



**ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF FORMOTEROL
FUMARATE AND BUDESONIDE IN PRESSURISED METER DOSE INHALER FORM
BY USING RP-HPLC**

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ABSTRACT

A reversed-phase-liquid chromatographic (RP-HPLC) method was developed for the simultaneous estimation of formoterol fumarate and budesonide in pressurised meter dose inhaler form. The method was carried out on a thermo hypersil C-18 column (250 mm × 4.60 mm i.d., particle size 5 μ m), with a mobile phase consisting of Methanol: buffer phosphate 40:60 v/v adjust the pH 3.6 at flow rate 0.5 ml/ min. The wavelength used for detection of formoterol fumarate and budesonide was 267 nm. The retention time of formoterol fumarate and budesonide were found to be 6.68 ± 0.10 min. and 5.84 ± 0.10 min. respectively. The method was validated in terms of linearity, range, accuracy, and precision, limit of detection (LOD) and limit of quantitation (LOQ) as per ICH guidelines. The method showed a good linearity in the concentration range of 17.5-52.5 μ g/ml for Formoterol fumarate and for Budesonide 6-18 μ g/ml. The % recoveries of Formoterol fumarate and Budesonide were found to be between 99.90-100.56% and 99.75-100.63% respectively. The % RSD for the method precision was found to be less than 2%. The proposed method can be successfully applied for the simultaneous estimation of Formoterol fumarate and Budesonide in combined tablet dosage form.

KEYWORDS: Formoterol fumarate, Budesonide RP-HPLC, Pressurised Metered Dose Inhaler, Author mail.

INTRODUCTION

Formoterol fumarate, N-[2-Hydroxy-5-[(1RS)-1-hydroxy-2-[[[(1RS)-2-(4-methoxyphenyl)-1-methylethyl] amino] ethyl] phenyl] formamide (E)-butenedioate dehydrate is a beta₂-adrenoceptor agonist and a bronchodilator. Budesonide, 16 α , 17-[(1RS)-butylidenebis(oxy)]-11 β , 21-dihydroxypregna-1,4-diene-3, 20-dione is a glucocorticoid. [figure 1 & Figure 2].

A pressurised meter dose formulation containing 6 mcg of formoterol fumarate and 400 mcg of budesonide per actuation is available with Cipla brand name Foracort inhaler 400. Formoterol fumarate and budesonide standard were checked as per, BP^[2] method. The present work describes the development of a simple, precise and accurate reverse phase HPLC method for the simultaneous estimation of formoterol fumarate and Budesonide in pressurised meter dose inhaler formulation.[7,8 & 9].

Foracort Inhaler is a medication that is a combination of a corticosteroid and a long-acting beta₂-adrenergic agonist. It is indicated for treatment of asthma. It is also used for the maintenance treatment of airflow obstruction

in patients with chronic obstructive pulmonary disease (COPD) including chronic bronchitis and emphysema.^[6,9] Formoterol fumarate has a molecular weight of 840.9, and its empirical formula is (C₁₉H₂₄N₂O₄)₂·C₄H₄O₄·2H₂O. Formoterol fumarate is a white to yellowish crystalline powder, which is freely soluble in glacial acetic acid, soluble in methanol, sparingly soluble in ethanol and isopropanol, slightly soluble in water and practically insoluble in acetone, ethyl acetate, and diethyl ether.[Figure 1].

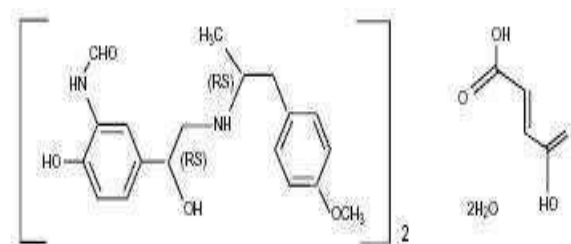


Fig. 1: Formoterol Fumarate.

Budesonide, is a corticosteroid designated chemically as (RS)-11 β , 16 α , 17, 21-tetrahydroxypregna-1, 4-diene-3, 20dione cyclic 16, 17-acetal with butyraldehyde. Budesonide is available as a mixture of two epimers

(22R and 22S). The empirical formula of budesonide is $C_{25}H_{34}O_6$ and its molecular weight is 430.5. Budesonide is a white to off-white, tasteless, odourless powder that is practically insoluble in water and heptane, sparingly soluble in ethanol, and freely soluble in chloroform.[figure 2].

