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Practical Manual of Instrumental Method of Analysis

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national and international Journal of repute, has more than 100 abstract in various Seminar & Conferences, published 40 books and serving as Executive Editor, International Journal of Pharmacy and Life Sciences, Reviewer of many Journals and Editorial Board Member of several International Journal viz., African Journals, American J. of Pharmacy etc. He has been awarded as Young Scientist award in 2018, Young Researcher award in 2018, Inspirational Teacher award in 2019, Young Achiever award in 2020, Eminient Researcher Award, 2024 & Best Innovator Award 2024 by various organizations. He has also received letter of appreciation by DHR Industrial, India for adopting his research work formula and methodology for formulation of herbal oil and shampoo in Nov, 2018. He has delivered more than 50 lectures in various Conferences & Seminar. Dr. Dwivedi has successfully organized 6 Seminar funded by MPCST & AERB; Workshop on Medicinal plants and completed two major research project funded by Omar Al-Mukhtar University, Al- Bayda, Libya & Research Cell, OUI. He has also Published 19 Indian Patent, granted with 1 Indian Patent, 1 Australian Patent, 14 Indian Design, 5 UK Design Patent & 1 Copyright. He has guided more than 35 Postgraduate students, awarded eight Ph.D and presently 2 students are working under him for their Doctorate. He has keen interest in Herbal Medicine, Documentation and Validation of herbal dosage form. He is involved in research on biological & pharmacological screening, formulation, standardization of herbals, cultivation practices of medicinal plants and phenological studies of herbs.

Preface

The rapid advancements in scientific instrumentation have revolutionized the field of chemical analysis, making it more precise, accurate, and efficient. *The Practical Manual of Instrumental Method of Analysis* has been designed to provide students, researchers, and professionals with a comprehensive understanding of various instrumental techniques used in analytical chemistry.

This manual serves as a practical guide to the fundamental principles, working mechanisms, and applications of modern analytical instruments. It includes step-by-step experimental procedures, sample preparation techniques, and data interpretation methods for key instrumental methods such as spectroscopy, chromatography, electrochemical analysis, and thermal analysis. The content is structured to ensure clarity and ease of use, making it suitable for both academic and industrial applications.

The manual aims to bridge the gap between theoretical knowledge and hands-on experience, helping users develop proficiency in using analytical instruments effectively. Each experiment is designed to enhance problem-solving skills, critical thinking, and scientific reasoning. Additionally, safety precautions and best practices are emphasized to ensure a responsible approach to laboratory work.

We hope that this manual serves as a valuable resource for students and professionals, fostering a deeper understanding of instrumental analytical techniques and their significance in scientific research and industry.

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Dr. Sumeet Dwivedi

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Chapter 1

Determination of Maximum Wavelength and Effect of Solvents on Absorption Maxima by Colorimeter

1 Introduction

To determine the λ max of $KMnO_4$ and to study the effect of solvent on absorption spectrum of $KMnO_4$.

2 Theory

The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length (cuvette length), Visible spectroscopy (colorimetry) can be used to determine the concentration of the absorber in a solution. The absorbance changes with concentration, A higher concentration of the coloured solution absorbs more light (and transmits less) than a solution of lower concentration.

According to Beer–Lambert law,

$$\log (I_o / I_t) = A = \varepsilon c l$$

where I_o and I_t are the incident and transmitted intensities,

A = absorbance and ε is a constant i.e. absorptivity (formerly called the extinction coefficient).

If the concentration is measured in $molL^{-1}$, the absorptivity is called the molar absorptivity .

$$A = \varepsilon c l$$

At constant length of solution

$$A \alpha c$$

3 Procedure

Determination of λ max

- 100 mg of $KMnO_4$ was weighed and dissolved in a 100 ml volumetric flask.
- The volume of the solution was added up to 100 ml with distilled water.
- From the solution above, 10 ml was taken and diluted to 100 ml with distilled water.
- Then from the second solution, 5 ml was taken and diluted to 10 ml with distilled water and a resulting solution of 50 µg/ml was obtained.

- The UV-Vis Spectrophotometer / colorimeter was switched on and allowed to stabilize for 15 minutes.
- The absorbance was set at zero by using distilled water as a blank.
- Then the transmittance was set to 100% by distilled water.
- The absorbance values of the resulting solution at different wavelengths were taken and tabulated.
- A graph of absorbance values against the wavelength was plotted.

4 Observations

Table 1.1: Wavelength vs Absorbance

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

5 Results

The λ max of potassium permanganate ($KMnO_4$) was found to be _____ nm.

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Chatwal, G.R. & Anand, S.K. (2012). IMOCA, Published by Meena Pandey for Himalaya publishing House, 5th Edition 2002.

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Chapter 2

Assay of Dextrose Injection

1 Introduction

To perform assay of Dextrose Injection I.P. by Colorimetry

2 Theory

Colorimetry or (colourimetry) is a technique used to determine the concentration of coloured compounds in solution. Colorimetry is the measurement of concentration of analyte in a solution by determining absorption of a particular wavelength. A colorimeter is a device used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light. To use this device, different solutions must be made, and a control (usually a mixture of distilled water and another solution) is first filled into a cuvette and placed inside a colorimeter to calibrate the machine. Only after the device has been calibrated you can use it to find the densities and/or concentrations of the other solutions. The technique is based upon Beer-Lambert's law. Overall the process is accomplished with the measurement of analyte in a colored solution or development of color that appears in the solution following reaction with specific reagent. In alkaline medium p-hydroxybenzoic acid hydrazide (PHBAH) reacts with dextrose that give products which can be assayed by colorimetry. Dextrose reacts with p-hydroxybenzoic acid hydrazide (PHBAH) reagent to give coloured product; the absorbance noted is proportional to concentration of sugar.

Operation:

- Turn on the colorimeter warm up for 15 min.
- Set zero.
- Set wavelength.
- Insert blank
- Set full scale.
- Insert unknown.
- Read % T or A.

3 Procedure

- Prepare glucose solution (stock) 300 mg %.
- Pipette 0, 0.5, 1.0, 2.0 and 2.5 ml of above solution in to clean corning test tubes of about 20ml of capacity.
- Add 3 ml of 3N NaOH to all the test tubes.
- Add 3ml of DNSA solution to all the test tubes.
- Add enough distilled water to make 10ml in each case.(4ml,3.5ml.3ml,2.5ml,2.0ml,1.5ml)
- Shake the test tubes to mix contents.
- Keep the test tubes in boiling water for 15 min. cool the test tubes.
- Read A or %T at 600nm.
- Dextrose inj. I.P.(25%w/v). Dilute 1 ml to 100ml. Take 1 ml and 2ml of diluted sample. Proceed from step (3) of procedure.

