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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF SALMETEROL XINAFOATE IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A RP-HPLC method has been developed for the estimation of Salmeterol Xinafoate in bulk and formulation. The Chromatographic separation has performed with Phenomenex, C8 column (150 x 4.6 mm, 5µm) and mobile phase, Acetonitrile: Water (10:90v/v). The flow rate was 1ml/min and eluent were monitored at 216 nm. The retention time of Salmeterol Xinafoate was 3.6 min. The method was found to be linear over a range of 5-25 µg/ml for Salmeterol Xinafoate with correlation coefficient (r^2= 0.9991). The validation results showed that the method is reproducible, precise and has satisfactory accuracy and linearity profile for the assay of Salmeterol Xinafoate. So, the methods can be successfully applied for the routine analysis of Salmeterol Xinafoate. The degradation studies indicated that Salmeterol Xinafoate showed degradation in acid.

KEYWORDS

Salmeterol Xinafoate, Stability Indicating Method, Validation and RP-HPLC.

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INTRODUCTION

Modern analytical methods of analysis are extremely sensitive, providing precise, accurate and detailed information from small samples of material. Most of the drugs in dosage form can be analyzed by RP-HPLC method. The several advantages like rapidity, specificity, accuracy, precision, and ease of automation in these methods. HPLC method eliminates tedious extraction and isolation procedures[1].

The purpose of stability testing is to provide evidence of how the quality of an API or FPP

(finished pharmaceutical product) varies with time under the influence of a variety of environmental factors such as temperature, humidity and light[2,3].

Chemically Salmeterol Xinafoate is (RS)-4-hydroxy-α′-[[[6-(4-phenyl butoxy) hexyl] amino] methyl]-1, 3-benzenedimethanol 1-hydroxy-2-naphthoate.[4]

Salmeterol Xinafoate is selective adrenergic beta-2 receptor agonist that functions as a bronchodilator when administered by inhalation. It is used to manage the symptoms of asthma and chronic obstructive pulmonary disease. It is formulated as its 1-hydoxy-2-napthoate (Xinafoate) salt. It causes bronchodilation by relaxing the smooth muscle in the airway so as to treat the exacerbation of asthma. The molecule initially diffuse into the plasma membrane of the lung cells, and then slowly release back outside the cell where they come into contact with the beta-2 adrenoreceptors, with the long carbon chain forming an anchor in the membrane[5].

Literature survey reveals that the Salmeterol Xinafoate has been estimated by Spectrophotometric techniques[6], tandem mass spectrometry[7]. LC/MS/MS[8], HPTLC[9], UPLC technique[10] and RP-HPLC[11].

The present work is concerned with development and validation of simple, precise and accurate stability indicating RP-HPLC method for determination of Salmeterol Xinafoate in the presence of their degradation product generated from forced degradation studies.

MATERIAL AND METHODS

Reagents and Chemicals

Salmeterol Xinafoate pure drug was gifted by Vamsi Labs Ltd, Solapur, Maharashtra, India.

The reagents used for the present study are as follows Acetonitrile LiChrosolv®, Methanol LiChrosolv®, Methanol GR Water LiChrosolv®, Sodium hydroxide, Hydrogen Peroxide I.P, ware of analytical grade from Merck Specialities Pvt. Ltd., Mumbai, India. and Hydrochloric acid from S.D Fine chem. Ltd., Mumbai, India.

Salmeterol rotacaps 50mcg strength were purchased from the local pharmacy in Solapur under commercially available brand name Serobid (Cipla).

Instrumentation

The method development and validation of RP-HPLC method were performed on Younglins acme 9000 HPLC system comprising of 4 Channel vacuum Degasser and Mixer SDV50A, Rheodyne sample injection port with 20 µl loop, using Security Guard Cartridges C8 (4x3mm) column with UV730D detector. The chromatogram was recorded with Autochro-3000 software. Shimadzu (Electronic Balance AY220) balance was used for weighing. Other instruments used were UV-Visible Double beam spectrophotometer make Shimadzu 1800, hot air oven (Lab Hosp.™).

