INTRODUCTION

Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of drugs. It deals with materials, dosage forms and more recently biological samples in support of biopharmaceutical and pharmacokinetic studies.

Analytical chemistry concerned with improved means of measuring the chemical composition of natural and artificial materials. The scope of analytical chemistry is very broad and embraces a wide range of manual chemical and instrumental analysis.

The technique of this science is used to identify the substance which may be present in a material and to determine substance, both qualitatively and quantitatively.

A qualitative method yields information about the identity of atomic or molecular species or the functional groups in the samples.

A quantitative method provides numerical information as to the relative amount of one or more of these compounds.

Analytical methods are often classified as being either classical or instrumental method.

CLASSICAL METHODS ARE

Gravimetry	The substance being determined is converted into an insoluble precipitate which is collected and weighed.
Titrimetric analysis	The substance is allowed to react with an appropriate reagent and the volume of solution needed for complete reaction is determined.
Volumetric analysis	The volume of gas evolved or absorbed in a chemical reaction is measured.

INSTRUMENTAL TECHNIQUES

Most instrumental techniques fit into one of these principal areas: - spectroscopy, electrochemical techniques and chromatography.

Spectroscopic techniques	UV and visible spectrophotometry Fluorescence and phosphorescence Atomic spectrophotometry Raman spectroscopy X-ray spectroscopy Radiochemical techniques including activation of analyte.
Chromatographic techniques	Gas chromatography HPLC
Miscellaneous techniques	Thermal analysis Mass spectrometry Kinetic techniques
hyphenated techniques	GC-MS ICP-MC GC-IR MS-MS LC-MS
Electrochemical techniques	Potentiometry Voltametry Stripping techniques Amperometric techniques Coulometry Conductance techniques Electrogravimetry

SPECTROSCOPIC TECHNIQUES :- Spectroscopic methods of analysis depends on measuring the amount of radiant energy of a particular wavelength absorbed by the sample or measuring the amount of radiant energy of a particular wavelength emitted

by the sample. Wavelengths involved include visible, ultraviolet or infrared spectrophotometry.

ULTRA-VIOLET AND VISIBLE SPECTROSCOPY

The technique of UV-visible spectrophotometry is one of the most frequently employed in pharmaceutical analysis.

The extent of absorption of radiation by a given absorbing system at a specific monochromatic wavelength is governed by two classical laws of absorptiometry.

Lambert's law

At a given concentration of a homogenous absorbing system, the intensity of a transmitted light decreases exponentially with increase in path length.

Beer's law

It is concerned with concentration. It states that transmittance decreases exponentially with increase in concentration of a homogenous system.

The combination of both laws gives Beer-Lamberts law,

$\log i_0/i = kcb$, where k=absorptivity of the system.

The assay of an absorbing substance may be quickly carried out by preparing a solution in a transparent solvent and measuring its absorbance at a suitable wavelength.

MULTICOMPONENT ANALYSIS

The different multicomponent analytical methods are

Using absorbance corrected for interference

If the identity, concentration and absorption of the absorbing interferents are known it is possible to calculate their contribution to the total absorbance of the mixture. The

concentration of the absorbing component of interest is then calculated from the corrected absorbance.

Assay after the solvent extraction of the solvent.

If the interference from other absorbing substance is large or if the contribution to the total absorbance can not to be calculated, it may be possible to separate the absorbing interferents from the analyte by solvent extraction procedures.

The concentration of the analyte can be obtained by simple measurement of absorbance of the extract containing the analyte.

Simultaneous equation method

In this method, sample containing two absorbing drugs (X and Y)each of which absorbing at the λ max of the other may be determined

The equation is : $A_1 = ax_1bc_x + ay_1bc_y$

$A_2 = ax_2bc_x + ay_2bc_y$

$ax_1$and ax_2 = absorptivity values of compound X at λ_1 and λ_2 respectively

ay_1 and ay_2 = absorptivity values of compound Y at λ_1 and λ_2 respectively

A_1 and A_2 = absorbance of diluted samples at λ_1 and λ_2 respectively

Absorbance ratio method

It depends on the property that, for a substance which obeys beer's law at all wavelengths, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length.

Derivative spectroscopy

Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum.

Geometric correction method

It is a mathematical correction procedure which reduces or eliminates the background irrelevant absorption that may be present in samples of biological origin.

Orthogonal polynomial method

The basis of the method is that an absorption spectrum may be represented in terms of orthogonal function

Experiment No : I

Date :

ESTIMATION OF SALICYLIC ACID BY COLORIMETRY

AIM: To estimate the amount salicylic acid in given sample by colorimetric method.

