"ACTIVE PHARMACEUTICAL INGREDIENTS AND IMPURITY PROFILING"

Ms. Kolekar Swapnali Dadaso.
Guide- Khatik R.V

Organization- Sarasam Collage Of Pharmacy , Palshiwadi, Tal-Baramati, Dist-Pune.

- **Abstract:**

Impurity profiling of pharmaceutical substances is an essential aspects of quality control, ensuring drug safety, efficacy and compliance with regulatory standards. This study focuses on the impurity profiling of benzocaine, a local anesthetic widely used in topical and oral formulations. The analysis aimed to identify and quantify potential impurities arising from synthesis, degradation, or storage.
Highperformance liquid chromatography (HPLC), coupled with UV detection, was employed as the primary analytical technique, supported by gas
chromatographymass spectroscopy (GC-MS) and infrared spectroscopy (IR) for structural elucidation of unknown impurities. The study revealed the presence of minor impurities . Method validation confirmed accuracy, precision, linearity, and robustness within acceptable limits as per ICH guidelines. The finding underscore the importance of routine impurity profiling in maintaining the pharmaceutical quality of benzocaine and preventing potential toxicological risks associated with impurity accumulation.

- **Objectives:**

1) To understand handling and precaution of chemical and safety requirements.
2) To synthesize Benzocaine from PABA.
3) To evaluate impurity profile by chromatography.
4) To characterized Benzocaine.

Modules-1: Laboratory Safety

❖ **Introduction of laboratory safety:**

Laboratory safety practices include appropriate facilities and equipment, adequate training, Personnel protective equipment, chemical management, standard operating procedures, waste handling, signage, proper laboratory practices and safe working conditions.

❖ **Basic Safety Rules:**

1) Know locations of laboratory safety showers, eyewash stations, and fire extinguishers.
The safety equipment may be located in the hallway near the laboratory entrance.
2) Know emergency exit routes.
3) Avoid skin and eye contact with chemicals.
4) Minimize all chemical exposures.
5) No horseplay will be tolerated.
6) Assume that all chemicals of unknown toxicity are highly toxic.
7) Post warming signs when unusual hazards, hazardous materials, hazardous equipment, or other special conditions are present.

8) Avoid distracting or starling persons working in the laboratory.

9) Computers and instrumentation should be labelled to indicate whether gloves should be worn or not. Inconsistent glove use around keyboards is a source of potential contamination.

10) Laboratory coats should not be stored in office or break room as this spreads contaminates to other areas.

11) All equipment should be regularly inspected for wear or deterioration.

12) Equipment should be maintained according to the manufacture's requirements and records of certification, maintenance, or repairs should be maintained for the life of the equipment.

❖ Introduction to the hazardous chemicals and msds:

A hazardous chemicals is a substance or mixture that may pose harm to human health, facilities/property, and the environment. It will have one or more of the following characteristics: Irritants. Corrosive. Harmful to health.

❖ Example of hazardous:

a) Drug

b) Paint

c) Cosmetic

d) Cleaning chemical

e) Gas cylinder

f) Detergent.

The MSDS lists the hazardous ingredients of a product, its physical and chemical characteristics (e.g. flammability, explosive properties), its effect on human health, the chemicals with which it can adversely react, handling precautions, the type of measures that can be used to control exposure, emergency and first…

A Material Safety Data Sheet (MSDS) provides basic information on a material or chemical product. A MSDS describes the property and potential hazards of the material , how to use it safely, and what to do in an emergency.

A safety data sheet (SDS), previously called a material safety data sheet (MSDS), is a document that provides critical information about hazardous chemicals. For example given below.

❖ Main purpose of an msds:

1) The chemical's identity and ingredients.

2) Health and physical hazards.

3) Safe handling and storage procedures.

4) Emergency procedures.

❖ Introduction to Handling of Chemicals and Safety Requirements:

Chemical handling involve the safe management of substances to prevent accidents and ensure a secure working environment.

Always wear appropriate personal protective equipment (PPE) such as gloves and goggles when working with chemicals. Familiarize yourself with properties and hazard of each chemical, using Material Safety Data Sheet (MSDS) as a reference.

Store chemicals in designated areas based on compatibility, and clearly label containers. Establish emergency procedures, including access to safety equipment and exists.

❖ **Safety Requirements:**

1) Lab coats
2) Gloves
3) Masks
4) Safety glasses
5) Guards
6) Shield 7) Goggles
8) Helmets.

