TECHNOCRATS

Lab Work Book of

Instrumental Methods of Analysis

(BP-705)

Department of Pharmacy

Lab Manual of

Instrumental Methods of Analysis

(BP-705)

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TECHNOCRATS
PUBLICATIONS

Lab Work Book
of

INSTRUMENTAL METHODS OF ANALYSIS

(BP-705)

(Strictly According to RGPV Syllabus)

Name : .....

Enrollment No. : .....

Institute : .....

Academic Session : .....

Department of Pharmacy

TECHNOCRATS
PUBLICATIONS

Vision of the Institute

To grow as an institute of Excellence for Pharmacy Education and Research and to serve the humanity by sowing the seeds of intellectual, cultural, ethical, and humane sensitivities in the students to develop a scientific temper, and to promote professional and technological expertise.

Mission of the Institute

M 1: To inculcate ethical, moral, cultural and professional values in students

M 2: To provide state of art infrastructure facilities to the staff and students so as to enable them to learn latest technological advancements

M 3: State of art learning of professionalism by the faculty and students

M 4: To produce well learned, devoted and proficient pharmacists

M 5: To make the students competent to meet the professional challenges of future

M 6: To develop entrepreneurship qualities and abilities in the students

PROGRAM OUTCOMES (POs)

1. **Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.
2. **Planning Abilities:** Demonstrate effective planning abilities including time management, resource management, delegation skills and organizational skills. Develop and implement plans and organize work to meet deadlines.
3. **Problem analysis:** Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, while solving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions.
4. **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of thelimitations.
5. **Leadership skills:** Understand and consider the human reaction to change, motivationissues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and well- being.
6. **Professional Identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).
7. **Pharmaceutical Ethics:** Honour personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.
8. **Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions.
9. **The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice.
10. **Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.
11. **Life-long learning:** Recognize the need for, and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

PEOs

PEO 1: To inculcate quality pharmacy education and training through innovative Teaching Learning Process.

PEO 2: To promote professionalism, team spirit, social and ethical commitment with effective interpersonal communication skills to boost leadership role assisting improvement in healthcare sector.

PEO 3: To enhance Industry-Institute-Interaction for industry oriented education and research, which will overcome healthcare problems of the society.

PEO 4: To adapt and implement best practices in the profession by enrichment of knowledge and skills in research and critical thinking

PEO 5: To generate potential knowledge pools with interpersonal and collaborative skills to identify, assess and formulate problems and execute the solution in closely related pharmaceutical industries and to nurture striving desire in students for higher education and career growth.

Course Outcomes (COs):

Student will be able to:

- CO1: Determine the percentage purity of Ascorbic acid by performing acid-base titration.
- CO2: Estimate the percentage purity of Ampicillin by their assay method.
- CO3: Calculate the purity of Metronidazole by performing assay.
- CO4: Estimate the percentage purity of ibuprofen by their volumetric titration.
- CO5: DDetermine the percentage purity of compounds by gravimetric analysis.

Index

Experiment No. 1

OBJECT :

To calibrate Ultra Violet Spectrophotometer.

REFERENCE:

Sharma BK, Instrumental method of chemical analysis, twenty third edition 2004, Goel publishing house, Page no. 46

REQUIREMENTS:

Glassware:- Volumetric Flask, Pipette, beaker

Chemicals:- Perchloric acid, Potassium dichromate, Sulphuric acid, Potassium chloride, Holmium Oxide, Toluene

THEORY:

Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared, but does not cover time-resolved spectroscopic techniques.

Spectrophotometry uses photometers that can measure a light beam's intensity as a function of its color (wavelength) known as spectrophotometers. Important features of spectrophotometers are spectral bandwidth, (the range of colors it can transmit through the test sample), and the percentage of sample-transmission, and the logarithmic range of sample-absorption and sometimes a percentage of reflectance measurement.

Ultraviolet–visible spectroscopy or **ultraviolet-visible spectrophotometry** (**UV-Vis** or **UV/Vis**) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared [NIR]) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state

Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons (i.e. lower energy gap between the HOMO and the LUMO), the longer the wavelength of light it can absorb.

