Quantitative GLC Determination of Conjugated Estrogens in Raw Materials and Finished Dosage Forms

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Abstract □ A GLC method is described for the quantitative analysis of conjugated estrogens. The procedure was used for the analysis of various estrogen mixtures obtained from formulations containing estrogen sodium sulfate salts. The method involves slurrying or dissolving a portion of the formulation in pH 5.2 buffer, hydrolysis with sulfatase enzyme, extraction with ethylene dichloride, and, finally, GLC separation and quantitation of the corresponding trimethylsilyl ethers on a 2.5% diethylene glycol succinate column.

Keyphrases □ Estrogen sodium sulfate salt formulations—enzymatic hydrolysis, GLC separation and analysis of conjugated estrogens □ Conjugated estrogen mixtures—enzymatic hydrolysis of estrogen sodium sulfate salt formulations, GLC separation and analysis □ GLC—separation, analysis, conjugated estrogens in estrogen sodium sulfate salt formulations

The analysis of conjugated estrogen mixtures has been the object of many studies. Various techniques such as TLC (1–3), liquid partition chromatography (4), colorimetric analysis (5), and GLC (6–9) have been used to separate and, in some cases, quantitate hydrolyzed estrogen mixtures of varying complexity. Gel permeation chromatography (10) also has been used in the partial resolution of some estrogen mixtures in conjugated form.

However, most reported methods are of limited use in completely separating and quantitating equine estrogen mixtures containing nine or more structurally similar estrogens. In many cases, these methods are not suitable for routine use because they may involve harsh hydrolysis techniques, lengthy extraction and purification steps, long elution times, and/or complex chemistry in the development of characteristic colors prior to quantitation.

These problems initiated the development of an assay method that would conveniently and completely hydrolyze the conjugated estrogens without creating artifacts and that would separate and quantitate the individual free estrogens. GLC appeared to offer the most promising method for separating and quantitating free estrogen mixtures. To accomplish this goal, it was necessary to develop a quantitative procedure for the complete conversion of the steroid conjugates to the free estrogens. After extensive investigation involving hot acid, solvolysis, and enzyme hydrolysis, the enzyme procedure was selected because it provided complete hydrolysis in the presence of tablet and other excipients without causing degradation.

Other criteria imposed and satisfied by this method are that it be relatively rapid, involve a limited number of steps, and offer a high degree of precision and accuracy. The method developed involves enzyme hydrolysis of the estrogen conjugates, a single extraction step, formation of the trimethylsilyl ether derivatives, and, finally, GLC separation on a very polar, selective column. The major estrogen peaks (up to 11) are then quantitated with disk or digital integration, using testosterone as the internal standard.

EXPERIMENTAL

Instrumentation—A research grade gas chromatograph[1] equipped with a U-shaped column and flame-ionization detector, together with a recorder[2] fitted with a disk integrator or equivalent, was used. A silanized (11) 1.8-m (6-ft) U-shaped glass column, 4 mm i.d., was packed with 2.5% diethylene glycol succinate[3] on 100–120 Gas Chrom Q[3]. This packing is prepared by using a 2.0–2.5% solution of the polyester in acetone and by making use of the absorptive properties of the solid support as described previously (12, 13).

Thorough degassing of the solid support under vacuum is also required to get a uniform liquid phase coating and to minimize tailing. The fluidized packing (14) is carefully packed into a column and conditioned with oxygen-free[4] nitrogen carrier gas at 200° overnight at 45 ml/min. Several injections of a silylating reagent[5] are made to achieve a stable baseline. After conditioning and when the column is not in use, the column oven temperature should be lowered to 175°.

Chromatographic Conditions—The following were utilized: column oven temperature, 195 ± 5°; detector temperature, 240°; injection port temperature, 225°; nitrogen flow rate, 45 ml/min; hydrogen flow rate, 40 ml/min; air flow rate, 1.8 S.C.F.H.; and range and attenuation, 100-10-1K (1×10^{-9} amp).

Reagents and Standards—The following required reagents and solvents were all of reagent grade quality or equivalent: 3A ethanol, ethylene dichloride, acetic acid, redistilled dry pyridine, anhydrous sodium sulfate, and anhydrous sodium acetate. The nine free estrogens used to determine chromatographic response factors relative to testosterone[6] were of reference standard quality[7].

