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Research Article

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Simultaneous determination of 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 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 [75:25 % (v/v)]. The detection was carried out at wavelength 225 nm. The mixture of buffer of pH 4.3 and acetonitrile [75:25% (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 guaiphenesin and salbutamol sulphate from combined dosage form.

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

INTRODUCTION

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 a HPLC [4] and spectrophotometric [5] methods for simultaneous determination of guaiphenesin and salbutamol sulphate in combined dosage form.

EXPERIMENTAL SECTION

Chemical and reagents

Reference standard of 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 [75:25 %(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.

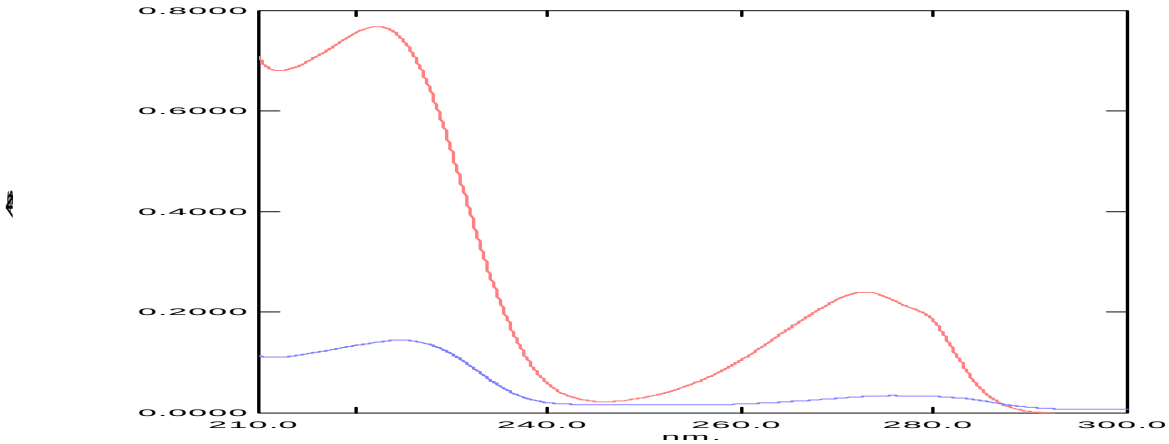
Preparation of Standard preparation
Standard solution
A 2 mg of salbutamol sulphate was weighed accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent [mixture of buffer of pH 4.3 and acetonitrile (75:25 % v/v)] was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 200 µg /ml of salbutamol sulphate respectively. It was stock solution A.

A 10 mg of guaiphenesin was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent [mixture of buffer of pH 4.3 and acetonitrile (75:25 % v/v)] was added and sonicated for 10 minutes, in this solution 1 ml of stock solution A was added and again sonicated for 10 minutes and the volume was adjusted up to the mark with diluent to give concentration as guaiphenesin 1000 µg /ml and 20 µg /ml of salbutamol sulphate respectively. The working standard solution was prepared by diluting 1 ml of 1000 µg /ml guaiphenesin and 20 µg /ml. of salbutamol sulphate solution to 10 ml with diluent to get concentration 100 µg /ml guaiphenesin and 2 µg /ml of salbutamol sulphate respectively.

Sample preparation
Pharmaceutical formulation equivalent to 100 mg of guaiphenesin and 2 mg of salbutamol sulphate were weighted accurately and transferred in 10 ml volumetric flask to give concentration as 10000 µg /ml guaiphenesin and 200 µg /ml. of salbutamol sulphate respectively. The working standard solution was prepared by diluting 1 ml 10000 µg /ml guaiphenesin and 200 µg /ml of salbutamol sulphate solution to 100 ml with diluent to get concentration 100 µg /ml guaiphenesin and 2 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 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 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, area and resolution 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 guaiphenesin and salbutamol sulphate

Name	Retention Time	Area	Area %	USP Plate Count	Symmetry	Resolution
Salbutamol sulphate	2.423	121262	1.64	1723	1.5317	-
Guaiphenesin	5.373	7256859	98.36	4845	1.3182	10.87

