Spectrophotometric Analysis

1

INTRODUCTION

Absorption spectrophotometry is an oldest method for quantitative analysis and structural elucidation. This is mainly concerned with the following regions of the spectrum — ultraviolet 100–400 nm, visible 400–800 nm and colorimetry. Colorimetry is concerned with the visible region of the spectrum. The instruments applied for measuring the absorption or emission of radiant energy from object are called by various names, such as

- Photometers,
- Colorimeters and
- Spectrophotometers.

Photometer. An instrument measures the ratio, or some functions of two, of the radiant power of two electromagnetic beams. Filters are used to isolate a narrow wavelength region and a photocell or phototube to measure the intensity of the radiation.

Colorimeter. Any instrument used for measuring absorption in the visible region is generally called a colorimeter. Some commercial filter photometers are called colorimeters.

Spectrophotometer. An instrument measures the ratio, or a function of two, of the radiant power of two electromagnetic beams over a large wavelength region. In this instrument is used monochromator instead of a filter. In addition, sensitive detectors like photomultipliers are used.

Theory of Spectrophotometric Analysis

Instrumentation

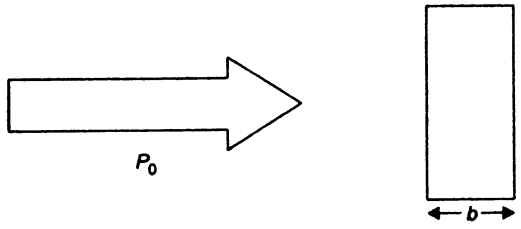
All photometers, colorimeters and spectrophotometers have the following basic components:

(*a*) Source
(*b*) Filter and monochromator
(*c*) Sample cell
(*d*) Detector

Laws Governing Absorption of Radiation

Many compounds absorb ultraviolet (UV) or visible light. The diagram below shows a beam of monochromatic radiation of radiant power P_0, directed at a sample solution.

Absorption takes place and the beam of radiation leaving the sample has radiant power P.



EXPERIMENT 1.2B

Date _____

ASSAY OF PARACETAMOL TABLETS I.P.

(Manual)

AIM

To determine the percentage purity of given sample of paracetamol.

PROCEDURE

1. Weigh and powder 20 tablets with the help of mortar and pestle.
2. Weigh accurately a quantity of the powdered tablets equivalent to 0.15 gm of Paracetamol.
3. Add 50 ml of 0.1M NaOH and dilute to 100 ml with distilled water.
4. Shake for 15 minute and add sufficient distilled water to produce 200 ml.
5. Mix well and filter the resulting solution with Whatmann filter paper.
6. Take 10 ml of this solution and dilute to 100 ml with distilled water.
7. To 10 ml of resulting solution, add 10 ml of 0.1M NaOH and dilute to 100 ml with distilled water and mix well.
8. Measure the absorbance of the resulting solution at 257 nm against blank solution.

Note: The solution ready for absorbance should be clear and should not contain any particle.

OBSERVATIONS

1. Weight variation test

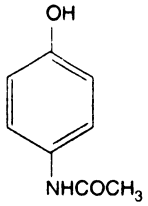
S. No.	Wt. of tablets (gm)	S. No.	Wt. of tablets (gm)
1		11	
2		12	
3		13	
4		14	
5		15	
6		16	
7		17	
8		18	
9		19	
10		20	
		Average weight (gm)	

2. Absorbance (A) =

ASSAY

Weigh accurately about 0.2 g, dissolve in 50 ml of anhydrous glacial acetic acid and carry out the Method A as given in I.P. 1996 (Appendix 3.45) for non-aqueous titration, determining the end-point potentiometrically. Perform a blank determination and make any necessary correction. Each ml of 0.1M perchloric acid is equivalent to **0.03181 g of $C_{14}H_{10}Cl_2NNaO_2$.**

ACETAMINOPHEN (PARACETAMOL)



Mol. Formula: $C_8H_9NO_2$ **Mol. Wt.:** 151.16

Paracetamol is 4-hydroxyacetanilide.

Category: Analgesic; antipyretic.

Description: White crystals or white crystalline powder.

Solubility: Freely soluble in ethanol (95%) and in acetone; sparingly soluble in water; very slightly soluble in dichloromethane and in ether.

