Table III—Purity of Metaraminol Bitartrate Raw Materials by HPLC and USP Methods

Sample[a]	Initial Concentration of Metaraminol, % w/v	Observed Results, % w/v		Purity of Metaraminol Bitartrate Raw Material, %	
		HPLC[b]	USP	HPLC	USP[c]
Raw material 1	1.065	1.037 ± 0.010	1.038	97.4	97.5
Raw material 2	1.019	0.994 ± 0.010	1.014	97.5	99.5
Raw material 3	1.001	0.980 ± 0.009	0.980	98.0	98.0

[a] The same solution of each raw material was used for both methods. [b] Result is based on two replicate injections. The value given is the mean ± *SD*. [c] The purity of each raw material also was determined by the USP XX procedure for metaraminol bitartrate raw material. The results for the three raw materials were 100.2, 100.5, and 99.6%, respectively.

an octadecylsilane column[8]. The degassed mobile phase [methanol–water (60:40) with 0.005 *M* dioctyl sodium sulfosuccinate and 10 ml of acetic acid/liter] was pumped through the column at a flow rate of 1.0 ml/min at ambient temperature until a stable baseline was obtained. Replicate 20-μl injections of the sample and standard solutions were made. The peaks were detected at 254 nm.

RESULTS AND DISCUSSION

Studies on metaraminol bitartrate preserved with methylparaben and propylparaben showed that these three drug components can be separated by HPLC and determined by UV absorption at 254 nm within 15 min. When injected into the chromatograph, methylparaben was eluted first, followed by propylparaben, butylparaben (the internal standard), and metaraminol at retention times of ~4.5, 6.3, 8.2, and 11.4 min, respectively (Fig. 1).

Standard solutions of metaraminol bitartrate, methylparaben, and propylparaben were chromatographed using the reversed-phase C_{18} column. The concentration of each component was determined by a programmable integrator[9]. A linear regression analysis of the data for six concentrations of metaraminol bitartrate, methylparaben, and propylparaben is shown in Table I. The data obtained show that metaraminol bitartrate, methylparaben, and propylparaben are linear to at least 25, 3.8, and 0.53 μg, respectively. A separate solution was run to determine the recovery and precision of the HPLC method. These data are shown in Table II.

The purity of three metaraminol bitartrate raw materials was determined by the HPLC method and the USP XX procedure as shown in Table III. The utility of HPLC in the analysis of metaraminol bitartrate is clearly demonstrated, and the results agree favorably with those obtained by the USP method.

[8] μBondapak C_{18} (<10 μm), 30-cm × 4-mm i.d. column, Waters Associates, Milford, Mass.

[9] Spectra-Physics System I.

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High-Pressure Liquid Chromatographic Assay of Quinestrol Tablets

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Received April 30, 1980, from the *Analytical Research Laboratories, Pharmaceutical Research Division, Warner-Lambert Company, Morris Plains, NJ 07950.* Accepted for publication June 19, 1980.

Abstract □ A specific assay for quinestrol was developed using high-pressure liquid chromatography. The estrogen was separated from tablet excipients on a chemically bonded hydrocarbon column utilizing acetonitrile–water as the mobile phase. Linearity studies were carried out using peak height measurements, and the detector response to the concentration of the steroid was confirmed. This procedure was rapid, accurate, precise, and specific for the assay of the synthetic estrogen in the presence of formulation excipients and structurally similar estrogens.

Keyphrases □ Quinestrol—high-pressure liquid chromatographic analysis, tablets □ High-pressure liquid chromatography—analysis, quinestrol tablets

The pink chromophore resulting from the reaction of quinestrol with methanolic sulfuric acid was shown to afford an accurate, precise, and selective colorimetric assay for quinestrol in pharmaceutical dosage forms (1). This method correlated with the GLC assay (2), which proved useful as a reference method but had disadvantages in speed and convenience. High-pressure liquid chromatography (HPLC) affords a simple alternative with advantages in speed, interlaboratory precision, and productivity.

