High-Pressure Liquid Chromatographic Evaluation of Aqueous Vehicles for Preparation of Prednisolone and Prednisone Liquid Dosage Forms

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Abstract □ A high-pressure liquid chromatographic method was developed that separates prednisolone from prednisone, prednisone from methylprednisolone succinate sodium, and hydrocortisone from hydrocortisone acetate or cortisone acetate. The common liquid dosage preservatives methylparaben, propylparaben, and sodium benzoate do not interfere with quantitative prednisolone, prednisone, and hydrocortisone determinations. The method was used to study prednisolone and prednisone stability in five aqueous vehicles (water, citrate buffer USP, 50% glycerin, 50% sorbitol, and 50% sucrose) containing 10% (v/v) ethanol. Prednisone crystallized out in all vehicles except glycerin, in which it appeared to be stable for at least 92 days. Prednisolone did not crystallize in any vehicle but decomposed quickly in citrate buffer. Sorbitol and glycerin appeared to be the best vehicles for prednisolone. The developed method was applied successfully to the quantitative determinations of prednisolone, prednisone, and hydrocortisone in commercial tablets.

Keyphrases □ Prednisolone—analysis, high-pressure liquid chromatography, liquid dosage forms, separation from prednisone □ Prednisone—analysis, high-pressure liquid chromatography, liquid dosage forms, separation from prednisolone □ High-pressure liquid chromatography—analysis, prednisolone and prednisone in liquid dosage forms □ Dosage forms, liquid—prednisolone and prednisone, separation by high-pressure liquid chromatography

A number of drugs are not available commercially in liquid dosage forms due to stability problems. However, physicians often prescribe some of these drugs for infants and small children who cannot swallow tablets or capsules. Furthermore, liquid dosage forms may be required for administration to adults through nasal gastric tubes.

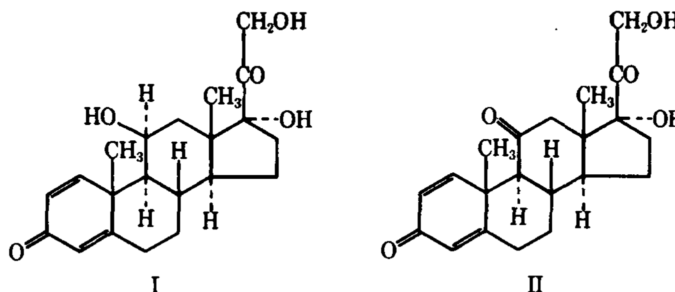
BACKGROUND

The effect of some solid buffering agents, *e.g.*, antacids, aluminum hydroxide, calcium carbonate, magnesium carbonate, magnesium oxide, and magnesium trisilicate, on prednisolone stability in aqueous suspensions was reported previously (1). The steroid was adsorbed by magnesium trisilicate and decomposed in the presence of magnesium carbonate. The other antacids had no effect on the stability. A later article (2) on base-catalyzed prednisolone decomposition reported that the steroid exhibited first-order degradation kinetics. A kinetic study (3) on fluprednisolone acetate stability in aqueous solution (pH 7.2) also indicated that the steroid could not be stabilized in a neutral aqueous solution.

Prednisone may change to prednisolone on standing in an aqueous solution[1]. Before vehicle evaluation, therefore, it was important to develop an appropriate analytical method to separate prednisolone from prednisone. The previously developed high-pressure liquid chromatographic (HPLC) technique for hydrocortisone (4) was not suitable since it did not separate prednisolone from prednisone. In this procedure, a column of semipolar packing material consisting of a monomolecular layer of cyanopropylsilane permanently bonded to silica was used.

The purposes of this study were to develop an appropriate analytical procedure to separate prednisone from prednisolone and to evaluate the effect of some aqueous vehicles [ethanol, citrate buffer USP (5), 50% glycerin, 50% sorbitol, and 50% sucrose] on prednisone and prednisolone stability.

