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RP-HPLC method for the simultaneous estimation of ambroxol hydrochloride and desloratadine in pure and dosage form

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ABSTRACT

A simple, fast, accurate, precise, method has been developed for the simultaneous estimation of Ambroxol hydrochloride and Desloratadine in pure and tablet dosage form by reversed-phase high performance liquid chromatography. The separation was carried out on C₁₈ Phenomenex column, using mobile phase consisting of a mixture of acetonitrile: potassium dihydrogen orthophosphate buffer in the ratio 25: 75 and pH adjusted to 3.9 using orthophosphoric acid. The flow rate was adjusted to 1 ml/min. the UV detection was carried out at a wavelength of 270 nm. The retention time of Ambroxol hydrochloride and Desloratadine was found to be 4.4 min and 6.6 min respectively. Linear response obtained for Ambroxol hydrochloride was in the concentration range 3-15 µg/ml ($r^2 = 0.999$) and Desloratadine in the range 0.2-1 µg/ml ($r^2 = 0.999$). The relative standard deviation in the tablets was found less than 2% for six replicates. The method was validated according to ICH guidelines with respect to linearity, precision, limit of detection, limit of quantitation, accuracy, ruggedness, and robustness. Thus, proposed method can be successfully applicable to the pharmaceutical preparation containing the above mentioned drugs without any interference of excipients.

Keywords: Ambroxol hydrochloride; Desloratadine; RP-HPLC;

INTRODUCTION

Ambroxol hydrochloride (AMB) is chemically trans-4-(2-amino-3,5-dibromobenzylamino)-cyclohexanol is a secretolytic agent used in the treatment of trachea bronchitis, emphysema with bronchitis pneumoconiosis, chronic inflammatory pulmonary conditions, bronchiectasis, bronchitis with bronchospasm asthma [1]. It is official in IP and BP [2, 3]. Literature survey also reveals, HPLC [4], Ultra Performance Liquid Chromatography (UPLC) [5], Spectrophotometric [6] and HPTLC [7] methods for determination of AMB with other drugs.

Desloratadine (DES) is chemically 8-chloro-6,11-dihydro-11-(4-piperidinylidene)- 5H benzo [5, 6] cyclohepta [1, 2-b] pyridine. It is used for the relief of perennial allergic rhinitis and for the symptomatic treatment of pruritus and urticaria associated with chronic idiopathic urticaria [8]. Desloratadine is not official in any pharmacopeia. Literature survey reveals HPTLC [9] and spectrophotometric [10,11] methods for the determination of DES. Literature survey also reveals RP-HPLC [12,13] methods for determination of DES with other drugs.

The combined dosage forms of AMB and DES are available in the market for the treatment of chronic asthma and chronic bronchitis. No official method is available for the simultaneous estimation of AMB and DES in their combined dosage forms. Hence, it has driven the authors to develop a method which is new, simple, precise, and

accurate for the simultaneous determination of both drugs in their pharmaceutical dosage forms. The chemical structure of ambroxol and desloratadine is given in Fig 1.

