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Different methods for the determination of gestodene, and cyproterone acetate in raw material and dosage forms

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Abstract

Four new precise accurate and selective methods have been developed for the determination of gestodene (I) and cyproterone acetate (II). The first method (A) depends on reaction of (I) and (II) with isoniazide in an acid medium and the colored products were measured at 378 and 400 nm, respectively. The second method (B) depends on the reaction of (I) and (II) with tetrazolium blue in an alkaline medium and the colored products were measured quantitatively at 515 and 520 nm, respectively. The optimum conditions for the analysis were studied. Both methods determined gestodene (I) in concentration range from 4 to 24 μg ml^{-1} with mean percentage recoveries 99.54% ± 1.20 and 99.63% ± 1.89 for method A and B, respectively. For cyproterone acetate, the concentration ranges were 4–36 and 8–40 μg ml^{-1} with mean percentage recoveries 99.94% ± 1.19 and 99.23% ± 2.00 for methods A and B, respectively. The third method (C) depends on the quantitative evaluation of (I) and (II) densitometrically using dichloroethane:methanol:water (95:5:0.2) as mobile phase and the chromatogram were scanned at 247 and 281 nm, respectively. Method (C) determines (I) and (II) in concentration ranges from 0.2 to 1.6 and 0.1–0.7 μg μl^{-1} using Hamilton syringe 10 μl, with mean percentage recoveries 99.94% ± 1.19, and 99.82% ± 1.75, respectively. The fourth method (D) is a first derivative one depends on measuring the D_1 value at 303 nm for (II) only in concentration range 10–20 μg ml^{-1} with mean percentage recoveries 99.95% ± 1.49.

Keywords: Colorimetric; Densitometry; Derivative spectrophotometry; Gestodene; Cyproterone acetate; Tablets

1. Introduction

Gestodene (I) ,17α-ethynyl-17B-hydroxy-18-methyl-4,15-estradien-3-one, is used as oral contraceptive in combination with ethinyl estradiol [1]. The methods reported for its determination include HPLC [2], radio-immunoassay [3], and voltametry [4].

Cyproterone acetate (II), 6-chloro-1,2α-methyl-ene-4,6-pregnadien-17α-ol-3,20-dione, is used in the treatment of acne in combination with ethinyl estradiol [1]. The methods reported for its determination include gas chromatography [5], HPLC [6], UV spectrophotometry [7,8] and identity by TLC [7,8].

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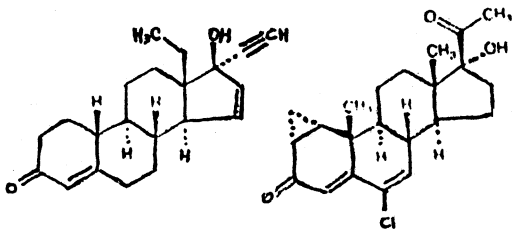
The strcutural formulae for (I) and (II) are shown as follows



(I) (II)

As shown the proposed methods have not been previously applied, consequently the present work describes new methods which are simple, less expensive, and not need complicated instruments.

2. Experimental

2.1. Apparatus

1. UV/VIS spectrophotometer SHIMADZU 1601 PC.
2. Densitometer — Dual wavelength SHIMADZU flying CS-9301 PC.
3. Thin layer chromatographic (TLC) plates, precoated with silica gel GF_{254}, 0.25 mm thickness. (E. Merck, Darmstadt, Germany).
4. Short wavelength lamp. (254 nm).

2.2. Material

2.2.1. Pure samples

Gestodene, working standard, kindly supplied by Schering AG (Germany, Allemagne). Its purity was found to be $99.74 \pm 1.09\%$ according to the reported method [2].

Cyproterone acetate, working standard, kindly supplied by Cid Co., Cairo, Egypt. Its purity was found to be $100.47 \pm 1.70\%$ according to the official method [7].

2.2.2. Market samples

Gynera tablet (*Schering*), *batch no*. 29. Each tablet was labeled to contain 0.075 mg gestodene, 0.03 mg ethinyl estradiol, 0.20 mg magnesium stearate, 0.06 mg calcium disodium edetate, 1.70 mg povidone 25 000, 15.50 mg starch, 37.43 mg lactose, 0.05 mg wax, 4.24 mg talc, 8.70 mg calcium carbonate, 2.18 mg polyethylene glycol 6000, 0.17 mg povidone 700 000, 19.60 mg saccharose.