Assay: Weigh accurately about 0.5 g, dissolve in a mixture of 10 ml of water and 50 ml of 1M sulphuric acid. Boil under reflux condenser for 1 hour, cool and dilute to 100.0 ml with water. To 20.0 ml of the solution, add 40 ml of water, 40 g of water in the form of ice, 15 ml of 2M hydrochloric acid and 0.1 ml of ferroin solution and titrate with 0.1 M cerric ammonium sulphate until yellow colour is produced. Perform a blank determination and make any necessary correction. Each ml of 0.1M cerric ammonium sulphate is equivalent to 0.00756 g of $C_8H_9NO_2$.

PROCEDURE

1. **For estimation of Paracetamol:**
   - **(*a*) Preparation of standard solution of Paracetamol (10 µg/ml)**
     - (*i*) Weigh accurately 100 mg of PCM powder and dissolve in 50 ml 0.1N HCl.
     - (*ii*) Make up the volume up to 100 ml by 0.1N HCl.
     - (*iii*) Take 10 ml of above solution and dilute to 100 ml with 0.1N HCl.
     - (*iv*) To get the resulting solution of 10 µg/ml concentration, take 10 ml and dilute to 100 ml with 0.1N HCl.
   - **(*b*) Preparation of sample solution of Paracetamol**
     - (*i*) Weigh and powder 20 tablets with the help of pestle mortar.
     - (*ii*) Weigh accurately a quantity of the powdered tablet equivalent to 0.100 gm of Paracetamol.
     - (*iii*) Extract the drug with three portions of 25–25 ml of 0.1N HCl.
     - (*iv*) Filter through Whatmann filter paper.
     - (*v*) Combine all the three filtrates and make up the volume up to 100 ml with 0.1N HCl.
     - (*vi*) Take 10 ml and dilute to 100 ml with 0.1N HCl.
     - (*vii*) To get the resulting solution, take 10 ml of above solution and dilute to 100 ml with 0.1N HCl.

     Measure the absorbance of both standard and sample at 244 nm and deduce the results by comparison.
2. **For estimation of Diclofenac sodium (DCLS)**
   - **(*a*) Preparation of standard solution of Diclofenac sodium (10 µg/ml)**
     - (*i*) Weigh accurately 100 mg of DCLS powder and dissolve in 50 ml 0.1N NaOH.
     - (*ii*) Make up the volume up to 100 ml by 0.1N NaOH.
     - (*iii*) Take 10 ml of above solution and dilute to 100 ml with 0.1N NaOH.
     - (*iv*) To get the resulting solution of 10 µg/ml concentration, take 10 ml and dilute to 100 ml with 0.1N NaOH.

EXPERIMENT 1.11A

Date _____

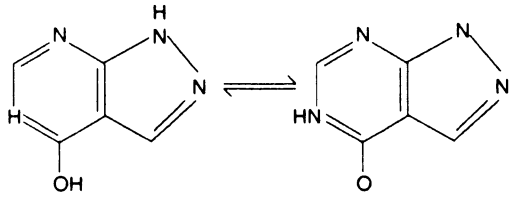
ASSAY OF ALLOPURINOL

AIM

To determine the percentage purity of given sample of allopurinol tablets I.P.

PRINCIPLE

ALLOPURINOL



Mol. Formula: $C_5H_4N_4O$ **Mol. Wt.:** 136.11

Allopurinol is a tautomeric mixture of 1*H*-pyrazolo [3,4-*d*]- pyrimidin-4-ol and 1,5-dihydro-4*H*-pyrazolo [3,4-*d*]-pyrimidin-4-one.

Category: Gout therapy.

Description: White or almost white, crystalline powder.

Solubility: Very slightly soluble in water and in ethanol (95%); practically insoluble in chloroform and in ether. It is soluble in dilute solutions of alkali hydroxides.

ASSAY

Weigh accurately about 0.2 g, dissolve with gentle heating, if necessary, in 50 ml of dimethylformamide and carry out the method A for non-aqueous titration given in I.P. 1996 (Appendix 3.45) using 0.1M tetrabutylammonium hydroxide as the titrant and determining the end-point potentiometrically. Each ml of 0.1M tetrabutylammonium hydroxide is equivalent to 0.01361 g of $C_5H_4N_4O$.

USES

Prophylaxis of gout and hyperuricaemia.

PROCEDURE

1. Weigh and powder 20 tablets with the help of mortar and pestle.
2. Weigh accurately a quantity of the powdered tablets equivalent to 0.100 gm of Allopurinol.
3. Add 10 ml of 0.1M NaOH and dilute 100 ml with 0.1M HCl.
4. Take 10 ml of the solution and dilute to 100 ml with 0.1M HCl.
5. Take 10 ml of resulting solution and again dilute to 100 ml with 0.1M HCl.
6. Measure the absorbance at 250 nm.