RESEARCH PAPER | Chemistry | Volume : 4 | Issue : 3 | Mar 2014 | ISSN - 2249-555X

Studies of Analytical Method Validation of Some Antiashthamatic Drugs for Futured Intended Analytical Application

KEYWORDS: Salbutamol, HPLC method, Method Development and Validation

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ABSTRACT *A simple, rapid and accurate RP-HPLC method was developed for the determination of salbutamol in pure and tablet dosage form by RP-HPLC method using C18 (Phenomenex, 250 x 4.6mm, 5µm) in isocratic mode. The mobile phase consisted of Acetonitrile, buffer and Tetrahydrofuran in the ratio of 70:20:10 (v/v) was used and maintain the pH 4.7. The flow rate was maintained at 1.0ml/min and the injection volume was 20 µl. Detection wavelength with UV detector at 225nm and run time was kept 05min. The retention time of salbutamol was 2.325 min. The validation of method was carried out utilizing ICH-guidelines. The described HPLC method was successfully employed for the analysis of pharmaceutical formulations.*

Introduction:

Salbutamol sulphate (SAL), bis [(1RS)-2-[(1, 1-dimethylethyl) amino]-1-[4-hydroxy-3-(hydroxymethyl) phenyl] ethanol] sulphate, is beta-adrenocepter agonist used for the relief of broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease[1]. It is official in Indian pharmacopoeia[2]. It is Used for the treatment of asthma[3,4]. Literature survey reveals that only few UV spcetrophotometric methods[5-9], HPTLC[10], RP-HPLC[11,12], TLC method[13] has been found to be reported for the quantitative estimation of Salbutamol Sulphate in bulk and pharmaceutical formulations. The proposed method was optimized and validated as per ICH guidelines[13].

Hence an attempt has been made to develop new HPLC methods for its estimation in bulk and pharmaceutical formulation with good accuracy, simplicity and precision.

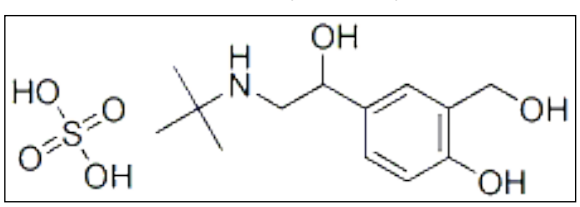

Fig. 1: Salbutamol sulphate

MATERIAL AND METHOD

Instrumentaation and Materials

The liquid chromatographic system consists of shimadzu with SPD-M10AVP detector. The analytes were monitered at 225nm. Chromatographic analysis was performed on Phenomenex C18 column having 250 mm× 4.6 mm and 5µm particle size. Chromatogram was automatically obtained by spinchrome system software.

Reagents and Materials

All chemicals and reagents were used of AR grade.

Chromatographic Conditions

The Phenomnex C18 column (250 x 4.6mm, 5µm) equilibrated with mobile phase Acetonitrile, buffer and Tetrahydrofuran in the ratio of 70:20:10 (v/v) was used and maintain the pH4.7. The flow rate was maintained at 1.0 mL/min. Detection wavelength with UV detector at 225 nm, and the injection volume was 20 µL and run time was kept 05 min.

Preparation of buffer solution

To prepare buffer solution take accurately 1.5ml of Orthophosphoric acid (H3PO4) and was dissolved in 750 mL HPLC grade water. After that its pH was adjusted to pH 4.7 with the help of Triethylamine.

Preparation of mobile phase

The mobile phase was prepared by taking 70% Acetonitrile and 20% Buffer and 10% Tetrahydrofuran. It was filtered through 0.45µ filter and degassed under ultrasonic bath prior use. The mobile phase was pumped through the column to stabilize the column.

Preparation of standard stock solution

26 mg Salbutamol sulphate was weighed accurately and it was dissolved in the mobile phase and after complete dissolution the volume was made up to 100 ml. The stock solution was prepared.

Preparation of working standard solution

From the standard stock solution, 5ml solution was pipette out in 50ml volumetric flask and volume was made up to the mark with mobile phase.

