thoroughly characterized to ascertain their identity, strength, quality, and purity. Testing requirements for the reference standards are more rigorous as compared to bulk drug substance. The purity correction factors for non-USP standards should be included in any method calculations.

Quantitation of actives in chromatographic methods is based on either external or internal standards. An external standard method is used when the standard is analyzed on a separate chromatogram from the sample. Quantitation in this case will be by comparison of the sample and reference standard responses, i.e., peak area or peak heights for HPLC and GC or spot intensity in TLC for a given analyte of interest. External standard methods are generally used for samples with a single target concentration and narrow concentration ranges (acceptance and release tests). Simple sample preparation procedures or longer run times for detection of extraneous peaks, e.g., impurity test, HPLC methods for stability, and TLC methods also use external standards. For internal standard methods, a compound of known purity is added directly to the sample. However, it must be ensured that the compound being used as an internal standard does not interfere with any analyte of interest or degradation products in the sample. The response ratio between internal standard and analyte of interest in the sample is compared to the ratio of the internal standard and the analyte in the standard that is used for quantitation purposes. Internal standard methods are widely used for quantitation in biological samples and for low and wide sample concentration ranges, e.g., in pharmacokinetics studies.

There are some basic points that should be addressed in the test method.

1. The sample and the standard should be prepared in the mobile phase. If this is not possible, then the level of organic solvent used in the preparation of the sample and the standard must be lower than that present in the mobile phase.
2. The sample and standard concentrations should be close to each other.
3. Sample preparations often require filtration prior to injection onto the system. Filtration removes particulate matter that may clog the column. However, analyte adsorption on the filter can take place. This adsorption effect is important for low-level impurities. Therefore, data to validate this aspect will be required.

In conclusion, method validation is a dynamic process and should not be considered a one-time situation. The design and validation of the method should be such that they ensure its ruggedness or robustness throughout the life of the method. The accuracy of the data is affected by variations in the manufacturing process, the preparation of samples in the laboratory, and the instrument performance. With a well-designed validation and tight chromatographic system suitability criteria, the reliability of the data can be significantly improved. Variations, except from the drug product manufacturing process, can and should be minimized. Good, reliable validated methods will generate data that is trustworthy.

REFERENCES

1. FDA. Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics, February 1987. Center for Drugs and Biologics, FDA, Department of Health and Human Services.