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Open Access Research Article

Development and Validation of Analytical Method for Simultaneous Estimation of Formoterol Fumarate Dihydrate and Fluticasone Propionate from Bulk and Dry Powder Inhaler Formulation

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ABSTRACT

A method was developed and validated for analysis of Formoterol Fumarate and Fluticasone Propionate in dry powder inhaler formulations. Separation was achieved on a HiQ Sil C18HS, 250×4.6 mm, 5 µm column using a mobile phase consisting of Acetonitrile: 0.01 M Ammonium Dihydrogen Phosphate solution (80:20 %v/v) at a flow rate of 1ml/min PDA detection at 215.0 nm. This method is validated according to ICH guidelines, which include linearity, precision, accuracy, specificity, robustness. The result obtained were within the acceptance criteria as per ICH guidelines.

Keywords: formoterol fumarate dihydrate, fluticasone propionate, buffer, HPLC.

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INTRODUCTION

Ultraviolet-Visible Absorption Spectroscopy:

This deals with the absorption of electromagnetic radiation in the wavelength region of 160 to 780 nm. UV absorption spectroscopy deals with absorption of light by a sample in the Ultra Violet (UV) region (190 - 380 nm), while Visible region absorption spectroscopy (colorimetric) deals with absorption of light by a sample in the Visible region (380 - 780nm). Absorption of UV – Visible light causes promotion of a valence electron from bonding to antibonding orbitals. There are 4 types of transitions observed in UV visible spectroscopy, $\sigma \rightarrow \sigma^*$, $\pi \rightarrow \pi^*$, $n \rightarrow \sigma^*$, and $n \rightarrow \pi^*$. It is not always necessary that the excitation of the electron take place from bonding orbital to anti-bonding orbital when the compound is exposed to UV visible light. The relation between the excitation coefficient and transition probability is given as;

$$E_{max} = 0.87 \times 10^{20} p \times a$$

Where,

E_{max} = excitation coefficient.

p = transition probability with values from 0 to 1.

a = target area of the absorbing system (Chromophore).

High Performance Liquid Chromatography

The Principle of Chromatographic Separation:

By classical definition, chromatography is a separation process that is achieved by distributing the substances to be separated between a moving phase and a stationary phase. Those substances distributed preferentially in the moving phase pass through the chromatographic system faster than those that are distributed preferentially in the stationary phase. As a consequence the substances are eluted from the column in reverse order of their distribution coefficient with respect to the stationary phase.

Instrumentation:

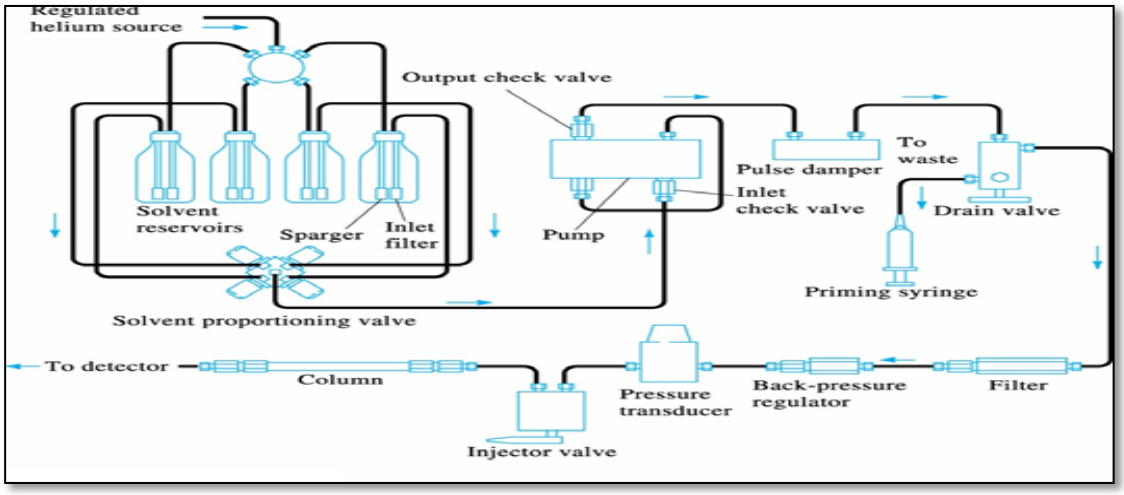

Figure 1: HPLC Instrumentation

MATERIAL AND METHODS

Formoterol fumarate dihydrate: Active pharmaceutical ingredient (API) was supplied by prerana enterprises (ahmednagar).

Fluticasone propionate: Active pharmaceutical ingredient (API) was supplied by prerana enterprises (ahmednagar)

All chemicals used throughout the work were of analytical grade and the solvents were of HPLC grade purchased from Merck, Mumbai.

Reagents and chemicals

Sr. No.	Name	Specification	Manufacturer/Supplier
1	Acetonitrile	HPLC grade	Merck
2	Methanol	HPLC grade	Merck
3	Orthophosphoric acid	A.R	Merck
4	Ammonium dihydrogen phosphate	A.R	Merck
5	Potassium dihydrogen phosphate	A.R	Merck
6	Sodium dihydrogen phosphate	A.R	Merck
7	Water	HPLC grade	Merck

Apparatus/Instruments Used:

Apparatus/Instruments

Sr. No	Name	Model	Manufacturer/Supplier
1	Weighing balance	AUX 220	Shimadzu
2	Digital pH meter	Eq610	EQUIP-TRONICS
3	Sonicator	Fast Clean	Ultrasonic Cleaner
4	HPLC	2075	JASCO
5	Column	HiQ Sil C18HS, 250×4.6mm, 5 µm	KYA TECH