Ensuring safety when working with chemicals involves adhering to specific requirements. Here are key safety measures:

❖ **Ensuring safety when working with chemicals involved adhering to specific requirements. Here are key safety measures:**

1. **Personal protective Equipment(PPE)**

-Wear appropriate PPE such as gloves, goggles, lab coats, and if needed respiratory protection.

-Ensure PPE is in good condition and replace it when damaged.

2. **Training:**

-Provide comprehensive training on chemical hazards, safe handling procedures, and emergency protocols.

-Regularly update training to keep employees informed about new chemicals or procedures.

3. **Ventilation:**

-Work in well-ventilated areas, and use fume hoods when dealing with volatile substances.

-Ensure ventilation system are in good working order.

4. **Emergency Equipment:**

-Have accessible emergency equipment, including eyewash stations, safety showers, and fire extinguishers.

-Conduct regular checks to ensure emergency equipment is functional.

5. **Labels & Signs:**

-Clearly label all chemical container with the appropriate hazard information.

-Use signage to indicate hazardous areas and emergency exits.

6. **Storage:**

-Store chemicals according to compatibility guidelines.
-Use designated storage areas and cabinets, and keep them locked when not in use.

➢ Module 2: Laboratory Techniques

1) General Techniques

FILTRATION:

Filtration involves separation of a solid from a liquid by passing the liquid through a porous material. In filtration, the porous filtering material can be a piece of cloth, paper, sintered glass, asbestos and so on. Filters of various pore size are available. If a filter paper has large pores, the liquid will pass through it more easily, and the filtration will be fast. However, solid particles of small size may also pass through the filter. Therefore, choice of the method of filtration and the filtering material depends on particle size of material to be retained on the filter paper.

Filtration Techniques:

1. Vacuum filtration
2. Gravity filtration
3. Hot filtration
4. Depth filtration
5. Multilayer filtration
6. Reverse osmosis

EXTRACTION:

Extraction in chemistry is a separation process consisting of the separation of a substance from a matrix. The distribution of a solute between two phases is an equilibrium condition described by partition theory. This is based on exactly how the analyte moves from the initial solvent into the extracting solvent.

Extraction Techniques:

1) Decoction
2) Maceration
3) Supercritical fluid extraction
4) Microwave assisted extraction 5) Soxhlet extraction 6) Reflux extraction.

- ***CRYSTALLIZATION:***

Crystallization, is the process of atoms or molecules arranging into a well-defined, rigid crystal lattice in order to minimize their energetic state. The small entity of a crystal lattice is called a unit cell, which can accept atoms or molecules to grow a macroscopic crystal.

- ***RECRYSTALLIZATION:***

Recrystallization is a purification technique foe solid compounds. To perform recrystallization , an impure solid compound is mixed with hot solvent to form a saturated solution. As this solution cools, the solubility of the compound decreases, and pure crystals grown from solution.

- ***DECOLORIZATION:***

Decolorization refers to the method of removing brightly colored organic impurities from the sample mixture. The procedure is typically administered within the solution phase after the solid product and impurity are dissolved during a suitable solvent.

2. Chromatography

Chromatography is a laboratory technique for the separation of a mixture into its components. The mixture is dissolved in a fluid solvent (gas or liquid) called the mobile phase, which carries it through a system (a column, a capillary tube, a plate, or a sheet) on which a material called the stationary phase is fixed.

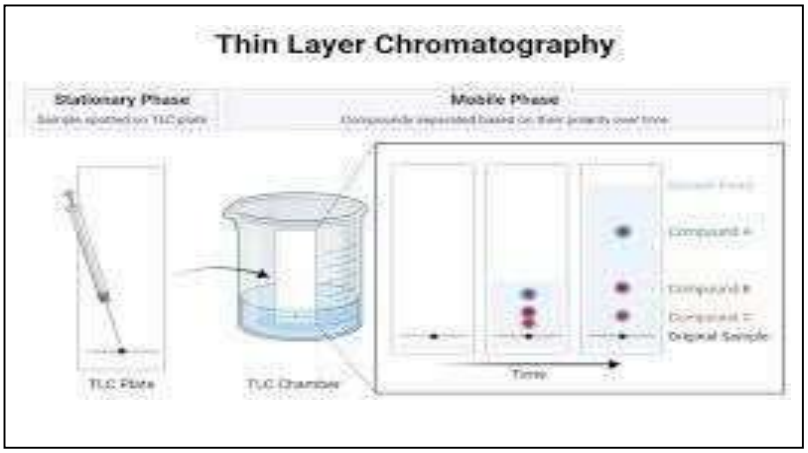
- **Types of Chromatography**

1) Thin layer chromatography:

- TLC is an example of adsorption chromatography, the stationary phase being a thin layer adsorbent held on a suitable backing. Separation of the compounds present in the plant extract depend on the difference in their adsorptive/desorptive behavior in respect of the stationary phase.

