

Wine

ブドウ酒

Wine is an alcoholic liquid obtained by fermenting the juice of the fruits of *Vitis vinifera* Linné (*Vitaceae*) or allied plants.

It contains not less than 11 vol% and not more than 14 vol% of ethanol (C_2H_6O : 46.07) (by specific gravity), and not less than 0.10 w/v% and not more than 0.40 w/v% of L-tartaric acid ($C_4H_6O_6$: 150.09).

It contains no artificial sweetener and no artificial coloring agent.

Description Wine is a light yellow or reddish purple to red-purple liquid. It has a characteristic and aromatic odor. It has a slightly astringent and faintly irritating taste.

Specific gravity d_{20}^{20} : 0.990 – 1.010

Optical rotation Boil 160 mL of Wine, neutralize with potassium hydroxide TS, and concentrate to 80 mL on a water bath. Cool, dilute with water to 160 mL, add 16 mL of lead subacetate TS, shake well, and filter. To 100 mL of the filtrate add 10 mL of a saturated solution of sodium sulfate decahydrate, shake well, filter, and use the filtrate as the sample solution. Allow 20 mL of the sample solution to stand for 24 hours, add 0.5 g of activated charcoal, shake, stopper, and allow to stand for 10 minutes. Filter, and observe the optical rotation of the filtrate in a 200-mm cell. Multiply the optical rotation observed by 1.21, and designate as the optical rotation of Wine: it is between -0.3° and $+0.3^\circ$.

Purity (1) Total acid [as L-tartaric acid ($C_4H_6O_6$)]—To exactly 10 mL of Wine add 250 mL of freshly boiled and cooled water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 1 mL of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 7.504 mg of $C_4H_6O_6$

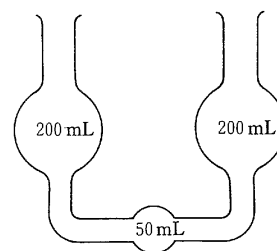
Total acid is not less than 0.40 w/v% and not more than 0.80 w/v%.

(2) Volatile acid [as acetic acid ($C_2H_4O_2$: 60.05)]—Transfer 100 mL of Wine to a beaker, add 1 mL of 1 mol/L sodium hydroxide VS and the same volume of 1 mol/L sodium hydroxide VS as that of 0.1 mol/L sodium hydroxide VS titrated in (1) to make the solution alkaline, and concentrate to 50 mL on a water bath. Cool, add water to make 100 mL, transfer to a 1000-mL distillation flask, containing previously added 100 g of sodium chloride. Wash the beaker with 100 mL of water, and combine the washings in the distillation flask. Add 5 mL of a solution of L-tartaric acid (3 in 20), and distil with steam cautiously to maintain the volume of the solution in the flask until 450 mL of the distillate is obtained for 45 minutes. Dilute the distillate to exactly 500 mL with water, and use this solution as the sample solution. Titrate a 250-mL portion of the sample solution with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 6.005 mg of $C_2H_4O_2$

The volatile acid is not more than 0.15 w/v%.

(3) Sulfur dioxide—Stopper a 750-mL round-bottomed flask with a stopper having two holes. Through one hole, insert a glass tube A extending nearly to the bottom of the flask. Through the other hole, insert a glass tube B ending to the neck of the flask. Connect the tube B to a Liebig's condenser, and the end of the condenser to a joint of which inner diameter is 5 mm at the lower end. Connect the other end of the joint with a holed rubber stopper to a U tube having three bulbs as shown in the Figure. Pass carbon dioxide



washed with a solution of potassium permanganate (3 in 100) through the tube A. Displace the air in the apparatus by carbon dioxide, and place 50 mL of a freshly prepared and diluted starch TS (1 in 5) and 1 g of potassium iodide in the U tube. From the other end of the U tube, add 1 to 2 drops of 0.01 mol/L iodine VS from a burette. While passing carbon dioxide, remove the stopper of the flask a little, add exactly 25 mL of Wine, 180 mL of freshly boiled and cooled water, 0.2 g of tannic acid, and 30 mL of phosphoric acid, and stopper again. Pass carbon dioxide for further 15 minutes, heat the distillation flask with caution so that 40 to 50 drops of the distillate may be obtained in 1 minute. When the color of starch TS in the U tube is discharged, add 0.01 mol/L iodine VS dropwise from a burette so that the color of the starch TS remains light blue to blue during the distillation. Read the volume of 0.01 mol/L iodine VS consumed when exactly 60 minutes have passed after the beginning of distillation. In this case, however, the coloration of starch TS produced by 1 drop of 0.01 mol/L iodine VS should persist at least for 1 minute.

