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Thin-layer chromatographic separation of equine estrogens on silica gel H–silver nitrate plates

Although the separation of estrogenic steroids, including equine estrogens, by thin-layer chromatography (TLC) has been extensively studied and reviewed[1], a satisfactory separation of the steroidal constituents of pregnant mares urine (estrone, equilin, equilenin and their corresponding pairs of 17-dihydroderivatives, in particular the 17α-alcohols) by TLC has not been reported.

The separation of compounds differing from each other only in degrees of unsaturation, using TLC plates impregnated with silver nitrate, has been reported[2–6]. We have extended this technique to the equine estrogens, in order to obtain a more satisfactory separation of the total mixture.

Preparation of TLC plates

Silica Gel G plates were prepared using Desaga equipment. Five plates (20 × 20 cm) were coated with a slurry of 40 g of Silica Gel G (Merck) with 80 ml of distilled water. After air-drying for 1 h, plates were stored in a cabinet until used.

For the preparation of Silica Gel H–silver nitrate plates five plates (20 × 20 cm) were coated with a slurry of 40 g of Silica Gel H with 100 ml of 7.5% aqueous silver nitrate. The plates (being constantly protected from light) were air-dried for at least 1 h, then heated at 105° for 20 min, cooled and stored in a desiccator. It was found preferable to use these plates on the day they were prepared, or the following day.

Procedure

The solvent system used was: cyclohexane–ethyl acetate (70:30). A solution of each of the compounds (Table I) was prepared in anhydrous ethanol (1 mg/ml).

Chromatography tanks were allowed to equilibrate with the solvent mixture for 1 h before use, and were lined with filter paper. Of each solution 1 μg was applied to each plate in a horizontal line at the origin (except α- and β-dihydro-equilenin on Silica Gel H–silver nitrate plates, for which 5 μg was necessary). Plates were developed to a height of 15 cm, three developments being required for Silica Gel G and four for Silica Gel H–silver nitrate, each time in a fresh tank, with air-drying of the plates between runs. Plates were lightly sprayed with 50% sulfuric acid in ethanol and heated for 10–15 min although α- and β-dihydroequilenin on Silica Gel H–silver nitrate were best viewed after 2–3 min, since they faded with further heating.

Results and discussion

Having examined all the more satisfactory reported separations[1] for equine estrogens, we found the best to be that shown in Fig. 1. The chief advantage of the Silica Gel G system was that many of the compounds gave distinctive colors (Table I), whilst on Silica Gel H–silver nitrate all compounds gave a yellowish brown color, except equilin, which had a greenish cast.

Since the separation achieved on Silica Gel G was not satisfactory for our purposes, systems based on silver nitrate-impregnated plates were developed, the best being shown in Fig. 2.

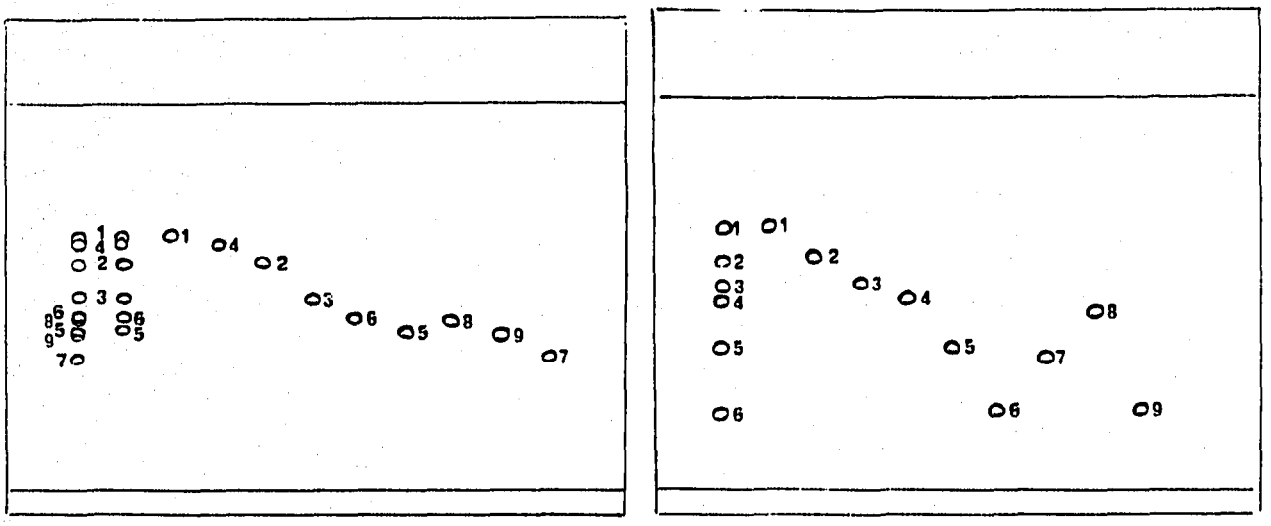

Fig. 1. Chromatogram on Silica Gel G. 1 = Estrone; 2 = equilenin; 3 = α-estradiol; 4 = equilin; 5 = α-dihydroequilenin; 6 = α-dihydroequilin; 7 = β-dihydroequilenin; 8 = β-estradiol; 9 = β-dihydroequilin.

Fig. 2. Chromatogram on Silica Gel H impregnated with silver nitrate. For numbering of spots, see the legend to Fig. 1.

TABLE I

R_F VALUES OF EQUINE ESTROGENS

Solvent system: cyclohexane–ethyl acetate (70:30). Three developments on Silica Gel G; four developments on Silica Gel H.

Compound	*Silica Gel H*		*Silica Gel G*	
	Impregnated with $AgNO_3$	*Plain*	*R_F*	*Colour*
Estrone	67	55	66	yellow
Equilin	48	52	64	green-yellow
Equilenin	50	48	50	pink
α-Estradiol	52	38	50	salmon
α-Dihydroequilin	10	34	45	orange
α-Dihydroequilenin	30	30	42	reddish
β-Estradiol	40	33	44	yellow
β-Dihydroequilin	21	28	41	yellow
β-Dihydroequilenin	34	23	36	pink

The most significant effect on silver nitrate-impregnated plates is that equilin and its derivatives have lower R_F values than on plain Silica Gel H, whereas the other six compounds have higher R_F values, comparable to their R_F values on Silica Gel G. Thus the π-complex of the isolated 7–8 double bond in the equilin with the

silver ion is much more significant than possible interactions of that ion with the aromatic ring which is common to all nine steroids.

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