Working Enzyme Solution—Sufficient enzyme concentrate[8] is taken and diluted to 50 ml with distilled water to give a working solution containing about 1000 ± 100 units of sulfatase enzyme/ml. All enzyme solutions should be stored at 4° until use.

Buffer Solution—The pH 5.2 buffer is prepared by combining 21.0 ml of 0.2 *M* acetic acid solution and 79.0 ml of 0.2 *M* sodium acetate solution. The solution should be checked on a pH meter and adjusted, if necessary, to pH 5.2 ± 0.1 unit.

Standard Steroid Solutions—Standard solutions of the steroids are prepared in 3A ethanol with the approximate desired concentrations (±10%) as follows: 17α-estradiol, 15 μg/ml; 17β-estradiol, 2 μg/ml; 17α-dihydroequilin, 45 μg/ml; 17β-dihydroequilin, 6 μg/ml; 17α-dihydroequilenin, 6 μg/ml; 17β-dihydroequilenin, 6 μg/ml; estrone, 180 μg/ml; equilin, 90 μg/ml; equilenin, 18 μg/ml; and testosterone, 100 μg/ml.

Standard Solution—One milliliter of each of the ten standards

[1] Bendix model 2500 and F&M model 400 were used in these studies.
[2] Honeywell 19 recorder.
[3] Hi-Eff 1B, Applied Science Laboratories, State College, Pa.
[4] Oxy-Sorb Trap, Regis Chemical Co., Morton Grove, Ill.
[5] Regisil [bis(trimethylsilyl)trifluoroacetamide plus 1% trimethylchlorosilane].
[6] Analytical grade, Nutritional Biochemicals Inc., Cleveland, Ohio.
[7] Ayerst Research Laboratories, Montreal, Quebec, Canada.
[8] Glusulase, Endo Laboratories, Garden City, N.Y.

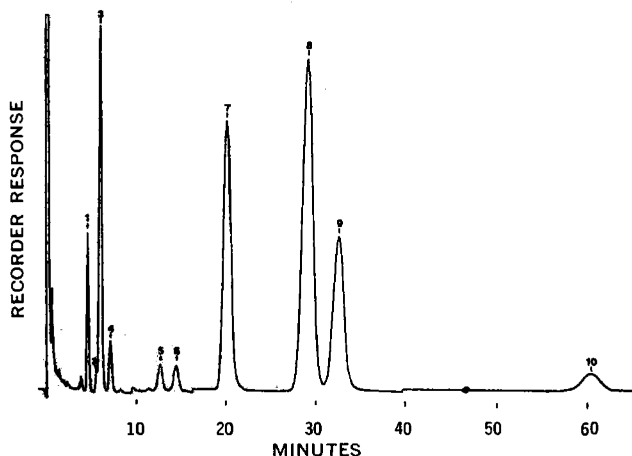


Figure 1—*Separation of the components of a nine-estrogen standard with testosterone as the trimethylsilyl ethers. The peaks with their relative retention times are: 1, 17α-estradiol (0.230); 2, 17β-estradiol (0.272); 3, 17α-dihydroequilin (0.299); 4, 17β-dihydroequilin (0.353); 5, 17α-dihydroequilenin (0.627); 6, 17β-dihydroequilenin (0.714); 7, testosterone (1.00); 8, estrone (1.452); 9, equilin (1.615); and 10, equilenin (2.988).*

is pipetted into a 12-ml centrifuge tube equipped with a Teflon-lined screw cap. Just prior to use, the solutions are evaporated to dryness at 40° in a water bath. The residue is dissolved in 15 μl of pyridine and 65 μl of silylating reagent[5], mixed well, and allowed to stand 15 min prior to injection. (The solution should be refrigerated when not in use and discarded after 24 hr.)

