

Potential Applications of Mathematics in Pharmaceutical Quality Control

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Abstract: This paper presents statistical analyses of two scenarios that are common in the pharmaceutical industry. The first analysis examines the performance of analytical methods that are used to test pharmaceutical products. The statistical model has been programmed into a Microsoft Excel application, which allows users to determine if a method is precise enough to measure whether or not a given batch of a drug product meets FDA specifications. The second analysis examines the possible advantage of varying the amount of drug substance (such as a powder) used to create a drug product (such as a capsule or tablet). In the current procedure, the amount of drug substance used to form a drug product is adjusted proportionally to the substance active ingredient concentration. A statistical analysis has been developed to evaluate this procedure, and recommendations for implementing a more rigorous procedure are given. Explanations of all statistical and mathematical methods are included.

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Problem 1: Analytical Method Performance

Introduction

Background

The objective of this problem was to quantify the precision of analytical methods used to test drug products, and to develop an application that can be used to determine whether or not an analytical method is precise enough. The workflow of a typical drug manufacturing process is shown schematically in Figure 1. When a batch of a drug product is manufactured, it must be tested to verify that it meets FDA specifications before it is released for distribution. The typical FDA product specification is that the product must contain between 90% and 110% of the active ingredient amount claimed on the label. Some analytical method is used to test a sufficient number of specimens from the batch, and the results are used to determine whether or not the batch meets product specifications. This project focuses on evaluating the precision of these analytical methods.

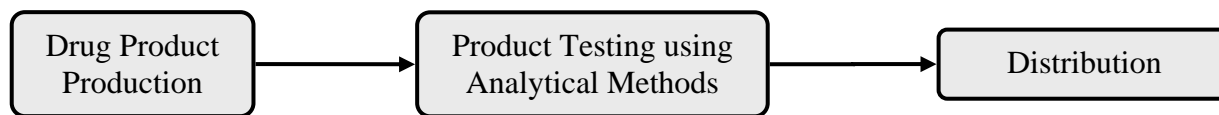


Figure 1. Before a drug product is distributed it must be tested using an analytical method.

Before any analytical method can be used to validate a drug product, the method itself must be proven to have sufficient precision. The analytical method must be precise enough to determine whether or not a drug product meets the FDA specification—that is, the method must be able to distinguish between a batch that falls within 90% and 110% of its label claim and a batch that does not. Although some measurement error is unavoidable, the analytical method error must not be so large that it cannot discern (with some acceptable level of confidence) whether or not a drug product batch meets FDA specifications.

When using an analytical method to measure a drug product, the following three errors contribute to the overall error of the measurement:

- *Method Error*, caused when a chemist or lab technician physically handles the drug product during the testing procedure (for instance, pipetting, grinding, dissolving, or filtering);
- *Instrument Error*, caused by the machines (such as high performance liquid chromatography machines, abbreviated HPLC) used to measure the drug product strength; and
- *Variation in Product Error*, which occurs in the drug product itself and originates from the production process.

The objective of the measurement process is to determine the variation in product error as accurately as possible, since this is the error that is actually present in the formulations that will be administered to patients. However, the variation in product error cannot be measured without somehow handling the product and using mechanical instruments. Therefore the method and

instrument errors distort the measurement of the variation in product error. This relationship is shown schematically in Figure 2.

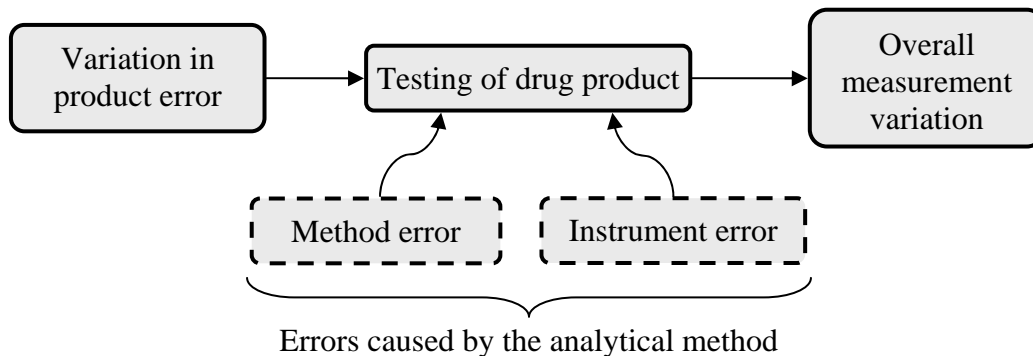


Figure 2. Analytical method precision is limited by method error and instrument error.

Of the three error types that contribute to the overall measurement variation of a practical analytical method, only the method and instrument errors are implicit properties of the analytical method itself, as shown in Figure 2. That is, the precision of the analytical method does not depend on the variation in product error. This is because the analytical method precision is limited only by the way specimens are physically handled (method error) and the precision of the machines involved (instrument error).

While neither the method nor instrument error depend on the variation in product error, it is not possible to measure only the method and instrument errors together. However, the instrument error can be measured separately using a procedure like the one shown in Figure 3. A large number of tablets are ground into a powder and dissolved together to create a single homogeneous solution. Then, multiple samples of that solution are injected into an HPLC (high performance liquid chromatography) machine to measure the active ingredient quantity present. Since the solution is homogeneous, the variation in the HPLC output is caused solely by instrument error. Thus the instrument error can be estimated by computing the sample standard deviation of these measurements.

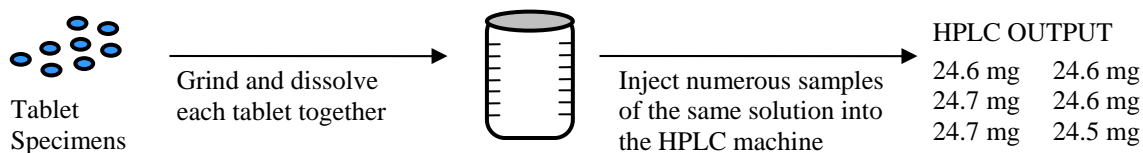


Figure 3. Procedure used to measure instrument error.

On the other hand, it is impossible to measure the method error separately from the variation in product error. This is because a collection of specimens must be used to obtain any measurements whatsoever, and the specimens will inevitably contain an unknown variation in product error. Therefore, in summary, the instrument error can be measured separately from the other errors, but the method error and the variation in product error cannot.

Because the method error cannot be measured independently, the total error caused by the analytical method (as shown in Figure 2) is always unknown—only the instrument error can be determined experimentally. However, it is possible to obtain an upper bound for the error caused by the analytical method using a procedure such as the one shown in Figure 4. In this experiment, several tablet specimens are ground into a powder, and the powder from each tablet is individually dissolved in a liquid solvent to form a solution. Again, the solutions are injected into an HPLC machine to measure their active ingredient concentrations. In this case, the HPLC outputs are affected by all three errors (variation in product, method, and instrument errors). This procedure gives an upper bound for the error caused by the analytical method, since the method and instrument errors are amplified by the variation in product error of the specimens.

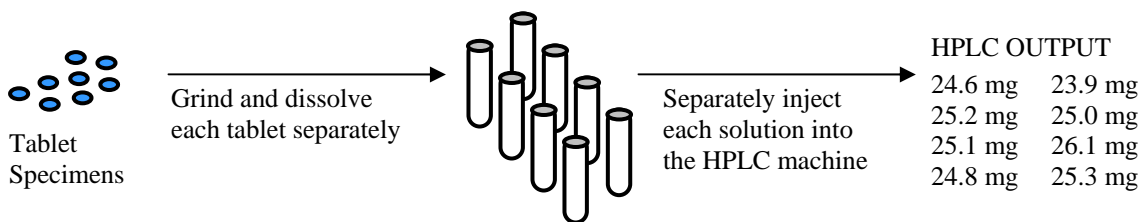


Figure 4. Procedure used to determine overall measurement error.

In summary, it is not possible to measure the variation in product error of a drug product batch without using some analytical method, and thereby introducing its associated method and instrument errors. On the other hand, it is impossible to quantify the error caused by an analytical method without using some collection of specimens, and thus introducing the variation in product error of those specimens. Therefore, it should be emphasized that *the processes of drug product validation and analytical method validation are inexorably linked*. It is impossible to quantify one of the processes without considering the other.

