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# HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF HYDROCORTISONE AND METHYLPREDNISOLONE AND THEIR HEMISUCCINATE SALTS

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## SUMMARY

A reversed-phase high-performance liquid chromatographic (HPLC) procedure for the determination of hydrocortisone hemisuccinate and methylprednisolone hemisuccinate and their corresponding free steroids in experimental, lyophilized products of the hemiesters is described. The method is suitable as a stability-indicating assay.

HPLC was performed on a 5-$\mu$m ODS column, using a mobile phase of 2% glacial acetic acid, 35–40% acetonitrile and 65–60% water and UV detection (242 nm). A comparison of this HPLC method with the U.S.P. blue tetrazolium assay was made. For freshly prepared samples, the sum of the hemisuccinate ester and the 21-hydroxycorticosteroid as determined by HPLC approximates the blue tetrazolium assay values.

## INTRODUCTION

The sodium salts of hydrocortisone hemisuccinate (HCHS) and methylprednisolone hemisuccinate (MPHS) are water-soluble esters of hydrocortisone (HC) and methylprednisolone (MP). After parenteral administration of a solution of these drugs, they are hydrolyzed to the pharmacologically active 21-hydroxycorticosteroids, HC and MP.

*In vitro*, an alkaline solution of 21-hydroxycorticosteroid HS esters readily decomposes to produce the 21-hydroxycorticosteriod and other degradation products[1]. Thus, dosage forms of the hemiesters must be lyophilized and reconstituted into parenteral solution only prior to use. However, even in the dried form, these products are moisture-labile.

The compendial assay for HC sodium succinate or MP sodium succinate is a blue tetrazolium assay[2]. This is a general procedure for the quantitative assay of assay of any steroid possessing the reducing $\alpha$-ketol group. It also measures any steroid which can be hydrolyzed in base to an $\alpha$-ketol and because it is performed

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under alkaline conditions, both the 21-hydroxycorticosteroid and its 21-hemisuccinate ester are measured. The method is not specific and, therefore, it is not a stability-indicating assay[3].

Several methods have been described in the literature to measure specifically the concentration of the hemiester of the 21-hydroxycorticosteroid or the 21-hydroxy-corticosteroid. These assay procedures consist of a blue tetrazolium reaction preceded by a separation step, either thin-layer chromatography[4] or alkaline extraction[5]. A photometric method for determining only the 21-hydroxycorticosteroid[6] is based on a condensation of the glyoxal obtained by cupric acetate oxidation of 21-hydroxy-corticosteroid with phenylhydrazine. The 21-hemiesters do not react. All of these procedures require several manipulative steps for the analysis. Also, a separate procedure is needed for the 21-hydroxycorticosteroid and its 21-hemisuccinate ester. Several high-performance liquid chromatographic (HPLC) methods have been described for the separation of mixtures of steroids in pharmaceutical preparations[7–9] and in biological fluids[10–13]. However, few of these methods were developed into a stability-indicating method for dosage forms of steroids.

The method described in this report uses reversed-phase HPLC to determine HC and MP and their respective HS salts in lyophilized dosage forms. Our method is a stability-indicating assay for the HS esters of HC and MP.

## EXPERIMENTAL

### *Apparatus*

A Spectra-Physics Chromatronix Model 3500 high-performance liquid chromatograph (Santa Clara, Calif., U.S.A.), equipped with a variable-wavelength spectrophotometric detector, Chromatrix Model 770, and a Hewlett-Packard Model 7130A strip-chart recorder was used. A Spectra-Physics sample injection valve with a 100-$\mu$l sampling loop was used to introduce the samples. Peak areas were determined with a Spectra-Physics Autolab minigrator.

### *Column*

A $C_{18}$ reversed-phase packing material from Spectra-Physics (Spherisorb 5 $\mu$m ODS; 3 $\times$ 250 mm) was used.

### *Operating conditions*

The variable UV detector was maintained at 242 nm. The sensitivity of the detector was 0.2 a.u./10 mV output. The flow-rate was held constant at about 1 ml/min. The column pressure was between 2000–3000 p.s.i.

### *Mobile phase*

The mobile phase was a mixture of acetonitrile (distilled-in-glass spectroscopic grade, Burdick & Jackson, Muskegon, Mich., U.S.A.), distilled water and glacial acetic acid (Mallinckrodt, St. Louis, Mo., U.S.A.). The ratio of the mobile phase mixture was dependent upon the hemiester and parent steroid being assayed and the column used. For the determinations of HC and HCHS, the ratio of acetonitrile–water–glacial acetic acid was 35:65:2. For the determination of MP and MPHS, the ratio of acetonitrile–water–glacial acetic acid was 40:60:2. The mobile phase was

prepared by mixing exact volumes of acetonitrile, filtered distilled water and glacial acetic acid. The mixture was stirred and degassed.

