TABLE I.—RELATIVE ABSORBANCE[a] OF REACTION PRODUCTS OF CALCIUM AND SODIUM PANTOTHENATE

Determination No.	Assay 1 CaPan	Assay 1 NaPan Equivalent	Assay 2 CaPan	Assay 2 NaPan Equivalent	Assay 3 CaPan	Assay 3 NaPan Equivalent
1	145	129	149	141	145	156
2	144	132	147	148	149	152
3	138	131	144	141	143	146
4	133	137	141	153	163	150
5	146	125	154	137	138	160
Mean	141.2	130.8	147.0	144.0	147.6	152.8
Std. Error	2.5	2.0	2.2	2.9	4.2	2.4

[a] Absorbance × 100 per mg.

TABLE II.—RECOVERY OF SODIUM *d*-PANTOTHENATE ADDED TO A COMPLEX VITAMIN PREPARATION

Sodium *d*-Pantothenate, mg./ml. Added	Found	Recovered
0	0.25	...
0.50	0.83	0.58
1.00	1.38	1.13
2.00	2.20	1.95

pantothenate added to a liquid elixir containing a mixture of the B vitamins and yeast extracts, as shown in Table II, indicated a recovery of 97–116%.

Samples of typical multivitamin preparations containing sodium *d*-pantothenate were analyzed, using calcium *d*-pantothenate as a reference standard. Three of these samples were in a lyophilized form. The results, shown in Table III, indicated a reasonable mean overage of 20% of the declared potency.

It may be concluded that the spectrophotometric method developed for calcium *d*-pantothenate is equally applicable to the determination of its sodium salt in pharmaceutical products, and that calcium *d*-pantothenate may be used as a reference standard.

TABLE III.—SODIUM *d*-PANTOTHENATE CONTENT OF MULTIVITAMIN PREPARATIONS

Sample	Mg. per ml. or Vial — Label Claim	Found
1. Injectable, lyophilized, B vitamins,[a] C, gentisic acid ethanolamide	20	22.7
2. Injectable, lyophilized, B vitamins,[a] C	5	7.13
3. Injectable, lyophilized, B vitamins,[a] C	5	5.20
4. Liquid drops, B vitamins,[a] A, C, D	8.33	10.2

[a] Including thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, niacinamide, vitamin B_{12}, and sodium *d*-pantothenate.

REFERENCE

(1) Panalaks, T., and Campbell, J. A., *Anal. Chem.*, 34, 64(1962).

Thin-Layer Chromatographic Identity Test for Steroids in Sesame Oil Preparations

By B. P. KORZUN and S. BRODY

A thin-layer chromatographic method is described as a rapid identity test for methandrostenolone,[1] estradiol dipropionate,[2] testosterone propionate,[3] desoxycorticosterone acetate,[4] and aldosterone acetate in sesame oil preparations. Preliminary extractions are not necessary to obtain desired results.

IT WAS desirable to obtain a rapid method for identifying steroids and decomposition products in sesame oil preparations. Attempts to apply paper chromatography failed because the sesame oil interfered, and the low concentration of steroid present did not give discernible spots with the usual reagents. Thin-layer chromatography, a technique described by Stahl (1, 2), using silica gel G as the adsorbent gave good results. The steroid is well separated from the oil and is easily detected with a modified Le Rosen reagent (3). The time required to complete the test is one to two hours.

Received August 1, 1962, from the Research Department, CIBA Pharmaceutical Co., Summit, N. J.
Accepted for publication August 21, 1962.

[1] Marketed as Dianabol by CIBA Pharmaceutical Co.
[2] Marketed as Ovocylin by CIBA Pharmaceutical Co.
[3] Marketed as Perandren by CIBA Pharmaceutical Co.
[4] Marketed as Percorten by CIBA Pharmaceutical Co.

EXPERIMENTAL

Apparatus.—The apparatus for coating the glass plates and other accessories is commercially available from Research Specialties Co., Richmond, Calif.; Arthur H. Thomas Co., Philadelphia, Pa.; and Desaga, Heidelberg, Germany. The adsorbent used is silica gel G.[5]

Preparation of Plates.—A slurry of 30 Gm. of silica gel G and 60 ml. of water is prepared and poured into applicator and spread over the plates.

