A QUANTITATIVE CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF PURITY OF GRISEOFULVIN

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A chromatographic assay for the determination of griseofulvin is described, employing a hexane: methanol: chloroform: water partition system supported on Celite. Chromatographic separation is followed by ultra-violet measurement of the eluate fractions at 291 mμ. The system enables the determination of griseofulvin to be made in the presence of tetrahydrogriseofulvin, dehydrogriseofulvin, dihydrogriseofulvin, isogriseofulvin, dechlorogriseofulvin and griseofulvic acid.

METHODS for the determination of griseofulvin in fermenter broth fail to differentiate completely between griseofulvin and structurally similar contaminants which are also present (unpublished observations). The assay of broth samples is normally undertaken with the object of following the progress of the fermentation where this lack of specificity is not a serious handicap.

The presence of small amounts of these closely related substances in the final product would, however, go undetected by such methods and it was therefore considered desirable that a specific assay for griseofulvin be developed.

EXPERIMENTAL

The physical properties of griseofulvin and of some of the likely contaminants are listed in Table I. In view of their close similarity,

TABLE I

PHYSICAL PROPERTIES OF GRISEOFULVIN AND SOME LIKELY CONTAMINANTS

Compound	Melting-point °C	Ultra-violet absorption λmax mμ	Log ε	Infra-red absorption C=O region νmax. cm.$^{-1}$	Specific rotation $[\alpha]^{20}_D$
Griseofulvin	216	324 291 236	3·79 4·38 4·36	1,653 1,701	+356°
Dihydrogriseofulvin ..	198	323 288 234	3·70 4·31 4·10	1,680 1,715	−20°
Tetrahydrogriseofulvin	181	322 287 234	3·68 4·28 4·10	1,700	−34°
Dechlorogriseofulvin	179	288 250	4·40 4·18	1,652 1,695	+400°
Isogriseofulvin ..	199	325 291 262 234	3·70 4·31 4·39 4·31	1,654 1,696	+215°
Griseofulvic Acid ..	255–258	326 291 267 236	3·72 4·40 4·30 4·17	1,654 1,670 1,695	+399°

preliminary separation of the griseofulvin is obviously essential in any analysis specific to this substance. Previous experience with partition chromatography on Celite suggested a possible solution to the separation of griseofulvin from other likely impurities.

The solvent system used was obtained by shaking together methanol: water: hexane: chloroform (8:2:9:1). The lower layer of the mixture was employed as stationary phase supported on the Celite column packing. The upper layer was used to develop the column. The progress of the chromatogram was followed by measuring the extinction of successive 10 ml. fractions of column eluate at 291 mμ, the curve relating extinction to volume of eluate for pure griseofulvin is shown in Fig. 1.

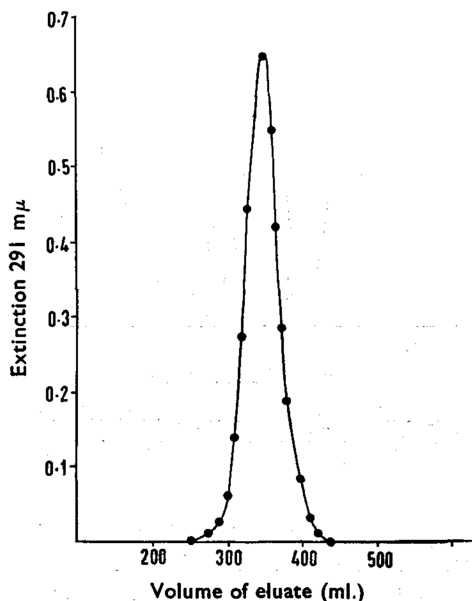

FIG. 1. Curve relating extinction with volume of eluate for pure griseofulvin.

Reagents

Prepared Celite. Stir Celite 545 (500 g.) intermittently for 12 hr. with concentrated hydrochloric acid (2 litres). Decant the hydrochloric acid and suspend the residue in 1 litre of water. Filter through a Buchner funnel, wash the residue with water until free from acid, wash with methanol (500 ml.) then methanol/ethyl acetate (1:1) (1 litre). Dry the residue in an oven at 100°. Store in well stoppered jars.

Procedure

Preparation of solvent system. Shake together methanol A.R. (800 ml.), water (200 ml.), n-hexane (900 ml.) and chloroform A.R. (100 ml.) and allow to separate. The upper layer is the eluent phase; the lower, the stationary phase.

Preparation of sample and standard. Dissolve about 20 mg. accurately weighed in the stationary phase and dilute to 25 ml. in a volumetric flask with the same mixture. Prepare a standard solution of pure griseofulvin in a similar manner.

