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Note

Comparison of detection systems for the high-performance liquid chromatographic analysis of complex triglyceride mixtures

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Until recently liquid chromatographic studies of triglycerides have been limited to the preparation of derivatives of the component fatty acids and their determination, whilst analysis of the actual triglycerides was restricted to their quantification as a group, when separated from mono- and diglycerides and free fatty acids. The separation and analysis of individual triglycerides *per se* is more difficult and requires a suitable stationary phase and a suitable detector. The two most widely used liquid chromatographic methods for separating triglycerides are argentation chromatography, where the triglycerides are separated on the basis of unsaturation[1,2], and non-aqueous reversed-phase (NARP) chromatography, where separation is on the basis of equivalent carbon number[2,3]. For simple oils, with few triglycerides, separation is easily achieved by these methods using isocratic elution: however, for more complex mixtures, such as butter[4–7], gradient elution is necessary and this limits the choice of detector.

There are many different types of detectors available, each with its own limitations[8], and the following four are commonly used for lipid analyses:

(a) Refractive index (RI) detector. This cannot normally be used with gradient elution and thus optimum separation is not always achieved[6,9,10]. Sensitivity is also relatively poor.

(b) Ultraviolet (UV) detector. The absorption region 200 to 230 nm (ester bond) is used to detect triglycerides, however, many suitable solvents for the chromatographic separation (*e.g.* acetone) also absorb in this region and cannot be used[6].

(c) Transport flame ionisation or moving-wire detector. This detector is no longer commercially available. It could be used with any volatile solvent, and hence solvent gradients. However, it lacked sensitivity as the majority of the eluate was not retained for detection[8,10]. A new flame ionisation detector (FID), Tracor Model 945, is now on the market; however, we did not have one for comparison.

(d) Infrared detector. This detector can be used with gradient elution, although some baseline drift occurs in the absorption region used for lipids (5.75 μm, ester carbonyl region)[8,11].

A fifth type of detector, which is relatively inexpensive, has recently been described as potentially suitable for the detection of triglycerides[12]. This is the mass detector, which detects solutes on the basis of light scattering, after nebulisation of the eluate and removal of solvent by evaporation[13]. It is compatible with gradient

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elution and for non-aqueous volatile solvents gives no baseline drift. Its main limitation is that the sample must be less volatile than the solvent.

Extensive studies on an experimental light-scattering detector, which utilised a laser light source, have been published[6,7]. However, to our knowledge no comparative data are available for the commercially available mass detector.

EXPERIMENTAL

A Gilson Model 704 gradient chromatograph with computerised integration and data handling was used (Anachem, Luton, U.K.). Detection systems used were: a mass detector Model 750/14, a refractive index detector Model 750/13 (both from Applied Chromatography Systems, Luton, U.K.) and a Cecil CE212 variable wavelength UV monitor (Cecil Instruments, Cambridge, U.K.). Injection was achieved through a Rheodyne valve Model 7125 (fitted with a 20-μl loop) into two Spherisorb-5-ODS 2 (Phase Separations, Gwent, U.K.) columns in series (50 × 4.6 I.D. and 250 × 4.6 mm I.D.) which had been slurry-packed with acetone in our own laboratories. All solvents were of HPLC grade (Rathburn Chemicals, Walkerburn, U.K.) except for AR grade acetone used with the mass detector (Fisons, Loughborough, U.K.).

The butter (dutch, unsalted) was prepared for analysis by melting at 40°C and filtering hot through glass fibre filters (GF/A, Whatman; supplied by Fisons, Loughborough, U.K.).

For RI detection elution was isocratic using acetone–acetonitrile (65:35, v/v). For UV and mass detection, gradient elution with ethanol–acetonitrile (for UV and mass detector) or acetone–acetonitrile (for mass detection) was used. The gradient (20 to 90% in 55 min then 90 to 100% in 5 min) was followed by a re-equilibration cycle (100 to 20% in 5 min then 20% for 15 min). Injections of 15 μl of 8.449% butter dissolved in acetone were made in each case[14].

RESULTS AND DISCUSSION

A sample of butter was prepared as described above and chromatographed using three different detectors: (a) RI; (b) UV; (c) mass detection.

