RESEARCH PAPER

Advanced AQbD-Based HPLC Method for the Synchronized Quantification of Salbutamol Sulfate and Budesonide in Bulk and Tablet using Green Chemistry Approach

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Abstract

The present study aimed to develop, validate, and perform force degradation studies for simultaneous estimation of Salbutamol Sulfate(SS) and Budesonide(BUD) using RP-HPLC. The objective of the method was to fulfill the current pharmaceutical industry's requirements in bulk and pharmaceutical dosage forms for cost and time reduction. The method was developed using Agilent 1260 Infinity II model HPLC with DAD detector and Agilent zorbax bonus RP (250 mm × 4.6 mm, 5 µm) column. The optimized mobile phase developed for the study was the combination of 0.1% trifluoroacetic acid and acetonitrile in a ratio of 45:55%*v/v*. at a flow rate of 0.7 ml/min and wavelength 238 nm with a run time of 15 minutes. The optimized method simultaneously estimated SS and BUD at 2.64 mins and 9.04 mins, respectively. The method showed linearity in the range of 7.2-10.8 µg/ml for SS and 6.4-7.2 µg/ml BUD for following Lamberts -beer law. Both instrument and method precision were under the accepted criteria limits. The relative standard deviation for salbutamol sulfate at 80%, 100%, and 120% was found to be 0.42%, 0.14%, and 0.28% respectively; and for budesonide was found to be 0.31%, 0.11%, and 0.09% respectively. The LOD & LOQ for SS and BUD were estimated from the data as 0.29 µg/ml and 0.89 µg/ml respectively for SS, and 0.16 µg/ml and 0.49 µg/ml for BUD. Both SS and BUD showed the highest acid-induced degradation, base-induced, and oxidation, during force degradation studies. Thermal and photolysis degradation was found to be absent in both drugs. Thus the developed method was declared as Precise and robust for regular drug analysis in plain and combination of drugs.

Keywords: Salbutamol Sulfate, Budesonide, RP-HPLC, Robustness, %RSD, Precision, LOD, LOQ, Accuracy, linearity, Forced degradation

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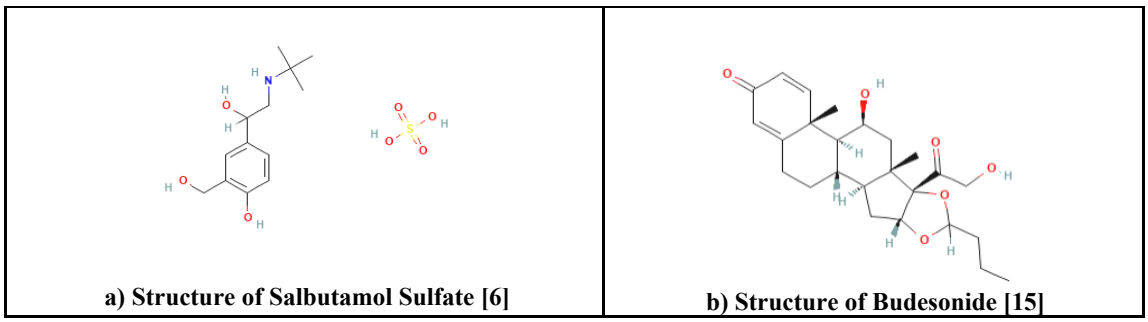
1. INTRODUCTION

Pharmaceutical compounding has experienced a significant surge in usage in recent years for the preparation of pharmaceuticals that are not readily available in the market. The management of asthma, which involves the narrowing of the airways (bronchoconstriction), often includes the administration of albuterol or salbutamol (SS) and a solution comprising budesonide(BUD). Currently, the Indian market is devoid of nebulizer solutions having a combination of both salbutamol sulfate and budesonide. However, in the US market, comprising a product with the brand name Airspura that combines both drugs known by the name albuterol sulfate), having a mode of action with a short-acting -adrenergic agonist acting on beta 2 receptor, showing its presence in airway path of smooth muscles with budesonide, an inhaled corticosteroid.[1,2] The studies indicated that the United States in January 2023[3] approved a combination of salbutamol sulfate and budesonide for medicinal use. It was considered to be the first medicine in the US- market available as an inhaled corticosteroid as a relief treatment for asthma, rather than as a controller. Salbutamol, also known as albuterol, The IUPAC name of Salbutamol Sulfate is 4-[2-(tert-

butylamino)-1-hydroxyethyl]. Salbutamol is typically administered through an inhaler or nebulizer, but it is also available in tablet, liquid, and injectable forms. and it reacts with sulfuric acid to form 2-(hydroxymethyl)phenol[4]. It is a remedy, that makes a broad sense for the medium and large airways in the respiratory system. It exerts as a bronchodilator by activation of the β2 adrenergic receptors, resulting in the relaxation of smooth muscles. The drug provides excellent treatment for asthma patients, both for asthma attacks and exercise-induced bronchoconstriction, as well as chronic obstructive pulmonary disease (COPD). It also plays an important contribution in the management of high potassium levels in the blood[5]. Thus it can be used as medicine for hypertension as a repurposed drug.

