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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF PARTIAL GLYCERIDES AND OTHER TECHNICAL LIPID MIXTURES*

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SUMMARY

A high-performance liquid chromatographic (HPLC) technique for the analysis of partial glyceride mixtures is described and some technical details are discussed. Gradient elution HPLC with a "moving-wire" detector linked to a computer is used for quantitative analysis.

The method described has been applied to the analysis of mono-, di- and triglyceride mixtures, food emulsifiers, certain seed fats, and technical products such as polymer additives.

INTRODUCTION

Partial glyceride analysis is a problem in many areas of food chemistry, as well as in natural products, technical oleochemicals and emulsifier and polymer additive work. A number of thin-layer, enzymatic and gas chromatographic analytical methods are available but none is very satisfactory for quantitative analysis. We have developed high-performance liquid chromatographic (HPLC) methods for such analysis.

The development of HPLC for lipid analysis has been slow. This is caused in part by detector problems, by problems associated with various gradient elution requirements for the separation of mixtures of lipid classes covering a wide range of polarities, and by the fact that often an entire lipid class, consisting of a variety of different molecules, is eluted as one peak and needs to be quantitated as such[1].

Many workers during the past 10 to 15 years have devoted their time and imagination to the question of how to detect non-UV-absorbing lipids under conditions of gradient elution, and to the question of how to quantitate a "mixture peak" which consists of an entire lipid class. The ideal solution has not yet been found.

At the German research laboratory of Unilever, we have been using the so-called "moving-wire detector" which was originally developed as a monitor for the

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preparative column chromatography of lipids. In its methane conversion form[2] this detector is also useful as a quantitative detector for HPLC[3–5]. We are now using this detector for mono–diglyceride analyses.

EXPERIMENTAL

Fig. 1 is a schematic drawing of the equipment we have assembled for lipid class analyses by gradient elution HPLC[5]. It is similar to the equipment previously described[3–5] and consists of three solvent reservoirs, a solvent-gradient programmer with two solenoid valves (LKB Ultrograd), a high-frequency reciprocating pump (Model 196-100, Milton Roy) with pump stroke damping device, pre-column and pressure monitor, a sample injection port (Precision Sampling), the chromatographic column, a transport-flame-ionization detector of the so-called "moving-wire" type (Model LCM-2 Pye Unicam), a recorder (Model S, Servogor) and a connection to a calculating computer (Model 3352, Hewlett-Packard), via an analogue-to-digital converter.

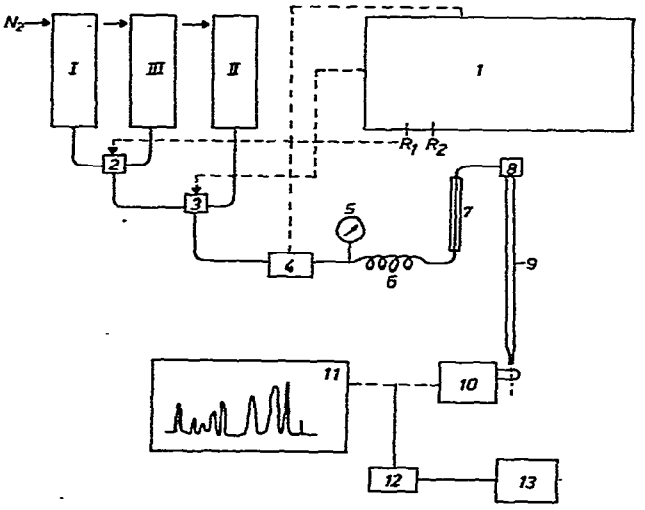

Fig. 1. Equipment for lipid class analyses by HPLC. I, II, III, Solvent reservoirs under slight N_2 pressure (*ca.* 0.5 atm). 1, Solvent gradient programmer (LKB Ultrograd). 2, 3, Solenoid valves controlled by gradient programmer. 4, Milton Roy 196-100 pump. 5, Pressure gauge. 6, Pulse damping coil. 7, Pre-column. 8, Precision Sampling injection head. 9, Separation column. 10, LCM-2 moving-wire detector. 11, Recorder. 12, ADC. 13, HP 3352 computer.

