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DEVELOPMENT AND VALIDATION OF DUAL WAVELENGTH UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF AMBROXOL HYDROCHLORIDE AND DESLORATADINE HYDROCHLORIDE IN THEIR COMBINED TABLET DOSAGE FORM

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical dual wavelength spectrophotometric method for the simultaneous determination of Ambroxol Hydrochloride and Desloratadine Hydrochloride in combined tablet dosage form. The principle for dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest". The method was based on determination of Ambroxol Hydrochloride at the absorbance difference between 253.2 nm and 258.5 nm and Desloratadine Hydrochloride at the absorbance difference between 301.2 nm and 314 nm. The linearity was obtained in the concentration range of 5 - 75µg/ml for both Ambroxol Hydrochloride and Desloratadine Hydrochloride. The method was successfully applied to pharmaceutical dosage form because no interference from the tablet excipients was found. The suitability of these methods for the quantitative determination of Ambroxol Hydrochloride and Desloratadine Hydrochloride was proved by validation and recovery study. The proposed methods were found to be simple and sensitive for the routine quality control application of Ambroxol Hydrochloride and Desloratadine Hydrochloride in pharmaceutical tablet dosage form.

INTRODUCTION: Ambroxol Hydrochloride (AMB) is chemically Trans – 4 – (2 – Amino - 3, 5 – dibromobenzylamino) – cyclohexanol [1] is a secretolytic agent used in the treatment of tracheobronchitis, emphysema with bronchitis pneumoconiosis, chronic inflammatory pulmonary conditions, bronchiectasis, bronchitis with bronchospasm asthma [2]. It is official in Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP). IP [1] describes High Performance Liquid Chromatography (HPLC) method and BP [3] describes HPLC, Spectrophotometric and High Performance Thin Layer Chromatography (HPTLC) method.

Literature survey also reveals Spectrophotometric [4-5], HPLC [6-7], Ultra Performance Liquid Chromatography (UPLC) [8] and HPTLC [9] methods for determination of AMB with other drugs. Desloratadine Hydrochloride (DES) is chemically 8-chloro-6, 11-dihydro-11-(4-piperdinylidene) - 5*H* benzo [5, 6] cyclohepta [1, 2-b] pyridine [10] is a second generation antihistaminic drug. It is used for the relief of symptoms of seasonal allergic rhinitis, perennial (non-seasonal) allergic rhinitis and for the symptomatic treatment of pruritus and urticaria (hives) associated with chronic idiopathic urticaria [11].

Desloratadine is not official in any pharmacopoeia. Literature survey reveals HPTLC [12] and Spectrophotometric [13-14] methods for the determination of DES. Literature survey also reveals RP-HPLC [15-17] methods for determination of DES with other drugs. The combined dosage forms of AMB and DES are available in the market for the prophylaxis and treatment of chronic asthma and chronic bronchitis. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of AMB and DES in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric or other method for simultaneous estimation of AMB and DES in combined dosage forms.

The present communication describes simple, sensitive, rapid, accurate and economical spectrophotometric method based on dual wavelength UV spectroscopy method for simultaneous estimation of both drugs in their combined tablet dosage form.

MATERIALS AND METHODS:

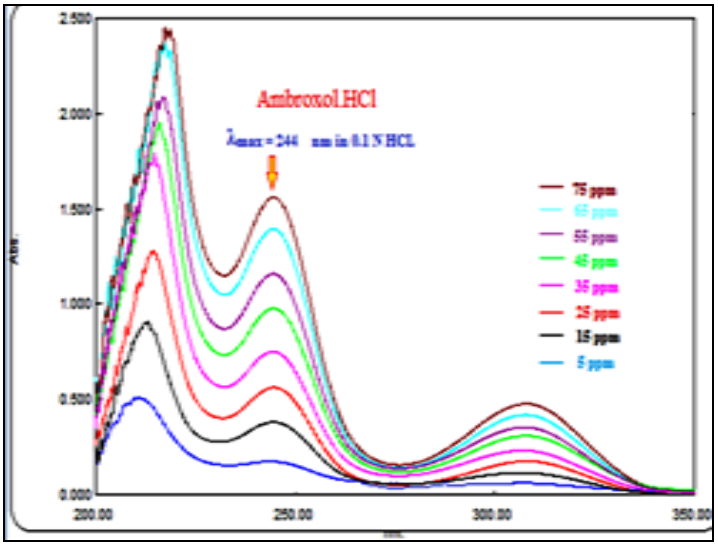
Apparatus: A double beam UV/Visible spectrophotometer (shimadzu model 1800, Japan) with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. An analytical balance (K.ROY instruments Pvt. Ltd., Varanasi, India); an ultrasonic bath (Janki Impex Pvt. Ltd., Ahmedabad, Gujarat, India) was used in the study.

