

## **Validation of analytical methods (2 days)**

### ***Objectives***

In general, the validation is the last step of the development phase of an analytical method before its use in routine analysis.

Based on scientific and regulatory requirements (European Directives, FDA, ICH, ISO,...), the validation should allow to evaluate the performances of the method regarding some parameters called “validation criteria” with the use of appropriate statistical tools.

In introduction, the regulatory documents will be presented, defining the various parts of chemical and pharmaceutical documentation you have to follow. Each criterion of validation will be treated by presenting the methodology and different statistical approaches which can be used for their determination.

In particular, we will examine the new approach based on Accuracy Profile and the total error of measurements. A critical analysis will show why the statistics reject a method which, a priori, seems acceptable or accept a method which does not seem to be acceptable. A brief reminder on the statistics will be carried out with the aim of better evaluating the advantages and the disadvantages of the various methodologies presented. Several practical examples will be studied in order to illustrate this methodology.

### ***Target audience***

This training is aimed at people involved in the validation of analytical methods, working in physico-chemical analysis laboratories in R&D or Quality Control, and in any pharmaceutical and chemicals industries.

## ***Program***

### **1. Basis of the analytical methods validation**

- Objectives of an analytical method and its validation
- Regulatory documents (ICH, ISO, FDA, ...)
- The validation criteria
- Acceptance criteria of a validation

### **2. Harmonised approach for the validation of analytical methods**

- Harmonisation of the criteria
- Total Error concept
- Validation Protocols
- Accuracy Profile:  $\beta$ -expectation tolerance interval and acceptance limits
- Risk Profile

### **3. Discussion on practical cases**

- Examples of validation of analytical methods;
- Possibility to discuss and compute examples from laboratories (if data are sent before the training)

## **Validation of Ligand-binding assays (2 days)**

### **Objectives:**

In introduction, the different types of ligand-binding assays are presented but the validation of definite and relative quantitative assays only is discussed in this training. The regulatory requirements are discussed and different designs of validation experiments are presented.

Most of the ligand-binding assays being based on a non-linear regression model, the basics of the non-linear curve fitting are reminded.

Validation criteria are then treated by presenting the methodology and different statistical approaches which can be used for their determination. In particular, measurement error based approaches like the Accuracy Profile and the Risk Profile are detailed.

Finally, several examples are studied for illustrate purpose.

### **Target audience:**

This training is dedicated to the scientists who validate definite and relative quantitative ligand-binding assays. These assays are frequently used to assess the efficacy of a compound of interest in drug discovery as well as to quantify a biomarker either during the pre-clinical development (animal matrix) or during the clinical development (human matrix).

**Program:****1. Introduction**

- Method classification
- Objectives of an analytical method and its validation
- Regulatory documents (ICH, ISO, FDA, ...)
- Design of experiment

**2. Curve fitting and quantification**

- Non-linear regression
- Inverse prediction

**3. Validation criteria**

- Linearity, trueness, precision, measurement error ( $\beta$ -expectation tolerance interval and risk assessment), limit of quantification, limit of detection

**4. Examples**

## **Analytical method transfer and methods comparison (2 days)**

### ***Objectives:***

Analytical method transfer between two laboratories is a complex process requiring critical steps. At the end of this process, demonstration must be made that the transferred analytical method is mastered by the receiving laboratory in comparison to pre-established acceptance criteria. The aim of this training is to elucidate the steps required for an analytical method transfer:

- The methodology to follow in order to make the adequate decision with all guarantees
- A reminder of the basic statistical tools
- The statistical approaches applicable and their pros and cons;

Another crucial step in any analytical method life cycle is the comparison of a candidate analytical method to a so called reference one. The different methodologies to compare analytical methods are reviewed, their conditions of applicability and their inconvenient. Examples will illustrate those different aspects.

