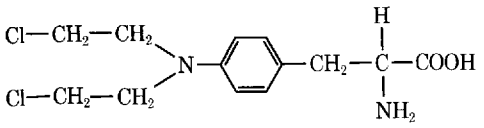
Qualitative and Quantitative Tests for Melphalan

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.

L - 3 - p - [BIS(2 - chloroethyl)amino]phenylalanine; $C_{13}H_{18}Cl_2N_2O_2$; mol. wt. 305.21. The structural formula of melphalan may be represented as



Physical Properties.—Melphalan occurs as an off-white to buff-colored powder with a faint odor. It is slightly soluble in methanol and in alcohol, and practically insoluble in water. It is soluble in dilute mineral acids.

Identity Tests.—Transfer 1 ml. of a 0.01% alcoholic solution of melphalan into a glass-stoppered test tube and add 1 ml. of U.S.P. phthalate buffer, pH 4.0, 1 ml. of a 5 in 100 solution of 4-(*p*-nitrobenzyl)pyridine (NBP) in acetone, and 1 ml. of saline T.S. Heat on a water bath at 80° for 20 min. and cool the solution quickly. Add 10 ml. of alcohol and 1 ml. of 0.1 *N* potassium hydroxide: a violet to red-violet color is produced.

A 1 in 100,000 solution of melphalan in alcohol exhibits an ultraviolet absorbance maximum at about 260 mμ [absorptivity (*a*) about 72] and a minimum at about 226 mμ. The spectrum is shown in Fig. 1.

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

Purity Tests.—Dry about 1 Gm. of melphalan, accurately weighed, *in vacuo* at 105° to constant weight: it loses not more than 7% of its weight.

Char about 1 Gm. of melphalan, 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%.

Determine the nitrogen content by the U.S.P. XVII nitrogen determination, method II, using about 325 mg. of melphalan, accurately weighed, and 0.1 *N* sulfuric acid for the titration. Each milliliter of 0.1 *N* sulfuric acid is equivalent to 1.401 mg. of nitrogen (N). The amount of nitrogen found is not less than 8.90% and not more than 9.45% calculated on a dried sample weight.

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Burroughs Wellcome & Co., Inc., Tuckahoe, N. Y., has cooperated by furnishing samples and data to aid in the development and preparation of this monograph.

Assay.—Transfer about 200 mg. of melphalan, accurately weighed, into a titrating beaker and dissolve in 20 ml. of 0.5 *N* sodium hydroxide. Cover the beaker with a watch glass and boil the solution for 30 min., adding water as necessary to maintain the volume. Cool, neutralize to phenolphthalein T.S. with acetic acid, add 1 ml. excess acetic acid, and titrate potentiometrically with 0.1 *N* silver nitrate. Each milliliter of 0.1 *N* silver nitrate is equivalent to 15.26 mg. of $C_{13}H_{18}Cl_2N_2O_2$. The amount of melphalan found is not less than 93% and not more than 100.5% calculated on the dried basis. (*Note.*—Based on the assay result, melphalan is factored to 100% for use in the tablet formulation.)

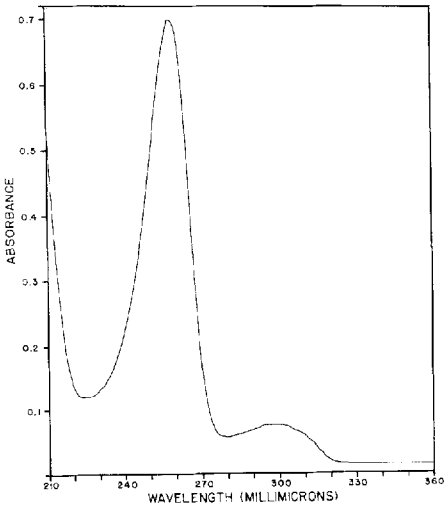

Fig. 1.—Ultraviolet absorption spectrum of melphalan in alcohol (10 mcg./ml.). Beckman model DK-2A spectrophotometer.

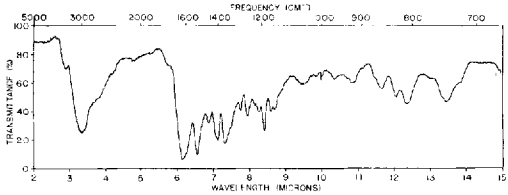

Fig. 2.—Infrared spectrum of melphalan in potassium bromide disk (0.5%). Perkin-Elmer model 21 spectrophotometer; sodium chloride prism.

