Spectrofluorimetric estimation of salbutamol sulphate in different dosage forms by formation of inclusion complex with β-cyclodextrin

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DOI: 10.4103/2229-4708.72231

Abstract

A simple, precise, reproducible and accurate spectrofluorimetric method for estimation of Salbutamol sulphate (SAL) in bulk drug and various dosage forms has been developed. This method is based on formation of inclusion complex of SAL in β-cyclodextrin (BCD) which gives fluorescence at excitation wavelength of 279.6 nm and emission wavelength of 609.8 nm in water. Formation of inclusion complex of drug with BCD enhances fluorescence intensity of drug leads to increased sensitivity. The developed method was validated according to ICH guidelines with respect to accuracy, precision, linearity, limit of detection, limit of quantification. Linearity was observed in the range of 4-20 μg/ml with correlation coefficient of 0.9982. The simplicity of the method permitted rapid analysis suitable for routine control. The developed method was successfully applied for the estimation of SAL in different marketed dosage forms like tablets, syrup and aerosol.

Key words: Salbutamol sulphate, β-cyclodextrin, spectrofluorometry, method validation pharmaceutical formulations

INTRODUCTION

Salbutamol Sulphate (SAL) is chemically (RS)-1-(4-hydroxy-3-hydroxy methyl phenyl)-2-(tert-butyl amino) ethanol sulphate. It is β_2-adrenoceptor agonist very widely used as bronchodilator in the treatment of asthma and seasonal allergies.[1] SAL is official in IP, BP and USP[2-4] and the official method for quantification of SAL bulk drug is non-aqueous titration, while for syrup and injection dosage form, colorimetric method is employed. Electrochemical techniques,[5-7] chromatographic methods,[8-10] spectrophotometric methods[11-15] and automated methods such as flow injection analysis[16,17] are also reported for detection of SAL in bulk drug and pharmaceutical formulation. The reported chromatographic methods for estimation of SAL are time consuming while official electrochemical and colorimetric methods are less selective and sensitive. Literature survey revealed that different analytical methods are available for estimation of SAL from different marketed formulations. Hence the aim of the research work is to develop a single analytical method applicable for estimation of SAL in various marketed formulations. The present research article describes the development and validation of spectrofluorimetric method for SAL using β-cyclodextrin (BCD) as an inclusion complex. The developed method was validated and was extended for the estimation of SAL in different pharmaceutical formulations.

MATERIALS AND METHODS

Materials and chemicals

Pure sample of SAL and BCD was gifted from Barouq Pharmaceuticals Ltd.

(Anand, India) and Roquette Ltd. (Mumbai, India), respectively. The pharmaceutical formulations of SAL like tablets, syrup and aerosol (all with brand name ASTHALIN, Cipla Pharmaceuticals Ltd. India) were purchased from local market. Sulphuric acid (98%), ethanol, diethyl ether and dimethylsulphoxide (DMSO) were used of analytical grade. Distilled water was used throughout the study.

Instrument

Spectrofluorimetric measurements were carried out using Spectrofluorometer FP 6500 with 1 cm quartz cell (JASCO Corporation, Japan).

Analytical methodology for estimation of SAL in Pharmaceutical Formulations

Standard preparation

SAL (20 mg) was accurately weighed and transferred into 200 ml volumetric flask and diluted up to mark with water. An aliquot (1 ml) was further diluted with water in 10 ml volumetric flask, to obtain final concentration 10 μg/ml.

BCD (50 mg) was weighed accurately and transfer into 50 ml volumetric flask and diluted up to mark with water to obtain concentration 1 mg/ml.

Preparation of inclusion complex

Accurately weighed 20 mg of SAL was dissolved in 5 ml of DMSO. BCD solution (1 mg/ml) was added with continuous agitation to prepare different ratios of SAL: BCD in different proportion from 1: 0.5 to 1: 2.0, it was then diluted with water to obtain concentration of 100 μg/ml SAL in BCD inclusion complex. An aliquot (1ml) was further diluted with water in 10 ml volumetric flask to obtain final concentration 10 μg/ml.