[1] J. T. Slattery, School of Pharmacy, University of New York at Buffalo, Buffalo, N.Y., personal communication



EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents were USP, NF, or ACS grade and were used without further purification. Prednisolone[2] (I), prednisone[2] (II), hydrocortisone[2], hydrocortisone acetate[3], and cortisone acetate[4] were used as received. Methylprednisolone succinate sodium[2] powder for injection USP also was used without further treatment.

Apparatus—The high-pressure liquid chromatograph[5] was equipped with a fixed wavelength (254 nm) detector, a recorder[6], and an integrator[7].

Column—The nonpolar prepacked column[8] consisted of a mono-

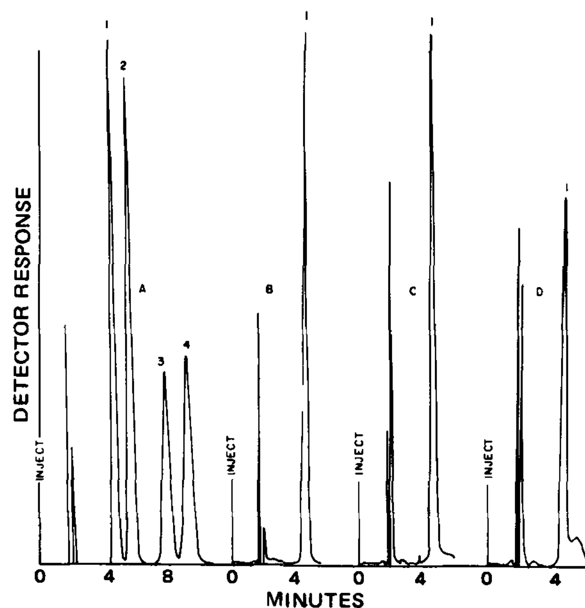


Figure 1—*Sample chromatograms. Key: peaks 1–4 in A, prednisone, prednisolone, methylprednisolone succinate sodium, and hydrocortisone acetate, respectively; and peak 1 in B–D, prednisone in a standard solution, in 50% glycerin (92 day old), and in 50% sugar (92 day old), respectively. For chromatographic conditions, see text.*

[2] The Upjohn Co., Kalamazoo, Mich.
[3] Premo Pharmaceutical Laboratories, South Hackensack, N.J.
[4] Merck & Co., Rahway, N.J.
[5] Waters ALC 202 equipped with U6K universal injector.
[6] Omniscribe 5213-12 equipped with an integrator.
[7] Autolab Minigrator, Spectra-Physics, Santa Clara, Calif.
[8] Waters μBondapak/C_{18}.

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Table I—Assay Results and pH Values of Aqueous Solutions

Vehicle	Active Ingredient	Percent Retained after				pH (±0.05)	
		2 Days	30 Days	61 Days	92 Days	Initial	Final
Water	I	100.6	99.6	99.8	96.5	6.1	6.2
Citrate buffer	I	99.8	84.4	77.7	62.9	4.5	4.4
50% Glycerin	I	99.3	100.1	100.0	99.6	5.5	5.5
50% Sorbitol	I	98.9	99.5	99.5	99.0	5.4	5.3
50% Sucrose	I	100.4	99.3	93.9	93.3	6.0	6.0
Water	II	66.3[a]	—	—	—	6.0	—
Citrate buffer	II	62.8[a]	—	—	—	4.4	—
50% Glycerin	II	100.6	99.4	99.6	99.7	5.5	5.7
50% Sorbitol	II	81.6[a]	—	—	—	5.5	—
50% Sucrose	II	101.2	83.3[a]	—	69.3[a,b]	6.0	—

[a] Prednisone crystallized and, therefore, was not followed. [b] Done only to determine if II had changed to I (see Fig. 1D and *Discussion*).

molecular layer of octadecyltrichlorosilane permanently bonded to silica (30 cm and 4 mm i.d.).

Chromatographic Conditions—The solvent was 0.01 M KH_2PO_4 in water containing 50% (v/v) methanol. The temperature was ambient, the flow rate was 1.6 ml/min, and the chart speed was 30.5 cm/hr. The attenuation unit for full-scale deflection was 0.04.