Budesonide is a medication classified as a corticosteroid. Its IUPAC name is (1S,2S,4R,8S,9S,11S,12S,13R)-11-hydroxy-8-(2-hydroxyacetyl)-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo[10.8.0]icosa-14,17-dien-16-one.[6] This drug is available in various forms, including inhaler, nebulization solution, tablet, nasal spray, and rectal administration[7,8]. The inhaled form is considered for the long-term treatment of asthma and chronic obstructive pulmonary disease (COPD). [9,10] The nasal spray is prescribed for allergic rhinitis and nasal polyps[11]. Extended-release tablets or capsules and rectal formulations are used to treat inflammatory bowel diseases such as Crohn's disease, ulcerative colitis, and microscopic colitis. [12-14]

As per the literature study, various HPLC methods are available for eluting single drug but not in combinations. We are the first to estimate these medications in combination using RP-HPLC.[15-25] This combined approach can be utilized to detect compounded formulations for the benefit of patient safety.[26] Therefore, the objective of this study was to simultaneously determine the concentrations of SS and BUD in a combination pharmaceutical dose form. The structure of both drugs is indicated in Figure. 1, and Table 1 given below shows the limits for HPLC analysis as per ICH guidelines.



a) Structure of Salbutamol Sulfate [6] **b) Structure of Budesonide [15]**

Figure 1: Structure of SS (a) and BUD (b)

Table 1: Critical Analytical Attributes

Parameter	Limits
Theoretical Plates(TP)	More than 2000
Asymmetry	Not More than 2
Tailing Factor	Not More than 2.0
Run time	Not More than 20 minutes
Resolution	Not Less than 2.0

2. MATERIAL AND METHOD

2.1Chemicals and Reagents

Complimentary samples of SS and BUD were made available by Aadhaar Life Sciences Pvt. Ltd. India, HPLC-grade acetonitrile (Qualigens) and AR-grade trifluoroacetic acid (Molychem) were purchased from a local supplier, and internal Milli-Q system for HPLC water.

2.2. Instrumentation

HPLC is made with Agilent 1260 Infinity II, which was equipped with a quaternary pump and DAD detector with the software Openlab Ezchrom. Wet

chemistry was conducted using the lab man ultrasonicator and the analytical balance.

2.3 Selection of mobile phase:

Depending on the literature search the mobile phase comprising 0.1% Trifluoroacetic acid and Acetonitrile (ACN) was selected for the HPLC analysis of SS & BUD to gain better results, various ratios with 50:50, 65:35, and 70:30 were tried.

2.4 DEVELOPMENT AND OPTIMIZATION OF THE METHOD USING AQBD DESIGN:

2.4.1. Analytical Target Profile (ATP)

ATP sets the performance standard needed to carry out the analytical method, which must be achieved for its intended use, to meet the acceptance criteria of industry and regulatory bodies. This includes validation parameters such as specificity, precision, accuracy, and robustness.

2.4.2. Critical Analytical Attributes (CAA) & Critical Method Parameters (CMP)

The suggested HPLC method recognized the retention duration, theoretical plates, peak purity, and asymmetry (Table 1) as critical analytical attributes (CAA). The CMPs are the method parameters or the inputs that directly affect the analytical target profile (ATP). The composition of the mobile phase and the flow rate were identified as two critical method parameters that needed to be controlled to maintain the acceptable response range of ATP. The Table. 2 signifies the effect of CMP on CAA.

Table 2: Effect of CMP and CAA

CMP	CAA				
	Retention time	Theoretical Plate	Peak Asymmetry	Resolution	Peak Purity
Buffer Strength	Medium	Medium	Low	Low-medium	Low
Organic solvent concentration	Medium	Medium	Low - Medium	Low	Low
Mobile phase composition	High	High	Medium	High	Low
Column	Low	Low	Low	Low	Low
Injection volume	Low	Low-Medium	High	Low	Low
Wavelength	Low	Low	Low	Low	Low
Flow rate	High	Low-medium	Low	Low-medium	Low

Mobile phase composition showed a direct impact on the retention time (RT), Theoretical plate (TP), and Asymmetric (ASY) due to the change in the polarity of the solvent mixture towards the drug's polarity. Flow rate affects retention time and has a low to medium impact on TP and Resolution, and showed an inverse relationship with flow rate, and retention time, also slow elution may cause fronting or tailing of the peaks. Buffer strength and organic solvent have a medium impact on RT and TP as they can alter peak shapes and height, and also increase or decrease the retention time. However, this can be controlled with the initial development phase. Other attributes have a low impact on the critical analytical attributes and therefore can be controlled.

2.4.3. Critical Method Material Attributes (CMMA)

Risk factors about manufacturing processes or preparation variables are termed CMMA. These factors, which experimenters can directly control, have a low to medium impact on CQAs. Therefore, the reagents used in the procedure should be of compendial grade, and the glassware should be Type A. Identifying these factors is crucial in the QbD process to determine which parameters should be used as input variables to control CQAs and keep them within the specified range for quality and safety.

2.5 Selection of Wavelength:

For observing maximum absorbance, a diluent solution (50:50 %v/v) of 0.1% TFA and ACN was scanned on a UV Spectrophotometer in the range 190 to 400 nm.

2.6. HPLC Method Development

For method development various trials were carried out to obtain the peaks of SS and BUD

simultaneously and based on these trial data, Design Expert Software version 13.0 was employed to refine the method.

2.6.1. Preparation of Mobile phase

A 0.1% Trifluoroacetic acid (TFA) buffer was prepared by adding 1 ml of TFA to 1000 ml of HPLC-grade water. Then, 450 ml of this 0.1% TFA buffer was mixed with 550 ml of Acetonitrile. The mixture was filtered through a 0.45 µm nylon membrane and sonicated to degassed.

2.6.2. Preparation of Diluent

A mixture with 50:50% v/v (0.1% TFA and ACN) was used as diluent.

2.6.3. Preparation of Standard Solution

A standard solution was prepared with a concentration of Salbutamol at 9 µg/ml & Budesonide at 8 µg/ml.

