Physicochemical Properties of Amphoteric β-Lactam Antibiotics II: Solubility and Dissolution Behavior of Aminocephalosporins as a Function of pH

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Abstract □ The solubility of aminocephalosporins in aqueous solution at 37° and an ionic strength of 0.5 exhibited U-shaped curves against pH. At their isoelectric pH, cephradine monohydrate was the most soluble, followed by cephalexin monohydrate and cephaloglycin dihydrate, with intrinsic solubilities of 26.0, 17.2, and 14.8 mg/ml, respectively. The dissolution rate constants from the rotating disk were also determined as a function of the pH of the dissolution medium and interpreted reasonably by the simultaneous dissociation equilibrium reaction and the diffusion kinetics model. Energies for the solubility and dissolution were determined for these three aminocephalosporins.

Keyphrases □ β-Lactam antibiotics—various aminocephalosporins, solubility and dissolution, effect of pH □ Aminocephalosporins, various—solubility and dissolution, effect of pH □ Solubility—various aminocephalosporins, effect of pH □ Dissolution—various aminocephalosporins, effect of pH □ Antibacterials—various aminocephalosporins, solubility and dissolution, effect of pH

The solubility and dissolution rate of drugs ingested orally are important physicochemical properties for understanding their bioavailability and the rate-limiting step in absorption. Previously (1), the dissolution behavior of aminopenicillins such as ampicillin, amoxicillin, and cyclacillin, which are used therapeutically by the oral route, was described. It was concluded that the dissolution of these antibiotics follows diffusion-controlled kinetics over the entire pH range and cannot become the rate-limiting step of their GI absorption, consistent with the previous claim (2) given for ampicillin.

The present paper describes the solubility and kinetics of the dissolution rate of some orally effective aminocephalosporins, cephalexin, cephradine, and cephaloglycin, as a function of the dissolution medium pH.

EXPERIMENTAL

Materials—Cephalexin monohydrate[1] (925 μg/mg), cephradine monohydrate[2] (952 μg/mg), and cephaloglycin dihydrate[1] (962 μg/mg) were used as supplied. All chemicals were the highest grade available commercially.

Procedure—Procedures for the determination of pKa values, diffusion coefficients, solubility, and dissolution rates were described previously (1). Aminocephalosporins were determined by UV spectrophotometric measurement[3] at 260 nm. All experimental conditions were chosen from previously published kinetic data (3) so that the degradation of antibiotics was within 5%.

RESULTS AND DISCUSSION

Solubility–pH Profile—The solubility of aminocephalosporins was determined as a function of the solution pH at 37° and $\mu = 0.5$ (Scheme I). The relationship between the solubility and pH showed U-shaped curves with the minimum solubility near the respective isoelectric point (Fig. 1), similar to those of aminopenicillins (1, 4).

Table I—Intrinsic Solubility and Dissociation Constants of Aminocephalosporins and Aminopenicillins[a] at 37° and $\mu = 0.5$

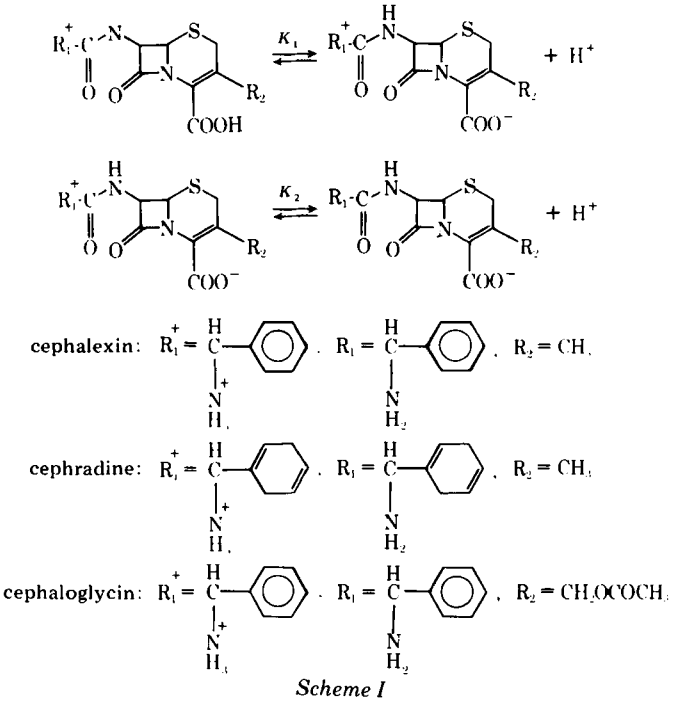
Antibiotic	Molecular Weight	Solubility[b], $10^2 \times C_0$, M (mg/ml)	Dissociation Constants[c] pK_1	pK_2
Cephradine monohydrate	367.4	7.08 (26.0)	2.63	7.35
Cephalexin monohydrate	365.4	4.72 (17.2)	2.67	6.96
Cephaloglycin dihydrate	441.4	3.36 (14.8)	2.03	6.89
Ampicillin trihydrate	403.4	2.23 (9.0)	2.67	6.95
Ampicillin anhydrate	349.4	3.97 (13.9)		
Amoxicillin trihydrate	419.4	1.30 (5.4)	2.67	7.11
Cyclacillin anhydrate	341.4	8.90 (30.4)	2.64	7.18

