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Colorimetric determination of parasiticides hycanthone and metronidazole

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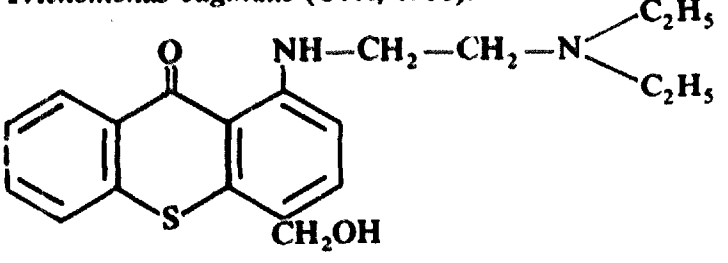
Summary

Sensitive, simple and accurate methods are described for the colorimetric determination of hycanthone and metronidazole. The methods are based on the reaction of hycanthone with 2,4-dinitrophenylhydrazine and the separated hydrazone when treated with ethanolic potassium hydroxide solution, gives a wine red colour. Metronidazole on reduction with zinc and hydrochloric acid forms complex with *p*-dimethylaminocinnamaldehyde which is highly coloured. The methods are applicable to microgram amounts and can be used successfully for the determination of hycanthone and metronidazole in dosage forms.

Introduction

Hycanthone is schistosomicide which is an active metabolite of lucanthone. It is most useful against *Schistosoma haematobium* and *S. mansoni* (Osol, 1980). Metronidazole exhibits broad antiprotozoal activity against *Entameba histolytica* and *Trichomonas vaginalis* (Osol, 1980).



Hycanthone

1-{[2-(diethylamino)ethyl]amino}-4-hydroxymethylthioxanthen-9-one

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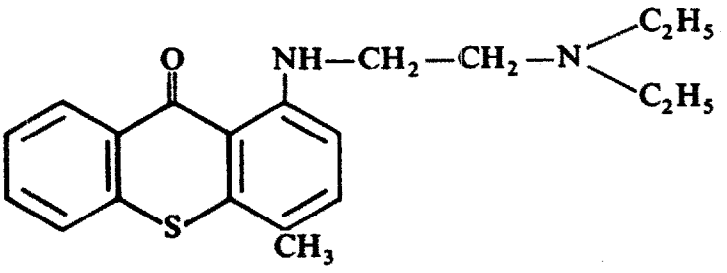
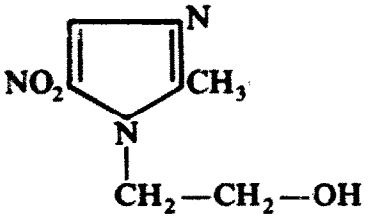


Lucanthone

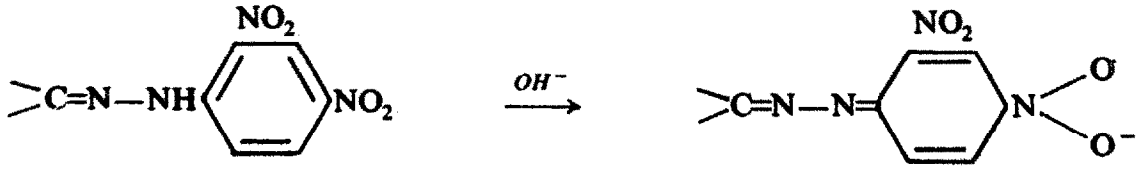
1-{[2-(diethylamino)ethyl]amino}-4-methylthioxanthen-9-one



