INSTRUMENTAL METHODS OF ANALYSIS

PRACTICAL LAB MANUAL

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

S.A.RAJA PHARMACY COLLEGE

RAJA NAGER, VADAKANGULAM-627116

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LABORATORY CERTIFICATE

This is to certify that Smt./ Sri …… has satisfactory completed the course of experiments in ……..... practical, prescribed by the Pharmacy Council Of India RGUHS for the …….. course in the laboratory of this college in the year

Date: **Signature of the Teacher In-charge**

Head of the Department

Name:…….

Reg.No. …….

Examination centre ……

Date of the Practical Examination……..

PARTICULARS OF THE EXPERIMENTS PERFORMED

Sl.No	DATE	NAME OF THE EXPERIMENTS	P.No.
1.		Introduction of U.v. spectroscopy	
2.		To carry out the estimation of ibuprofen in the given sample by UV spectroscopy.	
3.		To Estimation Of Paracetamol by U.V. Method	
4.		To estimate the amount of diclofenac present in given sample.	
5.		To estimate the amount of ampicillin present in the given sample.	
6.		To carry out the estimation of given amoxicillin capsule by double beam spectroscopy.	
7.		Simultaneous estimation of aspirin and caffeine from a given sample of combination formulation.	
8.		Simultaneous estimation of paracetamol and nimesulide by UV-Visible Spectroscopy method.	
9.		Simultaneous estimation of paracetamol and diclofenac in a combination formulation using UV-visible spectroscopy.	
10.		To carry out the simultaneous estimation of paracetamol and ibuprofen .	
11.		Introduction ofchromatography.	
12.		To separate and identify the amino acids by paper chromatography (ascending type).	
13.		To identify the given mixture of amino acid by circular [or] radial chromatography.	
14.		To carry out the separation and identification of aminoacid by thinlayer chromatography.	

15.		To carry out the separation and identification of agivenmixtureof the aminoacids by column chromatography.	
16.		Introducton of H.P.L.C.	
17.		To determine retention time (t_r) and AUC for a given sample of diclofenac sodium by HPLC method (qualitative analysis).	
18.		Estimation of Diclofenac sodium from given sample by HPLC method (quantitative analysis).	

Date:

Introduction To U.V –Visible Spectroscopy

The absorption of UV radiation which ranges from 200-400 nm .

The absorption of visible radiation which ranges from 400-800 nm .

In both UV and visible valence electrons absorb the energy thereby the molecule undergoes transition from ground state to excited state.

Instruments which measure the ratio of the intensity of two beams of light in the UV-visible region are called ultraviolet visible spectrophotometers.

Spectroscopy is a branch of science deals with the study of interaction of electromagnetic radiation with matter.

The energy of a EMR can be given by the following equation.

$E = h\nu$

Where , E = energy of radiation

h = plank's constant(6.624×10^{-34})

ν = frequency

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

$= h.c.\lambda^{-1}$

While, frequency $\nu = c/\lambda$

Where, c = Velocity of light in vaccume

λ = Wave length

When EMR travels, a medium containing atoms, molecules or ions where there absorption of energy, intensity of emergent light (intensity) of incident light.

DISCUSSION:-

Spectroscopy is one of the most atomic and molecular structure and is used in the analysis of wide range of sample. The study of spectroscopy can be carried out under the following headings.

ATOMIC SPECTROSCOPY:-

This deals with the interaction of EMR with atoms which are most commonly in their lowest energy state called the ground state.

MOLECULAR SPECTROSCOPY:-

This deals with the interaction of EMR with molecule. This results in transition between rational and vibration energy level in edition to electronic transition. Molecular spectra extends from the visible through infra red into microwave region.

RATION (MICRO-WAVE) SPECTRA:-

This spectra rises due to transition between the rotational energy of gaseous molecule on the absorption of radiation following in the microwave region. This spectra are shown by molecule which posses a dipole movement e.g. - HCl, CO, H_2O, vapours, NO, etc.

VIBRATIONAL – ROTATIONAL SPECTRA:-

These spectra arises due to transition included between vibrational energy level of the molecule of the absorption at radiation belonging to IR. IR spectra is shown by molecule when vibrational motion is accompanied by a change in the dipole movement of molecules.

NUCLEAR MAGNETIC SPECTRA:-

It arises due to transition included between the molecule spin energy level of a molecule in applied magnetic field.

ELECTRONIC SPECTRA:-

It arises due to electronic transition in a molecule by absorption radiation following in visible and UV region.

ELECTRONIC SPIN RESONANCE:-

ESR results from transitions induce between electron spin energy of molecules in an applied magnetic field.

LAW OF PHOTOMETRY:-

1) **BEER'S LAW:-**

It states that when monochromatic light passes through a transparent medium containing sample. The rate of decreasing the intensity is directly proportional to the increase in the concentration of absorbance species.

$$I_t = I_0 10^{-kc} \ldots\ldots\ldots\ldots (1)$$

Where, I_t = intensity by travelled light

I_0 = intensity of incident light

k = constant

C = conc. of medium.

It states that when monochromatic light passes through at transparent medium, the decrease in the intensity of transmitted medium radiation is directly proportional to the increase in thickness of medium .

$I_t = I_0 10^{-kt}$……..(2)

Combining (1) & (2)

$I_t = I_0.10^{-kct}$

$I_t = I_0.10^{-act}$

Above equation is called as beer's – lambart's law and can be written as follows.

$\log I_0/I_t = act$

$A = act$

Where,

A = Absorbance

a = molecular absorptivity

t = thickness or pathlength of medium

c = $conc^n$ of medium

Experiment No:1 Date:

ESTIMATION OF IBUPROFEN

AIM:-To carry out the estimation of ibuprofen in the given sample by UV spectroscopy.

REQUIREMENT:-Beaker, volumetric flask, measuring cylinder, pipette (10ml, 1ml)

PRINCIPLE OF UV SPECTROSCOPY:

The type of electrons present in any molecule may be conveniently classified as

σ electron: present in saturated compounds such electrons do not absorb near UV, but absorb vacuum UV radiation.

π electron: present in unsaturated compound example: double bond or triple bond.

n electron: these are non bonded electrons which are not involved in any bonding example: lone pair of electrons

Any molecule has either n electron, π electron or σ electron or combination of these electrons. These bonding and nonbonding electrons absorb the characteristics UV radiation and undergoes transition from ground state to excited state by the characteristic absorption peaks, the nature of the electron present and hence the molecular structure can be elucidated.

1. LAMBART'S LAW:-

This law can be stated as follows when a beam of light as allowed to pass through a transparent medium, the rate of decrease of intensity with thickness of medium is directly proportional to intensity of light.

Mathematically, the lambart's law may be stated as follows.

$-dI/dt = KI$……(1)

Where, I = intensity of incident light of wave length (λ)

t = thickness of medium

k = Proportionality constant

Integrating equation & putting $I= I_0$ & t=0

$In\ I_0/I_t = kt$

$I=I_0.e^{-kt}$

$I_t= I_0 10^{-kt}$ (changing equation to natural log)

Where, k = 1/ 2.303

2. BEER'S LAW:-

The intensity of beam of monochromatic light decreases exponentially with increase in concentration by absorbing substance arithmetically.

$I_t = I_0 e^{-k'c}$

$I_t = I_0 e^{-kc}$

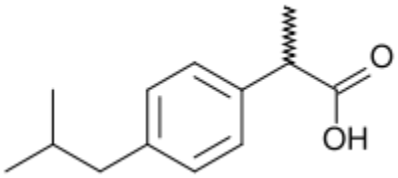
Combining equation,

$I_t = I_0.10^{-act}$

$\log I_t/I_0 = act$…….(3)

Its called beer's- lambart's law.

