People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research

Djilali Bounaama University Khemis Miliana
Faculty of Matter Sciences and Computer Science
Department of Chemistry

Handout

Drug Analysis and Control

"Course Material and Evaluation Exercises"

Prepared by:
Dr. Meriem FIZIR

Academic Year: 2025-2026

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Preface

This course handout for ***Drug Analysis and Control*** is designed for second-year Master's students in Pharmaceutical Chemistry, and is also suitable for those in Pharmaceutical Engineering and Pharmacy programs.

The content integrates fundamental chemical principles with their specialized applications to ensure the safety, efficacy, and quality of medicinal products. The knowledge presented in these chapters forms the essential foundation supporting the pharmaceutical industry's commitment to public health. Drawing on several years of teaching experience in this field, this handout aims to provide students with comprehensive support to master the essential concepts of pharmaceutical quality control.

This handout has been developed in accordance with the official program curriculum. It guides students from broad regulatory frameworks to the detailed application of analytical techniques for specific drug classes.

The essential context is first established in Chapters I and II, which examine the State System Structure in Quality Control and the rigorous preparation of technical documentation for standards. The core principles of pharmaceutical analysis are systematically presented in Chapter III, detailing specific characteristics, essential criteria, method validation parameters, and the general principles for establishing the authenticity of medicinal substances. Chapter IV discusses the types of technical analysis within a pharmaceutical complex. This theoretical foundation is complemented by the practical aspects covered in Chapter V: Sample Selection, emphasizing that the validity of any sophisticated analysis depends entirely on obtaining representative samples.

A comprehensive examination of modern pharmaceutical quality control methods is provided in Chapters VI through X. Chapter VI discusses sterilization methods for microbiological safety; Chapter VII presents ash analysis for purity assessment; Chapter VIII details physical characterization methods, including melting point and optical rotation determination; Chapter IX covers chemical analysis techniques through titrimetric methods and functional group identification; and Chapter X explores advanced physicochemical instrumentation, including

spectral, chromatographic, and electrochemical techniques that form the core of modern quality control laboratories. Subsequent chapters (XI-XIV) apply these methods to specific drug classes, including aliphatic, aromatic, heterocyclic, and alkaloid compounds.

Upon completion of this course, students will be equipped not only to perform these analyses but also to critically evaluate analytical data, develop robust quality control protocols, and contribute to advancements in pharmaceutical sciences.

The final assessment consists of two components, each worth 50% of the final grade: a practical work assessment (comprising mini-projects, attendance, and participation) and a final written exam covering all course material from the semester. A minimum overall grade of 10 out of 20 is required to pass the module.

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CHAPTER I: CONCEPT OF STATE SYSTEM STRUCTURE IN DRUG QUALITY CONTROL

1. Objectives

By the end of this chapter, the student will be able to:

- Explore the state system structure in drug quality control
- Examine quality management systems in the pharmaceutical industry
- Understand the role of pharmacopoeias
- Develop knowledge of quality documentation and standards

2. Introduction

Every medicine is characterized by special requirements of efficiency and safety, which determine its quality. High-grade medicines and their active substances always comply with all regulatory requirements. Medicine quality control is carried out during the whole manufacturing process (at every stage), also when releasing the drug on the market and during its circulation there.

3. Concept of state system structure in drug quality control

The concept of state system structure in drug quality control refers to the organization and management of quality control processes for drugs. This includes the establishment of protocols and procedures for testing, analysis, and evaluation of drug products to ensure that they meet the required standards for safety, efficacy, and quality. The state system structure may involve the use of various analytical techniques and technologies, such as chromatography, spectroscopy, and microbiological methods, as well as the implementation of quality management systems and regulatory frameworks to monitor and control drug quality throughout the entire supply chain. The ultimate goal of the state system structure in drug quality control is to safeguard public health by ensuring that drugs are safe, effective, and of high quality.

4. Quality management systems

The process of standardization and quality control is implemented in three main areas: identification of medicines, purity assessment (absence of impurities) and assay. Drugs quality indicators together with the methods of their analysis are stated in special regulatory documents (RD). The main one is Pharmacopoeia. There are other several quality management systems used in drug quality control. Here are a few examples:

4.1. Good Manufacturing Practice (GMP)

GMP is a set of guidelines that ensure that pharmaceutical products are consistently manufactured and controlled according to quality standards. GMP covers all aspects of the manufacturing process, including the facilities, equipment, personnel, documentation, and procedures.

4.2. International Organization for Standardization ISO 9001

ISO 9001 is a quality management system standard that can be applied to any industry, including the pharmaceutical industry. ISO 9001 provides a framework for implementing a quality management system that focuses on customer satisfaction, continuous improvement, and the prevention of defects.

4.3. Pharmacopeial standards

Pharmacopeial standards are a set of standards for the identity, strength, quality, and purity of drugs and drug ingredients. These standards are developed by organizations such as the United States Pharmacopeia (USP) and the European Pharmacopoeia (Ph. Eur.) and are used by regulatory authorities and pharmaceutical companies to ensure the quality of drugs.

4.4. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines

The ICH is a global organization that brings together regulatory authorities and the pharmaceutical industry to develop and harmonize guidelines for the development, registration, and post-approval of pharmaceutical products. The ICH guidelines provide a framework for ensuring the quality, safety, and efficacy of drugs by establishing standards for the design, conduct, and reporting of clinical trials, as well as for the manufacturing and quality control of drug products. The guidelines cover various aspects of drug development and quality control, including stability testing, impurity testing, analytical methods validation, and risk management. By implementing the ICH guidelines, pharmaceutical companies can

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ensure that their products meet the required standards for quality and safety, and regulatory authorities can rely on a common set of standards for evaluating drug products.

5. Common approaches for quality control of drugs: Pharmacopoeia

Pharmacopoeia, or pharmacopoea1 (literally,"drug-making"), in its modern technical sense, is a book containing directions for the identification of samples and the preparation of compound medicines It is published by the authority of a government or a medical or pharmaceutical society. Descriptions of preparations are called ***monographs***.

Pharmacopoeia is a collection of official documents (standards and regulations) that sets quality standards for pharmaceutical substances (active pharmaceutical ingredients – API), excipients, diagnostic and medicinal products in different pharmaceutical forms. The provisions of Pharmacopoeia are based on the achievements of pharmaceutical chemistry and pharmaceutical analysis, its criteria, methods and techniques.

Pharmacopeia has two main sections: **general chapters and specific monographs**.

Monographs: A detailed written study of a single specialized subject or an aspect of it.

Specific monographs. Specific monographs are devoted to individual substances or finished dosage forms of medicines (USP, JP).

Pharmacopoeia monograph is the standard of quality for specific (individual) drug.

The general chapters contain information about the methods of analysis, reagents, etc. For example, there are a lot of general chapters in Ph.Eur: Methods of Analysis (Apparatus. Physical and physicochemical methods. Identification. Limit tests. Assays. Biological tests. Biological assays) – Methods in pharmacognosy – Pharmaceutical technical procedures – Materials for Containers–Allergen products – Dosage Forms – Essential oils – Extract – Herbal drugs – Products of fermentation – Radiopharmaceutical preparations – Recombinant DNA technology – Substances for pharmaceutical use – Vaccines for human – Vaccines for veterinary use – Vegetable fatty oils.

5.1. The State Pharmacopeia

The State Pharmacopeia is the official document, which is under state supervision and its requirements are mandatory for all institutions in the state, engaged in the manufacture, storage and use of medicines, including herbal (medicinal plant materials).

In the world pharmaceutical practice, the recognized leaders among the pharmacopoeias are the **Pharmacopoeia of Europe, the United States pharmacopoeia and Japan pharmacopoeia**.

The Convention developed and the circle of members Pharmacopoeia expanded. Today its membership includes 36 countries and 22 observers. Russia has observer status. Members of *the European Pharmacopoeia Commission are:*

Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Montenegro, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, the former Yugoslav Republic of Macedonia, Turkey, United Kingdom and the European Union.

The observers to the European Pharmacopoeia Commission are:

Albania, Algeria, Australia, Belarus, Brazil, Canada, China, Georgia, Israel, Madagascar, Malaysia, Morocco, Republic of Kazakhstan, Russian Federation, Senegal, Syria, Tunisia, Ukraine,United States of America and WHO (World Health Organization).

5.1.1. Pharmacopoeia of Europe

Now a new Ph. Eur. edition is published every three years and additional Updates – every few months. The 7th edition of Ph. Eur. has come into force since 2011. The European Pharmacopoeia has a classic structure. But its peculiarity is that, unlike the USP and BP, it does not contain monographs for dosage forms. It presents the monographs only for substances. The European Pharmacopoeia is published by the Directorate for ***the Quality of Medicines & HealthCare of the Council of Europe*** (EDQM). Commission of European Pharmacopoeia, which includes all members and observers, also regulates work on Ph. Eur. Its functions include the work program adoption, definition of expert groups as well as pharmacopoeia texts approval.

After each monograph has been approved, special group of experts regulates it. The proposals from national delegations, expert groups, and pharmaceutical industry representatives replenish Ph. Eur. The decision on new monograph inclusion depends on the therapeutic effect of drug, widespread of it use, on the number of countries that have approved it and on its proven quality.

5.1.2. United States pharmacopoeia

The first edition of the United States Pharmacopoeia – National Formulary (USP – NF) was published in 1820. Current edition is USP 42-NF 37 (2019). It came into force on May 1, 2011. This is the most dynamically developing Pharmacopoeial standard. This document, as its name implies, is really a collection that includes two different standards: Pharmacopeia and National Formulary. The latter pertains to some excipients and other substances that are not drugs.

5.1.3. Japanese Pharmacopoeia (JP)

JP is another leading international pharmacopoeia. The new edition is published every five years. Current edition (JP 17) came in 2016. JP is published not only in Japanese but also in English.

5.1.4. British Pharmacopoeia (BP)

BP has been published since 1864, the current edition – BP 2019. BP operates in the United Kingdom simultaneously with the European Pharmacopoeia. BP monographs for substances are practically the same as the corresponding Ph. Eur. monographs. BP also contains monographs for finished dosage forms of medicines.

5.2. Harmonization of pharmacopoeias

Increased facilities for travel have brought into greater prominence the importance of an approach to uniformity in the formulae of the more powerful remedies, in order to avoid danger to patients when a prescription is dispensed in a different country from that in which it was written. The first attempts were made by international pharmaceutical and medical conferences to settle a basis on which an international pharmacopoeia could be prepared, but due to national jealousies and the attempt to include too many preparations nothing has yet been achieved.

Globalization processes indicated the need for harmonization of medicines quality requirements. Harmonisation of various countries pharmacopoeia requirements is determined by ICH (www.ich.org) – the International Conference on Harmonization of Medicines Quality and the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. The World Health Organization and three leading Pharmacopeias (Ph. Eur, USP, and JP) are directly involved in ICH activity. The purpose of ICH is to develop common requirements for standardization, quality control, efficiency, safety, production and registration of medicines. Representatives from three leading Pharmacopoeias meet twice a year since 1990 in the Pharmacopoeial Discussion Group to try to work towards «compendial harmonisation»'. Specific monographs are proposed, and if accepted, proceed through the stages of review and consultation. Adoption of a common monograph provides a common set of tests and specifications for a specific material. Not surprisingly, this is a slow process

6. Order of development of technical documentation of standards in pharmacy

The order of development of technical documentation of standards in pharmacy generally follows the same steps as described earlier. Here are the general steps:

Identification of the need: The first step is to identify the need for a standard in a specific area of pharmacy, such as manufacturing, quality control, distribution, use, or disposal of drugs.

Development of the draft standard: Based on the identified need, a draft standard is developed. This draft describes the objectives, scope, definitions, requirements, and recommendations for the specific area of pharmacy concerned.

Consultation: The draft standard is then subjected to consultation with relevant stakeholders, such as manufacturers, regulators, pharmacists, healthcare professionals, patient associations, and consumers. Comments and suggestions are gathered and taken into account to improve the draft standard.

Revision: Based on the received comments, the draft standard is revised and modified if necessary to reflect the concerns and comments of the stakeholders.

Adoption: Once the draft standard is finalized, it is submitted to a vote by the members of the standardizationcommitteeforadoption**.**

Publication: Once adopted, the standard is published and made available to the public. It can be used as a reference for the pharmaceutical industry, regulators, healthcare professionals, and patients.

Update: Like all standards, pharmaceutical standards are regularly reviewed and updated to reflect technological advancements and changes in industry requirements. Updates are generally made according to a defined schedule and after consultation with relevant stakeholders.

These steps may vary depending on the specific needs of the pharmaceutical industry and the procedures followed by the standardization body responsible for its development.

CHAPTER II: QUALITY CONTROL OF DRUGS IN PHARMACIES

1. Objectives

By the end of this chapter, the student will be able to:

- Define the core concepts of quality, quality assurance, and quality control.
- Outline the ten fundamental principles of Good Laboratory Practices and their significance in ensuring quality in pharmaceutical research and development.
- Describe the role and importance of the European Pharmacopoeia in setting quality standards for medicines and protecting public health.
- Differentiate between the roles and responsibilities of Quality Control and Quality Assurance within the pharmaceutical quality management system.

2. Introduction

This chapter introduces the fundamental principles of quality control for drugs in pharmacies. It begins by defining key concepts like "quality" and "quality assurance" according to international standards. The chapter then explores the critical frameworks that ensure drug safety and efficacy, including Good Laboratory Practices (GLP) for research and the standards of the European Pharmacopoeia for quality control. Finally, it distinguishes between the roles of Quality Control (QC) and Quality Assurance (QA), highlighting their interconnected responsibilities in guaranteeing that every pharmaceutical product is effective, safe, and reproducible.

3. Definition of quality

The official ISO definition: "Quality is the set of properties and characteristics of a product, process or service that gives it the ability to satisfy expressed or implied needs." (Paula Marc).

4. Definition of quality assurance

According to *ISO standard 8402-94*, quality assurance is "All the pre-established and systematic activities implemented within the quality system, and demonstrated as needed, to provide appropriate confidence that an entity will meet the requirements for quality."

5. Quality in the pharmaceutical industry

The rules to be followed in the pharmaceutical field to obtain a quality product, in this case the drug, are described in Good Manufacturing Practices (GMP). GMP describes the means, organization and controls to be put in place. The goal of the pharmaceutical industry is to produce a quality drug, through thorough clinical and preclinical studies, controlled production, in order to obtain a sufficient benefit/risk ratio to satisfy the patient. A quality drug can be described as:

- Effective: with the required and sufficient therapeutic effect
- Safe: the patient's health must not be jeopardized
- Controlled by a quality system: which guarantees its reproducibility

All these aspects are included in the Marketing Authorization (MA) dossier, which is a kind of product identity card, as it combines the efficacy and safety of the drug (through clinical and preclinical trials) and the quality (through the controls put in place by the manufacturer). These elements assure us that the drug is reproducible and of quality, and that the manufacturer has an effective quality system.

6. Good laboratory practices (GLP)

Since 1978, "Good Laboratory Practices" have regulated quality assurance in pharmaceutical research and development, aiming to prevent health scandals in the pharmaceutical industry. Issued worldwide, the guidelines they contain are now recognized internationally. Although GLP measures depend on the size of companies and institutes, certain standards must be respected in all laboratory activities. GLP-related operations can only be authorized if all controls have been carried out and fully documented.

6.1. GLP principles

As shown in **Fig.II.1**. The basic principles of GLP include:

- Organization and personnel of the test facility
- Quality assurance program
- Facilities
- Equipment, materials and reagents

- Test systems
- Test and reference items
- Standard operating procedures
- Performance of the study
- Reporting the study results
- Storage and retention of records and materials

Fig.II.1 GLP principles.

Chapter 1: Organization and personnel of the test facility

- Management: must ensure compliance with GLP principles in the test facility
- Study director: solely responsible for the control of the study and assumes responsibility for the overall conduct of the study and the final report
- Principal investigator: will ensure that the phases of the study delegated to them are carried out in accordance with the applicable GLP principles
- Study personnel: are familiar with the test protocol, implement it, document the results and report any deviations from the protocol

Chapter 2: Quality assurance program

- Deviations
- Non-conformities and corrective actions
- Amendments

Chapter 3: Facilities

- Facilities and premises
- Equipment and apparatus
- Electronic data and validation of computerized systems
- Archiving

Chapter 4: Apparatus, materials and reagents

Chapter 5: Test systems

- Management of the test items: test and reference items
- Management of reagents
- Test systems and specimens

Chapter 6: Test and reference items

Chapter 7: Standard operating procedures

- Documentary organization and traceability
- Data and record management
- Raw data: recording, validation

Chapter 8: Performance of the study

- Implementation and conduct of studies
- The different phases of the study
- Documentation of activities
- Study monitoring
- Management of unforeseen events and decisions during the study
- Management of study data
- Closure of the study

Chapter 9: Reporting the study results

Chapter 10: Storage and retention of records and materials

7. The European pharmacopoeia

7.1. Definition of the European pharmacopoeia

The European Pharmacopoeia (Ph. Eur.) is a unique reference work for quality control of medicines. The official standards published provide a scientific basis for quality control throughout the life of medicines. They concern the qualitative and quantitative composition and the tests to be carried out on the medicines, on the raw materials used in their production and on the synthesis intermediates. All producers of medicines and/or pharmaceutical substances must apply these quality standards in order to market their products in the signatory states of the Convention.

7.2. The role of the European pharmacopoeia

The role of the European Pharmacopoeia is to contribute to the protection of public health through the development of recognized common specifications relating to the quality of the medicinal product and its components. These specifications must be appropriate since they constitute, for the patient, one of the fundamental guarantees regarding the safe use of medicines. In addition, their existence facilitates the free movement of medicines within Europe and beyond.

The monographs and other texts of the European Pharmacopoeia are developed to meet the needs of:

- Regulatory authorities.
- Services responsible for quality control of medicines and their constituents.
- Manufacturers of medicines and their various components.

The European Pharmacopoeia is widely used internationally. Globalization and the expansion of international trade in the pharmaceutical field have increased the need to develop quality standards of global scope, and the Commission works closely with all users of the Pharmacopoeia worldwide.

8. Definition of quality control

Quality Control is part of Good Manufacturing Practice which is concerned with: sampling, specifications and testing, and the organisation, documentation and release procedures which ensure that the necessary and relevant tests are actually carried out and that materials are not released for use, nor products released for sale or supply, until their quality has been judged to be satisfactory.

Quality control refers to the sum of the procedures undertaken to ensure the identity and purity of a particular pharmaceutical.

- Manufacturers of drugs shall have a quality control systems which is designed to ensure that products are manufactured under adequate conditions and in accordance with procedures so as to meet established specifications,

• Quality control is not confined to laboratory operations, but must involve in all decisions which may concern the quality of the product.

- To meet this purpose, Quality Control Department should be appropriate and independent.

8.1. Quality management in the drug industry

In the drug industry at large, quality management is usually defined as the aspect of management function that determines and implements the "quality policy", i.e. The overall intention and direction of an organization regarding quality, as formally expressed and authorized by top management (**Fig.II.2**).

Fig.II.2 Quality relationship.

8.1.1. Quality assurance

− A wide-ranging concept which covers all matters which individually or collectively influence the quality of a product.

− The sum total of the organized arrangements to ensure that the products are of the quality required.

− Quality Assurance therefore incorporates Good Manufacturing Practice plus other factors outside the scope of these Guidelines, such as product design and development.

According to *ISO 9000*: QA is a part of quality management focused on providing confidence that quality requirements will be fulfilled. Whereas, QC is a part of quality management focused on fulfilling quality requirement.

- Within an organization, quality assurance serves as a management tool. In contractual situations, quality assurance also serves to generate confidence in the supplier. The concepts of quality assurance, GMP and quality control are interrelated aspects of quality management. They are described here in order to emphasize their relationship and their fundamental importance to the production and control of pharmaceutical products

8.1.2. Quality control responsibilities

- Perform testing of finished product
- Control of quality of the product on a daily basis at the company.

8.1.3. Quality assurance responsibilities

- Define quality standards and metrics
- Review quality standards and metrics by working closely with all departments
- Perform in-process checks
- Help in the prevention of defects
- Perform self-inspection
- Prepare documents
- Help in preparing a quality improvement plan

Fig.II.3 shows the difference between quality control and quality assurance.

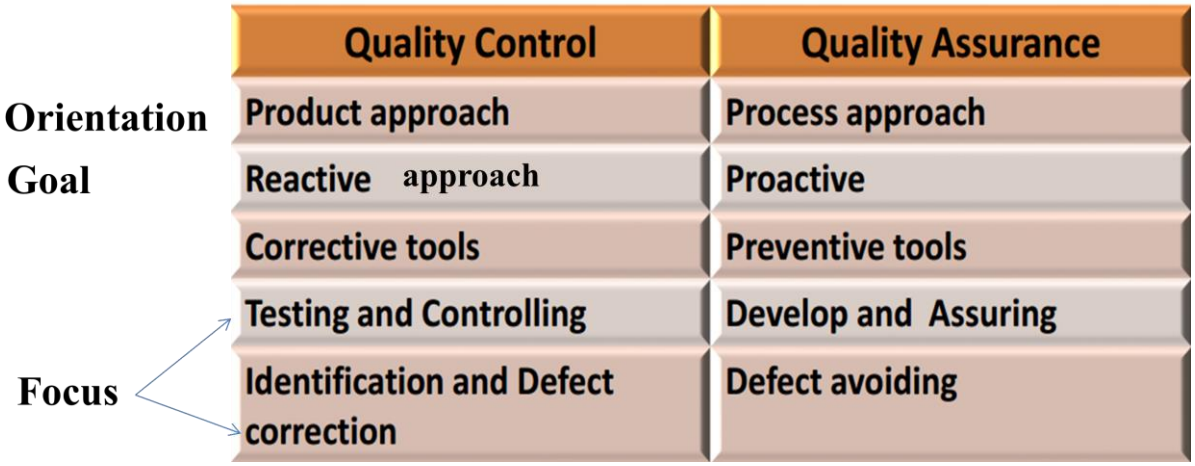


Fig.II.3 Difference between quality control and quality assurance.

CHAPTER III : PARTICULARITIES OF PHARMACEUTICAL ANALYSIS AND THE ESSENTIAL CRITERIA

1. Objective

By the end of this chapter, the student will be able to:

- Get an idea about the rules of pharmaceutical analysis data treatment and reporting, the scope and process of validation of analytical procedures
- Consolidate knowledge in problem solving and laboratory work

2. Introduction

Pharmaceutical analysis involves the analytical signals treatment and transformation into data concerning the nature and amount of a substance, its chemical structure or spatial location of the sample - important components that require statistical treatment to have any real value. According to pharmacopoeial and GMP requirements, the analytical procedures used for drug quality control must be validated to confirm experimentally that they provide relevant and reliable information about the object of analysis and are suitable for their intended purpose. All quantitative tests, including impurities limit tests, should be validated, as should identification tests if it is necessary to confirm their specificity. The validation of analytical procedures is regulated by various national and international standards (ICH), which evaluate characteristics such as accuracy, precision, specificity, detection limit, quantitation limit, linearity, range, and robustness. Strict adherence to these validation parameters is essential to ensure the reliability and integrity of pharmaceutical analysis, which is critical for product quality, safety, and efficacy.

Revalidation of an analytical procedure is performed in case of:

– changes in drug manufacturing process (the object of analysis);

– changes in the drug's composition;

– changes in the previously approved analytical procedure.

Trueness with regard to a set of measurements involves a combination of two components: Accuracy and Precision.

3. Essential criteria of pharmaceutical analysis

3.1. Accuracy

Accuracy is a crucial aspect of pharmaceutical analysis and refers to the closeness of the test result obtained by the analytical procedure to the true value **Fig. III.1**. The value of the bias (systematic error) is an indicator of the accuracy. An analytical procedure to be validated is considered accurate if the values accepted as conventionally true fall within the confidence intervals of the relevant average test results obtained by this procedure.

There are different approaches for assessing the accuracy of quantitative tests (assays):

a) Analysis by a validated procedure using Reference Standards (RS) or model mixtures to which known quantities (concentrations) of the drug substance to be analyzed are added. This allows the comparison of the measured values to the true/expected values.

b) Comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined. This provides a direct comparison between the two methods.

c) Consideration of the linearity of the proposed analytical procedure: if the absolute term of the equation $y = b\,x + a$ is not statistically different from zero, then the use of this procedure gives results free of bias.

These approaches provide a robust evaluation of the accuracy of the analytical procedure, which is essential for ensuring the reliability and integrity of pharmaceutical analysis. Maintaining high levels of accuracy is critical for product quality, safety, and efficacy.

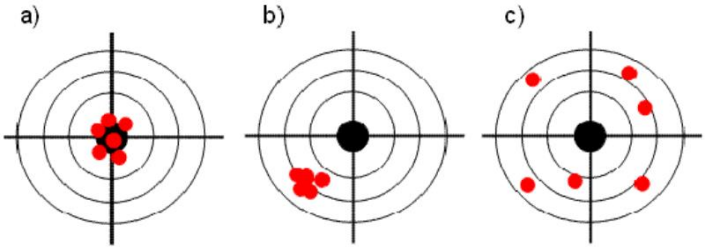


Fig. III.1 The accuracy and precision: a) and b) high precision; b) high accuracy in addition; c) the low accuracy and precision. Black circle indicates that the measured value is accepted to be true.

3.2. Precision

Precision is another crucial characteristic of pharmaceutical analysis, and it refers to the scattering of the results obtained by the analytical procedure around the average value. This characteristic depends only on random factors and is not connected to the true value being measured. The measure of this scattering is the standard deviation of the individual measurements obtained from a sufficiently large sample size.

Precision is assessed for all quantitative tests (assays) using the results of at least three determinations for three levels of values to be measured (lower, middle, and upper), which are within the range. In many cases, the precision can be evaluated based on the experimental data using the least squares method.

The precision study is performed using homogeneous samples and can be assessed in three ways:

1. **Repeatability:** This evaluates the precision under the same operating conditions over a short interval of time.
2. **Within-laboratory (intermediate) precision:** This evaluates the precision within the same laboratory, but under different conditions, such as different days, analysts, or equipment.
3. **Between-laboratory reproducibility (precision):** This evaluates the precision between different laboratories.

The results of these precision studies are usually characterized by the corresponding value of the standard deviation of the individual determinations, along with the number of degrees of freedom. The characteristic of an analytical procedure, connected with the possible identification or detection of analyte within the range of its small amounts is called Sensitivity and in the presence of other components – Specificity. Let's consider the basic numerical characteristics of sensitivity and specificity. Sensitivity is characterized by **Detection and Quantitation Limits.**

3.3. Detection Limit (Limit of Detection, LOD)

The Detection Limit is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. This means that limit tests can only confirm whether the analyte is present above or below a certain level, without providing a precise quantitative measurement.

The LOD is typically expressed as the concentration of the analyte (e.g., percentage, parts per billion) in the sample. It characterizes the procedure in terms of qualitative analysis.

For procedures with visual result assessment, the LOD is estimated by analyzing samples with known concentrations of the analyte to determine the minimum detectable amount.

For procedures with instrumental result assessment, the LOD can be calculated using either the signal-to-noise ratio or the standard deviation of the analytical signal and the slope of the calibration curve.

For the calculation using analytical signal/noise level ratio it is necessary to determine the minimum amount (concentration) of the analyte in the sample, in which the signal/noise ratio is in the range of **3 to 5**.

The equation **LOD = 3.3 × S/b** is commonly used, where S is the standard deviation of the analytical signal, and b is the sensitivity coefficient (slope of the calibration curve).

3.4. Quantitation Limit (Limit of Quantitation, LOQ)

The Quantitation Limit is the lowest amount of analyte in a sample that can be quantitatively determined with the required within-laboratory (intermediate) precision and accuracy. The

LOQ is particularly important for the determination of impurities and/or degradation products at low levels.

Similar to the LOD, the LOQ is expressed as the concentration of the analyte (e.g., percentage, parts per million) in the sample.

For procedures with visual result assessment, the LOQ is estimated by analyzing samples with various known concentrations of the analyte to determine the minimum value at which the analysis result can be obtained visually with the required accuracy and within-laboratory (intermediate) precision.

For procedures with instrumental result assessment, the LOQ can be calculated using either the signal-to-noise ratio or the standard deviation of the analytical signal and the slope of the calibration curve. For the calculation using analytical signal/noise level ratio it is necessary to determine the minimum amount (concentration) of the analyte in the sample, in which the **signal/noise ratio ≥10**

The equation **LOQ = 10 × S/b** is commonly used, where S is the standard deviation of the analytical signal, and b is the sensitivity coefficient (slope of the calibration curve).

3.5. Selectivity and Specificity

Selectivity - The proposed analytical procedure is considered selective if it allows for the quantitative determination of the analyte in a mixture of compounds with similar properties without bias.

Specificity - If the proposed procedure provides the ability to identify and determine quantitatively only the target analyte, then the procedure is considered specific.

For identification tests, the proposed procedure should provide reliable information on the presence of the drug substance in the API or dosage form, even in the presence of other excipients or ingredients.

For quantitative tests (assays), the procedure's specificity should be estimated experimentally to prove that the presence of related substances does not affect the result of the analysis.

Evaluation of specificity can be done in a few ways:

1. Analysis of model mixtures containing the analyte and known composition
2. Comparison of the results obtained using the proposed procedure to those of a second well-characterized and specific procedure

The specificity is also confirmed by analyzing blank samples without the analyte present. For example, the HPLC chromatograms **(Fig.III.2)** show that endogenous substances in the blood serum do not interfere with the identification of the target drug components.

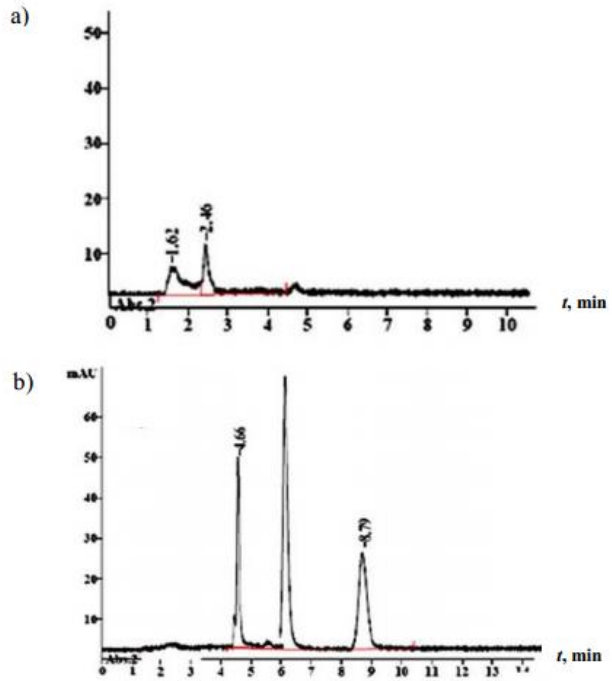


Fig.III.2 HPLC chromatograms of blood serum samples: a) sample without the analytes (blank test); b) spiked sample (standard addition of analytes).

3.6. Robustness

Robustness refers to the ability of a validated analytical procedure to remain unaffected by small but deliberate variations in the method parameters. In other words, it means the procedure should maintain the estimated characteristics (e.g. precision, accuracy) that were obtained under optimal (nominal) conditions, even when there are likely small deviations from those conditions.

3.7. Linearity

Linearity refers to the presence of a linear relationship between the analytical signal (e.g. peak area, absorbance) and the concentration (or amount) of the analyte in the sample, within a given range of the analytical procedure.

To evaluate the linearity of the procedure, you would experimentally measure the analytical signals for at least five samples with different amounts or concentrations of the analyte.

The correlation coefficient (r) should then be calculated. In analytical chemistry, a linear dependence is typically considered acceptable if $|\mathbf{r}| \geq \mathbf{0.99}$.

For the analysis of trace amounts, a slightly lower correlation coefficient of $|\mathbf{r}| \geq \mathbf{0.90}$ may be acceptable. The linearity can also be represented graphically as a function of the analytical signal versus the analyte concentration (**Fig.III.3**).

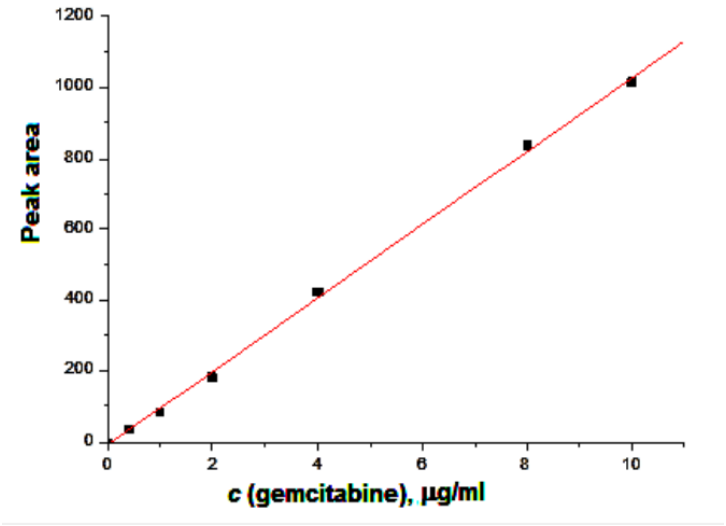


Fig.III.3 Dependence of the area of chromatographic peaks of the concentration of the active ingredient (gemcitabine) in the «Gemzar».

CHAPTER IV: TECHNICAL LABORATORY OPERATIONS IN A PHARMACEUTICAL COMPLEX

1. Objectives

Upon the completion of this chapter, the student will be able to:

- Explain the importance of pharmaceutical analysis in ensuring drug quality, safety, and efficacy.
- Identify and describe the four main stages of pharmaceutical analysis.
- Describe the design and purpose of stability studies.
- Describe the essential requirements for the organization and management of a quality control laboratory.
- Recognize and evaluate critical laboratory safety rules and procedures,

2. Introduction

Pharmaceutical quality control represents a critical discipline where scientific rigor and systematic management converge to ensure that every medicine released to the market is safe, effective, and of consistent quality. This field encompasses a comprehensive framework that spans the entire product lifecycle, beginning with rigorous analysis of raw materials and components that serves as the first line of defense in the quality system. The process continues with real-time in-process controls that monitor production at critical stages, followed by exhaustive finished product testing that constitutes the final verification before market release. Complementing these activities, stability testing provides ongoing assurance of product quality throughout its shelf life by systematically evaluating how drugs withstand environmental factors over time.

These analytical activities rely on sophisticated instrumentation including chromatographic methods like HPLC and GC for separation and quantification, spectroscopic techniques such as UV-Vis and FTIR for identification, and specialized physical testing equipment for assessing product performance characteristics. However, technical capability alone is insufficient without a robust organizational framework. Pharmaceutical quality control laboratories must operate as legally responsible entities with clear management structures,

competent personnel, and systems that ensure independence from commercial pressures. The foundation of reliable operations rests on a comprehensive quality management system that includes detailed quality manuals, standardized operating procedures, and systematic documentation control.

3. Types of technical analysis in a pharmaceutical complex

Technical analysis is integrated throughout the entire product lifecycle. Here are the main types, typically organized by the stage at which they are applied:

3.1. Analysis of raw materials and components

The analysis of raw materials and components serves as the first line of defense in the quality control system of a pharmaceutical complex. Its primary purpose is to ensure that all incoming materials including Active Pharmaceutical Ingredients, excipients (inactive ingredients), packaging materials, and solvents are suitable for their intended use before they are released into the manufacturing process. This is achieved through a battery of common tests designed to comprehensively characterize each material. These tests typically include identification to confirm the correct substance is present using techniques like FTIR or pharmacopoeial methods; assay and potency testing to quantify the strength of the API; purity and impurity profiling to detect and quantify contaminants such as related substances or residual solvents using HPLC or GC; and an assessment of key physicochemical properties like particle size, density, pH, and water content, which are critical for ensuring consistent product performance and stability.

3.2. In-process controls (IPC)

In-process controls are a series of checks performed during the manufacturing process to monitor and control production in real-time. The purpose of IPC is to ensure that the product meets its predefined specifications at critical intermediate steps, allowing for immediate adjustments to be made before the batch is completed. This proactive approach prevents the propagation of errors and is fundamental to building quality into the product. Typical elements tested include intermediate products, blend uniformity, and key process parameters. Common tests involve checking *blend uniformity* to ensure a homogeneous powder mix before tablet compression; monitoring *weight variation* of tablets as they are being compressed; controlling *pH and temperature* during chemical synthesis or in liquid

formulations; and assessing *solution clarity and color* for injectable products to guarantee purity and the absence of visible particulates.

3.3. Finished product testing (release testing)

Finished product testing, also known as release testing, constitutes the final and comprehensive analysis of the drug product in its market container. Its critical purpose is to confirm that the final dosage form—whether a tablet, capsule, injection, or cream—complies with all established release specifications before it is approved for distribution to the market. This testing verifies the identity, strength, quality, and purity of the product. A battery of common tests is performed, including *identification* to confirm the presence of the correct Active Pharmaceutical Ingredient; *assay* to precisely measure the amount of API per dosage unit; *content uniformity* to ensure consistency of the API across individual units; and *dissolution testing* to measure the release rate of the API, which is critical for predicting its absorption in the body. Furthermore, *microbiological testing* is conducted to check microbial limits or sterility, and specific *tests on the dosage form* itself, such as hardness and friability for tablets, sterility for injectables, and leak testing for packages, are performed to ensure overall integrity and performance.

3.4. Stability testing

Stability testing is an ongoing program that runs in parallel to a product's market life. Its primary purpose is to determine the product's shelf life (expiration date) and recommend appropriate storage conditions by systematically evaluating how its quality changes over time under the influence of various environmental factors like temperature and humidity. This is achieved by storing product batches in stability chambers under controlled conditions such as 25 °C/60% relative humidity or more accelerated conditions like 40 °C/75% relative humidity and testing them at predetermined intervals throughout their lifespan, for example at 0, 3, 6, 9, 12, 18, 24, and 36 months. The key parameters monitored during this testing include the assay of the active ingredient, the formation of degradation products (impurities), dissolution performance, physical appearance, and microbiological quality to ensure the product remains safe, effective, and of high quality until its expiration date.

4. Analytical techniques

The types of analysis mentioned above rely on a suite of sophisticated instruments:

- **Chromatography:** To separate, identify, and quantify components in a mixture.
  - HPLC: The workhorse for assay and impurity testing.
  - GC: For volatile compounds and residual solvents.
- **Spectroscopy:** For identification and quantification.
  - UV-Vis Spectroscopy: Often used for assay.
  - FTIR Spectroscopy: Primarily for identification.
  - Atomic Absorption (AA)**:** For elemental impurities and heavy metals.
- **Physical Testing:**
  - Dissolution Test Apparatus: To measure drug release.
  - Friability and Hardness Testers: For tablet quality.

5. Good practices applicable to pharmaceutical quality control laboratories

Pharmaceutical quality control analyses generally involve performing repetitive tests on samples of APIs or a limited number of pharmaceutical products. In contrast, national quality control laboratories must be capable of handling a much larger number of pharmaceutical substances and products and, therefore, must implement a greater variety of analytical methods. The following text addresses specific recommendations for national pharmaceutical quality control laboratories. Quality control laboratories may perform some or all activities in this field, for example, sampling, analysis of APIs, excipients, packaging materials and/or pharmaceutical products, stability testing, analysis against specifications, and investigative analysis.

