Quantitative Dexamethasone and Dexamethasone Sodium Phosphate Determinations in Pharmaceutical Dosage Forms by High-Pressure Liquid Chromatography

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Abstract □ A high-pressure liquid chromatographic procedure for quantitative dexamethasone and dexamethasone sodium phosphate determinations in all types of commercially available pharmaceutical dosage forms was developed. The method also separates dexamethasone from its phosphate salt and separates dexamethasone or its salt from a number of inactive ingredients such as benzoic acid, benzyl alcohol, some colors, creatinine, and parabens. Inactive ingredient concentrations may be estimated without additional cost. Part of the parabens present in the commercial injections may be adsorbed by the rubber closures.

Keyphrases □ High-pressure liquid chromatography—analysis, dexamethasone and dexamethasone sodium phosphate, various pharmaceutical dosage forms □ Dexamethasone—analysis, high-pressure liquid chromatography, various pharmaceutical dosage forms □ Dexamethasone sodium phosphate—analysis, high-pressure liquid chromatography, various pharmaceutical dosage forms

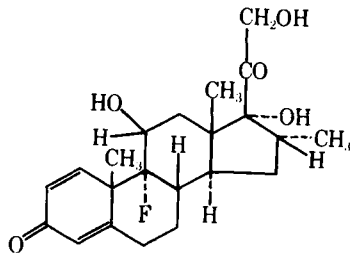
The USP methods (1) for quantitative dexamethasone (I) and dexamethasone sodium phosphate (II) determinations in pharmaceutical dosage forms (elixir, injections, ointment, ophthalmic solution, and tablets) are tedious and time consuming. The USP methods involve many different preliminary extraction procedures and then reactions with blue tetrazolium at room temperature for 45 min or with phenylhydrazine at 60° for 2 hr. For testing the injection, a 2-hr preliminary treatment with alkaline phosphatase is required to free the base before reacting with blue tetrazolium.

Obviously, the analyst has to use different techniques to analyze similar active ingredients. Moreover, determining the presence of dexamethasone base in dexamethasone phosphate salt involves an additional and different method.

A high-pressure liquid chromatographic (HPLC) procedure for quantitative dexamethasone determination in creams and ointments was reported (2). In this method, the mobile phase was 0.2% acetonitrile in hexane, and the column was β,β'-oxydipropionitrile on Zipax (2). Application of this method to other pharmaceutical dosage forms, such as an elixir containing colors and benzoic acid and creams and injections containing dexamethasone sodium phosphate and parabens, was not reported.

Another HPLC procedure for quantitative dexamethasone determination in milk (3) involved a complicated preliminary extraction. The HPLC method reported (4) for quantitative plasma dexamethasone determination also involved complicated preliminary extraction. Furthermore, these methods (3, 4) were not investigated to determine their applicability to pharmaceutical dosage forms with numerous inactive ingredients.

This paper describes a simple HPLC procedure for the quantitation of dexamethasone and its phosphate salt in all pharmaceutical dosage forms available commercially which also separates dexamethasone base from its salt. The HPLC procedure was preferred because it can often assay some inactive ingredients without additional cost.



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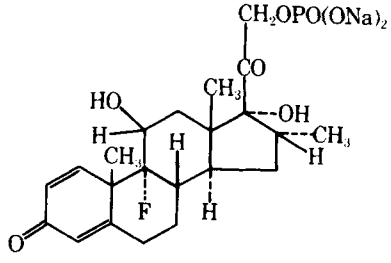

II

EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents were USP, NF, and ACS grade and were used without further purification. Dexamethasone[1] (I) and dexamethasone sodium phosphate[1] (II) were used as received.

Apparatus—The high-pressure liquid chromatograph[2] was equipped with a fixed wavelength (254 nm) detector, a recorder[3], and an integrator[4].

Column—The nonpolar prepacked column[5] consisted of a monomolecular layer of octadecyltrichlorosilane permanently bonded to silica (30 cm long and 4 mm i.d.).

