East Point College of Pharmacy

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

Approved
by
Pharmacy Council of India, New Delhi

Affiliated
to
Rajiv Gandhi University of Health Sciences
Karnataka
Bengaluru – 560041
India

LAB MANUAL

MODERN PHARMACEUTICAL ANALYSIS PRACTICAL

M. PHARM 1st SEMESTER

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M. Pharmacy (Pharmaceutics)	
Programme Outcome (PO)	
PO1	An ability to independently carry out research /investigation and development work.
PO2	An ability to write and present a substantial technical report/document
PO3	Students should be able to demonstrate a degree of mastery over the area as per the specialization of the program. The mastery should be at a level higher than the requirements in the appropriate bachelor's program
PO4	Graduates will demonstrate comprehensive knowledge and practical skills in advanced pharmaceutical development, encompassing drug analysis, drug formulation, and evaluation of novel drug delivery systems.
PO5	Students will acquire a deep understanding of regulatory processes and compliance, preparing dossiers for submission to regulatory agencies worldwide. They will navigate the intricacies of innovator and generic drug concepts, ensure adherence to global guidelines, and exhibit expertise in Biopharmaceutics& Pharmacokinetics.
PO6	Graduates will integrate technological advancements into pharmaceutical research and development, utilizing computational modelling, design of experiments, and prototype modelling.

Programme Specific Outcomes (PSO)	
PSO1	Apply appropriate tools and techniques for design and development of Pharmaceutical Dosage forms, cosmeceuticals and drug delivery systems
PSO2	Comprehend the pharmacokinetic parameters of drugs, dose calculations and biopharmaceutical approaches in problem solving
PSO3	Acquaint knowledge on investigational new drugs and regulatory submissions

Course Outcomes (CO's)	
Code: MPH101P Modern Pharmaceutical Analysis Practical	
CO 1	Understand the principles, procedures and applications of different analytical techniques
CO 2	Determine the structure of various categories of drugs by interpreting the results and data obtained from a variety of analytical techniques such as UV, visible and IR spectroscopic techniques
CO 3	Separate the components of chemical mixture by different chromatographic techniques like paper, TLC, HPLC and electrophoresis
CO 4	Perform skillfully in all their laboratory performances as per prescribed analytical guidelines

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INTRODUCTION

U.V. SPECTROSCOPY

Spectroscopy is the branch of science which deals with the study of interactions of Electromagnetic radiation with matter. The most important consequence of such interaction is that energy is absorbed or emitted by the matter in discrete amounts called quanta. The Absorption or Emission processes are known throughout the EM spectrum ranging from the gamma region to the radio region.

For UV and Visible spectrum Electronic transition occurs in the range 200-800 nm and involves the promotion of electrons to higher energy level.

Range of UV – Visible Spectra:-

Far UV Near UV Visible

150nm ---------- 200nm ---------- 400nm --------- VIBGYOR ---------- 800nm.

A record of the amount of light absorbed by the sample as a function of wavelength is called an Absorption spectrum generally consists of Absorption bands.

Properties of Electromagnetic Radiation:-

- Electromagnetic radiation is a form of energy that is transmitted through space at an enormous velocity.
- EMR does not require any supporting media as it readily passes through a vacuum than in supporting media.

- The most common example of EMR, is light which occupies only a small portion of EMR.
- EMR is said to have a dual nature exhibiting both wave and particle characteristic. This duality is useful for the quantitative description of any phenomena. This duality is not confined to the visible portion of EMR, but can be demonstrated for the whole region of EMR.

Wave properties of EMR: As the name implies an EMR is an alternating electrical and associated magnetic force field in space. Thus, EMR has an Electric component and a Magnetic component. The two components oscillate in planes I^r to each other and to the direction of propagation of the radiation.

The figure is a vector representation of Electromagnetic Radiation moving along X- axis. The electric field varies in the direction of the Y axis and the corresponding magnetic field varies in the direction of Z-axis.

The velocity of EMR in vacuum is independent of frequency and has the value of 3×10^8 m/sec in contrast to other wave phenomena such as sound. However, EMR doesn't require any supporting media, for its transmission & it readily passes through vacuum.

Beer's law:-It states that "The intensity of a beam of monochromatic light increases exponentially with the increase in the concentration of absorbing species arithmetically".

$$di/dc \alpha I$$

$$di/dc = KI$$

$di/dc = Kdc$

$I_n I = Kc + b \rightarrow ?$

On integration, b is constant

When, concentration = 0, there is no absorbance

Hence, $I = I_o$.

Substituting in Equation 1:

$I_n I = ke - I_n I_o$

$I_n I_o - I_n I = kc$

Substituting the value of b, in equation 1:

$I_o / I = ec^{kc}$

$I / I_o = ec^{-kc}$

$I = I_o e^{-kc}$ ------ > Beer's law equation

Lambert's law:-The lambert's law states that the rate of decrease of intensity with the thickness of the medium is directly proportional to the intensity of incident light.

A = kct or A = ect → Mathematical

Equation for Beer – lambert's law -

where,

A – Absorbance /Extinction Co-efficient

E – Molecular extinction Co- efficient

E - Concentration of drug (m mol/lit)

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

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Wave Properties:-An Electromagnetic wave shows the following terms-

1.Wavelength:-Distance between two successive maxima on an Electromagnetic wave. It is denoted by **λ** and various units are used in meters, cm, mm, nm, um and A.

2.Frequency:-The number of wavelength units passing through a given point in unit time is called Frequency and it is denoted by **v**. Frequency is generally expressed in cycles/second, Hertz or Fresnel.

3.Wave Number:-Reciprocal of the wave length is called as wave number.

$$V = 1/\lambda$$

Units are cm^{-1} for which the name Kaiser denoted by (K) or Kilo Kaiser (KK) is used.

4.Velocity:-Velocity of EMR depends on the medium through which the radiation passes.

Units are cm/sec, m/sec, etc.

The velocity of the wave is the product of Wavelength and Frequency.

$$V = \lambda * v$$

Velocity is inversely proportional to the density of the medium. As density decreases velocity increases. Reaching constant maximum in a vacuum where $3 X 10^{10}$ cm/sec. since frequency must be constant these are related as

$$\lambda * v = C$$

C = Velocity of light in vacuum.

Particle Properties of These are the various wave properties like Refraction, Reflection and Reinforcement and Destruction Interference. But the wave nature fails to explain phenomenon like Photo Electric effect, etc.

In order to explain such a phenomenon it is assumed that the EMR consist of a stream of discrete particles of pure energy called quanta/photon. Photons have definite energy and travel in direction of propagation of radiation beam with an equal velocity of light. The energy of photon is proportional to the frequency of radiation and is related as:

$$\mathbf{E = h\ v}$$

Where, E = Energy of photon in ergs

v = Frequency of EMR in cycles/second

H = Planck's constant = 6.624×10^{-27} ergs sec

= 6.624×10^{-34} Joules sec

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Laws Governing Absorption of Radiation:-

The two laws related to the absorption of radiation are:-

1. **Beer's law** (related to concentration of absorbing species)

2. **Lambert's law** (related to thickness/path length of absorbing species).

When light is incident upon a homogenous medium a part of incident light is reflected, a part of it is absorbed and the remaining is transmitted.

If, I_o = Incident light,

$I_o = I_a + I_t + I_r$

(Absorbed, Transmitted and Reflected light)

When comparison cell is used the valve of which is very small (about 4%) can be eliminated for air- glass interfaces thus the equation becomes.

$I_o = I_a + I_t$

Bouguer investigated the range of absorption of light with the thickness of medium and Lambert extended the concept.

Electrons present in organic molecules:-There are three types of electrons present

1) Sigma (σ) electron.
2) Pi (л) electron.
3) Non – bonding (n) electron.

Sigma (σ) electron:-

▪ Associated with the saturated bonds.

▪ The σ electrons are tightly held because of strong bonds.

▪ Compounds containing σ bonds do not absorb in near UV region but absorbed in vacuum region because energy produced by near UV region is insufficient to excite the electron.

Example: the electron in the single valence bonds between C-C, C-H, and O-H.

Pi (л) electron:-

▪ These electrons are involved in unsaturated compounds.

▪ Electrons are localized in a direction perpendicular to the nuclear axis.

▪ These bonds are weak bonds, easily excited by UV rays.

▪ They contain groups like > C = C < and -C – N.

Example: alkenes, alkynes and aromatic compounds.

Non – bonding (n) electron:-

▪ These electrons are less firmly held.

▪ Found in Nitrogen, Oxygen, Sulphur and Halogen.

▪ They are not involved in the bonding between the atoms in molecules.

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Electronic transition in U.V. region:-

There are four types of transitions:-

1) σ → σ*
2) n →σ*
3) л → л*
4) n → л*

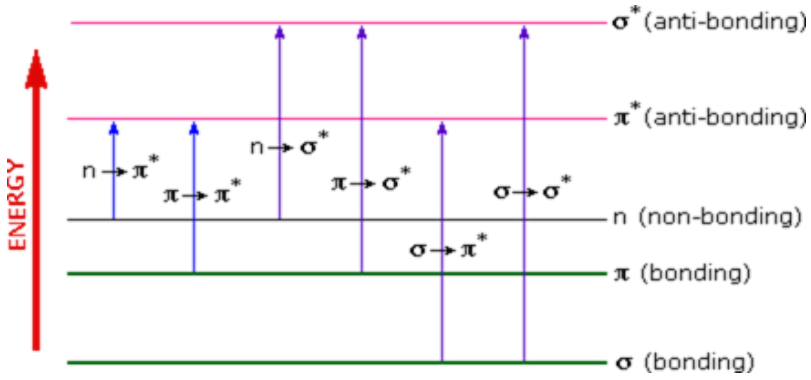

Diagram showing energy required by different transitions

σ → σ* transition:-

▪ Transition of an electron from a bonding sigma orbital of a molecule to the higher energy antibonding sigma orbital.

▪ Energy required for this transition is very high because sigma bonds are strong bonds.

▪ This transition occurs in those compounds in which all the electrons are involved in sigma bonds & there is no lone pair of electrons.

▪ This transition is studied in vacuum ultra violet region below 200nm because it requires very short wavelength

л → л* transition:-

▪ The transition corresponds to the promotion of an electron from a bonding л orbital to an antibonding л* orbital and available in compounds with unsaturated centers such as alkenes, carbonyl compounds etc.

▪ The excitation of л electrons require lesser energy then n → л* transition.

▪ These types of transition occur at a longer wave length.

▪ Compounds containing double bonds or triple bonds undergo л → л*. Such as aromatic compounds, alkenes, alkynes, carbonyl compounds such as aldehyde and ketones.

▪ Bands attributed to л → л* transition is also called as **K – bands**.

n → σ* transition:-

▪ n → σ* transition is seen in saturated compounds containing atoms with unshared electron pair (such as Sulphur , Oxygen, Nitrogen, Halogen or Non – bonding electrons).

▪ n → σ* transition needs less energy then σ → σ* transition.

▪ This transition is studied in near ultraviolet region(150 – 250 nm).

n → л* transition:-

▪ Bands attributed to n → л* transition is also called as **R – bands** .

▪ In n → л* transition an electron of unshared electron pair on a hetero atom such as Oxygen, Nitrogen or Sulphur is excited to л* antibonding electron.

▪ This transition requires least amount of energy then all other transitions.

▪ This transition gives rise to an absorption band at longer wavelength.

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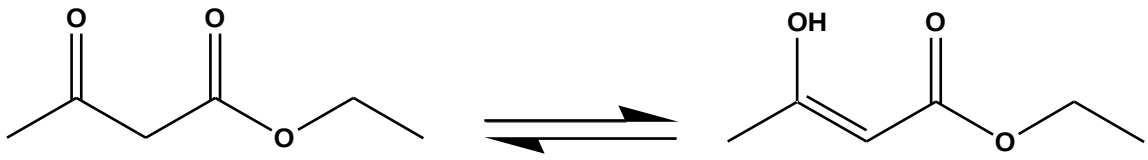
Applications of UV Spectroscopy:-

1. Keto – Enol Tautomerism:-

UV Spectroscopy can be used to determine the percent of various Keto & Enol forms present in a tautomeric equilibrium. The best example is ethyl acetoacetate where the equilibrium is –



Keto form
λ max = 275nm

Enol form
λ max = 244nm

Keto form has absorption maxima at 275 nm and e =16. This has only the weak n ----- π^* band because of the isolated carbonyl group.

Enol form has absorption maxima at 244 nm and e =16000. One can measure the proportions of tautomer's present in the ethyl aceto acetate.

This method is used for the determination of percentage of Enol in ethers & alcohols.

2. Determination of Molecular weight:-Example – Amines.

A known weight of amines is taken and converted to amine picrate and absorbance of the solution is found. By using the equation, the molecular weight of amines can be calculated:

A = act

a = A/CT

= a * molecular weight.

Therefore, molecular weight = e/a.

