

Validation Viewpoint

Accuracy is one of several data elements required for assay validation. In a regulated pharmaceutical laboratory, determining accuracy can take on many different forms. This month's installment of "Validation Viewpoint" focuses on ways to measure and document accuracy for typical pharmaceutical formulations, including synthetic drugs and bioanalytical samples.

**Michael Swartz and
Ira Krull**
Validation Viewpoint Editors

Analytical Method Validation: Accuracy in Quantitation

In past "Validation Viewpoint" columns and other publications, we have discussed different methods of quantitation as a distinct and decided part of method validation (1–4). And while many factors are taken into account when validating a method, the determination of the method's accuracy is an integral part of validating any quantitative method.

Accuracy is defined as the closeness of the test results obtained by the method of interest to the true value, demonstrated across the range of analyte levels expected to be found in samples during routine analyses (5). The determination of accuracy varies according to the method's intended purpose, and whether the sample is a drug substance, the final drug product, or related impurities. In general, accuracy is determined for drug substances by comparison with standard reference materials; for drug products by the analysis of synthetic mixtures spiked with known quantities of components; and for impurities, it is determined by the analysis of samples (either drug substance or drug product) spiked with known amounts of impurities. For drug substances and drug products, accuracy also can be inferred in some instances once precision, linearity, and specificity have been established.

In most cases, accuracy requires a priori knowledge of the level of analyte present in simulated drug products, or in synthetic mixtures spiked with known amounts or quantities of the components of interest (analytes). When standards are available, it is sufficient to demonstrate that results are unaffected by using spiked samples. International Conference on Harmonization (ICH) documents suggest a minimum of nine determinations over a minimum of three concentration levels covering the specified range (for example, three concen-

trations with three replicates each) (6).

However, in situations in which the actual level is not known, or if standards of the analyte of interest are not available, then accuracy can be determined only by comparison with a second validated method, if one exists.

Measuring and Documenting Accuracy

The United States Pharmacopeia (USP) and the ICH guidelines categorize methods and require different validation schemes for each category (5,6). Depending upon the nature of the specific method, accuracy is a required data element in all but one category: identification. Accuracy can be measured, represented, or reported many different ways. It can be reported as percent recovery (ideally 100%, but at least reproducible), as a correlation coefficient (versus a currently accepted method of analysis for the same analyte in the same sample types or matrices), as percent relative error, or as the difference (error) between the mean value and true value, with confidence intervals.

Different ways of calculating and reporting accuracy are illustrated by example in Figure 1. To determine the correlation coefficient r , the agreement between the amount found in method 1 (perhaps a new method) is compared with that found in method 2 (an accepted or validated method whose accuracy has already been demonstrated). If the newer method shows the same accuracy as the currently accepted method, that is the correlation coefficient r , approaches 1.00, the new method of analysis is exhibiting a high degree of accuracy for those specific samples. The individual values and the averages for each of the methods are used to calculate the correlation coefficient, as shown in Figure 1a.

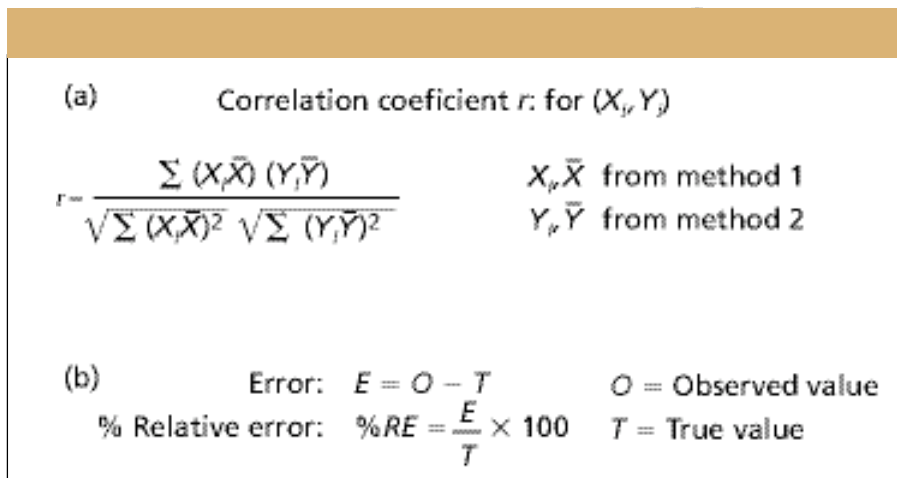


Figure 1: Determining (a) the correlation coefficient and (b) the percent relative error.

