CHAPTER 2: Main Elements of an Analytical Strategy

I. Analytical strategy in pharmaceutical quality control

Quality control (QC) is a fundamental component of pharmaceutical engineering. It ensures that medicines are **safe, effective, and compliant with regulatory standards**. QC is primarily based on analytical chemistry and complementary disciplines (biology, microbiology).

Two main types of control are performed:

1. Qualitative Control

- Used to confirm identity and conformity of substances.

Examples:

- Identification by IR, HPLC, UV
- Organoleptic evaluation (color, odor, appearance)

2. Quantitative Control

Used to measure numerical parameters such as:

- Assay of the Active Pharmaceutical Ingredient (API)
- Impurity levels
- Water content
- Average mass
- Dissolution rate

Some parameters (e.g., solubility, flowability) are qualitative by nature but expressed with numerical limits in pharmacopoeias to reduce subjectivity.

Before analysis, **sample preparation and extraction** are essential and depend on:

- Nature of the API
- Type of matrix (tablet, capsule, solution, etc.)

II. Main Analytical Techniques in Pharmaceutical QC

- **Chromatography** (TLC, HPLC, GC): separation, identification, impurity profiling
- **Spectroscopic techniques** (IR, NMR, MS): structural characterization
- **UV–Visible spectrophotometry**: quantitative analysis (190–800 nm)
- **Atomic absorption spectrometry (AAS)**: metal ion analysis

Raw materials are controlled according to pharmacopoeial standards, while finished products follow USP or validated internal methods if no official monograph exists.

III. Designing an Analytical Strategy

When establishing an analytical strategy, five key questions must be addressed:

1. What should be analyzed?
2. Which reference standard applies?
3. What analytical parameters must be evaluated?
4. Which analytical procedure should be used?
5. What are the acceptance criteria?

1) What Should Be Analyzed?

Key considerations:

- Dosage form (solid, liquid, semi-solid)
- Physicochemical properties
- Chemical or biological molecule?
- Stability (thermosensitive, photosensitive, hydrolysable?)
- Solubility (hydrophilic or lipophilic?)
- Presence of enantiomers (requires specific method)

These factors guide method selection and sample preparation.

2) Reference Standards

The primary references in pharmaceutical QC are pharmacopoeias such as:

- European Pharmacopoeia
- United States Pharmacopeia
- Methods described in official monographs are considered validated.
- No additional validation is required unless specified.
- If no monograph exists, internal specifications must be developed and the analytical method must be fully validated.

3) Analytical Parameters

A. Raw Materials (Pharmacopoeial Structure)

1. Characteristics
2. Identification
3. Tests
4. Assay

B. Finished Products

1. Appearance
2. Average mass
3. Tests (water content, pH, dissolution, disintegration, content uniformity, etc.)

4. API identification and assay
5. Assay of critical excipients (e.g., preservatives)
6. Impurity identification and quantification

4) Selection of the Analytical Procedure

Each parameter requires a defined analytical procedure including:

- Reagents
- Reference standards
- Sample preparation
- Equipment and consumables (e.g., HPLC column)
- Calculation formulas
- Acceptance limits

Simple Parameters

- **Appearance**: visual inspection
- **Average mass**: precision balance (10–20 units), % deviation limits

Complex Parameters

- Water content
- Dissolution
- Content uniformity
- Assay and impurity profiling

5) Establishing Acceptance Criteria

A. Pharmacopoeial Products

Follow official monograph (procedure + limits).

B. Non-Pharmacopoeial Products

Require:

- Method development (often HPLC-based)
- Optimization of mobile phase, column, detection conditions
- Full analytical validation

Specific Cases

- **APIs**: Typically 95–105% (according to ICH guidelines, adjustable if justified).
- **Excipients with known effect**: Limits based on toxicity and formulation requirements.
- **Impurities**: Controlled according to ICH thresholds (reporting, identification, qualification).

