reached 60 min postdosing, and the decrease in plasma level between 3 and 12 hr was not a simple monoexponential decline. These results indicate that the method is applicable for determination of plasma concentrations of I in humans at this therapeutic dose.

DISCUSSION

The results demonstrate that GLC with a flame-ionization detector is very effective for the determination of the lipophilic drug, I, in biological fluids in the low nanogram per milliliter concentration range. Most of the endogenous material and one metabolite of I did not interfere. A minor interference peak from a blank human plasma extract was sometimes observed, but its amount was insignificant (<1% of the internal standard peak).

The lower limit of sensitivity is 20 ng/ml with a 2-ml plasma sample; however, the sensitivity can be improved to 10 ng/ml by employing larger plasma samples.

Both the accuracy and the precision of the method are good. The 20-ng/ml value shown in Table II has a larger relative standard deviation than the other three. One of the five values is approximately 60% lower than the mean of the other four values. If the 13.5-ng/ml value had been excluded, the relative standard error would have been 6%. This value is well within the range of the other relative standard errors. The 13.5-ng/ml value was probably due to random error. Since this analytical method is intended for samples from biological experiments, the levels of precision and accuracy are more than adequate. For 32 samples, including six standards, the total extraction and analysis time is 8 hr. Close to 500 human plasma samples have been analyzed successfully by this method over 1 year.

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Differential Pulse Polarographic Determination of Clorazepate Monopotassium and Dipotassium

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Abstract □ Polarographic investigation of clorazepate monopotassium and dipotassium showed two cathodic waves at about −1.28 and −1.66 v. The cathodic wave associated with clorazepate monopotassium or dipotassium at about −1.66 v was a pH-independent, diffusion-controlled wave. This wave was used to develop a specific stability-indicating procedure for clorazepate monopotassium and dipotassium in the presence of their degradation products, namely, nordiazepam, 2-amino-5-chlorobenzophenone, and glycine. The method involves a 10^{-2} *M* LiOH–10^{-1} *M* LiCl extraction of the active ingredient from the formulation, filtration, dilution with the same supporting electrolyte, and then use of the standard addition technique for drug quantitation in capsules. Typical formulation excipients did not interfere with the analysis. Accuracy and precision of the procedure were 99.55 ± 0.68%.

Keyphrases □ Clorazepate monopotassium and dipotassium—differential pulse polarographic analyses in pharmaceutical preparations □ Polarography, differential pulse—analyses, clorazepate monopotassium and dipotassium in pharmaceutical preparations □ Tranquilizers—clorazepate monopotassium and dipotassium, differential pulse polarographic analyses in pharmaceutical preparations

Several methods have been reported for the quantitative analysis of clorazepate monopotassium (I) and dipotassium (II) and their capsule formulations, including UV spectrophotometry (1), fluorometry (2), colorimetry (3), potentiometry (4), GLC (2, 5), TLC (1), and high-pressure liquid chromatography (6). One method utilized ac and differential pulse polarography for the assay of clorazepate dipotassium in its capsules (7). In this polarographic procedure, the clorazepate dipotassium was dissolved in an acetate buffer containing 10% dimethylformamide to give *N*-desmethyldiazepam after 10 min. Then quantitation was achieved through measurement of the reducible double bond C=N moiety.

The reported procedures lack specificity and, consequently, cannot be used for stability studies. In this study, differential pulse polarography was applied to achieve a specific stability-indicating procedure for the analysis of clorazepate monopotassium or dipotassium in capsule formulations.

EXPERIMENTAL

Apparatus—A polarograph[1] was equipped with a 4.0 *N* saturated calomel fiber junction reference electrode, a dropping mercury electrode at 40 cm (62 cm corrected), a platinum wire auxiliary electrode, and a drop timer[2]. An *x–y* recorder[3] was attached to the polarograph.

Reagents and Solutions—All chemicals were reagent grade[4]. The potency and purity of clorazepate monopotassium and dipotassium reference standards[5] were 94.27 and 99.77%, respectively, as ascertained by the manufacturers' physicochemical and analytical procedures.

Working standards contained 40.0 and 51.0 mg of I and II/100 ml, respectively.

The supporting electrolyte was 10^{-2} *M* LiOH–10^{-1} *M* LiCl.

[1] Model 174, Princeton Applied Research, Princeton, N.J.
[2] Model 170, Princeton Applied Research, Princeton, N.J.
[3] Model 2000, Houston Co., Houston, Tex.
[4] Fisher Scientific Co., Fair Lawn, N.J.
[5] Abbott Laboratories, North Chicago, Ill.

