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SIMPLE METHOD DEVELOPMENT AND ITS VALIDATION FOR COMBINATION OF BUDESONIDE AND SALBUTAMOL SULPHATE USING HPLC

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ABSTRACT

Salbutamol Sulphate and Budesonideare available in the market as a combination for the treatment of asthma. The developed chromatographic system depends on usingHiQ sil C8 column (4.6 mm x 250 mm). The mobile phase was prepared by mixing Acetonitrile: 0.02M of Phosphate buffer (pH 3) 50:50v/v at a flow rate of 1 ml/min detection at 228 nm, the injection volume 20 µl. The method satisfied linearity with regression coefficient $R^2$0.9965 for Salbutamol Sulphateand $R^2$0.9993 for Budesonide in the range 5-25 µg/ml for Salbutamol Sulphate and 2-10 µg/ml for Budesonide. TheLOD and LOQ; were 0.23 and0.70µg/ml for Salbutamol Sulphate and 0.02 and0.06µg/ml for Budesonide. The method showed satisfying results.

Keywords: Validation, Combination, Salbutamol Sulphate,Budesonide, HPLC

INTRODUCTION

Salbutamol sulphate (SAL) is also known as (RS)-4-[2-(tert-Butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol, Budesonide (BUD) also known as 11β,21-dihydroxy-16α,17α-(butylidenebis(oxy))pregna-1,4-diene-3,20-

dione, is a synthetic pregnane steroid and non-halogenated cyclic ketal corticosteroid **[1]**. Salbutamol or albuterol, a moderately selective beta (2)-receptor agonist similar in structure to terbutaline, is widely used as a bronchodilator to manage asthma and other chronic obstructive airway diseases. Salbutamol belongs to the class of bronchodilators that work by relaxing muscles and widening the airways of the lungs. Thus, it makes breathing easier. Budesonide belongs to the class of corticosteroids that works by acting inside cells of the nasal lining and stopping the release of certain chemicals in the body that cause inflammatory reactions. Thereby, provides relief from sneezing, runny or blocked nose, and sinus discomfort[**2, 3]**.

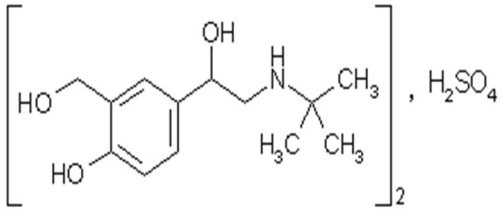

Figure 1: Salbutamol Sulphate

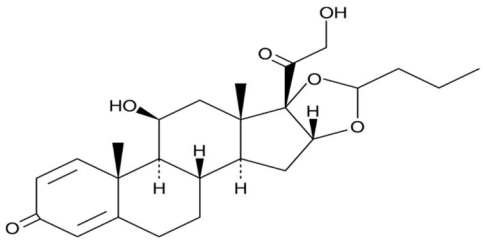

Figure 2. BUDESONIDE

A detailed literature search indicates that there are a few HPLC methods **[4-10]**, few UPLC methods **[11, 12]**, few HPTLC methods **[13-17]**, few LC-MS methods **[18-20]**, and few UV methods **[21]**, reported for single and combination of SAL and BUD. To develop a simple method, the proposed work was carried out and developed a rapid method for the combination of these two drugs. Also, the mobile phase used in the proposed work Acetonitrile: 0.02M of Phosphate buffer (pH 3) 50:50 v/v indicates that the method developed compared to the methods found in other literature where a four to five-component mobile phase was used.

MATERIALS AND METHODS

Chemicals and Reagents

Budesonide and salbutamol sulphate were gifts from NATCO Pharmaceutical in Hyderabad and RALINGTON Pharma LLP in Gujarat, respectively. The following items were bought from Loba Chemie Pvt. Ltd., Mumbai, India: methanol (HPLC grade), hydrochloric acid (AR grade), potassium dihydrogen phosphate, 30% v/v hydrogen peroxide (AR grade), and sodium hydroxide. Using the Extrapure Lab Link water purifier system, water with a conductivity of less than 0.05 S cm-1 was obtained for use in HPLC.

