product should not be assumed hypertonic with respect to blood. All aqueous glycerin and propylene glycol solutions studied in this investigation were hypotonic with respect to rabbit and human erythrocyte membranes.

Sodium chloride is effective in preventing hemolysis of rabbit and human erythrocytes in glycerin and propylene glycol solutions when the alcohols were in hypo-osmotic concentrations (1). Zanowiak and Husa (8) reported that 0.6% sodium chloride present in 10% glycerin and propylene glycol solutions prevented hemolysis of rabbit and human erythrocytes. The present investigation shows the presence of sodium chloride will prevent hemolysis of rabbit and human erythrocytes in aqueous solutions containing 5–90% glycerin. Sodium chloride prevented hemolysis of rabbit and human erythrocytes in aqueous propylene glycol solutions as long as the glycol concentration did not exceed 40–45%. Water–propylene glycol solutions containing more than 40–45% propylene glycol could not be made isotonic to rabbit and human erythrocyte membranes by the addition of sodium chloride. These data demonstrate that propylene glycol has greater hemolytic activity than glycerin. Jacobs, *et al.* (14), reported that each successive hydroxyl group added to the propane molecule decreased the rate of penetration of the alcohol into ox and rabbit erythrocytes.

Brittain and D'Arcy (15) have reported on the *in vivo* hematological effects in the rabbit following the intravenous injection of aqueous solutions containing different concentrations of propylene glycol in normal saline. Although the fragility of the red blood cells was not affected by different concentrations of propylene glycol, there was a marked decrease in blood clotting time with a corresponding increase in platelet count after the injection of 50% propylene glycol. The effect of 25% propylene glycol was considerably less. In this investigation the addition of sodium chloride to 30–40% propylene glycol solutions prevented hemolysis of rabbit and human erythrocytes, while the addition of sodium chloride to 50% propylene glycol solutions did not prevent complete hemolysis of these erythrocytes.

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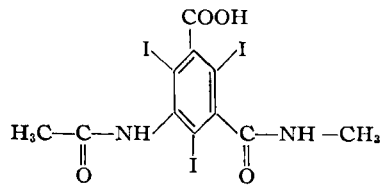
Drug Standards

Qualitative and Quantitative Tests for Iothalamic Acid

Provisional, unofficial monographs are developed by the Drug Standards Laboratory, in cooperation with the manufacturers of the drugs concerned, for publication in the *Journal of Pharmaceutical Sciences*. The ready availability of this information affords discriminating medical and pharmaceutical practitioners with an added basis for confidence in the quality of new drug products generally, and of those covered by the monographs particularly. Such monographs will appear on drugs representing new chemical entities for which suitable identity tests and assay procedures are not available in the published literature. The purity and assay limits reported for the drugs and their dosage forms are based on observations made on samples representative of commercial production and are considered to be reasonable within expected analytical and manufacturing variation.

5 - Acetamido - 2,4,6 - triiodo - *N* - methylisophthalamic acid, $C_{11}H_9I_3N_2O_4$; M.W. 613.92; 62.01% iodine. The structural formula of iothalamic acid may be represented as follows:



Received August 27, 1963, from the Drug Standards Laboratory, American Pharmaceutical Association Foundation, Washington, D. C. 20037.
Accepted for publication October 2, 1963.
This monograph was developed by Robert R. Stark. Mallinckrodt Chemical Works has cooperated by furnishing samples and data to aid in its development and preparation.

Physical Properties.—Iothalamic acid occurs as a white, odorless, bulky crystalline powder. In a

melting point capillary it darkens at about 275° and decomposes with evolution of iodine fumes at 281–285° (U.S.P. XVI Class Ia). It is slightly soluble in water and in alcohol, and is practically insoluble in chloroform. It is soluble in solutions of alkali hydroxides.

Identity Tests.—Heat about 500 mg. of iothalamic acid in a porcelain crucible over a free flame: violet vapors of iodine are evolved.

A 1:100,000 solution of iothalamic acid in alcohol exhibits an ultraviolet absorbance maximum at about 242 mμ [absorptivity (1%, 1 cm.) about 530]. The spectrum is shown in Fig. 1.

The infrared spectrum of a 0.5% dispersion of iothalamic acid in potassium bromide, in a disk of about 0.82-mm. thickness is shown in Fig. 2.

Purity Tests.—Dry about 1 Gm. of iothalamic acid, accurately weighed, at 105° for 18 hours: it loses not more than 1.0% of its weight.

Char about 1 Gm. of iothalamic acid, accurately weighed, cool the residue, add 1 ml. of sulfuric acid, heat cautiously until evolution of sulfur trioxide ceases, ignite, cool, and weigh: the residue does not exceed 0.1%.

Add about 200 mg. of iothalamic acid to a mixture of 2 ml. of water and 2 ml. of chloroform, shake vigorously, and allow the layers to separate: the chloroform layer remains colorless (absence of free iodine).

