short communications

Determination of ethinyloestradiol in the presence of norethisterone by derivative-difference spectrophotometry

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Introduction

Various methods have been reported for the determination of ethinyloestradiol (EO) in contraceptive tablets. These include spectrofluorometric,[1,2] high pressure liquid chromatographic,[3] thin layer chromatographic,[4] and colorimetric methods.[5,6] Corti *et al.* used second derivative UV-spectrophotometry for the determination of binary mixtures of oestrogens and progestogens with a relative standard deviation of less than 5%.[7] Ebel *et al.* reported several methods for determining EO in oral contraceptive tablets including the use of direct, differential, derivative UV-spectrophotometry and orthogonal polynomial methods.[8] The authors reported that the latter method gave the most reliable results.

Recently, it has been shown that the application of derivative techniques to spectrophotometry is very useful in resolving spectral overlap and in cancelling irrelevant absorption from the secondary sample ingredient.[9,10] However, in certain circumstances, the derivative technique cannot cope with the level of interference, especially when the irrelevant absorption is linearly dependent upon the spectrum of the pure compound, a situation in which differential spectrophotometry could be helpful.

Changes in pH have been more exploited than any other change of chemical conditions in applying the differential method, especially for compounds showing bathochromic or hypsochromic shift. If such chemical shifts occur, the presence of a pH-insensitive irrelevant absorption (Z) may be cancelled by changing the solvent from 'a' to 'b'. Thus, $\Delta A = (A_a+Z) - (A_b+Z)$. However, in certain conditions, the irrelevant absorption may give such a heavy contribution that it is only partially cancelled, *i.e.* $\Delta A = (A_a-A_b)+Z'$ where $Z' = Z_a-Z_b$ and is less than Z_a and Z_b. In such a case, the application of a derivative technique may compensate for Z'. This will be the major advantage for the application of derivative-difference spectrophotometry over the use of each technique alone. Despite this advantage relatively few applications of derivative-difference spectrophotometry have been published.[11,12]

This paper reports a simple, rapid and accurate method for the direct determination of EO in the presence of norethisterone (NE) in a ratio of 1 : 20 using a new approach which is based on the use of derivative-difference spectrophotometry.

Methods

APPARATUS

A Perkin-Elmer (Überlingen, FRG) Model 550S UV-VIS spectrophotometer with a fixed slit width (2 nm) and a Hitachi Model (Tokyo, Japan) 561 recorder were used. The spectra of test and reference solutions were recorded in 1 cm quartz cells over the range 340 to 220 nm. Suitable settings are: scan speed 120 nm/min; chart speed 60 mm/min; derivative mode D_1 (first derivative) and D_2 (second derivative); ordinate maximum and minimum approx. 0.2 for D_1 and approx. 0.01 for D_2; response time 10 s. For differential measurement, the zero set of the instrument was adjusted by placing a cuvette filled with methanol in

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Abstract

A simple and direct method for the determination of ethinyloestradiol as a minor component in the presence of norethisterone and in oral contraceptive tablets is presented. The method is based on measuring the signal intensity, $d\Delta A/d\lambda$ and $d^2\Delta A/d\lambda^2$, of the generated first and second derivative spectra of the Δ-absorbance curves obtained by measuring solutions in methanol and methanol–sodium hydroxide at certain wavelengths. The method has been applied to the determination of ethinyloestradiol in oral contraceptive tablets, with a coefficient of variation of less than 2%.

the reference compartment and the other cuvette filled with methanol–NaOH solution in the sample compartment. After this adjustment the hormone solution in both solvents was measured in the same order *i.e.* the hormone solution in methanol was placed in the reference compartment and the hormone solution in methanol–NaOH was placed in the sample compartment.

REAGENTS AND MATERIALS

Samples of EO and NE were kindly provided by CID Company, Giza, Egypt. Methanol–NaOH solution was prepared by mixing 1 ml 1 N NaOH solution and 9 ml methanol. Anovlar® 1 contraceptive tablets (CID Company) were labelled to contain EO 0.05 mg and NE 1.0 mg per tablet. All reagents and chemicals used were analytical reagent grade. EO solution was prepared by dissolving 40 mg EO in 50 ml methanol and a 10 ml portion of this solution was further diluted to 50 ml with methanol.

DETERMINATION OF ETHINYLOESTRADIOL

Two 5 ml portions of the hormone solution were transferred into two separate 10 ml volumetric flasks (A and B). To flask B, 1 ml 1 N NaOH aqueous solution was added and both flasks A and B were completed to volume with methanol. The first derivative (D_1) and second derivative (D_2) of the zero order spectra for B were