Each mL of 0.01 mol/L iodine VS = 0.6406 mg of SO_2

The amount of sulfur dioxide (SO_2 : 64.06) does not exceed 7.5 mg.

(4) Total sulfuric acid—Transfer 10 mL of Wine to a beaker, boil, and add 50 mL of a solution prepared by dissolving 5.608 g of barium chloride dihydrate in 50 mL of hydrochloric acid and water to make 1000 mL. Cover the beaker, and heat on a water bath for 2 hours, supplying the water lost by distillation. Cool, centrifuge, and decant the supernatant liquid in another beaker. To this solution add 1 to 2 drops of dilute sulfuric acid, and allow to stand for 1 hour: a white precipitate is formed.

(5) Arsenic—Evaporate 10 mL of Wine on a water bath to dryness. Prepare the test solution with the residue according to Method 3, and perform the test using Apparatus B (not more than 0.2 ppm).

(6) Glycerin—Pipet 100 mL of Wine into a 150-mL porcelain dish, and concentrate on a water bath to 10 mL. Add 1 g of sea sand (No. 1), and make the solution strongly alkaline by adding a solution prepared by dissolving 4 g of calcium hydroxide in 6 mL of water. Heat on a water bath with

constant stirring and pushing down any material adhering to the wall of the dish until the contents of the dish become soft masses. Cool, add 5 mL of ethanol (99.5), and grind to a grue-like substance. Heat on a water bath, add 10 to 20 mL of ethanol (99.5) while agitating, boil, and transfer to a 100-mL volumetric flask. Wash the dish with seven 10-mL portions of hot ethanol (99.5), combine the washings with the contents of the flask, cool, and add ethanol (99.5) to make exactly 100 mL. Filter through a dry filter paper, evaporate 90 mL of the filtrate on a water bath, taking care not to boil the solution during the evaporation. Dissolve the residue in a small amount of ethanol (99.5), transfer to a 50-mL glass-stoppered volumetric cylinder, wash with several portions of ethanol (99.5), and add the washings to the solution in the cylinder to make 15 mL. Add three 7.5-mL portions of dehydrated diethyl ether, shake vigorously each time, and allow to stand. When the solution becomes quite clear, transfer to a tared, flat weighing bottle. Wash the volumetric cylinder with 5 mL of a mixture of dehydrated diethyl ether and ethanol (99.5) (3:2). Transfer the washings to the weighing bottle, and evaporate carefully on a water bath. When the liquid becomes sticky, dry at 105°C for 1 hour, and cool in a desiccator (silica gel), and weigh: the mass of the residue is not less than 0.45 g and not more than 0.90 g.

(7) Reducing sugars—To a 25-mL portion of the sample solution obtained in the Optical rotation add 50 mL of boiling Fehling's TS, and heat for exactly 2 minutes. Filter the separated precipitates by a tared filter containing asbestos mat by suction, wash successively with hot water, with ethanol (95) and with diethyl ether, and continue to dry the precipitates by suction. Heat the filter gently at first, and then strongly until the precipitates become completely black. Cool the precipitates in a desiccator (silica gel), and weigh as copper (II) oxide: the mass of cupric oxide does not exceed 0.325 g.

(8) Sucrose—Transfer a 50-mL portion of the sample solution obtained in the Optical rotation to a 100-mL flask, neutralize with diluted hydrochloric acid (1 in 30), followed by further addition of 5 mL of diluted hydrochloric acid (1 in 30). Heat in a water bath for 30 minutes, cool, neutralize with a solution of potassium hydroxide (1 in 100), add 4 drops of sodium carbonate TS, filter into a 100-mL volumetric flask, wash with water, combine the washings with the filtrate, and add water to make 100 mL. To 25 mL of this solution add 50 mL of boiling Fehling's TS, and proceed as directed in (7), and weigh as copper (II) oxide. From the number obtained by multiplying the mass (g) of copper (II) oxide by 2, deduct the amount (g) of copper (II) oxide determined in (7), and multiply again the number so obtained by 1.2: the number obtained does not exceed 0.104 (g).