For most amine drugs, e of amine picrate's at 380nm is 13, 400.

Therefore, Molecular weight = 13,400/a

= 13,400 * Ct/A

Where, A = Absorbance

C = Concentration using path length't'

3. Quantitative Analysis:-In UV Spectroscopy quantitative analysis can be determined by the following methods. Specifically by:-

a. Using $E^{1\%}_{1cm}$ values.

b. $E^{1\%}_{1cm}$ not available, but raw material is available.

c. Single standard or direct comparison method.

d. Calibration curve method/multiple standard method.

Example –

- Spectrophotometric Estimation of dopinirole hydrochloride in tablets.

Maximum absorption occurs at 250nm.

- Simultaneous derivative and Multi component, Spectrophotometric Determination of Drotaverine Hydrochloride and Mefenamic acid in tablets.

4. Qualitative Analysis and Purification control:-It is also used in Qualitative analysis & Purification control in Dye stuffs & Pharmaceutical industries.

For identification of compounds containing unbounded electrons (n-electrons) or those with conjugated double bond system (Π electrons) a comparison is done by comparing the absorption spectrum with the spectra of known compounds.

In UV the absorption spectrum is influenced by the whole molecule as well as by particular groups containing the electrons. This makes it difficult to identify a particular group so this method is not as useful as the NMR method.

For purification control the presence of impurities can be done by UV spectroscopy which is considered as the best method for such detection. This method is considered superior to other methods because the bands due to impurities are very intense.

In manufacture of nylon it is described that the starting material are very pure and the purity of those materials could be checked by UV Spectroscopy. This helps in getting a product of high quality.

5. Calculations of Dissociation constants of acids & Bases:-To make such calculation, let us consider an acid HA.

The acid HA and dissolution in water dissociates to form H_2O & A^- ions.

$$HA + H_2O \rightarrow H_2O + A^-$$

Then by definition dissociation constant (Ka) for this acid is given by:

$$K_a = {}^a H_3O + {}^a A^-/HA$$

For every dilute solution it becomes:

$$K_a = [H_3O^+][\ A^-]\ /\ [HA]$$

Take logarithm on both sides we get:

$$- \log Ka = - \log [H_3O^+] - \log [A^-] + [HA]$$

$$p^{ka} = p^H - \log\ [A^-]/[HA]$$

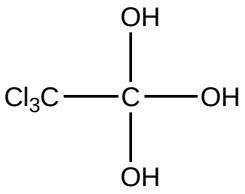
$$p^{ka} = p^{H} + \log [HA] / [A^-]$$

Thus, if the ratio HA/H^- is known at any particular pH then we can calculate Pka value.

Then, find the ratio HA/H^- Spectrometrically, a graph is plotted between Absorbance & Wavelength at different PH Value.

Change in absorbance of the system (HA+A) as the pH of the system is increased from 2 to 4.

6. <u>Structure of chloral:</u>-The possible structure for chloral is:



[I]

CCl_3CHO

[II]

When the UV spectra of chloral was recorded in hexane and an absorption band was observed at 298nm (e=33), but when its UV spectrum was recorded in aqueous solution no band was observed from this it was confirmed that the actual structure of chloral is I and not II.

7. <u>Detection of functional groups</u>:-The technique is applied to detect the presence or absence of Chromophore. The absence of a band at a particular wavelength may be regarded as evidence for a specific compound. A little information can be drawn from UV spectrum, if the molecular is very complicated and if the spectrum is transparent above 200 nm it shows absence of conjugation, a carbonyl group, benzene or aromatic compounds or iodo atoms. An isolated double bond or some other atoms or groups may be present,

it means that no definite conclusions can be drawn if the molecule absorbs below 200nm.

Chromophore	Examples	Solvent	Λ max	€ max
Alkenes	$C_6H_{13}CH=CH_2$	n- heptane	177	13,000
Alkynes	C_5H_{11} C=C-CH_3	n- heptane	178	10,000
Carboxyls	CH_3COCH_3	n- heptane	196	2,000
Carboxyl	CH_3COOH	Ethanol	225	160
Amide	CH_3CONH_2	water	186	1000

8. <u>Chemical kinetics:</u>-UV spectroscopy can be used to study the kinetics of reaction. In order to determine the kinetics of the reaction the change in concentration of either a reactant or product with the time is measured. Absorbance is directly proportional to concentration, UV spectroscopy can be used to follow the reaction course. The method is based upon the fact that one of the reactants or products exhibiting suitable absorption in the UV region is not overlapped by absorption due to other species present. This method can be employed to study such rates which must be relatively slow.

The UV spectrophotometry is also used to study the fast reactions by following a Stop-flow method. In this method, two solutions enter through X and Y. Then these are allowed to pass through reaction chamber B, and the flow of the mixed solution is stopped by piston D. The absorbance of any species which absorbs in the UV region is measured by UV spectrum with suitable adjustment of flow rates; reaction with half-lives down to milliseconds can be studied.

In the Stop – flow method, photomultiplier is used as a detector whose output is displayed on the screen with a time base.

Important terms in UV spectroscopy:-

1. **Chromophore:-** It is defined as any group that exhibits absorption of Electromagnetic radiation in the Visible or Ultraviolet region.

   Ex - Ethylenic, acetylenic, carbonyls, acids, esters, nitrile group.

2. **Auxochromes**:-It is a group which itself does not act as a chromophore but when attached to a chromophore it shifts the absorption maximum towards a longer wavelength along with an increase in the intensity of absorption.

   Ex - OH, - NH2, - OR – NHR & NR2

3. **Bathochromic shift or Red shift**:-It involves the shift of absorption maximum towards longer wavelength because of the presence of certain groups such as OH and NH_2 or by change of solvent.

   Ex - ethylene shows **л → л*** transition at 170nm whereas 1.3 – butadiene shows at 217nm.

4. **Hypsochromic shift or Blue shift**:- It involves the shift of absorption maximum towards a shorter wavelength and may be caused by the removal of conjugation in the system or by a change of solvent. It is caused by changing the polarity of the solvent.

   Ex - In aniline, λmax is 280nm because the pair of electrons on the nitrogen atom is in conjugation with the Π bond system of the benzene ring in acidic solutions. A blue shift is caused and absorption takes place at λmax of 200mn.

5. **Hyperchoromic effect**: -It involves an increase in the intensity of absorption and is usually brought about by the introduction of an autochrome.

   Ex – Introduction of methyl group in position 2 of pyridine increase from 2750 to 3560 nm.

6. **Hypochromic effect**:-It involves a decrease in the intensity of absorption and is brought by groups which are able to distort the geometry of the molecule.

   Ex – When a methyl group is introduced in position 2 of biphenyl group, hypochromic effect occurs because of distortion caused by methyl group.

7. **Transmittance (T)**:-It is a measure of the quantity of light which is not absorbed by the radiation transmitted by the solution.

8. **Absorbance (A)**:-The absorbance 'A' of a solution is given by the equation:

$$A = \log_{10} T$$

$$A = \log pH$$

Instrumentation:- Different instruments used are colorimeters and spectrophotometers.

The different components are:

1. Source of light – The most commonly used radiation sources are Hydrogen or Deuterium lamps, Xenon discharge lamp & Mercury arc. In all sources, excitation is done by passing electrons through a gas and then collision between electron and gas molecules may result in electronic, vibration, and rotational excitation in gas molecules.

When the pressure of gas is low only a line spectrum are emitted. But if the pressure is high a bond spectra and continuous spectra will be emitted.

Ideal characteristics:-

a. Must be stable.

b. Must be of sufficient intensity for the transmitted energy to be detected at the end of Optical path.

c. Must supply continuous radiation over the entire wavelength region in which it is used.

2. Filter and Monochromators:-Generally they are termed as Wavelength Selectors. The wavelength selectors restrict the radiation being measured to a narrow band. Such devices greatly enhance both the selectivity and sensitivity of the instrument.

Filters:-It is of 2 types - Interference filter and Absorption filters.

Filters operate by absorbing all but a restricted band of radiation from a continuous source.

Monochromators:-It is used for UV, Visible and IR radiation and all are similar in construction i.e.; they employ slit, lenses, mirrors, windows,

dispersing devices. The materials from which these components are fabricated depend upon the wavelength region to be used.

Components are - Entrance slit, Collimating lens /Mirrors, Prism/ Grating and Focusing elements.

Types of Monochromators:-

a. Gratings – 2 types (i) Diffraction grating (ii) Transmission gratings.

b. Prisms – 2 types (i) Refractive prism (ii) Reflective prisms.

3. Sample cells:-They are also called as Sample containers or Cuvettes. For UV that is below 350nm, quartz or fused silica can be used. Glass is not used for UV region as glass absorbs UV radiation. The most common cell length or path length for UV & visible is 1cm.

4. Solvents:-It plays as an important role in UV spectroscopy since compound peak could be obscured by solvent peak. Hence the solvent is selected in such a way that it neither absorbs nor affects the absorption of the sample.

Different solvents like - Water – 191nm, Cyclohexane – 195nm, Methanol – 203 nm, Ethanol – 20lnm, Ether – 215nm, Chloroform- 230 nm and Carbon Tetrachloride- 255nm.

5.Recording system:-T signal from the detectors is finally received by the recorder system.

References:-

1. Skoog, West, Holler, Crouch: "Fundamental of Analytical Chemistry" 8th edition – page no. (3-4, 192, 784, 785, 789-790)
2. B.K Sharma: "Instrumental Method of Chemical Analysis" 13th edition – page no. (40, 41, 51, 88).

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Experiment. No:01

DETERMINATION OF λMAX OF PARACETAMOL

AIM: To determine the λ max of Paracetamol by UV spectroscopy.

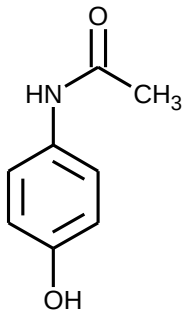
Requirements:-

Chemicals:-Paracetamol, Methanol.

Apparatus:-Volumetric flasks, Pipettes, UV Instrument.

Principle:-UV spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200 nm to 400 nm. Compounds that are colorless absorb radiation in the UV region. In both UV as well as Visible Spectroscopy only valence electrons absorb energy by the molecules undergoing transition from ground state to excited state. This absorption is characteristic and depends on the nature of the electrons present. The intensity of absorption depends on the concentration and path length.

In the present experiment, the Paracetamol determination can be done by UV method at a particular wavelength.



PARACETAMOL

Two types of transitions are take place in paracetamol.

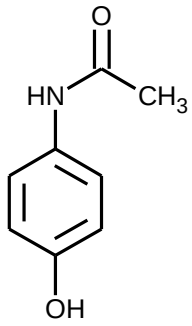
n $\rightarrow \Pi^*$ = Due to presence of lone pair of nitrogen, it has lower energy.

$\Pi \rightarrow \Pi^*$ = Due to the presence of C = C, it has higher energy than $n \rightarrow \Pi^*$ which gives the absorption maximum at 257 nm.

Procedure:-

1. Accurately 50mg of paracetamol was weighed & mixed with 50ml of methanol in the volumetric flask (1mg/ml =1000µg/ml).
2. From the above solution 1, 2, 3 & 4 ml was pipette out and volume was made up with methanol solvent. This gives 4, 8, 12 and 16 µg/ml in l0ml volumetric flasks.
3. The absorbance value was noted in UV region.
4. The graph was plotted against concentration Vs Absorbance.

Discussion:-



PARACETAMOL

Chemical name :- 4 (P) – hydroxy acetanilide

Molecular formula :- $C_8H_9O_2N$

Molecular weight :- 151.16.

Category :- Analgesic & Anti Pyretic agents

Description :- white crystals or white in colour.

Solubility : - Freely soluble in Ethanol (95%) and in Acetone, Sparingly soluble in water, Very slightly soluble in ether and dichloro methane.

Standards :- contains < 99.0% and > 101.0%

Storage :- stored in well- closed light resistant containers.

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Paracetamol (Acetaminophen) is the de-ethylated active metabolite of phenacetin and it is used since 1950.

Pharmacological Actions:-

- The central Analgesic action of Paracetamol is like Aspirin i.e., it raises pain threshold, but has weak Peripheral Anti-Inflammatory component. Paracetamol is a good and promptly acting Antipyretic.
- It is a poor inhibitor of PG synthesis in peripheral tissues, but more active on Cox in brain.
- In contrast to aspirin, paracetamol does not stimulate respiration or it has no effect on CVS.
- Gastric irritation is insignificant – bleeding and mucosal erosion occurs rarely only in overdose. It does not affect platelet function or clotting factors.

Pharmacokinetics:-It is well absorbed orally, only about 1/3 is protein bound in plasma & it is uniformly distributed in the body. It is conjugated with Glucuronic acid, Sulfate and is excreted rapidly in urine. Plasma half life is 2-3 hours.

Adverse effects:-In antipyretic doses, the drug is safe and well tolerated. Nausea, Rashes and Leucopenia occurs.

