# East Point College of Pharmacy

East Point Campus, Jnana Prabha, Virgo
Nagar PostBengaluru – 560049, Karnataka

**Approved**
***by***
**Pharmacy Council of India, New Delhi**

**Affiliated**
***to***
**Rajiv Gandhi University of Health SciencesKarnataka**
**Bengaluru – 560041 India**

# *LAB MANUAL*

## INSTRUMENTAL METHOD OF ANALYSIS

***B. PHARM 7th SEMESTER***

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Bengaluru – 560049, Karnataka

# B Pharmacy

## Program Outcomes (PO's)

**PO 1- Pharmacy Knowledge**

Possess knowledge and comprehension of the core and basic knowledge associated with theprofession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.

**PO 2- Planning Abilities**

Demonstrate effective planning abilities including time management, resource management,delegation skills and organizational skills. Develop and implement plans and organize workto meet deadlines.

**PO 3- Problem analysis**

Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, whilesolving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions

**PO 4- Modern tool usage**

Learn, select, and apply appropriate methods and procedures, resources, and modernpharmacy-related computing tools with an understanding of the limitations.

**PO 5- Leadership skills**

Understand and consider the human reaction to change, motivation issues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and wellbeing.

**PO 6- Professional Identity**

Understand, analyse and communicate the value of their professional roles in society (e.g.health care professionals, promoters of health, educators, managers, employers, employees).

**PO 7- Pharmaceutical Ethics**

Honor personal values and apply ethical principles in professional and social contexts. Demonstrate behaviour that recognizes cultural and personal variability in values,communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions

**PO 8- Communication**

Communicate effectively with the pharmacy community and with society at large, such as,being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions

**PO 9- The Pharmacist and society**

Apply reasoning informed by the contextual knowledge to assess societal, health, safety andlegal issues and the consequent responsibilities relevant to the professional pharmacy practice.

**PO 10- Environment and sustainability**

Understand the impact of the professional pharmacy solutions in societal and environmentalcontexts, and demonstrate the knowledge of, and need for sustainable development.

**PO 11- Life-long learning**

Recognize the need for, and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-access and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

| Programme Specific Outcomes (PSO's) | |
|---|---|
| **PSO 1** | Acquire a thorough foundational knowledge in pharmaceutical sciences, including pharmacology, pharmaceutics, medicinal chemistry, and pharmacognosy, to excel in further academic pursuits |
| **PSO 2** | Gain expertise in the application of contemporary pharmaceutical techniques and technologies, enhancing employability across various sectors including the pharmaceutical industry, academia, and research institutions. |
| **PSO 3** | Equip with entrepreneurial skills and knowledge of pharmaceutical business management, including market analysis, product development, regulatory affairs, and financial planning, to initiate and run successful ventures in the pharmacy sector |

| Course Outcomes (CO's) | |
|---|---|
| **Code: BP705P Instrumental Method of Analysis** | |
| **CO 1** | Comprehend the fundamental principles underlying the interaction between matter and electromagnetic radiation, as well as various chromatographic separation techniques. Explore theirdiverse applications in drug analysis |
| **CO 2** | Perform quantitative and qualitative analysis of drugs using various analytical instrument |
| **CO 3** | Understand the chromatography separation and analysis of drugs |
| **CO 4** | To understand the knowledge through oral assessment |

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Bengaluru – 560049, Karnataka

# INDEX


| Sl. No | Experiments |
|---|---|
| 1. | Separation and identification of Amino Acids by Ascending Paper Chromatography |
| 2. | Separation and identification of Amino Acids by Radial Paper Chromatography |
| 3. | Separate and identify alkaloids by thin-layer chromatography |
| 4. | Demonstration experiment on HPLC |
| 5. | Determine $\lambda_{max}$ of $KMnO_4$ solution. |
| 6. | Determination of λ MAX of paracetamol solution by UV- Visible spectrophotometer |
| 7. | Effect of solvents on absorption maxima of deltiazem HCl |
| 8. | Estimation of salicylic acid by Colorimetry |
| 9. | Estimation of paracetamol in tablets by using standard |
| 10. | Estimation of paracetamol by using calibration curve method |
| 11. | Assay of chloramphenicol capsules by using UV- visible spectrophotometric method |
| 12. | Effect of PH on absorption maxima of paracetamol |
| 13. | Effect of solvent on absorption maxima of paracetamol |
| 14. | Estimation of Quinine sulphate by Fluorimetry |
| 15. | Estimation Of Sodium Concentration by Flame Photometry |
| 16. | Estimation Of Potassium Concentration by Flame Photometry |
| 17. | Potentiometric titration of Strong Acid Vs Strong Base |

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# LABORATORY SAFETY GUIDELINES

## Laboratory safety

When working in a chemical laboratory we handle several chemicals with more or less adverse effects to human health, and we perform experiments that have a number of potential hazards associated with them. Thus, a chemical laboratory can be a dangerous place to work in. With proper care, strictly following all precautionary measures, however, practically all accidents can be prevented.

It is the prevention of accidents and damages posed by the specialty of the chemical laboratory experiments that requires you to follow the instructor's advice as well as keep the laboratory order during work in the laboratory. You should never forget that your carelessness or negligence can threatennot only your own safety but that of your classmates working around you.

This section has guidelines that are essential to perform your experiments safely, without accidents.

## Preparation in advance

Read through the descriptions of the experiments carefully. If necessary, do study the theoretical background of the experiments from your textbook(s). After understanding, write down the outline of the experiments to be performed in your laboratory notebook. If any item is still unclear, do ask your instructor before starting the work.

Prepare your notebook before the laboratory practice. Besides description of the outline of the experiments, preliminary preparation should also include a list of the work you did prior to the start ofpractical work.

## Laboratory rules

Always wear laboratory coat and shoes in the laboratory. Sandals and open-toed shoes offerinadequate protection against spilled chemicals or broken glass.

Always maintain a disciplined attitude in the laboratory. Careless acts are strictly prohibited. Mostof the serious accidents are due to carelessness and negligence.

Never undertake any unauthorised experiment or variations of those described in the laboratory manual.

Maintain an orderly, clean laboratory desk and cabinet. Immediately clean up all chemical spills fromthe bench and wipe them off the outer surface of the reagent bottles with a dry cloth.

Drinking, or eating is not permitted during the laboratory practice. Do not bring other belongings than your notebook, stationery, and laboratory manual into the laboratory. Other properties should beplaced into the cupboard at the corridor.

Be aware of your bench neighbours' activities. If necessary, warn them of improper techniques or unsafe manipulation.

## Laboratory safety and accident protection

At the end of the laboratory session, completely clean all glassware and equipment, and clean them with a dry cloth. After putting back all your labware into your cabinet, lock it carefully. Always wash your hands with soap before leaving the laboratory.

## Handling chemicals and glassware

At the beginning of the laboratory practices, the instructor holds a short introduction when all questions related to the experimental procedures can be discussed.

Perform each experiment alone. During your work always keep your laboratory notebook at hand to record the results of the experiments you perform.

Handle all chemicals used in the experiments with great care. Never taste, smell, or touch a chemicalor solution unless specifically directed to do so.

Avoid direct contact with all chemicals. Hands contaminated with potentially harmful chemicals may cause severe eye or skin irritations.

Reactions involving strong acids, strong bases, or chemicals with an unpleasant odor should be performed under the ventilating hood. If necessary, safety glasses or goggles should be worn.

When checking the odor of a substance, be careful not to inhale very much of the material. Never hold your nose directly over the container and inhale deeply.

When experimenting, check the label on the bottle twice to make sure that you use the correct reagent. The wrong reagent can lead to accidents or "inexplicable" results in your experiments.

Do not use a larger number of reagents than the experiment calls for. Do not return any reagent to the reagent bottle! There is always the chance that you accidentally pour back some foreign substance which may react with the chemical in the bottle in an explosive manner.

Do not insert your pipette, glass rod, or spatula into the reagent bottles; you may introduce impurities that could spoil the experiment for the person using the stock reagent after you.

Always mix reagents slowly. Pour concentrated solutions slowly and stirring it continuously into water or a less concentrated solution. This is especially important when diluting concentrated sulphuric acid.

Discard waste or excess chemicals as directed by your laboratory instructor.

Using clean glassware is the basic requirement of any laboratory work. Clean all glassware with a test-tube brush and a detergent, using tap water. Rinse first with tap water and then with distilled water. If dry glassware is needed, dry the wet one in drying oven, or rinse with acetone and air dry it.

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**Experiment No: 01**

## SEPARATION AND IDENTIFICATION OF AMINO ACIDS BY ASCENDING PAPER CHROMATOGRAPHY

**Aim**: To separate and identify the given amino acids by Ascending Paper Chromatography.

**Apparatus and glassware:** Chromatographic chamber, spraying gun, capillary tubes and Whatman-grade filter paper

**Chemicals:**

**Solvent System**: N-butanol, Acetic acid and Water

**Visualizing Agent**: Ninhydrin solution

**Standard references**: Amino acids

**Principle**:

The principle involved is **partition**, where the substances are distributed or partitioned between two liquid phases. One phase is the water which is held in pores of filter paper used and the other phase is that of the mobile phase which moves over the paper. The compounds in the mixture get separated due to differences in their affinity towards water (in the stationary phase) and mobile phase during the movement of the mobile phase under the capillary action of pores in the paper

The principle can also be adsorption chromatography between solid and liquid phases, where in the stationary phase is the solid surface of paper and the liquid phase is of mobile phase. But most of the applications of paper chromatography work on the principle of partition chromatography.e. partitioned between to liquid phases. Identification of amino acids in the given mixture is determined by the $R_f$ value

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

$R_f$ value is less than one for all compounds.

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**Preparation of solutions**:

Solution A: 0.1 gm of Methionine in required quantity of suitable solvent
Solution B: 0.1 gm of Valine in required quantity of suitable solvent
Solution C: 0.1 gm of in required quantity of suitable solvent Solution
D: unknown mixture
Mobile phase: N-butanol: Acetic acid: Water (4: 1: 5) Ninhydrin
Solution: 0.2% (200mg in 100ml of N-butanol)

**Procedure**:

a. **Preparation of mobile phase**: 40 ml of N-butanol, 10 ml of acetic acid and 50 ml of water were taken in a beaker and mixed well.
b. **Preparation of sample**:0.1 gm of ariginine,0.1 gm of valine and 0.1 gm of methionine were taken separately and dissolved in required quantity of ethanol.
c. **Application of sample**: One drop of individual sample solutions were applied to chromatographic paper with capillary tube.