6. Critical Elements of an Analytical Strategy

Two essential pillars:

1. **Analytical Method Validation**
    - Specificity
    - Accuracy
    - Precision
    - Linearity
    - Robustness
    - Detection/quantification limits
2. **Equipment Qualification & Calibration**
    - Installation Qualification (IQ)
    - Operational Qualification (OQ)
    - Performance Qualification (PQ)

II. Analysis of Analytical Technique Performance

Two fundamental pillars ensure the reliability of an analytical strategy in pharmaceutical engineering:

1. Analytical method validation
2. Equipment qualification and calibration

II.1. Analytical Method Validation

According to GMP, validation is a documented process that provides a high level of assurance that a method consistently produces results meeting predefined acceptance criteria.
In simple terms, under the same conditions, the method must produce consistent and reliable results.

Scope

Validation applies to:

- Manufacturing processes
- Cleaning processes
- Analytical methods

Here, the focus is on analytical methods.

II.2. Regulatory Framework

- Methods must be validated according to GMP.
- Pharmacopoeial methods (e.g., European Pharmacopoeia, United States Pharmacopeia) are considered validated but must be verified under real laboratory conditions.
- Validation must follow International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines.
- Equipment must be qualified before method validation.

II.3. Documentation

Two key documents are required:

- **Validation Protocol** (planning document)
- **Validation Report** (results and conclusion)

II.4. Main Validation Parameters

1. **Specificity** – Ability to measure the analyte without interference.
2. **Accuracy** – Closeness to the true value.
3. **Precision**
    - Repeatability
    - Intermediate precision
    - Reproducibility
4. **LOD** – Lowest detectable amount.
5. **LOQ** – Lowest quantifiable amount with acceptable precision.
6. **Linearity** – Proportionality between concentration and response ($r \approx 1$).
7. **Range** – Concentration interval where method remains valid.
8. **Robustness** – Resistance to small variations (pH, temperature, flow rate).

Validation ensures that the method is reliable, reproducible, and suitable for its intended purpose.

II.5. Equipment Qualification

Qualification is documented proof that equipment is properly installed, operates correctly, and performs as expected.

It is part of validation but does not replace process validation.

II.5.1. Types of Qualification

- **DQ (Design Qualification)** – Design suitability
- **IQ (Installation Qualification)** – Correct installation
- **OQ (Operational Qualification)** – Proper functioning within limits
- **PQ (Performance Qualification)** – Consistent performance in real conditions

II.6. Calibration

Calibration is the comparison of a measuring instrument with a traceable reference standard.

According to GMP:

- Critical instruments must be calibrated periodically.
- Certified reference standards must be used.
- Records must be maintained.
- Non-compliant instruments must not be used.

Key Concepts

- **Adjustment** – Technical correction to reduce measurement error.
- **Measurement uncertainty** – Degree of doubt in a measurement result.
- **Traceability** – Link of measurement results to national/international standards.

Qualification vs Calibration

- Qualification → Equipment and systems
- Calibration → Measuring instruments

Both are essential to ensure reliability of analytical results.

III. Analytical Objectives in the Pharmaceutical Field

In pharmaceutical sciences, analytical chemistry serves two major purposes.

III.1. During Drug Research and Development

At the stage of discovery and development of a therapeutic molecule, analytical techniques are used to:

- Confirm the **chemical structure** of the molecule
- Determine its **physical, chemical, and biological properties**
- Ensure its identity and characterize its behavior

This phase focuses mainly on structural elucidation and physicochemical characterization.

III. 2. During Therapeutic Use (Quality Control Stage)

Once the molecule is formulated and marketed as a medicine, analytical objectives change. It becomes necessary to verify that:

- The medicine contains the **declared active substance** (qualitative analysis using selective identification reactions)
- The substance is **pure**, free from synthesis intermediates, impurities, or degradation products
- The **amount of active ingredient** corresponds to the labeled claim (quantitative analysis)

Specific Challenges in Drug Analysis

Analytical methods used for the pure raw material (active pharmaceutical ingredient) are not always suitable for the finished pharmaceutical product. This is due to:

- Detection limit constraints requiring micro-methods
- Interference from excipients
- Presence of other active substances in combination products
- Lack of sufficient specificity in some techniques

Therefore, drug analysis is often complex and requires a wide range of analytical techniques. In pharmaceutical chemistry, various methods are employed depending on the objective, whether structural characterization, purity assessment, or dosage determination.

