Potential Source of Error in Official Diazepam Assays

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Abstract □ A possible source of interference by a benzophenone hydrolysis product with the USP XIX spectrophotometric determination of diazepam in dosage forms is reported. A minor adaptation of the official assay procedures is briefly proposed as one method to correct this error.

Keyphrases □ Diazepam—spectrophotometric analysis in dosage forms, official method adapted to correct for potential interference by hydrolysis product □ Spectrophotometry—analysis, diazepam in dosage forms, official method adapted to correct for potential interference by hydrolysis product □ Hydrolysis product—diazepam, official spectrophotometric analysis in dosage forms adapted to correct for potential interference □ Sedatives—diazepam, spectrophotometric analysis in dosage forms, official method adapted to correct for potential interference by hydrolysis product

Recently, possible mechanisms for the hydrolysis of the 1,4-benzodiazepines oxazepam and diazepam (1) and chlordiazepoxide (2) to benzophenone final products were reported. 2-Methylamino-5-chlorobenzophenone and glycine were confirmed (1) as the previously reported final products of diazepam (3), but the sole, scant hydrolysis intermediate was not isolated. Depending on conditions, other medicinally useful 1,4-benzodiazepines, *e.g.*, flurazepam and clorazepate, yield benzophenone, acridone, and unspecified hydrolysis products (4, 5).

The estimation of a benzophenone degradation product in the presence of an intact 1,4-benzodiazepine derivative might be useful in the evaluation of both commercial and extemporaneous dosage forms of these drugs. A recent investigation (6), which included the estimation of diazepam and its benzophenone hydrolysis product in extemporaneous oral liquid products prepared from diazepam tablets, was the impetus for this report. An earlier clinical study using noncommercial diazepam suspensions cited no attempt to determine the stability or dispersion uniformity of the drug (7).

EXPERIMENTAL

Analytical standard quality diazepam[1] (I), mol. wt. 284.74, and 2-methylamino-5-chlorobenzophenone[2] (II), mol. wt. 245.71, were used without further purification. Diazepam injection[3] (III), 5 mg/ml, was obtained commercially. Spectra and absorbance values were determined with a recording spectrophotometer[4], and analytical grade reagents were used throughout.

Spectra of I and II were determined in alcohol USP and in acidic alcohol[5]. Beer's law plots for I and II in both solvents were linear over the 0.5–50.0-μg/ml concentration range. Concentrations of II and III were quantitatively adjusted in certain samples by adding an appropriate volume of an alcohol solution of II, 1.0 mg/ml, to the required volume of III. Otherwise, the official assay for diazepam injection (8) or the proposed method was followed.

[1] RO 5-2807, lot 545055, Hoffmann-La Roche, Nutley, N.J.
[2] RO 5-4365, lot PP-4, Hoffmann-La Roche, Nutley, N.J.
[3] Valium injection, 5 mg/ml, lot 0676-04066 (expires Apr. 1, 1978), Hoffmann-La Roche, Nutley, N.J.
[4] Beckman DB-GT grating spectrophotometer, Beckman Instruments, Fullerton, Calif.
[5] Sulfuric acid–alcohol (1:350), USP XIX (8).

DISCUSSION

From Fig. 1, it is evident that II in alcohol may be determined at approximately 405 nm without interference from I; I absorbs below 350 nm, reaching a maximum at approximately 315 nm. However, the absorbances of I and II in acidic alcohol overlap at the official wavelengths of 285 ± 2 and 368 ± 2 nm specified for diazepam tablets and injection, respectively (8).

Samples 6–8 (Table I) consisted of adequate II and III to simulate samples of diazepam injection in which approximately 5, 12.5, and 25%, respectively, of the diazepam had previously degraded to II. From the last column of Table II, it may be concluded that, according to the official method, Samples 6–8 conformed to official content standards by containing 90–110% of the labeled amounts of diazepam (8). However, the theoretical assay error values in Table II show that only Sample 6 meets the 90–110% diazepam content limits.

The pH-dependent separation of I and II by extraction of aqueous samples with chloroform was not effective. The pKa of diazepam was reported as 3.4 (9) and 3.5[6]. The pKa of II is probably very near this point because 1-methylamino-4-chlorobenzene has a pKa of about 4.3 (10), which would be lowered by the addition of an electron-withdrawing benzoyl radical *ortho* to the methylamino group.

