Nirma Univ J Pharm Sci; 2023, 10(1) 87-94

ISSN 2348 –4012

RESEARCH ARTICLE

VALIDATION AND ASSESSMENT OF STABILITY INDICATING RP-HPLC METHOD FOR SALBUTAMOL SULFATE IN INHALATIONAL SOLUTION

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Abstract

RP-HPLC SIAM was developed and validated for the assessment of salbutamol sulfate in inhalational solution on an adopted compendial method. The method was tested for its feasibility and specificity to separate the principal component, related substances and impurities from the prepared in-house formulation undergoing stability studies. The studies elaborate feasibility testing of adopted method optimization of forced degradation studies to achieve 10-30% degradation of API as such and in formulation. The API and formulation were exposed to stressful condition for 7 days and they were analyzed using a waters symmetry C_8 (150mm X 3.9mm, 5µm) column at 25°C on a flow rate of 1.0mL/min for over 50 minutes. The mobile phase was prepared by mixing 220 mL acetonitrile and 780 mL phosphate buffer (pH 3.65). Detection wavelength used was 220nm. The optimized method was validated as per the ICH guidelines(Q2A).

Keywords: Salbutamol sulfate, RP-HPLC, SIAM, Forced degradation

INTRODUCTION

Salbutamol sulfate is a beta-2 adrenergic receptor agonist class of drug used as bronchodilator in treatment of asthma. The IUPAC name of salbutamol is 4-[2-(*tert*-butylamino)-1-hydroxyethyl]-2(hydroxymethyl)phenol-sulfate (Figure 1).

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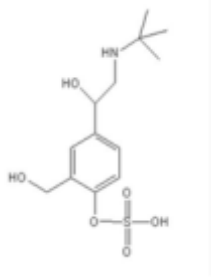

Figure 1. Structure of Salbutamol

It is used as a bronchodilator in management of asthma and various chronic obstructive respiratory disorders. R-isomer show potent broncho-dilatory activity whereas S-isomer increases reactivity of bronchi. It stimulates beta-2 adrenergic cyclase which catalyzes enzyme adenylate cyclase which catalyzes ATP conversion into cAMP which stimulates cAMP resulting into relaxation of bronchial muscles and reliving spasm. Salbutamol sulfate is administered either by oral or nasal route. It is available in tablets, capsules, metered dose inhalers and nebulizer solution dosage form in varying strength of 0.5mg/mL to 5mg/mL with pH of inhalational formulations between 3 to 4 [1][2].

EXPERIMENTAL

Materials

Salbutamol Sulphate (99% purity) was gifted by Aculife healthcare Pvt. Ltd. (Ahmedabad, India). HPLC grade acetonitrile was procured from Finar Chemicals (Ahmedabad, India). Analytical reagent grade sodium hydroxide, hydrochloric acid, hydrogen peroxide was procured from Rankem (Mumbai, India). HPLC grade sodium heptane sulphonic acid and potassium dihydrogen ortho phosphate was procured from Finar Chemicals (Ahmedabad, India). MilliQ water purified by Milli Q water purification system (Merck Germany) was used for the preparation of various solutions.

Instrumentation

The HPLC system LC-2010 HT with SPD-M20A diode array detector (Shimadzu, Kyoto, Japan) was used. A reversed phase Waters Symmetry C_8 (150mm x 3.9mm; 5µm) column was used for separation of compounds. The chromatographic data was recorded on Lenovo computer system with lab solution data acquiring software (Shimadzu, Kyoto, Japan). Photo stability chamber (Thermolab Scientific equipment, Palghar, India) was used for the experiment.

Forced degradation study

Forced degradation study of salbutamol sulfate API and inhalation formulation was carried under conditions such as oxidative, photolytic, hydrolysis and thermal degradation. Oxidation was carried out by employing 3% hydrogen peroxide solution, for thermal degradation, samples were kept at 70°C. For photo degradation, samples were exposed to ultra violet light for 20 watt hours and for 1.2 million lux hours. Hydrolysis of the samples was carried for acid hydrolysis and base hydrolysis by using 0.1M HCl solution for acid and 0.1M

NaOH solution for base hydrolysis respectively. The studies were carried out for 7 days for oxidation, thermal and hydrolysis degradation whereas the samples were exposed to photo degradation until the programmed exposure ended in photostability chamber. The optimized forced degradation conditions are outlined in Table 1 [3] [4][5][6].