Structure of Ibuprofen:



PROCEDURE:-

Preparation of standard solution:-

Weigh accurately 100 mg of ibuprofen powder, add 0.1 NaOH to dissolve & make up volume upto 100 ml and make different concentration 5,10,15,20,25,30 μg/ml.

Preparation of sample solution:-

Weigh accurately 10 mg of powder drug & dilute it to 100 ml to give 100 μg/ml concentration. Take 1.5 ml of above solution & dilute to 10 ml with 0.1N NaOH to give concentration of 15 μg/ml.

Find out λ_{max} for standard & carryout absorbance value for each sample.

REPORT:-

The λ_{max} value for ibuprofen found to be ……nm.

The unknown concentration was found to be…… μg/ml.The concentration of ibuprofen in tablet was found to be ……

Experiment No:2 Date:

ESTIMATION OF PARACETAMOL

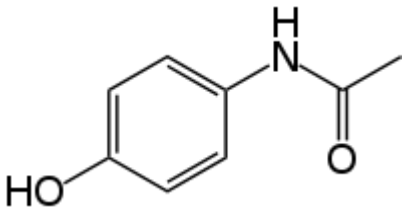
Aim: To Estimation Of Paracetamol by U.V. Method

References: I.P. 1996. vol-I, Organic Spectroscopy, 3rd edition by William Kemp.

Apparatus: Volumetric flask, pipette, beaker, measuring cylinder.

Chemicals: Paracetamol (drug), 0.1 N HCl.

Structure of paracetamol:



Pharmaceutical uses of paracetamol:

analgesic for headache, musculoskeletal pain, etc

Procedure:

1) Preparation of stock solution: Dissolve 100 mg of Paracetamol (drug) in sufficient 0.1 N HCl to produce 100 ml. From above stock solution 1ml is taken & again diluted to 25 ml – 40 μg/ml which is the final stock solution. Take 1ml,2ml,3,4,5ml and make it upto 10ml, To get 4, 8, 12, 16, 20μg/ml. Respectively

2) Preparation of sample solution: Dissolve the given sample in 100ml 0.1N HCl. Take 1ml of this solution and make the volume upto 25ml with 0.1N HCl and estimate the amount of pracetamol in the given sample at wavelength 244 nm.

Report: The amount of pracetamol in given sample was found to be……

Experiment No:3 **Date:**

ESTIMATION OF CAFFEINE

AIM:- To carry out the estimation of caffeine from a given sample by U.V spectroscopy.

REQUIREMENT:- Pipette, beaker, volumetric flask, digital balance, spatula , funnel.

REAGENT:- 7% of ammonia solution, Distilled water.

STRUCTURE OF CAFFEINE:-

PHARMACEUTICAL USES OF CAFFEINE:

Bronchial asthma and COPD

PROCEDURE:-

⇒**ESTIMATION OF CAFFEINE:-**

⇒**Standard Solution**:- Make different concentration of 4,8,12,16,20 µg/ml and measure absorbance at λ_{max} 272 nm. Draw a standard plot of absorbance v/s concentration.

- **Sample Preparation**:- Weigh powder accurately eq. to 30 mg of caffeine in 2 ml of distilled water. Dilute 0.5 ml of the suitable to produce final concentration (µg/ml) of caffeine citrate. Measure absorbance at 272 nm taking water as a blank.

- **REPORT:-** The amount of Caffine citrate in given sample was found to be……

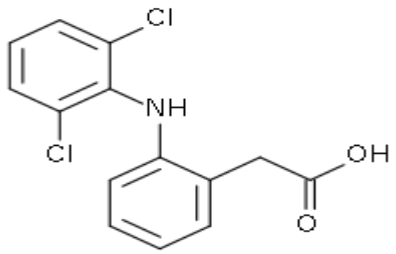
Experiment No:4 Date:

ESTIMATION OF DICLOFENAC SODIUM

AIM:- To estimate the amount of diclofenac present in given sample.

REQUIREMENT:- Beaker, volumetric flask, stirrer, digital balance, funnel, NaOH, standard diclofenac sodium.

STRUCTURE OF DICLOFENAC :-



PHARMACEUTICAL USES DICLOFENAC SODIUM:-

Short term analgesics and long term anti inflammatory activity

PROCEDURE:-

Preparation of standard solution:-

Weigh accurately 100 mg of diclofenac sodium, dissolve & make up the volume to 100 ml with 0.1 N NaOH, make different concentration 4, 8, 12, 16, 20 μg/ml. Plot the standard graph.

Sample Solution :-

Weigh accurately given sample & dilute with 0.1N NaOH & prepare 8 μg/ml concentration..

REPORT:-

The amount of diclofenac sodium in the sample was found to be_____mg.

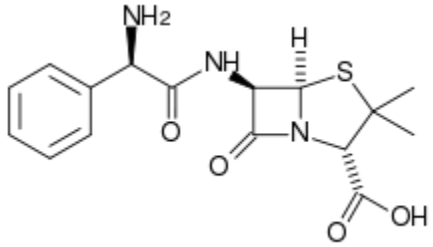
Experiment No:5 Date:

ESTIMATION OF AMPICILLIN

AIM:- To estimate the amount of ampicillin present in the given sample.

REQUIREMENT:- Beaker, volumetric flask, stirrer, digital balance, funnel, fehling's A& B, standard ampicillin.

STRUCTURE OF AMPICILLIN:-



PHARMACEUTICAL USES OF AMPICILLIN:-

Urinary tract infection, respiratory tract infection, meningitis, gonorrhoea

PROCEDURE:-

Preparation of standard solution:-

Weigh 50 mg of ampicillin, dissolved in water & make up the volume 50 ml & make up different concentration 10, 20,30, 40, 50ml. To 2 ml of standard solution, add 3 to 5 ml of water, then add 0.5 ml of fehling's solution (A & B). After 1 min. measure the absorbance in visible spectroscopy.

Preparation of sample solution:-

Accurately weigh 50 mg of powdered ampicillin & 40 ml of water, Shake & make up the volume to 50 ml, to produce 1µg/ml.

Fehling's solution:-

Prepare by stirring weighed i.e. 2 ml of fehling's solution A & B & diluting to 50 ml with water.

REPORT:-

The given sample found to be_____.

Experiment no:6 Date:

ESTIMATION OF AMOXYCILLIN

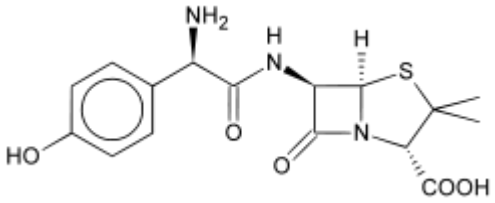
AIM:- To carry out the estimation of given amoxicillin capsule by double beam spectroscopy.

REQUIREMENT:-

Reagents- PDAB (P-Dimethyl- amino- benzyl, distilled water)

Apparatus- pipette, beaker, volumetric flask, measuring cylinder.

STRUCTURE OF AMOXYCILLIN:-



PHARMACEUTICAL USES OF AMOXYCILLIN:-

Urinary tract infection, respiratory tract infection, meningitis, gonorrhoea

PROCEDURE:-

Weigh accurately 400 gm of PDBA, add 10 ml of ethyl alcohol & 2 ml of concentrated H_2SO_4 & dilute to 50 ml with water.

Standard solution –

1 mg/ml of amoxicillin in water.

