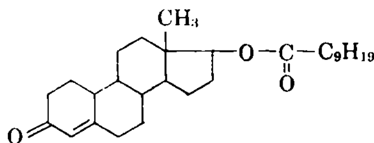
Qualitative and Quantitative Tests for Nandrolone Decanoate

Provisional, unofficial monographs are developed by the Drug Standards Laboratory, in cooperation with the manufacturers of the drugs concerned, for publication in the *Journal of Pharmaceutical Sciences*. The ready availability of this information affords discriminating medical and pharmaceutical practitioners with an added basis for confidence in the quality of new drug products generally, and of those covered by the monographs particularly. Such monographs will appear on drugs representing new chemical entities for which suitable identity tests and assay procedures are not available in the published literature. The purity and assay limits reported for the drugs and their dosage forms are based on observations made on samples representative of commercial production and are considered to be reasonable within expected analytical and manufacturing variation. *Drug Standards Laboratory*

19-Nor-Δ^4-androstene-17β-ol-3-one decanoate; $C_{28}H_{44}O_3$; M.W. 428.66. The structural formula of nandrolone decanoate may be represented as follows:



Physical Properties.—Nandrolone decanoate occurs as a white to creamy white crystalline powder which is odorless or may have a slight odor, m.p. 33–37° (U.S.P. XVI, Class Ia). It is very soluble in acetone, in alcohol, in dioxane, and in methanol, and is practically insoluble in water.

Identity Tests.—A 1:100,000 solution of nandrolone decanoate in alcohol exhibits an ultraviolet absorbance maximum at about 239 mμ [absorptivity (1%, 1 cm.) about 400]. The ultraviolet absorption spectrum is shown in Fig. 1.

The infrared spectrum of a 0.5% dispersion of nandrolone decanoate in potassium bromide in a disk of about 0.82-mm. thickness is shown in Fig. 2.

Mark a 4 × 19-in. strip of filter paper (Whatman No. 1 or the equivalent) with a fold line 3 in. from one end. Mark the starting line 2$^1/_4$ in. below the fold line. Impregnate the strip with immobile solvent consisting of a 2:1:3 mixture of propylene glycol, 2-phenoxyethanol, and methanol. Blot the strip with dry filter paper and hang it in a hood for a few minutes to dry. Prepare 1% solutions of nandrolone decanoate (*Test preparation*) and nandrolone decanoate reference standard (*Standard preparation*) in methanol and spot the paper at the starting line as follows: Apply 5.0 μl. of the *Test preparation* (50 mcg.) to one spot, 5.0 μl. of the *Standard preparation* (50 mcg.) to a second spot, and 2.5 μl. each of the *Test preparation* and of the *Standard preparation* to a third spot. Place the strip in a tank arranged for descending chromatography, add an excess of n-heptane to the bottom of the tank, and equilibrate the system for 1 hour. Add the mobile solvent, consisting of a 2:1:200 mixture of propylene glycol, 2-phenoxyethanol, and n-heptane, seal the tank, and allow the solvent front to pass to the bottom of the strip. Remove the strip from the tank, and hang it in a hood for a few minutes to air-dry. Heat the strip in an oven at 100° for 30 minutes, allow it to cool to room temperature, and pass it through a 0.25% solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid. Alternatively, spray the strip with the reagent by means of a suitable atomizer Red spots appear slowly at room temperature. The spot obtained with the *Test preparation* agrees in color and R_f value with that obtained with the *Standard preparation*. The chromatogram obtained with the mixed spot is monodisperse.

Purity Tests.—Dry about 250 mg. of nandrolone decanoate, accurately weighed, in a vacuum desiccator over phosphorus pentoxide for 4 hours. The loss in weight does not exceed 0.5%.

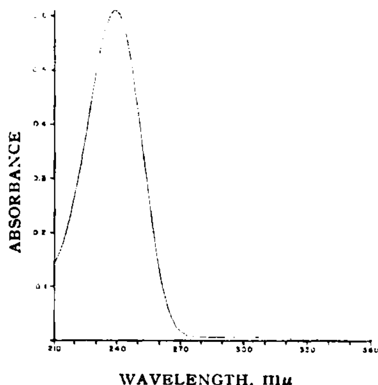


Fig. 1.—Ultraviolet absorption spectrum of nandrolone decanoate in alcohol (15 mcg. per ml.); Beckman model DK-2A spectrophotometer.

Received August 27, 1963, from the Drug Standards Laboratory, American Pharmaceutical Association Foundation, Washington, D.C.

Accepted for publication October 14, 1963.

Organon, Inc., has cooperated by furnishing samples and data to aid in the development and preparation of this monograph.

