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Note

Argentation thin-layer chromatography of fatty acid methyl esters on Silufol®$_{UV\ 254}$ sheets

Č. MICHALEC, J. REINIŠOVÁ and Z. KOLMAN

Laboratory of Protein Metabolism, Faculty of General Medicine, Charles University, Prague (Czechoslovakia)

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A number of thin-layer chromatographic (TLC) techniques have been used in order to identify unsaturated higher fatty acids in various fractions of animal and plant sphingolipids. One of the most effective methods for the separation of unsaturated fatty acid methyl esters is argentation TLC. Several excellent reviews of the subject have been published[1–3].

In the present study, a micro-chromatographic method was developed for the separation of unsaturated fatty acid methyl esters on Silufol®$_{UV\ 254}$ sheets impregnated with Ag^+ ions. These pre-coated silica gel sheets were used successfully in our laboratory for the differentiation of other lipids, *e.g.*, long-chain bases and fatty aldehydes[4–6].

EXPERIMENTAL

Materials

All saturated and unsaturated fatty acids were commercial products of various origins. The *trans*-isomers (*trans*-9-22:1) brassidate and (*trans*-9-18:1) elaidate were kindly donated by Doc. Ing. V. Koman (Slovak Chemical Polytechnic High School, Bratislava, Czechoslovakia).

The fatty acids were isolated from human adult brain and sheep erythrocyte sphingomyelins after enzymic cleavage with phosphatidylcholine cholinephosphohydrolase (E.C. 3.1.4.3) to ceramides and subsequent alkaline hydrolysis. The methyl esters were prepared by reaction with 5% hydrochloric acid in absolute methanol for 18–20 h at 65–70°.

Chromatography and detection

Silufol®$_{UV\ 254}$ sheets Kavalier, (Glassworks Votice, Czechoslovakia; Series 052168; 7.5 × 4 cm or 7.5 × 7.5 cm) were impregnated with a 5% aqueous solution of silver nitrate[4]. Chromatography was carried out by multiple development as described by Morris *et al.*[7] at labory temperature using the following solvent systems:

(a) *n*-hexane–benzene (65:35);
(b) *n*-hexane–benzene (50:50);
(c) benzene–methanol (99:1).

The spots were located by spraying the chromatogram with a 5% solution of molybdophosphoric acid in approximatively 4% ethanolic hydrochloric acid, followed by heating at 100–110° for 10–15 min. Blue spots of the esters appeared on a light yellow-green backround[8].

RESULTS AND DISCUSSION

A typical scheme for the separation of individual fatty acid methyl esters on Silufol$_{UV\ 254}$ sheets impregnated with silver nitrate is given in Fig. 1. In this system, saturated esters have the highest mobilities and in most experiments a double spot was obtained. According to our experience on the formation of such spots, a difference in the chain-length is responsible. It seems that the acids with more than 22 carbon atoms in the molecule have slightly higher mobilities than homologous acids with a smaller number of carbon atoms (*e.g.*, stearic or palmitic acid).

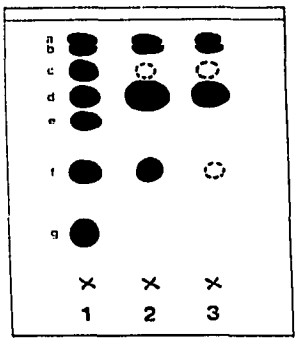

Fig. 1. Separation of fatty acid methyl esters on Silufol®$_{UV\ 254}$ sheets impregnated with silver nitrate in *n*-hexane–benzene (65:35). The chromatogram was developed three times (to half, three quarters and the full height of the sheet). 1 = Standard mixture of fatty acid methyl esters; 2 = esters of sheep erythrocyte sphingomyelins; 3 = esters of human adult brain sphingomyelins. a = Saturated C-22 and higher esters; b = saturated C-20 and lower esters; c = (*trans*-9-22:1)brassidate; d = (*trans*-9-18:1)elaidate + (*cis*-9-24:1)nervonate; e = (*cis*-9-22:1)erucate; f = (*cis*-9-18:1)oleate; g = (*cis*-9, *trans*-12-18:2)linoleate. Black spots = major components; dotted spots = minor components.

The resolution of esters according to the degree of unsaturation is very clear. *Cis*- and *trans*-isomers are also well separated, although some of them have the same position on the chromatogram (*e.g.*, elaidate and nervonate).

Solvent systems (a) and (b) are very useful for the characterization of fatty acid patterns in sphingomyelins isolated from various biological materials (Figs. 2 and 3). In addition, these systems could also be used for the identification of unsubstituted fatty acids in human brain cerebrosides and sulphatides (Fig. 5). System (c) facilitates the separation of acids with a larger number of double bonds, *e.g.*, linoleate and linolenate. In this case, the esters with higher mobilities than elaidate and oleate remain unseparated (Fig. 4).