Roos (3) described an HPLC method for ethinyl estradiol, estrone, estradiol, and estradiol esters as their dansyl

Table I—Peak Height *versus* Concentration of Quinestrol

Concentration, μg/ml	Peak Height, mm
6.8	26
13.6	52
20.4	80
34.0	130

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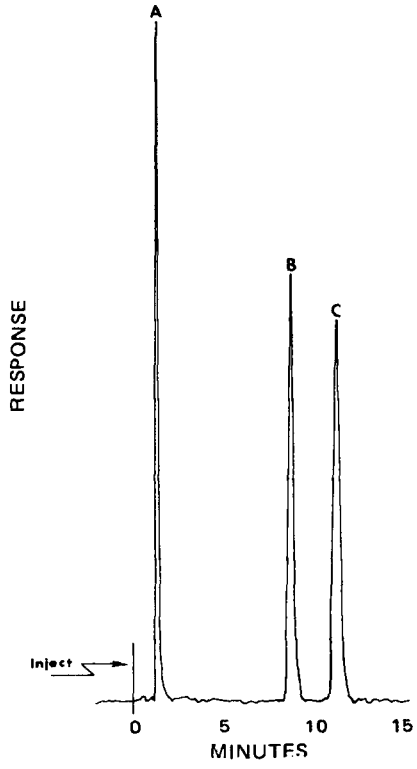

Figure 1—*Liquid chromatogram of quinestrol and related estrogens. Key: A, ethinyl estradiol and estrone; B, quinestrol; and C, estrone 3-cyclopentyl ether.*

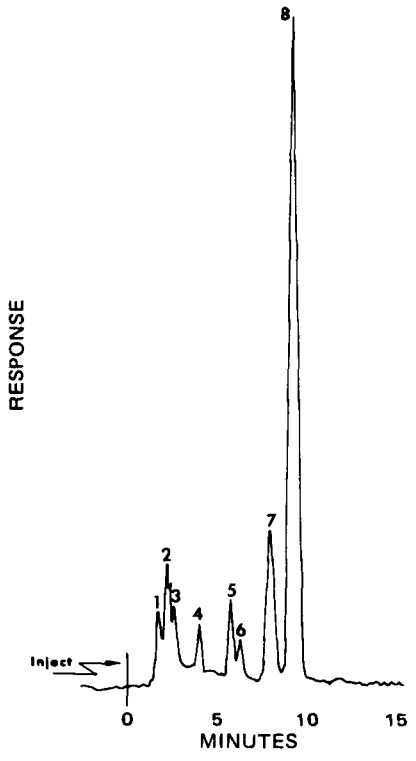

Figure 2—*Liquid chromatogram of decomposed quinestrol. Key: 1–7, unidentified peaks; and 8, quinestrol.*

derivatives, using UV detection at 254 nm or fluorescence detection. The HPLC method reported here uses detection of the estrogen at 281 nm without derivatization. Adequate sensitivity and excellent precision and recovery were obtained.

EXPERIMENTAL

Apparatus—The liquid chromatograph included a pump[1], an automatic injector[2] with a fixed-volume 50-μl loop, a stainless steel 4.6-mm × 25-cm column packed with bonded octadecylsilane on silica[3] (5–6-μm particles), a variable-wavelength detector[4] set at 281 nm, and a strip-chart recorder[5].

Mobile Phase—UV grade acetonitrile[6] was passed through a membrane[7]. Distilled water was passed through a membrane filter[8], and the filtered acetonitrile and water were mixed (80:20) and degassed.

Standard Preparation—About 10 mg of quinestrol USP reference standard was weighed accurately, transferred to a 50-ml volumetric flask, and dissolved in acetonitrile. The solution was diluted to volume, and 5.0 ml of this solution was diluted to 50 ml with the mobile phase in a volumetric flask to obtain a concentration of ~20 μg/ml.

Assay Preparation—Not less than 20 tablets were weighed and pulverized. A portion of the powder equivalent to ~100 μg of quinestrol was weighed, transferred to a 5-ml volumetric flask, suspended in 1 ml of water, and heated at steam bath temperature for ~5 min. The solution was diluted to volume with acetonitrile and mixed on a vibrating device[9] for ~2 min.