4 Observations

Table 2.1 Conc. of standard stock Glucose solution -300mg%

Sr. no.	Stock sample	Conc. µg/ml	A	%T
1.	0.0 ml	-		
2.	0.5 ml	150		
3.	1.0 ml	300		
4.	1.5 ml	450		
5.	2.0 ml	600		
6.	2.5 ml	750		
7.	Unknown			

5 Results

Given unknown solution contain---------- mg/ml of Glucose.

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Hobart H. Willard, Lynne L. Merritt jr., John A. Dean and Frank A. Settle jr. (1986). Instrumental Methods of Analysis, Sixth Edition, Wadsworth Publishing Company, Florence.

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Chapter 3

Estimate the amount of Sulphanilamide by Colorimetry

1 Introduction

To estimate the amount of sulphanilamide by Colorimetry

2 Theory

Sulphanilamide is an example of antifungal agent. One of the methods used for the estimation of sulfanilamide involves diazotization of the drug with nitrous acid followed by coupling with NED reagent to produce pink colored chromogen with a λmax value of 550nm. The absorbance at 550nm is measured using a suitable colorimeter against a reagent blank. A standard plot with unknown concentration of glucose is first made from which known concentration can be easily found out.

Operation:

- Turn on the colorimeter warm up for 15 min.
- Set zero.
- Set wavelength.
- Insert blank
- Set full scale.
- Insert unknown.
- Read % T or A.

3 Procedure

- In to the series of test tubes transfer 1ml, 2ml, 3ml, 4ml and 5ml of working std solution (10µg/ml) of sulphanilamide and 1ml 5N HCl and 1 ml of $NaNO_2$ solution to each container.
- Mix keeps aside for 2 min. add 1 ml of ammonium sulphamate solution and finally add 1 ml of NED reagent.

- Adjust the total volume in each test tube to 10 ml with remaining vol of distilled water.
- Measure the absorbance values at 550nm using a colorimeter.
- Enter the values in a tabular form.
- Construct a plot (abs Vs Conc.) from the values obtained.
- Checkout the absorbance of unknown sample also, and extrapolate it on X axis in the graph and note the conc. of unknown sample.

4 Observations

Table 3.1 Concentration and absorbance

Sr. no.	Conc. µg/ml	Absorbance at 550nm
1.	10	
2.	10	
3.	20	
4.	30	
5.	40	
6.	50	
7.	Unknown	

5 Results

The amount of sulphanilamide in the given unknown solution was found to be

----------µg/ml.

References

Hobart H. Willard, Lynne L. Merritt jr., John A. Dean and Frank A. Settle jr. (1986). Instrumental Methods of Analysis, Sixth Edition, Wadsworth Publishing Company, Florence.

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Chapter 4

Simultaneous Equation Method using UV-Visible Spectroscopy

1 Introduction

To determine the concentration of Ibuprofen and Paracetamol in an unknown mixture by using simultaneous equation method

2 Theory

Simultaneous equation method

If a sample contains two absorbing drugs (x and y) each of which absorb at the λ max of the other, it may be possible to determine both drugs by the technique of simultaneous equation method (Vierodt's method) Provided that certain criteria apply.

The Information required is;

a) Absorptivities of X at lambda 1 and lambda 2, ax1 and ax2 respectively.

b) Absorptivities of Y at lambda 1 and lambda 2, ay1 and ay2 respectively.

c) The absorbances of diluted sample at λ1 and λ2, A1 and A2 respectively.

Let Cx and Cy be the concentrations of x and y respectively in a diluted sample.

ax1 = absorptivity of paracetamol at λ1

ax2 = absorptivity of paracetamol at λ2

ay1 = absorptivity of Ibuprofen at λ1

ay2 = absorptivity of Ibuprofen at λ2

A1 = Absorbance of Mixture at λ1

A2 = Absorbance of Mixture at λ2

Cx = A2 ay1-A1ay2 / ax2 ay1- ax1 ay2

Cy = A1 ax2-A2ax1 / ax2 ay1- ax1ay2

3 Procedure

Selection of solvent- Ibuprofen and paracetamol were soluble in 0.1 N NaOH and solutions were stable. Prepare 0.1N NaOH solution by dissolving 4 gm NaOH in 100 ml of water in a volumetric flask.

Preparation of API Dilution from stock solutions:

- Weigh accurately 100mg of paracetamol & 100 mg Ibuprofen and transfer in different volumetric flask.
- Dissolve in 100 ml of 0.1 N NaOH (1000µg/ml)
- Take 10 ml of above solution and dilute to100 ml with 0.1 N NaOH (100µg/ml)
- Again 10ml of above solution dilute to 100ml with 0.1 N NaOH (10µg/ml)
- Pipette out 5, 10, 15, 20, 25 ml of above diluted solution (in different volumetric flask)in to a set of 10 ml volumetric flask and make up to 10 ml with 0.1 N NaOH to get concentration of 0.5, 1, 1.5, 2, 2.5, 3 µg/ml respectively.

Preparation of Tablet 1000 mg (500 mg Paracetamol, 325 mg Ibuprofen, 175 mg excipients) dilution.

- Weigh accurately 20 tablets of marketed formulation.
- Calculate average weight and prepare fine powder.
- Weigh powdered tablet equivalent to 100 mg of paracetamol content in the tablet and dissolve in 100 ml of 0.1 N NaOH (1000µg/ml)

  *Approx 1000 mg tablet contains 500 mg paracetamol, therefore weight equivalent to 100 mg of paracetamol = 1000 x 100 /500 = 200 mg
- Take 10 ml of above solution and dilute to100 ml with 0.1 N NaOH (100µg/ml)
- Again 2 ml of above solution dilute to 100ml with 0.1 N NaOH (20µg/ml)

Determination of Absorbance at analytical wavelength:

λ1 for Paracetamol = 257 nm

λ2 for Ibuprofen = 222 nm

Determination of absorptivity:

absorptivity = Absorbance/ Conc. (µg/ml)

4 Observations

Table 4.1 Drug and their absorbance at λ1=257; λ2=222 and absorptivity

S. No.	Name of Drug	Concentration (c) µg/ml	Absorbance (A)		Absorptivity	
			λ1 = 257 nm	λ2 = 222 nm	ax = A/c at 257 nm	ax = A/c at 222 nm

1	**Paracetamol**	5				
		10				
		15				
		20				
		25				
		Mean			**ax_1**	**ax_2**
S. No.	**Name of Drug**	**Concentration (c)** µg/ml	**Absorbance (A)**		**Absorptivity**	
			λ1 = 257 nm	λ2 = 222 nm	ay = A/c at 257 nm	ay = A/c at 222 nm
2	**Ibuprofen**	5				
		10				
		15				
		20				
		25				
		Mean			**ay_1**	**ay_2**
3	**Tablet**		**A_1**	**A2**		

