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

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ABSTRACT

A simple, rapid and accurate high performance liquid chromatography method is described for simultaneous determination of ambroxal hydrochloride, guaiphenesin 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 of pH 4.3 and acetonitrile [72:28 % (v/v)]. The detection was carried out at wavelength 230 nm. The mixture of buffer of pH 4.3 and acetonitrile [72:28% (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 ambroxal hydrochloride, guaiphenesin and salbutamol sulphate from combined dosage form.

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

INTRODUCTION

Ambroxal hydrochloride, is trans-4-[(2Amino-3,5-dibromobenzyl)amino] cyclohexanol. It shows molecular formula as $C_{13}H_{18}Br_2N_2O.HCl$ with molecular weight 414.57. It is official in BP [1] and IP [2]. Ambroxal is a metabolite of bromhexine. It is an expectoration improver and mucolytic agent used in the treatment of acute and chronic disorders characterized by the production of excess or thick mucus.

Guaiphenesin is, 3-(2-Methoxyphenoxy)-1,2-propanediol. It shows molecular formula as $C_{10}H_{10}O_4$ with molecular weight as 198.2. It is official in BP[1] and IP[2] and USP[3] is used to increase the volume and reduce the viscosity of tenacious sputum and is used as expectorant for productive cough.

Salbutamol sulphate is, chemically known as bis [(1RS)-2-[(1, 1-Di-methyl-ethyl) amino]-1-[4-hydroxy-3-(hydroxyl methyl) phenyl] ethanol] sulphate, 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 [4] for simultaneous determination of ambroxal hydrochloride, guaiphenesin and salbutamol sulphate in combined dosage form. Combination of ambroxal hydrochloride, guaiphenesin and salbutamol sulphate is used for the treatment of asthma and bronchitis.

MATERIALS AND METHODS

Chemical and reagents

Reference standard of ambroxal hydrochloride, guaiphenesin and salbutamol sulphate were 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 4.3 and acetonitrile [72:28 %(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 30 mg of standard ambroxal hydrochloride, 50 mg of guaiphenesin 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 4.3 and acetonitrile (72:28 % v/v)] was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 3000 µg /ml of ambroxal hydrochloride, guaiphenesin 5000 µg /ml and 200 µg /ml of salbutamol sulphate respectively. The working standard solution was prepared by diluting 1 ml of 3000 µg /ml of Ambroxal hydrochloride, 5000 µg /ml guaiphenesin and 200 µg /ml. of salbutamol sulphate solution to 10 ml with diluent to get concentration 300 µg /ml of ambroxal hydrochloride, 500 µg /ml guaiphenesin and 20 µg /ml of salbutamol sulphate respectively.

