

Research Article

Analytical Method Development and Validation of RP-HPLC Method for Simultaneous Estimation of Levosalbutamol Hydrochloride and Budesonide in Bulk and Pharmaceutical Dosage Form

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A simple, precise, accurate RP-HPLC method with DAD detector has been developed and subsequently validated for the simultaneous estimation of Levosalbutamol Hydrochloride and Budesonide in pure and pharmaceutical dosage form. The estimation was carried out on Zorbax Bonus-RP (4.6x 250mm 5µm), at 1 ml/min flow rate, detection wavelength is 239 nm, mobile phase containing 0.1% Perchloric Acid: Acetonitrile in ratio of 35:65. The retention times of Levosalbutamol Hydrochloride and Budesonide were 2.34 min & 5.78 min respectively. The concentration range was found to be linear 10 to 15 µg/ml for Levosalbutamol Hydrochloride and 0.4-6 µg/ml for Budesonide. The correlation coefficient (r^2) was found to be 0.999 for both the drugs. LOD and LOQ value for Levosalbutamol Hydrochloride was found to be 0.39 µg/mL and 1.18 µg/mL, LOD and LOQ value for Budesonide was found to be 0.09 µg/mL and 0.27 µg/mL respectively. The %RSD values were less than 2 for both the drugs. Assay for Levosalbutamol Hydrochloride and Budesonide was found to be 100.01 & 100% respectively. The method was validated for linearity, accuracy, precision, robustness, LOD, LOQ as per ICH guidelines. The developed method was successfully used for the quantitative analysis of commercial available dosage form.

Keywords: Levosalbutamol Hydrochloride, Budesonide, RP-HPLC, Linearity, Precision.

INTRODUCTION

Levalbuterol Hydrochloride (also known as Levosalbutamol Hydrochloride) is the R-enantiomer of short acting β_2 -adrenergic receptor agonist of Salbutamol (Albuterol). Chemically it is designated as (R)- α 1-[[[(1,1-dimethylethyl) amino] methyl]-4-hydroxy-1,3-benzenedimethanol hydrochloride. Chemical structure of Levalbuterol Hydrochloride is shown in Figure no.1. Levalbuterol leads to activation

of β_2 -adrenergic receptors on airways smooth muscle resulting in muscle relaxation and bronchodilatation. Levalbuterol relaxes the smooth muscle of all airways, from the trachea to the terminal bronchioles. Levalbuterol Hydrochloride is a highly selective β_2 agonist; cardiac side effects are less prominent. Selectivity is further increased by inhaling the drugs. Inhaled Levalbuterol Hydrochloride is used to abort and terminate attacks of asthma. [1,2]

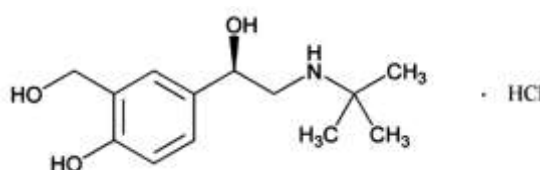


Figure no. 1: Chemical structure of Levosalbutamol Hydrochloride

Budesonide is designated chemically as (RS)-11- β , 16- α , 17, 21-tetrahydroxypregna-1, 4-diene-3,20-dione cyclic 16, 17-acetalbutyraldehyde. [3] Chemical structure of Budesonide is shown in Figure no. 2. Budesonide is a second-generation glucocorticoid, exhibits high affinity to the corticosteroid receptors with a high ratio of topical to systemic anti-inflammatory activity. Glucocorticoids have effects such as gluconeogenesis, proteolysis, lipolysis, suppression of inflammation and immune responses. Budesonide exhibits potent topical anti-inflammatory effects as a glucocorticoid and undergoes significant

first-pass metabolism, making it well-suited for localized IBD therapy. Beyond IBD, has also been employed in treating various clinical conditions, including asthma [4], allergic rhinitis [5], and eosinophilic esophagitis [6], owing to its potent local anti-inflammatory action and favorable degradation in the upper gastrointestinal tract, which restricts its effective delivery to the colon [7]. The therapeutic success of Budesonide in ulcerative colitis hinges on overcoming GI barriers through the development of advanced colon-targeted delivery systems that enable localized, controlled release at the site of inflammation [8].

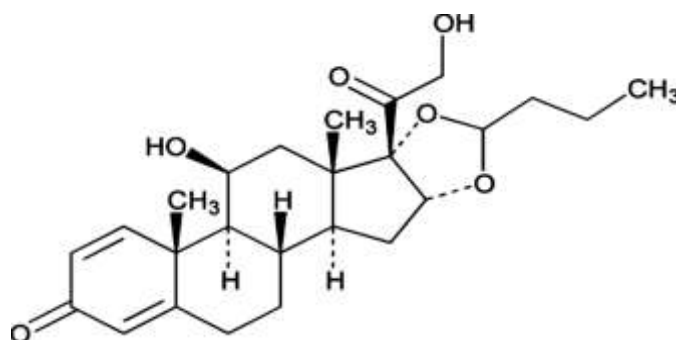


Figure no. 2: Chemical structure of Budesonide

The literature review described HPLC, UV-Visible spectrophotometric, LC-MS method for determination of Budesonide individually and combination with other drugs in their pharmaceutical dosage form. [9-14]