(a) Refractive index detection

The most widely used solvent system for triglycerides appears to be acetone–acetonitrile[14,15]. This combination is compatible with refractive index detection when used isocratically. Various concentrations of acetone in acetonitrile were tested and 65% (v/v) (Fig. 1) was found to produce the best, albeit unsatisfactory separation.

This isocratic separation was compared with the gradient separations achieved with UV and mass detection. For simpler triglyceride separations isocratic elution with refractive index detection can produce very acceptable results[3].

(b) UV detection

UV detection is compatible with gradient elution, provided that the solvents used do not absorb at the detection wavelengths. The ester bond absorption region (200–230 nm) was used, and for this reason the use of acetone was precluded. A

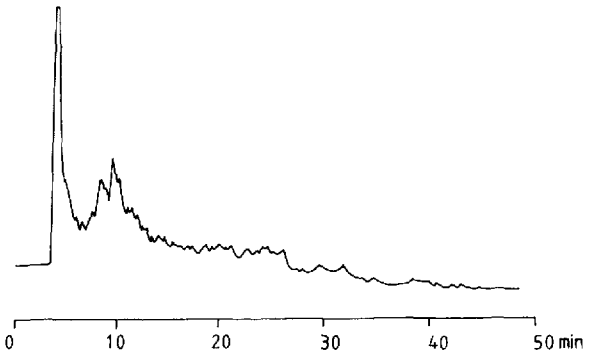

Fig. 1. Liquid chromatographic separation of butter triglycerides with refractive index detection. Column, Spherisorb-5-ODS 2; detector, × 1; temperature, ambient; mobile phase, acetone–acetonitrile (65:35); flow-rate, 1.5 ml min^{-1}.

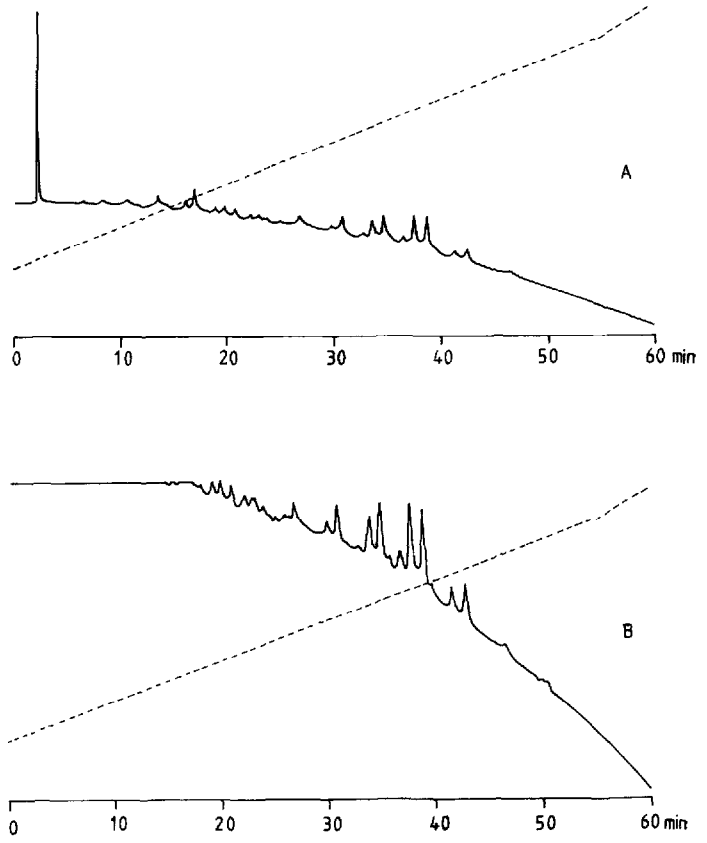

Fig. 2. Liquid chromatographic separation of butter triglycerides with UV detection at 225 nm. Column, Spherisorb-5-ODS 2; detector, 0.02 a.u.f.s.; temperature, ambient; solvent gradient from 20% to 100% ethanol in acetonitrile; flow-rate, 1.5 ml min^{-1}. (A) original chromatogram. (B) computer-enhanced chromatogram.

gradient of ethanol in acetonitrile (20 to 100%, v/v) was used and the UV detector was connected in series with the mass detector. Sensitivity was low and some baseline drift occurred (Fig. 2A). Special data-handling facilities on the Gilson system allowed the plot to be scaled-up (Fig. 2B), which gave a better visual display but did not improve sensitivity, in relation to the baseline drift.

