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Development and Validation of RP-HPLC Method for Estimation of Formoterol Fumarate and Budesonide in Pharmaceutical Dosage Form

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ABSTRACT

The main objective of the present work was to develop a simple, precise, specific & stability indicating RP-HPLC method for simultaneous estimation of Formoterol Fumarate and Budesonide in bulk tablet dosage form. Chromatographic separation of Formoterol Fumarate and Budesonide was achieved on waters symmetryC18 (4.6*150mm) 5µm and the mobile phase containing water ethanol in the ratio of 30:70 v/v as mobile phase. The flow rate was 1.0 ml/min, detection was carried out by absorption at 298 nm u sing a photodiode array detector. The RT of Formoterol Fumarate and Budesonide is found to be 1.59 and 3.52 min respectively. . The linearity of the method was excellent over the range 10- 60µg/ml and 10-100 µg/ml for Formoterol Fumarate and Budesonide respectively. The correlation coefficient was found to be 0.999. The proposed method was validated according to ICH guidelines. And it was found to be suitable accurate and sensible method for quantitative analysis of drug from Dosage form and study of its stability.

Keywords: RP-HPLC, Dosage forms, validation, of Formoterol Fumarate and Budesonide

INTRODUCTION:

Chronic obstructive pulmonary disease (COPD) is a type of obstructive lung disease. It is characterized by long-term breathing problems and poor airflow. The main symptoms of COPD include shortness of breath and cough with sputum production. COPD is a progressive disease, meaning it typically worsens over time. The term "chronic bronchitis" is also used to define a productive cough that is present for at least three months each year for two to three years.

Formoterol Fumarate Dihydrate is a bronchodilator and Budesonide is anti-inflammatory drugs available in different combinations in multiple Aerosol dosage forms to treat COPD, Asthma and Chronic bronchitis. Both drugs are available in white to almost white powder form. A bronchodilator is a substance that dilates the bronchi and bronchioles, decreasing resistance in the respiratory airway and increasing airflow to the lungs while Anti Inflammatory drug is used for the treatment of inflammation occurred on respiratory tract.[1-7]

Inhalation suspension dosage forms are utilised as life saving formulations and administered with the help of different types of nebulizers. These dosage forms are mainly anti asthmatics and requires very low dose ranging from 5mcg to 400mcg per dose. So method development for analysis of these drugs is very challenging job.

In the present study, a developed HPLC method was validated for the determination of a lower concentration of Fumarate and Budesonide drugs in Inhalation suspension pharmaceutical dosage form. The proposed analytical method was found to be precise, repeatable, linear, accurate, rugged, robust and specific. RP-HPLC method for the combined determination of Formoterol Fumarate and Budesonide is considered the main contribution of this study.[8-14]

Material and Methods:

Preparation of mobile phase:

A Combination of Potassium dihydrogen orthophosphate buffer (pH-4.5)(*refer 7.2.1.1*)and Acetonitrile was mixed in the ratio of 30:70, The pH was adjusted to 4.5 with Orthophosphoric acid and filtered through 0.45 µm membrane filter.

This prepared solution was used as mobile phase. This solution was also used for specificity blank solution.

Preparation of standard solution of Formoterol Fumarate and Budesonide fortrials:

Standard solution of Formoterol Fumarate and Budesonide were prepared by dissolving 10 mg of each drug in 10 mL of mobile phase. Further dilution was made by adding 1 mL of the stock solution to 10 mL standard flask and making up the volume with the mobile phase.

Preparation of Solutions for assay

Preparation of the Formoterol Fumarate and Budesonide standard and samplesolution

Sample solution preparation

22 mg of Formoterol Fumarate and Budesonide capsule powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, 2 ml of diluent was added and sonicate to dissolve it completely and making volume up to the
mark with the same solvent(Stock solution). Further pipette out 0.2 ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

VALIDATION OF HPLC FOR METHOD DEVELOPMENT

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Table 6.14: Results for system suitability ofFormoterol Fumarate

Injection	RT(min)	Peak area	TP	TR
1	2.095	127651	1564.31	1.29
2	2.095	127376	1634.55	1.31
3	2.095	128904	1563.37	1.31
4	2.096	126372	1612.78	1.29
5	2.096	128143	1560.59	1.31
6	2.098	127305	1624.48	1.29
Mean		127625	--	--
SD		853.0	--	--
%RSD		0.7	--	--

Table 6.15:Results for system suitability of Budesonide

Injection	RT(min)	Peak area	TP	TF
1	4.337	434309	4325.32	1.18
2	4.344	436839	4242.75	1.18
3	4.346	436814	4282.56	1.18
4	4.348	435350	4384.18	1.18
5	4.353	435462	4322.24	1.18
6	4.354	439095	4348.18	1.19
Mean		436311.4	-	-
SD		1669.5	-	-
%RSD		0.4	-	-

Acceptance criteria

The % RSD for the retention times of Formoterol Fumarateand Budesonide Peaksfrom 6 replicate injections of each Standard solution should be not more than 2.0 % The number of theoretical plates (N) for the Formoterol Fumarate and Budesonide peaks is not less than 2000.

The Tailing factor (T) for the Formoterol Fumarate and Budesonide peak is not morethan 2.0.

Observation:

The % RSD for the retention times and peak area of Formoterol Fumarate and Budesonide were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit.

Specificity

Preparation of blank solution. The Mobile phase, Potassium dihydrogen phosphatebuffer pH - 4.5) :Acetonitrile (30:70) was taken as blank solution.

Preparation of standard solution

The above prepared solutions were injected and the chromatograms were recorded for the same. The chromatogram for blank is shown figure 6.12.The chromatogram for the standard solution is given in figure 6.14 and the results of the chromatogram are given the chromatogram for the test sample i.e tablet sample is given and the resultsof the chromatogram are given in figure 6.13.

