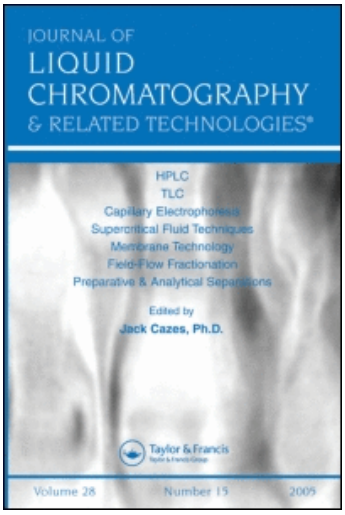

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Ethynylestradiol and Norethindrone in Synthetic Intestinal Fluid and in Timed-Release Oral Formulations

Nollie F. Swynnerton[a]; Joseph B. Fischer[a]
[a] Southwest Research Institute, San Antonio, Texas

To cite this Article Swynnerton, Nollie F. and Fischer, Joseph B.(1980) 'Determination of Ethynylestradiol and Norethindrone in Synthetic Intestinal Fluid and in Timed-Release Oral Formulations', Journal of Liquid Chromatography & Related Technologies, 3: 8, 1195 — 1204
To link to this Article: DOI: 10.1080/01483918008064751
URL: http://dx.doi.org/10.1080/01483918008064751

JOURNAL OF LIQUID CHROMATOGRAPHY, 3(8), 1195-1204 (1980)

DETERMINATION OF ETHYNYLESTRADIOL AND NORETHINDRONE IN SYNTHETIC INTESTINAL FLUID AND IN TIMED-RELEASE ORAL FORMULATIONS

Nollie F. Swynnerton and Joseph B. Fischer
Southwest Research Institute
6220 Culebra Road
San Antonio, Texas 78284

ABSTRACT

High performance liquid chromatographic methods are described for the rapid assay of the antifertility steroids ethynylestradiol and norethindrone in sustained-release oral formulations. A novel method is reported for isolation of these hormones from the synthetic intestinal fluid used for in-vitro release rate studies.

INTRODUCTION

The incorporation of presently-marketed contraceptive preparations into orally active sustained-release formulations holds the promise of minimizing daily peaks of the drug and reducing body burden while maintaining contraceptive efficacy. In the development of such formulations, in-vitro release rate procedures (1) utilizing synthetic intestinal fluids (2) allow comparison of experimental capsules prior to pharmacokinetic studies.

The quantitative determination of antifertility steroids, (mainly ethynylestradiol, EE) has been carried out using a variety of methods, including radiochemical GLC (3) and high-performance liquid chromatography (4,5). Bagon and Hammond (4) separated EE from other steroids in contraceptive tablets

using a reverse-phase system and UV detection at ca. 212 nm. Roos (5) measured estrogens in pharmaceutical tablet and injectable dosage forms by HPLC separation of their dansyl derivatives and fluorescence detection. The latter method uses a silica gel column.

The present study was conducted to develop analytical procedures for the rapid and precise quantitation of EE and norethindrone (NET) in microsphere form and in "synthetic intestinal fluid."

MATERIALS

A Waters Associates Model 244 liquid chromatograph equipped with a Waters U6K injector and a Waters Model 440 UV detector was used for the development work. Detection was at 254 nm (NET) and 280 nm (EE). Routine analyses are now done on an Altex Model 330 liquid chromatograph fitted with a Rheodyne 7105 injector and an Altex UV detector. Columns used were:

(1) μBondapak C_{18}, 30 cm x 4.6 mm I.D., Waters Associates, Milford, MA.

(2) μPorasil, 30 cm x 4.6 mm I.D., Waters Associates, Milford, MA.

(3) Partisil PXS 10/25, 25 cm x 4.6 mm I.D., Whatman, Inc., Clifton, NJ.

(4) Ultrasphere-Si (5 μm), 15 cm x 4.6 mm I.D., Altex Scientific Inc., Berkeley, CA.