Instrumentation and chromatographic condition

On a JASCO HPLC system, model PU 2080 Plus pump with Rheodyne sample injection port (20 L), the samples were analyzed. HiQ Sil C8 (4.6 mm x 250 mm) column was used for the experiment, and salbutamol and budesonide were detected using PDA detectors (MD 2010) and Borwin chromatography software (version 1.5) at a wavelength of 228 nm. Acetonitrile: 0.02M of Phosphate Buffer (pH 3) 50: 50 v/v at the flow rate of 1 mL/min, used in isocratic mode, was chosen as the mobile phase's optimal composition. Other equipment used in the investigation included a hot air oven (Kumar Laboratory Oven), a vacuum pump (JET-VAC-J1), a photostability chamber (Newtronic), and a UV-visible spectrophotometer (JascoV-730). Optimized chromatographic conditions are shown in **Table 1**.

Table 1: Optimized chromatographic conditions

Parameters	OptimizedCondition
Instrument	JASCO HPLC System
Detector	PDAdetector
Column	HiQ sil C8 (4.6mm x 250mm)
MobilePhase	Acetonitrile:0.02M of Phosphate Buffer (pH 3) 50:50 v/v
SampleVolume	20µl
Typeofelution	Isocraticelution
FlowRate	1ml/min
DetectionWavelength	228nm
RunTime	7 Minutes

Mobile phase optimization

To achieve optimal chromatographic conditions, various columns were tried out such as Agilent C8 and Thermo scientific BDS Hypersil^{TM}C8 Columns. Initially, Methanol:Water (70: 30 v/v) was tried but tailing was found of SAL and theoretical plates were not sufficient for BUD. Significant resolution and desired system suitability parameter were obtained by using HiQ sil C8 column, Acetonitrile:0.02M of Phosphate Buffer (pH 3) 50:50 v/v at flow rate 1 ml/min with detection at 228 nm, the injection volume 20 µl.

Preparation of mobile phase

Acetonitrile and 0.02M of Phosphate Buffer (pH 3) were taken in the ratio 50:50 (v/v) and filtered through a 0.45 µm membrane filter. Then it was sonicated for 15 min in the ultrasonicate bath.

Preparation of standard stock solution

Accurately weighed 25 mg BUD was transferred into a 25 ml volumetric flask, and the volume was made up with Methanol, to get

standard stock solution 1000µg/ml (Solution A).

Accurately measure 25 mg SAL was transferred into a 25 ml volumetric flask, and the volume was made up with Methanol, to get standard stock solution 1000µg/ml (Solution B).

Preparation of sample solution

For the preparation of the working standard, 1 ml of stock solution (Solution A) was diluted with methanol to get 100 µg/ml as a working solution. Pipetted out 1 ml of BUD standard solution (100 µg/ml) into the volumetric flask and added methanol up to 10 ml to obtain BUD solution (10 µg/ml).

For the preparation of the working standard, 1 ml of stock solution (Solution B) was diluted with methanol to get 100 µg/ml as aworking solution. Pipetted out 1 ml of SAL standard solution (100 µg/ml) into the volumetric flask and added methanol up to 10 mlto obtain SAL solution (10 µg/ml).

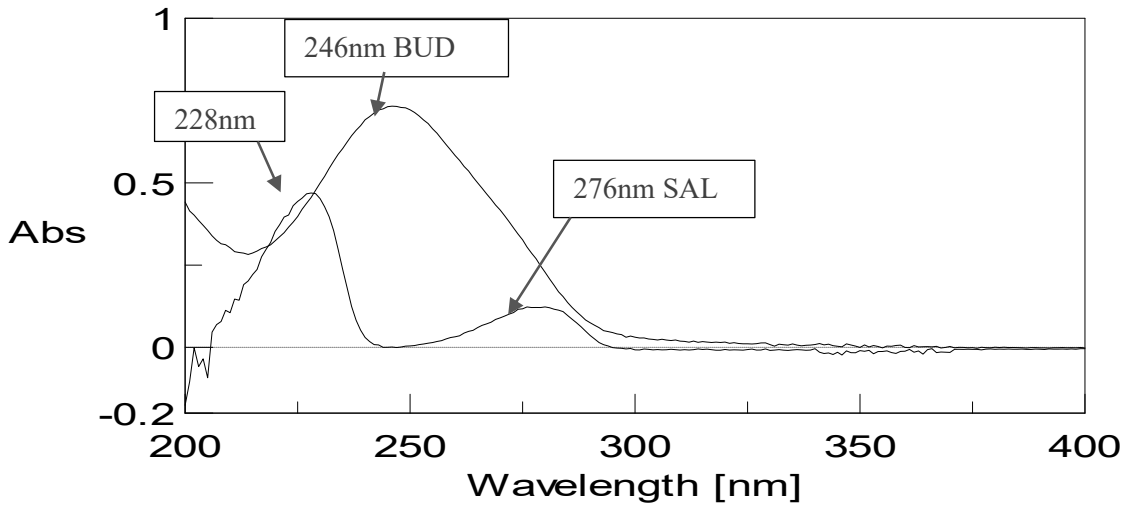

Figure 3: Spectral overlay of Salbutamol Sulphate and Budesonide

Method validation Parameters [22]

The validation of the HPLC method was carried out according to International Conference on Harmonization covering parameters Range linearity, detection limit, quantification limit, repeatability, intermediate precision and accuracy, robustness and specificity.

