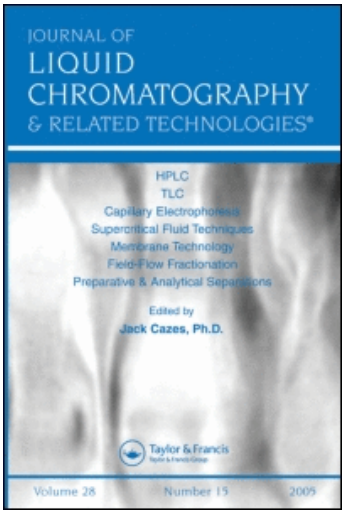

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Evaluation of an HPLC Test for Impurities in the Corticosteroid, 11β,21-Dihydroxy-pregna-1,4-diene-3,20-dione[17a,16a–d]2′-methyloxazoline-21-acetate

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ABSTRACT

A high performance liquid chromatographic (HPLC) procedure was developed and evaluated for the determination of impurity levels in bulk 11β,21-dihydroxy-pregna-1,4-diene-3,20-dione[17a,16a–d]2′-methyl-oxazoline-21-acetate, a corticosteroid compound. A gradient program, with a mobile phase of 0.025 M sodium phosphate buffer in acetonitrile/water (apparent pH 6.9), was used with a Hypersil ODS column. The acetonitrile composition was increased linearly from 15% to 50% over a 40 min period and held at 50% for 10 min. Ultraviolet detection at 245 nm was used to quantify all components. The procedure was validated for accuracy using spiked levels (0.1–1.4% w/w) with three suspected known impurities. A multi-day repeatability study, using

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three different Hypersil ODS columns, showed consistent impurity quantification results with one batch lot of the compound.

Key Words: Impurity test; Gradient HPLC; Steroid; Drug analysis.

INTRODUCTION

High performance liquid chromatography (HPLC) is the most widely used separation technique for testing the impurities in bulk drug within the modern pharmaceutical industry, and is evidenced both by the number of methods published in the literature and the current USP.[1] The main advantages of HPLC include its ability to have reproducible and reliable analysis and its reliance on the solubility of the analyte. These general characteristics of HPLC make it well suited for the analysis of steroid compounds.[2] Gas chromatography (GC) requires thermal volatility and thermal stability of analytes, which is not always practical for a test method, and GC analysis of steroid compounds has been reviewed in the literature.[3] The use of derivatives to gain volatility in GC analysis can make for a more complex test procedure and, perhaps, a less rugged or robust test procedure. HPLC also offers useful detection systems. Ultraviolet detection is generally inexpensive for routine analysis and is well suited for many steroidal and pharmaceutical compounds, which have UV chromophores. Mass spectrometric detectors, although expensive, can be used to supplement information, such as confirming the identity of impurities detected by an HPLC separation.

Reversed-phase HPLC has often been used extensively in the analysis of steroid compounds in the literature, and C18 columns have often been used,[4–9] which was chosen for this work. 11β,21-Dihydroxy-pregna-1,4-diene-3,20-dione[17a,16a–d]2′-methyloxazoline-21-acetate [Fig. 1(A)] is a glucocorticoid drug under study, and exhibits many of the properties of prednisone. This is an area of active research of therapeutic drugs; new corticosteroid drugs, having fewer side effects, are highly desirable. Prolonged therapy with corticosteroids may result in suppression of pituitary-adrenal function,[10] or result in loss of bone mass.[11] It was the objective of the current reported work to develop and validate[12] an HPLC test method, to have in place to evaluate different bulk lots of the test drug. Ultimately, a standard analytical Hypersil ODS (25 cm × 4.6 mm ID) column was used with a gradient mobile phase composed of phosphate buffer and acetonitrile as the organic modifier. The gradient mode of separation was chosen to reduce analysis time and improve the peak shape of the analytes. Since UV chromophores were present in the drug compound and the known suspected

