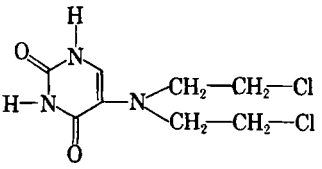
Qualitative and Quantitative Tests for Uracil Mustard

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-[BIS(2-CHLOROETHYL)AMINO] URACIL; $C_8H_{11}Cl_2N_3O_2$; mol. wt. 252.10. The structural formula of uracil mustard may be represented as



Physical Properties.—Uracil mustard occurs as a cream-white, odorless, crystalline powder, and melts at about 200° dec., U.S.P. XVI Class Ia. It is slightly soluble in acetone and in alcohol, very slightly soluble in water, and practically insoluble in benzene and in chloroform.

Identity Tests.—Mix 5 mg. of uracil mustard and 50 mg. of benzoin (benzoylphenylcarbinol) in a 10 × 75 mm. test tube and cover the tube opening with a moistened strip of Congo red test paper. Immerse the tube into an oil bath at 170°: the test paper begins to turn blue within 1 minute.

A 1 in 40,000 solution of uracil mustard in alcohol exhibits an ultraviolet absorbance maximum at about 256 mμ [absorptivity (1%, 1 cm.) about 226], a shoulder at about 295 mμ, and a minimum at about 233 mμ. The spectrum is shown in Fig. 1.

The infrared spectrum of a 0.5% dispersion of uracil mustard in postassium bromide, in a disk of about 0.82 mm. thickness, is shown in Fig. 2.

Purity Tests.—Dry about 1 Gm. of uracil mustard, accurately weighed, *in vacuo* over silica gel for 18 hours: it loses not more than 0.5% of its weight.

Char about 0.1 Gm. of uracil mustard, 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%.

Dissolve about 20 mg. of uracil mustard in 10 ml. of alcohol, add 2 drops of nitric acid and 1 ml. of silver nitrate T.S.: no opalescence is produced (absence of chloride ion).

Place about 500 mg. of finely powdered potassium nitrate in the cup of a Parr peroxide bomb, add about 250 mg. of finely powdered sucrose, and mix with a glass rod. Transfer to the cup about 250 mg. of finely powdered uracil mustard, accurately weighed, and again mix. Place a 5-Gm. portion of sodium peroxide in the cup, mix, and add a 10-Gm. portion of the peroxide, mixing the entire contents of

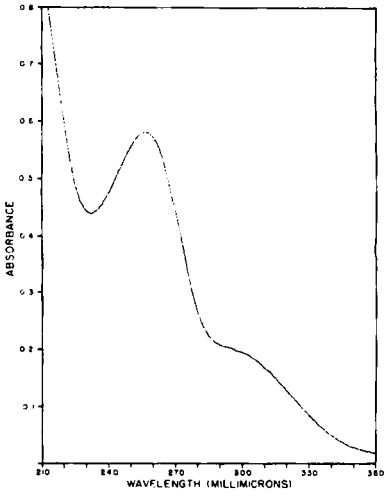


Fig. 1.—Ultraviolet absorption spectrum of uracil mustard in alcohol (25 mcg. per ml.). Beckman model DK-2A spectrophotometer.

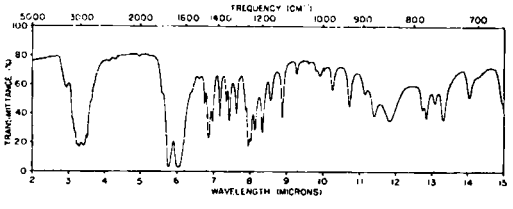


Fig. 2.—Infrared spectrum of uracil mustard in potassium bromide disk (0.5%). Perkin-Elmer model 21 spectrophotometer; sodium chloride prism.