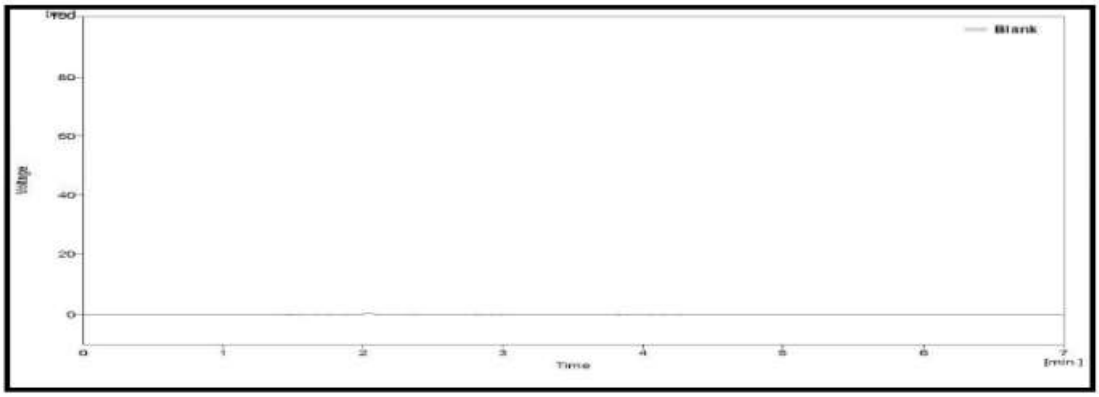


Fig. 1: Chromatogram of blank

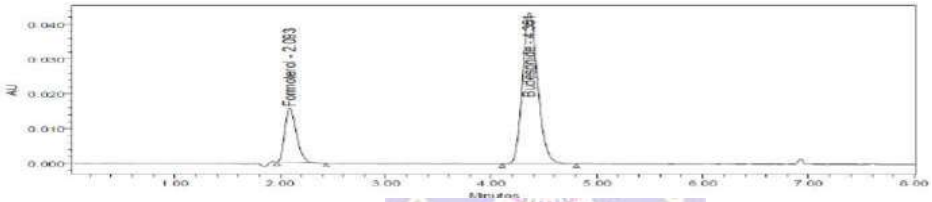


Fig. 2**:**Chromatogram for specificity of FF and BU sample

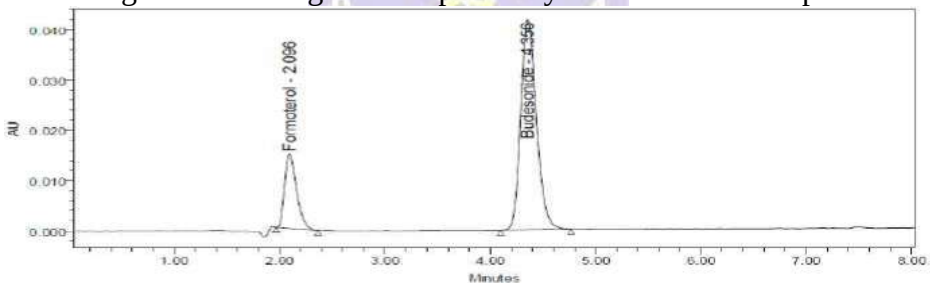


Fig. 6.14:Chromatogram for Specificity of FF and BUstandard

Table.1: For Specificity of Formoterol Fumarate and Budesonide sample

Drug	RT(min)	Peak Area	TF	Efficiency	Resolution
FF	2.093	120404	1.35	2729	-
BU	4.361	436416	1.19	4422	9.5

Table 2: Specificity of FormoterolFumarate and Budesonide standard

Drug	Retention time (min)	Peak Area	TF	Efficiency	Resolution
FF	2.096	116063	1.40	2572	
BU	4.356	428498	1.21	4211	9.2

Observation:

It was observed from the above data, diluent or excipient peaks are not interfering with the Formoterol Fumarate and Budesonide peaks.

Linearity and range

Preparation of standard stock solution Preparation of working standard solution

The working standard solution was prepared from the standard stock solution .The prepared working standard solutions were injected and the chromatograms were recorded for the same as shown in

figures.6.15-6.

From the obtained data, a graph between the concentration of the drug and peak area are plotted based on data given in figure .for Formoterol Fumarate and Budesonide respectively and the linearity graphs are given below

Table.3: Preparations for Linearity

Preparations	Volume from Standard stocktransferred in ml	Volume made up in ml (with diluent)	Concentration ofsolution(µg /ml)	
			FORMO	BUDESO
Preparation 1	0.1	10	10	15
Preparation 2	0.2	10	20	30
Preparation 3	0.3	10	30	45
Preparation 4	0.4	10	40	60
Preparation 5	0.5	10	50	75

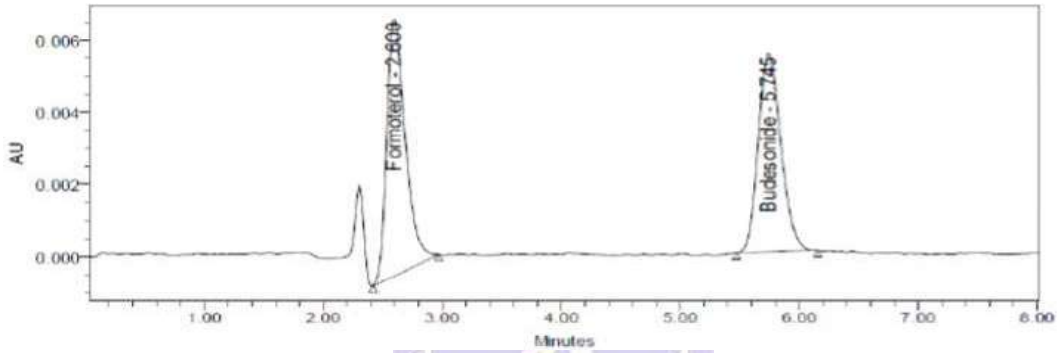


Fig. 3*:* Chromatogram of Formoterol Fumarate and Budesonide preparation 1

Table: 4: Results of preparation-1 for Linearity

Drugss	RT (min)	Peak Area	TF	Efficiency	Resolution
FF	2.600	76879	1.29	2353.34	-
B	5.745	72549	1.18	4293.09	9.75

