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NOTE

Development and Validation of Spectrophotometric Methods for the Estimation of Itraconazole

T.K. MURTHY, M.N. REDDY*, Y. SRINIVASA RAO, K. SRINIVASU and D.G. SANKAR

Pharmaceutical Analysis Division, Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530 003, India

Three simple spectrophotometric methods (A, B and C) have been developed for the determination of itraconazole in pure and in its pharmaceutical formulations. Method A is based on the formation of blood red colored complex with ferric chloride and 2,2′-bipyridine having absorption maximum at 525 nm. In method B, itraconazole forms green coloured complex with ferric chloride and potassium ferricyanide exhibiting maximum absorption at 725 nm, whereas method C is based on the oxidation of the drug with a known excess of oxidant. potassium permanganate ($KMnO_4$). The excess permanganate is determined using the dye, Fast Green FCF (FGFCF) at 625 nm. The results obtained are reproducible and are statistically validated.

Itraconazole (ITCZ) is a broad-spectrum triazole antifungal agent used to treat fungal infections. It acts by inhibiting fungal cytochrome P-450 and sterol C-14 α-demethylation that results in inhibition of ergosterol synthesis, and chemically it is 4-[4-[4-[4-[[2-(2A-dichlorophenyl)-2-(1H-1,2,4-triazol-1-yl-methyl)-1,3-dioxolan-4-yl methoxy]-phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(1-methyl propyl)-1,2,4-trazol-3-one[1]. Few HPLC[2-4] methods have been reported for the determination of ITCZ in serum and plasma. So far no spectrophotometric methods have been developed for ITCZ; so the authors have made an attempt and succeeded in developing three simple sensitive and reproducible spectrophotometric methods (A, B and C) for the determiantion of ITCZ. In method A, ITCZ reacts with $FeCl_3$ and 2,2′-bipyridine and forms a blood red coloured complex having absorption maximum at 525 nm. In method B, it reacts with $FeCl_3$ and potassium ferricyanide forms a green colored chromogen exhibiting maximum absorption at 725 nm, whereas in method C the drug is treated with a known excess of potassium permanganate, and the excess permanganate remaining is estimated by utilizing its ability to oxidize the dye (Fast Green FCF) and thereby decrease the colour intensity of the solution of a known strength of the dye measured at 625 nm. The decrease in the colour intensity of the dye solution is proportional to the concentration of the drug under estimation.

All the chemicals used were of analytical grade. Ferric chloride (3.3×10^{-3} M and 0.1 M), 2,2′-bipyridine (1.0×10^{-2} M), potassium ferricyanide (0.1 %), $KMnO_4$ (2.0×10^{-3} M) in 2 M H_2SO_4, Fast Green FCF (FGFCF) (1.23×10^{-4} M) in 1 M H_2SO_4, Na_2SO_4 (1 M) in distilled water were prepared. The commercially

available capsules were procured from the local market. Spectral and absorbance measurements were made on Systronics UV-Visible spectrophotometer model 117 with 10 mm-matcheds quartz cells.

Standard and sample solutions: About 10 mg of ITCZ (pure or formulation) was accurately weighed and dissolved in 1 mL of dichloromethane and made up to 100 mL with methanol. The above stock solution was further diluted with 0.1 N HCl to get a working standard solution of 25 μg/mL for methods A and B and 20 μg/mL for method C.

Method A: Aliquots of working standard solution of ITCZ ranging from 1.0 to 5.0 mL (1 mL = 25 μg) were transferrred into a series of 10 mL volumetric flasks. To that 0.5 mL of $FeCl_3$ (3.3×10^{-3} M) and 1.5 mL of 2,2′-bipyridine (1.0×10^{-2} M) were successively added. Then the flasks were set aside for 15 min at room temperature and the final volume was brought to 10 mL with distilled water. The absorbance of the blood red colored species formed was measured at 525 nm against reagent blank and the amount of ITCZ present in the sample solution was computed from its calibration curve.

Method B: To a series of 10 mL graduated test tubes aliquot sample of working standard solutions ITCZ ranging from 0.25 to 2.5 mL (1 mL = 25 μg) were transferred, then 1.5 mL of ferric chloride (0.1 M) and 1.0 mL of potassium ferricyanide (0.1 %) were added and kept aside at room temperature for 30 min for complete color development. Appropriate quantity of distilled water was added to all the test tubes to make the volume up to 10 mL in each. The absorbance of green colored chromogen formed was measured at 725 nm against reagent blank. The amount of ITCZ present in the sample solution was computed from its calibration curve.

Method C: Aliquots of standard drug solution (0.25 to 1.5 mL, 1 mL = 20 μg) were taken into a series of 25 mL calibrated tubes. To each of these tubes, 0.5 mL of $KMnO_4$ solution was added and the total volume in each was brought to 10 mL with distilled water and kept aside for 15 min at room temperature. Then 4.0 mL of FGFCF solution and 4 mL of sodium sulfate were added successively. After 10 min, the volume was made up to the mark with distilled water. The absorbance was measured at 625 nm against distilled water. A blank experiment was carried out in a similar manner omitting the drug. The decrease in absorbance corresponding to the drug was obtained by substracting the absorbance of the blank from that of the standard solution. The amount of drug present in the sample solution was computed from the standard carlibration graph.

The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar extinction coefficient, per cent relative standard deviation (calculated from the eight measurements containing 3/4th of the amount of the upper Beer's law limits of ITCZ), % range of error (0.05 to 0.01 confidence limits) were calculated for both the methods and the results are summarized in Table-1. The values obtained for the determination of ITCZ in several pharmaceutical formulations (capsules) by the proposed and reported methods are compared in Table-2.

TABLE-1
OPTICAL CHARACTERISTICS AND PRECISION

Parameters	Method A	Method B	Method C
Beer's law limit (μg/mL)	2.5–12.5	0.60–7.5	0.2–1.2
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.01612	0.00781	0.001298
Molar extinction coefficient (1 mole^{-1} cm^{-1})	4.3749×10^4	0.9032×10^5	5.4334×10^5
% Relative standard deviation	0.8065	0.7055	0.7301
% Range of error:			
0.05 confidence limits	± 0.6743	± 0.5899	± 0.6105
0.01 confidence limits	± 0.9977	± 0.8727	± 0.9032
Correlation coefficient	0.99976	0.99983	0.9998
Regression equation (Y*):			
Slope (a)	0.0060	0.3223	0.0294
Intercept (b)	0.0044	0.0009	0.00542

Y* = b + aC, where "C" is concentration in μg/mL and Y is absorbance unit.

TABLE-2
ESTIMATION OF ITRACONAZOLE IN PHARMACEUTICAL FORMULATIONS

Sample	Label amount (mg)	Amount obtained (mg) Proposed methods			Per cent recovery of the proposed methods		
		A	B	C	A	B	C
1	100	99.8	101.2	99.2	99.8	101.2	99.2
2	100	100.7	100.1	100.4	100.7	100.1	100.4
3	100	100.6	100.5	99.7	100.6	100.5	99.7

To evaluate the validity and reproducibility of the methods, known amount of pure drug was added to the previously analysed pharmaceutical preparations and the mixtures were analysed by proposed methods and the per cent recoveries are given in Table-2. Interference studies reveal that the common excipients and other additives usually present in the dosage form did not interfere in the proposed methods. In conclusion the proposed methods are simple, sensitive and accurate and can be used for the routine determination of ITCZ in bulk as well as in its pharmaceutical preparations.

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