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Keyphrases

Terpin hydrate-codeine elixir—analysis
GLC—analysis
Benzoic acid—internal standard, terpin hydrate
Cholestane—internal standard, codeine
Isothermal, programmed temperature—GLC

Technical Articles

Automated Colorimetric Analysis of Ethinyl Estradiol and Mestranol in Pharmaceutical Tablets

By WILLIAM F. BEYER

An automated colorimetric procedure is described for the determination of ethinyl estradiol and ethinyl estradiol-3-methyl ether in pharmaceutical tablets. Chloroform solutions of the estrogen are automatically extracted with alcohol–sulfuric acid and analyzed at a rate of 20 samples/hr. The relative standard deviation of repetitive sampling of tablet extracts was less than 1 percent, and Beer's law was obeyed over the range of 0.40–8.00 mcg./ml. of sample solution. The procedure is applicable to unit dosage assays and to assays requiring composite samples of pulverized tablets or numerous whole tablets.

NUMEROUS METHODS have been reported for the determination of ethinyl estradiol (EE) or its 3-methyl ether derivative (EE-3ME) from tablets (1–5). The majority of these procedures depends upon the spectrophotometric analysis of sulfuric acid-induced color, and involve somewhat lengthy separative techniques.

A recent publication of Khoury and Cali (6) describes an automated assay for EE and EE-3ME based on the fluorescence exhibited by the estrogens in 90% sulfuric acid. The necessity of assaying large numbers of tablets for EE in combination with nonestrogenic steroids also prompted the investigation of automation in these laboratories. A relatively simple, automated method has been developed with instruments normally found in laboratories employing automatic analysis—sampler, proportioning pump, heating bath, spectrophotometer, and recorder. The procedure is based on a manual method developed in these laboratories (7), a modification and quantitation of the USP XVII sulfuric acid identification test for EE (1). The red color formed when sulfuric acid is added to a chloroform solution of EE is extractable with sulfuric acid and exhibits a maximum absorption at 518 mμ. The color develops rapidly and is

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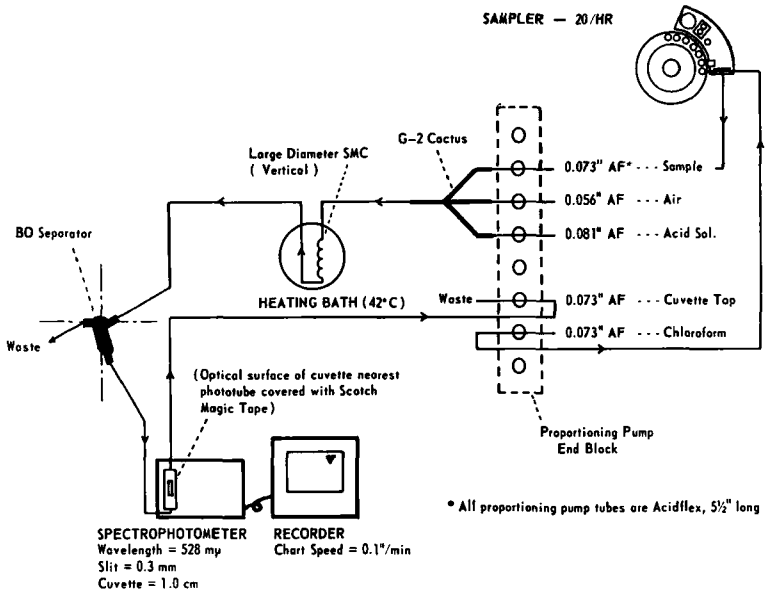

Fig. 1—Flow diagram for the automated determination of ethinyl estradiol and ethinyl estradiol-3-methyl ether, at wavelengths of 528 and 538 mμ, respectively.

stable at room temperature for at least 15 hr. Although the reaction is not influenced by the volume of chloroform, the color is enhanced by the small amount of alcohol used as a preservative in chloroform. The interference of nonestrogenic steroids present in tablets in combination with the estrogen is eliminated by correcting for absorbances due to nonestrogenic steroids.

