# Simultaneous Determination of Dexamethasone and Betamethasone in Pharmaceuticals by Reversed-Phase HPLC

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## Key Words

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## Summary

An HPLC method for the simultaneous determination of the glucocorticoids betamethasone and dexamethasone is described. The method based on the separation of these compounds using a binary water-tetrahydrofuran mobile phase and a reversed-phase Hypersil $C_{18}$ column, was applied to the determination of betamethasone and dexamethasone in tablets.

## Introduction

Dexamethasone (DM) and betamethasone (BM) are highly potent synthetic glucocorticoids. The structural differences between these epimeric compounds are the different configurations of the methyl group on C-16 (Figure 1). They are widely used as anti-inflammatory, antiallergic agents and in the treatment of adrenal cortex insufficiency [1]. Corticosteroids are also used as adulterants in locally produced herb extracts and in certain homeopathic drugs [2]. In many countries anabolizing compounds have been used for cattle. Recently, the use of these compounds has decreased because of many routine analyses for them. One explanation could be the use of new anabolic steroids or other xenobiotic products such as corticoids in cattle production because of their ability to promote water retention in the body [3].
On the other hand, BM is more expensive than DM. For this reason, sometimes BM is substituted by DM and their simultaneous determination is necessary.

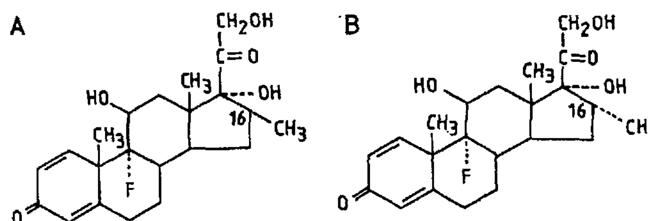


**Figure 1**
Structures of (A) betamethasone, (B) dexamethasone.

Several HPLC methods for individual determination of DM or BM and related compounds in different types of sample are described in the literature [4–7], including the official methods of the United States Pharmacopeia [8]. However, HPLC methods not involving derivatization have not been reported for the simultaneous determination of DM and BM. In previous papers two HPLC methods were developed for determining these compounds involving derivatization procedures for the epimers with the homochiral reagent, N-carbobenzoxyl-L-phenylalanine in the presence of an activating agent [9], or with 1-ethoxy-4-(dichloro-s-triazinyl)-naphthalene [10].
Recently, an optimization study of the separation of a sample containing natural and synthetic corticosteroids for screening purposes has been developed [11]. On this basis, a rapid, sensitive and selective method for the simultaneous determination of DM and BM using MPL as internal standard has been developed and used to determine these compounds in commercial tablets.

## Experimental

### Chemicals and Reagents

Dexamethasone (DM), betamethasone (BM), and methylprednisolone (MPL, internal standard) were from Sigma (St. Louis, MO, USA). HPLC-grade tetrahydrofuran, methanol and acetonitrile were from Promochem (Wesel, Germany). Water was purified with a Milli-Q system (Millipore, Molsheim, France). Millipore 0.45 µm Nylon filters (Bedford, MA, USA).



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### Apparatus

The chromatographic system consisted of the following components, all from LDC Analytical (Riviera Beach, FL, USA): a ConstaMetric 4100 solvent delivery system, a spectromonitor 5000 photodiode-array detector covering the range 190–360 nm, interfaced to a computer for data acquisition and a recorder model CI 4100 data module. A Rheodyne 20 µl loop injector (Cotati, CA, USA), and a Jones Chromatography block, heated series 7960, for thermostating columns in the range 30–60 °C (Seagate Technology, Scotts Valley, CA, USA) were also used. The following reversed-phase columns: 5 µm Hypersil ODS (250 × 4.6 mm i.d.) from Phenomenex (Torrance, CA, USA), 5 µm Spherisorb ODS (150 × 4.6 mm i.d.) and a polymeric 5 µm PRP-1 (150 × 4.1 mm i.d.) from Hamilton (Reno, NV, USA) were used. A sonication bath, Ultrasons P from Selecta (Barcelona, Spain), was also used.

### Mobile Phase

The mobile phase was prepared by mixing Milli-Q water with HPLC solvents in a required volume ratio, by programming the pump. HPLC solvents and water were previously filtered under vacuum through 0.45 µm nylon filters and degassed using helium spare.

### Sample Preparation

Ten tablets of BM [(ca. 0.5 mg per tablet (200 mg)] or DM [ca. 1.0 mg per tablet (100 mg)] were each weighed and pulverized. A 100 mg or 40 mg sample of the fine powder for analysing BM or DM was weighed and transferred into a 25 ml volumetric flask, and completed to volume with methanol. These solutions contained 5 µg $ml^{-1}$ or 10 µg $ml^{-1}$ MPL (IS) respectively. These mixtures were mixed and sonicated at 40 °C for 20 min. After cooling, an aliquot of the suspension containing BM was placed in a stoppered centrifuge tube. After centrifugation at 3700 g for 3 min, 20 µl of the supernate were injected into the HPLC system. 5 ml of the mixture containing DM was diluted twice and processed under BM conditions.

