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Research Article

Analytical Method Development and Validation of Formoterol Fumarate Dihydrate By Chromatographic and Spectrophotometric Techniques

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ABSTRACT

Three simple, precise and economical methods have been developed and validated for estimation of Formoterol Fumarate Dihydrate in bulk and in pharmaceutical dosage form. Method A is simple UV spectrophotometric method for estimation of drug in phosphate buffer (pH 3) at 214 nm. Linearity was found in the conc. range of 2-12 µg/ml while the detection and quantitation limits were found to be 0.11 and 0.32 µg/ml respectively. Method B is the first- order derivative spectrometric technique performed at 229 nm, in which linearity was found in the conc. range of 2-12 µg/ml while the detection and quantitation limits were found to be 0.14 and 0.44 µg/ml respectively. Method C is RP-HPLC method in which separation was achieved by gradient elution using an InertsilC_{18} (150 × 4.6 mm) column, a mobile phase consisting of sodium phosphate buffer (0.01M; pH 3.0): acetonitrile (70:30 *v/v*), a flow rate of 1.0 ml/min and UV detection at 214 nm. The linearity was obtained in the conc. range of 2-7 µg/ml while the detection and quantitation limits were found to be 0.04 and 0.14 µg/ml respectively. All the three methods were validated successfully across the guidelines in accordance with ICH. Thus, the proposed methods can be used for routine analysis of formoterol fumarate dihydrate as it does not showed any interference of excipients when estimated in pharmaceutical formulations.

Keywords: Formoterol fumarate, spectrophotometric, chromatographic, validation, ICH guidelines.

INTRODUCTION

Formoterol fumarate dihydrate (FFD) is a long-acting (12 hours) β2-agonist. Beta-blockers block the therapeutic effects of β2-agonist and may produce bronchospasm in patients with asthma and chronic obstructive pulmonary disease[1]. Inhaled FFD works like other β-agonists, causing bronchodilation through relaxation of the smooth muscle in the airway so as to treat the exacerbation of asthma. It is a white to yellowish crystalline powder, which is freely soluble in glacial acetic acid, soluble in methanol, sparingly soluble in ethanol, slightly soluble in water, and practically insoluble in acetone, ethyl acetate, and diethyl ether.

Structurally, formoterol fumarate dihydrate; [Fig. 1] ((RR)-(±)-N-[2-hydroxy-5-[1-hydroxy-2-[[2(4methoxypheny)- 1-methylethyl] amino] ethyl]phenyl] formamide) is a phenyl ethylamine derivative with one phenolic hydroxyl and one secondary amino group, and is widely marketed as a racemate of the enantiomers, which have the RR+SS configuration[2]. The bronchodilation activity of formoterollie with the (R, R) enantiomer and the (S, S) enantiomer does not exert any contractile effects when present in the racemate.

There have been various methods reported for the estimation of FFD as single drug and in combination with the other drugs[3-4]. However, in the available methods, the utilization of solvents was more, which leads to an uneconomic affair [5]. Moreover, till date no single UV spectrophotometric method was reported for its estimation in bulk and pharmaceutical dosage form using buffer as a solvent which is quite economic one. So in the present study an attempt was made to develop the more economical spectrometric and chromatographic methods for estimation of FFD in pharmaceutical dosage form so that it can be use for routine analysis of drug.

EXPERIMENTAL

Instrumentation–

The Shimadzu UV double beam spectrophotometer with the pair of matched quartz cell and UV Probe software was used in the current study. The chromatographic separation was achieved[6] by using Agilent system consisting photodiode array detector, Inertsil C_{18} (150 × 4.6 mm) column.

Chemicals –

The gift sample of Formoterol fumarate dihydrate was obtained. Analytical grade solvents and reagents were purchased from Merck specialties Pvt. Ltd. Mumbai (India). Double distilled water filtered through the membrane filter was used. Foradil capsules each containing 6 mg of active constitution were purchased from the local market.

Preparation of Buffer solution

The 1.56 gm of NaH_2PO_4 $2H_2O$ was weighed and dissolved in 1000 ml double distilled water. The pH of this solution was adjusted to 3 by drop wise addition of

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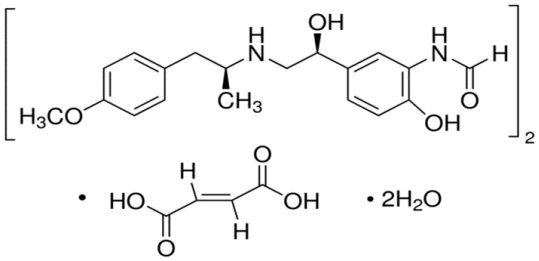

Figure 1: Chemical structure of FFD.