Chromatographic Conditions—The chromatographic 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 units for full-scale deflection were 0.04 for I and 0.1 for dexamethasone phosphate (III), which was made using an equivalent quantity of II.

Assay Solutions—The elixir (label claim of 0.1 mg of I/ml) was diluted from 5.0 to 25.0 ml with water. Injections (label claim of 4 mg of III/ml from an equivalent amount of II) were diluted from 2.0 to 100.0 ml with water. The ophthalmic solution (label claim of 0.1% of III) was diluted from 8.0 to 100 ml with water.

Extraction of III from Ointment (Label Claim of 0.05% of III)—Ointment (0.8 g) was dissolved in 25 ml of methylene chloride in a separator and extracted with 25.0 ml of water. The clear water layer was

[1] Merck Sharp & Dohme, West Point, Pa.
[2] Waters ALC 202 equipped with U6K universal injector.
[3] Omniscribe 5213-12 equipped with an integrator.
[4] Autolab Minigrator, Spectra-Physics, Santa Clara, Calif.
[5] μBondapak C_{18}, Waters Associates, Milford, Mass.

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Table I—Assay Results

Product	Results, % of label claim
Synthetic injection 1 with parabens	100.5
Synthetic injection 2 with benzyl alcohol	99.8
Commercial injections	
1	99.6[a]
2	100.1[b]
3	99.1[c]
Commercial elixir[d]	99.8
Commercial ointment[e]	106.9[f]
Ophthalmic drops[g]	109.3[h]
Commercial tablets	
1 (0.75 mg/tablet, blue)	98.3[i]
2 (4.0 mg/tablet, white)	99.4[i]

[a] With parabens similar to synthetic solution 1. The results on parabens were estimated to be only ~80% of the claim. To estimate parabens, the attenuated units for full-scale deflection were 0.2 for methylparaben and 0.04 for propylparaben. [b] Same as footnote *a*, except that the results on parabens were about 76% of the label claim. [c] With benzyl alcohol similar to synthetic solution 2. The result on benzyl alcohol was estimated (using peak 1 in Fig. 1C) to be 99.2% of the label claim. [d] Also contained red color and 0.1% benzoic acid as preservative. [e] White petrolatum and mineral oil base. [f] The result using the blue tetrazolium method as provided by the manufacturer was 105.0%. [g] Also contained creatinine, sodium borate, sodium citrate, sodium bisulfite (0.32%), phenylethyl alcohol (0.25%), and benzalkonium chloride (0.02%). [h] The result using the blue tetrazolium method as provided by the manufacturer was 110.0%. [i] The results with the blue tetrazolium method were between 302 and 425%.

assayed using attenuation units for full-scale deflection of 0.04 instead of 0.1. The standard solution contained 16 μg of III/ml.

Extraction of I from Tablets (Label Claim of Sample 1, 0.75 mg of I/Tablet; Label Claim of Sample 2, 4 mg of I/Tablet)—Ten tablets were weighed and ground to a fine powder. Powder representing 2.0 mg of the active ingredient was weighed accurately and transferred to a 150-ml beaker. Ethanol (80 ml) was added, heated just to boiling, cooled, and brought to volume (100.0 ml) with alcohol. The solution was filtered, the first 20 ml of filtrate was rejected, and a sample was collected for analysis.

Standard Solutions—Two milliliters of a stock I solution (1 mg/ml) in alcohol was diluted to 100.0 ml with water for the elixir or with alcohol for the tablets. Ten milliliters of a stock II solution (contained an equivalent amount of 0.8 mg of III/ml of water) was diluted to 100.0 ml