5.1. Organization and management of a pharmaceutical quality control laboratory

The laboratory, or the organization to which it belongs, must be a legally authorized entity that can be held legally responsible.

➢ The laboratory must be organized and must operate in a manner that satisfies the standards set out in this directive.

➢ The laboratory must:

a) Have managerial and technical staff with the authority and resources necessary to perform their duties and to identify instances of deviation from the quality management system or the methods for performing analyses and/or calibrations, validations, and verifications, and to initiate actions to prevent or minimize such deviations;

b) Make provisions to ensure that its management and staff are shielded from any commercial, political, financial, and other pressures, or conflicts of interest that could adversely affect the quality of their work;

c) Have established a policy and procedure guaranteeing the confidentiality of:
— information contained in marketing authorizations,
— the transmission of results or reports, and
— the protection of data in records (on paper or in electronic format);

d) Define, using organizational charts, the laboratory's organizational and managerial structure, its place within the parent organization (e.g., the ministry or the National Regulatory Authority if it is a national pharmaceutical quality control laboratory), and the relationships between management, technical operations, support services, and the quality management system;

e) Specify the responsibilities, reporting lines, and relationships between different members of the managerial, operational, and quality control staff involved in the quality of analyses and/or calibrations, validations, and verifications;

f) Ensure a clear allocation of responsibilities, including the designation of specific units responsible for particular types of medicines;

g) Appoint deputies/assistants for key managerial staff and specialized scientific personnel;

h) Provide adequate supervision of staff, including trainees, by persons thoroughly familiar with the methods and procedures for analysis and/or calibration, validation, and verification, as well as their purpose and the evaluation of results;

i) Ensure management has overall responsibility for technical operations and the resources needed to guarantee the required quality of the laboratory's operations;

j) Appoint, from among the staff, a quality manager who, independent of other duties, will ensure compliance with the quality management system. This staff member must have direct access to the highest level of management where decisions are made concerning laboratory policies or resources;

k) Ensure sufficient flow of information among staff at all levels. Staff must be aware of the relevance and importance of their activities;

l) Ensure the traceability of samples, from receipt through all stages of analysis, to the execution of the analysis report;

m) Maintain an up-to-date collection of all specifications and related documents (in paper or electronic form) applied in the laboratory;

n) Define appropriate safety procedures.

- The laboratory must maintain a registration system with the following functions:

a) Receiving, distributing, and monitoring the dispatch of samples to the various units;
b) Keeping records of all incoming samples and associated documents.

- In a large laboratory, communication and coordination must be ensured between different staff members analyzing the same sample in different units.

5.2. Quality management system

- The management of the laboratory or organization must define, implement, and maintain a quality management system appropriate to the scope of its activities, including the type, range, and volume of the analysis and/or calibration, validation, and verification work it undertakes. The laboratory management must ensure that its policies, systems, programs, methods, and procedures are described to the extent necessary to enable the laboratory to ensure the quality of the analysis results it produces. The documentation used in this quality management system must be communicated to, available to, understood by, and implemented by the relevant personnel. The elements of this system must be documented, for example in the form of a quality manual, for the organization as a whole and/or for a laboratory within it.
- The quality manual must include at a minimum:

a) A quality policy statement, including at least:

i) A statement of management's intentions regarding the level of service provided.

ii) A commitment to define, implement, and maintain an effective quality management system.

iii) The management's commitment to professional good practices and the quality of analyses, calibrations, validations, and verifications.

iv) The management's commitment to comply with the content of this directive.

v) The requirement for all personnel involved in analysis and calibration activities within the laboratory to be thoroughly familiar with the quality documentation and to implement the policies and procedures in their work.

b) The laboratory's structure (organizational chart).

c) The operational and functional activities relating to quality, so that the scope and limits of responsibilities are clearly defined.

d) A description of the structure of the documentation used in the laboratory's quality management system.

e) Internal general quality management procedures.

f) References to specific methods for each test.

g) Information on the required qualifications, experience, and appropriate competencies for personnel.

h) Information on the initial and continuous training of personnel.

i) A policy on internal and external audits.

j) A policy on the application and verification of corrective and preventive actions.

k) A policy on the handling of complaints.

l) A management policy for the review of the quality management system.

m) A policy for the selection, definition, and approval of analytical methods.

n) A policy on the handling of out-of-specification (OOS) results.

o) A policy on the use of appropriate reference substances and materials.

p) A policy on participation in appropriate proficiency testing schemes, collaborative trials, and performance assessments (applicable to national pharmaceutical quality control laboratories, but also potentially to other laboratories).

q) A policy for the selection of service providers and suppliers.

- The laboratory must define, apply, and maintain authorized, written Standard Operating Procedures (SOPs) covering, but not limited to, administrative and technical operations, such as:

a) Personnel matters, qualifications, training, attire, and hygiene.

b) Change control.

c) Internal audits.

d) Handling of complaints.

e) Application and verification of corrective and preventive actions.

f) Purchasing and receipt of goods (e.g., samples, reagents).

g) Sourcing, preparation, and control of reference substances and materials.

h) Internal labeling, quarantine, and storage of goods.

i) Equipment qualification.

j) Equipment calibration.

k) Preventive maintenance and verification of instruments and equipment.

l) Sampling, if performed by the laboratory, and visual inspection.

m) Analysis of samples with descriptions of the methods and equipment used.

n) Out-of-specification and atypical results.

o) Validation of analytical methods.

p) Cleaning of laboratory facilities, including benches, equipment, workstations, clean (aseptic) rooms, and glassware.

q) Monitoring of environmental conditions, e.g., temperature and humidity.

r) Monitoring of storage conditions.

s) Disposal of reagents, solvents, and samples.

t) Safety measures.

➢ The laboratory's activities must be systematically and periodically reviewed (through internal audits and, where applicable, external audits or inspections) to verify compliance with the requirements of the quality management system and to apply, if necessary, corrective and preventive actions. Audits must be performed by trained and qualified personnel independent of the activity being examined. The quality manager is responsible for planning and organizing internal audits covering all elements of the quality management system. These reviews must be recorded, with full details of any corrective and preventive actions taken.

➢ Management must regularly review (at least annually) quality-related matters, including:

a) Reports from internal and external audits or inspections and any required follow-up to correct deficiencies.

b) The results of investigations conducted following the receipt of complaints, or of doubtful (atypical) or aberrant results from collaborative trials and/or proficiency tests.

c) Corrective actions applied and preventive measures introduced as a result of these investigations.

5.3. Presentation of documentation in complex laboratories

➢ Documentation is an essential part of the quality management system. The laboratory must establish and maintain procedures to control and review all documents (produced internally and from external sources) that form part of the quality documentation. A controlled document list, identifying the version status and distribution, should be established and readily available.

➢ The procedures must ensure that:

a) Each document, whether technical or quality-related, has a unique identification number, a version number, and an effective date.

b) Authorized and appropriate procedures are available at all necessary locations, for example, near instruments.

c) Documents are kept up-to-date and reviewed as necessary.

d) Any obsolete document is withdrawn and replaced by the revised and authorized document with immediate effect.

e) Any revised document contains references to the previous version.

f) Obsolete and old documents are retained in the archives to ensure traceability in the evolution of methods; all copies [designated for use] are destroyed.

g) All relevant personnel are trained on new and revised procedures.

h) Quality documentation, including records, is retained for a minimum of five years.

➢ A change control system must be established to inform staff of new or revised methods. This system must ensure that:

a) Revised documents are prepared by the originator, or a person in the same role, reviewed and approved at the same level as the original document, and then distributed by the quality manager (quality unit).

b) Personnel confirm, by signature, that they have acknowledged the applicable changes and their implementation date.

5.4. Working methods in pharmaceutical laboratories

5.4.1. Sample reception and management

The process begins with the receipt of samples, which may be intended for compliance testing or investigative testing. Compliance testing typically involves samples collected systematically, those suspected of non-conformity, or those submitted for marketing authorization. Investigative samples, often from customs or police, concern suspicious or counterfeit products. An essential practice is to divide each sample into three equal parts: the

first for immediate analysis, the second for confirmation if needed, and the third for archiving in case of dispute. Each sample must be accompanied by a standardized analysis request form containing all critical information such as origin, complete description, batch number, and specifications to be used.

5.4.2. Traceability and documentation

Traceability is fundamental throughout the entire process. Upon arrival, samples receive a unique registration number that is affixed to each container. A register is maintained to record this number, the date of receipt, and the sending unit. Staff immediately performs a visual inspection to verify labeling conformity and the sample's condition. Any anomaly is documented without delay. The analysis worksheet is the central traceability document, serving as objective evidence to demonstrate that the analysis was performed in accordance with requirements. It must contain all information on the methods used, results obtained, and conclusions, with strict rules for error correction.

5.4.3. Validation and execution of analyses

All analytical methods must be validated to demonstrate their suitability for the intended use. This validation follows a rigorous protocol evaluating parameters such as specificity, accuracy, and precision. Pharmacopoeial methods, while considered validated, require verification of their applicability to the specific product. Before each analysis, system suitability tests are performed to ensure equipment is functioning properly. During analysis, any deviation from the method must be approved and documented. Results are examined statistically, and any questionable value triggers a thorough investigation according to a defined process.

5.4.4. Management of results and archiving

The evaluation of results involves a complete review of all data. In the case of an out-of-specification (OOS) result, a rigorous investigation is conducted, including verification of calculations, calibration, and reagents used. If no error is identified, a confirmation analysis by another analyst is required. Final conclusions are recorded in an analysis report and a certificate of analysis for compliant batches. Archiving is a crucial step: samples are stored in their original packaging in sufficient quantity to allow for at least two re-analyses, while all documents, including analysis worksheets and raw data, are stored securely for a defined minimum period, thereby ensuring complete traceability and full transparency in the analytical process.

5.5. Laboratory safety rules

➢ General and specific safety instructions, commensurate with the identified risks, must be available to every member of staff and regularly supplemented as appropriate (e.g., through written instructions, posters, audiovisual materials, and sometimes seminars).

➢ General safety rules, in accordance with national regulations and procedures, normally include:

a) Making safety data sheets available to staff before performing analyses.

b) Prohibiting smoking, eating, and drinking in the laboratory.

c) Staff being thoroughly familiar with the use of fire-fighting equipment, such as extinguishers, blankets, gas masks, etc.

d) Wearing laboratory coats or other protective clothing, including eye protection.

e) Taking special precautions appropriate for handling, for example, highly active, infectious, or volatile substances.

f) Handling highly toxic and/or genotoxic samples in a specially designed facility to avoid the risk of contamination.

g) Fully labeling all chemical containers with clearly visible warnings (e.g., "Poison," "Flammable," "Radioactive") whenever necessary.

h) Ensuring sufficient electrical insulation and anti-spark systems for all wiring and electrical equipment, including refrigerators.

i) Observing safety rules when handling compressed gas cylinders and ensuring staff are familiar with the identification color codes.

j) Staff being acutely aware of the need to avoid working alone in the laboratory.

k) Providing first aid equipment and training staff in first aid techniques, emergency care, and the use of antidotes.

➢ Protective clothing must be available, including eye protection, masks, and gloves. Safety showers must be installed. Rubber bulbs must be used with pipettes and manual siphons. Staff must know how to handle laboratory glassware, corrosive reagents, and

solvents safely, and specifically know how to use safety collectors or containers to prevent accidental spills. Warnings, instructions, and precautions must be provided when work involves violent, uncontrollable, or hazardous reactions due to the handling of specific reagents (e.g., mixing water and acids, acetone-chloroform and ammonia), flammable materials, oxidizing or radioactive agents, and especially biological products, such as infectious agents. Peroxide-free solvents should be used. Staff must be familiar with methods for the safe disposal of unwanted corrosive or hazardous materials by neutralization or deactivation, as well as the complete and safe disposal of mercury and its salts.

- Toxic or hazardous products must be properly marked and labeled, but no other chemical or biological product should be considered inherently safe. Unnecessary contact with reagents, especially solvents and their vapors, must be avoided. The use of known carcinogens and mutagens must be limited or entirely excluded if required by national regulations. Replacing toxic solvents and reagents with less toxic products or limiting their use should always be the goal, particularly when developing new techniques.

CHAPTER V : SAMPLING OPERATIONS

1. Objectives

By the end of this chapter, the student will be able to:

- Define sampling and explain its critical importance in the overall validity of pharmaceutical analysis.
- Identify the roles and necessary qualifications of a competent sampler.
- Differentiate between key sampling terms.
- Outline the general rules for sampling, including the requirements for dedicated facilities, health and safety protocols, and the proper use of sampling tools.
- Describe the correct procedures and essential precautions for conducting a sampling operation.
- Explain the appropriate methods for packaging, labeling, and storing different types of samples.

2. Introduction

Sampling comprises the operations designed to select a portion of a pharmaceutical product for a defined purpose. The sampling procedure should be appropriate to the purpose of sampling, to the type of controls intended to be applied to the samples and to the material to be sampled. All operations related to sampling should be performed with care, using proper equipment and tools. Any contamination of the sample by dust or other foreign material is liable to jeopardize the validity of the subsequent analyses.

• Sample selection in drug analysis

The selection of one or more appropriate samples from a larger quantity of material is a very important step in chemical analysis. This is rarely a simple matter. If the final results obtained are expected to have practical value, it is preferable that the sampling steps be carried out by or under the direction of a qualified sampler who understands the general context of the analysis. This person should be an experienced analyst or someone specially trained in sampling. When it is impossible to use the services of such qualified persons to collect samples, the laboratory is encouraged to work with the client to obtain advice and possibly

practical assistance, to ensure sampling is as appropriate as possible. It is a very common pitfall to underestimate the importance of the sampling procedure and to entrust it to an employee who is neither qualified nor trained for this task.

- ## Termes and types of samples

One of the best approaches to sampling terminology is provided in the recommendations published by IUPAC. The following definitions have been used as proposed by IUPAC.

Sample

A portion of a material collected according to a defined sampling procedure. The size of any sample should be sufficient to allow all anticipated test procedures to be carried out, including all repetitions and retention samples. If the quantity of material available is not sufficient for the intended analyses and for the retention samples, the inspector should record that the sampled material is the available sample (see Sampling record) and the evaluation of the results should take account of the limitations that arise from the insufficient sample size.

Sampler

Person responsible for performing the sampling operations.

Sampling method

That part of the sampling procedure dealing with the method prescribed for with drawing samples.

Batch

A quantity of any drug produced during a given cycle of manufacture. If the manufacturing process is continuous, the batch originates in a defined period of time during which the manufacturing conditions are stable and have not been modified.

Available sample

Whatever total quantity of sample materials is available.

Original sample

Sample collected directly from the material.

Final sample

Sample ready for the application of the test procedure.

Retention sample

Sample collected as part of the original sampling process and reserved for future testing. The size of a retention sample should be sufficient to allow for at least two confirmatory analyses. In some cases statutory regulations may require one or more retention samples, each of which should be separately identified, packaged and sealed.

Combined sample

Sample resulting from combining all or parts of two or more samples of the material.

Consignment

The quantity of a bulk starting material, or of a drug product, made by one manufacturer or supplied by an agent, and supplied at one time in response to a particular request or order.

Homogeneity

A material is regarded as homogeneous when it is all of the same origin (e.g.from the same batch) and as non-homogeneous when it is of differing origins.

Uniformity

A starting material may be considered uniform when samples drawn from different layers do not show significant differences in the quality control tests which would result in non-conformity with specifications. The following materials may be considered uniform unless there are signs to the contrary: organic and inorganic chemicals; purified natural products; various processed natural products such as fatty oils and essential oils; and plant extracts. The assumption of uniformity is strengthened by homogeneity, i.e. when the consignment is derived from a single batch.

Sampling plan

Description of the location, number of units and/or quantity of material that should be collected, and associated acceptance criteria.

Sampling record

Written record of the sampling operations carried out on a particular material for a defined purpose. The sampling record should contain the batch number, date and place of sampling, reference to the sampling protocol used, a description of the containers and of the materials sampled, notes on possible abnormalities, together with any other relevant observations, and the name and signature of the inspector.

Fig.V.1 shows the different classed of materials to be samples in the pharmaceutical industry.

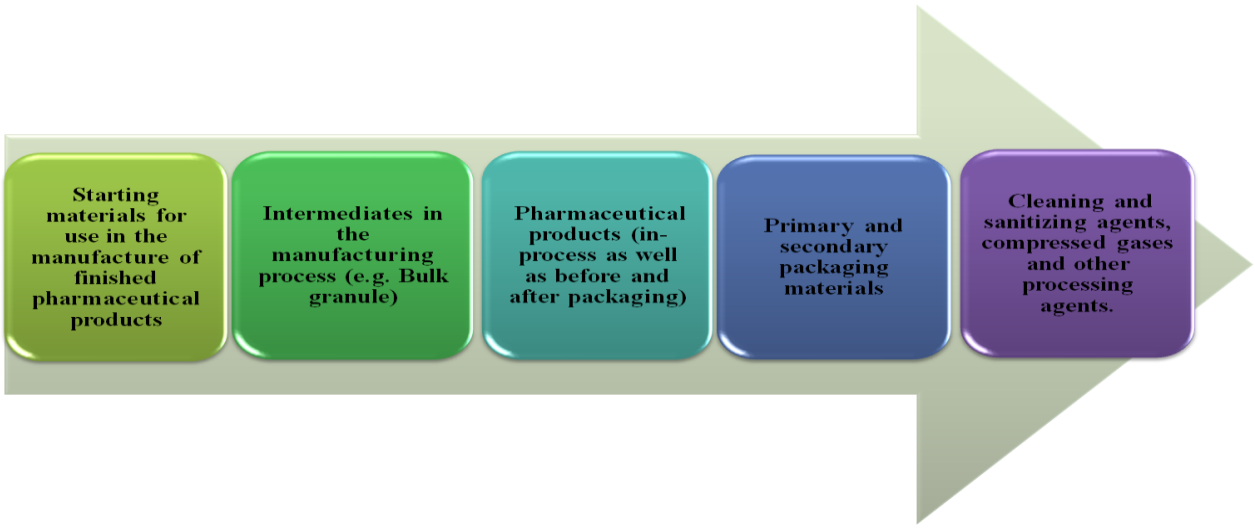


Fig.V.1 Different classes of materials to be samples in the pharmaceutical industry.

5. General rules

5.1. Sampling facilities

Where possible, sampling should be performed in an area designed for and dedicated to this purpose (**Fig.V.2)**, although this will not be possible where samples are required to be taken from a production line (e.g. in-process control samples). The area in which the sample was taken should be recorded in the sampling record.

Sampling from large containers of starting material or bulk products can present difficulties. Whenever possible, this work should be carried out in a separate, closed cubicle within the warehouse, to reduce the risk of contamination (e.g. by dust) of either the sample or the materials remaining in the container, or of cross-contamination.

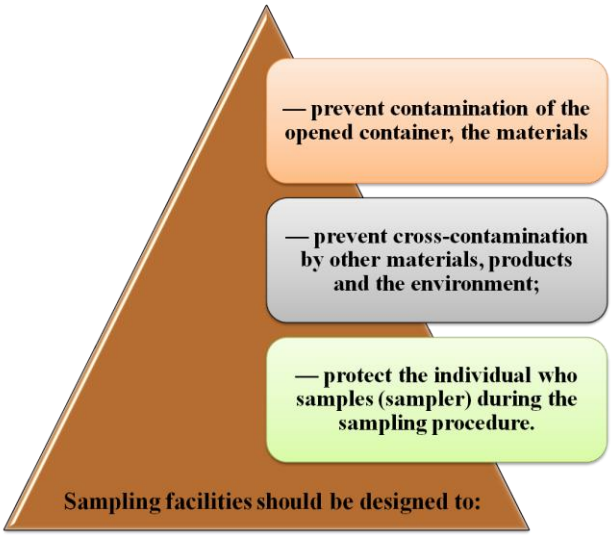


Fig.V.2 Sampling facilities requirements.

5.2. Health and safety

- The sampler should:

✓ read the relevant health and safety information (e.g. the safety data sheet for a pharmaceutical product and related materials) before sampling the material. The information should include necessary safety precautions and requirements for both the operator and the environment.

✓ wear appropriate protective clothing for the task. If specific safety precautions are required, such as the use of respiratory equipment, the sampler should be properly trained in its use.

✓ have safe access to and egress from the place where the sample is taken. The sample storage areas should have adequate light and ventilation and should be arranged to satisfy the requirements for safety as well as any special ones arising from the characteristics of the material being sampled.

- Care should be taken to guard against collapse of stacked containers or solids in bulk.

- For the sampling of products, the responsible person should have at his or her disposal all the tools (**Fig.V.3)** needed to open the containers.

✓ All sampling tools and implements should be made of inert materials and kept scrupulously clean. After use or before reuse, they should be thoroughly washed, rinsed with water or suitable solvent, and dried. They should be stored in clean

conditions. Adequate washing facilities should be provided in, or in close proximity to, the sampling area, otherwise samplers will need to bring separate clean sets of implements for sampling each product.

✓ The cleaning procedure used for all sampling tools and implements should be documented and recorded.

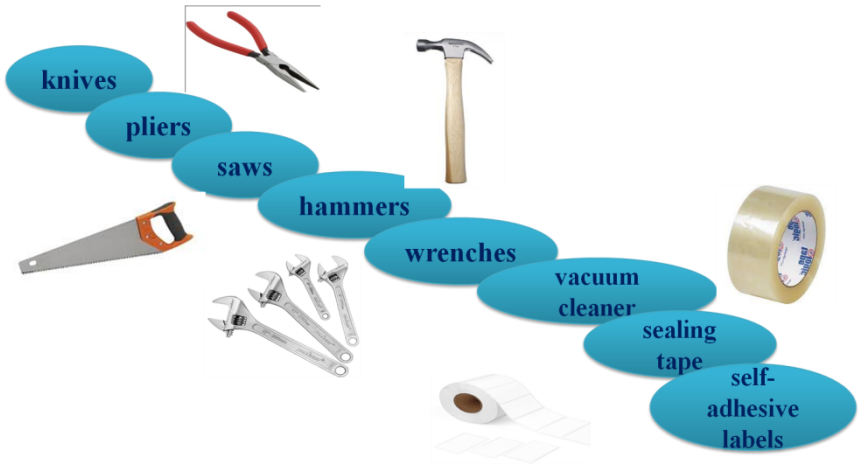


Fig.V.3 Sampling tools.

5.3. Sampling operation and precautions

- Sampling procedure should include the details of the health and safety aspects of sampling.
- Sampling procedure should ensure that representative samples are taken in sufficient quantity for testing in accordance with specifications.
- Samples should never be returned to the bulk.
- The sampling process should be appropriately supervised and documented.
- During the sampling procedure, attention should be paid to any signs of nonconformity of the material (differences in shape, size or color of particles in crystalline, granular or powdered solid substances; moist crusts on hygroscopic substances; deposits of solid pharmaceutical product in liquid or semi-liquid products; and stratification of liquid products. Such changes, some of which may be readily reversible, can occur during prolonged storage or exposure to extreme temperatures during transportation).

- **Packaging, labeling and storage of samples**

The container used to store a sample:

- Should also protect the sample from light, air and moisture, as required by the storage directions for the pharmaceutical product or related material sampled.
- Liquid samples should be transported in suitable bottles closed by screw tops with inert liners that provide a good vapor-proof (moisture-proof) seal for the contents.
- Suitable screw-top jars (**Fig.V.4)** in exceptional cases only should be used for solid or semi-solid pharmaceutical products.

Fig.V.4 Screw-top jars and screw tops.

Solid dosage forms such as tablets or granules should be protected during transit, either by totally filling the container with the product or by filling any residual space with a suitable material. All samples should be packaged adequately and transported in such a way as to avoid breakage and contamination during transport.

Labeling of samples should provide appropriate details, including the batch number and, if known, the container number from which the sample was taken, the amount taken and for what purpose. Labels should be applied at the time of sampling. The container used to store the sample should also be properly labeled with appropriate details such as sample type, name of material, identification code, batch/lot number, code, quantity, date of sampling, storage conditions, handling precautions and container number.

CHAPITRE VI : STERILZATION

1. Objectives

By the end of this chapter, the student will be able to:

- Define and explain the core concept of sterilization.
- Classify the different sterilization methods.

2. Introduction

Sterilization is a term referring to any process that eliminates (removes) or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media. Sterilization can be achieved by applying heat, chemicals, irradiation, high pressure, or filtration.

3. Sterilization methods

Sterilization methods are fundamentally categorized into two overarching principles: destruction-based methods, which actively kill microorganisms, and elimination-based methods, which physically remove them. These principles are executed through physical and chemical means (**Fig.VI.1)**.

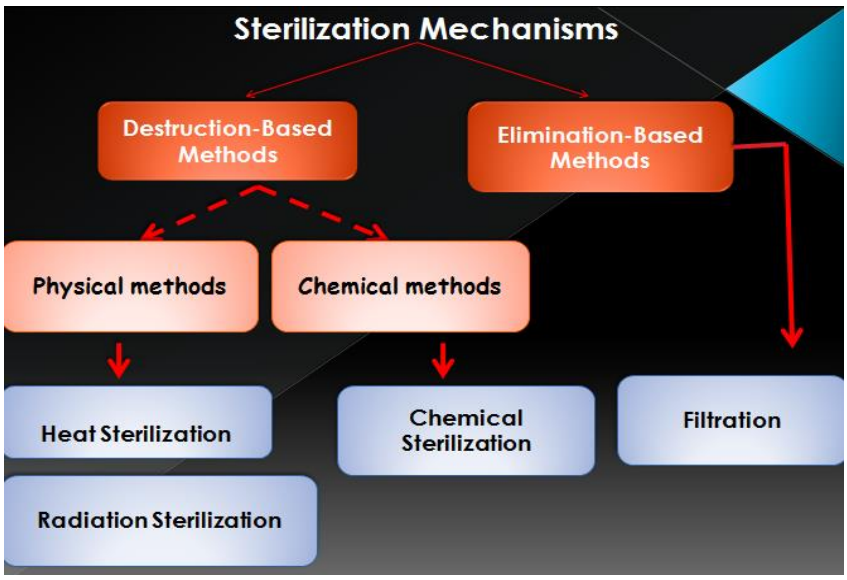


Fig.VI.1 Sterilization mechanism.

3.1. Destruction based methods

These methods work by actively destroying or inactivating microorganisms at the molecular or structural level. They cause irreparable damage to essential microbial components like proteins, DNA, and cell membranes, leading to cell death.

3.1.1. Heat (Dry and moist)

Heat is considered to be most reliable method of sterilization of objects that can withstand heat.

A. Dry heat

Dry heat is one such method, whose primary mechanism of action is the oxidation of microbial cell components, effectively burning them. It is applied through several techniques.

Flaming (**Fig**.**V.2**) is a common practice for the direct sterilization of small items like inoculating loops, wires, and spatulas.

For larger or more complex items, a **hot air oven** (**Fig**.**V.2**) is used, typically operating at standard cycles of 160 °C for 2 hours or 170 °C for 1 hour. This method is commonly employed to sterilize glassware, such as Petri dishes and test tubes, as well as metallic instruments like scalpels and forceps.

Fig.**V.2** Dry heat sterilization.

B. Moist heat

The sterilization method of choice in most laboratories is autoclave (**Fig.V.3**). It uses pressurized steam to heat the material to be sterilized. This is a very effective way to kill all microbes, spores and viruses, but some specific bugs require particularly high temperatures or incubation times. Autoclaves kill microorganisms by hydrolyzing and coagulating cellular proteins. This is efficiently achieved by applying intense heat in the presence of water. Intense heat comes from steam. Compressed steam has a high latent heat. It retains 7 times more heat than water at the same temperature. This heat is released upon contact with the colder surface of the material to be sterilized, allowing rapid heat release and good penetration into high density materials. At these temperatures, water does a great job of hydrolyzing proteins.

Fig.V.3 Autoclave.

3.1.2. Radiation

There are two main types of radiation used for microbial control, both of which typically destroy nucleic acids.

a) Non-ionizing Radiation

- **UV Radiation** has a germicidal effect on microorganisms. However, it has limited penetration in air, so sterilization is only effective in a reasonably small area around the lamp.

It is a relatively safe method and is useful for sterilizing small, enclosed spaces like laminar flow hoods (**Fig.V.4**).

- **Common uses:** Surface disinfection in hospitals, operating theatres, and laboratories.

b) Ionizing Radiation

- **X-rays and gamma rays** are far more penetrating than UV light. This makes them more hazardous to handle but also highly effective for the large-scale, "cold" sterilization of items like plastic syringes during manufacturing. This type of radiation uses high energy to cause direct breaks in DNA strands and generates free radicals that damage other cellular structures.

Fig.V.4 Sterilization by UV.

3.1.3. Gas Sterilization

Ethylene oxide (**Fig.V.5)** gas is a low-temperature alkylating agent used to sterilize heat-sensitive objects that would be damaged by steam sterilization. Its common applications include medical and pharmaceutical products, as well as plastic containers. Formaldehyde, a water-soluble gas often supplied as a 35% solution in water known as formalin, is another effective sterilant. It acts as a broad-spectrum germicide against bacteria, fungi, viruses, and even endospores through a mechanism of protein denaturation. It is commonly used for the sterilization of surgical instruments. In contrast, alcohols such as ethanol (80%), propanol (60%), and isopropanol (70%) are primarily used as disinfectants rather than sterilants. Their action, which also involves protein denaturation, is quite effective against bacteria and fungi but less so against viruses, and they are entirely ineffective against bacterial spores. Consequently, their most common uses are for the surgical and hygienic disinfection of skin and hands.

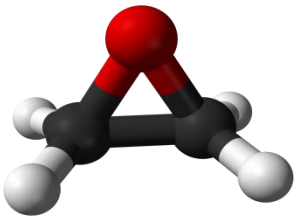


Fig.V.5 Ethylene oxide.

3.2. Elimination based methods

These methods work by physically separating and removing microorganisms from the product or medium. They do not necessarily kill the microbes; instead, they are mechanically separated and retained by a barrier.

3.2.1. Filtration

Membrane filters with a pore size of 0.22 µm are commonly used as sterilizing filters. However, macromolecules, such as proteins and peptides, can be damaged during filtration due to shear stress, leading to an alteration of their three-dimensional structure. In some cases, the formulation can affect the integrity of the filter and cause clogging. Furthermore, some filters adsorb the drug product. Therefore, drug interactions with the filter material are carefully studied before implementing this sterilization method. Common filter materials include nylon and Teflon.

CHAPTER VII: ASH ANALYSIS

1. Objectives

By the end of this chapter, the student will be able to:

- Understand the principles and significance of ash values in quality control.
- Perform ash determination techniques.

2. Introduction

Total ash and acid-insoluble ash are fundamental purity tests for pharmaceuticals and herbal medicines. While total ash measures all inorganic residues (both natural and contaminants), acid-insoluble ash specifically detects adulterants like silica or sand. This chapter covers the principles, standard methods, and step-by-step techniques to perform these analyses accurately.

3. Ash values

The in-organic content remaining after incinerating a crude drug is called as ash content. An ash value implies the naturally inherent inorganic salts or those imparted from external sources. Official ash values are chiefly used for quality confirmation of powdered drugs. Determination of ash value helps in knowing absence of mineral matter that is accidentally introduced from earth, sand, floor sweepings, absence of other parts of plant, absence of adulterated and exhausted drug, and absence of materials that possess with stone cells, starch which modify the value.

Total ash value is used for ensuring quality of drugs, which possess with little calcium oxalate (ex: ginger). Acid-insoluble ash is referred if calcium oxalate present is high. It is believed that acid-insoluble ash gives different values due to varying conditions of ignition. Treatment of ash with hydrochloric acid leaves only silica which is imparted from soil and hence acid-insoluble ash is preferred. Water soluble ash is the difference between total ash and water insoluble residue.

3.1. Determination of total ash

3.1.1. Principle

Principle involved is that when a known weight of feed is ignited to ash, the weight of ash thus obtained is expressed in terms of percentage.

3.1.2. Apparatus

- Silica crucible
- Tongs
- Weighing balance
- Electrical bunsen burner
- Muffle furnace
- Desiccator
- Asbestos sheet

3.1.3. Procedure

1. Find out the weight of a clean dry crucible.
2. Place about 2 g of sample and weigh this to find out accurate weight of the sample taken.
3. Carefully place the weighed crucible over electric burner. The crucible should be partially opened.
4. The sample will get charred with initial expulsion of smoke.
5. Place the crucible in a muffle furnace (**Fig.VII.1**) and heat to 600 ºC. Keep it for 2 hours. At this temperature all organic matter will be burnt leaving behind minerals.
6. Remove the crucible from the furnace carefully and cool it in a dessicator to room temperature and weight again.

The percentage ash was calculated using this equation.

$$\%\text{Total Ash} = (W_1 - W_0)/(W_2 - W_0) \times 100$$

Where

W_0 = Weight of empty crucible

W_1 = Weight of crucible + dry sample

W_2 = Weight of crucible + ash sample

What is obtained after complete combustion of a sample is total ash. It comprises of two portions: The portion that is soluble in dilute acids contains all essential minerals and that is

the useful portion of the ash. Other portion, insoluble in dilute acids consists of mainly sand and silica. For the most part, it represents impurity or adulteration.

3.2. Determination of acid insoluble ash

The ash obtained described as total ash was boiled for 5 min. with 25 cm3 of 10 % dilute hydrochloric acid. The insoluble matter was collected on an ash-less filter paper and washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Fig.VII.1 Muffle furnace.

3.3. Sulphated ash analysis

The sulfated ash test is a critical method for evaluating non-volatile inorganic impurities in unorganized drugs (e.g., resins, gums) and organic substances. This procedure involves igniting the sample with sulfuric acid, which decomposes organic matter and oxidizes residual materials, leaving only stable sulfate salts of cationic impurities. Compared to other ash tests, this method offers superior reproducibility due to the thermal stability of inorganic sulfates at high temperatures. By quantifying non-volatile residues after ignition, the test provides a reliable measure of inorganic contaminants in accordance with pharmacopeial standards.

3.3.1. Procedure

a) Method A

Accurately weigh about 1 g of the substance, or the quantity specified in the monograph, into a suitable crucible (usually platinum) and moisten with sulfuric acid (~1760 g/l) Test Solution (TS). Heat gently to remove the excess of acid and ignite at about 800 °C until all the black

particles have disappeared; again moisten with sulfuric acid (~1760 g/l) TS and reignite. Add a small amount of ammonium carbonate R and ignite to constant weight.

b) Method B

Ignite a suitable crucible (for example silica, platinum, quartz or porcelain) at 550 °C to 650 °C for 30 minutes, cool the crucible in a desiccator (silica gel or other suitable desiccant) and weigh it accurately.

Take the amount of test sample specified in the individual monograph in the crucible and weigh the crucible accurately.

Moisten the sample with a small amount (usually 1 mL) of sulfuric acid (~1760 g/l) TS, heat gently at a temperature as low as practicable until the sample is thoroughly charred.

After cooling moisten the residue with a small amount (usually 1 mL) of sulfuric acid (~1760 g/l) TS, heat gently until white fumes are no longer evolved and ignite at 550 °C to 650 °C until the residue is completely incinerated.

Ensure that flames are not produced at any time during the procedure.

Cool the crucible in a desiccator (silica gel or other suitable desiccant), weigh accurately and calculate the percentage of residue.

Unless otherwise specified, if the amount of residue so obtained exceeds the limit specified in the individual monograph, repeat the moistening with sulfuric acid, heating and ignition as before, using a 30 minute ignition period, until two consecutive weighings of the residue do not differ by more than 0.5 mg or until the percentage of residue complies with the limit in the individual monograph.

CHAPTER VIII: PHYSICAL ANALYSIS METHODS FOR CHEMICAL PREPARATIONS IN PHARMACY

1. Objectives

Upon completing this chapter, the student will be able to:

- Explain the fundamental principle and pharmacopoeial procedure for determining the colour of liquids and its significance in purity assessment.
- Differentiate between mass density and relative density, and describe the pycnometer method for their determination.
- Define melting point, boiling point, and congealing point, and relate their determination to the identification and purity testing of pharmaceutical substances.
- Compare the principles of Loss on Drying and Karl Fischer titration for water content determination, identifying the advantages and limitations of each.
- Apply the principles of Polarimetry to calculate specific rotation and explain its critical role in characterizing chiral active pharmaceutical ingredients.
- Describe the principle of refractive index measurement and its application in identifying liquid substances and testing their purity

2. Introduction

Physical analysis methods form the first line of assessment in pharmaceutical quality control, providing fundamental, rapid, and often non-destructive means of evaluating chemical substances. These techniques focus on measuring the intrinsic physical properties of a material such as its color, density, melting point, and optical characteristics rather than its chemical reactions. This chapter details the standard procedures for these essential tests, which are critical for confirming identity, assessing purity, and ensuring batch-to-bustch consistency. From the visual assessment of a solution's colour to the precise determination of a compound's specific rotation, these methods are indispensable for verifying that raw materials and finished products comply with stringent pharmacopoeial standards before they are released for therapeutic use.

3. Colour of liquids

3.1.Definition

The test for colour of liquids is carried out by comparing the test solution prepared as specified in the monograph with a standard colour solution indicated in the monograph. The composition of the standard colour solution is selected depending on the hue and intensity of the colour of the test solution corresponding to the limits permitted in the specifications.

3.2. Recommended procedure

Unless otherwise specified in the monograph, carry out the comparison in flat-bottomed tubes of transparent glass that are matched as closely as possible in internal diameter and in all other respects (tubes of about 16 mm internal diameter are suitable). Use 10 m1 of the test solution and 10 m1 of the standard colour solution; the depth of liquid should be about 50 mm. The colour of the test solution is not more intense than the standard colour when viewed down the vertical axis of the tubes in diffused light against a white background.

4. Determination of mass density and relative density

4.1. Definition

The mass density (ρ) of a substance is the mass of one unit volume of the substance. In terms of SI base units, mass density is expressed in kilograms per cubic metre. However, in the International Pharmacopoeia, the mass density is expressed in kilograms per liter (which is equivalent to grams per milliliter) at a temperature of 20 °C (ρ20) and it is corrected for buoyancy (i.e. reduced to vacuum conditions).

For pharmacopoeial purposes, the mass density of liquids is not measured directly but calculated from their relative density. The relative density d^{20}_{20} is the ratio of the mass of the substance in air at 20 °C to that of an equal volume of water at the same temperature. A relative density less than 1 means the substance is less dense than the reference and will float in water, while a relative density greater than 1 means the substance is denser and will sink.