**Procedure of development of chromatogram**

- Take a Whatman filter paper and draw a thin straight line of about 2cm from the bottom of the paper.
- Mark four points with equidistance on the straight line and number them.
- Prepare sample solutions and place a drop of these solutions on the straight line by using capillary tube.
- Hang the chromatographic paper in chamber containing mobile phase, Run the chromatogram till the mobile phase travels on ¾$^{th}$ of the chromatographic paper.
- Remove the paper from the chamber and mark the solvent distance with a pencil and dry in air for 15 minutes
- Now spray ninhydrin solution to the chromatogram and dry it in oven for 10-15 minutes
- Measure the distance of purple color spots from the baseline and also the distance of travel by mobile phase.
- Calculate the $R_f$ values for the identification of amino acids in the given mixture

$R_f$ = Distance traveled by solute/ Distance traveled by solvent

## Observation:

| S.No | Aminoacid | Distance travelled By amino acid | Distance travelled By solvent front | $R_f$ value |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |

## Report:

The $R_f$ values of **standard or reference samples** are: Arginine:

Valine:

Methionine:

$R_f$ Vlues of amino acids in the mixture are: 2. Based

on the $R_f$ Values, the mixture was found

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**Experiment No: 02**

## SEPARATION AND IDENTIFICATION OF AMINO ACIDS BY RADIAL PAPER CHROMATOGRAPHY

**Aim**: To separate and identify the given amino acids by Radial Paper Chromatography.

**Apparatus and glass ware:** **Chemicals:** Chromatographic chamber, spraying gun, capillary tubes and whatman grade filter paper

**Chemicals:**

**Solvent System**: N-butanol, Acetic acid and Water (**BAW System)**

**Visualizing Agent**: Ninhydrin solution

**Standard references**: Aminoacids

**Principle**:

The principle involved is partition. where in the substances are distributed or partitioned between to liquid phases. One phase is the water which is held in pores of filter paper used and other phase is that of mobile phase which moves over the paper. The compounds in the mixture get separated due to differences in their affinity towards water (in the stationary phase) and mobile phase during the movement of the mobile phase under the capillary action of pores in the paper

The principle can also be adsorption chromatography between solid and liquid phases, where in the stationary phase is the solid surface of paper and the liquid phase is of mobile phase. But most of the applications of paper chromatography work on the principle of partition chromatography

i.e. partitioned between to liquid phases. Identification of amino acids in the given mixture is determined by $R_f$ value.

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

$R_f$ value is less than one for all compounds.

**Preparation of solutions**:

- Solution A: 0.1 gm of Methionine in required quantity of suitable solvent
- Solution B: 0.1 gm of Valine in required quantity of suitable solvent
- Solution C: 0.1 gm of Methionine in required quantity of suitable solvent
- Solution D: unknown mixture
- Mobile phase: N-butanol: Acetic acid: Water (4: 1: 5)
- Ninhydrin Solution: 0.2% (200mg in 100ml of N-butanol)

**Procedure**:

a. **Preparation of mobile phase**: 40 ml of N-butanol, 10 ml of acetic acid and 50 ml of water were taken in a beaker and mixed well.

b. **Preparation of sample**:0.1 gm of arginine,0.1 gm of valine and 0.1 gm of methionine were taken separately and dissolved in required quantity of ethanol.

c. **Application of sample**: One drop of individual sample solutions were applied to chromatographic paper with capillary tube.

**Procedure of development of chromatogram**

Take a Whatman filter paper and draw a round circle in the middle of paper and make a wick exactly in the centre of the circle.

- Mark four points with equidistance on the circle line and number them.
- Prepare sample solutions and place a drop of these solutions on the points using capillary tube.
- Dip the wick of chromatographic paper in chamber containing mobile phase.
- Run the chromatogram till the mobile phase travels ¾$^{th}$ of the chromatographic paper.
- Remove the paper from the chamber and mark the distance travelled by solvent with a pencil and dry in air for 15 minutes
- Now spray ninhydrin solution to the chromatogram and dry it in oven for 10-15 minutes
- Measure the distance of purple colour spots, from the baseline and also the distance of travelling of mobile phase.
- Calculate the $R_f$ values for the identification of amino acids in the given mixture

  $R_f$ = Distance travelled by solute/ Distance travelled by solvent

## Observation:

| S.No | Aminoacid | Distance travelled By amino acid | Distance travelled By solvent front | $R_f$ value |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |

## Report:

The $R_f$ values of **standard or reference samples** are:

Arginine:

Valine:

Methionine:

$R_f$ Vlues of amino acids in the mixture are Based on

the $R_f$ Values, the mixture was found

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**Experiment No: 03**

## SEPARATION AND IDENTIFICATION OF ALKALOIDS BY THIN-LAYER CHROMATOGRAPHY

**Aim**: To separate and identify alkaloids by thin-layer chromatography

**Apparatus and chemicals:** Chromatographic chamber, TLC plates, capillaries, dryer, spray gun

**Adsorbent**: Silica gel G

**Mobile phase:** Methanol, Ammonia

**Visualizing agent**: Dragendroff's reagent

**Standard references**: Ephedrine, Atropine and Quinine

**Principle**:

TLC is based on the principle of adsorption. The stationary phase used in TLC is adsorbents like silica gel coated onto a inert solid support such as glass plate, mobile phase is either single solvent or mixture of solvents based on the chemical nature of sample i.e. polarity. Sample should be dissolve in mobile phase. Silicagel contains some free Si-OH groups these groups form hydrogen bonds or other vanderwaal interactions with the analyte components, thus adsorption takes place. Identification of amino acids in the given mixture is determined by $R_f$ value.

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

**Preparation of solutions**:

Solution A: 0.1 g of Atropine in required quantity of ethanol Solution

B: 0.1 g of Quinine in required quantity of ethanol Solution C: 0.1 g

of A & B

Mobile phase: Methanol: Ammonia (200: 3)

Visualizing Agent: Dragendroff's reagent

**Procedure**:

a. **Preparation of mobile phase:** Take 200 ml of methanol and 3 ml of ammonia in a beaker and mix well, keep a side for 20-30 min to get saturation of mobile phase

b. **Preparation of sample:** Take 0.1 g of ephedrine, 0.1 g of atropine and 0.1 g of quinine in individual separate volumetric flasks and dissolved in required quantity of methanol.

**Application of sample**: Make a straight horizontal line 1cm above from the bottom of TLC plate. Apply one drop of individual sample solutions to respective spots on TLC plate with capillary tube.

**Procedure for development of TLC plate**

- Sample solutions are spotted on the plate by using capillary tubes. After drying of spots TLC plate has to dip in the development chambers which contain mobile phase.

**Note: Dip the plate in such a way that the straight line on the TLC plate should not be immersed.**

- Remove the plate from the chamber and mark the distance travelled by solvent with a pencil and dry in air for 5 minutes
- The sample s were visualized using Dragendroff's reagent after getting the solvent front.
- TLC plates were dried in an oven to visualize the samples
- The $R_f$ values were measured and reported.

**Report:** The $R_f$ values of **standard reference samples** are: Ephedrine

Atropine:

Quinine:

$R_f$ values of alkaloids in the mixture are: 1. 2.

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**Experiment No: 04**

## HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Aim: It is a technique in analytical chemistry used to separate the components in a mixture, to identify each component, and to quantify each component. The placement(injection) of a small volume of a liquid sample into a tube packed with porous particles (stationary phase). Where individual components of a sample are transported along the column by a liquid moved by pressure. This is called High-Performance Liquid Chromatography as this technique is simple and able to detect components at nanogram level.

It is also called High Pressure Liquid Chromatography as mobile phase is pumped with high pressures

Principle involved in separation by HPLC

- The separation of compounds is due to their relative differences in travel through the column on application of pressure exerted through mobile phase or carrying liquid
- The compounds of the mixture travel with different rates due to their relative affinities with the solvent and stationary phase.
- Compound with higher affinity towards stationary phase of the column travels slowly and vice-versa.
- The above principle is similar to that of column chromatography but in HPLC, the separation is more effective due to greater surface area achieved due to very small particle size of stationary phase in comparison to that used in column chromatography.

Types of HPLC techniques

Based on modes of chromatography

1. Normal phase: Stationary phase is polar, mobile phase is non polar
2. Reverse phase: Stationary phase is non polar, mobile phase is polar

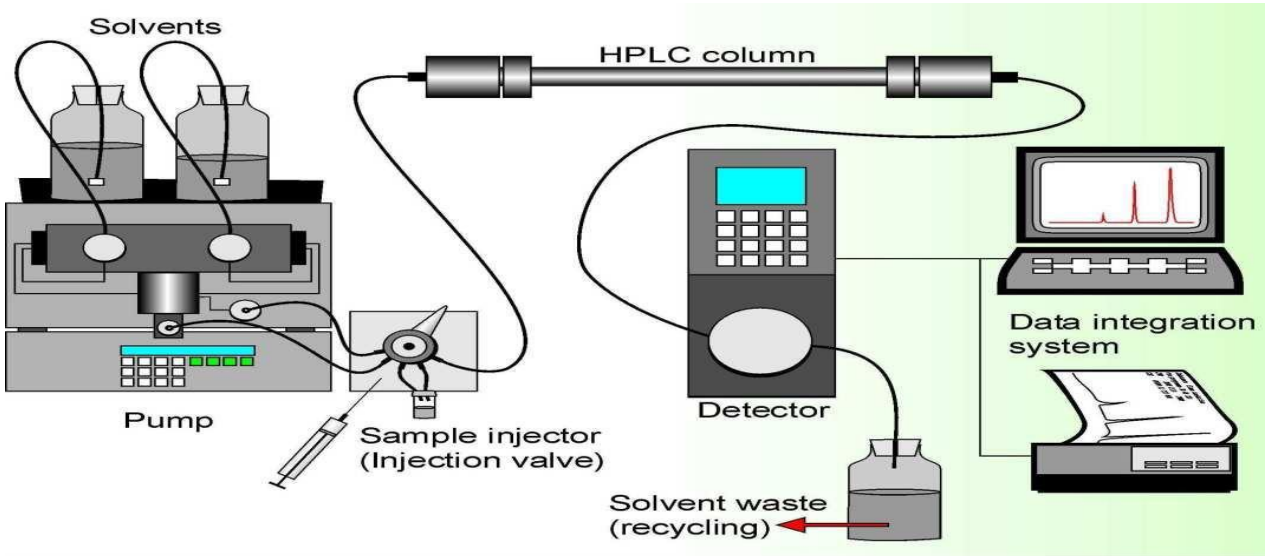
Different columns used include ODS, $C_{18}$, $C_8$

Based on elution technique

1. Isocratic separation: Polarity of mobile phase is same in entire procedure
2. Gradient separation: Polarity of mobile phase is gradually increasing, to get better separation.

