Journal of Chromatography, 285 (1984) 178–181
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,394

Note

Influence of injection solvent on the reversed-phase chromatography of triglycerides

MARIA TSIMIDOU and ROBERT MACRAE*
Department of Food Science, University of Reading, London Road, Reading RG1 5AQ (U.K.)
(Received October 20th, 1983)

The influence of the injection (or sample) solvent on chromatograms produced using high-performance liquid chromatography (HPLC) under both normal and reversed-phase conditions has been extensively documented[1,2]. However, in most cases these reports refer only to minor reductions in column efficiency or to distortion of the early part of the chromatogram[3]. In the present study, where reversed-phase chromatography is being used to characterise the triglyceride composition of olive oils, it has been found that the injection solvent, and also its volume, has a large effect on the entire chromatogram. Ideally the injection solvent should be identical to the initial mobile phase, or possibly more polar in the case of reversed-phase chromatography to provide on-column concentration. With complex mixtures of triglycerides there is also a problem of solubility. For example where a sample contains both low-molecular-weight unsaturated triglycerides and high-molecular-weight saturated triglycerides a wide range solvent gradient will be required. This means that the initial polar solvent, which should also ideally provide the injection solvent, will not be able to dissolve the fully saturated (hydrophobic) triglycerides. Thus a compromise for injection solvent polarity is required, between compatibility with the mobile phase and adequate sample solubility. The problem is highlighted with gradient elution but is also important under isocratic conditions. A review of the published work on the reversed-phase chromatography of triglycerides shows that acetone–acetonitrile mixtures are the most commonly used mobile phases[3–7]. Yet at the same time a vast range of injection solvents have been used including chloroform[3,6], acetone[3,7], tetrahydrofuran[5] and toluene[8]. Of these chloroform appears to have been the most widely used, and indeed publications are still appearing showing its use. However, under many conditions it produces severe distortion of the resulting chromatograms. Furthermore those workers who are now using a more suitable injection solvent have not emphasised its importance. This effect has been investigated for a number of oils in the present study.

EXPERIMENTAL

An Applied Chromatography Systems (Luton, U.K.) gradient chromatograph (Model 750) with a mass detector (Model 750/14) were used[9]. The detector settings employed were: evaporation temperature, 40°C; photomultiplier, 2; attenuation,

0021-9673/84/$03.00

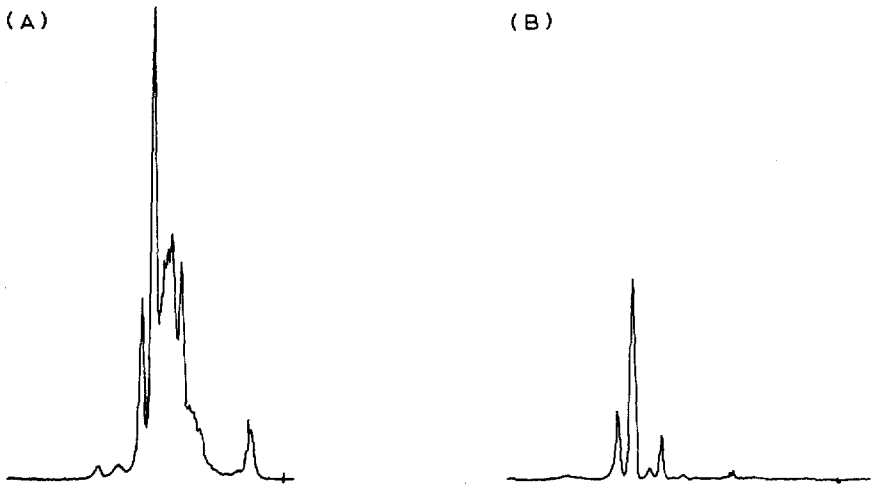

Fig. 1. The effect of injection volume (chloroform) on the resolution of olive oil: (A) 20 μl; (B) 5 μl.

× 1. Injection was achieved via a Rheodyne injection valve (Model 7120, 20-μl loop) and chromatograms were recorded and integrated with a Hewlett-Packard integrator (Model 3390A). The chromatographic column (250 × 4.6 mm I.D.) was packed with Spherisorb 5 ODS in our laboratory as a slurry in acetone at 6000 p.s.i. Acetone (S.L.R.) was obtained from Fisons (Loughborough, U.K.) and acetonitrile (HPLC grade) from Rathburn Chemicals (Walkerburn, U.K.). A mobile phase of acetone–acetonitrile (6:4, v/v) was used throughout this work (1 ml min^{-1}).

