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Development and validation of stability indicating area under curve method for simultaneous estimation of ambroxol hydrochloride and loratadine in bulk and tablet dosage form

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ABSTRACT

The objective of present work was to develop a simple, accurate, rapid and precise Stability Indicating UV-Spectrophotometric method for simultaneous estimation of Ambroxol hydrochloride (AMB) and Loratadine (LORA) in bulk and tablet Pharmaceutical dosage form using Area Under Curve (AUC) method. It involves measurement of area under curve in the range of 310-320 nm for AMB and 240-250 nm for LORA in methanol. The linearity was observed in the concentration range of 12-72µg/ml for AMB and 1-6µg/ml for LORA with a correlation coefficient (r^2) of 0.999 for AMB and 0.998 for LORA respectively. Assay results of marketed formulation were found to be 98.85% for AMB and 100.11% for LORA. Satisfactory values of percent recovery indicated accuracy of the method. The value of % RSD for Intra-day and Inter-day precision were found to be 1.1718 and 0.2018 for AMB and 0.6159 and 1.3696 for LORA respectively. Limit of Detection and Quantitation was found to be 0.2207µg/ml and 0.6688µg/ml for AMB and 0.6648µg/ml and 1.6548µg/ml for LORA respectively. The proposed method was successfully applied for quantitative detection of AMB and LORA in pharmaceutical dosage form. The method was validated according to ICH guidelines.

Keywords: Ambroxol Hydrochloride, Loratadine, Area under Curve, UV-Spectroscopy, Stability indicating.

INTRODUCTION

Ambroxol (AMB) is chemically Trans-4-(2–Amino–3, 5–dibromo benzylamine)–cyclohexanol [1] (Fig. 1) is a mucolytic agent [2] and used as a bronchosecretolytic and expectorant drug. It stimulates the transportation of the viscous secretion in the respiratory organs and reduces the stand stillness of the secretion [3]. It is official in Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP) [4] IP describes High Performance Liquid Chromatography (HPLC) method and BP describes Spectrophotometric, High Performance Liquid Chromatography (HPLC) and High Performance Thin Layer chromatography (HPTLC) method. Loratadine (LORA) is a tricyclic antihistamine, which acts as a selective inverse agonist of peripheral histamine H1-receptors [5]. It is chemically Ethyl-4-(8-chloro-5,6dihydro-11H-benxo [5,6] cyclohepta [1,2 b] pyridine-11-ylidene)-1-piperidine carboxylate (Fig. 2), is a long acting antihistamine drug[6]. It is official in USP [7], BP and IP. Loratadine is given orally, is well absorbed from the gastrointestinal tract and has rapid first pass hepatic metabolism, it is metabolized by isoenzymes of the cytochrome P450 system, including CYP2D6 and to a lesser extent, several others. Loratadine is almost totally (97-99%) bound to plasma proteins [8].

According to literature many methods have been described for the determination of AMB by RP-HPLC [2, 9-11], Spectrophotometric [3,12,18,19], HPLC [3,19,20], HPTLC [2] and Gas-LC [16] and Loratadine by RP-HPLC [9-11,17], Spectrophotometric [8,12,13,17], HPLC [5,6,14], LC/MS [15], Individually and in combinations with other drugs from bulk drugs and pharmaceutical formulations. However, there is no UV Spectroscopy by Stability Indicating Area under curve method reported for the simultaneous estimation of these drugs in combined dosage forms. Fixed dose combination containing Ambroxol (60mg) and Loratadine (5mg) is available in the tablet form in the market. The aim of this work was to develop a Stability indicating UV Spectroscopy Area under curve method for the simultaneous determination of Ambroxol hydrochloride and Loratadine in pharmaceutical dosage form. The AUC method was validated following the ICH guidelines [21].

