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Liquid-Chromatographic Assay of Dexamethasone in Plasma

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We report a simple, efficient method for removing dexamethasone from plasma by use of solid-phase extraction columns. The dexamethasone is then quantified by "high-performance" liquid chromatography with ultraviolet detection at 254 nm. Dexamethasone concentrations and peak-height ratios were linearly related over the range 5 to 200 μg/L. The lower limit of sensitivity was 5 μg/L. The average recoveries of dexamethasone and methylprednisolone (internal standard) were 72 and 71%, respectively. This procedure offers improved efficiency over that of previously described methods by decreasing analytical time and improving sample cleanup. Sensitive, specific, and relatively inexpensive, this method is suitable for most clinical applications.

Additional Keyphrases: sample preparation • corticosteroids • methylprednisolone as internal standard

Dexamethasone (9-fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione) is a synthetic corticosteroid that is important in treating many allergic and inflammatory conditions (1, 2). In moderate to large doses, dexamethasone is also an effective anti-emetic in the treatment of chemotherapy-induced nausea and vomiting in cancer patients (3–5). High doses are routinely used, but the therapeutic plasma concentrations for this indication have not been defined.

Radioimmunoassay procedures, the most commonly used techniques for determining dexamethasone in biological fluids, require the time-consuming and expensive preparation of a specific antibody (6–8). Recently, several investigators have described methods for dexamethasone analysis in human plasma in which normal or reversed-phase "high-pressure" liquid chromatography (HPLC) is used (9–10). These procedures are indeed specific and sensitive but are complicated and time-consuming, requiring between two and five extraction steps before analysis. Here we report a reversed-phase HPLC method with ultraviolet detection in which the dexamethasone is extracted from plasma on solid-phase extraction columns.

Materials and Methods

Reagents. Dexamethasone, methylprednisolone, and triethylamine were obtained from Sigma Chemical Company, St. Louis, MO, and used as received. Acetonitrile for use in the HPLC separation was "distilled-in-glass" grade, from MCB Reagents, Cincinnati, OH 45212. Chloroform (from MCB) and methanol (from Burdick and Jackson Laboratories, Inc., Muskegon, MI) for use in the extraction procedure were analytical grade. Filtered de-ionized water was supplied by a water-purification system (Corning Glass Works, Corning, NY). Silica and C18 Bond-Elut extraction columns (both 1-mL capacity) were from Analytichem International, Harbor City, CA.

Apparatus. A Model 210 sample-injection valve and a Model 112 solvent-delivery module (both from Beckman Instruments, Fullerton, CA 92634) were used with the HPLC to monitor the column effluent. We used a Beckman Model 160 absorbance detector set at 254 nm and a sensitivity of 0.01 A full scale. Peak heights were measured and recorded with a Model 3390A reporting integrator (Hewlett Packard, Avondale, PA 19311). The 150 × 4.6 mm column was prepacked with "ultrasphere" octadecysila, of 5 μm average particle diameter (Altex, Berkeley, CA).

Procedures. The HPLC solvent was prepared by mixing 280 mL of acetonitrile with 720 mL of de-ionized water. We then added 200 μL of triethylamine to each liter of solution. The final mixture was passed through a 0.5-μm (pore-size) filter (Millipore Corp., Bedford, MA), then degassed by sonicking under reduced pressure for 10 min. A flow rate of 1.5 mL/min at 1.9 kPa was maintained. Chart speed was 20 cm/h. All procedures were carried out at room temperature.

Silica extraction columns were connected in series above the C18 columns with a fitted Bond-Elut adaptor. On the day before the analysis, each set of columns was washed with 2 mL of methanol followed by 2 mL of de-ionized water. On the day of the procedure, the columns were prepared by washing with 3 mL of methanol, then with 4 mL of de-ionized water. To each sample and standard, we added 76 ng of methylprednisolone (internal standard) in 38 μL of water. We aspirated fluids and samples through the columns under reduced pressure, using a 10-place vacuum manifold (Vac-Elut; Analytichem International). The samples consisted of 0.5 to 2.0 mL of plasma, applied after dilution with de-
ionized water to a total volume of 1.0–4.0 mL. To prevent drug loss, we rinsed the test tubes that had contained the samples with 0.5 mL of de-ionized water and also passed this through the extraction system. After the sample had passed through the columns, the silica phase was removed. Both the silica and the C18 Bond-Elut columns were reused one time after successive washings with 5 mL of chloroform, 5 mL of methanol, and 3 mL of de-ionized water.

We then washed the C18 columns with 3 mL of aqueous methanol (50 mL of methanol per liter), then with 4 mL of chloroform to remove any interfering substances that might remain on the column. Finally, we eluted the drug and internal standard with 2 mL of methanol/chloroform (3/1 by vol). The last eluent was evaporated in a "Meyer N-Evap" evaporator (Organomation, South Berlin, MA 01549-0159) at 36 °C, under nitrogen; the residue was dissolved in 50–100 µL of mobile phase, and a 50-µL aliquot was injected onto the HPLC column for quantification.

**Calibration and reproducibility.** A standard curve was prepared from data on standards containing 0, 5, 10, 25, 50, 75, 100, 150, and 200 ng of dexamethasone per milliliter of plasma (prepared from stock solutions containing 10 and 100 ng/50 µL of water). We added to each sample a constant amount, 76 ng, of methylprednisolone as the internal standard, 38 µL of an aqueous solution containing 100 ng/50 µL. Pooled plasma was extracted as previously outlined.