Metronidazole

2-methyl-5-nitroimidazole-1-ethanol

No literature is available concerning the determination of hycanthone; all methods found dealt with the analysis of lucanthone. Numerous colorimetric methods for the analysis of metronidazole have been described. These involve the use of a variety of chromogenic reagents after reducing the nitro group. These chromogenic reagents include *p*-dimethylaminobenzaldehyde (Piere et al., 1969), diazotization and coupling with Bratton-Marshall reagent (Lau Edward et al., 1969), strong alkali solution and vanillin (Sanghair and Chandramoham, 1974). A UV absorption method is used for analyzing metronidazole in different solvents and at different wavelengths of maximum absorption (Sandor and Eva, 1970; Gagolkin and Grin, 1972; Puryak and Kurinnaya, 1970; Kompantseva and Vergeickik, 1973). Meanwhile gas liquid chromatographic (Wood, 1975), polarographic (Brooks et al., 1976) and gravimetric methods (Inamdar and Mody, 1977) have been mentioned in the literature. This paper reports the determination of hycanthone by reacting with 2,4-dinitrophenylhydrazine, the isolation of the product and measurement of the absorption of the red colour (λ_{max} 450 nm) produced with alcoholic potassium hydroxide solution. It has been reported by Connors (1975) that when 2,4-dinotrophenylhydrazone are treated with alkali, a red colour is produced probably as a result of resonance delocalization.



Although numerous colorimetric methods are available for metronidazole as well as several alternative instrumental methods, the need still found for the development of another colorimetric method that is sensitive, simple and accurate. The developed method depends on reducing the nitro to amino group, reacting with *p*-dimethylaminocinnamaldehyde and measuring the highly coloured Schiff's base produced at λ_{max} 510 nm. These methods are simple not only in theory but also in their technique and proved to be applicable to microgram amounts and can be used successfully for the determination of hycanthone and metronidazole in pure and dosage forms.

Materials and methods

Apparatus. Pye Unicam Spectrophotometer SP 600 was used in this study.

Materials and reagents. All chemicals used were either of analytical or pharmaceutical grade. Distilled water was used throughout.

I. For hycanthone by 2,4-dinitrophenylhydrazine method

(1) Hycanthone mesylate pure and Etrenol vials [1], supplied by Winthrop Products, New York, U.S.A.

(2) A 2,4-dinitrophenylhydrazine reagent: prepared by dissolving 0.2 g of 2,4-dinitrophenylhydrazine in 1 ml sulphuric acid, adding 1.5 ml water dropwise with swirling until solution is complete. To this warm solution add 10 ml of 95% pure ethanol.

(3) Pure ethanol: it is prepared by refluxing ethanol 99.5% for 30 min with potassium hydroxide and zinc granules followed by distillation.

(4) Ethanolic potassium hydroxide: 10% solution in 80% alcohol.

II. For metronidazole by p-dimethylaminocinnamaldehyde

(1) Metronidazole pure, flagyl vaginal tablets [2], and flagyl suspension [3] supplied by Specia Societé Parisienne D'expansion Chimique 21 Rue Jean Cougon, Paris.

(2) *p*-Dimethylaminocinnamaldehyde† reagent 0.2% solution: prepared by dissolving 0.2 g *p*-dimethylaminocinnamaldehyde and 2 g trichloroacetic acid in 100 ml methanol.

General procedure

Determination of hycanthone mesylate with 2,4-dinitrophenylhydrazine

To 10 ml of hycanthone mesylate solution (1 ml = 1 mg) add with constant

[1] Etrenol (brand of hycanthone as mesylate) powder vials of 200 mg base, 0-S057.

[2] Flagyl vaginal tablets each contains 0.5 g metronidazole Code No. 600-5B_2.

[3] Flagyl suspension contains 4 p100 of benzoyl metronidazole equivalent to 2.5 p100 of metronidazole.

[4] *p*-Dimethylaminocinnamaldehyde supplied by Merck-Schuchardt: Schuchardt, 8011 Hohenbrunn bei München.

stirring 3 ml of 2,4-dinitrophenylhydrazine reagent. Raise the temperature to boiling for about 1 min. The solution became cloudy and the orange precipitate of hydrazone was left to settle out at room temperature for 30 min. The precipitate was collected, washed with water and allowed to dry on filter pump. Dissolve in chloroform–alcohol mixture in a ratio of 1:1.5 in a 250 ml volumetric flask. An aliquot of this solution containing 0.02–0.4 mg hycanthone mesylate, was placed in a series of test tubes and evaporated to dryness on water-bath. To the residue add 1 ml ethanolic potassium hydroxide solution and transfer the solution to 10 ml volumetric flask and dilute to volume with ethanol. The absorption of the red solution was measured at 450 nm against a blank.

