Qualitative and Quantitative Tests for Acetohexamide

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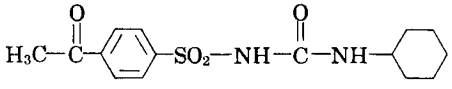
Provisional, unofficial monographs are developed by the Drug Standards Laboratory, in cooperation with the manufacturers of the drug concerned, for publication in the *Journal of Pharmaceutical Sciences*. The ready availability of this information affords discriminating medical and pharmaceutical practitioners 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.

1 - [(*p* - ACETYLPHENYL)SULFONYL] - 3 - CYCLOHEXYLUREA; $C_{15}H_{20}N_2O_4S$; mol. wt. 324.40. The structural formula of acetohexamide may be represented as:



Physical Properties—Acetohexamide occurs as a practically odorless, white, crystalline powder, m.p 184–189° (U.S.P. class Ia). It is practically insoluble in water and in ether, and slightly soluble in alcohol and in chloroform. It is soluble in pyridine and in dilute solutions of alkali hydroxides.

Identity Tests—A 1 in 100,000 solution of acetohexamide in 0.01 *N* sodium hydroxide exhibits an ultraviolet absorbance maximum at about 249 mμ [absorptivity (*a*) about 43]. The spectrum is shown in Fig. 1.

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

Dissolve about 100 mg. of 2,4-dinitrophenylhydrazine in 1 ml. of sulfuric acid and cautiously add 5 ml. of alcohol. Cool, filter if necessary to obtain a clear solution, add 5 ml. of a solution of acetohexamide in hot alcohol (1 in 200), and warm on a steam bath: an orangish yellow precipitate is formed.

Purity Tests—Dry about 1 Gm. of acetohexamide, accurately weighed, at 105° for 3 hr.: it loses not more than 1% of its weight.

Char about 1 Gm. of acetohexamide, 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 heavy metals content of acetohexamide by the U.S.P. heavy metals test, method II: the heavy metals limit for acetohexamide is 20 p.p.m.

Assay—Transfer about 300 mg. of acetohexamide, accurately weighed, to a 125-ml. conical flask, and dissolve in 40 ml. of dimethylformamide. Add 5 drops of thymol blue T.S., and titrate with 0.1 *N* sodium methoxide, using a magnetic stirrer and

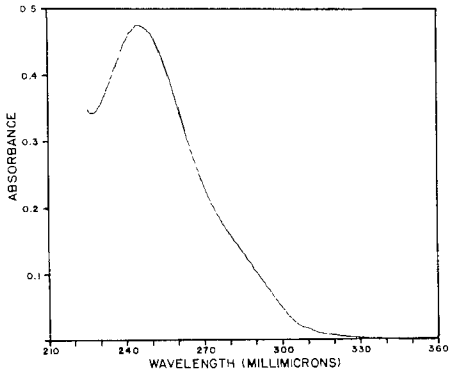


Fig. 1—Ultraviolet absorption spectrum of acetohexamide in 0.01 N sodium hydroxide (10 mcg./ml.); Beckman model DK-2A spectrophotometer.

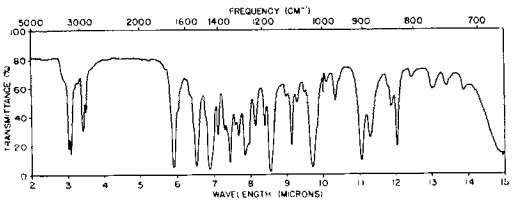


Fig. 2—Infrared spectrum of acetohexamide in potassium bromide disk (0.5%); Perkin-Elmer model 21 spectrophotometer, sodium chloride prism.

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taking precautions against absorption of atmospheric carbon dioxide. Perform a blank determination, and make any necessary correction. Each milliliter of 0.1 *N* sodium methoxide is equivalent to 32.44 mg. of $C_{15}H_{20}N_2O_4S$. The amount of acetohexamide found is not less than 97% and not more than 101%, calculated on the dried basis.

DOSAGE FORMS OF ACETOHEXAMIDE

Acetohexamide Tablets

Identity Tests—The final solution prepared from the tablet sample in the *Assay* exhibits an absorbance maximum and minimum at the same wavelengths as the acetohexamide standard solution.

Assay—Weigh and finely powder not less than 20 acetohexamide tablets. Weigh accurately a portion of the powder, equivalent to about 500 mg. of acetohexamide, and transfer to a 100-ml. volumetric flask. Add about 60 ml. of 0.1 *N* sodium hydroxide and shake for 30 min. Dilute to volume with water, and mix. Filter the solution, discarding the first portion of filtrate, and transfer 20.0 ml. into a 125-ml. separator. Add 2 ml. of diluted hydrochloric acid and extract with four 40-ml. portions of chloroform, filtering each portion through chloroform-washed paper into a 200-ml. volumetric flask. Make to volume with chloroform, and mix. Pipet 20.0 ml. of this solution into a suitable beaker and evaporate to dryness on a steam bath. Transfer the residue quantitatively, with the aid of 0.1 *N* sodium hydroxide, into a 100-ml. volumetric flask, add alkaline solution to volume, and mix. Dilute 10.0 ml. of this solution to 100.0 ml. with water, and mix. Concomitantly determine the absorbance of this solution and of a standard solution of acetohexamide, in the same medium, at a concentration of about 10 mcg./ml., in 1-cm. cells, at the maximum at about 249 mμ, with a suitable spectrophotometer, using 0.01 *N* sodium hydroxide as the blank. Calculate the quantity, in milligrams, of $C_{15}H_{20}N_2O_4S$ in the portion of tablets taken by the formula $50C \times (A_u/A_s)$, in which C is the exact concentration of the standard solution, in micrograms per milliliter, A_u is the absorbance of the sample solution, and A_s is the absorbance of the acetohexamide standard solution. The amount of acetohexamide found is not less than 90.0% and not more than 110.0% of the labeled amount.

DISCUSSION

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

Acetohexamide[1] is an oral hypoglycemic agent effective in the treatment and management of selected patients with stable diabetes mellitus. The reduction of blood sugar levels by sulfonylurea drugs is achieved primarily by stimulating the release of endogenous insulin and secondarily by inhibition of glucose formation from liver glycogen.

Identity Tests—The qualitative tests included for acetohexamide provide suitable differentiation from official sulfonylurea compounds. The ultraviolet absorption maximum of acetohexamide in alkaline solution is exhibited at about 249 mμ as compared to 230 and 228 mμ for chlorpropamide and tolbutamide, respectively, in the same medium. The formation of the hydrazone derivative with acetohexamide which does not occur with chlorpropamide nor tolbutamide constitutes a rapid distinctive test.

Quantitative Tests—The nonaqueous titration of acetohexamide with sodium methoxide using thymol blue T.S. gave an average value of 100.2 ± 0.5%.[2] Satisfactory recoveries can be obtained by dissolving acetohexamide in a known excess of 0.1 *N* sodium hydroxide and back titration of the excess base with 0.1 *N* hydrochloric acid using phenolphthalein T.S. Neutralized alcohol is added prior to the back titration to retard precipitation of acetohexamide at the end point. Spectrophotometric analyses of commercial tablets labeled to contain 250 and 500 mg. of acetohexamide gave average values of 102.2 ± 0.7%[2] and 100.6 ± 0.4%[2] of the labeled amounts, respectively.

[1] Marketed as Dymelor by Eli Lilly and Co., Indianapolis, Ind.

[2] Maximum deviation from the mean value.