RESEARCH ARTICLE

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DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF AMBROXOL HYDROCHLORIDE AND DESLORATADINE HYDROCHLORIDE IN COMBINED TABLET DOSAGE FORM

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Abstract: The present manuscript describe simple, sensitive, rapid, accurate, precise and economical first order derivative spectrophotometric method for the simultaneous determination of Ambroxol Hydrochloride and Desloratadine Hydrochloride in combined tablet dosage form. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectra were obtained in 0.1 N Hydrochloric acid and the determinations were made at 256 nm (ZCP of Desloratadine Hydrochloride) for Ambroxol Hydrochloride and 308 nm (ZCP of Ambroxol Hydrochloride) for Desloratadine Hydrochloride. The linearity was obtained in the concentration range of 10 - 80 μg/ml for Ambroxol Hydrochloride and 5 - 40 μg/ml for Desloratadine Hydrochloride. The mean % recovery was 98.84 – 100.05% and 99.49 – 101.62% for Ambroxol Hydrochloride and Desloratadine Hydrochloride, respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of Ambroxol Hydrochloride and Desloratadine Hydrochloride in pharmaceutical combined tablet dosage form. The results of analysis have been validated statistically and by recovery studies.

Keywords: Ambroxol Hydrochloride, Desloratadine Hydrochloride, First order derivative spectrophotometric method, 0.1 N Hydrochloride acid, Mucolytic agent.

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 Thin Layer Chromatography (TLC) 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 1st order derivative 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.

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.

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.

Methodology

The standard solutions of AMB (10 μg/ml) and DES (10 μg/ml) were scanned separately in the UV range of 200 - 400 nm. The zero-order spectra thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 0.1 nm. The two spectra were overlain and it appeared that AMB showed zero crossing at 232, 244 nm, 276 nm, 308 nm, while DES showed zero crossing at 256, 282, 330 nm. At the zero crossing point (ZCP) of AMB (308 nm), DES showed a significance first-derivative absorbance, whereas at the ZCP of DES (256 nm), AMB showed a significance first-derivative absorbance. Hence 256 and 308 nm was selected as analytical wavelengths for determination of AMB and DES, respectively. These two wavelengths can be employed for the determination of AMB and DES without any interference from the other drug in their combined dosage formulations.
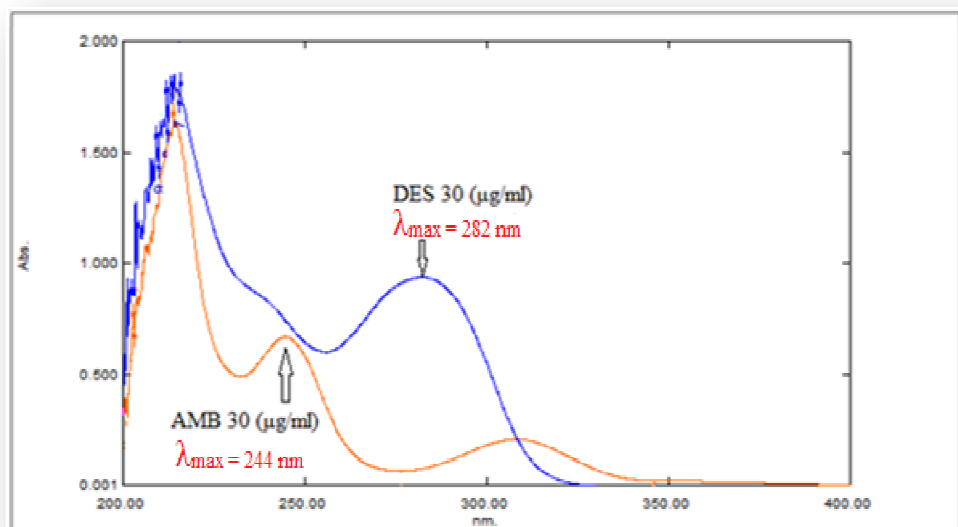
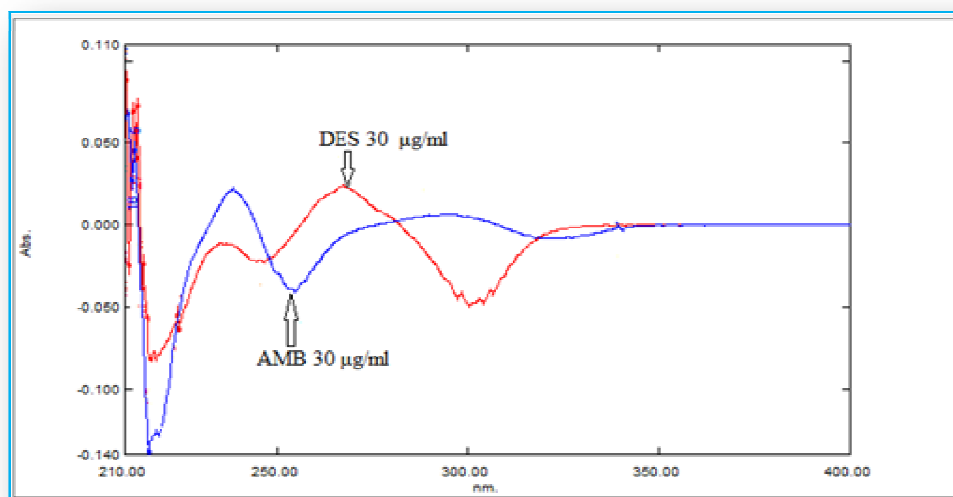Figure 1. Overlain zero-order absorption spectra of AMB and DES in 0.1 N HCl

