(1979).
(12) P. N. Prasad and R. V. Smith, *ibid.*, **71**, 4646 (1979).
(13) P. N. Prasad and E. D. Stevens, *ibid.*, **66**, 862 (1977).
(14) J. C. Bellows, E. D. Stevens, and P. N. Prasad, *Acta Crystallogr. Sect. B*, **34**, 3256 (1978).
(15) K. C. Cheng and E. Shefter, *Int. J. Pharm.*, **2**, 81 (1979).

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High-Performance Liquid Chromatographic Analysis of Clorazepate Dipotassium and Monopotassium in Solid Dosage Forms

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Abstract □ Methodology for the quantitative determination of clorazepate dipotassium and monopotassium in solid dosage forms was developed. Clorazepate was resolved from its degradation products, making the analysis specific and stability indicating. Analytical separation was performed on a octadecylsilylated silica column. Clorazepate was extracted from the dosage forms with 0.04% NaOH and chromatographed with aqueous 0.005 *M* tetra-*n*-butylammonium ion (pH 7.5)–acetonitrile (70:30) as the eluent. The analysis was completed in ~20 min with a precision of <2.4% *RSD*.

Keyphrases □ High-performance liquid chromatography—analysis of clorazepate dipotassium and monopotassium in solid dosage forms □ Clorazepate, dipotassium and monopotassium—high-performance liquid chromatographic analysis, solid dosage forms □ Tranquilizers—clorazepate dipotassium and monopotassium, high-performance liquid chromatographic analysis, solid dosage forms

In the past, clorazepate (I) primarily was determined chromatographically as its primary degradation product and major metabolite nordiazepam (II). Clorazepate decarboxylates to give nordiazepam; in aqueous systems (pH 2–11), the transformation is unimolecular with respect to clorazepate. Nordiazepam can undergo further metabolic transformations to oxazepam and the glucuronide in urine (1).

GLC has been used extensively (2–6) for the analysis of clorazepate as nordiazepam in biological fluids. Similarly, nordiazepam has been determined in plasma and urine by high-performance liquid chromatography (HPLC) in both reversed-phase (7–11) and normal-phase (12, 13) chromatographic modes.

Recently, a reversed-phase HPLC system for the qualitative identification of clorazepate in oral dosage forms was reported (14). With aqueous phosphate buffer (pH 8) and methanol (1:2 and 3:4) as the eluent, clorazepate was chromatographed intact on an octadecylsilylated column both with and without tetra-*n*-butylammonium ion (0.005 *M*) as an ion-pairing counterion. This paper reports the quantitation of clorazepate in its dosage forms by a similar procedure used in these laboratories for several years. The procedure has been applied to eight formulations of clorazepate (monopotassium and dipotassium), both capsules and tablets, and is specific and stability indicating.

EXPERIMENTAL

Reagents and Chemicals—Acetonitrile[1], tetra-*n*-butylammonium hydroxide[2], phosphoric acid[3], and sodium hydroxide[4] were used as received. 2,6-Dimethylaniline[5] was converted to the hydrochloride by acidification of a hexane[4] solution of the amine (10% v/v) with concentrated hydrochloric acid[6]. The precipitated amine salt was filtered, washed with hexane, and dried for use as the internal standard. Distilled water was used for all aqueous reagent and mobile phase preparations.

Apparatus—The liquid chromatograph consisted of a pump[7], a loop-type injector[8], a variable-wavelength UV detector[9], and a recorder/data handling system[10] for quantitative work. The 30-cm × 4-mm i.d. column contained an octadecylsilylated silica material[11].

Mobile Phase—A 0.005 *M* aqueous solution of tetra-*n*-butylammonium ion was prepared from 5.0 ml of tetra-*n*-butylammonium hydroxide in 1 liter of water, and the pH was adjusted[12] to 7.5 with phosphoric acid. The mobile phase was prepared by diluting 300 ml of acetonitrile to 1 liter with 0.005 *M* tetra-*n*-butylammonium-ion solution. The eluent was filtered[13] through a 0.45-μm membrane filter[14], stirred magnetically, and degassed under vacuum.