The detection reagent used is more satisfactory than an alcoholic solution of molybdophosphoric acid without addition of hydrochloric acid, because the difference

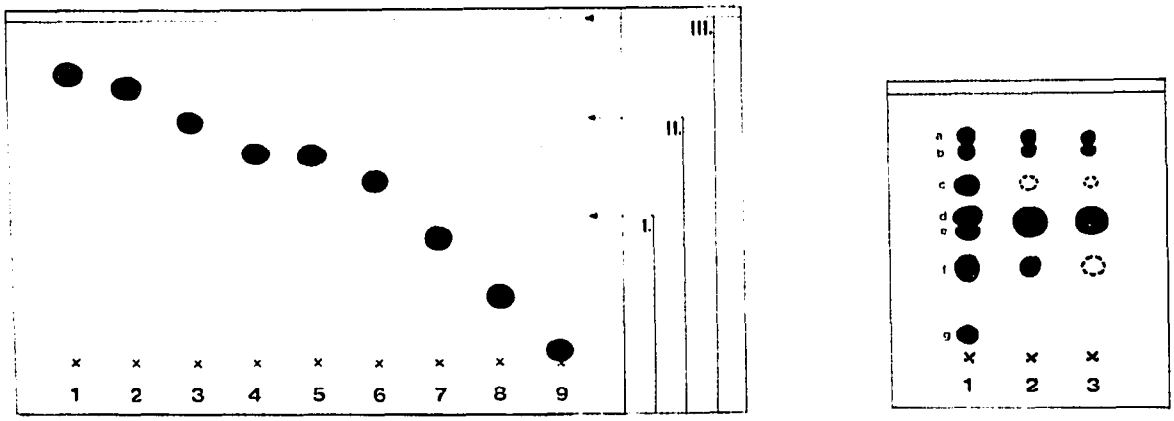

Fig. 2. Separation of individual fatty acid methyl esters on Silufol®$_{UV\ 254}$ sheets impregnated with silver nitrate in *n*-hexane–benzene (65:35). Experimental conditions as in Fig. 1. 1 = Lignocerate (24:0); 2 = palmitate (16:0); 3 = (*trans*-9-22:1)brassidate; 4 = (*trans*-9-18:1)elaidate; 5 = (*cis*-9-24:1)nervonate; 6 = (*cis*-9-22:1)erucate; 7 = (*cis*-9-18:1)oleate; 8 = (*cis*-9, *trans*-12-18:2)linoleate; 9 = (*cis*-9, *cis*-12, *cis*-15-18:3)linolenate. I, Development to half of the chromatogram; II, development to three quarters; III, development to full height.

Fig. 3. Separation of fatty acid methyl esters on Silufol®$_{UV\ 254}$ sheets impregnated with silver nitrate in *n*-hexane–benzene (50:50). Experimental conditions as in Fig. 1. 1 = Standard mixture of esters (designation of spots as in Fig. 1); 2 = esters of sheep erythrocyte sphingomyelins; 3 = esters of human adult brain sphingomyelins.

Fig. 4. Separation of fatty acid methyl esters on Silufol®$_{UV\ 254}$ sheets impregnated with silver nitrate in benzene–methanol (99:1). Experimental conditions as in Fig. 1. 1 = Standard mixture of esters (designation of spots as in Fig. 1); 2 = esters of rape seed oil; 3 = esters of linseed oil. a = Saturated C-22 and higher esters; b = saturated C-20 and lower esters; c = (*trans*-9-22:1)brassidate; d = (*trans*-9-18:1)elaidate + (*cis*-9-24:1)nervonate; e = (*cis*-9-22:1)erucate + (*cis*-9-18:1)oleate; f = (*cis*-9, *trans*-12-18:2)linoleate; g = (*cis*-9, *cis*-12, *cis*-15-18:3)linolenate.

Fig. 5. Separation of fatty acid methyl esters on Silufol®$_{UV\ 254}$ sheets impregnated with silver nitrate in *n*-hexane–benzene (65:35). Experimental conditions as in Fig. 1. 1 = Standard mixture of esters (designation of spots as in Fig. 1); 2 = esters of unsubstituted fatty acids of human adult brain cerebroside fraction; 3 = esters of unsubstituted fatty acids of human adult brain sulphatide fraction.

between the coloration of the spots and the background is much more distinct, especially on layers impregnated with Ag^+ ions.

We conclude that Silufol® UV_{254} sheets are very suitable for the simple and rapid qualitative micro-separation of fatty acid methyl esters in standard mixtures and in lipid fractions isolated from biological sources.

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