Determination of metronidazole with p-dimethylaminocinnamaldehyde

Transfer 50 mg metronidazole to 100 ml conical flask, add 20 ml water, 5 ml hydrochloric acid and 2 g zinc dust. Allow the reduction to proceed for about 1 h, filter, wash with water, collect the filtrate and washings in a 100 ml volumetric flask and complete to volume with water. An aliquot of this solution containing 0.05–0.4 mg metronidazole was placed in a series of test tubes, add 4 ml of *p*-dimethylaminocinnamaldehyde reagent solution and heat for about 20 min at 60–70°C. Cool and complete to 25 ml with methanol in a volumetric flask. Determine the absorbances at 510 nm against a blank.

Application to pharmaceutical preparations

Etrenol vials. The general procedure for the determination of hycanthone mesylate was carried out without pretreatment.

Flagyl vaginal tablets. Weigh 20 tablets, powder them and transfer a weight equivalent to 100 mg metronidazole to a 50 ml volumetric flask, add 5 ml hydrochloric acid, add sufficient water and dissolve as completely as possible. Complete to volume with water. Transfer to centrifuge tube and centrifuge for about 10 min. An aliquot of this solution equivalent to 50 mg metronidazole was transferred to 100 ml conical flask and complete as mentioned under the general procedure.

Flagyl suspension. An aliquot of the suspension equivalent to 100 mg metronidazole was diluted to 50 ml with water in a volumetric flask after adding 5 ml hydrochloric acid. Filter in a dry flask and dry funnel. Transfer an aliquot of the filtrate equivalent to 50 mg metronidazole to a 100 ml conical flask and complete as mentioned under general procedure.

Results and discussion

Hycanthone yields an insoluble hydrazone on reacting with 2,4-dinitrophenylhydrazine. An alcohol–chloroform mixture in the ratio of 1.5:1 was used because of its greater solvating capacity. The orange yellow colour of the hydrazone is converted to a deep red colour by adding ethanolic potassium hydroxide solution. The hydrazone in alkali hydroxide exhibits maximum absorption at 450 nm. The amount of ethanolic potassium hydroxide does not greatly affect the

TABLE I

EFFECT OF AMOUNT OF ETHANOLIC POTASSIUM HYDROXIDE SOLUTION ON THE ABSORBANCE OF 2,4-DINITROPHENYLHYDRAZONE

Amoutn of alc. KOH (ml)	Absorbances	Average absorbances
1	0.185–0.193–0.189	0.189
3	0.195–0.19 –0.185	0.19
5	0.185–0.185–0.185	0.185

intensity of the colour as shown in Table 1, so that 1–3 ml may be used to develop the red colour. The relationship between absorbance at 450 nm and concentration was quite linear up to 400 μg hycanthone mesylate/10 ml as shown in Fig. 1. Hycanthone mesylate was accurately determined by this method with a mean per cent recovery of 100.18 as shown in Table 2. The method was applied successfully for the determination of etrenol vials with a mean per cent recovery of added hycanthone mesylate 99.7 as illustrated in Table 3.

