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NEW VALIDATED VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF SALBUTAMOL SULPHATE IN BULK AND DOSAGE FORM

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Abstract

The present study describes a simple, accurate, precise and cost effective Visible-Spectrophotometric method for the estimation of Salbutamol sulphate. The stock solution of Salbutamol sulphate was prepared by using distilled water as solvent. This method based on the complex formation when the Salbutamol sulphate was treated with FC (Folin ciocalteu) reagent diluted with water (1:3) in presence 0.1N NaOH solution. The complex is bluish green in colour and has the maximum absorbance at 640nm. The developed method obeys the beer's law in the concentration range of 10-50µg/ml. The method was validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. This method can be used for the determination of Salbutamol sulphate in quality control of formulation without interference of the excipients.

Keywords: Salbutamol sulphate; Visible-spectrophotometry; FC (Folin -ciocalteu) reagent.

Introduction

Salbutamol sulphate, *RS*-[4-[2-(*tert*-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol] Sulphate is a short acting β_2-adrenergic receptor agonist used for the relief of Broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease [1]. It is formulated as 1:1 sulphate salt.

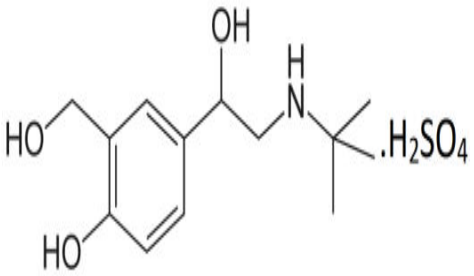

Fig: 1. Molecular structure of Salbutamol Sulphate.

It is a recently introduced azapeptide inhibitor of HIV-1 Protease. It is formulating as 1:1 sulphate salt. The drug is official in European Pharmacopoeia [2], which describes a potentiometric titration in non-aqueous medium, British Pharmacoeia [3] and Indian Pharmacopoeia [4]. Literature survey revealed that Salbutamol was quantitatively assayed different analytical techniques like HPLC [5]-[28], LC-MS/MS [29]- [32], Potentiometry [33], Voltametric [34], UV [35]-[56] TLC [57]-[58], Titrimetric method [59].

The Salbutamol Sulphate was assayed by different visible spectrophotometric methods based on reactions such as redox [60]-[61], reduction followed by chelation [62], oxidative coupling [63]-[64], diazotization and coupling [65]-[66], nitrosation [67], nitration [68], nitration followed by Meisenheiner complex formation [69] and charge-transfer complex formation [70]. However, many of these procedures suffer from some disadvantage, such as poor sensitivity, heating or extraction step, critical working conditions or the use of organic solvents, and are hence unsatisfactory for routine analysis. The only visual titrimetric method [71] reported employs NBS as the oxidimetric titrant in the presence of potassium bromide and using methyl red as indicator. However, the method is applicable over a macro scale. Recently, Issa *et al*. [72] have reported a conductometric titration method using phosphotungstic and phosphomolybdic acids as titrants. The methods employ N-bromosuccinimide [73], bromate–bromide solution[74] as an oxidizing agents, rhodamine-B and methylene blue dyes[73]-[74] as reagents for spectrophotometric analysis. Diazotised o-nitroaniline (DONA) [75] & diazotised p-nitroaniline (DPNA) [76] as a for colour formation, Continuous and Stop flow methods [77] & Spectroflourometric Estimations [78].

Even these procedures are time consuming and less sensitive, the aim of this work is to develop and validate a simple, accurate and low cost analytical method by using visible spectrophotometry for the estimation of Salbutamol sulphate in bulk and pharmaceutical dosage forms.

Materials and Methods

Pharmaceutical grade Salbutamol sulphate was supplied by Hetero Drugs Ltd., Hyderabad, India. The glacial acetic acid was purchased from Fisher scientific and commercially available tablets ASTHALIN-4 (equivalent to 4 mg of Salbutamol sulphate) manufactured by Cipla were purchased from market for analysis.

