THIN-LAYER CHROMATOGRAPHY OF STEROIDS ON MICROPLATES WITH FIXED SILICA GEL

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Chromatography in a thin layer of adsorbent is widely used for the analysis of steroid compounds both in the laboratory and in clinical practice [1-3]. The method of chromatography on microplates with a bound layer of adsorbent developed by Peifer in 1962 has speeded up analysis still more and has made it simpler and more economical [4]. This method has made it possible to operate with small amounts of substance (0.1-1 γ) which is particularly important in the analysis of biological materials and the study of enzymatic processes.

We have used Russian silica gel of types KSK and KSM to prepare microplates by Peifer's method and have used it for the chromatographic analysis of sterols, bile acids, and other steroid compounds.

A solution of iodine in potassium iodide, Lugol's reagent [5-7], was used to detect the steroids. When 0.15-1 γ of the substance was deposited on a plate, well-defined spots were obtained (see table).

The usual solvent systems were used for chromatography. The layer of silica gel (1.8-2 mg/cm^2) on the plates was extremely strong and withstood spraying with various reagents. A time of 10-15 min was required for the solvent to travel from the start to the front (60-65 mm) on these plates. This method can be used to detect and separate a number of substances. However, no connection between the structure of the steroids and their coloration on treatment with Lugol's reagent is found. Thus, of the 11 steroids investigated at a concentration of 0.4 γ, only cholest-4-enone, cholest-5-enone, and cholest-anone gave a yellow coloration. The other steroids were uncolored even at a concentration of 0.8 γ.

A majority of the acids investigated formed whitish spots and only cholic acid and its methyl ester gave blue spots. The esters of the other acids were colored yellow. The method described permits several substances of similar mobilities to be separated. Thus, 11α- and 16α-hydroxyprogesterones are colored blue and 15α-hydroxyprogesterone yellow.

As in paper chromatography, treatment of the spots with ether enhances the sensitivity of the reaction. Good results are also obtained by spraying the spots with a solution of vanillin in sulfuric acid [8] and with a solution of 2,4-dinitrophenylhydrazine. The method of determining the Δ^4- and $\Delta^{1,4}$-3-keto groupings by treatment with a solution of isonicotinic acid hydrazide (INAH) proposed by Smith and Foell [9] can also be used with silica gel microplates. For example, on being sprayed with a weak solution of INAH androstenedione gives a yellow coloration after 5-10 min, and androsta-1,4-dienedione in a concentration of 0.8 g shows up 1-1.5 hr after spraying with a concentrated solution of INAH and in a concentration of 0.15-0.3 γ only after 10-15 hr.

We have used the method of chromatographing steroids on microplates that has been described above for monitoring the course of microbiological conversions of steroid compounds and chemical reactions.

Experimental

Preparation of the silica gel. Granulated silica gel of type KSK or KSM previously ground in a coffee mill was placed in a glass column, washed with concentrated hydrochloric acid until the reaction for iron was negative, then with tap water to neutrality, with distilled water until the reaction for chlorine ion was negative and, finally, with methanol. The silica gel was dried in air and ground for 1-1.5 hr in a ball mill, the fraction with grain dimensions less than 30 μ being taken and dried for 48 hr at 120° C.

Preparation of the plates. A mixture of 30 g of silica gel, 1.5 g of dry sieved (through a 250-mesh sieve) medicinal gypsum, and 160 ml of chloroform was stirred vigorously with a magnetic stirrer for 30-40 min until a uniform suspension had been formed. A dry plate (microscope slide 2.6 × 7.6 cm) that had been washed with chromic acid mixture was immersed in the suspension and immediately withdrawn, the excess of suspension being allowed to drain off, and after the evaporation of the chloroform the silica gel was removed from one of the sides of the plate. The plates can be used without additional activation. On storage in a dry place protected from dust, they do not require activation for several months.

Chromatography. A Petri dish with a ground cover was used as the chamber. Solutions of the steroids in chloroform (0.5 mg/ml) were deposited by means of a calibrated micropipet.

R_S Values of some Steroid Compounds on Microplates with a Fixed Layer of KSK Silica Gel
(Visualization with Lugol's Reagent)

