TABLE 2: THE EFFECT OF AUXINS ON BIOPRODUCTION OF HYPERICIN

Hormone	Amount of Hypericine (w/w on dry wt. basis)
Dicamba	0.024%
2,4-D	0.028%

Average of 3 readings, 2,4-D is 2,4-Dichlorophenoxyacetic acid. Dicamba is 3,6-dichloro-o-ansic acid.

have to be developed before commercialization of this technique for the production of hypericin from tissue cultures.

ACKNOWLEDGEMENTS

N. Sulochana Rani is thankful to University Grants Commission for the award of Junior Research Fellowship.

REFERENCES

1. Wilkinson. J.A., In; IBC 5th Annual Conference. London, 1998, 784.
2. Maisenbacher, P. and Kover. K.A., **Planta Med.**,1992. 58. 291.
3. Ernst. E., **Phytomedicine**, 1995, 2, 67.
4. Bombardelli. E. and Morazzoni. P., **Fitoterapia**, 1995, 1, 67.
5. Ciddi. V and Kokate. C.K., **Indian Drugs**, 1997, 34, 354.
6. Dias, A.C.P., Francisco. A., Tomas. B., Fernandes. F. and Federico. F., **Phytochemistry.** 1998, 48, 1165.
7. Cardoso. M.A. and De Oilveira. D.E., **Plant Cell Tissue Organ Cult.**, 1996, 44, 91.
8. Zdunek. K and Alfermann. A. W., **Planta Med.** 1992, 58, A621.
9. Ishiguro. K., Nakajima. M., Fukumoto., H. and Isoi., K., **Phytochemistry.**, 1995, 39,903.
10. Karting. T., Gobel. I. and Hetdel. B., **Planta Med.**, 1996, 62, 1.
11. Bhojwani, S.S. and Razdan. M.K., In; Plant Tissue Culture : Theory and Practice. Elsevier. Amsterdam. 1983, 37.

Spectrophotometric Methods for the Estimation of Satranidazole in Pharmaceutical Formulations

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Accepted 24 July 2001
Revised 17 July 2001
Received 23 October 2000

Two spectrophotometric methods (I and II) in the visible region have been developed for the estimation of satranidazole in bulk drug and pharmaceutical formulations. Methods I and II are based on the reaction of reduced satranidazole with *p*-dimethylaminobenzaldehyde (PDAB) and *p*-dimethylaminocinnamaldehyde (PDACA) in acidic conditions to form orange red and purple coloured chromogens with absorption maxima at 511 nm and 568 nm respectively. The reduction of satranidazole was carried out with zinc granules and 4 N hydrochloric acid at room temperature in ethanol. Beer's law was obeyed in the concentration range of 10-50 µg/ml for both the methods. The results of analysis have been validated statistically and by recovery studies. The methods were found to be simple, rapid, accurate, reproducible and economic. The results are comparable with those obtained using UV spectrophotometric methods in alcohol at 315 nm.

Satranidazole[1], 1-methylsulphonyl-3-(1-methyl-5-nitro-2-imidazolyl)-2-imidazoli dinone[2], is one of the large series of nitroimidazoles with a potent antiprotozoal activity[3], against *E. hystolytica, T. vaginalis* and *Giardia.* Satranidazole is chemically different from metronidazole

*For correspondence

and other commercially available nitroimidazoles. It is not offical in any phamacopoeia and analytical reports are not found for its estimation in the literature.

