Comparison of an Ultraviolet Spectrophotometric Assay and a Gas-Liquid Chromatographic Assay for 17α-Ethynylestradiol 3-Methyl Ether in Tablets

By ARVIN P. SHROFF and JOSEPH GRODSKY

Ultraviolet spectrophotometric and gas-liquid chromatographic methods for the analysis of tablets containing 17α-ethynylestradiol 3-methyl ether and 17α-ethynyl-19-nortestosterone have been developed. The results obtained by the two methods are compared. The gas-liquid chromatographic method is found to be superior to the ultraviolet method, especially on aged tablets. A complex from tablet excipients was found to interfere with the ultraviolet method and was identified by gas-liquid chromatography.

MORE THAN 5 million women in the U. S. and about 2 million women overseas are now using oral contraceptives. These oral contraceptives are composed of estrogen–progestin mixtures. Of the seven U. S. companies which offer 11 different dosage forms, eight forms contain the estrogen, 17α-ethynylestradiol 3-methyl ether (mestranol). This paper describes the development of ultraviolet spectrophotometric and gas-liquid chromatographic methods for the analysis of mestranol in tablets containing the estrogen and norethindrone and gives the results so obtained.

The steroidal estrogens, having the characteristic phenolic A-ring, have been quantitatively assayed by utilizing the well-known Kober reaction or various modifications thereof (1–4). Colorimetric determinations (5–11) based on the coupling of the phenolic hydroxyl group with diazotized amines and spectrofluorometric methods (12–14) have been reported for a number of estrogens. All these methods, though sensitive, lack to some degree the specificity required in the presence of other steroids and tablet excipients for a rapid and accurate analytical method.

Recently, a number of other methods have been employed including ion exchange (15) and paper chromatography (16). The inaccuracy of the latter method has been discussed by Schulz (17).

Klein and co-workers (18) have described the quantitative analysis of ethynylestradiol and methyltestosterone by ultraviolet spectrophotometry. Ethynylestradiol and mestranol have also been quantitatively determined by gas-liquid chromatography (17, 19, 20). The authors failed to duplicate the mestranol determination by GLC using the reported (17) parameters since the mestranol always decomposed in part to estrone methyl ether, thus making quantitation impossible.

This investigation describes an ultraviolet spectrophotometric assay and a gas-liquid chromatographic assay for mestranol. Both assays are performed in the presence of 17α-ethynyl-19-nortestosterone (norethindrone). In the GLC determination an XE-60 coated column and an internal standard was used. The steroids do not undergo any decomposition and can be separated without prior acylation or trimethylsilyl ether formation. Practical separation (by GLC) of steroids and a nonsteroidal substance having an U.V. interfering chromophore has also been achieved. The advantages and disadvantages of the two assay methods are also discussed.

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EXPERIMENTAL

U.V. Assay Procedure

Apparatus—A Beckman model DU spectrophotometer with 1-cm. cells and a Cary recording spectrophotometer model 11.

Mestranol Standard Solution—Five milligrams of mestranol was accurately weighed into a 100-ml. volumetric flask and diluted to mark with spectral grade methylcyclohexane.

Method of Analysis for Standard Mestranol—The absorbance (at slit width 0.15 mm.) of the standard mestranol solution against spectral grade methylcyclohexane was measured at 316, 302, 287.7, and 278 mμ. The absorbance at 316 and 302 mμ was zero and the exact wavelengths of the peaks at 287.7 and 278 mμ were determined and their absorbance measured. The absorptivity (a) and the ratio of the two peaks (R) were calculated as follows:

$$a = \frac{A_{287.7} \times 100 \text{ ml.}}{\text{wt. in mg.}} \qquad R = \frac{A_{278}}{A_{287.7}}$$