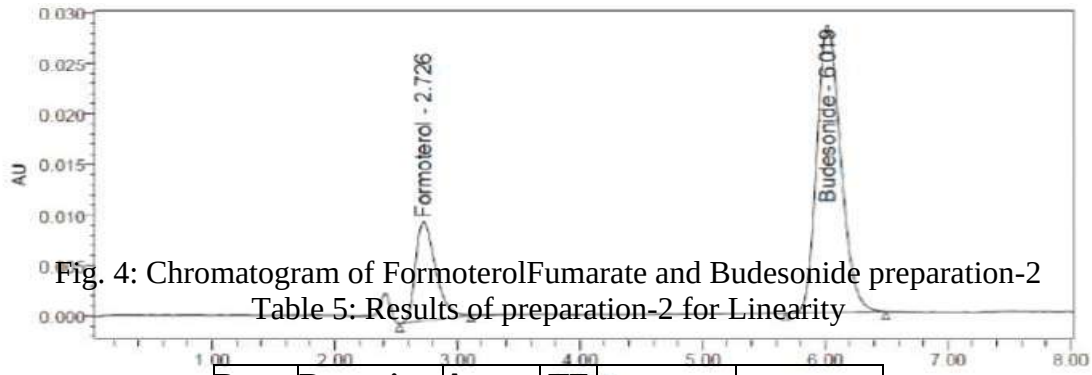


Fig. 4: Chromatogram of FormoterolFumarate and Budesonide preparation-2

Table 5: Results of preparation-2 for Linearity

Drugs	Retention time (min)	Area	TF	Efficiency	Resolution
FF	2.690	174699	1.36	2463.36	-
BU	5.891	578153	1.21	4398.06	9.89

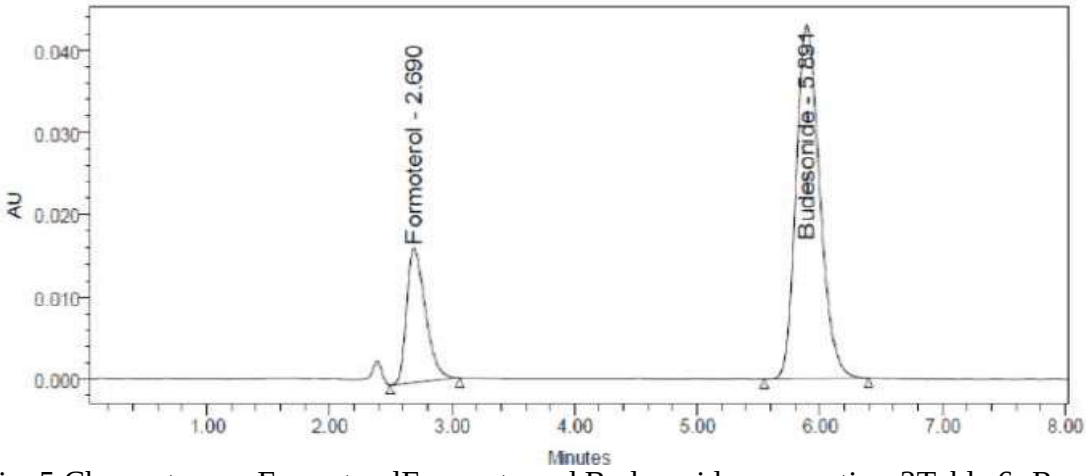


Fig. 5:ChromatogramFormoterolFumarate and Budesonide preparation-3Table 6*:* Results of preparation-3 for Linearity

Drug	RT (min)	Peak Area	TF	Efficiency	Resolution
FF	2.726	110214	1.33	2376.70	-
BU	6.019	384068	1.21	4557.69	9.94

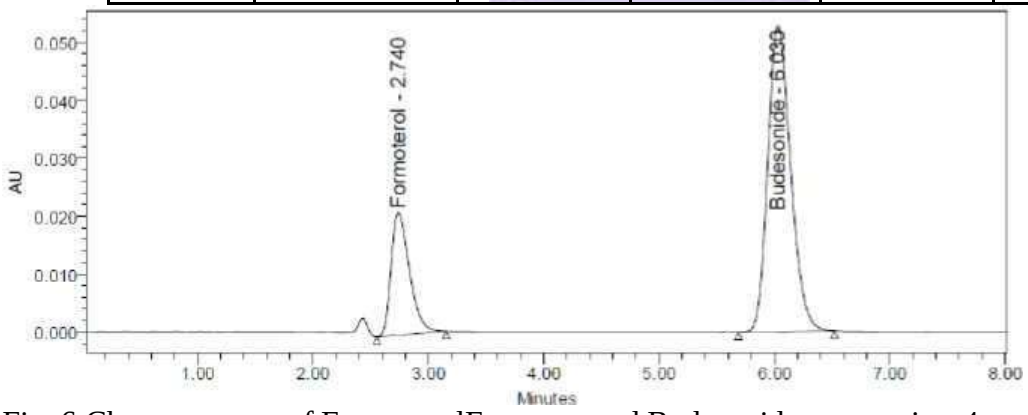


Fig. 6:Chromatogram of FormoterolFumarate and Budesonide preparation-4

Table. 6.22:Results of preparation-4 for Linearity

Drug	RT(min)	Area	TF	Efficiency	Resolution
FF	2.740	233585	1.39	2440.18	-
BU	6.030	726703	1.21	4407.77	9.86

Fig. 7:Chromatogram Formoterol Fumarate and Budesonide preparation-5

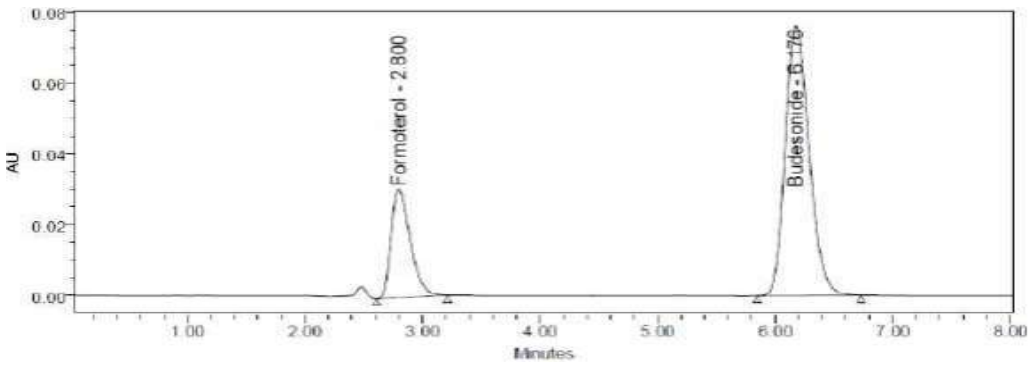
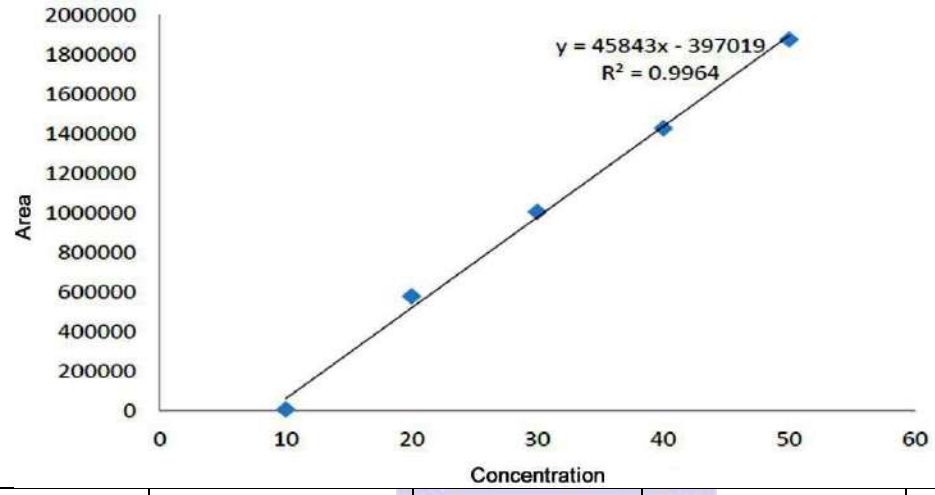


