

PHARMACEUTICAL ANALYSIS

Method of Analysis and Assay: Miscellaneous Methods of Analysis

Dr. M. Shahar Yar
Lecturer
Dept. of Pharmaceutical Chemistry
Faculty of Pharmacy
Jamia Hamdard
Hamdard Nagar
New Delhi- 110062

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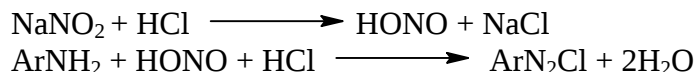
Keywords

Diazotization titrations, Kjeldahl's nitrogen determination, Karl Fisher titrimetry, Determination of alcohol in liquid galanicals, Oxygen flask combustion method

Diazotization Titration or Nitrite Titration

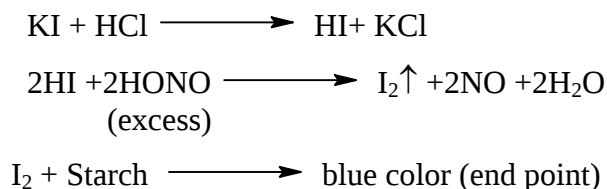
Diazotization is used in the analysis of aromatic compounds containing an amino group in the molecules. This analysis is based on the reaction between aromatic primary amine ($-\text{NH}_2$), HONO , in presence of excess mineral or inorganic acids.

NaNO_2 is generally used in direct method of diazotization. It gives HNO_2 in acidic solution. Many aromatic primary amine with free $-\text{NH}_2$ group can be analyzed quantitatively by measuring the volume of NaNO_2 solution required to convert them into diazonium salts. The typical reaction of diazotization reaction in presence of HCl is given as



When all the aromatic amine has reacted with NaNO_2 then next portion (excess) of NaNO_2 added to the solution under test, converted to HONO that remain in the solution and can be detected by the starch paper or paste as an external indicator. This appearance of free HNO_2 in solution indicates that diazotization reaction is complete and equivalence point is attained.

Starch iodide paper or paste is the starch solution plus equal volume of 5% KI solution in H_2O . Starch iodide paste with reaction mixture is as follows-



The liberated I_2 reacts with starch to give blue colour. The diazotization proceeds quantitatively only in presence of inorganic acid. It is important to check the acidity at the end of the titration. If there is no excess of acid present, starch-iodide paper will not detect excess HNO_2 and so will not indicate the end point.

Condition for diazotization

- (1) Rate of titration
- (2) Temperature of the solution

(1) **Rate of titration:** Different amino compound react with HONO at different rates. NaNO_2 added from the burette needs time to react with amino group accumulating in the solution. Amines are classified as rapidly slowly diazotisable depending on the rate of conversion into azo compounds. Slow diazotisable compounds include compounds that contain sulpha groups, nitrous oxide group, or carboxylic group in aromatic ring besides aromatic ring.

Example: - isomeric nitro aniline, sulphanilic acid and anthranilic acid.

Fast diazotisable compounds do not contain any substituent group other than amino group but some times they may contain $-\text{CH}_3$ or $-\text{OH}$ group along with NH_2 group. Example: - aniline, toluidine and aminophenol.

Adding KBr to the solution can increase the rate of titration. In case of slowly diazotisable compound sufficient time (5-10 min) should be given to the solution under test. So, that last portion of nitrous acid could fully react with amine.

If the test for end point determination is carried out immediately after addition of the sodium nitrite, the nitrous acid so formed has not yet entered into the reaction with amine will be taken as excess nitrous acid, indicate the end point has reached. By sufficient stirring and slow addition of NaNO_2 and keeping in view the time factor, a large excess of NaNO_2 is not allowed to build up. It is also suggested that the burette tip is placed below the surface of reaction mixture to prevent the loss of HNO_2 .

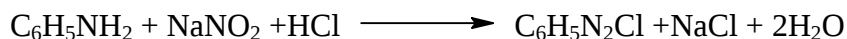
(2)Temperature: The diazonium compounds so formed are instable and readily decompose at elevated temperature. This can lead to side reaction and give wrong result to eliminate this titration as carried out at low temperature ($0-5^\circ\text{C}$), optimum temperature for most amine is $10-15^\circ\text{C}$, when they form relatively stable diazo compounds.

End point determination: - is done by two methods.