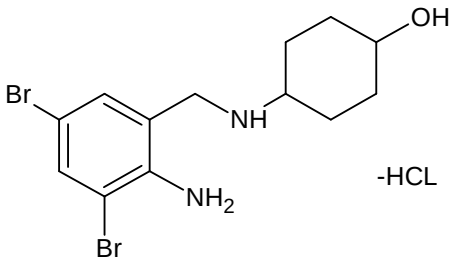

Fig. 1: Ambroxol hydrochloride

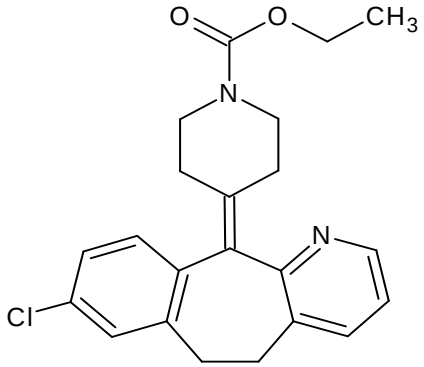

Fig. 2: Loratadine

MATERIALS AND METHODS

Chemicals and reagents

Ambroxol hydrochloride and Loratadine were obtained as a gift sample from Ami Life sciences Pvt. Ltd., Baroda and Vasudha Pharma Chem. Ltd., Hyderabad, respectively. Lorfast-AM tablet (AMB 60mg and LORA 5mg) in combined dosage form was procured from local market. All solvents and chemicals were purchased from Rankem Pvt. Ltd., Mumbai, India and were of analytical grade.

Instruments and apparatus

Double beam UV-visible Spectrophotometer (Shimadzu, Model: UV-1800) having two quartz cells with 1cm light path with UV probe software, Electronic analytical weighing balance (Anamed AA-2200) and Ultrasonic Bath (HMG India CD-4820), were also used. All the glasswares (Borosil*) were calibrated before use.

Preparation of standard stock solutions

Accurately weighed 10mg of AMB and 10mg of LORA were transferred into two separate 50ml volumetric flask, dissolve and volume made up to mark with methanol and sonicated for 20min to give solutions containing 200µg/ml for both AMB and LORA respectively. The stock solutions of both the drugs were further diluted separately with solvent to obtain 10µg/ml solution each and scanned in spectrum mode from 200-400nm.

Detection of wavelength

The drug solutions were scanned between the range of 200-400nm. The Area Under Curve (AUC) is good determined at both the selected analytical wavelength ranges were selected as 310-320nm for AMB and 240-250nm for LORA respectively (fig.3, 4). The overlain spectrum of both the drugs was shown in fig. 5.