Method of Analysis for Mestranol-Containing Tablets—A number of tablets containing about 200 mcg. of mestranol was put into a 29.5 × 80-mm. vial and treated with 4.0 ml. of water and 4.0 ml. of methylcyclohexane. The vial was shaken on a Burrell wrist-action shaker for 15–30 min. The mixture was centrifuged and a portion of the clear upper layer was transferred to a 1-cm. cell. The absorb-

ance (at slit width 0.15 mm.) was read for the sample against spectral grade methylcyclohexane at 316, 302, 287.7, and 278 mμ. Two methods of calculation were used as follows: (*a*) algebraically, a straight line was extended through 316 and 302 mμ and the peak height at 287.7 mμ was measured from this base line. The micrograms of mestranol per tablet was calculated by the following formula (see Eq. 2 for the algebraic derivation)

$$[A^{\text{obs.}}_{287.7} - 2A_{302} + A^{\text{obs.}}_{316}] \times \frac{4.0 \text{ ml.} \times 1000}{a \times \text{No. of tablets}}$$

(*b*) Algebraically, a straight line was drawn from 302 mμ at a slope to make the ratio of A_{278} and $A_{287.7}$ parallel to the ratio R determined in the standard mestranol curve. The micrograms of mestranol per tablet was calculated by the following formula (see Eq. 11 for the algebraic derivation):

$$\frac{0.714}{1.714 - R}\,[2.4A^{\text{obs.}}_{287.7} - 1.4A^{\text{obs.}}_{278} - A^{\text{obs.}}_{302}] \times \frac{4.0 \text{ ml.} \times 1000}{a \times \text{No. of tablets}}$$

The average of the two values was used. If the difference between the two values was greater than 0.006%, then it was presumed that there was too much extraneous base line absorption and another assay was used.

GLC Assay Procedure

Instrument—F and M model 1609 with modified injection port. The modified injection port was made by F and M Scientific, Avondale, Pa., to eliminate the potential for inlet sample splitting and thus create less dead volume.

Column—A stainless steel column 21 × 1/4 in. i.d., packed with coated support, was employed.

Liquid Phase—A silicone gum nitrile (*e.g.*, F and M Scientific Corp. No. LP 125, designated as XE-60) was used.

Inert Support—Untreated siliconized diatomaceous earth[1] was suitable as support material. The column packing was prepared by coating the inert support with 4% XE-60 using distilled acetone as the solvent for the application of the liquid phase.

Column Conditioning—The stainless steel packed column was conditioned by passing nitrogen carrier gas for about 12–18 hr. at a rate of 72 ml./min. at a column temperature of 240°.

Instrumental Parameters—In the development of the analytical method a column temperature of 195°, inlet port temperature of 242°, and a detector temperature of about 300° were used. The flow rate was between 70–75 ml./min. for the carrier gas, nitrogen.

Mestranol Standard—Throughout this work commercial mestranol was used which has an *a*-value at 278 mμ of 6.5, in distilled ethyl alcohol.

Internal Standard—Throughout this work, estrone U.S.P. XVI was used.

Preparation of Column Packing—In a 500-ml. round-bottom flask, place 1 Gm. of XE-60 dissolved in 100 ml. of acetone. To this add 25.0 Gm. of untreated siliconized diatomaceous earth 60/80 mesh (F and M Scientific Corp. No. ST120) and evaporate the solvent under reduced pressure (preferably a rotary flash evaporator). Heat the powder on a water bath for about 2 hr. Collect the material which falls off with gentle tapping. The yield is about 24 Gm.

[1] Diatoport-S.

Standard Mestranol Solution—Weigh accurately about 25.0 mg. of reference standard mestranol in a 50-ml. volumetric flask and dilute to mark with ethyl alcohol.

Standard Estrone Solution—Place exactly 50.0 mg. of estrone U.S.P. XVI in a 50-ml. volumetric flask and dilute to mark with ethyl alcohol.