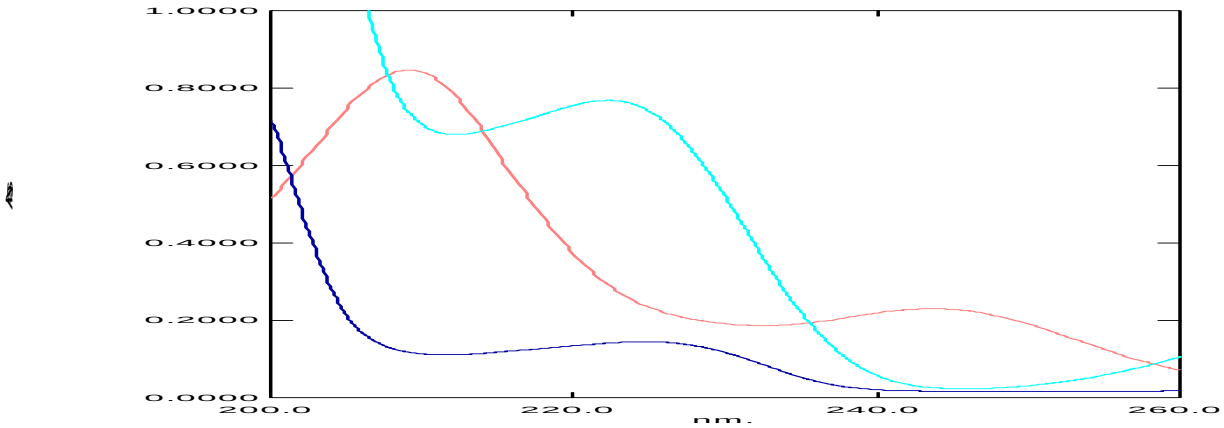
Sample preparation

Pharmaceutical formulation equivalent to 30 mg of standard Ambroxal hydrochloride, 50 mg of guaiphenesin and 2 mg of salbutamol sulphate were weighted accurately and transferred in 10 ml volumetric flask to give concentration as 3000 µg /ml of ambroxal hydrochloride, 5000 µg /ml guaiphenesin and 200 µg /ml. of salbutamol sulphate respectively. The working standard solution was prepared by diluting 1 ml of 3000 µg /ml of Ambroxal hydrochloride, 5000 µg /ml Guaiphenesin and 200 µg /ml of salbutamol sulphate solution to 10 ml with diluent to get concentration 300 µg /ml of Ambroxal hydrochloride, 500 µg /ml guaiphenesin 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 4.3 and acetonitrile (75:25 % v/v). The buffer was mixtures of 0.1 % (v/v) tri-ethyl amine adjusted the pH 4.3 with ortho-phosphoric acid. The flow rate of the mobile phase was adjusted to 1 ml /min. The detection was carried out at wavelength 230 nm. (Fig.1) The injection volume of the standard and sample solution was set at 1.0 µl.

Figure 1: Overlay UV spectra of ambroxal hydrochloride, guaiphenesin 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 Ambroxal hydrochloride, Guaiphenesin and salbutamol sulphate

Name	Retention Time	Area	Area %	USP Plate Count	Symmetry	Resolution
Salbutamol sulphate	2.320	572399	1.98	1942	1.59	-
Guaiphenesin	4.357	18251098	63.24	5376	1.52	9.09
Ambroxal hydrochloride,	5.080	10034417	34.77	2179	1.75	1.75

Specificity

Specificity is the ability of the method to resolve the active ingredients. Hence blank, standard Ambroxal hydrochloride, Guaiphenesin 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 Ambroxal hydrochloride, Guaiphenesin and salbutamol sulphate (standard)

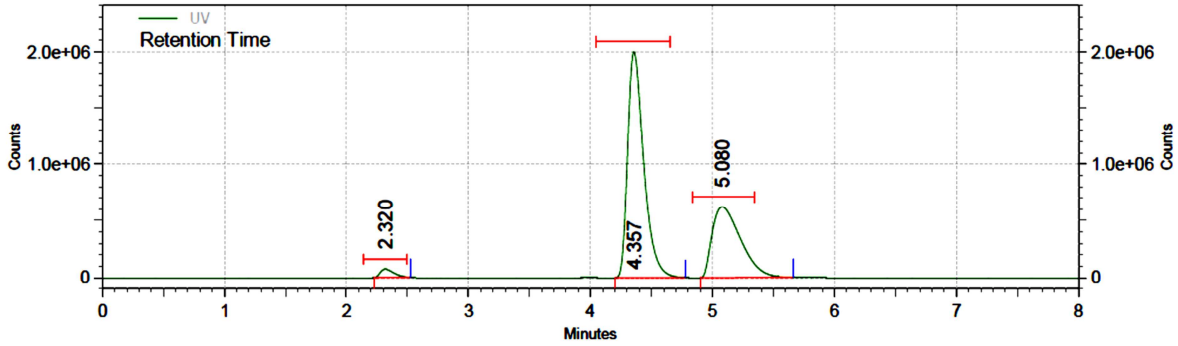


Figure 3: Typical chromatogram of Ambroxal hydrochloride, Guaiphenesin and salbutamol sulphate (sample)

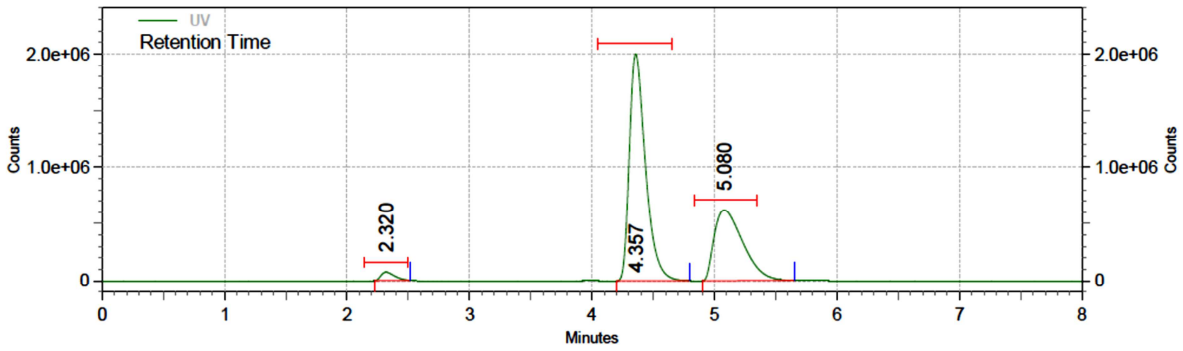


Table 2: Statistical evaluation of the data subjected to regression analysis

Parameters	Salbutamol sulphate	Guaiphenesin	Ambroxal hydrochloride
Correlation Coefficient (r)	0.9999	0.9999	0.9998
% Intercept (y)	83.769	243125	369298
Slope (m)	24428	32025	35364

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.

