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Simultaneous determination of bromhexine hydrochloride and salbutamol sulphate in pharmaceutical dosage by reverse phase high performance liquid chromatography

Rajan V. Rele.

Central research laboratory, D.G. Ruparel College, Matunga, Mumbai

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ABSTRACT

A simple, rapid and accurate high performance liquid chromatography method is described for simultaneous determination of bromhexine hydrochloride and salbutamol sulphate from active pharmaceutical ingredients. The separation of drug was achieved on Zorbax Eclipse C18 (250 x 4.6 mm i.d.) with 5 µ particle size column showed most favorable chromatographic pattern over the other columns. The mobile phase consisted of a mixture of buffer and acetonitrile (65:35 % (v/v)). The buffer was mixtures of 0.1 % (v/v) tri-ethyl amine solution adjusted the pH 3.0 with ortho-phosphoric acid. The detection was carried out at wavelength 225 nm. The mixture of buffer of pH 3.0 and acetonitrile (65:35% v/v) was used as a diluent. The method was validated for system suitability, linearity, accuracy, precision, robustness, stability of sample solution. The method has been successfully used to analyze bromhexine hydrochloride from active pharmaceutical ingredients.

Keywords: Bromhexine hydrochloride, Salbutamol sulphate, Acetonitrile, tri-ethyl amine, ortho phosphoric acid.

INTRODUCTION

Bromhexine Hydrochloride is chemically named 2-amino-3,5- dibromo-N-cyclohexyl-N-methyl benzenemethanamine hydrochloride, is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus . The drug is official in IP [1] and BP [2].

Salbutamol sulphate is, chemically known as bis [(1RS)-2-[(1, 1-Di-methyl-ethyl) amino]-1-[4-hydroxy-3-(hydroxyl methyl) phenyl] ethanol] sulphate. It is beta-adrenoceptor agonist used for the relief of broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease. The drug is official in Indian pharmacopoeia [1]. Literature survey reveals HPLC [3] method for simultaneous determination of bromhexine hydrochloride and salbutamol sulphate in combined dosage form. Combination of bromhexine hydrochloride and salbutamol sulphate is used for the treatment of asthma and bronchitis.

MATERIALS AND METHODS

Chemical and reagents

Reference standard of bromhexine hydrochloride was obtained from reputed firm with certificate of analysis. Tri-ethylamine, acetonitrile and ortho phosphoric acid were used of analytical grade and the HPLC grade water was

used from Millipore. Standard and sample solutions were prepared in diluent [mixture of buffer of pH 3.0 and acetonitrile (65:35 %(v/v)].

Instrumentation
The HPLC system used was MERCK Hitachi HPLC system equipped with auto sampler (D 7200 separation module) and UV detector (D- 7400). The chromatogram was recorded and peaks quantified by means of PC based EZ Chrom Elite software.

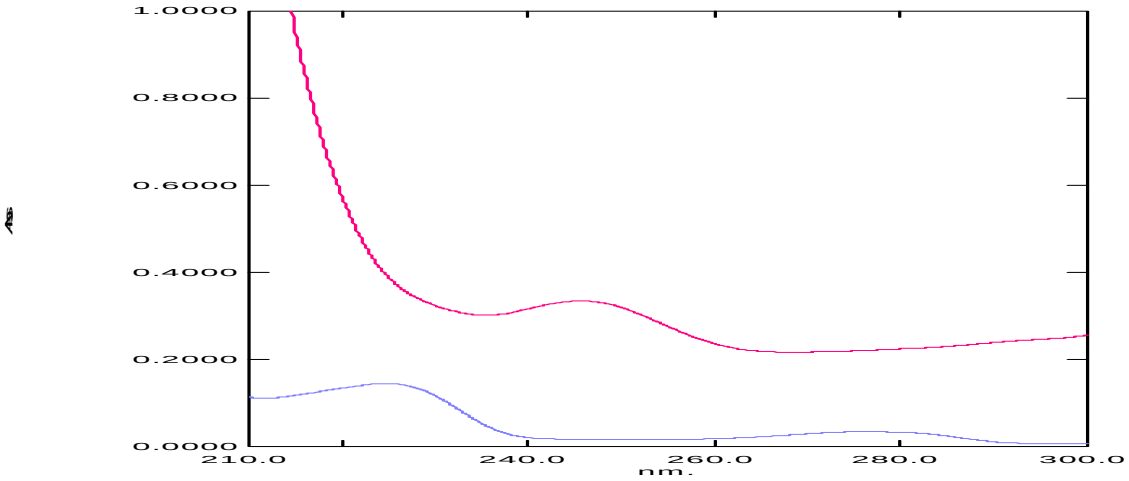
A SHIMADZU analytical balance (0.01 mg) was used.

Preparation of Standard preparation
Standard solution
A 8 mg of standard bromhexine hydrochloride and 2 mg of salbutamol sulphate were weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent [mixture of buffer of pH 3.0 and acetonitrile [65:35 % (v/v)] was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 800 µg /ml of bromhexine hydrochloride and 200 µg /ml of salbutamol sulphate respectively. The working standard solution was prepared by diluting 1 ml of 300 µg /ml of bromhexine hydrochloride and 20 µg /ml. of salbutamol sulphate solution to 10 ml with diluent to get concentration 30 µg /ml of bromhexine hydrochloride and 2 µg /ml of salbutamol sulphate respectively.