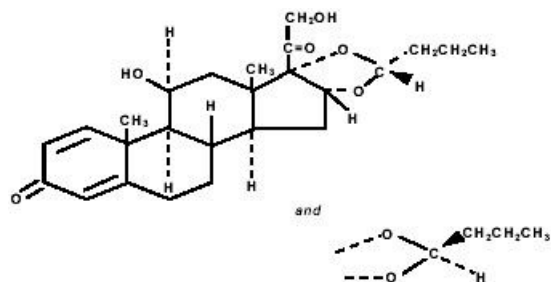


Fig 2 Budesonide (Epimer A and Epimer B).

The retention time of formoterol fumarate and budesonide was found to be 6.69 and 5.83min respectively. The method has been validated for linearity, accuracy and precision. Linearity for formoterol fumarate and budesonide were in the range of 17.5-52.5ug/ml and 6-18 μ g/ml. The mean recoveries obtained for formoterol fumarate and budesonide were 100.53% and 99.96%, respectively. The developed method was found to be accurate, precise, selective and rapid for the simultaneous estimation of formoterol fumarate and budesonide in pressurised meter dose inhaler.

MATERIALS AND METHODS

Reagents and chemicals

The working standard of API's, formoterol fumarate dihydrate and budesonide were provided by Ultratech India ltd. HPLC grade solvents, Methanol and water of 'Merck' were used for the analysis. B u d a m a t e - 4 0 0 C a p s u l e s pressurised meter dose inhaler of lupin was purchased from market which claimed to contain formoterol fumarate 6 mcg and budesonide 4 00 mcg per actuation.

Instrumentation

The HPLC system (Thermo) consisted of a U.V. Visible detector, column used was octadecylsilyl silica gel for chromatography R (5 μ m) with a pore size of 10 nm, column size: 1 = 0.15 m, \varnothing = 4.6 mm of (Peerless, Chromatopak), at column temperature: 30 °C. pH meter of Labindia make.

Chromatographic conditions

The chromatographic analysis was performed on Chromatopak Peerless -C18 analytical column with a mobile phase composed of M e t h a n o l: buffer (40:60 v/v) (buffer pH 3.0, adjusted with orthophosphoric acid) and was isocratically eluted at a flow rate of 0.5 mL min⁻¹. Column oven temperature was 30°C. A small sample volume of 5 μ L was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 269 nm and the total run time as 8 min.

Preparation of Buffer Solution

Buffer solution was prepared by dissolving 1.38 g of sodium dihydrogen phosphate and 1.22gm decane sulphonic acid in 1Ltr std volumetric flask, dissolved with HPLC grade water, pH adjusted to 3.0 with orthophosphoric acid.

Preparation of Standard Stock Solution

6.0 mg of formoterol fumarate was weighed accurately and transferred in 100ml volumetric flask and the volume was adjusted to the mark with the mobile phase. From the above solution 10mL was pipetted out in 100mL volumetric flask and adjusted to the mark with mobile phase. This is Solution (A). (Concentration 6 ppm) 20.0 mg of budesonide was weighed accurately in 100.0 mL volumetric flask, and volume was adjusted with mobile phase up to the mark. This is solution (B). (Concentration 200 ppm).

Standard Solution

10 mL of solution A and 20mL of solution B was transferred to a 50 mL volumetric flask and the volume was adjusted to the mark with mobile phase to give 1.2 mcg/ml of formoterol fumarate and 80mcg/ml of budesonide.

Analysis of pressurized metered dose inhaler (formulation) Sample Preparation Formoterol fumarate and budesonide pressurised metered dose inhaler (FB-pMDI)

10 actuations from Budetrol Cap were carefully taken (equivalent to 60 ppm of formoterol fumarate and 4000 ppm of budesonide) in a 100 mL beaker filled with 20 mL mobile phase and a teflon disk having 0.5 mm hole at the centre. This solution is transferred to 50mL volumetric flask, sonicated for 15 min and cooled to room temperature. The volume was made up with mobile phase. Final concentration formoterol fumarate is 1.2 ppm and budesonide is 80 ppm^[5] [Refer figure 3 for A typical chromatogram.].