The term "relative density" d^{20}_{20} is equivalent to the formerly used term "specific gravity determined at 20 °C". The relative density d^{20}_{4} denotes the ratio of the mass of the substance in air at 20 °C to that of an equal volume of water at 4 °C. As the relative density of water at 20 °C is 0.998234, these values are related by the following equation:

$$d_4^{20} = 0.998234\, d_{20}^{20}.$$

4.2. Recommended procedure

Determine the relative density (d^{20}_{20}) using a hydrostatic balance (only when the precision indicated in the monograph is three decimal digits) or a pycnometer. If the value of mass density ρ20 (in kg/l or g/mL) is referred to in the monograph, carry out the measurement of relative density and from the value obtained calculate the mass density according to the formula:

$$\varrho_{20} = 0.99703\, d_{20}^{20} + 0.0012$$

4.2.1. Use of a pycnometer

Use a pycnometer of suitable form of a capacity of not less than 5 mL. Weigh accurately the empty, dry pycnometer (**Fig.VIII.1),** and fill it with the test liquid brought previously to a temperature of about 20 °C. Hold the filled pycnometer at a temperature of 20 ± 1 °C for about 30 minutes, adjust the liquid to the mark using, if necessary, a small strip of filter-paper to remove the excess and to wipe the inlet from the inside, and weigh accurately. Calculate the weight of the liquid in the pycnometer. Remove the liquid, clean and dry the pycnometer, repeat the measurement with carbon-dioxide-free water R, also at 20 ± 1 °C, and calculate the weight of water in the pycnometer. The ratio of the weights of the test liquid and of water gives the relative density d^{20}_{20}.

Fig.VIII.1 Empty glass pycnometer and filled glass pycnometer**.**

5. Determination of melting temperature, congealing point, and boiling point

5.1. Determination of melting points

5.1.1. Definition

The melting point (m.p) ℃ of a compound is the temperature at which it changes from a solid to a liquid. Since this requires that the intermolecular forces that hold the solid together have to be overcome. The melting point is a physical property (melting point, boiling point, density, solubility, etc.) often used to identify compounds. The presence of impurities in a substance results in a more or less pronounced lowering of its melting point. Even more significant is that impurities present in the substance may cause its melting range to be extended. In most cases where melting behavior is used as a criterion of purity the *International Pharmacopoeia* therefore prescribes determination of the melting range rather than the melting point.

Melting point range: The interval between the temperature at which a solid sample just begins to turn to liquid and the temperature at which the entire sample becomes liquid. or range of temperatures in which the first crystal starts to melt until the temperature at which the last crystal just disappears

The statement in a monograph "*melting range a-b "C"* means that the melting range determined by the method below must fall within these limits.

The melting temperature of a substance is the corrected temperature at which it is completely melted as shown by the disappearance of the solid phase.

A. Why measure melting points?

There are various methods of chemical and physical analysis used to differentiate, identify, and classify materials. Measurement of the melting point is, among other things, one of these standard laboratory procedures. It is an experimental and easily performed method of physical analysis used to find out the identity, the purity, and the thermal stability of a material.

- **Identification**

Pure materials have exactly defined melting points which can be obtained from reference tables. Thus, the identity of a material can be determined by measuring its melting point: One needs only to compare the melting point of the substance as determined in the test with the values in the technical literature. Of course, determination of the melting point alone is not yet enough for the clear identification of a substance. There may be several substances with the same melting point. In such cases, the shift in the mixed melting point can provide an indication about the definitive identification of the material.

- **Purity**

Even slight impurities in a material cause a lowering of its melting point or at least a widening of its melting range (the material melts within a range of temperatures and not at a precisely definable melting point).

- **Thermal stability**

Many materials change at high temperatures, e.g., decomposition or discoloration. Measurement of the melting point is one method that can be used to determine how much the material can be heated without causing chemical changes. This value is useful as an indicator for the thermal stability of a substance and can be used to suggest a possible temperature for drying.

5.1.2. Principles and methods of melting point determination

A. Determining the melting point in the capillary tube

Melting temperature determined using the capillary method is that temperature at which the last solid particle from a compact column of the substance in the melting point tube goes over into the liquid phase. The determination thus uses the so-called «clear» melting point since this represents an unmistakable criterion for recording the melting point.

The standard capillary method described in the pharmacopoeias starts with a heated liquid bath equipped with a thermometer for measuring the temperature. A glass capillary tube containing the substance to be determined is introduced into this liquid in such a way that the substance in the capillary tube is located close to the mercury reservoir on the thermometer. At the clear melting point, the temperature reading on thermometer is taken. The capillary method described in the pharmacopoeias starts from visual detection of the melting point. More modern instruments for melting point determination enable detection of the melting

point not only in the traditional way, visually, but also automatically. **Fig. VIII.2** shows two possible instruments that operate using the «bath method» described in the pharmacopoeias.

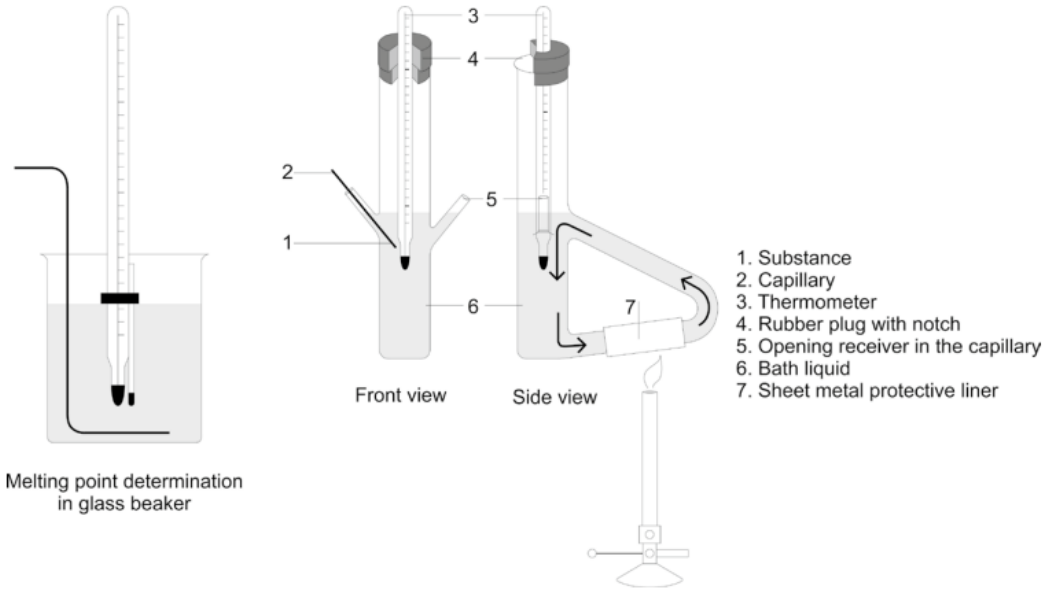


Fig.VIII.2 Melting point determination in the glass beaker and in the Thiele apparatus.

B. Immediate melting point

Some materials decompose during the melting process or show a tendency toward polymorphous modifications. The capillary method is not suitable for use with such substances, which is why the pharmacopoeia provides for using the flash melting point in such cases. In this method, the temperature does not affect the substance at all prior to its reaching its melting point. This method uses a heatable metal block (e.g., of brass) that is heated up evenly. A few crystals of the substance to be determined are scattered on this block at regular intervals. The first temperature at which the substance melts immediately on contact with the metal block is read off as T1. The heating of the block is discontinued, and while it is cooling down, small samples of the substance are again scattered on the block at regular intervals. T2 is the temperature at which the substance stops melting immediately on contact with the metal. The immediate melting point is then obtained from the formula. The Kofler heating stand is particularly well-suited for series measurement of the immediate flash point where no special requirements are made with regard to accuracy of measurement. **Fig.VIII.3** and **Fig.VIII.4** show the various instruments available.

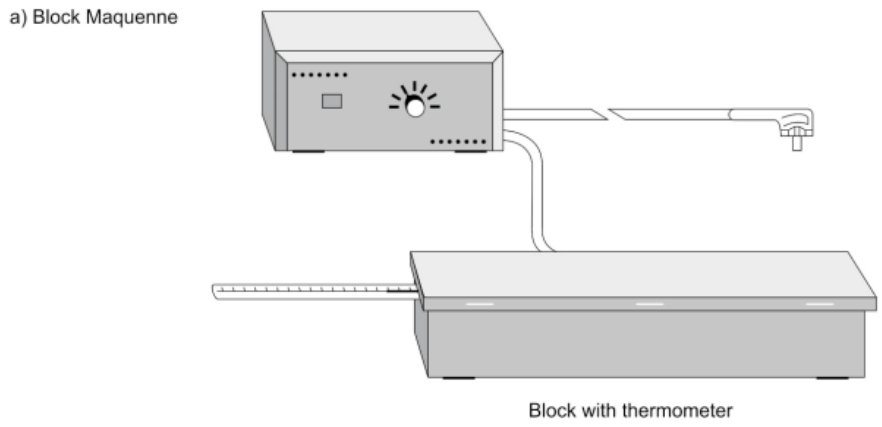


Fig.VIII.3 Metal block instruments for determining the immediate melting point: Block Maquenne.

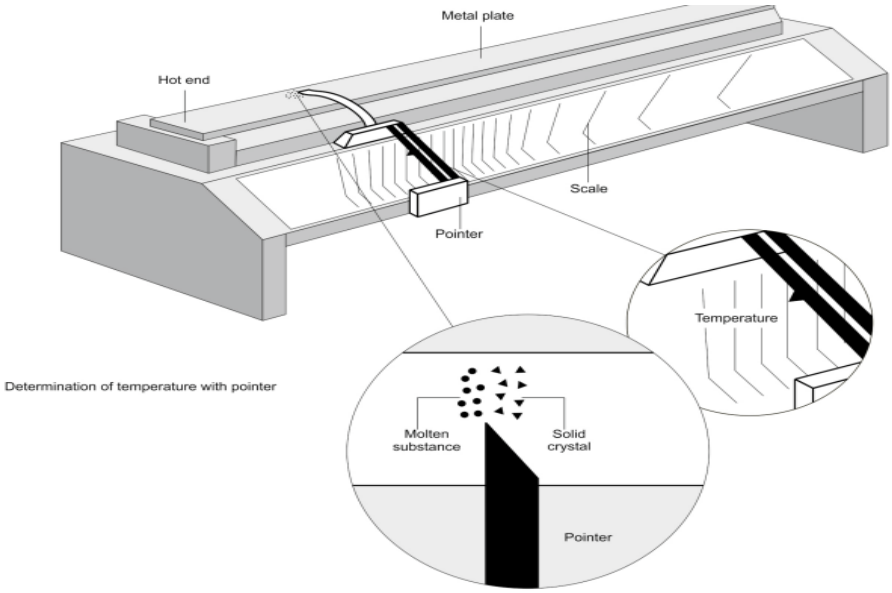


Fig.VIII.4 Metal block instruments for determining the immediate melting point: Kofler heating.

5.2. Determination of boiling points

The boiling point of an organic liquid is the temperature at which its vapour pressure equals the atmospheric pressure over the liquid or it is the temperature at which the vapour and liquid phases are in equilibrium at a given pressure. The boiling point is considered as a criterion of purity of a compound and is useful for identification of organic compounds.

Similar to the melting point the boiling point may be sharp or may vary over a temperature range. Pure liquids have sharp boiling points while mixtures show a boiling point range. The atmospheric pressure plays an important role in determination of the boiling point correctly. Reduction of the pressure leads to a decrease or a depression in the boiling point and vice versa.

5.2.1. Apparatus and experimental procedures

A suitable apparatus for the determination consists of a vessel with appropriate liquid, a source of heat, and a thermometer, a thin-walled test-tube of glass of external diameter about 4 mm and length suitable for the apparatus used and a thin-walled capillary tube of glass of internal diameter not exceeding 1 mm, which should be closed by fusing about 2 mm from one end (**Fig.VIII.5**).

Attach a clean and empty test tube to a thermometer with sewing thread. Put an empty capillary tube in the test tube so that the open end of capillary is down.

Ensure that the temperature of the paraffin oil is below 50 °C. Place 2-3 mL of sample in the test tube.

Turn on the hot plate and use a clean glass rod to stir the paraffin oil to ensure a uniform heat distribution.

Record the temperature when rapid air bubbles come out from the capillary. At this stage, the vapor pressure of the unknown inside the capillary is higher than the atmospheric pressure.

Turn off the hot plate and carefully insert a ceramic tile between the beaker and the hotplate. Alternatively, you may replace the hot plate with the one that has not been used. However, the thermometer bulb and the content in the test tube should be submerged in the paraffin oil at all times.

As the temperature decreases, air bubbling will gradually slow down. Record the temperature when you see the last bubble come out and some liquid goes into the capillary.

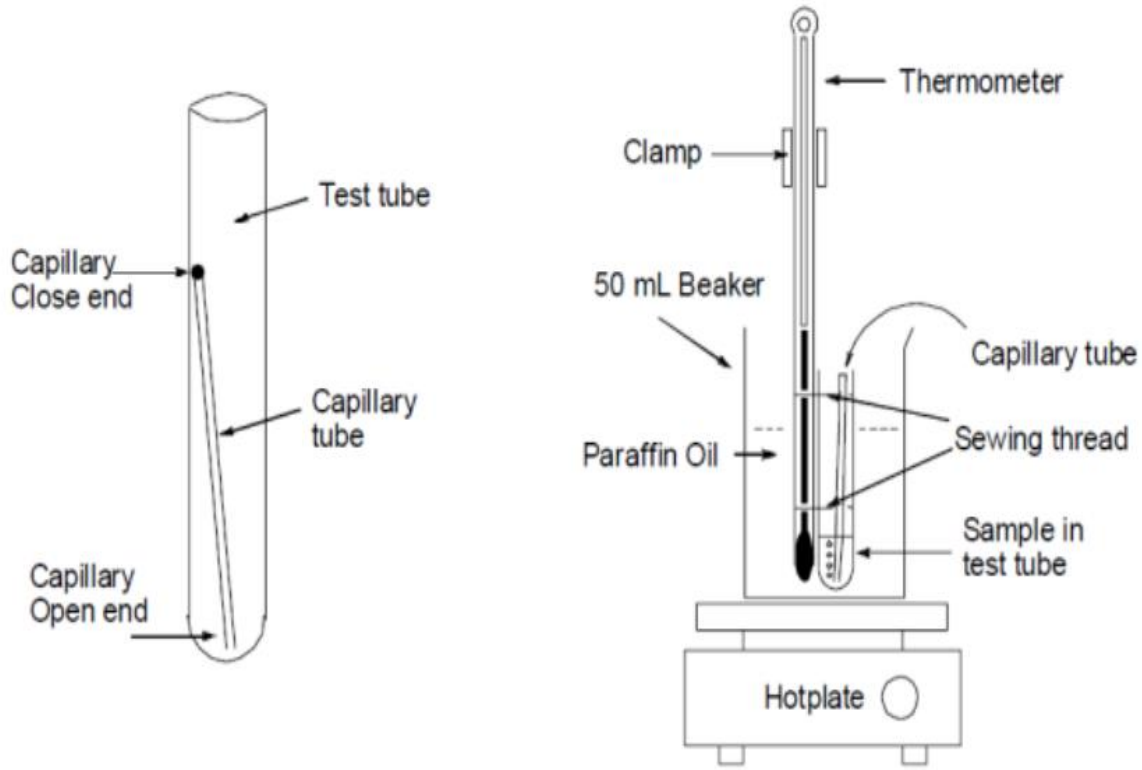


Fig.VIII.5 Apparatus set up of boiling point determination experiment.

5.3. Determination of congealing point

The congealing point of a liquid or of a melted solid is the highest temperature at which it solidifies. The congealing point of the liquid is the same as the melting temperature of the solid, but since the liquid may be cooled to a temperature below its congealing point without assuming the solid form, the method described below is used to determine the congealing point of a liquid or of a melted solid.

5.3.1. Apparatus and experimental procedures

A suitable apparatus consists of a test-tube of about 2 cm internal diameter and about 10 cm in length, suspended by means of a bored cork inside a larger tube, about 3 cm in diameter and 12 cm in length, a vessel with water or suitable freezing mixture, and an accurately standardized thermometer.

Unless otherwise specified in the monograph, place in the inner test- tube about 10 m1 of the liquid, or 10 g of the melted solid, to be tested and cool together the inner and the outer tubes in water or in a suitable freezing mixture to a temperature about 5 °C below the expected congealing point of the liquid; with the thermometer gently stir the liquid until it begins to solidify. At first there is a gradual fall in temperature. Then, as the solid phase forms, the

temperature remains constant for some time or rises before becoming constant. The highest temperature observed is regarded as the congealing point. If the liquid does not start to congeal within 2 °C of the expected temperature, congelation may be induced by adding a small crystal of the substance to the liquid or by rubbing the inner walls of the test-tube with the thermometer.

- ## Determination of water content in pharmaceutical substances

The accurate determination of water content is critical in pharmaceuticals as it can affect a drug's stability, shelf life, efficacy, crystallinity, and processing behavior.

6.1. Drying methods (Thermogravimetric Methods)

These methods involve the removal of water from the sample, and the result is calculated based on the mass loss. A key limitation is that they are *non-selective;* any volatile material lost during drying (e.g., solvents, volatile oils, decomposition products) will be measured as "water."

6.1.1. Direct determination

- **Principle:** The sample is heated, and the released water vapor is carried by a dry gas into a tube containing a chemical desiccant (like phosphorus pentoxide, P_2O_5 or anhydrous magnesium perchlorate). The water is trapped, and the increase in mass of this tube is the mass of the water.
- **Procedure :**

1. The absorption tube is weighed.
2. The sample is placed in a heated chamber, and a dry inert gas is passed over it.
3. The volatile components, including water, are swept into the absorption tube where water is selectively trapped.
4. The absorption tube is weighed again.

- **Calculation:**

% Water = (Mass gained by absorption tube / Initial sample mass) × 100

6.1.2. Indirect determination: Loss on Drying (LOD)

- **Principle:** The sample is weighed, subjected to a precisely defined drying process and then weighed again. The mass loss is reported as "Loss on Drying."
- **Procedure:**

1. The sample is placed in a tared (pre-weighed) dish.
2. It is dried under specified conditions (e.g., 105°C for 2 hours) in an **oven**.
3. The dish is cooled in a desiccator (to prevent moisture reabsorption from the air) and reweighed.
4. This process may be repeated until constant mass is achieved.

- **Calculation:**

$$\% \text{ LOD} = [(\text{Initial mass} - \text{Final mass}) / \text{Initial sample mass}] \times 100$$

This is the reference (standard) method in many pharmacopoeias for substances that are stable and do not contain other volatiles. The conditions (temperature, time, pressure) are strictly defined for each monograph.

6.2. Selective (Chemical) methods

These methods are specific for water and do not measure other volatile substances.

6.2.1. Karl Fischer (KF) titration

This is the most widely used and precise method for direct water quantification in pharmaceuticals.

- **Principle:** A reaction based on the reduction of iodine by sulfur dioxide in the presence of water. The reaction requires a buffer (to maintain pH) and a solvent (to dissolve the sample).
  - The fundamental reaction is:

$$I_2 + SO_2 + 2H_2O \rightarrow 2HI + H_2SO_4$$

  - This reaction only proceeds stoichiometrically in the presence of water.

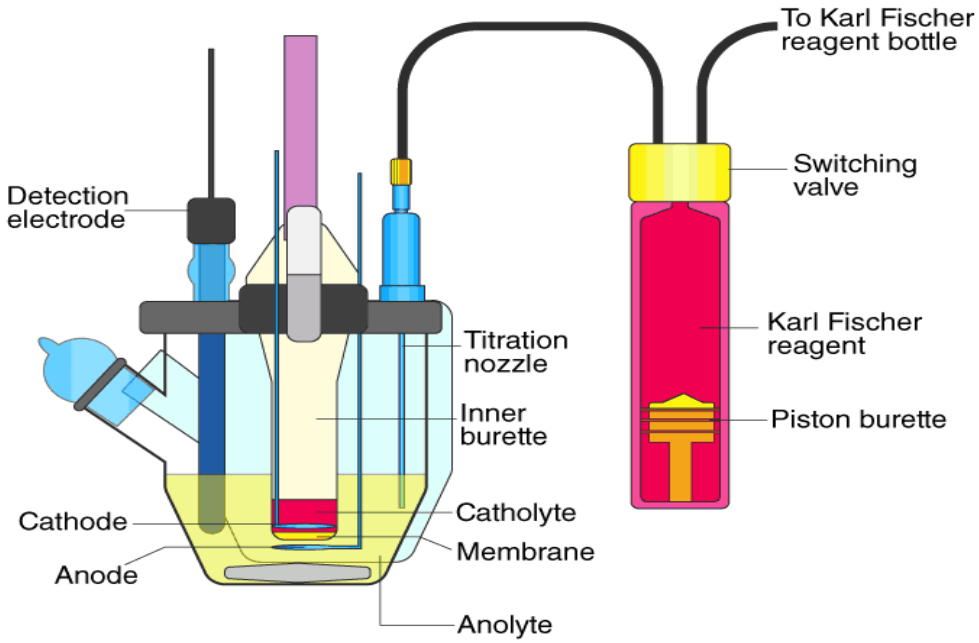


Fig.VIII.6 Karl Fischer titration.

- ## Determination of optical rotation and specific rotation

7.1. Generality

Optical isomers, or enantiomers, have the same sequence of atoms and bonds but are different in their 3D shape. Two enantiomers are non-superimposable mirror images of one another (i.e., chiral), with the most common cited example being our hands (**Fig.VIII.7**). Optical isomers have basically the same properties (melting points, boiling points, etc.) but there are a few exceptions (uses in biological mechanisms and optical activity). There are drugs, called enantiopure drugs, which have different effects based on whether the drug is a racemic mixture or purely one enantiomer. For example, d-ethambutol treats tuberculosis, while l-ethambutol causes blindness. Optical isomers differ in a property called optical activity, in which a sample rotates the plane of polarization of a polarized light beam passing through.

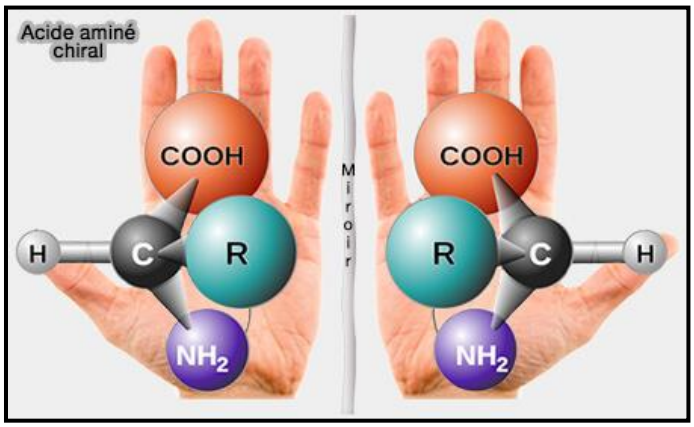


Fig.VIII.7 Chiral molecule.

7.2. Polarimetry

It is the measurement and interpretation of the polarization of electromagnetic waves that have traveled through some material in order to characterize that object. Polarimetry is a sensitive, nondestructive technique for measuring the optical activity exhibited by inorganic and organic compounds. Some chemical substances are optically active, and polarized (unidirectional) light will rotate either to the left (counter-clockwise) or right (clockwise) when passed through these substances. The amount by which the light is rotated is known as the angle of rotation. A polarimeter is a scientific instrument used to measure the angle of rotation caused by passing polarized light through an optically active substance (**Fig.VIII.8**).

Fig.VIII.8 An automatic digital polarimeter.

7.3. Measuring optical rotation

The sample is usually prepared as a tube where the optically active substance is dissolved in an optically inactive chemical such as distilled water, ethanol, and methanol. Optically active

samples, such as solutions of chiral molecules, causes rotation of the polarization of plane polarized light as it passes through the sample (**Fig.VIII.9**). In an ordinary light, the vibrations occur in all planes perpendicular to direction of propagation. When it is allowed to pass through a Nicol prism then its vibrations in all directions except the direction of axis of the prism are cut off. The light emerging out of the prism is said to be plane polarized because its vibration is in one direction. If two Nicol prisms are placed with their polarization planes parallel to each other, then the light rays emerging out of the first prism will enter the second prism. As a result, complete bright light is observed. If the second prism is rotated by an angle of 90°, the light emerging from the first prism is stopped by the second prism due to which complete dark or no light region is observed.

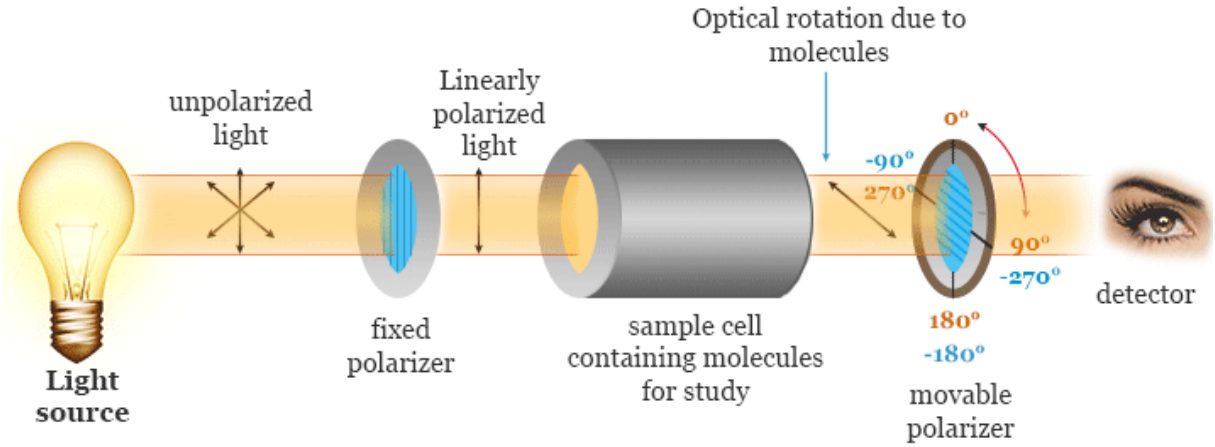
In a polarimeter (**Fig**.**VIII.9**), plane-polarized light is introduced to a tube (typically 10 cm in length) containing a solution with the substance to be measured. If the substance is optical inactive, the plane of the polarized light will not change in orientation and the observer will read an angle of α= 0o. If the compound in the Polarimetry cell was optical active, the plane of the light would be rotated on its way through the tube. The observed rotation is a result of the different components of the plane polarized light interacting differently with the chiral center. In order to observe the maximum brightness, the observer (person or instrument) will have to rotate the axis of the analyzer back, either clockwise or counterclockwise direction depending on the nature of the compound. For clockwise direction, the rotation (in degrees) is defined as positive («+») and called dextrorotatory (from the Latin: dexter = right). In contrast, the counterclockwise direction is defined as negative («-») and called levorotatory (from the Latin laevus=left).

The specific rotation of the sample may then be calculated. Temperature can affect the rotation of light, which should be accounted for in the calculations.

$$[\alpha]=\alpha / l \times c$$

Where:

$[\alpha]$ is the specific rotation (in $°\cdot mL\cdot dm^{-1}\cdot g^{-1}$), T is the temperature (in °C), λ is the wavelength of light (in nm), α is the measured optical rotation (in degrees), l is the length of the polarimeter tube (optical path length in dm), and c is the mass concentration of the solution (in g/mL)



FigVIII.9 Circuit to explain the rotation of polarized light plane as it passes through the optically active sample.

7.4. Calibration

Polarimeter can be calibrated or at least verified by measuring a quartz plate, which is constructed to always read at a certain angle of rotation (usually +34°, but +17° and +8,5° are also popular depending on the sample). Quartz plates are preferred by many users because solid samples are much less affected by variations in temperature, and do not needs to be mixed on-demand like sucrose solutions.

• Determination of refractive index

8.1. Definition

The refractive index (n) of a substance is the ratio of the velocity of light in a vacuum to its velocity in the substance which means it describes how light, or any other radiation, propagates through that medium. It varies with the wavelength of the light used in its measurement and with the temperature. It is therefore necessary to specify these conditions**.** It is defined as

$$n = c / \upsilon$$

Where c is the speed of light in vacuum and υ is the phase velocity in the substance.

For example, the refractive index of water is 1,33, meaning that light travels 1,33 times as fast in vacuum as it does in water.

The measurement of the refractive index is employed for pharmacopoeial purposes mainly to establish the identity of liquid substances. It may also be used to test the purity of such substances. The accuracy of the measurement should be related to the requirements of the monograph. For pharmacopoeial purposes it is usually adequate to express the refractive index to three decimal places.

3.4. Apparatus

Refractometers (**Fig.VIII.10)** are instruments that measure the bending of light as it crosses an interface between dissimilar substances and converts the bending light rays into a useful scale. A refractometer is a laboratory or field device for the measurement of an index of refraction.

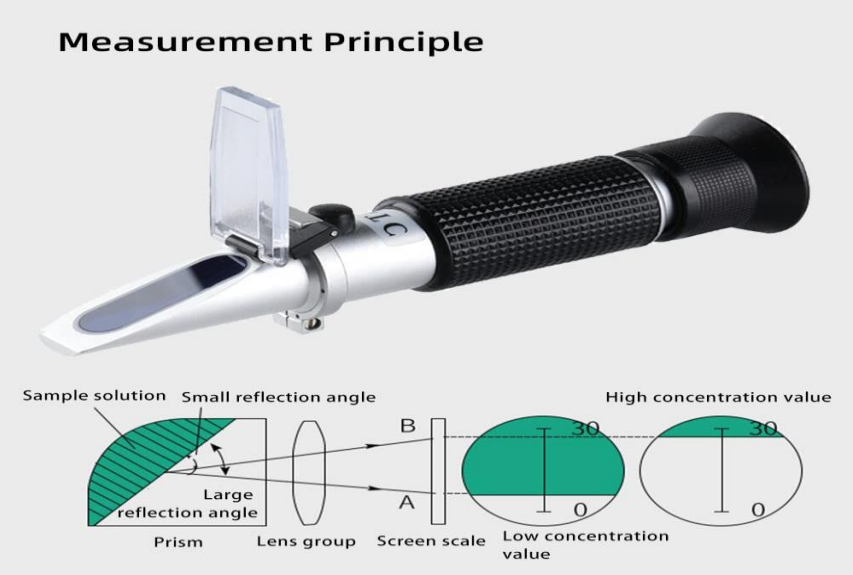


Fig.VIII.10 Refractometer.

Commercial refractometers are normally constructed for use with white light but are calibrated to give the refractive index in terms of the sodium light of wavelength 589.3 nm. The optical parts of the apparatus should be kept brilliantly clean. The working surfaces of prisms should be free from scratches.

The instrument should be calibrated against a standard provided by the manufacturer; the temperature control of the liquid being examined and the cleanliness of the prism should be checked frequently by deter- mining the refractive index of distilled water, which is 1.3330 at 20 °C and 1.3325 at 25 °C.

CHAPTER IX: CHEMICAL ANALYSIS METHODS FOR CHEMICAL PREPARATIONS IN PHARMACY

1. Objectives

Upon the completion of this course, the student will be able to:

- Form a system of knowledge about the chemical pharmacopoeial methods for drugs quality control.

2. Introduction

Pharmaceutical analysis relies heavily on chemical methods to ensure the identity, purity, quality, and stability of drugs. These methods, rooted in classical analytical chemistry, remain indispensable in modern laboratories due to their accuracy, cost-effectiveness, and alignment with global pharmacopeial standards like the USP and EP. This chapter focuses on three foundational chemical techniques: titrimetric methods for quantification, the oxygen flask method for elemental analysis, and chemical identification tests for verifying substance identity.

4. Titrimetric Methods

3.1. Generality

Titrimetric analysis represents a foundational chemical method based on the precise measurement of reagent volumes required to complete a chemical reaction with an analyte. This technique relies on well-defined stoichiometric relationships and characteristic endpoint detection to deliver accurate quantitative results. As a classical analytical approach, it remains widely valued for its robustness, cost-effectiveness, and compliance with international pharmacopeial standards. The methodology encompasses several reaction types including acid-base, redox, complexometric, and non-aqueous titrations, each serving specific analytical purposes. Particularly in the examination of fats and oils, titrimetry provides critical chemical parameters such as acid value, peroxide value, saponification value, and iodine value through characteristic chemical reactions that reveal material composition, stability, and purity. This

chemical method continues to form an essential component of analytical protocols where specific, reproducible, and economically efficient quantitative analysis is required.

3.2. Definition

The term titrimetric analysis refers to quantitative chemical analysis carried out by determining the volume of a solution of accurately known concentration, which is required to react quantitatively with a measured volume of a solution of a substance to be determined. The solution of accurately known concentration is called standard solution. The term volumetric analysis was used for this form of quantitative determination but it has now been replaced by titrimetric analysis. In titrimetric analysis the reagent of known concentration is called titrant and the substance being titrated is termed the titrand.

3.2.1. Definition of some terms

Titration

Titration is the process in which the standard reagent is added to a solution of an analyte until the reaction between the analyte and reagent is complete.

Equivalence point and End point

The equivalence point of a titration is a theoretical point that cannot be determined experimentally. Instead, we can only estimate its position by observing some physical change associated with the condition of equivalence. This change is called the end point for titration.

Titration error

The difference between the observed end point and the true equivalence point in a titration.

$$TE = Vep - Veq$$

Indicators

Indicators are often added to analyte solution in order to give an observable physical change (end point) at or near the equivalence point. In other wards indicator is a compound having a physical property (usually color) that changes abruptly near the equivalence point of a chemical reaction. Indicator selection also depends on the type of reaction: acid-base indicators – phenolphthalein, methyl red, methyl orange, phenol red, and others; starch solution is used in iodometric titrations, tropeolin OO for diazotisation titrations, in

argentimetric titrations solutions K_2CrO_4, $FeSO_4$, etc. are used; murexide is applied in complexometric titrations.

3.3. End points in volumetric analysis

Detection of an end point involves the observation of some property of the solution that change in a characteristic way at or near the equivalent point. The properties that have been used for this purpose are numerous and varied; they include **(Fig.IX.1)**:

1. Color due to the reagent, the substance being determined, or an indicator substance.

2. Turbidity changes resulting from the formation or disappearance of solid phase.

3. Electric conductivity of the solution.

4. Electric potential between a pair of electrodes immersed in the solution.

5. Refractive index of the solution.

6. Temperature of the solution.

7. Electric current passing through the solution.

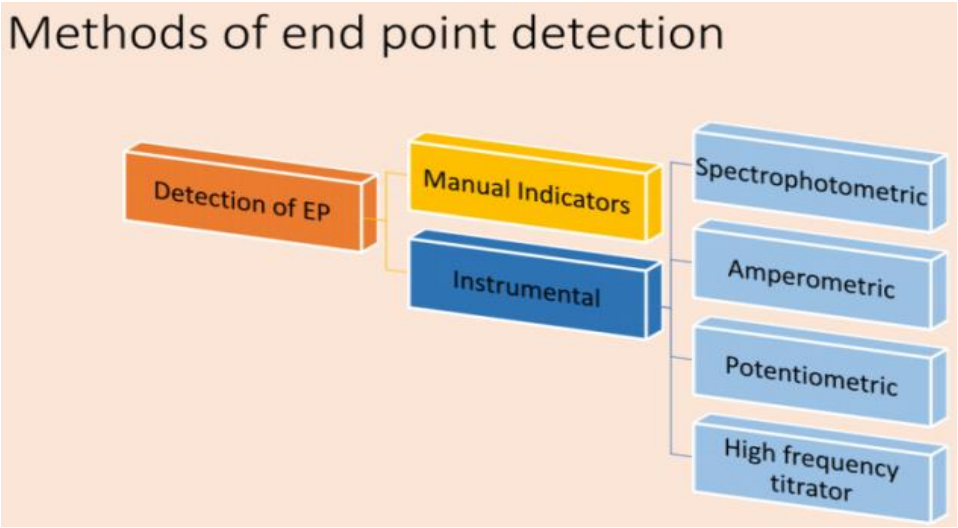


Fig.IX.1 Methods of end point detection.

3.4. Classification of reaction in titrimetric analysis

In titrimetric (or volumetric) analysis, the volume of a standard solution of known concentration (the **titrant**) required to react completely with an analyte is measured. The fundamental basis for classifying these methods is the **type of chemical reaction** that occurs between the titrant and the analyte. The four main classes are presented in **Fig.IX.2**.

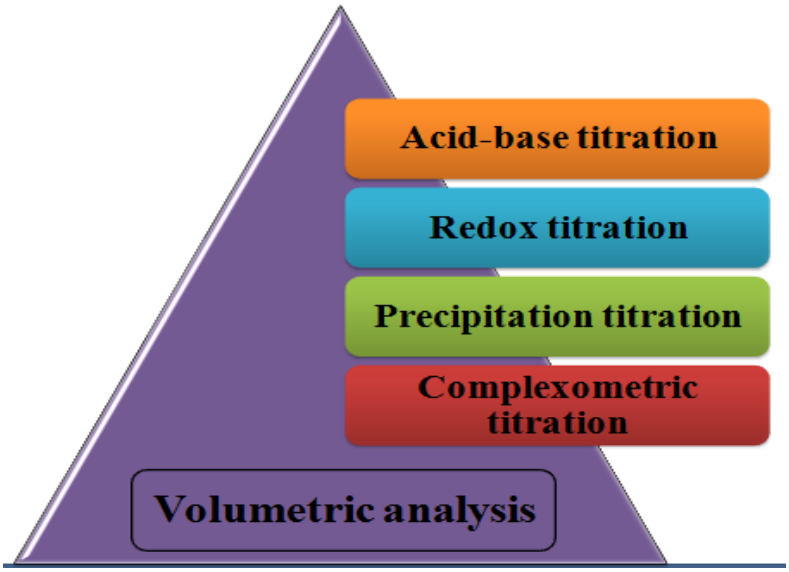


Fig.IX.2 Classification of volumetric analysis.

3.4.1. Acid-base titration

An acid base titration is the determination of the concentration of an acid or base by exactly neutralizing the acid or base with an acid or base of known concentration. This allows for quantitative analysis of the concentration of an unknown acid or base solution. It's also known as Neutralization titration.

Example:

$$HCl + NaOH \longrightarrow NaCl + H_2O$$

$$CH_3COOH + NaOH \longrightarrow CH_3COONa + H_2O$$

The objective of carry out acid base titration is to determine equivalent quantity of other substance required for neutralization.

3.4.2. Redox titration

Oxidation: It can be defined as loss of electrons or increase in oxygen content.

Reduction: It can be defined as gain of electrons or increase of hydrogen content.

Oxidizing agent: substance which get reduced.

Reducing agent: substance which get oxidized.

Both processes are combined and occur together so we combine them in one word as REDOX reaction.

Reaction of ferrous ion with ceric ion:

$$Fe^{2+} + Ce^{4+} \longrightarrow Fe^{3+} + Ce^{3+}$$

$Fe^{2+} - e \rightleftharpoons Fe^{3+}$ (Loss of electrons: **Oxidation**)

$Ce^{4+} + e \rightleftharpoons Ce^{3+}$ (Gain of electrons: **Reduction**)

Remarks: In every redox reaction, both reduction and oxidation must occur. Substance that gives electrons is the reducing agent or reductant. Substance that accepts electrons is the oxidizing agent or oxidant.

3.4.3. Precipitation titration

Precipitation Reactions

Precipitation is the formation of a solid in a solution. Solid formed is called the precipitate. A precipitation reaction occurs when water solutions of two different ionic compounds are mixed and an insoluble solid separates out of solution. The precipitate is itself ionic; the cation comes from one solution and the anion from another.

