Qualitative and Quantitative Tests for Furosemide

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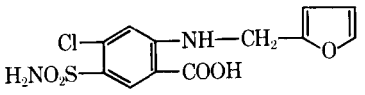
Provisional, unofficial monographs are developed by the Drug Standards Laboratory, in cooperation with the manufacturers of the drug concerned, for publication in the *Journal of Pharmaceutical Sciences*. The ready availability of this information affords discriminating medical and pharmaceutical practitioners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

4-CHLORO - *N* - FURFURYL - 5 -SULFAMOYLANTHRANILIC ACID; $C_{12}H_{11}ClN_2O_5S$; mol. wt. 330.75. The structural formula of furosemide may be represented as:



Physical Properties—Furosemide occurs as a white to slightly yellow crystalline, odorless powder. It is practically insoluble in water, sparingly soluble in alcohol, soluble in methanol, very slightly soluble in chloroform, and slightly soluble in ether. It is freely soluble in acetone, in dimethylformamide, and in solutions of alkali hydroxides.

Identity Tests—Dissolve about 5 mg. of furosemide in 10 ml. of methanol. Transfer 1 ml. to a flask, add 10 ml. of 2 *N* hydrochloric acid, and reflux for 15 min. on a steam bath. Cool, add 15 ml. of sodium hydroxide T. S. and 5 ml. of sodium nitrite solution (1 in 1000). Allow the mixture to stand 3 min., add 5 ml. of ammonium sulfamate solution (1 in 200), mix well, and add 5 ml. of *N*-(1-naphthyl)-ethylenediamine dihydrochloride solution (1 in 1000): a red to red-violet color is produced.

A 1 in 200,000 solution of furosemide in methanol exhibits ultraviolet absorbance maxima at about 234 mμ, 274 mμ [absorptivity (*a*) about 66], and 338 mμ and absorbance minima at about 247 and 293 mμ. The spectrum is shown in Fig. 1.

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

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

Char about 1 Gm. of furosemide, accurately weighed, in a tared crucible, 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 furosemide by the USP heavy metals test, method II: the heavy metals limit for furosemide is 20 p.p.m.

Assay—Transfer about 600 mg. of furosemide, accurately weighed, to a 125-ml. conical flask, and dissolve in 50 ml. of methanol. Add 3 drops of bromothymol blue T. S. and titrate with 0.1 *N* sodium hydroxide to a blue end point. Perform a blank determination, and make any necessary correction. Each milliliter of 0.1 *N* sodium hydroxide is

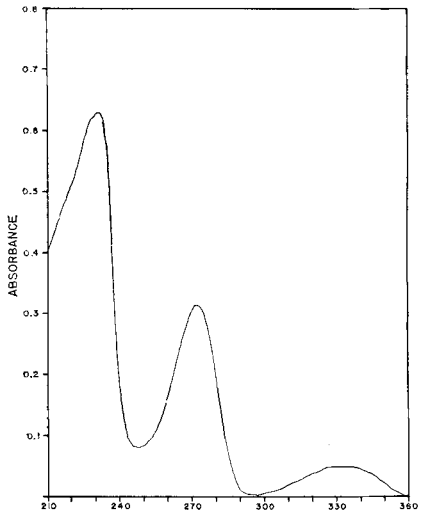


Fig 1—Ultraviolet absorption spectrum of furosemide in methanol (5 mcg./ml.); Beckman model DK-2A spectrophotometer.

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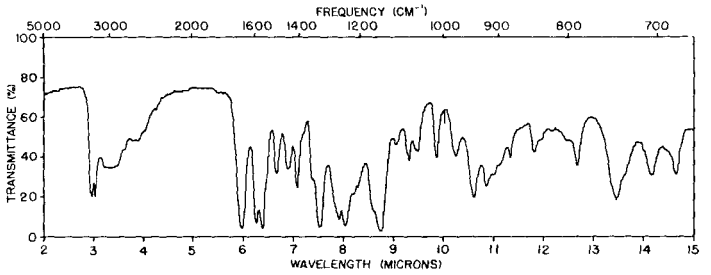

Fig 2—Infrared spectrum of furosemide in potassium bromide disk (0.5%); Perkin-Elmer model 21 spectrophotometer, sodium chloride prism.

equivalent to 33.07 mg. of $C_{12}H_{11}ClN_2O_5S$. The amount of furosemide found is not less than 98% and not more than 101% of the weight of the sample taken.