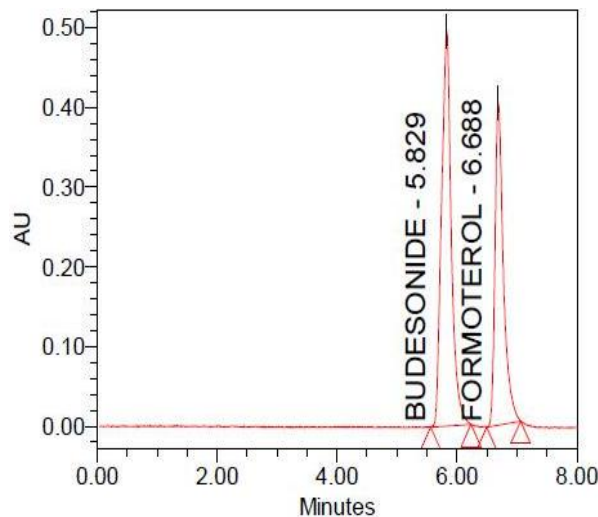


Figure 3: Typical HPLC chromatogram of Formoterol Fumarate and Budesonide.

RESULTS AND DISCUSSION

Method development

The objective of this study was to develop a method for estimation of formoterol fumarate and budesonide combination under isocratic conditions. The mobile phase used was the mixture of methanol with buffer in different ratios. The mixture of methanol: buffer (pH 3.0) in the ratio of [40:60] (v/v) was proved to be most effective mixture than the other mixtures used for better elution. The flow rates tested were 0.5, 1.0 and 1.5 mL. Among them, flow rate of 0.5 mL was selected for the assay because of better elution of the peak. The column oven temperature selected as 30°C for better peak shape and elution of peak. The above mentioned

chromatographic conditions proved to provide a better and symmetric elution of combined mixture of formoterol fumarate and budesonide in a reasonable time of 6.68 & 5.84 min. The optimum wave length for detection was 269 nm and no indigenous interfering compounds were eluted at the retention times of the drugs. The peak purity for formoterol fumarate and budesonide were found to be above 99.9% without interference of other compounds, impurities etc.

System suitability parameters observed during analysis

At the chromatographic conditions selected for the system suitability parameters for HPLC were in Table-1.

Table 1: System Suitability Parameters.

S. NO.	Drug name	Rt	Theoretical plates	Resolution	Tailing factor	%RSD
1	BUD	5.84	5280	NA	1.134	0.1
2	FOR	6.68	11774	3.025	1.571	0.1

METHOD VALIDATION

SPECIFICITY

Specificity is the ability of the method to measure the analytes response in the presence of its potential impurities, excipients and diluents. Interference of impurities and excipients present in the formulation and interference of diluents in the elution of the drugs was checked. The specificity of the HPLC method was determined by comparing the chromatograms of the standard and sample solutions.

LINEARITY

Linearity is studied to determine the range over which analyte response is a linear function of concentration. This study was performed by preparing standard solutions of different concentrations and analyses were performed five times. The responses were measured as peak area. The calibration curves were obtained by plotting peak area against concentration.

PRECISION

The precision of an analytical method is the closeness of replicate results obtained from analysis of the same homogeneous sample. Precision was considered at two levels, i.e. repeatability and Intermediate precision. Results from determination of repeatability and intermediate precision were expressed as SD and % RSD.

Repeatability was studied by carrying out system precision and Method Precision. System Precision was determined from results for six replicate of synthetic mixture and Method Precision is for formulation Mixture. Intermediate precision was studied by carrying out intraday precision and Interday precision. Intraday precision of the developed method was evaluated by analyzing Combined working standard solutions containing BUD (6-18 µg/ml) and FOR (17.5-52.5 µg/ml) 3 times on the same day. Each concentration was prepared in triplicate. Interday was determined by

analyzing Combined working standard solutions containing BUD (6-18 µg/ml) and FOR (17.5-52.5 µg/ml) on the three different days.

ACCURACY

The accuracy of an analytical is the closeness of results obtained by that method to the true method value for the sample. It is expressed as recovery (%), which is determined by the standard addition method. Samples were spiked with the standard containing 50, 100, and 150% was performed in experiment concentration of sample solution and analyzed. The triplicate. Recovery (%) and RSD (%) were calculated for each concentration.

LIMITS OF DETECTION AND LIMIT OF QUANTITATION

The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method linearity. It may be calculated as

$$\text{LOD} = 3.3 \times (\text{SD} / \text{Slope}) \quad \text{LOQ} = 10 \times (\text{SD} / \text{Slope})$$

Where,

SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

ROBUSTNESS

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by changing following parameters one by one.

Change in the ratio of component in the mobile phase.

Change in pH of Buffer by ± 0.2 units (pH 2.8 and pH 3.2)

Change in flow rate by ± 0.2 ml/minute (0.3ml/minute and 0.7ml/minute)

After each change, sample solution was injected and % assay with system suitability parameters were checked.