[a] Values determined previously (1). [b] Defined by Eq. 1. [c] Determined by the potentiometric method.

The total solubility, C_T, as a function of the solution pH can be expressed as:

$$C_T = C_0\left(\frac{a_{H^+}}{K_1} + 1 + \frac{K_2}{a_{H^+}}\right) \qquad \text{(Eq. 1)}$$

where C_0 is the intrinsic solubility of amphoteric cephalosporins with the electrically neutral zwitterion, a_{H^+} is the hydrogen-ion activity of the solution, and K_1 and K_2 are dissociation constants for 4-carboxylic acid and the conjugated acid of the α-amino group, respectively. The C_0 values estimated from the solubility near the isoelectric point, $pI = \frac{1}{2}(pK_1 + pK_2)$, and the dissociation constants determined by a potentiometric method (1) under the same experimental conditions are listed in Table I. As seen in Fig. 1, the theoretical curves generated from Eq. 1 fit rea-



Scheme I

[1] Shionogi & Co., Osaka, Japan.
[2] Sankyo Co., Tokyo, Japan.
[3] Model UV-200S double-beam recording spectrophotometer, Shimadzu Seisakusho, Kyoto, Japan.

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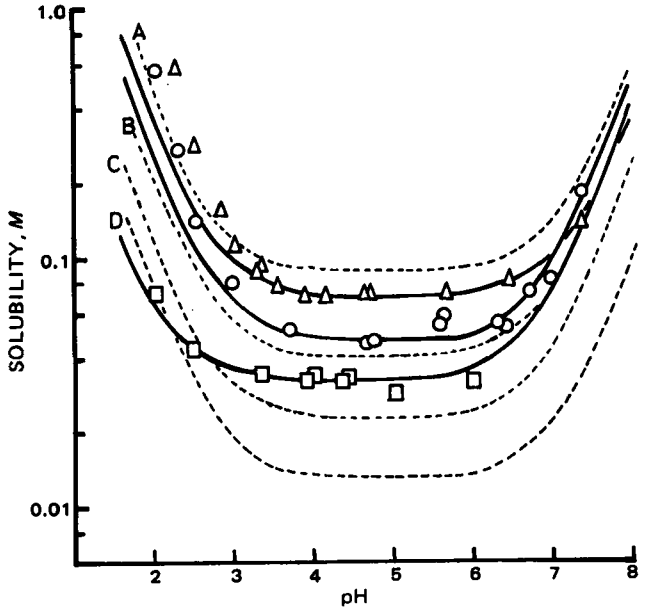

Figure 1—*Solubility–pH profiles of aminocephalosporins at 37° and μ = 0.5. The points are experimental values. The solid curves were generated from Eq. 1 and the parameters listed in Table I. Key: ○, cephalexin monohydrate; △, cephradine monohydrate; and □, cephaloglycin dihydrate. The dotted lines are theoretical curves (1) for the solubility of aminopenicillins at 37° and μ = 0.5. Key: A, cyclacillin anhydrate; B, ampicillin anhydrate; C, ampicillin trihydrate; and D, amoxicillin trihydrate.*

sonably well with experimental points for all aminocephalosporins.

In the pH range below 7, cephradine monohydrate was the most soluble, followed by cephalexin monohydrate; cephaloglycin dihydrate exhibited the lowest solubility. For comparison, the solubilities of several aminopenicillins determined under the same conditions (1) are also shown in Fig. 1 and Table I.