- TLC involves a thin layer of adsorbent, mixed with a binder such as CaSo4, which is spread on a glass plate and allow to dry.

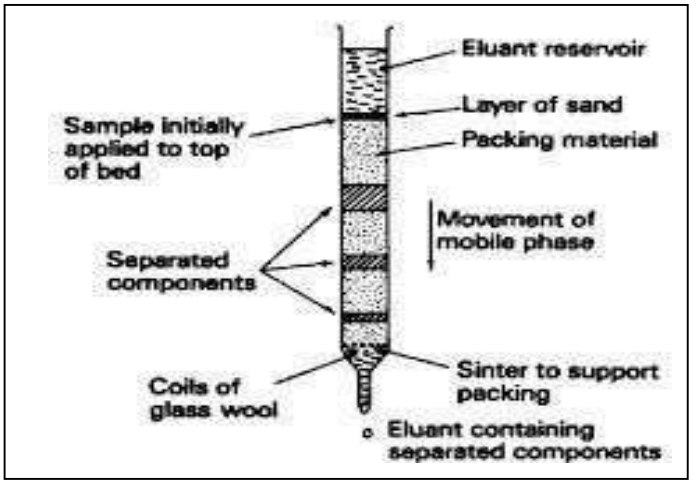
- The plant mixture to be separated is applied as a spot near the base of the plate, which is then placed is closed glass tank containing a layer of developing solvent.



2) Column Chromatography:

It is a method used to purify individual chemical compounds from mixtures of compounds the principle of separation is adsorption. The classical preparative chromatography column, is a glass tube with a diameter from 5 mm to 50 mm and a height of 5cm to 1m with a tap and some kind of filter (a glass frit or glass wool plug- to prevent the loss of stationary phase) at the bottom.

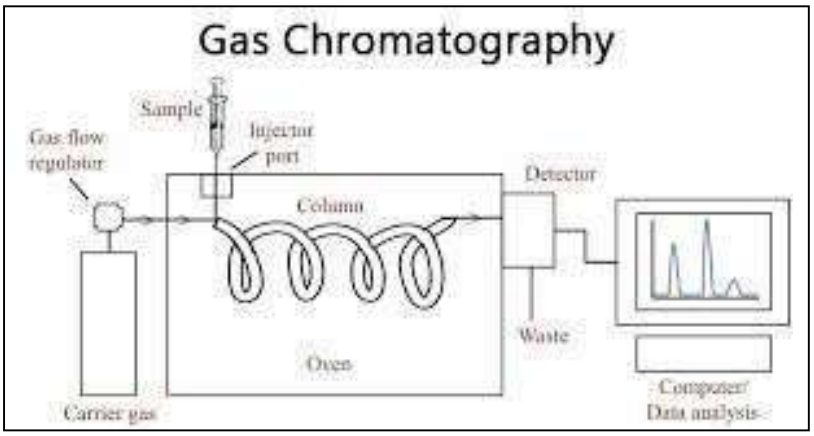
Column Chromatography



3) Gas Chromatography (GC):

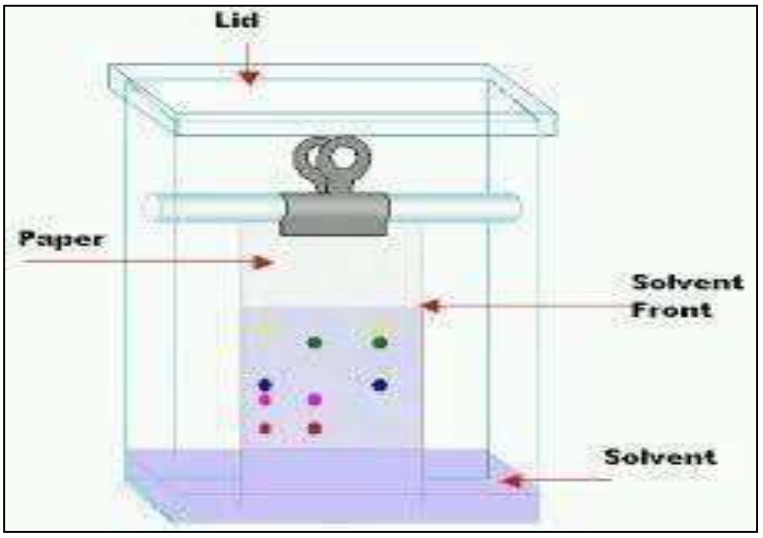
- It is an analytical technique for separating compounds based primarily on their volatilities.
- GC provides both qualitative and quantitative information for dividing compounds present in a sample.