4 Calculation

$Cx=A_2ay_1-A_1ay_2/Ax_2ay_1-ax_1ay_2$
$Cy=A_1ax_2-A_2x_1/ax_2ay_1-ax_1ay_2$
A1 = Absorbance of mixture at λ_1 =257nm
A2 = Absorbance of mixture at λ_2 =222nm
Cx =concentration of Paracetamol Cy =Concentration of Ibuprofen
ax_1= Absorptivity of Paracetamol at λ_1 = Absorbance of Paracetamol at λ_1 / Conc. of Paracetamol
$ax_{2=}$ Absorptivity of Paracetamol at λ_2= Absorbance of Paracetamol at λ_2 / Conc. of Ibuprofen
$a_{y1=}$ Absorptivity of Ibuprofen at λ_1= Absorbance of Ibuprofen at λ_1 / Conc. of Paracetamol
$a_{y2=}$ Absorptivity of Ibuprofen at λ_2= Absorbance of Ibuprofen at λ_2 / Conc. of Paracetamol

5 Results

The concentration of unknown mixture by simultaneous equation method was found to

be

1) Ibuprofen……µg/ml

2) Paracetamol…….µg/ml

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Chapter 5

Assay of Paracetamol by UV-Visible Spectroscopy

1 Introduction

To report the amount of Paracetamol present in the given tablet by UV Spectroscopy (Assay of paracetamol by U.V. spectroscopy)

2 Theory

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 ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagneticatoms and molecules undergoelectronic transitions. Absorption spectroscopy 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 spectrum, atoms and molecules undergo electronic transitions. Absorption spectroscopy 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.

3 Procedure

- Weigh and powder 20 tablets, take the powder equivalent to 0.15gm of paracetamol, accurately weighed, add 50 ml of 0.1N NaOH and dilute to 100ml with water.
- Shake the mixture for 15 min. and add sufficient water to produce 200ml. The resulting solution is mixed and filtered.
- Take 10ml of above filtrate and dilute to 100ml with water.
- Take 10ml of resulting solution add 10ml of 0.1N NaOH and make up volume to 100ml with water. Mix well.
- Measure the absorbance of the resulting solution at 257nm and calculated the concentration of paracetamol by taking 715 as $A_{1cm}^{1\%}$(abs)

4 Observations

Average weight of paracetamol tablet=……..gm

Label claim = 500mg paracetamol

……gm of paracetamol tablet powder contain 0.5gm (500mg) of paracetamol

X gm of paracetamol tablet powder contain 0.15gm (150mg) of paracetamol.

5 Calculation

Label claim of given tablet = _____ gm

Weight of twenty tablets = _____ gm

Average weight of each tablet = _____ gm-------- (A)

Average weight of each tablet (A) contains = 0.500 gm of paracetamol

x gm of powder contains = 0.15gm of paracetamol

x = 0.15 * (A) / 0.500 = ---------- Test weight (gm)

Test Absorbance at wavelength 257 nm = ----------

Specific absorbance = 715

$$\text{Assay (\% Purity)} = \frac{\text{Test absorbance}}{\text{Specific absorbance}} \times \frac{1}{100} \times \frac{\text{Test dilution}}{\text{Test weight (g)}} \times 100$$

6 Results

The percentage purity of Paracetamol tablet I.P. was found to be ……%.

7 References

Chatwal, G.R. & Anand, S.K. (2012). IMOCA, Published by Meena Pandey for Himalaya publishing House, 5th Edition 2002.

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Chapter 6

Assay of Quinine sulphate by Fluorimetry

1 Introduction

To perform the assay of Quinine sulphate by Fluorimetry.

2 Theory

Fluorescence is the emission of visible light by a substance that has absorbed light of a different wavelength. The emitted photon has a longer wavelength and lower energy. As the excitation of the molecule is due to the absorption of a photon (light), these types of luminescence are called photoluminescence. Fluorescence is also defined as the radiation emitted in the transition of a molecule from a singlet excited state to a singlet ground state. Quinine sulphate is fluorescent in acidic solutions like 0.1 N Sulphuric acid. Sensitivity of analysis being very high (0.1µg/ml), and above method is widely used for routine studies experiment involves dissolution of known quantity of quinine salt in 0.1 N Sulphuric acid followed by reading fluorescence (F) with a suitable fluorimeter. Its excitation and emission wavelength are 365 nm and 459 nm respectively.

3 Procedure

Preparation of 0.1 N H_2SO_4:-

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

Preparation of standard Quinine sulphate solution

- Weigh accurately 100mg of Quinine sulphate powdered drug.
- Dissolve in 100 ml of 0.1 N conc. H_2SO_4 (1000µg/ml)
- Take 10 ml of above solution and dilute to100 ml with 0.1N H_2SO_4 (100µg/ml)
- Again 10ml of above solution dilute to 100ml with 0.1N H_2SO_4 (10µg/ml)
- Pipette out 0.5, 1, 1.5, 2, 2.5, 3 ml of above diluted standard quinine sulphate solution in to a set of 10 ml volumetric flask and make up to 10 ml with 0.1N H_2SO_4 to get concentration of 0.5, 1, 1.5, 2, 2.5, 3 µg/ml respectively.

Preparation of Sample solution: Tablets-

- Weight 20 tablets and reduce to fine powder.
- Weight accurately a quantity of powder equivalent to 100 mg of Quinine sulphate and transfer into 100 ml volumetric flask and dissolve in 100 ml of 0.1 N conc. H_2SO_4.
- Take 10 ml of above solution and dilute to100 ml with 0.1N H_2SO_4.
- Read the fluorescence.

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

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

➢ Set the fluorescence intensity to 0% by using 0.1N H2SO4 as blank and 100% by using highest concentration of the standard solution (3 µg/ml). Measure the fluorescence of serial dilutions & sample solution and plot the calibration curve (fluorescence intensity Vs concentration).

4 Observations

Table: 6.1 : Concentration vs Fluorescence

Sr.no.	Concentration (µg/ml)	% Fluorescence intensity
1.	Blank	
2.	0.5	
3.	1	
4.	1.5	
5.	2	
6.	2.5	
7.	3	
7.	Sample	

5 Results

(1) Unknown solution contains ……µg/ml of quinine salt.

6 References

Hobart H. Willard, Lynne L. Merritt jr., John A. Dean and Frank A. Settle jr. (1986). Instrumental Methods of Analysis, Sixth Edition, Wadsworth Publishing Company, Florence.

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Chapter 7

Quenching effect on Fluorescence of Quinine sulphate solution

1 Introduction

To study the Quenching effect due to the presence of varying concentrations of iodide ions on the fluorescence of Quinine sulphate solution.

2 Theory

Fluorescence is the emission of visible light by a substance that has absorbed light of a different wavelength. The emitted photon has a longer wavelength and lower energy. As the excitation of the molecule is due to the absorption of a photon (light), these types of luminescence are called photoluminescence. Fluorescence is also defined as the radiation emitted in the transition of a molecule from a singlet excited state to a singlet ground state. Quenching is a process fluorescent intensity of a compound is reduced due to the presence of certain ions or molecule in the medium. The energy is transferred from the excited molecule to the quenching particle due to collision. Halides like Cl, I cause dissipation of excitation energy due to collision quenching. These effects may be due to various factors like Concentration, pH, presence of specific chemical substances, temperature, viscosity, etc.