Minulet tablet (*Wyeth*), *batch no*. *2730*. Each tablet was labeled to contain 0.075 mg gestodene 0.03 mg ethinyl estradiol, 0.20 mg magnesium stearate, 0.06 mg calcium disodium edetate, 1.70 mg polyvinyl pyrrolidone, 15.50 mg maize starch, 37.43 mg lactose, 19.66 mg sucrose, 0.17 mg polyvinyl pyrrolidone, 2.18 mg polyethylene glycol, 8.69 mg calcium carbonate, 4.24 mg talc,0.05 mg wax.

Diane tablet (*Cid Co*.) , *batch no*. *299101*. Each tablet was labeled to contain 2 mg cyproterone acetate, 0.035 mg ethinyl estradiol, 2.10 mg polyvinyl pyrrolidone, 2.14 mg polyethylene glycol.

2.2.3. Reagents and chemicals

All reagents and chemicals used were of analytical grade and were used without further purification, the solvents were of spectroscopic grade.

1. Ethanol (Analar, BDH, England).
2. Methanol (Analar, BDH, England).
3. 0.1 N Hydrochloric acid methanolic solution (Riedel-de Haën-Germany).
4. 0.2 M Isonicotinic acid hydrazide (Isoniazide), (BDH, England) was prepared by dissolving 27.5 mg in 10 ml methanol containing 0.1 ml of 0.1 N hydrochloric acid , freshly prepared.
5. 0.1 N Tetramethyl ammonium hydroxide (Switzerland, Fluka AG), was prepared by diluting 1 ml of 1 N solution to 10 ml with ethanol.
6. 0.5% Tetrazolium blue in ethanol, (Prolabo.): warm if necessary in hot water bath. It must be freshly prepared and protected from light.

2.2.4. Standard stock solution

Standard stock solutions were stable for at least 1 week, when preserved in a refrigerator.

Gestodene (I) standard solutions:

1. 200 μg ml^{-1} in methanol for method A.
2. 200 μg ml^{-1} in ethanol for method B.

3. 2 mg ml^{-1} in methanol for method C.

Cyproterone acetate (II) standard solutions:

1. 200 μg ml^{-1} in methanol for method A.
2. 200 μg ml^{-1} in ethanol for method B.
3. 1 mg ml^{-1} in methanol for method C and D.

2.3. Procedures

2.3.1. Construction of calibration curves for gestodene

2.3.1.1. For method A. Transfer accurately different aliquots of the standard stock solution (200 μg ml^{-1}) equivalent to 100–600 μg into 25 ml volumetric flask, add 2 ml isoniazide reagent and complete to volume with methanol. Leave in the dark for 60 min, and measure the absorbance at 378 nm against a blank prepared similarly. Construct the calibration curve and calculate the regression equation.

2.3.1.2. For method B. Transfer accurately different aliquots of the standard stock solution (200 μg ml^{-1}) equivalent to 100–600 μg into 25 ml volumetric flasks add 2 ml of tetrazolium blue reagent, followed by 2 ml of tetramethylammonium hydroxide and complete to volume with ethanol. Leave in the dark for 90 min and measure the absorbance at 515 nm against a blank prepared similarly. Construct the calibration curve and calculate the regression equation.

2.3.1.3. For method C. From a standard stock solution (2 mg ml^{-1}), apply 0.2–1.6 μg μl^{-1} using Hamilton syringe 10 μl to a thin layer chromatographic plate (20 × 20 cm). Spots were spaced 2 cm apart from each other and 1.5 cm apart from the bottom edge of the plate. Place the plate in a chromatographic tank containing the developing mobile phase dichloroethane:meth-anol:water (95:5:0.2) and develop for a distance of about 16 cm. Dry the plate at room temperature, then detect the spots under UV lamp 254 nm and scan at 247 nm. Plot the calibration curve representing the relationship between the area under the peak and the concentration and calculate the regression equation.