2.7. METHOD VALIDATION

2.7.1. Specificity

Diluent, as well as individual standard solutions (9 µg/ml) of SS and (8 µg/ml) of BUD, were injected into the injection port of HPLC to ensure that the blank peak should not interfere with the major analyte peaks.

2.7.2. System Suitability

First, the system was tested to determine its suitability. As per ICH guidelines, the theoretical plate count should be not less than 2000, asymmetry not more than 2.0, and resolution not less than 2.

2.7.3. Accuracy

Three replicate injections were performed at 80%, 100%, and 120% concerning standard concentration and calculated drug concentration, recovery percentage, and % RSD of recovery less than 2.

2.7.4. Precision

To find out the precision of the Instrument and method, the injection was performed in six replicates to ensure system suitability and relative standard deviation. It should be less than 2 as per ICH Q2 R1 guidelines.

2.7.5. Linearity

Five-point linearity was analyzed and plotted calibration curve for linear regression coefficient (R^2) (not less than 0.999).

2.7.6. LOD and LOQ

Limits of detection (LOD) and quantification (LOQ) represent the capacity of the method to detect and quantify the smallest analyte amounts.

$$LOD = \frac{3.3 \times Std.Error\ of\ Intercept}{Coefficients\ of\ X\ Variable\ 1}$$

$$LOQ = \frac{10 \times Std.Error\ of\ Intercept}{Coefficients\ of\ X\ Variable\ 1}$$

2.7.7. Robustness

The robustness study involved changing the column temperature by a range of ± 2˚C and the wavelength by a range of ± 2 nm.

2.7.8. Inter-day & Intraday Precision:

For carrying out intraday precision, the working standard solution was injected in the morning and evening on the same day and inter-day precision on a different day. The solution stability acceptance criteria of %RSD of peak area should be not less than 2.

2.7.9. Forced Degradation

Forced degradation studies were conducted to determine the chemical behavior of the molecule under various stress conditions, such as heat, light, oxidation, pH extremes, and humidity, and to find out probable impurities and degradants in the medication. as outlined in Table 3.

Table 3: Stress stability studies conditions

Degradation Condition	Strength and procedure	
	Salbutamol Sulfate	**Budesonide**
Acid	9 mg SS + 1N Hydrochloric Acid (1ml), kept at room temperature for 30 min, further 1 ml of this solution was diluted to 100 ml using a diluent.	8 mg BUD + 1N Hydrochloric Acid (1ml), kept at room temperature for 30 min, further 1 ml of this solution was diluted to 100 ml using diluent.
Base	9 mg SS + 1.0 N Sodium Hydroxide (1ml), kept at room temperature for 30 min, further 1 ml of this solution was diluted to 100 ml using diluent.	8 mg BUD + 1.0 N Sodium Hydroxide (1ml), kept at room temperature for 30 min, further 1 ml of this solution was diluted to 100 ml using diluent.
Peroxide	9 mg SS + 30% Hydrogen peroxide (1 ml),	8 mg BUD + 30% Hydrogen peroxide

	kept at room temperature for 30 min. Take 1 ml dilute to 100 ml using diluent	(1ml), kept at room temperature for 30 min. Furthermore, 1 ml of this solution was diluted to 100 ml using diluent.
Heat (Dry)	9 mg SS heat at a temperature of 105°C kept for 5 hours in an oven, diluted to 10 ml, further again 1 ml diluted to 100 ml using diluent.	8 mg BUD heat at a temperature of 105°C kept for 5 hours in an oven, diluted to 10 ml, further again 1 ml diluted to 100 ml using diluent.
UV	9 mg SS was kept in a UV cabinet for photolysis for 8 hours, diluted 10 ml further, and diluted 1 to 100 ml using a diluent.	8 mg BUD was kept in a UV cabinet for photolysis for 8 hours, diluted 10 ml further, diluted 1 to 100 ml using a diluent

3. RESULTS AND DISCUSSION

3.1. Wvelength Selection

After scanning in the 200 – 400 UV range. The maximum absorption for SS and BUD were found to be at 238 nm as shown in Figure 2.

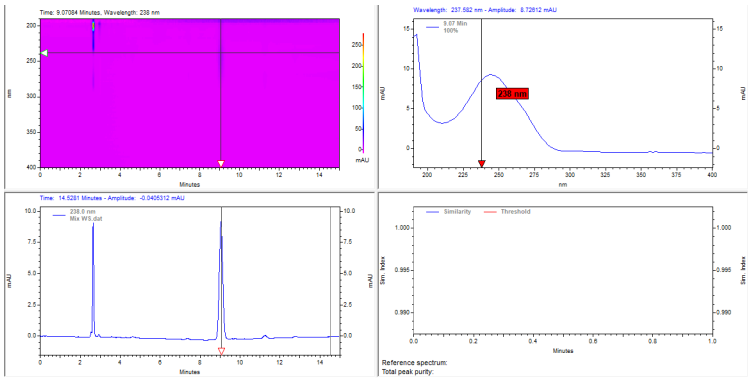


Figure 2: Wavelength Selection of Salbutamol Sulfate and Budesonide

3.2. Method Development

Several trials were conducted using Agilent's Zorbax Bonus RP (250 x 4.6 mm, 5µ), with TFA (0.1%, 0.15%, and 0.05%) and ACN as the mobile phase. Adjusting the mobile phase ratios, the initial peak chromatogram showed retention times (RT) of SS and BUD at 2.29 min and 5.20 min, respectively, with theoretical plates exceeding 5000, peak purity of 1.0, asymmetry less than 1.2, and resolution for BUD greater than 15. A randomized central composite design expert model was used with %*v/v* mobile phase and flow rate as variables, and theoretical plates and retention time as responses. The software generated nine runs with varying flow rates and Mobile phase concentrations, as shown in Table 4 under the AQbD Runs column. The analysis was conducted accordingly, and the results are detailed in Table 4 under the AQbD Results column.