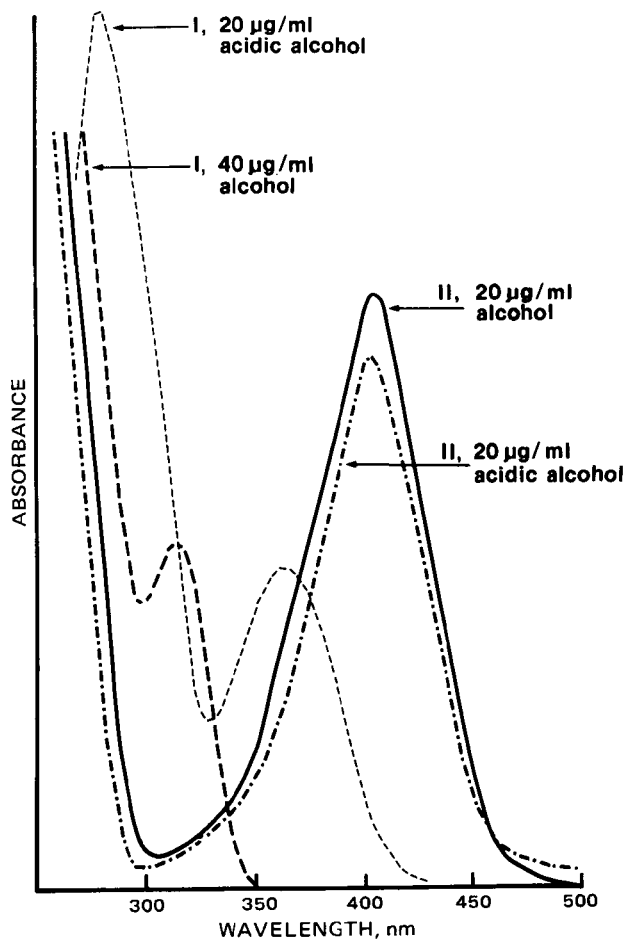

Figure 1—*Spectra of I and II in alcohol and acidic alcohol.*

[6] Dr. W. E. Scott, Hoffmann-La Roche, Nutley, N.J., Aug. 1975, personal communication.

Table I—Assay Data for Samples of I–III Determined Spectrophotometrically According to the USP XIX Procedure for III

Assay Sample[a]	Known or Labeled Quantity in Samples, mg			Absorbance, nm	
	I	II	III[b]	283	370
1	10.0	—	—	0.40	0.14
2	—	—	10.0	0.43	0.14
3	40.0	—	—	—[c]	0.56
4	—	—	40.0	—[c]	0.58
5	—	10.0	—	0.10	0.11
6	—	0.4	9.5	—[c]	0.57
7	—	1.1	8.8	—[c]	0.54
8	—	2.2	7.5	—[c]	0.51

[a] Average of three trials. [b] Volume of III expressed as diazepam content in milligrams. [c] Value exceeded scale denominations of instrument in settings used.

Table II—Assay Data for Simulated Degraded Samples of Diazepam Obtained by USP XIX Procedures

Sample	Diazepam Content, μg/ml		Error in Diazepam Content, %	
	Actual[a]	Found	Actual[b]	Found[b]
6	38.0	40.7	−5	1.8
7	35.0	38.6	−12.5	−3.5
8	30.0	36.4	−25	−9.0

[a] Based on values reported in Table I. [b] [(Content found − 40)/40] × 100.

The official assay procedures for diazepam dosage forms do not seem capable of adequately differentiating intact diazepam (I) from its final aromatic hydrolysis product (II). This is not surprising since many spectrophotometric procedures are somewhat nonspecific, being based on functional group analysis. However, the present ambiguity may be alleviated by the proposed method involving the simultaneous determination of two separate samples of diazepam dosage forms.

The first sample is processed according to official specifications (8), which permit greater sensitivity in determining diazepam spectrophotometrically. The second sample is treated accordingly, except that the chloroform extract residue is reconstituted with alcohol and the absorbance is read at 405 nm. This absorbance value multiplied by the value[7] 9.667 or 19.314 yields a quantitative estimate, in milligrams, of the amount of diazepam present as II in the original injection or tablet samples, respectively.

Neither glycine nor the yellow coloring material present in the only source of 5-mg diazepam tablets[8] marketed in the United States interferes with the determination of II. Interference by intermediate hydrolysis products other than II was not investigated.

[7] Calculated from Beer's law data for II in alcohol determined at 405 nm, dilution factors in the USP XIX procedures (8), and the formula weight ratio, 284.74 to 245.71, of I to II.

[8] Valium tablets, 5 mg, lot 6373-12225 (expires Jan. 1, 1981), Hoffmann–La Roche, Nutley, N.J.

Table III—Assay Data for Simulated Degraded Samples of Diazepam Determined According to the Proposed Method

Sample	Diazepam Content, μg/ml		Error in Diazepam Content, %	
	Actual[a]	Found[b]	Average	Range
6	38.0	39.1	2.9	2.3–3.1
7	35.0	35.6	1.7	1.2–2.5
8	30.0	30.9	3.0	2.6–3.3

[a] Based on values reported in Table I. [b] Average diazepam content corrected for interference of II with the USP XIX determination of III.

The assay results reported in Table II substantiate that significant error in the diazepam content of specimens also containing its major hydrolysis product, II, may not be detected by USP XIX assay procedures. In contrast, the data in Table III support the capability of the proposed method to quantitate diazepam accurately under these conditions.

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