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Key Principles and Lifecycle Considerations for Pharmacopoeial Analytical Procedures

Mihaela Buda, EDQM

CASSS CMC Strategy Forum Europe, Basel, 20-22 October 2025

European Pharmacopoeia:
Documentary and reference standards

Legally binding in the **40 signatory parties** of the Ph. Eur. Convention and used as a reference **worldwide**; **33 observers** from all continents

About **2 900 documentary standards** for the quality control of medicines covering the whole manufacturing process

All stages of the **life cycle** of a medicine from development to production and market surveillance

About **3 200 reference standards** shipped to **127 countries**

Laboratory, production, storage and distribution

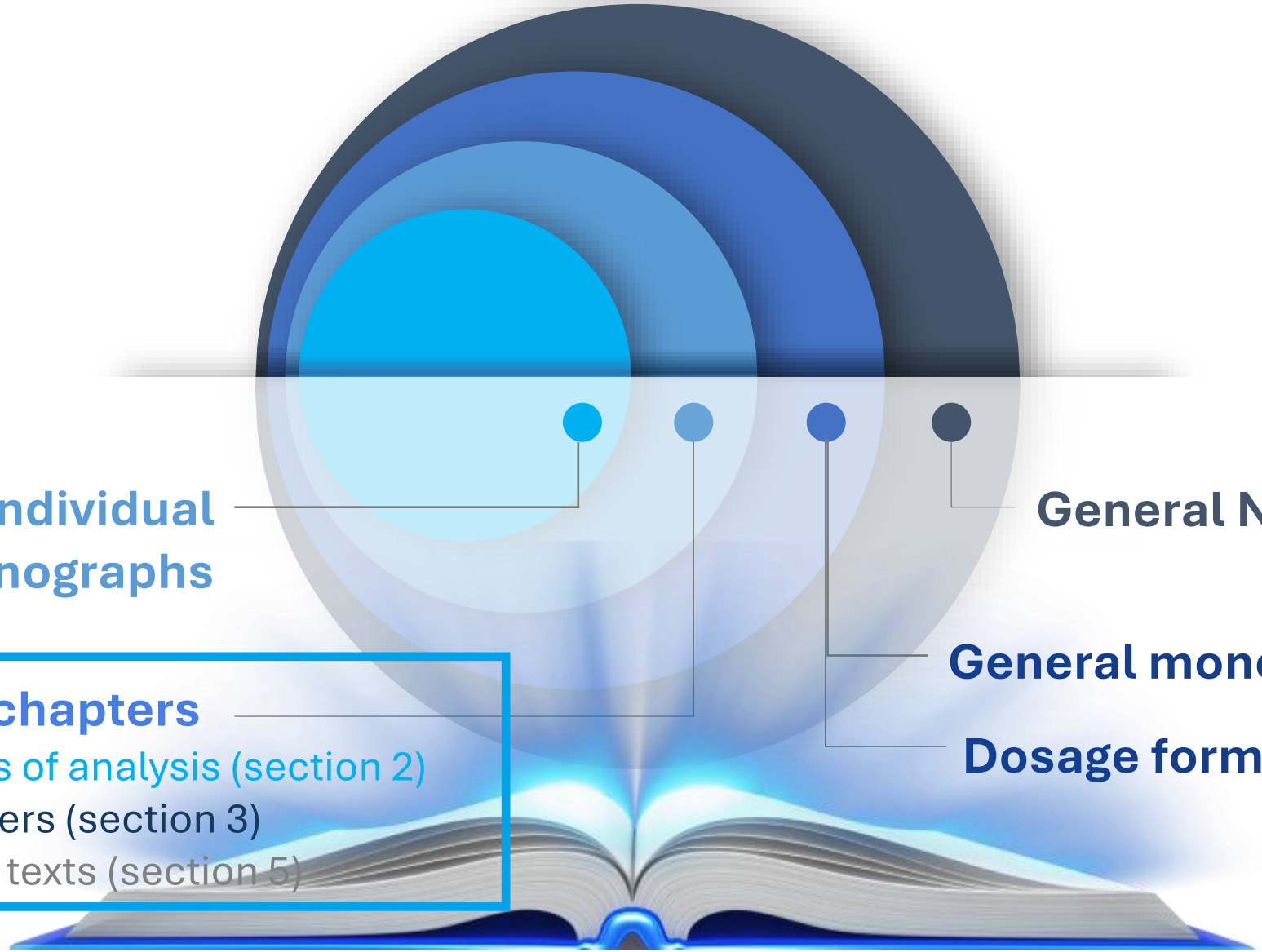
European Pharmacopoeia Commission – treaty-based body - and its experts' groups

Biological Standardisation Steering Committee

PUBLIC HEALTH IMPACT

- Ensures quality and safety of medicinal products
- Facilitates their free movement in Europe and beyond

Ph. Eur.: Content and Structure



Individual monographs

General Notices

General monographs

Dosage form monographs

General chapters

- Methods of analysis (section 2)
- Containers (section 3)
- General texts (section 5)

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Ph. Eur. General Chapters

Methods of analysis (section 2)

- Provide general requirements for equipment, equipment qualification or calibration
- Avoid repeating standard procedures or requirements in each monograph
- Provide standard analytical procedures that may be used when there is no monograph (with product-specific validation)
- Become mandatory when referred to in a monograph, unless otherwise stated (mandatory also when referred to in another general chapter that is itself referred to in a monograph, unless otherwise stated)
- Provide a harmonized analytical framework, common analytical expectations; widely applicable
- May include product-specific considerations; serve as a starting point for development of product-specific analytical procedures

General texts (section 5)

- Often published for information and guidance
- Become mandatory when referred to in a monograph
- Specific to certain topics (e.g. microbiology, chemometrics)
- Reproduce principles of regulatory guidelines
- May provide a non-mandatory framework of recommendations

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Ph. Eur. Methods of Analysis: Evolution Toward Advanced Analytical Concepts

Traditional methods:

- Official test procedures
- Fully prescriptive - test conditions, SST
- Apply as is (with confirmation of suitability for a given article)

2.5.33. TOTAL PROTEIN

Many of the assay methods described in this chap
performed using kits from commercial sources.

