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NEWER RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF FORMOTEROL FUMARATE AND BUDESONIDE IN DOSAGE FORM

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Abstract:

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validation of Formoterol Fumarate and Budesonide, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Gemini C18 (4.6×250mm) 5µ column using a mixture of Methanol: TEA Buffer (65:35 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 230nm. The retention time of the Formoterol Fumarate and Budesonide was 2.121, 3.643 ±0.02min respectively. The method produce linear responses in the concentration range of 10-50mg/ml of Formoterol Fumarate and 20-100mg/ml of Budesonide. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: *Formoterol Fumarate, Budesonide, RP-HPLC, validation.*

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INTRODUCTION[1]:

Formoterol oral inhalation is used to control wheezing, shortness of breath, and chest tightness caused by chronic obstructive pulmonary disease. Formoterol (FF), N-[2-hydroxy-5-[(1 R)-1-hydroxy-2-[[(2 R)-1-(4-methoxyphenyl)propan-2-yl]amino]ethyl]phenyl]formamide, is a long-acting β2-adrenoceptor agonist[1] A new combination dosage form containing formoterol fumarate and budesonide is indicated for the treatment of asthma and chronic obstructive pulmonary disease COPD[2,3]

It is commonly used by inhalation to treat asthma, intranasal for allergic rhinitis or by oral administration to treat inflammatory bowel disease. The high potency is due to its high affinity to the steroid receptor and rapid conversion to metabolites with minimal or no steroid activity.[4]

Inhalation of the two drugs as one dose in combination inhalers has been shown to be more clinically effective.[5]

MATERIALS AND METHODS[2]:

Formoterol Fumarate from Sura labs, Budesonide from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck.

HPLC METHOD DEVELOPMENT:[3]

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Formoterol Fumarate and Budesonide 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 0.3 ml of Formoterol Fumarate and 0.6ml of Budesonide from the above 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, Methanol: Phosphate buffer and ACN: Water with varying proportions. Finally, the mobile phase was optimized to TEA buffer (pH 4.0), Methanol in proportion 65:35 v/v respectively.

Optimization of Column:

The method was performed with various C18columns like Symmetry, X terra and ODS column. Phenomenex Gemini C18 (4.6×250mm) 5µ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:[4]

Instrument used	:	Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature	:	40ºC
Column	:	Phenomenex Gemini C18 (4.6×250mm) 5µ
Mobile phase	:	Methanol: TEA Buffer (65:35 v/v)
Flow rate	:	1ml/min
Wavelength	:	230nm
Injection volume	:	10µl
Run time	:	6minutes

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE[5]:

Preparation of Triethylamine buffer (pH-4.0):

Take 6.0ml of Triethylamine in to 750ml of HPLC water in a 1000ml volumetric flask and mix well. Make up the volume up to mark with water and adjust the pH to 4.0 by using Orthophosphoric acid, filter and sonicate.

Preparation of mobile phase:

Accurately measured 350 ml (35%) of TEA buffer and 650 ml of HPLC Methanol (65%) were mixed and degassed in a digital ultrasonicater for 10 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[6]:

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

Table 1: Optimized Chromatogram (Standard)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Formoterol Fumarate	2.121	406433	77644	1.2	4009	
2	Budesonide	3.643	1592811	251532	1.1	7849	9.8

Optimized Chromatogram

Table 2: Optimized Chromatogram (Sample)

S.no	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Formoterol Fumarate	2.142	403871	77464	1.2	4136	
2	Budesonide	3.649	1573821	259361	1.1	7812	10.3

System suitability:

Table 3: Results of system suitability for Formoterol Fumarate

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Formoterol Fumarate	2.152	382726	70725	5271	1.2
2	Formoterol Fumarate	2.157	382621	70625	5928	1.2
3	Formoterol Fumarate	2.141	389172	70617	5283	1.2
4	Formoterol Fumarate	2.133	384152	70718	5763	1.2
5	Formoterol Fumarate	2.166	389721	70172	6222	1.2
Mean			385678.4			
Std. Dev.			3497.932			
% RSD			0.906956			

