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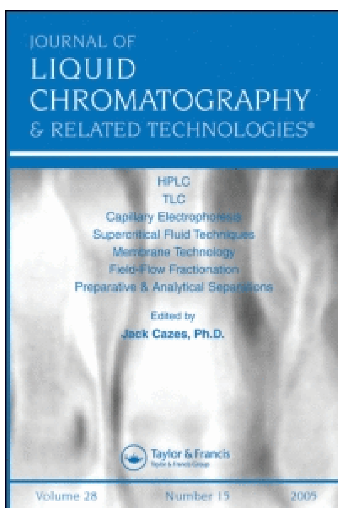

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THE USE OF HPLC TO SEPARATE TRIGLYCERIDES IN CONFECTIONERY FATS USING ULTRA VIOLET DETECTION

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ABSTRACT

The triglycerides of confectionery fats have been separated by reverse phase HPLC using mixtures of either acetonitrile and tetrahydrofuran or acetonitrile and methyl tertiary butyl ether using UV detection at 237 nanometres. The method has been applied to samples of cocoa butter, cocoa butter equivalents, milk fat and other vegetable fats. The fat from a milk chocolate has been separated by the above system.

INTRODUCTION

The reversed phase HPLC column has rapidly become the method of choice for most HPLC separations. This type of column offers stability and selectivity in a way

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that few alternatives can compete with. A number of authors have used reversed phase HPLC to separate fats (1, 2, 3, 4, 5). A particular problem of fat HPLC is that most fats are largely saturated and do not have strong absorbances in the ultra violet. Some authors have used systems using refractive index detection to surmount this problem. While refractive index detection can be satisfactory for a number of separations and can be made to work satisfactorily on isocratic separations the impossibility of using refractive index detection and gradient elution means that it is very much harder to develop methods for a separation where refractive index detection is used. Bearing this in mind, it is a considerable advantage to use ultra violet detection at least in the methods development stage. If ultra violet detection is to be used in fat HPLC it is necessary to find solvents in which the fat will dissolve, and, are still transparent to ultra violet light. Some authors have used acetone as a major component of their solvent. This is, of course, totally incompatible with the use of ultra violet detection. In this work it has been found that by using mixtures based on 60% acetonitrile and 40% of either tetrahydrofuran (THF) or methyl tertiary butyl ether (MTBE) it is possible to detect the triglycerides at 237 nanometres. This sort of wavelength makes less requirements of the solvent system and is less susceptible to interference from small quantities of aromatic impurities than shorter wavelengths. One problem in using THF is that although this material

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itself is reasonably transparent in the ultra violet the material is normally supplied with an anti-oxidant, such as butylated hydroxyanisole (BHA). This material is a strong UV absorber, and is normally incorporated into the THF to act as an anti-oxidant and to prevent the formation of peroxides. THF to be used with ultra violet detection should be of a grade devoid of such additives. Unfortunately in this form THF is unstable and readily forms peroxides, this has a number of consequences. The THF must be kept under an inert gas, such as nitrogen or helium, and the formation of peroxides reduces its transparency to ultra violet light, also it is extremely hazardous to recover material from unstabilised THF because of the risk of explosions from the peroxides. Another problem in the use of THF is its great solvent power for plastic materials. The HPLC used must not contain any incompletely fluorinated material which will dissolve in THF. Some HPLC pumps have valves which contain such material and are unsuitable for use with THF. The Spectra Physics HPLC used in this work is particularly suitable for use with THF, since the pump only contains stainless steel, ruby and PTFE and the helium degas system in this instrument provides an extremely convenient method of keeping oxygen out of the THF. The methyl tertiary butyl ether was investigated because it was hoped to have the beneficial solvent properties of THF with none of the concommitent disadvantages.

The column used in this work should be a highly hydrophobic material (6). The performance in this type

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of work where the solvent used is entirely non aqueous is entirely different to that obtained in the usual water methanol or water acetonitrile mixtures. The non aqueous solvents used in this type of chromatography are, of course, considerably less polar than the water based mixtures mentioned previously. For this reason a C18 type material, preferably with a heavy loading of organic groups, is much to be preferred. Otherwise there is actually a risk that the column could be less polar than the solvent mixture being used.

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As this type of column separates essentially by a partition rather than absorbtion mechanism, the separation of assymetric from symetric triglycerides is not to be expected. But, the degree of resolution available should enable separations to be performed of triglycerides on a basis of equivalent carbon number and even within each equivalent carbon number (7).

METHODS

All the samples under study were dissolved in the less polar solvent, that is either THF or MTBE. The resulting solution was then filtered using a Gelman sciences 0.45 micron CR type PTFE filter. The filtered solution was then injected in to the chromatograph. The chocolate samples were prepared by dissolving the chocolate either in THF or MTBE and the resulting solution was again filtered as previously.

