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METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF BUDESONIDE, FORMOTEROL FUMARATE BY USING RP-HPLC

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Abstract:
A new, simple, precise, rapid, selective and stability reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the simultaneous quantification of Budesonide and Formoterol Fumarate in pure form and its pharmaceutical dosage form. The method is based on Phenomenex Gemini C18 (4.6×250mm) 5µ column. The separation is achieved using isocratic elution by Methanol: TEA Buffer in the ratio of 65:35% v/v, pumped at flow rate 1.0mL/min and UV detection at 230nm. The column is maintained at 40°C throughout the analysis. The total run time is about 6min. The method is validated for specificity, accuracy, precision and linearity, robustness and ruggedness, system suitability, limit of detection and limit of quantitation as per International conference of harmonization (ICH) Guidelines. The method is accurate and linear for quantification of Budesonide and Formoterol Fumarate between 10 - 50µg/mL and 20 - 100µg/mL respectively. Further, satisfactory results are also established in terms of mean percent- age recovery (100.37% for Budesonide and 100.34% for Formoterol Fumarate, intra-day and inter-day precision (<2%) and robustness. The advantages of this method are good resolution with sharper peaks and sufficient precision. The results indicate that the method is suitable for the routine quality control testing of marketed tablet formulations.

Keywords: *Budesonide and Formoterol Fumarate, RP-HPLC, ICH Guidelines, Accuracy, Precision.*

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INTRODUCTION:

Analytic method development and validation are key elements of any pharmaceutical development program. HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products.

Method development:

Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use" [1-2]. Understanding of the physical and chemical characteristics of drug allows one to select the most appropriate high performance liquid chromatography method development from the available vast literature. Information concerning the sample, for example, molecular mass, structure and functionality, pKa values and UV spectra, solubility of compound should be compiled. The requirement of removal of insoluble impurities by filtration, centrifugation, dilution or concentration to control the concentration, extraction (liquid or solid phase), derivatization for detection etc. should be checked. For pure compound, the sample solubility should be identified whether it is organic solvent soluble or water soluble, as this helps to select the best mobile phase and column to be used in HPLC method development.

Method development in HPLC can be laborious and time consuming. Chromatographers may spend many hours trying to optimize a separation on a column to accomplish the goals. Even among reversed phase columns, there is astonishing diversity, owing to differences in both base silica and bonded phase characteristics. Many of these show unique selectivity. What is needed is a more informed decision making process for column selection that may be used before the chromatographer enters the laboratory. The method of column selection presented here involves a minimal investment in time initially, with the potential of saving many hours in the laboratory.

Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods that support safety and characterization studies or evaluations of drug performance are also to be evaluated. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate the potential degradation of the API in the presence of formulation excipients [3, 4].

The three critical components for a HPLC method are: sample preparation (% organic, pH, shaking/sonication, sample size, sample age) analysis conditions (%organic, pH, flow rate, temperature, wavelength, and column age), and standardization (integration, wavelength, standard concentration, and response factor correction). During the preliminary method development stage, all individual components should be investigated before the final method optimization. This gives the scientist a chance to critically evaluate the method performance in each component and streamline the final method optimization [5]. The percentage of time spent on each stage is proposed to ensure the scientist will allocate sufficient time to different steps. In this approach, the three critical components for a HPLC method (sample preparation, HPLC analysis and standardization) will first be investigated individually [6-8].

The degraded drug samples obtained are subjected to preliminary chromatographic separation to study the number and types of degradation products formed under various conditions [9]. Scouting experiments are run and then conditions are chosen for further optimization [10]. Resolving power, specificity, and speed are key chromatographic method attributes to keep in mind during method development [11]. Selectivity can be manipulated by combination of different factors like solvent composition, type of stationary phase, mobile phase, buffers and pH. Changing solvents and stationary phases are the most comfortable approaches to achieve the separation. The proper range of pH is an important tool for separation of ionizable compounds. Acidic compounds are retained at low pH while basic compounds are more retained at higher pH. The neutral compounds remain unaffected. The pH range 4-8 is not generally employed because slight change in pH in this range would result in a dramatic shift in retention time. However, by operating at pH extremes (2-4 or 8-10), not only is there a 10-30 fold difference in retention time that can be exploited in method development but also the method can be made more robust which is a desirable outcome with validation

in minutes [12,13]. Various steps for HPLC method development are given below.