1. **Reagents and Materials:** AMB and DES bulk powder was kindly gifted by Cadila Pharmaceuticals Ltd. Ahmedabad, Gujarat, India and Sun Pharmaceutical Ltd., Halol, Panchmahal, Gujarat, India respectively. The commercial fixed dose combination product Dyl Ax (AMB – 75 mg, DES – 5 mg) was procured from the local market which is manufactured by Ajanta Pharma Limited (APL). 0.1 N Hydrochloride acid (HCl) solution is used as solvent for the preparation of different concentration of both drugs AMB and DES.

2. **Preparation of standard stock solutions:** An accurately weighed quantity of AMB (100 mg) and DES (100 mg) were transferred to a separate 100 ml volumetric flask and 50 ml 0.1 N HCl is added to both volumetric flasks. Volume was adjusted up to the mark with 0.1 N HCl to obtain standard solution having concentration of AMB (1000 µg/ml) and DES (1000 µg/ml). 10 ml solution of AMB (1000 µg/ml) and DES (1000 µg/ml) were transferred to a separate 100 ml volumetric flask and diluted up to concentration of AMB (100 µg/ml) and DES (100 µg/ml) with 0.1 N HCl.

3. **Methodology:** The working standard solutions of AMB and DES were prepared separately in 25 ml volumetric flask using 0.1 N HCl as a solvent. They were scanned in the UV range of 200-400 nm. From the overlain spectra, four wavelengths 253.2 nm (λ_1), 258.5 nm (λ_2), 301.2 nm (λ_3) and 314 nm (λ_4) were selected for quantitation of both the drugs by proposed dual wavelength spectrophotometric method. The quantitative determination of AMB is carried out by measuring the absorbance difference value at between 253.2 nm and 258.5 nm where DES has same absorbance at both the wavelengths.

   The absorbance difference between 253.2 nm and 258.5 nm is directly proportional to concentration of AMB. The quantitative determination of DES is carried out by measuring the absorbance difference value at 301.2 nm and 314 nm where AMB has same absorbance at both the wavelengths. The absorbance difference between 301.2 nm and 314 nm is directly proportional to concentration of DES.



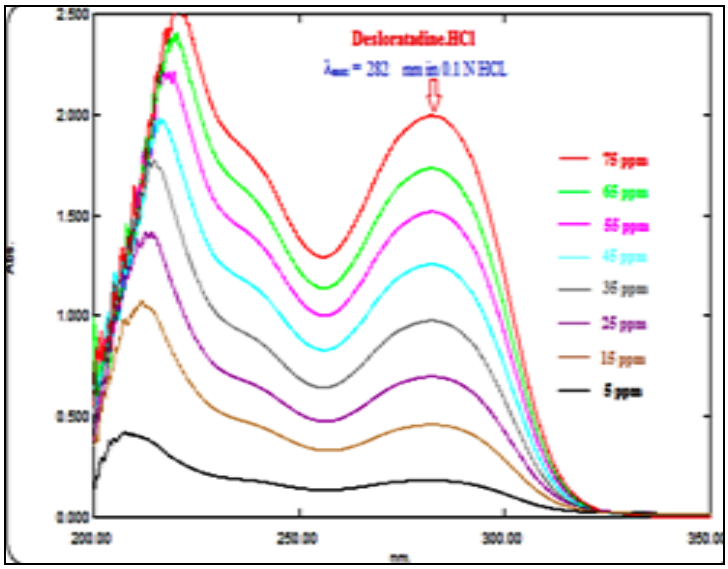
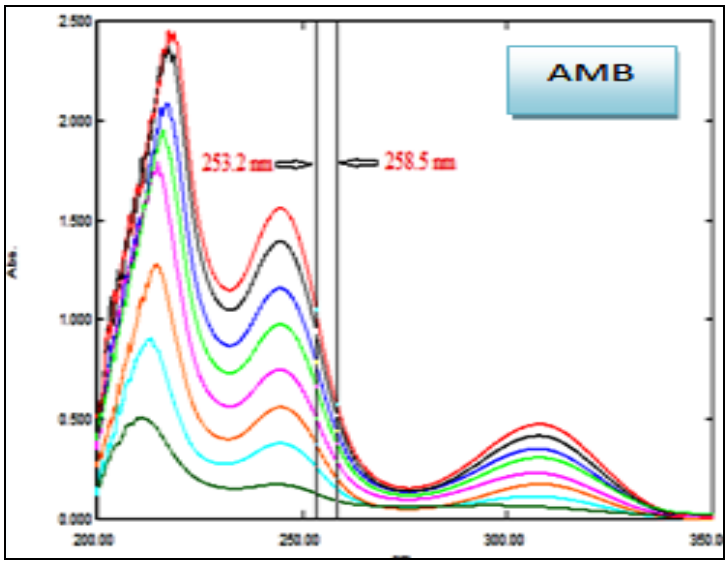


