CHROMATOGRAPHY OF SERUM LIPID FRACTIONS ON A THIN LAYER OF Al_2O_3

A. VACÍKOVÁ, V. FELT AND J. MALÍKOVÁ
Research Institute of Endocrinology, Prague (Czechoslovakia)

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Chromatography on a thin layer of silica gel with a binder introduced by STAHL[1-6], was used with satisfactory results for the separation of lipids from human blood serum[7], brain[8] and various oils and fats[9]. Silica gel layers impregnated with silicon permitted the separation of homologues of higher fatty acids and their esters respectively[10]. Recently MOTTIER's method[11] has been employed more extensively, where for the separation of different substances a thin layer of Al_2O_3 without any binder is used (so-called spread-layer chromatography).

In the present work the method of spread-layer chromatography with Al_2O_3 as adsorbent is used for the quantitative separation of lipids from human serum.

EXPERIMENTAL

Materials

Aluminium oxide, analytical grade (Lachema, n.e., ČSSR), washed with distilled water and dried, was used. Its pH was adjusted with hydrochloric acid to 4 and the activity (according to BROCKMANN[12]) to IV.

As standards the following preparations were used: cholesterol (Chemofarma, n.e., ČSSR), cholesterol palmitate (Merck, GFR), palmitic acid (Lachema, n.e., ČSSR), crude lecithin purified ten times by repeated precipitation according to JOHNSON *et al.*[13]. The above substances were dissolved in chloroform and applied to the chromatoplates in amounts of 1 mg (in palmitic acid 1 mmole). Further BLOOR's[14] extract from 50 ml human serum was used. The extract was evaporated and re-extracted with 50 ml petroleum ether (b.p. 60°). For estimations 1 ml of this extract was always used. The other chemical substances used were analytical grade with the exception of heptane which was purified by distillation.

Preparation of the spread layer. Aluminium oxide was poured on a glass plate 16 cm × 23 cm and was smoothed with a rubber-tipped rod to form a homogeneous layer 1–1.5 mm thick. The chromatograms were developed in glass chambers by the ascending method at an angle of 30°.

Method

Two cm from the edge of the chromatogram a mixture of standards and samples to be analysed was applied in the form of a strip. The chromatograms were developed in a system of petroleum ether–ether (95:5). Afterwards the chromatoplates were removed and dried at room temperature. The detection was carried out either under

ultraviolet light at 365 mμ or by spraying the chromatogram with a solution of bromthymol blue[8]. In this system cholesterol esters and triglycerides were separated. Phospholipids, fatty acids and cholesterol remained at the start. Cholesterol ester fractions and triglyceride fractions were removed from the layer by a vacuum pump and eluted from the adsorbent[15]. The cholesterol esters were eluted with 4 ml chloroform and the absorbent rinsed with 3 × 2 ml chloroform. The combined eluates were evaporated to dryness and cholesterol was estimated by ABELL's method[16]. Triglycerides were similarly eluted with ethanol–ether (1:1). In the residue after evaporation triglycerides were estimated using STERN AND SHAPIRO's method[17] or they were first subjected to hydrolysis with 50% KOH and after neutralisation the liberated fatty acids were estimated by TROUT's method[18]. (It cannot be ruled out that in the triglyceride fraction mono- and diglycerides were also present.)

From the remaining chromatoplate part of the Al_2O_3 layer was removed from the site of the triglyceride fraction to the front. The surface of the glass plate was supplemented by new aluminium oxide and smoothed to form a homogeneous layer.

