Stability-Indicating High-Performance Liquid Chromatographic Assay for Oxazepam Tablets and Capsules

VAN D. REIF ˣ **and NICHOLAS J. DEANGELIS**

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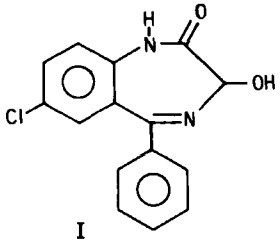
Abstract □ A stability-indicating high-performance liquid chromatographic (HPLC) assay for oxazepam in capsules and tablets was developed. The material was extracted with 2% aqueous methanol and chromatographed on a C18 reverse-phase column, which was eluted with methanol–water–acetic acid (60:40:1). A wavelength of 254 was used for detection. This assay separated oxazepam from all degradation products mentioned in the literature or observed in stress-degraded samples. Degradation products could be detected at the 0.1% level. Degradation of oxazepam to 6-chloro-4-phenyl-2-quinazolinecarboxaldehyde and 2-amino-5-chlorobenzophenone was observed after either acid or base treatment. Acidic conditions also afforded 2′-benzoyl-4′-chloroglyoxanilide and 6-chloro-4-phenyl-2(1*H*)-quinazolinone.

Keyphrases □ Oxazepam—analysis, high-performance liquid chromatography, stability, tablets, capsules □ High-performance liquid chromatography—oxazepam capsules and tablets, stability-indicating analysis □ Sedatives—oxazepam, high-performance liquid chromatographic analysis, tablets, capsules, stability indicating

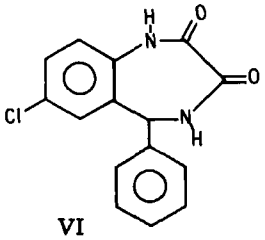
Various methods have been described for the determination of oxazepam (I), a 1,4-benzodiazepine tranquilizer. The current official method (1) employs UV spectrophotometric determination after extraction with ethanol. Increased selectivity was observed using polarographic detection (2, 3), which is dependent on reduction of the intact 4,5-azomethine bond. During GLC analysis, oxazepam decomposes to 6-chloro-4-phenyl-2-quinazolinecarboxaldehyde, II (4, 5). GLC methods, therefore, have been based on measurement of either II or 2-amino-5-chlorobenzophenone (III), which is formed by prior hydrolysis of oxazepam (6). High-performance liquid chromatographic (HPLC) methods have been described for detection and quantitation of I in biological fluids (7–11) and in mixtures of benzodiazepines (12–15). However, a selective chromatographic method for dosage forms in the presence of degradation products has not been published. This report describes the development of a specific and precise HPLC method for oxazepam tablets and capsules. Degradation products were isolated and identified to validate method specificity.

The hydrolysis of I to a benzophenone (III) was reported in 1964 (16). Formation of the aldehyde (II) after heat treatment (4, 5) and under acidic conditions (17) has been described, along with proposed mechanisms. Han *et al.* (18) reported the tentative identification of two intermediates of oxazepam hydrolysis. The interpretation of kinetic data obtained by UV measurements was, in part, substantiated by the isolation of these components. One, a prominent acid degradation product (IV) was designated as the initial product of 4,5-azomethine hydrolysis. The other (V) was predominant in base, but formed also in acid, and was designated as the product of 1,2-amide bond hydrolysis. However, neither degradation product when isolated, gave a mass spectral parent peak that corresponded to the mass of the assigned structure.

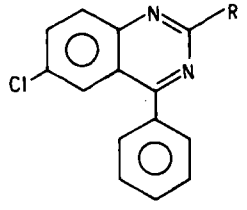
Two potential degradation products, 7-chloro-5-phenyl-4,5-dihydro-2*H*-benzodiazepine-2,3-(1*H*)-dione (VI) and 6-chloro-3,4-dihydro-4-phenyl-2-quinazolinecarboxylic acid (VII), were prepared from I by treatment with strong base (19). Two other potential degradation products, 2′-benzoyl-4′-chloroglyoxanilide (VIII) and 6-chloro-4-phenyl-2(1*H*)-quinazolinone (IX), have been reported as metabolites (20).