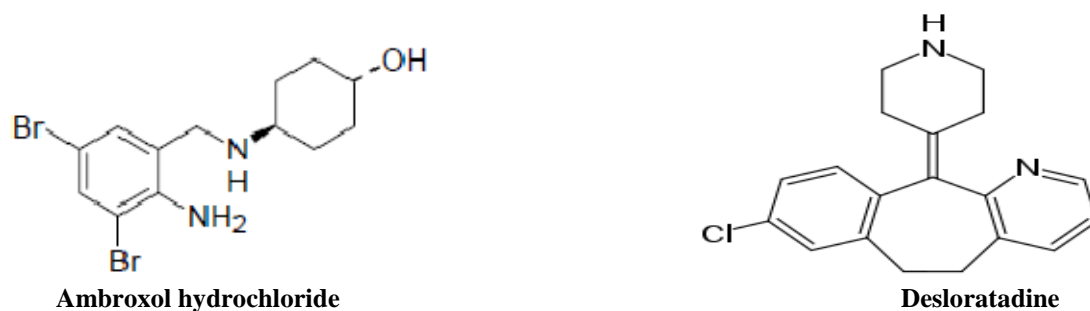


Fig 1 The chemical structure of Ambroxol and Desloratadine

MATERIALS AND METHODS

Chemicals

Active pharmaceutical ingredients of AMB and DES were received as gift samples from East India Pharmaceuticals, Kolkata and Sang Rose Laboratories Pvt. Ltd Mavelikkara, respectively. Methanol (HPLC grade), potassium dihydrogen orthophosphate (AR), ortho phosphoric acid (AR) and water (HPLC grade) were procured from Merck Chemicals, India. Tablet containing AMB and DES (75: 5 mg) were procured from local pharmacy retail shop (Dyl-AX manufactured by Ajanta Pharmaceuticals).

Equipment and chromatographic conditions

The HPLC system used was a Shimadzu HPLC system equipped with a Rheodyne injector (20 μ l) and UV detector. Chromatographic separation was carried isocratically at room temperature with a C₁₈ Phenomenex column (250 mm \times 4.6 mm i.d., 5 μ m). The mobile phase consisted of acetonitrile and potassium dihydrogen orthophosphate buffer in the ratio 25: 75, pH adjusted to 3.9 using orthophosphoric acid. The mobile phase was premixed and filtered through a 0.45 μ m nylon filter and degassed. The injection volume was 20 μ l and eluted at a flow rate of 1 ml/min. The detection wavelength was 270 nm.

Preparation of standard stock solution

Standard stock solutions (100 μ g/ml) of AMB and DES were prepared by dissolving accurately weighed 10 mg of each drug separately in mobile phase in 100 ml volumetric flask and filtered through 0.45 μ nylon filter. The working standard solutions of these drugs were further diluted with mobile phase to get required concentration of AMB (9 μ g/ml) and DES (0.6 μ g/ml).

Preparation of tablet solution

20 Dyl Ax tablets (Avrg weight 0.143g) each containing 75mg and 5 mg of Desloratadine was ground in to a fine powder using a clean glass mortar and pestle. Equivalent weight of tablet powder 0.178 is transferred in to 100ml volumetric flask dissolved in 10 ml HPLC grade methanol, sonicated for 30 mins and made up to 100ml with methanol. Further dilutions were made so as to get the final solution made with mobile phase, contained 75 and 5 μ g/ml of AMB and DES respectively.

Method validation

The method of analysis was validated as per the recommendations of ICH for the parameters like linearity, accuracy, limit of detection, limit of quantitation, intra-day and inter-day precision, repeatability and robustness [14, 15].

To establish the linearity, a series of dilutions ranging from 3-15 μ g/ml for AMB and 0.2-1 μ g/ml for DES were prepared separately and calibration graph was plotted between the mean peak area Vs respective concentration and regression equation was derived.

The ICH guideline defines specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. For this diluent was used as blank. Standard and sample were prepared as per test procedure, and checked for the interference of blank with the analyte peak. In the case of assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients.

The accuracy refers to the correctness of a single measurement. Accuracy is determined by comparing the measurement against the true or accepted value. The accuracy of the method was studied by recovery experiments. The recovery experiments were determined at three levels, via 50%, 100% and 150% of the selected concentration. Test solutions were injected in 6 times for each spike and the assay was performed as per the test method. The results are reported in term of percent recovery.

Precision of estimation of AMB and DES by proposed method was ascertained by different levels, i.e. method, system, inter-day and intra-day. Repeatability was studied by carrying out system precision and method precision. System precision was determined with six replicate of standard mixture and method precision was for formulation mixture. Intra-day precision of the developed method was evaluated by analyzing samples of 100% concentration in triplicates on same day. Inter-day precision was determined from the same concentration for three consecutive days. The relative standard deviation (% RSD) was determined to assess the precision of the assay and it was found to be not more than 2.0 %.

The limit of detection (LOD) and limit of quantitation (LOQ) was calculated for the proposed method, which was based on the standard deviation of the y intercept and the slope of the calibration curves. LOD and LOQ were calculated using following formulae: $LOD = 3.3(SD)/S$ and $LOQ = 10(SD)/S$. Where, SD = standard deviation of response (peak area) and S = slope of the calibration curve.

