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## Note

# Simultaneous separation of estrogens and androgens using thin-layer chromatography

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Although the metabolism of testosterone has been extensively studied in many of the male target tissues[1,2], the metabolism of androgens in the uterus has only recently become of interest. A testosterone receptor has been described in the rat uterus[3], necessitating the study of testosterone metabolism in the uterus. My laboratory[4,5] has studied the translocation of the uterine cytoplasmic estrogen receptor to the nucleus by testosterone and 5α-dihydrotestosterone. These studies also required the ability to detect any conversion of androgens to estrogens. Most investigators have used gas chromatography[6], multiphase thin-layer chromatography (TLC)[7], or liquid–liquid partition column chromatography[8] to separate the major androgens from estrogens. This paper reports a simple and rapid TLC method utilizing only one solvent system to separate simultaneously three major androgens and three major estrogens.

## MATERIALS AND METHODS

Estradiol-17β, estrone, estriol, testosterone, 5α-dihydrotestosterone, and androstanediol* were all obtained from Sigma (St. Louis, Mo., U.S.A.). 5 μg each of the above steroids were spotted together on Kontes Quantum 20×20 cm LQDF silica gel-coated glass plates (Kontes Glass, Vineland, N.J., U.S.A.) containing a preabsorbent. The plates were preconditioned for 30 min with sulfuric acid solutions to create different humidities (see Table II). Several solvent systems, humidities, and times were tested at 23° using the Camag Vario-KS-chamber (Camag, New Berlin, Wisc., U.S.A.) TLC equipment. This system allows for testing various solvents and/or humidities simultaneously on one chromatogram. After air drying, the plates were developed in iodine vapor.

## RESULTS AND DISCUSSION

Several solvent systems were tested in order to separate the androgens from

* Estradiol-17β = 3,17β-dihydroxy-1,3,5(10)-estratriene; estrone = 3-hydroxy-1,3,5(10)-estratrien-17-one; estriol = 3,16α,17β-trihydroxy-1,3,5(10)-estratriene; testosterone = 17β-hydroxy-androst-4-en-3-one; 5α-dihydrotestosterone = 17β-hydroxy-5α-androstan-3-one; androstanediol = 3α,17β-dihydroxy-5α-androstane.

TABLE I

$R_F$ VALUES OBTAINED FOR THE MAJOR ANDROGENS AND ESTROGENS USING VARIOUS SOLVENT SYSTEMS

The steroids (5–10 μg) were spotted together on Kontes silica gel plates, preconditioned for 30 min at 32% humidity, and TLC was performed for 90 min. Detection was by iodine vapor. Solvent systems: (A) chloroform–ethyl acetate (90:10); (B) chloroform–ethyl acetate (80:20); (C) chloroform–ethyl acetate (50:50); (D) chloroform–methanol (98:2); (E) benzene–ethyl acetate (60:20).

| *Steroid* | *Solvent system* | | | | |
|---|---|---|---|---|---|
| | *A* | *B* | *C* | *D* | *E* |
| Estriol | 0.01 | 0.05 | 0.19 | 0.09 | 0.04 |
| Androstanediol | 0.23 | 0.37 | 0.66 | 0.41 | 0.29 |
| Testosterone | 0.33 | 0.48 | 0.68 | 0.46 | 0.35 |
| Estradiol-17β | 0.33 | 0.53 | 0.77 | 0.43 | 0.50 |
| 5α-Dihydrotestosterone | 0.40 | 0.60 | 0.79 | 0.47 | 0.49 |
| Estrone | 0.49 | 0.75 | 0.92 | 0.49 | 0.72 |

the estrogens. Only those solvent systems which at least separated the estrogens or the androgens are reported here. Table I demonstrates that only one solvent system, *viz.* chloroform–ethyl acetate (80:20), completely separated the six compounds into discrete bands. The $R_F$ values obtained for the various compounds are given, and it should be noted that the Kontes plates with the preabsorbent gave excellent separations since the samples ran as discrete narrow bands and not as spots. Whereas many solvent systems separated either the estrogens or the androgens alone, only the one solvent system allowed for the simultaneous separation of all six compounds. Table II demonstrates that chloroform–ethyl acetate (80:20) gave the best separation with higher humidities (32% or 72%) as opposed to a low humidity (9%), thereby indicating that humidity also affected the separation of the steroids. The report of this simple and rapid technique for the separation of major estrogens from major androgens allows investigators in the area of reproductive biology to quickly analyze

TABLE II

$R_F$ VALUES OBTAINED FOR THE MAJOR ANDROGENS AND ESTROGENS USING VARIOUS HUMIDITIES

Kontes silica gel plates were preconditioned for 30 min with varying humidities prior to addition of the solvent (chloroform–ethyl acetate, 80:20) and TLC was performed for 100 min. Detection was by iodine vapor. Varying % humidities were prepared by using mixtures of concentrated $H_2SO_4$–water as follows: 9% (1:0.6), 32% (1:1.47), and 72% (1:3.65).

| *Steroid* | *Humidity (%)* | | |
|---|---|---|---|
| | *9* | *32* | *72* |
| Estriol | 0.08 | 0.08 | 0.11 |
| Androstanediol | 0.44 | 0.41 | 0.48 |
| Testosterone | 0.48 | 0.47 | 0.53 |
| Estradiol-17β | 0.51 | 0.52 | 0.57 |
| 5α-Dihydrotestosterone | 0.58 | 0.57 | 0.62 |
| Estrone | 0.69 | 0.73 | 0.74 |

whether uterine or other tissue incubations with radiolabeled testosterone or 5α-dihydrotestosterone result in conversion of these androgens to any of the known major estrogens, since the steroid bands obtained on TLC with carrier steroids can be extracted and counted in a liquid scintillation system for quantitation.

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REFERENCES

1 N. Bruchovsky, *Endocrinology*, 89 (1971) 1212.
2 J. D. Wilson and R. E. Gloyna, *Rec. Prog. Hormone Res.*, 26 (1970) 309.
3 G. Giannopoulos, *Biochem. Biophys. Res. Commun.*, 44 (1971) 943.
4 T. S. Ruh, S. G. Wassilak and M. F. Ruh, *Steroids*, 25 (1975) 257.
5 T. S. Ruh and M. F. Ruh, *Endocrinology*, 97 (1975) 1002.
6 H. H. Wotiz and S. J. Clark, *Methods Enzymol.*, 15 (1969) 158.
7 B. P. Lisboa, *Methods Enzymol.*, 15 (1969) 3.
8 P. K. Siiteri, *Methods Enzymol.*, 36 (1975) 485.