Figure 2. Overlain first-order derivative spectra of AMB and DES in 0.1 N HCl

METHOD VALIDATION

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

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 10 - 80 μg/ml for AMB and 5 - 40 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 10, 20, 30, 40, 50, 60, 70 and 80 µg/ml AMB and 5, 10, 15, 20, 25, 30, 35 and 40 µg/ml. These solutions scanned separately in the UV range of 200 - 400 nm. First-derivative absorbance (D1) was measured at 256 nm for AMB and 308 nm for DES. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

Method precision (repeatability)

The precision of this method was checked by repeated scanning and measurement of absorbance of solution (n = 6) for AMB (10, 20, 30, 40, 50, 60, 70 and 80µg/ml) and DES (5, 10, 15, 20, 25, 30, 35 and 40 µg/ml) without changing the parameter of the first-derivative spectrophotometry method.

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 (30, 40, 50

µg/ml for AMB and 25, 30, 35 µg/ml for DES). The result was reported in terms of relative standard deviation (% RSD).

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 were added at 80, 100 and 120 % for AMB and 80, 100 and 120% for DES to prequantified sample solutions of AMB and DES (30µg/ml and 30µg/ml 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 256 nm and 308 nm for quantification of AMB and DES, respectively. 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, and zero-order spectra (Figure 1) thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 0.1 nm. The two derivative spectra showed significance absorbance at 256 nm (ZCP of DES) for AMB and 308 nm (ZCP of AMB) for DES. First-derivative absorbance (D1) was recorded 256 nm for AMB and 308 nm for DES (Figure 2). First derivative spectra give good quantitative determination of both the drugs at their respective wavelength without any interference from the other drug in their combined dosage formulations.

Linear correlation was obtained between absorbance and concentration of AMB and DES in the concentration ranges of 10 - 80 μg/ml and 5 - 40 μg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression (Table 1). The RSD values for AMB and DES for repeatability were found to be -0.68 and -0.81%, respectively (Table 1). The relative standard deviation (less than 2 %) indicates that the proposed method is repeatable. The RSD values of interday (-0.91 to -1.29 % and -1.53 to -1.95%) and intraday (-0.65 to -0.91% and -1.51 to -2.01%) for AMB and DES, respectively, reveal that the proposed method is precise (Table 1). LOD values for AMB and DES were found to be -3.29 and -1.41 μg/ml, respectively and LOQ values for AMB and DES were found to be -9.98 and -4.27 μg/ml, respectively (Table 1). These data show that proposed method is sensitive for the determination of AMB and DES.

The recovery experiment was performed by the standard addition method. The mean % recoveries were 98.84 – 100.04% and 99.49 – 101.62% for AMB and DES, respectively (Table 2). The results of recovery studies indicate that the proposed method is accurate. The proposed validated method was successfully applied to determine AMB and DES in their combined dosage form. The results obtained for AMB and DES were

comparable with the corresponding labelled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of AMB and DES in pharmaceutical dosage forms.

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 10 - 80 μg/ml and 5 - 40 μg/ml for AMB and DES, respectively with co-efficient of correlation, $(r^2) = 0.99766$ and $(r^2) = 0.99829$ for AMB and DES, respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. This is also a cost effective method. The additives usually present in the pharmaceutical formulation of the assayed sample did not interfere with determination of AMB and DES. The method can be used for the routine analysis of the AMB and DES in combined dosage form without any interference of excipients.

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Table 1.

Regression analysis data and summary of validation parameters for the proposed method

Parameters	First-derivative UV Spectrophotometry	
	AMB	DES
Concentration range (µg/ml)	10 - 80	5 - 40
Slope	-0.00128	-0.00107
Intercept	0.000324	-0.00108
Correlation Coefficient (r^2)	0.99766	0.99829
Accuracy (% recovery) (n = 6)	98.84 – 100.05	99.49 – 101.62
Repeatability (%RSD, n = 6)	0.68	0.81
Interday (n = 3) (%RSD)	-0.90 to -1.29	-1.53 to -1.95
Intraday (n = 3) (%RSD)	-0.65 to -0.91	-1.51 to -2.01
LOD (µg/ml)	-3.29	-1.40
LOQ (µg/ml)	-9.98	-4.27

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) ± S.D
AMB	0 %	30	0	29.65	98.84 ± 0.40
	80 %	30	24	54.03	100.05 ± 0.12
	100 %	30	30	59.81	99.68 ± 0.99
	120 %	30	36	65.58	99.36 ± 0.75
DES	0 %	30	0	30.05	100.17 ± 0.35
	80%	30	24	54.88	101.62 ± 0.85
	100 %	30	30	59.69	99.49 ± 0.81
	120 %	30	36	66.23	100.35 ± 0.12

Table 3.

Analysis of AMB and DES by proposed method

Tablet	Label claim (mg)		Amount found (mg)		% Label claim	
	AMB	DES	AMB	DES	AMB	DES
I	75	5	76.30	5.078	101.74 ± 1.02	101.56 ± 0.98

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