present study reports simultaneous estimation of Diphenhydramine and Bromhexine by RP-HPLC in tablet dosage form.

MATERIALS AND METHODS

Materials

Combination Diphenhydramine and Bromhexine tablets (Histachlor Oyster Labs Limited) received from spectrum lab, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents were obtained from Rankem Laboratories Pvt Ltd [7-10].

Instruments

Electronics Balance-Denver, pH meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Diphenhydramine and Bromhexine solutions.

Methods

Diluent

Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions

Accurately weighed 25mg of Diphenhydramine, 8mg of Bromhexine and transferred to 10ml 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. (2500µg/ml of Diphenhydramine and 800µg/ml Bromhexine)

Preparation of Standard working solutions (100% solution)

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (250µg/ml of Diphenhydramine and 80µg/ml of Bromhexine)

Preparation of Sample stock solutions

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 10ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (2500µg/ml of Diphenhydramine and 800µg/ml of Bromhexine)

Preparation of Sample working solutions (100% solution)

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (250µg/ml of Diphenhydramine and 80µg/ml of Bromhexine)

Preparation of buffer

0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Buffer: 0.01N Potassium dihyrogen ortho phosphate

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.0 with dil. Orthophosphoric acid solution.

Method development was done by changing various, mobile phase ratios, buffers etc.

RESULT AND DISCUSSION

Optimized method

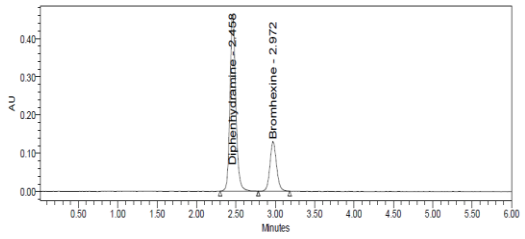

Figure 3: Optimized Chromatogram

Observation

Diphenhydramine and Bromhexine were eluted at 2.458 min and 2.972 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated [11-13].

System suitability

All the system suitability parameters were within the range and satisfactory as per ICH guidelines

Table: 1 System suitability parameters for Diphenhydramine and Bromhexine

S no	Diphenhydramine			Bromhexine		
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing
1	2.456	5716	1.16	2.969	6061	1.11
2	2.456	5716	1.16	2.969	6061	1.11
3	2.458	5769	1.18	2.972	6370	1.1
4	2.458	5757	1.17	2.972	6386	1.09
5	2.465	5673	1.13	2.978	6229	1.1
6	2.465	5679	1.13	2.978	6229	1.1

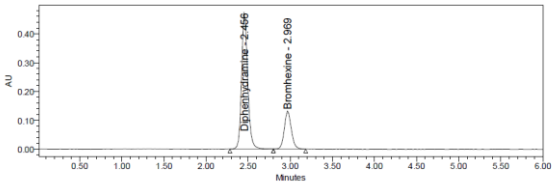

Figure 4: System suitability Chromatogram

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Specificity

Retention times of Diphenhydramine and Bromhexine were 2.458 min and 2.972 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

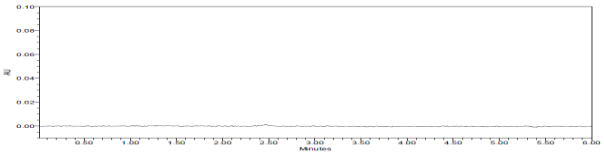

Figure 5: Chromatogram of blank

Linearity

Six linear concentrations of Diphenhydramine (62.5-375/ml) and Bromhexine (20-120µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Diphenhydramine was y = 9539.x +42940 and of Bromhexine was y = 9765x + 8034 Correlation coefficient obtained was 0.999 for the two drugs.

Table 2: Linearity table for Diphenhydramine and Bromohexine

Diphenhydramine		Bromhexine	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
62.5	653277	20	207024
125	1283232	40	414399
187.5	1849097	60	594388
250	2396559	80	767086
312.5	3029852	100	994188
375	3609261	120	1180466