$KCl + AgNO_3 \rightleftharpoons AgCl + KNO_3$

Cl^- solution — *Precipitating agent* — *White precipitate*

Precipitation titration

Precipitation titration is a titration method based on the formation of precipitate, which is slightly soluble. The basic requirements are:

• The reaction must be sufficiently rapid and complete, lead to a product of reproducible composition and of low solubility.

• And a method must exist to locate the end point.

- ***Argentometric titration:***

Titrations involving silver are termed argentometric, from the Latin name for silver, argentum. The major precipitation reaction used is that of silver with a range of anions. These anions include:

• Halides (Cl^- , Br^- , I^-).

• Pseudohalides (S^{2-} , HS^-, CN^-, SCN^-).

3.4.4. Complexometric titration

a. Complexometry

A volumetric titration involves the formation of soluble complex between metal ion (as acceptor) and ligand (as donor) to form coordination bonds. (A titration based on the formation of a coordination complex is known as a complexometric titration). Complexometric titrations are particularly useful for the determination of a mixture of different metal ions in solution. The metal ion is known as Central metal atom. The anion or neutral molecule is known as Ligand (L). The metallic ion (atom) has stable electronic configuration. It forms additional completed shells by accepting electron pairs from donor atoms.

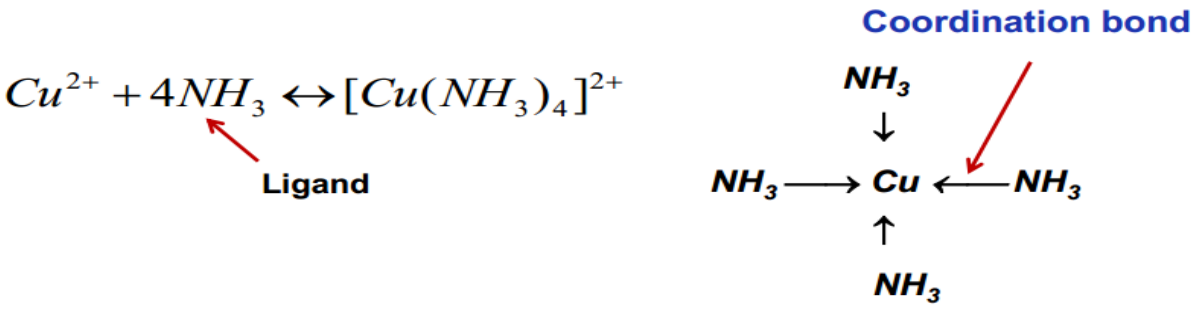


Fig.IX.3 Complex**.**

b. Titration with multidentate complexers (Chelating Agents)

Ethylene diamine tetra acetic acid (EDTA) possess enough donor atoms to fill the whole coordination sphere of metal ions in one step (**Fig.IX.4**).

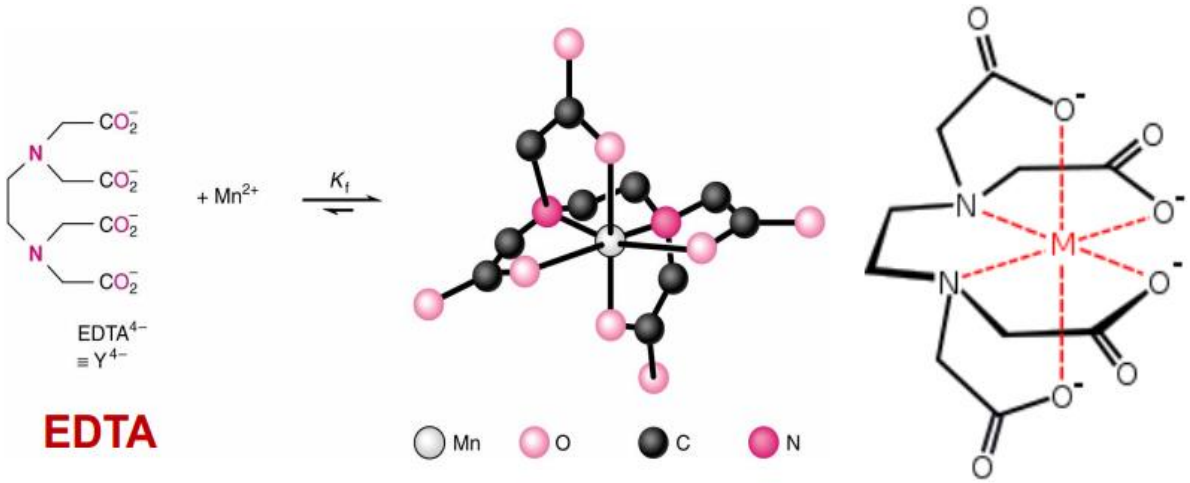


Fig.IX.4 The three-dimensional structure of the 1:1 metal-EDTA chelate with Mn^{2+}.

c. Detection of end point: use metal ion indicators

Indicator is a dye which is capable of acting as a chelating agent to give a dye-metal complex.The latter is different in colour from the dye itself and also has a low stability constant than the chelate-metal complex. The colour of the solution, therefore, remains that of the dye complex until the end point, when an equivalent amount of sodium EDTA has been added. As soon as there is the slightest excess of EDTA, the metal-dye complex decomposes to producefree dye; this is accomplished by a change in colour.Metal indicators must comply with the following requirements:

• Metal-indicator complex must be less stable than the metal-EDTA complex.

• Binding between metal and indicator must not be too weak. It has to avoid EDTA replacing at the beginning of the titration.

• In general, the metal-indicator complex should be 10 to 100 times less stable than the metal-titrant complex.

• Colour of the indicator and the metal complexed indicator must be sufficiently different.

Examples of metal ion indicators

1. Eriochrome black T (EBT)

It can be represented by H_2In. The color of Indicator changes with the change of pH. EBT contains 2 replaceable phenolic hydrogens (**Fig.IX.5**).

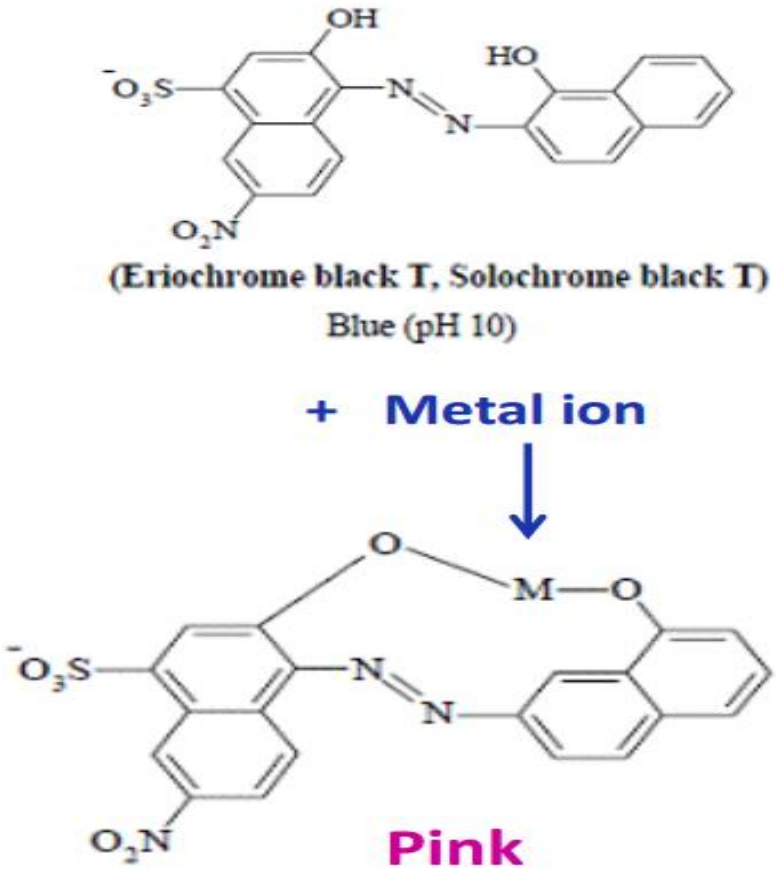


Fig.IX.5 Eriochrome black T.

2. Murexide

Ammonium salt of purpuric acid and its anion has the following structure. Murexide is used for the direct titration of calcium at pH=10, the end point changes from pink to violet (**Fig.IX.6**).

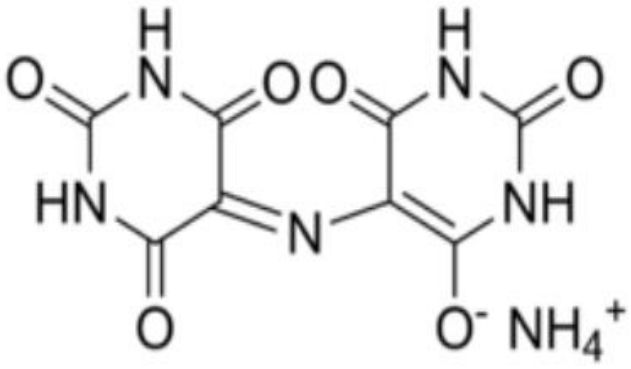


Fig.IX.6 Murexide.

d. Examples: direct determination of water hardnessof metal ion indicators

•Water hardness is due to the presence of Ca^{2+}& Mg^{2+}salts.

•EDTA forms complex with Ca^{2+}& Mg^{2+}

•Ca-EDTA complex is more stable than Mg-EDTA complex.

•At pH 12 EDTA forms complex with Ca^{2+} only.

Total Ca^{2+}& Mg^{2+}

•Total $Ca^{2+}$and Mg^{2+}determined by titration with EDTA at pH 10 using ammonia buffer and EBT as indicator. Upon titration with EDTA, Ca^{2+} will be chelated first, then Mg^{2+}.

For Ca^{2+} Only

Direct titration with EDTA at pH 12 using 8% NaOH and Murexide. Mg^{2+} is precipitated as $Mg(OH)_2$leaving $Ca^{2+}$to be titrated with EDTA.

For Mg^{2+}

Total – Ca^{2+}= Mg^{2+}

4. Analysis of oils and fats in the pharmaceutical industry

In the pharmaceutical industry, oils and fats are analyzed for key chemical properties to ensure their purity, stability, and quality for use in formulations such as emulsions, softgel

capsules, and topical products. Critical analytical parameters include fatty acid profiles, peroxide value, acidity, saponification value, and iodine value.

4.1. Acid Value

The acid value is defined as the number of milligrams of potassium hydroxide (KOH) required to neutralize the free fatty acids present in 1 gram of fat or oil. This value is determined by dissolving a weighed quantity of the sample in neutral ethanol and titrating it against a standard KOH solution using phenolphthalein as an indicator. The acid value indicates the amount of free fatty acids present, which is a marker of freshness. A higher acid value suggests hydrolysis (often due to moisture or enzymatic action) and is an indicator of rancidity, typically resulting from improper storage conditions.

4.2. Saponification Value

The alkaline hydrolysis of a fat or oil is called saponification, as it produces soap (the sodium or potassium salt of fatty acids). This reaction is used to determine the saponification value, defined as the number of milligrams of KOH required to completely saponify 1 gram of oil or fat.This value represents the amount of KOH needed to neutralize the fatty acids released from the complete hydrolysis of the triglycerides. The saponification value is inversely related to the average molecular weight of the fat: a higher molecular weight results in a lower saponification value, and vice versa. It provides insight into the average chain length of the fatty acids in the sample.

3. Iodine Value (Iodine Number)

The degree of unsaturation in a fat or oil is measured by its iodine value. The absorption of halogen is proportional to the number of carbon-carbon double bonds present in the fatty acid chains. The iodine value is defined as the number of grams of iodine (or iodine equivalent, from reagents like ICl or IBr) absorbed by 100 grams of fat or oil.

Therefore, a higher number of double bonds (greater unsaturation) corresponds to a higher iodine value. This value is crucial for assessing the oxidative stability of an oil; oils with high iodine values (e.g., linseed oil) are more susceptible to oxidation and rancidity.

4. Acetyl Value

The acetyl value is a measure of the free hydroxyl groups (e.g., from hydroxy acids like ricinoleic acid in castor oil) present in an oil or fat. It is defined as the number of milligrams

of KOH required to neutralize the acetic acid produced by the hydrolysis of 1 gram of the acetylated fat. This value is determined by first acetylating the sample (reacting it with acetic anhydride) to esterify all free hydroxyl groups. The acetyl value helps in characterizing oils that contain hydroxy fatty acids.

5. Ester Value

The ester value represents the proportion of the material that is present in esterified form (primarily triglycerides). It is defined as the number of milligrams of KOH required to saponify the esters present in 1 gram of a substance. If the saponification value and acid value have been determined, the ester value can be easily calculated from the difference:

Ester Value = Saponification Value − Acid Value

5. The oxygen flask method

5.1. Definition

The oxygen flask combustion (OFC) method was invented by Wolfgang Schöniger in 1955. It is a useful technique for the estimation of certain elements, such as halogens (chlorine, bromine, iodine, and fluorine) and sulfur, in organic compounds within pharmaceuticals. In this technique, the sample is combusted in a sealed flask filled with oxygen. The combustion converts the elements into water-soluble inorganic ions (e.g., Cl^-, Br^-, SO_4^{2-}). These ions are absorbed in a solution and quantified via titration or other analytical methods. The technique is also known as the Schöniger oxygen-flask method.

5.2. Apparatus and reagents

The apparatus (**Fig.IX.6)** for this method consists of a thick-walled conical flask, typically made of borosilicate glass, with a capacity of 500–1000 mL and a ground-glass stopper to ensure an airtight seal. A platinum or quartz sample holder is attached to the stopper, as these materials resist high temperatures and do not interfere with the combustion. Key reagents include high-purity oxygen to maintain an oxygen-rich environment and an appropriate absorbing solution, such as sodium hydroxide for halogens or hydrogen peroxide for sulfur, which captures and converts the combustion products into analyzable ions. Ashless filter paper is used to wrap the sample to prevent introducing external contaminants.

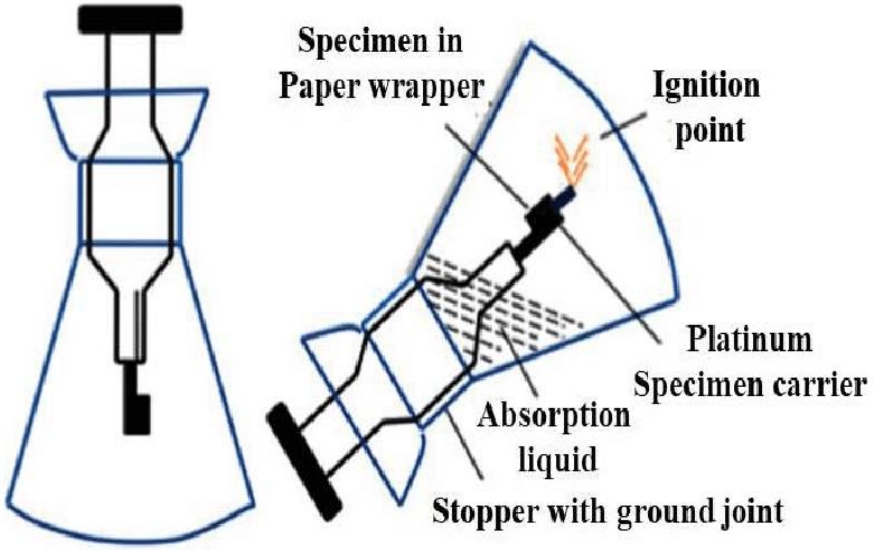


Fig.IX.6 Oxygen-flask method.

5.3. Procedure

The procedure begins with weighing a small sample, typically 5–20 mg, and wrapping it in ashless filter paper. The wrapped sample is placed in the platinum holder, and the absorbing solution is added to the flask. The flask is flushed with pure oxygen to displace any air, ensuring efficient combustion. The sample is ignited, and the stopper is swiftly inserted to seal the flask. During combustion, the sample burns completely, and the resulting gases are absorbed into the solution. After cooling, the flask is shaken to ensure complete absorption of the combustion products, and the solution is then analyzed for the ions of interest.

5.4. Applications in pharmaceuticals

In pharmaceuticals, the Oxygen Flask Method is widely used for quantifying halogens in active pharmaceutical ingredients, such as chlorine in chlorothiazide or fluorine in fluoroquinolones. It is also employed for sulfur determination in compounds like penicillins and cephalosporins. Additionally, the method supports limit tests for halogen and sulfur impurities, as mandated by pharmacopeias like the European Pharmacopoeia and the United States Pharmacopeia, ensuring the purity and safety of pharmaceutical products.

5.2. Determination of bromine and chlorine via oxygen flask combustion

5.2.1. Principle

The organic sample is combusted in a sealed flask filled with oxygen. Bromine and chlorine atoms covalently bound in the molecule are quantitatively converted to their corresponding hydrogen halides (HBr, HCl) and halogens (Br_2, Cl_2). These combustion products are absorbed in a suitable solution, where they are oxidized to bromate and chlorate, or directly reduced to bromide (Br^-) and chloride (Cl^-) ions. These ions are then quantified by titration. The choice of absorption solution is critical to ensure complete recovery and avoid errors.

- **For Simultaneous Br/Cl Determination:** A mixture of sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) is most common. NaOH neutralizes the acidic hydrogen halides. H_2O_2 reduces any free halogens (Br_2, Cl_2) to halides and oxidizes sulfur interferences to sulfate.

5.2.2. Procedure

Precisely weigh 5-20 mg of the organic sample and wrap it in an ashless filter paper. Place the wrapped sample in the platinum gauze carrier attached to the flask's stopper. Add the absorption solution to the bottom of the flask. Flush the flask thoroughly with a stream of pure oxygen for 10-15 seconds. Ignite the tip of the paper and immediately insert the stopper into the flask, ensuring a tight seal. Invert the flask to allow the absorbing solution to form a liquid seal. Allow the combustion to complete and the flask to cool. Shake the flask vigorously for 1-2 minutes to ensure complete absorption of all combustion products. After combustion, the halides in the absorbing solution are quantified. The most common method is argentometric titration (using silver nitrate, **see Section.3.4**).

Evaluation Exercise

Briefly, discuss according to the EU Ph the determination procedure of fluorine, sulfure, and iodine using the oxygen flask method**.**

5. Chemical identification tests

5.1. Definition

Chemical identification tests are analytical procedures that use specific, observable chemical reactions to confirm the identity of a substance. These tests rely on the principle that functional groups, ions, or molecules undergo predictable reactions producing visible changes (e.g., color formation, precipitate, gas evolution) to verify their presence. They are qualitative or semi-quantitative and are foundational in pharmacopeial compliance for raw materials, APIs, and excipients.

5.2. Examples of common tests

5.2.1. Ferric chloride test for phenols

The ferric chloride test is used to identify phenolic compounds. When a sample containing phenol is mixed with a neutral ferric chloride ($FeCl_3$) solution, it forms a complex with Fe^{3+} ions, resulting in a distinct color change. The color varies depending on the specific phenol ranging from violet (e.g., salicylic acid) to blue, green, or red. This test is particularly useful in pharmaceuticals to detect phenolic APIs or degradation products; for example, aspirin hydrolyzes to salicylic acid, which yields a violet color with $FeCl_3$.

5.2.2. Silver nitrate test for halides

The silver nitrate test detects halide ions (Cl^-, Br^-, I^-) in a sample. When silver nitrate ($AgNO_3$) solution is added to a halide-containing sample, it forms insoluble silver halide precipitates: white for chloride (AgCl), pale yellow for bromide (AgBr), and yellow for iodide (AgI). The precipitates can be further distinguished by their solubility in ammonia. This test is critical for identifying halogenated APIs (e.g., chlorothiazide) or inorganic halides in excipients.

CHAPTER X: PHYSICO-CHEMICAL ANALYSIS METHODS FOR DRUG QUALITY CONTROL

2. Objective

At the end of this chapter, the student will be able to:

- Learn the theoretical and practical foundations of ultraviolet and visible spectroscopy.
- Assess the quality of medicine using UV/Visible spectroscopy.
- Learn the theoretical and practical foundations of infrared spectroscopy.
- Create a system of knowledge about the Atomic adsorption spectroscopy as pharmacopoeial method of medicines quality control.

3. Introduction

Physico-chemical analysis methods are essential tools in drug quality control, ensuring the safety, efficacy, and consistency of pharmaceutical products. These techniques evaluate critical attributes, including the identity, purity, strength, and stability of active ingredients and finished drugs. Ranging from classical titrimetry to advanced instrumental techniques, physico-chemical analysis forms the scientific foundation for guaranteeing that all medicines meet required quality standards. This chapter will focus on three pivotal instrumental categories: spectral, chromatographic, and electrochemical methods.

4. Spectral methods for drug quality control

3.1. Ultraviolet and visible spectrophotometry

3.1.1. Generality

The spectrophotometer has well been called the workhorse of the modern laboratory. In particular, ultraviolet and visible spectrophotometry is the method of choice in most laboratories concerned with the identification and measurement of organic and inorganic compounds in a wide range of products, including, pharmaceuticals. In every branch of molecular biology, medicine and the life sciences, the spectrophotometer is an essential aid to

both research and routine control. Modern spectrophotometers are quick, accurate and reliable and make only small demands on the time and skills of the operator.

3.1.2. The electromagnetic spectrum

Man lives in an environment that is permanently exposed to naturally occurring electromagnetic radiation, some of which he detects with his own senses. Radiant heat from the sun is recognised by the body as warmth while the eye responds to light to give the power of sight. But the visible spectrum, that part of the whole spread of wavelength to which the human eye is sensitive, is a very small part of the total range.

The familiar rainbow colors extend in one direction beyond red through infrared to microwaves and radio waves (increasing wavelength) and in the other direction past violet to ultraviolet and then, with progressively diminishing wavelength, via X-rays and gamma rays to cosmic rays (**Fig.X.1**).

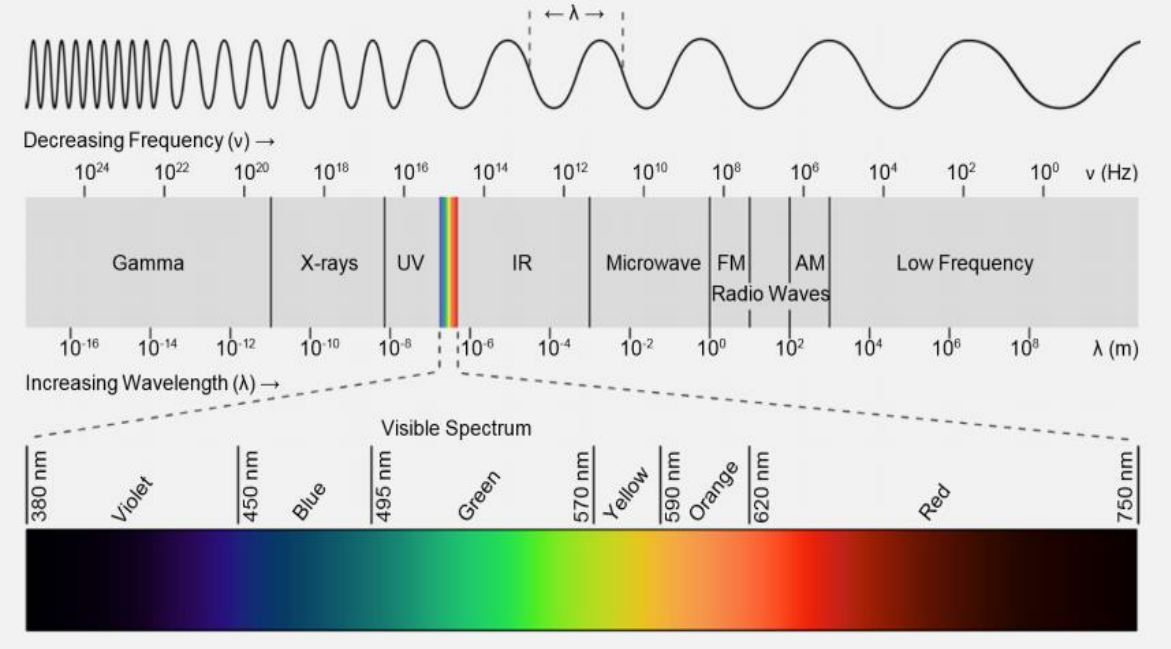


Fig.X.1 The electromagnetic spectrum.

All electromagnetic radiation travels at a fixed speed of 3 × 10^10 cm per sec which is the speed of light, c, in a vacuum. The distance between two peaks along the line of travel is the wavelength, λ, and the number of peaks passing a point in unit time is the frequency, ν, usually expressed in cycles per second (hertz) (**Fig.X.2**).

The arithmetic relationship of these three quantities is expressed by:

$$c = \lambda \cdot \nu$$

The laws of quantum mechanics may be applied to photons to show that:

$$E = h \cdot \nu$$

Where E is the energy of the radiation; ν – the frequency and his Planck's constant.

Combining these two equations:

$$E = h \cdot c / \lambda.$$

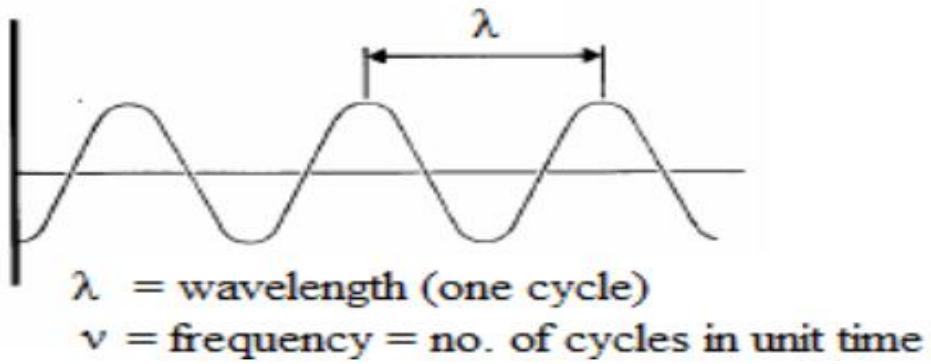


Fig.X.2 Wavelength and frequency**.**

In the visible region it is convenient to define wavelength in nanometers (nm), that is in units of 10^{-9} meters. The visible spectrum is usually considered to be 380–770 nm and the ultraviolet region is normally defined as 200–380 nm.

3.1.3. Specific absorption

Because each electron in a molecule has unique ground state energy, and because the discrete levels to which it may jump are also unique, it follows that there will be a finite and predictable set of transitions possible for the electrons of a given molecule. Each of the transitions, or jumps, requires the absorption of a quantum of energy and if that energy is derived from electromagnetic radiation there will be a direct and permanent relationship between the wavelength of the radiation and the particular transition that it stimulates. That relationship is known as specific absorption and a plot of those points along the wavelength scale at which a given substance shows absorption «peaks», or maxima, is called an absorption spectrum (**Fig.X.3**).

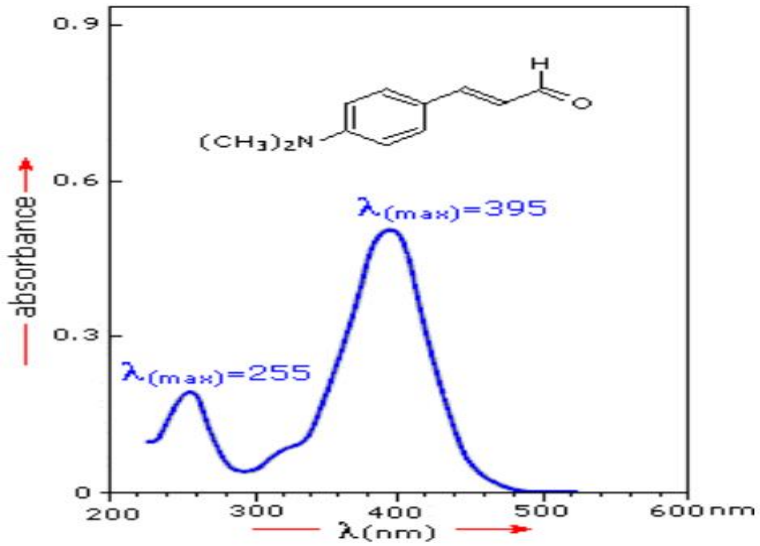


Fig.X.3 Typical absorption spectrum in UV/visible region.

The chemical group most strongly influencing molecular absorption characteristics is called a **chromophore**. Chromophores which can be detected by UV/Vis spectrophotometers always involve a multiple bond (such as C = C, C = O or C ≡N) and may be conjugated with other groups to form complex chromophores. A typical example is the benzene ring which has an absorption peak at 254 nm. Increasingly complex chromophores move the associated absorption peak towards longer wavelengths and generally increase the absorption at the maxima.

3.1.4. Absorption and concentration

Fig.X.4 shows the absorption of radiation by a solution containing absorbing compound.

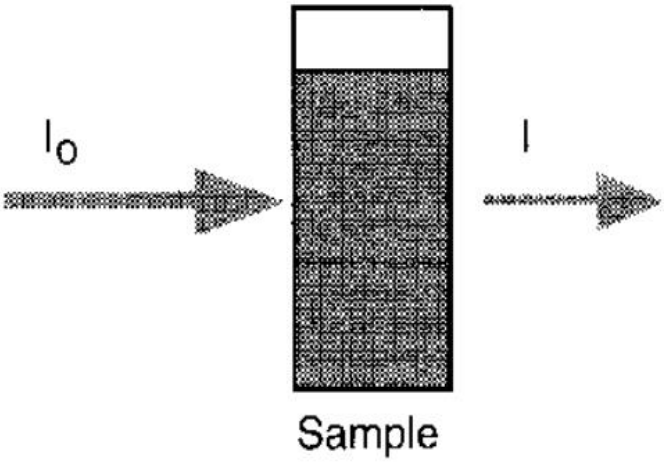


Fig.X.4 Absorption of light by a solution.

For analytical purposes, two main propositions define the laws of light absorption.

- **Lambert's Law.**

The proportion of incident light absorbed by a transparent medium is independent of the intensity of the light (provided that there is no other physical or chemical change to the medium). Therefore, successive layers of equal thickness will transmit an equal proportion of the incident energy. Lambert's law is expressed by:

$$I/I_0 = T$$

Where I is the intensity of the transmitted light; I_0– the intensity of the incident light; T– the Transmittance.

- **Beer's Law.**

The absorption of light is directly proportional to both the concentration of the absorbing medium and the thickness of the medium in the light path.

A combination of the two laws (known jointly as the Beer Lambert Law) defines the relationship between absorbance (A) and transmittance (T):

$$A = \log I0/I = \log 1/T = -\log T = \varepsilon \cdot C \cdot l$$

Where

A is absorbance (no unit of measurement); ε– molar absorptivity ($dm^3mol^{-1}cm^{-1}$); C– molar concentration (mol dm^{-3}); l – path length (cm).

3.1.3. Instrumentation

The minimum requirements of an instrument to study absorption spectra (a spectrophotometer) are shown below (**Fig.X.5**) and include:

1) A source of radiation of appropriate wavelengths;

2) A means of isolating light of a single wavelength and getting it to the sample compartment – monochromator and optical geometry;

3) A means of introducing the test sample into the light beam – sample handling;

4) A means of detecting and measuring the light intensity.

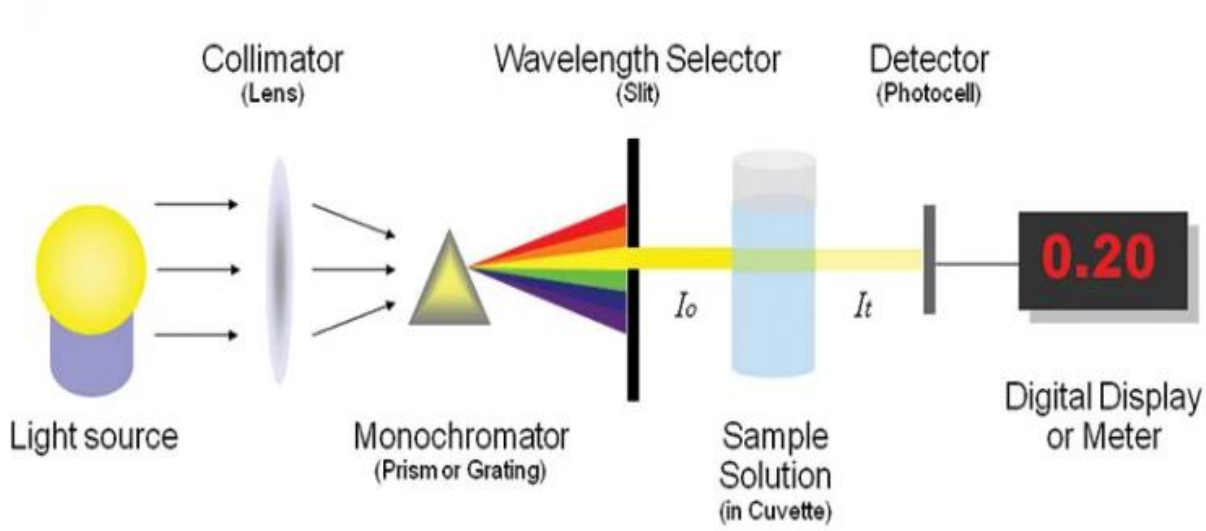


Fig.X.5 Basic construction of a spectrophotometer**.**

❖ Sources

The ideal light source would yield a constant intensity over all wavelengths with low noise and long-term stability. Unfortunately, however, such a source does not exist. Two sources are commonly used in UV-visible spectrophotometers

- The first source, the deuterium arc lamp, yields a good intensity continuum in the UV region and provides useful intensity in the visible region (see **Fig.X.6**). Although modern deuterium arc lamps have low noise, noise from the lamp is often the limiting factor in overall instrument noise performance. Over time, the intensity of light from a deuterium arc lamp decreases steadily. Such a lamp typically has a half-life (the time required for the intensity to fall to half of its initial value) of approximately 1,000 h.

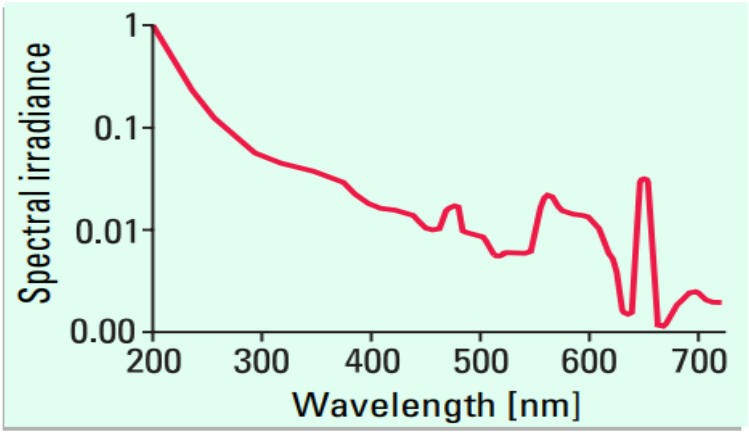


Fig.X.6 Intensity spectrum of the deuterium arc lamp.

- The second source, the tungsten-halogen lamp (see **Fig.X.7**), yields good intensity over part of the UV spectrum and over the entire visible range. This type of lamp has very low noise and low drift and typically has a useful life of 10,000 h. Most spectrophotometers used to measure the UV-visible range contain both types of lamps. In such instruments, either a source selector is used to switch between the lamps as appropriate, or the light from the two sources is mixed to yield a single broadband source.

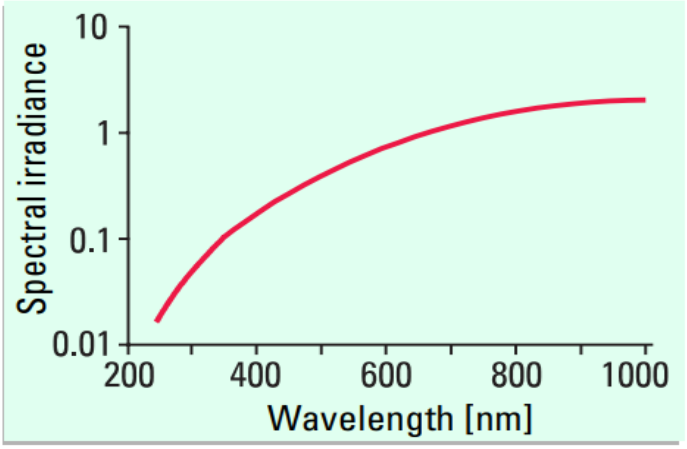


Fig.X.7 Intensity spectrum of the tungsten-halogen lamp.

- An alternate light source is the xenon lamp (see **Fig.X.8**), which yields a good continuum over the entire UV and visible regions. However, because the noise from currently available xenon lamps is significantly worse than that from deuterium or tungsten lamps, xenon lamps are used only for applications such as diffuse reflectance measurements, in which high intensity is the primary concern.

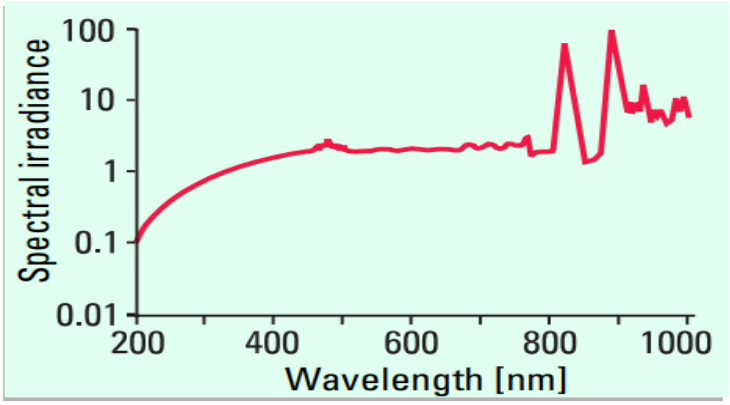


Fig.X.8 Intensity spectrum of the xenon lamp.

- Ultraviolet light is generally derived from a deuterium arc that provides emission of high intensity and adequate continuity in the 190–380 nm range.
- Visible light is normally supplied by a tungsten lamp or, in modern systems, by a tungsten-halogen.

❖ Dispersion devices

Dispersion devices cause different wavelengths of light to be dispersed at different angles. When combined with an appropriate exit slit, these devices can be used to select a particular wavelength (or, more precisely, a narrow waveband) of light from a continuous source. Two types of dispersion devices, prisms and holographic gratings, are commonly used in UV-visible spectrophotometers.

A prism generates a rainbow from sunlight. This same principle is used in spectrophotometers. Prisms are simple and inexpensive, but the resulting dispersion is angularly nonlinear (see **Fig.X.9a**). Moreover, the angle of dispersion is temperature sensitive.

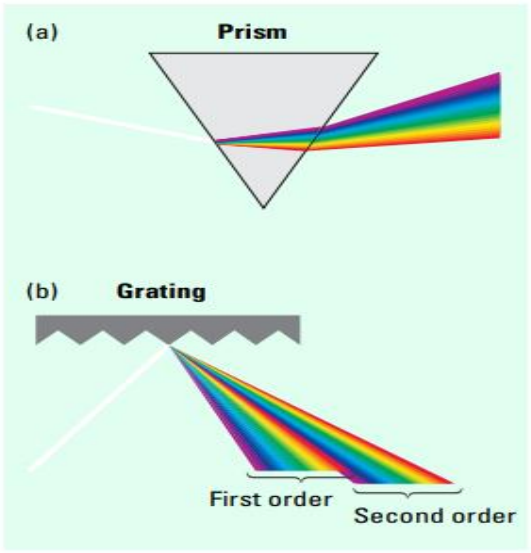


Fig.X.9 Dispersion devices.