Preparation of Sample Extracts—*Tablets*—Randomly select 10 tablets from the sample and weigh to the nearest 0.1 mg. Place them in a mill[9] with one large and one small steel ball and mill them for 3 min. Collect the powder in an amber bottle and weigh a portion to the nearest 0.1 mg into a 60-ml (2-oz) extraction bottle fitted with a Teflon-lined screw cap. The sample weight should be approximately equivalent to one tablet so that from 0.3 to 2.5 mg of conjugated estrogens is taken for assay.

Add 15 ml of pH 5.2 buffer to the bottle and cap tightly. Shake mechanically for 0.5 hr, sonify for 30 sec in a sonic bath, and shake for an additional 0.5 hr. Add approximately 1000 units of sulfatase enzyme to the bottle and incubate for 20 min with shaking in a 50 ± 2° water bath. Immediately, without cooling the enzyme hydrolysate, pipet 15 ml of ethylene dichloride into the bottle, cap tightly, and shake mechanically for 15 min. Centrifuge the bottle at ~2000 rpm for 10 min and repeat if necessary to obtain a clear lower layer. Remove as much of this ethylene dichloride layer as possible and filter through a small amount of sodium sulfate (~5 g) in a 3.5-cm plastic funnel, containing a glass wool plug, into a 12-ml centrifuge tube.

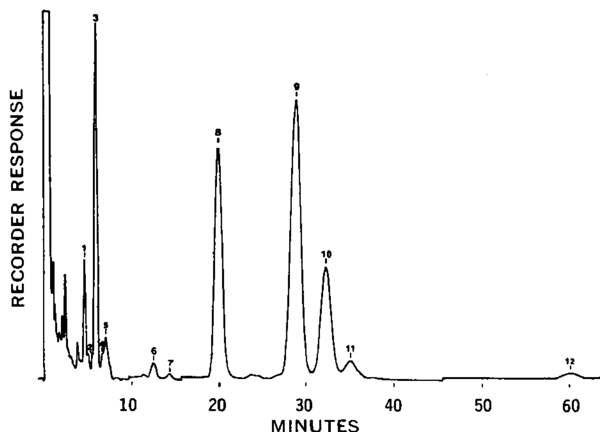


Figure 2—*Separation of a mixture of estrogen trimethylsilyl ethers derived from Sample A (see Table II). The peaks with their relative retention times are: 1, 17α-estradiol (0.235); 2, 17β-estradiol (0.280); 3, 17α-dihydroequilin (0.304); 4, iso-17α-dihydroequilin (0.337); 5, 17β-dihydroequilin (0.356); 6, 17α-dihydroequilenin (0.624); 7, 17β-dihydroequilenin (0.715); 8, testosterone (1.00); 9, estrone (1.443); 10, equilin (1.604); 11, $\Delta^{8,9}$-dehydroestrone (1.743); and 12, equilenin (2.980).*

[9] Spex.

Table I—Characteristics of Different Batches of Diethylene Glycol Succinate Liquid Phase

Measurement	Batch 1922	Batch 1989
IR, cm^{-1}	3530, 3460, 3260, 2960, 2880	3550, 3470, 2970, 2900
TGA	Two-step weight loss curve	Single-step weight loss curve
Viscosity[a], centistokes	0.646	0.739
Titration[b], mEq H^+/g	1.00	0.143
Water[c], %	1.03	0.86

[a] These are relative measurements obtained on a 5% solution of polymer in acetone at 25° using a Ubbelohde viscometer, model 1B-290. [b] The polymer was dissolved in dimethylformamide and titrated with tetrabutylammonium hydroxide in benzene–methanol (8:1). [c] The Karl Fischer method was used.

Pipet a known aliquot containing 200–500 μg of free estrogens into another 12-ml centrifuge tube fitted with a Teflon-lined screw cap. Add 1 ml of internal standard solution and evaporate to dryness at 40° in a water bath using nitrogen. To this dry residue, add 15 μl of dry pyridine and 65 μl of silylating reagent[5]. Mix thoroughly and allow the tube to stand 15 min prior to injection. The solution is stable and may be stored in a refrigerator up to 24 hr in case a reassay is required.