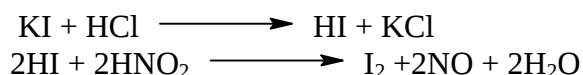
- Visual end point determination method
- Electrometric end point determination method.

(a) Visual end point determination method:

Principle: - aromatic primary amine reacts with sodium nitrite in acid solution (i.e, nitrous acid) to form diazonium salts.



Under controlled condition reaction is quantitative and can be used for the determination of most substance containing a free primary amino group as in sulphanilamide and other sulpha drugs. Observation of end point depends upon the detection of small excess of nitrous acid, which is then present. This can be demonstrated visually by using starch iodide paste as external indicator.



The liberated iodine (I_2) reacts with starch to give blue colours.

Method: - weigh 2.5 gm of sample; transfer it into a 250 ml flask. Add 50 ml of concentrated HCl and 5 gm of KBr, adjust the final volume to 250 ml. pipette out 50 ml of this solution into a conical flask cool the solution so that the temperature is maintained between 10 to 15°C . Titrate the solution with N/10 NaNO_2 and shaking continuously until distinct blue color is produced on a small piece of starch iodide paper. The reaction is slow initially but behaves normal as the reaction proceeds.

(b) Electrometric End Point determination method:

Principle- End point may be detected electrometrically using a pair of bright platinum electrodes immersed in the titration liquid. Electrode polarization occurs in the titration liquid. Electrode polarization occurs when a small voltage (30-50 mV) is applied across the electrodes and no

current flows through the sensitive galvanometer included in the circuit, during the course of titration. Liberation of excess of nitrous acid at the end point depolarizes the electrode, current flows and full deflection in galvanometer needle is observed. This is known as the dead stop end point.

The electrode must be clean other wise the end point is sluggish cleaning can be accomplished by immersing the electrodes in boiling nitric acid containing a little ferric chloride for about 30 sec and then washing with water. The blank determination is necessary, the difference between the two titrations represent the volume of sodium nitrite solution equivalent to the aromatic amine.

Method : Weigh accurately the sample (about 0.5 gm) and transfer into a 250 ml beaker and dissolve in the HCl 10 ml and water 75 ml. insert a pair of bright platinum electrodes into the solution, connected through a sensitive galvanometer and suitable potentiometer at a 2V battery in such a way as to produce a potential drop between 30-50 mV across the electrodes.

Titrate slowly with N/10 NaNO_2 with continuous stirring until a permanent deflection of the galvanometer is observed at the end point.

Application of diazotization titration: An important pharmaceutical application of sodium nitrite titration is the analysis of sulphonamides by diazotization of primary aromatic amino group usually present in this class of drugs. Several sulphonamides require the formation of primary amine prior to diazotization step.

Example – phthalylsulphathiazole and succinyl sulphathiazole are first hydrolyzed to give primary aromatic amine and determined by NaNO_2 titration method.

List of compounds assayed by sodium nitrite titration:

- (1) Dapsone
- (2) Benzocaine
- (3) Procaine hydrochloride
- (4) Calcium amino salicylate
- (5) Sodium amino salicylate
- (6) Sulphacetamide tablet
- (7) Sulphadoxine
- (8) All sulpha drugs

Karl Fischer Titration

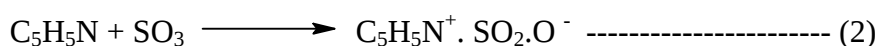
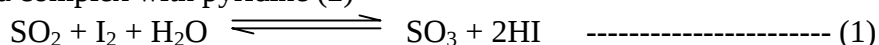
Introduction: Aquametry can be defined as quantitative determination of water in pharmaceuticals.

The Karl Fischer Titration (KFT) technique was introduced in 1935, for the determination of small amounts of water in a variety of organic and inorganic solid and liquid samples. The fundamental principle behind it is based on the Bunsen Reaction between iodine and sulfur dioxide in an aqueous medium. Karl Fischer discovered that this reaction could be modified for

the determination of water in a non-aqueous system containing an excess of sulfur dioxide. He used a primary alcohol (methanol) as the solvent, and a base (pyridine) as the buffering agent.

Background: Water, in small amount, is generally controlled by a loss on drying under specified conditions. When present in appreciable amount, the water content may often be readily determined by titration with KARL FISSHER REAGENT, which contains iodine, sulphur dioxide, anhydrous methanol and anhydrous pyridine.

