(5) Mitchell, L. D., *Nature*, **170**, 621(1952).
(6) Folin, O., and Ciocalteu, V., *J. Biol. Chem.*, **73**, 627 (1927).
(7) Snyder, L. R., *Anal. Chem.*, **39**, 705(1967).
(8) Stahl, E., "Thin-Layer Chromatography," Academic Press Inc., New York, N. Y., 1965, p. 484.

Keyphrases

Diethylstilbestrol (DES)—analysis
Nitrofurazone–DES suppositories—analysis
Column chromatography—separation
TLC—identity
Densitometer—analysis

Qualitative and Quantitative Tests for Allopurinol

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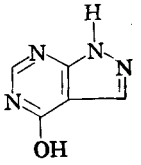
Provisional, unofficial monographs are developed by the Drug Standards Laboratory, in cooperation with the manufacturers of the drug concerned, for publication in the *Journal of Pharmaceutical Sciences*. The ready availability of this information affords discriminating medical and pharmaceutical practitioners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

1H-PYRAZOLO [3,4-*d*]-pyrimidin-4-ol; $C_5H_4N_4O$; mol. wt. 136.11. The structural formula of allopurinol may be represented as



Physical Properties—Allopurinol occurs as a white to off-white, practically odorless powder and melts above 300°. It is very slightly soluble in water and in alcohol, and practically insoluble in chloroform and in ether. It is soluble in dimethylformamide and in dilute solutions of alkali hydroxides.

Identity Tests—A 1 in 100,000 solution of allopurinol in 0.1 *N* hydrochloric acid exhibits an ultraviolet absorbance maximum at about 250 mμ [absorptivity (*a*) about 56] and a minimum at about 231 mμ. The spectrum is shown in Fig. 1.

The infrared spectrum of a 0.3% dispersion of allopurinol in potassium bromide, in a disk of about 0.82 mm. thickness, is shown in Fig. 2.

Purity Tests—Dry about 1 Gm. of allopurinol, accurately weighed, in vacuum at 105° for 5 hr.: it loses not more than 1% of its weight.

Dissolve about 10 mg. of allopurinol in 1 ml. of 0.1 *N* sodium hydroxide. Arrange for ascending

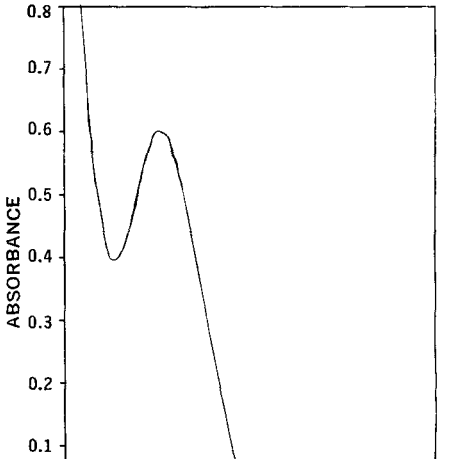


Fig. 1—Ultraviolet absorption spectrum of allopurinol in 0.1 N hydrochloric acid (10 mcg./ml.); Beckman model DK-2A spectrophotometer.

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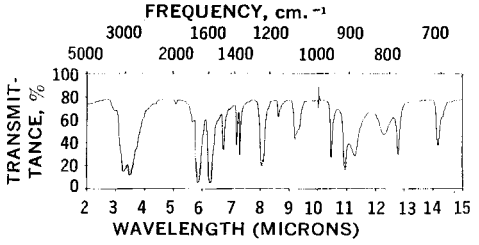

Fig. 2—Infrared spectrum of allopurinol potassium bromide disk (0.3%); Perkin-Elmer model 21 spectrophotometer, sodium chloride prism.

paper chromatography using a strip of paper (Whatman No. 1 filter paper) about 40 cm. in length. Spot 0.005 ml. on the starting line of the chromatographic paper, and suspend the paper in a chromatographic chamber equilibrated with a mobile solvent consisting of ammonium sulfate solution (1 in 20)-isopropanol (95:5). Proceed with the chromatography until the solvent front has advanced about 25 cm., remove the paper, dry, and examine under ultraviolet light: only one spot is visible in the chromatogram.

Assay—Transfer about 300 mg. of allopurinol, accurately weighed, to a 125-ml. conical flask, and dissolve in 60 ml. of dimethylformamide. Add 5 drops of thymol blue T. S., and titrate with 0.1 *N* sodium methoxide, using a magnetic stirrer and 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 13.61 mg. of $C_5H_4N_4O$. The amount of allopurinol found is not less than 97% and not more than 102%, calculated on the dried basis.

DOSAGE FORMS OF ALLOPURINOL

Allopurinol Tablets—Identity Test—The final solution prepared from the tablet sample in the *Assay* exhibits an ultraviolet maximum and minimum at the same wavelengths as the allopurinol standard solution.

Assay—Weigh and finely powder not less than 20 allopurinol tablets. Weigh accurately a portion of the powder, equivalent to about 100 mg. of allopurinol, and transfer to a 100-ml. volumetric flask. Add 10 ml. of 0.1 *N* sodium hydroxide, shake for about 5 min., dilute to volume with 0.1 *N* hydrochloric acid, and mix. Filter the solution, discarding the first portion of filtrate, and dilute a portion of the clear filtrate quantitatively and stepwise with 0.1 *N* hydrochloric acid to obtain a solution having a concentration of the equivalent of 10 mcg. of allopurinol/ml. Concomitantly determine the absorbance of the final solution and of a standard solution of allopurinol, in the same medium, at a concentration of about 10 mcg./ml., in 1-cm. cells, at the maximum at about 250 mμ, with a suitable spectrophotometer, using 0.1 *N* hydrochloric acid as the blank. Calculate the quantity, in milligrams, of $C_5H_4N_4O$ in the portion of tablets taken by the formula $10\ C \times (A_u/A_s)$, 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 allopurinol standard solution. The amount of allopurinol found is not less than 93% and not more than 107% of the labeled amount.

DISCUSSION

USP and NF terminology for solubility, melting range, reagents, *etc.*, has been used wherever feasible.

Allopurinol[1] is an oral uricosuric agent effective in the treatment of hyperuricemia associated with gout and other conditions. Its action differs from that of other uricosuric agents which lower the serum uric acid level by increasing urinary excretion of uric acid. Allopurinol reduces both the serum and urinary uric acid levels by blocking the formation of uric acid.

Identity Tests—An additional identification test for allopurinol is obtained by comparing absorbances of the hydrochloric acid solution at 231 mμ (minimum) and at 250 mμ (maximum). The ratio A_{231}/A_{250} is between 0.5 and 0.62. A ratio of 0.57 was calculated from absorbance data in the ultraviolet absorption identification of allopurinol tablets. A 1 in 100,000 solution of allopurinol in pH 11 buffer solution exhibits an ultraviolet absorbance maximum at about 262 mμ and a minimum at about 235 mμ.

Quantitative Methods—Nonaqueous titration of allopurinol with sodium methoxide using thymol blue T.S. gave an average value of 98.5 ± 0.4%.[2] Spectrophotometric analysis of commercial allopurinol tablets (100 mg.) gave an average value of 100.3 ± 0.6%[2] of the labeled amount.

[1] Marketed as Zyloprim by Burroughs Wellcome & Co., Inc., Tuckahoe, N. Y.

[2] Maximum deviation from the mean value.

Keyphrases

Allopurinol—analysis
IR spectrophotometry—identity
Paper chromatography—purity test
Sodium methoxide titration—analysis
Allopurinol tablets—analysis
UV spectrophotometry—analysis, identity