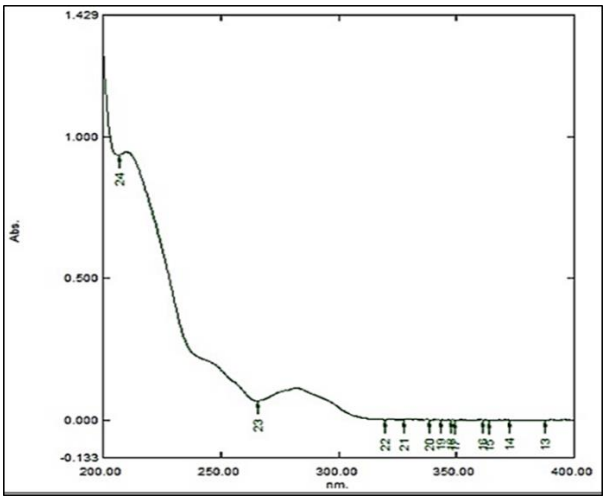

Figure 2: UV spectrum of Formoterol fumarate dihydrate showing λ max at 214 nm.

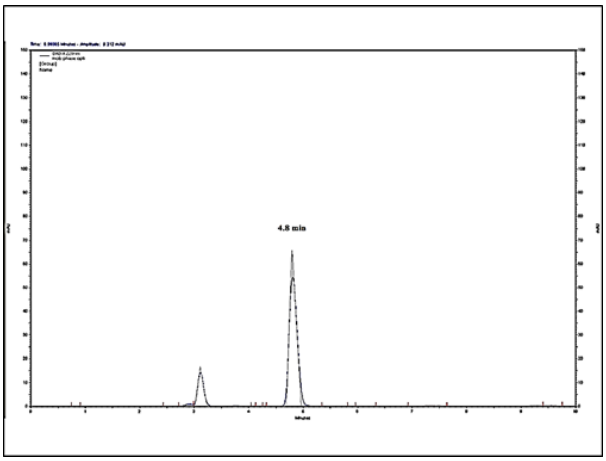

Figure 3: Chromatograph of Formoterol fumarate dihydrate showing retention time 4.8 min.

sufficient amount of ortho phosphoric acid. The buffer is then filtered through a 0.45µm membrane filter and further sonicated.

Preparation of standard stock solution

The 10 mg of FFD was weighed and transferred to the 100 ml volumetric flask, to this 70 ml buffer solution was added, mixed well, sonicated, and the volume was made up to the mark by adding same solvent to obtain the concentration of 100 µg/ml.

Method A: Zero order UV-Visible spectrophotometric method

The solution with concentration of 10 µg/ml was prepared by diluting 1 ml of standard stock solution with 10 ml of buffer solution. This solution was scanned in the UV-region 400 nm -200 nm. Maximum absorbance was obtained at 214 nm as shown in Fig. 2.

Method B: First order UV-Visible spectrophotometric method

The zero order spectrum (λ max 214 nm) was derivatized into first order spectrum (Δ λ = 4, scaling factor = 10) and amplitude of the trough was recorded at 229 nm. The linearity curve was plotted concentration *vs* amplitude of the trough.

Method C: RP-HPLC method

Separately injected 20µl of the blank, standard and sample solution into the chromatographic system, chromatographs were recorded and the peak areas were measured. Different trials were taken for optimization of chromatographic conditions by varying the constitutions of the components of mobile phase. Initially the pH of the buffer was set at higher side in which the peak was appeared to be broad and showed unacceptable tailing. Then it was reduced to pH 3, but still it shows the merging of peaks. Finally the ratio of mobile phase was adjusted to phosphate buffer: ACN (70:30 *v/v*) showed excellent well resolved peak at retention time 4.8 min [Fig. 3].

Validation of proposed methods

The proposed method was validated across the various parameters like linearity, accuracy, precision, ruggedness, sensitivity, repeatability, bulk and pharmaceutical formulation assay[7].

Linearity

The aliquots from concentration 2-12 µg/ml were prepared from stock solution and analyzed to evaluate the linearity of the methods A and B while for method C, the linearity range selected was 2- 7 µg/ml.

Accuracy

To the pre-analyzed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80%, 100% and 120 % and the solutions were re-analyzed by proposed methods.