Calibration curves were constructed by calculating the ratio of the peak height of each injection to that of the internal standard and plotting the ratio against the amount of dexamethasone added to the sample. By least-squares linear regression analysis, we determined the slope, y-intercept, and correlation coefficients. The response of the HPLC system was linearly related to concentration in the range 5–200 µg/L.

To assess analytical recovery of dexamethasone, we compared the height of the chromatographic peak for plasma samples to which 100 ng of dexamethasone had been added with that for a direct injection of aqueous standard. Assay precision was determined by successive sampling of dexamethasone-supplemented pooled plasma. To study the specificity of the method, we analyzed aliquots of various commonly administered drugs, both directly and after completion of the extraction procedure.

**Results and Discussion**

Figure 1 depicts chromatograms of a blank plasma extract and of plasma with 10 and 100 µg of dexamethasone per liter. Retention times are: dexamethasone, 12.0 min; internal standard, 10.0 min. Analysis of the blank plasma indicated no significant interference from endogenous compounds. Concentrations (x) and peak-height ratios (y) of dexamethasone to methylprednisolone were linearly related for all dexamethasone concentrations investigated, 5 to 200 µg/L (r² = 0.9984, y = 0.0106x – 0.0130). The average analytical recoveries from 10 separate plasma samples containing 100 µg of dexamethasone and 76 µg of methylprednisolone per milliliter were 72% (range 60–90%) and 71% (range 56–93%), respectively.

Results (in micrograms per liter) of within-day precision studies (n = 10) were as follows. For 10: mean 10.87, SD 1.06, CV 9.74; for 100: mean 101.5, SD 3.46, CV 3.41%. We determined between-day precision by analyzing 10 samples during one month. CVs for 30, 80, and 160 µg/L were 4.47, 5.44, and 5.20%, respectively. None of the following drugs interfered with the assay of dexamethasone: amikacin, aminophylline, bleomycin, cefoxitin, cephalothin, cimetidine, clindamycin, cyclophosphamide, digoxin, diphenhydramine, doxorubicin, droperidol, 5-fluourouracil, fluphenazine, furosemide, gentamicin, hydroxyzine, imipramine, methotrexate, metoclopramide, penicillin G, prochlorperazine, promethazine, thiethylperazine, ticarcillin, tobramycin, and vincristine. As little as 10 ng of dexamethasone (extracted from 2 mL of plasma) could be measured accurately; thus the lower sensitivity limit of the procedure is 5 µg/L, sensitive enough for studies of healthy volunteers and patients receiving oral and parenteral doses of 4 and 10 mg of dexamethasone. Figure 2 shows a typical concentration–time profile of dexamethasone in plasma after oral and intravenous administration.
intravenous administration to a healthy man.

Although methylprednisolone possesses pharmacological properties similar to those of dexamethasone, it is unlikely that these two agents would deliberately be used in combination in treating a patient. Therefore, we believe it is a good choice for use as an internal standard. The chromatographic procedure described differs from those previously reported by the use of a fast, efficient, and inexpensive solid-phase extraction step. The process takes approximately 40 min to the completion of the drying step, so that about 20 samples of plasma can be analyzed within an 8-h work day. We believe this procedure is suitable for clinical applications and offers significant advantages over previously described methods.

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References


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An Automated Liquid-Chromatographic System for Convenient Determination of Glycated Hemoglobin \( \text{A}_{1c} \)

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For quantification of glycated hemoglobin (HbA₁c), we compared an automated system (Pharmacia Fast Protein Liquid Chromatography), which separates and determines HbA₁c with a commercial disposable-minicolumn kit (Boehringer-Mannheim), which separates total HbA₁. We studied 41 diabetic women and 79 apparently normal women on their first postpartum day. The automated method was more precise (within-run CV 0.98–4.16%) than the kit method (within-run CV 3.67–7.77%). Results by the two methods correlated well for both control (\( p < 0.001 \)) and diabetic (\( p < 0.05 \)) groups. Values for HbA₁c correlated significantly with fasting blood-glucose concentrations in controls and diabetics (intraassay correlation coefficient \( r = 0.622 \) and 0.865, respectively, \( p < 0.001 \)) as well as with 1- and 2-h values for glucose after a 75-g glucose load in the control group (\( r = 0.549 \) and 0.846, respectively, \( p < 0.001 \)). For HbA₁c, no such significant correlation was found except with values for fasting blood glucose in diabetics (\( r = 0.745 \), \( p < 0.001 \)).

Evaluation of HbA₁c was a more sensitive index of glycemic status. The automated system is convenient, reliable, and easily operated.

Additional Keyphrases: diabetes · glucose tolerance test · pregnancy · fetal hemoglobin (HbF)

Minor variants of adult hemoglobin (HbA₁) have been identified as hemoglobin A₁A, A₁B, and A₁C according to their order of elution on cation-exchange chromatography (1). Hemoglobin A₁C (HbA₁C) is similar to HbA₁ except that glucose is linked to the amino-terminal valine of the \( \beta \)-chain by means of a Schiff base (2). The Schiff base adduct slowly undergoes the Amadori rearrangement to stable ketoamine (3–5). "Glycated hemoglobin" is the term recently applied to describe this nonenzymatic linkage (7). The N-terminal valine is not the only glycation site of HbA₁. Ketoamine linkages have also been found with \( \varepsilon \)-amino groups of lysine residues in both \( \alpha \) - and \( \beta \)-chains of HbA₁ (8). However, glycation of N-terminal valine causes the molecule to undergo a change in its isoelectric point, which allows it to be separated from other hemoglobins. On the other hand, hemoglobin A₁C has been further separated into two components, A₁A₁ and A₁B₁, which co-chromatograph with hemo-

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