Stability-indicating RP-HPLC Method for Estimation of Salmeterol xinafoate in Bulk and in Pharmaceutical Formulation

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ABSTRACT

A new simple, precise, accurate and selective RP-HPLC method has been developed and validated for estimation of salmeterol xinafoate in pharmaceutical formulation. The method was carried out on a Qualisil RP C-18 (250 mm x 4.6 mm, 5 µm) column with a mobile phase consisting of buffer: acetonitrile: methanol (45:25:30 v/v/v), pH adjusted to 6 with diammonium hydrogen orthophosphate and flow rate of 0.5 mL/min. Detection was carried out at 250 nm. The retention time for salmeterol xinafoate was found to be 7.41 min. The salmeterol xinafoate followed linearity in the concentration range of 1- 6 µg/mL (r^2= 0.9984). The amount of the drug estimated by proposed method was found to be in good agreement with label claim. The developed method was validated for sensitivity, accuracy, precision, ruggedness and robustness. The LOD and LOQ were found to be 0.10 and 0.29 µg. Salmeterol xinafoate was subjected to acid and alkali hydrolysis, oxidation and thermal degradation. The drug undergoes degradation under acidic, basic, oxidation and thermal conditions. This indicates that the drug is susceptible to acid, base, oxidation and thermal conditions. The degraded product was well resolved from the pure drug with significantly different Retention Time. The proposed method can be used for routine analysis of salmeterol xinafoate in bulk drug and pharmaceutical formulation.

Keywords: *Salmeterol xinafoate, RP-HPLC Validation, methanol, Stability; Degradation.*

INTRODUCTION

Salmeterol xinafoate 4-Hydroxy-[6-4phenylbutoxy)hexyl]amino]-methyl]-1, 3-benzenedimethanol, 1-hydroxyl-2-napthoate. It is a salmeterol is a long-acting beta2-adrenergic receptor agonist. In addition it is a drug commonly used for Prevention of exercise-induced bronchospasm. Salmeterol xinafoate as prescribed for asthma and chronic obstructive pulmonary. The drug is official in British Pharmacopeia[1]. Literature survey revealed that one HPLC method is reported for Simultaneous determination of salmeterol xinafoate and Fluticasone Propionate in bulk powder and Seritide [2], analysis using High Performance Liquid Chromatographic and Spectrophotometric Method [3] and one SP-HPLC method is developed [4]. Therefore, the main objective of this work is to develop simple and economical RP-HPLC method for salmeterol xinafoate. The second objective is to validate the method as per the ICH guidelines [5-7].

EXPERIMENTAL

Chemicals

Salmeterol xinafoate is obtained from, Galaxo SmithKline group india as a gift sample. Methanol (HPLC Grade) was purchased from Merck (India) Ltd., Worli, Mumbai, India. Salmeterol xinafoate 0.1mg per 10 mL.

Instrumentation and Chromatographic Conditions

Comprising G 1311A solvent delivery system (pump), G1315 diode array detector, UV detector and a Rheodyne injector with 20 µL loop. EZ Chrome Elite was used as a data processer. A Qualisil BDS C-18 column (250 mm x 4.6 mm i.d., 5-µm) was used for chromatographic separation under suitable conditions. The mobile phase consists of buffer: methanol: acetonitrile (45:25:30 *v/v/v*), pH adjusted to 6 with diammonium hydrogen phosphate at a flow rate of 0.5 mL/min and the run time was 10 min. Before analysis both the mobile phase and sample solution was filtered through a 0.45 µm membrane filter and degassed for 15 min in an ultrasonicator. The detection of the drug was carried out at 250 nm. The UV-spectra of the drug in methanol is shown in Fig.2**.**

