**ELEMENTAL ANALYSIS: QUANTITATIVE DETERMINATION OF CARBON, HYDROGEN, OXYGEN, NITROGEN ELEMENTS, ALCOHOL AND ALKALOIDAL CONTENT**

**Elemental analysis** is a process where a sample of some material (e.g., soil, waste or drinking water, bodily fluids, minerals, chemical compounds and Pharmaceuticals) is analyzed for its elemental and sometimes isotopic composition. Elemental analysis can be qualitative (determining what elements are present), and it can be quantitative (determining how much of each are present).

**Quantitative Analysis**

Determination of the relative proportions (as percentages) of various elements present in an organic compound is called quantitative analysis of the compound. The percentages of the various elements present in the compound are estimated by suitable chemical methods as described.

1. **Estimation of Carbon and Hydrogen**

Both carbon and hydrogen present in an organic compound are estimated in one experiment by 'Liebig's method'.

**Principle:** These elements are estimated by its combustion in an atmosphere of pure oxygen to form carbon dioxide (CO2) and water (H2O).





**Apparatus**

The apparatus used for the estimation of carbon and hydrogen consists of a long hard-glass combustion tube packed with copper oxide and coarse copper oxide. Left end of the combustion tube is connected to the oxygen supply, while at the other end; two pre-weighed absorption tubes are placed. One of the absorption tubes is packed with magnesium perchlorate which absorbs water vapor, and the other is packed with KOH or asbestos which absorbs CO2.



**Fig: 1** Apparatus for the estimating carbon and hydrogen

**Procedure**

A small quantity (0.2 to 0.3 g) of the organic compound is placed in a porcelain / platinum boat. The boat is placed in the combustion tube and is heated at 400o to 450oC. Pure dry oxygen is slowly passed through the tube. Combustion is complete in 8 to 10 hours. It results in the formation of CO2 and H2O from the carbon and hydrogen in the compound. They are absorbed in the absorption tubes at the end. The absorption tubes are removed and weighed to determine the amount of CO2 and H2O formed in the reaction. The masses and the percentages of C and H in the compound are calculated as follows.

**Calculations**

Let,

The mass of the organic compound taken be = W g

Increase in the mass of magnesium perchlorate tube = W1 g

Increase in the mass of the KOH tube = W2 g

So,

Mass of water formed = W1 g

Mass of carbon dioxide formed = W2 g



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1. **Estimation of Nitrogen**

Nitrogen can be estimated by either of the two methods:

* Dumas' method
* Kjeldahl's method

**Dumas' method for estimation of Nitrogen:**

A known mass of the organic compound is heated with cupric oxide in an atmosphere of carbon dioxide. The carbon and hydrogen in the compound are oxidised to carbon dioxide and water respectively, while nitrogen is set free. Any oxide of nitrogen produced during this process, is reduced back to free nitrogen by heated copper gauze. The gaseous mixture consisting of CO2, H2O and N2 is collected over an aqueous solution of potassium hydroxide. All the gases except nitrogen are absorbed by the solution. The volume of gas (nitrogen) collected is measured. From the volume of nitrogen obtained the percentage of nitrogen in the compound is calculated.



**Fig: 2** Apparatus for the estimation of nitrogen by Dumas' method

**Calculations**

Let,

The mass of the organic compound taken be = W g

Volume of nitrogen collected = V1 g

Atmospheric pressure = P mm Hg

Temperature at which gas is collected = T1 K

Therefore,

Pressure of the N2 gas, P1 = (P - p) mm of Hg





 (1 mol of N2 = 28 g = 22400 mL)

Therefore,



**Kjeldahl's method for estimation of Nitrogen:**

Kjeldahl's method is a faster method than Dumas' method. However, this method is used only for those organic compounds that are converted quantitatively to ammonium sulphate on heating strongly with concentrated sulphuric acid.

Kjeldahl's method cannot be used for the organic compounds,

* Containing nitrogen in the ring, e.g., pyridine, quinoline etc.
* Containing nitro (-NO2) and diazo (-N = N-) groups.

Kjeldahl's method involves two steps: Digestion and Distillation

1. **Digestion:** A known mass (0.3 to 0.5 g) of the given organic compound is digested with concentrated H2SO4, in presence of a small quantity of potassium sulphate and copper sulphate in a Kjeldahl's flask. Potassium sulphate raises the boiling point of sulphuric acid and copper sulphate catalyzes the digestion. In 3 to 4 hours, the organic compound is completely decomposed to form ammonium sulphate.



1. **Distillation:** The digested reaction mixture, on cooling, is transferred to a round bottomed distillation flask, and distilled with a concentrated alkali solution (NaOH). Ammonia produced is absorbed in a known volume of HCl solution of a known strength.





The un-neutralised HCl is then back-titrated against a standard alkali. From the acid consumed, the amount of ammonia produced and hence the mass of nitrogen is calculated.



