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

Effect of Sampling and Bulk Mix Heterogeneity on Tablet Variation

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The coulometric bromine oxidation procedure for *o*-methoxy-α-methylphenethylhydrazine has been applied to the analysis of two experimental tablet preparations and one lot of bulk drug. The results show that the variation from tablet to tablet is significantly greater than the error in the assay, that random sampling of bulk drug should be rigidly controlled, that the micro-heterogeneity of a tablet mix may exceed the variability among tablets. The present investigation points out the need for statistically designed studies to determine the uniformity of the drug content.

One of the major production and control interests in the pharmaceutical industry is the preparation of reproducible dosage forms. The variables encountered are the degree of homogeneity of the bulk drug and the pharmaceutical mixture of the drug and excipient, the reproducibility of the dose per unit dosage form, and the relation of these factors to the error in the assay of bulk drug, formulation mix, and final dosage form.

This paper considers the detailed design and statistical evaluation of data obtained on an exploratory batch of tablets to delineate completely the above stated problems.

A coulometric titration procedure with electrolytically generated bromine had been devised to assay the pharmacologically active drug, *o*-methoxy-α-methylphenethylhydrazine in acetic acid-water-methanol and is reported in the literature (1).

The statistical methods are as given in standard texts (2) and the prior paper in this series (3).

EXPERIMENTAL

The coulometric titration with electrolytically generated bromine of *o*-methoxy-α-methylphenethylhydrazine, I, has been published by Olson (1) and the procedures used in these studies were identical with those described. For single tablet assay, the tablet was thoroughly ground, and weighed portions of this mix were analyzed. Analysis of I without excipients and with the excipients of tablet preparations A and B shows no interference with the assay procedure (Table I). The consistent positive error in these assays has been shown to be due to slight hydrazine contamination.

The details of the statistical designs for the studies

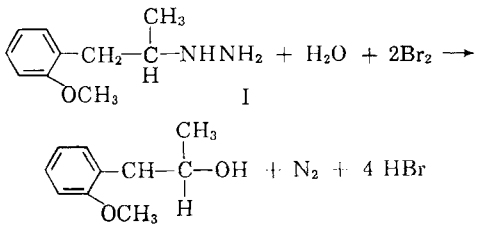

TABLE I.—EFFECT OF EXCIPIENT ON ASSAY OF DRUG

Excipient/12 mg. of Drug	% Drug[a]
None	105.14
	105.09
Preparation A	
8 mg. citric acid	
14.5 mg. starch	106.21
210 mg. terra alba	105.18
3.0 mg. calcium stearate	
Preparation B	
8.0 mg. citric acid	
19.5 mg. starch	
180 mg. mannitol	105.04
4.0 mg. calcium stearate	106.18
5.0 mg. talc	

[a] Hydrazine contaminant gives positive error (1).

TABLE II.—PER CENT *o*-METHOXY-α-METHYLPHENETHYLHYDRAZINE IN 25 SAMPLES OF LOT A[a]

Day	Sample 1	2	3	4	5
1	101.85	100.82	102.54	101.21	102.75
	104.46	101.22	101.75	101.91	102.19
2	102.87	101.50	102.85	102.35	102.56
	102.09	102.22	102.53	102.28	102.41
3	103.36	100.30	100.18	107.67	97.84
	101.43	101.00	97.21	99.77	99.79
4	100.28	100.66	98.95	100.25	97.88
	103.78	101.23	101.79	100.88	101.46
5	100.58	101.75	105.58	103.15	103.69
	102.32	103.44	103.31	102.48	103.53
			Average	101.518	

[a] The request was for random samples of the lot. The duplicates are from a blended, homogenized sample. Five samples were run on each of five successive days.