(9) Benzoic acid, cinnamic acid and salicylic acid—Transfer exactly 50 mL of the sample solution obtained in (2) to a separator, add 10 g of sodium chloride and 2 mL of dilute hydrochloric acid, and extract with three 10-mL portions of diethyl ether. Combine the diethyl ether extracts, wash with two 5-mL portions of water, and extract with three 10-mL portions of 0.1 mol/L sodium hydroxide VS. Combine the alkaline extracts, evaporate the diethyl ether by warming on a water bath, cool, neutralize with 1 mol/L hydrochloric acid VS, and add 5 mL of potassium chloride-hydrochloric acid buffer solution and water to make exactly 50 mL. Perform the test as directed under the Ultraviolet-visible Spectrophotometry with this solution, using a solution prepared

in the same manner instead of the sample solution as the blank: the absorbance does not exceed 0.15 at a wavelength between 220 nm and 340 nm.

(10) Boric acid—Transfer 50 mL of Wine to a porcelain dish, add 5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite: a half portion of the residue does not respond to Qualitative Tests (1) for borate. Dissolve another half portion of the residue in 5 mL of hydrochloric acid: it does not respond to Qualitative Tests (2) for borate.

(11) Methanol—Wine meets the requirements of the Methanol Test, when proceeding with exactly 1 mL of ethanol layer obtained by Method 1 of the Alcohol Number Determination and distilling without adding water after shaking with 0.5 g of calcium carbonate.

(12) Formaldehyde—To 25 mL of Wine add 5 g of sodium chloride and 0.2 g of L-tartaric acid, distil, and obtain 15 mL of the distillate. To 5 mL of the distillate add 5 mL of acetyl acetone TS, mix, and heat on a water bath for 10 minutes: the solution has no more color than that of the following control solution.

Control solution: Using 5 mL of water instead of the distillate, perform the test in the same manner.

Extract content 1.9 – 3.5 w/v% Pipet 25 mL of Wine to a 200-mL tared beaker containing 10 g of sea sand (No. 1), previously dried at 105°C for 2.5 hours, and evaporate to dryness on a water bath. Dry the residue at 105°C for 2 hours, cool in a desiccator (silica gel), and weigh.

Total ash 0.13 – 0.40 w/v% Pipet 50 mL of Wine to a tared porcelain dish, and evaporate to dryness on a water bath. Ignite the residue to constant mass, cool, and weigh.

Assay (1) Ethanol—Pipet Wine into a 100-mL volumetric flask at 15°C, transfer to a 300- to 500-mL flask, and wash this volumetric flask with two 15-mL portions of water. Add the washings to the sample in the flask, connect the flask to a distillation tube having a trap, and distil using the volumetric flask as a receiver. When about 80 mL of the distillate is obtained (it takes about 20 minutes), stop the distillation, allow to stand in water at 15°C for 30 minutes, and add water to make exactly 100 mL. Shake well, and determine the specific gravity at 15°C according to Method 3 under the Specific Gravity: the specific gravity d_{15}^{15} is between 0.982 and 0.985.

(2) L-Tartaric acid—Pipet 100 mL of Wine, add 2 mL of acetic acid (100), 0.5 mL of a solution of potassium acetate (1 in 5) and 15 g of powdered potassium chloride, and shake vigorously to dissolve as much as possible. Add 10 mL of ethanol (95), rub the inner wall of the beaker strongly for 1 minute to induce the crystallization, and allow to stand between 0°C and 5°C for more than 15 hours. Filter the crystals by suction, wash successively the beaker and the crystals with 3-mL portions of a solution prepared by dissolving 15 g of powdered potassium chloride in 120 mL of diluted ethanol (1 in 6), and repeat the washings five times. Transfer the crystals together with the filter paper to a beaker, wash the filter with 50 mL of hot water, combine the washings in the beaker, and dissolve the crystals by heating. Titrate the solution with 0.2 mol/L sodium hydroxide VS immediately (indicator: 1 mL of phenolphthalein TS). The number obtained by adding 0.75 to the amount (mL) of 0.2 mol/L sodium hydroxide VS consumed represents the amount (mL) of 0.2 mol/L sodium hydroxide VS consumed.

Each mL of 0.2 mol/L sodium hydroxide VS
= 30.018 mg of C₄H₆O₆

Containers and storage Containers—Tight containers.

Dried Yeast

乾燥酵母

Dried Yeast is dried and powdered cells of yeast belonging to *Saccharomyces*.