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

PRINCIPLE

The increasing concentrations of salicylic acid are treated with 1% ferric ~~chloride~~ ~~$FeCl_3$~~ $Fe(NO_3)_3$ Nitrate reagent (1g ~~$FeCl_3$~~ $Fe(NO_3)_3$ in 100ml of ~~1% Hydrochloric acid~~ 0.01N HNO_3). 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

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. A series of standard solutions were prepared in five numbered volumetric flasks. 5 ml from each standard was taken and transferred to labeled 10ml test tubes. 5 ml of unknown sample was transferred to test tube no 6. Add 5 ml of distilled water to 7th test tube which serves as blank. 2ml of ferric nitrate was added to each test tube and absorbance was measured at 540nm, which is proportional to concentration. The concentration of unknown was determined, from calibration curve.

REPORT

The concentration of salicylic acid in the given sample was found to be - - - -

~~The concentration of salicylic acid in the given sample was found to be~~

Experiment No : 11

Date :

EFFECT OF pH UPON THE ABSORPTION SPECTRUM OF GIVEN COMPOUND

AIM

To show the effect of pH upon the absorption spectrum of the given compound (sulphanilamide)

REQUIREMENTS

UV-Visible spectrophotometer, volumetric flasks, 0.1N NaOH, 0.1N HCl

PRINCIPLE

UV spectroscopy involves the measurement of the amount of ultraviolet or visible radiation absorbed by a substance in solution. Absorption of UV and visible radiation of the electromagnetic spectrum induces electronic transitions in a molecule and is associated with vibrational and rotational transitions.

The position and intensity of absorption is modified by structural changes or changes in solvent properties such as pH, polarity. Changes in spectra can also be produced by fully unsaturated groups attached to a chromophoric system and these are called auxochrome. The effects produced may be bathochromic shift, hypsochromic shift, and hyperchromic or hypochromic effect. The effect of change in pH in the solvent used for the UV measurements is very striking.

In sulphanilamide the primary amino group is a powerful auxochrome when attached directly to benzene system. In alkaline solution it is retained as the auxochrome. But in acidic solution the $-NH_2$ group is replaced by $-NH_3^+$ which is less efficient as auxochrome. So change in pH to acidic condition shifts the absorption maxima to lower wavelength (Blue shift or hypsochromic shift)

PROCEDURE

1. **Preparation of sulphanilamide in HCl**: weigh accurately 100mg of sulphanilamide and transfer to 100ml standard flask. Dissolve in little of HCl and make upto volume with HCl. Pipette out 1ml from this solution and transfer to 100ml standard flask and make up to the volume to 100ml with HCl

   Preparation of sulphanilamide in NaOH: weigh accurately 100mg of sulphanilamide and transfer to 100ml standard flask. Dissolve in little of 0.1M NaOH and make up to volume with NaOH. Pipette out 1ml from this solution and transfer to 100ml standard flask and make up to the volume to 100ml with NaOH
2. Switch on the UV –visible photometer and allow it to warm for about 15min.
3. Adjust the instrument to 100% T or zero absorbance using 0.1M HCl as blank for acidic solution and 0.1M NaOH for alkaline solution
4. Measure the absorbance of both solution in the range 225 -275 nm at a bandwidth of 10nm
5. Adjust the instrument to 100% T or zero absorbance after each change in wavelength
6. Plot the graph by taking absorbance on Y axis and wavelength on X axis
7. Calculate the absorption maxima from the plot

REPORT

Absorption maxima of sulphanilamide in alkaline and acidic solution was measured and found that as the pH changes from alkaline to acidic condition absorption maxima shifted to lower wavelength, from ------- to --------.

REFERENCE

1. A.H Beckett , J.B Stenlake, Practical pharmaceutical chemistry, fourth edition-part II, page No 320-321, 328

Experiment No :

Date :

EFFECT OF SOLVENT UPON THE ABSORPTION SPECTRUM OF PHENOL

AIM

To show the effect of solvent upon the absorption spectrum of phenol

REQUIREMENTS

UV-Visible spectrophotometer, volumetric flasks, phenol, water, cyclohexane

PRINCIPLE

UV spectroscopy involves the measurement of the amount of ultraviolet or visible radiation absorbed by a substance in solution. Absorption of UV and visible radiation of UV and visible radiation of the electromagnetic spectrum induces electronic transitions in a molecule and is associated with vibrational and rotational transitions. The position and intensity of absorption is modified by structural changes or changes in solvent properties such as pH, polarity.

A suitable solvent for the UV spectroscopy should meet the following requirements

- Should not itself absorb radiations in the region under investigation
- Should be less polar so that it has minimum interaction with the solute molecules

The Position and intensity of the absorption maxima also influenced by the nature of the solvent. When a group is more polar in the ground state than in the excited state, then increasing the polarity of the solvent stabilizes the non-bonding electrons in the ground state because of hydrogen bonding. Thus absorption is shifted to lower wavelength.

