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Simultaneous determination of ambroxal hydrochloride and guaiphenesin 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 determination of ambroxal hydrochloride and guaiphenesin from active pharmaceutical ingredients. The separation of drug was achieved on BDS Hypersil C18 (150 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 (75:25 % v/v). The buffer was mixtures of 0.1 % (v/v) tri-ethyl amine solution adjusted the pH 3.5 with acetic acid. The detection was carried out at wavelength 230 nm. The mixture of water and acetonitrile (50:50% 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 from active pharmaceutical ingredients.

Keywords: Ambroxal hydrochloride, Guaiphenesin, Acetonitrile, tri-ethyl amine, acetic 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.

In literature survey reveals UV spectrophotometric [4], HPLC[5-7] and TLC[8] for simultaneous determination of ambroxal hydrochloride and guaiphenesin in combined dosage form.

MATERIALS AND METHODS

Chemical and reagents

Reference standard of ambroxal hydrochloride was obtained from reputed firm with certificate of analysis. Tri-ethylamine, acetonitrile and acetic 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 water and acetonitrile (50:50 % 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 3 mg of standard ambroxal hydrochloride and 10 mg of guaiphenesin were weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent(water and acetonitrile (50:50% v/v)) was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 300 µg /ml of ambroxal hydrochloride and 1000 µg /ml. of guaiphenesin respectively. The working standard solution was prepared by diluting 1 ml of 300 µg /ml of ambroxal hydrochloride and 1000 µg /ml. of guaiphenesin solution to 10 ml with diluent to get concentration 30 µg /ml of ambroxal hydrochloride and 100 µg /ml of guaiphenesin respectively.

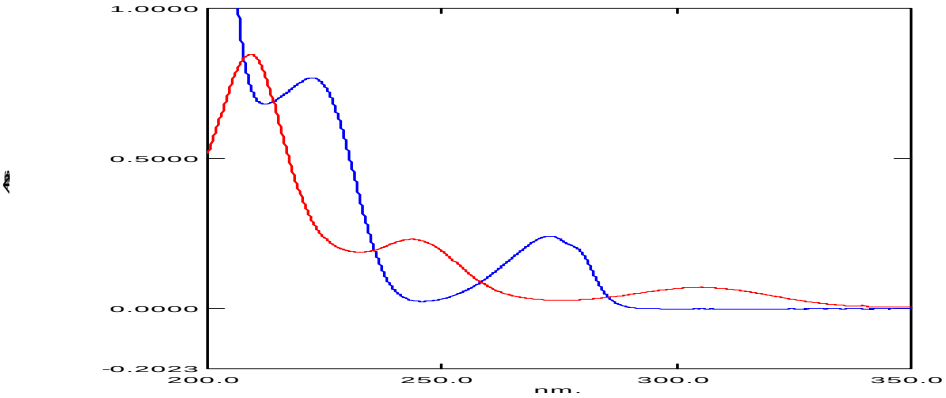
Sample preparation

Twenty tablets were weighed accurately and average weight of each tablet was determined. A powder equivalent to 3 mg of standard ambroxal hydrochloride and 10 mg of guaiphenesin were weighted accurately and transferred in 10 ml volumetric flask. It was dissolved in small quantity of diluent. It was sonicated for 15 minutes and further diluted to volume using diluent to give concentration as 300 µg /ml of ambroxal hydrochloride and 1000 µg /ml. of guaiphenesin respectively. The working standard solution was prepared by diluting 1 ml of 300 µg /ml of ambroxal hydrochloride and 1000 µg /ml of guaiphenesin solution to 10 ml with diluent to get concentration 30 µg /ml of ambroxal hydrochloride and 100 µg /ml. of guaiphenesin respectively.

Chromatographic condition

Chromatographic separation was performed at ambient temperature on a reverse phase BDS Hypersil C18 (150 x 4.6 mm i.d.) with 5 µ particle size column. The mobile phase was a mixture of buffer of pH 3.5 and acetonitrile (75:25 % v/v). The buffer was mixtures of 0.1 % (v/v) tri-ethyl amine adjusted the pH 3.5 with acetic acid. The flow rate of the mobile phase was adjusted to 1 ml /min. The detection was carried out at wavelength 230 nm. (Fig. no.1) The injection volume of the standard and sample solution was set at 1.0 µl.

Figure 1: Overlay UV spectra of ambroxal hydrochloride and guaiphenesin



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 and guaiphenesin

Name	Retention Time	Area	Area %	USP Plate Count	Symmetry	Resolution
Guaiphenesin	3.407	5366720	79.78	2317	1.22	-
Ambroxal hydrochloride	5.810	1359988	20.22	3462	0.97	7.09

Specificity

Specificity is the ability of the method to resolve the active ingredients. Hence blank, standard ambroxal 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 ambroxal hydrochloride (standard)