Sample solution –

Weigh accurately equivalent 25 mg of amoxycillin & 15 ml of water & heat on water bath to dissolve & adjust to 25 ml with water.

To 2 ml of the sample & standard, add 4 ml of PDBA reagent & heat on water bath at 60°C for 1 hr cool to room temperature & adjust the volume to 10 ml with water. Measure the absorbance of both sample & standard at 410 nm. The method obeys beer's –lambart's law in concentration reagent of 10-600 µg/ml.

REPORT:- The given sample was found to be_____ µg/ml.The concentration of amoxicillin in tablet was found to be_____ µg/ml &_____%.

QUANTITATIVE ANALYSIS

1. Calibration curve method: In a single standard method, when error is introduced in preparing the solution or measurement of absorbance, the error in result would be greater eliminate or to minimize this error, we can use calibration curve method. In calibration curve method, a calibration curve is plotted using concentration Vs absorbance value of 5 or more standard solutions. A straight line is drawn either through maximum no. of points or in such a way that there is equal magnitude of positive and negative errors. From the absorbance of the sample solution and using the calibration curve, the concentration of the drug ,amount and the percentage purity can be calculated.

2. single standard or direct comparison method: In this method, the absorbance of a standard solution of known concentration and a sample solution is measured. The concentration of unknown can be calculated using the formula.

$$A_1 = €c_1t$$

$$A_2 = €c_2t$$

Where,

A_1, A_2 – Absorbance of standard & sample

c_1, c_2 – Concentration of standard & sample

€ – Molecular extinction coefficient

t – Path length (1 cm)

On dividing, we get

$$A_1/A_2 = €c_1t /€c_2t$$

$$A_1/A_2 = c_1/c_2$$

$$c_2 = c_1A2/A1$$

Since, c_1, A_1 and A_2 are known, c_2 can be calculated.

3.Using $E^{1\%}_{1cm}$ values: This method can be used for estimation from formulation or raw material, when reference standard is available. $E^{1\%}_{1cm}$ means the absorbance of 1% w/v solution, using a path length of 1 cm. $E^{1\%}_{1cm}$ at a wavelength is a constant value for each drug and can be seen in Pharmacopoeias and standard books on the subject.

SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION

when no region can be found out free from overlapping spectra of two chromophores (groups that produce color in a compound), it is still possible to devise a method based on measurement at two or more wavelengths. Two dissimilar chromophores have different powers of radiation absorption at some or several points in their absorption spectra. If, therefore measurements are made on an unknown solution at two wavelengths where the absorptivities of the two components are different, it is possible to set up two independent equations and solve them simultaneously for the two unknown concentrations. First, it is necessary to select two points on the wavelength scale where the ratio of the molar absorptivities is maximal. Next, it is necessary to calculate the molar absorptivity for each component at each wavelength selected.Thus, two simultaneous equations may be written: $C_1(\epsilon_1)\lambda_1 + C_2(\epsilon_2)\,\lambda_1 = A\,\lambda_1$

$$C_1(\epsilon_1)\lambda_2 + C_2(\epsilon_2)\,\lambda_2 = A\,\lambda_2$$

The equations are solved for the concentration of each component

Experiment No:7 Date:

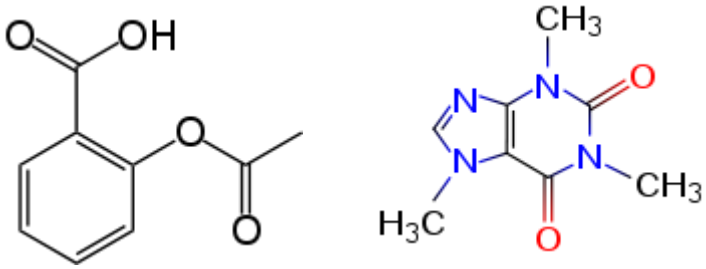
SIMULTANEOUS ESTIMATION OF ASPIRIN AND CAFFEINE

AIM:- Simultaneous estimation of aspirin and caffeine from a given sample of combination formulation.

REQUIREMENT:- Pipette, beaker, volumetric flask, digital balance, spatula , funnel.

REAGENT:- 7% of ammonia solution, Distilled water.

STRUCTURE OF ASPIRIN AND CAFFEINE:



PROCEDURE:-

⇒**ESTIMATION OF CAFFEINE:-**

- **Standard Solution**:- Make different concentration of 4,8,12,16,20 µg/ml and measure absorbance at λ_{max} 272 nm. Draw a standard plot of absorbance v/s concentration.
- **Sample Preparation**:- Weigh powder accurately eq. to 30 mg of caffeine in 2 ml of distilled water by gentle shaking and filter thorough a sintered glass funnel. Retain the residue for estimation of aspirin. Dilute 0.5 ml of the filtrate suitable to produce final concentration (µg/ml) of caffeine citrate. Measure absorbance at 272 nm taking water as a blank.

⇒**ESTIMATION OF ASPIRIN :-**

- **Standard Solution:-** Weigh accurately 100mg of aspirin, dissolve and dilute to 100 ml by 7% ammonia solution, make proper dilution to produce 4, 8, 12, 16, 20 µg/ml concentration solution , measure absorbance of each solution and plot standard graph.

⇒**PREPARATION OF SAMPLE:-** Dissolve entire insoluble residue obtained above in 6 ml of 7% ammonia solution to produce concentration of about 10 µg/ml. Measure absorbance at 240 nm using 7% ammonia solution as a blank.

REPORT:-

The given sample contains _____of Aspirin and_____ of caffeine.

Experiment No:8 Date:

SIMULTANEOUS ESTIMATION OF PARACETAMOL AND NIMESULIDE

AIM:- Simultaneous estimation of paracetamol and nimesulide by UV-Visible Spectroscopy method.

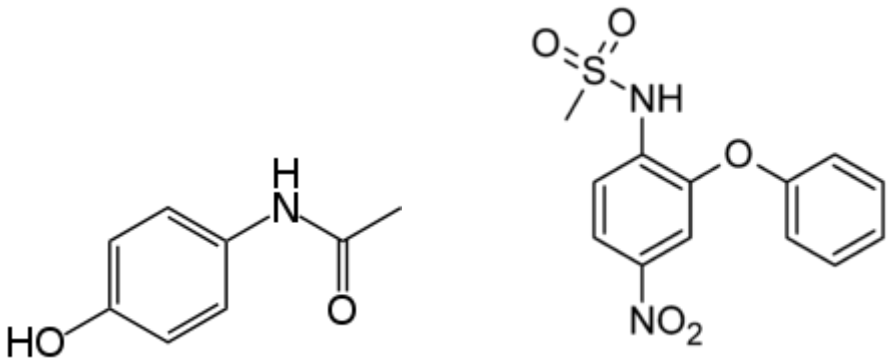
REQUIREMENT:- Beaker, volumetric flask, pipette, digital balance, spatula.

REAGENTS:- 0.1N HCl,

0.1N NaOH,

Distilled water.

STRUCTURE OF PARACETAMOL AND NIMESULIDE:



PROCEDURE:-

⇒ **Preparation of standard solution of PCM & Nimesulide:-**

Weigh accurately quantity of PCM & nimesulide & dilute it with 0.1 N HCl and 0.1 N NaOH respectively, to yield a final solution of 4,8,12,16,20 µg/ml. Measure absorbance for each and plot a standard graph of absorbance v/s concentration.

⇒ **Preparation of sample solution:-**

Take accurately weighed quantity of tablet powder eq. to 100 mg PCM, dissolve in 0.1 N NaOH in 10 ml volumetric flask, make up the volume to 100 ml, filtrate it. Filtrate was dissolved in 0.1 N HCl and make up the volume up to 100 ml. Analyse both drug by UV-spectroscopy.

