Comparative Utility of the Silyl Ether and Acetate Ester Derivatives of Ethinyl Estradiol for Its Quantitative Gas Chromatographic Determination

Sir:

A recent paper (1) in *J. Pharm. Sci.*, comparing the U.S.P. method with a gas chromatographic determination of ethinyl estradiol, contains several allusions to our paper on the gas chromatographic assay of the estrogen (2), claiming certain advantages for the choice of the trimethyl silyl ether derivative over our use of the acetate derivative. We should like to offer a rebuttal on several of these comparisons.

Boughton, Bryant, Ludwig, and Timma (1) inferred from our work that, "The ethinyl estradiol appears to have been uncontaminated with other steroids . . . ." Since the extraction procedure reported by these authors is essentially identical with ours, one would hardly expect interference in one procedure and not the other. In point of fact, however, the samples on which we reported contained 10 to 20 times the concentration of ethinyl estradiol of an experimental progestational steroid, which resulted in no interference.

The authors (1) further claimed that evaporation of acetic anhydride necessary in our procedure introduces a possible source of error, presumably circumvented by substitution of silylation in their procedure for acetylation in ours. This contention is contradicted by comparison of our reported precision of $\pm 1.7\%$ with the standard deviations of $\pm 1.5\%$ found for the silylation method, which are not statistically different.

The statement that ". . . the authors wished to analyze for at least 50% less ethinyl estradiol than Talmage, Penner, and Geller had analyzed, and for our purposes the acetate derivative did not give sufficient response on the chromatograph" (1) is perhaps the most misleading. We did not find it necessary to use maximum sensitivity in our procedure; using the acetate derivative, sensitivity could easily have been increased by a factor of 10. We have chromatographed unmodified ethinyl estradiol, its silyl ether, and its acetate ester at the same concentration using instrumental conditions previously described (2). The chromatograms shown in Fig. 1 indicate that little sensitivity gain is afforded by use of the silyl ether in preference to the acetate ester derivative; however, it is interesting to note the increase in retention time with increase in molecular weight.

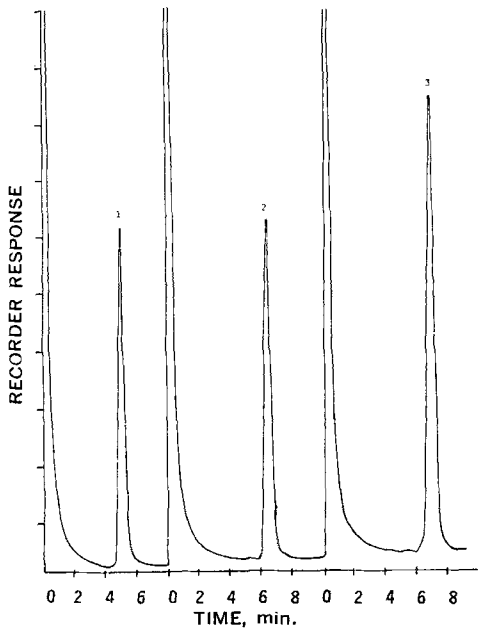

Fig. 1—Comparative chromatogram. Key: 1, ethinyl estradiol; 2, ethinyl estradiol, acetate ester; 3, ethinyl estradiol, silyl ether.

The results shown in Fig. 1 indicate that ethinyl estradiol can be determined by gas chromatography without formation of derivatives. We were aware of this during our work with the acetate ester, but found better precision using the derivative. Subsequent to this work, however, it was determined that the unmodified ethinyl estradiol can be estimated with good precision and accuracy if the column is saturated with the estrogen before quantitative samples are run. We interpret this as saturating the active sites on the column, thus making them unavailable for irreversible adsorption.

(1) Boughton, O. D., Bryant, R., Ludwig, W. S., and Timma, D. L., *J. Pharm. Sci.*, **55**, 951(1966).
(2) Talmage, J. M., Penner, M. H., and Geller, M., *ibid.*, **54**, 1194(1965).

JOSEPH M. TALMAGE
MELVIN H. PENNER

Pharmaceutical Research and Development Laboratories
Warner-Lambert Research Institute
Morris Plains, NJ 07950

Received December 30, 1966.
Accepted for publication March 1, 1967.