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Development and validation of RP-HPLC method for estimation of formoterol fumarate and budesonide in pressurised meter dose inhaler form

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ABSTRACT

A reverse phase high performance liquid chromatography method was developed for the simultaneous estimation of Formoterol Fumarate and Budesonide in pressurised metered dose inhaler. The separation was achieved by octadecyl silica gel column (C_{18}) and buffer of sodium dihydrogen phosphate and decane sulphonic acid in combination with acetonitrile as eluent, at a flow rate of 2 ml/min. Detection was carried out at 220 nm. Method was validated as per ICH guidelines and found to be stable, indicating its usefulness for analysis of said drug combination [1].

Key words: RP-HPLC Pressurised Metered Dose Inhaler.

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 a, 17-[(1RS)-butylidenebis(oxy)]-11 ß, 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 $(C_{19}H_{24}N_2O_4)_2{\bullet}C_4H_4O_4{\bullet}2H_2O$. 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]

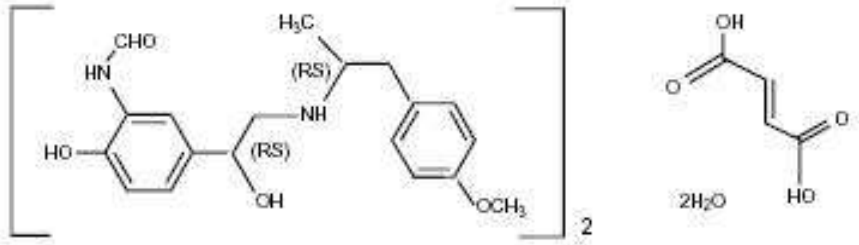

Fig 1 Formoterol Fumarate

Budesonide, is a corticosteroid designated chemically as (RS)-11β, 16α, 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].

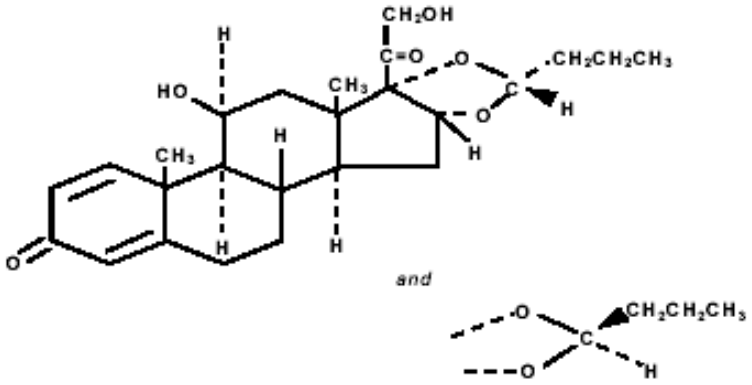

Fig 2 Budesonide (Epimer A and Epimer B)

The retention time of formoterol fumarate and budesonide was found to be 3.54 and epimer A 13.09, epimer B 14.33 min respectively. The method has been validated for linearity, accuracy and precision.

Linearity for formoterol fumarate and budesonide were in the range of 0.96-1.44ug/ml and 64-96 µg/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, acetonitrile and water of 'Merck' were used for the analysis. Foracort inhaler - 400 pressurised meter dose inhaler of Cilpa was purchased from market which claimed to contain formoterol fumarate 6 mcg and budesonide 400 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 µm) with a pore size of 10 nm, column size: l = 0.15 m, Ø = 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 buffer: acetonitrile (65:35v/v) (buffer pH 3.0, adjusted with orthophosphoric acid) and was isocratically eluted at a flow rate of 2.0 mL min^{-1}. Column oven temperature was 30ºC. A small sample volume of 200 µL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 220 nm and the total run time was 25 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 weighted 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 Foracort-400 (FB-pMDI) 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.]

