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THE VALIDATION OF ANALYTICAL PROCEDURES USED IN THE  
EXAMINATION OF PHARMACEUTICAL MATERIALS

Comments or observations on this draft document are kindly invited and should be forwarded to WHO, Pharmaceuticals, attention: Dr A. Mechkovski, 1211 Geneva 27, Switzerland, before 15 August 1989.

1. What is Analytical Procedures Validation?

The continued efficacy and safety of a medicinal product can often only be assured by analytical monitoring of its quality. The identity, purity, strength and overall quality of a medicine must therefore be capable of assessment throughout its storage, distribution and use. This objective can only be achieved if pertinent specifications are applied based on validated procedures that will demonstrate the relationship in quality between the material under examination and that initially subjected to toxicological and pharmacological evaluation. That is, sound quality control depends on the use of valid analytical procedures. For this reason it is important that any analytical procedure proposed for a particular active ingredient or dosage form should be systematically evaluated so as to demonstrate that it is scientifically sound under the conditions in which it is to be applied. This process of evaluation is known as analytical validation.

The principal purpose of analytical validation is to ensure that the procedure under consideration is capable of objective application to give reproducible and reliable results that are adequate for the intended purpose of the procedure. To achieve this objective it will be necessary to properly define the conditions under which the procedure is to be used and to indicate the purpose for which it is intended.

Validation is necessary whether the procedure is intended to be applied within a manufacturing company, within an official control laboratory or whether it is intended to be included in a pharmacopoeia.

The considerations set out in the present paper are confined to a consideration of procedures based on chemical and physio-chemical attributes. Many of the points made will be equally applicable to microbiological and biological procedures but they are not primarily intended for that purpose.

2. Presentation of data for either registration or pharmacopoeial purposes

It is recommended that any data presented in support of a specification proposed for a particular active ingredient or dosage form should be provided under three main headings:

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- (a) A section devoted to justification of the proposed test procedure in comparison with other possible approaches. Where an unusual procedure is to be proposed this section should also discuss the scientific basis of the procedure. Where the procedure is being proposed to replace an existing one comparative data should be applied.
- (b) A section providing a description of the procedure giving as much detail as is deemed necessary to allow properly trained workers to carry it out in a reliable manner. This section should also define (either in detail or by reference to readily-available published texts) the reagents required and should give details concerning the availability of any reference substances required. Where calculation of results is based on the application of well-established principles of analytical chemistry it should not be necessary to provide formulae for calculation of results. Where, however, the methodology is complex a full formula for calculation of results, with all terms defined, should be included.
- (c) A section providing the validation data (see below). Each analytical performance characteristic that is applicable to the particular procedure defined (see Section 4) should be discussed and supported by experimental data. Where data presented for registration purposes relies on established pharmacopoeial methodology, the need for supporting validation data may be considerably reduced on the assumption that the pharmacopoeial procedures will have been properly validated before being included in the pharmacopoeia. However, evidence that the pharmacopoeial procedure is applicable to the material under test may well be required especially for dosage forms.

### 3. Analytical characteristics that may be applicable

Not all characteristics listed below will be applicable to every test procedure or to every particular material. Much will depend on the purpose for which the procedure is required. This aspect of validation is discussed in Section 4 below.

The characteristics that may be required are listed and defined below, each with an indication as to how they may be determined.

- (a) Accuracy of the procedure. The accuracy of a procedure relates to the closeness of results obtained by the procedure to the true value. It is sometimes possible to obtain an indication of the accuracy of the procedure by determining the percentage recovery by the assay of known added amounts of the material to be analysed (the analyte).  
The accuracy of a procedure may be determined by applying the procedure to samples of the material to be examined that have been prepared with quantitative accuracy. The carefully prepared samples so examined should include materials to which the analyte has been added in quantities both above and below the expected range of values. Samples with quantities of the analyte that are 10 % above or below the expected range of claims are usually suitable.
- (b) Precision of the procedure. The precision of a procedure relates to the degree of agreement among individual test results. It is measured by the scatter of individual results from the mean and usually expressed as the Standard Deviation or as the Coefficient of Variation (Relative Standard Deviation) when the complete procedure is applied repeatedly to separate samples drawn from the same homogeneous batch of material.

**Repeatability.** (within laboratory variation) Precision of the procedure when repeated by same analyst under some set of conditions (same reagents, equipment, settings, same laboratory, etc.) - only difference is sample. The repeatability of a procedure is assessed by carrying out complete separate determinations on separate samples of the same homogeneous batch of material and thus provides a measure of the precision of the procedure under normal operating conditions.

**Reproducibility.** (between laboratory variation) Precision of the procedure when carried out by different analyst under slightly different conditions as different reagents, equipment, settings, usually in different laboratory.