Table 4: Results of system suitability for Budesonide

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Budesonide	3.674	1562821	227365	5827	1.1	10.1
2	Budesonide	3.631	1562726	226748	6183	1.1	10.1
3	Budesonide	3.625	1567361	227163	5029	1.1	10.1
4	Budesonide	3.692	1562811	226948	4920	1.1	10.1
5	Budesonide	3.629	1563816	226452	5183	1.1	10.1
Mean			1563907				
Std. Dev.			1982.03				
% RSD			0.126736				

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Assay (Standard):

Table 5: Peak results for assay standard of Formoterol Fumarate

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Formoterol Fumarate	2.152	406538	77074	1.2	4009	1
2	Formoterol Fumarate	2.198	409975	76001	1.2	4136	2
3	Formoterol Fumarate	2.179	402283	76048	1.2	5263	3

Table 6: Peak results for assay standard of Budesonide

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Budesonide	3.646	1609924	251956	1.1	7849	1
2	Budesonide	3.604	1601840	246020	1.1	7819	2
3	Budesonide	3.610	1602832	248287	1.1	7826	3

Assay (Sample):

Table 7: Peak results for Assay sample of Formoterol Fumarate

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Formoterol Fumarate	2.152	406538	77074	1.2	4009	1
2	Formoterol Fumarate	2.150	409975	76001	1.2	4136	2
3	Formoterol Fumarate	2.187	402911	77823	1.2	5173	3

Table 8: Peak results for Assay sample of Budesonide

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Budesonide	3.646	1609924	251956	1.1	7849	1

2	Budesonide	3.651	1601840	246020	1.1	7819	2
3	Budesonide	3.601	1603821	240291	1.1	6812	3

%ASSAY =

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

=1605195 /1604865 ×10/60×60/0.0254×99.5/100×0.0382/15×100

= 99.7%

The % purity of Formoterol Fumarate and Budesonide in pharmaceutical dosage form was found to be 99.7%

LINEARITY

Table 9: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY OF FORMOTEROL FUMARATE:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	10	135005
66	20	277120
100	30	405128
133	40	534643
166	50	672357

Table 10: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY OF BUDESONIDE:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	20	469094
66	40	1149397
100	60	1657592
133	80	2150412
166	100	2748444

REPEATABILITY

Table 11: Results of repeatability for Formoterol Fumarate:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing	%Assay
1	Formoterol Fumarate	2.157	400459	70717	1.2	4987	99%
2	Formoterol Fumarate	2.159	402118	71819	1.2	5019	99.4%
3	Formoterol Fumarate	2.186	405412	73930	1.2	5126	100%
4	Formoterol Fumarate	2.160	406506	73333	1.3	4999	100%
5	Formoterol Fumarate	2.170	407673	72623	1.2	5214	100%
Mean			404433.6				
Std.dev			2716.809				
%RSD			0.671757				

Table 12: Results of repeatability for Budesonide:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing	%Assay
1	Budesonide	3.603	1617864	226985	1.1	7045	98.7%
2	Budesonide	3.608	1618493	234764	1.1	7399	98.8%
3	Budesonide	3.600	1628262	227712	1.2	7159	99.4%
4	Budesonide	3.696	1615796	235459	1.1	7896	98.6%
5	Budesonide	3.629	1619626	242158	1.1	7965	98.8%
Mean			1620008				
Std.dev			4310.623				
%RSD			0.266086				

Intermediate precision:

Table 13: Results of Intermediate precision Day 1 for Formoterol Fumarate

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	%Assay
1	Formoterol Fumarate	2.198	405262	70572	5672	1.2	100%
2	Formoterol Fumarate	2.196	405637	70516	5639	1.2	100%
3	Formoterol Fumarate	2.160	405628	70572	6183	1.2	100%
4	Formoterol Fumarate	2.160	405647	70372	5923	1.2	100%
5	Formoterol Fumarate	2.160	405948	70592	6739	1.2	100%
6	Formoterol Fumarate	2.186	408732	70526	5837	1.2	100%
Mean			406142.3				
Std. Dev.			1287.197				
% RSD			0.316933				

