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Note

Comparative study of the applications of gas–liquid chromatography and high-performance liquid chromatography to the analysis of norethandrolone

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Norethandrolone (17α-ethyl-17β-hydroxyestr-4-en-3-one) is an anabolic agent with an anabolic–androgenic quotient of *ca.* 3 (ref. 1) and with anabolic activity by the oral route comparable to that of methyltestosterone.

The B.P. (1973) assay procedure[2] involves extraction of the drug from tablets with boiling methanol, followed by dilution and spectrophotometric determination at *ca.* 240 nm. Such a procedure does not take into account interference from other Δ^4-3-ketosteroids, unlike the NF XIII (1975) method[3], which contains a limit test (based on TLC) for foreign related steroids. The assay procedure in the NF XIII is based on the condensation of norethandrolone with isonicotinyl hydrazide in acidic solution. In the course of efforts in this laboratory to develop general methods for the analysis of anabolic steroids in pharmaceutical dosage forms, the application of gas–liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) to the analysis of norethandrolone has been studied.

EXPERIMENTAL

GLC conditions

A Bendix Model 2500 gas chromatograph equipped with a flame ionization detector and fitted with a 1.8 m × 0.63 cm O.D. glass U-shaped column was used. The column packing was 3% of OV-17 (phenyl-methyl silicone) coated on Chromosorb W-HP (100–120 mesh) by the funnel coating technique[4]. The injection port and detector block temperatures were both set at 270°. Hydrogen and air flow-rates were 30 and 500 ml, respectively. The column temperature was set at 260° ± 5°, and the nitrogen flow-rate at 40 ± 5 ml/min in order to obtain retention times of *ca.* 4.5 and 13.0 ± 0.5 min for the internal standard and norethandrolone, respectively. Peak areas and retention times were determined by using a Hewlett-Packard integrator (model 3385A Automation System.)

Assay procedure

Internal standard solution. A solution of diethylstilbestrol was prepared in aqueous 70% (v/v) methanol at a concentration of 1 mg/ml.

Standard preparation. A 1 mg/ml solution of norethandrolone (B.P., authentic specimen) was prepared in internal standard solution.

Sample preparation. Not less than 20 tablets were weighed and finely powdered. An amount of powder equivalent to one tablet (10 mg of norethandrolone) was accurately weighed into a 15-ml centrifuge tube equipped with a Teflon -lined cap, and 10 ml of internal standard solution were added. The tube was capped and immersed for 15 min in an ultrasonic bath (Mettler Model ME 4.6), then the tube was shaken for 15 min and centrifuged to obtain a clear solution.

Procedure. A 2.5-μl aliquot of standard preparation and of sample preparation were successively injected into the gas chromatograph. The area ratio of norethandrolone to the internal standard was calculated for both standard preparation and sample preparation, and the quantity of norethandrolone per tablet was calculated from the following formula:

$$C = 10 \times C_s \times \frac{Ru}{Rs} \times \frac{Wt}{Wu}$$

where C is the mg of norethandrolone per tablet, C_s is the concentration of norethandrolone in the standard preparation (mg/ml), Ru is the area ratio for the sample preparation, Rs is the area ratio for the standard preparation, Wu is the weight of sample taken, and Wt is the average weight per tablet.

HPLC conditions

A modular HPLC system consisting of a Laboratory Data Control Constametric II pump operating in the constant-flow mode, a Schoeffel model SF 700 variable-wavelength detector fixed at 240 nm, a Rheodyne septumless valve injector (Model 7120) equipped with a 20-μl loop, and a Brownlee Laboratory RP-18 (10 μm) column 25.0 cm × 4.6 mm I.D. were used. All solvents were of HPLC grade, except the water, which was twice distilled from glass. Solvent mixtures were freshly prepared and filtered through a 0.45-μm Millipore membrane (type HA) before use. Aqueous 70% (v/v) methanol was used as mobile phase (flow-rate 3 ml/min). The peak height was measured from a recorder (Hewlett-Packard model 7132A).

Assay procedure

Internal standard solution. A solution of biphenyl in aqueous 70% (v/v) methanol at a concentration of 0.68 mg/ml was used.

Standard preparation. A solution of norethandrolone (B.P., authentic specimen) was prepared in internal standard solution at a concentration of 1 mg/ml of norethandrolone.

Sample preparation. As described under GLC sample preparation above.

Procedure. A 20-μl aliquot of standard preparation and of sample preparation were successively injected into the chromatograph via the 20-μl loop injector. The peak-height ratio of norethandrolone to the internal standard was calculated for both standard preparation and sample preparation, and the quantity of norethandrolone per tablet was calculated using the formula described previously, but with peak heights in place of peak areas.

RESULTS AND DISCUSSION

The linearity of response to norethandrolone concentration was checked with

standard curves, and was found to be linear for each technique at the concentration levels described in the Experimental Section. The ranges of linearity were 0.5–3.0 μg injected (GLC) and 4.0–24.0 μg injected (HPLC). The correlation coefficients (r) obtained were 0.9996 (GLC) and 0.9998 (HPLC).

Norethandrolone was resolved from the potentially present related steroids norethindrone and 17α-vinyl-19-nortestosterone[5]. Although fully resolved by each technique greater separation was obtained using HPLC (see Table I).

TABLE I

RETENTION TIMES OF NORETHANDROLONE AND RELATED COMPOUNDS BY GLC AND HPLC

Compound	*Retention time in GLC (min)*	*Retention time in HPLC (min)*
Norethandrolone	12.9	8.0
Norethindrone	10.9	3.2
17α-Vinyl-19-nortestosterone	11.6	5.2
Diethylstilbestrol	4.4	—
Biphenyl	—	11.0

Both chromatographic procedures have a better reproducibility than the B.P. (1973) method[2]. The fact that results from the chromatographic procedures are slightly lower than those of the B.P. method can probably be attributed to the greater specificity of the chromatographic methods. However, differences between the three methods (Table II) are not significant. No related steroids could be found in any of the commercial lots studied by either GLC or HPLC. Both techniques are fast and specific, and should easily be adopted for single-tablet analysis, since each possesses adequate sensitivity.

TABLE II

QUANTITATIVE ANALYSIS OF NORETHANDROLONE TABLETS

Method	*Potency* (%)*	*Standard deviation***
GLC	97.1	±1.4
HPLC	98.1	±1.3
B.P. 1973	98.8	±2.0

* Percentage of label claim.
** From 10 determinations.

CONCLUSION

Both GLC and HPLC may be used for the analysis of norethandrolone tablets, since good correlation is obtained between the two techniques, and also with a less specific spectrophotometric method.

REFERENCES

1 J. A. Vida, *Androgens and Anabolic Agents*, Academic Press, New York, 1969.
2 *British Pharmacopoeia*, Pharmaceutical Press, London, 1973, p. 323.
3 *The National Formulary XIII*, Mack Publishing Co., Easton, Pa., 1970, p. 487.
4 H. M. McNair and E. J. Bonelli, *Basic Gas Chromatography*, Varian Aerograph, Walnut Creek, Calif., 1969, p. 65.
5 F. B. Colton, L. N. Nysted, B. Riegel and A. L. Raymond, *J. Amer. Chem. Soc.*, 79 (1954) 112.