Experimental

Development and Optimization of HPLC Method for Formoterol Fumarate Dihydrate and Fluticasone Propionate

Method I - Simultaneous Equation Method

- **Selection of solvent:** Methanol is selected as a solvent
- **Selection of analytical wavelength:**

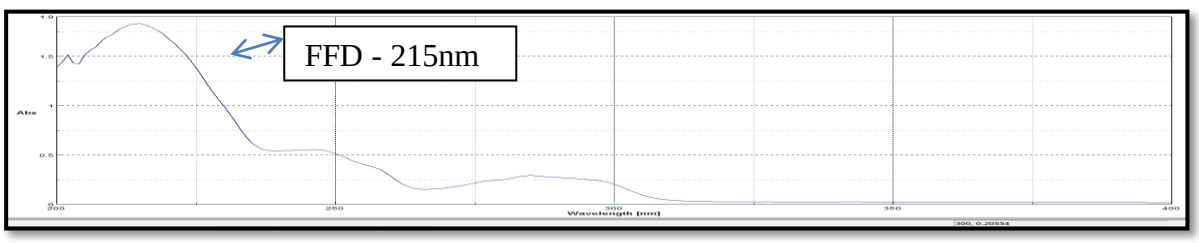

Figure 2: UV spectra of FFD in methanol

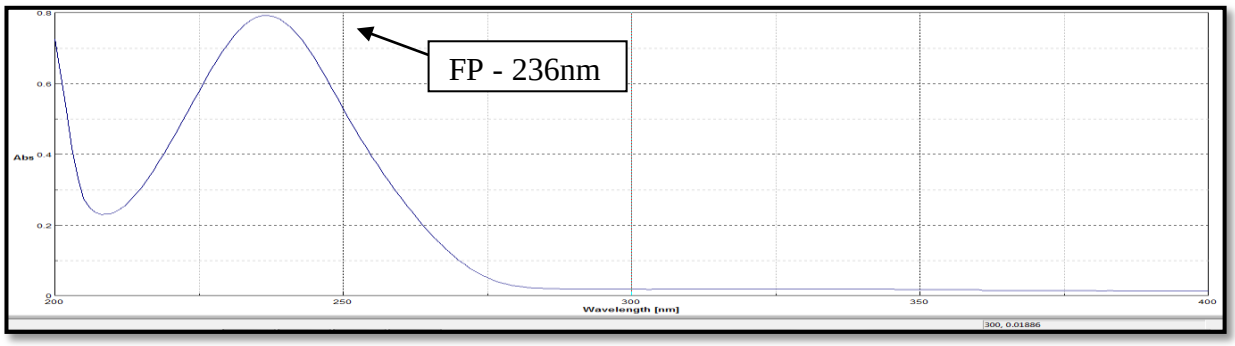

Figure 3: UV spectra of FP in methanol

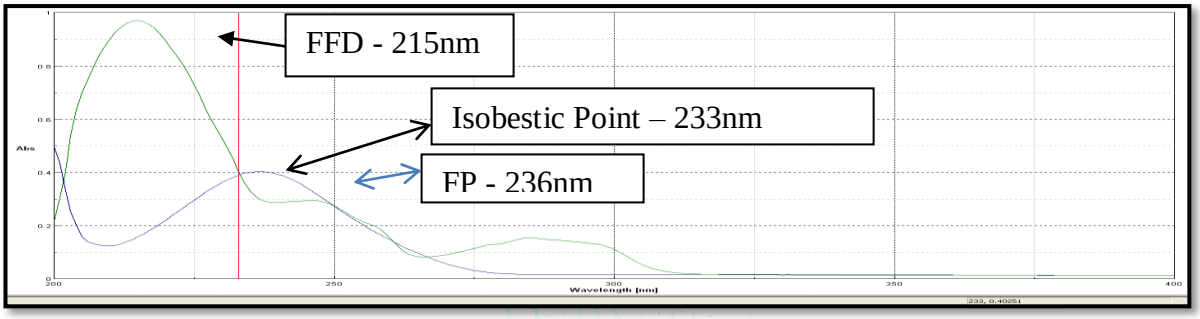

Figure 4: Overlain UV spectra of FFD & FP in methanol

- **Selection of linearity range:**

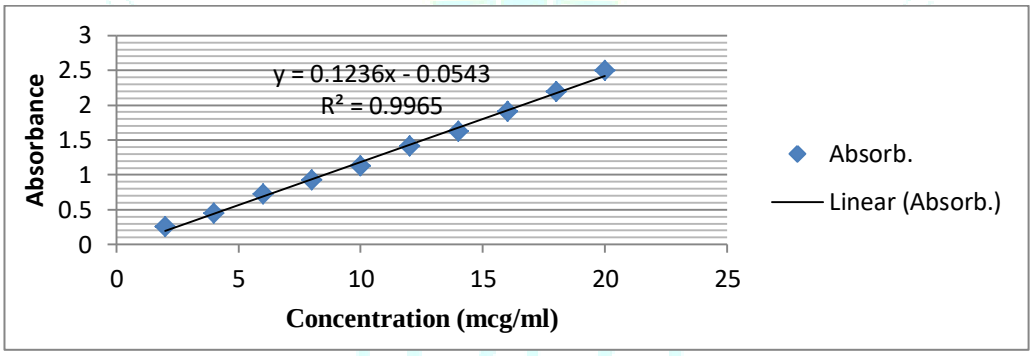

Figure 5: Standard calibration curve for FFD at 215 nm

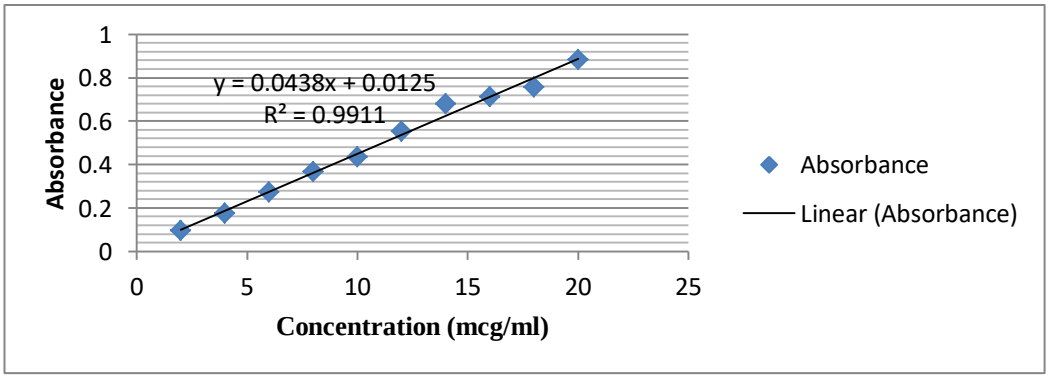

Figure 6: Standard calibration curve for FP at 236nm

- **Determination of absorptivity at analytical wavelengths:**
- **Standard absorptivity values of FFD and FP**

Drug	λ1=215	λ2=236
Formoterol Fumarate Dihydrate	ax1= 9.7137	ax2 = 2.961
Fluticasone Propionate	ay1 = 1.5829	ay2 = 4.0373

- **Analysis of Marketed formulation:**

Table 1: Analysis of marketed formulation

Sr. no.	Capsule components	Label Claim (mcg)	% of Amount found*	S.D*	%R.S.D. *
1	FFD	6	99.60	1.5857	1.5919
2	FP	100	101.18	1.2494	1.2348

* denotes average of three determinations.

Validation of simultaneous equation method:

a. Precision:

Table 2: Precision Study data

	Intraday study				Inter day study		
Drug	**% of Amount found***	**S.D.***	**% RSD***	**Drug**	**% of Amount found***	**S.D.***	**% RSD***
FFD	99.87	1.6310	1.63300	FFD	99.97	1.65210	1.65300
FP	100.12	1.63100	1.62901	FP	100.22	1.63600	1.63501

b. Accuracy:

Table 3: Recovery study data

Level of recovery	Drug	By Simultaneous Equation	
		% Recovery *	S.D.*
80 %	FFD	99.63	0.5431
	FP	100.36	0.59002
100 %	FFD	99.67	0.613807
	FP	100.32	0.613808
120 %	FFD	100.70	1.2997
	FP	99.29	1.2998

