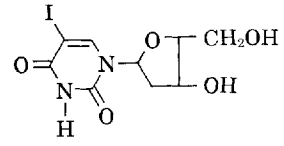
Qualitative and Quantitative Tests for Idoxuridine

Provisional, unofficial monographs are developed by the Drug Standards Laboratory, in cooperation with the manufacturers of the drug 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-IODO-2′-DEOXYURIDINE; $C_9H_{11}IN_2O_4$; mol. wt. 354.10. The structural formula of idoxuridine may be represented as



Physical Properties.—Idoxuridine occurs as a white, odorless, crystalline powder. In a melting point capillary it darkens and then decomposes with evolution of iodine fumes at 180–185° (U.S.P., class I*a*). It is slightly soluble in water and in alcohol, and is practically insoluble in chloroform and in ether. A 1% solution in 1 *N* NaOH is dextrorotatory.

Identity Tests.—Heat about 100 mg. of idoxuridine in a porcelain crucible over a free flame: violet vapors of iodine are evolved.

Dissolve 100 mg. of idoxuridine in 100 ml. of a 1 in 4 solution of isopropylamine in methanol. To 5 ml. of this solution add 5 ml. of chloroform and 0.2 ml. of a freshly prepared 1 in 100 solution of cobaltous acetate in methanol: a violet to blue-violet color is produced.

A 1:50,000 solution of idoxuridine in 0.1 *N* sodium hydroxide exhibits an ultraviolet absorbance maximum at about 280 mμ [absorptivity (*a*) about 16.0] and a minimum at about 253 mμ. The spectrum is shown in Fig. 1.

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

Purity Tests.—Char about 1 Gm. of idoxuridine, 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 0.5%. Retain the residue for the heavy metals test.

Dissolve the sulfated ash obtained from 1 Gm. of idoxuridine in a small volume of hot nitric acid and evaporate to dryness on a steam bath. Dissolve the residue in 2 ml. of diluted acetic acid, dilute to 25 ml. with water, and determine the heavy metals content of this solution by the U.S.P. XVII heavy metals test, method I: the heavy metals limit for idoxuridine is 20 p.p.m.

Assay.—*Iodine.*—Transfer about 75 mg. of idoxuridine, accurately weighed, into a large nickel crucible. Add 1 Gm. of anhydrous potassium carbonate powder and mix thoroughly with the sample using a dry glass rod. Gently tap the crucible to compact the mixture. Overlay the mixture with 12 Gm. of anhydrous potassium carbonate powder and again tap the crucible to compact the material. Ignite the contents of the uncovered crucible for 30 min. at 525° in a muffle furnace preheated to that temperature. Cool the crucible and transfer the contents to a 400-ml. beaker, rinsing the crucible with several portions of water, and adding the rinsings to the beaker. Add about 50 ml. of water to the beaker, heat gently, and break up the melt with the aid of a stirring rod. Filter the suspension through paper,

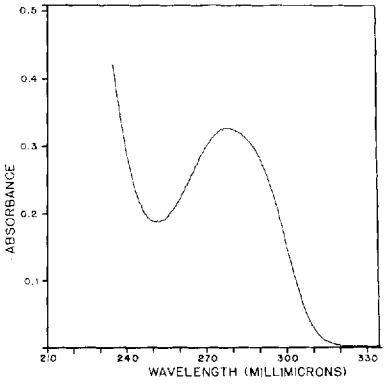


Fig. 1.—Ultraviolet absorption spectrum of idoxuridine in 0.1 *N* sodium hydroxide (20 mcg./ml.); Beckman model DK-2A spectrophotometer.

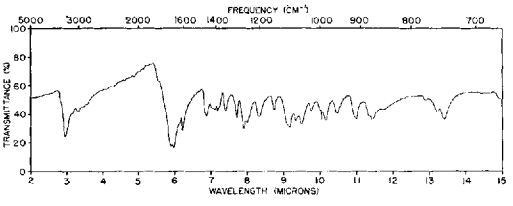


Fig. 2.—Infrared spectrum of idoxuridine in potassium bromide disk (0.5%); Perkin-Elmer model 21 spectrophotometer, sodium chloride prism.