PROCEDURE:

Calibration

Calibration of UV-VIS Spectrophotometer is done in four steps.
A] Control of Wave length
B] Control of Absorbance
C] Limit of Stray Light
D] Resolution Power

Control of Wave length

1. Weigh accurately 1.0 gm of Holmium Oxide and dissolve it in 1.4 M Perchloric acid solution. Make up to 25 ml with the same solvent.
2. Select the method file of CONTROL OF WAVE LENGTH in the instrument.
3. After selecting the file press Reference button for baseline correction.
4. Then fill the Cuvette with 1.4M Perchloric acid and put in the sample cubicle and press reference to zero.
5. After auto zero put the Holmium perchlorate solution in sample cubicle then press start key.
6. Scan it and verify the wavelength using absorption maxima of Holmium Perchlorate solution

Control of Absorbance

1. Dry a quantity of the Potassium dichromate by heating to constant weight at 130°C.
2. Weigh accurately about 60 mg of dried potassium dichromate and dissolve it in 0.005M sulphuric acid solution. Make upto 1000 ml with the same solvent. Mark the solution as (A).
3. Weigh accurately about 60 mg of dried potassium dichromate and dissolve it in 0.005M sulphuric acid solution. Make up to 100 ml with the same solvent. Mark the solution as (B).
4. Select the method file of CONTROL OF ABSORBANCE in the instrument.
5. After selecting the file press Reference button for baseline correction.
6. Then fill the Cuvette with 0.005M Sulphuric acid for blank and put in both sample cubicle and press reference to zero.
7. After auto zero put the Potassium Dichromate solution labeled as solution ‘A’ in sample cubicle then press start key taking absorbance individually for first four wavelength mentioned
8. Now take absorbance at 430 nm for solution ‘B’.
9. Note the absorption maxima of Potassium Dichromate solution at different wave length and calculate the absorbance, tolerance

Limit of Stray light

1. Dry sume quantity of the Potassium chloride by heating to constant weight at 130°C.
2. Weigh accurately 1.20 g of dried potassium chloride and dissolve it in 50 ml distilled water. Make upto 100 ml with the same solvent.
3. Select the method file of LIMIT OF STRAY LIGHT in the instrument.
4. After selecting the file press Reference button for baseline correction.
5. Check the absorbance of above solution using water as a blank at 200 nm.
6. Absorbance should be greater than 2.0

Resolution power

1. Prepare 0.02%v/v solution of Toluene in Hexane UV.
2. Select the method file of RESOLUTION POWER in the instrument.
3. After selecting the file press Reference button for baseline correction.
4. Measure the absorbance of above solution at 266 nm and 269 nm using Hexane UV as blank solution.
5. The ratio of absorbance maxima at 269 nm to that of 266 nm minima should be more than 1.5
6. Note down the report in the internal calibration certificate and in Instrument Logbook.

OBSERVATION:

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RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.1 What are the principle of Ultraviolet–visible spectroscopy.

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Q.2 What is wavelength.

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Q.3 What is lambert beer law.

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Q.4 What is Normality

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Q.5 How to make 1 N sulfuric acid solution.

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Experiment No. 2

OBJECT:

To verify the Beer,s Lamberts Law with Benzoic acid and find out the concentration of the given unknown solution .

REFERENCE:

Sharma BK, Instrumental method of chemical analysis, twenty third edition 2004, Goel publishing house, Page no. 49-50

REQUIREMENTS:

Glassware:- Volumetric Flask, Pipette, beaker

Chemicals:- Benzoic acid,

THEORY:

The **Beer–Lambert law**, also known as **Beer's law**, the **Lambert–Beer law**, relates the attenuation of light to the properties of the material through which the light is traveling. The law is commonly applied to chemical analysis measurements and used in understanding attenuation in physical optics, for photons, neutrons or rarefied gases.

