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RESEARCH PAPER

A Stability-Indicating HPLC Method to Determine Cyproterone Acetate in Tablet Formulations

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ABSTRACT

A simple and accurate liquid chromatographic method was developed to estimate cyproterone acetate (CA) in pharmaceuticals. The drug was chromatographed on a reversed-phase C18 column. Eluents were monitored at a wavelength of 254 nm utilizing a mixture (60:40) of acetonitrile and water. Solution concentrations were measured on a weight basis to avoid the use of an internal standard. The method was statistically validated for linearity, accuracy, precision, and selectivity. Due to its simplicity and accuracy, we believe that the method can be used for routine quality control analysis. No specific sample preparation is required except for the use of a column guard and a suitable prefilter attached to the syringe.
Key Words: *Cyproterone acetate; HPLC method; Stability indicating; Tablets.*

INTRODUCTION

Cyproterone acetate (6-chloro-1β,2β-dihydro-17α-hydroxy-3′*H*-cyclopropa[1,2]-pregna-1,4,6-triene-3,20-dione acetate) (CA) has antiandrogenic and some progestogenic properties. It is used for the control of libido in severe hypersexuality or sexual deviation in adult males. It is also used in males for the palliative treatment of prostatic carcinoma and may be prescribed jointly with ethinylestradiol in females for the control of severe acne and idiopathic hirsutism. Cyproterone acetate is poorly absorbed from the gastrointestinal tract. It is rapidly metabolized and slowly excreted in feces and urine (1).

A method for the determination of this drug is de-

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scribed in the Royal Spanish Pharmacopeia (2), but there are no formulated preparations of CA. The Spanish pharmacopoeia specifies spectrophotometry at 282 nm for this determination and a high-performance liquid chromatography (HPLC) method for the determination of related substances. Although many HPLC methods for the assay of CA in biological fluids have been described (3–7), only a few were found in the literature for the determination of CA in tablets (8,9).

This report describes the development and validation of a stability-indicating method for the assay of CA tablets.

EXPERIMENTAL

Instrumentation

The HPLC system consisted of a dual-piston reciprocating Spectra Physics pump (model ISO chromatographic LC pump), a UV-Vis Hewlett Packard detector (model 1050, Japan), a Hewlett Packard integrator (series 3395, California), and a Rheodyne injector (model 7125, California).

Materials, Reagents, and Chemicals

The authentic working standard for CA was developed locally using a crystallizing technique and was donated by Laboratorios Kampel Martian (Buenos Aires, Argentina). The solvent was HPLC grade water. HPLC grade was obtained by distillation and passed through a 0.45-μm membrane filter.

Two local commercial tablet formulations were used. Lot 1 contained 50 mg of CA in a matrix of lactose monohydrate, sodium lauryl sulfate, sodium starch glycolate, microcrystalline cellulose, sodium crosscarmelose, hydroxypropylmethylcellulose, talc, magnesium stearate, and colloidal silicon dioxide. Lot 2 had the same amount of CA in sodium starch glycolate, microcrystalline cellulose, cellactose, and magnesium stearate.

Chromatographic Conditions

The experiment was performed on a LiChroCART® 250*4 mm HPLC LiChrosorb® 100 RP-18 cartridge (10 μm) (Merck, Darmstadt, Germany), coupled with a column guard of LiChroCART 4*4 mm LiChrosorb RP-18 (10 μm) (Merck).

The mobile phase consisted of 60% acetonitrile and 40% water. The mobile phase was filtered through a nylon membrane (pore size 0.45 μm) (Micron Separations N04SP04700) and degassed before use. Chromatography was performed at room temperature using a 1.2-ml/min flow rate and a 20-min run time. The column was used at room temperature. In these conditions, CA retention time t_R was roughly 12 min. Detector sensitivity was set at 1 aufs, and eluents were monitored at 254 nm. CA exhibited absorbance maxima in the region of 282 nm. Detection at 254 nm was selected in an effort to promote the sensitivity of the method for the potential presence of unknown degradation products. The volume of each injection was 20 μl.

Working Standard Solution

A standard solution of CA, 0.5 mg/ml, was prepared in the mobile phase.

Sample Solution

For the sample solution, 20 tablets were weighed and crushed to a fine powder. Powder samples equivalent to 1 tablet were placed in a 100-ml volumetric flask, 70 ml of mobile phase were added, and the flask was kept in an ultrasonic bath for 5 min. The extraction process employed moderate sonication (5 min) to avoid exposing the samples to heat generated from prolonged treatments. The mixture was then diluted to 100 ml with mobile phase, thoroughly mixed, and filtered through Whatman no. 42 paper.