c. Ruggedness:

Table 4: Ruggedness Data (Analyst to analyst)

Drug	Concentration (µg/ml)	Analyst I		Analyst II	
		% of Amount found*	%RSD*	% of Amount found*	%RSD*
FFD	6	99.60	1.5919	100.12	1.62901
FP	100	101.18	1.2348	99.87	1.63300

d. Limit of detection (LOD) and Limit of quantitation (LOQ):

Parameter	FFD	FP
*L.O.D. (µg/ml)	1.5	2.10
*L.O.Q. (µg/ml)	1.3	2.0

Method II - Q Analysis or Absorbance Ratio Method

- **Preparation of Standard Stock Solutions:**
- **Selection of linearity range:**

Linearity range was found to be 2-20 for both FFD & FP.

Procedure for analysis:

- **Analysis of formulation:**

Table 5: Analysis of marketed formulation

Sr. no.	Capsule components	Label Claim (mcg)	% of Amount found*	S.D*	%R.S.D.*
1	FFD	6	100.86	1.03890	1.03002
2	FP	100	98.32	0.2869	.0291801

- **Validation of absorbance ratio method:**

1. Precision:

Table 6: Precision Study data

	Intraday study				Inter day study		
Drug	% of Amount found*	S.D.*	% RSD*	Drug	% of Amount found*	S.D.	% RSD*
FFD	100.05	1.7937	1.7928	FFD	100.12	1.6937	1.6928
FP	99.94	1.79398	1.7947	FP	99.89	1.69398	1.6947

*Mean of six estimation

2. Accuracy:

Table 7: Recovery study data

Level of recovery	Drug	By Absorbance ratio method	
		Recovery %*	S.D.*
80 %	FFD	99.63	1.2675
	FP	100.36	1.2685
100 %	FFD	100.26	0.9679
	FP	99.73	0.9689
120 %	FFD	99.74	1.6937
	FP	100.25	1.6938

3. Ruggedness:

Table 8: Ruggedness Data

Drug	Concentration (µg/ml)	Analyst I*	%RSD	Analyst II *	%RSD
FFD	6	100.05	1.7928	100.12	1.6928
FP	100	99.94	1.7947	99.89	1.6947

*Mean of three observations

4. Limit of detection (LOD) and limit of quantitation (LOQ):

Parameter	FFD	FP
*L.O.D. (µg/ml)	1.6	2.2
*L.O.Q. (µg/ml)	1.2	2.0

Method –III First Order Derivative Method

- **Preparation of standard stock solutions:**
- **Selection of analytical wavelength ranges:**