Procedure—The mobile phase was pumped through the column at a flow rate of 2 ml/min until a stable baseline was obtained. Fifty microliters of the assay preparation was injected by means of a precise loop injector, and the chromatogram was allowed to develop for 15 min. The procedure was repeated using the standard preparation. The peak corresponding to quinestrol eluted at ~8.5 min. The quantity of quinestrol was calculated from $5C(H_U/H_S)$, where C is the exact concentration (micrograms per milliliter) of the standard preparation and H_U and H_S are the peak heights in the chromatograms from the assay preparation and the standard preparation, respectively.

System Suitability—The relative standard deviation of the peak heights obtained after six successive injections of the standard preparation should not exceed ±1.0%. The number of theoretical plates should be no less than 5000, and the retention times for quinestrol and ethinyl estradiol should be ~8.5 and 2.0 min, respectively.

RESULTS AND DISCUSSION

Linearity—A straight line intercepting the origin was obtained by plotting the peak height against concentration (Table I).

Sensitivity—The quinestrol peak was ~0.35 full scale with a recorder sensitivity of 0.02 aufs and was adequate for quinestrol dosage forms. Sensitivity could be quadrupled using detection at 225 nm; however, at low wavelengths, detector sensitivity appeared to be more dependent on the light source age and detector cell cleanliness than at the wavelength chosen.

Recovery—The recovery of quinestrol was determined by adding known amounts of the estrogen to placebo tablets and assaying by the described procedure. Duplicate trials at levels representing 80, 100, and 120% of the claim for 100-μg tablets gave recoveries of 100.9, 101.8, and 99.8%, respectively. Six duplicate recovery experiments at 100.0% of the label claim by the same procedure gave recoveries of 98.8, 101.2, 100.0, 102.5, 102.5, and 99.4% of the added amounts, with an average of 100.7% and a relative standard deviation of ±1.6%.

Criteria for System Suitability—It is useful to establish criteria for system suitability on HPLC methods since the performance of any particular instrumental assembly may change over time and several different instruments may be employed. Excellent precision has been obtained in these laboratories using a loop injector, obviating the need to select an internal standard that could interfere with detection and estimation of a synthesis precursor, process contaminant, or degradation product. The actual precision of six replicate injections of the standard preparation was ±0.57%. The number of theoretical plates calculated for the column was 7530; this column gave satisfactory separations for the drug and related compounds. The requirement of not less than 5000 theoretical plates for system suitability was derived from a policy of setting this value at 70% of that obtained during method development.

Separation of Quinestrol from Other Estrogens—A chromatogram was run under the described conditions using 25 μg/ml of ethinyl estradiol, estrone, estrone 3-cyclopentyl ether, and quinestrol in the mobile

[1] Waters Associates model 6000A.
[2] Perkin-Elmer LC-420.
[3] Zorbax ODS, DuPont.
[4] Perkin-Elmer LC-55.
[5] Honeywell Elektronik 196.
[6] Burdick & Jackson Laboratories.
[7] Millipore FH, 0.5 μm.
[8] Millipore HA, 0.45 μm.
[9] Vortex Genie.

phase. Estrone, the starting material in the synthesis of quinestrol, and ethinyl estradiol, a possible process contaminant, were not resolved from each other and had a retention time of 2 min. Estrone 3-cyclopentyl ether, the penultimate intermediate in quinestrol synthesis, eluted at 10.8 min and was well separated from the quinestrol peak at 8.5 min (Fig. 1).

Selectivity of Assay in Presence of Decomposition Products—A 1-g sample of quinestrol was heated in an open vial at 125° for 96 hr. This procedure was shown previously to produce a similar TLC pattern to that obtained with quinestrol tablets that had been exposed to air for several months. (Quinestrol in open containers is susceptible to autoxidation, which involved an induction period of over 1 year at room temperature. None of the decomposition products has the same mobility as the synthesis precursors and process contaminants. One of the two major decomposition products isolated by preparative TLC had an NMR spectrum consistent with the structure of 6β-hydroxyquinestrol[10].) The brown material was pulverized, and a 50-mg portion was dissolved in acetonitrile and diluted to 50.0 ml with that solvent. A 5-ml aliquot was diluted further to 50.0 ml with the mobile phase and subjected to chromatography as described. The chromatogram (Fig. 2) exhibited unidentified peaks with retention times of ~1.7, 2.2, 2.4, 4.0, 5.7, 6.3, and 8.0 min, along with the peak for undegraded quinestrol at 8.5 min. The quantity of undegraded quinestrol was estimated as 57.7% by this HPLC method and 58.9% by the colorimetric method (1).

