Simultaneous Determination of Estradiol and Medroxyprogesterone Acetate in Pharmaceutical Formulations by Second-Derivative Spectrophotometry

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This paper reports a simple and fast method for the simultaneous determination of estradiol (ED) and medroxyprogesterone acetate (MP) in pharmaceutical formulations by second-derivative spectrophotometry. Methanol was used to extract the drugs from formulations, and subsequently the extracts were evaluated directly by derivative spectrophotometry. The drugs were determined simultaneously by using the graphic method at 297.4 nm for ED and the zero-crossing method at 273.4 nm for MP. If both compounds are present together in a sample, it is possible to quantitate one in the presence of the other. The best signal-to-noise ratio was found when the second derivative of the spectrum was used. The linear ranges for determination of the drugs were 4.7×10^{-6} to 1.6×10^{-4} and 7.2×10^{-6} to 2.0×10^{-4} mol/L for ED and MP, respectively. The ingredients commonly found in commercial pharmaceutical formulations do not interfere with the determination. Chemical and spectral variables were optimized for the determination of both analytes. Good levels of repeatability (relative standard deviation), 1.4 and 1.9%, were obtained for ED and MP, respectively. The proposed method was applied to the determination of these drugs in pharmaceutical formulations.

The normal levels of the female hormones estradiol (ED) and medroxyprogesterone acetate (MP) in women can vary because of aging or abnormalities associated with the reproductive organs. Consequently, ED and MP are prescribed individually or in combination to adjust these hormonal levels. ED is the most important of the estrogens produced by the ovaries. These hormones are the steroids responsible for growth, uterine function, and secondary sexual characteristics. The effects of ED are based on the union of the estrogen with specific receptors and the subsequent synthesis of specific proteins. MP, on the other hand, is a female hormone used for the treatment of amenorrhea (lack of menstrual flow), abnormal bleeding of the uterus, or endometriosis caused by an abnormality in the internal membrane of the uterus.

During recent years, concern has risen over the potential pollution of waterways with estrogenic compounds, including steroidal hormones from human and animal sources. The most likely source of steroidal hormone contamination is the incomplete removal of these compounds in wastewater treatment systems (1). Likewise, the effects of the intermittent exposure of live organisms in the environment (fish, animals, plants, etc.) to estrogenic compounds are unknown, particularly for ED (2).

Analytical methods have been reported for the determination of ED together with other drugs: for example, ED and norgestrel in contraceptive tablets by reversed-phase liquid chromatography (RP–LC; 3); ED, zidovudine, chloramphenicol, and endogenous urine materials by LC (4); and ED with estrone in human hair by gas chromatography/mass spectrometry (GC/MS; 5). For the determination of MP together with other drugs, it is possible to mention only the quantitation of hydroxyprogesterone hexanoate, progesterone, and MP in mixtures by RP–LC (6). On the other hand, there are many examples of the isolated determination of these drugs: MP acetate in serum by LC with peroxyoxalate chemiluminescence detection using a fluorogenic reagent (7), MP in tablets by LC (Novapak C_{18}; 8), MP in human plasma by LC (column packed with 5 μm Spherisorb 5ODS2; 9), MP in human serum by GC/MS (10), etc.

The traditional techniques used to identify and monitor the use of these drugs have been enzyme-linked inmunosorbent assay (ELISA) and radioimmunoassay (RIA). Both RIA and ELISA methods for ED (11–14) and MP (15, 16) have been reported.

Because of the lack of published methods for the simultaneous determination of both compounds, together with the need for such methods, we developed a simple, rapid, inexpensive, sensitive, and selective method of analysis for that purpose.