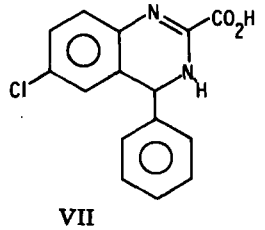
I



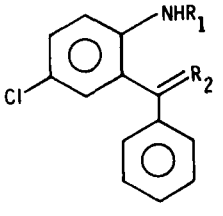
VI



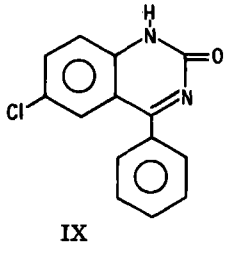
II: R = CHO
X: R = CH_2OH



VII



III: R_1 = H R_2 = O
VIII: R_1 = COCHO R_2 = O
IV: R_1 = COCHOH—NH_2 R_2 = O
V: R_1 = H R_2 = N—CHOH—CO_2H



IX

EXPERIMENTAL

Materials—The following reference compounds were synthesized according to literature procedures: II (17); VI and VII (19); and (IX) (21). Compounds VIII[1] and X[2] were obtained in-house. The mass spectra for X gave the predicted monochlorinated parent peaks at *m/z* 270 and 272, and the melting point was the same as reported (22). The melting point and IR spectra for VIII corresponded to reported data (23). Compounds I[3] and III[4] were obtained commercially.

[1] C. O. Tio, Drug Disposition Section, Wyeth Laboratories, Inc.
[2] C. L. Robinson, Chemical Development Section, Wyeth Laboratories, Inc.
[3] Wyeth Laboratories, Inc., Philadelphia, Pa.
[4] Aldrich Chemical Co., Milwaukee, Wis.

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Table I—Chromatographic Separation of Impurities and Degradation Products

Compound	HPLC[a] Retention Time, min	TLC[b] R_f
VI (Dione)	3.6	0.20
VII (Carboxylic Acid)	4.5	0
I (Oxazepam)	5.6	0.22
IX (Quinazolinone)	8.2	0.51[c]
VIII (Glyoxanilide)	11.2	0.54
II (Aldehyde)	11.8	0.76
X (Alcohol)	12.4	0.68[c]
III (Benzophenone)	18.4	0.83[d]

[a] Assay system, Chromegabond C18, E. S. Industries. [b] Chloroform–toluene–methanol (52:45:7); silica gel GF, visualization by shortwave UV. [c] Distinguished by blue fluorescence under long wavelength UV. [d] Distinguished by yellow spot with visible light.

Oxazepam Degradation—Hydrolysis conditions and TLC procedures were similar to those reported previously (18). The following 0.1 *M* buffers at 80° were used: hydrochloric acid, pH 5.5 phosphate, pH 3.2 acetate, and sodium hydroxide. Aliquots of hydrolyzed solutions were also withdrawn and analyzed directly by HPLC. TLC spots were extracted with methanol and were analyzed by HPLC and mass spectra for further identification.

Assay—The chromatograph[5] was equipped with a UV detector[6] set at 254 nm. A fixed-loop septumless injector[7] with a 10-μl loop was used in conjunction with 10-μm microparticulate reverse-phase columns[8], 25–30 cm × 4.6-mm i.d. The mobile phase consisted of methanol–water–acetic acid (60:40:1) with a 2-ml/min flow rate. An electronic integrator[9] was used for area determinations; peak heights were determined manually.

The system suitability was tested by dissolving ~10 mg of oxazepam and 15 mg of II in 250 ml of methanol; 10 μl of this solution was chromatographed using the assay conditions. The resolution factor (24) between oxazepam and II is ≥5.0. Unsuitable resolution can often be improved by a slight reduction of the mobile phase methanol concentration.