Organic solvents were purchased from Burdick and Jackson, Muskegon, MI (Distilled-In-Glass grade). Water was purified with a Millipore MILLI-Q™ Water Purification System. Degassing of the mobile phases was done under water aspiration using ultrasonic agitation.

Cartridges filled with C_{18} reverse-phase packing (SEP-PAK™) were purchased from Waters Associates.

"Synthetic intestinal fluid" was prepared by dissolving 13.6 g monobasic sodium phosphate and 76 mL 1N sodium hydroxide in deionized water and diluting to 2 liters. The pH of the

resulting solution was adjusted to 7.5 ± 0.1 with 1N sodium hydroxide when necessary (2).

METHODS

In-Vitro Release

The method employed was based on that reported by the National Formulary XIII, entitled "Release Tablets and Capsules -- In-Vitro Test Procedure," with minor modifications (1).

For each microsphere formulation to be tested, five samples of microspheres (each equivalent to ≈ 100 µg steroid) were weighed into glass bottles, and 60 mL synthetic intestinal fluid was added to each bottle. The capped vessels were then rotated in a 37°C bath. At timed intervals, samples were removed, the microspheres separated from the solution using a fine mesh stainless steel screen and the solution filtered under reduced pressure through a 0.45 µm Millipore Type HA filter. The microspheres were rinsed with water and dried in air at 50% relative humidity.

Sample Preparation

Microspheres were assayed for residual steroid by dissolving weighed samples (≈ 50 mg) in 1.0 mL chloroform and injecting 20-µL aliquots onto the HPLC column.

Synthetic intestinal fluid was analyzed for released steroid using the following procedure. A 50.0-mL aliquot of the filtered fluid was transferred by pipette to a 50-cc syringe body which had been fitted with a C_{18} SEP-PAK™ cartridge and clamped upright as shown in Figure 1. The fluid was forced through the cartridge using argon at 6 psi. This was accomplished by holding to the top of the syringe an inverted #7 rubber stopper containing the argon line. Flow rate was held at approximately 20 mL/min. The aqueous effluent was discarded. The steroid was then eluted from the cartridge into a 10-mL vial with 3 mL of tetrahydrofuran. This solution was taken to dryness at 55°C under a stream of argon, and the residue was taken up in 1.0 mL of chloroform. Aliquots of this solution were injected onto the HPLC column.

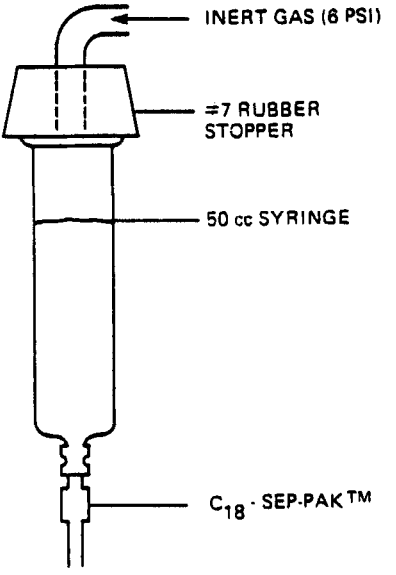

FIGURE 1. EXTRACTION APPARATUS

HPLC Analysis

Chloroform solutions containing EE or NET were injected from a fixed 20-µL loop onto a column containing fully porous microparticulate silica gel (See Discussion). A mobile phase of 6% absolute ethanol in heptane and a flow rate of 1.5 mL/min eluted all materials in less than 10 min with any of the columns tested (representative chromatograms are shown in Figure 2). Detection was by UV at 254 nm (NET) or 280 nm (EE). A linear regression of the peak height vs concentration data obtained from injections of standard solutions provided a response factor (slope) for the quantitation of the steroid-containing samples. Figure 3 shows typical calibration curves.