When the group is more polar in the excited state, then absorption gets shifted to longer wavelength with increase in polarity of the solvent which helps stabilizing the non-bonding electrons in the excited state.

The increase in polarity of the solvent generally shifts $n \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ bands to shorter wavelengths and $\pi \rightarrow \pi^*$ bands to longer wavelengths.

In cyclohexane little interaction between solvent and solute occurs and vibrational fine structure is observed. In water salvation of the solute and hydrogen bonding are possible so that the fine structure is almost eliminated, only the band envelope is obtained.

PROCEDURE

Prepare 0.002%w/v solution of phenol in a) water and b) cyclohexane. Record the absorption spectrum of each solution over the range 200-300nm using 1cm cells and the appropriate solvent as blank.

REPORT

Absorption spectra of 0.002% w/v solution of phenol in water and cyclohexane was measured and found that as polarity increases fine structure of spectra disappears

REFERENCE

2. A.H Beckett , J.B stenlake, Practical pharmaceutical chemistry, fourth edition-part II, page No 307, 327
3. Gurdeep R. Chatwal, Sham K.Anand, Instrumental methods of chemical analysis, Page No 2.160, 2.161

Experiment No : III

Date :

DETERMINATION OF λ MAX OF PARACETAMOL SOLUTION BY UV-VISIBLE SPECTROPHOTOMETER

Aim: To determine λ_{max} of paracetamol solution

Apparatus: 100ml volumetric flasks, NaOH, water

Chemicals: paracetamol, Sodium Hydroxide

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

λ_{max} is defined as 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. Chromophore is a functional group or a part of the molecule responsible for the absorption of electromagnetic radiation at a specific frequency. λ_{max} is characteristic of a compound and provides information of the electronic transitions 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 λ_{max}.

NOTE: λ_{max} *is* independent from the concentration of the analyte.

PROCEDURE

- 100mg of paracetamol was weighed and transferred to 100 ml volumetric flask and add 30 ml NaOH. Shake well and volume made upto 100 ml with distilled water
- Dilute 5 ml of this solution to 50 ml with distilled water
- Again pipette out 5 ml and transferred to 50 ml volumetric flask and add 5 ml NaOH and make up to the mark with distilled water.
- Prepare a blank in the same manner omitting the drug
- Take the solution of paracetamol in cuvette and scan from 200-400 nm.
- Plot a spectrum of paracetamol with wavelength on X-axis and absorbance on Y axis.
- Find out the wavelength at which maximum absorption takesplace. It is known as λ_{max}

Report:

The λ_{max} of paracetamol was found to be ..... nm

The λ_{max} of paracetamol was found to be ..... nm.

Experiment No : IV

Date :

ASSAY OF PARACETAMOL BY UV SPECTROSCOPY

AIM

To determine the percentage content of paracetamol by using $E_{1cm}^{1\% \text{ value}}$

PRINCIPLE

Paracetamol is chemically known as 4-acetamino phenol or para acetamido phenol. It has analgesic and antipyretic action. The assay of paracetamol is performed by technique of UV spectroscopy using solvent such as water, ether, n-butanol, ethanol etc. Paracetamol is insoluble in water. 0.1 M alkali such as sodium hydroxide is used to ionize the paracetamol into p-acetamino phenolate ion and λ max shifts bathochromically in aqueous solution from 243 to 257 nm.

The principle of assay of paracetamol is based on absorption spectrophotometry. The absorption of radiation is due to the fact that paracetamol contains electrons which can be raised to higher level by absorption of energy. The required energy can be supplied by UV radiation. It has conjugated double bonds. The absorption by paracetamol is greatly affected as it contains Chromophore benzene ring, NH_2 Groups acts as the Chromophore, whereas -OH groups acts as the auxochrome.

Assay involves the measurement of the amount of UV radiation absorbed by the substance in solution. The concentration is linearly related to absorbance as given by Beer's law.

$A = -\log T = \log I_0 / I = abc$

A-absorbance

T-transmittance

I_0 –incident radiant power

I-transmitted radiant power

b- Path length of sample

C-concentration of absorber

The assay is carried out by preparing solution of paracetamol in water and measuring its absorbance at 257 nm. The concentration is then calculated from the measured absorbance by the use of standard absorptivity value. The absorbance of 1% solution through a path length of 1cm was found to be 715at 257nm.

PROCEDURE

Weighed and powdered 20 tablets.Weighed accurately a quantity of the powder equivalent to about 0.18 gm of paracetamol. Added 50 ml of 0.1N NaOH, diluted with 100 ml of water shaked for about 15min and added sufficient water to produce 250 ml. Mixed, filtered and diluted to 10 ml of the filtrate to 100ml with water. To 10ml of the resulting solution add 10ml 0.1N NaOH. Diluted to 100ml with water and mixed. Measure absorbance of 1cm layer of resulting solution at the maximum of 257nm. Calculated the content of paracetamol by using 215 as value of specific absorbance.

