OC = operating characteristic curve
R_C = consumer's risk (set at a 10% level)
R_P = producer's risk (set at a 95% level)
CV = coefficient of variation [$CV = (s/m) \cdot 100$]
s = sample standard deviation
m = sample mean
μ = population or true mean
T = prescribed value
L = limit symmetrically set about the mean
k = fraction of the mean by which limits are expressed ($L = km$)
u = factor for calculating CV from k when a normal distribution is assumed ($CV = uk$)

Outsiders = specimens outside mean $\pm L$
Insiders = specimens inside mean $\pm L$

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Quantitative Gas-Liquid Chromatographic Determination of Estrone in Dermatological Products

PRAMOD P. KARKHANIS and JON R. ANFINSEN

Abstract □ A gas-liquid chromatographic procedure employing an internal-external standard ratioing technique is described for the analyses of estrone in dermatological preparations. The analysis of a cream or lotion is performed by the addition of an internal standard, extraction of sample with 10% sodium hydroxide, filtration, adjustment of the filtrate pH to 9–9.5, and chromatography on a 3% OV-1 column.

Keyphrases □ Estrone dermatological products—analysis □ Extraction procedure, estrone—internal–external standard ratioing technique □ GLC—analysis □ Equilenin solution—internal standard

Estrone has been incorporated in creams primarily for the treatment of senile vaginitis, pruritus vulvae, leukoplakia vulvae, and in emollients for the relief of local antikeratotic and trophic therapy in skin of the climacteric. In addition to the base, these preparations frequently contain vitamin A, hydrocortisone, and pyrilamine maleate for local antihistaminic and analgesic effect.

Several chemical methods for the estrone are found in the literature (1–5). However, due to the small amount of the steroid and the interference from the other ingredients in these pharmaceutical preparations, the results obtained with some of these methods were unreliable. The biological assay of estrone (6), based on the cellular change in the vagina of the spayed mouse or rat, gave erratic results.

Kroman et al. have quantitatively determined the concentration of estrone in the human plasma using a combination of chemical extraction and gas chromatography (7) and Wotiz and Chattoraj have described a method to determine estrone in low- and high-titer urine employing TLC and gas chromatography (8).

A GLC procedure has been described for ethinyl estradiol in both sesame oil solutions and solid dosage forms, using estrone as an internal standard, by Talmage et al. (9); Boughton et al. have determined ethinyl estradiol in tablets and granulations by gas chromatography using estrone as an internal standard (10). The proposed method, with a simple clean-up procedure, allows the separation and determination of estrone by gas chromatography while eliminating interferences from excipients commonly present in the creams and lotions.

EXPERIMENTAL

Instrument—Hewlett-Packard 5754A research chromatograph equipped with Hewlett-Packard 3370A electronic integrator and Honeywell Electronic 16 recorder.

Column—A 1.22-m. (4-ft.) helical glass column, 4 mm. i.d.

Liquid Phase—Three percent OV-1 on diatomite aggregate[1] (HP), 80–100 mesh (Supelco, Inc., Bellefonte, Pa.). The column is conditioned overnight at 300° with a helium flow rate of 45 ml./min.

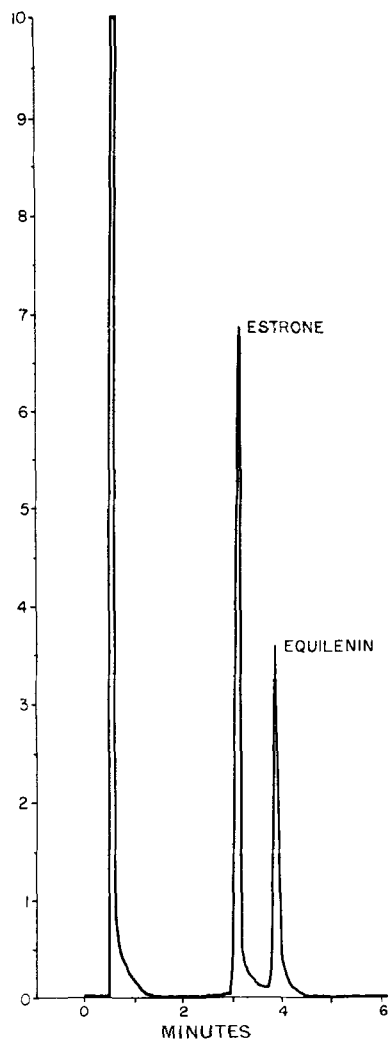[1] Chromosorb G, Johns-Manville Products Corp., New York, N. Y.