Preparation of chromatographic column. Mix prepared Celite (15 g.) with stationary phase (7·5 ml.) and pack into a chromatographic column (70 cm. in length 2·2 cm. internal diam. fitted with a sinter plate) in portions of about 3 g., packing down firmly with a tamper between each addition. To a further 2 g. of prepared Celite add sample solution

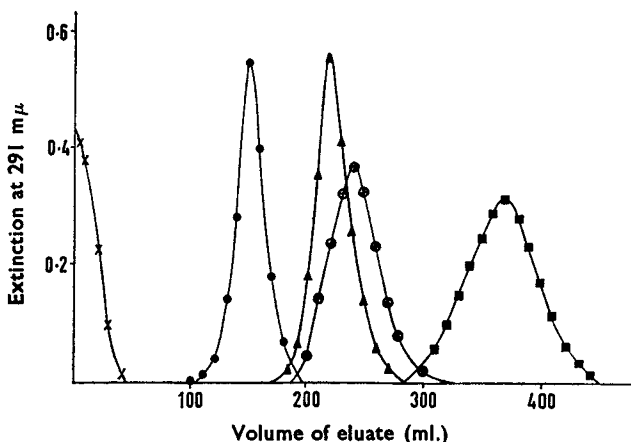

Fig. 2. Curves relating extinction volume for griseofulvin and some commonly encountered impurities. ×——× Tetrahydrogriseofulvin. ●——● Dihydrogriseofulvin. ▲——▲ Isogriseofulvin. ○——○ Dechlorogriseofulvin. ■——■ Griseofulvin.

(1·0 ml.), mix thoroughly and transfer the mix quantitatively to the top of the stationary phase in the column. Carefully add eluent phase until the stationary phase is covered to a depth of about 50 cm. and adjust the flow of eluate from the column to about 10 ml./75 sec. Collect 50 successive 10 ml. fractions of eluate in 6 in. × 1 in. stoppered test tubes. Measure the extinction at 291 mμ of each fraction against eluent phase in the reference cell. Use 1 cm. cells. Repeat the chromatogram using 1·0 ml. of standard griseofulvin solution.

Then, per cent griseofulvin in the sample $= \frac{\epsilon_a \cdot w_s \cdot 100}{\epsilon_s \cdot w_a}$ where ϵ_a and ϵ_s are the sums of extinction values under the sample and standard peaks respectively: w_a = weight of sample (mg.). w_s = weight of standard griseofulvin (mg.)

Samples of tetrahydrogriseofulvin, dihydrogriseofulvin, dehydrogriseofulvin, isogriseofulvin, dechlorogriseofulvin and griseofulvic acid were assayed as described above and the relationships between extinction at 291 mμ and volume of eluate for the less polar group of compounds are illustrated in Fig. 2. It is clear that none of the above compounds

interferes with the assay of griseofulvin by the method described and this was confirmed by the assay of artificial mixtures of pure griseofulvin with each of the above mentioned impurities. Quantitative recovery of the griseofulvin was obtained in every instance.

TABLE II

COMPARISON OF RESULTS OBTAINED USING THE PROPOSED METHOD WITH THOSE OF THE DIRECT ULTRA-VIOLET ASSAY

Sample	Griseofulvin content per cent w/w		Major impurities detected
	Proposed method	U.V. method (B.P. 1963)	
Pharmaceutical Grade Griseofulvin			
1	97·0	98·2	
2	99·5	99·0	
3	97·0	97·6	
4	100·0	100·2	
5	99·6	100·0	
6	98·4	99·0	
7	99·5	98·6	
Crude Griseofulvin			
8	96·0	96·6	Dechlorogriseofulvin
9	90·5	97·8	,,
10	95·0	97·2	,,
11	96·0	97·2	,,
12	96·0	97·5	Dechlorogriseofulvin + Dehydrogriseofulvin
Griseofulvin Residues			
13	51·7	95·0	Dechlorogriseofulvin + Dihydrogriseofulvin
14	81·0	98·0	Dechlorogriseofulvin
15	83·5	91·5	,,
16	44·0	87·2	Griseofulvic Acid
17	55·0	100·0	Dehydrogriseofulvin

RESULTS AND DISCUSSION

The proposed method has been applied to a series of samples of both pharmaceutical and crude grades of griseofulvin, and to material isolated from mother liquors at various stages of the purification process. The results compared with those using the direct ultra-violet absorption procedure proposed for the B.P. 1963 are shown in Table II and illustrate the value of the method for both control of pharmaceutical grade griseofulvin and in process development studies. Much information is gained about the nature and quantity of any impurity present and although the method as described is not recommended for the accurate determination of trace impurities in the pharmaceutical grade, simple adaptations of the technique that make this possible will be the subject of a further publication.

The papers were presented by MR. HOLBROOK.