DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 17(2), 233-244 (1991)

CONTENT UNIFORMITY: SEPARATION AND QUANTITATION OF SOURCES OF DOSE VARIATION

W. C. Stagner[1], B. J. Cerimele[2], and G. H. Fricke[3]

[1]Pharmaceutics Department, Glaxo Inc., Research Triangle Park, North Carolina

[2]Department of Pharmacology, School of Medicine, Indiana University, Indianapolis, Indiana

[3]Department of Chemistry, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana

ABSTRACT

A propagation of error formula is presented which describes a mathematical relationship between uniformity of dosage units (dose variation) and the individual sources of variability. Using this propagation of error formula and a minor modification of the USP Content Uniformity test procedure, one can assign dose variation to weight variation, blend heterogeneity and assay imprecision without compromising the information required by the USP XXII. This formula separates and quantitates the sources of dose variation and defines the manner in which weight variation, blend heterogeneity and assay imprecision are propagated. By knowing how the dose variation is propagated, specific measures can be taken to improve the uniformity of dosage units.

The propagation of error formula can also be used to calculate *a priori* estimates of dose variation by choosing reasonable or expected variances for weight variation, blend heterogeneity and assay imprecision.

Such calculations can save hours of fruitless effort which attempt to improve the content uniformity without considering how dose variation is propagated and to what extent the expected dose variation can be improved.

INTRODUCTION

The USP XXII Content Uniformity test measures the variation in drug quantity among individual dosage units. Discounting the variation arising from sampling error, one can intuitively attribute the reported Content Uniformity to weight variation, blend heterogeneity and assay imprecision. The USP XXII Content Uniformity test procedure[1] does not separate or quantitate these sources of variation and, therefore, is of little value to formulators or production support personnel who are interested in obtaining a desired level of content uniformity. To achieve a desired content uniformity, it would be advantageous to know to what extent weight variation, blend heterogeneity and assay imprecision contribute to dose variability so that specific measures could be taken to improve the uniformity of the dosage units.

In a series of articles, Sampson and co-workers[2,3,4] used a statistical approach to separate and quantitate sources of tablet dose variation. The total dose variability was separated into two sources: weight variation and blend non-uniformity. This information was used to set tolerance limits on the dose. In this article, a propagation of error formula is presented which describes the mathematical relationship between dose variation, weight variation, blend heterogeneity and assay imprecision. In addition, a minor modification of the USP XXII Content Uniformity test procedure is described which provides the necessary data for the propagation of error formula and retains the required USP information.

The propagation of error formula that is presented has two main applications. First, the pharmaceutical scientist can use the propagation of error formula to determine the primary source(s) of dose variation of

finished products. Once the measured dose variation has been partitioned into weight variation, blend heterogeneity and assay imprecision, the proportion of the total variation arising from each source can be quantified. If it is desirable to improve the uniformity of the dosage units, this information can be used as a tool to help direct efforts to decrease dose variation. Therefore, if weight variation is identified as the major source of dose variation, one could direct efforts to improve weight variation. Likewise, if assay imprecision is the major source of the measured dose variation, one could focus attention on improving the assay methodology. In these cases, the propagation of error formula can be used as a tool to identify the source(s) of variation that need to be improved to reduce the measured dose variability.

The second main use of the propagation of error formula is to provide a priori estimates of the expected dosage unit variation. This is accomplished simply by substituting reasonable or expected estimates for weight variation, blend heterogeneity and assay imprecision into the formula.

PROPAGATION OF ERROR FORMULA

The "law of propagation of error" is a tool that has been used by scientists for many years to calculate the random uncertainty associated with physical measurements.[5] In this paper, the sources of variability which contribute to dose variability are separated and quantitated by a propagation of error formula. It is assumed that the variables in the general mathematical relationship,

$$D = PW \tag{1}$$

identify the major contributions to dose variation where D (dose) is drug weight/dosage unit, P is drug weight/unit weight (proportion of drug in formulation mixture), and W is unit weight/dosage unit. "Dosage unit" refers to those dosage forms discussed under Uniformity of Dosage Units USP

XXII. "Unit weight" requires further definition depending on the dosage form. For uncoated tablets, the definition of "unit weight" is unambiguous and refers to the tablet weight. For coated tablets, hard capsules, soft capsules, powders and suspensions in single-unit containers, "unit weight" is defined as the core tablet weight or fill-weight. In all cases the variables P and W are assumed to be independent.