**Components of HPLC system**

- ✓ Mobile phase reservoirs
- ✓ Degassing units
- ✓ HPLC Pumps
- ✓ Mixing valves
- ✓ Sample injector (manual or auto)
- ✓ Guard columns
- ✓ Column
- ✓ Column ovens
- ✓ Detector
- ✓ Recorder and integrator
- ✓ Mobile phase waste container



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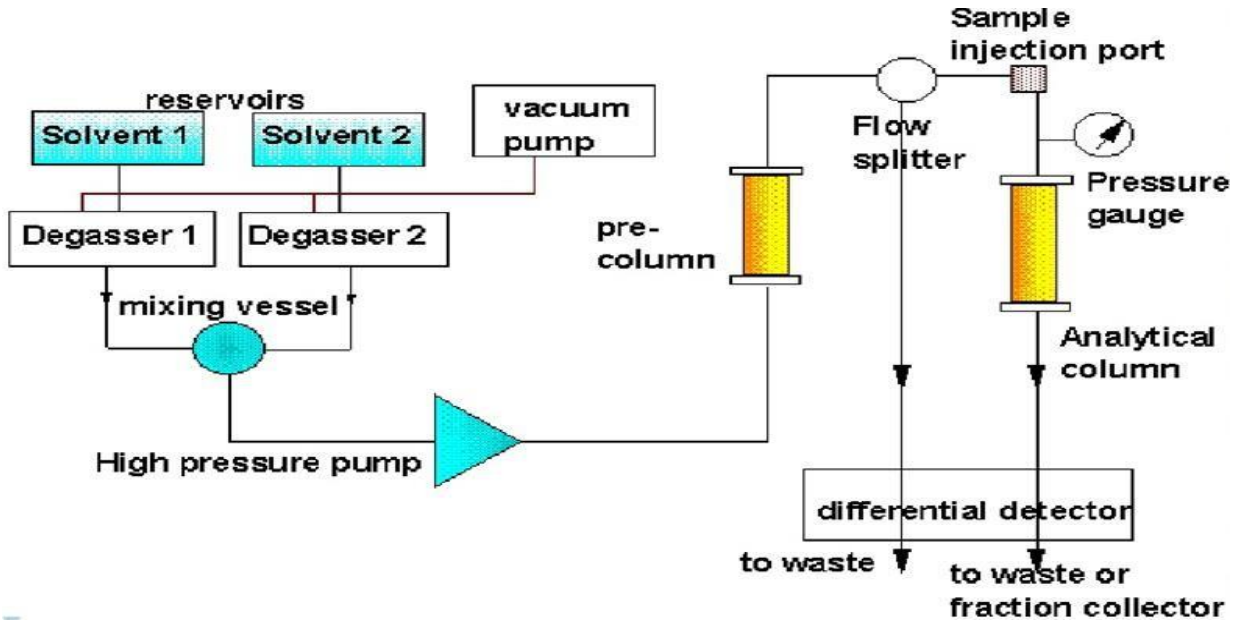
**BLOCK DIAGRAM OF HPLC**




**Degassing units**

- Degassing of the mobile phase can be done with reservoir of inert gases He or $N_2$
- By applying vacuum
- Ultrasonication (converts ultra high frequency to mechanical vibrations)

**SOLVENT DELIVERY PUMPS**

To produce an appropriate pressure to push solvent into the column.

A pump capable of pumping solvent up to a pressure of 4000 psi and at flows of up to 10 ml/min

Types of pumps:

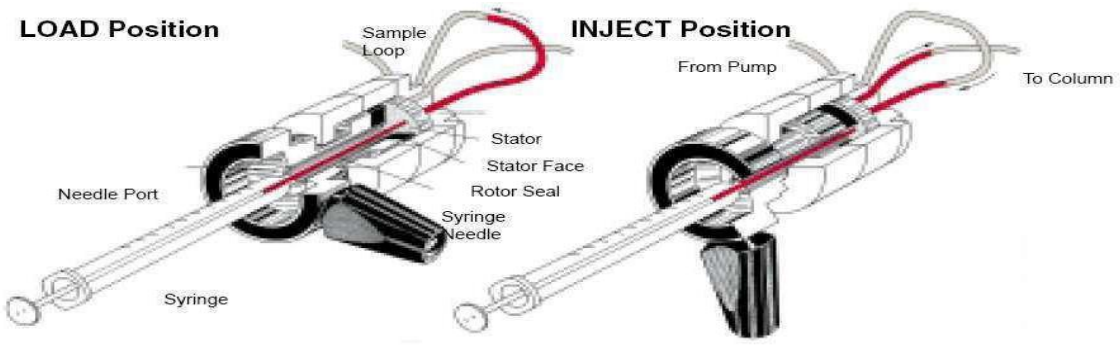
Direct Gas-Pressure pumps

- Syringe type pumps
- Pneumatic pumps
- Reciprocating pumps

Reciprocating pumps are widely used as they maintain accurate flow rate

**Solvent delivery systems**

**Injection systems**




**Rheodyne Inject**

- Isocratic systems
- Low pressure gradient systems
- High pressure gradient systems
- Rheodyne injectors
- Syringe injectors

The heart of the system is the column. Many different reverse phase columns will provide excellent specificity for any particular separation. It is therefore best to routinely attempt separations with a standard C8 or C18 column (e.g. Zorbax RX C8) and determine if it provides good separation.

Types of columns include:

**Guard column:** Protect the analytical column from impurities and foreign substances, no

separation will be occurred in guard column

**Analytical column: Separation of compounds will take place in analytical column.**

- Length (5-15 cm) much shorter than GC column
- Diameter (4 mm to 50mm)
- Particle size (3- 10 mm)

Based up on the type of column material columns are classified into\

- Normal phase columns
- Reverse phase columns
- Size exclusion columns
- Ion exchange columns
- Chiral columns

## HPLC Detectors

The **HPLC detector**, located at the end of the column, must register the presence of various components of the sample, but must not detect the solvent. For that reason there is no universal detector that works for all separations. A common HPLC detector is a **UV absorption detector**, as most medium to large molecules absorb UV radiation. Detectors that measure fluorescence and refractive index are also used for special applications. A relatively new development is the combination of an HPLC separation with an **NMR detector**. This allows the pure components of the sample to be identified and quantified by nuclear magnetic resonance after having been separated by HPLC, in one integrated process.

List of most common HPLC detectors

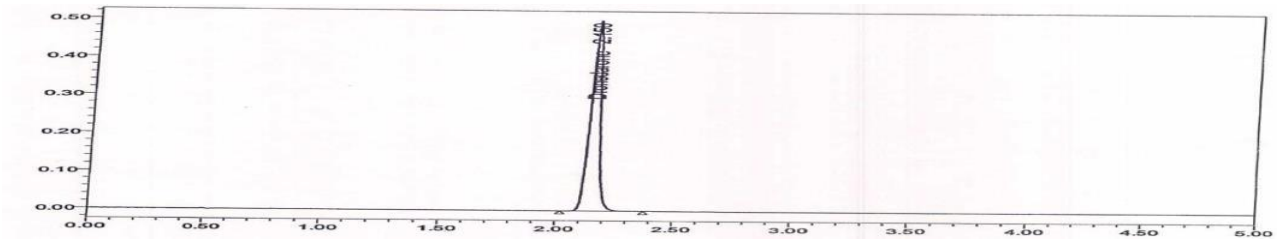
- UV-Visible
- Refractive Index
- Fluorescence
- Conductivity (for ion chromatography)
- Photodiode array detector & Amperometric detector
- Mass spectroscopy detectors
- NMR detectors

**RECORDERS&INTEGRATORS**

**Recorders**: Used to record the responses

**Integrators:** Used to data processing, record individual peaks with $R_t$, height, width of peaks, peak area, % of area

**Typical HPLC chromatogram**




**APPICATIONS OF HPLC**

- Pharmaceutical field
- Chemical and Petrochemical industry
- Forensic studies
- Biochemical separations
- Food analysis
- Qualitative analysis : Checking the purity of a compound
- Quantitative Analysis
    - ✓ Direct Comparison method
    - ✓ Calibration curve method
    - ✓ Internal standard method
- HPLC is used to analyze raw materials and finished products to assure that pre-established quality levels are being met.
- Multi component analysis
- Isolation and identification of drugs
- Stability studie

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**Experiment No: 05**

## DETERMINATION OF $\lambda_{MAX}$ OF $KMNO_4$ BY COLORIMETRY

**Aim:** To determine $\lambda_{max}$ of $KMnO_4$ solution.

**Instrument:** colorimeter

**Apparatus:** 100ml volumetric flask, test tubes,

beakers **Chemicals:** $KMnO_4$ solution, distilled

water **Principle:**

Spectroscopy is the tool for the study of atomic and molecular structure. It deals with the interaction of electronic radiation with matter involving the measurement and interpretation of the extension of absorption or emission of electromagnetic radiation by molecule.

$\lambda_{max}$ is defined as the wavelength at which maximum absorption of radiation takes place. The extent to which a sample absorbs light depends strongly upon the wavelength of light and the type of chromophore present in the analyte. A chromophore is a functional group or a part of the molecule responsible for the absorption of electromagnetic radiation at a specific frequency. $\lambda_{max}$ is characteristic of a compound and provides information of the electronic transitions occur in the analyte. To obtain the highest sensitivity and to minimize deviations from Beer's Law, analytical measurements are made using light with a wavelength of $\lambda_{max}$.