Spectrofluorometric determination

For selection of excitation and emission wavelength of inclusion complex, excitation spectra was scanned between 220-400 nm, while emission spectra was scanned between 400-700 nm. The wavelengths selected for analysis was 279.6 nm as excitation wavelength and 609.8 nm as emission wavelength. Fluorescence intensity of standard and sample solutions determined at selected excitation and emission wavelength.

Sample preparation

Tablet dosage form

Twenty tablets were weighed and crushed. Tablet powder equivalent to 20 mg of SAL was accurately weighed and transferred to volumetric flask; 10 ml water was added into crushed powder and was sonicated for 20 minutes. Above solution was filtered using whatman filter paper 41. Filtrate was collected in crucible and was allowed to evaporate in vacuum dryer until the constant weight was obtained. Collected dry powder was dissolved in 5 ml DMSO and mixed with BCD solution (1 mg/ml, 24 ml) with continuous agitation and kept aside for 20 min and diluted up to 250 ml with water. An aliquot was further diluted with water to obtain final concentration 9.6 μg/ml.

Syrup dosage form

Ten ml of syrup containing 4 mg of SAL was accurately pipetted out and mixed with 25 ml 0.05 M H_2SO_4. Aqueous solution was extracted twice with 50 ml diethyl ether. Aqueous extract was collected in 250 ml volumetric flask. Ether extract was washed with 10 ml water. All aqueous extract was collected together and passed through charcoal to remove coloring matter. DMSO (5 ml) was added in aqueous solution and then BCD solution (1mg/ml, 4.8 ml) was added with continuous agitation and kept aside for 20 min. Volume was adjusted upto the mark with water in 250 ml volumetric flask. An aliquot was further diluted with water to obtain final concentration 9.6 μg/ml.

Aerosol dosage form

For assay of SAL from aerosol dosage form (200 μg/dose), pressurized container removed from the actuator. All the labels and markings present on the container were removed with a suitable solvent. Container was placed in the plastic bag and cooled at least -20 ^{0}C for 24 hrs in deep freezer. Small hole was carefully pierced on the shoulder of the container. Propellant was allowed to evaporate (about 3 hrs) and then top was removed. The top and valve of the open container were washed with little ethanol. All the component of the container put in ethanol (about 20 ml) and sonicated for 15 minutes. Combined alcoholic extract was evaporated into vacuum dryer to obtained constant weight. Collected dry powder was dissolved in 5 ml DMSO and further treated same as given under tablet dosage form to prepare inclusion complex and further diluted to prepare final concentration 9.6 μg/ml.

Sample solutions of all pharmaceutical formulations of SAL were analysed as described under spectrofluorometric determination.

Method validation

The developed method was validated in terms

of linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy.

For linearity and range, SAL: BCD inclusion complex (1:1) was prepared in concentration range of 4-20 µg/ml and then fluorescence intensity of the prepared samples was measured.

The LOD and LOQ were determined from the slope values and standard deviation of intercept of linearity study.

The precision study was performed by intraday and interday precision, where analytical experiment was repeated three times in a day and on three different days using three different concentrations (2, 8, 15 µg/ml) and the results were evaluated for RSD of the assay results obtained in the two conditions.

Accuracy of the method was evaluated by recovery study where standard SAL solution was spiked at three different levels (80 %, 100 % and 120 %) and the amount recovered was found out.

RESULTS AND DISCUSSION

Method development

SAL is freely soluble in water but it gives very less fluorescence due to quenching effect [Figures 1a and b]. Literature survey revealed that BCD forms inclusion complex with benzene derivatives and decreases quenching effect.[18] Formation of inclusion complex of SAL with BCD did not show any significant wavelength shift but it enhanced peak intensity. This may be because the hydrophobic cavity of BCD increases solubility, decrease quenching effect and improve fluorescence intensity; hence BCD was choosen as complexing agent in this experiment. Different complexation methods were used to prepare SAL: BCD inclusion complex. The inclusion complex ratio was also optimized and it was observed that 1:1 ratio of SAL and BCD gave maximum fluorescence intensity [Figure 2]. From the scanned spectra it was observed that SAL gives maximum absorbance at 279.6 nm as excitation wavelength and emission wavelength observed was 609.8 nm [Figures 3a and b].