FORMULATION

To determine the content of BUD and FOR in marketed tablet dosage form. Sample solutions were prepared and analyzed

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

Column chemistry, solvent selectivity (solvent type), solvent strength (volume fraction of organic solvent(s) in the mobile phase), additive strength, detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. Several mobile phase compositions were tried to resolve the peaks of BUD and FOR. The optimum mobile phase containing methanol and phosphate buffer pH 3 (40:60 v/v) was selected because it could resolve the peaks of BUD (RT = 5.84 ± 0.10 min) and FOR (RT = 6.68 ± 0.10 min) with a resolution factor of 3.025. Quantification was achieved with UV detection at 269 nm on the basis of peak area at 0.5 ml/min flow rate. A typical HPLC chromatogram obtained during simultaneous determination of BUD and FOR is given in (Figure 4).

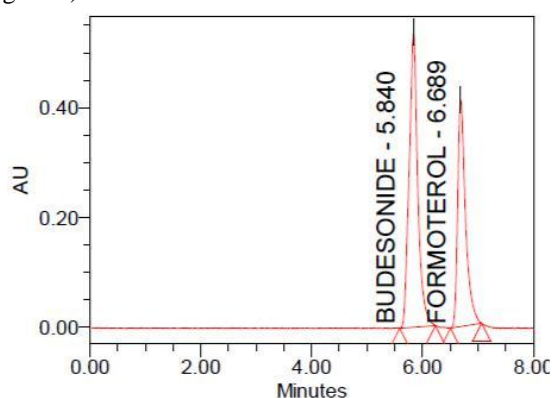
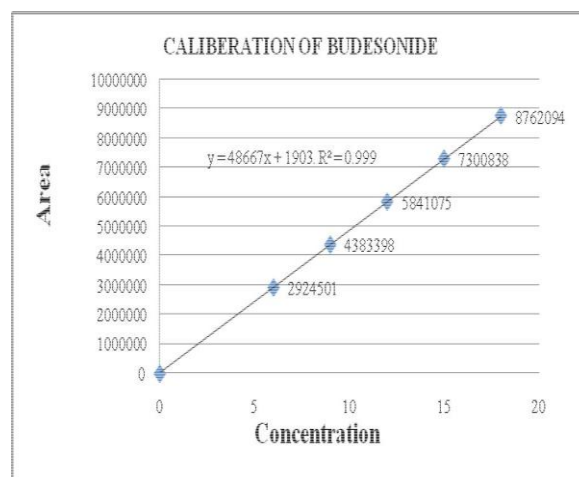
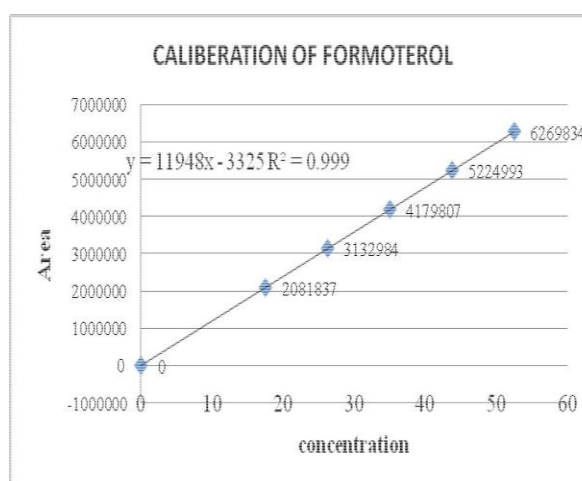


Figure:4: chromatogram for marketed sample.



CALIBERATION CURVE FOR BUDESONIDE



CALIBERATION CURVE FOR FORMOTEROL FUMARATE

METHOD VALIDATION

LINEARITY AND RANGE

Different concentrations (6-18 µg/ml of BUD and 17.5-52.5 µg/ml of FOR) of the mixture of two drugs were prepared for linearity studies. The calibration curves obtained by plotting peak area against concentration showed linear relationship over a concentration range of 6-18 µg/ml of BUD and 17.5-52.5 µg/ml of FOR. The linear regression equations for BUD and FOR were found to be $y = 48667x + 1903$ and $y = 11948x - 3325$ respectively. The regression coefficient values (r^2) were found to be 0.999 and 0.9993 for BUD and FOR respectively indicating a high degree of linearity. Calibration curves of BUD and FOR are shown in Figure 4 and 5 respectively. Regression characteristics of the proposed HPLC method are given in Table 2.

Table 2. Regression characteristics of the proposed HPLC method.