*Standards*

HC, MP, HCHS and MPHS standard stock solutions were prepared to contain 500 μg/ml of U.S.P. reference standard material in anhydrous methanol. These stock solutions were diluted with 5% acetic acid solution to contain 40, 50 and 60 μg/ml of hemiester and 1, 1.5 and 2.5 μg/ml of the 21-hydroxysteroid.

*Calibration curves*

Least-squares linear regression analysis was used to calculate the concentration of HC and its HS ester as well as MP and its HS ester. Either peak area or peak height were used in the regression analysis.

*Analysis of unit dosage*

Lyophilized products were reconstituted with bacteriostatic water for injection per package directions. An aliquot of the solution was diluted with distilled water (1:100) and then an aliquot of this solution was diluted with 5% acetic acid to a concentration of approximately 50 μg/ml. A 100-μl volume of this solution was injected onto the column via the sampling loop. The chromatogram was recorded and the peak areas or peak heights were determined.

## RESULTS AND DISCUSSION

The retention of the 21-hydroxycorticosteroids as well as their 21-hemiesters is quite sensitive to the acetonitrile–water ratio in the mobile phase. To optimize the separation and number of samples assayed per hour, the mobile phase acetonitrile–water ratio was adjusted to give a retention time of about 4 min for the first peak. The value of $\alpha$ (separation factor) for HC and HCHS was 1.8. The value of $\alpha$ for MP and MPHS was 1.5. A typical chromatogram for HC and HCHS standards and a commercial product is shown in Fig. 1. A typical chromatogram for MP and MPHS standard and a commercial product is shown in Fig. 2.

The precision of this method was determined by assaying samples of known concentrations of the 21-hydroxycorticosteroids and their 21-hemisuccinate esters. All samples studied contained up to 12% methanol. The results of this study for HCHS are given in Table I. The average difference (μg/ml) between the calculated concentrations and the known concentration was −0.06 using peak area and 0.01 using peak height.

Results of the precision study for HC are also given in Table I. The average difference between the calculated concentrations and the known concentration was 0.00 using peak area and 0.01 using peak height. The sensitivity of this method under the conditions described in the experimental section was determined to be 0.10 μg/ml for HC, which corresponds to 0.25% degradation.

The precision study results for MPHS are given in Table II. The average difference (μg/ml) between the calculated concentrations and the known concentrations was 0.00 using peak area and 0.12 using peak height.

The results of the precision study for MP are also given in Table II. The

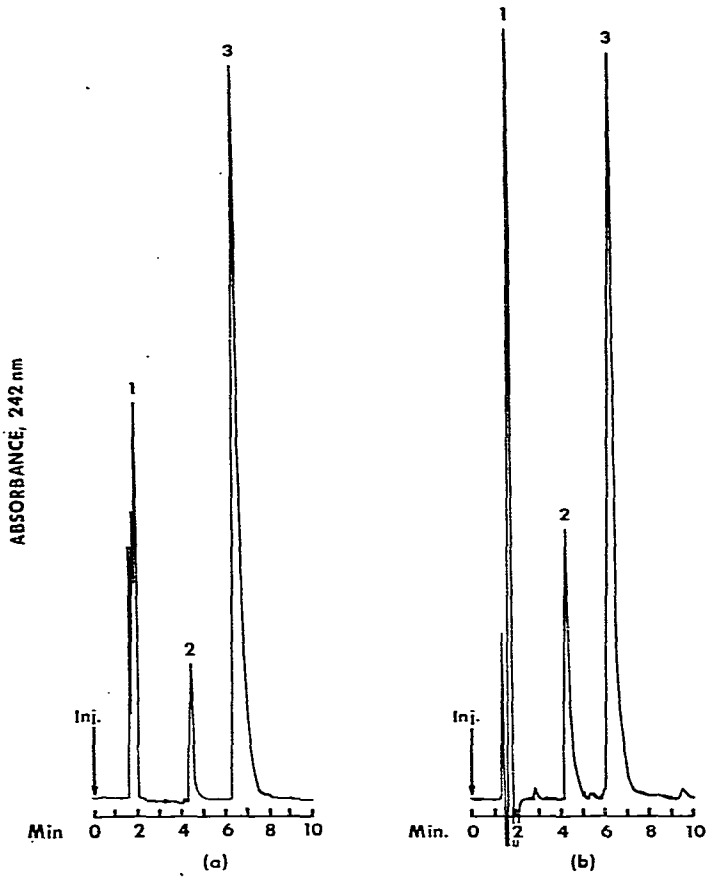


Fig. 1. Chromatogram of standards (a) and commercial sample of HC sodium succinate for injection, U.S.P. (b). 1 = Solvent front; 2 = HC (attenuated ×1) and 3 = HCHS (attenuated ×8).

average difference between the calculated concentration sand the known concentrations was —0.02 using peak area and 0.00 using peak height. The sensitivity of this method under the conditions described in the experimental section was determined to be 0.10 μg/ml for MP, which corresponds to 0.25% degradation.