REPORT:

The percentage purity of the given sample of Paracetamol was found to be..... %w/w

Experiment No : VI

Date :

ASSAY OF METFORMIN TABLETS I.P

AIM

To determine the percentage content of Metformin tablets by using $E_{1cm}^{1\%\ value}$

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

Chemicals : Metformin Tablets, Water

PRINCIPLE

Assay involves the measurement of the amount of UV radiation absorbed by the substance in solution. The concentration is linearly related to absorbance as given by Beer's law.

$A = -\log T = \log I_0 / I = abc$

A-absorbance

T-transmittance

I_0 –incident radiant power

I-transmitted radiant power

b- Path length of sample

C-concentration of absorber

PROCEDURE

Weigh and powder 20 tablet. Weigh a quantity of powder equivalent to 0.1g Metfromin Hydrochloride. Shake with 70ml of water for 15 minutes. Dilute to 100 ml with water and filter. Diluted 10 ml of the filtrate to 100ml with water. Further dilute 10ml to 100 ml. Measure the absorbance the absorbance of resulting solution at 232 nm. Calculate the content of Metfromin Hydrochloride taking 798 as a value of $A_{1\%}^{1cm}$ at maximum at about 232 nm.

REPORT

The percentage purity of the given sample of Metformin was found to be..... %w/w

Experiment No :

Date :

ASSAY OF FUROSEMIDE TABLETS

AIM

To determine the percentage purity of the given sample of furosemide

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

Chemicals: Furosemide Tablets, NaOH

Principle

Furosemide is 4-chloro-N-furfuryl-5-sulphamoyl - anthranilic acid. Furosemide is a loop diuretic. The estimation is based on Beer-Lamberts law. In this reaction, sodium hydroxide reacts with carbonyl group present in the benzene ring.Hence - NH_2 and - CH_2 act as auxochromes which enhances the absorption due to chromophores like benzene and furan rings.

According to Beer lambert's law,

A = abc, where

A → Absorbance

a → Absorptivity

b → Pathlength

c → Concentration

Procedure

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 0.2gm of furosemide and shake with 300ml of 0.1N NaOH for 10 minutes. Add sufficient 0.1N NaOH to produce 500ml and filter. Dilute 5ml to 500 ml with 0.1N NaOH and measure the extinction of a 1cm layer of the resulting solution at the maximum at about 271nm. Calculate the content of $C_{12}H_{11}ClN_2O_5S$, taking 580 as the value of E (1%, 1cm) at the maximum at about 271nm.

Reference

Indian Pharmacopoeia, 1996

Report

The % purity of furosemide tablets was found to be.

Experiment No :

Date :

ASSAY OF CHLORAMPHENICOL CAPSULES BY UV SPECTROSCOPY

AIM

To determine the amount of chloramphenicol present in the given sample

APPRATUS : Mortar and pestle, beaker, standard flasks, funnel

CHEMICALS : Chloramphenicol capsules, water

PRINCIPLE

Chloramphenicol exists in two isomeric forms like dextro and levo due to the presence of atomic molecule. Nitro group and chorine atom show absorption maxima at 278nm using water as solvent. Maximum absorption of chloramphenicol occurs in UV region at 278nm. Chloramphenicol absorbs UV radiation and undergoes transition from ground state to excited state. The transition of electron take place as $n \rightarrow \Pi^*$, $n \rightarrow \sigma^*$, $\Pi \rightarrow \Pi^*$, $\sigma \rightarrow \sigma^*$, the absorbed energy is directly proportional to the concentration of chloramphenicol in solution. The assay is carried out by UV-Spectroscopy based on Beer - Lambert's Law.

$A = abc,$

A → Absorbance

a → Absorptivity

b → pathlength

c → Concentration

Absorptivity 'a' can also be expressed as follows ie.; a= , means the absorbance of 1% W/V solution using a path length of 1cm. $A^{1\%}_{1cm}$ at a wavelength is a Constant value for each drug. This value is useful in estimating the concentration of drug in the sample formulations and solutions. λ_{max} is the wavelength at which maximum absorption taken place. The value of λ_{max} is constant for particular substances.

PROCEDURE

Weigh accurately a quantity of the mixed contents of 20 capsules equivalent to about 0.2 g of Chloramphenicol, dissolve in 800 ml of *water,* warming if necessary to effect solution and add sufficient *water* to produce 1000ml. Dilute 10ml of this solution to 100ml with *water* and measure the *absorbance* of the resulting solution at the maximum at about 278 nm. Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$, taking 297 as the value of at the maximum of about 278 nm.