Dissolve 500 mg. of iothalamic acid in 20 ml. of water containing 2–3 drops of stronger ammonia

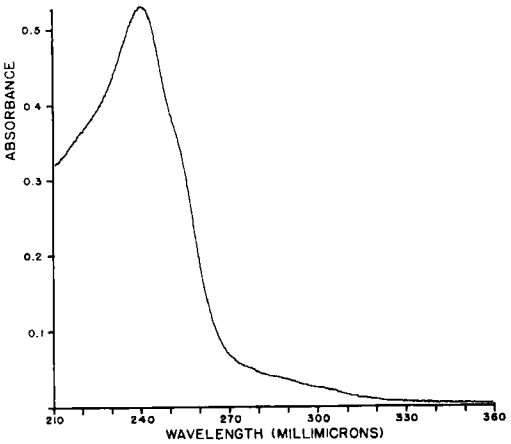


Fig. 1.—Ultraviolet absorption spectrum of iothalamic acid in alcohol (10 mcg. per ml.); Beckman model DK-2A spectrophotometer.

water contained in a 150-ml. glass-stoppered flask. Add 5 ml. of diluted nitric acid, shake vigorously, and filter the mixture through chloride-free paper. Add 2 ml. of silver nitrate T.S. to the filtrate: the solution shows no more turbidity than that produced by 0.01 mg. of chloride ion (Cl) in an equal volume of solution containing 5 ml. of diluted nitric acid and 2 ml. of silver nitrate T.S. (about 20 parts per million).

Transfer 1 Gm. of iothalamic acid to a 250-ml. beaker, add 15 ml. of nitric acid, and evaporate almost to dryness on a hot plate. Cool, add 10 ml. of nitric acid and 5 ml. of perchloric acid, and evaporate to dryness. Cool, add 2 ml. of hydrochloric acid, and rinse down the inner walls of the beaker with a few milliliters of water. Evaporate to dryness, cool, add another 2 ml. of hydrochloric acid, and again rinse down the inner walls of the beaker with a few milliliters of water. Evaporate to dryness, and bake the residue for a few minutes. Cool, add 10 ml. of water, 1 drop of phenolphthalein T.S., and sodium hydroxide T.S., dropwise, until the solution just turns pink. Add 1*N* hydrochloric acid dropwise until the color is discharged, add 1 ml. of diluted acetic acid, and dilute to 25 ml. with water. Prepare a control containing 20 mcg. of lead (Pb) and 1 ml. of diluted acetic acid in 25 ml. Add a small amount of activated charcoal (Darco G-60 is satisfactory) to each solution, and filter through retentive paper into 50-ml. color comparison tubes. Wash with a few ml. of water, dilute the solutions to 40 ml., and add 10 ml. of hydrogen sulfide T.S. to each solution: any brown color produced in the sample solution is not darker than that in the control solution: the heavy metals limit for iothalamic acid is 20 parts per million.

Assay.—(*Iodine*).—Transfer about 300 mg. of iothalamic acid, previously dried at 105° for 18 hours and accurately weighed, to a glass-stoppered 250-ml. flask, add 30 ml. of sodium hydroxide solution (1 in 20) and 500 mg. of powdered zinc. Connect the flask to a reflux condenser and reflux the mixture for 30 minutes. Cool to room temperature, and wash the condenser with 20 ml. of water. Filter the mixture and wash the flask and the filter with small portions of water, adding the washings to the filtrate. Add to the filtrate 5 ml. of glacial acetic acid and 1 ml. of a 1 in 1000 solution of tetrabromophenolphthalein ethyl ester in glacial acetic acid, and titrate with 0.05 *N* silver nitrate until the color of the yellow precipitate just changes to green. Each milliliter of 0.05*N* silver

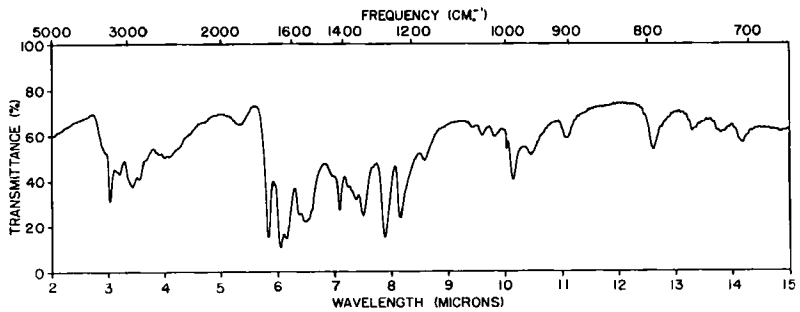


Fig. 2.—Infrared spectrum of iothalamic acid in potassium bromide disk (0.5%); Perkin-Elmer model 21 spectrophotometer, sodium chloride prism.

nitrate is equivalent to 6.345 mg. of iodine (I). The amount of iodine found is not less than 61.2% and not more than 62.8% of the weight of the sample taken.

(*Iothalamic Acid*).—Transfer about 2 Gm. of iothalamic acid, previously dried at 105° for 18 hours and accurately weighed, to a 250-ml. conical flask, and add 50.0 ml. of 0.1*N* sodium hydroxide. Swirl the flask to dissolve the sample, add 3 drops of phenolphthalein T.S., and titrate the excess base with 0.1 *N* hydrochloric acid. Each ml. of 0.1 *N* sodium hydroxide consumed is equivalent to 61.39 mg. of $C_{11}H_9I_3N_2O_4$. The amount of iothalamic acid found is not less than 99.0% and not more than 101.0% of the weight of the sample taken.

