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Original Article

DEVELOPMENT OF NEW ANALYTICAL METHOD AND ITS VALIDATION FOR THE DETERMINATION OF SALBUTAMOL SULPHATE IN BULK AND MARKETED FORMULATIONS

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ABSTRACT

Objective: The present study aims to develop a simple, sensitive, specific, and validated colorimetric method for the quantitative estimation of Salbutamol Sulphate in bulk and pharmaceutical dosage form.

Method: A simple colorimetric method for the determination of Salbutamol Sulphate in pure as well as in its dosage form has been developed. The method is based on formation of a blue coloured chromogen by the interaction of Salbutamol Sulphate with Folin–Ciocalteu reagent under basic condition and the maximum absorbance of the developed chromogen was found. The developed method is validated in terms of Linearity, Accuracy, Precision, Limit of Detection, Limit of Quantitation, as per ICH Guidelines.

Results: The λ_{max} was found to be 723nm for assay. Linearity was found in the concentration range of 10-50 µg/ml respectively with a correlation coefficient of 0.9979. The regression equation was found as y=bx+a: 0.0199x+0.0208.

Conclusion: The proposed method makes use of simple reagent, which an ordinary analytical laboratory can afford. The method was found to be simple, precise, economic and less time consuming. The method has been statistically evaluated and results obtained are accurate, precise and insensitive and free from the interferences of other additives present in the formulation.

Keywords: Salbutamol Sulphate, Colorimetric method, Folin–Ciocalteu reagent, Validation, λ_{max}.

INTRODUCTION

Spectrometric methods are a large group of analytical methods that are based on atomic and molecular spectroscopy[1]. Colorimetric assays generally consists of adding a reagent to the assay preparation or to the substance being tested, to produce a color that is compared with that of a standard preparation that has been prepared simultaneously and contains approximate quantity of the reference standard[2]. In general, in analysis the first step is to determine the nature of the sample that is complete qualitative information and then further proceed for quantitative information by accuracy, LOD, LOQ etc [3]. Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use [4]. The parameters for method validation have been defined in different working groups of national and international committees and are described in the literature [5]. Salbutamol Sulphate is a beta-2-adrenoceptor agonist and bronchodilator. Chemically Salbutamol Sulphate is Bis[(1RS)-2-[(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanol] sulphate (fig. 1). Salbutamol is a beta-(2)-adrenergic agonist and thus it stimulates beta-(2)-adrenergic receptors. Binding of Salbutamol to beta-(2)-receptors in the lungs results in relaxation of bronchial smooth muscles. It is believed that Salbutamol increases cAMP production by activating adenylate cyclase, and the actions of Salbutamol are mediated by cAMP. Increased intracellular cyclic AMP increases the activity of cAMP-dependent protein kinase A, which inhibits the phosphorylation of myosin and lowers intracellular calcium concentrations. A lowered intracellular calcium concentration leads to a smooth muscle relaxation and bronchodilation. In addition to bronchodilation, Salbutamol inhibits the release of bronchoconstricting agents from mast cells, inhibits microvascular leakage, and enhances mucociliary clearance. It is freely soluble in water, practically insoluble or very slightly soluble in ethanol (96 per cent) and in methylene chloride[6,7,8]. The literature survey reveals that few methods are reported for the determination of Salbutamol Sulphate such as Thin layer chromatography, RP isocratic HPLC, UHPLC etc[9].[10,11,12].[13,14]. Validation characteristics evaluated are Specificity, Accuracy, Precision, Limit of detection, Limit of quantitation, Linearity, Range, Ruggedness and Robustness. The method is based on formation of a blue coloured chromogen by the interaction of Salbutamol Sulphate with Folin–Ciocalteu reagent under basic condition.

MATERIALS AND METHODS

Shimadzu UV spectrophotometer 1700 and JascoV-630 spectrophotometer with 1 cm matched quartz cells was used for all spectral and absorbance measurements. Pure drug was procured from Yarrow Chem Products Ltd, Mumbai as a gift sample. Basic apparatus like calibrated volumetric flasks, pipette, beakers and graduated pipettes were used.

Experimental

Preparation of stock solutions

100 mg standard Salbutamol Sulphate was weighed and transferred to a 100 ml volumetric flask and dissolved in distilled water. The flask was shaken and volume was made up to the mark with distilled water to get a concentration of 1000 µg/ml (Stock solution A). From this stock solution A, 10 ml solution was pipetted out and placed into 100 ml volumetric flask. The volume was made up to mark with distilled water to get a concentration of 100µg/ml (Stock solution B).