Chromatographic Conditions—The temperature was ambient, and the flow rate was ~1.8 ml/min. The detector sensitivity was set at 0.2 au (230 nm). The injector loop size was 20 μl, and the chart speed was 0.2 cm/min.

Sample Solvent—Sodium hydroxide (0.04% w/v) was filtered[13] through a 0.45-μm membrane filter[14].

Internal Standard—2,6-Dimethylaniline hydrochloride (30 mg) was dissolved in, and diluted to 100.0 ml with, the sample solvent.

Reference Standard—A solution containing 50–60 μg of clorazepate reference standard[15]/ml was prepared by dissolving it in, and diluting appropriately with, the sample solvent. An ultrasonic water bath[16] was employed to aid dissolution. A 5.0-ml portion of this solution and 2.0 ml of the internal standard solution were diluted to 10.0 ml with the sample

[1] Distilled-in-glass, Burdick & Jackson Laboratories, Muskegon, Mich.
[2] Titration grade, 1.0 *M* in methanol, Southwestern Analytical Chemicals, Austin, Tex.
[3] Reagent grade (85%), J. T. Baker Chemical Co., Phillipsburg, N.J.
[4] AR grade, Mallinckrodt, Paris, Ky.
[5] Eastman Organic Chemicals, Rochester, N.Y.
[6] Baker analyzed, J. T. Baker Chemical Co., Phillipsburg, N.J.
[7] Model M6000A, Waters Associates, Milford, Mass.
[8] Model 7120, Rheodyne, Berkeley, Calif.
[9] Model 450, Waters Associates, Milford, Mass.
[10] Model 3385A, Hewlett-Packard Corp., Rolling Meadows, Ill.
[11] μBondapak C_{18}, Waters Associates, Milford, Mass.
[12] Model 10 pH meter, Corning Scientific, Medfield, Mass.
[13] Millipore Corp., Bedford, Mass.
[14] Flotronics Division, Selas Corp., Huntingdon Valley, Pa.
[15] Clorazepate dipotassium (lot 72-307-CA) or clorazepate monopotassium (lot 64-922-AL), House Reference Standards, Abbott Laboratories, North Chicago, Ill.
[16] Bransonic 32, Branson Cleaning Equipment Co., Shelton, Conn.

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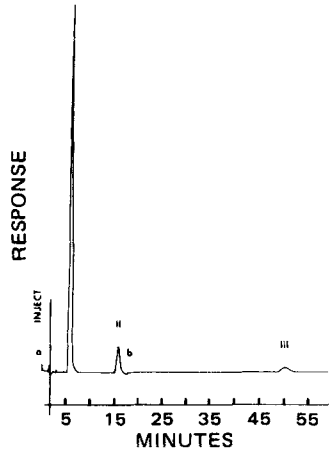


Figure 1—*Chromatogram of a mixture of 49.8 μg of clorazepate (I)/ml, 2.25 μg of nordiazepam (II)/ml, and 2.16 μg of 2-amino-5-chlorobenzophenone (III)/ml (a at 2.0 ml/min and b at 3.0 ml/min).*

solvent, giving 25–30 μg of clorazepate/ml and 60 μg of internal standard/ml in the final solution.

Sample Preparation—Sample preparations were similar for all dosage forms but varied slightly between capsule and tablet formulations.

Clorazepate Capsules—The average capsule fill weight was determined by weighing 20 filled capsules, emptying the contents as completely as possible, and reweighing the emptied shells.

