

The physicochemical and biological evaluation of erythromycin nicotinate showed promising results for potential use and further studies on animals and human beings be undertaken to prove its suitability for clinical use.

#### REFERENCES

1. Laurence, D.R. and Bennett, P.N., In; Clinical Pharmacology, 6th Edn., ELBS, Edinburgh, 1987, 230.
2. Garrod, L.P., Lambert, H.P. and Grady, P.O., In; Antibiotics and Chemotherapy, 4th Edn, Churchill Livingstone, Edinburgh, 1973.
3. Josselyn, L.E. and Sylvester, J.C., *Antibiot. Chemother.*, 1952, 3, 63.
4. Kirby, W.M.M., Maple, F.M. and Leary, B.O., *Antibiot. Chemother.*, 1953, 3, 437.
5. Griffith, R.S. *Antibiot. Anna.*, 1955, 269.
6. Walasek, O.F., *U.S. patent No. 2,881,163*, 1959.
7. Stephens, V.C., Conine, J.W. and Murphy, H.W., *J. Amer. Pharm. Assn.*, 1959, 48, 620.
8. Bray and Stephens, *U.S. patent No. 3,000, 874*, 1961.
9. Shepher, J. T., *U.S. patent No. 2,852, 429*, 1958.
10. Hoffhine, C.E. (Jr.), *U.S. patent No. 2,761, 859*, 1956.
11. Stephens, V.C., *U.S. patent No. 2,993, 833*, 1961.
12. Stephens, V.C. and Conine, J.W., *Antibiot. Ann.*, 1958-1959, 346.
13. Goswami, B.B., Manna, P.K. and Basu, S.K., *Arzneim. Forsch.*, 1995, 45, 813.
14. Manna, P.K., Basu, S.K. and Goswami, B.B., *Drug Develop. Ind. Pharm.*, 1995, 21, 2097.
15. Mohanta, G.P. and Basu, S.K., *Indian Drugs*, 1995, 32, 190.
16. Manna, P.K. and Basu, S.K., *Drug Develop. Ind. Pharm.*, 1998, 24, 879.
17. Manna, P.K. and Basu, S.K., *Drug Develop. Ind. Pharm.*, 1999, 25, 391.
18. Dutta, S.K. and Basu, S.K., *Indian patent No. 142, 584*, 1977.
19. Dutta, S.K. and Basu, S.K., *U.S. patent No. 4,137, 379*, 1979.
20. Marsh, J.R. and Weiss, P.G., *J. Assoc. Anal. Chem.*, 1967, 50, 457.
21. Grove, D.C. and Randall, W.A., In; Assay Methods of Antibiotics-A laboratory Manual, Medical Encyclopedia, New York, 1955.
22. Hale, L.J. and Inkely, G.W., *Lab. Pract.*, 1965, 14, 452.
23. Litchfield, J.T. (Jr.) and Wilcoxon, F., *J. Pharmacol. Expt. Ther.*, 1949, 96, 99.
24. Ghosh, M.N., In; Fundamental of Experimental Pharmacology, Scientific Book Agency, Calcutta, 1971, 112.

---

## Spectrophotometric Estimation of Itraconazole in Pharmaceutical Formulations

---

T. K. MURTHY, M. N. REDDY, Y. SRINIVASA RAO AND D. G. SANKAR\*  
Pharmaceutical of Analysis Division, Department of Pharmaceutical Sciences,  
Andhra University, Visakhapatnam-530 003.

Accepted 27 March 2002

Revised 18 March 2002

Received 7 April 2001

**Two simple spectrophotometric methods (A and B) 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 1,10-phenanthroline having absorption maximum at 520 nm, where as in method B, itraconazole forms a green colored complex with ferric chloride and MBTH reagent exhibiting maximum absorption at 630 nm. The chromogens obey Beer's law in the concentration ranges of 1.2 to 7.5 µg/ml and 2.5 to 20 µg/ml for methods A and B, respectively. The results obtained are reproducible and are statistically validated.**

Itraconazole (ITCZ) is a broad-spectrum triazole anti-fungal agent used to treat fungal infections. It acts by inhibiting fungal cytochrome P-450 and sterol C-14  $\alpha$ -

demethylation that results in inhibition of ergosterol synthesis, and chemically it is 4-[4-[4-[(2-(2A-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-ylmethoxy]-phenyl)-1-piperazinyl] phenyl]-2,4-dihydro-2-(1-

\*For correspondence

methylpropyl)-1,2,4-triazol-3-one<sup>1</sup>. Few HPLC<sup>2-4</sup> 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 two simple sensitive and reproducible spectrophotometric methods (A and B) for the determination of ITCZ. In method A, ITCZ reacts with FeCl<sub>3</sub> and 1,10-phenanthroline and forms a blood red coloured complex having absorption maximum at 520 nm. In method B, it reacts with FeCl<sub>3</sub> and MBTH reagent and forms a green colored chromogen exhibiting maximum absorption at 630 nm.

All the chemicals used were of analytical grade. Ferric chloride (0.033 M), 1,10-phenanthroline (0.1 M), MBTH (0.2%) (all reagents were procured from Loba Chemie, Mumbai) were prepared in distilled water. The commercially available capsules were procured from the local market. Spectral and absorbance measurements were made on a Systronics UV/Vis spectrophotometer model 117 with 10 mm matched quartz cells.

