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Method Development and Validation of Salmeterol xinofoate by HPLC

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Abstract:- ***A rapid and precise high performance liquid chromatography method has been developed for the validation of Salmeterol xinofoate in its pure dosage form. The separation was carried out on Agilent Zorbax Bonus RP- (250mm ×4.6mm 5µ) column with a mobile phase consisting of 0.1% Formic acid: Acetonitrile in the ratio of 64:36 v/v as a mobile phase and flow rate is 1ml/min. The detection was carried out at wavelength 234nm. The column thermostatically controlled at 30℃ The retention time of Salmeterol was found to be 1.96 min. The Salmeterol xinofoate followed linearity in the concentration range of 40-60µg/mL with r^2= 0.999. The developed method was validated for sensitivity, accuracy and precision. The sample was scanned from 200-400nm with PDA detector. The % recovery of sample was found to be. The LOD and LOQ of the Salmeterol xinofoate was found to be 2.67µg/ml and 8.08µg/ml respectively. The suitability of this HPLC method for quantitative estimation of Salmeterol xinofoate was proved by validation by the requirements of ICH guidelines.***

Keywords:- Salmeterol xinofoate, HPLC, Validation, ICH guidelines.

Introduction: -

Chemically Salmeterol xinofoate is (RS) -4 -hydroxy -α1- [[[6 -(4-phenylbutoxy) hexyl] amino] methyl] -1, 3 -benzenedi methanol 1 -hydroxy -2 -naphthoate. Salmeterol xinofoate drug come under bronchodilator category. The chemical formula of Salmeterol xinofoate is $C_{25}H_{37}NO_4$, $C_{11}H_8O_3$ and molar mass is 603.756g/mol. Salmeterol xinifoate is official in I.P. It is freely soluble in methanol. Salmeterol xinofoate powder is white in colour [1]. Literature survey reveals that some analytical methods are reported for the determination of Salmeterol xinofoate HPLC [2-4], SIM RP-HPLC [5], UPLC [6], HPTLC [7] and UV spectrophotometric [8]. Its salt is used in the treatment of asthma and chronic obstructive pulmonary diseases [9]. Salmeterol xinofoate dissociates into solution yield Salmeterol base and hydroxynaphthoate and display poor aqueous solubility [10]. Salmeterol is a weak base with an ionizable phenol [11]. The combination of Salmeterol xinofoate and fluticasone propionate is used against

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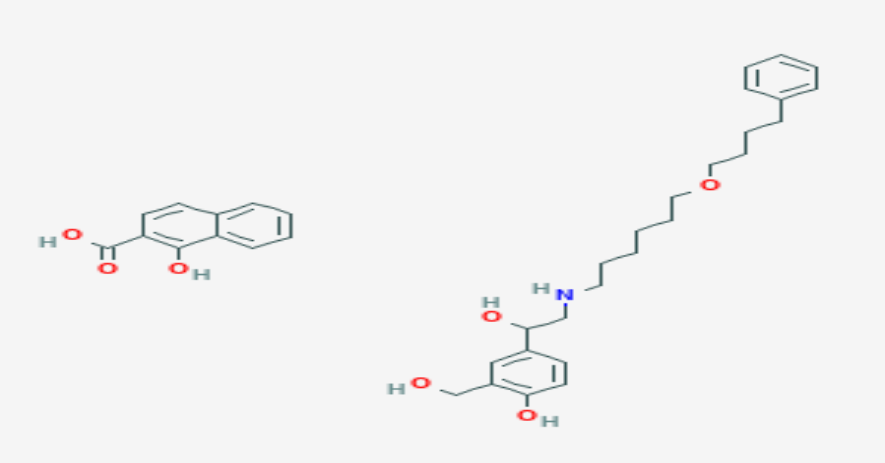
bronchoconstriction which is occurring in asthma [12]. Salmeterol xinofoate is used in prevention of asthma symptoms. It is available as a powder inhaler that releases powdered form of drug. Combination of inhaled corticosteroid and Salmeterol has a synergistic action and reduces frequency of asthma attack [13]. The main objective of this work is to develop simple and economical HPLC method for determination of Salmeterol xinofoate. The second objective is to validation of the method as per ICH guidelines. [14-15].



Chemical structure of Salmeterol xinofoate

Molecular formula: - $C_{36}H_{45}NO_7$

Molecular weight: - 603.756g/mol

Material and Method:

Salmeterol xinofoate was obtained from Vamsi Labs Ltd, Solapur as a gift sample. The serobid rotacaps was used as pharmaceutical dosage form for the study. It was purchased from local pharmacy, Sangola.

