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## Thin-layer chromatographic separation of conjugated estrogens on Silica Gel G–silver nitrate plates

In attempting the thin-layer chromatographic (TLC) separation of conjugated estrogens, we examined the reported systems for separation of steroid conjugates[1–10]. Because these systems showed no promise, we tried silver nitrate impregnated plates which had proven useful for equine estrogens[11].

*Experimental*

*Preparation of TLC plates.* Five plates (20 × 20 cm) were coated with a slurry of 40 g of Silica Gel G with 100 ml of 15% aqueous silver nitrate, using a plexiglas TLC applicator and levelling board purchased from Applied Science Laboratories, Inc. The plates were air-dried (away from light) and stored in a desiccator.

*Procedure.* A chromatography tank was lined with filter paper and allowed to equilibrate for at least 1 h with the solvent system isooctane–chloroform–ethanol (40:70:18).

TABLE I

$R_F$ × 100 VALUES OF CONJUGATED ESTROGENS

TLC plates: Silica Gel G impregnated with silver nitrate. Solvent system: isooctane–chloroform–ethanol (40:70:18) (4 developments).

| *No.* | *Compound* | $R_F$ × 100 *value* |
|---|---|---|
| 1 | Sodium estrone sulfate | 65 |
| 2 | Sodium equilenin sulfate | 61 |
| 3 | Sodium equilin sulfate | 57 |
| 4 | Sodium α-estradiol sulfate | 53 |
| 5 | Sodium α-dihydroequilenin sulfate | 42 |
| 6 | Sodium α-dihydroequilin sulfate | 30 |
| 7 | Sodium β-estradiol sulfate | 51 |
| 8 | Sodium β-dihydroequilin sulfate | 30 |

A solution of each of the compounds (Table I) was prepared in water (2 mg/ml). A 4 μg spot of each compound, as well as a mixture of compounds Nos. 1–6, was applied in a horizontal line at the origin. Plates were developed four times to a height of 15 cm. They were air-dried between developments and returned to the same tank. Plates were sprayed lightly with 50% sulfuric acid in ethanol and heated for 10–15 min at 100°.

*Results and discussion*

As indicated in Fig. 1, the system completely separates compounds 1 to 6 inclusive. The chief disadvantage is that the β compounds, 7 and 8, are not completely separated from the corresponding α compounds (Nos. 4 and 6). However, for practical purposes, the system is a useful one.

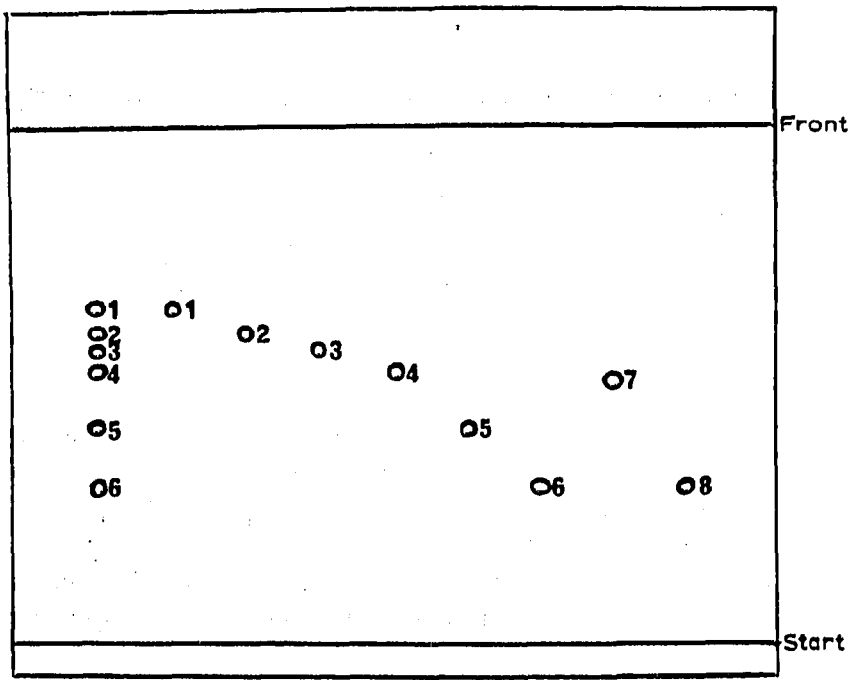


Fig. 1. Thin-layer chromatogram of conjugated estrogens. For explanation of numbers see Table I.

All compounds gave a discreet spot, yellowish brown in color except $\alpha$-estradiol sulfate which was pinkish brown. The background was very white and did not darken when the sprayed plate was stored in a cupboard for one week.

Under the same conditions, the free phenols ran close to the solvent front, well separated from the conjugated estrogens. When the compounds were spotted on a plain Silica Gel G plate, there was no separation, $R_F \times 100$ values were much lower (8 to 18), and the spots tended to streak and diffuse.

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