FIGURE 1: OVERLAIN ZERO-ORDER ABSORPTION SPECTRA OF AMB AND DES IN 0.1 N HCl



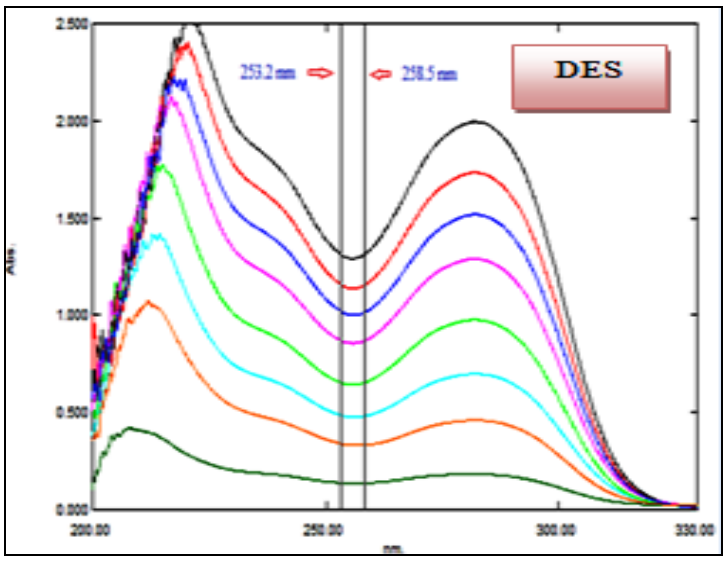
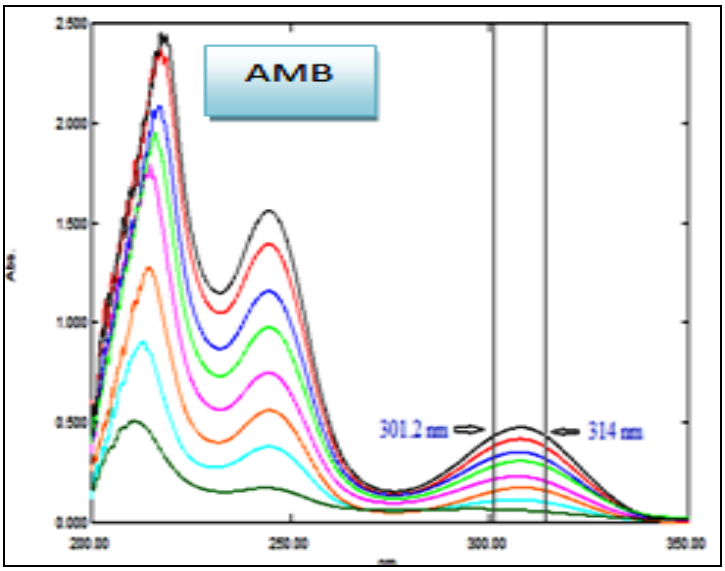


FIGURE 2: SPECTRA OF AMB AND DES FOR DIFFERENT CONC. AT 253.2nm AND 258.5 nm WHERE DES HAS SAME ABSORBANCE AND AMB HAS DIFFERENT ABSORBANCE