Therefore, when using the correlation coefficient approach, it is not 100% necessary to have samples of known concentration, as only the results of two different methods are being compared.

Error and percent relative error (%RE), illustrated in Figure 1b, are yet another approach to demonstrating accuracy. Relative error is determined by knowing the true value (T) and then determining the

observed value (O). The ratio of E/T multiplied by 100 yields %RE. When the error is small, the analytical method is at its most accurate. Therefore, for the highest accuracy possible, both the error and the percent relative error would approach zero in an ideal situation.

In Table I, a set of typical results is summarized using different target concentrations of the final drug product formulation.

These results illustrate yet another way of measuring or reporting accuracy by percent recovery. The spiked concentration (milligrams per milliliter) and the measured or determined concentration (milligrams per milliliter) are used to calculate percent recovery (% recovery = measured concentration/spiked concentration \times 100). To generate the data in Table I, five different sample matrices (placebos) were spiked at different concentrations, homogenized, and then analyzed individually in triplicate ($n = 3$). In the Table I example, results show the percent recoveries range from 98.0% to 103%, with an average recovery of 99.52%, a standard deviation of 1.99, and a relative standard deviation of 2.00. These results are an indication of good accuracy for the final method being validated. Note that to derive this accuracy data, the exact (precise) amount of spiked concentrations (milligrams per milliliter) for the various samples assayed must be known. Known concentrations are the ideal approach to demonstrate accuracy in quantitation, assuming, of course, that the scientist can indeed obtain a true placebo of the final sample composition and at the correct