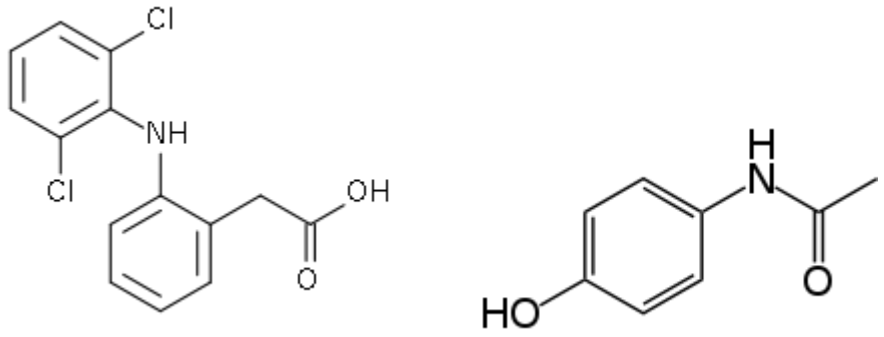
REPORT:-Given sample of tablet contains _____of PARACETAMOL and _____ of NIMESULIDE of labeledvalue.

Experiment No:9 Date:

SIMULTANEOUS ESTIMATION OF DICLOFENAC AND PARACETAMOL

AIM:- Simultaneous estimation of paracetamol and diclofenac in a combination formulation using UV-visible spectroscopy.

STRUCTURE OF DICLOFENAC AND PARACETAMOL:



PROCEDURE:-

⇒ESTIMATION OF PARACETAMOL:-

- **Standard Solution:-** 10 µg/ml of PCM in 0.1 N HCl
- **Sample Solution:-** Weigh accurately powder sample eq. to about 100 mg of PCM. Carry out the drug extraction with twice 25 ml portion of 0.1 N HCl. Filter through C_4 sintered glass funnel. Combine the filtrate to 100 ml with acid to get final concentration of above 10 µg/ml, dilute the filtrate further approximately.

  Measure the extraction of both sample and standard solution at λ_{max} for about 244 nm.

⇒ESTIMATION OF DICLOFENAC SODIUM:-

- **Standard Solution:-** 100 µg/ml

- **Sample Solution:-** Entire residue left after the extraction of paracetamol as described above is used for estimation of diclofenac. Transfer the residue left in conical flask and on top of sintered funnel quantitatively to 100 ml volumetric flask with the help of 0.1 N NaOH. Make up the volume and carry out further dilution approx. with NaOH to get final concentration of 100 µg/ml.

  Measure the absorbance of standard and sample at about 276 nm using 0.1 N NaOH as a blank.

  REPORT:-

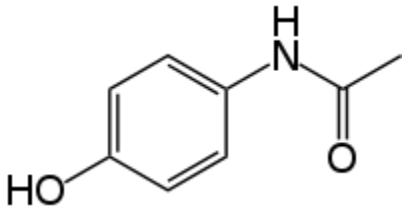
  The given sample of tablet contains _____of paracetamol and _____ of diclofenac sodium.

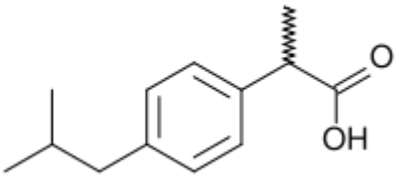
Experiment No:10 Date:

SIMULTANEOUS ESTIMATION OF PARACETAMOL AND IBUPROFEN

AIM:- To carry out the simultaneous estimation of paracetamol and ibuprofen .

STRUCTURE OF PARACETAMOL AND IBUPROFEN:





PROCEDURE:-

⇒ **ESTIMATION OF PARACETAMOL:-**

- **Standard Solution:-** 10 µg/ml of PCM in 0.1 N HCl.
- **Sample Solution:-** Weigh accurately exact quantity of sample eq. to 100 mg of paracetamol. Extract the drug and filter through C_4 sintered glass funnel. Measure the extinction of both solution at λ_{max} of about 244 nm using HCl as a blank.

⇒ **ESTIMATION OF IBUPROFEN:-**

- **Standard Solution:-** 250 µg/ml of ibuprofen in methanol.
- **Sample solution:-** Dissolve the entire residue obtained in the estimation of PCM in methanol to make 10 ml. Further dilution are done approximately and step wise with methanol to get the final concentration of 250 µg/ml. Measure of both sample and standard solution at a maximum of about 267 nm using methanol as a bank.

REPORT:-

The given sample contains _____mg of paracetamol and _____mg of ibuprofen.

CHROMATOGRAPHY

Chromatography is a powerful separation method that finds application in all branches of science ,chromatography was invented and named by the Russian botanist Mikhail Tswett at the beginning of the twentieth century .

He employed the technique to separate various plant pigment such as chlorophyll and xanthophylls.

The name chromatography (greek chroma –colour and graphy –writing)means colour writing.

It is used to resolve a multicomponent mixture of trace,minor or major constituents into its individual fractions. Final identification requires confirmation by some other analytical procedures, such as infrared spectroscopy ,NMR or mass spectrometry qualitative analysis can be carried out by measuring the area of the chromatographic peak. Hence chromatography can be used for qualitative analysis.

Definition_: chromatography may be regarded as an analytical technique employed for purification and separation of organic and inorganic subtrates.chromatography is mainly based upon then principle that all the substances have a tendency of adhering to the substrates with definite forces which is a combination of adsorption, partition and ion exchange.

Classification in chromatography:

Based on the nature of the fixed and moving phase different types of chromatography are as follows

1. Adsorption chromatography
2. Partition chromatography
3. Gas chromatography
4. Ion exchange chromatography
5. Exclusion chromatography

1. Adsorption chromatography;

It is a technique in which the stationary phase is solid(ex :alumina or silica) and the mobile phase is either a gas or liquid. Separation takes place when one component of a two component mixture is strongly adsorbed than the other by solid stationary phase ,the adsorbed components are then eluted by passing suitable solvents through the column. The commonly used adsorbents for column adsorption chromatography are sucrose, cellulose .starch ,calcium carbonate,calcium sulphate, silica gel,magnesium carbonate, charcoal etc.

2. Partition chromatography:

In this technique the stationary phase is a liquid, frequently water held on a suitable inert porous solid such as cellulose. The mobile

phase can be gas or liquid mixture. In this case the solute gets distributed between the fixed liquid and the moving liquid (solvent).

Ex: paper chromatography is a special type of partition chromatography in which the adsorbent column is a paper strip.

The commonly used solvent systems are

Stationary phase: Water

Water + acid

Water + alkali

Water + buffer

Alcohols

Formamide

Glycols.

Mobile phase:

Alcohols (n-butanol)

Isobutanol

Hydrocarbons(benzene,toluene,hexane)

Chloroform

Ethyl acetate

3. **Gas chromatography (Gc)**

When the moving phase is a mixture of gases it is called gas chromatography or vapor phase chromatography

The carrier gases generally used are helium and nitrogen.

Gas liquid chromatography :

The mobile phase is a gas and the stationary phase is a thin layer of nonvolatile liquid bound to a solid support

Gas solid chromatography :

This utilizes the solid adsorbent as the stationary phase and adsorption process takes place.

Advantages of GC :

The technique has strong separation power and even complex mixtures can be resolved in to constituents.

The sensitivity of the method is quite high ,it is a micro method and only a few milligram of the sample is sufficient for analysis.