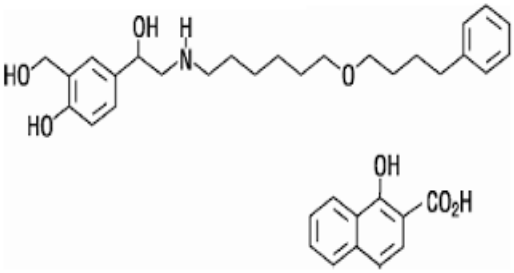

Fig. 1. *Chemical structure of Salmeterol xinafoate.*

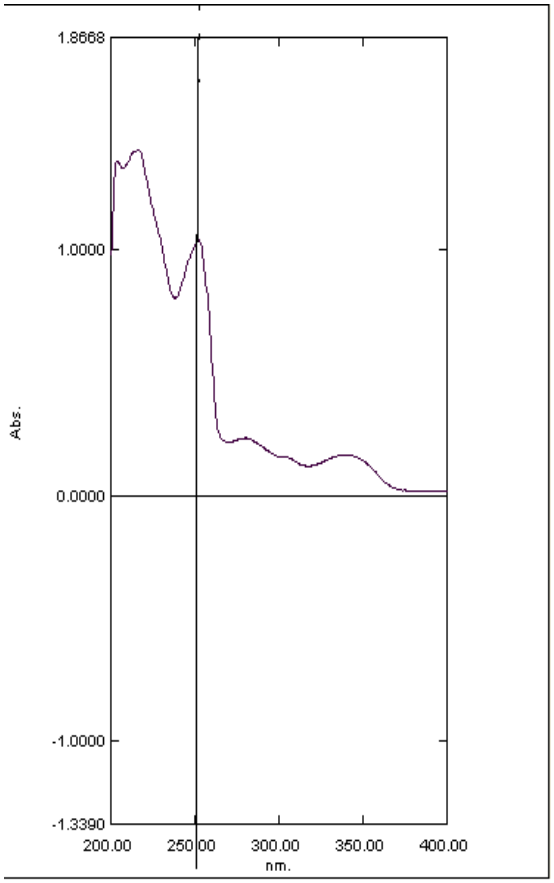

Fig. 2. *UV spectra of salmeterol xinofoate at 250*

Preparation of Stock Standard Solution and Calibration

Stock standard solution was prepared by dissolving 0.1 mg of EL in 10 mL methanol that gives concentration of 10 µg/mL From stock standard solution, aliquots of 1, 2, 3, 4, 5 and 6µL was taken in 10 mL volumetric flasks with the help of micro pipette and diluted up to the mark with mobile phase previously filtered and sonicated such that to obtained concentration of EL in the range 1 – 6 µg/mL. Volume of 20 µL of each sample was injected with the help of hamilton syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area *versus* the drug concentration.

Analysis of marketed formulation

Salmeterol xinafoate infusion contained 1ml /mL of salmetrol xinafoate in 10ml of the infusion. From this 0.1ml of the solution was taken in 10ml of volumetric flask to give 10µg/mL of concentration. From this 60µL was taken with micro pipette and was further diluted with mobile phase to get final concentration of 6 µg/mL. This was analyzed by proposed method and amount of EL was determined.

Method Validation

The HPLC method was validated in accordance with ICH guidelines [5- 7].

Precision:

The precision of the method was studied as intra-day, inter-day and repeatability of sample injections. Intra-day precision was determined by analysis of the solution three times on the same day. Inter-day precision was assessed by analysis of the solution on three different days over a period of one week. Repeatability of sample injections was performed by injecting same concentration of the drugs for six times and effects on peak areas were examined.

Specificity and Selectivity:

Specificity of the method was ascertained by analyzing drug standard and sample. The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. The method is quite selective. There was no other interfering peak around the retention time of salmeterol xinafoate; also the base line did not show any significant noise.

Accuracy:

The accuracy of the method was studied by recovery study. To the pre anlaysed sample solution (2 µg/mL of EL) a known quantity of EL was added at 80, 100 and 120 % level and analyzed by the proposed RP-HPLC method.

Sensitivity:

Sensitivity of the proposed method was estimated in terms of Limit of Detection

(LOD) and Limit of Quantitation (LOQ). LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, where ASD is the average standard deviation and S is the slope of the line.

Robustness:

Robustness of the method was studied by making deliberate changes in few parameters *viz*. variation of flow rate, mobile phase composition and change in pH. The effects on the results were studied by injecting 2 µg/mL of EL.

Ruggedness:

From the stock solution, sample solution of EL (2 µg/mL) was prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions, six times.

Forced degradation of Salmeterol Xinafoate

Acid and base induced degradation:

The 10 mg of salmeterol xinafoate was separately dissolved in 5 ml of methanolic solution of 1 N HCl and 2 N NaOH. These solutions were kept for 40 min and 20 min at 70^{0}C temperature in the dark in order to exclude the possible degradative effect of light. The 1ml of above solutions was taken and neutralized, then diluted up to 10 ml with mobile phase. The resultant solution were inject in HPLC column. The chromatograms were run as described in Section 2.2.

