Colorimetric Determination of Betamethasone Benzoate in Topical Gel Preparation by Lewbart–Mattox Method

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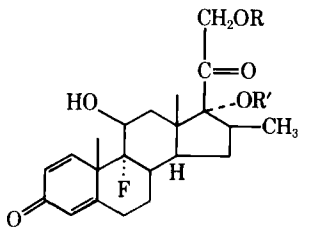
Abstract □ Betamethasone benzoate, a 17-substituted ester, is determined by preliminary oxidation of the 17α-ketol function (−$COCH_2OH$) to a glyoxal (−COCHO), which is then condensed with phenylhydrazine in acid to provide a yellow chromophore. The method is selective for betamethasone benzoate in the presence of its acyl migration product, the 21-benzoate, which is the only degradation product detected in formulations. Observations on the chemistry and scope of the method are presented.

Keyphrases □ Betamethasone benzoate—topical gel preparation, colorimetric determination by Lewbart–Mattox method □ Lewbart–Mattox method—colorimetric determination of betamethasone benzoate in topical gel preparation □ Colorimetry—determination, betamethasone benzoate in topical gel preparation by Lewbart–Mattox method

Betamethasone benzoate[1] (II) is the 17-substituted ester of betamethasone (I). It is a potent new synthetic corticosteroid and has been formulated in topical gel preparations. Long-term stability studies on these preparations, monitored by TLC, have shown only small increases in the concentration of betamethasone 21-benzoate (III), which, along with betamethasone alcohol, may be detectable in samples of the drug substance as a synthesis process impurity. Recent kinetic studies, using temperatures as high as 85°, confirmed that acyl migration to the 21-benzoate is the only important degradation route for the steroid.

A convenient colorimetric assay for gel preparations of betamethasone benzoate, selective in the presence of its rearrangement product, was developed. The method is based on chemistry described by Lewbart and Mattox (1), requiring preliminary oxidation of the 17-ketol function (−$COCH_2OH$) to a glyoxal (−COCHO), followed by reaction with acidic phenylhydrazine to form a Porter–Silber chromophore. The use of this method in the analysis of pharmaceuticals apparently has not been reported previously. A description of the procedure and observations on its scope are presented.



I: R = R′ = H
II: R = H, R′ = COC_6H_5
III: R = COC_6H_5, R′ = H

[1] USAN name for 9-fluoro-11β-17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-benzoate.

EXPERIMENTAL

Materials—Betamethasone benzoate[2], betamethasone[2], betamethasone 21-benzoate[2], betamethasone benzoate reference standard[3], and corticosterone[4] were used. Other steroids tested were of USP or NF grade. The assays were performed on clear gel preparations[5], which declare 0.025% betamethasone benzoate, carboxyvinyl polymer, disodium edetate, propylene glycol, diisopropanolamine, purified water, and alcohol.

Assay—*Sulfuric Acid Reagent*—Cautiously add 80 ml of concentrated sulfuric acid to 20 ml of water.

Cupric Acetate Solution—Dissolve about 100 mg of cupric acetate monohydrate in methanol and dilute to 100 ml. The solution is usable for at least 1 week, despite possible color changes and precipitation.

Methanol–Acid—Add 2 volumes of the sulfuric acid reagent to 1 volume of methanol.

Phenylhydrazine Reagent—Prepare fresh as needed by dissolving 20 mg of colorless phenylhydrazine hydrochloride in 20 ml of the sulfuric acid reagent.

Standard Preparation—Dissolve an accurately weighed amount of betamethasone benzoate reference standard in methanol and dilute quantitatively and stepwise to obtain a solution containing about 50 μg/ml.

Assay Preparation—Transfer an accurately weighed portion of the gel, equivalent to about 0.5 mg of betamethasone benzoate, to a 125-ml separator containing 20 ml of water. Add 1 ml of saturated sodium acetate solution, mix thoroughly, and extract with three 25-ml portions of chloroform, shaking about 2 min with each portion.

