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DEVELOPMENT AND VALIDATION OF THE UV-SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF SALMETEROL XINAFOATE IN API AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A rapid, simple, selective, sensitive, precise and specific UV Spectrophotometric method has been developed for the determination of Salmeterol Xinafoate in API and pharmaceutical dosage form. Salmeterol Xinafoate standard solution was scanned in the UV range (200-400nm) in 1cm quartz cell in a double beam UV Spectrophotometer. The spectrophotometric detection was carried out at an absorption maximum of 254 nm using Acetonitrile:Methanol (50:50) as a solvent. The method was validated for specificity, linearity, accuracy, precision, robustness and ruggedness. The detector response for the Salmeterol Xinafoate was linear over the selected concentration range 2-12 µg /ml with a correlation coefficient of 0.999 and equation for the regression curve was found to be *y=0.0474x+ 0.0401*. The accuracy was between 98.7-102%. The precision (%RSD) among six samples preparation was 0.459 %. The LOD and LOQ are 0.1056 And 0.3200 µg /ml respectively. Statistical analysis proved that the methods are repeatable and specific for the determination of the said drug. These methods can be adopted in the routine assay analysis of Salmeterol Xinafoate in API and pharmaceutical dosage form.

KEYWORDS: Salmeterol Xinafoate, UV Spectrophotometer, Acetonitrile: Methanol (50:50), Method Validation.

INTRODUCTION

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.[3]

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.[4]

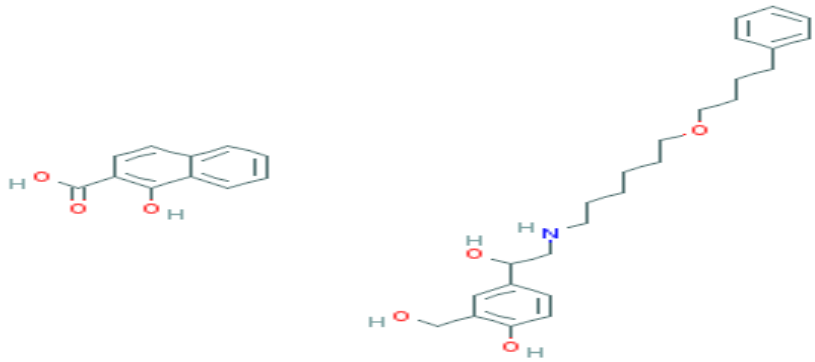

Figure. 1: Chemical structure of Salmeterol Xinafoate.

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

Salmeterol Xinafoate it is a white powder which is freely soluble in methanol, Acetonitrile, slightly soluble in anhydrous ethanol, practically insoluble in water and methylene chloride.

Literature survey reveals that the Salmeterol Xinafoate has been estimated by Spectrophotometric techniques[5], LC/MS/MS[6], HPTLC[7], RP-HPLC[8] and HPLC with fluorescence detection.[9]

The present work is a simple, sensitive, accurate and precise Spectrophometric Method for the estimation of Salmeterol Xinafoate in API and its Pharmaceutical Dosage Forms with the help of, Acetonitrile: Methanol (50:50) solvent.

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MATERIALS AND METHODS

Instruments: For weighing, a calibrated weighing balance (Shimadzu) of 1mg sensitivity was used. A Systronic UV-visible double beam spectrophotometer- 2201 was used. All the glass wares and were made of borosilicate and were calibrated.

Chemicals

API- Salmeterol Xinafoate pure drug was gifted by Vamsi Labs Ltd, Solapur.

Salmeterol rotacaps 50mcg strength were purchased from the local pharmacy in Solapur under commercially available brand name Serobid (Cipla), Acetonitrile : Methanol (50:50) LR was used in this study.

UV Spectroscopic Method

Solvent Selection

Salmeterol Xinafoate is soluble in Acetonitrile : Methanol (50:50) so, Aetonitrile and methanol is used as the solvent.