For these reasons, most modern spectrophotometers contain holographic gratings instead of prisms. These devices are made from glass blanks, onto which very narrow grooves are ruled. Traditionally, this task was done mechanically, but modern production methods use a holographic optical process. The dimensions of the grooves are of the same order as the

wavelength of light to be dispersed. Finally, an aluminum coating is applied to create a reflecting source. Light falling on the grating is reflected at different angles, depending on the wavelength. Holographic gratings yield a linear angular dispersion with wavelength and are temperature insensitive. However, they reflect light in different orders, which overlap (see **Fig.X.9b**). As a result, filters must be used to ensure that only the light from the desired reflection order reaches the detector. A concave grating disperses and focuses light simultaneously.

A monochromator consists of an entrance slit, a dispersion device, and an exit slit. Ideally, the output from a monochromator is monochromatic light. In practice, however, the output is always a band, optimally symmetrical in shape. The width of the band at half its height is the instrumental bandwidth (IBW).

❖ Sample handling

Cuvettes (**Fig.X.10)** are typically made of glass or UV grade silica (according to the wavelength range of interest). The holder that locates the cuvette in the light beam must ensure precise and reproducible location with respect to the beam. The most commonly used cuvette has a light path length of 10 mm,

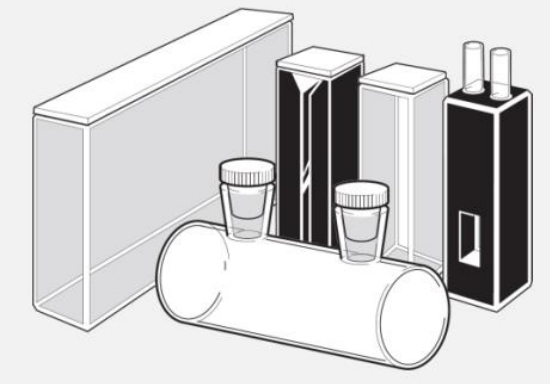


Fig.X.10 Sample Cuvettes.

❖ Detectors

A detector converts a light signal into an electrical signal. Ideally, it should give a linear response over a wide range with low noise and high sensitivity. Spectrophotometers normally contain either a photomultiplier tube detector or a photodiode detector.

❖ **Measuring Systems**

The primary function of a spectrophotometer ends with the provision of a signal (normally an electrical voltage) that is proportional to the absorption by a sample at a given wavelength. The signal handling and measuring systems can be as simple as an amplifier and a meter or as elaborate as a personal computer and printer, depending on the application.

3.2. Infrared (IR) spectroscopy

Infrared spectroscopy is a technique based on the vibrations of the atoms of a molecule. An infrared spectrum is commonly obtained by passing infrared radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration of a part of a sample molecule.

Infrared spectroscopy is certainly one of the most important analytical techniques available to today's scientists. One of the great advantages of infrared spectroscopy is that virtually any sample in virtually any state may be studied. Liquids, solutions, pastes, powders, films, fibres, gases and surfaces can all be examined with a judicious choice of sampling technique. The term «infrared» covers the range of the electromagnetic spectrum between 0,78 and 1000 μm (**Fig.X.11**). It is suitable to divide the infrared region into three sections; near, mid and far infrared. The most useful for chemical structure determination is the middle IR region, which lies between 4000– 670 cm^{-1}.

Table.X.1 Sub divisions of infrared region**.**

Region	Wavenumber range cm^{-1}
Near	12800-4000
Middle	4000-200
Far	200-10

In the context of infrared spectroscopy, wavelength is measured in «wavenumbers», which have the units cm^{-1}. Wavenumber is defined like this:

$$\textbf{Wavenumber} = 1/\lambda$$

3.2.1. Energy of a molecule

Energy of molecule consists of translational, rotational, vibrational and electronic energy. Translation energy of a molecule is associated with the movement of the molecule as a whole, for example in a gas. Rotational energy is related to the rotation of the molecule, whereas vibrational energy is associated with the vibration of atoms within the molecule. Finally, electronic energy is related to the energy of the molecule's electrons. Like radiant energy, the energy of a molecule is quantized too and a molecule can exist only in certain discrete energy levels. Within an electronic energy level, a molecule has many possible vibrational energy levels. To raise the electronic energy state of a molecule from the ground state to the excited state will cost more energy than to raise the vibrational energy state. A simplified representation of the quantized electronic and vibrational energy levels of a molecule can be found in **Fig.X.11.**

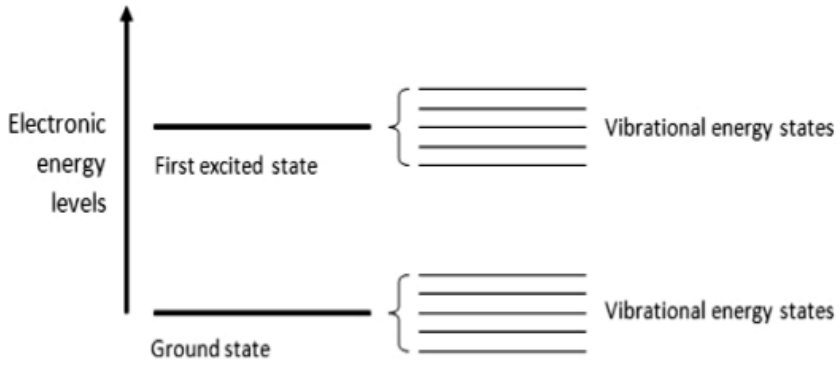


Fig.X.11 A schematic representation of the quantized electronic and vibrational energy levels of a molecule: to raise the electronic energy state of a molecule from the ground state to the excited state will cost more energy than to raise the vibrational energy state.

3.2.2. Interaction of light and molecules

IR radiation does not have enough energy to induce electronic transitions as seen with UV and visible light. Absorption of IR is restricted to excite vibrational and rotational states of a molecule. Even though the total charge on a molecule is zero, the nature of chemical bonds is such that the positive and negative charges do not necessarily overlap in this case. Such molecules are said to be polar because they possess a permanent dipole moment. For a molecule to absorb IR, the vibrations or rotations within a molecule must cause a net change in the dipole moment of the molecule. The alternating electrical field of the radiation interacts

with fluctuations in the dipole moment of the molecule. If the frequency of the radiation matches the vibrational frequency of the molecule then radiation will be absorbed, causing a change in the amplitude of molecular vibration. The result of IR absorption is heating of the matter since it increases molecular vibrational energy (**Fig.X.12**).

Molecular vibrations give rise to absorption bands throughout most of the IR region of the spectrum. The far IR, lying adjacent to the microwave region, has low energy and may be used for rotational spectroscopy.

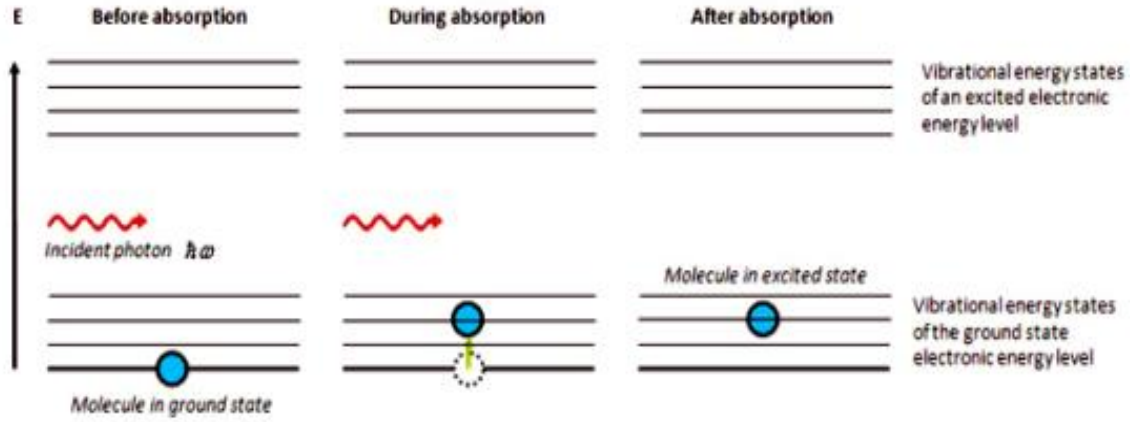


Fig.X.12 Energy levels of a molecule during the absorption of a photon.

3.2.3. Older Technology

The original infrared instruments were of the dispersive type. These instruments separated the individual frequencies of energy emitted from the infrared source. This was accomplished by the use of a prism or grating. An infrared prism works exactly the same as a visible prism which separates visible light into its colors (frequencies). A grating is a more modern dispersive element which better separates the frequencies of infrared energy. The detector measures the amount of energy at each frequency which has passed through the sample. This results in a spectrum which is a plot of intensity vs. frequency. Fourier transform infrared spectroscopy is preferred over dispersive or filter methods of infrared spectral analysis for several reasons:

• It is a non-destructive technique.

• It provides a precise measurement method which requires no external calibration.

• It can increase speed, collecting a scan every second.

• It can increase sensitivity – one second scans can be co-added together to ratio out random noise.

• It has greater optical throughput.

• It is mechanically simple with only one moving part.

3.2.4. Fourier Transform Infrared (FT-IR) spectrometry

FT-IR spectrometry was developed to overcome the slow scanning limitations of dispersive instruments. It uses an interferometer to simultaneously encode all infrared frequencies into a rapid signal called an interferogram. A computer then applies a Fourier transformation to decode this signal, generating the interpretable frequency spectrum for analysis.

3.2.5. The Sample analysis process

The normal instrumental process is as follows (**Fig.X.13-14**):

1. **The Source**: Infrared energy is emitted from a glowing black-body source. This beam passes through an aperture which controls the amount of energy presented to the sample (and, ultimately, to the detector).

2. **The Interferometer**: The beam enters the interferometer where the "spectral encoding" takes place. The resulting interferogram signal then exits the interferometer.

3. **The Sample**: The beam enters the sample compartment where it is transmitted through or reflected off of the surface of the sample, depending on the type of analysis being accomplished. This is where specific frequencies of energy, which are uniquely characteristic of the sample, are absorbed.

4. **The Detector**: The beam finally passes to the detector for final measurement. The detectors used are specially designed to measure the special interferogram signal.

5. **The Computer**: The measured signal is digitized and sent to the computer where the Fourier transformation takes place. The final infrared spectrum is then presented to the user for interpretation and any further manipulation.

Because there needs to be a relative scale for the absorption intensity, a background spectrum must also be measured. This is normally a measurement with no sample in the beam. This can

be compared to the measurement with the sample in the beam to determine the "percent transmittance." This technique results in a spectrum which has all of the instrumental characteristics removed. Thus, all spectral features which are present are strictly due to the sample. A single background measurement can be used for many sample measurements because this spectrum is characteristic of the instrument itself.

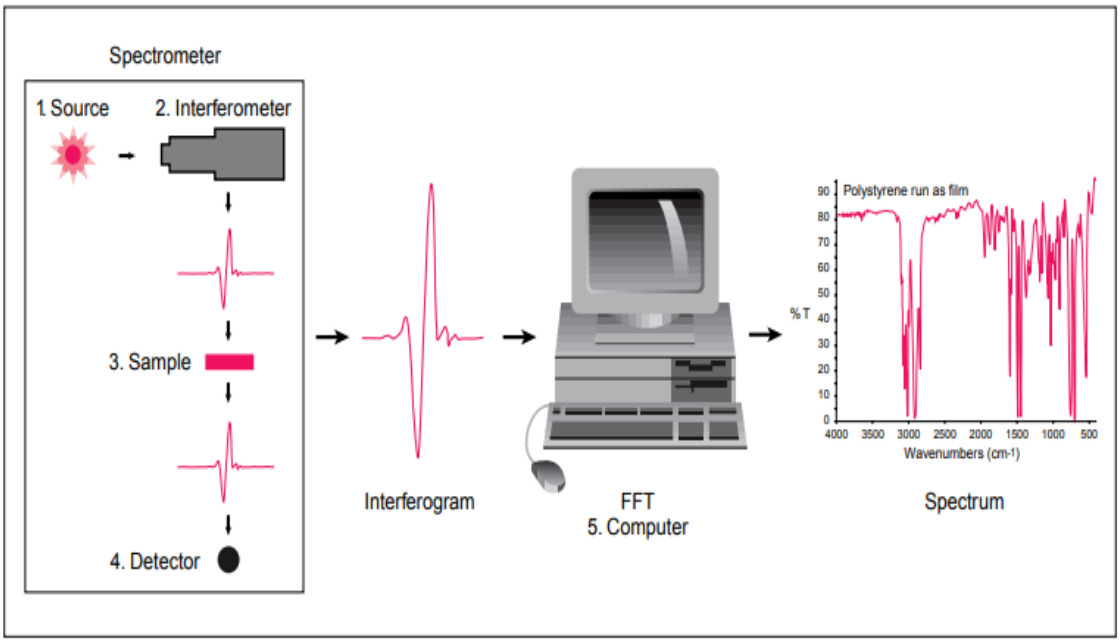


Fig.X.13 FT-IR Instrumental process.

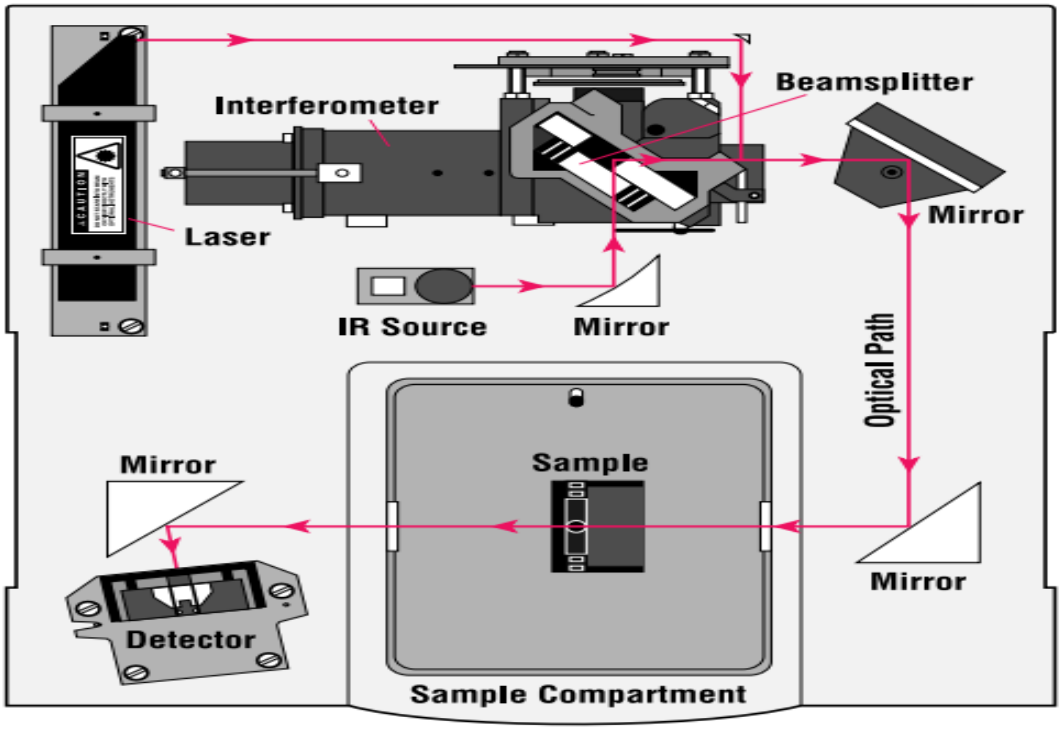


Fig.X.14 Detailed FT-IR Instrumental process.

3.2.6. An example of IR spectrum analysis

Fig.X.15 illustrates the diffuse reflectance infrared spectrum of acetylsalicylic acid (aspirin).

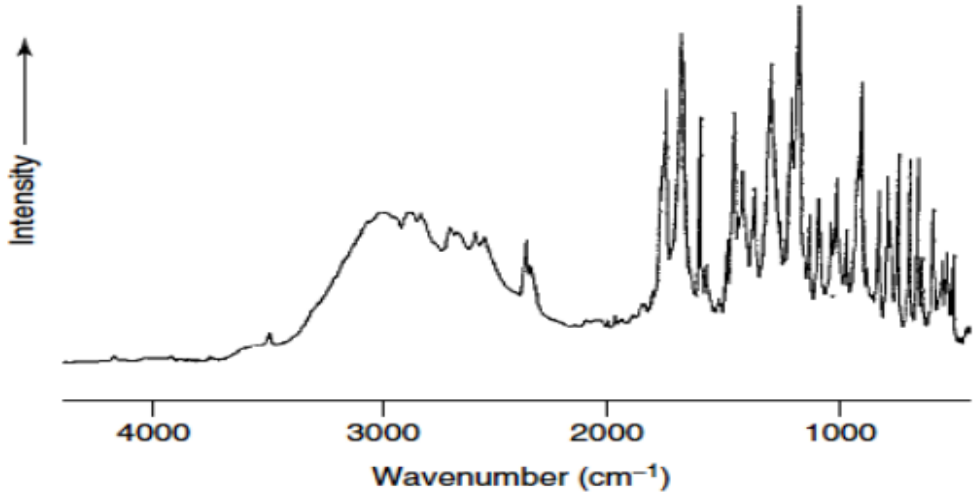


Fig.X.15 Diffuse reflectance IR spectrum of acetylsalicylic acid.

Such a spectrum may be used to identify the functional groups present in this molecule (**Fig.X.16**).

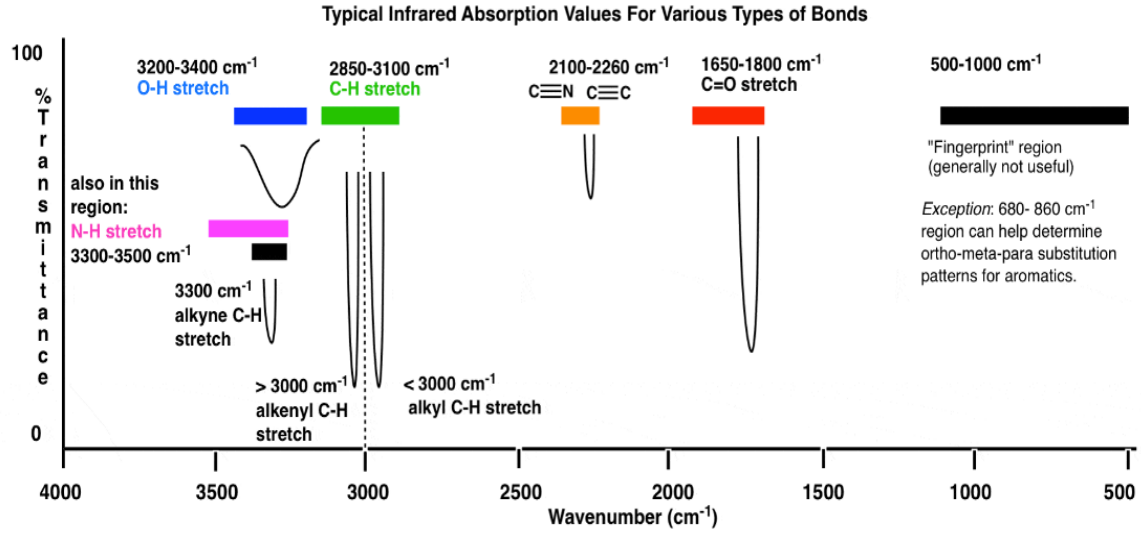


Fig.X.16 Characteristic IR absorption frequencies of organic functional groups.

The presence of O–H groups in acetylsalicylic acid is indicated by a broad band in the 3400–3300 cm^{-1} region and C–H stretching bands overlap with this band in the 3000–2800 cm^{-1} range. The spectrum shows two strong C = O stretching bands at 1780, 1750 cm^{-1}, so indicating that the molecule contains carbonyl groups in different environments. The spectrum also shows strong bands at 1150 and 1100 cm^{-1} due to C–O stretching: the 1150 cm^{-1} band is due to the presence of a C–O–H group, while the 1100 cm^{-1} band is due to a C–O–C group in the structure. There is also evidence of a benzene ring, including a series of characteristic bands in the 800–500 cm^{-1} range due to C–H stretching in the aromatic ring. In summary, acetylsalicylic acid contains O–H, C = O, C–O–C, C–O–H and aliphatic and aromatic C–H groups.

The acetylsalicylic acid structure is illustrated in **Fig.X.17**, hence confirming the presence of these functional groups.

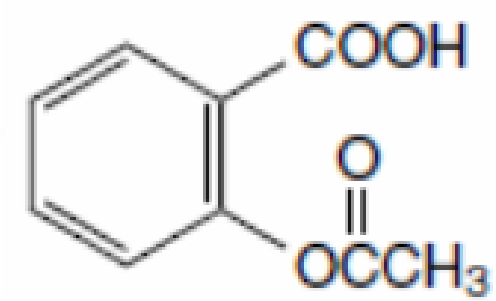


Fig.X.17 Structure of acetylsalicylic acid.

3.3. Atomic absorption spectroscopy (AAS)

Atomic absorption spectroscopy is a spectroanalytical procedure for the quantitative determination of chemical elements employing the absorption of optical radiation (light) by free atoms in the gaseous. Atomic absorption spectrometry was first used as an analytical technique, and the underlying principles were established in the second half of the 19th century by R.W. Bunsen and G.R. Kirchhoff, both professors at the University of Heidelberg, Germany. In analytical chemistry the technique is used for determining the concentration of a particular element (the analyte) in a sample to be analyzed. AAS can be used to determine over 70 different elements in solution or directly in solid samples employed in pharmacology, biophysics and toxicology research. The method's intrinsic sensitivity, specificity, andaccuracy make it invaluable in metallurgy, pharmaceutical sciences, environmental analysis, and other sectors; it also spurs improvements in industrial processes, research, and quality control procedures.

3.3.1 Theory of absorption by atoms

Atomic Absorption Spectroscopy quantifies element concentration by measuring the absorption of light by free atoms. A cathode lamp emits element-specific light, which is absorbed when atoms in a flame or furnace are elevated to a higher energy state. This absorption is directly proportional to the element's concentration, enabling precise quantitative analysis.

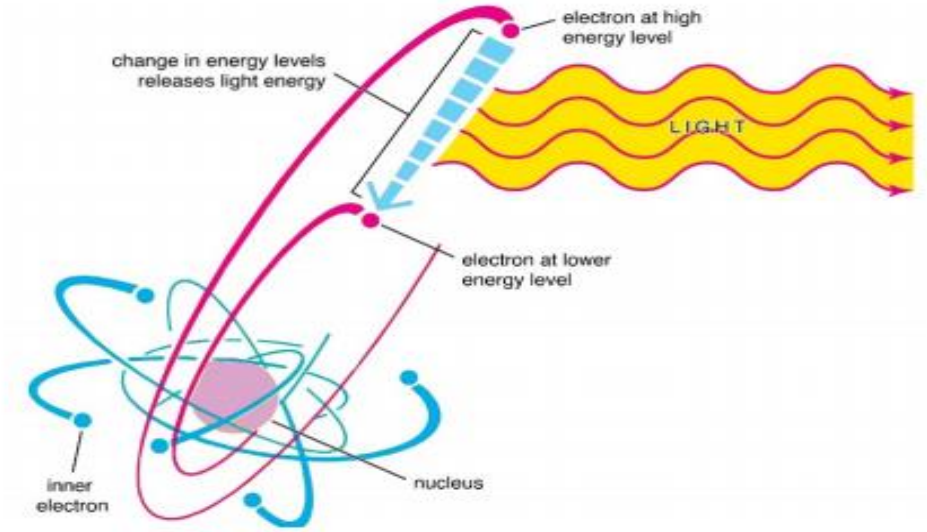


Fig.X.18 The process of atomic absorption.

3.3.2. Instrumentation and components

Hollow Cathode Lamp (HCL): The main light source in an AAS is the HCL, which emits light at wavelengths particular to the object of interest. It is composed of a target element cathode encased inside a glass tube that is low-pressured and contains an inert gas (often neon or argon). The target element's atoms are excited by electrons released from the cathode whenever a high voltage is applied across the anode and cathode, leading to the emission of distinctive radiation.

Monochromator: The chosen wavelength of light that the HCL emits for analysis is chosen by the monochromator. It usually comprises a prism or diffraction grating that splits the light produced into its individual wavelengths. A particular wavelength matching to the element's resonance line is separated and directed toward the sample by turning or modifying the grating or prism.

Sample Introduction System: To atomize and introduce the specimen onto its light path for analysis, the sample introduction mechanism is essential. The sample is inhaled through an ignited object (which is frequently an aperture or cup burner) in flame AAS, where the heat of the flame atomizes, vaporizes, and then excites the sample. A small quantity of sample solution is put onto a graphite tube in the graphite furnace AAS, and the tube is subsequently warmed to high temperatures, atomizing and excitivizing the sample.

Detector: Following contact with the sample, the detector calculates the transmitted light's intensity. AAS frequently uses photomultiplier tubes (PMTs) as detectors because of their high sensitivity and quick reaction times. The light intensity is transformed by the detector toward a signal of electricity, which is subsequently processed and examined to ascertain the sample's absorbance.

Automation and Control Systems: In order to improve the efficiency and repeat ability of analyses, modern AAS devices frequently include automation capabilities and computer control systems. Throughput can be increased and operator mistakes can be decreased by using automated sample changers, which enable sequential analysis of manysamples without the need for manual intervention. To guarantee optimal performance and precision, computer control systems allow for the exact modification of equipment parameters such wavelength selection, slit width, and lamp current.

Accessories and Optional Components: AAS equipment may be made more capable and versatile by adding different alternative parts and accessories. These might include programs that perform data processing and analysis, thermostat systems like a flame or graphite furnace, and background correction systems (such as the deuterium / Zeeman effect correction). To maximize instrument performance, guarantee precise measurements, and successfully interpret experimental data, one must have a thorough understanding of the instruments and components of AAS. Each part is essential to the instrument's overall operation, which ultimately determines the tool's analytical potential and practicality in a range of settings. Instrumentation and components of AAS are shown in **Fig.X.19**.

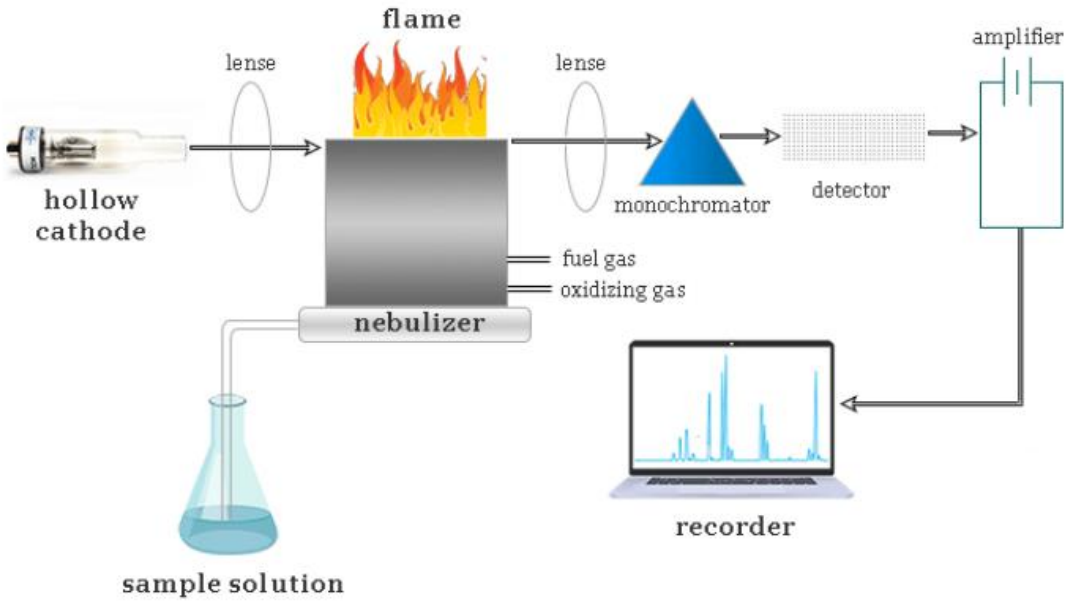


Fig.X.19 Instrumentation and components of AAS.

3.3.3. Sample preparation techniques

Sample preparation strategies are critical to achieving precise and dependable findings with atomic absorption spectroscopy (AAS). The basic goal of preparing a sample is to transform the sample into a format appropriate for analysis while reducing disturbances and matrix effects. Some common sample preparation procedures in AAS include:

Dissolution: Solid samples are frequently dissolved in suitable solvents to ensure uniform dispersion for the chemical in solution. Digestion with acids or microwave-

assisted digestion procedures can be used to break down complicated matrices and improve analyte recovery.

Dilution: Samples with high analyte concentrations may need to be diluted to put them within the instrument's linear range. Dilution also helps to reduce matrix influences and interferences, resulting in precise measurement.

Filtration: Filtration is used for eliminating suspended particles and insoluble contaminants from sample solutions, preventing nebulizer clogging and interfering alongside the atomization process in flame AAS.

Centrifugation: Centrifugation separates intractable constituents or portions separate the sample solution, making it easier to collect supernatants for examination.

Extraction: To separate the substance being studied through interference species or complex matrices, liquid-liquid or solid-phase extraction procedures can be used, increasing selectivity and sensitivity.

3.3.4. Calibration and standardization methods

Calibration and standardization are critical procedures in the technique of atomic absorption spectroscopy to enable accurate and consistent elemental concentration measurements in samples. Calibration is the process of creating a curve for calibration using standard solutions with known analyte concentrations of substances, whereas standardization guarantees that the instrument operates within prescribed parameters. Some popular calibration and standardization procedures in AAS are:

External Calibration: External calibration involves preparing and analyzing an array of typical solutions with known analyte concentrations using an AAS instrument. The absorbance values regarding the benchmarks are displayed against their concentrations to create a calibration curve. The amount present of the component in unidentified specimens is calculated by extrapolating the absorbance measurements over the calibration curve.

Internal Calibration: Internal calibration entails adding an established amount of a standard substance to the two parts the standard solution and the sample. The absorption proportion of the metaboliteto the standard solution is subsequently compared against the amount present ratio, therefore removing any inaccuracies caused by instrument parameter fluctuations and sample handling.

Instrument Standardization: Instrument standardization entails testing and modifying instrument settings to achieve precise andrepeatable readings. This might involve assessing lamp intensity, wavelength correctness, background security,and detector sensitivity. To ensure analytical accuracy and precision, equipment performances should be standardized on a regular basis using approved materials for reference and validation processes. AAS provides the preciseness, accuracy, and dependability of quantitative elements analysis across a wide range of specimen matrices and applications by using suitable calibration and standardisation procedures. These techniques are critical for confirming analytical data, meeting regulatory criteria, and providing transparency in analytical measurements.

3.3.5. Applications in pharmaceutical analysis:

AAS is used in pharmaceutical analysis for determination of metal residues in drugs remaining from the manufacturing process, for assay ions (Ca^{2+}, Mg^{2+}) in haemodialysis fluid, zinc (Zn^{2+}) in insulin, etc. In Ph. Eur. AAS is used in a number of limit tests for metallic impurities, e.g.: magnesium and strontium in calcium acetate; palladium in carbenicillin sodium and lead in bismuth subgalate. It is also used to assay metals in a number of other preparations: zinc in zinc insulin suspension and tetracosactrin zinc injection; copper and iron in ascorbic acid; zinc in acetylcysteine; lead in bismuthsubcarbonte; silver in cisplatinum; lead in oxyprenaolol; aluminium in albumin solution and calcium, magnesium, mercury and zinc in water used for diluting haemodialysis solutions.

5. Chromatographic methods for drug quality control

In the rapidly evolving landscape of pharmacy and nursing, chromatographic techniques play a pivotal role in pharmaceutical analysis. Chromatography, as a powerful separation and identification tool, is indispensable in the pharmaceutical industry for ensuring the quality, safety, and efficacy of drugs and formulations. The chapter introduces the fundamental principles of chromatography, highlighting its differential interaction between the stationary and mobile phases, leading to precise separation of complex mixtures. Subsequently, the instrumentation and key parameters affecting separation in prominent techniques like High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and Thin Layer Chromatography (TLC) are explored. Additionally, the chapter discusses other important chromatographic techniques, including Size Exclusion Chromatography and Ion Exchange Chromatography, each

offering unique advantages in pharmaceutical analysis. The significance of method validation to ensure the accuracy and reliability of results is emphasized throughout the chapter. By providing valuable insights into cutt ing-edge advancements and best practices, this chapter equips students with the knowledge needed to leverage chromatographic techniques effectively in pharmaceutical research and quality control.

4.1. Chromatography principle

Chromatography is based on the principle where molecules are transferred between the mobile phase and the stationary phase. It occurs due to the absorbance or partition of molecules present in the mixture which we want to separate. The rate of travel of individual solute molecules through a column or thin layer is not uniform. It is directly related to the partition of the molecules between the mobile phase and stationary phase.

4.2. Stationary phase in chromatography

The stationary phase may be a column **(Fig.X.20)**, plate, or paper in chromatographic technique. It is a solid phase or a liquid phase coated on the surface of a solid phase.

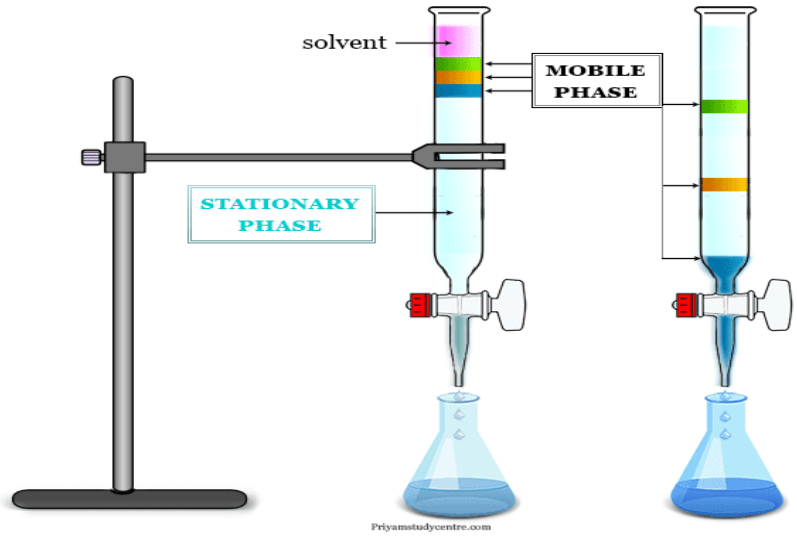


Fig.X.20 Stationary phase.

Depending upon the nature of the stationary phase, three chromatographic techniques have been used widely in the laboratory. These techniques are,

- Liquid-solid absorption or column chromatography (LSC)

- Paper chromatography (PC)
- Thin-layer chromatography (TLC)

4.3. Mobile phase in chromatography

The mobile phase (liquid or gas) moves through the stationary phase when different components are separated due to different rates of migration.

1. If the mobile phase is liquid, it is called liquid chromatography (LC).
2. If the mobile phase is gas then it is called gas chromatography (GC).

The migration of molecules depends on their affinity to the stationary and mobile phases. Therefore, the components which are attached less by the stationary phase will move faster in mobile phases.

4.4. High performance liquid chromatography (HPLC)

HPLC is the most widely used chromatographic technique for analysis of drugs and pharmaceutical formulations. It offers high resolving power, sensitivity, accuracy and reproducibility. HPLC has advantages like high selectivity, sensitivity and speed of analysis which make it an invaluable technique for pharmaceutical analysis and quality control applications. Developing robust and optimized HPLC methods is an essential part of drug development and product lifecycle management.

4.4.1. Principle of HPLC

HPLC is a type of partition chromatography that utilizes the partitioning of substances between the stationary and mobile phases. The mobile phase consists of a solvent or a mixture of solvents that moves through the column carrying the sample when a pump exerts pressure. The stationary phase is a solid material, such as silica, packed in a column or cartridge. When the sample passes through the column, the stationary phase adsorbs the components of the sample with different affinities, resulting in a separation of the components. The basis of HPLC is that molecules will move through the stationary phase at different speeds, depending on their relative affinity for the stationary phase. Different compounds travel at different speeds, which results in the separation of the compounds. The rate of movement of the components is determined by the relative affinities of the analyte molecules for the mobile

and stationary phases. Compounds with a high affinity for the stationary phase will travel slower and separate morethan less strongly adsorbed molecules.

4.4.2. Instrumentation of HPLC System

A basic HPLC system (**Fig.X.21**) consists of high pressure pumps, injection system, chromatographic column, a detector like Ultraviolet (UV), Photo Diode Array (PDA), Evaporative light scattering detector (ELSD), etc., and data acquisition and processing system.

Solvent delivery pump: It is used to maintain a constant flow rate of the mobile phase through the HPLC system. The purpose of the pump is to force the mobile phase through the column while maintaining a specific flow rate.

Injector: An HPLC injector allows the introduction of samples onto the column. The role of the injector has a lot of significance because direct injection of the sample is not suggested as the working pressure of the HPLC is adequately high that we cannot inject the sample into the mobile phase. Care must be taken while injecting the sample. Points that must be kept in mind like introducing a sample without air bubbles, a sample introduced with constant pressure and flow rate, injection volume of the sample is in microliters, and the sample must be free from any particulate matter. An advanced injector known as Auto-sampler injector is used to deliver an aliquot of sample to the HPLC column. It is high-tech automation equipment with high precision, variable volume, and long-term reliability.

HPLC Column: The HPLC column is an integral part of the HPLC system that performs the critical task of separating molecular compounds during analysis. Several types of columns are used in the pharmaceutical industry; however, the most commonly used ones are C18 and C8 columns.

Detector: Detectors are used to sense the presence of separated compounds as they leave the column. The separates are monitored and expressed electronically by the detectors. Detectors used shall be sensitive, have good stability, reproducibility, inexpensive, non-destructive, highly reliable, and withstand temperatures ranging up to 400 °C. Detectors measure the difference in some physical properties of the solute in the mobile phase compared to the mobile phase alone.

Computer Data Station (Recorder) Electronic data signals expressed by the detectors are interpreted and processed into a meaningful inference in the form of chromatograms. They record the baseline and all the peaks obtained with respect to time.

Degasser: The mixing of liquids involves the entrapment of gases like oxygen, which contributes to noise and causes an unstable baseline. Degasser is a high-efficiency in-line system designed to remove dissolved gases from a solvent.