MATERIAL AND METHODS:

1.2.1 Instrumentation Chemicals and reagents:

The HPLC system consisted of Agilent connected with PDA detector. Levosalbutamol Hydrochloride and Budesonide standard gift samples were provided by Aadhaar Life Science Pvt. Ltd. Solapur. Methanol, acetonitrile and water (HPLC grade) were purchased from Merck Chemical Company. Sodium hexane Sulphonic acid, glacial acetic acid (Analytical grade) and 0.22 μ m pump Nylon filter were purchased from S.D. Fine Chemicals Ltd., Mumbai. The Whatman filter paper No. 41 was obtained from Modern Science Lab. All other reagents used were analytical grade. All the glassware used were borosilicate glass. Respules formulations (Budesal respules) were obtained from the local market.

1.2.2 Chromatographic Conditions:

- Oven Temp: 30°C
- Flow rate: 1 ml/min.
- Mobile Phase: 0.1% Perchloric Acid: Acetonitrile (35: 65, % v/v)
- Preparation of Buffer:** In 1000 ml HPLC water, 1 ml of Perchloric Acid was added and mixed well and filtered through 0.45-micron membrane filter and sonicated to degas for 10 minutes.
- Runtime: 10 minutes
- Injection Volume: 10 μ l
- Wavelength: 239 nm
- Diluent: 0.1% Perchloric Acid: Acetonitrile (50: 50, % v/v)
- Column: Zorbax Bonus-RP (4.6x 250mm 5 μ m)

1.3 Standard Preparation:

a. Levosalbutamol hydrochloride Standard Stock solution-I (SSS-I):

i. Prepare a Standard Stock Solution (SSS-I) of by adding 12.5 mg of Levosalbutamol hydrochloride in 100 ml volumetric flask & add 50 ml diluent, sonicate for 5 minutes and make the volume to 100 ml with diluent. (Conc. of Levosalbutamol hydrochloride in SSS-I = 125 µg/ml).

b. Budesonide Standard Stock solution-II (SSS-II)

ii. Prepare a Standard Stock Solution (SSS-II) of by adding 5 mg of Budesonide in 100 ml volumetric flask & add 50 ml diluent, sonicate for 5 minutes and make the volume to 100 ml with diluent. (Conc. of Budesonide in SSS-II = 50 µg/ml).

c. Then add 1.0 ml of SSS-I and 1.0 ml of SSS-II in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Levosalbutamolhydrochloride = 12.5 µg/ml and Conc. of Budesonide = 5 µg/ml)

1.4 Preparation of Drug Product sample solution:

The drug product sample solution was prepared by taking sample equivalent to 1.25 mg of Levosalbutamol hydrochloride and 0.5 mg of Budesonide accurately in 100 ml volumetric flask and 50-70 diluent was added to it and sonicated for 5 minutes and made up to the mark with diluent.

1.5 Selection of Wavelength:

The sample was scanned from 190-400 nm with DAD detector. The Wavelength selected for analysis chosen was 239 nm on the basis of isosbestic point.

1.6 Method Validation:

a. Specificity & Assay:

i. Individual sample of Blank, Levosalbutamol hydrochloride working standard (12.5 µg/ml), Budesonide working standard (5 µg/ml), Mixture working standard and Drug product of was prepared and peaks were for identified from Retention Time.

ii. % Assay was calculated as follows:

$$\% \text{ Assay} = \frac{\text{Sample area}}{\text{Standard area}} \times 100$$

b. Repeatability & System Suitability:

i. A single working standard was prepared as described in section 2 and 6 injections were made from same solution and checked for system suitability.

ii. System suitability parameters are as below:

1. Retention Time,
2. Theoretical plates,
3. Asymmetry (Tailing factor),
4. Resolution.

c. Linearity & Range:

i) 5 samples of varying concentrations ranging from 80-120% were prepared.

ii) The concentrations are given below

Table no.: 1. Concentration for linearity Study of for HPLC

% Level	Levosalbutamol hydrochloride Conc. (µg/ml)	Budesonide Conc. (µg/ml)
80	10	4.0
90	11.25	4.5
100	12.5	5.0
110	13.75	5.5
120	15	6.0

iii. The sample preparations are given as below;

X ml of Levosalbutamol hydrochloride and Y ml of Budesonide standard solution was added to 10 ml diluent to make up the concentrations given above:

X ml of SSS-I	X ml of SSS-II	Diluted to
0.8	0.8	10 ml
0.9	0.9	10 ml
1.0	1.0	10 ml
1.1	1.1	10 ml
1.2	1.2	10 ml

d. Accuracy:

i. Samples were prepared of 80%, 100% and 120% concentration by spiking the same amount of concentration given in table for Linearity.

ii. Samples were injected in triplicate to calculate % RSD.

iii. % Recovery was also calculated.

e. LOD/ LOQ:

Was calculated by using ANOVA technique.

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

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

f. Robustness:

i. The Robustness was performed by changing the column temperature and Wavelength by $\pm 2^\circ\text{C}$ and $\pm 2\text{ nm}$.

ii. Each Sample was injected and % RSD of peak area was calculated at each condition.