METHOD 1

2.9.20. PARTICULATE CONTAMINATION: VISIBLE PARTICLES

Particulate contamination consists of mobile undissolved substances, other than gas bubbles, unintentionally present in liquid preparations.

The test is intended to provide a simple procedure for the visual assessment of the quality of liquid preparations, if applicable after reconstitution, as regards visible particles.

2.6.14. BACTERIAL ENDOTOXINS(1)

The test for bacterial endotoxins (BET) is used to detect or quantify endotoxins from gram-negative

Methodology-based chapters:

- Provide core principles and scientific frameworks; “toolbox”
- Guide method development and validation
- Form the basis for product-specific/ platform analytical procedures

2.2.46. CHROMATOGRAPHIC SEPARATION TECHNIQUES(1)

INTRODUCTION

Chromatographic separation techniques are multi-stage separation methods in which the components of a sample are distributed between 2 phases, one of which is stationary, while the ot

2.2.47. CAPILLARY ELECTROPHORESIS(1)

GENERAL PRINCIPLES

Capillary electrophoresis is a physical method of analysis based on t
charged analytes dissolved in an electrolyte solution, under the influ

2.6.34. HOST-CELL PROTEIN ASSAYS

This general chapter provides guidance for the development and validation of host-cell protein (HCP) assays used to test products exclude t the comp

2.6.35. QUANTIFICATION AND CHARACTERISATION OF RESIDUAL HOST-CELL DNA

This general chapter describes analytical methods that may be used to measure the content and to characterise the size of residual host-cell DNA in biological products produced in cell substrates. It does not exclude the use of alternative approaches that are acceptable to the competent authority.

Multi-product/product-class general methods:

- Established through multi-lab, multi-product studies
- Standardised, “ready-to-use” analytical procedures
- Validation required if applied to substances outside of the scope covered by the initial validation

2.7.26. CELL-BASED ASSAYS FOR POTENCY DETERMINATION OF TNF-ALPHA ANTAGONISTS

The assays described (Procedures A, B, C and D) have been validated for the potency determination of specific TNF-alpha antagonist substances (i.e. Procedure A – etanercept; Procedure B – infliximab; Procedure C – certolizumab pegol and Procedure D – adalimumab). Whereas full validation

Examples

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Ph. Eur. General Method ≠ "Platform Analytical Procedure"

Ph. Eur. general method of analysis

- Standardised, consensus-based analytical procedures/methodologies
- Based on widely used techniques, supported by validated analytical procedures, historical data and well-established scientific knowledge
- Reflects the one-size-fits-all nature of the technique/analytical procedure, suitable for broad categories of substances/dosage forms or analytical needs
- Represents the "official method" when referenced in a monograph
  → **public standard**

Platform analytical procedure

- "…Suitable to test quality attributes of different products without significant change to its operational conditions, system suitability and reporting structure. […] can be used to analyse molecules that are sufficiently alike with respect to the attributes that the platform analytical procedure is intended to measure." [ICH Q2(R2)]
- "When an established platform analytical procedure is used for a new purpose, validation testing can be abbreviated, if scientifically justified." [ICHQ2(R2)]

Example in ICH Q2(R2) / Q14 Training Module 7

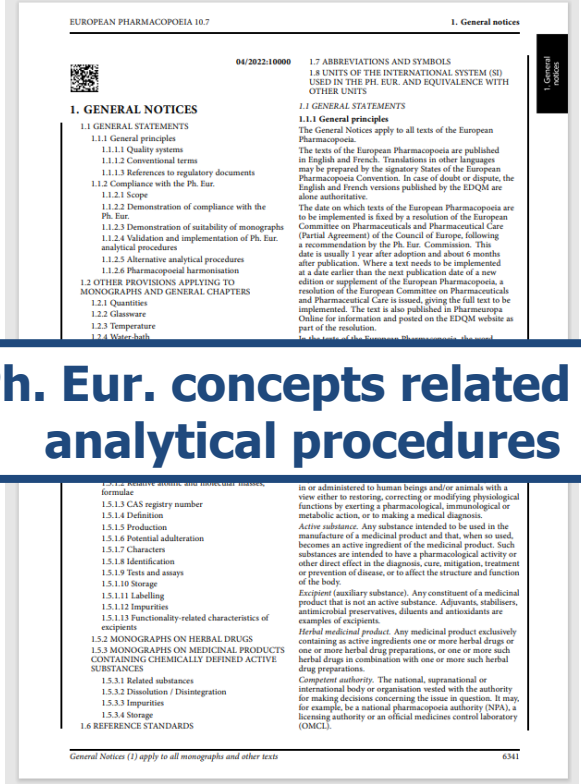
Ph. Eur. Methods of Analysis: Product-specific Application