Requirements for good method development:

Choosing the appropriate HPLC column:

C_{18} columns are the commonly used columns in HPLC method analysis. C_8 or Octyl bonded phases are also used occasionally. Like C_{18}, they are non-polar, but not as hydrophobic. Therefore, retention times for hydrophobic compounds are typically shorter. Also, they may show somewhat different selectivity than C_{18} due to increased base silica exposure unique selectivity results in proton interaction of the bonded phase with electron deficient functional groups of solute molecules.

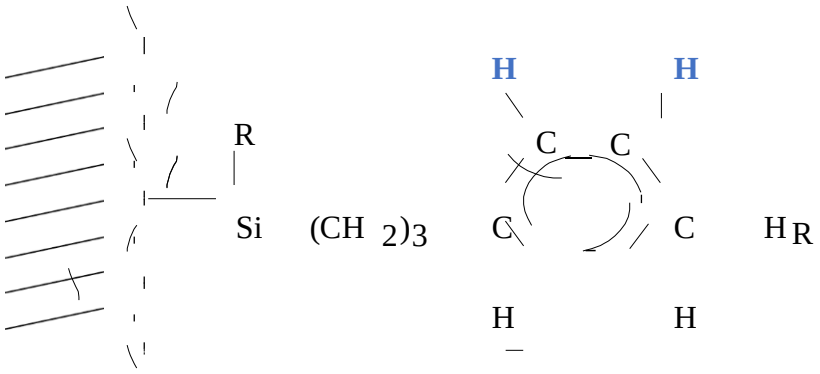

Figure: Chemical structure of C_{18}column

Retention is a mixed mechanism, resulting from both hydrophobic interactions and dipole interactions of the bonded phase C□N group with solute amino groups or p - p interactions with sites of unsaturation. It is the best for polar organic compounds and is versatile enough for use in both normal and reversed phase modes.

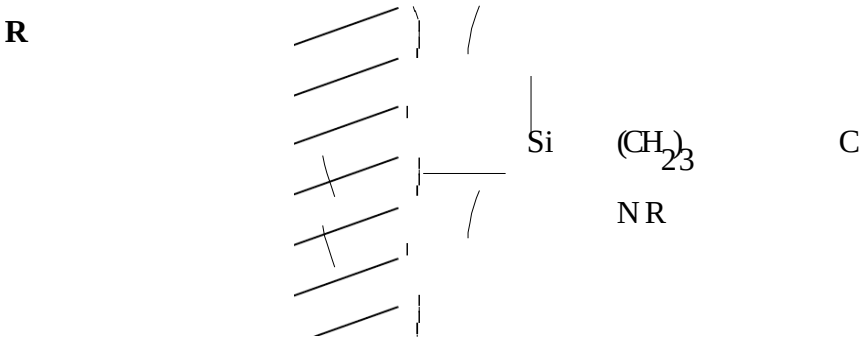

Figure: Chemical structure of -CN column

Each bonded phase has unique selectivity for certain sample types. For example: to separate toluene and ethyl benzene (differ by only one -CH_2- unit), we would choose a C18 bonded phase. Further, we would want to narrow the decision to a particular packing material that shows good or excellent retention of such hydrophobic compounds (i.e. high % carbon load) to be able to maximize the particular separation. The effects of surface area and carbon load are discussed in the next section. The stationary phase must be able to "hold on" to the two compounds long enough to resolve them by differential migration.

Table: Physical properties of default and optimum columns

	Default Column	Optimum Column
Column Bed Dimensions	150 x 4.6mm	250 x 4.6mm
Particle Size	5µm	3* or 5µm
Surface Area	2 200m /g	2 >200m /g
Pore Size	100Å	100Å
Carbon Load	10%	16 - 20%
Bonding Type	Monomeric	Mono- or Polymeric
Base Material	Silica	Silica
Particle Shape	Spherical	Spherical

MATERIALS AND METHODS:

Chemical-Brand Names,Budesonid-Sura labs, Formoterol Fumarate-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck.