Statistical Viewpoint

Industry experience shows that the three errors described above are each normally distributed. Also, the variation in product error originates when the product is manufactured, whereas the method and instrument errors originate when the product is measured, as already mentioned in Figure 2. Thus, it is clear that the variation in product error is independent of the method and instrument errors. Furthermore, the method and instrument errors are independent of one another, since for all practical cases the instrument precision is not affected by the prior handling of the drug product. Therefore, it is reasonable to assume that the variation in product error, method error, and instrument error are all independent and normally distributed.

When an analytical method is to be validated, the goal is to measure the total error caused by the analytical method (as shown in Figure 2). A sample of specimens from several batches of the same type of drug product is used to obtain an upper bound for the analytical method error by performing experiments, such as the one shown in Figure 4. As mentioned earlier, this is an upper bound for the total analytical method error, since the specimens used inherently contain variation in product error.

Once these experiments are performed, the probability density function (PDF) for the measured active ingredient quantity x of the specimens in the sample is determined. This PDF represents the contributions of the method error and instrument error, in addition to the variation in product error. As all of these errors are normally distributed, the PDF for the overall measurement variation is also normally distributed. The sample mean and sample standard deviation are computed from the experimental data, and the results are used to generate the graph of the PDF, as shown in Figure 5.

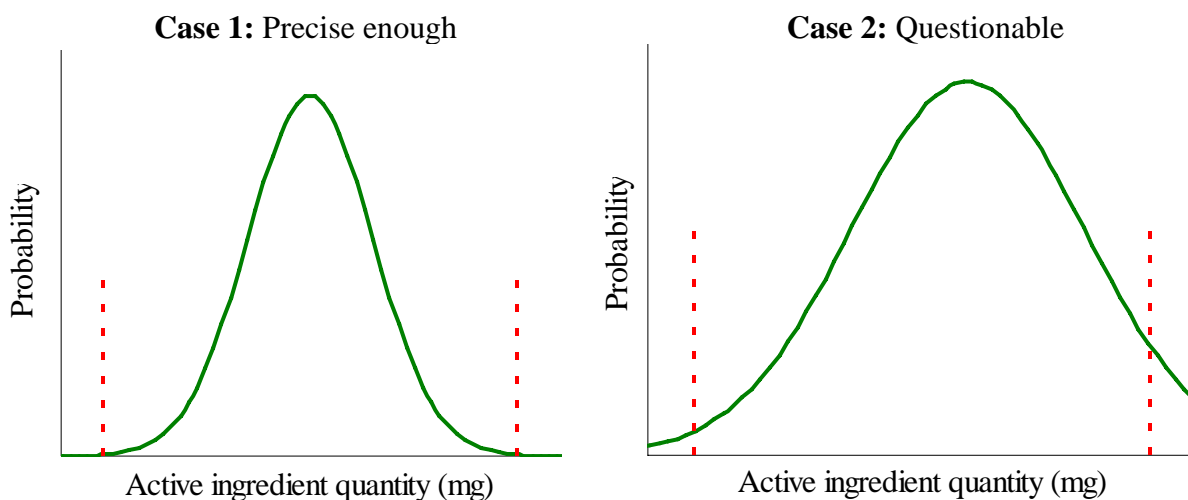


Figure 5. At least 95% of the area under the curve must lie within the spec range for the method to be validated.

For an analytical method to be validated using a 95% confidence level, at least 95% of the area under the graph of its PDF must lie within the specification range. For example, it is clear that the analytical method whose PDF is shown in the left-hand graph of Figure 5 can be validated, since at least 95% of the area under the curve lies within the specification range shown by the dashed lines. On the other hand, the analytical method whose PDF is shown in the right-hand graph is questionable at best.

Methods that are too imprecise to distinguish between acceptable and unacceptable batches cannot be used to validate drug products, so there is certainly an incentive to implement precise analytical methods. On the other hand, it would not be efficient to use a method that is far more precise than necessary. Therefore, the objective of this project was to find a means of quantifying analytical method precision, so that this relationship can be better understood.

To accomplish this goal, an Excel application was developed that enables users to determine whether or not a method is precise enough by inputting the relevant statistical parameters. The analytical method precision is quantified by introducing a *modified process performance index*, which allows users to easily compare the practicality of different methods. Graphical representations of the results are also generated by the application, including plots similar to the one shown in Figure 5. The statistical methods that are programmed into the application are explained in the following analysis section.

Analysis

The three errors mentioned above (product variation, method, and instrument errors) are assumed to be independent and normally distributed, so that

$$X_{\text{Product Variation}} \sim N(\mu_X, \sigma_X^2), \quad (1a)$$

$$Y_{\text{Method}} \sim N(\mu_Y, \sigma_Y^2), \quad (1b)$$

$$Z_{\text{Instrument}} \sim N(\mu_Z, \sigma_Z^2), \quad (1c)$$

where each μ and σ denotes the mean and standard deviation for the respective error type. Using normal probability theory [1], the total error is

$$E = (X + Y) + Z \sim N(\mu_E, \sigma_E^2), \quad (2)$$

where

$$\mu_E = (\mu_X + \mu_Y) + \mu_Z \quad (3a)$$

and

$$\sigma_E^2 = (\sigma_X^2 + \sigma_Y^2) + \sigma_Z^2. \quad (3b)$$

Although the product variation and method errors are independent, neither can be measured separately, as discussed in the Introduction. Therefore, these two errors are grouped together in equations (2), (3a) and (3b). Also, based upon industry practices, the means for the method and instrument errors are assumed to be zero, since any method or instrument that introduces a systematic error would not be used. Therefore, $\mu_Y = \mu_Z = 0$, and (3a) is reduced to

$$\mu_E = \mu_X \quad (3c)$$

to describe practical analytical methods.

To validate an analytical method, a collection of specimens from several batches is gathered, and the analytical method is used to measure the active ingredient quantity of the specimens (as shown in Figure 4). Then the sample mean \bar{x} and sample standard deviation $\hat{\sigma}_E$ are calculated. These two parameters are the experimental values for $\mu_T + \mu_E$ and σ_E as defined in (3a) and (3b). In practice, the relative standard deviation

$$RSD_E = \frac{\hat{\sigma}_E}{\bar{x}} \quad (3d)$$

is used to express the overall error standard deviation relative to the sample mean. The Excel application allows users to input RSD_E , which is converted to $\hat{\sigma}_E$ for use in further calculations. Then the desired confidence interval for the target mean of the active ingredient amount μ_T can be computed from

$$\bar{x} \pm z_{\alpha/2} \frac{\hat{\sigma}_E}{\sqrt{n}}. \quad (4a)$$

Here, $z_{\alpha/2}$ represents the value of the standard normal cumulative distribution function for which the probability (or equivalently, the area under the normal curve) is $\alpha/2$, where

$$\alpha = 1 - C/100 \quad (4b)$$

and C is the desired confidence level (for example, $C = 95$ for 95% confidence). In the Excel application, $z_{\alpha/2}$ is computed by the NORMSINV function [3]. Since the confidence interval given by (4a) describes the precision of one analytical method and not the active ingredient quantity of the individual specimens, the sample size n is one in every case. For instance, using ten samples of six tablets each to obtain \bar{x} and $\hat{\sigma}_E$ would constitute one analytical method validation experiment, and thus $n = 1$, not 60. Using $n = 60$ would give a confidence interval for the active ingredient quantity of the tablets, rather than the precision of the analytical method. Therefore, (4a) reduces to

$$\bar{x} \pm z_{\alpha/2} \cdot \hat{\sigma}_E \quad (4c)$$

It should also be noted that the confidence interval does not directly depend on the parameters for the instrument error, because the overall standard deviation $\hat{\sigma}_E$ already accounts for the instrument and method errors, as shown in (3b).

The confidence interval given by (4c) describes the target mean μ_T , or in other words, the intended quantity of active ingredient. The underlying assumption is that the population means μ_B of the various batches of drug product are normally distributed with mean μ_T . That is, even though the population mean of each individual batch may be slightly different than μ_T , if there are no persistent systematic errors in the production processes, then

$$\mu_T = \frac{1}{N} \sum_{k=1}^N \mu_{B,k} \quad (5)$$

where $\mu_{B,k}$ is the population mean of the k^{th} batch and N is the total number of batches. Since extreme care is taken to eliminate systematic errors at every stage of drug manufacturing, this is a very reasonable assumption.

