GLC Analysis of Griseofulvin: A Collaborative Study

MICHEL MARGOSIS

Abstract □ A GLC method for the assay of griseofulvin in bulk and dosage forms was subjected to a wide and rigorous collaborative study. An overall recovery of 99.52 ± 2.33% for three samples from 19 participating laboratories was obtained. The success of this study is ascribed to the fact that strict performance requirements are specified for the operating system.

Keyphrases □ Griseofulvin—GLC analysis, collaborative study □ GLC—analysis, griseofulvin, collaborative study

The GLC method for the assay of griseofulvin in pharmaceutical preparations reported previously (1) was shown to be useful in characterizing the drug and has proved to be more specific, accurate, and reliable than the current official UV and microbial assay methods (2). As in the preceding study of chloramphenicol (3), it appeared advisable to subject this method to a wide collaborative study encompassing industry and academia, as well as national and supranational organizations, to demonstrate its merits and validate its practical applicability.

EXPERIMENTAL

Separate vials containing three "unknown" samples and one internal standard were sent to 24 laboratories[1]. The first unknown sample, Sample 1, was a commercial bulk, nominally pure; unknown Sample 2 was a commercial tablet; unknown Sample 3 was a tablet specifically prepared with known excipients for this study. The nominal values of the griseofulvin concentration in these samples were obtained from the manufacturers when applicable and were verified independently by the official UV procedure and this GLC procedure. These concentration values were then established at 100.00, 75.58, and 45.99% for Samples 1, 2, and 3, respectively.

Additional bulk materials were provided as working reference material and to allow the analyst to become familiar with the method and to optimize the instrumental conditions.

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Table I—Results for Griseofulvin Sample 1 (Bulk)

Laboratory	Number of Runs	Recovery Mean, %	Coefficient of Variation
1	8	100.28	3.32
2	4	100.23	0.21
3	12	97.91	2.45
4	4	101.05	0.98
5	4	96.52	2.26
6	2	98.21	0.04
7	8	100.34	0.72
8	4	97.98	0.75
9	4	95.65	1.05
10	4	100.09	0.40
11	2	98.79	1.45
12	4	100.97	0.81
13	10	99.74	0.65
14	4	99.49	1.14
15	4	98.93	1.33
16	6	99.23	2.29
17	4	96.96	1.42
18	12	99.33	2.29
19	4	99.41	1.22
Mean		99.01	1.50

The protocol of analysis was basically similar to that cited previously (1) and employed the identical curing and conditioning treatment recommended for practically all silicone columns. The calculations for potency (assay of griseofulvin), efficiency, resolution factor, and tailing factor were identical to those used in the collaborative study of chloramphenicol (3).

The analyst was requested to run duplicate injections of two separate weighings of each test sample and to compare the factor calculated from each to the average value obtained from the standard. When the coefficient of variation for five replicate injections of a standard solution exceeded a 2% limit, the entire system was to be tuned-up.

RESULTS[2]

Calculations and results from each collaborator were verified when sufficient data were available. These results include those submitted by the participants of this study and those calculated by the author from available chromatograms. Several sets of results were rejected outright. In two cases, precision values exceeding 5% in the replications of the standard were obtained. All results from another collaborator exceeded an acceptable sum rank limit by the Youden test (4), indicating a gross systematic error.

The final results were normalized to the nominal "labeled" concentration of the sample and are summarized in Tables I–IV for each sample and for the sum of the three samples. In Table V, "WD mean" is the result obtained through the weighted analysis of all observations from each collaborator, inclusive of "within sample" errors. "Mean" in Tables I–IV is the result obtained from the calculated mean from each collaborator; thus equal weight was attributed to each collaborator. The mean percent recoveries for Samples 1, 2, and 3 were 99.01 ± 1.50, 100.17 ± 2.05, and 99.37 ± 3.10, respectively, with an overall mean recovery of 99.52 ± 2.33% from 57 calculated means.

In Table V, results obtained from measurements by electronic integration are shown for comparison as well as to demonstrate the difference between the digital *versus* the combined digital and manual techniques. As expected, results derived from electronic

[2] All calculations, data processing, and statistical analyses were performed through the APL time-sharing system of an IBM 370/155 computer.