2.3.2. For dosage forms

2.3.2.1. For method A. Weigh and powder 20 tablets then accurately weigh an amount of powder equivalent to 1 mg gestodene, dissolve in 4 × 20 ml methanol and filter each time. Evaporate the methanolic solution under nitrogen till about 1 ml and transfer quantitatively into 5 ml volumetric flask and complete to volume with the same solvent (200 μg ml^{-1}). Proceed as under construction of calibration curve (Method A), starting with the words 'Transfer accurately different aliquots…'. Calculate the concentration from the regression equation, results obtained are shown in Table 3.

2.3.2.2. For method B. Powder 20 tablets and accurately weigh an amount of the powder equivalent to 1 mg gestodene, dissolve in 50 ml distilled water, filter and wash the filter paper with 2 × 10 ml distilled water. Dissolve the residue in 4 × 20 ml ethanol, and proceed as under Section 2.3.2.1, starting with the words 'Evaporate under nitrogen,' then proceed as under construction of calibration curve (Method B), with the exception that the blank contains the same concentration of ethinyl estradiol as that of the tablet solution in its final volume for each measurement. Calculate the concentration from the regression equation and results obtained are shown in Table 3.

2.3.2.3. For method C. Proceed as under method A, then apply as under construction of calibration curve for method C starting with the word 'apply accurately 0.2–1.6 μg μl^{-1}, using Hamilton syringe 10 μl. Calculate the concentration from the regression equation and results obtained are shown in Table 3.

2.3.3. Construction of calibration curves for cyproterone acetate

2.3.3.1. For method A. Transfer different aliquots of the standard stock solution (200 μg ml^{-1}) equivalent to 100–900 μg into 25 ml volumetric flask and add 5 ml of the isoniazide solution. Leave in the dark for 120 min and measure the absorbance at 400 nm against a blank prepared similarly. Construct the calibration curve and calculate the regression equation.

2.3.3.2. For method B. Transfer different aliquots of the standard stock solution (200 μg ml^{-1}) equivalent to 200–1000 μg in 25 ml volumetric flask and add 5 ml of tetrazoliun blue reagent, followed by 5 ml tetramethyl ammonium hydroxide and complete to volume with ethanol. Leave at 50°C for 90 min and measure the absorbance at 520 nm against a blank prepared similarly. Construct the calibration curve and calculate the regression equation.

2.3.3.3. For method C. Transfer different aliquots of the standard stock solution (1 mg ml^{-1}) equivalent to 0.1–0.7 μg μl^{-1} using Hamilton syringe 10 μl to a thin layer chromatographic plate (20 × 20 cm). Place the plate in a chromatographic tank using mobile phase dichloroethane:methanol:water (95:5:0.2) and leave for a distance of about 16 cm. Dry the plate at room temperature, then detect the spots under UV lamp at 254 nm and scan at 281 nm. Construct the calibration curve and calculate the regression equation.

2.3.3.4. For method D. Transfer accurately different aliquots of the standard stock solution (1 mg ml^{-1}) equivalent to 100–200 μg to 10 ml volumetric flask, and complete to volume with methanol. Measure the D_1 value at 303 nm against methanol as a blank and plot the calibration curve representing the relationship between the measured D_1 value and the corresponding concentration.

2.3.4. For dosage forms

2.3.4.1. For method A. Weigh and powder 10 tablets then weigh accurately an amount of the powder equivalent to 10 mg cyproterone acetate and dissolve quantitatively in 30 ml methanol. Filter in 50 ml volumetric flask, wash the filter paper and complete to the mark with the same solvent. Proceed as under construction of calibration curve for (Method A), calculate the concentration from the regression equation, results obtained are shown in Table 4.

2.3.4.2. For method B. Remove the coat of 10 tablets with distilled water and dry between filter papers. Weigh and powder then weigh accurately an amount of the powder equivalent to 10 mg cyproterone acetate, dissolve in 2 × 25 ml water to remove povidone and filter. Dissolve the residue in 2 × 25 ml methanol, transfer quantitatively into 50 ml volumetric flask and complete to the mark with methanol. Proceed as under construction of calibration curve (Method B) against blank containing the same concentration of ethinyl estradiol equivalent to the test solution. Calculate the concentration from the regression equation, results obtained are shown in Table 4.