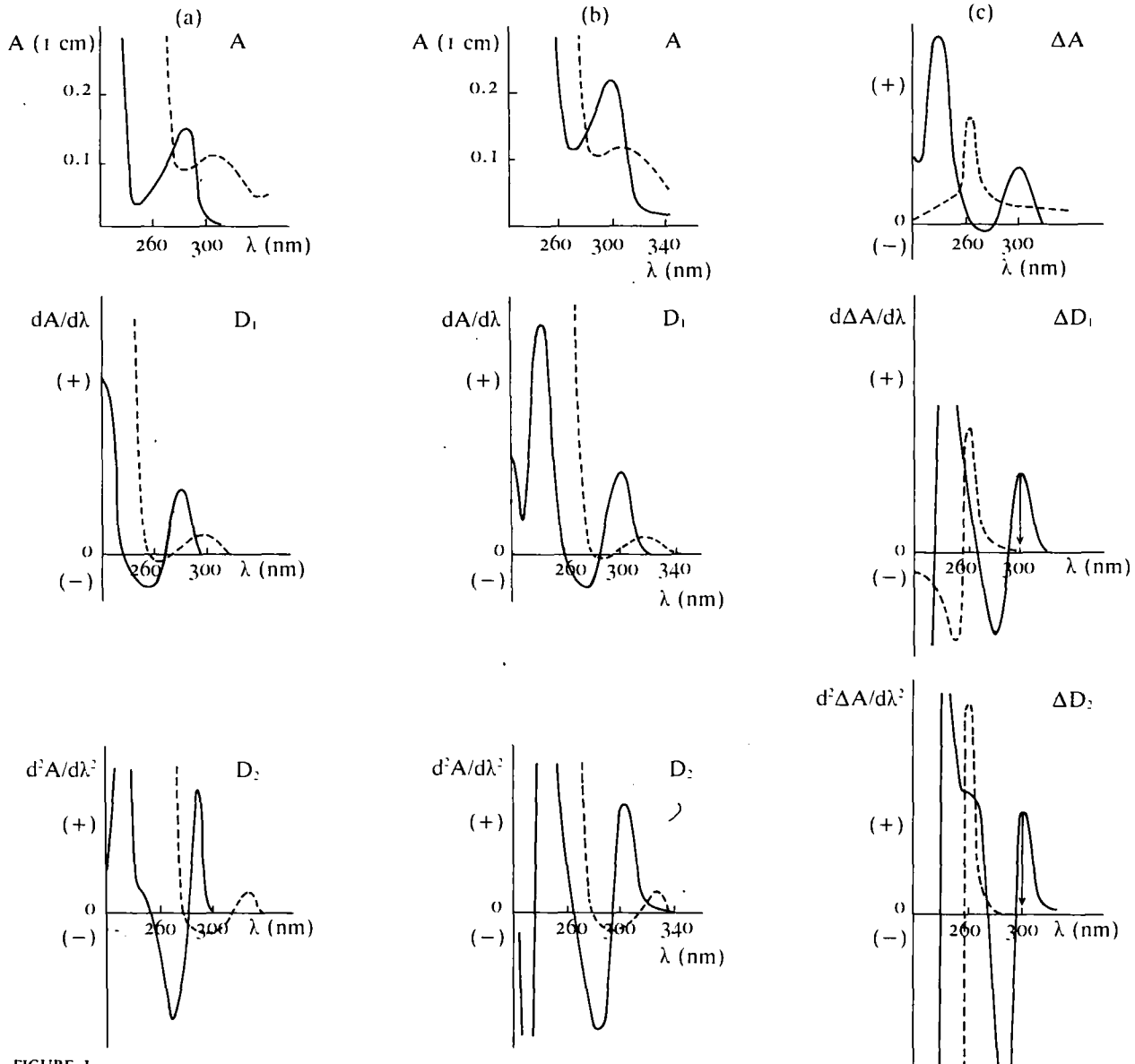

FIGURE 1

Zero order absorption spectra (A) of 67.5 μmol/l ethinyloestradiol (——) and 1.34 mol/l norethisterone (----) and their derivative modes (D_1 and D_2) in methanol (a) and in methanol–NaOH solution (b) as well as differential spectra (ΔA) and their derivative modes (ΔD_1 and ΔD_2) in methanol and methanol–NaOH solution (c). Arrows indicate the chosen ΔD_1 and ΔD_2 peaks

recorded in duplicate (without refilling the cells) against a methanol–NaOH solution as blank. The absolute peak amplitudes (peak to zero line) D_1 and D_2 were measured at 300 and 304 nm respectively (Fig. 1b).

For differential measurement, solution A was placed in the reference cell and solution B in the sample cell and the Δ absorbance ($\Delta A = A_b - A_a$), the absolute value (peak to zero line) for ΔD_1 ($=d\Delta A/d\lambda$) and ΔD_2 ($=d^2\Delta A/d\lambda^2$) were measured at 295, 301 and 304 nm, respectively (Fig. 1c). The concentration of the drug was calculated from the corresponding calibration graphs prepared using a reference EO.

PROCEDURE FOR CONTRACEPTIVE TABLETS

100 Tablets were decoated by washing each with a few drops of water, and, after drying in a desiccator, were weighed and powdered. A quantity of the powdered tablets equivalent to 4 mg EO was transferred into a small beaker with the aid of 5 ml distilled water. The content of the beaker was warmed for about 5 min in a waterbath. 30 ml Methanol was added with stirring for a further 5 min. The solution was cooled, transferred into a 50 ml calibrated flask by washing through a filter paper and, next, the flask was filled to the mark with methanol. The procedure described above was followed.