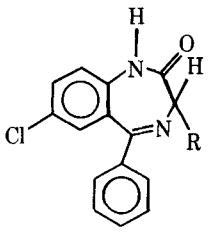
Four capsules then were weighed, the contents were transferred to a 250-ml volumetric flask, and the emptied shells were reweighed to obtain the sample weight. Approximately 200 ml of the sample solvent was added, and the flask was placed in an ultrasonic bath[16] for 5 min. The sample was diluted to volume with the sample solvent and mixed. A portion of the sample then was filtered[17] through a 0.4–0.5-μm membrane filter[18] and diluted according to dose strength with the sample solvent as follows: 3.0–3.75 mg, no dilution; 6.5–7.5 mg, 25.0 ml to 50.0 ml; and 13–15 mg, 25.0 ml to 100.0 ml. A 5.0-ml portion of the last solution and 2.0 ml of the internal standard solution were diluted with the sample solvent to 10.0 ml.

Clorazepate Tablets—The number of tablets equivalent to 112.5 mg (for 11.25- and 22.5-mg tablets) or 75 mg (for 3.75-, 7.5-, and 15.0-mg tablets) of clorazepate dipotassium were placed in a 500-ml tall form beaker, and 200.0 ml of the sample solvent was added. The sample then was homogenized[19] at ~6000 rpm for 3.0 min. A portion of the blended solution was filtered[17] through a 0.4–0.5-μm membrane filter[18] and diluted according to dose strength with the sample solvent as follows: 11.25 and 22.5 mg, 5.0 ml to 50.0 ml; and 3.75, 7.5, and 15.0 mg, 15.0 ml to 100.0 ml. A 5.0-ml portion of the last solution and 2.0 ml of the internal standard solution were diluted with the sample solvent to 10.0 ml.

Analysis and Calculation—Twenty microliters of both the standard and sample preparations were injected. Normal run time was ~20 min since nordiazepam sometimes can be observed at 14–16 min. Multiple standard preparations were analyzed, and their response (R_s) was averaged for calculation purposes:

$$R_s = \left(\frac{A_2}{A_1}\right)_{std}(C) \qquad \text{(Eq. 1)}$$

where A_1 is the peak area of clorazepate, A_2 is the peak area of the internal standard, and C is the concentration of clorazepate in the standard



I: R = COOK (monopotassium)
I·KOH: R = COOK·KOH (dipotassium)
II: R = H

[17] Swinnex 25, Millipore Corp., Bedford, Mass.
[18] A 25-mm polycarbonate membrane, Nuclepore Corp., Pleasanton, Calif.
[19] Polytron homogenizer, Brinkmann Instruments, Westbury, N.Y.

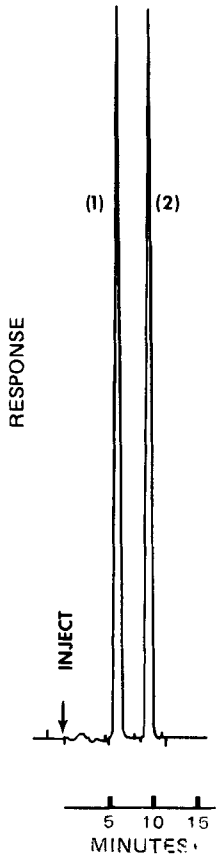


Figure 2—*Typical chromatogram of a sample preparation. Key: 1, clorazepate and 2, internal standard.*

(milligrams per milliliter).

For capsules:

$$\frac{\text{mg of clorazepate}}{\text{capsule}} = \left(\frac{A_1}{A_2}\right)_{spl} \times R_s \times 500 \times DF \times \frac{W_1}{W_2} \qquad \text{(Eq. 2)}$$

where DF is the appropriate dilution factor from the procedure and W_1 and W_2 are the average capsule fill weight and individual sample weight, respectively, in milligrams.

For tablets:

$$\frac{\text{mg of clorazepate}}{\text{tablet}} = \left(\frac{A_1}{A_2}\right)_{spl} \times R_s \times 400 \times \frac{DF}{N} \qquad \text{(Eq. 3)}$$

where N is the number of tablets used in the sample preparation.