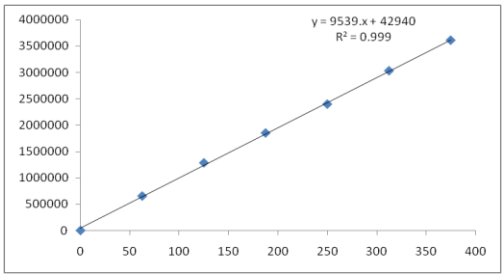

Figure 6: Calibration curve of Diphenhydramine

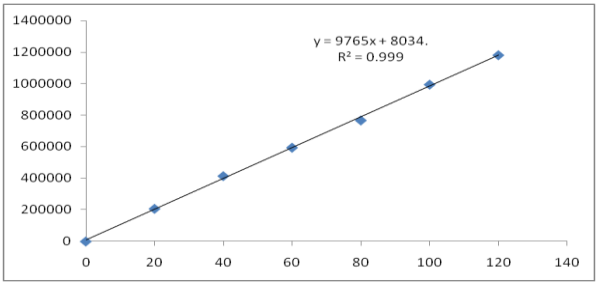

Figure 7: Calibration curve of Bromhexine

Precision

System Precision: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.3% 0.2% respectively for Diphenhydramine and Bromhexine. As the limit of Precision was less than "2" the system precision was passed in this method (Figure 1- Figure 7).

Table 3: System precision table of Diphenhydramine and Bromhexine

S. No	Area of Diphenhydramine	Area of Bromhexine
1	2389976	760114
2	2382256	761254
3	2370867	763684
4	2385746	762691
5	2388631	763872
6	2380954	764192
Mean	2383072	762635
S.D	6925.8	1632.2
%RSD	0.3	0.2

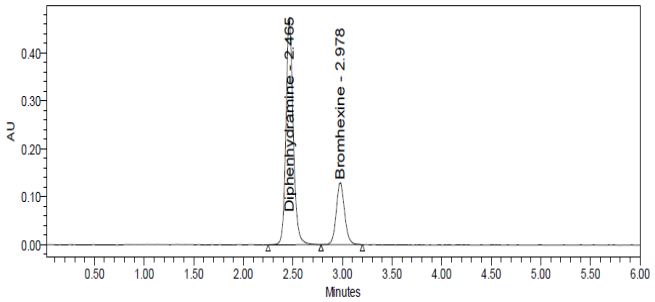

Figure 8: System precision chromatogram

Repeatability

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 0.3% respectively for Diphenhydramine and Bromhexine. As the limit of Precision was less than "2" the system precision was passed in this method.

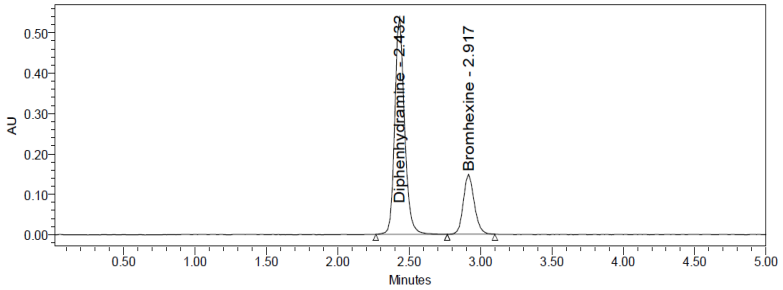

Figure 9: Repeatability chromatogram

Table 4: Repeatability table of Diphenhydramine and Bromhexine

S. No	Area of Diphenhydramine	Area of Bromhexine
1	2375405	760462
2	2361582	760702
3	2352717	762728
4	2388878	762293
5	2375021	761496
6	2365100	766017
Mean	2369784	762283
S.D	12681.5	2028.6
%RSD	0.5	0.3

Intermediate precision (Day_ Day Precision)

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 1.2% and 0.3% respectively for Diphenhydramine and Bromhexine. As the limit of Precision was less than "2" the system precision was passed in this method.

Table 5: Intermediate precision table of Diphenhydramine and Bromhexine

S. No	Area of Diphenhydramine	Area of Bromhexine
1	2295635	765308
2	2251596	760614
3	2287512	761673
4	2304762	760091
5	2301136	761269
6	2336636	760082
Mean	2296213	761506
S.D	27561.1	1968
%RSD	1.2	0.3

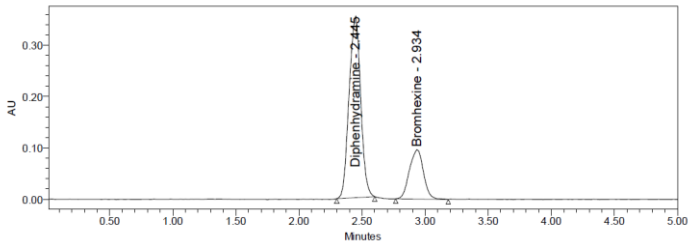

Figure 10: Inter Day precision Chromatogram

Accuracy

Three levels of Accuracy samples were prepared by standard addition method (Figure 8-Figure 14). Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.20% and 99.40% for Diphenhydramine and Bromhexine respectively (Table 1-Table 4).