REPORT:

The percentage purity of the given sample of chloramphenicol was found to be..... .....%w/w

Experiment No :

Date :

ESTIMATION OF QUININE SULPHATE BY FLUORIMETRY

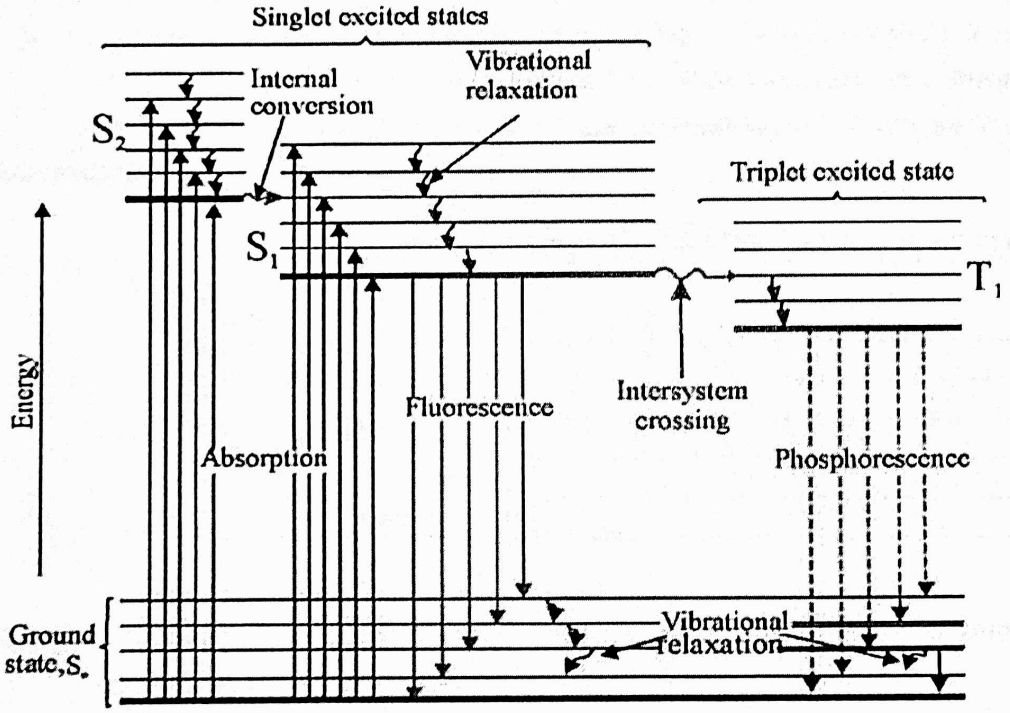
Aim: To estimate the amount of Quinine sulphate present in given sample by fluorimetry.

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 form 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 above diluted standard quinine sulphate solution in to a set of 10 ml volumetric flask and make up to 10 ml with 0.1N H_2SO_4 to get concentrationof 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 highest concentration of the standard solution.Measure the fluorescence of serial dilutionsand and plot the calibration curve (fluorescence intensity Vs concentration).

Observation:

S.No	Concentration (µg/ml)	% Fluoroscence 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 ……..

Experiment No :

Date :

EFFECT OF QUENCHING ON THE FLUORESCENCE OF QUININE SULPHATE

AIM: To estimate the effect quenching on the fluorescence of Quinine sulphate by Fluorimetry.

REQUIREMENTS: Quinine sulphate , Fluorimeter, Sulphuric acid, KI.

APPARATUS: Pipette, volumetric flask, measuring cylinder.

PRINCIPLE

The reduction in fluorescence intensity due to certain ions is known as quenching. These processes can occur during the excited state or they may occur due to formation of complexes in the ground state. Quenching is due to various factors like pH, concentration, temperature, viscosity, and presence of certain ions like halides.

Quenching mainly is of four types:

1. Self quenching
2. Chemical quenching
3. Collisional quenching
4. Static quenching

Presence of halides leads to quenching due to Collision.

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 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**) from this finallyprepare **1µg/ml** solution.

Preparation of KI solution

- Weigh accurately 100mg of KI, 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)**
- In six 10 ml volumetric flasks, take 1ml of **1µg/ml** solution in each.
- Add 1,2,3,4 and 5ml of KI solution in each flask. Make up the volume with 0.1N H_2SO_4
- Switch on the instrument and stabilize for 10-15min.
- Set excitation and emission filters at the wavelengths about 365 and 459nm respectively.
- Set the fluorescence intensity to 0% by using 0.1N H_2SO_4 as blank and 100% by using highest concentration of the standard solution (not containing KI). Measure the fluorescence of serial dilutions and plot a graph between (volume of KI Vs fluorescence intensity).