HPLC METHOD DEVELOPMENT FOR SX

Preparation of Standard Stock Solution

Standard Stock Solution of SX

10mg of standard SX was weighed and transferred to a 10ml volumetric flask then dissolved in the methanol LiChrosolv®. The volume was made up to the mark with same solvent to obtain conc. of 1000µg/ml of SX. From the resulting solution 1 ml was diluted to 10 ml with the same solvent to obtain conc. of 100µg/ml of SX, and labeled as 'Std Stock SX'.

Selection of Analytical Wavelength

To investigate the appropriate wavelength for determination of SX, the solution in the mobile phase was scanned in the range of 200-400nm.

Selection of Mobile Phase

Solution of SX (10µg/ml) was prepared in methanol and filtered through syringe filter, then injected into the HPLC system, after the column saturated with mobile phase and constant back pressure. The solution was analyzed using different combinations of Acetonitrile: Methanol: Water (5:20:75), Methanol: Water (30:70, 15; 85, 20:80), Acetonitrile: Water (50:50, 30:70, 10:90 at flow rate of 1ml/min for 10 to 20 10 min at 216nm.

Chromatographic Conditions

Analytical Column

Phenomenex C8 column (150 mm × 4.6 mm, 5 µm)

Mobile Phase

Acetonitrile: Water (10:90)

Flow Rate

1ml/min

Injection Volume
20 µl
Detection Wavelength
216nm.

FORCED DEGRADATION STUDY

Preparation of stock solution of SX
1 ml of Std stock SX (100µg/ml) solution diluted with mobile phase up-to 10ml concentration to get solution of 10µg/ml. This solution was filtered through syringe filter and injected in HPLC. The chromatogram was recorded and Peak area of drug was noted.

Acid hydrolysis
In 1ml stock solution 1ml 1N HCL was added, This solution was subjected for stress by heating at 60^0C for 30 min. After heating neutralized this solution of 1N NaOH and diluted up to 10ml with mobile phase solution. The solution was filtered through 0.45µm syringe filter, injected in HPLC. The chromatogram was recorded and Peak area of drug, degradation products were noted and amount of drug degraded was calculated.

Alkaline hydrolysis
In 1ml stock solution 1ml of 1N NaOH was added, this solution was subjected for stress by heating at 60^0C for 10 min. After heating neutralize this solution with 1N HCL and dilute up to 10ml with mobile phase. The solution was filtered through syringe filter, injected in HPLC. The chromatogram was recorded and Peak area of drug, degradation products were noted and amount of drug degraded was calculated.

Oxidation
1ml of 30% hydrogen peroxide diluted up to 10ml with methanol. From that 0.1ml was transferred in 1ml stock solution, this solution was subjected for stress by keeping at room temperature for 10min.The solution was diluted up to 10ml with mobile phase concentration of solution 20µg/ml. The solution was filtered through syringe filter, injected in HPLC. The chromatogram was recorded and Peak area of drug, degradation products were noted and amount of drug degraded was calculated.

Thermal
Standard Salmeterol Xinafoate was spread uniformly on petriplate and plate kept in Hot air oven at 80 ^{0}C 48 hrs. Powder equivalent to 10mg was dissolved in 10ml methanol to get concentration 1000µg/ml. 0.2ml of resulting solution diluted up to 10ml with mobile phase to obtain concentration 20µg/ml. The solution was filtered through syringe filter injected through HPLC system. The **c**hromatogram was recorded peak area of drug recorded and amount of drug degraded was calculated.

VALIDATION OF RP-HPLC METHOD

Specificity
The chromatogram of standard solution of SX was compared with chromatogram of SX with degradants.