Acute Paracetamol poisoning:-It occurs specially in small children who have low Hepatic Glucoronide conjugation ability. In large doses, serious toxicity can occur.

Mechanism:-N- acetyl – P- benzo quinoneimine is a highly reactive arylating minor metabolite of Paracetamol which is detoxified by conjugation with Glutathione.

Uses:-

▪ Paracetamol is most commonly used as Analgesic for headache, Musculoskeletal pain, Dysmenorrhoea, etc.

▪ It is first choice of Analgesic for Osteoarthritis and Antipyretic.

Dose:-

▪ 0.5 - lg TDS to Infants (50 mg), children 1-3 yrs (80-160 mg), 4-8 yrs (240-320 mg), 9-12 yrs (300-600 mg).

Other trade names of paracetamol:-Crocin, Metacin, Paracin, Ultragin, Pyrigesic, Calpol, Neomol, Fevastin, Febrinil.

Report:-The absorption maxima of given sample of paracetamol was found to be …… and absorbance was …… .

References:-

1. IP volume-II 1996 Pg No- 554-555
2. Essentials of Medical pharmacology by K.D.Tripathi.
3. B.K Sharma: “Instrumental Method of Chemical Analysis” 13th edition

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Experiment.No:02

Determination Of Absorbance, Absorptivity, Molar Absorptivity & Specific Absorbance

AIM: To carry out the absorbance of paracetamol and to calculate the absorptivity, specific absorbance & molar absorptivity.

References:-1. Indian pharmacopoeia 1996, Pg no. 556.

2. Practical Pharmaceutical Chemistry by A.H.Beckett and J.B.Stenlake Vol-II.4th Edition.

Apparatus Used:-Volumetric flask, beaker, funnel, pipette, cuvettes and glass rod.

Chemicals Required:-Paracetamol reference standard, 0.1M Sodium Hydroxide and Distilled water.

Principle:

Laws governing absorption of radiation:-

The two laws relating to the absorption of radiation are:

1. Beer's law (related to the absorption of species).
2. Lambert's law (related to thickness / path length of absorbing species).

1)Beer's law:-The intensity of a beam of monochromatic light decreases exponentially with increase in the concentration of absorbing species arithmetically.

$I = I_0 e^{-kc}$

I = Intensity of incident light

I_0 = Intensity of absorbed light.

C = Concentration.

2)Lambert's law:-When monochromatic light passes through a transparent medium, the rate of decrease of intensity with the thickness of the medium is directly proportional to the intensity of the incident light.

I)Absorbance(A):-It is the logarithm to the base 10 of the reciprocal of the transmittance.

$$A = \log_{10} 1/T$$

II)Absorptivity(a):-It is the ratio of the absorbance (A) to the product of concentration (c), in µg / ml and length of optical path (b) in cm.

$$a = A / b\ c$$

III)Molar absorptivity/ Molar extinction co-efficient(ε):-Absorbance of a sample, of concentration 1 mole /litre, present in a cell of path length one cm is called *molar absorptivity.*

$$\varepsilon = A/ b\ c$$

IV)Specific absorbance ($A^{1\%}$1cm):-Absorbance (of light) per unit path length (usually in centimeters) and per unit of mass concentration (gm %).

$$A^{1\%}\ 1cm = A / b\ c$$

Absorption is an extensive property of a substance, whereas absorptivity is an intensive property. If there is a change in concentration of the solution and the thickness of container the value of molar absorptivity will remain constant within Beer's law range. But the absorbance will change significantly. The value of molar absorptivity will be different wavelength.

Procedure:-

Preparation of solutions:-Preparation of 0.1M NaOH:-

4gms of sodium hydroxide made up to 1000ml gives 0.1M NaOH.

Preparation of standard solutions:-

Stock solution I:-(1mg/ml solution) or (1000mcg/ml)

Accurately weigh 50mg of Paracetamol into a 50ml of volumetric flask, add 0.1M NaOH, dissolve well and make up to mark with 0.1M NaOH.

Stock solution II:-(100µg/ml solution)

Pipette out 10ml of stock solution-I into a 100ml volumetric flask and make up to mark with 0.1M NaOH.

Preparation of serial dilutions:-Pipette out 1,2,3,4 and 5ml of stock solution-II into five different 10ml volumetric flasks and make up to mark with 0.1M NaOH to get 10, 20, 30, 40 and 50µg/ml solutions respectively.

1) Perform baseline correction with blank.
2) Determine the λ_{max} of paracetamol in 0.1M NaOH by spectrum scan of any of the dilutions as sample & blank solution as reference.
3) Determine the absorbance of serial dilutions using 0.1M NaOH as blank. Calculate a, ε and $A^{1\%}$ 1cm by using the formulas given above.

Observation:-

Sl.no	Conc(µg/ml)	Absorbance (A)	Absorptivity (a)	Molar absorptivity (ε)	$A^{1\%}1cm$
1.					
2.					
3.					
4.					
5.					

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Experiment.No:03

Determination Of Amax Of Nimesulide

AIM: To determine the λ max of Nimesulide by UV Spectrophotometry method.

Requirements:-

Chemicals:-Nimesulide, Sodium Hydroxide.

Apparatus:-Volumetric flasks, Pipettes, Beakers, UV instrument.

Principle:-UV Spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200nm to 400nm. Compounds which are colorless absorb radiation in UV region. In both UV as well as visible spectroscopy only the valence electrons absorb energy their by the molecules undergoes transition from ground state to excited state. This absorption is characteristic and depends on the nature of electrons present. The intensity of absorption depends on the concentration and path length.

In the determination of λ max of Nimesulide, Π → Π * and n → Π * transitions occur. Due to the presence of C = C, Π → Π * transition occurs which has higher energy.

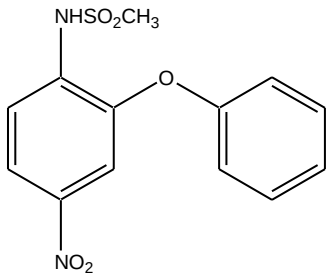
Whereas n → Π *, due to the presence of lone pair of nitrogen it exists in higher energy. Nitro group of chromophore is present which has a strong E-max value which gives the absorption maxima at 394 nm.

Procedure:-

1. Accurately weigh 50 mg of Nimesulide and is dissolved in 50 ml of Sodium hydroxide (1000 µg/ml).
2. From the above solution, 5ml was taken and diluted to 50ml with Sodium hydroxide (100 µg/ml).
3. Further from 100 µg/ml, 1ml is diluted to l0 ml with Sodium Hydroxide to give 10 µg/ml.
4. 1ml of solution was pipetted out from 10 µg/ml and made upto 10 ml volume in 10 ml volumetric flask.
5. The Absorbance was noted in UV region.
6. The graph was plotted against Concentration versus Absorbance.

Discussion:-

Chemical Structure:-



Molecular formula:-$C_{13}H_{12}O_5N_2S$

It is a group of preferential Cox – 2 Inhibitor.

Nimesulide is a newer NSAID and it is a relatively weak inhibitor of PG synthesis and there is some evidence to indicate relative Cox -2 selectivity. Anti-Inflammatory action may be exerted by other mechanisms as well ex – reduced generation of superoxide by neutrophils, inhibition of PAF synthesis

and TNF_{α} release, free radical scavenging, inhibitor of metalloproteinase activity in cartilage.

The Analgesic, Antipyretic and Anti-Inflammatory activity of Nimesulide has been rated comparable to other NSAIDs.

Pharmacokinetics:-It is completely absorbed orally, 99% plasma protein bound, extensively metabolized and excreted mainly in urine and it has a half life time of 2- 5 hours.

Adverse effects:-

Gastro intestinal - EPI gastralgia, Heart burn, nausea and loose motions.

Dermatological - Rash, Pruritus, Central Somnolence, Dizziness.

Italian studies has shown that ulcer complications are prevalent with others NSAID'S.

Uses:-

▪ Used primarily for short lasting painful inflammatory conditions like sports injuries, sinusitis & other Ear –nose – throat disorders, dental surgery, bussitis, low backache, dysmenorrhea, osteoarthritis & for fever

Dose:-100 mg tablet, 50 mg 15 ml suspension

Other trade names:-Nimulid, Nimegeric & Nimodol.

Report:-The λ max and Absorbance of the given sample was to be

References:-

1. K.D.Tripathi: Essentials of Medical pharmacology: pg: 169.
2. B.K Sharma: "Instrumental Method of Chemical Analysis" 13th edition

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Experiment.No:04

Determination Of Λmax Of Ibuprofen

AIM: To determine the λmax of Ibuprofen by UV Spectrophotometry.

Requirements:-

Chemicals:-Ibuprofen, Sodium hydroxide.

Apparatus:-Volumetric flasks, Pipette, UV instrument.

Principle:-UV spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200 nm to 400nm. Compounds which are colorless absorb radiation in the UV region. In both UV as well Visible Spectroscopy only valence electrons absorb energy, their by the molecules undergoes transition from ground state to exited state. This absorption is characteristic and depends on the nature of electrons present. The intensity of absorption depends on the concentration and path length.

In the determination of λ max of Ibuprofen, Π → Π * and n → Π* transitions occurs. Due to the presence of C = C, Π → Π * transition occurs which has higher energy. Whereas n → Π*, due to the presence of lone pair of nitrogen it exists in higher energy. Nitro group of chromophore is present which has a strong λmax value.

Transitions are forbidden and hence these are of low intensity which gives the absorption maxima at 264 nm.

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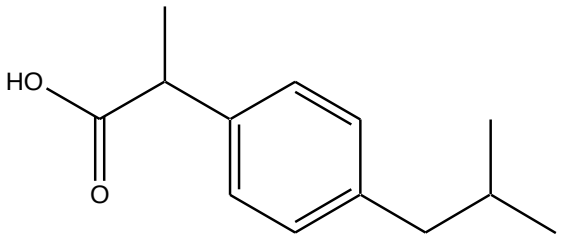
Procedure:-

1. Accurately weigh 50 mg of Ibuprofen and it is dissolved in 50 ml of 0.1N Sodium Hydroxide (1000 µg/ml).
2. From the above level, 5 ml was taken and diluted to 50 ml with Sodium Hydroxide (100 µg/ml).
3. Further from 100 µg/ml, 1ml is diluted to l0 ml with Sodium Hydroxide to give 10 µg/ml.
4. The absorbance is noted in UV region.
5. The graph was plotted against Concentration versus Absorbance.

Discussion:-

Chemical structure:-



Ibuprofen

Chemicals name :-(RS) -2- (4 – isobutyl phenyl) prop ionic acid.

Molecular formula :-$C_{12}H_{18}o_2$

Molecular Weight :-206.28

Category :-Anti- inflammatory agent

Solubility :-Freely soluble in Acetone, Chloroform, Ethanol (95%), Ether and practically insoluble in water. It dissolves in dilute solution of alkaline Hydroxides and Carbonates.

Standards :-contains<98.5% & > 101.0%

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Ibuprofen is a Propionic acid derivatives and it has been rated as the safest conventional NSAID by the spontaneous adverse drug reaction reporting system in U.K.

Pharmacokinetics:-It enters brain, synovial fluid and cross placenta. They are largely metabolized in liver by hydroxylation and glucoronide conjugation and excreted in urine as well as bile pigments. It's plasma half life is 2 hours.

Uses:-▪ It is used as a simple Analgesic and Antipyretic in the same way as low dose of aspirin.

▪ Particularly effective in Dysmennorrhoea in which the action is clearly due to PG synthesis inhibition. It is available as an 'Over the Counter' drug.

▪ Used widely in Rheumatoid arthritis, Osteoarthritis and other disorders.

Dose:-400 – 800 mg IDS.

Other trade names:-Bruffen, Emflam, Ibusynth, Ibugesic.

Report:-The λ max and Absorbance of the given sample of Ibuprofen was found to be ----

References:-

1. IP vol – 1 1996 Pg 387-388.
2. K.D.Tripathi: Essentials of Medical pharmacology.
3. B.K Sharma: "Instrumental Method of Chemical Analysis" 13th edition.

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Experinment.No:05

Determination Of Λ Max Of Caffeine

Aim:-To determine the λmax of Caffeine by UV Spectrophotometry.

Requirements:-

Chemicals:-Caffeine, Methanol.

Apparatus:-Volumetric flasks, Pipettes. Beakers, UV instrument.

Principle:-UV spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200nm to 400nm. Compounds which are colorless absorb radiation in the UV region. In the UV as well as Visible spectroscopy only valence electrons absorb the energy their by the molecules undergoes transition from ground state to excited state. This absorption is characteristic and depends on the nature of electrons present. The intensity of absorption is characteristic and depends on the concentration and path length.

In Caffeine , n and Π electrons are present. Due to the presence of unsaturation Π→ Π * transition occurs which occurs at higher intensity. Due to the presence of heteroatom, lone pair of electrons n→ Π * transition occurs. In this, the transitions are forbidden and are having low intensity ethylene group of chromophore is present which has a λmax value of 1.000 and 8.000 which gives the absorption maxima at 278nm.