Table1: Stress conditions employed in forced degradation studies

Degradation type	Condition	Study duration
Hydrolysis	0.1M Acid	7 Days
	0.1M Base	7 Days
Oxidative	3% H_2O_2	7 Days
Photo	200-watt hours & 1.2 Million lux hours	Until completion of both the exposure parameters (approx. 6 days)
Thermal	70°C	7 Days

Chromatographic parameters

Waters symmetry C_8 (150mm x 3.9mm, 5µm) column was used for the elution of the analytes. Elution was carried out at 25°C oven temperature, with mobile phase flow rate 1.0 mL/min. 20 µL sample was injected into the system and run time of chromatographic elution was 50min. The column eluent was monitored at 220nm [7][8].

Solution preparation

Buffer was prepared by taking 2.87 gm of sodium heptane sulphonate and 2.5gm of potassium dihydrogen ortho phosphate and dissolving it in 1000 mL water. The content was mixed well and pH was adjusted to 3.65 with 2M orthophosphoric acid. Mobile phase was prepared by taking mixture of 220mL acetonitrile and 780 mL of buffer. The mobile phase mixture was sonicated to degas before use. Mobile phase was also used as a diluent for various solution preparation. Standard solution of drug was prepared by taking 20.0 mg of working standard of salbutamol sulphate and transferring it into a 50 mL volumetric flask. Few mL of mobile phase diluent was added to it and drug was dissolved completely using sonication. 3.0 mL of this solution was taken and diluted up to 20 mL with diluent. From this 20 mL solution, 1.0 mL was taken and diluted up to 10 mL with diluent. The final solution was labelled as standard solution.

Drug sample preparation was carried out by taking 5 mL of sample solution and diluting it up to 20 mL using diluent.

Placebo / blank preparation was carried out by accurately transferring 5mL diluent to various 20 mL volumetric flasks and adding each of degrading solutions i.e. 0.1 M HCl, 0.1M NaOH, 3% H_2O_2 in an individual flask. The solutions were kept for 7 days in the same experimental conditions. For photo and thermal degradation blank samples, the diluent solution was kept as such in a flask under defined experimental conditions.

Method validation

System suitability

To check the system suitability of the method a standard solution of 3 µg/mL of the salbutamol sulphate was prepared as per the method mentioned above six replicate injections were injected in HPLC.

Specificity

Specificity was carried out by injecting 3 µg/mL standard salbutamol solution, placebo and sample solution as per the experimental method. The peaks were analyzed using PDA detector. Standard impurities were spiked in the standard solution and peaks due to the impurities and solution of principle component were identified.

Linearity

Linearity of the method was check at 5 levels 25% to 150% for concentration range from 1.250 µg/mL to 3.750 µg/mL. Linearity solutions were prepared as per table 2. A linearity curve was plotted for concentration Vs peak area at each level. A linearity stock solution was prepared by diluting 7.5 mL of principal standard solution to 30 mL using diluent.

Table2. Linearity solution preparation

Linearity level	Volume of linearity stock	Dilution	Concentration of solution (µg/mL)
Level-1 (25%)	2	10	1.250
Level-2 (50%)	3	10	1.875
Level-3 (100%)	4	10	2.500
Level-4 (125%)	5	10	3.125
Level-5 (150%)	6	10	3.750

Precision

For method precision standard, sample and placebo solutions were prepared as per the method and injected in HPLC system. Six replicates of standard solutions were injected to establish system % RSD, NTEP, tailing factor and difference between retention time. %RSD of six results for each individual impurity and total impurity were calculated.

Accuracy

Accuracy of the method was studied at 3 levels from 50% to 100% for concentration range from 0.25% to 1.0 %. Each set was injected as triplicate and % recovery of impurity D, mean % recovery and %RSD for each level was calculated.

LOD and LOQ

Three sets of LOD and LOQ at 0.025% and 0.050 % concentrations were prepared and injected and S/N ratio of 3 and 10 were determined for LOD and LOQ respectively.

Robustness

Robustness study was performed for variation in column oven temperature, variation in flow rate, variation in pH of buffer in mobile phase and variation in composition by ±5% change in above all conditions [9].

RESULTS AND DISCUSSION

An ICH guideline Q2A was followed for validation and assessment of the method. To validate the method all recommended parameters such as system suitability, specificity, linearity, limit of detection, limit of quantification, robustness, precision and accuracy of the method were analyzed and evaluated. Which gave a documented evidence that the developed method is accurate and give precise results being robust to ±5% variation in methods parameters such that they do not affect the specificity of the method and gives accurate and precise results within the range. The linearity of the method lies in range of 1.254-3.762 µg/mL with regression coefficient to be 0.99 and equation of linearity be y = 36852x + 776 (Figure 2).