Once again, the objective of this analysis is to quantify analytical method precision. One way this can be done is by determining the maximum overall error standard deviation for which the analytical method is still precise enough to be used. The maximum allowable overall error standard deviation corresponds to the situation when the confidence interval given by equation (4c) coincides with the FDA product specification range. The FDA requires that

$$LSL \leq \mu_B \leq USL \quad (6a)$$

where

$$\begin{aligned} LSL &= (1 - S)\mu_T, \\ USL &= (1 + S)\mu_T, \end{aligned} \quad (6b)$$

and S is the product specification. For instance, $S = 0.1$ for a specification range of 90% to 110% of μ_T . Therefore, the maximum allowable value of the overall error standard deviation is the largest possible value of $\hat{\sigma}_{E,\max}$ that satisfies

$$\frac{1}{\hat{\sigma}_{E,\max}} \frac{1}{\sqrt{2\pi}} \int_{LSL}^{USL} \exp\left[-\frac{(x-\bar{x})^2}{2\hat{\sigma}_{E,\max}^2}\right] dx \geq \frac{C}{100}, \quad (7)$$

where LSL and USL are the lower and upper specification limits as defined above [4]. The graphical interpretation of (7) is that $\hat{\sigma}_{E,\max}$ is the value of the overall error standard deviation for which at least C percent (for instance, 95%) of the area under the normal curve lies between the LSL and USL . In terms of $z_{\alpha/2}$, the analytical solution for $\hat{\sigma}_{E,\max}$ must satisfy the inequality

$$LSL \leq \bar{x} - z_{\alpha/2} \hat{\sigma}_{E,\max} < \bar{x} + z_{\alpha/2} \hat{\sigma}_{E,\max} \leq USL, \quad (8)$$

where the left hand equality holds when $LSL \leq \bar{x} \leq \mu_T$ and the right hand equality holds when $\mu_T \leq \bar{x} \leq USL$. Therefore, $\hat{\sigma}_{E,\max}$ is given by

$$\hat{\sigma}_{E,\max} = \begin{cases} \frac{\bar{x} - LSL}{z_{\alpha/2}} & \text{if } LSL \leq \bar{x} \leq \mu_T \\ \frac{USL - \bar{x}}{z_{\alpha/2}} & \text{if } \mu_T < \bar{x} \leq USL. \end{cases} \quad (9)$$

In the extreme case that $\bar{x} < LSL$ or $\bar{x} > USL$, no further analysis is reasonable, because in this instance, at least 50% of the area under the curve would not fall within the specification range.

An analytical method is precise enough if the experimental value for the overall error standard deviation $\hat{\sigma}_E$ is smaller than $\hat{\sigma}_{E,\max}$. In practice, $\hat{\sigma}_E$ can be determined using a collection of specimens from several different batches as shown in Figure 4.

Yet another way of quantifying the precision of an analytical method is to introduce a *modified process performance index*, which is calculated by

$$P_{pk} \equiv \frac{\min\{USL - \bar{x}, \bar{x} - LSL\}}{z_{\alpha/2} \hat{\sigma}_E}. \quad (10)$$

This definition of P_{pk} was modified from the standard definition used in Six-Sigma quality control applications. For most applications, it is customary to use 3σ instead of $z_{\alpha/2} \hat{\sigma}_E$ in (10), which would correspond to a confidence level of approximately 99.7% [2]. The denominator shown in (10) is used to reflect the level of confidence specified by the user. An acceptable analytical method is one with a P_{pk} greater than 1.0, whereas a method with a P_{pk} below 1.0 is unacceptable. In comparing two or more analytical methods using sample collections with the same \bar{x} , the method with the largest P_{pk} value is the most precise. For more information on the process performance index, please refer to any text on six-sigma methodology.

Example Outputs from the Excel Application

The first example considers the input values given in Table 1A. The application results indicate that this method is precise enough to be of practical use. This is shown in Table 1B, which indicates the method is precise enough since $P_{pk} > 1$, and because the actual RSD_E is smaller than the maximum allowable relative standard deviation, $RSD_{E,max}$. Also, Figure 6 shows that the method is precise enough, since the 95% confidence interval of (23.2 mg, 26.8 mg) lies within the specification range. The two PDFs graphed in Figure 6 can also be compared to make this conclusion. The actual PDF is plotted using \bar{x} and $\hat{\sigma}_E$ for the mean and standard deviation, whereas the critical PDF is plotted using \bar{x} and $\hat{\sigma}_{E,max}$. Since the peak of the actual PDF is above the peak of the critical PDF, this analytical method is precise enough.

Table 1A. Input Parameters for Example 1.

| | |
|--------------------|------------|
| μ_T | 25 mg |
| \bar{x} | 25 mg |
| FDA specifications | 90% – 110% |
| Confidence level | 95% |
| RSD_E | 3.6% |

Table 1B. Numerical Outputs for Example 1.

| | |
|---------------|-------|
| $RSD_{E,max}$ | 5.10% |
| P_{pk} | 1.42 |

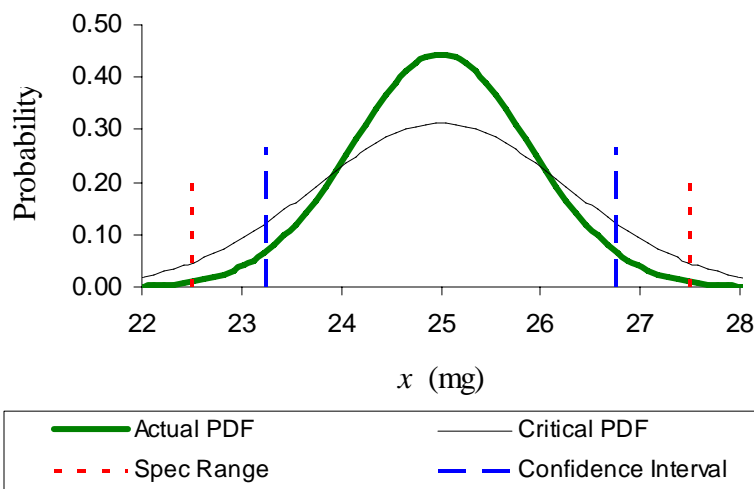


Figure 6. The analytical method whose PDF is shown here is precise enough.

Example 2 demonstrates a method that is nearly too imprecise, whose parameters are shown in Table 2A. This example method has an RSD_E larger than the method from the previous example, but it has the same sample mean. The results show that this method is precise enough, but the level of precision is marginally sufficient. Table 2B indicates that the P_{pk} is nearly 1.0, and that $RSD_{E,max} = 5.10\%$, which is only 0.1% larger than RSD_E , and thus there is little room for additional error. Also, the confidence interval in Figure 7 nearly overlaps the specification range, and the peak of the actual PDF is marginally higher than that of the critical PDF.

Table 2A. Input Parameters for Example 2.

| | |
|--------------------|------------|
| μ_T | 25 mg |
| \bar{x} | 25 mg |
| FDA specifications | 90% – 110% |
| Confidence level | 95% |
| RSD_E | 5.0% |

Table 2B. Numerical Outputs for Example 2.

| | |
|---------------|-------|
| $RSD_{E,max}$ | 5.10% |
| P_{pk} | 1.02 |

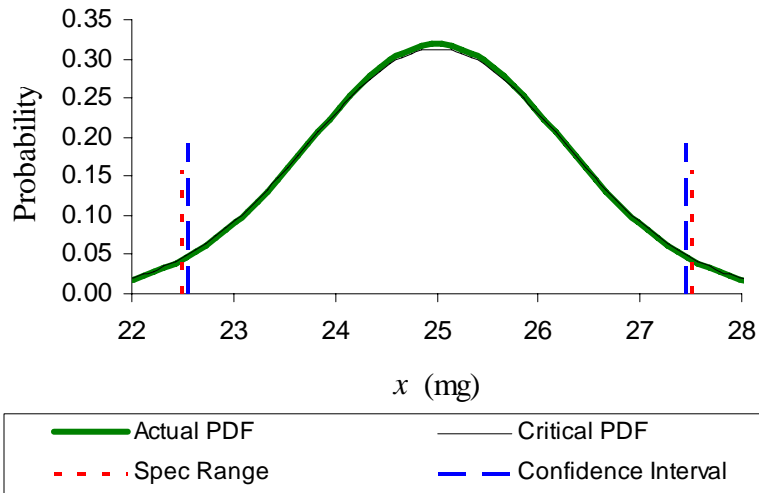


Figure 7. The precision of this analytical method is marginally sufficient.