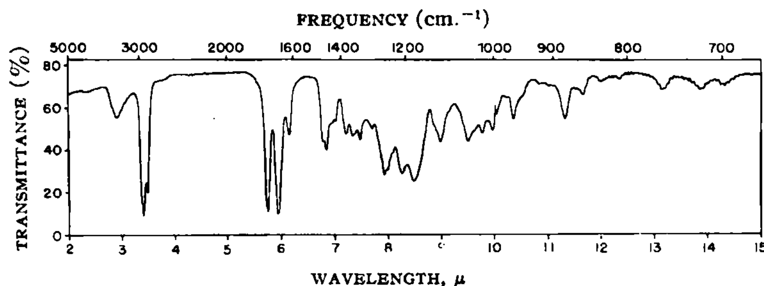


Fig 2.—Infrared spectrum of nandrolone decanoate in potassium bromide disk (0.5%); Perkin-Elmer model 21 spectrophotometer, sodium chloride prism.

Determine the optical rotation of a 1% solution of nandrolone decanoate in dioxane by the method of U.S.P. XVI. The specific rotation is between +32 and +36°.

Prepare a 2% solution of nandrolone decanoate in dioxane: the solution is clear and is free of mechanical impurities.

Assay

Assay Preparation.—Transfer about 25 mg. of nandrolone decanoate, accurately weighed, to a 100-ml. volumetric flask, dilute to volume with methanol, and mix. Transfer 10.0 ml. of this solution to a 100-ml. volumetric flask, dilute to volume with methanol, and mix.

Isoniazid Reagent.—Transfer 500 mg. of isoniazid to a 500-ml. volumetric flask, dissolve it in methanol, add 0.625 ml. of hydrochloric acid, dilute to volume with methanol, and mix.

Procedure.—Pipet 5 ml. of the *Assay Preparation* into a 10-ml. volumetric flask, add isoniazid reagent to volume, and mix. Allow to stand for 1 hour with occasional shaking. Determine the absorbance in a 1-cm. cell at 380 mμ with a suitable spectrophotometer *versus* a blank prepared by diluting isoniazid reagent with an equal volume of methanol. Calculate the quantity, in mg., of $C_{28}H_{44}O_3$ in the portion of nandrolone decanoate taken by the formula $W \times A_u/A_s$, where A_u is the absorbance of the solution, A_s is the absorbance of a similarly prepared solution of nandrolone decanoate reference standard, and W is the weight of nandrolone decanoate reference standard taken. The amount of nandrolone decanoate found, on the anhydrous basis, is not less than 96.0% and not more than 104.0% of $C_{28}H_{44}O_3$.

DOSAGE FORMS OF NANDROLONE DECANOATE

Nandrolone Decanoate Injection

A sterile solution of nandrolone decanoate in vegetable oil.

Identity Test.—Prepare a 2 × 19-in. strip of filter paper as in the assay for nandrolone decanoate injection. Apply 10 μl. each of the *Assay preparation* and of the *Standard preparation* to the starting line, $^3/_4$ in. apart, and develop the chromatogram in the same manner as in the assay. After drying at 100° for 30 minutes, allow the strip to cool to room temperature, and pass it through a 0.25% solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid. Alternatively, spray the strip with the reagent by means of a suitable atomizer. Red spots appear slowly at room temperature. The spot obtained with the *Assay preparation* agrees in color and R_f value with that obtained with the *Standard preparation*. No secondary spots appear.

Assay.—*Standard preparation.*—Dissolve about 50 mg. of nandrolone decanoate reference standard, accurately weighed, in sufficient acetone to make 10.0 ml., and mix.

Assay preparation.—By means of a pipet calibrated "to contain," transfer a volume of nandrolone decanoate injection, equivalent to about 50 mg. of nandrolone decanoate, to a 10-ml. volumetric flask, rinse the pipet with small portions of acetone, dilute to volume with acetone, and mix.

Isoniazid reagent.—Transfer 500 mg. of isoniazid to a 1000-ml. volumetric flask, dissolve it in methanol, add 0.625 ml. of hydrochloric acid, dilute to volume with methanol, and mix.

Procedure.—Prepare six $1^1/_2$ × 19-in. strips of filter paper (Whatman No. 1 or the equivalent) as follows. Mark the fold line 3 in. from one end, and the starting line $2^1/_4$ in. below the fold line. Wash the strips twice with methanol and hang them in a hood to air-dry. Impregnate the strips with immobile solvent, consisting of a 1:1:2 mixture of propylene glycol, 2-phenoxyethanol, and methanol. Blot the strips with dry filter paper, and hang them in a hood for a few minutes to dry.

By means of micropipets, spot three of the strips at the starting line with 10.0 μl. of the *Standard preparation* and the other three with 10.0 μl. of the *Assay preparation*. Place the strips in a tank arranged for descending chromatography, add an excess of n-heptane to the bottom of the tank, and equilibrate the system for 1 hour. Add the mobile solvent, consisting of a 1:1:200 mixture of propylene glycol, 2-phenoxyethanol, and n-heptane, seal the tank, and allow the chromatogram to develop until the solvent front reaches the end of the paper (2–3 hours). Remove the strips from the tank, hang them in a hood for 15 minutes to air-dry, and heat them in an oven at 100° for 30 minutes. By means of a short wavelength ultraviolet lamp (maximum emission at about 254 mμ), locate the positions of the spots, which appear dark purple against a light purple background. Outline the spots lightly with pencil, and cut the strips transversely at a distance of 3 cm. above and below the center of the spot. For the blank determinations, cut a second 6-cm. segment from the portion of each strip which lies immediately below the excised segment. Roll the twelve 6-cm. segments and place each in an 18 × 150-mm. test tube. To each tube add 4.0 ml. of isoniazid reagent, thoroughly wetting the paper. Close the tubes and allow them to stand for 1 hour at room temperature with occasional agitation. Decant the contents of each test tube into separate 15-ml. centrifuge tubes, and centrifuge at 5000