Table 7: Linearity data for preparation 5

Drug	RT (min)	Peak Area	TF	Efficiency	R esolution
FF	2.800	344428	1.40	2434.75	-
BU	6.176	1075106	1.22	4392.20	9.90

Table 8*:* Results Linearity data of Formoterol Fumarate and Budesonide



S.NO.	Conc(µg)	Area	Conc(µg)	Area
1.	15	76879	10	72549
2.	30	174699	20	578153
3.	45	110214	30	384068
4.	60	233585	40	726703
5.	75	344428	50	1075106

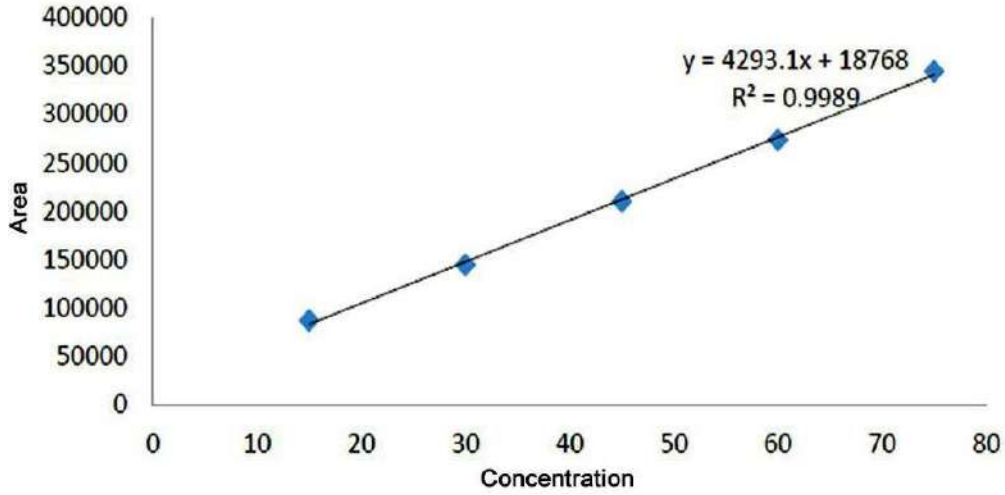


Fig.9:Linearity graph of Formoterol Fumarate

Fig.6.21*:* Linearity graph of Budesonide

Acceptance criteria

The relationship between the concentration (in %) of Formoterol Fumarate and Budesonide and area of Formoterol Fumarate and Budesonide should be linear in the specified range and the correlation should not be less than 0.99.

Observation

The correlation coefficient for linear curve obtained between concentration vs. Area for standard

preparations Formoterol Fumarate and Budesonide is 0.998 and 0.996. The relationship between the concentration of Formoterol Fumarate and Budesonide and area of Formoterol Fumarate and Budesonide is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre-analysed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%, 150%.

Preparation of standard stock solution (spikingPreparation of Test stock solution

Preparation of sample solutions

A. For preparation of 50% solution (with respect to target assayconcentration)

5 mg of budesonide and 5 mg of Formoterol working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with The same solvent (Stock Solution).Further pipette out 1 ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

B. For preparation of 100% solution (with respect to target assayconcentration)

10 mg of budesonide and 10 mg of formoterolworking standards were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1 ml of above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

C. For preparation of 150% solution (with respect to target assayconcentration)

15 mg of budesonide and 15 mg of formoterol working standards into a 10ml clean dry volumetric flask add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipetteout 1 ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark withdiluent.

Procedure

The standard solutions of accuracy 50%, 100% and 150%were injected into chromatographic system. Calculate the amount found and amount added for budesonide and Formoterol. Calculated the individual % recovery and mean % recovery values of each.

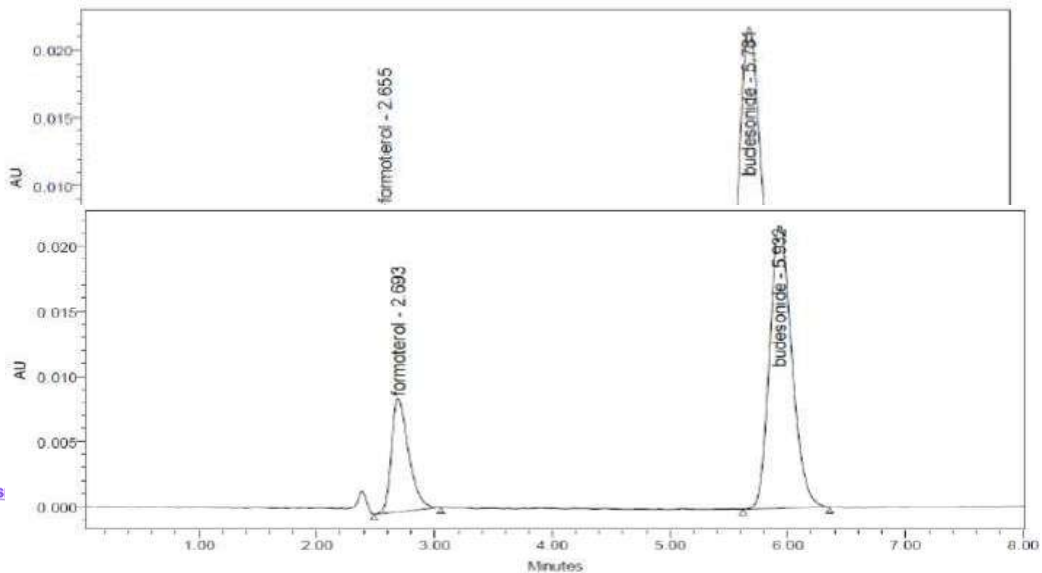
Procedure

The standard solutions of accuracy 50%, 100% and 150%were injected into chromatographic system. Calculate the amount found and amount added for budesonide and formoteroland calculate the individual % recovery and mean %recovery values.

