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INVESTIGATION OF HEATED AND OXIDIZED OILS AND FATS BY GRADIENT ELUTION LIQUID CHROMATOGRAPHY

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SUMMARY

The major classes of artefacts in frying oils can be analyzed by liquid chromatography, where oils with different pre-treatments show characteristic fingerprint chromatograms. Gradient elution programmes are also useful for the analysis of technical polar glycerides and seed oils containing oxygenated fatty acids.

Using a UV and a moving-wire detector in series, it can be shown that in heated and oxidized oils, UV-absorbing substances are found primarily among the most polar fractions.

The moving-wire detector also permits the routine "total artefacts" analysis of heated and oxidized oils. The steady increase of the total artefacts peak with heating time is demonstrated.

INTRODUCTION

The question of the exact time when a used frying oil must be discarded is still under discussion[1–3] and both the food industry and government agencies require analytical methods for the evaluation of heated and oxidized oils. In our laboratory, investigations have been carried out using paper[4], thin-layer[5,6], gel permeation[7,8] and liquid chromatographic (LC)[9–11] techniques with the aim of obtaining specific information on edible oils and fats that have been heated, oxidized or used for deep-frying.

When fats are heated in the presence of air and foods, as during the deep-frying process, three major classes of artefacts are formed[3,11]: oxidation products, such as oxidized triglycerides; polymerization products, *i.e.*, dimeric and higher polymeric triglycerides (which in turn oxidize easily); and products of hydrolysis, *e.g.*, diglycerides and free fatty acids. Most of these compounds are more polar than the parent triglycerides so that they can be separated from them by adsorption or partition LC .

In used frying fats and autoxidized fats, compounds with conjugated diene groups are present, which can be detected by measuring their UV absorption. However, many of the separated zones in lipids and fatty chemicals do not absorb sufficiently in the UV region so that more general methods of detection are required. On the other hand, gradient elution techniques are always necessary because used

frying fats contain components of widely differing polarities, as is also the case with many biological lipid fractions[12].

When one has to use a variety of solvents and solvent mixtures, refractive index (RI) detectors are impractical. RI detectors are widely used for separation methods in which only one solvent is used, as, for instance, in gel permeation chromatography[7,8].

Both UV and RI detectors are useful for quantitative work, but only for samples of which the composition is known. One has to know the UV absorbance or the refractive index of all of the components in order to be able to calculate the weight-percentage composition of the mixture from the chromatographic peaks obtained. When one peak is a mixture of two or more compounds of widely differing UV absorbances or refractive indices and when the percentage composition of this peak (*i.e.*, the ratio of the compounds in the peak) changes from sample to sample, then no quantitative information can be obtained from either the UV or the RI detector.

Detectors involving the principle of solute detection after complete removal of the solvent are more generally applicable. However, their reproducibility of detection is not as good as that of UV or RI detectors, and for many applications they may not be sensitive enough. The main advantages of the so-called solute transport or moving-wire LC detector are a good sensitivity for lipids and other aliphatic and alicyclic compounds that are often not active in the UV region, and the fact that there is no great difference in the response of this detector to the substances involved in the present work, so that mixture peaks can be quantified. Moreover, this detector is not influenced by gas bubbles or by changes (even large changes) in the solvent composition.

MATERIALS AND EQUIPMENT

The equipment used has been described in principle elsewhere[9,13].

The solvents used were of analytical-reagent grade (E. Merck, G.F.R.) and were used as received. Gradients were formed by means of an Ultrograd master unit with two magnetic valves[13] from mixtures of heptane (H), diisopropyl ether (D) or diethyl ether (Et_2O) and ethanol (E) with or without a small percentage of water (W).

The Ultrograd programmes were cut out of black paper to yield either rather step-wise solvent changes (Fig. 1) or similar continuous gradients (Fig. 2, top right).