Specificity

Peak purity profiling studies were carried out for evaluating the specificity of the method. Peak purity for the drug peak of the assay was monitored using HPLC PDA software. It compares the UV spectrum at peak start, midpoint, and peak end.

Linearity &Range

It was said the method is linear if there is a good proportionality between the response (peak area) and working concentration starting from the lowest point in the tested range till the highest point and the R^2 should be ≥ 0.99. Linear regression equation:

Y = mx + c

Where, Y= Peak area, X= Concentration (%), mis the slope and c is the intercept.

Precision

The method precision was studied by repeatability and intermediate precision studies. In the repeatability study, the injection of six replicates of the standard solution was done on the same day after some time intervals. In intermediate precision, injection of six replicates of the standard solution was injected into the HPLC system on three consecutive days. The% RSD was calculated.

Assay

The marketed product Budesal was assayed. The label claim states each respule contains Budesal (1.25 mL SAL and 0.5 mL BUD). For sample preparation, accurately measure the marketed product which is equivalent to 1 mL of drug content, and transferred to a10 mL volumetric flask and completely dissolved in 10 mL methanol. Pipette out 1 mL from the medium and transfer into the volumetric flask. Then added mobile phase up to the volume respectively—the2replicatesofthe same concentration and injected into the HPLC system.

Accuracy

Accuracy was determined by the method of standard addition. A known amount of API to beanalyzed was added to the marketed formulation of Budesal each respule contains (1.25 ml SAL and 0.5 ml BUD). In the assay solution, the pure drug was spiked at 80%, 100%, and 120% levels. The 2 replicates of 3 concentrations were evaluated to calculate % recovery.

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

The LOD and LOQ were calculated using equations, LOD= 3.3 x σ/S; LOQ =10 x σ/S, respectively where σ is the standard deviation of the Y-intercept and S is the slope of the calibration curve.

Robustness

To evaluate the robustness of the method the chromatographic conditions were deliberately varied and the degree of reproducibility was evaluated. Robustness was carried out on standard drug solutions. The robustness of the method was carried out by changing the flow rate by ± 0.05 ml/min, changing mobile phase composition by ±2ml, and changing in

wavelength by ±2 nm. One factor at a time was varied.

RESULTS AND DISCUSSION

Specificity

Specificity was monitored by peak purity studies fora sample and it was found to be more than 0.995 as shown in Table 2. The values indicate that the method is specific.

Linearity and range

Linearity was observed in the range of 5-25 µg/ml and 2-10 µg/ml SAL and BUD respectively. The correlation coefficient was found 0.9965 with the equation of y = 34134x + 46028 for SAL and the linearity was observed over a range of 2-10 µg/ml and the correlation coefficient was found 0.9993 with the equation of y = 27054x + 24428 for BUD. The overlay of linearity is shown in **Figure 4**. The calibration curve is shown in **Figure 5 and Figure 6**.

Assay

Assay was carried out using the marketed formulation Budesal. The drug content in respules was found to be100.39 ± 0.71% for SAL and 100.84± 0.51% for BUD respectively. Shown in **Table 3**.

Accuracy

The % mean recovery was found to be 101.35 % for SAL and 102.52 % BUD respectively, which indicated that the proposed method is accurate for the estimation of the drug in the respules dosage form. The percent recovery for SAL and BUD was found to be in range as shown in **Table 4**.

Precision

Repeatability and Intermediate precision were performed. The % RSD was found to be 0.99 and 1.17% for SAL and 0.24% and 0.45% for BUD respectively.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ were calculated using equations, LOD= 3.3 x σ/S; LOQ =10 x σ/S, respectively where σ is the standard deviation of the Y-intercept and S is the slope of the calibration curve. LOD and LOQ was shown in **Table 5** respectively.

Robustness

It was observed that there were no marked changes in the peak areas, The percentage RSD was found to be less than 2% which indicates that the method is robust. For results of robustness are shown in **Table 6**.

