COLORIMETRIC ESTIMATION OF DITHRANOL

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Dithranol in glacial acetic acid when reacted with solution of sodium nitrite develops an orange yellow colour. The optimum conditions for this reaction were exploited for the quantitative estimation of dithranol alone or in ointments. Some of the common ingredients such as salicylic acid, benzoic acid, zinc oxide and boric acid usually present in dithranol ointment were found not to interfere. The method is simple and accurate and results are reproducible within $\pm$ 2 per cent.

DITHRANOL is a parasiticide and is used in treatment of psoriasis, ringworm infections and other chronic dermatoses. Though dithranol and its ointments are official in the British Pharmacopoeia, 1958, and Indian Pharmacopoeia, 1955, and also in U.S. N.F. but under the name anthralin, its assay method is available in N.F. only. This method of Auerbach[1] is based on ultra-violet absorption and was preferred to the method described by the Council of Pharmacy and Chemistry[2]. Review of the literature shows that little work has been done on the colorimetric estimation of dithranol.

Dithranol is generally incorporated in an ointment either alone or with zinc oxide, boric acid, salicylic acid and benzoic acid. While engaged in manufacture of these products, the need for a rapid but simple method for estimation of dithranol was felt. A simple test was developed in which dithranol in glacial acetic acid when reacted with a solution of sodium nitrite gave an orange yellow colour. Optimum conditions for development of this colour were studied and a colorimetric method for its quantitative estimation had been devised.

EXPERIMENTAL

Reagents: Glacial acetic acid B.P.; sodium nitrite reagents, 5 per cent w/v solution of reagent quality sodium nitrite in water; standard dithranol solution, sufficient Dithranol B.P. was dissolved in glacial acetic acid to give 0·004 per cent w/v solution.

Instrument: Measurements were made with a Spekker absorptiometer, type H760, using a 2 cm. cell, heat absorbing filters H697, and Ilford spectrum filter 602.

Light Absorption Characteristics of the Colour Developed

The light absorption of orange yellow colour produced by the reaction of dithranol and sodium nitrite was measured using the Spekker with Ilford Spectrum filters 601 to 608 from 400 to 700 mμ. The maximum absorption was obtained using filter 602 having wavelength 450 to 500 mμ which was therefore selected for the assay purpose.

Effect of Time and Temperature on Development of Colour

The colour development was slow at room temperature (29°) but rapid at 100°. The maximum intensity was reached after 2 minutes'

heating and then faded at the rate of about 3 per cent per minute (Fig. 1). Hence the reaction mixture should be heated for exactly 2 minutes with occasional shaking.

Effect of Concentration of Sodium Nitrite Reagent

Experiments were made to find the optimum concentration of sodium nitrite solution which would give maximum colour development. The findings are recorded in Figure 2. Concentrations higher than 4 per cent gave the same intensity of colour and so for our assay 5 per cent sodium nitrite was used.

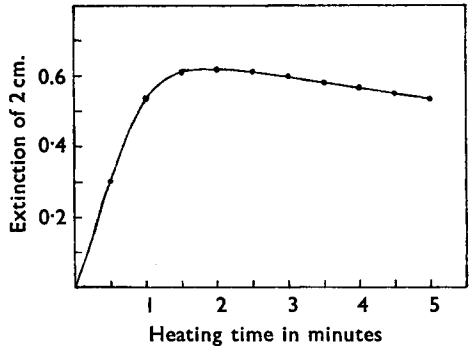

FIG. 1. Plot of absorbance of final solution against time of reaction at 100°. Initial concentration of dithranol was 150 μg. in reaction mixture. Filter used Ilford Spectrum 602.

Standard Curve and Compliance with the Beer-Lambert Law

To a series of 25 ml. volumetric flasks was transferred 1, 2, 3, 4 and 5 ml. of standard dithranol solution equivalent to 40, 80, 120, 160 and 200 μg. of dithranol respectively. The volume was made to 5 ml., if necessary with glacial acetic acid. Sodium nitrite reagent 1·0 ml. was added and the mixture heated in a boiling water bath for exactly 2 minutes with occasional shaking; the mixture was then cooled immediately to room temperature and the volume made up with glacial acetic acid. The colour was measured within 10 minutes in a Spekker absorptiometer, taking as zero the absorbance of a blank similarly treated but replacing sodium nitrite reagent by 1 ml. of distilled water. A graph representing optical density versus concentration was plotted, and found to be linear over a wide range of concentration, thus the reaction complies with the Beer-Lambert law.