- Compounds move through a GC column as a gases, either because the compounds are normally gases or they can be heated and vaporized into gases state.
- The differential partitioning into the stationary phase allows the compounds to be separated in time and space.



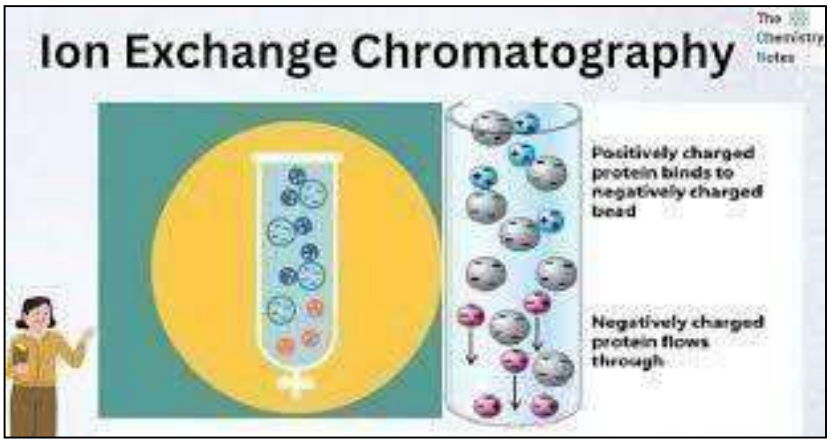
4) Paper Chromatography:

- The principle is partition mainly the stationary phase is moisture present in the cellulose fibers and mobile vary as we using. The components separated based on their solubility.
- The ratio between the distance travelled on the paper by a component of the test solution and the distance travelled by the solvent is termed the RF value. Under standard conditions, this is a constant for the particular compound. oIn practice however, variations of the RF value often occur and it is best to run a reference compound alongside the unknown mixtures.



5) Ion-Exchange Chromatography:

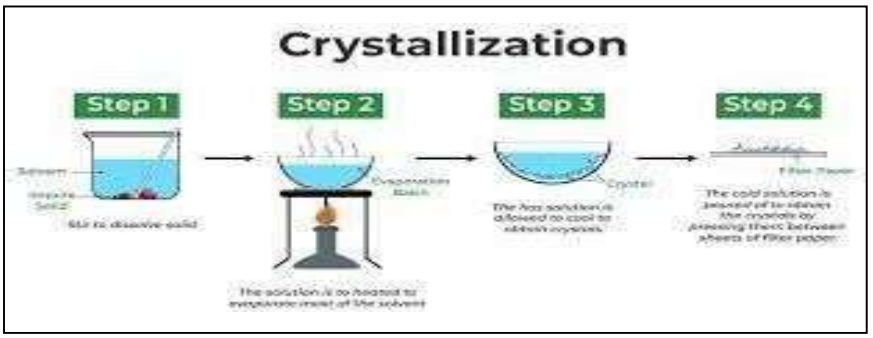
Ion-exchange chromatography allows the separation of ions and polar molecules based on their affinity to the ion exchanger. Thus, it can be used for almost any kind of charged molecule including large proteins, small nucleotides, and amino acids.



3. Crystallization

o Crystallization is the process by which solid form, where the atoms or molecules are highly organized into a structure known as crystal.

