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RESEARCH PAPER

Sampling Bias in Blending Validation and a Different Approach to Homogeneity Assessment

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ABSTRACT

Sampling of batches studied for validation is reported. A thief particularly suited for granules, rather than cohesive powders, was used in the study. It is shown, as has been demonstrated in the past, that traditional 1× to 3× thief sampling of a blend is biased, and that the bias decreases as the sample size increases. It is shown that taking 50 samples of tablets after blending and testing this subpopulation for normality is a discriminating manner of testing for homogeneity. As a criterion, it is better than sampling at mixer or drum stage would be even if an unbiased sampling device were available.

Key Words: *Blending; Normal distribution; Sampling; Sampling bias; Thief.*

INTRODUCTION

The inadequacy of the 3× sampling scheme dictated by Judge Wohlin in the Barr case has been reported several times in the past (1–5). Allegedly, the small cavity prevents representative sampling. Intensified sampling plans (2,3) for blending validation have been suggested in the literature, but their acceptance by the Food and Drug Administration (FDA) has not been forthcoming.

There are several purposes for this article. One is to demonstrate, by a modified spoon sampling method (6), that thief sampling is, indeed, biased. The intent is also to show that the bias decreases as the sample size increases. Also, as pointed out by previous authors (2,3), the best sampling device of the blending train (blender, conveyance, drumming, hoppering, and delivery to the feed frame) is the tablet itself. Finally, it is a goal of this article to present a new, different, and possibly more sensitive intensified sampling plan for blending validation.

MATERIALS AND METHODS

Batches of a melt-granulated product containing 15 mg of drug per 480-mg tablet were made in sizes of 170

kg. The granulation equipment was a 600-L T. K. Fielder high-shear mixer. The blending was carried out in a 300-L Englesman drum mixer. Optimization was carried out during the development, a point dealt with below.

During the development of the upscaled product, optimization and manufacturing changes were effected. In the following, the original process is referred to as process A, and the later, revised process is called process B. Blending validation was carried out on the original process; after the change, it was then repeated on process B.

After final mixing with talc, the batches were subjected to sampling using a thief of the construction shown in Fig. 1 (Powder and Granule Sampler Type 1030A Sampler, Sampling Systems, Units 8 and 9, Coleshil Trading Est., Coleshil Wan Wicks B46 1HT, England). This thief differs from most other types of thief in that it is particularly well adapted to sampling granules. Side-entry thieves are the most common, but bottom-entry plug thieves (e.g., as described by Orr and Shotton in Ref. 7) are probably better thieves when powders are cohesive. However, when powders are granular and free flowing, a plug is difficult to produce in the bottom cavity, and other means (see Fig. 1) are necessary to harvest a representative (it is hoped) sample. Other thieves (Accutrol, AccuSampler, Accutrol Co., Inc., 1106 General Lafayette Blvd., West Chester, PA 19382) (7,8) of more recent vintage have been reported to have advantages in this respect, but were not tried in this study.

As shown in Fig. 1a, the thief consists of a sampling core surrounded by a movable cylinder. At the time of insertion, the level of the tip of the sampling core is at C; as the thief is inserted, disturbance of the powder occurs at the area BC. When the point of sampling is reached (Fig. 1b), the sampling core is pushed down. This allows powder to flow into the volume CDEF, and it is noted that the perturbation of the bed from which this sample is taken is rather small. The outer shell is then pushed down (Fig. 1c), capturing the sample, and the thief is then removed.

This thief probably produces more representative samples than side-entry thieves, although this is speculative, and it is not the intent here to prove this. The point is that bottom-entry ports do not contain the space on the side of the thief where unblended powder may accumulate. It also takes a less-disturbed sample since the sample is taken from below the thief, that is, in a space as yet undisturbed by the sides of the thief. It should be pointed out, however, that samples were taken from the same spot several times, so that some disturbance, even with this arrangement, is likely to occur.

The samples (1×) were taken by thief in a triangular, centric scheme as shown in Fig. 2. Samples were taken at three levels: bottom, middle, and top of the drum. At each level, samples were taken from the points of a triangle, as well as from the center. By changing to the next level, the triangle was rotated by 180°.