Figure 1—*A typical chromatograph.*

Operating Conditions—Hydrogen pressure 14 p.s.i.; air pressure, 30 p.s.i.; helium flow rate, 70 ml./min.; column oven temperature, 245°; detector temperature, 290°; injection port temperature, 290°; detector, flame ionization.

Reagents and Solutions—Estrone NF[2]; equilenin (K and K Laboratories, Inc.); chloroform NF; sodium hydroxide 10% aqueous solution; 6 N sulfuric acid.

Standard Estrone Solution—Weigh accurately 50.0 mg. of estrone NF into a 25-ml. volumetric flask. Dissolve in and make to volume with methanol–methylene chloride (1:1).

Internal Standard Equilenin Solution—Weigh accurately 50.0 mg. of equilenin into a 50-ml. volumetric flask. Dissolve in and make to volume with methanol.

Working Standard Solution—Pipet 1 ml. each of standard and internal standard solutions into a small stoppered flask. Evaporate the solvent to dryness on a steam bath. To the residue add 0.5 ml. of chloroform. Mix well.

Preparation of Sample Solution—Into a glass or polypropylene stoppered tube, weigh accurately an amount of sample containing approximately 1.0 mg. of estrone. With a pipet add 1 ml. of internal standard solution followed by 20 ml. of 10% sodium hydroxide solution. Heat on a steam bath for about 5 min. and then shake the tube on a mechanical shaker for about 10–15 min. or until the sample is well dispersed. Cool to room temperature. Filter the solution under vacuum through a 0.64-cm. (0.25-in.) layer of diatomaceous earth[3] spread over glass-fiber filter paper (2.1 cm.)

in a Gooch crucible. Rinse the tube with 10 ml. of water and filter the rinsing.

Transfer the filtrate to a 100-ml. beaker; rinse the vacuum flask with 10 ml. of water and transfer it to the beaker. Adjust the pH of the solution to 9–9.5 with 6 N sulfuric acid while mechanically stirring. Transfer this solution to a 125-ml. separator and extract three times with 25 ml. chloroform. Collect the chloroform layer and discard the aqueous layer after the third extraction. Filter the chloroform extract through about 5–6 g. of anhydrous sodium sulfate over a glass-fiber filter paper into a 125-ml. flask. Wash with 10 ml. chloroform. Evaporate the solvent to about 10 ml. on a Rinco evaporator. Transfer the solution to a small tube, rinse the flask with 5 ml. of chloroform, and transfer it to the tube. Then evaporate the solvent to about 1 ml. by heating in a stream of nitrogen. This is the working sample solution.

Chromatography—Balance the instrument after it has reached the operating temperatures and then set the range at 10^2 and attenuator to 8. Inject aproximately 3–4 μl. of working standard solution and working sample solution, using a 10-μl. Hamilton syringe. The estrone peak has a retention time of about 3.0 min., whereas the equilenin peak has a retention time of about 4.0 min. The area under each peak is determined with an electronic integrator or by employing a manual technique (11).