Table 6: Accuracy table of Diphenhydramine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	125	123.46	98.77	99.20%
	125	124.53	99.63	
	125	124.96	99.97	
100%	250	247	98.8	
	250	247.7	99.08	
	250	247.07	98.83	
150%	375	371.87	99.17	
	375	372.19	99.25	
	375	372.48	99.33	

Table 7: Accuracy table of Bromhexine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	40	39.91	99.76	99.40%
	40	40.2	100.5	
	40	39.27	98.19	
100%	80	78.85	98.56	
	80	79.45	99.31	
	80	79.41	99.26	
150%	120	118.91	99.09	
	120	119.82	99.85	
	120	120.04	100.04	

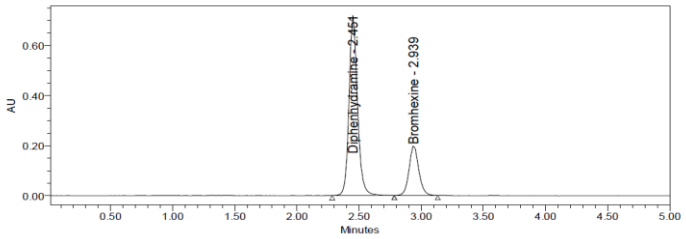

Figure 11: Accuracy 50% Chromatogram of Diphenhydramine and Bromhexine

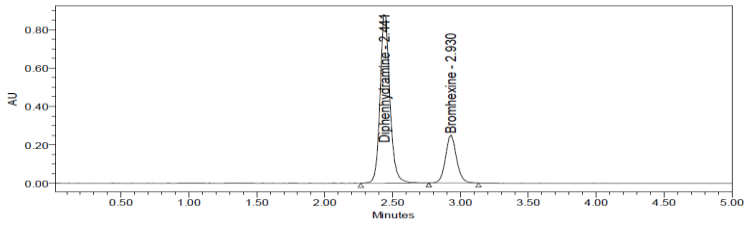

Figure 12: Accuracy 100% Chromatogram of Diphenhydramine and Bromhexine

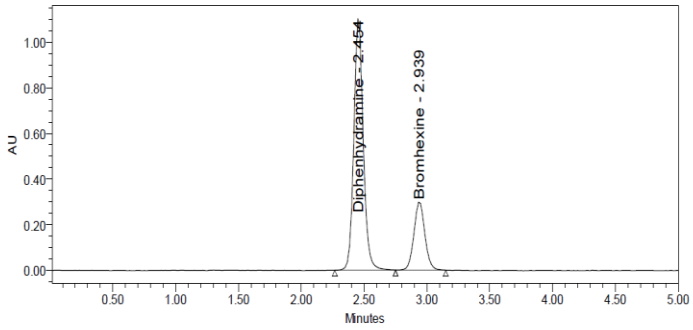

Figure 13: Accuracy 150% Chromatogram of Diphenhydramine and Bromhexine

Sensitivity

Table 8: Sensitivity table of Diphenhydramine and Bromhexine

Molecule	LOD	LOQ
Diphenhydramine	0.07	0.2
Bromhexine	0.11	0.33

Robustness

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55B:45A), mobile phase plus (45B:55A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table 5-Table 8).

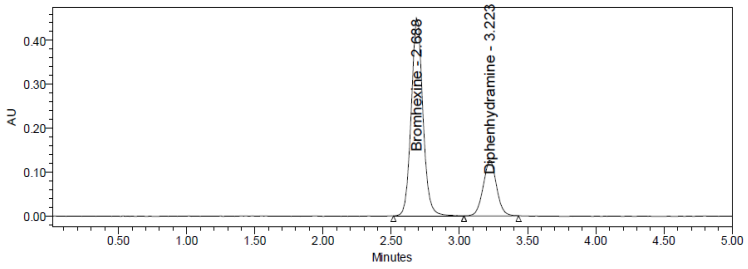

Figure 14: Flow minus Chromatogram of Diphenhydramine and Bromhexine

Table 9: Robustness data for Diphenhydramine and Bromhexine

S.no	Condition	%RSD of Diphenhydramine	%RSD of Bromhexine
1	Flow rate (-) 1.1ml/min	0.2	0.5
2	Flow rate (+) 1.3ml/min	0.5	0.5
3	Mobile phase (-) 55B:45A	0.4	0.8
4	Mobile phase (+) 45B:55A	0.7	1.1
5	Temperature (-) 25°C	0.3	0.6
6	Temperature (+) 35°C	1.2	1