Metronidazole is analyzed through its nitro group after its reduction and the product forms a highly coloured Schiff's base with *p*-dimethylaminocinnamaldehyde

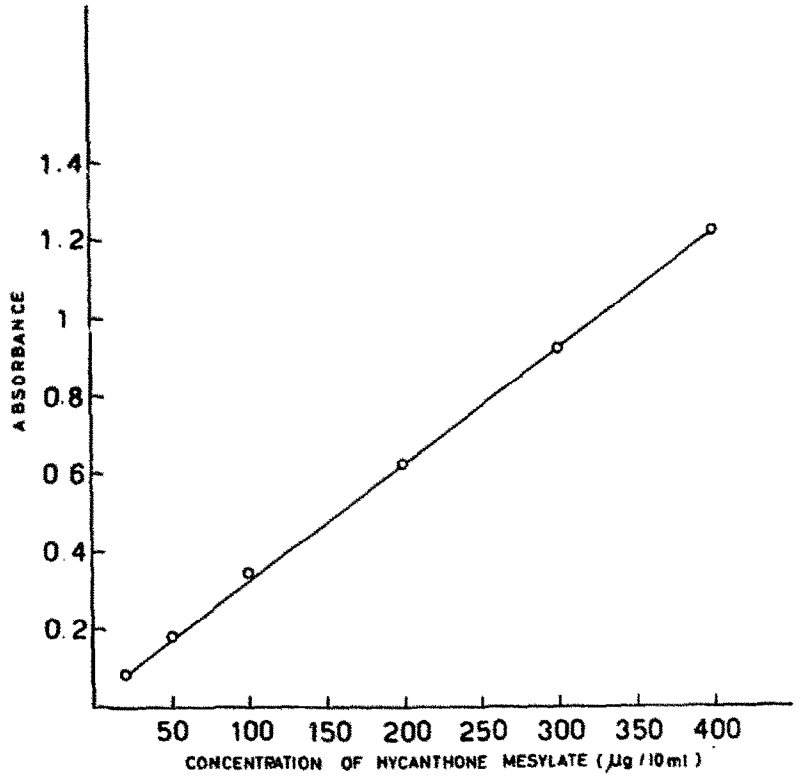


Fig. 1. Calibration curve for the determination of hycanthone mesylate with 2,4-dinitrophenylhydrazine.

TABLE 2

DETERMINATION OF HYCANTHONE MESYLATE WITH 2,4-dinitrophenylhydrazine

Amount of hycanthone mesylate		Recovery (%)
Taken (μg/10 ml)	Found (μg/10 ml)	
20	19.76	98.8
50	50.50	101.0
100	101.00	101.0
300	306.00	102.0
400	392.40	98.1
Mean		100.18
S.D.		±1.60
S.E.		±0.71

Each value given is the average of 3 experiments.

in presence of trichloroacetic acid in methanol.

$$RNH_2 + OHCC_6H_5 \rightarrow RN{=}CHC_6H_5 + H_2O$$

The optimum conditions were carefully studied and found to be 4 ml reagent as shown in Fig. 2 and heating for 20 min at 70–80°C as found in Fig. 3. The colour produced acquired maximum absorption at 510 nm and proved to be stable for about 2 h. The obedience to Beer's law is achieved at 50–400 μg metronidazole/25 ml as noticed in Fig. 4.

Table 4 shows that the mean per cent recovery of added metronidazole is 101.2 and 99.7 when the method is applied for the analysis of flagyl vaginal tablets and

TABLE 3

DETERMINATION OF ETRENOL VIALS WITH 2,4-DINITROPHENYLHYDRAZINE

Amount of hycanthone mesylate			Recovery (%)
Taken (μg/10 ml)	Added authentic (μg/10 ml)	Recovery added authentic	
100	50	49.3	98.6
200	100	101.0	101.0
250	100	99.5	99.5
	Mean	99.7	
	S.D.	±1.2	
	S.E.	±0.69	

Each value given is the average of 3 experiments.