Labindia-3000 double beam UV-Visible spectrophotometer with 1cm path length supported Labindia UV-3000+ operated by UV-Win 5.2.0.1104 software was used for spectral measurements with 1 cm matched quartz cells. LC/GC balance was used for all weighing.

Method Development

Preparation of Stock Solution:

Weigh accurately 25mg of Salbutamol sulphate and transferred to 25mL volumetric flask. Then add 5mL of Distilled Water to dissolve the drug. Then the final volume was made up to the mark with distilled water.

Preparation of Working Standard Solution:

From stock solution 10mL was further diluted to 100mL with distilled water to get the solution having concentration 100µg/ml.

Optimization of method

The objective is to optimize the assay method for colorimetric estimation of Salbutamol sulphate by using FC reagent based on the literature survey made and methods given in official pharmacopoeias. The trials were done by changing the ratio of FC reagent and water to prepare the reagent and volume of regent added.

Determination of λmax

From the above working standard solution, 5mL was pipette out into a 10mL volumetric flask. Then add 1mL of 0.1N NaOH solution and 0.5mL of FC (Folin ciocalteu) reagent and allowed to stand for 10 min to complete the reaction. Then final volume was made up to the mark with distilled water to prepare a concentration of 50µg/ml. Then the bluish green colour complex solution was scanned in UV-VIS Spectrophotometer in the range 800-400nm against blank and the wavelength corresponding to maximum absorbance (λmax) was found to be 640nm (fig: 3.5.2).

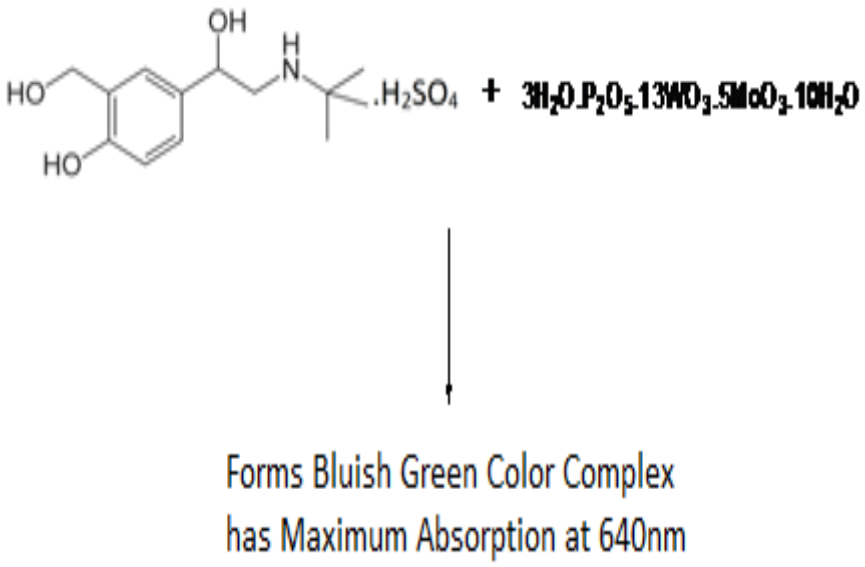

Fig: 2. Reaction of FC with Salbutamol Sulphate.

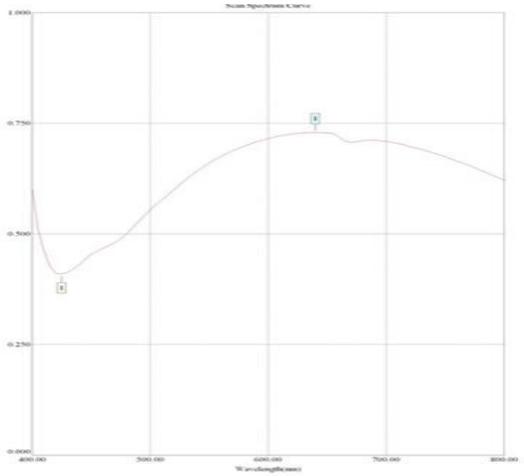

Fig: 3. Visible spectrum of Salbutamol sulphate (50µg/ml) using FC.

