International Journal of Pharmaceutics & Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF FORMOTEROL FUMARATE IN MARKETED FORMULATION BY USING RP-HPLC

Nisha Manjhi* Narottam Singh, Muraree Lal, Munesh Singh Bhadouria, Avinash K Kondalkar

Sun Institute of Pharmaceutical Education & Research (SIPER), Lahar

***Correspondence Info:**
Nisha Manjhi
Sun Institute of Pharmaceutical Education & Research (SIPER), Lahar
Email:
nishamanjhi01@gmail.com

***Article History:**

Received: 21/05/2023
Revised: 04/06/2023
Accepted: 20/06/2023

ABSTRACT

In the present research work, a successful attempt was made for Validated HPLC method development for the estimation of Formoterol fumarate in marketed formulation which was developed by experimentation based on thorough literature survey and ascertained by statistical parameters of sampling. Liquid chromatographic system from waters comprising of manual injector, Waters 515 binary pump for constant flow and constant pressure delivery and U.V. detector connected to data ace software controlling the instrumentation as well as processing Methanol: Acetonitrile in the ratio of 50:50 v/v at a flow rate of 1.0 ml min^{-1}. A thermo C-18 column (4.6 x 250mm, 5µ particle size) was used as the stationary phase, 300nm was selected as the detection wavelength for UV-vis. detector. Proposed method was found to be linear in the range of 1-5µg/ml Formoterol fumarate with the correlation coefficient near to one respectively. The validation and the reliability of proposed method were assessed by recovery study. The recovery of added standards (80%, 100% 120%) was ranging from 99.20±0.230 to 99.77±0.040%, for Formoterol fumarate. The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in marketed formulation.

Key words: Formoterol fumarate, Method development, HPLC, Validation.

INTRODUCTION:

Analytical method development and validation plays an important role in the discovery, development and manufacture of pharmaceuticals. These methods used to ensure the identity, purity, potency and performance of drug products. There are many factors to consider when developing methods. The initially collect the information about the analyte's physiochemical properties (pKa, logP, solubility) and determining which mode of detection would be suitable for analysis. The majority of the analytical development effort goes into validating a stability indicating HPLC method. The goal of the HPLC method is to try and separate quantify the main active drug, any reaction impurities, all available synthetic intermediates and degradants (Sethi, 1996; Davidson *et al.*, 1989; Jeffery *et al.*, 1989).

Analysis can be divided in to two classes, i.e. Qualitative analysis and Quantitative analysis. Qualitative analysis gives an indication of the identity of the chemical species in the sample. Quantitative analysis estimates, how much quantity is present in a mixture. Modern analytical chemistry is functioning by instrumental analysis. Separation of components in a mixture is based on their interaction between a stationary and a mobile phase. These interaction differences are achieved based on the properties such as polarity, electric charge (for ionic compounds), pH, functional groups and size of the molecule (Swarbrick *et al.*, 1998; Sahu *et al.*, 2006).

High-performance liquid chromatography is a separation technique based on a solid stationary phase and a liquid mobile phase. Separations are achieved by partition, adsorption, or ion exchange processes, depending upon the type of stationary phase used (Jain *et al.*, 2009; Khan and Jain, 2006).

Most of the drugs in multicomponent dosage forms can be analyzed by HPLC method for the reason that of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC method eliminates tiresome extraction and isolation procedures. HPLC method development is not very difficult when literature reference for the same or similar compounds to be analyzed can be found. Literature survey revealed that various analytical methods like HPLC have been reported for the determination of Formoterol fumarate and either individually or combination with some other drugs, but no stability indicating HPLC method was reported for estimation of Formoterol fumarate in dosage forms. The aim of this study is to develop and validate a reliable and accurate HPLC method for the estimation of Formoterol fumarate.

MATERIALS AND METHODS

Selection of Mobile Phase

Initially to estimate Formoterol fumarate in fix dosage form number of mobile phase in different ratio were tried.

Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol in the ratio of 50:50 v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Preparation of standard stock solution Accurately weighed 10 mg of Formoterol fumarate was transferred into 50 ml volumetric flasks and dissolved in 10 ml of methanol, then volume was made up to 50 ml with acetonitrile and vortex it to get complete dissolution of drug. Stand it aside for few minute, Concentration of Formoterol fumarate was 200µg/ml. (Stock- A)

Preparation of Sub Stock Solution 5 ml of solution was taken from stock-A of Formoterol fumarate transferred into 10 ml volumetric flask separately and diluted up to 10 ml with diluent (methanol) to give concentration of 100µg/ml (Stock-B).

