specimen holder made of aluminum or glass. As a rule, the orientation of sample crystallites have to be randomized before packaging. The specimen may be ground in an agate mortar to a fine powder in order to randomize the orientation of crystallites. However, this grinding method is sometimes inappropriate due to the physical characteristics of a specimen or the measurement object.

In setting up the specimen and apparatus, coplanarity of the specimen surface with the specimen holder surface and the setting of the specimen holder at the position of symmetric reflection geometry have to be assured. Further it should be noted that the grinding procedure may affect the crystallinity of the specimen and the packaging pressure on the specimen holder may induce orientation of the crystallites.

Identification and/or Judgement

Identification of the specimen with the standard material can be accomplished by comparing the X-ray powder diffraction patterns with each other. Judgement of polymorphism and crystalline solvates can be done by comparison of the diffraction pattern obtained for the specimen with that of the reference material or the same material measured previously.

Comparison of two X-ray diffraction patterns should be based on the intensity ratio of diffracted peaks, and the interplanar spacings d. The intensity ratio is defined by the ratio of the peak intensity of a particular diffraction angle to the intensity of the standard peak, for which the strongest maxima in the diffraction pattern is usually selected. However, the diffraction angle 2θ can be used as a basis for the identification, where the same wavelength of the radiation beam is utilized for the diffraction measurement of the sample and reference material. The scanning angle range for diffraction measurement is usually between 5° and 40° for ordinary organic substances, except where it is specified in particular *Monographs*. Based on the obtained X-ray diffraction patterns, the identification of a specimen with a standard material can be confirmed, if the diffraction pattern for the specimen gives diffraction peaks of the same intensity at the same diffraction angle 2θ, as those of the standard. If two powder crystallites ascribed to the same substance have the same crystal form, the X-ray diffraction angles should agree within $\pm$ 0.2°.

Assay

A quantitative analysis by X-ray powder diffraction does not give a sufficiently precise result. Thus, the quantitative application of this method is limited to a few analytical problems: numerical estimation of degree of polymorphism, solvation number for crystalline solvates, and degree of crystallinity.

For a quantitative analysis of polymorphism and/or solvate, an appropriate diffraction peak has to be selected. Usually, the calibration curve method can be applied to the quantitative estimation by the X-ray analysis. Before measurement of the diffraction intensity for a sample specimen at a selected diffraction peak, a calibration curve must be prepared under the same conditions, using a series of standard samples containing known amounts of the objective substance.

Alternatively the internal standard method can also be effective in place of the above standard method. A known amount of internal standard is usually added to weighed amounts of a sample to be analyzed. Diffraction intensity ratios of the specimen to the internal standard are measured. Separately, a calibration curve for the intensity ratio against the mixing ratio of the reference material to the internal standard are prepared under the same conditions. By using the calibration curve, a quantitative analysis is possible in X-ray powder diffraction measurement. If more than two diffraction peaks ascribed to different lattice planes (hkl) are used, the influence of orientation of crystallites can be detected. The internal standard should have approximately the same density as the specimen and similar absorption characteristics with regard to the X-ray beam. Further the diffraction peak given by the standard should not overlap with that of the specimen to be analyzed.

Caution: Handle the apparatus with great care since X-ray may affect the human health.

70. Reference Standards; Reagents, Test Solutions; Standard Solutions for Volumetric Analysis; Standard Solutions; Matching Fluids for Color; Optical Filters for Wavelength and Transmission Rate Calibration; and Measuring Instruments, Appliances

Reference standards are substances which are prepared to have definite purity or definite biological action, and are used when drugs are tested physically, chemically or biologically.

Reagents are chemicals which are used in the tests of the Japanese Pharmacopoeia. Those reagents described as standard substances for volumetric analysis, special class, first class or for water determination in the Pharmacopoeia conform to the specifications of standard reagents for volumetric analysis, special class ones, first class ones, or for water determination ones of the Japanese Industrial Standards, respectively, and the testing procedures refer to the Japanese Industrial Standards. When the name of a reagent used in the Pharmacopoeia differs from that used in the Japanese Industrial Standards, the latter is shown together. When there appears the indication, "Same as the namesake monograph," the reagent conforms to the specification of the individual monograph. As to reagents for which the testing procedures are given, proceed as directed for the testing procedures of the Pharmacopoeia.

Test solutions are solutions which are prepared to be used in the tests of the Pharmacopoeia.

Standard solutions for volumetric analysis are solutions of reagents of precisely known concentrations intended primarily for use in quantitative determinations.

