tion responds to the *Thin-layer Chromatographic Identification Test* ⟨201⟩, the Standard solution being prepared at a concentration of 1 mg of USP Diatrizoic Acid RS per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

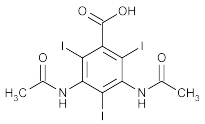
B: Evaporate a volume of Solution, equivalent to about 500 mg of diatrizoate sodium, to dr yness, and heat the residue so obtained in a suitable crucible: violet vapors are evolved.

pH ⟨791⟩: between 4.5 and 7.5.

Iodine and iodide—Using as the *Test preparation* a volume of Solution equivalent to 2.0 g of diatrizoate sodium and diluting it with water to 24 mL in a 50-mL centrifuge tube provided with a stopper, proceed as directed for *Procedure* in the test for *Iodine and iodide* under *Diatrizoate Meglumine*.

Assay—Pipet a volume of Solution, equivalent to about 400 mg of diatrizoate sodium, into a 125-mL conical flask. Add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the yellow precipitate just turns green. Each mL of 0.05 N silver nitrate is equivalent to 10.60 mg of $C_{11}H_8I_3N_2NaO_4$.

Diatrizoic Acid



$C_{11}H_9I_3N_2O_4$ (anhydrous) 613.91
Benzoic acid, 3,5-bis(acetylamino)-2,4,6-triiodo-.
3,5-Diacetamido-2,4,6-triiodobenzoic acid [117-96-4].
Dihydrate 649.95 [50978-11-5].

» Diatrizoic Acid is anhydrous or contains two molecules of water of hydration. It contains not less than 98.0 per cent and not more than 102.0 percent of $C_{11}H_9I_3N_2O_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers. Store at room temperature.

Labeling—Label it to indicate whether it is anhydrous or hydrous.

USP Reference standards ⟨11⟩**—**
USP Diatrizoic Acid RS
USP Diatrizoic Acid Related Compound A RS
5-Acetamido-3-amino-2,4,6-triiodobenzoic acid.
$C_9H_7I_3N_2O_3$ 571.88

Identification—

A: It responds to the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, the test solution and the Standard solution being prepared at a concentration of 1 mg per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

B: Heat about 500 mg in a suitable crucible: violet vapors are evolved.

Water, *Method I* ⟨921⟩**:** not more than 1.0% (anhydrous form), and between 4.5% and 7.0% (hydrous form).

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Iodine and iodide—

Test preparation—Suspend 10.0 g in 10 mL of water, and add in small portions, with stirring, 1.5 mL of sodium hydroxide solution (2 in 5). When solution is complete, adjust to a pH between 7.0 and 7.5 with a dilute solution (1 in 125) of sodium hydroxide or hydrochloric acid, and dilute with water to 20 mL.

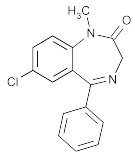
Procedure—Dilute 4.0 mL of *Test preparation* with 20 mL of water in a 50-mL centrifuge tube provided with a stopper, and proceed as directed for *Procedure* under *Diatrizoate Meglumine*.

Heavy metals ⟨231⟩**—**To a 50-mL color-comparison tube transfer 2.0 mL of solution prepared as directed for *Test preparation* in the test for *Iodine and iodide,* add 5 mL of 1 N sodium hydroxide, dilute with water to 40 mL, and mix. Using this as the *Test preparation,* proceed as directed in the test for *Heavy metals* under *Diatrizoate Meglumine:* the limit is 0.002%.

Free aromatic amine—Transfer 1.0 g to a 50-mL volumetric flask, and add 12.5 mL of water and 2.5 mL of 1 N sodium hydroxide. Proceed as directed in the test for *Free aromatic amine* under *Diatrizoate Meglumine,* beginning with "To a second 50-mL volumetric flask transfer 4 mL of water." The absorbance of the solution from the Diatrizoic Acid is not greater than that of the Standard solution (0.05%).

Assay—Transfer about 300 mg of Diatrizoic Acid, accurately weighed, to a glass-stoppered, 125-mL conical flask, and proceed as directed in the *Assay* under *Diatrizoate Meglumine,* beginning with "add 30 mL of 1.25 N sodium hydroxide." Each mL of 0.05 N silver nitrate is equivalent to 10.23 mg of $C_{11}H_9I_3N_2O_4$.

