LABORATORY DATA MANAGEMENT GUIDANCE

Analytical Procedure Lifecycle Management

(APLM)

Name and Role	Date
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Document Revision History

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V 0.2	30-Mar-2017	Structural template for review at AQCG Board meeting	Template
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Expert Drafting Group

This guideline is the result of a collaborative effort involving;

- members of the ECA AQCG board in the first instance
- colleagues on the USP Validation and Verification Panel and the USP Statistics Subcommittee.

Those involved in ECA AQCG board were;06-Jul-18

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Key References

1. ICH Q2(R1), *Validation of Analytical Procedures: Text and Methodology*, November 2005
2. ICH Q8(R2), *Pharmaceutical Development*, August 2009
3. ICH Q9, *Quality Risk Management*, November 2005
4. ICH Q6A, *Specifications: Test Procedures and Acceptance Criteria For New Drug Substances and New Drug Products: Chemical Substances*, October 1999
5. ICH Q10, *Pharmaceutical Quality System*, June 2008
6. ICH Q12: *Technical and Regulatory Considerations for Pharmaceutical Product Lifecycle Management,* Step 1 Core guideline, June 2017
7. USP 40 (2018), General Chapter <1224>, Transfer of analytical procedures
8. USP 40, (2018), General Chapter <1225>, Validation of analytical procedures
9. USP 40, (2018), General Chapter <1226>, Verification of analytical procedures
10. *Lifecycle Management of Analytical Procedures: Method Development, Procedure Performance Qualification, and Procedure Performance Verification*; **Pharmacopeial Forum 39(5)** 2013
11. *Fitness for use: Decision rules and Target Measurement Uncertainty*; **Pharmacopeial Forum 42(2)** 2016
12. *Measurement Uncertainty for the Pharmaceutical Industry*, **Pharmacopeial Forum 44(1)** 2018
13. *Analytical Control Strategy*; **Pharmacopeial Forum 42(5)** 2016
14. *Analytical Target Profile: Structure and Application throughout the analytical lifecycle*; **Pharmacopeial Forum 42(5)** 2016
15. *General Chapter <1210>: Statistical tools for Procedure Validation*; **Pharmacopeial Forum 42(5)** 2016
16. *Proposed New USP General Chapter: The Analytical Procedure Lifecycle <1220>*; **Pharmacopeial Forum 43(1)** 2017
17. *FDA Analytical Procedures and Methods Validation for Drugs and Biologics Guidance for Industry*; July 2015
18. *PDA Technical Report 57-2 Analytical Method Development and Qualification for Biotechnology Products, 2012*
19. *MHLW/PMDA and FDA Joint Proposal: Analytical Procedure Development and Revision of Q2(R1) Analytical Validation, 28-March-2017*
20. *ICH Outline of Concept Paper Analytical Procedure Development and Revision of Q2 (R1) Analytical Validation June 2018*

Quality involvement/Responsibilities

The extent of Quality oversight is dependent on individual company requirements. Organisation and nomenclature of Quality Control and Assurance functions and assignment of responsibilities are also highly company specific. This Guideline does not dictate or recommend specific steps that must be supervised by specific quality functions other than those required by regulation. Therefore, the term Quality Unit (QU) as used in the revised chapter 1 of EU GMP Guide, is used here.

Introduction and Rationale for this Guideline

The current regulatory guidance relating to analytical procedure validation predominately resides in ICH Q2(R1)[1] and the United States Pharmacopeia (USP) General Chapters <1224>, <1225> and <1226>.[2] and FDA Analytical Procedures and Methods Validation for Drugs and Biologics Guidance for Industry[3]. Currently these are based upon traditional analytical concepts for chromatographic procedures and generally view validation as a point in time isolated activity rather than considering the whole lifecycle of a procedure development and use. In 2011, FDA revised their process validation guidelines to transform validation into a lifecycle process as opposed to an activity.[4] This approach is consistent with EU GMP Annex 15. It is therefore desirable to use a similar model for analytical procedures. The USP has been working for some years on a new General Chapter <1220> and has recently published a draft proposal[5].

The purpose of applying lifecycle principles to analytical procedures is to holistically align analytical procedure variability with the requirements of the product to be tested and to improve the reliability of the procedure by understanding, reducing, and controlling sources of variability.[6]

Enhanced understanding of variables that affect the performance of an analytical procedure provides greater assurance that the quality attributes of the tested product can be reliably assessed. The lifecycle management process provides a framework for defining the criteria for and development of an analytical procedure that meets the acceptance criteria[5].

A high level overview of the process is shown in **Figure 1**.

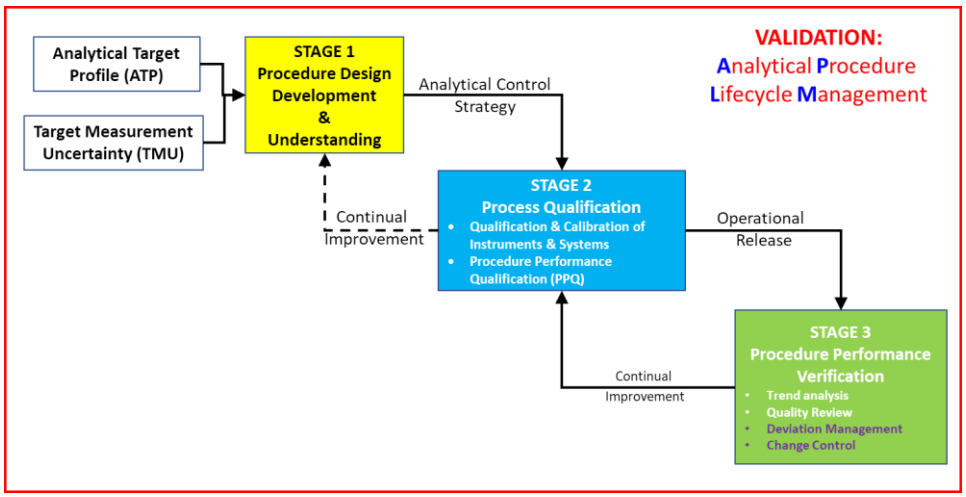

Figure 1 Overview of the Analytical Procedure Lifecycle Model

[1] ICH Q2(R1), Validation of Analytical Procedures: Text and Methodology, November 2005

[2] USP 40 (2018), General Chapter <1224>, Transfer of analytical procedures; General Chapter <1225>, Validation of analytical procedures; General Chapter <1226>, Verification of analytical procedures

[3] FDA, Analytical Procedures and Methods Validation for Drugs and Biologics Guidance for Industry; July 2015

[4] FDA, Guidance for Industry Process Validation: General Principles and Practices, Revision 1, January 2011

[5] Proposed New USP General Chapter: The Analytical Procedure Lifecycle <1220>; Pharmacopeial Forum 43(1) 2017

[6] *Lifecycle Management of Analytical Procedures: Method Development, Procedure Performance Qualification, and Procedure Performance Verification*; **Pharmacopeial Forum 39**(5) 2013,

The purpose of this ECA AQCG guideline is to provide;

1. An overview and framework for a lifecycle approach to the qualification of analytical procedures as part of a lifecycle approach which is consistent with the principles of the FDA process validation guideline and regulatory expectations.
2. Descriptions and recommendations regarding the staged activities in the lifecycle.
3. Input for the forthcoming revision of ICH Q2(R1), the new Q14 and support of the on-going development of USP General Chapter <1220> on Analytical Procedure Lifecycle
4. Guidance on the application of the lifecycle approach for existing methods and procedures.
5. A focus for debate regarding the practical implementation of APLM within the industry

This guideline is intended to apply to chemical methods of analysis for both univariate methods such as HPLC, UV spectrophotometric assays for example and multivariate methods for identification of materials using FTIR, FTNIR and Raman spectroscopies for example. Univariate is a term commonly used in statistics to describe a type of data which consists of observations on only a single characteristic or attribute whereas multivariate encompasses the simultaneous observation and analysis of more than one outcome variable

These principles also apply to biopharmaceutical assay methods.
Biological and microbiological methods are not explicitly covered by this guideline.

Limitations of the current ICH Q2(R1)

The ICH guideline Q2 (R1) describes a general validation protocol for the validation of chromatographic procedures with respect to assay, determination of impurities and limit tests. Furthermore, the guideline defines a terminology for all described validation parameters and many of the principles are still applicable in the lifecycle approach.

However, the current ICH validation guideline does not include

- validation parameters for other analytical techniques, e.g. NIR or procedures for the determination of elemental impurities, e.g. ICP-OES, ICP-MS.
- acknowledgement of the fact that the reportable result has an uncertainty budget associated with the precision and accuracy of the method.
- adequate terminology e.g. the term “specificity” is not appropriate as the text describes more “selectivity”, i.e. identification of the analyte in the presence of matrix components.
- guidance on how to set appropriate acceptance criteria for the validation parameters
- information with respect to requirements for a stability-indicating procedure are also missing from the current guidance. adequately description of non linear fitting models.
- With respect to “linearity”, two fundamentally different aspects are confused.
    1. The “ability to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample” refers to the reportable result of the analytical procedure but is already included in accuracy.
    2. The relationship of “signals as a function of analyte concentration” refers to the analytical response function, i.e. the calibration model, which is often the linear one described in the guideline but may assume any other mathematical model.
- the examples given for the calculation of detection and quantification limits are based on the slope of a linear calibration model and thus cannot be used when the calibration model is non-linear.
- A redefinition of the term for validation is required because “validation” does not only address the determination of each validation parameter at a single point in time with respect to its variability but should also be extended to a continuous process in order to control the analytical procedure under life-cycle conditions.

All these limitations will be discussed in this guideline and the technical aspects will be included in an appendix.

Limitations of USP General Chapters <1224>, <1225> and <1226>

General chapter <1225> on validation of analytical procedures was a precursor to ICH Q2(R1) and was written more than 20 years ago with a focus on chromatographic methods. General chapters <1224> on transfer of analytical procedures and <1226> on verification of compendial procedures are more recent and were written in response to user needs. As these three general chapters were written separately there is no overall consistent lifecycle approach. The intention by USP is to write a new general chapter <1220> which covers the analytical lifecycle and all the topics in the previous three general chapters.

Importance of adopting an APLM approach in the context of Data Governance

The current regulatory climate has highlighted deficiencies and concerns regarding data integrity and security in laboratories in a global context. These observational findings cover both fraudulent activities and bad practices. The lifecycle management approach for analytical procedures is an essential part of ensuring that analytical data are 'fit for intended purpose'. The APLM approach provides an efficient and effective process for delivering this requirement.

Data integrity and security is concerned with more than just the generation and security of correct numbers. It is concerned with the totality of arrangements under a QMS to ensure that data, irrespective of the format in which they are generated, recorded, processed, retained and used to ensure a complete, consistent and accurate record throughout the data life cycle in accordance with the ALCOA+ model[7].

Data governance is the term used for the overall management strategy to ensure data integrity and security. Functional aspects within the laboratory and the quality function must be under control otherwise data integrity could be compromised despite the best efforts of all staff. The foundation of data governance is the engagement and involvement of executive and senior management throughout any organisation. Therefore, there must be management leadership, data governance and data integrity policies that cascade down to laboratory data integrity procedures together with data integrity training at all levels.

Figure 2 illustrates the 3 main elements of data governance. Whilst this guideline is primarily concerned with the technical and procedural control aspects, it is essential that the other two components, a strong and effective QMS and an ethical and supportive management culture are in place.

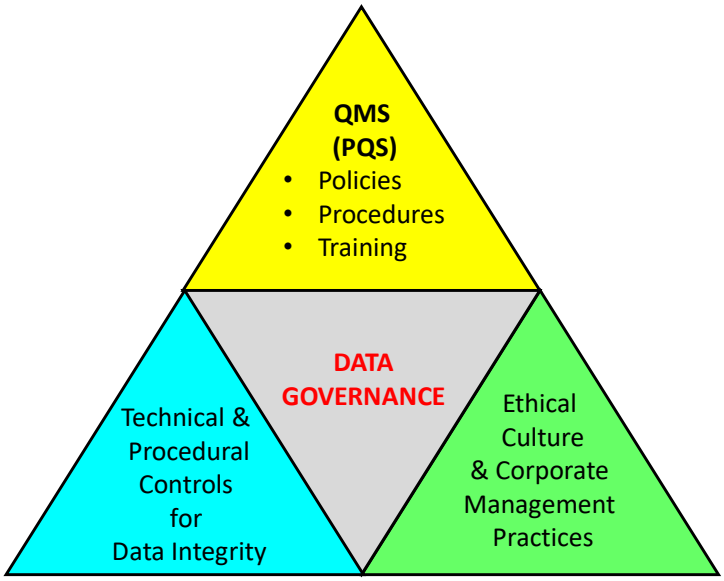

Figure 2 Data Governance structure for the regulated laboratory

[7] Data Governance and Data Integrity Guideline; v2.0, Jan 2018

The main elements of data integrity have been well defined in the US regulation 21CFR 211 for many years. For example

- § 211.68 requires that backup data are exact and complete, and secure from alteration, inadvertent erasures, or loss
- § 212.110(b) requires that data be stored to prevent deterioration or loss
- §§ 211.100 and 211.160 require that certain activities be documented at the time of performance and that laboratory controls be scientifically sound
- § 211.160(b)(4) requires that the calibration of instruments… at suitable intervals in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for remedial action in the event accuracy and/or precision limits are not met.
- § 211.180 requires true copies or other accurate reproductions of the original records; and
- §§ 211.188, 211.194, and 212.60(g) require complete information, complete data derived from all tests, complete record of all data, and complete records of all tests performed.

The integrity of the primary analytical record is dependent on many factors but the analytical procedure and it's on going control is critical.

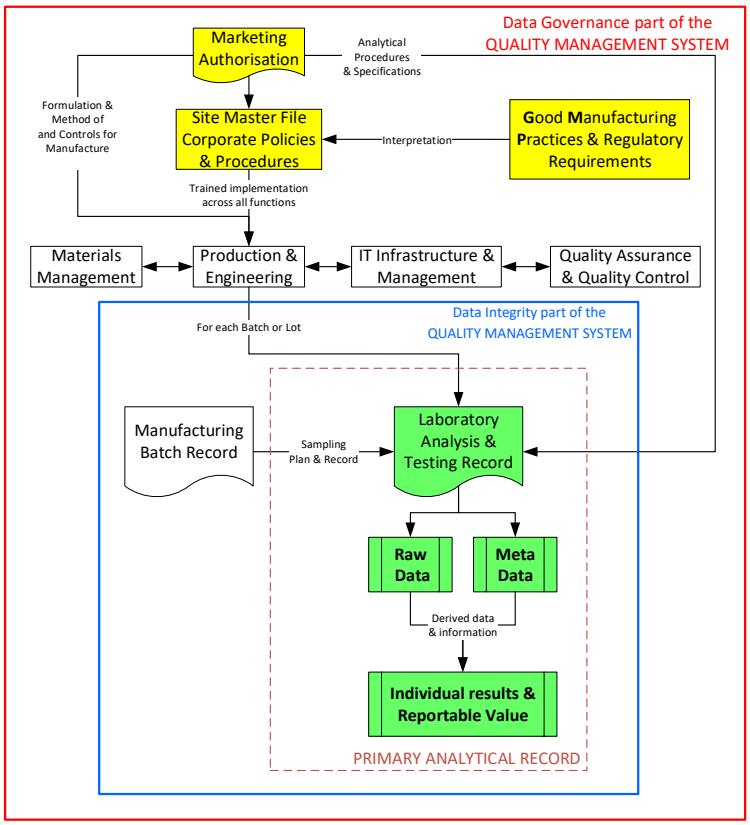

Figure 3 The primary analytical record in the context of Data Governance and Data Integrity[8]

[8] C. Burgess, R.D. McDowall, What's In a Name?, **LCGC Europe, 28(11)** 621–626, 2015,

This guideline forms part of a set of ECA Guidelines and are available on the member's area of the ECA website for download. Previous relevant guidelines are;

- OOS Guideline, v2.1, August 2013
- OOE & OOT Guideline; v1.1 November 2016
- Data Governance and Data Integrity Guideline; v2.0 January 2017

Principles of Analytical Procedure Lifecycle Management (APLM)

The overall lifecycle management three stage approach to analytical procedures is illustrated in **Figure 4**

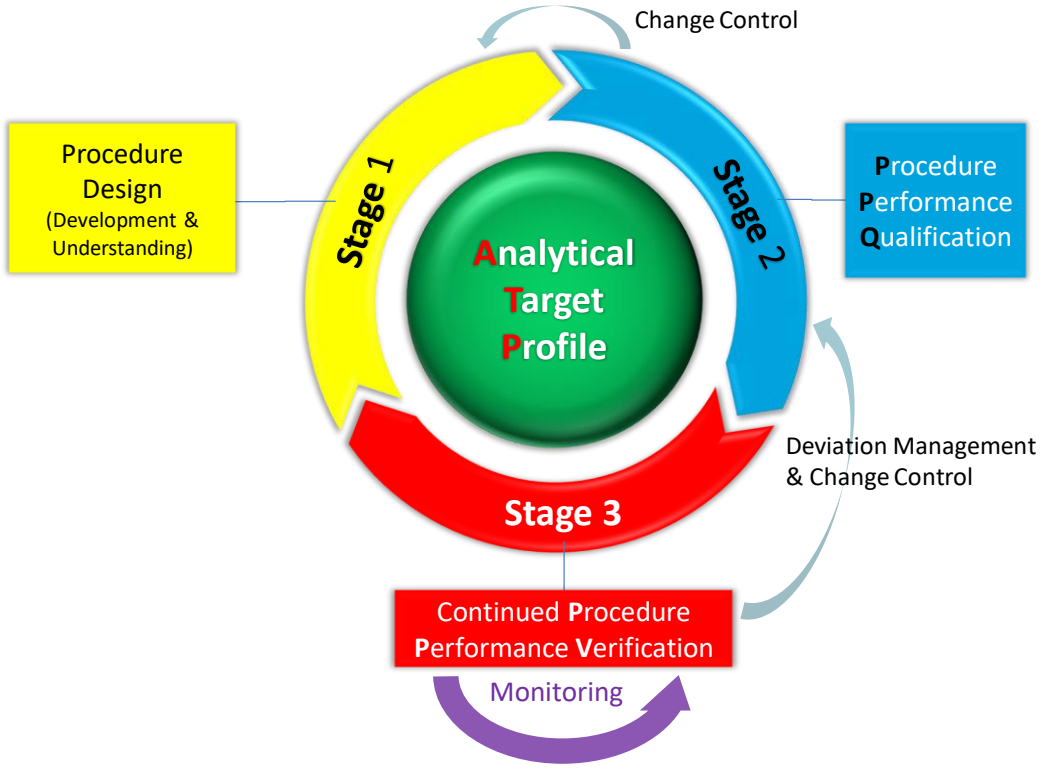

Figure 4 Overall lifecycle management approach for analytical procedures (APLM)

Any analytical procedure must be shown to be fit for its intended purpose before use. The current process of demonstrating this 'fitness for purpose' in analytical laboratories takes place is by way of a documented validation study as required by ICH Q2(R1) and USP <1225> and, if required, a verification under actual conditions of use, USP <1226>, or transfer process, USP <1224>, to demonstrate the procedure performs appropriately in the laboratory in which it will be routinely used. Hence these activities are seen to be discrete activities disconnected from development activities and rigidly controlled. These documents are guidance documents but have acquired the status or expectation of mandatory regulatory requirements.

Many of the elements necessary for the APLM are already in place in the current process but are not linked in a lifecycle manner. **Figure 5** shows the current process flows for both Pharmacopeial and User developed procedures.