Linearity
From the 'Std Stock SX' (100µg/ml) solution 0.5, 1, 1.5, 2 and 2.5 ml were transferred in a series of 10ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain the concentration of 5, 10, 15, 20, and 25 µg/ml of SX.
The solutions were filtered through syringe filter and 20µl injected into the HPLC system and their chromatogram were recorded for 10mins. Under the chromatographic conditions as described above after getting a stable baseline. Peak areas were recorded for all the peaks. Calibration curves of SX was constructed by plotting the peak area of SX *v/s* Conc. of SX. The correlation coefficient (r^2) of least square linear regression for SX was calculated.

Range
The range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the curve. The correlation coefficient (r^2) of least square linear regression for SX was calculated.

Accuracy
0.1ml Sample Solution was transferred to four different 10 ml volumetric flasks and 0, 0.8, 1, 1.2 ml of (SX 100 µg/ml) Standard solution was added and volume adjusted to 10 ml with methanol. All the solution were filtered through syringe filter and injected into the HPLC system and chromatograms

were recorded under the same chromatographic conditions after getting a stable baseline. Peak area was recorded for all the peaks. From above data percentage recoveries were calculated.

Precision

The precision of an analytical method was studied by performing Repeatability.

Repeatability

From the 'Std Stock SX' (100µg/ml) solution, 1ml was transferred in 10ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain the conc. of 10µg/ml of SX. The solution was filtered through syringe filter and 20µl injected into the HPLC system and its chromatogram was recorded under the same chromatographic conditions after getting a stable baseline. Peak area was recorded. The procedure was repeated for six times.

Limit of Detection

LOD calculated by the following formulae.

LOD = 3.3(SD/S)

Where, SD- Standard deviation; S- Slope of Curve

Limit of Quantitation

LOQ calculated by the following formulae.

LOQ = 10(SD/S)

Where, SD- Standard deviation; S- Slope of Curve

Robustness

Combined standard solution of (10µg/ml) was prepared and analyzed at different flow rates (0.9, 1.0, 1.1 ml/min) and different wavelengths (214, 216, 218nm) separately.

System Suitability

Sample solutions of SX (10µg/ml) were prepared and analyzed six times.

Assay of Capsule Dosage Form

Quantity of the contents of the capsules (Serobid rotacaps) were weighed. An accurately weighed contents of the capsules equivalent to 10 µg of SX was transferred to 10 ml volumetric flask and dissolved in methanol and made the volume with methanol up to 10 ml (Sample Solution) and filtered through syringe filter, injected in to the HPLC system and chromatograms was recorded under the same chromatographic conditions after getting a stable baseline. Peak areas were recorded and percentage of SX was calculated.

RESULTS AND DISCUSSION

RP-HPLC Method for SX

Selection of wavelength

SX shows the maximum absorbance at 216nm. Hence, HPLC analysis was carried out at 216nm.

Selection of Mobile phase

(C8 Column, at 1ml/min flow rate, detection wavelength is 216nm, mobile phase ratio containing Acetonitrile: Water (10:90) respectively)

After several permutations and combinations of mobile solvents with stationary phase C8, the above method has been optimized i.e. Acetonitrile: Water (10:90) respectively using C8 column which has given good resolution, capacity factor, etc.

Identification of Peak

With above optimized conditions SX was eluted at 3.6 min. SX eluted within 10 min which will reduce the analysis time and cost.

RESULT OF STRESS DEGRADATION STUDY OF SX

Degradation Chromatogram of SX

SX Standard (API)

Acid Induced Degradation

For acid hydrolysis, API solution was treated with 1N HCL and solution heated on water bath at 60ºC for 30 min. A degree of degradation was achieved in 1N HCL and degradants appeared at 1.7. The percent degradation of drug was found to be 15.95%.

Alkaline Induced degradation

For alkaline hydrolysis, API solution was treated with 0.1N NaOH and solution heated on water bath at 60ºC for 10 min. The percent degradation of drug was found 43.2%.

Oxidation Induced degradation

For Oxidation degradation, API solution was treated with 6% H_2O_2 at RT for 10 min. The percent degradation of drug was found 54.41%.

Thermal Induced degradation

For thermal degradation, API SX was subjected in hot air oven for 60^0 for 48hrs.No sufficient degradation was observed therefore drug said to be stable for thermal degradation.