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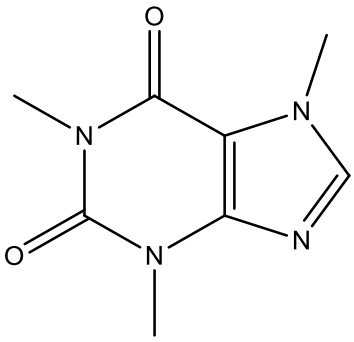
Procedure:-

1. Accurately weigh 50mg of caffeine and is dissolved in 50ml of methanol (1000 ug/ml).
2. From the above solution, 5ml was diluted to 50 ml with methanol (100 ug/ml).
3. From the above solution, 1 ml was diluted to 10ml with methanol to give 10 ug/ml.
4. The absorbance was recorded in the UV region.
5. The graph was plotted against Concentration versus Absorbance.

Discussion:-

Chemical Structure:-



caffeine

Chemical name :- 3,7 – Dihydro–1.3,7,-trimethyl–1H–purine–2.6–dione

Molecular formula :– $C_8 H_{10} N_4 O_2$

Molecular weight :– 194

Category :– CNS Stimulant

Description :– silky white crystals, odorless, sublimes readily.

Solubility :– freely soluble in chloroform, inhibits water and sparingly soluble in water and Ethanol(95%)

Standards :– contains <98.5% and > 101.5%

Storage :– store in a tightly closed containers

It is a methyl Xanthine derivative. Caffeine is usually prepared from Thea sinensis, Coffee Arabia, Cola acaminata and Theobroma cacao.

Pharmacological actions:-

▪ CNS actions:-Caffeine is a CNS stimulant, 10 ml dose affects the higher centres and 150- 250 mg produces a sense of well being, alertness and fatigue.

▪ CVS actions:-It directly stimulates the heart and increases the force of myocardial contractions.

▪ Kidney:-It has minimal action.

▪ Skeletal muscles:-It enhances the contractile power.

▪ Stomach:-It enhances secretion of acid and pepsin in stomach on parenteral injection.

Pharmacokinetics:-Caffeine has poor water solubility and rapidly but irregularly absorbs after oral administration. It is less than 50% bound to plasma proteins and volume of distributions is 0.05 l/kg and gets completely metabolized in liver by Demethylation and Oxidation and is finally excreted in urine. Plasma half life is 3-6 hours in adults.

Adverse effects:-Gastric irritation, Aneuria, Vomiting, Nervousness, insomnia, Agitation and rise in body temperature.

Uses:-

▪ In Analgesic mixture

▪ Migraine – used in combination with Ergotamine

▪ To counteract hypnotic overdose – caffeine & sodium benzoate injection.

▪ Apnoea in premature infants - as alternative to theophylline

Dose:-300 to 600 mg

Other trade names in combination with ergotamine:-

▪ Cafergot - Caffeine 100 mg + ergotamine 1mg tablet.

▪ Micropyrin - Caffeine 20 mg + aspirin 350 mg tablet.

Report:-The λmax and Absorbance of the given sample of Caffeine was found to be

References:-

1) IP Vol – 2 1996 Pg – 121 -122.
2) Essentials of Medical pharmacology by K.D.Tripathi.
3) B.K Sharma: "Instrumental Method of Chemical Analysis" 13th edition.

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Experinment.No:06

Estimation Of Paracetamol By U.V. Spectroscopy

AIM: To carry the estimation of Paracetamol in the given sample by U.V. spectroscopy.

Requirements:-

Chemicals:-Paracetamol, 0.1 N Sodium Hydroxide.

Apparatus:-Volumetric flasks, Pipette, Weighing balance, Measuring cylinder, Cuvettes, Funnel and Beaker.

Principle:-UV spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200 nm to 400nm. Compounds which are colorless absorb radiation in UV range. In this spectroscopy only valence electrons absorb energy, their by valence electrons undergo transition from ground state to exited state. This absorption is characteristic and depends on the concentration and path length.

In the present experiment, it is estimated spectrophotometrically. Paracetamol is taken and dissolved in 0.1 M Sodium Hydroxide in which sodium salt of Paracetamol is formed from which a series of known concentration are prepared. The Absorbance of unknown and series of known concentration was noted at 257 nm

Procedure:-

1. Accurately weigh 50 mg of Paracetamol and is dissolved in 0.1M Sodium Hydroxide and the volume was made up to 50ml with 0.1M Sodium Hydroxide (1000 µg/ml).

2. From the above solution, 5 ml was diluted to 50 ml with 0.1M Sodium Hydroxide (100 µg/ml).
3. Further from the above stock solution 0.4, 0.8, 1.2, 1.6 and 2.0 ml was diluted with 0.1M Sodium Hydroxide to give 4, 8, 12, 16 and 20 µg/ml concentration and unknown sample was also given.
4. Absorbance was measured at 257 nm.
5. Graph was plotted by taking Concentration (x-axis) versus Absorbance (y-axis).
6. Then the given unknown sample was made to 10ml and the concentration was calculated by intra-polating the graph.

Report:-The concentration of the given unknown sample no…… was found to be…….µg/ml and the percentage purity was found to be…….%.

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Experinment.No:07

Estimation Of Nimesulide By U.V. Spectroscopy

AIM: To carry out the estimation of Nimesulide in the given sample by U.V. spectroscopy.

Requirements:-

Chemicals:-Nimesulide, 0.5 N Sodium Hydroxide, distilled water.

Apparatus:-Volumetric flask, Pipettes, Beaker, Measuring cylinder, UV instrument.

Principle:-UV spectroscopy is concerned with the study of absorption of UV radiation ranging from 200nm to 400nm. Compounds which are colorless absorb radiation in UV region. In both UV as well as Visible spectroscopy only the valence electrons absorb energy, their by the molecules undergoes transition from ground state to excited state. This absorption is characteristic and depends on the nature of electrons present. The intensity of absorption depends on the concentration and path length.

In this experiment Nimesulide is estimated Spectrophotometrically. It has n → Π* and Π → Π* transition. Nimesulide is taken and dissolved in 0.1 N Sodium Hydroxide in which a series of known concentration are prepared. The absorbance of sample and series of known concentration was noted at 396nm.

Procedure:-

1. Accurately weigh 50mg of Nimesulide and is dissolved in 0.1N Sodium Hydroxide (1000 µg/ml).
2. From the above solution, 5 ml was diluted to 50ml with 0.1 N Sodium Hydroxide (100 µg/ml).
3. From the above solution 1 ml, 2 ml, 3 ml, 4 ml and 5 ml was pipetted out and is diluted with 0.1 N Sodium Hydroxide which gives 10, 20, 30, 40 and 50 µg/ml concentration.
4. Absorbance was measured at 396 nm.
5. The graph was plotted by taking Concentration verses Absorbance.
6. Then the given unknown sample was made to 10 ml and concentration was calculated by interpolating the graph.

Report:-The concentration of the given unknown sample no……was found to be .....µg/ml and the percentage purity was found to be ……%.

Reference:-

1. M.S.Yadav:-A Text book of spectroscopy

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Experinment.No:08

Estimation Of Sulphanilamide By Uv Spectrophotometry

AIM: To carry out the estimation of Sulphanilamide in the given sample by UV Spectroscopy method.

Requirements:-

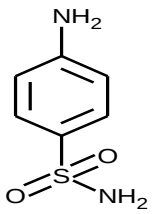
Chemicals:-Sulphanilamide, 1M Hydrochloric acid, Distilled water.

Apparatus:-Volumetric flasks, pipettes, beaker, UV Instrument.

Principle:-UV Spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200 nm to 400 nm. Compounds which are colorless absorb radiation in UV region. In both UV as well as in Visible spectroscopy only the valence electrons absorb energy their by the molecules undergoes transition from ground state to excited state. This absorption is characteristic and depends on the nature of electrons present. The intensity of absorption depends on the concentrates and path length. In this experiment Sulphanilamide is estimated Spectrophotometrically. It involves

n → Π * and Π → Π * transitions. Sulphanilamide is weighed and dissolved in 1M Hydrochloric acid in which a series of known concentration are prepared. The Absorbance of sample and series of known concentration was noted at 264 nm.

Sulphanilamide:-



SULPHANILAMIDE

It contains sigma, n and π electrons. Transitions occurs below 200nm, hence it is invisible. Due to the presence of unsaturation, Π → Π * transition occurs at

high intensity. Due to the presence of Nitrogen and Sulphur atoms $n \rightarrow \Pi^*$ transition occurs.

Procedure:-

1. Accurately weigh 50 mg of Sulphanilamide and is dissolved in 1M Hydrochloric acid (1000µg/ml).
2. From the above solution, 5ml was diluted in 50 ml of 1M Hydrochloric acid (100µg/ml).
3. From the above solution 1, 2, 3, 4, 5 and 6 ml was pipetted out and is diluted with 1M Hydrochloric acid which gives 4, 8, 12, 16, 20 and 24 µg/ml concentration and unknown sample was given.
4. Absorbance was measured at 264 nm.
5. Graph was plotted by taking Concentration versus Absorbance.
6. Then the given unknown sample was made upto 10 ml and the concentration was calculated by interpolating the graph.
7. Finally the percentage purity of the sample was calculated.

Discussion:-Sulphanilamides were the first Antimicrobial agents (AMAS) which are effective against pyrogenic bacterial infections. Prontosil was one of the dyes included by Domagk to treat experimental streptococcal infection in mice and found it to be highly effective.

By 1937 it became clear that Prontosil was broken down in the body to release Sulphanilamide which was the active Antibacterial agent.

Chemistry:-All Sulphanilamides are considered to be the derivatives of Sulfonamides (p-amino benzene sulfonamide). A free amino group in the para position (N_4) is required for Antibacterial activity. Anti bacterial Spectrum –

Sulphanilamide are primarily bacteriostatic against many gram positive and gram negative bacteria. However, Bactericidal may be attained in urine.

MOA of Sulfanilamide:-

1. PABA in small quantities antagonizes the antibacterial action of Sulfonamides.
2. Only those microbes which synthesize their own Folic acid (FA) and can't take it from the medium are susceptible to Sulphanilamide.

Pharmacokinetics:-They are rapidly and completely get absorbed from G.I.T. Extent of plasma protein binding differs considerably (10 – 95 %) among different members. They are widely distributed in the body – enter serious cavities easily.

The primary pathway of metabolism is acetylation at N_4 by Non-Microsomal enzyme, primarily in liver. There are slow and fast acetylators, but the difference is mostly insufficient to be clinically significant.

They are excreted mainly by the kidney through Glomerular Filtration.

Adverse effects:-Nausea, Vomiting and Epigastric pain are seen. Crystalluria is dose related, Rectal irritation, Haematuria and aneuria are rare now. Presence in urine can be minimized by taking plenty of fluids. Hypersensitivity reactions occur in 2-5% patients. Hepatitis occurs in 0.1% patients.

Uses:-Systemic use of Sulphanilamides alone is rare now -

- Suppressive therapy of Chronic Urinary Tract Infection.
- Streptococcal Pharyngitis.

- Gum Infection.
- Combined form used for Antibacterial Infections.

Report:-The concentration of the given sample was…….. ug/ml and percentage purity was found to be …… %.

Reference:-

1. K.D.Tripathi: Essentials of Medical pharmacology.

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Experinment.No:09

Estimation Of Sulphanilamide By Colorimetry

AIM: To estimate Sulphanilamide by Colorimetry using N-1-napthyl ethylene diamine dihydrochloride (BMR Reagent).

Requirements:-

Chemicals:-Sulphanilamide, 0.5% Bratton Marshall Reagent (BMR), 0.1 N Sodium Nitrite, 0.5% Ammonium Sulphamate and Hydrochloric acid.

Apparatus:-Beaker, Volumetric flasks, Pipette, Measuring cylinder, Colorimeter, Ice bath.

Principle:-The compound containing primary amino group is subjected to Diazotization. Diazotization is brought about by Nitrous acid which is formed by reaction between Hydrochloric acid and Sodium Nitrite. Nitrous acid is produced in excess so that all primary amino groups are diazotized. The diazotized product is coupled with BMR reagent. This results in the formation of a purple colour. It has an absorption maxima at 545 nm. So intensity of colour is measured at 545 nm in Colorimetry.

The Colorimetry is concerned with the study of absorption of Visible radiation whose range is from 400 – 800 nm. Any colored substance will absorb radiation in this wavelength region. The excess of Nitrous acid should be destroyed otherwise it will interfere with coupling reaction so hence Ammonium Sulphamate is used that results in evolution of Nitrogen.

Discussion:-The compound Sulphanilamide exhibits a structural similarity to PABA. Sulphanilamide by virtue of their structural similarity to PABA competes with the substitute for the later in the bacterial metabolism.