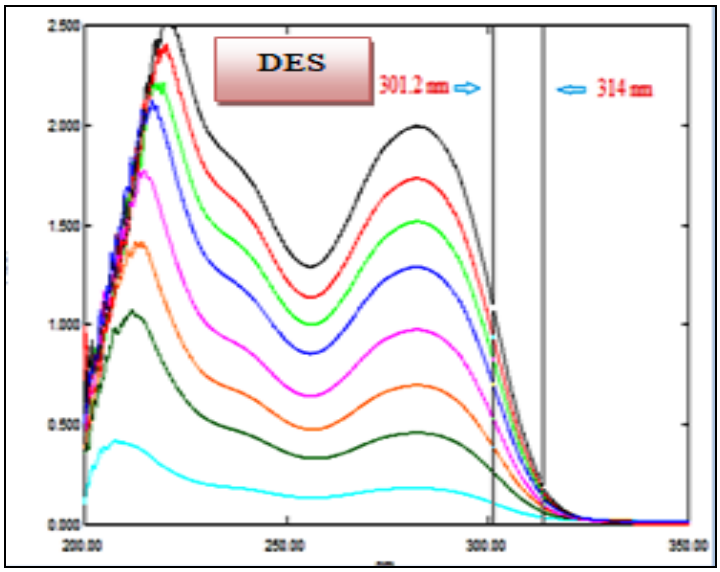


FIGURE 3: SPECTRA OF AMB AND DES FOR DIFFERENT CONCENTRATION AT 301.2 nm AND 314 nm WHERE AMB HAS SAME ABSORBANCE AND DES HAS DIFFERENT ABSORBANCE

Validation of the Proposed Method: The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [18].

1. **Linearity (Calibration curve):** The calibration curves were plotted over a concentration range of 5 - 75 µg/ml for AMB and 5 - 75 µg/ml for DES. Appropriate volume of aliquot from standard stock solution AMB (100 µg/ml) and DES (100 µg/ml) was transferred to different volumetric flasks of 25 ml capacity. The volume was adjusted to the mark with the 0.1 N HCl to obtain concentration of 5, 15, 25, 35, 45, 55, 65 and 75 µg/ml AMB and 5, 15, 25, 35, 45, 55, 65 and 75 µg/ml. These solutions scanned separately in the UV range of 200 - 400 nm.

2. The absorbances of the solutions were measured at 253.2 nm (λ_1), 258.5 nm (λ2), 301.2 nm (λ3) and 314 nm (λ4). The difference in absorbance between 253.2 nm (λ_1) and 258.5 nm (λ_2) is due to the AMB and was plotted against AMB concentration (µg/ml). The difference in absorbance between 301.2 nm (λ_3) and 314 nm (λ_4) is due to the DES and was plotted against DES concentration (µg/ml) and two different regression equations were obtained.

3. **Method precision (repeatability):** The precision of this method was checked by repeated scanning and measurement of absorbance of solution (n = 6) for AMB (5, 15, 25, 35, 45, 55, 65 and 75 µg/ml) and DES (5, 15, 25, 35, 45, 55, 65 and 75 µg/ml) without changing the parameter of the proposed spectrophotometry method.

4. **Intermediate precision (reproducibility):** The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of AMB and DES (25, 35, 45 µg/ml for AMB and 25, 35, 45 µg/ml for DES). The result was reported in terms of relative standard deviation (% RSD).

5. **Accuracy (recovery study):** The accuracy of the method was determined by calculating recovery of AMB and DES by the standard addition method. Known amounts of standard solutions of AMB and DES were added at 100, 120 and 140 % level to prequantified sample solutions of AMB and DES (30µg/ml and 30µg/ml for AMB and DES, respectively). The amounts of AMB and DES were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for three times.

Analysis of AMB and DES in Combined Tablet Dosage Form: Twenty Tablets were weighed and powdered. The powder equivalent to 75 mg of AMB and 5 mg of DES was transferred to a 100 ml volumetric flask. 0.1 N HCl (50 ml) was added to it and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with 0.1 N HCl. This solution is expected to contain 750 µg/ml of AMB and 50 µg/ml of DES. This solution (10 ml) was taken in to a 100 ml volumetric flask and the volume was adjusted up to mark with 0.1 N HCl to get a concentration of AMB (75 µg/ml) and DES (5 µg/ml). The responses of the sample solution were measured at 253.2 nm (λ_1), 258.5 nm (λ_2), 301.2 nm (λ_3) and 314 nm (λ_4) for quantification of AMB and DES. The amounts of the AMB and DES present in the sample solution were calculated by fitting the responses into the regression equation for AMB and DES in the proposed method.

RESULTS AND DISCUSSION: The standard solutions of AMB and DES were scanned separately in the UV range 200 – 400 nm. From the overlain spectra of both drugs, four specific wavelengths are selected. The absorbance at 253.2 nm (λ_1) and 258.5 nm (λ_2) wavelengths was found to be with same absorbance for DES. The difference in absorbance at these two wavelengths ($A_{258.5} - A_{253.2}$) cancels out the contribution of absorbance of DES. These two selected wavelengths were employed to determine the concentration of AMB.