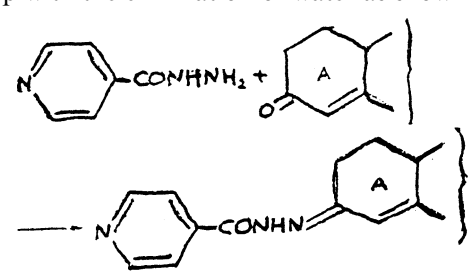
2.3.4.3. For method C. Proceed as under (Method A) for the preparation of tablet solution then apply as under construction of calibration curve for (Method C). Calculate the concentration from the regression equation, results obtained are shown in Table 4.

2.3.4.4. For method D. Weigh and powder 10 tablets and weigh accurately an amount of powder equivalent to 10 mg cyproterone acetate. Dissolve in 2 × 25 ml methanol and filter. Evaporate the methanol till about 5 ml, transfer quantitatively into 10 ml volumetric flask and complete to volume with the same solvent. Proceed as under construction of calibration curve for (Method D). Results obtained are shown in Table 4.

2.3.5. Results and discussion

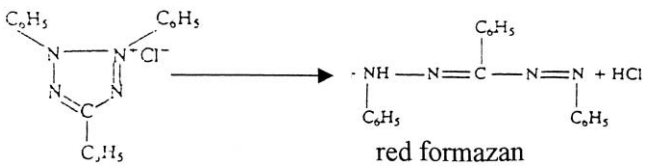
2.3.5.1. For isoniazide method A. The ketonic group at C_3 in association with the Δ^4 in ring A was an important structural feature of the biologically active corticosteroids [9]. A number of hydrazine and hydrazide reagents have been described for the colorimetric assay of ketosteroids [10].

Isoniazide reacts with 4 en-3 one in acidic medium to catalyze the condensation and protonates the hydrazone to produce yellow color with λ_{max} around 400 nm. The reaction involves the nucleophilic attack by the amine on the carbonyl group with the elimination of water as shown [10]:

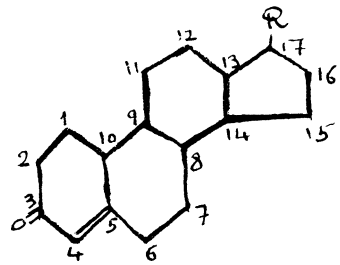


The reaction conditions were optimized with regard to the volume of the reagent, effect of temperature and time of the reaction as shown in Table 1. Under the chosen favorable conditions of reaction Beer's law was found to be valid over the concentration ranges of 4 to 24, and 4–36 μg ml^{-1} with mean percentage recoveries of 99.54 and 99.94%, and relative standard deviation of 1.20 and 1.19% for I and II, respectively as shown in Table 2.

2.3.5.2. For tetrazalium blue method B. There is a reducing character toward tetrazolium blue of a number of Δ^4-3-ketosteroids with a hydroxyl or keto group in various position of the molecule. This function depends on their location and configuration give rise to color formation with varied rates and intensities [9].



The red formazan developed in highly alkaline solution with tetramethyl ammonium hydroxide at 515 and 520 nm for I and II, respectively as shown.



Verification of Beers' Law showed obedience in concentration ranges 4–24 μg ml^{-1} and 8–40 μg ml^{-1} with mean percentage recoveries of 99.63 and 99.23% and relative standard deviation of 1.89 and 2.00% for I and II, respectively, as shown in Table 2. Ethinyl estradiol presents as a second hormone in both pharmaceutical formulations of I and II, and this interferes with tetrazolium blue reaction, so it must be added to the blank to cancel its effect. Povidone presents as excipients in pharmaceutical dosage forms of cited drugs and this also interferes due to the presence of ketonic group in its structure. To overcome this interference, povidone in the powdered tablets was extracted first with distilled water, and then dissolved in ethanol to volume.