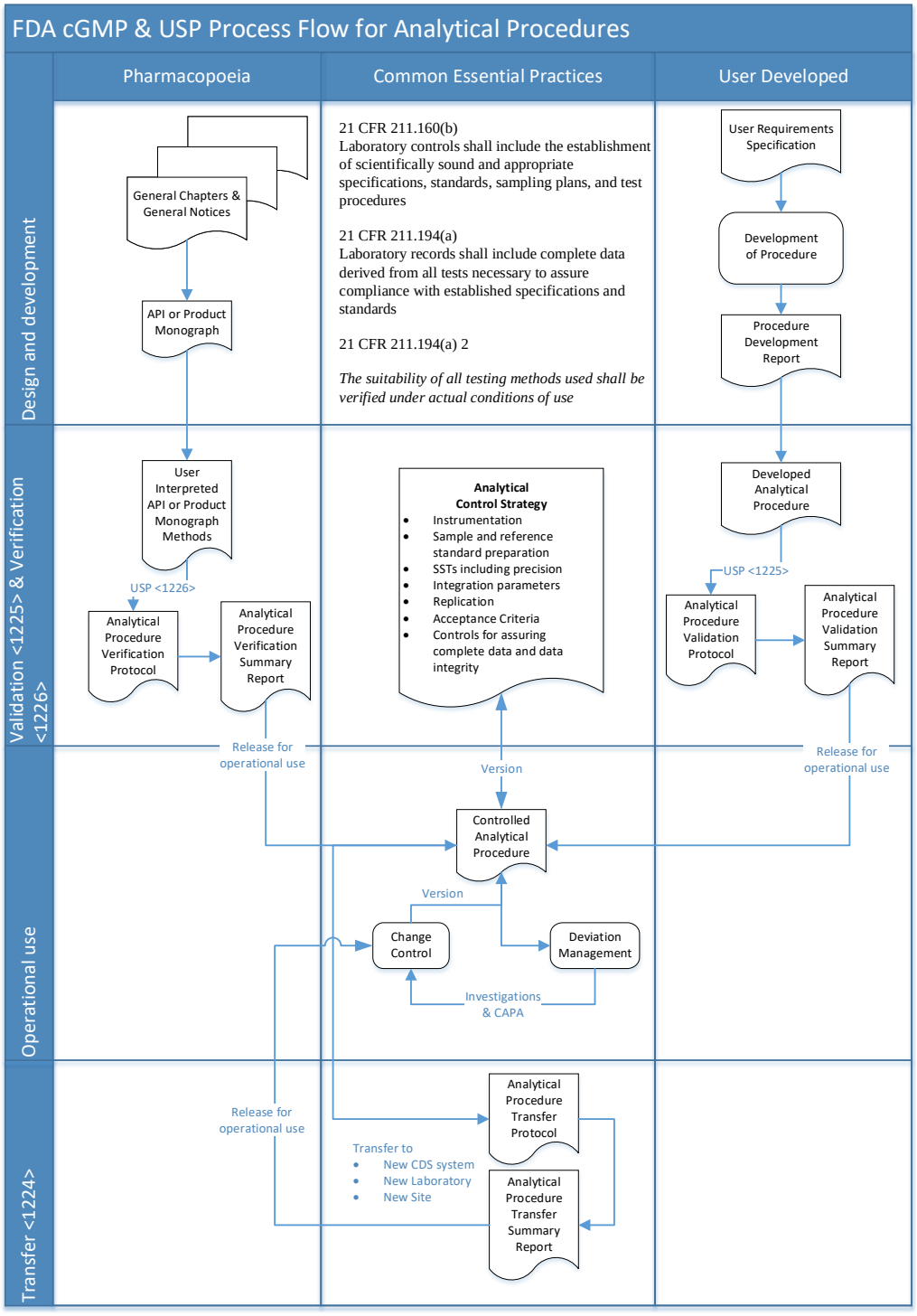

Figure 5 Current process flows for analytical procedure design to meet FDA cGMP and USP requirements

The lifecycle concept of validation described in FDAs Guidance on process validation[9], and now reinforced in ICH Q12, is applicable to analytical procedures if we consider that an analytical procedure is a process and the output of this process is the reportable result, that is, the value that will be compared to the specification or acceptance criterion[10].

The overall lifecycle from specification of the purpose (the ATP) through design and development (Stage 1), qualification for routine use (confirmation of 'fitness for purpose' Stage 2) and on-going satisfactory performance (Stage 3) is illustrated in **Figure 4**.

Each stage may be subdivided into specific subject areas;

Stage 1: Procedure Design and Development

- Knowledge gathering
- Selection of appropriate analytical methodology capable of meeting the ATP
- Forced degradation studies for API and formulated products
- Risk assessment
- Design of experiments and modelling to establish a method operable design region MODR (equivalent to the design space in-process validation)
- Analytical control strategy
- Initial replication strategy
- Knowledge management and Transfer

Stage 2: Procedure Performance Qualification

- Qualification study under a procedure performance protocol
- Confirmation of the analytical control strategy and finalised replication strategy
- Release for operational use

Stage 3: Procedure Performance Verification

- Trend analysis of critical procedure parameters
- Change control
- Deviation management

Regulatory requirements for on-going performance verification (Stage 3) are already defined under both US & EU GMPs.

21 CFR§ 211.180(e) (and EU GMP Part I chapter 6.9) requires that an ongoing program to collect and analyse product and process data that relates to product quality must be established. Annex 15 of EU GMP has similar requirements. The data collected should include relevant process trends and quality of incoming materials or components, in-process material, and finished products. The data should be statistically trended and reviewed by trained personnel. The information collected should verify that the quality attributes are being appropriately controlled throughout the process.

[9] FDA Guidance for Industry, Process Validation: General Principles and Practices, January 2011 revision 1

[10] USP40/NF35, General Notices 7.10. Interpretation of Requirements

Under the EU GMPs, manufacturers should monitor product quality, as part of Product Quality Review, to ensure that a state of control is maintained throughout the product lifecycle with the relevant process trends evaluated. Statistical tools should be used, where appropriate, to support any conclusions with regard to the variability and capability of a given process and ensure a state of control.

All investigations pertaining to the batch being certified (including out of specification and adverse trend investigations) must have been completed to a sufficient level prior to certification

In addition, USP General Chapter <1226>; Verification of Pharmacopeial Procedures and the FDA Analytical Procedures and Methods Validation for Drugs and Biologics Guidance for Industry, specify that the suitability of all testing procedures used shall be verified under actual conditions of use quoting 21 CFR§ 194(a)(2).

These requirements align with ICH Q10's (Pharmaceutical Quality System) continual improvement of process performance and product quality section as shown in Figure 6 and specifically as part of the process performance and product quality monitoring system.

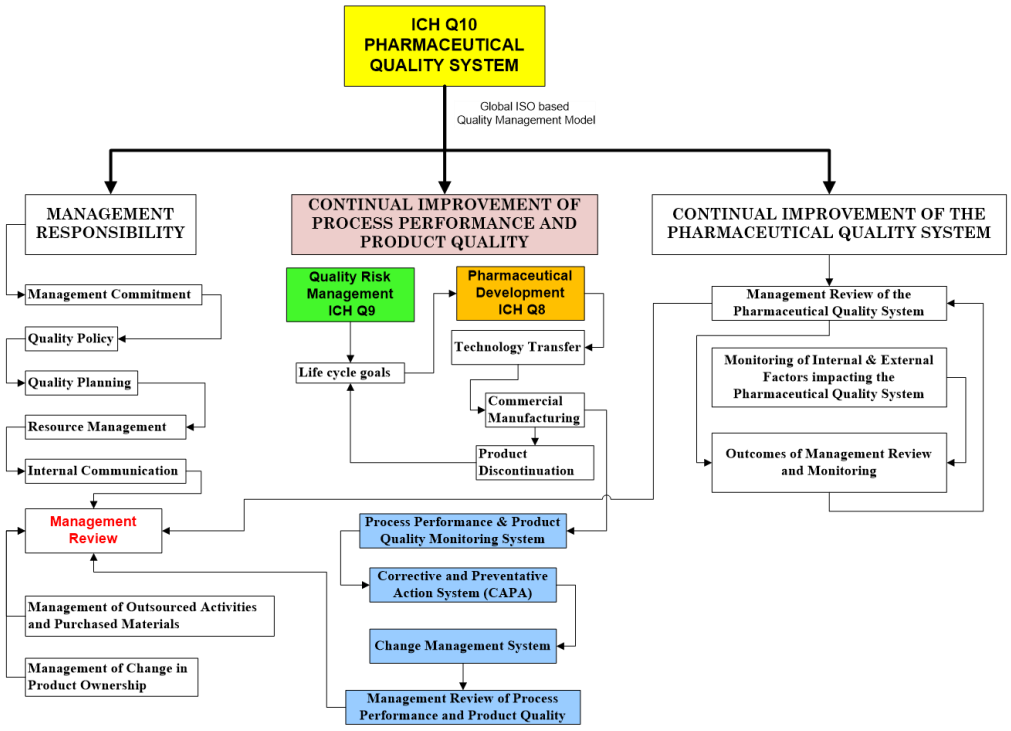

Figure 6 Pictorial representation of ICH Q10 Pharmaceutical Quality System

In other words, a State of Control is established and confirmed during Stages 1 & 2 of the APLM, in which the set of controls (The Analytical Control Strategy) consistently provides procedure performance assurance and is verified during Stage 3.

The draft ICH Q12[11] uses the term Established Conditions (ECs) and discusses them in relation to analytical procedures in section 3.2.3.2. and in 8.1 regarding a structured approach to analytical procedure changes over the lifecycle.

ECs related to analytical procedures should include elements which assure performance of the procedure. Appropriate justification should be provided to support the identification of ECs for analytical procedures. The extent of ECs could vary based on the method complexity, development and control approaches.

- *Where the relationship between method parameters and method performance has not been fully studied at the time of submission, ECs will incorporate the details of operational parameters including system suitability.*
- *When there is an increased understanding of the relationship between method parameters and method performance defined by a systematic development approach including robustness studies, ECs are focused on method-specific performance criteria (e.g., specificity, accuracy, precision) rather than a detailed description of the analytical procedure*[11].

ICH Q12 is therefore preparing the way for performance based procedures, as proposed by USP in 2009 [12, 13],to be developed.

In addition, the PDA (Parenteral Drug Association) proposed a similar but less rigorous lifecycle model in 2012 for biotechnology products.
Their process flow is illustrated in Figure 7.

[11] ICH Q12: Technical and Regulatory Considerations for Pharmaceutical Product Lifecycle Management Step 1, Core guideline, June 2017, section 3.2.3.2

[12] R.L. Williams, D.R. Abernethy, W.F. Koch, W.W. Hauck, and T.L. Cecil, **Pharmacopeial Forum 35(3)** 2009, Stimuli to the Revision Process: Performance-based Monographs

[13] W.W. Hauck, A.J. DeStefano, T.L. Cecil, D.R. Abrnethy, W.F. Koch, R.L. Williams, **Pharmacopeial Forum 35(3)** 2009, Stimuli to the Revision Process: Acceptable, Equivalent, or Better: Approaches for Alternatives to Official Compendial Procedures

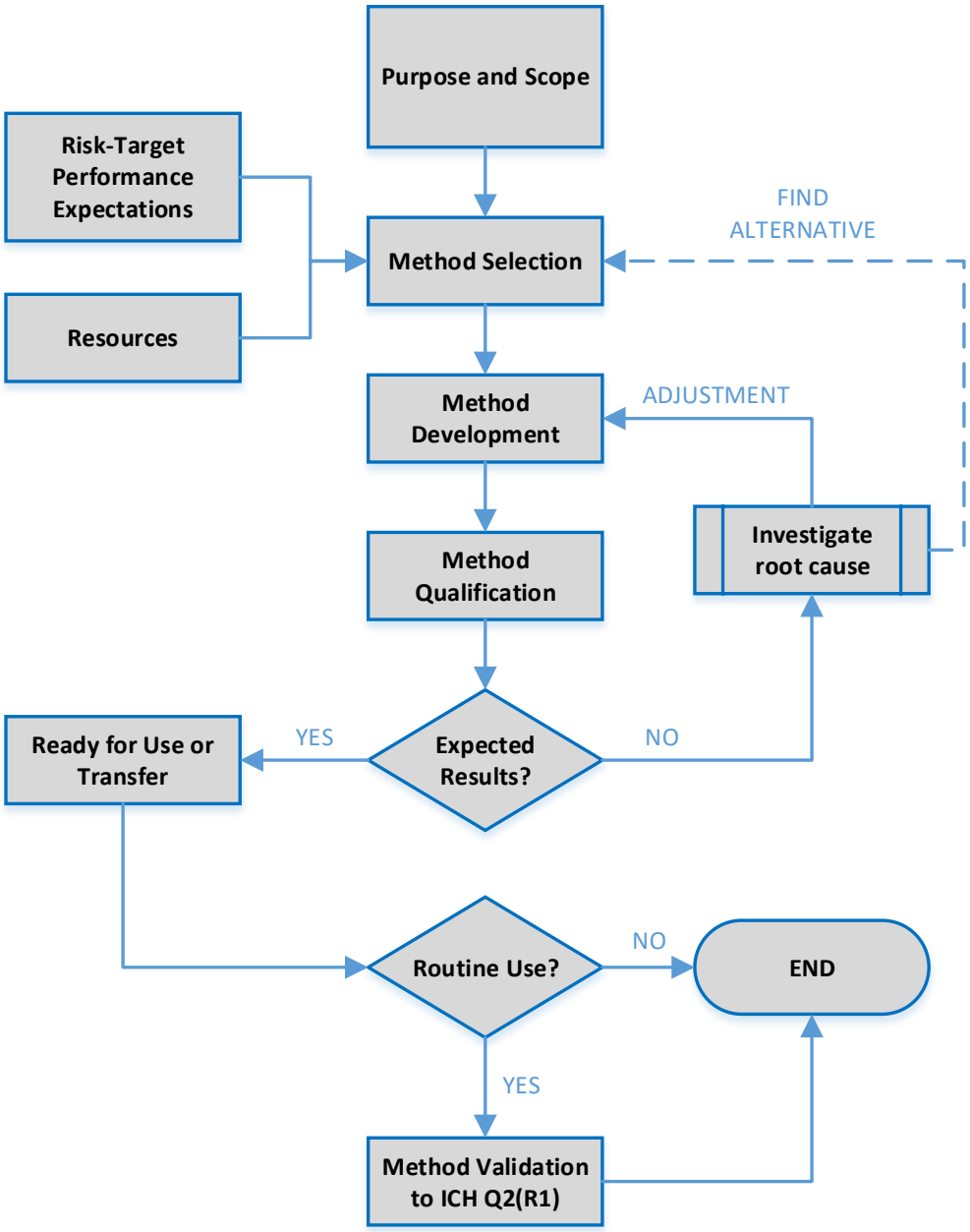

Figure 7 PDA 2012 development and qualification lifecycle for biotechnology products (adapted and redrawn from Figure 1.2-1 [14])

[14] PDA Technical Report 57-2 Analytical Method Development and Qualification for Biotechnology Products, 2012

Specifications, Acceptance Criteria and Decision Rules

Specifications are a prerequisite for the definition of the quality attributes or characteristics of the drug substance/drug product and includes test parameters and acceptance criteria. Typical test parameters in a specification are

- Identification
  - E.g. structural properties
- Purity (active ingredient)
  - Quantitative tests
- Safety
  - Limit tests for impurities (e.g. residual solvents and elemental impurities)
  - Quantitative tests for impurities
  - Microbiological contamination (e.g. endotoxin)
  - Sterility
- Potency
  - Content/Concentration (calculated)
  - Assay
  - Functional activity (e.g. enzymatic or binding activity)

Justification of the acceptance criteria in the specifications are mainly based on experimental work during development of the product and knowledge of the safety parameters during clinical trials. With a lifecycle approach, specifications evolve with the knowledge of the product as it is developed through the clinical phases. This is further illustrated in Figure 8 in the following section. The specification is finalised before registration of the product.[15]

Standards for drug substances and drug products are currently primarily set by regulation without explicit regard for metrological uncertainty. Pharmacopeial limits are not specifications but standards which define acceptance criteria that every sample tested must meet within shelf life under appropriate storage conditions [16].

Therefore, Pharmacopeial limits should not be used as a release testing for a batch or lot as they relate only to the sample tested in accordance with the monograph[17].

A major reason for adopting the lifecycle approach to an analytical procedure and its acceptance criteria is to ensure that the reportable result is fit for use. The analytical target profile (ATP) concisely defines the requirements for a reportable result to be fit for use.

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[15] ICH Q6A, Specifications: Test Procedures And Acceptance Criteria For New Drug Substances And New Drug Products: Chemical Substances, October 1999

[16] L.D. Torbeck, *In Defense of USP Singlet Testing*, ***Pharmaceutical Technology***, February, 38-40, 2005,

[17] C. Burgess, *Singlet Determination Revisited; Is there a difference between a Specification or a Standard?*. ***Pharmaceutical Technology***, **41**(10) 2014

The Decision Rules (DRs) define the use of the reportable result, and can provide the information, such as acceptable probabilities, needed to set the target measurement uncertainty (TMU), which is defined in the *International Vocabulary of Metrology*[18] as a "measurement uncertainty (MU) specified as an upper limit and decided on the basis of intended use of measurement results.

DRs give a procedure for the acceptance or rejection of a product based on the measurement result (reportable value), its uncertainty, and the specification limit or limits[19]. Additionally, DRs can take into account an acceptable level with regard to the probability of making a wrong decision.

The wrong decision can lead to accepting an out-of-specification (OOS) reportable result which is not true or rejecting an OOS reportable result which is true, or a false failure or a missed fault[20].

Defining the requirement; The Analytical Target Profile (ATP)

The ultimate purpose of the analytical procedure is to obtain a reportable result that will tell us something about the quality of the product and/or the process step from where the sample is taken. The test result or reportable result will then be used to make decisions on the batch or process step. Therefore, it is important to design an analytical procedure that will give the 'right' answer to the question asked. Defining the question (i.e. the requirement) is the essential first step in starting the process of designing the analytical procedure. The formulation of the ATP is a critical step of the APLM. This section provides an overview of the ATP process and more detail is provided in Key Reference 14 on page 6.

It is important to note that the requirement is independent of the analytical technique and it is based on product and process understanding (knowledge gathering). The establishment of a predefined objective and purpose of the analytical procedure with predefined performance requirements is gathered in the Analytical Target Profile (ATP).

In determining the ATP the following aspects will need be considered;

- The sample to be tested
- The matrix/components of the sample
- The content range of analyte in the sample. In-process, drug substance or drug product may have different concentration levels of the analyte to be measured
- What is the permitted error and the risk of not meeting specifications. The ATP must consider the acceptable level of risk based on patient safety and product efficacy.

[18] ISO International vocabulary of metrology - Basic and general concepts and associated terms (VIM), ***ISO/IEC Guide 99:2007***. International Organization for Standardization, Geneva; 2007.

[19] C. Burgess *Using the guard band to determine a risk-based specification*. ***Pharmaceutical Technology***, **38**(10) 2014

[20] C. Burgess, P. Curry, D.J. LeBlond, G.S. Gratzl, E. Kovacs, G.P. Martin, P.L. McGregor, P. Nethercote, H. Pappa, J.Weitzel, *Fitness for Use: Decision Rules and Target Measurement Uncertainty*, ***Pharmacopeial Forum***, **42(2)** 2016

By way of an example, suppose that it is decided that it is necessary to travel to London from Copenhagen. This can be done in several ways; fly, sail, go by car, train, bus, bicycle and even walk or use a combination of transport options. Therefore, the first part of the ATP is defining the purpose; It is necessary to travel to London in this example.

The second part in defining the ATP is "the how-to". I need to travel from Copenhagen to London because of a dinner at 19:00 today. This requirement limits my transportation options to only flying, because any of the other options will not get me to London in time. However, if the requirement was stated "I want to travel from Copenhagen to London in the most cost effective manner" choosing to fly may not be the best option. We can continue to build the requirement by stating that the dinner is at a busy restaurant that will not hold the reservation if I am more than 10 minutes late. Then we might want to take an earlier flight so we are more likely to get to the restaurant in time. All these requirements and constraints are necessary to establish the ATP and in order to select and design an analytical procedure that is capable of providing the performance needed to meet the ATP criteria. This concept is also described in various Quality by Design papers [21], [22].

Establishing Target Measurement Uncertainty (TMU)

On the basis that all measurements and processes are subject to error and no measurement is exact.

- The true value cannot be known unless it was measured an infinite number of times which of course, is not practical
- Every measurement is subject to some uncertainty.
- A measurement result is only complete if it is accompanied by a statement of the uncertainty in the measurement.

It is the measured value and the uncertainty associated with it which are used in the reportable result.

Following the Journey analogy , the TMU is the error associated with the transportation. It includes, for example, flight scheduled duration and unforeseen delays due to traffic conditions.

[21] P Borman, P. Nethercote, M. Chatfield, D. Thompson, K. Truman. *The Application of Quality by Design to Analytical Methods*, **Pharmaceutical Technology** ,**31(**10), 2007

[22] G.L. Reid, J. Morgado, K. Barnett, B. Harrington, J Wang, J.Harwood, David Fortin. *Analytical Quality by Design (AQbD) in Pharmaceutical Development*. **American Pharmaceutical Review**. 2013

The TMU is an essential element of the ATP and should take into consideration the different aspects as illustrated in Figure 8.

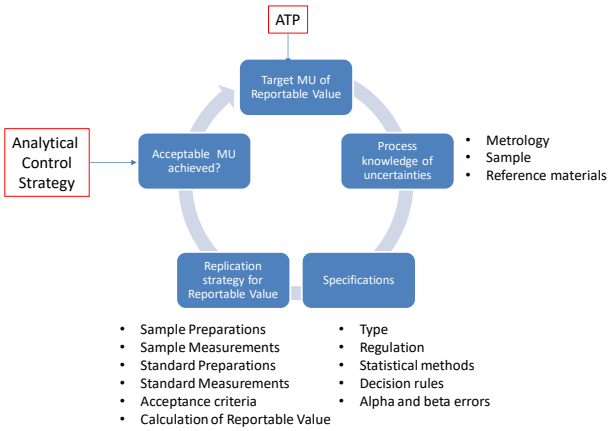


Figure 8 Deming cycle for TMU and the ATP [23]

The TMU is calculated using the model of a normal distribution that has a central value. In practice, this value may not be centred on the midpoint in a specification range. The commercial manufacturing range may also not be centred and/or the analytical procedure may have a bias. Both of these instances will impact the setting of the TMU.

Technical details of setting the TMU and the associated Decision Rules are beyond the technical scope of this guideline and reference is made to more detailed discussion papers on this topic [24],[25],[26].