3 Procedure

Preparation of 0.1 N H2SO4:-

- Pipette out 4.9 ml of conc H2SO4 to 100 ml of water and then make it up to 1000 ml with water.

Preparation of standard Quinine sulphate solution

- Weigh accurately 100 mg of Quinine sulphate powdered drug and dissolve in 100 ml of 0.1 N H_2SO_4 (1000µg/ml).
- Take 10 ml of above solution and dilute to100 ml with 0.1N H_2SO_4 (100µg/ml).
- For final concentration again add 1 ml of above solution and dilute to 100 ml with 0.1 N H_2SO_4 (10 µg/ml).

Preparation of Potassium Iodide (KI) solution

- Weigh accurately 100 mg of KI and dissolve in 100 ml of 0.1 N H_2SO_4 (1000 µg/ml).
- Take 10 ml of above solution and dilute to100 ml with 0.1 N H_2SO_4 (100 µg/ml)
- For final conc. Again add 1 ml of above solution and dilute to 100 ml with 0.1 N H_2SO_4 (10 µg/mL)

Determination of Quenching effect

- In six 10 ml volumetric flasks, take 1 ml of standard quinine sulphate solution.
- Add 1, 2, 3, 4 and 5ml of KI solution in each flask. Make up the volume with 0.1N H_2SO_4.
- Switch on the instrument and stabilize for 10-15min.
- Set the fluorescence intensity to 0% by using 0.1N H_2SO_4 as blank and 100% by using highest concentration of the standard solution (not containing KI). Measure the fluorescence of serial dilutions and plot a graph between (volume of KI vs fluorescence intensity).

Table 7.1: Different concentrations of Quinine Sulphate and Potassium iodide

Volumetric Flask no.	1	2	3	4	5	6
ml of Quinine solution	1	1	1	1	1	1
ml of KI solution	0	1	2	3	4	5
ml of 0.1 N H_2SO_4	9	8	7	6	5	4

4 Observations

Table: 7.2: Concentration vs Flourescence

Sr.no.	Volume of KI (ml)	% **Fluoroscence intensity**
1.	Blank (0.1 N H_2SO_4)	0 %
2.	1	
3.	2	
4.	3	
5.	4	
6.	5	
7.	Quinine Sulphate (10 µg/ml)	100 %

5 Results

A decrease in fluorescence intensity shall be observed for quinine solution with the addition of potassium iodide solution.

6 References

Hobart H. Willard, Lynne L. Merritt jr., John A. Dean and Frank A. Settle jr. (1986). Instrumental Methods of Analysis, Sixth Edition, Wadsworth Publishing Company, Florence.

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Chapter 8

Concentration of Sodium ions by Flame Photometer

1 Introduction

To determine the concentration of sodium ions by flame photometer.

2 Theory

Flame photometry is also known as atomic emission spectrometry. It works on the basis of heating the metal, in this case sodium, such that the atoms of the metal travel from ground state to their excited state. The atoms then return to their ground state and release their energy as photons of ultraviolet radiation. The wavelength of the UV radiation is then measured. When a liquid sample consisting a metallic salt solution is introduced into a flame the solvent gets evaporated and the solid salt gets converted to its gaseous state. The dissociation of either portion or total gaseous molecules give rise to neutral atoms (or) free radicals. The neutral atoms are excited by the thermal energy of the flame which are unstable . They instantly emit photons and return to it's ground state .The measurement of photons (emitted radiations forms the fundamental basis of flame photometry. The intensity of radiation emitted by depends upon proportion of thermally exited atoms.

3 Procedure

- Weigh accurately 100 mg of sodium chloride powdered drug and dissolve in 100 ml of distilled water. (1000 µg/ml)
- Take 10 ml of above solution and dilute to100 ml with distilled water (100 µg/ml)
- Pipette out 5, 10, 20, 30, 40, 50 ml of above diluted standard solution in to a set of 100 ml volumetric flask and make up to 100 ml with to get concentration of 5,10, 20, 30,40, 50 µg/ml respectively.
- Switch on the instrument, select sodium filter and stabilize for 10-15min.
- Set the gas in flame in order to get the non-luminous flame and air pressure at 0.4 to 0.5 Kg/cm^2
- Set the flame intensity to 0% by using distilled water as blank and 100% by using highest concentration of the standard solution (50 µg/ml).

- Measure the % flame intensity of serial dilutions and plot the calibration curve (% intensity Vs concentration).
- Find out the concentration of unknown sample from calibration curve by extrapolating.

4 Observations

Table: 8.1 concentration Vs Emission intensity

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

5 Results

The unknown solution contains ……µg/ml of Sodium.

6 References

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Chapter 9

Concentration of Potassium ions by Flame Photometer

1 Introduction

To determine the concentration of Potassium ions by flame photometer.

2 Theory

Flame photometry is also known as atomic emission spectrometry. It works on the basis of heating the metal, in this case sodium, such that the atoms of the metal travel from ground state to their excited state. The atoms then return to their ground state and release their energy as photons of ultraviolet radiation. The wavelength of the UV radiation is then measured. When a liquid sample consisting a metallic salt solution is introduced into a flame the solvent gets evaporated and the solid salt gets converted to its gaseous state. The dissociation of either portion or total gaseous molecules give rise to neutral atoms (or) free radicals. The neutral atoms are excited by the thermal energy of the flame which are unstable . They instantly emit photons and return to it's ground state .The measurement of photons (emitted radiations forms the fundamental basis of flame photometry. The intensity of radiation emitted by depends upon proportion of thermally exited atoms.

3 Procedure

- Weigh accurately 100 mg of potassium chloride powdered drug and dissolve in 100 ml of distilled water. (1000 µg/ml)
- Take 10 ml of above solution and dilute to 100 ml with distilled water (100 µg/ml)
- Pipette out 5, 10, 20, 30, 40, 50 ml of above diluted standard solution in to a set of 100 ml volumetric flask and make up to 100 ml with to get concentration of 5,10, 20, 30,40, 50 µg/ml respectively.
- Switch on the instrument, select sodium filter and stabilize for 10-15min.
- Set the gas in flame in order to get the non-luminous flame and air pressure at 0.4 to 0.5 Kg/cm^2.
- Set the flame intensity to 0% by using distilled water as blank and 100% by using highest concentration of the standard solution (50 µg/ml).

- Measure the % flame intensity of serial dilutions and plot the calibration curve (% intensity Vs concentration).
- Find out the concentration of unknown sample from calibration curve by extrapolating.

4 Observations

Table: 8.1 concentration Vs Emission intensity

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

5 Results

The unknown solution contains ……µg/ml of Potassium.