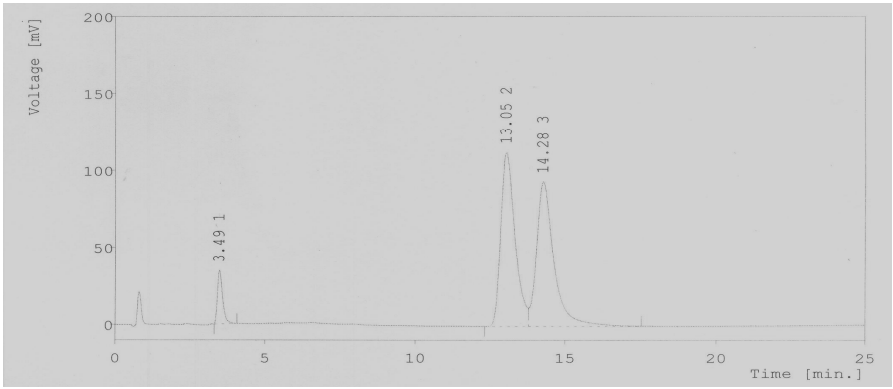

Figure 3 : Typical HPLC chromatogram of Formoterol Fumarate and Budesonide

Retention time for Formoterol Fumarate - 3.49
Retention Time for Budesonide (Epimer A – 13.05
Retention time for Budesonide (Epimer B) – 14.28
(For calculation purpose, summation of areas of epimer A and epimer B was considered)

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 acetonitrile with buffer in different ratios. The mixture of acetonitrile: buffer (pH 3.0) in the ratio of [35:65] (*v/v*) was proved to be most effective mixture than the other mixtures used for better elution. The flow rates tested were 1.5, 2.0 and 2.5 mL. Among them, flow rate of 2.0 mL was selected for the assay because of better elution of the peak. The column oven temperature selected as 30ºC 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 3.49, 13.05 & 14.28 min. The optimum wave length for detection was 220 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,

1) Theoretical plates (n) were found to be about 2800 for formoterol fumarate and about 6700 for budesonide.
2) Asymmetry (T) was 1.750 for formoterol fumarate and 1.99 for budesonide.
3) The retention time for formoterol fumarate is 3.5 ±0.5 min and for budesonide (epimer A 13.0 ±0.5 min, epimer B 14.30 ±0.5 min) were found comparable in both standard and sample solutions. Refer Figure 8 and Figure 9 for HPLC chromatograms for standard and sample solutions respectively.[3]

Method validation
Method validation was conducted according to ICH guidelines. Assay performance was evaluated by intraday and inter day (two different days) precision which is determined from replicate analysis of samples. Analysis of six different sample solutions was performed in the same day for intraday precision. Linearity was carried out for FB-pMDI over the concentration range of 0.96 ppm to 1.44 ppm for formoterol fumarate and 64 ppm to 96 ppm for budesonide. Accuracy of the method was tested by preparing three solutions of three different concentrations ranging from (90% to 110%) of sample and standard. The percentage recovery of sample is determined by comparing against standard solution. The precision was expressed in terms of RSD from mean intra and inter day assays. Robustness was tested by analysis of variations in analytical condition. Influence of mobile phase composition and pH were evaluated. The chromatographic parameters monitored were % assay, peak retention time, tailing factor and theoretical plate number.[4]

RESULTS AND DISCUSSION

Method Development
Changes in the analytical procedure were tested. Mobile phases with different compositions were tried. The pH value of the buffer was adjusted with orthophosphoric acid in the range 2.8 to 3.2. Change in Flow rate was also evaluated.

After selecting the best conditions based on peak performance, with isocratic elution the retention time of the proposed API's noted as follows:

i) Individual Analysis of API's: Formoterol fumarate and budesonide were eluted at 3.54 min and epimer A 13.09 min, epimer B 14.33 min.[Refer figure 6 & figure 7]

ii) Analysis of standard solution: Formoterol fumarate and budesonide were eluted at 3.54 min and epimer A 13.06 min, epimer B 14.31 min. [Refer figure 8]

iii) Analysis of FB-pMDI: Formoterol fumarate and budesonide were eluted at 3.53 min and epimer A 13.11 min, epimer B 14.35 min. [Refer figure 9]

Validation of the Method
The method was validated, in accordance with ICH guidelines, for specificity, linearity, accuracy, precision, ruggedness, and robustness.[1]