Results and discussion

Figure 1 (a, b and c) shows the zero-order absorption spectra of 67.5 μmol/l EO and 1.34 mol/l NE (ratio 1 : 20, which simulates the tablet ratio) and their derivative modes (D_1 and D_2) as well as the differential spectra (ΔA) and their derivative modes (ΔD_1 and ΔD_2) measured in methanol and methanol–NaOH solution.

Being phenolic, EO possesses a bathochromic shift with hyperchromic effect when the methanolic solution is made alkaline (Fig. 1, a and b) and NE was found to contribute significantly for both zero-order spectra. On the other hand NE gave a positive contribution to the D_1 signal of EO at 284 and 300 nm in methanol and methanol–NaOH solution, respectively, and a negative contribution to the D_2 signal of EO at 287 and 304 nm in methanol and methanol–NaOH solution, respectively. This means that the application of direct A_{max}, first and/or second derivative modes methods is susceptible to erroneous results. Furthermore, the ΔA curve for NE (Fig. 1c) possesses a positive contribution to the ΔA curve of EO. However, NE possesses a nil contribution to the ΔD_1 and ΔD_2 signals of EO at 301 and 304 nm, respectively. This led us to use the ΔD_1 and ΔD_2 values to determine EO.

Under the described experimental conditions, a linear correlation was obtained between ΔD_1 and ΔD_2 with the concentration C of EO in the range 60-400 μmol/l. The two regression equations were found to be:

$$\Delta D_1 = -0.0034 + 6.809 \times 10^{-4} C \quad (r = 0.9998)$$

$$\Delta D_2 = -0.0002 + 0.472 \times 10^{-4} C \quad (r = 0.9998)$$

Replicate determination of EO using ΔD_1 and ΔD_2 methods gave relative standard deviations of 2.06 and 1.45% (5 separate determinations), respectively.

For assessing the potential feasibility for the assay of EO in the presence of NE, synthetic mixtures have been prepared from authentic drugs in the ratios stated in Table 1, and analysed by measuring D_1 and D_2 (in methanol–NaOH solution) and ΔA, ΔD_1 and ΔD_2 at the specified wavelengths. The results obtained are presented in Table 1. From these results the following can be concluded.

- The higher results obtained using the ΔA and D_1 methods were due to the positive contribution of NE to the spectra of EO in the vicinity 280-320 nm. The low results obtained using the D_2 method were due to the negative contribution of the NE D_2-signal in the range 280-310 nm.
- The ΔD_1 and ΔD_2 gave generally more accurate and precise results.

It was found that the ratio 1 : 40 (EO : NE) is the maximum concentration of NE at which EO can still be assayed accurately using the proposed methods.

The proposed ΔD_1 and ΔD_2 methods were applied to the determination of EO in contraceptive tablets (Anovlar® 1) and the mean percentages found ± SD (five separate determinations) were 100.2 ± 1.73 and 99.0 ± 0.89, respectively.

TABLE 1

Determination of ethinyloestradiol (EO) in the presence of norethisterone (NE) by different methods

Sample	Ratio of added EO to NE	Recovery (%)				
		ΔA (295 nm)	D_1 (300 nm)	D_2 (304 nm)	ΔD_1 (301 nm)	ΔD_2 (304 nm)
1	1 : 32	184.8	146.3	85.0	99.4	100.7
2	1 : 24	149.1	128.0	91.1	100.1	100.5
3	1 : 20	157.6	120.0	95.2	100.6	100.3
4	1 : 16	132.3	116.3	96.6	100.0	100.2
5	1 : 10	146.7	113.5	98.9	97.8	98.9
6	1 : 8	118.2	108.8	98.6	99.1	100.1
7	1 : 6	133.3	106.5	99.0	99.5	99.0

TABLE II
Recovery of added ethinyloestradiol (EO) to the contraceptive tablets

Sample	EO concentration in tablet sample* (μmol/l)	Added EO (μmol/l)	Recovery (%)	
			ΔD_1	ΔD_2
1	27.0	168	100.0	100.0
2	40.5	168	99.4	100.1
3	54.0	168	99.5	100.1
4	71.0	168	100.0	99.5
5	108.0	168	99.7	100.9

*Calculated from the nominal content of Anovlar® 1 tablets.

The possibility of interfering constituents in tablets must not be overlooked. Therefore, the standard addition principle was used to evaluate the accuracy of the proposed methods for such samples and to test for interference from the tablet constituents. The recoveries for added EO to the tablet sample (in quantities presented in Table II) varied from 99.4 to 100.0 and from 99.5 to 100.9, using the ΔD_1 and ΔD_2 methods, respectively. Although first and second derivative modes of the Δ absorption curves gave comparable results (Table I). In general, the use of higher derivative order (second or higher order) may be preferable in improving the resolution of overlapping peaks and in discriminating against the spectral background.[9]

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