Sulphanilamide inhibits the enzyme Folic acid synthetase which is involved in the conversion of PABA to Folic acid

Sulphanilamide shows allergic reactions like skin rashes, fever, etc. It shows crystalline due to the precipitation of the acetylated form of Sulphanilamides.

Procedure:-

1. 250 mg of Bratton Marshall reagent was weighed in electronic balance & dissolved in 1M Hydrochloric acid and the volume was made upto 50ml.
2. Stock of l mg/ml concentration was prepared by weighing 50mg of pure Sulphanilamide and 50ml by IM Hydrochloric acid.
3. From this stock solution, dilute in such a way to obtain 0.4, 0.8, 1.2, 1.6 and 2.0 µg/ml concentration in a 10ml volumetric flask.
4. To each volumetric flask, 1 ml of Hydrochloric acid was added and followed by 0.4 ml of 0.1N Sodium Nitrite was added and shaken for 5 minutes for complete Diazotization and kept in ice bath for 10–15 minutes.
5. Then 0.8ml of 0.5% Ammonium Sulphamate was added and mixed well.
6. The 0.4ml of 0.5% Bratton Marshall reagent was added and mixed well and made upto 10 ml with distilled water.
7. The Colorimeter was stabilized and made upto zero by using blank solution.
8. Absorbance of different concentrations was taken at 570nm.
9. Graph was plotted by extrapolating the reading of unknown concentration and it is obtained.

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Report:-Sulphanilamide was estimated by Colorimetry and the concentration of the given unknown sample was found to be

Reference:-

1. A.H. Beckett and J.B. Stenlake, Practical Pharmaceutical chemistry.

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Experinemt.No:10

Assay Of Sulphanilamide By Potentiometry

AIM: To estimate the Sulphanilamide by Potentiometry.

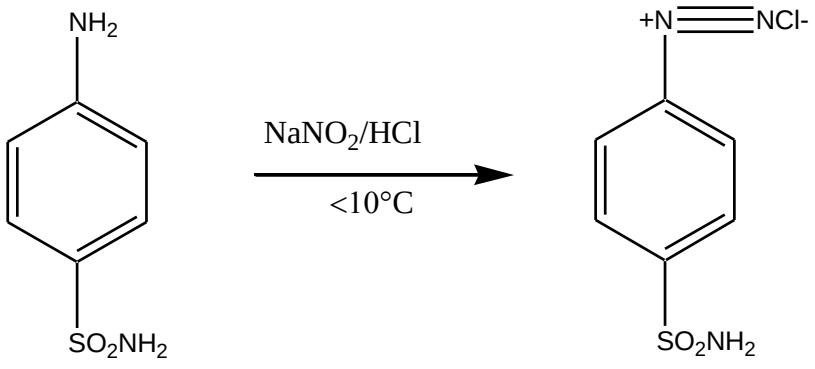
Requirements:-

Chemicals:-Sulphanilamide, Sodium nitrite, concentrated Hydrochloric acid, Distilled water.

Apparatus:-Beaker, Magnetic stirrer, Platinum electrode, Burette, Pipette, Potentiometer, Measuring cylinder, Tissue paper.

Principle:-Potentiometry a branch of Electrochemistry deals with the study and measurement of electrode potentials. Oxidation - Reduction, Precipitation and Neutralizations reactions involve marked change in the potential and the end point can be carried out Potentiometry. It helps in precise location of the end point.

Reaction:- $NaNO_2 + HCl \rightarrow HNO_2 + NaCl$



Sulphanilamide Diazonium Salt

Excess of Nitrous acid changes electrode potential.

Procedure:-

1. The pure sample of drug was weighed i.e., 200 mg in an electronic balance and was transferred to a beaker. To this, 20ml of concentrated Hydrochloric acid and 25 ml of distilled water was added (if needed it can be more diluted so that volume error can be minimized).
2. After preparing the stock solution, Potentiometer is switched on. Then the instrument was kept on for 15 minutes for stabilization.
3. Put the stirrer in the solution and keep the beaker on a magnetic stirrer.
4. The stirrer speed and sensitivity was adjusted and electrodes were dipped in the solution.
5. Then the end point was selected when one drop of Sodium Nitrite has given full deflection.
6. The process was repeated using standard Sulphanilic acid and the titration was carried out in duplicate to get concordant values.

Factor:-1 ml of 0.1 M Sodium Nitrite – 17.22 mg of Sulphanilamide.

▪ 0.1M Sodium Nitrite - 7.5g of Sodium Nitrite was dissolved in 100ml Distilled water.

Standardization of Sodium Nitrite:-0.3 g of Sulphanilic acid in 50 ml of 2M Hydrochloric acid was taken and 3gms of Potassium Bromide was added, cooled in ice and titrated with 0.1 M Sodium Nitrite to determine the end point electro chemically (Potentiometrically).

Discussion:-Potentiometric method of analysis consists of the measurement of the EMF of chemical cells using a pair of Indicator electrode and Reference electrode. The potential of the solution depends on the nature and the

concentration of the ions of the drug substances. The potential is measured in milli volt using a Potentiometer.

$$E_{cell} = E_{ref} + e_{ind} + e_{jnc}$$

An electrode such as platinum is immersed in a solution of an oxidized to reduced species. A general expression of this phenomenon is given by the Nernst Equation:

$$E = E^0\ 0.0591/N \log [\ A_{Reduction} / A_{Oxidation}\ ]$$

where, E= Standard Potential of the metal

N = Valency of ions.

A = Activity of Oxidized or Reduced species

Potentiometric methods - 2 types of Electrochemical analysis:-

1. The direct measurement of potential of an Indicator electrode with respect to the Reference electrode from which the calculation of the activity or concentration of the ion of interest can be achieved.
2. Potentiometric titration involves the measurement of the changes in the EMF of the cell brought about by adding a titrant (i.e., the monitoring of the potential serves only to locate the equivalence point of a titration).

Electrodes of the First kind:-These are methods which are in contact with its own ions.

Eg – Zn/Zn^{2+} electrode.

Electrodes of the second kind:-These consists of the metal part covered by a layer of one of its slightly soluble salt and immersed in an acid on a salt solution with same ion. They include Oxide electrode and Specific – Ion electrode.

Glass – Membrane electrodes:-These are of following types -

1. Hydrogen ion – sensitive glass electrodes.
2. Metal- ion sensitive glass electrodes
3. Liquid membrane electrodes
4. Precipitate and solid state electrodes
5. Transistor electrodes

Classes of potentiometric Analysis - Measurement of pH:-

Determination of activity of H+ is done by Potentiometrically or by direct reading with a pH meter. A circuit is designed to give meter needle deflect as a function of pH. The following types of titrations can be done by potentiometric methods:

1. Acid base titrations (Aqueous and Non – Aqueous)
2. Redox titration
3. Diazotization titration
4. Precipitation titration
5. Complexometric titrations
6. Dead-stop end point technique

Different Electrodes used:-

1. Indicator Electrodes.
   Ex- Antimony – Antimony electrode
2. Glass electrodes

3. Reference Electrodes
   - Ex – Hydrogen electrode
   - Saturated calomel electrode
   - Silver –silver chloride Electrode
4. Hydrogen Electrode

Report:-The weight of Sulphanilamide in the given sample no……was found to be…….gms.

References:-

1. Laboratory hand book of instrumental drug analysis by B.G.nagavi.
2. Quantitative chemical analysis by Vogel's.
3. Text book of Pharmaceutical analysis by Dr.S.Ravishankar.

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Experinment.No:11

Estimation Of Paracetamol Tablets

AIM: To determine the percentage purity of the given Paracetamol tablets.

Requirements:-

Chemicals:-Paracetamol tablets, 0.1 N Sodium Hydroxide, Distilled water.

Apparatus:-Volumetric flask (100 ml) , Measuring cylinder (100ml), Beaker (250 ml), Pipettes (1.0 ml), Funnel, Glass rod, Beaker(100ml).

Principle:-Chemically, Paracetamol is 4-hydroxy acetanilide. Paracetamol is estimated Specrophotometrically. Here sodium salt of paracetamol is formed, from which a series of known concentration are prepared and the Absorbance is found at 257nm. Since it has a conjugated diene structure and also a Chromophore, it shows maximum Absorbance at 257nm.

Procedure:-

1. 50 mg of Paracetamol was accurately weighed into 50ml of 1N Sodium Hydroxide solution to obtain 1mg/ml (1000µg/ml) solution.
2. From above stock solution prepare 2, 4, 6, 8 and 10 µg/ml solutions in a 10 ml volumetric flask.
3. Check the absorbance for all the dilutions at 248nm.
4. Check the unknown sample also in the same wavelength.
5. Calculate the concentration of given unknown sample by Calibration method, Single point method, $E^{1\%}_{1cm}$ and Percentage purity.

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Discussion:-

Usual Strength :- 300mg and 500mg

Storage :- Store in a well- closed and light resistant containers

Standards :- < 95.0 % and > 105.0%

Report:-The percentage purity of the given sample of paracetamol tablet was found to be ……. **%.**

References:-

1. IP Vol – II Pg – 556
2. Gurudeep R Chatwal: Instrumental Methods of Chemical analysis.

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Experinment.No:12

Estimation Of Nimesulide Tablets

AIM: To determine the percentage purity of the given Nimesulide tablets.

Requirements:-

Chemicals:-Nimesulide tablets, 0.1 N Sodium Hydroxide, Distilled water.

Apparatus:-Volumetric flask, Measuring cylinder, Beaker, Pipettes.

Principle:-UV spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200 nm to 400 nm. Compounds which are colorless absorb radiation in UV region. In both UV as well as Visible spectroscopy only the valence electrons absorbs energy their by the molecules undergoes transition from ground state to excited state. This absorption is characteristic and depends on the nature of electrons present. The intensity of absorption depends on the concentration and path length.

In Nimesulide, Π → Π* and n → Π* transition occurs. Nimesulide is estimated Spectrophotometrically and series of known concentrations are prepared and the Absorbance is found at 396 nm.

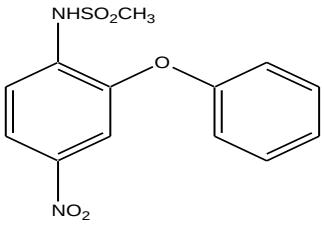
Procedure:-

1. 50 mg of Nimesulide was accurately weighed into 50ml of 1N Sodium Hydroxide solution to obtain 1mg/ml (1000µg/ml) solution.
2. From above stock solution prepare 2, 4, 6, 8 and 10 µg/ml solutions in a 10ml volumetric flask.
3. Check the absorbance at all dilutions at 396nm.
4. Check the unknown sample also in the same wavelength.

5. Calculate the concentration of given unknown sample by Calibration method, Single point method, $E^{1\%}_{1cm}$ and Percentage purity.

Discussion:-

Chemical Structure:-



Molecular formula :– $C_{13}H_{12}O_5N_2$ S.

It is a preferential COX – 2 Inhibitor.

N- Arylsulfonamides:-Lead structures for this class of selective COX-2 agents were also compounds developed as Anti- Inflammatory agents before the discovery of the COX-2 isoform. Nimesulide was identified as an NSAID Anti-Inflammatory drug with weak Prostaglandin synthetase activity (COX-1) but potent in vivo in the carrageen an – induced edema model.

An analogue of Nimesulide, from Taisho was shown to be a Selective COX-2 inhibitor. This Class has a characteristic acidic proton by virtue of the N- Arylsulfonamide or Methane Sulfonanilide.

Adverse effects:-Gastrointestinal, Dermatological & Central effects are seen. Gastric tolerability of Nimesulide is better, but a recent Italian study has shown that ulcer complications are as prevalent as with other NSAID'S. There is also no proof that renal complications are missing. Haematuria is reported in few children. Recently several instances of Hepatic failure have been associated nimesulide and it has been withdrawn in Spain, Finland and Turkey. Its use in

children is banned in Portugal and Israel considering that it has not been marketed in many countries having effective ADR monitoring like UK, USA, Australia, Canada and the overall safety of this drug especially in children has been questioned.

However, most asthmatics and those who develop bronchospasm or intolerance to Aspirin and other NSAID's do not cross react with Nimesulide. Its specific usefulness appears to be only in such patients.

Uses:-

- In sinusitis and other Ear-Nose-Throat disorders
- Dental surgery
- Bursitis
- Low backache
- Dysmenorrhea
- Post-operative pain
- Osteoarthritis and
- Fever

Dose:-100 mg BD

Other brand names:-Nimulid, Nimegisic, Nimodol 100mg tablet, 50 mg/5ml suspension.

Report:-The percentage purity of the given sample of Nimesulide tablet was found to be …….. %.

References:-

1. Burger: Medicinal chemistry & drug discovery: pg – 241- 242.
2. K.D.Tripathi: Essentials of Medical pharmacology: pg: 179.