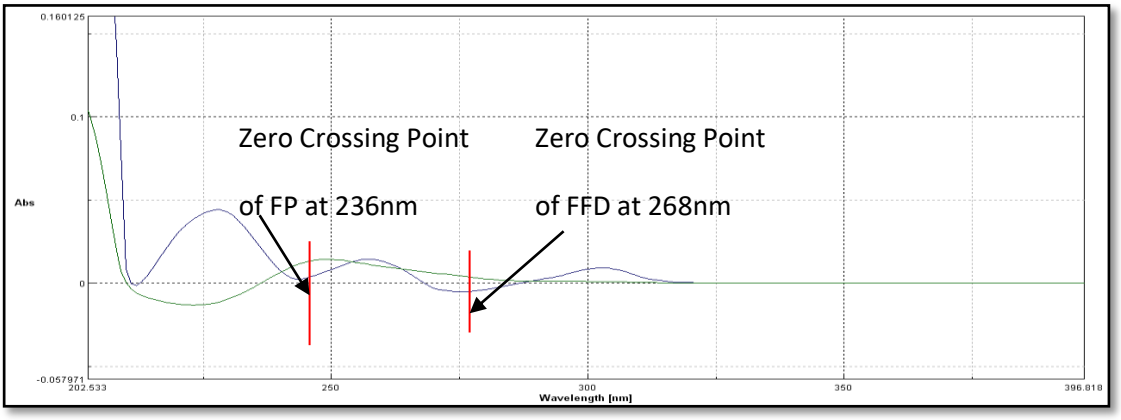

Figure 7: Overlain derivative spectra of FFD and FP

➢ **Selection of linear concentration ranges:**

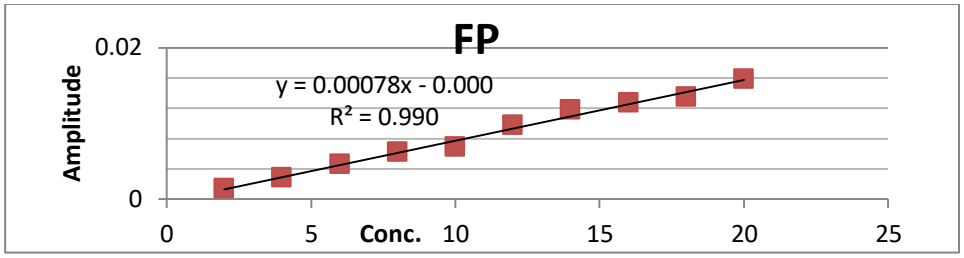

Figure 8: Calibration curves for FP

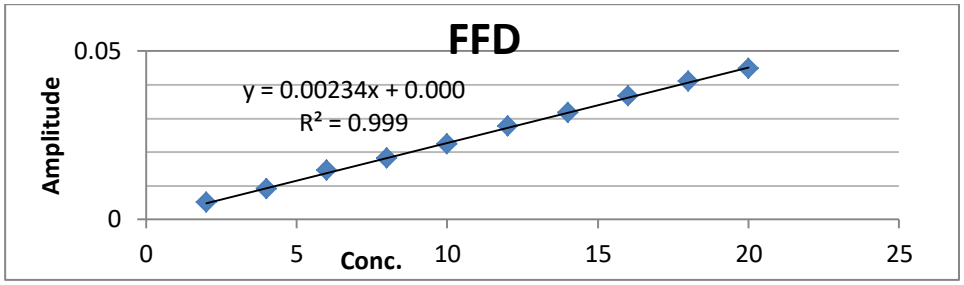

Figure 9: Calibration curves for FFD

Table 9: Parameters for calibration curves:

Parameters	FFD	FP
	At 236 nm	At 268 nm
Linearity range (µg/ml)	2-20	2-20
*Slope	0.00234	0.00078
*Intercept	0.000	0.000
*Regression coefficient (r^2)	0.999	0.990

➢ **Determination of coefficient of absorptivities (dA/dλ) at analytical wavelength:**

The standard Absorptivity values of drugs at the selected wavelengths are:

Table 10: Standard absorptivity values of FFD and FP

Drug	λ1=236	λ2=268
Formoterol Fumarate Dihydrate	ax1= 2.2264 X 10^{-2}	ax2 = 9.50734X 10^{-5}
Fluticasone Propionate	ay1 = -3.5103 X 10^{-4}	ay2 = -6.88466X 10^{-3}

➢ **Analysis of marketed formulation:**

Table 11: Results of analysis of marketed formulation

Sr. no.	Capsule components	Label Claim (mcg)	% amount found*	S.D*	%R.S.D.*
1	FFD	6	100.25	1.462	1.473
2	FP	100	98.40	1.356	1.383

* Average of six determinations

➢ **Method validation:**

6.1C.6.1. Precision:

6.1C.6.1.1. Repeatability:

Studies were carried out as described in Method I. The standard deviation (S.D.), % relative standard deviation (%R.S.D.) and standard error (S.E.) were calculated.

6.1C.6.1.2. Intermediate precision (Intra-day and inter-day precision):

The Intra and inter-day precision was determined as mentioned in method I. The S.D., % R.S.D. and S.E. were calculated and are shown in Table No.12.

Table 12: Statistical evaluation for precision studies

Precision Parameter	% Mean*		S.D.*		% R.S.D.*	
	FFD	FP	FFD	FP	FFD	FP
Intra-day	100.28	99.41	0.6123	1.0303	0.61059	0.0555
Inter-day	99.92	99.95	0.1199	0.0991	0.1199	1.04695

*Average of six determinations

6.1C.6.2. Accuracy:

Table 13: Results of recovery studies

Level of % Recovery	%* Mean Recovery		S.D.*		%R.S.D.*	
	FFD	FP	FFD	FP	FFD	FP
80	100.08	9989	0.1916	0.2929	0.1915	0.2936
100	99.56	99.84	0.02524	0.05571	0.2535	0.0558
120	100.04	101.05	0.1100	0.1438	0.1099	0.1423

*Average of three determinations

6.1C.6.3. Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Table 14: LOD and LOQ values

Sr. No.	Component	*LOD (µg/ml)	*LOQ (µg/ml)
1.	FFD	1.2	2.0
2.	FP	1.5	2.4

6.2.4. Method Development for Formoterol Fumarate Dihydrate and Fluticasone Propionate:

Selection of Solvent – Mobile Phase was used as diluents for dilutions.

