M: The amount (g) of the chemically pure substance corresponding to 1 mL of the standard solution for volumetric analysis in the case of $f = 1$.

Procedure

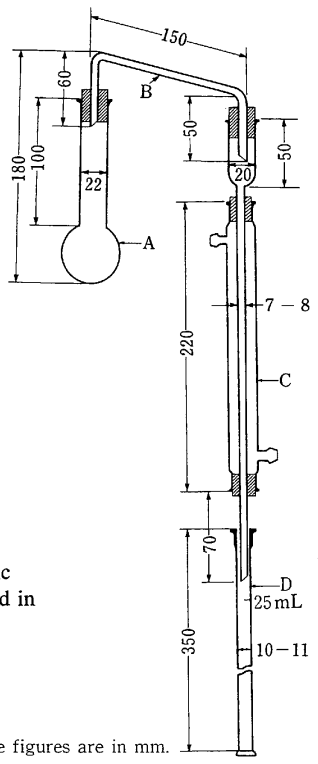
Under the conditions directed in the monograph, proceed as follows. Dry and weigh accurately the sample, and transfer it into a volumetric flask. Add V' mL of the standard solution for volumetric analysis with a pipet, and if directed in the monograph, add the specified solvent with a pipet. Then add the pH indicator, and dissolve the sample in water to make a definite volume. Use a spectrophotometer, and read the absorbances, A_1 and A_2, of this solution at λ_1 and λ_2 in a 10-mm cell, unless otherwise specified, at ordinary temperature using water as a blank. Calculate the value of r by using the formula. Estimate the value of x, using the obtained value of r and the x-r curve obtained from the table of relationships between x and r described in the monograph. Calculate the amount (g) of the chemically pure substance in the sample taken.

2. Alcohol Number Determination

The Alcohol Number Determination represents the number of milliliters of ethanol at 15°C obtained from 10 mL of tincture or other preparations containing ethanol by the following procedures.

Method 1 Distilling method

This is a method to determine the Alcohol Number by reading the number of milliliters of ethanol distillate at 15°C obtained from 10 mL of a sample measured at 15°C by the following procedures.



A: Distilling flask (50 mL)
B: Delivery tube
C: Condenser
D: Glass-stoppered volumetric cylinder (25 mL, graduated in 0.1 mL)

The figures are in mm.

Ethanol content in the sample (vol%)	Distillate to be collected (mL)
above 80	13
80 - 70	12
70 - 60	11
60 - 50	10
50 - 40	9
40 - 30	8
below 30	7

(1) Apparatus

Use hard glass apparatus as illustrated herein. Ground glass may be used for the joints.

(2) Reagent

Alkaline phenolphthalein solution: To 1 g of phenolphthalein add 7 mL of sodium hydroxide TS and water to make 100 mL.

(3) Procedure

Transfer 10 mL of the sample preparation, accurately measured at 15 ± 2°C, to the distilling flask A, add 5 mL of water and boiling chips. Distil ethanol carefully into the glass-stoppered, volumetric cylinder D.

By reference to the following Table, a suitable volume of distillate (mL) should be collected, according to the content of ethanol in the sample preparation.

Prevent bumping during distillation by rendering the sample strongly acidic with phosphoric acid or sulfuric acid, or by adding a small amount of paraffin, beeswax or silicone resin before starting the distillation.

When the samples contain the following substances, carry out pretreatment as follows before distillation.

(i) Glycerin: Add sufficient water to the sample so that the residue in the distilling flask, after distillation, contains at least 50% of water.

(ii) Iodine: Decolorize the sample with zinc powder.

(iii) Volatile substances: Preparations containing appreciable proportions of essential oil, chloroform, diethyl ether or camphor require treatment as follows. Mix 10 mL of the sample, accurately measured, with 10 mL of saturated sodium chloride solution in a separator, add 10 mL of petroleum benzin, and shake. Collect the separated aqueous layer. The petroleum benzin layer was extracted with two 5 mL portions of saturated sodium chloride solution. Combine the aqueous layers, and distill. According to the ethanol content in the sample, collect a volume of distillate 2 to 3 mL more than that shown in the above Table.

(iv) Other substances: Render preparations containing free ammonia slightly acidic with dilute sulfuric acid. If volatile acids are present, render the preparation slightly alkaline with sodium hydroxide TS, and if the preparations contain soap along with volatile substances, decompose the soap with an excess of dilute sulfuric acid before the extraction with petroleum benzin in the treatment described in (iii).

To the distillate add 4 to 6 g of potassium carbonate and 1 to 2 drops of alkaline phenolphthalein solution, and shake vigorously. If the aqueous layer shows no white turbidity, agitate the distillate with additional potassium carbonate. After allowing to stand in water at 15 ± 2°C for 30 minutes, read the volume of the upper reddish ethanol layer in mL, and regard it as the Alcohol Number. If there is no clear boundary surface between these two layers, shake vigorously

affer addition of a few drops of water, then observe in the same manner.