Preparation of reagents and chemicals

a) Preparation of 1N NaOH

4 gm of NaOH was dissolved in 100 ml of distilled water.

b) Preparation of 2N FCR

50 ml of standard phenol reagent was taken and diluted to 100 ml with distilled water.

Determination of [λ_{max}]

Selection of analytical wavelength

To a 10 ml volumetric flask, 1 ml of drug solution (100µg/ml) from Stock solution B was added. To the flask 0.8 ml of 2N FCR solution

and 6 ml of distilled water was added. The volumetric flask was shaken well for at least 5 min followed by addition of 1.5 ml of 1N NaOH solution. The volume was made up to the mark and stored for 15 min in the dark. The absorbance reading was taken against the blank. The solution was scanned in UV spectrophotometer between 800-400 nm. Absorption spectra obtained showed the absorption maxima [λ_{max}] 723 nm, which was selected as wavelength for analytical measurements. Absorption maxima obtained is given in fig. 2

Preparation of Standard curve

A series of concentration of 10-50µg/ml were prepared of drug solution from Stock Solution (B) in 10 ml volumetric flasks by adding 1 ml, 2 ml, 3 ml, 4 ml and 5 ml respectively. To each appropriately labeled flask 0.8 ml of 2N FCR solution and 6 ml of distilled water was added. The volumetric flasks were shaken well for at least 5 min followed by addition of 1.5 ml of 1N NaOH solution. The volume was made up to the mark and stored for 15 min in the dark. The absorbance was measured at 723 nm against the reagent blank. The result was recorded in table 1 and graph is given in fig. 3.

Method validation

1) Linearity

Linearity was determined over the range of 10–50 µg/ml for Salbutamol Sulphate. Accurately measured Stock solution B of Salbutamol Sulphate (1, 2, 3, 4, and 5 ml) was transferred to one set of a series of 10 ml volumetric flasks. To each appropriately labeled flask 0.8 ml of 2N FCR solution and 6 ml of distilled water was added. The volumetric flasks were shaken well for at least 5 min followed by addition of 1.5 ml of 1N NaOH solution. The volume was made up to the mark and stored for 15 min in the dark. A spectrum was recorded by placing drug solutions and diluent in sample and reference cells respectively. The absorbance was measured at 723 nm (Peak maxima) and was plotted vs. concentration to give calibration curve, and regression equation and correlation coefficient was calculated and presented in table 2 and fig. **3.** The calibration curve of amplitude of absorbance against concentration of the drug showed linearity.

2) Sensitivity

The sensitivity of the proposed method for measurement of Salbutamol Sulphate was estimated in terms of limit of detection [LOD] and limit of quantification [LOQ]. The LOD and LOQ were calculated by using the slope and SD of response. The mean slope value and SD of response were obtained after plotting six calibration curves. The LOD and LOQ obtained are reported in table 2**.**

3) Precision

The precision of the method was established by system precision and method precision. System Precision was subjected to intraday and inter-day variation studies.

a) System Precision

Intraday precision was determined by using three different levels of drug concentrations (10, 20, 30 µg/ml) prepared from Stock solution B and each level was analyzed three times in a day. Same procedure was followed for three different days to study the Inter-day precision. Data obtained are given in the table 2**.**

b) Method Precision

Method precision was determined by using sample solution of drug concentrations (10, 20, 30, 40 and 50µg/ml) and it was analyzed six times in a day by the same analyst. Data obtained are given in the table 2**.**

4) Accuracy

Recovery studies by the standard addition method were performed to study the accuracy of the proposed method. Preanalysed samples of Salbutamol Sulphate (10 µg/ml) were spiked with 80, 100 and 120 % extra Salbutamol Sulphate standard and the mixture were analyzed with the proposed method. Accuracy was assessed as the % Recovery at each concentration level. Data obtained from accuracy study are given in table 2**.**

5) Ruggedness

To establish ruggedness of the proposed method, assays for two different concentrations of Salbutamol Sulphate tablets were performed by two different analysts. The results of assays are represented as % Recovery with SD and % RSD showing the ruggedness of the proposed method are illustrated in table 2**.**

6) Robustness

The absorbance readings of 10µg/ml were measured at different laboratory using different spectrophotometer by another analyst and the %RSD values obtained to verify their robustness. Data obtained are given in the table 2**.**

7) Specificity

Refers to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behavior. The data obtained are given in the table 3**.**

Stability of colour

A 10 µg/ml concentration of Salbutamol Sulphate was prepared as a working solution and the absorbance was recorded after a period of every 5 minutes in a time span of 90 minutes. The data obtained are shown in the table 4 and fig. 4

