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Spectrophotometric Estimation of Formoterol Fumerate in Pharmaceutical Formulations

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Three simple, accurate, rapid and sensitive methods (A, B and C) have been developed for the estimation of formoterol fumerate in its pharmaceutical dosage form. The method A is based on the formation of blue coloured chromogen, due to reaction of formoterol fumerate with Folin Ciocalteu reagent in the presence of alkali, which exhibits λ_{max} at 730 nm. Method B is based on the reduction of ferric ions of the reagent ferric chloride to ferrous ions by the drug, which further in the presence of potassium ferricyanide as oxidizing agent produces blue coloured chromogen measured at 700 nm, against reagent blank. The method C is based on the formation of red coloured chromogen with ferric chloride and 1,10-phenanthroline, which shows absorption maximum at 520 nm. The absorbance-concentration plot is linear over the range of 1-12 mcg/mL for method A, 1-10 mcg/mL for method B and 1-12 mcg/mL for method C. Results of analysis for all the methods were validated statistically and by recovery studies. The proposed methods are economical and sensitive for the estimation of formoterol fumerate in bulk drug and in its formulations (Rotacap).

Key Words: UV-Visible spectrophotometry, Formoterol fumerate, Folin Ciocalteu reagent, 1,10-Phenanthroline.

INTRODUCTION

Formoterol fumerate[1] (Fig. 1) is chemically (±)-2'-hydroxy-5'-[(RS)-1-hydroxy-2-{(RS)-*p*-methoxy-α-methylphenyl]amino}ethyl] formanilide fumerate. It is official in Japanese and European pharmacopoeias. It is a long acting and potent β_2 agonist, with duration of action up to 12 h. The literature survey reveals that the drug is determined by using HPLC[2], gas chromatography[3], capillary electrophoresis[4] and very spectrophotometric

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methods[5]. The present study describes simple, sensitive, accurate, rapid and economical spectrophotometric methods A, B and C for the estimation of formoterol fumerate in its formulations (Rotacap).

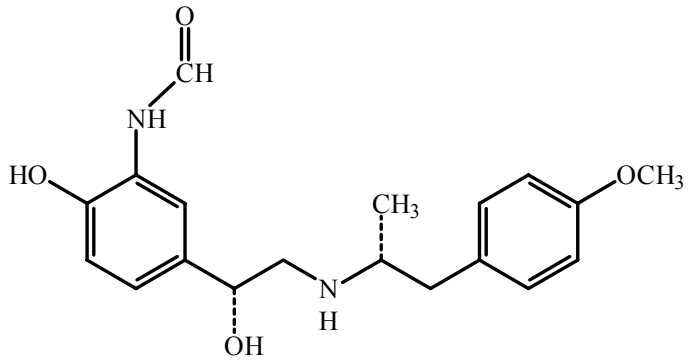

Fig. 1. Structure of formoterol

EXPERIMENTAL

Elico Ultraviolet-Visible double beam spectrophotometer SL-164 with 1 cm matched quartz cells was used for all spectral measurements.

All the chemicals used were of analytical reagent grade: (1) **Folin Ciocalteu reagent (FC reagent):** It is prepared in 1:2 with distilled water. (2) **Sodium carbonate (10 % w/v):** 10 g of sodium carbonate was dissolved in 100 mL of distilled water. (3) **Ferric chloride (0.3 % w/v):** 300 mg of ferric chloride was dissolved in 100 mL of distilled water. (4) **Potassium ferricyanide (0.2 % w/v):** 200 mg of potassium ferricyanide was dissolved in 100 mL of distilled water. (5) **1,10-Phenanthroline (0.01 M):** 198 mg of 1,10-phenanthroline was dissolved in 100 mL of warm water. (6) **Ferric chloride (0.003 M):** 80 mg of ferric chloride was dissolved in 100 mL of distilled water.

Procedure: Standard stock solution was prepared by dissolving 10 mg of formoterol fumerate in 1.5 mL of 10 % hydrochloric acid. The volume was made upto 10 mL with distilled water to get a concentration of 1000 mcg/mL. This was further diluted to get the working standard solution.

Assay procedure

Method A: Aliquots of standard drug solution of formoterol fumerate 0.5 - 6.0 mL (20 mcg/mL) were taken and transferred into series of graduated test tubes. To each test tube 2 mL of 10 % sodium carbonate solution and 2 mL of FC reagent was added. After thorough shaking, the test tubes were set aside for 10 min, for the reaction to complete. The volumes in

each test tube were adjusted to 10 mL with distilled water. The absorbances of the solutions were measured at 730 nm against reagent blank and the calibration curve was plotted. Similarly the absorbance of the sample solution was measured and the amount of formoterol fumerate was determined by referring to the calibration curve.