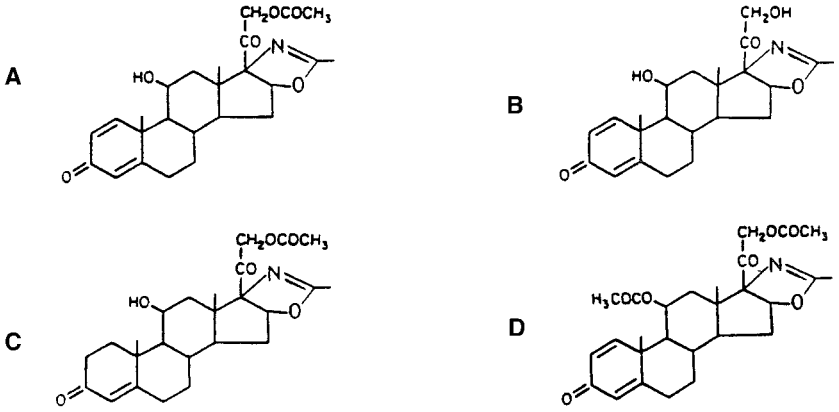

Figure 1. The structure of (A) 11β,21-dihydroxy-pregna-1,4-diene-3,20-dione [17a,16a–d]2′-methyloxazoline-21-acetate, the drug substance and three of the suspected known impurities, (B) the 21-alcohol analog (RRT = 0.70 for the developed chromatographic system), (C) the Δ-4 analog (RRT = 1.02), and (D) the 11-acetate analog impurity (RRT = 1.15).

impurities (Fig. 1), UV detection was chosen as the detection system for this routine test procedure. Three possible impurities were of concern to this study. The 21-alcohol analog [Fig. 1(B)], the Δ-4 analog [Fig. 1(C)], and the 11-acetate analog [Fig. 1(D)] of the parent compound were included in the validation work of this test procedure. The validation results for this analysis procedure, including accuracy and run-to-run reproducibility and repeatability are discussed.

EXPERIMENTAL

Reagents

High purity HPLC water was provided by a Barnstead (Boston, MA) NANOpure system, followed with an ultraviolet radiation treatment by a Barnstead ORGANICpure system. HPLC grade acetonitrile was purchased from Burdick & Jackson (Muskegon, MI). Concentrated phosphoric acid and sodium hydroxide were ACS grade (Fisher Scientific, Fair Lawn, NJ). The corticosteroid drug, 11β,21-dihydroxy-pregna-1,4-diene-3,20-dione [17a,16a–d] 2′-methyloxazoline-21-acetate, and related analogs, which were known possible impurities (see Fig. 1), were obtained "in-house."

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Chromatographic Conditions and Apparatus

A Spectra-Physics (San Jose, CA) Model SP8800 liquid chromatograph equipped with a Rheodyne (Cotati, CA) Model 7010 injector valve and an Applied Biosystems (PE Biosystem, Norwalk, CT) Model 757 detector was used for all HPLC experiments. A Hypersil (Thermo-Hypersil-Keystone Bellefonte, PA) ODS 5 μm particle, 250 mm × 4.6 mm type column was used. Mobile phase A consisted of 15/85 acetonitrile/water (v/v) made 0.025 M in sodium phosphate buffer (2.9 g of concentrated phosphoric acid (85%) were added to each liter of mobile phase and 10 M sodium hydroxide was added dropwise to obtain apparent pH 6.9). Mobile phase B consisted of 50/50 acetonitrile/water (v/v) made 0.025 M in sodium phosphate buffer (apparent pH 6.9 made as described previously). The gradient program was linear, starting at 100% A to 100% B during a 40 min period, and then a hold at 100% B for 10 min was used. A 3 min return ramp to mobile phase A was followed by a 10 min equilibration time at 100% A before the next chromatographic run. The flow rate was 1.1 mL/min and detection was at 245 nm. Sample solution injection size was 20 μL. The sample solution concentration was 0.25 mg/mL prepared in acetonitrile. A reference solution at 1% concentration (a 1 to 100 dilution in acetonitrile or approximately 0.025 mg/mL) was made for each sample weight. The 1% reference solution was injected and chromatographed, then followed by the corresponding sample solution.