The next two (somewhat hypothetical) examples illustrate the possibility that the tested specimens do not have a sample mean equal to the target mean—that is, $\bar{x} \neq \mu_T$. Therefore the PDFs shown in Figure 8 are off center. In example 3, the method illustrated is still precise enough, whereas in example 4, it is not. However, the method in example 4 would be precise enough if either the sample mean were closer to the target mean, or if RSD_E were smaller.

Table 3A. Input Parameters for Examples 3 and 4.

| Parameters | Example 3 | Example 4 |
|--------------------|------------|------------|
| μ_T | 25 mg | 25 mg |
| \bar{x} | 24 mg | 25.6 mg |
| FDA specifications | 90% – 110% | 90% – 110% |
| Confidence level | 95% | 95% |
| RSD_E | 2.5% | 4.0% |

Table 3B. Numerical Outputs for Examples 3 and 4.

| Parameters | Example 3 | Example 4 |
|---------------|-----------|-----------|
| $RSD_{E,max}$ | 3.19% | 3.79% |
| P_{pk} | 1.28 | 0.95 |

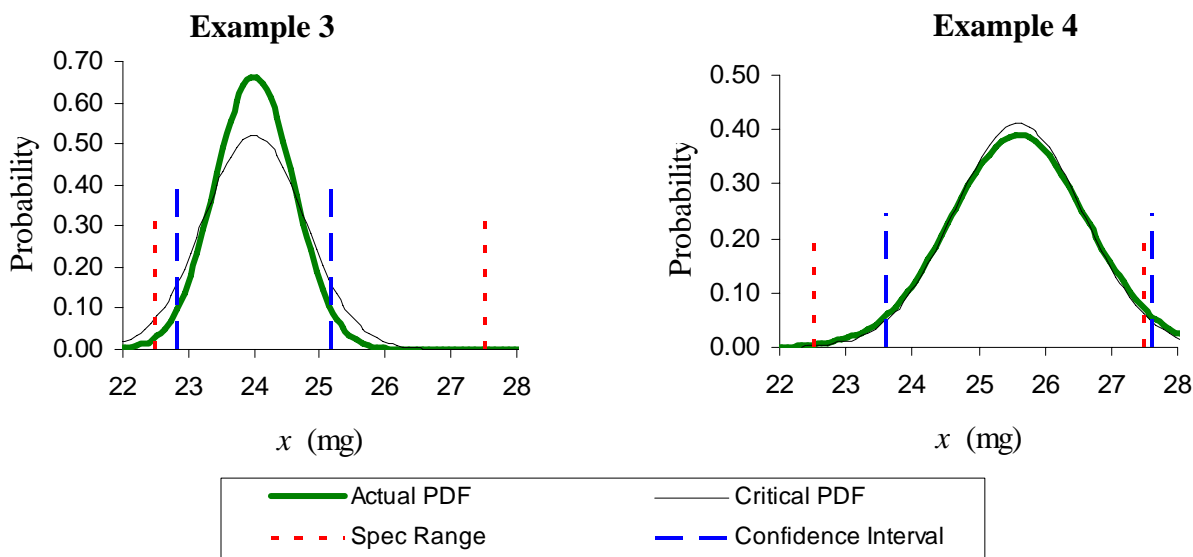


Figure 8. The method from example 3 is precise enough, whereas the method from example 4 is not.

Discussion

Remarks on the Statistical Analysis

Several important assumptions were made during the development of the analysis. The primary assumption was that the various measurement errors are independent and normally distributed. This assumption is reasonable, since the method used to measure a drug product is not affected by the process used to manufacture that drug product, as explained in the Introduction. Since industry experience indicates that all the errors are normally distributed, the desired confidence intervals were obtained using normal probability theory. Since all three errors are independent, their means and variances can be added to obtain the mean and variance of the overall error, as shown in (3a) and (3b). The overall error standard deviation is then used to form the confidence interval given by (4a), and this result forms the basis for all the graphical representations of the analytical method precision described later in the analysis. Therefore, the validity of analysis (and therefore, the accuracy of the Excel application) is justified, provided that the method, instrument, and variation in product errors are normally distributed and independent.

Since the entire analysis relies on this assumption, care must be taken if the application is ever used to characterize new analytical methods. In particular, sufficient testing should be done to verify that the errors associated with any new prospective analytical methods are independent and normally distributed. Also, if evidence were to arise that suggests some method has an error pattern which follows some other probability density function besides the normal distribution, an investigation may be in order.

It should be also be emphasized that in (4a), the sample size n is equal to one, since the confidence interval describes the analytical method rather than the tested specimens. In other words, setting $n = 1$ reflects the confidence in *each measurement* obtained using the analytical method. This can also be understood by reasoning that the precision limitations that inherently belong to the analytical method in question should not depend on the number of specimens used to test it. That is, an analytical method certainly has some inherent precision limitations, and those limitations certainly cannot be reduced by using the method to test more specimens.

Remarks on the Example Results

The analytical methods shown in examples 1 and 3 are clearly precise enough, whereas the method shown in example 4 was not. The method in example 2 was statistically precise enough, but from a practical standpoint it is questionable at best. These results can be quantified using the modified process performance index as described earlier. Since the values for the methods in examples 1, 2, and 3 are larger than 1.0, these methods are precise enough. However, the P_{pk} values for the methods from examples 1 and 3 were significantly larger than 1.0, indicating that the methods were precise enough to be of practical use. By contrast, the P_{pk} value for example 2 was very close to 1.0, so the practicality of this method is questionable. The P_{pk} value for example 4 indicates that this analytical method is too imprecise to be used.

As mentioned in the Analysis, the precision of two different analytical methods (for measuring the same drug product) can be compared using their P_{pk} values, provided that the sample means of the specimens used in the method validation experiments are the same. So, for example, since the sample means were 25.0 mg in both examples 1 and 2, these two analytical

methods can be compared using their P_{pk} values, and in that sense, the method from example 1 is more precise as it has a larger P_{pk} . On the other hand, the analytical methods from examples 3 and 4 should not be compared on the basis of their P_{pk} values, since the sample means from those examples were different. However, these two methods (and for that matter, any two methods) could be compared based on their relative standard deviations, and in that sense, the analytical method from example 3 is more precise than that of example 4. The fact that \bar{x} for example 3 is farther away from the target mean than the \bar{x} of example 4 has nothing to do with the precision of those analytical methods. Again, analytical method precision does not depend on the collection of specimens used to test the analytical method.

The maximum allowable RSD values given by the Excel application must be interpreted carefully to avoid confusing the overall measurement error with the overall error belonging only to the analytical method itself, as shown in Figure 2. As mentioned in the introduction, the errors inherent to the analytical method (that is, the method and instrument errors) cannot be measured together without introducing variation in product error. Thus, the standard deviations obtained from experiments such as the one shown in Figure 4 (and thus, the RSD_E input value and $RSD_{E,max}$ output value of the Excel application) correspond to the overall measurement error, which includes the variation in product error of the samples used to perform the experiment.

A useful advantage of reporting maximum allowable error in the form of the relative standard deviation $RSD_{E,max}$ is that this value is constant with respect to the particular drug product under consideration when all other variables are held fixed. That is, the application outputs indicate that $RSD_{E,max} = 5.1\%$ regardless of the target mean, provided that the sample mean is equal to the target mean, and the specification range and confidence level are also fixed. On the other hand, the (non-relative) standard deviation increases with the target mean (since, for instance, a standard deviation of 5 mg would be unacceptable for a 10 mg drug product, but acceptable for an 800 mg drug product). Thus, using RSD to report the maximum allowable error provides a convenient measure for analytical method precision that is invariant with respect to the target mean of the drug product.

Remarks on Method Validation Procedures

Since the input and output RSD_E values in the application inherently contain variation in product error, the application results will be most realistic if the samples used to obtain \bar{x} and RSD_E are obtained from many different batches (of the same drug product). If the experiments were done using samples from a single batch, the application results would reflect the variation in product error of *only* that particular batch. This leaves the possibilities that this batch is either unusually precise or unusually imprecise. This is undesirable, since the application would give results that are either too conservative if the selected batch were unusually precise, or too strict if the selected batch were unusually imprecise. Sampling from a sufficiently large number of batches would give results that reflect the *average* variation in product error that is typically present in batches of the drug product. In addition to this advantage, there is another possible benefit in sampling from different batches. Suppose that a sufficiently large sample were gathered, which consisted of several specimens from different batches (of the same drug product). Further suppose that the sample mean of this collection differed appreciably from the target mean. This would suggest some kind of systematic error might be occurring in the drug production process.