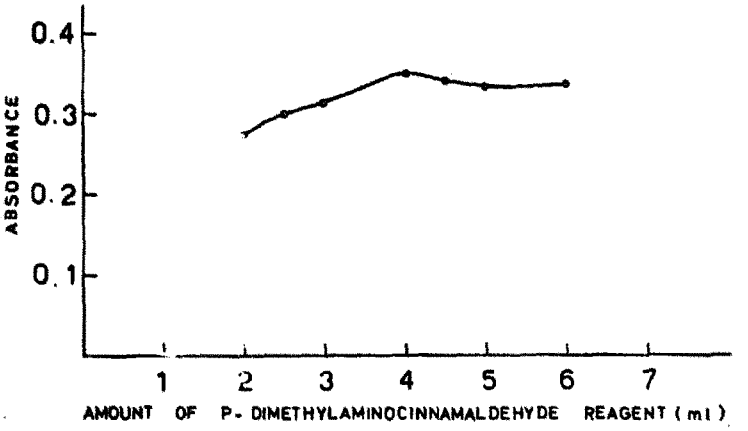


Fig. 2. Determination of amount of *p*-dimethylaminocinnamaldehyde reagent.

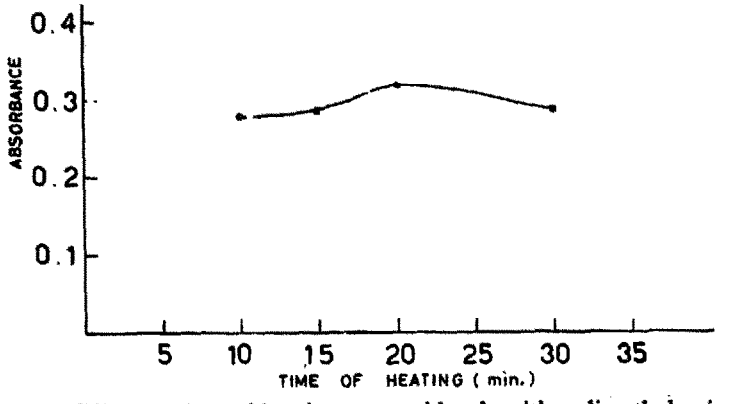


Fig. 3. Effect of time of heating metronidazole with *p*-dimethylaminocinnamaldehyde.

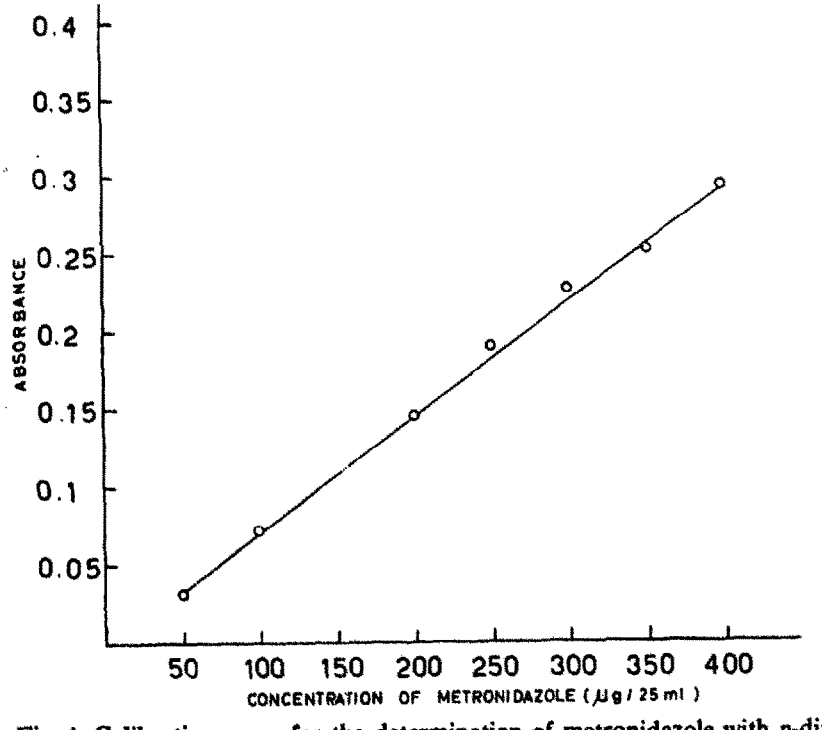


Fig. 4. Calibration curve for the determination of metronidazole with *p*-dimethylaminocinnamaldehyde.