Diazepam



$C_{16}H_{13}ClN_2O$ 284.75
2*H*-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-1-methyl-5-phenyl-.
7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one [439-14-5].

» Diazepam contains not less than 95.0 per cent and not more than 105.0 per cent of $C_{16}H_{13}ClN_2O$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩**—**
USP Diazepam RS
USP Diazepam Related Compound A RS
2-Methylamino-5-chlorobenzophenone.
$C_{14}H_{12}ClNO$ 245.71
USP Diazepam Related Compound B RS
3-Amino-6-chloro-1-methyl-4-phenylcarbostyril.
$C_{16}H_{13}ClN_2O$ 284.74
USP Nordazepam RS
7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one.
$C_{15}H_{11}ClN_2O$ 270.72

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—

Test solution: 5 mg per mL, in acetone.

Developing solvent system: a mixture of ethyl acetate and *n*-heptane (1:1).

Procedure—Proceed as directed in the chapter except use an unsaturated developing chamber.

Melting range, *Class I* ⟨741⟩**:** between 131° and 135°.

Loss on drying ⟨731⟩**—**Dry it in vacuum over phosphorus pentoxide at 60° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Heavy metals, *Method II* ⟨231⟩**:** 0.002%.

Related compounds—

Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay*.

Standard solution—Dissolve accurately weighed quantities of USP Diazepam Related Compound B RS, USP Diazepam Related Compound A RS, and USP Nordazepam RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having known concentrations of about 1 µg per mL, 0.1 µg per mL, and 3 µg per mL, respectively.

Test solution—Transfer about 10 mg of Diazepam, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of diazepam related compound B, diazepam related compound A, and nordazepam in the portion of Diazepam taken by the formula:

$$(C_R / W)(r_U / r_S)$$

in which C_R is the concentration, in µg per mL, of USP Diazepam Related Compound B RS, USP Diazepam Related Compound A RS, or USP Nordazepam RS in the *Standard solution; W* is the weight, in mg, of Diazepam taken to prepare the *Test solution;* and r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.01% of diazepam related compound A, not more than 0.1% of diazepam related compound B, and not more than 0.3% of nordazepam are found.

Calculate the percentage of any other impurity in the portion of Diazepam taken by the formula:

$$(C_S / W)(r_i / r_S)$$

in which C_S is the concentration, in µg per mL, of USP Diazepam Related Compound B RS in the *Standard solution;* r_i is the peak response for any other impurity obtained from the *Test solution;* and r_S is the peak response of USP Diazepam Related Compound B RS obtained from the *Standard solution:* not more than 0.1% of any other impurity is found; and not more than 1.0% of the total impurities is found.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, and methanol (2:2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve suitable quantities of USP Nordazepam RS and USP Diazepam RS in methanol, using sonication if necessary, to obtain a solution containing about 0.1 mg of each per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Diazepam RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Transfer about 10 mg of Diazepam, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.76 for nordazepam and 1.0 for diazepam; the resolution, *R,* between nordazepam and diazepam is not less than 4; the column efficiency is not less than 5000 theoretical plates for the diazepam peak; the tailing factor for diazepam is not more than 2.0; and the relative standard deviation for the diazepam peak for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{16}H_{13}ClN_2O$ in the portion of Diazepam taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Diazepam RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Diazepam Capsules

» Diazepam Capsules contain not less than 90.0 percent and not more than 110.0 per cent of the labeled amount of diazepam ($C_{16}H_{13}ClN_2O$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩**—**
USP Diazepam RS
USP Nordazepam RS
7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one.
$C_{15}H_{11}ClN_2O$ 270.72

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

B: Transfer an accurately weighed amount of Capsule contents, equivalent to about 10 mg of diazepam, to a 50-mL centrifuge tube, and add 2 mL of acetone. Place the centrifuge tube in an ultrasonic bath for 5 minutes, and centrifuge. Using 100 µL of the supernatant as the test solution, and 100 µL of a solution of USP Diazepam RS in acetone containing 5 mg per mL as the Standard solution, proceed as directed for *Identification* test *B* under *Diazepam*.

Dissolution ⟨711⟩**—**

Medium: 0.01 N hydrochloric acid; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{16}H_{13}ClN_2O$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 284 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Diazepam RS in the same *Medium*.

Tolerances—Not less than 85% *(Q)* of the labeled amount of $C_{16}H_{13}ClN_2O$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase, System suitability solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Diazepam*.