MATERIALS

The THF and MTBE were obtained either from Fisons or from Rathburn Chemicals Ltd. The pure triglyceride standards were obtained from Applied Science laboratories. The filters were obtained from Gelman Sciences Ltd. The column was a 25 cm x 4.6 cm column supplied by Chrompack, packed with CP C18 ten micron.

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The chromatograph used was a Spectra-Physics SP8000 used with an SP8400 UV/visible detector. The SP8000 was equipped for gradient elution and with a helium degas system. It was also equipped to use the SP8400 to scan peaks if necessary. Also the SP8000 data system was inserted.

RESULTS

Figure 1 shows a sample of cocoa butter run using a mixture of acetonitrile and THF. The three major triglyceride peaks can clearly be seen cf (1). Figure 2 shows a chromatogram of a standard sample of glycerol 1,3 dipalmitoyl-2-oleate (POP). The peak near 15 minutes is likely to be POP. Interestingly, the standard material apparently contains the same impurity as the equivalent peak in the cocoa butter possibly having been prepared from that source. In (1) a similar

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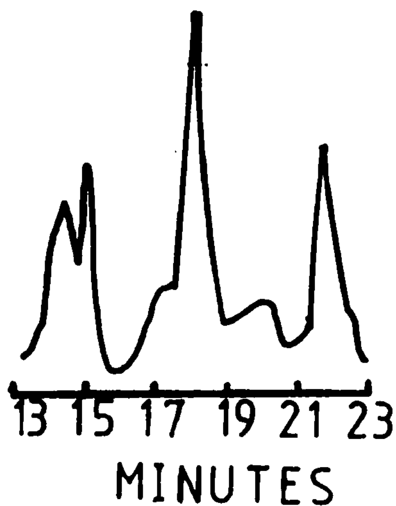

FIGURE 1
Example of cocoa butter run using 60% acetonitrile, 40% THF at 1 ml/minute. Column Chrompack C18 25 cm x 4.6 mm wavelength 237 nm.

FIGURE 2
Glycerol-2-oleat-1,3 dipalmitate, conditions as figure 1.

peak was identified as a glycerol palmitoyl dioleate. Figure 3 shows a chromatogram of glycerol-2-oleat-1,3-distearate from which it can be deduced that this material is the third major peak in cocoa butter. These results are very similar to those in (1) which were obtained on a different column with an acetone/acetonitrile mobile phase and refractive index detection. Figures 4, 5, 6 and 7 show chromatograms of various fats sold as cocoa butter equivalents (CBEs). These fats have physical properties very similar to cocoa butter but differ in the ratios of the different triglycerides as can be seen. Figure 8 shows another cocoa butter and gives some idea of the sort of variability inherent in cocoa butter cf (4). Figure 9 shows the same cocoa butter as in 8 run in a mixture of acetonitrile 60%, MTBE 40%. If this chromatogram is compared with figure (1) it can be seen that very similar results can be obtained by substituting MTBE for THF. Figure 10 shows a toffee fat run in the same solvent as Figure 9, this gives quite a different chromatogram as it has a totally different pattern of triglycerides. Figure 11 shows the pattern of butter fat which is radically different. Figure 12 is the fat recovered from a milk chocolate run in the THF/acetonitrile solvent. This clearly shows the cocoa butter triglycerides and the material from the butter fat present. Some of the early peaks are likely to be intensely ultraviolet absorbing material from the chocolate.

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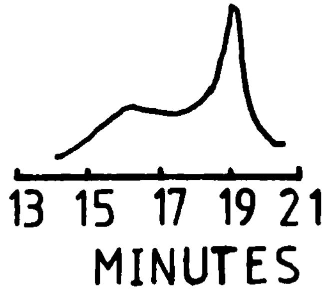

FIGURE 3
Glycerol-2-oleat-1,3-distearate conditions as figure 1.

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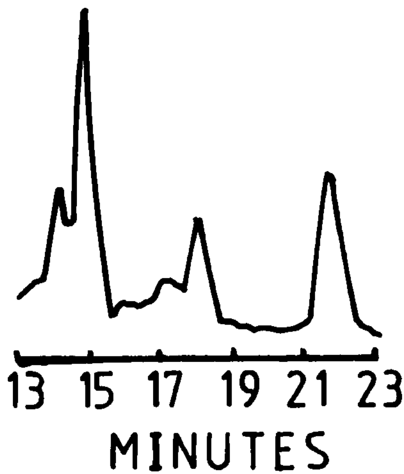

FIGURE 4
A Cocoa Butter Equivalent (CBE), conditions as figure 1.