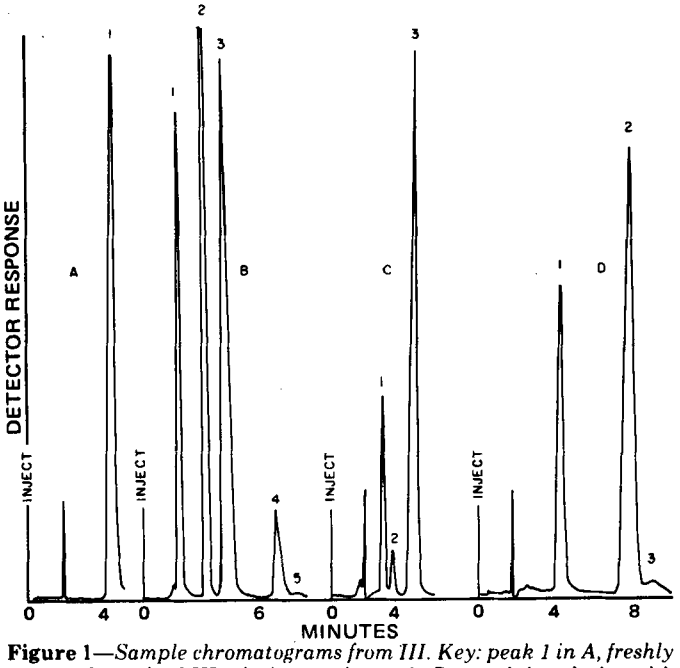

Figure 1—*Sample chromatograms from III. Key: peak 1 in A, freshly prepared standard III solution; peaks 1–5 in B, creatinine eluting with the solvent, methylparaben (out of scale when using 0.1 aufs), III, propylparaben, and a small quantity of a decomposition product, respectively (the chromatogram was obtained from a commercial injection sample); peaks 1 and 2 in C, benzyl alcohol present in a commercial injection from a different manufacturer; peak 3 in C, III; peak 1 in D, III in a 53-day-old aqueous solution of III (160 μg/ml); and peaks 2 and 3 in D, decomposition products. For chromatographic conditions, see text.*

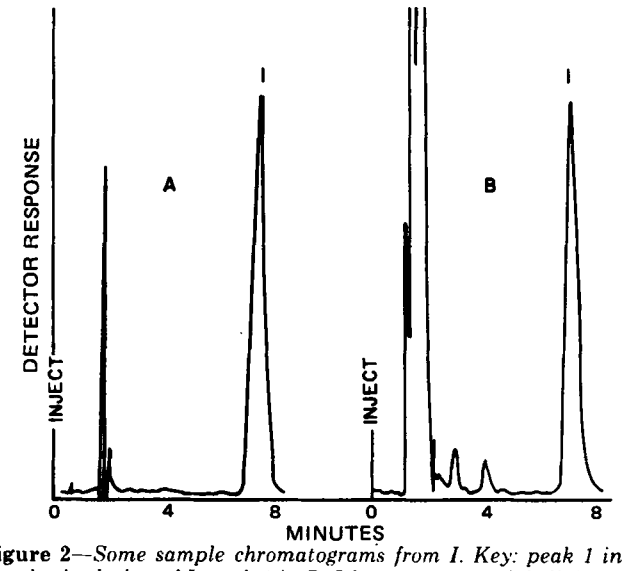

Figure 2—*Some sample chromatograms from I. Key: peak 1 in A, standard solution of I; peak 1 in B, I from a commercial elixir; other peaks, red color and benzoic acid (which comes out with the solvent). For chromatographic conditions, see text.*

with water. For the ointment, it was diluted further, 10.0 ml to 50.0 ml with water. Fresh II solutions were prepared every 4 hr.

Solutions Similar to Two Commercial Injections—One solution contained, per milliliter, 4.0 mg of III, 8 mg of creatinine, 10 mg of sodium citrate, 1.0 mg of sodium bisulfite, 1.5 mg of methylparaben, and 0.2 mg of propylparaben. The second contained, per milliliter, 4.0 mg of III, 1.0 mg of sodium sulfite, and 10 mg of benzyl alcohol. These solutions were diluted from 2.0 to 100.0 ml with water for quantitative analysis.

Assay—An aliquot (20.0 μl) was injected into the chromatograph as described. For comparison, an identical standard solution volume was injected after the sample was eluted.