Table 2: Specificity studies

Drug	Sr. No	Parameter	Peak purity (purity front)	Peak purity (purity tailing)
Salbutamol Sulphate	1	Standard	995.40	998.55
	2	Sample	999.20	997.50
Budesonide	1	Standard	998.50	999.25
	2	Sample	999.20	998.70

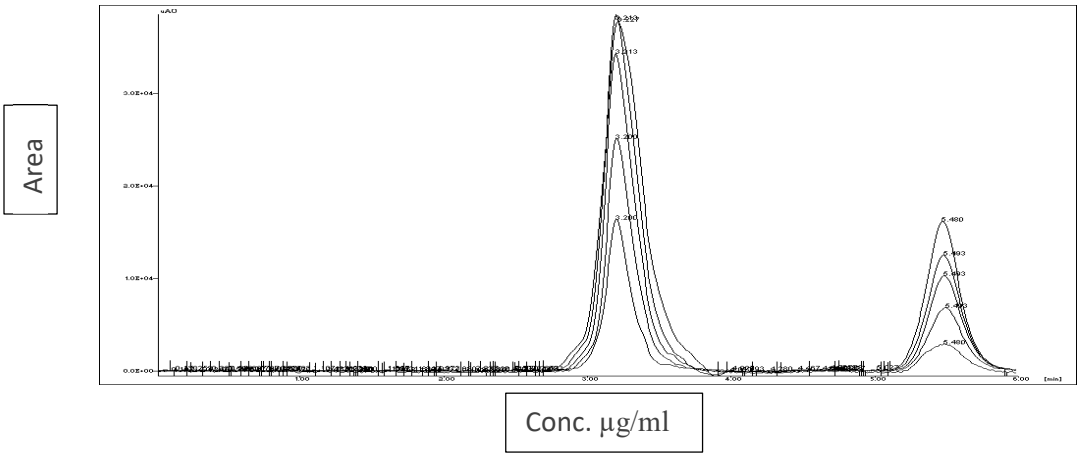

Figure 4: Overlay of chromatogram for standard Linearity

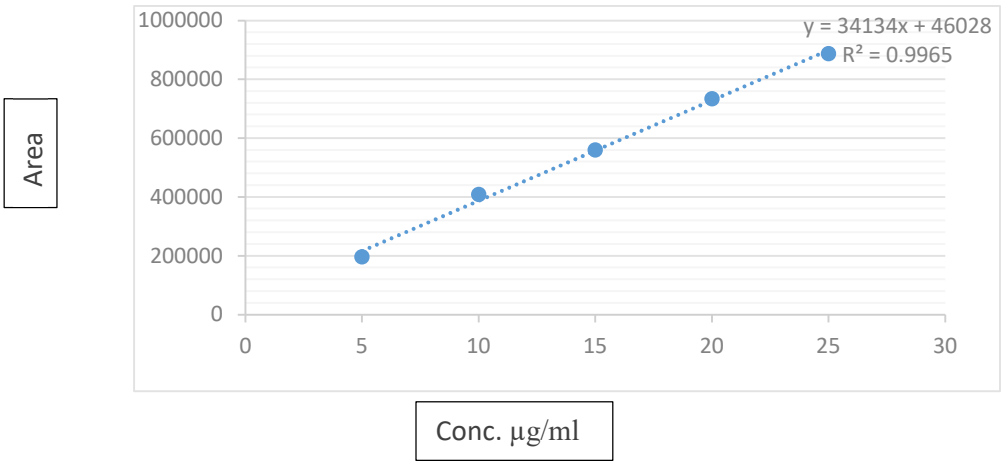

Figure 5: Calibration curve of Salbutamol Sulphate (5-25 µg/ml)

