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SAMPLING IN BLENDING VALIDATION

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ABSTRACT

It is shown by a short statistical experiment, that sampling size in blending validation prior to the point where blending is as complete as it can be, is related to the size of the sample by being inverse function of the weight of the sample. The use of small thieves of the size of the dosage unit are apt to cause mechanical separation, and bias the results from the validation experiments. If the powder mixture is adequatly blended, then scaling can be carried out by considering the mass to be of a binomial distribution.

The article is a preliminary article and will be followed by work done on larger matrices. It is noted that it is educational in nature, attempting to show in an easy manner what the parameters used and what the effects are. In the work of blending, experimental results usually have large variation, and for such considerations, simulation, as presented here, is a more fruitful approach.

INTRODUCTION

The theoretical aspects of blending have been investigated by several authors, whereas actual experimental papers are few and far between.[1,2] The rate of blending[3,4] and the extent to which it

has been carried forward are always judged by sampling the mixer in various locations, assaying the samples taken, and calculating the mean and standard deviation.

Of late there has been a tendency by federal agencies to request that blending validation be carried out using samples the size of a dosage unit. Although this might seem to simplify the translation of results directly to what might be expected in the filling (or tableting) operation, the actual mechanics of taking such small samples is liable to introduce bias.

Although the blend may be considered multinomial and treated statistically on that basis, the following paper shows by an easy-to-understand example, what effect the sample size really has on the observed standard deviation in a blend which is not quite mixed. "Fully" mixed blends, as shall be seen shortly, are best assessed by how close the standard deviation is to the smallest possible.

EXPERIMENTAL

To demonstrate the effect of reasonable sample size on standard deviations obtained from a blender, the following, simulatory approach was taken:

It is envisaged that a mix meant to be finished into N units of dosage form of weight M, consists of N cells, each weighing M grams and containing C grams of drug substance. A simulation experiment would then remove Q cells (if the sample size was M) calculate the mean and standard deviation, and repeat this a certain number of times. The experiment could then be repeated with other sample sizes.

A set of "assays" of a dosage form containing 100 mg of drug per 1 g of granulate or powder were generated in this study. These numbers were arranged at random in an array, which is the type of box shown in Table. 1. It is meant to represent a blender with ten layers and ten columns. This allows simulation sampling by coordinates, so that, for instance, if a sample with coordinates 59 were to be sampled, then one would seek out column 5 and line 9, and arrive at the "assay" 97.

The product is assumed to have a dose weight of 1 g, so that the blender represents 100 doses. The whole population of 100 numbers has a mean of 99.95 and a standard deviation of 6.54 (mg or % RSD). It is specifically chosen so that it is slightly higher, standard deviation-wise, than that allowed by USP content uniformity test (6%), i.e. it would be a sample chosen close to but shorter than what is condisered the minimum blending endpoint (6%).

Table 1. "Mixer" with a heterogeneous blend with a mean of 100 mg/g of drug substance and a standard deviation of 6.54%. Numbers are random between 88 and 112 (i.e. a square distribution)

Column #->	0	1	2	3	4	5	6	7	8	9
Row										
0	108	109	109	107	105	106	103	105	106	102
1	88	90	92	91	94	95	97	89	93	98
2	102	104	103	101	101	99	97	103	101	94
3	106	102	103	105	106	109	107	106	108	110
4	104	109	109	105	106	110	112	107	111	112
5	94	96	95	97	100	101	105	95	102	100
6	102	98	99	104	94	93	90	103	92	94
7	91	95	94	91	97	90	93	90	93	91
8	105	105	105	108	109	100	98	104	98	99
9	100	92	91	91	88	97	98	98	96	100

A random number table was chosen[5] to "sample" the mixer. The numbers in the first column of Table 1 resulted. These are the first set of numbers in the reference table (Daniel, Table G).

In the first scheme, 1 gram samples were chosen, by selecting 100 random numbers, and positioning the assay in that coordinate of the mixer. The random numbers and the assays resulting are shown in the first two columns of Table II.

