Selection, Evaluation, and Control of the Assay of the Pharmaceutical Product I

Reproducibilities of Assay and Drug Recovery from Dosage Forms

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Studies have been conducted on the variation of fill weight and drug content among units of hard filled capsules, soft elastic capsules, and compressed tablets. The cases in point were exploratory dosage forms of the antibiotic fumagillin. Consideration of the dosage form and the excipients dictated the methodology. Statistical treatment of the data has permitted conclusions as to optimum amounts of solvent for the separation of the active ingredient, the number of dosage units necessary to assay within a desired confidence limit, the reproducibility of fill weight among units, and the homogeneity of the fill mix. The evaluation of the variability among the data also permitted the most economic utilization of assay facilities in stability studies.

A BASIC FUNCTION of the control laboratories in the pharmaceutical industry is to assay and control the final product so that it conforms to those specifications which have been established to insure that (*a*) the composition is and remains as specified, (*b*) the pharmaceutical utility is retained, and (*c*) the pharmaceutical elegance of color, taste, and form persists; all for the duration of the pharmaceutical lifetime of such a product.

Routine assay procedures for final products must be carefully designed so that they are simple, efficient, and economical. These considerations necessitate the application of statistical techniques for the proper evaluation of assay procedures and the determination of errors of assay. Thus, the degree of confidence in product composition, stability estimates, and biological potency may be ascertained.

This paper considers the fundamental problems of recovery of drug from the final pharmaceutical dosage form, the number of such dosage forms to be used in assay, and the variability in content that may be encountered among units of the same dosage form. Dosage forms considered are hard filled capsules (HFC), soft elastic capsules (SEC), and compressed tablets (CT). The procedures of the simple statistics used are easily followed from many of the fine texts available (1).

The particular drug considered in these studies was the antibiotic fumagillin (2–4) in exploratory preparations of HFC, SEC, and CT. The initial reason for this comparative study was to establish the minimum and most economical procedures for the evaluation of the drug's stability in its final dosage form.

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EXPERIMENTAL

The ultraviolet spectrophotometric absorbance of fumagillin at 351 mμ in 95% ethanol-water was chosen as the method of determining fumagillin content since these values have been correlated with biological activity (2). Photolytic, oxidative, and thermal degradation of fumagillin in solution and as crystalline material diminishes the absorbance at 351 mμ (2–4). The Beckman ultraviolet spectrophotometer, model DU, was used.

The procedures devised for the spectrophotometric determination of fumagillin content follow.

Hard Filled Capsules.—The theoretical composition of the HFC was 12 mg. fumagillin, 10 mg. ascorbic acid, 226 mg. lactose, 1.3 mg. calcium stearate, with a fill weight of 259 mg. An initial study was conducted on 10 capsules where each capsule was weighed on an analytical balance and subsequently emptied into a beaker by tapping or gouging with the end of a fine spatula. The capsule was washed twice with 95% ethanol, the spatula was rinsed with

TABLE I.—FILL WEIGHT AND SPECTROPHOTOMETRIC ASSAY OF FUMAGILLIN CONTENTS OF HARD FILLED CAPSULES

Capsule No.[a]	Wt. of Fill (*W*)	Absorbance (*A*)[b] at 351 mμ	*A*/*W*
1	0.2613	0.454	1.7375
2	0.2597	0.410	1.5787
3	0.2562	0.379	1.4793
4	0.2576	0.380	1.4752
5	0.2572	0.323	1.2558
6	0.2535	0.377	1.4872
7	0.2576	0.373	1.4480
8	0.2671	0.517	1.9356
9	0.2588	0.477	1.8431
10	0.2579	0.477	1.8496

[a] A 10-ml. quantity of 95% ethanol was used to extract fumagillin from each of capsules 1–7; 50 ml. from each of capsules 8–10. [b] Measured at 4×10^{-4} capsule contents per ml. final solution.

solvent, and all washings and contents were collected so that a total of 10 ml. solvent to extract was used in seven cases and a total of 50 ml. was used to extract in the other three cases. The material was allowed to settle and the contents of the beaker were decanted through No. 2 Whatman filter paper. The residue was washed with two 2-ml. portions of ethanol, filtered, and the filtrate was made up to 100 ml. in a volumetric flask with 95% ethanol. A 10-ml. aliquot of this solution was made up to 250 ml. The result was that there was 4×10^{-4} of the original capsule per ml. of final solution. This concentration could be spectrophotometrically read in the appropriate absorbance range (*ca.* 0.5) at 351 mμ. The weight of the empty capsule was determined after drying at room temperature so that the net weight of fill could be obtained. These data are given in Table I.