Standard solutions are solutions to be used as the bases for comparison in the tests of the Pharmacopoeia.

Matching fluids for color are used as references for comparison of colors in the tests of the Pharmacopoeia.

Measuring instruments are instruments or machines used for the measurements in the tests of the Pharmacopoeia.

Appliances are instruments designed to render the condi-

tions of the tests as constant as possible in the tests of the Pharmacopoeia.

(1) Reference Standards

The Japanese Pharmacopoeia Reference Standards are as follows:

Acetaminophene, Alprostadil, Amikacin Sulfate, *p*-Aminobenzoyl Glutamic Acid, Amitriptyline Hydrochloride, Amoxicillin, Amphotericin B, Ampicillin, Anhydrous Lactose, Ascorbic Acid, Aspirin, Aspoxicillin, Atropine Sulfate, Azathioprine, Aztreonam, Bacampicillin Hydrochloride, Baclofen, Baicalin, Berberine Chloride, Betamethasone, Betamethasone Dipropionate, Betamethasone Sodium Phosphate, Betamethasone Valerate, Bisacodyl, Caffeine, Calcium Folinate, Calcium Oxalate Monohydrate, Camostat Mesilate, *d*-Camphor, *dl*-Camphor, Carbidopa, Cefadroxil, Cefalexin, Cefapirin Sodium, Cefatrizine Propylene Glycolate, Cefazolin, Cefcapene Pivoxil Hydrochloride, Cefdinir, Cefditoren Pivoxil, Cefepime Dihydrochloride, Cefetamet Pivoxil Hydrochloride, Cefixime, Cefmetazole, Cefminox Sodium, Cefoperazone, Cefoselis Sulfate, Cefotiam Hydrochloride, Cefozopran Hydrochloride, Cefpirome Sulfate, Cefradine, Cefsulodin Sodium, Ceftazidime, Ceftibuten Hydrochloride, Ceftizoxime, Ceftriaxone Sodium, Cefuroxime Sodium, Cellulose Phthalate Acetate, Chlordiazepoxide, Chlormadinone, Chlorpheniramine Maleate, Cholecalciferol, Chorionic Gonadotrophin, Clarithromycin, Cloxacillin Sodium, Ciclosporin, Clofibrate, Clomifene Citrate, Colistin Sodium Methanesulfonate, Cortisone Acetate, Cyanocobalamin, Cycloserine, Deferoxamine Mesilate, Deslanoside, Dexamethasone, Diclofenamide, Dicloxacillin Sodium, Diethylcarbamazine Citrate, Digitalis, Digitoxin, Digoxin, Dihydroergotoxine Mesilate, Dobutamine Hydrochloride, Drostanolone Propionate, Edrophonium Chloride, Elcatonin, Endotoxin 100, Endotoxin 10000, Epinephrine Bitartrate, Epitiostanol, Ergocalciferol, Ergometrine Maleate, Erythromycin, Estradiol Benzoate, Estriol, Ethenzamide, Ethinyl Estradiol, Ethyl Aminobenzoate, Faropenem Sodium, Fluocinolone Acetonide, Fluocinonide, Fluorometholone, Fluoxymesterone, Folic Acid, Fosfestrol, Fosfomycin Phenethylammonium, Fursultiamine Hydrochloride, Gabexate Mesilate, Gitoxin, Glycyrrhizinic Acid, Guaifenesin, Heparin Sodium, High Molecular Mass Urokinase, Human Insulin, Hydrochlorothiazide, Hydrocortisone, Hydrocortisone Acetate, Hydrocortisone Sodium Phosphate, Hydrocortisone Succinate, Hydroxypropylmethylcellulose Phthalate, Idarubicin Hydrochloride, Idoxuridine, Imipramine Hydrochloride, Indomethacin, Insulin, Isepamicin Sulfate, Josamycin, Kallidinogenase, Kitasamycin, Lactose, Lactulose, Lanatoside C, Lithium Clavulanate, Loxoprofen, Maltose, Mecobalamin, Menatetrenone, Meropenem Trihydrate, Mestranol, Methotrexate, Methoxsalen, Methyldopa, Methyltestosterone, Metildigoxin, Mexiletine Hydrochloride, Midecamycin, Midecamycin Acetate, Minocycline Hydrochloride, Mupirocin Lithium, Neostigmine Methylsulfate, Netilmicin Sulfate, Nicotinamide, Nicotinic Acid, Norepinephrine Bitartrate, Norgestrel, Nystatin, Paeoniflorin, Panipenem, Perphenazine, Piperacillin, Posterior Pituitary, Potassium Sucrose Octasulfate, Povidone, Prednisolone, Prednisolone Acetate, Prednisolone Succinate, Primidone, Probeneside, Prochlorperazine Maleate, Progesterone, Protamine Sulfate, Pyridoxine Hydrochloride, Reserpine, Retinol Acetate for Thin-layer Chromatography, Retinol Palmitate for Thin-layer Chromatography, Riboflavin, Rokitamycin, Roxithromycin, Saccharated Pepsin, Scopolamine Hydrobromide, Secretin, Serum Gonadotrophin, Sisomicin Sulfate, Spironolactone, Sulbactam, Sulfadiazine Silver, Sulfinpyrazone, Sultamicillin Tosilate, Swertiamarin, Teicoplanin, Testosterone Propionate, Tetracycline Hydrochloride, Thiamine Hydrochloride, Thrombin, Ticarcillin Sodium, Tocopherol, Tocopherol Acetate, Tocopherol Nicotinate, Tocopherol Succinate, Tolazamide, Tolbutamide, Tolnaftate, Triamcinolone, Triamcinolone Acetonide, Trihexyphenidyl Hydrochloride, Tubocurarine Chloride, Tyrosine, Ubidecarenone, Ulinastatin, Vinblastine Sulfate, Zinostatin Stimalamer.