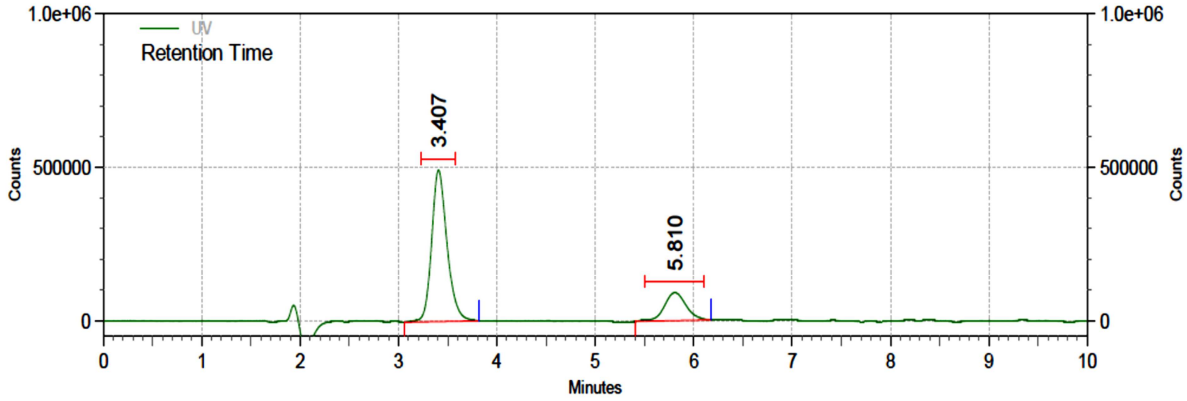
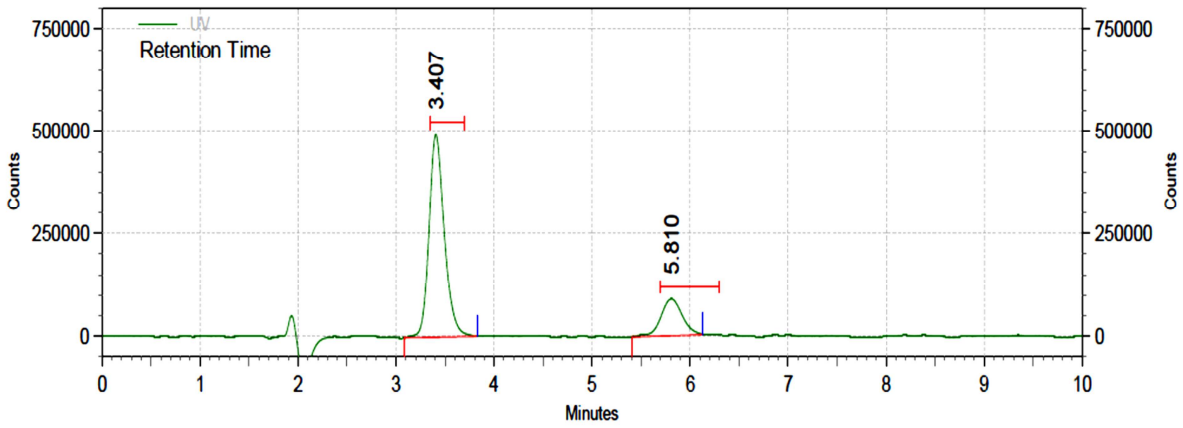


Figure 3: Typical chromatogram of ambroxal hydrochloride (sample)



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	Ambroxal hydrochloride	Guaiphenesin
Correlation Coefficient (r)	0.9978	0.9993
% Intercept (y)	432668	57337
Slope (m)	49547	42923

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 ambroxal hydrochloride

level	test	wt in mg	area	quantity added in µg /ml	quantity recovered in µg /ml	% recovery	mean recovery
80%	1	10.32	4389465	80.16	81.32	101.45	**101.41**
	2	10.36	4363444	80.16	80.84	100.85	
	3	10.39	4409641	80.16	81.70	101.92	
100%	1	10.23	5357748	100.2	99.26	99.07	**99.16**
	2	10.26	5384848	100.2	99.77	99.57	
	3	10.41	5346329	100.2	99.05	98.85	
120%	1	10.36	6383784	120.24	118.27	98.36	**98.41**
	2	10.45	6377922	120.24	118.16	98.27	
	3	10.11	6399279	120.24	118.56	98.60	

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	3.22	1083204	25.68	25.72	100.15	**99.63**
	2	3.19	1080530	25.68	25.65	99.90	
	3	3.22	1069107	25.68	25.38	98.84	
100%	1	3.12	1330154	32.1	31.58	98.38	**99.83**
	2	3.15	1360416	32.1	32.30	100.62	
	3	3.26	1358697	32.1	32.26	100.49	
120%	1	3.24	1645646	38.52	39.07	101.43	**100.86**
	2	3.18	1651367	38.52	39.21	101.78	
	3	3.19	1612241	38.52	38.28	99.37	

Precision

The method precision was established by carrying out the analysis of ambroxal hydrochloride and guaiphenesin. 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 ambroxal hydrochloride

wt of test	Area	% assay
10.28	5368508	98.97
10.17	5373458	100.14
10.32	5419048	99.52
10.35	5412983	99.12
10.33	5442591	99.85
10.41	5441486	99.07
Mean Assay		**99.45**
SD		**0.474**
RSD		**0.476**

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

wt of test	Area	% assay
3.20	1383020	99.41
3.21	1383519	99.14
3.35	1461159	100.33
3.33	1450391	100.19
3.19	1397887	100.80
3.26	1413885	99.76
Mean Assay		**99.94**
SD		**0.616**
RSD		**0.616**

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 3 mg of standard ambroxal hydrochloride and 10 mg of guaiphenesin were weighted accurately and transferred in 10 ml volumetric flask to give concentration as 300 µg /ml of ambroxal hydrochloride and 1000 µg /ml. of guaiphenesin respectively. The working standard solution was prepared by diluting 1 ml of 300 µg /ml of ambroxal hydrochloride and 1000 µg /ml. of guaiphenesin solution to 10 ml with diluent to get concentration 30 µg /ml of ambroxal hydrochloride and 100 µg /ml. of guaiphenesin 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 ambroxal hydrochloride 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 ambroxal hydrochloride 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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