4. Ion exchange chromatography

Ion exchange is a process in which an interchange of ions of like signs takes place between a solution and essentially in soluble solid (ion exchanger) in contact with the solution .In ion exchange chromatography a reversible exchange of ions is possible between ions in a liquid phase (mobile phase) of a solid, insoluble substances containing ionic sites (ion exchange resin). Ion exchange resin consists of beads of highly polymerized cross linked organic material containing large number of acidic or basic groups.

5. Exclusion chromatography :

It is a chromatography process in which separation of sample component takes place according to molecular size . The two types of exclusion chromatography techniques are

- Gel permeation
- Sieving separation

The other types of chromatograph include

- Thin layer chromatography
- High pressure or(performance) liquid chromatography
- High pressure thin layer chromatography (HPTLC)
- Counter current chromatography

Theories of chromatography ;

- Plate theory
- Rate theory

Plate theory

Plate theory was developed by Martin and singe , according to this theory a chromatographic column consist of series of discrete yet continuous horizontal layer which are termed as the theoretical plates An equilibration of the solute between the stationary and mobile phase takes place at each of these plates . Migration of solute occurs by a series of stepwise transfer between one plates to the other. The efficiency of separation in a chromatographic column gets increases as the no of theoretical plate increases.

No of theoretical plates is given by $N = L / H$

Where L = length of the column

H= height equivalent to theoretical plate

Rate theory

Rate theory is able to explain the effect of variables such as mobile phase velocity and adsorbabilities which determine the width of an elution band it also relates the effect of these variables on the time taken by a solute to make its appearance at the end of the column. If a

molecule is attached to the stationary phase its migration down the column is temporarily stopped , but the zone passes on that is one molecule make it immobilized temporarily on the column while the molecule migrate . A particle can migrate only if it is present in the mobile phase and as a result migration down the column is highly irregular thus some solute molecules may migrate rapidly where as others may lags behind, the net result of all these random individual process the width of the zone gets increased as it migrates down the column because more time is needed for migration to take place hence the zone width is directly related to residence or retention time on the column and inversely proportional to the mobile phase velocity.

Development of the chromatogram

Three methods have been used to develop chromatogram , they are frontal analysis , elution analysis ,and displacement analysis .

Frontal analysis

This method was developed by Tisellius . It consists of passing the sample solution continuously through the adsorbent column . This makes the active centres of the adsorbent column being occupied by the more strongly adsorbed solutes. The less strongly adsorbed solutes are adsorbed down the column or collect in the migrating solvent front . the solvent issues out first from the column exit followed by the least

adsorbed solute . Other solutes emerge one after another depending upon their degree of adsorbtivity

Elution analysis

This technique is most widely used to develop chromatograms . In this technique a small quantity of sample solution is introduced at the top of the column, pure solvent is than poured down the column , this give rise to differential migration of solutes in the mobile phase , each solute emerges from the column exit depending on its partition coefficient . If the partition coffiecient for the solute in the samples are sufficiently different the mixtures separates out in to the bands which migrate at different rates.

Displacement analysis

In this methiod, a small quantity of a sample solution is first introduced at the top of the column . the component of the mixtrure are than separated by running a solution of substances which is more strongly adsorbed than any off the component of the mixture . the substance thus run is termed as displacing agent.

Composition of what Mann filter paper:

Alpha cellulose	98.99 %
Beta cellulose	0.3 -1%

Pentosans - 0.4-0.8%

Ether soluble matter 0.015-0.02%

Ash 0.07-0.01%

Mobile phases for paper chromatography:

Isopropranol - Ammonia –water

n-butanol – acetic acid – water

Water- phenol.

Formamide – chloroform

Formamide – chloroform – benzene

Formamide - benzene – cyclo hexane

Locating or visualizing agents ;

Ninhydrin reagent – for detection of amino acids , amino sugar ,

Aniline phthalate - for detection of reducing sugars

Antimony trichloride in $CHCl_3$ - for steroids and glycosides

Dragendroff s reagent – alkaloids

Bromocresol purple – halogen ions (except Flourine) dicarboxylic acids

Bromocresol green – carboxylic acids

Experiment No:11 Date:

PAPER CHROMATOGRAPHY

AIM: To separate and identify the amino acids by paper chromatography (ascending type).

REQUIREMENTS: Whatmann filter paper grade-1, paper chromatography chamber, capillary tube, test tubes, standard amino acid solutions, sample amino acid solution, Ninhydrin reagent.

PRINCIPLE: Paper chromatography is based on the principle of partition in which the substances are distributed between two liquids, i.e., one is the stationary liquid (usually water) which is held in the fibers of the paper and called the stationary phase, the other is the moving liquid or developing solvent and called the moving phase / mobile phase. The components of the mixture to be separated migrate at different rates and appear as spots at different points on the paper.

In this technique, a drop of the test solution is applied as a small spot on the filter paper and the spot is dried. The paper is kept in a closed chamber and the edge of the filter paper is dipped into a solvent called developing solvent. As soon as the liquid gets through its capillary axis and when it reaches the spot of the test solution (a mixture of two or more substances), the various substances are moved by solvent system at various speeds. When the solvent moves these

substances to a suitable height, the paper is dried and the various spots are visualized by suitable reagents called visualizing reagents. The movement of the substance relative to the solvent is expressed in terms of R_f values which are a migration parameter.

DISCUSSION: The analysis of unknown substances by the flow of solvent on a filter paper is known as paper chromatography. This technique proceeds by mechanism which is partly partition (distribution) and partly adsorption. The constituents of a mixture are distributed between the water held in the filter paper and organic solvent.

TYPES: Ascending chromatography, descending chromatography, ascending-descending chromatography, radial chromatography, two-dimensional chromatography.

Migration parameters: The position of migrated spots on the chromatogram is indicated by different terms such as R_f, R_x, R_m, and R_c. These parameters are quantitative and qualitative characteristic of a substance.R is a function of the partition co-efficient. It is a constant for a given substance, provided the conditions of the chromatographic system are kept constant with respect to temperature, type of paper, duration, and direction of development, etc.

R_f=Distance traveled by the solute from the origin line

Distance traveled by the solvent from the origin line

R_x=Distance traveled by the substance from the origin line

Distance traveled by the standard substance 'x' from origin line

R_m=log $(1/R_F - 1)$

In some cases, the solvent front runs off the end of filter paper, the movement of a substance in such cases is expressed as R_x.

- R_m is used when R_f values of chemically related compounds are very close.

Types:

(1) Partition chromatography (paper used).

(2) Paper adsorption chromatography (paper first impregnated with an adsorbent, like silica or alumina).

PROCEDURE:

- Take a sheet of Whatmann paper draw a line 2-4cm above the bottom boundary.
- Apply 5 micro liters of amino acid solutions at an interval of 1-2cm.
- Dry this in a stream of hot air.
- Place this paper onto the paper chromatography chamber, saturated with solvent system.

- Allow the edge of the paper to touch the solvent.
- Run the chromatogram until the solvent has reached 3/4th of the strip for 1hr.
- Now take out the paper and dry it in a stream of hot air.
- Visualize the spots by spraying Ninhydrin reagent on the paper and dry it.
- Calculate the R_f value and report it.

APPLICATIONS:

(1) **Purity of the sample:** Purity of the sample is routinely carried out for this direct comparison of the sample and authentic sample is done, if the impurity present is shown as extra spots and this can be detected very easily.

(2) **Examination of the reaction:** To assess whether the reaction is complete or otherwise, this method is also used in checking other separation process and purification process like distillation, molecular distillation.