**NOTE**: $\lambda_{max}$ *is* independent from the concentration of the analyte.

**Procedure:**

- Prepare a solution of 100 µg/ml of $KMnO_4$
- Switch on the instrument and warm up for 15 minutes.
- Using distilled water as blank adjust to 100% transmittance or zero absorbance.
- Take the solution of $KMnO_4$ in cuvette and measure the absorbance from 400-800 nm with interval of 40nm.
- Plot the graph between wavelength $v_s$ absorbance and note the wavelength at which maximum absorbance is observed.

## Observation:

| S.No | Wavelength (nm) | Absorbance |
|---|---|---|
| 1 | 400 | |
| 2 | 440 | |
| 3 | 480 | |
| 4 | 520 | |
| 5 | 560 | |
| 6 | 600 | |
| 7 | 640 | |
| 8 | 680 | |
| 9 | 720 | |
| 10 | 760 | |
| 11 | 800 | |

## Report:

The $\lambda_{max}$ of potassium permanganate ($KMnO_4$) was found to be …… nm.

## Reference:

A Practical Approach to Pharmaceutical Analysis, Nema et al, CBS Publications, P5-6.

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**Experiment No: 06**

## DETERMINATION OF $\lambda_{MAX}$ OF PARACETAMOL SOLUTION BY UV-VISIBLE SPECTROPHOTOMETER

**Aim:** To determine $\lambda_{max}$ of paracetamol solution

**Instrument:** UV-Visible Spectrophotometer **Apparatus:**

100ml volumetric flask, test tubes, beakers **Chemicals:**

paracetamol, distilled water

**Principle:**

Spectroscopy is the tool for the study of atomic and molecular structure. It deals with the interaction of electronic radiation with matter involving the measurement and interpretation of the extension of absorption or emission of electromagnetic radiation by molecule.

$\lambda_{max}$ is defined as the wavelength at which maximum absorption of radiation takes place. The extent to which a sample absorbs light depends strongly upon the wavelength of light and type of chromophore present in the analyte. A chromophore is a functional group or a part of the molecule responsible for the absorption of electromagnetic radiation at a specific frequency. $\lambda_{max}$ is characteristic of a compound and provides information of the electronic transitions that occurs in the analyte. In order to obtain the highest sensitivity and to minimize deviations from Beer's Law, analytical measurements are made using light with a wavelength of $\lambda_{max}$.

**Procedure:**

- Prepare a solution of 100 µg/ml of paracetamol.
- Switch on the instrument and warm up for 15 minutes.
- Using distilled water as blank adjust to 100% transmittance or zero absorbance.
- Take the solution of paracetamol in cuvette and scan from 200-400 nm.
- Repeat its for 3-6 times and consider the wavelength at which maximum absorption of radiation takes place. It is known as $\lambda_{max.}$

**Report:** **The $\lambda_{max}$ of potassium paracetamol was found to be …… nm.**

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**Experiment No: 07**

## EFFECT OF SOLVENTS ON ABSORPTION MAXIMA OF DILTIAZEM HCl

**Aim:** To determine the effect of solvents on absorption maxima of Diltiazem HCl

**Apparatus:** Volumetric flask, pipette, beaker, glass rod

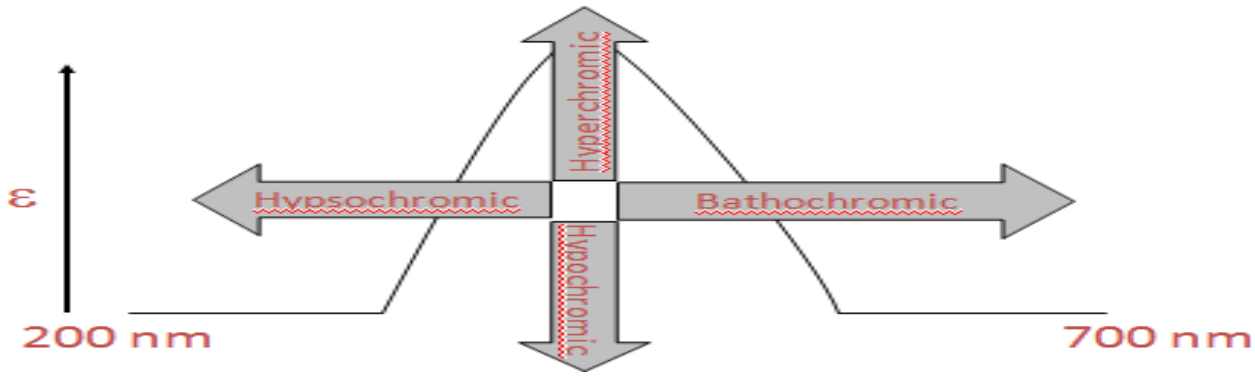
**Chemicals:** Deltiazem HCl, distilled water, methanol, NaoH

**Principle:**

$\lambda_{max}$ is a constant value in a particular solvent as the solvent system varies, the $\lambda_{max}$ also varies, The shift in absorption maxima depends on the polarity of solvent and the pH of the solvent.

Molecule which undergo π-π* transition, the π* state is more polar and stabilized more in polar solvent relative to nonpolar, thus in going from nonpolar to polar solvent there is a **red shift or bathochromic shift** (increase in $\lambda_{max}$).

For n-π* transition, the n state is much more easily stabilized by polar solvent (H-bonds and association), so in going from nonpolar to polar solvent there is a **blue shift or hypsochromic shift (**decrease in $\lambda_{max}$).



## Procedure:

Weigh accurately 100mg of deltiazem HCl powder and add distilled water to make it 100ml in a volumetric flask, shake well and take 10 ml of above solution and dilute to 100ml with distilled water. Scan the above solution to get the spectrum against the distilled water as blank. Similarly make the Diltiazem HCl solution by using methanol and NaOH as solvents. Scan the above solution to get the spectrum against the methanol and NaOH as blanks. Finally overlap all the spectra and observe the shift in $\lambda_{max}$

## Report:

The absorption maxima using water was found at nm

The absorption maxima using methanol was found at nm

The absorption maxima using NaOH was found at nm

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**Experiment No: 08**

## ESTIMATION OF SALICYLIC ACID BY COLORIMETRY

**AIM:** To estimate the amount of Salicylic acid in a given sample by colorimetric method.

**CHEMICALS:** Salicylic acid, Hydrochloric acid (1%v/v), Ferric chloride, Distilled water.

**PRINCIPLE:**

The increasing concentrations of salicylic acid is treated with 1% ferric chloride reagent (1g $FeCl_3$ in 100 mL of 1% Hydrochloric acid). The free phenolic hydroxyl group present in salicylic acid reacts with the reagent and forms a violet-colored complex i.e., ferric salicylate which is proportional to the concentration of salicylic acid.

**PROCEDURE:** A ferric chloride reagent is prepared by adding 1 gm of $FeCl_3$ to 100 ml of 1% HCl (1mL concentrated hydrochloric acid added to 100mL of distilled water). Stock solution of salicylic acid (1mg/ml) is prepared by dissolving 100 mg of salicylic acid in few ml of methanol and made up to 100 ml with distilled water in a volumetric flask. 10 ml of this stock solution is diluted with 100 ml distilled water to get 100 µg/ml salicylic acid solution. Take the respective samples in each test tube, add the reagent and distilled water to make total volume of 10 ml (as per mentioned in table) and measure the absorbance of the violet colored complex usingUV-Visible spectrophotometer or colorimetry at wavelength of 525 nm against blank sample (without salicylic acid).

| Volume of stock solution (mL) | Volume of reagent (mL) | Distilled water to make 10 mL | Concentration of Salicylic acid (µg/mL) | Absorbance |
|---|---|---|---|---|
| 0 | 1 | 9 | 0 | 0 |
| 1 | 1 | 8 | 10 | |
| 2 | 1 | 7 | 20 | |
| 3 | 1 | 6 | 30 | |
| 4 | 1 | 5 | 40 | |
| 5 | 1 | 4 | 50 | |
| 6 | 1 | 3 | 60 | |

Plot a graph taking concentration on the X-axis and observed absorbance values on the Y-axis, draw a best-fit line, and record the r2 value (regression coefficient) and equation of a straight line.

**REPORT:**

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## EXPERIMENT NO: 09

## ESTIMATION OF PARACETAMOL IN TABLETS BY USING STANDARD ABSORPTIVITY VALUE

**Aim:** To estimate the percentage purity of Paracetamol percent in the given sample

**Apparatus:** Mortar and pestle, beaker, standard flasks, funnel

**Chemicals:** Paracetamol tablets, water, NaOH

**Principle:**

Estimation of paracetamol by UV- Visible spectrophotometer depends on the Beer's -Lambert's law

**Beer's law**: States that "the intensity of a beam of monochromatic light decreases exponentially with increase in the concentration of absorbing species arithmetically"

$-dI / dc \alpha I$

**Lambert's law**: states that the rate of decrease of intensity (monochromatic light) with the thickness of the medium is directly proportional to the intensity of incident light

$-dI / dt \alpha I$

**Beer – lamberts law**

$I = I_o e^{-kct}$

$I = I_o 10^{-kct}$ (converting natural algorithm to base 10)

$I / I_o = 10^{-kct}$ (rearranging terms)

$I_o / I = 10^{kct}$ (inverse on both side)

$\text{Log } I_o / I = kct$ (taking log on both sides) --- Equation 4

It can be learnt that transmittance (T) = $I / I_o$ and Absorbance (A) = $\log 1 / T$

Hence $A = \log 1 / I / I_o$

$A = \log I_o / I$ --- Equation 5, by Using Equation 4 & 5 , Since $A = \log I_o / I$ and $\log I_o / I = Kct$

we can infer that $A = Kct$ (instead of K, we can use ε)

**$A = \varepsilon ct$ or $A = act$ or $A = A^{1\%}_{1cm} ct$**

Where:

A = Absorbance or optical density

ε = Molecular extinction coefficient

c = Concentration of the drug (mol/lit)

t = Path length (normally 10mm or 1cm)

Paracetemol is chemically known as Para-acetyl-aminophenol. Estimation of Paracetemol concentration in tablets can be alone done by U.V. spectrophotometry by using standard Absorptivity value ($A^{1\%}_{1cm}$). In this the absorbance of the diluted sample solution is observed at 257nm. From this calculate the concentration of Paracetemol taking 715 on $A^{1\%}_{1cm}$ value at maximum of about 257nm

**Procedure:**

- Weigh and powder 20 tablets
- Take the powder equivalent to 0.15gm
- Add 50ml of 0.1 M NaOH and dilute to 100ml with water
- Shake the mixture for 15 minutes and add sufficient water to produce 200ml
- The resulting solution is mixed and filtered
- Take 10ml of above solution and dilute to 100ml with water
- To 10ml of resulting solution add 10ml of 0.1 N NaOH
- Make up the volume to 100ml with water and mix it
- Measure the absorbance of resulting solution at 257nm by using 715 as $A^{1\%}_{1cm}$ value

**Report:**

The purity of Paracetamol in the given sample of tablet powder was found to be

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**Experiment No: 10**

## ESTIMATION OF PARACETAMOL BY USING CALIBRATION CURVE METHOD

**AIM:** To estimate the percentage purity of Paracetamol present in the given sample

**APPRATUS:** Beaker, glass rod, volumetric flask, measuring cylinder, pipette

**CHEMICALS:** Paracetamol powder, Paracetamol tablets, NaOH, water

**PRINCIPLE:**

The assay of light absorbing substance may be quickly carried out by using Beer – Lamberts Law.