IV. Analytical Approach for the Identification of a Pharmaceutical Substance

The identification of a pharmaceutical substance follows a structured analytical strategy based on **organoleptic evaluation** and **physicochemical constants**.

Preliminary tests are performed using **simple, rapid, and specific methods** on samples taken from different containers of the same received batch. The objective is to confirm that the substance corresponds to the name stated on the label.

- These methods confirm identity but **do not guarantee purity**.

1. Organoleptic Analysis

Organoleptic evaluation involves sensory examination of:

- **Appearance**
- **Color**

- **Odor**
- **Taste (limited use)**
- **Solubility behavior**

These characteristics allow rapid recognition of the substance and may provide preliminary information about purity (presence of foreign matter, mold, deterioration, or falsification). They are always described in pharmacopoeial monographs.

1.1 Appearance

- Liquids: clarity and fluidity
- Powders and pastes: homogeneity
- Solids: crystal shape and size

1.2 Color

A color change may indicate degradation due to poor storage or expiration.
Example: Most ferrous salts are green (e.g., ferrous sulfate), while others may present different colors (e.g., fumarate: brown; oxalate: yellow).

1.3 Odor

- Often characteristic for natural products.
- An abnormal odor may reveal contamination or degradation.

Some substances can be smelled safely (e.g., acetone), whereas others require caution (e.g., acetic acid, ammonia, formaldehyde).

1.4 Taste

Some substances have a characteristic taste (e.g., quinine is intensely bitter). However, taste testing is no longer recommended in official pharmacopoeias due to safety and liability concerns. It must be used with extreme caution, especially for toxic substances.
Example: A laboratory mistakenly received sodium fluoride (toxic, salty taste) instead of glucose (sweet), illustrating the importance of careful identification.

Physicochemical Constants

Physicochemical parameters are essential for confirming the identity and assessing the quality of a pharmaceutical substance.

1. Phase Transition Temperatures

• Melting Point (MP)

- A characteristic property of solid compounds.
- Impurities generally lower and broaden the melting point range.
- Measured using devices such as the Kofler bench with temperature calibration.

• Boiling Point (BP)

- Determined according to pharmacopoeial methods for liquid substances.

2. Solubility

Solubility is the ability of a solute to dissolve in a solvent to form a homogeneous solution.

Substances are classified as:

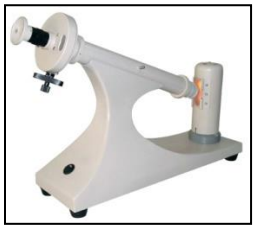
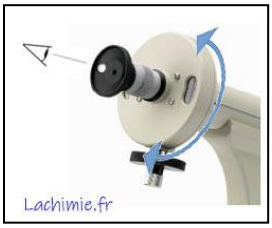
- Very soluble
- Soluble
- Slightly soluble
- Insoluble

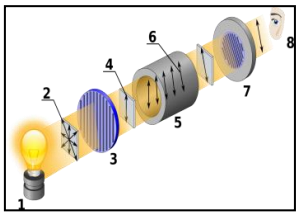
3. Optical Rotation

Certain compounds (e.g., amino acids, sugars, chiral molecules) can rotate plane-polarized light.

- ***Measured using a polarimeter.***

This parameter confirms identity and stereochemical purity.



4. Refractive Index

The refractive index measures how light propagates through a medium.

It is dimensionless and determined using a refractometer.

Used to verify identity, purity, or concentration (especially for oils and pure liquids).

5. Chemical Indices

Used mainly for fatty substances to assess quality:

- Acid value
- Saponification value
- Peroxide value

6. Relative Density

Density is measured using a pycnometer (for solids or liquids).

It helps differentiate substances.

Example:

- Mercury or barium salts are very heavy.
- Basic magnesium carbonate is particularly light.

V. Analytical Approach for the Quantitative Determination of a Pharmaceutical Substance

The quantitative determination (assay) of pharmaceutical substances depends on the matrix analyzed:

- **Raw materials (active ingredients)**
- **Finished pharmaceutical forms**
- **Biological fluids**

Each requires different analytical performance criteria.