To evaluate robustness of a HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate ± 0.2 , pH of mobile phase ± 0.2 and wave length ± 2 nm units.

To check the system suitability, six replicate injections of mixed standard solution were injected and parameters such as, resolution, capacity factor, tailing factor, theoretical plate, and asymmetry factor of the peaks were calculated.

RESULTS AND DISCUSSION

The proposed method for simultaneous estimation of AMB and DES in bulk as well as in pharmaceutical preparation was found to be simple, accurate, economical and rapid. The method was validated as per the ICH guidelines.

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of acetonitrile and potassium dihydrogen orthophosphate buffer in the ratio 25: 75, pH adjusted to 3.9 using orthophosphoric acid, at a flow rate of 1 ml/min. The optimum wavelength for detection was set at 270 nm at which much better detector responses for both drugs were obtained. The retention time for AMB and DES was found to be 4.3 min and 6.6 min respectively.

System suitability testing

To know reproducibility of the method, system suitability test was employed to establish the parameters such as retention time, tailing factor, etc. The results obtained for system suitability are summarized in Table 1.

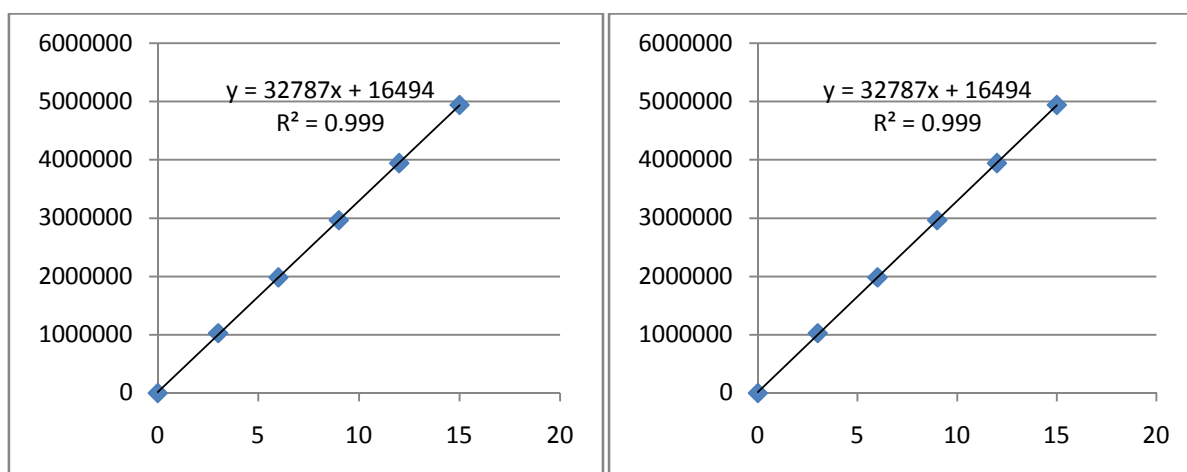


Fig 2 (a) Linearity graph of ambroxol

Fig 2 (b) Linearity graph of desloatadine

Linearity

AMB and DES showed a linearity of response between 3-15 µg/ml and 0.2-1 µg/ml with a correlation coefficient of 0.999 and 0.999 respectively. The results obtained for linearity of AMB and DES is summarized in Table 1 and represented in Fig 2 (a) and Fig 2 (b).

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The sensitivity of method is described in terms of LOD and LOQ. LOD and LOQ values for AMB were found to be 0.20 µg/ml and 0.60 µg/ml and that for DES were found to be 0.02 µg/ml and 0.07 µg/ml respectively. The results of LOD and LOQ studies are shown in Table 1.

Precision

The precision of this method was determined by intra-day and inter-day precision. The % RSD was found less than 2; this indicates that the method is precise. The results of precision studies are shown in Table 1.

Repeatability

The experimental values obtained for the repeatability of AMB and DES in samples is presented in Table 3. The result obtained shows % RSD < 2, indicating good repeatability of method.

