but heparitin sulphate and keratosulphate can be differentiated by analysis of the carbohydrate components.

The disadvantage of this method, however, is that chondroitin sulphates A, B and C cannot be separated from each other; though iduronic acid of chondroitin sulphate B and glucuronic acid of chondroitin sulphate A and C can be differentiated by gas chromatography. The quantitative determination of the uronic acids by this method is under investigation.

This procedure is useful for the qualitative and semiquantitative analysis of the tissue polysaccharides, when the individual electrophoretic fractions are available in amounts of 5–50 μg.

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Quantitative determination of griseofulvin by gas–liquid chromatography

Several methods have been described for the determination of griseofulvin in many types of mixtures. The use of a spectrophotometric method[1] is relatively non-specific and therefore an indirect method[2], based on the conversion of griseofulvin to isogriseofulvin, was devised for quantitating griseofulvin in the presence of structurally similar contaminants. There is a spectrophotofluorometric assay[3,4] which is rather more specific and has been used extensively for detecting and estimating griseofulvin in biological fluids. In this assay, however, aspirin, salicylic acid and quinine are likely to interfere[5]. Recently a liquid–solid chromatographic method[6], although the procedure is tedious and time consuming, has been reported for the direct analysis of griseofulvin in complex fermenter broths.

In this study, a new gas chromatographic method was successfully developed for

the rapid, accurate, and direct determination of griseofulvin in pharmaceutical preparations.

Experimental

Apparatus and materials. A Shimadzu Model GC-1B gas chromatograph equipped with a differential hydrogen flame ionization detection system was used for this study. Packings were 1.5 % SE-30 (methyl silicone; General Electric Co.) on acid-washed and silanized Chromosorb W, 80- to 100-mesh (Johns-Manville Co.), and 1.5 % QF-1 (fluorinated alkyl silicone; Dow Corning Corp.) on acid-washed and silanized Anakrom, 80- to 100-mesh (Analabs Inc.), both prepared by the solution-coating technique[7]. A 150 cm (75 cm × 2) × 4 mm I.D. U-shaped stainless steel column was packed in a vertical position by tapping and was preconditioned overnight at 260° before use. Operating conditions were as follows: column and injection port temperature, 230°; detector temperature, 240°; nitrogen as carrier gas at 17.5 ml per min (2 kg per sq.cm) at inlet. Samples of 1 to 2 μl were injected with a 10.0 μl Hamilton syringe.

Calibration curve. A series of synthetic mixtures was prepared for injection by accurately adding 1 to 8 mg of pure griseofulvin, λ_{max}^{EtOH} 291 mμ ($E_{1cm}^{1\%}$ 708) to 1 ml of a solution containing 2 mg per ml diphenyl phthalate (internal standard) in acetone. At the fixed sensitivity and range of the instrument, approximately 1 μl of each mixture was injected into the chromatograph. The peak areas were determined by planimeter and/or by triangulation. By plotting the weight ratios against the peak area ratios of griseofulvin to diphenyl phthalate, a straight line passing through origin was obtained for the calibration curve.

Sample preparation. A quantity of suspension or a finely pulverized sample equivalent to 3 to 15 mg of griseofulvin was extracted twice with 20 ml of ether (for a suspension) or of acetone (for a powder) by shaking vigorously for 2 min. The extract was filtered through filter paper which was then washed with 10 ml of solvent. The filtrate was evaporated to dryness on a hot plate and the residue was dissolved in exactly 2 ml of a 2 mg/ml acetone solution of diphenyl phthalate.

Approximately 1 to 2 μl of solution was injected, the ratio of the peak areas again determined, and the amount of griseofulvin was calculated by comparison with the calibration curve.

TABLE I

RETENTION DATA FOR GRISEOFULVINS

Conditions the same as for Fig. 1.

	Relative retention times	
	1.5% SE-30	*1.5% QF-1*
Diphenyl phthalate (internal standard)	1.00*	1.00**
Griseofulvin	1.84	3.25
Isogriseofulvin	2.40	5.15

* Retention time: 5.5 min.
** Retention time: 5.2 min.

Results and discussion

The gas chromatography of griseofulvins without extensive pretreatment was successful on thin film columns of the silicone polymer type. A mixture of griseofulvin, isogriseofulvin, and diphenyl phthalate as the internal standard could be eluted with a satisfactory resolution factor (Table I) both on a 1.5 % SE-30 or on a 1.5 % QF-1 column. A typical chromatogram is reproduced in Fig. 1, in which each peak corresponds to 2 to 5 μg of the samples.