Prerequisites for the APLM

The analysis may be regarded as process whereby the inputs of samples, standards, reagents etc are converted via an analytical procedure into reportable results from transformed measurement data. Like any process, there are controllable and uncontrollable factors requiring risk mitigation. Such a process is illustrated in Figure 9.

[23] J. Weitzel, Private Communication

[24] *Fitness for use: Decision rules and Target Measurement Uncertainty*; **Pharmacopeial Forum 42(2)** 2016

[25] C. Burgess *The Basics of Measurement Uncertainty in Pharma Analysis*. ***Pharmaceutical Technology***, **37**(9) 2013

[26] Eurachem, *Quantifying Uncertainty in Analytical Measurement*, 3rd Edition, 2012

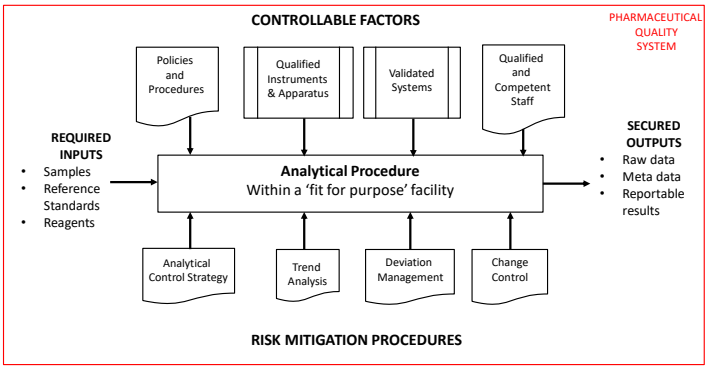


Figure 9 Overview of an analytical process flow

As a precursor to the development and qualification of an analytical procedure, certain elements need to be in place. This section briefly describes them and references more detailed guidances. Given the importance of data governance and data integrity in a regulatory context, it is useful to note the 4 layers of analytical data integrity lifecycle shown in Figure 10. Stages 1 and 2 in this APLM guideline are primarily concerned with Level 2 assuming the prerequisites of Level 1 and the Foundation.

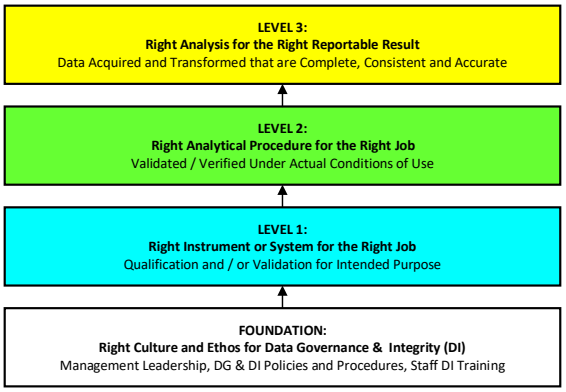


Figure 10 Analytical Data Integrity Model [27]

Data Governance and Data Security is the subject of another ECA Guideline where these topics are discussed in more detail.[28] Stage 3, Performance Verification maps more clearly to Level 3 in the Analytical Data Integrity model.

Analytical Instrument Qualification

It is a basic tenet for all analytical scientists that, before the commencement of an analytical procedure, they must ensure the suitability and proper operation of any instrument or system that is part of the measurement process in order to demonstrate 'fitness for purpose'. This challenge is becoming increasingly difficult as spectroscopic

[27] R.D. McDowall, ***Validation of Chromatography Data Systems***, 2nd Edition, Figure 7.5, Royal Society of Chemistry 2017

[28] ECA Data Governance and Data Integrity Guideline; v2.0 October 2017

applications increase[29], [30], [31].and specialist knowledge in the laboratory becomes rarer. This is particularly true for newer sensor technologies such as those used in PAT.

A large variety of analytical instruments, ranging from simple apparatus to complex computerized systems are used in the pharmaceutical industry to acquire data to help ensure that products meet their specifications. The majority of these instruments combine a metrological function with software control.
As a precursor to the development and qualification of an analytical procedure, any analytical instrument or system needs to have its "fitness for purpose" established. Regulatory guidance in this matter can be found in USP General Chapter <1058>[32] and additionally for metrological and calibration guidance in the EDQM's technical guide for the ELABORATION OF MONOGRAPHS[33].

There are many ways of demonstrating that an instrument is qualified and under control and these can include qualification, calibration, validation, and maintenance. In order to assure "fitness for purpose" an integrated approach based upon a risk assessment is recommended.

USP General Chapter <1058> provides general guidance in a scientific, risk-based approach for carrying out an analytical instrument qualification (AIQ). Detailed instrument operating parameters to be qualified are found in the respective general chapters for specific instrument types. It is left to each laboratory to justify and document its specific approaches. The instrument owners/users and their management are responsible for assuring their instruments are suitably qualified and calibrated.

Analytical instrument qualification is not a single continuous process, but instead results from several discrete activities over the life time of the instrument. For convenience, these activities can be grouped into four phases: design qualification (DQ), installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ). Some AIQ activities cover more than one qualification phase, and analysts potentially could perform them during more than one of the phases. However, in many instances there is need for specific order to the AIQ activities; for example, installation qualification must occur first in order to initiate other qualification activities. All AIQ activities should be defined and documented.

Routine analytical tests do not constitute OQ testing. OQ tests are specifically designed to verify the instrument's operation according to specifications in the user's environment as documented in the DQ and repeating the testing at regular intervals may not be required. However, when the instrument undergoes major repairs or modifications,

[29] C. Burgess, J Hammond, *Standards in spectroscopy and spectrometry for the 21st Century; Establishing trust in measurement*, ***Spectroscopy Europe***,**20**(5), 24-26, 2008

[30] C. Burgess, J Hammond, *Exploding the Myths – an update on UV-Visible Spectrometry*, ***Spectroscopy Europe***,**21**(1), 22-24, 2009

[31] C. Burgess, J Hammond, *Exploring the 'forgotten' region – an update on NIR Spectrometry*, ***Spectroscopy Europe***,**21**(1), 19-22, 2009

[32] USP 40 2017 (1st supplement), General Chapter <1058>, ANALYTICAL INSTRUMENT QUALIFICATION

[33] Technical guide for the ELABORATION OF MONOGRAPHS, 7th Edition, EDQM, 2015

relevant OQ and/or PQ tests should be repeated to verify whether the instrument continues to operate satisfactorily. If an instrument is moved to another location, an assessment should be made of what, if any, OQ test should be repeated.

Computerised System Validation (CSV)

There is an increasing inability to separate the hardware and software parts of modern analytical instruments and systems. In many instances the software is needed to qualify the instrument and the instrument operation is essential when validating the software. Therefore, to avoid overlapping and potential duplication, software validation and instrument qualification can be integrated into a single activity.

Software used for analytical work can be classified into four categories: firmware; instrument control, data acquisition, and processing software; and standalone software.

Where applicable, OQ testing should include critical elements of the configured application software to show that the whole system works as intended. Functions to be tested are functions applicable to data capture, analysis of data, and reporting results under actual conditions of use and security and access control and audit trail. The user should apply risk assessment methodologies and leverage the supplier's software testing to focus the OQ testing effort.

Detailed advice regarding CSV may be found in the GAMP Good Practice Guide[34], GAMP 5[35] and the PIC/S Guide[36].

In addition, there has been a proposal to harmonise the GAMP and USP <1058> approaches[37]. In the proposal, the data quality triangle is expanded to include more detail as well as the responsibilities of the user with respect to the 4Qs model and the responsibility of the instrument manufacturer and supplier see Figure 11.

Both the GAMP® 5 / Laboratory GPG and USP<1058> present a risk-based approach (using categorization) for compliant laboratory computerized systems (one based on software and one on hardware) and are designed to ensure that laboratory computerized systems are fit for purpose and operated in a controlled manner to produce the expected results. In addition, the proposal maps the GAMP®5 and USP <1058> in order to provide a harmonised approach (Figure 12).

[34] GAMP® ***Good Practice Guide: Testing GxP Systems***, 2nd Edition, ISPE, December 2012

[35] GAMP® 5: ***A Risk-Based Approach to Compliant GxP Computerized Systems***, ISPE, 2008

[36] Pharmaceutical Inspection Convention / Pharmaceutical Inspection Co-operation Scheme, ***Computerised Systems in GXP Environments (PI-011-3)***, 2007

[37] L. Schuessler, M.E. Newton, P. Smith, C. Burgess and R.D. McDowall, ***Pharmaceutical Engineering***, Jan/Feb, 46-56, 2014,

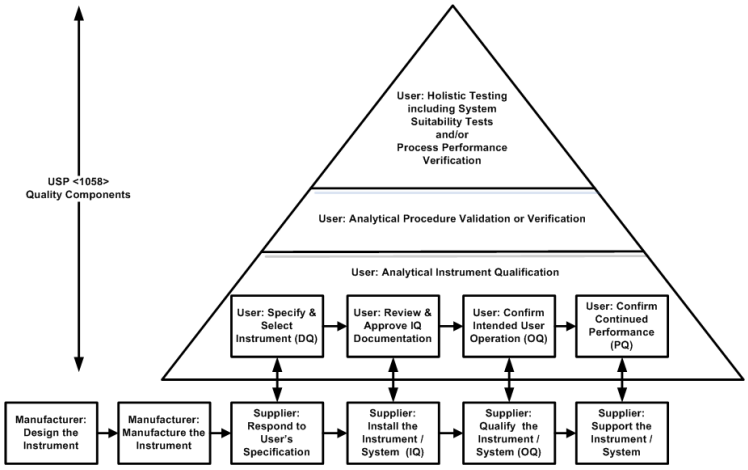


Figure 11 The Proposed update to the USP <1058> Data Quality Triangle[37]

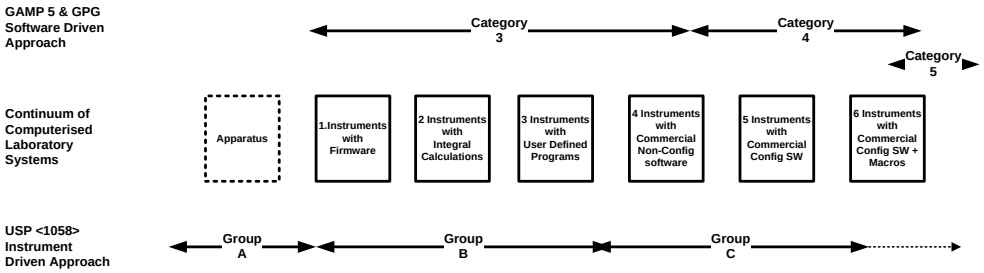


Figure 12 Mapping USP<1058> Instrument Groups and GAMP® 5 Software Categories

Reference Standards

Traceable reference standards are an essential prerequisite for assuring freedom from bias when developing and using analytical procedures. Standards are required for identity and purity of chemical entities as well as for calibration of instruments and systems. Data must be available to establish that the analytical procedures used in testing meet proper standards of accuracy, sensitivity, specificity, and reproducibility and are suitable for their intended purpose[38] and [39]. Specific holistic testing standards should also be developed by the user to ensure instrument, system and procedure suitability under routine conditions of use[40]. In the glossary of EU GMP[41], the role of reference standards in calibration is defined as;

*'The set of operations which establish, under specified conditions, the **relationship** between values indicated by a measuring instrument or measuring system, or values represented by a material measure, and the **corresponding known values of a reference standard'***

A range of reference materials is available for example see Figure 13.

[38] 21 CFR 211.165(e) and 211.194(a)(2)

[39] ***FDA Analytical Procedures and Methods Validation for Drugs and Biologics Guidance for Industry***, July 2015

[40] W B Furman, T P Layloff & R T Tetzlaff, ***J AOAC International***, 77(5) 1314-1317 (1994)

[41] https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/pdfs-en/glos4en200408_en.pdf

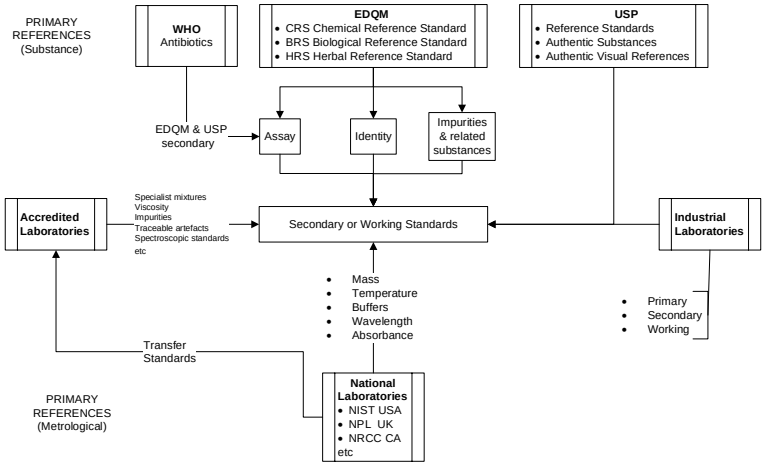


Figure 13 Examples of traceable reference materials employed in pharmaceutical analysis

Reference substances may play also an important role e.g. for the determination of a correction factor.
In many cases separate analytical procedures might be necessary in order to characterize a reference standard in a sufficient manner. Also, these analytical procedures should be scientifically sound e.g. using a reduced validation program shown to be 'fit for purpose' which could e.g. precision, linearity, and QL for the determination of an impurity.

Secondary or working standards should be qualified using appropriate statistical evaluation methods for example ISO Guide 80[42]

Sampling and Sample Management

The importance of correct sampling cannot be overemphasised[43]. Based upon the testing of a sample, the purpose of analysis is to be able to predict the properties of the batch or lot. As Deming[44] reminds us '*The object of taking data is to provide a basis for action*'.

If the laboratory test sample is not representative of the batch or bulk material, it will not be possible to relate the analytical result measured as an estimate of the analyte concentration in the original material, no matter how good the analytical procedure is nor how carefully the analysis is performed. It is important to understand that sampling is always an error generating process and that although the reportable result may be dependent upon the analytical method, it will *always* be dependent upon the sampling process[45].

A guideline for sampling principles and practices has been published by WHO in 2005[46] and from the food industry, AFFCO[47]. However, the sections dealing with reduced sampling plans in the WHO guidance have to be read very carefully to avoid misinterpretation.

Training of analytical staff

An adequate number of personnel with the necessary qualifications and practical experience in analytical chemistry are available in the laboratory. It is a requirement under the GMPs that all staff are suitable qualified and competent by ensuring that the required initial and continuing training of personnel is carried out and adapted according to need. In addition, the skill base in the laboratory should cover the statistical aspects of data evaluation and interpretation

[42] ISO Guide 80, Guidance for the in-house preparation of quality control materials (QCMs), 2014

[43] L D Torbeck, *Representative Sampling: Understanding the differences between convenience, target, and self-selected samples*, ***Pharmaceutical Technology***, **36**(4), 38-40, 2012

[44] W Edwards Deming, ***Statistical Adjustment of Data***, 1943

[45] C Burgess, ***Valid Analytical Methods and Procedures***, The Royal Society of Chemistry, 2000

[46] World Health Organization, *WHO guidelines for sampling of pharmaceutical products and related materials,* ***WHO Technical Report Series, No. 929***, 61-, Annex 4, 61-93, 2005

[47] Sample and Sample Handling Working Group, Good Samples, FDA, AAFCO, AFDO,APHL and Industry, October 2015 http:///www.aafco.org/Punlications/GOODSamples

The practical effectiveness of training should be periodically assessed. Training programmes should be available and approved by management as appropriate. Training records should be kept. Training requirements cover technical and operational aspects as well as GMP needs under the QMS.

Application of AQbD Principles in Analytical Procedures Lifecycle

The analytical procedure is a process and needs to be thought of in a process-wise way in order to design and control the analytical procedure. The principles of QbD developed for manufacturing processes can well be applied to the design of analytical procedures to ensure that they are robust, will be successfully transferred from development to the manufacturing facility and deliver quality reportable results.[48,49] A comparison of the application of QbD to production and analytical processes is shown in Figure 14 and Table 1.

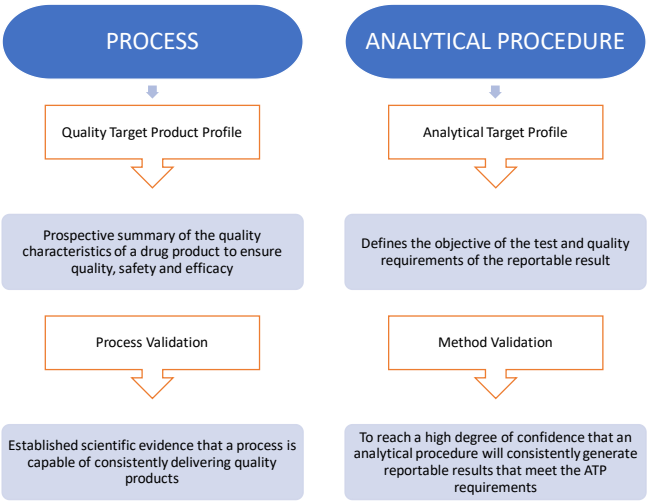


Figure 14 QbD comparative workflows for Production and Analytical applications

[48] P. Nethercote, J.Ermer, *Quality by Design for analytical methods: implications for method validation and transfer*, ***Pharmaceutical Technology***, **36**(10), 74–79, 2012

[49] D.Thompson, K. Truman, *The application of Quality by Design to analytical methods,* ***Pharmaceutical Technology***, **31**(10), 142–152, 2007

QbD for Manufacturing Process		QbD for Analytical Procedures	
QTPP	A prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality	ATP	ATP is a predefined written record of the requirements for the quality of the reportable test result that the analytical procedure must generate, in order for the procedure to be considered fit for purpose throughout its lifecycle. Sometimes referred as URS or functional requirements for test methods
Process Development Activity	The activities performed to develop a manufacturing process which will produce a product that meets the requirements of the QTTP.	Analytical Development Activity	The activities performed to develop an analytical measurement procedure which will produce a reportable value that meets the requirements of the ATP.
Process Control Strategy	A set of controls, on process parameters and attributes, facility and equipment operating conditions, in-process controls, product specifications, and the associated methods and frequency of monitoring and control	Analytical Control Strategy	A set of controls, on procedure parameters and attributes, facility and equipment operating conditions, system suitability tests, data quality, acceptance criteria, replication levels, monitoring and control.
Process Qualification	Demonstration that the manufacturing process in the commercial facility produces product that meets the specification	Procedure Performance Qualification	Demonstration that the procedure operated in a routine laboratory produces a reportable value that meets the data quality specification.
Continued Process Verification	ICH Q8(R2) describes CPV as an approach to process validation that includes the continuous monitoring and evaluation of manufacturing process performance (Q8, Q9 & Q10 Questions and Answers, Appendix Q & A's from training sessions (FDA, July 2012)	Process Performance Verification:	Ongoing assurance is gained during routine commercial use that the analytical procedure remains in a state of control during its lifecycle.

Table 1 Comparison of activities and nomenclature for QbD for Manufacturing Processes and Analytical Procedures

Application of Quality Risk Management principles over the Analytical Procedures Lifecycle

The principles of the Quality Risk Management (QRM) should be applied through all the stages of the analytical lifecycle. It starts with the identification of the method variables and evaluation of the risks to be investigated in DoE and robustness studies and continues through the design phase and transfer phase to establishment of the analytical procedure in the final QC lab. Risk evaluations should be revisited whenever a problem occurs (non-conformity, OOS or trends). In the context of analytical procedures, the "product" is the reportable result and the risks are evaluated in relation to the impact on the quality of the reportable result.

The QRM for an analytical procedure is a systematic process for assessment, evaluation, control and review of the quality of the reportable result. Risk Management shall be carried out in order to ensure that design of an analytical procedure is in accordance with the ATP and the TMU.

The risk management process[50,51] should include the activities shown in **Figure 15**.

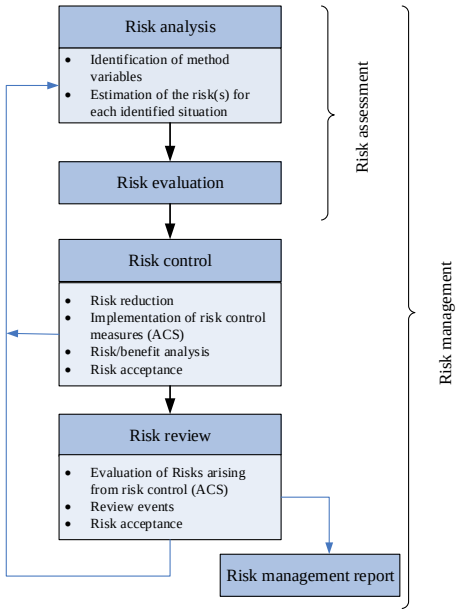


Figure 15 QRM Process Flow

The process of evaluating measurement uncertainty includes risk analysis. The Eurachem guide[52] gives good guidance on this topic including identifying the MU component. Significant risks need to be mitigated using the analytical control strategy.

An example of risk management is given in Appendix 1 Risk Management.

[50] ICH Guideline, Q9, Quality Risk Management,2005

[51] ISO Guide 14971:2012, Application of risk management to medical devices

[52] Eurachem Guide for Quantifying Uncertainty in Analytical Measurement, 3rd Edition, 2012, https://www.eurachem.org/index.php/publications/guides/quam

.