For 50 % Recovery

Fig. 10: Chromatogram of 50% recovery – injection 1

Fig. 11: Chromatogram of 50% recovery – injection 2



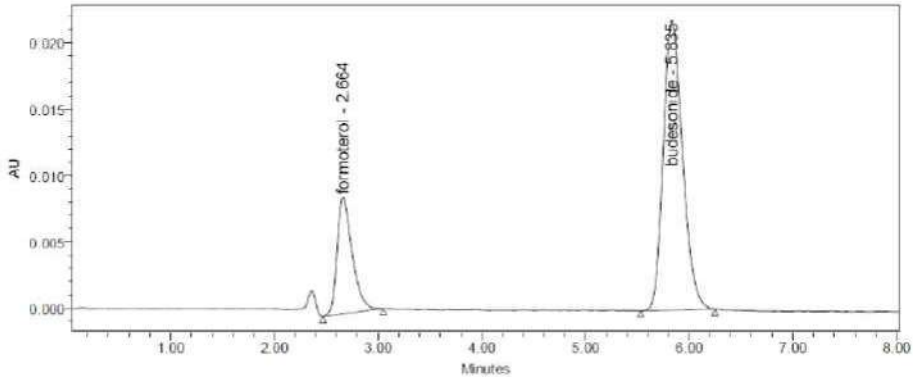


Fig.12: Chromatogram of 50% recovery – injection 3

Table 8:Results for 50% Recovery

Injection	FF		BU	
	RT	Area	RT	Area
1	2.655	87403	5.781	278081
2	2.693	88028	5.932	285613
3	2.664	88863	5.835	282085
Avg	2.6706	88098	5.849	281926.33

For 100 % Recovery

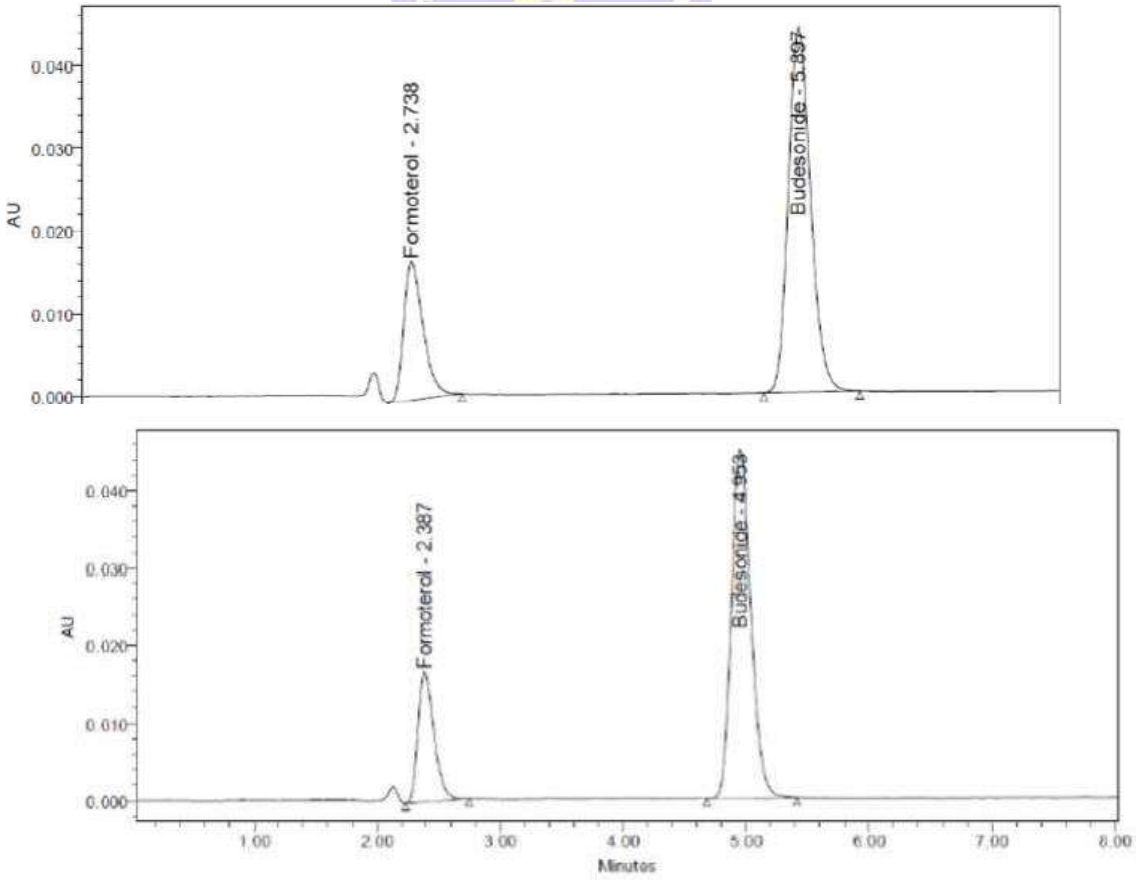


Fig. 13: Chromatogram of 100% recovery – injection 1

Fig. 14: Chromatogram of 100% recovery – injection 2

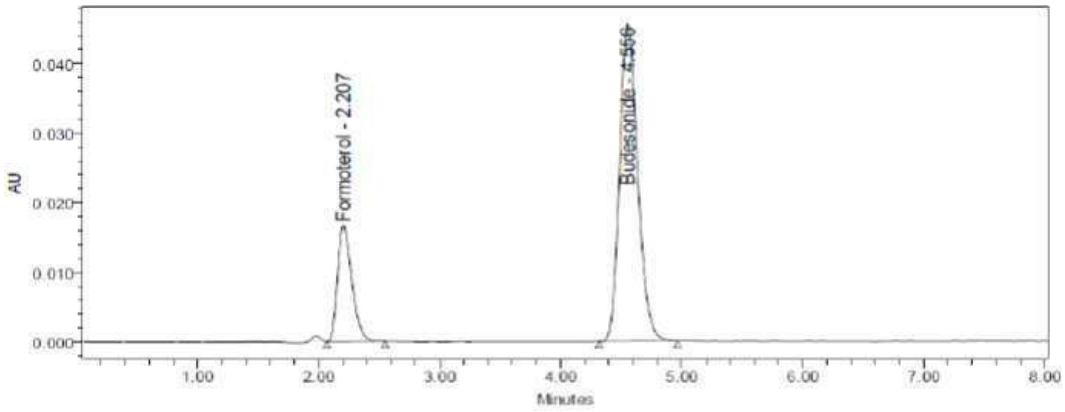


Fig. 15:Chromatogram of 100% recovery – injection 3Table 9: Results for 100% Recovery

