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STRESS DEGRADATION STUDIES AND DEVELOPMENT OF STABILITY INDICATING ASSAY METHOD FOR SIMULTANEOUS ESTIMATION OF AMBROXOL HYDROCHLORIDE AND SALBUTAMOL SULPHATE IN BULK AND ITS FORMULATION

L. R. Gandhi*, A. Sharma

NIMS University, Shobha Nagar, Jaipur

ABSTRACT

Simple, rapid, precise, and accurate, reversed phase high performance liquid chromatographic method was developed and validated for simultaneous determination of Salbutamol Sulphate (SALB) and Ambroxol Hydrochloride (AMBR). The chromatographic separation was achieved on Grace 4.6 (id) x 250 mm column at detector wavelength of 224 nm, using an mobile phase consisting of phosphate buffer and acetonitrile in a ratio of 50:50 at pH 3.0 with a flow rate 1 ml/min. The retention time for Salbutamol Sulphate (SALB) and Ambroxol Hydrochloride (AMBR) were found to be 3.8 and 6.2 respectively. The method showed adequate precision with a relative standard deviation (RSD) smaller than 3%. The accuracy was analyzed by adding a standard drug and good recovery values were obtained for all drug concentration used. The HPLC method developed in this study showed specificity and selectivity with linearity in the working range and good precision and accuracy, making it very suitable for quantification of Salbutamol Sulphate and Ambroxol Hydrochloride in tablet dosage form. Degradation studies were carried out under conditions of Alkali Degradation, Acid Degradation, dry heat Degradation, Peroxide Degradation, and UV light and the drug substances were degraded in all conditions. Force degradation studies shows all the degradant peak obtained during degradation were well resolved from main peak of the drugs. The analytical procedure is reliable and offers not only advantages in terms of speed but also met the regulatory requirements for specificity, Linearity, LOD, LOQ, Precision, accuracy.

KEY WORDS: Salbutamol Sulphate, Ambroxol Hydrochloride, Acetonitrile, Methanol etc.

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***Address for Correspondence**
L. R. Gandhi, Department of Pharmaceutical Sciences, NIMS University, Shobha Nagar, Delhi Highway, Jaipur - India

INTRODUCTION[1-6]:

Ambroxol Hydrochloride (AMBR) is a secretolytic agent used in the treatment of respiratory diseases associated with viscid or excessive mucus. It stimulates the transportation of theviscous secretion in the respiratory organs and reduces the stand stillness of the secretion. It is also anti-inflammatory, reducing redness in a sore throat. (Figure 1) Salbutamol Sulphate (SALB) is a short-acting, selective beta 2-adrenergic receptor agonist used in the treatment of asthma and COPD (figure 2)

Several HPLC methods have been reported in the literature for quantitative determination AMBR alone or with other drug combination and in human serum or other biological fluid. Similarly with SALB various

methods were reported[9-15]. The object of the present work was to develop and validate a simple, economic, rapid, precise, isocratic method with good sensitivity for simultaneous determination of AMBR and SALB in accordance with ICH guideline. Degradation studies were carried out under conditions of Alkali Degradation, Acid Degradation, Dry Heat Degradation, Peroxide Degradation, and UV light and the drug substances were degraded in all conditions[7]. Force degradation studies shows all the degradant peak obtained during degradation were well resolved from main peak of the drugs. The propose method was successfully applied for quality control during manufacture of product.