Since preliminary investigations indicated that peak areas were directly related to concentrations (range of 0.2–0.6 μg for I, 0.5–2.0 μg for III for injections and ophthalmic solutions, and 0.1–0.4 μg of III for ointment), the results were calculated using the following equation:

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

where A_a is the assay solution peak area and A_s is the standard solution peak area. The results are presented in Figs. 1 and 2 and Table I.

For comparison, the commercial tablets also were assayed by the blue tetrazolium method as described in the USP (5) using the assay solution described (Table I).

DISCUSSION

The results indicate (Table I) that a single and simple assay can be used for the quantitative determinations of I and III in elixir, injections, ointment, ophthalmic solution, and tablets. The relative percent standard deviations based on five standard solution injections were 2.3 and 2.1 for I and III, respectively.

The USP assay methods (1) involve different preliminary treatments depending on the dosage form. These treatments are tedious and time consuming. After the preliminary treatment, color development by reaction with either blue tetrazolium or phenylhydrazine is also time consuming.

The standard III solution must be prepared fresh since it decomposes on standing. The single wash procedure with methylene chloride recommended in the USP to remove I from III extracted only ~97% of the total I present. The mixture contained 120 μg of III/ml and 40 μg of I/ml in water. A 10.0-ml quantity of the aqueous solution was extracted with 25 ml of methylene chloride. This estimation was possible since the developed procedure separates I and III very efficiently (see Figs. 1A and 2A for retention times).

There was no interference from: (*a*) red color and benzoic acid present in the elixir (Fig. 2B); (*b*) creatinine, parabens, and benzyl alcohol present in the injections (Figs. 1B and 1C); (*c*) phenylethyl alcohol present in ophthalmic solution; and (*d*) blue color and other inactive ingredients present in the tablets. With the tablets, there was significant interference

with the blue tetrazolium procedure (Table I, footnote *i*), presumably due to the heat used to extract the active ingredient. An extraction procedure using cold alcohol gave inconsistent and sometimes low results.

Another advantage of the developed method is that some inactive ingredients can be estimated without additional cost. For example, in commercial injections, parabens were estimated to be only between 76 and 80% of the label claim (Table I, footnotes *a* and *b*). This finding is presumably due to paraben adsorption onto the rubber closures. This problem was not recorded with benzyl alcohol (Table I, footnote *c*) as the preservative.

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Terpenoid Biotransformation in Mammals II: Biotransformation of *dl*-Camphene in Rabbits

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Abstract □ The biotransformation of *dl*-camphene in rabbits was investigated. Four neutral metabolites, 6-*exo*-hydroxycamphene, 10-hydroxycamphene, and diastereoisomers of camphene-2,10-glycol, were identified and two alcohols, 7-hydroxycamphene and 3-hydroxytricyclene, were estimated by IR, UV, NMR, and mass spectra and chemical degradations. The formation of these compounds can be explained through a homoallylic oxidation or an epoxide formation.

Keyphrases □ Camphene—biotransformation in rabbits, four urinary metabolites identified by spectrometry and chemical degradations □ Biotransformation—camphene in rabbits, four urinary metabolites identified by spectrometry and chemical degradations □ Choleretic activity—camphene, biotransformation in rabbits, four urinary metabolites identified by spectrometry and chemical degradations

During investigations into terpenoid detoxification in mammals, the biotransformation of 3-carene and α- and β-pinenes having a *gem*-dimethyl group on the three- or four-membered ring, respectively, was reported (1). In this paper, the biotransformation of camphene in rabbits is reported. Camphene has a *gem*-dimethyl group on the five-membered ring.

Camphene is found in the essential oils of most plants, and conifers containing this compound are often damaged worldwide by field animals. The choleretic activity of camphene in rats has been studied (2). The present investigation was carried out to clarify camphene biotransformation in mammals with respect to xenobiotics.

RESULTS

Characterization and Identification of Free Neutral Metabolites—TLC and GLC revealed the products in the urinary extracts as shown in Fig. 1. The metabolites were column chromatographed on silica gel in *n*-hexane with gradually increasing amounts of ethyl acetate. When necessary, metabolites were isolated by preparative GLC.