Chromatographic condition:

An Agilent Zorbax Bonus RP-(250mm× 4.6mm, 5µ) column was used for the chromatographic separation under suitable condition. The mobile phase consisting of 0.1% Formic acid: Acetonitrile in the ratio 64:34v/v with a flow rate 1ml/min and run time was 10 minutes. The detection of drug was carried at 234nm.

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Preparation of standard solution:

a. Standard stock solution –i (sss-i):

- Initially prepare a standard stock solution of by adding 5 mg of Salmeterol xinofoate in 10ml volumetric flask and add 5ml diluents, mix for two minutes and make the volume to 10ml with diluents. (Conc. of Salmeterol xinofoate = 500µg/ml.
- Then add 1.0ml of SSS-I in 10ml volumetric flask and add 5ml diluents and vortex and make up the volume with diluents. (Conc. of Salmeterol xinofoate).

Selection of Detection Wavelength:

From the standard stock solution dilution are made by using solvent. The sample was scanned over the range 200-400nm with PDA detector.

Table 1. Chromatographic condition

Column temperature	30℃
Flow rate	1.0ml/min
Mobile phase	0.1% formic acid: acetonitrile (64:36)
Runtime	10 minutes
Injection volume	10µl
Wavelength	234nm
Diluents	0.1% FA: ACN
column	Agilent Zorbax Bonus-RP
R.T. of salmeterol xinofoate	1.96 Minutes

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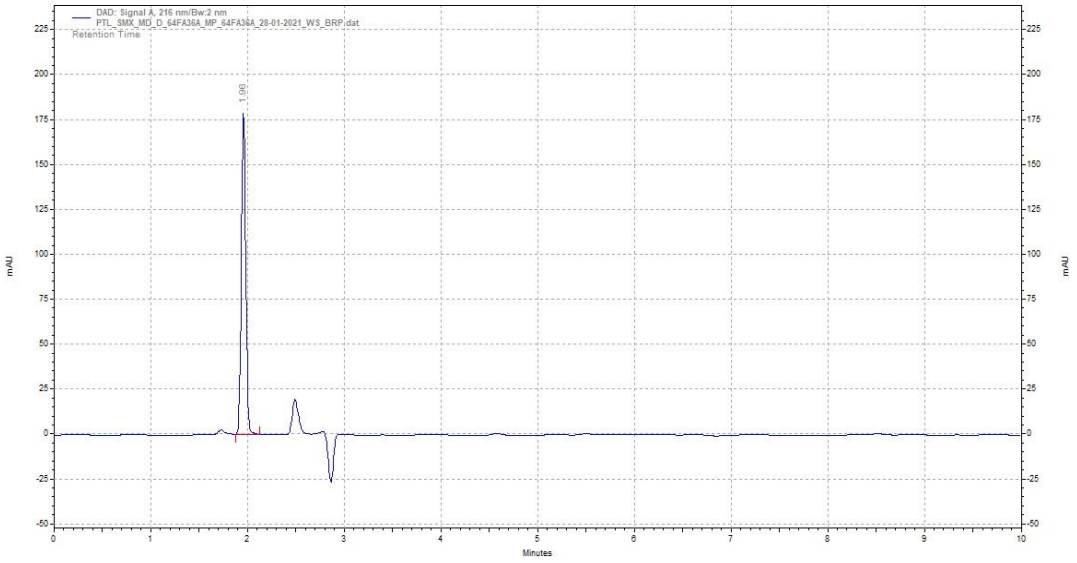

