

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF FORMOTEROL BULK DRUG AND ITS PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Three simple, sensitive, selective, accurate, precise and economical methods (method A, B and C) have been developed for the quantitative estimation of Formoterol in bulk drug and its pharmaceutical formulations. Methods were based on the formation of coloured chromogens. Which were measured at 520nm, 756nm, and 618 nm. Linearity range was found to be 2-10 µg/ml in all the three methods, the results of analysis for both the methods have been validated statistically and by recovery studies. The proposed methods are economical and sensitive for estimation of Formoterol in bulk drug and its rotacaps dosage form.

Key words: Formoterol, 2, 2'- Bipyridyl, Potassium ferericycnide ferric chloride, Gibb's reagent visible spectroscopy

INTRODUCTION

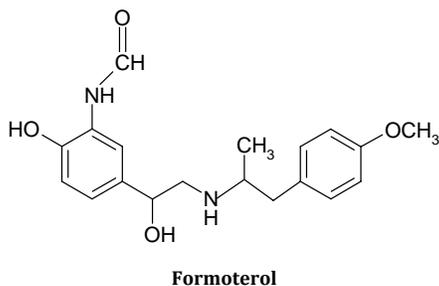
Formoterol¹⁻² is a long-acting beta2-agonist used in the management of asthma and/or chronic obstructive pulmonary disease (COPD). Inhaled formoterol works like other beta2-agonists, causing bronchodilatation by relaxing the smooth muscle in the airway so as to treat the exacerbation of asthma.

N-[2-hydroxy-5-[1-hydroxy-2-[1-(4-methoxyphenyl)propan-2-ylamino] ethyl] phenyl] formamide phenylethylamine derivative with one phenolic hydroxyl and one secondary amino group.

Objective

The aim of this study Formoterol fumarate is latest anti asthmatic drug. It is available in rotacap dosage form. It is official in Indian pharmacopoeia.³ and British pharmacopoeia⁴. The literature survey reveals that one spectrophotometric⁵, few HPLC Chromatographic⁶ analytical methods have been reported for determination of formoterol in rotacaps. In present investigation we have developed a simple isocratic RP-HPLC method for quantitative estimation of Formoterol fumarate in bulk drug and pharmaceutical formulations with high accuracy and precision.

Hence in the present work spectrophotometric methods has been developed for the estimation of Formoterol using 2,2'- Bipyridyl in presence of Ferric chloride in method A, Potassium Ferericycnide in presence of FeCl₃ in method B. and Gibb's reagent in presence Borax in method C. The above methods are simple, sensitive, accurate and precise and can be used for the routine quality control of this drug in bulk as well as in pharmaceutical formulation.

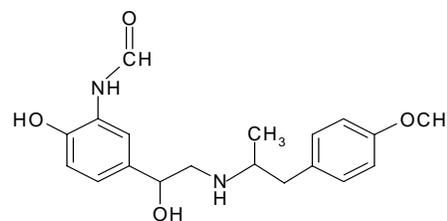


MATERIALS AND METHODS

Three simple and sensitive spetrophotomtrtic methods (A,B, and C) have been developed for the quantitative estimation of Formoterol

by using 2,2'- Bipyridyl, Potassium Ferericycnide, and Gibb's reagent at room temperature colures stable for 2 hours.

Method A



It is based on formation of stable orange colored chromogen when Formoterol is treated with 2,2'- Bipyridyl in presence of Fe²⁺ resulted from oxidation of drug with Fe³⁺. The orange colour chromogen exhibited absorption maximum at 520 nm and obeyed beer's law in the concentration range of 2 - 10 µg/m. The reaction mechanism for method A is shown in (Figure 1).

