Simultaneous Determination of Cyproterone Acetate and Ethinylestradiol in Tablets by Derivative Spectrophotometry[1)]

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Derivative spectrophotometry offers a useful approach for the analysis of drugs in multi-component mixtures. In this study a third-derivative spectrophotometric method was used for simultaneous determination of cyproterone acetate and ethinylestradiol using the zero-crossing technique. The measurements were carried out at wavelengths of 316 and 226 nm for cyproterone acetate and ethinylestradiol respectively. The method was found to be linear (r^2>0.999) in the range of 0.5—6 mg/100 ml for cyproterone acetate in the presence of 35 μg/100 ml ethinylestsradiol at 316 nm. The same linear correlation (r^2>0.999) was obtained in the range of 10—80 μg/100 ml of ethinylestradiol in the presence of 2 mg/100 ml of cyproterone acetate at 226 nm. The limit of determination was 0.5 mg/100 ml and 10 μg/100 ml for cyproterone acetate and ethinylestradiol respectively. The method was successfully applied for simultaneous determination of cyproterone acetate and ethinylestradiol in pharmaceutical preparations without any interferences from excipients.

Key words cyproterone acetate; ethinylestradiol; derivative spectrophotometry; pharmaceutical preparation

A combination of cyproterone acetate (2 mg) and ethinylestradiol (35 μg) is commonly used for the treatment of androgen-dependent acne, seborrhoea, alopecia and hirsutism in females and as oral contraceptive in patients with androgenic symptoms.[2)] The combination of these drugs is also used in polycystic ovary syndrome.[3,4)] Literature survey in well-known Pharmacoepias showed no official procedure for simultaneous determination of cyproterone acetate and ethinylestradiol in pharmaceutical preparations. Direct UV–visible spectrophotometric method is not suitable for simultaneous determination of cyproterone acetate and ethinylestradiol due to their spectral overlapping. Application of derivative technique of spectrophotometry offers a powerful tool for quantitative analysis of multi-component mixtures.[5—9)] In the last decades, this technique has rapidly gained application in the field of pharmaceutical analysis to overcome the problem of interference, due to substances other than analytes, commonly present in pharmaceutical formulations or for combination of two or more drug substances.[10—17)]

Lack of any published method for simultaneous spectrophotometric determination of cyproterone acetate and ethinylestradiol, therefore, provoked us to investigate the application of derivative spectrophotometry for simultaneous determination of these compounds in pharmaceutical dosage forms using zero-crossing method.

Experimental

Materials and Methods Cyproterone acetate and ethinylestradiol were from Cipex (Handelsges, Wien, Austria) and obtained from Abouraihan Pharmaceutical Company (Tehran, Iran). Methanol was of analytical grade and obtained from Merck (Darmstadt, Germany).

Apparatus Absorption and derivative spectra were recorded in 1 cm quartz cells using a Shimadzu UV-160 double beam UV–visible spectrophotometer (Shimadzu, Kyoto, Japan) with a fixed bandwidth (2 nm) and data processing capacity. The zero-order absorption spectra were recorded over the wavelength range 200—400 nm, against a solvent blank. The derivative spectra were obtained over the same wavelength range at different slit width ($\Delta\lambda$). The ordinate, maximum and minimum, was adjusted to the magnitude of derivative values.

Standard and Calibration Solutions Standard stock solution of cyproterone acetate was prepared by dissolving 100 mg of cyproterone acetate in 100 ml methanol. Two stock solutions of ethinylestradiol were prepared by dissolving 100 and 35 mg of ethinylesradiol in 100 ml methanol respectively. Two other solutions of ethinylestradiol (1 mg/100 ml, 3.5 mg/100 ml) were prepared from the stock solutions.

Accurate volumes of the standard stock solutions were transferred into two sets of 100 ml calibrated flasks. The first series contained a constant quantity of ethinylestradiol (35 μg/100 ml) and varying concentrations of cyproterone acetate (0.5—6 mg/100 ml). The second series contained a constant amount of cyproterone acetate (2 mg/100 ml) and varying concentrations of ethinylestradiol (10—80 μg/100 ml).

Pharmaceutical Tablet Formulation To evaluate the validity of the proposed method, two commercial dosage forms (tablet) of this drug were used: (1) Cyproterone compound (Abouraihan Pharmaceutical Company, Tehran, Iran, Batch No: 002) and (2) Diane 35 (Schering AG, Germany, Batch No: 0202). Both products contained 2 mg of cyproterone acetate and 35 μg of ethinylestradiol.

The excipients to make up the total weight of the tablets were magnesium stearate, sodium starch glycolate, starch, sodium croscarmelose, sodium lauryl sulfate, polyvinylpyrolidine and aerosyl.

Spectrophotometric Measurements Zero-order spectra of standard solutions of cyproterone acetate (2 mg/100 ml) and ethinylestradiol (35 μg/100 ml) *versus* their solvent blanks were recorded in the range of 200—400 nm. The third-order derivative spectra of standard solutions of each drug were obtained in the same range of wavelength against their blanks. The values of D_3 amplitudes for cyproterone acetate in the presence of ethinylestradiol and *vice versa* were measured at 316 nm (zero-crossing of ethinylestradiol) and 226 nm (zero-crossing of cyproterone acetate) respectively.