Observation:

S.No	Vol. of KI added	% Fluoroscence intensity
1	0	
2	1	
3	2	
4	3	
5	4	
6	5	

Experiment No :

Date :

ESTIMATION OF POTASSIUM CONCENTRATION BY FLAME PHOTOMETRY

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

REQUIREMENTS: Potassium chloride, Distilled water, Flame Photometer.

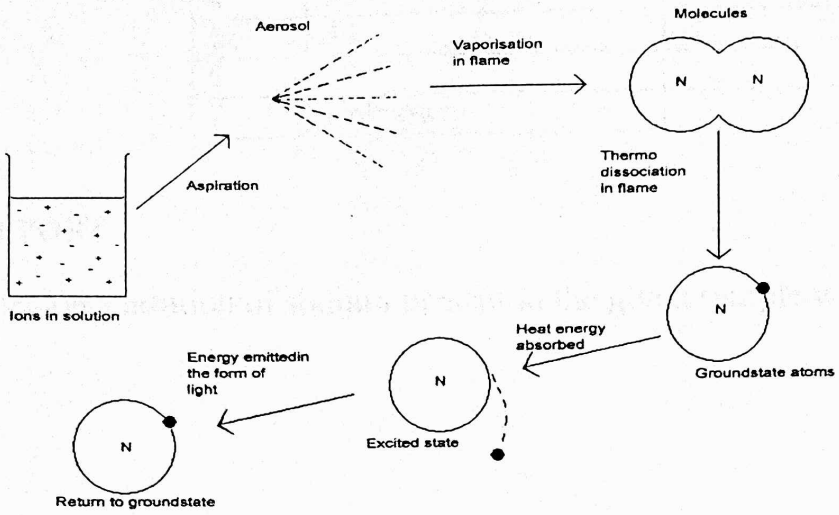
APPARATUS: Pipette, volumetric flask, measuring cylinder.

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 groundstate to their excited state. The atoms then return to their ground state and release their energy asphotons 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 flamephotometry



The intensity of radiation emitted by depends upon proportion of thermally excited 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 sodium filter and stabilize for 10-15min.
- Set the gas in flame in order 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 highest concentration of the standard solution. Measure the % flame intesity of serial dilutions and plot the calibration curve (% intensity Vs concentration).
- Find out the concentration of unknown sample from 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

Experiment No :

Date :

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.

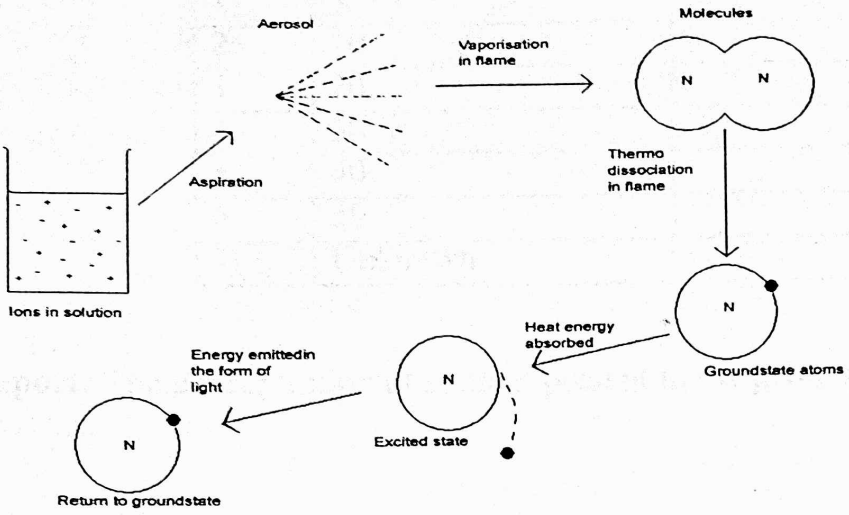
APPARATUS: Pipette, volumetric flask, measuring cylinder.

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 asphotons 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 flamephotometry



The intensity of radiation emitted by depends upon proportion of thermally excited 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 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 sodium filter and stabilize for 10-15min.
- Set the gas in flame in order 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 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 unknown sample from 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 ……..

CHROMATOGRAPHY

It is a physical separation method in which the components to be separated are selectively distributed between two immiscible phases a mobile phase flow

through a stationary phase bed.

Various techniques of chromatography

- Absorption chromatography
- A solid stationary phase and liquid or gaseous mobile phase
- Partition chromatography
- A liquid stationary phase and a liquid or gaseous mobile phase.
- Ion-exchange chromatography

A solid polymeric stationary phase containing replaceable ions and an ionic liquid mobile phase.

