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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD FOR ESTIMATION OF SALMETEROL XINAFOATE

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ABSTRACT: Simple, rapid validated stability-indicating HPLC method for estimation of Salmeterol xinafoate was successfully developed. The separation was achieved by using a mobile phase of buffer: methanol in the ratio of 60:40 v/v using HiQ SiL C18 column (150 × 4.6 mm i.d. 5 µm) at 1.2 mL/min as flow rate. The detection was carried out at 252 nm using a PDA detector. The retention time observed was 11.89 ± 0.3 min. This drug was subjected to stress conditions as per ICH Q1A (R2). Linearity was found to be in the concentration range of 10-50 µg/mL with r^2 = 0.9958. The suitability of this HPLC method for quantitative estimation of Salmeterol xinafoate was proved by validation by the requirements of ICH guidelines Q2A (R1).

INTRODUCTION: Chemically Salmeterol xinafoate is (RS) -4 -hydroxy -α1-[[[6 -(4-phenyl-butoxy) hexyl] amino] methyl] -1, 3 -benzenedi methanol 1 -hydroxy -2 -naphthoate. Salmeterol xinafoate belongs to bronchodilator drugs category. The chemical formula is $C_{25}H_{37}NO_4$, $C_{11}H_8O_3$ and molar mass is 603.756 g/mol. Salmeterol xinafoate is official in IP 2014. It is freely soluble in methanol. Salmeterol xinafoate is white to off-white powder [1]. Literature survey reveals few analytical methods are reported for the determination of Salmeterol xinafoate *viz*. HPLC [2], [3, 4], SIM RP-HPLC [5], UPLC [6], HPTLC [7], UV-spectrophotometric [8].

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The ICH guideline for stability testing of new drug substances and products requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions to determine the stability of the molecule [9]. There is only one stability indicating Column Chromato-graphic method; therefore the aim of the present work is to develop an accurate, specific, and reproducible stability indicating HPLC-PDA method for determination of Salmeterol xinafoate and to develop a validated stability-indicating assay method.

MATERIALS AND METHODS:

Reagents and Chemicals: The working standard Salmeterol xinafoate was provided by NATCO Pharma (Hyderabad, India). The reagents used for the present study are as follows methanol HPLC grade (MeOH), distilled water, hydrochloric acid

(HCl), sodium hydroxide (NaOH), 6% w/v hydrogen peroxide (H_2O_2) were of analytical grade from Loba Chemie Pvt. Ltd., Mumbai, India.

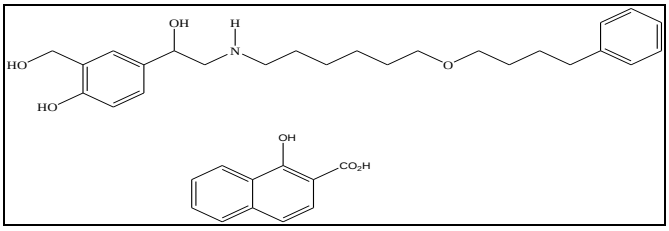

FIG. 1: STRUCTURE OF SALMETEROL XINAFOATE

Instrumentation: The method development and validation of RP-HPLC method were performed on JASCO HPLC system comprising of model PU 2080 Plus pump, Rheodyne sample injection port with 20 µl loop, using HiQ SiL C18 Column with MD 2010 PDA detector. The chromatogram was recorded with Borwin- PDA software (version 1.5). Shimadzu (Model AY-120) balance was used for weighing. Other instruments used were UV-Visible Double beam spectrophotometer make Jasco Model V-730, Elga Lab water purification system (PURELAB UHQ-II), hot air oven (Kumar Laboratory Oven), Photostability chamber (Make Newtronic. Model IC DAC version 1.2).

Preparation of Stock Solution: Standard stock solution of Salmeterol xinafoate was prepared by dissolving 10 mg of drug in 10 ml of methanol to get a concentration of 1000 µg/mL. It was suitably diluted with mobile phase to get concentration of solution 500 µg/mL working standard solution was prepared to contain Linearity range 10-50 µg/mL using the mobile phase as a solvent.

Selection of Detection Wavelength: From standard stock solution, appropriate dilution was made using Methanol and scanned over the range of 200-400 nm.