Specificity
Specificity is the ability to unequivocally assess the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. Specificity of an analytical method is its ability to measure accurately and specifically the analyte of interest without interference from the blank and placebo. Specificity of the peak purity of FB-pMDI was assessed by comparing the retention time of formoterol fumarate and budesonide in standard and sample and good correlation was obtained. The peak found pure in both standard and sample solution. Also there were no peaks when the placebo and blank were injected and no interferences, hence the method is specific. System suitability parameter also satisfied with respect to % RSD for replicate injection of standard, tailing factor and theoretical plates for formoterol fumarate and budesonide peak. Refer Table 1. Refer figure 4, 5, 6, 7, 8 & 9 for blank placebo ,standard & sample solutions chromatograph respectively.[6]

Table 1

	Standard analysis solution (20 ppm Formoterol Fumarate+ 8ppm Budesonide		Sample solution (10 act diluted in 25 mL)	
	FF	BU	FF	BU
Retention Time in minute	3.50	13.08	3.54	13.06
Theoretical plates (More than 2000)	2947	37947	2859	3589
Peak Purity	Peak Purity Index : 1.00	Peak Purity Index : 1.00	Peak Purity Index : 1.00	Peak Purity Index : 1.00
Blank/Placebo Interference	Not detected	Not detected	Not detected	Not detected
Average Area	419.56	7484.46	415.95	7487.76
% RSD peak area (NMT 2.0 %)	0.62%	0.20%	1.22%	0.12%