Classic spectrophotometry permits only the determination of ED in the presence of MP, but it is not an appropriate tech-

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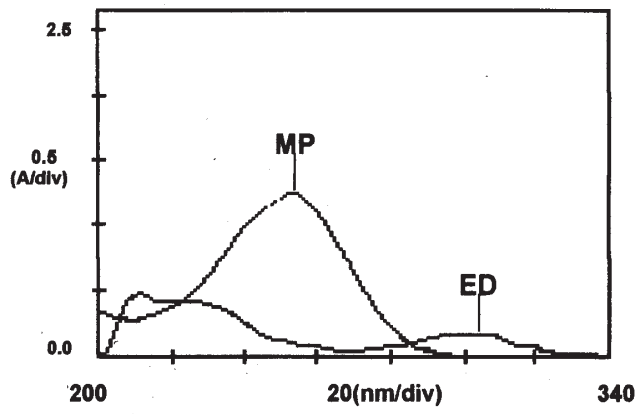

Figure 1. Absorption spectra of ED (1.0×10^{-4} mol/L) and MP (1.0×10^{-4} mol/L) measured versus methanol.

nique for the simultaneous determination of both drugs because the absorption bands of MP overlap those of ED between 200 and 275 nm. In order to enhance the detectability of minor spectral features and to perform this simultaneous determination without previous separation, we used derivative spectrophotometry, which consists of the differentiation of a normal spectrum. This technique has been used directly for the simultaneous determination of inorganic (17, 18) and organic (19–22) compounds in many kinds of matrixes.

It is necessary to highlight the importance of the development of this method, because the simultaneous determination of these drugs has not yet been reported in the *U.S. Pharmacopeia* (23).

This paper describes the development of the proposed method for the simultaneous determination of both drugs by second-derivative spectrophotometry. It was necessary to optimize the solvent and the spectral variables to obtain precise procedures and accurate results by the proposed method, which was applied to the analysis of 2 widely prescribed pharmaceutical formulations containing both drugs.

Experimental

Instrumentation

A Shimadzu UV-160 spectrophotometer with 10 mm quartz cells was used to obtain measurements of the absorbance and derivative spectra. For all the solutions tested, the first-, second-, third-, and fourth-derivative spectra were recorded over a range of 340–200 nm vs solvents. The derivative spectra were obtained digitally by software incorporated

