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Research Article

HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF SALBUTAMOL SULPHATE

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ABSTRACT

Analytical method development helps to understand the critical process parameters and to minimize their influence on accuracy and precision. High Performance Liquid Chromatography is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. The new *HPLC* method was developed and validated for estimation of salbutamol sulphate in pharmaceutical dosage form. This method has provided adequate separation for salbutamol sulphate from their impurities and degradation products. Separation was obtained by using a Kromasil (125 * 4.0nm) C-18 column at 40°C temperature and using a mobile phase buffer (pH 3.7). Acetonitrile in the ratio of (815:185) at a flow rate 1.0 mL/min and wavelength for detection was 210 nm. Three replicate samples were taken to calculate % assay of ipratropium bromide and salbutamol sulphate. The % mean assay of the drug was found to be 116.4%. The linearity of developed method was achieved in the range of 1-25 µg/mL (r^2=0.9999) for salbutamol sulphate. The proposed analytical method development and estimation of salbutamol sulphate and its validation by *HPLC* is accurate, precise, simple, selective, sensitive and rapid, hence this method can be effectively used for analysis of salbutamol sulphate in pharmaceutical dosage form.

Keywords: Salbutamol sulphate, HPLC, Method development, Validation.

1. INTRODUCTION

The number of drugs being introduced into pharmaceutical market is increasing every year. These drugs may be either new chemical entities or variants of existing drug. Most of the drugs are introduced in the market but its inclusion in pharmacopoeias is much delayed. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these circumstances, standard analytical procedures for these drugs may not be available in the pharmacopoeias. Therefore, it becomes necessary to develop newer analytical methods for such drugs.

There are many reasons such as: regulatory requirements, good science, and quality control requirements, for the need to validate the analytical procedures [1, 2].

Salbutamol sulphate chemically known as 4-[2-(tert-butyl amino)- 1-hydroxyethyl]-2 hydroxymethyl) phenol sulfuric acid belongs to the class of medicines known as short acting beta-2 adrenergic agonist.

It is used in the treatment of bronchospasm in bronchial asthma, chronic bronchitis and chronic obstructive pulmonary disease.

Salbutamol sulphate relaxes the smooth muscles of all airways, from the trachea to the terminal bronchioles, thus making breathing easier [3-5]. Literature assessment showed that various analytical methods have been reported for the assessment of salbutamol sulphate for routine quality control analysis, related substances and impurity determinations in dosage forms containing salbutamol sulphate [6-8], but do not fulfill regulatory requirements. An attempt has been made to develop a new HPLC method for determination of salbutamol sulphate. The aim of present study is to develop and validate the HPLC method that is simple, accurate, rapid, précise, sensitive and reliable for the estimation of salbutamol sulphate as per ICH guideline.

2. MATERIALS AND METHODS

2.1. Materials

Salbutamol sulphate pure drug was used and received as gift sample from Cadila Healthcare Limited, Gujarat. HPLC grade Acetonitrile (ACN), Potassium dihydrogen phosphate, Heptanes Sulphonic acid sodium salt, O-

phosphoric acid were used and procured from Merck, India. The water for HPLC grade was prepared by triple glass distillation and filtered through a nylon 0.22 µm-47 mm membrane filter.

2.2. Development of *HPLC* Method for analysis of salbutamol sulphate

2.2.1. Selection of detection wavelength

The sensitivity of *HPLC* method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected. In the present study, standard solution of salbutamol sulphate was scanned in the range of 200-400 nm wavelengths. The drug showed absorbance at 210 nm. Hence, the 210 nm wavelength was chosen for estimation of salbutamol sulphate in dosage forms.

2.2.2. Selection of chromatographic condition

Appropriate selection of the *HPLC* method depends on the nature of the sample which is ionic or ionizable or neutral molecule, its molecular weight and solubility. *HPLC* was chosen for the initial separation because of its simplicity and suitability. To optimize the condition of chromatography, the effect of chromatographic variables such as: mobile phase, pH, flow rate and solvent ratio were studied, and the chromatographic parameters such as capacity factor, asymmetric factor, resolution and column efficiency were calculated. The condition that gave the best resolution, symmetry and capacity factor was selected for estimation.