A similar experiment was conducted on each of six HFC except that the volume of 95% ethanol used to extract the fumagillin was varied. See Table II.

TABLE II.—ABSORBANCES OF HFC FUMAGILLIN AT 351 mμ EXTRACTED WITH VARYING AMOUNTS OF 95% ETHANOL

Capsule No.	95% Ethanol Used in Extraction, ml.	Absorbance at 351 mμ[a]
11	10	0.418
12	20	0.442
13	30	0.430
14	40	0.479
15	50	0.484
16	75	0.493
17	100	0.489

[a] At 4×10^{-4} capsule contents per ml. of final solution.

Soft Elastic Capsules.—The theoretical composition of the SEC was 12 mg. fumagillin, 10 mg. ascorbic acid, 160 mg. peanut oil, 5.27 mg. Cotoflake, with a fill weight of 196 mg. Each of 10 capsules was weighed on an analytical balance and cut almost in half over a beaker with a sharp, clean razor blade. Extreme care was taken to get no oil on the fingers. The entire capsule was dropped into the skellysolve B rinsings of the razor blade edge (*ca.* 15 ml.) and swirled to remove the contents. The SEC shell was removed and rinsed with a few ml. of fresh skellysolve B which was then added to the contents of the beaker. The shell was dried in a hood at room temperature and re-weighed to obtain the net weight of the fill. The skellysolve B was evaporated from the beaker in the hood at room temperature, and 50 or 100 ml. of 95% ethanol was added to attempt dissolution. Subsequent filtration and preparation of the solution for spectrophotometric assay was the same as for that previously given for the HFC with 4×10^{-4} of the original capsule contents per ml. of final solution. The data are given in Table III.

Compressed Tablets.—The theoretical composition of the CT was 12 mg. fumagillin, 10 mg. ascorbic acid, 226 mg. lactose, 9.8 mg. sucrose, 6.5 mg. starch, 2.3 mg. calcium stearate, with a weight of 277 mg. Each tablet was weighed on an analytical balance and dropped into 95% ethanol. The tablet was extracted with various amounts of ethanol as specified in Table IV. Subsequent filtration and preparation of the solution for spectrophotometric assay was the same as for that previously given for the HFC with 4×10^{-4} of the original tablet per ml. of final solution. The data are given in Table IV.

TABLE III.—FILL WEIGHT AND SPECTROPHOTOMETRIC ASSAY OF FUMAGILLIN CONTENTS OF SOFT ELASTIC CAPSULES

Capsule No.[a]	Wt. of Fill (W)	Absorbance (A)[b] at 351 mμ	A/W
1	0.2011	0.542	2.7001
2	0.2030	0.533	2.6256
3	0.1929	0.510	2.6439
4	0.1698[c]	0.447	2.6325
5	0.2012	0.556	2.7634
6	0.2120	0.591	2.7877
7	0.1993	0.507	2.5439
8	0.2043	0.552	2.7019
9	0.1979	0.608	3.0723
10	0.2024	0.575	2.8409

[a] A 50-ml. quantity of 95% ethanol was used to extract fumagillin from the contents of each of capsules 1–5; 100 ml. from contents of each of capsules 6–10. [b] At 4×10^{-4} capsule contents per ml. final solution. [c] This datum is low; it may be queried if this is a maverick or if it has a real probability of occurrence. See text.

TABLE IV.—COMPRESSED TABLET WEIGHT AND SPECTROPHOTOMETRIC ASSAY OF FUMAGILLIN CONTENT

Tablet No.[a]	Wt. of Tablet (W)	Absorbance (A)[b] at 351 mμ	A/W
1	0.2737	0.460	1.680
2	0.2832	0.472	1.667
3	0.2795	0.464	1.660
4	0.2816	0.475	1.687
5	0.2761	0.470	1.702
6	0.2808	0.475	1.692
7	0.2778	0.475	1.710
8	0.2760	0.472	1.710
9	0.2799	0.481	1.718

[a] A 50-ml. quantity of 95% ethanol was used to extract fumagillin from each of tablets 1–3; 75 ml. from each of tablets 4–6; and 100 ml. from each of tablets 7–9. [b] At 4×10^{-4} tablet per ml. final solution.

TABLE V.—ANALYSIS OF VARIANCE OF ABSORBANCE AT 351 mμ PER GM. TABLET WEIGHT (A/W) FOR VARYING AMOUNTS OF ETHANOL EXTRACTION[a]

Source	Sum of Squares	Degrees of Freedom	Variance
Among extractions	0.0030481	2	0.001524
Within extractions	0.0002208	6	0.0000368
Total	0.0032689	8	0.0004086

[a] Quantities of 50, 75, and 100 ml.