Using the propagation of error method, a Taylor series expansion is applied to eq. 1 which yields an exact formula for the dose variance σ_D^2:

$$\sigma_D^2 = \mu_P^2\sigma_W^2 + \mu_W^2\sigma_P^2 + \sigma_P^2\sigma_W^2 \quad (2)$$

If $\sigma_D/\mu_D \leq 0.078$ (the acceptable USP Content Uniformity relative standard deviation is 0.078), it can be shown that the, $\sigma_P^2\sigma_W^2$ term in eq. 2 accounts for only approximately 0.15% of the total dose variance (see Appendix I). Therefore, for convenience the $\sigma_P^2\sigma_W^2$ term is considered negligible and is set to zero. Substituting sample estimators into eq. 2, one obtains the approximate formula for the sample dose variance:

$$s_D^2 = \bar{P}^2 s_W^2 + \bar{W}^2 s_P^2 \quad (3)$$

Equation 3 describes the variability in D due to P and W. Measurement error in P and W will also contribute to the sample dose variance. The measurement errors in weighing are known to be quite small; however, the measurement error in P resulting from assay imprecision can be a significant source of variation. It is assumed that the variance of P (proportion of drug in the formulation mixture) is equal to the sum of the variances due to blend heterogeneity, $s_{P_h}^2$, and assay imprecision, $s_{P_a}^2$:

$$s_P^2 = s_{P_h}^2 + s_{P_a}^2 \quad (4)$$

If $s_{P_a}^2$ and s_P^2 are independently determined, then $s_{P_h}^2$ can be calculated from eq. 4. The sample assay variance can be obtained from the assay validation information while s_P^2 is obtained from a modified USP Content

Uniformity test procedure which will be discussed later. By substituting eq. 4 into eq. 3, the propagation of error formula becomes:

$$s_D^2 = \bar{P}^2 s_W^2 + \bar{W}^2 s_{P_h}^2 + \bar{W}^2 s_{P_a}^2 \quad (5)$$

Thus, the variance in dose is propagated as the sum of contributions of weight variation ($\bar{P}^2 s_W^2$), blend heterogeneity ($\bar{W}^2 s_{P_h}^2$), and assay imprecision ($\bar{W}^2 s_{P_a}^2$). The percent variance in dose due to each variable can be calculated.

MODIFIED USP CONTENT UNIFORMITY TEST PROCEDURE

The USP allows the use of two methods to demonstrate uniformity of dosage units - weight variation or content uniformity. Since the content uniformity method has a more universal application, it was the method chosen for modification. The USP Content Uniformity test procedure specifies that 10 dosage units be individually assayed as directed in the assay monograph. The USP procedure does not require that each assay be associated with a specific uncoated tablet weight or unit fill-weight. The proposed modification for uncoated tablets requires that 20 tablets be individually weighed. Ten of these tablets are to be assayed and a record kept of the assay and corresponding tablet weight. From this information the sample mean ($\bar{P}$) and sample variance (s_P^2) for the proportion of drug weight per unit weight can be calculated. The weights of the other 10 tablets are used to provide an independent measure of the sample mean tablet weight ($\bar{W}$) and the sample weight variance (s_W^2).

The proposed modification for other dosage units requires that the contents be carefully emptied and the fill-weight carefully determined. The contents of 10 units are assayed and a record kept of the assay and corresponding fill-weight. From this information, the sample mean ($\bar{P}$) and variance (s_P^2) for the proportion of drug weight per fill-weight can be calculated. The fill-weights of another 10 units are used to independently calculate the sample mean ($\bar{W}$) and variance (s_W^2) of the unit fill-