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the cup thoroughly with the glass rod. Brush any particles clinging to the glass rod into the cup with a camel hair brush. Cover the cup and shake for 1 to 2 minutes, tapping the cup sharply several times to loosen any material clinging to the sides or bottom of the cup. Remove the cover, brush into the cup any particles adhering to the cover or to the rim of the cup, place the electric ignition head on the cup, and tap the base sharply on a hard surface to compact the charge. Assemble the bomb and ignite, observing the precautions given under *Sulfur and Halogen Determination*, U.S.P. XVI, p. 908, and the instructions in the bomb manufacturer's manual. After the bomb has cooled, rinse the exterior of the cup with water and dismantle the bomb. Immerse the ignition head into a 1-L. beaker containing about 100 ml. of hot water to dissolve any of the fusion mixture which may be adhering to its underside and rinse the head with hot water, catching the rinsings in the beaker. With a pair of tongs lay the fusion cup on its side in the same beaker of hot water, covering it immediately with a watch glass. After the fusion mixture has dissolved, remove the cup and rinse it into the beaker with hot water. Allow the solution to cool, add 50 ml. of dilute nitric acid (1 in 1), slowly with stirring, and filter, washing the filter with water. To the filtrate add 500 mg. of hydrazine sulfate and, very slowly with stirring, add 25 ml. of 0.1 *N* silver nitrate, avoiding exposure to strong light. Filter the mixture through a tared sintered-glass crucible of medium porosity, wash the precipitate with 300 ml. of water in divided portions, dry at 140° for 1 hour, cool, and weigh. The weight of the dried precipitate, multiplied by 0.2474, represents the weight of chlorine in the sample. The chlorine content is not less than 27.6% and not more than 28.7%, calculated on the dried basis.

Assay

Transfer about 200 mg. of uracil mustard, previously dried *in vacuo* over silica gel for 18 hours and accurately weighed, to a 100-ml. volumetric flask, dilute to volume with alcohol, and mix. Pipet 20 ml. of this solution into a 50-ml. volumetric flask containing 20 ml. of water, mix, add 1.0 ml. of 5% acetic acid solution, and mix. Transfer 6.0 ml. of 8-hydroxyquinoline T.S. to the flask, mix, add 3.0 ml. of sodium carbonate solution (1 in 10), dilute to volume with water, mix, and allow to stand 150 minutes. Determine the absorbance of this solution and of a similar solution prepared with uracil mustard reference standard in 1-cm. cells with a suitable spectrophotometer at about 466 mμ, using a reagent blank to set the instrument. Record the absorbance of the sample solution as A_u and that of the standard solution as A_s and calculate the amount of $C_8H_{11}Cl_2N_3O_2$, in milligrams, in the amount of sample taken by the formula $W(A_u/A_s)$, where W is the exact weight of uracil mustard reference standard taken. The amount of uracil mustard found is not less than 97.0% and not more than 103.0% of the weight of the sample taken.

DOSAGE FORMS OF URACIL MUSTARD

Uracil Mustard Capsules

Identity Test.—Transfer the contents of two uracil mustard capsules to a 100-ml. volumetric flask, add alcohol to volume, and mix. Centrifuge a portion of this mixture until clear. The ultraviolet absorption spectrum of the solution exhibits a maximum at 256 ± 3 mμ, a shoulder at 295 ± 3 mμ, and a minimum at 233 ± 3 mμ, comparable to that of a similar solution of uracil mustard reference standard.

Assay.—*Standard Preparation.*—Transfer about 50 mg. of uracil mustard reference standard, previously dried *in vacuo* over silica gel for 18 hours and accurately weighed, to a 50-ml. volumetric flask, dissolve in alcohol, dilute to volume with alcohol, and mix.

Assay Preparation.—Transfer as completely as possible the contents of not less than 20 uracil mustard capsules to a tared weighing bottle and weigh accurately. Transfer to a glass-stoppered centrifuge tube an amount of the mixed powder, equivalent to about 10 mg. of uracil mustard and accurately weighed, add 10.0 ml. of alcohol, mix thoroughly by inversion, and centrifuge.

Procedure.—Pipet 4 ml. of water into each of three glass-stoppered test tubes. Pipet 4 ml. of the *Assay Preparation* and of the *Standard Preparation*, respectively, into separate tubes; prepare a blank by adding 4.0 ml. of alcohol to the remaining tube and mix. To each tube add 1.0 ml. of 5% acetic acid and 2.0 ml. of 8-hydroxyquinoline T.S., mixing after each addition. Add 3.0 ml. of sodium carbonate (1 in 10) to each tube, mix well, and allow to stand for 150 minutes. Filter the assay sample through dry filter paper and determine the absorbances of the solutions in 1-cm. cells using a suitable spectrophotometer at the wavelength of maximum absorbance at about 466 mμ using the blank to set the instrument at zero absorbance. Record the absorbance of the solution from the *Standard Preparation* as A_s and that from the *Assay Preparation* as A_u and calulate the weight, in milligrams, of $C_8H_{11}Cl_2N_3O_2$ in the portion of uracil mustard capsules taken by the formula $10C(A_u/A_s)$, where C is the concentration, in milligrams per milliliter, of uracil mustard reference standard in the *Standard Preparation*. The amount of uracil mustard found 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.