### Chromatographic Analysis

Once the column had been conditioned with mobile phase, chromatograms were obtained at 30 °C. A methanolic solution containing DM, BM and MPL (IS) or an appropriate mixture of them in the range 2–10 µg $ml^{-1}$ was injected (20 µl). The flow-rates were: 0.5 ml $min^{-1}$ when using the Hamilton PRP-1, 1.2 ml $min^{-1}$ for Hypersil and 1.0 ml $min^{-1}$ for Spherisorb columns. Detection was at 245 nm.

## Results and Discussion

### Optimization of Mobile Phases and Columns

In a previous paper, DM and BM were separated from other corticoids contained in a sample under different isocratic conditions for screening purposes [11]. With the aim of improving these separations for simultaneous determination of DM and BM using MPL as IS, an optimization study was carried out on Spherisorb, Hypersil and polymeric PRP-1 combined with isocratic mobile phases: $H_2O$-methanol, $H_2O$-acetonitrile and $H_2O$-tetrahydrofuran. Only retention factors, k, were considered in the range 1–20. With different $H_2O$-methanol mixtures no separation was achieved in any case. However, using various $H_2O$-acetonitrile mixtures separation only occurred on the Hamilton column. Optimum separation was with $H_2O$-acetonitrile 76:24 (v/v), but was not baseline. k values of these compounds are given in Table I. When $H_2O$-tetrahydrofuran mixtures were used, separations were achieved with all three columns (Figure 2). The optimum composition of the mobile phase was the same in all cases and k values are in Table I. Water-tetrahydrofuran and the Hypersil column were finally selected for further applications. Resolution values, $R_s$, for DM and BM for the columns and mobile phases studied are in Table I.

### Calibration Graphs

Standards containing mixtures of DM, BM and MPL (IS) were prepared at five different concentrations in the range 2.00–10.0 µg $ml^{-1}$ using 5.0 µg $ml^{-1}$ MPL. These solutions were separated with $H_2O$-tetrahydrofuran (72:28, v/v) at 1.2 ml $min^{-1}$, a Hypersil column and UV-DAD detection at 245 nm. The results were analyzed by linear regression. Plotting the ratio of corticosteroid peak area to IP (IS) (PAR) against the concentration (x) of each corticosteroid, the calibration

**Table I.** Retention factors, k, for DM, BM and MPL obtained with various optimum mobile phases and reversed-phase packings, resolution values, $R_s$, for DM and BM. Mobile phases: **A** = $H_2O$-acetonitrile (76:24, v/v) and **B** = $H_2O$-tetrahydrofuran (76:24, v/v).

| Mobile phase | Columns | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Spherisorb ODS | | | | Hypseril ODS | | | | PRP-1 | | | |
| | MPL | BM | DM | $R_s$ | MPL | BM | DM | $R_s$ | MPL | BM | DM | $R_s$ |
| A | – | – | – | – | – | – | – | – | 9.70 | 11.62 | 12.56 | 0.68 |
| B | 11.96 | 13.72 | 15.25 | 1.2 | 8.73 | 9.94 | 11.12 | 1.0 | 12.01 | 15.12 | 16.86 | 1.0 |

