Analysis of Tablets Containing Estradiol Valerate and Norethisterone by HPLC

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ABSTRACT: OBJECTIVE To develop a HPLC method for determination of estradiol valerate and norethisterone in compound tablets. **METHOD** The chromatographic system consisted of Zorbax CN column and mobile phase of MeCN-H_2O (47 : 53), with a detection wavelength at 280 nm. Progesterone was as the internal standard. **RESULTS** The average (n = 9) recovery both for estradiol valerate and norethisterone was 100.6 % with RSD 0.9 % and 100.2 % with RSD 0.8 %, respectively. Three batches of samples were analyzed, and satisfactory results were achieved. **CONCLUSION** Without complicated sampling procedure, the method was simple, rapid and accurate for the assay of the components in this pharmaceutical formulation.

KEY WORDS: estradiol valerate; norethisterone; HPLC

高效液相色谱法测定复方戊酸雌二醇片中戊酸雌二醇和炔诺酮的含量

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摘要:目的 建立用高效液相色谱法测定复方制剂中戊酸雌二醇和炔诺酮含量的方法。方法 用氰基键合硅胶为固定相,乙腈/水(47:53)为流动相,黄体酮为内标,UV检测波长为 280 nm。结果 该方法回收率为戊酸雌二醇 100.6 %, RSD = 0.9 %(n = 9),炔诺酮 100.2 %, RSD = 0.8 %(n = 9);测定了三批样品,结果满意。结论 该法不需经复杂的样品处理,简便、快速,准确可靠,可作为该复方制剂的含量测定方法。

关键词:戊酸雌二醇;炔诺酮;HPLC
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Exogenous estrogen therapy has being used for many years to prevent and treat the systemic involutional affection, such as coronary heart diseases and osteoporosis, which would occur in some of the postmenopausal women. It has been proved that this therapy plays an important part in improving the living quality of the menopausal women. As taking estrogen alone without progesterone for long term may increase the danger of suffering from the endometrial carcinoma for some women, progesterone has been added for the therapy to reduce the danger. The compound tablet dosage form containing estradiol valerate and norethiserone has recently been developed. However, there are no published methods yet for the determination of the two components simultaneously.

This report details the development of a reversed-phase HPLC method, which is internally standardized, for the determination of the two components in the pharmaceutical dosage form. The procedure has a high degree of simplicity, specificity, and accuracy, and is suitable for the assay of the formulation.

1 Experimental

1.1 Chemicals and reagents

Reference standards of estradiol valerate (EV) and norethisterone (NET) were Ch.P. quality. Progesterone (PG) was obtained from sigma (St. Louis, NO, USA), which was used as the internal standard. Acetonitrile was HPLC grade (Fisher Scientific Company). Water used in the study was redistilled. Samples were from a pharmaceutical company as the trial product, containing EV 2 mg and NET 0.7 mg each tablet.

1.2 Instrumentation

The HPLC analysis was performed by a Waters 510 (Waters Associates, Milford, Mass, USA) instrument, equipped with 484 UV-detector, and a N2000 Chromatogram Processor (Zhejiang University, Hangzhou, China). The column used was Zorbax CN (5μm), 250 mm × 4.6 mm I.D. (Du Pont

Company, USA).

1.3 Chromatographic conditions

Separation was performed at room temperature. Acetonitrile-water (47∶53) was used as the mobile phase. The flow rate was 1.0 mL/min. The UV detection wavelength was 280nm. The injection volume was 15μL.

1.4 Preparation of solutions

1.4.1 Internal standard solution

Progesterone (30 mg) was accurately weighed into a 100 mL volumetric flask and dissolved in 50 mL acetonitrile. Once dissolved, the resulting solution was diluted to volume with water.

1.4.2 Stock solution

The reference standards of estradiol valerate (40 mg) and norethisterone (14 mg) were accurately weighed into a 100-mL volumetric flask, dissolved and diluted as described above (1.4.1). 5 mL of the solution was transferred to a 25-mL volumetric flask. Internal standard solution (5 mL) was added to this flask, and diluted to volume with 50 % aqueous acetonitrile.

1.4.3 Sample solution

A tablet mass equivalent to 2 mg of EV and 0.7 mg of NET was accurately weighed into a 25-mL volumetric flask. 5 mL of acetonitrile was added. After mixing in an ultrasonic bath for 2 minutes, 5 mL of internal standard solution was added, and then diluted to volume with 33 % aqueous acetonitrile, and filtered through a 0.45-μm filter.

2 Results and discussion

2.1 Chromatography

A good chromatographic separation of the two components and the internal standard was achieved (Fig. 1A). Previous HPLC separations of the compound preparations containing EV and other progesterone s[1,2] were performed on the columns packed with the silane bonded stationary phase. We tried C_{18} column to separate NET and EV firstly in this work. It was found that the two components differed on the retention characteristics greatly, the relative retention (α) was 4.6, and the capacity factor (k') of NET was only 1 when the retention time of EV was at about 15 min, while with CN column the data of α was about 2, and the k' of NET was 2 when retention time of EV was about 10 min. This indicated that a suitable chromatographic separation was obtained by using CN column for this formulation within a reasonable analysis time. As the internal standard, progesterone appeared between NET and EV, the resolutions (R) were as follow: $R_{(NET,PG)} = 4.5$, $R_{(PG,EV)} = 7.3$.