Table 4: Optimization of Method development using AQbd design

AQBD Runs			AQBD Results							
Run	Mobile Phase (0.1%TFA:CAN) (%*V/V*)	Flow rate (ml/min)	Retention Time		Theoretical Plates		Asymmetry		Resolution	
			SS	BUD	SS	BUD	SS	BUD	SS	BUD
1	35:65	0.7	2.62	6.71	9802	7131	1.14	0.94	0.00	19.30
2	35:65	0.9	2.03	5.22	7483	6593	1.11	0.97	0.00	18.15
3	**45:55**	**0.7**	**2.64**	**9.04**	**10208**	**12114**	**1.13**	**1.05**	**0.00**	**29.56**
4	45;55	0.8	2.31	7.90	9052	11586	1.21	1.06	0.00	28.64
5	40:60	0.7	2.63	7.67	9850	9248	1.11	1.00	0.00	23.73

6	35:65	0.8	2.29	5.87	8469	6807	1.15	0.97	0.00	18.67
7	45:55	0.9	2.05	7.03	7721	11179	1.14	1.04	0.00	27.78
8	40:60	0.8	2.30	6.67	8647	8723	1.06	1.00	0.00	22.73
9	40:60	0.9	2.04	5.94	7622	8477	1.06	1.02	0.00	22.19

After the completion of elution, it was manifested that the mobile phase having 0.1% TFA and Acetonitrile in the ratio of 45:55%v/v, flow rate 0.7 ml/min i.e. trial run no. 3 as shown in figure 3, showed the highest theoretical plate for SS and BUD with highest resolution for BUD, with run time under 15 minutes was selected as the lead chromatographic condition.

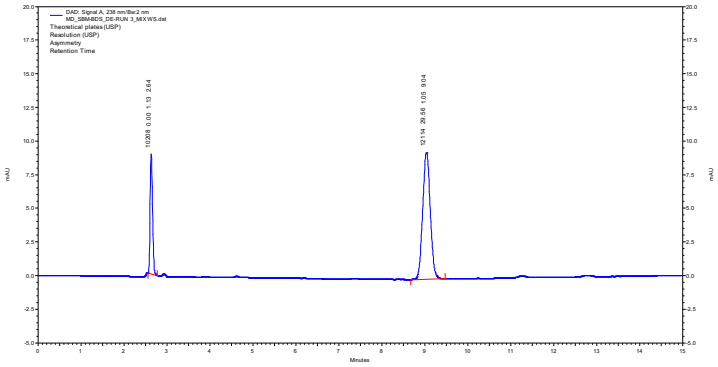


Figure 3: Final chromatogram after optimization

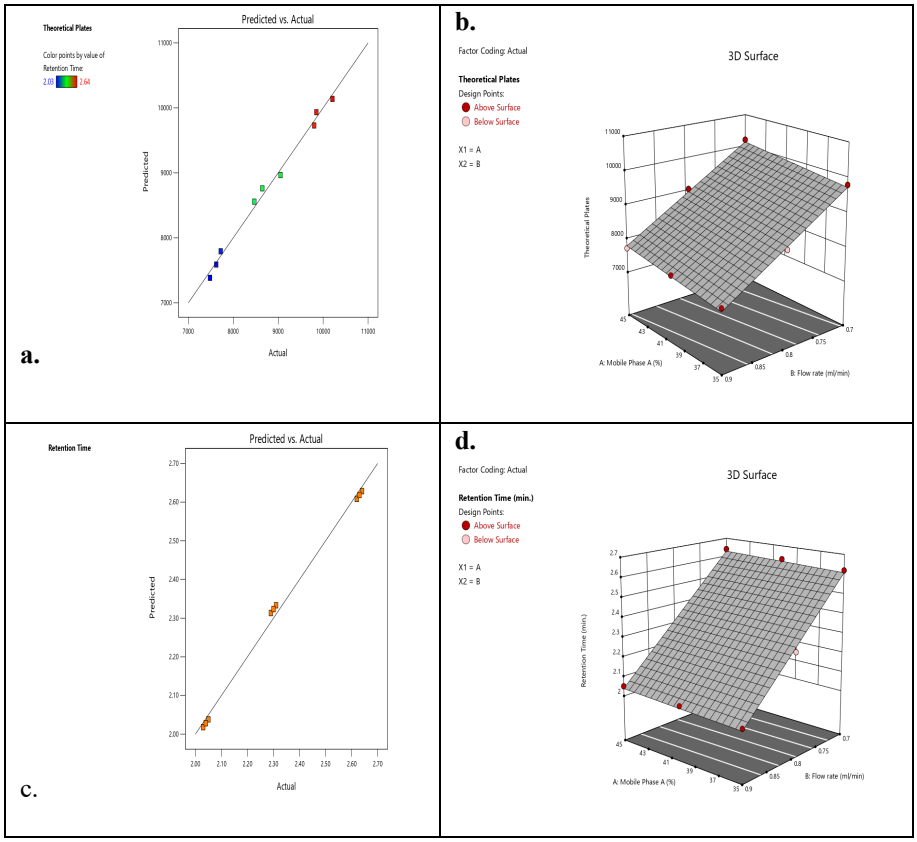


Figure 4: Salbutamol sulfate actual vs predicted graph and 3D surface plots for Theoretical plate (a & b) and retention time (c & d)