RESULTS AND DISCUSSION

Early in the in-vitro release rate studies, EE and NET were isolated from the synthetic intestinal fluid by extraction with dichloromethane. Removal was shown to be quantitative. After

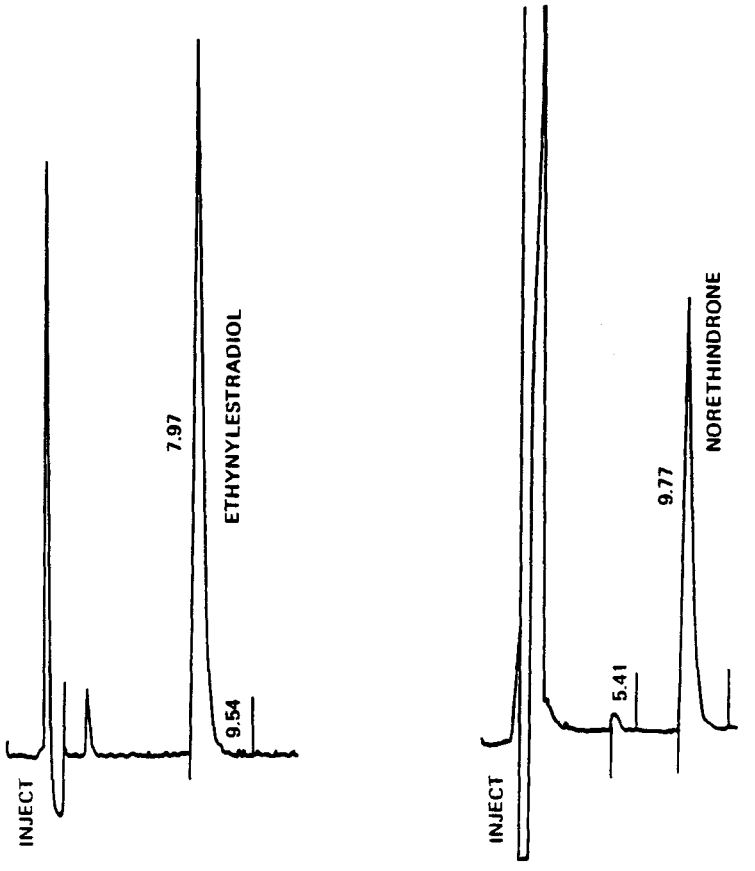

FIGURE 2. REPRESENTATIVE CHROMATOGRAMS OF ETHYNYLESTRADIOL AND NORETHINDRONE

the solvent was evaporated, the residue was taken up in 1-propanol and analyzed by HPLC using reverse-phase techniques (Column 1, Materials Section). In order to more efficiently process the large number of samples generated in this study, several modifications to the early procedures were made. It was found that EE and NET could be quantitatively removed from the synthetic intestinal fluid by passing the fluid through a SEP-PAK™, a

