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Draft document for comment

World Health Organization

GUIDELINES ON VALIDATION – APPENDIX 4
ANALYTICAL METHOD VALIDATION
(June 2016)

DRAFT FOR COMMENTS

Should you have any comments on the attached text, please send these to Dr S. Kopp, Group Lead, Medicines Quality Assurance, Technologies, Standards and Norms (kopps@who.int) with a copy to Ms Marie Gaspard (gaspardm@who.int) by **30 July 2016**.

Medicines Quality Assurance working documents will be sent out electronically only and will also be placed on the Medicines website for comment under "Current projects". If you do not already receive our draft working documents please let us have your email address (to bonnyw@who.int) and we will add it to our electronic mailing list.

SCHEDULE FOR THE PROPOSED ADOPTION PROCESS OF DOCUMENT QAS/16.671:

GUIDELINES ON VALIDATION – APPENDIX 4
ANALYTICAL METHOD VALIDATION

Discussion of proposed need for revision in view of the current trends in validation during informal consultation on data management, bioequivalence, GMP and medicines' inspection	29 June–1 July 2015
Preparation of draft proposal for revision of the main text and several appendices by specialists in collaboration with the Medicines Quality Assurance Group and Prequalification Team (PQT)-Inspections, based on the feedback received during the meeting and from PQT-Inspections, draft proposals developed on the various topics by specialists, as identified in the individual working documents.	July 2015–April 2016
Presentation of the progress made to the fiftieth meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations	12–16 October 2015
Discussion at the informal consultation on good practices for health products manufacture and inspection, Geneva	4–6 April 2016
Preparation of revised text by Ms S. Croft, member of the PQT-Inspections Team, and review by Dr A.J. van Zyl, participant at the above-mentioned consultation, based on the feedback received during the informal consultation by the meeting participants and members of PQT-Inspections	May 2016
Circulation of revised working document for public consultation	June 2016
Consolidation of comments received and review of feedback	August–September 2016
Presentation to the fifty-first meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations	17–21 October 2016
Any other follow-up action as required	…

Background information

The need for revision of the published *Supplementary guidelines on good manufacturing practices: validation (1)* was identified by the Prequalification of Medicines Programme and a draft document was circulated for comment in early 2013. The focus of the revision was the Appendix on non-sterile process validation (Appendix 7), which had been revised and was adopted by the Committee at its forty-ninth meeting in October 2014.

The main text was sent out for consultation as *Working document QAS/15.639* entitled *"Guidelines on Validation*" which constitute the general principles of the new guidance on validation.

The draft on the specific topics, the appendices to this main text, will follow. One of them, i.e. e ***Analytical method validation,*** *constitutes this working document.*

The following is an overview on the appendices that are intended to complement the general text on validation:

Appendix 1
Validation of heating, ventilation and air-conditioning systems
→ will be replaced by cross-reference to WHO Guidelines on GMP for HVAC systems for considerations in qualification of HVAC systems
(update - working document QAS/15.639/Rev.1)

Appendix 2
Validation of water systems for pharmaceutical use
→ will be replaced by cross-reference to *WHO Guidelines on water for pharmaceutical use for consideration in qualification of water purification systems*

Appendix 3
Cleaning validation – consensus to retain

Appendix 4
Analytical method validation – updated text proposed in this working document

Appendix 5
Validation of computerized systems – (update – see working document QAS/16.667)

Appendix 6
Qualification of systems and equipment – update in process

Appendix 7
Non-sterile process validation – update already published as Annex 3, WHO Technical Report Series, No. 992, 2015

APPENDIX 4
ANALYTICAL METHOD VALIDATION

1. PRINCIPLE

1.1 This appendix presents some information on the characteristics that should be considered during validation of analytical methods. Approaches other than those specified in this appendix may be followed and may be acceptable. Manufacturers should choose the validation protocol and procedures most suitable for testing of their product.

1.2 The manufacturer should demonstrate (through validation) that the analytical procedure is suitable for its intended purpose.

1.3 Analytical methods, whether or not they indicate stability, should be validated.

1.4 The analytical method should be validated by research and development before being transferred to the quality control unit when appropriate.

1.5 The recommendations as provided for in good laboratory practices and guidelines for transfer of technology should be considered, where applicable, when analytical method validation is organized and planned.