6 References

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Chapter 10

Chloride and Sulphate limits in Calcium Gluconate by Nephlo Turbidometry

1 Introduction

To determine chloride and sulphate limits in Calcium Gluconate by Nephlo Turbidometry.

2 Theory

Turbidometry is a branch of Spectrophotometry in which transmitted light is measured after radiant energy passes through a turbid solution or suspension. Colorimeter and spectrophotometers can be employed for turbidimetric measurements.Itobyes standard Lambert-Beer law.

$S=\log P_O/P$

Where,

S= Turbidance

Po= Intensity of incident radiation

P= Intensity of Transmitted radiation

3 Procedure

(For chloride determination):

Standard turbidity-

(1) Measure 1 ml of 0.02N HCl in to clean 50ml volumetric flask.

(2) Add 35 ml of distilled water, free chloride after testing.

(3) Add 1 ml of conc. HNO_3.

(4) Add sufficient distilled water to produce 50ml.

(5) Stopper and invert several times.

(6) Allow to stand for 5 min protected from direct sun light.

(7) Fill a Nephlo-Turbidimeter test tube to the mark with the suspension.

(8) Wipe test tube clean, insert, read NTU(NephloTuridometric unit)

NTU_{std} Cl^- = 100or 1000

Sample Turbidity-

Step1-2 Weigh about 1gm of sample exactly (wt= ……..) and transfer via solutions in 36 ml distilled water in to a clean 50ml volumetric flask.

Step3-9 same as for standard.

NTU sample Cl^-=

Calculations-

USP Limits- A 1gm sample of calcium gluconate shows no more Cl^- than that corresponds to 1 ml of 0.02N HCl (700ppm)

Ionic Cl^-= 35.453

0.002/1000X35.453=0.00070906 gm Cl^-in 1.000gm.

Calcium gluconate is 709.06 ppm.

Weight sample Cl^-= NTU sample Cl^-/NTU std Cl^- x Weight $_{std}$ Cl^-

= ……..gm

…….gm/wt sample gm, Represents (……..)ppm.

Procedure (For Chloride determination):

Standard turbidity-

Measure 1 ml of 0.02 N H_2SO_4 in to a clean 50ml volumetric flask.

Add 35 ml of distilled water, free sulphate after testing.

Add 1 ml of Dil HCl.

Add 3 ml of $BaCl_2$.

Allow to stand for 10 min protected from direct sun light.

Fill a Nephlo-Turbidimeter test tube to the mark with the suspension.

Wipe test tube clean, insert, read NTU.

NTU_{std} SO_4^{2-}

Sample Turbidity

Step 1-2 Weigh about 2gm of sample exactly (Wt=…….) and transfer via solutions in 36 ml distilled water in to a clean 50ml volumetric flask.

Step 3-9 Same as for standard.

4 Observations

USP limits: A 2 gm sample of calcium gluconate shows no more SO_4^{2-} than that corresponds to 1ml of 0.002N H_2SO_4 (400ppm)

Ionic SO_4^{2-} = 96.0616

0.02/1000x 96.616/2= 0.000960616 gm SO_4^{2-}in 2.000gm

Calcium gluconate is 480.308ppm.

Weight $_{sample}$ SO_4^{2-}= NTU $_{sample}$ SO_4^{2-}/ NTU $_{std}$ SO_4^{2-}xWeight $_{std}$$SO_4^{2-}$

= ……gm

…….gm ---------- represents (…….)ppm.

Wt. sample gm

5 References

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Chapter 11

Paper chromatography of Amino acids

1 Introduction

To separate and identify the given mixture of Amino acids by paper chromatography.

2 Theory

The chromatographic techniques used to separate out mixtures of different substances into their discrete components. All forms of chromatographic techniques work on the same principle. They all have basic requirements of stationary phase (a solid or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase tides through the stationary phase and carries the components of the mixture with it. Different components travel at different rates based on their affinities toward stationary phase and mobile phase. In paper chromatography, the stationary phase is a very uniform adsorbent paper and the mobile phase is a suitable liquid solvent or mixture of solvents. The principle involved is partition, where the substances are distributed or partitioned between to liquid phases. One phase is the water which is held in pores of filter paper used and other phase is that of mobile phase which moves over the paper. The compounds in the mixture get separated due to differences in their affinity towards water (in stationary phase) and mobile phase during the movement of mobile phase under the capillary action of pores in the paper. Identification of amino acids in the given mixture is determined by Rf value

$$\textbf{Rf value} = \frac{\text{Distance travelled by solute from origin}}{\text{Distance travelled by solvent from origin}}$$

3 Procedure

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

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

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

d. Preparation of spraying reagent: Ninhydrin Solution, Ethanolic: Dissolve 0.1 g of *ninhydrin* in 50 ml of *ethanol* (95 *per cent)* and add 10 ml of *glacial acetic acid.*

Procedure of development of chromatogram

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

4 Observations

Table 11.1 : R_f values

S. No.	Amino Acid	Distance travelled by amino acid	Distance travelled by solvent	R_f value
1	Valine			
2	Arginine			
3	Methionine			
4	Tryptophan			

5 Results

Rf value of Valine was found to be:…….

Rf value of Arginine was found to be:…….

Rf value of Methionine was found to be:…….

Rf value of Tryptophan was found to be:…….

6 References

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Chapter 12

Thin Layer Chromatography of Sugars

1 Introduction

To separate and identify the given mixture of sugars by Thin layer chromatography.

2 Theory

A thin layer of adsorbent material, such as silica gel or alumina, is coated onto a solid support, usually a glass or plastic plate. This thin layer acts as the stationary phase. The sample mixture to be analyzed is spotted near the bottom of the plate. The separation begins when the plate is placed in a developing chamber with a suitable solvent called the mobile phase. The mobile phase moves up through capillary action, carrying the sample components. Each component interacts differently with the stationary and mobile phases as it moves up the plate. The interactions between the components and these two phases result in differential migration rates. Compounds with stronger interactions with the stationary phase will move more slowly than those with weaker interactions. It leads to the separation of individual components on the TLC plate.

Rf= Distance travelled by solute from origin/ Distance travelled by solvent from origin

3 Procedure

Preparation of mobile phase: Mobile system consists of a mixture of chloroform, acetic acid, and water (3:3.5:0.5) by volume, respectively

Preparation of TLC Plate: Thick layers (1-2 mm) of silica gel can be prepared by slurring silica gel G with water in ratio 25:40. The layers are air dried for about leman and then activated by heating in an over at about 11 for 2 hrs.

Preparation of sample: Take 0.1 g of Glucose, 0.1 g of Sucrose and 0.1 g of Xylose in individual separate volumetric flasks and dissolved in required quantity of methanol.

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

Preparation of spraying reagent: Dragendorff Reagent : 1 ml of Dragendorff solution is mixed with 2 ml of glacial acetic acid and 10 ml of water.