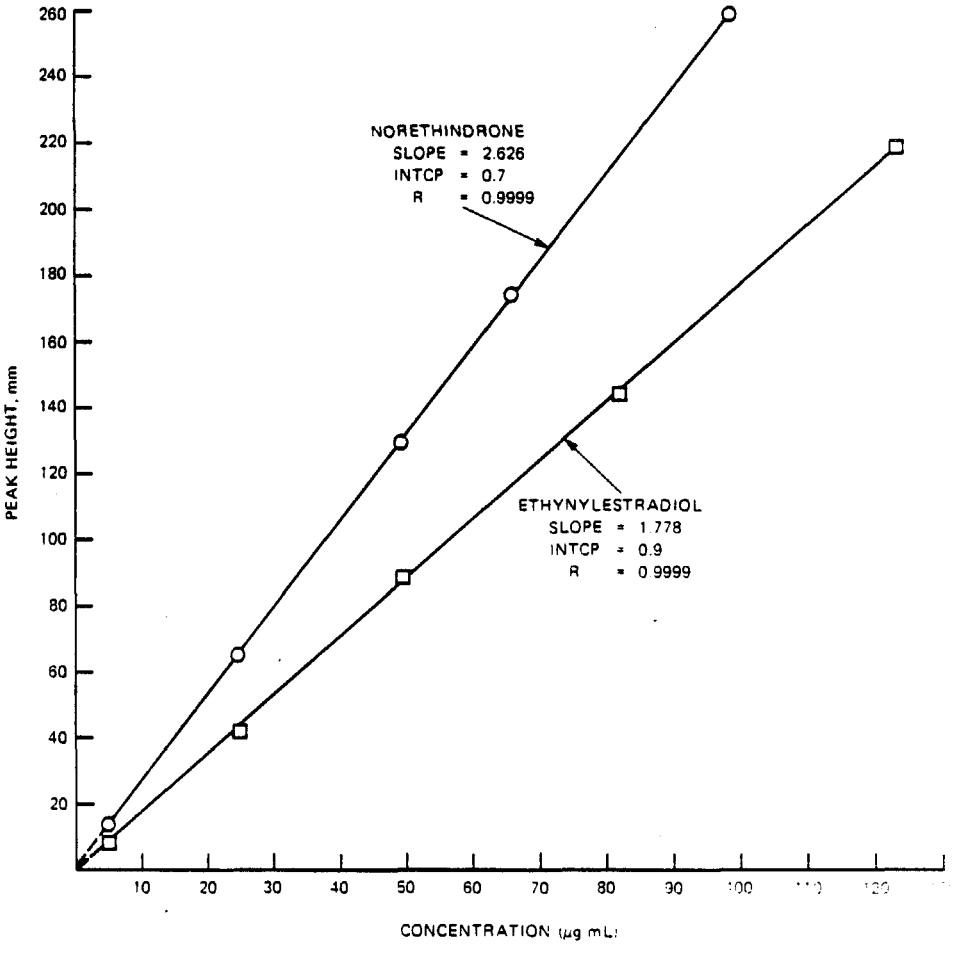

FIGURE 3. CALIBRATION CURVES FOR ETHYNYLESTRADIOL AND NORETHINDRONE

cartridge containing C_{18} reverse-phase material. A simple device used for this step is shown in Figure 1. The fluid was placed in the syringe body and forced through the cartridge with an inert gas at a pressure of 6 psi. This gave a flow rate of ≈ 20 mL/min. At higher flows, removal of steroid was incomplete. This device prevents the loss of material associated with the use of a plunger. In addition, residual aqueous solution may be

