then be possible to predict the effect of chain length on solubility with respect to the dielectric constant.

It is felt that these rather simply determined dielectric solubility profiles may have the characteristic of being able to give significant and interpretable results with respect to solubility phenomena. Only after a large number of type compounds and derivatives have been studied can trends be established in the position of solubility maxima and the concomitant magnitude of solubility.

REFERENCES

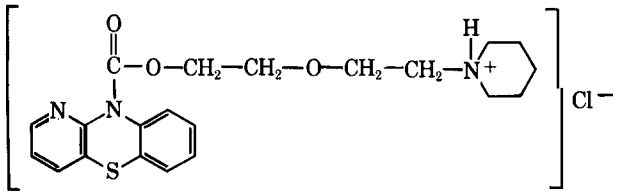
(1) Paruta, A. N., Sciarrone, B. J., and Lordi, N. G., *J. Pharm Sci.*, **51**, 704(1962).
(2) Lordi, N. G., *et al.*, *ibid.*, **53**, 463(1964).
(3) Paruta, A. N., *ibid.*, **53**, 1252(1964).
(4) Paruta, A. N., Sciarrone, B. J., and Lordi, N. G., *ibid.*, **53**, 1349(1964).
(5) *Ibid.*, **54**, 838(1965).
(6) *Ibid.*, **54**, 1325(1965).
(7) Smith, G., and Mitchell, J. I., *Pharm. J.*, **1962**, 137.
(8) Restaino, F. A., and Martin, A. N., *J. Pharm. Sci.*, **53**, 636(1964).
(9) Gorman, W. G., and Hall, G. D., *ibid.*, **53**, 1017(1964).

Drug Standards

Qualitative and Quantitative Tests for Pipazethate Hydrochloride

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.

2-(2-PIPERIDINOETHOXY)ETHYL-10 H-[3,2-*b*][1,4] pyridobenzothiazine-10-carboxylate hydrochloride; $C_{21}H_{26}ClN_3O_3S$; mol. wt. 435.97. The structural formula of pipazethate hydrochloride may be represented as



Physical Properties.—Pipazethate hydrochloride occurs as a white crystalline powder and melts at about 162° (U.S.P. XVI, class I). It is very soluble in water, and freely soluble in alcohol, and in methanol. The pH of a 2% aqueous solution of pipazethate hydrochloride is about 5.4.

Identity Tests.—Dissolve about 100 mg. of pipazethate hydrochloride in 3 ml. of water and add 1 ml. of nitric acid: a reddish color, gradually changing to amber and to yellow, is produced.

Dissolve about 100 mg. of pipazethate hydrochloride in 3 ml. of water, add ammonia T.S. until basic,

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E. R. Squibb and Sons, New York, N. Y., has cooperated by furnishing samples and data to aid in the development and preparation of this monograph.

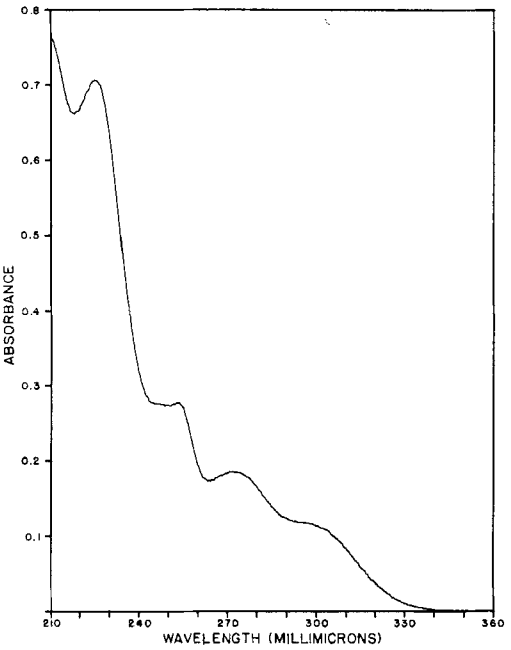

Fig. 1.—Ultraviolet absorption spectrum of pipazethate hydrochloride in methanol (16 mcg./ml.); Beckman model DK-2A spectrophotometer.

and filter. Acidify the filtrate with diluted nitric acid, and add 1 ml. of silver nitrate T.S.: a white precipitate forms, which is insoluble in diluted nitric acid, but soluble in ammonia T.S. (presence of chloride).

Dissolve about 50 mg. of pipazethate hydrochloride in 1 ml. of alcohol, transfer a drop of the solution to a piece of filter paper, and allow the alcohol to evaporate. Examine the residue under a short wavelength ultraviolet lamp (maximum emission at about 254 mμ): a white fluorescence is observed.