Procedure of development of chromatogram

- Take a TLC Plate and draw a thin straight line of about 2 cm from the bottom of the plate on back side.
- Mark three points with equidistance on the straight line and number them.
- Prepare sample solutions and place a drop of these solutions on the straight line by using capillary tube.
- Dip the chromatographic paper in chamber containing mobile phase, in such a way that the straight line on the TLC plate should not be immersed.
- Run the chromatogram till the mobile phase travels on ¾th of the chromatographic paper.
- Remove the plate from the chamber and mark the solvent distance with a pencil and dry in air for 15 minutes
- The sample's were visualized using Dragendroff's reagent after getting the solvent front.
- TLC plates were dried in an oven to visualize the samples
- Calculate the Rf values for the identification of sugar in the given mixture.

4 Observations

Table 12.1: R_f values

S. No.	Sugar	Distance travelled by sugar	Distance travelled by solvent	Rf value
1	Glucose			
2	Sucrose			
3	Xylose			

5 Results

Chlorophylls, Xanthophylls and Carotenoids in leaves were separated by Column Chromatography.

6 References

Khopkar S.M. (2002). Analytical chemistry, Published by New Age International (P) Ltd Publishers , 1st edition.

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Chapter 13

Column Chromatography

1 Introduction

To separate Chlorophylls, Xanthophylls and Carotenoids in Leaves by Column Chromatography.

2 Theory

Column chromatography is a technique which can be applied to the separation of many complex mixtures. Thgsample solution is applied to the top of the column. The mobile phase flows down through the column filled with the stationary phase material. The green leaves of plants contain a number of pigments viz: chlorophyll-a, chlorophyll-b, xanthophylls and carotenes. The success of a separation by column chromatography depends on the choice of the stationary and mobile phases. The stationary phase material is filled in a column. Any of the three possible mechanisms: partition, adsorption or ion exchange can be employed by the use of a particular type of the stationary phase inside the column. For example, for the separation based on adsorption an adsorbent is packed in the column. The choice of the mobile phase depends on the nature of the substance and how strongly it is adsorbed. In a number of cases such as alumina and silica gel as the adsorbent, the mobile phase is generally a non-polar solvent such as petrol and benzene because polar groups such as hydroxyl-(OH) group in water and in ethanol would cause desorption. Eluents containing two or more solvents may be used for better results.

3 Procedure

Preparation of the Extract:

- Take 5-10 g of fresh green leaves cut it up into fine pieces in a mortar, grind for about 30 seconds, add 10 ml of ethyl alcohol and 20 ml of petroleum ether, grind again.
- Decant the liquid into a separatory funnel after filtering through glass wool placed in an ordinary funnel.
- Add 10 ml alcohol and 20 ml petroleum ether again to the mortar containing leaves, grind and transfer the liquid after decantation to the separatory funnel containing the first fraction. Shake gently.
- A light green emulsion may form, if shaken vigorously. Allow to settle the layers.

- The bottom layer is water-ethanol layer and the upper layer of petroleum-ether contains grass extract. Remove the aqueous layer.
- Transfer the extract to a clean and dry test tube, cover it and take it for chromatography.

Preparation of column:

- Take a glass column or a burette of about 20 cm in length and 7-8 mm diameter tube.
- Place a small wad of cotton wool as the column support. Pack the column with anhydrous calcium carbonate (dried by heating in a china dish over a burner), tap it regularly with a glass rod.
- Add the adsorbent in small portions and gently press down until a column of 8-10 cm has been uniformly packed.
- Place a small wad of cotton wool at the top of the calcium carbonate column and use it for chromatography.
- Take the uniformly packed column containing calcium carbonate and fix it in a stand vertically.
- Take 1-2 cm3 of dried extract of leaves, drip into the column in the form of a thin layer of solution, allow to run evenly into the adsorbent untill a green zone 3-4 mm deep is formed at the top of the column. This is known as the loading of the sample.
- Add the developer (benzene) to the column and allay the developer through the column packing till separate bands are observed.
- Note the colour of different bands and their order.
- If extra time is available, continue the passage of developer and collect the different coloured substances in fractions, noting the volume eluted by a measuring cylinder.

4 Observations

The bands observed on the column are of different colours. The uppermost thin yellowish green zone is chlorophyll-b, below this the bluish green zone of chlorphyll-a, next orange-yellow zone contains xanthopylls and the lowest orange zone contains carotenes. The carotenes are least adsorbed by the adsorbent and can be easily washed out of the column.

5 Results

Chlorophylls, Xanthophylls and Carotenoids in leaves were separated by Column Chromatography.

6 References

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Chapter 14

Demonstration experiment on HPLC

1 Introduction

To explain principal and demonstrate working of HPLC

2 Theory

High performance liquid chromatography (HPLC) is a chromatographic technique that uses a solvent under pressure to separate, identify, or quantify substances in a mixture. An analytical separation technique that involves the high-pressure flow of a liquid through a column that contains the stationary phase.

Liquid chromatography is a separation technique that involves the placement (injection) of a small volume of liquid sample into a tube packed with porous particles (stationary phase) where individual components of the sample are transported along the packed tube (column) by a liquid moved by gravity. The components of the sample are separated from one another by the column packing that involves various chemical and/or physical interactions between their molecules and the packing particles. The separated components are collected at the exit of this column and identified by an external measurement technique, such as a spectrophotometer that measures the intensity of the colour, or by another device that can measure their amount. The components of the sample are separated from one another by the column packing that involves various chemical and/or physical interactions between their molecules and the packing particles. The separated components are collected at the exit of this column and identified by an external measurement technique, such as a spectrophotometer that measures the intensity of the color, or by another device that can measure their amount.

3 Principle

The purification takes place in a separation column between a stationary and a mobile phase. The stationary phase is a granular material with very small porous particles in a separation column. The mobile phase, on the other hand, is a solvent or solvent mixture which is forced at high pressure through the separation column. Via a valve with a connected sample loop, i.e. a small tube or a capillary made of stainless steel, the sample is injected into the mobile phase flow from the pump to the separation column using a syringe. Subsequently, the individual components of the sample migrate through the column at different rates because they are retained to a varying degree by interactions

with the stationary phase. After leaving the column, the individual substances are detected by a suitable detector and passed on as a signal to the HPLC software on the computer. At the end of this operation/run, a chromatogram in the HPLC software on the computer is obtained. The chromatogram allows the identification and quantification of the different substances.

Types of HPLC

- **Normal phase:** Column packing is polar (e.g silica) and the mobile phase is non-polar. It is used for water-sensitive compounds, geometric isomers, cis-trans isomers, and chiral compounds.
- **Reverse phase:** The column packing is non-polar (e.g C18), the mobile phase is water and miscible solvent (e.g methanol). It can be used for polar, non-polar, ionizable, and ionic samples.
- **Ion exchange:** Column packing contains ionic groups, and the mobile phase is buffer. It is used to separate anions and cations.
- **Size exclusion:** Molecules diffuse into pores of a porous medium and are separated according to their relative size to the pore size. Large molecules elute first and smaller molecules elute later.

