

Reverse Phase High Performance Liquid Chromatography Analysis of Formoterol Fumarate in Bulk and Its Pharmaceutical Formulations

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A sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the assay of formoterol fumarate in bulk and its pharmaceutical formulations. The separation was achieved by isocratic elution using a hypersil BDS C-18 (250 × 4.6 mm) column, using bambuterol as internal standard in a mobile phase consisting of ammonium acetate (50 mM, pH 5.0)-ethanol (60 : 40, v/v), a flow rate of 1.0 mL/min and UV detection at 242 nm. The detection and quantitation limits were 0.05 and 0.15 µg/mL, respectively, while the linear range of detection was between 0.1 and 100 µg/mL. The results of analysis were treated statistically, as per International Conference on Harmonization (ICH) guidelines for validation of analytical procedures and by recovery studies. The study shows that the proposed method is an accurate, linear and sensitive assay method for formoterol fumarate in bulk and its pharmaceutical formulations.

Key Words: RP-HPLC, Formoterol fumarate, Validation, Pharmaceutical formulation.

INTRODUCTION

Formoterol (FOR) is a long-acting beta-2-adrenoreceptor agonist with demonstrated bronchodilatory effects and rapid onset of action¹. As a result of its prolonged duration of action, formoterol appears to be more effective than shorter-acting beta-2-agonist in the treatment of nocturnal and exercise-induced asthma^{2,3}. Structurally, formoterol, ((RR)-(±)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]ethyl]phenyl] formamide) is a phenylethylamine 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. The antibronchoconstrictor activity of formoterol lies with the (R, R) enantiomer and the (S, S) enantiomer does not exert any contractile effect when present in the racemate⁴.

There have been limited reports on chromatographic assay of FOR in bulk drugs. Thus Graham *et al.*⁵ described a liquid chromatographic assay method for FOR in which the drug and a related compound formoterol fumarate acetamide analog were nearly baseline separated on a short octyl bonded silica column using water-acetonitrile-trifluoroacetic acid (800 : 200 : 0.5, v/v/v) as the mobile phase. Despite its high sensitivity, the method appears not to be a good choice for potency assay of formoterol fumarate in bulk drug because of the poor hydrolytic stability

of drug in low pH environment ($\text{pH} < 4.0$)⁶. Other chromatographic techniques available in literature are primarily developed for the determination of FOR in biological fluids⁷⁻¹⁰. Beside the non-specific potentiometric titration method described in the Japanese Pharmacopoeia (JP) monograph for the drug¹¹, our literature searches did not reveal the existence of standard methods of pharmacopoeias for potency assay of formoterol fumarate in active pharmaceutical ingredients. Consequently, alternate simple, fast and specific chromatographic method assay of formoterol fumarate in bulk drugs must be developed and validated because of the increasing attention to develop the drug in various dosage forms for the treatment of asthma.

EXPERIMENTAL

Quantitative HPLC was performed on a gradient high pressure liquid chromatograph (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength programmable diode array detector SPD-M10AS VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu). The HPLC system was equipped with the software "Class-VP series (Shimadzu)".

Ammonium acetate (Qualigens) and ethanol (Qualigens) are commercially procured.

Preparation of standard drug solution: A stock solution (1 mg/mL) of pure drug was prepared by dissolving 100 mg of FOR and 100 mg of bambuterol (internal standard) separately in 100 mL volumetric flasks containing 70 mL of ethanol, sonicated for at least 15 min and then made up to volume with ethanol. Daily working standard solutions of FOR and internal standard were prepared by suitable dilution of the stock solution with appropriate mobile phase.

Method development: To develop an HPLC method for the analysis of formoterol fumarate in formulations, different solvent systems were used. The criteria employed for assessing the suitability of a particular solvent system for the drug were cost, time required for analysis, sensitivity of the assay, solvent noise, preparatory steps involved and use of the same solvent system for extraction of the drug from the formulation excipients matrix for estimation of the drug content.

Preparation of standard curve: Composition and flow rate of the mobile phase was programmed from motor pump and the mobile phase ammonium acetate : ethanol (60 : 40 v/v) was passed through the 0.45 μm membrane filter using Millipore HPLC solvent filtration assembly and delivered at 1.0 mL/min for column stabilization. During this period, the baseline was continuously monitored. The wavelength of detection was selected at 242 nm. The prepared dilutions containing concentrations of formoterol fumarate in the range 0.1–100 $\mu\text{g/mL}$ and fixed concentration (5 $\mu\text{g/mL}$) of internal standard (bambuterol) were injected into the chromatograph. The peak area ratios to the internal standard were calculated. The stability of the solution of formoterol fumarate during analysis was determined by repeated analysis of samples during the course of the experiment on the same day and also on different days after storing at laboratory

bench conditions and in the refrigerator. Chromatogram parameters, retention time and asymmetry factor were standardized.

