Stability Assay for Prazepam and Related Drugs

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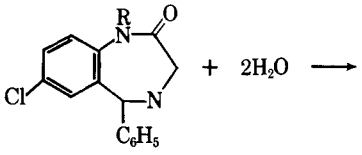
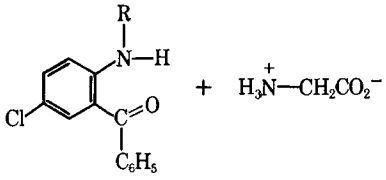
The observation that a pronounced difference in spectra of prazepam and its hydrolysis products can be produced by protonation was exploited in the development of an ultraviolet spectrometric stability assay for the drug in tablet and capsule dosage forms. The modified spectrophotometric assay provides sensitivity equivalent to that of a polarographic assay, and its precision and convenience are superior. The procedure applies equally to diazepam dosage forms.

PRAZEPAM (1) is a new psychotropic drug structurally related to diazepam. The compounds differ chemically in the alkyl substituent at the 1-position of the diazepin ring, and their analytical chemistry is closely similar. The only route of degradation observed with these benzodiazepine drugs has been hydrolysis to a benzophenone derivative and glycine. The reaction is illustrated below, where I is prazepam when R is cyclopropylmethyl; diazepam when R is methyl. A suitable assay for use in stability studies should thus discriminate between I and the 2-alkylamino-5-chlorobenzophenone (II).



I



II

Oelschläger, Volke, and Kurek (2) reported that diazepam and its hydrolysis products exhibit closely similar ultraviolet spectra in methanol solution, with absorption maxima at 230 mμ and 236 mμ, respectively, and similar absorptivities. On the basis of this limitation, they developed an oscillographic polarographic determination for the drug in the presence of its degradation products. Polarographic methods have also been reported for diazepam by Senkowski *et al.* (3) and Cimbura and Gupta (4).

Examination of the spectra of prazepam and its benzophenone hydrolysis product showed that one could measure the latter selectively in methanol solution at 408 mμ in the violet; however, it was deemed preferable to combine the stability determination with a content assay by measuring the intact drug instead of the decomposition product. Use of the 365 mμ absorbance maximum in acid–methanol was found to provide a direct measure of unhydrolyzed prazepam in dosage forms. The method was compared with conventional polarography and found to be superior in precision and convenience.

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EXPERIMENTAL

Equipment and Supplies—A Beckman DU spectrophotometer and a Beckman DK-2 recording instrument were employed with 1-cm. silica cells. A Sargent model XXI polarograph equipped with a dropping mercury electrode (DME) and a saturated calomel reference electrode (SCE) was used with the variable instrumental parameters set as follows: span, −1.0 v.; percent EMF applied, 0–50; initial, −0.6 v.; DME (−); d.c. EMF, 1.5 v. span; initial EMF, additive; sensitivity, 0.010 μamp./mm.; drop rate, 1/3–4 sec. damping, off. $E_{1/2}$-values and diffusion currents were determined by the method described by Meites (5).

A 4.08% potassium biphthalate solution was used as buffer, polysorbate 80[1] as maximum suppressor, and 2 *M* potassium chloride as the supporting electrolyte in the polarographic work. Methanol and a mixture of 3 vol. of absolute methanol diluted to 100 vol. with concentrated sulfuric acid (acid–methanol reagent) were the spectrophotometric solvents.

Spectrometric Procedure—Accurately weigh a sample corresponding to about 5 mg. of prazepam into a 100-ml. volumetric flask. Add about 50 ml. of acid–methanol reagent, mix, and dilute to the mark with acid–methanol. Filter the mixture, and determine the absorbance at the maximum, about 365 mμ. Concomitantly determine the absorbance of a dilution of reference prazepam containing about 5 mg./100 ml., accurately known, in the same solvent and at the same wavelength. Designating the absorbance of the sample A_u, the absorbance of the standard, A_s, and the concentration of the standard in mg./100.0 ml. as *C:*

prazepam, mg./cap. (tab.) =

$$A_u/A_s \times C \times \frac{\text{mg. av. cap. (tab.) wt.}}{\text{mg. sample wt.}}$$

Polarographic Method—Accurately weigh an amount of sample equivalent to about 5 mg. of prazepam in a 100-ml. volumetric flask. Add exactly 20.0 ml. of absolute methanol and shake the flask mechanically for 30 min. Add exactly 25.0 ml. of biphthalate, 1 drop of polysorbate 80, and 5.0 ml. of 2 *M* potassium chloride, and dilute the solution to the mark with distilled water. Filter the mixture through paper, discarding the first 10–15 ml. of filtrate. Rinse the polarographic cell with portions of filtrate three times, then fill the cell and deaerate with nitrogen. Insert the DME, passing nitrogen over the surface of the solution, and

[1] Marketed as Tween 80 by Atlas Chemical Industries, Wilmington, Del.

allow the cell to equilibrate 3 min. Record the polarogram. Prazepam exhibits an $E^1/_2$ of −0.83 to −0.84 v.; the $E^1/_2$ of the decomposition product is about −0.94 v.