Of course, in most practical situations this would not be the case, but sampling from different batches is one possible way to verify that such errors are not occurring. For both of these reasons, the application inputs should be determined using samples from different batches of the same drug product.

The methods from examples 3 and 4 are somewhat hypothetical in nature, since it is unlikely that the sample mean \bar{x} would vary from the target mean by as much as 20% of the specification range in practical situations. However, these examples do point out that the maximum allowable overall error standard deviation for the analytical method depends on the *sample mean drift*,

$$\delta = |\bar{x} - \mu_T|, \quad (11)$$

or in other words, the distance of the sample mean from the target mean. This relationship is exemplified in Table 4. The analytical methods from examples 1, 4, and 3 have sample means that are increasingly farther away from the target mean, and therefore their maximum allowable overall error standard deviation decreases. This can also be interpreted graphically, as shown in Figure 5, in which the maximum allowable overall error standard deviation is shown by double sided arrows. It is apparent that the allowable standard deviation is much smaller in the second case, where the sample mean drift is much larger.

Table 4. Relationship between sample mean drift and allowable error.

| Parameter | Example 1 | Example 4 | Example 3 |
|-------------------------|-----------|-----------|-----------|
| \bar{x} | 25.0 mg | 25.6 mg | 24.0 mg |
| δ | 0.0 mg | 0.6 mg | 1.0 mg |
| $\hat{\sigma}_{E,\max}$ | 1.275 mg | 0.969 mg | 0.765 mg |

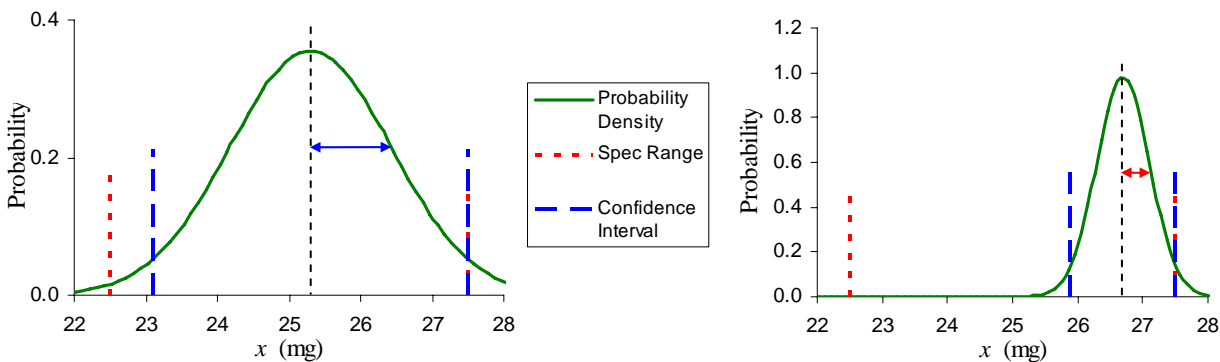


Figure 5. The maximum allowable standard deviation decreases as the sample mean drift increases.

Although sample mean drifts of the magnitude shown in the right hand graph of Figure 5 are very unlikely to occur in practice, small sample mean drifts such as the one shown in the left hand graph of Figure 5 are certainly plausible. This further reinforces the advantage of sampling from *different* batches when method validation experiments are performed. If all the samples were obtained from the same batch of drug product, the sample mean of that particular batch would be meaningless, since there is a good possibility that the sample mean of a *typical* batch is

different than that of the batch used for the method validation experiment. One possible (but somewhat questionable) response to this situation is to neglect the batch sample mean drift, and in the Excel application, input the intended active ingredient quantity (the target mean) instead of the experimentally determined value for \bar{x} . This amounts to assuming that the *average* sample mean drift of *all* drug batches is zero, even though the particular batch used for method validation had a nonzero sample mean drift. Equivalently, this asserts that no systematic errors ever occur in the final stages of the drug production process (such as capsule filling processes). In that regard, this assumption is somewhat reasonable, since care is taken to eliminate systematic errors. On the other hand, making this assumption is unnecessary if the sample specimens are obtained from different batches. Thus, from a statistical standpoint, the latter approach is certainly preferred.

Conclusions

The following conclusions are supported by the results of this project.

- Because method error and variation in product error cannot be measured separately, using the overall measurement error from an analytical method test is the best way to quantify the precision of that analytical method.
- When the FDA specification range is 90% - 110% of the target mean, and when the sample mean is equal to the target mean, the maximum allowable overall error relative standard deviation for an analytical method is 5.1%. This holds true for any drug product, no matter what value of μ_T is used.
- Samples used for analytical method precision testing should be taken from different batches (of the same drug product). The analysis discussed above was made under the assumption that sampling occurs across batches, and therefore may not apply directly to analytical methods tested with samples from one batch of drug product.
- The Excel application outputs can be used to compare two or more analytical methods. In the case where the sample means of two methods are equal (in other words, $\bar{x}_1 = \bar{x}_2$), then the more precise method is the one with the larger P_{pk} value. If $\bar{x}_1 \neq \bar{x}_2$, then the P_{pk} values of the two methods cannot be compared.

Problem 2: Responding to Fluctuation in Drug Substance

Introduction

This problem examines the process in which a formulated drug substance (such as a powder) is used to create a finished drug product (by filling the powder into capsules, for example). A typical process of this sort is shown schematically in Figure 6. Before the powder is filled into capsules, it is measured using some kind of analytical method to determine its active ingredient concentration. Then the filling set point (that is, the amount of powder to be filled into each capsule) is calculated based on the measured active ingredient concentration of the powder. The goal in determining the set point is to fill exactly the intended amount of active ingredient into each capsule, and thereby make up for any possible fluctuation in the drug substance strength. So for instance, if the analytical method indicates that the powder strength is slightly less than intended, the filling set point is increased.

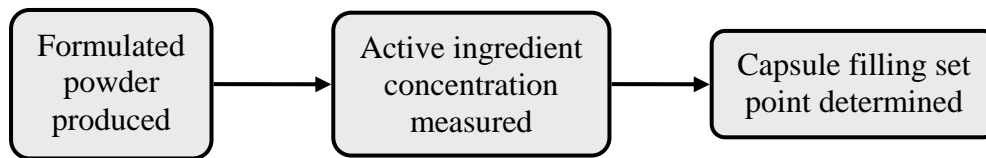


Figure 6. Before a drug product is distributed it must be tested using an analytical method.

Presently, the set point of the filling process is adjusted proportionally depending on the measured active ingredient concentration of the powder. For example, suppose the finished drug product is to be a capsule containing 25 mg of active ingredient, and suppose that the intended powder concentration is 10 mg of active ingredient per gram of powder, or 10 mg/g. Then in the ideal case when the powder concentration is exactly 10 mg/g, the set point of the filling process would be 2.5 grams. To detect and respond to non-ideal cases in which the powder strength is not exactly 10 mg/g, an analytical method is used to take several measurements of the powder, and the measurements are averaged to obtain an estimate for the true powder strength. If the average measured concentration were 9.5 mg/g, then the set point of the filling process would be increased to

$$\frac{25 \text{ mg}}{9.5 \text{ mg/g}} = 2.63 \text{ g}, \quad (12)$$

so that (hopefully) each capsule would contain exactly 25 mg of active ingredient after it is filled with 2.63 g of powder.

This project seeks to improve the simple set point determination method shown by (12), as there are several other variables that this approach does not take into account. In particular, the drug substance measurements used to obtain the average measured concentration certainly have a standard deviation associated with them, which is not considered in (12). The error types that contribute to the overall standard deviation of the measurements include the following:

- *Variation in Substance Error*, due to possible non-homogeneity of the drug substance;
- *Method Error*, which occurs when the drug substance is handled (the same as before); and

- *Instrument Error*, due to the machines used to obtain the active ingredient concentration measurements (also the same as before).

In addition to errors that affect the drug substance measurements, there are also limitations associated with the capsule filling process, which include the following:

- *Set Point Precision*, which is the maximum level of accuracy to which the set point can be programmed into the machines that fill the capsules; and
- *Filling Error*, which includes any random errors associated with the processes and machines used to fill the powder into the capsules.