DOSAGE FORMS OF IOTHALAMATE SALTS

Meglumine Iothalamate Injection

A sterile solution of iothalamic acid in water for injection, prepared with the aid of meglumine (*N*-methylglucamine); $C_{18}H_{26}I_3N_3O_9$; M.W. 809.14.

Physical Properties.—Meglumine iothalamate injection is a clear, pale yellow, slightly viscous liquid. The pH is between 6.8 and 7.5.

Identity Tests.—Dilute a volume of meglumine iothalamate injection, equivalent to about 2 Gm. of meglumine iothalamate, with water to 100 ml., add 5 ml. of dilute hydrochloric acid, mix, and filter off the precipitated iothalamic acid. Wash the precipitate with four 10-ml. portions of water, and dry at 105° for 4 hours: the dried residue responds to the chemical and infrared identity tests for iothalamic acid.

Purity Tests.—Transfer a volume of meglumine iothalamate injection, equivalent to 1 Gm. of meglumine iothalamate, to a 250-ml. beaker. Proceed as directed in the heavy metals test in the monograph for iothalamic acid, beginning with ". . .add 15 ml. of nitric acid. . . ." The heavy metals limit for meglumine iothalamate is 20 parts per million.

To about 2 ml. of meglumine iothalamate injection add a few drops of starch T.S.: no blue color is produced (absence of free iodine).

Assay.—(*Meglumine Iothalamate*).—Pipet a volume of meglumine iothalamate injection, equivalent to about 3 Gm. of meglumine iothalamate, into a 100-ml. volumetric flask, add water to volume, and mix. Pipet 10 ml. of the solution into a 250-ml. glass-stoppered flask and proceed as directed in the *Assay for Iodine* under iothalamic acid, beginning with ". . .add 30 ml. of sodium hydroxide solution (1 in 20). . . ." Each milliliter of 0.05 *N* silver nitrate is equivalent to 13.49 mg. of $C_{18}H_{26}I_3N_3O_9$. The amount of meglumine iothalamate found is not less than 96.0% and not more than 104.0% of the labeled amount.

Sodium Iothalamate Injection

A sterile solution of iothalamic acid in water for injection, prepared with the aid of sodium hydroxide.

Physical Properties.—Sodium iothalamate injection is a clear, pale yellow, slightly viscous liquid. The pH is between 6.8 and 7.5; $C_{11}H_8I_3N_2NaO_4$; M. W. 635.90.

Identity Tests.—The solution responds to the identity tests for meglumine iothalamate injection and to the U.S.P. XVI flame test for sodium.

Purity Tests.—Determine the heavy metals content by the procedure for meglumine iothalamate injection. The heavy metals limit for sodium iothalamate is 20 parts per million.

To about 2 ml. of sodium iothalamate injection add a few drops of starch T.S.: no blue color is produced (absence of free iodine).

Assay.—(*Sodium Iothalamate*).—Assay sodium iothalamate injection by the procedure for meglumine iothalamate injection. Each milliliter of 0.05 *N* silver nitrate is equivalent to 10.60 mg. of $C_{11}H_8I_3N_2NaO_4$. The amount of sodium iothalamate found is not less than 97.5% and not more than 102.5% of the labeled amount.

DISCUSSION

U.S.P. and N.F. terminology for solubility, melting range, reagents, etc., have been used wherever feasible.

Iothalamic acid, synthesized by Hoey, *et al.* (1), is a radiographic contrast medium. Solutions of its salts are used for intravenous urography (meglumine iothalamate)[1] and for intravascular angiocardiography and aortography (sodium iothalamate).[2] Chemically, these solutions are similar to methylglucamine diatrizoate injection U.S.P. XVI, and sodium diatrizoate injection U.S.P. XVI, respectively, and the tests and standards in the above monographs are also similar to those in the official monographs.

Identity Tests.—Because of similarity of structure, identity tests based upon evolution of iodine vapor and comparison of the ultraviolet absorption spectrum with that of a reference standard are not sufficient to identify iothalamic acid or its salts. Comparison of the infrared spectrum with that produced by reference material under identical conditions provides a satisfactory test.

Quantitative Methods.—The quantitative methods provided are simple enough to perform and possess sufficient accuracy and precision for their intended purposes. Acidimetric determination of iothalamic acid gave an average value of 100.3 ± 0.2%.[3] Argentimetric determination of this compound gave an average value equivalent to 61.1 ± 0.1% iodine. Analysis of commercial preparations gave average values of 98.3 ± 0.1% and 97.7 ± 0.1% for meglumine iothalamate and sodium iothalamate injections, respectively.

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[1] The tradename for meglumine iothalamate injection is Conray.

[2] The tradename for sodium iothalamate injection is Angio-Conray.

[3] Maximum deviation from the mean value.