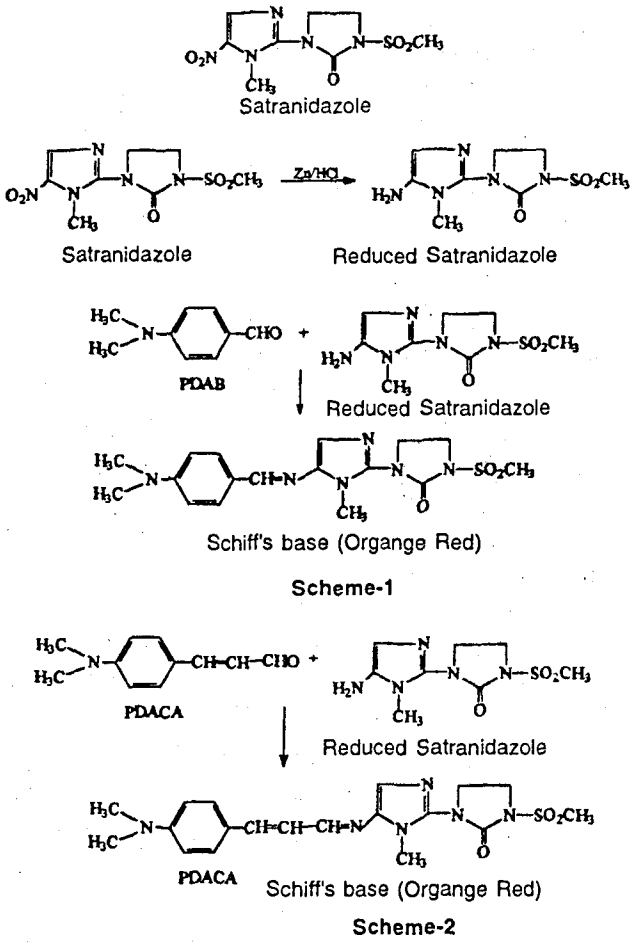
Two simple and sensitive spectrophotometric methods for the quantitative estimation of satranidazole have been developed after converting it to its reduced form, 1-methylsulphonyl-3-(1-methyl-5-amino-2-imidazolyl)-2-imidazolidinone by using zinc granules and 4N hydrochloric acid in ethanol at room temperature.

Two methods (I and II) developed are based on formation of coloured Schiff's bases in acidic condition. Method I is based on the reaction of reduced satranidazole with p-dimethylaminobenzaldehyde (PDAB) to form an orange red coloured chromogen with an absorption maximum (λ max) 511 nm. Method II is based on the condensation of reduced satranidazole with p-dimethylaminocinnamaldehyde (PDACA) to form a purple coloured chromogen with an absorption maximum (λ max) of 568 nm, and obeyed Beer's Law in the concentration range of 10-50 µg/ml for method I and method II, respectively. These methods have been successfully extended to the pharmaceutical preparations (tablets) containing satranidazole.

A Systronics model 119 UV/visible spectrophotometer with 1 cm matched quartz cells was used. *p*-Dimethylaminobenzaldehyde and *p*-dimethylaminocinnamaldehyde were obtained from Loba Chem. Pvt. Ltd, Mumbai and were purified by crystallisation. Satranidazole in bulk and in tablet formulations was obtained as gift sample from Alkem Laboratories Ltd. Mumbai.

About 100 mg of satranidazole (pure or formulation) was accurately weighed and dissolved in 20 ml of alcohol. The alcoholic solution of satranidazole was treated with 10 ml of 4 N hydrochloric acid and 0.75 g of zinc granules were added in portions while shaking. After standing for one hour at room temperature, the solution was filtered through cotton wool. The residue was washed with 10 ml portions of alcohol three times and the total volume of the filtrate was made up to 100 ml with alcohol. The final concentration of reduced satranidazole was made to 100 µg/ml (Method I and II). If the alcoholic sample solutions were diluted with distilled water, small particles were found to appear; therefore directly alcoholic solutions were used for estimation.

In method I, fresh aliquots of reduced satranidazole



Scheme-1

Scheme-2

ranging from 1-5 ml (1 ml=100 µg) were transferred into a series of 10 ml volumetric flasks. To each of the above aliquots 1 ml of PDAB (0.1%) was added and heated at 60-70° for 10 min. After cooling, the volume was brought up to the mark with alcohol and the absorbance of the orange red coloured species was measured at 511 nm against reagent blank. The colored species was stable for more than 2 h. The amount of satranidazole was computed from calibration curve. Colour was found to be stable up to 80°, at alkaline pH colour starts fading.

In method II, fresh aliquots of reduced satranidazole ranging from 1-5 ml (1 ml=100 µg) were transferred into a series of 10 ml volumetric flasks. To each of above aliquots 1 ml PDACA (0.1%) was added and heated at 60-70° for 20 min. After cooling, the volume was brought up to the mark with alcohol and the absorbance of the purple coloured species was measured at 568 nm against reagent blank. The coloured species was stable for more than 1 h. The amount of satranidazole was computed

TABLE 1: OPTICAL CHARACTERISTICS AND PRECISION

	PDAB	PDACA
λ max (nm)	511	568
Beer's law limits (μg/ml)(C)	10-50	10-50
Molar absorptivity (lit. moles^{-1} cm^{-1})	2.05×10^5	1.40×10^5
Sandell's sensitivity (μg/cm^2 -0.001 absorption units)	0.052	0.020
Regression equation (bC+a)		
Slope (b)	3.6×10^{-2}	9.42×10^{-2}
Intercept (a)	0.051×10^{-2}	0.034×10^{-2}
Correlation co-efficient (r)	0.9997	0.9996
%RSD	1.223	1.522
Range of errors** Confidence limits with 0.05 level	± 1.30	± 1.36