PROCEDURE:

1. Prepare 1000 ml of N/1000 Benzoic acid and as a stock solution.
2. From the stock solution prepare 100 ml of each benzoic acid solution of 2, 4,6,8,10 ml respectively.
3. Determine absorbance of each solution at 250 nm plot the graph b/w conc. And absorbance and shows beers law is obeyed.
4. From the stock solution, prepare another Solution of unknown conc. determines the absorbance of the solution find out the concentration of the solution from the graph obtained in solution-2.

OBSERVATION:

SN	CONCENTRATION	ABSORBANCE
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RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.1 What is beer law.

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Q.2 How to make 1 N Benzoic acid solution

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Q.3 What is stock solution.

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Q.4 What is bathochromic shift

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Q.5 What is chromophor.

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Experiment No. 3

OBJECT:

To perform the assay of Paracetamol tablet I.P.

REFERENCE:

Beckett AH, Stenlke JB, practical pharmaceutical chemistry, fourth edition 2004, CBS publishers anddistributors new delhi, Page No. 275

REQUIREMENTS:

Glassware:- Volumetric Flask, Pipette, beaker

Chemicals:- Paracetamol tablet, sodium Hydroxide

THEORY:

Paracetamol, also known as acetaminophen or APAP, is a widely used over-the-counter pain medication and medication to reduce fever. It is commonly used to help with headaches, other minor aches and pains, and is a major ingredient in many cold medications. In combination with opioid analgesics, paracetamol is used in the management of more severe pain such as post-surgical and cancer pain. Though paracetamol is used to treat inflammatory pain, it is not classified as an NSAID because it exhibits only weak anti-inflammatory activity.

While generally safe for use at recommended doses, even small overdoses can be fatal. Compared to other over-the-counter pain relievers, paracetamol is significantly more toxic in overdose but may be less toxic when used chronically at recommended doses. Paracetamol is the active metabolite of phenacetin and acetanilide, both once popular as analgesics and antipyretics in their own right. However, unlike phenacetin, acetanilide and their combinations, paracetamol is not considered carcinogenic at therapeutic doses. Paracetamol is classified as a mild analgesic.

PROCEDURE:

1. 20 tablets were weighed and powdered.
2. A quantity of the powder equivalent to abt 0.15 gm of paracetamol was weighed accurately and 50 ml of 0.1 M NaOH was added to it.
3. It was then diluted with 100 ml water and shaken for 15 mins.

4. Sufficient water was added to produce 200 ml.
5. It was mixed, filtered and 10 ml of filtrate was diluted with 100 ml of distilled water.
6. To the 10 ml of the resulting solution 10 ml of 0.1 m NaOH was added and diluted to 100 ml with water & mixed.
7. The absorbance of the resulting solution was measured at the maximum at about 257 nm.
8. The content of the paracetamol was calculated taking 715 as the value of A (1%, 1 cm) at the maximum at about 257 nm.

Formula:- A= A 1%/1cm bc

OBSERVATION:

SN	CONCENTRATION	ABSORBANCE
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RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.1 What is transition state.

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Q.2 How to make 0.1 M sodium hydroxide solution

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Q.3 What is λmax of Paracetamol.

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Q.4 What is the category of Paracetamol

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Q.5 What is auxochrome.

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Experiment No. 4

OBJECT:

To perform the assay of ATENOLOL tablet I.P. by using uv spectrophotometery.

REFERENCE:

Sharma BK, Instrumental method of chemical analysis, twenty third edition 2004, Goel publishing house, Page no. 49-51

REQUIREMENTS:

Glassware:- Volumetric Flask, Pipette, beaker

Chemicals:- Atenolol tablet, Methanol

THEORY:

spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared, but does not cover time-resolved spectroscopic techniques.

