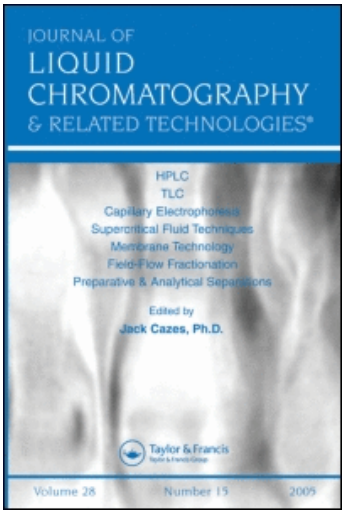

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HPLC Assay for Meprednisone in Tablets

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Abstract: A high performance liquid chromatographic (HPLC) method is presented for the determination of meprednisone in the presence of its degradation products using a reverse phase C_{18} column at ambient temperature with mobile phase consisting of acetonitrile:0.04 M dibasic sodium phosphate, pH adjusted to 7.0 (45:65, v/v). The flow rate was 1.3 mL/min. Quantitation was achieved with UV detection at 245 nm based on peak area. The method was developed and validated for the determination of meprednisone in tablets. The proposed method was validated for selectivity, linearity, accuracy, and robustness. The method was found to be suitable for the quality control of meprednisone in tablets as well as the stability indicating studies.

Keywords: Assay, HPLC, Meprednisone, Stability indicating method

INTRODUCTION

Meprednisone,pregna-1,4-diene-3,11,20-trione,17,21-dihydroxy-16methyl-(16β) is a corticosteroid with mainly glucocorticoid activity (Figure 1). It has been given by mouth as either the free alcohol or the acetate and by injection as the sodium hemisuccinate.[1]

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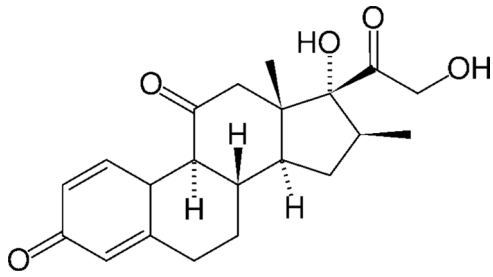

Figure 1. Meprednisone.

The method was validated by following the analytical performance parameters suggested by the International Conference on Harmonization (ICH).[2]

The literature survey revealed some high performance liquid chromatographic methods for meprednisone determination in pharmaceuticals (gradient system for corticosteroids) or as internal standard.[3,4] Other analytical techniques such as TLC has also been described.[5] An UV spectroscopy method for steroids is described in United States Pharmacopeia.[6]

The present manuscript describes a simple, rapid, precise, and accurate isocratic reversed phase HPLC method for the determination of meprednisone in the tablet dosage form in the presence of its degradation products.

EXPERIMENTAL

Chemicals and Reagents

Meprednisone (100.3%) was obtained from Tianjin Tianmao Technology Development Corp. Ltd. (Tianjun, China).

A commercial tablet formulation was studied. Its composition was meprednisone in a matrix of lactose, starch, magnesium stearate, silicon dioxide, and microcrystalline cellulose.

Acetonitrile used was HPLC grade, J. T. Baker, (Estado de Mexico, México), dibasic sodium phosphate AR Grade, J. T. Baker, (Estado de Mexico, México). Distilled water was passed through a 0.45 μm membrane filter.

Equipment

The HPLC system consisted of a dual piston reciprocating Spectra Physics pump (Irvine, CA, United States, Model ISO Chrom. LC pump),

a UV-Vis Hewlett Packard detector (Model 1050), a Hewlett Packard integrator (Loveland, CO, United States, Series 3395), and a Rheodyne injector (Model 7125).

Chromatographic Conditions

The analytical column was a reversed phase C_{18} column (Inerstil ODS-3, GL Sciences Inc.) (250×4.6 mm, 5 μm). The separation was carried out under isocratic elution with acetonitrile:0.04 M dibasic sodium phosphate, pH adjusted to 7.0 (45:65, v/v). The flow rate was 1.3 mL/min. The wavelength was monitored at 245 nm, and the injection volume was 20 μL. The HPLC was operated at ambient temperature. Under these conditions, the retention time (t_R) of meprednisone was approximately 6 min.