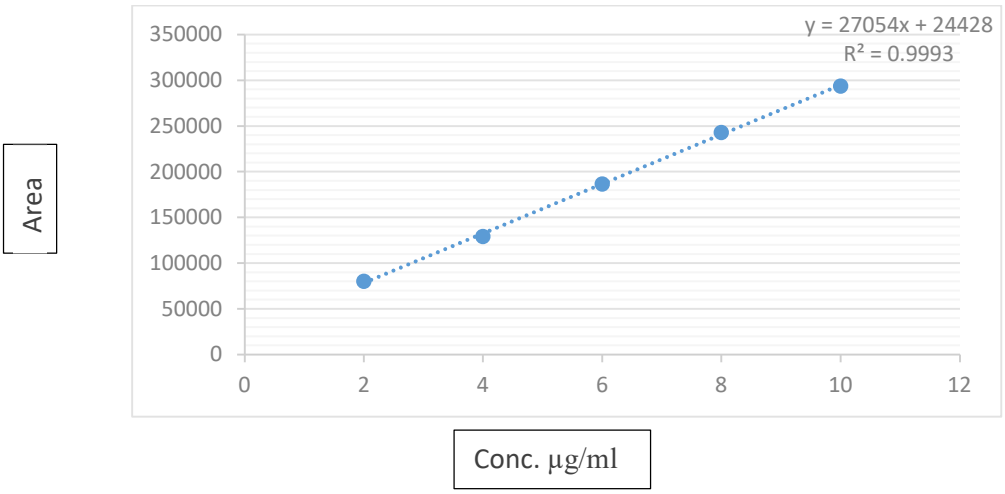

Figure 6: Calibration curve of Budesonide (2-10 µg/ml)

Table 3: Assay

Drug	Amount Recovered (µg/mL)	% Drug content	Mean ± % RSD
Salbutamol Sulphate	9.988	99.88	100.39 ± 0.71
	10.09	100.9	
Budesonide	3.957	98.92	100.84± 0.51
	4.111	102.77	

Table 4: Recovery studies

Drug	Sr. No	Amount from marketed formulation (µg/mL)	Amount of standard added	The total amount of the drug (µg/mL)	Amount recovered	% recovery
Salbutamol Sulphate	1	10	8	18	17.78	98.98
	2	10	10	20	20.11	100.59
	3	10	12	22	22.29	101.35
Budesonide	1	4	3.2	7.2	19.45	99.56
	2	4	4	8	22.12	101.22
	3	4	4.8	8.8	23.81	102.12

Table 5: LOD and LOQ

Drug	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
SAL	0.23	0.70
BUD	0.02	0.06

Table 6: Robustness

Parameters	Conditions	%RSD Salbutamol Sulphate	%RSD Budesonide
Mobile Phase ratio Acetonitrile: 0.02 M of Phosphate Buffer (pH 3)	(48: 52)	1.44	0.83
	(52:48)	1.01	0.78
Flowrate	0.95mL/min	1.52	1.04
	1.05mL/min	1.87	1.41
Wavelength	226nm	1.37	0.98
	230nm	1.47	1.28
Buffer pH	2.8	1.29	1.01
	3.2	1.78	1.65

Table 7: Summary of validation parameters

Sr. No	Validation Parameter		Salbutamol Sulphate	Budesonide
1	Linearity		y = 34134x + 46028 R^2 = 0.9965	y=27054x+ 24428 R^2 = 0.9993
2	Range		5-25 µg/mL	2-10 µg/mL
3	Precision (%RSD)	Repeatability	0.99	0.24
		Intermediate	1.17	0.45
4	Accuracy (% recovery)	80%level	98.82	99.56
		100%level	100.59	101.22
		120%level	101.35	102.12
5	Assay%		100.39	100.84
6	LOD		0.23 µg/mL	0.02 µg/mL
7	LOQ		0.70 µg/mL	0.06 µg/mL
8	Robustness		Robust	
9	Specificity		Specific	

DISCUSSION

As per the literature, the survey revealed that numerous methods reported in previous studies utilized mobile phases composed of four to five components. These methods also exhibited long run times per injection. However, in the proposed method, the focus was on developing a rapid and simple approach, particularly concerning mobile phase preparation and minimizing run time. By streamlining the mobile phase composition, the proposed method offers a more straightforward and efficient process for preparing the mobile phase. This simplification can contribute to time savings during method development and routine analysis. Additionally, the reduced number of components in the mobile phase can potentially enhance method robustness and reduce the likelihood of interference from impurities or other matrix components. One of the key advantages of the proposed method is its low run-time per injection. The shortened run time allows for increased sample throughput, making it more suitable for high-volume analysis scenarios. This is especially beneficial in laboratories that handle a large number of samples daily, as it enables faster data generation and result reporting. Furthermore, the simplicity and rapidity of the proposed method development conditions highlight its feasibility for routine analysis. The method can be easily implemented and adopted by analysts without extensive method optimization or complex instrument setups. This can save valuable time and resources in both method development and routine analysis phases.

CONCLUSION

This developed HPLC method is simple, and rapid for routine quantitative analysis of SAL and BUD as bulk drugs and in the dosage form without the interference of commonly used excipients. The developed method was validated as per ICH guidelines. The peak purity value was found within the limit confirming the specificity of the developed method. Thus, this method can conveniently be used for quantitative analysis of SAL and BUD on a routine basis.

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