Standard Solutions—The stock solution of each hormone was prepared by dissolving 100.0 mg of hormone in enough ethanol to make 100.0 ml. The standard solutions were prepared by diluting 2.0 ml of the stock solution to 100.0 ml with water. For tablet analysis, the standard solution was prepared by diluting with ethanol. A standard solution containing the four hormones, I, II, methylprednisolone succinate sodium, and hydrocortisone acetate, also was prepared by diluting with water.

Aqueous Solutions of I and II—Five aqueous solutions each of I and II were prepared. The stock solutions of I and II were prepared by dissolving 500.0 mg of drug in enough ethanol to make 100.0 ml. A 10.0-ml quantity of a stock solution was brought to volume (100.0 ml) either with water or with another appropriate solution, a 50% aqueous solution of glycerin (v/v), a 50% solution of sorbitol in water, a 50% solution of sucrose in water, or citrate buffer USP (5). The citrate buffer pH was 4.4. All solutions contained 0.1% sodium benzoate as a preservative.

Assay Solutions—A 2.0-ml quantity of the aqueous solution was diluted to 50.0 ml with water.

Assay Solutions from Commercial Tablets—Ten tablets were weighed and ground to a fine powder. A quantity of the powder representing 2.0 mg of the active ingredient was weighed accurately and transferred to a 150-ml beaker. Then 80 ml of ethanol was added, and the mixture was heated just to boiling. The mixture was cooled, brought to 100.0 ml with ethanol, and filtered. The first 20 ml of the filtrate was rejected, and then samples were collected for analysis.

After the initial assays using the method discussed below and the determination of pH values (after diluting 1:10 with water), the aqueous solutions of I and II were stored at room temperature. After appropriate storage periods, the aqueous solutions were assayed again and pH values also were determined.

Assay—Twenty microliters of the assay solution was injected into the chromatograph using the described conditions. For comparison, an identical volume of the standard solution was injected after the assay was eluted.

Since preliminary investigations on each hormone indicated that Beer's law was followed in the 0.2–2.5-μg range, the results were calculated as follows:

$$\frac{A_a}{A_s} \times 100 = \text{percent of label claim} \qquad \text{(Eq. 1)}$$

where A_a is the peak area of the assay solution and A_s is the peak area of the standard solution (Figs. 1 and 2 and Tables I and II).

Table II—Tablet Results

Tablet	Active Ingredient per Tablet	Color	Assay Results, % of claim: HPLC Method	Assay Results, % of claim: Blue Tetrazolium Method
1	5 mg of I	Yellow	100.5	295.3
2	5 mg of II	White	101.3	348.6
3	5 mg of hydrocortisone	White	99.8	>386

For comparison, the commercial tablets were also assayed (Table II) using the USP blue tetrazolium method (6). For this method, the same assay solution as already described was used.

DISCUSSION

The developed HPLC method separated I from II (Fig. 1A) efficiently. Other systems tried without success were[9]: (*a*) 0.01 M KH_2PO_4 in water containing 20% (v/v) methanol; (*b*) same as *a* with 2% (v/v) acetic acid (final pH 2.6); (*c*) same as *b* with 0.005 M heptanesulfonic acid sodium; (*d*) 0.01 M KH_2PO_4 in water containing 20% (v/v) ethanol (pH 7.8 adjusted with 1:100 phosphoric acid in water); (*e*) 0.005 M $(NH_4)_2HPO_4$ in 20% (v/v) ethanol in water; (*f*) 0.005 M tetrabutylammonium hydroxide in 20% (v/v) ethanol in water (pH 7.8 adjusted with 1:100 phosphoric acid in water); and (*g*) 0.005 M $(NH_4)_2HPO_4$ in water containing 10% (v/v) methanol, acetonitrile, or a combination of both (5% each).