At the concentrations used for the assay of the hemiesters methanol appears to have little effect upon peak height or area. However, at the lower concentrations (0.2–3.0 μg/ml) of the 21-hydroxycorticosteroids, the lack of methanol in the commercial sample preparation resulted in a 6% negative bias.

Our HPLC method showed good day-to-day reproducibility. The results of the analysis of three experimental lyophilized preparations on three consecutive days on HC sodium succinate for injection, U.S.P., are shown in Table III. The slight decrease in hemiester concentration and increase in 21-hydroxysteroid may be due to hydrolysis of the sample during the three days, even though the samples were diluted with 5% acetic acid solution and stored at 5°. It should be noted that the sum of HCHS and HC decreases over the 3-day period, indicating further decom-

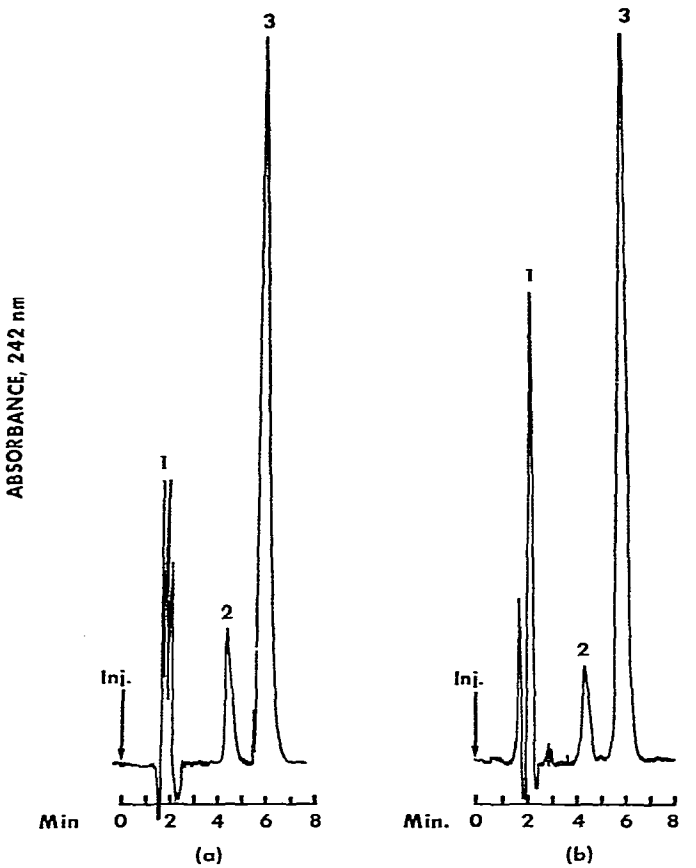


Fig. 2. Chromatogram of standards (a) and commercial sample of MP sodium succinate for injection, U.S.P. (b). 1 = Solvent front, 2 = MP (attenuated ×1) and 3 = MPHS (attenuated ×8).

TABLE I

PRECISION STUDY FOR HC AND HCHS

$N$ = number of assays.

| *Theory (μg/ml)* | *Found (μg/ml), determined by* | | | |
|---|---|---|---|---|
| | *N* | *Peak area (mean ± standard deviation)* | *N* | *Peak height (mean ± standard deviation)* |
| *HCHS* | | | | |
| 30.27 | 3 | 30.20 ± 0.24 | 3 | 30.42 ± 0.61 |
| 45.40 | 6 | 45.48 ± 0.53 | 4 | 45.98 ± 1.15 |
| 50.44 | 2 | 50.37 ± 0.53 | 2 | 49.61 ± 0.54 |
| 65.57 | 3 | 65.38 ± 1.50 | 3 | 65.70 ± 1.17 |
| *HC* | | | | |
| 0.26 | 3 | 0.24 ± 0.02 | 3 | 0.24 ± 0.01 |
| 1.79 | 6 | 1.80 ± 0.03 | 5 | 1.79 ± 0.03 |
| 2.55 | 3 | 2.56 ± 0.15 | 2 | 2.62 ± 0.03 |
| 3.83 | 3 | 3.81 ± 0.04 | 3 | 3.84 ± 0.05 |

TABLE II

PRECISION STUDY FOR MP AND MPHS

$N$ = number of assays.