2.2.3. Selection of mobile phase ratio

The solution containing standard of salbutamol sulphate was chromatographed over mobile phases having different ratio of buffer and acetonitrile, at different pH values.

2.2.4. Selection of suitable column

For *HPLC* method, various columns are available but the main aim was to resolve the drug in the presence of degradation products and other impurities. So the Kromasil C18 column was selected over the other columns. Kromasil C18 column has embedded polar groups which are more stable at lower pH and high carbon loads, provides high peak purity and more retention to polar drugs and facilitates the separation of impurity peaks within a very short run time [9-11].

2.3. Assay of salbutamol sulphate by developed *HPLC* method

A buffer solution (pH 3.7) was prepared by dissolving 2.5 gm of potassium dihydrogen phosphate and 2.87 gm of Heptan sulphonic acid sodium salt in 1000 ml in Milli-Q water; pH was adjusted to 3.7 with diluted o-ophosphoric acid and filtered. The mixture of Buffer and ACN in the ratio of 815:185 and 815:150 were used as mobile phase and diluent, respectively. Standard stock solution of salbutamol sulphate was prepared by accurately weighing 25 mg of salbutamol sulphate, which was transferred into a 100 ml volumetric flask, sonicated to dissolve and diluted to make up the volume with diluent and mixed well. The sample was assayed by using developed HPLC method.

2.4. Forced degradation study

In order to prove the analytical method selectivity, the API of salbutamol sulphate was studied under various stressed conditions to perform forced degradation study. Stress study was carried out under the condition of acid/base hydrolysis, oxidation and thermal, as mentioned in ICH Q1A (R2). From the Forced degradation study, the optimized conditions for stress testing were selected.

2.5. Validation of *HPLC* Method for analysis of salbutamol sulphate

Validation was done as per ICH guideline Q2 (R1). The developed *HPLC* method was validated with respect to parameters such as linearity, precision, accuracy, specificity, robustness and solution stability.

2.5.1. Linearity

Linearity was determined at six levels over the range of 10% to 200%. A standard linearity solution was prepared in different concentrations of 10%, 20%, 40%, 100%, 150% and 200% of the test solution [12, 13].

2.5.2. Precision

Six different test samples were prepared from salbutamol sulphate. Samples were prepared under the same condition and of same concentration. Then they were injected and analysed on same day. Results related to method precision of salbutamol sulphate were recorded [14, 15].

2.5.3. Accuracy

The accuracy of the method was performed at four levels in the range of 10-200% of the working concentration of sample. Calculated amount of salbutamol sulphate working standards were added in placebo to prepare

10%, 40%, 100% and 200% levels of the working concentration. Each level was prepared in triplicate manner. Results of accuracy study of salbutamol sulphate were recorded [16-18].

2.5.4. Robustness

Parameters such as: flow rate, minor components in the mobile phase, column temperature, and mobile phase pH were changed one by one and their effect was observed on system suitability test and assay. Results of robustness study of salbutamol sulphate were recorded [14].

2.5.5. Stability of Analytical Solution

Stability period for standard solution was determined by keeping the solution for 24 hours at room temperature. After 4, 8, 12, 16, 20, 24 hours, the solutions were analysed. The insignificant changes (<2%) were observed for the chromatographic responses for the solution analysed, relative to freshly prepared standard. The stability period of salbutamol sulphate was determined and recorded [15].