Use of pharmacopoeial general method

Scenario A

General method referred to in a monograph

Ph. Eur. General Notices


EUROPEAN PHARMACOPOEIA 10.7 — 1. General notices

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1. GENERAL NOTICES

1.1 GENERAL STATEMENTS
1.1.1 General principles
1.1.1.1 Quality systems
1.1.1.2 Conventional terms
1.1.1.3 References to regulatory documents
1.1.2 Compliance with the Ph. Eur.
1.1.2.1 Scope
1.1.2.2 Demonstration of compliance with the Ph. Eur.
1.1.2.3 Demonstration of suitability of monographs
1.1.2.4 Validation and implementation of Ph. Eur. analytical procedures
1.1.2.5 Alternative analytical procedures
1.1.2.6 Pharmacopoeial harmonisation
1.2 OTHER PROVISIONS APPLYING TO MONOGRAPHS AND GENERAL CHAPTERS
1.2.1 Quantities
1.2.2 Glassware
1.2.3 Temperature
1.2.4 Water-bath
1.3.1.2 Relative atomic and molecular masses; formulae
1.3.1.3 CAS registry number
1.3.1.4 Definition
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1.3.1.9 Tests and assays
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1.5.2 MONOGRAPHS ON HERBAL DRUGS
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1.5.3.1 Related substances
1.5.3.2 Dissolution / Disintegration
1.5.3.3 Impurities
1.5.3.4 Storage
1.6 REFERENCE STANDARDS

1.7 ABBREVIATIONS AND SYMBOLS
1.8 UNITS OF THE INTERNATIONAL SYSTEM (SI) USED IN THE PH. EUR. AND EQUIVALENCE WITH OTHER UNITS

1.1 GENERAL STATEMENTS

1.1.1 General principles

The General Notices apply to all texts of the European Pharmacopoeia.

The texts of the European Pharmacopoeia are published in English and French. Translations in other languages may be prepared by the signatory States of the European Pharmacopoeia Convention. In case of doubt or dispute, the English and French versions published by the EDQM are alone authoritative.

The date on which texts of the European Pharmacopoeia are to be implemented is fixed by a resolution of the European Committee on Pharmaceuticals and Pharmaceutical Care (Partial Agreement) of the Council of Europe, following a recommendation by the Ph. Eur. Commission. This date is usually 1 year after adoption and about 6 months after publication. Where a text needs to be implemented at a date earlier than the next publication date of a new edition or supplement of the European Pharmacopoeia, a resolution of the European Committee on Pharmaceuticals and Pharmaceutical Care is issued, giving the full text to be implemented. The text is also published in Pharmeuropa Online for information and posted on the EDQM website as part of the resolution.

in or administered to human beings and/or animals with a view either to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis.

Active substance. Any substance intended to be used in the manufacture of a medicinal product and that, when so used, becomes an active ingredient of the medicinal product. Such substances are intended to have a pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease, or to affect the structure and function of the body.

Excipient (auxiliary substance). Any constituent of a medicinal product that is not an active substance. Adjuvants, stabilisers, antimicrobial preservatives, diluents and antioxidants are examples of excipients.

Herbal medicinal product. Any medicinal product exclusively containing as active ingredients one or more herbal drugs or one or more herbal drug preparations, or one or more such herbal drugs in combination with one or more such herbal drug preparations.

Competent authority. The national, supranational or international body or organisation vested with the authority for making decisions concerning the issue in question. It may, for example, be a national pharmacopoeia authority (NPA), a licensing authority or an official medicines control laboratory (OMCL).

General Notices (1) apply to all monographs and other texts — 6341

Ph. Eur. concepts related to analytical procedures

General method applied for quality control of substances or medicinal products not covered in Ph. Eur. monographs

"stand-alone"

Validation for specific product required
(ICH Q2 Guideline on *Validation of analytical procedures*)

Subject to approval by the competent authority
as part of the assessment of market authorisation application

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Ph. Eur. Concepts Related to Analytical Procedures

- **Ph. Eur. Chapter 1 General Notices:**

1.1.2.4 Validation and implementation of Ph. Eur. analytical procedures

The analytical procedures given in an individual monograph have been **validated** in accordance with accepted scientific practice and recommendations on analytical validation. Unless otherwise stated in the individual monograph or in the corresponding general chapter, validation of these procedures by the user is not required.