4 Instrumentation

Major components:

A) Solvent or mobile phase

- Usually, a mixture of an organic solvent (Ex. methanol, IPA) and water.
- Solvent polarity affects the separation process. ❖
- Sometimes buffered - keeps solutes in electrically neutral form.
- Mobile phase must be filtered (to prevent tiny solids from depositing at the column head) and degassed as bubbles could interfere with detection.

B) Pump

- Role is to pump the solvent at a high pressure (usually from 1000 to 6000 psi) through the packed column
- Pump Module–types:
    - Isocratic pump -delivers constant mobile phase composition; •solvent must be pre-mixed; •lowest cost pump
    - Gradient pump -delivers variable mobile phase composition; •can be used to mix and deliver an isocratic mobile phase or a gradient

mobile phase. a. Binary gradient pump –delivers two solvents. b. Quaternary gradient pump –four solvents.

C) Sample introduction system

- Usually a loop injector .
- Introduces the injected sample to the flowing mobile phase
- Automated injectors are common

D) Column

- A small metal tube (typically 5 to 30 cm long; 1-5 mm i.d.) that contains the stationary phase
- Role is to separate the components of a mixture
- Highly efficient separations achieved in HPLC due to interactions of both mobile phase and stationary phase with the components of a mixture.

E) Detector

- The detector can see (detect) the individual molecules that come out (elute) from the column.
- A detector serves to measure the amount of those molecules so that the chemist can quantitatively analyze the sample components.
- The detector provides an output to a recorder or computer that results in the liquid chromatogram (i.e., the graph of the detector response).
- UV detectors , fluorescent detector

F) Computer

- Frequently called the data system, the computer not only controls all the modules of the HPLC instrument, but it takes the signal from the detector.
- Uses it to determine the time of elution (retention time) of the sample components (qualitative analysis) and the amount of sample (quantitative analysis)

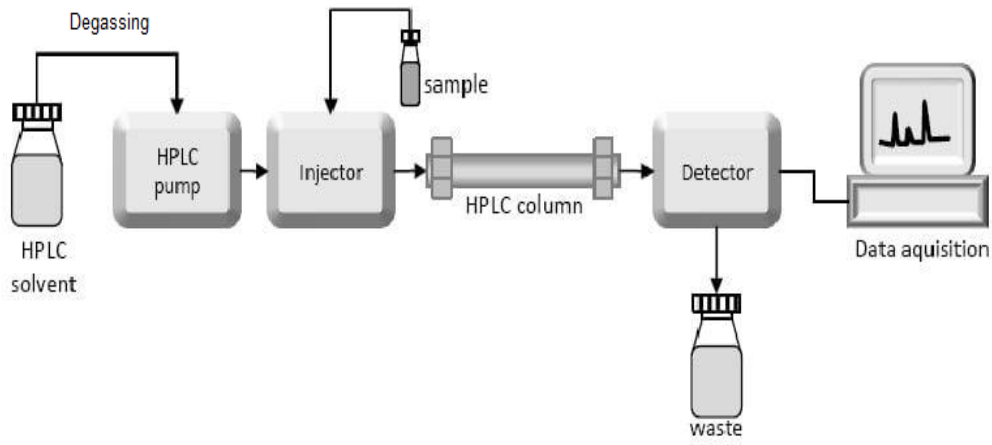

Fig. 14.1: HPLC Instrumentation

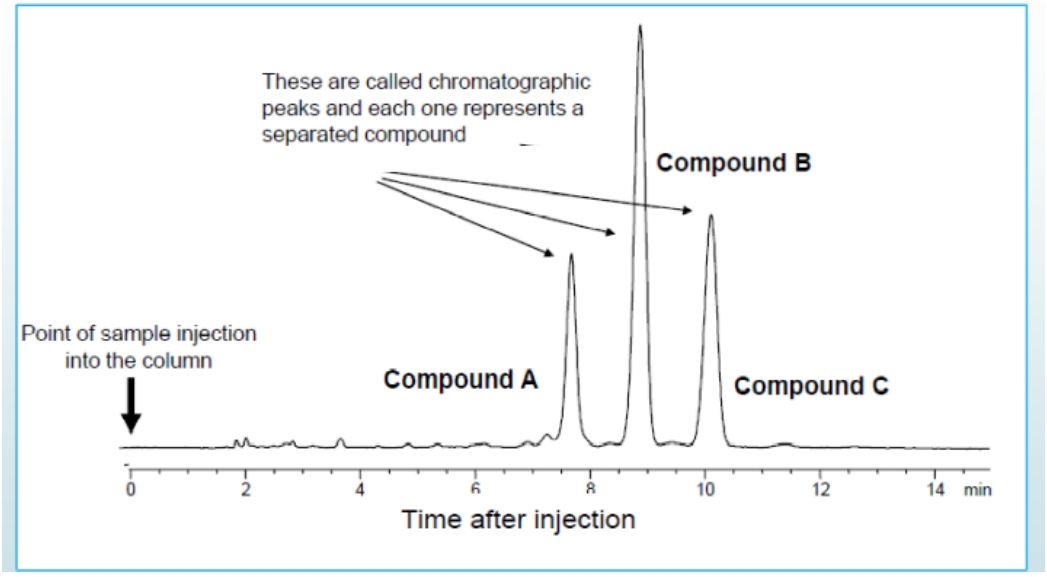

Fig. 14.2: A Chromatogram

5 Working

- The injection of a small volume of liquid sample into a tube packed with tiny particles (3 to 5 micron (μm) in diameter called the stationary phase).
- Where individual components of the sample are moved down the packed tube (column) with a liquid (mobile phase) forced through the column by high pressure delivered by a pump.
- These components are separated from one another by the column packing that involves various chemical and/or physical interactions between their molecules and the packing particles.
- These separated components are detected at the exit of this tube (column) by a flow-through device (detector) that measures their amount.
- An output from this detector is called a "liquid chromatogram".
- In principle, LC and HPLC work the same way except the speed, efficiency, sensitivity and ease of operation of HPLC is vastly superior.

6 Results

The principal and working of HPLC was demonstrated.

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Chapter 14

Demonstration experiment on GC-MS

1 Introduction

To explain principal and demonstrate working of GC-MS

2 Theory

GC-MS (Gas Chromatography-Mass Spectrometry) is a hybrid analytical technique that combines Gas Chromatography (GC) for separating volatile compounds and Mass Spectrometry (MS) for identifying and quantifying them. This combination allows for highly sensitive and specific analysis of complex mixtures.

Gas Chromatography operates on the principle of differential partitioning of compounds between a mobile phase (carrier gas) and a stationary phase (column coating) based on their volatility and interaction with the column material.