Spiked sample solutions containing the parent corticosteroid compound (11β,21-dihydroxy-pregna-1,4-diene- 3,20-dione[17a, 16a–d]2′-methyloxazoline-21-acetate) and the three related analogs (Fig. 1) were prepared at the 0.1%, 0.2%, 0.5%, 1.0%, and 1.4% (w/w) equivalent level. Response factors were determined by comparing the peak area of 0.025 mg/mL level solutions of each known component to the same concentration of parent compound. A spiked sample solution containing 1% (w/w) Δ-4 analog [Fig. 1(C)] was used to calculate resolution between the parent and analog peaks on different Hypersil ODS columns used during this study.

Calculations

The peak areas for all impurity peaks and the peak area of the 1% reference peak for the drug are determined by integration. The known impurities % (w/w) were determined by the following equation:

$$\frac{1}{F} * \frac{A_i}{A_r + (\sum A_i/100)} = \%\ (\mathrm{w/w}) \quad \text{for the known components in the sample}$$

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where F is the response factor of the identified component [$F = 1.0$ for unknown components for % A/A. The parent steroid drug has a response factor of 1.0, its 21-alcohol analog has a response factor of 0.92, the Δ-4 analog equals 0.97 and the 11-acetate analog's response factor equals 0.91. The response factor is for % w/w.] A_i is the area of the impurity peak; ΣA_i is the sum of the areas of all the impurity peaks in the chromatogram; and A_r is the area of the drug peak in the %1 reference chromatogram.

Resolution between the parent steroid and the Δ-4 analog peaks was calculated by the traditional equation:

$$R_s = \frac{2(t_2 - t_1)}{(w_1 - w_2)}$$

where t is the retention time of the peak and w is peak width at the baseline.

DISCUSSION

The separation of the three impurity analytes from the parent, as well as the low level unknown impurities, was easily accomplished using this chromatographic procedure. Chromatograms of one batch lot of the corticosteroid compound is shown in Fig. 2; both a chromatogram of the sample solution and the 1% level reference solution are displayed. Three impurities were detected, all at relatively low levels. The two peaks preceding the parent peak are unknown; the peak eluting after the parent compound corresponds to the retention time of the 11-acetate analog [identification was subsequently verified by spiked solutions]. Figure 3 shows two chromatograms using the same batch lot of corticosteroid compound spiked at 0.1% and 0.5% (w/w), with the three known impurities. Resolution of the Δ-4 analog [Fig. 1(C)] from the tail of the parent peak was acceptable with three different Hypersil ODS columns used in the development of this procedure. Calculated resolution of a 1% spiked level solution was consistently between 1.0 and 1.2. This was considered adequate resolution for estimation of the Δ-4 analog impurity. Other reversed-phase HPLC columns were evaluated during the early development phase of this study. A Vydac 201HS54 column (Grace Vydac, Hesperia, CA), a Spherisorb ODS and Spherisorb C6 column (Alltech Associates, Deerfield, IL), each having the same 250 mm × 4.5 mm (ID) and 5 μm particle size, were evaluated for separating the target analytes. None performed as well as the Hypersil ODS column finally used in resolving the Δ-4 analog from the parent compound.