For example, if the set point precision were ± 0.0001 g, the filling process could be programmed to place 2.6316 g of powder into each capsule. On the other hand, if the set point precision were only ± 0.1 g, then 2.6 g would be the logical choice for the set point. After considering the possible effects of all these additional factors, it seems clear that the simple calculation from (12) does not account for all the relevant variables. Therefore, the objective of this project was to develop a more rigorous approach for calculating the filling set point, and to determine the circumstances under which adjusting the set point is beneficial. A preliminary statistical analysis for this problem is detailed in the following section, and recommendations for extending this work are subsequently given.

Analysis and Discussion

As in Problem 1, industry experience indicates that the four errors mentioned above (variation in substance, method, instrument, and filling errors) are all normally distributed. Therefore,

$$S_{\text{Substance Variation}} \sim N(\mu_S, \sigma_S^2), \quad (13a)$$

$$P_{\text{Method}} \sim N(\mu_P, \sigma_P^2), \quad (13b)$$

$$Q_{\text{Instrument}} \sim N(\mu_Q, \sigma_Q^2), \quad (13c)$$

and

$$F_{\text{Filling}} \sim N(\mu_F, \sigma_F^2), \quad (13d)$$

where each μ and σ denote the mean and standard deviation for the given error.

Several reasonable simplifications can be introduced so that these random variables reflect the true nature of the errors. As in Problem 1, great care is taken to ensure that no method, instrument, or machine that produces a systematic error is used. For instance, it is never permissible to use a capsule filling machine that slightly overfills each capsule on average, or an HPLC machine that slightly underreports concentrations on average. Therefore,

$$\mu_P = \mu_Q = \mu_F = 0 \quad (14)$$

for all practical scenarios. Additionally, typical drug substances are homogeneous mixtures—for instance, if the formulated drug substance in question is a powder, then the active ingredient concentration of the powder is assumed to be homogeneous throughout each batch (or barrel).

Therefore in every case, $\sigma_S = 0$. On the other hand, the powder strength may differ from the intended strength, so $\mu_S \neq 0$. With these simplifications, equations (13a-d) simplify to

$$S_{\text{Substance Variation}} \sim N(\mu_S, 0), \quad (15a)$$

$$P_{\text{Method}} \sim N(0, \sigma_P^2), \quad (15b)$$

$$Q_{\text{Instrument}} \sim N(0, \sigma_Q^2), \quad (15c)$$

and

$$F_{\text{Filling}} \sim N(0, \sigma_F^2). \quad (15d)$$

The standard deviations σ_Q and σ_F of the instrument and filling errors can be measured, so these parameters are known. Furthermore, probability theory can be used to obtain some additional information relating S , P , and Q . As mentioned previously, when a batch of formulated powder is created and before it is filled into capsules, its active ingredient concentration is measured. The total error M associated with this measurement is

$$M = (S + P) + Q, \quad (16)$$

where S and P are grouped to emphasize the fact that they cannot be independently measured, as discussed in the introduction to Problem 1. Since all three errors on the right hand side of (16) are normally distributed,

$$M \sim N(\mu_M, \sigma_M^2), \quad (17)$$

where

$$\begin{aligned} \mu_M &= \mu_S + \mu_P + \mu_Q \\ &= \mu_S + 0 + 0 \end{aligned} \quad (18a)$$

and

$$\begin{aligned} \sigma_M^2 &= \sigma_S^2 + \sigma_P^2 + \sigma_Q^2 \\ &= 0 + \sigma_P^2 + \sigma_Q^2 \end{aligned} \quad (18b)$$

Therefore,

$$M \sim N(\mu_S, \sigma_P^2 + \sigma_Q^2). \quad (19)$$

From these results, the confidence interval for the target powder concentration μ_{pT} is

$$\bar{x}_p \pm z_{\alpha/2} \frac{\hat{\sigma}_M}{\sqrt{n}}, \quad (20a)$$

where \bar{x}_p and $\hat{\sigma}_M$ are the sample mean and sample standard deviation of the n powder measurements and $z_{\alpha/2}$ is the same as before. Also, since $\sigma_S = 0$, the true powder concentration is the sum of the target μ_{pT} and the mean μ_S of the variation in substance error, and the

experimental value for this true concentration $\mu_{pT} + \mu_S$ is \bar{x}_p . For instance, in the introductory example, $\mu_{pT} = 10$ mg/g and $\bar{x}_p = 9.5$ mg/g. In this regard, the interpretation of (20) is that with (say) 95% confidence, the actual concentration $\mu_{pT} + \mu_S$ of the powder falls inside

$$\left(\bar{x}_p - z_{\alpha/2} \frac{\hat{\sigma}_M}{\sqrt{n}}, \bar{x}_p + z_{\alpha/2} \frac{\hat{\sigma}_M}{\sqrt{n}} \right). \quad (20b)$$

Since the powder itself is homogeneous, each capsule will be filled with precisely the same substance, so the confidence interval given by (20a) does not *directly* relate to the amount of active ingredient that will be present in each capsule after they are filled. The variation of the filling process itself is described by the filling error F , so on average, the amount in mg of active ingredient present in each capsule is

$$(SP + \mu_F)(\mu_{pT} + \mu_S) = SP(\mu_{pT} + \mu_S), \quad (21)$$

since the mean of the filling error is assumed to be zero. Here, SP denotes the set point chosen for the filling process in grams of total powder (per capsule). Additionally, the confidence interval for the amount of total powder filled into any given capsule is

$$SP \pm z_{\alpha/2} \hat{\sigma}_F, \quad (22)$$

where $\hat{\sigma}_F$ is an experimental value for σ_F that could be found by weighing the capsules after they are filled. Here, the sample size is taken as 1 since this confidence interval describes the amount of powder filled into any one arbitrarily selected capsule, and no division by \sqrt{n} is used. Roughly speaking, a confidence interval for the *average* amount of active ingredient that will be present in each capsule could be found by applying (20a) and (22) to describe the confidence in the overall average result given by (21). However, an overall standard deviation would first need to be determined by combining the filling error with the various measurement errors.

To relate the measurement errors to the filling error quantitatively, some kind of scaling factor is needed, since these two types of errors have different units. The measurement errors S , P , and Q have units of mg/g, or more explicitly,

$$S [=] P [=] Q [=] \frac{\text{mg active ingredient}}{\text{g total powder}}, \quad (23a)$$

where the symbol [=] stands for “has the same units as” or “has units of.” However, the filling error F describes the quantity in grams of total powder filled into each capsule, so

$$F [=] \text{g total powder}. \quad (23b)$$

Thus, F may be scaled so that it has the same units as S , P , and Q by noting that

$$F \cdot \frac{\mu_{pT}}{SP_0} [=] \text{g total powder} \cdot \frac{\text{mg active ingredient} / \text{g total powder}}{\text{g total powder}}, \quad (24a)$$

where SP_0 is the ideal set point (2.5 g in the example). The ideal set point is given by

$$SP_0 = \frac{\mu_{cT}}{\mu_{pT}}, \quad (24b)$$

where μ_{cT} is the target mean of the active ingredient quantity in milligrams present in each capsule (25 mg in the example). Thus, (24a) rearranges to

$$F \cdot \frac{\mu_{pT}^2}{\mu_{cT}} [=] \frac{\text{mg active ingredient}}{\text{g total powder}}, \quad (24c)$$

which has the same units as S , P , and Q .

Now that a means of scaling the filling error has been developed, focus may be returned to applying the results of (20a) and (22) to (21), in hopes of finding a confidence interval for the average quantity of active ingredient present in each capsule. Since the filling and measurement errors are all normally distributed,

$$\sigma_C = \sqrt{\sigma_M^2 + \frac{\mu_{pT}^2 \sigma_F^2}{\mu_{cT}}}, \quad (25)$$

where σ_C represents the standard deviation of the composite measurement and filling errors. Applying this result to (21), the confidence interval for the population mean μ_c of the active ingredient quantity (in milligrams) present in the finished capsules is

$$SP \cdot \bar{x}_p \pm z_{\alpha/2} \sqrt{\frac{\hat{\sigma}_C}{n}}, \quad (26)$$

where $\hat{\sigma}_C$ is an experimentally determined value for σ_C found by using the measured values $\hat{\sigma}_M$ and $\hat{\sigma}_F$ for σ_M and σ_F in (25).