Table II—Results for Griseofulvin Sample 2 (Tablets)

Laboratory	Number of Runs	Recovery Mean, %	Coefficient of Variation
1	8	102.61	2.26
2	4	100.64	0.05
3	12	99.94	3.57
4	6	101.55	1.38
5	4	96.49	2.66
6	4	103.29	0.71
7	8	99.23	2.74
8	4	96.72	0.54
9	4	101.46	0.33
10	4	101.26	0.53
11	2	97.95	2.76
12	4	100.64	0.52
13	6	97.64	0.59
14	4	100.43	0.71
15	4	102.56	1.57
16	5	99.31	1.05
17	4	101.49	3.01
18	8	97.87	2.71
19	4	102.19	1.90
Mean		100.17	2.05

Table III—Results for Griseofulvin Sample 3 (Tablets)

Laboratory	Number of Runs	Recovery Mean, %	Coefficient of Variation
1	8	99.99	3.76
2	4	99.23	0.31
3	12	100.85	3.73
4	4	102.61	0.71
5	4	97.42	8.70
6	4	103.79	0.97
7	8	99.17	3.62
8	4	105.97	4.38
9	4	101.79	0.68
10	4	99.91	0.87
11	2	93.74	1.08
12	4	100.91	1.69
13	6	100.16	0.36
14	4	94.67	1.33
15	4	99.60	1.31
16	8	99.19	2.54
17	4	97.10	1.21
18	8	95.87	7.13
19	4	96.09	1.72
Mean		99.37	3.10

measurements were noticeably more precise, as indicated by the values of the range, variances, and standard deviations. All quantitative measurements were accomplished by one or more of six different procedures: electronic digital integration (11 sets), triangulation (six sets), peak height (four sets), disk integration (two sets), planimetry (one set), and cut and weigh (one set). These 25 sets of measurements were partitioned among the appropriate 19 participating laboratories as tabulated.

A computer plot of the results *versus* the frequency of results for each sample group numbering about 100, as well as for the combined groups of values totaling 303, yielded the typical S-shaped curve. A good fit to a normal distribution was indicated by the χ-square test for the results for each and for the combined samples. Because of its simplicity, a nonparametric sign test was performed; this indicated no detectable differences between the three samples at any confidence level.

The variances within each sample were found to be homogeneous by Bartlett's test, indicating similar reliability or precision from each collaborator at the 95% confidence level. Athough this test was not considered to be overly reliable within several sets of results because of the scarcity of observations, it may serve to attest that precision was more adversely affected by the manual techniques of peak measurements. A one-way analysis of variance was performed on all results calculated from each sample, and collaborator means were significantly different at $p < 0.05$ for all samples. However, when this analysis was performed on the means from each sample from each collaborator, no significant difference was noted between the laboratories ($F = 1.17$). This result was further verified in a two-way analysis of variance of the means, which indicated no significant differences between sample means ($F = 1.38$) and between laboratory means ($F = 1.26$).

A paired t-test was also employed to ascertain whether a statistically significant difference existed between the three samples. It was used to compare Samples 1 to 2, 2 to 3, and 1 to 3, but no such difference was detected at the 95% confidence level. A similar test comparing multiple groups of values (the Hotelling T square test) did indicate that the difference was not significant at the $p < 0.05$ level, but the calculation of this probability showed it to be near borderline (F of probability = 0.055, significant <0.05).

As mentioned before, Sample 3, made especially for this study, was formulated to contain 110 mg of griseofulvin in a 240-mg tablet containing lactose, starch, and magnesium stearate. The tablet weight varied somewhat (coefficient of variation = 1.25%), and the assay results on a per tablet basis gave a corresponding variation. To minimize this source of variation, the assay results in this study are reported on the basis of weight percent of granulation, which is indeed shown to be quite uniform.

Dechlorogriseofulvin was detected in the chromatograms by each collaborator but, because of its low concentration, it could be quantitated only by those 11 participants employing an electronic digital integrator. A 95% confidence limit of the mean for this component was calculated at 0.51–0.76% for the standard, 0.73–0.97% for Sample 1, 0.81–1.20% for Sample 2, 0.47–0.85% for Sample 3, or 0.70–0.87% for the combined total. The coefficient of variation for this measurement was estimated at about 30%, which may be acceptable for substances at this low concentration level and should also prove useful as an identity test for the product.

DISCUSSION

As in the earlier collaborative study, characteristics of column and operating conditions varied substantially from one laboratory to the next. Fifteen different models of GLC instruments from eight manufacturers were used; coiled or U-shaped columns ranged from 0.6 to 2 m in length and from 2 to 6 mm in diameter. Various supports were used with loading of 1–3% OV-17 in all but one case where 5% SE-30 was used. Except in one instance where argon was used, helium and nitrogen were used by about the same number of laboratories, with flow rates from 20 to 100 ml/min at column temperatures ranging from 245 to 280°.