The HPLC method separated I from its salt, methylprednisolone succinate sodium (Fig. 1A). It also separated hydrocortisone from hydrocortisone acetate and from cortisone acetate. Hydrocortisone did not separate from I, and hydrocortisone acetate did not separate from cortisone acetate. The common preservatives methylparaben, propylparaben, and sodium benzoate did not interfere with the determinations of I, II, and hydrocortisone. The relative percent standard deviations based on five injections of the standard solutions were 0.64, 0.84, and 1.8 for I, II, and hydrocortisone, respectively.

Due to the poor solubility of II, partial crystallization occurred in all of the vehicles except 50% (v/v) glycerin. In glycerin, there was no II decomposition (Table I) in 92 days. Chromatograms from a standard so-

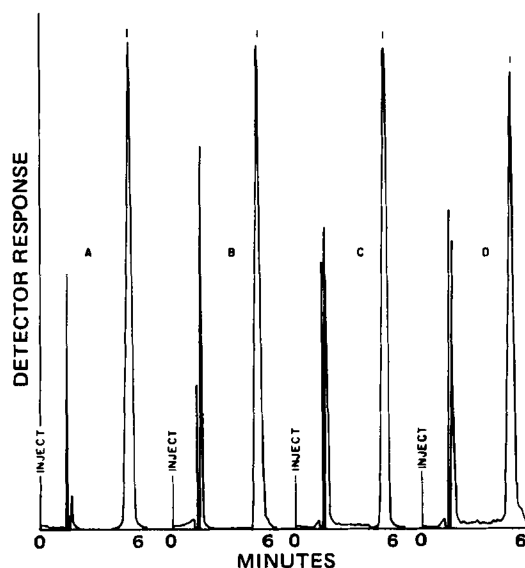

Figure 2—*Sample chromatograms from prednisolone. Key: peak 1 in A–D, prednisolone in a standard solution, in 50% glycerin, in 50% sorbitol, and in 50% sucrose, respectively; and B, C, and D, 92-day-old samples. For chromatographic conditions, see text.*

[9] Using Waters μBondapak/CN.

lution (Fig. 1B) and from a glycerin solution (Fig. 1C) were similar. In the presence of sugar, besides crystallization (Fig. 1B), II may be partially reduced to I (Figs. 1B and 1D).

Prednisolone did not crystallize in any vehicle investigated. It decomposed fast in the presence of citrate buffer. The percent retained after 92 days was 62.9 (Table I). Some decomposition also was recorded in water and in 50% aqueous sucrose (Table I and Fig. 2D). Both 50% (v/v) glycerin in water and 50% aqueous sorbitol appear to be excellent I vehicles (Table I). The peak obtained from a I solution in sorbitol (Fig. 2C) compared better with the standard (Fig. 1A) than did the peak from a glycerin solution (Fig. 2B), which had a slight shoulder, perhaps a sign of slight decomposition. All solutions studied contained 10% (v/v) ethanol and 0.1% sodium benzoate.

The developed method also was suitable for the quantitative determinations of I, II, and hydrocortisone in commercial tablets (Table II). The results obtained with the blue tetrazolium method were very high, presumably due to interference from some inactive ingredients. When the active ingredients were extracted with ethanol without heat, inconsistent and sometimes low results were obtained. Therefore, the mixture was heated to extract the active ingredient quantitatively.

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(3) E. H. Jensen and D. J. Lamb, *J. Pharm. Sci.*, **53**, 402 (1964).

(4) V. D. Gupta, *ibid.*, **67**, 299 (1978).

(5) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 458.

(6) *Ibid.*, p. 238.

ACKNOWLEDGMENTS

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Fluorometric Determination of Chlorzoxazone in Tablets and Biological Fluids

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Received December 4, 1978, from the *Department of Medicinal Chemistry, School of Pharmacy, University of Georgia, Athens, GA 30602.* Accepted for publication December 27, 1978.