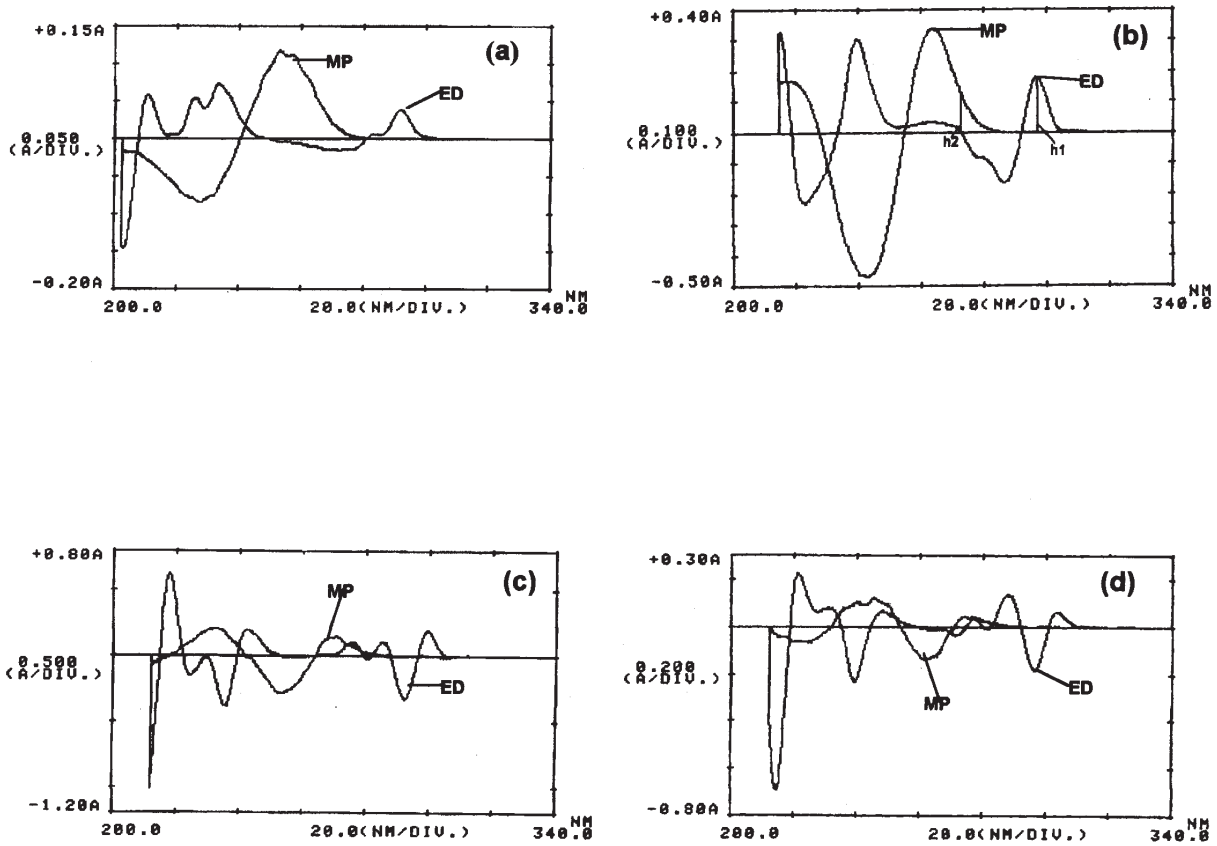

Figure 2. Derivative spectra of ED (1.0×10^{-4} mol/L) and MP (1.0×10^{-4} mol/L) measured versus methanol: (a) first-derivative spectra; (b) second-derivative spectra; (c) third-derivative spectra, and (d) fourth-derivative spectra.

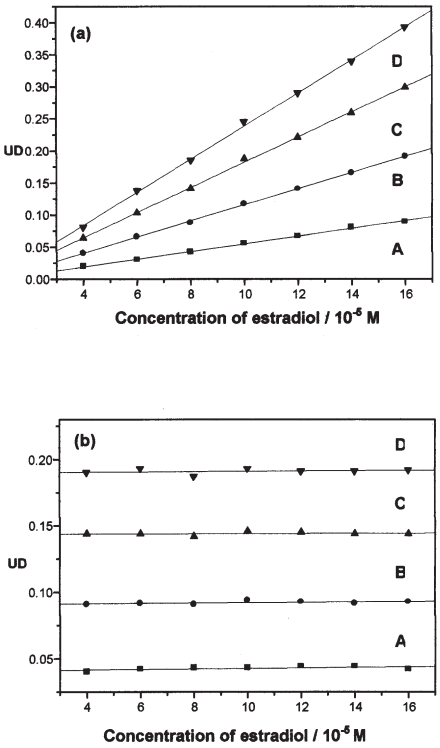

Figure 3. (a) Calibration graphs prepared from second-derivative spectra of ED in the presence of MP at 6.0×10^{-5} mol/L at different $\Delta\lambda$ values: (A) $\Delta\lambda$ = 2.8 nm, (B) $\Delta\lambda$ = 3.5 nm, (C) $\Delta\lambda$ = 4.2 nm, and (D) $\Delta\lambda$ = 4.9 nm at 297.4 nm; (b) effect of ED concentration on the signal of MP at 6.0×10^{-5} mol/L, obtained at 273.4 nm by second-derivative spectrophotometry, at different $\Delta\lambda$ values: (A) $\Delta\lambda$ = 2.8 nm, (B) $\Delta\lambda$ = 3.5 nm, (C) $\Delta\lambda$ = 4.2 nm, and (D) $\Delta\lambda$ = 4.9 nm. DU: derivative unit.