In principle, the set point should be adjusted when, with a sufficient level of confidence, there exists some value of SP different than SP_0 for which μ_c is closer to μ_{cT} than it would if SP_0 were used instead. The confidence interval given by (26) could potentially be used to determine whether or not such a value of SP exists. Additionally, the set point precision issue mentioned in the introduction needs to be addressed. Since it is not possible to program a filling machine with unlimited precision (for instance, a set point of 2.63159043 g is not practical), SP must be regarded as a discrete variable, rather than a continuous variable. It is highly likely that the statistical analysis presented here, or perhaps an extension of it, could be programmed into an Excel application, which could in turn be used to determine the optimal SP for a given set of measurement error and filling process parameters.

Recommendations for Future Work

Problem 1: Analytical Method Precision

Since the method error of an analytical method cannot be measured separately, the overall measurement error is the best feasible way to measure and quantify analytical method precision. However, if a means of estimating the method error were ever developed, the analysis presented in this report could be extended, and a clearer picture of the relationship between analytical method validation and drug product validation could be obtained. Therefore, it is advisable to consider in detail the various processes that contribute to method error (such as grinding tablets or pipetting solvents), and investigate any possible ways that the errors caused by these processes could be measured. Is there one particular contributor to method error that is much more significant than all the others? If so, could a sufficiently accurate estimate of the method error be found by assuming that only this primary contributor is important, and by neglecting the other contributions? If such an estimate were possible, the method error variance could be used in (3b) to determine the variance of the variation in product error itself. Such a discovery could possibly expediate the drug validation process significantly by quantitatively linking analytical method precision and drug product validation.

Problem 2: Responding to Fluctuation in Drug Substance

The preliminary analysis developed in this work should be implemented into a spreadsheet or some other kind of numerical simulation. Typical values for the measurement and filling error parameters should be used, and various values of the set point SP could be attempted. For each set point, the resulting distribution of capsule active ingredient quantity could be determined, and it is likely that such an approach would lead to a more rigorous procedure for determining the filling process set point. Extending the statistical analysis might also lead to a closed formula for the optimal set point in terms of measurable error parameters.

References

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Appendix – User Guide

Configuring Excel

The file `Pfizer_Model.xls` contains macros, so Excel must be configured to enable macros to run before the file is opened. This can be done using the following steps:

1. Start Microsoft Excel (but not by opening the `Pfizer_Model.xls` file). If a new workbook called Book1 is opened automatically, close its window (but not the entire Excel application window) by clicking the Close Window box, or by selecting Close from the File menu.
2. Pull down the Tools menu, point to Macro, and select Security. The Security dialog box appears. Click the Security Level tab.
3. By default, Excel has the Security Level set to High. To use all features of the application, the Security Level must be set to Medium or Low. The Medium setting is recommended. Click the radio button for the desired setting, and then click OK.
4. Open the file `Pfizer_Model.xls`. If the Medium security setting was selected, a Security Warning dialog box similar to the one shown in Figure A1 will appear (this is normal). Click the Enable Macros button.

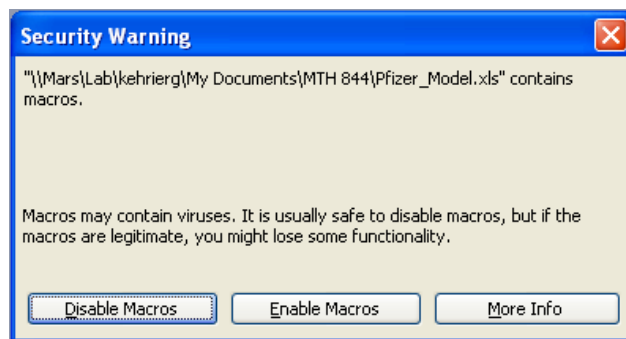


Figure A1. This Security Warning dialog box is normal.

The Security Level will remain the same even after the `Pfizer_Model.xls` file is closed, so only Step 4 needs to be repeated each time the file is opened.

Application Organization

The application consists of two worksheets, `AnalyticalMethod` and `DataPoints`. The `AnalyticalMethod` sheet contains all inputs and outputs, so it is the only sheet that ordinarily needs to be used. The `DataPoints` sheet simply performs the calculations needed to form the graphs on the `AnalyticalMethod` sheet, so it does not need to be modified. The cell colors on both worksheets are used to communicate the following:

- Blue cells are input parameters, which are designed to be changed as the user desires.
- Black cells are either headings or calculations, which do not need to be modified.
- Green and red cells are outputs. Green indicates a favorable result, whereas red indicates an undesirable result or error.

The AnalyticalMethod sheet appears when the file is first opened. This worksheet consists of four sections, as shown in Figure A2. The first (Table 1) allows the user to input the analytical method parameters, the second (Table 2) shows the intermediate calculations, the third (Table 3) consists of the numerical results, and the fourth (Figure 1) shows the graphical results. Each of these sections is explained in the following paragraphs.

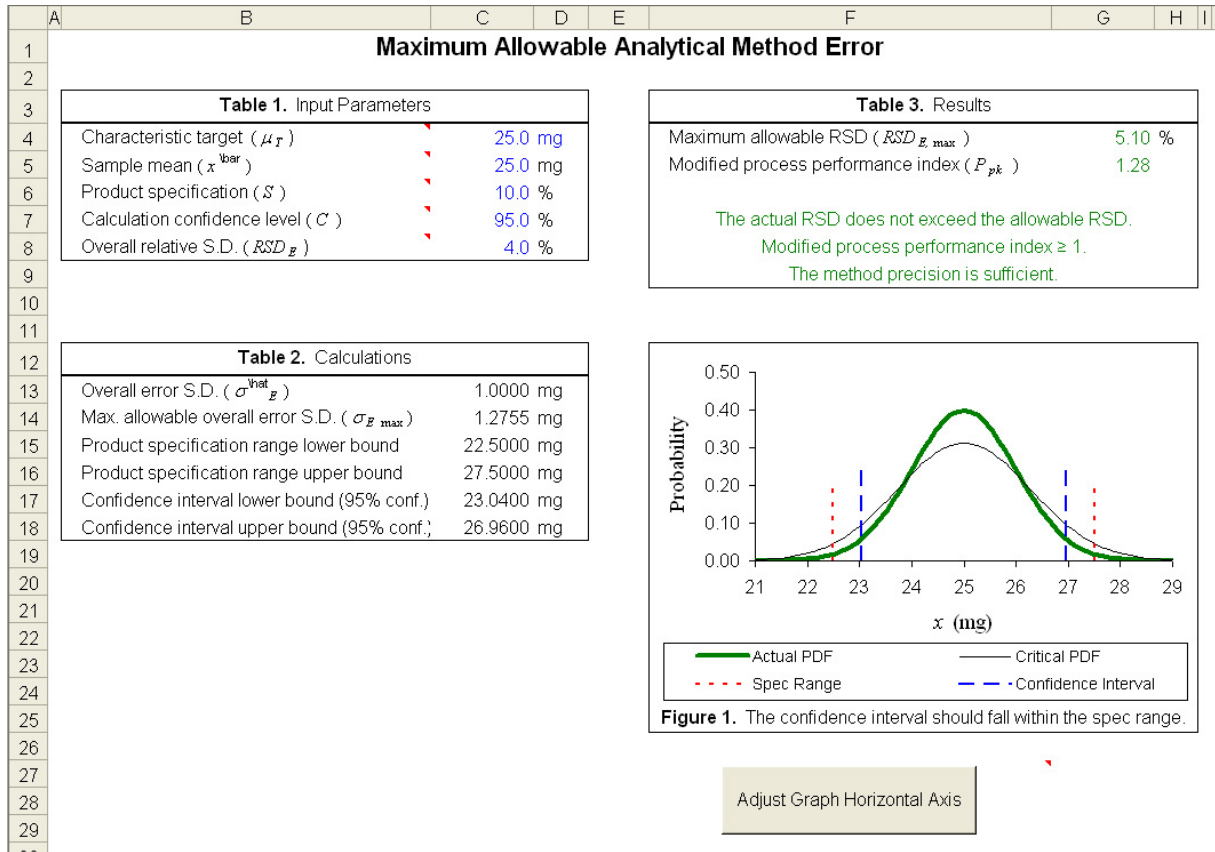


Figure A2. Screen shot of the AnalyticalMethod worksheet.