FF-Formoterol Fumarate. BU : Budesonide. RSD : Relative Standard Deviation

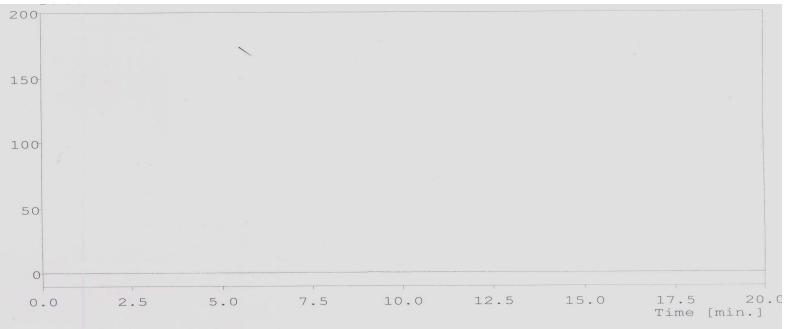

Figure 4 : HPLC chromatogram of Blank solution

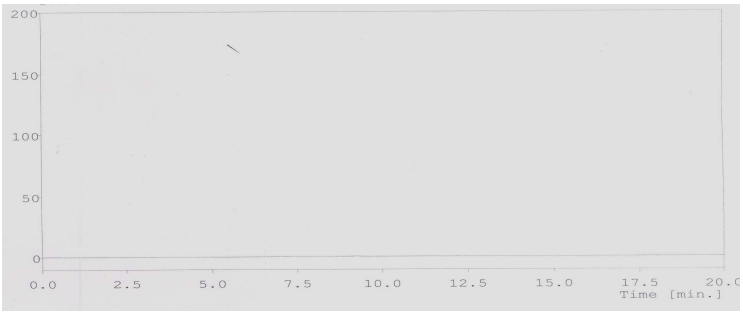

Figure 5 : HPLC chromatogram of Placebo solution

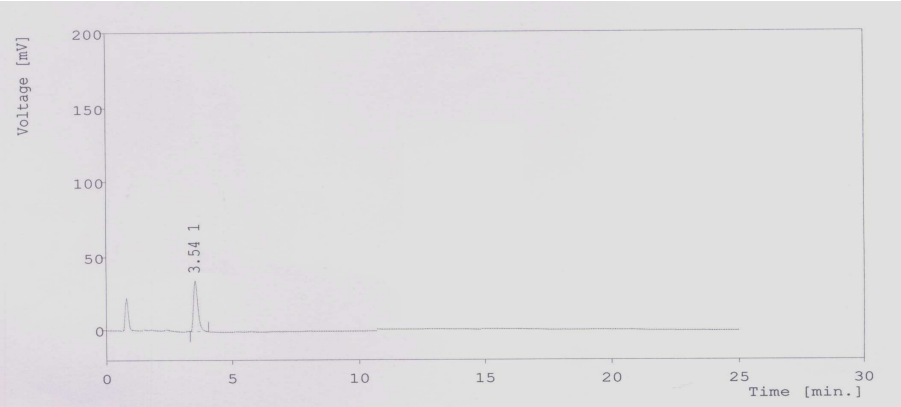

Figure 6 : HPLC chromatogram of Formoterol Fumarate solution

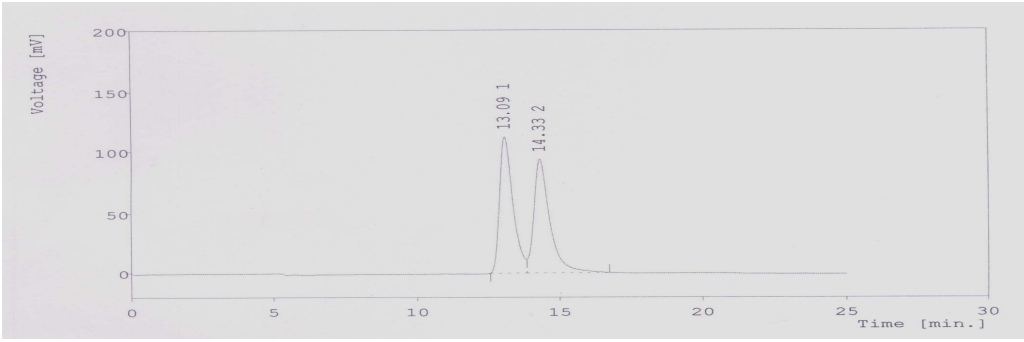

Figure 7: HPLC chromatogram of Budesonide solution

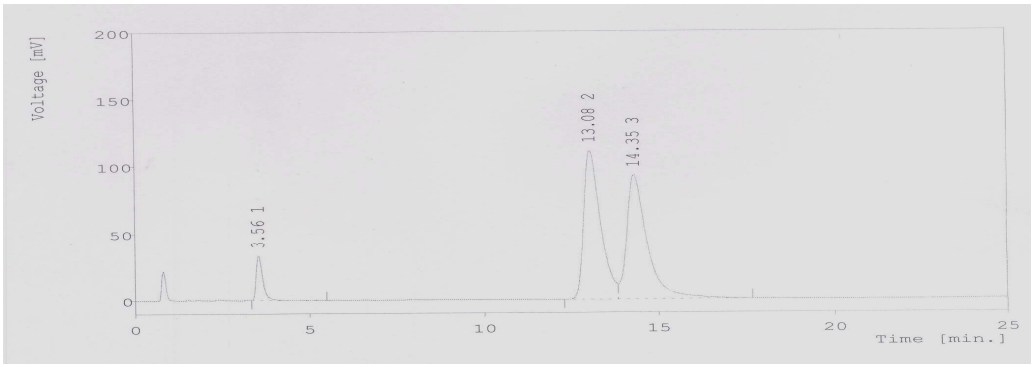

Figure 8 : HPLC chromatogram of standard analysis solution

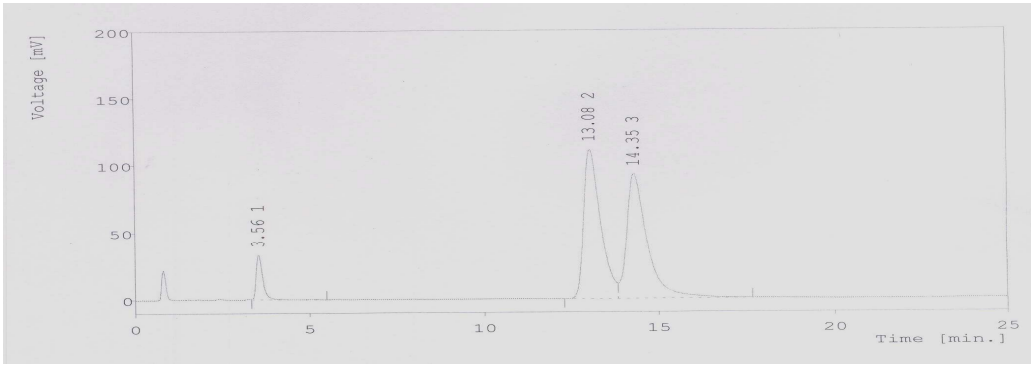

Figure 9 : HPLC chromatogram of in sample solution

Linearity

Linearity was assessed with the aid of serially diluted calibration solutions as mentioned (Refer. Tables 2 & 3). The Standard and sample were injected separately. Calibration graphs were plotted on the basis of triplicate analysis of each calibration solutions. Linear correlations were obtained over the range studied, with correlation coefficients of 0.99907 $\geq$ 0. 99 for formoterol fumarate and 0.9953 $\geq$ budesonide. In case of sample (FB–pMDI), slope = 438.66 and R^2=0.9941 for formoterol fumarate and slope = 57.90 and R^2=0.9902 for budesonide (Refer table 4). Refer figures 10 and 11 for Linearity correlation graph and figures (12a, 12b ,12c, 12d &,12e)for linearity HPLC chromatograph.

Sample stock solution :

