

A RAPID METHOD FOR THE DETERMINATION OF GRISEOFULVIN IN FERMENTER BROTH

BY A. HOLBROOK, F. BAILEY AND GRETA M. BAILEY

*From the Pharmaceutical Department, Imperial Chemical Industries Limited
Pharmaceuticals Division, Alderley Park, Cheshire*

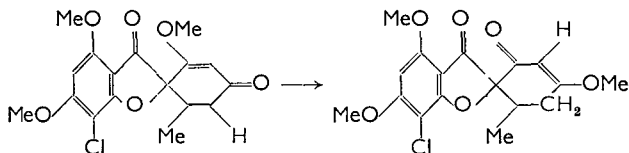
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A method has been devised for the determination of griseofulvin in fermenter broths. This is based on the conversion of griseofulvin to isogriseofulvin with methane sulphonic acid in methanol. The reaction is followed by measuring the resulting change in ultra-violet absorption. The method is rapid, accurate and suited to the routine examination of a wide variety of samples.

GRISEOFULVIN in methanol shows two absorption maxima at $236 m\mu$ ($\epsilon = 22,900$) and $291 m\mu$ ($\epsilon = 24,000$). For the estimation of griseofulvin the method of Ashton and Tootill (1956) utilises the peak at $291 m\mu$ and applies a seven-point statistically derived correction to allow for irrelevant absorption in the fermenter broth extract. The procedure requires lengthy evaluation of the experimental results and is ill suited to the daily examination of large numbers of samples. More serious, however, is the failure to differentiate between griseofulvin and such closely related compounds as dihydro-, dehydro-, tetrahydro- and dechlorogriseofulvin, griseofulvic acid and isogriseofulvin, all of which are returned as griseofulvin and all of which have been isolated from fermenter broth (unpublished observations). The present work was undertaken with the object of developing a rapid and simple assay specific for griseofulvin and applicable to a wide range of fermenter broth samples, including those typifying complex media and fermentation conditions.

EXPERIMENTAL

The change from griseofulvin to isogriseofulvin may be shown thus:



and a process for the isomerisation using hydrochloric acid in methanol has been described by Grove, MacMillan, Mullholland and Thorold-Rogers (1952). Griseofulvin and its isomer show significant differences in absorption between 260 and $280 m\mu$ (Fig. 1) and an assay based on this conversion is obviously possible if a reasonable and consistent proportion of the griseofulvin is capable of being converted to its isomer.

Untreated fermenter broth is unsuitable for spectrophotometric measurement and extraction of griseofulvin therefrom is necessary. Of

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various solvents tried, ethyl acetate was selected, as it had satisfactory optical transmission between 260–280 $m\mu$ and griseofulvin was reasonably soluble in it. For the isomerisation of griseofulvin to isogriseofulvin ethanolic solutions of hydrochloric acid, sulphuric acid and methane sulphonic acid all gave complete conversion of 1 mg. of griseofulvin in ethyl acetate in under 1 hr. at 20° and on account of the speed of reaction and ease of preparation, 2N methane sulphonic acid was chosen for routine use.

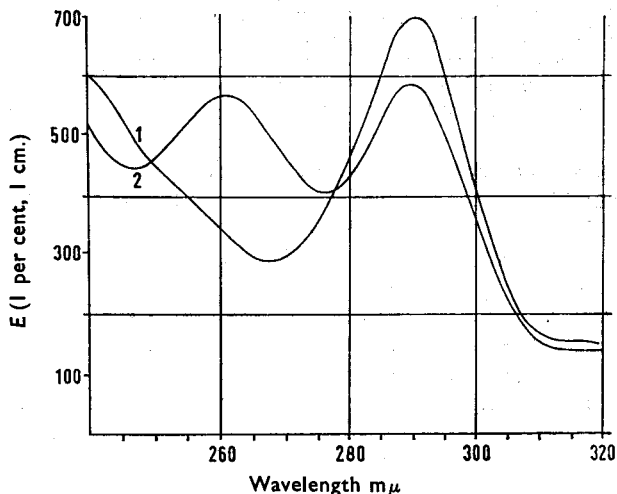


FIG. 1. Ultra-violet absorption curves for griseofulvin (1) and isogriseofulvin (2) in methanol.