3. RESULT AND DISCUSSION

The main objective of this work was to develop and validate *HPLC* method for estimation of salbutamol sulphate in dosage form. Analytical method development for estimation of salbutamol sulphate and its validation by *HPLC*, various mobile phases like water, methanol, and different pH buffer solutions were tried in various proportions. Varied temperatures, different flow rate and different columns were tried and finally optimized chromatographic conditions are shown below:

- Stationary phase: Kromasil 100-5 C18 (125 mm x 4.0 mm) 5 µm
- Mobile phase: Buffer: Acetonitrile (815:185)
- Flow rate: 1.0 mL/min
- Injection volume: 50 µL
- Column temperature: 40°C
- Detection: At 210 nm with UV-visible detector.

The forced degradation study was successfully applied for the development of this method. Optimized conditions of forced degradation study are presented in Table 1. The method has provided adequate separation for salbutamol sulphate from their impurities and degradation products. Separation was achieved by using a Kromasil (125 * 4.0nm) C 18 column at 40°C temperature and using a mobile phase Buffer (pH 3.7): Acetonitrile in the ratio of (815:185) at a flow rate 1.0 mL/min and wavelength for detection was 210 nm. The stress study of salbutamol sulphate was carried out as per ICH guideline Q1A (R2). The specificity of the method was proved by doing forced degradation study of salbutamol sulphate API and its formulation. Forced degradation study and peak purity data (Table 2) proved the selectivity of the above-developed method. The method is specific for the estimation of salbutamol sulphate in dosage form. The % assay of salbutamol sulphate were calculated by taking 3 replicates of test sample and % mean assay of salbutamol sulphate were found to be 116.4%.

Table 1: Optimized conditions for forced degradation study of salbutamol sulphate

Stress Type	Stress Condition
Acidic	2 mL of 1N HCl for 1 hour at 80°C
Alkali	2 mL of 0.1N NaOH for 60 min at 80°C
Thermal	80°C for 1 hour
Peroxide	2 mL of 5 % H_2O_2 for 1 hour at 80°C

Table 2: Forced degradation data (% degradation and peak purity index) of salbutamol sulphate

Condition	% Degradation	Peak Purity Index
As such	0.0	999.232
Acid	0.0	999.246
Alkali	2.48	999.379
Thermal	0.0	999.955
Peroxide	0.0	999.346

Table 3: Linearity data of salbutamol sulphate

Linearity Range	Stock (A) solution to be taken (mL)	Dilute to volume with diluents (mL)	Final concentration of Salbutamol Sulphate (µg/mL)	Area
10%	0.5	100	1.250	93624
20%	1.0	100	2.500	201276
40%	2.0	100	5.000	388634
100%	5.0	100	12.475	934746
150%	7.5	100	18.750	1394727
200%	10.0	100	24.625	1837371

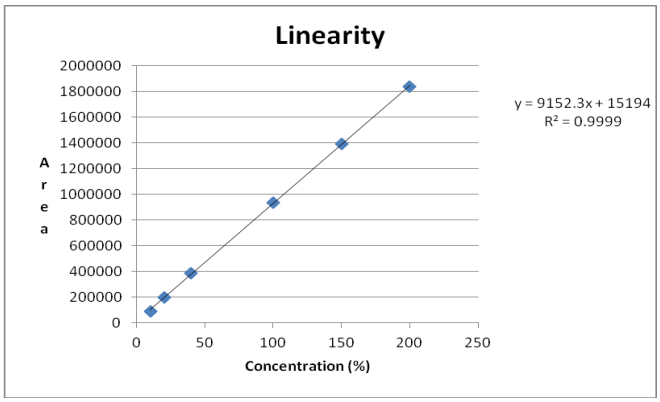

Fig. 1: Linearity of salbutamol sulphate

Table 4: Method precision data of salbutamol sulphate

Set No.	% Assay	% Assay Mean	% RSD
1	117.1	116.4	0.7
2	117.2		
3	117.3		
4	116.4		
5	115.3		
6	115.4		