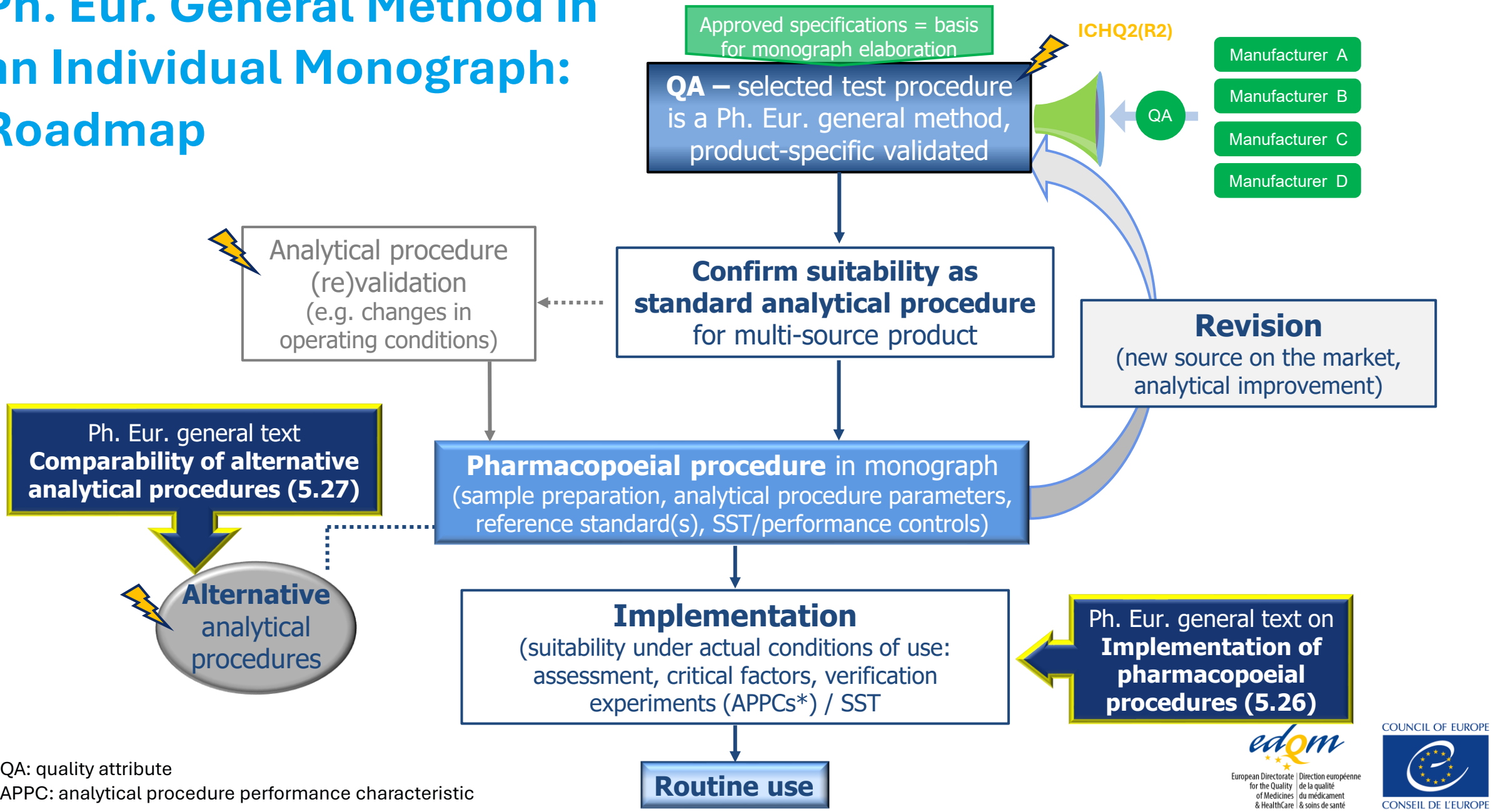
When **implementing** a Ph. Eur. analytical procedure, the user must assess whether and to what extent its suitability under the actual conditions of use needs to be demonstrated according to relevant monographs, general chapters and quality systems.

1.1.2.5 Alternative analytical procedures

*The tests and assays described are the official analytical procedures upon which the **standards** of the Ph. Eur. are based. With the **agreement of the competent authority**, alternative analytical procedures may be used for control purposes, provided that they enable an **unequivocal decision** to be made as to whether compliance with the standards of the monographs would be achieved if the official procedures were used. In the event of **doubt or dispute**, the analytical procedures of the Ph. Eur. are **alone authoritative**.*

In this context, the term 'Ph. Eur. analytical procedure' refers to an analytical procedure given in a monograph.

Ph. Eur. General Method in an Individual Monograph: Roadmap



QA: quality attribute
APPC: analytical procedure performance characteristic

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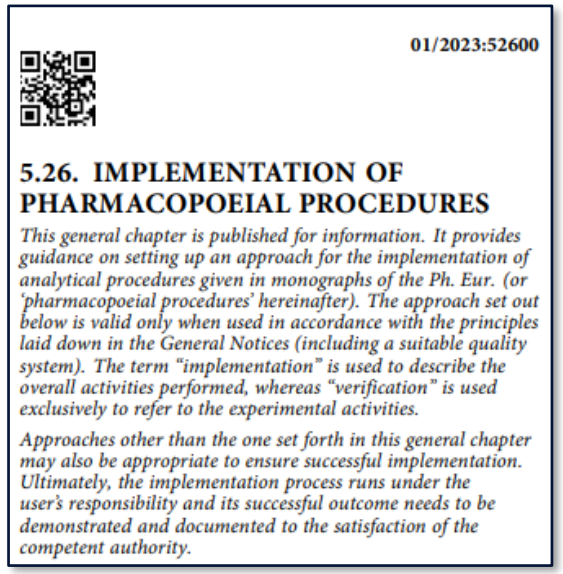
General Text 5.26: Implementation Process



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5.26. IMPLEMENTATION OF PHARMACOPOEIAL PROCEDURES

This general chapter is published for information. It provides guidance on setting up an approach for the implementation of analytical procedures given in monographs of the Ph. Eur. (or 'pharmacopoeial procedures' hereinafter). The approach set out below is valid only when used in accordance with the principles laid down in the General Notices (including a suitable quality system). The term "implementation" is used to describe the overall activities performed, whereas "verification" is used exclusively to refer to the experimental activities.

Approaches other than the one set forth in this general chapter may also be appropriate to ensure successful implementation. Ultimately, the implementation process runs under the user's responsibility and its successful outcome needs to be demonstrated and documented to the satisfaction of the competent authority.

STEP 1

Implementation assessment

- To identify any **critical factors** related to the actual conditions of use that may affect the performance of the pharmacopoeial procedure:
  - composition of the article under test
  - complexity of the sample preparation
  - reagents required to run the procedure
  - laboratory equipment required to run the procedure
  - laboratory environment
- Carried out in conjunction with provisions given in monographs and relevant general chapters (e.g. suitability requirements or any other described performance tests)

Critical factors?