Table 1 Method validation and system suitability parameters

Parameter	AMB	DES
Linearity range	3-15 µg/ml	0.2-1 µg/ml
Correlation coefficient (r)	0.999	0.999
Limit of detection	0.20 µg/ml	0.02 µg/ml
Limit of quantitation	0.60	0.07
Precision	Inter-day (% RSD)	0.12
	Intra-day (% RSD)	0.16
Retention time (min)	4.3	6.6
Tailing factor	1.162	0.357

Accuracy

The accuracy was evaluated by the recovery of AMB and DES at three different levels (50, 100 and 150 %). The percentage recovery was found to be 100 % and 99 % for AMB and DES respectively, % RSD was found to be less than 2, ensuring that the method is accurate. The results of accuracy studies are shown in Table 2.

Robustness

Robustness of the method was carried out by deliberately made small change in the flow rate ± 0.2 , pH of mobile phase ± 0.2 and wave length ± 2 nm units. The results of robustness studies are shown in Table 4.

Table 2 Results of recovery study of AMB and DES

Formulation	Label claim (mg/tab)	Amount added (%)	Total amount added (mg)	% Recovery \pm SD	% RSD
AMB	75 mg	50 %	0.095	100.29 \pm 0.52	0.520
		100 %	0.190	99.68 \pm 0.06	0.065
		150 %	0.286	99.43 \pm 0.17	0.174
DES	5 mg	50 %	1.43	99.72 \pm 0.84	0.838
		100 %	2.86	99.33 \pm 0.23	0.227
		150 %	4.29	99.37 \pm 0.20	0.203

Average of six determinations, SD is standard deviation and % RSD is relative standard deviation

Table 3 Result of repeatability study of AMB and DES

Parameter	System precision		Method precision	
	AMB	DES	AMB	DES
Mean	2959589	206205.3	2958286	205910
SD	4879.59	240.597	3511.10	619.73
% RSD	0.164	0.116	0.118	0.30

Average of six determinations, SD is standard deviation and % RSD is relative standard deviation

Specificity

Specificity was observed that the excipients present in the formulation and diluents did not interfere with detection of AMB and DES.

Table 4 Result of robustness study

Parameter	Variation	SD		% RSD	
		AMB	DES	AMB	DES
Flow rate (ml/min) \pm 0.2	0.8	0.165	0.0086	0.374	0.130
	1.0	0.042	0.0323	0.096	0.494
	1.2	0.0539	0.023	1.304	0.357
pH \pm 0.2	3.7	0.0122	0.0299	0.282	0.455
	3.9	0.0849	0.0437	0.020	0.666
	4.1	0.0296	0.0383	0.682	0.583
Wavelength(peak area)	268	4839.85	529.987	0.163	0.257
	270	3113.87	226.89	0.190	0.300
	272	5499.65	619.73	0.100	0.110

Average of six determinations, SD is standard deviation and % RSD is relative standard deviation

Table 5 Result of assay of tablet formulation

Formulation	Label claim (mg/tab)	Amount found (mg)	% Label claim (Mean \pm SD)
AMB	75 mg	75.10 mg	100.17 \pm 0.12
DES	5 mg	4.96 mg	99.88 \pm 0.14