Preparation of a Typical Standard Curve—Pipet into three different vials exactly 1.0 ml. of the standard estrone solution. Evaporate to dryness over a steam bath and under a stream of nitrogen. To each vial add 0.80 ml. (about 400 mcg.), 1.0 ml. (about 500 mcg.), and 1.2 ml. (about 600 mcg.) of standard mestranol solution, respectively. Evaporate once again to dryness over a steam bath and under a stream of nitrogen. Dissolve each residue in 0.5 ml. of distilled acetone. Inject separately 6–7 μl. of each sample in the GC and adjust the attenuator to 320. Elute for at least 1 hr. Measure the areas of the two peaks separately after drawing a base line tangent to both minima of each peak. The area is obtained by multiplying the height of the peak by the width at one-half the peak height. The major peak close to the solvent front corresponds to mestranol and the one farthest away corresponds to estrone. A standard curve is obtained by plotting the ratio of the areas under mestranol and estrone *versus* the weight of mestranol and drawing the best straight line.

Analysis of Tablets—Place a number of tablets equivalent to about 1,000 mcg. of mestranol in a 29.5 × 80-mm. vial. To this add 10 ml. of distilled water and 10 ml. of methylcyclohexane (spectroquality reagent) by means of a pipet. Clamp the vial to a Burrel wrist-action type shaker and shake vigorously for 0.5 hr. and then centrifuge for 2 min. Pipet 5.0 ml. of the supernatant solution into a 21 × 50-mm. vial and evaporate to dryness over a steam bath and a stream of nitrogen. Add exactly 1.0 ml. of the standard estrone solution and once again evaporate as above. Treat this residue with 0.5 ml. of acetone and inject (6–7 μl.) into the GC. The attenuator is adjusted to 320. After at least 1 hr. the areas corresponding to the peaks of mestranol and estrone are calculated as indicated previously. (*Note:* There is an additional major peak due to norethindrone which appears between mestranol and estrone.) Using the ratio of these areas (mestranol/estrone) on the standard curve, find the amount of mestranol.

ALGEBRAIC DERIVATION FOR U.V. CALCULATIONS

Figure 1 represents an ultraviolet spectrum of norethindrone and Figs. 2 and 3 represent typical expanded scale spectra of mestranol. The algebraically extended lines in Figs. 2 and 3 are hypothetically magnified to explain the derivation for the two formulas used in mestranol calculation.

According to Fig. 2:

$$A^{\text{corr.}}_{287.7} = A^{\text{obs.}}_{287.7} - \text{PN} - A^{\text{obs.}}_{302} \qquad \text{(Eq. 1)}$$

Since triangle MPN = Δ302M316

$$\text{PN} = A^{\text{obs.}}_{302} - A^{\text{obs.}}_{316}$$

therefore,

$$A^{corr.}_{287.7} = A^{obs.}_{287.7} - 2A^{obs.}_{302} + A^{obs.}_{316} \quad \text{(Eq. 2)}$$

In Fig. 3:

$$A^{corr.}_{287.7} = A^{obs.}_{287.7} - X - A^{obs.}_{302} \quad \text{(Eq. 3)}$$

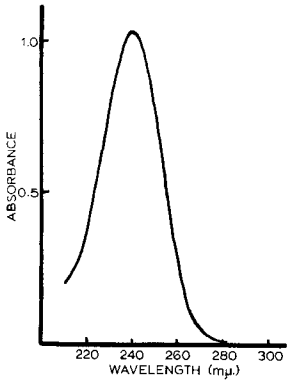

Fig. 1—U.V. absorption spectrum of norethindrone.

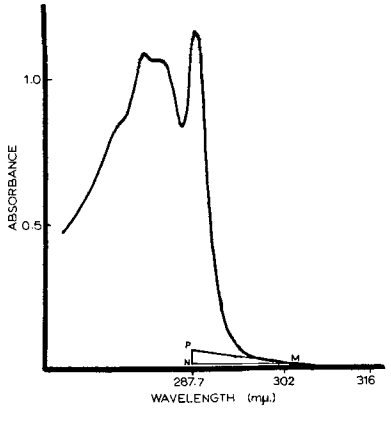

Fig. 2—U.V. spectrum of mestranol shown with the base line correction for Eq. 2.