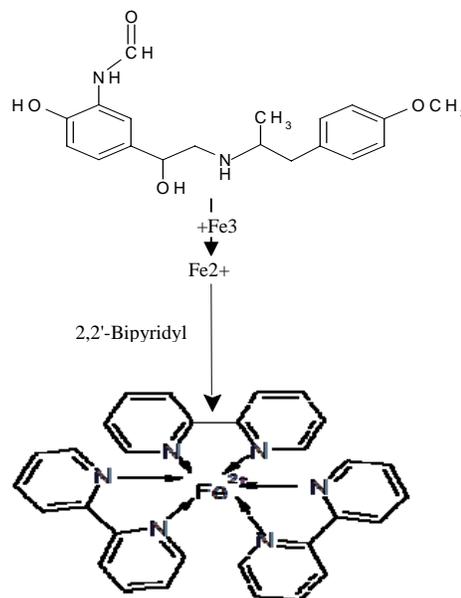


Fig. 1: Orange colour chromogen λ max 520nm

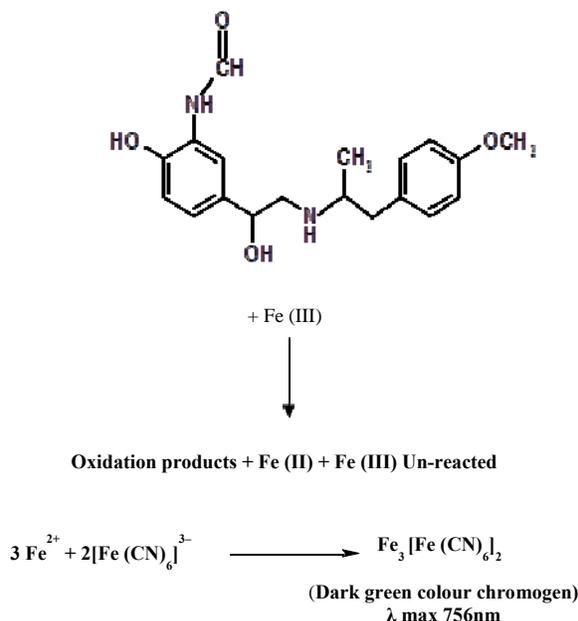


Fig. 2:

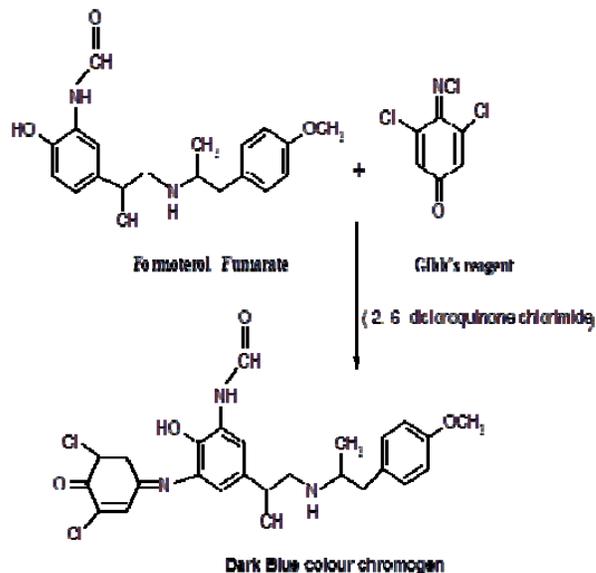


Fig. 3:

Method B

Formoterol reduces ferric chloride to ferrous forms which in turn forms complex with Potassium ferricyanide to give dark green colour chromogen complex. That exhibited absorption maximum at 756nm and obeyed Beer's law in the concentration range of 2 - 10 $\mu\text{g/ml}$. The reaction mechanism for method B is shown in (Figure 2).

Method C

Formoterol Forms dark blue colored chromogen with Gibb's reagent (2,6 dichloroquinone chlorimide) in alkaline PH That exhibited absorption maximum at 618 nm and obeyed Beer's law in the concentration range of 2 - 10 $\mu\text{g/ml}$. The reaction mechanism for method C is shown in (Figure 3).

EXPERIMENTAL

A 119 UV/visible double beam spectrophotometer With 1 cm - matched quartz cell, was used for all spectral measurements, scanning range of 190- 380nm for UV range and 380-800 nm for visible range were used. All the chemicals and reagents used were of analytical reagent grade from S.D. Fine chemicals, Mumbai. Formoterol was gifted by cipla Pharmaceuticals Pvt Ltd, goa.

Preparation of standard

Accurately weighed 100 mg of Formoterol (bulk drug) was dissolved in 40 ml of distilled methanol and volume was made up to 100 ml with distilled methanol (i.e. 1000 $\mu\text{g/ml}$). The final concentration of formoterol fumarate was brought to (100 $\mu\text{g/ml}$) with distilled methanol.

Preparation of sample

A total 20 rotacaps each from different batches (1 rotacap contains only 12 μg) were dissolved in a mixture of 1ml of sodium hydroxide and 4 ml of distilled methanol and filtered through Whatman No. 41 filtrate paper. The residue was washed thoroughly with distilled methanol into a 10ml volumetric flask and volume was made up to the mark with distilled methanol (24 $\mu\text{g/ml}$).

PRECEDURE**Method A**

Aliquots of Formoterol ranging from 0.2 - 1.0 mL (1 ml-100 $\mu\text{g/ml}$) were transferred into a series of 10 mL volumetric flasks to provide final concentration range of 2 - 10 $\mu\text{g/ml}$. To each flask of 0.5ml of a Ferric chlorride (0.5%) solution and 1 ml of 2,2'- Bipyridyl (0.2%) reagent were added. The flask were heated at 80 $^{\circ}\text{C}$ for 20 minutes The solution was cooled and then in each tube were made up to the mark with distilled methanol The absorbance of orange colored chromogen was measured at 520 nm against the reagent blank. The amount of Formoterol present in the sample solution was computed from its calibration curve.

