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## Note

# Separation of certain triglyceride isomers by argentation thin-layer chromatography with flame ionisation detection by the Iatroscan TH 10

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Several chromatographic techniques have been used for triglyceride separation. Non-aqueous reversed-phase high-performance liquid chromatography (HPLC) on $C_{18}$ columns has now been developed to such a degree that separation of triglycerides can be achieved not just according to equivalent carbon number (ECN) but peaks of each ECN can be resolved into their constituent carbon number[1–3]. However, resolution of glyceride isomers differing only in the positions of the acyl group [*e.g. rac* 1,3-distearoyl, 2-oleoylglycerol (SOS) and 1,2-distearoyl, 3-oleoylglycerol (SSO)], cannot be achieved by HPLC on $C_{18}$ columns, and is only possible on silver nitrate impregnated columns at subambient temperatures[4]. This is a laborious and expensive procedure and column life is very short.

Dallas and Padley[5] successfully separated symmetrical (*e.g.* SOS) and unsymmetrical (*e.g.* SSO) mono-unsaturated triglycerides on a silica gel thin-layer chromatography (Si-TLC) plate impregnated with silver nitrate using chloroform–toluene as a developing solvent. However, as with all TLC techniques, quantification of the components cannot readily be achieved.

The separation of lipid classes using the Iatroscan has been extensively reported in the literature[6–12]. However, separation of lipids, particularly triglycerides, according to their degree of unsaturation has been less extensively reported. Sebedio *et al.*[13] used silver nitrate impregnated Chromarods to separate fatty acid methyl esters according to double bond configuration. The same authors[14] also separated mono acid triglycerides and studied response factors and the effect of column re-use on these factors. Other authors[15,16] have applied the system to intact triglycerides. This paper describes our attempts to achieve separations of mono-unsaturated triglyceride positional isomers using the Iatroscan system, which had previously only carried out by argentation Si-TLC.

## EXPERIMENTAL

### *Equipment*

The Iatroscan TH10 and all ancillary equipment (Chromarods, frame, tanks, spotting guide) were obtained from Iatron's U.K. agent, Trivector Scientific, Sandy, U.K. Silica gel SII (high-performance) Chromarods were employed throughout.

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*Materials*

All lipid standards were obtained from Sigma Chemicals, Poole, U.K., except for mixed acid triglycerides which were synthesised in-house. Solvents, all analytical grade, were obtained from BDH, Poole, U.K., who also supplied the silver nitrate (analytical grade).

*Method*

Chromarods were activated by passing through a flame ionisation detector, then impregnated with silver ions by immersion for 15 min in a 2.5% (w/v) solution of silver nitrate in acetonitrile. They were then removed from the solution, drained, dried and re-activated in an oven at 120°C for 3 h. Samples were applied and rods developed in the solvent under investigation over their whole length. Following use, the rods were cleaned by soaking overnight in concentrated nitric acid, which was then removed by immersion sequentially in two portions of distilled water followed by rinsing with acetone. The rod preparation procedure is broadly the method described by Sebedio *et al.*[13].

For triglyceride separations, the following solvent systems were studied: (1) chloroform*–toluene (50:50) (The solvent system employed by Dallas and Padley[5] for TLC separations of this type); (2) chloroform*; (3) chloroform; (4) dichloromethane; (5) benzene (single development); (6) benzene (triple development); (7) chloroform*–benzene–diethyl ether (70:30:1.5).

Where the chloroform in the above list is marked* it was purified to remove ethanol (added as stabiliser to most commercial chloroform) by triple extraction with 0.2 volumes of water, followed by drying (with calcium sulphate) and distilling. Otherwise chloroform was as received, *i.e.* containing 1–2% (v/v) ethanol.

For all analyses, 2-$\mu$l samples were applied as a 1% (m/v) solution in dichloromethane (selected because of its volatility), using a 10-$\mu$l Hamilton GC syringe. Rods were rotated during application to ensure even distribution of the sample.

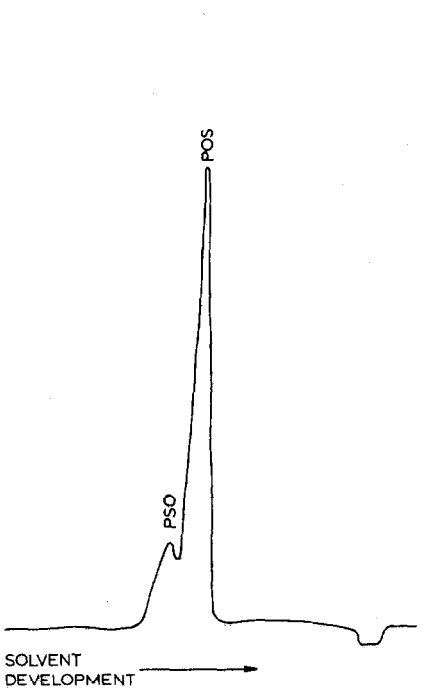
## RESULTS AND DISCUSSION

Impregnation of Chromarods with copper sulphate had previously been shown to shorten the life of the rods[15]. For this application, however, rods could be repeatedly re-used for argentation separations, generally without significant loss of resolution, by washing off the silver nitrate in concentrated nitric acid, drying and re-impregnating with silver nitrate as before. However, once silver-impregnated, rods could not be completely washed free of silver salts, even with overnight soaking in aqua regia, so they could not therefore be used subsequently for conventional adsorption type separations, which would require the rods to be free of silver salts.