Table 5: Results of accuracy data of salbutamol sulphate

For Salbutamol Sulphate					
Level	**Amount of Drug added (µg/mL)**	**Amount of Drug recovered (µg/mL)**	**Recovery (%)**	**Mean (%)**	**% RSD**
10 %	1.250	1.250	100.0	100.1	0.1
	1.250	1.252	100.2		
	1.250	1.250	100.0		
40 %	5.000	5.100	102.0	102.0	0.0
	5.000	5.100	102.0		
	5.000	5.100	102.0		
100 %	12.500	12.475	99.8	99.8	0.0
	12.500	12.475	99.8		
	12.500	12.475	99.8		
200%	25.000	24.600	98.4	98.4	0.1
	25.000	24.600	98.4		
	25.000	24.625	98.5		

Above developed method was validated according to ICH guideline parameters like linearity, precision, accuracy, robustness and solution stability and their results complies with official standards. The linearity of developed method was achieved in the range of 1-25 µg/mL (r^2=0.9999) for salbutamol sulphate, shown in Table 3 and presented in Figure 1. The plot was found to be linear with a correlation co-efficient for the analyte salbutamol is 0.9999 (1.000) and the correlation co-efficient was not less than 0.999.

The result of method precision shown in Table 4 indicates that developed method was precise and the study concludes repeatability (precision) of the test result obtained by this method. Accuracy of developed method shows that no determination was outside the range of 98-102 % of label claim and % RSD was not more than 2.0 %, results are presented in Table 5.

The developed method shows robust result (Table 6) at different deliberate changes in chromatograph conditions and % RSD was found not more than 2.0 %. Results of stability of both standard and sample solution of salbutamol sulphate were determined. Table 7 shows that the peak area difference of salbutamol for both standard and sample solution was not more than ± 2.0 % from the initial peak area for the accepted storage time i.e. up to 36 hours. The standard solution and sample solution was stable up to 36 hours.

The results show that all validation parameters of developed method lie within its specific acceptance criteria (Table 8).

Table 6: Results of robustness study

Compound	% RSD		
	Normal Condition	Changed Condition	
Temperature	**Normal**	**(-5°C)**	**(+5°C)**
Ipratropium Bromide	0.2	0.4	0.4
Salbutamol Sulphate	0.1	0.4	0.2
pH	**Normal**	**(-0.2 unit)**	**(+0.2 unit)**
Ipratropium Bromide	0.2	0.3	0.3
Salbutamol Sulphate	0.1	0.1	0.1
Flow Rate	**Normal**	**(-10%)**	**(+10%)**
Ipratropium Bromide	0.2	0.3	1.2
Salbutamol Sulphate	0.1	0.2	0.1
Mobile phase ratio	**Normal**	**(-10%)**	**(+10%)**
Ipratropium Bromide	0.2	0.5	0.2
Salbutamol Sulphate	0.1	0.6	0.3

Table 7: Results of stability of standard and sample solution of salbutamol sulphate

Time (hour)	Area		% Difference	
	Standard	Sample	Standard	Sample
0 (Initial)	934472	1178986	-	-
12	931750	1174592	-0.3	-0.4
18	933812	1180139	-0.1	0.1
24	927458	1170410	-0.8	-0.7
30	928763	1173852	-0.6	-0.4
36	922022	1170868	-1.3	-0.7

Table 8: Compiled results of various validation parameters

Salbutamol Sulphate	
Linearity and Range	Correlation coefficient: 0.9999 (1.000) Range: 1.0 to 25.0 µg/mL
Method precision (n = 6) (Repeatability)	RSD: 0.7 %
% Mean recovery	98.0 – 102.0%
Robustness: Change in Flow rate (+/- 10%), mobile phase ratio (+/- 10 %), column temperature (+/- 5°C), pH(+/-0.2)	Method shows robust result at different deliberates changes in chromatograph conditions
System suitability	Meets the system suitability criteria

4. CONCLUSIONS

The HPLC method developed and validated in this work proved to be simple, fast, accurate, precise and sensitive. The method described affords quantification of salbutamol sulphate. There is also simplicity of sample preparation and the low costs of solvents used. So the method described is specific. Hence, this method can be used for analysis of salbutamol sulphate in pharmaceutical dosage form.

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