Method B

Aliquots of Formoterol ranging from 0.2 - 1 mL (1 ml-100 $\mu\text{g/ml}$) were transferred into a series of 10 mL volumetric flasks to provide final concentration range of 2 - 10 $\mu\text{g/ml}$. To each flask 0.5 ml of aqueous Ferric chloride (0.5 %) solution and 1 ml of Potassium ferricyanide (0.1%) were add. After 10minutes at room temperature, the volume was brought upto the mark with distilled methanol. The absorbance of dark green colored chromogen was measured at 756nm against the reagent blank. The amount of Formoterol present in the sample solution was computed from its calibration curve.

Method C

Aliquots of Formoterol ranging from 0.2 - 1 mL (1 ml-100 $\mu\text{g/ml}$) were transferred into a series of 10 mL volumetric flasks to provide final concentration range of 2 - 10 $\mu\text{g/ml}$. To each flask aqueous solution of 1.0ml borax (Disodium tetra borate 0.2%) and 1.0ml of Gibb's reagent (0.1 %) solution of were add. After 10minutes at room temperature, the volume was brought upto the mark with distilled methanol. The absorbance of blue colored chromogen was measured at 618nm against the reagent blank. The amount of Formoterol present in the sample solution was computed from its calibration curve.

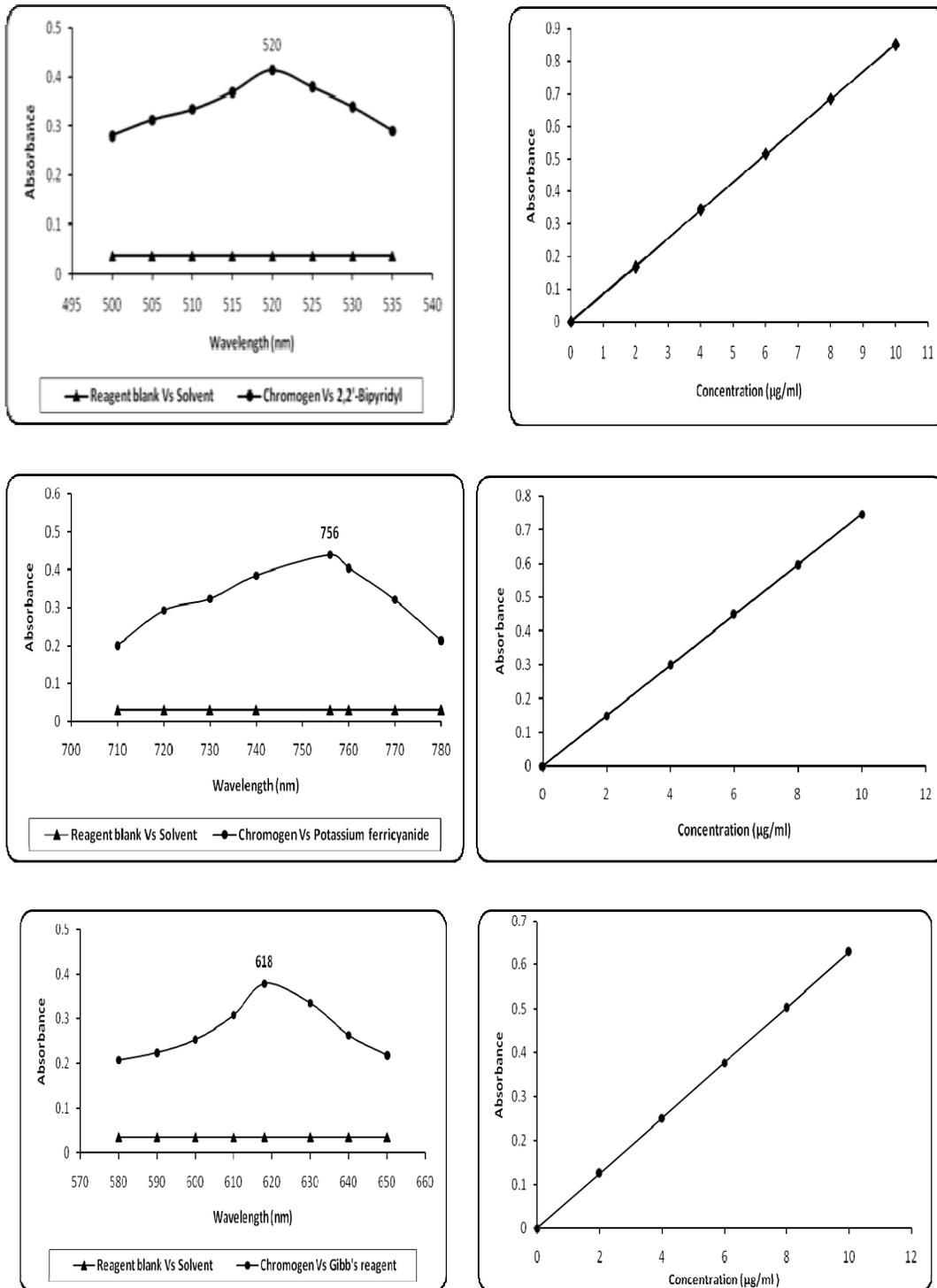


Table 2: Assay and recovery of Formoterol in pharmaceutical dosage form

Pharmaceutical dosage form	Labelled Amount	Amount found by proposed methods			Reference method (UV method)	Recovery of proposed methods (%)		
		A	B	C		A	B	C
B ₁	12 µg	11.89	11.8	11.79	11.98	98.79	98.89	99.21.
B ₂	12 µg	11.88	11.94	11.88	11.96	98.88	98.92	99.35

B₁, B₂ are rotacaps from same manufacturers, average of 5 determinations (12 µg of Formoterol was added and recovered).

Table 1: Optical Characteristics and precision

Parameters	Method-A	Method-B	Method-c
λ_{\max} (nm)	520	756	618
Beer's law limits ($\mu\text{g/ml}$) (c)	2-10	2-10	2-10
Color	Orange	Green	Blue
Molar absorptivity ($\text{lit/mol}^{-1} \text{cm}^{-1}$)	5.6211×10^4	5.1342×10^4	5.05768×10^4
Limit of Detection (LOD/ mcgml^{-1})	0.04374	0.03528	0.039
Limit of Quantification (LOQ/ mcgml^{-1})	0.1325	0.1066	0.120
Sandell's sensitivity ($\mu\text{g/ml}/0.001$ abs units)	0.0140	0.0120	0.0150
Regression equation (Y*)			
Slope (b)	0.06983	0.07492	0.062768
Intercept (a)	3.314×10^{-4}	-2.49×10^{-4}	3.96×10^{-4}
Standard error of estimation (Se)	0.0006745	0.0002672	0.000356
Correlation coefficient (r)	0.9999	1.0025	0.9999
% RSD	0.221	0.223	0.201
Confidence limits with 0.05 level \pm	0.00082	0.00105	0.0006756
Confidence limits with 0.01 level \pm	0.00034	0.00154	0.0000998
0% Error in bulk Samples***	0.31	0.12	0.23

* $Y=bC+a$, where C is the concentration of Formoterol in $\mu\text{g/ml}$ and Y is the absorbance at the respective maximum absorbency, **Average for eight determination, ***Average for three determination