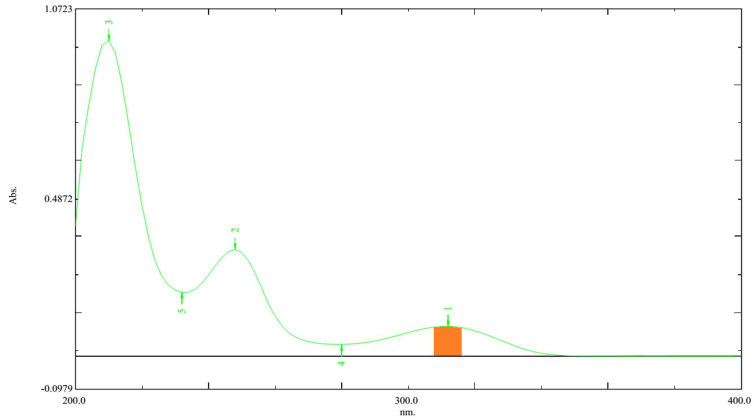

Fig. 3: AUC of Ambroxol Hydrochloride

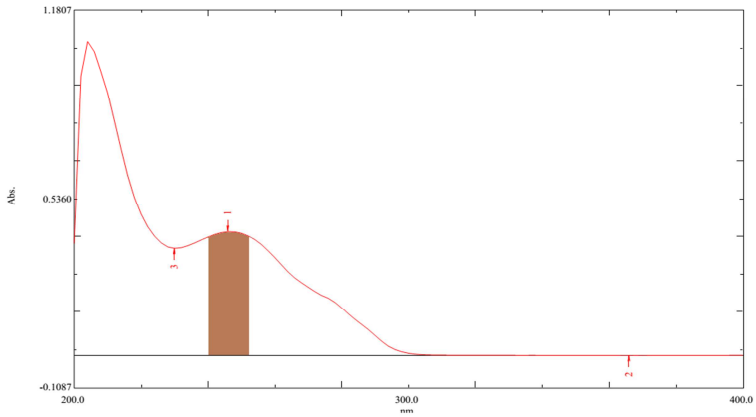

Fig. 4: AUC of Loratadine

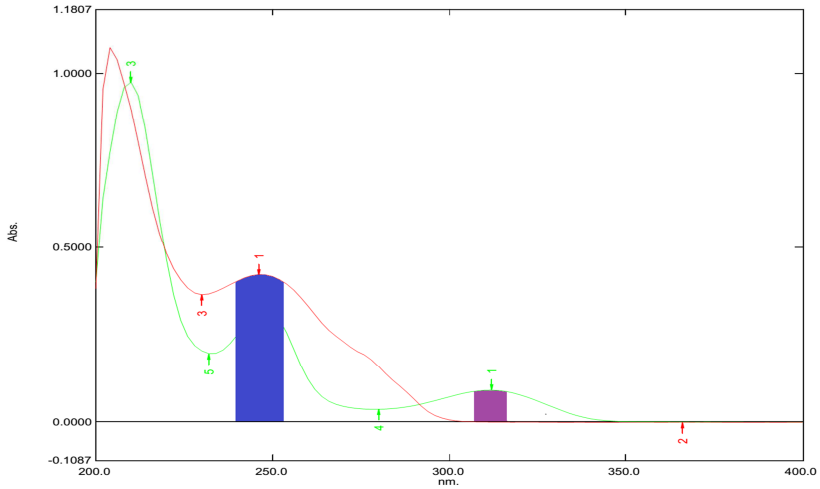

Fig. 5: Overlain Spectrum of AMB and LORA

Preparation of calibration curve

The above stock solution, working standard solution of drugs were prepared by appropriate dilution and were then scanned in the range of 200-400nm against diluents as blank. A series of dilution were prepared for standard solutions AMB and LORA 12-72µg/ml and 1-6µg/ml respectively. The absorbance curves (λmax) were found to be 310-320nm and 240-250nm for AMB and LORA. The calibration curve was plotted absorbance Vs concentration (fig. 6, 7).

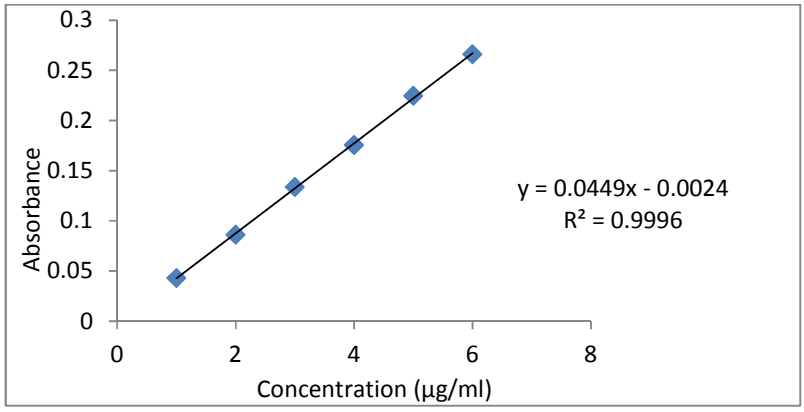

Fig. 6: Calibration curve of AMB

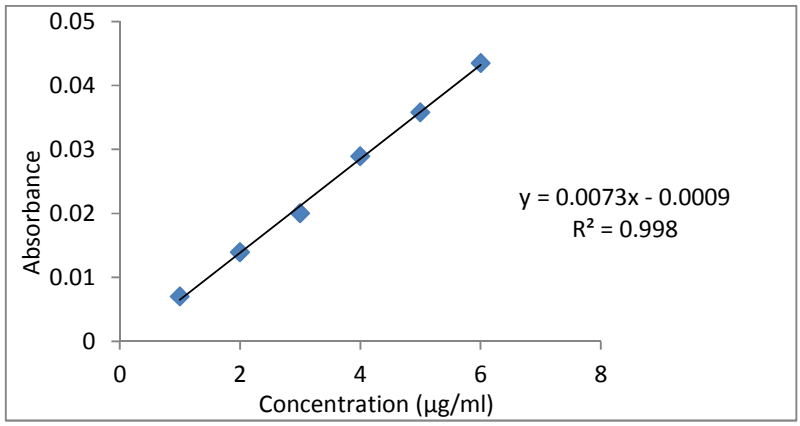

Fig. 7: Calibration curve of LORA

Area Under Curve

In this method area calculation involves calculation of integrated value of absorbance with respect to wavelength in indicated region of wavelengths. Area calculation processing item calculates the area bounded by the curve and horizontal axis [22-23]. Here horizontal axis represents baseline.

$$\text{Area calculation: } (\alpha+\beta) = \int_{\lambda 2}^{\lambda 1} A d\lambda$$

Whereas, α is area of portion bounded by curve data and a straight line connecting the start and end point, β is area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ1 and λ2 are wavelengths representing start and end point of curve region [24]. In this study area was integrated between wavelength ranges from 310-320nm for AMB and 240-250nm for LORA.