Injectable Vials—Carefully remove the caps from five lyophilized vials and add 5 ml of water to each vial. Recap, shake to dissolve the cake, pipet 2 ml from each vial into a 25-ml volumetric flask, and then dilute to volume. Pipet 1 ml into a 60-ml (2-oz) extraction bottle fitted with a Teflon-lined screw cap and add 15 ml of pH 5.2 buffer and 1000 units of sulfatase enzyme. Proceed with the hydrolysis and remaining steps described under *Tablets*.

Raw Materials—Given an approximate potency for the powdered sample, weigh one sample containing about 7.5 mg of conjugated estrogens into a 120-ml (4-oz) extraction bottle fitted with a Teflon-lined screw cap. Pipet 75 ml of pH 5.2 buffer into the bottle and shake mechanically for 10 min. Sonify the sample for 0.5 min in a sonic bath and then shake for an additional 5 min. Immediately pipet 15 ml of uniform suspension into a 60-ml (2-oz) extraction bottle. Add 1000 units of sulfatase enzyme and proceed with the hydrolysis and remaining steps under *Tablets*.

Instrument Standardization—Once the instrument has reached its operating temperature, the column should be conditioned with several injections of silylating reagent until a stable baseline is obtained. Inject about 2–4 μl of silylated standard and adjust the sample size and/or instrument attenuation so the major peaks (17α-dihydroequilin, testosterone, and estrone) are greater than 70% of full-scale deflection. To establish the identity of the standard estrogen peaks, compare their relative retention times (RRT) *versus* testosterone with those indicated in Fig. 1.

Measure accurately the areas of the estrogen and testosterone peaks using disk integration. Assume an exponential decay of the solvent peak and draw in this curve to meet the observed baseline points after 17α-estradiol and after 17β-dihydroequilin. Drop perpendiculars from each valley between the pertinent peaks to the projected baseline. This procedure will allow proper allocation of the peak areas to the estrogens involved.

Using the measured peak areas and known concentrations, calculate a response factor (RF) for each of the nine estrogens as follows:

$$RF = \left(\frac{A_t}{C_t}\right)\left(\frac{C_e}{A_e}\right) \quad \text{(Eq. 1)}$$

where A_t = area of testosterone peak, C_t = micrograms of internal standard (testosterone) in standard, C_e = micrograms of estrogen in standard, and A_e = area of estrogen peak.

The standard is injected again following the last sample scan of

Table II—Compositions of Various Commercial Samples Containing Mixtures of Estrogen Sodium Sulfate Salts[a]

Component[b]	Sample											
	A[c]	B[c]	C[c]	D[c]	E[d]	F[d]	G[d]	H[e]	I[f]	J[g]	K[h]	L[h]
I	0.063	0.002	0.005	0.048	0.004	—	0.009	—	0.12	0.85	2.70	1.77
II	0.009	0.003	0.229	—	0.003	0.022	0.005	—	0.05	0.14	—	0.29
III	0.217	0.039	—	0.071	0.007	—	0.080	—	0.69	3.43	0.40	6.78
IV	0.028	0.005	—	—	—	—	0.011	—	0.11	0.37	—	0.94
V	0.019	0.013	—	0.036	0.006	—	0.006	—	—	0.49	2.60	0.58
VI	0.007	—	—	—	—	—	—	—	—	0.13	—	0.29
VII	0.747	0.786	0.806	0.653	0.488	0.792	0.612	0.788	13.21	13.74	271.0	22.82
VIII	0.395	0.335	0.329	0.330	0.100	0.040	0.028	0.116	6.75	7.17	27.7	12.11
IX	0.028	0.037	0.122	0.036	0.052	0.023	—	0.017	0.58	0.82	2.9	0.69
Total	1.513	1.240	1.491	1.174	0.660	0.877	0.751	0.921	21.51	27.14	307.3	46.27

[a] These numbers represent an average of two separate determinations. [b] All components are expressed as the estrogen sodium sulfate salts. [c] Values are reported as milligrams per tablet, and samples have a label claim of 1.25 mg of conjugated estrogens/tablet. [d] Samples have a label claim of 0.625 mg of conjugated estrogens/tablet, and all values are reported as milligrams per tablet. [e] Sample of international origin has a label claim of 1.00 mg of conjugated estrogens/tablet with values as milligrams per tablet. [f] An international sample with a label claim of 20 mg/vial with all values reported as milligrams per vial. [g] A sample of international origin with a label claim of 25 mg/vial with values reported as milligrams per vial. [h] Powdered raw material samples with values reported as milligrams per gram.

the day, and the RF's are calculated and averaged with the first run. The average RF's are then used in subsequent sample calculations.