A detection wavelength of 245 nm was chosen for this method for several reasons. The λ_{max} for the parent compound in mobile phase A was 245 nm,

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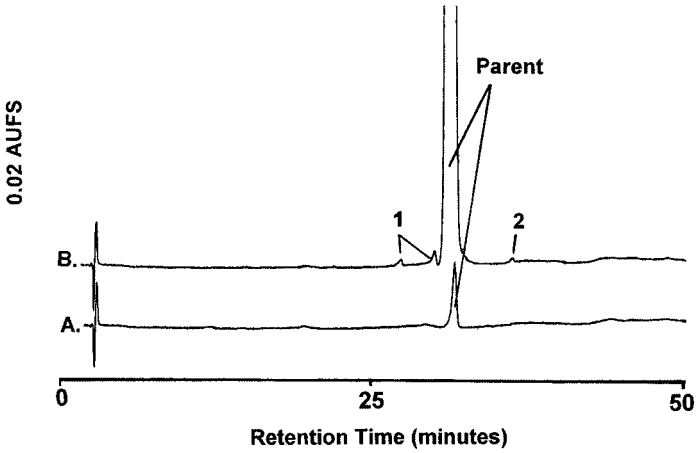

Figure 2. A chromatogram (A) of the 1% level reference solution and a chromatogram (B) of a sample batch of the steroid drug. The concentration of the drug in the sample solution is approximately 0.25 mg/mL; the reference solution is approximately 0.0025 mg/mL. The trace unknown impurities, peaks 1, in this sample lot are 0.07% and 0.14% (*A*/*A*). Trace impurity peak 2 is the 11-acetate analog and is less than 0.05% (*A*/*A*) in this batch lot of corticosteroid drug.

and this wavelength was very near the relative λ_{max} of the three analog compounds studied. All had similar UV maxima between 243 and 245 nm. The ultraviolet spectra of the Δ-4 and 11-acetate analogs showed a minimal absorbance near 210 nm, so lower detection wavelengths were not appropriate for this impurity test. Also, the use of a lower wavelength, such as 210 nm, gave a poor baseline response over the course of the gradient run. Response factors relative to the parent corticosteroid compound were calculated for each of the analog compounds, and found to be between 0.91 and 0.97 of the parent. Response factors were used for the calculation of recovery using the known spiked solutions.

The accuracy of this method to estimate the level of known impurities was verified by chromatographing solutions containing known levels of the three analog compounds. Spike levels of 0.1%, 0.2%, 0.5%, 1.0%, and 1.4% (w/w) in the parent solution were run. The HPLC method was found to be accurate as the data in Table 1 clearly show. Using two different Hypersil ODS columns, the measured level of any of the three impurities was acceptable for the study. The batch lot of corticosteroid drug compound used in the study, had very low levels of impurities, as is shown at the zero spike level in Table 1. The largest impurity was the Δ-4 analog of the parent drug, and it had an average level of 0.12% (w/w) at the zero spike level, as is reported in

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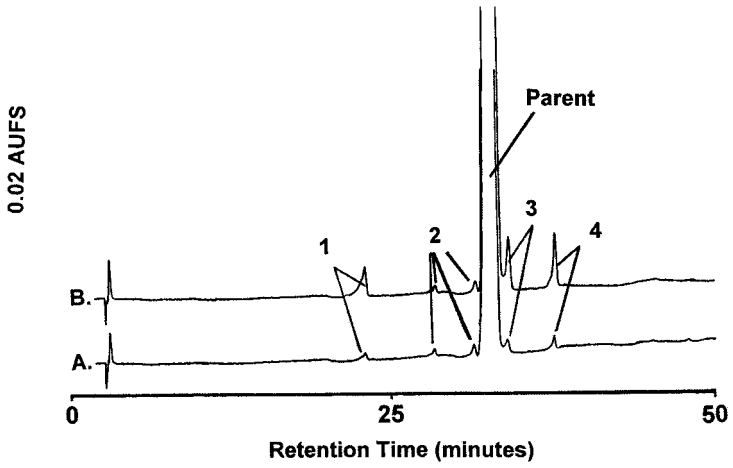

Figure 3. Chromatograms of spiked sample solutions containing 0.1% (A) and 0.5% (w/w) (B) of the three known impurities. Peak 1 is the 21-alcohol analog, peaks 2 are unknown trace impurities, peak 3 is the Δ-4 analog, and peak 4 is the 11-acetate analog impurity. The trace unknown impurities, peaks labeled as 2, were described in Fig. 2 and are at 0.07% and 0.14% (*A*/*A*) in this batch lot of corticosteroid drug.