The columns were made of regular glass tubing, *ca.* 40–50 cm long and 4 mm I.D. Columns for frontal elution "total artefacts" chromatograms were shorter (20–25 cm). The columns were filled dry under suction, either with Merckogel as described earlier[13] or with Porasil A (Waters Ass., Mass., U.S.A.; formerly indicated as Porasil 60). Columns were filled in sections, *ca.* 5 mm high at a time, while tapping the column wall in three or four directions.

When a simultaneous recording of the UV absorption was required, a Uvicord II UV monitor (LKB Instruments, Sweden) equipped with a 50-μl cell was built in series with the moving-wire detector (Fig. 2). In this case, a small separate flow resistance was installed at a point between the UV and moving-wire detectors so that gas bubbles would form only at a point beyond the UV cell. Alternatively, we also used pumps with de-gassed solvents. The chromatograms were recorded with a Philips PM 8010 two-pen recorder.

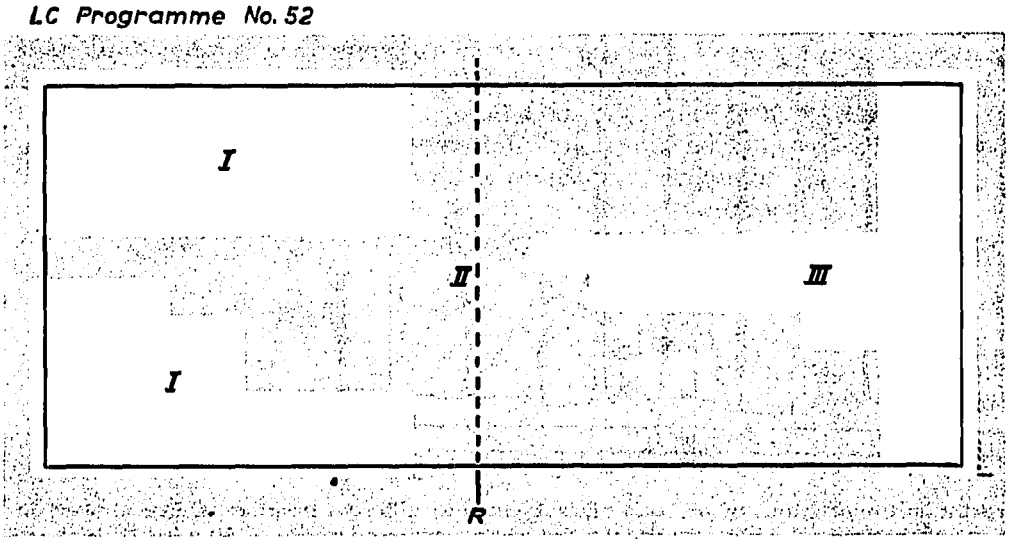

Fig. 1. A step-wise gradient programme for the Ultrograd main unit. The programme is cut out of black paper. Valve 2 (Fig. 2) is switched whenever the light point sensor, in its *y*-movement, crosses the black–white borderline. Rider R switches valve 1. *Cf.*, other programmes shown in refs. 10, 11 and 13. I = H; II = H (saturated with water):D = 50:50; III = H:D:E = 40:50:10.