6.2.4.1. Preparation of stock solutions of standard:

The standard FFD, 10 mg and FP, 100 mg were dissolved separately in diluent in separate 100 ml volumetric flasks and volume was made with the same solvent to give stock solutions of 100µg/ml for FFD & 1000 µg/ml for FP.

6.2.4.2. Selection of analytical wavelength:

6.2.4.3. Optimization of mobile phase:

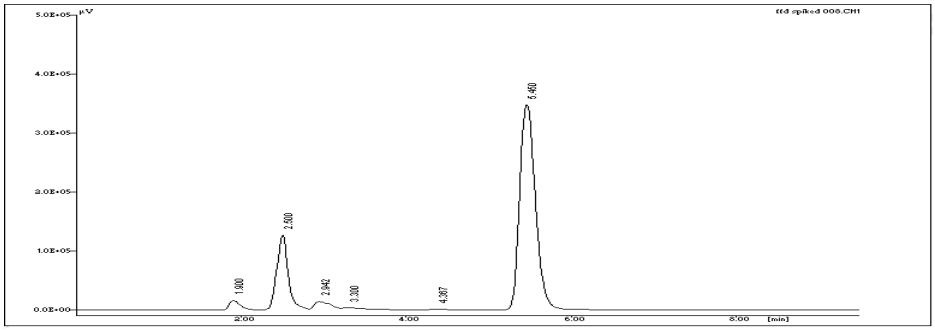

Figure 10: Chromatographic conditions 1 – Mobile phase - Water:ACN(30:70)

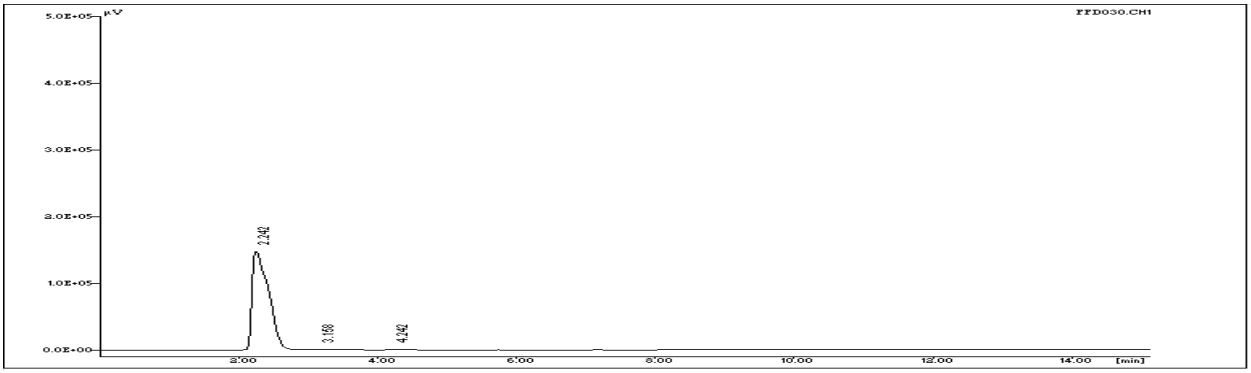

Figure 11: Chromatographic conditions 2 - Mobile phase - ACN: Methanol 70:30)

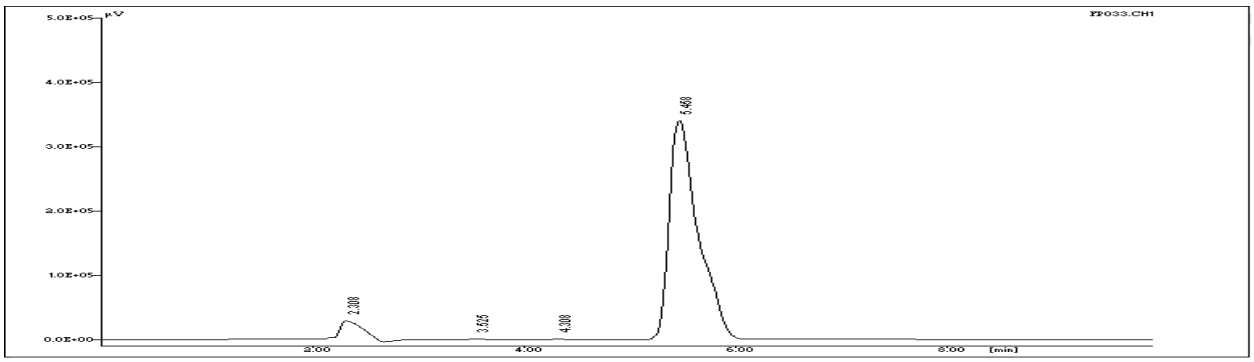

Figure 12: Chromatographic conditions 3 - Mobile phase - ACN:Buffer(potassium dihydrogen phosphate) (70:30)

Table 15: Optimized chromatographic conditions for HPLC method

HPLC Column	HiQ Sil C18HS, 250×4.6mm, 5 µm
Column temperature	Ambient temperature
Mobile Phase	Acetonitrile: 0.01 M Ammonium Dihydrogen Phosphate solution (80:20 %v/v)
Flow rate programming	Flow rate of 1ml/min
Detection wavelength	215.0 nm
Injection volume	20 µl
Run time	15 min