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SPECTROPHOTOMETRIC SIMULTANEOUS ESTIMATION METHODS FOR MULTICOMPONENT SAMPLES

The Spectrophotometric assay of drugs rarely involves the measurement of Absorbance of sample containing only one absorbing component. The pharmaceutical analyst frequently encounters situation where the concentrations of one or more substances are required in samples and are known to contain other absorbing substances which potentially interfere in the assay.

The basis of all the Spectrophotometric techniques for Multi-Component samples is the property that all wavelengths:-

(a)The Absorbance of a solution is the sum of absorbance's of the individual components; or

(b) The measured Absorbance is the difference between the total absorbance of the solution in the sample cell and that of the solution in the reference (blank) cell.

In multi-component formulations the concentration of the absorbing substance is calculated from the measured absorbance using one of the following procedures:-

(a) Assay of a Single-Component sample:-The concentration of a component in a sample which contains other absorbing substances may be determined by a simple Spectrophotometric measurement of Absorbance, provided that the other components have a sufficient small absorbance at the wavelength of measurement.

(b) Assay using Absorbance corrected for interference:-If the identity, concentration and absorptivity of the absorbing interference are known, it is possible to calculate their contribution to the total absorbance of a mixture.

(c) Simultaneous equation method:-If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λ_{max} of the other, it may be possible to determine both drugs by the technique of Simultaneous equations (Vierodt's method).

$$Cx = \frac{A_2\, a_{y1} - A_2\, a_{y2}}{A_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

$$Cy = \frac{A_1\, a_{x2} - A_2\, a_{x1}}{A_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

where ,

a) The absorptivity of X at λ_1 and λ_2 are a_{x1} and a_{x2} respectively.

b) The absorptivity of Y at λ_1 and λ_2 are a_{y1} and a_{y2} respectively.

c) The Absorbance's of the diluted sample at λ_1 and λ_2 are A_1 and A_2 respectively.

Criteria for obtaining maximum precision based upon absorbance ratios, that place limits on the relative concentrations of the components of the mixture.

$$\frac{A_1 / A_2}{a_{x2} / a_{x1}} \quad \text{and} \quad \frac{a_{y2} / a_{y1}}{A_2 / A_1}$$

The criteria are that the ratios should lie outside the range 0.1- 2.0 for the precise determination of Y and X respectively. These criteria are satisfied only when the λ max of the two components are reasonably dissimilar. An additional criterion is that the two components do not interact chemically.

To reduce the random errors during measurements, sometimes instead of carrying out analysis of two components at two wavelengths, it is carried out at 3 or 4 wavelengths. The equations will no longer have a unique solution but the best solution can be found out by the least square criterion.

(d) Absorbance ratio method:-The Absorbance ratio method is a modification of the Simultaneous equations procedure. It depends upon the property that a substance obeys Beer's law at all wavelengths. Q-analysis is based on the relationship between absorbance ratio value of a binary mixture and relative concentrations of such a mixture. The ratio of two absorbance determined on the same solution at two different wavelengths is constant. This constant was termed as "Hufner's Quotient' or Q-value which is independent of concentration and solution thickness eg-two different dilutions of the same substances give the same absorbance ratio A_1/ A_2. In USP this ratio is referred to as a Q value.

In the Quantitative assay of two components in a mixture by the Absorbance ratio method, absorbance are measured at two wavelengths, one being the λ max of one of the components (λ_2) and the other being a wavelength of equal absorptivity of the two components (λ_1), at Iso - absorptive point.

$$Cx = \quad Qm - Qy / \ Qx - Qy \ . \ A_1/ a_{x1}$$

Equation gives the concentration of X in terms of absorbance ratio, the absorbance of the mixture and the absorptivity of the compounds at the Iso-absorptive wavelengths. Accurate dilutions of the sample solution and the standard solutions of X and Y are necessary for the accurate measurement of A_1 and A_2 respectively.

(e) Geometric correction method:-A number of mathematical correction procedures have been developed which reduce or eliminate the background irrelevant absorption that may be present in samples of biological origin. The simplest procedure is the three point geometric procedure which may be applied if the irrelevant absorption is linear at the three wavelengths selected. If the wavelengths λ_1, λ_2 and λ_3 are selected so that the background absorbances B_1, B_2 and B_3 are linear, then the corrected absorbance D of the drug may be calculated from the three absorbances A_1, A_2 and A_3 of the sample solution at λ_1, λ_2 and λ_3 respectively as follows.

Let ${}_vD$ and ${}_wD$ be the absorbance of the drug alone in the sample solution at λ_1 and λ_3 respectively, i.e., v and w are the absorbance ratios of ${}_vD/D$ and ${}_wD/D$ respectively.

$$B_1 = A_1 - {}_vD$$

$$B_2 = A_2 - D$$

$$B_3 = A_3 - {}_wD$$

Let y and z be the wavelength intervals $(\lambda_2 - \lambda_1)$ and $(\lambda_3 - \lambda_2)$ respectively.

$$D = y(A_2 - A_3) + z(A_2 - A_1) / y(1-w) + z(1-v)$$

This is a general equation which may be applied in any situation where A_1, A_2 and A_3 of the sample, the wavelength intervals y and z and the absorbance ratio v and w are known.

(f) Orthogonal polynomial method:-The technique of orthogonal polynomials is another mathematical correction procedure which involves more complex calculations than the Three-point correction procedure. The basis of the method is that an absorption spectrum may be represented in terms of orthogonal functions as follows:

$$A(\lambda) = p\,P(\lambda) + p_1\,P_1(\lambda) + p_2\,P_2(\lambda) \ldots\ldots p_n\,P_n(\lambda)$$

Where, A denotes the absorbance at wavelength λ belonging to a set of n+1 equally spaced wavelengths at which the orthogonal polynomials, $P(\lambda)$, $P_1(\lambda)$, $P_2(\lambda)$ ….. $P_n(\lambda)$ are each defined.

The accuracy of the orthogonal functions procedure depends on the correct choice of the polynomial order and the set of the wavelengths. Usually, quadratic or cubic polynomials are selected depending on the shape of the absorption spectra of the drug and the irrelevant absorption. The set of the wavelengths is defined by the number of wavelengths, the interval and the mean wavelength of the set (λ m). Approximately linear irrelevant absorption is normally eliminated using six to eight wavelengths, although many more up to 20, wavelengths may be required if the irrelevant absorption contains high-frequency components. The wavelengths interval and λ m are best obtained from a Absorption curve.

This is a plot of the absorptivity coefficient for a specified order of polynomial, a specified number of wavelengths and a specified wavelength interval against the λ m of the set of wavelengths. The optimum set of wavelengths corresponds

with a maximum or minimum in the convoluted curve of the analyte and with a coefficient of zero in the convoluted curve of the irrelevant absorption. In favorable circumstances the concentration of an absorbing drug in admixture with another may be calculated if the correct choice of polynomial parameters is made, thereby eliminating the contribution of the drug from the polynomial of the mixture.

(g)Differential spectrophotometry:-Differential spectroscopy provides a sensitive method for detecting small changes in the environment of a chromophore or it can be used to demonstrate ionization of a chromophore leading to identification and quantitation of various components in a mixture. The selectivity and accuracy of Spectrophotometric analysis of samples containing absorbing interference may be markedly improved by the technique of difference spectrophotometry. The essential feature of a difference spectrophotometric assay is that the measured value is the difference absorbance (Δ A) between two equimolar solutions of the analyte in different forms which exhibit different spectral characteristics.

The criteria for applying difference spectrophotometry to the assay of a substance in the presence of other absorbing substances are that :-

A) Reproducible changes may be induced in the spectrum of the analyte by the addition of one or more reagents.

B) The absorbance of the interfering substances is not altered by the reagents.

The simplest and most commonly employed technique for altering the spectral properties of the analyte properties of the analyte is the adjustment of the pH by means of aqueous solutions of acid, alkali or buffers. The Ultraviolet-Visible absorption spectra of many substances containing ionisable functional groups

e.g. phenols, aromatic carboxylic acids and amines, are dependent on the state of ionization of the functional groups and consequently on the pH of the solution.

If the individual absorbances, A_{alk} and A_{acid} are proportional to the concentration of the analyte and path length, then the Δ A also obeys the Beer-Lamberts law and a modified equation may be derived:

$$\Delta A = \Delta abc$$

where, Δ = Difference Absorptivity of the substance at the wavelength of measurement.

If one or more absorbing substances are present in the sample at the analytical absorbance A_x in the alkaline and acidic solutions, its interference in the spectrophotometric measurement is eliminated.

$$\Delta A = (A_{alk} + A_x) - (A_{acid} + A_x)$$

The selectivity of the ΔA procedure depends on the correct choice of the pH values to induce the spectral change of the analyte without altering the absorbance of the interfering components of the sample. The use of 0.1M Sodium hydroxide and 0.1M Hydrochloric acid induces the Δ A of the analyte which is convenient and satisfactory when the irrelevant absorption arises from pH-insensitive substances. Unwanted absorption from pH-sensitive components of the sample may also be eliminated if the pK_a values of the analyte and interference differ by more than 4.

(h) Derivative spectrophotometry:-Direct Spectrophotometric determination of Multicomponent formulation is often complicated by interference from formulation matrix and spectral overlapping. Such interferences can be treated in many ways like solving two Simultaneous equations, using absorbance ratios

at certain wavelengths but still it give erroneous results[21]. Other approaches include PH induced differential least squares[22] and orthogonal function methods[23]. Also the compensation technique can be used to detect and eliminate unwanted or irrelevant absorption.

Derivative Spectrophotometry is a useful means of resolving two overlapping spectra and eliminating matrix interferences or interferences due to an indistinct shoulder on side of an absorption band[24]. Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, Zeroth order or D spectrum. The absorbance of a sample is differentiated with respect to wavelength λ to generate first, second or higher order derivative:

$$[A] = f(\lambda): \text{zero order}$$

$$[dA/d\lambda] = f(\lambda): \text{first order}$$

$$[d^2A/d\lambda^2] = f(\lambda): \text{second order}$$

The first derivative spectrum of an absorption band is characterized by a maximum, a minimum and a cross-over point at the λ max of the absorption band. The second derivative spectrum is characterized by two satellite maxima and an inverted band of which the minimum corresponds to the λ max of the fundamental band. The spectral transformation confers two principal advantages on derivative spectrophotometry, firstly an even order spectrum is of narrower spectral bandwidth than its fundamental spectrum, secondly derivative spectrophotometry discriminates in favor of substances of narrow spectral bandwidth substances. The enhanced resolution and bandwidth discrimination increases with increasing derivative order. However, it is also

found that the concomitant increase in electronic noise inherent in the generation of the higher order spectra and the consequent reduction of the signal to noise ratio place serious practical limitations on the higher order spectra.

The important features of derivative techniques include enhanced information content, discrimination against back ground noise and greater selectivity in quantitative analysis. It can be used for detection and determination of impurities in drugs, chemicals and also in food additives and industrial wastes[25].

(i) Least square approximation:-Occasionally one finds it advisable to admit that experimental measurements are not as accurate as might be desired, but are subject to random errors. An answer with higher probably accuracy can be obtained if excess experimental information is applied. Instead of carrying out analysis of two components at two wavelengths, it is carried out at three or four wavelengths. If it is carried out at three wavelengths the problem becomes the solution of three equations in two unknowns. This can not be carried out by any other method. The best solution is the least square criterion, which is found by multiplying by transpose of the absorptivity matrix.

This now gives two equations in two unknowns, such that the solution to these two equations is also the optimum solution to the three original equations. The method yields a higher precision of determinations for systems whose absorption spectra are very similar. With increasing diversity of the absorption curves, the efficiency of the method of measurements taken at a large number of wavelengths i.e. the method of an over-determined system of linear equations decreases and for systems with highly diversified curves it may even deteriorate the precision of the determination. All the foregoing methods of calculating the content of individual component in a multicomponent analysis fail to use the entire information capacity of the spectrophotometric method of analysis. Only

the method that stores the whole spectra of standard substance in the computer memory and uses the algorithm matching the absorption spectrum of the sample with the spectrum obtained mathematically by adding up the individual spectra of components makes a full use of the information load of the spectrophotometric method. This is also the operating principle of advanced design UV-Visible spectrophotometers equipped with multicomponent analysis program.

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Experinment.No:13

Simultaneous Estimation Of Paracetamol And Nimesulide

AIM: To carry out the Simultaneous estimation of Paracetamol and Nimesulide by UV method.

Requirements:-

Apparatus:-UV spectrometer, beaker, Volumetric flasks, pipettes, Measuring cylinder.

Chemicals:-Paracetamol, Nimesulide, Methanol.

Principle:-

Simultaneous equation method:-If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λ_{max} of the other, it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method).

$$Cx = \frac{A_2\, a_{y1} - A_2\, a_{y2}}{A_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

$$Cy = \frac{A_1\, a_{x2} - A_2\, a_{x1}}{A_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

where,

a) The absorptivity of X at λ_1 and λ_2 are a_{x1} and a_{x2} respectively.

b) The absorptivity of Y at λ_1 and λ_2 are a_{y1} and a_{y2} respectively.

c) The absorbances of the diluted sample at λ_1 and λ_2 are A_1 and A_2 respectively.