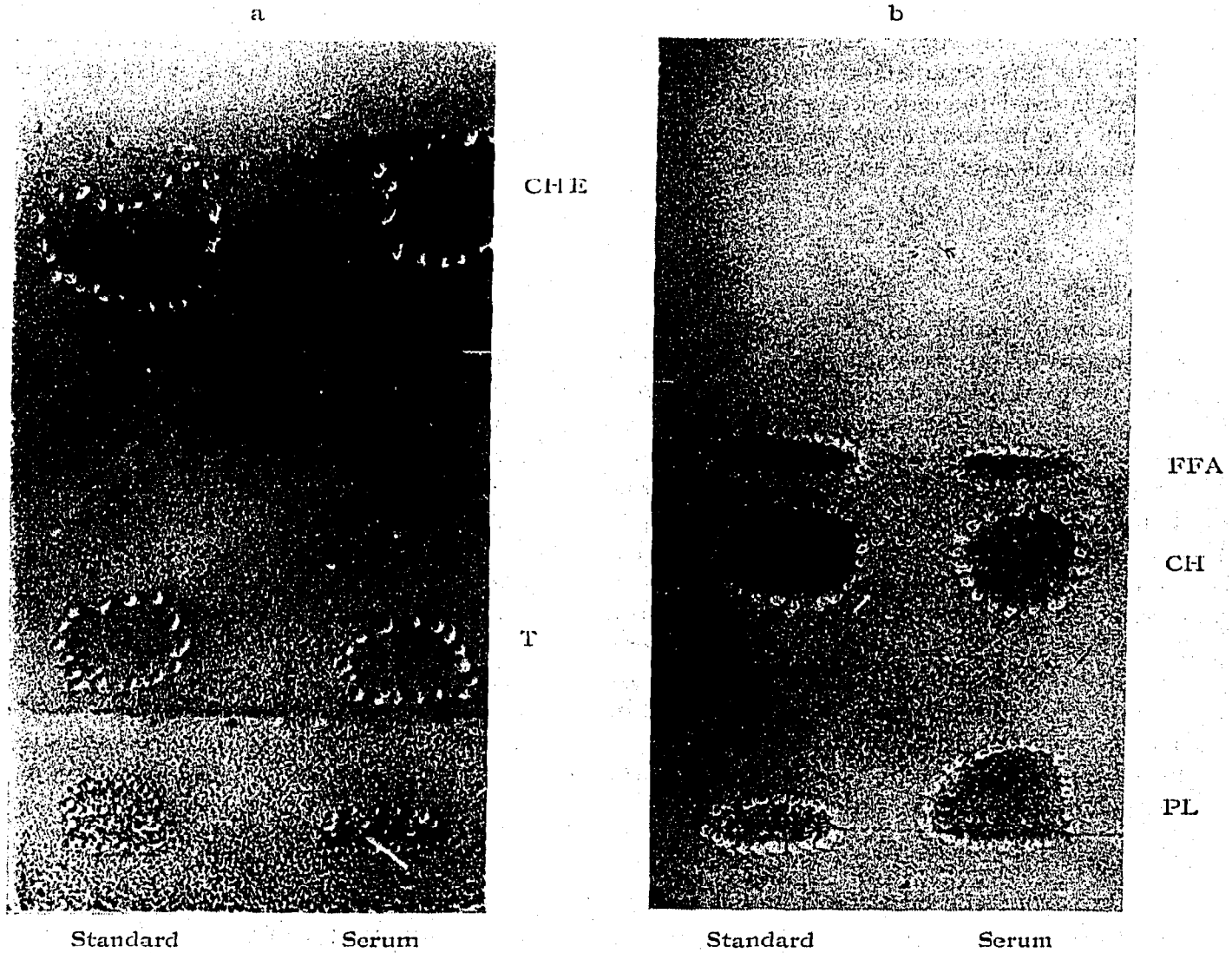

Fig. 1. Samples of chromatograms. (a) System 1: petroleum ether–ether (95:5). (b) System 2: petroleum ether–ether–acetic acid (94.5:5:0.5).

The chromatoplate prepared in this manner was developed in a system of petroleum ether–ether–acetic acid (94.5:5:0.5)*. The subsequent procedure was the same as above. In this system fatty acids, cholesterol and phospholipids were separated. The fractions were removed by a vacuum pump from the layer and eluted. Cholesterol was eluted and estimated as for the cholesterol esters. Fatty acids were eluted by the extraction mixture according to TROUT *et al.*, with sulphuric acid and water and estimated by titration. Phospholipids were subjected to hydrolysis (as in the case of the triglycerides) and the liberated fatty acids were estimated also by TROUT's method.

The separation of lipid fractions in the above systems is apparent from the photographs (Fig. 1).

RESULTS

In the tables individual lipid fractions are given. The following abbreviations are used:

CH	= cholesterol	FFA	= free fatty acids
CHE	= cholesterol esters	PL	= phospholipids
T	= triglycerides	FA	= total fatty acids

The R_F values of lipid fractions in individual systems are summarized in Table I. In the above method the recovery estimated is given in Table II.

TABLE I

COMPOSITION OF SOLVENT SYSTEMS AND R_F VALUES OF LIPID FRACTIONS

(Activity of Al_2O_3 IV, pH 4)

Solvent system	*Composition*	R_F				
		CH	*CHE*	*T*	*FFA*	*PL*
Petroleum ether–ether	95:5	0	0.91	0.30	0	0
Petroleum ether–ethanol	98:2	0.10	0.87	0.38	0.06	0
Petroleum ether–ether–acetic acid*	94.5:5:0.5	0.35	—	—	0.47	0
Heptane–acetic acid	98:2	0.29	—	—	0.37	0

TABLE II

RECOVERY OF LIPID FRACTIONS AFTER CHROMATOGRAPHY ON A SPREAD LAYER OF Al_2O_3

Sample	*Lipid fractions in percentage by weight*				
	CH	*CHE*	*T*	*FFA*	*PL*
1	96	97	99	93	105
2	96	98	102	113	102
3	97	95	105	81	102
4	93	103	101	91	94
5	94	89	101	103	105
6				111	
7				101	
8				101	
9				101	
Average	95	96	102	98	102

* The composition of this system must be modified for some commercial types of Al_2O_3. It proved useful to raise the ether content and the acetic acid content.