VALIDATION OF HPLC METHOD

Specificity

The chromatogram of standard solution of SX was compared with chromatogram of SX with degradants indicating that there is no interference of degradants.

Linearity

Range

The range of analytical methods was decided from the interval between upper and lower level of calibration curve by plotting the curve. The range is from 5-25 % of the test concentration.

Accuracy

Precision

The precision, evaluated as the repeatability of the method, was calculated as %RSD values for six determinations of peak area ratio, performed on the same day and under the same experimental condition.

Limit of Detection

Detection limit is calculated based on standard deviation of response and slope.

Limit of Quantification

Quantification limit is calculated based on standard deviation of response an

Robustness

The robustness was investigated by achieving deliberate changes in flow rate by ±1 units from 0.9 to 1.1ml/min and change in wavelength by ±2nm that is at 214 to 218nm Robustness of the method was carried out at concentration of 10µg/ml. and then T,Rs and N were evaluated. The system suitability parameters remained unaffected over deliberate small changes in the chromatoghraphic system, illustrating that the method was robust over an acceptable working range of its HPLC operational parameters.

System Suitability Testing

Study of resolution, tailing factor and capacity factor shows system is suitable for this method.

Assay of Capsule dosage forms

Table No.1: Optimization of Chromatographic Conditions

S.No	Mobile Phase Ratio	RT (min)	Area (mVs)	Tailing Factor	Theoretical Plates	Resolution
1	50:00:50:00	1.1	605	1.55	646	0.0
2	30:00:70:00	1.1	598	1.25	960	0.0
3	00:30:70:00	1.8	239	1.81	856	2.6
4	00:15:85:00	6.8	260	0.75	1482	2.2
5	00:20:80:00	4.8	210	0.68	847	7.0
6	05:20:75:00	1.8	463	1.86	2035	4.3
7	10:00:90:00	3.6	745	0.64	2132	4.0

Table No.2: Result of Stress Degradation Study of SX (API)

	Degradation Mode	Condition	% Assay of active ingredient	% Degrandants	Retention time of Degradation product found
SX (API)	Acid Hydrolysis	1N HCL, at 60^0C, 30min	84.05	15.95	1.7, 7.3
	Alkaline Hydrolysis	1N NaOH, at 60^0C, 10 min	56.80	43.2	2.0
	Oxidation Hydrolysis	H_2O_2, at RT 10 min	45.59	54.41	1.3
	Thermal	80^0C, 48hrs	56.94	43.06	1.1

Table No.3: Chromatographic Response on SX at various linearity levels

S.No	Conc. of SX (µg/ml)	Peak Area (mV)
1	5	394
2	10	712
3	15	1067
4	20	1445
5	25	1806

Table No.4: Linear regression analysis of calibration curve for SX

S.No	Parameters	SX
1	Slope	71.14
2	Intercept	17.7
3	Correlation Coefficient (r^2)	0.9991

Table No.5: Range for RP-HPLC Method

S.No	Parameter	SX
1	Linearity Range (µg/ml)	5-25

Table No.6: Accuracy study for SX

S.No	Level of % Recovery	Amount of Sample	Amount of Std Drug added (µg/ml)	Total Amount found (µg/ml)	Amount Recovered (µg/ml)	%Recovery
1	0	0.1	-	0.09	-	-
2	80	0.1	0.8	8.41	8.32	83
3	100	0.1	1	10.12	10.3	101
4	120	0.1	1.2	12.73	12.64	105

Table No.7: Precision study for SX

Precision	Analytical Response(Peak area)						% RSD
	1	2	3	4	5	6	
Repeatability	712	720	729	731	742	745	1.72