2.2 UV detection wavelengh

The UV λmax of EV is at 280 nm with minor absorbance, while its content is two times more than NET; and the λmax of NET is at 240nm, which has reasonable absorbency at 280 nm either. In respect of these reasons, 280 nm was selected as the detective wavelength for the components detected simultaneously. Satisfactory result has been obtained.

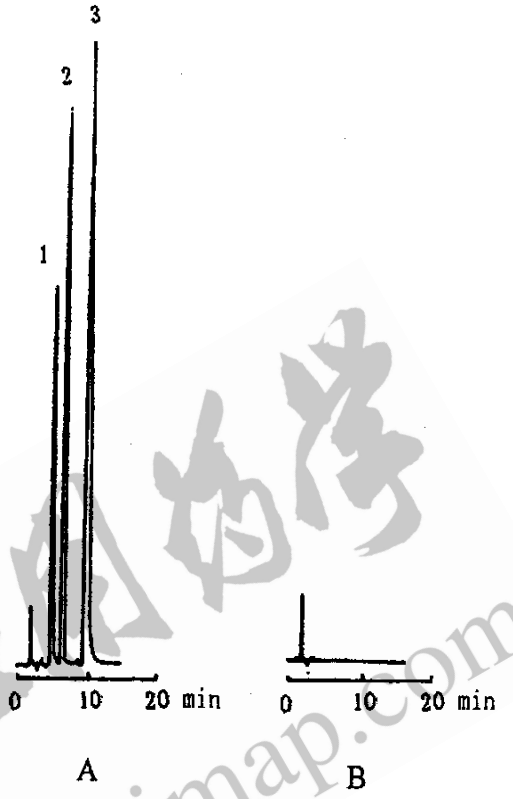

Fig 1 HPLC chromatograms. (A) tablet sample with the internal standard of PG and (B) extract of blank excipient

Peak numbers: 1. NET (5.0 min), 2. PG(6.5 min), 3. EV(9.9 min)

2.3 Linearity and recovery

Linearity of the assay for both NET and EV was determined with 5 data points over the ranges of EV 50 ~ 120μg·mL^{-1}, NET18 ~ 40μg·mL^{-1}. Calibration curves showed a linear correlation between peak area ratio (y) of NET or EV against the internal standard and the concentration of the drugs (x).

The linear calibration equation was $y = -0.006 + 22.60x$ ($r = 0.9997$) for EV and $y = 0.011 + 15.83x$ ($r = 0.9998$) for NET.

The accuracy of the assay method was also examined. Samples were prepared covering the range of 70 ~ 140 % of the theoretical dosage (EV/NET/each: 2 mg/0.7 mg) at three different levels as shown in Table 1. About 1.4 mg、2 mg、2.8 mg of EV were firstly weighed into 25 mL flask respectively, and then added the corresponding amount of NET solution. Afterwards, appropriate weights of excipients (about 50 mg) were mixed with the known amounts of NET and EV given above in the flasks. These samples were analyzed according to the described method. The results presented in Table 1 showed that excellent

recoveries were obtained for both of the components from the tablet matrix at all levels.

Tab 1 Recoveries of NET and EV from the tablet matrix

表 1 回收率试验结果

Added, mg		Found, mg		Recovery, %		Mean, %		RSD%	
NET	EV	NET	EV	NET	EV	NET	EV	NET	EV
0.559	1.40	0.563	1.42	100.7	101.4				
0.559	1.44	0.567	1.43	101.4	99.3	100.5	100.8	1.0	1.3
0.559	1.78	0.556	1.81	99.5	101.7				
0.742	2.09	0.737	2.09	99.3	100.0				
0.742	2.09	0.740	2.11	99.7	101.0	100.0	100.5	0.8	0.5
0.742	2.10	0.750	2.11	100.9	100.5				
0.925	2.79	0.918	2.82	99.2	101.1				
0.925	2.84	0.934	2.82	101.0	99.3	100.1	100.6	0.9	1.1
0.925	2.92	0.927	2.96	100.2	101.4				
Mean recovery for three concentrations (n = 9), %						100.2	100.6	0.8	0.9

2.4 Stability of analytical solution

10 HPLC injections were made within 8 hours to evaluate the stability of the solution. The RSD of the results of EV and NET were 0.98 % and 0.64 % respectively.

2.5 Specificity

Blank excipient was analyzed with the method. The chromatogram (Figure 1B) demonstrated that the HPLC assay results would not be affected by the presence of the excipient.

2.6 Sample assay

Considering the above results, the developed HPLC procedure afforded a basis for the rapid and accurate quantitative analysis of the dosage form. The assays from three representative production batches of tablets were proved to be valid (Table 2).

Tab 2 Analysis of three production batches of NET and EV (n = 3)

表 2 样品中戊酸雌二醇和炔诺酮含量测定结果(n = 3)

Batch	Content, %		RSD, %	
	EV	NET	EV	NET
A	100.2	98.6	1.2	1.3
B	104.7	99.9	0.7	0.9
C	103.4	100.8	1.6	0.4

As EV is not easily dissolved in this HPLC fluent, 5 mL of acetonitrile was added to the powder first to let the substance dissolved completely. Afterwards, adjusted the portion of acetonitrile to the same as the fluent.

参考文献

[1] Chinese Pharmacopoeia, 2000, Vol.2[S].2000: 503.

[2] Jiang LX, Wang ZJ, Matlin SA. HPLC analysis of injectable contraceptive preparation containing norethisterone enanthate and estradiol valerate[J]. J Liq Chromatogr, 1990, 17: 3473.

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