**Beer's law**: States that "the intensity of a beam of monochromatic light decreases exponentially with increase in the concentration of absorbing species arithmetically" - $dI / dc \alpha I$

**Lambert's law**: states that the rate of decrease of intensity (monochromatic light) with the thickness of the medium is directly proportional to the intensity of incident light

$-dI / dt \alpha I$

**Beer – lamberts law**

$I = I_o e^{-kct}$

$I = I_o 10^{-kct}$ (converting natural algorithm to base 10) $I / I_o = 10^{-kct}$ (rearranging terms)

$I_o / I = 10^{kct}$ (inverse on both side)

$\log I_o / I = kct$ (taking log on both sides) --- Equation 4

It can be learnt that transmittance (T) = $I / I_o$ and Absorbance (A) = $\log 1 / T$

Hence $A = \log 1 / I / I_o$

$A = \log I_o / I$ --- Equation 5, by Using Equation 4 & 5, Since $A = \log I_o / I$ and $\log I_o / I = Kct$ we can infer that A= Kct (instead of K, we can use ε)

**$A = \varepsilon ct$ or $A = act$ or $A = A^{1\%}_{1cm} ct$**

Where:

A = Absorbance or optical density

ε = Molecular extinction coefficient

c = Concentration of the drug (mol/lit)

t = Path length (normally 10mm or 1cm)

Paracetamol is chemically known as *N*-(4-hydroxyphenyl) acetamide. Estimation of paracetamol concentration in tablet can be done by UV- spectrophotometry using calibration curve method. In this the absorbance of the diluted sample solution is observed at 257nm. From this concentration is calculated.

**CALIBRATION CURVE:** It is a standard graph or linear plot of serial standard dilutions.Graph plotted between concentration(x-axis) Vs response(Y-axis). The curve should be passed through the origin and $r^2$ value should be greater than 0.99 and less than 1.

## PROCEDURE:

**Preparation of stock solution:** 100mg of paracetamol powder is dissolved in 100ml of 0.1N NaOH (1mg/ml)

**Working standard:** From the stock solution, 10 ml is taken and diluted to 100 ml with 0.1N NaOH (100µg/ml)

**Dilutions of Linearity Range:** From the working standard 1ml,2ml.3ml, 4ml,5ml is taken into individual volumetric flasks (10ml) and make up the volume by usimg 0.1N NaOH to get the concentrations about 10,20.30,40,50 µg/ml respectively.

**Estimation of amount of Pracetamol in tablets:**

- Weigh and powder 20 tablets
- Take the powder equivalent to 0.15gm

- Add 50ml of 0.1 M NaOH and dilute to 100ml with water
- Shake the mixture for 15 minutes and add sufficient water to produce 200ml
- The resulting solution is mixed and filtered
- Take 10ml of above solution and dilute to 100ml with water
- To 10ml of resulting solution add 10ml of 0.1 N NaOH
- Make up the volume to 100ml with water and mix it
- Measure the absorbance of resulting solution at 257nm

**REPORT:**

The purity of Paracetemol in the given sample of tablet powder was found to be

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**Experiment No: 11**

## ASSAY OF CHLORAMPHENICOL CAPSULES BY USING UV-VISIBLE SPECTROPHOTOMETRIC METHOD

**Aim:** To estimate the percentage purity of chloramphenicol percent in the given capsules

**Appratus:** Mortar and pestle, beaker, standard flasks, funnel

**Chemicals:** Chloramphenicol capsules, water

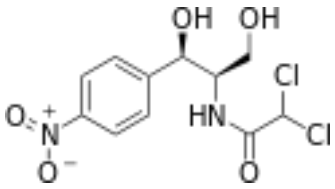
**Principle:**

The assay of an absorbing substance may be quickly carried out by using Beer – Lamberts Law.

$I = I_o\ e^{-kct}$

**$A = \varepsilon\ ct$**

Where: A = Absorbance or optical density. ε = Molecular extinction coefficient, c = Concentration of the drug (mol/lit) ,t = Path length (normally 10mm or 1cm)




Chloramphenicol is chemically known as 2,2-dichloro-N-[(1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl) propan-2-yl] acetamide. Estimation of chloramphenicol concentration in capsule can be alone done by U.V. spectrophotometry by using standard Absorptivity value ($A^{1\%}_{1cm}$). In this the absorbance of the diluted sample solution is observed at 278nm. From this calculate the concentration of chloramphenicol by taking 298 as $A^{1\%}_{1cm}$ value.

**Procedure:**

- Weigh 20 capsules and mix the contents.
- Take the powder equivalent to 0.200gm of chloramphenicol
- Add 500ml of distilled water, warm gently to get a clear solution and dilute to 1000ml with distilled water
- Take 10ml of above solution and dilute to 100ml with distilled water
- To 10ml of resulting solution add 10ml of 0.1 N NaOH
- Measure the absorbance of resulting solution at 278 nm by using 298 as $A^{1\%}_{1cm}$ value

**Report:**

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**Experiment No: 12**

## EFFECT OF $P^H$ ON ABSORPTION MAXIMA OF PARACETAMOL

**AIM:** To determine the effect of $P^H$ on $\lambda_{max}$ of paracetamol **APPRATUS:** Volumetric flask, pipette, glass rod, beaker **CHEMICALS:** Buffer – 4, 7, 9 capsules, Paracetamol, distilled water **PRINCIPLE:**

High pH solutions absorb at higher wavelengths. Whereas low pH solutions absorb at lower wavelengths.

$C_6H_5O_H \longrightarrow C_6H_5O^- + H^+$

In the given equation when the equilibrium of the reaction lies to the right, then the wavelength of the absorbance would decrease, because the concentration of $H^+$ has increased (ie lower pH). The increased $H^+$ concentration stabilize the ground state as opposed to the excited electronic state leads to decrease in wavelength (hypsochromic (blue) shift), which in turn is an increase in energy. In other words, more energy is required for an electronic excitiation to occur (stabilised ground state by the $H^+$).

**PROCEDURE:**

- Weigh accurately 100mg of Paracetamol powder and add it to $P^H$-4 buffer in 100ml volumetric flask
- Shake well and mask it as solution A
- Similarly add 100mg of Paracetamol powder to $P^H$ – 9 and $P^H$ -7 buffer in 100ml volumetric flask
- Shake well and mark it as solution B and C respectively
- Prepare blank solutions of $P^H$ 4, 7, 9
- Solution A is taken and spectrum is plotted by U.V. Visible spectrophotometer and $\lambda_{max}$ is obtained against the blank $P^H$ – 4
- Similarly plot for the solution B and C against $P^H$ – 9 and $P^H$ -7 as blank
- Overlap spectra and observe the shift in $\lambda_{max}$ and report it

**REPORT:** **In neutral $P^H$ – $\lambda_{max}$ –nm In acidic $P^H$ – $\lambda_{max}$ –nm In alkaline $P^H$ – $\lambda_{max}$ –nm**

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**Experiment No: 13**

## EFFECT OF SOLVENT ON ABSORPTION MAXIMA OF PARACETEMOL

### PROCEDURE:

Weigh accurately 100mg of Paracetemol powder and add distilled water to make it 100ml in a volumetric flask, shake well and scan the above solution to get spectrum against the distilled water as blank. Similarly make the paracetamol solution by using methanol as solvent. Scan the above solution to get spectrum against the distilled methanol as blank. Finally overlap both the spectra and observe the shift in $\lambda_{max}$

### REPORT:

The absorption maxima using methanol was found at higher wavelength.

## IR INTERPRETATION

Infrared spectroscopy (IR) measures the bond vibration frequencies in a molecule and is used to determine the functional groups and bonds present in chemical substance. The infrared region of the spectrum encompasses radiation with wave numbers ranging from about 12,500 to 50$cm^{-1}$ (or) wave lengths from 0.8 to 200µ.

The infrared region constitutes 3 parts

- ✓ The near IR (0.8 -2.5 µm) (12,500-4000 $cm^{-1}$)
- ✓ The middle IR (2.5 -15 µm) (4000-667 $cm^{-1}$)
- ✓ The far IR (15-200 µm) (667-50 $cm^{-1}$)

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## PRINCIPLE:

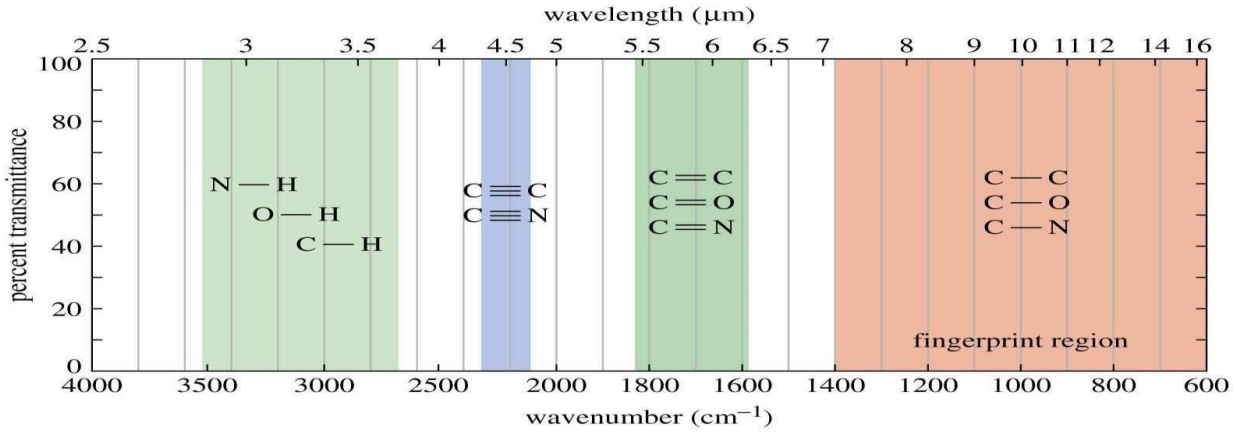
The absorption of infrared radiation leads to the excitation of molecules and changes in the shape of the molecule because of stretching of bonds, bending of bonds, or internal rotation around single bonds. Vibrational transitions which are accompanied by a change in the dipole moment of the molecule are called infrared active transitions and those which are not accompanied by a change in dipole moment are not IR active transitions.