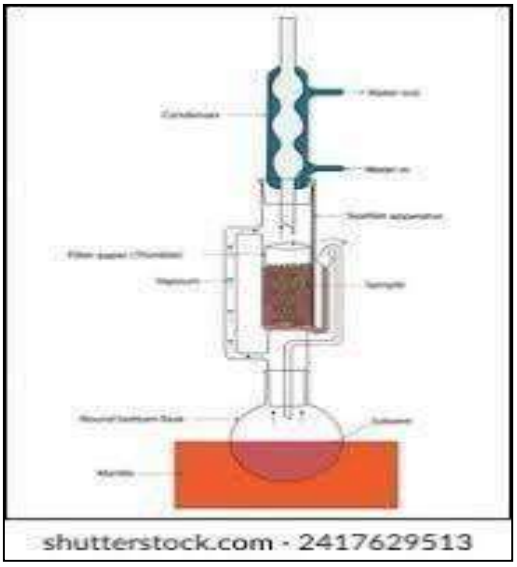
- Some ways by which crystals form are precipitating from a solution, freezing, or more rarely deposition directly from a gas. Attributes of the resulting crystals depend largely on factors such as temperature, air pressure, cooling rate, and in the case of liquid crystals, time of fluid evaporation.



4. Extraction

o Extraction in a chemistry is a separation process consisting of the separation of a substance from a matrix. The distribution of a solute between two phases is an equilibrium condition describe by partition theory. This is based on exactly how the analyte moves from the initial solvent into the solvent.

o The term washing may also be used to refer to an extraction in which impurities are extracted from the solvent containing the desired compounds.



- **Types of Extraction:**

i) Liquid-liquid extraction

a) Acid-base extraction
b) Supercritical fluid extraction

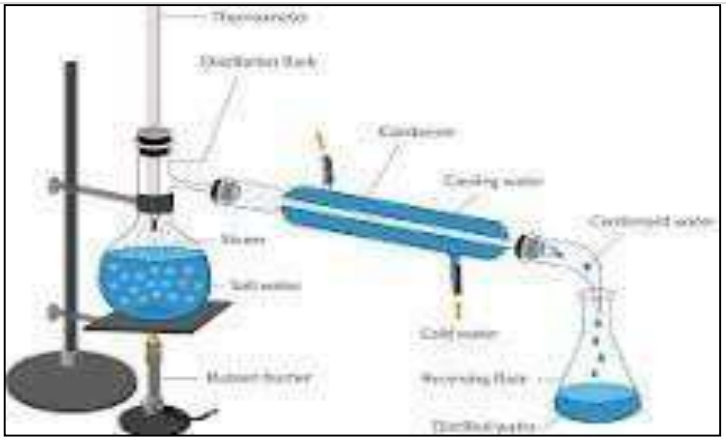
ii) Solid-Liquid extraction

a) Solid phase extraction
b) Maceration
c) Ultrasound-assisted extraction
d) Microwave-assisted extraction
e) Heat reflux extraction
f) Instant controlled pressure drop extraction (Detent instantanee controlee)

iii) Persraction.

5. Distillation

o Distillation, also classical distillation, is the process of separating the component substance of a liquid mixture of two or more chemically discrete substances; the separation process is realize by way of the selective boiling of the mixture and the condensation of the vapours in a still



Types of Distillation

1) Simple Distillation:

In simple distillation, the vapour is immediately channelled into a condenser. Consequently, the distillate is not pure but rather its composition is identical to the composition of the vapours at the given temperature and pressure.

2) Fractional Distillation:

Fractional distillation is separation of a mixture into its component parts, or fractions. Chemical compounds are separated by heating them to a temperature at which one or more fractions of the mixture will vaporize. It uses distillation to fractionate.

3) Vacuum Distillation:

Vacuum distillation or distillation under reduced pressure is a type of distillation perform under reduced pressure, which allows the purification of compounds not readily distillate at ambient pressure or simply to save time or energy. This technique separates compounds based on differences in their boiling points.

4) Steam Distillation:

Steam distillation is a separation process that consist of distilling water together with other volatile and non-volatile components. The steam from the boiling water

carries the vapour of the volatiles to a condenser, both are cooled and return to the liquid and solid state, while the non-volatile residues remain behind the boiling container.

5) Rotary Evaporation:

Rotary evaporation is the process of reducing the volume of a solvent by distributing it as a thin film across the interior of a vessel at elevated temperature and reduced pressure. This promotes the rapid removal of excess solvent from less volatile samples.

6) Other Techniques:

Melting Point:

The milting point of a substances is the temperature at which it changes state from solid to liquid. At the melting point the solid and liquid phase exist in equilibrium.

a) Boiling Point:

The boiling of a substances is the temperature at which the vapour pressure of a liquid equals the pressure surrounding the liquid and the liquid changes into a vapour.

b) Sublimation:

Sublimation is the transition of a substances directly from the solid to the gas state, without passing through the liquid state.

c) Chemical Tests:

In chemistry, a chemical test is a qualitative or quantitative procedure designed to identify, quantify, or characterise a chemical compound or chemical group.