NO

Procedure may be used in the implementing laboratory **without** any specific **verification experiments**

YES

Procedure may be used provided a set of **verification experiments** evaluating the impact of identified critical factors on selected APPCs is **performed**

VERIFICATION

STEP 2

General Text 5.26: Implementation Process

STEP 2 - VERFICATION EXPERIMENTS

- To **demonstrate** that the implementation is feasible
- **Relevant APPCs** are assessed and verified depending on the objective of the analytical procedure.

Verification plan

- Experiments required to verify **critical APPCs** together with the corresponding acceptance criteria defined by the user
- Suitability tests prescribed in an individual monograph and/or relevant general chapter can be used as a partial or full verification of the corresponding APPCs

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5.26. IMPLEMENTATION OF PHARMACOPOEIAL PROCEDURES

Table 5.26.-1. – *Relevant APPCs to be recommended for verification based on the intended use of the procedure*

Intended use / APPCs	Identification	Testing for impurities		Assay - content/potency - dissolution (measurement only)	Other quantitative tests
		Limit test	Quantitative test		
Accuracy	○	○	○	◗	◗
Precision					
- Repeatability	○	○	●	●	●
- Intermediate precision	○	○	◗	◗	◗
Specificity/Selectivity	◗	●	●	●	◗
Sensitivity	○	●	●	○	◗
Linearity	○	○	○	◗	◗
Range	○	○	○	◗	◗
Robustness	○	○	◗	◗	◗

● *signifies that this characteristic should be experimentally verified.*
◗ *signifies that this characteristic should be experimentally verified, if impacted by critical factors from the actual conditions of use in the implementing laboratory.*
○ *signifies that this characteristic is typically not relevant for purposes of verification.*

Compliance with pre-defined acceptance criteria demonstrates that implementation of the pharmacopoeial procedure for a given article is feasible.

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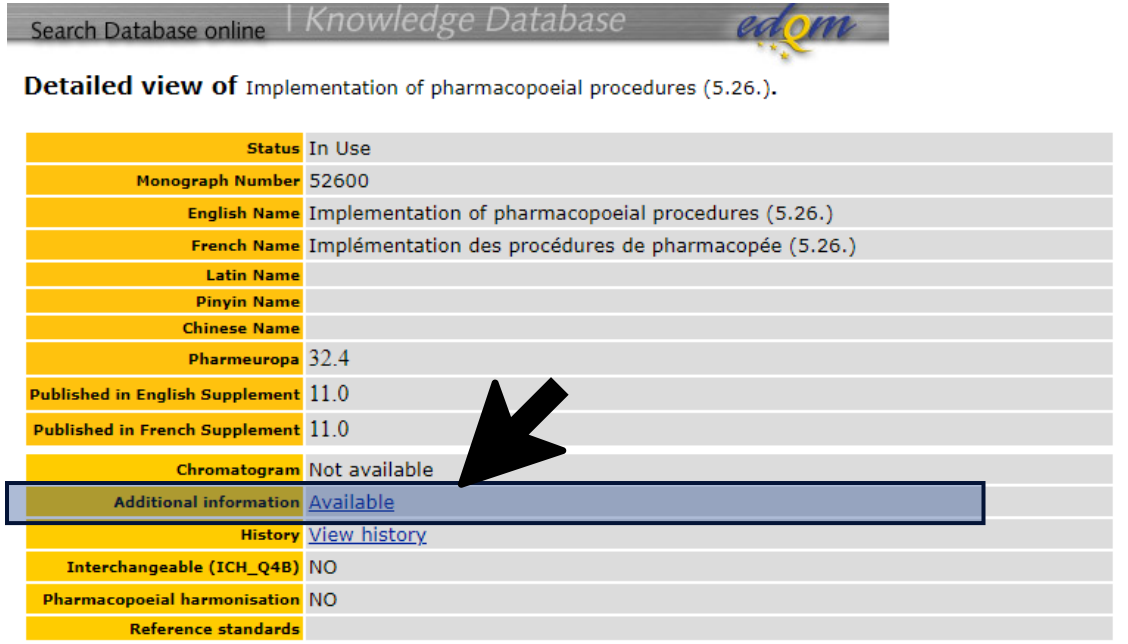
Implementation of Pharmacopoeial Procedures (5.26)

- **Examples of implementation** of pharmacopoeial procedures according to 5.26:
  - for **illustrative purposes** only
  - "Ultimately, the **implementation process** runs under the **user's responsibility** and its successful outcome needs to be demonstrated and documented to the satisfaction of the competent authority."



Selected examples

Pharmacopoeial procedure	Ph. Eur. monograph	Ph. Eur. General chapter
Identification by IR	0559, Mannitol (07/2019)	2.2.24. Absorption spectrophotometry, infrared
Related substances test by LC-UV	2986, Deferiprone tablets (01/2022)	2.2.29 Liquid chromatography 2.2.46 Chromatographic separation techniques
Potency by cell-based assay	2928, Infliximab concentrated solution (04/2023)	2.7.26 Cell-based assays for potency determination of TNF-alpha antagonists, Procedure B

Key Aspects of General Chapter 5.27

Framework

- Published for information
- Guidance on possible approaches
- No new requirements introduced
- ‘Comparability’ ≠ ‘equality’

Scope

- Cases where a pharmacopoeial (official) analytical procedure, as referenced in an individual monograph, would be replaced by an alternative (“in-house”) analytical procedure
- Applies to qualitative and quantitative analytical procedures

Not in scope

- Development of new analytical procedures
- Application of pharmacopoeial analytical procedures to articles not covered by Ph. Eur.