Formula:

Table no. 2 Column Oven Temperature Robustness Study.

Condition	Increased	Normal	Decreased
Column Oven Temperature	32°C	30°C	28°C
Wavelength	266 nm	264 nm	262 nm

g. Intra & Inter-day Precision:

i) Single mixture working standard and drug product was prepared and injected twice in a day at different time intervals to evaluate intra-day precision.

ii) Same mixture working standard was analysed on second day to evaluate the inter-day precision.

iii) % RSD of peak was calculated at each interval and stability of solutions were estimated.

RESULT AND DISCUSSION:

i) Selection of analytical wavelength:

The sample was scanned from 190-400 nm with DAD detector. The Wavelength selected for analysis chosen was 239 nm on the basis of isosbestic point.

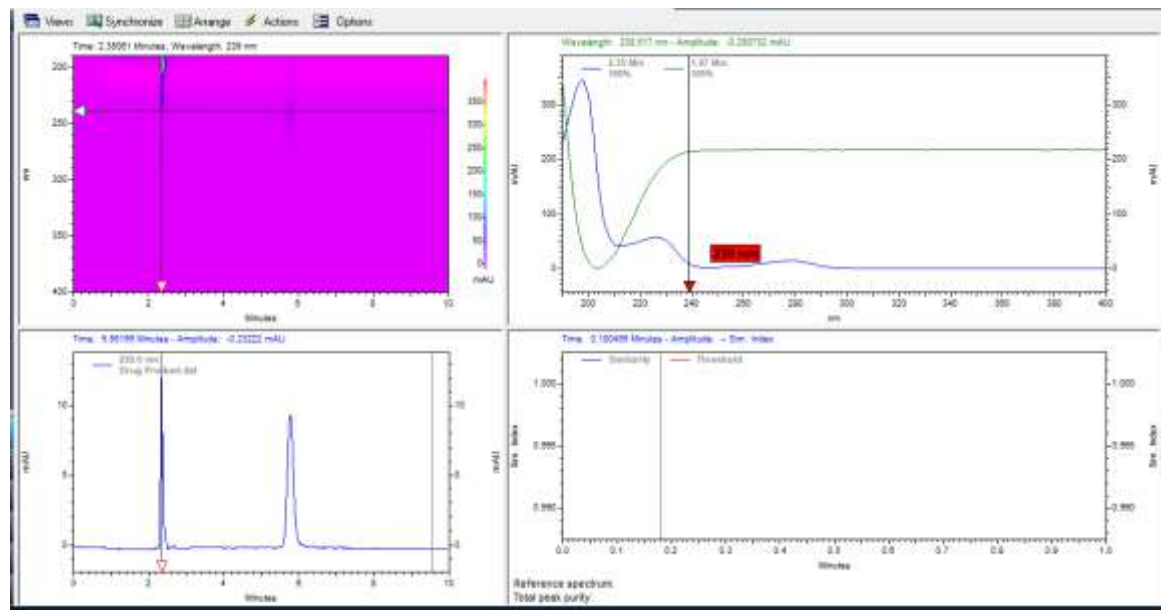


Figure no.3: Spectrum of Levosalbutamol Hydrochloride and Budesonide between 200-400 nm in mobile phase

Levosalbutamol Hydrochloride RT 2.34 min and Budesonide RT 5.78 min show the maximum absorbance at 264 nm. Hence, HPLC analysis was carried out at 264 nm. (Figure.1)

Table no. 1Details of Various trial of mobile phase for mixture containing Levosalbutamol Hydrochloride and Budesonide.

Trial No.	Mobile Phase	Ratio	Wavelength	Levosalbutamol Hydrochloride			Budesonide		
				RT	TP	Asymmetry	RT	TP	Asymmetry
1	0.1% Perchloric Acid: Acetonitrile	50-50	250 nm	1.97	6501	1.20	8.87	9221	1.15
2	0.1% Perchloric Acid: Acetonitrile	40-60	239 nm	1.89	6839	1.34	5.27	7049	1.14
3	0.1% Perchloric Acid: Acetonitrile	40-60	239 nm	2.32	8287	2.17	5.21	5184	1.06
4	0.1% Perchloric Acid: Acetonitrile	35-65	239 nm	2.34	8839	1.23	5.78	6103	1.13

Final Method: Zorbax Bonus-RP (4.6x 250mm 5um), at 1 ml/min flow rate, detection wavelength is 239 nm, mobile phase containing 0.1% Perchloric Acid: Acetonitrile in ratio of 35:65.

