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Reversed Phase High Performance Liquid Chromatography Method for Determination of Ambroxal Hydrochloride from Active Pharmaceutical dosage form

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ABSTRACT

A simple, rapid and accurate high performance liquid chromatography method is described for determination of ambroxal hydrochloride 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 (70:30 % v/v). The buffer was mixtures of 0.1 % tri-ethyl amine solution adjusted the pH 4.0 with acetic acid. The detection was carried out at wavelength 250 nm. The mixture of buffer of pH 4 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, 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. A literature survey reveals a spectrophotometric[3-7], HPLC[8-13] and miscellaneous[14-20] methods.

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 EZChrom Elite software.

A SHIMADZU analytical balance (0.01 mg) was used.

Preparation of Standard preparation

Standard solution

A 10 mg of standard ambroxal hydrochloride was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent [mixture of water and acetonitrile (50:50 % v/v)] to give concentration as 1000 µg /ml. The working standard solution was prepared by diluting 1 ml of 1000 µg /ml solution to 10 ml with diluent to get concentration 100 µg /ml.

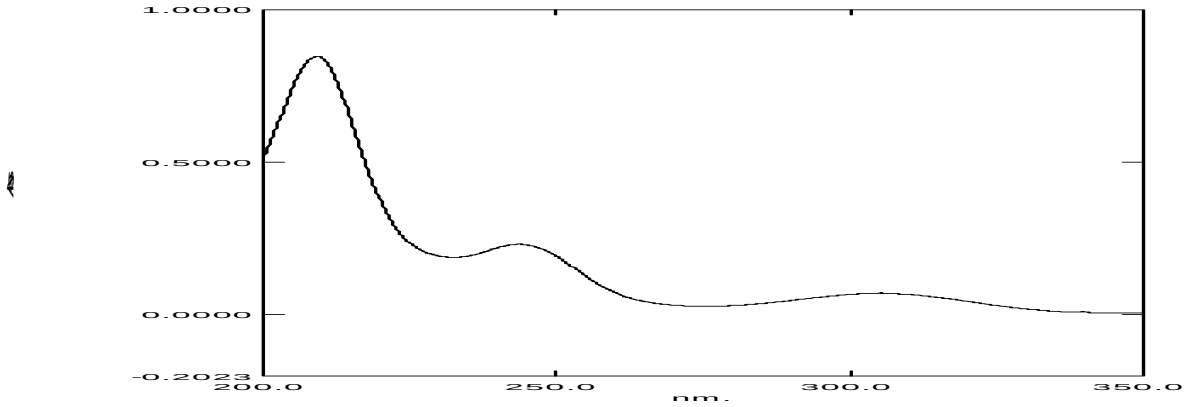
Sample preparation

Twenty tablets were weighed accurately and average weight of each tablet was determined. A powdered tablet equivalent to 10 mg of ambroxal hydrochloride sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent [mixture of 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 1000 µg /ml. The sample solution was prepared by diluting 1 ml of 1000 µg/ml solution to 10 ml with diluent to get concentration 100 µg /ml.

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 4.0 and acetonitrile (70:30 % v/v). The buffer was mixtures of 0.1 % tri-ethyl amine adjusted the pH 4.0 with acetic acid. The flow rate of the mobile phase was adjusted to 1 ml /min. The detection was carried out at wavelength 250 nm. (Fig.1) The injection volume of the standard and sample solution was set at 1.0 µl.

Figure 1: UV spectra of ambroxal hydrochloride



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

Retention Time	Area	Area %	USP Plate Count	Symmetry
4.190	4659098	100	2699	1.54

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)

