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A column chromatographic method for the fractionation of fish neutral lipids

In our studies on the chemical composition of fish lipids we required a method which would fractionate the neutral fraction efficiently, quickly and with minimum oxidative changes.

A number of methods for the column separation of neutral lipid classes of the type encountered in fish have been reported[1–5], but we have found that these methods gave incomplete separation and sometimes required up to 66 h to complete. In lengthy fractionations of this nature the very unsaturated fish lipids tended to oxidise and give misleading results.

Thin-layer chromatographic methods have also been used for this purpose[6–8] but with the mixtures that we wished to fractionate unacceptable levels of oxidation occurred even in the presence of antioxidant[9]. Only a relatively low loading of the lipid mixture could be applied to the plate if refractionation of the various classes was to be avoided and this then provided insufficient amounts for further analysis of the minor lipid components.

To overcome some of these difficulties a column chromatographic technique has been developed for the routine analysis of neutral fish lipids. The method is efficient and has been in use for a period of some three years in our laboratories.

Experimental and results

All solvents used were of analytical reagent grade. The Silica Gel G (E. Merck, Darmstadt, G.F.R.) used contained organic materials which interfered with the chromatographic separations. These impurities were removed by washing a dry packed column of the adsorbent with a chloroform–methanol (2:1) mixture until the dark band that developed on addition of the solvent was eluted. The column was then washed with acetone and the silica gel heated overnight in an oven at 110°. If the gel was not used immediately it was stored in a vacuum desiccator and prior to its subsequent use it was heated for 1 h at 110°.

The actual chromatographic column was prepared by pouring a suspension of Silica Gel G (20 g) in benzene into an 18-mm diameter glass column. This gave a bed of approximately 125 mm in length, the top of which was covered with a washed filter paper disc. The lipid mixture (100–200 mg) was applied to the column in a minimum volume of benzene and was then eluted with the solvents shown in Table I.

TABLE I

ELUTION PATTERN OF A STANDARD LIPID MIXTURE

Solvent	*Volume used*	*Solvents*	*Lipid eluted*
A	30 ml	Benzene	Hydrocarbon
A	30 ml	Benzene	Sterol ester
B	70 ml	Benzene–ether–ethanol (80:19:1)	Triglyceride
B	55 ml	Benzene–ether–ethanol (80:19:1)	Diglyceride
B	45 ml	Benzene–ether–ethanol (80:19:1)	Sterol
C	70 ml	Benzene–ether–ethanol (30:70:3)	—
D	70 ml	Ether	Monoglyceride
E	80 ml	Methanol	Free fatty acid

A flow rate of approximately 1.5 ml/min was obtained when the column head was maintained at a pressure of 3 p.s.i. with oxygen free nitrogen.

Before arriving at these solvent systems the suitability of a number of solvents was examined. This was expedited by observing the effect of the particular solvent on the elution of a lipid mixture applied to thin-layer plates.

As shown in Table I good separation of the major lipid components could be obtained. Unfortunately if the combined amount of diglyceride and sterol exceeded 5 % of the total oil, separation of these two components was not complete. For examination of the fatty acid components of the classes present, separation of these two lipid classes is not essential and under these circumstances the amount of benzene used in the first solvent elution can be reduced to 60 ml. Such a separation of a standard mixture is shown in Fig. 1. Fig. 2 depicts a thin-layer chromatogram of the various lipid fractions obtained from the neutral lipids of the ratfish (*Chimaera monstrosa* L). The hydrocarbons present elute at the solvent front prior to any wax, sterol and