Other regions in the infrared spectrum are

Group frequency region (2.5 – 8.0 µm) (4000 - 1300 $cm^{-1}$)

Fingerprint region (8.0 - 25 µm) (1300 – 400 $cm^{-1}$)

- ✓ 4000 □ 2500 $cm^{-1}$: Absorbance of single bonds formed by hydrogen and other elements e.g. O□H, N□H,C□H
- ✓ 2500 □ 2000 $cm^{-1}$: Absorbance of triple bonds e.g. C≡C, C≡N
- ✓ 2000 □ 1500 $cm^{-1}$: Absorbance of double bonds e.g. C=C, C=O
- ✓ 1500 □ 400 $cm^{-1}$: This region often consists of many different, complicated bands. This part of the spectrum is unique to each compound and is often called the ***fingerprint*** region. It is rarely used for the identification of particular functional groups



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## BASE VALUES
(+/- 10 $cm^{-1}$)

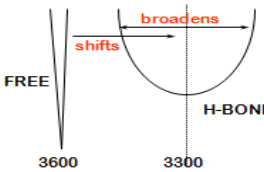
| | |
|---|---|
| O-H | 3600 |
| N-H | 3400 |
| C-H | 3000 |
| C≡N | 2250 |
| C≡C | 2150 |
| C=O | 1715 |
| C=C | 1650 |
| C–O | ~1100 |

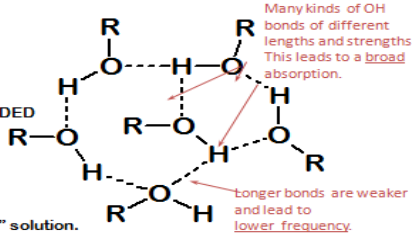
These are the minimum number of values to memorize.

large range

### O-H STRETCH

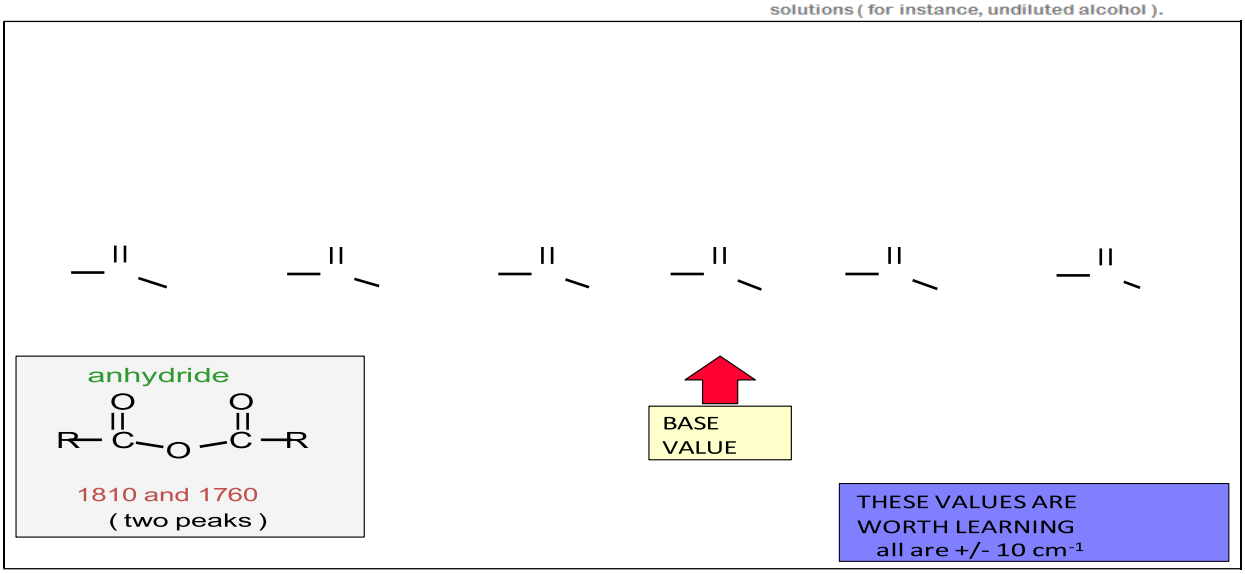
The O-H stretching region
- O-H 3600 $cm^{-1}$ (alcohol, free)
- O-H 3300 $cm^{-1}$ (alcohols & acids, H-bonding)



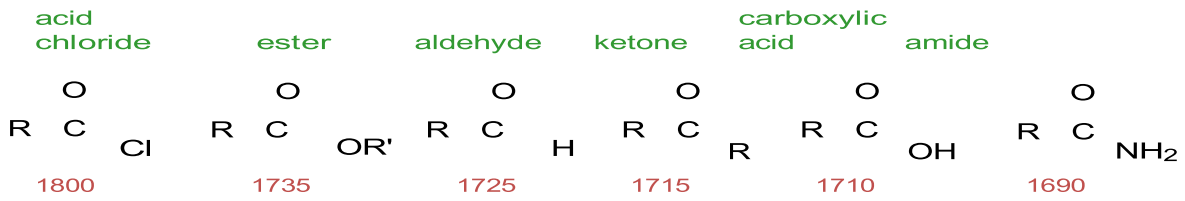




"Neat" solution.

Hydrogen bonding occurs in concentrated solutions ( for instance, undiluted alcohol ).




## C=O IS SENSITIVE TO ITS ENVIRONMENT




## C=O IS SENSITIVE TO ITS ENVIRONMENT

EACH DIFFERENT KIND OF C=O COMES AT A DIFFERENT FREQUENCY

| Bond | Type of Compound | Frequency Range, $cm^{-1}$ | Intensity |
|---|---|---|---|
| C—H | Alkanes | 2850–2970 | Strong |
| | | 1340–1470 | Strong |
| C—H | Alkenes $\left(>C{=}C<^{H}\right)$ | 3010–3095 | Medium |
| | | 675–995 | Strong |
| C—H | Alkynes (—C≡C—H) | 3300 | Strong |
| C—H | Aromatic rings | 3010–3100 | Medium |
| | | 690–900 | Strong |
| O—H | Monomeric alcohols, phenols | 3590–3650 | Variable |
| | Hydrogen-bonded alcohols, phenols | 3200–3600 | Variable, sometimes broad |
| | Monomeric carboxylic acids | 3500–3650 | Medium |
| | Hydrogen-bonded carboxylic acids | 2500–2700 | Broad |
| N—H | Amines, amides | 3300–3500 | Medium |
| C=C | Alkenes | 1610–1680 | Variable |
| C=C | Aromatic rings | 1500–1600 | Variable |

| Bond | Type of Compound | Frequency Range, $cm^{-1}$ | Intensity |
|---|---|---|---|
| C≡C | Alkynes | 2100–2260 | Variable |
| C—N | Amines, amides | 1180–1360 | Strong |
| C≡N | Nitriles | 2210–2280 | Strong |
| C—O | Alcohols, ethers, carboxylic acids, esters | 1050–1300 | Strong |
| C=O | Aldehydes, ketones, carboxylic acids, esters | 1690–1760 | Strong |
| $NO_2$ | Nitro compounds | 1500–1570 | Strong |
| | | 1300–1370 | Strong |

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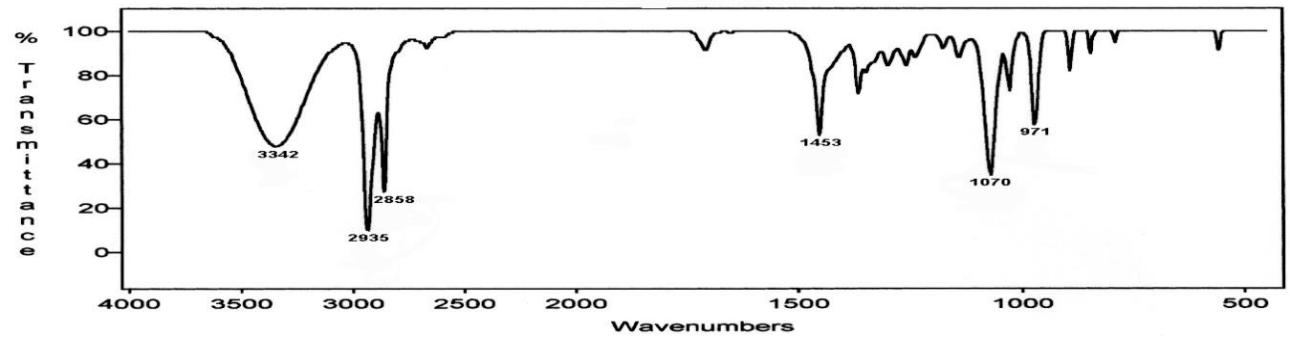


