Spectrophotofluorometric Determination of Mestranol in Oral Contraceptive Tablets

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Abstract □ A rapid, reliable fluorometric method has been developed for assaying 17α-ethynylestradiol-3-methyl ether (mestranol) in some oral contraceptive tablets. The method utilizes the native fluorescence of mestranol. No separations are required and the method is applicable in the presence of norethynodrel, ethynodiol diacetate, or norethindrone. Chlormadinone acetate interferes in the procedure. The method is sensitive (0.8–1.0 mcg./ml.) and applicable to single-tablet analyses. The accuracy under the conditions studied was ±1.29%, and the precision ranged from ±0.896 to ±1.73%.

Keyphrases □ Mestranol determination—tablets □ UV spectrophotometric, fluorometric, colorimetric analyses, mestranol—comparison □ Fluorometry—analysis

The estrogenic component of oral contraceptives is usually 17α-ethynylestradiol-3-methyl ether (mestranol). Because of its low dosage in tablets, mestranol is difficult to assay. UV (1–3), GLC (4–8), and colorimetric (9–14) methods have been proposed but all lack specificity or are unsuitable for routine analysis.

Several fluorometric procedures have also been reported (13, 15–20). The procedures involved induced fluorescence where reagent preparation, temperature, and time are all very critical for reproducibility. Use of fluorescence does increase sensitivity, but the methods currently available suffer the same limitations and drawbacks as colorimetric methods.

Duggan *et al.* (21) reported that natural estrogens possess native fluorescence. Mestranol, a synthetic estrogen, also possesses native fluorescence, whereas the more common progestins do not fluoresce appreciably. To date, methods that utilize native fluorescence (22) usually require internal standards and excipient blanks which are not readily available except to the manufacturer. Conceivably, each commercial product could have different excipients which would preclude the use of a "universal excipient blank."

The proposed assay procedure overcomes these limitations and is applicable to a variety of commercial products. No internal standards or excipient blanks are required, no preliminary separations are necessary, and the method is suitable for single-tablet analysis.

EXPERIMENTAL

Apparatus—A recording spectrophotofluorometer[1] with 1-cm. cells was used. The following instrument parameters were employed: xenon lamp; meter multiplier, 0.01; sensitivity, 45–50; 1P21 photomultiplier; slit arrangement No. 4; excitation wavelength, about 284 mμ; and emission wavelength, about 327 mμ.

Standard Preparation—Prepare a solution containing 0.8–1.0 mcg. of mestranol[2] per ml. in absolute ethanol.

[1] Aminco-Bowman spectrophotofluorometer, American Instrument Co., Inc., Silver Spring, Md.
[2] Syntex Labs, Inc., Palo Alto, Calif.

Table I—Fluorometric Assay of Commercial Tablets Containing Mestranol

Mestranol Found, mg./Tablet (Mestranol Declared, 0.1 mg./Tablet)		
Sample 1[a]	Sample 2[a]	Sample 3[b]
0.101	0.0997	0.0979 0.100
0.101	0.100	0.0986 0.100
0.105	0.0984	0.100 0.100
0.104	0.103	0.101 0.0995
0.103	0.101	0.0991 0.100
0.101	0.100	0.0985
Av. = 0.102	Av. = 0.100	Av. = 0.0995
SD = ±1.73%	*SD* = ±1.59%	*SD* = ±0.896%

[a] Samples 1 and 2 also contain norethindrone, 2 mg./tablet. [b] Sample 3 also contains norethynodrel, 2.5 mg./tablet.

Procedure—Rinse all glassware with ethanol prior to use. Grind at least 20 tablets to pass a 60-mesh sieve. Accurately weigh a portion of powder equivalent to one tablet, and transfer to a 50-ml. centrifuge tube (for single-tablet analysis, place tablet in centrifuge tube and crush with glass rod). Pipet 20 ml. of absolute ethanol into the tube, stopper securely, and shake on a mechanical shaker for 30 min. Centrifuge to clarify the solution. Pipet an aliquot of the supernatant, equivalent to 20–25 mcg. of mestranol, into a 25-ml. volumetric flask, and dilute to volume with absolute ethanol.

Fluorescence Determination—Adjust the spectrophotofluorometer to read 70% emission, at 327 mμ, with the standard solution. Determine the fluorescence spectra of the standard and sample solutions by scanning from 285 to 400 mμ. Make calculations using the maximum emission at about 327 mμ. Use absolute ethanol as a blank and correct for it.