Precision

The intra-day precision was studied by analyzing 4, 6, and 8 µg/ml of FFD solutions three times a day (i.e. morning, afternoon, evening) and inter-day precision was studied by analyzing these solutions daily for three consecutive days for methods A and B while concentration of 3, 4, and 5 µg/ml was taken for analysis by method C.

Ruggedness

The ruggedness of method was studied for the conc. 6 µg/ml of FFD (method A) and 4 µg/ml (method C) by analysing 6 aliquots from homogenous slot by two analysts using same operational and environmental conditions.

Sensitivity

The sensitivity of method was determined in terms of limit of detection (LOD) and limit of quantitation (LOQ). LOD was calculated by using formula LOD = 3.3 × σ / S. Where, σ = the standard deviation of the response and S = the slope of the calibration curve. LOQ was calculated by formula LOQ = 10 × σ / S. Where, σ = the standard

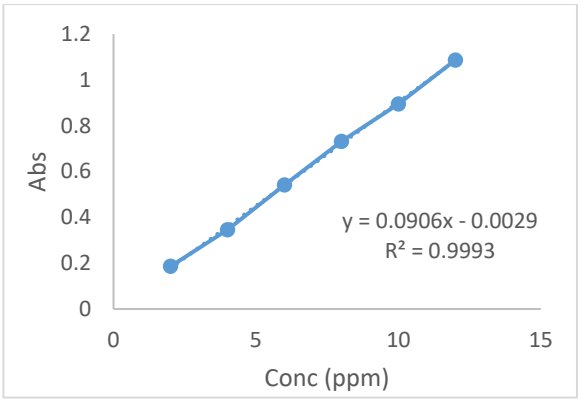

Figure 4a: Linearity curve for Formoterol fumarate dihydrate by zero order estimation.

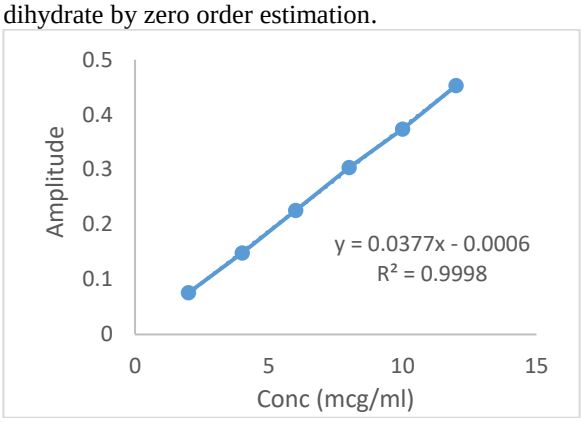

Figure 4b: Linearity curve for Formoterol fumarate dihydrate by first order estimation.

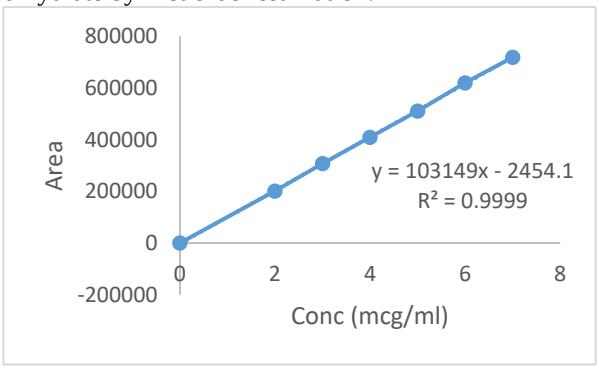

Figure 5: Linearity curve for Formoterol fumarate dihydrate by RP-HPLC.

Table 1: Linearity studies of Formoterol Fumarate Dihydrate..

Method A & B		Method C		
Conc. (µg/ml)	Absorbance	Amplitude	Conc. (µg/ml	Area
2	0.186	0.078	2	200577
4	0.346	0.147	3	307564
6	0.542	0.226	4	409459
8	0.731	0.304	5	510641
10	0.895	0.374	6	620648
12	1.087	0.453	7	718948

deviation of the response and S = the slope of the calibration curve.

Repeatability

The repeatability of method was determined by analyzing six solutions of FFD having concentration 6 µg/ml and 4 µg/ml for spectroscopic and chromatographic methods respectively.

Determination of Formoterol Fumarate Dihydrate in bulk

In spectroscopic methods A and B; the aliquots of 6 µg/ml solution were prepared from standard stock solution. The resulting solution was scanned at 214nm (for zero order) and amplitude was measured at 229nm (for first order) respectively. The concentrations of the drug were calculated from linear regression equations. In Chromatographic method C, the aliquots of 6 µg/ml solution were prepared from standard stock solution. The resulting solution was injected in system and concentration of the drug calculated from linear regression equation.

