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Quantitative Determination of Chlorthalidone in Pharmaceutical Dosage Forms by High-Pressure Liquid Chromatography

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Received October 25, 1977, from the *Department of Analytical Research and Development, USV Pharmaceutical Corporation, Tuckahoe, NY 10707.* Accepted for publication May 29, 1978.

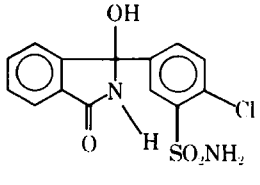
Abstract □ A reliable and selective high-pressure liquid chromatographic procedure for the quantitative determination of chlorthalidone in pharmaceutical dosage forms is described. A comparison of this stability-indicating procedure with the USP spectrophotometric assay is presented for chlorthalidone tablets and chlorthalidone tablets containing reserpine.

Keyphrases □ Chlorthalidone—high-pressure liquid chromatographic analysis in pharmaceutical dosage forms □ High-pressure liquid chromatography—analysis, chlorthalidone in pharmaceutical dosage forms □ Diuretics—chlorthalidone, high-pressure liquid chromatographic analysis in pharmaceutical dosage forms

Chlorthalidone (I), 2-chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl)benzenesulfonamide, is an oral antihypertensive–diuretic administered alone and in combination with reserpine in tablets[1]. Several methods have been reported for the determination of chlorthalidone in biological media (1–4) and for its chromatographic separation and detection in the presence of other substances (5, 6). At present, there is no simple direct method for the quantitative and stability determinations of chlorthalidone in pharmaceutical dosage forms.

The USP method (7) consists of solvent extraction of the powdered tablet material and subsequent quantitative comparison of the UV absorption of a solution of the extracted chlorthalidone with the absorption of a solution of USP reference standard chlorthalidone run concomitantly. The compendial method lacks analytical specificity and selectivity for the active drug substance and is, therefore, not a reliable stability-indicating assay.

This report describes a high-pressure liquid chromatographic (HPLC) procedure that is simple, direct, and specific for chlorthalidone in tablets as the single active drug substance and in combination with reserpine.



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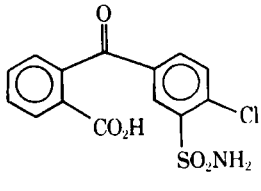

II

[1] Marketed by the USV Pharmaceutical Corp. under the trade name of Hygroton and in combination with reserpine under the trade name of Regroton.

EXPERIMENTAL

Reagents—Commercial distilled-in-glass solvents[2] and analytical reagent grade acetic acid[3] were used without additional purification.

Apparatus—A high-pressure liquid chromatograph[4], interfaced to an electronic integrator[5], was equipped with a fixed wavelength (254 nm) UV absorption detector and a constant volume injection valve[6].

Column—The column consisted of a stainless steel tube (1m × 2.2 mm i.d.) prepacked with a polyamide-coated stationary phase[7].

External Standard Solution—The external standard solution was prepared by accurately weighing approximately 50 mg of chlorthalidone reference standard[8], transferring the sample to a 25.0-ml volumetric flask, and dissolving and diluting it to volume with acetonitrile–water (9:1 v/v).

Sample Preparation—Twenty tablets were weighed and ground to a fine powder. An accurately weighed sample of the ground powder, equivalent to approximately 50 mg of chlorthalidone, was extracted with 25.0 ml of acetonitrile–water (9:1 v/v) in a 50-ml screw-capped centrifuge tube by vigorous agitation on a mixer[9] for 15 min. Then the sample was centrifuged, and the supernate was saved for chromatographic analysis.

Assay—The mobile phase, 2-propanol–acetic acid–water–*n*-hexane (30:1.5:0.5:68 v/v) was pumped through the column at a flow rate of 2 ml/min with a column head pressure of approximately 35 kg/cm². The fixed wavelength detector was operated at an attenuation of 0.32 aufs, and the column oven was maintained at 35° throughout the analysis.