(2) Reagents, Test Solutions

Absorbent cotton [Same as the namesake monograph in Part II]

Absorbent gauze [Same as the namesake monograph in Part II]

Acenaphthene $C_{12}H_{10}$ White to pale yellowish white crystals or crystalline powder, having a characteristic aroma. Freely soluble in diethyl ether and in chloroform, soluble in acetonitrile, sparingly soluble in methanol, and practically insoluble in water.

Identification—Determine the infrared absorption spectrum of acenaphthene according to the paste method under the Infrared Spectrophotometry, with 5 mg of acenaphthene: it exhibits absorption at the wave numbers of about 1605 cm^{-1}, 840 cm^{-1}, 785 cm^{-1} and 750 cm^{-1}.

Melting point: 93 - 96°C

Purity—Dissolve 0.1 g of acenaphthene in 5 mL of chloroform, and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under the Gas Chromatography according to the following conditions. Measure each peak area by the automatic integration method, and calculate the amount of acenaphthene by the area percentage method: it shows a purity of not less than 98.0%.

Operating conditions

Detector: Hydrogen flame-ionization detector

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with 150- to 180-μm siliceous earth for gas chromatography coated with 10% of polyethylene glycol 20 M.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen

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

Detection sensitivity: Adjust the detection sensitivity so that the peak height of acenaphthene obtained from 2 μL of the solution prepared by adding chloroform to 1.0 mL of the sample solution to make 100 mL is 5% to 15% of the full scale.

Time span of measurement: About 3 times as long as the

retention time of acenaphthene after the solvent peak.

Residue on ignition—Not more than 0.10% (1 g).

Acetaldehyde CH_3CHO [K 8030, First class]

Acetaldehyde for assay Distil 100 mL of acetaldehyde under reduced pressure, discard the first 20 mL of the distillate, and use the subsequent. Prepare before use.

Acetanilide $C_8H_9NO_2$ White, crystals or crystalline powder.

Melting point: 114 - 117°C

***p*-Acetanisidide** $C_9H_{11}NO_2$ White to purplish white, crystals or crystalline powder, having a characteristic odor.

It is freely soluble in ethanol (95) and in acetonitrile, and very slightly soluble in water.

Melting point: 126 - 132°C

Content: not less than 98.0%. Assay—Dissolve 0.1 g of *p*-acetanisidide in 5 mL of chloroform. Perform the test with 2 μL of this solution as directed under the Gas Chromatography according to the following conditions, and determine the area of each peak by the automatic integration method.

$$\text{Content} = \frac{\text{peak area of } p\text{-acetanisidide}}{\text{total of all peak areas}} \times 100$$

Operating conditions

Detector: Hydrogen flame-ionization detector

Column: A glass tube 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography coated with alkylene glycol phthalate ester for gas chromatography in 1% (177–250 μm in particle diameter).

Column temperature: A constant temperature of about 210°C

Carrier gas: Nitrogen

Flow rate: Adjust to a constant flow rate of between 30 and 50 mL per minute and so that the retention time of *p*-acetanisidide is between 11 and 14 minutes.

Time span of measurement: About 3 times as long as the retention time of *p*-acetanisidide after the solvent peak.