Assay of tablet dosage form

Twenty tablets were accurately weighed and average weight was calculated. These tablets were crushed and powdered in mortal. Powder equivalent to 60mg of AMB and 5mg of LORA was weighed accurately and transferred into a 50ml volumetric flask. It was dissolved with 40ml methanol and contents were sonicated for about 30min and diluted up to mark with methanol. The solution was filtered using Whatmann filter paper (No.41). The solution was further diluted with methanol to get a final concentration of 12µg/ml of AMB and 1µg/ml of LORA. This solution was integrated at wavelength range of 310-320 for AMB and 240-250 for LORA (Table-1).

METHOD VALIDATION

Validation of an analytical procedure is the process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirements for the intended analytical application. The proposed method has been extensively validated according to ICH guidelines.

Linearity and range

The working standard solutions were prepared by diluting stock standard solution with methanol to give a concentration range of 12-72µg/ml for AMB and 1-6µg/ml for LORA. The absorption spectra of above solutions were recorded in the range of 400-200nm using Methanol as blank. Area determined as both wavelengths ranges 310-320nm and 240-250nm for AMB and LORA respectively. The relationship between area under curve (as a dependant variable) and concentration of standard working solution (as an independent variable) were established by simple linear regression method. The regression equation was obtained and this relationship is presented in the calibration curve (Fig. 6, 7). The range of solution has been decided according to correlation coefficient of regression equation.

Precision

The precision of an analytical procedure express the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Intra-day and Inter-day precision was studied by AUC determined at wavelength range between 310-320nm and 240-250nm for AMB (12µg/ml) and LORA (1µg/ml) were for intra-day concentration at six independent series in the same day and inter-day concentration on three subsequent days %RSD was calculated and it was within limit less than 2 (Table-2).

Accuracy

The accuracy of an analytical procedure express the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. Accuracy often expressed as % Recovery by the same assay of known, added amount of analyte by standard addition method. Known amount of standard solution of AMB were added at 80%, 100% and 120% to pre-quantified sample solution of AMB (12µg/ml) and LORA (1µg/ml). The amount of AMB and LORA were estimated from straight line equation of Calibration curve. Three determinations at each level were performed and results were expressed as % RSD (Table-3,4).

Limit of detection (LOD) and Limit of quantitation (LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessary quantitated as an exact value. The quantitation limit of an individual analyte in a sample which can be quantitatively determined with suitable precision and accuracy[21]. The LOD and LOQ were determined by using the formula as,

$$LOD = 3.3 (SD/Slope)$$
$$LOQ = 10 (SD/Slope)$$

Where, S is average value of slope of calibration plots and SD is calculated using values of y intercepts of regression equation.

FORCED DEGRADATION STUDIES

Specificity of the method was determined by calculating percent amount of possible degradation products produced during the force degradation study. The stress conditions applied for degradation study involved acid, base, neutral, sunlight, thermal, UV photolysis, and oxidative degradation for find out the stability nature of the drug. The degradation samples were prepared by taking suitable aliquots of the drug and drug product solution and then undertaking the respective stress testing procedures for each solution. After the fixed time period the treated drug solutions were diluted with solvent. For every stress condition three solutions were prepared as 12µg/ml of AMB and 1µg/ml LORA. The specific stress conditions and results are mentioned in the (Table-6).

Table 1: Assay of Tablet Dosage Forms

Amount of Drug (µg/ml)		Amount Obtained (µg/ml)		% Assay		Mean % Assay	SD		% RSD	
AMB	LORA	AMB	LORA	AMB	LORA	AMB	LORA	AMB	LORA	AMB	LORA
60	5	59.33	5.03	98.88	100.60	98.85	100.11	0.4337	1.0708	0.4387	1.0696
60	5	59.05	5.01	98.41	100.20						
60	5	59.03	5.08	98.38	101.60						
60	5	59.08	4.97	98.46	99.40						
60	5	59.47	4.94	99.11	98.80						
60	5	59.66	4.95	99.43	99.00						

**n=6*

Table 2: Precision Data of AMB and LORA

Drug	AMB		LORA	
Parameters	**Intra-day precision**	**Inter-day precision**	**Intra-day precision**	**Inter-day precision**
Sample solution concentration (µg/ml)	12	12	1	1
Area Under Curve (Mean ± S.D.)*	0.1792 ± 0.0021	0.1739 ± 0.0011	1.1045 ± 0.0161	1.1027 ± 0.0151
% RSD	1.1718	0.2018	0.6159	1.3696

**n=6*

Table 3: Accuracy Data of AMB

Level of Recovery	Sample conc. (µg/ml)	Standard added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Average	SD	% RSD
80%	60	48	106.97	99.05	100.20	1.0136	1.0115
	60	48	109.01	100.94			
	60	48	108.68	100.63			
100%	60	60	120.64	100.54	99.99	1.4438	1.4439
	60	60	118.03	98.36			
	60	60	121.30	101.09			
120%	60	72	131.03	99.27	99.99	1.0518	1.0519
	60	72	133.58	101.20			
	60	72	131.35	99.51			