RESULTS AND DISCUSSION

Clorazepate (I) decarboxylates readily to give nordiazepam (II) as the primary degradation product. Further acidic hydrolysis produces 2-amino-5-chlorobenzophenone (III). Other minor degradation products have been observed but at such low levels as to not be of concern in this assay.

HPLC provided the technique by which clorazepate could be separated from its degradation products for quantitation. With an octadecylsilylated stationary phase and methanol–water mixtures, poor peak shapes and retention were obtained for clorazepate. However, clorazepate chromatographed well when an eluent containing aqueous tetra-*n*-butylammonium ion (pH 7.5) and acetonitrile was employed. The use of tetra-*n*-butylammonium ion in the HPLC of weakly acidic species was previously reviewed (15, 16).

Figure 1 shows a chromatogram of clorazepate, nordiazepam, and 2-amino-5-chlorobenzophenone mixture under chromatographic conditions similar to those described here. Clorazepate was resolved from its degradation products, making the assay both specific and stability indicating.

The sample solvent (0.04% NaOH) was chosen to improve the stability of the clorazepate solutions during the time required to perform the analysis. At the pH of this solvent (~11.3), clorazepate was stable for at least 3–4 hr, after which time a gradual decline in the assay value was observed. Samples for analysis were prepared and analyzed within a few hours.

Table I—Clorazepate Analysis in Dosage Forms

Formulation	Dose, mg	Standard Addition Recovery, %[a]	Precision mg ± *SD*	Precision *RSD*, %
Clorazepate monopotassium capsules	3.25		3.38 ± 0.05	1.52
	6.25	97.9–100.2	6.49 ± 0.07	1.09
	13.0		12.82 ± 0.18	1.42
Clorazepate dipotassium capsules				
A	3.0	97.9–101.5	2.95 ± 0.07	2.36
B	3.75			
	7.5	96.3–100.5	7.56 ± 0.12	1.64
	15.0		14.78 ± 0.25	1.71
C	3.75			
	7.5	98.8–102.1	7.19 ± 0.09	1.32
	15.0			
D	7.5	98.9–101.0	7.49 ± 0.09	1.18
E	7.5	100.7–101.2	7.30 ± 0.11	1.50
Clorazepate dipotassium tablets	11.25	99.7–100.4	10.98 ± 0.09	0.84
	22.5		22.01 ± 0.27	1.25
	3.75		3.68 ± 0.02	0.51
	7.5	98.7–102.1	7.67 ± 0.05	0.68
	15.0		15.06 ± 0.18	1.21

[a] Clorazepate reference standard.

All samples analyzed in this work were routine research and quality assurance samples from in-house sources. A typical chromatogram for the analysis of a formulation is presented in Fig. 2. Nordiazepam sometimes was observed as a very small peak at ~14–16 min, in which case the total analysis time was ~20 min. The long retention time of nordiazepam prevented its quantitation under these conditions at the normally low levels (<1%) encountered in the formulations. 2-Amino-5-chlorobenzophenone and other related impurities were present at such low levels as not to be observed at all.

Figure 3 is a chromatogram of a sample that had been intentionally degraded at elevated temperature. The intact clorazepate assay was 15.5%. A large peak also was observed for nordiazepam, indicating that this procedure detects decomposition and is stability indicating.

Linearity—A plot of the ratio of the clorazepate peak area to the internal standard peak area *versus* the clorazepate concentration in the solution was linear to at least 0.3 mg/ml and passed essentially through the origin ($r > 0.999$). The analytical concentration of clorazepate was 0.025–0.030 mg/ml.

Recovery—A known amount of clorazepate was added to the various formulations to check the recovery of standard additions. Additions at 50, 100, and 150% of label claim were added to at least one dose strength within each formulation. Weighed amounts of clorazepate standard were added to either placebo formulations or previously assayed dosages containing the active ingredient. The preparations then were analyzed as described (Table I). Recoveries ranged from 96.3 to 102.1%.