Preparation of Standard Stock Solution

The standard stock solution Salmeterol Xinafoate (SX) was prepared by transferring accurately weighed 10 mg of Salmeterol Xinafoate into 10 ml volumetric flask containing Acetonitrile: Methanol (50:50), dissolved properly. Then volume was made up to the mark by using Acetonitrile: Methanol (50:50) to give a concentration of 1000 µg / ml. From this, 1ml of the solution was transferred to a 10 ml volumetric flask and make up the volume with Acetonitrile : Methanol (50:50) to give a concentration of 100 µg/ml which is a standard stock solution and it is further diluted with Acetonitrile : Methanol (50:50) to get concentration range of 2-12 µg/ml.

Determination of Absorption Maxima

The standard stock solution of 10µg/ml was scanned in the range of 200-400 nm to determine the wavelength of Maximum Absorption. The drug showed Absorption maxima at 254 nm.

Preparation of Calibration Curve

For the preparation of calibration curve, the concentration of 2-12 µg/ml were prepared by pipetting out 0.2, 0.4, 0.6, 0.8, 1 and 1.2 ml of the 100 µg/ml solution into 10 ml volumetric flasks and made up the volume with Acetonitrile : Methanol (50:50).

The absorbance of each solution was measured at 254 nm against Acetonitrile : Methanol (50:50) as blank. Calibration curve of the Salmeterol Xinafoate was plotted by taking the absorbance obtained on the y-axis and concentration of the solution on the x-axis. The curve showed linearity in the range of 2-12 µg/ml with correlation coefficient 0.999.

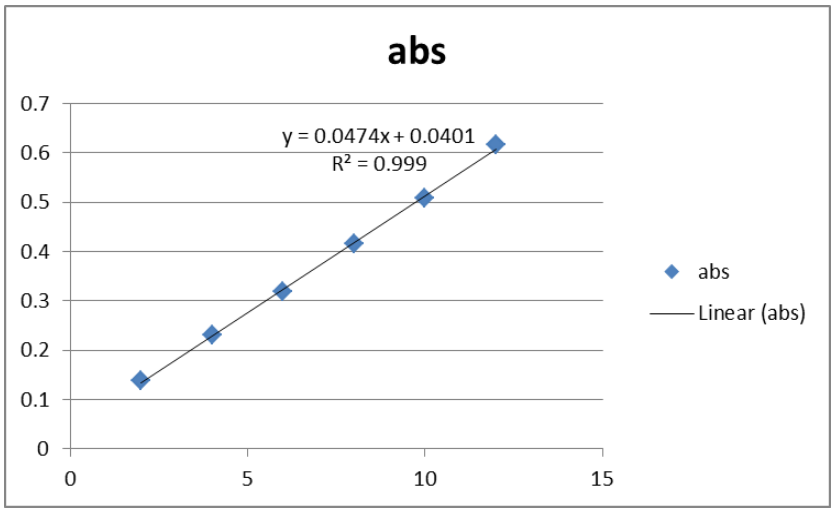

Figure. 2: Calibration curve of Salmeterol Xinafoate (SX).

Quantitative Analysis of Capsule Dosage Form

20 capsules of marketed formulation of SX 50 µg (serobid rotacaps) respectively were weighed;their average weights determined. The correct amount of drug powder equivalent to label claim was weighed and transferred to 10 ml volumetric flask, dissolved in Acetonitrile:Methanol (50:50) and sonicated for 15min. The volume was then made up to the mark using same solvent. The resultant solution was filtered through 0.45 µ membrane filter This solution was filtered through filter paper to remove some un- dissolved excipients. The filtrate was having concentration 50 µg/ml for SX. After filtration, from this 2.4 ml was taken and diluted to 10 ml with Acetonitrile:Methanol (50:50) which gives 12 µg/ml solution and

Absorbance of this sample solutions was recorded at 254nm ((λmax of SX) and concentration of drug in the sample were determined the absorbance of the solution was measured at 254 nm.