Optimized Chromatographic Conditions: The various mobile phases tried consisted of phosphate buffer pH 5: ACN: MeOH, water: ACN, ACN: MeOH, MeOH (0.05M HSASS): ACN, phosphate buffer (pH 7): ACN, ammonium acetate (pH 3): ACN, water: ACN: MeOH, potassium dihydrogen phosphate buffer pH 5 with 10M KOH: methanol. The optimized mobile phase consisted of buffer: methanol in the ratio of 60:40 v/v was chosen as the mobile phase, which gave good resolution and acceptable peak parameters. It was then filtered through a 0.45 µm membrane filter using vacuum filtration assembly. They were then sonicated using an ultrasonic water bath for 10 min.

Stress Degradation Studies of Bulk Drug: The forced degradation studies were carried out on bulk drug substance to prove the stability-indicating property and selectivity of the developed method. The API was subjected to hydrolysis under different pH, oxidative, thermal and photolytic conditions. Optimization of conditions was done by changing the strength of reagent and the duration of exposure to achieve degradation in the 10-30% range.

Acid Treatment: 1 ml working standard solution of Salmeterol xinafoate (500 µg/mL) was mixed with 1 ml of 1N hydrochloric acid (HCl) and 8 ml of mobile phase to get a final concentration of 50 µg/mL and solution was kept at room temperature for overnight.

Alkali Treatment: 1 ml working standard solution of Salmeterol xinafoate (500 µg/mL) was mixed with 1 ml of 1 N sodium hydroxide (NaOH) and 8ml of mobile phase to get a final concentration of 50 µg/mL and the solution was kept at room temperature for overnight.

Oxidative Degradation: 1 ml working standard solution of Salmeterol xinafoate (500 µg/mL) was mixed with 1 ml of 6% w/v hydrogen peroxide (H_2O_2) and 8 ml of mobile phase to get a final concentration of 50 µg/mL and the solution was kept at room temperature for overnight.

Thermal Degradation: Thermal degradation was performed by keeping the drug in an oven at 80 ºC for a period of 2 h. A sample was withdrawn, weighed and dissolved in the mobile phase to get the solution of 50 µg/mL.

Photolytic Degradation: Salmeterol xinafoate was exposed to UV light (200-watt-hours/square meter) and cool white fluorescent light (1.2 million lux hours). The sample was weighed, dissolved in mobile phase and diluted to a concentration of 50 µg/mL.

RESULTS AND DISCUSSION:

Selection of Analytical Wavelength: It was observed that Salmeterol xinafoate showed

considerable absorbance at 252 nm; hence this wavelength was chosen for detection.

The result of Forced Degradation Studies: Degradation was seen in acidic, alkaline and oxidation condition. No degradation was seen in Thermal and photolytic conditions for pure Salmeterol xinafoate in HPLC. Also, there is no separate degradation peak in any condition. It was confirmed by multi-wavelength analysis peak purity comparison. These degradation studies indicated that Salmeterol xinafoate was susceptible to hydrolysis under acidic, basic pH and also to oxidative conditions. Relatively stable in other stress conditions.

TABLE 1: CHROMATOGRAM AND SYSTEM SUITABILITY PARAMETER OF DRUG

Name	RT (min)	Conc. (µg/mL)	Area	Theoretical plates	Asymmetry	Resolution
Salmeterol xinafoate	12.16	50	1949235.47	4838.56	1.46	1.86

TABLE 2: OPTIMIZATION OF CHROMATOGRAPHIC CONDITION

S. no.	Parameter	Conditions used for Analysis
1	Column	HIQ SIL C18 column (150 × 4.6 mm i.d, 5 µm)
2	Mobile phase	Potassium dihydrogen phosphate buffer: Methanol:: 60:40 v/v
3	Flow rate	1.2 mL/min
4	Detection wavelength	252 nm
5	Sample volume	20 µl
6	Column temperature	Room temperature

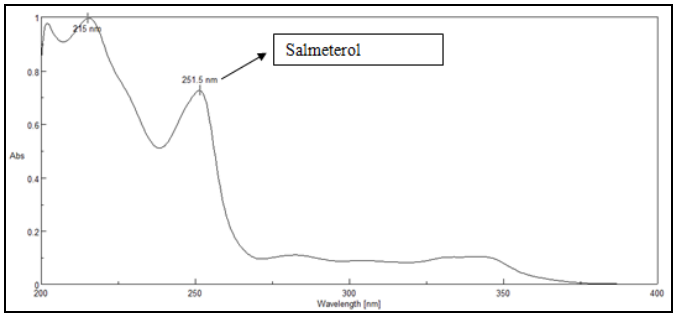

FIG. 2: UV SPECTRUM OF SALMETEROL XINAFOATE (10ppm)