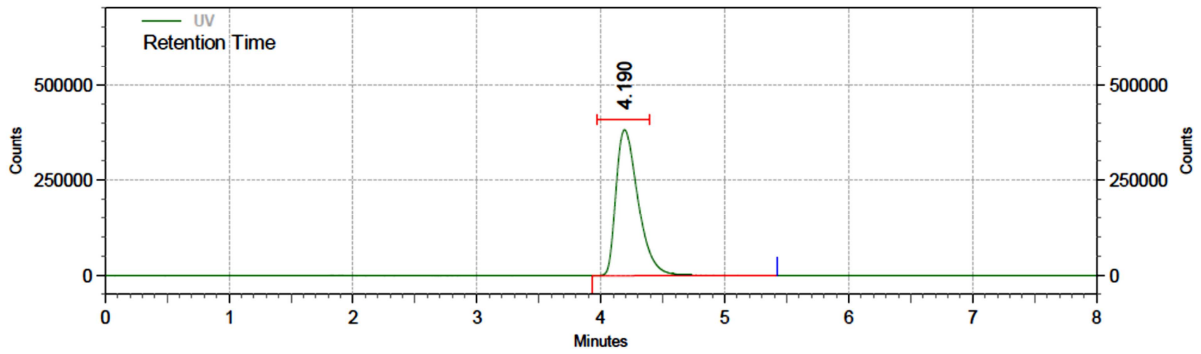
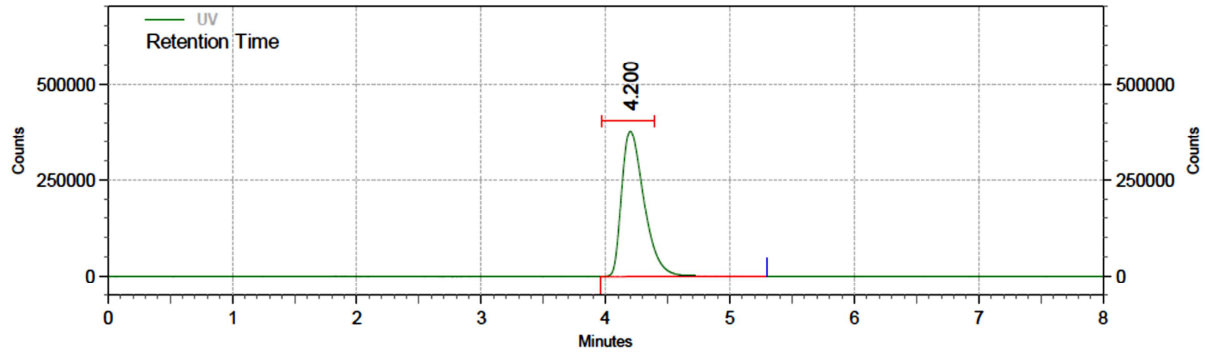


Figure 3: Typical chromatogram of ambroxal hydrochloride (sample)



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	Values
Correlation Coefficient (r)	0.9996
% Intercept (y)	107470
Slope (m)	46039

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

Level	Test	Weight in mg	Area	Quantity added in µg /ml	Quantity recovered in µg /ml	% Recovery	Mean recovery
80%	1	10.31	3826840	83.04	83.93	101.07	**101.34**
	2	10.36	3861179	83.04	84.68	101.98	
	3	10.32	3822676	83.04	83.84	100.96	
100%	1	10.25	4657647	103.8	102.15	98.41	**98.33**
	2	10.21	4659098	103.8	102.18	98.44	
	3	10.13	4645437	103.8	101.88	98.15	
150%	1	10.22	5748964	124.56	126.08	101.22	**101.16**
	2	10.29	5733494	124.56	125.75	100.95	
	3	10.36	5753696	124.56	126.19	101.31	
					Mean of all recovery		**100.28**

Accuracy
The accuracy method was determined by applying proposed method to synthetic mixture containing known amount of drug corresponding to 80 %, 100 % and 150 %. 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.

Precision
The method precision was established by carrying out the analysis of ambroxal 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.

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

Test	**Weight of test**	**Area**	**% assay**
Solution-1	10.29	4733345	99.14
Solution-2	10.35	4722725	99.50
Solution-3	10.41	4729086	100.21
Solution-4	10.22	4740570	98.62
Solution-5	10.32	4717378	99.10
Solution-6	10.41	4710056	99.81
	Mean Assay		**99.39**
	SD		**0.565**
	RSD		**0.569**

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. Powder equivalent to 10 mg of ambroxal hydrochloride sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml diluent was added and sonicated for 10 min to dissolve it. Further volume was made up to the mark with the diluent to give 1000 µg /ml. Further the 1 ml of this solution was diluted to 10 ml with diluent to give 100 µg /ml of ambroxal hydrochloride. 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. It indicates the amount of ambroxal hydrochloride 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 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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