Sample preparation
Twenty tablets were weighed accurately and average weight of each tablet was determined. A powder equivalent to 8 mg of standard bromhexine hydrochloride and 2 mg of salbutamol sulphate were weighted accurately and transferred in 10 ml volumetric flask to give concentration as 800 µg /ml of bromhexine hydrochloride and 200 µg /ml. of salbutamol sulphate respectively. The working standard solution was prepared by diluting 1 ml of 800 µg /ml of bromhexine hydrochloride and 200 µg /ml of salbutamol sulphate solution to 10 ml with diluent to get concentration 80 µg /ml of bromhexine hydrochloride and 20 µg /ml. of salbutamol sulphate respectively.

Chromatographic condition
Chromatographic separation was performed at ambient temperature on a reverse phase Zorbax Eclipse C18 (250 x 4.6 mm i.d.) with 5 µ particle size column. The mobile phase was a mixture of buffer of pH 3.0 and acetonitrile [65:35 % (v/v)]. The buffer was mixtures of 0.1 % (v/v) tri-ethyl amine adjusted the pH 3.0 with ortho-phosphoric acid. The flow rate of the mobile phase was adjusted to 1 ml /min. The detection was carried out at wavelength 225 nm. (Fig.1) The injection volume of the standard and sample solution was set at 1.0 µl.

Figure 1: Overlay UV spectra of bromhexine hydrochloride and salbutamol sulphate



Method validation

System suitability

System performances of developed HPLC method were determined by injecting standard solutions. Parameter such as theoretical plates (N), symmetry, and area were determined. The results are shown in table 1 which indicates good performance of the system.

Table 1: System suitability parameters evaluated on standard solution of Bromhexine hydrochloride and salbutamol sulphate

Name	Retention Time	Area	Area %	USP Plate Count	Symmetry	Resolution
Salbutamol sulphate	2.180	1119915	15.36	1534	1.78	-
Bromhexine hydrochloride	9.960	6173106	84.64	4682	1.32	19.33

Specificity

Specificity is the ability of the method to resolve the active ingredients. Hence blank, standard bromhexine hydrochloride was injected to prove specificity. The typical chromatogram of the standard and sample assayed are given in figure 2 and 3 respectively.

Figure 2: Typical chromatogram of Salbutamol sulphate and bromhexine hydrochloride (standard)

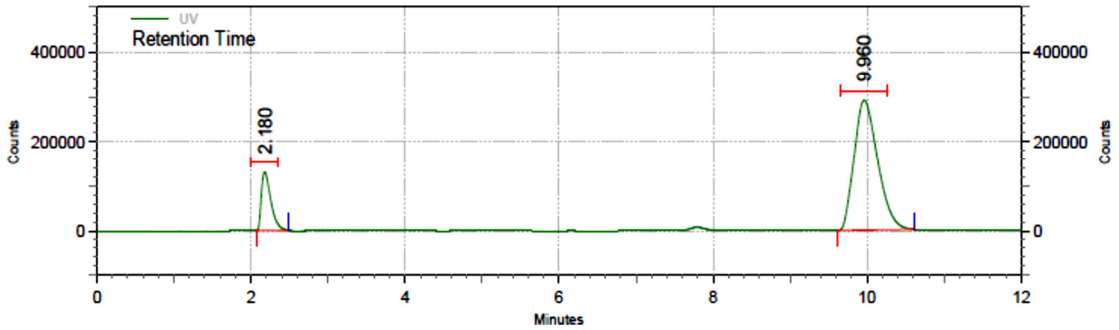
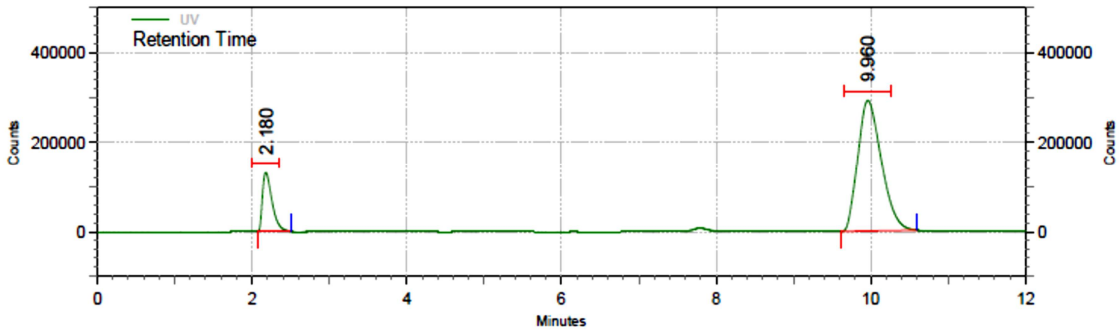


Figure 3: Typical chromatogram of salbutamol sulphate and bromhexine hydrochloride (sample)



Linearity

Under the experimental conditions described above, linear calibration curve were obtained throughout the concentration range studied. Regression analysis was done on the peak area (y) v/s concentration (x). The regression analysis data obtained is tabulated in table no. 2.