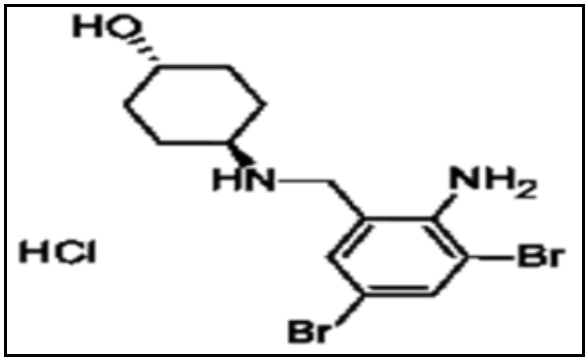

Figure 1: Ambroxol Hydrochloride

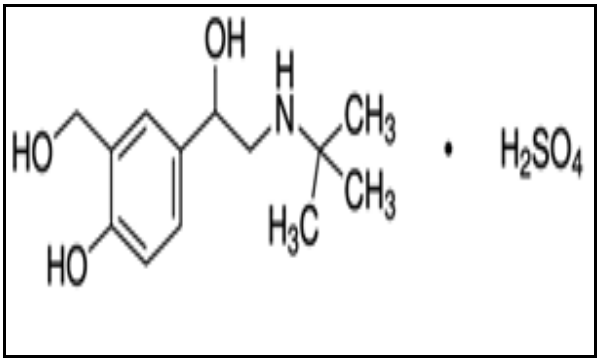

Figure 2: Salbutamol Sulphate

MATERIALS AND METHODS

Chemical, Reagents and Solution

AMBR and SALB gift sample from Leben Lab.Pvt. Akola, India the formulation of this drug is buy from local market i.e. Salmucolite tablet. HPLC grade Methanol, Acetonitrile and Water from Loba chemical.

The stock standard solutions of AMBR and SALB were freshly prepared by dissolving 10 mg of each drug in 10 ml Methanol. The stock standard solutions were further diluted with Methanol to obtain a concentration of 50µg/ml of both AMBR and SALB. The λmax was determined on Shimadzu UV-Visible spectrophotometer (Model UV-1601) in the range 200-400 nm using Methanol as blank. The solution of mixture exhibited maxima at about 224 nm.

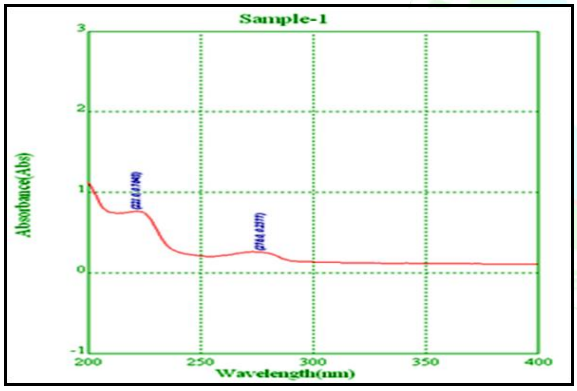

Figure 3: UV spectra of SALB

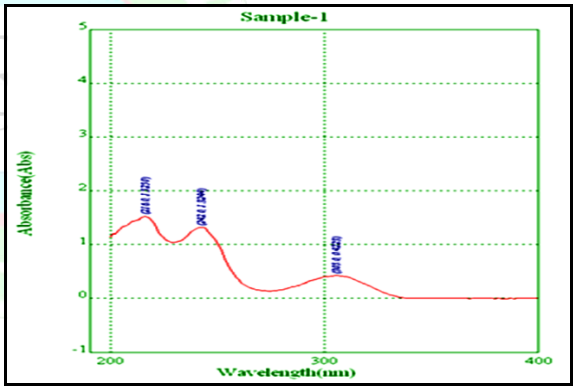

Figure : UV spectra of AMBR

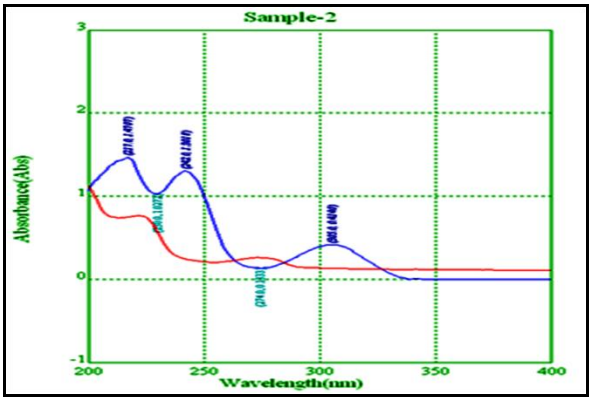

Figure 5: Overlain spectra of SALB & AMBR

HPLC Instrumentation and Chromatographic condition:

After determination of λmax of mixture 224 nm wavelength selected for evaluation of chromatographic parameter. The chromatographic separation was achieved Grace 4.6 (id) x 250 mm column. The standard solution containing mixture of AMBR & SALB was run and different individual solvents as well as combinations of solvents have been tried to get a good separation and stable peak. Each mobile phase was filtered through Whatman filter paper No. 42. well resolved peaks with symmetry within limits and significant Based on sample solubility & stability, various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic conditions. From various mobile phases tried, mobile phase containing. Phosphate buffer:Acetonitrile (50:50), pH 3.0 flow 1ml/ min was selected, since it gives sharp reproducible retention time for AMBR & SALB.(Figure06)