		FF	BU	
Injection				
	RT	**Area**	**RT**	**Area**
1	2.738	183067	5.897	552361
2	2.387	151053	4.953	490353
3	2.207	136633	4.556	469094
Avg	2.444	156917.66	5.135	503939.33

For 150 % Recovery

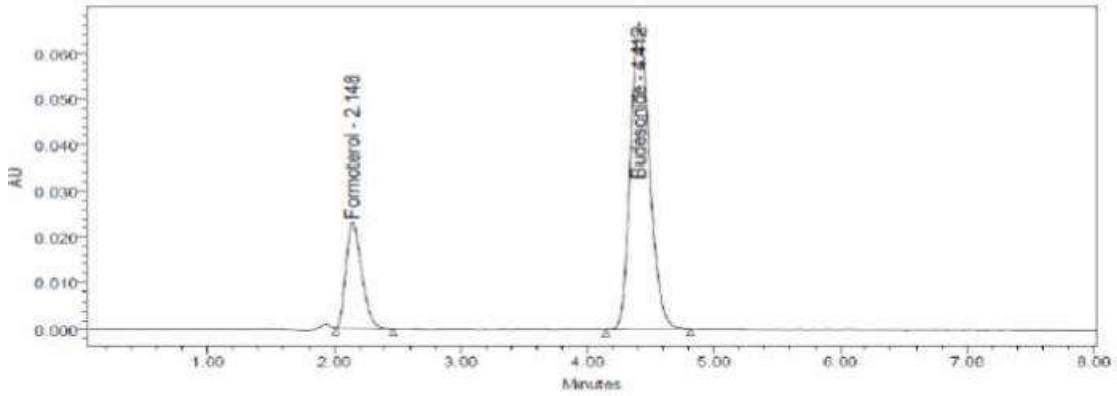


Fig.16 : Chromatogram of 150% recovery – injection 1

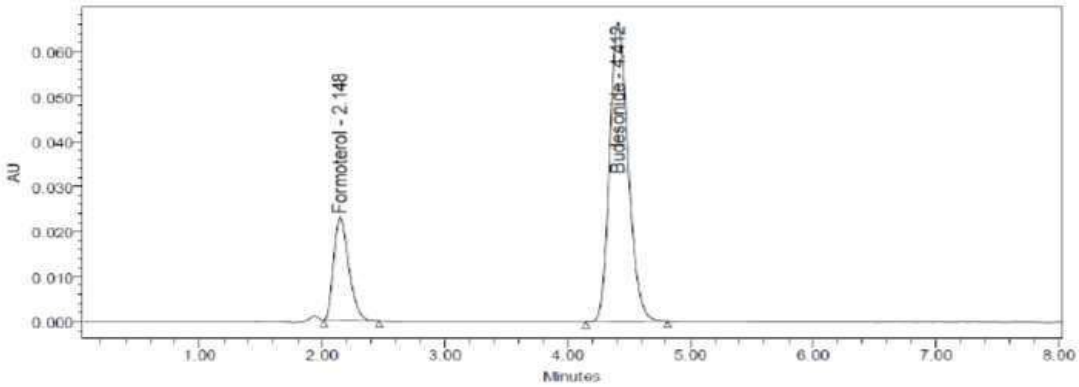


Fig. 17: Chromatogram of 150% recovery – injection 2

Fig.18: Chromatogram of 100% recovery – injection 3

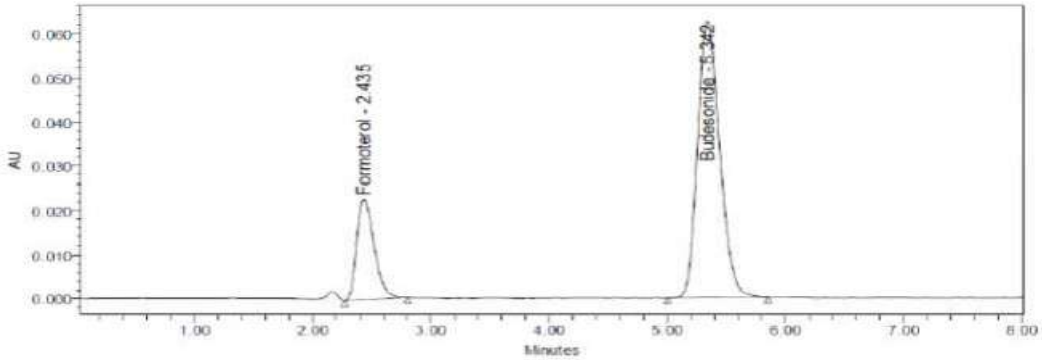


Table 10: Results for 150% Recovery

Injection	FF		BU	
	RT	Area	RT	Area
1	2.148	196994	4.412	697285
2	2.148	196994	4.412	697285
3	2.435	235736	5.342	815472
Avg	2.243	209908	4.722	736680.666

Table 11:Results for Recovery of Formoterol Fumarate

Concentration	Area	Amount added(µg / mL)	Amount found(µg/ mL)	% Recovery	% meanRecovery
50	88098	50(µg/ mL)	49.26	98.52	
100	156917.66	100(µg/ mL)	99.75	99.75	98.82
150	209908	150(µg/ mL)	147.38	98.2	

Table 12: Results for 150% Recovery

Concentration	Area	Amount added(µg/ mL)	Amount found(µg/ mL)	% Recovery	% mean Recovery
50	281926	50(µg/ mL)	48.75	97.5	
100	503939	100(µg/ mL)	102.25	102.5	99.6
150	736680	150(µg/ mL)	148.36	98.8	

Acceptance criteria

The % recovery of Formoterol Fumarate and Budesonide should lie between98% and 102%. The RSD of all the recovery values should not be more than 2.0%.