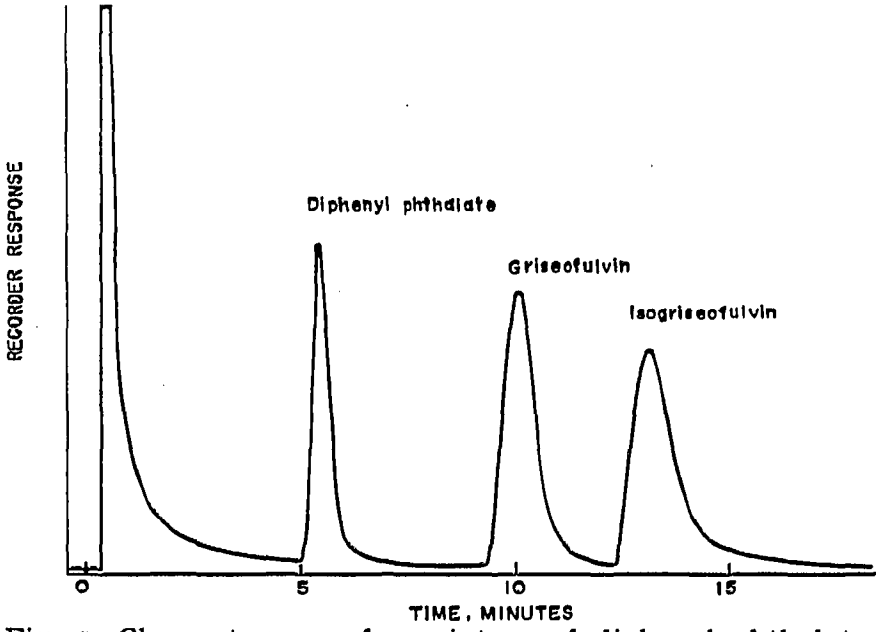

Fig. 1. Chromatogram of a mixture of diphenyl phthalate, griseofulvin, and isogriseofulvin. Conditions: 150 × 0.4 cm I.D. stainless steel column packed with 1.5 % SE-30 on 80- to 100-mesh Chromosorb W; 230°; 17.5 ml per min (2 kg per sq. cm) nitrogen; hydrogen flame ionization detection system.

Lack of thermal degradation of griseofulvin was evidenced by the fact that a single reproducible and almost symmetrical peak was obtained and also by the fact that the effluent collected at the outlet of the column presented the same ultraviolet and infrared spectra (Fig. 2) as the starting material.

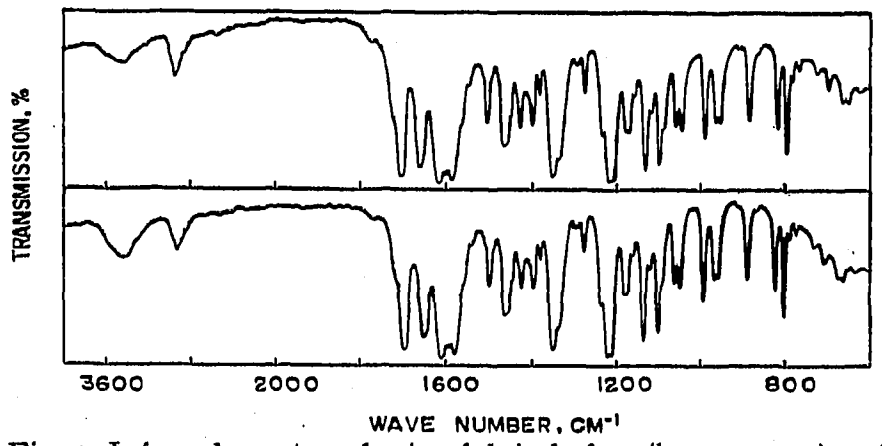

Fig. 2. Infrared spectra of griseofulvin before (lower curve) and after (upper curve) chromatography in a KBr disc.

Interfering peaks were not found in the pharmaceutical preparations examined for griseofulvin. Six synthetic pulverized mixtures were prepared of griseofulvin in milk sugar to study the recovery of a sample subjected to this proposed procedure. Recovery values determined on a SE-30 column are shown in Table II and the average overall recovery of 3.29 to 14.03 mg of griseofulvin added to 0.5 g of milk sugar was 98.9 % with a standard deviation of ± 3.1 % based on peak area measurement.

TABLE II

RECOVERY OF GRISEOFULVIN ADDED TO MILK SUGAR*

Added (mg)	*Found (mg)*	*Recovery (%)*
3.29	3.34	101.5
4.77	4.81	100.8
7.15	6.77	94.8
8.33	8.55	102.6
12.64	12.24	96.8
14.03	13.60	96.9
Average		98.9
Standard deviation		±3.1

* In separate experiments the precision was found to be ± 0.9 %.

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