means that aggregation of polysilicic acid as well as polymerization of monosilicic acid in the pH 2 solution is strongly retarded.

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Resolution of some closely related prednisolone derivatives by thin-layer chromatography

One of the most important tests of steroidal drugs is the related foreign steroids test[1–3]. In this test use is made of thin-layer chromatography (TLC) to detect the presence of structurally related compounds that may exist in the therapeutically active steroid. As a result of the interest in this laboratory in the determination of steroid purity and absence of closely related compounds by TLC, solvent systems were reported previously for the separation of some estrogens[4] and some closely related hydroxycorticosteroids[5]. The semiquantitation of the closely related steroids as well as the estimation of flurandrenolone acetonide purity by TLC, were also achieved[6]. Several TLC systems for cortical steroids have been reported[7–10]. None of these were found to be completely adequate for the complete resolution of a mixture of closely related prednisolone derivatives in which we were interested. This paper describes a new developing system—and its application—for the complete resolution of this particular mixture by TLC on silica gel.

Materials

Reagents. All solvents and chemicals were reagent grade.

Developing system. Ethyl propionate.

Spray reagent. Methanolic sulfuric acid, prepared as mentioned previously[4].

Equipment. Pre-coated 250 μ thin-layer plates (Silica Gel F_{254}) supplied by Brinkmann Instruments Inc. Micro-pipets, Microcaps (Drummond Scientific Compa-

ny). Chromato-vue equipped with short and long wavelength UV lamp (about 254 and 366 mμ, respectively) by Ultra-Violet Products Inc.

Procedure

The TLC chamber was lined with filter paper and allowed to equilibrate with the developing solvent for 30 min to achieve complete saturation. The solutions of the prednisolone derivatives (Table I) made up (1 mg/ml) in a mixture of chloroform-methanol (1:1) were spotted on the plate using a micro-pipet. Spots of 5 μg steroid were used. The plate was developed in the chamber allowing the solvent front to travel 15 cm after passing through the point of application. It was then removed from the chamber and the solvent allowed to evaporate at room temperature (25°) for 5 min. A second development of the plate in the same developing solvent was necessary to obtain adequate resolution of the zones. The plate was allowed to dry again at room temperature and the spots were detected using a short wavelength UV light and marked.

Results and discussion

Each of the prednisolone derivatives listed in Table I was run singly and in combination with other compounds listed. Measurements were made from the point of application to the center of the spot and the R_F value of each steroid was calculated as the observed movement of the spot after the second run divided by the distance of a single development (15 cm) (ref. 18). The average value for five determinations of the R_F (× 100) for each of these steroids is given in Table I.

TABLE I

R_F (× 100) VALUES FOR THE PREDNISOLONE DERIVATIVES DEVELOPED TWICE BY ETHYL PROPIONATE
Each figure represents the average of five independent determinations.

No.	*Steroid*[a]	*R_F (× 100)*
1	6α-Fluoro-16α-methyl prednisolone (paramethasone)[17]	21
2	6α-Fluoro-16α-methyl prednisolone-21-acetate (paramethasone acetate)[17]	51
3	6α-Fluoro-16α-methyl prednisolone-11,21-diacetate	57
4	6α-Fluoro-16α-methyl prednisone-21-acetate	63

[a] All steroids in this work were obtained from Syntex Corporation, Palo Alto, Calif., U.S.A.

The solvent system reported in this paper has been used satisfactorily in this laboratory for the semiquantitative determination of the closely related foreign steroids (Table I, Nos. 1, 3 and 4) that may be present in the raw material of paramethasone acetate. In this case the TLC plate was divided into four equal sections. One and 3 μg of each of the related foreign steroids were applied on sections 1 and 3, respectively. On section 2, 100 μg of paramethasone acetate sample was applied. Section 4 was considered as the plate blank. The plate was developed and visualized as mentioned before. For the evaluation of the plate in order to determine the percentage of related foreign steroids, each extra spot—in section 2—other than the main compound was compared with the spot having the same R_F value (mobility) in

sections 1 and 3 containing, respectively, 1 and 3% level of related foreign steroids. The visual semiquantitation under short wavelength UV light is facilitated by having the sample between two levels of related foreign steroids, namely 1 and 3%. It was found that better detection of the 1 μg spot was achieved by applying the spray reagent then by heating at 105° for 5 min and observing the plate under long wavelength UV light.

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