Table 1: Results obtained in the determination of SX in dosage form

formulation	Label claim	Amount taken	Amount found	Assay %
Serobid Rotacaps	50mcg	12 µg/ml	11.8µg/ml	98%

Method Validation

The developed method was validated as per ICH guidelines for the following parameters:

1. Linearity: 0. 2, 0.4, 0.6, 0.8, 1, 1.2 ml of standard SX solution was transferred into a series of 10 ml volumetric flasks. The volume was made up to the mark with Acetonitrile : Methanol (50:50) to obtain the concentration of 2, 4, 6, 8, 10, 12µg/ml. Then absorption of these solutions was recorded and the graph was plotted of absorption against concentration. The correlation coefficient (r^2) of least square linear regression of SX was calculated.

2. Range: The Range of the analytical method was decided from the interval between upper and lower level of calibration curve by plotting curve.

3. Accuracy: Recovery study was carried out by the standard addition method by adding a known amount of SX to the pre-analyzed sample at three different concentration levels that is 80%, 100%, 120% of assay concentration and percent recovery were calculated. 0.5 ml of capsule solution was transferred to 4 different 10 ml volumetric flasks (labelled as blank, 80%, 100%, 120%) separately and 0, 0.3, 0.5, 0.7 ml of 100 µg/ml standard solution was added respectively and the volume was made up to the mark with Acetonitrile : Methanol (50:50). Absorbances were noted for these samples. Standard deviation and % RSD was calculated. Accuracy is reported as % recovery, which was calculated from the expression as equation given below

% Recovery = Observed value / True value ×100

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same sample under the prescribed conditions. The precision of the method was determined in terms of repeatability and intra-day and inter-day precisions. Intra-day and inter-day precision (Intermediate Precision)

Intraday precision was determined by analyzing the drugs at concentrations (6 µg/ml) and each concentration for three times, on the same day. Inter-day precision was determined similarly, but the analysis being carried out daily, for two consecutive days.

Repeatability

Repeatability of the method was determined by analyzing six samples of same concentrations of the drug (6 µg/ml). Absorbance of each was measured.

4. Robustness: The robustness of the developed method is its capacity to remain unaffected by small changes in altered conditions. To determine the robustness of the method, the wavelength of analysis was deliberate and the assay was evaluated. The effect of detection wavelength was studied at ±5 nm.

5. Ruggedness: Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

6. Limit of Detection: Detection limit was determined based on the standard deviation of absorbance of same concentration that is a standard solution of SX (6µg/ml) and LOD calculated by LOD = 3.3 (SD/S) Where, SD- standard deviation; S= slope of the curve.

7. Limit of Quantification: Quantification limit was determined based on the standard deviation of peak area of same concentration that is standard solution SX (6µg/ml) prepared six times and LOQ calculated by LOD = 10(SD/S) Where, SD= standard deviation; S= slope of Curve.

RESULT

Determination of wavelength of maximum absorption the wavelength of maximum absorption was found to be 254 nm.

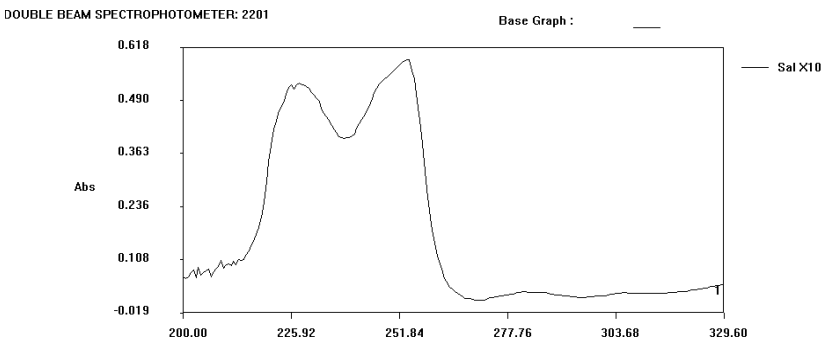

Figure. 3: Wavelength of maximum absorption of Salmeterol Xinafoate.

Linearity: The linearity of this method was determined at ranges from 2-12 µg/ml for Salmeterol Xinafoate. The regression equation was found to be *Y=0.0474x +0.0401*, R^2=0.999.

Table 2: Linearity table.