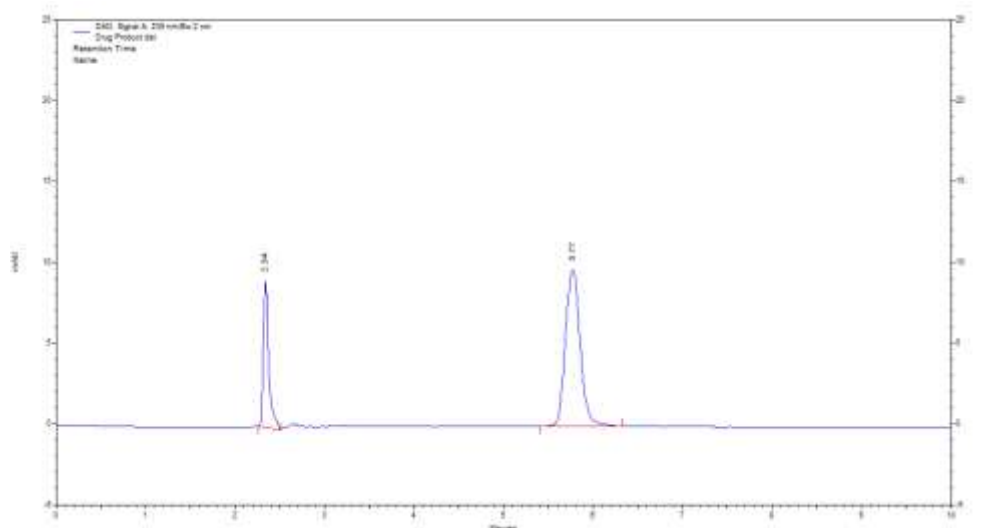


Figure no.4: Chromatogram of Standard Mixture of Levosalbutamol Hydrochloride and Budesonide in optimized chromatographic conditions.

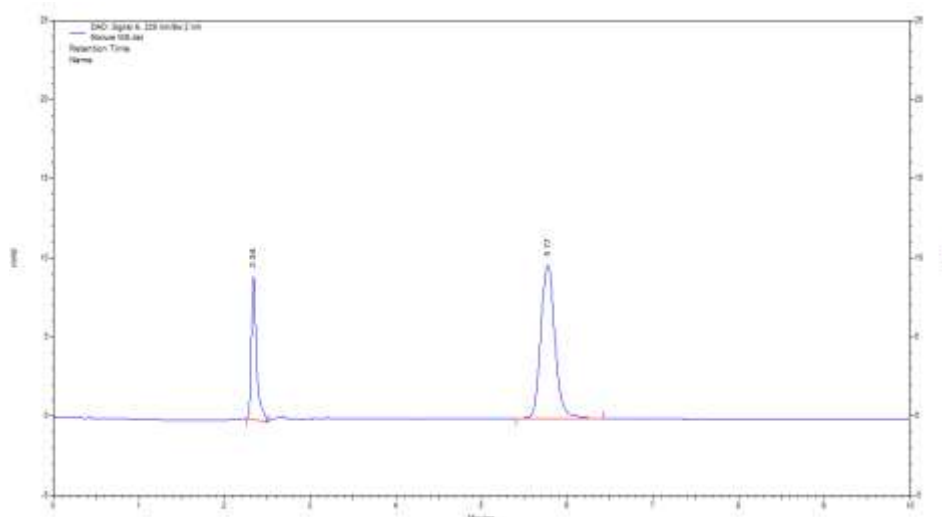


Figure no.5: Chromatogram of Sample of Levosalbutamol Hydrochloride and Budesonide in optimized chromatographic conditions.

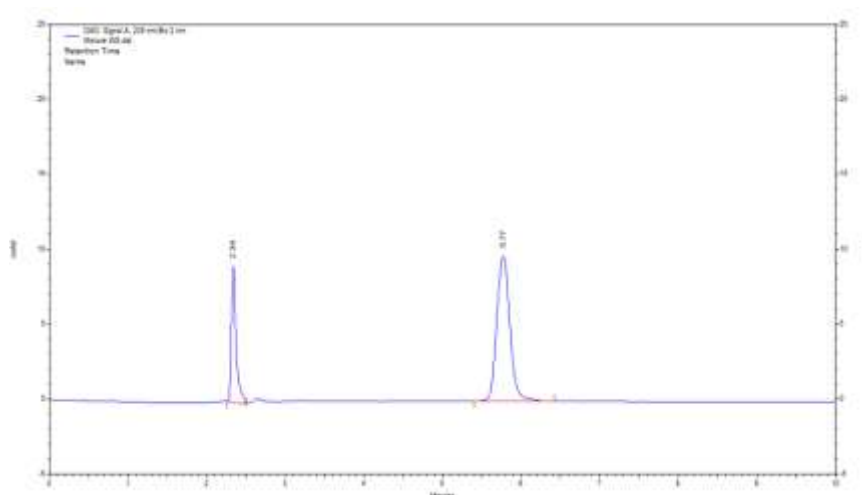
Table no. 2: Details of chromatogram of standard mixture containing Levosalbutamol Hydrochloride and Budesonide.

Sr. No	Name of drug	RT (min)	Plates	Tailing factor
1.	Levosalbutamol Hydrochloride	2.34 ± 0.5 min	8839	1.20
2.	Budesonide	5.78 ± 0.5 min	6103	1.33

iii) Analysis of tablet formulation: -

Table no.3 Analysis of marketed formulation.

