Qualitative and Quantitative Tests for Nalidixic Acid

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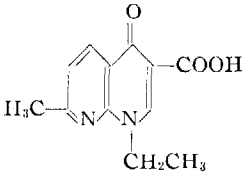
Provisional, unofficial monographs are developed by the Drug Standards Laboratory, in cooperation with the manufacturers of the drug concerned, for publication in the *Journal of Pharmaceutical Sciences*. The ready availability of this information affords discriminating medical and pharmaceutical practitioners 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-ETHYL-7-METHYL-1,8-NAPHTHYRIDIN-4-ONE-3-CARBOXYLIC ACID; $C_{12}H_{12}N_2O_3$; mol. wt. 232.24. The structural formula of nalidixic acid may be represented as:



Physical Properties.—Nalidixic acid occurs as a white to slightly yellow crystalline, odorless powder, m.p. 225–231°, U.S.P. class *Ia*. It is practically insoluble in water, slightly soluble in alcohol, soluble in chloroform, and very slightly soluble in ether. It is soluble in solutions of fixed alkali hydroxides and carbonates.

Identity Tests.—A 1 in 200,000 solution of nalidixic acid in 0.1 *N* sodium hydroxide exhibits ultraviolet absorbance maxima at about 332 and 258 mμ [absorptivity (*a*) about 111] and minima at about 276 and 236 mμ. The spectrum is shown in Fig. 1.

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

Purity Tests.—Dry about 1 Gm. of nalidixic acid, accurately weighed, at 105° for 2 hr.: it loses not more than 0.5% of its weight.

Char about 1 Gm. of nalidixic acid, 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%. Retain the residue for the heavy metals test.

Dissolve the sulfated ash obtained from 1 Gm. of nalidixic acid in a small volume of hot nitric acid and evaporate to dryness on a steam bath. Dissolve the residue in 2 ml. of diluted acetic acid, dilute to 25 ml. with water, and determine the heavy metals content of this solution by the U.S.P. heavy metals test, method I: the heavy metals limit for nalidixic acid is 20 p.p.m.

Determine the nitrogen content of nalidixic acid by the U.S.P. nitrogen determination, method II: not less than 11.77% and not more than 12.37% of nitrogen (N) is found.

Assay.—Transfer about 250 mg. of nalidixic acid, accurately weighed, to a 125-ml. conical flask, and dissolve in 30 ml. of dimethylformamide which has been previously neutralized to thymolphthalein T.S. Titrate with 0.1 *N* lithium methoxide, using a magnetic stirrer and taking precautions against the absorption of atmospheric carbon dioxide. Each milliliter of 0.1 *N* lithium methoxide is equivalent to 23.22 mg. of $C_{12}H_{12}N_2O_3$. The amount of nalidixic acid found is not less than 98% and not more than 102% of the weight of the sample taken.

DOSAGE FORMS OF NALIDIXIC ACID

Nalidixic Acid Tablets

Identity Tests.—The ultraviolet absorption spectrum of the sample solution obtained in the *Assay* exhibits maxima and minima at the same wavelengths as that of the *Standard Preparation*.

Transfer a sample of finely powdered tablets, equivalent to about 100 mg. of nalidixic acid to a glass-stoppered conical flask. Add 50 ml. of chloroform and shake for 15 min. Filter through paper and evaporate the chloroform solution on a steam bath to dryness. Dry the residue at 105° to constant weight: the resulting residue of nalidixic acid melts between 225° and 231°.

Assay.—*Standard Preparation.*—Dissolve about 100 mg. of nalidixic acid, accurately weighed, in 200.0 ml. of chloroform. Transfer 2.0 ml. of this solution to a 200-ml. volumetric flask, dilute to volume with chloroform, and mix.

Procedure.—Weigh and finely powder not less than 20 nalidixic acid tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg. of nalidixic acid, to a 125-ml.

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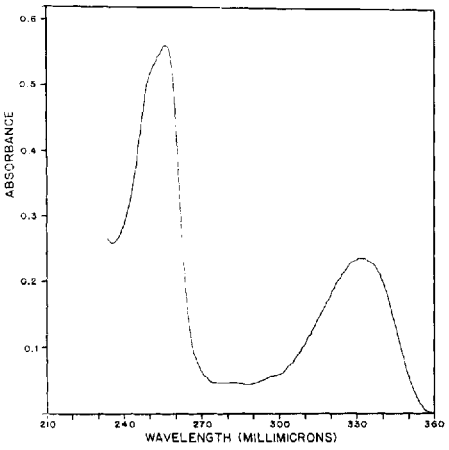