CALCULATIONS AND RESULTS

The data of Table I indicate a large difference in measured absorbance between HF capsules 1–7 (average $A/W = \bar{x}_1 = 1.4945$ for $n_1 = 7$) extracted with 10 ml. 95% ethanol and capsules 8–10 (average $A/W = \bar{x}_2 = 1.8761$ for $n_2 = 3$) extracted with 50 ml. 95% ethanol. Application of the t test confirms this difference. The calculated t is $(\bar{x}_2 - \bar{x}_1)/\sigma \cdot \sqrt{n_1 n_2/(n_1 + n_2)} = 3.73$ at 8 degrees of freedom. This exceeds the critical value from the t tables, i.e., 2.31 for 5% probability of no difference between the extraction procedures. It is thus necessary to

TABLE VI.—SUMMARY OF REPRODUCIBILITY OF PHARMACEUTICAL PREPARATIONS OF FUMAGILLIN

	HFC	SEC	CT
Fill wt. and 95% confidence limits of one tablet or capsule ($\bar{x} \pm t\sigma$)[a]	259 ± 8 mg. (259)	202 ± 12 mg. (196)[c]	279 ± 7 mg. (277)
% Deviation of single unit from mean, 100 ($\sigma/\bar{x}$)	1.39%	2.56%[c]	1.09%
Absorbance at 351 mμ and 95% confidence limits ($\bar{x} \pm t\sigma$)	0.483 ± 0.017[b]	0.553 ± 0.080	0.472 ± 0.015
% Deviation of single absorbance from mean, 100 ($\sigma/\bar{x}$)	1.39%[b]	6.21%	1.34%

[a] Theoretical fill weight given in parentheses. [b] Includes only those HFC extracted with 40 or more ml. of 95% ethanol. [c] SEC No. 4 in Table III is excluded.

determine the minimum amount of 95% ethanol necessary for complete extraction of fumagillin from the contents of the hard filled capsules. These data are reported in Table II and readily show that 40 or more ml. of 95% ethanol is necessary for relatively complete extraction of fumagillin from one capsule.

From the data of Table III we have, for capsules numbered 1 through 5, the average $A/W = \bar{x}_1 = 2.6731$ for $n_1 = 5$, and for capsules numbered 6 through 10, the average $A/W = x_2 = 2.7893$ for $n_2 = 5$. The calculated t for 8 degrees of freedom does not exceed the Table's value of t. It is concluded that either 50 or 100 ml. of 95% ethanol will give satisfactory extraction of fumagillin from the contents of each soft elastic capsule.

The data of Table IV for compressed tablets were obtained from extractions with 50, 75, and 100 ml. of 95% ethanol. An analysis of variance of these data calculated as absorbance per unit weight of fill, A/W, is given in Table V. The calculated F value for the ratio of variance among extractions to variance within extractions is 41 for 2 and 6 degrees of freedom. The F value at the 1% level from the tables is 10.9. It can be concluded that the variation among volumes of extraction is highly significant with respect to the variation within extraction by the same volume of 95% ethanol. This does not necessarily mean that the use of 100 ml. rather than 50 ml. of ethanol introduces a large error in this case, only that our methods of measurement are precise enough to evaluate the differences. In fact, the difference in extractability is extremely small, percentage wise, only 2.5% between the mean A/W values for 50 ml. and 100 ml. ethanol extractions (see Table IV).

The per cent standard deviation, $100 \times \sigma/\sqrt{(A/W)}$, among all the tablets is 1.2%. This includes the possible heterogeneity of fumagillin in the tablet mix, the procedural error, and the variation among volumes of ethanol used in extraction.

The reproducibility of the fill weight and the absorbance of extracted fumagillin in each of the HFC, SEC, and CT preparations of fumagillin are summarized in Table VI. The 1.4% probable deviation in absorbance from capsule to capsule, HFC, is the same as the estimated probable deviation in fill weight, which indicates that the fill may be of homogeneous fumagillin content as a first approximation provided that sufficient volumes of extracting solvent are used. This is confirmed by the consistency of the absorbances of capsules 14 through 17 in Table II.

DISCUSSION

These investigations and the simple statistics involved contribute a great deal of information. They permit an evaluation of the best methodology for the assay of the pertinent component of a finished pharmaceutical.

The fundamental step is to separate the drug from the assay-interfering substances of the final product and prepare it appropriately for assay. In the cases of the HFC and SEC preparations, this involved a physical manipulation of each unit. Spectrophotometric assay procedures necessitated the preparation of homogeneous solutions. Alcoholic solution with subsequent filtration was a satisfactory method to separate the fumagillin from the excipients of the HFC fill and the tablets.

The oil base and nature of the SEC demanded the use of an oil-miscible solvent for separation and skellysolve B was an appropriate choice.