Similarly, the absorbance at 301.2 nm (λ_3) and 314 nm (λ_4) wavelengths was found to be with same absorbance for AMB. The difference in absorbance at these two wavelengths ($A_{301.2} - A_{314}$) cancels out the contribution of absorbance of AMB. These two selected wavelengths were employed to determine the concentration of DES.

The proposed method was found to be simple, sensitive, rapid, accurate, precise and economic for the routine simultaneous estimation of two drugs. The linearity range for AMB and DES were found to be 5 - 75 µg/ml and 5 - 75 µg/ml respectively. Regression analysis data and summary of all validation parameters is given in **Table 1**. Accuracy was determined by calculating the recovery and the mean was determined in **Table 2**.

For the recovery study of DES, the 0% solution contained 30µg/ml of AMB and 2µg/ml of DES. The higher concentration of AMB interfered in calculation of DES, so the standard amount of 28µg/ml of DES was added to 0% solution for making solution of DES 30µg/ml that is equal to the concentration of AMB (30µg/ml). Then accuracy study was carried out for 100, 120 and 140% level for DES.

Thus, by applying spiking of 28µg/ml of DES in 0% solution, the interference by AMB is omitted in accuracy study of DES. Precision was calculated as repeatability (% RSD) and intra and inter day variation (% RSD) for both the drugs. The LOD and LOQ were found to be 1.61 and 4.89µg/ml respectively for AMB and 1.60 and 4.86 µg/ml respectively for DES indicates sensitivity of the proposed method. The method was successfully used to determine the amounts of AMB and DES present in tablets (**Table 3**).

The results obtained are in good agreement with the corresponding labelled amount. By observing the validation parameters, the method was found to be sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these drugs in combinations.

TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR THE PROPOSED METHOD

Parameters	Dual wavelength Spectroscopy method	
	AMB	DES
Concentration Range (µg/ml)	5 - 75	5 - 75
Slope (m)	0.00607	0.01175
Intercept (c)	0.01819	0.01679
Correlation Coefficient (r^2)	0.99932	0.99933
Accuracy (% recovery) (n = 3)	98.66 – 100.30 %	98.03 – 100.18%
Repeatability (%RSD) (n = 6)	0.74 %	0.54 %
Interday (n = 3) (%RSD)	1.10 – 1.30 %	0.62 – 0.69 %
Intraday(n = 3) (%RSD)	0.52 – 0.88 %	0.18 – 0.51 %
LOD (µg/ml)	1.61 µg/ml	1.60 µg/ml
LOQ (µg/ml)	4.89 µg/ml	4.86 µg/ml

TABLE 2: RECOVERY DATA OF PROPOSED METHOD

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	Amount Recovered (µg/ml) (n=3)	% Recovery (n=3)
AMB	0 %	30	0	29.64	98.80 ± 0.89
	100 %	30	30	60.01	100.01 ± 0.20
	120 %	30	36	66.20	100.30 ± 0.11
	140 %	30	42	71.04	98.66 ± 0.92
DES	0 %	30	0	30.04	100.10 ± 0.30
	100%	30	30	58.82	98.03 ± 0.68
	120 %	30	36	66.06	100.00 ± 0.10
	140 %	30	42	72.13	100.18 ± 0.23

TABLE 3: ANALYSIS OF AMB AND DES BY PROPOSED METHOD

	Label claim (mg)		Amount taken (µg/ml)		Amount Recovered (µg/ml) (n=3)		% Label claim	
Tablet	AMB	DES	AMB	DES	AMB	DES	AMB	DES
Dyl - AX	75	5	75	5	74.91	4.98	99.88 ± 0.87	99.60 ± 0.58

CONCLUSION: Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 5 - 75 µg/ml and 5 - 75 µg/ml for AMB and DES, respectively with co-efficient of correlation, (r^2) = 0.99932 and (r^2) = 0.99933 for AMB and DES, respectively. The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of AMB and DES in tablet dosage form. The method utilizes easily available and cheap solvent for analysis of AMB and DES hence, the method was also economic for estimation of AMB and DES from tablet dosage form. The common excipients and other additives are usually present in the tablet dosage form do not interfere in the analysis of AMB and DES in method, hence it can be conveniently adopted for routine quality control.

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