A 1:60,000 solution of pipazethate hydrochloride in methanol exhibits ultraviolet absorbance maxima at about 273, 254, and 226 mμ, with a shoulder at about 295 mμ [absorptivity (1%, 1 cm.) at 273 mμ about 110]. The spectrum is shown in Fig. 1.

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

Purity Tests.—Dry about 1 Gm. of pipazethate hydrochloride, accurately weighed, at 105° for 2 hr.: it loses not more than 1.0% of its weight.

Char about 1 Gm. of pipazethate hydrochloride, 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 0.25%.

Determine the heavy metals content of pipazethate hydrochloride by the U.S.P. XVI heavy metals test, method II: the heavy metals limit for pipazethate hydrochloride is 30 p.p.m.

Assay.—*Chloride.—Preparation and Standardization of 0.01 N Mercuric Nitrate.*—Dissolve about 3.3 Gm. of anhydrous mercuric nitrate in sufficient 0.01 *N* nitric acid to make 1000 ml. Transfer about 40 mg. of reagent grade potassium chloride, previously dried at 110° for 3 hr. and accurately weighed, to a 100-ml. volumetric flask, dilute to volume with water, and mix. Pipet 15 ml. into a 250-ml. beaker and proceed as directed in the assay, beginning with "To the solution add 20 ml. of alcohol . . . ." Each milliliter of 0.01 *N* mercuric nitrate is equivalent to 0.7456 mg. of KCl.

Transfer about 30 mg. of pipazethate hydrochloride, accurately weighed, to a 250-ml. beaker and dissolve it in 15 ml. of water. To the solution add 20 ml. of alcohol and 5 drops of alcoholic bromophenol blue solution (1 in 2000). Adjust the solution to a yellow color by the addition of 0.1 *N* nitric acid and add 0.5 ml. in excess. Add 5 drops of an alcoholic solution of diphenylcarbazide (1 in 200) and titrate, by means of a microburet, to a purple end point with 0.01 *N* mercuric nitrate. Perform a blank titration with the same reagents and in the same manner and apply any necessary corrections. Each milliliter of 0.01 *N* mercuric nitrate is equivalent to 0.7091 mg. of chloride (Cl). The amount of chloride found, on the anhydrous basis, is not less than 7.89% and not more than 8.38% of the weight of the sample taken.

Pipazethate Hydrochloride.—Transfer about 500 mg. of pipazethate hydrochloride, accurately weighed, to a 200-ml. tall form beaker, and dissolve it in 40 ml. of glacial acetic acid. Add 40 ml. of chloroform and 10 ml. of mercuric acetate T.S., and titrate potentiometrically with 0.1 *N* acetous perchloric acid, taking the second break in the titration curve as the end point. Each milliliter of 0.1 *N* acetous perchloric acid is equivalent to 21.80 mg. of $C_{21}H_{26}ClN_3O_3S$. The amount of pipazethate hydrochloride found, on the anhydrous basis, is not less than 98.0% and not more than 102.0% of the weight of the sample taken.

Pipazethate Hydrochloride.—Alternate Method.—Transfer about 40 mg. of pipazethate hydrochloride, accurately weighed, to a 100-ml. volumetric flask, add methanol to volume, and mix. Pipet 10 ml. of this solution into a 100-ml. volumetric flask, add methanol to volume, and mix. In a similar manner, prepare a standard solution of pipazethate hydrochloride reference standard in methanol of known concentration containing about 40 mcg./ml. Determine the absorbances of both solutions in 1-cm. cells at 273 mμ with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg., of $C_{21}H_{26}ClN_3O_3S$ in the portion of pipazethate hydrochloride taken by the formula $C(A_u/A_s)$, in which C is the concentration, in mcg./ml., of pipazethate hydrochloride reference standard

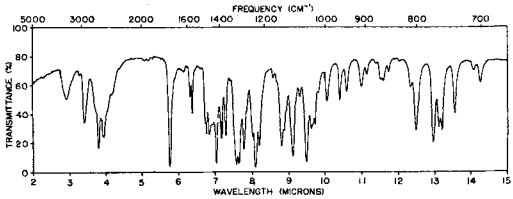

Fig. 2.—Infrared spectrum of pipazethate hydrochloride in potassium bromide disk (0.5%); Perkin-Elmer model 21 spectrophotometer, sodium chloride prism.

in the standard solution, A_u is the absorbance of the pipazethate hydrochloride solution, and A_s is the absorbance of the standard solution. The amount of pipazethate hydrochloride found, on the anhydrous basis, is not less than 98.0% and not more than 102.0% of the weight of the sample taken.

