International Journal of Pharmaceutics, 67 (1991) 211–214

ADONIS 037851739100064T

IJP 02279

Some considerations on the analytical method for dissolution of conjugated estrogen tablets

Syang Y. Su [1], Gerald K. Shiu [1], John E. Simmons [2] and Jerome P. Skelly [3]

[1] *Biopharmaceutics Research Branch, Center for Drug Evaluation and Research (CDER), FDA, Washington, DC 20204 (U.S.A.),* [2] *Division of Cardio-Renal Drug Products, FDA, 5600 Fishers Lane, Rockville, MD 20855 (U.S.A.) and* [3] *Office of Research Resources, CDER, FDA, 5600 Fishers Lane, Rockville, MD 20855 (U.S.A.)*

(Received 16 April 1990)
(Accepted 16 August 1990)

Key words: Conjugated estrogen tablet; Dissolution; Filter absorption/adsorption; HPLC analysis

Summary

Dissolution samples of conjugated estrogens were prepared for HPLC analysis by filtration and centrifugation. Polyethylene filters were found to be far less retentive of conjugated estrogens than nylon membranes. Samples clarified by centrifugation alone caused rapid HPLC column fouling. Several RP-HPLC columns were evaluated with respect to separation and quantitation of estrone sulfate, equilin sulfate, impurities and contaminants — a resolution factor of 1.5 or greater is recommended. Sample injection volume was reduced to 20 μl for dissolution monitoring of 1.25 and 2.5 mg strength tablets — far less than that required for existing assays (200–500 μl). Dissolution samples of conjugated estrogens should be analyzed soon after collection or refrigerated to retard degradation.

Orally administered conjugated estrogen tablets remain a popular therapy for postmenopausal/post-ovariectomy deficiencies, certain cancers and various pregnancy/delivery related conditions. The use of estrogen harvested from pregnant mare's urine predates FDA existence. Yet, to date, there are no compendial in vitro release rate standards for conjugated estrogen tablets.

As described in U.S.P. XXI (1986a) the tablets should contain not less than 73% and not more than 95% of total sodium sulfate salts of estrone, equilin and some other minor compounds such as 17-α-dihydroequilin, etc. The ratio of equilin to estrone salts should be between 0.35 and 0.65.

The first standard dissolution test method proposed in 1986 was based on work carried out at the U.S.P. Drug Research and Testing Laboratories (*Pharm. Forum*, 1986). The method employed U.S.P. apparatus 1 (rotating basket) and in 500 ml of pH 6.8 phosphate-buffered dissolution media maintained at 50 °C. Samples were analyzed via RP-HPLC. Release rates were slow and no reference standards, except the tablet itself, were used in the method.

The most recently proposed method employed a disintegration apparatus (*Pharm. Forum*, 1988) and simulated gastric fluid (SGF) without enzyme. Again powdered tablets were used as reference standard and analysis was via RP-HPLC.

Correspondence: S.Y. Su, Biopharmaceutics Research Branch, Center for Drug Evaluation and Research (CDER), FDA, Washington, DC 20204, U.S.A.

There has been a great deal of controversy concerning the equivalence of several generic forms of conjugated estrogens to the innovator product Premarin (Ayerst). In this submission we examine some of the problems associated with the analysis of conjugated estrogens in dissolution media and attempt to address the current controversy concerning standard methodology for the in vitro characterization of conjugated estrogens released from tablets.

Estrone sulfate, equilin sulfate and 17-α-dihydroequilin sulfate were purchased from Diosynth Labs (Oss, The Netherlands). They were used to confirm the identification of these three conjugated estrogens. Conjugated estrogen tablets studied include those of Ayerst (New York, NY), Zenith (Northvale, NJ) and Duramed Labs (Cincinnati, OH). All chemicals and solvents used were either reagent grade or HPLC grade. The simulated gastric fluid (SGF) was prepared as per U.S.P. XXI (1986b).

Filtration materials studied were pre-pleated paper filters no. 588 (8–12 μm pore size, Schleicher & Schuell Inc., Keene, NH), 25 mm nylon-66 filters (0.2 μm pore size, Thomson Instrument Co., Springfield, VA) and polyethylene pipet tip filters (20 μm pore size, Centaur West, Inc., Sparks, NV).

Dissolution samples were filtered through nylon membranes using a syringe or a pipet equipped with polyethylene filter tips and collected in 1 ml increments for analysis.