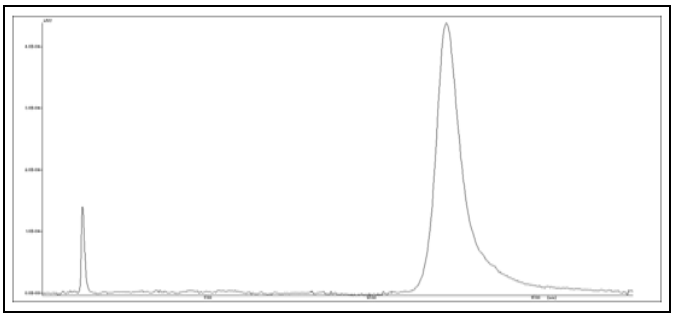

FIG. 3: CHROMATOGRAM OF STANDARD SALMETEROL XINAFOATE (50ppm)

TABLE 3: RESULTS OF THE STRESS DEGRADATION STUDIES FOR STANDARD SALMETEROL XINAFOATE

S. no.	Parameters	Condition	% Recovery
1	Initial	No treatment kept overnight	-
2	Acid hydrolysis	1 N HCl overnight at RT	80.56
3	Alkali hydrolysis	1N NaOH overnight at RT	66.77
4	Oxidative stress degradation	6% H_2O_2 overnight at RT	71.00
5	Neutral hydrolysis	H_2O	113.3
5	Photolytic	The UV light (200 watt-hours/square meter)	97.06
	degradation	cool white fluoro light (1.2 million lux hours)	107.47
6	Dry heat degradation	Hot air oven at 60 ˚C at 8 h	95.45

Validation of the Method: [10] The method was validated for various parameters by ICH guideline.

Specificity: Peak purity profiling studies ascertained the specificity of the method. The peak purity values were found to be more than 990, indicating the non-interference of any other peak of degradation product or impurity

Linearity: Calibration curve was obtained in the range 10-50 µg/mL, peak area was recorded. Standard calibration graph was plotted of peak area *vs.* concentration injected. The equation of calibration curve found to be Y = 37986x + 49376 having coefficient of correlation (r^2) = 0.9958 shown in **Fig. 5**.

Range: The linearity range was found to be 10-50 µg/mL of Salmeterol xinafoate.

Assay: The assay was carried out using a marketed formulation. Salmeterol xinafoate is available in the market as Serobid rotacaps 50 mcg capsule for 75 rupees. 100 mcg Salmeterol xinafoate

equivalent powder was weighed. And dissolve in 4 ml methanol sonicate for 10 min. Centrifuge the mixture and filter through membrane filter paper. Then dilution was made to get a concentration of 20 µg/mL. The assay was found to be 99.75% for Salmeterol xinafoate.

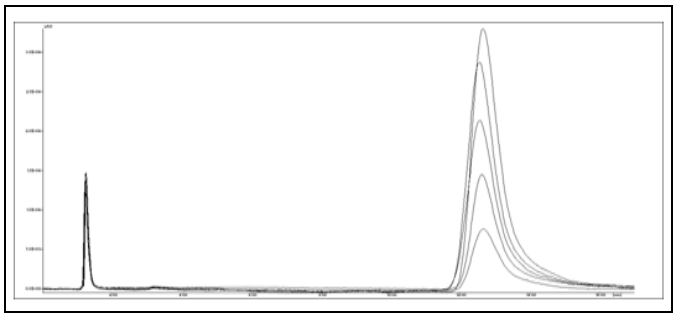

FIG. 4: CHROMATOGRAM OF CALIBRATION CURVE OF SALMETEROL XINAFOATE (10-50 mg/µL)