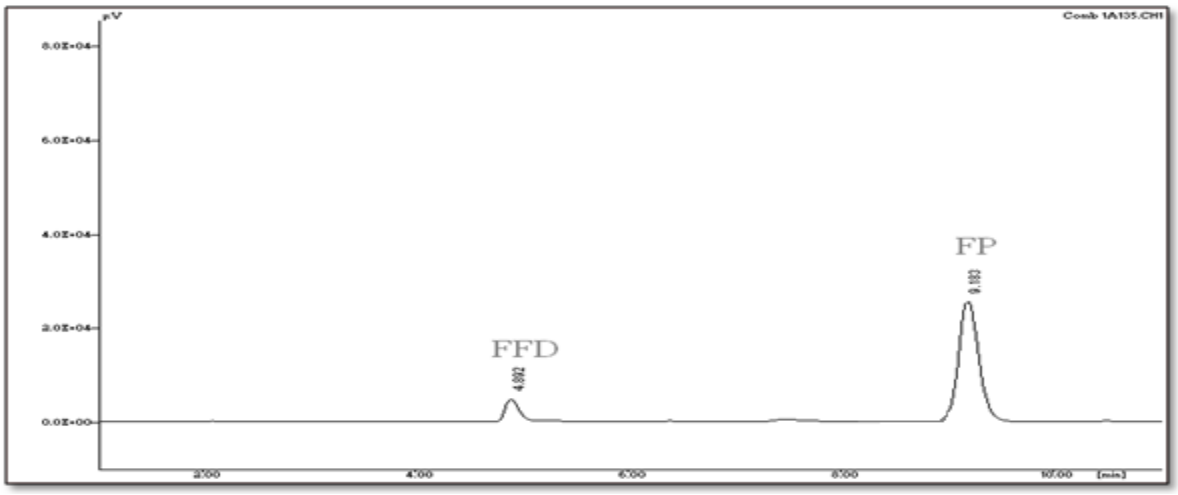

Figure 13: Typical chromatogram of Combination of FFD & FP obtained in Mobile Phase - Acetonitrile: 0.01 M Ammonium Dihydrogen Phosphate solution

6.2.5. Preparation of standard calibration curves of FFD and FP:

Standard calibration data for FFD

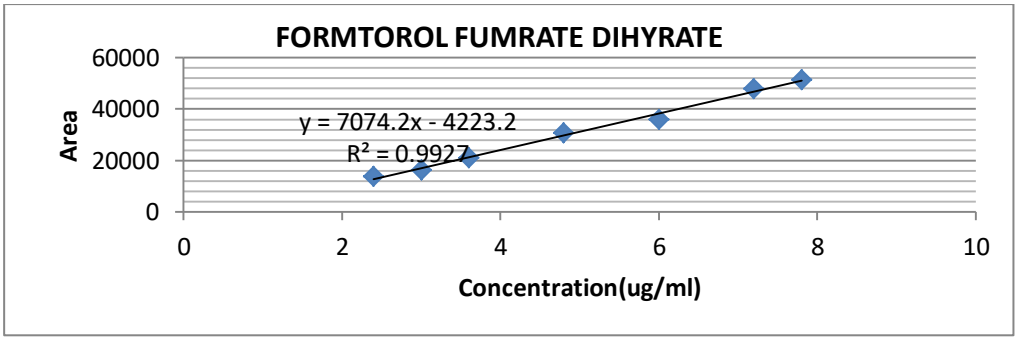

Figure 14: Standard calibration curve for FFD

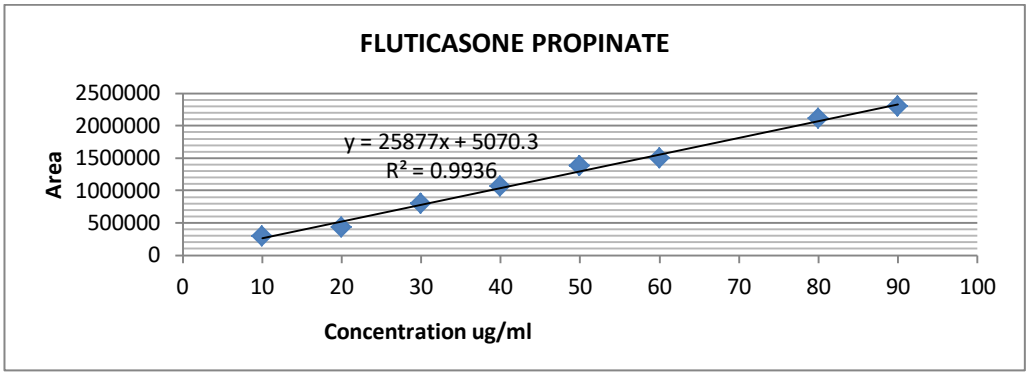

Figure 15: Standard Calibration curve for FP

6.2.6. Analysis of the marketed formulation:

Table 16: Results of analysis of capsule formulation by HPLC method

Sr. No.	Drugs	Label Claim (mcg/cap)	Amount Found* (mcg/cap)	% of Amount found*
1	FFD	6	5.92	98.67
2	FP	100	101.39	101.39