Analysis of tablet formulation

Twenty tablets (Asthalin-4) were weighed, triturated, mixed thoroughly and average weight of tablet was calculated. Accurately weighed quantity of tablet powder equivalent to 4mg of SAL was transferred to 100ml volumetric flask, it was dissolved in mobile phase and sonicate for 10min. The resultant solution was filtered through 0.45µ filter. 15ml of resultant solution further diluted to 25ml with mobile phase and injected to HPLC system (Table 1).

Sample	Label claim	RP-HPLC % estimated ± S.D.	% RSD
SAL	4mg	99.15±0.6859	0.6918

Table 1: Analysis data of Salbutamol Sulphate

VALIDATION OF METHOD DEVELOPED BY RP-HPLC

Linearity

Different standard solution were prepared by diluting standard stock solution with mobile phase in concentration 80%, 100%, 120% injected and chromatogram were taken under standard chromatographic conditions. The calibration curve data are shown in Fig 2.

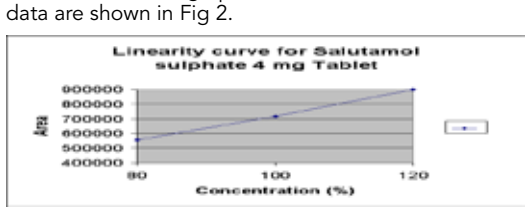

Fig. 2: Calibration curve of SAL

Volume : 4 | Issue : 3 | Mar 2014 | ISSN - 2249-555X

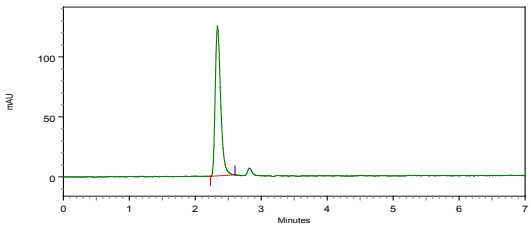

Fig. 3: Chromatogram of SAL

Accuracy

Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 80%, 100% and 120% to the pre analyzed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated which sense to confirmation that the proposed method was accurate.

S. No.	Concentration	% Recovery
1	80%	99.49
2	100%	98.51
3	120%	98.58
Mean		98.86 %
SD		0.335514 %

Table. 2: Accuracy

Precision

Repeatability

Repeatability was assessed using six determinations at 100 percent of the test concentration i.e. 100µg/ml of SAL. Data were subjected to statistical treatment for the calculated of SD and RSD. The data shown in table no. 3.

S. No	% Assay
1	98.39 %
2	98.59 %
3	99.23 %
4	98.78 %
5	99.78 %
6	100.11 %
Mean	99.15 %
SD	0.685993 %
%RSD	0.691897 %

Table 3: Precision (Repeatability)

Robustness

The robustness was determined by injecting triplicate injections of standard and three sample solutions in single at each different condition with respect to control condition. Robustness of the method was checked by varying the instrumental conditions. Flow rate(±0.2ml/min) and temperature (±2°C). Sample solution was injected in each condition.

RESULT & DISCUSSION

Linearity and Range

The method was found to be linear. In the linearity study, regression equation and coefficient of correlation for SAL was found to be r^2 =0.9992.

Accuracy

The mean recovery was found to be 98.86%. The limit for mean recovery is 80-120%. Thus the method was found to be accurate.

Precision

Repeatability

The repeatability study which was conducted on the solution having the concentration for SAL showed a RSD of 0.691% for SAL. It was concluded that the analytical technique showed good repeatability.

Robustness

This method is robust for the analysis of SAL within the specified range of deviations in the experimental conditions.

Assay

The present content of SAL in tablet was found to be 99.15%

CONCLUSION

The developed method was validated as per ICH guideline and was found to be within the prescribed limit. It concludes that the developed methods are simple, accurate, sensitive and precise and suitable for both authentic and pharmaceutical dosage form.

ACKNOWLEDGMENTS

The authors are thankful to Pramukh Swami Science and H D Patel Arts College, Kadi, Gujarat for providing working facilities.

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