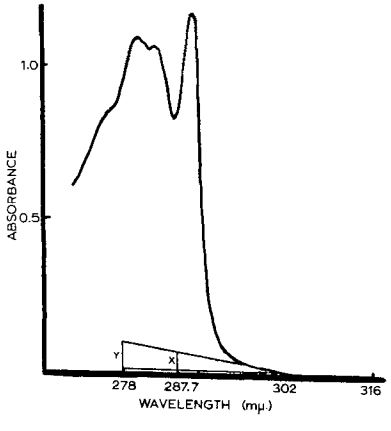

Fig. 3—U.V. spectrum of mestranol shown with base line correction for Eq. 11.

and

$$A^{corr.}_{278} = A^{obs.}_{278} - Y - A^{obs.}_{302} \quad \text{(Eq. 4)}$$

where

$$Y = \frac{302\ m\mu - 278\ m\mu}{302\ m\mu - 288\ m\mu} \times X \text{ (similar triangles)}$$

or

$$Y = 1.714\ x$$

Also Eq. 4 is equal to:

$$RA^{obs.}_{287.7} - RX - RA^{obs.}_{302} \quad \text{(Eq. 5)}$$

where R is the ratio of $A^{corr.}_{278}$ to $A^{corr.}_{287.7}$ and is obtained from a standard curve. By substituting the value of Y in Eq. 5 one obtains:

$$A^{obs.}_{278} - 1.714x - A^{obs.}_{302} = RA^{obs.}_{287.7} - RX - RA^{obs.}_{302} \quad \text{(Eq. 6)}$$

or

$$(1.714 - R)X = A^{obs.}_{278} - RA^{obs.}_{287.7} - A^{obs.}_{302} + RA^{obs}_{302}$$

and

$$X = \frac{A^{obs.}_{278} - RA^{obs.}_{287.7} - A^{obs.}_{302} + RA^{obs}_{302}}{1.714 - R}$$

Substituting the value of X in Eq. 3 gives:

$$A^{corr.}_{287.7} = A^{obs.}_{287.7} - \left\{\frac{A^{obs.}_{278} + RA^{obs.}_{287.7} + A^{obs.}_{302} - RA^{obs.}_{302}}{1.714 - R}\right\} - A^{obs.}_{302} \quad \text{(Eq. 7)}$$

Multiplying Eq. 7 by $(1.714 - R)$ gives:

$$(1.714 - R)A^{corr.}_{287.7} = 1.714^{obs.}_{287.7} - RA^{obs.}_{287.7} - 1.714A^{obs.}_{302} + RA^{obs.}_{302} - A^{obs.}_{278} + RA^{obs.}_{287.7} + A^{obs.}_{302} + RA^{obs.}_{302} \quad \text{(Eq. 8)}$$

Solving Eq. 8 gives:

$$(1.714 - R)A^{corr.}_{287.7} = 1.714A^{obs.}_{287.7} - 0.714A^{obs.}_{302} - A^{obs.}_{278} \quad \text{(Eq. 9)}$$

Dividing Eq. 9 by (0.714) gives:

$$\left(\frac{1.714 - R}{0.714}\right)A^{corr.}_{287.7} = 2.4A^{obs.}_{287.7} - 1.4A^{obs.}_{278} - A^{obs.}_{302} \quad \text{(Eq. 10)}$$

or

$$A^{corr.}_{287.7} = \frac{0.714}{1.714 - R}\left[2.4A^{obs.}_{287.7} - 1.4A^{obs.}_{278} - A^{obs.}_{302}\right] \quad \text{(Eq. 11)}$$

RESULTS AND DISCUSSION

Tables I through IV summarize the data obtained in determining the accuracy, precision, and standard deviation for the ultraviolet spectrophotometric and gas-liquid chromatographic methods. The data indicate that both methods are in excellent agreement and can be used for routine assays. Therefore, several samples of formulation, prepared for clinical study, were subjected to the improved extraction procedure and analyzed by these two methods. The excipients of these formulated tablets consisted of lactose, cornstarch, magnesium stearate, and polyvinylpyrrolidone.