Parameter	BUDESONIDE	FORMOTEROL FUMARATE
Range (µg/ml)	6-18	17.5-52.5
Regression coefficient (r ²)	0.9996	0.9993
Slope	48667	11948
Intercept	1903	3325

PRECISION

The system and method precision showed a % RSD of 0.03% for BUD & 0.83% for FOR and 1.22% for BUD & 1.05% for FOR respectively. The intraday precision having %RSD of (1.13- 1.29%) of BUD and (1.04-1.11%) for FOR. Likewise, the interday precision showed a %RSD (1.37-1.54%) of BUD and (1.27-1.44%) for FOR. All data of precision are shown in Table 2.

ACCURACY

Recovery studies were carried out by applying the standard addition method. It was carried out by spiking the already analyzed sample of the tablets with the standard of BUD and FOR which contains concentration of 50, 100 and 150% of concentration of already analyzed sample. The average % recoveries for BUD and

FOR in marketed formulation were found to be between 99.38-100.83% and 100.63- 100.21 % for BUD and FOR respectively. The results revealed that there was no interference of excipients. The results of accuracy are shown in Table 2.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ)

The limit of detection and limit of quantification were found to be 0.146 and 0.487 μ g/ml for BUD and 0.104 and 0.348 μ g/ml for FOR. The values indicate that the method is sensitive

ROBUSTNESS

The proposed method was found to be robust enough (% RSD < 2.) to withstand such slight changes and allow routine analysis of the sample.

The result of robustness is shown in Table 3.

Table 3. Summary of Validation parameters for proposed method.

Parameters	BUD	FOR
(1) Beer's Law Limit (μ g/ml)	6-18	17.5-52.5
(2) Regression equation	$y = 48667x + 1903$	$y = 11948x + 3325$
(3) Correlation coefficient (r^2)	0.9996	0.9993
		(4) Precision
System Precision (% RSD)	0.68	0.83
Method Precision (% RSD)	1.22	1.05
Intraday precision (% RSD)	1.59	0.69
Interday precision (% RSD)	0.31	1.51
	(5)	Accuracy (% Recovery)
50%	100.57 \pm 0.75	100.21 \pm 0.75
100%	100.39 \pm 1.34	100.12 \pm 0.59
150%	99.38 \pm 0.72	100.63 \pm 1.24
(6) LOD (μ g/ml)	0.146	0.104
(7) LOQ (μ g/ml)	0.487	0.348
(8) Robustness		
Change in organic phase(35:65v/v)	0.35	0.03
Change in organic phase(45:55v/v)	0.49	1.45
Change in pH (pH 2.8)	1.35	1.53
Change in pH (pH 3.2)	1.62	1.64
Change in flow rate(0.3 ml/minute)	1.40	0.88
Change in flow rate(0.7 ml/minute)	1.03	1.58

ANALYSIS OF MARKETED FORMULATION

Analysis of marketed tablet (ETOVA-MR) was carried out using optimized mobile phase and HPLC conditions. The % drug content of tablets obtained by the proposed method for BUD and FOR was found to be 100.64 and 99.59 respectively. This showed that the estimation of dosage forms was accurate within the acceptance level of 95% to 105%. The results are given in Table 4.

Table 4: Analysis of marketed Formulation.

Drug	Label Claim (mg)	Found Quantity (mg) Mean \pm SD	% Amount Found Mean \pm SD
Budesonide	400	399.49 \pm 2.38	99.70 % \pm 1.09
Formoterol	6	5.96 \pm 0.05	98.01% \pm 1.26

*Average of three determination

SYSTEM SUITABILITY PARAMETERS

For system suitability parameters, seven replicate injections of mixed standard solution were injected and parameters such as the resolution, theoretical plate and asymmetry factor of the peaks were calculated. The results are shown in Table 5.

Table 5. System suitability Parameter.

Parameter	Formoterol	Budesonide
Resolution		3.025
Asymmetry Factor	0.947	0.930
Theoretical Plates	5280	11774

CONCLUSION

The proposed RP- HPLC method was found suitable for simultaneous estimation of Budesonide and Formoterol in their combined marketed tablet formulation. The method gave good resolution for both the drugs with a short analysis time below 8 minutes. The developed method was validated and it was found to be novel, simple, precise, accurate, and sensitive. The good % recovery in tablet forms suggests that the excipients present in the dosage forms have no interference in the determination. The %RSD was also less than 2% showing high degree of precision of the proposed method. The developed method can be used for routine analysis of Budesonide and Formoterol in combined dosage form.

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