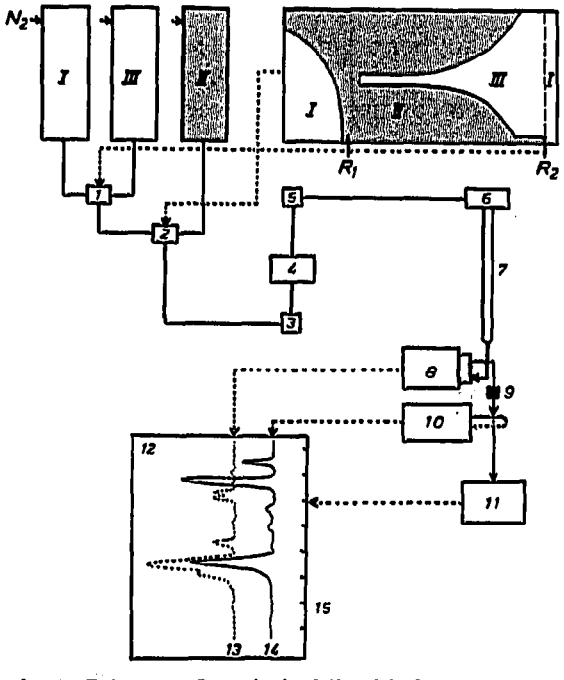

Fig. 2. Scheme of analytical liquid chromatograph with solvent programming for three solvents and UV and moving-wire detectors in series. I, II, III = pressurized solvent reservoirs and corresponding Ultrograd programme sections. 1 = Valve switched by riders R_1, R_2; 2 = valve switched by black–white programme sections; 3 = mixing device (not used with low flow-rates); 4 = pump (Milton Roy 196-100 or Waters Model 6000); 5 = pulsation damping device; 6 = injection head; 7 = column; 8 = UV detector with quartz cell; 9 = separate flow resistance, producing bubbles of N_2 gas; 10 = moving-wire detector; 11 = fraction collector with 5-ml siphon, producing "counts"; 12 = two-pen recorder; 13 = UV trace; 14 = "Wire" trace; 15 = siphon counts.

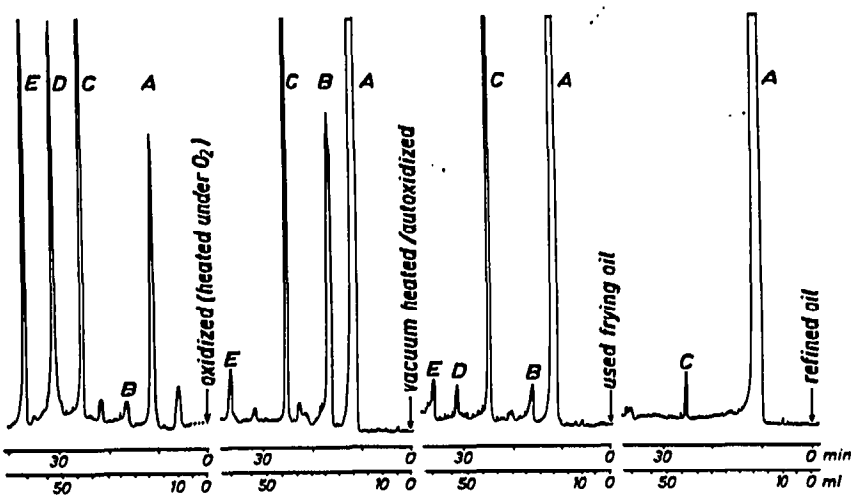

Fig. 3. Fingerprint chromatograms of soybean oils subjected to different treatments. For a more detailed description of the peaks A to E, see the text. Solvent programme of Fig. 1, 30 min; N_2 pressure 1.1 atm. No pump was used. Column: Porasil A of 75–125-μm grain size, 50 cm long, cooled to 5–10° below room temperature. Moving-wire detector, attenuation 2 × 10. Column rinsing: 4 min, D:E:W = 50:49:1; 4 min, solvent II; 12 min, solvent I (*cf.*, Fig. 1).

RESULTS AND DISCUSSION

Fig. 3 shows gradient chromatograms of soybean oil subjected to different treatments: fresh or refined oil; an oil that had been used for a fish-frying experiment; an oil that had been heated under vacuum and then further autoxidized upon standing; and an oil that had been extensively oxidized. The four oils were analyzed using the same column and solvent programme (Fig. 1) with equipment similar to that shown in Fig. 2. The separated peaks were detected with a moving-wire detector only; no UV detector and no pump were used, but instead, the pressure on the solvent reservoirs was increased.

In Fig. 3, peak A represents non-polar triglycerides and is the main peak in the refined oil. When such an oil is oxidized, peak C will increase. When the oil is heated for a longer period of time in the absence of oxygen, peak B will become larger. Peak B in the third oil is caused mainly by non-polar dimeric triglycerides. In an extensively oxidized oil, as in the one to the left, peaks D and E will also become large.