A Beckman automated System Gold HPLC (San Ramon, CA), Beckman UV detector (205 nm, 0.01 AUFS), a Hewlett Packard integrator (Avondale, PA) and a Nelson data system (Perkin-Elmer Co., Norwalk, CT) were employed for this study. The mobile phase (*Pharm. Forum*, 1986) was 25 : 75 acetonitrile/0.025 M KH_2PO_4 at an optimized flow rate of 1.2 ml/min.

LC columns studied included Beckman Ultrasphere 5 μm C-18 high resolution 4.6 mm × 25 and 15 cm columns (San Ramon, CA), a Brownlee ODS Spheri-5 4.6 mm × 22 cm column (Santa Clara, CA), a Burdick & Jackson ODS 5 μm 4.6 mm × 25 cm column (Baxter Co., McGaw Park, IL) and a Waters μ-Bondapak C-18 4.6 mm × 30 cm column (Bedford, MA).

The dissolution apparatus was a Distek System 2100 (Monmouth Junction, NJ).

The filters used to clean up dissolution samples prior to HPLC analysis were found to retain conjugated estrogens. The degree of retention was dependent on the filter material and the pH and/or composition of the dissolution medium (Fig. 1). Reliable analysis of dissolution samples required the selection of non-absorbing filter material and the determination of optimal collection fractions.

A comparison of percent recoveries of estrone sulfate in dissolution samples prepared by centrifugation alone with filtration through nylon-66 or PE-20 filters is illustrated in Fig. 1. Powders of Ayerst conjugated estrogens tablets were dissolved in pH 6.8 phosphate-buffered media and pH 1.2 SGF media for these studies. The percent recovery was calculated by comparing peak areas of estrone sulfate in the filtrate to that in supernatant after centrifugation at 1800 rpm for 10 min.

A consistent recovery of estrone sulfate was observed for the polyethylene pipet filter (PE-20) after collection of the third milliliter of filtrate (the first 2 ml were discarded) using either media (curves a and b, Fig. 1). Recoveries of estrone sulfate dissolved in SGF media declined perceptibly after the 13 ml of filtrate (curve a, Fig. 1). Polyethylene filters were suitable for the filtration

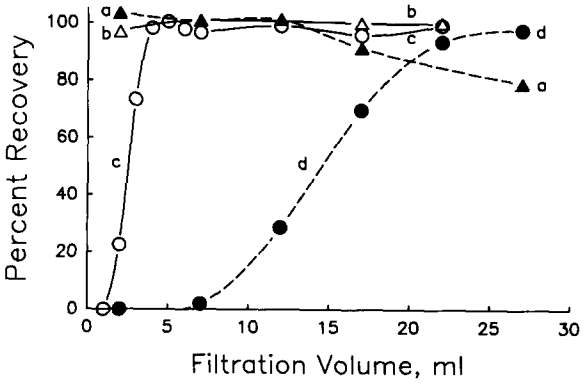

Fig. 1. Recovery of estrone sulfate in two media from two types of filters. Percent recovery = (peak area of estrone sulfate by filtration)/(peak area of estrone sulfate by centrifugation at about 1800 rpm); filtration volume = the volume of dissolution media passed through the filter (in ml). Curves: (a) PE-20, pH 1.2 simulated gastric fluid (SGF); (b) PE-20, pH 6.8 phosphate buffer; (c) nylon-66, pH 6.8 phosphate buffer; (d) nylon-66, pH 1.2 SGF.

of dissolution samples in both media, if the 2–13th ml of filtrates were collected for analysis.

A significant loss of estrone sulfate due to binding was observed when nylon-66 membranes were used. Data indicated that the first 5 ml of filtrate contained no estrone sulfate when pH 6.8 buffered medium was used (curve c, Fig. 1) and when SGF was used at least 25 ml of filtrates could be discarded before reasonable recoveries were obtained (curve d, Fig. 1). The nylon-66 membranes were unsuitable for filtration of SGF samples, but could be used for pH 6.8 medium if the first 5 ml were discarded.

Direct injection of samples prepared by centrifugation (approx. 1800 rpm) is not recommended for routine sample analysis because the HPLC guard columns were easily clogged by small particles in the supernatant. A rapid rise in back pressure was observed which necessitated guard column cleaning or replacement. Reduction of injection volume to 20 μl of conjugated estrogens solutions was sufficient to get satisfactory peak detection for analysis of dissolution samples (1.25 & 2.5 mg tablets) under the chromatographic conditions described. Injection of a larger volume 200–500 μl, as proposed previously (*Pharm. Forum*, 1986, 1988), needlessly shortens the life of the column and can be avoided.