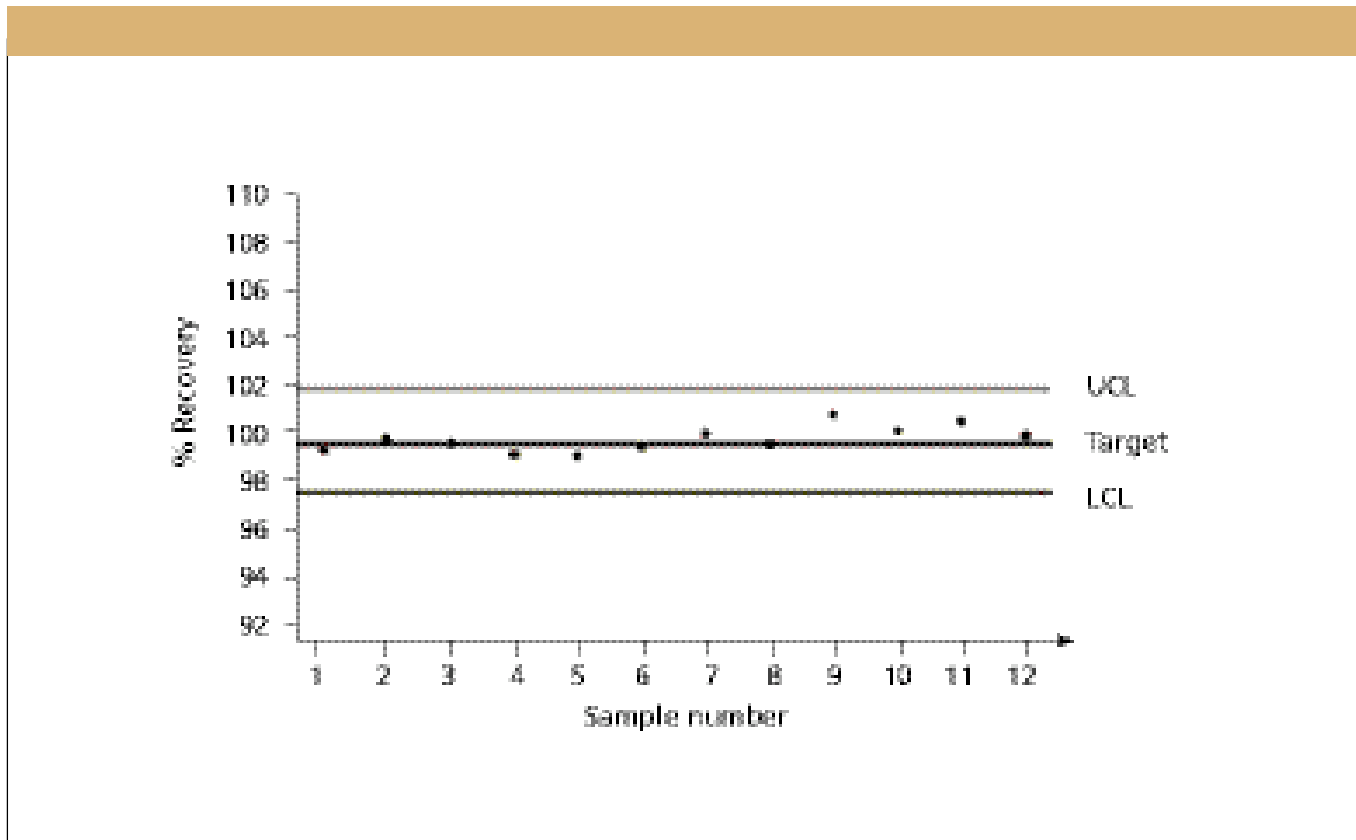


Figure 2: Control chart showing the upper control limit and lower control limit versus the mean, or target value, representing the accuracy of the method.

Table I: Example accuracy data and calculations

Target Concentration	Spiked Concentration (mg/mL)	Measured Concentration (mg/mL) Avg	Percentage (%)
50%	0.50	0.49	98.0
75%	0.75	0.74	98.7
100%	1.00	1.03	103.0
125%	1.25	1.24	99.2
150%	1.50	1.48	98.7
Average recovery:	99.52%		
Standard deviation:	1.99		
Relative standard deviation	2.00		

levels or amounts to be found in real samples.

The examples in Figure 1 and Table I illustrate common approaches to determine accuracy for drug substances, drug products, and impurities.

Determining the True Value

One of the major underlying issues encountered in determining accuracy is a general lack of knowledge of the true value of analyte or impurities in a given sample. In its simplest fashion, accuracy requires knowledge of the true value or spiked concentration in contrived, quality control samples. The best way to prepare a quality control sample is to take a true placebo and spike it with known amounts of a 100% pure standard of the analyte of interest, as was done to generate the data shown in Table I.

Once we know what is present in a real sample, regardless of origin (tablets, biological matrices, pond water, and so forth), all that is needed is to prepare a placebo to mimic the sample without adding the analyte of interest. Or, we can remove the analyte of interest from a real sample, demonstrate it is absent, and then take what remains as the placebo for further analyses.

We also could determine the true value by using a second validated method of analysis on a real sample or samples, and then use that as the true value to determine accuracy using the equation for error or %RE in Figure 1.

For bioanalytical methods (methods that are used to quantify drugs and their metabolites in a biological matrix, such as blood, plasma, serum, or urine), once validated for routine use, system suitability, and quality control (QC) samples also are used to monitor the system and ongoing accuracy and precision, and to accept or reject results. QC samples are prepared sep-

arately and analyzed with true unknowns at intervals according to the number of unknown samples to be analyzed (7,8).

Control charts are a common way to monitor ongoing results, and an example is presented in Figure 2. Method specifications dictate upper and lower control limits (UCL and LCL, respectively) established about the mean, based upon statistically valid confidence intervals. Modern chromatographic data systems are capable of plotting data in this format automatically.

In spiking experiments and the preparation of QC samples, reference standards — or standard reference materials (SRMs) — must be used. A reference standard is a highly purified compound that is well characterized. Because chromatographic methods rely heavily upon reference standards to provide accurate data, the quality and purity of the reference standard should be well documented. There are two categories of reference standards: USP reference standards that do not need characterization and noncompendial standards. Noncompendial standards need to be of the highest purity that can be obtained by reasonable effort and should be thoroughly characterized to assure identity, strength, quality, and purity. Reference standards are not always available for every analyte of interest. According to the USP: “. . . in the absence of other information, it may be necessary to calculate the amount of an impurity based on comparison of its response to that of the drug substance; the ratio of responses of equal amounts of the impurity and the drug substance (response factor) should be used if known” (5). In other words, it is perfectly acceptable to quantitate an impurity against the drug substance if a suitable impurity reference standard is not available. However, it is strongly recommended that authentic standards are either synthesized or isolated for all analytes of interest.