Sample ID	Levosalmolbutamol hydrochloride			Budesonide		
	RT	Area	% Assay	RT	Area	% Assay
LSB WS	2.34	79249	-	-	-	-
BDS WS	-	-	-	5.77	237150	-
MIX WS	2.34	79253	-	5.77	237253	-
Drug Product	2.34	79258	100.01	5.77	237257	100.00

**Figure no 6. Chromatogram of Levosalbutamol Hydrochloride and Budesonide in tablet formulation.**

Amount of drug present in the marketed formulation was calculated using RP-HPLC. Amount of Levosalbutamol Hydrochloride and Budesonide was found to be 100.01 & 100% respectively. This method can be employed for routine analysis of Levosalbutamol Hydrochloride and Budesonide. The result of assay of marketed formulation are given in Table 7.10. The separation was achieved by Zorbax Bonus-RP (4.6x 250mm 5um) column, at 1 ml/min flow rate, detection wavelength is 239 nm, mobile phase containing 0.1% Perchloric Acid: Acetonitrile in ratio of 35:65. The detection was carried out at 239 nm.

The retention time of Levosalbutamol Hydrochloride and Budesonide was found to be 2.34 ± 0.5 min and 5.78 ± 0.5 min respectively.

After establishing the chromatographic conditions, analysis of tablet formulation was done. The results are given in (Table 3& Figure 6)

1.8) Validation Of RP-HPLC Method: [15-19]

A. Linearity: Different concentration of solution prepared for Linearity of both Levosalbutamol Hydrochloride and Budesonide are shown in (Table - 4 and Table -5) calibration curves are shown in Figure 7& 8 respectively.

Table no.4 Linearity dilutions for Levosalbutamol Hydrochloride.

Levosalmolbutamol Hydrochloride		
% Level	Conc. (µg/ml)	Area
80	10	65055
90	11.25	71849
100	12.5	79249
110	13.75	85776
120	15	92885

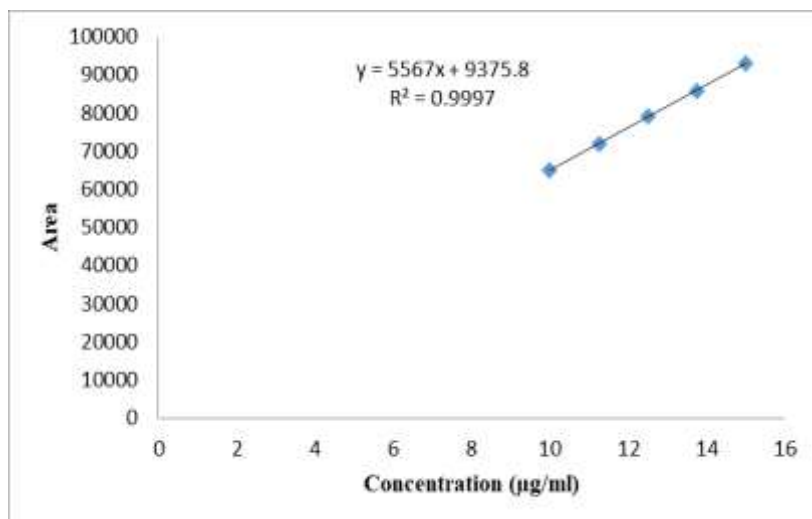


Figure no.7 Calibration curve of Levosalbutamol Hydrochloride.

Table no.5 Linearity dilutions for Hydrochlorothiazide.

Budesonide		
% Level	Conc. (µg/ml)	Area
80	4	189932
90	4.5	214000
100	5	237150
110	5.5	261584
120	6	284498

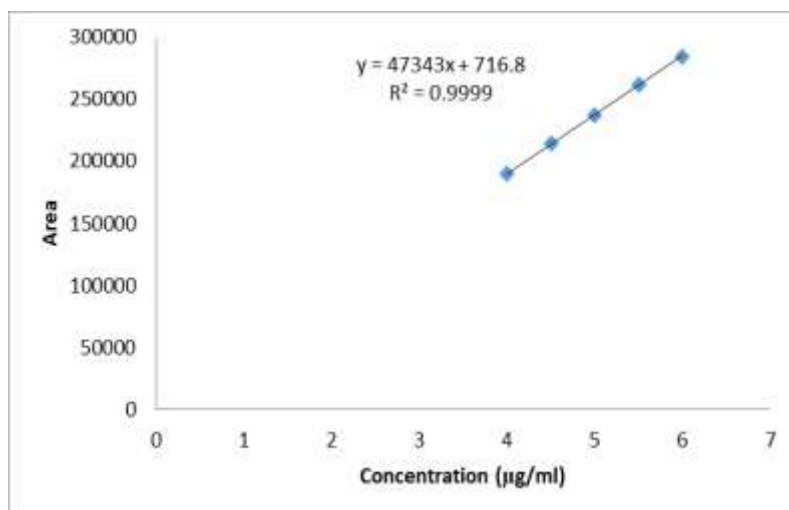


Figure no.8 Calibration curve of Hydrochlorothiazide.

According to ICH guideline linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of an analyte. Linearity was studied by plotting a graph of area v/s concentration. A series standard solution of Levosalbutamol Hydrochloride and Budesonide were prepared in the concentration range of 10 µg/ml to 15 µg/mL and 4 µg/mL to 6

µg/mL respectively with linearity range 80-120% for both the drug and is shown in Table 4 and 5. The regression coefficient (r^2) of Levosalbutamol Hydrochloride was found to be 0.999 & for Budesonide regression coefficient (r^2) was found to be 0.999. The equation of regression line for Levosalbutamol Hydrochloride was found to be $y = 5567x + 9375.8$ for Budesonide was found to be

$y=47343x+716.8$ Linearity graph of Levosalbutamol Hydrochloride and Budesonide shown in figure 8&9 respectively.