Hplc method development:

Trails :

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Budesonide and Formoterol Fumarate working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Budesonide and 0.3ml of the Formoterol Fumarate stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate Buffer in proportion 45:55 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Phenomenex Luna C18 (4.6×250mm, 5µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 40ºC
Column : Phenomenex Gemini C18 (4.6×250mm) 5µ
Buffer : Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.
pH : 4.6
Mobile phase : Methanol: TEA Buffer (65:35 v/v)
Flow rate : 1ml/min
Wavelength : 230nm
Injection volume : 10 µl
Run time : 6 min

Validation

Preparation of buffer and mobile phase:

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-4.6):

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of mobile phase:
Accurately measured 450 ml (45%) of Methanol, 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.
Diluent Preparation:
The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION:

Optimized Chromatogram (Standard)

Mobile phase ratio : Methanol: TEA Buffer (65:35 v/v)
Column : Phenomenex Gemini C18 (4.6×250mm) 5µ
Column temperature : 40ºC
Wavelength : 230nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 6minutes

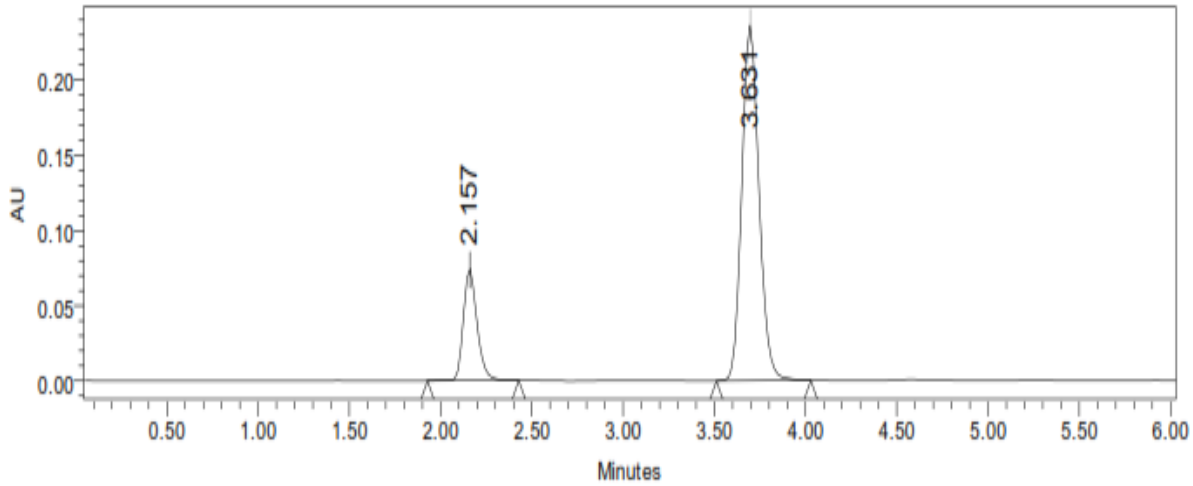

Figure-: Optimized Chromatogram (Standard)

Table-: Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Budesonide	2.157	526540	78565	1.63	5850	
2	Formoterol Fumarate	3.631	1645876	265841	1.49	7966	9.0

Observation: From the above chromatogram it was observed that the Budesonide and Formoterol Fumarate peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.
Optimized Chromatogram (Sample)