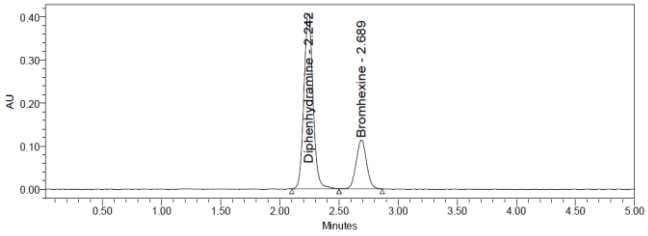

Figure 15: Flow plus Chromatogram of Diphenhydramine and Bromhexine

Assay

Oyster Labs Limited, bearing the label claims Diphenhydramine 25mg, Bromhexine 8mg (Histachlor). Assay was performed with the above formulation. Average % Assay for Diphenhydramine and Bromhexine obtained was 99.24and 99.75% respectively.

Table 10: Assay Data of Diphenhydramine

S.no	Standard Area	Sample area	% Assay
1	2389976	2375405	99.48
2	2382256	2361582	98.9
3	2370867	2352717	98.53
4	2385746	2388878	100.04
5	2388631	2375021	99.46
6	2380954	2365100	99.05
Avg	2383072	2369784	99.24
Stdev	6925.8	12681.5	0.53
%RSD	0.3	0.5	0.54

Table 11: Assay Data of Bromhexine

S.no	Standard Area	Sample area	% Assay
1	760114	760462	99.52
2	761254	760702	99.55
3	763684	762728	99.81
4	762691	762293	99.76
5	763872	761496	99.65
6	764192	766017	100.24
Avg	762635	762283	99.75
Stdev	1632.2	2028.6	0.3
%RSD	0.2	0.3	0.3

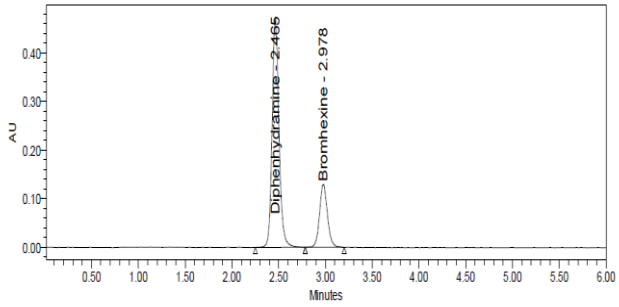

Figure 16: Chromatogram of working standard solution

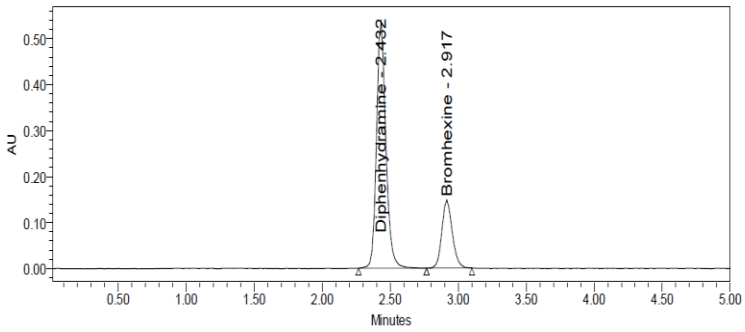

Figure 17: Chromatogram of working sample solution

DEGRADATION

Degradation Studies

Degradation studies were performed with the formulation and the degraded samples were injected (Figure 15-Figure 21). Assay of the injected samples was calculated and all the samples passed the limits of degradation (Table 9-Table 13).