Chromatographic Analysis—Inject a portion of silylated sample. The injection size and/or instrument attenuation should again be adjusted to keep the major peaks greater than 70% of full scale. The sample estrogen peaks can be identified by comparing their RRT's *versus* testosterone with those indicated on the sample GLC scans (Figs. 2–4). Accurately measure the areas of the estrogen and testosterone peaks using disk integration.

For the peak clusters that are not completely resolved, the areas are allocated by dropping a perpendicular from the minimum to a baseline projected across the base of the peak group. For the quantitation of two naturally occurring double bond isomers, iso-17α-dihydroequilin and $\Delta^{8,9}$-dehydroestrone, two approaches can be taken. They can be listed separately and quantitative values can be reported for each, or their areas can be added to the areas of 17α-dihydroequilin and equilin, respectively, since they are structurally closely related. For this study the second approach is used since it allows a more direct comparison of the GLC values obtained here with those values obtained using an official method (17). With this procedure, a single injection from each of four samples can be assayed in between two injections of the standard on a single column in 1 day.

The weight of the individual conjugated estrogens present in the samples is calculated from the response factors, the sample peak areas, and the constant; the weights of the individual species are then summed to get the total weight of conjugated estrogens present in the sample as follows:

$$\text{weight conjugated estrogens} = \text{constant}\,(\mathrm{RF_e})(A_e) = \frac{\text{mg conjugated estrogens}}{\text{unit dosage form (tablet, vial, grams)}} \quad \text{(Eq. 2)}$$

and:

$$\text{constant} = \frac{15\text{ ml }(1.38)(10^{-3}\text{ mg}/\mu\text{g})(C_t)}{x\text{ ml }(y)(A_t)}\,(\text{dilution factor}) \quad \text{(Eq. 3)}$$

where:

- 15 ml = extract volume of ethylene dichloride
- 1.38 = factor for converting "free" estrogens into their corresponding estrogen sodium sulfate salts
- 10^{-3} mg/μg = conversion factor for micrograms to milligrams
- C_t = micrograms of testosterone in sample
- dilution factor = inverse of any additional dilution of any sample
- x ml = number of milliliters of ethylene dichloride extract taken for assay
- y = equivalent number of dosage form units taken into the assay (tablets, vials, grams)
- A_t = area of testosterone peak in sample

RESULTS AND DISCUSSION

Sample Preparation—During the early phases of this development work, attempts were made to extract the estrogen conjugates from tablet or formulation excipients. Initial results from recovery studies of estrone sodium sulfate from placebo tablets showed some small and unreproducible losses. Also, the multiple extractions required proved tedious and time consuming. Therefore, the technique of suspending a portion of the powdered formulation in buffer, in the presence of sulfatase enzyme, was tried. This straightforward simple procedure was found to give consistent, quantitative recoveries.

Various studies with mineral acid hydrolysis of estrogen conjugates (15, 16) have shown that side reactions and steroidal transformations frequently occur. Since interest was in measuring the original components of the mixture without creating artifacts during workup, acid hydrolysis was not used.

The level of enzyme necessary to achieve complete hydrolysis in the presence of excipients was also studied. Earlier work with very high levels of enzyme indicated that protein binding of the estrogens and thick emulsions would be problems. The lower levels of enzyme used in this method (~1000 units of arylsulfatase/0.3–2.5 mg of conjugated estrogens) give complete hydrolysis of the conjugated estrogens within a short time without causing sample preparation problems. A more detailed study of the enzyme procedure will appear elsewhere.

