Applications of Procedure—Both the USP XVII procedure for vitamin D assay and the present modified procedure have been applied to (*a*) samples of vitamin E only, (*b*) a laboratory mixture of vitamins A, D, and E, (*c*) multivitamin tablets and drops, the latter including samples aged at 45°. In all cases the equivalent of 2,000 USP units of vitamin D based on label claim was taken as the original sample and the entire 2,000 units taken for the first column chromatography in both procedures.

RESULTS

The results are summarized in Table I. When only vitamin E is carried through the USP vitamin D assay procedure, a significant amount of brown color is obtained and measured as vitamin D. The additional chromatography on secondary magnesium phosphate in the modified procedure reduces this nonspecific blank to a very low level. In the model mixture of vitamins A, D, and E, the USP method overestimated the 400 units of vitamin D by 45–74% in the presence of 15 mg. of α-tocopheryl acetate. Recoveries in the two trials of the modified procedure with this combination of vitamins were 100 and 101%. Similarly, with multivitamin products the USP method overestimates the vitamin D content to a considerable degree (130–210 USP units on a total of 500–600 units). The results by the modified procedure are close to the actual levels of addition of vitamin D.

The magnitude of the interference due to tocopherol in the USP assay for vitamin D is not sharply reproducible from one sample or product to the next. On the basis of the results in Table I, it does not appear that a generally applicable correction to the USP assay value could be calculated based on the relative proportions of tocopherol and vitamin D and the dilutions used. Preliminary removal of the tocopherol *via* column chromatography on secondary magnesium phosphate provides an effective and reliable method of removing this source of interference.

REFERENCES

(1) "Recommended Dietary Allowances," 6th rev. ed., Publication 1146, Food and Nutrition Board, National Academy of Sciences, National Research Council, Washington, D. C., 1964.
(2) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965, p. 891.
(3) Bro-Rasmussen, F., and Hjarde, W., *Acta Chem. Scand.*, **11**, 34, 44(1957).
(4) Mulder, F. J., de Vries, E. J., and Keuning, K. J., *Pharm. Weekblad*, **100**, 1457(1965).

Keyphrases

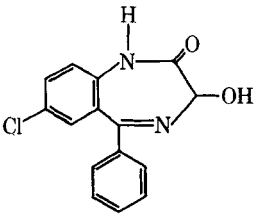
Vitamin D analysis
Vitamin E—interference elimination
Column chromatography—separation
Magnesium phosphate, secondary—column adsorbent
UV light—vitamin A fluorescence
Colorimetry—analysis

Qualitative and Quantitative Tests for Oxazepam

By EDWARD F. SALIM*, J. L. DEUBLE†, and G. PAPARIELLO†

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.

7-CHLORO-1,3-DIHYDRO-3-HYDROXY-5-PHENYL-2H-1,4-BENZODIAZEPIN-2-ONE; $C_{15}H_{11}ClN_2O_2$; mol. wt. 286.72. The structural formula of oxazepam may be represented as I.



I

Physical Properties—Oxazepam occurs as a creamy white to pale yellow, practically odorless powder. It is practically insoluble in water, slightly

Received May 24, 1967, from the *Drug Standards Laboratory, American Pharmaceutical Association Foundation, Washington, DC 20037
Accepted for publication September 20, 1967.
† Wyeth Laboratories, Philadelphia, PA 19101. Wyeth Laboratories has cooperated by furnishing samples and data to aid in the development and preparation of this monograph.
The Drug Standards Laboratory gratefully acknowledges the assistance of Miss Carolyn Damon and Miss Hannah Klein.

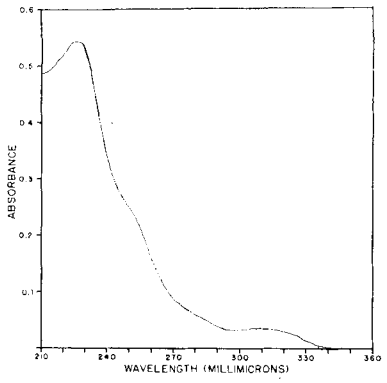


Fig. 1—Ultraviolet absorption spectrum of oxazepam in alcohol (4 mcg./ml.); Beckman model DK-2A spectrophotometer.

soluble in alcohol and in chloroform, and very slightly soluble in ether. The pH of a 2% suspension in carbon dioxide-free water is between 4.8 and 7.0.