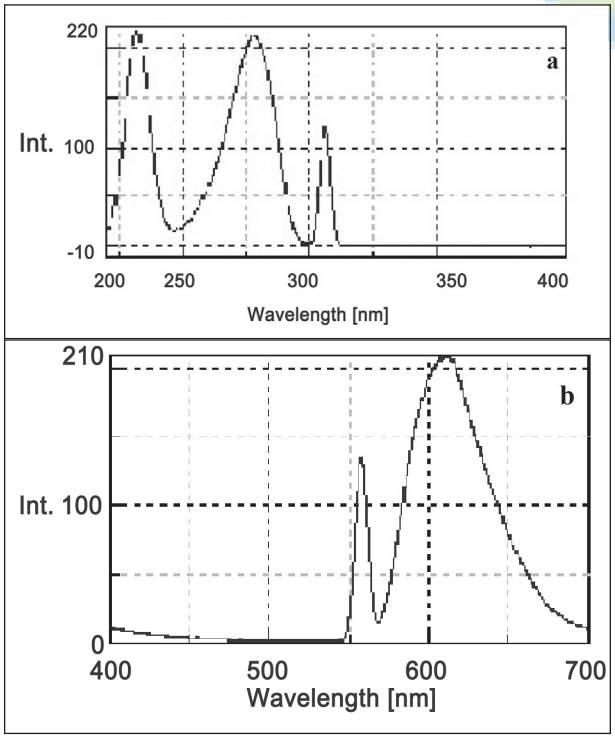

Figure 1: Spectrofluorimetric Spectra of SAL before complexation (a) Excitation spectra (b) Emission spectra

Method validation

The developed method was validated for linearity and range, precision, LOD, LOQ, accuracy as per ICH guidelines for analytical method validation.[19] The SAL: BCD inclusion complex showed linear response in the concentration range of 4-20 µg/ml with good correlation coefficient (r) value of 0.9982. Similarly the method showed good precision when it was performed on the different intervals on same day (% RSD 0.67) and on different days (% RSD 1.16). The results of linearity, LOD, LOQ and precision study are given in table 1. The

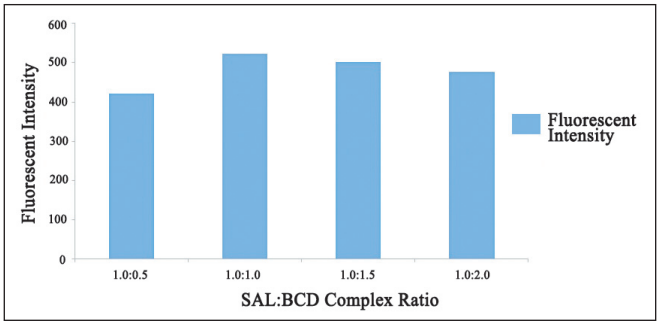

Figure 2: Effect of different ratios of SAL:BCD on fluorescent intensity

Table 1: Summary of results of method validation of SAL by proposed method

Validation parameter	Results
Linearity range (µg/ml)	4–20
Regression equation	y = 41.89x + 57.84
Correlation coefficient (r)	0.9982
Intra-day precision (RSD) (n = 3)	0.67%
Inter-day precision (RSD) (n = 3)	1.16%
LOD (µg/ml)	0.60
LOQ (µg/ml)	1.81