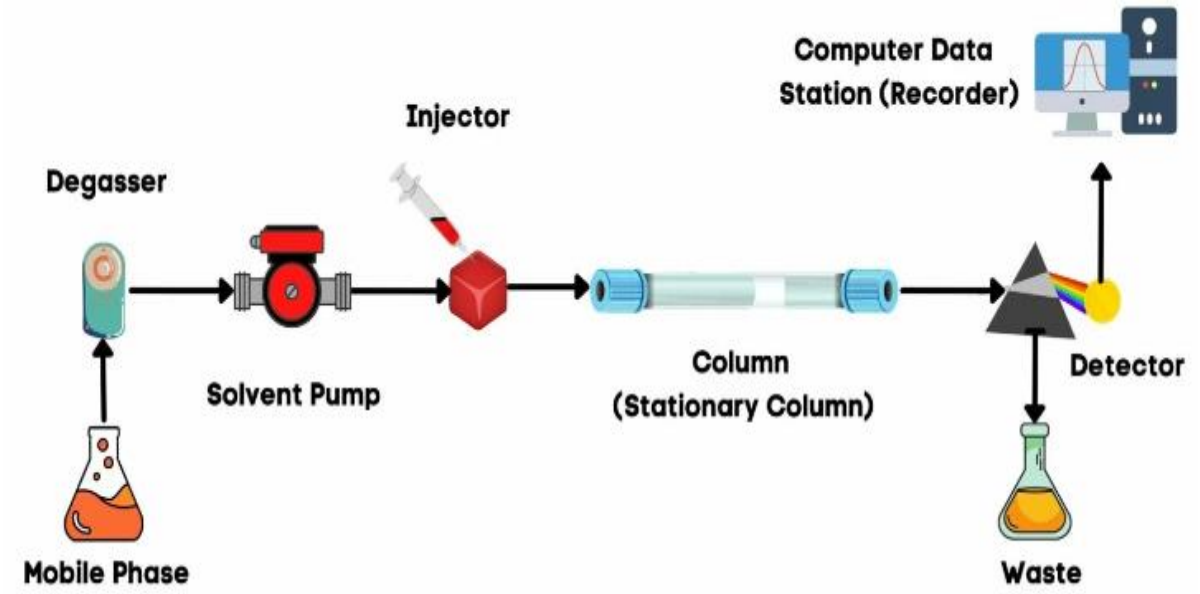


Fig.X.21 High performance liquid chromatography**.**

4.4.3. Types of High-Performance Liquid Chromatography

a. Normal Phase chromatography:

This chromatography type uses columns packed with a polar stationary phase and a nonpolar or moderately polar mobile phase to separate polar compounds. The polar stationary phase includes silica, alumina, or polar-bonded phase, whereas the nonpolar mobile phase includes hexane, heptane, etc. This method separates analytes based on polarity. Less polar solutes move the fastest and therefore exit the column and are detected first, followed by solutes of increasing polarity, which move more slowly.

b. Reverse Phase Chromatography:

This chromatography type uses columns packed with a nonpolar stationary phase and a moderately polar, aqueous mobile phase to separate polar, nonpolar, ionic, and ionizable compounds. It works on the principle of hydrophobic interactions. In this chromatography, the surface of the column stationary phase is covalently bound with alkyl or aromatic ligands to provide a hydrophobic surface. Hydrophobic solutes present in the mobile phase tend to get bound to the stationary phase via hydrophobic interactions, forming the basics of separation.

c. Ion Exchange Chromatography:

Ion exchange chromatography (IEX) is a chromatographic separation method based on the protein's net charge. IEX separates molecules by their surface charge, a property that can vary vastly between different proteins.

d. Size Exclusion Chromatography:

Size Exclusion Chromatography is a method where molecules in a solution are separated by their size and molecular weight. The separation is achieved by the attraction between solute ions and the charged sites bound to the stationary phase. It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers.

4.4.4. Parameters affecting HPLC separation

Following are the parameters that affect HPLC separation:

- Nature of stationary and mobile phases
- pH, ionic strength and temperature of mobile phase
- Flow rate of mobile phase
- Column dimensions

4.4.5. Applications of HPLC in pharmaceutical analysis

The applications of HPLC in pharmaceutical analysis are:

- Determination of drug content and impurities in samples
- Simultaneous determination of multiple drug components
- Determination of drugs in biological samples like plasma and urine
- Separation of enantiomers for chiral drugs
- Stability-indicating assays for drug degradation products.

4.5. Gas chromatography (GC)

Gas chromatography in analytical chemistry is a common type of chromatography analysis process where the mobile phase is a gas and the solute of the analyzed sample is separated as a vapour. The mobile phase which we usually use in gas chromatography is noble gas or an unreactive gas such as helium, argon, nitrogen, and hydrogen. The main working principle of gas solid chromatography (GSC) or gas liquid chromatography (GLC) instrumentation is based on the movement of different components of the sample through a stationary phase present in the column. A gas chromatography system provides great advantages in testing the purity of substances or the separation of a particular component from a mixture of analyzed samples.

4.5.1. Principle of GC

The main principle of gas chromatography instrumentation is based on the movement of different components of the sample through a stationary phase present in the column. The component of a sample that has a greater affinity for the stationary phase spends more time in the column. Therefore, it elutes later and has a longer retention time than the component that has a lower affinity for the stationary phase.

Separation in gas chromatography is feasible by partitioning the sample between a mobile gas phase and a thin layer stationary phase of nonvolatile, high boiling liquid held on the solid support. The idea of fractioning gases by passage over solid or immobilized gases was first introduced in 1941 and became popular after 1955.

- A sample is injected into a heating block where the compound is readily vaporized. The sample vapour is carried by the carrier gas into the column inlet.
- The solute is absorbed in the head of the column by the stationary phase. However, it travels at its own rate through the column according to its partition coefficient value.
- Solutes are eluted according to their partition coefficient and entered into the detector.
- In the detector, solutes give a series of signals resulting from concentration changes and different rates of elution.
- A plot or chromatogram of time against the composition of the carrier gas stream appears in the recorder of the GC machine. The peaks of the plot give the quantitative data in the gas chromatogram.

4.5.2. Instrumentation of GC System

A good gas chromatography machine contains the following important components,

1. Pressure regulator
2. Sample injection port
3. GC column
4. Stationary phase
5. Mobile phase
6. Detector
7. Signal recorder

The instrumentation of the gas chromatography machine is given in **Fig.X.22**.

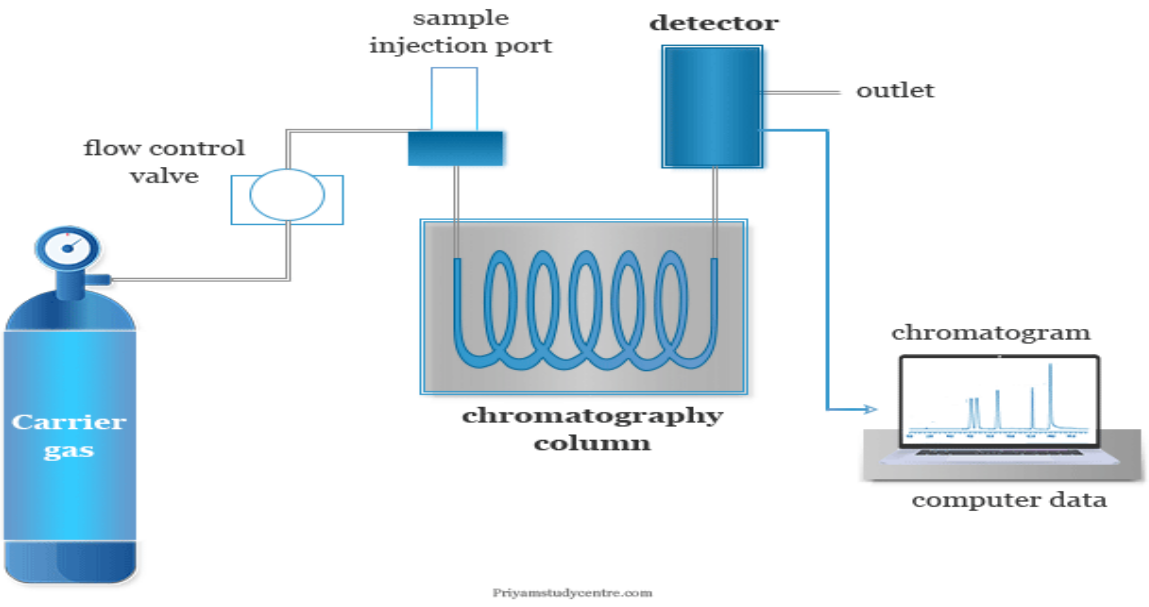


Fig.X.22 Gas chromatography machine.

a. Pressure Regulator

Pressure is adjusted within the limits of 1 to 4 atmospheres while the flow control valve measures 1 to 1000 liters per minute of gas. A trap containing a molecular sieve is used in GC instrumentation to filter out contaminating impurities.

b. Sample Injection Port

Samples are injected by a microsyringe through a self-sealing silicon rubber septum in a heated metal. Generally, the metal bock is heated by an electrical heater fitted in the GC instrument.

c. Gas Chromatography Column

The gas chromatography column can be made by tubing coiled into an open spiral. Copper or stainless steel is used because they are stable during high-temperature operation. The choice of the column depends on the sample which we analyzed. The column in the gas chromatography instrument contains an electrically controlled oven. The velocity of the carrier gas flow rate depends on the inner diameter of the chromatographic column. The usual size of the column is 2 meters

d. Mobile Phase

The mobile phase is a chemically inert gas that is used to carry analyte molecules through the stationary phase of a heated column. The mobile phase which we usually use in GC instrumentation is noble gas or an unreactive gas such as helium, argon, nitrogen, and hydrogen.

e. Detector

The detector can detect the arrival of separated components coming from the column to provide an electrical signal. Pressure and temperature detectors are the two major groups of detectors used in GC analysis. The detector in gas chromatography instrumentation is situated near the column to avoid the condensation of liquids or to detect the sample before decomposition. In a packed column GC instrumentation, we used mostly a thermal conductivity detector (TCD) or a flame ionization detector (FID). Among these TCD is the most popular.

4.5.3. Stationary phase in gas chromatography

Gas liquid chromatography can be available in almost an infinite variety of liquid partition materials. The liquid or stationary phase in gas chromatography can be divided into nonpolar, intermediate polarity, polar carbowaxes, and hydrogen bonding compounds like glycol. The maximum temperature of the stationary phase can be determined by its volatility. The excess

volatility of the stationary phase can shorten the life of the column. Loading of the column by stationary phase can be expressed by percentage of weight. For example, 15% means, a 100 g column has 15 g of stationary phase.

4.5.4. Parameters affecting GC separation

The parameters affecting GC separation are: nature and flow rate of carrier gas, temperature and pressure of column, nature of stationary and coated phases, column dimensions.

4.5.5. Applications of GC in pharmaceutical analysis

- Analysis of thermal stability and degradation products of drugs
- Analysis of organic volatile impurities in drugs and formulations
- Determination of residual solvents and pesticide residues
- Chiral separation of drug enantiomers
- Detection of counterfeit and substandard drugs

4.6. Thin layer chromatography (TLC)

TLC is a simple, cost-effective and rapid technique for preliminary separation and analysis of drugs. Thin layer chromatography in analytical chemistry is a chromatography principle or technique, which we use to separate or identify non-volatile mixtures on a small scale. In the TLC technique, the adsorbent is coated on a glass plate which acts as a stationary phase. Usually, we used alumina or silica gel with little calcium sulfate for the production of thin layer chromatography plates. The solvent or mobile phase which we used in thin layer chromatography is a very important step. Compared to paper chromatography, it uses a very small amount of samples for quantitative analysis with a lower detection limit. The TLC technique is very useful for the analysis of a large number of compounds. Therefore, compounds that are not volatile or too labile for liquid or gas chromatography can be analyzed by the TCL technique. Though it lacks sensitivity and resolution compared to HPLC and GC, TLC finds practical applications as an initial step in method development and quality control testing.

4.6.1. Principle of TLC

The thin layer chromatography can be performed on the TLC plate. A TLC plate is a sheet of aluminum foil, plastics, or glass that supports the stationary phase or a thin layer of solid

absorbent. Silica or alumina are used commonly for making solid absorbents. The mobile phase in the TLC technique is a solvent or a mixture of solvents.

After sporting the TLC plate, it is kept in a closed jar or beaker (TLC chamber) containing the eluent or mobile phase. The solvent rises up the TLC plate by capillary action. The solvent also drags the sample mixture along with it.

The component of the sample is attracted by the polar sites of the absorbent surface by the electrostatic force of attraction. The solvent also interacts with the components of absorbents. Depending on the polarity and interaction of solute, solvent, and absorbent, different components of the mixture move at different rates. When the solvent almost reaches 90 percent of the length of the TLC plate, it is taken out from the TLC chamber. The TLC plate is allowed to dry by evaporating the solvent and the components of the mixture are marked under the ultraviolet (UV) light. Therefore, the individual components are identified by calculating the retardation factor (R_f) and comparing it with the standard substances.

4.6.2. Solvent used in thin layer chromatography

The compounds in the spot will move up on the TLC plate depending on the solvent used in the mobile phase. Polar solvents will drug polar substances while nonpolar solvents can help to move nonpolar compounds with them.

- **Very polar solvents**: Methanol > ethanol > isopropanol
- **Moderately polar solvents**: Acetonitrile > ethyl acetate > chloroform > dichloro methane > diethyl ether > toluene
- **Nonpolar solvents**: Cyclohenane, petroleum ether, hexane, pentane.

The choice of solvent system and composition of thin layer plate determined the principle of TLC. The solvent used in the TCL technique should be non polar and volatile. The eluting powers of the common solvents are, Pentane < hexane < carbon tetrachloride < chloroform < diethyl ether < ethyl acetate < acetone < ethanol < methanol < water.

4.6.3. Instrumentation of TLC

The basic components of a TLC system are: TLC plates coated with adsorbent like silica gel, mobile phase solvents for development, developing chamber, and UV lamp or staining reagents for visualization.

a. Thin Layer Chromatography Plates

Thin layer chromatography or TLC plate is a glass plate that has three different sizes 25 × 75 mm, 5 × 20 cm, and 20 × 20 cm. We used alumina or silica gel with little calcium sulfate for the coating of TLC plates. Cellulose powder and diatomaceous earth can also be used for coating TCL plates.

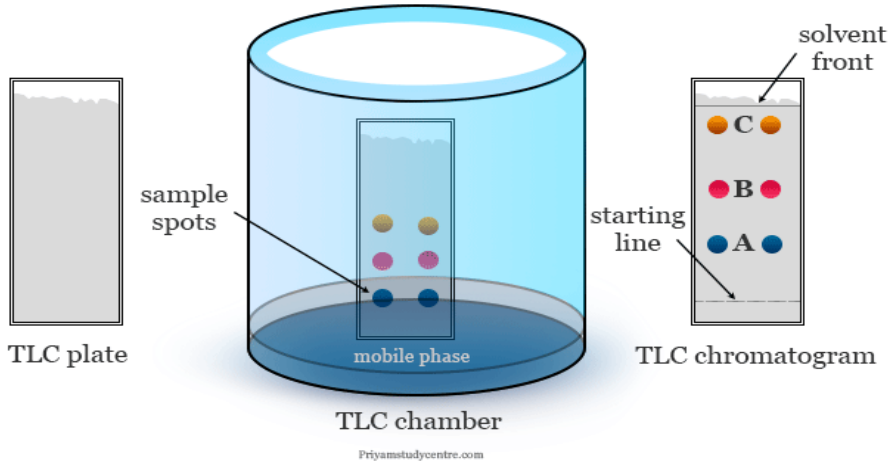


Fig.X.23 Thin Layer Chromatography.

b. Chromatogram

When the spot becomes dry, the plate is put into the chamber which contains a suitable solvent with its saturated vapour. The absorbent layer must be under the solvent and spots must be above the solvent. When the solvent front travels at least three fourth of the length of the plate, a chromatogram is developed.

c. Identification of components

Components of colour compounds mixture are directly identified by colour spots in the chromatogram. Components of colorless compounds can be identified by UV light on the dried plate to obtain fluorescence or by placing it into an iodine chamber or spring with a suitable reagent to give coloured spots. After making the spot, the R_f values are calculated.

4.6.4. Applications of TLC in pharmaceutical analysis

- Quick screening and identification of compounds in complex mixtures
- Determination of R_f values for standardization and method development
- Purity testing of drug substances and formulations such as analgesics, anesthetics, hypnotics, anticonvulsants, sedatives, tranquilizers, etc
- Separation of isomers and enantiomers
- Identification of degradation products

4.7. Paper Chromatography

Paper chromatography in analytical chemistry is a technique that we used for the identification and separation of coloured samples. In paper chromatography experiments, a mixture of substances is located and identified by the flow of a mixture of two solvents which is immiscible or partially miscible on specially designed Whatman filter paper. The solvent rises up on paper owing to capillary action. Paper chromatography experiment is used mainly for the separation or identification of amino acids, dyes, or sugars. Presently, the paper chromatography procedure is used as a teaching tool in labs due to the discovery of other chromatography methods such as thin-layer chromatography, ion-exchange chromatography. In 1944 Consden, Gordon, and Martin first introduced the paper chromatography technique.

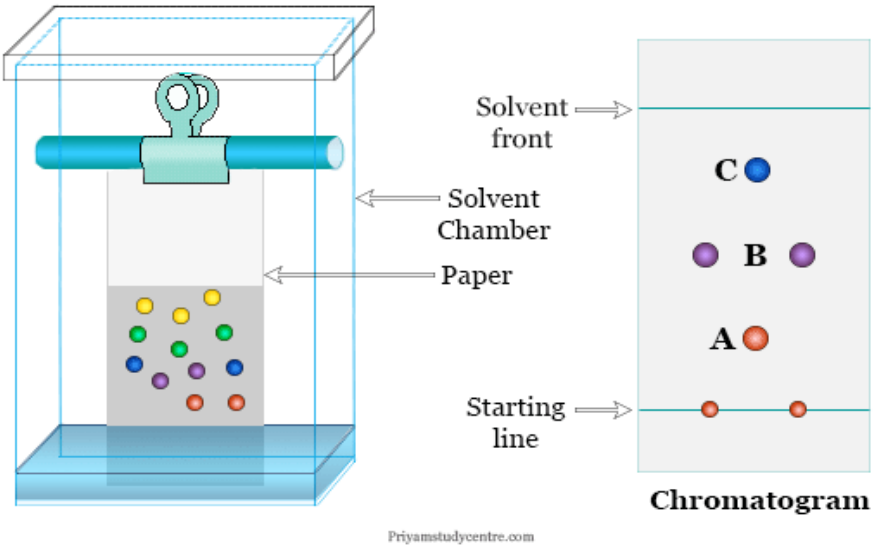


Fig.X.24 Paper chromatography.

4.7.1. Paper chromatography principle

- In paper chromatography experiments, a drop of mixed solution is placed near one short end of rectangular filter paper by a microsyringe or capillary tube. The spot on the paper is allowed to dry.
- The spotted filter paper is suspended vertically in the solvent chamber where the spot just touches solvent levels. Simply, the filter paper below the spot dipped into the solvent.
- When the solvent front moves upward and passes the spot, it carries the different components upwards. The rates of upward capacity depend on the nature of substances, the polarity of the solvents, and the size of the molecules.
- The solvent front is allowed to move upward until it travels 3/4 of the height of the filter paper.

a. What is R_f Value?

The relative rate of movement of solute and solvent is called the R_f value. It is the ratio of the distance traveled by the sample component and the distance traveled by the solvent.R_f value is constant for a given substance under given conditions. Therefore, various components can be identified by their R_f value in paper chromatography. R_f value has no unit but varies with the change of solvent.

4.7.2. Material required in paper chromatography

- Whatman filter paper strip.
- A mixture of unknown amino acids is dissolved in 10 ml of water.
- Solution of glycine: 5 mg of glycine in 1 ml of water.
- Ninhydrin spray: 200 mg of ninhydrin is dissolved in 99 ml of n-butanol and 1 ml of acetic acid.
- Solvent: n-butanol, acetic acid, and water = 80 ml, 20 ml, and 100 ml.

4.7.3. Paper chromatography procedure

A line parallel to the short end of the chromatography paper sheet is drowned by a pencil about 10 cm apart. Two points are marked on the Whatman filter paper strip.

The mixture of amino acids is spotted on one mark with no more than 5 mm diameter. Similarly, the second mark is spotted by the standard solution of glycine. The spot is allowed to dry.

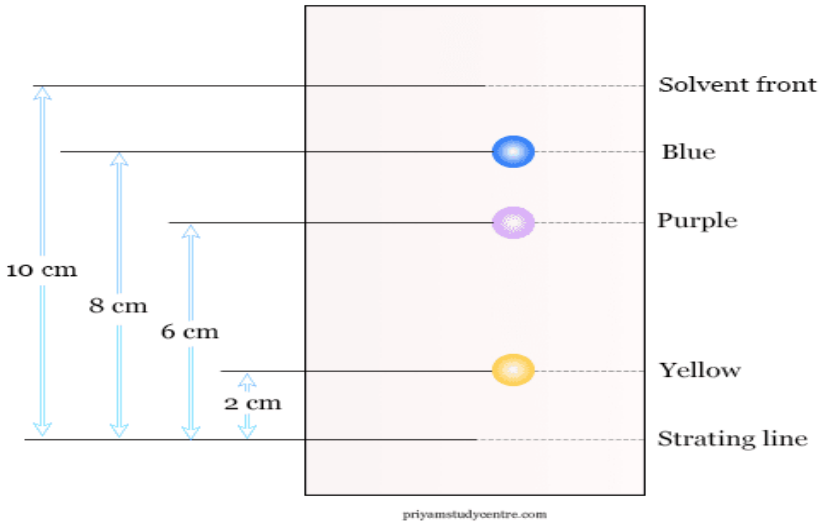


Fig.X.25 Paper chromatography.

The developing solvent is placed in a clean dry glass chamber of a paper chromatographic instrument. The glass chamber is covered with a glass plate having a hole in the middle.

The paper strip hangs from a wire hook dipped into the solution where the spots are just above the solvent front. The solvent rises owing to capillary action on the paper strip. When it almost reaches the point of suspension from the wire hook, the paper strip is carefully taken out from the chamber.

4.7.4. Uses of paper chromatography

It is used mainly for the identification of substances such as amino acids, sugars, dyes, etc. The separations by the paper chromatography principle are possible because of the continuous partitioning of substances between the aqueous phase and organic mobile phase running down the paper.

Solute migration is initiated from a small narrow spot. Surface tension is the driving force for capillary action. Therefore, a liquid-liquid partition is predominant in the mechanism of paper chromatography.

4.8. Other chromatographic techniques

4.8.1. Size exclusion chromatography

Also known as gel permeation chromatography. Separation is based on size rather than interactions. Large molecules are excluded and elute first while smaller molecules penetrate into pores and elute later. It is used for:

- Determining molecular weight distribution of polymers
- Purification of macromolecules

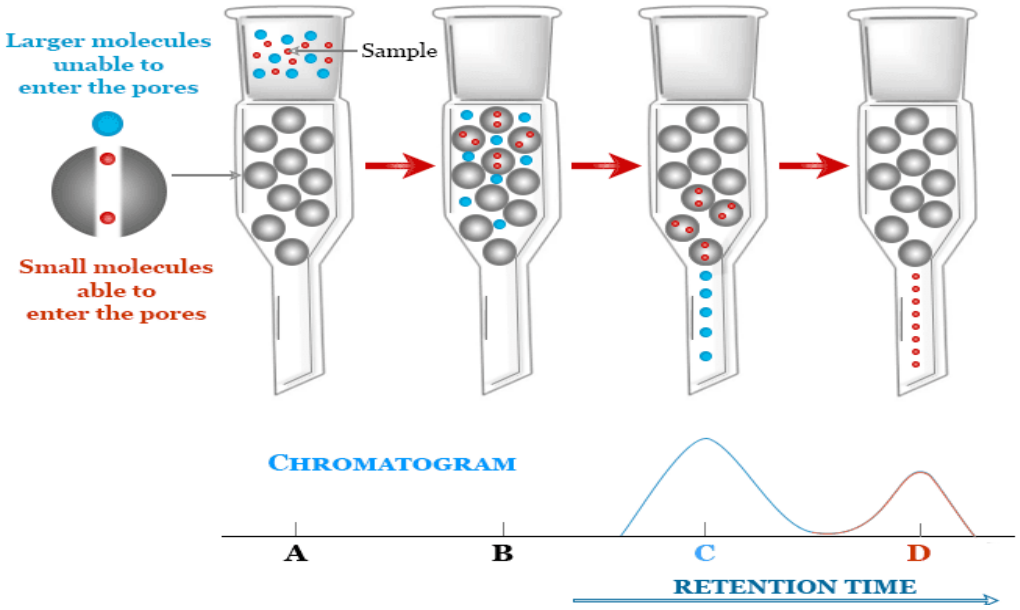


Fig.X.26 Size exclusion chromatography.

4.8.2. Ion exchange chromatography

Separation based on ionic interactions between sample ions and oppositely charged stationary phase. This technique is used for:

- Determination of ionic impurities
- Purification of chiral drugs by changing buffer pH and salt concentration

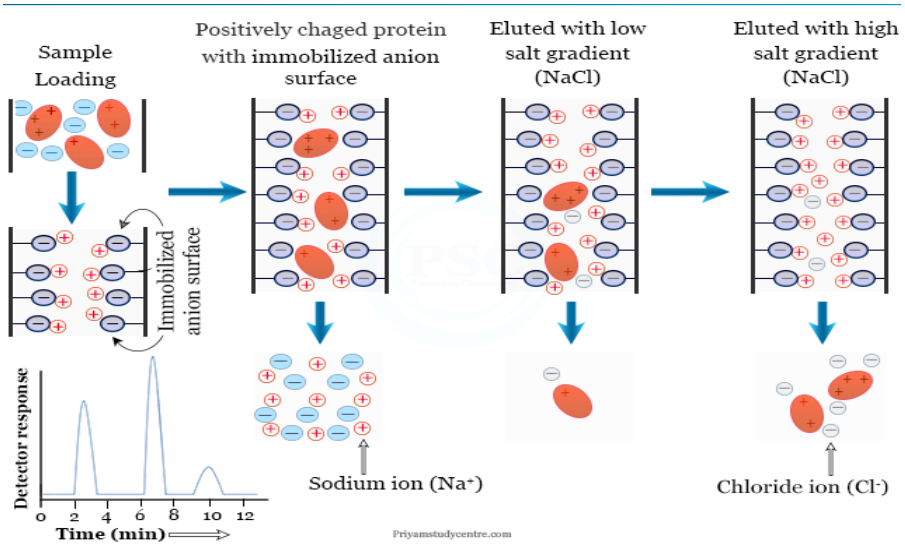


Fig.X.27 Ion Exchange Chromatography.

4.8.3. Super critical fluid chromatography

This technique uses supercritical carbon dioxide as mobile phase instead of liquids. The advantages of this method are:

- ❖ Higher diffusivity and lower viscosity for better separation
- ❖ Greens chemistry technique
- ❖ Used for analysis of thermally labile compounds

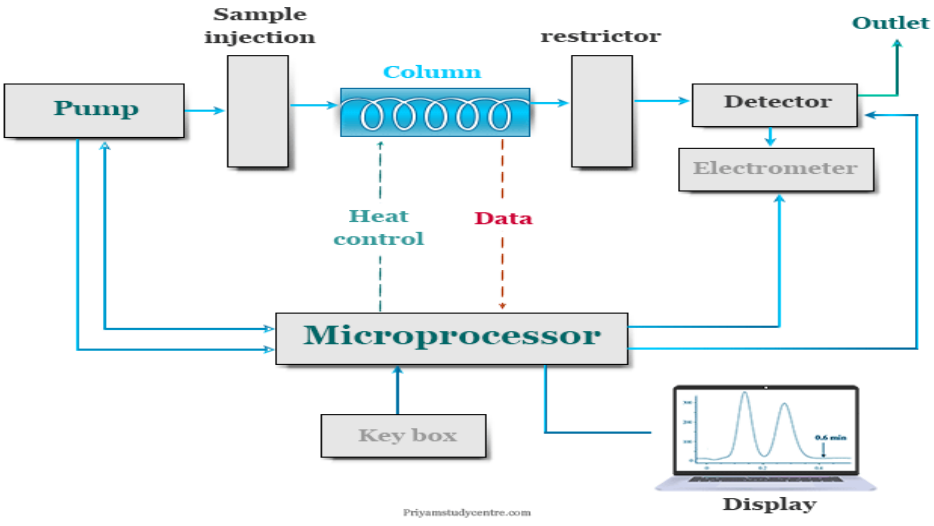


Fig.X.28 Super critical fluid chromatography.

4.8.4. Chiral chromatography

This chromatographic technique uses Enantioselective separation of chiral drugs using chiral stationary phases or chiral mobile phases. It is used for:

- Enantiomeric purity testing of chiral drugs
- Separation of enantiomers for development of single enantiomer drugs

These chromatographic techniques offer various separation mechanisms and advantages that complement HPLC and GC. They find applications in specific areas like determination of impurities, purification of samples and chiral separations which are important tasks in pharmaceutical analysis.

4.9. Method validation for chromatographic techniques

- Specificity: It is defined as the ability of method to measure analyte response in the presence of interferences. Specificity is assessed by comparing chromatograms of blank, standard and sample.
- Accuracy: Accuracy is closeness of test results to the true value. It is determined by recovery studies at multiple concentration levels.
- Precision: Precision is the degree of reproducibility of test results under normal operation. It is assessed by repeatability and intermediate precision.
- Limit of Detection (LOD) and Quantification (LOQ): The smallest concentration that can be reliably detected is called as LOD and while that can be quantified is called LOQ by the method. It is determined from the calibration curve.
- Linearity and Range: Method's ability to obtain test results proportional to concentration within a given range. It is established by analyzing standards at multiple concentration levels.
- Robustness: Robustness is the method's capacity to remain steady with small alterations in parameters. It is assessed by deliberately changing conditions and analyzing the impact on method performance.

Thorough method validation as per regulatory guidelines is essential to demonstrate that a chromatographic technique will consistentl y provide reliable results for its intended use. The various parameters ensure the method is specific, accurate, precise and rugged enough for quantitative analysis of drugs and impurities .

6. Electrochemical methods

Electrochemical method has emerged as a critical tool in the pharmaceutical industry, offering several advantages over traditional techniques like spectrophotometry and chromatography. One of its key benefits is its high sensitivity and selectivity, which enable the detection of trace amounts of drugs, metabolites, and impurities. Unlike chromatography, which often requires extensive sample preparation and expensive solvents, electroanalytical methods operate with minimal sample volumes and offer rapid and cost-effective analysis. Additionally, electroanalysis provides real-time monitoring, which is particularly useful for therapeutic drug monitoring and point-of-care diagnostics. This chapter explores the principles, techniques, and applications of electroanalysis in pharmaceuticals.

5.1. Principles of electrochemical methods

Electrochemical methods rely on measuring electrical characteristics, including current, voltage, and charge. The analyte and electrode interaction under an applied voltage is the foundation of these approaches, as shown in **Fig.X.29**. The redox processes occurring at the electrode surface are critical for the detection and quantification of analytes.

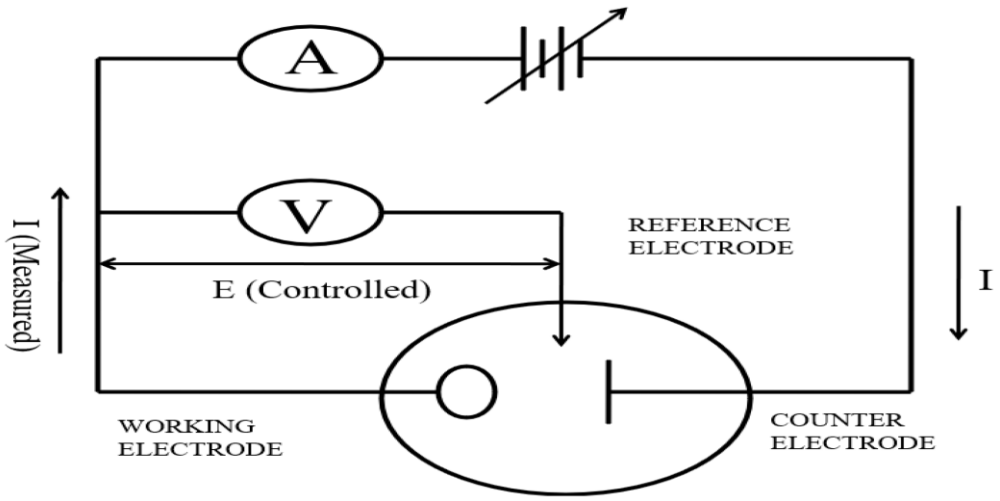


Fig X.29. A simplified circuit for cyclic voltammetry. Where A is an Ammeter to measure the current (**I**) flowing through the circuit. V is a Voltmeter used to measure and control the potential difference (**E**) between the working and reference electrodes. Working Electrode is the electrode where the electrochemical reaction of interest occurs while Reference Electrode provides a stable reference potential against which the working electrode's potential is controlled. Counter Electrode also known as the auxiliary electrode, completes the circuit and allows current to flow.

5.2. Electrochemical techniques

Electrochemical techniques are a group of analytical methods based on the measurement of electrical properties (like current, potential, or conductivity) in an electrochemical cell. They are prized for their sensitivity, selectivity, and applicability to a wide range of samples**,** from pharmaceuticals to environmental monitoring. These techniques can be broadly categorized based on what is measured and controlled.

5.2.1. Voltammetry

a. **Principle**

Voltammetry involves measuring the current that flows in an electrochemical cell as a function of an applied voltage**.** . It is well known for its sensitivity and capacity to provide extensive information on the electrochemical behavior of the analytes, including redox potentials and reaction kinetics.This category includes techniques such as differential pulse voltammetry (DPV, cyclic voltammetry (CV)and square wave voltammetry(SWV). The main distinction within voltammetry lies in how the voltage is applied.

b. **Specialized voltammetric techniques**

- **Polarography:** A historical type of voltammetry that uses a dropping mercury electrode. It is the foundation for many modern voltammetric methods.
- **Amperometry:** A subset of voltammetry where the current is measured at a single, fixed potential over time. Often used in biosensors and detection systems (e.g., glucose meters) where a stable current is proportional to concentration after an initial voltammetric characterization.

5.2.2. Potentiometry

a. **Principle**

Potentiometry involves measuring the potential (voltage) of an electrochemical cell at zero current (i.e., under conditions of no current flow). This measured potential is related to the concentration of an ion in solution.

b. **Key component: ion-selective electrodes (ISEs)**

Potentiometric measurements commonly use Ion-Selective Electrodes**,** which are designed to be sensitive to a specific ion. The most common ISE is the pH electrode, which is sensitive to H^+ ions. Ion-Selective Electrodes are crucial for measuring ion concentrations (like Na^+, K^+, Ca^{2+}, Cl^-) in pharmaceutical formulations, clinical blood tests, and environmental samples.

5.2.3. Conductometry

a. **Principle**

Conductometry measures the ability of an electrolyte solution to carry an electric current**,** known as its electrical conductivity**.** The conductivity is directly related to the total concentration of ions present. It is used for:

- Monitoring water purity.
- Tracking the progress of a reaction involving ions (e.g., titration).
- Various industrial and clinical applications where a quick measurement of total ion content is needed.

5.3. Practical Applications

Electroanalysis is an indispensable tool in the pharmaceutical industry, offering high sensitivity, selectivity, and rapid response times for various analytical applications. Its versatility allows for the precise measurement of drug compounds, impurities, and metabolic byproducts.

5.3.1. Drug discovery and development

Electroanalytical techniques aid in screening potential drug candidates by evaluating their redox properties, stability, and bioavailability, thereby accelerating the early-stage development process.

5.3.2. Quality control and assurance

Differential pulse voltammetry and related techniques enable the precise quantification of active pharmaceutical ingredients and the detection of impurities, ensuring compliance with regulatory standards.

5.3.3. Pharmacokinetics and metabolism studies

Real-time monitoring of drug and metabolite concentrations in biological fluids using amperometric biosensors provides critical data for determining optimal dosage regimens and administration routes.

5.3.4. Therapeutic drug monitoring

Electroanalytical methods facilitate personalized medicine by continuously tracking patient drug levels, optimizing treatment effectiveness, and minimizing adverse effects.

5.3.5. Environmental monitoring

The detection of pharmaceutical residues in environmental samples, such as wastewater and surface water, is crucial for assessing their ecological impact. Square wave voltammetry allows for the highly sensitive identification of trace contaminants.

5.3.6. Counterfeit drug detection

Electroanalysis offers a rapid and reliable approach for counterfeit drug detection by identifying variations in active pharmaceutical ingredients and excipients through electrochemical fingerprinting. Portable electrochemical sensors further enable on-site testing, aiding regulatory bodies and pharmacies in ensuring drug authenticity and patient safety.

CHAPTER XI: ANALYSIS AND CONTROL OF ALIPHATIC PHARMACEUTICAL PREPARATIONS

1. Objectives

By the end of this chapter, the student will be able to:

- Categorize the fundamental roles of aliphatic compounds specifically halogenated derivatives, alcohols, ethers, and urea derivatives as Active Pharmaceutical Ingredients, excipients, and impurities in pharmaceutical formulations.
- Design a strategic analytical workflow for the quality control of a pharmaceutical preparation containing aliphatic compounds, ensuring identity, potency, purity, and safety in compliance with regulatory standards.

2. Introduction

Aliphatic compounds, characterized by carbon atoms arranged in straight or branched chains, or non-aromatic rings, form a foundational class of molecules in pharmaceutical science. Unlike their aromatic counterparts with rigid, delocalized electron systems, aliphatic molecules exhibit greater conformational flexibility, which directly influences their physicochemical properties, biological interactions, and analytical behavior. This category encompasses a vast range of substances, from simple hydrocarbon gases to complex macromolecules. The analysis of these preparations is critical, as their properties can dictate drug delivery, stability, and efficacy. This chapter is dedicated to the analytical strategies for four critically important sub-classes of aliphatic pharmaceuticals: **alcohols, ethers, halogenated derivatives, and urea derivatives**.

3. Analysis of halogenated derivatives

Halogenated derivatives, organic compounds containing one or more atoms of fluorine (F), chlorine (Cl), bromine (Br), or iodine (I), hold a position of critical importance in modern pharmaceuticals. The introduction of a halogen atom is a fundamental strategy in medicinal chemistry, primarily used to modulate a drug molecule's properties. Halogens can influence lipophilicity, metabolic stability, receptor binding affinity, and overall pharmacokinetic

profile. Consequently, a vast number of blockbuster drugs, from antibiotics and antidepressants to chemotherapeutic agents, are halogenated. However, this very utility introduces significant analytical and regulatory challenges, as many halogenated compounds can be toxic, genotoxic, or persistent environmental pollutants. This chapter details the analytical strategies employed to ensure the safety, quality, and efficacy of halogen-containing pharmaceuticals.

3.1.Roles in pharmaceutical formulations

Halogenated derivatives play a dual and critical role in pharmaceutical formulations, serving both as essential Active Pharmaceutical Ingredients and as significant impurities requiring strict control. As APIs, specific halogens are strategically incorporated into drug molecules to enhance their properties: chlorine is found in antibiotics like Ciprofloxacin and antihistamines like Loratadine; fluorine, commonly used to block metabolically labile sites, is a key feature in drugs such as the antidepressant Fluoxetine, the cholesterol-lowering Atorvastatin, and the anticancer agent 5-Fluorouracil; bromine appears in antihistamines like Dexbrompheniramine; and iodine is crucial in thyroid hormones like Levothyroxine and iodinated contrast agents for medical imaging. Conversely, the analysis of these compounds is often driven by their role as impurities, including highly reactive alkyl halides (e.g., alkyl chlorides, bromides) classified as Potential Genotoxic Impurities (PGIs) that must be controlled at very low levels, halogen-containing counterions of elemental impurity catalysts (e.g., Palladium, Nickel), and various halogenated acids or other unwanted derivatives formed through hydrolysis or oxidative degradation.