the table. Furthermore, the spikes of the Δ-4 analog impurity demonstrated accurate recovery; this was with columns having calculated resolution between it and the parent peak of 1.0–1.2. The repeatability of this test procedure was demonstrated by analyzing the same batch lot of synthesized corticosteroid drug five times on four separate trial days, using three different Hypersil ODS columns. The total impurity level was consistent, estimated to be between 0.36% and 0.41% (*A*/*A*) over the study period (Table 2), and had an average recovery of 0.39% (*A*/*A*). The three major impurities are also listed in Table 2, and shows repeatability for the sample analyzed. Linearity of response for the parent compound was also verified. Chromatographed solutions, having concentrations of 0.00026, 0.0026, 0.026, 0.26, and 0.36 mg/mL (0.1–140% concentration of the normal working range of the sample solution), gave peak area responses which were linear. The *y*-intercept of the five point peak area response curve was nearly zero and the correlation coefficient was 1.0.

Finally, this procedure required the use of acetonitrile to be used to dissolve the parent corticosteroid compound. Degradation of the corticosteroid to the 21-alcohol analog was noticed when using aqueous solvent systems to dissolve the sample. Also, opening of the oxazoline ring is possible. Sample solutions held for 24 hr in acetonitrile did show the development of a small degradation peak at relative retention time (RRT) 1.08 at 0.1–0.2% (*A*/*A*)

Table 1. Known impurity recovery study using spiked solutions (% w/w).

Level of spike	Impurity analog	Actual weighed spike level	Measured level[a]		Average recovery
			Column 1	Column 2	
0.0	21-alcohol	0	0.01	0.05	0.03
	Δ-4	0	0.13	0.11	0.12
	11-acetate	0	0.03	0.02	0.03
0.1	21-alcohol	0.10	0.12	0.10	0.11
	Δ-4	0.10	0.23	0.21	0.22
	11-acetate	0.10	0.12	0.11	0.12
0.2	21-alcohol	0.20	0.22	0.21	0.22
	Δ-4	0.20	0.32	0.33	0.33
	11-acetate	0.20	0.22	0.30	0.26
0.5	21-alcohol	0.49	0.48	0.43	0.46
	Δ-4	0.49	0.57	0.59	0.58
	11-acetate	0.49	0.48	0.50	0.49
1.0	21-alcohol	0.98	0.95	1.04	1.00
	Δ-4	1.00	1.03	1.13	1.08
	11-acetate	0.99	0.91	1.00	0.96
1.4	21-alcohol	1.42	1.41	1.46	1.44
	Δ-4	1.43	1.47	1.52	1.50
	11-acetate	1.42	1.32	1.31	1.32

[a]Corrected by response factors.

levels. That degradation product has not yet been identified. If the sample solution is chromatographed within 6 hr after preparation, degradation peaks will not be detected. This is a reasonable working time to prepare and chromatograph the samples.

Planned future work will include identification of unknown impurities, as well as the evaluation of additional synthetic batch lots of the corticosteroid drug. This is outside the scope of this manuscript and the immediate need of a validated test method for the current impurity compounds available. The identification of impurities, along with future availability of actual reference compounds, will greatly advance the ability to monitor bulk production lots of this corticosteroid compound.

CONCLUSIONS

An HPLC procedure to measure the known impurities of 11β,21-dihydroxy-pregna-1,4-diene-3,20-dione [17a, 16a–d]2′-methyloxazoline-21-acetate bulk

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Table 2. Repeatability study of the test procedure.