Standard Solutions

A standard stock solution of meprednisone was prepared at a concentration of 0.4 mg/mL in diluents, which was a mixture of acetonitrile and water (80:20, v/v). The standard solution was obtained by diluting the standard stock solution with diluent to obtain a solution containing 16 μg/mL of meprednisone.

Sample Preparation

Twenty tablets were weighed, finely powered, and an accurately weighed powered sample equivalent to one tablet was transferred to a 25 mL volumetric flask in 20 mL of diluents, and the flask was kept in an ultrasonic bath for 5 min. The contents of the flask were then diluted to 25 mL with diluents and thoroughly mixed. A 1 mL aliquot of the solution was transferred to a 100 mL volumetric flask. The sample was diluted to volume with diluents and filtered through a 0.2 μm nylon membrane (25 mm disposable filter; Cat. N° Y02025WPH microclar, Buenos Aires, Argentina).

Method Validation

System Suitability

Relative standard deviations (RSD) values of the peak area, tailing factor, retention time, and resolution were the chromatographic parameters selected for the system suitability test.[6]

Specificity

Forced degradation studies were performed to evaluate the specificity of the method. Degraded samples were prepared by refluxing 0.4 mg/mL meprednisone working standard with acid (1 N hydrochloric acid), base (1 N NaOH), water, 30% hydrogen peroxide, and refluxing for at least 30 min. The drug was subjected to thermal degradation (either in the solid state or in solution) in an open container in an oven at 110°C for 24 h and photochemical degradation (a solution was transferred to a container and exposed to daylight for 24 h). After degradation treatment, samples were allowed to cool at room temperature and diluted, if necessary, to the same concentration as that of the standard solution, after being neutralized. After degradation, samples were analyzed using the methodology and the chromatographic conditions described.

Linearity

Linearity solutions were prepared at six concentrations levels from 30% to 150% of analyte concentration.

Precision

Precision of the method was checked by carrying out six independent assays of meprednisone test samples against the qualified working standard. Intermediate precision was performed by analyzing the samples by two different analysts on different days.

Accuracy

The accuracy was evaluated by the recovery studies at concentration levels of 80, 100, and 120% (3 samples each). Twenty tablets from the same lot of a commercial formulation were emptied in a mortar. The amount of meprednisone recovered in relation to the results obtained in the intermediate precision study was calculated.

Robustness

Robustness was performed by deliberately changing the chromatographic conditions. The organic strength was varied by ±10%, while pH was varied by ±0.5 units.

Area, RSD, retention time, tailing, and resolution were evaluated.

RESULTS AND DISCUSSION

The described reversed phase liquid chromatography method was developed to provide a rapid quality control determination of meprednisone in tablets. Validation of the method was performed according to ICH. This method uses a simple mobile phase. All samples were analyzed using the assay chromatographic conditions described.

The analytical column was equilibrated with the eluting solvent system used. After an acceptable stable baseline was achieved, the standards and then the samples were analyzed.

System Suitability

System suitability results were calculated according to the USP 29 <621> from typical chromatograms. Instrument precision as determined by six successive injections of the standard preparation provided a relative standard deviation (RSD) below 1.5%. Peak asymmetry or tailing factor, T, was calculated as $T = W_{0.05}/2f$; where $W_{0.05}$ is the distance from the leading edge to the tailing edge of the peak, measured at 5% of the peak height from the baseline and f is the distance from the peak maximum to the leading edge of the peak. The tailing factor did not exceed 1.5. The resolution between meprednisone and its degradation product should be greater than 1.5.

Stability of the standard solution and sample preparation was studied by injecting the prepared solution at periodic intervals into the chromatographic system up to about 24 hours stored at room temperature and refrigerated. The solutions maintained at least 98.9% and 99.4% of their initial concentration under the test conditions.