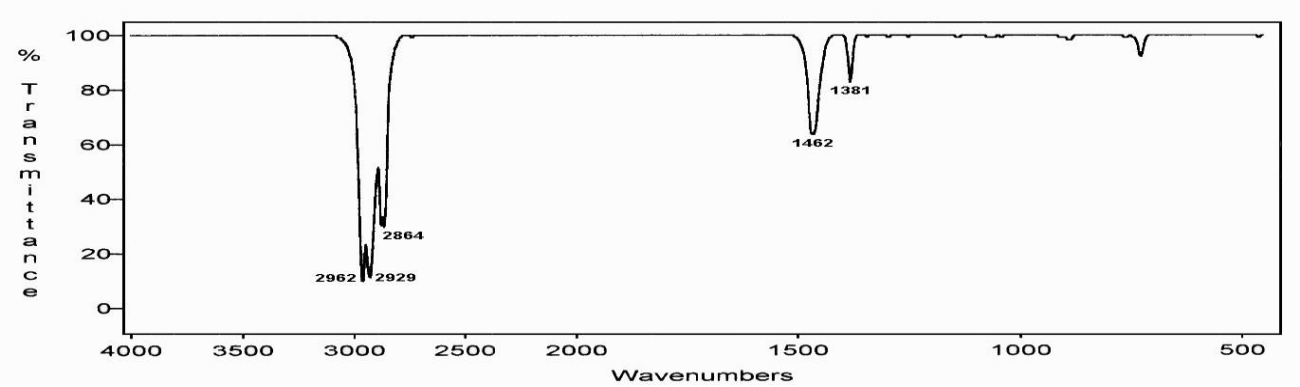


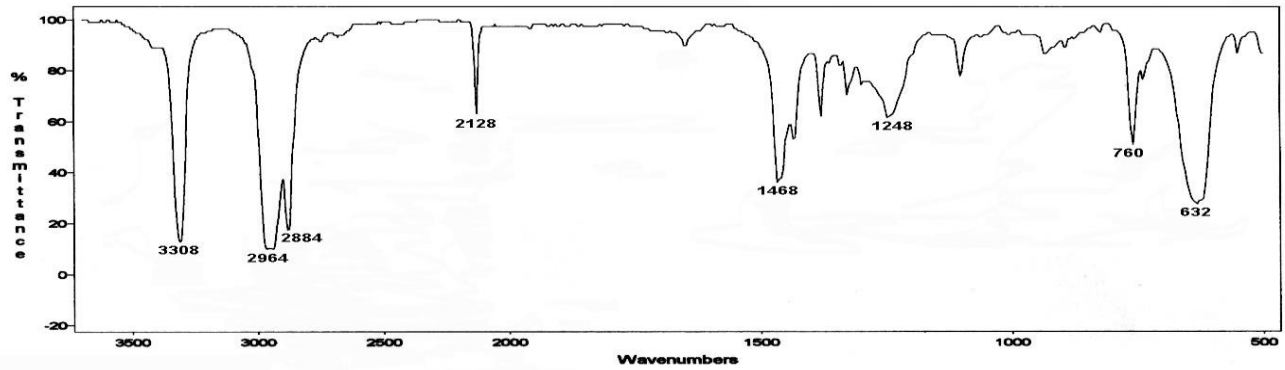


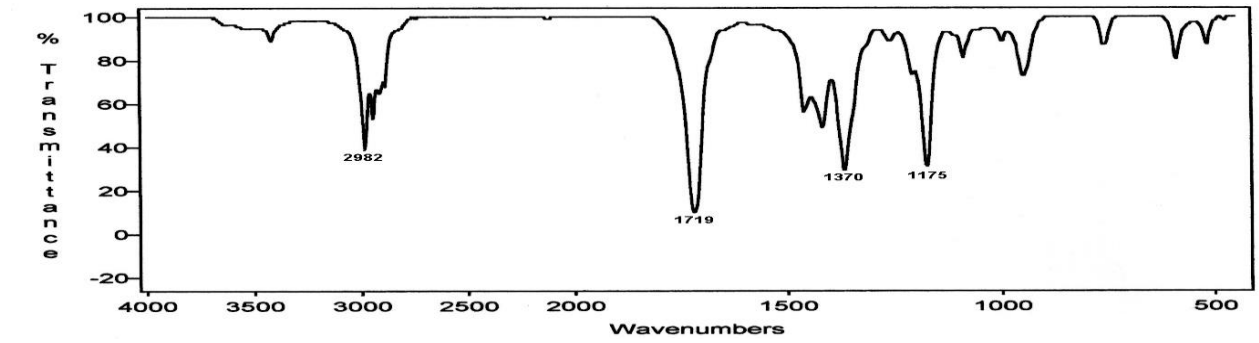

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**Experiment No: 14**

# ESTIMATION OF QUININE SULPHATE BY FLUORIMETRY

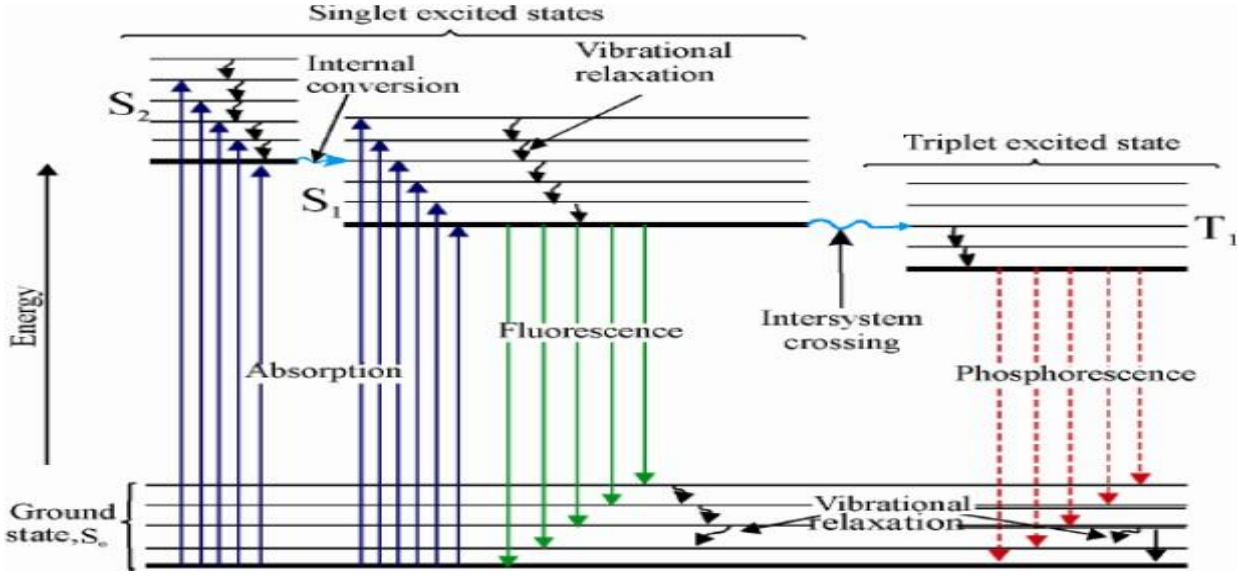
**Aim**: To estimate the amount of Quinine sulphate present in given sample by fluorimetry.

**Requirements:** Quinine sulphate, Fluorimeter, Sulphuric acid.

**Apparatus:** Pipette, volumetric flask, measuring cylinder.

**Principle:**

Fluorescence is the emission of visible light by a substance that has absorbed light of a different wavelength. The emitted photon has a longer wavelength and lower energy. As the excitation of the molecule is due to the absorption of a photon (light), these types of luminescence are called photoluminescence. Fluorescence is also defined as the radiation emitted in the transition of a molecule from a singlet excited state to a singlet ground state.



Fluorescence is also defined as the radiation emitted in the transition of a molecule from a singlet excited state to a singlet ground state. The intensity of the emitted radiation proportional to concentration of sample. such measurement forms the basis of a sensitive method of analysis called the fluorometry. Fluorometric methods of analysis have found application in many situations of pharmaceutical interest such as in analysis of riboflavin, thiamine,reserpine, quinine sulphate in drug dosage forms.

**Procedure;**

**Preparation of 0.1 N $H_2SO_4$:**

Pipette out 2.7 ml of conc $H_2SO_4$ to 100 ml of water and then make it up to 1000 ml with water.

Preparation of standard Quinine sulphate solution

- Weigh accurately 100mg of Quinine sulphate powdered drug
- Dissolve in 100 ml of 0.1 N conc $H_2SO_4$ to get 1mg/ml
- Take 10 ml of above solution and dilute to100 ml with 0.1N $H_2SO_4$(**100µg/ml)**
- Again, 10ml of above solution dilute to 100ml with 0.1N $H_2SO_4$(**10µg/ml)**
- Pipette out 0.5,1, 1.5, 2,2.5,3 ml of the above diluted standard quinine sulphate solution into a set of 10 ml volumetric flask and make up to 10 ml with 0.1N $H_2SO_4$ to get the concentration of 0.5,1,1.5,2,2.5,3 µg/ml respectively.

➢ Switch on the instrument and stabilize for 10-15min.

➢ Set excitation and emissiom filters at the wavelengths 365 and 459nm respectively.

➢ Set the fluorescence intensity to 0% by using 0.1N $H_2SO_4$ as blank and 100% by using the highest concentration of the standard solution. Measure the fluorescence of serial dilutions and plot the calibration curve (fluorescence intensity Vs concentration).

**Observation:**

| S.No | Concentration (µg/ml) | % Fluorescence intensity |
|---|---|---|
| 1 | 0.5 | |
| 2 | 1 | |
| 3 | 1.5 | |
| 4 | 2 | |
| 5 | 2.5 | |
| 6 | 3 | |
| 7 | Unknown | |

**Report:** The amount of Quinine sulphate present in the given sample was found to be ……..

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**Experiment No: 15**

## ESTIMATION OF SODIUM CONCENTRATION BY FLAME PHOTOMETRY

**Aim**: To estimate the concentration of sodium present in given sample by flame photometry.

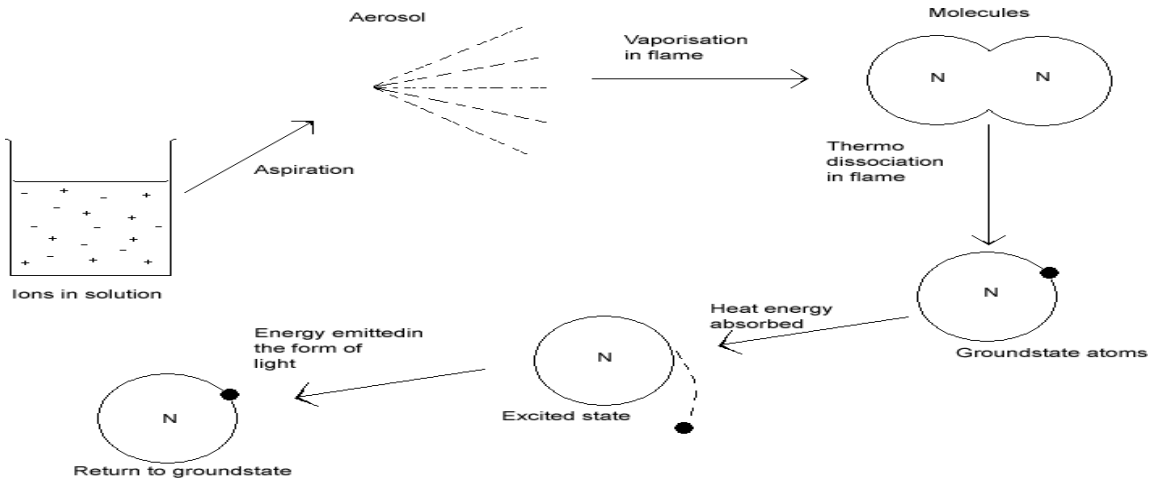
**Requirements:** Sodium chloride, Distlled water, Flame Photometer.