Table 4: Accuracy Data of LORA

Level of Recovery	Sample conc. (µg/ml)	Standard added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Average	SD	% RSD
80%	5	4	9.10	101.12	100.04	1.0750	1.0745
	5	4	8.90	98.97			
	5	4	9.00	100.05			
100%	5	5	9.96	99.69	100.02	0.3089	0.3088
	5	5	10.03	100.30			
	5	5	10.00	100.08			
120%	5	6	11.06	100.56	99.89	0.7312	0.7320
	5	6	11.13	99.11			
	5	6	11.00	100.00			

Table 5: Summary of Validation Parameters

Parameter	Results	
	AMB	**LORA**
λ max (nm)	310-320	240-250
Linearity Range (µg/ml)	12-72	1-6
Regression Equation (y=mx+c)	y=0.044x+(-0.002)	y=0.007x+(-0.00)
Slope (m)	0.044	0.007
Intercept (c)	-0.002	-0.00
Correlation Coefficient (R^2)	0.999	0.998
	Precision (% R.S.D.*)	
Intra-day	1.1718	0.6159
Inter-day	0.2018	1.3696
Accuracy (Mean % Recovery)	100.06	99.98
LOD	0.2207	0.6648
LOQ	0.6688	1.6548

**n=6*

Table 6: Forced Degradation Study

Sr. No.	Stress Conditions	% Degradation		% Assay	
		AMB	LORA	AMB	LORA
1.	Acid hydrolysis (0.1N HCL, 60°C, 4hrs)	6.74	7.14	93.26	92.86
2.	Alkaline hydrolysis (0.1N NaOH, 60°C, 4hrs)	7.90	4.28	92.1	95.72
3.	Neutral hydrolysis (H_2O, 60°C, 4hrs)	14.18	14.28	85.82	85.72
4.	Oxidative degradation (3% H_2O_2, 60°C, 4hrs)	9.06	17.14	90.94	82.86
5.	Photolytic degradation (UV-radiation, 4hrs)	10.69	15.71	89.31	84.29
6.	Thermal degradation (80°C, 2hrs)	15.81	11.42	84.19	88.58
7.	Sunlight degradation (keep under sunlight, 4hrs)	12.09	8.57	87.91	91.43

RESULTS AND DISCUSSION

The summary of validation parameters for the proposed analytical spectrophotometric method is given in table no. 5. Here, value of R^2 was very close to1 (Fig. 6,7) which suggest that the developed method is following linearity in the concentration range 12-72µg/ml for AMB and 1-6µg/ml for LORA respectively. Results obtained by assay of Ambroxol hydrochloride and Loratadine tablet dosage form indicate that applicability of developed methods to the tablets, as an average amount founds were 98.85 with low % RSD (0.4387) for AMB and 100.11 with low % RSD (1.0696) for LORA. Percent relative standard deviation (%RSD) values for the intra-day and inter-day precision were 1.1718 and 0.2018 for AMB and 0.6159 and 1.3696 for LORA respectively, which is under accepted range. This show the developed method is precise. LOD and LOQ values suggests the lowest amount of drug that can be detected using this analytical procedure is 0.2207µg/ml for AMB and 0.6648µg/ml for LORA and lowest amount of drug in a sample that can be quantitatively determined 0.6648µg/ml for AMB and 1.6548µg/ml for LORA respectively. The percent (%) recovery was found in the ranges of 99.99 to 100.20% (with mean 100.06%) for AMB and 99.89 to 100.04% (with mean 99.98%) for LORA. Results of the recovery studies indicated good accuracy of the method. There was no interference from the excipients of tablet formulation. The validation parameters are summarized in Table 5.The results of all forced degradation studies were found in the range (6.74%-15.81% for AMB) and (4.28%-17.14% for LORA) respectively.

CONCLUSION

All above factors leads to conclude that the developed spectrophotometric method is precise, accurate and linear over the concentration range from 12-72µg/ml for AMB and 1-6µg/ml for LORA; all the analytical reagents used have excellent shelf life, inexpensive and are available easily in any analytical laboratory. Developed method can be applied successfully for the estimation of Ambroxol hydrochloride and Loratadine in bulk and pharmaceutical formulation. Therefore, these methods can be recommended for the routine analysis of Ambroxol hydrochloride and Loratadine in quality control and clinical laboratories. The result of stress degradation studies shows that the drug undergoes degradation.

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