RESULTS AND DISCUSSION

Progestins commonly associated with mestranol in contraceptive tablets exhibit negligible native fluorescence. However, because of their relatively high concentrations in tablets as compared to mestranol (as much as 25:1), concentration quenching will occur. This could affect the fluorescence of mestranol but is effectively negated by diluting samples to large volumes. Recovery samples containing norethindrone (a progestin) and mestranol gave low recoveries for mestranol when the norethindrone concentration in the final solution was 100 mcg./ml. or more. This same sample gave 100% recovery when the norethindrone concentration was 50 mcg./ml. or less.

The accuracy of the method was demonstrated by five assays of a synthetic mix. The average mestranol recovery was 99.8%, with a standard deviation of ±1.29%.

Table I gives results of the fluorometric assay for three commercial products. Samples 1 and 2 were labeled to contain 2 mg. of norethindrone and 0.1 mg. of mestranol per tablet; Sample 3 was labeled to contain 2.5 mg. of norethynodrel and 0.1 mg. of mestranol per tablet. The standard deviation ranged from ±0.869 to ±1.73%.

Table II compares the data obtained by UV (23) and colorimetric (12) methods with results by the present fluorometric method. Results by the fluorometric method compare favorably to those by the colorimetric method. The samples represent four different manufacturers.

Mestranol in combination with chlormadinone acetate could not be determined by the fluorometric procedure without prior separation, because the excitation wavelength for mestranol (about 284 mμ) is too close to the absorption peak for chlormadinone acetate (283.5 mμ).

Table II—Comparison of Assay Methods for Mestranol in Commercial Samples

Sample	Progestin Present	Mestranol Declared, mg.	% of Amount Declared by— UV	Color	Fluorometric
1	Norethynodrel	0.1	85.1[a]	99.0[a]	100
2	Ethynodiol diacetate	0.1	98.0	—[b]	104
3	Norethindrone	0.1	100	99.0	101
4	Norethindrone	0.1	95.4	100	99.7
5A[c]	—	0.08	102	102	102
5B[c]	Chlormadinone acetate	0.08	101[a]	—[b]	—[b]

[a] Corrected for interference in the spectrum. [b] Interferences prevented accurate calculations. [c] Sequential-type tablets.

The method is also applicable to single-tablet analyses. Assay values for 10 tablets ranged from 98.5 to 105% of declared values.

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Assay of Emetine Hydrochloride Injection

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Abstract □ Data are presented for the DAB 7 assay of emetine hydrochloride and for quantitative assay of emetine hydrochloride injection by a method previously reported for injections of amin-salts. The method involves nonaqueous titration of a chloroform. eluted sample from a magnesium oxide–siliceous earth mixture Thermogravimetric data show that emetine hydrochloride forms no stable hydrates, that water can be lost even at room temperature, and that loss still continues slowly beyond the usual drying temperature so that it is difficult to render the material anhydrous. For these reasons, it is suggested that emetine hydrochloride and emetine hydrochloride injection be labeled with the content of anhydrous emetine hydrochloride. Permissible variation should be ±1% for emetine hydrochloride and ±5% for the injection.

Keyphrases □ Emetine HCl injection—analysis □ Potentiometric titration—analysis □ Titrimetry—analysis

Emetine Hydrochloride Injection USP XVII has a peculiar definition in that "it contains an amount of anhydrous emetine hydrochloride ($C_{29}H_{40}N_2O_4 \cdot 2HCl$) equivalent to not less than 84% and not more than 94% of the labeled amount of emetine hydrochloride" (1). Thus it is the only pharmaceutical preparation formulated at less than 100% of label claim. This peculiar definition is necessitated by the official definition of Emetine Hydrochloride USP XVII as a hydrate of uncertain composition, which "contains not less than 98.0% and not more than 101.5% of $C_{29}H_{40}N_2O_4 \cdot 2HCl$, calculated on the anhydrous basis" (2).

Water is determined as follows: "Dry it at 105° for 2 hr.: it loses not less than 8% and not more than 14% of its weight" (2). The average water content of the solid is thus 11%, which corresponds to the average requirement for the injection of 89% of anhydrous material.

An attempt was made to assay emetine hydrochloride injection by a previously proposed method (3). The method involves distributing the sample over a mixture of magnesium oxide and purified diatomaceous earth held on a sintered-glass filtering funnel, eluting the