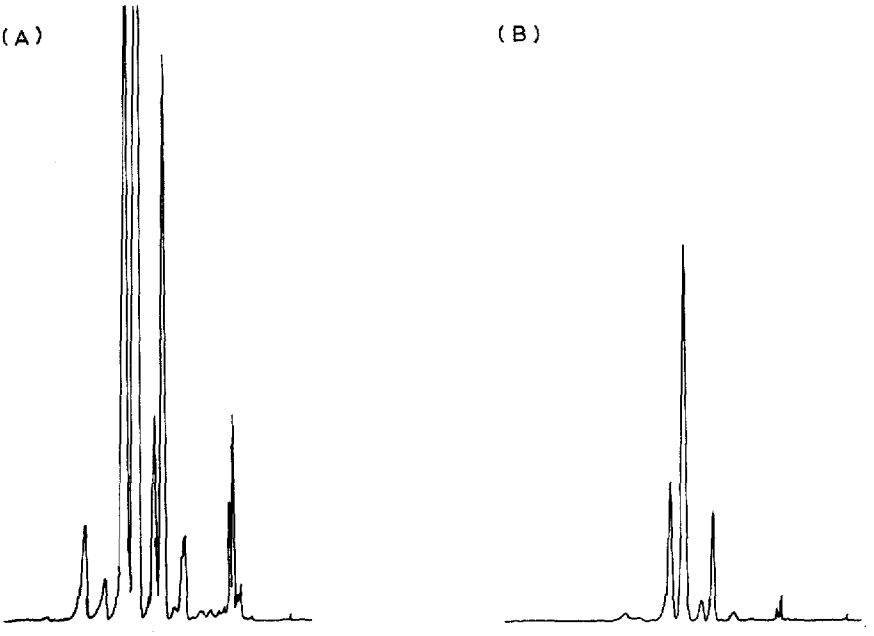

Fig. 2. The effect of injection volume (acetone) on the resolution of olive oil: (A) 20 μl; (B) 5 μl.