Module 3: API Technology

1. Overview of API, API Intermediates and fine chemicals industry:

- **Defination:**

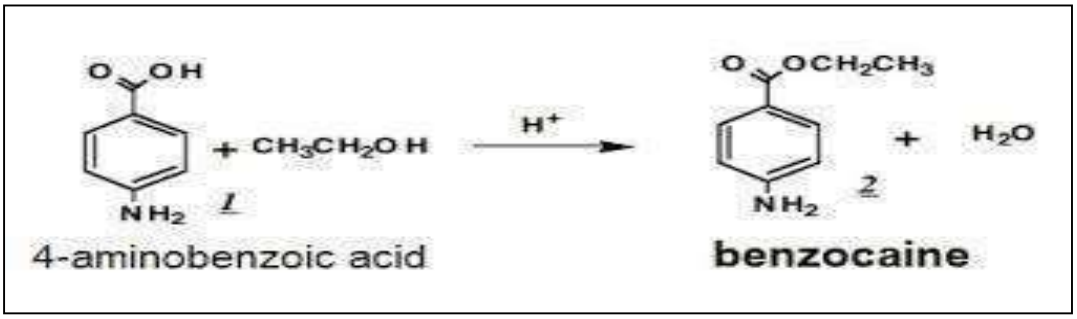
An active ingredients is any ingredient that provides biologically active or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to effect the structure or any function of the body of

humans or animals.

- **API Intermediate:**

An API intermediate is an chemically active substance that is responsible for the intended pharmacological activity of a medication. This ingredients are typically synthesized through chemical process or derived from natural sources.

- **Unit Process in Synthesis:**



- **Procedure:**

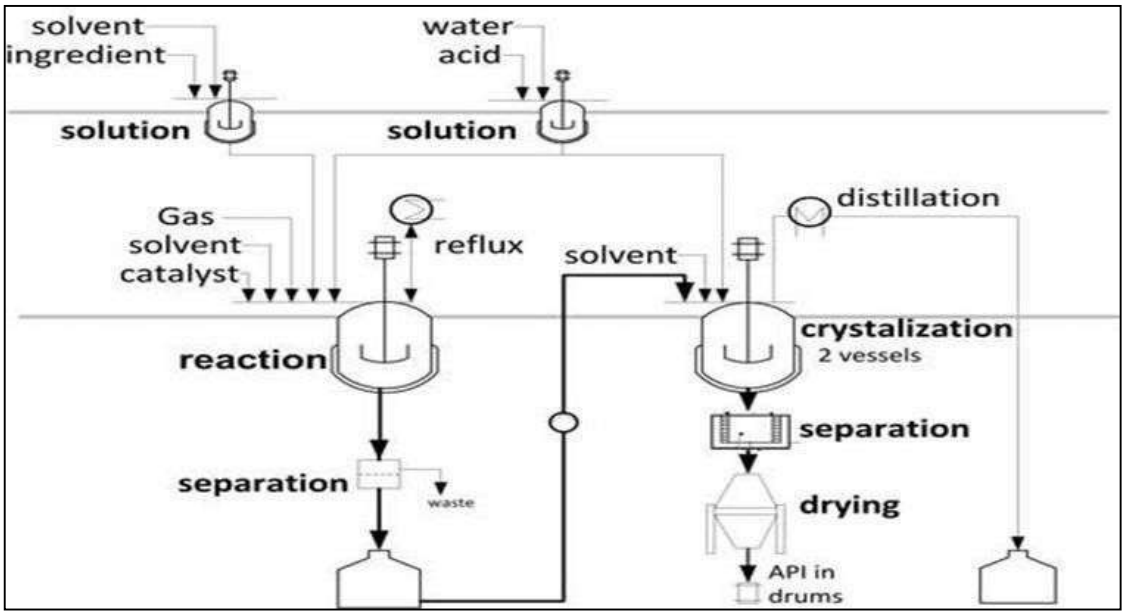
1) Clean dry 250ml round bottom flask was taken and 3gm of p-amino benzoic acid, 20ml 95% ethanol was added to it.
2) The flask was shaken thoroughly so as to dissolve the benzoic acid in ethanol.
3) The flask was cooled slightly and 3ml conc. H_2SO_4 was added.
4) The flask was attached to reflux condenser for 2 to 3 hrs.
5) The flask was cooled and reaction mixture was poured into 50ml ice cold water with constant stirring.
6) Saturated solution of sodium carbonate was added with stirring till solution became neutral.
7) As soon as solution became neutral benzocaine precipitate out.
8) The result product was filtered and was crystallized from ehanol.