Uracil mustard,[1] synthesized by Lyttle and Petering (1), is an orally active alkylating agent of the nitrogen mustard class which is used in the palliative treatment of certain neoplasms affecting the reticuloendothelial system.

Identity Tests.—Identification of uracil mustard as an aliphatic halogen compound is based on its reaction with molten benzoin (2). The reaction yields a halogen hydride which can be detected readily in the gas phase by the action on Congo paper. The ultraviolet absorption spectrum for uracil mustard cannot be used alone for identification since an intermediate in its synthesis, 5-[bis-(2-hydroxyethyl)amino]uracil, has a similar spectrum with a nearly identical molar absorptivity. However, these identity tests together with the infrared spectrum provide a satisfactory identification of uracil mustard.

[1] Marketed only under the generic name.

Quantitative Methods.—The chloride content of uracil mustard determined by a sodium peroxide fusion and gravimetric analysis gave an average value of 28.0 ± 0.3%.[2] The colorimetric analysis of uracil mustard using 8-hydroxyquinoline reagent in alkaline solution serves to differentiate uracil mustard and the 5-[bis(2-hydroxyethyl)amino] uracil intermediate. Conformity to Beer's law was observed from 10–40 mg. of uracil mustard per 50 ml. of reaction solution. Analysis of commercial uracil mustard capsules by this method gave an average value of 96.2 ± 4.3%.[2] The colorimetric method of Petering and Van Giessen (3) provides an alternate quantitative procedure and an additional identity test for uracil mustard and related alkylating agents. This method employs the reaction of alkylating agents with 4-(*p*-nitrobenzyl)pyridine (NBP) producing a chromophore with a maximum at about 600 mμ and may be adaptable to the capsule formulation, although limited investigation in this laboratory did not yield quantitative results.

[2] Maximum deviation from the mean value.

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Technical Articles

Experiences with Unit-to-Unit Variations in Tablets

By LEON LACHMAN and HANNA D. SYLWESTROWICZ

The literature reports on the subject of interunit dosage and weight variations of tablets indicate that past studies were mainly concerned with analyzing the finished product to detect gross departures from the desired quality. These studies, however, were cognizant of the several stages of manufacture before the compression step could contribute to the end tablet variability. In this investigation, the variability in several stages of tablet manufacturing (dry mixing, granulating, lubricating, and tableting) was evaluated for a particular tablet formulation. The importance of employing correct sampling procedures to obtain random samples to be used for the accurate estimation of the sources of variability and product uniformity is illustrated. The relationship that exists between tablet weights and drug concentration was determined. Since systematic sampling is commonly employed in the in-process control of tableting operations, the information that can be gained from this type of sampling procedure and random sampling is presented and discussed.

THE TERM "unit-to-unit variation of potency" of solid dosage forms has developed considerable significance in recent years. Several reports have appeared in the literature on this subject (1–5). It has been demonstrated (1, 2) that substantial variations can exist in the potency between individual tablets which would not be detected by test methods devised to determine average drug content.

Realizing the importance of this situation, the Quality Control Section of the Pharmaceutical Manufacturers Association initiated an extensive study of this problem. As a result of this study, recommendations have been submitted to the United States Pharmacopeia and National Formulary revision committees that these compendia include a two-limit attribute statistical test in their specifications for tablet composition-uniformity to measure intertablet dosage uniformity (6).

Tablets have received the most attention and study with regard to interunit dosage variation (1–5) because they are the most acceptable dosage form on the U. S. market for the administration of orally effective therapeutic agents and account for a major part of the pharmaceutical sales.

Although these studies were cognizant of the several stages of manufacture before the compression step could contribute to the over-all end variability of the drug concentration in the tablets, none of these investigators studied in detail the variability of each of these steps; instead, they sampled the finished product to detect gross departures from the desired quality. However, to insure acceptable quality of the finished product, the successive phases of the manufacturing process must be evaluated to

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