Combine the extracts in a second separator, wash with one 10-ml portion of water, and filter the extracts through paper into a conical flask with the aid of several small portions of chloroform. Evaporate the chloroform nearly to dryness on the steam bath with the aid of a current of air, and remove the last traces of solvent without heating. Dissolve the residue in exactly 10.0 ml of methanol.

Procedure—Transfer two 1-ml portions of the assay preparation and two 1-ml portions of the standard preparation to separate 15-ml glass-stoppered tubes. Add 0.1 ml of the cupric acetate solution to each tube, mix, and let stand open to air for 20 min with occasional mixing. Place the tubes in an ice bath, and add 2.0 ml of the phenylhydrazine reagent to one tube from the assay preparation and one from the standard preparation, allowing the reagent to flow down the sidewall of the tube without mixing. Similarly, add 2.0 ml of the sulfuric acid reagent to the remaining tubes, which serve as blanks.

Stopper the tubes, mix the contents carefully (heat is evolved), and heat the tubes in a constant-temperature bath at 60° for 15 min. Cool the tubes, add 2.0 ml of methanol–acid to each, and mix. Then determine the absorbances of the solutions from assay and standard preparations, against their respective blanks, in 1-cm cells at the wavelength of maximum absorbance at about 425 nm.

[2] Obtained from the Warner-Vister Steroid Research Institute, Casatenovo (Como), Italy.

[3] Prepared by the Analytical and Physical Chemistry Department, Warner-Lambert Research Institute.

[4] Pregnen-11β,21-diol-3,20-dione, Mann Research Laboratories.

[5] Benisone Gel, Warner-Chilcott Laboratories, Morris Plains, NJ 07950; Flurobate Gel, Texas Pharmacal Co., San Antonio, TX 78215

Table I—Data from Reactions in Thermostatted Cells for Various Corticosteroids

Steroid	Temperature	Time to Constant A	a, liters/g cm	ϵ, liters/mole cm
Betamethasone benzoate	25°	20 hr	29.5	14,600
	60°	15 min	25.3	12,600
Corticosterone	25°	15 min	15.9	5,500
Hydrocortisone	25°	70 min	14.4	6,200
Triamcinolone acetonide	25°	60 min	9.45	4,100

Calculate the percentage of betamethasone benzoate, by weight, from the formula $(C/W)(A_U/A_S)$, where C is the concentration, in micrograms per milliliter, of betamethasone benzoate reference standard in the standard preparation; W is the weight, in milligrams, of the portion of gel taken; and A_U and A_S are the absorbances of the solutions from the assay and standard preparations, respectively.

Chromatographic Method—Evaporate 4.0 ml of the assay preparation to dryness in a conical tube with the aid of a current of nitrogen, and take up the residue in 0.2 ml of a mixture of chloroform–methanol (7:3). Prepare comparison solutions, in the same solvent mixture, containing 0.2 mg of betamethasone/ml, 0.2 mg of betamethasone 21-benzoate/ml, and 1.0 mg of betamethasone benzoate reference standard/ml. Activate a 20 × 20-cm TLC plate, coated with a 0.25-mm layer of silica gel GF, by heating at 105° for 30 min. Mark equidistant points 2 cm above one edge of the plate, and apply the test solution and standards, all in 5-μl increments, according to the following scheme:

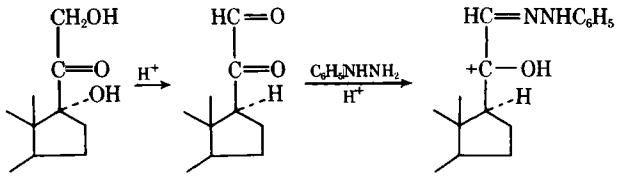
	solutions spotted, μl				
points	1	2	3	4	5
betamethasone	5	10	—	—	25
betamethasone 21-benzoate	5	10	—	—	25
betamethasone benzoate	90	80	—	100	50
reference standard	—	—	100	—	—
test solution					

Develop the plate in a chamber previously equilibrated with a solvent mixture of benzene–acetone–methanol (75:25:4) until it has ascended about 15 cm, remove the plate, and examine under shortwave UV light. The R_f values are about 0.4 for betamethasone, 0.6 for betamethasone benzoate, and 0.7 for betamethasone 21-benzoate. Estimate the concentration of betamethasone and betamethasone 21-benzoate in the test solution (lane 3) by comparison with the corresponding standards in lane 1 (1%), lane 2 (2%), and lane 5 (5%), viewing the plate under shortwave UV light.