2. GENERAL

2.1 There should be specifications for both materials and products. The tests to be performed should be described in the documentation on standard test methods.

2.2 Specifications and standard test methods in pharmacopoeias ("pharmacopoeial methods"), or suitably developed specifications or test methods ("non-pharmacopoeial methods") as approved by the national regulatory authority (NRA) may be used.

2.3 Well-characterized reference materials, with documented purity, should be used in analysis.

2.4 The most common analytical procedures include identification tests, assay of drug substances and pharmaceutical products, quantitative tests for content of impurities and limit

tests for impurities. Other analytical procedures include dissolution testing and determination of particle size.

2.5 The results of analytical procedures should be accurate, legible, contemporaneous, original, reliable and reproducible. All results should be archived for an appropriate period of time as defined by the laboratory and be in compliance with NRA requirements.

2.6 The procedure should become part of a continuous verification procedure to demonstrate that it meets the predefined criteria over the life of the procedure.

2.7 Trend analysis and risk assessment should be considered at intervals to ensure that the method is appropriate for its intended application.

2.8 Changes to methods should be managed in accordance with the authorized change control procedure. The variability of reference materials and other factors such as changes in the process for synthesis of the drug substance, changes in the composition of the finished product, changes in the analytical procedure, when analytical methods are transferred from one laboratory to another (when method transfer is not possible) or when major pieces of equipment instruments change should be considered. These should be understood, controlled and, where possible, reduced. Verification or revalidation should be considered where appropriate.

2.9 The scope of verification or degree of revalidation depend on the nature of the change(s) and the outcome of risk assessment.

2.10 There should be evidence that the analysts, who are responsible for certain tests, are appropriately qualified to perform those analyses ("analyst proficiency").

2.11 The data obtained during method validation and verification should be considered covered by good anything practices (GxP) requirements and are expected to follow the principles of good data and record management practices *(2)*. Their associated metadata are also expected to be retained and subjected to good data and record management practices.

2.12 When computerized systems are used to obtain and process data relating to method validation and verification, they should comply to the principles enunciated in Appendix 5 – Validation of computerized systems.

2.13 Adequate attention should be paid to the method of sample preparation. The description of this step should be as detailed as possible, especially if it can have a significant impact on tests results (e.g. particular attention should be paid to details such as sonication time, sonication bath temperature and mixing and to samples where demixing is known to occur).

2.14 Failures occurring during method validation, and how these were overcome, should be included in the method validation report – it is not acceptable to present only the passing results as it will give a biased imaged on the reliability of the method and on how it should be applied.

3. PHARMACOPOEIAL METHODS

3.1 When pharmacopoeial methods are used, evidence should be available to prove that such methods are suitable for routine use in the laboratory (verification).

3.2 Pharmacopoeial methods used for determination of content or impurities in pharmaceutical products should also have been demonstrated to be specific with respect to the substance under consideration (no placebo interference).

4. NON-PHARMACOPOEIAL METHODS

4.1 Non-pharmacopoeial methods should be appropriately validated.

5. METHOD VALIDATION

5.1 Validation should be performed in accordance with the validation protocol. The protocol should include procedures and acceptance criteria for all characteristics. The results should be documented in the validation report.

5.2 Justification should be provided when non-pharmacopoeial methods are used if pharmacopoeial methods are available. Justification should include data such as comparisons with the pharmacopoeial or other methods.

5.3 Standard test methods should be described in detail and should provide sufficient information to allow properly trained analysts to perform the analysis in a reliable manner. As a minimum, the description should include the chromatographic conditions (in the case of chromatographic tests), reagents needed, reference standards, the formulae for the calculation of results and system suitability tests.

6. METHOD VERIFICATION

6.1 Method verification consists of partial validation. It should be performed for already validated analytical methods under the following circumstances:

(a) when an already validated method is used on a product for the first time (e.g. in case of a change in active pharmaceutical ingredient (API) supplier, change in the method of synthesis or after reformulation of a drug product);
(b) when an already validated method is used for the first time in a laboratory (in some cases, method transfer may be preferable).

6.2 Method verification may include only the validation characteristics of relevance to the particular change. For instance, in the case of a change in API supplier, the only expected difference would be in the impurity profile or solubility of the API, and therefore, for a related substances method, there should be an appropriate verification that the method is able to detect and quantitate all potential impurities, even the late eluting ones. Specificity should be among the tests considered (see sections 9 and 10 below for more detail).