Guidance recommendations for the 3 stages of the APLM

Stage 1: Procedure Design and Development

Overview

Once the ATP and the TMU have been established, the analytical technique can be selected that provides the performance needed to meet the ATP requirements. The technique selected should ideally be available both in development and the production sites. The staff must have the necessary expertise to run the technique during routine analysis. Typical analytical techniques that are required by the specifications ICH (Q6A and B) include those designed to evaluate physical and chemical/biological properties of the drug substance or drug product including identity, assay (quantity and potency), purity and impurities and safety (for sterile products).

Some of the techniques may also be required to demonstrate stability indicating properties in which case, the ATP should also include that the analytical procedure must be able to detect physical or chemical changes due to storage conditions.

A typical process flow for APLM for Stage 1 is shown in Figure 16.Figure 16

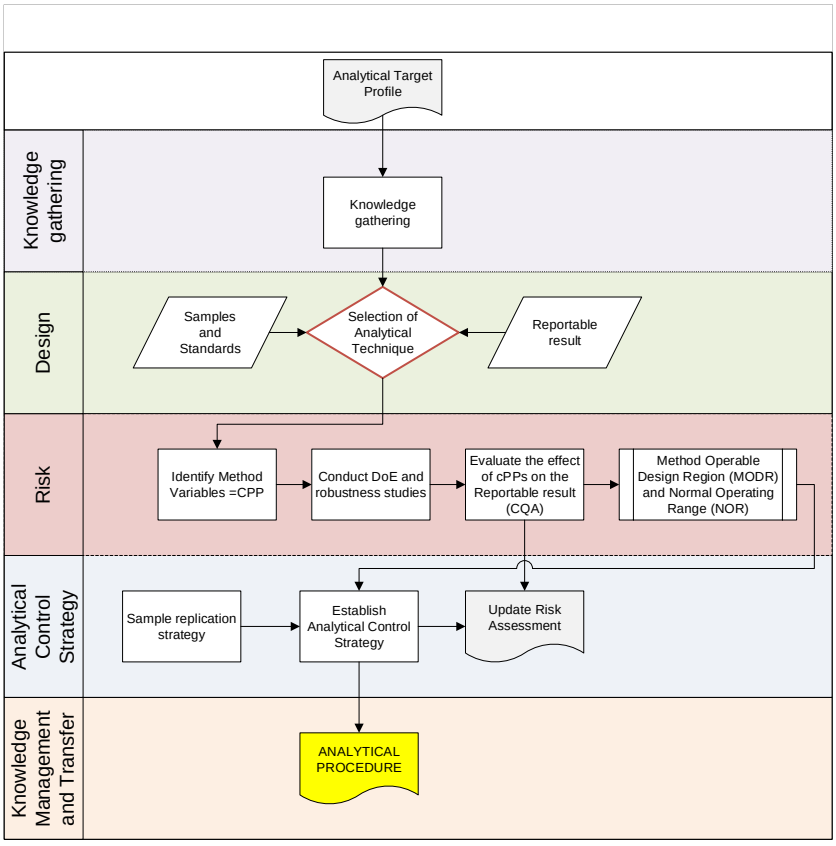


Figure 16 APLM for Stage 1 prior to Process Performance Qualification PPQ

However, it should be recognised that this is an iterative process which includes the ATP itself. It may be that knowledge and understanding gained over the development lifecycle may require modification of the ATP prior to Stage 2. This stage of the iterative process is shown in Figure 17.

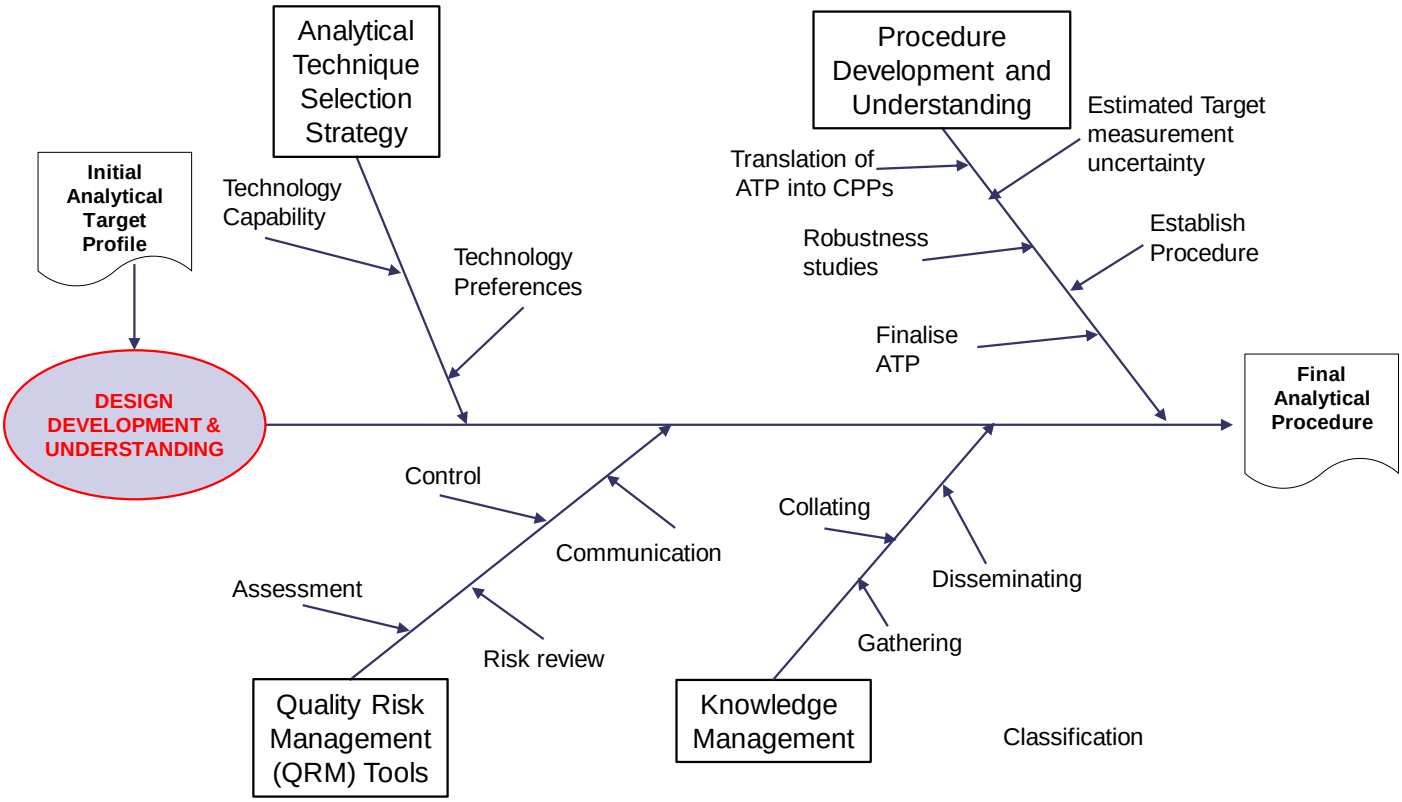


Figure 17 Stage 1 Process of APLM

As part of process development and understanding and guided by the risk assessment, the effect of analytical variables will need to be investigated over the proposed operational range. A powerful methodology to investigate the effect of these variables is Design of Experiments. It is a systematic method to determine the relationship between the variables of an analytical procedure and their impact on the analytical result. In a DoE, method variables are varied simultaneously in a structured way using, for example, a factorial design. This methodology allows for a significant reduction in the number of experiments needed to establish size of effects and interactions between the factors. Once all method variables have been identified, investigated and assessed, those evaluated as critical need to be controlled.

Variables that are known or found to impact the quality of the reportable result are to be controlled and their variance contribution minimised or limited to ranges where the impact is reduced to an acceptable level. Hence, DoEs are useful to establish the 'design space' or boundaries of the analytical procedure allowing a control strategy to limit impacts on the reportable result.

Procedure development and gaining understanding

Application of lifecycle management concepts to analytical procedures is based on QbD and provides an opportunity to use the knowledge gained from the application of scientific approaches and apply that knowledge to reportable values generated when using the analytical procedure. The concept of QbD is understood as a systematic approach that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management (ICH Q8). The quality risk management (QRM) for an analytical procedure is a systematic process for the assessment, control, communication, and review of risk to the quality of the reportable value across the analytical procedure lifecycle. It is important to understand and control sources of variability to ensure that measurement uncertainty is aligned with the decisions that will be made using results generated by an analytical procedure[53]. It is critical for procedure performance that the requirements of the decision rule and ATP (TMU) are met and continue to be met.

The benefits of this approach are;

- that the effect of bias and precision are considered together.
- that the significance of the value of a parameter, eg. linearity, calibration, bias etc. can be quantitatively assessed by comparing it with the TMU and decision rule defined in the ATP.

According to the proposed chapter on Analytical Lifecycle Procedure <1220> the following table provides an example on the impact on bias as well as on precision.

Table 2 Relevant parameters for Target Measurement Uncertainty

Target Measurement Uncertainty	
Bias	**Precision**
Selectivity	Sample preparation
Linearity	Weighing
Extraction efficiency	Instrument
Filter recovery	Integration
Detector wavelength	Background noise
Solution stability	Replicate strategy
Analyte solubility	Analyst

In the sections below, the different analytical procedure qualification attributes are described and should be established during stage 1 and confirmed in stage 2. These attributes are further described with examples in Appendix 2 Critical Analytical Performance Attributes.

[53] Proposed New USP General Chapter: Analytical Lifecycle Procedure <1220>, **Pharmacopeial Forum 43(1)** 2017

Selectivity (Specificity)

See Appendix 2; 2.1 Selectivity (Specificity)

Goodness of fit (Linearity)

Examination of the analyte response function should be performed over the specified operational range in order to check if the chosen calibration model it fit for purpose. If the signal is directly proportional to the respective concentration then a linear model is appropriate. In case signal is not directly proportional, non linear models might be appropriate.

Demonstration that the chosen calibration model, irrespectively of linear or non-linear model, will fit the data is required.

See Appendices 4 and 5.

Accuracy and Recovery over an operational range

According to ICH Q2 (R1) the accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.
See also Appendix 2; 2.4 Accuracy

Quantitation & Detection limits

See Appendix 2; 2.5 Quantitation Limit QL and 2.6 Detection Limit DL

Precision

"Precision" is one of the most important parameters to study in order to gain information about the variability of the analytical procedure.

According to the definition in the ICH Q2 (R1) Guideline the precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision, under ICH, is considered at three levels: repeatability, intermediate precision and reproducibility.

However, it is also necessary to include the system or instrument precision into this precision hierarchy. The number of measurement replicates plays an important role, especially, with respect to the discussion when "atypical results" might occur in a quality control testing. See also Appendix 2; 2.7 Precision

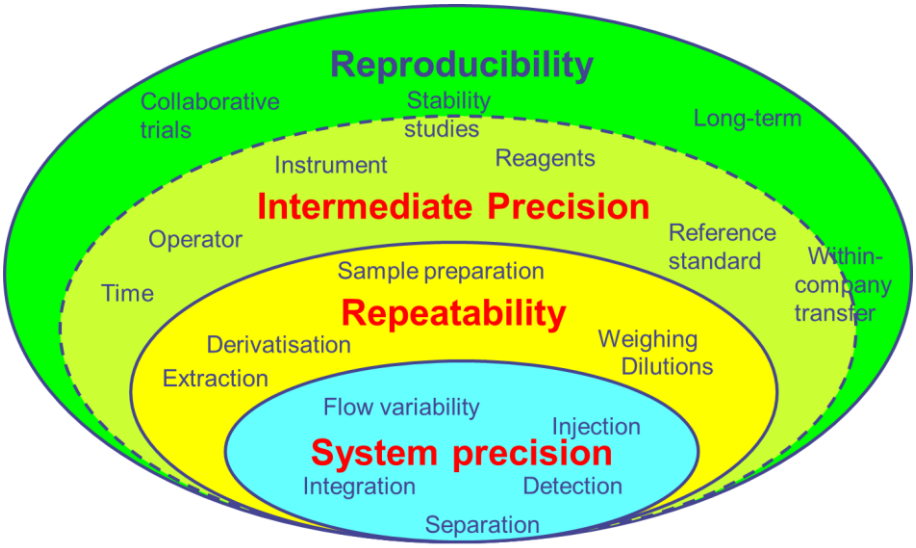


Figure 18 Precision levels with illustrative variance contributions [54]

Precision of the Reportable Result

As the reportable result is compared to the specification limits, this precision is relevant for the suitability of the analytical procedure. A replication strategy (i.e. the number of injections/measurements, sample preparation, and series) may be applied to reduce the random variability of the mean (reportable result). The intermediate precision corresponds to the precision of a single reportable result. It should be noted that increasing the number of replicates will only reduce the random variability corresponding to the step that is replicated.

Trueness

According to ISO guide 3534, trueness can be defined as the closeness of agreement between an average value obtained from a large series of measurements and an accepted reference value whereby trueness implies the lack of bias.

Establishing an Analytical Control Strategy

The analytical procedure is a process that can be defined in inputs (materials and equipment) and outputs (Reportable test result).

An example of a simplified process flow is shown in Figure 19

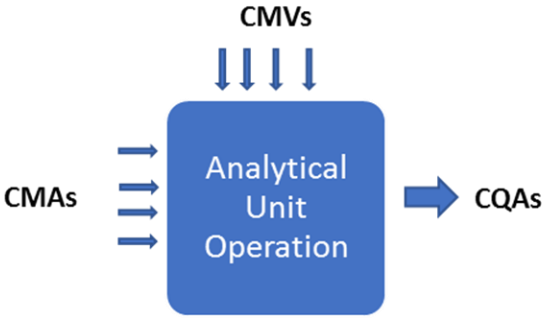
[54] Reference 39 Figure 5.6

Figure 19 Example of a simplified process flow for a spectrophotometric analysis

Defining the inputs for each process step and evaluation of the potential impact of each input variable on the reportable result (ie the output) is performed during the risk assessment. Once the critical input variables have been identified, variation in these should be mitigated via an analytical procedure control strategy.

An analytical procedure variable is critical when it can have a significant impact on the reportable result and therefore it presents a significant risk to the procedure failing the ATP.

Under this definition, the performance of an analytical procedure depends on its Critical Method Variables (CMVs) and the Critical Input Material (CMs) as illustrated in Figure 20.



CMA (Critical Material Attribute)	CMV (Critical Method Variable)	CQA (Critical Quality Attribute)
Reagents	Temperature	Accuracy
Standards	Wavelength	Precision
Buffers	Pipetting mode	
Solvents	Software (protocol)	

Figure 20 Relationships between CMVs and CQAs

The Analytical Control Strategy, ACS, is a planned set of controls, derived from understanding the requirements for fitness for purpose of the reportable value, the understanding of the analytical procedure as a process, and the management of risk, that assures the performance of the procedure and the quality of the reportable value, in alignment with the ATP, on an ongoing basis.

The ACS, will in addition, include selection of performance indicators which are intended to provide a means of verifying that the procedure is performing as expected (system suitability tests).

The Analytical Control Strategy is the mechanism for mitigating the risks identified. Typically, this covers 4 main areas as shown in Figure 21.

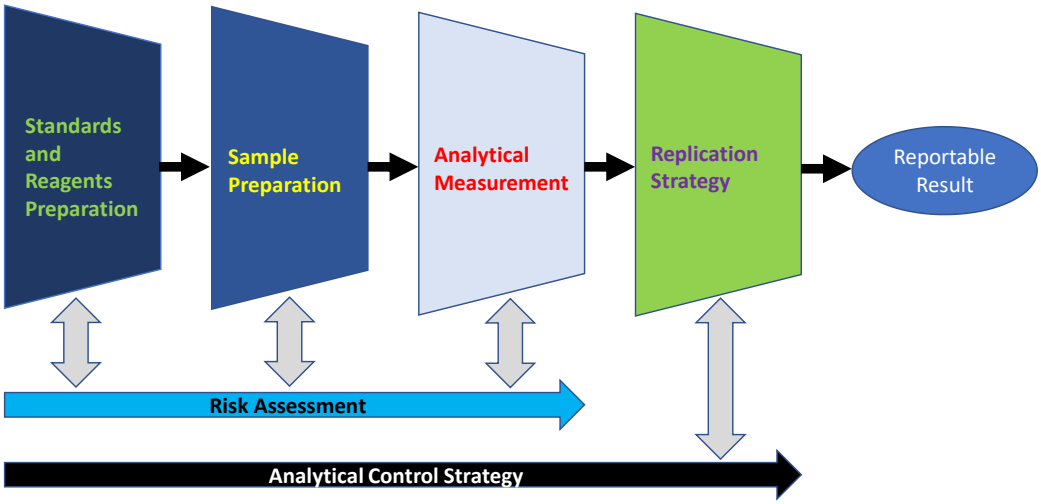


Figure 21 Elements of an Analytical Control Strategy (adapted and redrawn from [55])

A **unit operation** is any part of potentially multiple-step process which can be considered to have a single function with clearly defined boundary. Since the unit operations are sequential, and some of the process steps are linked by input-output relationships, it is important to consider controls for unit operations that have an impact on downstream processing and/or end-product quality.

Examples of controlling CMAs:

- Setting a material specification based on the effect on the Reportable result.
- Effect of material variability, including supplier variations is understood and controlled.
- Follow special requirements for the sample mixing (e.g. vortex or bend-over), storage (e.g. time, temperature, light), etc.
- Procedural control via detailed description on handling of materials

Examples of controlling sample preparation unit operations:

- Procedural control via detailed description of sample preparation steps with e.g. tolerance intervals for weighing and volume measurements
- Effect of variability of pipetting modes is understood and controlled.

Examples of controlling measurement unit operations:

- Procedural control via detailed description of measurement steps
- Instrument settings
- Calibration model
- System Suitability Tests (SST)
- Control samples

[55] E.Kovacs et al, *Analytical Control Strategy*; ***Pharmacopeial Forum* 42**(5) 2016

Establishing a Replication Strategy

Neither Risk nor Variability can be completely eliminated but they may be reduced to an acceptable level. This can be achieved by introducing a replication strategy which should be established by performing appropriate precision studies.

The impact of increasing the replicates on the precision of the reportable value depends on the corresponding variance contribution, because increasing the number of injections will have no impact on the variance of the sample preparation, for example. In this instance, it is important to perform a designed precision qualification study.

Documenting the Analytical Procedure

It is essential that sufficient information is contained in the written analytical procedure to allow a competent analyst to conduct the analysis specified and establish that it is 'fit for intended use'.

Typically, a version controlled procedure would contain, as appropriate;

1. Scope and applicability of the procedure with description of
    - Samples including stability and storage with any special requirements
    - Analytes
    - Ranges
2. Description and principle of the method especially if non standard
3. Instruments and systems required
    - Specification & range of operability
    - Calibration and qualification and validation needs
4. Reference standards and reagents
    - Specification
    - Preparation
    - Storage & expiry dating
    - Qualification procedure for critical reagents
5. Analytical procedure
    - Preparation of samples
    - Preparation of standards
    - Critical Procedure Parameters and Analytical Control Strategy
    - System Suitability Tests and acceptance criteria
    - Detailed description of all steps including critical instrumental parameters and typical outputs; chromatograms, spectra, etc.
    - Method of measurement with specified replication
6. Calculation of results and reportable values
    - Rounding and significant figures
    - Calibration model
    - Calculation formulae to be used
    - Evaluation of acceptance criteria
7. Special training requirements
8. References and bibliography

Health and Safety information should also be included if not documented separately.

Stage2: Procedure Performance Qualification

Introduction

ICHQ2 describes what characteristics\attributes of a method should be considered in validation eg specificity, precision etc. PPQ is the name given to the process of performing experiments that confirm that a method performs satisfactorily in the end user laboratory as shown in Figure 4. It has to be demonstrated that the final analytical procedure is 'fit for the intended purpose' and also will remain 'fit for the intended purpose' in the future.

With respect to the definition of the finalised analytical target profile (ATP) which defines the objective of the test and quality requirements for the reportable result, it has to be demonstrated that the analytical procedure, used under operating conditions, will meet the respective requirements.