Rate of Color Development—The time required to attain constant absorbance in the chromogenic reaction was determined for betamethasone benzoate and some other corticosteroids, selected for comparison, in 1-cm cells in the thermostatted[6] cell compartment of a double-beam spectrophotometer[7], set at 425 nm and equipped with a strip-chart recorder[8]. Test solutions, phenylhydrazine reagent, and sulfuric acid reagent were chilled in an ice bath before mixing in the spectrophotometer cell, and the absorbances were recorded without further dilution with methanol–acid.

RESULTS AND DISCUSSION

Chemistry of Colorimetric Method—The Porter–Silber (2) reaction, production of a yellow chromophore by reaction of a corticosteroid with phenylhydrazine in sulfuric acid, is restricted to corticosteroids with a 17-dihydroxyacetone side chain. The reaction has been shown (3, 4) to proceed in two stages: *(a)* the acid-catalyzed rearrangement of the dihydroxyacetone moiety to a 17-deoxy-20-one-21-ol, and *(b)* condensation of this glyoxal with phenylhydrazine to form a 21-phenylhydrazone (Scheme I).



Scheme I

Although many esters of corticosteroids, *e.g.*, hydrocortisone 21-acetate, can be determined by the Porter–Silber reaction, neither betamethasone benzoate nor its 21-benzoate isomer gives any color in the reaction, indicating that the ester is stable to hydrolysis under the strongly acid reaction conditions.

Lewbart and Mattox (1) extended the Porter–Silber reaction to the detection and microdetermination of pure 17-deoxy-α-ketolic steroids. Their modification consists of producing the requisite glyoxal reactant by oxidation with cupric acetate, followed by reaction with phenylhydrazine (Scheme II).

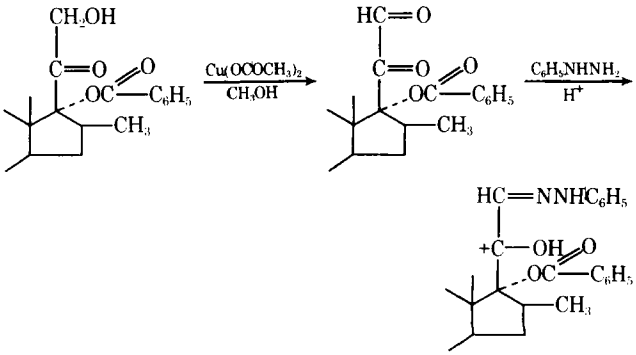
Betamethasone 21-benzoate, since it cannot be oxidized to a glyoxal, does not interfere. Betamethasone alcohol is chromogenic in the reaction; however, the molar absorptivity of the color produced with it is only about 40% that of betamethasone benzoate. Betamethasone undergoes the normal Porter–Silber reaction; however, it gives only about one-tenth the absorptivity without preliminary cupric acetate oxidation that it provides in the assay method, all other reaction parameters being identical.

Stability of Betamethasone Benzoate—Gardi (5) reviewed the chemistry of 17-monoesters of corticosteroids. He noted that these compounds undergo facile rearrangement to 21-esters. Studies on a number of different formulations of betamethasone benzoate revealed acyl migration to the 21-benzoate as the only degradation route detectable. Since the 21-benzoate does not interfere in the colorimetric assay, the method is stability indicating.

Two additional routes of decomposition were considered when these studies were initiated: ester hydrolysis and α-ketol group oxidation. Neither has been observed. Betamethasone, which might be formed by ester hydrolysis, has been estimated at less than 1–2% of initial betamethasone benzoate concentration throughout stability studies of its formulations. Increases in betamethasone concentration have been seen in high temperature kinetic studies on gels only when more than half of the 17-benzoate rearranged. As noted, the benzoate ester is stable to the strong acid medium of the Porter–Silber reaction. No evidence of oxidation has been discerned during 5 years of stability studies on diverse formulations.