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collecting the filtrate in a 250-ml. volumetric flask. Wash the beaker and filter paper with hot water until near the mark. Cool to room temperature, dilute to volume with water, and mix well. Pipet a 50-ml. aliquot of this solution into a 500-ml. conical flask and add 150 ml. of water. Add methyl orange T.S., then add phosphoric acid to produce a definite pink color. Add 10 ml. of bromine T.S. (sufficient amount to impart a definite yellow color to the solution) and several glass beads and heat to boiling. Boil the solution until it is colorless, rinse the inside walls of the flask with water, and boil an additional 5 min. Stopper the flask loosely, and cool to room temperature in a cold water bath. Add 5 ml. of phenol solution (1 in 20), again rinse the walls of the flask, and allow to stand 5 min. Add 5 ml. of potassium iodide solution (1 in 10) and titrate immediately with 0.05 *N* sodium thiosulfate, adding 3 ml. of starch T.S. as the end point is neared. Perform a blank determination and make any necessary correction. Each milliliter of 0.05 *N* sodium thiosulfate is equivalent to 1.058 mg. of iodine (I). The amount of iodine found is not less than 34.7% and not more than 36.6% of the weight of the sample taken.

Idoxuridine.—Dissolve about 500 mg. of idoxuridine, accurately weighed, in 40 ml. of dimethylformamide which has been previously neutralized to thymol blue T.S. Titrate with 0.1 *N* sodium methoxide to a blue end point. Each milliliter of 0.1 *N* sodium methoxide is equivalent to 35.41 mg. of $C_9H_{11}IN_2O_5$. The amount of idoxuridine found is not less than 98.0% and not more than 101.0% of the weight of the sample taken.

DOSAGE FORMS OF IDOXURIDINE

Idoxuridine Ophthalmic Solution

A sterile solution of idoxuridine in water. The solution may be rendered isotonic by addition of appropriate agents.

Identity Tests.—Evaporate 5 ml. of idoxuridine ophthalmic solution to dryness with the aid of a stream of air, add 2 ml. of alcohol, and again evaporate to dryness. To the residue add 5 ml. of a 1 in 4 solution of isopropylamine in methanol. If the residue is not completely soluble in this solution, the use of a stirring rod will aid in dissolving the idoxuridine present. Add 5 ml. of chloroform and 0.2 ml. of a freshly prepared 1 in 100 solution of cobaltous acetate in methanol: a violet to blue-violet color is produced.

Purity Tests.—To about 10 ml. of idoxuridine ophthalmic solution add a few drops of starch T.S.: no blue color is produced (absence of free iodine).

Assay.—To 4 Gm. of purified siliceous earth in a 4-oz. glass mortar add 4 ml. of water and incorporate by kneading thoroughly with a flexible spatula blade until the mixture is fluffy. Transfer to a chromatographic tube (19 × 200 mm.) equipped with a Teflon stopcock, and tamp gently to compress the material to a uniform mass. Transfer 2 Gm. of purified siliceous earth to the glass mortar, add 2.0 ml. of idoxuridine ophthalmic solution, mix as before, and transfer to the tube without tamping. Transfer 2 Gm. of purified siliceous earth to the same mortar, add 2 ml. of water, mix well, transfer about half of the mixture of the tube, and tamp down gently until the column appears uniform. Transfer the remaining portion of the tube, tamp as before, and cover with a small pledget of glass wool. Elute with chloroform–*n*-butanol (5:1) at a flow rate of approximately 1 ml./min., discarding the first 15–20 ml. of eluant. Collect the next 80 ml. of eluant in a 100-ml. volumetric flask, dilute to volume with the eluting solvent, and mix. Concomitantly determine the absorbance of this solution and of a standard solution of idoxuridine,[1] in the same medium, at a concentration of about 20 mcg./ml., in 1-cm. cells, at 292 and at 310 mμ, with a suitable spectrophotometer, using chloroform–*n*-butanol (5:1) as the blank. Calculate the quantity, in mg., of $C_9H_{11}IN_2O_5$ in the volume of ophthalmic solution taken by the formula $0.1C \times (A_{u292} - A_{u310})/(A_{s292} - A_{s310})$ in which C is the exact concentration of the standard solution, in mcg./ml., A_u is the absorbance of the sample solution, and A_s is the absorbance of the standard solution. The amount of idoxuridine is not less than 90% and not more than 110% of the labeled amount.

DISCUSSION

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

Idoxuridine,[2] synthesized by Prusoff (1), is an antiviral agent indicated in the treatment of corneal erosions caused by the herpes simplex virus. The effectiveness of idoxuridine in the treatment of herpes simplex keratitis has been attributed to its similarity of structure to thymidine; idoxuridine differs from thymidine only in the substitution of an iodine atom for a methyl group in the carbon-5 position of the uracil moiety. Administration of idoxuridine in the form of an ophthalmic solution results in a competition with thymidine for incorporation in the synthesis of deoxyribonucleic acid (DNA), the genetic material of herpes simplex virus which determines its ability to infect and reproduce. Incorporation of idoxuridine in the DNA synthesis results in the production of a "faulty" DNA, inhibiting reproduction of the invading virus.