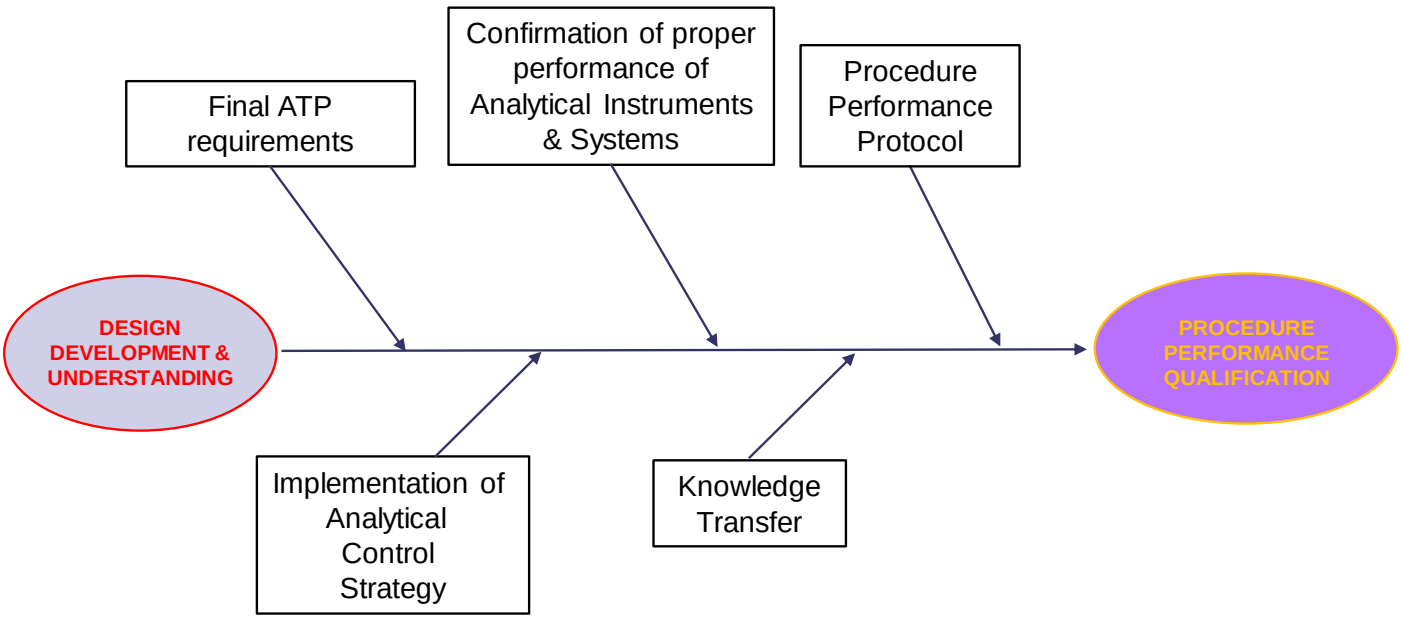


Figure 22 Transition from Stage 1 to Stage 2 of APLM

The procedure performance qualification should confirm that the analytical procedure is capable of producing accurate and reproducible data which consistently meets the ATP requirements.

Procedure performance qualification will include well designed studies in order to assess the variability of an analytical procedure in the end user laboratory.

The term procedure performance qualification (PPQ) covers the activities traditionally associated with analytical transfer as well as the implementation of compendial procedures (traditionally referred to as verification).

The proposed qualification procedure will ensure that the attributes which are considered under traditional ICH Q2 validation are addressed. However, the APLM approach focusses on generating evidence in Stage 1 that the sources of potential bias have been identified and removed as far as possible. These Stage 1 activities include ensuring selectivity (with forced degradation studies if the method is to be stability indicating) and defining a calibration model that ensures that the procedure has no bias over the intended range of use. The focus of the PPQ in Stage 2 is to identify if any bias is introduced by operating the procedure in a new laboratory and to gain an understanding of the overall precision of the procedure and ensure the Final ATP is met.

However verification is not required for basic compendial test procedures that are routinely performed such as loss on drying, residue on ignition, various wet chemical procedures such as acid value, and simple instrumental determinations such as pH measurements unless there is an indication that the compendial procedure is not appropriate for the article under test. It is assumed that analysts should have the appropriate experience, knowledge, and training to understand and be able to perform the written analytical procedure.

Qualification protocols are required during the introduction of the procedure to the end user laboratory to ensure 'fitness for purpose'. The general principles are outlined in USP General Chapter <1224>. The critical parameters to be confirmed may be a subset of those considered in Stage 2 (PPQ) for in-house procedures.

The Qualification protocol is a documented procedure that qualifies a laboratory to use an analytical procedure. This protocol ensures that the laboratory has the ability to perform the analytical procedure as intended.

The Performance Qualification Protocol contents should include;

- Scope and responsibilities
- Sample management
- Reagents, standards and instrument systems that will be used
- Detailed analytical procedure (or reference to it) together with the analytical control strategy including replication
- Experimental design to be used
- Acceptance criteria for all critical parameters
- Statistical methods to be used for the evaluation of data
- Recording and reporting of data requirements; paper and electronic
- Data integrity procedures

After execution of the protocol, the receiving unit prepares the PPQ report which;

- Summarises the lifecycle requirements of the protocol
- Documents any deviations with a justification and impact assessment
- Documents and evaluates the results obtained in relation to the acceptance criteria
- If all acceptance criteria are not met, the procedure cannot be considered qualified until effective remedial steps are adopted in order to meet the acceptance criteria

Stage 3: Procedure Performance Verification

This section focusses on the technical aspects of PPV. It is assumed that this activity is carried out under a QMS which has an efficient change control system and effective deviation management. As these aspects are essential elements of GMP they are not considered in more detail here. The purpose of PPV is to provide objective evidence that Analytical Procedures remain in a state of control or, if not, indicate when remedial actions are required to bring them back into a state of control.

Analytical Procedures as processes

Laboratory data quality management processes are a part of the overall Quality Management System as required by Chapter 1 of EU GMP and the FDA cGMPs as specified in 21 CFR §210 & §211. Analytical processes and procedures should be managed as part of a lifecycle concept. Laboratory data integrity and security are critical requirements under the GMPs. Such a process is illustrated in Figure 23.

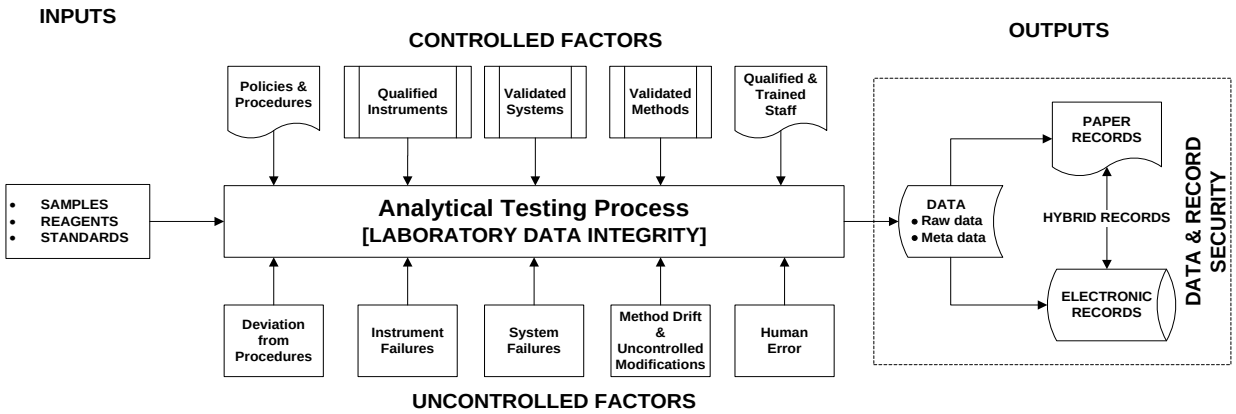


Figure 23 The Analytical Process

Requirements for routine monitoring of analytical procedures

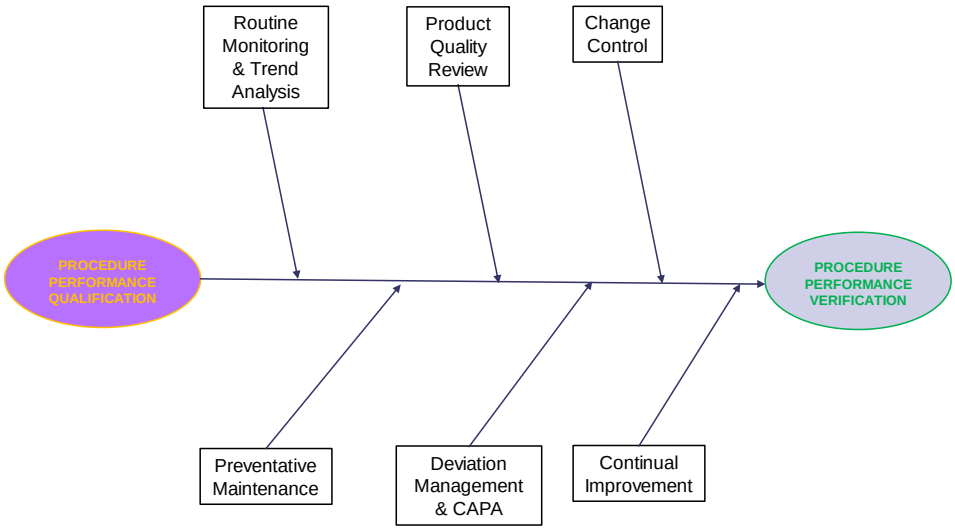


Figure 24 Transition from Stage 2 to Stage 3 of the APLM process

The purpose of Stage 3 is to generate data to show that the analytical process remains in a 'state of control' during the life cycle by;

1. verifying that the performance of the procedure or of appropriate steps within it (for example, injection and sample preparation variability) maintains an acceptable level over the procedure lifetime.
2. providing an early indication of potential procedure performance issues or adverse trends
3. identifying any changes required to the analytical procedure
4. providing confidence that the reportable result(s) generated are 'fit for purpose'.

The ongoing verification tasks should include;

1. an ongoing program to collect and analyze data that relate to analytical procedure performance
2. monitoring including tracking analytical results, system suitability failures, out-of-specification or out-of-trend investigations, stability trends, or other parameters as appropriate
3. a procedure if trend data indicate that an analytical procedure is not in control so that an investigation is performed with a goal of identifying the root cause
4. corrective and preventive actions in order to ensure that the analytical control strategy is updated or modified as required

Routine process monitoring tools

The control charting approach is based on the idea that no matter how well a process is designed there exists a certain amount of natural variability in output measurements. When the variation in process quality is due to random causes (process noise) alone, the process is said to be in statistical control. If the process variation includes both random and special causes of variation, the process is said to be out of statistical control. The purpose of any control chart is to detect the presence of special causes of variation or trends (OOT).

A trend is a sequence of time related events. Trend analysis refers to techniques for detecting an underlying pattern of behaviour in a time or batch sequence which would otherwise be partly or nearly completely hidden by noise. These techniques enable specific behaviours (OOT; Out of Trend) such as a shift, drift or excessive noise to be detected.

Analytical process data, where the expectation is that there is no trend, is monitored to confirm that it is under statistical control.

Trend analysis using statistical process control tools

Conceptually, a control chart is simply a plot of a response variable against time or analytical run whereby the degree of variation is predicted by the chosen distribution (mathematical model) around a mean or target value. Hence for a continuous variable which is assumed to be normally distributed the trend plot is shown as the normal distribution with respect to time. The decision rules regarding an out of trend result come from the likelihood of the pattern of responses or the distance from the target or mean value. An idealised concept is shown in Figure 25.

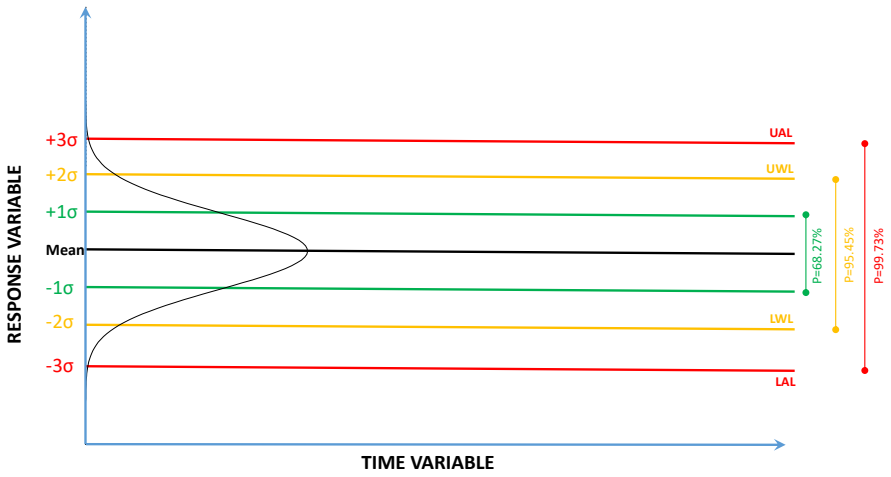


Figure 25 Idealised control chart for a normal distribution

A more detailed description of the tools and techniques applicable for Stage 3 together with commonly used decision rules are given in the ECA AQCG LABORATORY DATA MANAGEMENT GUIDANCE Out of Expectation (OOE) and Out of Trend (OOT) Results[56].

Process Quality Metrics

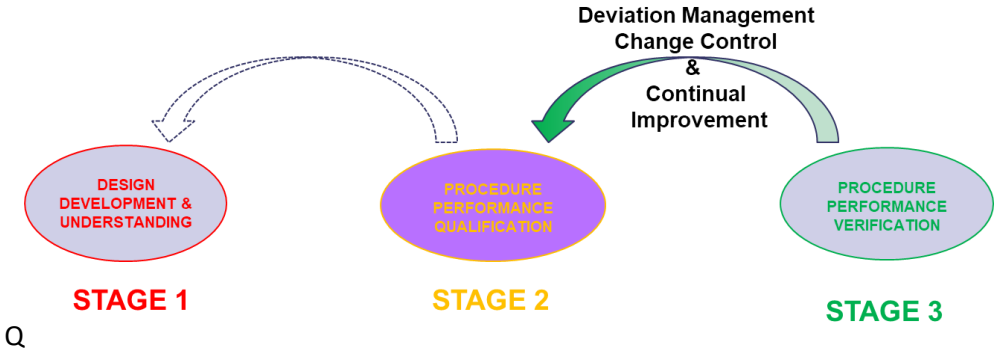
It is important to be able to quantify procedure performance and capability. An overview of a number of these measures is given in Appendix 6.

Application of APLM to existing analytical methods and procedures

There are a large number of existing legacy procedures which have not been developed in accordance with the principles and processes described in this guideline. These include the majority of Pharmacopeial methods. The question arises as to what needs to be done with such methodologies. In practice, Stage 3 of the lifecycle is the most important aspect for legacy procedures.

During the conduct of Product Quality Review (PQR), the performance evaluation of analytical methodologies is required especially with respect to deviations and change control[57].

The prioritisation of problematic procedures identification, as part of PQR, and their remediation strategies will be based upon a risk assessment and a business case.



Q

Figure 26 Remediation flow for problems identified in Stage 3

Issues may be resolvable by modifications to the analytical control strategy and minor technical changes triggering a repeat of PPQ. However, the possibility exists that a redevelopment or redesign is required in some instances necessitating a new Stage 1.

[56] ECA _AQCWG_ SOP 02_OOE & OOT_v1.1_November 2016_rev10 available from the member's area on the ECA website

[57] "The Rules Governing Medicinal Products in the European Union", Volume 4, Good Manufacturing Practice (GMP) Guidelines 2017, Chapter 1 Quality Management System 1.10; Product Quality Review

Technical Glossary

The analytical terms are defined primarily according to guidelines (ICH) and (FDA) and international standard (VIM) and the wording is taken from the references.

Analytical term	Definition	Reference
Abridged validation	Reduced validation performed on selected validation parameters	-
Acceptance criteria (validation)	Numerical limits, ranges, or other suitable measures for acceptance of the final test result of a validation parameter. Acceptance criteria are defined based on the product critical quality attributes, customer requirements, specifications (regulatory) and product and process tolerances.	-
Acceptance criteria (specification)	Numerical limits, ranges, or other suitable measures for acceptance of the final reportable results of analytical procedures.	FDA
Accuracy	The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. It is typically expressed as percent recovery or percent inaccuracy compared to the theoretical value. The error associated with inaccuracy is referred as Bias or systematic error.	ICH
Analyte	Is a substance or chemical constituent that is of interest in an analytical procedure	Wiki
Analytical Control Strategy (ACS)	Elimination or limitation of variables that are known or found to have an impact on the quality of the reportable result and selection of performance indicators which are intended to provide a means of verifying that the procedure is performing as expected ("system suitability" tests). The combination of input controls and performance indicators as components of a control strategy will make sure the risk of harm to the quality of the reportable value will not exceed the TMU thus ensuring that the ATP will be met on ongoing basis	
Analytical procedure (AP), Also called Test Method Procedure	The analytical procedure refers to the way of performing the analysis. It must describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus (equipment), generation of the calibration curve, use of the formulae for the calculation, etc. Also referred as Test Method Procedure.	ICH
Analytical run	The act of performing analytical tests according to the analytical procedure one time, which is one execution of the analytical procedure.	-
Analytical Target Profile (ATP)	ATP is a predefined written record of the requirements for the quality of the reportable test result that the analytical procedure must generate, in order for the procedure to be considered fit for purpose throughout its lifecycle of use. Sometimes referred as URS or functional requirements for test methods	USP
Blank	Sample with no analyte present. Often a sample of dilution buffer.	-
Blank signal (background signal)	Signal obtained from a phenomenon, body, or substance similar to the one under investigation, but for which a quantity of interest is supposed not to be present, or is not contributing to the signal.	VIM
Calibration curve	Expression of the relation between signal and corresponding quantity value.	VIM

Analytical term	Definition	Reference
Calibrators	Analyte in predefined solution which is used as a measurement base for similar substances	-
Co-validation	Validation performed in collaboration between departments/sites.	-
Critical Material	A material that should be controlled as its lot -to-lot variation may impact the reportable result. Determined during risk assessment.	
Critical Method Attributes (CMA)	Also, referred as Critical Quality Attributes (CQA). A method parameter that should be within an appropriate limit, range, or distribution to ensure the desired reportable test result. Typical CMAs are specificity, precision, accuracy and sensitivity.	-
Critical Method Variables (CMV)	Also referred as Critical Process Parameters (CPP). Method variables that have an impact on the critical method attributes and the reportable test result. Critical Method Variables are identified from a list of potential CMVs using risk assessment and experimental work. A CMV could be e.g. time or temperature in an analytical incubation step.	-
Continued Procedure Performance Verification (CPPV)	To assure continually that the performance of the analytical procedure remains in a 'state of control' (the validated state) during routine testing.	
Cross reactivity	Non-specific response of a test method to components in the sample other than the target analyte	-
Decision rule	A documented rule ... that describes how measurement uncertainty will be allocated with regard to accepting or rejecting a product according to its specification and the result of a measurement.	ASME B89.7.3.1-2001 (reaffirmed 2006)
Degradant	Components generated by degradation of the drug substance or product.	-
Design Space	Also referred as Operating Range or Method Operable Design Range (MODR) The multidimensional combination and interaction of input variables (e.g. materials) and method variables (e.g. temperature) that have been demonstrated to provide assurance of quality of the reportable result. Working within the design space is not considered as a change.	
Detection limit (DL)	The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Note: Detection limit is sometimes also referred to as limit of detection (LOD).	ICH
FMEA	Failure Modes and Effects Analysis	
Identification	To ensure the identity of an analyte.	ICH
Interference	Changed response as a function of the presence of non-target components (e.g. matrix) in the sample material/test sample.	-
Intermediate result	Result generated in the process from measurement result(s) to the final reportable result.	-
Intermediate precision	Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.	ICH
Intermediate precision condition	Condition of measurement, out of a set of conditions that includes the same measurement procedure, same location, and replicate measurements on the same or similar objects over an extended period of time but may include other conditions involving changes.	VIM
Linearity	The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.	ICH

Analytical term	Definition	Reference
Matrix	Components in the sample other than the analyte.	-
Measurement Uncertainty (MU)	Measurement error (i.e. uncertainty and bias) associated with the measured value and describes the difference between the true value and the measured value	
Precision	The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions (every measurement encompass all steps from sample preparation to final reportable result). Precision may be considered at three levels: • Repeatability • Intermediate precision • Reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.	ICH
Procedure Performance Qualification (PPQ)	To reach a high degree of confidence that an analytical procedure will consistently generate reportable results that meet the ATP requirements.	
Qualitative test result	A result reported as 'Yes/ no' or 'present/ not present results. Predominately used for identification purposes and often linked to a descriptive scale 'granulated', 'blue', 'diffuse lines'.	-
Quality by Design (QbD) of Methods	A systematic approach to development that begins with predefined objectives (ATP) and emphasizes product and process understanding and process control, based on sound science and quality risk management	
Quantitation limit (QL)	The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products. Note: Quantitation limit is sometimes referred to as limit of quantitation (LOQ).	ICH
Quantitative test result	A numerical test result that quantifies the analyte of interest in the sample.	-
Range (of the analytical procedure)	The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.	ICH
Repeatability	Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.	ICH
Repeatability condition	Condition of measurement, out of a set of conditions that includes the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time.	VIM
Replicates	Repeated measurements of a given sample.	-

Analytical term	Definition	Reference
Reportable result	The reportable result is the final test result which is reported for a sample. The reportable value, which often is a summary value for several individual determinations, is compared with the acceptance criteria. The reportable result might be obtained by replicate testing of the sample and includes calculating a mean based on intermediate results, multiplying by the dilution factor, rounding etc.	-
Reproducibility	Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardisation of methodology).	ICH
Robustness	The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.	ICH
Semi-quantitative test result	A test result reported as 'greater than', 'smaller than', or' equal to' a reference or a control analysed simultaneously with the sample. For some biological tests the scale used is a combination of both qualitative and semi-quantitative measures e.g. 'weak positive', 'dark blue'.	-
Sensitivity	May be expressed as Detection Limit and/or Quantitation Limit or by means of low control samples included during development and Method Design, as required by the type of Analytical Procedure.	
Specificity	Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).	ICH
Spiking	The addition of a small known amount of a known compound to a standard, sample, or placebo, typically for the purpose of confirming the performance of an analytical procedure or the calibration of an instrument.	-
System Suitability Tests (SST)	Tests 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 to be established for a particular procedure depend on the type of procedure being validated.	ICH
Test	General term for analytical procedures that provide information on a specific characteristic of the sample (e.g. identification test, purity test).	ICH
Target Measurement of Uncertainty (TMU)	Also referred to as Total (Analytical) Error or uncertainty. The maximum uncertainty that the data should have in order to maintain acceptable levels of confidence in data quality. The combined impact of precision and bias errors that can affect the accuracy of the test result.	-
Unit Operation	A unit operation is any part of potentially multiple-step process which can be considered to have a single function with clearly defined boundary	
Verification of Test Method	Demonstration that a previously validated method, normally well established, can meet the requirements e.g. for precision, accuracy for the intended use.	-

Appendix 1 Risk Management

1.1 Risk Assessment

This is a stage of learning and understanding what the hazards are (method variables) that influence the quality of the reportable result. There are two steps in Risk Assessment; Risk Analysis and Risk Evaluation:

1.1.1 Risk Analysis:

Systematic use of available information to identify all method variables and estimate the risks associated with those method variables. Experience and prior knowledge together with tools such as Ishikawa diagrams (Fishbone or Cause and Effect diagrams) are useful in identifying analytical method variables such as material variables related to reagents or reference standards, equipment variables, environment variables (e.g. temperature), training, procedural and measurement variables (e.g. instrument accuracy). Risk identification addresses the question: What might go wrong? Risk analysis estimates the risk associated with the identified hazards and addresses the questions: What is the likelihood (probability) it will go wrong? and Will I be able to detect (measure) if it goes wrong?