Key Components of GC:

- Sample Injection: The sample is introduced into the injector port, where it is vaporized.
- Carrier Gas: An inert gas (e.g., helium, nitrogen, or hydrogen) carries the sample through the column.
- Column & Stationary Phase: The column is coated with a liquid or polymer stationary phase that interacts with the sample components, affecting their retention time.
- Separation Mechanism:
    - Compounds with lower boiling points and weaker interactions with the stationary phase travel faster and elute earlier.
    - Compounds with higher boiling points and stronger interactions travel slower and elute later.
- Detection: As the separated components exit the column, they are detected, typically by a Flame Ionization Detector (FID) or Mass Spectrometer (MS) in GC-MS.

Mass Spectrometry (MS) Theory

Mass Spectrometry is based on the generation of ions, their separation according to their mass-to-charge ratio (m/z), and their detection to determine the molecular composition of the sample.

Key Steps in MS:

Ionization:

- The eluted compounds from the GC column enter the mass spectrometer and are ionized.
- The most common ionization method in GC-MS is Electron Ionization (EI), where high-energy electrons bombard the molecules, leading to fragmentation.

Fragmentation:

- The ionized molecules break into characteristic fragments, forming a unique mass spectrum for each compound.
- This fragmentation pattern helps in structural identification.

Mass Analysis:

- The mass spectrometer sorts the fragments based on their mass-to-charge ratio (m/z) using a mass analyzer.
- Common types of mass analyzers include:
  - Quadrupole: Uses electric fields to filter ions by m/z.
  - Time-of-Flight (TOF): Measures ion velocity to determine mass.
  - Ion Trap: Traps and sequentially ejects ions for detection.

Detection and Data Analysis:

- The separated ions are detected, and their intensities are recorded.
- A mass spectrum is generated, representing the molecular fingerprint of the compound.
- The obtained spectrum is compared with reference libraries for compound identification.

Combined GC-MS Functionality

- GC separates the compounds based on their volatility and polarity.
- MS identifies the separated compounds based on their mass spectra.
- The combination of these two techniques enhances both the sensitivity (ability to detect small quantities) and selectivity (ability to distinguish between similar compounds).

Advantages of GC-MS

- High sensitivity and specificity.
- Can analyze complex mixtures with high resolution.
- Allows qualitative (identification) and quantitative (concentration measurement) analysis.
- Used in diverse fields such as forensics, environmental analysis, food safety, and pharmaceuticals.

3 Principle

GC-MS (Gas Chromatography-Mass Spectrometry) is a powerful analytical technique that combines the separation capability of **Gas Chromatography (GC)** with the identification and quantification ability of **Mass Spectrometry (MS)**. This technique is widely used in fields such as pharmaceuticals, environmental analysis, forensic science, and food safety testing.

Gas Chromatography (GC) Principle:

- GC is used to separate volatile and semi-volatile compounds based on their boiling points and interaction with the stationary phase in a chromatographic column.
- The sample is vaporized and carried through the column by an inert **carrier gas** (e.g., helium, nitrogen).
- Components with lower boiling points and weaker interactions with the column elute first, while those with higher boiling points and stronger interactions elute later.

- A **detector** (such as a Flame Ionization Detector or a Mass Spectrometer) records the retention time of each compound.

Mass Spectrometry (MS) Principle:

- The separated components from GC enter the mass spectrometer, where they are **ionized** (usually by electron impact or chemical ionization).
- The ions are fragmented into characteristic mass-to-charge ratio (**m/z**) fragments.
- These fragments are detected and analyzed using a mass analyzer (e.g., Quadrupole, Time-of-Flight, or Ion Trap).
- The resulting **mass spectrum** provides a "fingerprint" of each compound, which can be compared with spectral libraries for identification.

4 Instrumentation

a) Sample Injector

- Introduces the sample into the GC system.
- Sample is vaporized instantly for separation.
- Common injection methods:
    - **Split Injection:** Only a portion of the sample enters the column, useful for concentrated samples.
    - **Splitless Injection:** The entire sample enters the column, used for trace-level analysis.
    - **Direct Injection:** Used for liquid samples without vaporization.

b) Carrier Gas System

- The mobile phase that carries the sample through the column.
- Common carrier gases: helium, nitrogen, or hydrogen (helium is preferred for high efficiency).
- A flow controller or pressure regulator ensures a steady gas flow rate.

c) GC Column and Oven

- Column: The heart of the GC system, responsible for separating sample components.
    - Made of fused silica or stainless steel.
    - Coated with a stationary phase (polysiloxane, polyethylene glycol, etc.).
    - Column length: 10–60 meters**, diameter:** 0.1–0.5 mm**.**
- **Oven:** Maintains a controlled temperature program to optimize separation.
    - Higher temperatures speed up elution, while lower temperatures improve resolution.

d) Detector (MS Interface)

- After separation, the analytes exit the GC column and enter the MS.
- The interface is heated (~250°C) to prevent condensation of compounds before ionization.

Mass Spectrometry (MS) Components

a) Ionization Source

- Converts neutral molecules into charged ions for mass analysis.
- Common ionization techniques:
    - **Electron Ionization (EI):** High-energy electrons (70 eV) ionize molecules, causing fragmentation.
    - **Chemical Ionization (CI):** Uses a reagent gas (e.g., methane, ammonia) to generate softer ionization with fewer fragments.

b) Mass Analyzer

- Separates ions based on their mass-to-charge ratio (m/z)**.**
- Common types:
    - **Quadrupole:** Uses electric fields to filter ions by m/z.
    - **Time-of-Flight (TOF):** Measures ion velocity to determine mass.
    - **Ion Trap:** Traps and sequentially ejects ions for detection.

c) Ion Detector

- Measures the abundance of ions.
- Converts ion signals into electrical signals for data processing.
- Common types:
    - **Electron Multiplier Detector (EMD)**
    - **Faraday Cup Detector**

Data System (Computer & Software)

- Processes and analyzes the detected ion signals.
- Generates mass spectra and chromatograms.
- Identifies compounds by comparing spectra with reference libraries (e.g., NIST, Wiley).

Schematic Diagram of GC-MS

Sample Injection → **2.** Vaporization → **3.** Separation in GC Column → **4.** Transfer to MS → **5.** Ionization → **6.** Mass Analysis → **7.** Detection & Data Analysis

5 Working

- The sample is introduced into the GC injector and vaporized.
- The GC column separates the components based on their volatility and interactions with the stationary phase.
- Separated components enter the MS ionization source, where they are ionized and fragmented.
- The mass analyzer sorts the fragments based on their m/z ratio.
- A detector records the ion intensities, generating a mass spectrum.
- The data is analyzed using software and compared with reference libraries for compound identification.

GC-MS is highly sensitive and selective, making it an essential tool for detecting trace-level compounds in complex mixtures.

6 Results

The principal and working of GC-MS was demonstrated.

7 References

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