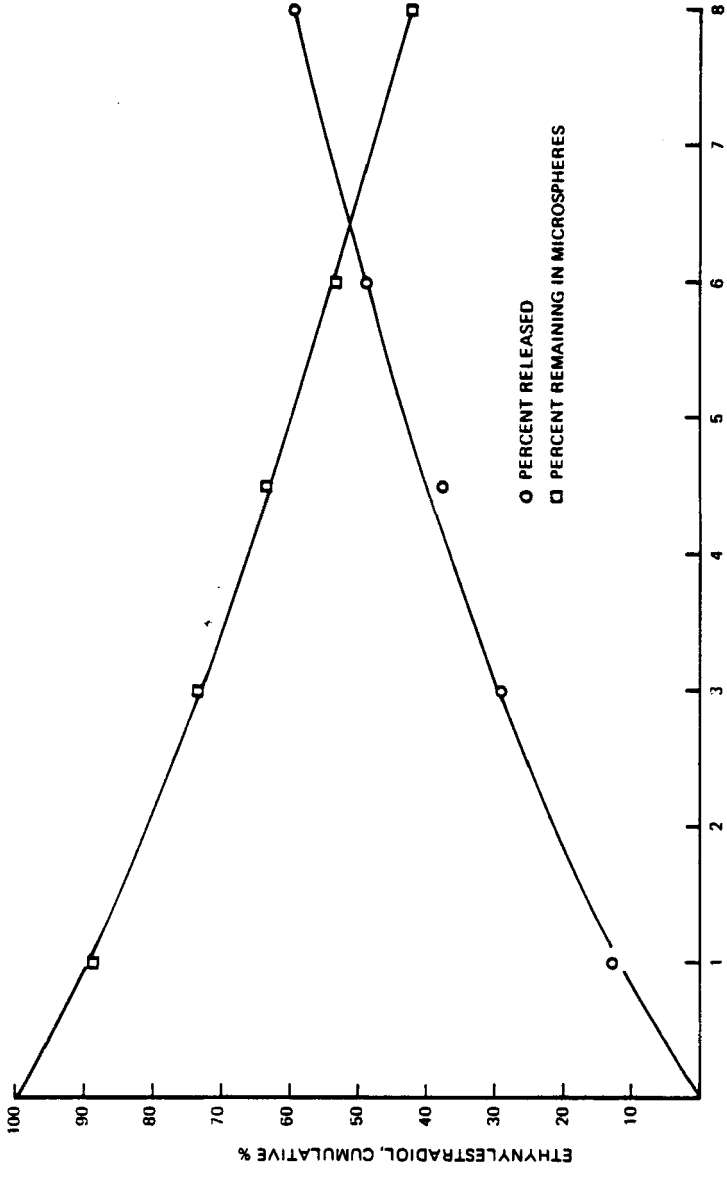

FIGURE 4. REPRESENTATIVE EE RELEASE RATE RESULTS

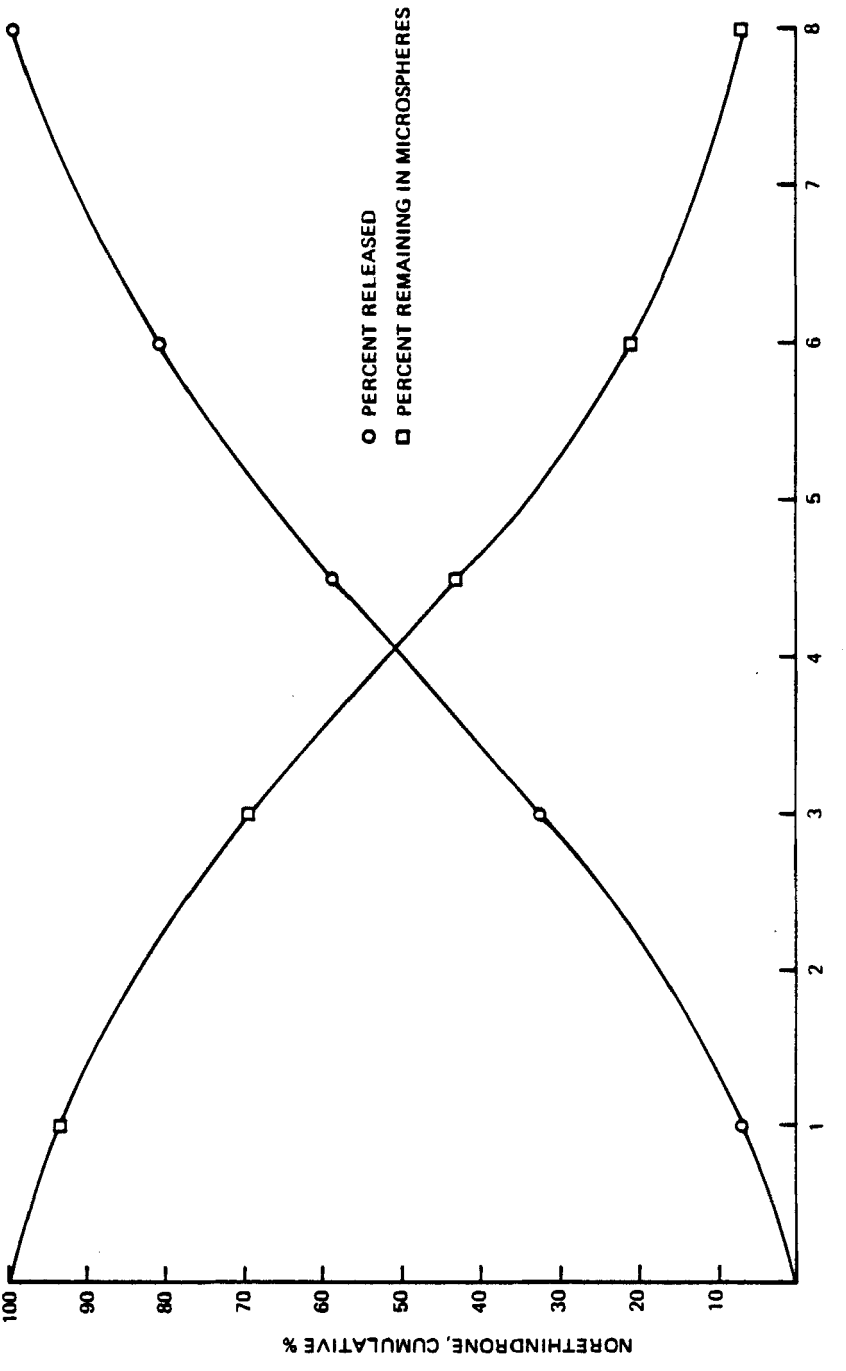

FIGURE 5. REPRESENTATIVE NET RELEASE RATE RESULTS

"blown-out," thereby reducing the amount of water which must be evaporated when the steroid is isolated.

Although a reverse-phase column (Column 1, Materials Section) could be used for the determination of EE and NET, a normal phase system was chosen for the following reasons. First, several of the formulations contained an unidentified excipient which had an absorbance at 254 nm and co-eluted with NET under all reverse-phase conditions studied. This interference was separated from NET by silica gel columns. Second, the fatty matrices of the microspheres are not soluble in solvents compatible with aqueous mobile phases but are completely soluble in the chlorinated solvents compatible with normal-phase systems. Therefore, the microsphere formulations may be assayed by simply injecting their solutions in chloroform directly onto a silica gel column. This permits an easy accountability determination for each release rate study, since the recovered microspheres may be quickly analyzed for remaining estrogen. Figures 4 and 5 show actual release rates for two experimental formulations. The accountability is merely the sum of the two curves at any time t.