Reversed phase ODS columns of two different column lengths have been proposed for the separation of conjugated estrogens (*Pharm. Forum*, 1986; *USP*, 1986a). We have examined the suitability of various reversed-phase columns for resolution of estrone sulfate, equilin sulfate and unknown peaks (Fig. 2A). In the Pharmacopeial Forum proposal a resolution (R) between equilin sulfate and estrone sulfate larger than 1 was recommended (*Pharm. Forum*, 1986, 1988), however, $R = 1.5$ was necessary for separation of these two sulfates and other tablet components. The resolution and separation factors (k') between estrone and equilin sulfates peaks were determined for the following columns: 1.87/1.13 (Waters), 1.16/1.15 (B&J), 1.25/1.14 (Brownlee), 3.37/1.15 (Beckman 25) and 1.8/1.15 (Beckman 15). A typical chromatogram of Ayerst conjugated estrogens using the Beckman 25 cm column is shown in Fig. 2A.

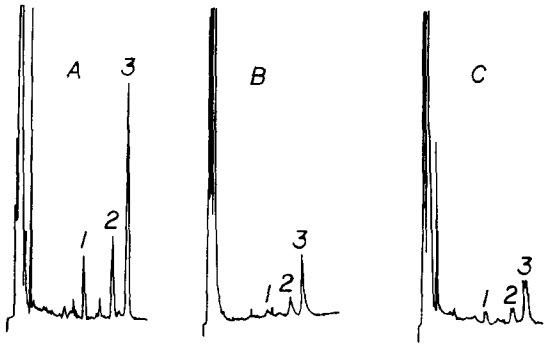

Fig. 2. Chromatograms of conjugated estrogen tablet dissolution samples. Instrumentation: Beckman System Gold liquid chromatograph with a 25 cm × 4.6 mm Ultrasphere 5 μm C-18 column, UV wavelength = 205 nm, 0.01 AUFS; mobile phase: 25 : 75 acetonitrile/KH_2PO_4 (as in *Pharm. Forum*, 1986, 1988), flow rate = 1.2 ml/min. Chromatograms: (A) using a fresh column or after regeneration; (B) after more than 100 injections; (C) additional injections on column pictured in (B) prior to regeneration. Peaks: (1) 17-α-dihydroequilin sulfate; (2) equilin sulfate; (3) estrone sulfate.

It should be noted that after approx. 200 injections of conjugated estrogen samples (20 μl), especially conjugates isolated from natural sources (e.g. Ayerst), the peaks were observed to broaden and split (Fig. 2B and C). One could speculate that either tablet contaminants accumulate on column or tablet excipients were retained on the stationary phase and affected the separation mechanism. This process was found to be reversible by conditioning the column with 0.005 M tetrabutylammonium salts in the mobile phase for about 4 h, then reequilibrating the columns with the mobile phase. Column efficiency was restored several times using this regeneration procedure.

Dissolution samples of conjugated estrogens tablets should be analyzed immediately after preparation. Samples may be refrigerated at 2° C or frozen, if they are not to be analyzed for more than 1 week, without affecting results. Dramatic reductions in peak areas were observed when samples became turbid following storage at room temperature.

After comparing data generated by both HP integrator and Nelson data system it was concluded that either peak area or peak height can be used for quantitation purposes.

In conclusion, the analysis of conjugated estrogens in dissolution media requires that the absorptive properties of the filtration materials and/or the nature of the sample contaminants must be fully understood. The HPLC column employed for separation and analysis must have a resolution greater than 1.5 to ensure precision and accuracy of the data. Injection volumes should be kept as low as possible, 20 μl in most cases, to extend column life and reduce variability in separations. Peak area integration was the preferred method for quantitation because it was less affected by peak asymmetry or nongaussian shape.

References

Pharmacopeial Forum, 12 (1986) 1892–1893.
Pharmacopeial Forum, 14 (1988) 4251–4252.
U.S. Pharmacopeia, 3rd Suppl., U.S. Pharmacopeial Convention, Rockville, MD, 1986a, p. 1991.
U.S. Pharmacopeia, 3rd Suppl., U.S. Pharmacopeial Convention, Rockville, MD, 1986b, p. 1424.