The calibration curves for derivative spectrophotometry were constructed by plotting the drug concentration *versus* the absorbance values of the third-derivative spectrum (D_3), at 316 nm for cyproterone acetate and at 226 nm for ethinylestradiol.

Linearity Calibration curves were constructed using six series of cyproterone acetate solutions among 0.5—6.0 mg/100 ml in the presence of 35 μg/100 ml ethinylestradiol. The same procedure was used for solutions contained ethinylestradiol (10—80 μg/100 ml) in the presence of 2 mg/100 ml of cyproterone acetate. The calibration curves were constructed and statistical analysis was performed.

Precision To establish the reliability of the proposed method, two series of solutions containing 0.5, 2.0 and 6.0 mg/100 ml of cyproterone acetate plus 35 μg/100 ml of ethinylestradiol and 10, 40 and 80 μg/100 ml of ethinylestradiol plus 2 mg/100 ml cyproterone acetate were prepared respectively and analyzed as discussed above. To evaluate repeatability of the analytical method three series of these synthetic mixtures were assessed in one day using their corresponding calibration curves. Admixtures of similar concentrations were analyzed on three different days to obtain reproducibility.

Accuracy For recovery assays various concentrations of cyproterone acetate (0.5, 2.0, 6.0 mg/100 ml) with ethinylestradiol (35 μg/100 ml) were an-

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alyzed by the proposed method and the percentage of deviation between added and measured concentrations was calculated. The same procedure was carried out for different concentrations of ethinylestradiol (10, 40, 80 μg/100 ml) with cyproterone acetate (2 mg/100 ml).

Analysis of Tablet Twenty tablets containing cyproterone acetate and ethinylestradiol were weighed and finely powdered. Appropriate amount of the powder equivalent to one tablet was accurately weighed, transferred in a 100.0 ml volumetric flask, dilluted with methanol, sonicated for 30 min and then adjusted to the mark with the same solvent. After centrifugation at 3000 rpm for 10 min, the clear layer of the centrifugate was used for the analysis. The general procedure were followed and the concentrations of cyproterone acetate and ethinylestradiol were calculated.

Results and Discussion

Derivative Spectrophotometric Method Zero-order absorption spectra of cyproterone acetate and ethinylestradiol showed overlapping spectra which prevent the direct simultaneous determination of this formulation (Fig. 1). Derivative spectrophotometry, based on a mathematical transformation of the zero-order curve into the derivative spectra can overcome this problem.[5,6] In this investigation the spectrophotometric parameters were optimized through derivative spectra of cyproterone acetate and ethinylestradiol at different orders and $\Delta\lambda$ values. The third-order derivative spectra traced with $\Delta\lambda=1.4$ nm ($n=2$) was used to resolve the spectral overlapping. Zero-crossing points of cyproterone acetate (226 nm) and ethinylestradiol (316 nm) as presented in Fig. 2 were used for simultaneous determination of these compounds.

Calibration Curves and Statistical Analysis The linearity of the present method was established from the third-derivative spectra by measuring the absorbance of standard solutions (repeated six times) containing the various concentrations of each compound with the constant concentration of the other one. The calibration curves were constructed by plotting the D_3 values against cyproterone acetate or ethinylestradiol concentration at the zero-crossing wavelength of ethinylestradiol (316 nm) or cyproterone acetate (226 nm) respectively. The results are summarized in Table 1. The linearity of the calibration curves and the adherence of the method to Beer's law are validated by the high value of the correlation coefficient and value of intercept on ordinate which is close to zero.

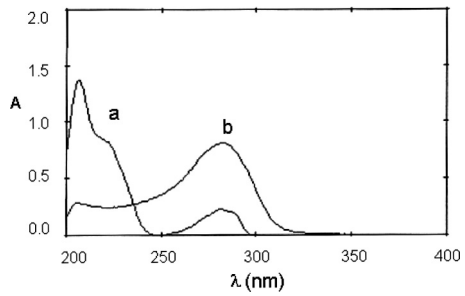

Fig. 1. Zero-Order Spectra of (a) Ethinylestradiol (150 μg/100 ml) and (b) Cyproterone Acetate (2 mg/100 ml)

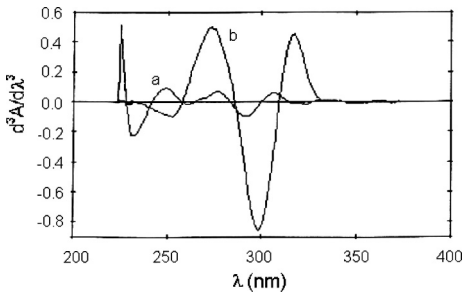

Fig. 2. Third-Derivative Spectra of (a) Ethinylestradiol (150 μg/100 ml) and (b) Cyproterone Acetate (2 mg/100 ml)

Sensitivity The limit of quantification with CV<2.13% was found to be 0.5 mg/100 ml and 10 μg/100 ml for cyproterone acetate and ethinylestradiol respectively.