Method B: Aliquots of standard drug solution of formoterol fumerate 0.5-5.0 mL (20 mcg/mL) were taken and transferred into series of graduated test tubes. To each test tube 1 mL of ferric chloride (0.3 %) and 0.5 mL of potassium ferricyanide (0.2 %) were added and thoroughly shaken and set aside for 5 min. The volume in each test tube was made upto 10 mL with distilled water. The absorbances of the solutions were measured at 700 nm against reagent blank, within 0.5 h and the calibration curve was plotted. Similarly the absorbance of the sample solution was measured and the amount of formoterol fumerate was determined by referring to the calibration curve.

Method C: Aliquots of standard drug solution of formoterol fumerate 0.5 - 6.0 mL (20 mcg/mL) were taken and transferred into series of graduated test tubes. To each test tube 2 mL of ferric chloride (0.003M) and 2 mL of 1,10-phenanthroline (0.01 M) were added. The test tubes were allowed to stand in water bath at 60ºC for 15 min. The test tubes were then cooled to room temperature and the solutions were made upto 10 mL with distilled water. The absorbance of the red coloured chromogen was measured at 520 nm against reagent blank and a calibration curve was constructed. The absorbance of the sample solution was measured and the amount of formoterol fumerate was determined by referring to the calibration curve.

The methods were extended for the determination of formoterol fumerate from rotacap formulations, strength 12 mcg (Foratec, Cipla). The total contents of 20 rotacaps were weighed, powdered and powder equivalent to 200 mcg was dissolved in 10 mL of distilled water in a volumetric flask. The above solution was analyzed as described, in the above-mentioned methods. The analysis procedure was repeated three times with rotacap formulations and the results of analysis are shown in Table-2.

Recovery studies: To ensure the accuracy and reproducibility of the results obtained, adding known amounts of pure drug to the previously analyzed formulated samples and these samples were reanalyzed by the proposed method and also performed recovery experiments. The percentage recoveries thus obtained were given in Table-2.

RESULTS AND DISCUSSION

In the present study, the method A involves quantitative reaction of the drug with FC reagent. The reaction is based on the reduction of

phosphomolybdotungestic acid, present in the FC reagent by formoterol fumerate in presence of 10 % sodium carbonate solution, thereby producing the reduced species of molybdenum blue, having characteristic blue colour with maximum absorbance of 730 nm. Stability study of the developed chromogen was carried out by measuring the absorbance values at time intervals of 15 min for 3 h and it was found to be stable for more than 3 h at room temperature. The linearity was found to be in the concentration range of 1-12 mcg/mL.

In the method B, the estimation is based on the reduction of ferric ions to ferrous ions by the drug, which further in the presence of oxidizing agent like potassium ferricyanide produces blue coloured chromogen having a maximum absorbance at 700 nm. The blue coloured chromogen was stable for 0.5 h at room temperature and obey's Beer's law in the concentration range of 1-10 mcg/mL.

The method C is based on the reduction of ferric chloride to ferrous form by the drug, which forms complex with 1,10-phenanthroline to yield red coloured chromogen, having absorbance maximum at 520 nm. The linearity was found to be in the concentration of 1-12 mcg/mL. The coloured chromogen was stable for 2 h.

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table-1.

The regression analysis using the method of least squares was made for slope (m), intercept (b) and correlation obtained from different concentrations and the results are summarized in Table-1.

TABLE-1
OPTICAL CHARACTERISTICS AND PRECISION DATA

Parameters	**Method A**	**Method B**	**Method C**
λ_{max} (nm)	730	700	520
Beer's law limits	1-12	1-10	1-12
Molar absorptivity (L/mol cm)	6.744×10^3	4.377×10^3	8.387×10^3
Sandell's sensitivity (micrograms/ cm^2/0.001 absorbance unit)	0.1242	0.0930	0.1003
Regression Equation* (Y)			
Slope (m)	0.008	0.01	0.01
Intercept (c)	- 0.0077	0.03	- 0.0073
Correlation Coefficient(r)	0.9999	0.9998	0.9989
Precision (% Relative Standard Deviation)	0.789	0.269	0.3302
Standard error of mean	0.0174	0.0142	0.0149

*Y = mx + c, where x is the concentration in micrograms/mL and Y is absorbance unit.

The reproducibility and precision of the methods are very good as shown by the low values of coefficient of variance (CV). The mean percentage recovery value of 99.1 % for method A, 100.5 % for method B and 100.2% for method C, indicates non-interferences from the formulation excipients. All the validated parameters are summarized in Table-2.

TABLE-2
ASSAY OF FORMOTEROL FUMERATE IN ROTACAP FORMULATIONS

Labelled amount (mcg)	Amount taken for 20 rotacaps (mcg)	Amount obtained (mcg)* by proposed method			Recovery by the proposed method (%)**		
		Method A	**Method B**	**Method C**	**Method A**	**Method B**	**Method C**
12	240	238.5	240.5	238.8	99.2	99.8	100.5
12	240	241.5	241.3	240.2	99.3	101.2	100.3
12	240	239.3	239.8	240.6	98.9	100.5	99.8

*Average of three determinations. ** After spiking the sample.

In conclusion, the proposed methods are simple, sensitive, accurate and economical for the routine estimation of formoterol fumerate in bulk and in its formulations (rotacaps).

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