RESULTS AND DISCUSSION

The optical characteristics such as Beers law limit, sandell's sensitivity, molar extinction coefficient, percent relative standard deviation (calculation from eight measurements containing $\frac{3}{4}$ th of the amount of the upper Beers law limit) were calculated. Regression Characteristics like slope, intercept, correlation coefficient and percentage range of errors (0.05 and 0.01 confidence limits), LOD, LOQ, Error's in bulk sample and standard error of estimation were calculated and are given in Table 1

Commercial formulation of Formoterol was successfully analyzed by proposed UV spectrophotometric methods in table 2 and results are calculated. To evaluate validity and reproducibility of the methods, fixed amounts of drug were added to the pre analyzed formulation. These results of percentage recovery are calculated. There is no interference of additive (lactose, starch, gelatin, talk) and excipients in proposed analytical methods. The proposed spectrophotometric methods for the estimation of Formoterol are simple, sensitive, accurate and precise and can be used for the routine quality control of this drug in bulk as well as in pharmaceutical formulations.

Three new, sensitive and most economical analytical colorimetric methods were developed for the estimation of formoterol in bulk and pharmaceutical dosage forms. These methods are validated in terms of sensitivity, accuracy and precision. The result were found to be accurate, and free from the interference of rotacaps excipients. The % recovery, linearity, and range, LOD and LOQ, sandell's sensitivity and molar absorptivity for formoterol are summarized in [table1and 2]. The reaction mechanisms for all methods are shown in Figure 1-3.

CONCLUSION

The UV spectroscopy methods and colorimetric methods demonstrated here in, are applicable to the estimation of formoterol in pure as well as dosage forms. In order ensure that the data is

generated, the above-mention methods would prove both accurate and precise. The experiments have been performed on calibrated equipments using suitable reference standards. To prove document their reliability, the method have been carried out a possible extent. The resultants expressed in [table1 and 2] are for spectrophotometric methods. In addition to positive requirements for analytical methods, the striking advantage of all the presently developed methods is that they are economical.

The proposed method are found to be simple, sensitive, selective, accurate, precise and economical, and can be used in the determination of formoterol in bulk drug and its pharmaceutical dosage forms (rotacaps) in a routine manner.

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