Specificity

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

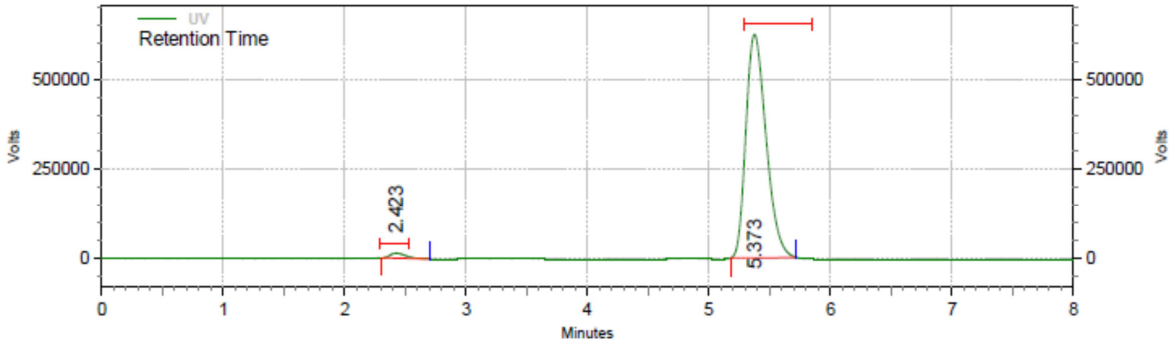
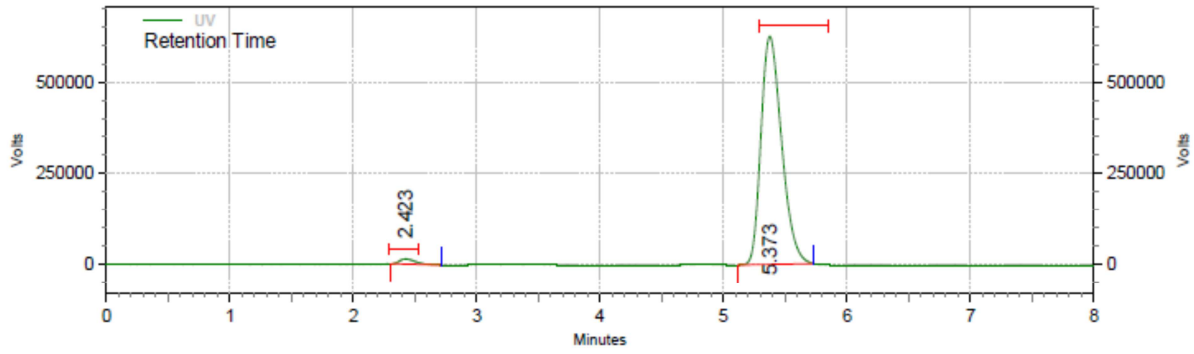


Figure 3: Typical chromatogram of guaiphenesin and salbutamol sulphate (sample)



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	Guaiphenesin
Correlation Coefficient (r)	0.9980	0.9999
% Intercept (y)	5114	75195
Slope (m)	5299	74078

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 salbutamol sulphate

level	test	wt in mg	area	quantity added in µg /ml	quantity recovered in µg /ml	% recovery	mean recovery
80%	1	1.62	97984	17.44	17.39	99.69	**99.25**
	2	1.65	97155	17.44	17.24	98.84	
	3	1.68	97532	17.44	17.30	99.23	
100%	1	2.09	123130	21.8	21.85	100.21	**99.97**
	2	2.15	122099	21.8	21.66	99.38	
	3	2.12	123270	21.8	21.87	100.33	
120%	1	2.44	141140	26.16	25.04	95.73	**97.47**
	2	2.46	144326	26.16	25.61	97.89	
	3	2.43	145666	26.16	25.85	98.80	
					Mean recover of all level		**98.90**

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	8.18	5753022	81.52	81.66	100.17	**100.47**
	2	8.05	5768118	81.52	81.87	100.43	
	3	8.12	5789419	81.52	82.17	100.80	
100%	1	10.16	7265671	101.9	103.13	101.20	**100.87**
	2	10.08	7255844	101.9	102.99	101.07	
	3	10.05	7202522	101.9	102.23	100.32	
120%	1	12.11	8715177	122.28	123.70	101.16	**101.13**
	2	12.07	8719112	122.28	123.76	101.21	
	3	12.03	8701722	122.28	123.51	101.01	
					Mean recover of all level		**100.82**

Precision

The method precision was established by carrying out the analysis of salbutamol sulphate and guaiphenesin and. 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.5, 6.

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

wt of test	Area	% assay
2.15	121262	100.07
2.14	121617	100.83
2.24	127609	101.08
2.22	126450	101.06
2.21	124931	100.30
2.22	125764	100.51
Mean Assay		**100.64**
SD		**0.415**
RSD		**0.413**

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

wt of test	Area	% assay
10.05	7256859	100.78
10.06	7256526	100.68
10.11	7271727	100.39
10.12	7280708	100.41
10.07	7105510	98.48
10.00	7106523	99.19
Mean Assay		**99.99**
SD		**0.933**
RSD		**0.933**

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, 100 mg guaiphenesin and 2 mg of salbutamol sulphate were weighted accurately and transferred in 10 ml volumetric flask to give concentration as 10000 µg /ml guaiphenesin and 200 µg /ml. of salbutamol sulphate respectively. The working standard solution was prepared by diluting 1 ml of 10000 µg /ml guaiphenesin and 200 µg /ml. of salbutamol sulphate solution to 100 ml with diluent to get concentration 100 µg /ml guaiphenesin and 2 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. 5, 6. It indicates the amount of salbutamol sulphate and guaiphenesin 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 salbutamol sulphate and guaiphenesin 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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