**Fig: 3** Apparatus for the estimation of nitrogen by Kjeldahl's method

**Calculation**

Let,

Mass of the organic compound = W g

Volume of the standard acid required for complete neutralization of the evolved ammonia = V mL

Normality of the standard solution of acid = N

From the law of equivalence (normality equation),

1000 mL of 1 N acid = 1000 mL of 1 N NH3 = 17g NH3 = 14 g nitrogen

Then,

V mL of N acid = V mL of NH3

NV milli equivalent of acid = NV milli equivalent of ammonia

Therefore,



Then,



Percentage of nitrogen in the sample =



1. **Determination of Oxygen:**

The percentage of oxygen in an organic compound is usually found by the difference between the total percentage (100) composition and the sum of percentages of all other elements. However, oxygen can also be estimated as follows:

A defined mass of an organic compound is decomposed by heating in a stream of Nitrogen gas. The mixture of gaseous product containing oxygen is passed over a red- hot coke when all the oxygen is converted to carbon mono oxide. Warm iodine peroxide (I2O5) is ten passed over carbon monoxide, when carbon mono oxide is oxidized to carbon dioxide producing iodine.

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Compound 2CO

I2O5+5CO I2 + 5CO2

The percentage of oxygen can be derived from the amount of carbon di oxide or iodine produced

**Calculation:**

Let the mass of organic compound taken be *m* g

Mass of carbon dioxide = m1g

44g carbon dioxide = 32 g oxygen

m1g carbon dioxide contains$\frac{32Xm1}{44}$ g O2

Therefore,

 Percentage of Oxygen= $\frac{32Xm1X100}{44Xm}\%$

Presently, estimation of elements in organic compounds is carried out by using micro quantities of substance and automatic experimental techniques. The elements carbon, hydrogen and nitrogen present in a compound is determined by an instrument CHN elemental analyzer.

1. **Determination of Alkaloids :**

Alkaloidsarebasic nitrogenous compound, contains one or more nitrogen in heterocyclic ring system having marked physiological action on human and animals when use in small quantities. They can be determined quantitatively by:

1. **Volumetric methods:** These are based on reaction of alkaloidal bases with acids (Acid-Base titration).They include:
2. **Aqueous titration:** This is carried by either:
* Direct titration of the alcoholic solution of the alkaloidal residue with standard acid, or
* Back titration by dissolving the residue in a known amount of standard acid and back titration of residual acid against standard alkali.
1. **Non-aqueous titration:** This method is suitable for determination of weak bases e.g. Caffeine.
2. **Gravimetric methods:** These methods are recommended for determination of:
* Very weak bases which cannot be determined by volumetric methods e.g. caffeine and colchicine.
* Mixtures of alkaloids that are obtained from the same plant but differ greatly in their molecular weight e.g. Cinchona and Rawolfia alkaloids.

They can be performed by either:

1. Direct Weighing of the alkaloidal mixtures
2. Precipitation of the total alkaloids and determination of the weight of the precipitate obtained.

The major drawbacks of the gravimetric methods are:

1. They are insensitive to microamounts of alkaloids.
2. They could not be applied in case of thermolabile and volatile alkaloids.
3. Lipophilic impurities in the residue are calculated as alkaloids.

**C. Colourimetric Method:**

 e.g. Morphine + NaNO2/HCl

 Ergot + p-dimethylaminobenzaldehyde

1. **Spectrophotometric Methods.**
2. **Polarimetric Method.**
3. **Fluorimetric Method.**
4. **Chromatographic Methods:**  including HPLC
5. **Determination of Alcohol**

 Ethyl alcohol is commonly known as alcohol. It is very widely used in pharmacy for various purposes. It serves as vehicle in pharmaceutical preparations and manufacture of various formulations. The amount and strength of alcohol used varies from preparation to preparation. In some preparations different strength of alcohol is used, while in others industrial methylated spirit is used. The denatured spirit or industrial methylated spirit contains upto 5 % methyl alcohol in alcohol. Detection of it is usually recommended in pharmacopoeias, since it is toxic and harmful to the health.

 Determination of alcohol content is very routinely carried out in pharmaceutical industry. Different methods are used for its determination. The methods adopted by pharmacopoeia of India are discussed below.

Traditionally, determination of alcohol in galenicals is carried out by distillation method followed by physical measurements of the distillate. During the distillation following steps are carried out which decides particular method to be used.

1. Distillation of all the ethyl alcohol from the sample.
2. Removal of volatile impurities and interfering materials.
3. Test on the distillate for identification of methyl alcohol or isopropyl alcohol; and dilution of distillate to record specific gravity to be in measurable range.

**Distillation Methods :** The basic principle of this method is the distillation of the liquid sample using an appropriate distillation assembly. The distillate is then analyzed either by determining specific gravity of distillate at specified temperature or by refractive index. In distillation method care is to be taken to minimize the loss of alcohol by evaporation. Three methods are used in distillation depending upon the nature of the sample. An appropriate treatment is given to the sample before placing it in distillation assembly. The distillation apparatus used in alcohol determination is described below.