Observation

The percentage mean recovery of Formoterol Fumarate and Budesonide is and respectively and the results were found to be within the limits.

Precision

Precision was determined by analyzing standard preparation of Formoterol Fumarate(50µg,ml) and Budesonide (2.5µgml) for six times. The chromatograms were recorded and the results were summarized in Table 6.30.

Table 13: Results for Precision

Injection	Formoterol Fumarate		Budesonide	
	RT	Area	RT	Area
1	2.092	132443	4.327	436949
2	2.093	130445	4.330	435877
3	2.094	128713	4.331	431699
4	2.094	128211	4.332	432385
5	2.095	132105	4.333	433739
6	2.096	126517	4.333	435272
Average		**1297389**		**434319.9**
SD		**2331.2**		**2058.8**
%RSD		**1.8**		**0.5**

Acceptance criteria

The % Relative standard deviation of Peak area of Formoterol Fumarate andBudesonide from the six replicate injections should be not more than 2.0%

Observation

Test results for Formoterol Fumarate and Budesonide are showing that the %RSD ofAssay results are within limits. The results were shown in table 6.30.

Limit of Detection (LOD)

The LOD for this method was found to be 0.655µg forFormoterolFumarate and1.201µg for Budesonide

Limit of Quatification

The slope S may be estimated from the calibration curve of the analyte.

The LOQ for this method was found to be 1.9 µg for Formotero l Fumarate and 3.642 µg for Budesonide

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared standard solution as per test method and injected in 5 replicate at different variable conditions like using different conditions like flow rate and temperature, wavelength, mobile phase organic composition. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. System suitability parameters were compared with that of method precision.

Acceptance criteria

The system suitability should pass as per the test method at variable conditions.

- **Variation in flow**

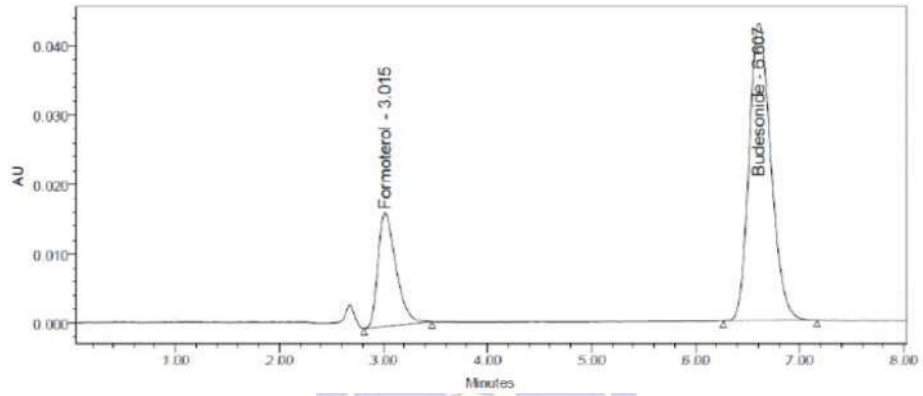


Fig. 19:Chromatogram of FF and BU for Robustness(Less flow 0.8 mL/min)

Table 14:Results of Formoterol Fumarate and Budesonide for Robustness(0.8ml/min)

Drug	RT(min)	Peak Area	TF	Efficiency	Resolution
FF	3.015	197661	1.40	2436.50	-
BU	6.607	625443	1.21	4740.46	10.08

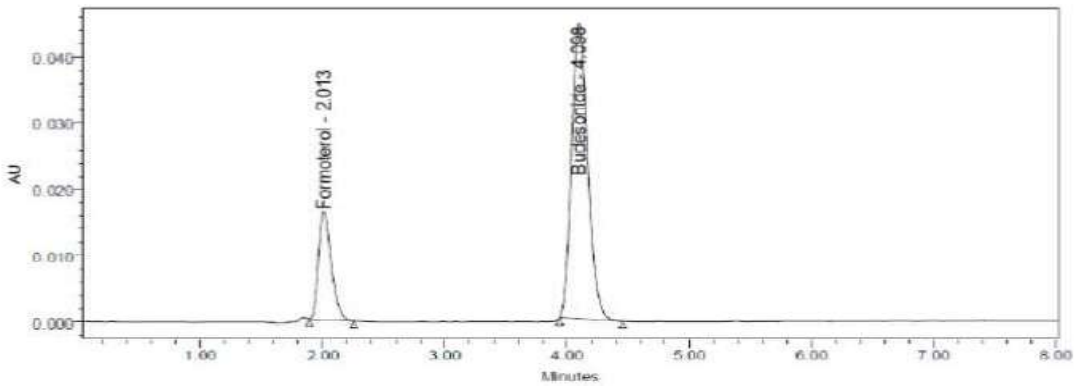


Fig. 20: Chromatogram of FormoterolFumarate and Budesonide for Robustness(More flow 1.2 mL/min)
Table 15: Results of FormoterolFumarate and Budesonide for Robustness (1.2mL/min)

Drug	RT (min)	Peak area	TF	Efficiency	Resolution
FF	2.013	118857	1.35	2750.35	--
BU	4.098	413383	1.21	44983	9031

- **Variation in Wave Length**

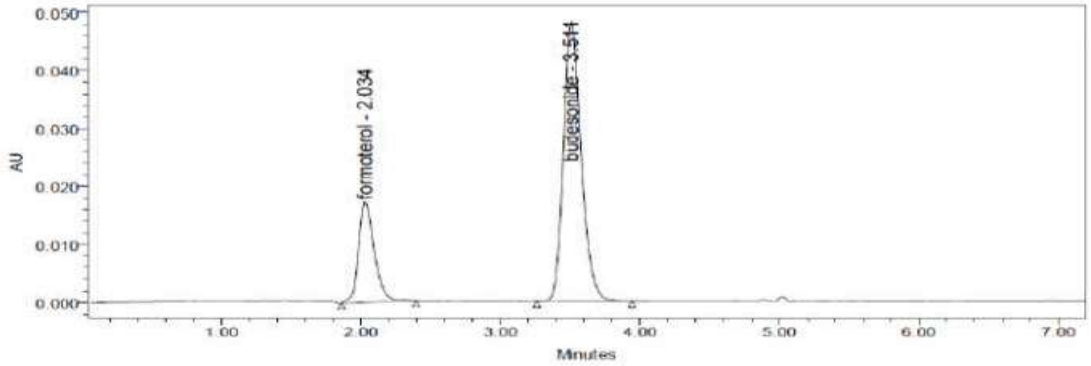


Fig. 21: Chromatogram ofFormoterolFumarate and Budesonide forRobustness (226nm)
Table 16: Results of Formoterol Fumarate and Budesonide for Robustness (226 nm**)**