Table No.8: Limit of Detection Data of SX

S.No		SX
1	LOD(µg/ml)	0.58

Table No.9: Limit of Quantification data of SX

S.No		SX
1	LOQ(µg/ml)	1.77

Table No.10: Result of Robustness Study: Variation in flow rate and wavelength

S.No	Conditions	Range Investigated	Retention Time (min)	Theoretical Plates(N)	Resolution	Tailing Factor(T)
1	Flow Rate (ml/min)	0.9	3.6	2142	3.0	0.62
		1.1	3.3	2717	2.4	0.61
2	Wavelength (nm)	214	3.5	2091	5.6	1.07
		218	3.7	2345	4.5	0.60

Table No.11: Results of System Suitability Parameters

S.No	Analyte	Retention Time (min)	Tailing Factor (T)	Theoretical Plates (N)	Resolution (R)
1	SX	3.6	0.64	2132	4
2	Required limits	--	T < 2	N > 2000	R >2

Table No.12: Assay of Capsule dosage forms

S.No	Drug Name	Label claim (µg)	% amount found in drug
1	SX	50	102%

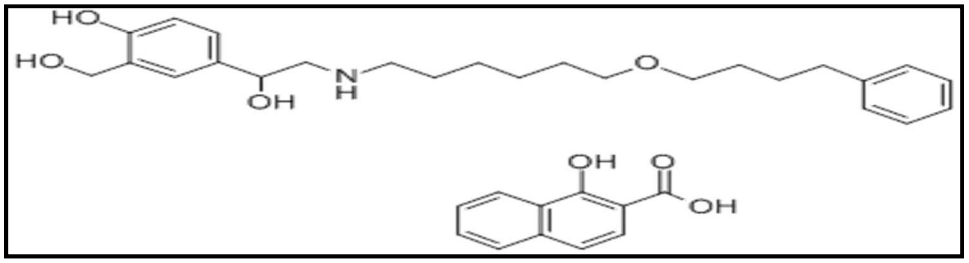

Figure No.1: Chemical structure of Salmeterol Xinafoate

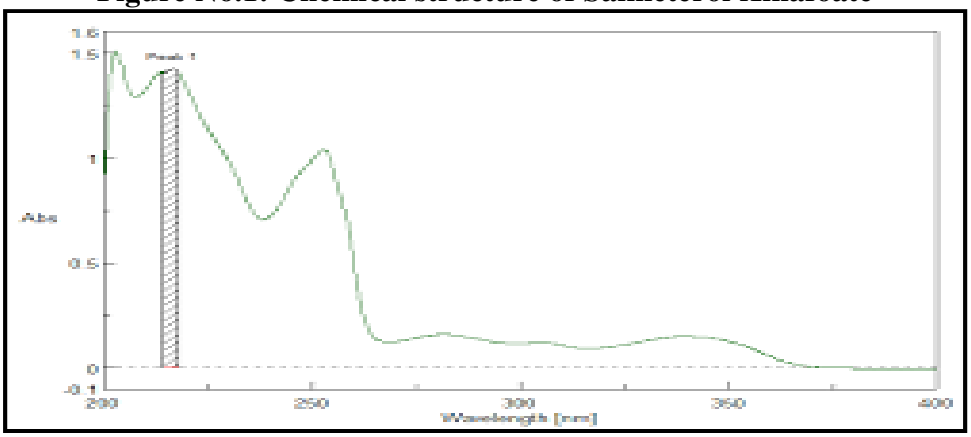

Figure No.2: UV spectrum of SX (10µg/ml) between 200-400nm in mobile phase

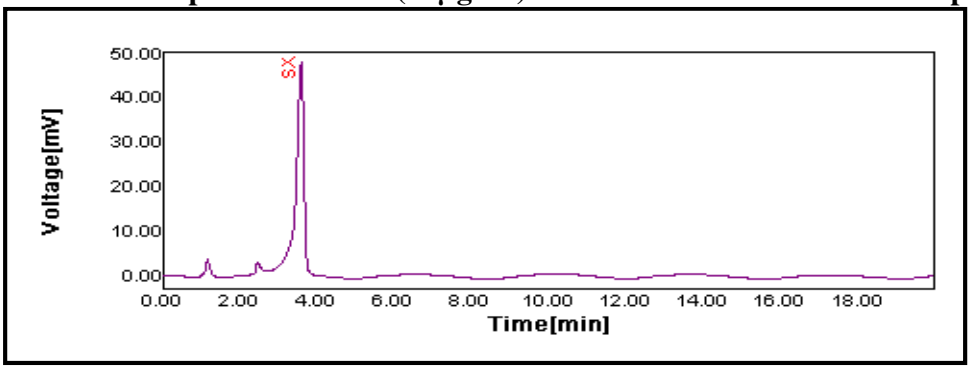

Figure No.3: Chromatogram of SX (10µg/ml) in optimized chromatographic conditions