[5] Manufactured according to specifications of E. Stahl by Merck, A. G., Darmstadt, Germany, and available through Research Specialties Co. and Terra Chemicals, Inc., New York.

The plates are then activated in an oven at 80° for 2 hours, and stored over a desiccant in an air-tight container.

Sample Application.—The samples are applied as a streak 2.5 cm. wide, 2.5 cm. apart, and 1.5 cm. from the bottom of the plate. This is accomplished with a micropipet just above the point of application and allowing the solution to be deposited uniformly as the pipet is passed along the 2.5 cm. length. The sample is applied in a volume sufficient to deposit 100 mcg. of the steroid on the plate. An equal volume of sesame oil used to prepare the sample is run as a reference and a 100 mcg. steroid reference sample is also applied. The concentration of steroid in the sesame oil preparation ranges from 0.5 mg./ml. to 50 mg./ml. Therefore, the reference samples of sesame oil must be applied at a volume equal to the volume required to apply 100 mcg. of steroid in the sample preparation.

Solvent Systems.—The developing chambers are lined with filter paper to saturate the chambers. The solvent for testosterone propionate and desoxycorticosterone acetate is chloroform. The solvent for estradiol dipropionate is benzene-methylene chloride (4:6); for aldosterone acetate the solvent is chloroform-ethyl acetate (7:3); and for methandrostenolone the solvent is chloroform-methanol (98:2). The length of development is 15 cm. from the point of application for all solvent systems. After the first development the solvent is dried and developed again.

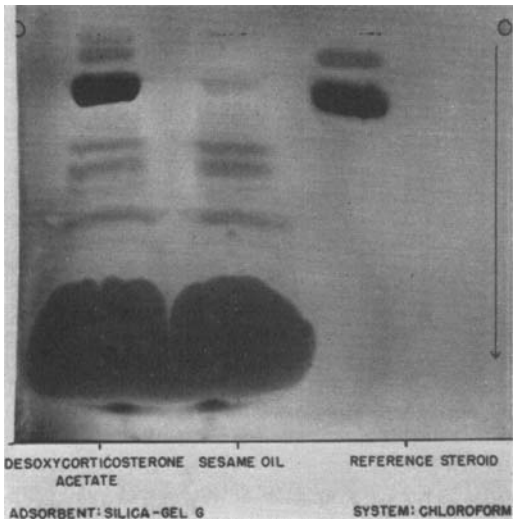

Fig. 1.

Detection.—The steroids are revealed by spraying the plate with a modified Le Rosen reagent (3). The reagent is prepared by dissolving 0.8 ml. formaldehyde (37%) in 10 ml. sulfuric acid and diluted with 3 ml. water. After being sprayed, the plate is heated in an oven at 80° for 5 to 10 minutes. The steroids produce visible colored spots and also produce fluorescent spots under an ultraviolet lamp (3660 Å.).

RESULTS

Figure 1 shows a typical separation of the steroid in sesame oil. The reference steroid shows another slower moving band which can be detected in the oil preparation. Also, another band is detected at the origin in the sample which is not detected in the sesame oil or the reference steroid and which could be a decomposition product of the steroid. Therefore, it is necessary to run a sesame oil reference sample to determine which bands are due to the steroid. Table I lists the R_f values and the colors produced after spraying with formalin reagent. The identity test can be accomplished in one to two hours.

TABLE I.

Steroid	R_f Value[a]	Color	
		Visible	Fluorescent
Methandrostenolone	0.40	Brown	Salmon pink
Estradiol dipropionate	0.45	Red	Orange
Testosterone propionate	0.40	Dark green	Pale pink
Desoxycorticosterone acetate	0.15	Blue	Red
Aldosterone acetate	0.30	Dark yellow	Yellowish green

[a] Value for steroid in solvent described.

REFERENCES

(1) Stahl, E., *Chemiker-Ztg.*, **82**, 323(1958).
(2) Stahl, E., *Pharm. Rundschau*, **1**, No. 2, 1(1959).
(3) Le Rosen, A. J., Moraveck, R. T., and Calton, J. K., *Anal. Chem.*, **24**, 1335(1952).