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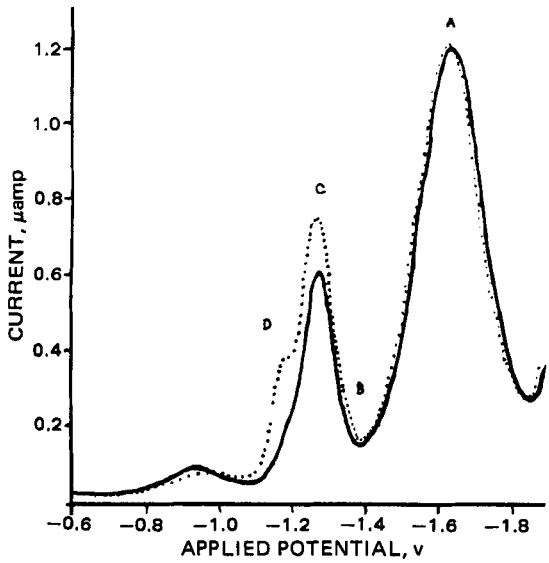

Figure 1—*Differential pulse polarogram for I, excipients, and its degradation products at a 10% level. Key: —, I reference standard and excipients; . . . ., I reference standard, excipients, and degradation products; A, I; B, 2-amino-5-chlorobenzophenone; C, increase in current due to glycine; and D, nordiazepam.*

Polarographic Conditions—The conditions were: drop time, 1 sec; scan rate, 2–5 mv/sec; display direction, positive; scan direction, negative; initial potential, −0.6 v; range, 3.0 v; sensitivity, 2–5 μafs; modulation amplitude, 25–50 mv; temperature, ambient; low pass filter, off; and output offset, off.

Polarographic Analysis of Samples—Prior to sample analysis, 50.0 ml of blank solution (10^{-2} *M* LiOH–10^{-1} *M* LiCl) was transferred into the polarographic cell, deaerated for 10 min with high purity nitrogen, and scanned from −0.6 to −2.0 v. The circuit was opened, and the cell was emptied. Then the cell was rinsed with distilled water and dried. With the previously described operating conditions, the blank polarogram should be an essentially straight, horizontal line over the whole range from −0.6 to −1.8 v.

The contents of five capsules were transferred into a 100-ml volumetric flask, about 75 ml of the supporting electrolyte solution was added, and the stoppered flask was mechanically shaken for 15 min. Then the solution was brought to volume with the same supporting electrolyte solution and filtered; the first 20 ml of filtrate was discarded. An aliquot, equivalent to 5.406 mg of I or 5.752 mg of II, of the filtered sample solution was transferred to the polarographic cell and adjusted to 45 ml with the supporting electrolyte solution. The solution was then deaerated with nitrogen for 10 min and allowed to stand for 1 min under a blanket of nitrogen. The differential pulse polarogram was recorded between −0.6 and −2.0 v. The resulting peak height at about −1.66 v was the sample peak height, *S*.

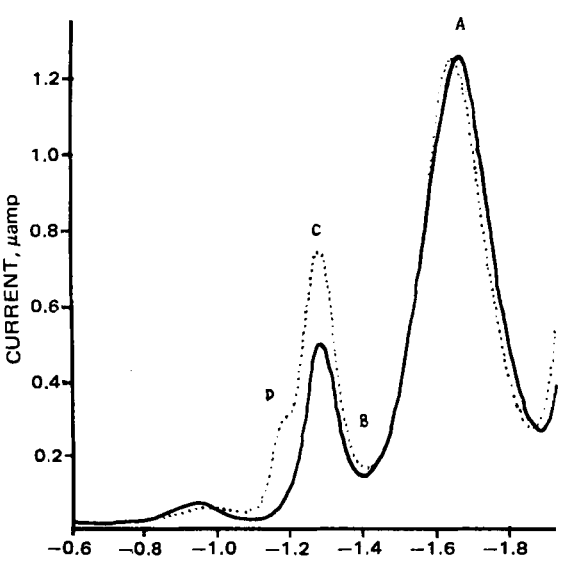

Figure 2—*Differential pulse polarogram of II, excipients, and its degradation products as a 10% level. Key: —, II reference standard and excipients; . . . ., II reference standard, excipients, and degradation products; A, II; B, 2-amino-5-chlorobenzophenone; C, increase in current due to glycine; and D, nordiazepam.*