5.27. COMPARABILITY OF ALTERNATIVE ANALYTICAL PROCEDURES

This general chapter is published for information. It
an alternative analytical procedure to a pharmacop
demonstrated. Other approaches to demonstrating c
The use of an alternative procedure is subject to autho
The final responsibility for the demonstration of compara
the successful outcome of the process needs to be demonstrated and documented to the satisfaction of the competent authority. Comparability must be maintained over the lifecycle of both the pharmacopoeial and alternative

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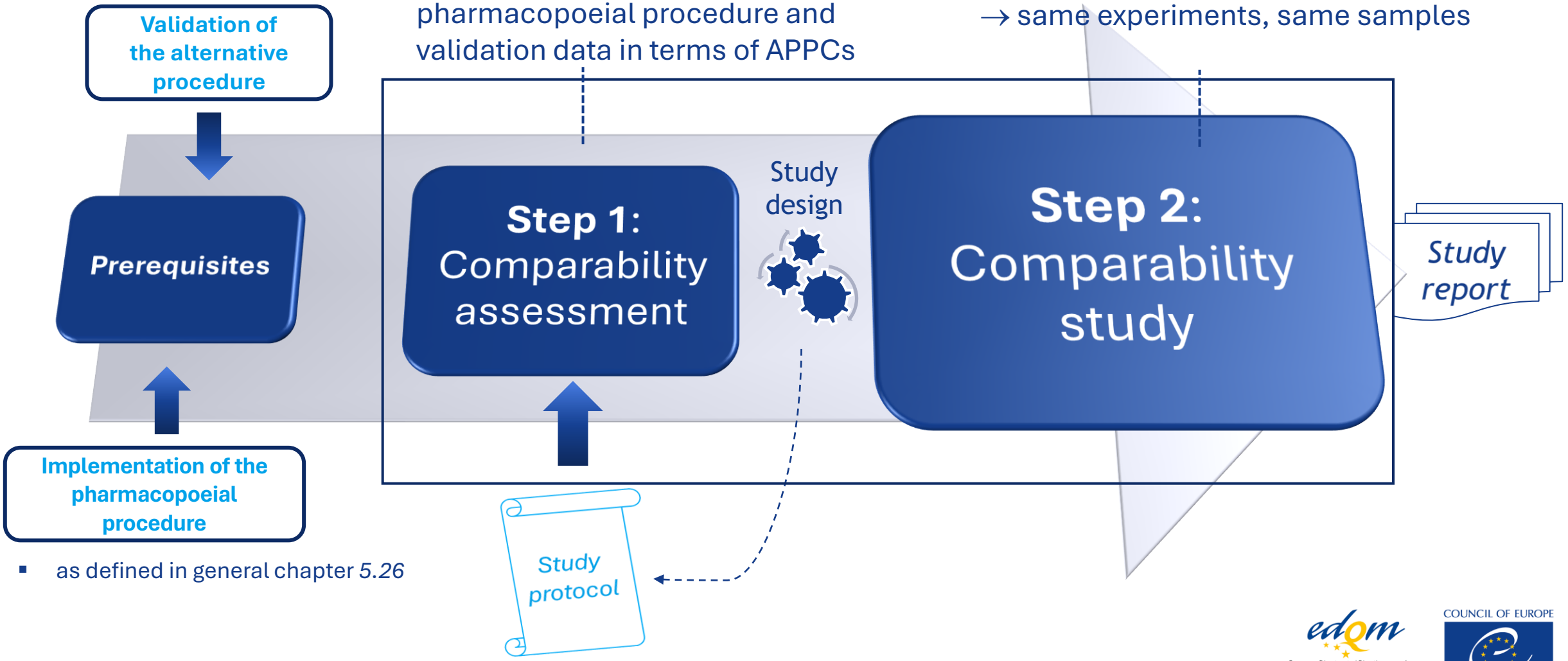
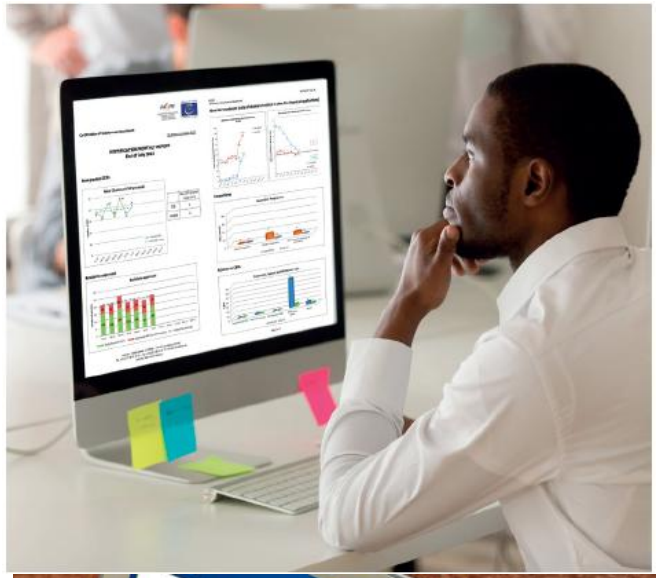
Process

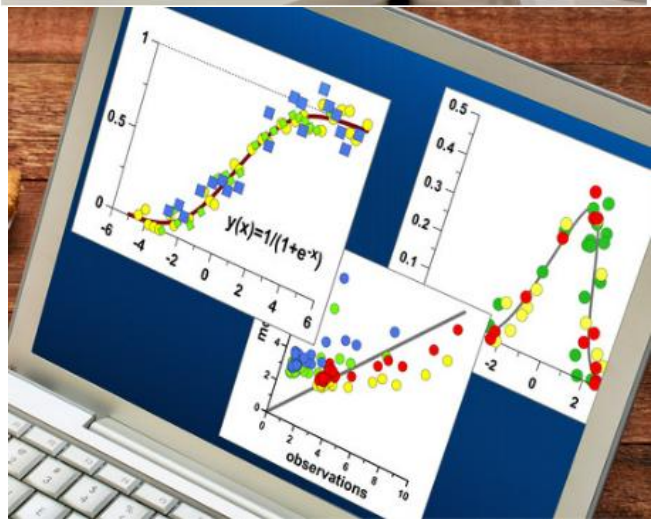
- **Comparison of data** obtained in the implementation of the pharmacopoeial procedure and validation data in terms of APPCs
- **Head-to-head testing**, with the aim of reaching the same analytical decision → same experiments, same samples