Abstract □ A fluorometric determination for chlorzoxazone was developed based on the intrinsic drug fluorescence in chloroform using excitation and emission wavelengths of 286 and 310 nm, respectively. A calibration curve for chlorzoxazone in chloroform gave a linear working range of 0.027–2.3 μg/ml (r = 0.9999) with the minimum detectability at 27 ng/ml. The procedure was applied to chlorzoxazone analysis in spiked plasma and urine samples. Minimum detectable drug levels in these samples were 60 and 130 ng/ml, respectively. Data revealed that chlorzoxazone could be determined in plasma and urine even in the presence of 20-fold molar excesses of its major metabolite, 6-hydroxychlorzoxaxone, and acetaminophen. The method also was applicable to chlorzoxazone analysis in a commercial dosage form containing acetaminophen.

Keyphrases □ Chlorzoxazone—analysis, fluorometry, in commercial tablets and biological fluids, in presence of hydroxychlorzoxazone and acetaminophen □ Fluorometry—analysis, chlorzoxazone in commercial tablets and biological fluids, in presence of hydroxychlorzoxazone and acetaminophen □ Muscle relaxants—chlorzoxazone, fluorometric analysis in commercial tablets and biological fluids, in presence of hydroxychlorzoxazone and acetaminophen

Chlorzoxazone (I) is one of the most useful skeletal muscle relaxants in the treatment of painful muscle spasm, especially in combination with acetaminophen, a product that has been among the top 100 drug products in the United States in recent years. Studies of chlorzoxazone metabolism in humans have shown that its major metabolite is 6-hydroxychlorzoxazone (II) (1, 2). The drug is rapidly absorbed from the GI tract and essentially disappears from the blood in 7–8 hr. Less than 1% of the intact drug is excreted in the urine.

Among the analytical methods reported for I analysis have been spectrophotometry (1), titrimetry (3), and GLC and TLC (4–6). Direct application of many procedures to the determination of the drug in biological fluids is lacking. In other instances, parameters such as accuracy, linearity, sensitivity, and detection limit were not evaluated for the reported methodology.

In this paper, the intrinsic fluorescence of I is presented as a suitable analytical technique for the determination of the drug in biological fluids. The method also is useful for analysis of I in tablets. The method described is sensitive for I in the nanogram per milliliter range and is free from interference from II and acetaminophen. A previous report examined the analysis of I using derivatization with various fluorogenic reagents (7).

EXPERIMENTAL

Apparatus—Fluorescence measurements were made with a spectrophotofluorometer[1] equipped with a corrected spectra accessory. The following settings were used: excitation and emission slits, 10 nm; amplifier sensitivity coarse, 0.1; and measurement mode in true emission. Clear fused quartz cells (12.5 × 45 mm) were used as sample cells for dosage form analysis. Quartz microcells[2] (path length 5 mm) were used for the biological fluid assays.

Reagents and Chemicals—Chlorzoxazone[3], 6-hydroxychlorzoxazone[3], and acetaminophen[4] were obtained as pure powders and were used without further purification in the preparation of stock solutions. All other reagents and chemicals were commercially available and were used as received.

Determination of Chlorzoxazone in Dosage Form—A tablet[5] containing 250 mg of chlorzoxazone and 300 mg of acetaminophen was suspended in 15 ml of distilled water contained in a 50-ml centrifuge tube. After 25 ml of ethyl acetate was added, the solution was mixed[6] for 5 min. An aliquot of the ethyl acetate extract corresponding to 100 mg of chlorzoxazone was transferred to a 50-ml volumetric flask and diluted to volume with ethyl acetate. One milliliter of this solution was trans-

[1] Model MPF-4, Perkin-Elmer Corp., Norwalk, Conn.
[2] Part No. 018-0056, Hitachi, Ltd., Tokyo, Japan.
[3] McNeil Pharmaceuticals, Fort Washington, Pa.
[4] Eastman Organic Chemicals, Rochester, N.Y.
[5] Parafon-Forte, McNeil Pharmaceuticals, Fort Washington, Pa.
[6] Vortex-Genie mixer, Scientific Industries, Bohemia, N.Y.

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