Quantitative Gas Liquid Chromatographic Determination of Ethinyl Estradiol

By JOSEPH M. TALMAGE, MELVIN H. PENNER, and MILTON GELLER

A gas liquid chromatographic procedure is described for ethinyl estradiol in both sesame oil solutions and solid dosage forms. The method involves preliminary separation of the active principle from the oily vehicle or tablet excipients, addition of an internal standard, acetylation, and chromatography on a 4 per cent SE-30 column. Quantitation is accomplished by integration of the areas under the peaks. Results indicate a precision of ±1 per cent with an accuracy of 100.2 ± 1.7 per cent. The accuracy and precision are superior to the iron phenol procedure described in the U.S.P. XVI. The chromatographic method allows improved specificity and simultaneous determination of decomposition products, if present.

A SENSITIVE analytical method is reported for the determination of ethinyl estradiol contained in sesame oil solutions and solid dosage forms. One of the most widely used methods for the quantitation of estrogens utilizes the Kober reaction or various modifications thereof (1–3). In order to apply a Kober-type reaction for the sesame oil dosage form, complete separation of the estrogen from the oil is essential. Exhaustive attempts to effect a complete separation by liquid-liquid extraction methods failed because of the formation of an interfering color. In addition to this difficulty, the standard deviation of the Kober method, as performed in this laboratory, has been found to be of the order of ±10%. The use of an ultraviolet spectroscopic method (4) was also thwarted because of an interfering chromophore from the incomplete separation of sesame oil from the estrogen.

Spectrofluorometry (5, 6), ion exchange (7), and recently a paper chromatographic method (8) have been described for the determination of ethinyl estradiol in various dosage forms. Gas liquid chromatography was considered to be a more desirable technique, since it is rapid and offers the advantage that quantitation is obtained concurrently with fractionation.

Since Vanden Heuvel *et al.* (9) first described a practical separation of steroids by gas liquid chromatography, the technique has been widely used. Estrogens have been separated without modification (10), as fully acetylated derivatives (11), and as the trimethylsilyl ethers (12).

In this investigation, the fully acetylated derivatives were used since Martin (13) has shown these to be thermally stable to 340° by infrared and ultraviolet studies and also as was demonstrated by the symmetry of the gas chromatographic peaks.

EXPERIMENTAL

Instrument.—Perkin-Elmer model 801 gas chromatograph equipped with dual hydrogen flame ionization detectors, model 194B printing integrator, and a Honeywell-Brown Electronik recorder.

Operating Conditions.—Hydrogen pressure, 17 p.s.i.; air pressure, 42 p.s.i.; helium flow rate, approximately 40 ml./min.; injector temperature, 280°; column oven temperature, 240°; detector temperature, 250°.

Column Description.—A 6-ft. helical glass column, 0.250-in. O.D., packed with 4% SE-30, 0.2% Epon 1001 on Chromosorb P, acid-washed HMDS treated 80/100 mesh, commercially available complete from the Perkin-Elmer Corp. The column is preconditioned before using by programming the temperature from ambient to 250° at 0.5°/min. with a helium flow rate of 40 ml./min.

Preparation of Standard.—Prepare a solution of estrone U.S.P. (internal standard) at a concentration of 0.5 mg./ml. in acetone.

Prepare a solution of ethinyl estradiol U.S.P. at a concentration of 0.2 mg./ml. in acetone. Into four appropriately marked vials, pipet 1, 2, 3, and 4 ml. of ethinyl estradiol standard and 1 ml. of estrone standard into each and evaporate to dryness on a steam bath in a stream of nitrogen.