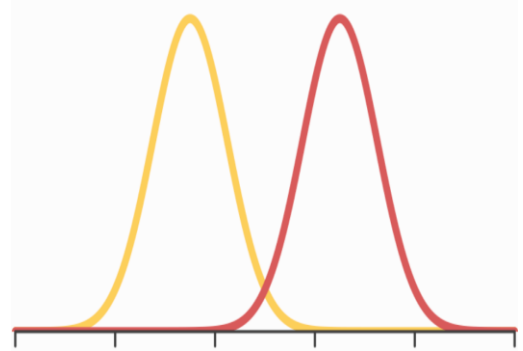
- as defined in general chapter *5.26*

Acceptance Criteria for Comparability





- Defined in the study design phase and stated in the study protocol
- **Equivalence margin:** the acceptable difference between the means of results from two procedures, which includes an acceptable confidence level
- Determined by a combination of scientific knowledge and statistical expertise
- For quantitative results: example (most commonly used approach) - comparison of two group means: TOST method
- **Pass/Fail criterion is key**



Online training

Webinar on new general chapter Comparability of alternative analytical procedures (5.27)

EUROPEAN PHARMACOPOEIA | 17/01/2024 | ON-DEMAND WEBINAR

Ph. Eur. General Analytical Procedures for mAb Analysis

‘Horizontal standards’ (general chapters)

I. Cell-based assays for potency determination of TNF-alpha antagonists (2.7.26)

III. Size-exclusion chromatography for recombinant therapeutic monoclonal antibodies (2.5.43)*

II. Capillary isoelectric focusing for recombinant therapeutic monoclonal antibodies (2.5.44)

* Under elaboration

Key Aspects

- Based on validated analytical procedures (mAb-specific), extended to a range of mAbs
- Evaluation of selected analytical procedures through **collaborative studies** (up to 20 laboratories), with the aim to demonstrate suitability as **multi-product procedures** for mAb analysis
- Standardised analytical procedures (well-defined conditions)
- Tools to control analytical procedure performance (including reference materials)

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Charge Heterogeneity

- The major sources of charge-related heterogeneity of therapeutic mAbs include post translational modifications such as glycosylation and C-terminal lysine clipping as well as chemical modifications such as oxidation and deamidation
- Potential impact on safety and efficacy
- Charge variant analysis by cIEF is commonly part of:
  - Characterisation studies
  - DS and DP release specifications (identity and purity) - comparison to a reference material

Major chemical degradation pathways which are a common source of charge-related heterogeneity of therapeutic IgG1 mAbs

Major chemical degradation pathways	Effect	Species formed
Sialylation	COOH addition	Acidic
Deamidation	COOH formation	Acidic
C-terminal lysine cleavage	Loss of NH2	Acidic
Adduct formation	COOH formation or loss of NH2	Acidic
Succinimide formation	Loss of COOH	Basic
Methionine, cysteine, lysine, histidine, tryptophan oxidation	Conformational change	Basic
Disulfide-mediated	Conformational change	Basic
Asialylation (terminal Galactose)	Loss of COOH	Basic
C-terminal lysine and glycine amidation	NH2 formation or loss of COOH	Basic

Khawli L. A. *et al.*, mAbs 2:6, 613-624; 2010

Establishment of Capillary IEF General Methods for MAbs

Preliminary experiments

Method verification

- Conventional cIEF method (initial validation)
- Same method run on both cIEF and icIEF systems using common samples

Discrepant results between conventional cIEF and icIEF in terms of pI values, profiles and resolution

Selection of candidate analytical procedure

- Conventional cIEF method
- Imaged cIEF method

- Use of separate, system-specific procedures
- Validation packages
- Re-evaluation of the factors known to have a significant impact on resolution
- Reduce inconsistencies in measured pI values

Preparatory work

Establish study strategy

- Elaboration of study protocol, including SST and system performance criteria
- Define sample panel and common internal controls
- Verification strategy (specificity, precision, reproducibility)