Calculations—Es = estrone peak area, Eq = equilenin peak area, A = micrograms of estrone in the working standard solution, and W = sample weight in grams:

$$\frac{A}{W} \times \frac{Es/Eq \text{ for sample}}{Es/Eq \text{ for std.}} = \text{mcg. estrone/g.} \qquad \text{(Eq. 1)}$$
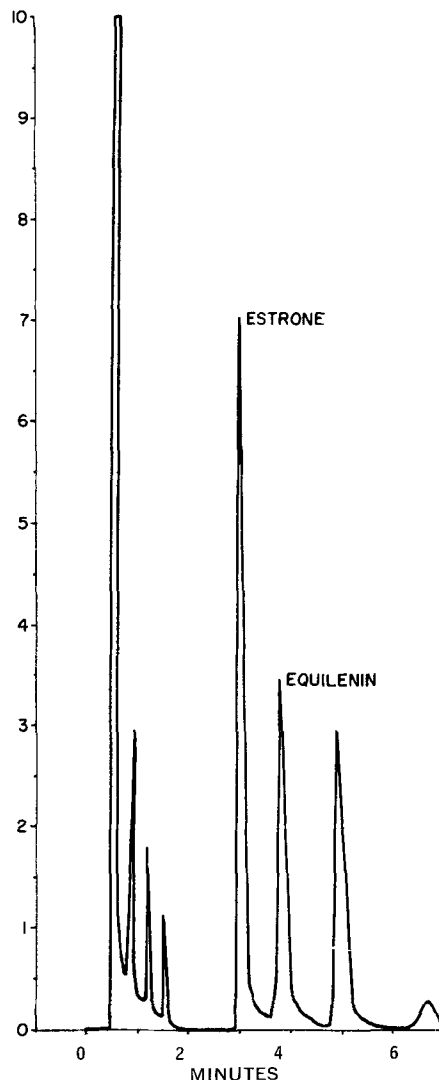

Figure 2—*A chromatograph obtained from the extract of dermatological preparation.*

Product	Label Claim, mcg. Estrone	Estrone Found, mcg./g.
Cream I	200.0	205.4
Cream II	200.0	202.9
Emollient	200.0	204.8

RESULTS AND DISCUSSION

The calibration curve for concentration of estrone *versus* integrated area of estrone/integrated area for equilenin gave a straight line. A typical chromatograph is shown in Fig. 1, and a chromatograph obtained from the extract of the dermatological preparation is shown in Fig. 2. Liquid phases such as SE-30, OV-1, and XE-60 were investigated. OV-1 [methyl silicone (Supelco, Inc.)] was found to be most satisfactory because of its stability at high temperatures.

The accuracy of a gas chromatographic procedure is dependent upon the ability to compensate for errors resulting from extraction, filtration, and injection. The internal–external ratioing technique makes it possible to minimize these errors when an internal standard with chemical properties similar to the compound being quantitated can be identified. The most important chemical similarity to achieve is that of partition coefficient since large errors are introduced by extraction losses. Equilenin was selected as an internal standard due to its very close structural relationship with estrone (Structures I and II) so that the losses incurred during the entire operation were compensated. The value of R, the response factor as described by Celeste and Turczan (12), was found constant; however, it should be noted that
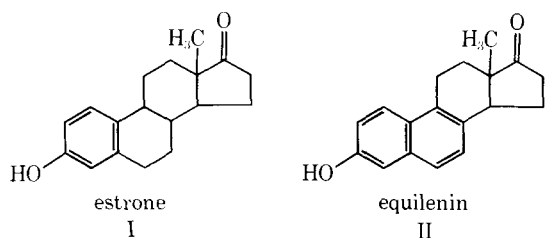
when employing the internal–external ratioing procedure, the determination of gas chromatographic response factor is not necessary.

The sample cream base formulated was composed of cetyl alcohol, stearyl alcohol, spermaceti, beeswax, white petrolatum, mineral oil, glycerin sodium lauryl sulfate, ascorbyl palmitate, BHT, BHA, and methyl- and propylparabens. Vitamin A palmitate and hydrocortisone alcohol were also present. To this cream base a fixed amount of internal standard of equilenin and known amounts of estrone at different levels were added. For 18 replicate samples, average recovery and coefficients of variation were found to be 98.9% and 1.1%, respectively. Three commercial preparations[4] were assayed for their estrone content. The results obtained are shown in Table I.

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H₃C O

HO

estrone

I

H₃C O

HO

equilenin

II