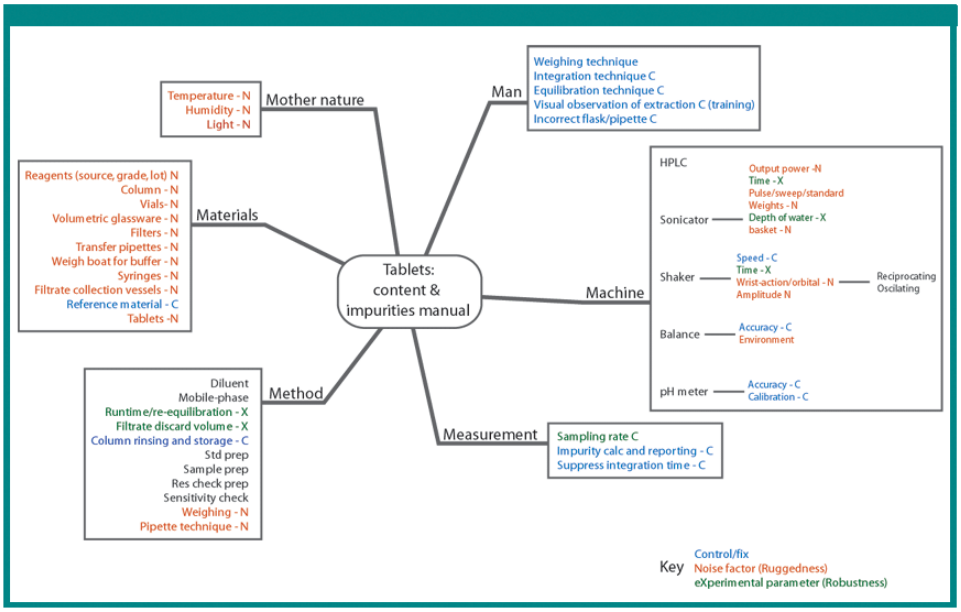


Figure 27 Example of risk identification and mapping taken from Borman et al[21]

1.1.2 Risk Evaluation:

This is the process of comparing the estimated risk against predetermined acceptability of the risk. Once the variables have been identified, these should be evaluated in relation to the impact on the quality of the reportable result, i.e. the CQAs. Critical quality attributes of the reportable result could be e.g. precision and accuracy. Risk evaluation addresses the question: Is the identify risk acceptable or does it require mitigation?

Risk evaluation and assessment can be performed using tools such as a priority matrix and Failure Mode and Effect Analysis (FMEA)

In the example shown in Figure 17**, a 3-factor FMEA** is used. The FMEA is based on the evaluation of the impact of each method variable on the **Severity** (S), as well as the **Weighing Factor** (W) on the method CQAs, the **Probability** (P) of Occurrence and **Detectability** (D) of the failure. Each of the factors can be scored as follows:

The weighing factor is defined in relation to each CQA (e.g. precision, accuracy and selectivity) and how important is the CQA for the quality of the final reportable result. The weighing factor is linked to the type of the analytical procedure as defined by ICH Q2 (Identification, limit test for impurities, quantitative test for impurities and Assay). For example, an analytical procedure used for Identification will have a weighing factor of 5 for the CQA Selectivity, whereas precision and accuracy are less important (weighing factor of 1). Thus, some method variables may impact the precision of the analytical procedure (severity 9) but may not be relevant for the overall impact on the reportable result.

Weighing Factor	**Description**
1	Small impact on overall quality of the reportable result
3	Intermediate impact on overall quality of the reportable result
5	High impact on overall quality of the reportable result

Severity = Effect of the method variable on the method CQAs (e.g. precision, accuracy and sensitivity)

Severity	**Importance**
1	The potential failure has a low impact on this method CQA - CQA is affected by a severe failure in method variable
3	The potential failure has an intermediate impact on this method CQA - The CQA factor is affected significantly by severe failure in method variable
9	The potential failure has a high impact on this method CQA - The CQA factor is affected significantly by minor failure (very sensitive to method variables)

The overall Severity (S) is calculated by the sum of (severity of each CQA (S_i) x weighing factor (W_i)) divided by the sum of weighing factors.

$$Severity = \frac{\sum_{i=1}^{CQA} S_i * W_i}{\sum_{i=1}^{CQA} W_i}$$

Probability = Likelihood of Occurrence of the failure. It is related to method knowledge and controls. Includes uncertainty for new processes or process changes

Score	Frequency of occurrence
1	Highly unlikely: Theoretically possible, but practically nearly impossible. This failure has never been observed in the past.
2	Remotely possible: The failure very rarely occurs. May have been observed in past analytical runs, but excellent measures are in place to prevent occurrence.
3	Occasional: Failure occurs from time-to-time, but not systematically. Measures are not fully effective to avoid failure.
4	Probable: Failure occurs with regular intervals. Measures are either not effective or non-existent.
5	Frequent: It is nearly certain that the failure will occur on a larger scale. No measures are in place to prevent failure.

Detectability = Ability to Detect a Failure. Appropriateness and capability of analytical procedure.

Score	Probability of detection
1	Very high: The failure is detected every time immediately after or during the analytical procedure step by a full automated process. The tested sample is automatically rejected.
2	High: The failure is detected shortly after or during the analytical procedure step. Human action or decision is involved in the testing process (although the actual test may be automatic).
3	Medium: The failure is not detected every time, or only a long time after the analytical procedure step has been completed (e.g. test taking a long time to perform such that the next analytical procedure step has already started).
4	Low The ACS measures in place are not appropriate to detect the failure.
5	Very low: Detection is impossible or not done.

The priority of the risk is defined by the combination of Severity, Probability and Detection. The overall Risk Priority Number (RPN) is calculated from S. P and D and normalized to 100.There has to be a predetermined acceptance criterion defined which triggers the need for a control or mitigation strategy

Risk Factor = Severity x Occurrence x Detectability, i.e.

RPN = S x P x D / 2,25

The following are considered hazardous situations:

- RPN >= 27
- (Weighting factor) x (Severity score) = 45

When the RPN number is higher than 27 there is a high average severity coupled with an occasional failure and medium detection. If the a CQA is weighted as having a high impact on overall quality of the reportable result, and the failure under analysis estimated to have a high impact on the CQA factor, then this failure is considered so critical irrespective of other factors that it should be escalated and proper measures identified that mitigate the failure. Risk Assessment should be conducted throughout the entire life cycle of the analytical procedure.

Analytical step ID	Analytical step	Potential Failure	Effect of failure	Selectivity	Sensitivity	Precision	Accuracy	Severity	Causes of failure	Mitigating actions currently in place: Preventative action	Mitigating actions currently in place: Testing action	Frequency of occurence	Detectability	RPN
Critical Quality Attributes (CQA's):														
Weighting factor				3	5	1	1							
				0: none 9: high								1: low 5: high	1: high 5: low	
1	Sample preparation with biotinylation	Too low biotin added	Can lead to lower OD response. Will affect test curves below the LOD. Thus, not possible to calculate purity	1	9	3	3	5.4	wrong calculation of amount of Biotin needed	Template has a check of calculations	Major errors can be captured by visual analysis of test curves and by comparing with previous batches	2	3	14
		Too much biotin added	Can lead to higher OD response. Will affect the reference and test material equally. Calculation of purity possible	1	1	1	1	1.0	wrong calculation of amount of Biotin needed	template has a check for calculations		2	3	3
			Can mask antigen binding sites. Possible false negative or low results	9	9	3	3	7.8	The amount of cross-reacting antibodies is not constant and thus some of them might be over biotinylated due to different biotin/antibody molar ratios	The overall molar ratio Biotin/Ab is kept constant via procedure in order to ensure consistency between tested batches.	N/A	2	4	28
2	Coating: Preparation of coating sample	Concentration too high >10 µg/mL	Can potentially lead to "loss-bound antigen" and non-effective binding of test sample. Failure will affect reference and test material equally. Calculation of purity possible	1	1	3	3	1.4	Error in preparation of coating layer solution	When the well is fully saturated, excess material will be removed in the subsequent washing step.	Major errors can be captured by visual analysis of test curves and by comparing with previous batches	2	2	2
		Concentration too low <2 µg/mL	Failure may affect reference and test material differently: 1)Not enough antigen to capture all reference antibody giving lower signal, 2) Not enough time/temperature to establish equilibrium with low antigen/test Ab	3	9	3	3	6.0	Error in preparation of coating layer solution	Concentration tested during assay development, securing that the chosen concentration gives robust results. template has a check for calculations		2	3	16
3	Addition of coat layer	Mix-up of antigens	Wrong cross-reacting curves or not on the expected postion on the plate	3	3	1	1	2.6	Error in addition of coating layer solution on the plate	PX129 template has a column to write the actual position where the antigen is added to the plate	Major errors can be captured by visual analysis of test curves and by comparing with previous batches	3	2	7

Figure 28 Example of a risk assessment using FMEA for an ELISA analytical procedure to measure low level of impurities (only the first 3 steps are illustrated)

1.2 Risk Control

After the Risk Assessment has been conducted, all identified risks should be reduced. Risk Control measures should be implemented to reduce each identified risk As Far As Possible (AFAP) or to an acceptable level.

All Risk Control options should be applied (if possible and still contributing to the risk reduction). The amount of effort used to mitigate the risk should be proportional to the importance of the risk and the benefit of the mitigation procedure. Implementation of the risk control measure and its effectiveness should be determined, verified and documented.

In the case that some of the risks cannot be mitigated or reduced to an acceptable level, the analytical procedure should be redesigned and the risk management process repeated.

1.3 Risk Review

After the Risk Control measures are applied, a Risk/Benefit analysis shall always be made. All residual risks shall be combined (accumulated) and the resulting risk shall be balanced against the benefit of analytical procedure. Risk review should be an on-going part of the quality management process. The performance of the analytical procedure should be reviewed on a regular basis and as part of the monitoring process in Stage 3.

1.4 Risk Report

The purpose of the risk report is to summarise the risk assessment and mitigation actions put in place.

Appendix 2 Critical Analytical Performance Attributes

The discussion on criticality begins in the development stage and it is important that new analytical procedures is appropriate for the intended use and should fulfil the scientific requirements[58] whilst keeping the respective business requirements in the focus, e.g. handling and costs.

For example, HPLC-UV/DAD is a technique which often allows a selective separation of impurities with a good sensitivity and the possibility to determine the variability by performing precision and/or accuracy experiments. However, this analytical technique also is time consuming with respect to the experimental work beginning with a sample preparation, the chromatographic procedure itself up to the evaluation of the analytical data.

On the other hand, NIR can in some instances be directly applied for a determination of an assay without a sample preparation obtaining analytical results immediately after the measurement. However NIR, does not allow the definition of variability based on precision and /or accuracy experiments in the same way as for HPLC.

Also in Stage 1, appropriate forced degradation studies should be performed especially for chromatographic procedures used for the determination of organic impurities and assay. Stress testing is necessary to establish a basis for sufficient selectivity which is a prerequisite for developing a stability indicating analytical procedures.

In addition, it should be taken into account that robustness testing is still part of stage 1 of the APLM process and may reveal critical aspects with respect to variability. In the case of HPLC, important method parameters include e.g. mobile phase composition, pH of mobile phase and column temperature. In the case of NIR, other parameters are likely to be critical such as sources of excipients in a drug product formulation in order to determine (e.g.) an assay of a drug product.

[58] J.Ermer, P Nethercote (Eds), ***Method Validation in Pharmaceutical Analysis***, 2nd Edition, Wiley, 192ff, 2015 (ISBN978-3-527-33563-3)

	Type of analytical procedure				
Characteristics	Identification	Testing for Impurities		Assay (dissolution (measurement only), content / potency)	Other analytical procedures, e.g. sulphated ash
		Quantitation	Limit test		
Accuracy	-	+	-	+	
Repeatability Precision	-	+	-	+	+
Intermediate Precision	-	+ (1)	-	+ (1)	
Specificity/ Selectivity (2)	+	+	+	+	
Detection Limit/Sensitivity	-	- (3)	+	-	
Quantitation Limit	-	+	-	-	
Goodness of fit/Linearity	-	+	-	+	
Stability/Forced degradation					
Range	-	+	-	+	

Table 3 Analytical Performance Characteristics required by procedure type to be confirmed during PPQ

Key to Table 2

- signifies that this characteristic is not normally evaluated

+ signifies that this characteristic is normally evaluated

(1) in cases where reproducibility has been performed, intermediate precision is not needed

(2) lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)

(3) may be needed in some cases

2.1 Selectivity (Specificity)

According to the current ICH Guideline Q2(R1), specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix etc.

However, there is much discussion with respect to the correctness of the term selectivity vs. specificity.

For chromatographic procedures it can be clearly stated that the selectivity can be described as the ability of the analytical procedure to separate all relevant impurities from the main component as well as from all other relevant analytes.

Also in case of ICP-OES or ICP-MS selectivity can be demonstrated using a "False-positive-test" in order to demonstrate the presence of elemental impurities using spiking experiments.

The definition of "specificity" is more difficult e.g. with respect to titration methods for which it only can be demonstrated that the titration is not influenced by any solvents or chemicals used for the respective application. In case of titration specificity can only be demonstrated by checking the influence of matrix, drug substance and drug product.

Identification

- To establish the identity of an analyte. Suitable tests should be able to discriminate between compounds of closely related structures which are likely to be present.

Examples for the identification of a drug substance are e.g. Infrared Spectroscopy (IR) identifying the drug substance using a comparable infrared spectrum of the certified reference standard or identifying characteristic wave numbers in the infrared spectrum.

Other identication tests are generally e.g. polarimetry, melting point or UV-spectra which are not considered citical with respect to qualfication.

In case of a drug product, identication may be simply carried out, e.g using liquid chromatography, by comparing the retention times with the certified reference standard.

Impurity profiling

Impurity profiling includes identification structure elucidation and quantitative determination of related organic impurities as well as degradation products in drug substances and pharmaceutical formulations. Impurity profiling is very important to not only identify relevant impurities but also potentially toxic impurities. Identification is generally performed by HPLC and should be supported by hyphenated and / or orthogonal techniques e.g. using HILIC, HPTLC or SFC.

2.1.1Stress testing

According the ICH guideline ICH-Q1A(R2): stress testing is defined as:
'Stress testing helps to determine the intrinsic stability of the molecule by establishing degradation pathways in order to identify the likely degradation products and validate the stability indicating power of the analytical procedure'

The FDA-Guideline on Analytical Procedures and Method Validation specifies :

"To demonstrate specificity of a stability-indicating test, … a combination of challenges should be performed. Some challenges include the use of samples spiked with target analytes and all known interferences; samples that have undergone various laboratory stress conditions; and actual product samples that are either aged or have been stored under accelerated temperature and humidity conditions."

Stress test conditions for the drug substance can be:

- with respect of variation of temperature and humidity:
  e.g. 25°C/ 60%RH, 25°C/75%RH, 30°C/70%RH, 40°C/75%RH, 40°C, 50°C, 60°C, 70°C
- pH - range might be varied in a more detailed plan, if necessary
  e.g. 0.1 M citric acid/0.2M disodium phosphate, pH adjusted with 0.01M NaOH)
- oxididation, e.g.using (0.3%)peroxide solutions
- photolysis according the ICH guideline Q1B (UV light exposure)

Storage time of the samples are dependent on the speed of degradation. This is generally between 2 days and up to 12 weeks. Ideally, 5 to 15 % degradation should be achieved in order to assess whether the analytical procedure is stability indicating.

Depending on the speed of degradation, it is recommended to perform the analytical determinations after at least four time points (incl. zero value) in order to get sufficient information.

Mass balance should be checked in order to compare the sum of degraded compounds with the main component.

The calculation of the mass balance using the sum of all impurities above a reporting threshold may be appropriate.

Photostability testing according to ICH Q1B[59] should be also performed for the drug product

- Tests on the exposed drug product outside of the immediate pack
- If necessary: Tests on the drug product in the immediate pack
- If necessary: Tests on the drug product in the marketed pack

Peak purity

Peak purity in HPLC can be checked by using a photo diode array detection. In this case peak purity means the spectral homogeneity of a chromatographic peak compared to a known component.

Alternative analytical procedures such as TLC, HPLC-MS can be used in order to check the selectivity of the main analyte and its impurities. Specialist techniques such as qNMR can also be used

[59] ICH Q1B, Stability Testing: Photostability Testing of New Drug Substances and Products, 1996

2.2 Setting acceptance criteria based on fitness for intended purpose

It is the aim of the APLM approach to set acceptance criteria based on how much uncertainty can be tolerated in the reportable result. Current guidance on analytical procedure validation often includes suggested criteria and experimental plans for attributes such as accuracy and precision. This section includes examples based on current guidance.

However, it must be emphasised that a fundamental aspect of the APLM approach is that acceptance criteria should be derived based on how much uncertainty in the reportable is acceptable and should not be based on arbitrary numbers.

Range:

According to the current ICH guideline the following ranges are defined and recommended:
The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

According to the ICH Guideline Q3A (R2), a reporting threshold (RTh) was introduced. ICH defined reporting thresholds above which the analytical result have to be reported quantitatively. As a consequence, a reporting threshold of 0.05 % means that amounts of ≤ 0.05 % are not reported. Therefore, the quantitation limit should be at least equal to or less than the RTh.

Furthermore, it should be pointed out that for elemental impurities as well as for residual solvents a different process compared to the determination for organic impurities should be set. The guidelines Q3C for residual solvents and Q3D for elemental impurities give limits with respect to the toxicological concern.

In other words, precision, accuracy and linearity should be demonstrated within or at the extremes of the specified range of the analyte in the sample matrix..

Assay:	80 – 120% of test concentration
Content uniformity:	70 – 130% or as *required*
Dissolution:	+/- 20% over the *specified* range
Impurities:	Reporting threshold to 120% of the specification (minimum requirement)

Note: It should be pointed out that, especially, for ICP-MS or ICP-OES larger ranges for the elemental impurities are described in the respective pharmacopoeias.

There are different recommendations to set acceptance criteria for impurities:

Ermer has proposed acceptance criteria [60] based on data from a book edited by J.M. Miller & J. B. Crowther[61] of concentration as a function of the reporting threshold and an average recovery compatible with the attainable precision.

Concentration range	Repeatability	Intermediate precision	Average recovery
< 2 x RTh	≤ 25.0 %	≤ 30.0 %	70.0 - 130.0 %
2 x RL – 10 x RTh	≤ 15.0 %	≤ 20.0 %	80.0 - 120.0 %
10 x RL – 20 x RTh	≤ 10.0 %	≤ 15.0 %	85.0 - 115.0 %
>20 x RTh	≤ 5.0 %	≤ 10.0 %	90.0 - 110.0 %

Another recommendation to provide acceptance criteria was described in the Australian TGA Guideline[62] for veterinary chemical products:

Accuracy: % Active/ impurity content	**Acceptable mean recovery**
≥ 10 %	98 – 102 %
≥ 1 %	90 – 110%
0.1 – 1 %	80 – 120%
< 0.1 %	75 – 125%
Analytes measured in samples	**Precision**
≥ 10,0 %	≤ 2 %
1.0 up to 10.0 %	≤ 5 %
0.1- up to 1.0 %	≤ 10 %
< 0.1 %	≤ 20

For a main component peak assay, the following acceptance criteria are suggested for guidance only:

HPLC:

Accuracy: 98.0 – 102.0 %

Repeatability: RSD ≤ 1.0 % (≤ 2.0 % in case of e.g. poor solubility of the drug substance may be accepted)

Intermediate precision: RSD ≤ 1.0 % (≤ 2.0 % in case of e.g. poor solubility of the drug substance may be accepted)

[60] J Ermer, P Nethercote (Ed.) , ***Method Validation in Pharmaceutical Analysis***, 2nd Edition, Wiley -VCH, page 106, 2015

[61] J.M. Miller, J. B. Crowther (Ed.), ***Analytical Chemistry in a GMP Environment, A Practical Guide***, John Wiley, N.Y. 2000, p.448-449:

[62] Guidelines for the Validation of Analytical Methods for Active Constituent, Agricultural and Veterinary Chemical Products; Australian Pesticides and Veterinary Medicines Authority, Oct. 2004

Titration, qNMR, NIR:

Accuracy: 99.0 – 101.0 %

Repeatability: RSD ≤ 1.0 %

Intermediate Precision: RSD ≤ 1.0 %

Acceptance criterion for each analytical procedure type should be justified.