After thief sampling had taken place, samples were taken during transfer to an intermediate bulk container, with the transfer carried out by manual scooping. Samples from the top were taken before the powder was transferred into the intermediate bulk container. Samples from the middle were taken at the point at which half of the granules had been transferred, and samples from the bot-

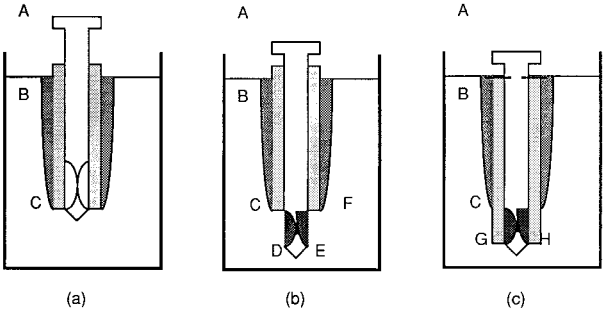

Figure 1. Schematic of thief used in this study. Note that the thief has a fixed sample size, and that a different thief is used for a different sample size.

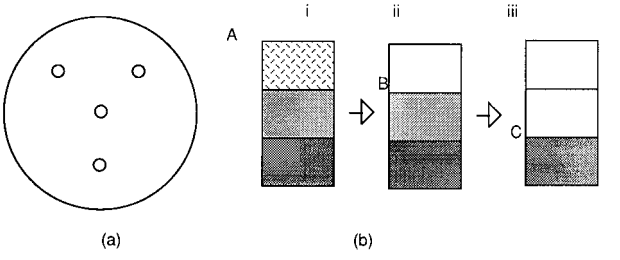

Figure 2. (a) Top view of sampling scheme: 3 samples are taken at each of three levels, in the positions shown, with a total of 12 samples taken. (b) Side view of sampling scheme: A represents the top sampling, one-third of the powder is then removed; spoon sampling is taken at the same points as in Fig. 2a at level B, and a third sampling is taken at level C.

Table 1

Samples of a Batch Taken by Thief

Sample size	0.5 g
Mean assay	82.7% LC
Relative standard deviation	6.3%

tom were taken when almost all the powder had been transferred.

Individual sampling and assaying was performed on 50 tablets made from the batch.

RESULTS AND DISCUSSION

Initial Process

The first sets of data presented in the following refer to the original manufacturing process, process A. The results from thieving (1× sampling) are shown in Table 1.

Samples of size 1× are about 0.5 g, and it might possibly be felt that the results would validate the blending since the relative standard deviation (RSD) of a 3× sample was 2%, that is, well below the required 5%. Theoretically, one could construct the sampling scheme by taking 3× samples and in this manner produce data that would validate the blending. However, the fact that the 1× sampling gave an RSD of 6.3% is of concern, as is the low mean assay of this sample size. The data, however, do demonstrate the influence of the size of the sample and hence are an example of the bias in the 1×-to-3× sampling scheme.

The point to be made here is that, although the sampling device may be somewhat inadequate, it is not tantamount to saying that a blend is satisfactory. There must be other means of ensuring adequacy of blending unless large type I or type II errors may give rise to faulty conclusions.

Bias of the Thief: (In-)Validation of Thief Sampling Versus Spoon Sampling

Spoon sampling has been reported in the past (1) as an unbiased method for taking samples. Table 2 shows the mean drug contents from 10 process A batches of 170 kg, and Table 3 shows the RSD values. The content uniformity results from the tablets produced are shown. The last presentation mode is discussed below.

It is noted that the results are satisfactory from a regulatory point of view, that is, all the relative standard deviations are below 5%, and it would appear that the process is in control. It is noted that the average drug contents in spoon samples are not significantly higher than in thief samples. There is, however, a difference in the standard deviations in Table 4, columns 2 and 3 (significant at the 95% level by *t* test).