Further evidence of the validity of the HPLC method and the colorimetric method as stability-indicating methods was obtained by dissolving the degraded quinestrol in the mobile phase to obtain a concentration of ~10 mg/ml, injecting 50 μl into the liquid chromatograph, and collecting the eluates corresponding to the decomposition products. These eluates were evaporated to dryness on the steam bath with a nitrogen stream, and the residue was dissolved in 2.0 ml of methanol. A 1-ml portion, corresponding to >100 μg of quinestrol degradation products, was tested by the colorimetric method (1) and gave no color with the methanol–sulfuric acid chromogenic reagent.

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COMMUNICATIONS

Human Plasma Levels of Propranolol: Fluorometric Measurement in a Hydrosolvatic System

Keyphrases □ Propranolol—fluorometric measurement of human plasma levels □ Spectrofluorometry—analysis, propranolol, human plasma □ Antiarrhythmic agents—propranolol, fluorometric analysis, human plasma

To the Editor:

We have developed a rapid, sensitive, fluorometric technique for directly measuring human plasma concentrations of total propranolol. As little as 0.3 ml of plasma is required. The plasma sample is diluted 1:1 with a hydrosolvatic solution, such as dimethyl sulfoxide–water (1:2), and the fluorescence is measured with no further sample workup. Plasma concentrations of propranolol free base also may be determined, but a larger sample volume and an additional extraction step are needed. The limiting concentration that can be detected is 10 ng/ml, and the standard curve in plasma is linear from 10 to 10,000 ng/ml in each case. The principal aromatic metabolite of propranolol, 4-hydroxypropranolol, does not interfere with the procedure since it emits at much longer wavelengths than the parent compound.

The technique may be used for plasma, aqueous protein solutions, or purely aqueous systems that do not contain chemicals or drugs emitting UV radiation at the same wavelength as propranolol, such as metoprolol, timolol, oxprenolol, and nadolol. However, this limitation does not apply to many commonly and concurrently administered cardiovascular drugs. For example, triamterene, hydrochlorothiazide, clonidine, hydralazine, prazosin, digoxin, and quinidine either do not or should not interfere with the developed procedure (Table I).

Table I—Fluorescence Maxima of Some Commonly and Concurrently Administered Cardiovascular Drugs Compared to Those of Propranolol (λ_{ex} 317 nm)

Drug	Fluorescence Maximum, nm
Propranolol	337
4-Hydroxypropranolol[a]	420
Hydrochlorothiazide	380[b,c]
Triamterene	NI[d]
Clonidine	390[b,c]
Hydralazine	NF[e]
Prazosin	387[b,c]
Digoxin	NF[f]
Quinidine	450[g]

[a] Furnished by T. Walle, Department of Pharmacology, Medical University of South Carolina. [b] Determined in this laboratory. [c] These agents did not contribute to the emission intensity of propranolol (λ 340) in either plasma or buffer at concentrations 10^4 times the limiting detectable concentration of propranolol free base. [d] NI = no interference, absorption maximum at 356 nm ("The Merck Index," 9th ed., Merck & Co., Rahway, N.J., 1976, p. 1233) indicates that its fluorescence will not interfere with the assay. [e] NF = no fluorescence. Taken from Ref. 13. [f] NF from Ref. 14. [g] Taken from Ref. 14.

Propranolol[1], hydrochlorothiazide[2], clonidine[3], and prazosin[4] were obtained commercially. A buffer (μ = 0.005, pH 7.4) was prepared from analytical grade 0.1 *N* KH_2PO_4[5], 0.1 *N* K_2HPO_4[5], and distilled, deionized water. All solvents were analytical grade and contained no fluorescent impurities.

Table II shows the excitation and emission spectral

[1] Ayerst Laboratories, New York, NY 10017.
[2] Merck Sharp and Dohme, West Point, PA 19486.
[3] Boehringer Ingelheim Ltd., Ridgefield, CT 06877.
[4] Pfizer Laboratories, New York, NY 10017.
[5] J. T. Baker Chemical Co., Phillipsburg, NJ 08865.

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