Three mixed solvents (I, II, and III) of increasing polarity are used to produce a sequence of two solvent gradients (from I to II and then from II to III), using the Ultrograd gradient programmer.

The sequence of solvents used in this investigation consisted of a mixture of carbon tetrachloride and iso-octane (34:66) in solvent reservoir I, chloroform, dioxane, and hexane (40:11:49) in reservoir II and chloroform, methanol, and diisopropyl ether (34:36:30) in reservoir III. Stainless steel columns were slurry-packed

with microparticulate silica gels such as LiChrosorb SI-60, 5 or 10 μm (Merck, Darmstadt, G.F.R.). Chloroform-containing solvents gave better resolution between free sterols and 1,2-diglycerides, owing to secondary solvent effects, and the microparticulate columns permitted electronic integration of the peak areas.

Fig. 2 shows one of the Ultrograd programme charts which we have developed for lipid class analysis. These charts of black paper are scanned by a photocell in the Ultrograd master unit, which switches the solenoid valves accordingly[6]. The charts are cut in such a way that column-rinsing periods are carried out automatically, usually first with solvent II and then with solvent I, before a new injection is made. The right-hand last portion of the chart represents the first part of the first gradient which is already stored in the pump, pulse damper and connecting tubing before the injection is made.

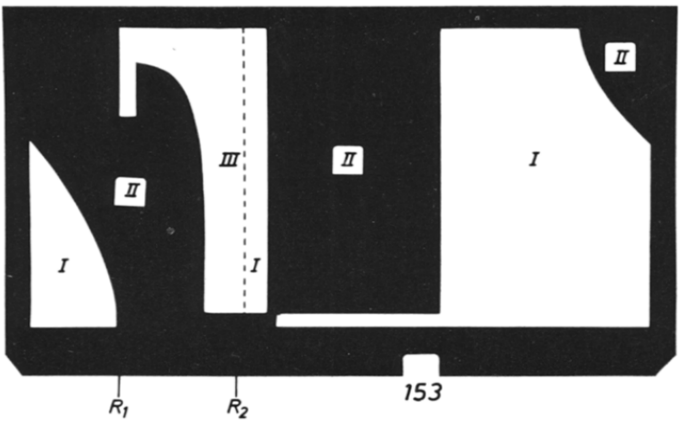

Fig. 2. Solvent programme chart for the Ultrograd. Programme no. 153, sequence of two solvent gradients plus two column-rinsing periods.

We have attempted to keep analysis times as short as 30 min, including both the sequence of two solvent gradients and the column-rinsing or regeneration periods. This short regeneration period for a silica gel column is in marked contrast to other observations[7]. Injections for quantitative analyses should, therefore, be made only during continuous operation of the column. Whenever the column has been standing idle for some time, it is re-started with a blank run.

The eluted lipid class peaks are detected and quantitated by the LCM-2 moving-wire detector, which is linked to a computer. Response factors are obtained from test chromatograms, and internal standards may be used where applicable. In a few cases area-percent calculations were done.

The LCM-2 detector response depends on a number of factors. The most important is solvent flow-rate, which may depend on column back-pressure if a reciprocating piston pump is used, and which may change during a gradient run. The calibration factors found for the individual partial glycerides will, therefore, depend not only on the carbon content of the lipid, but also on its position during gradient elution. Frequent recalibration of the whole set-up is a prerequisite for quantitative

work. This is done by injecting a quantitative test mixture, followed by an RC ("recalibrate") order to the computer. With this arrangement, the relative standard deviation (r.s.d.) of retention times is generally *ca.* 1–3% and that of peak areas *ca.* 3–7%[5].

RESULTS AND DISCUSSION

Fig. 3 shows typical lipid class chromatograms as obtained from partial glyceride test mixtures. Methyl oleate appears first, followed by triolein, 1,3-diglyceride, 1,2-diglyceride, and finally monoglyceride. The isomeric monoglycerides are eluted as one peak. Impregnation of the silica gel columns with H_3BO_3 has not yet been tried. It can be seen that the 1,2-isomer of the diglyceride, which is present in the saturated test mixture (18-0-A), is missing in the unsaturated mixture (18-1-A). Similarly, the concentration of the 1,2-dipalmitin is very low in a technical dipalmitin obtained from Fluka (Buchs, Switzerland) (Fig. 4) whereas the ratio of the 1,2- to the 1,3-isomers is usually between 1:3 and 1:5 in most technical partial glycerides and emulsifiers (Fig. 5).