Aliquots (10 μl) of the standard and sample solutions were injected in

[2] Burdick and Jackson Laboratories, Muskegon, Mich.
[3] Mallinckrodt, St. Louis, Mo.
[4] Model 830, E. I. DuPont de Nemours, Wilmington, Del.
[5] Autolab, System IV B, Spectra-Physics, Santa Clara, Calif.
[6] Six port equipped with 10-μl loop, Valco Instruments Co., Houston, Tex.
[7] Pellamidon, Whatman, Clifton, N.J.
[8] USP reference standard.
[9] Vortex Genie, Scientific Industries, Bohemia, N.Y.

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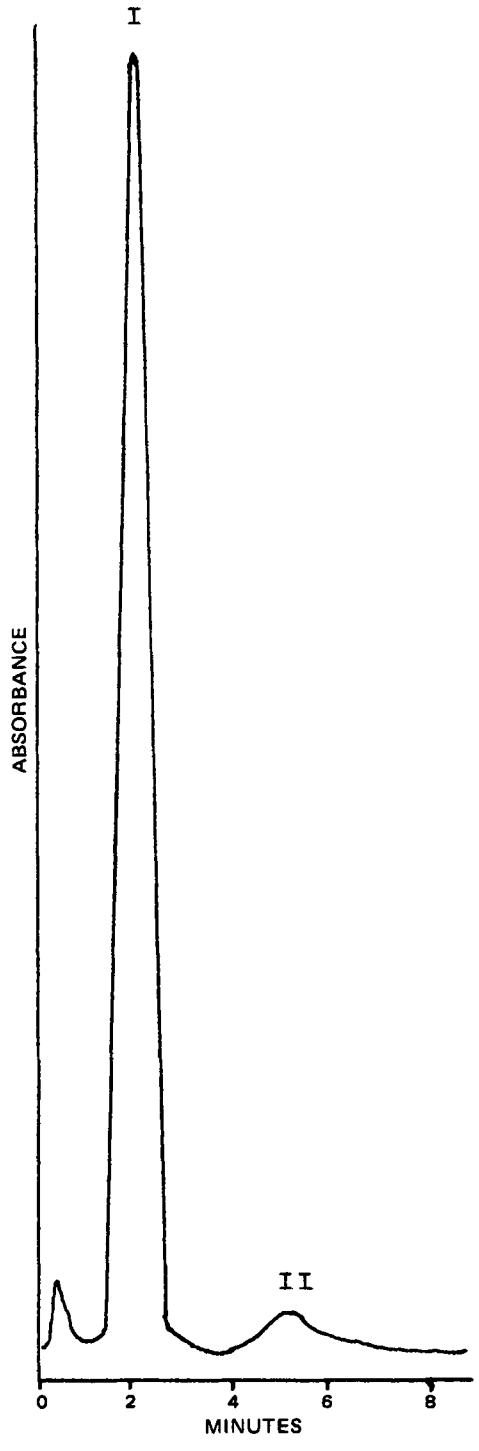

Figure 1—*Representative high-pressure liquid chromatogram.*

duplicate through the constant volume injection valve. Peak heights, determined manually, were used to calculate the average tablet content of chlorthalidone with the following equation:

$$\text{mg of chlorthalidone/tablet} = F \times H \times \frac{\text{average tablet weight (mg)}}{\text{weight of sample (mg)}} \quad \text{(Eq. 1)}$$

where H is the peak height of chlorthalidone in the sample solution and F is the ratio of the standard weight (milligrams) to the peak height of chlorthalidone in the standard solution.

Alternatively, satisfactory results were obtained on an electronic integrator programmed to measure peak area and to calculate the dosage of chlorthalidone per tablet.

Table I—Accuracy, Precision, and Percent Recovery of Chlorthalidone from Spiked Tablet Placebo by HPLC

Standard Mixture	Placebo, mg	Chlorthalidone, mg Added	Chlorthalidone, mg Found	Recovery, %
1	140	50.47	49.6	98.3
2	140	50.23	49.6	99.3
3	140	50.86	51.4	101.1
4	140	50.92	50.4	99.0
5	280	50.16	50.2	100.1
6	280	50.72	50.8	100.2
7	280	50.47	50.5	100.1
8	280	50.60	50.1	99.0
9	560	50.62	50.0	98.8
10	560	50.57	50.8	100.5
11	560	50.69	52.0	102.6
12	560	50.17	50.4	100.5
Average				100.0
Average deviation				0.89
SD				1.18

RESULTS AND DISCUSSION

The quantitative determination of chlorthalidone in experimental tablets with and without reserpine was accomplished by HPLC using a stainless steel column packed with a polyamide stationary phase. The polyamide column separates organic substances according to subtle differences in polarity and degree of hydrogen bonding (8).