About 100 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 50 µg/ml for method A and 100 µg/ml for method B.

Aliquots of working standard solution of ITCZ ranging from 0.25 to 1.5 ml (1 ml = 50 µg) were transferred in to a series of 10 ml volumetric flasks. To that 0.5 ml of FeCl<sub>3</sub> (0.033 M) and 2.0 ml of 1,10-phenanthroline (0.1 M) were successively added. Then the flasks were set aside for 20

min at room temperature. The final volume was brought to 10 ml with distilled water. The absorbance of the blood red coloured species formed was measured at 520 nm against reagent blank and the amount of ITCZ present in the sample solution was computed from its calibration curve.

To a series of 10 ml graduated test tubes aliquot sample of working standard solutions of ITCZ ranging from 0.25 to 2.0 ml (1 ml = 100 µg) were transferred. To these 1.5 ml of ferric chloride (0.033 M) and 1.5 ml of MBTH reagent (0.2%) were added and kept aside at room temperature for 5 min. 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 630 nm against reagent blank. The amount of ITCZ present in the sample solution was found out from its calibration curve.

The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar extinction coefficient, percent 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 method are compared in Table 2. To evaluate the validity and reproducibility of the methods, known amounts of pure drug was added to the previously analysed pharmaceutical preparations and the mixtures were analysed by proposed methods and the percent recoveries are given in Table 2.

TABLE 1: OPTICAL CHARACTERISTICS AND PRECISION.

Parameters	Method A	Method B
Beer's law limit (µg/ml)	1.2-7.5	2.5-20.0
Sandell's sensitivity (µg/cm <sup>2</sup> /0.001 absorbance unit)	0.0108	0.02245
Molar extinction coefficient (L.mole <sup>-1</sup> .cm <sup>-1</sup> )	6.4918x10 <sup>4</sup>	3.1424x10 <sup>4</sup>
% Relative standard deviation	0.5178	0.987
Correlation coefficient	0.9998	0.9999
Regression equation (Y*)		
Slope (a)	0.00924	0.00432
Intercept (b)	0.00401	0.00847

Y\*=b+aC, where C is the concentration in µg/ml and Y is absorbance unit.

TABLE 2: ESTIMATION OF ITRACONAZOLE IN PHARMACEUTICAL FORMULATIONS.

Sample	Labelled amount (mg)	Amount obtained (mg)		% recovery of the proposed method*	
		Proposed methods		A	B
		A	B		
1	100	99.8	101.0	100.04	100.21
2	100	100.2	99.6	99.86	100.17
3	100	99.7	100.1	99.94	98.99

\* Average of five determinations.

Interference studies revealed that the common excipients and other additives usually present in the dosage form such as parabens, lactose, sucrose, starch, sodium benzoate, sodium phosphate, calcium gluconate, gelatin, talc, magnesium stearate did not interfere in the proposed methods.

The blood red coloured complex formed in method A may be due to the fact that each of the two nitrogen atoms in 1,10-phenanthroline has an unshared pair of electrons that can be shared with Fe (II) ion [formed by reaction of ITCZ with Fe (III)]. Three such molecules of 1,10-phenanthroline attach themselves to the metallic ion (ferroin complex). In method B, under reaction conditions MBTH loses two electrons and one proton on oxidation with FeCl<sub>3</sub> to give an electrophilic intermediate, which has been postu-

lated to be the active coupling species. This intermediate couples with the drug molecule to form the green coloured complex. In conclusion, the methods developed are simple, sensitive, economical and accurate and can be used for the routine determination of ITCZ in bulk as well as in pharmaceutical preparations.

#### REFERENCES

1. William, K.N., In; Gennaro, A.R., Eds., Remington: The Science and Practice of Pharmacy, 19th Edn., Vol. II, Mack Publishing Company, Easton, Pennsylvania, 1995, 1330.
2. Bai, L., Lu, Y., Chen, X., Ha, Y. and Du, M., *Zhongguo Yiyuan Yaoxue Zazhi*, 1999, 19, 7.
3. Compas, D., Touw, D.J. and De Croede, P.N.F.C., *J. Chromatogr., Biomed. Appl.*, 1996, 687, 453.
4. Allenmark, S., Edebo, A. and Lindgren, K., *J. Chromatogr.*, 1990, 532, 203.

---

### Analgesic and Antipyretic Activity of *Pergularia extensa* in Rats

---

S. S. JALALPURE<sup>1</sup>, P. V. HABBU<sup>\*</sup>, M. B. PATIL<sup>1</sup>,  
R. V. KULKARNI<sup>2</sup>, C. C. SIMPI<sup>2</sup> AND C. C. PATIL<sup>2</sup>  
B. V. V. S's H. S. K. College of Pharmacy, Bagalkot-587 101.  
<sup>1</sup>K. L. E's College of Pharmacy, Belgaum-590 010.  
<sup>2</sup>B. L. D. E. A's College of Pharmacy, Bijapur-586 103.

Accepted 11 April 2002

Revised 3 April 2002

Received 30 June 2001

**The presence of flavanoids, steroids and saponins was detected in the preliminary phytochemical investigation of different leaf extracts of *Pergularia extensa*. The ethanolic extract and petro-**

**\*For correspondence**