In an older used frying oil, as in the second chromatogram from the right, most of the peroxides have already been decomposed, yielding monohydroxy compounds, which are accumulated in peak C. If, for some reason, large amounts of peroxides and epoxides are present, this result may be altered. Both peroxides and epoxides would appear shortly after B, or in the area between peaks B and C, under the conditions of the present programme.

In model experiments[11], we have synthesized a dimeric triolein and a triolein, in which one of the oleic acid residues is epoxidized. The retention of the dimeric triolein is identical with that of peak B, while the monoepoxytriolein appears shortly thereafter, almost coinciding with B.

We have also trapped the major peaks A to E by using the peak-trapping equip-

ment described earlier[13]. The molecular-weight distribution of the trapped peaks was then analyzed by gel permeation chromatography. Peak A showed a molecular weight of *ca.* 900, corresponding to a triglyceride, and B one of 1800, *i.e.*, a dimeric triglyceride. C had three maxima around 600, 900, and 1800, the main maximum being near 600. D and E showed essentially the same wide distribution between 600 and 6000, with smaller peaks around a molecular weight of 300.

Resolution in the polar artefacts area around peak C was not optimized in Fig. 3. Similar fingerprint chromatograms of used frying oils and other oils using hand-

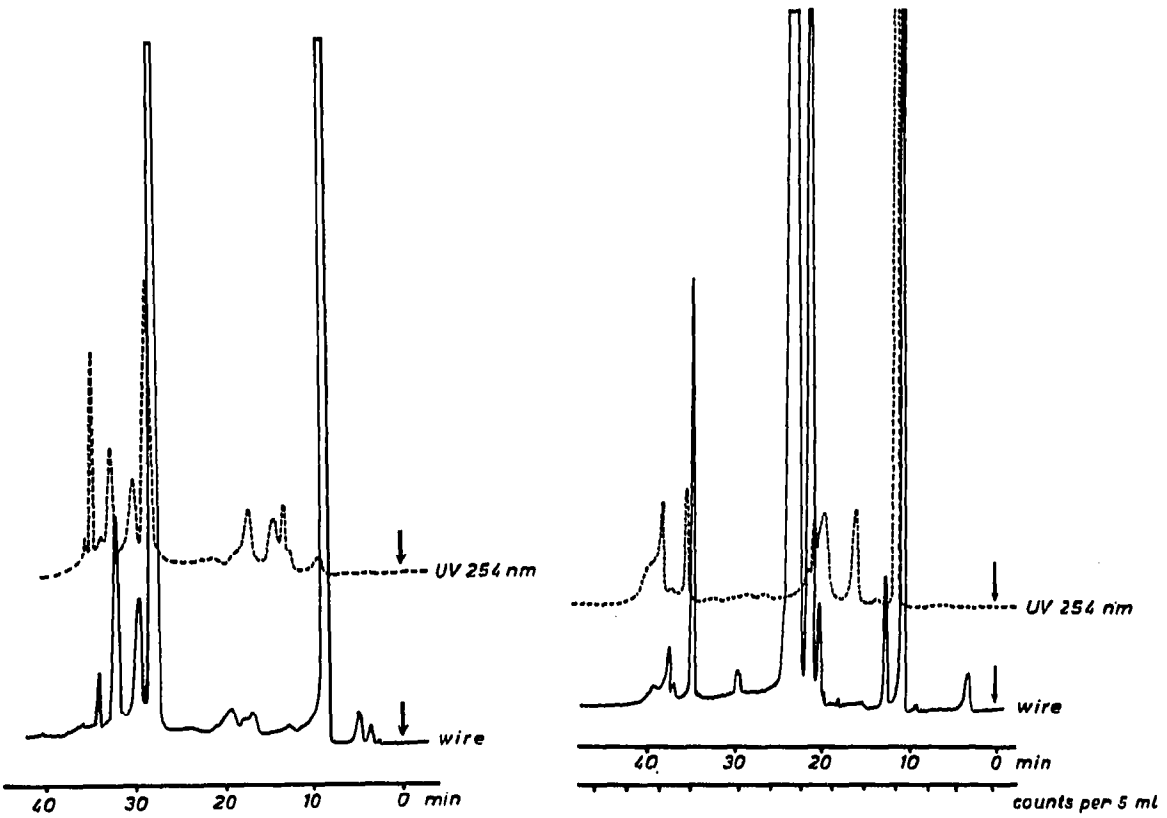

Fig. 4. Simultaneous recording of UV absorption in oxidized soybean oil. Column: Merckosorb SI 60, 10 μm, 35 cm. Hand-programmed step-wise elution using Waters Model 6000 pump set at 1.5 ml/min. Solvents (H:Et_2O:E): 2 × 3 ml each of 95:5:0 85:15:0, 69:30:1, 68:30:2, 66:30:4, 62:30:8 and 50:25:25; column rinsing with 2 × 3 ml of 85:15:0 and 2 × 3 ml plus 4 × 5 ml of 100:0:0. Uvicord II with 50-μl cell and moving-wire detector.