Construction of Calibration Curve:

From the working standard solution, pipette out 1mL, 2mL, 3mL, 4mL and 5mL into 10 ml volumetric flask individually, then add 1mL 0.1N NaOH solution and 0.5 ml of FC (Folin ciocalteu) reagent and makeup the final volume up to the mark with distilled water to produce 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, and 50µg/ml solutions respectively. Then measure the absorbance of these solutions at the λmax of 640nm against reagent blank. The calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis (in fig: 3.5.5). The curve showed linearity in the concentration range of 10-50µg/ml. The correlation coefficient (r^2) was found to be 0.9997.

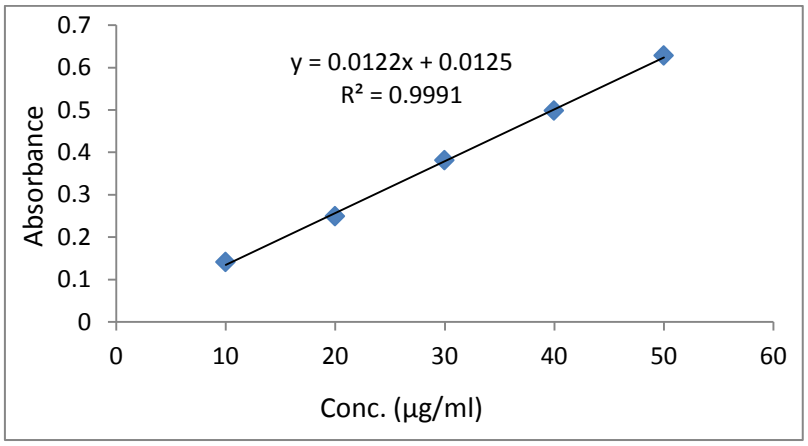

Fig: 4. Calibration curve of Salbutamol Sulphate.

Assay of Salbutamol sulphate tablets (ASTHALIN-4):

A quantity of powder equivalent to 25mg of Salbutamol sulphate was taken in a 25mL volumetric flask and it was dissolved in distilled water by shaking the flask for 3 to 5 minutes and diluted up to the mark distilled water. Then the solution was filtered using Whatmann filter paper No.40. From this filtrate, appropriate dilutions were made with addition of 1mL of 0.1 N NaOH solutions and 0.5 mL of FC (folin ciocalteu) reagent and distilled water to obtain the

desired concentration (30µg/ml). The absorbance of these solutions was measured at 640 nm against reagent blank and the result was indicated by % recovery given in Table1.

Table: 1. Analysis of Formulation.

DRUG	LABED CLAIM (mg/ Tablet)	AMOUNT* FOUND (mg/ Tablet)	% AMOUNT FOUND	%RSD
ASTHALIN-4	4	3.96	99.00	0.57

*Mean of three readings

Method Validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

The method was validated as per ICH guidelines [79] for different parameters like Linearity, Accuracy, Precision, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ).

Linearity

Various aliquots were prepared from the working standard solution (100µg/ml) ranging from 10-50µg/ml. The samples were scanned in UV-VIS Spectrophotometer using distilled water as blank. It was found that the selected drug shows linearity between the 10-50µg/ml (Table: 3).

Accuracy

The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation (ASTHALIN-4) was kept constant (20µg/ml) and the amount of pure drug was varied that is 16µg/ml, 20µg/ml and 24µg/ml. The solutions were prepared in triplicates and the accuracy was indicated by % recovery (table: 5).

Precision

Precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day variation study, six different solutions of same concentration that is 30µg/ml were prepared and analysed three times in a day i.e. morning, afternoon and evening and the absorbances were noted. The result was indicated by % RSD (table: 6). In the

inter-day variation study, 6 different solutions of same concentration (45µg/ml) were prepared and analysed three times for three consecutive days and the absorbances were noted. The result was indicated by % RSD (table: 7).