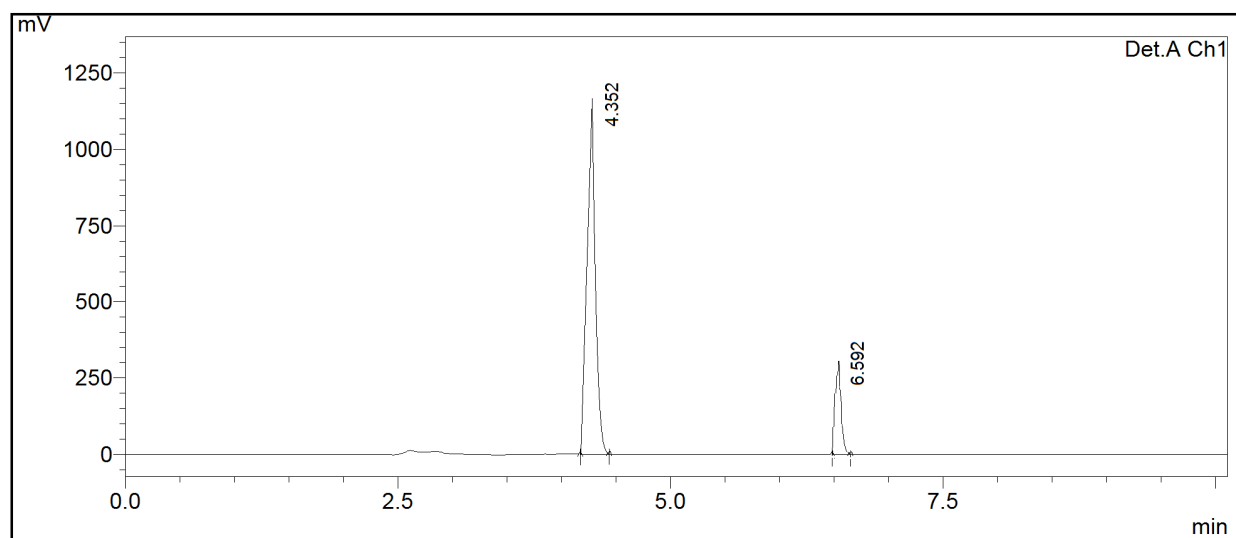


Fig 3 RP-HPLC chromatogram of standard ambroxol and desloratadine

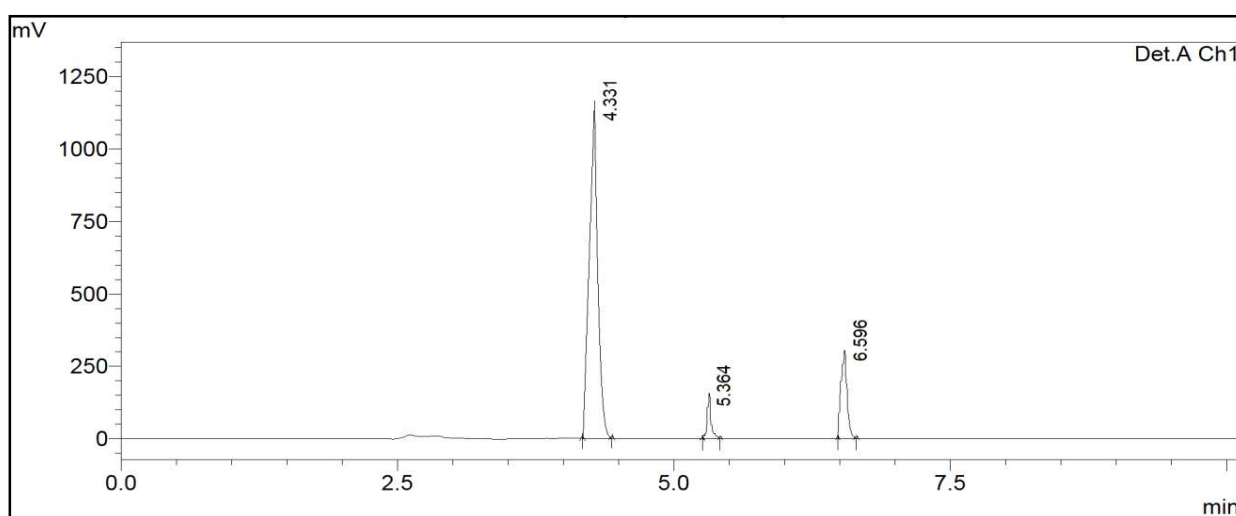


Fig 4 RP-HPLC chromatogram of ambroxol (RT 4.4) and desloratadine (RT 6.6) in tablet

Label claim recoveries from tablets

The proposed method was evaluated in the assay of commercially available tablets containing AMB (75 mg) and DES (5 mg). Six replicate determinations were carried out on an accurately weighted amount of the pulverized tablets equivalent to 0.190 mg of AMB and 2.86 mg of DES. The results of label claim studies are shown in Table 5. Chromatogram of the sample is shown in Fig 3 and Fig 4.

CONCLUSION

All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, rugged, and rapid as per the guidelines prescribed by ICH, and can be applied successfully for the estimation of AMB and DES in pharmaceutical formulations without interference and with good sensitivity; hence it can be used for the routine analysis in quality control department.

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