TABLE 1

Data Obtained from the Modified USP Content Uniformity Test Procedure

Ten Assayed Capsules			Ten Unassayed Capsules
Fill-Weight (mg/capsule)	Drug Weight (mg/capsule)	P Drug Weight/Fill-Weight (mg/mg)	W Fill-Weight (mg/capsule)
260.8	5.5	0.0211	248.5
258.2	5.3	0.0205	254.2
261.1	5.3	0.0203	255.4
248.9	4.9	0.0197	253.2
248.5	5.0	0.0201	260.7
257.8	5.4	0.0210	259.7
252.3	5.1	0.0202	248.4
251.0	5.1	0.0203	249.5
250.1	4.9	0.0196	257.6
254.1	5.3	0.0209	260.5
254.3 ± 4.8	5.2 ± 0.2	0.0204 ± 0.0005	254.8 ± 4.8

weights. This procedure provides all the necessary information for USP Content Uniformity testing and for further separation and quantitation of dose variation.

SEPARATION AND QUANTITATION OF SOURCES OF DOSE VARIATION

A capsule formulation is presented to illustrate how the modified USP Content Uniformity procedure is used in conjunction with the propagation of error formula to separate and quantitate the sources of dose variation. Example calculations are presented for a 250 mg capsule having a label claim of 5 mg drug. The data collected from the modified USP test procedure are given in Table 1.

From the analytical validation study, the assay relative standard deviation was reported to be 2.30 percent and the assay variance, $s^2_{P_a}$, was calculated to be 22.0×10^{-8} (mg drug/mg fill-weight)^2.

The terms in the propagation of error formula are evaluated:

$\bar{W}$ = 254.8 mg fill-weight/capsule from 10 unassayed capsules,

s^2_W = 23 (mg fill-weight/capsule)^2 from 10 unassayed capsules,

$\bar{P}$ = 0.0204 mg drug/mg fill-weight from 10 assayed capsules,

$s^2_P = 25 \times 10^{-8}$ (mg drug /mg fill-weight)^2 from 10 assayed

$s^2_{P_h} = s^2_P - s^2_{P_a} = 3 \times 10^{-8}$ (mg drug/mg fill-weight)^2

Substituting these values into eq. 5, the estimated dose variance is 2.6×10^{-2} (mg drug/capsule)^2. The percentage contributions due to weight variation, blend heterogeneity and assay imprecision are 38, 7, and 55 percent, respectively. These results suggest that improvement in assay precision would lead to the most significant reduction in the observed dose variation. On the other hand, attempts to improve blend homogeneity would result in only marginal improvement.

A PRIORI ESTIMATION OF EXPECTED DOSE VARIATION

The propagation of error formula can also be used as a tool to provide an *a priori* estimate of the expected dose variation. This is calculated simply by substituting estimates for weight variation, blend heterogeneity and assay imprecision into the propagation of error formula (eq. 5).

An *a priori* estimate for the expected dose variation for a bead-filled capsule formulation is presented. Given the amount of drug that could be coated onto a bead and the dose, it was determined that each capsule should contain 294 active beads to deliver an 8 mg dose. It was also determined that nonpareil beads would be added as filler to give a final fill-weight of 490 mg.

To estimate the expected dose variability, values for weight variation, blend heterogeneity and assay imprecision need to be assigned. From previous bead filling experience, the fill-weight variance s_W^2, was set at 35.7 $(mg/capsule)^2$ which corresponds to a percent relative standard deviation (RSD) = $\frac{\text{standard deviation (100)}}{\text{mean}} = \frac{5.97\ (100)}{490} = 1.22\%$.

Assay impression, $s_{P_a}^2$, was determined to be 1.67×10^{-7} (mg drug/mg fill-weight)^2 which corresponds to a RSD = 2.50%.

To estimate blend heterogeneity, the binary system was treated as a random mixture of drug/excipient particles having the same size, shape, and density. Moreover, the filling process was assumed to fill every capsule with the same number of beads. The number of drug-containing beads is treated as a random variable following a binomial distribution. Hence, the weight of drug is variable and the standard deviation of the drug weight proportion is approximated using the statistical formula[6]:

$$s = (Npq)^{0.5} \tag{6}$$

where

s = standard deviation in number of active beads

p = proportion of active beads, 0.300

q = proportion of inactive beads, 0.700

N = total number of beads in a filled capsule, 980

and s = 14.3 active beads.