Sr. No.	Conc.	Absorbance
1.	2	0.139
2.	4	0.231
3.	6	0.320
4.	8	0.415
5.	10	0.508
6.	12	0.617

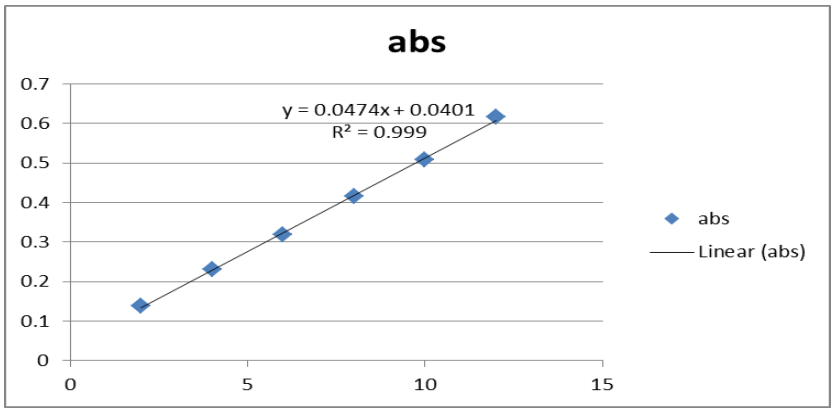

Figure 4: Linearity graph of Salmeterol Xinafoate.

The linearity for Salmeterol Xinafoate was found to be linear in the range of 2-12 µg/ml with R^2= 0.999 and the straight line equation as *y= 0.0474x+0.0401*.

Accuracy: The accuracy of the analytical method for Salmeterol Xinafoate was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 254 nm and results were expressed in terms of % recoveries.

Table 3: Table for accuracy

Sr.no.	Level of % Recovery	Amount of capsule sample (ml)	Amount of standard drug added (µg/ml)	Amount added (µg)	Amount found (µg/ml)	% Recovery
1	0	0.5	0	0	0	0
2	80	0.5	0.3	8	7.9	98.75%
3	100	0.5	0.5	10	10.1	101%
4	120	0.5	0.7	12	12.23	102%

Precision: The precision (measurement of intra-day, inter-day, repeatability) results showed good reproducibility with the relative standard deviation (% RSD) below 2.0 %. This indicated that method was highly precise.

Intra-day Precision

Table 4: Intra-day morning precision

Sr.No.	Concentration (µg/ml)	Absorbance	SD	% RSD
1	6	0.324		
2	6	0.323		
3	6	0.325	0.001366	0.420
4	6	0.325		
5	6	0.327		
6	6	0.324		
		$\bar{y}$ = 0.324667		

Table 5: Intra-day afternoon precision

Sr.No.	Concentration (µg/ml)	Absorbance	SD	% RSD
1	6	0.327		
2	6	0.328		
3	6	0.327	0.000983	0.299
4	6	0.327		
5	6	0.329		
6	6	0.329		
		$\bar{y}$ = 0.327833		

Table 6: Intra-day evening precision

Sr.No.	Concentration (µg/ml)	Absorbance	SD	% RSD
1	6	0.323		
2	6	0.336		
3	6	0.334	0.004708	1.41
4	6	0.332		
5	6	0.335		
6	6	0.331		
		$\bar{y}$ = 0.331833		

Inter-day Precision

Table 7: Inter-day morning precision study.

Sr.No.	Concentration (µg/ml)	Absorbance	SD	% RSD
1	6	0.345		
2	6	0.341		
3	6	0.34	0.00216	0.6309
4	6	0.341		
5	6	0.345		
6	6	0.342		
		$\bar{y}$ = 0.34233		

Table 8: Inter-day afternoon precision study

Sr.No.	Concentration (µg/ml)	Absorbance	SD	% RSD
1	6	0.347		
2	6	0.346		
3	6	0.346	0.001211	0.3506
4	6	0.344		
5	6	0.344		
6	6	0.345		
		$\bar{y}$ = 0.3453		