Details of the automated procedure and a comparison of assay results with the manual procedure are the subject of this report.

EXPERIMENTAL

Equipment—Automatic sampler, proportioning pump, and assorted tubing and glass fittings[1]; heating bath[2]; spectrophotometer[3]; recorder[4]; and 12 × 35-mm. glass shell vials.

Reagents—Chloroform, analytical grade; 10% 3A alcohol[5] in 90% sulfuric acid, analytical grade (v/v).

Standards—Chloroform stock solutions containing 100 mcg./ml. of USP ethinyl estradiol or ethinyl estradiol-3-methyl ether are refrigerated in tightly sealed volumetric flasks. The stock standards are brought to room temperature and diluted with chloroform to approximately 4 mcg./ml. just prior to use.

Interfering Nonestrogenic Steroids—Chloroform stock solutions of steroids present in tablet formulation in combination with EE or EE-3ME are refrigerated in tightly sealed volumetric flasks. Chloroform solutions of nonestrogenic steroids are prepared from the stock solutions, maintaining the ratio of estrogen to nonestrogen that exists in the tablets.

Tablets—Tablets are disintegrated by gentle shaking in polyethylene-stoppered conical flasks, containing a small amount of water and several glass beads. To completely disintegrate the tablets, the stoppers are loosened and the flasks are heated on a steam bath for approximately 15 min. After cooling to room temperature, chloroform is added at a ratio of 10 ml. for each ml. of water so that the final estrogen concentration is similar to that of the standard. For example in the assay of 0.05-mg. EE tablets, eight tablets are disintegrated with 10.0 ml. of water and extracted with 100.0 ml. of chloroform. The flasks are shaken vigorously for approximately 30 min. A portion of the chloroform phase is filtered directly into shell vials using a rapid flow filter paper such as Whatman No. 41. A layer of water (approximately 0.4 ml.) is added to each vial immediately after filtration to suppress evaporation of chloroform.

Procedure—The sampling probe of the automatic sampler is aligned so that satisfactory aspiration from the shell vials and from the rinsing compartment occurs. A zero base line is established with all instruments operating using the flow system of Fig. 1 with sample and rinse lines in chloroform and the remaining line in acidic alcohol. Sampling rate is set at 20/hr. with a sample–rinse ratio of 1:1. Before aspiration of samples, the flow cell is inverted and tapped to release chloroform or water droplets that may be trapped. Vials of the nonestrogenic steroid are sampled first if that particular steroid is present in the tablets in combination with the estrogen. Vials of duplicate EE or EE-3ME standards follow, with approximately 0.4 ml. of water added to vials of both the nonestrogenic steroid and standard. Additional standards are interspaced among test samples to compensate for instrumental variation. EE is measured at a wavelength of 528 mμ, EE-3ME at 538 mμ.

Calculations are based on the relative absorbance of standards and test samples with corrections made for the absorbance of the nonestrogenic steroid present in the tablet formulation.

RESULTS AND DISCUSSION

Virgorous shaking of tablets in chloroform follow-

[1] Technicon, Ardsley, N. Y.
[2] Tamson heating bath, Witt Sales, Cleveland, Ohio
[3] Hitachi-Perkin-Elmer 139 spectrophotometer with 1.0-cm. Thomas flow cell No. 9120-NO5, A. H. Thomas Co., Philadelphia, Pa.
[4] Sargent SRL recorder
[5] US Industrial Chemical Co., New York, N. Y. (100 gal. ethanol denatured with 5 gal. methanol).