There are, in some instances, SRMs of a specific analyte in a known sample matrix, such as mercury in tuna fish or insulin in a biofluid. These types of SRMs often are available commercially from various vendors or the government (National Institute of Standards and Technology, NIST, Washington, DC). Such SRMs come with a Certificate of Analysis (COA), guaranteeing that the reference material contains a guaranteed amount or level of analyte in a known sample matrix. Because the true value is provided on the COA, it is then only a matter of deriving the error or %RE for the new method of analysis being compared with the COA. However, it is not unusual to find that the exact SRMs to meet all expected needs are not always available. Sometimes SRMs must be prepared in-house or through an outside vendor or contractor. Services are available wherein an outside firm will provide a new SRM for the specific analyte of interest in the required sample matrix, though it is usually more costly than readily available SRMs.

What Happens if Two Methods Do Not Agree?

In general, “accepted methods” (for example, USP methods) are considered to be validated. However, some older methods might not have been validated according to today’s standards, or for the current intended purpose. It is common to run two methods and get results that do not agree to within experimental error. In this instance, the currently accepted method of analysis has to be shown to be invalid for one reason or another. Perhaps the sample matrix or the excipients are different or the specificity of the method is in question. In a regulated environment, regulators will use the accepted or validated method (if one exists) unless it is proven to be inappropriate. In this instance, the burden of proof is not on the regulators. Only by using placebo samples or SRMs at various analyte levels to show that the currently accepted method does not yield the true value as its observed value, can proof be certain. Because time always is of the essence, it is generally a good idea to use an officially sanctioned, accepted, or validated method whenever possible. This situation is particularly significant when utilizing the correlation coefficient method of demonstrating accuracy. It is important to be 100% certain that the currently accepted method is

indeed accurate for those very samples of current interest. It is also important to be certain that the accepted method was already fully validated, especially its quantitative aspects, according to current standards. Of course, this situation is avoided when using SRMs of known composition.

Conclusion

Accuracy is an essential part of overall method validation. One must recognize

that if a method has been applied to a given number of samples, even with good precision, without knowing the true value of analyte levels or using a direct comparison with at least one currently accepted, validated method, it is impossible to demonstrate true accuracy. And without proving accuracy, quantitative results cannot be obtained, and the method cannot be truly validated.

References

- (1) I.S. Krull and M.E. Swartz, *Am. Lab.* **6** (February, 2004).
- (2) I.S. Krull and M.E. Swartz, *LCGC* **16**(10), 922 (1998).
- (3) I.S. Krull and M.E. Swartz, *LCGC* **16**(12), 1084 (1998).
- (4) A.S. Rathore, R.R. Kurumbail, A.M. Lasdun, M.E. Swartz, and I.S. Krull, *LCGC* **20**(11), 1042 (2002).
- (5) The United States Pharmacopeia 25/National Formulary 20, Chapter 1225 (The United States Pharmacopeial Convention, Inc., Rockville, Maryland, 2002), pp. 2256–2259.
- (6) ICH Q2B: Validation of Analytical Procedures: Methodology (International Conference on Harmonization of Technical Requirements for the Registration of Drugs For Human Use, Geneva, Switzerland, May 1997), www.ich.org.
- (7) M.E. Swartz and I.S. Krull, *LCGC* **21**(2), 136 (2003).
- (8) Guidance for Industry, Bioanalytical Method Validation, U.S. Department of Health and Human Services FDA, May 2001 (www.fda.gov/guidance/index.htm).

Michael E. Swartz

"Validation Viewpoint" Co-Editor
Michael E. Swartz is a Principal Scientist at Waters Corp., Milford, Massachusetts, and a member of LCGC's editorial advisory board.



Ira S. Krull

"Validation Viewpoint" Co-Editor
Ira S. Krull is an Associate Professor of chemistry at Northeastern University, Boston, Massachusetts, and a member of LCGC's editorial advisory board.



The columnists regret that time constraints prevent them from responding to individual reader queries. However, readers are welcome to submit specific questions and problems, which the columnists may address in future columns. Direct correspondence about this column to "Validation Viewpoint," LCGC, Woodbridge Corporate Plaza, 485 Route 1 South, Building F, First Floor, Iselin, NJ 08830, e-mail lcgcedit@lcgc-mag.com.

ADVANS
COMMUNICATIONS

For Client Review Only. All Rights Reserved. Advanstar Communications Inc. 2005

ADVANS
COMMUNICATIONS

For Client Review Only. All Rights Reserved. Advanstar Communications Inc. 2005