**Apparatus:** Pipette, volumetric flask, measuring cylinder.

**Principle:** Flame photometry is also known as atomic emission spectrometry. It works on the basis of heating the metal, in this case sodium, such that the atoms of the metal travel from ground state to their excited state. The atoms then return to their ground state and release their energy as photons of ultraviolet radiation. The wavelength of the UV radiation is then measured. When a liquid sample consisting a metallic salt solution is introduced into a flame, the following steps takes place

- The solvent gets evaporated
- The solid salt gets converted to its gaseous state
- The dissociation of either portion or total gaseous molecules give rise to neutral atoms (or) free radicals
- The neutral atoms are excited by the thermal energy of the flame which are unstable
- They instantly emit photons and return to it's ground state

The measurement of photons (emitted radiations forms the fundamental basis of flame photometry




The intensity of radiation emitted by depends upon proportion of thermally exited atoms

**Procedure;**

- Weigh accurately 100mg of sodium chloride powdered drug
- Dissolve in 100 ml of distilled water to get 1mg/ml
- Take 10 ml of the above solution and dilute it to100 ml with distilled water (**100µg/ml)**
- Pipette out 5,10, and 20,30,40,50 ml of the above diluted standard solution into a set of 100 ml volumetric flasks and makeup to 100 ml to get concentrations of 5,10, 20, 30,40, and 50 µg/ml respectively.
- Switch on the instrument, select the sodium filter and stabilize for 10-15min.
- Set the gas in flame to get the non-luminous flame and air pressure at 0.4 to 0.5Kg/$cm^2$
- Set the flame intensity to 0% by using distilled water as blank and 100% by using the highest concentration of the standard solution. Measure the % flame intensity of serial dilutions and plot the calibration curve (% intensity Vs concentration).
- Find out the concentration of the unknown sample from the calibration curve.

**Observation:**

| S.No | Concentration (µg/ml) | % Flame intensity |
|---|---|---|
| 1 | 5 | |
| 2 | 10 | |
| 3 | 20 | |
| 4 | 30 | |
| 5 | 40 | |
| 6 | 50 | |
| 7 | Unknown | |

**Report:** The concentration of sodium present in the given sample was found to be ……..

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## Experiment No: 16
## ESTIMATION OF POTASSIUM CONCENTRATION BY FLAME PHOTOMETRY

**Aim**: To estimate the concentration of potassium present in given sample by flame photometry.

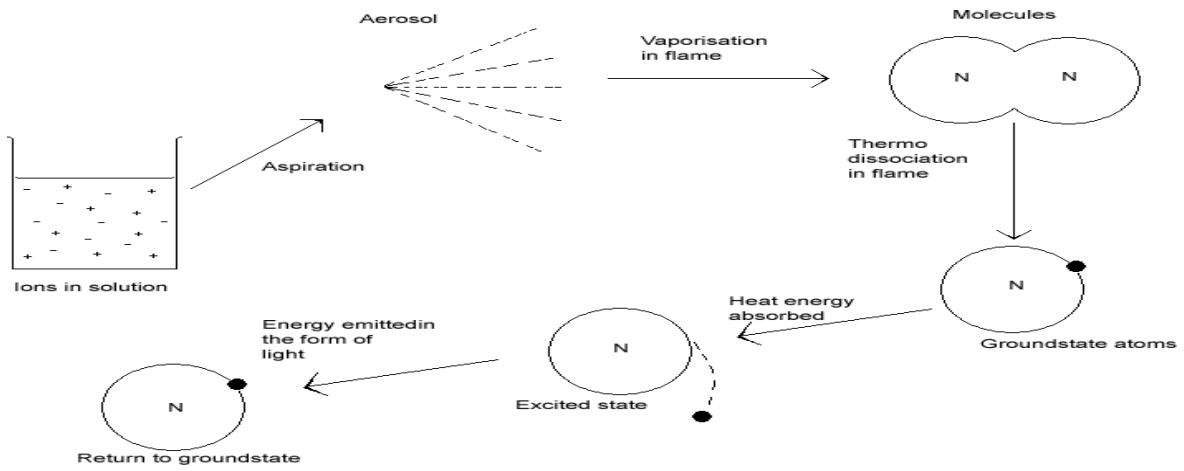
**Requirements:** Potassium chloride, Distlled water, Flame Photometer.

**Apparatus:** Pipette, volumetric flask, measuring cylinder.

**Principle:** Flame photometry is also known as atomic emission spectrometry. It works on the basis of heating the metal, in this case sodium, such that the atoms of the metal travel from ground state to their excited state. The atoms then return to their ground state and release their energy as photons of ultraviolet radiation. The wavelength of the UV radiation is then measured. When a liquid sample consisting a metallic salt solution is introduced into a flame, the following steps takes place

- The solvent gets evaporated
- The solid salt gets converted to its gaseous state
- The dissociation of either portion or total gaseous molecules give rise to neutral atoms (or) free radicals
- The neutral atoms are excited by the thermal energy of the flame which are unstable
- They instantly emit photons and return to it's ground state

The measurement of photons (emitted radiations forms the fundamental basis of flame photometry



The intensity of radiation emitted by depends upon proportion of thermally exited atoms

**Procedure;**

- Weigh accurately 100mg of potassium chloride powdered drug
- Dissolve in 100 ml of distilled water to get 1mg/ml
- Take 10 ml of above solution and dilute to100 ml with distilled water (**100µg/ml)**
- Pipette out 5,10, 20,30,40,50 ml of above diluted standard solution in to a set of 100 ml volumetric flask and make up to 100 ml with to get concentration of 5,10, 20, 30,40, 50 µg/ml respectively.
- Switch on the instrument, select the sodium filter and stabilize for 10-15min.
- Set the gas in flame to get the non-luminous flame and air pressure at 0.4 to 0.5Kg/$cm^2$
- Set the flame intensity to 0% by using distilled water as blank and 100% by using the highest concentration of the standard solution. Measure the % flame intensity of serial dilutions and plot the calibration curve (% intensity Vs concentration).
- Find out the concentration of the unknown sample from the calibration curve.

**Observation:**

| S.No | Concentration (µg/ml) | % Flame intensity |
|---|---|---|
| 1 | 5 | |
| 2 | 10 | |
| 3 | 20 | |
| 4 | 30 | |
| 5 | 40 | |
| 6 | 50 | |
| 7 | Unknown | |

**Report:** The concentration of sodium present in the given sample was found to be ……..

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**Experiment No: 17**

## POTENTIOMETRIC TITRATION OF STRONG ACID Vs STRONG BASE

**AIM**: To determine the exact equivalent point and normality of 0.1 N HCl by titrating it with 0.1N NaOH solution potentiometrically.

**CHEMICALS**: NaOH, distilled water, HCl.

**APPARATUS**: pH meter, potentiometer, Burette, Beaker, Electrodes, Measuring cylinder.

**PRINCIPLE**: Potentiometric determination of end point depends upon the potential across to suitable reference and indicator electrodes immersed in solution changes sharply at the end point. This change is similar to colour change by ion-by-ion indicator by visual method. Potentiometric endpoint determination is more accurate and these titrations are useful when there is no suitable indictor available. End point will be accurately noted after plotting a graph between volume of titrant vs potential developed.

**PROCEDURE:**

**Preparation of 0.1 N NaOH**: 0.4 g of NaOH dissolved in 100 ml of $H_2O$.

**Preparation of 0.1 N Oxalic acid**: 0.63 g of oxalic acid dissolved in 100 ml of water.

**Preparation of 0.1 N HCl**: 0.84 ml of conc. HCl is dissolved in 100 ml water.

**Standardization of 0.1 N NaOH:**

- Pipette out 20 ml of 0.1 N oxalic acid solution into beaker
- Dip the electrodes (saturated calomel electrode as reference electrode and glass electrode as indicator electrode) into the solution which is connected to a potentiometer.
- Take the prepared 0.1 N NaOH solution into a burette and add 0.5 ml of this to the beaker until the equivalent point of the titration is obtained.

- Pipette out 20 ml of standardised NaOH solution into a beaker and take the prepared HCl solution into a burette.
- Proceed the titration in the same way like oxalic acid.
- A graph is plotted between the volume of titrant add on X-axis and the potential difference on Y-axis in mV.

| S.No. | Vol of titrant | pH of solution | e.m.f |
|---|---|---|---|
| 1 | 0 ml | | |
| 2 | 1 ml | | |
| 3 | 2 ml | | |
| 4 | 3 ml | | |
| 5 | 4 ml | | |
| 6 | 5 ml | | |
| 7 | 6 ml | | |
| 8 | 7 ml | | |
| 9 | 8 ml | | |
| 10 | 9 ml | | |
| 11 | 10ml | | |

**REPORT**: The endpoint in potentiometric titration of strong acid $v_s$ strong base was found to be -------With pH---- and e.m.f was found to be-------

# Vision and Mission of the Institution

## Vision

The East Point College of Pharmacy aspires to be a globally acclaimed institution, **recognized** for **excellence in** pharmaceutical education, research and nurturing students for **holistic development.**

## Mission

**M1** Create pharmacy graduates through **quality education**

**M2** Promote innovation, **creativity**, and excellence **in teaching**, learning, and **research**

**M3** **Inspire** integrity, teamwork, critical thinking**, personal** development, and ethics in **students** and lay **the** foundation for lifelong learning

**M4** **Serve** the **healthcare, technological, scientific**, and **economic** needs of then **society**.