The standard was prepared by dissolving a quantity of oxazepam standard[10] in methanol–water (49:1) to give a known concentration of ~0.1 mg/ml. Samples were prepared by emptying ≥20 capsules and determining the average weight, or by weighing ≥20 finely powdered tablets. An accurately weighed portion of the powder containing ~25 mg of oxazepam was transferred to a 250-ml volumetric flask, 5 ml of water was added, and the flask was swirled to wet the powder. Approximately 200 ml of methanol was added, the solution was sonicated[11] for 5 min, stirred for 30 min, and diluted to volume with methanol. A portion was centrifuged to provide a clear solution for injection into the chromatograph.

Degradation Determination—Degradation was determined in the dosage forms with a 0.1% limit of detection. Methanol (10 ml) was added to a portion of capsule or tablet powder containing ~25 mg of oxazepam. The suspension was sonicated for 10 min and then centrifuged. Using the above HPLC conditions, 10 μl of the supernatant was injected into the chromatograph within 30 min after preparation. (After 1 hr, 0.1% of II, due to degradation of I in the methanol solution, may be detected.) The detector sensitivity was adjusted to give standard peak heights of ≥10 mm. Standards were prepared in methanol at a concentration of ~5 μg/ml.

RESULTS AND DISCUSSION

Oxazepam Degradation—With the use of similar hydrolysis and TLC conditions, three degradation products, comparable to those originally reported by Han *et al.* (18), were observed. The pH conditions necessary for their formation and the TLC R_f values matched the original data. As reported, the ultimate hydrolysis product, the benzophenone (III), was observed after either acid or base treatment. Consistent with the original results, a second degradation product, which formed with ease in acid, had a mass spectral parent peak at *m/z* 287. However, this substance was identified by TLC R_f as the glyoxanilide (VIII) and not as the initial hydrolysis product, IV, as originally stated. When eluted from the TLC plate, this acid degradation product also had the same HPLC retention time as VIII reference material (Table I), and its mass spectrum was the same as a metabolite previously identified (20) as VIII. The third degradation product, predominant in base, was identified as the aldehyde (II) by comparison of mass spectra and chromatographic retention with reference material (Table I). No degradation products were observed that could be identified as IV or V.

A yellow TLC band at R_f 0.93, identified as the Schiff-base dimer of II and III, was also observed in chloroform extracts of both acidic and basic hydrolyses solutions. A mass spectrum of the extracted TLC band gave peaks at *m/z* 231, 268, and 481, corresponding to parent peaks for

[5] Constametric II, Laboratory Data Control.
[6] Spectroflow Monitor SF-770, Schoeffel.
[7] Rheodyne 70-10.
[8] Chromegabond C18, E. S. Industries; μ-Bondapak C18, Waters Associates; Zorbax ODS (15 cm × 4.6 mm), DuPont; and Partisil ODS-3, Whatman.
[9] SP4100, Spectraphysics.
[10] USP Reference Standards, Rockville, MD 20852.
[11] Branson Ultrasonic Bath, Branson Cleaning Equipment Co., Shelton, Conn.

Table II—Method Reproducibility and Recovery

Oxazepam Product	Precision[a]
10-mg Capsule	98 ± 0.9 (height) 98 ± 1.3 (area)
30-mg Capsule	97 ± 1.9 (height) 97 ± 1.9 (area)
15-mg Tablet	99 ± 0.72 (height) 99 ± 0.35 (area)

	Recovery, %	
Composition[b]	10 parts oxazepam	30 parts oxazepam
A	99	101
B	99	101
C	101	99

[a] Average percent of label claim ± *RSD*; $n = 5$. [b] A: lactose–Ac Di Sol–stearic acid–methylcellulose (300:6:3:60) and oxazepam (10 or 30); B: dicalcium phosphate dihydrate–polacrilin potassium–starch–magnesium stearate (300:6:6:3) and oxazepam (10 or 30); C: Avicel–alginic acid–sodium starch glycolate–talc (300:6:6:3) and oxazepam (10 or 30).