Analysis of marketed formulation

Preparation of sample solution:

A tablet marketed formulation, Asthalin-2 (*Cipla Ltd.*) was taken for analysis. Tablet powder equivalent to 100 mg was weighed accurately and transferred into 100 ml volumetric, to this 70 ml of distilled water was added and the volumetric flask was vigorously shaken for about 20 minutes. Finally the volume was made up to the mark with distilled water to get 1000µg/ml concentration (Stock Solution A). The above solution was carefully filtered through Whatman filter paper (No. 41). From this solution 10 ml was pipetted into a 100 ml volumetric flask and made up to the mark with distilled water to obtain 100 µg/ml, (Stock Solution B)

Recovery experiments

From Stock solution B, 2 ml was pipetted out into a 10 ml volumetric flask. Then 0.8 ml of 2N FCR solution and 6 ml of distilled water was added. The volumetric flask was shaken well for at least 5 min followed by addition of 1.5 ml of 1N NaOH solution. The volume was made up to the mark and stored for 15 min in the dark. The absorbance was read at 723 nm against the reagent blank. The result was recorded in table 5.

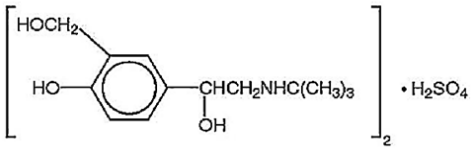

Fig. 1: Salbutamol Sulphate

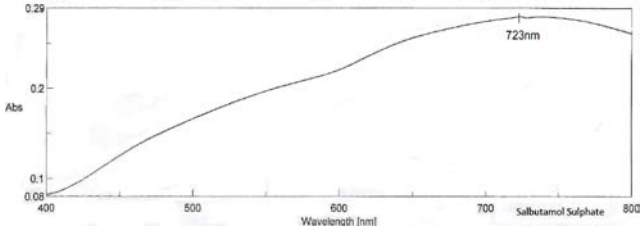

Fig. 2: λ_{max} of coloured species of Salbutamol Sulphate

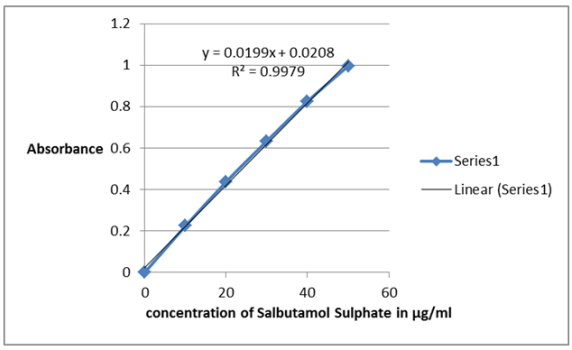

Fig. 3: Standard curve for Salbutamol Sulphate at 723 nm

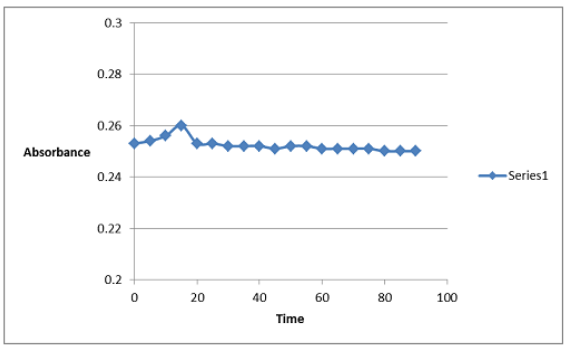

Fig. 4: Stability of colour

Table 1: Standard curve for salbutamol sulphate

S. No.	Volume of drug solution (ml)	Concentration in µg/ml	Absorbance at 723 nm mean±S. D. (n=6)
1	1 ml	10	0.224±0.002428
2	2 ml	20	0.437±0.003983
3	3 ml	30	0.634±0.003899
4	4 ml	40	0.825±0.004412
5	5 ml	50	0.996±0.001871