The reaction of Karl Fischer Titration is a three-stage process. The first stage involves the reaction of sulphur dioxide with water and iodine which reduces iodine (1), in second stage SO_3 forms a complex with pyridine (2)



The third stage involves reaction of pyridine complex with methanol to form stable complex (3).



The each molecule of iodine is equivalent to one molecule of water.

The reactive alcohol is typically methanol or 2-(2-Ethoxyethoxy) ethanol, also known as diethylene glycol monoethyl ether (DEGEE), or another suitable alcohol. Classic Karl Fisher reagents contained pyridine, a noxious carcinogen, as the base. The reagents most frequently used today are pyridine-free and contain imidazole or primary amines instead.

There are two important requirements if the above reactions are to be stoichiometric. Firstly, the alcohol used must completely etherify the sulphur dioxide and secondly the base must be of suitable strength to completely neutralize the acids produced during the reaction.

Preparation of Karl Fischer Reagent:

Method – Into a glass-stopper flask (about 750 ml capacity) place anhydrous methanol (400 ml, containing not more than 0.03 % water) and pure dry pyridine 80 mg. Immerse the flask in a freezing bath and slowly pass sulphur dioxide into a cold solution, with continuous agitation, until the increase in the weight is 20 gm. Care must be taken all time to prevent access of moisture. Finally add iodine 45 gm, shake to dissolve and allow the mixture to stand for 24 h before use. The reagent prepared in this manner will have water equivalent of 3.5 mg/ml approximately.

Standardisation: - Karl Fischer reagent is not stable and must be rigorously protected from both light and moisture. It must be standardized immediately before use by titration against a known amount of water, either as a standard water-methanol reagent or, preferably, as solid crystalline sodium tartrate dihydrate. Although the latter is almost insoluble in methanol, it yields its water of hydration to the solvent, and its precise water content can be determined by drying at 150° to constant weight.

How does it work?: Water and iodine are consumed in a 1:1 ratio in the above reaction. When all of the water present is consumed, the presence of excess iodine is detected by indicator

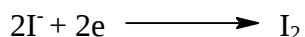
electrode that signals the end-point of the titration. The amount of water present in the sample is calculated, based on the concentration of iodine in the Karl Fisher titrating reagent (i.e., titer) and the amount of Karl Fisher Reagent consumed in the titration.

Role of pH : The rate of the reaction depends on the pH value of the solvent or working medium. The titration proceeds normally when pH is between 5 to 8. However, when the pH is lower than 5, the titration speed is very slow. On the other hand, when pH is higher than 8, titration rate is fast, only due to an interfering side reaction, which produces water, resulting in a vanishing endpoint. Thus, the optimal pH range for the Karl Fischer reaction is from 5 to 8, and highly acidic or basic samples need to be buffered to bring the overall pH into that range.

Karl Fischer Instrument: The Karl Fischer instrument used is the Mitsubishi Coulometric moisture meter Model CA-100.



Titration Method: Electrolytic oxidation is carried out in the analyte, which consists mainly of iodide, sulphur dioxide, bases, and solvents such as alcohols. Electrolytic oxidation proceeds when a sample is added to the analyte. The Karl Fischer reaction occurs to produce iodine.



Iodine is produced in proportion to the quantity of electricity so the water content can be estimated based on the coulombs required for electrolytic oxidation. A check solution containing a known amount of water is titrated at the beginning and the end of experiments to ensure that the KF instrument is functioning correctly.

Types of Karl Fischer Titrations:

1) Volumetric KFT: In volumetric Karl Fischer, iodine is added mechanically to a solvent containing the sample by the titrator's burette during the titration. Water is quantified on the basis of the volume of Karl Fischer reagent consumed. Volumetric analysis is best suited for determination of water content in the range of 100 ppm to 100%.

There are two main types of volumetric KFT reagent systems:

- a) In one-component volumetric KF, the titrating reagent (also known as a CombiTitrant, or a Composite) contains all of the chemicals needed for the Karl Fischer Reaction, namely iodine, sulfur dioxide, and the base, dissolved in a suitable alcohol. Methanol is typically used as the working medium in the titration cell. One-component volumetric reagents are easier to handle, and are usually less expensive than two-component reagents.
- b) In two-component volumetric KF, the titrating agent (usually known as the Titrant) contains only iodine and methanol, while the Solvent containing the other Karl Fischer Reaction

components is used as the working medium in the titration cell. Two-component reagents have better long-term stability and faster titration times than one-component reagents, but are usually more costly, and have lower solvent capacity.