Hydrogen peroxide-induced degradation:

The 10 mg of salmeterol xinafoate was separately dissolved in 5 ml of methanolic solution of hydrogen peroxide (6%, v/v). The solution was kept for 1 h at 80^{0}C temperature in the dark in order to exclude the possible degradative effect of light. The resultant solution were inject in HPLC column. The chromatograms were run as described in Section 2.2.

Photochemical Degradation:

The photochemical stability of the drug was also studied by exposing the stock solution to direct sunlight for 12 h. No degradation was observed. The resultant solution was applied in a HPLC column and chromatograms were run as described in section 2.2.

Dry heat degradation Product:

The powdered drug was stored at 90^{0}C for 24 h under dry heat condition. No degradation was observed. In all degradation studies, the average peak areas of salmeterol xinafoate after application of three replicates were obtained.

RESULTS AND DISCUSSION

Selection of Chromatographic Condition and Optimization of Mobile Phase

After trying columns containing different stationary phases, the final choice giving satisfactory resolution and run time was Qualisil BDS RP C-18 column (250 mm x 4.6 mm i.d.,5 µm). The mobile phase was chosen after several trials with buffer and acetonitrile in various proportions. A mobile phase consisted of buffer: acetonitrile (40:60 *v/v*) resolved peak with tailing. It was overcome by adjusting the pH of the mobile phase to 6 with diammonium hydrogen phosphaphate. Finally, buffer: methanol: acetonitrile (45:25:30 *v/v/v*), pH 6 was selected to achieve symmetrical peak. The effects of flow rates in the ranges of 0.4 to 1.1 mL/min were examined. A flow rate of 0.5 mL/min gave good results, system suitability parameter and reasonable retention time. The retention time of EL was observed 7.41 min at 250 nm wavelengths. The total time of analysis was less than 10 min. A typical chromatogram of the drug is shown in Fig. 3.

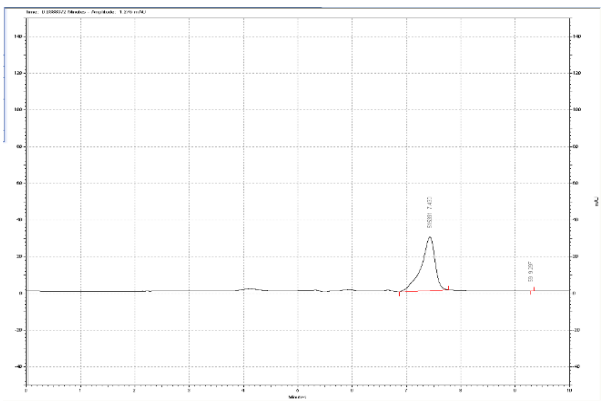

Fig.3. Chromatogram of standard salmeterol xinofoate.

Linearity

The linearity was determined for salmeterol xinafoate. Solution of the drug at six different concentrations was analyzed and calibration curve was constructed by plotting mean response factor against the respective concentration. The method was evaluated by determination of the correlation coefficient and intercept value.

Salmeterol xinafoate follows linearity in the concentration range of 1 - 6 µg/mL; respectively. The result is shown in Table 1.

Table 1: Linearity study of Salmeterol xinafoate

Sr. No.	Concentration of EL [µg/mL]	Mean peak area*	%R.S.D.
1	1	1034609.4 ± 7831.81	0.76
2	2	1980987.4± 13768.09	0.70
3	3	3051104.2 ± 59888.44	1.96
4	4	4008343.1 ± 116927.25	2.92
5	5	4638148.6 ± 92050.47	1.98
6	6	5434828.4 ± 105304.35	1.94

*average of six determination

Precision

The precision study was evaluated on the basis of % RSD value. The intra-day precision for salmeterol xinafoate was found to be in the range 0.53 – 1.22 and 0.74 – 2.08 %, respectively. The low values of % R.S.D. indicate high precision of the method. Results of precision study are shown in Table 2.

Table 2: Precision Studies (Intra-day and Inter-day)

Drug	Conc. [µg /mL]	Intra-day Amount found* [µg/mL]		Inter-day Amount found* [µg/mL]	
		Mean ± S.D.	% R.S.D. [n= 3]	Mean ± S.D.	% R.S.D . [n= 3]
SX	2	1040230 ± 5499	0.53	194549 ± 57325	2.19
	3	2963959 ±51748	1.75	293128 ± 34636	1.18
	4	388596 ±472602	1.22	389596 4 ± 5767	0.15

*****average of three determinations

Specificity and Selectivity

Specificity of the method was ascertained by comparing the chromatogram obtained from formulation and standard drug. The retention time of the standard drug and the drug from formulation was same, so the method was specific. The method was also specific and selective because there was no interference from excipients in the formulation. The method is quite selective. There was no other interfering peak around the retention time of salmeterol xinafoate; also the base line did not show any significant noise.