In Table III the values of lipid fractions from BLOOR's extract corresponding to 1 ml of serum are given.

The values of fatty acids in individual lipid components after fractionation were compared with the total value before fractionation (Table IV).

The method presented gives results which are in satisfactory agreement with the data of MAN AND ALBRINK[19] (Fig. 2).

TABLE III

CONTENT OF LIPID FRACTIONS IN EXTRACT FROM SERUM AFTER SEPARATION ON A SPREAD LAYER OF Al_2O_3

Sample	*Serum lipid fractions*				
	CH (mg)	*CHE (mg)*	*T (mg)*	*FFA (mequiv.)*	*PL (mequiv.)*
1	0.64	1.54	0.68	0.63	6.41
2	0.62	1.46	0.96	0.61	8.10
3	0.69	1.47	1.22	0.59	7.30
4	0.86	1.41	0.87	0.69	7.68
5	0.66	1.48		0.86	5.84
6	0.65	1.32		0.67	6.00
7	0.70	1.35		0.75	5.84
8	0.59	1.35		0.55	5.75
9	0.69	1.49		0.76	5.63
10		1.46			5.22
Average	0.66	1.43	0.93	0.68	6.38
Standard deviation	0.03	0.07	0.19	0.09	0.92

TABLE IV

COMPARISON OF LIPID COMPONENTS AFTER FRACTIONATION AND THEIR SUM WITHOUT FRACTIONATION (FA = 100%)

Lipid fraction	*Content of fraction*	
	mequiv.	*Percentage*
CHE	2.31	17.0
T	3.61	26.5
FFA	0.68	5.0
PL	6.38	46.9
Sum	12.98	95.4
FA	13.60	100.0

DISCUSSION

A method of chromatographic separation of lipid fractions which is rapid, simple and reliable and has sufficient separating capacity is still the object of research. In the paper presented, the authors describe optimal conditions for the use of Al_2O_3 for the separation of lipid fractions. Under the conditions described above (pH, activity of Al_2O_3) aluminium oxide is well suited for this purpose. Spread layers without a binder have some advantages as compared with thin layers with a binder. These include the easy and rapid preparation of chromatoplates, rapid development, rapid drying after

development and easy removal of the adsorbent with the separated fractions for elution. The easy preparation of the homogeneous layer makes it possible to remove the part of the layer containing the components separated after first development and to substitute a new layer of adsorbent. During development with a second system on a chromatoplate thus renewed the remaining components are separated.

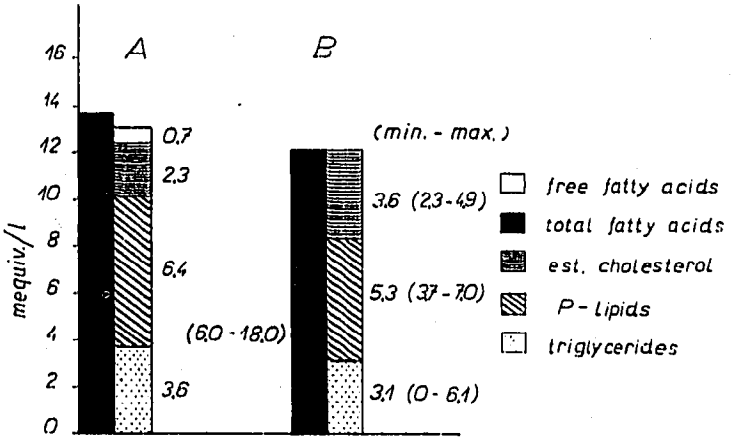

Fig. 2. Comparison of values of lipid components in human serum obtained (A) by the present method, (B) with values obtained by MAN AND ALBRINK.

The results obtained showed that by this method it is possible to achieve rapid quantitative separation of all lipid components of human serum. The recovery of this method is within the same range as that of other chromatographic methods. From Table IV it is apparent that the difference between the sum of fatty acids in individual lipid fractions and the total fatty acid content of the original extract is 4.6%. Comparison of the content of lipid fractions after separation on a spread layer with their estimation directly in the extract is usually not possible in view of the non-specificness of common analytical methods. Results obtained by the method submitted are in good agreement with the results obtained by a specific analytical method, where such a method exists (*e.g.* determination of lipid phosphorus).

By using chromatography on a spread layer the interference of phospholipid in estimation of FFA (free fatty acids) is excluded.

SUMMARY

Spread-layer chromatography on Al_2O_3 was modified for the separation of lipid fractions from human blood serum. The procedure recommended can be used for the separation and quantitative estimation of lipid fractions.

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