Metabolites 1-3 (M-I–M-III)—Peak 2 was isolated preparatively by GLC as one component (TLC and GLC) and had a fragrant odor; mass spectrum: *m/e* (%) 152 (M^+, $C_{10}H_{16}O$, 3), 134 (9), 119 (24), 108 (base), 93 (67), 72 (21), and 66 (28); IR: ν ($CHCl_3$) 3625, 3450, and 890 cm^{-1}. Its NMR spectrum showed two kinds of metabolites (M-I and M-II) in a 2:1 ratio. The major signal group assigned was: δ ($CDCl_3$) 4.84 and 4.63 (each 1H, s, *endo*-methylene), 3.80 [1H, q, $J_{5\text{-}exo\text{-}6\text{-}endo} = 7$, $J_{5\text{-}endo\text{-}6\text{-}endo} = 3$ Hz, —C*H*(OH)], 2.62 (1H, b, bridge head), 2.21 (1H, octet, $J_{5\text{-}exo\text{-}5\text{-}endo} = 13$, $J_{5\text{-}exo\text{-}6\text{-}endo} = 7$, $J_{5\text{-}exo\text{-}4} = 2$ Hz, *exo*-5), 1.89 (1H, b, *endo*-5), 1.63 (2H, b), and 0.98 and 1.04 (each 3H, *gem*-dimethyl). All of these M-I signals agreed well with those of the synthetic 6-*exo*-hydroxycamphene (I) (3).

The Jones oxidation of I yielded 6-oxocamphene [ν ($CHCl_3$) 1740 and 890 cm^{-1}] (4) with a minor ketone; mass spectrum (minor ketone): *m/e* (%) 150 (M^+, $C_{10}H_{14}O$, 25), 135 (12), 121 (15), 108 (41), 107 (98), 93 (base), 91 (54), and 79 (41); IR: ν ($CHCl_3$) 1740 and 890 cm^{-1}; NMR: δ ($CDCl_3$) 4.98 and 4.76 (each 1H, s), 3.08 (1H, b), and 1.16 and 1.07 (each 3H, s). These spectra revealed the minor ketone to be 7-oxocamphene. Therefore, 7-hydroxycamphene (II) (M-II) was estimated as the camphene metabolite.

The minor signal group found in the I NMR spectrum can be attributed to II: δ ($CDCl_3$) 4.73 and 4.48 (each 1H, s), 4.24 (1H, bs), 2.68 (1H, bs), and 1.04 (6H, s). The other minor ketone was obtained as the Jones oxidation product from M-III following M-I. Its spectra were considerably different from those of the above-mentioned ketones; mass spectrum: *m/e* (%) 150 (M^+, $C_{10}H_{14}O$, 7), 122 (16), 107 (base), 105 (12), 91 (28), and 79 (14); IR: ν ($CHCl_3$) 1740 cm^{-1}; NMR: δ ($CDCl_3$) 1.21, 1.04, and 1.00 (each 3H, s). Disappearance of the *endo*-methylene group suggests some camphene rearrangement. Accordingly, this ketone was assigned as 3-ketotricyclene

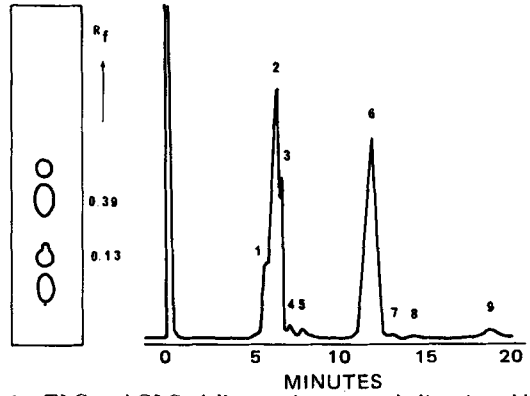

Figure 1—*TLC and GLC of* dl-*camphene metabolites in rabbits. The TLC solvent system was benzene–n-hexane–ethyl acetate (14:5:6); GLC utilized an SE-30 (5%) column.*

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