DOSAGE FORMS OF PIPAZETHATE HYDROCHLORIDE

Pipazethate Hydrochloride Tablets

Identity Tests.—Powder a suitable number of tablets and transfer an amount equivalent to about 50 mg. of pipazethate hydrochloride to a small separator. Add 2 ml. of water, swirl for a few minutes, and shake the sample with 10 ml. of ether. Allow the layers to separate and draw the aqueous phase into a centrifuge tube. Add 1 ml. of alcohol, mix, and centrifuge. Transfer a drop of the supernatant liquid to a piece of filter paper and allow the solvent to evaporate. Examine the residue under a short wavelength ultraviolet lamp (maximum emission at about 254 mμ): a white fluorescence is observed.

Assay.—*Pipazethate Hydrochloride.*—Weigh and finely powder not less than 20 tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg. of pipazethate hydrochloride, to a 100-ml. volumetric flask. Add about 40 ml. of water and 1 ml. of hydrochloric acid, stopper, and shake for about 10 min. Dilute to volume with water, and mix. Filter a portion of the solution, discarding the first 10 ml. of filtrate. Pipet 10 ml. of the filtrate into a 250-ml. separator, add 20 ml. of water, make alkaline with stronger ammonia water (about 5 drops are required), and extract with four 25-ml. portions of ether. Extract the combined ether extracts with four 25-ml. portions of dilute hydrochloric acid (1 in 100), collecting the aqueous extracts in a 250-ml. volumetric flask. Aerate to remove residual ether, add dilute hydrochloric acid (1 in 100) to volume, and mix. Dissolve a suitable quantity, accurately weighed, of pipazethate hydrochloride reference standard in dilute hydrochloric acid (1 in 100), and then dilute quantitatively and stepwise with the dilute acid to obtain a standard solution of known

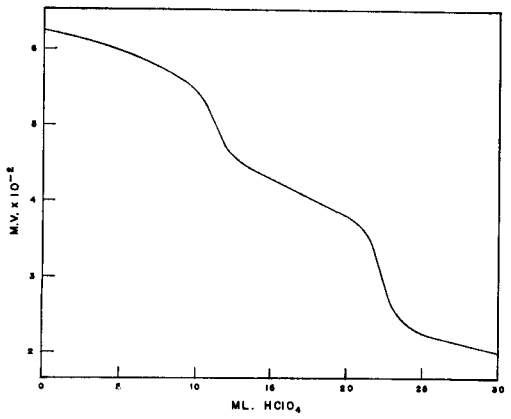

Fig. 3.—Potentiometric titration of pipazethate hydrochloride in a 4:4:1 mixture of glacial acetic acid–chloroform–mercuric acetate T.S. with 0.1 *N* acetous perchloric acid as titrant.

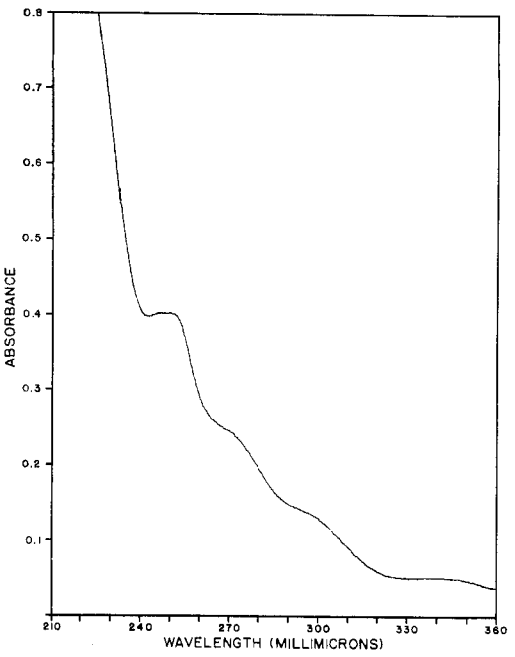

Fig. 4.—Ultraviolet absorption spectrum of pipazethate hydrochloride (20.5 mcg./ml.) in dilute hydrochloric acid (1 in 100) extracted from 10-mg. tablets.

concentration containing about 20 mcg./ml. Determine the absorbances of both solutions in 1-cm. cells at 251 mμ with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 100) as the blank. Calculate the quantity, in mg., of $C_{21}H_{26}ClN_3O_3S$ in the portion of the tablets taken by the formula $C(A_u/A_s)$, in which C is the concentration, in mcg./ml., of pipazethate hydrochloride reference standard in the standard solution, A_u is the absorbance of the solution from the tablets, and A_s is the absorbance of the standard solution. The amount of pipazethate hydrochloride 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., have been used wherever feasible.