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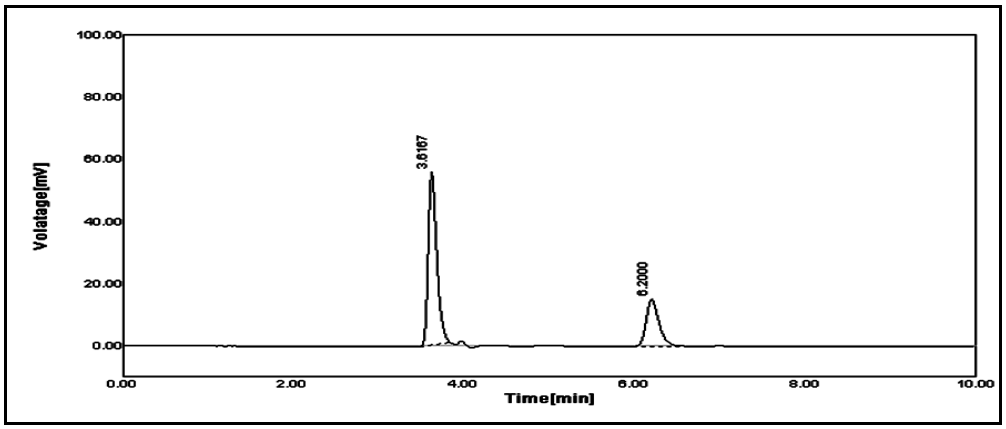

Figure.6: Chromatogram of AMBR & SALB

System suitability test:

System suitability is a Pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done[8]. Filtered mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. A 20µL std. drug solution was injected which was made in five replicates and the system suitability parameters were recorded as shown in Table1

Table.1: Summary of system suitability test result

Sr.no.	Parameter	SALB	AMBR
1.	Peak area	417.67	1355.2
2.	Retention time (min)	6.20	3.81
3.	Asymmetry	1.72	1.991
4.	Efficiency	60370.89	112295.1

Analysis of standard laboratory mixture and marketed formulation to see feasibility of the proposed methods.

Preparation of laboratory mixture (standard and sample): The standard solution of AMBR & SALB were prepare and mixed properly, also sample solution were prepare to obtain laboratory mixtures containing a concentration in a ratio of marketed formulation. The peak area of standard laboratory mixture and sample laboratory mixture was compared to obtain the concentration. The laboratory mixture and formulation Result were recorded and compare as shown in Table:2

Table 2: Summary of laboratory mixture and marketed formulation analysis

S.no.	Sample	Statistical data	% Estimation		% Recovery	
			SALB	**AMBR**	**SALB**	**AMBR**
1.	Standard Laboratory mixture	Mean	99.71	99.83	-	-
		S.D.	0.401	0.651	-	-
		C.V.	0.402	0.652	-	-
2.	Salmucolite	Mean	99.64	99.82	99.83	90.14
		S.D.	0.661	0.582	1.41	0.26
		C.V.	0.663	0.582	1.40	0.26

Method Validation

The analytical method was validated for various parameter according to ICH guideline[16]

Linearity: The linearity of the method was determined at five concentration level ranging from 2-10 µg/ml & 30-150 µg/ml. The graph plotted as the concentration of the drug Vs peak area depicted in figure 07 and 08.

Precision: Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method. Table no 03.

Accuracy - Accuracy of the proposed method was ascertained from the recovery studies by standard addition method. The result shows in table no.03 as bellow

Robustness: To evaluate the robustness of the method, the chromatographic condition were deliberately altered and the resolution between AMBR and SALB was evaluated. Table no. 04

The chromatographic condition selected on various flow rates, different pH were tried.

Ruggedness: The studies of ruggedness were carried out under two different conditions-

- Days (Interday & Intraday)
- Different Analyst.

The summary of result shows in table no 04.

Specificity: Specificity was measured as ability of the proposed method to obtain well separated peak for AMBR and SALB without any interference from component of matrix.

Mean retention time for

AMBR– 6.20

SALB – 3.81

The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix.

Force 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[16]. After the fixed time period the treated drug solutions were diluted with solvent results are mentioned in the table no.5 & 6.