2) Coulometric KFT: In coulometric Karl Fischer, iodine is generated electrochemically *in situ* during the titration. Water is quantified on the basis of the total charge passed (Q), as measured by current (amperes) and time (seconds), according to the following relationship:

$$Q = 1 \text{ C (Coulomb)} = 1 \text{ A} \times 1 \text{ s} \quad (\text{where } 1 \text{ mg H}_2\text{O} = 10.72 \text{ C})$$

Coulometry is best suited for determination of water content in the range of 1 ppm to 5%. There are two main types of coulometric KFT reagent systems:

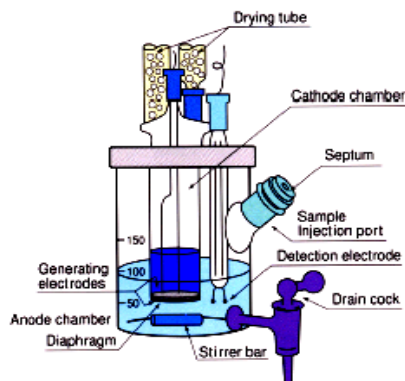
a) In conventional, or fritted-cell, coulometric KF, a diaphragm – or frit – separates the anode from the cathode that forms the electrolytic cell known as the generator electrode. The purpose of the frit is to prevent the iodine generated at the anode from being reduced back to iodide at the cathode instead of reacting with water.

b) In fritless-cell coulometric KF, an innovative cell design is used that through a combination of factors, but without a frit, makes it nearly impossible for iodine to reach the cathode and get reduced to iodide instead of reacting with water.

The advantages of the fritless cell include:

- Uses only one reagent
- Lower reagent cost
- Titration cell much easier to clean
- Reduced downtime
- Lower maintenance cost
- Long-term drift (background) value more stable
- Can use reagent longer without refilling
- Refilling of electrolyte suitable for automation
- Reduced downtime
- Increased lab safety

Titration Cell for Coulometric Titration



Working of volumetric titrator: The volumetric titrator performs the following three key functions:

- It dispenses KF titrating reagent containing iodine into the cell using the burette
- It detects the endpoint of the titration using the double platinum pin indicator electrode.
- It calculates the end result based on the volume of KF reagent dispensed using the On-board microprocessor.

Working of Coulometric Titrator: The titrator performs the following three key functions:

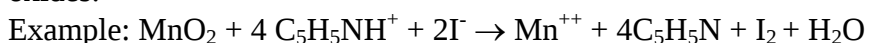
- 1) It generates iodine at the anode of the titration cell, instead of dispensing KF reagent as in volumetric titration
- 2) It detects the endpoint of the titration using the double platinum pin indicator electrode
- 3) It calculates the end result based on the total charge passed (Q), in Coulombs, using the on-board microprocessor

Sample size: The amount of sample used depends on the anticipated water content and the desired degree of accuracy. Refer to the following convenient reference table:

SAMPLE WATER CONTENT	VOLUMETRIC SAMPLE SIZE	COULOMETRIC SAMPLE SIZE
100%	0.02 to 0.05 g	NOT RECOMMENDED
50%	0.05 to 0.25 g	0.01 g
10% (100,000 PPM)	0.25 to 0.50 g	0.01 to 0.05 g
5% (50,000 PPM)	0.50 to 2.50 g	0.05 to 0.10 g
1% (10,000 PPM)	2.50 to 5.00 g	0.10 to 0.50 g
0.5% (5,000 PPM)	5.00 to 7.50 g	0.20 to 1.00 g
0.1% (1,000 PPM)	7.50 to 10.0 g	1.00 to 2.00 g
0.01% (100 PPM)	10.0 to 15.0 g	2.00 to 5.00 g
0.001 (10 PPM)	15.0 to 20.0 g	5.00 to 10.0 g
0.0001% (1 PPM)	NOT RECOMMENDED	10.0 g OR MORE

Limitation of Karl Fischer Titration: The Karl Fischer titration has a number of serious limitation due to possible interference to erroneous results, namely:

Oxidizing agents, for instance: chromates, Cu (II), Fe (III), peroxides, salts and higher oxides.