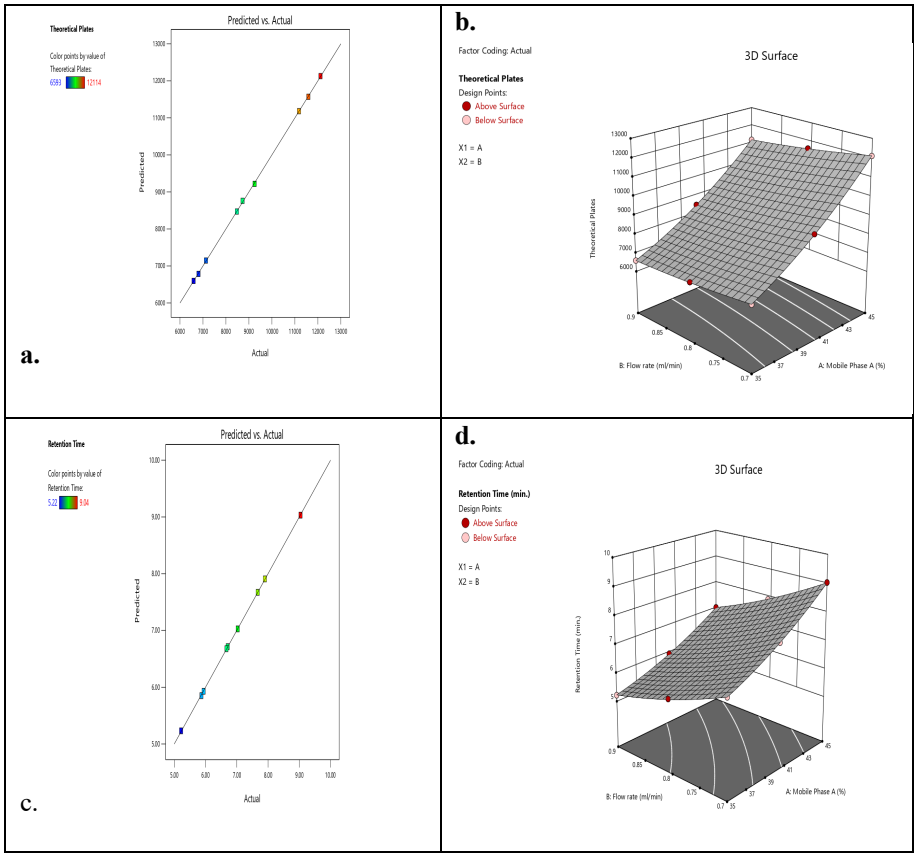


Figure 5: Budesonide actual vs predicted graph and 3D surface plots for Theoretical plate (a & b) and retention time (c & d)

Figures 4 & 5, clearly indicate that actual vs predicted values are quadratic fit with r^2 value above 0.998 for both drugs and based on the 3D surface graph showed in Figures 4 and 5, we can interpret that, with slower flow rate and higher mobile phase concentration used during method development, there is a significant increase in theoretical plates and eventually higher retention time of both drugs giving significantly good resolution between the two peaks. Table 5 shows the final chromatographic conditions for validation.

Table 5: Final Chromatographic Condition

Parameter	Condition
HPLC Instrument	Agilent 1260 Infinity II
Column	Agilent Zorbax Bonus RP (250 mm x 4.60 mm,5µm)
Wavelength	238 nm
Mobile Phase	45:55(0.1% TFA:ACN)
Diluent	0.1% Trifluoroacetic acid: Acetonitrile (50:50) v/v
Run time	15 minutes
Injection Volume	10 µL
Flow Rate	0.7 ml/min
Column oven Temperature	30°C (± 2°C allowed by Robustness)

3.3 Method Validation

3.3.1. Specificity

For confirming specificity, the retention times (RT) for SS and BUD were observed to be 2.65 minutes and 9.05 minutes, respectively, and no physical or chemical incompatibility was observed due to the use of diluent or both drugs as illustrated in Figure 6. The assay results for the marketed drug product were 99.29% and 99.81% for SS and BUD.