Robustness

Robustness of the method was determined by carrying out the analysis at five different wavelengths (i.e. 640±0.5nm). The respective absorbances were noted and the result was indicated by % RSD (table: 8).

Ruggedness

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbencies were noted. The result was indicated by % RSD (table: 8).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is the minimum concentration that can be able to detect by using the developed method. The limit of quantification (LOQ) is the minimum concentration that can be able to quantify or measured by the developed method.

Both values were calculated by using the formulae involving standard deviation of Y-intercepts and slope of calibration curve (table: 9).

$$LOD = 3.3 \times SD/S$$

$$LOQ = 10 \times SD/S$$

Where, SD = Standard deviation of Y-intercepts

S = mean value of slop

Results and Discussion

The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries (99.14% to 100.39%) of the drug were obtained at each added concentration, which indicates that the method was accurate.

The LOD and LOQ were found to be in sub-microgram level, which indicates the sensitivity of the method. The method was also found to be robust and rugged as indicated by the %RSD values which are less than 2%.

The results of assay show that the amount of drug was in good agreement with the label claim of the formulation as indicated by % amount found (99.71%). Summary of validation parameters of proposed method is shown in table: 2.

Table: 2. Summary of validation.

Parameter	Value
Linearity indicated by correlation coefficient	0.999
Precision indicated by %RSD	1.163
Accuracy indicated by % recovery	99.75
Limit of detection (LOD), µg /ml	0.97
Limit of quantification (LOQ), µg /ml	2.94
Linear regression equation	Y=0.017-0.003
Robustness indicated by %RSD	0.213
Ruggedness indicated by %RSD	1.194
Assay indicated by % purity	99.71

Table: 3. Linearity of Salbutamol Sulphate.

S. No.	Concentration (µg/ml)	Absorbance
1.	10	0.141
2.	20	0.249
3.	30	0.381
4.	40	0.498
5.	50	0.628

Table: 4. Optical characteristics of Salbutamol Sulphate.

Optical characteristics	Values
Beer's law limit (µg/ml)	10-50
Molar extinction coefficient (L/Mol/cm)	13.672533×10^4
Correlation coefficient (r^2)	0.999
Regression equation	y=0.012x+0.012
Slope (a)	0.012
Intercept (b)	0.012

Table: 5. Recovery studies of Salbutamol Sulphate.

Con.of (µg/ml)		% drug added	Amount recovered (µg/ml)*	% recovered	SD	%RSD
Tab	pure drug					
20	16	80	15.91	99.48	0.54	0.49
20	20	100	20.18	100.94	1.08	0.82
20	24	120	23.70	98.78.	0.28	0.27

* mean of three readings

Table: 6. Intra-day precision.

S. No.	Concentration (µg/ml)	Absorbances			Avg. % RSD
		Morning*	A.noon*	Evening*	
1.	30	0.390	0.387	0.0.379	1.186

* mean of six readings

Table: 7. Inter-day precision.

S. No.	Concentration (µg/ml)	Absorbances			Avg. % RSD
		Day 1*	Day 2*	Day 3*	
1.	30	0.388	0.384	0.379	1.079

* mean of six readings

Table: 8. Robustness and Ruggedness of Salbutamol Sulphate.

Parameter		% RSD*
Robustness	Change in λmax (± 0.5nm)	0.375
Ruggedness	1st analyst	0.394
	2nd analyst	0.383

* mean of three readings

Table: 9. LOD & LOQ of Salbutamol sulphate.

Standard	LOD (µg/ml)	LOQ (µg/ml)
Salbutamol sulphate	0.194	0.589

Conclusion

All the above factors lead to the conclusion that the proposed method is accurate, precise, simple, robust and cost effective and can be applied successfully for the estimation of Salbutamol Sulphate in bulk and pharmaceutical formulation.

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