Fig 1. Chromatogram of Standard Salmeterol Xinofoate

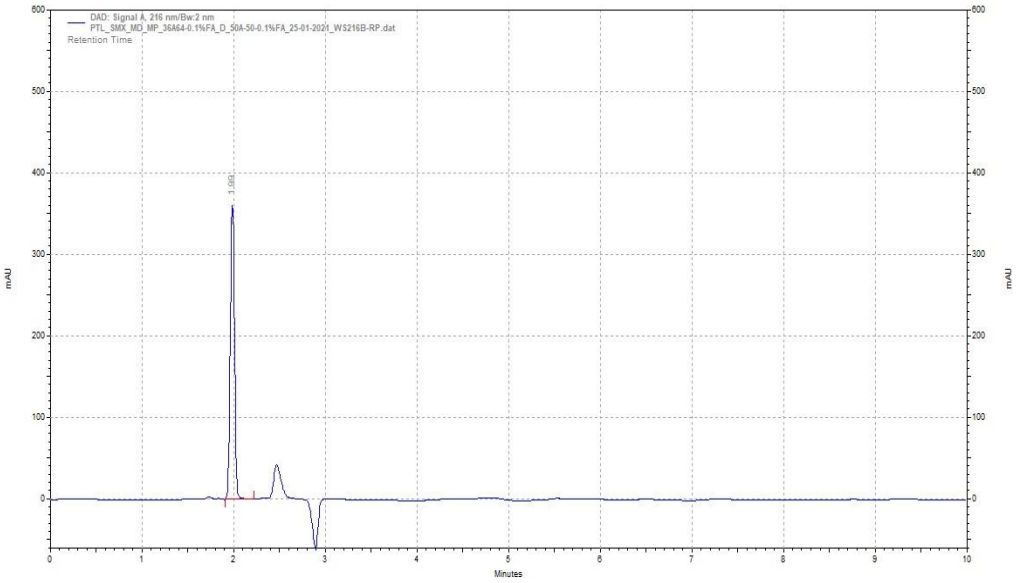

Fig 2. Chromatogram of Sample of Salmeterol Xinofoate

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Sample Preparation for Assay:

b. Capsule Sample Solution (CSS):

- 20 Capsule were weighed and average weight was calculated. Then the content was mixed in mortar and pestle.
- Powder weight equivalent to 500µg Salmeterol xinofoate was weighed into 10ml volumetric flask & add 5ml diluents, sonicate for 10 minutes and make the volume to 10ml with diluents. (Conc. of Salmeterol xinofoate = 50µg/ml.

Method validation:

Method validation is the process used to confirm that analytical procedure used for a specific test is suitable for its intended use. Various experiments are done with respect to various combinations of phases to optimize proper chromatographic condition. Typical parameters are verified like linearity, accuracy, range, precision etc. The proposed method has been developed and validated as per ICH guidelines.

Linearity:

Linearity of peak response for Salmeterol xinofoate was established in the range 80-120% with respect to sample concentration 40-60µg/ml was prepared. The linearity of this proposed method was evaluated by using calibration curve to calculate the coefficient of correlation, slope and intercept values.

i. 5 samples of varying concentrations ranging from 80-120% were made.
ii. the concentrations are given below.

% Level	Salmeterol xinafoate Conc. (µg/ml)
80	40
90	45
100	50
110	55
120	60

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iii. the sample preparations are given as below;
iv. X ml of Salmeterol xinafoate were added to 10 ml diluents to make up the concentrations given above

X ml of SSS-I	Diluted to
0.8	10 ml
0.9	10 ml
1	10 ml
1.1	10 ml
1.2	10 ml

Precision:

Precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies 5 injection of standard solution was made from that response factor and % RSD was calculated. In intra-day variation studies 5 injection of standard solution was made for 2 consecutive days and response factor and % RSD was calculated.

Accuracy:

The study of recovery of salmeterol xinofoate was at three different spike level like 80%, 100% and 120%. Samples were prepared. Salmeterol sample solution was made in duplicate for each spike level and assayed as per proposed method and % recoveries were calculated.

System suitability:

System suitability is integral part of method development and used to ensure adequate performance of chromatographic condition. System suitability parameters are as follows-

1. Retention time
2. Theoretical plates
3. Tailing factor.

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LOD and LOQ:

The residual standard deviation of regression line and slope of calibration curve were used to calculate the LOD and LOQ. Limit of detection (LOD) and Limit of quantification (LOQ) were calculated by following formula.

$$LOD = 3.3 * SE/ A \quad (1)$$

$$LOQ = 10 * SE/ A \quad (2)$$

Were,

SE = Standard error of Y intercept

A = Slope of calibration curve.

Table 2. Linearity dilutions:

Sr. No.	% Level	Volume of Salmeterol Xinofoate stock solution to be taken (ml)	Concentration of Salmeterol Xinofoate (µg/ml)	Diluted to volume (ml)
1	80	0.8	40	10
2	90	0.9	45	10
3	100	1	50	10
4	110	1.1	55	10
5	120	1.2	60	10

Table 3. Assay data of Salmeterol Xinofoate

Salmeterol Xinofoate		
Sample	**Working standard**	**Drug product**
Area	2488723	2471079
Assay	-	99.29

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Table 4. Linearity data of Salmeterol Xinofoate

% Level	Concentration (µg/ml)	Area
80	40	198098
90	45	2240953
100	50	2498998
110	55	2727441
120	60	2965460