(3) In chromatography, it is used for the identification of natural products like volatile oil, fixed oil, waxes, terpenes, alkaloids, glycosides, steroids, etc.,

(4) **Biochemical analysis:** Separation of biochemical metabolites / constituents from its body fluids, blood plasma serum, urine, etc.,

(5) For the detection of impurities in a pharmacopoeia drug analysis.

(6) Various drugs like hypnotics, sedatives, anticonvulsants, tranquilizers, local anesthetics, steroidal drugs have been tested qualitatively.

(7) Important in multicomponent separation and formulation.

REPORT: The R_f value of sample A was found to be …….. and …….**.** Therefore the sample contains

Experiment No:12 Date:

CIRCULAR PAPER CHROMATOGRAPHY

Aim: To identified the concentration of the given mixture of amino acid by circular [or] radial chromatography.

Reference: 1) Instrumental method of chemical analysis by G. Chatwal and P Anand 5th Edition page -585-590

2) Analytical chemistry by B. K. Sharma page -85-95.

Requirement: n-butanol : Glacial acetic acid : Water, 4 : 1: 5

0.2 % w/v ninhydrin in acetone and standard amino acid solution.

Apparatus: Separating funnel, petridish, sprayer, and capillary tube, whatman filter paper.

Principle: In this technique a circular piece of paper having a wick cut parallel to the radius from edge to center is used the center is deposited at the paper at the upper end of the wick and flow through the paper is placed on the edge of circular dish as a result the liquid ascend the wick and flow through the paper the flow of mobile phase is outward from a central part on spot

The process allowing the solvent to move along the filter paper is called development the separation of components after the development is

called resolution. The amount applied as a spot to be paper is called loading

For The Circular Paper Chromatograph

1. Choice Of Paper:

The paper is many grades of thickness and porosity in sheets. Suitable paper for a particular can only determined by a trial and error but whatmann No: 1 is of great importance and wide applicability for medium flow rate

2. Temperature Control: Temperature should be kept to a minimum either by insulating individual that with plastic foam [or] by thermostatically controlled cabinet

3. Application of samples: Sample can be applied by a capillary tube, platinum loop or quantitatively by a micropipette, graduated capillary or micro syringe. Several ampoules can be spotted at once by various automatic and semiautomatic device it is preferable to apply the sport in small volume the position of compound can be expressed by their retardation factor [R_f]

$[R_f]$ = Distance traveled by component from original line

Distance traveled by the solvent from origin line

RF value depends upon several factors: are

1. The nature of solvent.
2. The medium used quality of the paper.
3. The nature of mixture to be separated.
4. The temperature.
5. The size of the container in which the experiment is performed.

Procedure:

1. Prepare solution of 0.1mg / 100ml each of glycine, Leucine, alanine and mixture of glycine, Leucine,alanine.

2. The apparatus was fashioned equalized petridish and a filter disc.

3. The wick was made from a filter paper rolled into a cylinder 2-3 mm thick at the end in the form of a brush and inserted through a small note at the center of the paper.

4. The sample drop to be analyzed was placed on paper and is air dried the developing solvent was prepared n-butanol : GL acetic acid : water [4 : 1 : 5]

5. The was placed b/n two glass dishes the lower containing solvent the solvent which is already saturated for 1 hr.

6. The solvent rises by capillary and run the chromatogram compleate development and removes the paper and mark solvent front air dry the paper and spray the ninhydrin reagent thus purple spot identify the amino acid determine the [R_f] value.

Result: The Rf value of amino acid were found to be

Glycine-------

Leucine -------

Alanine----------

Unknown ---------

Conclusion: The unknown Rf value 1 and 2 matches with glycine and phenylalanine so hence the given mixture contains glycine and phenylalanine.

Experiment No:13 Date:

THIN LAYER CHROMATOGRAPHY

AIM: To carry out the separation and identification of amino acid by thin layer chromatography.

DISCUSSION: Thin layer chromatography usually employs solid layers which adhere to the glass plate, generally by virtue of a binding agent such as calcium, sulphate which is incorporated with this and particular advantage is that corrosive reagent which attack paper can be used for spraying. The prepared thin layer on glass is often called as chromates plate.

Chromates plates prepared by applying a uniform layer of an appropriate material in the form of an aqueous paste to clear glass plate.The usual sorbents include silica gel, alumina, keiselghur, and cellulose powder.

Silica gel is largely used because it can serve as a medium for the separation of both polar compounds and non polar compounds. Spots are placed at regular and equal distance. The mixture may be applied using a capillary tube. Development is carried out by ascending technique in a small glass jar.

PREPARATION OF SILICA GEL PLATE: Mix 2g of silica gel with 4ml of distilled water in a small conical flask and shake/mix well and spread over glass plate.

MATERIALS

Solvent system: - n-Butanol: Acetic acid: Water (8:2:2)

Visualizing agent: - Ninhydrin reagent

Amino acids: - Alanine, Valine, Leucine, and Glycine

PROCEDURE:

Preparation of the plates

The plates are prepared by pouring slurry of adsorbent (silica gel) in water with smooth consistency /by using a spreader.Allow the plates to stand for 5 minutes. Then dry the plates at 120 degrees Celsius for 30 minutes. Then allow to cool and set aside for exposure to the atmosphere for about 30 minutes.

Draw the baseline 1.5cm from the lower end of the glass plate. Mark the points equidistant from each other and spot the sample using capillary tube.

Preparation of the standard

Dissolve 5mg of each of the above amino acids separately in 0.25ml of water. Measure the volume of water using 1ml graduated pipette. Mix a drop of each solution to provide the test mixture dilute the remainder of each solute separately to 1ml to give solution of the respective amino acids. The latter will contain about 5mg/ml of each amino acid / ml.

Development of chromatography

After the application of the sample the plates are placed in previously saturated solvent system. The solvent is allowed to pass upto $3/4^{th}$ of the plate. Plate is then removed and air dried. Visualizing reagent (Ninhydrin reagent) is sprayed and plates are dried in hot air oven till the spots are developed. R_f value is calculated.

REPORT: The R_f value of the mixture of unknown sample was found to be ……. and ……..

Experiment No:14 Date:

COLUMN CHROMATOGRAPHY

AIM : To carry out the separation and identification of a given mixture of the amino acids by column chromatography

APPARATUS : Glass column with a stop cock

Beaker

Conical flask

TLC plate

Capillary tube

CHEMICALS : Benzene , silica gel , sand , glass wool , sintered glass

PRINCIPLE: Column chromatography is based on the principle of selective adsorption. Mixture to be separated is dissolved in a suitable solvent and allowed to pass through a tube containing the adsorbent. The component which has greater absorbing power is adsorbed in the upper part of the column. the net component is adsorbed in the lower portion of the column which has lesser adsorbing power than the first component, the process is continued, as a result the materials are partially separated and adsorbed in the various part of the column, the

initial separation of various component can be improved by passing either the original or some other suitable solvent slowly through the column Two procedure are used to separate various constituents

A) The various zones are cut with a knife at boundaries and the substance present in the zones is extracted with suitable solvent this process of recovery of constituents is known as elution.

B) After development the column may be washed with more solvent, now termed the eluant and each component is collected separately as it reaches the end of the column and is released.

DISSCUSION:

Solvent is selected based on the solubility relation of substances. The solvents commonly used as mobile phase are arranged according to there increasing eluting power .thus elutotropic series are obtained which vary with type of adsorbent used.