No.	Compound	Solvent system and R_S		$I_2 + KI$	
				Minimum conc., γ	Color
	R_S referred to cholesterol				
1	Cholesterol	1	1.0	0.15	W.
2	β-Sitosterol		1.0	0.15	
3	Stigmasterol		1.08	0.15	
4	Ergosterol		0.92	0.15	
5	Lanosterol		1.52	0.15	
6	Cholesterol acetate		2.48	0.15	
7	β-Sitosterol acetate		2.48	0.15	
8	β-Sitosterol benzoate		2.64	0.15	
9	Cholest-5-en-3-one		2.12	0.4	Y.
10	Cholest-4-en-3-one		1.48	0.4	
11	Cholestanone		2.16	0.4	
	Acids				
12	Cholic acid	2	0.13	0.15	B.
13	Deoxycholic acid		0.36	0.15	W.
14	Ursodeoxycholic acid		0.38	0.15	
15	Hyodeoxycholic acid		0.20	0.15	
16	Chenodeoxycholic acid		0.36	0.15	
17	Lithocholic acid		0.84	0.15	
18	Dehydrocholic acid		0.60	0.15	
19	3β-Hydroxychol-5-enoic acid		0.82	0.15	
20	3β-Hydroxyeti-5-enoic acid		0.80	0.15	
	Methyl ether-acid (salt)				
21	Methyl cholate	3	0.09	0.15	B.
22	Methyl deoxycholate		0.46	0.15	Y.
23	Methyl hyodeoxycholate		0.22	0.15	
24	Methyl lithocholate		0.91	0.15	
25	Methyl dehydrocholate		0.72	0.15	
26	Methyl 2β-hydroxyeti-5-enoate		0.90	0.15	
	Acids				
27	3β-Acetoxychol-5-enoic acid	4	1.08	0.15	W.
28	3β-Acetoxy-bis-norchol-5-enoic acid		1.16	0.15	
29	3β-Acetoxy-5α-bis-norcholanic acid		1.12	0.15	
30	3β-Acetoxy-5α-etianic acid		1.16	0.15	
31	Methyl cholest-5-en-27-oate		1.50	0.15	
32	(24–26)-Lactone of 3β-acetoxychol-5-enoic acid		0.72	0.15	
33	Dehydroepiandrosterone		0.55	0.4	
34	Androst-4-ene-3,17-dione		0.43	0.4	—
35	Androst-1,4-diene-3,17-dione		0.25	0.4	Y.
36	19-Norandrost-4-ene-3,17-dione		0.40	0.15	B.
37	19-Norandrost-4,9(10)-diene-3,17-dione		0.30	0.15	Y.
38	Androsta-4,6-diene-3,17-dione		0.51	0.15	
39	Testosterone		0.30	0.15	
40	19-Nortestosterone		0.23	0.15	B.
41	17β-Hydroxy-19-norandrosta-4,9(10)-dien-3-one		0.18	0.15	Y.
42	17β-Hydroxyandrost-5(10)-en-3-one		0.48	0.15	
43	17β-Hydroxyandrosta-4,6-dien-3-one		0.28	0.15	
44	Dihydroxytestosterone		0.50	0.15	
45	2-Methyldihydrotestosterone		0.75	0.8	
46	6β-Hydroxytestosterone		0.11	0.8	
47	Testosterone propionate		1.01	0.4	
48	Dehydroepiandrosterone acetate		1.09	0.4	

Continued

No.	Compound	Solvent system	R_s	I_2 + KI' Minimum conc., γ	I_2 + KI' Color
	R_s referred to cholesterol				
49	Estrone	5	1.09	0.15	W.
50	Estradiol	5	0.70	0.8	W.
51	Estrone methyl ether	5	1.25	0.15	Y.
52	Reichstein's substance S	5	0.73	0.15	Y.
53	Acetate of Reichstein's substance S	5	1.00	0.15	Y.
54	Corticosterone	5	0.58	0.15	Y.
55	Cortisone	5	0.58	0.15	B.
56	Hydrocortisone	5	0.42	0.15	—
57	Epihydrocortisone	5	0.25	0.8	—
58	Prednisone	5	0.42	0.15	Y.
59	Prednisolone	5	0.44	0.15	Y.
60	6β, 11α-Dihydroxypregn-4-ene-3, 20-dione	5	0.39	0.15	—
61	6β, 14α-Dihydroxypregn-4-ene-3, 20-dione	5	0.42	0.15	—
62	11α, 17α, 20β-Trihydroxypregn-4-ene-3, 20-dione	5	0.14	0.15	W.
63	17α, 20β, 21-Trihydroxypregn-4-en-3-one	5	0.21	0.15	Y.
	R_s referred to 11α-hydroxy progesterone				
64	Progesterone	6	2.2	0.4	Y.
65	11α-Hydroxyprogesterone	6	1.00	0.15	B.
66	15α-Hydroxyprogesterone	6	1.00	0.15	Y.
67	16α-Hydroxyprogesterone	6	1.00	0.15	B.
68	17α-Hydroxyprogesterone	6	2.00	0.15	DG
69	20β-Hydroxypregn-4-en-3-one	6	1.96	0.15	Y.
70	3β-Hydroxypregn-5-en-20-one	6	1.80	0.4	Y.
71	Deoxycorticosterone	6	1.73	0.15	Y.
72	11β-Hydroxyprogesterone	6	1.53	0.8	Y.
73	6-Oxoprogesterone	6	2.00	0.8	Y.
74	11-Oxoprogesterone	6	2.00	0.8	Y.
75	3β-Hydroxy-16α, 17α-epoxypregn-5-en-20-one	6	1.64	0.8	—
76	11α-Hydroxy-16α, 17α-epoxypregnene-3, 20-dione	6	1.34	0.8	—
77	11α-Hydroxyprogesterone acetate	6	2.00	0.8	Y.
78	17α-Hydroxyprogesterone acetate	6	2.40	0.4	Y.
79	3β-Hydroxypregn-5-en-20-one acetate	6	2.60	0.4	—
80	Deoxycorticosterone acetate	6	2.20	0.15	Y.
81	3β-Hydroxypregna-5, 16-dien-20-one	6	2.25	0.15	Y.
82	Hydroxy-16α-17α-epoxypregnenone acetate	6	2.60	0.15	Y.
83	16α, 17α-Epoxy-20-ethylenedioxy-3β-hydroxypregn-5-ene	6	2.11	0.15	Y.

Note: Solvent systems: 1) hexane–ethyl acetate (4:1); 2) the same (1:3); 3) the same (1:2); 4) the same (2:1); 5) Benzene–acetone (2:1); 6) the same (1:3). Colors: Y–yellow, B–blue, DG–dark gray, W–whitish or uncolored (detected by spraying with H_2SO_4 followed by heating).

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Summary

1. A method for chromatographing steroids on microplates with silica gel of types KSK and KSM has been developed.

2. The possibility of using a solution of iodine in potassium iodide to detect steroid compounds has been demonstrated and the sensitivity of this reaction has been determined.

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