Research Article

Development and Validation of Q-Absorbance Ratio Method for Simultaneous Estimation of Ambroxol and Desloratadine In Combined Tablet Dosage Form

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical Q-absorbance ratio method for the simultaneous determination of Ambroxol and Desloratadine in combined tablet dosage form. The spectrophotometric method was based on the determination of both the drugs at the λ_{max} and iso-absorptive point. The spectra were obtained in 0.1 N Hydrochloric acid and the determinations were made at 244.4 nm (λ_{max} of Ambroxol) and 308.3 nm (iso-absorptive point). The linearity was obtained in the concentration range of 5 - 75 µg/ml for both drug Ambroxol and Desloratadine. The mean % recovery was 99.70 ± 0.004 and 99.23 ± 1.32 for Ambroxol and Desloratadine, respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of Ambroxol and Desloratadine in combined tablet dosage form. The results of analysis have been validated statistically and by recovery studies.

Keywords: Ambroxol, Desloratadine, Q absorbance ratio method, 0.1 N Hydrochloric acid.

1. INTRODUCTION

Ambroxol (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 (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 Q absorbance ratio method for simultaneous estimation of both drugs in their combined tablet dosage form.

2. MATERIALS AND METHODS

2.1 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.

2.2 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.

2.3 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.

2.4 Development of the methods

Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an iso-absorptive point and other being the λ_{max} of one of the two components. From the overlay spectra of two drugs, it is evident that AMB and DES show an iso-absorptive point at 308.3 nm. The second wavelength used is 244.4 nm, which is the λ_{max} of AMB. Eight working standard solutions having concentration 5, 15, 25, 35, 45, 55, 65 and 75 µg/ml for both AMB and DES were prepared in 0.1 N HCL, and the absorbance at 308.3 nm and 244.4 nm were measured and absorbtivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations

$$C_X = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1/aX_1 \quad \text{.....(3)}$$

$$C_Y = (A_1/aX_1) - C_X \quad \text{.....(4)}$$

Where, A_1 and A_2 are absorbance of mixture at 244.4 nm and 308.3 nm; and aX_1 and aY_1 are absorptivities of AMB and DES at 244.4 nm; aX_2 and aY_2 are absorptivities of AMB and DES respectively at 308.3 nm; and $Q_M = A_2 / A_1$, $Q_X = aX_2 / aX_1$ and $Q_Y = aY_2 / aY_1$.

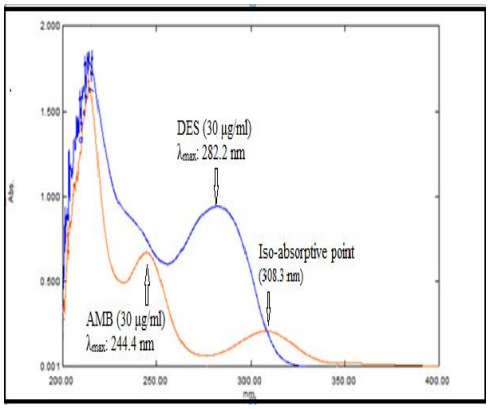

Fig. 1: Overlain absorption spectra of AMB (244.4 nm) and DES (282.2 nm) showing iso-absorptive point (308.3 nm) in 0.1 N HCL

3. METHOD VALIDATION

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

3.1 Linearity (calibration curve)

The calibration curves were plotted over a concentration range of 5 - 75 µg/ml for both AMB and DES. Accurately measured standard solutions of AMB and DES (0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5 and 7.5 ml) were transferred to a series of 10 ml of

volumetric flasks from the standard stock solution of AMB (100 µg/ml) and DES (100 µg/ml) respectively and diluted to the mark with 0.1 N HCl. Absorbance was measured at 244.4 nm and 308.3 nm. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

3.2 Method precision (Repeatability)

The precision of this method was checked by repeated scanning and measurement of absorbance of solution (n = 6) for AMB (35 µg/ml) and DES (35 µg/ml) without changing the parameter of the spectrophotometry method.

3.3 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).

3.4 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 both AMB (30 µg/ml) and DES (30 µg/ml). 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.

3.5 Analysis of 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 244.4 nm and 308.3 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.

4. RESULTS AND DISCUSSION

In absorbance ratio method (Q-analysis), the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 308.3 nm (iso-absorptive point) and 244.4 nm (λ-max of AMB) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of AMB (244.4 nm) and DES (282.2 nm) showing iso-absorptive point (308.3 nm) in 0.1 N HCL is shown in Figure 1.

The validation parameters were studied at all the wavelengths for the proposed method. Optical characteristics and summary of validation parameters for method 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. The method was successfully used to determine the amounts of AMB and DES present in the tablet dosage forms. The results obtained were in good agreement

with the corresponding labeled amount in Table 3. Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs (Table 1). By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these two drugs in combined dosage form.

5. CONCLUSION

In this proposed method the linearity is observed in the concentration range of 5 - 75 µg/ml with co-efficient of correlation, (r^2) = 0.999158 and (r^2) = 0.998128 for AMB and DES, respectively at 244.4 nm and (r^2) = 0.998468 at 308.3 nm. The result of the analysis of Pharmaceutical formulation by the proposed method is highly reproducible and reliable and is in good agreement with label claim of the drugs. The additive usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of AMB and DES. The method can be used for the routine analysis of 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	AMB	DES	AMB & DES
Wavelength range (nm)	244.4	244.4	308.3
Beer's law limit (µg/ml)	5 - 75	5 - 75	5 - 75
Slope	0.019996	0.020594	0.005999
Intercept	0.069518	0.085113	0.026673
Correlation Coefficient (r^2)	0.999158	0.998128	0.998468
Molar extinction co-efficient (l mol^{-1} cm^{-1})	8102.14	7077.81	2484.65 (AMB) 2042.53 (DES)
Repeatability (%RSD, n = 6)	0.78	1.62	1.83
Interday (n = 3) (%RSD)	0.075 – 0.612	0.072 – 0.199	0.183 – 0.665
Intraday (n = 3) (%RSD)	0.199 – 1.357	0.168 – 1.39	0.317 – 1.15
LOD (µg/ml)	1.8033	2.689	2.432
LOQ (µg/ml)	5.4645	8.1499	7.371

Table 2: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml) (n=3)	% Mean Recovery (n = 3)
AMB	0 %	30	0	29.88	99.60
	100 %	30	30	60.13	100.22
	120 %	30	36	65.88	99.82
	140 %	30	42	71.38	99.15
DES	0 %	30	0	29.95	99.84
	100%	30	30	58.36	97.268
	120 %	30	36	65.79	99.68
	140 %	30	42	72.10	100.14

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
I	75	5	75	5	74.47	4.77	99.29	95.50

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