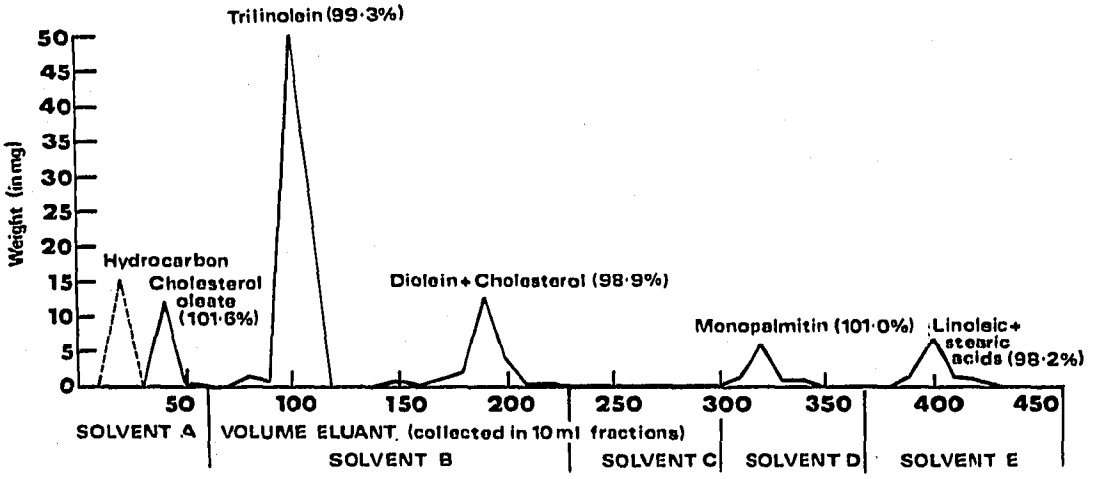

Fig. 1. Separation of a standard lipid mixture (percentage recoveries in brackets).

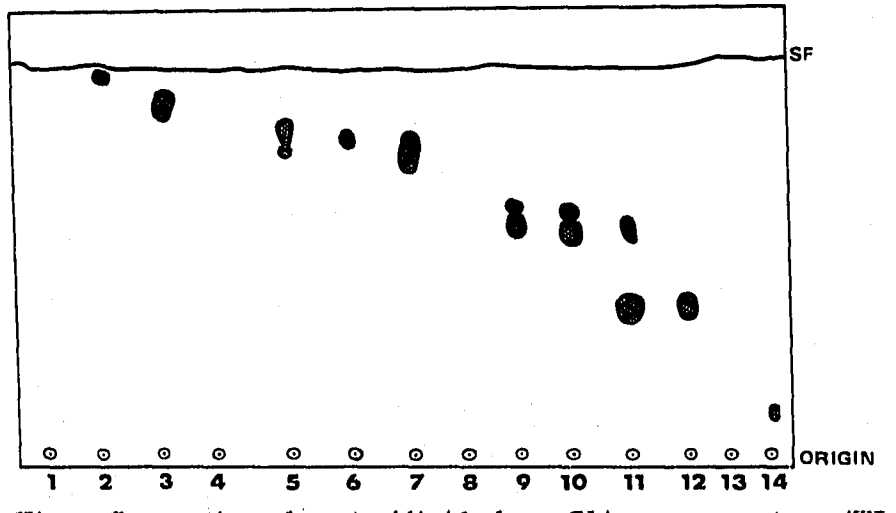

Fig. 2. Separation of neutral lipids from *Chimaera monstrosa* (TLC solvent system that of FREEMAN AND WEST[6]). 2 = hydrocarbon; 3 = sterol ester; 5–7 = alkenyl + alkyl diglyceride + triglyceride; 9,10 = diglyceride; 11,12 = sterol (plus a trace of diglyceride in 11) and 14 = monoglyceride.

vitamin A esters which may be present. These latter esters are not separated by this column. A partial separation of the alk-1-enyl diglycerides from alkyl diglycerides and triglycerides was obtained.

The total time required for the complete separation is in the region of 5 h. In practice the time is shortened by the removal of the free fatty acids by the method of McCARTHY AND DUTHIE[10] which allowed solvents C and D to be dispensed with and the separation completed within 3 h.

Recoveries were good, never falling below 98 %, and, as long as 0.01 % BHT was incorporated in the solvents, no oxidation occurred.

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