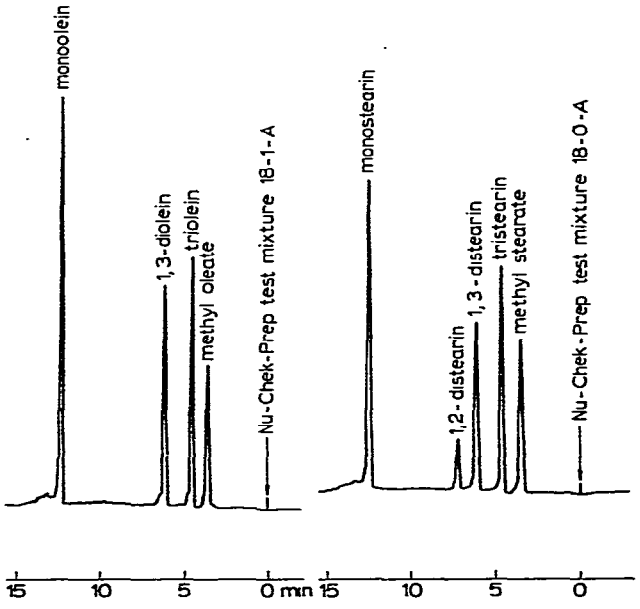

Fig. 3. HPLC chromatograms of partial glyceride test mixtures.

Fig. 5 shows HPLC chromatograms of three different partial glyceride food emulsifiers. Emulsifier No. 1 is a commercial "monodiglyceride" with a monoglyceride content of *ca.* 47%, and emulsifier No. 3 is a distilled monoglyceride with a monoglyceride content of 94.5%, also available commercially. Emulsifier No. 2 can be purchased as a mixture of a distilled monoglyceride with 20% vegetable oil.

Fig. 6 shows chromatograms of some specialty seed fats which are known to contain diglycerides, besides larger amounts of unsaponifiable. The considerable

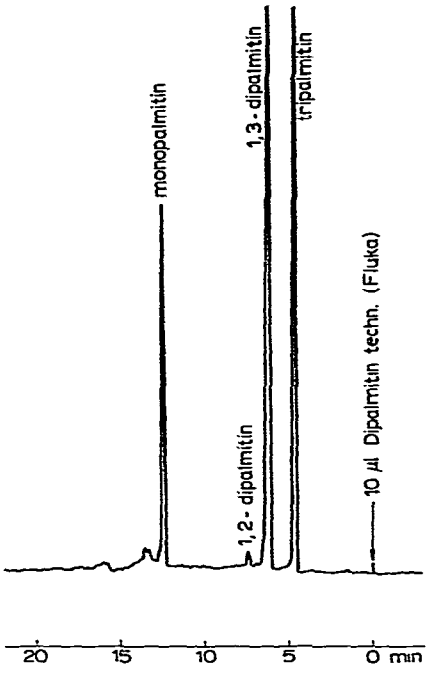

Fig. 4. Chromatogram of technical dipalmitin.