**Apparatus:**

The apparatus consists of four parts. Part A is a 500 ml capacity round bottom flask. To this is fitted distillation head a (B) with a steam trap. A vertical condenser (C) is fitted to the distillation head. The distillate is collected in 100 to 200 ml capacity volumetric flask (D). This flask is generally immersed in an ice-water mixture during distillation.

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**Fig. 4** Apparatus for determination of Alcohol

Amongst distillation methods, the following methods are followed:

 **Method-I:** This general method is used for normal samples, which do not need any special treatment. For working, place 25 ml of an accurately measured sample at 250C into distillation flask. Add about 150 ml of water, little pumic powder and distill. Collect not less than 90 ml of distillate in 100 ml volumetric flask placed in a beaker containing ice-water mixture. Adjust the temperature of distillate to 250C and refer the table given in standard books find the corresponding percent of alcohol. Multiply the volume by four to get alcohol content in the sample. If the sample gives frothing during distillation add few drops of liquid paraffin or silicon oil to prevent frothing. If the distillate appears turbid, follow Method-III. Turbidity usually occurs due to the presence of steam volatile acids during distillation.

**Method-II:** This method is used for the samples containing appreciable amounts of volatile matter. The volatile matter gets into distillate during distillation, and hence requires removal by extraction with suitable solvent.

 Place 25 ml of accurately measured sample at 250C into a 250 ml capacity separating funnel, add about 100 ml water. Saturate this mixture with solid sodium chloride and add 100 ml of hexane. Shake vigorously for 2-3 minutes, allow standing till two layers separate and run the lower layer into distillation flask. Wash the hexane (upper layer) with about 25 ml of sodium chloride solution and place it into the flask. Make the mixture in distillation flask just alkaline with 1 N sodium hydroxide using solid phenolphthalein as indicator. Add little piece of pumic and distill till 90 ml is collected in volumetric flask. Determine the alcohol content as per the procedure described in Method-I.

 **Method-III:** This method is similar Method-II in certain respects. If the sample contains methyl alcohol or isopropyl alcohol, test for their presence or absence in distillate is made. Sometimes sample preparations contain industrial methylated spirit (containing about 5 % methyl alcohol). This gives the colour due to oxidation of methyl alcohol into formaldehyde.

 Place 25 ml of accurately measured sample at 250C in distillation flask, and 150 ml water, little pumic powder and collect 100 ml distillate. Transfer this to separating funnel, add solid sodium chloride and extract with 100 ml hexane. Using the lower layer now proceed as per Method-II and find out the alcohol content.

Since many preparations containing alcohol are made using industrial methylated spirit or denatured spirit, detection for methyl alcohol and/or isopropyl alcohol is done on the distillate. For this two tests are carried out as follows:

 **1. For methyl alcohol:** Take 5 ml of distillate, add 1 drop of dilute phosphoric acid, 1 drop of potassium permanganate solution, mix and allow standing for one minute. Then add drop wise sufficient sodium bisulphate solution till permanganate colour is discharged. If slight brown colour is there then add one drop of dilute phosphoric acid. Now add 5 ml of freshly prepared acid solution and heat on water bath at 600C for ten minutes; no violet colour is produced.

This test is based upon the oxidation of methyl alcohol to formaldehyde, which forms Schiff’s base with chromotropic acid to give violet color.

 **2. For isopropyl alcohol:** To 1 ml of distillate add 2 ml mercuric sulphate solution and heat on water bath. No precipitate is formed. (Indicates absence of isopropyl alcohol).

 In the earlier editions of pharmacopoeia there was method-IV, which involved preliminary extraction of 25 ml of sample with 25 ml of sulphuric acid in water and extract with 100 ml light petroleum ether. After the petroleum ether extraction, the aqueous phase is transferred to the distillation flask and distillate is collected. Now-a-days this method is no longer adopted.

 **Gas-liquid Chromatographic Methods**

Now-a-days gas chromatography is widely used in pharmaceutical analysis. This method is adopted for certain specified samples containing alcohol. It gives accurate results rapidly.

A gas-liquid chromatography apparatus as described in pharmacopoeia or standard textbooks is used. Two methods are followed. In method I, solutions used are (a) solution containing 5 % V/V ethyl alcohol and 5 % V/V propyl alcohol as internal standard. (b) Sample solution is diluted such to contain between 4.0 ⎯ 6.0 % alcohol; (c) 5 % propyl alcohol is added to the dilute sample.

 For GLC, porapak Q or chromosorb 101 columns maintained at 1500C, nitrogen as carrier gas, flame ionization detector is used. Method-II is used for samples containing methyl alcohol. Since many preparations are made using industrial methylated spirit, which contains up to 5 % methyl alcohol, detection and determination of methyl alcohol peak is done using GLC.

 Determination of alcohol containing volatile oils, sample needs to be analyzed carefully. If the preparation containing alcohol is acidic, it should be neutralized with sodium hydroxide solution and if alkaline then neutralize with sulphuric acid before distillation. Furthermore if preparations contain chloroform besides ethyl alcohol then separation of chloroform during distillation need to be carried out using modified apparatus, which is described in pharmacopoeias and pharmaceutical codex.