The apparent equilibrium solubility observed over the temperature range of 25–60°, when plotted in the classical van't Hoff fashion, gave a reasonably good linear relationship (Fig. 2). The values of the heat of solution, ΔH_{sol}, for cephalexin monohydrate, cephradine monohydrate, and cephaloglycin dihydrate were calculated from the slopes in Fig. 2 to be 1.39, 1.58, and 1.87 kcal/mole, respectively.

Dissolution Rate–pH Profile—The dissolution rate of an antibiotic solid in aqueous solution can be represented by Eq. 2 at an earlier stage of dissolution (1):

$$\left(\frac{dC}{dt}\right)_0 = \frac{S}{V} k_T \qquad \text{(Eq. 2)}$$

where C is the concentration of the drug dissolved at time t, S is the area of the solid surface exposed to the solvent, V is the volume of the dissolution medium, and k_T is the dissolution rate constant in units of molar centimeters per second.

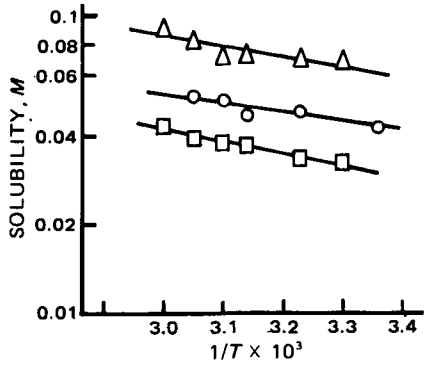

Figure 2—*Plots of solubilities of aminocephalosporins in 0.5 M KCl versus reciprocal of absolute temperature. Key: ○, cephalexin monohydrate; △, cephradine monohydrate; and □, cephaloglycin dihydrate.*

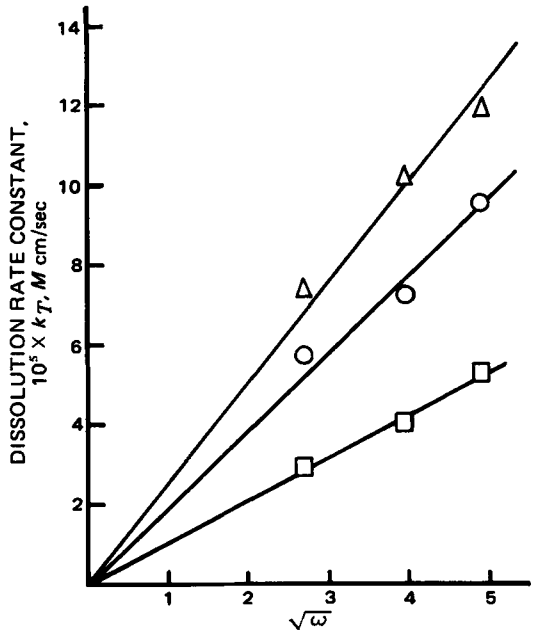

Figure 3—*Effect of stirring speed, $\sqrt{\omega}$, on the dissolution rate of aminocephalosporins in 0.5 M KCl at 37°. Key: ○, cephalexin monohydrate; △, cephradine monohydrate; and □, cephaloglycin dihydrate.*

If dissolution of aminocephalosporins from the rotating disk follows the rate-limiting diffusion, the dissolution rate constants at their isoelectric pH values can be predicted theoretically (5) as:

$$k_T = 0.620 D^{2/3} \nu^{-1/6} \omega^{1/2} C_0 \qquad \text{(Eq. 3)}$$

where D is the diffusion coefficient of a drug, ν is the kinematic viscosity of dissolution medium, and ω is the angular velocity of rotation. The dissolution rate constants, k_T, measured in 0.5 M KCl at 70, 150, and 228 rpm, were on the straight line against $\sqrt{\omega}$ (Fig. 3), apparently obeying