Table 9: Interday evening precision study

Sr.No.	Concentration (µg/ml)	Absorbance	SD	% RSD
1	6	0.353		
2	6	0.353		
3	6	0.353	0.000837	0.2374
4	6	0.353		
5	6	0.352		
6	6	0.351		
		$\bar{y}$ = 0.3525		

Repeatability

Table 10: Repeatability study

Sr.No.	Concentration (µg/ml)	Absorbance	SD	% RSD
1	6	0.329		
2	6	0.329		
3	6	0.33	0.001517	0.459
4	6	0.333		
5	6	0.331		
6	6	0.331		
		$\bar{y}$ = 0.365		

Limit of Detection

Table 11: For Limit of Detection

LOD (µg/ml)	**0.1056 µg/ml**

Limit of Quantification

Table 12: For Limit of Quantification

LOQ (µg/ml)	**0.3200 µg/ml**

Robustness (6µg)

Table 13: Robustness study

Sr.No.	Wavelength (nm)	Absorbance	SD	% RSD
1	247	0.68		
2	248	0.69		
3	249	0.313		
4	250	0.315	0.010266	3.28
5	251	0.317		
6	252	0.319		
7	253	0.319		
8	254	0.321		
9	255	0.318		
10	256	0.286		
		$\bar{y}$ =0.3125		

Ruggedness (6µg): Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Table no 14. For Ruggedness.

Analyst-1		
Concentration (µg/ml)	**Absorbance**	**Statistically analysis**
6	0.337	
6	0.337	Mean = 0.337167
6	0.336	SD = 0.001602
6	0.339	% RSD = 0.4751
6	0.339	
6	0.335	
Analyst-2		
6	0.351	
6	0.352	Mean = 0.3508
6	0.352	SD=0.001472
6	0.352	%RSD=0.4195
6	0.349	
6	0.349	

DISCUSSION

Preliminary Analysis of Salmeterol Xinafoate

Preliminary analysis of Salmetrol Xinafoate such as description, solubility was performed.

UV-spctrophotometry for Salmeterol Xinafoate

Salmeterol Xinafoate being UV absorbing has been successfully employed for its quantitative determination by UV Spectrophotometric method. Being soluble in Acetonitrile : Methanol (50:50), stock solutions and working standards were prepared in Acetonitrile : Methanol

(50:50) .The maximum wavelength of absorption of drug was determined by taking scan of the drug solution in the UV region (200-400 nm). The correlation of the standard curve for the drug was 0.999. The accuracy was from 98.75-102% at 254nm. The proposed method showed absorption maxima at 254nm and obeyed Beer's law in the concentration range of 2-12 µg/ml. The limit of detection (LOD) was found to be 0.1056 µg/ml and limit of quantification (LOQ) to be 0.3200 µg/ml respectively. All statistical data prove validity of the proposed method, which can be applied for routine analysis of Salmeterol Xinafoate.

Assay of Capsule formulation: Amount of drug present in Capsule formulation was calculated using equation at 254 nm, and *y=0.0474 xs+0.0401* and amount of Salmeterol Xinafoate were found to be 98% of label claim respectively. This method can be employed for routine analysis of Salmeterol Xinafoate.

Summary and conclusion: Summary of UV Spectrophotometeric Method of Salmeterol Xinafoate.

Table 15: For Summary

Sr. No.	Parameters	Values
1	Beer's Law limit (µg/ml)	2-12
2	Absorption maxima (nm)	254
3	Standard regression equation	0.0474x+0.0401
4	Correlation coefficient (R^2)	0.999
5	Accuracy	98.75-102%
6	Precision (% RSD) Repeatability	0.459
7	LOD (µg/ml)	0.1056
8	LOQ (µg/ml)	0.3200
9	Robustness (%RSD)	3.28
10	Ruggedness	0.4751 and 0.4195
11	Assay (%)	98%

CONCLUSION

The UV-Spectrophotometric method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of Salmeterol Xinafoate in API and its dosage form without any interference from the excipients. This method can be effectively applied for the routine analysis of Salmeterol Xinafoate in API. Its advantages are the low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

Abbreviations

UV-Ultra Violet

API- Active Pharmaceutical Ingredient

SX- Salmeterol Xinafoate

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