Table 2: Results from the accuracy study of SAL by standard addition method

Concentration of SAL in (µg/ml)	% addition w.r.t. test concentration	Fluorescence intensity	Amount of SAL recovered (µg/ml)	Recovery (%)
4	80	348.6738	7.25	100.69
	80	344.9235	7.17	99.58
	80	350.9282	7.30	101.39
4	100	381.9254	7.99	99.89
	100	387.5295	8.12	101.45
	100	377.1492	7.89	98.57
4	120	420.5925	8.84	100.55
	120	415.8293	8.74	99.35
	120	412.5254	8.66	98.52

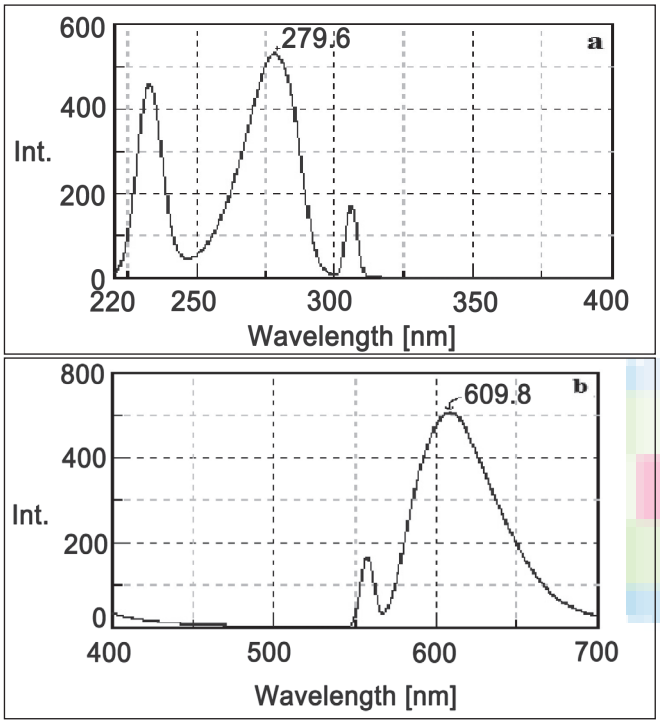

Figure 3: Spectrofluorimetric spectra of SAL: BCD inclusion complex (a) Excitation spectra showing λ_{Exmax} 279.6 nm (b) Emission spectra showing λ_{Emmax} 609.8 nm

accuracy study was performed by standard addition method. Table 2 shows the percentage of drug recovered (98.52–101.45%) which was in good agreement with the added amount and label claim. Recovery experiment indicated the absence of interference from commonly encountered pharmaceutical additives and excipients.

Method application

Different types of marketed formulations of SAL are available and according to that sample preparation can vary for estimation. Initially IP procedure was followed for sample preparation[2] and modification were incorporated as per developed method requirement. Assay results of SAL in all formulations are summarized in Table 3. The obtained results,

Table 3: Summary of results of assay of SAL from different dosage forms

Dosage forms[a]	Label claim	Amount recovered[b]	Assay found[c] (%)
Tablet	4 mg	3.94 ± 0.08 mg	98.54 ± 2.09
Syrup	4 mg/10ml	3.82 ± 0.06 mg	95.79 ± 1.41
Aerosol	200 µg/dose	206.81 ± 1.97 µg	103.40 ± 0.99

a = Brand names of all the dosage forms used in the study - ASTHALIN (Cipla pharmaceuticals Ltd. India) b = Mean and standard deviation of three determinations c = Mean assay and standard deviation of three results

indicates that the developed and validated method can be successfully applied for estimation of SAL from all type of marketed formulations like tablet, syrup and aerosol.

CONCLUSION

Drugs which show fluorescent quenching in aqueous solutions can be analyzed easily by preparing inclusion complex with BCD. The proposed and developed spectrofluorimetric method is simple, specific, accurate, precise, economical and rapid. The developed method allowed estimation of SAL from different marketed pharmaceutical dosage forms like tablet, syrup and aerosol without interference. The proposed procedure is useful for routine quality control of SAL in different pharmaceutical dosage forms.

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Source of Support: Nil, **Conflict of Interest:** None declared.