Dried Yeast contains not less than 400 mg of protein and not less than 100 µg of thiamine compounds [as thiamine hydrochloride (C₁₂H₁₇ClN₄OS.HCl: 337.27)] in each 1 g.

Description Dried Yeast occurs as a light yellowish white to brown powder. It has a characteristic odor and taste.

Identification Dried Yeast, when examined under a microscope, shows isolated cells, spheroidal or oval in shape, and 6 to 12 µm in length.

Purity (1) Rancidity—Dried Yeast is free from any unpleasant or rancid odor or taste.

(2) Starch—Add iodine TS to Dried Yeast, and examine microscopically: no or only a few granules are tinted blackish purple.

Loss on drying Not more than 8.0% (1 g, 100°C, 8 hours).

Total ash Not more than 9.0% (1 g, proceed as directed in the Total ash under the Crude Drugs).

Assay (1) Protein—Weigh accurately about 0.05 g of Dried Yeast and perform the test as directed under the Nitrogen Determination.

$$\begin{aligned} &\text{Amount (mg) of protein in 1 g of Dried Yeast} \\ &= \text{amount (mg) of nitrogen (N)} \\ &\quad \times 6.25 \times \frac{1}{\text{amount (g) of sample}} \end{aligned}$$

(2) Thiamine—Weigh accurately about 1 g of Dried Yeast, add 1 mL of dilute hydrochloric acid and 80 mL of water, and heat in a water bath at 80°C to 85°C for 30 minutes with occasional shaking. After cooling, add water to make exactly 100 mL, and centrifuge for 10 minutes. Pipet 4 mL of the supernatant liquid, add exactly 5 mL of acetic acid-sodium acetate TS and exactly 1 mL of enzyme TS, and allow to stand at 45°C to 50°C for 3 hours. Place exactly 2 mL of this solution onto a chromatographic column prepared by pouring 2.5 mL of a weakly acidic CM-bridged cellulose cation exchanger (H type) (40 to 110 µm in particle diameter) into a chromatographic tube about 1 cm in inside diameter and about 17 cm in length, and elute at the flow rate of about 0.5 mL per minute. Wash the upper part of the column with a small amount of water, and wash the column with two 10-mL portions of water at the flow rate of about 1 mL per minute. Elute the column with two 2.5-mL portions of diluted phosphoric acid (1 in 50) at the flow rate of about 0.5 mL per minute, and combine the eluate. To the eluate add exactly 1 mL of the internal standard solution and 0.01 g of sodium 1-octanesulfonate, and after dissolving, use this

solution as the sample solution. Separately, weigh accurately about 0.015 g of the Thiamine Hydrochloride Reference Standard (determine the water content in the same manner as for Thiamine Hydrochloride), dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 1 mL of the internal standard solution and 3 mL of the mobile phase, and use this solution as the standard solution. Perform the test with 200 µL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of thiamine to that of the internal standard.

$$\begin{aligned} &\text{Amount (µg) of thiamine in 1 g of Dried Yeast} \\ &= \text{amount (mg) of Thiamine Hydrochloride Reference} \\ &\quad \text{Standard, calculated on the anhydrous basis} \\ &\quad \times \frac{Q_T}{Q_S} \times \frac{1}{\text{amount (g) of the sample}} \times 12.5 \end{aligned}$$

Internal standard solution—Dissolve 0.01 g of phenacetin in acetonitrile to make 100 mL, and to 1 mL of this solution add diluted acetonitrile (1 in 5) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilylanized silica gel for liquid chromatography (5 to 10 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.7 g of potassium dihydrogenphosphate in 1000 mL of water, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). Dissolve 1.6 g of sodium 1-octanesulfonate in 800 mL of this solution, and add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of thiamine is about 8 minutes.

Selection of column: Proceed with 200 µL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of thiamine and the internal standard in this order with the resolution between these peaks being not less than 8.

Containers and storage Containers—Tight containers.

Zanthoxylum Fruit

Zanthoxyli Fructus

サンショウ

Zanthoxylum Fruit is the pericarps of the ripe fruit of *Zanthoxylum piperitum* De Candolle (*Rutaceae*), from which the seeds separated from the pericarps have been mostly removed.

Description Capsules of 2 or 3 flattened spheroidal mericarps, which are dehiscent in 2 pieces about 5 mm in diameter; the outer surface of pericarp, dark yellow-red to