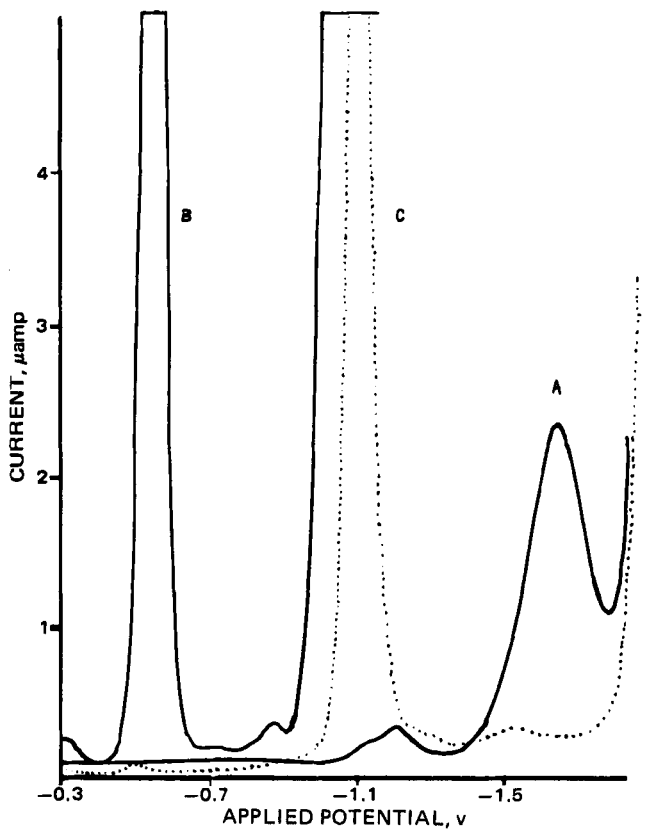

Figure 3—*Composite polarogram for the carboxylate functionality polarographic behavior of I. Polarograms of I reference standard and placebo were in 0.01* M *LiOH (A), 1.0* M *HCl (to pH 1) (B), and 1.0* M *LiOH (to pH 12) (C).*

A standard addition technique was used for quantitation. Without removing the cell, a 5.0-ml aliquot of standard solution was added. The cell was then again deaerated for 5 min with nitrogen and allowed to stand for 1 min under nitrogen, and a polarogram was recorded. The resulting peak height was the total peak height, *T*. Triplicate runs were carried out on each sample solution.

Calculations—Peak heights, *S* and *T*, were measured by drawing a horizontal line through the low point in the polarogram. The baseline was extrapolated so that it passed under the actual peak, and the peak height was then measured in millimeters:

$$\text{mg of I or II/capsule} = \frac{\text{peak height } S \times \text{clorazepate standard weight (mg)} \times \text{purity factor} \times \text{sample dilution factor}}{\left(\text{peak height } T \times \frac{50}{45} - \text{peak height } S\right) \times 100} \quad \text{(Eq. 1)}$$

RESULTS AND DISCUSSION

Determination of the polarographic behavior of I and II with typical excipients and degradation products was conducted using a 10^{-2} *M* LiOH–10^{-1} *M* LiCl solution as the supporting electrolyte. The polarograms for both I (Fig. 1) and II (Fig. 2) showed two major cathodic waves at about −1.28 and −1.66 v. The peak current for the second cathodic wave at about −1.66 v was unaffected by the presence of 10% levels of nordiazepam, 2-amino-5-chlorobenzophenone, and glycine. Nordiazepam produced a prewave on the first cathodic wave at about −1.16 v, 2-

Table I—Effect of Variation of pH on the Cathodic Waves at about −1.28 and −1.66 v ([LiCl] = 10^{-1} *M*)

	First Peak Potential at −1.28 v		Second Peak Potential at −1.66 v	
[LiOH], *M*	Peak Potential, v	Peak Height, mm	Peak Potential, v	Peak Height, mm
10^{-4}	−1.15	51.0	−1.62	43.0
10^{-3}	−1.18	28.5	−1.61	48.5
10^{-2}	−1.24	16.5	−1.62	48.5
10^{-1}	−1.28	7.9	−1.67	49.0

Table II—Effect of Variation of Ionic Strength on the Cathodic Waves at about −1.28 and −1.66 v

		First Peak Potential at −1.28 v		Second Peak Potential at −1.66 v	
[LiOH], *M*	[LiCl], *M*	Peak Potential, v	Peak Height, mm	Peak Potential, v	Peak Height, mm
10^{-2}	0	−1.24	5.5	−1.74	57.0
	10^{-2}	−1.29	6.0	−1.71	52.5
	10^{-1}	−1.24	16.5	−1.62	48.5
10^{-1}	0	−1.28	5.5	−1.71	52.0
	10^{-2}	−1.29	6.5	−1.70	51.0
	10^{-1}	−1.28	7.9	−1.67	48.0

amino-5-chlorobenzophenone produced a slight wave between the two major waves at about −1.42 v, and glycine increased the observed current at the first peak potential at about −1.28 v.