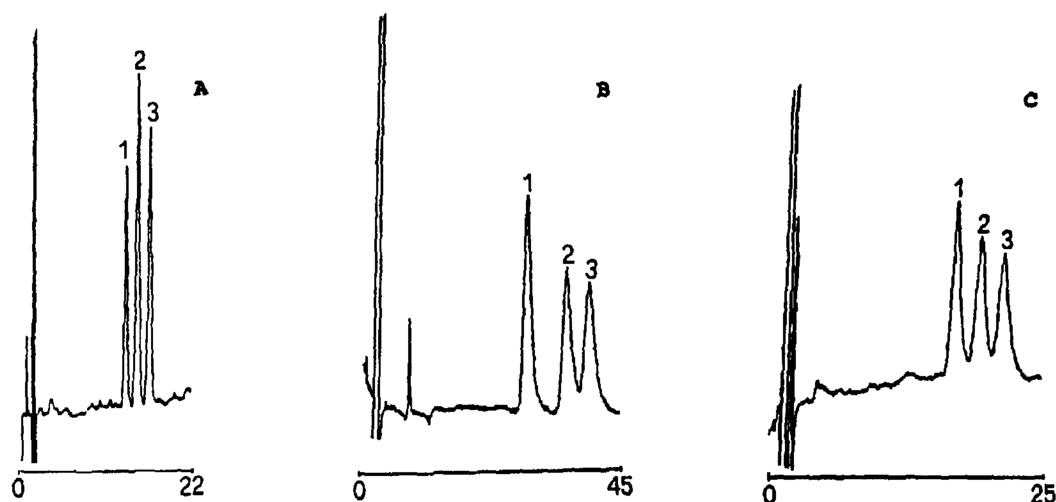


**Figure 2**

Separation of MPL, BM and DM eluted with $H_2O$-tetrahydrofuran (76:24, v/v). MPL (IS) (5 μg $ml^{-1}$). (A) Hypersil ODS column. BM and DM (8 μg $ml^{-1}$), (B) PRP-1 ODS column. BM and DM (5 μg $ml^{-1}$) and (C) Spherisorb ODS column. BM and DM (5 μg $ml^{-1}$). UV detection 245 nm. Peaks: **1** = MPL; **2** = BM; **3** = DM.

**Table II.** Linear regression equations (PAR = A + Bx) for DM and BM. PAR is peak area ratio to MPL (IS) 5 μg $ml^{-1}$; x = μg $ml^{-1}$ BM or BM; r = correlation coefficient.

| Compound | A | B | r | LD (ng) | RSD, % |
|---|---|---|---|---|---|
| 1. BM | – 0.029 | 0.203 | 0.999 | 0.12 | 3.1 |
| 2. DM | – 0.038 | 0.201 | 0.999 | 0.12 | 2.4 |

**Table III.** Determination of DM, BM, and mixtures in commercial tablets.

| Samples | Mean values, % BM | Mean values, % DM |
|---|---|---|
| BM tablets (n = 5) | 93.2 RSD: 1.8 | – |
| DM tablets (n = 5) | – | 98.2 RSD: 2.1 |
| mixtures (DM + BM) (n = 4) | 94.3 RSD: 2.2 | 97.5 RSD: 2.7 |

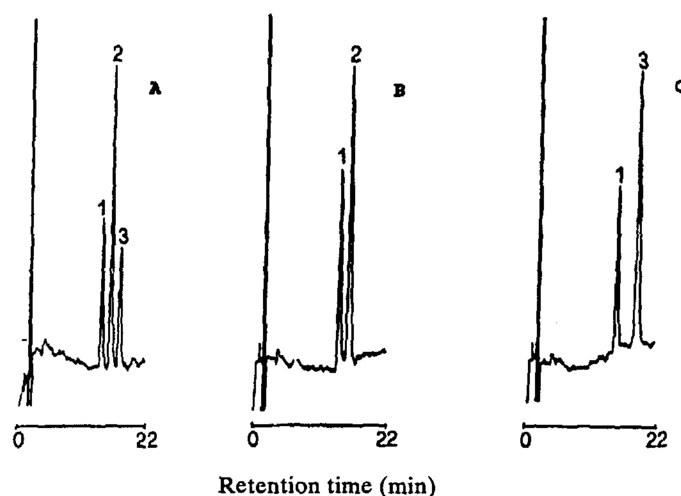


**Figure 3**

Typical chromatograms in commercial tablet analysis: (A) DM and BM mixture; (B) BM and, (C) DM peaks as Figure 2.

equations, PAR = A + B x (μg $ml^{-1}$), were obtained. In Table II parameters A (intercept), B (slope) and r (regression coefficient) are shown. In all cases the intercepts were not significantly different from zero.

## Precision, Selectivity and Detection Limits

The precision was examined by analyzing ten different samples of corticosteroids containing 5 μg $ml^{-1}$ each using the calibration graphs. The RSD for BM and DM is shown in Table II. The limits of detection (LD) obtained for a signal-to-noise ratio of 3 (S:N = 3) are also in Table II. Potential interference by other corticoids was investigated under working conditions, including the following: triamcinolone, prednisone, cortisone, prednisolone, corticosterone, 11α-hydroxy-progesterone, fludrocortisone, fludrocortisone acetate, methyl-prednisolone, triamcinolone-acetonide (TRA), deflazacort, cortisol, and 21-OH-deflazacort. Interference was found only with TRA because it coeluted with BM.

## Application

The present method was applied to the determination of DM and BM, and mixtures of these compounds in commercial tablets in quintuplicate (n = 5). Four differ-

ent mixtures of DM and BM were prepared by mixing different amounts of pulverized tablets. The total amount of these compounds remained constant and their concentrations were in the range 2–10 $\mu g\ ml^{-1}$. The results obtained are summarized in Table III. As it can be seen DM and BM lie within the range 90–110 % required by the current USP [8]. Typical chromatograms of DM, BM and their mixtures are shown in Figure 3.

From the results, it can be concluded that this method can be used for the individual determination of these drugs in bulk or pharmaceutical materials.

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