Simple statistical evaluations permitted the choice of optimum amounts of solvent to use in the assay procedures.

The single tablet or capsule technique clearly showed a high reproducibility of fumagillin content in a single HFC and tablet (Table VI).

The determination of variations in fumagillin content per unit dosage form, fill weight, and the ratio of these two values demonstrated that modern pharmaceutical processing may produce a high degree of homogeneity in the prefill mix and a high degree of reproducibility per unit HFC and tablet.

The knowledge of a standard deviation, σ, permits determination of the number, n, of units to be used to give a control value within the error range desired, i.e. $\sigma/\sqrt{n}$. For example, if 2% error were acceptable, the absorbance values from nine soft elastic capsules should be averaged which would reduce the 6% standard deviation of a single capsule cited in Table VI. This would be a valid procedure if the variation among capsules can be attributed solely to the error in the assay of a single capsule.

The greatest limitation on elegant stability studies on finished pharmaceutical products is the expense of assay. Certainly in the case of these HFC and CT preparations, assay of single unweighed units at timed intervals under accelerated or storage conditions would minimize cost and yet give excellent stability data.

The evaluation of the data for the SEC show that this preparation is not as suitable. The variation in reproducibility of fill weight is relatively high 2.6% (Table VI), and the total fumagillin content

varies even more (6.2%), even with the exclusion of the anomalous capsule No. 4 of low fill weight. Capsule No. 4 may either have a reasonable probability of occurrence or may be a maverick, possibly due to either improper weighing or being the first or last capsule prepared from the lot. Good control practice would demand repeat assays to clarify this.

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Preparation and Properties of New Gastric Antacids V

Aluminum-Magnesium Hydroxide Dried Gels

By STEWART M. BEEKMAN

The preparation of nine aluminum-magnesium hydroxide gels in the Al:Mg atomic ratio range of 2:1, 1:1, and 0.5:1 by a new process is described. The determination of the antacid activity by the modified *in vitro* method of Holbert, Noble, and Grote shows that dried gels stabilized with glycine or sorbitol have antacid characteristics similar to clinically proved liquid aluminum-magnesium hydroxide gel. Blends of the various components separately dried are shown to be relatively ineffective by comparison. The new blends of old antacids are predominantly simple mixtures and not new compounds. Unstabilized dried gels show a substantial aging effect.

In 1944 Rossett and Flexner (1) concluded that mixtures of milk of magnesia with aluminum hydroxide gel were more effective gastric antacids than aluminum hydroxide gel alone. They reached these conclusions as a result of *in vivo* experiments on humans together with extensive clinical experience. They suggested smaller and less frequent doses of the mixtures. They pointed out the avoidance of the undesirable high initial pH rise from the milk of magnesia alone, and the offsetting of the constipating effect of aluminum hydroxide. The outstanding clinical effectiveness of liquid aluminum-magnesium hydroxide gels have been reported by others (2–4) for the treatment of peptic ulcer, hyperacidity, heart burn, spasticity, and gastritis. Properly formulated they are very prompt and prolonged in antacid activity, maintain the gastric pH between 3 and 5, and do not exhibit significant diminution of antacid action on aging.

This investigation had as its purpose the preparation of aluminum-magnesium hydroxide gels in dry form which approaches the antacid activity of the clinically proved fluid gels.

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EXPERIMENTAL

Preparation of Dried Gels.—Three series of aluminum-magnesium hydroxides were prepared. In each series the amount of aluminum to magnesium was varied in the atomic ratios of 2:1, 1:1, and 0.5:1.

The first series (AMH) consisted solely of aluminum and magnesium hydroxide. The second series (AMHG) was formulated to contain approximately 20% of glycine in the dried gel. The third series (AMHS) was prepared to contain 20% of sorbitol.

The method of preparation of all nine dried gels was similar. Highly reactive aluminum hydroxide containing some carbonate and pure gelatinous magnesium hydroxide were separately precipitated, the combined slurries were filtered and washed free of soluble salts, the mixed hydrogels were subjected to highly intensive shear at room temperature and reduced to finely divided powder form by either spray drying or low temperature air drying together with fine pulverizing. Glycine crystals or sorbitol solution was added to the mixed hydrogels before milling.

Physical Properties.—All the dried gels are soft, white, smooth, fine, tasteless, and odorless powders which react readily with gastric strength acid-containing pepsin. The AMH dried gels are somewhat less dense than those containing sorbitol or glycine. The pH of aqueous suspensions of the AMHG series is about 9.0—the others are about 9.6. The carbonate (as CO_2) content ranges from about 5.0 to 12% depending mainly on the method of drying. Dried gels containing glycine or sorbitol are less