In a second scheme, 3 gram samples were chosen, in the third scheme 5 gram samples were taken, and in the fourth scheme 9 gram samples were taken. The manner in which these samples were drawn are shown in Fig. 1

This was done by choosing the same random numbers as shown in the first column of Table II, and including the two neighboring assays in the row of the random number. For instance in the third entry in the table, the random number is 73 and the "assay" of the one gram sample was 106. The average of entry 63 (107), 73 (106) and 83 (108) is 107, so this is the "assay"" of the (horizontal) three gram samples. For lines 9 and 0, the neighbors are considered to be in lines 0 and 9 respectively.

Table II. Results of simulation 1 g sampling of a population of N=100 with an average of 99.95 mg/g and a (relative standard deviation of 6.54 mg (%).

	One Gram Sample			Five Gram Sample		
Random Digit	Assay mg/g	Mean mg/g	SD mg(%) (RSD)	Assay mg/g	Mean mg/g	SD mg(%) (RSD)
85	102			100		
96	94			95.8		
73	106			107.2		
15	95	99.5	5.5	98.4	100.4	4.9
21	92			96.6		
45	100			97.6		
18	105			100.4		
50	99	99.0	5.4	100.2	9.2	1.8
85	102			100		
36	104			97		
09	100			101.2		
95	100	101.5	1.9	100.4	99.65	1.8
89	96			99.6		
23	103			104.4		
69	98			98.8		
62	97	98.5	3.1	100.6	100.8	2.5
67	93			96		
83	108			107.2		
56	93			93.6		
31	91	96.3	7.9	97	98.45	6.0

Table II. (Cont'd) Results of simulation 1 g sampling of a population of N=100 with an average of 99.95 mg/g and a (relative standard deviation of 6.54 mg (%).

	One Gram Sample			Five Gram Sample		
Random Digit	Assay mg/g	Mean mg/g	SD mg(%) (RSD)	Assay mg/g	Mean mg/g	SD mg(%) (RSD)
45	100			99.6		
01	88			97.2		
62	97			100.6		
07	91	94	5.5	96.8	98.55	1.8
48	109			100.4		
35	97			100.2		
14	109			104		
53	109	106	6.0	104.4	102.2	2.3
16	98			96.2		
49	88			98		
86	92			96.8		
34	105	95.8	7.5	104.4	98.85	3.8
87	93			92.8		
64	112			108.2		
31	91			97		
83	93	97.3	9.8	97.4	98.85	6.6
81	93			97.4		
59	97			97.8		
49	88			98		
58	100	94.5	5.2	98.8	98	5.9

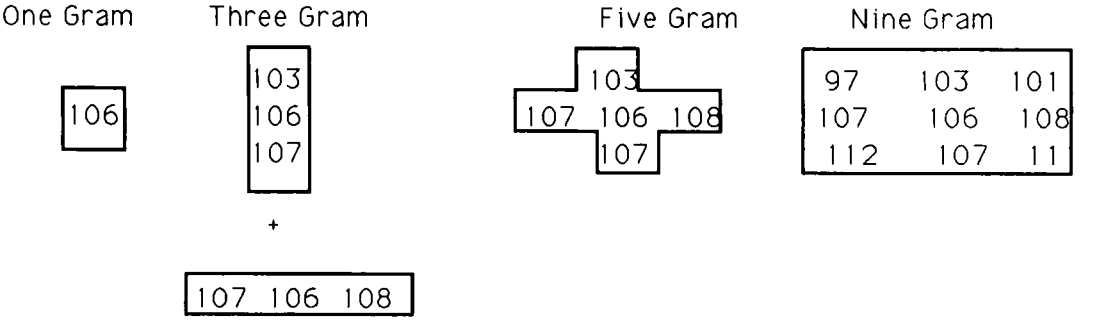

Fig. 1. Sampling scheme at spot #73 for one gram, three gram, five gram and nine gram samples. It is noted, that for symmetry reasons, both horizontal and vertical samples are taken in the case of the three gram sample. Hence there are 20 samples in this scheme, ten in the other three schemes.

