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The separation of some estrogens by thin layer chromatography

Many solvent systems for the separation and characterization of estrogens and their derivatives by thin layer chromatography (TLC) have been reported[1-5]. None of these were found to be completely adequate for the separation of a particular mixture of eight estrogens (Table I) in which we were interested. This paper describes a new developing system for the TLC of these estrogens.

The spray reagent used for the visualization of the estrogens was previously reported for the colorimetric[6,7] and fluorometric[7] determination of some estradiol derivatives. Visual inspection of the colored spots has been used for qualitative location, and densitometric scanning confirmed adequate resolution.

Experimental

Reagents. All chemicals used were reagent grade. The chloroform was washed with enough purified water to remove completely any ethanol present. It was then dried with anhydrous sodium sulfate. This washed chloroform was used within one month.

Developing system. Washed chloroform–glacial acetic acid (10:1).

Spray reagent. Transfer 30 ml of methanol to a 100 ml volumetric flask placed in an ice bath. Carefully add in small portions concentrated sulfuric acid with constant swirling. Adjust the solution to room temperature before adding the final portion of acid to bring it to the mark.

Equipment. Pre-coated 250 μ thin layer plates [Silica Gel F 254] supplied by Brinkmann Instruments Inc. Micropipets, Microcaps (Drummond Scientific Co.). Photovolt Densitometer model 530 attached to a Varicord recorder model 42-B, and Integraph integrator model 49 (Photovolt Corp., New York, N.Y.).

Procedure

The TLC chamber was lined with filter paper and allowed to equilibrate with the developing solvent for 30 min before use to achieve complete saturation[2]. A mixture of the eight estrogens (Table I) in methanol was prepared. Samples containing 1 and 2μg of each estrogen in the mixture were spotted on the plate using a micropipet. The plate was developed at room temperature allowing the solution front to move 15 cm from the point of application (about 75 min). The plate was removed from the chamber and dried at room temperature. For visualization of the estrogens the spray reagent was applied, and the plate was heated at 110° for 10 min[7].

Results and discussion

The color of the eight estrogens in visible light, and the fluorescence under long wavelength U.V. light (about 366 mμ) are recorded in Table I. Also included in the Table are the R_F($\times$100) values. The reported R_F value for each estrogen is the average of 5 independent determinations. Fig. 1 shows the developed chromatogram after visualization as mentioned above. It is apparent that the mixture of the eight estrogens was completely resolved. For further confirmation of the separation of the estrogen mixture, the plate with the colored spots was scanned (Fig. 2) using the Photo-

TABLE I

COLOR, FLUORESCENCE AND R_F (× 100) VALUES OF THE EIGHT ESTROGENS DEVELOPED IN WASHED CHLOROFORM–GLACIAL ACETIC ACID (10:1)

Each figure represents the average of 5 independent determinations.

Estrogen[a]	*Color in visible light*	*Fluorescence*	R_F (× 100)
(1) Estriol	violet	yellow	8
(2) Estriol-3-methyl ether	violet	dark yellow	17
(3) Estradiol	yellow-orange	yellow-green	43
(4) 17α-Ethinylestradiol	pink	pink	46
(5) Estrone	yellow-orange	yellow-green	55
(6) Estradiol-3-methyl ether	yellow-orange	yellow-green	62
(7) 17α-Ethinylestradiol-3-methyl ether	pink	pink	67
(8) Estrone-3-methyl ether	yellow-orange	yellow-green	75

[a] (1) and (2) obtained from Mann Research Labs. Inc., New York, N.Y., (3) from the National Formulary Reference Standard, others obtained from Syntex Corporations, Mexico.

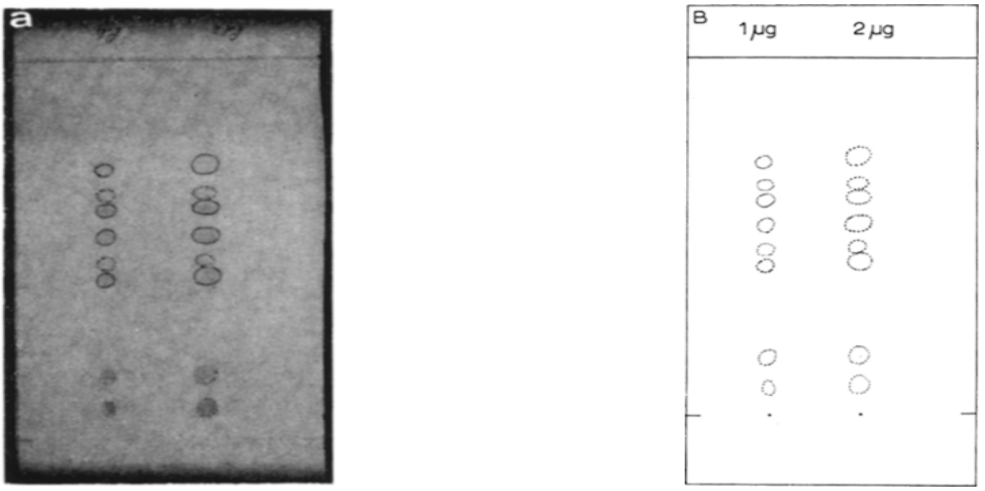

Fig. 1. The thin layer chromatogram showing the eight separated estrogens in the order as reported in Table I. Left lane corresponds to 1 µg, right lane 2µg of each estrogen. (a) Polaroid picture of the thin layer chromatogram; (B) diagram of thin layer chromatogram.

Fig. 2. Densitometric scan of TLC plate. Typical separation of the eight estrogen spots on the thin layer chromatogram with their corresponding recorded and integrated density curves. The peaks are numbered to correspond to Table I.

volt Densitometer equipped with an incandescent light source (without filter), range switch No. 3, and response No. 5.

This developing system has been used satisfactorily in this laboratory for the semi-quantitative measurement of the related foreign estrogens present in 17α-ethinylestradiol-3-methyl ether.

Preliminary experiments applying this system to estrogen level determinations in urine are promising, and will be reported later.

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Analytical and preparative thin-layer chromatography of flavonol glycosides and their acylated derivatives from pea tissues

It has been shown in recent years that developmental changes are often, in many plants, accompanied or preceded by quantitative or qualitative changes in their flavonoid composition[1–5]. Progress along this front has to some extent been inhibited by the time-consuming nature of the methods available for the separation of the many flavonoid components commonly found in higher plant tissues. Such a situation exists in *Pisum sativum* var. Alaska in which four closely related flavonol compounds show complex and transitory changes associated with developmental events[1]. The methods previously used for this study involved either two-dimensional paper chromatography[6], or selective adsorption on columns of borate-impregnated silica gel[1]. Neither of these methods enabled reliable separation of all the components. This paper describes a one-dimensional thin-layer chromatographic method which can be used for rapid analytical and preparative separation of all four components and which may be of use in other plant systems.

The flavonoids present in green pea leaves are kaempferol-3-triglucoside (KG); kaempferol-3-*p*-coumaroyltriglucoside (KGC); quercetin-3-triglucoside (QG); and quercetin-3-*p*-coumaroyltriglucoside (QGC). The separation is achieved on 20 × 20 cm plates using 0.25–1.0 mm layers of Silica Gel G (Merck) impregnated with a complexing anion, air dried and activated at 110° for 10 min. The anions investigated were borate,