Table 2: Statistical data for Salbutamol Sulphate by colorimetric method

Parameter	Salbutamol Sulphate at 723 nm
Linear Range(µg/ml)	10-50
Regression Equation* (y)	y=bx+a: 0.0199x+0.0208
Slope (b)	0.0199
Intercept (a)	0.0208
Correlation coefficient (R^2)	0.9979
Standard deviation of slope	0.0002429
Standard deviation of intercept	0.001943
Limit of Detection (µg/ml)	0.32220
Limit of Quantition(µg/ml)	0.97638
Precision:	
Method precision(n=6)	0.12-0.55
System precision (n=3)	
Intraday	0.29-0.58
Inter day	0.32-0.44
Robustness	0.40-0.62
Ruggedness:	
Analyst I	99.88±0.5392-100.40±0.6557
Analyst II	99.43±0.3055-100.05±0.3041
Accuracy (recovery, n=3)	1) At Level-1 (80%)= 99.70±0.4518 2) At Level 2(100%)= 99.50±0.2316
% Mean recovery	3) At Level-3(120%)= 99.84±0.3772

*y = bx+a where x is the concentration of Salbutamol Sulphate in µg/ml and y is the absorbance at the respective wavelength.

Table 3: Specificity

Study	Salbutamol Sulphate
Specificity	Specific

Table 4: Stability of colour

S. No.	Volume of drug solution in 10 ml volumetric flask (100µg/ml)	Time (Minutes)	Absorbance at 723 nm
1	1 ml	0	0.253
2	1 ml	5	0.254
3	1 ml	10	0.256
4	1 ml	15	0.260
5	1 ml	20	0.253
6	1 ml	25	0.253
7	1 ml	30	0.252
8	1 ml	35	0.252
9	1 ml	40	0.252
10	1 ml	45	0.251
11	1 ml	50	0.252
12	1 ml	55	0.252
13	1 ml	60	0.251
14	1 ml	65	0.251
15	1 ml	70	0.251
16	1 ml	75	0.251
17	1 ml	80	0.250
18	1 ml	85	0.250
19	1 ml	90	0.250

Table 5: Assay results of marketed formulation

Formulation	Actual concentration of Salbutamol Sulphate(µg/ml)	Amount obtained of Salbutamol Sulphate (µg/ml)	%Salbutamol Sulphate
Tablet	20 µg/ml	19.68 µg/ml	98.40%

RESULTS AND DISCUSSION

Proposed reaction

Folin–Ciocalteu reagent which is a mixture of tungstates and molybdates works on the mechanism of oxidation–reduction reaction. The method is based on the reduction of the mixture heteropolyphosphotungsates–molybdates by the phenolic group of Salbutamol Sulphate which results in the formation of blue coloured chromogen. The phenolic group reacts with Folin–Ciocalteu reagent only under basic conditions adjusted by Sodium Hydroxide solution. Salbutamol Sulphate brings about a reduction of 1or 2 or 3 oxygen atoms from tungstate and/or molybdate in Folin–Ciocalteu reagent there by producing one or more of the possible reduced species which have a characteristic intense blue color.

The mixed acids present in Folin Ciocalteau reagent involve the following chemical species.

$3H_2O{\cdot}P_2O_5{\cdot}13WO_3{\cdot}5MoO_3{\cdot}10H_2O$ and

$3H_2O{\cdot}P_2O_5{\cdot}14WO_3{\cdot}4MoO_3{\cdot}10H_2O$

Optical conditions, optical characteristics and statistical data of the regression equation in colorimetric method

The optical characteristics such as Beer's law limits, Limit of detection, Limit of quantitation, regression characteristics like slope (b), intercept (a), and correlation coefficient (R^2) using the method of least squares were calculated and the results was presented in table 2. The linearity was found in the concentration range 10-50 µg/ml. The method was validated for accuracy, precision, ruggedness and robustness. Values for both system precision and method precision in terms of % RSD were found to be less than 2, indicating that the method was accurate and precise. Ruggedness was studied with the help of two analysts. Robustness of the method was studied in two different laboratories using two different UV-visible spectrophotometers. The results did not show any statistical difference between operators and environmental conditions, indicating that method developed was rugged and robust. For stability of coloured species of Salbutamol Sulphate, the colour was found to be stable for a period of 90 minutes, showing highest absorbance at 15 minutes. The method developed was found to be simple, sensitive, accurate, precise and economical and can be used in the determination of Salbutamol Sulphate from pharmaceutical formulations in a routine manner.

CONCLUSION

For routine analytical purpose, it is always necessary to establish methods capable of analyzing huge number of samples in a short time period with due accuracy and precision. Few analytical methods appeared in the literature for the determination of Salbutamol Sulphate. Proposed method makes use of simple reagent, which an ordinary analytical laboratory can afford. The method was found to be simple, precise, economic and less time consuming. In the present investigation, colorimetric method for the quantitative estimation of Salbutamol Sulphate in bulk drug and pharmaceutical formulations has been developed.

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CONFLICT OF INTERESTS

Declared None

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