The peak potential observed at about −1.28 v is believed to be associated with the azomethine functionality (8). To establish that the cathodic wave at about −1.66 v is related to the carboxylate functionality on the intact drug molecule, the pH of the solution in the cell was adjusted to approximately pH 1 by the addition of 1.0 *N* HCl, and the polarogram for this solution was recorded. A single peak at −0.55 v was observed. Ten minutes after the addition of acid, the pH of the solution was returned to its initial pH 12 using 1.0 *M* LiOH and rescanned. The peak at about −1.66 v was no longer present, and the peak current at about −1.16 v corresponding to the nordiazepam increased significantly, indicating that complete degradation of the drug had occurred (Fig. 3). Addition of a 10-fold excess of the two possible carboxylate degradation materials, namely, carbonate and formate, produced a slight shift in peak potential, but peak current remained constant in both cases.

The behavior of the cathodic wave at about −1.66 v with variation of pH and ionic strength was studied with 8.10×10^{-5} *M* I. Basicity and supporting electrolyte concentrations were varied while polarograms were recorded in both dc and differential pulse modes in a range of 1.5 v. The range of pH studies was from pH 10 to 13 because of the relative instability of I and II in neutral or acidic media. There was no correlation between either differential pulse peak height or peak potential with the pH or ionic strength of the cathodic wave at about −1.66 v (Tables I and II).

Solutions of I in 10^{-2} *M* LiOH–10^{-1} *M* LiCl ranging from 5.04×10^{-5} to 1.90×10^{-3} *M* were used to study the effect of concentration on the cathodic wave at about −1.66 v. Polarograms were recorded in both dc and differential pulse modes. The peak current and differential pulse peak heights observed depicted the linearity of the polarographic response over the concentration range studied.

A 2.016×10^{-4} *M* solution of monopotassium clorazepate in 10^{-2} *M* LiOH–10^{-1} *M* LiCl was used to study the effect of the height of the mercury column on the cathodic wave at about −1.66 v. The height of the mercury column, corrected to include the distance to the capillary tip of the dropping mercury electrode, was varied from 28.2 to 101.9 cm. Polarograms were recorded in the dc mode with scan rate and sensitivity adjusted to give approximately the same millivolts per drop under all mercury heights (Table III). Graphical representation of current *versus* the square root of height showed a linear dependency.

Use of II instead of I in the polarographic investigation resulted only in a difference in peak current for the carboxylate functionality at about −1.66 v due to the higher molecular weight of the dipotassium salt.

Accuracy and precision of fortified sample studies are presented in Table IV.

The results obtained using the differential pulse polarographic procedure for the analysis of 3.25, 6.5, and 13.0 mg of I[6]/capsule and 3.75, 7.5, and 15.0 mg of II[7]/capsule are summarized in Table V.

Table III—Effect of Variation of Height of Mercury Column on the Cathodic Wave at about −1.66 v

Clorazepate Wave Current, μamp ($E_{1/2}$ = −1.66 v)	Corrected Height, cm	√Corrected Height, cm
0.429	28.2	5.31
0.543	43.0	6.56
0.634	57.9	7.61
0.728	73.9	8.60
0.776	88.1	9.39
0.843	101.9	10.09

Table IV—Accuracy and Precision of I and II Fortified Capsules

Added, mg/capsule		Percent[a]	
I	II	Recovery	±*SD*
3.25	—	99.7	1.4
6.5	—	100.4	1.8
13.0	—	99.8	1.0
—	3.75	99.2	1.3
—	7.50	99.8	0.8
—	15.0	98.4	2.8

[a] Mean of 10 results.

Table V—Analysis of I and II Capsules

Labeled, mg/capsule		Found, mg/capsule		
I	II	I[a]	II[b]	±*SD*
3.25	—	3.21	—	0.05
6.5	—	6.41	—	0.07
13.0	—	12.8	—	0.3
—	3.75	—	3.65	0.03
—	7.5	—	7.4	0.00
—	15.0	—	14.67	0.04

[a] Mean of six results. [b] Mean of three results.

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