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Note

Analysis of testolactone and its formulations by high-performance liquid chromatography

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Testolactone (D-homo-17a-oxaandrosta-1,4-diene-3,17-dione) is used in the adjunctive and palliative treatment of inoperable breast cancer in women[1]. Although structurally related to the androgens, it is essentially devoid of androgenic activity in therapeutic doses. It is obtained from a number of steroidal compounds (testosterone, progesterone, 11-deoxycortisol) by microbial transformation using, for example, *Cylindrocarpon radicicola* ATCC 11011 (ref. 2). Such starting materials are therefore potential impurities.

The compendial method[3] for testolactone raw material, suspension and tablets is colorimetric, based on condensation with isoniazid. It is, therefore, non-specific, since it would be interfered with by the above mentioned starting materials if they were present as impurities. To overcome this problem partially, The National Formulary[3] (NF) requires in the raw material monograph a test for foreign steroids and other impurities, which is based on thin-layer chromatography (TLC). There is no such requirement for the suspension or the tablets, and consequently a laboratory performing the NF analysis of testolactone in formulations would be unable to detect any impurities which might be present. To alleviate this problem, a high-performance liquid chromatographic (HPLC) procedure has been developed which allows the assay of the drug and the detection of starting materials.

EXPERIMENTAL

Apparatus

A modular HPLC system was used, consisting of a pump (Constametric II, Laboratory Data Control, Riviera Beach, FL, U.S.A.) operated at 1.75 ml/min, a variable-wavelength UV detector (Schoeffel Model SF 700, Westwood, NJ, U.S.A.) set at 240 nm and a loop injector (Rheodyne septumless valve injector Model 7120, Berkeley, CA, U.S.A.) equipped with a 10-μl loop. The column was a 250 × 4.6 mm I.D. commercially available octyl silane, chemically bonded to totally porous irregularly shaped 10 μm micro-silica particles (RP-8, Brownlee Labs., Santa Clara, CA U.S.A.).

Peak retention times and areas were obtained by the use of a reporting integrator (Automation System 3385A, Hewlett-Packard, Avondale, PA, U.S.A.).

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Reagents

Testolactone was NF reference standard. Propylparaben (Aldrich, Milwaukee, WI, U.S.A.) was recrystallized commercial grade. Methanol was HPLC grade (Fisher, Fair Lawn, NJ, U.S.A.). Water was double-distilled in glass.

Mobile phase

Methanol in water (55:45); previously filtered through a membrane (HA 0.45 μm, Millipore, Bedford, MA, U.S.A.) and degassed was used.

Internal standard solution

A solution of propylparaben in aqueous methanol (60% v/v) was prepared at a concentration of 1 mg/ml.

Standard preparation

About 20.0 mg of testolactone reference standard, accurately weighed, was transferred to a 100-ml volumetric flask and dissolved in 50 ml of methanol. Internal standard solution (20.0 ml) was added. While mixing, water was added to volume.

Sample preparation

Tablets. A portion, accurately weighed, of finely powdered tablets, equivalent to about 50 mg of testolactone was transferred to a 50-ml volumetric flask, to which was added 30 ml of methanol. The flask was stoppered and was vigorously shaken for 30 min. Water was added to volume and the contents were well mixed and filtered. A 20.0-ml portion of the filtrate was pipetted and transferred to a 100-ml volumetric flask. Internal standard solution (20.0 ml) was added and mobile phase was added to volume and the contents were mixed.

Suspension. An accurately measured volume of well mixed testolactone suspension, equivalent to 100 mg, was transferred to a 100-ml volumetric flask, to which was added 60 ml of methanol. The flask was made up to volume with water, and the contents were well mixed and filtered (if necessary). A 20.0-ml portion of the clear solution was treated as above (*Tablets*).

Procedure

A 10-μl portion of the standard preparation and the sample preparation was successively injected into the column via the 10-μl loop injector.

The ratio (R) of the area of testolactone to the area of internal standard was recorded for the standard preparation and sample preparation. The concentration of testolactone in sample preparation was obtained by the following formula:

$$C_u = C_s \cdot R_u / R_s$$

where C_u is concentration of testolactone in sample preparation, C_s is concentration of testolactone in standard preparation (mg/ml), R_u is ratio of areas for sample preparation and R_s is ratio of areas for standard preparation.

RESULTS AND DISCUSSION

Baseline resolution was achieved between solvent front, benzyl alcohol, testolactone internal standard and with the three potential synthesis residues, namely testosterone, progesterone and 11-deoxycortisol[2] (Table I). The limit of detectability of each impurity was found to be 0.1 % w/w of testolactone. No impurity was detected from either the tablets or the suspension. Sample preparations were relatively stable, and no detectable degradation products appeared within 48 h.

TABLE I

CAPACITY FACTORS OF TESTOLACTONE AND RELATED COMPOUNDS

Substance	$k'(t-t_0/t_0)$
Testolactone	1.89
Testosterone	14.35
Progesterone	32.27
11-Deoxycortisol	6.12
Propylparaben	4.78
Benzylalcohol*	1.05
Degradation product	3.69
Androsta-1,4-diene-3,17-dione**	5.35
Androsta-1,4-diene-17-B-ol-3-one**	8.51

* Benzylalcohol was included because it is present in the suspensions.

** These two compounds are included because they are potential impurities.

Linear response *versus* concentration was found up to a concentration of 3 μg injected. Within this range, the standard curve passed close to the origin and its correlation coefficient was nearly ideal (0.99996).

Quantitative analysis of two commercial formulations are compared in Table II to the NF colorimetric procedure. All results are within compendial limits (90–110% for tablets and 90–120% for suspensions). Coefficients of variation are excellent. The difference of about 2.0% between results is most likely explained by the inherent differences between the two techniques.

TABLE II

COMPARISON OF HPLC TO NF ASSAY PROCEDURE

Values are the average of five determinations.

Formulation	*HPLC (%)*	*NF (%)*
Testolactone tablet	99.0 ± 0.6	100.4 ± 0.6
Testolactone suspension	108.1 ± 1.1	109.9 ± 1.9

CONCLUSION

This HPLC procedure for the analysis of testolactone and its formulations is fast, specific and accurate.

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