1. Fundamental Analytical Criteria

1.1 Raw Materials (Active Pharmaceutical Ingredients – APIs)

- **Precision** is the primary requirement (purity determined within ±0.1%).
- **Accuracy** can be corrected using a correction factor.
- **Specificity** is supported by complementary identification and impurity testing.

1.2 Finished Pharmaceutical Forms

- Active ingredient content must comply with regulatory limits (generally ±5% according to European legislation).
- **Specificity** is essential due to excipients and combination products.
- **Sensitivity** must allow detection at therapeutic levels.

1.3 Biological Matrices

- The most important criteria are **specificity and sensitivity**.
- Precision within ±10% is generally acceptable.
- Matrix interference and metabolites must be considered.

2. Assay of Raw Materials

2.1 Determination of Mineral Elements

A. Cations

• ***Alkali metals (Na^+, K^+, Li^+, etc.)***

- Determined by **flame photometry (atomic emission spectrophotometry)**.
- Simple and specific.
- Based on resonance emission lines.

• ***Alkaline earth metals (Ca^{2+}, Mg^{2+}, Ba^{2+}, etc.)***

- Preferably determined by **atomic absorption spectrometry (AAS)**.
- Complexometry (EDTA titration) is also used.
- EDTA titration requires strict pH control (buffered medium).
- Can be direct or back titration.

• ***Transition metals (Fe, Cu, Zn, Mn, Pb, etc.)***

- Determined mainly by **atomic absorption spectrometry (AAS)**.
- AAS measures total elemental concentration (cannot distinguish Fe^{2+} from Fe^{3+}).
- For oxidation state determination → polarography or selective electrodes.
- Alternative methods:
    - Chelatometric titration (EDTA)
    - Redox titration

B. Anions

In pharmacopoeias, either the cation or the anion is assayed.

• ***Halides (Cl^-, Br^-, I^-)***

- Determined by **argentimetric titration** using $AgNO_3$.
- Detection: potentiometric (silver electrode).
- Mohr method (neutral medium, mainly for chlorides).
- Volhard method (acidic medium).

• ***Complex anions (SO_4^{2-}, NO_3^-, PO_4^{3-}, CN^-)***

- Rarely directly assayed in raw materials.
- Preferably, the corresponding cation is determined.

2.2 Assay of Organic Molecules (Raw Materials)

The analyst selects a reactive functional group suitable for precise titration.

• Acids

- Aqueous acidimetry (for sufficiently strong acids).
- Non-aqueous acidimetry (using solvents like ethanol or acetone).
- Titrant example: alcoholic KOH.

• Bases

- Aqueous titration (strong bases).
- Non-aqueous titration (weak bases).
    - Example: **Pifer and Wollish method**
    - Dissolution in acetic acid.
    - Titration with perchloric acid ($HClO_4$ 0.1N).
    - Indirect method.

• Carbonyl compounds (aldehydes, ketones)

- Reaction with hydroxylamine.
- Simple volumetric method.

• Polyols (e.g., sugars)

- Periodimetry (reference method).
- Fehling's solution (reducing sugars).
- Polarimetry (optically active sugars).

• Amino acids

- Acid function: Sorensen formol titration.
- Basic function: non-aqueous titration.

• Organic salts

- Organic base salts → non-aqueous titration.
- Organic acid salts → cation determination (complexometry, AAS, flame photometry).

3. Assay of Finished Pharmaceutical Forms

More complex due to excipients and combinations.

3.1 Mineral Components

- Complexometry (if concentration is high and single cation present).
- Spectrophotometry (colored complex formation).
- Flame photometry (alkali metals).
- Atomic absorption spectrometry.
- ICP (Inductively Coupled Plasma) for multi-element analysis.

3.2 Anions

- Volumetric methods (if single species).
- Ion-selective electrodes (F^-, NO_3^-, CN^-, etc.).
- Ion chromatography (simultaneous determination).

3.3 Organic Molecules (Most Common Case)

Due to interference from excipients, **chromatographic methods are often necessary**.

Acidic drugs

- UV spectrophotometry.
- Gas chromatography (GC).
- High-performance liquid chromatography (HPLC).

Basic drugs (majority of medicines)

- Ion-pair titration.
- UV spectrophotometry.
- GC or more commonly HPLC.