TABLE 4

DETERMINATION OF METRONIDAZOLE IN DOSAGE FORMS.

	Amount of metronidazole			Recovery %
	Taken (μg/25 ml)	Added authentic (μg/25 ml)	Recovery of added authentic	
Flagyl vaginal tablets	100	100	100	100
	200	100	102	102
	250	50	50.8	101.6
		Mean		10.2
		S.D.		±1.06
		S.E.		±0.61
Flagyl suspension	100	50	50	100.0
	200	100	99.3	99.3
	300	50	49.9	99.8
		Mean		99.7
		S.D.		±0.36
		S.E.		±0.21

Each value given is the average of 3 experiments.

flagyl suspension respectively. This developed method was compared with the alternatives and found that the Gagolkin and Grin (1972) method for spectrophotometric determination of metronidazole showed that Lambert Beer's law was obeyed over the concentration range 1.6–4.4 mg/100 ml. Breinlich (1964) used 0.2 mg–10 mg/100 ml metronidazole for its spectrophotometric determination. Lambert-Beer's law was obeyed in the developed method over the concentration range 0.2–1.6 mg/100 ml, i.e. the developed method shows higher sensitivity than the alternatives. The detection of the degradation products is still under investigation.

References

Breinlich, J., Analysis of nitrofuran drugs and metronidazole. Deut. Apotheker. Zg., 104 (17) (1964) 535; through Chem. Abstr., 61 (1964) 5459c.

Brooks, M.A., D'Arconte, L. and De Silva, J.A.F., Determination of nitroimidazole in biological fluids by differential pulse polarography. J. Pharm. Sci., 65 (1976) 112–114.

Connors, K.A., Text Book of Pharmaceutical Analysis, 2nd Edn., John Wiley, New York, 1975, p. 481; through Analyst, 105 (1980) 669–673.

Gagolkin, S.P. and Grin, V.A., Spectrophotometric determination of metronidazole and chlomizole. Farm. Zh. (Kiev), 27 (5) (1972) 76-7; through Chem. Abstr., 78 (1973) 33972g.

Inamdar, M.C. and Mody, S.D., Estimation of metronidazole. Indian J Pharm., 39 (5) (1977) 106–108.

Kompantseva, E.V. and Vergeickik, E.N., Differential Spectrophotometric determination of metronidazole. Farmatsiya, 22 (1973) 45–8.

Lau Edward, P.K., Yao, C., Lewis, Marvin and Senkowski, Bernard, Z., Colorimetric determination of some N-substituted nitroimidazoles. J. Pharm. Sci., 58 (1969) 55–7.

Osol, A., Remington's Pharmaceutical Sciences, 16th Edn., Mack Publishers, Easton, PA, 1980, pp. 1179–1182.

Piere, P., Bernard, D., Guy, L. and Suzette, P., Colorimetric determination of 1-(2-hydroxyethyl)-2-methyl -5-nitroimidazole (metronidazole). Chem. Abstr., 70 (1969) 60876v.

Puryak, V.P. and Kurinnaya, N.V., Spectrophotometric determination of metronidazole in preparations and tablets. Khim. Issled. Farm., (1970) 74-5; through Chem. Abstr., 76 (1972) 50013w.

Sandor, G. and Eva, C., Simultaneous spectrophotometric determination of metronidazole and its 4-nitro derivative. Proc. Anal. Chem. Conf., 3rd, 2 (1970) 265-71; through Chem. Abstr., 74 (1971) 130449s.

Sanghair, N.M. and Chandramoham, H., Methods of estimation of metronidazole. Indian J. Pharm., 36 (1974) 151–2.

Wood, N.F., GLC (gas liquid chromatographic) analysis of metronidazole in human plasma. J. Pharm. Sci., 64 (1975) 1048–1049.