Precision—Analytical precision for clorazepate was determined by replicate analysis of 10 individual sample preparations on two separate days (Table I). Different analysts also were involved in some cases. The relative standard deviations ranged from ±0.51 to ±2.36%.

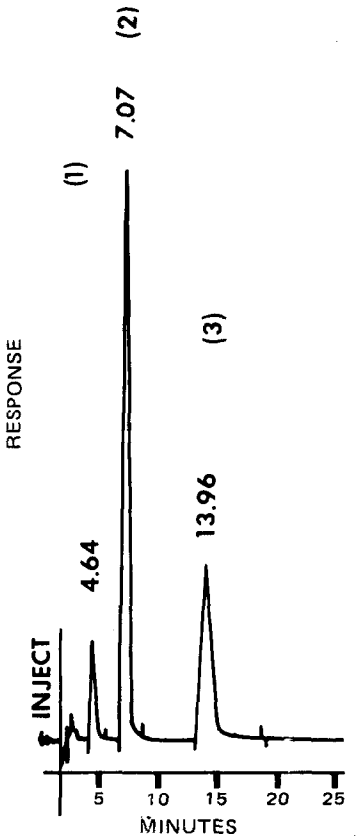


Figure 3—*Chromatogram for the analysis of a sample artificially degraded under accelerated conditions. Key: 1, clorazepate; 2, internal standard; and 3, nordiazepam.*

REFERENCES

(1) J. A. Raihle and V. E. Papendick, "Analytical Profiles of Drug Substances," vol. 4, K. Florey, Ed. Academic, New York, N.Y. 1975, pp. 91–112.

(2) D. M. Hailey, *J. Chromatogr.*, **98,** 527 (1974).

(3) C. W. Abruzzo, T. Macasieb, R. Weinfeld, J. A. Rider, and S. A. Kaplan, *J. Pharmacokinet. Biopharm.*, **5,** 377 (1977).

(4) A. H. C. Chun, P. J. Carrigan, D. J. Hoffman, R. P. Kershner, and J. D. Stuart, *Clin. Pharmacol. Ther.*, **22,** 329 (1977).

(5) P. J. Carrigan, G. C. Chao, W. M. Barker, D. J. Hoffman, and A. H. C. Chun, *J. Clin. Pharmacol.*, **17,** 18 (1977).

(6) M. A. Brooks, M. R. Hackman, R. E. Weinfeld, and T. Macasieb, *J. Chromatogr.*, **135,** 123 (1977).

(7) R. R. Brodie, L. F. Chasseaud, and T. Taylor, *ibid.*, **150,** 361 (1978).

(8) G. G. Skellern, J. Meier, B. I. Knight, and B. Whiting, *Br. J. Clin. Pharmacol.*, **5,** 483 (1978).

(9) N. Strojny, C. V. Puglisi, and J. A. F. DeSilva, *Anal. Lett.*, **B11,** 135 (1978).

(10) P. M. Kabra, G. L. Stevens, and L. J. Marton, *J. Chromatogr.*, **150,** 355 (1978).

(11) K. Hartzer and R. Barchet, *ibid.*, **132,** 83 (1977).

(12) R. J. Perchalski and B. J. Wilder, *Anal. Chem.*, **50,** 554 (1978).

(13) A. Bugge, *J. Chromatogr.*, **128,** 111 (1976).

(14) C. R. Clark and F. T. Noggle, Jr., *ibid.*, **188,** 426 (1980).

(15) R. Gloor and E. L. Johnson, *J. Chromatogr. Sci.*, **5,** 413 (1977).

(16) E. Tomlinson, T. M. Jefferies, and C. M. Riley, *J. Chromatogr.*, **159,** 315 (1978).

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