ing disintegration with a small amount of water, gave both a simple and efficient extraction of EE. The use of chloroform samples introduced difficulties; however, satisfactory procedures were developed that permitted their use. An attachment to hold glass shell vials in the sample plate of the automatic sampler and a glass cover plate for the sampler were constructed. Two metal disks were attached to the standard sample plate, below the plastic holder; one with forty 1.3-cm. (0.5-in.) holes to center the shell vials, the other without holes as a support for the containers. An exact duplicate of the original plastic cover plate was made using plate glass, to eliminate the effect of chloroform on the plate. Placing a layer of water on the vials of chloroform, eliminated the problem of chloroform evaporation. In-stream extraction of the estrogen in chloroform with sulfuric acid was aided by adding 10% water and 10% alcohol to the acid and by using a 42° water bath. Alcohol added to diluted sulfuric acid decreased the viscosity of the acid, giving a uniform flow of liquids and a satisfactory separation of acid–chloroform phases. The effect of striations caused by sulfuric acid passing through the flow cell was eliminated by applying Scotch Magic tape[6] to the optical surface of the flow cell nearest the photo tube in the manner described by Anderson *et al.* for the automated assay of vitamin A (8). Maximal absorption of EE and EE-3ME occurred at 528 and 538 mμ, respectively, verified with a recording spectrophotometer[7] after manually collecting acid from the exit port of the flow cell while sampling continuously.

Recordings of repetitive and continuous sampling of chloroform solutions of EE at a concentration of 3.0 mcg./ml. are shown in Fig. 2. A relative standard deviation for the 12 replicate analyses was 0.54% even though peak heights of individual samples did not reach that of continuous sampling and the base line was not reached during the rinse cycle. Recordings of various concentrations of EE (Fig. 3), with duplicate sampling at each level, show minimal interaction between subsequent samples. Beer's law was obeyed and a y intercept of −0.004 was calculated. The values gave a calculated slope of 0.999 when plotted on the ordinate against theoretical values on the abscissa.

The recovery of EE from pooled chloroform extracts of oral contraceptive tablets containing 0.05 mg. EE in combination with 10.0 mg. medroxyprogesterone acetate per tablet was tested. EE standard was added to varying quantities of tablet extracts, giving an average recovery of 100.2% (Table I), with a low of 99.6% and a high of 100.7%. Medroxyprogesterone acetate, the progestin in the tablet, did not interfere with the assay. Recovery studies of EE from tablets containing 0.02 mg. EE and 1.0 mg. fluoxymesterone, in which the anabolic androgen contributed approximately 3% of the absorbance, were carried out. Samples were prepared to contain tablet excipients, varying quantities of EE, and fluoxymesterone at a concentration equivalent to 50 times the level of EE used for assay purposes. Table II shows that it is possible to assay EE at a concentration of 75–125% of the labeled amount in the presence of the nonestrogenic steroid at label strength.

Numerous assays for EE in tablets containing medroxyprogesterone acetate or fluoxymesterone were carried out using automated and manual

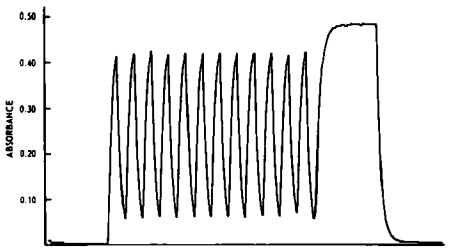

Fig. 2—Absorbance recordings of 3.0-mcg./ml. chloroform solution of ethinyl estradiol sampled continuously and repetitively.

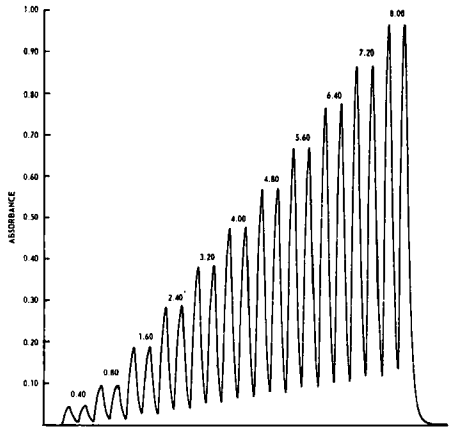

Fig. 3—Absorbance recordings of various mcg./ml. levels of ethinyl estradiol in chloroform.