Pipazethate hydrochloride[1] is a non-narcotic antitussive agent which is chemically, but not pharmacologically, related to the phenothiazine tranquilizers. It is a derivative of 1-azaphenothiazine.

Identity Tests.—A variety of identity tests have been included to aid in distinguishing pipazethate hydrochloride from members of the phenothiazine class. Other identity tests which may prove to be applicable to this compound may be found in a paper by Yung and Pernarowski (1).

Quantitative Methods.—The mercurimetric determination of chloride in the bulk material is a modification of the micromethod of Schales and Schales (2). Although these authors preferred the

[1] Marketed as Theratuss by E. R. Squibb and Sons, New York, N. Y.

use of diphenylcarbazone rather than diphenylcarbazide as the indicator, sharp end points were obtained with the latter in the solvent system described in this monograph. Undoubtedly, diphenylcarbazone can be used as well. The diphenylcarbazide solution should be protected from light and, preferably, should be freshly prepared as needed. Mercurimetric determination of pipazethate hydrochloride gave an average value of 99.4 ± 0.5%.[2]

The potentiometric nonaqueous titration method for pipazethate hydrochloride was developed after several solvent systems and indicator methods had been investigated. The solvent system finally chosen consisted of a 4:4:1 mixture of glacial acetic acid, chloroform, and mercuric acetate T.S. Titration with acetous perchloric acid resulted in a potentiometric titration curve which had two distinct breaks. This can be seen in Fig. 3. Titrations were performed with a Fisher titrimeter equipped with a glass and a sleeve-type calomel electrode. The breaks obtained in this solvent system were sharper than those obtained with 8:1 mixture of glacial acetic acid and mercuric acetate T.S. In both systems, however, cloudiness was noted due to the separation of an oil midway between the first and second titration breaks. No oil separation was obtained when the titration was performed in a 4:4:1 mixture of glacial acetic acid, acetonitrile, and mercuric acetate T.S. or in a 4:2:2:1 mixture of glacial acetic acid, chloroform, acetonitrile, and mercuric acetate T.S. The inclusion of acetonitrile, however, almost obliterated the second break in the curve. Metachromatic titrations with quinaldine red and methylrosaniline chloride as indicators were unsuccessful. The latter, in 8:1 glacial acetic acid–mercuric acetate T.S. mixture, turned from yellowish-green to yellow at a point approximately equivalent to the second potentiometric break, but the change was not sharp. Weaker indicators, such as Nile blue A or Sudan III (3) were not tried. Potentiometric nonaqueous titration of pipazethate hydrochloride gave an average value of 99.3 ± 0.2%.[2]

[2] Maximum deviation from the mean value.

The spectrophotometric determination of pipazethate hydrochloride in the bulk material is straightforward. In methanol, adherence to Beer's law was observed at 273 mμ as well as at 251 mμ. The higher wavelength was considered to be preferable for quantitative purposes in this case.

The spectrophotometric assay for pipazethate hydrochloride tablets was designed to eliminate ultraviolet-absorbing contaminants from the final dilution of the active ingredient. The extraction process, tested with the pure material, was found to be quantitative with an average efficiency of 99.8 ± 2.5%,[2] as determined from spectrophotometric measurements. The ultraviolet absorption curve of a tablet extract in dilute hydrochloric acid (1 in 100) is shown in Fig. 4. The absorption curve of an identical extract which had been purified by ether extraction was superimposable, except for a small reduction in absorbance in the region of 240 to 255 mμ. The extraction process removes interfering material to the extent of about 1.5% of the pipazethate hydrochloride content. Spectrophotometric assay of tablets labeled to contain 10 and 20 mg. of pipazethate hydrochloride gave average values of 100.8 ± 2.6%[2] and 100.6 ± 1.6%[2] of the labeled amounts, respectively.

REFERENCES

(1) Yung, D. K., and Pernarowski, M., *J. Pharm. Sci.*, **52**, 365(1963).

(2) Schales, O., and Schales, S. S., *J. Biol. Chem.*, **140**, 879 (1941).

(3) Bruckenstein, S., and Kolthoff, I. M., in Higuchi, T., and Brochmann-Hanssen, E., "Pharmaceutical Analysis," Interscience Publishers, Inc., New York-London, 1961, p. 820.