The term, $s_{P_h}^2$ (mg drug/mg fill-weight)^2, can be calculated from the standard deviation in number of active beads by:

$$s_{P_h}^2 = \left[\left(\frac{14.3 \text{ active beads}}{\text{capsule}}\right)\left(\frac{0.0272 \text{ mg drug}}{\text{active bead}}\right)\left(\frac{\text{capsule}}{490 \text{ mg fill-weight}}\right)\right]^2 =$$

$$6.30 \times 10^{-7} \left(\frac{\text{mg drug}}{\text{mg fill-weight}}\right)^2$$

This value of $s^2_{P_h}$ serves as an estimate for blend heterogeneity which can be substituted into the propagation of error formula to calculate the expected dose variation.

Substituting:

$$\bar{P} = 1.63 \times 10^{-2} \left(\frac{\text{mg drug}}{\text{mg fill-weight}}\right)$$

$$\bar{W} = 490 \frac{\text{mg fill-weight}}{\text{capsule}}$$

$$s^2_W = 35.7 \left(\frac{\text{mg fill-weight}}{\text{capsule}}\right)^2$$

$$s^2_{P_h} = 6.30 \times 10^{-7} \left(\frac{\text{mg drug}}{\text{mg fill-weight}}\right)^2$$

$$s^2_{P_a} = 1.67 \times 10^{-7} \left(\frac{\text{mg drug}}{\text{mg fill-weight}}\right)^2$$

into eq. 5 gives an *a priori* estimate of dose variance, s^2_D, of 0.201 $(\text{mg/capsule})^2$. This corresponds to a RSD of 5.60%. A batch of bead-filled capsules was prepared which attempted to match the weight variation, bead loading, proportion of active to nonpareil beads, and dose that was used to calculate the *a priori* expected dose variation. Five groups of 10 capsules per group were chosen randomly from the batch and were tested for content uniformity. The individual RSD values were 4.76, 5.36, 6.47, 6.68, and 4.23 percent. The calculated *a priori* RSD of 5.6 is in good agreement with observed values.

Given potential batch-to-batch variation and scale-up problems, it has been suggested that during development work a RSD limit of 3 percent or less be achieved.[7] The *a priori* calculations obtained from the propagation of error formula can be used to determine the primary source(s) of the expected dose variation. In this example, the percentage of the total dose variation due to weight variation, blend heterogeneity, and assay imprecision are 4.70, 75.3, and 20.0 percent, respectively. To decrease the RSD in dose variation in this example, one would need to improve the blend homogeneity and assay imprecision. Even if the assay variation is set to zero, the *a priori* estimate of dose RSD is approximately 5 percent

so time and effort would be better spent on improving the blend homogeneity. Since the blend heterogeneity was based on statistical predictions, changes in blender design, mixing time, or mixing processes would probably not lead to a further decrease in $s^2_{P_h}$. By using eq. 6 however, one can determine what effect increasing (p) has on the RSD in blend homogeneity. It can be shown that by increasing the weight proportion (p) of beads containing drug to 0.95, while maintaining the same degree of weight variation and assay imprecision, the expected RSD is reduced to 2.9% and the assay imprecision would be expected to account for approximately 75 percent of the dose variation. Such calculations can save hours of fruitless effort which attempt to improve the uniformity of dosage units without considering how the primary source(s) of variation are propagated and to what practical limits improvement could be expected.

DISCUSSION

In scientific experimentation, neither the physical quantity nor the associated variability of a physical measurement can be determined exactly. Thus, in cases where the variability of a physical measurement is of interest, the propagation of error formulas are only tools to help estimate the expected variation. It should be noted that the expected variability or uncertainty computed from the propagation of error formula developed for dose variation is at best an approximation and it is generally recognized that these equations do not represent all the contributions which could be responsible for the observed variability. However, if the propagation of error formula is used with this understanding, it can become a valuable tool to the pharmaceutical scientist.