Chromatographic conditions:

Parameters	Method
Stationary phase (column)	Hypersil BDS C-18 (250 × 4.6 mm, packed with 5 micron)
Mobile phase	Ammonium acetate (50 mM; pH 5.0) : Ethanol (60 : 40 v/v)
Flow rate (mL/min)	1.0
Column back pressure (psi)	1200
Run time (min)	20 min
Column temperature (°C)	Ambient
Volume of injection loop (μL)	20
Detection wavelength (nm)	242
Internal standard	Bambuterol
Drug RT (min)	10.42
Internal standard RT (min)	14.54

Method validation

Accuracy and precision: Five separate solutions of formoterol fumarate (4 μg/mL) standard and test solution were prepared in duplicate from freshly prepared stock solution and analyzed as per the procedure.

Linearity: Five separate series of solutions of the drug 0.1–100 μg/mL were prepared from the stock solution and analyzed.

Specificity: Series of five solutions of the drug in 4 μg/mL were prepared from the stock solution meant for the method validation and analyzed.

Limit of detection (LOD) and quantitation (LOQ): LOQ and LOD were calculated on the basis of signal to noise ratio. Experiments were performed to analyze the actual concentration that can be accurately quantified or detected by the method.

Ruggedness: It was determined for the method by varying the analyst, instrument and different columns of it for the proposed method.

Robustness: For determining the robustness of the method, the percentage of ethanol was varied (35, 40, 45%) and the effects on retention time and peak parameters were studied.

Estimation of formoterol from the commercial formulations by the proposed method: Commercially available capsules of formoterol fumarate were taken randomly from the Indian market for estimation of total drug content per capsule by the proposed method. 20 capsules were weighed and the contents were thoroughly mixed; aliquot amount (equivalent to 100 mg formoterol fumarate) was dissolved in 20 mL of ethanol. The weighed amount (100 mg) of active ingredient was extracted with ethanol and made up to get a stock solution of 1 mg/mL. This solution was filtered through a 0.45 μm membrane filter and was further diluted stepwise with mobile phase as under preparation of standard

solution to get the different concentrations required. The area under the curve, the drug content per capsule (on an average weight basis), was calculated. The results are tabulated in Table-3.

RESULTS AND DISCUSSION

For the determination of formoterol fumarate different mobile phases were employed. Initially, a mobile phase consisting of ammonium acetate : ethanol in the ratio of 40 : 60 was tried. Symmetry RP-C18 columns 242 nm was used. Early elution with tailing of peaks was observed in the above condition. Then the composition of mobile phase was changed to 50 : 50. Under these conditions broad peak shape and pronounced tailing was observed. For the same mobile phase, if the ratio was changed to 60 : 40, formoterol fumarate was eluted at around 10.42 min with symmetric peak shape.

A typical chromatogram for formoterol fumarate using C18 RP HPLC column with mobile phase, composed of ammonium acetate (60 : 40) at 1.0 mL/min flow rate is shown in Fig. 1. The λ_{\max} of detection was fixed at 242 nm so that there was less interference from mobile phase with highest sensitivity according to UV analysis.