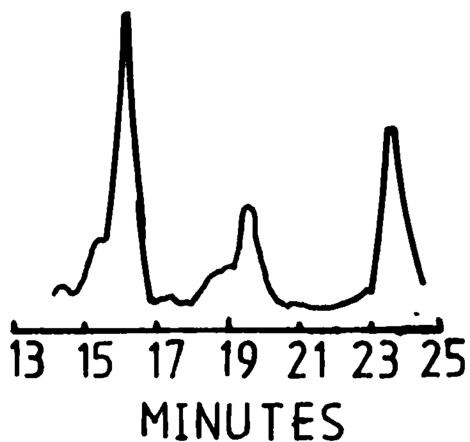

FIGURE 5
A Cocoa Butter Equivalent (CBE)

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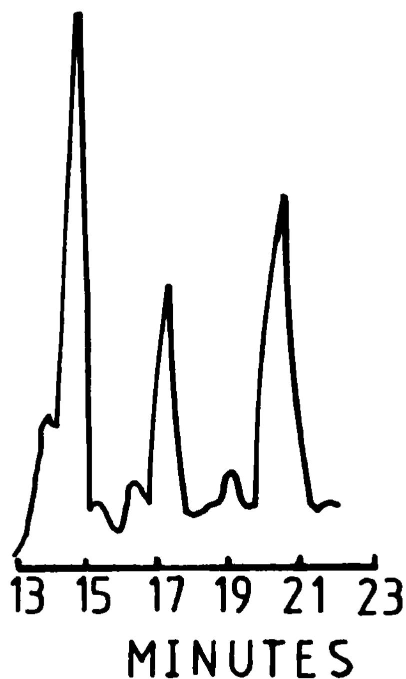

FIGURE 6
Another CBE, conditions as figure 1.

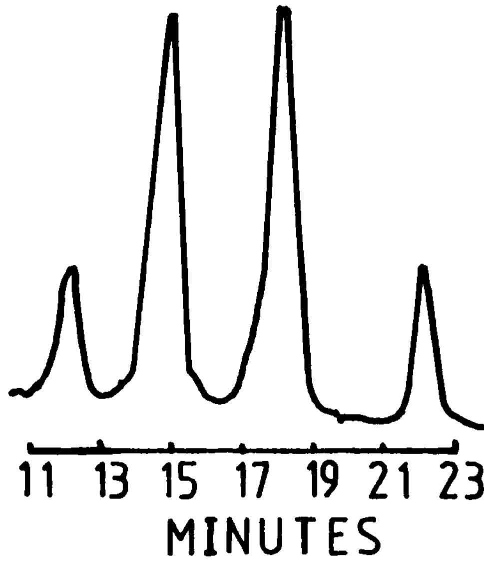

FIGURE 7
A CBE, conditions as figure 1.

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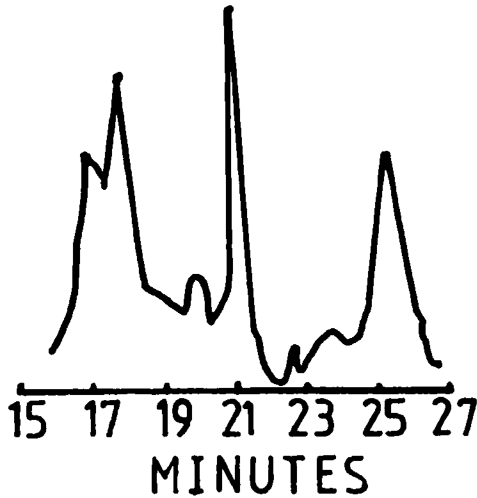

FIGURE 8
Another cocoa butter, conditions as figure 1.

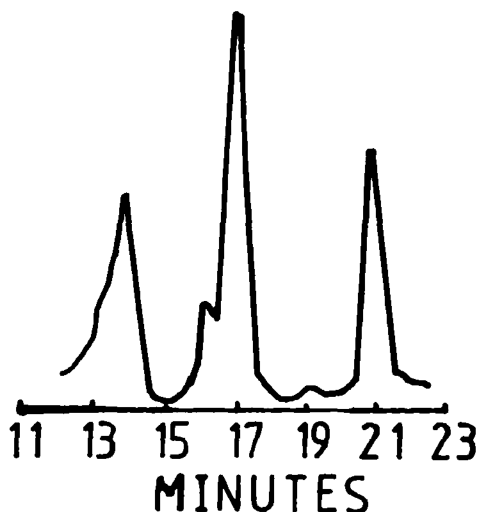

FIGURE 9
The same cocoa butter as in figure 8 run in 60% acetonitrile, 40% MTBE, other conditions as figure 1.

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DISCUSSION

Reversed phase HPLC can clearly separate triglycerides of the type used in confectionery fats relatively easily. This offers a considerable ability to the confectionery chemist to discriminate between different confectionery fats and even between different samples of the same confectionery fat. An important advantage of HPLC as a technique is that it is possible to separate material preparatively. Unfortunately this is particularly difficult to achieve when working with unstabilised THF. It was with this problem in mind that

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FIGURE 10
A toffee fat run as in figure 9.