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, 5.

Table 3: 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	2.32	441910	18.4	18.00	97.82	**98.70**
	2	2.29	444560	18.4	18.11	98.41	
	3	2.31	451175	18.4	18.38	99.88	
100%	1	2.28	564630	23	23.00	99.99	**100.39**
	2	2.26	569082	23	23.18	100.78	
	3	2.29	566973	23	23.09	100.41	
120%	1	2.27	675590	27.6	27.52	99.70	**100.06**
	2	2.32	678124	27.6	27.62	100.08	
	3	2.33	680298	27.6	27.71	100.40	

Table 4 : Statistical evaluation of the data subjected to accuracy of guaiphenesin

level	test	wt in mg	area	quantity added in µg /ml	quantity recovered in µg /ml	% recovery	mean recovery
80%	1	50.23	14514283	401.6	398.57	99.24	**99.48**
	2	50.19	14550321	401.6	399.56	99.49	
	3	50.32	14582739	401.6	400.45	99.71	
100%	1	50.15	18228449	502	500.56	99.71	**99.74**
	2	50.24	18234223	502	500.72	99.74	
	3	50.33	18239586	502	500.86	99.77	
120%	1	50.21	21687207	602.4	595.54	98.86	**98.89**
	2	50.31	21694184	602.4	595.73	98.89	
	3	50.29	21700347	602.4	595.90	98.92	

Table 5 : Statistical evaluation of the data subjected to accuracy of ambroxal hydrochloride

level	test	wt in mg	area	quantity added in µg /ml	quantity recovered in µg /ml	% recovery	mean recovery
80%	1	30.54	8071536	244.4	245.55	100.47	**100.71**
	2	30.42	8087036	244.4	246.02	100.66	
	3	30.35	8114243	244.4	246.85	101.00	
100%	1	30.51	10073241	305.5	306.44	100.31	**100.37**
	2	30.55	10079874	305.5	306.64	100.37	
	3	30.52	10086067	305.5	306.83	100.44	
120%	1	30.48	11918984	366.6	362.59	98.91	**98.97**
	2	30.46	11926506	366.6	362.82	98.97	
	3	10.11	11933664	366.6	363.04	99.03	

Precision

The method precision was established by carrying out the analysis of ambroxal hydrochloride, guaiphenesin and salbutamol sulphate. 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. 6, 7, 8.

Table 6: Statistical evaluation of the data subjected to method precision of salbutamol sulphate

Test	wt of test	Area	% assay
Test-1	2.3	572399	99.16
Test-2	2.29	577381	100.46
Test-3	2.3	576434	99.86
Test-4	2.29	571588	99.46
Test-5	2.27	575096	100.95
Test-6	2.3	579870	100.46
	Mean Assay		**100.06**
	SD		**0.681**
	RSD		**0.680**

Table 7: Statistical evaluation of the data subjected to method precision of guaiphenesin

Test	Weight of test	Area	% assay
Test-1	50.28	18251098	99.78
Test-2	50.25	18257044	99.84
Test-3	50.28	18249741	99.77
Test-4	50.3	18360111	100.33
Test-5	50.29	18361989	100.36
Test-6	50.26	18367890	100.46
	Mean Assay		**100.09**
	SD		**0.326**
	RSD		**0.326**

Table 8: Statistical evaluation of the data subjected to method precision of ambroxal hydrochloride

Test	Weight of test	Area	% assay
Test-1	30.28	10034417	100.81
Test-2	30.51	10042711	100.14
Test-3	30.32	10043703	100.77
Test-4	30.35	10047984	100.72
Test-5	30.53	10078830	100.43
Test-6	30.48	10057115	100.38
	Mean Assay		**100.54**
	SD		**0.269**
	RSD		**0.268**

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 $\pm$ 0.2 ml /min

Variation in mobile phase composition by $\pm$ 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 30 mg of standard ambroxal hydrochloride, 50 mg guaiphenesin and 2 mg of salbutamol sulphate were weighted accurately and transferred in 10 ml volumetric flask to give concentration as 3000 µg /ml of Ambroxal hydrochloride, 5000 µg /ml guaiphenesin and 200 µg /ml. of salbutamol sulphate respectively. The working standard solution was prepared by diluting 1 ml of 3000 µg /ml of ambroxal hydrochloride, 5000 µg /ml guaiphenesin and 200 µg /ml. of salbutamol sulphate solution to 10 ml with diluent to get concentration 300 µg /ml of Ambroxal hydrochloride, 500 µg /ml guaiphenesin 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. 6,7, 8. It indicates the amount of ambroxal hydrochloride, guaiphenesin 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 ambroxal hydrochloride, guaiphenesin 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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