STANDARD OPERATING PROCEDURE

Title: Method Validation

Effective Date: _____

Approvals (Signature and Date):

_____	_____	_____
Responsible Department Head	Technical Authority	QA/QC

1. PURPOSE

1.1 To establish standardized guidelines for designing and implementing a method validation protocol.

2. SCOPE

2.1 This procedure applies to the validation of all analytical methods used by the Quality Assurance/ Quality Control Department for lot release and stability testing. This SOP provides suggested guidelines for the design and implementation of method validation. Methods to be used by QA/QC for lot release and stability testing will be validated by the Analytical Methods Development group in R&D.

3. RESPONSIBILITY

3.1 It is the responsibility of the Analytical Methods Development group, or an appropriate technical authority, to draft written protocols for method validation.

3.2 Personnel in the Analytical Method Development group, or a group/technical authority so designated, are responsible for the implementation of method validation.

3.3 The Analytical Method Development Scientist, or a technical staff member reporting to the Analytical Method Development Scientist, will be responsible for writing the validation summary report.

3.4 Technical staff of the QC department will be responsible for assisting in specific areas of the method validation as defined by protocols associated with the validation.

3.5 The Analytical Method Development Scientist, working in conjunction with the QC manager and technical staff, is responsible for complete transfer of the method to QA/QC once validation is complete.

3.6 Any method validation performed on microbiological methods is the responsibility of QC Microbiology personnel.

4. REFERENCES

4.1 Analytical Chemistry of Therapeutic Proteins, Riggin, R, and Boddy, R., in Analytical Biotechnology - Capillary Electrophoresis and Chromatography, Horvath and Nikelly eds., American Chemical Society, Wsh. DC, 1990.

4.2 Validation of Analytical Methods by FDA Laboratories, Guerra, J., Pharmaceutical Technology, 1986.

4.3 *Submitting HPLC Methods to the Compendia and Regulatory Agencies,* Debesis, E. et. al., Pharmaceutical Technology, September 1982.

4.4 *Methods Validation, Instrument Calibration, and Inspectional Observations*, Bunch, E., and Johnson, R., paper presented at ASQC/FDA Laboratory CGMP II Seminar, March 19, 1993.

4.5 *Development, Validation, and Proper Use of HPLC Methods in the Drug Analytical Laboratory*, Bunch, E., paper presented at ASQC/FDA Laboratory CGMP II Seminar, March 19, 1993.

4.6 12-0015-SOP-1.0, QVD Protocol and Report Preparation

5. MATERIALS AND EQUIPMENT

5.1 A qualified reference or control lot of analyte is required to perform the tests in the validation procedure. The general approach to validating a method is to analyze reference standard material which is similar in all respects to test samples.

5.2 QVD protocol and report templates are available in the MS Word template directory at server path X:\WORD60\TEMPLATES as files "QVD-PROT.DOT" and "QVD-RPRT.DOT," respectively. New MS Word documents may be created from these templates by selecting the **File**, **New...** menu commands and then selecting either "QVD-PROT" or "QVD-RPRT" from the "New" dialog box.

6. HEALTH AND SAFETY CONSIDERATIONS

6.1 None

7. DOCUMENTATION REQUIREMENTS

7.1 Standard Operating Procedures describing the method to be validated.

7.2 A method validation protocol describing the procedure to be followed in validating that particular method. The protocol is assigned a QVD number by document control and is approved by appropriate department heads. Protocols should be written as described in 12-0015-SOP-1.0.

7.3 During the validation, all raw data is placed in a validation file or binder labeled with the QVD number of the validation. Upon completion of the validation, all raw data is attached to the summary report described in section 7.4 of this document.

7.4 A validation summary report containing the documentation from all tests is assembled upon completion of the validation. This summary report will describe the results of the validation and will state whether the method has been deemed acceptable for use as a QC assay. The summary report is assigned the same QVD number as in 7.2 and must also be approved by appropriate department heads. Summary reports should be written as described in 12-0015-SOP-1.0.

8. PROCEDURAL PRINCIPLES AND INTRODUCTION

8.1 The validation of an analytical method is the process of confirming, by thorough documented laboratory studies, that the performance characteristics meet the requirements for the intended application of the method. The validation process must provide documented evidence that the method is operating consistently and accurately, is sufficiently sensitive for its intended application, and is measuring what it is intended to measure. Analytical method validation generally concentrates on certain key performance parameters. These performance parameters are, to a large extent, common to all analytical methods. The approach to follow in adequately validating an analytical method is to perform a series of assays designed to examine each of these performance parameters. A brief description of each of the common performance parameters is provided below.

8.1.1 **Precision** is the degree of reproducibility of the method on multiple samplings of a test sample. It represents the distribution of individual test results around the mean and is generally expressed as the standard deviation or the coefficient of variation (%cv). Precision may refer to method precision (in which analyte is carried through the entire procedure, from sample preparation through measurement) or to system precision (which measures the precision of only a part of the method, e.g., precision of spectrophotometer readings on a single standard sample). Among those factors contributing to precision errors are reagents, analyst technique, and sample preparation. When determining the precision of a method or system, at least six replicate measurements on a standard reference sample are required to obtain statistically significant results. The variability of a method should also be examined on different days and by different operators, although this parameter is more appropriately included under the category of assay ruggedness.

8.1.2 **Accuracy** refers to the closeness of a measured value to the true or accepted value. It is a measure of the exactness of the analytical method. The accuracy of an analytical method is commonly assessed by performing spike/recovery experiments or by comparing the results of the method in question to the results obtained from a different standard method. In the spike/recovery method, a known amount of pure standard analyte is added to a sample mixture and the sample is then assayed. The difference between the spiked sample and the original sample is a measure of the amount of standard recovered by the assay. Accuracy can then be expressed in terms of the recovery of standard added to the sample. In the comparison method, if assaying for protein concentration, for example, the results obtained from optical density measurements at 280nm could be compared to the results obtained on the same sample from amino acid analysis, a commonly accepted standard assay procedure.

8.1.3 **Selectivity** or **Specificity** is the ability of the assay to accurately and precisely measure analyte in the presence of components which may interfere with such measurements. This represents the degree of interference by sample impurities, breakdown products, precursors or product excipients which may give a detectable signal in the assay. In a chromatographic assay, for example, potential impurities and degradation products must be demonstrated to be resolved from the analyte peak under the conditions of analysis and excipients must not interfere with the analysis of the analyte. One way to examine the specificity of the method is to perform accelerated aging studies in which a sample undergoes harsh treatment designed to force the appearance of degradation products. These degradation products should not interfere in the detection of the analyte. This is especially important if an assay is intended to be stability indicating. In this case, an accelerated aging study should be included in the specificity test.

8.1.4 **Limit of Detection** refers to the lowest level of analyte which can be detected in a sample but not necessarily quantitated. This value is commonly accepted to be twice the level of background or twice the signal to noise ratio. It may not be appropriate to examine this parameter for all assays. For example, if an assay is meant to measure the final product, limit of detection may not be of concern. If, however, an assay is intended to measure impurity levels, limit of detection will be of primary concern.

8.1.5 **Limit of Quantitation** is the lowest concentration of analyte that can be detected with acceptable precision and accuracy. This is usually taken as 10 times the signal to noise ratio. The limit of quantitation is also defined as the level of analyte below which the %cv of replicate measurements exceeds 10%. This parameter is of importance in any assay designed to quantitate the level of product in bulk or finished form.