Ultraviolet–visible spectroscopy or **ultraviolet-visible spectrophotometry** (**UV-Vis** or **UV/Vis**) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared [NIR]) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergoelectronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

PRINCIPLE:- Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons (i.e. lower energy gap between the HOMO and the LUMO), the longer the wavelength of light it can absorb

PROCEDURE:

1. 20 tablets are weighed and powdered.
2. A quantity of the powder equivalent to 0.2 gm of atenolol was weighed accurately.
3. It was transferred to a 500 ml volumetric flask and 300 ml methanol was added.
4. The resulting solution was heated to 60^0 C and shaken for 15 mins.
5. It was then cooled and diluted to 500 ml with methanol.
6. It was filtered through a sintered glass funnel.
7. A suitable volume of the filtrate was diluted with a sufficient methanol to produce a solution containing 0.01% w/v of atenolol.
8. The absorbance was measured with 275 nm.
9. The content was calculated taking 53.7 as the value A at the maximum at about 275 nm.

Formula:- A= A 1%/1cm bc

OBSERVATION:

SN	CONCENTRATION	ABSORBANCE
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RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.1 What are spectroscopic technique

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Q.2 Different types of spectroscopic technique .

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Q.3 What is HOMO & LUMO

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Q.4 What is W/V & V/V

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Q.5 Application of UV

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Experiment No. 5

OBJECT:

To perform the assay of PROPRANOLOL tablet I.P. by using uv spectrophotometery.

REFERENCE:

Beckett AH, Stenlke JB, practical pharmaceutical chemistry, fourth edition 2004, CBS publishers and distributors new delhi, Page No. 278

REQUIREMENTS:

Glassware:- Volumetric Flask, Pipette, beaker

Chemicals:- Propranolol tablet, Methanol

THEORY:

U**ltraviolet-visible spectrophotometry** (**UV-Vis** or **UV/Vis**) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared [NIR]) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

PRINCIPLE:

Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons (i.e. lower energy gap between the HOMO and the LUMO), the longer the wavelength of light it can absorb

PROCEDURE:

1. About 20 tablets were weighed and powdered and accurately a quantity of powder equivalent to 20 mg of propranolol was weighed.
2. It was shaken with 20 ml of water for 10 mins.
3. Methanol was added to produce 100 ml.
4. The resulting solution was filtered then diluted to 10 ml was taken and the volume is produced to 50 ml with methanol.
5. The absorbance was measured at 290 nm and the value of a is.

Formula:- A= A 1%/1cm bc

OBSERVATION:

SN	CONCENTRATION	ABSORBANCE
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RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.1 What is lambert beer law.

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Q.2 What is wavelength

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Q.3 What is absorbance

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Q.4 What are electon transition

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Q.5 What is hypsochromic shift.

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Experiment No. 6

OBJECT:

To perform the assay of phenolphthalein by colorimetry.

REFERENCE:

Delva rao G, Practical pharmaceutical analysis, second edition 2008, Birla publicationshouse, Page no.112

REQUIREMENTS:

Glassware:- Volumetric Flask, Pipette, beaker

Chemicals:- phenolphthalein, Methanol, glycine buffer

THEORY:

A **colorimeter** is a device used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light (not to be confused with the tristimulus **colorimeter** used to measure colors in general

Colorimetry is a technique "used to determine the concentration of colored compounds in solution." A colorimeter is a device used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light (not to be confused with the tristimulus colorimeter used to measure colors in general).

To use the colorimeter, different solutions must be made, including a control or reference of known concentration. With a visual colorimeter, for example the Duboscq colorimeter illustrated, the length of the light path through the solutions can be varied while filtered light transmitted through them is compared for a visual match. The concentration times path length is taken to be equal when the colors match, so the concentration of the unknown can be determined by simple proportions. Nessler tubes work on the same principle.

There are also electronic automated colorimeters; before these machines are used, they must be calibrated with a cuvette containing the control solution. The concentration of a sample can be calculated from the intensity of light before and after it passes through the sample by using the Beer–Lambert law. Photoelectric analyzers came to dominate in the 1960s.

The color or wavelength of the filter chosen for the colorimeter is extremely important, as the wavelength of light that is transmitted by the colorimeter has to be the same as that absorbed by the substance being measured. For example, the filter on a colorimeter might be set to red if the liquid is blue.