DOSAGE FORMS OF MELPHALAN

Melphalan Tablets

Identity Tests.—Transfer 2 ml. of the assay solution (5 mg. of melphalan in 100 ml.) into a glass-stoppered test tube and add 1 ml. of U.S.P. phthalate buffer, pH 4.0, 1 ml. of a 5 in 100 solution of 4-(*p*-nitrobenzyl) pyridine (NBP) in acetone, and 1 ml. of saline T.S. Heat on a water bath at 80° for 20 min. and cool the solution quickly. Add 10 ml. of alcohol and 1 ml. of 0.1 *N* potassium hydroxide: a violet to red-violet color is produced.

Assay.—*Standard Preparation.*—Transfer about 10 mg. of melphalan reference standard, accurately weighed, to a 100-ml. volumetric flask, dissolve in alcohol, dilute to volume with alcohol, and mix. Transfer 10.0 ml. of this solution to a second 100-ml. volumetric flask, dilute to volume with alcohol, and mix.

Procedure.—Weigh and finely powder not less than 20 melphalan tablets. Transfer to a 100-ml. volumetric flask an amount of powdered tablets, accurately weighed, equivalent to about 5 mg. of melphalan. Add 10 ml. of water, swirl the sample, then add 10 ml. of alcohol. Warm on a steam bath for about 2 min. with intermittent shaking and cool the solution. Add alcohol to volume and mix. Centrifuge a portion of the mixture, transfer 10.0 ml. of the clear liquid to a 50-ml. volumetric flask, make to volume with alcohol, and mix. Concomitantly, determine the absorbance of this solution and that of the *Standard Preparation* in 1-cm. cells, at the maximum at about 260 mμ with a suitable spectrophotometer, using alcohol as the blank. Calculate the quantity, in mg., of $C_{13}H_{18}Cl_2N_2O_2$ in the portion of the tablets taken by the formula $0.5C\ (A_u/A_s)$, where C is the exact concentration, in mcg./ml., of melphalan in the *Standard Preparation*, calculated on the dried basis, A_u is the absorbance of the solution from the tablets, and A_s is the absorbance of the *Standard Preparation*. The amount of melphalan found is not less than 93.0% and not more than 107.0% of the labeled amount.

DISCUSSION

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

Melphalan,[1] synthesized by Bergel and Stock (1), is an orally active alkylating agent of the nitrogen mustard class which is useful in the treatment of multiple myeloma. Early literature references to this compound may be found under the synonym sarcolysin.

Identity Tests.—The colorimetric identification of melphalan is based on the procedure of Petering and Van Giessen (2) for the determination of alkylating agents. An additional identification test for melphalan drug is obtained by comparing the absorbances of the alcoholic solution at 226 mμ (minimum) and at 260 mμ (maximum). The ratio A_{226}/A_{260} is about 0.15.

Quantitative Methods.—Argentimetric determination of melphalan gave an average value of 95.7 ± 0.2%.[2] The titration was conducted using a silver electrode and a calomel electrode modified to contain saturated potassium sulfate solution. A rapid, precise measure of the chloride content may be determined by the oxygen flask method included for butyl chloride, N.F. XII, First Supplement. Analysis of commercial melphalan tablets by the spectrophotometric method gave an average value of 101.7 ± 1.5%.[2]

REFERENCES

(1) Bergel, F., and Stock, J. A., *J. Chem. Soc.*, **1954**, 2409.
(2) Petering, H. G., and Van Giessen, G. J., *J. Pharm. Sci.*, **52**, 1159(1963).

[1] Marketed as Alkeran by Burroughs Wellcome & Co. Inc., Tuckahoe, N. Y.
[2] Maximum deviation from the mean value.

Technical Articles

Anhydrous Lactose in Direct Tablet Compression

By NICHOLAS H. BATUYIOS

The use of anhydrous lactose U.S.P. XVII, tablet grade, as a diluent in direct tablet compression was investigated. It was found that it possesses excellent tableting properties and can be run on a high-speed tablet machine. The placebo and active tablets produced were not affected by elevated temperatures, high humidity, and direct sunlight.

IN TERMS of economics and product stability, direct tablet compression offers distinct advantages over double compression, known also as "slugging," and the wet granulating method (1). Also, direct compression should produce tablets of faster dissolution rates because no colloidal binders, (*e.g.*, starch and gelatin) are used to envelop the granules.

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