The separation of chlorthalidone from its hydrolysis product (3), 4′-chloro-3′-sulfamoyl-2-benzophenone carboxylic acid (II), by the proposed method is shown in Fig. 1. The chromatogram was obtained by the analysis of a solution spiked with I and II. A trace amount of water was added to the described mobile phase to prevent unwanted effects caused by column dehydration. Acetic acid was necessary to control the extent of hydrogen bonding to the stationary phase, thereby providing a comparatively sharp symmetrical peak. Thus, by maintaining the recommended composition of the mobile phase, a reliable and quantitative separation of chlorthalidone was achieved. Since reserpine and normal tablet excipients have shorter retention times, they do not interfere with the analysis.

A statistical evaluation of this method is presented in Table I. The results on 12 consecutive analyses of standard mixtures, consisting of the addition of chlorthalidone to the tablet placebo, demonstrate its accuracy and precision. A linear relationship of sample size *versus* peak height was obtained over the concentration range of interest by varying the addition of chlorthalidone from 40 to 60 mg. Since no internal standard could be found, the concentrations of chlorthalidone in unknown samples were determined by comparison of peak heights of the drug in unknown samples with those in standard mixtures. Excellent results were obtained when the chromatographic injections were performed with a constant volume injection valve. The relative standard deviation for this procedure was 1.2%, and the repetitive analysis of a single solution of chlorthalidone gave a relative standard deviation of 0.89%.

Table II—Comparison between the HPLC and USP Methods on Chlorthalidone in Experimental Tablets

Sample[a]	Dosage Claimed, mg/tablet	Chlorthalidone Found, mg/tablet HPLC	Chlorthalidone Found, mg/tablet USP
1	12.5	12.8	12.4
2	25.0	25.2	24.2
3	50.0	50.3	50.8
4	100.0	99.1	100.0
5	75.0	74.6	74.8
6	100.0	98.8	100.0
7	100.0	101.4	100.0
8	100.0	101.2	100.7
9	100.0	99.0	100.6
10	50.0	50.3	50.8
11	50.0	49.1	51.8
12	50.0	50.5	51.0
13	50.0	50.3	50.6
14	50.0	49.2	50.8
15	50.0	50.0	50.9
16	50.0	50.2	51.3

[a] Experimental tablets without reserpine are Samples 1–12; experimental tablets with reserpine are Samples 13–16.

The experimental data in Table II were obtained on randomly selected samples of experimental tablets containing chlorthalidone with and without reserpine. The samples were analyzed in duplicate for chlorthalidone by the HPLC method and the conventional USP procedure. The results from both methods are comparable. However, since the HPLC method is stability indicating with respect to the hydrolysis product, II, this procedure is more reliable than the USP method and more accurately reflects the actual concentration of chlorthalidone per tablet dose.

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Anticonvulsant Activity of Alkyl-Substituted *N*-Benzylcyanoacetamides

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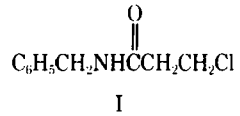
Received February 6, 1978, from the *School of Pharmacy, Auburn University, Auburn, AL 36830.* Accepted for publication June 1, 1978.

Abstract □ Thirteen new derivatives of 2-alkyl- and 2,2-dialkyl-*N*-benzylcyanoacetamide, a cyano analog of beclamide, were synthesized and tested for anticonvulsant activity. The unsubstituted compound was more active and more toxic than the derivatives. No activity was observed when the alkyl substituents in the symmetrically disubstituted derivatives contained a total of six or more carbon atoms or when benzyl was a substituent. The monosubstituted compounds were more toxic than the disubstituted compounds.