Reducing agent, such as: Sn (II) salts, sulphides and $\text{S}_2\text{O}_3^{-2}$.

Compound that have a tendency to form water with the ingredients of the KFR, for instances:

Basic oxides – eg. ZnO

Salts of weak oxy-acids –eg NaHCO_3

Uses of Karl Fischer Method: The method is used to control the water content of –

- Betamethasone sodium phosphate
- Cloxacillin sodium
- Cyclophosphamide
- Cyclophosphamide injections
- Prednisolone sodium phosphate
- Procaine penicillin
- Fortified procaine penicillin injection

Kjeldahl's Method of Nitrogen Determination

Although other chemical and physical method now exist for determination of organic nitrogen, the kjeldahl procedure is still used very extensively as it remain a highly reliable technique with well-established routines. The basic concept of the method is the digestion of organic material, e.g. proteins, using sulphuric acid and a catalyst to convert any organic nitrogen to ammonium sulphate in solution. By making the mixture alkaline, the liberated ammonia is steam-distilled and the resulting alkaline distillate is titrated with standard acid.

Procedure: - weigh out accurately part of the organic sample, sufficient to contain about 0.04 g of nitrogen, and place it in the long-necked Kjeldahl digestion flask. Add 0.7 gm of mercury (II) oxide, 15 g of potassium sulphate and 40 ml of concentrated sulphuric acid. Heat the flask gently in a slightly inclined position. Some frothing is likely to occur and may be controlled by the use of an anti foaming agent. When foaming ceases, boil the reactant for two hr. after cooling, add 200 ml of water and 25 ml of 0.5 M sodium thiosulphate solution and mix well. To the mixture add a few antibumping granules, then carefully pour sufficient 11 M NaOH solution inside of flask to make the mixture strongly alkaline (approximately 115 ml). Before mixing the reagents, connect the flask to a distillation apparatus in which the tip of the delivery tube is submerged just below the surface of a measured volume of 0.1 M HCl acid. Ensure the content of the distillation flask are well mixed, and then boil until at least 150 ml of liquid have been distilled into the receiver. Add methyl red indicator to the hydrochloric acid solution and titrate with 0.1 M sodium hydroxide (titration 'a' ml). Carry out a blank titration on an equal measured volume of the 0.1 M hydrochloric acid (titration 'b' ml).

Using the quantities and concentration given above, the percentage of nitrogen in the sample is given by

$$\frac{(b-a) \times 0.1 \times 14 \times 100}{\text{weight of sample (g)}}$$

Oxygen-Flask-Combustion Method

Schoniger first proposed the technique of oxygen flask combustion in 1955 for organically combined halogens. In a closed assembly, in presence of oxygen organic compound is burned, organic matter gets destroyed and halogen is released which is absorbed in an appropriate solution, which subsequently is analyzed. Besides halogen like fluorine, chlorine, bromine and iodine, sulphur is also determined by this method. This method is simple, rapid and accurate and can be applied to formulated products such as tablets, capsules, creams and ointments. However,

great care must be taken in carrying out the test especially during the combustion stage. Use of safety screen should be made while working.

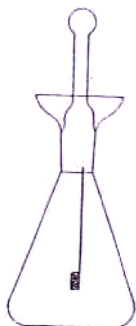


Fig.: Oxygen flask combustion apparatus

Apparatus: The apparatus (Above Figure) consists of a thick walled, 500 ml conical flask, fitted with a ground-glass stopper to which is fused a platinum wire about 13 cm long and 1 mm diameter. To this is attached a piece of platinum gauze for holding sample. The gauze is 2 cm x 1.5 cm in dimensions and is of No.36 sieve.

The general method is as follows:

Weigh accurately a suitable quantity of sample (about 20 mg) if solid on a halide free filter paper of 5 cm x 3 cm, wrap it and place in the platinum gauze sample holder. For ointments/cream weigh the sample on small size greaseproof paper, fold it and place in sample holder.

