Qualitative and Quantitative Tests for Cyclothiazide

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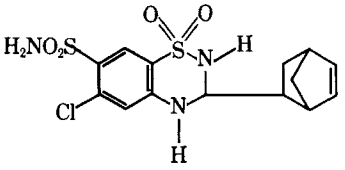
Provisional, unofficial monographs are developed by the Drug Standards Laboratory, in cooperation with the manufacturers of the drug concerned, for publication in the *Journal of Pharmaceutical Sciences*. The ready availability of this information affords discriminating medical and pharmaceutical practitioners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

6 - CHLORO - 3,4 - DIHYDRO - 3 - (5 -NORBORNEN-2 - YL) - 7 - SULFAMOYL - 1,2,4 - BENZOTHIADIAZINE-1,1-DIOXIDE; $C_{14}H_{16}ClN_3O_4S_2$; mol. wt. 389.88. The structural formula of cyclothiazide may be represented as:



Physical Properties—Cyclothiazide occurs as a white to nearly white, practically odorless powder, and melts with decomposition at about 220° (U.S.P., class I). It is practically insoluble in water and in chloroform, sparingly soluble in alcohol, and freely soluble in acetone and in methanol.

Identity Tests—A 1 in 100,000 solution of cyclothiazide in methanol exhibits ultraviolet absorbance maxima at about 271 mμ [absorptivity (*a*) about 57] and 315 mμ, and absorbance minima at about 242 and 295 mμ. The spectrum is shown in Fig. 1.

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

Transfer 20 mg. of cyclothiazide to a test tube and dissolve in 5 ml. of methanol. Add 1 ml. of hydrochloric acid and warm on a steam bath for 5 min.: the liquid acquires a blue color and a yellow fluorescence.

Purity Tests—Determine the water content of cyclothiazide by the titrimetric (Karl Fischer) method: not more than 1% is found.

Char about 1 Gm. of cyclothiazide, 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 0.2%.

Assay—Transfer about 400 mg. of cyclothiazide, accurately weighed, to a 125-ml. conical flask, and dissolve in 40 ml. of ethylenediamine. Add 5 drops of a solution of *o*-nitroaniline in benzene (1 in 100), and titrate with 0.1 *N* sodium methoxide, using a magnetic stirrer and taking precautions against absorption of atmospheric carbon dioxide as by the use of an atmosphere of nitrogen. Perform a blank determination and make any necessary correction. Each milliliter of 0.1 *N* sodium methoxide is equivalent to 19.49 mg. of $C_{14}H_{16}ClN_3O_4S_2$. The amount of cyclothiazide found is not less than 97% and not more than 101.5%, calculated on the anhydrous basis.

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DOSAGE FORMS OF CYCLOTHIAZIDE

Cyclothiazide Tablets

Identity Test—The solution prepared from the tablet sample in the *Assay* exhibits absorbance maxima and minima at the same wavelengths as the cyclothiazide standard solution.

Assay—Weigh and finely powder not less than 20 cyclothiazide tablets. Weigh accurately a portion of the powder, equivalent to about 2 mg. of cyclothiazide, and transfer to a separator. Add 5 ml. of water and extract with four 25-ml. portions of a mixture of chloroform–methanol (4:1), filtering each portion through chloroform-washed cotton into a suitable beaker. Evaporate the combined extracts on a steam bath in a current of air to dryness. Transfer the residue quantitatively with the aid of about 100 ml. of methanol to a 200-ml. volumetric flask, dilute to volume with methanol, and mix. Concomitantly determine the absorbance of this solution and of a standard solution of cyclothiazide, in the same medium, at a concentration of about 10 mcg./ml., in 1-cm. cells, at the maximum at about 271 mμ, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in milligrams, of $C_{14}H_{16}ClN_3O_4S_2$ in the portion of tablets taken by the formula $0.2C \times (A_u/A_s)$, 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 cyclothiazide standard solution. The amount of cyclothiazide 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.*, has been used wherever feasible.