PROCEDURE:

1. Weighed accurately about 50 mg of phenolphthalein and dissolve in methanol to make up the volume upto 50 ml.
2. Then 5 ml solution was diluted to 50 ml of methanol.
3. 5 ml of this solution was taken and evaporated to dryness.
4. The residue obtained was dissolved in glycine buffer pH 11.3 & volume was made up to 100 ml with same buffer solution.
5. Finally, the absorbance was measured in wavelength of abt 555 nm.
6. The content of phenolphthalein was calculated by taking 1055 as value of E 1% 1 cm.

Formula:- $A = A\ 1\%/1cm\ bc$

OBSERVATION:

SN	CONCENTRATION	ABSORBANCE
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RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.1 What is the principle of colorimetry.

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Q.2 What is buffer solution

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Q.3 What is buffer capacity

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Q.4 What are E 1% 1cm

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Q.5 How to make glycine buffer pH 11.3

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Experiment No. 7

OBJECT:

To perform the assay of ALBENDAZOLE TABLETS I.P. by using uv spectrophotometry.

REFERENCE:

Beckett AH, Stenlke JB, practical pharmaceutical chemistry, fourth edition 2004, CBS publishers and distrybutors new delhi, Page No. 275

REQUIREMENTS:

Glassware:- Volumetric Flask, Pipette, beaker

Chemicals:- Albendazole tablet, Methanol, Sodium Hydroxide

THEORY:

U**ltraviolet-visible spectrophotometry** (**UV-Vis** or **UV/Vis**) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared [NIR]) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergoelectronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

PRINCIPLE:

Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals The more easily excited the electrons (i.e. lower energy gap between the HOMO and the LUMO), the longer the wavelength of light it can absorb

PROCEDURE:

1. Weighed 20 tablets, powdered & weighed accurately a quantity of powder equivalent to about 0.05 gms.
2. 50 ml of methanol was added & shaken for 30 mins.
3. To this solution 0.1 m methanolic HCL was added to produce 100 ml.
4. It was then added, mixed & filtered and about 2 ml of the filtrate was taken and diluted with 0.1 m NaOH to produce 100 ml.
5. Absorbance was measured at 309 nm using 742 as the value of E 1% 1 cm.

Formula:- $A = A\,1\%/1cm\ bc$

OBSERVATION:

SN	CONCENTRATION	ABSORBANCE
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RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.1 Principle of UV spectroscopy.

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Q.2 What is non-bonding electrons.

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Q.3 How to make 0.1 M Sodium Hydroxide solution

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Q.4 What is Woodward fisher rule.

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Q.5 What is conjugated Dienes

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Experiment No. 8

OBJECT:

To perform the assay of bromohexane tablets I.P. using UV spectrophotometry.

REFERENCE:

Sharma BK, Instrumental method of chemical analysis, twenty third edition 2004, Goel publishing house, Page no. 49-51

REQUIREMENTS:

Glassware:- Volumetric Flask, Pipette, beaker

Chemicals:- Bromohexane tablet, Methanolic HCL, Sodium Hydroxide

THEORY:

Ultraviolet–visible spectroscopy or **ultraviolet-visible spectrophotometry** (**UV-Vis**) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared [NIR]) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergoelectronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

PRINCIPLE:

Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals The more easily excited the electrons (i.e. lower energy gap between the HOMO and the LUMO), the longer the wavelength of light it can absorb

PROCEDURE:

1. About 20 tablets were weighed & powdered.
2. Weighed accurately a quantity of powder equivalent to 8 mg of bromohexane HCL.
3. It was then shaken with 50 ml of 0.1 M methanolic HCL was added and produced to 100 ml and filtered.
4. Measure the absorbance at maximum at 317 nm.

Formula:- $A = A\ 1\%/1cm\ bc$

OBSERVATION:

SN	CONCENTRATION	ABSORBANCE
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RESULT AND DISCUSSION:-

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VIVA QUESTIONS

Q.1 What is standard solutions.

