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Short communication

Development and validation of a reversed-phase liquid chromatographic method for analysis of estradiol valerate and medroxyprogesterone acetate in a tablet formulation

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Abstract

A simple and accurate liquid chromatographic method was developed for estimation of estradiol valerate and medroxyprogesterone acetate in pharmaceuticals. Drugs were chromatographed on a reverse phase C18 column, using a mixture (30:70) of ammonium nitrate buffer and acetonitrile and eluants monitored at a wavelength of 280 nm. Solution concentrations were measured on a weight basis to avoid the use of an internal standard. The method was statistically validated for its linearity, accuracy, precision and selectivity. Due to its simplicity and accuracy, the authors believe that the method may be used for routine quality control analysis. It does not require any specific sample preparation except the use of a column guard before the analytical column and suitable prefilter attached to the syringe prior to injection.

Keywords: Estradiol valerate; Medroxyprogesterone acetate; Reversed-phase liquid chromatography

1. Introduction

The combination estrogen–gestagen is used for the treatment of estrogenic deficiency syndrome to control its symptoms (climateric syndrome), such as loss of bone minerals and development of heart diseases.

We have developed and validated a new chromatographic method for quantitation of this association in tablets. This method consists of a single operation, saving time and materials. Therefore, it differs from those methods found in the literature (all them by HPLC) [1–5] that require separate determinations for each drugs. Besides, as both drugs are quantified in the same chromatographic system, the risk of contamination with solvents is lower, protecting human health and the environment.

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Table 1
Assay precision

Estradiol valerate		Medroxyprogesterone acetate	
Injected (μg)	Average peak area response	Injected (μg)	Average peak area response
8.05	1781338	20.54	532777
	1781132		529919
	1779178		530598
	1780902		533617
	1795409		534077
	1785753		532866
	1787770		529903
	1782825		532883
	1803315		533905
	1782865		533513
RSD (%) =	0.4		0.3

RSD, Relative standard deviation.

2. Experimental

2.1. Materials and reagents

The working standards employed for estradiol valerate and medroxyprogesterone acetate were developed locally using a crystallizing technique. Ammonium nitrate was supplied by Merck (Darmstadt, Germany). Solvents were HPLC grade. Water HPLC grade was obtained by distillation and passed through a 0.45 micron membrane filter.

A commercial local tablet formulation was used. Its composition is estradiol valerate 2 mg, medroxyprogesterone acetate 5 mg, in a matrix of microcrystalline cellulose, starch and povidone.

2.2. Instrumentation

The HPLC system consisted of a dual piston reciprocating pump Spectra Physics (model ISO Chrom. LC pump), a detector UV-Vis Konik (model KNK-029-757), an integrator Spectra Physics (model SP 4600) and a Rheodyne injector (model 7125).

2.3. HPLC conditions

The experiment was performed on a LiChroCART^{R} 250×4 mm HPLC Cartridge LiChrospher^{R} 100 RP-18 (5 μm) Merck, coupled with a column guard of LiChroCART^{R} 4×4 mm LiChrosorb^{R} RP-18 (5 μm) Merck (Darmstadt, Germany). The mobile phase consisted of 30% of solution A and 70% of solution B. Solution A was 0.07 M ammonium nitrate buffer and solution B was acetonitrile. The mobile phase was filtered through a nylon membrane (pore size 0.45 μm) Micron Separations N04SP04700 and degassed. Chromatography was performed at room temperature using a flow rate of 2.0 ml min^{-1} and a run time of 15 min. Detector sensitivity was set at 0.2 a.u.f.s. and eluents were monitored at 280 nm. The volume of each injection was 20 μl. In these conditions medroxyprogesterone acetate retention time was ~ 4 min and estradiol valerate retention time was ~ 12 min.

2.4. Procedure

Solutions were prepared on a weight basis and volumetric flasks used as suitable containers in order 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.

Quantitation was accomplished using an external standard method.

