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STABILITY INDICATING TLC METHOD FOR THE QUANTIFICATION OF TINIDAZOLE IN PHARMACEUTICAL DOSAGE FORM - I.V. FLUID.

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A B S T R A C T

Tinidazole was separated from degradation products formed in large volume parenteral preparations by TLC method using Silica gel GF_{254} as stationary phase and Chloroform - Methanol (9:1) mixture as mobile phase. Subsequently extracted with ethyl alcohol and estimated by measuring absorbance at 310nm using standard calibration curve. Recovery was 98 to 102% and the co-efficient of variation was 2.1%.

I N T R O D U C T I O N

It is now well accepted[1] that Tinidazole, a 5 - Nitroimidazole derivative, has the antimicrobial actions of metronidazole and is used similarly in the treatment of susceptible protozoal infections and in the treatment and prophylaxis of anaerobic bacterial infections. Use of Tinidazole as intravenous infusion before, after and during surgery is going to be a regular practice in near future. In view of its expected increasing application,

it is of paramount interest not only to ascertain its stability, but a reliable assay method is also required.

The assay method for Tinidazole in different pharmaceutical formulations so far reported[2,3,4,5,6] involves spectrophotometric estimations in which interference due to the presence of common and expected degradation products (i.e. 2 - Methyl - 5 - nitroimidazole and Metronidazole at least in aqueous solution phase) can not be avoided. The reliable methods by using Gaschromatography and HPLC incurs huge cost. The proposed method is simple, involves low cost, reproducible and free from any interference of the degradation products and common additives of the formulation.

EXPERIMENTAL

Tinidazole (1- [2-Ethylsulfonyl] -2-methyl-5-nitroimidazole), used as the reference standard, was prepared by repeated crystallisation of technically pure compound from two different solvents (Ethanol and Benzene). The colourless crystals were of m.p. 127-8°C, λ max (95% EtoH)310 nm. (ϵ 8,925) and 230 nm. (ϵ 3,828). All other chemicals used are of A.R. grade. Glass plates were coated (250μ thickness) with silica Gel GF_{254} (E. Merck), dried at 110°C for one hour, cooled to room temperature in a desiccator and used on the same day. A mixture of chloroform-Methanol (9:1v/v) was used as mobile phase and after development spots were visualised under U-V light. Absorbance were measured in Hitachi 200-20 Model Spectrophotometer using 1cm matched quartz cells.

A stock solution of standard Tinidazole (5 mg/ml) was first prepared in 95% Ethanol. Aliquots from the properly diluted stock solution were so spotted that the amount of Tinidazole varied from 50 to 250μg per spot.

An unspotted space corresponding to the area alloted for each spot was left for preparing the blank. The plates were developed by the ascending technique upto 15cm at room temparature (28±2°C). The plates were removed, air dried, visualised under U-V lamp and marked. Each area corresponding to pure Tinidazole was directly scrapped off into a 15ml centrifuge tube, stirred well with 4ml 95% ethanol, centrifuged and the clear supernatant liquid transferred to a 10ml volumetric flask. Extraction was repeated twice and the volume was then made upto the mark. A similar extraction procedure was followed with identical area of the coating material alone for the preparation of sample blank. From the measured absorbances of the sample solutions at 310nm. against the blank a calibration curve was drawn which obeyed Beer's law satisfactorily in the specified region.

Tinidazole intravenous fluid normally[1] contain 0.2% w/v Tinidazole. Aliquots equivalent to about 100µg Tinidazole from a commercial product were directly applied and the procedure as described earlier was followed. Finally the Tinidazole content was computed from the absorbances at 310 nm. using the calibration curve. Results are tabulated in Table I.

To study the recovery label a Tinidazole IV solution (Sample A) was prepared in the laboratory containing 2mg standard Tinidazole per ml. along-with other common additives like buffering agents preservatives etc. To this solution different known quantities of standard Tinidazole added and subsequently analysed by the proposed method and results are in Table II.

RESULTS AND DISCUSSION

Large volume parenteral preparation of Tinidazole was found to undergo degradation on ageing or when subject

TABLE I

Expt. No.	Vol. of Tinidazole I.V. (Commercial product) spotted in ml.	% strength of Tinidazole in product	Average
1.	0.04	0.195	
2.	0.04	0.190	
3.	0.04	0.203	
4.	0.05	0.205	
5.	0.05	0.198	0.1983
6.	0.05	0.198	
7.	0.05	0.196	
8.	0.06	0.199	
9.	0.06	0.202	
10.	0.06	0.197	

TABLE II

Expt. No.	Vol. of Sample A spotted in ml.	Pure Tinidazole added in µg	Tinidazole found in µg	% Recovery
1.	0.03	0	59.8	-
2.	0.03	50	110.2	100.8
3.	0.03	100	158.9	99.1
4.	0.05	0	99.2	-
5.	0.05	50	150.0	101.6
6.	0.05	100	197.3	98.1

to drastic measures (i.e. steaming) such solutions on analysis by TLC revealed two sharp distinct spots major one corresponding to pure Tinidazole (Rf.-0.562) and minor one to pure Metronidazole or 2-Methyl-5-nitroimidazole (Rf.-0.385) as in the particular system Metronidazole and 2-Methyl-5-nitroimidazole gave the same Rf value. The adsorbent corresponding to the spot of Rf 0.385 was scrapped off, extracted with 95% ethanol and the ethanolic solution exhibited a single peak λmax at 300 nm. in U-V region though in the same solvent λ max for Metronidazole are at 310 & 230 nm. and λmax for 2-Methyl-5-nitroimidazole is at 298 nm. only. Further investigations are in progress to characterise this secondary spot thoroughly.

However, in the proposed method Tinidazole has been clearly separated from its common degradation products and no interference is also observed by additives used in this type of formulations. Values given in the Table I & Table II are the averages of triplicate determinations. Recovery was 98% to 102% and the coefficient of a variation calculated on data of Table I was 2.1%.

The method is specific, stability indicating, sensitive and accurate.

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