Fig. 1.—Ultraviolet absorption spectrum of nalidixic acid in 0.1 *N* sodium hydroxide (5 mcg./ml.); Beckman model DK-2A spectrophotometer.

separator containing 10 ml. of water. Completely extract the nalidixic acid with four 25-ml. portions of chloroform, filtering each portion into a 200-ml. volumetric flask, dilute to volume with chloroform, and mix. Pipet 2 ml. of this solution into a second 200-ml. volumetric flask, dilute to volume with chloroform, and mix. Concomitantly determine the absorbance of this solution and that of the *Standard Preparation* in 1-cm. cells at the wavelength of maximum absorbance at about 258 mμ with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg., of $C_{12}H_{12}N_2O_3$ in the portion of tablets taken by the formula $20\,C(A_u/A_s)$ in which C is the exact concentration, in mcg./ml., of nalidixic acid in the *Standard Preparation*, A_u is the absorbance of the solution from the tablets, and A_u is the absorbance of the solution from the tablets, and A_s is the absorbance of the *Standard Preparation*. The amount of nalidixic acid found is not less than 93% and not more than 107% of the labeled amount.

DISCUSSION

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

Nalidixic acid,[1] synthesized by Lesher and Gruett (1), is a systemic antibacterial agent which is highly effective against many Gram-negative bacteria and some Gram-positive organisms. It is particularly useful in genitourinary tract infections and can be used in other infections in which the causative agent is susceptible to the drug.

Quantitative Methods.—The nonaqueous titration of nalidixic acid with lithium methoxide using thymolphthalein T.S. gave an average value of 100.7 ± 0.1%.[2] The titration can be conducted using 0.1 *N* sodium methoxide with comparable results and thymol blue T.S. can be conveniently substituted as the visual indicator for either titrant.

[1] Marketed as NegGram by Winthrop Laboratories, New York, N. Y.

[2] Maximum deviation from the mean value.

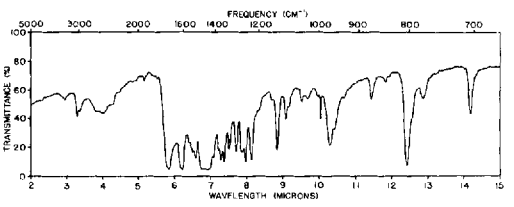

Fig. 2.—Infrared spectrum of nalidixic acid in potassium bromide disk (0.5%); Perkin-Elmer model 21 spectrophotometer, sodium chloride prism.

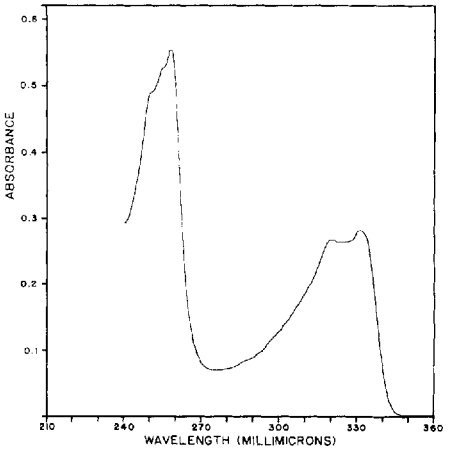

Fig. 3.—Ultraviolet absorption spectrum of nalidixic acid in chloroform (5 mcg./ml.); Beckman model DK-2A spectrophotometer.

The spectrophotometric assay for nalidixic acid tablets was designed to isolate the active ingredient prior to ultraviolet measurement. The extracted nalidixic acid is conveniently determined in chloroform solution at the absorbance maximum at either 258 or 332 mμ. The ultraviolet absorption spectrum of the *Standard Preparation* is shown in Fig. 3. Spectrophotometric analysis of commercial tablets gave an average value of 98.5 ± 0.5%[2] of the labeled amount.

An alternative method of analysis for the tablet formulations has been demonstrated by nonaqueous titration subsequent to chloroform extraction and evaporation to dryness. The residue representing about 250 mg. of nalidixic acid is dissolved in 30 ml. of dimethylformamide, previously neutralized to thymolphthalein T.S., and titrated with 0.1 *N* lithium methoxide in the manner noted for bulk nalidixic acid. An average recovery of 99.0 ± 0.4%[2] was obtained for the tablets by this procedure.

REFERENCE

(1) Lesher, G. Y., and Gruett, M. D., Belg. pat. 612,258 (July 3, 1962); through *Chem. Abstr.*, **58**, 7953(1963).