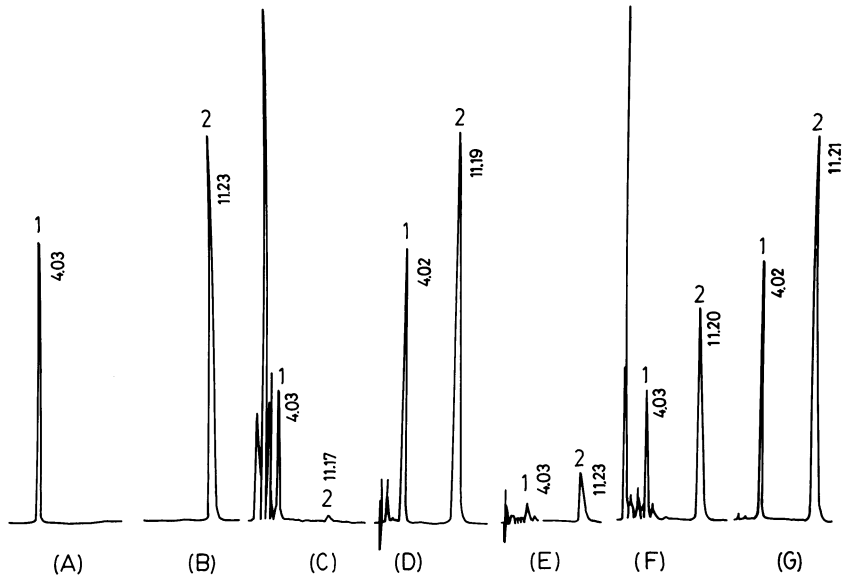

Fig. 1. Chromatograms of medroxyrogesterone acetate and estradiol valerate with their potential related substances. Peak identities: (1) medroxyprogesterone acetate, (2) estradiol valerate. (A) chromatogram of medroxyprogesterone acetate standard, (B) chromatogram of estradiol valerate standard, (C) chromatogram of alkaline degradation, (D) chromatogram of acid degradation, (E) chromatogram of reductive degradation, (F) chromatogram of oxidative degradation, (G) chromatogram of photolytic degradation.

2.5. System suitability

The chromatographic system was in agreement with the following parameters, calculated from six injections of a freshly prepared resolution test mixture: minimum of theoretical plates in the chromatographic column >2500 (plates m^{-1}), calculated on the basis of the estradiol valerate peak; the relative standard deviation (RSD) of estradiol valerate and medroxyprogesterone acetate peak areas of 1.0%, tailing factor for the medroxyprogesterone acetate and estradiol valerate peaks <1.5; resolution between medroxyprogesterone acetate and estradiol valerate peaks >2.

2.6. Preparation of the solutions

2.6.1. Reference solutions

Forty milligrams of estradiol valerate were taken in a 100 ml volumetric flask, dissolved in 70 ml of mobile phase, sonicated for about 15 min and then diluted to volume with mobile phase.

Fifty milligrams of medroxyprogesterone acetate were taken in a 50 ml volumetric flask, dissolved in 40 ml of mobile phase, sonicated for about 15 min and then diluted to volume with mobile phase.

2.6.2. Sample solution

Twenty tablets were weighed and finely powdered and an accurately weighed powder sample equivalent to two tablets was placed in a 10 ml volumetric flask. Eight milliliters of mobile phase were added and the flask was kept in an ultrasonic bath for 15 min. The mixture was then diluted to 10 ml with mobile phase, thoroughly mixed, and filtered through a Whatman No 42 paper.

2.6.3. Calibration solutions

Five solutions were prepared in mobile phase at concentrations ranging from 0.32 to 0.48 mg ml^{-1} for the study of estradiol valerate response linearity.

Five solutions were prepared in mobile phase at concentrations ranging from 0.80 and 1.20 mg ml^{-1} for the study of medroxyprogesterone acetate response linearity.

Table 2
Linearity data

Estradiol valerate			Medroxyprogesterone acetate		
Injected (μg)	Average peak area response	RSD (%)	Injected (μg)	Average peak area response	RSD (%)
9.864	2124868	0.5	24.648	636968	0.3
8.832	1953133	0.5	22.520	574687	0.4
8.048	1781812	0.4	20.540	532406	0.3
7.066	1560763	0.4	18.016	463390	0.3
6.576	1416831	0.3	16.432	425486	0.1
	Slope[a]	RSD	Intercept[b]	RSD	
Estradiol valerate	214657 ± 9313	0.03	30433 ± 76320	0.6	
Medroxyprogesterone acetate	25620 ± 472	0.06	4249 ± 9749	1.6	

[a] Confidence limits of the slope ($p = 0.05$).
[b] Confidence limits of the intercept ($p = 0.05$).
RSD, relative standard deviation.
Estradiol valerate: $Y = 2.15 \times 10^5\ X + 3.0 \times 10^4$.
Medroxyprogesterone acetate: $Y = 2.56 \times 10^4\ X + 4.0 \times 10^3$.