Preparation of Sample.—Transfer a quantity of oil solution or a finely powdered sample equivalent to about 0.5 mg. of ethinyl estradiol into a 125-ml. separator containing 50 ml. of heptane. Extract with 3 × 10 ml. of 10% NaOH, shaking vigorously for 2 min. during each extraction. Allow the layers to separate completely and withdraw the lower, alkaline layer into a second separator. Acidify with 10 ml. of H_2SO_4 (1:3), allow to cool, and extract with 3 × 25 ml. $CHCl_3$ shaking vigorously for 2

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TABLE I.—DETERMINATION OF THE REPRODUCIBILITY OF THE *R* VALUE

Estrone, mg.	Ethinyl Estradiol, mg.	(*A*)/(*B*)	*R* Value
0.544	0.201	0.297	0.803
0.544	0.201	0.302	0.815
0.544	0.402	0.605	0.816
0.544	0.603	0.905	0.814
0.544	0.804	1.229	0.825
$\bar{X}$ = 0.815			
σ = ±0.008			

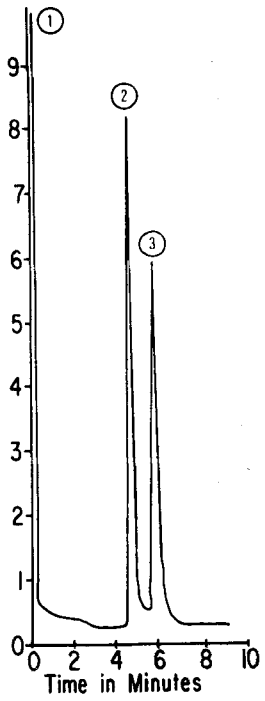

Fig. 1.—A typical chromatograph. Key: 1, solvent; 2, estrone; 3, ethinyl estradiol.

TABLE II.—DETERMINATION OF PER CENT RECOVERY

Sample	Theory, mg./ml.	Found, mg./ml.	Recovery, %
A	0.306	0.303	99.1
B	0.306	0.315	102.8
C	0.306	0.303	99.1
D	0.306	0.305	99.7
$\bar{X}$ = 100.2%			
σ = ±1.7%			

min. during each extraction. Wash the combined $CHCl_3$ extracts with 1 × 15 ml. of water, filter the $CHCl_3$ layer through a pledget of glass wool into a 150-ml. beaker, and wash the glass wool with 10 ml. of $CHCl_3$. Evaporate the $CHCl_3$ filtrate to about 2–3 ml. on a hot plate and transfer the solution quantitatively to a 15-ml. screw-cap vial using 3 × 2 ml. portions of $CHCl_3$ to effect the transfer. Evaporate the solution to dryness on the steam bath in a stream of nitrogen, taking extreme precautions to exclude steam from condensing on the inside of the vial. Pipet 1 ml. of estrone internal standard solution into the vial and evaporate to dryness on steam bath in a stream of nitrogen.

Acetylation.—To each vial, add 0.2 ml. of reagent grade pyridine and 1 ml. of reagent grade acetic anhydride. Swirl to dissolve the residue, cover with a piece of aluminum foil, and screw the cap on tightly. Heat at 70° for 30 min. Remove cap and evaporate the solution to dryness on a steam bath in a stream of nitrogen. Dissolve the residue in 0.5 ml. of CS_2.

Quantitation.—Set the attenuator to the ×10 position after the instrument has reached the operating temperatures, prime the column with 2 × 5 μl. of water followed by 2 μl. of acetone.

Inject approximately 1–2 μl. of each sample and standard solution, using a 10-μl. Hamilton syringe. The estrone (internal standard) peak has a retention time of about 4.4 min., while the retention time of ethinyl estradiol is about 5.9 min. The area under each peak is determined with the aid of the P.E. 194B printing integrator.