Drug	RT (min)	Peak Area	TF	Efficiency	Resolution
FF	2.034	133805	1.19	1708	-
BU	3.511	483920	1.21	3721	6.7

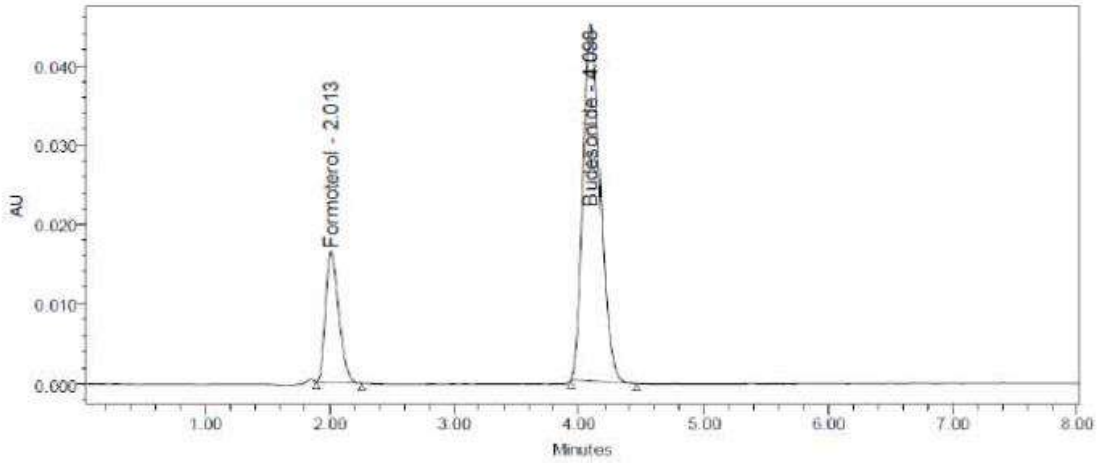


Fig.22:ChromatogramofFormoterolFumarate and Budesonide for Robustness (230nm)

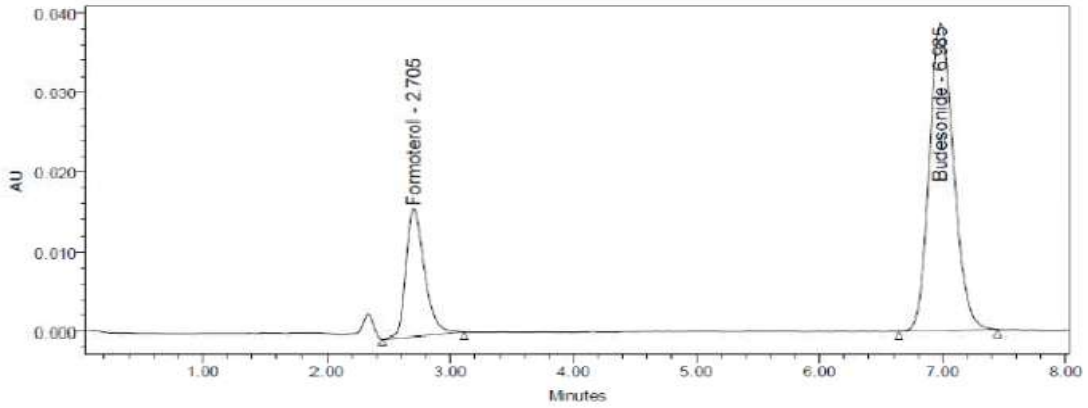


Table.17:Results of Formoterol Fumarate and Budesonide for Robustness (230nm)

Drug	RT (min)	Peak Area	TF	Efficiency	Resolution
FF	2.013	118857	1.35	2750.35	-
BU	4.098	413383	1.21	4454.13	9.31

Variation of mobile phase organic composition

Table 18:Results ofFormoterolFumarate and Budesonide for Robustness (lessorganic)

Drug	RT (min)	Peak Area	TF	Efficiency	Resolution -
FF	2.705	166234	1.28	1624.81	-
BU	6.985	527144	1.15	6001.97	13.38

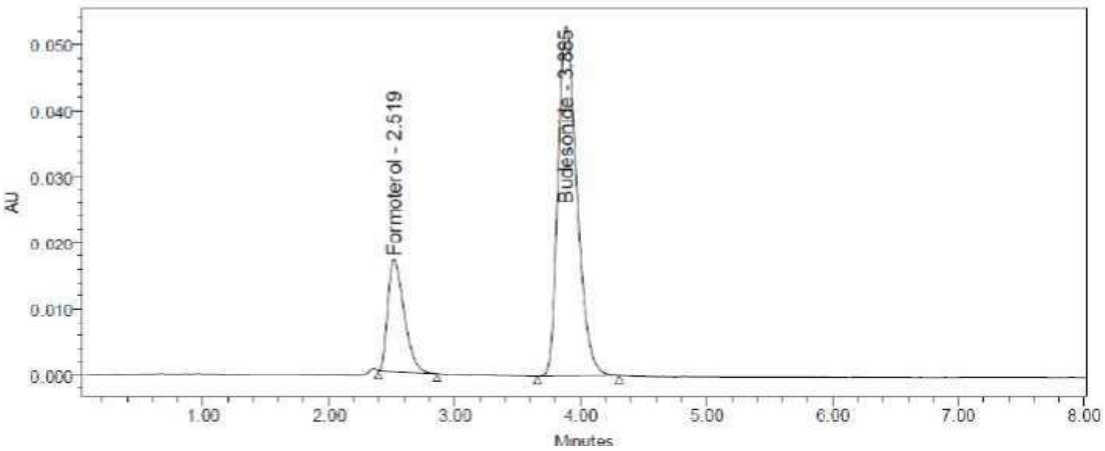


Fig. 23: Chromatogram of FormoterolFumarate and Budesonide forRobustness(MoreOrganic)

Conclusion:

present research work related to new method development of FormoterolFumarate and Budesonide was found satisfactory, simple, precise, accurate with good resolution, shorter retention time and among the other degradation products both FormoterolFumarate and Budesonide were well separated with all accurate results. Low limit of quantitation and limit of

detection makes this method suitable for use in quality control. The less retention time obtained for the both drugs which reduces the run time enhances the usage of this method.

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