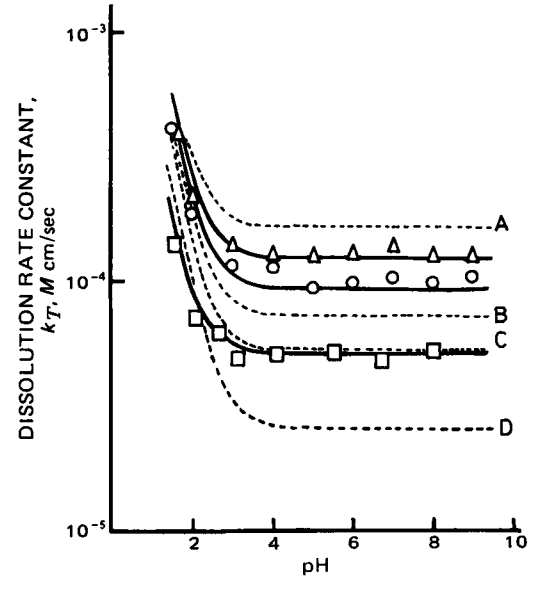

Figure 4—*Log k_T–pH profiles for the dissolution of aminocephalosporins at 228 rpm, 37°, and μ = 0.5. The points are experimental values, and the solid curves were generated from the theoretical equations listed in Table II. Key: ○, cephalexin monohydrate; △, cephradine monohydrate; and □, cephaloglycin dihydrate. The dotted lines are theoretical curves (1) for the dissolution rate constants of aminopenicillins at 228 rpm and μ = 0.5. Key: A, cyclacillin anhydrate; B, ampicillin anhydrate; C, ampicillin trihydrate; and D, amoxicillin trihydrate.*

Table II—Theoretical Equations[a] for the Dissolution Rate Constants at 228 rpm and Diffusion Coefficients of Aminocephalosporins at 37° and μ = 0.5

Aminocephalosporin	Theoretical Equation[b], k_T(theor.), M cm/sec	Diffusion Coefficients, $10^6 \times D$, cm²/sec: Dissolution Rate[c]	Diffusion Cell[d]
Cephradine monohydrate	$1.35 \times 10^{-2}(a_{H^+})_h + 1.24 \times 10^{-4} + 1.5 \times 10^{-2}(a_{OH^-})_h$	3.98	3.33
Cephalexin monohydrate	$1.22 \times 10^{-2}(a_{H^+})_h + 9.43 \times 10^{-5} + 1.5 \times 10^{-2}(a_{OH^-})_h$	4.90	3.23
Cephaloglycin dihydrate	$4.31 \times 10^{-3}(a_{H^+})_h + 5.19 \times 10^{-5} + 1.5 \times 10^{-2}(a_{OH^-})_h$	3.32	3.44

[a] Equation 4. [b] Calculated from Eqs. 4 and 5 with the parameters listed in Table I and those cited in the text. [c] Determined in 0.5 M KCl aqueous solution and used for the calculation of Eqs. 4 and 5. [d] Determined in 0.5 M KCl aqueous solution by means of a diffusion cell apparatus described previously (1) at the initial concentration of 0.01 M.

Eq. 3. Therefore, the diffusion coefficients of these antibiotics can be calculated from the slopes in Fig. 3 according to Eq. 3 by using the corresponding values of C_0 (Table I) and the viscosity of the solution, ν = 6.99×10^{-3} cm²/sec (6). The D values thus evaluated (Table II) are in good agreement with those determined by the diffusion cell method (1).

The dissolution rate constants, k_T, were determined as a function of the bulk solution pH maintained with a pH-stat. For comparison with the previous study on aminopenicillins (1), the experimental conditions were fixed at 228 rpm, 37°, and μ = 0.5 (Fig. 4).

The overall dissolution rate constants can be expressed theoretically as (1):

$$k_T = \frac{1}{h}\left[D_{H^+}\frac{D_{A^+}C_0}{D_{H^+}K_1 + D_{A^+}C_0}(a_{H^+})_h + D_{A^\pm}C_0 + D_{OH^-}\frac{D_{A^-}K_2C_0}{D_{OH^-}K_w + D_{A^-}K_2C_0}(a_{OH^-})_h \right] \quad \text{(Eq. 4)}$$

where subscripts A^+, $A^\pm$, and A^- represent the cationic, zwitterionic, and anionic species of amphoteric cephalosporins, respectively; D is the diffusion coefficient of the species indicated by the subscript; $(a_{H^+})_h$ and $(a_{OH^-})_h$ are the hydrogen-ion and hydroxide-ion activities, respectively, in the bulk solution; and h is the diffusion layer thickness calculated from (5):

$$h = 1.612 D^{1/3} \nu^{1/6} \omega^{-1/2} \quad \text{(Eq. 5)}$$

By assuming $D_{A^+} = D_{A^\pm} = D_{A^-}$ and employing $D_{H^+} = 4.12 \times 10^{-5}$ cm²/sec (7), $D_{OH^-} = 3.43 \times 10^{-5}$ cm²/sec (8, 9), $K_w = 2.38 \times 10^{-14}$ (10), and the parameters determined in this study (Table I), the theoretical dissolution rate constants, k_T (theor.), for the present experimental conditions can be derived from Eqs. 4 and 5 (Table II).