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TABLE III.—ANALYSIS OF VARIANCE OF TABLE II DATA[a]

Source of Variation	DF	SS	MS	Components of Variance	F Test
Total	49	166.96			
Among days	4	38.61	9.651	$\sigma^2 + 2\sigma_s^2 + 10\sigma_D^2$	$F = 3.15$ (5% F is 2.9); significant.
Within days	20	61.23	3.061	$\sigma^2 + 2\sigma_s^2$	Not significant
Between duplicates	25	67.12	2.684	σ^2	

Estimated variances

$\sigma^2 = 2.684$; $\sigma = 1.64$

$\sigma_s^2 = 0.189$; $\sigma_s = 0.43$

$\sigma_D^2 = 0.659$; $\sigma_D = 0.81$

$\sigma_t^2 = \sigma^2 + \sigma_s^2 + \sigma_D^2 = 3.532$; $\sigma_t = 1.88$

[a] Duplicate assays among and within days.

on the homogeneity of the bulk drug and tablets with the evaluation of assay errors are given in the next section.

RESULTS AND DISCUSSION

Evaluation of Variability among Assays and Days of Assay of the Bulk Drug.—The coulometric assay of *o*-methoxy-α-methylphenethylhydrazine in 25 samples of the bulk drug, lot A, are given in Table II. The high results (>100%) have been shown to be due to hydrazine contaminants (1). The request was made that this lot of bulk drug be randomly sampled. Each of the 25 samples was homogenized and micronized, and two assays were run on each sample. As received, 5 samples were run in duplicate on each of five days.

The Table III analysis of variance of the data of Table II demonstrated that the variation among the samples run on the same day is not significantly greater than the variations between duplicates. However, the variation among days is significantly greater than the variation among the daily samples. This implies a daily variation in the assay or a bias in the samples assigned to be assayed on a given day.

TABLE IV.—ASSAY DATA OF DRUG IN TABLETS OF PREPARATION A

Tablet No.	Tablet Wt., mg.	Replicate Assays mg. Drug/100 mg. of Tablets	
1	249.6	3.626	3.699
2	250.9	3.882	3.944
3	251.1	4.396	4.227
4	249.5	4.100	4.026
5	249.7	4.189	4.119
6	250.9	4.322	4.261
7	248.4	4.078	4.019
8	244.3	3.982	3.967
9	247.6	3.942	3.845
10	244.9	3.639	3.682
Average	248.7	4.000	

$\sigma^2 = 5.79$, $\sigma = 2.41$, % of mean = 0.969 for tablet wt.

Random Samples of Blend	
1	4.334
2	4.057
3	3.274
4	3.296
5	3.635
Average	3.719
σ^2	0.2191
σ	0.468
S. D.: % of Mean	12.6

TABLE V.—ASSAY DATA OF DRUG IN TABLETS OF PREPARATION B

Tablet No.	Tablet wt., mg.	Replicate Assays mg. Drug/100 mg. of Tablets	
1	230.2	4.042	3.826
2	232.3	4.406	4.372
3	228.0	4.358	4.382
4	231.5	4.322	4.284
5	226.1	4.185	4.095
6	229.0	4.012	3.881
7	230.0	4.178	4.134
8	228.1	4.167	4.173
9	236.1	4.469	4.694
Average	230.1	4.221	

$\sigma^2 = 8.553$, $\sigma = 2.93$, % of mean = 1.27 for tablet wt.

Random Samples of Blend	
1	4.573
2	4.937
3	3.900
4	4.825
5	4.818
Average	4.611
σ^2	0.1755
σ	0.419
S. D.: % of Mean	9.1

This latter could have been the case since no specific instructions as to how to sample randomly were given. For example, samples run on the first day may have been from the top fraction of the bulk drug, on the second day may have been from a second layer, etc. Future designs should be fully cognizant of this source of possible bias.

The estimated error in (standard deviation) per cent of the mean among samples within days is 1.64%. The variation ascribed among days is 0.81%. The standard deviation of any one sample (pooled among days and assays) may be estimated as 1.88%.