Collaborative study

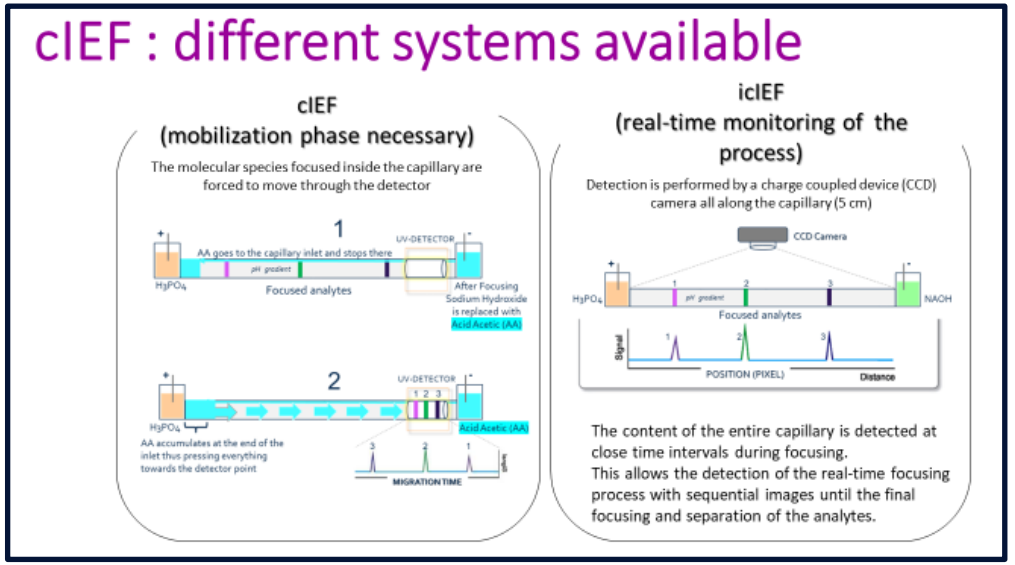
7 labs; 7 mAbs tested

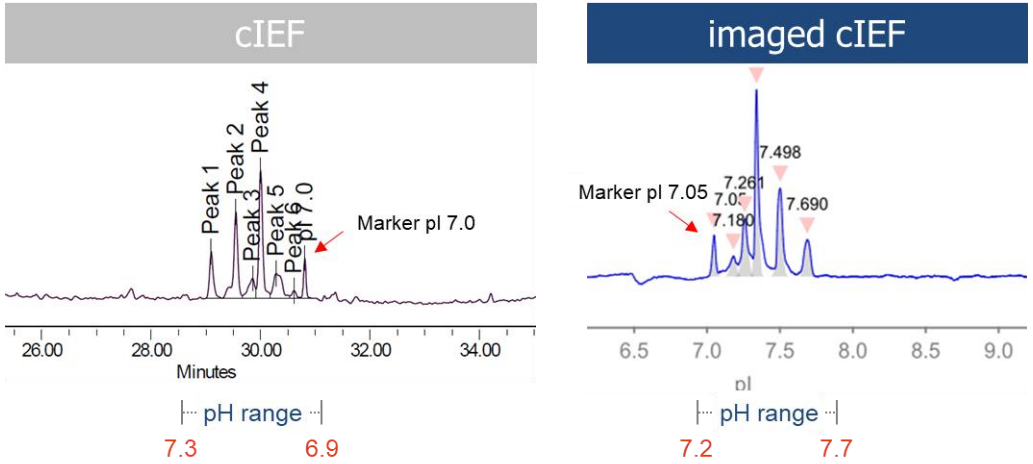
- Confirmed suitability, robustness and applicability of selected methods on a set of different mAbs
- Generated data to support elaboration of a general chapter

➢ Ascione A, Belfiore M, Vesterinen J, Buda M, Holtkamp W, Luciani F, *Charge heterogeneity of therapeutic monoclonal antibodies by different cIEF systems: views on the current situation*. mAbs, 2024;16(1)

Capillary IEF for mAbs: General Chapter 2.5.44

- Detailed description of two procedures based on:
  - **conventional** systems (Procedure A)
  - **imaged** systems (Procedure B)
- Test conditions described **may be used as is** or can be considered as starting conditions for the development of a product-specific procedure
- The **extent of analytical procedure optimisation** should be determined based on suitability for an individual mAb (case-by-case)
- **Validation needed for each mAb** to demonstrate suitability for the intended use and purpose (unless the specific procedure is described in an individual monograph)



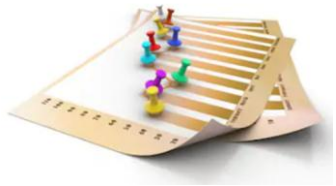


General Chapter 2.5.44: Outline

- **Introduction and scope** (including reference to general chapter *Capillary electrophoresis (2.2.47)*)
- **Principle** [traditional- and whole-column imaging cIEF]
- **Procedure** (materials/test and reference solutions; operating conditions):
  - Procedure A (two-step cIEF)
  - Procedure B (imaged cIEF)

Common sections

- **System performance** – *Ph. Eur. monoclonal antibody for system performance CRS*
- **System suitability** - pI markers
- **Assay acceptance criteria** - in-house reference preparation



- **Data analysis**
  - Identification of peaks
- **Results**:
  - Identification test
  - Quantitative test
- **General recommendations**
  - **Points to consider in analytical procedure development** – recommended steps:
    - testing of the default conditions
    - selection of carrier ampholytes and pI markers
    - increasing resolution
    - enzymatic treatment
  - **Validation**:
    - Qualitative analysis (identification)
    - Quantitative analysis (purity, stability and production consistency)

Key Takeaways

- ★ Ph. Eur. ‘methods of analysis ‘ provide a scientifically robust foundation, tools and practical guidance to further build on and support testing
  - Help establish an accepted and shared analytical language, contributing to standardisation through rationalisation of methodologies and common functionalities
- ★ Certain Ph. Eur. general analytical procedures are the result of collaborative studies involving industry, regulators and national control laboratories – ensuring robustness, reproducibility and broad applicability; help simplify and standardize QC testing
- ★ The evolution of general methods reflects progress and analytical innovation – from traditional tests (e.g. pH, microbiology, dissolution) to modern techniques (e.g. LC-MS, bioassays, high-throughput sequencing)
- ★ Their establishment is driven by standardisation needs, scientific advancements, regulatory alignment, harmonization efforts

Acknowledgments

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Francesca Luciani (ISS)

***

Members of the
Monoclonal Antibodies Working Party

***

All contributors to the Ph. Eur. activities – experts, study participants, providers of data/materials

***

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