Preparation of Solutions Used for Assay Validation

For the study of CA response linearity, six solutions were prepared in mobile phase at concentrations ranging from 50 μg/ml to 650 μg/ml.

System precision was evaluated by performing six consecutive injections of CA standard solution. Method precision was evaluated by six repeated assays of the same lot of two commercial formulations. Assay accuracy was assessed at 80%, 90%, 110%, and 120% CA by recovery experiments using tablets from the same lot of two commercial formulations.

Forced degradation studies were performed to provide an indication of the stability-indicating properties and specificity of the procedure. Degradation samples were prepared by transferring approximately 25 mg of sample into 50-ml volumetric flasks. Intentional degradation was attempted using acid, acid/zinc, base, hydrogen peroxide, heat, and light. After completing degradation treatments, samples were allowed to cool to room temperature and

Table 1

System Suitability Parameters

No.	Parameter	Value
1	Theoretical plates	>3500
2	Tailing factor	0.85
3	Capacity factor	5.91
4	SD	0.20

were prepared according to assay sample preparation after acid-base neutralization when required. Samples were analyzed against a control sample (lacking degradation treatment).

Procedure

Solutions were prepared on a weight basis, and volumetric flasks were used as suitable containers to minimize solvent evaporation. Prior to injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. Acceptable results for the number of theoretical plates, tailing factor, and precision, calculated using USP 23 equations (10), and detector linearity criteria were required before sample analysis. Quantification was accomplished using an external standard method. Each solution was injected in triplicate, and the relative standard deviation (RSD) was required to remain below 1.0% on CA peak area basis.

RESULTS AND DISCUSSION

System Suitability

A system suitability test was applied to a representative chromatogram to check various parameters, such as column efficiency, peak tailing, and capacity factor. The results obtained are shown in Table 1. If necessary, an internal progesterone (P) or hydrocortisone (H) standard may be used in a final concentration of 0.5 mg/ml. In these conditions, Pt_R was roughly 17 min, and hydrocortisone was 4 min; the resolution factor between CA and progesterone exceeded 3.4, and that between CA and hydrocortisone exceeded 8.7.

Selectivity

All samples were analyzed using the assay chromatographic conditions described. CA showed degradation products following oxidation, reduction, and alkaline and acid hydrolysis. The percentage of CA recovered is shown in Table 2. Degradation peaks, when observed, were resolved from the CA peak (Fig. 1).

Neither formulation ingredients nor degradation products interfered with CA quantification. No evidence of interactive degradation products was seen during evaluation.

Linearity

Six solutions containing CA at concentrations ranging from 50 μg/ml to 650 μg/ml were analyzed. The curve of the peak area versus concentration proved linear.

The regression line equation calculated by the least-squares method was $Y = 7.57 \times 10^6 X - 2.8 \times 10^5$ with a coefficient of correlation $r = .99994$, while intercept values were not significantly different from zero ($p = .05$) (Table 3).

Microsoft Excel software was used to plot the peak areas versus micrograms injected (Fig. 2).

Assay

The CA contents found in tablets by the proposed method are listed in Table 4. Low RSD values indicate that the method is precise and accurate.

Table 2

Degradation of Cyproterone Acetate

Condition	Time (hr)	% Recovery	RRT[a] of Degradation Products
Acid 1 N HCl, ref.	0.5	67.6	0.11, 0.30, 0.42, 0.61, 0.71
Base 1 N NaOH, ref.	0.5	49.5	0.11, 0.14, 0.18, 0.28, 0.38, 0.41, 0.55, 0.61
H_2O_2 100 vol, ref.	0.5	24.6	0.13, 0.29, 0.41, 0.61, 0.70
Acid 1 N HCl/zinc, ref.	0.5	7.2	0.11
Heat dry, 110°C	24	100.0	None detected
Daylight	24	100.0	None detected

[a] RRT = relative retention time; ref = refluxed.

