SHORTENING OF DEVELOPMENT TIME IN THIN-LAYER ADSORPTION CHROMATOGRAPHY

APPLICATION TO THE SEPARATION OF STEROIDS

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(Received February 8th, 1963)

One of the greatest advantages of thin-layer adsorption chromatography, described by STAHL[1], is the significant shortening of the time required for complete chromatographic analysis. With Merck's Silica Gel G, the time of development of thin-layer chromatograms varied from 20 to 120 min depending on the composition of the elution mixtures[1,2].

In our method of partition thin-layer chromatography[3], in which Celite 545 and Zaffaroni's solvent systems were used, the time of development was shortened to 3–7 min. Nevertheless the necessity for evaporating the slightly volatile components of Zaffaroni's stationary phases (formamide, glycols), prior to detection, prolonged the time of complete analysis.

In our present work we used mixtures of Silica Gel and Kieselguhr for coating the plates for thin-layer adsorption chromatography and were successful in effectively shortening the time required for complete chromatographic analysis. The addition of Kieselguhr to active adsorbents in order to increase the rates of flow from chromatographic columns has been previously described[4*].

We used Merck's Silica Gel G or Light's Silica Gel, specially prepared, (see Experimental) both mixed with 20 % or 50 % of Celite 545. Chromatograms were developed with various elution mixtures and the times taken by the front to move 10 cm were observed. The results are shown in Table I.

As can be seen, the addition of an equal amount of Celite 545 to Light's Silica Gel increased the rate of travel of the elution front about 3.5 times. In the case of Merck's Silica Gel G the increase was 2.5 times. Depending on the composition of elution mixtures, the time of development varied from 3–15 min (in one case only it was equal to 19 min).

As the elution mixtures commonly used in thin-layer adsorption chromatography consist mainly of low-boiling organic solvents, the time of drying is very short, *i.e.* 2–4 min and the complete chromatographic analysis can be carried out in about 20 min. It is particularly useful in cases of chemical or enzymic kinetic studies as for instance in biosyntheses of steroids.

In the bio-oxidation reaction of MAMOLI AND VERCELLONE[6] where 3,21 diacetoxy-

* Quite recently BENNETT AND HEFTMANN[5], have used plates coated with mixtures of Silica Gel G and Kieselguhr G (1 : 1) for better separation of C-25 sapogenins.

TABLE I

TIMES TAKEN BY THE LIQUID FRONT TO MOVE 10 CM FOR DIFFERENT ELUTION MIXTURES AND DIFFERENT ADSORPTION LAYERS

Elution mixture	*Adsorption layer*					
	Specially prepared Light's Silica Gel			*Merck's Silica Gel G*		
	No Celite added	*With Celite 545*		*No Celite added*	*With Celite 545*	
		4:1	*1:1*		*4:1*	*1:1*
Cyclohexane	54	38	19	32	29	15
Cyclohexane–benzene (1:1)	36	27	14	25	20	14
Toluene	33	27	11	20	16	9
Benzene	43	27	11	22	16	10
Toluene–ethyl acetate (9:1)	35	22	10	21	14	8
Toluene–ethyl acetate (1:1)	37	23	11	20	14	8
Ethyl acetate	31	23	11	20	17	8
Chloroform–ethyl acetate (1:1)	34	21	15	25	22	10

Δ^5-pregnen-17-ol-20-one was transformed into Reichstein "Substance S", using a mixture of Merck's Silica Gel G and Celite 545, we were able to give the semiquantitative results 35 min after taking a sample from the fermenter.

The addition of Celite 545 to the adsorbents changed only to a small extent the selectivity of the resolution of steroidal mixtures. The R_F values were increased, as we expected, because of the dilution of the active adsorbent in the layer.

A comparison of two chromatograms obtained by different methods is presented in Table II and Fig. 1.