50 actuations --> 50 mL of mobile phase ….(solution C)

Concentration of solution C

Formoterol fumarate 6mcg per actuation X 50 actuation equal to 300mcg/50mL= **6mcg/mL.**

Budesonide 400mcg per actuation X 50 actuation equal to 2000mcg/50mL= **400mcg/mL**

Table 2 linearity & Range study of Formoterol Fumarate

% conc.	Volume of sample stock solution ie solution C	Final Dilution (ml)	Area counts			Mean area counts	Relative standard deviation (%)
			1	2	3		
80 %	1.6	10 ml	316.47	321.56	321.74	319.92	0.93
90 %	1.8	10 ml	357.48	345.78	345.78	349.68	1.90
100 %	2.0	10 ml	421.74	433.55	420.33	425.20	1.70
110 %	2.2	10 ml	456.73	451.71	451.71	453.38	0.63
120 %	2.4	10 ml	508.64	508.64	515.43	510.90	0.76
Mean Relative Standard Deviation %							1.19%

Table 3 linearity & Range study of Budesonide

% conc.C	Volume of spl (ml)	Final Dilution (ml)	Area counts			Mean area counts	Relative standard deviation (%)
			1	2	3		
80 %	1.6	10 ml	6631.7	6672.45	6728.0	6677.38	0.72
90 %	1.8	10 ml	7479.87	7458.15	7458.15	7465.39	0.16
100 %	2.0	10 ml	7494.83	7512.17	7491.62	7499.54	0.14
110 %	2.2	10 ml	8402.55	8386.44	8386.44	8391.81	0.11
120 %	2.4	10 ml	9202.16	9202.16	9182.98	9195.76	0.12
Mean Relative Standard Deviation %							0.25%

Table 4 Result

	Formoterol Fumarate	**Budesonide**
Concentration range	0.96 -1.44 ppm	64-96 ppm
Correlation coefficient	0.9907	0.9953
Slope	438.66	57.90
R-square	0.9941	0.9902