The Precision study of Levosalbutamol Hydrochloride and Budesonide are shown Table 7.13 respectively.

B. Precision:

Table no. 6 Precision of Levosalbutamol Hydrochloride and Budesonide.

Levosolbutamol Hydrochloride				
Condition	Sample ID	RT	Area	% Assay
Morning	WS	2.34	79253	-
	DP	2.34	79258	100.01
Evening	WS	2.34	79245	-
	DP	2.34	79265	100.03
% RSD				0.01
Day 2	WS	2.34	79262	-
	DP	2.34	79264	100.00
% RSD				0.01

Budesonide				
Condition	Sample ID	RT	Area	% Assay
Morning	WS	5.77	237253	-
	DP	5.77	237257	100.00
Evening	WS	5.77	237345	-
	DP	5.77	237129	99.91
% RSD				0.07
Day 2	WS	5.77	237147	-
	DP	5.77	237045	99.96
% RSD				0.05

The Precision of test results is ensured by intraday and interday precision. Levosalbutamol Hydrochloride and Budesonide both had % RSD values less than 2. Results are shown in Table 6.

C. Accuracy: The accuracy study of Levosalbutamol Hydrochloride and Budesonide are shown in Table 7 and 8 respectively.

Table no. 7. Accuracy Study of Levosalbutamol Hydrochloride.

Levosolbutamol			
Std. wt. (mg)	% Purity	Std. Stock Conc. (µg/ml)	Working Std. Area
12.5	99.9	124.875	79253

Sample ID	Reps	Spiked Conc. (µg/ml)	Area	Amount Recovered (µg/ml)	% Recovery	AVG	STDEV	% RSD
80%	Rep 1	9.99	85055	13.40	134.15	134.14	0.035491	0.03
	Rep 2	9.99	85065	13.40	134.17			
	Rep 3	9.99	85022	13.40	134.10			
100%	Rep 1	12.49	79249	12.49	99.99	100.00	0.007025	0.01
	Rep 2	12.49	79256	12.49	100.00			
	Rep 3	12.49	79245	12.49	99.99			

120%	Rep 1	14.99	92885	14.64	97.67	97.67	0.007385	0.01
	Rep 2	14.99	92879	14.63	97.66			
	Rep 3	14.99	92893	14.64	97.68			

Table no.8 Accuracy Study of Budesonide.

Budesonide			
Std. wt. (mg)	% Purity	Std. Stock Conc. (µg/ml)	Working Std. Area
5	99.9	49.95	237253

Sample ID	Reps	Spiked Conc. (µg/ml)	Area	Amount Recovered (µg/ml)	% Recovery	AVG	STDEV	% RSD
80%	Rep 1	4.00	189932	4.00	100.07	100.05	0.027093	0.03
	Rep 2	4.00	189845	4.00	100.02			
	Rep 3	4.00	189936	4.00	100.07			
100%	Rep 1	5.00	237150	4.99	99.96	99.97	0.018571	0.02
	Rep 2	5.00	237230	4.99	99.99			
	Rep 3	5.00	237158	4.99	99.96			
120%	Rep 1	5.99	284498	5.99	99.93	99.92	0.006188	0.01
	Rep 2	5.99	284477	5.99	99.92			
	Rep 3	5.99	284463	5.99	99.92			

The method's accuracy defines how close the method's results are to the true value. The results of the accuracy testing revealed that the technique is accurate within acceptable ranges. When the % RSD for Levosalbutamol Hydrochloride and Budesonide is calculated, all of the results are within acceptable bounds. A maximum RSD of 2.0% indicated acceptable accuracy within the range. The results are shown in Table 7 and 8. According to the Accuracy

research, the percent recovery of Levosalbutamol Hydrochloride is 97.67-134.14 % and Budesonide is 99.92-100.05 %, both of which are within the ICH standards.

D. Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD and LOQ of Levosalbutamol Hydrochloride and Budesonide are shown in Table 9.

Table no.9 The LOD and LOQ of Levosalbutamol Hydrochloride and Budesonide.

Sr. No	Name of drug	LOD (µg/mL)	LOQ (µg/mL)
1.	Levosalbutamol Hydrochloride	0.39	1.18
2.	Budesonide	0.09	0.27

Sensitivity of the method was determined with respect to limit of detection (LOD) and the quantification limit of an individual analytical procedure is the lowest amount of an analyte in a sample which can be quantitatively determined with the suitable precision and accuracy. LOD and LOQ value for Levosalbutamol Hydrochloride was found to be 0.39 µg/mL and 1.18 µg/mL, LOD and LOQ value for

Budesonide was found to be 0.09 µg/mL and 0.27 µg/mL respectively. Results are shown in Table 9.

E. System suitability:

System suitability data of Levosalbutamol Hydrochloride and Budesonide given in below Table 10.