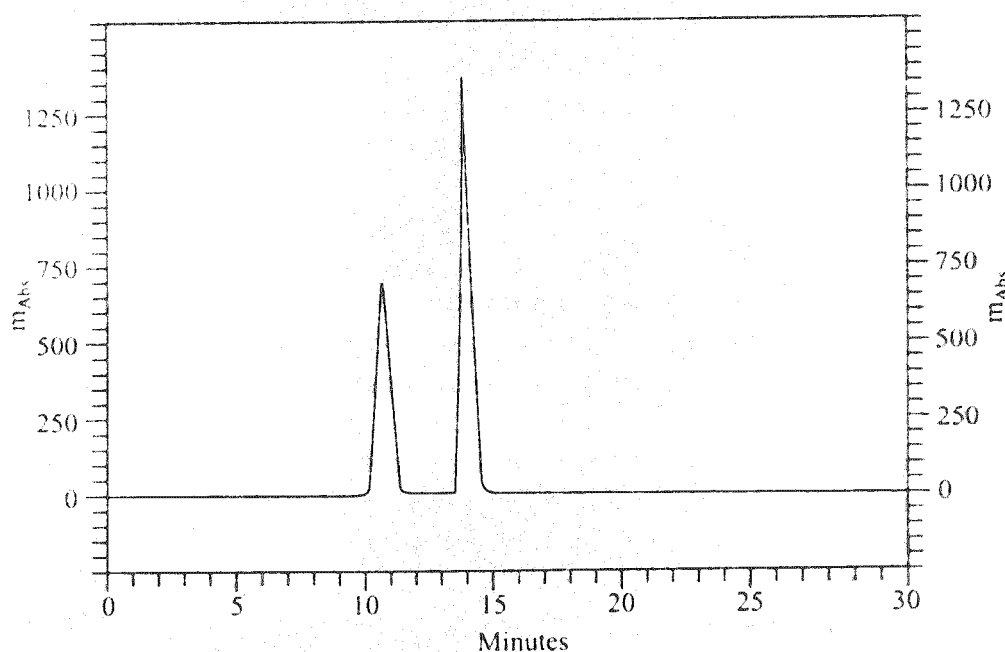


Fig. 1. Model chromatogram for formoterol fumarate (FOR)

The peak area ratio to the internal standard vs. concentration ($\mu\text{g/mL}$) was found to be linear. Values obtained for the calibration curve points and their standard deviation, coefficient of variance and standard error are presented in Table-1.

The linear regression equation obtained for the proposed methods was $Y = 0.3869X + 0.00449$, ($r = 0.9993$) where Y is the peak area ratio to the internal standard and X is the concentration of formoterol fumarate.

TABLE-1
OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY
OF THE PROPOSED HPLC METHOD FOR FORMOTEROL FUMARATE

Parameter	Method
Detection wavelength (nm)	242
Linearity range ($\mu\text{g/mL}$)	0.1-100
Detection limits ($\mu\text{g/mL}$)	0.03924
Regression equation ($Y = a + bC$)	
• Slope (b)	0.3869
Standard deviation of slope (S_b)	8.32×10^{-3}
Intercept (a)	0.00449
Standard deviation of intercept (S_a)	5.06×10^{-3}
Standard error of estimation (S_e)	6.44×10^{-3}
Correlation coefficient	0.9993

The developed method was validated according to the standard procedure¹² and the results obtained are presented in Table-2.

TABLE-2
VALIDATION REPORT FOR THE DETERMINATION OF FORMOTEROL FUMARATE
IN STANDARD SOLUTIONS BY HPLC METHOD

Analytical parameter	Results
Accuracy (%)	99.7 ± 0.28
Precision (%)	100.4 100.9 99.9 101.0 99.7 RSD* = 0.61
Linearity	0.1-100 ($\mu\text{g/mL}$)
Specificity	A 4 $\mu\text{g/mL}$ solution of formoterol fumarate will give an area of 347210.736 ± 0.35 at 242 nm using RP-C18 column in ammonium acetate : ethanol (60 : 40) mobile phase at a flow rate of 1.0 mL/min
Limit of detection	0.05 $\mu\text{g/mL}$
Limit of quantitation	0.15 $\mu\text{g/mL}$
Ruggedness (%)	98.95 ± 0.68

*Relative standard deviation

Recovery studies: The method was evaluated by estimation of FOR in pharmaceutical formulations by the proposed method and analysis of pure drug solution as reference. The results are presented in Table-3. The estimated drug content with low values of standard deviation established the precision of the proposed method. The accuracy of results of estimation was further tested by recovery experiments by adding known amount of pure drug to pre-analyzed

samples of the formulations. Common formulation excipients in the concentration normally used had no effect. Recovery experiments using the developed assay procedures further indicated the absorbance of interference from commonly encountered pharmaceutical excipients used in the selected formulations.

TABLE-3
ASSAY AND RECOVERY OF FORMOTEROL FUMARATE

Pharmaceutical formulation	Labelled amount (μg)	Amount obtained by proposed method	Recovery of proposed method (%)
*ROJACAP	12	11.97	99.75
*MDI	12	11.99	99.91

*Different manufacturers

Interference studies: The effect of wide range of excipients and other additives usually present in the formulation of formoterol fumarate in the determination under optimum conditions were investigated. The common excipients like starch, talc, magnesium stearate, methyl and propyl parabens, cellulose derivatives and propylene glycol have been added to the sample and injected. They have not disturbed the elution or quantification of drug or internal standard. In fact many have no absorption at this UV maximum.

Conclusion

The proposed method of estimation of formoterol was found to be accurate, precise and easy. The same recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. This method was used for routine analysis of formoterol in pure form and its formulations and can also be used for dissolution or similar studies.

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