Application of proposed methods to pharmaceutical formulation

The 100 Foradil capsules were taken and the contents were collected by removing the capsule shell and weighed. Concentration equivalent to 1.2 mg was weighed accurately and transferred to the 10 ml volumetric flask. To this 7 ml buffer solution was added, mixed and sonicated. Then the volume was made up to the mark by addition of same solvent to obtain desired conc. 120 µg/ml and it was filtered through 0.45 µm membrane filter. From this solution 0.5 ml was taken and diluted upto 10 ml with same solvent. The resulting solution was scanned at 214 nm (method A), amplitude was measured at 229nm (method B) and injected in the HPLC system (method C) respectively. The concentrations of the drug were calculated from linear regression equations.

Robustness

Robustness study was done by changing few chromatographic parameters deliberately as applicable for method C. The aliquots of 6 µg/ml were injected into the HPLC system by differing flow rates of mobile phase by ±0.2ml/min and pH of buffer in the mobile phase by ±0.2.

RESULTS AND DISCUSSION

The proposed methods (A, B and C) was validated across the various parameters like linearity, accuracy, precision, ruggedness, sensitivity, repeatability, bulk and pharmaceutical formulation assay as per ICH guidelines.

Linearity

The aliquots of 2-12 µg/ml were prepared from standard solution and scanned at 214 nm and 229 nm. The linear regression data for the calibration curves showed good linearity in the selected concentration range. The linear regression equation was found to be y = 0.0906x – 0.0029 and y = 0.0376x + 0.0003 for zero order and first order respectively while a good correlation coefficient (r^2 = 0.9993 & r^2 = 0.9996) for both the methods A and B [Fig. 4]. Also, the linear regression equation for method C was found to be y = 103780x – 5702.8 with r^2 = 0.9998 [Fig. 5]. The results of linearity are shown in Table 1.

Accuracy

The results of recovery studies shown in Table 2 indicate that the amount found was in between 98.79% to 99.45% for all the three methods with % R.S.D less than 2. This is an indicative that the proposed methods are accurate.

Table 2: Accuracy studies of Formoterol Fumarate Dihydrate.

Methods	at 80% % recovery* ± SD	% RSD	at 100% (n=3) % recovery* ± SD	% RSD	at 120% (n=3) % recovery* ± SD	% RSD
A	98.79± 0.35	0.35	99.45 ± 0.46	0.46	99.02± 0.31	0.32
B	99.42 ± 1.39	1.40	99.33 ± 0.44	0.44	99.15 ± 0.56	0.56
C	99.20 ± 0.02	0.44	99.11 ± 0.01	0.16	99.19 ± 0.05	0.11

*(n=3)

Table 3: Precision studies of Formoterol Fumarate Dihydrate.

Methods	Conc. (µg/ml)	Intra-day precision % Amount found*± SD	% RSD	Inter-day precision % Amount found*± SD	% RSD
A	4	98.91 ± 0.66	0.67	99.34 ± 0.66	0.67
	6	99.40 ± 0.47	0.47	99.27 ± 0.43	0.44
	8	99.67 ± 0.35	0.35	99.36 ± 0.21	0.21
B	4	99.60 ± 1.33	1.34	98.86 ± 1.38	1.40
	6	99.65 ± 1.29	1.30	99.45 ± 0.62	0.62
	8	99.63 ± 0.56	0.57	99.41 ± 0.62	0.62
C	3	98.94 ± 1.38	1.39	99.20 ± 1.36	1.37
	4	99.03 ± 1.23	1.25	98.89 ± 1.01	1.02
	5	99.26 ± 1.35	1.36	99.19 ± 0.93	0.94

*(n=3)

Table 4: Ruggedness studies Formoterol Fumarate Dihydrate.

Methods	Conc. (µg/ml)	Analyst I % Amount found*± SD	% RSD	Analyst II % Amount found*± SD	% RSD
A	6	99.68± 0.49	0.49	99.80± 0.47	0.47
B	6	99.52 ± 0.65	0.65	99.45 ± 0.77	0.78
C	4	99.57 ± 0.58	0.58	99.47 ± 1.07	1.07

*(n=6)

Table 5: Repeatability studies of Formoterol fumarate dehydrate.