Criteria for obtaining maximum precision, based upon absorbance ratios that place limits on the relative concentrations of the components of the mixture.

$$\frac{A_1 / A_2}{a_{x2} / a_{x1}} \quad \text{and} \quad \frac{a_{y2} / a_{y1}}{A_2 / A_1}$$

The criteria are that the ratios should lie outside the range 0.1- 2.0 for the precise determination of Y and X respectively. These criteria are satisfied only when the λ max of the two components are reasonably dissimilar. An additional criterion is that the two components do not interact chemically.

To reduce the random errors during measurements, sometimes instead of carrying out analysis of two components at two wavelengths, it is carried out at 3 or 4 wavelengths. The equations will no longer have a unique solution but the best solution can be finding out by the least square criterion.

Absorbances for these two compounds individually & in mixtures, obeys the beer's law provided at a absorption maxima at 252nm and 296nm respectively.

Procedure:-

1. Accurately 50 mg of each of Nimesulide and Paracetamol was weighed into two 50 ml volumetric flasks.
2. It was dissolved in small quantity of methanol and was diluted upto the mark with methanol which is considered as the stock-I solution (1000µg/ml).
3. From stock I solution, 5 ml was pipetted out into 50 ml volumetric flask which was diluted to 50 ml with methanol which is considered as the stock II solution (100µg/ml).

4. Further from the stock II solution 0.2, 0.4, 0.6, 0.8 and 1.0 ml of Paracetamol and 0.4, 0.8, 1.2, 1.6 and 2.0ml of Nimesulide was pipetted out into 10ml volumetric flasks and was made upto 10 ml with methanol.
5. The UV spectrometer was switched on and it was stabilized and baseline correction was done.
6. The Absorbance of Nimesulide and Paracetamol of various dilutions at 296 nm and 252 nm was noted down respectively.
7. The graph was plotted against Absorbance versus Concentration.
8. For Nimesulide & Paracetamol the E valve is calculated.

Discussion:-

Nimesulide:-

Molecular formula :– $C_{13}H_{12}O_5N_2$ S.

It is a group of preferential Cox-2 inhibitor. This newer NSAID is a relatively weak inhibitor of PG synthesis & there is some evidence to indicate relative COX-2 selectivity.

Anti-inflammatory action may be exerted by other mechanisms as well.

Ex- reduced generation of superoxide by neutrophils, inhibition of PAF synthesis and TNF, release, free radical scavenging, inhibition of metalloproteinase activity in cartilage.

The analgesic, antipyretic and anti-inflammatory activity of nimesulide has been dated comparable to other NSAID'S.

Pharmacokinetics:-It is completely absorbed orally 99% plasma protein bound, extensively metabolized & excreted mainly in urine within a half life time of 2.5 hours.

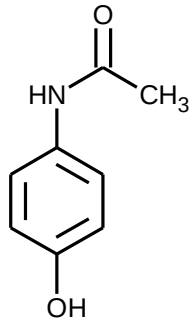
Adverse effects:-Epigastralgia, Heart burn, Nausea, loose motions, Dermatologically and it causes Rashes, Pruritis and Central – Somnolence, Dizziness.

Dose:-100 mg tablet, 50mg/5ml suspension.

Uses:-

- Sports injuries, sinusitis
- Other ENT disorders
- Dental surgery, bursitis
- Low backache
- Dysmenorrhea, Osteoarthritis & for fever.

Paracetamol:-



PARACETAMOL

Chemically it is a P- hydroxy acetanilide.

Molecular formula :- $C_8H_9O_2N$

Molecular weight :- 151.16.

Solubility :- Freely soluble in Ethanol, Acetone; Sparingly soluble in water and Very slightly soluble in ether & dichloromethane.

Colour :- white crystals or white in colour.

Category :- Analgesic & Antipyretic in nature

Storage :- stored in a well closed container.

Report:-The given sample no……..was found to contain……mg/ml of Paracetamol and …….. mg/ml of Nimesulide.

Reference:-

1. K.D.Tripathi: Essentials of Medical pharmacology.

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Experinment.No:14

Simultaneous Estimation Of Paracetamol And Ibuprofen

AIM: To carry out the Simultaneous estimation of Paracetamol and Ibuprofen.

Requirements:-

Apparatus:-UV Spectrometer, Volumetric flasks, Pipettes, Beakers.

Chemicals:-Paracetamol, Ibuprofen, 0.1 M Sodium Hydroxide.

Principle:-

Simultaneous equation method:-If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λ_{max} of the other, it may be possible to determine both drugs by the technique of Simultaneous equations (Vierodt's method).

$$Cx = \frac{A_2\, a_{y1} - A_2\, a_{y2}}{A_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

$$Cy = \frac{A_1\, a_{x2} - A_2\, a_{x1}}{A_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

where,

a) The absorptivity of X at λ_1 and λ_2 are a_{x1} and a_{x2} respectively.

b) The absorptivity of Y at λ_1 and λ_2 are a_{y1} and a_{y2} respectively.

c) The absorbances of the diluted sample at λ_1 and λ_2 are A_1 and A_2 respectively.

Criteria for obtaining maximum precision, based upon absorbance ratios that place limits on the relative concentrations of the components of the mixture.

$$\frac{A_1 / A_2}{a_{x2} / a_{x1}} \quad \text{and} \quad \frac{a_{y2} / a_{y1}}{A_2 / A_1}$$

The criteria are that the ratios should lie outside the range 0.1- 2.0 for the precise determination of Y and X respectively. These criteria are satisfied only when the λ max of the two components are reasonably dissimilar. An additional criterion is that the two components do not interact chemically.

To reduce the random errors during measurements, sometimes instead of carrying out analysis of two components at two wavelengths, it is carried out at 3 or 4 wavelengths. The equations will no longer have a unique solution but the best solution can be finding out by the least square criterion.

Absorbance for these two compounds individually & in mixtures, obeys the Beer's law provided at absorption maxima at 257 nm & 264nm respectively.

Preparation of 0.1 M Sodium Hydroxide:-

40 gm – 1000 ml distilled water → 1M Sodium Hydroxide.

4 gm – 1000 ml distilled water → 0.1M Sodium Hydroxide.

Procedure:-

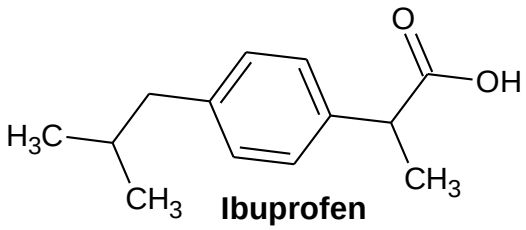
1. Accurately weigh 50 mg of Paracetamol and Ibuprofen into two 50 ml of volumetric flask and the volume is made upto 50 ml with Sodium Hydroxide (1000µg/ml – stock I).
2. Paracetamol and Ibuprofen was further diluted to get 100µg/ml (stock-II).
3. From stock-II of Paracetamol 0.4, 0.8, 1.2, 1.6 & 2.0 ml was pipetted to get the concentrations of 4, 8, 12, 16 & 20µg/ml.
4. From stock-II of Ibuprofen 1, 2, 3, 4 and 5 ml was pipetted to get the concentration of 100, 200, 300, 400 & 500µg/ml.

5. The UV spectrometer was switched on and it was stabilized and baseline correction was done.
6. The absorbance of Ibuprofen and Paracetamol of various dilutions at 264 nm and 257 nm was noted down respectively.
7. The graph was plotted against Absorbance Vs concentration.
8. For Ibuprofen & Paracetamol the E valve is calculated.

Discussion:-

Ibuprofen:-

Chemical structure:-



Ibuprofen

Chemical name :-(RS) – 2- (4- isobutyl phenyl) propionic acid.

Molecular Formula:-$C_{12}H_{18}O_2$

Molecular weight :-206.28.

Category :-Anti - inflammatory agent

Solubility :-Freely soluble in Acetone, Chloroform, Ethanol (95%), Ether and practically insoluble in water and it dissolves in dilute solutions of alkaline hydroxides and Carbonates.

Standards :-contains < 98.5% & > 101.0%

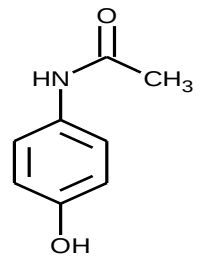
Dose :-400 – 800 mg TDS.

Ibuprofen is rated as the safest conventional NSAID by the spontaneous adverse drug RXU's reporting system in U.K.

Pharmacokinetics:-It enters brain, synovial fluid and cross placenta. They are largely metabolized in liver by hydroxylation & glucuronide conjugation and excreted in urine as well as bile.

Uses:-Analgesic & Antipyretic in the same may as low dose of aspirin.

Paracetamol:-



PARACETAMOL

Chemically it is a P- hydroxy acetanilide.

Molecular formula :- $C_8H_9O_2N$

Molecular weight :- 151.16.

Solubility :- Freely soluble in Ethanol, Acetone; Sparingly soluble in water and Very slightly soluble in ether & dichloromethane.

Colour :- white crystals or white in colour.

Category :- Analgesic & Antipyretic in nature

Storage :- stored in a well closed container.

Report:-The given sample no…… was found to contain……µg/ml of Paracetamol and ……µg/ml of Ibuprofen.

References:-

1. I.P volume II 1996.

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Experinment.No:15

Simultaneous Estimation Of Caffeine And Paracetamol

AIM: To carry out the Simultaneous estimation of Caffeine and Paracetamol by UV Spectroscopy.

Requirements:-

Chemicals:-0.lM Hydrochloric acid, Caffeine (standard), Paracetamol (standard), Distilled water.

Apparatus:-UV Spectrometer, Breaker, Volumetric flasks, Pipettes, Measuring cylinder.

Principle:-

Simultaneous equation method:-If a sample contains two absorbing drugs (X and Y) each of which absorbs at the l_{max} of the other, it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method).

$$Cx = \frac{A_2\ a_{y1} - A_2\ a_{y2}}{A_{x2}\ a_{y1} - a_{x1}\ a_{y2}}$$

$$Cy = \frac{A_1\ a_{x2} - A_2\ a_{x1}}{A_{x2}\ a_{y1} - a_{x1}\ a_{y2}}$$

where,

a) The absorptivity of X at λ_1 and λ_2 are a_{x1} and a_{x2} respectively.

b) The absorptivity of Y at λ_1 and λ_2 are a_{y1} and a_{y2} respectively.

c) The Absorbances of the diluted sample at λ_1 and λ_2 are A_1 and A_2 respectively.

Criteria for obtaining maximum precision, based upon absorbance ratios that place limits on the relative concentrations of the components of the mixture.

$$\frac{A_1 / A_2}{a_{x2} / a_{x1}} \quad \text{and} \quad \frac{a_{y2} / a_{y1}}{A_2 / A_1}$$

The criteria are that the ratios should lie outside the range 0.1- 2.0 for the precise determination of Y and X respectively. These criteria are satisfied only when the λ max of the two components are reasonably dissimilar. An additional criterion is that the two components do not interact chemically.

To reduce the random errors during measurements, sometimes instead of carrying out analysis of two components at two wavelengths, it is carried out at 3 or 4 wavelengths. The equations will no longer have a unique solution but the best solution can be finding out by the least square criterion.

Absorbance for these two compounds individually and in mixtures obeys the Beer's laws provided at a absorption maxima at 272 and 244nm respectively.

In this, the substance 1 (Caffeine) absorbs strongly at λ_1 (272 nm) and weakly at wavelength λ_2 (244 nm), whereas the substance 2 (Paracetamol) absorbs strongly at λ_2 (244 nm) and weakly at λ_1 (272 nm).

Procedure:-

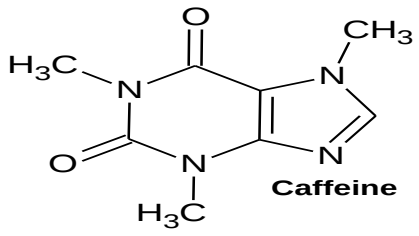
1. Accurately weigh 50 mg of each of Caffeine and Paracetamol into two 50 ml volumetric flasks separately and is diluted upto the mark with 0.1 M Hydrochloric acid (stock I solution).

2. From stock I solution, 5 ml was pipette out into 50 ml volumetric flask and is diluted to 50 ml with 0.1M Hydrochloric acid (stock II solution).
3. Further from the stock-II solution 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 ml was pipetted out into 10 ml volumetric flasks and was made upto 10 ml with 0.1 M Hydrochloric acid of each of Caffeine and Paracetamol separately.
4. The UV spectrometer was switched on and it is stabilized and baseline correction was done.
5. The Absorbance of Caffeine and Paracetamol of various dilutions at 272 nm and 244 nm was noted down respectively.
6. The graph was plotted against Absorbance versus Concentration.
7. For Caffeine and Paracetamol the E value is calculated.