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Q.2 What is π-electrons.

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Q.3 How to make 0.1 M Sodium Hydroxide solution

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Q.4 What is the test solution.

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Q.5 Application of UV spectroscopy.

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Experiment No. 9

OBJECT:

To calculate the Rf value of giving amino acid by ascending Paper Chromatography.

REFERENCE:

Delva rao G, Practical pharmaceutical analysis, second edition 2008, Birla publicationshouse, Page no.130

REQUIREMENTS:

Glassware:- TLC paper, capillary tube, TLC chamber, beaker,

Chemicals:- amino acid , Ninhydrin Solution, Methanol

THEORY:

Paper chromatography is an analytical method that is used to separate coloured chemicals or substances. This can also be used in secondary or primary colours in ink experiments. This method has been largely replaced by thin layer chromatography, but is still a powerful teaching tool.

Types:

1. Descending Paper Chromatography-In this type, development of the chromatogram is done by allowing the solvent to travel down the paper.

2. Ascending Paper Chromatography-Here the solvent travel upward direction of the Chromatographic paper. Both the Descending and Ascending Paper Chromatography are used for separation of Organic and Inorganic substances.

3. Ascending-Descending Paper Chromatography-It is the hybrid of both the above techniques. The upper part of the Ascending chromatography can be folded over a rod and allowing the paper to become descending after crossing the rod.

4. Radial Paper Chromatography-It is also called a Circular chromatography. Here a circular filter paper is taken and the sample is given at the center of the paper. After drying the spot the filter paper is tied horizontally on a Petridish containing solvent. So that Wick of the paper is dipped inside the solvent. The solvent rises through the wick and the component get separated in form of concentrate circular zone.

5. Two-Dimensional Paper Chromatography-In this technique a square or rectangular paper is used. Here the sample is applied to one of the corners and development is performed at right angle to the direction of first run.

PROCEDURE:-

1. Prepare a solution of Cystine 0.01% w/v.
2. Prepare a solution of Isolucine 0.01%w/v.
3. Prepare a solution of Glycine 0.01%w/v.
4. Prepare a solution of Methanol in Water a solvent system mobile Phase.
5. Then place a paper in solvent system for some time with the Spot of all the solution of amino acids .
6. Then dry the paper in air.
7. Prepare spraying reagent 2%w/v. (ninhydrine solution)
8. Then spray the reagent on paper and dried in Hot air oven.
9. Then find out conc. Spot and according to this calculate the Rf Value.

Formula:-

$$\frac{\text{Distance from Baseline travelled by Solute}}{\text{Distance from Baseline travelled by Solvent (Solvent Front)}}$$

OBSERVATION:

SN	Sample Name	Rf Value
1		
2		
3		
4		
5		
6		

RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.1 What is Chromatography.

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Q.2 Principle of Paper Chromatography.

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Q.3 What is Rf Value.

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Q.4 How to make 2% W/V ninhydin solution.

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Q.5 Different types of Chromatography.

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Experiment No. 10

OBJECT:

To separate the mixture of amino acids and calculate the Rf value by ascending Paper Chromatography.

REFERENCE:

Sharma BK, Instrumental method of chemical analysis, twenty third edition 2004, Goel publishing house, Page no. 230-233

REQUIREMENTS:

Glassware:- TLC paper, capillary tube, TLC chamber, beaker,

Chemicals:- amino acid , Ninhydrin Solution, Methanol

THEORY:

Paper chromatography is an analytical method that is used to separate coloured chemicals or substances. This can also be used in secondary or primary colours in ink experiments. This method has been largely replaced by thin layer chromatography, but is still a powerful teaching tool.

Paper chromatography is an analytical method that is used to separate coloured chemicals or substances. This can also be used in secondary or primary colours in ink experiments. This method has been largely replaced by thin layer chromatography, but is still a powerful teaching tool. **Double-way paper chromatography**, also called two-dimensional chromatography, involves using two solvents and rotating the paper 90° in between. This is useful for separating complex mixtures of compounds having similar polarity, for example, amino acids. If a filter paper is used, it should be of a high quality paper. The mobile phase is developing solutions that can travel up to the stationary phase carrying the sample along with it.