Table 12: Degradation Data of Diphenhydramine

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.77	0.159	0.361
2	Alkali	2.73	0.131	0.335
3	Oxidation	1.89	0.306	0.327
4	Thermal	0.97	0.159	0.358
5	UV	0.58	0.128	0.327
6	Water	0.64	0.306	0.325

Table 13: Degradation Data of Bromhexine

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.86	1.01	1.266
2	Alkali	2.9	0.781	0.971
3	Oxidation	1.88	0.73	0.903
4	Thermal	1	0.936	1.202
5	UV	0.81	0.764	0.956
6	Water	0.73	0.717	0.889

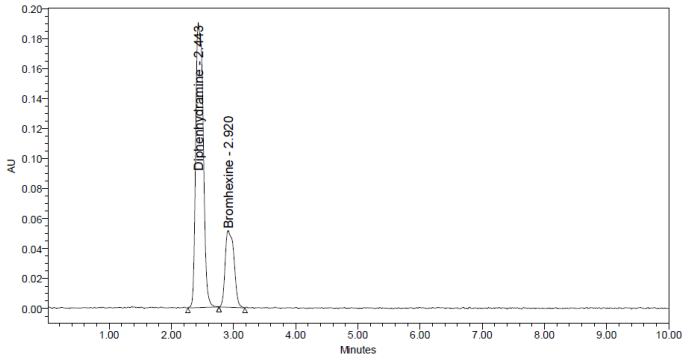

Figure 18: Acid chromatogram of Diphenhydramine and Bromhexine

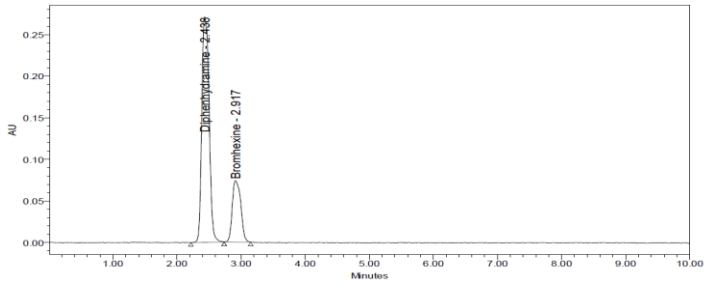

Figure 19:Base chromatogram of Diphenhydramine and Bromhexine

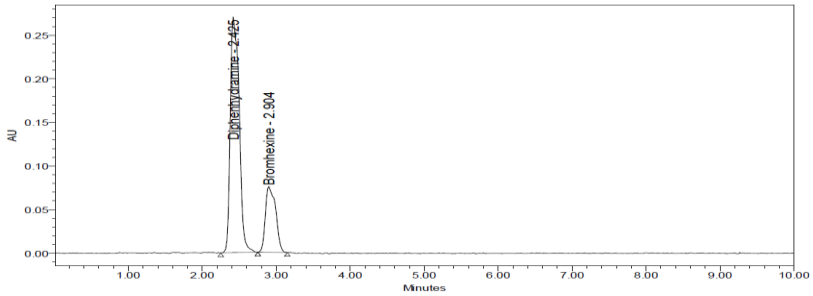

Figure 20: Peroxide chromatogram of Diphenhydramine and Bromhexine

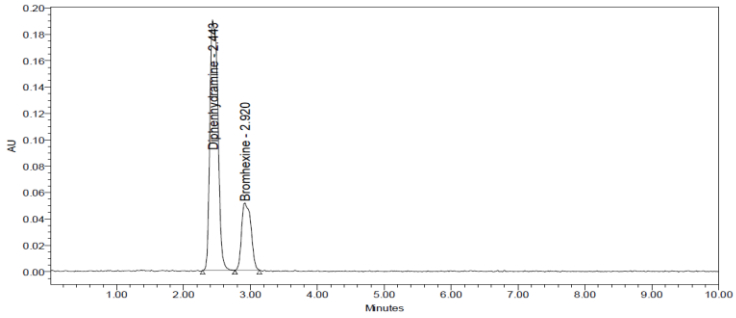

Figure 21: Thermal chromatogram of Diphenhydramine and Bromhexine

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Diphenhydramine and Bromhexine in Tablet dosage form. Retention time of Diphenhydramine and Bromhexine were found to be 2.458 min and 2.972. %RSD of the Diphenhydramine and Bromhexine were and found to be 0.5 and 0.3 respectively. %Recovery was obtained as 99.20% and 99.40% for Diphenhydramine and Bromhexine respectively. LOD, LOQ values obtained from regression equations of Diphenhydramine and Bromhexine were 0.07, 0.20 and 0.11, 0.33 respectively. Regression equation of Diphenhydramine is y = 9539x + 42940, and y = 9765x + 8034 of Bromhexine. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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