Table 2: Statistical evaluation of the data subjected to regression analysis

Parameters	Salbutamol sulphate	Bromhexine hydrochloride
Correlation Coefficient (r)	0.9997	0.9999
% Intercept (y)	15994	8649.7
Slope (m)	54705	78085

Accuracy

The accuracy method was determined by applying proposed method to synthetic mixture containing known amount of drug corresponding to 80 %, 100 % and 120 %. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis are enclosed under table no.3, 4.

Table 3: Statistical evaluation of the data subjected to accuracy of bromhexine hydrochloride

level	test	wt in mg	area	quantity added in µg/ ml	quantity recovered in µg/ ml	% recovery	mean recovery
80%	1	30.41	4939810	64.96	65.12	100.24	**100.49**
	2	30.52	4968177	64.96	65.49	100.81	
	3	30.45	4948863	64.96	65.23	100.42	
100%	1	30.46	6195994	81.2	81.67	100.58	**100.16**
	2	30.49	6177090	81.2	81.42	100.28	
	3	30.51	6135713	81.2	80.88	99.60	
120%	1	30.52	7325572	97.44	96.56	99.10	**99.34**
	2	30.50	7355572	97.44	96.96	99.51	
	3	30.49	7347753	97.44	96.86	99.40	
					Mean recovery of all level		**99.99**

Table 4 : Statistical evaluation of the data subjected to accuracy of salbutamol sulphate

level	test	wt in mg	area	quantity added in µg/ ml	quantity recovered in µg/ ml	% recovery	mean recovery
80%	1	2.11	903352	16.88	16.80	99.50	**99.25**
	2	2.13	891429	16.88	16.57	98.19	
	3	2.14	908400	16.88	16.89	100.06	
100%	1	2.13	1137857	21.1	21.16	100.26	**100.18**
	2	2.09	1148702	21.1	21.36	101.22	
	3	2.12	1124253	21.1	20.90	99.07	
120%	1	2.11	1345903	25.32	25.02	98.83	**99.36**
	2	2.13	1354065	25.32	25.18	99.43	
	3	2.11	1359306	25.32	25.27	99.82	
					Mean recovery of all level		**99.60**

Precision

The method precision was established by carrying out the analysis of bromhexine hydrochloride. The assay was carried out of the drug using analytical method in five replicates. The value of relative standard deviation lies well with the limits. The results of the same are tabulated in the table no. 4, 5.

Table 4: Statistical evaluation of the data subjected to method precision of bromhexine hydrochloride

Test	wt of test	Area	% assay
Test-1	8.17	6173106	100.09
Test-2	8.15	6170888	100.30
Test-3	8.20	6186547	99.94
Test-4	8.19	6195911	100.21
Test-5	8.12	6144595	100.24
Test-6	8.10	6152235	100.61
	Mean Assay		**100.23**
	SD		**0.226**
	RSD		**0.226**

Table 5: Statistical evaluation of the data subjected to method precision of salbutamol sulphate

Test	wt of test	Area	% assay
Test-1	2.1	1119915	99.15
Test-2	2.09	1122597	99.87
Test-3	2.13	1152560	100.61
Test-4	2.14	1158287	100.63
Test-5	2.12	1145044	100.42
Test-6	2.14	1142638	99.27
	Mean Assay		**99.99**
	SD		**0.665**
	RSD		**0.665**

Robustness
The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.
The typical variations are given below:
Variation in the flow rate by ± 0.2 ml /min
Variation in mobile phase composition by ± 2 %
Variation in wavelength ± 5 nm

The results of the analysis of the samples under the conditions of the above variation indicated the nature of robustness of the method.

Method application
Twenty tablets were weighed accurately and average weight of each tablet was determined. A powder equivalent to 8 mg of standard bromhexine hydrochloride and 2 mg of salbutamol sulphate were weighted accurately and transferred in 10 ml volumetric flask to give concentration as 800 µg /ml of bromhexine hydrochloride and 200 µg /ml. of salbutamol sulphate respectively. The working standard solution was prepared by diluting 1 ml of 800 µg /ml of bromhexine hydrochloride and 200 µg /ml. of salbutamol sulphate solution to 10 ml with diluent to get concentration 80 µg /ml of bromhexine hydrochloride and 20 µg /ml. of salbutamol sulphate respectively. From this solution 1.0 µl was injected specific conditions. The analyte peak was identified by comparison with that of respective standard. The (%) assay results were expressed in table no. 4, 5. It indicates the amount of bromhexine hydrochloride and salbutamol sulphate in the product meets the requirement.

RESULTS AND CONCLUSION

The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation. The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drug to the pre-analyzed active pharmaceutical ingredient and reanalyzing the mixture by proposed method. Thus the proposed RP-HPLC method is used for estimation of bromhexine hydrochloride and salbutamol sulphate from active pharmaceutical ingredient. It is more precise, accurate, linear, robust, simple and rapid method. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, active pharmaceutical ingredient and pharmaceutical formulation.

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