Polyols (sugars)

- Enzymatic methods (e.g., glucose).
- Periodimetry.
- HPLC with refractive index or polarimetric detection.

4. Assay in Biological Fluids

The most complex case.

Challenges:

- Separation from endogenous substances.
- Detection of metabolites.
- Very low concentrations.

Methods used:

- Immunological methods (ELISA, immunofluorescence).
- Chromatographic methods:
    - HPTLC (limited quantification).
    - GC.
    - HPLC (most widely used).

Selection between **GC** and **HPLC** depends on the physicochemical properties of the drug.

VI. Analytical Approach for the Detection of Impurities

After identification of the **active pharmaceutical ingredient (API)**, it is essential to verify that it is free from contaminants such as:

- Residual synthesis reagents
- By-products and intermediates
- Degradation products (due to improper storage)
- Residual solvents
- Inorganic contaminants

Impurities are classified into **three main categories**, with an additional concern for fraud:

1. **Organic impurities**
2. **Inorganic impurities**
3. **Residual solvents**
4. **Fraudulent substitutions (counterfeit-related impurities)**

1. Origin of Impurities

1.1 In Active Pharmaceutical Ingredients (APIs)

Impurities may arise from:

- Manufacturing processes
- Storage conditions
- Degradation (forced degradation studies)

A complete impurity profile must be established during development based on:

- Synthetic pathway analysis
- Degradation studies
- Stability testing

1.2 In Finished Products (FP)

According to **International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use ICH Q3B (R2)**, impurities in finished products mainly include:

- Degradation products of the API
- Degradation products arising from packaging interactions

2. Organic Impurities

These include:

- Starting materials
- Intermediates
- By-products
- Degradation products
- Isomers (including enantiomers)

They are often referred to as **"related substances."**

Example: Paracetamol

Paracetamol can degrade into 4-aminophenol, a nephrotoxic compound. Therefore, related substances testing are essential.

Isomeric Impurities

Chiral molecules may produce enantiomers with different pharmacological or toxicological properties.

Example: Levodopa

- The (S)-enantiomer is therapeutically active.
- The (R)-enantiomer may be toxic.

Therefore, enantiomeric purity must be controlled.

3. Inorganic Impurities

Include:

- Heavy metals
- Residual catalysts
- Chlorides, sulfates
- Filtration aids or charcoal residues

Heavy metals are critical due to toxicity (e.g., lead). Results are often expressed as ppm of lead equivalents.

Lead toxicity (saturnism in children) can cause severe neurological damage, especially in pregnant women and infants.

4. Residual Solvents

Residual solvents are organic or inorganic liquids used during synthesis or purification.

According to toxicity classification:

Class 1 – Solvents to Avoid

- Carcinogenic or environmentally hazardous
- Example: Benzene

Class 2 – Solvents with Restricted Use

- Toxic but acceptable within limits

- Example: Acetonitrile

Class 3 – Low Toxic Potential

- Low health risk
- Example: Acetic acid

Toxicological Concerns

Residual solvents may affect:

- Skin
- Central nervous system
- Liver and kidneys
- Fetal development (placental transfer)

They may also:

- Alter physicochemical properties of the API
- Affect container–content interaction
- Modify taste and odor of the final product

5. Fraud and Counterfeit Impurities

Some impurities result from intentional substitution.

Example: The contaminated heparin crisis (2007–2008)

Fraudulent substitution with oversulfated chondroitin sulfate led to approximately 130 deaths.

Such impurities are difficult to detect and require advanced analytical methods.

6. Main Analytical Methods for Impurity Detection

Thin Layer Chromatography (TLC)

- A pure substance gives one spot.
- Additional spots indicate impurities or degradation.

UV Spectrophotometry

- Increased absorbance may indicate impurities.
- Useful when API or impurity absorbs in UV.

Loss on Drying

- Detects water or volatile solvents.
- Important because moisture lowers API assay.

Sulfated Ash Test

- Measures inorganic residue in organic substances.

Heavy Metals Limit Test

- Pharmacopoeial procedures for detecting toxic metals.

Specific Impurity Testing

- Targeted analysis for known synthesis or degradation impurities.