Identity Tests—A 1 in 250,000 solution of oxazepam in alcohol exhibits an ultraviolet absorbance maximum at about 229 mμ [absorptivity (*a*) about 124]. The spectrum is shown in Fig. 1.

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

Purity Tests—Dry about 1 Gm. of oxazepam, accurately weighed, at 105° in vacuum, maintaining the pressure below 5 mm. of mercury, for 3 hr.; it loses not more than 2% of its weight.

Char about 1 Gm. of oxazepam, 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 0.3%.

Assay—Transfer about 400 mg. of oxazepam, accurately weighed, to a tall-form beaker, and dissolve in 100 ml. of dimethylformamide. Using a magnetic stirrer and taking precautions against absorption of atmospheric carbon dioxide in the sample and titrant, titrate the solution with 0.1 *N* tetrabutylammonium hydroxide [prepared in benzene–methanol (9:1)] to a potentiometric end point using glass *versus* calomel electrodes. Perform a blank determination, and make any necessary correction. Each milliliter of 0.1 *N* tetrabutylammonium hydroxide is equivalent to 28.67 mg. of $C_{15}H_{11}ClN_2O_2$. The amount of oxazepam found is not less than 98% and not more than 102%, calculated on the dried basis.

DOSAGE FORMS OF OXAZEPAM

Oxazepam Capsules

Identity Test—The ultraviolet absorption spectrum of the final solution prepared from the capsule sample in the *Assay* is similar to the spectrum obtained from the oxazepam standard solution.

Assay—Remove, as completely as possible, the contents of not less than 20 oxazepam capsules, and weigh. Transfer a quantity of mixed powder, equivalent to about 50 mg. of oxazepam, to a medium-porosity, sintered-glass funnel that is fitted into a small suction flask. Extract the sample with five 25-ml. portions of alcohol, transfer the combined

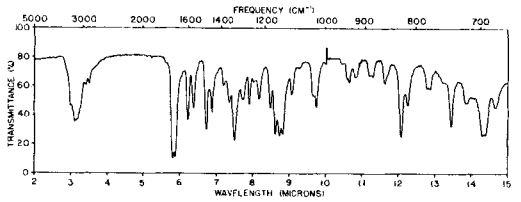


Fig. 2—Infrared spectrum of oxazepam in potassium bromide disk (0.5%); Perkin-Elmer model 21 spectrophotometer, sodium chloride prism.

extracts quantitatively to a 250-ml. volumetric flask, make to volume with alcohol, and mix. Transfer 2.0 ml. of the solution to a 100-ml. volumetric flask, dilute to volume with alcohol, and mix. Concomitantly determine the absorbance of this solution and of a standard solution of oxazepam, in the same medium, at a concentration of about 4 mcg./ml., in 1-cm. cells, at the maximum at about 229 mμ, with a suitable spectrophotometer, using alcohol as the blank. Calculate the quantity, in milligrams, of $C_{15}H_{11}ClN_2O_2$ in the portion of capsules taken by the formula $12.5 \times C(A_u/A_s)$, 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 oxazepam standard solution. The amount of oxazepam 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.

Oxazepam[1] is a psychotropic agent in the benzodiazepine class which is useful in the management and control of anxiety, tension, agitation, irritability and such related symptoms commonly seen in patients with a diagnosis of psychoneurotic or psychophysiological reaction. Oxazepam is structurally related to chlordiazepoxide and diazepam and the relative effectiveness of these compounds as tranquilizing agents has been reported (1, 2).

Identity Tests—The infrared spectrum of oxazepam constitutes an acceptable identification test for the compound. Ultraviolet absorption spectra in alcohol and 0.1 *N* alcoholic sulfuric acid can be used to differentiate the important benzodiazepine derivatives. The following tabulation indicates the wavelengths of maximum absorption for each compound in each solvent system.