*Average of three determinations

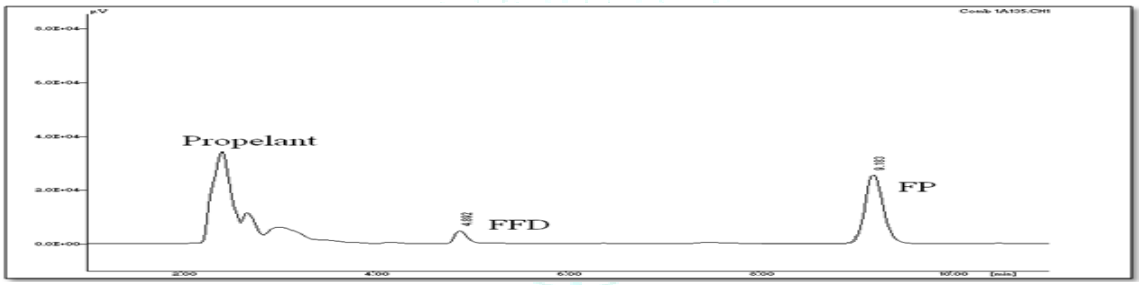

Figure 16: HPLC chromatogram of FFD and FP in capsule formulation

6.2.7. Method validation:

6.2.7.1. Linearity:

Table 17: Linear regression data for calibration curves of FFD and FP for HPLC method

Drugs	Linearity range (µg/ml)	Slope	y-intercept	Regression coefficient (r^2)
FFD	2.4-7.8	7074	4223	0.992
FP	10-90	25877	5070	0.993

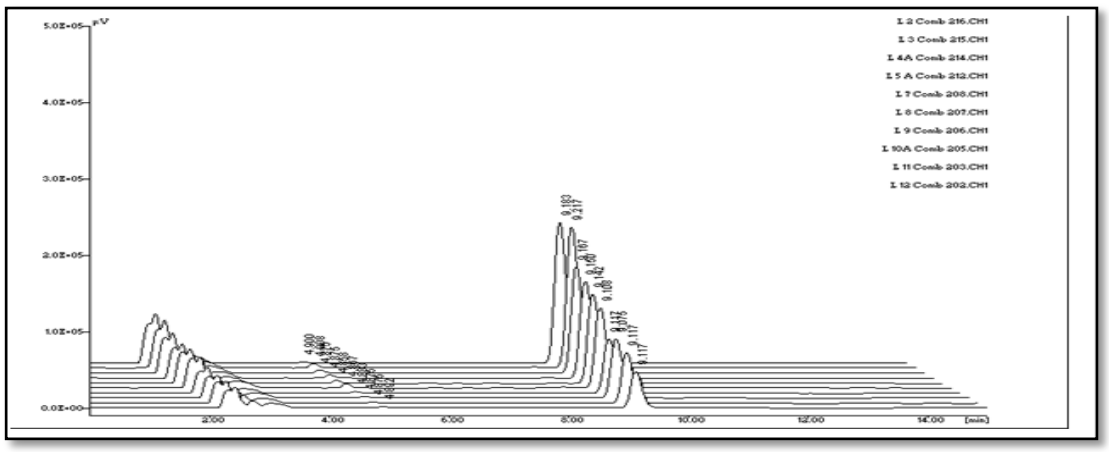

Figure 17: Chromatogram of FFD & FP Linearity

6.2.7.2. Precision:

Table 18: Statistical evaluation for precision studies

Precision Parameter	% Mean*		S.D.*		% R.S.D.*	
	FFD	FP	FFD	FP	FFD	FP
Intra-day	100.21	100.71	1.5834	1.9438	1.5800	1.9401
Inter-day	100.45	99.89	1.7762	1.5264	1.7768	1.5204

*Average of six determinations

6.2.7.3. Specificity:

The chromatogram of capsule sample showed only two peaks at retention time of 4.89 ± 0.02 and 9.18 ± 0.02 min for FFD and FP respectively (Fig. No. 25), indicating that there is no interference of the excipients present in the capsule formulation.

6.2.7.4. Accuracy:.

Table 19: Results of recovery studies for HPLC method

Level of % Recovery	Amount present (µg/ml)		Total amount recovered (µg/ml)		% Recovery	
	FFD	FP	FFD	FP	FFD	FP
80	10.8	180	10.87	181.81	100.70	101.00
	10.8	180	10.68	176.43	98.95	98.01
	10.8	180	10.82	184.23	100.24	100.20
100	12	200	11.76	200.29	98.04136	100.14
	12	200	12.28	201.92	101.1963	100.96
	12	200	12.33	200.61	101.5747	100.30
120	13.2	220	13.30	217.33	100.81	98.78
	13.2	220	13.04	224.01	98.79	101.82
	13.2	220	13.59	223.05	100.69	101.39

Table 20: Statistical validation of recovery data for HPLC method

Level of % Recovery	% Mean Recovery*		S. D.*		% R.S.D.*	
	FFD	FP	FFD	FP	FFD	FP
80	99.96	100.46	1.7862	1.5464	1.7868	1.5504
100	100.27	100.47	1.9400	0.4319	1.9347	0.4298
120	100.10	100.66	0.8292	1.3403	0.8283	1.3314

*Average of three determinations

6.2.7.5. Robustness:

Table 21: Results of robustness testing for HPLC method

Flow Rate (ml/min)	Retention time		Tailing factor	
	FFD	FP	FFD	FP
0.9	4.43	8.39	1.264	1,18
1.0	4.78	9.22	1.26	1.19
1.1	5.45	10.25	1.27	1.20
pH of Buffer				
3.4	3.98	9.08	1.31	1,20
3.5	4.78	9.22	1.26	1.19

6.2.7.6. Limit of Detection and Limit of Quantitation:

Table 22: LOD and LOQ values for HPLC method

Parameter	FFD	FP
*L.O.D. (mcg/ml)	0.730634	0.896917
*L.O.Q. (mcg/ml)	2.214043	2.717931

*Average of three determination

RESULTS AND DISCUSSION

UV SPECTROPHOTOMETRIC METHODS:

Table 23: Result & statistical validation data for marketed formulation by UV spectrophotometric methods