The limit of detection that can be reliably detected with a S/N ratio of 3 was found to be 100 μg/100 ml and 5 μg/100 ml for cyproterone acetate and ethinylestradiol respectively.

Accuracy and Precision The accuracy and precision were determined by using synthetic mixtures of cyproterone acetate and ethinylestradiol in the laboratory. The mean recoveries and CVs are illustrated in Tables 2 and 3. Data of these tables showed good accuracy and precision over the entire concentration range. The within-day and between-day variations showed coefficient of variation (CV%) values less than 2.00 and 2.13 for cyproterone acetate and ethinylestradiol respectively in all three selected concentrations. The

Table 1. Statistical Data of Calibration Curves of Cyproterone Acetate and Ethinylestradiol in Mixtures with Different Concentrations Using Third-Derivative Spectra

Parameters	Cyproterone acetate	Ethinylestradiol
Linearity	0.50—6.00 (mg/100 ml)	10.00—80.00 (μg/100 ml)
Regression equation	$y=0.216x+0.017$ [a)]	$y=3.70\times10^{-3}x-1.35\times10^{-2}$ [b)]
S.D. of slope	0.002	2.94×10^{-5}
R.S.D. of slope (%)	0.93	0.79
S.D. of intercept	0.004	4.19×10^{-3}
Correlation coefficient	0.9997	0.9997

a) $y=bx+a$, where x is the concentration of cyproterone acetate in mg/100 ml and y is the amplitude at the specified wavelength. *b*) $y=bx+a$, where x is the concentration of ethinylestradiol in μg/100 ml and y is the amplitude at the specified wavelength.

Table 2. Accuracy and Precision Data of Determination of Cyproterone Acetate (0.5—6 mg/100 ml) in the Presence of Ethinylestradiol (35 μg/100 ml) by Third-Derivative Spectrophotometry

Added cyproterone acetate (mg/100 ml)	Within-day ($n=3$)			Between-day ($n=9$)		
	Found (mg/100 ml)	CV (%)	Error (%)	Found (mg/100 ml)	CV (%)	Error (%)
0.50	0.50±0.01	2.00	0.00	0.50±0.01	2.00	0.00
2.00	2.03±0.02	0.99	1.50	2.04±0.02	0.98	2.00
6.00	5.98±0.06	1.99	−0.33	5.99±0.05	0.83	−0.17

Table 3. Accuracy and Precision Data of Determination of Ethinylestradiol (10—80 μg/100 ml) in the Presence of Cyproterone Acetate (2 mg/100 ml) by Third-Derivative Spectrophometry

Added ethinylestradiol (μg/100 ml)	Within-day ($n=3$)			Between-day ($n=9$)		
	Found (μg/100 ml)	CV (%)	Error(%) (%)	Found (μg/100 ml)	CV (%)	Error (%)
10.00	9.96±0.16	1.61	−0.40	9.84±0.21	2.13	−1.60
40.00	39.66±0.34	0.86	−0.85	40.06±0.75	1.87	0.15
80.00	80.12±0.98	1.22	0.15	80.19±0.76	0.95	0.24

Table 4. Results of the Analysis of Commercial Product Containing 2 mg Cyproterone Acetate and 35 μg Ethinylestradiol per Tablet by Third-Derivative Spectrophometry

Sample	Cyproterone acetate (mg)			Ethinylestradiol (μg)		
	Labelled	Found[a] (mean±S.D.)	Error (%)	Labelled	Found[a] (mean±S.D.)	Error (%)
Cyproterone compound	2.00	2.04±0.02	2.00	35.00	34.61±0.40	−1.11
Diane 35	2.00	2.03±0.02	1.50	35.00	35.70±0.38	2.00

a) The mean of ten determinations±S.D.

data indicate that the proposed derivative spectrophotometric method is highly precise during one run and between different runs.

The percentage of recovery in each case was calculated. The results obtained from the recoveries of both drugs (Tables 2, 3) showed excellent accuracy.

Specificity No interference was observed from the presence of starch, sodium croscarmelose, sodium starch glycolate, sodium lauryl sulfate, polyvinylpyrolidine, magnesium stearate, and aerosyl in the amounts commonly present in tablet dosage forms.

Stability Study of stability of cyproterone acetate and ethinylestradiol in solutions during the analytical method showed that analytes were stable for at least 24 h in solutions when protected from light.

Application The proposed method was successfully applied to the analysis of two pharmaceutical dosage forms. The results are summarized in Table 4. No interference from the sample matrix was observed. The results were in good agreement with the labelled content and the error of the determination does not exceed ±2%.

Conclusion

From the results of this study it may be concluded that the proposed third-derivative spectrophotometric method for simultaneous determination of cyproterone acetate and ethinylestradiol is a simple, rapid, practical, reliable and inexpensive method that may be used for routine analysis of cyproterone acetate and ethinylestradiol combination. Furthermore, no preliminary separation, as well as expensive and unavailable instrument is required.

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