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Note

High-performance liquid chromatographic analysis of estradiol valerate–testosterone enanthate in oily formulations

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Admixture of testosterone enanthate–estradiol valerate in oil is used in treatment of the menopausal syndrome in those patients not improved by estrogen alone and for the inhibition of lactation and relief of postpartum breast engorgement.

Extraction and analysis of steroid esters from vegetable oil has always presented considerable difficulty to the analyst. Isolation from oil has been reported using aqueous alcoholic or acetonitrile solutions[1–3], hexane–acetonitrile partition[4] and column partition chromatography[5,6]. All of these methods require a great deal of manipulation, leading to very long times of analysis.

EXPERIMENTAL

Apparatus

A fully automated high-performance liquid chromatography (HPLC) system (Model SP8100, Spectra-Physics, Santa Clara, CA, U.S.A.), operated at ambient temperature, consisting of solvent delivery system operated at 1.50 ml/min, an autosampler, an air-activated automatic injector equipped with a 20-μl loop, and a variable-wavelength UV detector (Model SP8400, Spectra-Physics) set at 210 nm (0.02 a.u.f.s.) was used. The column (250 × 4.6 mm I.D.) was octyl silane chemically bonded to totally-porous irregularly shaped silica particles (RP-8 on LiChrosorb 10 μm, Spectra-Physics).

Peak retention times and areas were obtained with a reporting integrator (Model SP4100, Spectra-Physics).

Reagents

Estradiol valerate and testosterone enanthate were U.S.P. reference standards.

Sesame oil (Fisher Scientific, Fairlawn, NJ, U.S.A.), acetonitrile (sample preparation) and carbon tetrachloride were reagent grade, acetonitrile (mobile phase) was HPLC grade (J. T. Baker, Phillipsburg, NJ, U.S.A.) and water was double-distilled in glass (house system).

Mobile phase

Acetonitrile and water [filtered through membranes (FH 0.2 μm and FA 0.45 μm, Millipore, Bedford, MA, U.S.A.) and degassed by sparging] were mixed by the solvent delivery system in a 70:30 ratio.

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Standard preparation

A mixed standard solution of estradiol valerate and testosterone enanthate was prepared in acetonitrile at the same concentration as in the sample preparation.

Sample preparation

The specific gravity of the sample was determined by weighing 10 ml in a tared volumetric flask at room temperature.

An accurately weighed amount of sample, equivalent to 1.0 ml, was transferred to a 100-ml volumetric flask. About 50 ml of carbon tetrachloride was added. The flask was swirled to dissolve the oil, then filled to volume with carbon tetrachloride, stoppered and mixed.

A 10-ml aliquot was passed through a silica cartridge (Sep-Pak, Waters Assoc., Milford, MA, U.S.A.) which was preconditioned by washing with 10 ml of carbon tetrachloride. The cartridge was washed with an additional 10 ml of carbon tetrachloride, with care being taken that the cartridge did not become dried out.

The active components were then eluted with acetonitrile, and the eluate was collected, and made up to volume through the cartridge, in a 10-ml volumetric flask, which was then stoppered and mixed.

Recovery study

A synthetic mixture was prepared by accurately weighing about 40 mg of estradiol valerate and about 900 mg of testosterone enanthate into a 10-ml volumetric flask. Sesame oil was then added to volume, and the contents of the flask were sonicated (Ultrasonic cleaner, Mettler Electronics, Anaheim, CA, U.S.A.) and mixed until all the solid materials had dissolved.

A volume of 1 ml of this synthetic mixture was treated as under *Sample preparation.*

Assay

Aliquots (20 μl) of the standard and sample preparations were successively injected into the chromatograph. Chromatograms were recorded and peak areas of estradiol valerate and testosterone enanthate were measured. The concentration of active ingredients in mg per ml were calculated using the following formula:

$$Cu = Cs \times \frac{Au}{As} \times \frac{Wsg}{Wu} \times 100$$

where Cu = concentration of active ingredient in mg/ml in the sample preparation; Cs = concentration of active ingredient in mg/ml in standard preparation; Au = peak area of active ingredient in sample preparation; As = peak area of active ingredient in standard preparation; Wsg = specific gravity of sample; Wu = weight of sample taken.