2.3 Analyte response function over an operational range (Calibration model)

The importance of using the correct calibration model cannot be overestimated. The critical aspect is ensuring that the experimental data have a good fit to the model chosen. This may be done by either by examination of the residuals or by statistical 'goodness of fit' criteria. The latter is the preferred option with multivariate spectroscopic data.

2.3.1 Linear calibration

Most HPLC applications are performed using a UV-detector for which a linear relationship is usually justified based upon the Beer-Lambert Law.

In such cases a single point calibration seems to be acceptable under the prerequisite that experimental data on accuracy confirms that there is no other impact on the measurement.

If linearity is to be performed, more data at the upper and lower specified limit should be generated in order to get more information with respect to the variability. Linearity can be well used for the support of correction or response factor e.g. for impurities in HPLC with respect to the y-intercept and the slope for the respective analyte. However, it is not advisable to evaluate an intercept over too large a calibration range.

Details of the calculations required are given in Appendix 4.

Sensitivity Analysis

For an area normalisation, the linearity should be evaluated over the whole range of the main component. In this case the intercept should be negligible.

One good method of detection of deviations from linearity in single point calibration is that of sensitivity analysis[63].

In theory, the peak area divided by the concentration over the operational range should be a constant. However, in practice, there will be some variation due to experimental noise.

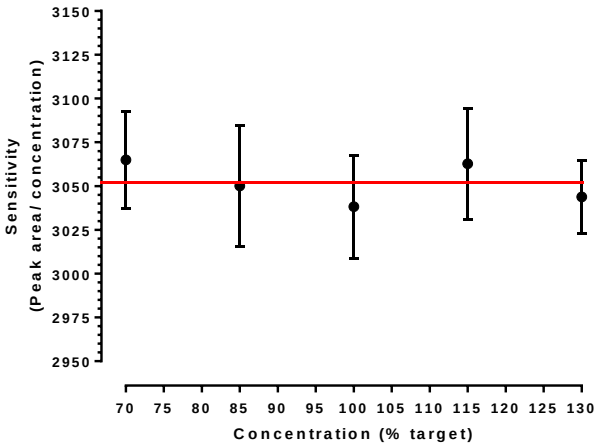


Figure 29 Example of a main peak sensitivity analysis over an analytical range of 70 to 130% of target

[63] J Emer, P Nethercote (Ed.) , ***Method Validation in Pharmaceutical Analysis***, 2nd Edition, Wiley -VCH, page 153, 2015

Figure 29 illustrates a well behaved linear calibration over the operational range using 6 replicates at each concentration. The data are evenly scattered around the mean ratio and without discernible trend.

By contrast, Figure 30 shows the dangers of extrapolating a linear model using 6 data points per concentration level, over a wide operational range. Over a smaller 60 to 120% range there is not a problem but over the full range there is both a trend, indicating nonlinearity and that the variances below the 0.1% concentration are much larger than those in the 60 to 120% range.

This trend will have an impact on the integrity of the reportable analytical result (≈3%) if main peak normalisation is used to quantify impurities at low concentration levels. Whilst this may not be of practical analytical significance, it is a data integrity issue. In addition, the inhomogeneity of variance across the operational range invalidates the use of ordinary linear least squares analysis.

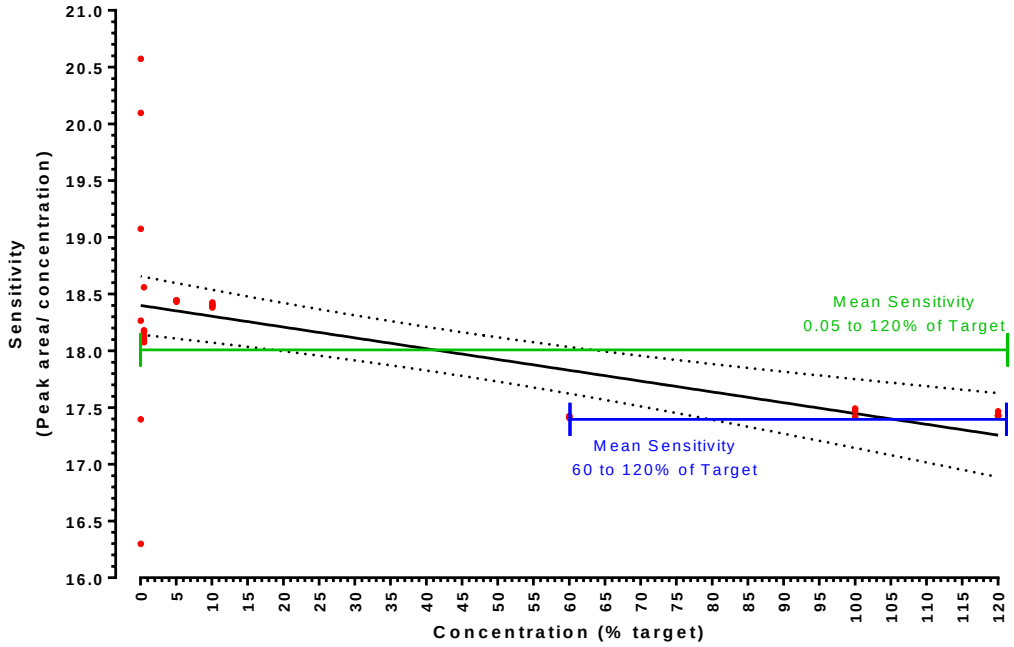


Figure 30 Example of a main peak sensitivity analysis over an analytical range of 0.05 to 120% of target

In case of very small concentrations of an analyte, standard addition techniques should be used, e.g. adding a reference standard to the sample.

2.3.2 Nonlinear calibration

Detection techniques other than UV such as conductivity detection may require the use of a non-linear calibration with e.g. a quadratic or sigmoid function. Therefore, this has to be evaluated and the number of calibration points (concentrations) deemed necessary for the establishment of the calibration function defined.

Some analytical techniques have univariate response functions which do not behave in a linear manner with respect to analyte concentration over the required measurement range. For example ion chromatographic detection. Under these circumstances, it may be possible to transform the data mathematically into a linear function and apply the linear least squares regression methodology described in the previous section.

However, transformation is not always possible or desirable[64]. Indeed it is not really appropriate to do so now as software packages handling non linear calibration models are readily available and should be used directly where possible. Examples include Weibull models for dissolution or sigmoidal models for ELISA etc. The same principles apply for the demonstration of 'goodness of fit' for non linear and linear models namely, residual sums of squares and distribution of residuals. The correlation coefficient may also give an indication of 'goodness of fit'.

Statistical software packages are readily available to perform non linear calibrations and statistical evaluation without the need for programming.

Multivariate spectroscopic techniques such as NIR and Raman usually requires more sophisticated calibration approaches such as PLS for example. For more details, specialist literature should be consulted.[65] [66].

An example of nonlinear calibration is given in Appendix 5.

[64] Dillard http://pubmedcentralcanada.ca/pmcc/articles/PMC2751416/pdf/12248_2008_Article_92260.pdf

[65] H Martens, T Naes, ***Multivariate Calibration***, Wiley 1989

[66] H Mark, J Workman, ***Statistics in Spectroscopy***, 2nd Edition, Academic Press 2003

2.4 Accuracy

Assay

Drug Substances (APIs)

- Accuracy can be performed using a minimum of 9 determinations (independent weighings) over a minimum of 3 concentration levels within the specified range (3 determinations per concentration level) or 6 determinations (independent weighings) at the target concentration.
- Standard deviation, coefficient of variation and the mean 95% confidence interval of the mean should be reported. The confidence interval of the mean should include 100% of the expected value. It may be possible that the coefficient of variation is very small and the 100 % value is not included. In such cases plausibility assessment should be applied. It is clear that this way of determination is no different to a linearity determination. However, some authorities may require this to be performed for an assay.
- Comparison of analytical result with a second, independent and well characterized (validated) analytical procedure
- Drug substance impurities: in this case spiking experiments at the respective levels should be applied.

Natural substances and products

Two possibilities can be proposed:

- The extract with a defined amount (e.g. a dried extract) is used and markers or lead substances are added to the extract which can be a pure extract or a finished product (to concentrations of e.g. 80, 100 and 120 % for an assay). For these determinations the full analytical sample preparation should be applied.

- Spiking of an extract or specific reference substances to a test sample. One possibility would to add the reference substance to a matrix in which a defined amount of the analyte already is present. The respective substance can be spiked e.g. to an amount of 50 % and adding reference substances to a total amount of e.g. 80 / 100 / 120 %.

- Prerequisite is in both cases is a well - defined matrix.

Drug Products

In case of a drug product the most common procedure is to spike an active drug substance to a matrix (placebo) with levels of 80, 100 and 120 % of the declared concentration. 3 determinations per level should be prepared.

Impurities

In case of impurities: the respective impurities should be spiked to a placebo sample also containing the drug substance (at a level of 100 %). Levels of impurities between the reporting threshold and 120 % of the acceptance criterion to be spiked to the placebo solution containing the drug substance.

2.5 Quantitation Limit QL

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter for quantitative assays with low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products.

Accepted approaches can be:

- Signal to noise ratio comparing measured signals from samples with known concentrations of the respective analyte with those of blank samples and be stablishing the minimum concentration at which the analyte can be reliably quantified. Recommended signal to noise ratio at the QL is 10:1.
- Standard deviation of the response according to;
  $QL = \frac{10\sigma}{s}$ where σ is the standard deviation of the response and s is slope of the linear calibration curve using the calibration curve in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of a regression line may be used as the standard deviation.
- The estimate of s may also be carried out as described in the following;
  - based on standard deviation of the blank measurement of the analytical background response is performed by analysing an appropriate number of blank samples and calculating the standard deviation of these responses.
  - based on the calibration curve using samples, containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

- Repeatability test (n at least 6) which can be also derived from precision and /or accuracy studies. The USP describes an approach in the draft of General Chapter <1210>[67].

The experimental quantitation limit should be below the reporting threshold also called in the Pharm. Eur. the disregard limit.

Especially, in case of repeatability experiments, it is advisable to repeat the experiments in case of chromatography using different detectors.

Setting a QL at 50% of the reporting threshold for an impurity gives a better safety margin for the approach of the real QL. Furthermore, it also might help to define a reference solution at the reporting threshold, which is required in the Pharm. Eur[68]. For unknown impurities the reference solution should be at 50% of the reporting threshold.

[67] USP General Chapter <1210>

[68] Pharm. Eur. General Chapter 2.2.4.6

Intermediate QL

In case of impurities which classified as toxic, according to the ICH M7 Guideline, the so-called real quantitation limit below the reporting threshold should be provided.

For this determination all factors which may vary or impact the future application s should be included in order to define the expected routine distribution of experimental QLs.

For the estimation of the QL including a safety margin, the intermediate QL may be derived from:

$$QL_{intermediate} = QL_{average} + 3.3\ s_{QL}$$

Measurement uncertainty and measurements at "natural limits"

As described in the last section, it won't be sufficient to determine the quantitation limit once.
With respect to measurement uncertainty, experimental errors such as random errors, systematic errors and independent (not correlated) errors should be taken into account.

Therefore, it is essential, to repeat the QL determinations under different conditions, especially, using different detectors. This might help to achieve a representative value which is a prerequisite for a robust analytical procedure.

2.6 Detection Limit DL

The detection limit of an individual analytical procedure is the lowest amount of an analyte in a sample which can be detected but not necessarily quantitated with any reasonable degree of precision.

Several approaches are possible also:

- Visual evaluation
- Signal to noise (3:1)
- Standard deviation (SD) of the response, of the blank or of y-intercepts of a linear regression line and slope

$$DL = \frac{3.3\sigma}{s}$$

Please note that DL is necessary for limit tests and semi-quantitative determinations (e.g. TLC).

In general it is not considered necessary with respect to quantitative determinations e.g. for organic impurities using HPLC but may be requested by some health authorities in case of a submission or change or variation.

2.7 Precision

2.7.1 System precision

The smallest variance contribution comes from the metrological uncertainty itself. This is found by repeated measurements of the same sample preparation by the same operator. It is not to be confused with repeatability.

2.7.2 Repeatability

Repeatability expresses the precision under the same operating conditions of a complete analytical procedure over a short interval of time. Repeatability is also termed intra-assay precision.

A minimum of 9 determinations covering the specified range for the procedure (e.g. 3 concentrations, 3 replicates each) should be performed or a minimum of 6 determinations at 100% of the test concentration which can also be carried out including a number of replicates.
For a main peak assay, where the analytical range is small 6 determinations at 100% is to be preferred as the 9 determinations over the range offer little advantage in the uncertainty of the standard deviation.

Assay

- The repeatability can be performed using a representative batch of drug substance.
- For drug product, repeatability should be carried out using a homogeneous sample wherever possible e.g. a powder prepared from a representative number of tablets.

Impurities

- Repeatability for drug substances can be performed using spiked solutions (e.g. of the specified impurities) or using a suitable sample solution containing the respective impurities.
- With respect to the concentration one possibility is to perform the spiking experiments at the reporting threshold in order to demonstrate the suitability of the analytical procedure. The acceptance criteria should be set with respect to the lowest quantifiable amount of analyte.
- For drug product, spiked samples should be used or samples containing the respective impurities can be used.

Please note that in case of more complex tests, e.g. content uniformity or dissolution, more determinations might be necessary

2.7.3 Intermediate Precision

Intermediate precision is an extension of repeatability which includes within-laboratories variations: Different days, different analysts, different equipment, different lots of materials and reagents etc. In this experiment the impact of so-called effects of random events onto the precision will be evaluated. The preparation of samples is identical to that described under repeatability.

In case of an HPLC an intermediate precision may contain the following variables:

- Analyst
- Calibration
- Instrument or system (laboratory)
- Column (batch)
- Time (days)
- Reagents

With respect intermediate precision, designed experiments should be performed with a sufficient minimum of degrees of freedom and there are sufficient data in order to correctly evaluate calculations with respect to injection repeatability, repeatability (e.g. based on 6 determinations per analyst) and the intermediate precision including the results obtained by different analysts. The use of a statistician is recommended for all but the simplest experimental designs.

2.7.4 Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardisation of methodology; round-robin tests). The number of samples tested per site may be dependent on the variability of the respective analytical procedure. Statistical approaches are required in order to evaluate if the transfer was successful based upon predetermined acceptance criteria.

Reproducibility is not required for a first submission, however, a transfer of analytical procedures between 2 sites have to be carefully documented (incl. protocol and report) and can become relevant for inspections.

2.8 System Suitability Tests

System suitability testing (SST) is an integral part of specific analytical procedures and are an essential component of the analytical control strategy. SSTs can be thought of as “in process checks” on the performance of the analytical procedure to ensure the ATP requirements are met.

- 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.
- SST Parameters to be established for a particular procedure depend on the type of procedure being validated. See pharmacopoeias for additional information.

The following SST parameters for chromatographic procedures are recommended in the pharmacopeia:

- Tailing Factor: should be 0.8 < T < 1.8 (according to the revised Pharm. Eur.). Tailing factor should only be applied for assay.
- Critical pair of peaks: should be derived from selectivity determinations already provided in Stage 1 and verified in Stage 2.
- The chromatographic resolution should be not less than 1.5.
- The relative standard deviation of the chromatographic system (Coefficient of variation) of the SST solution is defined in Pharm. Eur. , Chapter 2.2.46 as well as in USP <621> depending on the number of injections. However, a minimum of 6 injections are recommended in order to get a sufficiently reliable value.
- Sensitivity solution: which is e.g. described in JP should be about 0.1 % of the analyte with an recovery in a range of 75 – 125 %. However owing to the large variability of a single result at low concentrations, this is not a sensible parameter. Sensitivity is much better addressed by a S/N determination.

However, it is also possible to introduce an SST for other analytical procedures, e.g. in case of titration using a titrimetric standard before starting the titration of the test sample.

Appendix 3 Robustness

3.1 Design of Experiments (DOE)

Different models can be used in order to perform a DoE e.g. in order to optimize the chromatographic or sample preparation conditions:

Design of experiment (DoE) is an important tool in order obtain maximum of information about an analytical procedure whilst performing a minimum number of experiments. In order to perform these experiments, it should be pointed out that there are some limitations.

In DoE experiments, the analytical parameters tested should be cover the operational range.
One of the easiest possibilities is to perform a three factor two level full factorial design which may be visualised as a cube who's vertices indicate the combinations of the tested parameters. As a consequence, a minimum of 8 analytical determinations should be performed in order to get a maximum amount of information with respect to the impact of the tested parameters and their interactions. Details of suitable experimental designs can be found in standard textbooks and software packages[69],[70].

There are different software systems available in order to model the conditions being tested.
One of the most applied systems for chromatography is "Dry Lab®", a system which mainly works on the basis of have an average *k'* value which shows a linear relationship to the organic solvent concentration in e.g. reversed phase liquid chromatography. Furthermore, the program can be applied to other chromatographic procedures.
One of the most used applications within the robustness testing for chromatographic procedures is the variability of mobile phase composition (e.g. acetonitrile / methanol composition, pH of the mobile phase and column temperature) for the determination of assay or organic impurities.

Other optimisation software is available, e.g. Chromsword® which is based on the respective pK –values and also a suitable tool for the determination of the robustness, especially, with respect to the selection of the analytical HPLC column.

It should be pointed out that the results obtained by computer simulation models should always be verified experimentally.

[69] For example, D.C. Montgomery, ***Design and Analysis of Experiments***, 8th Edition, Wiley 2012 and the companion volume, ***Minitab Manual Design and Analysis of Experiments***

[70] Design-Expert version 10, Stat-Ease

3.2 Univariate and multivariate data

Many analytical procedures produce data which are univariate from a statistical point of view. That is to say that a test result is dependent on a single analytical metrological outcome. Examples include;

- Weights from analytical balance
- A pH measurement
- A melting point
- A peak area

Hence a simple statistical test, such as a *t* test, may be employed for comparison purposes with a standard value for example. In such instances univariate statistics are employed. Be aware, however, that statistical significance may not be the same as identifying analytically significant differences.

However, there are many analytical procedures which are inherently multivariate, i.e. there is no one to one correspondence between an analytical parameter and the analytical measurement.

Examples include;

- Spectral data, UV, NIR etc for identification in spectral libraries in qualitative analysis
- Spectral data, UV, NIR etc for development of calibration models in quantitative analysis
- Impurity profiles
- Design of Experiment data

Mathematical models are important tools for describing the analytical procedure and for the analytical instrumentation used in measurement. For this reason, it is scientifically sound practise for the chemometric methodologies to be employed during Stage 1 for compendial and pharmaceutical applications. Chemometrics is a chemical part of a discipline which applies the use of statistical methods in order to plan analytical experiments in an optimal design and to evaluate these data to get all relevant information with respect to e.g. robustness, variability and, if applicable, selectivity. Some examples of multivariate calibration in analytical procedures are given in [71].

[71] H Martens T Naes, ***Multivariate Calibration***, John Wiley 1989

Chromatographic procedures	Multivariate procedures are recommended with respect to chromatographic parameters, concentration of the analytes, sensitivity, selectivity and precision and consideration of discriminant analysis is recommended In case of non-linear relationship of the detection, cluster analysis can also be applied.
Spectroscopic methods: IR, NIR, Raman used for identification and assay	Multivariate procedures are recommended with respect to the knowledge in calibration on drug product composition (excipients, water etc.) for the determination of assay.
Determination of elemental impurities: ICP-MS ICP-OES	Multivariate procedures are recommended with respect to spectroscopic parameters, concentration of the analytes, sensitivity, selectivity and precision
AAS	Multivariate procedures are recommended e.g. with respect to non-linear calibration function
DSC	Multivariate procedures are recommended with respect to evaluating polymorphism which can provide information about a drug substance or an excipient

Table 4 Examples of multivariate procedures

Appendix 4 Linear Calibration Model

The most common calibration model or function in use in analytical procedures assumes that the analytical response is a linear function of the analyte concentration. Most chromatographic and spectrophotometric methods use this approach because the detection principle is based upon the Beer-Lambert Law which is a linear function.