Rf Value:

The retention factor (R_f) may be defined as the ratio of the distance traveled by the substance to the distance traveled by the solvent. R_f values are usually expressed as a fraction of two decimal places. If R_f value of a solution is zero, the solute remains in the stationary phase and thus it is immobile. If R_f value = 1 then the solute has no affinity for the stationary phase and travels with the solvent front. To calculate the R_f value, take the distance traveled by the substance divided by the distance traveled by the solvent (as mentioned earlier in terms of ratios).

PROCEDURE:

1. Prepare a solution of Cystine 0.01% w/v.
2. Prepare a solution of Isolucine 0.01%w/v.
3. Prepare a solution of Glycine 0.01%w/v.
4. Prepare a solution of unknown mixture from the above list-
5. Prepare a solution of Methanol in Water a solvent system mobile Phase.
6. Then place a paper in solvent system for some time with the Spot of all the solution of amino acid .
7. Then dry the paper in air.
8. Prepare spraying reagent 2% w/v (ninhydrine solution)
9. Then spray the reagent on paper and dried in Hot air oven.
10. Then find out conc. Spot and according to this calculate the Rf Value.

Formula:-

$$\frac{\text{Distance from Baseline travelled by Solute}}{\text{Distance from Baseline travelled by Solvent (Solvent Front)}}$$

OBSERVATION:

SN	Sample Name	Rf Value
1		
2		
3		
4		
5		
6		

RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.1 Principle of ascending Paper Chromatography.

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Q.2 What is Rf Value.

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Q.3 What is TLC.

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Q.4 What is Mobile & Stationary Phase.

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Q.5 Application of Chromatography.

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Experiment No. 11

OBJECT:

To find out the conc. of Salicylic acid in given sample using Colorimetry.

REFERENCE:

Chatwal GR, Anand SK, Instrumental method of chemical analysis,Fifth edition 2007, Himalaya publishing house, Page no. 2.108

REQUIREMENTS:

Glassware:- Volumetric Flask, Pipette, beaker

Chemicals:- Salicylic acid, Alcohol,

THEORY:

Colorimetry or **Colourimetry** is "the science and technology used to quantify and describe physically the human color perception." It is similar to spectrophotometry, but is distinguished by its interest in reducing spectra to the physical correlates of color perception, most often the CIE 1931 XYZ color space tristimulus values and related quantities. Colorimetric equipment is similar to that used in spectrophotometry. Some related equipment is also mentioned for completeness.

- A tristimulus colorimeter measures the tristimulus values of a color.
- A spectroradiometer measures the absolute spectral radiance (intensity) or irradiance of a light source
- A spectrophotometer measures the spectral reflectance, transmittance, or relative irradiance of a color sample.
- A spectrocolorimeter is a spectrophotometer that can calculate tristimulus values.
- A densitometer measures the degree of light passing through or reflected by a subject
- A color temperature meter measures the color temperature of an incident illuminant.

PROCEDURE:

1. Take 0.1g Salicylic acid in 10ml Alcohol.
2. Add about 100 ml distilled water.
3. Filter the resulting solution.
4. Add and divide filtrate in 5, 10, 15, 20,and 25ml in different volumetric flask.
5. Make up the volume upto 100ml each
6. Then immediately measure the absorbance using Colorimeter.
7. Plot a graph between concentration & absorbance
8. Calculate the concentration of absorbance on the graph.

OBSERVATION:

SN	CONCENTRATION	ABSORBANCE
1	**50 µg/ml**	
2	**100 µg/ml**	
3	**150 µg/ml**	
4	**200 µg/ml**	
5	**250 µg/ml**	

RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.1 Principle of colorimetry.

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Q.2 What is Wavelength.

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Q.3 Application of colorimetry

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Q.4 What is Normality

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Q.5 What is Absorbance.

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