The results for the 3 and for the 9 grams samples are not shown here. Table III shows the average assay and the standard deviation of sampling four (1) 1 gram samples, (b) 3 gram samples, (c) 5 gram samples and (d) 9 gram samples, and these are averaged below:

Table III. Averages and standard deviations for the data in Table II

Sample Size	Mean	sd	sd*	sd of sd
1 g	98.20	3.59	5.78	2.27
3g	99.45	2.30	4.78	2.31
5 g	99.5	1.3	3.74	1.95
9 g	100.2	0.95	3.10	1.29

*This is the average of the standard deviations shown, not the pooled standard deviation.

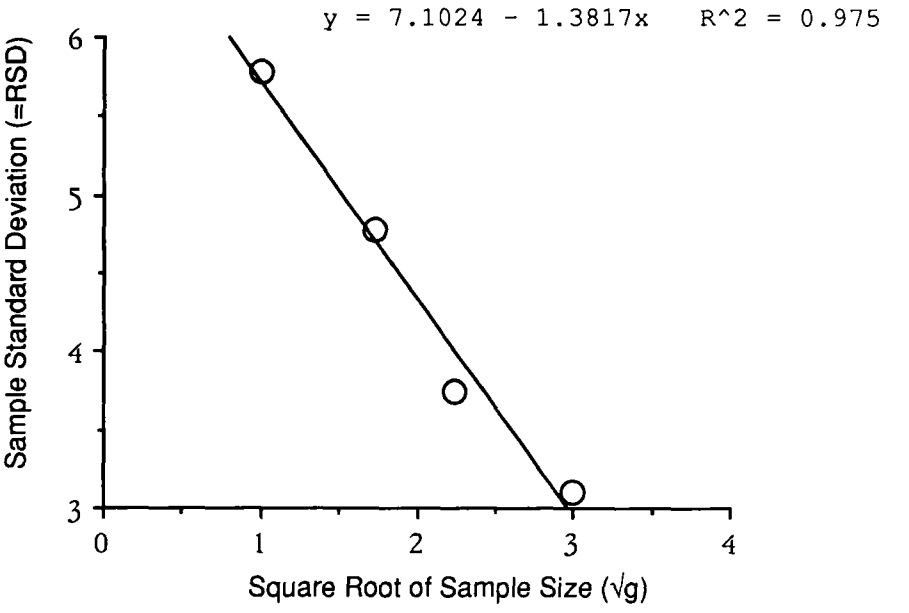

Fig. 2. Data from Table IV plotted versus the square root of the sample size.

RESULTS AND DISCUSSION

It is recalled, that in the filled (or tableted) dosage form, the figure of importance in the content uniformity test (aside from the integral spread) is the relative sample standard deviation (not the standard error of the mean).

It is the bolded, underlined figures in Table III that are the point of the exercise. When these are plotted versus the square root of the sample size, Fig. 2 results. The linearity is not surprising, because viewing the mixer as in Table 1, the samples of 3 essentially result in an average of 3, the samples of 5 an average of 5 and so on, so that the numbers, in that respect, represent standard errors of means, and as such should be inversely proportional to the number in the set. The point is, that in viewing the mixer as a certain number of dose units, and the sample size as a multiple of dose units, it is easy to see the relationship between sample size and standard deviation.

The main numerical advantage of the larger sample is that it gives a better estimate of the standard deviation and as also seen in the last column in Table III.

One of the problems that have arisen in sampling for the purpose of blender validation have been that samples of e.g. 30 grams have been taken. These samples have then been thoroughly mixed and a sample of the dose unit size (1 gram in the above sample) then *weighed out*, and assayed.

This adds an operation (subsampling) to the sampling scheme and can only confuse matters. In many instances there is actually segregation occurring while the analysts taps out the correct amount from a spatula onto weighing paper.

This problem would, of course, be avoided if the entire samples were assayed. It is not easy, in conventional fashion, to dissolve a sample of 30 grams, in many cases, but the analyst must be made aware of the fact that this is not a routine analysis, in fact it is, in general, only done once per product per mixer (and then, maybe, periodically, thereafter). Hence the extra effort is crucial to the carrying out of a meaningful experiment.