The theoretical curves calculated from these equations (Fig. 4) indicate reasonably good agreement with the experimental points for all aminocephalosporins. Figure 4 includes theoretical curves for the aminopenicillins from the previous report (1), showing almost similar dissolution rate behavior between aminopenicillins and aminocephalosporins with respect to the change in pH of the bulk dissolution medium.

Figure 5 shows plots of the logarithm of the apparent dissolution rate constants of aminocephalosporins in 0.5 M KCl at 228 rpm against the reciprocal of the absolute temperature. The heats of dissolution, ΔH_{dis}, calculated from the slope of these lines were 4.36, 4.76, and 6.09 kcal/mole for cephalexin monohydrate, cephradine monohydrate, and cephaloglycin dihydrate, respectively. These values are smaller than the 9.09 kcal/mole for ampicillin trihydrate (11) and the 9.67 kcal/mole for amoxicillin trihydrate (1) but higher than the 2.54 kcal/mole for ampicillin anhydrate (11).

By subtracting the heat of solubility, ΔH_{sol}, from that of dissolution, the intrinsic energy concerned with diffusion and viscosity of the solution can be obtained. The calculated energies, $\Delta H_{dis} - \Delta H_{sol}$, were 2.97, 3.18, and 4.22 kcal/mole for cephalexin monohydrate, cephradine monohydrate, and cephaloglycin dihydrate, respectively, comparable to the values of 3–4 kcal/mole (1, 11) obtained for amoxicillin trihydrate, ampicillin trihydrate, and ampicillin anhydrate. These energies are in the reasonable range of 3–5 kcal/mole reported (11) for diffusion processes.

The evidence obtained from the pH and temperature dependence of the dissolution rate constant strongly indicates that the dissolution of aminocephalosporins as well as aminopenicillins follows diffusion-controlled kinetics.

The dissolution rate of ampicillin anhydrate significantly decreased (1) with an increase in the ionic strength of the solution, probably as a result of the decrease in solubility (4). As seen in Fig. 6, the dissolution

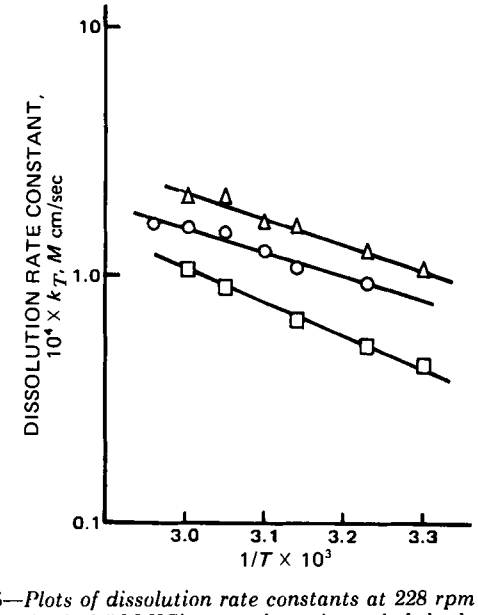

Figure 5—*Plots of dissolution rate constants at 228 rpm of aminocephalosporins in 0.5 M KCl versus the reciprocal of absolute temperature. Key: ○, cephalexin monohydrate; △, cephradine monohydrate; and □, cephaloglycin dihydrate.*

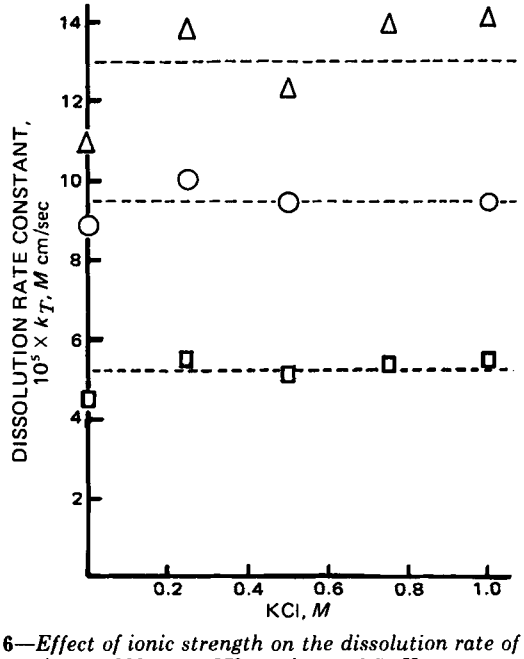

Figure 6—*Effect of ionic strength on the dissolution rate of aminocephalosporins at 228 rpm, 37°, and μ = 0.5. Key: ○, cephalexin monohydrate; △, cephradine monohydrate; and □, cephaloglycin dihydrate. The dotted lines are mean values of the data.*