Intensified sampling was carried out on the batches listed in Table 3 by taking six samples at the start, six midway through the process, and six at the end; the samples were taken at both maximum and minimum speeds. Tablets were tested for content uniformity (10 tablets on

Table 2

Comparison of Average Drug Content of Thief Versus Spoon Versus Tablet Samples

Granule/Tablet Lot No.	Blend Sampling (Thief)	Blend Sampling (Spoon)	Tablet Sample Content Uniformity (10 Tablets)	Tablet Sample Average of 50 Tablets	Final Tablets (Final Assay)
67/81	97.1	98.1	100.1	99.7	96.5
68/82	99.2	98.2	98.9	98.3	97.1
69/83	97.9	96.3	96.8	96.8	96.1
70/84	98.0	97.9	96.1	96.7	96.7
71/85	101.7	102.3	101.7	101.5	101.1
72/86	99.7	99.1	99.6	99.7	98.9
73/87	99.2	99.9	101.1	100.6	100.3
74/88	97.7	98.2	99.4	99.6	
75/89	95.9	98.8	99.2	99.7	98.4
Average ± SEM	98.5 ± 0.56	98.8 ± 0.55	99.2 ± 0.61		

Table 3

Comparison of Relative Standard Deviation (%) of Thief Versus Spoon Versus Tablet Samples

Granule/Tablet Lot No.	Blend Sample by Thief	Blend Sample by Spoon	Tablet Sample Content Uniformity (10 Tablets)	Tablet Sample Average of 50 Tablets
67/81	2.0	1.4	1.6	1.4
68/82	1.7	1.0	2.9	1.8
69/83	1.9	1.4	2.0	2.5
70/84	3.5	1.2	2.3	2.2
71/85	2.2	1.3	1.3	2.4
72/86	0.9	1.2	1.0	1.4
73/87	3.3	2.3	2.2	2.3
74/88	2.2	1.5	2.4	2.0
75/89	3.4	1.8	2.3	2.9

level 1 and 30 tablets on level 2). The data in Table 4 represent testing of more than 540 tablets. Mean values of the 6 × 10 tablets tested from each batch are shown. Values and conclusions from normal distribution testing of 50 tablets taken randomly are also shown.

Intensified Tablet Sampling: Adherence to Normal Distribution

The process to which the above applies is process A. At a given point in time, a process change was necessary, causing a need for renewed blending validation.

Mere meeting of regulatory standards, however, should not be the sole guiding principle in validation, and the concern in the results from process A was that the samples that were tested by intensified sampling were not normally distributed. The χ^2 tests on the batches in Table 3 were at the 95% confidence level.

Since the assay values of batches tested to excess in Table 4 were not normally distributed, the thought arose of using normal distribution of the tablets produced as an indication of adequate blending during validation. To ascertain adherence to normality, larger numbers of samples, of course, must be taken.

Table 4

Relative Standard Deviation of 6 Samples of 30 Tablets

Granule/Tablet Lot No.	Blend Sample Thief	Tablet Sample Average of 6 Content Uniformity Determinations	Average of 50 Tablets	Normality	Final Assay (%)
83/92	82.7/6.3	97.1/2.9	99.1/5.4	OK	100.1
84/93	89.2/1.6	98.7/3.1	98.9/4.9	−OK	98.5
85/94	84.0/13.2	98.1/4.1	97.7/10.7	−OK	97.7
86/95	92.5/8.4	97.7/3.2	97.4/4.8	OK	97.6
87/96	96.6/1.1	97.4/2.3	96.8/1.4	OK	97.6
82/97	93.9/2.6	97.3/3.6	99.0/3.6	OK	101.0
90/98	93.5/1.5		99.5/2.9	OK	101.5
91/99	92.9/4.4	99.4/2.4			97.8
89/100	86.5/7.6	98.3/2.4	98.2/5.3	−OK	98.7
88/101	95.2/4.0	99.7/1.7	97.5/1.6	OK	99.4