Preparation of Different Solution

0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the

solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, 5µg/ml for drug.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 1-5 µg/ml was prepared. All the solution were filtered through 0.2µm membrane filter and injected, chromatograms were recorded at 254nm and it was repeat for three times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

Validation of developed method

A. Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 1 to 5µg/ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure 6.5. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components (Lavanya *et al.*, 2013).

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The precision are established in three differences:

1. **Repeatability**

The repeatability was performed for five replicate at five concentrations in linearity range 1, 2, 3, 4 and 5 µg/ml for Formoterol fumarate indicates the precision under the same operating condition over short interval time.

2. **Intermidiate Precision**

a) Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days in five replicate at five concentrations.

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, methanol: acetonitrile (50:50 % v/v), to (45:55 % v/v).

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of marketed formulation

Amount equal to 1 mg of Formoterol fumarate Inhalation Solution was taken in 10ml volumetric flask. The volume is made up to the mark by mobile phase and filtered by whatmann filter paper (no.41) and the filtrate was used to prepare samples of 1µg/ml. This solution is injection 20µl and records the peak area and calculate percentage using caliberation curve method.

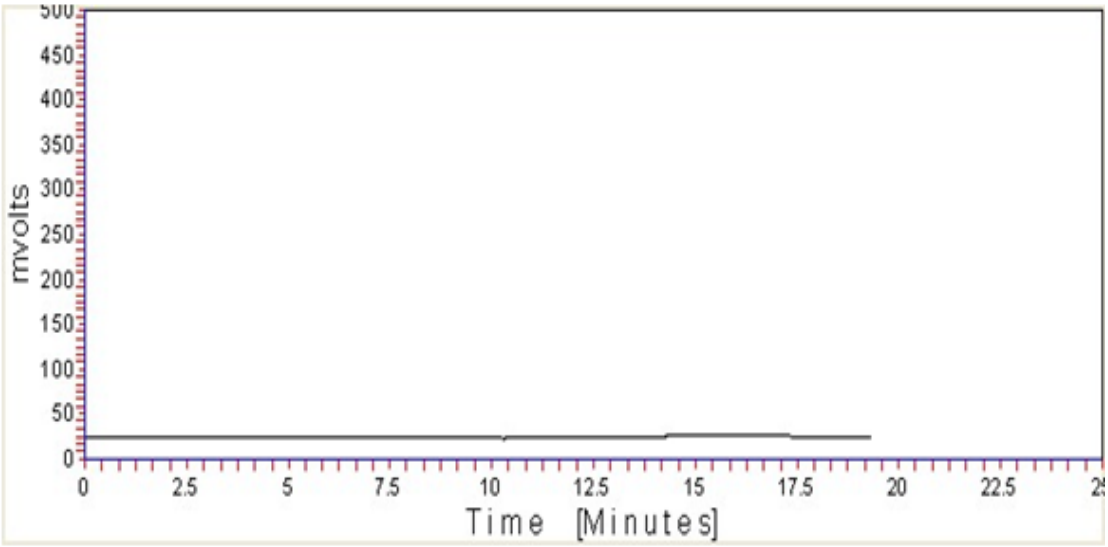

Figure 1: Chromatogram of blank diluent

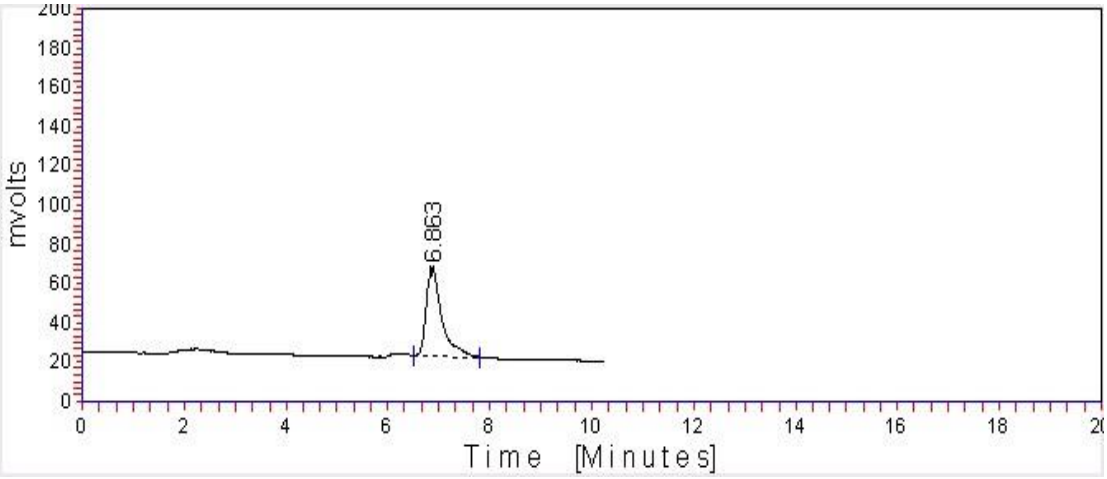

Figure 2: Chromatogram of pure drug

Table 1: Results of system suitability parameters

Parameters	Formoterol fumarate
No. of Theoretical Plates	3200.83
Tailing Factor	1.1467
Retention time	6.8615