Influence of water on the rate of isomerisation. It was found that water inhibited the rate of isomerisation to a marked degree, but the addition of anhydrous sodium sulphate to the sample before extraction overcame the difficulty.

TABLE I
INFLUENCE OF STRUCTURALLY RELATED IMPURITIES

Impurity	Assay by proposed method calculated as griseofulvin, per cent w/w	Assay by ultra-violet (B.P. 1963) method calculated as griseofulvin, per cent w/w
Isogriseofulvin	Nil	82
Tetrahydrogriseofulvin	Nil	80
Dihydrogriseofulvin	- 28.5	89
Dechlorogriseofulvin	110	100
Griseofulvic acid	- 25	100
Dehydrogriseofulvin	- 80	100

Influence of structurally similar impurities. Samples of impurities known to be present in fermenter broth were assayed by the proposed method. The results (Table I) show the method to be unaffected by either tetrahydro- and isogriseofulvin; the remaining impurities contribute some measure of interference, but with the exception of dechlorogriseofulvin,

the error introduced is far less than with the direct spectrophotometric procedure.

METHOD

Reagents

Standard griseofulvin solution. Dissolve pure griseofulvin (100 mg.) in ethyl acetate (100 ml.)

2N Methane sulphonic acid. Dissolve methane sulphonic acid (19.2 g.) in methanol A.R. (100 ml.)

Preparation of calibration graph. Transfer aliquots of 0.5, 1, 2, 3 and 4 ml. of standard griseofulvin solution to each of two groups of five 100 ml. volumetric flasks and add ethyl acetate to bring the volume in each flask to 5.0 ml. The first group of flasks represents the standard series; the second group the blank series. To each flask of the sample series, add 2N methane sulphonic acid (5.0 ml.). Mix and allow to stand at room temperature ($20^{\circ} \pm 3^{\circ}$) for 30 min. Dilute the contents of both series to 100 ml. with methanol A.R. and measure the extinction of each sample solution against the appropriate blank at $266 m\mu$, construct the calibration graph.

Sample assay. Transfer well mixed whole broth (2.5 g.) to a 150 ml. flask and add methanol A.R. (5.0 ml.) and anhydrous sodium sulphate (2 g.), stopper and shake vigorously to obtain an even dispersion. Add ethyl acetate (45.0 ml.), shake vigorously for 2 min. and filter. Transfer 5.0 ml. of the filtrate to each of two 100 ml. volumetric flasks and proceed as described in the preceding section.

RESULTS AND DISCUSSION

Samples chosen to cover a wide variation of media composition and fermenter conditions were analysed by the proposed method, both before and after fortification with known quantities of pure griseofulvin. The results shown in Table II illustrate quantitative recovery of the added griseofulvin. The proposed method gives results well within the range of accuracy usually considered acceptable for fermenter broth samples, and although not completely specific for griseofulvin, has considerable

TABLE II

RECOVERY EXPERIMENTS ON FERMENTER BROTH FORTIFIED WITH ADDED GRISEOFULVIN

Sample No.	Griseofulvin initially present mg.	Added mg.	Total mg.	Found mg.	Recovery per cent w/w
1	8.13	Nil	8.13	8.13	100
2	8.27	2.58	10.85	10.85	100
3	8.05	8.06	16.11	16.17	101
4	7.54	18.75	26.29	25.08	95.5
5	8.32	Nil	8.32	8.32	100
6	7.43	2.56	9.99	9.56	95.5
7	7.21	7.43	14.64	14.22	97
8	7.58	17.81	25.39	24.65	97
9	Nil	5.66	5.66	5.50	97
10	Nil	12.4	12.4	11.8	95
11	Nil	2.33	2.32	2.32	100
12	Nil	7.33	7.33	7.03	96
13	Nil	17.46	17.46	16.76	96

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advantage over previously published procedures both in day to day control of plant manufacture and in assessing the results of mutant screening experiments.

REFERENCES

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