**Robustness or Ruggedness.** This may be defined as the ability of the procedure to provide analytical results of acceptable accuracy and precision under changed conditions of test or environment. Robustness thus takes into account the variations that may be expected when the same defined procedure of analysis is employed in different laboratories, using different personnel, different reagents and different equipment. It is a measure of precision of the procedure under various sets of normal operational conditions. The robustness of a procedure may be assessed by obtaining analytical results on portions of the same homogeneous sample in different laboratories, using different analysts and using operational and environmental conditions that may differ yet are within the specified conditions laid down for the procedure.

- (c) **Linearity and range of the procedure.** The linearity of an analytical procedure is its ability to produce results that are directly or indirectly proportional to the concentration of analyte in the samples. The range of the procedure is an expression of the lowest and highest levels of analyte that have been demonstrated to be determinable with acceptable precision, accuracy, and linearity. These characteristics are determined by application of the procedure to a series of samples having analyte concentrations spanning the claimed range of the procedure. When the relationship between response and concentration is not linear, standardization may be provided by means of a calibration curve.
- (d) **Selectivity or Specificity.** The selectivity of a procedure is its ability to measure the analyte in a manner that is free from interference from other components that may be expected to be in the sample being examined (for example, impurities arising from manufacture or degradation or from the presence of other ingredients than the analyte, whether pharmaceutically active or inert. Selectivity (or lack of it) may be expressed in terms of bias of assay results obtained when the procedure is applied to the analyte in the presence of expected levels of other ingredients compared to results obtained on the same analyte without added substances. When other ingredients are all known and available selectivity may be determined by comparing test results obtained on the analyte with and without the addition of the potentially interfering materials. When such ingredients are either unidentified or unavailable a measure of selectivity can often be obtained by determining the recovery of standard addition of pure analyte to a material containing a constant level of the other ingredients.
- (e) **Sensitivity.** This is a general term sometimes used for the limit of detection or the limit of quantitation (see below).

- (f) **Limit of detection.** This may be defined as the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, under the stated experimental conditions. Such a limit is usually expressed in terms of the lowest concentration of analyte, for example as a percentage, or as parts per million, in the sample.

The determination of limit of detection will depend on the procedure of analysis to be used. Where the final measurement is based on an instrumental procedure due account will need to be taken of the signal to noise characteristics of the responses observed.

- (g) **Limit of quantitation.** This may be defined as the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the stated procedure is applied. The limit may be expressed in similar terms to those recommended for limit of detection (see 3.e above).

The limit of quantitation may be determined by analysis of samples containing diminishing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision are attainable. Where the final assessment is based on an instrumental procedure the magnitude of analytical background response may need to be assessed and taken into account. The limit of quantitation in many cases is approximately two times more than the limit of detection.

#### 4. What analytical characteristics are applicable in particular cases?

It is clear that not all of the various characteristics referred to in Section 3 will need to be considered in all cases. The particular characteristics applicable in specific instances will need to be considered on a case by case basis. As a guide, however, the following generalisations may assist.

Methods used for examination of pharmaceutical materials may be broadly classified as follows:

**Class A:**

Tests designed to establish identity, whether of bulk drug substances or to establish the presence of a particular ingredient in a finished dosage form.

**Class B:**

Methods designed to detect and quantitate impurities in a bulk drug substance or finished dosage form.

**Class C:**

Methods used to quantitatively assess a bulk drug substance or concentration of ingredient in a finished dosage form.

**Class D:**

Methods used to assess characteristics of finished dosage forms, such as dissolution profiles, content uniformity, etc.

The table given below offers guidelines as to which characteristics should be discussed in each case.

Table. Characteristics applicable to the various classes  
as defined above

	Class A	Class B	Class C	Class D
(a) Accuracy		X	X	X
(b) Precision		X	X	X
(c) Linearity and range		X	X	X
(d) Selectivity	X	X	X	
(e) Limit of detection	X	X		
(f) Limit of quantitation		X		
(g) Robustness	X	X	X	X

Notwithstanding the above generalisations there will clearly be occasions when certain characteristics marked as not being required above may be necessary. In addition, the purpose for which the submission is being made may have a bearing on the choice of characteristics and the extent to which they are invoked. For example, although Classes B, C and D are all referred to above as requiring consideration of precision, the stringency with which the consideration is applied may be different. For estimation of an impurity it may not be necessary to be as precise as for quantitative assessment of a bulk drug substance. By the same token a degree of bias may be acceptable in determination of accuracy for a uniformity of content test (Class D) that would not be permissible for quantitative assessment of concentration of ingredient in a finished dosage form (Class C).

Similarly a test designed to establish the identity of a new drug entity for which no previous data has been lodged may need to be considerably more searching than tests designed to verify the identity of a long-established drug substance to be included in a pharmacopoeia.

A different emphasis may be required for pharmacopoeial as opposed to registration purposes. For example, robustness is a critical characteristic for pharmacopoeial methodology but may be less significant for a manufacturer's release specification.

##### 5. Relevance for the International Pharmacopoeia

It is suggested that the general approach described in this document should be taken into consideration when new monographs, particularly those for dosage forms, are to be included in the International Pharmacopoeia. Full application of the validation process may not be necessary when the procedures to be used are based on similar approaches in other compendia that have been proven by long usage.

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