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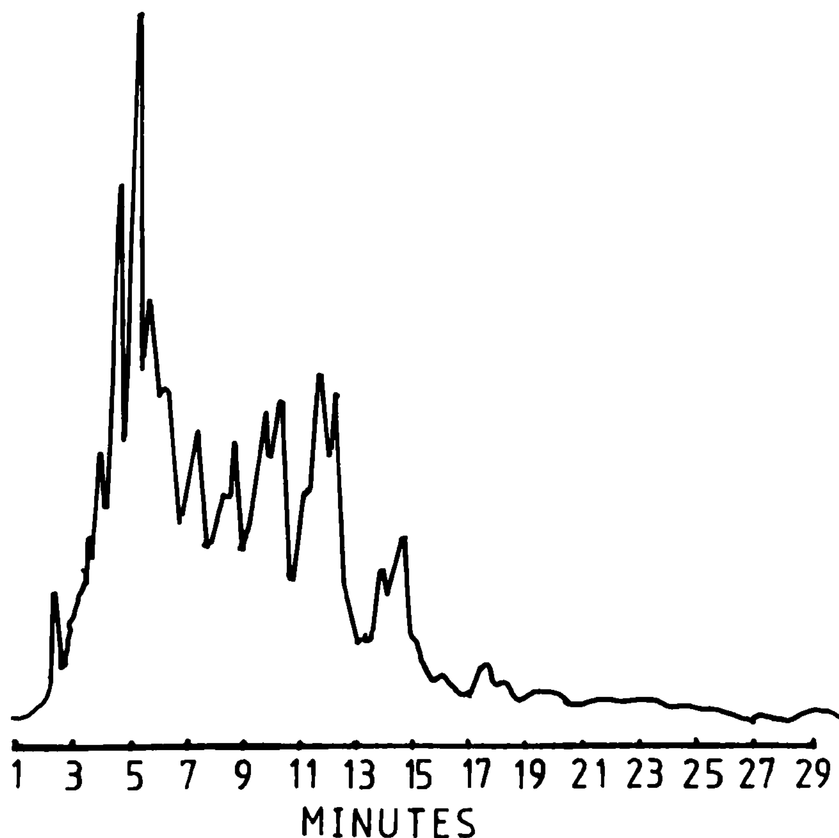

FIGURE 11
Butterfat run as in figure 10.

the use of MTBE was considered as a substitute for THF. MTBE has considerable working advantages in this work as it is stable, and does not require the use of an inert gas blanket. Nor does its UV absorbtion increase on storage, also it may readily and safely be recovered from preparative samples. It was found in this work that MTBE was a suitable replacement for THF.

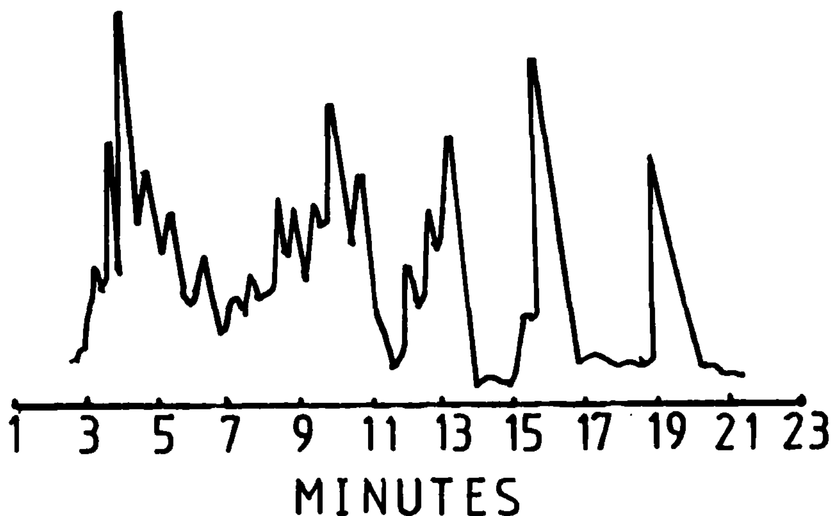

FIGURE 12
Fat from a milk chocolate run as figure 1.

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CONCLUSIONS

The separating power of reversed phase HPLC using non-aqueous solvents and UV detection has successfully been applied to separating triglycerides in confectionery fats. Very minimal sample preparation is required. MTBE is a safe and satisfactory alternative to THF for this work.

Note

In this work stearic acid is the C18:0 fatty acid, palmitic acid is the C16:0 fatty acid and oleic acid is

the C18:1-cis 9 fatty acid. In referring to triglycerides stearic is abbreviated to S. Palmitic to P and oleic to O.

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