Accuracy

The accuracy of the method studied at three different concentration levels i.e. 80%, 100% and 120% showed affordable % recoveries in the range of 98 - 100.31 % for salmeterol xinafoate. The results are shown in Table 3. The low value of % R.S.D. indicates accuracy of the method.

Table 3. Recovery Studies

Drug	Initial amount [µg/mL]	Amount added [µg/mL]	Amount recovered* ± S.D. [µg/mL]	% Recovery	% R.S.D.
SX	2	1.6	3.58 ± 0.01	100.77	0.88
	2	2	4.01 ± 0.05	98.12	2.67
	2	2.4	4.36 ± 0.03	100.31	1.14

* average of three determinations at each level

Sensitivity

The LOD for salmeterol xinafoate was found to be 0.10 µg and the LOQ for salmeterol xinafoate was found to be 0.29 µg. The low values of LOD and LOQ indicates adequate sensitivity of the method.

Robustness and Ruggedness study

Robustness of the method was studied by making deliberate changes in the chromatographic conditions and the effects on the results were examined. The content of the drugs were not adversely affected by these changes as evident from the low values of % relative standard deviation (less than 2 %) indicating robustness of the method. When the method was performed by two different analysts under the same experimental and environmental conditions it was found to be rugged.

ANALYSIS OF MARKETED FORMULATION

Six replicates of the sample solution (20 µL) were injected for quantitative analysis. The amount of salmeterol xinafoate estimated was found to be 99.60 %, respectively. A good separation and resolution of the drug indicates that there was no interference from the excipients commonly present in pharmaceutical formulation.

SYSTEM SUITABILITY TEST

According to USP, system suitability test are integral part of liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. Earlier prepared solution for chromatographic conditions was tested for system suitability testing. The results obtained from validation of the

method and system suitability studies are summarized in Table 4.

Table 4: Robustness of the method

Sr. No	Prameter	Asymmetry	S.D	%RSD
1	Flow rate	0.4	61731.10	2.17
		0.6	64307.69	2.27
2	Mobile phase volume (buffer: ACN: Methanol)	55:20:35	69314.45	2.48
		65:15:40	51936.34	1.86
3	Mobile phase =PH	6.3	78838.60	2.76
		5.3	83885.38	2.76
4	Column temp	25	60755.79	2.10
		35	64302.22	2.27

Force degradation

The chromatogram of the acid degraded samples for salmeterol xinafoate showed additional peak at retention time of 7.41 min (Fig. 4a), base degraded drug shows at 4.3min (Fig. 4b), hydrogen peroxide shows at 7.41min (Fig. 4c) respectively. The chromatograms of photo-degraded drugs and dry heat degraded drugs was not shows degradation. The spot of the degraded product was well resolved from the salmeterol xinafoate spot. In both cases, the concentration of the drug was changing from the initial concentration, indicating that salmeterol xinafoate undergoes degradation under acidic, basic and oxidative conditions. The results are listed in **Table 5.**

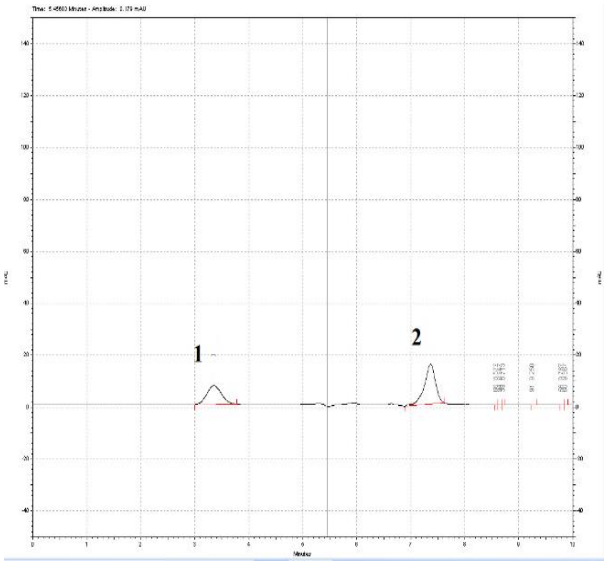

Fig. 4a: HPLC Chromatogram of acid (1 N HCl, 40min, 70^{0}C.) treated Salmeterol Xinafoate; peak 1(impurity) (R_t: 3.4min), peak 2(Salmeterol Xinafoate) (R_t: 7.4min).