Discussion:-

Caffeine:-

Chemical structure:-



Caffeine

Chemical name :- 3, 7–dihydro-1, 3, 7 – trimethyl-1H – purine – 2,6 – dione

Molecular formula :- $C_8H_{10}N_4O_2$

Molecular weight :- 194

Category :- CNS stimulant

Description :- Silky white crystals, odorless, sublimes readily.

Solubility :– Freely soluble in $CHCl_3$, boiling water and sparingly soluble in H_2O.

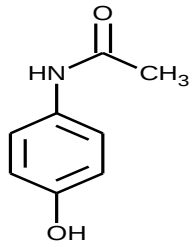
Standards :– contains < 98.5% and > 101.5%

Storage :– store in a tightly closed Containers

Caffeine is a methyl xanthene derivative

Paracetamol:-

Chemical structure:-



PARACETAMOL

Chemical name :- 4(P) – hydroxy acetanilide

Molecular formula :- $C_8H_9O_2N$

Molecular weight :- 151.16

Category :- Analgesic and Antipyretic.

Description :- white crystals or white in color

Solubility :- Freely soluble in Ethanol, Acetone; Sparingly soluble in water and Very slightly soluble in ether and dichloromethane

Storage :- stored in a well closed containers.

Paracetamol (Acetaminophen) is the deacetylated active metabolite of Phenacetin and it is used since 1950.

Dose :- 0.5 to 1 g (t.i.d)

Infants dose :- 50 mg

Children dose :- 80-160mg (1-3 yrs)

240-320mg (4-8yrs)

300-600mg (9-12yrs)

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Report:-The given sample no…… was found to contain …… µg/ml of Paracetamol and ……. µg/ml of Caffeine.

References:-

1. Caffeine - IP Volume II 1996 Pg no 121 to 122.
2. Paracetamol - IP volume II 1996 Pg no 554 to 555.

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CHROMATOGRAPHIC TECHNIQUES

Definition:-It is a technique where the separation of a mixture into individual components takes place by using a Stationary phase under the influence of a Mobile phase.

Classification:-

1. Types of chromatography.
2. Based upon separation

Types of Chromatography:-

1. Paper chromatography
2. Thin layer chromatography
3. Partition chromatography
4. Adsorption chromatography (or) Column chromatography.
5. Gas solid chromatography
6. Gas liquid chromatography
7. Ion exchange chromatography
8. High performance liquid chromatography.
9. High performance thin layer chromatography.

Based upon Separation:-

1. Adsorption chromatography.
2. Partition chromatography.

Principle of Separation:-

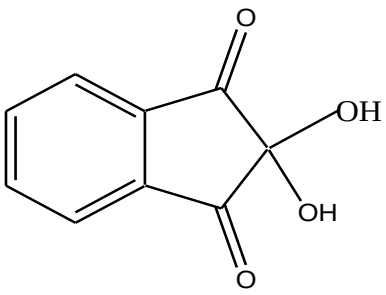
Adsorption:-One (or) more compounds are spotted on a thin layer of adsorbate coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action (against gravitational force). The components move according to their relative affinities towards the adsorbent .The component with

more affinity towards the stationary phase travels slower whereas the component with lesser affinity towards the stationary phase travels faster. Thus the components are eluted based upon the affinity of the components towards the stationary phase.

Partition:-When two immiscible liquids are present in a mixture of solutes then it is distributed according to their Partition co-efficient. When a mixture of compounds are dissolved in the mobile phase and passed through the column of liquid stationary phase then the component which is more soluble in stationary phase travels slower whereas the component which is more soluble in mobile phase travels faster. Here components are separated due to difference in the partition co-efficient.

Reagent used:-

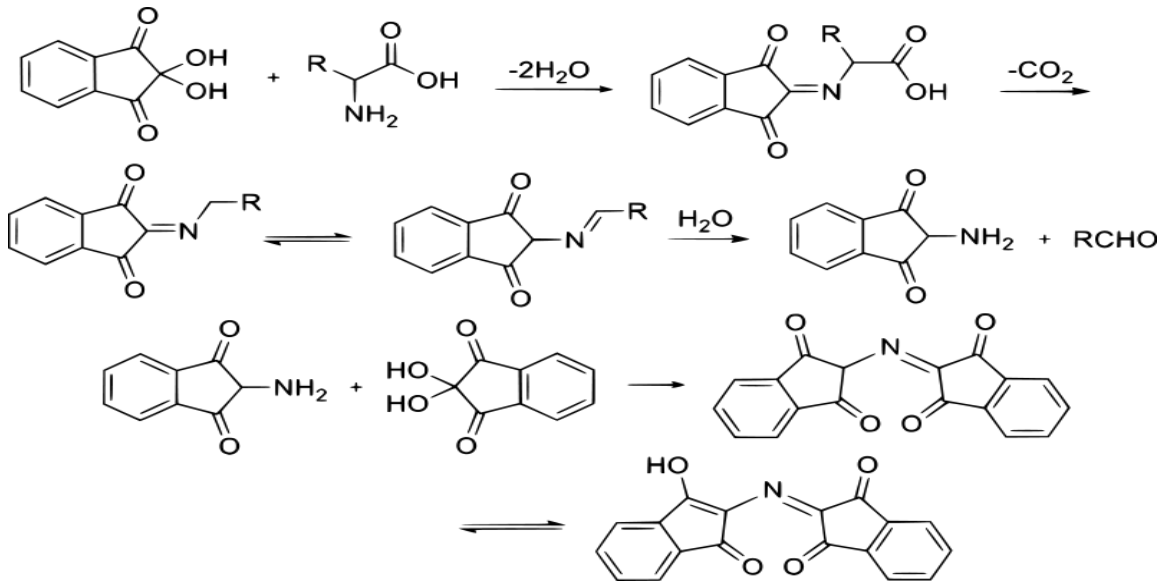
Ninhydrin reagent



2,2-dihydroxy indane 1,3-dione

Ninhydrin reacts with amines/proteins to form a deep blue /purple colour known as Ruhemann's Purple. Ninhydrin reagent is used in estimation of proteins. The amine is condensed with a molecule of ninhydrin to give a Schiff base. Thus, only ammonia and primary amines can be proceed by this step. At this step, there must also be an alpha proton for Schiff base transfer, so an amine adjacent to a tertiary carbon cannot be detected by the Ninhydrin test. The reaction of

Ninhydrin with secondary amines gives an Iminium salt, which is coloured and this is generally yellow-orange in colour.



Retardation Factor [R_f]:-In chromatography, the Retardation factor (R) is defined as the fraction of an analyte in the mobile phase of a chromatographic system. In planar chromatography in particular, the Retardation factor (sR_f) is defined as the ratio of the distance travelled by the centre of a spot to the distance travelled by the solvent front.

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Experinmen.No:16

Paper Chromatography

AIM: To carry out the Paper Chromatography of the given amino acid by Ascending method.

Requirements:-Whatmann's filter paper (rectangular in shape), Amino acid (sample), thread, rectangular Chromatographic glass chamber with glass lid, solvent system (n-Butanol : Acetic acid : Water), Detecting reagent (Ninhydrin in Acetone) and capillary tube.

Principle:-Paper Chromatography is based on the principle of Separation. The principle of separation is mainly due to Partition rather than Adsorption. In this method of Partition chromatograph, the substances are distributed between the two liquids i.e., one is stationary liquid (usually water) which is held in the fibres of paper and called the Stationary phase, the other is moving liquid and called the Mobile phase. The component of mixture to be separated migrates at different rates and appears as spot at different positions on the paper. The positions of migrated spots on the chromatograms are indicated by different terms such as R_f, R_x, R_m and R_c.

Retardation Factor (R_f):-'R' is related to the migration of the solute front relative to the solvent front as:-

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

'R' is the function of the Partition Co-efficient and it is constant for a given substance.

Procedure:-

1. The sample amino acids were prepared in water.
2. The chamber is saturated with Mobile phase (n-Butanol : Acetic acid : Water).
3. The whatmann's filter paper was taken.
4. By using the capillary tube, the amino acids are spotted on paper 1cm above the baseline.
5. The paper was placed in chamber by using a solvent system and is allowed to run up to ¾ the of paper length.
6. The paper was taken out of the chamber and dried at room temperature or it is air dried.
7. After the paper gets dried completely it is sprayed with Ninhydrin reagent.
8. R_f value of amino acids was calculated by using the formula:

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

Discussion:-

Ascending Paper Chromatography:-When the development of the paper is done by allowing the solvent to travel up the paper, it is known as ascending technique. In Ascending Chromatography, the mobile phase is placed in suitable container at the bottom of the chamber or in the chambers itself,

The samples are applied at a few centimetres from the bottom of edge of the paper suspended rolled into a cylinder, held together by staples, strings or plastic clips. This method is used for separation of organic and used for

separation of organic and inorganic substances but the ascending technique is preferred if the R_f values of various constituents are almost same.

Report:-The given sample no……of amino acids by Ascending Paper Chromatography was found to be ……. and …….

Reference:-

1. Gurudeep R Chatwal: Instrumental methods of chemical analysis.

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Experinment.No:17

Radial Paper Chromatography

AIM: To carry out the Radial Paper Chromatography of the given amino acid.

Requirements:-Whatmann's filter paper, cotton wig, Amino acid samples, petridish, Solvent system (n-Butanol : Acetic acid : Water), Detection reagent (Ninhydrin in acetone 0.2% w/v) and capillary tube.

Principle:-This technique is a type of Partition Chromatography in which the substances are distributed between the two liquids i.e., one is the stationary liquid which is held in the fibres of the paper and called the stationary phase. The other is the moving liquid or developing solvent and called the moving phase. The components of the mixture to be separated migrate at different rates and appear as spots at different points on the paper. The movement of substances relative to the solvent is expressed in terms of R_f values i.e., Migration parameters.

Procedure:-

1. A circular piece of whatmann filter paper was taken. At the centre of the paper a cotton wig is fixed.
2. The amino acid samples were spotted at the centre of the paper and at the upper end of the wig.
3. The paper was then laid on the edge of the circular disk with the wig dipping into the solvent at the bottom of the disk.
4. The liquid ascends by the wig and flows radially through the paper.
5. While moving, the liquid carries the compounds with it and thus chromatogram was developed.

6. The paper is air dried and Ninhydrin reagent is sprayed and again it was air dried.
7. The R_f valves of each amino acid sample was calculated.

Discussion:-

Radial paper chromatography:-This is also known as Circular Paper Chromatography. This makes use of radial development. In this technique, a circular filter paper is employed. Then the various material to be analyzed are placed at the center. After drying the spot, the paper is fixed horizontally on the petri dish possessing the solvent so that the tongue or the wick of the paper dips into the solvent.

Now cover the paper by means of petri dish cover. The solvent rises through the wick. When solvent front has moved through a sufficient large distance, the components get separated in the form of concentric circular zone as shown in the diagram.

Report:-The given sample no…… of amino acids by Radial Paper Chromatography method was found to contain …… and ……..

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Experinment.No:18

Thin Layer Chromatography Of Amino Acids

AIM: To identify the given unknown sample of Amino acids by calculating the Rf values by using Thin Layer Chromatography (TLC) method.

Requirements:-TLC chambers, TLC plates, Hot air oven.

Samples:-Valanine, Glycine, Alanine, Lucine, Isolucine, Tryptophan.

Solvent system:-n-Butanol : Acetic acid : Water (4 : 1 : 5).

Spraying reagent:-Ninhydrin reagent.

Principle:-Chromatography using thin layers of an adsorbent held on the glass plate or other supporting medium is known as Thin Layer Chromatography. A thin layer plate may be prepared by spreading aqueous slurry of finely ground adsorbent over the surface of a glass plate. Then the plates are activated at 105ºC for 30 minutes and are used. The selection of mobile phase depends upon type of constituents to be analysed and the spots are revealed by spraying with suitable detecting agent.

Procedure:-

1. Preparation of thin layers in plates by any one of the methods viz- pouring, dipping, spraying or spreading method.
2. Activation of adsorbent by drying the thin layer plate for 30 minutes and then it is dried in hot air oven at 105˚C for another 30 minutes.
3. Sample is applied with the help of capillary tube.
4. Plates are saturated in development chamber at an angle of 45˚
5. After the development it is dried and it is sprayed with Ninhydrin reagent.

Test solution:-It is dissolved in suitable solvent in order to obtain a concentrated solution.

Developing solvent:-n-Butanol : Acetic acid : Water (4 : 1 : 5).

Detecting reagent:-Ninhydrin reagent.

Report:-The given alkaloid samples, R_f values was found to be …….

Vision and Mission of the Institution

Vision

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

Mission

M1 Create pharmacy graduates through **quality education**

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

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

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