Of the seven solvent systems studied, six (*i.e.* all except dichloromethane) successfully resolved components based on their degree of unsaturation. Of these six, only chloroform–toluene gave no separation of symmetrical and unsymmetrical mono-(*cis*-)unsaturated triglycerides. Ethanol-free chloroform initially gave partial resolution of these isomers, but separation steadily deteriorated with subsequent runs and was not restored either by using new Chromarods or by further purification and stabilisation of the chloroform using 13X molecular sieve (which removes ethanol, water, hydrogen chloride and carbonyl chloride, the likely impurities). The best sep-

aration of the symmetrical and unsymmetrical monounsaturated isomers was achieved using chloroform–benzene–diethyl ether (70:30:1.5) as shown in Fig. 1, which showed a separation of *rac* 1-palmitoyl, 2-oleoyl, 3-stearoyl glycerol (POS) and its isomer PSO. This solvent gave marginally superior separation to benzene (triple development), and would be preferred to benzene because only one development was required. However it did not resolve trisaturated (SSS) and *trans* monounsaturated (STS) triglycerides. Fig. 2 shows an example of a separation of trimyristoyl glycerol (MMM) and 1-palmitoyl, 2-elaidoyl, 3-stearoyl glycerol (PES) in the ratio MMM: PES = 25:75, and shows only partial resolution. Toluene, in admixture with chloroform and diethyl ether, was evaluated as a substitute for benzene. Resolution was inferior to the mixture containing benzene, and the mixture was considered unsuitable.

Quantitatively the method was inferior to TLC (used in conjunction with scanning desitometry). A calibration of 1, 2, 5, 10 and 25% PSO in POS is shown in Fig. 3 and demonstrates that at the 25% level the method reported 17.1% PSO, *i.e.* the minor component was underestimated. The detection limit was about 5%, which was again poorer than TLC–densitometry. In addition, because resolution of isomers was not as good as can be achieved by TLC, integration of lesser peaks was less reliable.



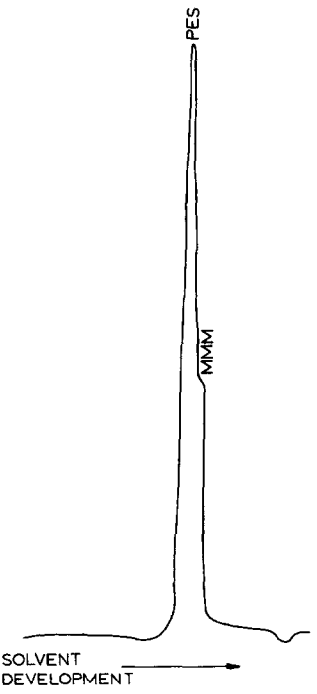




Fig. 1. Separations of POS and PSO on silver nitrate impregnated Chromarods (SII) using chloroform–benzene–diethyl ether (70:30:1.5, v/v/v).

Fig. 2. Separation of MMM and PES on silver nitrate impregnated Chromarods (SII) using chloroform–benzene–diethyl ether (70:30:1.5, v/v/v).

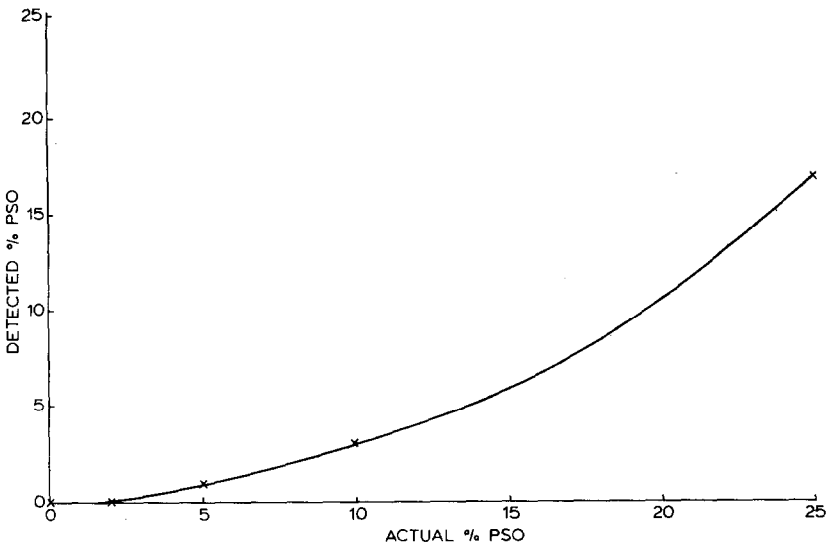



Fig. 3. Calibration of 0–25% PSO in POS on silver nitrate impregnated Chromarods (SII).

## CONCLUSIONS

The method described offered a rapid method of separating and estimating the relative proportions of triglycerides according to the number and position of the double bonds, and was capable of resolving the positional isomers of the mono-unsaturated triglycerides. The method was more rapid (both in terms of operator and total analysis time) than TLC–densitometry, but was less accurate.

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