AOAC Official Method 974.43
Ethinyl Estradiol
in Drugs

Spectrophotometric Method
First Action 1974
Final Action 1976

A. Reagents

(**a**) *Methanol–sulfuric acid*.—In ice bath, cautiously add chilled H_2SO_4 (≥95%) in small increments, with mixing, to 60 mL chilled anhydrous methanol in 200 mL volumetric flask. Cool to room temperature, dilute to volume with H_2SO_4, and mix. Reagent is stable at room temperature ca 1 month.

(**b**) *Washed chloroform*.—Vigorously shake ca 500 mL $CHCl_3$ with 30 mL H_2SO_4 (≥95%) in 1 L separator ca 2 min. Discard H_2SO_4 (bottom) layer. Wash $CHCl_3$ with 400 mL H_2O by shaking vigorously 1 min; discard H_2O. Repeat H_2O washing 3 times as above. Filter clear $CHCl_3$ layer through funnel containing pad of glass wool covered with ca 50 g granular anhydrous Na_2SO_4. Prepare fresh daily. Use same batch of washed $CHCl_3$ for all samples and standards throughout series.

(**c**) *Ethinyl estradiol standard solutions*.—(*1*) *Stock standard solution*.—0.8 mg/mL. Accurately weigh ca 40 mg USP Reference Standard ethinyl estradiol, dissolve in anhydrous methanol in 50 mL volumetric flask, dilute to volume with methanol, and mix. (*2*) *Intermediate standard solution*.—20 μg/mL. Pipet 5.0 mL stock standard solution into 200 mL volumetric flask, dilute to volume with isooctane, and mix. (*3*) *Working standard solution*.—4 μg/mL. Pipet 20 mL intermediate standard solution into 100 mL volumetric flask, dilute to volume with isooctane, and mix. (This solution is stable at room temperature ca 3 weeks.)

(**d**) *Diatomaceous earth*.—*See* **960.53B** (*see* 18.1.01).

B. Preparation of Column

Trap layer.—Transfer ca 5 g granular anhydrous Na_2SO_4 to 25 × 250 mm chromatographic tube containing pad of glass wool in base. Thoroughly mix 3 mL 10% NaOH solution with 3 g diatomaceous earth in 100 mL beaker. Transfer mixture to tube in 1 portion and tamp moderately.

Sample layer.—Accurately weigh portion of ground tablet composite containing ca 40 μg ethinyl estradiol into 100 mL beaker. Add 3 mL $CHCl_3$ and 2 mL H_2O, and stir frequently 2 min to dissolve maximum amount of sample. Mix with 4 g diatomaceous earth 1 min, transfer quantitatively to tube in 1 portion, and tamp moderately. Dry-wash beaker with ca 0.5 g diatomaceous earth and transfer wash to column. Wipe tamper, spatula, and beaker with glass wool and place glass wool on column.

C. Chromatography

Rinse tamper, spatula, and beaker with 25 mL isooctane and add rinse to column. Discard eluate. Using total of 55 mL $CHCl_3$–isooctane (1 + 9), repeat rinsing as above and discard eluate. Wash column with 15 mL isooctane and discard eluate. Finally, elute ethinyl estradiol with 50 mL washed $CHCl_3$, followed by 25 mL isooctane, collecting eluate in 250 mL separator.

D. Determination

Pipet 10 mL each of working standard solution and isooctane (reagent blank) into separate dry 250 mL separators. To each add 50 mL washed $CHCl_3$ and 15 mL isooctane, and mix gently. Pipet 10 mL methanol–H_2SO_4 into test solution, blank, and standard separators, letting pipet drain completely. Shake vigorously 4 min, and let layers separate ca 15 min; protect from strong light. Within 30 min, scan spectra between 700 and 500 nm of pink (lower) phases of standard and sample in 1 cm cells against reagent blank as reference, set at 0 at 700 nm.

$$\text{Ethinyl estradiol in final solution, } \mu g = (A/A') \times C \times 10 \text{ (mL)}$$

where A and A' refer to test and standard solutions, respectively, at maximum, ca 537 nm; and C = μg/mL standard solution.

Reference: *JAOAC* **57,** 747(1974).

CAS-57-63-6 (ethinyl estradiol)

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