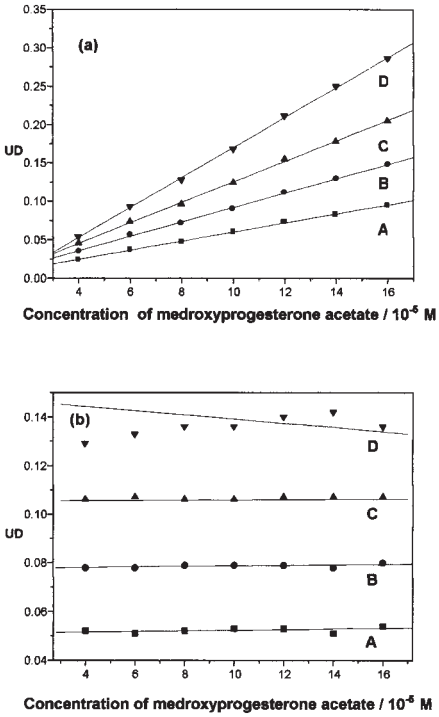

Figure 4. (a) Calibration graphs prepared from second-derivative spectra of MP in the presence of ED at 6.0×10^{-5} mol/L at different $\Delta\lambda$ values: (A) $\Delta\lambda$ = 2.8 nm, (B) $\Delta\lambda$ = 3.5 nm, (C) $\Delta\lambda$ = 4.2 nm, and (D) $\Delta\lambda$ = 4.9 nm at 273.4 nm; (b) effect of MP concentration on the signal of ED at 6.0×10^{-5} mol/L, obtained at 297.4 nm by second-derivative spectrophotometry, at different $\Delta\lambda$ values: (A) $\Delta\lambda$ = 2.8 nm, (B) $\Delta\lambda$ = 3.5 nm, (C) $\Delta\lambda$ = 4.2 nm, and (D) $\Delta\lambda$ = 4.9 nm. DU: derivative unit.

in the Shimadzu UV-160 spectrophotometer. A scan speed of 480 nm/min with $\Delta\lambda$ = 4.2 nm was used.

Materials and Reagents

All reagents were analytical reagent grade. ED and MP were provided by Laboratorio Chile (Santiago, Chile).

Stock solutions of MP and ED at 1.0×10^{-3} mol/L were prepared by dissolving 27.24 ± 0.01 and 38.65 ± 0.01 mg, respectively, in methanol and diluting each solution to 100 mL. Other concentrations were prepared by appropriate dilution with the same solvent. Tablets containing ED and MP were also dissolved in methanol.

Calibration Procedure for Determination of ED and MP in Mixtures

Aliquots of stock solutions of ED and MP were simultaneously diluted with methanol to concentrations within the range of 4.0×10^{-5} to 16.0×10^{-5} mol/L. The calibration graphs were prepared for each compound in the presence of the other compound at 6.0×10^{-5} mol/L. In all cases, the corresponding absolute values of the second-derivative spectra at 297.4 and 273.4 nm for MP and ED, respectively, were obtained, and the values were plotted vs the corresponding concentrations.

Procedure for Determination of ED and MP in Pharmaceutical Formulations

A total of 10 tablets of each formulation were weighed and powdered. A quantity of powder between 15 and 20 ± 0.01 mg

Table 1. Analytical parameters for the determination of ED and MP by second-derivative spectrophotometry

Analytical parameter	ED	MP
Detection limit, mol/L[a]	1.4×10^{-6}	2.2×10^{-6}
Quantitation limit, mol/L[b]	4.7×10^{-6}	7.2×10^{-6}
Determination range, mol/L	4.7×10^{-6} to 1.6×10^{-4}	7.2×10^{-6} to 2.0×10^{-4}
Repeatability, %[c]	1.4	1.9

[a] 3 σ criterion.

[b] 10 σ criterion.

[c] Relative standard deviation (n = 11).

Table 2. Recovery of ED and MP from different standard mixtures

	Stated concentration, mg/L		Concentration found, mg/L[a] (recovery, %)	
ED:MP ratio	ED	MP	ED	MP
1:1	12.0	12.0	12.2 ± 0.20 (101.4)	12.0 ± 0.20 (100.0)
2:1	24.0	12.0	24.6 ± 0.38 (102.5)	11.9 ± 0.19 (99.2)
3:1	36.0	12.0	36.8 ± 0.62 (102.2)	11.8 ± 0.18 (98.3)
4:1	47.9	12.0	49.1 ± 0.78 (102.5)	11.9 ± 0.20 (99.2)
1:2	12.0	24.0	11.8 ± 0.21 (98.3)	23.8 ± 0.37 (99.2)
1:2.5	12.0	30.0	12.10 ± 0.19 (100.8)	30.3 ± 0.54 (101.0)
1:3	12.0	36.0	12.3 ± 0.18 (102.5)	35.2 ± 0.61 (97.8)
1:4	12.0	48.0	11.8 ± 0.19 (98.3)	47.2 ± 0.79 (98.3)

[a] Each value is the average of 6 determinations.

for each formulation containing both compounds was accurately weighed, transferred to a 25 mL volumetric flask, and dissolved in methanol. The contents of the flask were diluted to volume with methanol, and the flask was shaken for 10 min. The contents of the flask were centrifuged, and the supernatant solution was evaluated by second-derivative spectrophotometry.