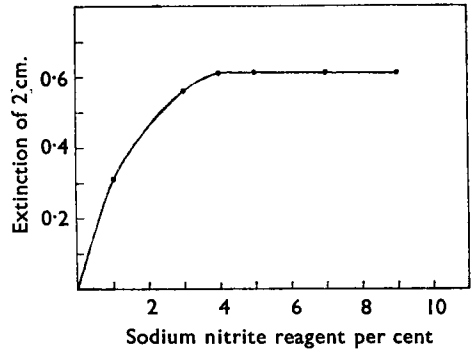

FIG. 2. Plot of absorbance of final solution againt 1 ml. of different strengths of reagent. The amount of dithranol present in reaction mixture was 150 μg. Time of heating 2 minutes. Filter used Ilford Spectrum 602.

Application of the Proposed Method to Dithranol in Ointments

An accurately weighed amount of the ointment is extracted with 20 ml. of hot glacial acetic acid, cooled and filtered. This hot glacial acetic acid

extraction is repeated three to four times. (A turbid filtrate may be obtained which will subsequently clear on further dilution.) The volume is made up to give a concentration of about 20 μg. of dithranol per ml. Take 5 ml. and develop the colour as described in the previous paragraph. (The turbidity likely to be produced on addition of sodium nitrite solution will subsequently dissolve on making the final volume before taking

TABLE I

ESTIMATION OF DITHRANOL FROM OINTMENTS

Sample	Weight taken g.	Theoretical amount of dithranol present per cent	Amount found per cent
Ointment of Dithranol B.P. ..	1·050	0·10	0·1007
" " " " ..	1·005	0·10	0·0997
" " " " ..	1·220	0·10	0·1016
Strong Ointment of Dithranol B.P.	0·988	1·0	1·002
" " " " "	1·223	1·0	1·005
" " " " "	1·105	1·0	1·015

TABLE II

RECOVERY OF DITHRANOL

Sample	Weight taken g.	Dithranol			Dithranol found mg.	Per cent recovery
		Present mg.	Added mg.	Total mg.		
Ointment of Dithranol B.P. ..	2·618	2·67	2·0	4·67	4·65	99·6
" " " ..	2·130	2·17	2·0	4·17	4·20	100·7
Strong Ointment of Dithranol B.P.	1·071	10·78	6·0	16·78	16·55	98·6
" " " " "	1·303	13·12	6·0	19·12	19·31	101·0

TABLE III

INTERFERENCE OF OTHER INGREDIENTS

Weight of Strong Ointment of Dithranol B.P. g.	Other ingredients added		Dithranol present in ointment mg.	Dithranol found mg.	Per cent recovery
1·005	Zinc oxide	100·0 mg.	10·12	10·22	101·0
1·108	Boric acid	100·0 mg.	11·16	11·02	98·8
1·025	Salicylic acid	50·0 mg.	10·32	10·22	99·0
1·010	Benzoic acid	50·0 mg.	10·17	10·27	101·0

readings.) The amount of dithranol can be calculated from the standard curve or from the optical density value of a known quantity of standard dithranol solution run simultaneously with the sample.

Using this procedure, carefully prepared samples of Ointment of Dithranol B.P. and Strong Ointment of Dithranol B.P. were analysed. The results are given in Table I and are comparable with the theoretical amount added and are reproducible within less than $\pm$ 2 per cent.

Recovery Experiments

Known amounts of dithranol were added to weighed quantities of previously analysed ointments and recoveries were found by following the method for ointment. The findings are recorded in Table II which indicate good recovery having a margin of error of less than $\pm$ 2 per cent.

Interference of Some Common Ingredients Dispensed with Dithranol in Ointments

To study the interference of some common ingredients which are likely to be present in such ointments, a weighed quantity of Strong Dithranol Ointment B.P. was taken and a known weight of each of the ingredients was added separately. The recovery was found by following the method for ointment. Results are given in Table III, which show that such ingredients do not interfere with the proposed method in the concentrations studied.

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References

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2. Council of Pharmacy and Chemistry, *J. Amer. med. Ass.*, 1944, **124**, 647.