3. Optimization of Organic Reactions and Process:

1) Overview
2) Method of choosing the right synthetic and scaling up root
3) Selection of reagent and raw material
4) Processing variable's impact on yield and product quality
5) Hazardous type in the manufacturing of API
6) Setup and isolation of the product
7) Scale up planning
8) Creating environmentally sustainable process
9) Reducing waste water on control

10) The kind of industrial units that pose a health risk and how to prevent them.

4. Industrial Process & Scale Up Techniques: Industrial Manufacturing Methods and Flow Charts of APIs:



5. Chirality in API Industry, Resolution of racemate, Asymmetric synthesis, few case Studies:

Drug that exhibit handedness are referred to as a chiral drugs. Chiral drug that are equimolar (1:1) mixture of enantiomers are called racemic drugs and these are obviously devoid of optical rotation. The most commonly encountered stereogenic unit , that confers chirality to drug molecules are stereogenic center.

- **Resolution of racemate:**

The process by which an optically active substance is transformed into the corresponding racemic modification is known as racemization. The converse process, by which a racemic modification is separated into the two enantiomers, is known as revolution.

- **Asymmetric Synthesis:**

Asymmetric synthesis is defined as a reaction in which a achiral unit is an assemble of substrate molecule is converted into a chiral unit in such a manner that unequal amounts of stereoisomers are produced.

6. Polymorphism in APIs:

Polymorphism is the formation of crystals for one molecule. There are several different crystal shapes for the same molecule with different crystal structure and different physical properties, such as melting points. Thus, this polymorph is different form of a solid with the molecular formula of the same compound from the crystals that are made, having different physical properties. So it can have a direct effect on the process and produce medicinal substances, such as stability of drug products, dissolution, and bioavailability.

7. APIs: Brief overview of QA/AC, GMP guideline in API manufacturing (ICH Q7,Q7A Q11)

- **Quality Assurance:**

Quality assurance can be defined as "part of quality management focused on providing confidence that quality requirement will be fulfilled." The confidence provided by quality assurance is twofold internally to management and externally to customers, government agencies, regulators, certifiers, and third parties.

- **Quality Control:**

Quality control (QC) is a procedure or set of procedures intended to ensure that a manufactured product or performed service adheres to a defined set of quality criteria or meets the requirements of the clients or customer. QC is similar to but, not identical with quality assurance (QA).

- **GMP guideline in API manufacturing:**

Good manufacturing practices (GMP) guidelines for Active Pharmaceutical
Ingredients (API) help ensure that APIs quality and purity standards. These guideline apply to all operations involve in the manufacturing of APIs including like Receiving materials, Production, Packaging, Labelling, Quality control, Release, Storage and Distribution.

- **ICH Q7:**

ICH Q7 provides guideline for good manufacturing practices for manufacturing active pharmaceutical ingredients. It is intended to help ensure APIs meet quality and purity requirements. The guideline apply to all stages of API manufacturing, including receiving, production, packaging, testing and distribution. It covers APIs made through chemical synthesis, extraction, fermentation and other processes. The guideline addressed requirements for facilities, equipment, personnel, documentation, material management, production control, validation, complaints, and recall to ensure quality management.

- **ICH Q7A:**

ICH Q7A means the good manufacturing practice guidance for active pharmaceutical ingredients developed under the auspices of the International Conference on Harmonization of Technical Requirements for Registration pf Pharmaceuticals for Human Use.

- **ICH Q11:**

The ICH Q11 general principles apply to selection of starting materials for linear or convergent synthesis. The ICH Q11 general principle should be applied independently to each branch of convergent synthesis, unless the point of convergence of braches occurs upstream of an appropriate starting material.

Module 4: Experimental Techniques

1) Purification and Impurity control APIs:

In process that use a series of chemical reaction step to synthesis the API, the removal of reaction by products, including colour bodies and metals, is critical to produce high quality of pharmaceuticals. The preferred method for removing residual metal crystals are distillation, crystallization and precipitation. A distillation collects the pure API as a distillate, leaving the non- volatile compound in a residue, while crystallization

and precipitation steps both generate solid material that can be physically removed by selecting a filtration step.