TABLE I—RECOVERY OF ETHINYL ESTRADIOL FROM CHLOROFORM EXTRACTS OF TABLETS CONTAINING ETHINYL ESTRADIOL AND MEDROXYPROGESTERONE ACETATE

EE Standard Added, mcg./ml.	EE from Tablets, mcg./ml.	Theoretical EE Present, mcg./ml.	Assayed EE, mcg./ml.	Recovery, %
2.60	0.25	2.85	2.87	100.7
2.60	0.50	3.10	3.13	100.6
2.60	1.00	3.60	3.61	100.3
2.60	1.50	4.10	4.11	100.2
2.60	2.00	4.60	4.59	99.8
2.60	2.50	5.10	5.08	99.6
			Av.	100.2

TABLE II—RECOVERY OF VARYING QUANTITIES OF ETHINYL ESTRADIOL FROM SAMPLES SPIKED WITH FLUOXYMESTERONE

EE Present, mcg./ml.	Fluoxymesterone Added, mcg./ml.	EE Assayed, mcg./ml.	Recovery, %
3.0	200	3.05	101.6
3.5	200	3.53	100.9
4.0	200	4.01	100.3
4.5	200	4.46	99.1
5.0	200	4.86	97.2
		Av.	99.8

[6] Minneapolis Mining and Manufacturing, St. Paul, Minn.
[7] Cary model 11, Cary Instruments, Applied Physics Corp., Monrovia, Calif.

Table III—Automated and Manual Ethinyl Estradiol Assays of Tablets Containing Ethinyl Estradiol and a Nonestrogenic Steroid

Lot No.	EE, mg./Tab. Automated	EE, mg./Tab. Manual
0.05 mg. Ethinyl Estradiol + 10.0 mg. Medroxyprogesterone Acetate		
1	0.050	0.050
2	0.049	0.050
3	0.050	0.049
4	0.048	0.049
5	0.047	0.051
6	0.050	0.048
7	0.049	0.049
8	0.047	0.050
9	0.049	0.048
10	0.048	0.050
0.02 mg. Ethinyl Estradiol + 1.0 mg. Fluoxymesterone		
11	0.019	0.019
12	0.019	0.020
13	0.021	0.020
14	0.021	0.022
15	0.020	0.020
16	0.020	0.019
17	0.019	0.020
18	0.017	0.020
19	0.020	0.021
20	0.021	0.021

procedures. Table III shows that satisfactory agreement occurred for the two methods of assay.

Progestins present in formulations of oral contraceptive tablets, in combination with EE-3ME interfered with the automated assay to varying degrees. The interference, however, was eliminated by correcting for the absorbance at 538 mμ, due to the progestin alone. Table IV gives results of tablet assays from five manufacturers.

SUMMARY

An automated colorimetric method has been described for the assay of ethinyl estradiol and ethinyl estradiol-3-methyl ether in tablets. Interferences due to nonestrogenic steroids also present in the tablets are eliminated by correcting for the absorbance due to the steroids. Following manual extraction of test preparations, chloroform samples are analyzed at a rate of 20/hr. The relative standard deviation is less than 1% and Beer's law is obeyed in the range of 0.4–8.0 mcg./ml. Satisfactory agreement of assay results was obtained for the automated and manual procedures.

Table IV—Ethinyl Estradiol-3-methyl Ether Assays of Oral Contraceptives from Various Manufacturers

Manufacturer	Progestin and Labeled Amount, mg.	EE-3ME Assay, mg./Tab.	% of Claim
A	Norethindrone, 1.0	0.047	94
B	Norethindrone, 2.0	0.077	96
C	Norethindrone, 2.0	0.096	96
D	Norethynodrel, 5.0	0.082	109
E	Chlormadinone acetate, 2.0	0.075	94

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Keyphrases

Mestranol—analysis
Automated analysis—tablets, steroid mixtures
Diagram—automated apparatus
Colorimetric analysis—spectrophotometer