Cyproterone acetate contains chloride in position six and this affects the rate of the reaction as it needs more time and higher temperature for methods A and B, respectively to enhance the reaction due to it's electron withdrawing property that affect the Δ^4-3 ketosteroid system [9]. Meth-

Table 1
Optimum conditions used for the proposed methods A and B

Parameters	Proposed methods			
	Gestodene		Cyproterone acetate	
	Isoniazide method	Tetrazolim blue method	Isoniazide method	Tetrazolim blue method
Amount of standard taken (μg)/25ml	100–600 μg	100–600 μg	100–900 μg	200–1000 μg
Amount of reagent (ml)	2	2	5	5
Heating temperature and time	At room temperature for 60 min	At room temperature for 90 min	At room temperature for 120 min	At 50°C for 90 min
λ_{max} (nm)	378	515	400	520
Stability of colored products (min)	30	30	30	30

Table 2
The spectral data for gestodene and cyproterone acetate using the proposed methods[a]

Parameters	Gestodene			Cyproterone acetate			
	Method A	Method B	Method C	Method A	Method B	Method C	Method D
Concentration ranges (μg ml^{-1})	4–24	4–24	0.2–1.6	4–36	8–40	0.1–0.7	10–20
Interept (a)	0.065	0.046	0.32	0.001	0.009	0.146	0.0007
Slope (b)	0.044	0.0362	6.59	0.0275	0.214	7.06	0.00176
Correlation coefficeint (r)	0.999	0.998	0.999	0.995	1.010	1.005	1.010
[b]Mean ± R.S.D.%	99.54 ± 1.20%	99.63 ± 1.89%	100.72 ± 1.90%	99.94 ± 1.19%	99.23 ± 2.00%	99.82 ± 1.75%	99.95 ± 1.49%
LOD (μg ml^{-1}) [12]	0.068	0.083		0.11	0.14		
LOQ (μg ml^{-1}) [13]	3.5−20	3.9−22		3.2−25	7.5−35		

[a] $A = a + bc$ (regression equation).
[b] The average of 5 determinations.

ods (A, B) are selective and specific for ketonic groups.

2.3.5.3. For method C. The present work is concerned with the application of a densitometric technique for the determination of I and II in the presence of ethinyl estradiol present in their dosage forms . Complete separation of I, II and ethinyl estradiol were obtained using dichlaroethane: methanol: water (95:5:0.2) as a mobile phase, where R_f. of gestodene, cyproterone acetate and ethinyl estradiol is 0.64, 0.74, 0.46, respectively.

The chromatograms can be scanned densitometrically at 247 and 280 nm for I and II, respectively.

By applying this technique, a linear correlation were obtained between the area under the peak and the concentration 0.2–1.6 and 0.1–0.7 μg μl^{-1} using Hamilton syringe 10 μl with mean percentage recoveries of 100.72 and 99.82% and relative standard deviation of 1.90 and 1.75% for I and II, respectively.

2.3.5.4. For method D. Zero order absorption spectra of cyproterone acetate (II) and ethinyl estradiol in methanol show certain overlapping which interfere with the direct determination of (II) as in Fig. 1.

A derivative spectrophotometer has been suggested to overcome this overlapping and it can be used as a well established technique for the determination of (II) in the presence of ethinyl estradiol Fig. 2.

By applying the first derivative technique a linear correlation was obtained between the D_1 values at 303 nm and the concentration over a range (10–20 μg ml^{-1}) with percentage recovery of 99.95% and relative standard deviation of 1.49%. This method cannot be used for determination of (I) in presence of ethinyl estradiol, because there is not any overlap between them (λ_{max} of (I) at 247 nm, and ethinyl estradiol at 281 nm). The proposed methods were applied for the determination of (I) and (II) in its dosage forms, and the results obtained were shown in Table 4. These methods (C, D) are specific for the cited drugs.

The validity of the proposed methods were assessed by applying the standard addition technique and the results were presented in Tables 3 and 4.