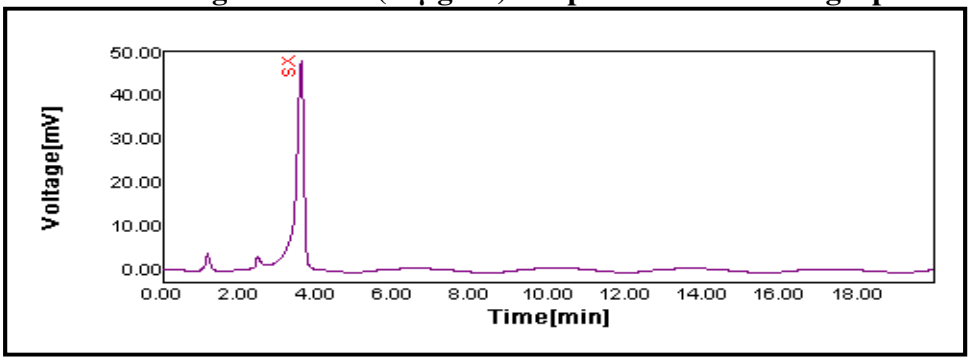

Figure No.4: Chromatogram of SX without treatment

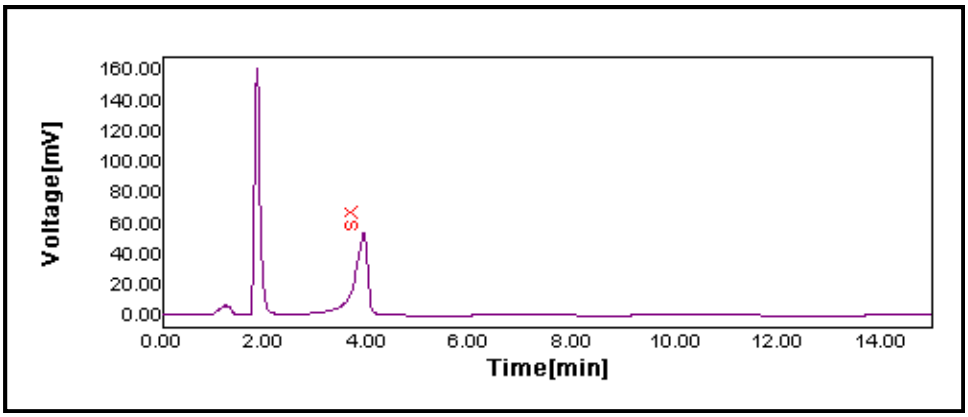

Figure No.5: Chromatogram 1N HCL treated with SX

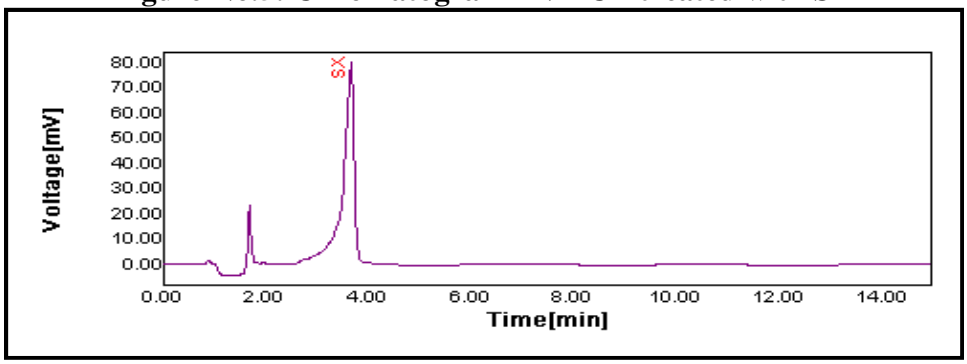

Figure No.6: Chromatogram 1N NaOH treated with SX

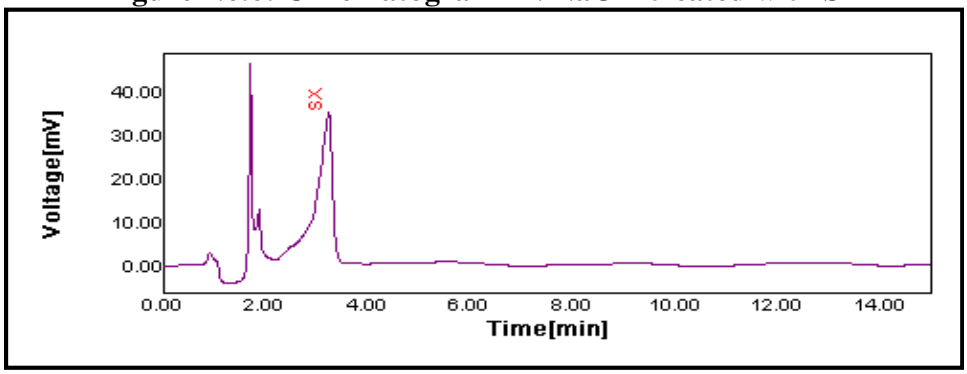

Figure No.7: Chromatogram of 6% H_2O_2 treated with SX

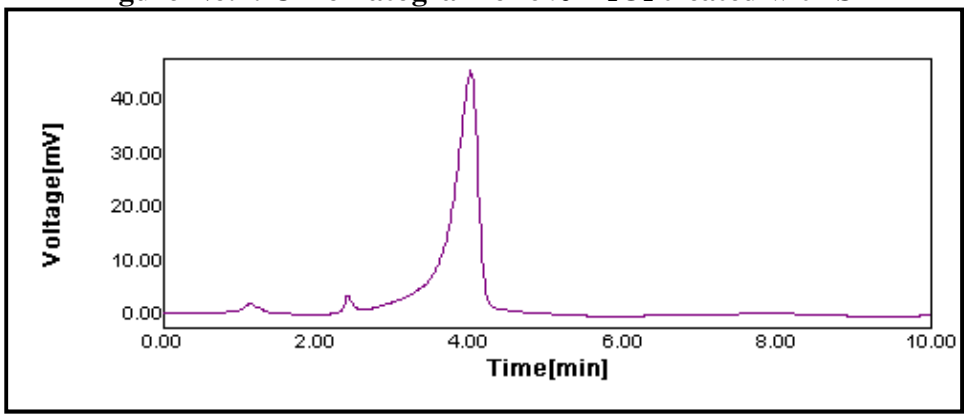

Figure No.8: Chromatogram of thermal treated with SX

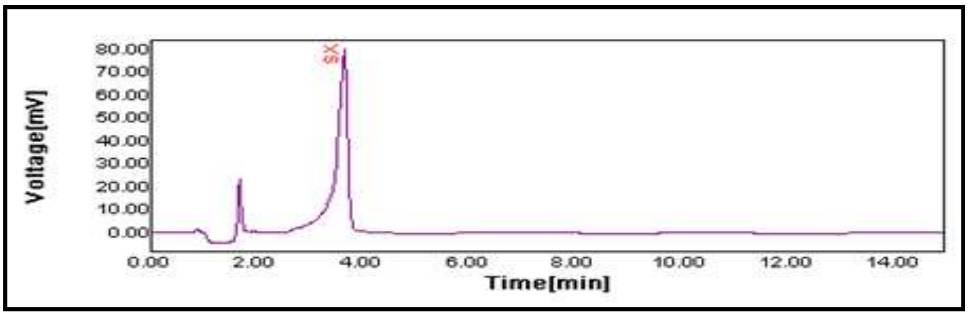

Figure No.9: Chromatogram of SX with degradants

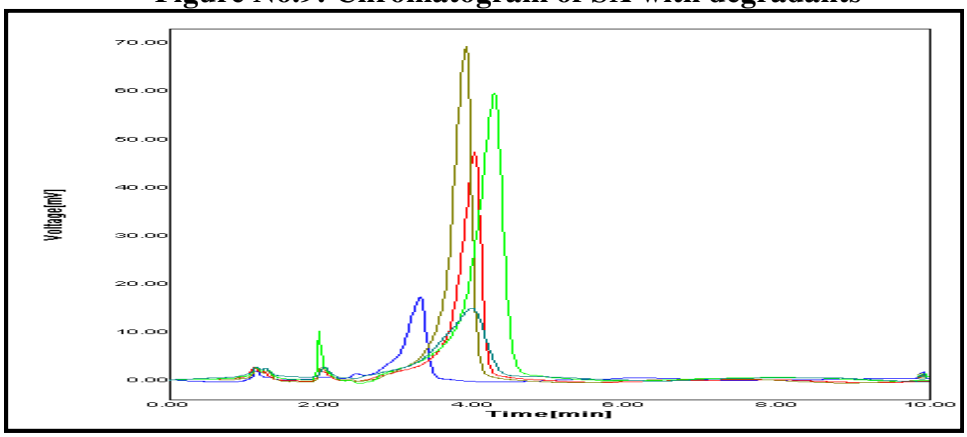

Figure No.10: Overlain chromatograms of serial dilutions of SX in optimized chromatographic conditions