Identity Tests.—Idoxuridine reacts with cobalt salts in nonaqueous alkaline medium to produce a colored complex. This color reaction is rather specific for compounds possessing the —CONHCO— or —CONHCS— group (2). Barbiturates, hydantoins, and xanthines are typical classes of compounds which produce colored complexes under similar conditions. The method may be adapted for quantitative determination of idoxuridine in the ophthalmic solution. The presence of isotonic agents such as sodium chloride which are relatively insoluble in the reaction solvents decreases the reproducibility of the method. The ultraviolet absorption spectrum may be run alternatively in acid solution with a maximum absorbance at about 288 mμ [absorptivity (a) about 21.5] and a minimum at about 248 mμ. These tests together

[1] Prepare a methanolic solution of idoxuridine in a concentration of 2 mg./ml. Dilute 1.0 ml. of this solution to 100.0 ml. with chloroform–*n*-butanol (5:1).

[2] Marketed as Dendrid by Alcon Laboratories, Fort Worth, Tex., as Herplex by Allergan Pharmaceuticals, Santa Ana, Calif., and as Stoxil by Smith Kline & French Laboratories, Philadelphia, Pa.

with the identity test based upon the evolution of iodine vapor and comparison of the infrared spectrum provide a satisfactory identification of idoxuridine.

Purity Tests.—Thin-layer or paper chromatographic procedures may be included for testing the purity of bulk idoxuridine. Comparison of idoxuridine to a reference standard is made by examination of developed chromatograms using an ultraviolet light and/or color producing reagents, *i.e.*, cysteine–sulfuric acid. The idoxuridine spot should be equivalent in position to the reference standard spot for idoxuridine, and no other spots at other positions should be visible. The spotting of control solutions containing the degradation products of idoxuridine (5-iodouracil, uracil, and deoxyuridine) will aid in detecting the position of extraneous spots on the chromatograms.

Quantitative Methods.—The quantitative determination of the iodine content of idoxuridine is similar to the official assay for sodium liothyronine (3) and gave an average value equivalent to 36.1 ± 0.1%[3] iodine. A rapid, precise measure of the iodine content may also be determined by the oxygen flask method (4–6). Nonaqueous titration of idoxuridine with sodium methoxide gave an average value of 99.8 ± 0.6%.[3] Azo violet indicator may also be used for the end point detection of the titration. Analysis of the sterile ophthalmic solutions by column partition chromatography was based on the procedure of Simpson and Zappala (7). Acid washed Celite 545 was used as the supporting phase without prior treatment. Celite 545 may be used as the adsorbant by prewashing an acidified column with organic solvents to remove extractable impurities. The organic solvents are then removed by oven drying. Incorporation of water or 0.1 *N* hydrochloric acid in the preparation of the Celite columns gave comparable results representing an average recovery of 97.4 ± 5.7%[3] of the theoretical amount of idoxuridine in the ophthalmic solutions.

The volume of eluting solvent included for the assay of idoxuridine ophthalmic solution gave quantitative recoveries with the Celite used. However, it should be noted that the elution rate for idoxuridine may vary from lot to lot of Celite which then necessitates a minor change in the volume of eluate collected. This should be demonstrated for each batch of Celite by a satisfactory recovery of a standard aqueous idoxuridine solution subjected to the column procedure.

[3] Maximum deviation from the mean value.

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Technical Articles

Automated Nephelometric Determination of Rat Liver Glycogen in Adrenal Steroid Bioassays

By WILLIAM F. BEYER

An automated procedure for the nephelometric determination of rat liver glycogen is described. Alkaline liver digests are mixed with 57.5 per cent alcohol and heated at 45°. Samples are analyzed at a rate of 60/hr. using an automatic sampler and analyzer (Technicon AutoAnalyzer) in conjunction with a commercially available fluorometer. The coefficient of variation for the automated procedure is approximately 1 per cent.

RAT LIVER glycogen is used as the criterion of response in the bioassay for endocrine principles of the adrenal cortex and also for synthetic steroids. The assay is based on the method of Pabst *et al.* (1) and is currently an official procedure for adrenal cortex injection as directed by N.F. XII (2). For the assay, livers of adrenalectomized rats, previously injected with test samples, are digested in hot 30% potassium hydroxide. After standing overnight at room temperature, the alkaline liver digests are diluted with water and glycogen is determined.

An automated procedure for glycogen has been described by Singer *et al.* (3) requiring manual deproteination with trichloroacetic acid. Glyco-

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