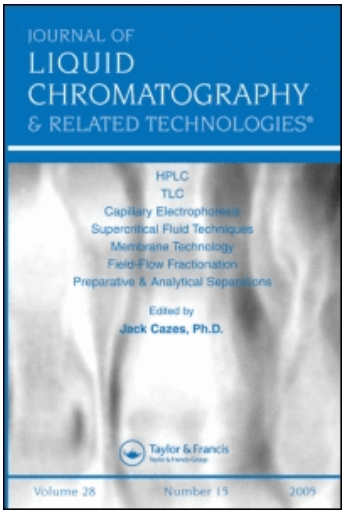

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HPLC ANALYSIS OF INJECTABLE CONTRACEPTIVE PREPARATION CONTAINING NORETHISTERONE ENANTHATE AND ESTRADIOL VALERATE

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ABSTRACT

A simple, accurate method for simultaneous determination of norethisterone enanthate (NET-EN) and estradiol valerate (EV) in the injectable is reported. An improved extraction procedure for steroid esters from the oily formulation was developed. Recoveries of 100.6 and 98.7% were obtained for NET-EN and EV.

INTRODUCTION

The once-monthly injectable, containing NET-EN (50 mg) and EV (5 mg) (Figure 1), is a widely used contraceptive for women. Side effects could be caused by excessive levels of the steroids or by the presence of some impurities. One batch (840601) of the injectable was suspected to have excessive amount of EV; it was therefore desirable to confirm this. There are neutralization [1]

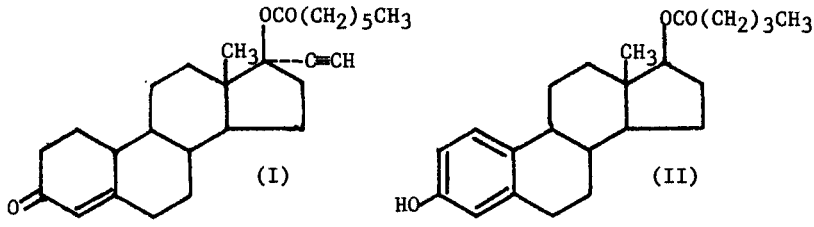

FIGURE 1. Structures of NET-EN (I) and EV (II).

and HPLC [2] methods for analysis of NET-EN alone in the composite injectable and, until now, there has been no report of a method for the quality control of EV in this injectable formulation.

Recently, we developed an HPLC method for simultaneous analysis of NET-EN and EV in the composite injectable. The method is rapid, simple, and has proven to be valid in terms of recovery, linearity and sample analysis.

EXPERIMENTAL

Materials

HPLC-grade methanol was obtained from Shanghai Wu-jin Chemical Plant, China. Redistilled water was freshly obtained from an automatic pure water distiller (Shanghai First Glassware Factory, China). The other reagents were AR-grade.

NET-EN and EV standards were obtained from Schering AG (West Berlin).

HPLC conditions

25uL of sample was injected into a 5um Zorbax-C_8 column (250 x 46mm I.D.) by a Shimadzu SIL-2AS autosampler, eluted with MeOH/H_2O (85/15) at 1.0 mL/min. Detection with a Shimadzu SPD-2AS UV monitor was at 222nm for both of the steroids. Column temperature was at about 15°C, detector sensitivity 0.32 AUFS.

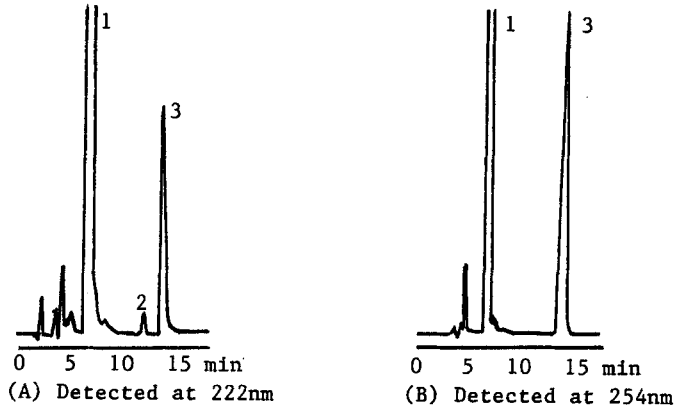

FIGURE 2. Typical chromatograms obtained from the sample at different wavelengths. Key: 1, benzyl benzoate; 2, EV; 3, NET-EN.

Preparation of standards

The mixed standards solution of NET-EN and EV used for content determination was prepared by accurately weighing 10mg of standard NET-EN into a 10mL volumetric flask and 1mg of standard EV into another 10mL flask, making them to volume with methanol, transfering 1.0mL from each flask to a third 10mL volumetric flask and making it to volume with methanol.

Assay procedure

Accurately weigh 20 mg of the injectable solution into a 10mL volumetric flask, fill with methanol. After mixing in an ultrasonic bath for 10 minutes, allow it to stand in refrigerator for about 4 hours to let the methanol solution clear. Take the supernatant solution for HPLC. Peak area is utilized for calculation.