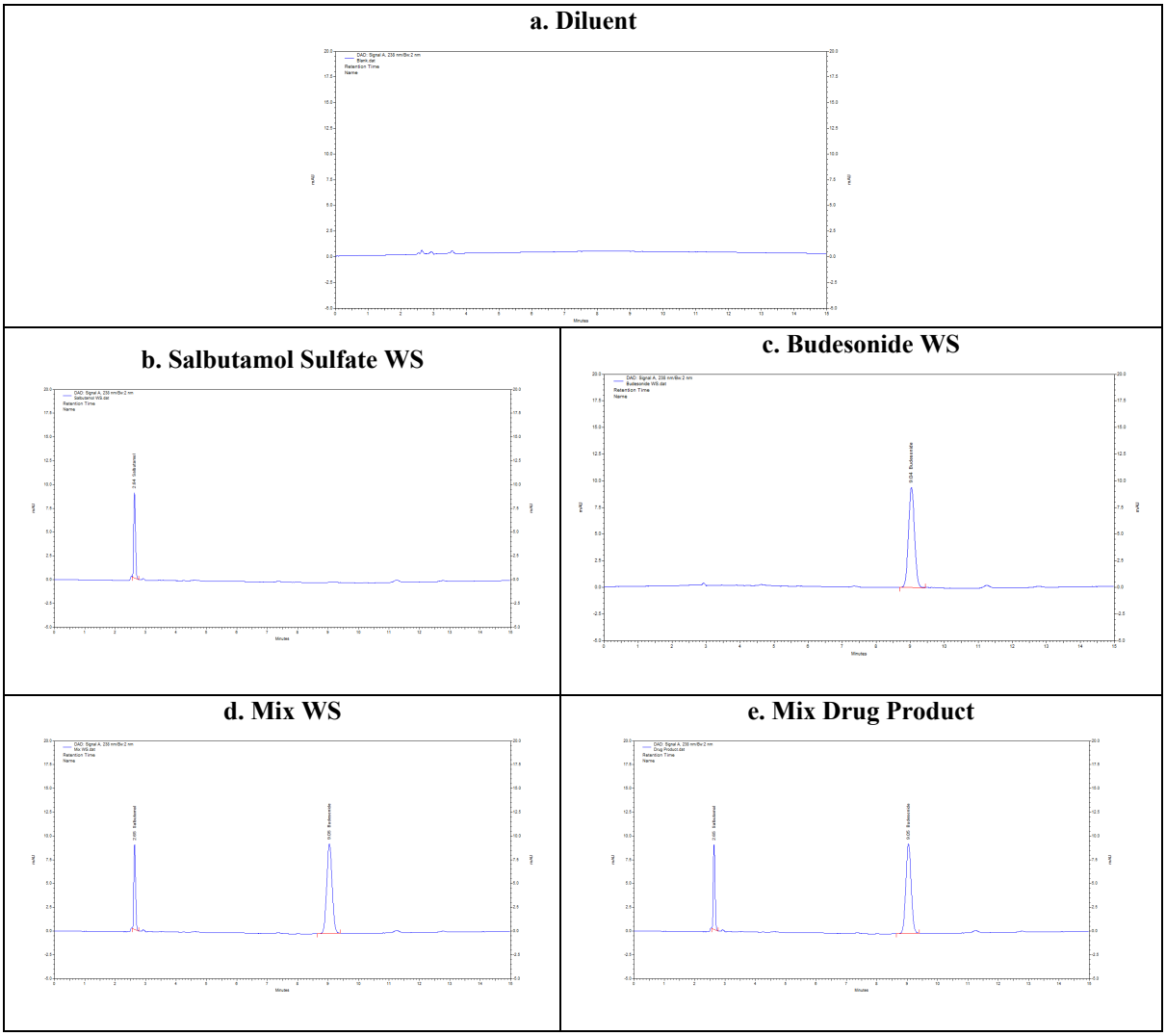


Figure 6: Chromatogram ID (a)Diluent, (b) SS Working Standard (c) BUD working standard, (d) Working Standard mixture of SS and BUD, (e) Drug Product of SS & BUD.

3.3.2. Precision (Instrument, Method, Intermediate, and System Suitability)

Precision studies carried out are indicated in Tables 6 & 7. The average % RSD for peak area for instrument precision, method precision, and intermediate precision for SS was found to be 0.22%, 0.22%, and 0.31%, respectively whereas found % RSD for BUD was 0.15%, 0.60%, and 0.019%, respectively. From %RSD results we conclude that the approach is highly precise and reliable performed by different analysts, and used for multiple sample preparations with the same concentration.

Table 6: System Suitability and Precision for Salbutamol Sulfate

SS System Suitability					**SS Peak Area**		
Sample ID	RT	TP	Asy	Resolution	Instrument Precision	Method Precision	Intermediate Precision
Rep 1	2.64	10177	1.11	0.00	73482	73374	73752
Rep 2	2.64	9752	1.12	0.00	73297	73221	73175

Rep 3	2.64	9345	1.05	0.00	73314	73452	73568
Rep 4	2.64	11208	1.08	0.00	73421	73568	73421
Rep 5	2.64	10254	1.13	0.00	73028	73125	73682
Rep 6	2.64	10337	1.09	0.00	73215	73337	73285
Average	2.64				73293	73337	73481
STDEV	0				160.543	160.929	226.742
%RSD	0				0.22	0.22	0.31

Table 7: System Suitability and Precision for Budesonide

BUD System Suitability					**BUD Peak Area**		
Sample ID	RT	TP	Asy	Resolution	Instrument Precision	Method Precision	Intermediate Precision
Rep 1	9.04	12050	1.05	29.45	242498	232219	245721
Rep 2	9.04	11684	1.03	29.45	241945	232487	246221
Rep 3	9.04	12754	1.02	29.45	242250	234752	245512
Rep 4	9.04	11257	1.04	29.45	241563	235595	245265
Rep 5	9.04	13025	1.07	29.45	241956	232848	246122
Rep 6	9.04	12575	1.08	29.45	242522	234575	246524
Average	9.04				242122	233746	245894
STDEV	0				371.247	1402.946	474.827
%RSD	0				0.15	0.60	0.19

Rt- Retention time, TP- Theoretical plates, Asy – Asymmetry

3.3.3. Linearity of Salbutamol Sulfate & Budesonide

Linearity studies were conducted for SS and BUD with concentrations ranging from 7.2-10.8 µg/ml and 6.4-7.2 µg/ml respectively, and the correlation coefficient was found to be 0.999 for both drugs. The linearity studies followed Lambert's- beer law as depicted in Table 8 and Figure 7

Table 8: Linearity data of NIR & RIT

Salbutamol Sulfate			**Budesonide**		
% Level	**Conc (ug/ml)**	**Area**	**% Level**	**Conc (ug/ml)**	**Area**
80	7.2	58624	80	6.4	194359
90	8.1	66289	90	7.2	217655
100	9.0	73482	100	8.0	242498
110	9.9	80592	110	8.8	266822
120	10.8	87614	120	9.6	291562
R^2= 0.999			R^2= 0.999		

