

An improved method for the gas chromatographic separation of estrogens*

The role of estrogens in human metabolism has emphasized the need for determining micro-quantities of these compounds. Methods previously described employing column partition chromatography¹, paper chromatography², a combination of countercurrent distribution and paper chromatography³ and acetylated compounds in gas chromatography⁴ have been either time consuming or insensitive. In a previous report from this laboratory⁵ the three estrogens: estrone, 17β -estradiol and estriol were successfully separated but the results presented in this report represent a superior separation of estrone and 17β -estradiol and a shorter retention time for estriol.

Experimental

The gas chromatographic separation of a synthetic mixture of estrone, 17β -estradiol and estriol was accomplished utilizing a Barber-Colman model 10 gas chromatograph equipped with a ⁹⁰Sr ionization detector.

A 6 ft. long, "U" shaped, pyrex glass column with an internal diameter of 5 mm

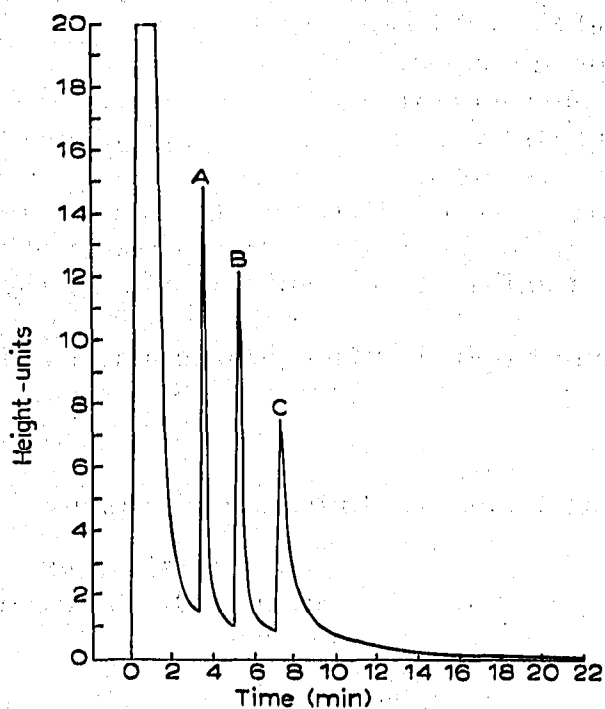


Fig. 1. Gas chromatographic separation of (A) $1.5 \mu\text{g}$ 17β -estradiol, (B) $1.5 \mu\text{g}$ estrone and (C) $5.0 \mu\text{g}$ estriol. Conditions: Column, 6 ft. 5 mm I.D. pyrex glass column; 3% QF-1 on 100/120 Gas Chrom-P; 243° , 60 ml/min flow rate. Detector, 272° . Flash heater, 303° .

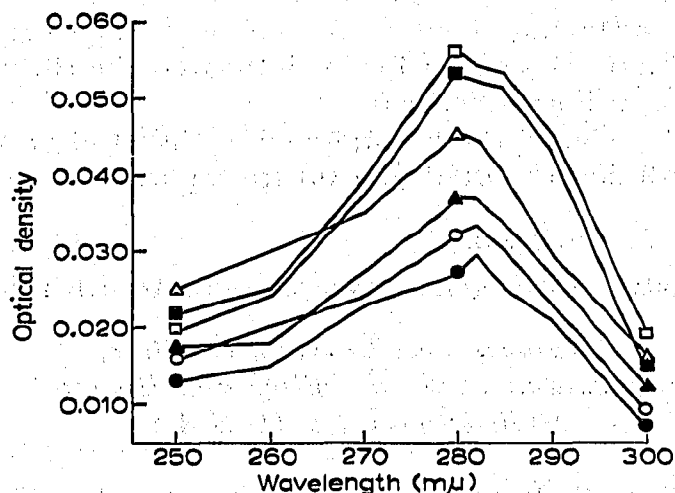


Fig. 2. Ultraviolet absorption spectrum for estrogens prior to and following passage through column. (Δ) $3 \mu\text{g}$ estrone before passing through column; (\blacktriangle) $3 \mu\text{g}$ estrone after passing through column; (\circ) $3 \mu\text{g}$ 17β -estradiol before passing through column; (\bullet) $3 \mu\text{g}$ 17β -estradiol after passing through column; (\square) $5 \mu\text{g}$ estriol before passing through column; (\blacksquare) $5 \mu\text{g}$ estriol after passing through column.

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was packed with a stationary phase of 3% QF-1 (fluorosilicone) on 100/120 Gas Chrom-P (Applied Science Laboratories, Inc., State College, Pa.). The temperature of the column was maintained at 243°, the detector at 272° and the "flash heater" at 303°. Argon was employed as the carrier gas and a flow rate of 60 ml/min was maintained. The column was previously conditioned, while vented to the air, at 253° and at the operating pressure of the carrier gas for 24 h.

The samples were introduced to the column with a 10 μ l microsyringe employing a spectroquality reagent grade of dioxane as the solvent.

Results and discussion

Two criteria were used to determine and prove the thermal stability of the estrogens in the gas chromatographic separation technique described. The first was an examination of the chromatogram over an extended length of time. Had any decomposition occurred, new peaks, skewing or broad peaks would have been observed. At no time were any of these conditions observed under the operating conditions described. The chromatogram of the three compounds is shown in Fig. 1. The second criterion was to determine the ultraviolet absorption spectra after passing the compounds through the column and comparing them to reference spectra. In all cases the absorption maxima were identical prior to and following passage through the column as may be observed in Fig. 2. This indicated thermal stability of the compounds.

The retention times of the three compounds are shown in Fig. 1.

Previous evidence for the gas chromatographic separation of estrogens has been presented by WOTIZ AND MARTIN⁴ and VANDENHEUVEL *et al.*⁶ but the problem of thermal instability was encountered in both reports and as a result separations were difficult. WOTIZ AND MARTIN⁴ found it necessary to acetylate the estrogens prior to introducing them to the columns. The symmetry of the peaks, as observed in our results as seen in Fig. 1, indicated the thermal stability of the compounds under the conditions described.

The methods employed in this study are being applied to biological material and will be presented in a future report.

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