Accurately weigh about 100 mg. of reference standard prazepam into a 100-ml. volumetric flask, add 50–60 ml. of absolute methanol, shake the mixture to dissolve the drug, and dilute to the mark with methanol. Pipet 4.0-ml. and 6.0-ml. volumes of the standard into appropriately labeled 100-ml. volumetric flasks, add 16.0 ml. and 14.0 ml. of absolute methanol to the two flasks to bring the volume in each to 20 ml., and proceed as described above for the sample, omitting filtration. Designate the concentration of the standard solution in mg./100 ml. as C, and perform the calculation using both values of C. Designating the diffusion current value of the sample as i_u and the diffusion current of the standard, i_s:

$$\text{prazepam, mg./cap. (tab.)} = C \times i_u/i_s \times \frac{\text{mg. av. cap. (tab.) wt.}}{\text{mg. sample wt.}}$$

RESULTS AND DISCUSSION

Validity of the Spectral Method—Ultraviolet spectral data obtained in methanol and in acid–methanol for prazepam, diazepam, and 2-cyclopropylmethylamino-5-chlorobenzophenone are displayed in Table I. Measurement of the absorbance at 365 mμ in acid–methanol provides a close approximation of the concentration of intact prazepam (or diazepam), since the hydrolysis product has less than 5% its absorptivity. A concomitant of the use of this wavelength is relative freedom from interference by dosage form excipients or many other drugs. The absorbance of prazepam solutions in the acid–methanol solvent was found to be stable for at least 2 hr. This is consistent with the observation of Cimbura and Gupta (4) on the relative stability of diazepam in acid solution. No degradation of prazepam was apparent by either the spectrometric or polarographic techniques in dosage forms stored up to 35 months at room temperature. In order to assess the validity of the method with degraded samples, mixtures of prazepam and molar equivalents of its degradation product were prepared to simulate various degrees of hydrolysis, and the mixtures were assayed for intact prazepam by spectrometry in acid–methanol. The results are presented in Table II.

TABLE I—ULTRAVIOLET SPECTRAL DATA FOR PRAZEPAM, DIAZEPAM, AND 2-CYCLOPROPYLMETHYLAMINO-5-CHLOROBENZOPHENONE

Compd.	λmax., mμ	ε, L./mole cm.	a, L./Gm. cm.
In Methanol			
Prazepam	227	33,350	102.5
	250i[a]	17,550	54.1
	313	2,160	6.7
Diazepam	227	32,500	114.1
	250i	16,700	58.5
	315	2,400	8.5
2-Cyclopropylmethylamino-5-chlorobenzophenone	235.5	24,800	86.7
	365	2,700	9.5
	408	7,100	24.8
In Acid–Methanol			
Prazepam	241	31,200	96.5
	285	16,300	50.2
	365	3,800	11.6
Diazepam	241	28,900	101.5
	283	13,000	45.6
	364	3,900	13.9
2-Cyclopropylmethylamino-5-chlorobenzophenone	257	12,700	44.2
	365	170	0.6

[a] Inflection.

TABLE II—RECOVERY OF PRAZEPAM FROM MIXTURES WITH ITS HYDROLYSIS PRODUCT

Prazepam	mg. Taken "Hydrolyzed Prazepam"	mg. Found Intact Prazepam
5.00	0	5.00, 5.00
4.75	0.25	4.77, 4.77
4.50	0.50	4.51, 4.50
4.00	1.00	3.97, 3.97
3.75	1.25	3.78, 3.77

The inherent advantage in precision of spectrometry over conventional polarography is demonstrated in Table III.

TABLE III—PRECISION OF ANALYTICAL METHODS FOR PRAZEPAM

Trial	Polarography $i_d \times 10^2$, μamp.[a]	Spectrometry $a_{365\ m\mu}$
1	77.04	11.51
2	79.09	11.51
3	79.27	11.50
4	81.09	11.48
5	74.60	11.75
6	73.12	11.51
Mean	77.37	11.54
Rel. S.D.	3.83%	0.90%

[a] Normalized to a concentration of 5.0 mg./100.0 ml.

SUMMARY

A simple spectrophotometric assay for the stability assessment of prazepam dosage forms has been described which uses the absorption maximum at 365 mμ of the protonated form of the drug. Recovery and precision data indicate the validity of the method in routine stability testing of prazepam formulations, and the superiority of the procedure to polarographic assay has been demonstrated. The method would be expected to be equally applicable to diazepam dosage forms because of the close structural relationship of the drugs.

REFERENCES

(1) McMillan, F. H., and Pattison, I., French pat. 1,394,287(April 2, 1965); through *Chem. Abstr.*, **63**, 8387*b* (1965); Wuest, H. M., U. S. pat. 3,192,200(June 29, 1965); through *Chem. Abstr.*, **63**, 11592*c*(1965).

(2) Oelschläger, H., Volke, J., and Kurek, E., *Arch. Pharm.*, **297**, 431(1964).

(3) Senkowski, B. Z., Levin, M. S., Urbigkit, J. R., and Wollish, E. G., *Anal. Chem.*, **36**, 1991(1964).

(4) Cimbura, C., and Gupta, R. C., *J. Forensic Sci.*, **10**, 282(1965).

(5) Meites, L., "Polarographic Techniques," Interscience Publishers, Inc., New York, N. Y., 1955, p. 60.