r.p.m. to remove any filter paper fibers. Decant the clear solutions into 1-cm. cells and determine their absorbances in a suitable spectrophotometer at 380 mμ *versus* the isoniazid reagent. Correct the absorbances for their respective blanks, and average the three absorbances obtained with the *Standard preparation* and the three obtained with the *Assay preparation*. Record the average absorbance obtained with the *Standard preparation* as A_s and that obtained with the *Assay preparation* as A_u and calculate the quantity, in milligrams, of $C_{28}H_{44}O_3$ in the volume of the sample taken by the formula $W \times A_u/A_s$, in which W represents the quantity, in milligrams, of nandrolone decanoate reference standard taken. The amount of nandrolone decanoate is not less than 90.0% and not more than 115.0% of the labeled amount of $C_{28}H_{44}O_3$.

DISCUSSION

U.S.P. and N.F. terminology for solubility, melting range, reagents, etc., have been used wherever feasible.

Nandrolone decanoate[1] is a long-acting anabolic agent which is chemically related to the androgenic steroids. Nandrolone differs from testosterone in the absence of a methyl group in the 19 position of the molecule.

Identity Tests.—Due to its possession of physical and chemical properties which are closely related to those of similar steroidal compounds, a specific paper chromatographic identity test is included in the monograph for the bulk material. A direct comparison of the R_f value (about 0.4 in the system used) with that of authentic material serves to aid in the identification of the compound. The 2,4-dinitrophenylhydrazine reagent which is used to locate the spots on the developed chromatogram further aids in identification by establishing the compound as a member of the ketosteroid class.

Quantitative Methods.—The quantitative methods included in the monograph were chosen from many which have been applied to this class of compounds. Due to the presence of an α,β-unsaturated carbonyl group in ring A of the steroid molecule, nandrolone decanoate may be assayed spectrophotometrically (1, 2), gravimetrically as the semicarbazone (3) or as the 2,4-dinitrophenylhydrazone (4), and colorimetrically following its reaction with isoniazid (5) or other reagents (6).

[1] Marketed as Deca Durabolin by Organon, Inc., West Orange, N.J.

The colorimetric determination of nandrolone decanoate with isoniazid (isonicotinic acid hydrazide) is suitably accurate and precise as an assay for the bulk material. Beer's law is obeyed for final solutions covering an approximate concentration range of 5–40 mcg. per ml. Hence, the reaction also possesses the sensitivity required of an assay method to be used in conjunction with paper chromatography.

The quantitative paper chromatographic assay for nandrolone decanoate in the dosage form is highly specific for the intact molecule. Washing of the paper with methanol prior to impregnation with the immobile phase removes traces of material which could later contribute to the absorbance measurements. Variations in blank absorbances were noted when separate paper strips were used for the blank determinations. These variations were reduced considerably by applying a blank correction obtained with a segment of each paper used in the assay. While this may not be an acceptable practice in many instances, no interfering absorbance due to artifacts was found in the segments used, and a general increase in precision was obtained. Where interference, due to decomposition products or other extraneous material in the dosage form exists, this method of blank determination should not be used. A discussion of the factors involved in this type of assay may be found in a recent publication by Roberts and Florey (7).

Analysis of a commercial preparation of nandrolone decanoate injection gave an average value of 97.4 ± 2.4%[2] of the labeled amount. A synthetic sample (similar in composition to the commercial product) containing 50 mg. per ml. of nandrolone decanoate plus 10% benzyl alcohol in sesame oil was prepared and assayed. An average recovery of 98.9 ± 3.2%[2] of the theoretical amount of nandrolone decanoate was obtained. Recoveries and precisions agree with published figures for a similar assay method (7).

REFERENCES

(1) Carol, J., *J. Assoc. Offic. Agr. Chemists*, **34**, 572(1951).
(2) Dorfman, L., *Chem. Revs.*, **53**, 47(1953).
(3) Madigan, J. J., Zeno, E. E., and Pheasant, R., *Anal. Chem.*, **23**, 1691(1951).
(4) Johnston, C. D., *Science*, **106**, 91(1947).
(5) Umberger, E. J., *Anal. Chem.*, **27**, 768(1955).
(6) Forist, A. A., and Johnson, J. L., in Higuchi, T., and Brochmann-Hanssen, E., "Pharmaceutical Analysis," Interscience Publishers, New York, N. Y., 1961, pp. 69–136.
(7) Roberts, H. R., and Florey, K., This Journal, **51**, 794 (1962).

[2] Maximum deviation from the mean value.