Method	Drug	Wavelength nm	Linearity range(µg/ml)	r^2	Mean %*	S.D.*	LOD (µg/ml)	LOQ (µg/ml)
Simultaneous equation method	FFD	215	2-20	0.996	99.87	1.6310	1.5	1.3
	FP	236	2-20	0.991	100.12	1.63100	2.10	2.0
Absorption ratio method	FFD	215	2-20	0.996	100.86	1.03890	1.6	1.2
	FP	233	2-20	0.991	98.32	0.2869	2.2	2.0
Ist order derivative	FFD	236	2-20	0.999	100.25	1.462	1.2	2.0
	FP	268	2-20	0.990	98.40	1.356	1.5	2.4

*Average of six determination

HPLC Method:

Table 24: Result & statistical validation data for marketed formulation by HPLC method

Drug	Wavelength nm	r^2	Linearity range (µg/ml)	Mean %*	S.D.*	LOD (mcg/ml)	LOQ (mcg/ml)
FFD	215	0.992	2.4-7.8	100.21	1.5834	0.730634	2.214043
FP	215	0.993	10-90	100.45	1.7762	0.896917	2.717931

*Average of six determination

SUMMARY AND CONCLUSION

Three UV spectrophotometric methods have been developed for simultaneous determination of Formoterol Fumarate Dihydrate & Fluticasone Propionate in dry powder Inhalation formulation. The first method employs simultaneous equations (Method I) which involve absorbance measurement at 215nm (λ max of FFD) and 236nm (λ max of FP). Second method involves absorbance ratio (Method II), absorbance measurement at 215nm (λ max of FFD) which takes advantage of the isobestic point at 233nm. Third method involves first order derivative spectroscopy which take advantage of zero crossing point at 236, 268nm respectively Formoterol Fumarate Dihydrate & Fluticasone Propionate.

The developed HPLC method is simple, sensitive and reproducible for the simultaneous determination of Formoterol Fumarate Dihydrate & Fluticasone Propionate in dry powder Inhalation formulation, without any interference from the excipients. The HPLC method includes use of reverse phase HiQ Sil C18HS, 250×4.6mm, 5µm column, at ambient temperature using a mobile phase consisting of Acetonitrile: 0.01 M Ammonium Dihydrogen Phosphate solution (80:20 %v/v) at pH 3.5 adjusted with *o*-phosphoric acid at 1ml/min flow rate. Retention time was found to be 4.89, 9.22 min for Formoterol Fumarate Dihydrate & Fluticasone Propionate, respectively. Quantization was achieved with UV detection at 215nm based on peak area with linear calibration curves at concentration range 2.4-7.8µg/ml (r^2 =0.992) for Formoterol Fumarate Dihydrate and 10-90µg/ml (r^2 =0.993) for Fluticasone Propionate.

The methods have been successively applied to simultaneous determination of Formoterol Fumarate Dihydrate & Fluticasone Propionate in dry powder Inhalation formulation. The methods were successfully validated as per ICH guidelines.

REFERENCES

1. Christen GD, Analytical Chemistry. Fifth edition, John Wiley and Sons, **2003**, 35-42, 131-132.
2. Mendham J, Denney RC, Barnes JD, Thomas M.Vogel's Textbook of Quantitative Analysis. Pearson Education, Singapore, **2003**, 8-9.
3. ICH, Q2 (R1). Validation of analytical procedures: text and methodology, International Conference on Harmonization, Geneva, **2005**, 1- 13.
4. FDA, International Conference on Harmonization: Draft Revised Guidance on Q1A(R) Stability Testing of New Drug Substances and Products, **2000** Federal Register 65 (78), 21446–21453[ICH Q1A(R)].
5. Rang, HP, Dale MM, Ritter JM Flower, R. J. Pharmacology, 6th edition, Published by Churchill Livingstone Elsevier, **2007**, 363-366 .
6. Indian Pharmacopoeia, Vol. II, Govt. of India, Ministry of Health and Family Welfare. New Delhi Published by The Controller of Publications, **2007**, 1142, 1144.
7. British Pharmacopoeia, Vol. I, Published by The Stationery Office on behalf of Medicines & Healthcare Products Regulatory Agency(MHRA), **2009**, 904-906, 913-915.
8. Martindale,The complete drug reference, publishesd by pharmaceutical press, 34^{rth} edition, **2005**, 786.1, 1102.3.
9. Lemke L, Williams DA, Roche VF, Zito SW, Foye's Principles of Medicinal Chemistry, 6th edition, Published by Lipincott Williams & Wilkins, **2008**, 900-901.
10. Munson JW, Pharmaceutical Analysis, Modern methods-Part B, International Medical book Distributors. Mumbai, **2001**, 51-54.
11. Sharma BK Instrumental Methods of Chemical Analysis, 25thedition, Goel Publication Co. Meerut, **1983**, 3- 6.
12. Skoog DA, Holler FJ, Crouch SR. Principle of Instrumental Analysis, 6th edition, Thomson Publications, India, **2007**, 1-3, 145-147, 180.
13. Chatwal, GR. Sharma A. Instrumental Methods of Chemical Analysis, 5th edition, Himalaya Publishing House, Delhi, **2004**, 1.1-1.5.
14. The Merck Index, 13th edition, Published by Merck Research Laboratories, Division of Merck & Co, INC Whitehouse station, NJ, 746(4237),753(4269).
15. Willard HH, Jr. Merritt LL, Dean JA, Jr. Settle, FA Instrumental Methods of Analysis, 7th edition, CBS Publishers and Distributors, Delhi, **2001**, 1-4.
16. Sethi PD, High Performance Liquid Chromatography, Quantitative Analysis of Pharmaceutical Formulations, 1st edition, CBS Publishers and Distributors, New Delhi, **2001**, 3-11, 116-120.