RESULTS AND DISCUSSIONS

The chromatogram (Fig. 1) was as expected with respect to the shape of the peaks (tailing factor[7] 1.20 and 1.5 for estradiol valerate and testosterone enanthate, respectively).

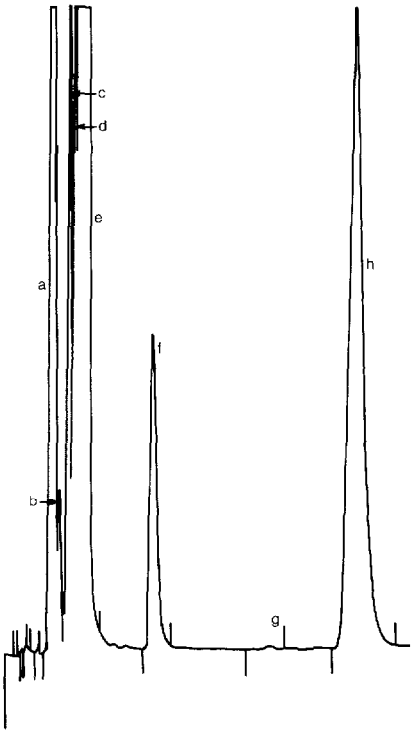

Fig. 1. Analysis of commercial formulation. Peaks: a = impurity in acetonitrile (reagent grade); b = chlorobutanol; c = sesamin; d = sesamolin; e = carbon tetrachloride; f = estradiol valerate; g = unknown; h = testosterone enanthate.

Complete baseline resolution was achieved between both the solvent front and estradiol valerate and between estradiol valerate and testosterone enanthate ($R \cdot 9.2$).

Table I shows retention times of active ingredients, extracting solvent, preservatives and components of sesame oil, as well as a potential internal standard decanophenone (Aldrich, Milwaukee, WI, U.S.A.).

TABLE I

RETENTION TIMES OF COMPOUNDS OF INTEREST

Name	*Retention time (min)*
Unknown in acetonitrile (reagent grade)	2.27
Benzyl alcohol	2.33
Chorobutanol	2.80
Sesamin*	3.23
Sesamolin*	3.48
Carbon tetrachloride	3.66
Estradiol valerate	7.70
Decanophenone**	11.65
Testosterone enanthate	18.28

* Components in sesame oil[2].

** Potential internal standard.

Response *versus* amount injected was found to be linear over the ranges 0.08–0.5 μg for estradiol valerate and 5–30 μg for testosterone enanthate. Within those ranges, standard curves passed close to the origin and the correlation coefficients were nearly ideal (Table II).

TABLE II

STANDARD CURVES

	Range (μg)	*Slope*	*Intercept*	*Corr. coeff.*
Estradiol valerate	0.08–0.5	304,000	1850	0.9995
Testosterone enanthate	5–30	14,296	−3090	0.9999

The silica cartridge extraction procedure was preferred to liquid–liquid extraction as it was faster (about 10 min compared to more than 1 h), requiring less manipulations. Overall time of analysis was therefore about 30 minutes. Accuracy and reproducibility were ascertained via a synthetic preparation, and recovery from this preparation (Table III) was found to be excellent.

TABLE III

RESULTS OF RECOVERY STUDY

Average of 5 determinations. R.S.D. = Relative standard deviation.

Compound	*Added (mg/ml)*	*Found (mg/ml)*	*Recovery (%)*	*R.S.D. (%)*
Estradiol valerate	4.011	3.958	98.68	0.39
Testosterone enanthate	91.722	91.905	100.20	0.37

Analyses of commercial formulations are presented in Table IV. All results are within regulatory limits (90–110%), with very good relative standard deviations (R.S.D.).

TABLE IV

RESULTS OF ANALYSIS OF COMMERCIAL FORMULATIONS

Average of 8 determinations.

	Estradiol valerate		*Testosterone enanthate*	
	% label	*R.S.D.*	*% label*	*R.S.D.*
Formulation No. 1	97.69	0.57	96.74	0.46
Formulation No. 2	92.94	0.64	98.90	0.78

CONCLUSION

This HPLC procedure for the analysis of estradiol valerate and testosterone enanthate formulated in oily solutions is fast, specific and accurate.

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