Method 2 Gas chromatography

This is a method to determine the alcohol number by determining ethanol (C_2H_5OH) content (vol%) from a sample measured at 15°C by the following procedures.

(1) Reagent

Ethanol for alcohol number: Ethanol (99.5) with determined ethanol (C_2H_5OH) content. The relation between specific gravity d_{15}^{15} of ethanol and content of ethanol (C_2H_5OH) is 0.797:99.46 vol%, 0.796:99.66 vol%, and 0.795:99.86 vol%.

(2) Preparation of sample solution and standard solution

Sample solution: Measure accurately a volume of sample at 15 ± 2°C equivalent to about 5 mL of ethanol (C_2H_5OH), and add water to make exactly 50 mL. Measure accurately 25 mL of this solution, add exactly 10 mL of the internal standard solution, and add water to make 100 mL.

Standard solution: Measure accurately 5 mL of ethanol for alcohol number at the same temperature as the sample, and add water to make exactly 50 mL. Measure accurately 25 mL of this solution, add exactly 10 mL of the internal standard solution, and add water to make 100 mL.

(3) Procedure

Place 25 mL each of the sample solution and the standard solution in a 100-mL, narrow-mouthed, cylindrical glass bottle sealed tightly with a rubber closure and aluminum band, immerse the bottle up to the neck in water, allowed to stand at room temperature for more than 1 hour in a room with little change in temperature, shake gently so as not to splash the solution on the closure, and allow to stand for 30 minutes. Perform the test with 1 mL each of the gas in the bottle with a syringe according to the Gas Chromatography under the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of ethanol to that of the internal standard.

$$\text{Alcohol number} = \frac{Q_T}{Q_S} \times \frac{5\ (\text{mL})}{\text{a volume (mL) of sample}} \times \frac{\text{ethanol } (C_2H_5OH) \text{ content (vol\%) of ethanol for alcohol number}}{9.406}$$

Internal standard solution—A solution of acetonitrile (6 in 100).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass tube about 3 mm in inside diameter and about 1.5 m in length, packed with 150- to 180-μm porous ethylvinylbenzene-divinylbenzene copolymer (mean pore size: 0.0075 μm, 500 – 600 m^2/g) for gas chromatography.

Column temperature: A constant temperature between 105°C and 115°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethanol is 5 to 10 minutes.

Selection of column: Proceed with 1 mL of the gas obtained from the standard solution in the bottle under the above operating conditions, and calculate the resolution. Use a column giving elution of ethanol and the internal standard in this order with the resolution between these peaks being not less than 2.0.

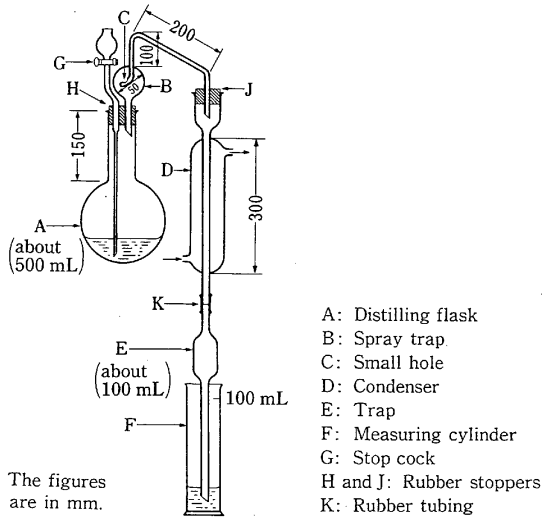
3. Ammonium Limit Test

The Ammonium Limit Test is a limit test for ammonium contained in drugs.

In each monograph, the permissible limit for ammonium (as NH_4^+) is described in terms of percentage (%) in parentheses.

Apparatus

Use a hard glass apparatus as illustrated in the figure; ground-glass joints may be used. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and for 30 to 60 minutes in water, and finally washed thoroughly with water before use.



Procedure

(1) Preparation of test solution and control solution—Unless otherwise specified, test solutions and control solution are prepared as directed in the following.

Place an amount of the sample, directed in the monograph, in the distilling flask A. Add 140 mL of water and 2 g of magnesium oxide, and connect the distillation apparatus. To the receiver F add 20 mL of boric acid solution (1 in 200) as an absorbing solution, and immerse the lower end of the condenser. Adjust the heating to give a rate of 5 to 7 mL per minute of distillate, and distill until the distillate measures 60 mL. Remove the receiver from the lower end of the condenser, rinsing the end part with a small quantity of water, add sufficient water to make 100 mL and designate it as the test solution.

Place a volume of Standard Ammonium Solution, directed in the monograph, in the distilling flask A, proceed as for the preparation of the test solution, and designate it as the control solution.

(2) Test of the test solution and the control solution—Unless otherwise specified, proceed as directed in the following.

Place 30 mL each of the test solution and the control solution in Nessler tubes, add 6.0 mL of phenol-sodium pentacyanonitrosylferrate (III) TS to each solution, and mix. Then add 4 mL of sodium hypochlorite-sodium hydroxide TS and water to make 50 mL, mix, and allow to stand for 60