Keyphrases □ *N*-Benzylcyanoacetamides, alkyl substituted—synthesized, evaluated for anticonvulsant activity in mice □ Anticonvulsant activity—alkyl-substituted *N*-benzylcyanoacetamides evaluated in mice □ Structure-activity relationships—*N*-benzylcyanoacetamides evaluated for anticonvulsant activity in mice

Although early reports (1–3) of the use of beclamide (I) in the treatment of grand mal and psychomotor seizures were promising, the usefulness of the agent has proved to be disappointing. Beclamide has the characteristic structural arrangement of the common antiepileptics (1), as shown in II. The fact that the α-carbon atom only bears a single substitution has been suggested as the reason for the variable results obtained clinically.

Schwartz *et al.* (4, 5) reported the anticonvulsant activity of a number of substituted cyanoacetamides. Although several C-monoalkyl cyanoacetamides were prepared as intermediates, only the C-dialkylated products were tested for anticonvulsant activity. Activity against electroshock, sedative activity, and toxicity increased with increasing hydrocarbon substitution on the α-carbon atom up to dipropyl, which was the most highly substituted compound tested (4). Also, *N*-methylation and *N*-dimethylation caused a significant decrease in potency, while



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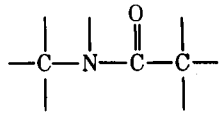

II

N-propylation and *N*-cyclization (pyrrolidine, piperidine, and morpholine) led to inactive compounds (4).

This report describes the preparation and activity of several substituted *N*-benzylcyanoacetamides as cyano analogs of beclamide. The substituents were chosen to study the effectiveness of substituting a nitrile group for a chloromethyl group in beclamide and the degree of hydrocarbon substitution that would retain activity in this series of cyanoacetamides.

EXPERIMENTAL[1]

2-Substituted *N*-Benzylcyanoacetamides (IV–XI and XVI)—These compounds were synthesized by a modification of the reported method (5). Compound III (6) (17.4 g, 0.10 mole) was added to a solution of potassium hydroxide (5.6 g, 0.10 mole, or 11.2 g, 0.20 mole) in formamide (50–75 ml). The mixture was stirred at ambient temperature for 1 hr, and the appropriate alkyl bromide (0.10 or 0.20 mole) was added.

For the preparation of the monosubstituted compounds, the mixture was stirred at ambient temperature for 3 hr; for the disubstituted derivatives, the mixture was refluxed for 1–3 hr. Then the reaction mixture was poured into ice water with stirring and filtered. The residue was recrystallized from the appropriate solvent (Table I) and activated charcoal.

The NMR spectra were consistent with the proposed structures. The NMR spectra of IV–XI showed the following common absorption peaks ($CDCl_3$): δ 7.15–7.25 (s, 5H or 10H, ArH) and 4.30–4.40 (d, 2H, NCH_2Ar) ppm. Additional absorption peaks were noted for: IV, 1.60 (s, 6H, CH_3) ppm; and V, VII, IX, and XI, 3.30–3.35 (m, 1H, CH) ppm. Depending on the alkyl substituent (V–X), the absorption in the hydrocarbon region ranged from δ 0.70 to 2.50 ppm with a multiplet splitting pattern. Each spectrum integrated for the correct number of protons. For XVI, the NMR spectrum showed the following absorption peaks ($CDCl_3$): δ 7.20 (s, 15H, ArH), 4.20 (d, 2H, NCH_2Ar), and 3.20 (q, 4H, CCH_2Ar) ppm.

***N*-Benzyl-2-benzyl-2-methylcyanoacetamide (XII)**—To a solution of potassium hydroxide (2.80 g, 0.05 mole) and formamide (10 ml) in dimethyl sulfoxide (30 ml) was added XI (13.2 g, 0.05 mole), and the mixture was stirred for 30 min. Potassium iodide (0.1 g) and methyl iodide

[1] Melting points were determined in open capillary tubes and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Atlanta, Ga. NMR data were recorded on a Varian Associates T-60A spectrophotometer.

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