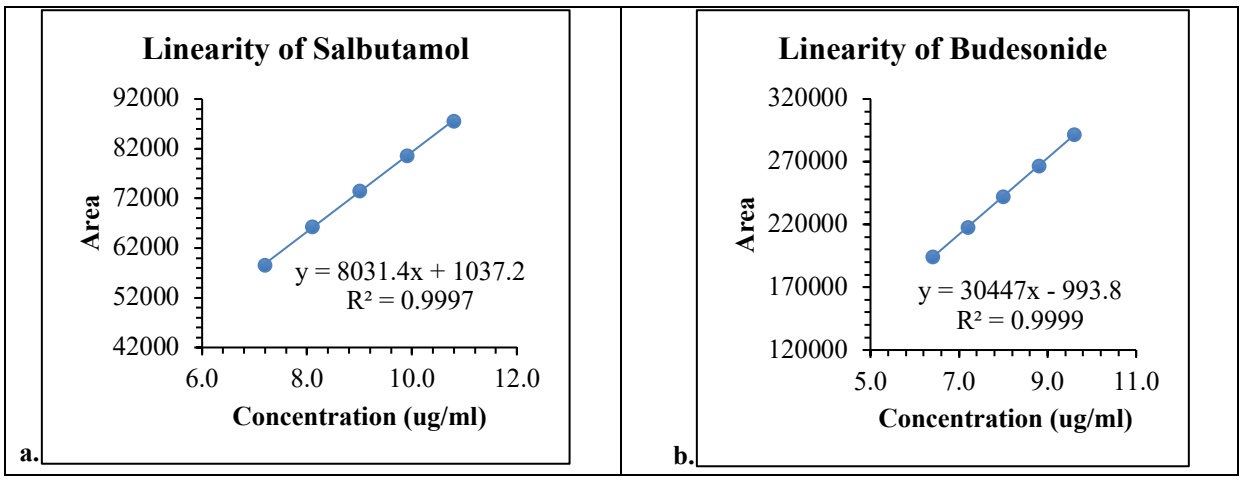


Figure 7: Linearity graph of a) Salbutamol Sulfate and b) Budesonide

3.3.4. LOD and LOQ for Salbutamol Sulfate and Budesonide

The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.29 µg/ml and 0.89 µg/ml for SS and 0.16 µg/ml and 0.49 µg/ml for BUD respectively. The LOD and LOQ were significantly low, suggesting the method to be very efficient in determining the least concentration of the drug. This value of LOD and LOQ will be useful in the future during cleaning validation in the industry which contributes Pharmaceutical companies to knowing if the manufactured vessel or equipment is free from API stains.

3.3.5. Accuracy

Accuracy for SS and BUD were conducted in triplicates and it was observed that the method was accurate for the range 80%, 100%, and 120%. The relative standard deviation for 80%, 100%, and 120% were 0.42%, 0.14%, and 0.28% respectively for SS and 0.31%, 0.11%, and 0.09% respectively for BUD as shown in Tables 9 and 10.

Table 9: Accuracy data for Salbutamol Sulfate

% Level	Spiked conc (ug/ml)	Reps	Area	Amount Recovered (ug/ml)	% Recovery	Average %	STDEV	% RSD
80%	7.19	Rep 1	58624	7.19	99.98	99.51	0.419652	0.42
		Rep 2	58158	7.13	99.19			
		Rep 3	58254	7.15	99.35			
100%	8.99	Rep 1	73482	9.01	100.26	100.10	0.139517	0.14
		Rep 2	73297	8.99	100.01			
		Rep 3	73314	8.99	100.03			
120%	10.79	Rep 1	87614	10.75	99.62	99.33	0.278863	0.28
		Rep 2	87125	10.69	99.06			
		Rep 3	87336	10.71	99.30			

Table No. 10. Accuracy data for Budesonide

% Level	Spiked conc. (ug/ml)	Reps	Area	Amount Recovered (ug/ml)	% Recovery	Average %	STDEV	% RSD
80%	6.39	Rep 1	194359	6.42	100.34	100.05	0.313273	0.31
		Rep 2	193865	6.40	100.09			

		Rep 3	193152	6.38	99.72			
100%	7.99	Rep 1	242498	8.00	100.16	100.04	0.114401	0.11
		Rep 2	241945	7.99	99.93			
		Rep 3	242250	8.00	100.05			
120%	9.59	Rep 1	291562	9.62	100.35	100.33	0.092982	0.09
		Rep 2	291741	9.63	100.41			
		Rep 3	291210	9.61	100.23			

3.3.6. Inter and Intraday Precision

The %RSD for SS & BUD for Intra & inter-day precision were observed to be 0.22% and 0.84% respectively.

3.7. Robustness

For conducting robustness the column oven temperature was changed at 28, 30, and 32°C. and the wavelength at 236, 238 and 240 nm separately. There was no significant change found in retention time or peak area of replicate injection for both the drugs as shown in Tables 11 and 12. The method was concluded to be robust with a change in column oven temperature and wavelength.