It is remarkable, in this connection, that conditions prescribed for blue tetrazolium colorimetry, which is an oxidation reaction, for betamethasone and its esters official in NF (6) require heating at 45 or 50° while the reaction is carried out at room temperature in the general method for corticosteroid drugs. One may speculate that the 16β-methyl group shields the ketol group from attack.

Scope of Reaction—Although betamethasone benzoate is converted smoothly to the glyoxal with cupric acetate, the chromogenic reaction occurs much more sluggishly with it than with any other corticosteroid tested. In experiments where the reaction was conducted in the thermostatted cell of a recording spectrophotom-



Scheme II

[6] A Haake circulating constant-temperature bath was used.
[7] Coleman model 124.
[8] Bausch & Lomb VOM 5.

Table II—Differential Absorbance Readings of Placebo Gel Extracts

Trial	1	2	3	4	5	6	Reagent Blank
Sample weight, g	1.9488	2.6514	2.0021	1.9674	1.7296	2.4704	–
A (425 nm)	0.026	0.024	0.018	0.022	0.020	0.019	0.023

eter, constant absorbance was obtained only after 20 hr at 25° or after 15 min at 60°; however, the absorptivity was lower for the heated sample (Table I).

The absorptivities obtained for betamethasone benzoate in these experiments were calculated using the nominal volume of 3.1 ml, and they are lower than is generally obtained by the procedure in the assay (see *Experimental*). Corticosterone, lacking a 17-hydroxy function, does not undergo the normal Porter–Silber reaction. Triamcinolone acetonide gives only about one-tenth the color obtained with hydrocortisone in the Porter–Silber reaction (7); however, it appears to react as readily as hydrocortisone under the Lewbart and Mattox conditions. The method appears to be applicable to any corticosteroid with a 20-one-21-ol side chain, but time and temperature parameters should be determined for each compound.

Recovery of Betamethasone Benzoate from Gels—Recovery experiments were performed by weighing 2-g portions of gel placebo formulations into separators containing 20 ml of water, adding exactly 1.0 ml of a methanol solution of betamethasone benzoate reference standard, containing 0.50 mg/ml, and following the assay procedure. Values of 102.1, 100.0, 101.2, 97.3, 101.2, and 102.9% of the amount added were obtained, averaging 100.8%. The relative standard deviation calculated for the six trials was 1.98%.

Absence of Excipient Interference—The proposed method affords a differential measurement of both sample and standard, using a reagent without phenylhydrazine as the blank. This technique compensates for any chromophores that might be induced by reagent acidity, but it does not correct for chromogenic reactions of phenylhydrazine with excipients. As a check on this point, six trials were made on placebo gel (Table II). It is evident that there is no relationship between the differential absorbance readings and the amount of gel taken nor any difference between these and the reagent blank. It was concluded, therefore, that there is no excipient interference in the method.

Linearity and Reproducibility—Plots of absorbance against concentration have been rectilinear when determined by any one chemist at one time. Absorptivities calculated for standards run by one chemist over several months ranged from 27.4 to 33.9, with a mean of 30.9 liters/g cm, in 16 determinations. Another chemist, in six trials at various times, obtained absorptivities ranging from 32.2 to 34.4, with a mean of 33.0. Six chemists, determining absorptivities on the same standard, obtained values ranging from 28.7 to 33.5, with a mean value of 31.5.

SUMMARY AND CONCLUSIONS

A novel and selective method for the colorimetric determination of betamethasone benzoate in a topical gel formulation is described. The procedure is derived from the Lewbart–Mattox (1) modification of the Porter–Silber reaction (2), which consists of preliminary oxidation of the 17α-ketol function with methanolic cupric acetate and condensation of the resulting glyoxal with phenylhydrazine. The method is selective for betamethasone benzoate in the presence of its 21-benzoate rearrangement product, the only decomposition product detected in stability studies of formulations. The glyoxal formed from betamethasone benzoate reacts much more slowly with Porter–Silber reagent than those from corticosterone, hydrocortisone, and triamcinolone acetonide. The method affords satisfactory recovery and precision values.

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