Calculations.—Using the method described by Celeste and Turczan (14), the calculations are as follows:

A.—For each of the standards, an *R* value is calculated as follows:

$$R = \frac{(A)(C)}{(B)(D)}$$

where

A = integrated area of ethinyl estradiol peak
B = integrated area of estrone peak
C = mg. of estrone added to each vial
D = mg. of ethinyl estradiol in each vial

B.—Quantitation of each sample is achieved *via* the following formulas:

mg. ethinyl estradiol/ml. oil =

$$\frac{(A)(C)}{(B)(R)} \times \frac{1}{(E)} \quad \text{(Eq. 1)}$$

TABLE III.—DETERMINATION OF ETHINYL ESTRADIOL IN DOSAGE FORMS

	Ethinyl Estradiol		
	Theory	Found	% of Theory
A, Sesame oil Solution I	0.300 mg./ml.	0.313 mg./ml.	104.3
		0.309 mg./ml.	103.0
B, Sesame oil Solution containing α-tocopherol	0.300 mg./ml.	0.299 mg./ml.	99.7
		0.303 mg./ml.	101.0
C, Sesame oil Solution II	0.300 mg./ml.	0.289 mg./ml.	96.3
		0.293 mg./ml.	97.7
D, Solid granulation I	91 mg./Gm.	89 mg./Gm.	97.8
E, Solid granulation II	91 mg./Gm.	90 mg./Gm.	98.9
F, Solid granulation III	91 mg./Gm.	93 mg./Gm.	102.2
$\bar{X}$ = 100.1%			
σ = ±2.7%			

where

A, B, and C have the previously noted connotations and

R = the average R value derived from the standard

E = ml. of oil sample taken for analysis

mg. ethinyl estradiol/Gm. =

$$\frac{(A)(C)}{(B)(R)} \times \frac{1}{(E)} \quad \text{(Eq. 2)}$$

where A, B, C, and R have the previously noted connotations and E = sample weight in Gm.

RESULTS AND DISCUSSION

Table I summarizes R values obtained in a typical calibration experiment. As can be seen, the standard deviation is approximately $\pm 1\%$. For comparison, peak height ratios were calculated, and the results indicate a standard deviation of $\pm 4.8\%$, illustrating the superior precision obtainable utilizing integrated areas. A typical chromatogram is reproduced in Fig. 1.

A solution was prepared of ethinyl estradiol in sesame oil to study the recovery of a sample subjected to this proposed procedure. The results of this experiment are shown in Table II.

Several samples of formulations prepared for clinical study have been analyzed by this technique. Data are summarized in Table III.

Although the authors have not encountered any examples of decomposition for ethinyl estradiol, the selectivity and sensitivity of the method appears adequate for stability studies. Certainly the method would be very sensitive to estrone, the most likely decomposition product.

SUMMARY

A quantitative gas liquid chromatographic procedure has been developed for the analysis of ethinyl estradiol using the internal standard technique. The precision of the gas chromatographic method is $\pm 1\%$, and the accuracy of the complete method is $100.2 \pm 1.7\%$. The method has been applied to oil solutions and solid granulations and permits a large number of replicate samples to be analyzed expeditiously with greater selectivity and sensitivity than previously reported methods.

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Technical Articles

Comparative Evaluation of Dextrose and Spray-Dried Lactose in Direct Compression Systems

By R. N. DUVALL, K. T. KOSHY, and R. E. DASHIELL

Many commercially available forms of dextrose have a distinct cost advantage over spray-dried lactose which is used extensively as a tablet excipient. The objective of this investigation was to compare the performance of a food grade dextrose with that of spray-dried lactose as an excipient in the direct compression of tablets. Several formulations were evaluated under accelerated stability conditions relative to changes in hardness, friability, disintegration time, and dissolution rate. Results indicated that dextrose can be partly or completely substituted for spray-dried lactose in some formulations. Dextrose was found to give less browning than spray-dried lactose in formulations containing no amines, whereas it gave more browning when amines were present.

IN RECENT years, interest in the direct compression of tablets has increased considerably. This technique is economical and enables one to tablet drugs which are not amenable to wet granulation procedures. The introduction of spray-dried lactose about 10 years ago and recent advances in tableting technology have lent considerable impetus toward the use of direct compaction methods. With today's potent medicinal agents, the active ingredient often makes up only

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