Evaluation of Variability among Tablets from Two Different Preparations, A and B.—Two tablet preparations were studied. The data are given in Tables IV and V and consist of the weight of 10 individual tablets and two replicate assays of these tablets. The tablets were halved and the halves randomized so that the operator had no knowledge of whether he was assaying the same or another tablet.

In addition, a sample of the bulk mix obtained prior to the tableting was strewn along the table top and at equal linear intervals, five samples were obtained and assayed.

TABLE VI.—ANALYSIS OF VARIANCE OF TABLE IV, PREPARATION A

Source of Variation	SS	DF	MS	Components of Variance	*F* Test
Among tablets	0.91408825	9	0.1015654	$2\sigma_1^2 + \sigma^2$	$F = 27.9$, significant at 0.001 level
Replicates	0.03774750	10	0.00377475	σ^2	
Total	0.95183575	19			

Estimated variances

$\sigma^2 = 0.0037748 \therefore \sigma = 0.0615$ S. D., % of mean = 1.54

$\sigma_1^2 = 0.0489 \therefore \sigma_1$ 0.221 S. D., % of mean = 5.52

$\sigma_t^2 = \sigma^2 + \sigma_1^2 = 0.0527 \therefore \sigma_1 = 0.230$ S. D., % of mean = 5.60

TABLE VII.—ANALYSIS OF VARIANCE OF TABLE V, PREPARATION B

Source of Variation	SS	DF	MS	Components of variance	*F* Test
Among tablets	0.716431	8	0.089533	$2\sigma_1^2 + \sigma^2$	$F = 12.6$ significant at <0.001 level
Replicates	0.063845	9	0.0070944	σ^2	
Total	0.780276	17			

Estimated variance

$\sigma^2 = 0.0070944 \therefore \sigma = 0.0843$, S. D., % of mean = 2.00

$\sigma_1^2 = 0.04123 \therefore \sigma_1 = 0.203$, S. D., % of mean = 4.80

$\sigma_t^2 = \sigma^2 + \sigma_1^2 = 0.04832 \therefore \sigma_t = 0.220$, S. D., % of mean = 5.21

The analyses of variance for the preparations are given in Tables VI and VII. The variation among tablets for the two preparations, A and B, was similar. The variations in drug assay (as standard deviation, per cent of mean) from tablet to tablet were ±5.5 and 4.8%, respectively. The respective estimates of assay error were 1.54 and 2.00%. These latter values agree well with the 1.64% among assays within days and the 1.88% among assay and days estimated from the previous study on the bulk drug.

The variations in tablet weight are small, ±1 and ±1.3%, respectively, for the two preparations.

It should be pointed out that the tablet-to-tablet variation of drug content is actually larger than that given in Tables IV and V, since the drug assay is given in terms of mg./100 mg. of tablet.

The blend prior to tableting shows an even greater heterogeneity than from tablet to tablet, 12.6 and 9.1%, respectively. This is significantly greater (by *t* test) than the variation among tablets. A valid explanation is that tableting tends to average out some of the inherent microscopic heterogeneity of the pretableting blend.

CONCLUSIONS

The variations in assay of *o*-methoxy-α-methylphenethylhydrazine from tablet to tablet far exceed the error in the assay for the preparations studied. This is highly significant. The drug potency varies 5% from tablet to tablet when considered on a per-unit weight basis Variation between the preparations shows no effect of the excipients. The assay error is only 1.5–2.0%.

The microscopic heterogeneity of the pretableting blend is significantly very much greater than that in the tablets and runs to ±9.1–12.6% in per cent standard deviation. (This includes assay error.)

Assay studies on the bulk drug substantiated the above assay error, 1.64% (per cent standard deviation). Significant variation in assay with days of assay may be attributed to possible bias in the sampling of drug from the bulk lot on the various days. Properly, this portion of the experiment should be repeated to substantiate or negate this possible source of variability.

Studies such as these serve an important function in that they may guide the proper future preparation of tablets to be used in clinical drug evaluation or to be placed on the market.

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