Gel chromatography

An inert gel which acts as a molecular sieve and a liquid mobile phase.

High performance liquid chromatography

HPLC utilizes a liquid mobile phase to separate the components of a mixture. The stationary phase can be a liquid or a solid phase.

TYPES

Types based on chromatographic modes

1. **NORMAL PHASE CHROMATOGRAPHY**
2. **ION-EXCHANGE CHROMATOGRAPHY**
3. **SIZE EXCLUSION CHROMATOGRAPHY**

4. REVERSE PHASE CHROMATOGRAPHY

NORMAL PHASE CHROMATOGRAPHY: - It is based on absorption or desorption of the analyte onto a polar stationary phase. It is particularly useful for the separation of non-polar compounds and isomers as well as for the fractionation of the complex samples by functional groups or for sample clean up.

ION-EXCHANGE CHROMATOGRAPHY:- In conventional ion exchange chromatography, electrostatic interactions between an analyte and stationary phase of opposite charge cause the analyte to absorb to the stationary phase. Common application is the analysis of ions and biological components such as amino acids, proteins/peptides and poly nucleotides.

SIZE-EXCLUSION CHROMATOGRAPHY: - It is a separation mode based solely on the analyte molecular size.

REVERSE-PHASE CHROMATOGRAPHY: - The separation is based on analyte partition coefficient between a polar mobile phase and no-polar stationary phase. The stationary phases are solid particles with bonding hydrophobic groups on silica support. It is suitable for analysis of polar, medium polar and some non-polar analytes.

Various column efficiency parameters in RP-HPLC include

- Resolution
- Separation factor
- Capacity factor
- Peak symmetry
- Number of theoretical plate
- Height equivalent to theoretical plate

QUANTITATIVE ANALYSIS

The basic theory for quantification involves the measurement of peak height peak area. To determine the concentration of a compound, the peak area or peak height is plotted versus the concentration.

The different calibration methods in quantitative analysis

- External standard method
- Internal standard method
- Standard addition method
- Area normalization method

EXTERNAL STANDARD METHOD:- A standard solution of known concentration of the compound of interest is preferred. A fixed amount, which should be similar in concentration to the unknown, is injected. Peak height or area is plotted versus the concentration of each compound.

Concentration unknown=area $_{unknown}$/area$_{known}$ xconcentration$_{known}$

INTERNAL STANDARD METHOD: - The internal standard method tends to yield the most accurate and precise results. In this method, an equal amount of internal standard, a compound that is not present in the sample is added to both sample and standard solutions.

Concentration unknown=area internal standard in known/area internal standard in unknown x area $_{unknown}$/area $_{known}$ x concentration $_{known}$

STANDARD ADDITION METHOD: - In this method area percentage of each peak is reported. For this, after completion of a run and the integration of all significant peaks in the chromatogram, the total peak area can then be calculated.

The area percentage of any individual peak is referred to as the area normalization or normalized peak area.

Experiment No :

Date :

SEPERATION AND IDENTIFICATION OF AMINO ACIDS BY ASCENDING PAPER CHROMATOGRAPHY

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

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

Chemicals:

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

Visualizing Agent: Ninhydrin solution

Standard references: Aminoacids- Phenyl Alanine, Glycine, Histidine

PRINCIPLE

When the development of paper is done by allowing the solvent to travel up the paper, it is known as ascending technique. It is a type of partition chromatography in which the substances are distributed between two liquids, one is the stationary liquid (usually water) which is held in the cellulose fibers of the paper and called stationary phase. The moving liquid or developing solvent is called the mobile phase. The components of the mixture to be separated migrate at different rates and appear as spots on different points on the paper.

In ascending chromatography the mobile phase is placed in the chamber. The samples are applied a few centimeters from the bottom edge of the paper suspended from a hook. The edge of the filter paper is dipped into solvent called developing solvent. The various components are moved by solvent system at various speeds. When the solvent has moved these substances to suitable height the paper is dried and various spots are visualized by suitable reagent called visualizing or spraying or chromogenic reagents. The movement of substances relative to the solvent is expressed in terms of R_f values. It is a constant for a given substance in a particular solvent system. The R_f value is always less than one.

Paper partition chromatography is a technique for the separation of amino acids. Amino acid sample is spotted on the paper followed by visualization using ninhydrin

which gives purple or brown colour spots. Amino acids react with ninhydrin to give hydantoin which condenses to give a purple colour.