Table 1: Input Parameters

The input section screen shot is shown below in Figure A3. This section allows the user to input the target mean μ_T , the measured sample mean \bar{x} , the product specification S in percent, the desired confidence level for the target mean in percent, and the measured overall relative standard deviation for the error, RSD_E , in percent. The unit of μ_T (mg by default) can also be changed, and if this is done, the rest of the units on the worksheet are automatically updated.

| Table 1. Input Parameters | |
|--------------------------------------|---------|
| Characteristic target (μ_T) | 25.0 mg |
| Sample mean (\bar{x}) | 25.0 mg |
| Product specification (S) | 10.0 % |
| Calculation confidence level (C) | 95.0 % |
| Overall relative S.D. (RSD_E) | 4.0 % |

Figure A3. Input section screen shot.

The sample mean \bar{x} must fall within the specification range in order for the calculations to be reasonable. Therefore, several cells will display error messages if the sample mean \bar{x} does not lie within the specification range. This situation usually occurs when the inputs are changed to represent a drug product with a different target mean. For example, if the default target mean of 25.0 is changed to 100.0, several cells will display “ERROR” until the sample mean is also updated, at which point the errors will disappear.

By default, the product specification is entered as a percentage S , and the application assumes that the specification range is centered around the target mean. For example, entering 10.0% corresponds to a specification range of 90% to 110% of the target mean. If the bounds of the specification range LSL and USL are known, the desired value of S to enter into the cell can be found by

$$S = \frac{USL - LSL}{2\mu_T} \cdot 100.$$

Table 2: Calculations

The intermediate results are shown in this section, whose screen shot is shown in Figure A4. Each of the calculations is explained in the following paragraphs.

| Table 2. Calculations | |
|---|------------|
| Overall error S.D. (σ_E^{hat}) | 1.0000 mg |
| Max. allowable overall error S.D. ($\sigma_{E\ max}$) | 1.2755 mg |
| Product specification range lower bound | 22.5000 mg |
| Product specification range upper bound | 27.5000 mg |
| Confidence interval lower bound (95% conf.) | 23.0400 mg |
| Confidence interval upper bound (95% conf.) | 26.9600 mg |

Figure A4. Calculations section screen shot.

The calculations begin by finding the overall error standard deviation (1.0000 mg as shown in Figure A4) using equation (3d) in the Analysis section of the report. Next, the maximum allowable overall error is found using (9), where $z_{\alpha/2}$ is computed by

$$ABS(NORMSINV((1 - C. \%) / 2)).$$

Here, $C.$ is the name of the cell containing the confidence level input. This computation makes use of the Excel function `NORMSINV`, which returns the value of the inverse of the standard normal cumulative distribution with mean zero and standard deviation one [3]. Since the range of any cumulative distribution function is $[0, 1]$, the value of $1 - C/100$ must fall between zero and one. This implies that the user must enter a value for C between 1 and 100.

The product specification range lower and upper bounds LSL and USL are obtained from equation (6b). The next two values, the confidence interval upper bound and lower bound, are computed using (4c), which is implemented in Excel by

$$\bar{x} + \text{NORMSINV}((1-C.)/2) * \text{sigma_E_hat} / \text{SQRT}(1),$$

or in other words,

$$\bar{x} \pm z_{\alpha/2} \cdot \frac{\hat{\sigma}_E}{\sqrt{1}}.$$

The square root of one is intentionally left in the formula to emphasize that the sample size n is equal to one in all cases. Again, this is because the confidence interval reflects the analytical method precision, which does not depend on the number of specimens tested. In other words, n represents the number of method validation experiments performed, as mentioned earlier.

Table 3: Results

The numerical results are shown in this section, as illustrated in Figure A5. First, the maximum allowable overall error of the analytical method is given in terms of the maximum relative standard deviation $RSD_{E,\max}$, which is computed by

$$RSD_{E,\max} = \frac{\hat{\sigma}_{E,\max}}{\bar{x}} \cdot 100.$$

The second output is the modified process performance index P_{pk} , which is calculated from (10).

As mentioned previously, any method with $P_{pk} > 1.0$ is an acceptable method. Also, if the experimental value (that is, the input value) of RSD_E is smaller than the maximum allowable RSD (that is, the output $RSD_{E,\max}$), then the analytical method is precise enough.

| Table 3. Results | |
|--|--------|
| Maximum allowable RSD ($RSD_{E,\max}$) | 5.10 % |
| Modified process performance index (P_{pk}) | 1.28 |
| <p>The actual RSD does not exceed the allowable RSD.</p> <p>Modified process performance index ≥ 1.</p> <p>The method precision is sufficient.</p> | |

Figure A5. Results section screen shot.

Figure 1: Graphical Outputs

This figure illustrates the relationship between the confidence interval for the target mean and the product specification range. A screen shot of this graph is shown in Figure A6. In order for the method to be precise enough, the confidence interval must lie completely within in the specification range. If either side of the confidence interval lies outside the specification range, then the method is not precise enough.

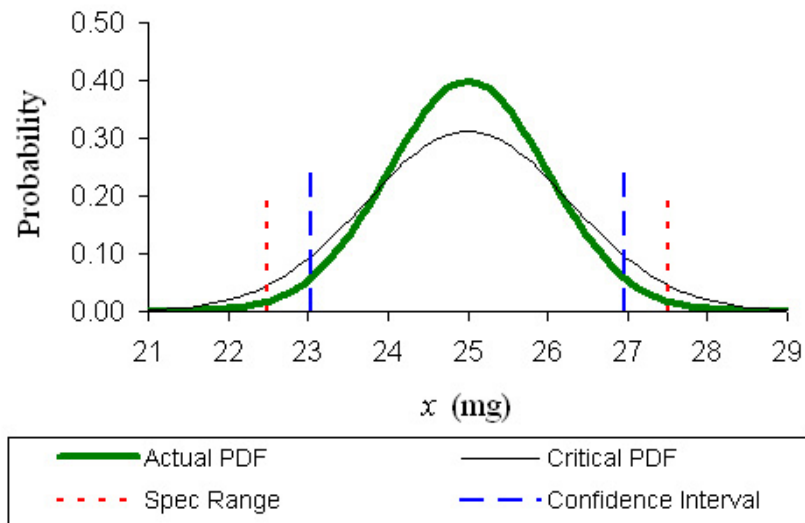


Figure A6. Screen shot of Figure 1 from the application.

If the input values for the target mean and sample mean are modified from the default value of 25.0, the horizontal axis of this graph will not be adjusted automatically. The standard Excel “auto-scale” feature is not used, because when it is enabled the graph is displayed using a minimum of zero on the horizontal axis. This is undesirable, as it makes the graph more difficult to interpret. To easily adjust the axis in a manner that is better suited for this application, the button labeled “Adjust Graph Horizontal Axis” was created. This button runs a macro which automatically modifies the axis settings depending on the target mean input value. This macro is designed to intelligently compute horizontal axis settings for target mean input values ranging from approximately 5 to 1000. For example, if the target mean and sample mean are changed to 50.0, the graph of the PDFs is not visible, but after clicking the Adjust Graph Horizontal Axis button, the graph is redrawn as shown in Figure A7. If desired, the user can view the source code of this macro by pressing Alt+F11 while the `Pfizer_Model.xls` file is open.

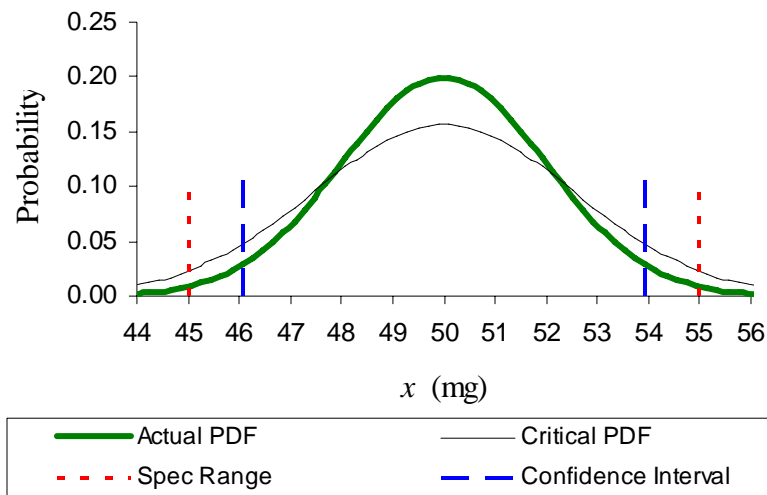


Figure A7. Screen shot of Figure 1 after the Adjust Graph Horizontal Axis button is used.