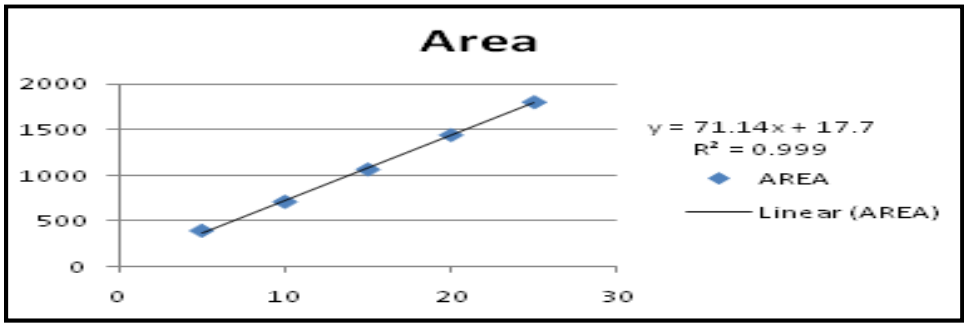

Figure No.11: Calibration curve of SX of RP-HPLC method

CONCLUSION

In conclusion, the proposed HPLC method is simple, accurate, reproducible method for estimation of SX in bulk and pharmaceutical formulation. The short chromatographic time makes this method suitable for processing of multiple samples in short time. The method shows no interference by the excipients. The statistical parameters and recovery data reveals the good accuracy and precision of the proposed method. Finally, since no pharmacopoeial method for determination of SX in bulk and pharmaceutical formulations have been reported yet, the proposed method could be useful and suitable for the estimation of the SX in bulk.

In the proposed study, stability indicating RP-HPLC method was developed for the estimation of SX validated as per ICH guidelines. The suitability of the method to study stability of SX under various forced degradation condition *viz,* acid, base, hydrogen peroxide, thermal degradation it can be conclude that method separates the drug from their degradation products it may be employed for analysis of stability samples of SX.

ABBREVIATIONS

UV-Ultra Violet
API- Active Pharmaceutical Ingredient
SX- Salmeterol Xinafoate

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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