rate of all types of aminocephalosporins was not significantly affected by a salt concentration.

From the present results, it is suggested that the dissolution of the aminocephalosporins studied cannot be a rate-limiting step in their absorption when they are ingested with sufficient water, as concluded previously (1) for aminopenicillins. The reduced bioavailability reported (12) for the least soluble cephaloglycin may be the result of the instability of the antibiotic at gastric and intestinal pH (3, 12) and of relatively slow GI membrane permeability of the dissolved cephaloglycin itself (13).

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Separation and Quantitation of Esterified Estrogens in Bulk Mixtures and Combination Drug Preparations Using High-Performance Liquid Chromatography

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Abstract □ A high-performance liquid chromatographic method for esterified estrogens is described. By using a facile acid hydrolysis extraction procedure for the sample preparation, the compounds are chromatographed as their free phenolic forms. The separation of structurally similar compounds, such as equilenin, equilin, estrone, and estradiol, was achieved with a reversed-phase column and a methanol–water mobile phase. Several samples of bulk mixtures and tablets were assayed; the results compared favorably with those obtained using the USP XIX method. The method was rapid, and the detector response was linear over a wide concentration range. A relative standard deviation of ±5% indicates the reliability and accuracy of the proposed method.

Keyphrases □ Estrogens, esterified—high-performance liquid chromatographic analysis in prepared solutions □ High-performance liquid chromatography—analysis, esterified estrogens in prepared solutions □ Hormones—esterified estrogens, high-performance liquid chromatographic analysis in prepared solutions

A combination drug preparation containing chlordiazepoxide base and esterified estrogens[1] is assayed for estrone and equilin by the USP colorimetric procedure. This modified Kober reaction procedure is quite lengthy (1) and complicated. Quantitation is concerned mainly with the proper ratio of the sulfate esters of estrone and equilin, calculated on the basis of the total estrogen content.

BACKGROUND

The present compendial analytical technique is remiss in that minor constituents of these complex estrogenic substances, such as equilenin, dihydroequilenin, dihydroequilin, and estradiol, are not differentiated readily (2–4). In an attempt to replace the cumbersome USP assay, many steroid separations have been reported.

In TLC, emphasis is placed on the use of silver nitrate-impregnated silica gel plates and a variety of solvent systems for resolution of the equine estrogens and their sulfates (5, 6).

Reversed-phase partition liquid chromatographic separation of estrone and equilin was achieved (7) by using an argentated mobile phase. The presence of small amounts of silver ion produced well-resolved equilin and estrone peaks (7).

Reported GLC determinations of urinary estrogens require preliminary purification and isolation on adsorption columns (8) and/or thin-layer plates (9, 10). These isolated free estrogens are derivatized subsequently to a more stable form amenable to the high temperatures encountered in GLC. Since these compounds are heat-labile substances, conversion to their silyl ethers is mandatory (11–13). It is this particular characteristic that makes open-column liquid chromatographic (14–16) and high-performance liquid chromatographic (HPLC) applications more practical and less time consuming (17–25).

Published HPLC determinations of conjugated and esterified estrogens in commercial dosage formulations are few (20, 22, 24). Most described methods are not applicable to this product because derivatizations with heat are used prior to the final chromatographic analysis (22–25).

These problems motivated the development of the assay described in this report. An analytical separation of the hydrolyzed esterified estrogens in various dosages was obtained by HPLC *via* a reversed-phase column and a methanol–water mobile phase.

Data obtained from statistical evaluations show that reliability and precision are attained using the method; the total estrogen content is easily quantitated by adding the individual estrogens.

EXPERIMENTAL

Apparatus—A constant pressure pump[2] was used in conjunction with

[1] Menrium, Hoffmann-La Roche, Nutley, N.J.

[2] Haskel pump in Dupont Instruments model 830 liquid chromatograph.

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