Selectivity

Degradation was indicated in the stressed sample by a decrease in the expected concentration of the drug and increased levels of degradation products. Meprednisone was degraded to different products under base, oxidation, and thermal degradation (solid and solution) (Table 1, Figure 2). In addition there was no any interference at retention time of meprednisone and its degradation product of placebo solution.

Linearity

The linearity of the method was determined by analysis of three replicates of six concentrations of standard solutions (range from 0.0970 and 0.4848 μg injected). The calibration curve showed good linearity over

Table 1. Selectivity: degradation conditions of meprednisone

Condition	Time (h)	Meprednisone (%)	RRT of degradation products
Acid (1 N HCl, reflux)	1.0	89.4	Non detectable
Base (1 N NaOH, reflux)	1.0	31.2	0.37
Hydrogen peroxide 30% (reflux)	1.0	71.2	1.16, 1.31, 2.17
Water (reflux)	1.0	94.7	Non detectable
Heat dry, 110°C (solution)	24	69.4	0.36, 0.40, 0.68, 0.85, 2.18
Heat dry, 110°C (solid)	24	88.1	0.3
Daylight exposure	24	92.9	Non detectable

*RRT, relative retention time.

the concentration range. The correlation coefficient (“r”) value was 0.9997. Typically, the regression equation for the calibration curve was found to be $y = 14997469.04x + 74809.5$. The linearity of the calibration graphs was validated by the high value of the correlation coefficient and the intercept value that was not statistically ($p = 0.05$) different from zero (Table 2).

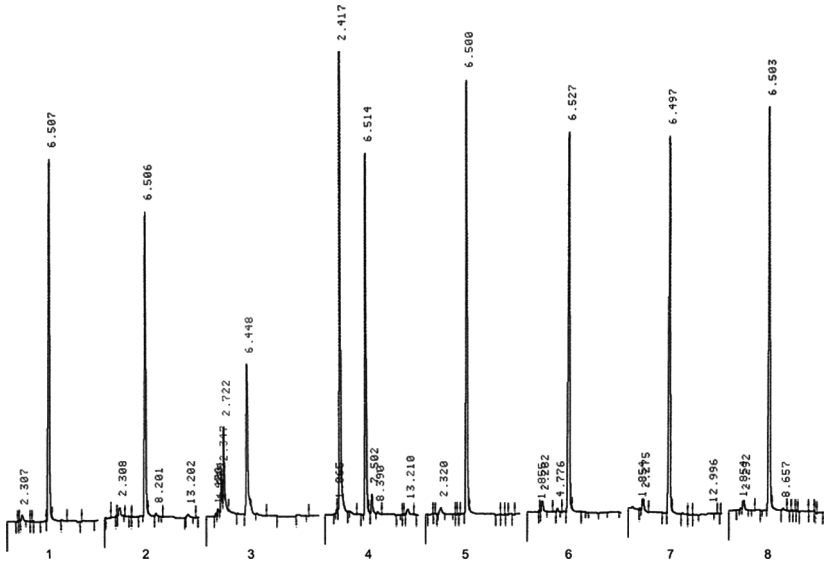

Figure 2. Chromatograms of meprednisone: 1, Standard; 2, Acid hydrolysis (1 N HCl, reflux, 1.0 h); 3, Alkaline hydrolysis (1 N NaOH, reflux, 1.0 h); 4, Oxidation (Hydrogen peroxide 30%, reflux, 1.0 h); 5, Hydrolysis (Water, reflux, 1.0 h); 6, Heat dry, 110°C (solution, 24 h); 7, Heat dry, 110°C (solid, 24 h); 8, Daylight exposure (24 h).

Table 2. Linearity data of meprednisone

% Of nominal value	Injected (μg)	Average peak area response	RSD
30	0.0970	1467570.00	1.53
50	0.1616	2522033.33	0.37
80	0.2600	4015821.33	0.28
100	0.3232	4944261.33	0.78
125	0.3878	5926263.67	0.51
150	0.4848	7284570.67	0.10
Slope[a]		$14997469.04 \pm 21265691.20$	
Intercept[b]		74809.51 ± 6685468.39	

[a]Confidence limits of the slope ($p = 0.05$).
[b]Confidence limits of the intercept ($p = 0.05$).