Table no. 10System suitability parameter of Levosalbutamol Hydrochloride.

Levosolbutamol Hydrochloride					
Sample ID	Area	RT	TP	Asymmetry	Resolution
100% Rep 1	79249	2.34	8715	1.19	0.00
100% Rep 2	79254	2.34	8724	1.20	0.00
100% Rep 3	79274	2.34	8745	1.24	0.00
100% Rep 4	79255	2.34	8766	1.30	0.00
100% Rep 5	79246	2.34	8723	1.26	0.00
100% Rep 6	79219	2.34	8792	1.23	0.00
AVG	79250	2.34			
STDEV	17.85217	0			
% RSD	0.02	0.00			

Table no. 11System suitability parameter of and Budesonide.

Budesonide					
Sample ID	Area	RT	TP	Asymmetry	Resolution
100% Rep 1	237150	5.77	6090	1.15	17.33
100% Rep 2	237156	5.77	6075	1.11	17.23
100% Rep 3	237268	5.77	6098	1.12	16.54
100% Rep 4	237256	5.77	6074	1.14	16.22
100% Rep 5	237085	5.77	6049	1.11	17.42
100% Rep 6	237169	5.77	6047	1.13	14.45
AVG	237181	5.77			
STDEV	69.48285	0			
% RSD	0.03	0.00			

The system, method, and column performance were validated by testing system suitability features. Six times, a standard solution of Levosalbutamol Hydrochloride and Budesonide was injected into the

system, and the system's suitable features were evaluated. Results are shown in Table 10 and 11.

F. Robustness: Robustness data of Levosalbutamol Hydrochloride and Budesonide given in below

Table no.12Robustness parameter of Levosalbutamol Hydrochloride.

Variation in Column temperature (Levosolbutamol Hydrochloride)							
Condition	Sample ID	RT	Area	% Assay	Average	STDEV	% RSD
28C	WS	2.34	79145	-	100.09	0.152636	0.15
	DP	2.34	79144	100.00			
30C	WS	2.34	79253	-			
	DP	2.34	79258	100.01			
32C	WS	2.34	79456	-			
	DP	2.34	79668	100.27			

Variation in wavelength ((Levosolbutamol Hydrochloride)							
Condition	Sample ID	RT	Area	% Assay	Average	STDEV	% RSD

237 nm	WS	2.34	79265	-	100.04	0.065602	0.07
	DP	2.34	79358	100.12			
239 nm	WS	2.34	79253	-			
	DP	2.34	79258	100.01			
241 nm	WS	2.34	79246	-			
	DP	2.34	79247	100.00			

Table no.13 Robustness parameter of Budesonide.

Variation in Column temperature (Budesonide)							
Condition	Sample ID	RT	Area	% Assay	Average	STDEV	% RSD
28C	WS	5.77	237125	-	100.00	0.01262	0.01
	DP	5.77	237145	100.01			
30C	WS	5.77	237253	-			
	DP	5.77	237257	100.00			
32C	WS	5.77	237456	-			
	DP	5.77	237418	99.98			

Variation in Column temperature (Budesonide)							
Condition	Sample ID	RT	Area	% Assay	Average	STDEV	% RSD
237 nm	WS	5.77	237845	-	99.95	0.095767	0.10
	DP	5.77	237456	99.84			
239 nm	WS	5.77	237253	-			
	DP	5.77	237257	100.00			
241 nm	WS	5.77	237252	-			
	DP	5.77	237259	100.00			

Robustness was investigated using various deliberate alterations in chromatographic settings, such as changes in column Condition like 28°C, 30°C and 32°C.

% RSD was shown to be less than 2% in the Levosalbutamol Hydrochloride and Budesonide robustness studies. As a result, it is strong and adheres to ICH criteria. Results are shown in Table 12 and 13.

CONCLUSION:

Levosalbutamol Hydrochloride and Budesonide may be easily and quickly quantified from their formulations using the RP-HPLC method that has been developed and validated. Every validation parameter was determined to be within the permissible ranges in compliance with ICH criteria. Regardless of the excipients present, the proposed method was found to be straightforward, accurate, exact, robust, and resilient. It was also shown to be

specific for the pharmaceuticals of interest. It can be applied to the regular examination of commercially available formulations.

REFERENCES

1. Joel GH, Lee EL. Goodman and Gilman's, The Pharmacological Basis of Therapeutics. 10th International ed. New York: McGraw Hill Publishers, Medical Publishing Division; 2001,717-722.
2. Tripathi KD. Essentials of Medical Pharmacology. 5th ed. New Delhi: Jaypee Brothers Medical Publishers; 2004,198-208.
3. O'Neil MJ. The Merck Index: - An Encyclopedia of Chemicals, Drugs and Biological. 14th ed. Whitehouse Station, NJ, USA: Merck and Co. Inc; 2006. 40, 240.
4. R. Oum, Y. Al Ayoub, F. Haddad, B. Aburas, A. Paradkar, M. Najafzadeh, M. T. Nasim, R.C. Gopalan, K.H. Assi, Development and evaluation of liquid formulations of chrysin and budesonide