| *Theory (μg/ml)* | *Found (μg/ml), determined by* | | | |
|---|---|---|---|---|
| | *N* | *Peak area (mean ± standard deviation)* | *N* | *Peak height (mean ± standard deviation)* |
| *MPHS* | | | | |
| 24.60 | 3 | 24.58 ± 0.16 | 3 | 24.58 ± 0.19 |
| 34.85 | 2 | 34.92 ± 0.18 | 1 | 35.32 |
| 41.00 | 10 | 40.71 ± 0.39 | 8 | 41.00 ± 0.62 |
| 53.30 | 4 | 53.53 ± 0.57 | 4 | 53.33 ± 0.42 |
| *MP* | | | | |
| 0.76 | 1 | 0.73 | 1 | 0.75 |
| 1.52 | 2 | 1.52 ± 0.04 | 1 | 1.52 |
| 2.03 | 3 | 1.98 ± 0.03 | 3 | 2.03 ± 0.03 |
| 2.54 | 6 | 2.54 ± 0.04 | 6 | 2.53 ± 0.04 |

TABLE III

REPRODUCIBILITY STUDY

| *Day* | *HCHS (μg/ml)* | | | *HC (μg/ml)* | | |
|---|---|---|---|---|---|---|
| | *Sample* | | | *Sample* | | |
| | *1* | *2* | *3* | *1* | *2* | *3* |
| 1 | 52.49 | 53.35 | 51.56 | 2.44 | 1.38 | 1.46 |
| 2 | 51.59 | 52.26 | 51.15 | 2.46 | 1.42 | 1.48 |
| 3 | 51.42 | 51.67 | 50.97 | 2.52 | 1.45 | 1.50 |

position of the hemiester or the 21-hydroxysteroid. This example illustrates the ability of this method to detect minute degradation.

A comparison of this method with the U.S.P. blue tetrazolium assay was made. Four experimental lots of HC sodium succinate for injection, U.S.P. and MP sodium succinate for injection, U.S.P. were assayed by both methods. Comparative results are given in Table IV. For the HPLC procedure, 9 vials were assayed individually (3 vials on 3 separate days) and the results were averaged. For the blue tetrazolium assay, 10 vials were assayed in one determination per U.S.P. directions.

The HPLC assay specifically determines the 21-hydroxycorticosteroid or its 21-hemisuccinate ester whereas the blue tetrazolium assay, U.S.P., measures the sum of all steroids possessing an α-ketol group, *i.e.*, 21-hydroxycorticosteroid plus its 21-hemisuccinate ester. As is shown in Table IV, the sum of the HS ester plus the 21-hydroxycorticosteroid approximates the blue tetrazolium assay values for fresh samples. The HPLC assay method is more specific than the U.S.P. blue tetrazolium method.

In conclusion, our HPLC method of determining HCHS and HC is an accurate, reproducible and stability-indicating assay. The same applies to the HPLC

TABLE IV

COMPARISON OF HPLC ASSAY WITH U.S.P. BLUE TETRAZOLIUM ASSAY

| *Sample* | *Lot* | *HPLC assay* | | *Total (%)* | *U.S.P. blue tetrazolium assay (%)* |
|---|---|---|---|---|---|
| | | *HCHS** | *HC*** | | |
| HC sodium succinate for injection, U.S.P. | 1 | 96.99 ± 3.44%*** | 1.39 ± 22%*** | 98.38 | 98.5§ |
| | 2 | 95.52 ± 1.82% | 0.81 ± 09% | 96.33 | 97.5 |
| | 3 | 99.78 ± 1.69% | 1.38 ± 15% | 101.16 | 101.3 |
| | 4 | 93.15 ± 1.63% | 2.77 ± 31% | 95.92 | 96.1 |
| | | *MPHS* | *MP* | | |
| MP sodium succinate for injection, U.S.P. | 1 | 96.67 ± 3.55%§§ | 1.37 ± 14%§§ | 98.04 | 101.7 |
| | 2 | 98.57 ± 1.07 | 2.49 ± 10 | 101.06 | 101.6 |
| | 3 | 98.59 ± 1.49 | 3.06 ± 08 | 101.65 | 101.2 |
| | 4 | 97.70 ± 0.92 | 3.48 ± 05 | 101.18 | 100.6 |

* Label claim (%).
** Equivalent label claim (%).
*** 9 Vials assayed (mean ± standard deviation).
§ 10 Vials assayed together.
§§ 3 Vials assayed (mean ± standard deviation).

method of determining MPHS and MP. Our method would be useful as the compendial assay for HC sodium succinate for injection, U.S.P. and MP sodium succinate for injection, U.S.P.

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