DOSAGE FORMS OF FUROSEMIDE

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

Assay—Weigh and finely powder not less than 20 furosemide tablets. Weigh accurately a portion of the powder, equivalent to about 200 mg. of furosemide, and transfer to a 100-ml. volumetric flask. Add about 50 ml. of 0.1 *N* sodium hydroxide and shake mechanically for 1 hr. Dilute to volume with the alkaline solution, and mix. Filter the solution, discarding the first portion of filtrate, and transfer 10.0 ml. into a small separator. Add 1 ml. of diluted hydrochloric acid and extract with four 25-ml. portions of a mixture of chloroform–acetone (4:1), filtering each portion through chloroform-washed paper 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. Dilute 10.0 ml. of this solution to 200.0 ml. with methanol, and mix. Concomitantly determine the absorbance of this solution and of a standard solution of furosemide, in the same medium at a concentration of about 5 mcg./ml., in 1-cm. cells, at the maximum at about 274 mμ, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in milligrams, of $C_{12}H_{11}ClN_2O_5S$ in the portion of tablets taken by the formula $40\ C \times (A_U/A_S)$, 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 furosemide standard solution. The amount of furosemide found is not less than 90% and not more than 110% of the labeled amount.

DISCUSSION

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

Furosemide[1] is an oral diuretic agent which is useful in the treatment of edema associated with congestive heart failure, cirrhosis of the liver, and renal disease, including the nephrotic syndrome. Chemically, it is distinct from organomercurials, thiazides, and other heterocyclic compounds employed as diuretics.

Identity Tests—Furosemide has been observed to melt between 205° and 207° [lit. (1) m.p. 206°] with decomposition (USP class Ia). Acid hydrolysis of furosemide yields a primary aromatic amine which is diazotized and coupled with Bratton-Marshall reagent to produce a red to red-violet color. This reaction which has been included as an identification test for furosemide has been reported by Hajdu and Haussler as a quantitative determination of the compound (2).

Quantitative Methods—Titration of furosemide with sodium hydroxide using bromothymol blue T.S. gave an average value of 100.1 ± 0.4%.[2] This determination involves neutralization of the carboxylic acid group in the molecule. A solution of furosemide in ethylenediamine titrated with 0.1 *N* sodium methoxide using azo violet indicator exhibits two acidic groups, the carboxylic acid and an acidic hydrogen. The latter is too weak an acid to titrate in aqueous solution but the acidity is promoted in a strongly basic nonaqueous medium so that titration is routine.

Analysis of commercial furosemide tablets (40 mg.) by the spectrophotometric method gave an average value of 99.2 ± 1.0%[2] of the labeled amount of furosemide. The fluorescence of furosemide in 0.01 *N* hydrochloric acid has been utilized as the basis of quantitative measurement by Hajdu and Haussler (2). Concentrations of 0.03–0.5 mcg./ml. of furosemide can be determined at the wavelength of maximum fluorescence at 410 mμ.

REFERENCES

(1) Sturm, K., Siedel, W., and Weyer, R., Ger. pat., 1,122,541 (Jan. 25, 1962); through *Chem. Abstr.*, **56**, 14032 (1962).

(2) Hajdu, P., and Haussler, A., *Arzneimittel-Forsch.*, **14**, 709(1964).

[1] Marketed as Lasix by Hoechst Pharmaceutical Company (formerly Lloyd Brothers, Inc.), Cincinnati, Ohio.

[2] Maximum deviation from the mean value.

Keyphrases

Furosemide—analysis
Identity test, color formation—furosemide
Purity test—furosemide
IR spectrophotometry—identity
Tablets, furosemide—analysis
UV spectrophotometry—analysis, identity