Approximately 45 tablet samples from almost as many different manufacturers were assayed with this method. For two samples, the enzyme levels specified here were not high enough to achieve

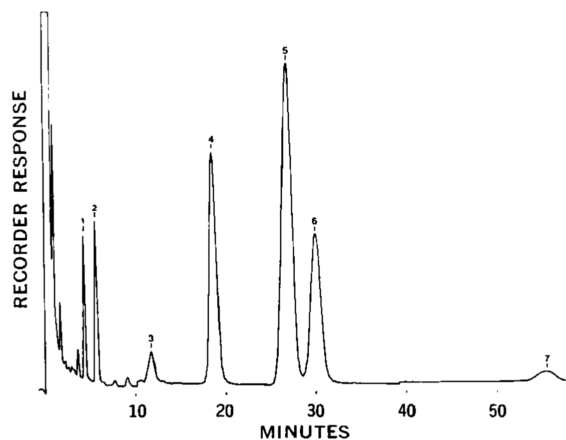
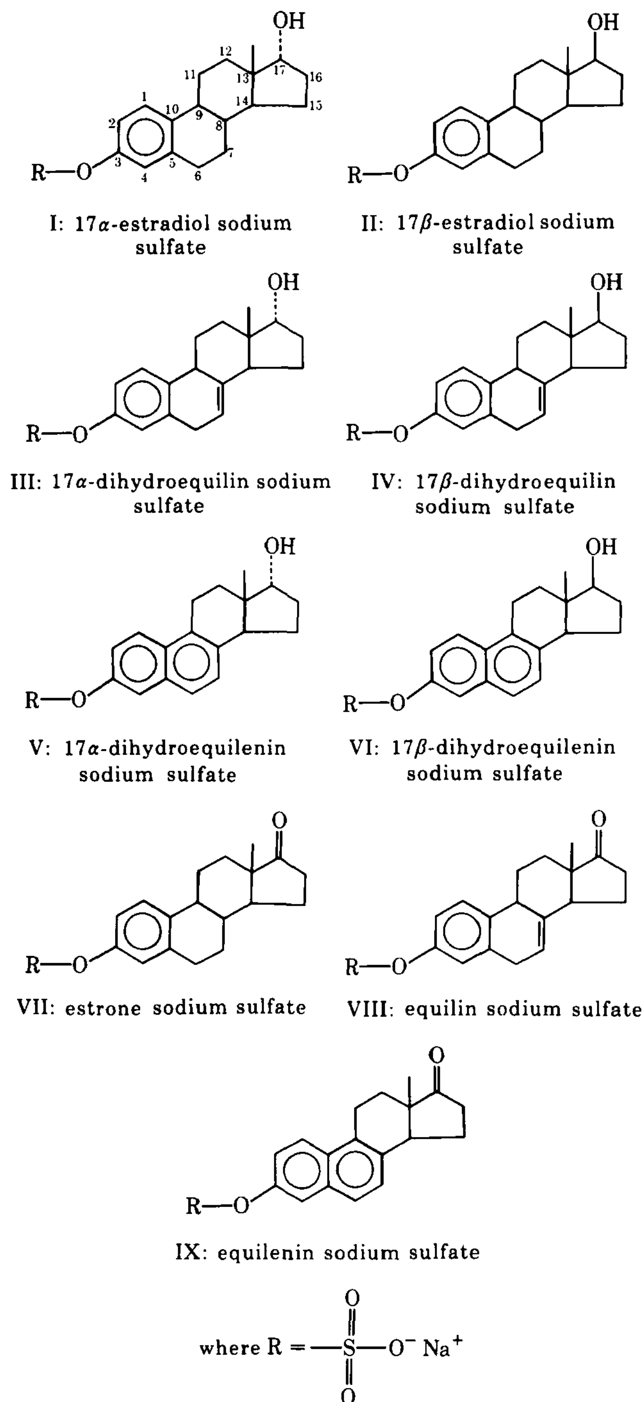


Figure 3—*Separation of a mixture of estrogen trimethylsilyl ethers derived from Sample D (see Table II). The peaks with their relative retention times are: 1, 17α-estradiol (0.233); 2, 17α-dihydroequilin (0.299); 3, 17α-dihydroequilenin (0.626); 4, testosterone (1.00); 5, estrone (1.441); 6, equilin (1.604); and 7, equilenin (2.957).*