**For eight measurements

TABLE 2: EVALUATION OF SATRANIDAZOLE IN PHARMACEUTICAL PREPARTIONS

Sample	Labelled Amount (mg)	Amount obtained (mg)			% Recovery*	
		Proposed method		Reference method		
		I	II	UV	I	II
T_1	300	298.76	299.73	299.51	99.39 ± 0.5	99.24 ± 0.06
T_2	300	298.32	298.80	300.30	98.08 ± 0.8	98.90 ± 0.7

* Mean and standard deviation of 8 determinations

from calibration curve. Colour was found to be stable even after 80°, at alkaline pH colour starts fading.

The results of the above methods are compared with the results obtained with UV spectrophotometric method. In UV method, solution of satranidazole in alcohol either pure or formulation (100 μg/ml) was prepared. Aliquots of satranidazole ranging from 0.2-1 ml (1ml=100 μg) were transferred into a series of 10 ml volumetric flasks. The volume was made up to the mark with alcohol and the absorbance of the solutions was measured at 315 nm against solvent blank. the amount of satranidazole was computed from calibration graph.

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 the slope (b), intercepts (a) and correlation(r) obtained from different concentrations and the results are summarized in Table 1. The percent relative standard deviation and percent range of error (0.05 level confidence limits) calculated from the eight measurements $^3/_4$ of the amount of upper Beer's Law limits in each method are summarized in Table 1. The results showed that the methods have reasonable precision. Results obtained with the proposed methods confirm the suitability of these

methods for pharmaceutical dosage forms. The other active ingredients and excipients usually present in pharmaceutical dosage forms did not interfere in the estimation when some commercial dosage forms (T_1 and T_2) were analysed by this method. The accuracy of the method was confirmed by the recovery studies, by adding a known amount of the pure drug to the formulation already analysed by this method and the analytical data is presented in Table 2. The methods reported here are found to be simple, sensitive, accurate and can be used in the determination of satranidazole from pharmaceutical dosage forms in a routine manner.

ACKNOWLEDGEMENTS

Authors are thankful to M/s Alkem Laboratories Ltd., Mumbai for their generous gift sample of satranidazole, Chairman, Department of Chemistry Gulbarga University, Gulbarga and Principal, H.K.E 's College of Pharmacy, Gulbarga for providing facilities for experimental work.

REFERENCES

1. Gowri Shankar, R.V., **J. Antimicrob. Chemother.**, 1985, 15, 463.
2. Nagarajan, K., **Indian J. Chem.**, 1982, 21B, 928.
3. Nagarajan, K., **Indian J. Exp. Biol.**, 1992, 30, 483.

Spectrophotometric Determination of Trimetazidine Dihydrochloride in Bulk and Solid Dosage Forms

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Accepted 25 July 2001
Revised 19 July 2001
Received 3 February 2001

A new simple, sensitive spectrophotometric method in ultraviolet region has been developed for the determination of trimetazidine dihydrochloride in bulk and in tablet dosage form. Trimetazidine dihydrochloride shows maximum absorbance at 270 nm. Beer's law was obeyed in the concentration range of 400-700 µg/ml. Results of the analysis were validated satistically and by recovery studies.

Chemically, trimetazidine dihydrochloride is 1[(2,3,4-trimethoxy pheny1) methy1] piperazine dihydrochloride, which is a unique antianginal and antiischemic agent[1] belonging to a new class of compounds called cytoprotectives that display antischemic effects without inducing haemodynamic changes and improve the status of the ischemic myocardium. A precise spectrophotometric method is developed for the determination of trimetazidine dihydrochloride in bulk and in solid dosage forms.

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Literature survey reveals that the drug is determined using HPLC[2] and GCMS[3] method in biological fluids. The present study describes a simple UV spectrophotometric method of determination of trimetazidine dihydrochloride in bulk as well as from solid dosage forms using distilled water as a solvent.

An Elico UV visible spectrophotometer-159 with 1 cm matched quartz cell was used. Pure trimetazidine dihydrochloride was obtained as a gift sample from Micro Labels Pondicherry. It's tablet formulations were obtained from market.

Trimetazidine dihydorichloride (10 mg) was accurately