6.3 Method verification is suitable in lieu of method validation for pharmacopoeial methods.

7. METHOD REVALIDATION

7.1 Methods should be maintained in a validated state over the life of the method (see point 2.6 above). Revalidation of an analytical procedure should be considered whenever there are changes made to the method, including:

- changes to the mobile phase (please refer to *The International Pharmacopoeia* and other pharmacopoeias for the acceptance limits beyond which revalidation must be performed);
- changes to the column;
- changes to the temperature of the column;
- changes to the concentration/composition of the sample and standards;
- changes to the detector (change in detector type, e.g. if going from ultraviolet (UV)-visible detection to fluorimetry, or wavelength of detection).

7.2 In case of repeated system suitability failures or when obtaining of doubtful results. In such cases an investigation of the root cause should be performed, the appropriate changes made and the method revalidated.

7.3 Periodic revalidation of analytical methods should be considered according to a period that is scientifically justifiable.

7.4 It is acceptable for revalidation to include only the validation characteristics of relevance to the particular change and method.

8. METHOD TRANSFER

8.1 During method transfer, documented evidence should be established to prove that a method has equivalent performance when used in a laboratory different from that where it has been originally validated.

8.2 Generally, it should be performed by comparing a set of results obtained by an analyst in one laboratory to that obtained by another analyst at the laboratory to which the method is being transferred.

8.3 The two sets of results should be statistically compared and the differences between the two sets of test results should be within an acceptable range.

8.4 Method transfer should be performed before testing of samples for obtaining critical data for a dossier, such as process validation or stability studies or applied for routine use.

8.5 A predefined protocol should be followed which includes at least: a title, objective, scope, responsibilities of the sending unit (SU) and the receiving unit (RU); a specification of materials and methods; the experimental design and acceptance criteria; documentation

(including information to be supplied with the results, and report forms to be used, if any); procedure for the handling of deviations; references; and details of reference samples (starting materials, intermediates and finished products). The protocol should be authorized and dated.

8.6 In the case of independent testing by a separate entity, such as a national quality control testing laboratory that is testing samples on its market, method transfer is not always possible. It is not considered an obligation but may be considered as an optional step when encountering difficulties in applying any particular method. See *WHO guidelines on transfer of technology in pharmaceutical technology* (3) for further reference.

9. CHARACTERISTICS OF ANALYTICAL PROCEDURES

9.1 Characteristics that should be considered during validation of analytical methods include:

- specificity;
- linearity;
- range;
- accuracy;
- precision;
- detection limit;
- quantitation limit;
- robustness.

This list should be considered typical but occasional exceptions should be dealt with on a case-by-case basis

9.1.1 *Accuracy* is the degree of agreement of test results with the true value, or the closeness of the results obtained by the procedure to the true value. It is normally established on samples of the material to be examined that have been prepared to quantitative accuracy. Accuracy should be established across the specified range of the analytical procedure.

Note: It is acceptable to use a "spiked" placebo where a known quantity or concentration of a reference material is used.

9.1.2 *Precision* is the degree of agreement among individual results. The complete procedure should be applied repeatedly to separate, identical samples drawn from the same homogeneous batch of material. It should be measured by the scatter of individual results from the mean (good grouping) and expressed as the relative standard deviation (RSD).

9.1.2.1 *Repeatability* should be assessed using a minimum of nine determinations covering the specified range for the procedure, e.g. three concentrations/three replicates each, or a minimum of six determinations at 100% of the test concentration.

9.1.2.2 *Intermediate precision* expresses within-laboratory variations (usually on different days, different analysts and different equipment). If reproducibility is assessed, a measure of intermediate precision is not required.

9.1.2.3 *Reproducibility* expresses *precision* between laboratories.

9.1.3 *Robustness* (or *ruggedness*) is the ability of the procedure to provide analytical results of acceptable accuracy and precision under a variety of conditions. The results from separate samples are influenced by changes in the operational or environmental conditions. Robustness should be considered during the development phase and should show the reliability of an analysis when deliberate variations are made in method parameters.

The verification of stability of analytical solutions is of particular importance.