Fig. 5. Chloroform extract of *Vernonia anthelmintica* seed. Programme of Fig. 1, 30 min but with solvents I = H; II = H:Et_2O = 70:30; III = H:Et_2O:E:W = 62:29:9:0.3. Equipment of Fig. 2, with 0.5 atm N_2 and Milton Roy pump, *ca.* 1.5 ml/min. No separate mixing device. Column: Porasil A, 37–75-μm grain size, *ca.* 40 cm. Column rinsing: 16 min, solvent II; 24 min, solvent I. The gap in the 5-ml siphon counts is caused by the Ultrograd reset movement, during which the pump is switched off. After this, the pump is switched on again for rinsing. Polar solvent III is in the tubing and column at this time, so that peaks may still be recorded at a time when rinsing solvent is already being fed to the pump.

programmed elution and gradient elution with other solvents have already been described[9].

When a UV detector is used in series with the moving-wire detector, it can be shown that UV-absorbing substances are found primarily among the most polar fractions, such as oxidized triglycerides.

It is well known[3,14] that the oxidation of the higher unsaturated fatty acids such as linoleic or linolenic acids leads to compounds that contain a conjugated diene group with UV absorption maxima near 232 nm. The increase in UV absorption at 232 nm is often used in the food industry as an indication of oil deterioration, *e.g.*, when checking raw materials. In both autoxidized oils and used frying oils, the main products that give rise to this UV absorption are triglycerides containing 9- and 13-hydroperoxy- and hydroxyoctadecadienoic acids[14–16], *i.e.*, they are more polar than the original triglycerides containing unsaturated fatty acids. When the hydroperoxides are decomposed, secondary oxidation products (*e.g.*, the hydroxy compounds) may accumulate.

Fig. 4 shows another example of a soybean oil that had been heated under oxygen, after which the UV absorption of the separated peaks was recorded. The very polar peaks are all active in the UV region, whereas the peaks of low polarity, including the unchanged triglyceride, are not. In this case, a step-wise solvent gradient was pumped through a Merckosorb column using a Waters Model 6000 two-piston pump. The Uvicord, like many other fixed-wavelength UV detectors, uses the Hg emission line at 254 nm. It would be advisable to build an equally simple UV monitor