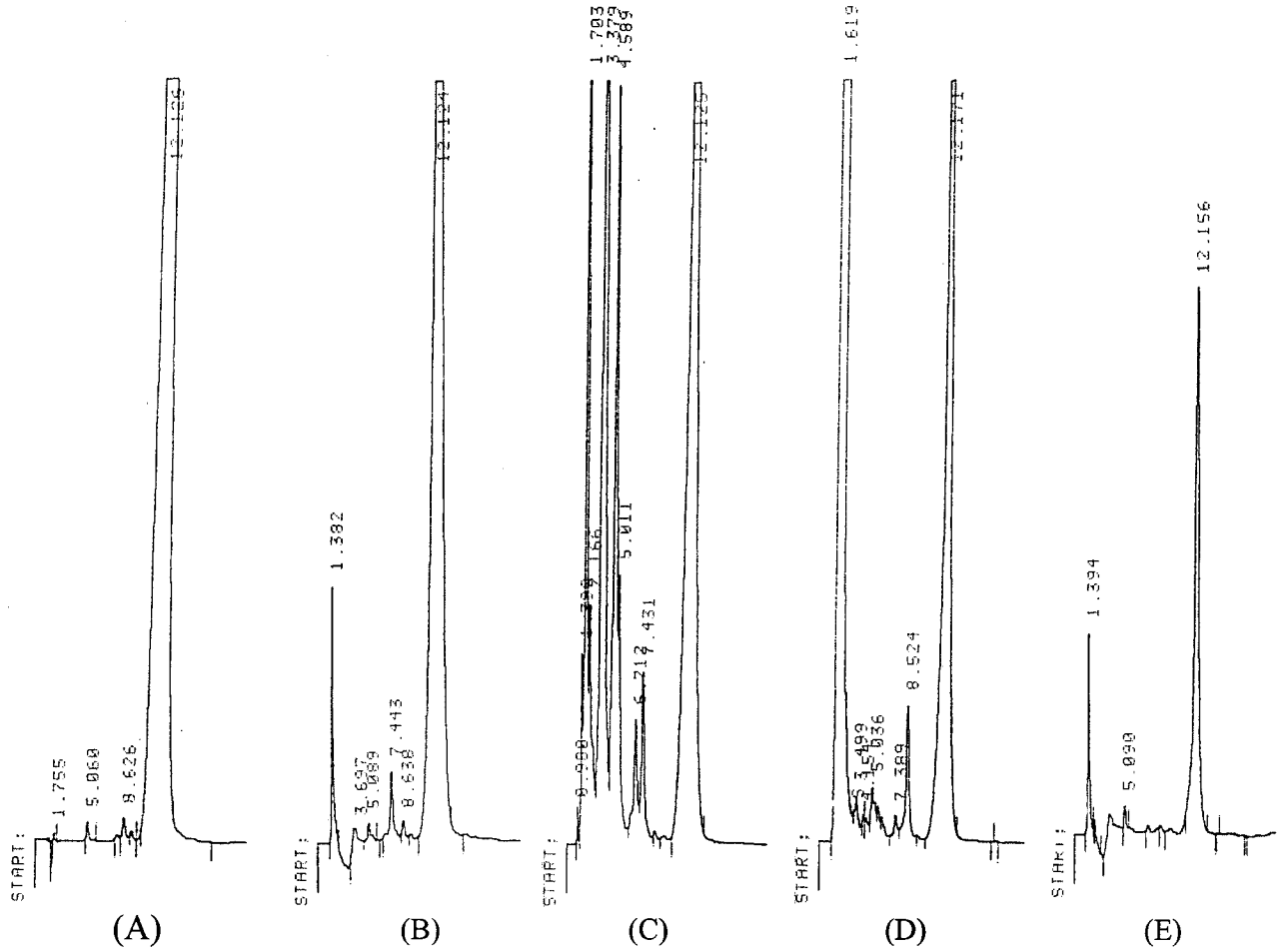

Figure 1. Chromatograms of (A) ciproterone acetate standard, (B) acid degradation, (C) alkaline degradation, (D) oxidative degradation, and (E) reductive degradation.

Table 3

Linearity Data

% w/w	Injected (μg)	Average Peak Area Response	RSD (%)
10	1.01	7,568,684	0.2
50	5.05	37,742,347	0.1
80	8.08	60,895,093	0.3
100	10.10	75,508,672	0.3
115	11.70	88,708,757	0.1
130	13.08	98,783,275	0.1
Slope[a]	7,566,199 ± 113,869		0.5
Intercept[b]	−281,373 ± 1,041,335		

[a] Confidence limits of the slope ($p = .05$).
[b] Confidence limits of the intercept ($p = .05$).

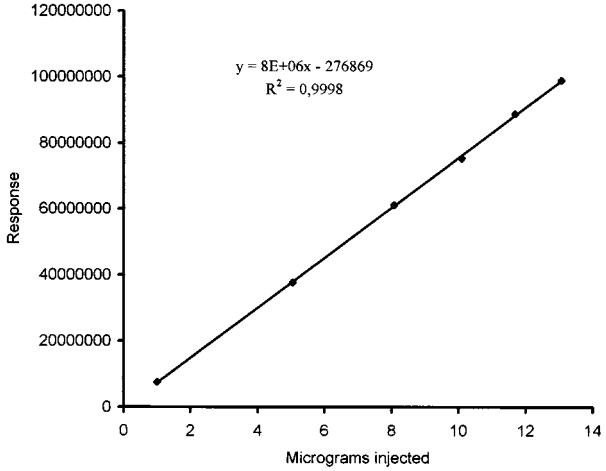

Figure 2. Linearity of peak areas versus micrograms injected.