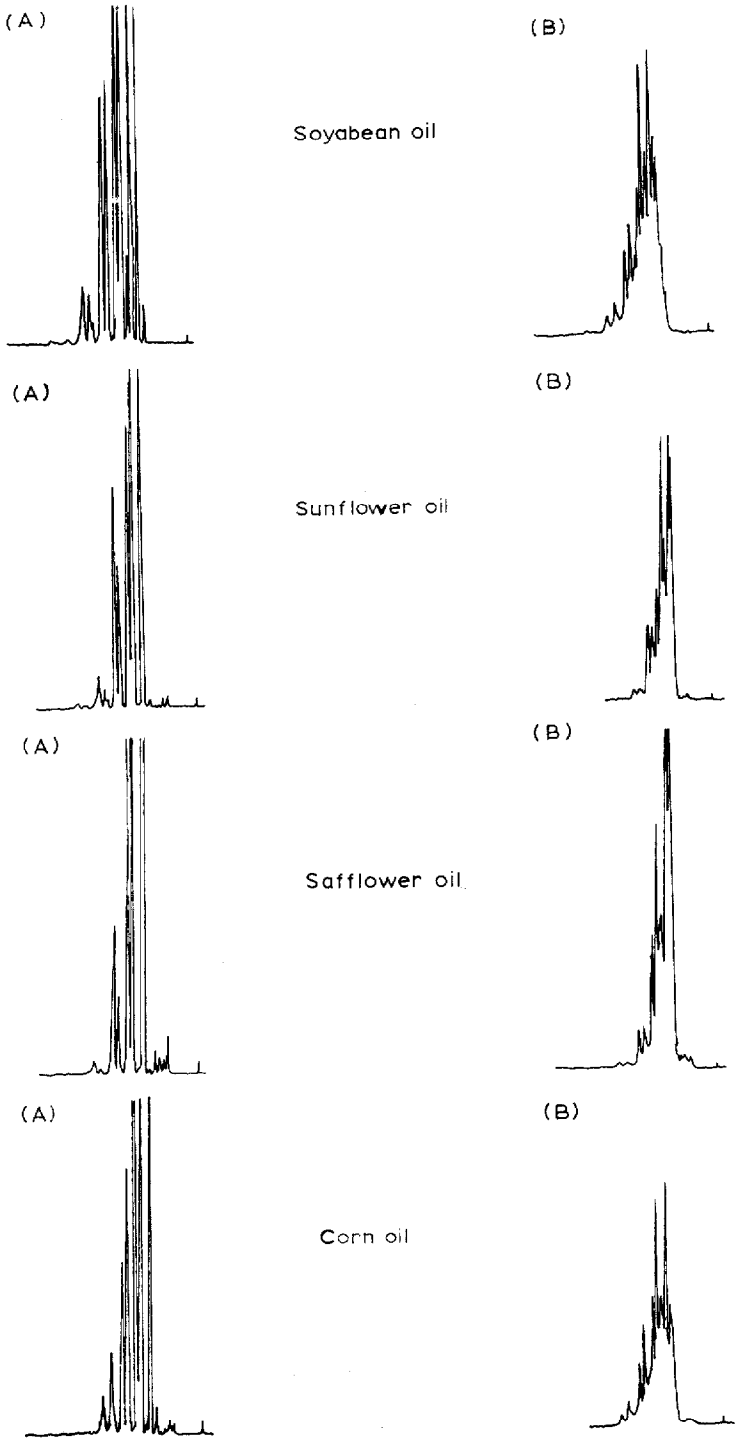

Fig. 3. The effect of injection solvent (20 μl) on the resolution of various oils: (A) acetone; (B) chloroform.

RESULTS AND DISCUSSION

The fact that the injection solvent may be causing problems was identified when double peaks were observed for standards dissolved in chloroform and eluted with a mobile phase of acetonitrile–acetone. Initially this was thought to be due to column overloading and indeed the problem disappeared when the injection volume (and hence the amount of standard) was reduced from 20 μl to 5 μl. However, this was subsequently shown to be due to reducing the amount of chloroform and not to reduction of the actual amount of triglyceride injected. This effect was then studied with olive oil samples. Reducing the injection volume (*i.e.* the amount of chloroform introduced into the system) greatly improved the resolution of the major components and also eliminated a number of non-reproducible artefacts, such as shoulders (Fig. 1). The next stage was to investigate alternative injection solvents to improve resolution further. The mobile phase itself (acetone–acetonitrile, 6:4, v/v) was found to be inadequate as a solvent at the sample concentrations used (*ca.* 5%, w/v), therefore acetone alone was used. In this case sample injections of 20 μl still produced excellent chromatograms in stark contrast to those achieved when chloroform was used (*cf.* Fig. 1(A) and Fig. 2(A)). This great difference is reduced when 5-μl injections of the two solvents are compared but nonetheless acetone still produces superior resolution (*cf.* Fig. 1(B) and Fig. 2(B).) Furthermore for certain applications, for example when minor components are of interest or when the technique is used preparatively, the use of large injection volumes is mandatory. Other solvents, of suitable polarity, such as diethyl ether and tetrahydrofuran were also investigated, but proved to be similar to chloroform, producing poor chromatograms when used with large injection volumes.

The possibility that olive oil, with its unique composition, was behaving atypically was disproved by extending the study to a wide range of oils; soyabean, safflower, sunflower and corn oils. In each case chloroform as an injection solvent produced significantly inferior resolution to that when acetone was employed (Fig. 3).

It is concluded that triglyceride analysis under reversed-phase conditions is particularly susceptible to the influence of the injection solvent. Chloroform, one of the most widely employed solvents, produces inferior resolution under all conditions and this is accentuated when large (10–20 μl) injection volumes are used. It is recommended that acetone be considered as a reliable alternative.

REFERENCES

1 Du Pont, *Liquid Chromatography Report, Get Better Performance from your HPLC System*, Wilmington, DE, 1978.
2 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley-Interscience, New York, 2nd ed., 1979, p. 290.
3 J-P. Goiffon, C. Reminiac and M. Olle, *Rev. Fr. Corps Gras*, 24 (1981) 169.
4 J. A. Bazard and M. A. Quedraogo, *J. Chromatogr.*, 196 (1980) 279.
5 A. H. El-Hamdy and E. G. Perkins, *J. Amer. Oil Chem. Soc.*, 59 (1981) 867.
6 B. Petersson, O. Podlaha and B. Toregard, *J. Amer. Oil Chem. Soc.*, 59 (1981) 1005.
7 M. W. Dong and J. L. Dicesare, *J. Amer. Oil Chem. Soc.*, 60 (1982) 788.
8 E. Schulte, *Lebensmittelchemie Gerichtl. Chem.*, 36 (1982) 88.
9 R. Macrae and J. Dick, *J. Chromatogr.*, 210 (1981) 138.