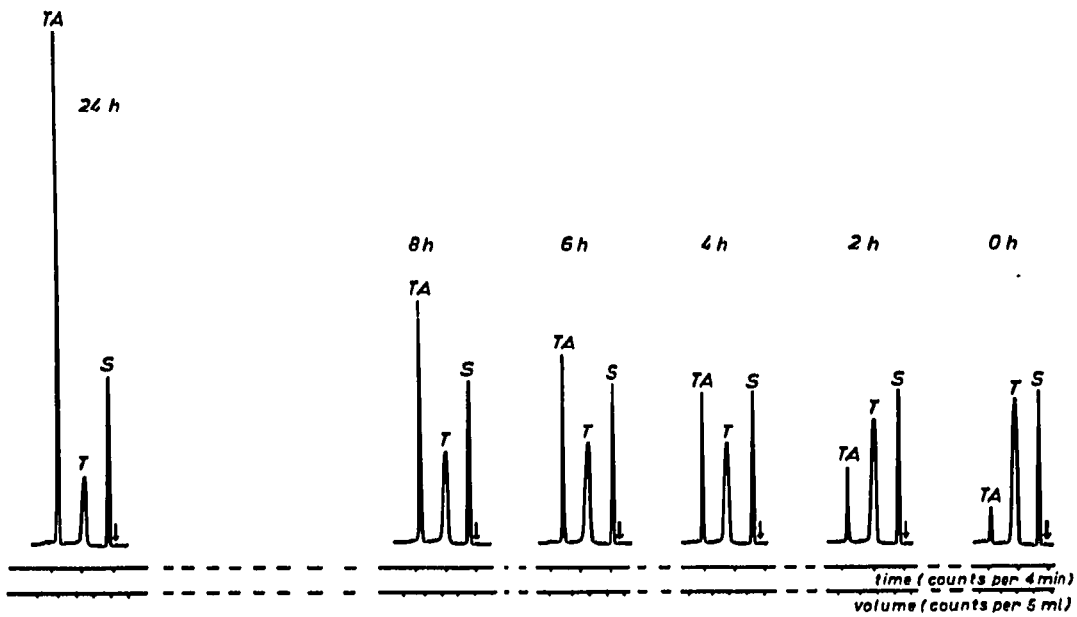

Fig. 6. "Total artefacts" chromatograms[11] for a series of sunflower oils heated under air in the laboratory under unfavourable conditions. Samples were taken after heating for 0, 2, 4, 6, 8 and 24 h. Owing to its non-Gaussian shape[10], the TA peak appears high and narrow while the central peak for unchanged triglycerides is much broader. S = squalane standard.

for 232 nm for the selective detection of the many different types of conjugated dienes[16] produced upon lipid autoxidation in foods.

Gradient elution chromatograms of technical epoxidized oils and of mixtures of mono- and diglycerides have already been described[9]. Another example of a fingerprint chromatogram is that of a seed oil containing oxygenated fatty acids. Fig. 5 shows the chromatogram of a chloroform extract of *Vernonia anthelmintica* seed containing mixed triglycerides of vernolic acid (12,13-epoxy-9-octadecenoic acid) mixed with other fatty acids.

The production of "polar materials" and "total artefacts" chromatograms by frontal elution and column conditioning has already been described[11]. Because the "total artefacts" peak combines dimeric triglycerides and oxidized triglycerides with diglycerides and free fatty acids, this peak becomes an extremely sensitive indicator for general oil degradation.

Fig. 6 shows this for a series of oils heated under air in the laboratory under unfavourable conditions. Both the decrease in the "unchanged triglycerides" peak T and the increase in the "total artefacts" peak TA can be clearly seen.

Based on our experience that the moving-wire detector has a similar response for all of the above-mentioned glyceride artefacts, we have attempted to quantitate the peaks and to express the results as area percentage values.

Fig. 7 shows the results for a series of model oils heated and oxidized in the laboratory.

Fig. 8a demonstrates that very little UV activity remains with the central triglyceride peak in "total artefacts" chromatograms. On the other hand, the main peak still contains some of the UV absorbing material, when only the very polar materials are separated. This UV absorption in the main peak is probably due to peroxides. Under the conditions of the second chromatogram (Fig. 8b), artefacts of low polarity are not separated from the main peak[11]. It can be shown that in samples