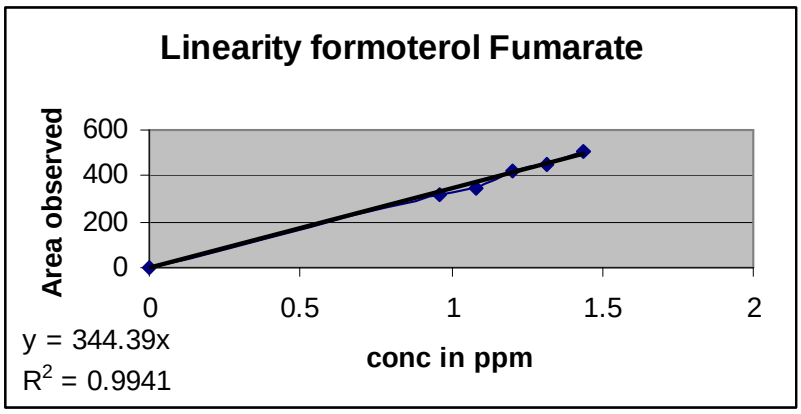

Figure 10: Linearity graph for Formoterol Fumarate

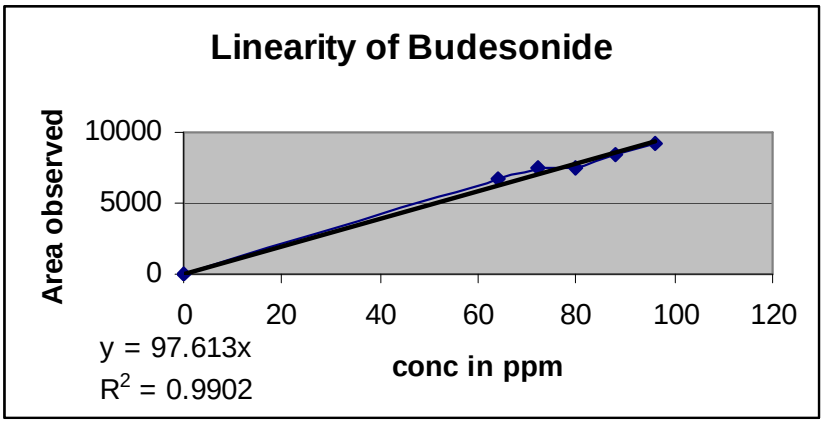

Figure 11: Linearity graph for Budesonide

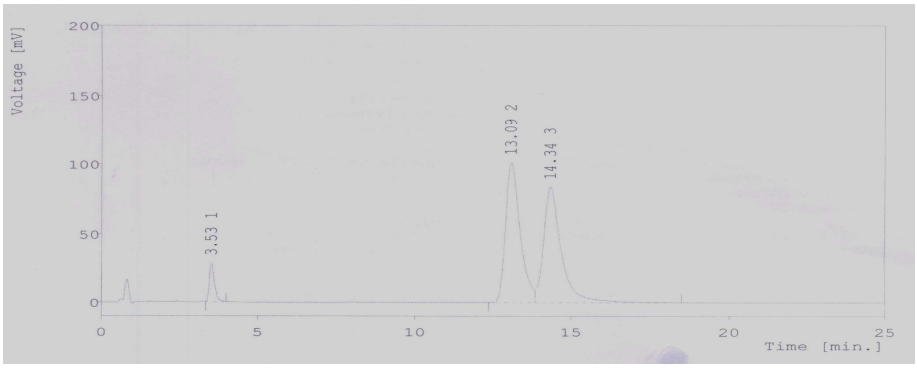

Figure 12 (a): Linearity 80%

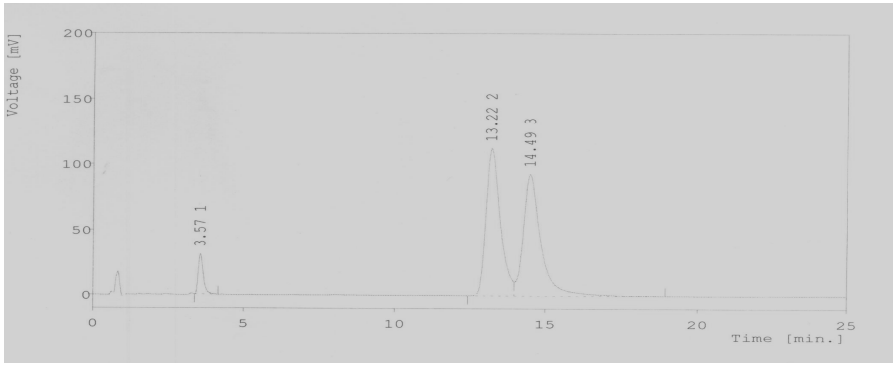

Figure 12 (b) : Linearity 90%

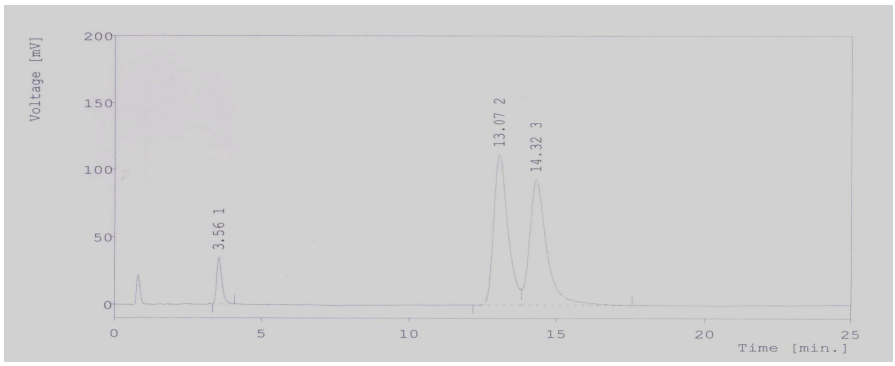

Figure 12 (c): Linearity 100%

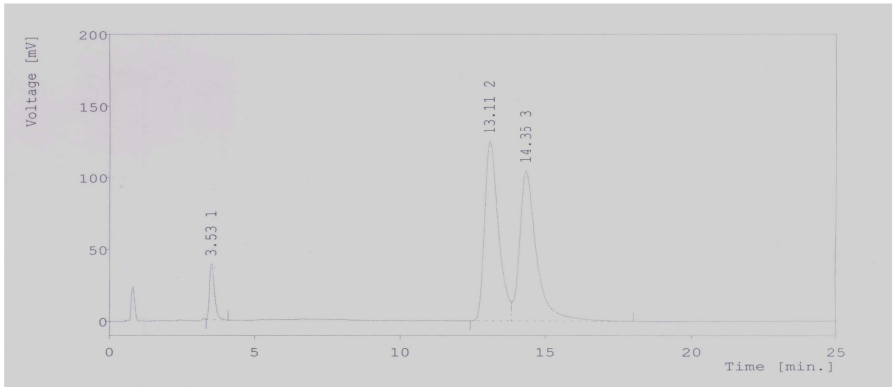

Figure 12 (d): Linearity 110%

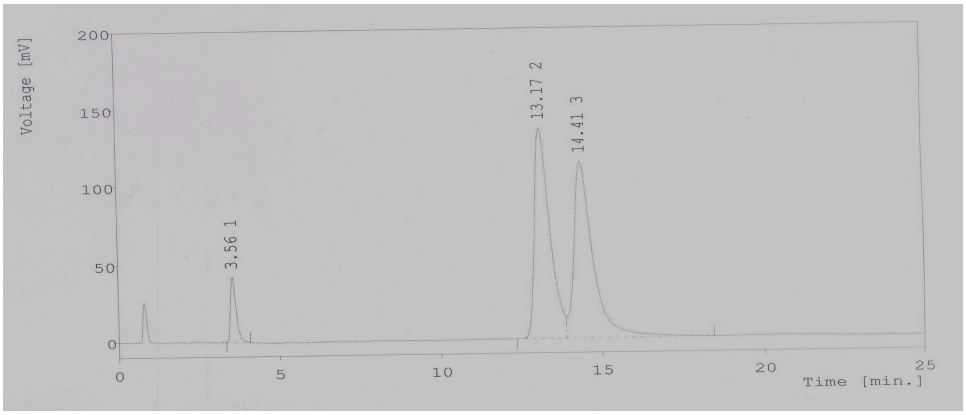

Figure 12 (e): Linearity 120%

Figure 12 (a, b, c, d & e): Linearity chromatograph for FB- pMDI

Precision

Precision was carried out for inter and intraday analysis for pressurised metered dose inhaler.

Precision was evaluated by carrying out six independent sample preparations of a single canister. The sample preparation for FB-pMDI was carried out in the same manner as described in sample preparation. Relative standard deviation (% RSD) was found to be less than 2.0%, which proves that the method is precise. Refer Table 5 & 6.

