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Note

Behaviour of synthetic corticoids in ointment on 3-cyanopropyltrichlorosilane in high-performance thin-layer chromatography

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In previous papers[1–4] we have considered the effect of the methylene chain length of amino chemically bonded silicas in high-performance thin-layer chromatography (HPTLC). Whereas there have been many reports on high-performance liquid chromatography (HPLC) on silica gels with chemically bonded cyanoalkyl groups, there are few reports on the effectiveness of cyanoalkyl bonded silicas in HPTLC[5–7]. Therefore, we have now studied the HPTLC separation of synthetic corticoids in ointment on 3-cyanopropyltrichlorosilane (3CPTS)-treated thin-layer plates.

EXPERIMENTAL

Chemical reagents and materials

Commercially available 10 cm × 10 cm thin-layer plates pre-coated with silica gel (silica gel 60, Art. 5631) were obtained from Merck (Darmstadt, F.R.G.). 3CPTS was obtained from Aldrich (Milwaukee, WI, U.S.A.). Most sample synthetic corticoids were from Tokyo Kasei (Tokyo, Japan) (Table I); other chemical reagents were from Wako (Osaka, Japan).

TABLE I

CORTICOIDS USED

No.	*Corticoid*
1	Prednisolone
2	Prednisolone valerate acetate
3	Hydrocortisone acetate
4	Dexamethasone
5	Betamethasone valerate
6	Betamethasone dipropionate
7	Fluocinolone acetonide
8	Beclometasone dipropionate
9	Prednisolone acetate
10	Hydrocortisone
11	Flumetasone pivalate

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Apparatus

The HPTLC measurements were carried out using a Shimadzu CS-910 TLC scanner equipped with a C-R1A Shimadzu Chromatopac, and a Shimadzu CS-930 TLC scanner equipped with a DR-2 Shimadzu Chromatopac.

Cyanoalkyl-treated plates

As described previously[8,9], eight dried plates of silica gel were immersed in 260 ml of a 1.92% toluene solution of 3CPTS. After standing for 72 h at room temperature, the plates were washed several times with toluene, chloroform and methanol, and then dried *in vacuo* at 70°C for 2 days, finally producing 3CPTS ($\equiv SiCH_2CH_2CH_2CN$)-treated plates for HPTLC.

Chromatography

Sample solutions dissolved in methanol were spotted 1.5 cm from one edge of the plate. The plate was developed to a distance of 6 cm from the origin at room temperature in a Camag Twin Through Chamber (10 cm × 10 cm). After drying, visualization of the corticoid spots with sulphuric acid was carried out according to the procedures of Carstensen[10] and Heftmann[11]. The plate was thoroughly dried, sprayed uniformly with 10% sulphuric acid solution and heated at 100°C for 10 min in an oven. The corticoids appeared as fluorescent violet spots on a dark white background, under UV irradiation with a Camag Reprostor system.

Densitometry

Quantitation of the fluorescence intensity of corticoid spots was carried out

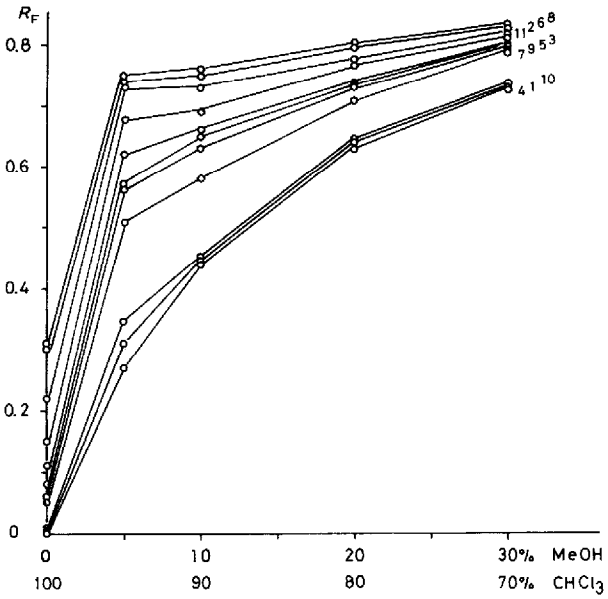

Fig. 1. The dependence of R_F on the mobile phase composition in the normal-phase mode. Stationary phase: 3CPTS-treated HPTLC plate. Migration distance; 6.0 cm in an unsaturated chamber. Sample: corticoids numbered as in Table I.

directly on the 3CPTS-treated plates with a Shimadzu CS-910 TLC scanner or a CS-930 TLC scanner. Integrated values of the fluorescence intensity were derived by use of a Shimadzu C-R1A Chromatopac or an DR-2 Chromatopac.