For the liquid samples use gelatin capsule or mix with methylcellulose powder and place on ash less grade paper. Insert one end of narrow strip of filter paper in the roll to serve as a fuse. Flush the flask with oxygen, moisten the neck with water and place specified quantity of absorbing liquid in the flask, fill it with oxygen and light the free end of paper strip and immediately insert stopper. Hold the stopper firmly during combustion of the sample, then shake the flask vigorously for about five minutes. Place few ml of water in the cup, carefully remove the stopper and rinse the stopper, platinum wire gauze with water. Then proceed for analysis for the particular ion or element.

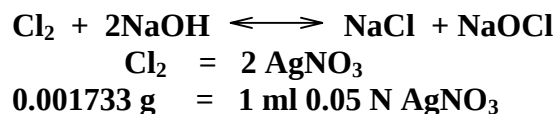
For fluorine: Twenty ml of water is used as absorbing liquid. After combustion of sample add sufficient water to produce 50 ml. To 2 ml of above solution, add 20 ml water, 10 ml alizarin fluorine blue solution, 3 ml of solution containing 2 per cent sodium acetate and 6 % w/v glacial acetic acid, 10 ml of cerous nitrate and sufficient water to produce 50 ml. Allow this to stand in dark for one hour and measure the extinction in 1 cm cell at 610 nm. Prepare the blank similarly, using 2 ml water instead of test solution. Calculate the fluorine content from calibration graph, prepared by using suitable quantity of sodium fluoride solution (22 mg/2 lit).

NOTE: *For Fluorine determination combustion is carried out in silica or soda glass flask. Borosilicate glass is affected by fluorine and should be avoided.*

For Chlorine: Absorbing liquid is 20 ml of 1 N sodium hydroxide solution. To the solution add 25 ml nitric acid, 10 ml or 0.1 N silver nitrate and titrate with 0.05 M ammonium thiocyanate using ferric ammonium sulphate solution as indicator. Carry out a blank titration. The difference

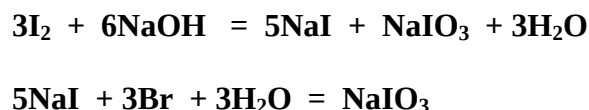
between two titrations represents the amount of 0.1 N silver nitrate required for the sample. Each ml of 0.1 N silver nitrate is equivalent to 0.001773 g of Cl.

Organically combined chlorine is converted to free chlorine during combustion and this is absorbed in sodium hydroxide to yield a mixture of sodium chloride and sodium hypochlorite. Addition of silver nitrate precipitates chloride as silver chloride and excess silver nitrate is back-titrated with ammonium thiocyanate solution.



For Bromine: Absorbing liquid is 15 ml of mixture of 1 volume of hydrogen peroxide (30%) and 9 volume of 1 N sulphuric acid. After the reaction is complete, add 5 ml of 2N nitric acid and 10 ml of 0.1 N silver nitrate and titrate with 0.05 N ammonium thiocyanate using ferric ammonium sulphate as indicator. A blank determination is carried. The difference between the titrations represents the number of ml of 0.05 N silver nitrate required for the sample. Each ml of 0.05 N silver nitrate is equivalent to 0.003995 g. of Br.

For Iodine: A mixture of 10 ml of water and 2 ml of 1 N sodium hydroxide is used as absorbing liquid. When the process is complete, add excess of acetic-bromine solution (5-10 ml) and allow to stand for two minutes. Remove the excess of bromine by the addition of formic acid (about 1 ml), drive off vapour of bromine with current of air. Add 1 g of potassium towards the end of titration. Each ml of 0.02 N sodium thiosulphate is equivalent to 0.000423 g of I



For Sulphur: There are two methods for sulphur determinations. Method I is for estimation of sulphur in the absence of halogens and phosphorous. Method II is for sulphur determination in presence of halogens or phosphorous. Both methods are similar to treatment upon combustion and differ in use of absorbing liquid and further treatment.

Method-I : Burn the specified quantity of sample material in the flask. 10 ml water containing 0.1 ml of hydrogen peroxide solution (30%) is used as absorbing liquid. When the process is complete, cool the solution in ice for 15 minutes. Boil the solution gently for 2 minutes, cool, add 50 ml of ethanolic-acetic-ammonia buffer (pH 3.7) and titrate with 0.05 M barium perchlorate using 0.3 ml alizarin red S as indicator until the solution becomes orange pink in colour. Each ml or 0.05 M barium perchlorate is equivalent to 0.001603 g of S.