Table 11: Robustness study - Change in Column oven temperature

Column Oven Temperature Variation							
Condition	**Sample ID**	**Salbutamol**			**Budesonide**		
		RT	**Area**	**% Assay**	**RT**	**Area**	**% Assay**
28°C	WS	2.65	73125	-	9.05	241572	-
	DP	2.65	72711	99.43	9.05	240912	99.73
30°C	WS	2.65	73482	-	9.05	242498	-
	DP	2.65	72958	99.29	9.05	242045	99.81
32°C	WS	2.65	73514	-	9.05	241954	-
	DP	2.65	73185	99.55	9.05	241221	99.70

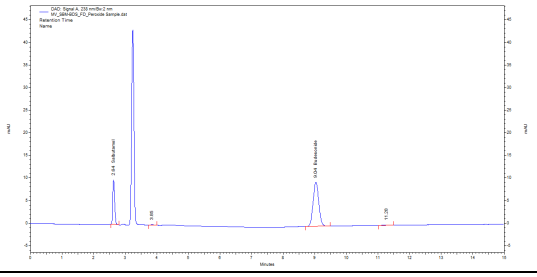
Table 12: Robustness study - Change in Wavelength

Wavelength Variation							
Condition	**Sample ID**	**Salbutamol**			**Budesonide**		
		RT	**Area**	**% Assay**	**RT**	**Area**	**% Assay**
236 nm	WS	2.65	73281	-	9.05	242155	-
	DP	2.65	72857	99.42	9.05	241345	99.67
238 nm	WS	2.65	73482	-	9.05	242498	-
	DP	2.65	72958	99.29	9.05	242045	99.81
240 nm	WS	2.65	73415	-	9.05	241874	-
	DP	2.65	72962	99.38	9.05	241185	99.72

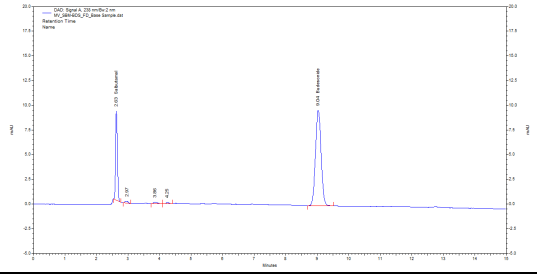
3.8 Forced Degradation:

The stress degradation experiment was conducted, and it revealed a significant degradation in acidic conditions. SS showed degradation in acidic conditions (9.59%), basic conditions (3.31%), and oxidative conditions (3.60). Budesonide showed degradation in acidic conditions (6.52%), basic conditions (1.03%), and oxidative conditions (6.38%). There were no significant degradants at dry heat degradation and photolysis for both drugs. Table 13 and Figure 8 show the forced degradation(deg) data and chromatograms.

Oxidation



Base Hydrolysis



Acid Hydrolysis

Table 13: Forced Degradation data for Salbutamol Sulfate & Budesonide

Condition	Salbutamol Sulfate			Budesonide		
	Peak Area	% Assay	% Deg	Peak Area	% Assay	% Deg
Control	76821	100.00	-	250424	100.00	-
Acid	69452	90.41	9.59	234091	93.48	6.52
Base	74281	96.69	3.31	247851	98.97	1.03
Peroxide	74054	96.40	3.60	234452	93.62	6.38
Photolytic	77391	100.74	No Deg	250627	100.08	No Deg
Dry Heat	76496	99.58	0.42	250978	100.22	No Deg

Deg: Degradation

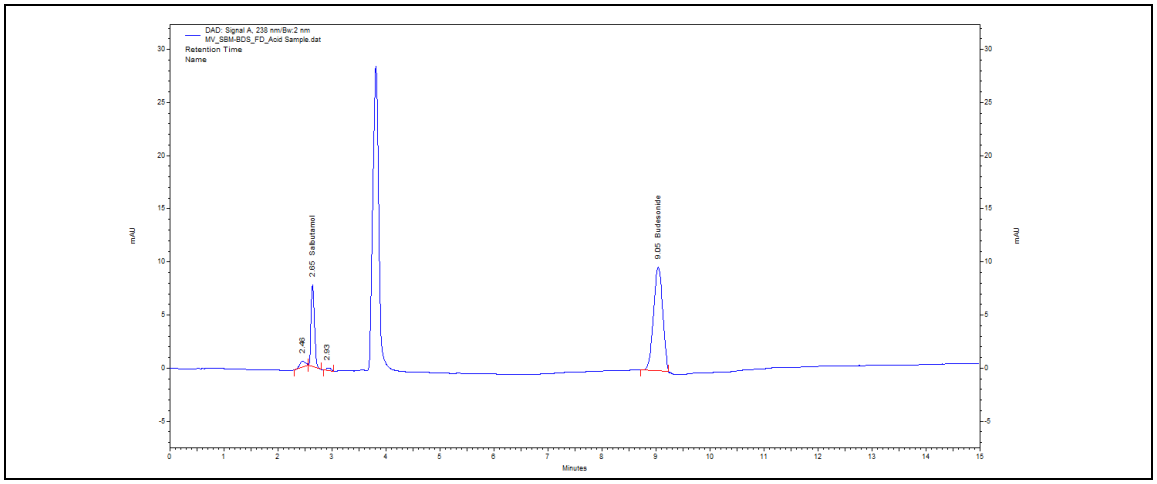


Figure 8: Simultaneous stressed condition chromatogram ID: a] Oxidation b] Base hydrolysis and c] Acid hydrolysis of SS and BUD

4. CONCLUSION

In this RP-HPLC study, a precise and accurate method was developed using the AQbD technique for estimating SS and BUD in bulk drugs and formulations. The method was validated for accuracy, precision, and robustness. Forced degradation studies showed that SS and BUD are highly susceptible to acidic conditions. The proposed method is deemed suitable due to its simplicity, reliability, sensitivity, speed, and selectivity for detecting very low concentrations which can be used as a reference in the quality control department of the pharma industry for cleaning validation. Validation data confirm that this method is accurate, precise, simple, economical, and can be used for routine analysis of SS and BUD in bulk and formulations.

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