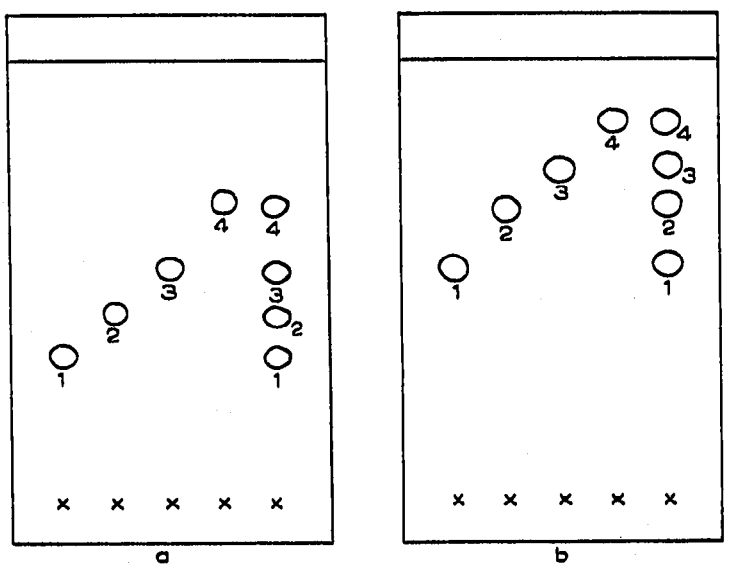

Fig. 1. Schematic view of the chromatograms described in Table II. Spot numerals correspond to the numerals of steroid substances in Table II. 30 γ of each steroid substances was applied. Detection with isonicotinic hydrazide. (a) Adsorbent Merck's Silica Gel G. Average diameter of spot 5 mm. Time of development 16–19 min. (b) Adsorbent mixture (1:1) of Merck's Silica Gel G and Celite 545. Average diameter of spot 6 mm. Time of development *ca.* 8 min.

TABLE II

COMPARISON OF THE R_F VALUES OF SOME STEROIDS ON THIN LAYERS OF SILICA GEL G AND A MIXTURE (1:1) OF SILICA GEL G AND CELITE 545

Elution mixture = Benzene–ethyl acetate (1:1)

No.	*Steroid*	*Thin layer of Silica Gel G*	*Thin layer of a mixture of Silica Gel G and Celite 545 (1:1)*
1.	Δ^1-Dehydromethyltestosterone	0.33	0.52
2.	Methyltestosterone	0.43	0.65
3.	Androstendion	0.53	0.74
4.	Methyltestosterone acetate	0.68	0.85

EXPERIMENTAL

(1) *Preparation of adsorbents*

Celite 545 (L. Light & Co, England) was ground in a ball mill and sifted through a sieve DIN 1171, 0.071 mm. Silica Gel (100/200 mesh) for chromatography (L. Light & Co, England) was ground in a ball mill and sifted through a sieve DIN 1171, 0.071 mm.

(2) *Preparation of plates*

Three plates of mirror glass (thickness 5 mm, width 100 mm, length 180 mm) were coated with a slurry of given alternative composition:

(a) 3.5 g of Silica Gel prepared as described in (1); 3.5 g of Celite prepared as described in (1); 0.4 g of gypsum sifted through a sieve DIN 1171, 0.071 mm; 20 ml of water.

(b) 3.5 g of Merck's Silica Gel G; 3.5 g of Celite 545 prepared as described in (1); 20 ml of water.

The slurry was spread on the plates by means of a glass rod. Uniform layers were afterwards obtained by shaking the plates by hand; the plates were dried horizontally in an oven while the temperature was raised gradually from 20° to 120° over a period of one h.

Comparative plates with pure adsorbents were made in a similar manner. The coating slurries were of the following compositions:

(c) 7 g of Silica Gel prepared as described in (1); 0.4 g of gypsum sifted through the sieve DIN 1171, 0.071 mm; 20 ml of water.

(d) 7 g of Merk's Silica Gel G; 20 ml of water.

SUMMARY

The application of mixtures (1:1) of Silica Gel and Celite 545 to adsorption thin-layer chromatography shortened the time of development 2.5 to 3.5 times as compared to chromatograms in which the pure absorbents were used. The addition of Celite to Silica Gel increased the R_F values but it had no influence on the selectivity of the steroid mixture resolution.

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