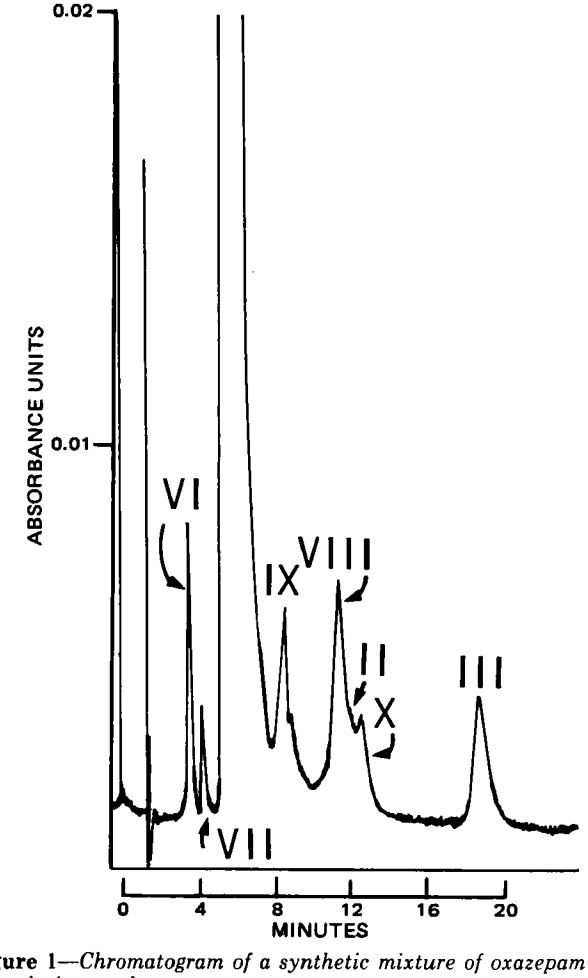

Figure 1—*Chromatogram of a synthetic mixture of oxazepam and degradation products.*

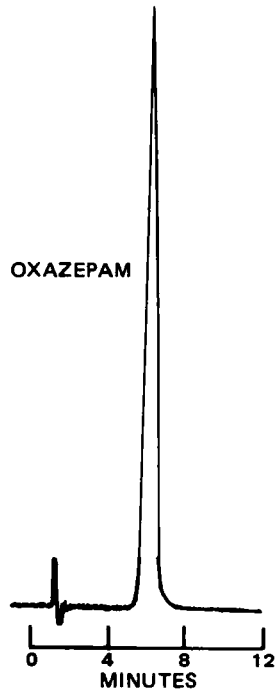

Figure 2—*Typical assay chromatogram.*

the benzophenone (III), the aldehyde (II), and the Schiff-base, respectively. Rechromatography of the extracted band gave, in addition to a spot at R_f 0.93, spots corresponding to II and III indicating that the dimer was not stable. HPLC gave only peaks corresponding to II and III. Further evidence for Schiff-base formation was the fact that the band at R_f 0.93 was obtained from chloroform standard solutions containing both II and III, but not from solutions containing II or III alone.

The quinazoline alcohol (X), the dione (VI), and the quinazolinecarboxylic acid (VII) were observed as relatively minor base-degradation products. The quinazolinone (IX) formed under either acidic or basic conditions. During method development, extraction of product composites with 0.1 *N* HCl–methanol (2:98) yielded low assays with rapid formation of IX.

The aldehyde, II, was the most prominent degradation product under stress conditions in the dosage forms. However, <0.5% was present in samples stored at room temperature for 5 years or at 45° for 6 months.

Assay—Precision data, obtained by replicate assays of commercial samples, and recovery data for synthetic mixtures of I and three combinations of commonly used excipients are reported in Table II. Placebo interference due to each mixture was <0.1% for the method. Linearity was checked by assaying solutions in the 0.06- to 0.12-mg/ml range. The chromatographic response was linear to the highest concentration tested.

The HPLC system separated I from all of the degradation products reported in the literature or observed in decomposed solutions (Table I). A separation of oxazepam from degradation products at levels of ~0.5% is shown in Fig. 1. Similar separations were achieved on the four different commercial columns tested, with the differences being minor variations in the resolution of individual impurities from each other. The columns tested passed the system suitability test. A typical assay chromatogram is shown in Fig. 2.

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