### ***Target audience :***

This training is dedicated to employees, responsible or technicians of quality control and analytical laboratories in R&D or production. Any individual implicated in analytical method transfer as well as quality assurance responsible can benefit from this training. The usual physico-chemical methods used in R&D and in production as well as the validation criteria are supposed known by the attendees.

**Program :**

**1. Why analytical method transfer?**

- Regulatory and scientific requirements,
- Transfer or on site revalidation?
- Objectives of transfer.

**2. Basic statistical tools**

- Descriptive Statistics: mean, variance, standard-deviation, relative standard deviation,...
- Distribution, confidence interval,
- Hypothesis testing.

**3. Statistical approaches for method transfer**

- Trueness: The descriptive approach, The difference approach, The equivalence approach,
- Precision: The descriptive approach, The equivalence approach,
- Accuracy of results: approach based on measurement total error,
- Experimental planning (number of series and replicates),
- Critic of the different approaches,
- Examples and simulations.

**4. Methods comparison**

- The diverse methodologies (Correlation, Bland-Altman, ...)
- Advantages and dis-advantages

**5. Study of real cases**

- Real cases describing the different statistical approaches reviewed,
- Discussion on the presentation of different scenarios.

## Uncertainty of measurement (2 days)

### **Objectives**

In R&D or in production, many decisions are based on quantitative analytical results.

Therefore, it is essential to trust in these results. This will be done by the estimation of the uncertainty of the result obtained.

It becomes a mandatory step in for any process in Quality Assurance whatever the references in application (BPL, BPF) but particularly for ISO 17025 norm.

For this, it is necessary to:

- identify the uncertainty root causes linked to the trials
- determine the methodology for the estimation of the uncertainty
- use the appropriate tools for the estimation of the uncertainty

This training aims to understand the uncertainty of measurement concept, to present the different approaches for the evaluation of the uncertainty applicable in analytical chemistry (GUM approach, ISO 5725, Barrwick et Ellison, Validation, Quality Control ...) and the expression of the result and its uncertainty.

The advantages and disadvantages of the different approaches will be presented through examples of analytical procedures.

The objective is to acquire the theory and the practice of the different ways of estimation of the uncertainty of measurement in analytical chemistry, with the target to be able afterwards to apply directly these methodologies in the laboratory.

## ***Target audience***

Technicians, researchers and manager from the analytical development laboratory.

Analysts involved in the conception, development and validation of analytical methods for chemical, pharmaceutical, food, cosmetic (...) industries.

## ***Program***

### **1. Definitions**

Why the Uncertainty?

Problems linked to the evaluation of the uncertainty

### **2. Reminder of basic notion of statistics**

The result

Variance and standard deviation

Confidence interval

Normal distribution

Random error / Systematic error.

### **3. Uncertainty notions**

Concept

Identification of the uncertainty root causes

Processus of the uncertainty estimation

### **4. Different approaches for the uncertainty estimation**

GUM method: Guide to the Expression of Uncertainty in Measurement

Validation

Robustness

ISO 5725 model

Evaluation of the uncertainty from the QC samples

### **5. Expression of the result and its uncertainty**

## **ROBUSTNESS (1,5 days)**

### ***Objectives:***

The robustness is a main quality of any analytical method. Required by regulatory (ICH, FDA, ...), scientific and economic necessities, must not only be seen as a criterion to fulfill but must be developed as soon as the development of the analytical method. It is highly critical for any method which has to be validated or transferred.

This training will:

- review the regulatory contexts (ICH, FDA, and European Union),
- identify the analytical method operating parameters to modify,
- use the design of experiments and walk through the appropriate analysis of the results: an experimental investment quickly beneficial by the development of robust analytical methods,
- The verification of the robustness, or of the limitations of a robust method also with the Design of Experiments to combine economy and efficiency.

It is essential to understand the place of the robustness in the whole life cycle of an analytical method.

### ***Target audience :***

- This training is dedicated to employees, responsible or technicians of quality control and analytical/bio-analytical laboratories in R&D for pharmaceutical industries or similar industries.
- Individual concerned by the development and the validation of analytical methods.