Results and Discussion

Solvent Effect

Methanol, acetonitrile, dioxane, and dimethylformamide (DMF) were tested as solvents to study their effect on the spectral behavior of ED and MP. Also, for each solvent the effect of the concentrations of the analytes on spectral behavior was assessed. Methanol and acetonitrile were found to be the best solvents, because the analytes produced spectral bands of considerable sensitivity, and increment in their concentrations did not alter the shape of the bands. DMF and dioxane were eliminated, because the signals were small and not well defined. Methanol was selected arbitrarily as the solvent for this work, but this determination is also possible with acetonitrile as the solvent.

Spectral Features

To perform the simultaneous determination, the spectral signals were evaluated directly from methanol solutions containing both ED and MP. In this solvent, ED can be determined easily, because in the range of 290–300 nm only ED absorbs, even if MP is present (Figure 1). Conversely, as Figure 1 shows, direct determination of MP presents more difficulties, because the spectral band of MP totally overlaps the first band of ED. In this context, ED can be determined in the presence of MP by classic spectrophotometry. However, when this technique is used, MP can be determined only by using a system of equations or a previous separation of both compounds. It is for this reason that derivative spectrophotometry was selected, because this technique permits the resolution and direct determination of constituents in a mixture.

Two modes of digital derivative spectrophotometry may be used. In the first mode, proposed by Savitzky and Golay (24), the derivative corresponds to $A/\Delta\lambda$, where A is the absorbance, which must be measured as fixed points at uniform intervals on the abscissa chosen. In this type of digital derivative spectrophotometry, a computerized least-squares procedure for smoothing and differentiating the numerical spectral data is used. Under these conditions, a higher signal-to-noise (S/N) ratio is obtained. In the second mode, the derivatives correspond to $\Delta A/\Delta\lambda$, the derivation process is also computerized, and a 2-column table is used. In this derivative type, $\Delta\lambda$ values can be varied, and this mode is very useful when the spectra strongly overlap. The latter alternative was used in this work.

Selection of Spectral Variables

Derivative order.—The derivative order, analytical wavelengths, and $\Delta\lambda$ value were optimized to obtain maximum resolution, sensitivity, and reproducibility. To choose the optimum derivative order, the first-, second-, third-, and fourth-derivative spectra of separate solutions of ED and MP were recorded versus methanol. As shown in Figure 2, the first- and second-derivative spectra could be used for this simultaneous determination, because both are well defined, and these derivatives present characteristic wavelengths for the determination of each compound. However, the ED signal is 5 times higher when the second derivative is used instead of the first derivative, and the MP signals are similar in both cases. Because typical pharmaceutical formulations contain 2 mg ED and 5 mg MP, that is, the ED content is 0.4 of the MP content, the second derivative was selected for the determination. This derivative order also produces a high S/N ratio.

Analytical wavelengths.—Analytical wavelengths were selected by recording the second-derivative spectra of separate solutions of ED and MP, each at a concentration of 1.0×10^{-4} mol/L (Figure 2). Figure 2 shows that the graphical

method can be used for the determination of ED at 297.4 nm. At this wavelength, the distance h_1 is proportional only to the concentration of ED. Similarly, for the measurement of the derivative spectrum at an abscissa value of 273.4 nm, h_2, corresponding to the zero-crossing point of the derivative spectrum of ED, can be used satisfactorily to determine MP (Figure 2). Another zero-crossing point at 237.5 nm was discarded because, in this area, the shape of the spectrum is affected by the ED concentration, which alters the value of the zero-crossing point. Furthermore, at both wavelengths selected, the analytical signal is not dependent on the concentration of the other compound.