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The system precision is a measure of the method variability that can be expected for a given analyst performing the analysis and was determined by performing eight replicate analyses of the same working solution. The relative standard deviation (RSD) obtained was 1.15.

The precision is usually expressed as the RSD of a series of measurements. The RSD of peak area response and retention time showed the satisfactory repeatability of the system (<1%). The intra-day precision was performed by assaying the samples on two different days by two different analysts. The results were given both individually and as the average. For each precision assays the results were as follows: mean values 41.22 and 41.16 mg/tablet, RSD 1.73% and 1.86%. Test "*t*" comparing two samples with 95% confidence for 10 degrees of freedom disclosed that both results were not significantly different *inter se* ($t_{n-2,\ \alpha:0.05}$) = 2.23 (Table 3).

Accuracy

Accuracy of the method was calculated by recovery studies with 9 samples of one commercial formulation studied ($n = 3$ for 80%, 100%, and 120%) indicated that the mean recovery was 100.00%.

Method accuracy was also demonstrated by plotting the amount (expressed in mg) of meprednisone found against the amount present in mg. Linear regression analysis rendered slopes not significantly

Table 3. Precision of the assay method for methocarbamol

Analyst 1			Analyst 2		
Sample N°	mg per tablet	RSD (%)	Sample N°	mg per tablet	RSD (%)
1	41.14	1.22	1	41.21	0.90
2	41.14	1.22	2	41.47	0.90
3	41.07	1.22	3	41.36	0.90
4	40.20	1.22	4	41.00	0.90
5	42.01	1.22	5	42.09	0.90
6	41.79	1.22	6	39.83	0.90
Mean	41.22	1.73	Mean	41.16	1.86

different from 1 (t test $p = 0.05$), intercepts not significantly different from zero (t test $p = 0.05$) and $r = 0.9983$, the RSD was 1.43. Also studied was the experimental t of the recovery percentage of which the value was 1.571, being far below the 2.306 established in the tabulated t (95% level of probability, 8 d.f) (Table 4).

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Robustness of the method was investigated under a variety of conditions including changes of pH and percentage of acetonitrile in the mobile phase.

Table 4. Recovery analysis of meprednisone

Nominal value (%)	Added amount (mg)	Found amount (mg)	Recovery (%)	Average recovery (n = 3)	RSD (%)
80	32.57	32.40	99.48	98.92	1.72
	32.54	32.56	100.06		
	32.34	31.45	97.22		
100	41.01	41.25	100.59	99.60	1.34
	40.63	40.40	99.43		
	41.42	40.91	98.77		
120	47.78	46.91	98.18	99.23	1.41
	48.97	48.61	99.26		
	48.78	48.90	100.25		
Mean (n = 9)				99.25	1.43

Table 5. Robustness

Mobile phase	RT Meprednisone impurity (minutes)	RT Resolution meprednisone (minutes)	Tailing
Acetonitrile:buffer (45:65, v/v) pH: 7.0	7.645	6.558 1.98	1.30
Acetonitrile:buffer (45:65, v/v) pH: 6.5	7.882	6.725 2.10	1.30
Acetonitrile:buffer (45:65, v/v) pH: 7.5	7.535	6.448 1.98	1.00
Acetonitrile:buffer (55:65, v/v) pH: 7.0	5.142	5.918 1.72	1.00
Acetonitrile:buffer (45:75, v/v) pH: 7.0	9.125	7.770 2.08	1.00

To verify, the separation of meprednisone and its nearer impurity under isocratic conditions were investigated. The effect on retention time, resolution, and tailing factor could be seen in Table 5. An increase of buffer content resulted in longer retention time and an increase in acetonitrile proportion reduces retention time and resolution. It was found that retention time of meprednisone was not significantly affected by changes of different pHs.

CONCLUSIONS

A simple, specific, linear, precise, and accurate RP-HPLC method has been developed and validated for quantitative determination of meprednisone in tablets. The method is very simple and specific, as the peak is well separated from its impurities and excipient peaks with a total runtime of 10 min, which makes it especially suitable for routine quality control analysis work.

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