Table 5

χ^2 Values of 50 Tablet Assays Each from 10 Batches Made with Improved Processes

Granule/Tablet Lot No.	Blend Sample by Spoon	Average of 50 Tablets	Normality	Content Uniformity	Final Assay (% Label Claim)
130/114	99.1/2.1	101.4	OK		97.9
131/115	98.4/1.5	99.5	OK		98.0
132/116	98.9/0.9	99.9	OK	99.1/1.2	99.2
133/117	99.5/1.1	99.9	OK	100.1/1.5	98.1
134/118	98.7/1.6	99.3	(OK	98.9/1.3	97.3
135/119	98.3/1.6	101.1	OK	98.3/1.7	97.4
136/120	98.1/0.9	98.2	OK	97.8/1.8	97.7
137/121	99.4/1.8	100.0	−OK	108.5/2.7	98.3
145/122	98.8/1.0	99.0	OK	96.7/1.3	98.9
146/123	98.4/1.0	99.0	OK	97.4/1.4	98.2

To this end, based on previous experience, the adequacy of blending was assessed by taking 50 tablet samples, assaying the individual units, and testing the numbers for normalcy.

It may be worthwhile to examine the sources of variance of the produced tablets. Suppose the blending were perfect and the unit contained N particles, a fraction x of which were drug. If there were no other source of variation, then the assay numbers should be binomially distributed (10) with a relative standard deviation s_s of

$$s_s = 100\{(1 - x)/(Nx)\}^{1/2} \quad (1)$$

Since N is large in this case, this number would be small, and the binomial distribution would approach normalcy.

Other sources of variation would be (*a*) inadequate blending, with a standard deviation of s_m; (*b*) assay with a standard deviation of s_a; (*c*) tablet weight with a standard deviation of s_b. The last two would be normally distributed, so the actual assays of the tablets should be normally distributed provided s_p is small, that is, the batch is well blended. The inadequacy of thieving does not play a part here because the sampling is carried out at the tableting station.

Table 5 shows the results from 50-tablet testing for blending validation of 10 batches made with processes improved over process A.

The 99.5% critical upper χ^2 limit for six degrees of freedom is 18.5, and only one batch (121) exceeded this number. Hence, this batch is the only batch that may not be considered normally distributed.

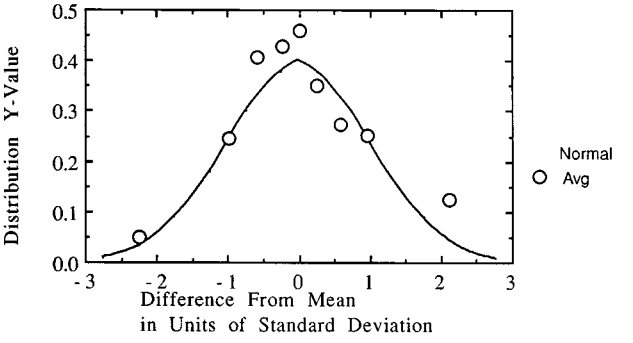

Figure 3. Distribution *Y* values as a function of distance from mean.

For the purpose of Fig. 3, the results from these batches have been normalized by calculating averages and by expressing the interval midpoints as deviations from the mean in units of standard deviation. The distribution ordinates *Y* were calculated by dividing the frequency by the interval length in units of standard deviation. The average values are shown in Fig. 3 and are compared with the *Y* values for the normal distribution. It is seen that the 10 batches (as also shown in Table 5) may be considered normally distributed.

CONCLUSIONS

1. A thief especially designed for granular materials was used for a melt-granulated product. The per-

formance in one process was marginal, but in another process was fair. Spoon sampling was employed to gauge the performance of the thief.

2. Batches made with a different process met both thief, spoon, and content uniformity requirements, and when 50 tablets were tested for content uniformity, all but 1 were found to be normally distributed.
3. It is suggested that a good (but stringent) criterion for homogeneity of a product would be sampling 50 (or more) tablets, assaying them individually, and testing the results for adherence to normal distribution. Of 10 large-scale batches made and tested in this manner, 9 were normally distributed at the tableting stage, and 1 was not. The last batch still adhered to regulatory requirements.

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