2) Biologicals (Biotechnological Products) as APIs:

A biological is as active pharmaceutical ingredients (API) produced by, derived from or based on chemical components from biological systems. Many biologics are polymers of fundamental building blocks such as sugars (e.g. polysaccharides such as LMWH or antigenic components of Pneumococcal polysaccharide vaccines), amino acids (e.g. monoclonal antibodies such as Humira and Rituxan), and nucleic acids.

3) Regulations for Biologicals:

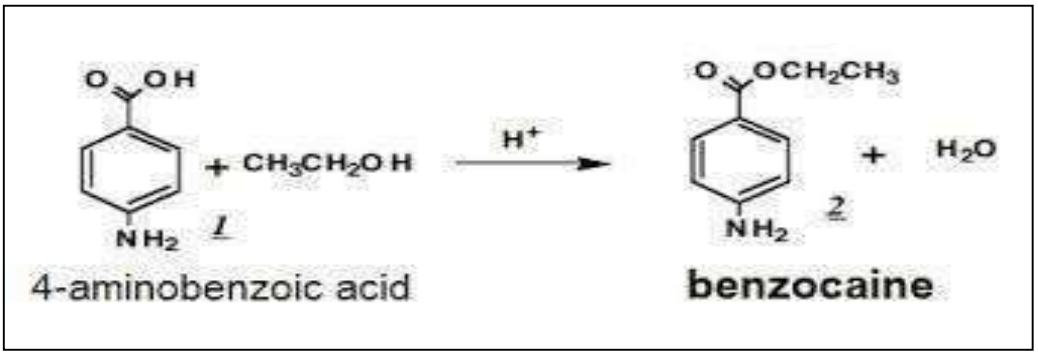
The import of drug including biologicals are regulated under the provisions of drugs and cosmetic act 1940 and drugs and cosmetic rules 1945. The import and manufacture of biologicals without a licence is an offence under the act. As such there is no separate definition of biosimilar in the act and rules, however, CDSCO and department of biotechnology published guidelines on regulation of similar biologic in 2012 which gives detailed account of regulatory exceptions for the licencing of recombinant DNA products.

4) Packaging and Repackaging of APIs:

The packaging of API automates the creation and configuration of Cloud Shell blueprint packages. Using packages, blueprints can be shared between Cloud Shell deployments and exported locally for backup purposes. With the packaging API, you can also create new blueprints and modify the setting the data model (families, models, attributes, drivers, etc.) of new or existing blueprints before packaging. This process includes repackaging high-quality APIs into new holding containers from within a clean, safe and verified Biosafety Cabinet.

Module 5: Report Writing

- **Reaction:**



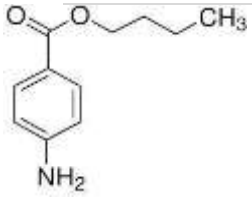
- **Procedure:**

1) Clean dry 250ml round bottom flask was taken and 3gm of p-amino benzoic acid, 20ml 95% ethanol was added.
2) The flask was shaken thoroughly so as to dissolve the benzoic acid in ethanol.
3) The flask was cooled slightly and 3ml of conc. H_2SO_4 was added.
4) The flask was attached to reflux condenser for 2 to 3 hrs.
5) The flask was cooled and reaction mixture was poured into 50ml ice cold water with constant stirring.

6) Saturated solution of sodium carbonate was added with stirring till solution became neutral.
7) As soon as solution became neutral benzocaine precipitate out.
8) The resulted product was filtered and was recrystallized from ethanol.

- **Result:** The result of synthesis of Benzocaine was found to be:

1. Theoretical yield: 3.6 gm
2. Practical yield: 2.6 gm
3. % Practical yield: 72.2%
4. Melting point: 91 ^{0}c
5. RF value: 0.34 (Mobile phase= Ethanol: Diethyl ether) (60:40)
6. Chemical name: Ethyl p-Amino benzoate
7. IUPAC name: Ethyl 4-aminobenzoate
8. Structure:



- **Outcomes:**

After the completion of report of Benzocaine, I understood

1) The handling and precaution of chemicals and safety requirements.
2) Various reactions observed in synthesis process.
3) The various reactions and their separation and purification of active pharmaceutical ingredients.
4) Biological as APIs.

- **Conclusion:**

According to domain Active Pharmaceutical Ingredients and Impurity Profiling Benzocaine is synthesized and purified recrystallization technique. Impurity profiling is done by using TLC and Melting point. As per Melting point and RF value it is found that impurities in synthesized Benzocaine found limit.

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