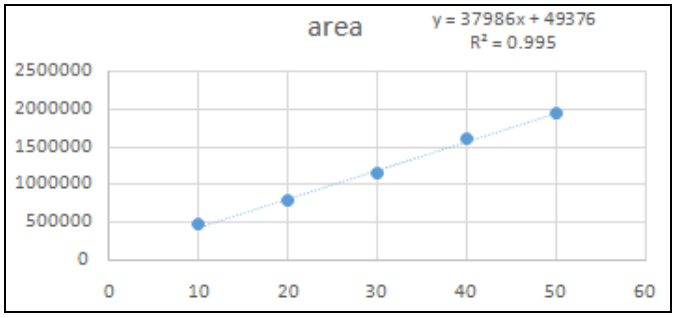

FIG. 5: CALIBRATION CURVE FOR SALMETEROL XINAFOATE AT 252 nm

Accuracy: To check the accuracy of the method, recovery studies were carried out by adding the standard drug to blank blend at three different levels 80, 100 and 120%. The results of the recovery studies indicated that the method is accurate for estimation of the drug in the blend. The results obtained are shown in **Table 4**.

TABLE 4: RECOVERY STUDIES FOR SALMETEROL XINAFOATE

Level (%)	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery
80	18	18.06	100.35
100	20	19.79	98.95
120	22	22.23	101.06

Precision: The intra-day and inter-day precision of the HPLC method are shown in **Table 5**. Results expressed in terms of % RSD, which describes intra-day and inter-day variation of Salmeterol xinafoate at a concentration of 10 µg/mL (n= 6).

TABLE 5: INTRA-DAY AND INTER-DAY PRECISION

S. no.	Amount (µg/mL)	Intra-day	SD	% RSD	Inter-day	SD	% RSD
1	10	466780.938	5412.67	1.17	459626.427	5172.60	1.11
2	10	453479.303			468876.155		
3	10	465406.473			467521.875		
4	10	455712.297			459958.249		
5	10	456714.108			458591.209		
6	10	460218.821			469735.486		

Limit of Detection (LOD) and Limit of Quantification (LOQ): The limit of detection and limit of quantification is the lowest concentration of an analyte in a sample which can be detected and quantified with acceptable accuracy and precision. The LOD and LOQ of the developed method were calculated using the formula as given below.

$$\text{Limit of Detection} = 3.3*\sigma / S$$

$$\text{Limit of Quantitation} = 10*\sigma / S$$

Where, σ = standard deviation of the response at the lowest concentration, S = slope of the calibration curve.

LOD and LOQ were found to be 0.40 µg/mL and 1.24 µg/mL respectively, which show the sufficient sensitivity of the method.

Robustness: Peak area checked after flow rate variation, change in mobile phase composition and change in pH by varying the amount of potassium dihydrogen phosphate buffer were well within the limit, indicating that the proposed method is robust under given set of defined experimental conditions.

Solution Stability: The Standard stock solutions of Salmeterol xinafoate were found to be stable for 48h if stored at room temperature.

TABLE 6: SUMMARY OF VALIDATION STUDY

S. no.	Validation Parameters	Salmeterol xinafoate
1	Linearity Equation	Y = 37986x+49376
	(r^2)	r^2 = 0.9958
2	Range	10-50 µg/mL
3	Precision (% RSD)	
	Interday	1.11
	Intraday	1.17
4	Accuracy	
	80%	100.35
	100%	98.95
	120%	101.06
5	Limit of Detection (µg/mL)	0.40
6	Limit of Quantitation (µg/mL)	1.24
7	Specificity	Specific
8	Robustness	Robust
9	Solution stability	Stable

DISCUSSION: The HPLC method was developed using an isocratic system with a run time 17 min. The assay method in Indian Pharmacopoeia (2014) is based on a gradient system with a run time 60 min. As per the literature survey, degradation pattern reported in acid, base, and oxidation is completely different. In other condition, like neutral hydrolysis, photodegradation and thermal, no visible degradation was seen. P. S. Jain *et al*., have reported stability-indicating RP-HPLC method for estimation of Salmeterol xinafoate in bulk and a pharmaceutical formulation. In this work, they have used refluxing at 70 ºC as the condition for hydrolytic and oxidative degradation. Photo degradation has been carried out using ordinary sunlight. In the present work, hydrolytic degradation was carried out at room temperature; photostability chamber with UV as well as fluorescent light was used for photodegradation studies. There was a peculiar observation of an increase in absorptivity upon the heating the solution. Thus our observation does not match with the ones reported by P. S. Jain.

CONCLUSION: The developed method is simple, rapid and stability indicating. It may be used to monitor stability of Salmeterol xinafoate.

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CONFLICT OF INTEREST: Authors have no conflicts of interest to declare.

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