3.2.Analysis methods

The analysis of halogenated compounds leverages techniques that are particularly sensitive to the presence of the halogen atom. The choice depends on the analyte's volatility, polarity, and the required level of detection.

3.2.1. Gas Chromatography

Gas Chromatography is highly effective for volatile halogenated compounds. While Flame Ionization Detection (FID) can be used, the method of choice for trace analysis is Gas Chromatography with Electron Capture Detection (GC-ECD). The ECD is exceptionally sensitive to compounds with high electron affinity, such as those containing chlorine, bromine, or iodine, offering detection limits in the parts-per-trillion range. This makes it ideal

for monitoring halogenated residual solvents like chloroform or dichloromethane. For identification, GC-MS is used, where halogens provide characteristic isotope patterns in the mass spectrum that aid in confirmation.

3.2.2. High-Performance Liquid Chromatography

HPLC is the primary technique for non-volatile halogenated APIs and their related substances. Standard UV/PDA detection is commonly employed, as many halogenated compounds have good chromophores. However, for the detection of trace-level halogenated impurities, especially Potential Genotoxic Impurities (PGIs), coupling to Mass Spectrometry (LC-MS) is essential. Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) provides the superior sensitivity and selectivity required to detect and quantify these hazardous compounds at the strict ppm or ppb levels.

3.2.3. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

It represents a technique of paramount importance for halogen-specific detection, particularly for fluorine and chlorine, which are difficult to analyze by conventional MS. In this approach, the HPLC effluent is introduced into the ICP torch, which atomizes and ionizes the elements. The mass spectrometer then detects specific ions. HPLC-ICP-MS is a powerful hyphenated technique used for "halogen screening," allowing for the sensitive and specific detection of any molecule containing a halogen atom, regardless of its structure, making it invaluable for profiling unknown halogenated degradation products.

3.2.4. Nuclear Magnetic Resonance (NMR) Spectroscopy

This method provides definitive structural confirmation. The presence of halogens can directly influence the chemical shift of nearby protons and carbons due to their electronegativity. Furthermore, ^{19}F **NMR** is an exceptionally powerful and direct probe for characterizing fluorinated pharmaceuticals, as fluorine-100% naturally abundant and gives a strong NMR signal, providing direct insight into the integrity of the fluorinated part of the molecule.

4. Analysis of alcohols and ethers

Alcohols and ethers are two fundamental functional groups in organic chemistry with immense significance in the pharmaceutical industry. They are not just common ingredients in drug substances and excipients but also potential impurities or degradation products that must be carefully monitored. The analysis of these compounds is critical for ensuring drug safety, efficacy, quality, and stability.

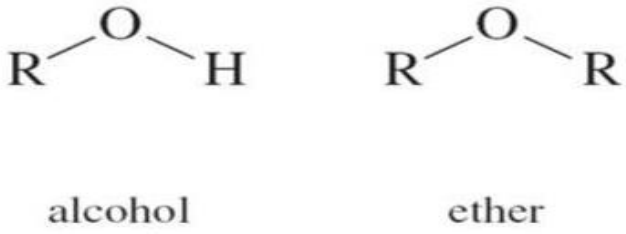


Fig.XI.1 Alcohols and ethers.

4.1. Roles in pharmaceutical formulations

4.1.1. Alcohols

Alcohols serve multiple roles in pharmaceutical preparations:

a. **Active Pharmaceutical Ingredients:** Many drugs contain alcohol functional groups.

- **Menthol:** A cyclic alcohol used in topical analgesics and decongestants for its cooling effect.
- **Cholesterol:** A steroid alcohol essential for cell membrane integrity and a precursor for steroid hormones.
- **Erythromycin:** A macrolide antibiotic containing multiple alcohol groups.
- **Salbutamol (Albuterol):** A bronchodilator containing a secondary alcohol group.

b. **Excipients (Inactive Ingredients):** Alcohols are widely used as solvents, preservatives, and co-solvents.

- **Ethanol:** Used as a solvent for APIs with poor water solubility, a preservative, and a penetration enhancer in topical products.

- **Glycerol (Glycerin):** Used as a humectant, sweetener, and solvent in syrups and elixirs.
- **Propylene Glycol:** A common solvent and stabilizer in oral, topical, and injectable formulations.
- **Benzyl Alcohol:** Used as a preservative in injectables and a local anesthetic.

c. **Impurities and degradation products:**

- **Residual Solvents:** Methanol and ethanol can be residual solvents from the synthesis of an API. Their levels are strictly controlled by ICH guidelines.
- **Degradation Products:** Oxidation of active molecules can lead to the formation of alcohols, or dehydration of alcohols can form alkenes or ethers.

4.1.2. Ethers

Ethers are less common as excipients but are crucial as APIs and synthetic intermediates.

a. Active Pharmaceutical Ingredients:

- **Diethyl Ether:** Historically used as a general anesthetic.
- **Polyethylene Glycol (PEG):** While a polyether, it's a crucial excipient. Many modern drugs, including anticancer agents, are PEGylated (covalently attached to PEG chains) to improve solubility and circulation time.

a. Excipients:

- **Polyethylene Glycol (PEG):** Used extensively as a solvent, plasticizer, lubricant, and base in suppositories and ointments.
- **Polysorbates (Tweens):** Surfactants and emulsifiers containing polyether chains, vital for stabilizing emulsions and protein-based therapeutics.

b. Impurities and degradation products:

- **Genotoxic Impurities:** Certain alkyl ethers can be potential genotoxic impurities (GTIs) formed during synthesis. For example, alkyl halides reacting with alcohols can form ethers, which must be controlled at very low levels (ppm).

- **Peroxides:** A significant hazard with low molecular weight ethers like diethyl ether. They can form explosive peroxides upon exposure to air and light. Analysis of peroxide content is critical for safety.

4.2.Analysis methods

4.2.1. Gas Chromatography

It is the premier technique for analyzing volatile alcohols and ethers. In pharmaceutical analysis, GC is indispensable for Residual Solvent Testing, where volatile impurities like methanol, ethanol, or diethyl ether must be quantified according to strict regulatory limits. This is typically performed using **Static Headspace-GC** (**Fig.X.29**), where a sample is heated in a sealed vial, and the vapor phase is injected, protecting the instrument from non-volatile matrix components. Detection is commonly achieved with a Flame Ionization Detector (FID) for robust quantification or Mass Spectrometry (GC-MS) for definitive identification of unknown volatile impurities.

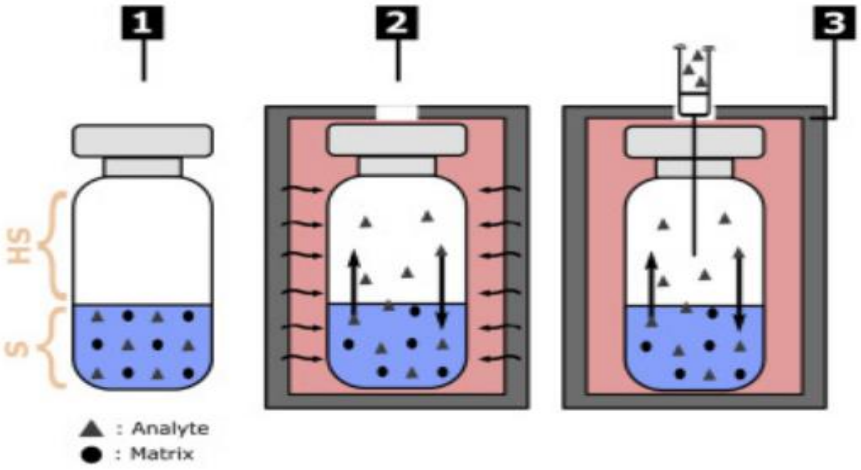


Fig.XI.1 Basic principle of static headspace sampling. 1) Sample is placed in a headspace vial. 2) Mechanical agitation and/or temperature regulation. 3) Sample collection from the headspace using a gas-tight syringe.

4.2.2. High-performance liquid chromatography

It serves as the workhorse for non-volatile, thermally unstable, or polar molecules containing alcohol or ether functional groups. This technique separates compounds based on their polarity and affinity for the chromatographic column under high pressure. HPLC is primarily

used for the assay and purity testing of Active Pharmaceutical Ingredients like Guaifenesin or Erythromycin, and for excipients like Polyethylene Glycol (PEG). A critical application is the development of stability-indicating methods, which are validated to separate and quantify the main active compound from its degradation products.

4.2.3. Fourier-transform infrared spectroscopy

It is a rapid and essential tool for identity testing, as mandated in many pharmacopoeial monographs. It identifies functional groups through their characteristic absorption of infrared light: alcohols show a broad O-H stretch (~3200-3600 cm^{-1}) and a strong C-O stretch (~1000-1260 cm^{-1}), while ethers display a C-O-C stretch (~1060-1150 cm^{-1}) and, critically, no O-H band.

4.2.4. Mass Spectrometry

It is the definitive tool for determining molecular weight and elucidating fragmentation patterns. When coupled with a separation technique like GC or LC, it acts as a powerful detector that provides both qualitative and quantitative data. In the analysis of alcohols and ethers, MS is crucial for identifying unknown impurities and degradation products. By analyzing the mass-to-charge ratio of molecular ions and their characteristic fragments—such as the loss of a water molecule from an alcohol or cleavage adjacent to the oxygen in an ether

5. Analysis of urea derivatives.

Urea derivatives represent a therapeutically vital class of aliphatic compounds characterized by the presence of the urea functional group (–N–C(=O)–N–). While urea itself (H_2N–C(=O)–NH_2) is a simple diamide of carbonic acid, its derivatives form the cornerstone of numerous major drug classes. The urea moiety is a key pharmacophore, contributing to strong hydrogen bonding, which is critical for target binding and imparting favorable physicochemical properties. The analysis of these compounds is paramount, as they can function as Active Pharmaceutical Ingredients, prodrugs, or critical impurities, each requiring specific and validated analytical methods to ensure identity, potency, purity, and safety.

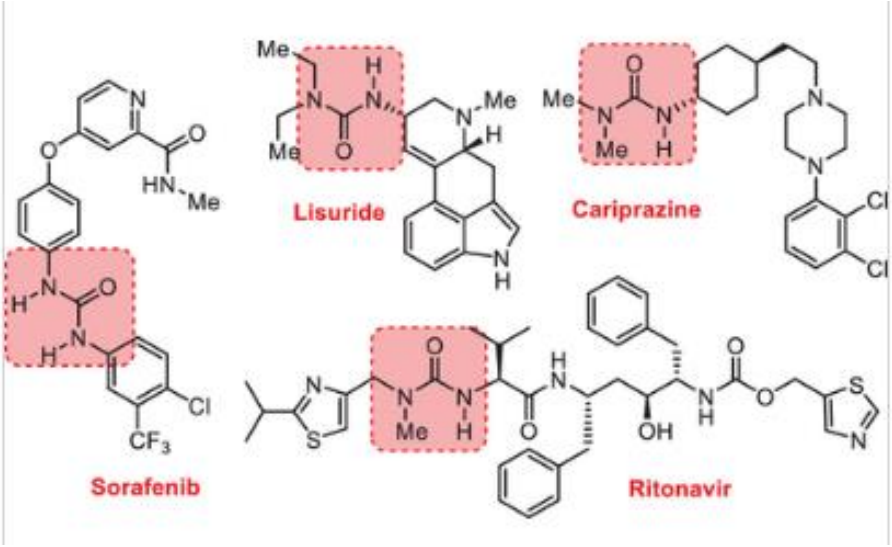


Fig.XI.2 Urea derivatives.

5.1. Major classes of urea-based pharmaceuticals

Urea derivatives are found in a wide array of therapeutic areas:

- **Anticonvulsants:** Phenytoin and its derivatives, where the urea moiety is incorporated into a heterocyclic hydantoin ring.
- **Antipsychotics / Anxiolytics:** Carbamazepine (a tricyclic compound with a urea group in a carbamazepine ring) and Buspirone.
- **Diuretics:** Acetazolamide and related compounds, which often contain a sulfonamide group in addition to the urea functionality.
- **Chemotherapeutic Agents:** Hydroxyurea, a simple yet critical derivative used in the treatment of sickle cell disease and certain cancers.
- **Dermatological Agents:** Urea itself is used in high concentrations as a keratolytic agent. Dexpanthenol is also a common derivative.

5.2. Analysis methods

5.2.1. High-performance liquid chromatography

High-Performance Liquid Chromatography serves as the primary analytical technique for urea derivatives, with Reversed-Phase (C18) chromatography being the most common mode, often employing buffered mobile phases to control ionization and enhance chromatographic peak shape. The selection of the detection system is critical and is dictated by the specific analyte's properties: Ultraviolet/Photodiode Array (UV/PDA) detection is suitable for derivatives

possessing aromatic rings or conjugated systems, such as Phenytoin and Carbamazepine, whereas Charged Aerosol Detection (CAD) or Evaporative Light Scattering (ELSD) are crucial for quantifying non-UV absorbing derivatives like urea and hydroxyurea, as these universal detectors provide a robust response independent of chromophore presence. For definitive identification, comprehensive impurity profiling, and sensitive bioanalysis, Mass Spectrometry (MS/MS) is employed due to its superior sensitivity and selectivity.

5.2.2. Gas chromatography

Limited to thermally stable and volatile urea derivatives. Often requires derivatization (e.g., silylation) to increase volatility and reduce polarity. Typically coupled with Flame Ionization (GC-FID) or Mass Spectrometry (GC-MS).

5.2.3. Fourier-transform infrared spectroscopy

Fourier-Transform Infrared Spectroscopy is a fundamental technique for the identity testing of urea derivatives, as mandated in many pharmacopoeial monographs, with identification based on their characteristic absorption bands: a broad N-H stretch around 3200-3500 cm^{-1}, a strong C=O stretch (Amide I band) around 1620-1680 cm^{-1} for the urea carbonyl, and an N-H bending band (Amide II band) around 1560-1640 cm^{-1}.

CHAPTER XII: ANALYSIS AND CONTROL OF AROMATIC PHARMACEUTICAL PREPARATIONS

1. Objectives

Upon completing this chapter, the student will be able to:

- Identify the main classes of aromatic pharmaceuticals and their key functional groups.
- Select appropriate analytical techniques for different quality control purposes.
- Explain how the chemical structure of an aromatic compound influences its analysis.

2. Introduction

Aromatic compounds, characterized by the presence of one or more benzene rings, form a cornerstone of modern therapeutics. This class encompasses a vast and diverse array of active pharmaceutical ingredients, including non-steroidal anti-inflammatory drugs (NSAIDs), local anesthetics, barbiturates, and numerous cardiovascular and psychotropic agents. The very stability and reactivity conferred by the aromatic ring make these compounds both therapeutically invaluable and analytically challenging.

The analysis and control of aromatic pharmaceutical preparations is therefore a critical discipline within pharmaceutical chemistry, dedicated to ensuring the safety, efficacy, and quality of these essential medicines. This field moves beyond simply confirming the identity of the active molecule to encompass a comprehensive assessment of its purity, stability, and final dosage form performance. This chapter is dedicated to the analytical strategies for three critically important sub-classes of aromatics pharmaceuticals: **phenol, aniline, benzoic acid, and salicylic derivatives**.

3. Analysis of phenol and aniline derivatives

Phenol and aniline derivatives constitute a foundational class of aromatic pharmaceuticals where biological activity is intrinsically linked to the aromatic ring and its substituents. The phenol functional group (–OH directly attached to the benzene ring) and the aniline functional group ($–NH_2$ attached to the benzene ring) are key pharmacophores. Phenols often exhibit

antiseptic, disinfectant, and antioxidant properties, while anilines serve as crucial synthetic precursors for many drugs, including sulfonamides and acetaminophen. Their analysis is critical as they can function as APIs, preservatives, or potentially toxic impurities and degradation products.

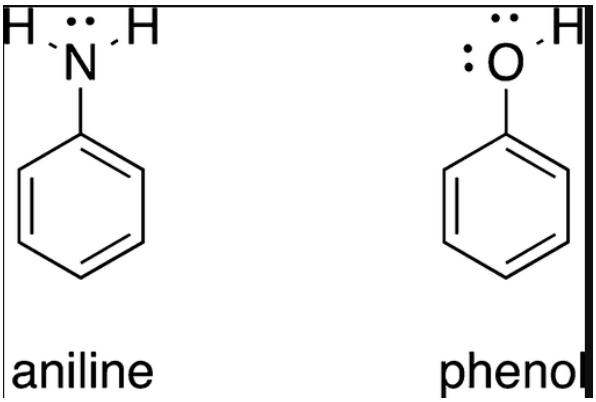


Fig.XII.1 Phenol and aniline.

2.1. Analysis techniques

2.1.1. High-performance liquid chromatography

High-Performance Liquid Chromatography with UV or Photodiode Array detection stands as the dominant technique for the assay and impurity profiling of phenol and aniline derivatives. The development of an effective HPLC method is critically dependent on the precise control of the mobile phase pH, which is used to manipulate the ionization state of the analytes' functional groups. For phenolic compounds, which are weakly acidic, employing a mobile phase with a pH significantly below their pKa (typically in the range of pH 2-4) ensures they remain in a neutral, unionized form, thereby promoting strong retention on standard reversed-phase C18 columns. Conversely, for basic aniline derivatives, using a mobile phase pH below their pKa (around pH 3-4) protonates the amino group, forming a cationic anilinium species that can improve chromatographic peak shape and retention; at a higher pH, the neutral form prevails, increasing lipophilicity. This technique is routinely applied for the accurate quantification of APIs like paracetamol (an aniline derivative) and propofol (a phenol derivative), as well as for monitoring their related substances and degradation products to ensure product stability and purity.

2.1.2. Gas chromatography-mass spectrometry

Gas Chromatography-Mass Spectrometry plays a specific role in the analysis of volatile phenol and aniline derivatives and is particularly valuable for identifying volatile impurities. Its applications include the analysis of volatile phenol-based compounds such as creosote components and certain preservatives like ortho-phenylphenol. Furthermore, GC-MS is indispensable for detecting and identifying volatile synthetic impurities or decomposition products within complex pharmaceutical matrices. A significant limitation of this technique, however, is its requirement that analytes be both thermally stable and volatile without decomposing in the injection port or column. For many less volatile phenols or anilines, this often necessitates a derivatization step to increase their volatility and thermal stability before analysis can be performed.

2.1.3. Titrimetry

Titrimetry represents a classical approach for the assay of basic aniline derivatives, leveraging their fundamental chemical properties. The principle involves non-aqueous titration, where an acidic titrant such as perchloric acid in glacial acetic acid is used to determine the purity of aniline-based APIs by exploiting their inherent basicity in a non-aqueous medium. While this method provides a robust means of quantifying the main component, its current status is that it has been largely superseded by more informative chromatographic techniques for comprehensive impurity profiling. Despite this, it remains an official compendial method for the assay of pure substance batches in various pharmacopoeias.

2.1.4. Fourier-transform infrared spectroscopy

Fourier-Transform Infrared Spectroscopy serves as a primary tool for identity testing, as mandated in many pharmacopoeial monographs. The identification is based on characteristic absorption bands unique to each functional group. For phenols, this includes a broad O-H stretching band observed between 3200-3600 cm^{-1}, alongside aromatic C=C stretches appearing near 1500 and 1600 cm^{-1}. For anilines, the spectrum typically shows N-H stretching vibrations, usually presenting as two bands in the 3300-3500 cm^{-1} region for primary amines, in addition to the same diagnostic aromatic C=C stretches.

2.1.5. Nuclear magnetic resonance spectroscopy

Nuclear Magnetic Resonance Spectroscopy is the definitive technique for structural elucidation and confirmation of phenol and aniline derivatives. In ^1H NMR spectroscopy, characteristic chemical shifts provide clear evidence of these functional groups. The phenolic -OH proton appears as a broad singlet over a wide range, typically δ 5-9 ppm, while the $-NH_2$ protons of anilines appear as a broad singlet in the δ 3-5 ppm region; both of these signals are exchangeable with deuterium from D_2O. Beyond these functional group identifiers, the pattern, chemical shifts, and coupling constants of the aromatic ring protons are highly diagnostic, allowing for the precise determination of the substitution pattern—whether ortho, meta, or para—on the benzene ring, which is crucial for confirming the identity of isomeric compounds.

4. Analysis of benzoic acid and its derivatives

Benzoic acid and its derivatives are characterized by a carboxylic acid group directly attached to a benzene ring. This structure confers both aromatic character and acidity, leading to diverse pharmaceutical applications. Benzoic acid itself is a classic preservative, while its derivatives, particularly the para-aminobenzoic acid (PABA) core, are essential for sulfonamide antibiotics and local anesthetics like procaine.

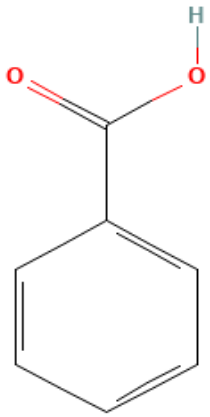


Fig.XII.2 Benzoic acid.

4.1. Analytical techniques

4.1.1. High-performance liquid chromatography with uv or photodiode array detection

serves as the principal technique for the analysis of benzoic acid derivatives. Reversed-phase chromatography, typically using a C18 column, is the standard mode of operation. A critical aspect of method development involves the use of acidic mobile phases, such as those containing 0.1% formic acid or phosphoric acid. The low pH of these mobile phases functions to suppress the ionization of the carboxylic acid group, ensuring the analyte remains in its neutral form. This suppression significantly improves chromatographic performance by enhancing peak shape and increasing retention on the hydrophobic stationary phase, leading to more robust and reproducible assays for both the active ingredient and its related substances.

4.1.2. Titrimetry

It represents a classical yet reliable method for the quantitative assay of pure benzoic acid. This approach involves a direct acid-base titration, which can be performed in either an aqueous or, more commonly, a non-aqueous medium. The method directly exploits the inherent acidity of the carboxylic acid functional group, using a standardized basic titrant to determine the substance's purity. While this technique provides accurate results for the bulk substance, it lacks the specificity to distinguish the parent acid from potential interfering impurities or degradation products, which is a key advantage of chromatographic methods.

4.1.3. Spectroscopic techniques

provide essential information for the identification and structural confirmation of benzoic acid derivatives. Fourier-Transform Infrared Spectroscopy is highly effective for identity testing, revealing characteristic functional group absorptions. For the carboxylic acid, this includes a very broad O-H stretching band in the 2500-3300 cm^{-1} region and a strong carbonyl (C=O) stretch at approximately 1690 cm^{-1}. In contrast, ester derivatives like the parabens exhibit a sharper, more distinct carbonyl stretch at a higher frequency, around 1720 cm^{-1}. For definitive structural analysis, Nuclear Magnetic Resonance Spectroscopy is employed. In a ^1H NMR spectrum, the acidic proton of the carboxylic acid group appears as a characteristically very broad singlet in the downfield region of δ ~11-12 ppm. Furthermore, the number, chemical shifts, and splitting patterns of the aromatic protons provide diagnostic information for determining the specific substitution pattern on the benzene ring.

5. Analysis of salicylic acid and its derivatives

Salicylic acid (2-hydroxybenzoic acid) is a fat-soluble organic acid that exists naturally in holly and salicylic. It is an ancient medicine with excellent exfoliation, pore cleaning ability, and high security, and the stimulation effect on the skin is lower than the fruit acid. It has an essential function in the medical esthetics profession. Salicylic acid is extensively used as a disinfectant preservative in the pharmaceutical industry. Salicylic acid can be used as a pharmaceutical intermediate to synthesize salazosulfanilamidum, aspirin, natrii salicylas, salicylamide and other sodium salicylates. Aspirin is a white crystal powder, slightly soluble in water and easily soluble in ethanol. It is a derivative of salicylic acid. Under humid acid-base conditions, aspirin is prone to degradation and generates free salicylic acid. If the residual amount of salicylic acid exceeds the limit value, patients who take it are likely to experience severe gastrointestinal irritation. As salicylic acid is one of the intermediates in the aspirin synthesis pathway, monitoring its content can effectively evaluate the completeness of the synthesis reaction and examine the control effect of the production process. Therefore, the importance of detecting salicylic acid in aspirin is crucial.

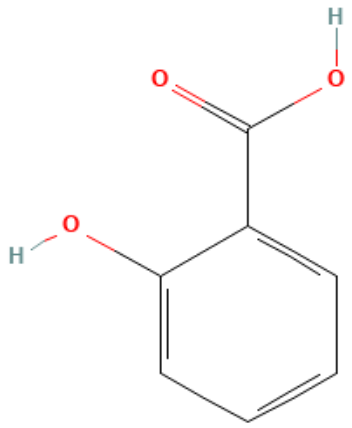


Fig.XII.3 Salicylic acid.

5.1. Analytical techniques

5.1.1. Titrimetric method

The titrimetric method is a classical approach based on the neutralization of the monobasic carboxylic acid group in salicylic acid with a standard basic solution. In a procedure based on the USP, a known, accurately weighed quantity of the sample is dissolved in a mixture of neutralized alcohol and water. This solution is then titrated with a 0.1 N sodium hydroxide (NaOH) volumetric solution, using phenolphthalein as an indicator; the endpoint is marked by

the first appearance of a faint pink color that persists for at least 30 seconds. The percentage purity is calculated using the formula: `

$$\% \text{ Purity} = (V \times N \times \text{Eq. Wt.} \times 100) / W`$$

where V is the volume of NaOH consumed in mL, N is its normality, Eq. Wt. is the equivalent weight of salicylic acid (138.12 g/mol), and W is the weight of the sample in mg.

The primary advantages of this method are its simplicity, rapidity, low cost, and robustness. However, a significant limitation is its lack of specificity, as any acidic impurity in the sample will interfere and lead to an overestimation of purity, making it unsuitable for impurity testing.

5.1.2. Spectrophotometric method

The spectrophotometric method leverages the fact that the phenolic group in salicylic acid forms a colored complex with ferric ions (Fe^{3+}), which exhibits a strong absorption maximum in the visible region around 530 nm, allowing for quantitative analysis. In practice, a sample solution is prepared by extracting the drug from its formulation, after which a dilute solution of ferric chloride ($FeCl_3$) is added to an aliquot of both the sample and standard solutions. The intensity of the developed violet color is then measured at 530 nm using a UV-Vis spectrophotometer, and the concentration of the unknown is determined by comparing its absorbance to a calibration curve prepared with salicylic acid reference standards. This method offers greater sensitivity than titration and is relatively simple, making it well-suited for quantifying low-level impurities. Its limitations include susceptibility to interference from other phenolic compounds or excipients and a sensitivity to experimental conditions such as pH, temperature, and reaction time.

5.1.3. High-performance liquid chromatography - the reference method

High-performance liquid chromatography is the reference method for this analysis, operating on the principle of separating compounds based on their differential partitioning between a stationary and a mobile phase. The most common mode is reverse-phase HPLC, which employs a non-polar stationary phase, such as a C18 column, and a polar mobile phase. Typical conditions for Aspirin impurity testing, as per the USP, involve a C8 or C18 column (250 mm x 4.6 mm, 5 µm), a mobile phase comprising a buffer and Acetonitril, a flow rate of 1.0 - 2.0 mL/min, and UV detection at 280-305 nm. HPLC is the preferred technique due to its exceptional specificity, which allows for the excellent separation of salicylic acid from

Aspirin, excipients, and other potential impurities. It also provides high sensitivity, capable of detecting salicylic acid at levels as low as 0.03% relative to Aspirin, and offers superior accuracy, precision, and automation, making it ideal for high-throughput quality control laboratories.

5.2. Practical application: HPLC analysis of salicylic acid in aspirin tablets

The practical application of HPLC for analyzing salicylic acid in Aspirin tablets involves a systematic workflow for impurity testing. It begins with standard solution preparation, where approximately 10 mg of salicylic acid reference standard is accurately weighed, dissolved, and diluted with the mobile phase to create a stock solution, which is then further diluted to a final concentration representing the specification limit. For the sample solution preparation, not less than 20 tablets are weighed and finely powdered, after which a portion equivalent to the weight of one tablet is accurately weighed, transferred to a volumetric flask, and dissolved in the mobile phase with the aid of sonication before being diluted to volume and filtered. The chromatographic analysis then proceeds with system suitability tests performed by injecting the standard solution to ensure the system meets performance criteria; following this, the standard and sample solutions are injected separately.

The percentage of salicylic acid in the sample is calculated using the formula:

$$\% \text{ Salicylic Acid} = (A_u / A_s) \times (C_s / C_u) \times 100`$$

where `A_u` and `A_s` are the peak areas of salicylic acid from the sample and standard injections, respectively, and `C_s` and `C_u` are the concentrations of the standard and sample preparations, respectively.

Chapter XIII: Analysis and Control of Heterocyclic Pharmaceutical Preparations

1. Objective

This chapter aims to:

- To identify and apply appropriate analytical techniques for the characterization, identification, and purity assessment of heterocyclic pharmaceuticals, with a focus on single-heteroatom systems and pyridine derivatives.
- To establish control strategies that ensure the safety, efficacy, and quality of heterocyclic drug preparations

2. Introduction

Heterocyclic compounds, also known as heterocycles, are cyclic compounds in which the ring members contain atoms of at least two different elements. Heterocycles are regarded as one of the most prominent and diverse class of compounds. They are amongst the largest and most varied families of organic compounds. The reason for such variety may be explained by the relatively simple requirements that must be met for classification as a heterocycle. The enormous degree of structural variety amongst heterocycles can be traced back to different factors including number of atoms in the ring, ring saturation or unsaturation, the type of heteroatoms in the ring, presence of multiple heteroatoms in the ring, structural isomers, and if the ring is fused or shares its atoms with another ring. Inorganic heterocycles, *e.g.* bicyclo[3.3.1]tetrasiloxane, are less common. The most frequently reported non-carbon atoms in heterocycles are nitrogen, oxygen, and sulfur.-It has been estimated that nitrogen heterocycles are present in 59–82% of small molecule drugs-

Due to their frequent presence within drug active substances, there is substantial interest in heterocycles. As heterocycles are so common across all drugs, they are seen in medicines that treat a wide range of therapeutic areas. The curiosity in heterocycle containing compounds is because they are not limited to one or two diseases or disorders, and they are present in treatments for a range of diseases such as cancer, infections, hormone disorders, and cardiovascular diseases.

3. Common structural types of heterocycles

The heterocycles with the structures analogous to that of benzene but with a heteroatom replacing at least one carbon atom of the benzene ring are called aromatic heterocycles, for example, pyridine. There are other analogous heterocycles where more than one carbon atom of the benzene ring are replaced by heteroatoms, for example, pyridazine, pyrimidine, pyrazine, 1,2,3-triazine, 1,2,4-triazine, 1,3,5-triazine, and 1,2,4,5-tetrazine.

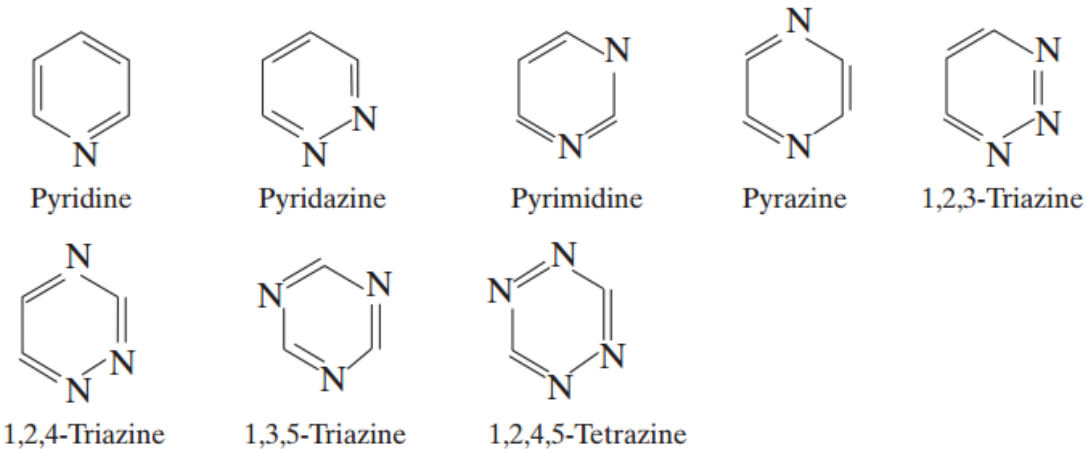


Fig.XIII.1 Heterocycles.

All of the above heterocycles are six-atom, six-π-electron aromatic heterocycles.

4. Analysis and control of heterocyclic pharmaceutical preparations with a single heteroatom

The analysis and control of heterocyclic pharmaceutical preparations containing a single heteroatom are fundamental to ensuring drug safety and efficacy, as these rings typically incorporating nitrogen, oxygen, or sulfur are pivotal for drug-receptor binding and defining key physicochemical properties.

A comprehensive analytical strategy employs a suite of techniques; chromatographic methods like HPLC are the workhorse for separating the active ingredient from its impurities and assessing potency, while spectroscopic tools such as Mass Spectrometry and NMR provide definitive structural confirmation and identity of the heterocyclic system. This analytical data directly informs the control strategy, which involves establishing strict specifications for the drug substance and final product. These specifications govern critical quality attributes like

purity, strength, and the presence of related substances, ensuring that every batch consistently meets pre-defined standards for identity, safety, and performance, despite potential challenges like polymorphism or hygroscopicity introduced by the heteroatom.

5. Analysis of pyridine derivatives

The pyridine ring is a "privileged scaffold" in medicinal chemistry, isosteric with benzene but possessing a basic nitrogen atom. This nitrogen imbues pyridine derivatives with several key properties: moderate water solubility (when protonated), the ability to form hydrogen bonds, and a defined dipole moment. Its stability and versatility have led to its incorporation into drugs across virtually all therapeutic areas, including cardiovascular, anti-infective, and anti-inflammatory medicines.

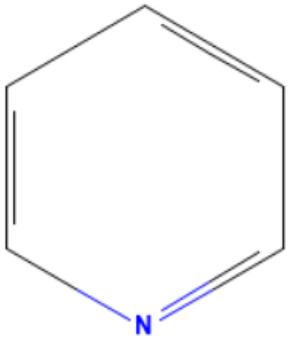


Fig.XIII.2 Pyridine.

5.1. Analytical techniques

The comprehensive analysis of pyridine derivatives relies on a suite of spectroscopic and diffraction techniques to elucidate their structure and properties. Infrared (IR) spectroscopy serves as a fundamental tool for identifying characteristic functional groups attached to the pyridine ring. The compound's electronic structure and optical properties are probed using UV-Vis absorption spectroscopy, while its fluorescent behavior is characterized through fluorescence spectroscopy; by recording emission spectra in solvents of varying polarity, researchers can interpret the effects of different substituents on the pyridine core. For definitive, unambiguous confirmation of molecular geometry and solid-state structure, single-crystal X-ray diffraction is employed, providing atomic-level resolution of the pyridine derivative. This multi-technique approach, often complemented by other methods, ensures a

full understanding of the identity, purity, and photophysical characteristics of these pharmaceutically relevant molecules.

CHAPTER XIV: ANALYSIS OF ALKALOIDS

1. Objectives

Upon the completion of this chapter, the student will be able to :

- Define alkaloid.
- Perform a standard identification test for alkaloids using Dragendorff's reagent.

2. Introduction

Alkaloids are a very large class of substances with a somewhat vague definition. Traditionally alkaloids have been identified with the use of four analytical reagents:

• Mayer’s reagent, a potassiomercuric iodide solution

•Wagner’s reagent, a solution of iodine in potassium iodide

• Hager’s reagent, a picric acid solution

• Dragendorff’s reagent, a potassium bismuth iodide solution

One definition of an alkaloid is that it is a substance that gives a positive reaction to the above reagents. Another definition is given by Trease and Evans: “Typical alkaloids are derived from plant sources, they are basic, they contain one or more nitrogen atoms (usually in a heterocyclic ring) and they usually have a marked physiological action on man or animal.” From this definition, a few general properties can be expected. Owing to the nitrogen atom they are basic (alkaloids means alkalilike), and therefore they have a higher water solubility at low pH than at high pH. Besides alkaloids constitute a large group with a variety of properties. Usually alkaloids are distinguished through a classification based on the amino acid from which they are derived, for example the ornithine alkaloids. The chemical diversity of the alkaloid group can be illustrated by the structures of morphine (**Fig.XIV.1**) and hyoscyamine (**Fig**.**XIV.2**) given below. Both of them are substances used in therapy. At the present about 10 monographs make reference to alkaloids.

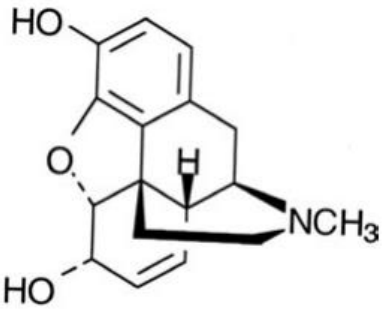


Fig.XIV.1 Morpine.

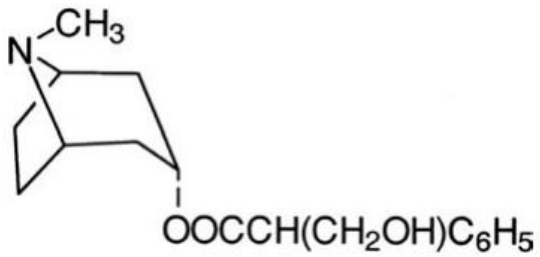


Fig.XIV.2 Hydroscyamine.

3. Identification test

The identification test of the European Pharmacopoeia makes use of the Dragendorff's reagent, which is a solution of potassium iodobismuthate, $KBiI_4$.

A test solution is prepared by dissolving a few milligrams of the substance to be examined, or the prescribed quantity, in 5 ml of water R. When dissolved, dilute hydrochloric acid R is added until an acid reaction occurs. Then 1 ml of potassium iodobismuthate solution R is added, an orange or orange-red precipitate is formed immediately.

The acid reaction occurs, as defined in the test, when methyl red changes from yellow to orange or red at about 5.1 pH or when bromothymol blue changes from blue to yellow at about 7.1 pH. This means that most tertiary and quaternary amines are found in the ionic acid form, and this allows the thereby cationic alkaloid to precipitate with anionic iodobismuthate. The salt is believed to be constituted not of monomeric entities, of for example one nitrogen base and one iodobismuthate, but of oligomeric cations and anions.

$$(alkaloid^+)_m\ (BiI_4^-)_m$$

A positive reaction is obtained with many tertiary and quaternary amines, but not with most primary and secondary amines. All the alkaloids described in monographs making reference to alkaloids are tertiary and quaternary. Obviously, alkaloids are not the only group of substances containing tertiary and quaternary amine groups, so not much specificity can be claimed.

Most of the literature dealing with the specificity of the Dragendorff,s reagent concentrates on the risk of false positive reactions by other secondary metabolites potentially present in plant

material. Here cumarines, hydroxyflavones, some triterpenes, and cardenolides could be mentioned. Even proteins can sometimes give positive reactions.