Table 4

Precision of the Assay Method

	Lot 1		Lot 2	
Sample No.	CA (%)	RSD (%)	CA (%)	RSD (%)
1	107.0	0.4	105.1	0.9
2	106.4	0.0	101.7	0.9
3	107.9	0.3	104.8	0.9
4	106.8	0.3	101.7	0.7
5	105.9	0.4	103.5	0.9
6	107.2	0.4	104.5	0.7
Means	106.9	0.6	103.6	1.5

Table 5

Recovery Analysis[a]

% w/w	Amount Present (mg)	Amount Found (mg)	Recovered (%)	Average Recovered ($n = 3$)	RSD (%)
Lot 1					
	21.32	21.10	99.0		
80	21.12	20.83	98.6	98.7	0.2
	22.50	22.18	98.6		
	24.60	24.40	99.2		
90	24.80	24.10	97.2	98.1	1.0
	24.20	23.70	97.9		
	29.40	29.60	100.7		
110	29.80	29.69	99.6	99.9	0.7
	29.90	29.70	99.3		
	32.02	32.14	100.4		
120	32.91	32.20	97.8	99.8	1.7
	32.20	32.55	101.1		
Means ($n = 12$)				99.1	1.2
Lot 2					
	20.74	20.74	100.0		
80	21.13	21.02	99.5	100.7	1.7
	21.45	22.01	102.6		
	23.81	23.60	99.1		
90	23.95	23.76	99.2	99.8	1.1
	23.46	23.70	101.0		
	29.07	28.89	99.4		
110	29.70	28.87	97.2	99.6	2.5
	29.82	30.44	102.1		
	31.30	31.17	99.6		
120	31.28	30.66	98.0	98.4	1.1
	31.43	30.64	97.5		
Means ($n = 12$)				99.6	1.7

[a] Accuracy acceptance criteria, 97.0% to 103.0%; precision acceptance criteria, 3% within each level.

Accuracy and Precision

Recovery data obtained from the study of lot 1 ranged from 97.2% to 101.1%, with a mean value of 99.1% ($n = 12$) and RSD of 1.2%. Lot 2 data ranged from 97.2% to 102.6%, with a mean value of 99.6% ($n = 12$) and RSD of 1.7% (Table 5).

The mean t value versus the true value with 98% confidence shows that the experimental average was not significantly different from the true value (t_{n-1}, α: 0.02) of 2.718, for 11 degrees of freedom, for each lot. The mean t value was calculated by the equation

$$t = \frac{|100 - R|\sqrt{n}}{\text{RSD}}$$

where R is the mean value.

Method accuracy was demonstrated by plotting the amount of CA found (expressed in milligrams) against the amount present in both lots. Linear regression analysis rendered slopes not significantly different from 1 (t test, $p = .05$), intercepts were not significantly different from zero (t test, $p = .05$), $r = .9977$ for lot 1, and $r = .9952$ for lot 2.

The CA recovery achieved shows that there was no interference from excipients present in the tablets.

Precision was considered at two levels of International Conference of Harmonisation (ICH) suggestions (11): repeatability and intermediate precision. Repeatability was evaluated by analyzing six replicate injections of CA reference solution, giving an RSD of 0.2% and minimal variation in retention time.

Intermediate precision was determined by carrying out two accuracy assays on two lots of commercial formulations 1 week apart by two different operators with the same equipment (Table 5). For each accuracy assay, the results were as follows: mean values 99.1% and 99.6%, standard deviations 1.2 and 1.7, and RSD 1.2% and 1.7%. The t test comparing two sample means with 95% confidence for 22 degrees of freedom disclosed that both results were not significantly different inter se (t_{n-2}, α: 0.05) = 2.074.

Stability of Sample Solution

The sample solution injected after 8 hr failed to show any appreciable change.

CONCLUSIONS

The linearity of CA peak area responses was demonstrated from approximately 10% to 130% of the 0.5-mg/ml working analytical concentration by a correlation coefficient of 0.9999 and y intercept of 0.5%. The precision of CA chromatographic response was calculated from six replicate injections of the same solution prepared at the nominal analytical concentration, and it showed an RSD of 0.2%.

According to recovery studies performed at 80%, 90%, 110%, and 120% of the analytical concentration, the extraction of the active component was shown to be quantitative.

Selectivity was demonstrated, showing that the CA peak was free of interference from degradation products, indicating that the proposed method can be used in a stability assay. The proposed reversed-phase HPLC method is simple, precise, rapid, and selective for the determination of CA and may be employed for its assay in dosage formulations.

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