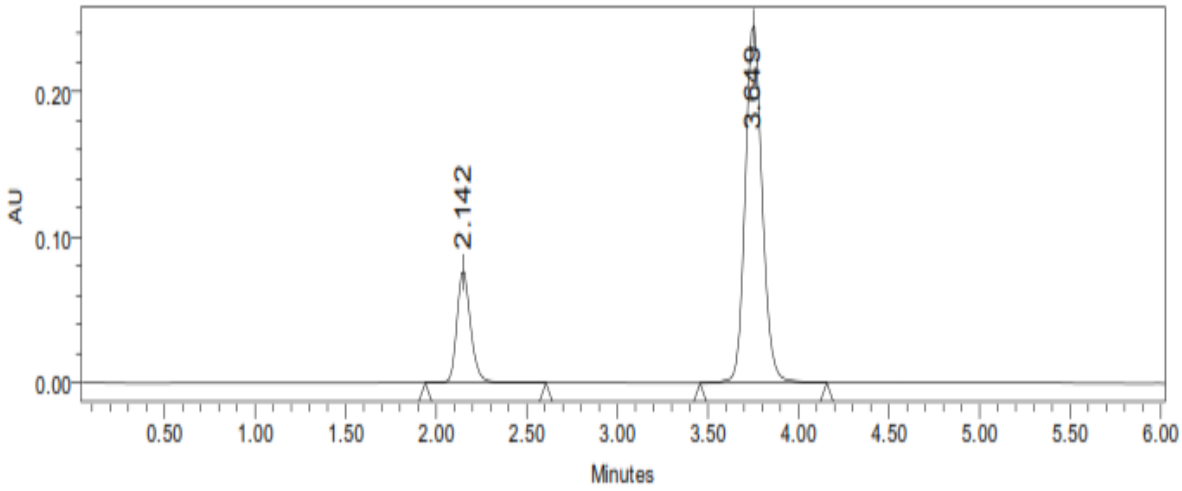

Figure-: Optimized Chromatogram (Sample)

Table: Optimized Chromatogram (Sample)

S.No.	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Budesonide	2.142	538955	7965	1.64	5985	
2	Formoterol Fumarate	3.649	1658746	275855	1.40	8055	10.2

Acceptance Criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.

System Suitability:

Table-: Results of system suitability for Budesonide

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Budesonide	2.152	526855	78560	1.64	5855
2	Budesonide	2.157	528795	78546	1.64	5873
3	Budesonide	2.141	526599	78955	1.64	5860
4	Budesonide	2.133	524876	78225	1.64	5898
5	Budesonide	2.166	526585	78966	1.61	5828
Mean			526741.5			
Std. Dev.			1392.399			
% RSD			0.264341			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table-: Results of system suitability for Formoterol Fumarate

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Formoterol Fumarate	3.674	1645986	268543	5868	1.47	10.02
2	Formoterol Fumarate	3.631	1648570	267855	5876	1.48	10.00
3	Formoterol Fumarate	3.625	1645730	268599	5863	1.46	9.98
4	Formoterol Fumarate	3.692	1645286	268746	5825	1.47	10.02
5	Formoterol Fumarate	3.629	1648599	268599	5825	1.45	10.03
Mean			1646838				
Std. Dev.			1618.326				
% RSD			0.098268				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Standard):

Table-: Peak results for assay standard of Budesonide

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Budesonide	2.152	526596	78568	1.64	5895	1
2	Budesonide	2.198	524657	78495	1.65	5878	2
3	Budesonide	2.179	528475	78458	1.61	5896	3

Table-: Peak results for assay standard of Formoterol Fumarate

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Formoterol Fumarate	3.646	1648545	265844	1.47	8013	1
2	Formoterol Fumarate	3.604	1648599	265419	1.48	7956	2
3	Formoterol Fumarate	3.610	1648576	265366	1.49	7988	3

Assay sample:

Table-: Peak results for Assay sample of Budesonide

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Budesonide	2.152	536597	79858	1.65	5968	1
2	Budesonide	2.150	536588	79266	1.66	5998	2
3	Budesonide	2.187	534659	79897	1.66	5987	3

Table-: Peak results for Assay sample of Formoterol Fumarate

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Formoterol Fumarate	3.646	1658953	278597	1.48	8015	1
2	Formoterol Fumarate	3.651	1658955	276985	1.47	8042	2
3	Formoterol Fumarate	3.601	1653658	275848	1.48	807	3

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Budesonide and Formoterol Fumaratein pharmaceutical dosage form was found to be 99.63%.

Linearity:

Chromatographic data for linearity study of budesonide:

Concentration µg/ml	Average Peak Area
10	185689
20	349852
30	521541
40	685986
50	848265