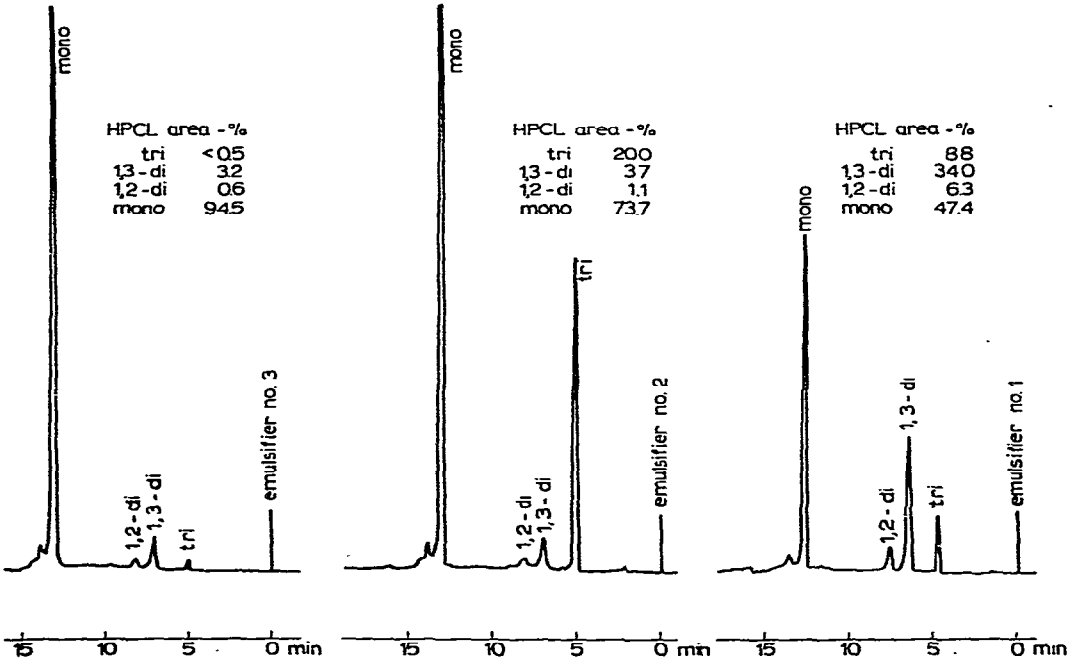

Fig. 5. Chromatograms of three different commercially available partial glyceride food emulsifiers.

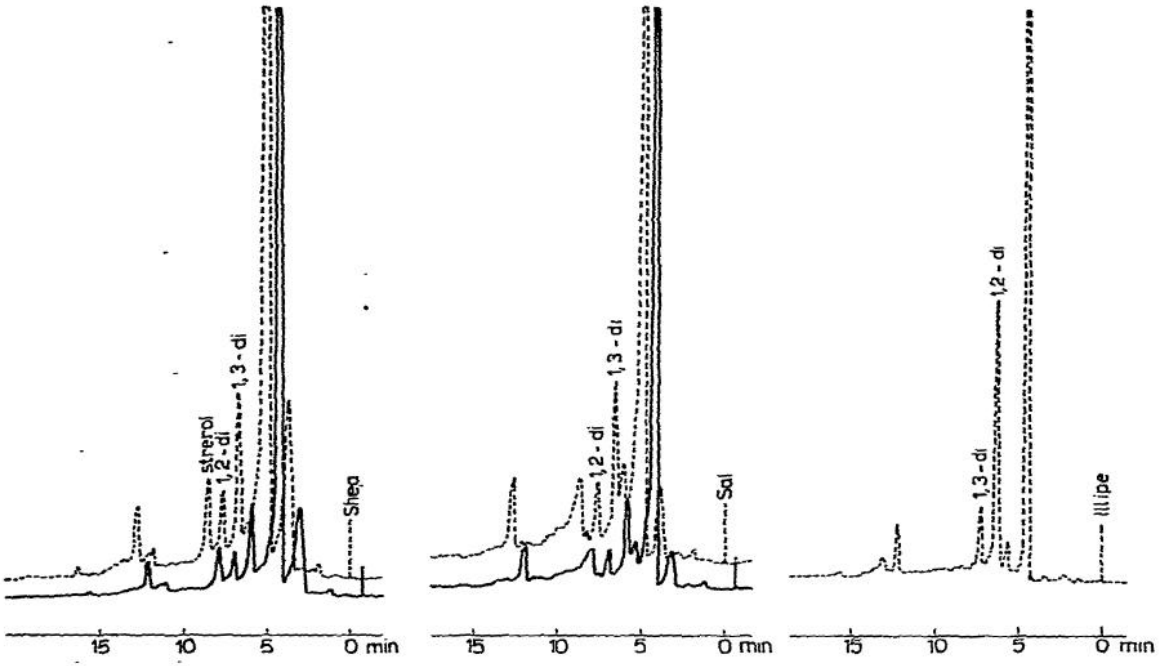

Fig. 6. Chromatograms indicating the presence of diglycerides in samples of Shea, Sal and Illipe fats. Upper and lower traces differ by an attenuation factor of 2.