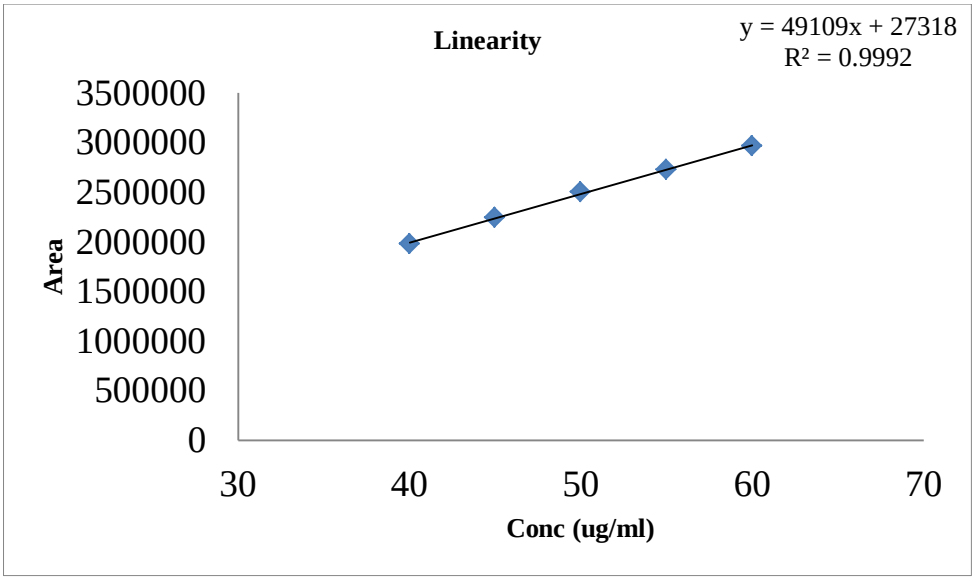

Fig. 3. Linearity graph of Salmeterol Xinofoate

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Table 5. Precision data of Salmeterol xinofoate

Salmeterol Xinofoate		
Concentration of sample (µg/ml)	**Sample ID**	**Area**
45	Rep 1	2498998
45	Rep 2	2575174
45	Rep 3	2491224
45	Rep 4	2497985
45	Rep 5	2480232
Average		2488723
STDEV		10645.28
RSD		0.43

Table 6. Accuracy data of Salmeterol xinofoate

Sample ID	Reps	Spiked conc. (µg/ml)	Area	Amt. Recovered (µg/ml)	% Recovery	Average	STDEV	RSD
80 %	Rep 1	39.99	1980980	39.79	99.50	99.69	0.186421	0.19
	Rep 2	39.99	1986229	39.89	99.76			
100 %	Rep 1	49.99	2498998	50.20	100.41	99.93	0.6769	0.68
	Rep 2	49.99	2475174	49.72	99.46			
120 %	Rep 1	59.99	2965460	59.56	99.30	99.51	0.3071	0.31
	Rep 2	59.99	2978431	59.82	99.73			

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Table 7. System suitability parameters

Parameter	Salmeterol Xinofoate
Retention time	1.96 minute
Theoretical plates	8342
Tailing factor	1.07
Resolution	0.00

Table 8. LOD and LOQ of Salmeterol xinofoate

Drug	LOD (µg/ml)	LOQ (µg/ml)
Salmeterol Xinofoate	2.67µg/ml	8.08µg/ml

Result and Discussion:

Assay:

The % assay of Salmeterol xinofoate was found to be 99.29 % as shown in table 1.

Linearity:

Five series of standard solution were prepared to assess the linearity range. The calibration curve plotted as peak area verses concentration of the standard solutions. The linearity range of Salmeterol xinafoate in the concentration range of 40-60µg/mL and r^2 = 0.999. Calibration data is presented in table 4. calibration curve is shown in fig.3.

Precision:

The method was found to be accurate and precise and indicated by recoveries studies and % RSD not more than 2. The result of precision is shown in table 5.

Accuracy:

The accuracy of the method was studied at three different concentrations i.e. 80%, 100% and 120% showed affordable % recoveries in the range of 99.30-100.41% for salmeterol xinofoate. The results of accuracy are shown in table 6. The low value of % RSD indicates accuracy of the method.

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System Suitability:

According to USP system suitability is the integral part of liquid chromatographic method. They are used to verify the various parameter of chromatographic system are represented in table 7.

LOD and LOQ:

The LOD for Salmeterol xinofoate was found to be 2.67µg/ml and LOQ for Salmeterol xinofoate was found to be 8.08µg/ml. The low value of LOD and LOQ indicate high sensitivity of method. The result of LOD and LOQ of salmeterol xinofoate shown in table 8.

Conclusions:

The developed HPLC method was simple, precise, economical and reproducible and can be used for the determination of Salmeterol xinofoate. The method was validated as per ICH guidelines. The solvent system used in this method is economical. The % RSD value not more than 2 hence, the method is highly precise. This method can be used for routine analysis of Salmeterol xinofoate in its pharmaceutical dosage form.

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