Table 14: Results of Intermediate precision Day 1 for Budesonide

S.No	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution	%Assay
1	Budesonide	3.623	1608292	235473	5372	1.1	10.1	98%
2	Budesonide	3.611	1609283	235938	5927	1.1	10.1	98.2%
3	Budesonide	3.696	1617836	235738	6129	1.1	10.1	98.7%
4	Budesonide	3.696	1619743	235963	5284	1.1	10.1	99.7%
5	Budesonide	3.696	1614262	231938	5284	1.1	10.1	98.5%
6	Budesonide	3.642	1608471	235948	6347	1.1	10.1	98.2%
Mean			1611315					
Std. Dev.			6077.093					
% RSD			0.377151					

Table 15: Results of Intermediate precision Day 2 for Formoterol Fumarate

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	%Assay
1	Formoterol Fumarate	2.198	405423	70572	5672	1.2	100%
2	Formoterol Fumarate	2.196	405927	70516	5639	1.2	100%
3	Formoterol Fumarate	2.178	405029	70572	6183	1.2	100%
4	Formoterol Fumarate	2.142	405432	70372	5923	1.2	100%
5	Formoterol Fumarate	2.177	405062	70592	6739	1.2	100%
6	Formoterol Fumarate	2.177	408417	70526	5837	1.2	101%
Mean			405881.7				
Std. Dev.			1283.857				
% RSD			0.316313				

Table 16: Results of Intermediate precision Day 2 for Budesonide

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution	%Assay
1	Budesonide	3.611	1638732	244384	5363	1.1	10.1	100%
2	Budesonide	3.623	1637438	235827	6282	1.1	10.1	100%
3	Budesonide	3.684	1638474	236382	5938	1.1	10.1	100%
4	Budesonide	3.697	1634273	239183	6194	1.1	10.1	99.7%
5	Budesonide	3.684	1636372	231931	5402	1.1	10.1	99.8%
6	Budesonide	3.684	1639283	234356	5837	1.1	10.1	100%
Mean			1637429					
Std. Dev.			1860.366					
% RSD			0.113615					

ACCURACY:

Table 17: The accuracy results for Formoterol Fumarate

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	201472.3	15	14.8	98.6	99.7%
100%	406193	30	30.1	100.3	
150%	607144	45	45.1	100.2	

Table 18: The accuracy results for Budesonide

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	826527.7	30	30.5	101.6	99.6%
100%	1622241	6	59.4	99	
150%	2422702	90	88.4	98.2	

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

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

FORMOTEROL FUMARATE

Result: = 3.3×4269.822/13396

= 1.05µg/ml

BUDESONIDE

Result: =3.3×57796.93/27563

= 6.9µg/ml

QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

FORMOTEROL FUMARATE

Result: =10×4269.822/13396

=3.1µg/ml

BUDESONIDE

Result: =10×57796.93/27563

=20.9µg/ml

Robustness

Table 19: Results for Robustness Formoterol Fumarate

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	406433	2.121	4009	1.2
Less Flow rate of 0.9 mL/min	398841	2.210	3800.8	0.9
More Flow rate of 1.1 mL/min	389947	2.184	4800.8	
Less organic phase	413898	2.200	4890.8	0.9
More Organic phase	389578	2.172	4190.8	0.7

Table 20: 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	1592811	3.643	7849	1.1
Less Flow rate of 0.9 mL/min	1613422	4.498	3312.2	0.9
More Flow rate of 1.1 mL/min	1619138	3.505	4312.2	0.8
Less organic phase	1616104	4.504	4392.2	0.9
More organic phase	1623185	3.512	4292.2	0.9

Acceptance criteria:

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

CONCLUSION[7]:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Formoterol Fumarate and Budesonide in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Formoterol Fumarate and Budesonide are freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Triethylamine Buffer 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 Formoterol Fumarate and Budesonide in bulk drug and in Pharmaceutical dosage forms.

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