Acetate buffer solution, pH 4.5 Dissolve 63 g of anhydrous sodium acetate in a suitable amount of water, add 90 mL of acetic acid (100) and water to make 1000 mL.

Acetate buffer solution, pH 5.5 Dissolve 2.72 g of soduim acetate trihydrate in water to make 1000 mL, and adjust the pH to 5.5 with diluted acetic acid (100) (3 in 2500).

Acetic acid See acetic acid (31).

Acetic acid-ammonium acetate buffer solution, pH 3.0 Add acetic acid (31) to ammonium acetate TS, and adjust the pH to 3.0.

Acetic acid-ammonium acetate buffer solution, pH 4.5 Dissolve 77 g of ammonium acetate in about 200 mL of water, adjust the pH to 4.5 by adding acetic acid (100), and add water to make 1000 mL.

Acetic acid-ammonium acetate buffer solution, pH 4.8 Dissolve 77 g of ammonium acetate in about 200 mL of water, and add 57 mL of acetic acid (100) and water to make 1000 mL.

Acetic acid, dilute Dilute 6 g of acetic acid (100) with water to make 100 mL (1 mol/L).

Acetic acid for nonaqueous titration [K 8355, Special class. meeting with following requirement.]

Purity Acetic anhydride—Dissolve 1.0 g of aniline in acetic acid for nonaqueous titration to make 100 mL, and use this solution as the sample solution. Pipet 25 mL of the sample solution, titrate with 0.1 mol/L perchloric acid VS, and designate the consumed volume as A (mL). A is not less than 26 mL. Pipet 25 mL of the sample solution, add 75 mL of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid VS, and designate the consumed volume as B (mL) (potentiometric titration). A − B is not more than 0.1 (mL) (not more than 0.001 g/dL).

Acetic acid, glacial See acetic acid (100).

Acetic acid-potassium acetate buffer solution, pH 4.3 Dissolve 14 g of potassium acetate in 20.5 mL of acetic acid (100), and add water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, pH 4.0 Dissolve 5.44 g of sodium acetate trihydrate in 900 mL of water, adjust the pH to 4.0 by adding acetic acid (100) dropwise, and add water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, pH 4.5 To 80 mL of sodium acetate TS add 120 mL of dilute acetic acid and water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, pH 4.5, for iron limit test Dissolve 75.4 mL of acetic acid (100) and 111 g of sodium acetate trihydrate in 1000 mL of water.

Acetic acid-sodium acetate buffer solution, pH 4.7 Dissolve 27.2 g of sodium acetate trihydrate in 900 mL of water, adjust the pH to 4.7 by adding acetic acid (100) dropwise, and add water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, pH 5.0 To 140 mL of sodium acetate TS add 60 mL of dilute acetic acid and water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, pH 5.5 Dissolve 20 g of sodium acetate trihydrate in 80 mL of water, adjust the pH to 5.5 by adding acetic acid (100) dropwise, and add water to make 100 mL.

Acetic acid-sodium acetate buffer solution, pH 5.6 Dissolve 12 g of sodium acetate trihydrate in 0.66 mL of acetic acid (100) and water to make 100 mL.

1 mol/L Acetic acid-sodium acetate buffer solution, pH 5.0 To sodium acetate TS add dilute acetic acid, and adjust the pH to 5.0.

0.1 mol/L Acetic acid-sodium acetate buffer solution, pH 4.0 Dissolve 13.61 g of soduim acetate trihydrate in 750 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL.

Acetic acid-sodium acetate TS Mix 17 mL of 1 mol/L sodium hydroxide VS with 40 mL of dilute acetic acid, and add water to make 100 mL.

6 mol/L Acetic acid TS Dilute 36 g of acetic acid (100) with water to make 100 mL.

Acetic acid (100) CH_3COOH [K 8355, Acetic Acid, Special class]

Acetic acid (100)-sulfuric acid TS To 5 mL of acetic acid (100) add cautiously 5 mL of sulfuric acid while cooling in an ice bath, and mix.

Acetic acid (31) Dilute 31.0 g of acetic acide (100) with water to make 100 mL (5 mol/L).

Acetic anhydride $(CH_3CO)_2O$ [K 8886, Special class]

Acetic anhydride-pyridine TS Place 25 g of acetic anhydride in a 100 mL volumetric flask, add pyridine to make 100 mL, and mix well. Preserve in light-resistant containers, protected from air. This solution may be used even if it becomes colored during storage.