Sample number	Column	Total impurities (area%)	Three major impurities detected area% (RRT)[a]
1	1	0.36	0.07 (0.85), 0.13 (0.94), 0.09 (1.02)
2	2	0.40	0.08 (0.85), 0.14 (0.94), 0.10 (1.02)
3	2	0.41	0.08 (0.85), 0.17 (0.94), 0.10 (1.02)
4	2	0.41	0.07 (0.85), 0.16 (0.94), 0.12 (1.02)
5	3	0.37	0.06 (0.85), 0.12 (0.94), 0.11 (1.02)
Mean		0.39	0.07 (0.85), 0.14 (0.94), 0.10 (1.02)

[a]RRT is the relative retention time with the parent drug peak.

compound was developed and found to be both accurate and reproducible. A Hypersil ODS column with dimensions of $250 \times 4.6\,mm^2$ (ID) was used in a gradient system of acetonitrile/water mobile phase and 0.025 M phosphate buffer at apparent pH 6.9. The organic content was varied from 15% (v/v) initially to a final content of 50% (v/v). This chromatographic system easily separated the three known impurities from the parent compound. Recovery of spiked sample solution from 0.1% to 1.4% (w/w) of these impurities gave accurate results. Three different Hypersil ODS columns gave reproducible results for impurity levels of one sample batch lot of the corticosteroid compound. In this sample, total impurities were consistently measured near 0.4% (A/A) using the developed HPLC procedure.

REFERENCES

1. *The 2002 United States Pharmacopoeia XXXV and National Formulary XX*; The United States Pharmacopoeia Convention, Inc.: Rockville, Maryland, 2001.
2. Volin, P. High performance liquid chromatographic analysis of corticosteroids. J. Chromatogr. B **1995**, *671* (1–2), 319–340.
3. Indrayanto, G.; Yuwono, M. Steriod analysis by gas chromatography. Encyclopedia Chromatogr. **2003**, *9*, 1–5.

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4. Shah, J.A.; Weber, D.J. Simultaneous determination of methylprednisolone and methylprednisolone 21-[8-[methyl-(8-methyl-(2-sulfonethyl)-amino]-8-oxooctanote]sodium salt in human urine by high performance liquid chromatography with ultraviolet detection. J. Chromatogr. **1989**, *434* (1), 125–254.
5. Gradner, R.S.; Walker, M.; Hollingsbee, D.A. A sensitive high performance liquid chromatographic method for the assessment of percutaneous absorption of topical corticosteroids. J. Pharmaceut. Biomed. B. **1990**, *8* (8–12), 1083–1085.
6. Marini, R.D.; Pantella, A.; Bimazubute, M.A.; Chiap, P.; Hubert, P.; Crommen, J. Optimisation and validation of a generic method for the LC assay of six corticosteroids and salicylic acid in dermopharmaceutical forms. Chromatographia **2002**, *5* (5–6), 263–269.
7. McBride, J.H.; Rodgerson, D.O.; Park, S.S.; Reyes, A.F. Rapid Liquid Chromatographic method for simultaneous determination of plasma prednisone, prednisolone and cortisol in pediatric renal-transplant recipients. Clin. Chem. **1991**, *37* (5), 643–646.
8. Shalaby, A.; Shahjahan, M. Improved high performance liquid chromatographic method for the determination of some corticosteroids. J. Liq. Chromatogr. **1991**, *14* (7), 1267–1274.
9. Hajkova, R.; Solich, P.; Dvorak, J.; Sicha, J. Simultaneous determination of methylparaben, propylparaben, hydrocortisone acetate and its degradation products in a topical cream by RP-HPLC. J. Pharm. Biomed. Anal. **2003**, *32* (4–5), 921–927.
10. Walsh, P.M. Ed. *Physicians' Desk Reference*, 57th Ed.; Thomson PDR: Montvale, NJ, 2003; 2005–2006.
11. Sweetman, S.C. Ed. *Martindale: The Complete Drug Reference*, 33rd Ed.; Pharmaceutical Press: London, 2002; 1039–1040.
12. Green, M.J. A practical guide to analytical method validation. Anal. Chem. **1996**, *68*, 305A–309A.

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