RESULTS AND DISCUSSION

A good HPLC seperation was achieved for quantitative analysis (Figure 2A).

TABLE 1

Recoveries from Prepared Injectable Solution

Amount added (mg)		Amount found (mg)*		Recoveries (%)	
NET-EN	EV	NET-EN	EV	NET-EN	EV
1.049	0.1049	1.061	0.1030	101.2	98.2
1.253	0.1253	1.246	0.1253	99.4	100.0
1.194	0.1194	1.206	0.1190	101.0	99.7
1.054	0.1054	1.060	0.1020	100.6	96.8
Average				100.6	98.6
RSD%				0.801	1.497

* Based on three replicate determinations for each sample

TABLE 2

Linearities of NET-EN (n=5) and EV (n=6)

	Intercept	Slope	Correlation coefficient
NET-EN	2603	528802	0.9999
EV	139	574629	0.9994

TABLE 3

Analysis of Three Production Batches of NET-EN and EV

Batch No	Ampule No	Content* %				Average	RSD
		1	2	3	4	%	%
8303292	EV	100.62	97.70	96.88	100.74	98.99	2.01
	NET-EN	94.54	94.16	95.48	95.14	94.85	0.78
8508173	EV	101.64	99.40	100.84	99.34	100.31	1.12
	NET-EN	98.36	96.14	98.32	99.68	98.12	1.49
830327	EV	96.98	102.00	97.00	97.80	98.45	2.44
	NET-EN	93.64	94.30	93.90	94.62	94.12	0.46

* Based on three replicate determinations for each sample

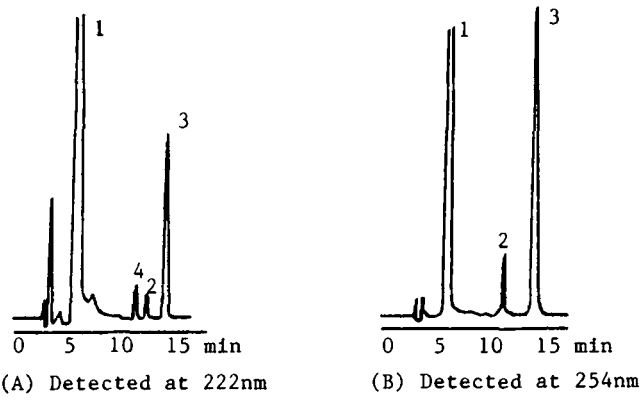

FIGURE 3. HPLC trace of the batch 840601. Key: 1, benzyl benzoate; 2, EV; 3, NET-EN; 4, unknown compound.

Previous HPLC detection [2] for the injectable was performed at 254nm. At that wavelength, EV could not be detected (Figure 2B). The analytical wavelength (222nm) we selected maximized the EV detector response and also was suitable for NET-EN (Figure 2A). The analysis of NET-EN and EV from samples was proven to be valid; the recoveries of both steroids were shown to be excellent. The linearity in the region of the working concentrations was also documented.

The recoveries from samples were shown by the prepared standard injectable (EV, NET-EN, the peanut oil and other ingradients: benzyl benzoate and benzyl alcohol as claimed) according to the assay procedure. An improved extraction procedure for steroids from the oily injectable was developed. It is more rapid, economical and simpler than extracting with ethanol and water several times[2] or use of a solid phase cartridge [3]. (Table 1).

The linearities in the region of the working concentration proved to be excellent too. Solutions, five for NET-EN and six for EV, varying in concentration from 50% to 150%, as claimed on the

label, were injected into the LC, three injections being made for each concentration (Table 2).

Considering the above results, the developed HPLC procedure afforded a basis for the rapid quantitative analysis of the injectable. The assays from three representative production batches (4 ampules for each batch) of injectables were proved to be valid (Table 3).

The batch that was suspected to contain an excessive amount of EV was also analyzed. The EV was 96% of the theoretical content and there was an unknown peak of impurity before the peak of EV (Figure 3A). When wavelength was changed from 222 to 254nm, which was the wavelength utilized in an earlier study [2], the peak of EV disappeared, and the impurity peak became larger, as did the the NET-EN peak (Figure 3B). Low temperature (less than 20°C) is very important for separating the impurity peak from the EV peak. Consequently, higher HPLC analytical results for EV were obtained in the summer, if temperature control was not used.

CONCLUSION

The validity of the described procedure has been proven for the samples. The assay method is simple, rapid and well suited for laboratories doing routine analysis of the injectable.

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REFERENCES

1. Analysis Department of the Institute of Medicine Research, Hangzhou, China, Testing standard for once-monthly injectable containing norethisterone enanthate and estradiol valerate (unpublished), 1983.

2. Liu, L., Wang, J., Jiang, L., Zhou, R., HPLC of norethisterone enanthate in the composite contraceptive injectable, Modern Applied Pharmacy, 3, 8, 1987.

3. Carignan, G., Lodge, B.A., Skakum, W., HPLC analysis of estradiol valerate and testosterone enanthate in oily formulations, J. Chromatogr., 301, 292, 1984.