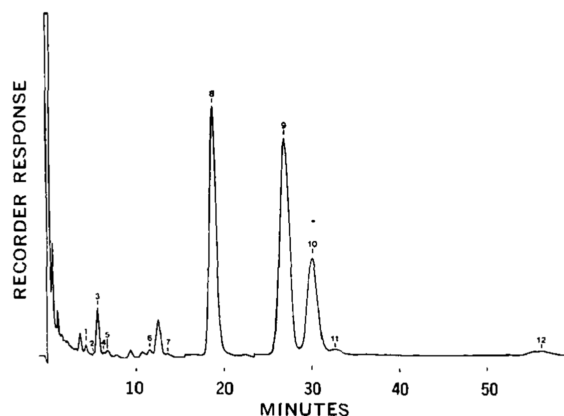


Figure 4—*Separation of a mixture of estrogen trimethylsilyl ethers derived from Sample I (see Table II). The peaks with their relative retention times are: 1, 17α-estradiol (0.232); 2, 17β-estradiol (0.281); 3, 17α-dihydroequilin (0.300); 4, iso-17α-dihydroequilin (0.333); 5, 17β-dihydroequilin (0.355); 6, 17α-dihydroequilenin (0.609); 7, 17β-dihydroequilenin (0.721); 8, testosterone (1.00); 9, estrone (1.427); 10, equilin (1.592); 11, $\Delta^{8,9}$-dehydroestrone (1.734); and 12, equilenin (2.969).*

complete hydrolysis, because of the presence of certain tablet excipients that inhibited enzyme activity. A 5–20-fold increase in enzyme activity was necessary to get complete hydrolysis and consistent recoveries in these two instances. Phosphate, a known inhibitor of sulfatase activity, was found in these two samples.

Chromatography—A systematic study of a large number of liquid phases was carried out in an attempt to resolve complex estrogen mixtures of the type obtained from pregnant mare's urine. These mixtures are known to contain estrone, equilin, equilenin, 17α- and 17β-estradiols, 17α- and 17β-dihydroequilins, and 17α- and 17β-dihydroequilenins (1); the structures of these steroids as the sodium sulfate salts are as shown (I–IX).

Only two commercial liquid phases were found, both of which were polyesters (ethylene glycol succinate and diethylene glycol succinate), that did separate the major components of the estrogen mixture. The diethylene glycol succinate, however, offered the most promise because of its slightly improved thermal stability and its ability to perform the separation of the estrogen trimethylsilyl ethers in a reasonable time. Two major estrogens, estrone and equilin, from urinary equine extracts are almost completely resolved on this column, and excellent quantitative results can be obtained. To the knowledge of the authors, a quantitative GLC method that allows the direct determination of estrone and equilin simultaneously has not been reported previously.

The repeated preparation of diethylene glycol succinate columns showed that not all columns gave the same degree of peak resolution and peak symmetry. Some difference existed between the batches of liquid phase obtained from the same source. Therefore, IR, viscosity, thermogravimetric analysis (TGA), titration, and water measurements were made in an attempt to pinpoint the factors accounting for variations in the quality of the batches of polymer tested. The results are summarized in Table I for two batches of diethylene glycol succinate, one of which gave an excellent column packing (Batch 1922) and the other a poor packing (Batch 1989).

Interpretation of the data in Table I allows several conclusions to be made about the relative differences between the two batches of liquid phase tested. The batch of diethylene glycol succinate giving a high quality packing is more acidic (by both IR and titration) and has a lower viscosity; furthermore, the two-step TGA curve suggests that a wide range of molecular weight polymer units is present since a weight loss occurs at a temperature believed to be below the degradation temperature (~225°). These techniques and relative values are now being used on a routine basis to evaluate new batches of liquid phase prior to use.

In an attempt to control operator performance in reproducibly preparing the packings, TGA is used to monitor all new column packings. An acceptable weight range for the liquid phase loading varies from about 2.0 to 2.5%.

Quantitation—The quantitative composition of 12 different commercial samples containing conjugated estrogens is found in Table II. The table includes results for tablets of varying strengths together with raw material samples; the numbers represent an average of two individual determinations. The data clearly show the gravimetric quantities of all individual estrogenic components present in the samples for the first time. By summing these values, a total potency value is obtained which may differ significantly and is usually higher than the potency value obtained using an official method (17).