The solvents used in chromatography have three functions to perform

1. Serve to introduce the mixture to the column

2. Effect the process of development by which zones of chromatography are separated to their fullest extent

3. Used to remove the required content of each zone from the mechanically separated parts of the column or from the column as a

whole after it is partly developed. The solvents used for this purpose are called eluants. Solvents: petroleum ether

- Cyclohexane
- Carbontetrachloride
- Trichloroethylene
- Benzene
- Chloroform
- Absolute alcohol
- Ethyl acetate
- Pyridine

PROCEDURE

1) Initially TLC was performed for the selection of solvent system

2) The sample was dissolved in suitable solvent, using a capillary tube a small amount of sample was applied on TLC plate.

3) The TLC plate was developed in different solvent system air dried and observed under uv light

4) The solvent which shows sufficient separation between the spots was selected as mobile phase (benzene)

5) Silica gel was mixed with the mobile phase and then filled in to the column

6) Sand was washed with the mobile phase and added in to the column to form a layer on the top of the adsorbent

7) Sample was mixed with mobile phase and introduced in to the column.

8) Mobile phase level was maintained above the adsorbent level

9) Eluent was collected from the bottom and presence of individual constituents was determined by performing TLC and observing the plates in UV

10) A series of eluent were collected and evaporated to get the pure sample .

REPORT

Date:

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Introduction

High-performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by injecting a plug of the sample mixture onto the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

PARAMETERS used in HPLC

RETENTION TIME (tR): is the time between sample injection (time zero) and the appearance of the band maximum; when all conditions are held constant, the retention time for a given peak (or compound) remains constant.

RETENTION VOLUME: It is the volume of mobile phase required to elute50% of the component from the column

Retention volume= retention time x flow rate

Separation factor: SEPARATION FACTOR (a): defined for two adjacent bands as the ratio of k' for the second band divided by k' for the first band; when the separation factor equals 1.00, the two bands are on top of each other and completely unseparated. Changing the experimental conditions can increase a and permit the two bands to be separated. Also sometimes called

RELATIVE RETENTION

RESOLUTION (Rs): defines how well separated two adjacent bands are. Larger values of resolution mean better separation.

THEORIES OF HPLC

PLATE THEORY:

According to this theory, the chromatographic column consists of continuous, narrow, horizontal plates of equal units called as

"Theoretical plates". Though these units are hypothetical, they give rise to a very useful method for the practical measurement of column efficiency.

Height equivalent to theoretical plates (HETP):

It is the *length of the column* necessary to obtain the equilibrium of the solute particles between the two phases & is given by

HETP = length of the column / no. of theoretical plates

Where L= length of the chromatographic column

N= no. of theoretical plates

HETP is given by *Van Demeter equation:*

HETP=A+B/u + Cu

Where,

A=eddy diffusion term or multiple path diffusion which arises due to *packing of the Column.* This is unaffected by the carrier gas velocity or flow rate. This can be minimized by uniformity in packing

B= longitudinal diffusion term or molecular diffusion which depends on flow rate

C= effect of mass transfer which depends on flow rate.

u= flow rate or velocity of the mobile phase

Resolution (R):

It is defined as the *distance between two adjacent peak maxima divided by their average peak width.*

N, HETP, and R are preferred as the measures of the column efficiency.

Retention time (Rt) :

It is the *difference in time between the point of injection and appearance of peak maxima.*

 It is the time required for 50% of a component to be eluted from the column.

 Measured in *min or sec*

RATE THEORY:

Plate theory failed to explain the ways to improve the performance of the column, which the rate theory did. This theory explained the fact that the mobile phase flows continuously & that the solute particles

are constantly being transported & partitioned in the column. It can be explained by Van Deemeter equation:

TYPES OF HPLC TECHNIQUES

A) Based on modes of chromatography

1. Normal phase mode

2. Reverse phase mode

B) Based on principle of chromatography

1. Adsorption chromatography

2. Ion exchange chromatography

3. Size exclusion/gel permeation chromatography

4. Affinity chromatography

5. Chiral chromatography

C) Based on elution techniques

1. Isocratic separation

2. Gradient separation

D) Based on the scale of operation

1. Analytical HPLC

2. Preparative HPLC

E) Based on the type of Analysis

1. Qualitative analysis

2. Quantitative analysis

F) Based on the internal diameter of the column employed for HPLC

1. Based on modes of chromatography- Interaction or Affinity between polar-polar and nonpolar-nonpolar

is more whereas the interaction affinity between the polar-no polar is less. A. Normal phase chromatography. Separation of polar analytes by partitioning onto **a** polar bonded stationary phase In this the stationary phase is polar (e.g. silica gel) and the mobile phase is non-polar. In this technique the non-polar compounds travel faster and are eluted first this is because **of** the less affinity between the

Solute and the stationary phase. Polar compounds are retained for a longer time in the column because of more affinity towards the

Stationary phase and take more time to be eluted from the column**.** This becomes a disadvantage and the process cannot be applied

for the pharmaceutical products as most of the drug molecules are polar in nature and takes longer time to be eluted and detected As a

result this technique is not widely used in Pharmacy

B. **Reversed Phase** chromatography: Separation of non-polar analytes by partitioning **onto a** non-polar, bonded stationary phase.

In reverse phase chromatography, a non-polar stationary phase and polar mobile phase is used. Hence, the polar components get

Eluted & first and the non-polar components are retained for a longer time. Since most of the drugs and pharmaceuticals are polar

In nature, they arc not retained ***for*** a longer time and are eluted faster, which is advantageous

B. Based on **principle of chromatography:**

1. Ion-exchange chromatography. The principle of separation is ion-exchange, which is reversible exchange of functional group In ion exchange chromatography, an ion exchange resin is used to separate a mixture **of** similar charged ions**.** For cations a cation exchange resin is used for anions, an anion exchange resin is used.

2. **Affinity chromatography:** uses the affinity of the sample with specific stationary phases. This technique is mostly used in the flied

of biotechnology, microbiology**,** biochemistry etc.

2. Based on elution techniques:

 Isocratic elution: Here the same mobile phase combination is used throughout. The process of separation. The same polarity or elution strength is maintained throughout the process.

 Gradient elution: Here a mobile phase combination of lower polarity or eluti0n strength is used followed by gradually increasing the polarity or elution strength.

3. Based on the scale of operation:

Analytica1 HPLC: used only for the analytical purpose and th recovery of the sample is not done since the qun1ity of sample used is very small usually in micrograms. *Preparative HPLC*: here the individual fractions of pure compound

Can be collected using fraction collector and the collected samples can be reused Eg.separations of few grams of a mixture.

Based on the type of analysis:

a. Quantitave analysis: is used to identify the compound, detect impurities and to find the number of components. It is done by the *retention time* values.

b.Quantitative analysis: is done to detect the amount of individual or several components in a mixture. It is done by comparing the peak area of standard and sample

Principle of separation in HPLC:

When a mixture of components is introduced into a HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slowest the component which has less affinity towards the stationary

phase travels faster. Since no two components have the same affinity towards the stationary

phase, the components are separated.

Stationary Phases: Stationary phases are particles which are usually about 1 to 20 μm average diameter ***(often*** **irregularly shaped).**

Polar ("Normal" Phase):

o Silica, alumina

o Cyano, amino or diol terminations on the bonded phase *f* Non-Polar("Reversed Phase")

o, C18 to about C8 terminations on the bonded phase

o Phenyl and cyano terminations on the bonded phase

NOTE

1. Either silica or bonded silica materials should not be used above pH8 because silica itself begins to dissolve at around pH 8.