Table 2: Results of linearity of Formoterol fumarate

Parameter	Formoterol fumarate
Conentration(µg/ml)	1-5
Correlation Coefficient (r^2)*	0.999
Slope (m)*	132.5
Intercept (c)*	1.949

Table 3: Results of recovery study

% Level	% Mean±SD*
80%	99.48±0.480
100%	99.20±0.230
120%	99.77±0.040

***** Value of three replicate and three concentrations

Table 4: Results of Precision and Robustness

Parameter	% MEAN±SD*
Repeatability	97.89±0.05
Intermediate precision	
Day to day precision	97.53±0.05
Robustness	97.64±0.03

***** Value of five replicate and five concentrations

Table 5: Assay of tablet formulation

S. No.	Parameter	Formoterol fumarate
1.	Mean	99.96
2.	S. D.	0.225
3.	% RSD	0.246

RESULTS AND DISCUSSION

The RP-HPLC method was developed for estimation of Formoterol fumarate in bulk and tablets dosage form by isocratically using methanol: Acetonitrile in the ratio of 50:50 v/v as mobile phase, Thermo C-18 column (4.6 x 250mm, 5µparticle size) column as stationary phase and chromatogram was recorded at 254nm. Then developed method was validated by using various parameters.

The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 10µg/ml of Formoterol fumarate were injected separately and chromatogram was recorded.

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatogram was recorded.

Specificity of the method was determined and the peaks of diluent, mobile phase and excipient of tablets did not interfere with standard peaks Formoterol fumarate. The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less then 2 indicate the accuracy of method.

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less then 2 indicate the precision of method. Result of precision shown in table 4. The robustness of developed method was checked by changing in the deliberate variation in solvent. Detection limit and quantitation limit of described method were observed as 0.15 µg/ml, and 0.45 µg/ml respectively based on the SD of response and slope, which meet the requirement of new method. The results of the analysis of tablets formulation were reported. The assay value of drugs was close to 100, SD and % RSD are less then 2 indicate the no interference of excipient in the estimation of drug.

CONCLUSION

The proposed methods were found to be linear in the range of 1-5 µg/ml with correlation coefficient close to one. Precision was determined by repeatability, Intermediate precision and reproducibility of the drugs. The robustness of developed method was checked by changing in the deliberate variation in solvent. The result obtained shows the developed method to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value of SD and % RSD less than 2), Precise and can be successfully employed in the routine analysis of these drugs in bulk drug as well as in tablet dosage form. The Simplicity, Rapidly and Reproducibility of the proposed method completely fulfill the objective of this research work.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Sethi, P.D. (1996). *HPLC: Quantitative Analysis of Pharmaceutical Formulation*. CBS Publishers and Distributors: New Delhi, pp. 113–202.
- Davidson, A.G., Beckett, A.H. & Stenlake, J.B. (1989). *Practical Pharmaceutical Chemistry*, 4th edn. CBS Publishers and Distributors: New Delhi, pp. 276–299.
- Jeffery, G.H., Bassett, J., Mendham, J. & Denrey, R.C.; Vogel's Textbook of Quantitative Chemical Analysis (1989), 5th edn. Longman Group Uk Ltd: England, pp. 6–14.
- Swarbrick, J.B. & James, C. (1998). *Encyclopedia of Pharmaceutical Technology*, Vol. I. Marcel Dekker, Inc.: New York, USA, pp. 217–224.
- Sahu, R., Nagar, P. & Jain, D. (2006). *Indian Journal of Pharmaceutical Sciences*, 68, 503–506.
- Jain, N., Jain, R., Swami, H. & Pandey, S. (2009). *Indian Journal of Pharmacy and Pharmaceutical Sciences*, 1, 189–191.
- Khan, M.R. & Jain, D. (2006). *Indian Journal of Pharmaceutical Sciences*, 64, 546–548.
- Lavanya, G., Sunil, M., Eswarudu, M.M., Eswaraiah, M.C., Harisudha, K. & Spandana, B.N. (2013) Analytical method validation: An updated review. *International Journal of Pharmaceutical Sciences and Research*, 4, 1280.