Table 3
Results of the recovery analysis of estradiol valerate

Amount of drug added (mg)	Amount recovered (mg per tablet)	Recovery (%)	RSD ($n = 3$)
1.67	1.65	98.8	1.0
1.66	1.68	101.2	0.9
1.64	1.63	99.4	0.5
2.20	2.16	98.2	0.1
1.95	1.90	97.4	0.4
2.35	2.35	100.0	0.1
2.60	2.54	97.7	0.7
2.37	2.34	98.7	0.6
2.40	2.33	97.1	0.1

RSD, relative standard deviation.

2.6.4. Selectivity

Method selectivity was determined by degrading estradiol valerate and medroxyprogesterone acetate as follows:

A mixture of estradiol valerate and medroxyprogesterone acetate was subjected to thermal (in an oven at 110°C for 1 h) and photochemical degradation (in an open container exposed to daylight for 24 h).

Forty milligrams of estradiol valerate and 100 mg of medroxyprogesterone acetate were dissolved in 25 ml of: water, HCl 1 N, NaOH 1 N, HCl 1 N/Zn and H_2O_2 100 vol, refluxed for at least 15 min and degradation was monitored as a function of time. Each solution was neutralized and suitably diluted with mobile phase in a 100 ml volumetric flask.

2.6.5. Accuracy

The accuracy of the assay was assessed by fortifying placebo tablets with known amounts of estradiol valerate and medroxyprogesterone acetate at 80, 100 and 120% of sample solution concentrations.

Table 4
Results of the recovery analysis of medroxyprogesterone acetate

Amount of drug added (mg)	Amount recovered (mg per tablet)	Recovery (%)	RSD ($n = 3$)
4.16	4.17	100.2	1.0
4.19	4.26	101.7	0.9
4.22	4.19	99.3	0.7
4.95	4.97	100.4	0.1
5.15	5.18	100.6	1.1
5.55	5.57	100.4	0.2
5.80	5.68	97.9	0.1
5.86	5.76	98.3	0.9
6.19	6.31	101.9	0.1

RSD, relative standard deviation.

3. Results and discussion

3.1. Precision

The variation in retention time among ten replicate injections of estradiol valerate reference solution and medroxyprogesterone acetate reference solution was very low, rendering a RSD of 0.4 and 0.3%, respectively (Table 1).

3.2. Selectivity

Neither formulation ingredients nor degradation products interfered with quantitation of estradiol valerate and medroxyprogesterone acetate.

All samples were analyzed using the assay chromatographic conditions described. No evidence of interactive degradation products was seen during evaluation.

However, estradiol valerate and medroxyprogesterone acetate showed degradation products afterwards alkaline and acid hydrolysis, reduction, oxidation and photolysis.

Selectivity was demonstrated showing that estradiol valerate and medroxyprogesterone acetate peaks were free of interference from degradation products indicating that the proposed method can be used in a stability assay (Fig. 1).

3.3. Linearity

Curves of peak areas versus concentration proved linear.

Regression lines calculated by the least-squares method were $Y = 2.15 \times 10^5\ X + 3.0 \times 10^4$ with a coefficient of correlation $r = 0.9960$ for estradiol valerate and $Y = 2.56 \times 10^4\ X + 4.0 \times 10^3$ with a coefficient of correlation $r = 0.9991$ for medroxyprogesterone acetate with confidence intervals at $p = 0.05$ for both drugs (Table 2).

3.4. Accuracy

Recovery data from the study of estradiol valerate was within the range 97.1–101.2 and RSD was 1.3%. Overall average recovery yields were 98.7% ($n = 9$) (Table 3).

Figures for medroxyprogesterone acetate were within the range 97.9–101.9 and RSD was 1.4%. Overall average recovery yields were 100.1% ($n = 9$) (Table 4).

Since the results obtained were within the acceptable $\pm$ 3% range, the method was deemed to be accurate.

4. Conclusion

The results of validation showed that the

method was unaffected by assay time. The method has been shown to be specific and to yield results similar to existing methods for individual components. The proposed RP-HPLC method is simple, rapid, precise, accurate and selective for the determination of estradiol valerate and medroxyprogesterone acetate and can be employed for their assay in dosage forms and stability studies. Use of the combined method is thus more efficient than analysis of estradiol valerate and medroxyprogesterone acetate using separate methods.

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