2. Chemically bonded materials also get cleaved off at pH 2.0

3. Adsorbed compounds can usually be removed from silica columns by flushing with methanol or water and from bonded phase columns by flushing with methanol or dichloromethane

4. In Adsorption chromatography, there is no additional phase on the stationary phase particles (Silica, alumina)

5. In Partition chromatography, the stationary phase is coated on to (often bonded) a solid support (silica, alumina, divinylbenzene resin)

The Mobile Phase in HPLC

Must do the following

1. solvate the analyte molecules and the solvent they are in

2. be suitable for the analyte to transfer "back and forth" between during the separation process

3. Must be compatible with the instrument (pumps, seals, mixing, detector, etc)

4. compatible with the stationary phase

5. readily available (often use liters/day)

6. of adequate purity Spectroscopic and trace-composition usually

7. Not too compressible (causes pump/flow problems)

Note:

1*. Solvent purity is very important in preparative HPLC where the sample s usually recovered* by *the evaporation of the solvent.*

2. *In order to achieve reproducibility in adsorption chromatography, the Water content of the mobile phase must be controlled carefully.*

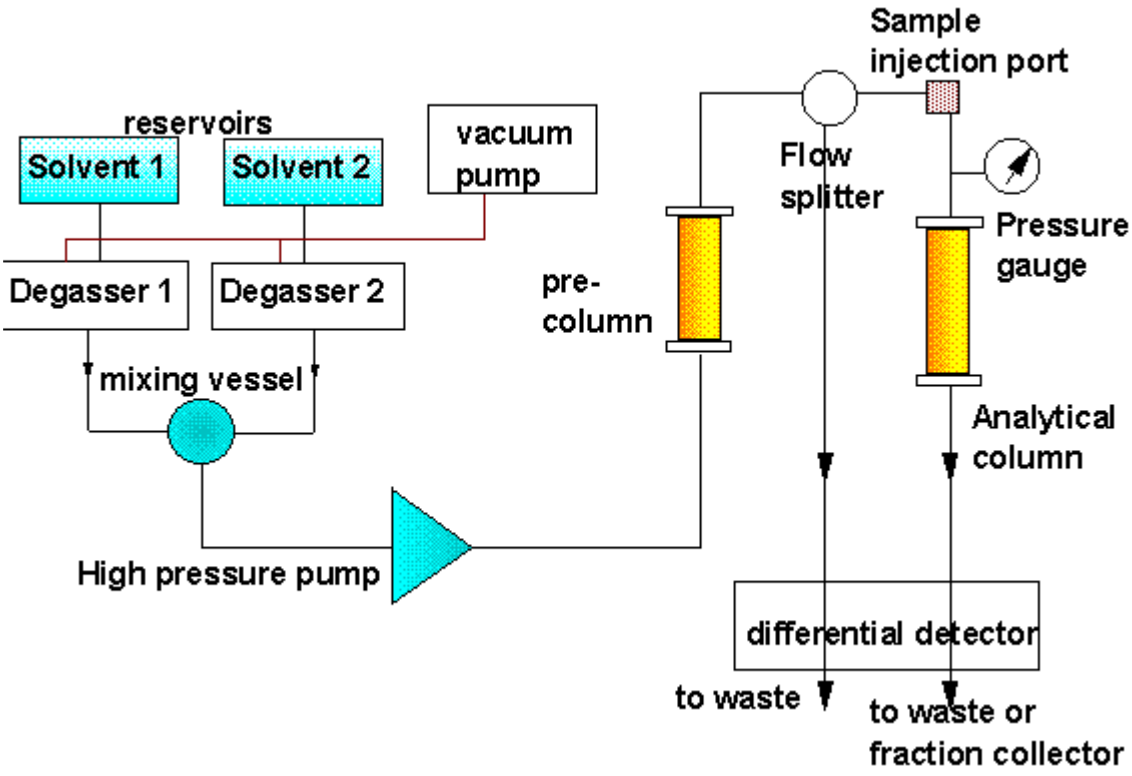
3. because most part of the chromatograph in contact with the mobile phase is constructed of stainless steel or Teflon, there are few restrictions of the mobile phase. However, halide ions should be avoided because these ions attack stainless steel, especially at low pH values.

4. Algal growth may result from the prolonged use of the biological buffer solutions such as citrate and these usually have to be

discarded

5. While working with buffer solutions and other potentially corrosive eluents, the system should be flushed out with water or a

water-methanol mixture when not in use. This is advantageous for both the chromatograph as well as column packing material.



INSTUMENTATION OF HPLC

1. Degassing system

2. Pump solvent delivery system

3. Check valves

4. Pulse damper

5. Pre-columns

6. Guard column

7. Flow splitter

8. Auto sampler

9. Sample injection port

10.Column

11.Detector

APPLICATIONS OF HPLC

1. Quality control testing of drugs

2. In Qualitative & Quantitave analysis

3. Therapeutic monitoring of drug metabolism studies

4. Separation & control of impurities

5. In analysis of biological fluids

6. Stability studies

7. Study of metabolic pathways in basic biochemical pathways

8. Separation of positional isomers, enantiomers, Optical isomers

9. Industrial applications

a. Determination of synthetic intermediates ex: atenolol

b. In determining traces of impurity ex:Tolnafate

c. Stability studies ex Acyclovir

Experiment No:15 Date:

DETERMINATION OF RETENTION TIME AND AREA UNDER CURVE FOR A GIVEN DRUG SAMPLE BY HPLC METHOD

AIM: To determine retention time (t_r) and AUC for a given sample of diclofenac sodium by HPLC method (qualitative analysis).

PRINCIPLE: Chromatography is a physical method of separation of different kinds of compounds from complex mixture; uses two mutually insoluble/immiscible phases- stationary phase and mobile phase, the latter runs over the former.

High pressure liquid chromatography (HPLC) uses a high pressure in order to pass the mobile phase through uniformly packed stationary phase column, at sufficient velocity and flow rates, and can be operated at various pressures as high as 1500psi.

At such high pressure, resolution property of chromatographic system changes; hence, also sometimes, known as high performance liquid chromatography (as performances of stationary and mobile phases change at high pressure).

Retention time (t_r): It is the time required by the given sample to elute out of the column with the mobile phase. The sample is injected to the mobile phase through the injection port before the column.

In HPLC, t_r is related parameter to, R_f (retention factor) in paper chromatography and TLC.

INSTRUMENTATION:

(1) Mobile phase reservoir

(2) Mobile phase delivery system , consists of:

- Pumps
- Pubic damping system
- Flow regulator
- Mixing chamber
- Deaeration and filters

(3) Sample injection system

(4) Column

- Guard column
- Main column
- Oven (thermostatic chamber)

(5) Detector

(6) Data processor, recorder and display.

REPORT: Retention time for diclofenac sodium was found to be…… and AUC was found to be……

Experiment No:16 Date:

ESTIMATION OF DICLOFENAC SODIUM BY HPLC

AIM: Estimation of Diclofenac sodium from given sample by HPLC method (quantitative analysis).

CHROMATOGRAPHIC CONDITION:

HPLC instrument: Water

Detector : Dual wavelength detector

Column : C_{18} reverse phase

(4.9mm*250mm)

Temperature : Ambient temperature

Mobile phase : Methanol

Flow rate : 1ml/min

Inlet pressure : 1824 psi

Volume injected : 30 micro liters

PROCEDURE:

Preparation of standard solution and standard curve of diclofenac sodium:

- Standard solution having concentration ranging from 5, 10, 15, 20, 25 micro grams/ml is prepared.
- Each sample is injected into column and AUC is calculated.
- A standard plot of AUC vs. concentration was prepared, from which concentration of the unknown sample can be determined.

REPORT: Concentration of Diclofenac sodium in the given sample is found to be……..