Methods	Amount taken (µg/ml)	Amount found*	% Amount found*± SD	% RSD
A	6	5.98	99.77 ± 0.49	0.49
B	6	5.94	99.15 ± 0.62	0.63
C	4	3.96	98.91 ± 1.24	1.26

*(n=6)

Table 6: Analysis of Formoterol fumarate dihydrate in bulk.

Methods	Amount taken (µg/ml)	Amount found*	% Amount found*± SD	% RSD
A	6	6.01	100.21 ± 0.55	0.54
B	6	5.95	99.23 ± 0.65	0.65
C	6	5.98	99.65 ± 1.12	1.12

*(n=6)

Precision

The precision of the developed method was expressed in terms of % RSD as intraday and inter-day precision by analyzing 4, 6, and 8 µg/ml solutions (method A and B) and 3, 4, and 5 µg/ml (method C). The % RSD found to be less than 2, indicating that the method is precise for the determination of the drugs in formulation. The results are tabulated in Table 3.

Table 7: Analysis of Formoterol fumarate dihydrate in capsule formulation.

Methods	Amount taken (µg/ml)	Amount found*	% Amount found*± SD	% RSD
A	6	5.98	99.71 ± 0.39	0.39
B	6	5.95	99.23 ± 0.43	0.43
C	6	5.97	99.46 ± 0.87	0.88

*(n=6)

Ruggedness

Ruggedness of the proposed methods was determined for selected concentrations. The amount found was close to 100% and % RSD was found to be less than 2, indicating that the method is rugged. The detail results are tabulated as in Table 4.

Repeatability

The mean of all estimations in repeatability indicate that the amount found was in between 98% to 102% with % RSD less than 2. Thus it showed that the repeatability studies are complying for the zero order, first order and RP-HPLC methods. The results are tabulated in Table 5.

Analysis of Formoterol fumarate dihydrate in bulk

The aliquots of 6 µg/ml solution were prepared from standard stock solution. The resulting solution was scanned at 214 nm (method A) and amplitude was measured at 229 nm (method B) respectively. After injecting the sample solution in the HPLC system (method C), the concentrations of the drug were calculated from

Table 7: Sensitivity studies of Formoterol fumarate dehydrate.

Methods	Linear regression equation	LOD (µg/ml)	LOQ(µg/ml)
A	y = 0.0906x – 0.0029 (r^2 = 0.9993)	0.11	0.32
B	y = 0.0376x + 0.0003 (r^2 = 0.9996)	0.14	0.44

Table 9: Robustness studies of Formoterol fumarate dehydrate.

Parameters	Amount taken (µg/ml)	Variations	Mean area ± SD
Flow rate of mobile phase	6	0.8 ml/min	401828±1.69
		1.0 ml/min	404829 ± 1.10
		1.2 ml/min	401870 ± 1.27
pH of buffer in mobile phase	6	Buffer pH 2.8	386457 ± 1.45
		Buffer pH 3.0	408277 ± 0.39
		Buffer pH 3.2	398724 ± 0.31

linear regression equation. The results of bulk analysis indicate that amount found was almost 100% and % RSD is within the acceptable range as shown in Table 6.

Analysis of FFD in formulation

The concentration of the drug was calculated in the pharmaceutical capsule formulation from linear regression equations and the results obtained are shown in Table 7. The obtained results signifies no interference of excipients from the formulation as the amount found is more than 99% with % RSD less than 2.

Sensitivity

The different aliquots were prepared in between concentration 2-4 µg/ml and scanned at 214nm (method A) and amplitude were measured at 229nm (method B). The LOD and LOQ for FFD were calculated and the results obtained are shown in Table 8. The LOD & LOQ values are in good agreement with the standards indicating that the proposed methods A and B are sensitive.

Robustness

The results of robustness study indicate that the method is highly robust. The result obtained by changing the flow rate of mobile phase and concentration of buffer is within the highly acceptable range. The overall results are tabulated in Table 9

Thus both the spectroscopic methods (A & B) are found to advantageous and can be applicable to capsule dosage form which is more suitable as compare to inhalation[8] and same is applicable to method C i.e. RP-HPLC as reported earlier[9].

CONCLUSION

The spectrophotometric and chromatographic methods are developed and validated successfully for estimation of Formoterol fumarate dihydrate. The estimation was done by taking bulk as well as in pharmaceutical capsule formulation. The results of the analysis of pharmaceutical formulation by the proposed methods are reproducible and reliable. This indicates that there is no interference of excipients. All these developed spectrophotometric and chromatographic methods are simple, accurate, precise, robust and economical and can be used for routine analysis of selected drug candidate.

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