The results of the ultraviolet assay on tablets stored under various conditions are listed in Table V. The ultraviolet assay data on the original granulations included in the table agree with the theoretical amount of mestranol added. It is evident from the results that, on storage, there is an apparent decrease in mestranol content. This decrease could be either the result of an incomplete extraction or partial mestranol degradation or to extraneous material which gives a faulty algebraic base line. The latter enters into the calculation and could be responsible for low results.

To determine if mestranol was incompletely extracted or was partially degraded the gas-liquid chromatographic technique was used. Both pure and extracted mestranol showed no decomposition on the 4% XE-60 column. The tablets from the same lot used in the U.V. assay were subjected to the extraction procedure and analysis. The analysis by GLC was carried out using an internal standard technique. The data are summarized in Table VI. It is evident from the results that mestranol does not undergo any decomposition on storage, and that complete extraction was achieved.

The question of extraneous material being extracted from the tablets was also answered by the GLC technique. During the different GLC assays on aged tablets prominent unidentified peaks near the solvent front (Fig. 4) were noticed. These were negligible with fresh tablets. It had also been established that these peaks were not the normal degradation products of mestranol, norethindrone, and estrone. Working with the excipients of the tablets, these peaks were later identified as being generated from a complex formed between polyvinylpyrrolidone and magnesium stearate. This complex has an absorption in the ultraviolet which distorts the base line and invalidates both correction factors. The low results obtained in Table V are thus attributed to these base line distortions.

A comparison of the two methods suggests that the ultraviolet assay is not reliable for aged tablets because of the interfering chromophore. However, it is quite suitable for fresh tablets. The GLC assay is sensitive for both fresh and aged tablets. An additional advantage of the GLC technique is that it offers quantitation concurrently with fractionation, the latter being important in aged tablets. In conclusion, this study points out that mestranol does not undergo any degradation at various storage conditions.

TABLE I—ACCURACY OF THE U.V. METHOD OF ANALYSIS[a]

No.	Mestranol Added, mcg.	Mestranol Recovered,[b] mcg.	% Recovery
1	198.8	193.5	97.3
2	198.8	192.5	96.8
3	198.8	193.0	97.1
4	198.8	193.5	97.3
5	198.8	202.5	102.0

[a] $\bar{X}$ = 98.1%; σ = ±2.2%. [b] Average of two calculations.

SUMMARY

1. Ultraviolet spectrophotometric and gas-liquid chromatographic assay methods are described and compared.

2. The gas-liquid chromatographic assay method is shown to be sensitive for mestranol in both fresh and aged tablets.

3. A complex between polyvinylpyrrolidone and magnesium stearate is identified by GLC.

4. Mestranol does not undergo any decomposition when stored under various conditions.

TABLE III—ACCURACY OF THE GLC METHOD[a]

No.	Mestranol Added, mcg.	Mestranol Recovered, mcg.	% Recovery
1	493	481	97.6
2	493	495	100.4
3	493	485	98.4
4	500	495	99.0
5	500	501	100.2

[a] $\bar{X}$ = 99.1%; σ = ±1.2%.

TABLE IV—PRECISION OF THE GLC METHOD[a]

No.	1	2	3	4
Mestranol assayed, mcg.	103.7	106.4	105.6	102.0
No.	5	6	7	8
Mestranol assayed, mcg.	104.2	102.0	103.8	105.6

[a] $\bar{X}$ = 104.2; σ = ±1.64%.

TABLE V—MESTRANOL ASSAY OF VARIOUS TABLETS BY U.V.