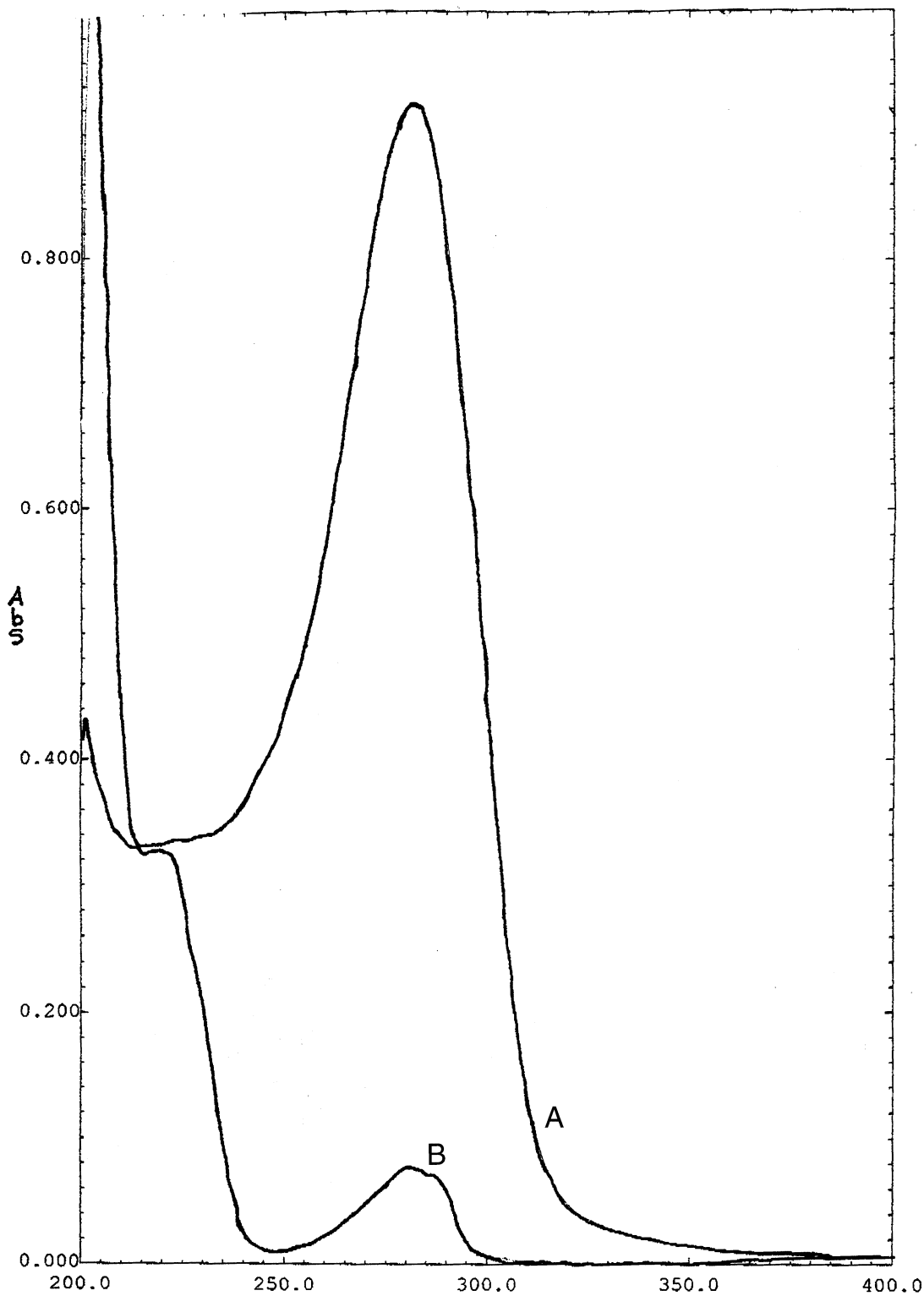


Fig. 1. Zero order absorption spectra of (A) cyproterone acetate (2 μg ml^{-1}) (B) ethinyl estradiol (1 μg ml^{-1}) in methanol.