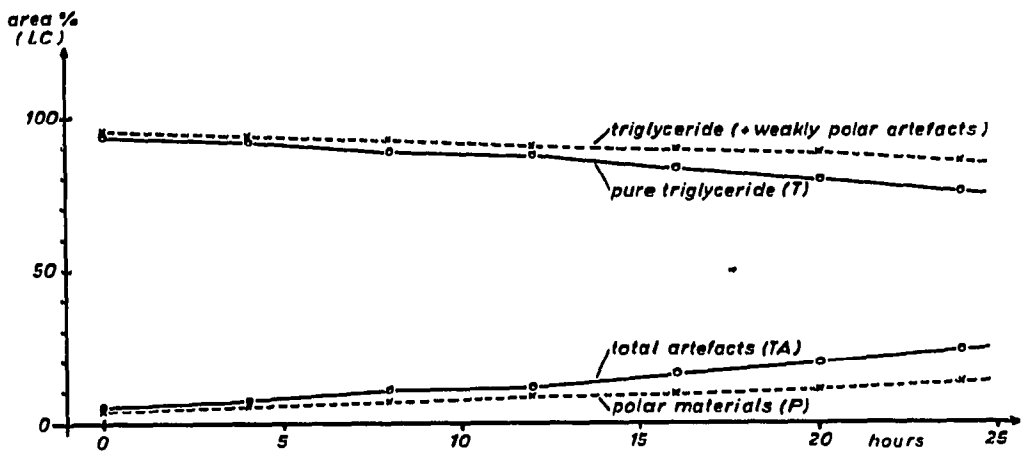

Fig. 7. Decrease of triglycerides and increase of artefacts in a partially hardened groundnut fat heated under air in the laboratory. Based on the assumption of equal detector response of the moving-wire detector, peak areas were determined and the results expressed as area percent (LC). Samples were taken after heating for 0, 4, 8, 12, 16, 20 and 24 h and analyzed both for "total artefacts" and "polar materials"[10,11].

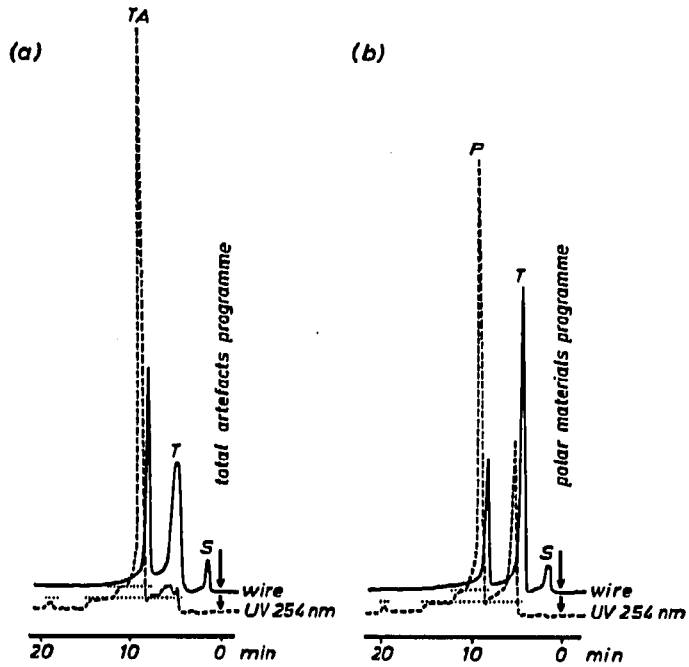

Fig. 8. Artefacts chromatograms[11] with UV recording. (a) In the "total artefacts" chromatogram most of the material absorbing at 254 nm is accumulated in the "total artefacts" peak (TA). Very little UV activity remains with the central peak (T). (b) When the column is programmed for "polar materials" (P), peak T also contains a noticeable amount of UV-absorbing materials. It is presumed that this is due to the presence of hydroperoxides[16] and similar weakly polar substances which are not separated from peak T under these conditions. The only difference between the two types of chromatograms (a) and (b) is in the preceding column equilibration[11] (not shown here). Solvents, column and programme as described in ref. 11 (*cf.* Figs. 1 and 4 of ref. 11). Horizontal dotted lines indicate UV background of solvents.

taken at constant time intervals from an oil that is being heated in the presence of air, the UV peak in the centre remains fairly constant, while the UV and moving-wire peaks for very polar materials increase. At the same time, the moving-wire peak in the centre decreases.

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