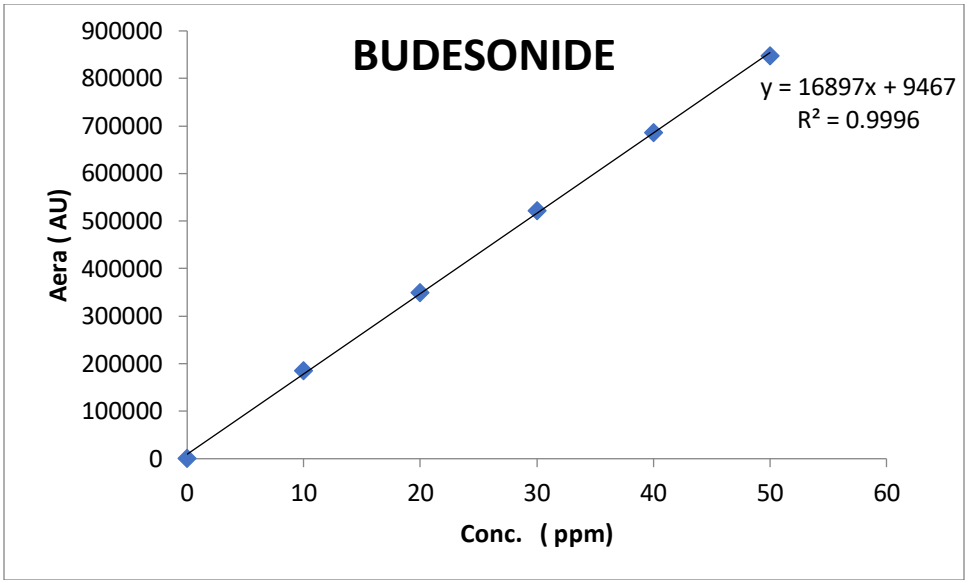

Fig-: Calibration Curve of Budesonide

Chromatographic data for linearity study of formoterol fumarate:

Concentration µg/ml	Average Peak Area
20	665985
40	1298698
60	1927852
80	2548545
100	3162468

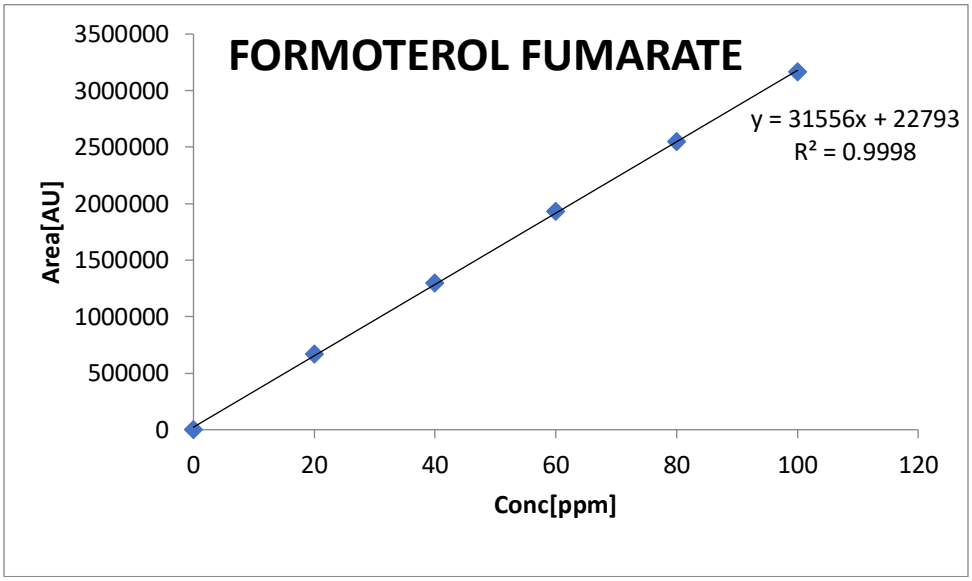

Fig-: Calibration Curve of Formoterol Fumarate

Repeatability:

Table-: Results of Repeatability for Budesonide:

S. No.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Budesonide	2.157	526855	78568	5868	1.63
2	Budesonide	2.159	523658	78468	5875	1.62
3	Budesonide	2.186	523855	78526	5897	1.63
4	Budesonide	2.160	523484	78547	5817	1.63
5	Budesonide	2.170	523484	78595	5870	1.64
Mean			524267.9			
Std.dev			1453.804			
%RSD			0.277303			

Acceptance Criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table-: Results of repeatability for Formoterol Fumarate:

S. No.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Formoterol Fumarate	3.603	1645878	265844	7986	5868
2	Formoterol Fumarate	3.608	1648579	265488	7965	5848
3	Formoterol Fumarate	3.600	1645984	265987	7914	5876
4	Formoterol Fumarate	3.696	1648758	265479	7926	5875
5	Formoterol Fumarate	3.629	1648573	265423	7963	5828
Mean			1647556			
Std.dev			1483.604			
%RSD			0.090040			