Although wide flexibility was allowed in the selection of the analytical system, control of the method was attained by demanding that it meet certain performance requirements after subjecting the column to the proper temperature curing, sample conditioning, and suitability test as outlined in the protocol of analysis. These requirements included a measure of efficiency, resolution, a symmetry factor, and a maximum coefficient of variation of a number of replicate injections.

Table IV—Combined Results for Three Griseofulvin Samples

Laboratory	Number of Runs	Recovery Mean, %	Coefficient of Variation
1	24	100.96	3.25
2	12	100.03	0.65
3	36	99.57	3.45
4	14	101.71	1.21
5	12	96.81	4.94
6	10	102.48	2.32
7	24	99.58	2.59
8	12	100.22	4.93
9	12	99.64	3.03
10	12	100.42	0.84
11	6	96.82	2.91
12	12	100.84	1.03
13	22	99.28	1.18
14	12	98.20	2.86
15	12	100.36	2.08
16	19	99.24	2.05
17	12	98.51	2.92
18	28	97.93	4.35
19	12	99.23	3.02
Mean		99.52	2.33

Table V—Summary of Results[a]

Sample	A	B	C	D	AE	BE	CE	DE
Number of collaborators	19	19	19	19	11	11	11	11
Sample size	104	99	100	303	48	47	48	143
WD mean	99.11	100.14	99.49	99.57	99.50	99.74	99.96	99.73
Median	99.64	100.52	99.51	99.92	99.96	100.26	99.73	99.99
95% lower confidence limit	98.70	99.59	98.66	99.22	99.06	99.10	98.93	99.32
95% upper confidence limit	99.52	100.69	100.32	99.93	99.94	100.38	100.98	100.15
Maximum	104.03	106.91	111.48	111.48	103.22	104.31	111.48	111.48
Minimum	92.78	92.67	82.71	82.71	95.30	95.66	93.02	93.02
Range	11.25	14.24	28.77	28.77	7.92	8.65	18.46	18.46
Variance	4.47	7.51	17.45	9.86	2.29	4.75	12.43	6.45
SD	2.11	2.74	4.18	3.14	1.51	2.18	3.53	2.54

[a] Labels A, B, and C are Samples 1, 2, and 3, respectively, and D is the combined value of all three, calculated by all methods of integration; those labeled with E are the corresponding results derived from electronic integration.

The efficiencies of the columns used in this study ranged from a minimum of about 300 to a maximum of 2000 theoretical plates/m with a mean of 993 ± 37 plates/m. The symmetry factor on the OV-17 phase was calculated to be from 0.7 to 1.3 with most from 0.9 to 1.15; with the SE-30 phase, a factor of 1.6 was obtained. Although the retention volume is a more characteristic qualitative property of the solute, the operating conditions in the different systems used were too variable and almost impossible to replicate for accurate measurement. In all cases but one, the resolution factor exceeded a value of 4 with most factors lying between 6 and 8.

The internal standard may be used quite effectively in lieu of the retention volume for identification of the drug, particularly when compared to the behavior of an authentic sample. In this study, the retention time of griseofulvin relative to tetraphenylcyclopentadienone ranged from 1.87 to 2.12 with a mean of 2.01 ± 0.06 (2.9%) (95% confidence interval: 1.98–2.04) in 19 cases where OV-17 was used; with SE-30, the relative retention time was 2.95.

The statistical difference noted in the one-way analysis of variance of all observations was likely due to the overloading of the chromatographic column, which was noted by some analysts during the study. This overloading may be avoided by injecting about 1 μg of solute either by using a reduced volume or by diluting the final solution about 10-fold. This decrease in the amount of injected solute improves the overall performance characteristics of the column, as typically demonstrated by one collaborator who increased the efficiency of the column threefold and greatly improved the symmetry of the peaks.

One participant had great difficulty in the elution of the internal standard because of condensation in the exit line. However, another participant employing an identical chromatograph, which does not normally possess the capability of separate heating of the exit port, obtained adequate and reproducible data. It is presumed that this result was achieved through a combination of higher temperatures and higher carrier gas flow rates. This was the only major handicap reported.

CONCLUSION

The participants in this collaborative study proved this GLC method for griseofulvin to be reliable and efficient. The results reported, analyzed, and discussed amply validate the method. Therefore, because of its demonstrated superiority, this method has been proposed for inclusion in the "Code of Federal Regulations" and is recommended for primary compendial and regulatory usage.

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