Table 5 : Precision for Formoterol Fumarate in FB-pMDI sample Method Precision - Intermediate Precision

Sr. No.	Formoterol Fumarate in Pressurised meter dose (Canister) 1.2 ppm	
	Method Precision	Intermediate Precision
1	421.74	433.55
2	433.55	427.36
3	420.33	424.21
4	411.85	422.50
5	415.13	423.34
6	416.63	423.37
Mean	**419.87**	425.72
SD	**7.59**	4.191
RSD	**1.80%**	**0.98**
Mean	**422.79**	
SD	**4.13**	
RSD	**0.97**	

Table 6 Precision for Budesonide in FB-pMDI sample Method Precision - Intermediate Precision

Sr. No.	Budesonide in Pressurised meter dose (Canister) 80ppm	
	Method Precision	Intermediate Precision
1	7494.83	7512.16
2	7512.16	7523.32
3	7491.62	7541.20
4	7490.89	7520.31
5	7494.49	7497.25
6	7466.94	7510.32
Mean	**7491.82**	**7517.42**
SD	**14.49**	**14.78**
RSD	**0.19%**	**0.19**
Mean	**7504.62**	
SD	**4.13**	
RSD	**0.97%**	

Accuracy (Recovery studies)

To check the degree of accuracy of the method, three solutions of different concentrations were prepared and injected in triplet both for standard and sample (90%, 100% and 110%). Areas were compared to find % recovery of sample at the same corresponding concentration.[Refer Table 7,8 & 9]

Table 7 : Standard Solution preparation & Area

% of Standard solution	Solution A in mL	Solution B in mL	Dilution volume in mL	Average Area of Formoterol Fumarate	Average Area of Budesonide
90%	1.8	3.6	10	357.48	6719.87
				345.78	6729.87
				357.48	6658.16
100%	2.0	4.0	10	411.85	7490.90
				415.13	7494.49
				416.63	7466.94
110%	2.2	4.4	10	456.73	8402.55
				456.73	8402.55
				451.71	8386.44

Table 8 Sample Solution preparation & Area

% of Sample solution	Number of Actuation	Dilution volume in mL	Average Area of Formoterol Fumarate	Average Area of Budesonide
90%	9 actuations	50	357.48	6692.05
			353.82	6690.84
			357.48	6695.72
100%	10 actuations	50	433.55	7512.18
			415.13	7494.49
			414.28	7477.57
110%	11 actuations	50	452.24	8394.08
			451.25	8380.07
			452.24	8394.08

Table 9 Percentage Recovery

	% Recovery	
Level	Formoterol Fumarate	Budesonide
90 %	100.75	99.85
100 %	101.55	100.14
110 %	99.30	99.90
Mean	100.53	99.96
% RSD	**1.13**	**0.15**

Robustness

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in optimized method parameters were done. The effect of change in mobile phase composition, change in pH of mobile phase, tailing factor and theoretical plates were studied. The method was found to be unaffected by small changes in mobile phase composition and change in pH. The results are described in Table 10.

Table 10

	Buffer pH 2.8		Buffer pH 3.2		Mobile phase composition Buffer: Acetonitrile [70:30]		Mobile phase composition Buffer : Acetonitrile[60:40]	
	FF	BU	FF	BU	FF	BU	FF	BU
Theoretical plates	2880	3600	2949	3695	2202	3809	2137	3802
Retention Time	3.56	13.08	3.50	13.08	3.59	13.28	3.53	13.09
Average Area	422.76	7474.15	419.74	7522.26	325.75	7477.83	319.20	6621.32
% RSD	**0.22%**	**0.52%**	**0.51%**	**0.11%**	**0.46%**	**0.13%**	**0.75%**	**0.16%**

FF- Formoterol Fumarate
BU – Budesonide

CONCLUSION

Considering the efficiency of HPLC, attempt has been made to develop simple, accurate, precise, rapid and economic method for simultaneous estimation of formoterol fumarate and budesonide in pressurized metered dose inhaler dosage form. Thus method described enables the quantification of both the API's. The advantages lie in the simplicity of sample preparation and the low costs of reagents used.

Experimental results were indicative of satisfactory precision and reproducible. Hence, above described method can be successfully implemented for the quantitative determination of API's formoterol fumarate and budesonide in pressurised metered dose inhaler in regular quality control department analysis.

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