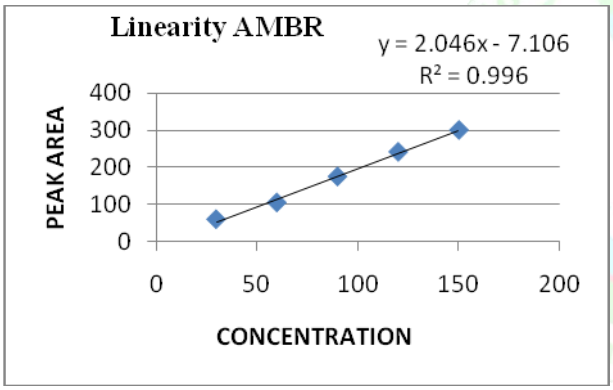

Figure7: Standard calibration curve of AMBR

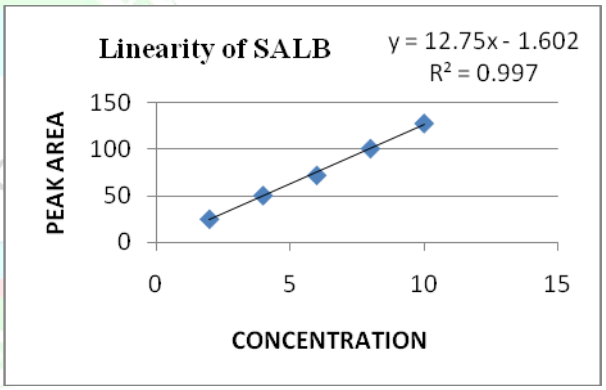

Figure 8: Standard calibration curve of SALB

Table 3: Summary of Precision &accuracy study of SALB and AMBR

Parameter	**Statistical data**	Result	
		SALB	**AMBR**
Precision		99.64	99.82
	S.D.	0.661	0.582
	C.V.	0.663	0.582
Recovery		100.1	100.2
	S.D.	0.5646	0.3076
	C.V.	0.5323	0.2900

Table 4: Summary of results of Ruggedness by RP-HPLC method

Parameter	Statistical data	% Estimation	
		SALB	AMBR
Interday		101.08	100.0
	S.D.	0.666	0.446
	C.V.	0.659	0.440
Intraday	Mean	101.15	100.78
	S.D.	0.9657	1.06
	C.V.	0.9642	1.053
Different analyst	Mean	99.81	99.75
	S.D.	0.41709	0.35118
	C.V.	0.41941	0.35127

RESULTS AND DISCUSSION

A RP-HPLC method was developed and validated for the determination of Salbutamol Sulphate (SALB) and Ambroxol Hydrochloride (AMBR) in tablet dosage form. The mobile phase that was found to be most suitable was Phosphate buffer Acetonitrile (50:50 pH 3.0), flow 1 ml/ min the wavelength 224nm was selected for the evaluation of the chromatogram of both drugs. The selection of the wavelength was based on the λ max obtained by scanning of standard laboratory mixture. This system gave good resolution and optimum retention time with appropriate tailing factor (<2). The mean values of system suitability test result are depicted in Table no 01.

After establishing the chromatographic conditions, standard laboratory mixture was prepared and analysed. It gave accurate, reliable results and was extended for estimation of drugs in marketed tablet formulation. The summary of results of laboratory mixture and marketed formulation are given in the Table. No.02. Degradation studies were carried out under conditions of Alkali Degradation, Acid Degradation, dry heat Degradation, Peroxide Degradation, and UV light and the drug substances were degraded in all conditions. Force degradation studies shows all the degradant peak obtained during degradation were well resolved from main peak of the drugs. The summary of stress degradation shown in table no 05 & 06

Table 5: Stressed Degradation studies of SALB

Sr. No.	Injection	%Assay	% Degradation	Purity Angle	Purity Threshold	Purity Flag
1	Acid Degradation	95.01	4.99	1.387	2.332	No
2	Base Degradation	93.79	6.19	0.301	0.438	No
3	Peroxide Degradation	92.18	7.79	0.711	0.884	No
4	Thermal Degradation	98.09	1.89	1.358	1.582	No
5	UV Degradation	98.60	1.37	0.815	1.637	No

Table 6: Stressed Degradation studies of AMBR

Sr. No.	Injection	%Assay	% Degradation	Purity Angle	Purity Threshold	Purity Flag
1	Acid Degradation	93.8	6.1	0.189	0.399	No
2	Base Degradation	94.70	5.26	0.181	0.939	No
3	Peroxide Degradation	93	6.99	0.684	0.896	No
4	Thermal Degradation	97.79	2.19	0.201	0.405	No
5	UV Degradation	99	0.99	0.198	0.404	No

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