	Wavelength, mμ	
	Alcohol	0.1 *N* Alcoholic Sulfuric Acid
Oxazepam	229	229
Diazepam	227	235, 280
Chlordiazepoxide hydrochloride	243, 262	247, 310

Quantitative Tests—Potentiometric nonaqueous titration of oxazepam with tetrabutylammonium hydroxide gave an average value of 98.9 ± 0.4%.[2] Preparation, standardization, and storage of the titrant have been detailed by Cundiff and Markunas (3). Determination of the end point potentiometrically is preferred to a visual titration with thymol

[1] Marketed as Serax by Wyeth Laboratories, Philadelphia, Pa.

blue T.S. since the indicator change which is somewhat gradual produces a greater variation in results. Similar titrations using azo violet or thymolphthalein were noted to give sharper end points but in each case a green color was observed at the stoichiometric point. The initial color of these indicators when added to a dimethylformamide solution of oxazepam is yellow and this leads to a green end point instead of the characteristic blue associated with the indicators.

Analysis of commercial 10-mg. oxazepam capsules by the spectrophotometric method gave a value of 97.8 ± 0.6%[2] of the labeled amount. A spectrophotometric approach can be used for analysis of bulk oxazepam provided suitable reference standard material is available for absorbance measurement.

[2] Maximum deviation from the mean value.

REFERENCES

(1) Geller, I., *Arch. Intern. Pharmacodyn.*, **149**, 243(1964).
(2) Klupp, H., and Kaehling, J., *Arzneimittel-Forsch.*, **15**, 359(1965).
(3) Cundiff, R. H., and Markunas, P. C., *Anal. Chem.*, **28**, 792(1956).

Keyphrases

Oxazepam
UV spectrophotometry–structure
IR spectrophotometry–structure
Potentiometric titration–analysis
Tetrabutylammonium hydroxide–titrant

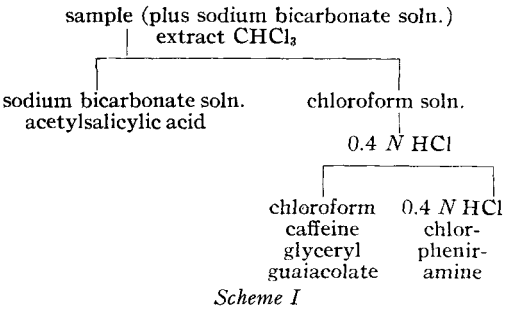
Paper Chromatographic Assay of Glyceryl Guaiacolate in a Pharmaceutical Formulation

By S. AHUJA*

A preliminary extraction scheme, which permits the separation of glyceryl guaiacolate [3-(*o*-methoxy-phenoxy)-1,2-propanediol] and caffeine from acetylsalicylic acid and chlorpheniramine maleate and other tablet excipients, has been developed. The separation of glyceryl guaiacolate and caffeine is achieved by paper chromatography. Glyceryl guaiacolate is then assayed on the basis of its absorbance in the UV region, after elution from paper chromatographic strips.

IN THIS LABORATORY, a simple and specific method was desired for assaying glyceryl guaiacolate in tablets containing glyceryl guaiacolate, acetylsalicylic acid, caffeine, and chlorpheniramine maleate in addition to the usual tablet excipients. Glyceryl guaiacolate can be determined titrimetrically (1, 2) or by gas chromatography of its acetate derivative (3). Giebelmann (4) described paper chromatographic methods (by the ascending technique) for qualitative detection of glyceryl guaiacolate. Since paper chromatography provides a good combination of a simple and specific method, this technique was investigated. This report describes a quantitative method for determination of glyceryl guaiacolate with paper chromatography (by the descending technique) with a newly developed solvent system. This solvent system provides good separations within 3 hr. Successful separation of the various constituents of the formulation is shown in Scheme I.

Glyceryl guaiacolate and caffeine are separated by paper chromatography and glyceryl guaiacolate is assayed spectrophotometrically after elution from paper chromatographic strips.



Scheme I

EXPERIMENTAL

Materials and Methods—An accurately weighed sample (equivalent to 195 mg. of glyceryl guaiacolate) is transferred to a separator. Sodium bicarbonate solution (25 ml. of 4% solution) is added and the separator is shaken well to ensure dissolution of the sample. This solution is extracted with chloroform (30 ml.) and the chloroform extract is transferred to another separator and shaken with 0.4 *N* hydrochloric acid (25 ml.). The chloroform extract is saved for paper chromatographic assay of glyceryl guaiacolate. This process is repeated

Received July 10, 1967, from Lederle Laboratories, Pearl River, NY 10965
Accepted for publication September 19, 1967.
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