Other characteristics of robustness include extraction time. In the case of liquid chromatography, robustness testing may also include verification of the impact of changes in pH, temperature and flow rate (see ICH Q2 – Validation of Analytical Procedures, Step 4, for further details).

9.1.3.1 Factors that can have an effect on robustness when performing chromatographic analysis include:

- stability of test and standard samples and solutions;
- reagents (e.g. different suppliers);
- different columns (e.g. different lots and/or suppliers);
- extraction time;
- variations of pH of a mobile phase;
- variations in mobile phase composition;
- temperature;
- flow rate.

9.1.4 *Linearity* indicates the ability to produce results that are directly proportional to the concentration of the analyte in samples. A series of samples should be prepared in which the analyte concentrations span the claimed range of the procedure. If there is a linear relationship, test results should be evaluated by appropriate statistical methods. A minimum of five concentrations should be used.

9.1.5 *Range* is an expression of the lowest and highest levels of analyte that have been demonstrated to be determinable for the product. The specified range is normally derived from linearity studies.

9.1.6 *Specificity* (*selectivity*) is the ability to measure unequivocally the desired analyte in the presence of components such as excipients and impurities that may also be expected to be present. An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and assay.

9.1.7 *Detection limit* (*limit of detection*) is the smallest quantity of an analyte that can be detected, and not necessarily determined, in a quantitative fashion. Approaches may include instrumental or non-instrumental procedures and could include those based on:

- visual evaluation;
- signal to noise ratio;
- standard deviation of the response and the slope;
- standard deviation of the blank;
- calibration curve.

9.1.8 *Quantitation limit* (*limit of quantitation*) is the lowest concentration of an analyte in a sample that may be determined with acceptable accuracy and precision. Approaches may include instrumental or non-instrumental procedures and could include those based on:

- visual evaluation;
- signal to noise ratio;
- standard deviation of the response and the slope;
- standard deviation of the blank;
- calibration curve.

9.2 Characteristics (including tests) that should be considered when using different types of analytical procedures are summarized in Table 1.

Table1. Characteristics to consider during analytical validation

Type of analytical procedure	Identification	Testing for impurities	Testing for impurities	Assay — dissolution (measurement only) — content/potency
Characteristics		Quantitative tests	Limit tests	
Accuracy	–	+	–	+
Precision				
Repeatability	–	+	–	+
Intermediate precision[a]	–	+	–	+
Specificity	+	+	+	+
Detection limit	–	–[b]	+	–
Quantitation limit	–	+	–	–
Linearity	–	+	–	+
Range	–	+	–	+

– Characteristic is normally not evaluated;
+ Characteristic should normally be evaluated.
[a] In cases where a reproducibility study has been performed, intermediate precision is not needed.
[b] May be needed in some cases.

Statistical analysis used to evaluate validation characteristics against predetermined acceptance criteria should be appropriate for the intended evaluation. Appropriately validated software should be used. An appropriate number of samples to provide adequate statistical power and range should be considered.

9.3 System suitability testing

Note: System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be

analysed constitute an integral system that can be evaluated as such. System suitability test parameters that need to be established for a particular procedure depend on the type of procedure being evaluated, for instance, a resolution test for a high-performance liquid chromatography (HPLC) procedure.

9.3.1 The suitability of the entire system should be confirmed prior to and during method validation tests as well as during the test of samples.

9.3.2 System suitability runs should include only established standards or reference materials of known concentration to provide an appropriate comparator for the potential variability of the instrument.

9.3.3 Where a sample is used for system suitability or a trial run, written procedures should be established and followed and the results of all such trial runs be included in the results and data review process. A sample can be used only if it is a well characterized material. Characterization in such a case should be performed prior to the use of this sample as part of system suitability testing. The sample material or product under test should not be used for trial run purposes or to evaluate suitability of the system (see *WHO guidelines on good data and record management practices* (2).

References

1. *Supplementary guidelines on good manufacturing practices: validation* (WHO Technical Report Series, No. 937, 2006, Annex 4).

2. *WHO Guidelines on good data and record management practices* (WHO Technical Report Series, No. 996, 2016, Annex 5).

3. *WHO guidelines on transfer of technology in pharmaceutical technology* (WHO Technical Report Series, No. 961, 2011, Annex 7).

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