Examples of the two main uses of the propagation of error formula for dose variation have been presented. The formula can be used to ascertain the primary source(s) of dose variation of a finished product or it can be used to estimate a priori the expected dose variation. With minimal additional record keeping, the modified USP Content Uniformity test procedure

allows one to use the propagation of error formula for finished products. The sample size of 10 was chosen because this is currently the number of test units required by the USP. However, the number of units can be larger or smaller than the 10 specified in the modified method. Obviously, the larger the sample size the more reliable the variability estimate will become.

ACKNOWLEDGEMENT

The authors thank Dr. Al Childers for his technical review and suggestions.

REFERENCES

1. The United States Pharmacopeia, 22nd Rev., and the National Formulary, 16th Rev., The United States Pharmacopeial Convention Inc., Rockville, MD, p. 1277 (1989).

2. J.P. Comer, H.L. Breunig and C.B. Sampson, J. Pharm. Sci., 59, 211 (1970).

3. C.B. Sampson, H.L. Breunig, J.P. Comer and D.E. Broadlick, J. Pharm. Sci., 59, 1653 (1970).

4. I.J. Hall and C.B. Sampson, Biometrics, 29, 109 (1973).

5. H.H. Ku, J. Res. of. N.B.S., 70C, 331 (1965).

6. J.T. Carstensen, "Solid Pharmaceutics: Mechanical Properties and Rate Phenomena," Academic Press, Madison, Wisconsin, p. 117, 1980.

7. J. Ryder, Int. J. Pharm. Tech. and Prod. Mfg., 1, 14 (1979).

APPENDIX I

Contribution of the $\sigma_P^2\sigma_W^2$ Term to Dose Variance

$$\text{Given } \sigma_D^2 = \underbrace{\mu_P^2\sigma_W^2}_{\text{Term(1)}} + \underbrace{\mu_W^2\sigma_P^2}_{\text{Term(2)}} + \underbrace{\sigma_P^2\sigma_W^2}_{\text{Term(3)}} \quad (1)$$

where μ_P and μ_W are population means and σ_P and σ_W are population variances of P and W.

In the simplest case, if σ_P^2 or σ_W^2 are zero; the $\sigma_P^2\sigma_W^2$ term, will not contribute to the dose variance, σ_D^2. Term(1) or Term(2) will account for the total contribution to the dose variance.

If σ_P^2 and σ_W^2 are non-zero, contribution of Term(3) can be estimated using the following assumptions:

1. Set $(\sigma_D/\mu_D)^2 = (0.078)^2$; where the acceptable USP Content Uniformity relative standard deviation limit is ≤ 0.078.
2. The largest contribution of the $\sigma_P^2\sigma_W^2$ term results when the Term(1) = Term(2).

Dividing eq. 1 by μ_D^2 gives:

$$\frac{\sigma_D^2}{\mu_D^2} = (0.078)^2 = \frac{\mu_P^2\sigma_W^2 + \mu_W^2\sigma_P^2 + \sigma_P^2\sigma_W^2}{\mu_P^2\mu_W^2} \tag{2}$$

$$(0.078)^2 = \underbrace{\frac{\sigma_W^2}{\mu_W^2}}_{\text{Term(1)}} + \underbrace{\frac{\sigma_P^2}{\mu_P^2}}_{\text{Term(2)}} + \underbrace{\frac{\sigma_P^2\sigma_W^2}{\mu_P^2\mu_W^2}}_{\text{Term(3)}} \tag{3}$$

when Term(1) = Term(2)

$$(0.078)^2 = 2\left(\frac{\sigma_W^2}{\mu_W^2}\right) + \frac{\sigma_W^4}{\mu_W^4} \tag{4}$$

solving eq. 4, $\sigma_W/\mu_W = 0.055$.

The relative contribution (RC) of the $\sigma_P^2\sigma_W^2$ term in eq. 3 can now be calculated by substituting $\sigma_W/\mu_W = \sigma_P/\mu_P = 0.055$.

$$RC = \frac{\text{Term(3)}}{\text{Term(1)} + \text{Term(2)} + \text{Term(3)}}$$

$RC = 1.5 \times 10^{-3}$ or 0.15%

Therefore, the largest expected relative contribution of the $\sigma_P^2\sigma_W^2$ term is 0.15%.