As mentioned the problem is confounded by the fact that the standard deviations have variances of their own. The sum of squares (normalized) from which they are derived, are distributed by χ^2 and this makes for a broad interval. This is manifest in the spread of the values of the standard deviations in the experiments shown. However the population standard deviation is within the predicted limits of each of the standard deviations stated. Herein lies one of the advantages of taking larger samples: the estimate of the population variance becomes much more precise.

FDA has of late required that samples of the size of the dose unit be taken. Here it should be noted that the actual sampling operation is one carried out by the use of a thief. The thief consists of two concentric tubes, one (the inner) with a smaller ID that the outer tube. Holes in the tube can either align or not, and in the latter case the inside compartments of the inside tube are not accessible. By turning the outside tube to make it align with the inner, a connection is made between the outside (the powder mixture) and the inside compartment.

The larger the sample that flows into the thief the more it will resemble the powder at the point of sampling. Smaller samples give rise to perturbation in composition due to segregation in flow.

The proof of good blending, in the long run, lies in the fact that a company may have a long history of a drug product where the content uniformity of the finished product is within SUP limits. One can then conclude that the process is in control. That is not to say that blender validation should not be carried out but that for existing products the product content uniformity is, in the long run, the better indicator that the process is in control.

If the standard deviations are fairly small, then sampling two sample sizes (e.g. 25 g and 10 g) and assuming inverse square root dependence on sample weight may allow for calculation of what the standard deviation would have been at dose unit weight, by the procedure of Fig. 1. Again, lack of precision of the standard deviations may hamper the procedure at times.

If validation is done at points only close to "complete" blending times, then the standard deviation of a dose unit weight can be calculated by assuming that a binomial distribution holds. The "best" relative standard deviation which can be attained of a sample with N particles is given by:

$$s_\infty = 100 \left[(1-x)/Nx \right]^{1/2} \qquad (1)$$

where x is the fraction of the number of particles that are drug particles, and (1-x) is the number of particles that are excipient particles. In such calculations, particles smaller than 5 μm are usually disregarded since the will orderly adhere to larger particles. A liberal interpretation of Eq. 1 is that if a standard deviation of s' is found in a larger sample containing N' particles and if the dose unit contains N particles, then the standard deviation of the dose unit would be:

$$s = s'[N'/N]^{1/2} \qquad (2)$$

To make use of this, it is necessary to know the mean diameter of the drug substance and excipients, the true density of the drug substance and that of the excipients.

CONCLUSION

1. It is concluded that the best scientific manner in which to sample a blender in validation experiments is to use an adequately sized thief, to take two samples of different size (10 and 25 grams for instance) and to assay the entire sample, i.e. not to subsample. From this a plot like shown in Fig. 1 can be used to estimate what the standard deviation would have been at a sample size equaling that of the dose unit. The general problem with such a procedure is the (lack of) precision of the standard deviation in general.

2. Alternately, if the standard deviations are small, then the standard deviation at unit dose size can be calculated approximately

from assumption of the binomial theorem holding (Eq. 2). If the theoretically smallest deviation lies in the 95% confidence interval of the standard deviation obtained, then it could be concluded that mixing is adequate.

3. Attempting to obtain the standard deviation of a sample of unit dose size is, however, not of much use. The blending validation is a validation of an operation, not a product, and the final analysis rests with consistent good performance of content uniformity.

REFERENCES

1. Patel, M., Mixing of Pharmaceutical Granulation, Ph.D. Thesis, U.W. School of Pharmacy, 1976
2. Carstensen, J.T. and Patel, M.,R. (1977), Powder Technol., 17, 273
3. Carstensen, J.T., (1980) Solid Pharmaceutics: Mechanical Properties and Rate Phenomena, Academic Press, N.Y., pg. 114-123
4 Carstensen, J.T., (1992) Pharmaceutical Principles of Solid Dosage Forms, Technomic Press, pg. 21-30
5 Daniel, W.W. Biostatistics, Wiley, N.Y., 1978, Table G.