Procedure

A 1-g amount of ointment was blended four times using 30 ml of methanol, warmed in a hot-bath at 50°C for 5 min and then cooled in the icebox for 30 min at 5°C. The organic phase was dried and made up to volume with methanol in a 30-ml volumetric flask.

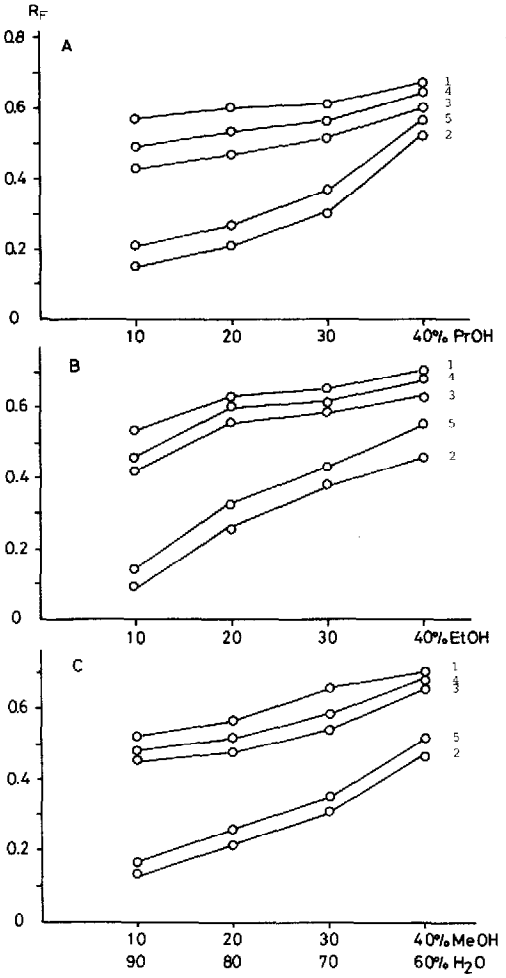

Fig. 2. The dependence of R_F on the mobile phase composition for corticoids on the 3CPTS-treated HPTLC plate in reversed-phase systems. Migration distance: 6.0 cm in an unsaturated chamber. Mobile phases: (A) propanol–water; (B) ethanol–water; (C) methanol–water. Sample: corticoids numbered as in Table I.

RESULTS AND DISCUSSION

Using the densitometric recordings of the fluorescence intensity, the relationship between the treatment temperature and the treatment time for each corticoid on the 3CPTS-treated plate was studied. A temperature of 100°C and a time of 10 min were found to be the most suitable. Synthetic corticoids were then investigated on the 3CPTS-treated HPTLC plate, using chloroform and chloroform–methanol as eluents. Fig. 1 shows the dependence of R_F on the mobile-phase composition. The 3CPTS-treated HPTLC plate, having a weakly polar stationary phase, may be suitable for separating strongly polar substances under mild elution conditions.

Fig. 2 shows the dependence of R_F for corticoids on the 3CPTS-treated HPTLC plate in reversed-phase systems, using methanol–water, ethanol–water or propanol–water as eluent. Because of the pK value of the cyano group in aqueous

TABLE II

RECOVERY OF CORTICOIDS FROM COMMERCIAL OINTMENTS ACCORDING TO THE DESCRIBED METHOD

C.V. = Coefficient of variation.

Sample No. (n = 5)	*Amount (g)*	*Corticoid added (mg)*	*Corticoid found (mg)*	*Recovery (%)*	*Corticoid content (%)*	*C.V. (%)*
1	1.01	0	4.99			
		5.01	9.92–9.95	98.4–99.0	0.49	0.33
2	0.99	0	2.98			
		3.00	5.91–5.97	97.7–99.7	0.30	1.10
3	1.02	0	9.99			
		10.02	19.85–19.98	98.4–99.7	0.98	0.71
4	1.01	0	0.49			
		0.51	0.99–1.00	98.0–100.0	0.05	1.10
5	1.02	0	1.20			
		1.20	2.43–2.39	95.0–99.2	0.12	2.30
6	1.00	0	0.63			
		0.64	1.25–1.26	96.9–98.4	0.06	0.79
7	1.03	0	0.24			
		0.25	0.48–0.49	96.0–100.0	0.02	2.19
8	0.99	0	0.24			
		0.25	0.48–0.49	96.0–100.0	0.02	2.17
9	1.01	0	5.00			
		5.03	9.91–10.00	97.6–99.4	0.50	0.99
10	1.01	0	9.98			
		10.01	19.85–19.93	98.6–99.4	0.99	0.44
11	1.02	0	0.19			
		0.22	0.40–0.41	95.5–100.0	0.02	2.42

media, the 3CPTS-treated HPTLC plate may be regarded as a weakly basic ion exchanger.

Table II shows the recovery of corticoids from commercial ointments according to this method.

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