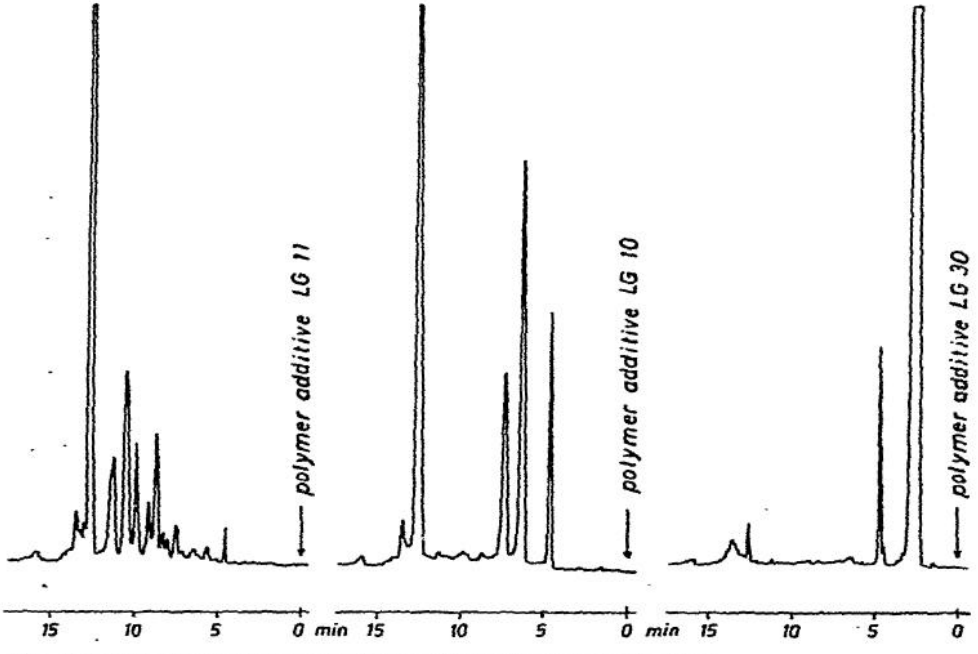

Fig. 7. HPLC chromatograms of three different polymer additives.

overlap between the diglyceride peaks and portions of the unsaponifiable indicates that the method is unsatisfactory for the analysis of such fats.

Fig. 7 shows the results of applying this technique to the characterization of oleochemical polymer additives. Additive LG 30 is a long-chain wax ester, and LG 10 is a partial glyceride mixture. The composition of additive LG 11 is unknown, but the analysis showed that it is neither a wax ester nor a partial glyceride. It may be an epoxidized or ethoxylated oil or a castor oil derivative.

The usefulness of this technique is further demonstrated by Fig. 8, which is the chromatogram of a test mixture consisting of a hydrocarbon, a wax ester, fatty acid methyl ester, triglyceride, partial glycerides, monoglyceride diacetate and cholesterol. The technique has recently been employed for analyses of sebum, serum lipids and other lipid classes of medical interest[5].

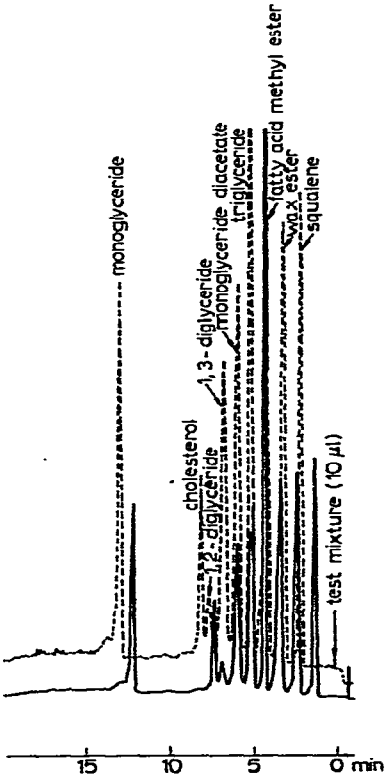

Fig. 8. Chromatogram of a test mixture composed of lipid classes similar to those found in human surface fat, or sebum[5]. Upper and lower traces differ by an attenuation factor of 2

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