Intermediate precision:

Table-: Results of Intermediate precision for Budesonide

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Budesonide	2.198	536597	79585	5964	1.65
2	Budesonide	2.196	536986	79686	5979	1.64
3	Budesonide	2.160	534588	79655	5948	1.65
4	Budesonide	2.160	536984	79844	5983	1.64
5	Budesonide	2.160	536986	79863	5970	1.66
6	Budesonide	2.186	538567	79686	5969	1.65
Mean			536784.6			
Std. Dev.			1277.908			
% RSD			0.238066			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Table-: Results of Intermediate precision for Formoterol Fumarate

S.No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Formoterol Fumarate	3.623	1658255	266599	8035	1.59	10.05
2	Formoterol Fumarate	3.611	1659873	266472	8044	1.52	10.05
3	Formoterol Fumarate	3.696	1653588	266959	8076	1.59	10.06
4	Formoterol Fumarate	3.696	1658459	266450	8048	1.51	10.07
5	Formoterol Fumarate	3.696	1653653	266354	8068	1.59	10.04
6	Formoterol Fumarate	3.642	1652394	266955	8025	1.52	10.08
Mean			1656036				
Std. Dev.			3175.804				
% RSD			0.191770				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Table-: Results of Intermediate precision Day 2 for Budesonide

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Budesonide	2.198	519688	77858	5748	1.60
2	Budesonide	2.196	518956	77984	5791	1.61
3	Budesonide	2.178	519857	77855	5747	1.62
4	Budesonide	2.142	519856	77868	5748	1.62
5	Budesonide	2.177	519868	77934	5717	1.60
6	Budesonide	2.177	519688	77953	5794	1.69
Mean			519652.6			
Std. Dev.			351.0977			
% RSD			0.067563			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Table-: Results of Intermediate precision Day 2 for Formoterol Fumarate

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Formoterol Fumarate	3.611	1638599	256984	7967	1.46	9.91
2	Formoterol Fumarate	3.623	1637848	257588	7951	1.47	9.92
3	Formoterol Fumarate	3.684	1635983	256984	7933	1.45	9.91
4	Formoterol Fumarate	3.697	1636597	254612	7985	1.48	9.91
5	Formoterol Fumarate	3.684	1635873	258488	7923	1.49	9.90
6	Formoterol Fumarate	3.684	1635983	259860	7914	1.48	9.90
Mean			1636815				
Std. Dev.			1145.884				
% RSD			0.070008				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Accuracy:

Table-: The accuracy results for Budesonide

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	263571	14	15.037	100.254%	100.38%
100%	518870.4	31	30.148	100.491%	
150%	772572.4	46	45.161	100.362%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

Table-: The accuracy results for Formoterol Fumarate

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	972935.8	31	30.101	100.364%	100.35%
100%	1919311	61	60.101	100.165%	
150%	2877021	91	90.448	100.499%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table-: Results for Robustness

Budesonide

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	526542	2.158	5858	1.61
Less Flow rate of 0.9 mL/min	589565	2.211	5634	1.62
More Flow rate of 1.1 mL/min	515244	2.185	5568	1.63
Less organic phase	502658	2.201	5155	1.64
More Organic phase	526486	2.171	5364	1.63

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Formoterol Fumarate

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1645874	3.642	7964	1.44
Less Flow rate of 0.9 mL/min	1635983	4.497	7855	1.45
More Flow rate of 1.1 mL/min	1624589	3.504	7424	1.44
Less organic phase	1652835	4.503	7620	1.46
More organic phase	1625543	3.511	7581	1.41

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Budesonide and Formoterol Fumarate in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps.

Budesonide was found to be soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide; it is very slightly soluble in water, slightly soluble in Acetonitrile and ethanol, sparingly soluble in methanol, practically insoluble in toluene.

Formoterol Fumarate was found to be very slightly soluble in water (0.9 mg/mL). Formoterol Fumarate is soluble in methanol (ca. 60 mg/mL), sparingly soluble in ethanol (ca. 10 mg/mL), very slightly soluble in isopropanol (<1 mg/mL), and very slightly soluble in acetone.

Methanol: TEA Buffer (65:35 v/v) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Budesonide and Formoterol Fumarate in bulk drug and in pharmaceutical dosage forms.

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