Preparation of solutions:

Solution A: 0.5 gm of Phenyl Alanine in required quantity of suitable solvent
Solution B: 0.5 gm of Glyicine in required quantity of suitable solvent
Solution C: 0.5 gm of Histidine in required quantity of suitable solvent
Solution D: Unknown mixture

Procedure for Development of Chromatogram

- Prepare the chamber by placing the mobile phase (n-butanol :glacial acetic acid: water in the ratio 4: 1: 5) in the bottom to a depth of about 1cm and line the tank with filter paper
- Take a what man filter paper of 25 X 5 cm and draw a thin straight line about 3 cm above the bottom edge of the paper
- Fold the paper at right angles to the line, into cylindrical form and hold the edges together with clips – don't write
- Mark 4 points at equal distances on the straight line and put the first letter of amino acid to be applied on that spot
- With the help of a capillary tube apply the samples of amino acids on respective spots Place
- ~~Hang~~ the paper in the chromatographic chamber having the mobile phase
- Allow the solvent to run up the paper to about ¾ th of height of the paper.
- Remove the chromatogram from the chamber and mark the solvent front.
- Dry the chromatogram
- Spray ninhydrin solution over the chromatogram
- Dry the chromatogram in an oven at 60°C for 10-15 min. purple spots will develop.
- Calculate the R_f values and carry out the qualitative analysis
- R_f value = Distance travelled by the solute from the baseline / Distance travelled by the solvent front

Observation: Write on the left side.

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

Report:

The R_f values of **reference samples** are:

Phenyl Alanine

Glycine

Histidine

R_f Values of amino acids in the mixture are:

Based on the R_f Values, the mixture was found to contain

REFERENCE

4. Practical pharmaceutical analysis , Dr.G.Devala Rao, page No : 130
5. Gurdeep R. Chatwal, Sham K.Anand, Instrumental methods of chemical analysis, Page No 2.598
6. A.H Beckett , J.B stenlake, Practical pharmaceutical chemistry, fourth edition-part II, page No 107-109

Experiment No :

Date :

SEPARATION AND IDENTIFICATION OF AMINO ACIDS BY CIRCULAR PAPER CHROMATOGRAPHY

AIM: To perform the separation and identification of amino acids by circular paper chromatography

APPARATUS: Chromatographic chamber, sprayer, beaker, capillary tubes

MATERIALS: What man No.1 filter paper, ninhydrin, n-butanol, glacial acetic acid, water, amino acids (Phenyl alanine, glycine, histidine)

PRINCIPLE

It is a type of partition chromatography in which the substances are distributed between two liquids, one is the stationary liquid (usually water) which is held in the cellulose fibers of the paper and called stationary phase. The moving liquid or developing solvent is called the mobile phase. The components of the mixture to be separated migrate at different rates and appear as spots on different points on the paper.

Circular paper chromatography is also known as radial paper chromatography. This makes use of radial development. In this technique circular filter paper is employed. Then the material to be analyzed is placed at the centre. After drying the spots the paper is fixed horizontally on the Petri-dish possessing the solvent so that the tongue or wick of the paper dips into the solvent. Cover the paper by means of Petri-dish cover. The solvent rises through the tongue or wick. When solvent front has moved through a sufficient large distance, the components get separated in the form of concentric zones.

Amino acids are detected using ninydrin as the visualizing agent. Amino acids react with ninhydrin to give hydantoin which condenses to give a purple colour. \

PROCEDURE

- Obtain a petri-dish and a circular chromatographic paper, slightly larger than the diameter of the dish
- Determine the centre of the paper and through it divide the whole circle into approximately four equal segments.

- Draw a circle of about 1.5cm in diameter.
- Put the first letter of amino acid to be applied in that sector.
- Punch a small hole at the centre of the paper for insertion of a wick , made from a small piece of rolled up filter paper.
- Apply the sample on the penciled circle in its proper sector
- Dry the spots
- When the paper become dry, open the dish and place the paper on the dish
- Wick is introduced through the central hole into the solvent
- Cover the upper half of the petri-dish and allow the solvent front to advance in the paper mainly to the rim of the dish.
- Remove the cover and take out the developed chromatographic paper and mark the position of the solvent front. Dry the paper at $105^{o}C$ for 5-10 minutes in an oven
- Spray ninhydrin reagent
- Dry again
- Mark the centre of the arc obtained for each amino acid and calculate R_f values for each amino acid
- R_f value = $\frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}}$

Observation:

S.No	Aminoacid	Distance travelled By amino acid	Distance travelled By solvent front	Rf value

Report:

The Rf values of **reference samples** are:

Rf Values of amino acids in the mixture are:

Based on the Rf Values, the mixture was found to contain

REFERENCE

7. Gurdeep R. Chatwal, Sham K.Anand, Instrumental methods of chemical analysis, Page No 2.598
8. A.H Beckett , J.B stenlake, Practical pharmaceutical chemistry, fourth edition-part II, page No 107-109
9. Pharmaceutical analysis-II, Instrumental methods, P.C Kamboj page No 229