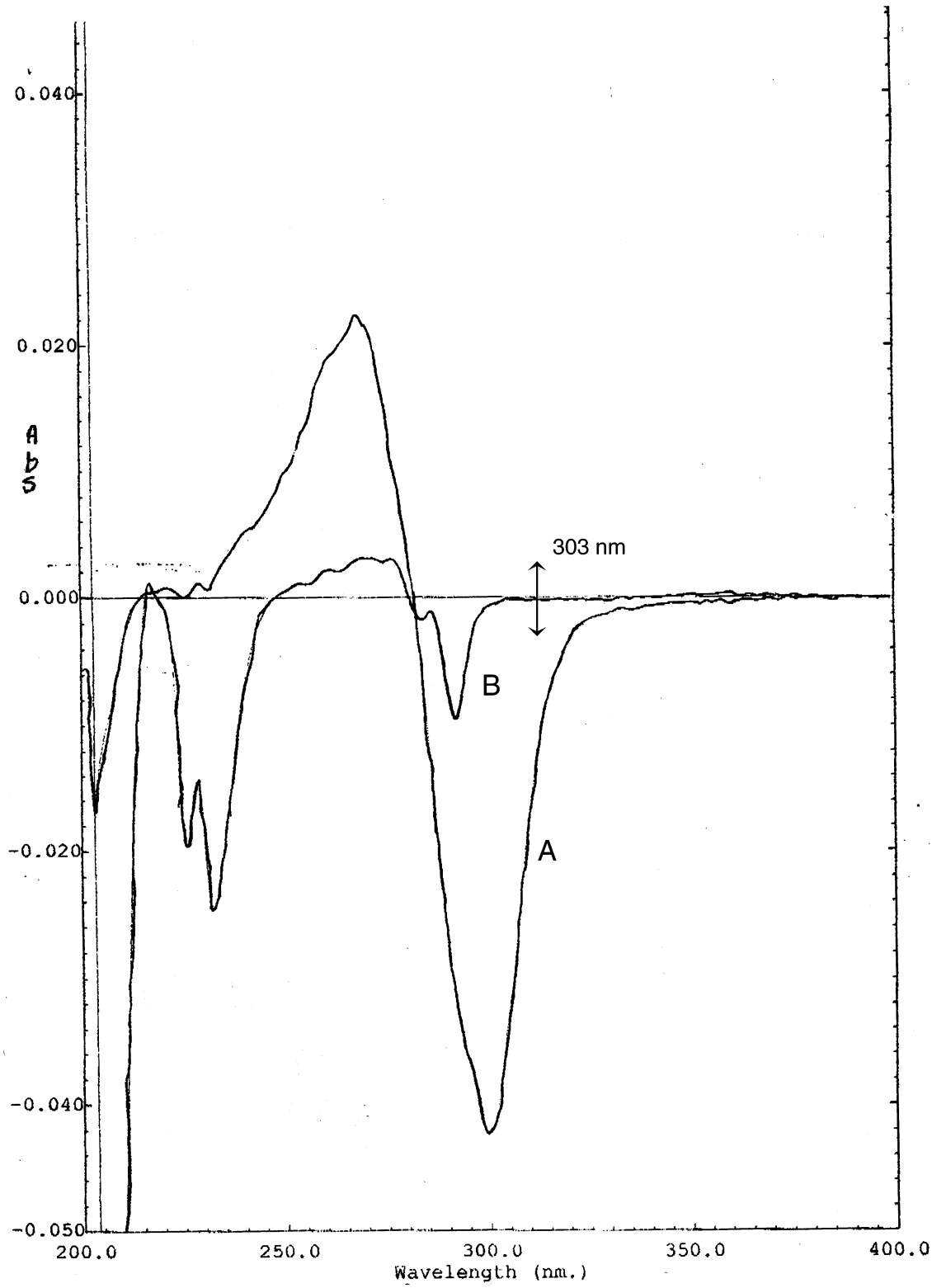


Fig. 2. First derivative spectra of (A) cyproterone acetate (2 μg ml^{-1}) and (B) ethinyl estradiol (1 μg ml^{-1}) in methanol at D_1 value 303 nm.

Table 3
Results obtained in the determination of gestodene in pharmaceutical dosage form using the proposed methods and compared with the reported one

Preparation	Method A		Method B		Method C		Reported method [2]
	Found (%)	Recovery (%)[b]	Found (%)	Recovery (%)	Found (%)	Recovery (%)	
Gynera tablet 0.075 mg gestodene 0.03 mg ethinyl estradiol/Tablet B.N.: 29	99.66 ± 0.96	100.44 ± 1.19	100.76 ± 0.56	99.58 ± 0.82	101.45 ± 0.98	99.28 ± 1.11	100.09 ± 1.01
		$F = 1.43$ (6.39)[a] $t = 0.55$ (2.306)		$F = 1.52$ (6.39) $t = 0.96$ (2.306)		$F = 1.22$ (6.39) $t = 1.35$ (2.306)	
Minulet tablet 0.075 mg gestodene 0.03 mg ethinyl estradiol/Tablet B.N.: 2730	99.68 ± 0.41	100.48 ± 0.61	100.94 ± 0.049	100.04 ± 1.19	99.40 ± 0.62	100.09 ± 0.49	100.09 ± 1.01
		$F = 2.70$ (6.39) $t = 1.03$ (2.306)		$F = 1.40$ (6.39) $t = 0.32$ (2.306)		$F = 4.21$ (6.39) $t = 0.005$ (2.306)	