The usual calculation approach adopted is the method of ordinary least squares (OLS) whereby the sums of the squares of the deviations from the predicted line are minimised. However, the OLS statistical approach assumes that all the errors are contained in the response variable, Y, and the concentration variable, X, is error free. As all measurements are subject to error, the relationship between Y, the response variable, and X the concentration is given by;

$$Y = \beta_0 + \beta_1 X + \varepsilon$$

However, the true values of β_0 the intercept and β_1 , the slope are not known but estimated from the calibration model such that

$$Y = b_0 + b_1 X$$

where Y is the calculated value of the response variable, *Y,* based on the least squares estimates of the true values of the slope b_1 and the intercept b_0

Standard texts, such as Draper and Smith[72] , should be consulted for more technical and statistical details of the least squares approach for linear regression.
The calibration model adopted should be shown to be 'fit for purpose' by demonstrating that the model fits the data well to predetermined acceptable extent.

The best method for doing this is by examining the pattern of residuals, r_i, given by $r_i = (Y_i - Y_i)$ for each of the calibration points over the operational range for the fitted line.

A good fit to the linear model is shown by there being a random scatter around zero according to the variability expected for the given experimental conditions but without discernible shape. The recommended minimum number of data points is 5 standard concentrations spanning the analytical operational range. Ideally these should be independently prepared and if practicable in duplicate to allow sufficient data points to clearly indicate the presence or absence of a pattern.

Traditionally, the correlation coefficient, R, has been used to indicate the quality of fit. This statistic has been misinterpreted to mean a measure of a 'good' straight line. It is not. It is a measure of the degree to which the variability of the calibration data is accounted for by the linear model. In other words, R measures the strength of the linear correlation between the variables X & Y. A perfect straight line has a value of unity for R.

[72] N.R. Draper and H. Smith, ***Applied Regression Analysis***, 3rd Edition, John Wiley & Sons, New York, 1998.

The equation for determining R is given by;

$$R = \sqrt{\frac{SS_{XY}^2}{SS_X . SS_Y}}$$

where *SS* stands for the sum of squares for the variables *X*, *Y* and *XY*.

Hence it is clear that to claim that for a calibration with a value for *R* of 0.99 means it is a 'better' straight line than one with a value of *R* of 0.98 is not correct. This is demonstrated graphically and statistically in a paper by Anscombe[73].

The adequacy of the calibration model should also be demonstrated using residual sum of squares or mean square error;

$$\text{Mean Square Error } MSE = \frac{SS_Y - b_1 SS_{XY}}{n-2}$$

As the purpose of the linear model is one of inverse regression ie predicting a value of sample concentration X_s from a response of Y_s, the 95% prediction interval is $\pm t_{0.05,n-2)} s_{X_s}$ where

$$s_{X_s} = \frac{MSE}{b_1} \sqrt{\left(\frac{1}{m} + \frac{1}{n} + \frac{\left(Y_s - \bar{Y}\right)^2}{b_1^2 \sum \left(X_s - \bar{X}\right)^2} \right)}$$

This prediction interval shown here is only an approximation but is commonly used in practice as the exact solution is much more complex[74]. m is the number of replicate measurements for n samples. Hence the value of s_{X_s} determines if the target measurement uncertainty of the ATP is likely to be met. However, this is only takes into account the variability of calibration.

[73] J Anscombe, Graphs in Statistical Analysis, ***American Statistician***, 27, 17-21 (1973)

[74] Reference 29 page 84

Appendix 5 An example of a Nonlinear Calibration Model

Standard concentration and a regression model for fitting a curve to calibration data should be established during method development and confirmed during validation. Design and analysis recommendations for the assessment of standard curve data and a regression model are summarized in Table I.

Method Development

The number of standard points and replicates should be large enough to provide a reliable assessment of all competing regression models. Calibrators should span the anticipated concentration range for diluted study samples with concentrations approximately evenly spaced on a logarithmic scale (if a logistic model is applied). An example of an ELISA standard curve is given in Fig. 1A. The absorbance responses should be fitted to the calibrator values.

It is recommended that standard curve concentration response data from a minimum of 3 independent analytical runs be analysed when establishing a calibration model. Duplicate curves should be included in each run to permit an evaluation of the intra-batch standard curve repeatability. The appropriateness of a model is judged by analysis of the Relative Error (RE) of the differences between the actual and model calculated standard points, where the RE for a point is obtained by subtracting the nominal concentration from the calculated concentration and dividing the difference by the nominal concentration. As a general rule, the absolute RE for each standard point should be ≤20% for most points within a curve.

For a model to be considered acceptable, the accumulated back-calculated values from all curves should have an absolute mean RE of ≤10% and a coefficient of variation (CV) of ≤15% for all concentrations within the anticipated measurement range. A trend in the mean RE across calibrator concentrations, i.e., calculated concentrations consistently above or consistently below the nominal concentrations, is evidence for a lack-of-fit that can invalidate a model. An example of a relative error plot is given in Fig. 1B.

Method Validation

Once the standard curve and model are established, validation of the calibration model is performed by analysing data from a minimum of 6 runs of at least 6 non-zero calibrators in duplicate within the anticipated range. For the curve within a run to be acceptable, the %RE of the back-calculated value for at least 75% of the standard points, not including anchor points, should be within 20% of the nominal concentration, except at the QL where the value should be within 25%. Anchor points or calibrators that are outside the expected range of quantification can be included to facilitate curve fitting.

Model confirmation should precede the reporting of analytical results for validation samples.

Table I. Standard Curve Assessment Criteria. Adapted from Binodh DeSilva, et al.[75]

Assessment topic	Method Development	Method Validation
Design		
1. Number of runs	≥3	≥6
2. Number of replicate curves/run	≥2	≥1
3. Number of standard pints/curve	≥8	≥6
4. Number of replicates/point	≥2	≥2
5. Spacing of standard points	Approximately equally spaced on a logarithmic scale	
Analysis		
6. Compute performance statistics		
a. Intra-curve	%CV of replicates; %RE of back-calculated standard point	
b. Inter-curve	Mean %RE and %CV of back-calculated standard	
7. Compare statistics to target limits		
a. %CV of replicate points (intra-curve)	Specify criteria for responses or back calculated concentration values	
b. %RE of standard point (intra-curve)	± 20	± 20 (±25 at QL)
c. Inter-curve mean %RE for each calibrator	± 10	≤ 15 (≤ 20 at QL)
d. Inter-curve %CV for each calibrator	≤ 15	≤ 15 (≤ 20 at QL)

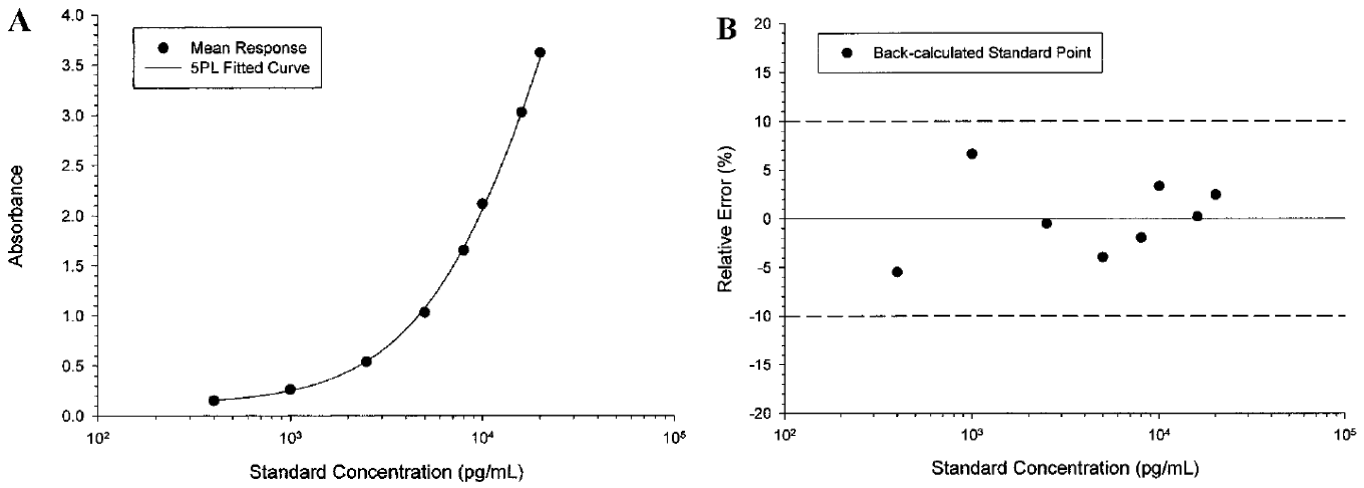


Fig. 1. A, Illustration of a five-parameter logistic (5PL) weighted nonlinear least squares regression curve.
B, Illustration of the percent relative errors in back-calculated concentrations for standard points in Figure 1A

[75] **Binodh DeSilva,et al.** Recommendations for the Bioanalytical Method Validation of Ligand-binding Assays to Support Pharmacokinetic Assessments of Macromolecules, Pharmaceutical Research, Vol. 20, No. 11, November 2003

Appendix 6 Process Performance and Capability Metrics

Performance metrics for processes are an area of much regulatory interest currently. However there isn't always a readily available clear definition of what is needed and guidance from regulators is not always consistent. The basics which were first set out by Shewhart[76] nearly 90 years ago and, in this appendix, relate them to some modern process performance and capability indices.

Definitions are important in providing a consistent nomenclature and the global ISO standard 3534-2:2006[77] will be used.

All process measurement results are subject to variations that come from a variety of sources[78]. However there are only two types as defined by Shewhart namely Common Cause variation and Special Cause variation.

Common Cause variation is the inherent noise in a process over time due to random effects and hence predictable within statistically derived limits. By definition, a process which contains only common cause variation is said to be in statistical control.

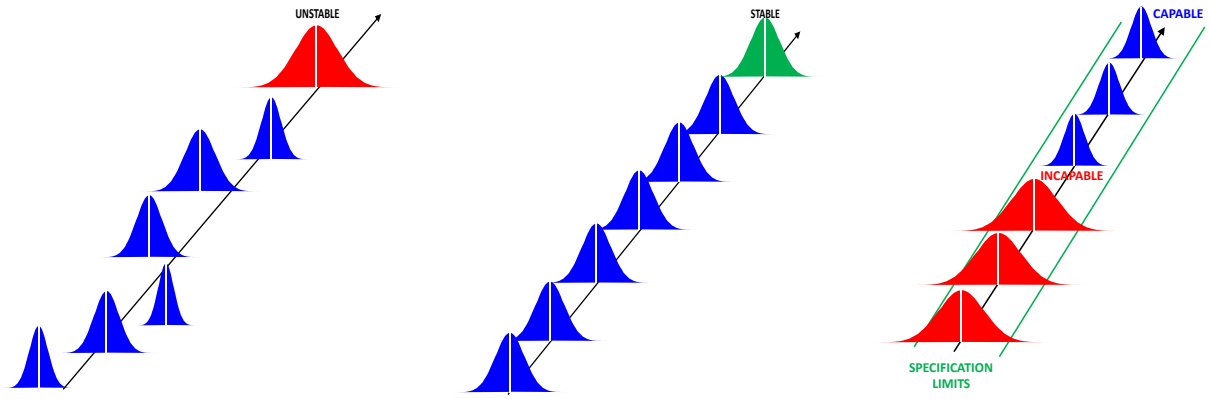
Special Cause variation occurs because of specific circumstances that are not always present manifesting themselves by, for example, by a shift or drift in the process mean or excessive noise. If a process contains special cause variation, it is unstable from a statistical point of view and the overall variation observed contains both common and special cause components. Control charts are designed to detect the presence of special causes of variation.

The normal distribution is characterised by two parameters; a measure of location (the arithmetic mean or average) and a measure of dispersion (the standard deviation). If a process is unstable it means that both of these parameters could be or are changing in an uncontrolled manner Figure 31 (a)).

[76] W A Shewart, Economic Control of Quality of Manufactured Product, Van Nostrand, 1931

[77] ISO 3534-2:2006; Statistics — Vocabulary and symbols — Part 2: Applied statistics

[78] C Burgess, Reportable Values: Where is the Variation Coming From?, ***Pharmaceutical Technology 42(5) 2018***



(a) An unstable process

(b) A stable process

(c) Stable Processes; Capable and Incapable

Figure 31 Process Stability and Capability[79]

The task is to bring these two parameters into a state of statistical control. This would entail ensuring that the mean and the standard deviations were not varying significantly. This ideal situation is illustrated in (Figure 31(b)). This process would then be said to be under statistical control i.e. no special cause variation and stable common cause variation. In this state, the process is amenable to the tools of Statistical Process Control (SPC). However, a stable process may not be statistically capable of meeting the specification limits. Figure 31(c) illustrates this showing that the red process albeit stable is incapable. The desired state is to arrive at the blue capable state.

Capability is assessed using a family of quality metrics or indices called process performance and capability indices.

1. Quality metrics for process performance & capability

There are a variety of performance indices for processes in regular use. However, only 4 will be discussed, P_p, P_{pk}, C_p and $C_{pk.}$ The definition and meaning of these 4 will be defined later. Of these 4, only two have any practical relevance, P_{pk}, and C_{pk}. The other two are of theoretical interest as they do not occur in practice other than by chance.

2. Process Performance

P_p, a process performance index, relates to the output performance of a process, irrespective if it is in control or not, with the specification assuming that the long term mean will be on the target for the product (an unbiased process).

The index is defined as a ratio of the difference between the upper and lower specification limits (called the specified tolerance in ISO) and the 99.73% probability of a value lying within ±3 standard deviations from the target (called the reference interval in ISO). Hence it can be said that this index would represent what the customer actually receives from the overall process.

[79] Adapted & Redrawn from QMS – Process Validation Guidance GHTF/SG3/N99-10:2004 (Edition 2) Annex A Statistical methods and tools for process validation; http://www.imdrf.org/documents/doc-ghtf-sg3.asp

$$P_p = \frac{U - L}{6S_t} \quad (1.1)$$

The overall standard deviation, S_t, is calculated from the usual formula for a sample standard deviation

$$S_t = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(X_i - \bar{X})^2} \quad (1.2)$$

Where $\bar{X}$ is the mean of the *N* data points.

Values for P_p of 1.33 or more would indicate a highly capable process. A value of less than 1 would indicate an incapable process which would lead to out of specification results.

As it is highly improbable that processes are unbiased, a practical process performance index, would need to take this bias into account when assessing process performance. This is done by calculating the upper and lower process performance indices P_{pkU} and P_{pkL} using not the target but the actual observed mean to calculate the from

$$P_{pkU} = \frac{U - \bar{X}}{3S_t} \text{ and } P_{pkL} = \frac{\bar{X} - L}{3S_t} \quad (1.3)$$

Hence the process performance index, P_{pk} is given by the smaller of the two values above.

$$P_{pk} = \min\left[\frac{U - \bar{X}}{3S_t}, \frac{\bar{X} - L}{3S_t}\right] \quad (1.4)$$

3. Process Capability

Process capability refers to the performance of the process when it is operating under statistical control. Two capability indices are usually computed: C_p and C_{pk} in a similar way as was described with P_p and P_{pk}. However, C_p measures the **potential** capability in the process, if the process was centred, while C_{pk} measures the actual capability in a process which is off-centre or biased. If a process is centred, then $C_p = C_{pk}$.

$$C_{pk} = \min\left[\frac{U - \bar{X}}{3S_w}, \frac{\bar{X} - L}{3S_w}\right] \quad (1.5)$$

The critical thing to note is that whilst the formulae for P_{pk} and C_{pk} look very similar, the standard deviation used to calculate the reference interval for C_{pk} is not S_t but S_w.

S_w is the within batch standard deviation (called the within sub group standard deviation in ISO) not the overall process standard deviation. It is usually estimated from a Shewhart mean and range control chart using the formula

$$S_w \approx \frac{\overline{R}}{d_2} \text{ where } \overline{R} \text{ is the mean range of the subgroups and } d_2 \text{ is a constant based on the subgroup size and may be found in many Statistical Process Control books} \quad (1.6)$$

Typical values for C_p and C_{pk} are 0.5 to 1 for incapable processes, 1 to 2 for capable processes and >2 for highly capable processes.

A word of caution is necessary in interpreting C_{pk} values. C_{pk} analysis requires a normal underlying distribution and a demonstrated state of statistical process control. When reporting a C_{pk} value, a 95 or 99% confidence interval should always be reported because this takes into account the sample size used in the calculation.[80,81].

The confidence interval is extremely important because it is not always recognised that for reasonably small confidence intervals around C_{pk} values the number of data points needs to be large. Figure 32 shows that to have a 95% confidence interval in C_{pk} of 1.33 ±10% requires in excess of 200 data points. One commonly used approximation formula (see footnote reference 79) for the confidence interval is;

$$C_{pk} = C_{pk} \pm Z_{\alpha/2}\sqrt{\frac{1}{9n} + \frac{C_{pk}^2}{2n-2}} \quad (1.7)$$

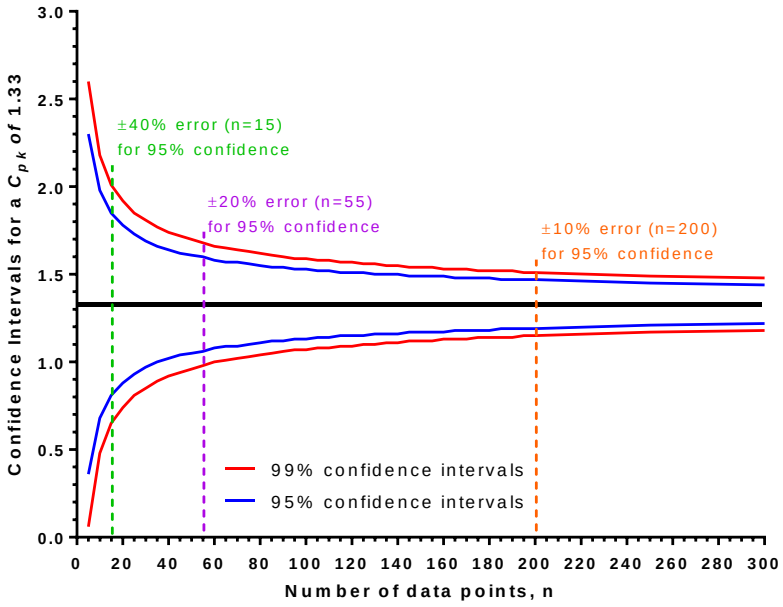


Figure 32 Confidence intervals as a function of sample size for C_{pk} of 1.33

Hence the use of C_{pk} values for comparison of performance needs to be interpreted with great care when n is small!

[80] ASTM E2281-15, ***Standard Practice for Process and Measurement Capability Indices***

[81] S. Klotz and N.L. Johnson, ***Process Capability Indices,*** Chapman & Hall/CRC, 1993

4. Example of Process Performance and Process Capability

It has been shown how to differentiate between process performance and process capability. However equations are not normally as clear as an example. Figure 33 shows data from 157 batches of a product with a target of 7.0 and upper and lower specification limits ± 1.5. The data are nicely normally distributed as can be seen from the normal probability plot but the long term mean of 7.4 is biased high.

However the process capability C_{pk} is excellent at 1.31 and even with the bias would be unlikely to produce OOS results due to common cause variation (red curve).

Unfortunately the process suffers from considerable special cause variation, the dashed black curve, with P_{pk} being an unacceptable 0.74 because the overall batch standard deviation (S_t) is 0.49 whereas the within batch standard deviation (S_w) used to calculate C_{pk} is much smaller at 0.28.

Note that if the mean bias were removed, P_p would be a more acceptable 1.02. However it would probably require a process change(s) to remove or minimise the special cause variation(s) to approach a truly capable process.

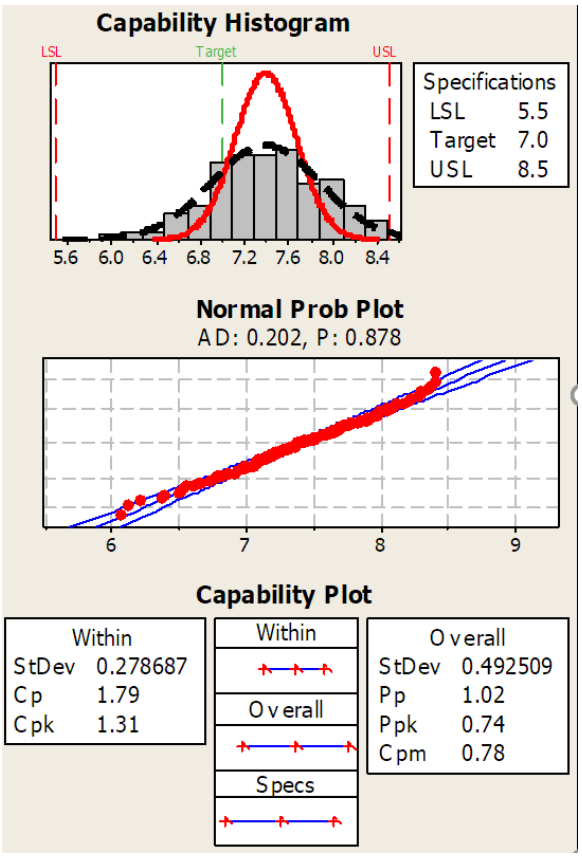


Figure 33 Example of process performance and capability plots (Minitab 17)