Cyclothiazide[1] is an oral diuretic agent useful in

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

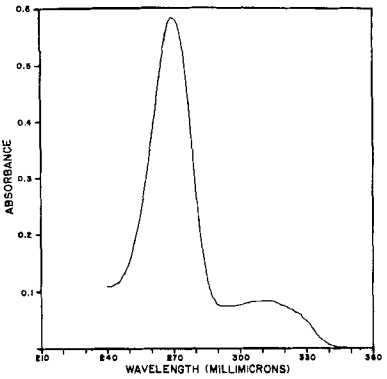

Fig. 1—Ultraviolet absorption spectrum of cyclothiazide in methanol (10 mcg./ml.); Beckman model DK-2A spectrophotometer.

conditions in which increased urinary excretion of sodium chloride and water is desirable. It is also effective for reduction of hypertension, alone or in combination with other antihypertensive agents.

Identity Tests—The identity tests included for the drug substance are satisfactory for distinguishing cyclothiazide from official compounds of similar structure. The ultraviolet absorption spectrum of cyclothiazide is significantly different from the spectrum of chlorothiazide in methanol (single absorbance maximum at 280 mμ) for identification purposes. The color and fluorescence observed when cyclothiazide is warmed in hydrochloric acid solution are not characteristic of chlorothiazide or hydrochlorothiazide and constitute a differentiating test.

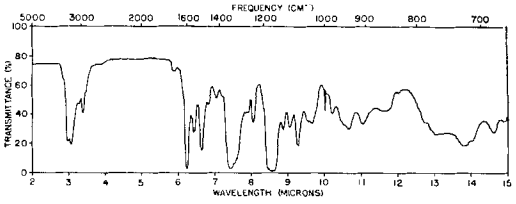

Fig. 2—Infrared spectrum of cyclothiazide in potassium bromide disk (0.5%); Perkin-Elmer model 21 spectrophotometer, sodium chloride prism.

Quantitative Tests—The nonaqueous titration of cyclothiazide with sodium methoxide using *o*-nitroaniline as the indicator gave an average value of 99.4 ± 0.3%.[2] The assay recoveries are calculated on an equivalent based on two titratable groups in the molecule for the conditions specified. Cyclothiazide dissolved in dimethylformamide and titrated with 0.1 *N* sodium methoxide to a thymol blue end point exhibits only one acidic group and a corresponding equivalent of 38.99 mg./ml. of titrant. Analysis of commercial tablets by the spectrophotometric method gave an average value of 99.2 ± 1.2%[2] of the labeled amount of cyclothiazide. The suitability of the procedure was verified by an average recovery of 100.3 ± 0.6%[2] of a standard cyclothiazide solution carried through the extractive steps as included in the tablet assay.

[2] Maximum deviation from the mean value.

Colorimetric Determination of Iodochlorhydroxyquin and Diiodohydroxyquin

By JOHN J. WINDHEUSER and DIAN Y. CHU

A method has been developed for the extraction and determination of 8-hydroxyquin derivatives utilizing cupric ion as a chelating agent. The method is highly sensitive and simple to carry out. The effect of time, pH, and cupric ion concentration has been investigated.

ALTHOUGH introduced into use as medicinal agents more than a half century ago, the halogenated derivatives of 8-hydroxyquinoline are still of significance today. The analysis of these drugs has been carried out by a number of methods. Fresenius (1) reported a colorimetric method by formation of a colored complex with ferric ion in a glacial acetic acid. The system suffered from a marked sensitivity to variation in the moisture content of the system. Haskins and Luttermaser (2) modified the system by dissolving the drugs or dried extracts from urine in 2-methoxymethanol[1] forming ferric complexes which were reported to be less sensitive to moisture variations. Both systems had the problem of interference by other phenolic compounds which might be present in the systems. Official compendia have utilized extraction techniques followed by halogen determination (3), precipitation as a metal chelate (4), and ultraviolet absorption (5). The halogen determination and metal chelate precipitation methods lack the sensitivity of the spectrophotometric methods. On the other hand, the direct spectrophotometric method suffers from interference by other absorbing species.

In this communication the development of an analytical procedure based on the formation of a

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[1] Marketed as Methyl Cellusolve by Union Carbide Corp., New York, N.Y.