**Program :**

**1. Introduction**

- Why robustness?
- Validation of analytical methods: reminder.

**2. Regulatory environment**

- Recommendations: ICH, European Union, FDA, ...

**3. Robustness: definition**

- What is robustness?
- Difference between Robustness and Reproducibility

**4. Optimisation of the robustness of methods**

- The different type of analytical methods,
- Life cycle of a method,
- General principles of the optimization of robustness,
- Modelization of the main characteristics of a method,
- Identification of the adequate factors for robustness,
- Sources of variations,
- Variability and sensibility to variations of an analytical method during its operating conditions,
- Design of experiments in optimization:
  - General principles: responses, levels, factors, selection of the type of design,
  - Applications.

**5. Verification of robustness**

- General principles,
- Choice of experimental limits,
- Choice of important criteria for the robustness,
- Experimental Design for the verification: general principles and applications,
- Determination of the limits of a method.

**6. Study of real cases**

## Connecting the dots from the development of an analytical method to the decision it permits.

---

*Overview/Content of the 2-day training provided by Arlenda SA, Belgium*

### **DAY 1**

#### **Start with the end in mind**

**9h00-10h30**

**Concepts:**

- Purpose of an analytical method
- Objective of an analytical method
- Objective of validation of an analytical method (pre-study)
- Introducing Design Space concept
- Component of Design Space: input, quality, assurance
- Validation as a specific case of Design Space
- Assurance and objectives of development
- Quality, specifications and objectives of an analytical method
- Regulatory requirements

**10h30-10h45: coffee break**

**10h45-12h30**

**Practically:**

- **e-noval** SW demonstration and utilization
- Interpretation of **e-noval** output

**12h30-13h30: Lunch**

#### **Life-cycle of analytical methods I: Development of an HPLC method**

**13h30-15h00**

**Concepts:**

- Useful statistical concepts: model, DOE and Design Space
- Design of experiment: DOE for HPLC
- Modeling an HPLC: an overview
- Quality by Design for HPLC methods
- Robust and Optimal HPLC methods
- Design Space of an HPLC
- The route to automated development
- Automated reading of chromatograms
- Bayesian modeling

**15h00-15h15**

**Practically:**

- Current developments ongoing

**15h15-15h30: coffee break**

**Life-Cycle of analytical methods II: from validation to routine**

**15h30-16h30**

**Concepts:**

- From chromatographic performance to quantitative performance
- Robustness, Design Space and system suitability
- Design space and intermediate precision
- Defining QC specifications limits from
- Pre-study vs in-study validation
- Tolerance intervals are predictive
- Role of DOE during validation

**16h30-17h00**

**Practically:**

- **e-noval** SW for in-study validation

## **DAY 2**

### **Life-Cycle of analytical methods III: from routine to transfer**

**9h00-10h30**

**Concepts:**

- Objectives of an HPLC method transfer
- Transfer as a special case of Validation
- Transfer and robustness
- Design space to facilitate transfer

**10h30-10h45: coffee break**

**10h45-12h30:**

**Practically:**

- **Transval** for seamless transfer: demonstration and utilization

**12h30-13h30: Lunch**

### **Use of analytical method: the case of stability studies**

**13h30-15h00**

**Concepts:**

- Objective of stability
- Role of HPLC results for stability studies
- Understanding variance components of analytical methods
- Impact of method performance on stability study design
- Runs and replicates for stability studies depending on method
- DOE to cope smartly with method performances in stability

**15h00-15h15: coffee break**

### **Ending with the start: connecting the dots**

**15h15-16h30**

**Concepts:**

- Objective: individual or mean?
- Aligning validation and routine
- Understanding role and consequences of results provided
- Measurements vs results
- Promote the added value of results
- Integrate end-user of results in specification definitions

### **Conclusions – Questions and answers**

**16h30-17h00**