[a] Figures between parenthesis are the corresponding theoretical values of F and t at $P = 0.05$.
[b] The average of 6 determinations.

Table 4
Results obtained in the determination of cyproterone acetate in pharmaceutical dosage form using the proposed methods and compared with the official one

Preparation	Method A		Method B		Method C		Method D		Official method [7]
	Found (%)	Recovery, (%)[b]	Found (%)	Recovery (%)	Found (%)	Recovery (%)	Found (%)	Recovery (%)	
Diane Tablet 2 mg Cyproterone acetate and 0.03 mg ethinyl estradiol/tablet B.N.: 299101	98.96 ± 0.98	100.25 ± 0.83	100.29 ± 1.37	100.27 ± 0.79	99.53 ± 0.49	99.71 ± 0.96	98.62 ± 0.53	99.79 ± 1.39	99.97 ± 0.69
		$F = 1.48$ (6.39)[a] $t = 0.62$ (2.306)		$F = 1.30$ (6.39) $t = 0.7$ (2.306)		$F = 1.93$ (6.39) $t = 0.53$ (2.306)		$F = 4$ (6.39) $t = 0.28$ (2.306)	

[a] Figures between parenthesis are the corresponding theoretical values of F and t at $P = 0.05$.
[b] The average of 6 determinations.

Table 5
Statistical comparison between the results of analysis of gestodene and cyproterne acetate in pure form using the proposed and the official method[c]

	Gestodene				Cyproterone acetate				
	Method A	Method B	Method C	Reported method[2][b]	Method A	Method B	Method C	Method D	Official method [7][c]
Mean[d] ± R.S.D.	99.54 ± 1.2 0	99.83 ± 1.89	99.94 ± 1.19	99.74 ± 1.09	99.94 ± 1.90	99.23 ± 2	99.82 ± 1.75	99.95 ± 1.49	100.47 ± 1.70
Variance	1.44	3.53	3.69	1.19	1.19	4.08	3.06	2.22	2.89
N 5	5	5	5	5	0,0	5	5	5	5
F	1.42 (6.39)[a]	2.99 (6.39)	3.10 (6.39)		2.43 (6.39)	1.41 (6.39)	1.06 (6.39)	1.30 (6.39)	
t	1.58 (2.306)	0.11 (2.306)	0.99 (2.306)		0.59 (2.306)	1.05 (2.306)	0.62 (2.306)	0.52 (2.306)	

[a] The figures between parenthesis are the theoretical values of F and t at $P = 0.05$.
[b] [2]HPLC method for the determination of gestodene using aquous 70% methanol as mobile phase , spherisorb ODS and detection at 242 nm.
[c] [7] Spectrophotometric method for the determination of cyproterone acetate, using methanol as solvent, λ_{max} at 282, $E^{1\%}_{km}$ 414.
[d] The average of 5 determination.

Table 5 shows statistical comparison of the results obtained by applying the proposed, reported and official methods.

The data permit one to conclude with 95% confidence, that there is no significant difference between the proposed and reported methods.

The results of the proposed methods were repeated inter and intraday, show their reproducibility.

3. Conclusion

The suggested methods have the advantages of being simple, accurate, sensitive and suitable for routine analysis in control laboratories. The colorimetric methods have almost the same sensitivity. TLC method is simple, and need no previous separation from the estradiol and can detect 0.1–1.6 μg μl^{-1} for both drugs.

First derivative has the advantage of sensitivity, simplicity and reproducibility. All these methods can be used as general methods for the determination of gestodene and cyproterone acetate in bulk and pharmaceutical formulations. They are convenient for quality control and routine analysis of these drugs. The studied drugs are highly stable [11], So the proposed methods are not used as stability indicating assays.

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