When judging the specificity of the reagent and its usefulness in relations to analysis on pure active pharmaceutical ingredients, one should keep in mind that the original purpose of the reagent was somewhat different. It was used, together with other reagents, to screen whether a plant might contain something that could prove to be an alkaloid.

EVALUATION EXERCISES

Chapter I

Choose the single best answer for each question.

1. What is the ultimate goal of the state system structure in drug quality control?

A) To increase the profitability of pharmaceutical companies.

B) To safeguard public health by ensuring drugs are safe, effective, and of high quality.

C) To reduce the time it takes for new drugs to reach the market.

D) To promote international trade of pharmaceutical products.

2. Which of the following is a set of guidelines that ensures pharmaceutical products are consistently manufactured and controlled according to quality standards?

A) ISO 9001

B) ICH Guidelines

C) Good Manufacturing Practice (GMP)

D) Pharmacopeial Standards

3. The main regulatory document that sets official quality standards for pharmaceutical substances is the:

A) International Council for Harmonisation (ICH) Guideline

B) Good Manufacturing Practice (GMP) guide

C) Pharmacopoeia

D) ISO 9001 Certificate

4. Which organization is a global body that brings together regulatory authorities and the pharmaceutical industry to harmonize technical requirements?

A) World Health Organization (WHO)

B) European Directorate for the Quality of Medicines & HealthCare (EDQM)

C) The International Council for Harmonisation (ICH)

D) Pharmacopoeial Discussion Group (PDG)

5. A pharmacopoeial monograph is best defined as:

A) A general guideline for analytical methods.

B) The standard of quality for a specific individual drug or substance.

C) A list of approved excipients.

D) A document describing manufacturing facility requirements.

6. Which of the following is a key difference between the European Pharmacopoeia (Ph. Eur.) and the British Pharmacopoeia (BP)?

A) The Ph. Eur. is published in English, while the BP is not.

B) The Ph. Eur. contains monographs for finished dosage forms, while the BP does not.

C) The BP operates in the UK alongside the Ph. Eur. and contains monographs for finished dosage forms.

D) The Ph. Eur. is mandatory, while the BP is only a recommendation.

7. The process of aligning the quality requirements of different countries' pharmacopoeias is known as:

A) Standardization

B) Validation

C) Harmonization

D) Legislation

8. Which of the following is NOT typically one of the three main areas of drug quality control implementation?

A) Identification of medicines

B) Marketing and advertising

C) Purity assessment (absence of impurities)

D) Assay (quantification of the active substance)

9. The first step in developing a new technical standard for pharmacy is:

A) Adoption by a voting committee.

B) Publication of the final document.

C) Identification of a need in a specific area.

D) Consultation with stakeholders.

10. Which of these pharmacopoeias is published every three years with frequent updates?

A) The United States Pharmacopeia (USP)

B) The Japanese Pharmacopoeia (JP)

C) The European Pharmacopoeia (Ph. Eur.)

D) The British Pharmacopoeia (BP)

11. The "Pharmacopoeial Discussion Group" is primarily concerned with:

A) Regulating drug prices in member countries.

B) Harmonizing monographs between Ph. Eur., USP, and JP.

C) Writing the ICH guidelines.

D) Inspecting manufacturing plants for GMP compliance.

12. What is the primary focus of ISO 9001 as a Quality Management System?

A) It provides specific analytical methods for drug testing.

B) It is a framework focused on customer satisfaction and continuous improvement.

C) It gives detailed rules for the safe handling of cytotoxic drugs.

D) It sets the legal limits for drug impurities.

13. In the context of pharmacopoeias, "General Chapters" typically contain information on:

A) Only monographs for active pharmaceutical ingredients (APIs).

B) The financial reports of the publishing organization.

C) Methods of analysis, reagents, and pharmaceutical technical procedures.

D) The personal details of the committee members.

14. Why is the harmonization of pharmacopoeias considered a "slow process"?

A) Because it requires agreement between independent, sovereign nations and expert bodies.

B) Because the documents are written in different languages.

C) Because there is a lack of interest from the pharmaceutical industry.

D) Because the scientific methods change too quickly.

15. Which quality system provides a framework for ensuring quality throughout the entire product lifecycle, from development to manufacturing?

A) GMP only
B) ICH Guidelines
C) Pharmacopoeial Standards only
D) ISO 9001 only

Answer

1. **B** To safeguard public health by ensuring drugs are safe, effective, and of high quality.
2. **C** Good Manufacturing Practice (GMP)
3. **C** Pharmacopoeia
4. **C** The International Council for Harmonisation (ICH)
5. **B** The standard of quality for a specific individual drug or substance.
6. **C** The BP operates in the UK alongside the Ph. Eur. and contains monographs for finished dosage forms.
7. **C** Harmonization
8. **B** Marketing and advertising
9. **C** Identification of a need in a specific area.
10. **C** The European Pharmacopoeia (Ph. Eur.)
11. **B** Harmonizing monographs between Ph. Eur., USP, and JP.
12. **B** It is a framework focused on customer satisfaction and continuous improvement.
13. **C** Methods of analysis, reagents, and pharmaceutical technical procedures.
14. **A** Because it requires agreement between independent, sovereign nations and expert bodies.
15. **B** ICH Guidelines (They cover development (QbD), stability (Q1A), impurities (Q3), etc., providing a comprehensive lifecycle framework).

Chapter II

1. According to the ISO definition provided in the chapter, "quality" is best defined as:

A) The absence of defects in a final product.

B) The ability of a product to be sold at a competitive price.

C) The set of properties and characteristics that give an entity the ability to satisfy needs.

D) Compliance with Good Manufacturing Practices (GMP).

2. The primary goal of Good Laboratory Practices (GLP) is to:

A) Regulate the marketing and sales of pharmaceutical products.

B) Ensure the quality and integrity of safety data generated during pharmaceutical research and development.

C) Replace the need for a Quality Control department in a manufacturing plant.

D) Standardize the clinical administration of drugs to patients.

3. Who has sole responsibility for the overall conduct of a study and its final report under GLP?

A) The Principal Investigator

B) The Quality Assurance Auditor

C) The Study Director

D) The Laboratory Technician

4. The European Pharmacopoeia (Ph. Eur.) is a unique reference work that provides:

A) Clinical guidelines for doctors on how to prescribe medicines.

B) Official, common quality standards for medicines and their components in signatory states.

C) Pricing information for pharmaceutical products across Europe.

D) The legal framework for patenting new drugs.

5. According to the chapter, a key role of the European Pharmacopoeia is to:

A) Fund pharmaceutical research and development.

B) Conduct inspections of manufacturing facilities.

C) Facilitate the free movement of medicines by providing common quality specifications.

D) Set the maximum retail prices for all medicines in Europe.

6. Quality Control (QC), as part of GMP, is specifically concerned with:

A) Setting the overall quality policy for the company.

B) Product design and development.

C) Sampling, specifications, testing, and release procedures.

D) Generating confidence in the supplier in contractual situations.

7. Which of the following statements best describes the relationship between Quality Assurance (QA) and Quality Control (QC)?

A) QA and QC are the same thing and the terms can be used interchangeably.

B) QC is a broader concept that encompasses all QA activities.

C) QA is a proactive, process-oriented function, while QC is a reactive, product-oriented function.

D) QA is only responsible for the final testing of the product before release.

8. Which task would typically fall under the responsibility of Quality Assurance (QA) rather than Quality Control (QC)?

A) Performing chemical testing on a raw material batch.

B) Reviewing and approving a batch record before product release.

C) Measuring the dissolution rate of a finished tablet.

D) Calibrating a laboratory HPLC instrument.

9. According to the definitions in the chapter, which concept is the *broadest* and incorporates GMP plus other factors like product design?

A) Quality Control

B) Quality Assurance

C) Good Laboratory Practices

D) The European Pharmacopoeia

10. The "Marketing Authorization (MA) dossier" is described as a product's identity card because it:

A) Contains the chemical structure of the active ingredient.

B) Combines evidence of efficacy, safety, and quality to ensure the drug is reproducible.

C) Lists all the pharmacies where the drug can be sold.

D) Is written in a standardized format for all countries.

11. Which of the following is NOT listed as one of the ten fundamental chapters of GLP principles?

A) Storage and retention of records

B) Financial auditing of the study

C) Standard operating procedures

D) Test systems

12. The standards of the European Pharmacopoeia are designed to meet the needs of all the following EXCEPT:

A) Regulatory authorities

B) Pharmaceutical manufacturers

C) Hospital pharmacists for patient counseling

D) Quality control services

13. The chapter defines a "quality drug" by all the following characteristics EXCEPT:

A) Effective

B) Low-cost

C) Safe

D) Reproducible

14. A key principle of GLP is that operations can only be authorized:

A) After the study director has obtained ethical approval.

B) If they are the most cost-effective option available.

C) if all controls have been carried out and fully documented.

D) Once the final report has been written.

15. The main purpose of distinguishing between QA and QC responsibilities is to:

A) Create a clear separation so that the two departments do not communicate.

B) Ensure that quality is built into processes (QA) and verified in the product (QC).

C) Make QC responsible for all documentation and QA for all testing.

D) Assign all preventative activities to the QC department.

Answer

1. **C** The set of properties and characteristics that give an entity the ability to satisfy needs.

2. **B** Ensure the quality and integrity of safety data generated during pharmaceutical research and development.
3. **C** The Study Director
4. **B** Official, common quality standards for medicines and their components in signatory states.
5. **C** Facilitate the free movement of medicines by providing common quality specifications.
6. **C** Sampling, specifications, testing, and release procedures.
7. **C** QA is a proactive, process-oriented function, while QC is a reactive, product-oriented function.
8. **B** Reviewing and approving a batch record before product release. (This is a process assurance/auditing function).
9. **B** Quality Assurance
10. **B** Combines evidence of efficacy, safety, and quality to ensure the drug is reproducible.
11. **B** Financial auditing of the study
12. **C** Hospital pharmacists for patient counseling
13. **B** Low-cost
14. **C** if all controls have been carried out and fully documented.
15. **B** Ensure that quality is built into processes (QA) and verified in the product (QC).

Chapter III

1. According to ICH guidelines, which of the following validation parameters measures the closeness of the test result to the true value and is an indicator of the absence of systematic error (bias)?

A) Precision

B) Robustness

C) Accuracy

D) Linearity

2. In the context of method validation, a laboratory needs to assess the scattering of results around the mean value when the analysis is performed on different days by different analysts. Which type of precision is being evaluated?

A) Repeatability

B) Specificity

C) Within-laboratory (intermediate) precision

D) Reproducibility

3. The Limit of Quantitation (LOQ) is best defined as the lowest amount of an analyte that can be:

A) Detected, but not necessarily quantified, under the stated experimental conditions.

B) Quantitatively determined with acceptable precision and accuracy.

C) Distinguished from noise with a signal-to-noise ratio of 3:1.

D) Measured without interference from other components in the sample.

4. An analytical chemist is developing a new HPLC method to measure a drug in the presence of its known degradation products. The parameter that demonstrates the method's ability to measure the drug accurately without interference from these products is called:

A) Robustness

B) Linearity

C) Precision

D) Specificity

5. During the validation of an analytical procedure, a scientist makes small, deliberate changes to the mobile phase pH and column temperature to see if the results are significantly affected. The validation parameter being studied through this experiment is:

A) Accuracy

B) Robustness

C) Limit of Detection (LOD)

D) Linearity

Answer

1. C) Accuracy.
2. C) Within-laboratory (intermediate) precision.
3. B) Quantitatively determined with acceptable precision and accuracy.
4. D) Specificity.
5. B) Robustness

Chapter IV

1. What is the primary purpose of analyzing raw materials and components in a pharmaceutical complex?

A) To determine the final product's shelf life.

B) To ensure all incoming materials are suitable for use before they enter the manufacturing process.

C) To monitor and adjust the production process in real-time.

D) To confirm the identity of the finished product before it is released to the market.

2. During the manufacturing of tablets, checks for blend uniformity and tablet weight variation are performed. These are classic examples of:

A) Stability Testing

B) Raw Material Analysis

C) In-Process Controls (IPC)

D) Finished Product Testing

3. Which of the following best describes the core principle of technical analysis as stated in the chapter?

A) Quality is most efficiently tested into the product at the final stage.

B) Quality must be built into the product, and technical analysis provides the data to prove it.

C) The cost of analysis should be minimized to ensure product affordability.

D) Technical analysis is primarily an ethical requirement, with limited impact on product quality.

4. A comprehensive testing program stores finished drug products at 40°C and 75% relative humidity, testing them at intervals over two years to establish an expiration date. This is known as:

A) Raw Material Analysis

B) In-Process Control (IPC)

C) Stability Testing

D) Microbiological Testing

5. Which type of technical analysis is considered the final and comprehensive check to confirm a drug product meets all specifications before it is approved for distribution?

A) Analysis of Raw Materials

B) In-Process Controls (IPC)

C) Stability Testing

D) Finished Product Testing (Release Testing)

Answer

1. B) To ensure all incoming materials are suitable for use before they enter the manufacturing process.
2. C) In-Process Controls (IPC).
3. B) Quality must be built into the product, and technical analysis provides the data to prove it.
4. C) Stability Testing.
5. D) Finished Product Testing (Release Testing).

Chapter V

1. According to the chapter, what is a common pitfall that can jeopardize the entire analytical process?

A) Using automated sampling tools instead of manual ones.

B) Underestimating the importance of sampling and entrusting it to an unqualified person.

C) Taking samples that are too large for the available containers.

D) Performing the sampling operation too quickly.

2. A portion of a material collected directly from a bulk container according to a defined procedure is known as the:

A) Final Sample

B) Combined Sample

C) Original Sample

D) Retention Sample

3. What is the primary purpose of a "Retention Sample"?

A) To be used for the immediate application of the test procedure.

B) To be combined with samples from other batches for efficiency.

C) To be reserved for future confirmatory testing, with a size sufficient for at least two full analyses.

D) To be returned to the bulk material if the initial tests are satisfactory.

4. Which of the following is a critical rule to follow during the sampling operation itself?

A) Samples that appear uncontaminated can be returned to the bulk to avoid waste.

B) The sampling process should be focused solely on speed to maintain production efficiency.

C) Representative samples should be taken in sufficient quantity for testing, and samples should never be returned to the bulk.

D) Health and safety precautions are only necessary when sampling hazardous chemicals.

5. For which of the following reasons is it recommended that sampling from large containers be performed in a separate, closed cubicle?

A) To provide a more comfortable working environment for the sampler.

B) To reduce the risk of contaminating the sample or the bulk material, and to prevent cross-contamination.

C) To store sampling tools more efficiently.

D) To keep the sampling procedure confidential from other production staff.

Answer

1. B) Underestimating the importance of sampling and entrusting it to an unqualified person.
2. C) Original Sample
3. C) To be reserved for future confirmatory testing, with a size sufficient for at least two full analyses.
4. C) Representative samples should be taken in sufficient quantity for testing, and samples should never be returned to the bulk.
5. B) To reduce the risk of contaminating the sample or the bulk material, and to prevent cross-contamination.

Chapter VI

1. What is the core principle of destruction-based sterilization methods?

A) They physically separate microorganisms from a product using a barrier.

B) They actively destroy or inactivate microorganisms by causing irreparable damage to essential cell components.

C) They create an environment unsuitable for microbial growth by removing nutrients.

D) They work by cooling materials to temperatures that halt all microbial metabolism.

2. Which of the following is a key advantage of using an autoclave for moist heat sterilization?

A) It operates at low temperatures, making it suitable for all heat-sensitive materials.

B) The pressurized steam allows for rapid heat release and good penetration into dense materials.

C) It uses dry heat, which prevents the corrosion of metal instruments.

D) It is primarily used for the sterilization of large, enclosed spaces like rooms.

3. A pharmaceutical company needs to sterilize a batch of pre-filled plastic syringes without exposing them to high heat. Which method would be most appropriate?

A) Dry heat in a hot air oven at 160°C for 2 hours.

B) Moist heat sterilization in an autoclave.

C) Ionizing radiation (e.g., gamma rays).

D) Filtration through a 0.22 μm membrane filter.

4. What is a significant limitation of using membrane filtration as a sterilization method for certain drug products?

A) It cannot effectively remove bacterial spores.

B) It may cause shear stress that damages macromolecules like proteins.

C) It requires extremely high temperatures that can degrade the product.

D) It leaves a toxic residue on the sterilized items.

5. How do non-ionizing radiation methods, like UV light, primarily achieve their germicidal effect?

A) By generating free radicals that damage multiple cellular structures.

B) By hydrolyzing and coagulating cellular proteins.

C) By alkylating cellular DNA and proteins.

D) By destroying nucleic acids, though with limited penetration power.

Answer

1. B) They actively destroy or inactivate microorganisms by causing irreparable damage to essential cell components.
2. B) The pressurized steam allows for rapid heat release and good penetration into dense materials.
3. C) Ionizing radiation (e.g., gamma rays).
4. B) It may cause shear stress that damages macromolecules like proteins.
5. D) By destroying nucleic acids, though with limited penetration power.

Chapter VII

1. What is the primary purpose of determining the total ash value of a crude drug?

A) To measure the exact amount of active organic compounds.

B) To quantify the total inorganic mineral content, which helps confirm quality and detect adulteration.

C) To calculate the water-soluble vitamin content.

D) To determine the drug's solubility in acidic media.

2. When is the acid-insoluble ash test preferred over the total ash test?

A) When the drug has a very low total mineral content.

B) When the drug contains a high amount of calcium oxalate, which is soluble in acid.

C) When the drug is intended for parenteral administration.

D) When a rapid, less precise analysis is acceptable.

3. In the total ash determination procedure, why is the sample heated in a muffle furnace at 600°C?

A) To gently dry the sample without charring it.

B) To ensure all organic matter is completely burned off, leaving only inorganic residue.

C) To sterilize the sample before analysis.

D) To volatilize all inorganic salts for easier measurement.

4. A key advantage of the sulphated ash test over the standard total ash test is its:

A) Ability to measure volatile organic impurities.

B) Use of a lower temperature, which preserves the drug's active ingredients.

C) Superior reproducibility due to the formation of stable sulfate salts.

D) Simplicity and shorter analysis time.

5. What does the "acid-insoluble ash" primarily consist of?

A) Essential minerals like potassium and sodium.

B) Calcium oxalate crystals.

C) Silica and sand, which are considered impurities.

D) Water-soluble salts.

Answer

1. B) To quantify the total inorganic mineral content, which helps confirm quality and detect adulteration.
2. B) When the drug contains a high amount of calcium oxalate, which is soluble in acid.
3. B) To ensure all organic matter is completely burned off, leaving only inorganic residue.
4. C) Superior reproducibility due to the formation of stable sulfate salts.
5. C) Silica and sand, which are considered impurities.

Chapter VIII

1. When determining the colour of a liquid according to the pharmacopoeial procedure, how is the comparison between the test solution and the standard solution performed?

A) The solutions are compared in spectrometric cuvettes at a wavelength of 450 nm.

B) The solutions are viewed down the vertical axis of matched tubes in diffused light against a white background.

C) The solutions are heated to 50°C and the colour intensity is measured with a colorimeter.

D) The solutions are evaporated to dryness and the residue is compared.

2. What is the primary limitation of the "Loss on Drying" (LOD) method for determining water content?

A) It is not suitable for solid substances.

B) It is a selective method that only measures water.

C) It is non-selective, as any volatile material lost during drying will be measured as water.

D) It requires expensive and complex instrumentation.

3. The presence of impurities in a solid pharmaceutical substance typically has what effect on its melting point?

A) It raises the melting point and narrows the melting range.

B) It has no effect on the melting point if the impurities are organic.

C) It lowers the melting point and broadens the melting range.

D) It causes the substance to decompose before melting.

4. The formula for specific rotation is $[\alpha] = \alpha / (l \times c)$. What does the variable 'l' represent in this equation?

A) The wavelength of light used, in nanometers.

B) The mass concentration of the solution, in g/mL.

C) The length of the polarimeter tube (optical path length), in decimeters.

D) The measured optical rotation, in degrees.

5. What is the main application of refractive index measurement in pharmaceutical quality control?

A) To determine the particle size distribution of a powdered drug.

B) To establish the identity and test the purity of liquid substances.

C) To measure the water content in a hygroscopic substance.

D) To quantify the amount of active ingredient in a tablet.

Answer

1. B) The solutions are viewed down the vertical axis of matched tubes in diffused light against a white background.
2. C) It is non-selective, as any volatile material lost during drying will be measured as water.
3. C) It lowers the melting point and broadens the melting range.
4. C) The length of the polarimeter tube (optical path length), in decimeters.
5. B) To establish the identity and test the purity of liquid substances.

Chapter IX

1. What is the primary purpose of titrimetric analysis in pharmaceutical quality control?

A) To separate mixtures of different metal ions.

B) To provide a quantitative determination of an analyte based on the volume of a reagent of known concentration.

C) To identify the presence of specific functional groups through color changes.

D) To measure the unsaturation level in fats and oils.

2. In a titration, what is the term for the theoretical point where the amount of titrant added is stoichiometrically equivalent to the amount of analyte?

A) The End Point

B) The Titration Error

C) The Equivalence Point

D) The Indicator Point

3. The Saponification Value of a fat or oil provides information about:

A) The amount of free fatty acids, indicating rancidity.

B) The degree of unsaturation in the fatty acid chains.

C) The average molecular weight (or chain length) of the fatty acids.

D) The number of free hydroxyl groups present.

4. The Oxygen Flask (Schöniger) Method is primarily used for the quantitative determination of which elements in organic pharmaceuticals?

A) Carbon and Hydrogen

B) Nitrogen and Oxygen

C) Halogens and Sulfur

D) Phosphorus and Potassium

5. In complexometric titration with EDTA, what is the critical role of a metal-ion indicator like Eriochrome Black T (EBT)?

A) It acts as a buffer to maintain a constant pH throughout the titration.

B) It forms a colored complex with the metal ion that is less stable than the metal-EDTA complex.

C) It oxidizes the metal ion to a higher oxidation state for easier chelation.

D) It precipitates the metal ion to signal the end of the reaction.

6. A higher Iodine Value in a pharmaceutical oil indicates:

A) A higher average molecular weight of the fatty acids.

B) A greater proportion of free fatty acids due to hydrolysis.

C) A greater degree of unsaturation, making it more prone to oxidation.

D) A higher content of esterified glycerides.

7. The Ferric Chloride test is a classical chemical identification test used to detect the presence of:

A) Halide ions (Cl^-, Br^-, I^-)

B) Phenolic compounds

C) Carbonyl groups in aldehydes

D) Sulfate ions

8. In the context of precipitation titrations, what is the term for titrations that specifically involve silver nitrate to determine anions like chloride?

A) Complexometric Titrations

B) Redox Titrations

C) Argentometric Titrations

D) Acid-Base Titrations

9. If the Saponification Value of an oil is 190 and its Acid Value is 5, what is its Ester Value?

A) 195

B) 38

C) 185

D) 200

10. What is the key function of hydrogen peroxide (H_2O_2) in the absorption solution used for the simultaneous determination of bromine and chlorine via the Oxygen Flask Method?

A) To neutralize the acidic hydrogen halides (HBr, HCl).

B) To reduce any free halogens (Br_2, Cl_2) to halides (Br^-, Cl^-).

C) To complex with the metal ions for better absorption.

D) To initiate the combustion of the sample.

Answer

1. B) To provide a quantitative determination of an analyte based on the volume of a reagent of known concentration.
2. C) The Equivalence Point
3. C) The average molecular weight (or chain length) of the fatty acids.
4. C) Halogens and Sulfur
5. B) It forms a colored complex with the metal ion that is less stable than the metal-EDTA complex.
6. C) A greater degree of unsaturation, making it more prone to oxidation.
7. B) Phenolic compounds
8. C) Argentometric Titrations
9. C) 185 (Calculated as Ester Value = Saponification Value - Acid Value; 190 - 5 = 185.)
10. B) To reduce any free halogens (Br_2, Cl_2) to halides (Br^-, Cl^-).

Chapter X

1. According to the Beer-Lambert Law, what is the relationship between absorbance (A), concentration (C), and path length (l)?

A) A is inversely proportional to both C and l.

B) A is directly proportional to both C and l.

C) A is directly proportional to C but independent of l.

D) A is directly proportional to l but independent of C.

2. In a UV-Vis spectrophotometer, which component is responsible for isolating a narrow band of wavelengths from the broad-spectrum source?

A) The Deuterium Arc Lamp

B) The Cuvette

C) The Monochromator

D) The Photomultiplier Tube Detector

3. For a molecule to absorb infrared radiation and exhibit a peak in an IR spectrum, what must occur during the molecular vibration?

A) The vibration must cause a net change in the dipole moment of the molecule.

B) The vibration must involve the breaking of a chemical bond.

C) The molecule must be in an excited electronic state.

D) The vibration must change the molecule's color.

4. What is the primary advantage of Fourier Transform Infrared (FT-IR) spectrometry over older dispersive IR instruments?

A) It uses a prism instead of a grating, making it less expensive.

B) It measures all infrared frequencies simultaneously, drastically reducing acquisition time.

C) It does not require a background spectrum for calibration.

D) It can only analyze solid samples, simplifying the procedure.

5. In Atomic Absorption Spectroscopy (AAS), what is the specific function of the Hollow Cathode Lamp (HCL)?

A) To atomize the liquid sample into a fine mist.

B) To provide a broad spectrum of visible light for detection.

C) To emit light at characteristic wavelengths of the element being analyzed.

D) To separate the different wavelengths of light after they pass through the sample.

6. Which chromatographic technique is most suitable for the separation of a mixture based primarily on the size of the molecules?

A) Ion Exchange Chromatography

B) Size Exclusion Chromatography

C) Reverse Phase Chromatography

D) Normal Phase Chromatography

7. In High-Performance Liquid Chromatography (HPLC), what is the main purpose of the degasser?

A) To heat the mobile phase to improve separation.

B) To remove dissolved gases from the solvent to prevent baseline noise.

C) To inject the sample into the high-pressure stream.

D) To detect the separated compounds as they leave the column.

8. Gas Chromatography (GC) is particularly well-suited for the analysis of which type of compounds?

A) Thermally stable and volatile compounds.

B) Large, polar biomolecules like proteins.

C) Inorganic metal ions.

D) Compounds with high molecular weight and low volatility.

9. In Thin Layer Chromatography (TLC), what does the Rf value represent?

A) The refractive index of the solvent.

B) The ratio of the distance traveled by the solute to the distance traveled by the solvent front.

C) The retention time of the compound on the plate.

D) The polarity of the stationary phase.

10. Which electrochemical technique involves measuring the current that flows as a function of an applied voltage and is known for its high sensitivity?

A) Potentiometry

B) Conductometry

C) Voltammetry

D) Titrimetry

11. The "specific absorption" of a molecule in UV-Vis spectroscopy is due to:

A) The vibration of atoms in the molecule.

B) The excitation of electrons from a ground state to a higher energy state.

C) The rotation of the entire molecule.

D) The presence of dissolved gases in the solvent.

12. A key application of Infrared (IR) Spectroscopy in pharmaceutical analysis is:

A) Quantifying trace metal impurities in a drug substance.

B) Determining the molecular weight distribution of a polymer.

C) Identifying functional groups and confirming the identity of a compound.

D) Measuring the concentration of an active ingredient in a solution.

13. In Reverse Phase HPLC, the stationary phase is typically:

A) Polar (e.g., silica), and the mobile phase is non-polar.

B) Non-polar (e.g., C18), and the mobile phase is a polar, aqueous solvent.

C) An ion-exchange resin, and the mobile phase is a buffer.

D) A size-exclusion gel, and the mobile phase is an organic solvent.

14. What is the role of the flame or graphite furnace in Atomic Absorption Spectroscopy (AAS)?

A) To provide a source of light at specific wavelengths.

B) To convert the sample into free atoms in the gaseous state.

C) To separate the different elements in the sample.

D) To detect the amount of light absorbed.

15. Which parameter in chromatography is defined as the method's ability to distinguish the analyte from other components in the sample?

A) Accuracy

B) Precision

C) Specificity

D) Robustness

16. The main principle of Paper Chromatography involves:

A) Partitioning of the sample between a stationary liquid phase (water in paper) and a mobile liquid phase.

B) Adsorption of the sample onto a solid stationary phase like alumina.

C) Separation based on the ionic charge of the molecules.

D) Separation due to differences in molecular size.

17. Which electrochemical technique is characterized by measuring the potential (voltage) of a cell under conditions of zero current and is often used with ion-selective electrodes?

A) Voltammetry

B) Amperometry

C) Potentiometry

D) Conductometry

18. A major application of Gas Chromatography (GC) in pharmaceutical quality control is:

A) The analysis of residual solvents and volatile organic impurities.

B) The separation of enantiomers using a chiral stationary phase.

C) The determination of water content in a solid drug.

D) The identification of inorganic salts in a formulation.

19. In the context of method validation for chromatographic techniques, what does "LOD" stand for?

A) Level of Detection

B) Limit of Dilution

C) Limit of Detection

D) Linear Optical Density

20. Which spectroscopic method would be most appropriate for rapidly checking the identity of a raw material API by comparing its spectrum to a reference standard in a pharmacopoeia?

A) Atomic Absorption Spectroscopy (AAS)

B) UV-Vis Spectrophotometry

C) Fourier Transform Infrared (FT-IR) Spectroscopy

D) Gas Chromatography (GC)

Answer

1. B) A is directly proportional to both C and l. (From the equation $A = \varepsilon * C * l$)
2. C) The Monochromator
3. A) The vibration must cause a net change in the dipole moment of the molecule.
4. B) It measures all infrared frequencies simultaneously, drastically reducing acquisition time.
5. C) To emit light at characteristic wavelengths of the element being analyzed.
6. B) Size Exclusion Chromatography
7. B) To remove dissolved gases from the solvent to prevent baseline noise.
8. A) Thermally stable and volatile compounds.
9. B) The ratio of the distance traveled by the solute to the distance traveled by the solvent front.
10. C) Voltammetry
11. B) The excitation of electrons from a ground state to a higher energy state.
12. C) Identifying functional groups and confirming the identity of a compound.
13. B) Non-polar (e.g., C18), and the mobile phase is a polar, aqueous solvent.
14. B) To convert the sample into free atoms in the gaseous state.
15. C) Specificity.
16. A) Partitioning of the sample between a stationary liquid phase (water in paper) and a mobile liquid phase.
17. C) Potentiometry
18. A) The analysis of residual solvents and volatile organic impurities.
19. C) Limit of Detection
20. C) Fourier Transform Infrared (FT-IR) Spectroscopy

Chapter XI

1. A key reason for incorporating halogen atoms (like fluorine or chlorine) into an Active Pharmaceutical Ingredient (API) is to:

A) Increase the molecule's water solubility and decrease its lipophilicity.

B) Modulate its lipophilicity, metabolic stability, and receptor binding affinity.

C) Ensure the molecule will be detectable by UV spectroscopy.

D) Make the molecule more volatile for analysis by Gas Chromatography.

2. Which analytical technique is considered the premier choice for the sensitive and specific detection of trace-level Potential Genotoxic Impurities (PGIs) that are alkyl halides?

A) Gas Chromatography with Flame Ionization Detection (GC-FID)

B) High-Performance Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS)

C) Fourier-Transform Infrared Spectroscopy (FTIR)

D) Nuclear Magnetic Resonance (NMR) Spectroscopy

3. For the analysis of a residual solvent like methanol in a solid drug product, which technique is most suitable?

A) Reversed-Phase HPLC with UV detection

B) Static Headspace Gas Chromatography (HS-GC)

C) Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

D) Potentiometry with an ion-selective electrode

4. The urea functional group in pharmaceuticals like hydroxyurea is best characterized for identity confirmation by FTIR due to its characteristic absorption bands for:

A) A broad O-H stretch and a C-O stretch.

B) A C-O-C stretch and the absence of an O-H band.

C) A broad N-H stretch and a strong C=O (carbonyl) stretch.

D) A strong C≡N stretch and aromatic C-H bends.

5. Which of the following is a critical safety-related analytical test specifically associated with low molecular weight ethers like diethyl ether?

A) Testing for heavy metal contamination.

B) Determination of peroxide content.

C) Measurement of optical rotation.

D) Assay for water content by Karl Fischer titration.

Answer

1. B) Modulate its lipophilicity, metabolic stability, and receptor binding affinity.
2. B) High-Performance Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS)
3. B) Static Headspace Gas Chromatography (HS-GC)
4. C) A broad N-H stretch and a strong C=O (carbonyl) stretch.
5. B) Determination of peroxide content.

Chapter XII

1. Why is precise control of mobile phase pH critical in the HPLC analysis of phenol and aniline derivatives?

A) To prevent degradation of the C18 column.

B) To manipulate the ionization state of the functional groups and control their retention.

C) To increase the volatility of the analytes for better detection.

D) To ensure the formation of a colored complex for UV detection.

2. Which technique is considered the definitive method for structural elucidation and confirming the substitution pattern on the benzene ring of an aromatic pharmaceutical?

A) Gas Chromatography-Mass Spectrometry (GC-MS)

B) Titrimetry

C) Fourier-Transform Infrared Spectroscopy (FTIR)

D) Nuclear Magnetic Resonance (NMR) Spectroscopy

3. A key advantage of using an acidic mobile phase in the HPLC analysis of benzoic acid derivatives is that it:

A) Ionizes the carboxylic acid group, making it more water-soluble.

B) Suppresses the ionization of the carboxylic acid, improving peak shape and retention.

C) Forms a complex with the analyte, enhancing UV detection.

D) Prevents the degradation of the aromatic ring.

4. What is the primary limitation of the classical titrimetric method for assaying salicylic acid?

A) It is too expensive and complex for routine use.

B) It lacks the specificity to distinguish the acid from other acidic impurities.

C) It cannot be automated for high-throughput analysis.

D) It has poor sensitivity and cannot detect low concentrations.

5. The spectrophotometric method for salicylic acid quantification is based on the formation of a colored complex with which ion?

A) Silver ion (Ag^+)

B) Ferric ion (Fe^{3+})

C) Sodium ion (Na^{+})

D) Potassium ion (K^{+})

Answer

1. B) To manipulate the ionization state of the functional groups and control their retention.
2. D) Nuclear Magnetic Resonance (NMR) Spectroscopy
3. B) Suppresses the ionization of the carboxylic acid, improving peak shape and retention.
4. B) It lacks the specificity to distinguish the acid from other acidic impurities.
5. B) Ferric ion (Fe^{3+})

Chapter XIII

1. What is the primary reason for the enormous structural variety found among heterocyclic compounds?

a) Their exclusive presence in small-molecule drugs.

b) The strict and complex requirements for their classification.

c) Factors like ring size, saturation, type and number of heteroatoms, and ring fusion.

d) Their unique property of being isosteric with benzene.

2. According to the text, what is the estimated prevalence of nitrogen-containing heterocycles in small-molecule drugs?

a) They are less common than inorganic heterocycles.

b) They are present in 59–82% of small-molecule drugs.

c) They are found only in treatments for cancer and cardiovascular diseases.

d) Their prevalence is not specified, but they are considered "privileged scaffolds."

3. Pyridine is considered a "privileged scaffold" in medicinal chemistry. Which of the following statements does NOT describe a key property of the pyridine ring?

a) It is isosteric with benzene.

b) It possesses a basic nitrogen atom that can be protonated.

c) It is a six-atom, six-π-electron aromatic system.

d) It is highly unstable and reactive, making it challenging to incorporate into drugs.

4. A comprehensive analytical strategy for heterocyclic pharmaceuticals uses a suite of techniques. Which technique pairing is correctly matched with its primary role in analysis?

a) HPLC & NMR: Used primarily for determining the compound's fluorescent behavior.

b) Mass Spectrometry & UV-Vis: Used for definitive structural confirmation and identity.

c) HPLC & Mass Spectrometry: Used for separation/potency assessment and structural confirmation, respectively.

d) IR Spectroscopy & X-ray Diffraction: Used exclusively for assessing purity and strength of the final product.

5. Which combination of analytical techniques would be most suitable for elucidating the full photophysical characteristics (e.g., electronic structure and fluorescence) of a newly synthesized pyridine derivative?

a) HPLC and Mass Spectrometry

b) UV-Vis Absorption and Fluorescence Spectroscopy

c) Single-crystal X-ray Diffraction and IR Spectroscopy

d) NMR and Chromatographic methods

Answer:

1. c
2. b
3. d
4. c
5. b

Chapter XIV

1. According to the definition provided by Trease and Evans, which of the following is a characteristic property of a typical alkaloid?

A) It is always synthesized in a laboratory.

B) It is acidic and contains a carboxylic acid group.

C) It is basic and contains one or more nitrogen atoms.

D) It has no physiological effect on humans or animals.

2. What is the visual indication of a positive reaction in the standard alkaloid identification test using Dragendorff's reagent?

A) The formation of a blue-colored complex.

B) The immediate formation of an orange or orange-red precipitate.

C) The evolution of a gas.

D) A change in the solution from colorless to yellow.

3. Why is dilute hydrochloric acid added to the test solution before adding Dragendorff's reagent?

A) To neutralize the basicity of the alkaloid.

B) To ensure the alkaloid is in its cationic (ionized) form for precipitation.

C) To dissolve the alkaloid completely in a non-aqueous medium.

D) To degrade any potential interfering substances.

4. The Dragendorff's reagent test is most reliably positive for which types of nitrogenous compounds?

A) Primary and secondary amines.

B) Most proteins and amino acids.

C) Tertiary and quaternary amines (like most alkaloids).

D) Simple ammonium salts.

5. What is a key limitation regarding the specificity of the Dragendorff's reagent test?

A) It is too specific and often fails to detect known alkaloids.

B) It can give false positive reactions with other plant metabolites like coumarins and flavonoids.

C) It only works with synthetic pharmaceutical compounds.

D) The precipitate formed is soluble in water.

Answer

1. C) It is basic and contains one or more nitrogen atoms.
2. B) The immediate formation of an orange or orange-red precipitate.
3. B) To ensure the alkaloid is in its cationic (ionized) form for precipitation.
4. C) Tertiary and quaternary amines (like most alkaloids).
5. B) It can give false positive reactions with other plant metabolites like coumarins and flavonoids.

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EXTRAIT DU PV
DE LA REUNION ORDINAIRE DU CONSEIL SCIENTIFIQUE
Du 02/02/2026

Objet : : Expertise de polycopié pédagogique

En l'an deux mille vingt-six (2026), le lundi 02 février à 13 h 30, une réunion ordinaire du Conseil Scientifique de la Faculté des Sciences de la Matière et de l'Informatique s'est tenue dans la salle de réunion de la faculté (Bloc B).

Suite aux rapports favorables reçus de la part des experts cités ci-après concernant l'expertise du polycopié pédagogique, le CSF a prononcé favorablement pour la conformité du polycopié pédagogique en vue de préparer son professorat.

- **Auteur du polycopié** : Dr. FIZIR Meriem (MCA)
- **Intitulé du polycopié** : Drug Analysis and Control « Course Material and Evaluation Exercises »
- **Destiné aux étudiants de** : 2ème année Master Chimie Pharmaceutique.
- **Experts du polycopié** :

• REZALA Houria	MCA	UDB-Khemis Miliana
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