Value of $\Delta\lambda$.—For the selection of the $\Delta\lambda$ value for differentiation, a series of second-derivative spectra of mixtures of MP at 6×10^{-5} mol/L and ED at concentrations ranging from 4.0×10^{-5} to 16×10^{-5} mol/L were evaluated at 297.4 nm by using different $\Delta\lambda$ values (Figure 3, a and b). Similarly, the second-derivative spectra of mixtures of ED at 6.0×10^{-5} mol/L and MP at concentrations ranging from 4.0×10^{-5} to 16×10^{-5} mol/L were evaluated at 273.4 nm (Figure 4, a and b).

Figures 3a and 4a show that good calibration lines are obtained for both compounds over the $\Delta\lambda$ range of 2.8–4.9 nm. However, in addition to a good calibration line, the signals for each compound must not be affected by the other compound; this is possible only when $\Delta\lambda$ is between 2.8 and 4.2 nm (Figures 3b and 4b). To obtain a higher sensitivity, 4.2 nm was selected as the optimum $\Delta\lambda$ value. Good reproducibility and a high S/N ratio were also obtained when the $\Delta\lambda$ value of 4.2 nm was used in the simultaneous determination of ED and MP.

Analytical Parameters

Calibration graphs were obtained by plotting the second-derivative value for ED (λ = 297.4 nm) h_1 and for MP (λ = 273.4 nm) h_2, with $\Delta\lambda$ = 4.2 nm, versus analyte concentration. The linear regression equations and the correlation coefficients calculated for mixtures of ED and MP were h_1 = 2000C(M) – 0.014 and r = 0.999 for ED and h_2 = 1300C(M) – 0.009 and r = 0.999 for MP, where h is in derivative units and C(M) corresponds to the analyte concentration in mol/L. All analytical parameters are shown in Table 1.

To establish the ratios at which one analyte can be accurately measured in the presence of the other, the recoveries of ED and MP were determined from analyses of samples containing standard solutions of mixtures of the analytes in different concentration ratios. The results are shown in Table 2. The content of each compound can be determined if the concentration ratio is between 1:4 and 4:1 for ED:MP, which includes the ratio normally used in commercial formulations.

Practical Application

The accuracy of the method was determined by analysis of synthetic formulations containing 2 mg ED, 5 mg MP, and 50 mg excipients (magnesium stearate + gelatin, approximately 3–5%; lactose–starch 95%). The recoveries were 101.5 ± 0.5 and 103.4 ± 0.4% for ED and MP, respectively. These results indicate that common excipients normally found in tablets do not interfere in the proposed method.

Table 3. Simultaneous determination of ED and MP in pharmaceutical formulations by second-derivative spectrophotometry

	Amount found per tablet, mg[a]	
Dosage form	ED	MP
Primaquin MP (Laboratorio Recalcine)	1.93 ± 0.03	4.95 ± 0.09
Enadiol MP (Laboratorio Chile)	1.95 ± 0.04	4.93 ± 0.08

[a] Each value is the average of 6 determinations.

However, it was still necessary to ensure that no product of degradation of the components or of the active drugs in pharmaceutical formulations interfered in the simultaneous determination proposed for ED and MP. One portion of each tablet formulation was diluted individually in methanol, so that the solution contained ED at 2×10^{-5} mol/L and MP at 5×10^{-5} mol/L. These solutions were stored in amber container flasks. An aliquot of each solution was taken every hour for 12 h for analysis by second-derivative spectrophotometry. In all cases, the spectra and the second derivative showed no change in the form, height, and wavelength maxima of the bands. Furthermore, the h_1 and h_2 values did not change, indicating that there is no interference by the components of the tablets, under the experimental conditions of the proposed method.

The pharmaceutical formulations Primaquin MP and Enadiol MP were analyzed by the proposed method. The labeled amounts of ED and MP are 2.0 and 5.0 mg, respectively, for both formulations; however, the labeled amounts of excipients for Primaquin MP and Enadiol MP are 50.0 and 97.2 mg, respectively. The amounts of the analytes found for these different formulations are shown in Table 3.

Acknowledgment

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