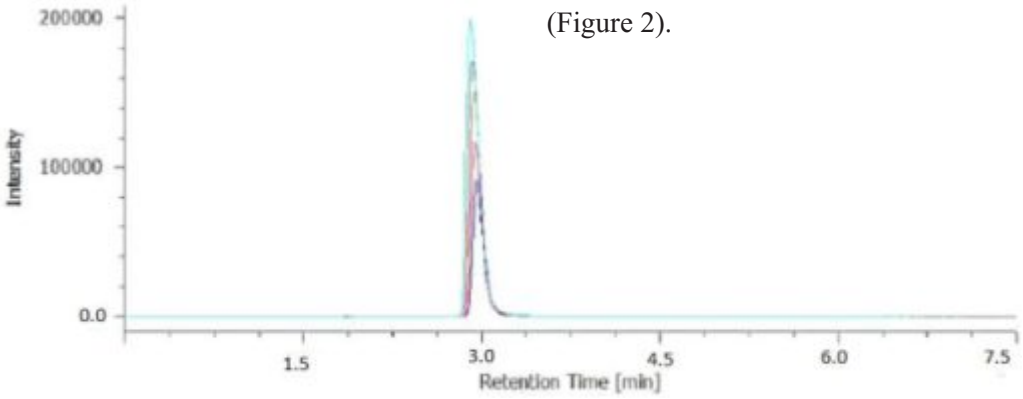

Figure 2. Linearity chromatogram for salbutamol sulphate (1.254-3.762 µg/mL)

The method was found to be accurate with recovery range of 98.2-100.4% calculated on results obtained in triplicate injection of three accuracy level at 50, 100 and 150% accuracy levels. The method passes intraday precision with %RSD for the peak area of the impurity D, max unknown impurity and total impurities is found to be 0.10%, 0.00% and 0.20% respectively and intermediate precision with %RSD for the peak area of the impurity D, max unknown impurity and total impurities is found to be 0.37%, 1.04% and 0.38% respectively. Mass balance and total impurity for API and sample is given in Table 3 and Table 4 respectively.

Table 3. Total impurities and mass balance for API

Conditions	Assay %	Total Impurity %	Total Assay %	As Such Assay %	Mass Balance %
Acid	102.2	0.069	102.27	99.4	2.87
Alkali	91.0	2.8.1	93.80	99.4	-5.60
Peroxide	100.5	0..92	100.59	99.4	1.19
Photo	101.4	0.081	101.48	99.4	2.08
Thermal	101.8	0.108	101.91	99.4	2.51

Table 4. Total impurities and mass balance for sample

Conditions	Assay %	Total Impurity %	Total Assay %	As Such Assay %	Mass Balance %
Acid	100.3	0.207	100.51	100.4	0.11
Alkali	98.1	2.104	100.20	100.4	-0.20
Peroxide	98.4	0.593	98.99	100.4	-1.14
Photo	98.9	0.356	99.26	100.4	-1.14
Thermal	97.7	0.355	98.06	100.4	-2.34

Range of the method rom the results obtained in linearity and accuracy study, it is concluded that the analytical procedure is precise, accurate and linear from 0.13% to 0.752% for determination of related substances of salbutamol sample solution.

CONCLUSION

Safety and efficacy of any drug substance or product highly depends upon the chemical stability of the pharmaceutical molecule which is of great concern in

terms of impurity and its self-life. A method indicating stability of the preparation capable of separating all the process and product related impurities from the principle component is developed and validated for the cause. The study shows that our previous method is successfully extended to determine salbutamol in aqueous solution as pharmaceutical preparation. The method was found to be robust for the experimental operating conditions over range of ±5% of the optimized conditions. The method is precise and accurate as on repeated performance it is giving reproducible results precisely and accurately. From the results obtained in validation studies it can be said that method is fit for use in quality control department for routine analysis and as method for analysis of sample in batch processing. The forced degradation study of API and finished product shows that there is no such potential impurity generated in the samples which shows that the formulation is well optimized and stable and also withholds with the stressful conditions exposed. The method is employed in separation and detection gives feasible results and from the forced degradation study and results obtained in validation it can be concluded that the method is stability indicating and separates analytes accurately.

CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

ACKNOWLEDGEMENT

The authors are thankful to Nirma University and Aculife Healthcare Private Limited, Ahmedabad for providing necessary chemicals, reagents and infrastructure facility.

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