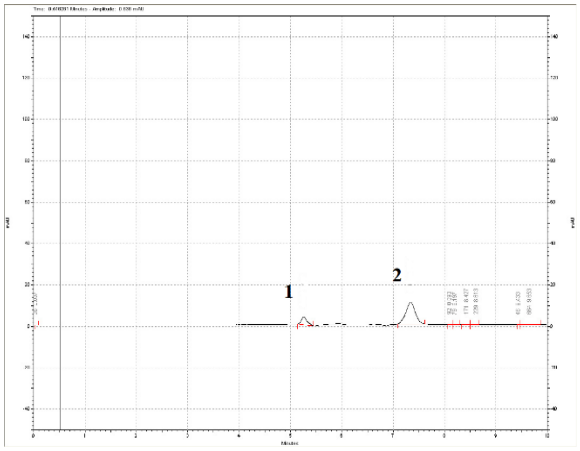

Fig. 4a: HPLC Chromatogram of base (2 N NaOH, 20min, 70^{0}C.) treated Salmeterol Xinafoate; peak 1(impurity) (R_t: 5.3min), peak 2(Salmeterol Xinafoate) (R_t: 7.4min).

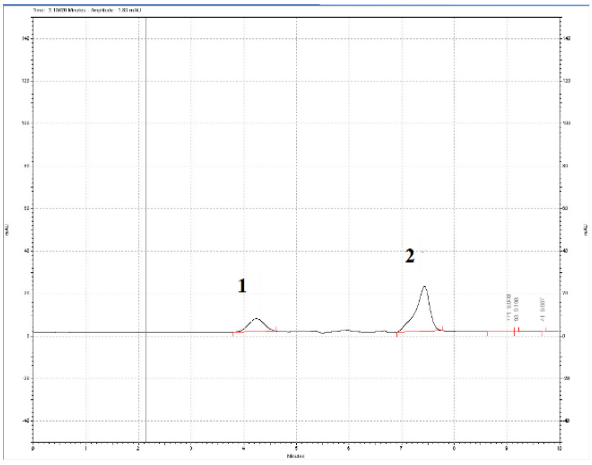

Fig. 4a: HPLC Chromatogram of Hydrogen Peroxide (6%v/v H_2O_2, 1hr, 80^{0}C.) treated Salmeterol Xinafoate; peak 1(impurity) (R_t: 4.2min), peak 2(Salmeterol Xinafoate) (R_t: 7.4min).

Table 5: Summary of Validation Parameter and System Suitability Study

Parameter	Salmeterol xinofoate
Linearity range [µg/mL]	1 – 6 µg/mL
Correlation coefficient	0.9980
LOD [µg]	0.10
LOQ [µg]	0.29
% Recovery [n = 9]	98.12
Analyst I [n = 6]	98.34
Analyst II [n =6]	98.56
Precision [% R.S.D.]	1.86
Repeatability of Injection [n = 6]	0.51
Intra-day [n = 3]	0.15 – 1.56
Inter-day [n = 3]	0.51 – 1.99
Robustness	Robust
Specificity	Specific
Retention time [t_R]	7.41
Theoretical plates [N]	9650
Capacity factor [k']	0.61
Asymmetry [T]	1.04

Table 6. Degradation of Salmeterol Xinafoate

Sr. No.	Sample exposure condition	No. of degradation product (RT)	Salmeterol Xinafoate remained(10ppm) (±drug remained)	Recovery (%)
1	1N HCL, 40 min, 70^0C.	1(3.4min)	1.39	13.86
2	2N NaOH, 20 min, 70^0C.	1(5.3min)	0.93	9.27
3	6% v/v H_2O_2, 1h, 80^0C	1(4.2min)	4.88	48.82
4	Heat, 24h, 90^0C	-	-	-
5	Photo, 6h	-	-	-

CONCLUSION

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately rugged and robust and can be used for determination of Salmeterol xinofoate in pharmaceutical formulation. The method can be used to determine the purity of the drug available from the various sources by detecting the related impurities. As the method separates the drug from its degradation products, it can be employed as a stability indicating one. The method was validated as per ICH guidelines.

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