Normal phase columns (See Materials Section) were obtained prepacked from three suppliers and gave essentially equivalent results. The shortest column, 15 cm x 4.6 mm I.D. (Column 4) containing 5-μm spherical silica gel, gave the sharpest peaks. This gave an increase in "apparent sensitivity" which may be of some value when very low concentrations are being measured.

ACKNOWLEDGMENT

This project has been funded at least in part with Federal funds from the Department of Health, Education, and Welfare under contract number NO1-HD-7-2831. The contents of this publication do not necessarily reflect the views or policies of the Department of Health, Education, and Welfare, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

REFERENCES

1. "Release Tablets and Capsules - In-Vitro Test Procedure," The National Formulary XIII, The American Pharmaceutical Association, Washington, D.C., 1970, p. 882.

2. "The United States Pharmacopeia." XVIII, Mack Publishing Co., Easton, Pa., 1970, p. 1027.

3. Zuleski, F.R., Loh, A., and Di Carlo, F.J., "Determination of Ethinyl Estradiol in Human Urine by Radiochemical GLC," J. Pharm. Sci., 67, 1138, (1978).

4. Bagon, K.R., and Hammond, E.W., "Determination of Ethinyl-oestradiol in Single Tablets and Its Separation from Other Steroids by High-Performance Liquid Chromatography," Analyst, 103, 156 (1978).

5. Roos, R.W., "High-Pressure Liquid Chromatographic Analysis of Estrogens in Pharmaceuticals by Measurement of Their Dansyl Derivatives," J. Pharm. Sci., 67, 1735 (1978).