(c) Mass detection

The mass detector is compatible with gradient elution and can be used with any volatile solvent, except that explosive or highly toxic solvents should be avoided. When explosive solvents are used, nitrogen should be used as the carrier gas in place of air. Gradients of ethanol in acetonitrile (Fig. 3) in series with the UV detector (corresponding UV chromatogram, Fig. 2) and acetone in acetonitrile (Fig. 4) were used (20 to 100%, v/v, in each case). Good separation and responses were observed with both solvent systems, with the acetone–acetonitrile gradient giving a slightly better separation and slightly increased peak heights for late eluting peaks.

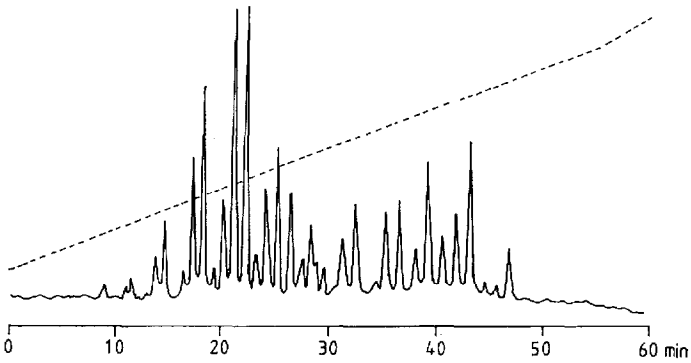

Fig. 3. Liquid chromatographic separation of butter triglycerides with mass detection. Column, Spherisorb-5-ODS 2; detector, × 1; photomultiplier setting, × 2; evaporator setting, 30°C; air flow, 22 p.s.i.; solvent gradient from 20% to 100% ethanol in acetonitrile; flow-rate, 1.5 ml min^{-1}.

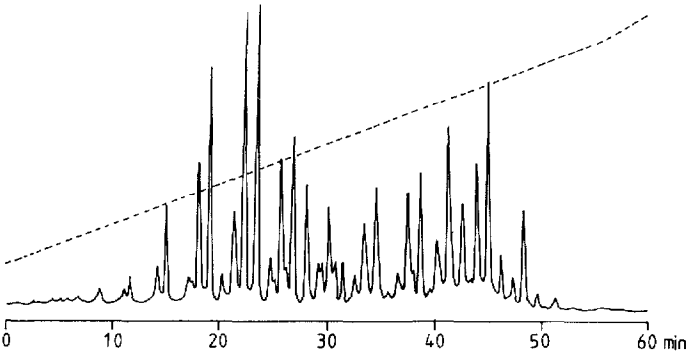

Fig. 4. Liquid chromatographic separation of butter triglycerides with mass detection. Column, Spherisorb-5-ODS 2; detector, × 1; photomultiplier setting, × 2; evaporator setting, 30°C; air flow, 22 p.s.i.; solvent gradient from 20% to 100% acetone in acetonitrile; flow-rate, 1.5 ml min^{-1}.

Comparison of Figs. 2 and 3 shows that even though UV is compatible with gradient elution, the mass detector produces better chromatograms, and unlike the UV does not exhibit baseline drift. Furthermore, the mass detector can be used with acetone, which in combination with acetonitrile allows separation of components that co-elute with other components in the ethanol–acetonitrile system (Figs. 3 and 4).

The gradient compatibility and insensitivity to eluting solvent composition make the mass detector a promising detector for use with triglycerides in complex mixtures. In this respect it is similar to the now obsolete moving-wire detector (*i.e.*, solvent was evaporated before detection in both cases) and this facility for gradient elution with any (non-explosive) volatile solvent combination makes the mass detector a very suitable replacement for the moving-wire detector. Also, despite the inherent sensitivity of FID the mass detector should be more sensitive than the moving-wire detector, because in the former all the solute is detected, whereas in the latter a moving wire is coated by a stream of eluate passing from the column and the majority of this eluate falls to waste without coating the wire (which subsequently passes into the detection chamber). Thus, only a small proportion of the eluate is analysed in the moving-wire detector as compared to the total eluate on the mass detector.

Preliminary work on linearity and response of the mass detector indicates that the eluting solvent may have a significant effect on the detector response. This phenomenon is currently under further investigation.

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