Acetone CH_3COCH_3 [K 8034, Special class]

Acetone for nonaqueous titration Add potassium permanganate to acetone in small portions, and shake. When the mixture keeps its purple color after standing for 2 to 3 days, distil, and dehydrate with freshly ignited anhydrous potassium carbonate. Distil by using a fractionating column under protection from moisture, and collect the fraction distilling at 56°C.

Acetone for purity of crude drug [K 8034, Special class] Use acetone meeting the following additional specification. Evaporate 300.0 mL of acetone to be tested in vacuum at a temperature not higher than 40°C, add the acetone to make exactly 1 mL, and use this solution as the sample solution. Separately, dissolve 2.0 mg of γ-BHC in hexane for purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, and add hexane for purity of crude drug to make exactly 100 mL. Further pipet 2 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution I. Perform the test with 1 μL each of the sample solution and the standard solution I as directed under the Gas Chromatography according to the following operating conditions, and determine each peak area by the automatic integration method: the total area of peaks other than the solvent peak from the sample solution is not larger than the peak area of γ-BHC from the standard solution I.

Operating conditions

Proceed the operating conditions in the Purity (3) under Powdered Ginseng except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution I, add hexane for purity of crude drug to make exactly 20 mL, and use this solution as the standard solution II. Adjust the detection sensitivity so that the peak area of γ-BHC obtained from 1 μL of the standard solution II can be measured by the automatic integration method, and the peak height of γ-BHC from 1 μL of the standard solution I is about 20% of the full scale.

Time span of measurement: About three times as long as the retention time of γ-BHC after the solvent peak.

Acetonitrile for liquid chromatography CH_3CN Colorless and clear liquid. Mixable with water.

Purity Ultraviolet light absorbing substances—Determine the absorbances at the following wavelengths as directed under the Ultraviolet-visible Spectrophotometry, using water as the control: not more than 0.07 at 200 nm, not more than 0.046 at 210 nm, not more than 0.027 at 220 nm, not more than 0.014 at 230 nm and not more than 0.009 at 240 nm.

Acetonitrile CH_3CN [K 8032, Special class]

Acetrizoic acid $C_9H_6I_3NO_3$ White powder.

Purity Related substances—Dissolve 0.06 g of acetrizoic acid in a solution of meglumine (3 in 1000) to make 100 mL. To 10 mL of this solution add water to make 100 mL, and use this solution as the sample solution. Proceed the test with 5 μL of the sample solution as directed in the Assay under Meglumine Sodium Amidotrizoate Injection: any peaks other than the principal peak are not observed.

Acetylacetone $CH_3COCH_2COCH_3$ [K 8027, Special class]

Acetylacetone TS Dissolve 150 g of ammonium acetate in a sufficient quantity of water, and add 3 mL of acetic acid (100), 2 mL of acetylacetone and water to make 1000 mL. Prepare before use.

Acetylene See dissolved acetylene.

Acidic ferric chloride TS See iron (III) chloride TS, acidic.

Acidic potassium chloride TS See potassium chloride TS, acidic.

Acidic potassium permanganate TS See potassium permanganate TS, acidic.

Acidic stannous chloride TS See tin (II) chloride TS, acidic.

Acid-treated gelatin See gelatin, acid-treated.

Acrinol $C_{15}H_{15}N_3O.C_3H_6O_3.H_2O$ [Same as the namesake monograph]

Acrylamide $CH_2CHCONH_2$ Pale yellow crystalline powder.

Melting point: 83 - 86°C

Content: not less than 97.0%.

Activated alumina Aluminum oxide with specially strong adsorptive activity.

Activated charcoal [Same as the monograph in Part II, Medicinal Carbon]

Adipic acid $C_4H_8(COOH)_2$ White crystals or crystalline powder. Freely soluble in ethanol (95), and sparingly soluble in water.

Melting point: 151 - 154°C

Content: not less than 98.0%. Assay—Weigh accurately about 1 g of adipic acid, and 100 mL of water, dissolve by warming, cool, and titrate with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 73.07 mg of $C_6H_{10}O_4$

Agar [K 8263, Special class. Same as the monograph in Part II, Agar or Agar Powder. Loss on drying is not more than 15%.]

Agar medium, ordinary See ordinary agar medium.

Agar slant Dispense portions of about 10 mL of ordinary agar medium into test tubes, and sterilize by autoclaving. Before the medium congeals, allow to stand in a slanting position, and solidify. When the coagulating water is lost, reprepare by dissolving with the aid of heat.

Ajmaline for assay $C_{20}H_{26}N_2O_2$ [Same as the monograph Ajmaline. When dried, it contains not less than