SUMMARY

A GLC method was developed for the quantitative analysis of complex mixtures containing conjugated estrogens. The method was used on both raw materials and finished dosage forms. It allows, for the first time, a complete delineation and quantitation of the conjugated estrogens present in extracts derived from preg-

nant mare's urine. Since the quality of the diethylene glycol succinate liquid phase and packed column is critical to the success of the method, details are provided relating to their use and conditioning.

Preliminary collaborative testing is underway to evaluate the use of this procedure as a routine control method. This method has also been used for the analysis of free estrogens, which might be present in formulations containing conjugated estrogens, by simply omitting the hydrolysis step and carrying out the balance of the procedure.

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ACKNOWLEDGMENTS AND ADDRESSES

Received July 5, 1973, from the *Analytical Research and Development Laboratory, Ayerst Laboratories, Inc., Rouses Point, NY 12979*

Accepted for publication November 25, 1974.

This work was carried out in the Analytical Research and Development Laboratory, Rouses Point, N.Y., and in the Analytical Research Department, Quebec, Canada, of Ayerst Laboratories, Inc.

The authors thank Mr. R. Daley for the IR scans, Mr. F. Gemmill for the TGA scans, Mr. M. Reimringer for the viscosity measurements, Mr. F. DiBernardo for the titration and water analyses, and Dr. G. Myers for supplying reference estrogen compounds. They also thank Dr. C. Warner for his constructive comments and criticisms of this paper and for his continued support and advice.

[x] To whom inquiries should be directed.

Analysis of Pholcodine in Cough Preparations

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Abstract □ A rapid and simple method for the assay of pholcodine in various syrup and linctus formulations, based on color reaction with *p*-dimethylaminobenzaldehyde, is suggested. At specified conditions, the results obtained show good reproducibility.

Keyphrases □ Pholcodine—analysis in cough preparations □ Antitussives—analysis of pholcodine in cough preparations □ *p*-Dimethylaminobenzaldehyde—reagent, analysis of pholcodine in cough preparations

Pholcodine resembles codeine in its action as a cough suppressant for the relief of unproductive cough. It has some pharmacological advantages over codeine including the absence of side effects such as constipation and other withdrawal symptoms (1).

Although pholcodine, like codeine, is a common component in syrups and linctus formulations, little has been written concerning the assay methods of pholcodine in pharmaceutical preparations. It is often the sole active component in a preparation[1] but may be combined with ephedrine hydrochloride, papaverine hydrochloride, promethazine, *etc.*

[1] Examples for such preparations are Copholco (Wade Co.), Falcodyl (Norton), Sancos (Sandoz), *etc.*

DISCUSSION

Pholcodine linctus formulations have appeared in the BPC (2). The official assay procedure involves extraction with chloroform from an alkaline solution followed by nonaqueous titration. The accuracy of the method depends on the purity of inactive components, such as sucrose, sorbitol, glycerol, citric acid, and chloroform spirit, used in the formulation. Since these components constitute the majority of the linctus weight, any impurity may introduce error. Theoretically, this difficulty would be overcome by running a blank linctus containing all components except pholcodine. However, since there are so many formulations and variations, running a blank cannot be considered a practical solution. This method also suffers from the use of a relatively dilute titrant (0.02 N $HClO_4$) which affects the sharpness of the end-point.

No method or modification has been proposed (2) for the assay of pholcodine combined with other drugs. The nonaqueous titration cannot be applied, since the free bases of most drugs commonly formulated with pholcodine are miscible in chloroform and would be titrated simultaneously with the pholcodine. This is the case with ephedrine, pseudoephedrine, oxomemazine, promethazine, codeine, promazine, papaverine, chlorpheniramine, trimeprazine, *etc.* Other methods of analysis of pholcodine in syrups are the reaction with hexamethylenetetramine–lactose reagent (3) (ephedrine interferes) and TLC (4, 5).

The method proposed in this paper utilizes the reaction of pholcodine with *p*-dimethylaminobenzaldehyde in strong acidic medium. Under the specified conditions, reproducible results are obtained in the assay of pholcodine when formulated alone or in combination with other drugs. The present method also has the