- for asthma and COPD; evaluation of the formulations' genotoxicity and anti-inflammatory activity, *J. Drug Deliv. Sci. Technol.* 105 (2025) 106567.
5. G. Asadi, P. Feizollahi, M. Rajabinejad, S. Falahi, F. RezaeiVarmaziar, E. Faryadi, A. GorginKaraji, F. Salari, A. Rezaieamaneh, Comparison of the efficacy of combined budesonide and fexofenadine versus combined fluticasone propionate and fexofenadine on the expression of class-4 semaphorins and their receptors in the peripheral blood cells of patients with allergic rhinitis, *Heliyon.* 10 (2024) e22924.
 6. E.S. Dellon, D.A. Katzka, V.A. Mukkada, M.H. Collins, G.W. Falk, C.A. Richmond, B. Terreri, M. Thakur, M. Boules, B. Goodwin, I. Hirano, Long-term safety and efficacy of Budesonide oral suspension for eosinophilic esophagitis: a 4-year, phase 3, open-label study, *Clin. Gastroenterol. Hepatol.* (2025), <https://doi.org/10.1016/j.cgh.2024.12.024>.
 7. M. Peng, D. Song, X. Ling, W. Jiang, Y. Zhang, Y. Yang, J. Le, Using thermal forced degradation approach for impurity profiling of budesonide solution-formulated metered dose inhalation with implementation of LC-QTOFMS and HPLC-UV, *J. Pharm. Biomed. Anal.* 208 (2022) 114445, <https://doi.org/10.1016/j.jpba.2021.114445>.
 8. H.S. Sardou, M. Abbaspour, A. Akhgari, P. Kesharwani, A. Sahebkar, Colon- targeted delivery systems of budesonide as second-line therapy in inflammatory bowel disease, *J. Drug Deliv. Sci. Technol.* 93 (2024) 105472, <https://doi.org/10.1016/j.jddst.2024.105472>.
 9. Mallikarjuna GM, Ramakrishna SA, Putta RK. Development and validation of selective UV spectrophotometric analytical method for Budesonide pure sample. *Journal of Applied Pharmaceutical Science.* 2011; 1 (7): 158-161.
 10. Gupta M, Bhargava HN. Development and validation of a high-performance liquid chromatographic method for the analysis of Budesonide. *Journal of Pharmaceutical and Biomedical Analysis.* 2006; 40 (2): 423-428.
 11. Hou S, Hindle M, Byron PR. A stability-indicating HPLC assay method for Budesonide. *Journal of Pharmaceutical and Biomedical Analysis.* 2001; 24 (21): 371-380.
 12. Hou S, Hindle M, Byron PR. Chromatographic and mass spectral characterization of Budesonide and a series of structurally related corticosteroids using LC-MS. *Journal of Pharmaceutical and Biomedical Analysis.* 2005; 39 (2005): 196-205.
 13. Faouzi MA, Dine T, Luyckx M, Brunet C, Gressier B, Cazin M, Wallaert B, Cazin JC. HPLC method for the determination of Budesonide in bronchoalveolar lavage of asthmatic patients. *Journal of Chromatography B: Biomedical Sciences and Applications.* 1995; 664 (2): 463-467.
 14. Panda SS, Beravenkata RK, Mohanta G. Stabilityindicating RP-HPLC method for simultaneous estimation of Levalbuterol Sulphate and Theophylline in combined dosage form. *Brazilian Journal of Pharmaceutical Sciences.* 2013. 49(3). 475-490.
 15. Amanda T. Barden*, a, Bruna L. Piccolia, Nadia M. Volpatoa, Martin Steppea, Second-Order Derivative UV Spectrophotometric and RP-HPLC Methods for the Analysis of Vildagliptin and Application for Dissolution Study, *Drug Anal Res*, 2018; 02, n.1, 46-53.
 16. Ramzia I. El-Bagary, Ehab F. Elkady, Bassam M. Ayoub, Liquid Chromatographic Methods for the Determination of Vildagliptin in the Presence of its Synthetic Intermediate and the Simultaneous Determination of Pioglitazone Hydrochloride and Metformin Hydrochloride, *Int J Biomed Sci* 2011; 7 (3): 201-208.
 17. Mohammed M. Amin, Salah A. Abdel-Aziz, Samia M. Mostafa and Sobhy M. El-Adl, Optimization and Validation of HPLC Method for Simultaneous Determination of Vildagliptin, Pioglitazone Hydrochloride and Glimepiride in Bulk and Tablets, *Journal of Pharmacy and Biological Sciences*, (Mar. - Apr.2017), Volume 12, Issue 2 Ver. IV ,18-27.
 18. Pintu Prajapati1*, Bageshree Ranal, VeeraShakar Pulusu2 and Shailesh Shah, Method operable design region for robust RP-HPLC analysis of pioglitazone hydrochloride and teneligliptin hydrobromide hydrate: incorporating hybrid principles of white analytical chemistry and design of experiments, *Future Journal of Pharmaceutical Sciences* (2023) 9:93.
 19. Jagdale. S. C, Patil. S, Kuchekar. B. S and Chabukswar. A, Preparation and characterization

of metformin hydrochloride — compritol 888 ato
solid dispersion, Journal of young pharmacists,
2011, 3, 197-204.

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