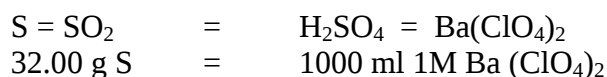
Method-II: Burn the specified quantity of sample as usual in flask. Absorbing fluid is 15 ml water containing 1 ml of hydrogen peroxide solution (10%). When the process is complete, boil solution for 10 minutes, cool and add 60 ml ethanol. Titrate the mixture with 0.01 M barium perchlorate, used as titrant and 0.1 ml of 0.2 % thoron and 0.1 ml of 0.0125 % methylene blue as indicator until the colour changes from yellow to pink.

Each ml of 0.01 M barium perchlorate is equivalent to 0.00032 g of S.

If the determinations as under Method-I and II are performed at different temperatures, the titrant volumes are corrected by the formula.

$$\begin{array}{rcll} \text{Where,} & V_c & = & V [1+0.008(t_1-t_2)] \\ & V_c & = & \text{the corrected volume of titrant} \\ & V & = & \text{the volume of titrant used} \\ & t_1 & = & \text{temperature of titrant during standardization} \\ & t_2 & = & \text{temperature of titrant during determination} \end{array}$$

During combustion, sulphur is converted to sulphur dioxide, which upon oxidation with hydrogen peroxide is transformed into sulphuric acid.



The following pharmacopoeial drugs are assayed by oxygen flask combustion method.

S.No.	Name	Identification	Absorbing liquid
1.	Diazepam	Chlorine	
2.	Diiodohydroxy quinoline	Iodine	
3.	Diiodohydroxy quinoline Tablets	Iodine	10 ml H ₂ O + 2ml 1N NaOH
4.	Diodone injection	Iodine	10 ml H ₂ O + 2ml 1N NaOH
5.	Ethacrynic acid	Chlorine	
6.	Flurouracil	Fluorine	
7.	Idoxuridine	Iodine	10 ml H ₂ O + 2ml 1N NaOH
8.	Niclosamide	Chlorine	1 N NaOH
9.	Quinodochlor	Chlorine and Iodine	5 ml 1% NaOH + 3 ml saturated sulphur solution
10.	Sulphobromophthalein sodium	Sulphur and Bromine	
11.	Throxine sodium	Iodine	

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 a ice-water mixture during distillation.

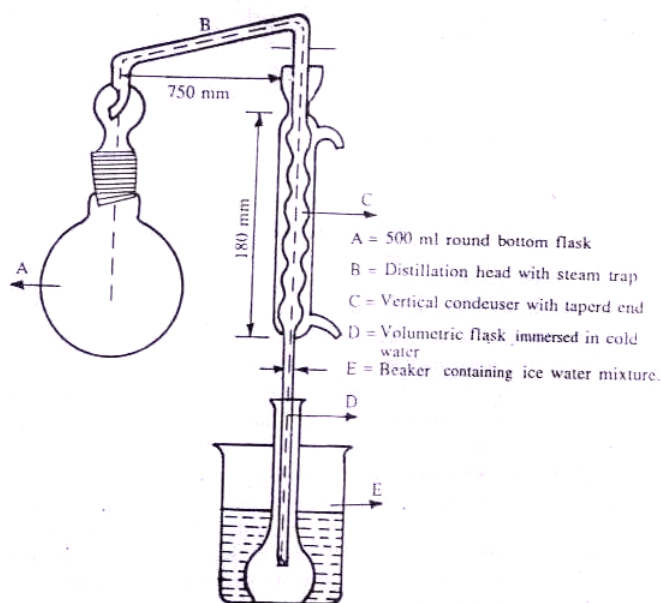


Fig.: Apparatus for Alcohol determination

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 25°C 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 25°C 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 25°C 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 25°C 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 60°C 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.

Suggested Readings:

- 1) K A Connors, A Text Book of Pharmaceutical Analysis, Wiley-Intersciences, New York.
- 2) A H Backett and J B Stenlake, Practical Pharmaceutical Chemistry, Vol.I and II, The Athlone Press of The University of London.
- 3) Pharmacopoeia of India, Govt.of India, Ministry of Health, Delhi.
- 4) J Bassett, R C Denney, G H Jeffery, J Mendham, Vogel's Textbook of Quantitative Inorganic Analysis, The ELBS and Longman, London.