Lot	Storage[a] Conditions	Original Granulation[b] U.V. Assay, mcg./Tablet	mcg./Tablet by U.V.	% Recovery
A	6 wk. at 100/80	51.1	42.5	83.2
B	6 wk. at 100/80	51.3	44.3	86.4
C	3 mo. at 100/80	51.3	42.8	83.4
D	6 mo. at 37°	80.0	71.7	89.6
E	7.5 mo. at RT	49.9	43.6	87.4
F	12 mo. at RT	56.0	51.2	91.4
G	12 mo. at RT	56.0	51.2	91.4
H	13 mo. at RT	51.5	45.6	88.5
I	18 mo. at RT	57.6	48.9	84.5

[a] 100/80 refers to 100°F. and 80% humidity. RT refers to room temperature. [b] Values within acceptable region of the theoretical value.

TABLE II—PRECISION OF THE U.V. METHOD OF ASSAY[a]

No.	1	2	3	4	5	6	7	8	9	10
Mestranol assayed, mcg.	98.6	100.1	101.4	101.7	101.4	99.6	99.2	98.9	101.0	99.7

[a] $\bar{X}$ = 100.16; σ = ±1.14.

TABLE VI—MESTRANOL ASSAY OF VARIOUS TABLETS BY GLC

Lot	Storage Conditions[a]	Original Granulation[b] U.V. Assay, mcg./ Tablet	mcg./ Tablet by GLC	% Recovery
A	6 wk. at 100/80	51.1	48.0	94
B	6 wk. at 100/80	51.3	51.8	100.9
C	3 mo. at 100/80	51.3	51.3	100.0
D	6 mo. at 37°	80.0	84.3	105.4
E	7.5 mo. at RT	49.9	51.6	103.4
F	12 mo. at RT	56.0	55.1	98.4
G	12 mo. at RT	56.0	55.0	98.2
H	13 mo. at RT	51.5	52.2	101.4
I	18 mo. at RT	57.6	56.0	97.2

[a] 100/80 refers to 100°F. and 80% humidity. RT refers to room temperature. [b] Within acceptable region of the theoretical value.

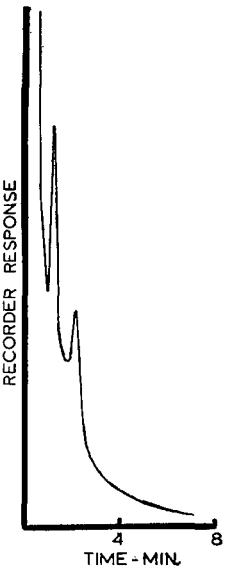

Fig. 4—GLC separation of a complex between polyvinylpyrrolidone and magnesium stearate.

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Diffusion of Drugs Across the Isolated Mesentery

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In order to obtain knowledge of the diffusion rates of pentobarbital, salicylic acid, and urea across the peritoneal membrane under ideal conditions, experiments with isolated rabbit mesentery were conducted. Diffusion coefficients of the drugs were applied to evaluate rates of dialysis at various conditions of pH. Pentobarbital and salicylic acid diffused across the isolated mesentery in both ionic and nonionic form, with no significant difference in rate due to pH. *In vivo* tests also make it appear that the ionized form of drugs may diffuse across the peritoneal membrane. These results suggest that the research for agents to promote dialysis should include substances other than alkalizing agents.

THE REMOVAL of drugs in poisonings by peritoneal dialysis has been the subject of many studies. Salicylates and barbiturates have been most commonly investigated due to the frequency with which they are involved in poisoning cases. The rates at which these drugs have been removed by peritoneal dialysis with the usual dialysis fluids have not been encouraging, and it is desirable that improved fluids and techniques be developed for this purpose. To proceed logically in this effort a more thorough knowledge is required of the mechanism by which drugs pass the membrane and the maximum rates which might be attained.

Berndt and Gosselin (1–3) indicated that the diffusion of ions and molecules through the peritoneal membrane may take place through pores.

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